The 31st International Carbohydrate Symposium

July 14-19, 2024

Shanghai, China

Shanghai Institute of Oragnic Chemistry, Chinese Academy of Sciences

Book of Abstracts

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14-19 July, 2024

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17:45-18:25	ICO Young Researcher Award Lecture
18:30-21:00	Welcome Reception

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11:00-11:15	Billy Wai-Lung Ng	Guozhi Xiao	Zhongping Tan	Maria Panova
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11:30-11:45	Flaviana Di Lorenzo	Chengli Zong	Yun Ge	Aïcha Abdallah
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14:15-14:30	Yukari Fujimoto	Kaimeng Liu	Atit Silsirivanit	Akihiro Ishiwata
14:30-14:45	Roberto Mastio	Lina Cui	Qiang Zhu	Zhenghao Zhai
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17:00-17:15	Marta Artola	Jia Niu	Yuanyuan Ruan	Yiqun Geng
17:15-17:30	Junpei Abe	Stefan Jopp	Juan Wei	Roberta Marchetti
17:30-17:45	Alberto Fernandez-Tejada	Feng Liu	Peng Dai	Myles Poulin
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11:00-11:15	Xuechen Li	Polina Abronina	Fei He	Michal Hricovini
11:15-11:30	Chao Cai	Xiang-Guo Hu	Yanzhuang Wang	Wenfeng Liao
11:30-11:45	Tayyab Gulzar	LiyaYang	Chunxia Li	Emanuella Fiandra
11:45-12:00	Dahai Ding	Maksim Romaniuk	Pablo Valverde	SergioAdorna Sánchez
12:00-13:30	Lunch			
13:30-14:00	Mariela Segura	Nikolay E. Nifantiev	Sin-Hyeog Im	Rachel Auzély-Velty
14:00-14:15	Hui Cai	You Yang	Meixia Li	Guillaume Pierre
14:15-14:30	Atsushi Shimoyama	Hai Dong	Mengxia Yan	Elisabete Coelho
14:30-14:45	Calum Haydon	Bogdan Novosad	Liang Wang	Chunlin Xu
14:45-15:00	Callum Johnson	Jennifer Johns	Shang-Chuen Wu	Gordon Boehlich
15:00-16:30	Coffee break & Posters			
16:30-17:00	James Paulson	Peng Wu	Alba Silipo	Manuel A. Coimbra
17:00-17:15	Ning Wang	Ming Joo Koh	Jinghua Yang	Yuxin Pei
17:15-17:30	Zeshi Li	Gang He	Jolanta Lukasiewicz	José García Fernández
17:30-17:45	Natasha Hatton	Yan-Ting Kuo	Yunlong Chen	Feiyang Wang
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10:00-10:30	Coffee break			
10:30-11:00	Xinshan Ye	Srinivas Hotha	Francisco Corzana	Pavla Bojarová
11:00-11:15	Alla Zamyatina	Clay Bennett	Hongjun Yu	Vittorio Bordoni
11:15-11:30	Jun Guo	Giuseppe Stefanetti	Lu Zhang	Xia Zou
11:30-11:45	Shuqin Xu	Oana Popa	Marlene Vuillemin	José López-Fernández
11:45-12:00	Qiang Ma	Xin Zhou	Ferran Nieto-Fabregat	Jihong Lu
12:00-13:30	Lunch			
13:30-14:00	Peter R. Andreana	Hien M. Nguyen	Yan Liu	Laura Russo
14:00-14:15	Sean Wu	Aniko Borbas	Peng Wang	Kevin Pagel
14:15-14:30	Nian Wu	Yuanwei Dai	Yang Ji	Jaroslav Katrlík
14:30-14:45	Kodai Sueyoshi	Haoru Zhuang	Kun Han	Xue Sun
14:45-15:00	Laurence Mulard	Toshiki Nokami	Andreea Cislaru	Yue Li
15:00-16:30	Coffee break & Posters			
16:30-17:00	David Bonnaffé	Dawen Niu	Heinz Läubli	June Ereño Orbea
17:00-17:15	Yi-Xian Li	Mingyi Wu	Zengqi Tan	Jin Yu
17:15-17:30	Shuyao Wang	Leonid Kononov	Li Chen	Nafisa Tursumamat
17:30-17:45	XiaoJun Li	Bodhayan Biswas	Yi-Shi Liu	Junhao Huang
17:45-18:00	Marcello Mercogliano	Elena Stepanova	Aldo Borjas	Xue Zhang

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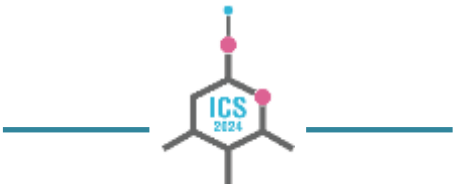
09:00-9:15	Yuan Chen	Zimin Zheng	Heng Yin	Shuo Huang
09:15-9:30	Emily Harlin	Yuntao Zhu	Liang Wu	Lifeng Sun
09:30-09:45	Jie Yu	Xiaoyang Hou	Erika Staudacher	Chunsheng Jin
09:45-10:00	Yue Yuan	Maria JesusMoure Garcia	Yan Wu	Kai Zhu
10:00-10:30	Coffee break			
10:30-11:00	Yasuhiro Kajihara	Ratmir Derda	Vered Padler-Karavani	Zbigniew Kaczynski
11:00-11:15	Carlo Unverzagt	Shuaishuai Wang	Dongmei Zhang	Erianna IsabelaAlvarado Melendez
11:15-11:30	Florian Küllmer	Peng Peng	Cristina Di Carluccio	Si Liu
11:30-11:45	Tianlu Li	Yongyong Wan	Vladimir Bokun	Xue Zhao
11:45-12:00	Shengyang Wang	Boqiao Fu	Tania Gerpe Amor	Gael Vos
12:00-13:30	Lunch			
13:30-14:15	Jesús Jiménez-Barbero			
14:15-15:00	Peter Seeberger			
15:00-16:00	Closing ceremony & Poster awards			

Friday, 19 July 2024
Excursion & Departure



Award Lectures

Activity-Based Glycosidase Profiling

Herman S. Overkleeft[a]

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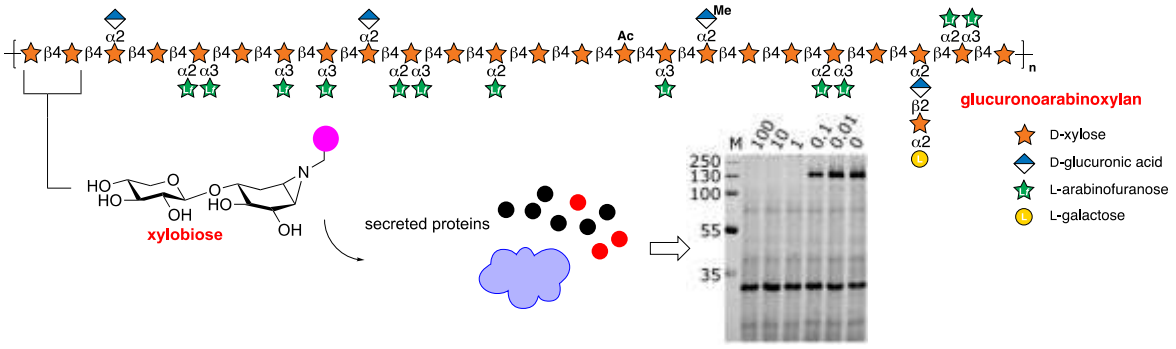
Activity-based protein profiling (ABPP) is a rapidly emerging field in chemical biology research. Enzymes that employ a mechanism in processing their substrate that involves formation of a covalent enzyme-intermediate adduct can be blocked by mechanism-based suicide inhibitors: compounds that react within the enzyme active site to form a covalent and irreversible adduct. Introduction of a reporter moiety (the purple bulb in the below picture) yields an activity-based probe (ABP) through which enzyme activities are discovered (comparative ABPP) and the efficacy of enzyme inhibitors analysed (competitive ABPP). Our work on ABPP development focuses on retaining glycosidases: hydrolytic enzymes able to cleave interglycosidic linkages and that do so through the formation of covalent enzyme-substrate intermediates. Configurational and functional analogues of the natural product and mechanism-based retaining beta-glucosidase inhibitor, cyclophellitol, prove to be highly versatile tools to study retaining glycosidases of various nature and origin in relation to human health and disease, but also in the field of biotechnology. In this lecture our results in studying mammalian[1,2] and microbial[3,4] retaining exo- and endoglycosidases will be presented.



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Structural Investigation Of Inhibitors Of Human Heparinase

Zach Armstrong

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Heparan sulfate proteoglycans (HSPGs) mediate essential interactions throughout the extracellular matrix (ECM), providing signals that regulate cellular growth and development. Altered HSPG composition during tumorigenesis strongly aids cancer progression. Heparanase (HPSE) is the principal enzyme responsible for extracellular heparan sulfate catabolism and is markedly up-regulated in aggressive cancers. HPSE overactivity degrades HSPGs within the ECM, facilitating metastatic dissemination and releasing mitogens that drive cellular proliferation. Reducing extracellular HPSE activity reduces cancer growth, but few effective inhibitors are known, and none are clinically approved. Here we present structural investigations into the binding of new classes of heparanase inhibitors that function in an unprecedented fashion. This has allowed the rationalization of inhibitor mechanisms and a path forward toward new HPSE inhibitors to treat cancer.

Plenary Lectures

Sugars & Proteins

Benjamin G. Davis,[a]

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[b] Department of Chemistry and Pharmacology, University of Oxford, ben.davis@chem.ox.ac.uk

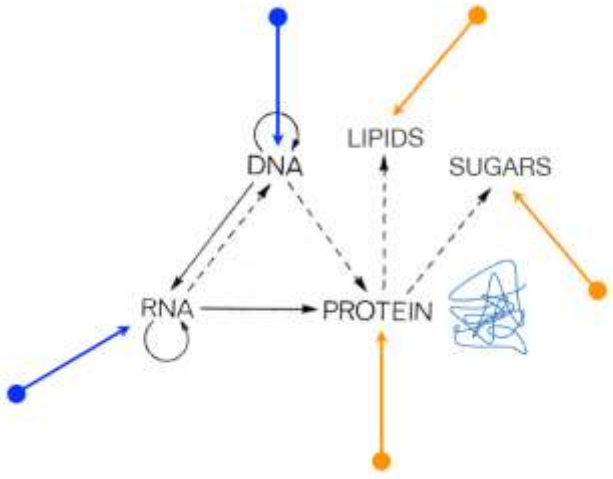
Our work studies the interplay of biomolecules – proteins, sugars, lipids and their modifications. Synthetic Biology's development at the start of this century may be compared with Synthetic Organic Chemistry's expansion at the start of the last; after decades of isolation, identification, analysis and functional confirmation, the future logical and free-ranging redesign of biomacromolecules offers tantalizing opportunities to dissect mechanism and control function in physiology and biology.

This lecture will cover past and emerging areas in our group in the manipulation of biomolecules with an emphasis on new bond-forming and bond-breaking processes compatible with biology and using those to understand molecular mechanisms.

:

(i) New methods: the development of precise methods that may be applied to biology at a post-translational level, generating minimal 'scars' or 'traces' (ideally 'trace'-less), could allow broad control of function. This will allow applications beyond simple 'labeling biology' or retrieval biology'. The development of chemo- and regio-selective methods with potential to post translationally 'edit' biology in this way, applied under benign conditions to redesign and reprogramme the structure and function of biomolecules, will be presented.

(ii) 'Synthetic Biologics' and their applications: biomimicry; functional recapitulation; effector [drug/agrochemical/gene/radio-dose] delivery; selective protein degradation; inhibitors of pathogen interactions; non-invasive presymptopmatic disease diagnosis; probes and modulators of *in vitro* and *in vivo* function illustrate possible resulting technologies,



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Innate Immunity To Bacterial Lps And Adp-Heptose: Pyroptosis And Beyond

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Lipopolysaccharide (LPS), the major cell-wall component of Gram-negative bacteria, is recognized in the host cytosol by mouse caspase-11 and human caspase-4/5 that bind directly to the lipid A moiety of LPS. Like caspase-1 activation by the canonical inflammasome, caspase-11/4/5 activation by cytosolic LPS induces pyroptosis by cleaving Gasdermin D (GSDMD), a member of the recently identified gasdermin family of pore-forming proteins. The cleavage releases the autoinhibition of the N-terminal domain of GSDMD that bears an intrinsic pore-forming activity and moves to and perforate the plasma membrane. This so-called noncanonical inflammasome, when overactivated, determines LPS-induced septic shock. Meanwhile, ADP-heptose, the precursor for LPS inner core oligosaccharide, is recognized in host cytosol by a novel kinase receptor ALPK1. ADP-heptose-activated ALPK1 potently stimulates NF-κB-dependent inflammatory responses for antibacterial immune defenses. In this talk, I will discuss our recent progresses on the noncanonical inflammasome pathway and address the following important questions. 1) Mechanistically, how does LPS bind and cause caspase-4/11 activation? 2) How do LPS-activated caspase-11/4 specifically recognize and cleave GSDMD to cause pyroptosis? 3) The function of the LPS-sensing noncanonical inflammasome in antibacterial defenses and the underlying immunological mechanism. 4) I will also briefly discuss the ALPK1 innate immune pathway activated by bacterial ADP-heptose.

Chemo-Enzymatic Synthesis of Complex Glycans to Probe-Host-Virus Interactions

Geert-Jan Boons[a,b]*

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Eukaryotic cells are covered by a dense layer of glycans that are expressed in tissue- and cell-specific manners and can differ considerably even between closely related species. Many pathogens, including respiratory- and enteric viruses, have proteins on their surface that bind to glycans on host cells to initiate infection. The glycan-binding specificities of pathogens and host glycan expression patterns are expected to be critical determinants of host range, cell tropism and pathogenesis. Despite the critical role of virus-glycan interactions, studying these biomolecules has been challenging due to the structural diversity of glycans, which are not genetically hardwired and cannot be easily probed using conventional experimental methods. To adress this difficiency, we have developed chemoenzymatic methodologies to prepare large panels of biologically relevant glycans[1] that have been used to develop designer glycan microarrays to probed receptor binding properties of respiratory viruses. To validate the array data, cell surface engineering strategies were developed to place synthetic glycans on the surface of cells for gain of function studies. The synthetic glycans have also been employed to develop ion-mobility mass spectrometry (IM-MS) approaches to determine exact structures of glycans in biological samples, which guided the selection of targets for synthesis.[2] It was found that for human A/H3N2 viruses, not only the sialoside type but also the length of the LacNAc chain and presentation at a specific antenna determines receptor binding properties.[3,4] In the case of beta-corona viruses, host-specific patterns of receptor recognition were found and revealed that two distinct human viruses uniquely bind 9-*O*-acetylated α2,8-linked disialoside. Immunofluorescence and cell entry studies support that such a glycotope as part of a ganglioside is a functional receptor for human coronaviruses.

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Glycans in Immunity: Elucidation From Synthetic Chemistry

Koichi Fukase

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Natural glycans function as recognition molecules, and glycan-mediated recognition is closely associated with immune responses. The challenge in glycoscience arises from the structural complexity and heterogeneity of natural glycans. We have identified the active glycan structures in innate immunity by synthesizing various homogeneous substructures.

We have investigated lipopolysaccharides (LPS) and their active component, lipid A, from Gram-negative bacteria, which exhibit potent immunostimulatory effects, including the effect of enhancing the vaccine efficacy as an adjuvant. However, these compounds are also known as endotoxins and present challenges due to their toxic effects, which are a result of induced inflammation. In pursuit of developing safe and effective adjuvants, we explored lipid A from symbiotic bacteria that have co-evolved with hosts, as well as lipid A derived from fermentative bacteria with dietary exposure [1, 2]. We demonstrated that synthetic *Alcaligenes faecalis* lipid A is an excellent adjuvant candidate for mucosal vaccines, exhibiting low inflammatory properties.

We have also investigated the efficient synthesis of *N*-glycans, which play crucial roles in immune regulation. We proposed the diacetyl strategy as an effective method for synthesizing NHAc-containing glycans, including *N*-glycans. Since compounds containing NHAc form intermolecular hydrogen bonds in organic solvents, significantly reducing the reactivity for glycosylation, we found that protecting NHAc as an imide (NAc_2) dramatically accelerates glycosylation reactions. Consequently, we succeeded in the chemical synthesis of fully sialylated tetra-branched *N*-glycans using the diacetyl strategy [3]. We further suggested that N-glycans exhibit emergent functionality based on multivalent recognition, with a focus on major chemical modifications such as core fucose, sialic acid, and polylactosamine.

Many natural glycans comprise complex systems with multiple active structures within the molecule, and emergent higher-order functions can arise synergistically through collective interactions of multiple molecules. Through a conjugation-based approach, we have also succeeded in creating immunoregulatory conjugated glycans. We synthesized conjugates of glycans or their dendrimers with tumor-targeting antibodies, enhancing cytotoxicity against tumors (CDC) [4, 5]. Additionally, we developed a method to introduce caged glycan antigens into cancer cells, releasing the glycans upon light irradiation to induce immune responses [6]. We also developed self-adjuvanting vaccines, which are composed of antigens and adjuvants and effectively induce antigen-specific immune responses, utilizing covalent conjugation and lipid nanoparticle formulation as key strategies [7].

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Identifying Appropriate Bio-Entities To See Glycans

Junhao Huang[1], Chuangye Yan[1], Nieng Yan[1,2,3]

[1]School of Life Sciences, Tsinghua University
[2]Shenzhen Bay Laboratory
[3]Shenzhen Medical Academy of Research and Translation (SMART)

Technological breakthroughs in cryo-EM and AI-driven structure prediction have enabled realtively convenient structural determination of previously intractable macromolecules. However, carbohydrates remain to be challenging for structural biology. Recently, we determined high-resolution cryo-EM structure of native mastigonemes isolated from *Chlamydomonas reinhardtii*. Unexpectedly, we observed a substantial amount of well-ordered arabino- and galactoglycans O-linked to hydroxyproline (HyP), contributing to 20-25% of the molecular mass of a mastigoneme stem. Additionally, we identified a previously uncharacterized glycoprotein, named Mstax, which constitutes the central shaft of a mastigoneme. The HyP-rich and heavily glycosylated Mstax protein comprises ~8000 residues and includes a PKD2-like transmembrane domain. Our study demonstrates the feasibility of resolving high-resolution glycan structures when appropriate research targets are identified

Chemical Tools For Glycobiology

Valentin Wittmann

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The elucidation of the numerous biological functions of carbohydrates benefits enormously from the development of chemical tools designed to achieve tight binding of carbohydrates to proteins and RNA, to synthesize glycoconjugates, and to trace carbohydrates within cells.

We developed combinatorial approaches to identify high-affinity multivalent lectin ligands and studied their binding mechanisms by numerous analytical methods including X-ray crystallography and EPR spectroscopy. From the lessons learned, we were able to develop a new design of multivalent lectin ligands, termed inline lectin ligands (iLecs). iLecs lead to exceptionally high binding affinities without concurrent precipitation of proteins due to crosslinking.[1]

The *glmS* riboswitch is a motif found in 5′-untranslated regions of bacterial mRNA that controls the synthesis of glucosamine-6-phosphate (GlcN6P), an essential building block of the bacterial cell wall. Activation of the *glmS* riboswitch by GlcN6P mimics interferes with the ability of bacteria to synthesize its cell wall. We developed a variety of GlcN6P mimics including carbasugars, thiasugars, and phosphonate mimics. Some of the compounds displayed antimicrobial activity and are promising lead structure for the development of future antibiotics with a potentially novel mode of action.[2]

Conjugation of unprotected (reducing) carbohydrates to surfaces or probes by chemoselective ligation reactions is indispensable for the elucidation of their biological functions. We studied the kinetics of the oxyamine ligation by real-time NMR spectroscopy and could show that the reaction rate is significantly increased (up to 500-fold) without the need for a catalyst when starting with glycosyl amines.[3]

Metabolic glycoengineering (MGE) is now a well-established approach to study the biological roles of carbohydrates.[4] We applied the inverse-electron-demand Diels-Alder (IEDDA) reaction in MGE to achieve the labeling of glycoproteins in and on cells. In combination with copper-free click chemistry and the photoclick reaction, we achieved a dual- and even triple-orthogonal labeling of glycans. Visualization of protein-specific glycosylation within living cells was possible using confocal FLIM-FRET microscopy. To study protein-O-GlcNAcylation, we developed dienophile-modified glucosamine-1-phosphate derivatives that do not lead to non-specific labeling by the recently reported *S*-glyco modification.[5]

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Breaking The Limits In Understanding Glycan Recognition By NMR

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Molecular recognition by specific targets is at the heart of the life processes. The interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization and tissue maturation to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, unravelling the structural and conformational factors and the physicochemical features that rule the interactions of these molecules is of paramount interest.
Solution NMR is unique in providing stereochemical and conformational information. Given the inherent flexibility and dynamic properties of glycans, we use NMR as key tool for deducing at atomic resolution molecular recognition processes in which glycans are involved, also assisted by a variety of synthetic, molecular biology, computational and biophysical techniques.
This presentation is focused on the application of state-of-the-art NMR methods both from the ligand and receptor's perspective to study molecular recognition processes between receptors of biomedical interest and glycans. As recent examples, key details of glycan recognition by these receptors will be shown, with special emphasis in the interactions of the human blood antigens with galectins, of sialoglycans and mimeticswith Siglecs, and of the spike glycoprotein of SARS CoV-2 with immune lectins and cell glycans.[1-6]

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Automated Optimization Of Glycosylation Reactions Accelerates Automated Glycan Assembly Of Complex Glycans

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Following its discovery over two decades ago,[1] automated glycan assembly (AGA)[2] has been developed into a robust and reliable technology that allows for preparation of diverse oligo- and polysaccharides[3] on a solid support employing a synthesizer.[4] Microwave-assisted[5] or Peltier-heating and cooling[6] accelerates capping, deprotection and functionalization steps of AGA. A better understanding of glycosylation reactions is needed in order to optimize coupling steps and thereby shorten overall assembly times. We developed a continuous flow set-up to optimize glycosylations using minimal amounts of material while achieving high reproducibility.[7] The data obtained helped us to quantitate 13 parameters that influence glycosylations and enabled the use of machine learning techniques as a basis for predicting glycosylation outcomes.[8] Reactivity and optimal glycosylation temperatures are correlated in order to further accelerate AGA.[9, 10] Access to ever more complex glycans including cis-linked polysaccharides[11] and complex N-glycans[12] are enabling fundamental investigations into the structure and function of polysaccharide materials, vaccines and diagnostics that will be discussed as well.

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Invited Lectures

Core Fucosylation And Hepatitis C Virus

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Aberrant N-glycosylation has been implicated in viral diseases. Alpha-(1,6)-fucosyltransferase (FUT8) is the sole enzyme responsible for core fucosylation of N-glycans during glycoprotein biosynthesis. Here we find that multiple viral envelope proteins, including Hepatitis C Virus (HCV)-E2,Vesicular stomatitis virus (VSV)-G, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-Spike and human immunodeficiency virus (HIV)-gp120, enhance FUT8 expression and core fucosylation. HCV-E2 manipulates host transcription factor SNAIL to induce FUT8 expression through EGFR-AKT-SNAIL activation. These aberrant increased-FUT8 expression promotesTRIM40-mediated RIG-I K48-ubiquitination and suppresses the antiviral interferon (IFN)-I response through core fucosylated-EGFR-JAK1-STAT3-RIG-Isignaling. FUT8 inhibitor 2FF, N-glycosylation site-specific mutation (Q352AT) of EGFR, and tissue-targeted Fut8 silencing significantly increase antiviral IFN-I responses and suppress RNA viral replication, suggesting that core fucosylation mediated by FUT8 is critical for antiviral innate immunity. These findings reveal an immune evasion mechanism in which virus-inducedFUT8 suppresses endogenous RIG-I-mediated antiviral defenses by enhancing core fucosylated EGFR-mediated activation.

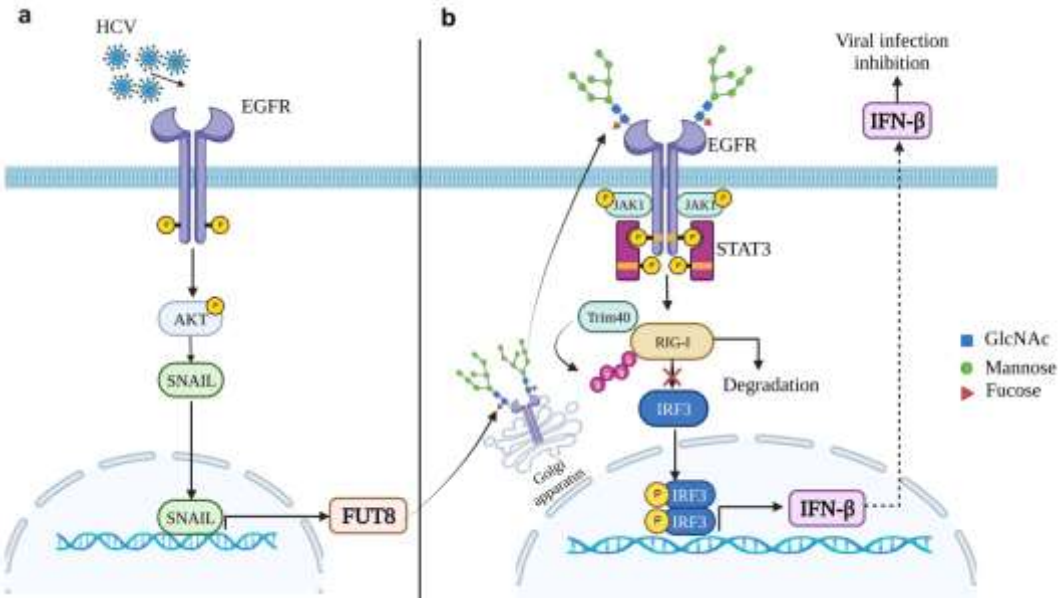

Fig. 1. Diagram showing that HCV upregulated-FUT8 promotes core fucosylated-EGFR-STAT3 and RIG-I degradation.

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Labeling And Imaging Of Glycans In Cells, Animals, And Clinical Specimens

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As one of the major biomacromolecules, glycans mediate various important physiological and pathological processes. On the other hand, glycans are highly complex and heterogeneous. Unlike nucleic acids and proteins, powerful tools for deciphering the biological function of glycans (i.e., glycocode) are relatively lacking. To address these challenges, chemistry and biology have been integrated to enable probing glycans in ways not possible before. For example, metabolic glycan labeling based on bioorthogonal chemistry and click chemistry has emerged as a central tool for glycan imaging and glycoproteomic profiling. Our group has developed various chemical tools for glycan labeling and analysis, with an emphasis on probing glycosylation dynamics in vivo and in tissue samples from human patients. We are particularly interested in how glycosylation regulates the physiology and pathology in the brain and during development. In this talk, I will introduce some of the recent progresses in this direction.

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Acceptor Reactivity In SN1, SN2 And Frontface-SN2 Glycosylation Reactions

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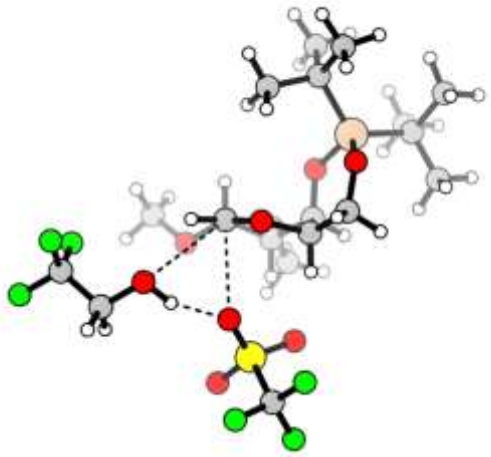
Synthetic oligosaccharides are invaluable as tool compounds to unravel the role of glycans and glycoconjugates in biological processes, and they can be used as components of synthetic vaccines and in the development of carbohydrate-based drugs. To expediate the synthesis of well-defined glycans for biochemical and biomedical studies, a better understanding of the glycosylation reaction, the reaction used to join two carbohydrate building blocks, is imperative. The huge structural variety in both donor and acceptor building blocks makes that is often impossible to translate glycosylation methodology from one system to another without loss of chemical yield or erosion of stereoselectivity.

In most glycosylation systems, *in situ* generated anomeric triflates act as leaving groups and our current understanding of the glycosylation reaction mechanism uses a continuum of reaction mechanisms in between the S_N1 and S_N2 extremes to explain the stereochemical outcome. Unexpectedly, we recently identified a glycosylation system of which the outcome can best be described using a front-face substitution mechanism of an anomeric triflate. This surprising reaction mechanism has so far has been overlooked in describing chemical glycosylation reactions, but it represents a common reaction mechanism used by retaining glycosyl transferases to substitute nucleotide diphosphates from natural sugar nucleotide diphosphate donors.



This presentation will describe how structural changes in the alcohol nucleophile impact the continuum of reaction mechanisms, including the front-face S_N2 substitution mechanism, and how this affects the stereochemical course of glycosylation reactions. Using systematic sets of substrates, we have been able to delineate structure-reactivity-stereoselectivity principles and pinpoint the stereoelectronic effects at play in glycosylation reactions. These can be applied in the development of effective routes of synthesis to assemble complex bacterial glycans, that can be used to map host-pathogen interactions at the molecular level.

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From Stereocontrolled Glycosylation To Automated Chemical Synthesis Of Glycans

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From the building blocks of nature to disease-battling pharmaceuticals, carbohydrates have had a profound impact on evolution, society, economy, and human health. Numerous applications of these essential biomolecules in many areas of science and technology exist, most of which can be found at the forefront of therapeutic agent and diagnostic platform development. Although carbohydrates are desirable for the pharmaceutical and biomedical communities, these molecules are very challenging targets for chemists because of the need for functionalization, protecting and leaving group manipulations, controlling anomeric stereoselectivity, separation, and analysis. The development of practical methods for the synthesis of building blocks, chemical glycosylation, and glycan assembly represent demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical synthesis of glycans. These tools will be discussed in light of recent results related to the development of new glycosylation reactions,[1-3] methods for controlling stereoselectivity,[4-5] and HPLC-based automated synthesis.[6-7] The effectiveness of methods developed will be illustrated by the synthesis of glycopharmaceuticals.[8-9] This work has been generously supported by the National Institutes of Health and the National Science Foundation.

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A Comprehensive Synthetic Library Of Poly-*N*-Acetyl Glucosamines Enabled Vaccine Against Lethal Challenges Of *Staphylococcus Aureus*

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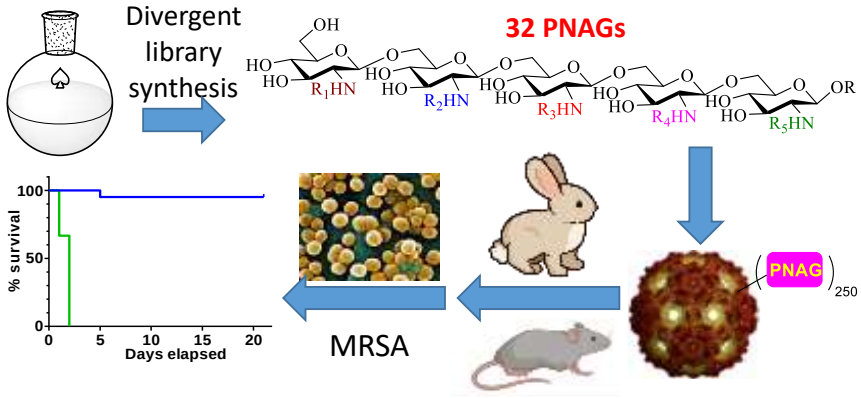
Poly-β-(1−6)-*N*-acetylglucosamine (PNAG) is an important vaccine target, expressed on many human pathogens. A critical hurdle in developing PNAG based vaccine is that the impacts of the number and the position of free amine *vs N*-acetylation on its antigenicity are not well understood. In this presentation, we will present a divergent strategy to synthesize a comprehensive library of 32 PNAG pentasaccharides. This library enables the identification of PNAG sequences with specific patterns of free amines as epitopes for vaccines against *Staphylococcus aureus* (*S. aureus*), an important human pathogen. Active vaccination with the conjugate of discovered PNAG epitope with mutant bacteriophage Qβ as a vaccine carrier as well as passive vaccination with diluted rabbit antisera provides mice with near complete protection against infections by *S. aureus* including methicillin-resistant *S. aureus* (MRSA). Thus, the comprehensive PNAG pentasaccharide library is an exciting tool to empower the design of next generation vaccines.[1]



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De Novo Asymmetric Synthesis Of Carbohydrate Containing Natural Product: Application To Medicinal Chemistry

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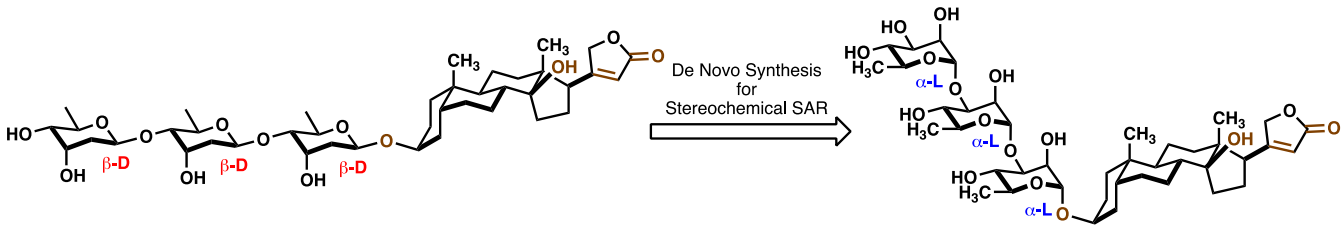
As part of chemistry's never-ending desire to mimic the elegance of nature, organic chemists have pursued novel methods for the synthesis of natural products. Our contribution to this mission involves the use of asymmetric catalysis for the enantioselective synthesis natural products from achiral starting materials. We refer to this approach as De Novo Asymmetric Synthesis. In this regard, we have been working to develop practical catalytic asymmetric approaches for the synthesis and study of stereochemically complex carbohydrate containing natural products. The stereochemical flexibility this approach engenders, allows us to carry out novel Stereochemical-Structure Activity Relationship (S-SAR) studies on these natural products with an emphasis on the carbohydrate portion of the molecule. For example, we have successfully used the tools of catalytic asymmetric synthesis to systematically map the stereochemical aspects of the structure activity relationship for the oligosaccharide portion of the cardiac glycosides. This effort and its application for oligosaccharide synthesis and related medicinal SAR-chemistry studies will be discussed.

Scheme 1: De Novo Asymmetric Synthesis for the S-SAR-Studies



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Chemoenzymatic Synthesis Of Mucin Tandem Repeat Glycopeptides To Map Bacteria Lectin And Mucinase Interactions

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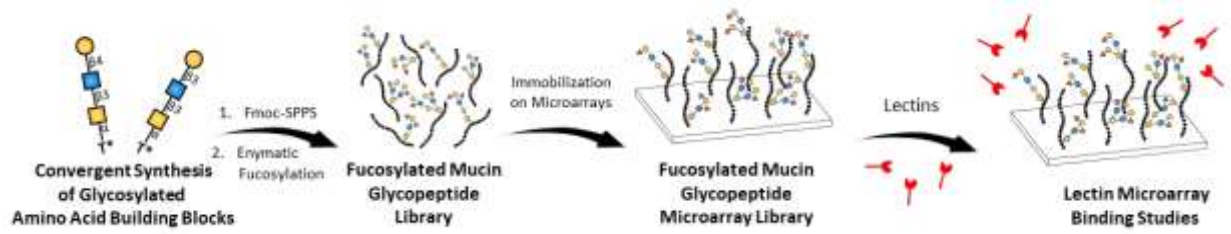
Mucins are densely *O*-glycosylated membrane-bound and secreted proteins ubiquitously found on the epithelial cell surface.[1] In the intestines the membrane-bound mucins are coated with an inner and outer mucus layer. The mucus consist to a large extend of secreted mucins and serve as a protective barrier, which keeps bacteria on distance from the epithelial tissue.[2] However, the microbiota have co-evolved with the human host and developed strategies to feed, degrade and penetrate the mucus barrier and thereby enable adhesion to carbohydrate ligands on the host cell-surface using lectins.

In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria lectins and carbohydrate ligands presented on mucin peptide backbones. Mapping of the glycan interactions of bacteria CBMs from mucin degrading enzymes (mucinases) is another key interest. In my presentation examples of chemoenzymatic synthesis of mucin tandem repeat glycopeptide libraries will be described. This includes synthesis of mucin core and extended core glycopeptides, which were enzymatically modified to form different Lewis antigens, including Lewis a, Lewis x, or H-type motifs as well as bi-fucosylated Lewis b and Lewis y structures. [4 The glycopeptide library was immobilized on microarray slides and applied to study interactions with fucose binding lectins including the toxin A of *Clostridium difficile* (TcdA), a bacterium that causes gastrointestinal disorders.[3] Different mucin glycopeptide libraries containing fucosylated and sialylated epitopes, LacNAc- or LacdiNAc extended core structures and tumor-associated ST-, T-, STn- and Tn-antigens were further employed to evaluate binding preferences of gut bacteria mucinase CBMs. Examples from these studies will be given at ICS2024.



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O-Glycosylation As A Host Defense Mechanism Against Sars-Cov-2 Infection

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SARS-CoV-2 is the causative pathogen of COVID-19, a global pandemic that has already claimed over 6.8 million lives worldwide. Unfortunately, there is still no effective strategy to eradicate the virus. It remains crucial to understand the infectious mechanism of the virus and to explore potential strategies to fight viral infection. As enveloped viruses hijack host cell's secretory machinery to synthesize viral proteins and to assemble virions, they are inevitably modified by host cell's glycosylation enzymes. However, the potential functions of glycosylation, especially O-GalNAc type glycosylation (O-glycosylation) on viral proteins, are not well studied. Using a SimpleCell-based glycoprofiling strategy, we uncovered clustered O-glycosylations near the furin cleavage site of the SARS-CoV-2 spike protein. We show that sequential glycosylation by GalNAc-T3 and T7 inhibits furin processing, suppresses the incorporation of the spike protein into virus-like-particles and affect viral infection. Mechanistic analysis reveals that the assembly of the spike protein depends on furin cleavage of spike protein and the interactions between cleaved spike and the membrane protein of SARS-CoV-2, suggesting a novel mechanism for furin-facilitated spike activation. Interestingly, viral evolution results in changes in furin site O-glycosylation. Mutations in the spike protein of the alpha and delta variants of the virus confer resistance against glycosylation by GalNAc-T3 and T7. In the omicron variant, additional mutations reverse this resistance, making the spike protein susceptible to glycosylation in vitro and sensitive to GalNAc-T3 and T7 expression in human lung cells. Our findings highlight the role of O-glycosylation as a defense mechanism employed by host cells against SARS-CoV-2 and shed light on the evolutionary interplay between the host and the virus.

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The Structural Characteristics And Significance Of *N*-Glycans In Cancer Stem Cells

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Cancer stem cells (CSCs) play a crucial role in tumor formation, progression, immune evasion and recurrence [1-2]. Membrane protein markers provide us with the molecular basis for sorting and targeting cancer stem cells. Analyzing the glycan structure characteristics of cancer stem cell membrane proteins can help discover the specific markers of cancer stem cells. Studying the glycan structures of membrane proteins in CSCs and their significance not only helps identify specific markers of CSCs but also provides new perspectives and tools for developing targeted therapeutic strategies. Our research focus on "The Structural Characteristics and Significance of Membrane Protein Glycans in Cancer Stem Cells" has led to the following major findings: ① CD133 modified by high mannose-type *N*-glycans is a specific marker of cancer stem cells. The low expression of glycosyltransferases such as mannosidase MAN1A1 leads to the formation of high-mannose *N*-glycan structures on CD133 in glioma stem cells. ② High mannose-type *N*-glycans enhance autophagy, thereby maintaining the self-renewal and tumorigenic ability of cancer stem cells. ③ High-mannose *N*-glycans on the surface of liver cancer stem cells bind to high-mannose receptors on lymphatic endothelial cells, promoting the transcription and secretion of IL-17A in lymphatic endothelial cells. This interaction promotes the self-renewal and immune evasion of liver cancer stem cells. Our research elucidates the functions and mechanisms of high-mannose *N*-glycans in CSC self-renewal and microenvironment interactions. These findings provide characteristic markers for the screening and targeted therapy of CSCs. The related research results have been published in prestigious international journals such as Advanced Science, Cell Reports, and Journal of Hepatology. This research was supported by the National Key R&D Program of China (2016YFA0501300), the National Natural Science Foundation of China (32071273, 82073273), the Shanghai Natural Science Foundation (23ZR1413900), and the Shanghai Biomedical Science and Technology Support Project (23S11900100).

Keywords: Cancer stem cell, *N*-glycan, CD133, Microenvironment

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Synthetic Peptidoglycans That Regulate Bacterial Cell Wall Biogenesis

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Peptidoglycan forms the fundamental structure of every bacterial cell wall, positioning it as a prime target for developing innovative bacterial-targeting agents. In my presentation, I will discuss our latest findings on anhydro-MurNAc[1], biohybrid peptidoglycan oligomers (PGO)[2], and lipid II and IV molecules.[3,4] Our research highlights the innovative incorporation of an anhydromuramyl moiety into peptidoglycan strands by transglycosylases, which effectively terminates glycan chain extension. These findings open up possibilities for using anhydromuro- peptides as a new class of peptidoglycan-terminating inhibitors, offering fresh strategies for antibacterial agent development (Figure). Moreover, we have observed high efficiency in the metabolic labeling of both gram-positive and gram-negative bacteria with PGOs. This approach not only facilitates the metabolic labeling but also the engineering of live bacterial cells by intercepting their enzymatic assembly with synthetic substrates.

This research not only enhances our understanding of bacterial cell wall mechanics but also sets the stage for significant advancements in antibacterial treatments.

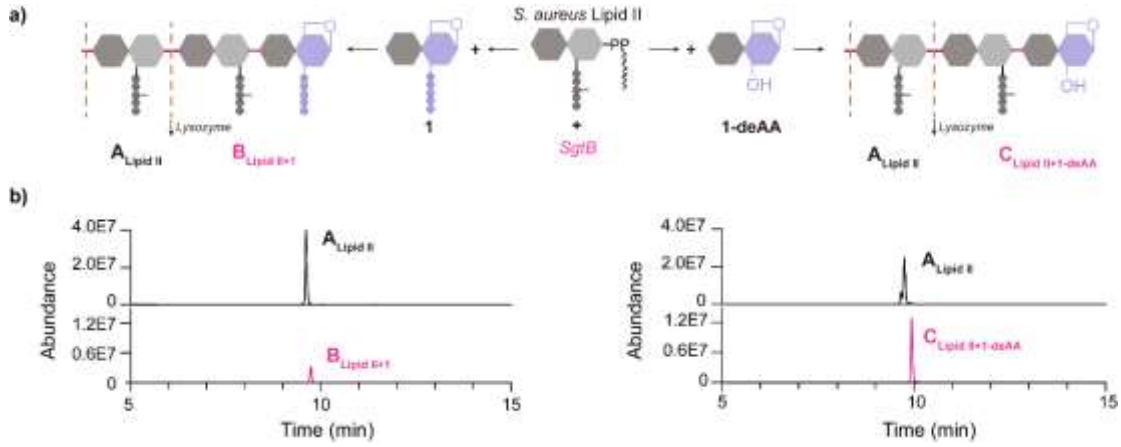

Figure. (a) Schematic of assay to analyze the structural requirement of TGase acceptor. The SgtB can utilize both **1** and its analogue **1-deAA** without pentapeptide as acceptors, which leads to two degradation products $\mathbf{B}_{\text{Lipid II + 1}}$ and $\mathbf{C}_{\text{Lipid II + 1-deAA}}$. (b) LC-HRMS EICs of TGase reactions containing **1** (left) and **1-deAA** (right) with *S. aureus* Lipid II show significantly higher TGase reactivity of **1-deAA**. $\mathbf{A}_{\text{Lipid II}}$: 626.2886 m/z; $\mathbf{B}_{\text{Lipid II + 1}}$: 1091.4977 m/z; $\mathbf{C}_{\text{Lipid II + 1-deAA}}$: 856.3733 m/z.

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In Silico Studies Of The Carbohydrate Interactions And Carbohydrate Processing Enzymes

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Carbohydrates are ubiquitously present in all cells in the variety of forms e.g. glyco-conjugates, playing pivotal role in a plethora of the biological processes. These glyco-conjugates are formed by glycosyltransferases which add saccharides onto proteins, lipids, sugars etc.

There are several ways how carbohydrates may interact with their receptors (e.g. classical hydrogen bonds, through metal ions as Ca(II)). The CH-π interactions that occur between carbohydrates and aromatic amino-acids are also strongly involved in carbohydrate-recognition process.

We have introduced systematic DFT and high-level ab initio study of CH-π interaction features between benzene as the simplest representative of aromatic moiety in proteins and selected saccharides. Nevertheless, also condensed aromatic moieties as Trp residues are responsible for the CH-π-mediated recognition of carbohydrate molecules, so the study has been extended to describe the degree of additivity of the CH-π interaction analyzing the interaction energy of carbohydrate-benzene complexes. Knowledge from the systematic study we have applied to biologically relevant systems where we have focused on RSL and AAL lectins. Both lectins bind predominantly L-fucose. We have studied this interaction by combination of the experimental approach combined with high level computational methods. Experimentally measured binding affinities were compared with computed carbohydrate-aromatic acid residue interaction energies.

Knowing of the reaction mechanism and ligand interaction of the carbohydrate processing ezymes have significant influence in the understanding of their function. In one of our studies we have explored the reaction mechanisms of O-GlcNAc transferase (OGTs), where GlcNAc is transferred to –OH group of Ser/Thr of the proteins. The fault in this mechanism has been linked to diabetes, alzheimer's, etc, hence there is a need to investigate the processes in-depth. Three different reaction mechanisms were proposed based on available OGTs crystal structures suggesting different catalytic base to abstract proton from acceptor Ser, 1) Histidine, 2) α-phosphate, and 3) water molecule. We have used the advanced QM/MM computational methods to find the most probable OGT reaction mechanism.

The interactions of the plant polysaccharides with the transglycosylase xyloglucan endtransglycosylase (XET), an enzyme which primary catalyze the cleavage of xyloglucan or cellulose followed by transfer of polysaccharide fragment with former nonreducing end to another molecule of xyloglucan or cellulose. IN this study we have prepared a homology model of the protein and His94, Ala104, Gln108, Lys234 and Lys237 were identified as amino acid residues most probably responsible for enzyme specificity related to neutral saccharides. This was proved also experimentally by site directed mutagenesis. Acceptor substrate docking was performed in the active site of XET model followed by molecular dynamics simulations and binding free energy calculations using MM(GB/PB)SA method were estimated and compared with the experimental enzymatic activities of XET.

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Sulfosugar Catabolism: Enzymes, Pathways And Probes

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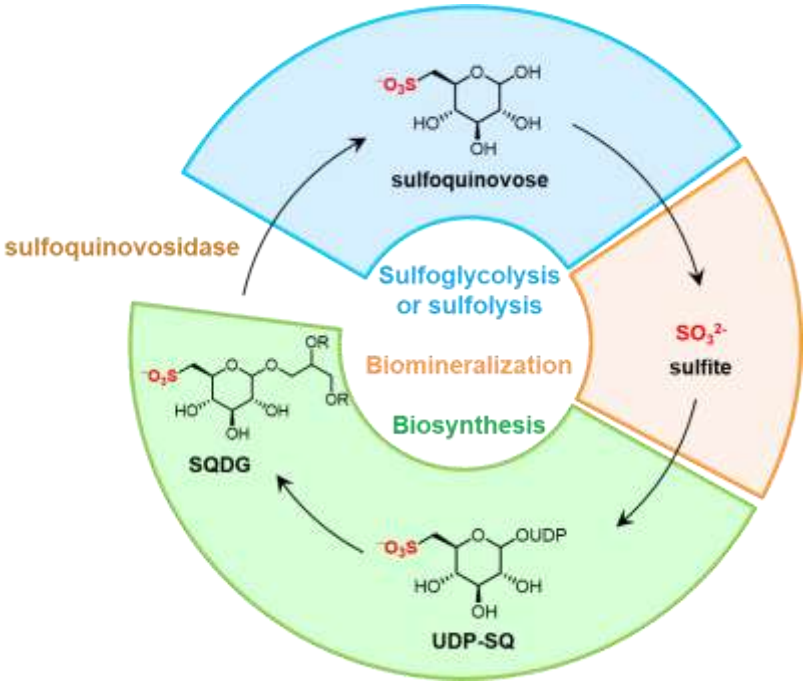
Sulfoquinovose (SQ) is a sulfosugar that is the anionic head group of sulfolipids found in algae, plants, and cyanobacteria [1]. It is a significant biosulfur reservoir within photosynthetic tissues and organelles, and it is estimated that 10 billion tonnes of SQ are produced annually. SQ is broken down through the microbial pathways of sulfoglycolysis and SQ sulfolysis [2,3]. Knowledge of these pathways provides insights into the biogeochemical sulfur cycle and microbial ecology in our seas and soils.

Over the last decade my group has conducted broad-ranging collaborative studies into the chemistry, biochemistry, microbiology, and structural biology of SQ breakdown, and have sought to exploit these findings to develop new chemical biology tools. In this talk I will describe the discovery of specialized glycosidases termed sulfoquinovosidases that release SQ from sulfolipids [4,5], a new pathway for SQ degradation that achieves the cleavage of the distinctive carbon-sulfur bond [6], and the development of synthetic tools [7] that can be used to study and exploit the new enzymes and proteins that we have discovered.



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Lectins And Glycomimetics In Infectious Diseases

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Lectins are exploited for initial infection by numerous pathogens such as bacteria, viruses and parasites.[1] In this talk, I will present our work on the synthesis of glycomimetics for bacterial and viral lectins and their biological evaluation. Bacterial biofilms are a severe problem for therapy. The Gram-negative bacterium P. aeruginosa is a critical bacterial pathogen as defined by the WHO priority pathogen list. This bacterium is difficult to treat due to excessive development of resistance to antibiotics and its abundant biofilm formation. The latter is a major resistance determinant of this pathogen since the biofilm shields embedded bacteria from chemotherapy and host defence. Therefore, several approaches to identify new anti-infectives against this bacterium aim to block biofilm formation. P. aeruginosa utilises the two lectins LecA (PA-IL) and LecB (PA-IIL) for initial adhesion to the host, for biofilm formation and as virulence factors. These are promising drug targets that are addressed in our research for therapeutics, diagnostics and conjugates.[2-5] Various other ESKPAE pathogens have lectins that may serve as drug targets in the future.

Sialic acids are recognized by numerous viruses, especially those with pandemic potential such as influenza and coronaviruses. Our work on the generation of sialic acid mimetics and their implementation into glycan arrays for the analysis of viral lectin binding will be discussed.

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Streptococcus Suis Interactions With B Lymphocytes: Uncovering The Role Of Igm And Capsular Polysaccharide

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Streptococcus suis is an encapsulated bacterium that can cause severe invasive diseases in pigs and it is considered an emerging zoonotic threat. The bacterial capsular polysaccharide (CPS) is a critical virulence factor that provides resistance against host immune cells. The antigenicity of the CPS defines 29 distinct serotypes of *S. suis*, with some serotypes being more commonly associated with clinical disease than others. For instance, the serotype 2 is the most prevalent worldwide in both, pigs and humans.

The adaptive humoral response is the result of a communication network between antigen presenting cells (APC), T cells and B cells. Although antibodies play a useful role in the elimination of *S. suis*, no commercial vaccine is available to prevent infections. Reports indicate that encapsulated *S. suis* can interfere with optimal APC and T cell functions. However, the interactions between *S. suis* and B cells are largely unknown. Our recent work aimed to characterize the development of the adaptive humoral immune response by evaluating germinal center (GC) B cell dynamics and the production and role of antibodies induced following *S. suis* infections in a mouse model. We found that mice infected with *S. suis* developed GC that peaked 13-21 days post-infection. GC further increased and persisted upon periodic reinfection that mimics real life conditions in swine farms. Anti-*S. suis* IgM and several IgG subclasses were produced, whereas antibodies against the *S. suis* capsular polysaccharide (CPS) were largely IgM. Somatic hypermutation or isotype switching was dispensable for controlling the infection or anti-CPS IgM production. Depletion of total IgG from the WT mouse sera had no effect on bacterial killing *in vitro*. However, T cell-deficient mice were unable to control bacteremia, producing optimal anti-CPS IgM or eliciting antibodies with opsonophagocytic activity. SAP deficiency, which prevents GC formation but not extrafollicular B cell responses, ablated anti *S. suis*-IgG production but maintained IgM production and eliminated the infection. In contrast, B cell deficient mice were unable to control bacteremia. Collectively, our results indicate that a GC-independent but T cell-dependent germline IgM being the major effective antibody specificity. Our results further highlight the importance IgM and mainly anti-CPS antibodies in clearing *S. suis* infections and provide insight for future development of *S. suis* vaccines.

In order to determine if the structure of the CPS influences antigen recognition by antibodies, serotype-switched mutants of *S. suis* serotype 2 were employed to compare the role played by the CPS structures of serotypes 2, 3, 4, 7, 8, 9 and 14, since the only difference between these strains is the type of CPS expressed. Results suggest that the different CPS structures of *S. suis* provide varying levels of protection by influencing antigen availability and elimination by the host immune system. This finding is of importance for vaccine development and highlights the need to closely monitor cross-protection when designing *S. suis* vaccines since the CPS structure might eventually affect the efficacy of vaccines targeting subcapsular antigens at the bacterial surface.

Restricted Receptor Specificity Of Recent H3N2 Influenza Viruses Shifts Tropism To Ciliated Epithelial Cells

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Influenza A viruses contain two surface glycoproteins required for infection and transmission: hemagglutinin (HA) and neuraminidase (NA). HA mediates virus attachment to sialic acid- containing glycan receptors on the host cell surface, and NA known as the 'receptor destroying' enzyme, releases new viral particles from the cell by removing sialic acids that would otherwise serve as receptors for the HA. Human influenza pandemics occur when a new influenza strain from birds or swine acquires the ability to transmit in an immunologically naïve human population, causing severe disease worldwide. Once in the human population, the virus mutates to avoid the human immune response while maintaining fitness to transmit and infect. It is well documented that the HA of human influenza viruses bind to receptors with sialic acids linked α2- 6 to galactose (human-type receptors), while the HA of avian viruses binds to receptors with sialic acids linked α2-3 to galactose (avian-type receptors). The longest circulating human influenza strain is the H3N2 virus, which entered the human population from an avian virus in the pandemic of 1968, and acquired the human-type receptor specificity needed for transmission in the human population. In recent years, the H3NA virus has evolved a strong preference for sialylated glycans with poly-LacNAc repeats or "extended glycans." To better understand this change in receptor specificity in the context of natural receptors, we have analyzed the glycome of human airway epithelial cells and found that such extended receptors are indeed present on ciliated epithelial cells. Using a synthetic glycan library representing the diversity of N-glycans in the airway glycome, we find that the extended glycan chains are required for binding the HA of recent H3N2 viruses. Surprisingly, this restricted receptor specificity causes the HA to preferentially bind to ciliated epithelial cells in contrast to HA from earlier strains, suggesting a shift in tropism to ciliated epithelial cells. (Funded in part by NIH grant R01AI114730, and CEIRR contract 75N93021C00015).

Synthetic Glyco-Tools For Exploring And Exploiting The Glycome

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The stereoselective synthesis of glycosides remains one of the biggest challenges in carbohydrate chemistry.[1] The chemical synthesis of complex carbohydrates generally involves the coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric centre, with a suitably protected glycosyl acceptor (R-OH). In many instances, these reactions lead to a mixture of two stereoisomers.

Towards this goal, our group has endeavoured to develop catalytic and stereoselective methods to address this important synthetic challenge.[2] Recent years have seen a steady increase in the application of transition metal catalysis applied to oligosaccharide synthesis,[3] since the reaction conditions are mild and the careful choice of catalyst can offer significant improvements over traditional methods in terms of atom economy, high yields and control of anomeric selectivity.

Herein, we will discuss the application of transition metal catalysis in the stereoselective synthesis of deoxy glycosides, including the α,α-stereoselective synthesis of trehalose derivates and their application as selective probes of bacteria detection. Moreover, I will also disclose the development of imidazolium-based MS labels and their applications to expedite oligosaccharide synthesis and enzyme discovery in glycobiology.[4]

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Role Of Glycoligand Presentation For Efficient Recognition

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Efficient methods are still required to assess the fine carbohydrate specificity of carbohydrate-recognizing proteins. A number of publications report contradictory results about the specificity of known antibodies and lectins. In some cases they report totally or partially mismatched conclusions, but mainly – overestimated specificities. In this lecture several recent examples of such cases will be discussed, with the emphasis on the importance of using synthetic oligosaccharides of strictly known structure as reference antigens. The significance of density, equimolarity and efficient spatial presentation of the assayed glycoligand, as well as its multimericity will also be discussed on the examples of the carbohydrate specificity of Abs and DC-SIGN immune lectin.

Acknowledgments: The synthesis of oligosaccharides and screening on glycoarrays was supported by the Russian Science Foundation (grant 19-73-30017-P to NEN). JJB and AA acknowledge Agencia Estatal de Investigación of Spain (MCIN/AEI/10.13039/501100011033) for grants PDI2021-1237810B-C21 and CEX2021-001136-S; CIBERES, an initiative of Instituto de Salud Carlos III (ISCIII, Madrid, Spain); and the European Research Council (RECGLYCANMR, Advanced Grant No. 788143).

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Degrading The Inhibitory Siglec-7 And -9 For Cancer Immunotherapy

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The development of immune checkpoint inhibitors based on anti-CTLA-4, anti-PD-1/PD-L1 has revolutionized cancer treatment. However, more than 50% of cancer patients fail to respond to to such treatment, suggesting the existence of other immune checkpoints orthogonal to these well-established ones. Siglecs (sialic acid-binding immunoglobulin-like lectins) have recently been described as glyco-immune checkpoints. We discovered that Siglec-7 and -9, which are constitutively expressed on myeloid cells such as monocytes and macrophages, are involved in immunosuppression in the tumor microenvironment (TME). Siglec-7/9 degradation, particularly in combination with anti-CTLA-4, enhanced T cell cytokine production and cytolytic activities and reshaped the TME, leading to excellent tumor control in multiple murine solid tumor models. Collectively, these data highlight the potential of Siglec-7/-9 depletion as a potential intervention that can be combined with the FDA-approved checkpoint blockade to reprogram TME for cancer immunotherapy.

Toward Fidning A Cure For Ngly1 Deficiency

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The cytoplasmic peptide:*N*-glycanase (PNGase; NGLY1 in humans) is a deglycosylating enzyme widely conserved in eukaryotes[1]. This enzyme is involved in the degradation of misfolded *N*-glycosylated proteins destined for proteasomal degradation in the cytosol, a process called endoplasmic reticulum-associated degradation (ERAD). The discovery of NGLY1 deficiency, a human genetic disorder bearing mutations in the *NGLY1* gene, has led to rapid research progress on this protein[2]. We have been analyzing various *Ngly1*-KO animals (mice/rats/flies), and have identified genetic factor(s) greatly affecting the phenotypic consequences of these animals[3, 4]. It is particularly worth noting that additional KO of *Fbs2*, a glycan-recognizing E3 ubiquitin ligase subunit, can rescue the embryonic-lethal phenotype of *Ngly1*-KO mice, and the *Ngly1 Fbs2* double-KO mice exhibited no obvious defect on their motor functions[4]. These genes can thus be regarded as a promising drug target for NGLY1 deficiency. Moreover, recent evidence also suggested that AAV9-based gene therapy could be a viable therapeutic option for NGLY1 deficiency[5]. In this lecture, I will summarize the most recent progress of our efforts to find a cure for NGLY1 deficiency[6] as well as the development of the facile assay method for NGLY1 activity potentially of use for early diagnosis of this disease[7-9].

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Structure And Functional Correlation Of Beta-Glucan In Immune Modulation

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Microorganism-derived polysaccharides, such as β-glucan, exhibit diverse immunoregulatory functions that either stimulate or modulate the immune system. β-glucan, notably abundant in the fungal cell walls and also found in some lactic acid bacterial capsules, presents in complex and diverse molecular structures. This polysaccharide is well-documented for its role in enhancing pro-inflammatory responses, which has led to its therapeutic application in treating infectious diseases and as an adjuvant in cancer therapy. Additionally, growing evidence supports β-glucan's capability to exert anti-inflammatory effects within immune responses.

In our recent investigations, we have specifically focused on the role of polysaccharides derived from commensal microorganisms in modulating immune responses, examining how their chemical structures correlate with their immunological impacts. Through detailed structural analysis, we discovered that β-1-6 glucans, sourced from commensal yeast or *Bifidobacterium bifidum*, predominantly induce anti-inflammatory responses. This is in contrast to beta-1-3 glucans, which are more commonly associated with enhanced immune stimulation. Our findings further reveal that recognizing these polysaccharides by specific pattern recognition receptors, such as TLR2 and Dectin-1 on dendritic cells, plays a crucial role in their function. β-1-6 glucans interact with these receptors to promote the secretion of the anti-inflammatory cytokine IL-10, facilitating the development of immunoregulatory T cells (Tregs). On the other hand, β-1-3 glucans potentiate the immune system's pro-inflammatory properties by different receptor-mediated pathways.

The complexity of these interactions highlights the critical roles that microorganism-derived polysaccharides play in immune modulation. These molecules activate diverse signaling pathways significantly affecting the body's immune responses. Therefore, understanding the specific chemical structures of these polysaccharides is essential for leveraging their properties to stimulate or down-regulate inflammatory responses effectively under various health conditions. This nuanced approach could pave the way for more targeted and effective therapeutic strategies that harness the full potential of immunomodulatory polysaccharides.

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Carbohydrates As Keywords In The Molecular Dialogue

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The evaluation of the structure of biomolecules built up of carbohydrates is a very challenging task due to the inherent complexity of sugar chemistry, which also impairs any computerized/automated approach. Nevertheless, this is a fundamental mission devoted to understanding interaction events at atomic level, including host-guest cross-talk. The combined use of complementary, biophysical approaches, including NMR spectroscopy, computational and biophysical techniques, native MS, together with immunological experiments is essential to unravel structure, properties, functions of glycans and understanding the mechanisms at the basis of recognition of the sugar code. In this talk, I will give a special focus to the description of bacterial glycocode, either as beneficial mediator of host homeostasis and immune system development or when harmful to the host. I will describe the chemical glyco-features located on bacterial cell surface able to tune eukaryotic immune responses..

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Iminosugar-Based Glycomimetics As Probes For Ligand-Directed Glycosidase Profiling

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Activity based protein profiling (ABPP) is a versatile tool for evaluating enzyme activity rather than their abundance in living systems and complex environments [1]. With respect to carbohydrate processing enzymes (CPE), several versatile and efficient ABPP strategies have been introduced [2]. One cachet of the majority of these strategies is that the respective enzyme undergoes labelling by covalent binding of the probe to the active site. We are interested in a complementary method by applying the ligand-directed chemistry (LDC) for protein labelling of CPEs. This strategy, introduced by Hamachi and coworkers, allows for labelling of the respective enzyme in a certain proximity to the active site [3].

We have designed and synthesised iminoalditol based probes featuring the respective components for LDC labelling [4]. Biological evaluations of these probes have been conducted with two model enzymes, β-glucosidase from almonds (*Prunus dulcis*, PdGH1) and β-glucosidase from *Thermotoga maritima* (TmGH1).

Details about the synthesis of iminoalditol based glycomimetics and biological evaluation as probes for LCD labelling of CPEs will be presented.

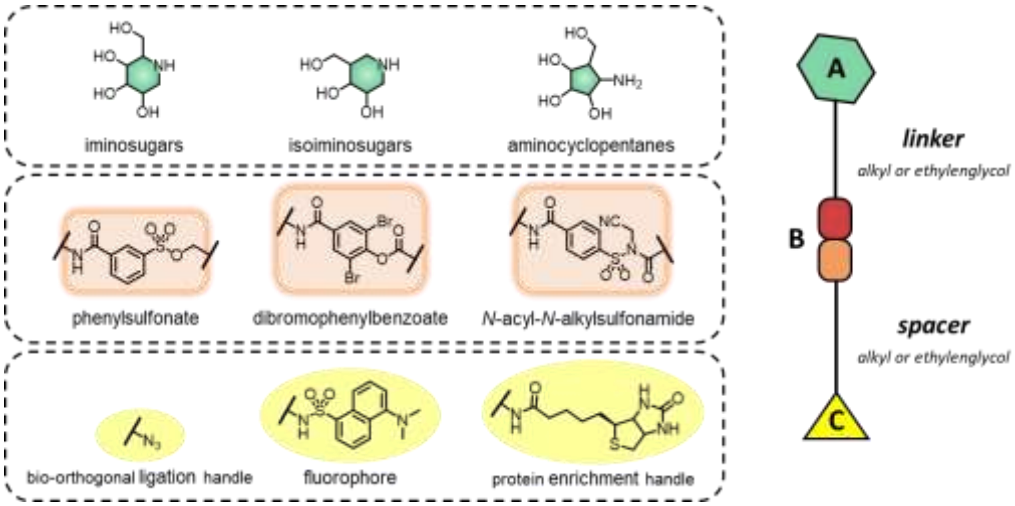

Figure 1: Building block concept for ligand directed chemistry (LDC) probes targeting glycoside hydrolases. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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Polysaccharide Hydrogels: From Controlled Synthesis To *In Vivo* Evaluation As Theranostic Agent For Regenerative Medicine Applications

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Hydrogel scaffolds that can repair damaged biological tissues have great potential for the treatment of injuries and diseases. Among the many potential building blocks of these three-dimensional networks, glycosaminoglycans (GAGs), as important components of the extracellular matrix (ECM) in animal tissues, have attracted great attention to design biomimetic hydrogels for *in vivo* applications.[1] For some time now, we have been interested in the design of injectable hydrogels based on hyaluronic acid (HA), a major GAG of the ECM, for biomedical applications. Herein, we will report original boronate ester-crosslinked HA hydrogels by tailoring the structure of the phenylboronic acid derivative.[2-4] We will show how the binding mode of boronic acids to saccharide moieties grafted on HA affects the mechanical properties of the hydrogels, which paves the way for the rational design of injectable self-healing hydrogels with tailored mechanical properties. Example of applications of these hydrogels as scaffolding material for treating neurological and degenerative joint diseases using small animal models will be presented.[5] In particular, we will show that the specific labeling of hydrogels enabled to monitor not only their delivery to target tissues through small-diameter needle but also their retention for several weeks post-administration using non-invasive imaging tools. Investigation of their therapeutic effect together with their *in vivo* fate indicate that these new HA hydrogels appear as a promising candidate for precision and regenerative medicine.

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Strategies To Recover Polysaccharides And Oligosaccharides With Prebiotic Activity From Agri-Food Byproducts

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There are a high number of unexploited sources of prebiotic molecules due the lack of strategies for its recovery from insoluble matrices, such as agri-food byproducts. These by-products are rich in polysaccharides that compose the dietary fiber, although insoluble. Soluble polysaccharides and oligosaccharides are prebiotic compounds that promote the growth of beneficial gut bacteria and contribute to improved health. Industrial juice production results in juices with moderate amounts of soluble dietary fiber because most polysaccharides that constitute the dietary fiber are retained in the pomace. Incorporation of glucanases and pectinases into the juice production process can therefore enrich the juice with soluble fibre and possible prebiotic effects. Pear and apple cloudy juice was only composed of 5 % and 4 % of fibre (dry basis), respectively, mainly pectic polysaccharides. Pear and apple polysaccharides (mostly insoluble fibre) are retained in pomace (41-37%) after the industrial juice processing. Pear pomace is richer in xyloglucans and apple pomace richer in cellulose and pectic polysaccharides. Hydrolysis of apple pomace with endo- and exo-cellulase released a high amount of material (63.9 %), composed of monosaccharides and a variety of cellulose and xyloglucan oligosaccharides. Hydrolysis with pectin lyase, polygalacturonase, and pectin methylesterase hydrolysis release 57.7% material composed of pectic polysaccharides and derived oligosaccharides. In lignified by-product materials, more drastic strategies need to be used to recover polysaccharides and oligosaccharides with prebiotic activity. Pine nut skin, a by-product obtained during pine nut processing, is a source of insoluble dietary fiber[1]. Subcritical water extraction allowed to obtain an extract rich in phenolic compounds and mono- and oligosaccharides, and an extract rich in pectic polysaccharides and xyloglucans[2]. The fermentation of mono- and oligosaccharides and phenolic compounds did not cause a significant microbial shift upon fermentation. Nonetheless, it stimulated significantly ($p< 0.05$) the growth of the prebiotic *Bifidobacterium adolescentis* and the short-chain fatty acids production, specifically acetate and propionate. Pectic polysaccharides and xyloglucans had an impact on the microbiota composition similar ($p< 0.05$) to fructooligosaccharides used as positive control, also increasing the abundance of *B. adolescentis*. The core microbiome upon these polysaccharides' fermentation was composed of multiple butyrate producers, which lead to an increase ($p< 0.05$) of butyrate concentration. Besides, it was observed a microbial utilization selectivity towards xyloglucans in detriment to rhamnose-containing pectic polysaccharides and type II arabinogalactans.

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Donor Preactivation-Based Glycan Assembly: From Manual To Automated Synthesis

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Carbohydrates paly important roles in life science. However, due to the intrinsic complexity and heterogeneity of carbohydrate structures, furnishing pure and structurally well-defined glycans for functional studies is a formidable task, especially for homogeneous large-size glycans. To address this issue, we have developed a donor preactivation-based one-pot glycosylation strategy enabling multiple sequential glycosylations in a single reaction vessel.[1] Using this protocol, a range of glycans including tumor-associated carbohydrate antigens, various glycosaminoglycans, complex *N*-glycans and diverse bacterial glycans have been synthesized manually. Gratifyingly, the synthesis of mycobacterial arabinogalactan containing 92 monosaccharide units has been achieved, which created a precedent in the field of polysaccharide synthesis.[2] Recently, the synthesis of a highly branched arabinogalactan from traditional Chinese medicine featuring 140 monosaccharide units has been also accomplished to evaluate its anti-pancreatic cancer activity.[3] Based on the preactivation protocol, an automated solution-phase synthesizer has been developed.[4] Using this synthesizer, a library of oligosaccharides covering various glycoforms and glycosidic linkages was assembled rapidly. The automated synthesis of a fully protected fondaparinux pentasaccharide was realized on the gram scale. Furthermore, the automated synthesis of large-size polysaccharides was performed, allowing the assembly of arabinans up to an astonishing 1,080-mer using the automated multiplicative synthesis strategy.

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Microwave-Based Glycosylation Methodology Leads To Novel Immunotherapies

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Neutral glycosylation, using microwave irradiation, is novel in the realm of carbohydrate chemistry. Providing mild reaction conditions, decreased reaction times and enabling good yields with high purity are the major advantages of microwave promoted reactions. However, only very few reports have been described. A Microwave Labile protecting group (MWLPG) will be discussed that gives good yields and high alpha selectivity with simple to complex glycosyl acceptors. In contrast to conventional glycosylation, this methodology aims to function without the use of chemical promoters such as Lewis or Bronsted acids. Furthermore, our strategy for immunotherapeutics involves the use of a novel capsular polysaccharide as a “carrier”. Zwitterionic polysaccharides (ZPSs), are unique in that they are known to activate CD4+ T-cells through the MHC II complex in the absence of peptide(s), lipids or proteins. Furthermore, they induce IgM to IgG class switching, invoke immune memory and essentially have challenged the paradigm of carbohydrate immune processing as T-cell independent antigens. Polysaccharide A1 (PS A1) is one of the most well-studied ZPSs and it is expressed on the surface of Gram-negative obligate anaerobic bacterium, Bacteroides fragilis ATCC 25285/NCTC 9343. In order to further our understanding, key immunological mechanisms, well-characterized synthetic constructs of designed oligomeric lengths are of high importance and substantial value for a variety of reasons. This talk will therefore focus on chemical methodology for oligosaccharide synthesis and evaluation of synthetic PS A1 conjugates thereof.

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Heparan Sulfate Fragments Synthesis And Functionalization: Allyl As Janus Group

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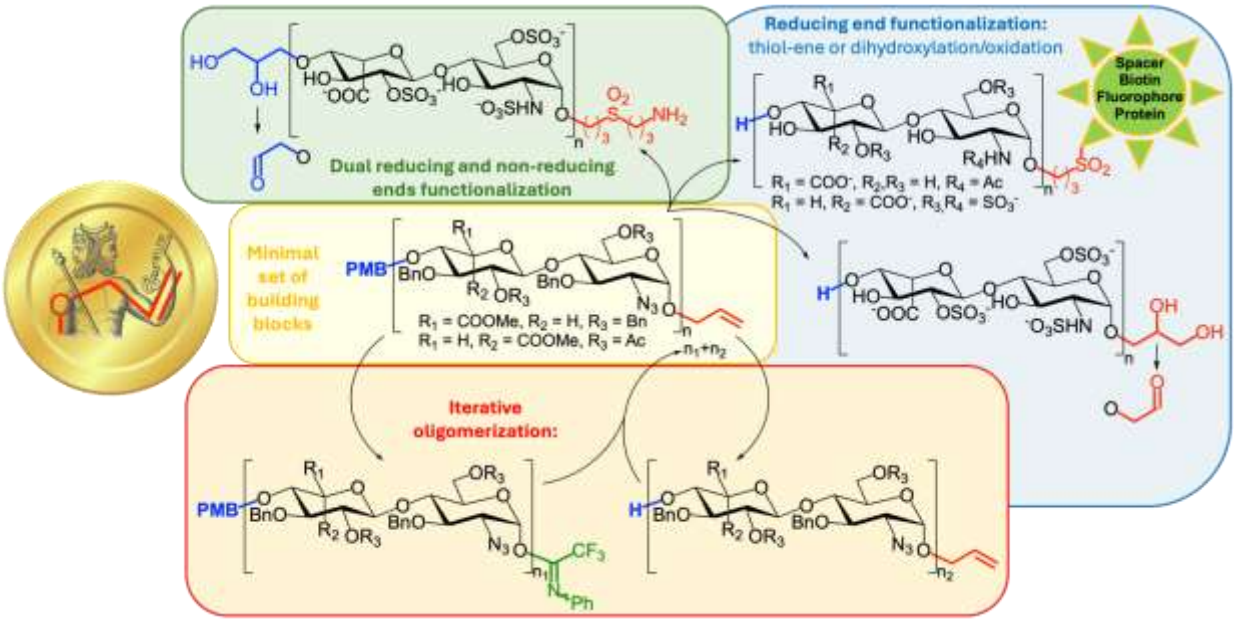
To address Heparan Sulfate (HS) biology and interactome, we have developed highly efficient modular syntheses of HS fragments of various size and structures. The exquisite orthogonality of the allyl and PMB groups allowed performing iterative oligomerization and structure diversification from a minimal set of disaccharide building blocks.[1] Importantly, from the very beginning of our studies, the allyl group was also chosen to be used as functionalization point once the oligosaccharide synthesis completed.[2] To this aim, we first used the thiol-ene reaction, for which we developed new "repairing conditions",[3] then used a dihydroxylation/oxidative cleavage strategy leading to a shorter and more rigid linker between the HS fragment and the functionalizing moiety. In this way we prepared: nanomolar inhibitors of IFN-γ proinflammatory activity,[4] nanomolar HIV-entry inhibitors,[5] Alexa Fluor 430 tagged heparosan dp8,[6,7] biotin tagged HS oligosaccharides... Finally, we developed a PMB/allyl interconversion methodology that allowed us to functionalize also the non-reducing end of synthetic HS fragments.

Taken together our studies clearly establish over the years the benefit of exploiting the Janus nature of the allyl group for efficient iterative syntheses **and** functionalization of HS oligosaccharides.



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Cister: A Chemical Glycan Editing Sequence

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Existing strategies for the syntheses of oligosaccharides follow convergent, divergent, chemical or enzymatic routes.[1] Contrary to these, nucleic acids enjoy the unique advantage of PCR, polymerases, and editing methods such as CRISPR[2] which have advanced the field to an unprecedented level. Progress in the synthesis of glycoconjugates is lagging behind due to inherent challenges in their syntheses.[3]

In this talk, the chemical editing of oligosaccharides viz. Cut-Insert-Stitch Editing Reaction (**CIStER**) sequence that stands on the subtle reactivity patterns will be presented (Figure 1).[4]

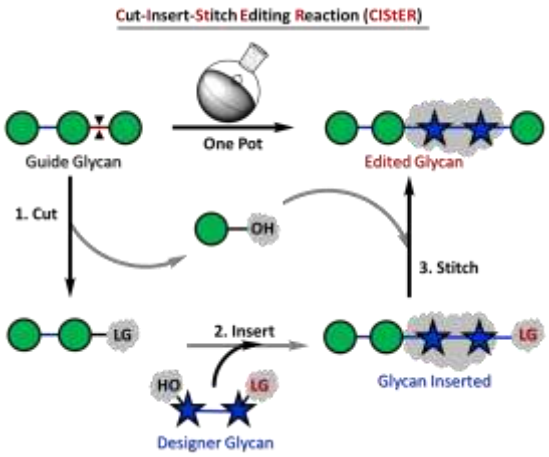

Figure 1. CIStER sequence

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Development Of Catalytic Stereoselective Glycosylations For The Synthesis And Evaluation Of Carbohydrate Vaccine Adjuvants

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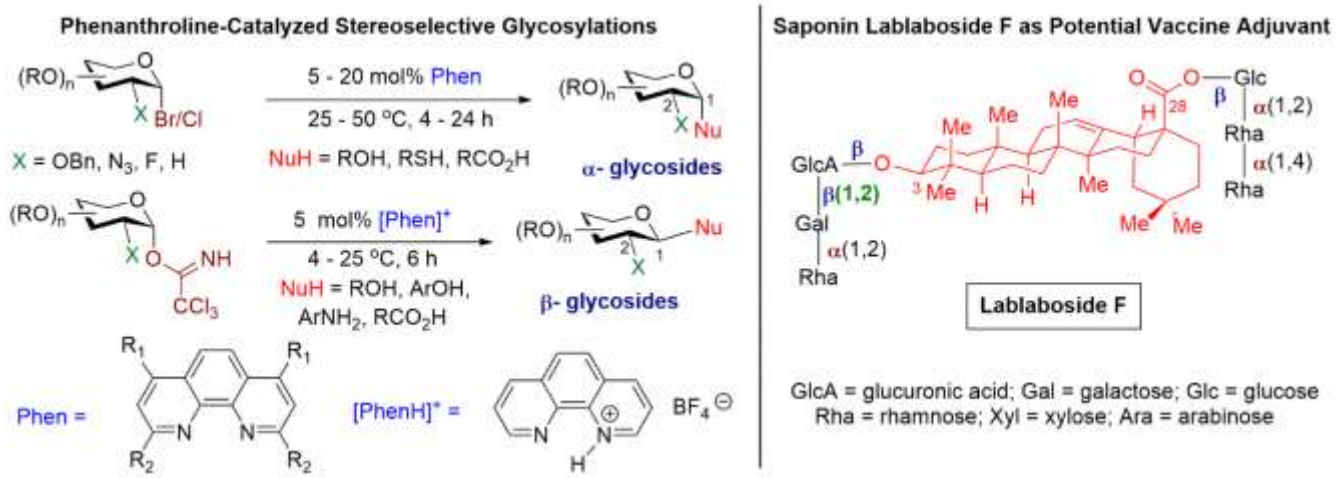
Although numerous classes of vaccine adjuvants have been developed over the past several decades, the choice of adjuvants for human vaccines remains limited because of the lack of available safe and effective adjuvants. Saponins are a class of sugar molecules that possess adjuvant activity. QS-21, a purified triterpene glycoside from a saponin mixture extracted from *Quillaja saponnaria tree*, exhibits adjuvant activity and has been used in HIV, antiviral, and anticancer vaccine trials.[1] However, there are several liabilities associated with its clinical use. First, QS-21 potency is dose-dependent and the required dose utilized cancer patients cannot exceed 100 μg. Second, it degrades in a matter of days at 25°C storage at pH 7.4 to produce toxic by-products with no adjuvant activity.[2] This led to the search for novel vaccine adjuvants with an extensive safety record. We have chemically synthesized a less toxic saponin, lablaboside F, and its analogs.[3] In the process, we developed the novel glycosylation methods[4] for the assembly of lablaboside F and its analogs. We recently evaluated these synthetic compounds as potential alternatives to QS-21. We discovered that one of synthetic lablaboside F analogs elicited increased production of OVA-specific total IgG antibodies 2 weeks post-boost compared to control mice when administered with an OVA peptide antigen intramuscularly into C57BL/6 mice using a prime-boost strategy. In addition, this synthetic compound exhibited decreased cellular toxicity 24 hour post-treatment compared to QS-21 *in vitro*. Interestingly, this potential vaccine adjuvant neither activated NF-κB alone nor induced significant production of the pro-inflammatory factor cytokine IL-1β with MPLA 24 hours post-treatment. These results suggest that this synthetic lablaboside analog does not activate the NLRP3 inflammasome, in contrast to QS-21. Overall, our data illustrate that novel saponin derivatives safely elicit humoral immunity through an NLRP3 inflammasome-independent mechanism and could potentially serve as subunit vaccine adjuvant alternatives to QS-21.



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Glycosylation Initiated By Non-Ionic Processes

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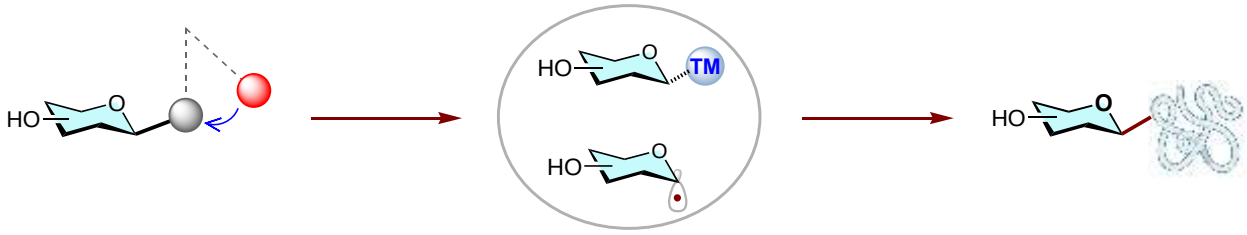
Glycosylation methods initiated by non-ionic processes have significant, but underexplored potential. In order to better exploit the power the such glycosylation methods, we have developed a range of bench stable glycosyl donors that can be readily prepared from simple starting materials. These donors can be activated to form glycosyl radicals or glycosyl transition metal complexes under mild conditions. Enabled by the use of these donors, we have developed a series of glycosylation methods and accomplished synthesis of diverse glycoconjugates that were previously difficult to access. In many cases, no protecting group on glycosyl donors is required. Moreover, it allows the utilization of external, delicate reagents or catalysts to control stereoselectivity, which is another essential task in carbohydrate synthesis.



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The Use Of Artificial Glycopeptides For Cancer Vaccines And Diagnostic Tools

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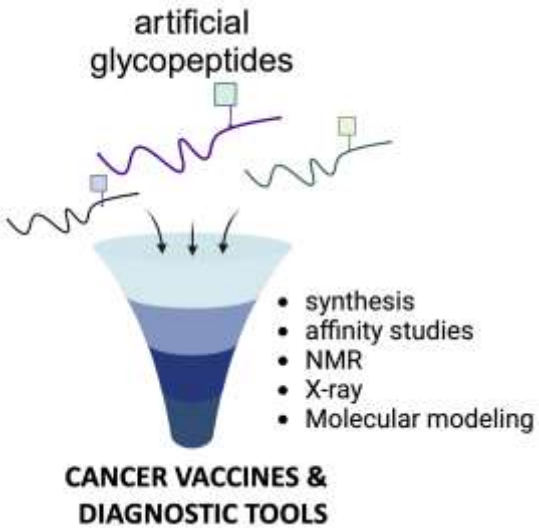
The glycoprotein mucin-1 (MUC1) is located on the surface of epithelial cells. In healthy tissue, MUC1 displays complex oligosaccharides characterized by branched and extended chains of glycans. In contrast, in cancer cells, glycosylated MUC1 residues exhibit altered *O*-glycan profiles where only simple or truncated glycan structures such as GalNAc (*N*-acetylgalactosamine) are attached to the MUC1 peptide scaffold. This change in glycosylation profile exposes different antigens of MUC1 that are otherwise hidden, such as the peptide sequence APDTRP or tumor-associated carbohydrate antigens, including the Tn antigens (α-*O*-GalNAc-S/T). Studies have shown that cancer patients can develop anti-MUC1 antibodies in the early stages of the disease, likely in response to these abnormal glycosylation patterns. Therefore, MUC1 is a promising target for cancer vaccine development and cancer diagnosis. [1,2]



Based on this premise, we have synthesized artificial MUC1 glycopeptides containing unnatural amino acids or modified carbohydrates. Our approach involves structure-based design to optimize the immunogenicity and stability of the glycopeptides using techniques such as NMR (nuclear magnetic resonance), molecular dynamics simulations and X-ray crystallography. These modified glycopeptides are then conjugated to gold nanoparticles or carrier proteins and serve either as cancer vaccines that elicit a robust immune response in mice or as diagnostic tools. [3,4] This presentation will describe several examples of these applications.

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Glycan Microarrays: Decoding Microbe-Host Interactions

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The interactions of glycans with their binding partners, governed by an intricate 'glyco-code', influence many of the key biological processes in all living organisms. As prominent host cell surface molecules, glycans mediate cell adhesion and trigger cell signaling, and serve as attachment sites for microbes playing a critical role in colonization and infection. As protective barriers and nutrient reservoirs, glycans such as those decorating mucins and proteoglycans at the mucosal surface, shape the dynamic interplay between the hosts and microbiota. Conversely, microbial glycans are recognized by proteins of the host immune system where they play crucial roles in triggering both innate and adaptive immune responses. Decoding these complex glycan-mediated microbe-host interactions holds immense potential for advancing therapeutics and vaccine designs.

Glycan microarray technologies, first introduced in 2002 in our group by Professor Ten Feizi and colleagues at Imperial College London (ICL), have gained momentum internationally and have revolutionized approaches to the molecular dissection of glycan-protein interactions. The ICL Carbohydrate Microarray Facility, since its foundation in 2012, has become an internationally leading operation, enabling collaborative studies on diverse glycan-mediated interactions involving the broad biomedical community (https://www.imperial.ac.uk/glycosciences/carbohydrate-microarray-facility/). Among the recent discoveries made through collaborative studies are the distinct glycan binding specificities of innate immune lectins that recognize different cell surface components of fungi [1], the recognition of glycosaminoglycans (GAGs) by human pathogenic viruses, such as adenoviruses and Chikungunya virus [2,3], and the binding to sialyl glycans by a minor pilin PilC of *Streptococcus sanguinis* type 4 pili (T4P) which provides novel functional and structural insights into the T4P-mediated host cell adhesion mechanisms [4].

In this communication, I will give highlights of our recent contributions to the unraveling of glycan-mediated interactions at the host-microbiota interface. I will discuss the latest advances at the ICL Facility in generating *epithelial O-glycome* arrays as a much-needed resource for microbial interaction studies, and the application of glycan microarrays in the analysis of glycan binding by whole bacterial cells that reside in the female reproductive tract with implications in preterm birth.

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Glyco-Engineering The Tumor Microenvironment Enhances Anti-Cancer Immunity

Heinz Läubli, MD PhD

Recent research has revealed that alterations in glycans can suppress cancer immune responses. For instance, suppressive myeloid cells have been shown to create an immunosuppressive microenvironment through the engagement of Siglec receptors. Our studies have demonstrated that we can effectively reduce myeloid cell-mediated immune suppression by applying sialidase within the tumor microenvironment. Moreover, we discovered that local desialylation can induce systemic anti-tumor immune responses by enhancing the cross-presentation capabilities of conventional dendritic cells and bolstering distant CD8 T cell-mediated anti-tumor immunity. Additionally, we achieved even greater anti-tumor responses when sialidase delivery was combined with fucosidase delivery in the tumor microenvironment. Overall, our findings indicate that glyco-engineering is a viable approach to enhancing anti-cancer immunity.

Galectins As A Therapeutic Target Of Synthetic Glycoconjugates

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Multivalent presentation of glycans on suitable carriers can multiply the biological potency of the synthetic glycoconjugate by several orders of magnitude [1]. One group of experimental protein targets with high biomedical significance are galectins – soluble human β-galactoside-binding lectins associated with tumor growth, angiogenesis, tumor cell migration, and evasion from the host immune system [2]. Besides cancer, galectins participate in pathological fibrotization related to pulmonary hypertension [3]. Selective multivalent glycoconjugates with a high affinity and selectivity can bind and/or inhibit overexpressed galectins, serving as prospective agents for both biomedical research and clinical applications.

To examine the potential of synthetic glycoconjugates to target galectins, we conjugated tailored glycomimetics and poly-LacNAc-type oligosaccharides to two multivalent carriers that featured high biocompatibility, non-toxicity, non-immunogenicity, renal clearance, and the ability to penetrate into cells. A library of glycopolymers based on *N*-hydroxypropylmethacrylamide exhibited antiproliferative, antimigratory, antiangiogenic, and immunoprotective properties in cell culture assays [2], and accummulation in the tumor. Hydrophilic glycopolymer inhibitors of galectin-3 based on polyoxazoline decreased the expression of markers of tissue remodeling in cardiac fibroblasts and pulmonary artery smooth muscle cells. In a biodistribution and pharmacokinetics study in rats, they accumulated in the heart and lungs, which are most affected by pulmonary hypertension [3]. These results demonstrate the potential of carbohydrate-loaded glycoconjugates as therapeutics of galectin-associated pathologies.

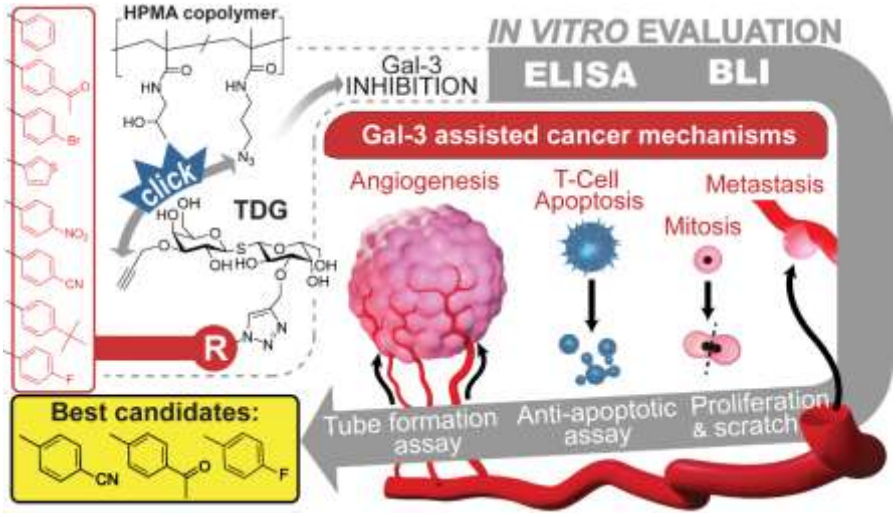

Figure. Synthetic glycopolymers as anti-cancer agents.

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Cell Fate Inducing Glycobiomaterials

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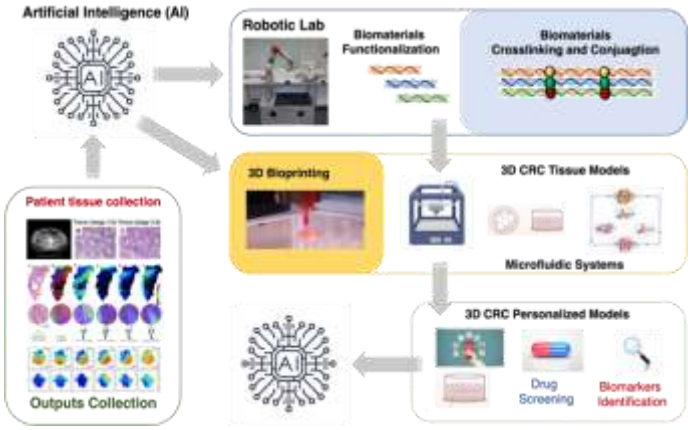
The development of human tissue mimetics is crucial for the advancement of healthcare management through the generation of in vitro organs for drug screening and tissue engineering. To this purpose hydrogels mimicking the Extracellular Matrix (ECM) morphology and signaling capacity must be generated [1,2]. The ECM glycosignature has a key role in the cell fate modulation, which is mediated by specific and multivarious interactions with cell receptors [3,4]. The generation of multifunctional glycobiomaterials employable in the design of functional organ-like constructs still represents an open challenge in the field. With the advent of Artificial Intelligence (AI) algorithms and automated manufacturing systems like 3D printing, it is now possible to control the formulation of glyco-biomaterials, limiting the combinatorial and artisanal chemical approach still utilized. Here in this talk, I will present my recent efforts to generate smart multifunctional biomaterials with patient required properties using also AI algorithms.



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Unveiling Siglec Interactions: Insights Into Glycan And Antibody Binding Mechanisms

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Hypersialylation, an aberrant increase in the expression of sialic acid, profoundly impacts tumor cell interactions with their microenvironment. Siglecs, immune receptors that recognize cell surface sialic acids, play a pivotal role in immune surveillance within the tumor microenvironment. Our group has studied the interaction between glycans containing sialic acids (sialoglycans) and Siglec receptors on immune cells, demonstrating that this pathway can be targeted to regulate immune responses and control tumor growth. Leveraging structural biology techniques, including X-ray crystallography, NMR, and molecular dynamics, we have scrutinized the molecular details governing their specificities for sialoglycans[1-6]. This information offers opportunities for developing novel molecules targeting Siglecs through modified sialic acids. Our research also focuses on uncovering structural insights on anti-Siglec antibodies to refine antibody-based therapies.

I thank AEI & MICIU (Spain), EU-GlycoTwining, EU-Glytunes, and BBVA Leonardo for funding, and all my collaborators and colleagues who are contributing to this research: Klaudia Sobczak, Iker Oyenarte, Unai Atxabal, Andrea Fernández, Pablo Valverde, Eunate Valdaliso, M Pia Lenza, M Elena Laugieri, Leire Egia-Mendikute, Asier Antoñana, Alexandre Bosch, Asis Palazón, and Jesús Jiménez-Barbero.

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Diversity-Oriented Synthesis And Ms/Ms-Based Analysis Of Glycosphingolipids

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Glycosphingolipids (GSLs) are the major glycolipids and a key component of the cell membrane in higher animals, which play a pivotal role in many physiological and pathological processes. For example, over 80% of the glycans in the glycocalyx of brain cells are from GSLs, and GSLs are directly involved in brain debelopment and functions such as cellular recognition, adhesion and signal transduction, cognition and memory, as well as an array of human dieseases like cancer, Alzheimer's disease, Parkinson's disease, and lysosomal storage disorders (LSDs). However, due to their complex and diverse structures, GSLs are difficult to isolate from natural sources and GSL characterization is very challenging. This has hindered in-depth investigations of GSLs.

To address these issues, we have developed a novel, diversity-oriented synthetic strategy for GSLs and GSL derivatives (Figure 1A).[1] This strategy is highlighted by using a simple lactoside containing the core structures of GSL glycan and lipid as a universal starting material to access various GSLs upon stepwise elongation of the glycan through either chemical or enzymatic glycosylations followed by chemoselective on-site lipid remodeling. The strategy enables a three-way diversification of the glycan and lipid moieties to rapidly generate both natural and functionalized GSLs, which are useful for various investigations and applications. Using natural GSLs as standards, we have also established a new MS/MS-based strategy for GSL characterization and GSL-omics analysis (Figure 1B).[2] This strategy is based upon two-stage matching of the MS/MS spectra of experimental and reference GSLs. During the first stage, carbohydrate fragment ions are matched to give GSL species identification; in the second stage, remaining glycolipid fragment ions are characterized by the rule-based matching method for lipid identification. This strategy uses a limited reference library containing the MS/MS spectrum of only one lipid form for each GSL species to identify all other lipid forms. It has been used to analyze brain GSLs to demonstrate its applicability to, for example, profiling of biological GSLs and discovery of new GSL markers related to human diseases. Synthetic GSL derivatives have also been employed to investigate the functional mechanisms of GSLs, such as their interaction with membrane proteins for signaling transduction.

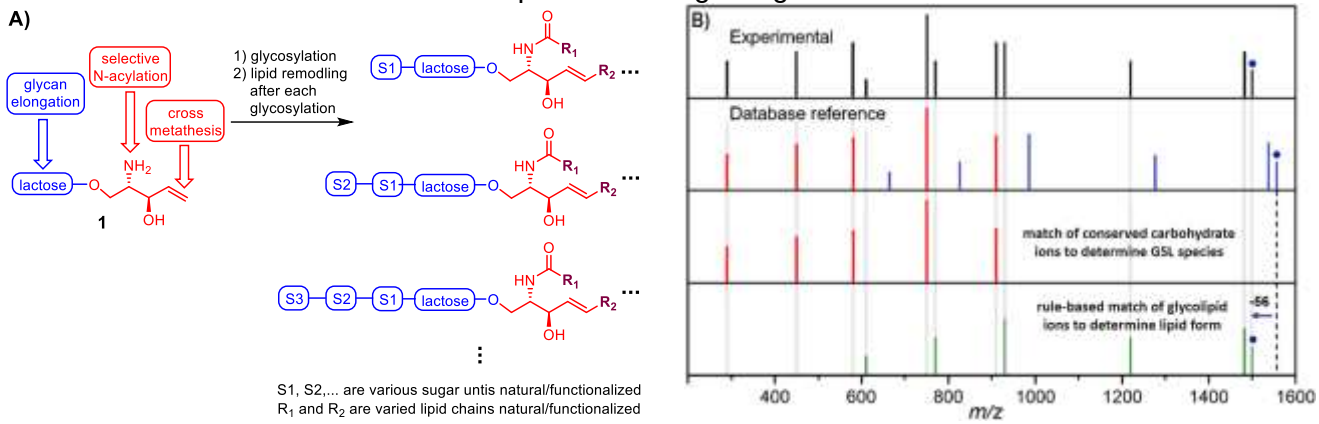

Figure 1. **A**) A diversity-oriented strategy for GSL synthesis through three-way diversification of the glycan and lipid moieties. **B**) Characterization of GSLs through two-stage matching of experimental and reference MS/MS spectra.

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Reinforcement Of Protein-Protein Interaction By Glycosylation

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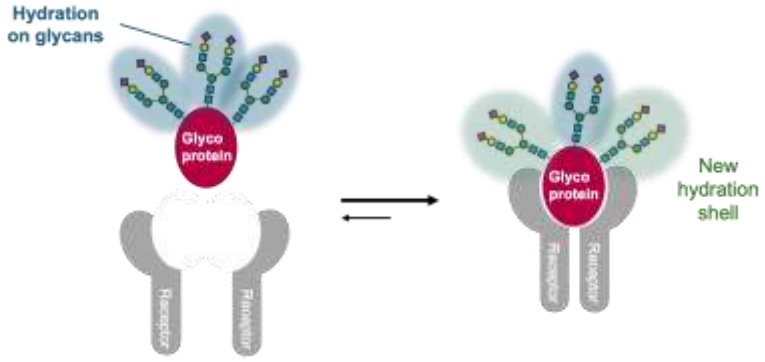
Proteins are ubiquitously glycosylated and play essential roles in many biological events. It is known that the glycans employ a unique hydration property. Therefore, glycans and their unique hydration properties may enhance glycoprotein functions. Although almost all biological events in the extracellular area are initiated by protein-protein interactions, little is known about the correlation between glycan hydration and protein functions. To address this fundamental question, we carried out total chemical syntheses of homogeneous glycoproteins and functional analyses of their hydration function. We synthesized erythropoietin glycoforms, cytokines, antifreeze glycoprotein and small proteins with varying glycosylation positions and glycan structures. We used our original new NMR techniques to analyze the interaction between water molecules and a glycan. Along with NMR measurements, ITC, SPR, and hydrogen-deuterium exchanging experiments were conducted toward homogeneous glycoproteins. Based on these results, we hypothesize that the unique hydration functions of glycans may accelerate and stabilize the protein-protein interactions.



Glycomimetic-Based Cell-Specific Transdermal Delivery Of Mrna And Proteins To Langerhans Cells

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The ability to target hepatocytes using trivalent presentations of GalNAc has revolutionized therapeutic approaches. This innovation has paved the way for the recent approval of several RNA-based drugs, including Givlaari, Oxlumo, Leqvio, Amvuttra, and Rivfloza. These successes highlight the power of carbohydrate-based delivery systems in achieving cell-specific delivery, a cornerstone of many groundbreaking nucleic acid-based medicines, including gene editing technologies. This targeted delivery approach represents a significant advancement in precision medicine.

In this work, we present the discovery and development of a novel glycomimetic small molecule specifically designed to bind to Langerin, a C-type lectin receptor expressed on Langerhans cells (LCs) [1,2]. LCs, antigen-presenting cells residing in the epidermis, play a critical role in orchestrating systemic immune responses against invading pathogens. Additionally, under normal conditions, they contribute to maintaining peripheral T cell tolerance. Given their strategic location in human skin, our glycomimetic-driven active targeting approach demonstrates exceptional capabilities in reaching LCs [3,4]. Ex vivo and in vivo investigations have confirmed the efficacy of our platform technology in targeted therapeutic delivery, ultimately leading to enhanced immune responses. These findings emphasize the transformative potential of this glycomimetic platform for efficient and targeted delivery of therapeutic payloads specifically to LCs.

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Deciphering *In Vivo* Glycobiology With Genetically-Encoded, Multivalent Liquid Glycan Array (Liga) And Liquid Lectin Array (Lila)

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A major barrier to studying the role of glycans *in vivo* is the fundamental lack of one-to-one correspondence between sequence of DNA and structures of carbohydrates. Investigations of carbohydrates, thus, cannot rely on DNA sequencing directly. To solve this challenge, we introduce genetically-encoded platform technologies termed Liquid Glycan Array (LiGA) [1,2,3] and Liquid Lectin Array (LiLA) [4]. Glycan arrays, made by ligation of carbohydrates to glass or bead surfaces and complementary array of lectins on glass slides are the workhorse tools in glycobiology. These technologies cannot measure interactions of glycans and GBPs in their natural environment on the surface of cells in tissues *in vivo*. In contrast, Liquid arrays introduce one-to-one correspondence between DNA sequence and carbohydrate structure of glycan or glycan binding protein displayed on phage. They enable unsupervised profiling of interactions of glycans with receptors on the surface of cells *ex vivo* and *in vivo*.

LiGA is produced by chemical [1] and chemoenzymatic ligation [2] of carbohydrates to bacteriophage (phage) particles. Genetically encoded, monodisperse carriers based on 700 mm long M13 phage particles display 50-1000 copes of glycans on their surface. The identity and presentation (density) of glycans are encoded by the DNA barcode inside the phage genome. LiLA in turn is produced by enzymatic ligation of lectins to bacteriophage using SpyCatcher-SpyTag technology [3,5]. LiGA and LiLA uncovered an optimal structure/density combination for recognition for a wide collection of lectins and immune lectin proteins (DC-SIGN and Siglec family proteins) expressed on live cells [1,2,4]. LiLA can detect presence of specific glycoisoforms on live cells. Injection of the LiGA or LiLA into mice identified glycan:GBP interactions necessary for homing to specific organs. This work provides an unprecedented quantitative evaluation of the interaction of complex glycans with GBPs *in vitro* and *in vivo*.

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Structure-Specific Analysis Of Glycosylation Modification Using Mass Spectrometry

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Glycosylation is a common modification on a variety of biomolecules including nucleic acids, proteins and lipids. In contrast to the single structures of small-molecule modifications such as methylation, acetylation and phosphorylation, glycosylation has tens of thousands of structures coming from multiple structural dimensions including sequence, linkage, anomer, and conformation. This talk will introduce our developments of structure-specific analysis of glycosylation on the backbone molecules of RNAs, proteins and lipids using mass spectrometry. Structure-specific qualitative and quantitative analysis of monosaccharide sequence structure isomers (including fucose core/branch/terminal position isomers) as well as sialic acid linkage isomers (α2,3 vs. α2,6) is achieved with in silico construction of theoretical glycans together with chemical derivatization, chromatographic separation, and/or tandem MS/MS. Both sequence and linkage structure isomers are widely identified; in malignant diseases, both sequence- and linkage-dependent differential expression (including the extreme combination of up-down or down-up pair) of glycosylation are extensively observed, which not only justifies upstream structure-specific characterization of glycosylation, but also downstream mechanistic and functional studies as well as final biomedical applications in terms of diagnostic and prognostic biomarkers, drug targets and drugs.

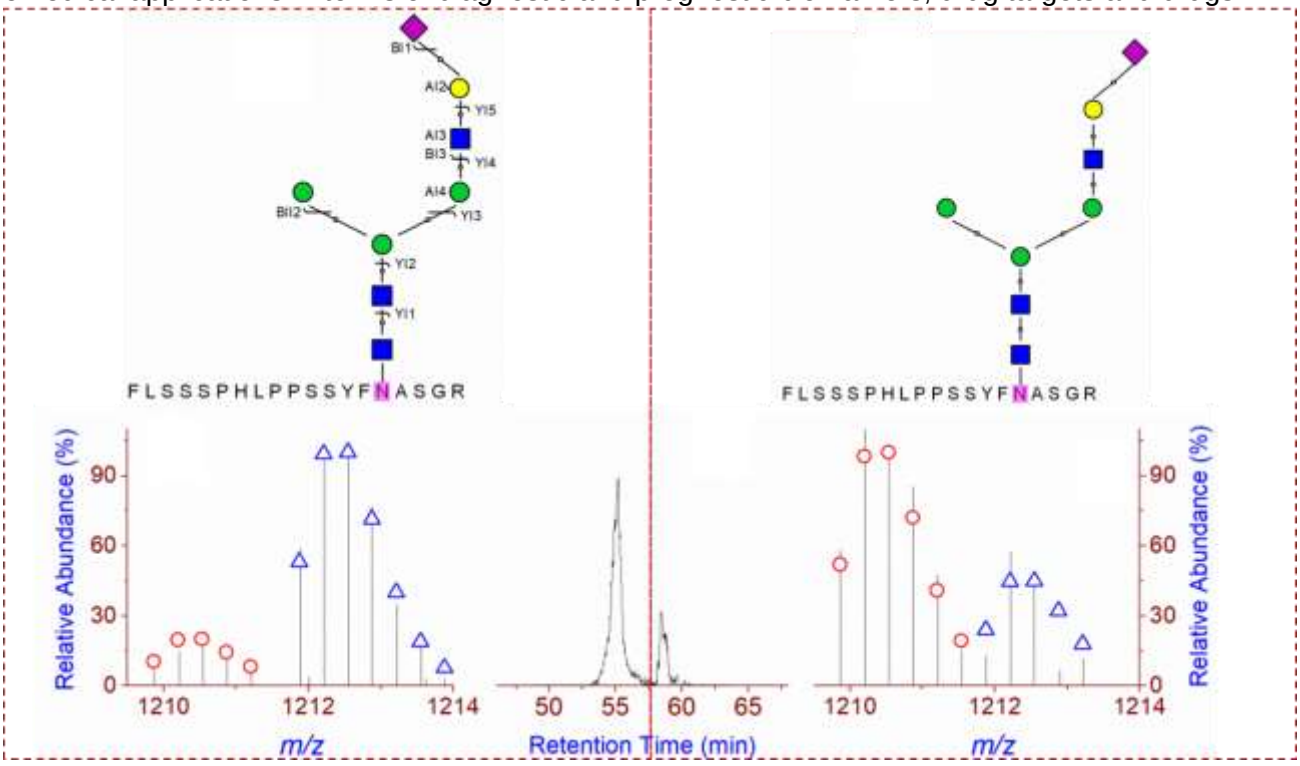

Figure 1. Sialic acid linkage-specific differential expression with α2,3 up-regulated (left panel) and α2,6 down-regulated in hepatocellular carcinoma HepG2 cells (relative to normal liver LO2 cells).

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Immunotherapy Targeting Cancer Glycosylation

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Immunotherapy aims to boost host immune response relying on specificity of antibodies and T cells, and their ability to distinguish between cancer and normal cells. Despite the phenomenal progress in this field, cancer remains a major cause of death, mainly due to difficulties in identifying potent target antigens. Carbohydrate chains (glycans) ubiquitously occupy the surface of cells, but cancer cells express aberrant glycans. We designed cancer immunotherapy targeting glycan-neoantigens, through an innovative interdisciplinary approach. The efficacy and immune responses were investigated systematically in vitro and in vivo for optimized glycotherapy. This approach holds promise as a new frontier in the fight against cancer.

CARBOHYDRATE MODIFICATION ON NATURAL PRODUCTS, PEPTIDES AND PROTEINS FOR DRUG RESEARCH

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Carbohydrate is a common structural motif in many clinic drugs and plays an important role in druggability. We sought to develop efficient carbohydrate modification chemistry on natural products, peptides, and proteins for drug discovery. In particular, we introduce extra sugar motifs onto vancomycin scaffold to enhance the antibacterial activity and reduce the toxicity to find better antibiotics against drug-resistant bacteria. We also engineered the glycan of IgG monoclonal antibodies to develop new generation antibody-drug conjugates for cancer therapy. New chemistry of N-terminal tryptophan modification with saccharides is also report for biomolecule conjugation. These data provide the concept and techniques for carbohydrate-containing drug discovery.

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Chemical Structures Of O-Polysaccharids Isolated From Phytopatogenic Bacteria

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The genera *Dickeya* and *Pectobacterium* contain economically important plant pathogenic bacteria. These bacteria cause symptoms of soft rot and blackleg in potatoes and a variety of ornamentals, herbs and vegetables. Phytopathogens are able to penetrate host tissues through the efficient production of plant cell wall degrading enzymes with pectinolytic, cellulolytic, proteolytic and lipolytic properties. Another very important virulence factor for these microorganisms is lipopolysaccharide (LPS). It is involved in bacterial adhesion to plant tissues and also interacts with host defence systems.

LPS is an important pathogenic agent with varying toxicity depending on its chemical structure. LPS is composed of a lipid A (endotoxic fragment), a core (regulates the toxicity of lipid A) and an O-specific polysaccharide (OPS), which has strong antigenic properties and is one of the most highly expressed components of the cell wall of Gram-positive bacteria.

In the study, the chemical structures of selected OPSs isolated from selected bacteria of the genera *Dickeya* and *Pectobacterium* were determined[1-6]. The knowledge of the OPS structure of different phytopathogens enhances the understanding of the molecular mechanisms of bacterial-host interactions and may lead to the development of new species-specific identification methods as well as ways to prevent plant diseases that contribute to high economic losses.

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Oral Lectures

Targeted Protein O-GlcNAcylation Using Bifunctional Small Molecules

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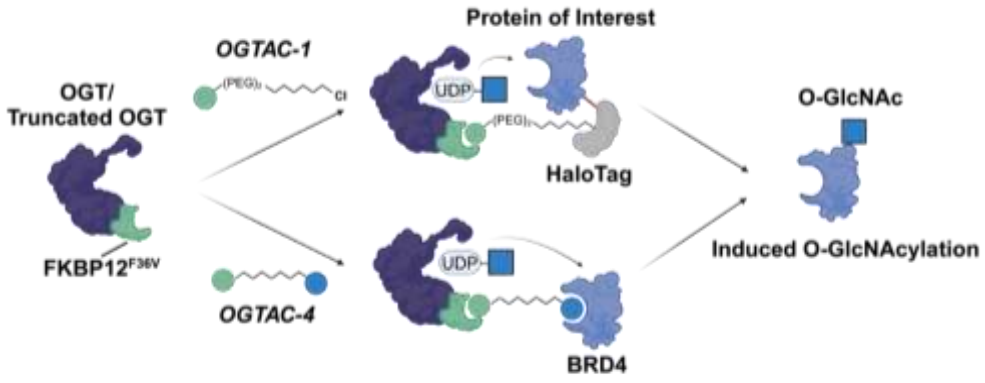
Protein O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) plays a significant role in the regulation of transcription, metabolism, cell signaling, protein stability, and nucleocytoplasmic trafficking.[1] Abnormal O-GlcNAcylation was reported to reprogram cellular metabolism to favor cancer proliferation.[2] Of note, both hyper- and hypo-O-GlcNAcylation are associated with tumorigenesis and resistance to anti-cancer therapy, suggesting that O-GlcNAc homeostasis is one of the underappreciated hallmarks of cancers.[3,4]

However, the lack of precise approaches for the manipulation of protein-specific O-GlcNAcylation greatly hindered the comprehensive dissection of its functions in human diseases. To address this, we have developed heterobifunctional small molecules, named O-GlcNAcylation TArgeting Chimeras (OGTACs), which enable protein-specific O-GlcNAcylation in living cells.[5] OGTACs promote O-GlcNAcylation of proteins such as BRD4, CK2α, and EZH2 *in cellulo* by recruiting FKBP12^{F36V}-fused O-GlcNAc transferase (OGT), with temporal, magnitude, and reversible control. Overall, the OGTACs represent a promising approach for inducing protein-specific O-GlcNAcylation, thus enabling functional dissection and offering new directions for O-GlcNAc-targeting therapeutic development.



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Rational Design Of Mucin Glycopeptides As Tumor Immunotherapeutic Agents

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Tumor-Associated Carbohydrate Antigens (TACAs) are aberrant glycan structures displayed on tumor cell-surface proteins and lipids. They are directly or indirectly responsible for many tumor characteristics, such as aggressiveness and metastatic potential. Additionally, their altered structures contribute to the host's antitumor immune response, and thus they have been employed as immunogens in the design of tumor vaccines. Our interests lie in O-glycosylation, which refers to reducing-end GalNAc glycans attached through an alpha linkage to the oxygen of serine (S) and threonine (T) residues. These are primarily displayed on mucins, large cell surface proteins that form a protective layer on tissue epithelia. The extracellular domains of mucins are made up of tandem repeat (TR) peptide sequences containing many S, T and proline (P) residues, where most all of the S/T residues are O-glycosylated. In particular, Mucin-4 (MUC4) is overexpressed on pancreatic ductal adenocarcinoma (PDAC) cells but not normal tissues. Taking advantage of this differential expression, our lab seeks to develop multivalent inhibitors and vaccine constructs based on gold nanoparticles (AuNPs). Vaccination of mice with several MUC4 TR glycopeptides containing the Thomsen-Friedenreich TACA disaccharide (viz., Galβ1-3GalNAc-α-*O*-S/T) coated on AuNPs uncovered a lead sequence to which we developed a monoclonal antibody (referred to as F5). F5 shows specificity for PDAC cells and tissues *in vitro* and showed $MUC4^{+}$-specific biodistribution to cell and patient derived xenografts *in vivo*. This led us to develop an antibody-drug conjugate (ADC) with a protease-cleavable linker that is able to target MUC4+tumors *in vivo* and show therapeutic efficacy. To better understand the substrate specificity of the MUC4 TR sequence, we enzymatically glycosylated the TR with ppGalNAc-transferase 1, the most promiscuous of the 20 mammalian ppGalNAc transferases, which are responsible for adding the first GalNAc residue to S/T residues in the biosynthesis of extracellular O-glycans. This specificity allowed us to determine the most prominent residues for glycosylation as a prelude to the production of better immunotherapies. We chemically synthesized each of these sequences, characterized their structures by NMR spectroscopy and embarked upon vaccination studies in hopes to produce even more potent and tumor-specific antibody and T-cell responses. This talk will highlight the rationale for our approach, detail the biological activity of each of our glycopeptides, and report preliminary data on the efficacy of both ADCs and CAR-T cells based on F5.

Lipopolysaccharides From Microbiota: Fantastic "Structures" And Where To Find Them

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The Gut Microbiota is an essential actor in the modern concept of human health driving many host physiological and pathological processes, including immune system modulation.[1] Initial sensing of microbes by the host immune surveillance is mediated by the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPSs), which are typically conserved among bacteria, i.e. they are shared by both commensal, mutualistic and pathogenic microbes inhabiting our intestines. [1] Due to its chemical structure, LPS is considered a potent elicitor of immune inflammatory reactions in mammals, being usually associated to perilous bacteria and detrimental outcomes for human health. [2] Nevertheless, LPS also decorates the membrane of harmless and beneficial Gram-negative bacteria composing our gut microbiota.[2] How LPS is tolerated and remains (apparently) silent in the gut is a major unsolved question representing a frontier in our understanding of innate immunity.

Deciphering the chemical structure and immunological properties of LPS from gut microbes, especially of those able to establish a neutral or beneficial relationship with the human host, is of paramount importance in biology, with tremendous repercussions for basic and clinical domains of biomedicine. In this frame, a detailed structure to function study focused on LPS from gut microbiota will give insights in the mechanisms at the basis of host-microbe crosstalk, both at the intestinal and systemic level. This will provide, in parallel, priceless information about how gut microbes modulate immune response through their LPS, thus resulting in an unprecedented improvement of the knowledge of the immune system.[1,2] Overall, the structural and functional information as well as the chemical tools that can be delivered by analyzing LPS from microbiota will result in advances beyond the state-of-the-art in the biomedical field, as they furnish a starting point (i) to create novel immune-therapeutics and (ii) to identify new biomarkers with diagnostic, prognostic, and/or predictive value in the frame of immune-mediated pathologies.

In this communication, I will show recent results about the chemical structure and immunological properties of LPS from a specific gut commensal, *Veillonella parvula*.[3] In particular, I will show that this bacterium produces an unprecedented LPS chemical structure and I will discuss about the structural moieties responsible for the observed immunomodulatory properties. In parallel, I will also show the potential of gut microbiota LPS as predictive biomarkers in food allergy and as a target of intervention to limit disease burden.[4]

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Development Of A Mucin O-Glycan Library For Investigating Pathogen Virulence

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The mucosal layer lining the epithelial surfaces of the respiratory, gastrointestinal, and urogenital tracts is widely recognized for its significant influence on the microbiome and serves as an initial defense against infections. Several recent studies have reported on the ability of mucin glycoproteins and their associated O-glycans to downregulate the expression of virulence-associated genes in diverse cross-kingdom pathogens. However, native mucin glycans are structurally diverse and the isolation of pure, defined individual structures in suitable amounts has proven extremely challenging due to their similar physical and chemical characteristics. This has prevented the study of the virulence attenuating properties of individual glycan structures. Therefore, to elucidate which specific glycans are responsible, we have been developing a convergent and scalable approach to obtain a comprehensive library of structurally defined mucin O-glycans in sufficient quantity and quality (>30 mg of target glycan). An initial library of core 1 & core 2-type methyl glycosides was first established[1] and has been successfully used to identify discrete glycan structures responsible for virulence attenuation in fungal pathogen *Candida albicans*[2,3] and prominent Gram-negative bacterial pathogen *Vibrio cholerae*.[4]

In subsequent work, we have recently expanded the mucin glycan library to include core 3 & core 4-type structures.[5] These compounds were synthesized as methyl α-glycosides retaining the stereochemistry of the natural GalNAc-Ser/Thr linkage in mucins. The strategy of the glycan assembly was based on building blocks developed in our previous work, aiming to maximize convergence in our process. Our focus primarily centered on core 3, core 3-Gal (type I), core 3-Gal (type II) and core 4 methyl glycoside derivatives. These structures provide a further expansion of our mucin glycan library to assess the virulence attenuating capabilities of individual glycans.

As the molecular mechanisms through which mucin O-glycans regulate pathogen virulence remain poorly understood, the development of synthetic methods to generate a comprehensive library of mucin O-glycans in sufficient quantity and purity will enable exploration of these processes and subsequently the development of novel therapeutic strategies.

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Developing Rhamnose-Modified Biomolecule For Cancer Immunotherapy By Recruiting Natural Antibody

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Abstract：Endogenous antibodies are naturally occurring antibodies present in the human circulatory system, targeting antigens such as the dinitrophenyl (DNP) group, galactose-α-(1,3)-galactose (αGal), and rhamnose (Rha).[1-3] Harnessing these antibodies for specific disease cell targeting has the potential to enable adaptive immunities for selective and effective therapy.[4-7] In this study, we designed and synthesized a series of multivalent rhamnose (Rha)-modified nanobody conjugates and investigated their antitumor activities, as well as their potential to overcome cetuximab resistance.

Structure-activity relationship studies revealed that the multivalent conjugate **D5**, which carried sixteen Rha haptens, was able to trigger the most potent Fc-mediated innate immunity to promote cancer cell death, highlighting its potential for cancer immunotherapy. Notably, **D5** exhibited complete resistance to enzymatic degradation by the serine protease (PRSS), which is commonly found in the tumor microenvironment of metastatic colorectal cancer (mCRC) patients and can degrade cetuximab. In contrast, cetuximab lacked the ability to induce complement-dependent cytotoxicity (CDC) due to the presence of complement regulatory proteins on tumor cells, while **D5** demonstrated robust in vitro CDC cytotoxicity. The remarkable antitumor activity of conjugate **D5** was demonstrated in a cetuximab-resistant tumor cell derived xenograft mice model as well.

These results highlight the potential of Rha-Nanobody (Nb) conjugates as a promising therapeutic strategy for treating cetuximab-resistant tumors. By enhancing innate Fc effector immunity and promoting the recruitment of endogenous antibodies to facilitate cancer cell clearance by innate immune cells, Rha-Nb conjugates offer a novel approach to address cetuximab resistance and improve therapeutic outcomes.[8]

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Synthesis And Immunomodulatory Functions Of Glycolipids Via C-Type Lectin Receptors And Lipid Antigen Presentation

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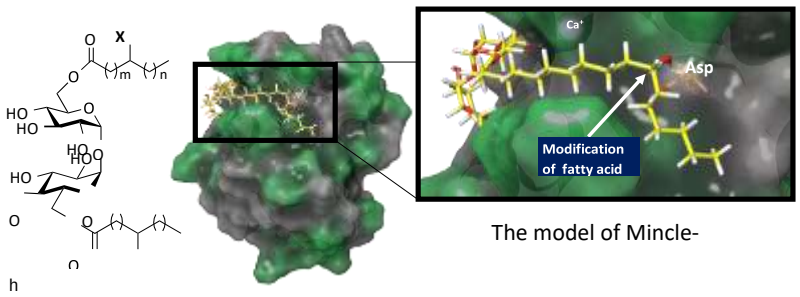
An innate immune receptor, macrophage-inducible C-type lectin (Mincle) receptor, recognizes various microbial and endougenous glycolipids, leading to signal transduction and activation of the innate immune system. The ligands include trehalose dimycolate (TDM) from *Mycobacterium tuberculosis* and -GlcCer from damaged endogenous cells. Some of Mincle ligands also interact with lipid antigen presenting molecules such as CD1d.

Although several types of Mincle ligands have been reported along with importance of the lipid moiety, the detailed recognition mechanism of the hydrophobic part of the Mincle ligands is not fully understood. We thus synthesized various glycolipids focusing on the structures of the lipid moiety with utilizing several headgroups including trehalose, -mannosyloxy-mannitol or -GlcCer backbone. After establishing the synthetic methods for -mannosyloxymannitol glycolipid “44-2” and their analogues,[1] similarly modified lipid moiety (e.g. 10-hydroxy stearic acid and analogues) was applied for the design and synthesis of trehalose diester analogues,[2] with linking to the strong immune modulators of lipid- modified GalCers.[3] We also synthesized and analyzed GlcCer’s structure-activity relationships. Hence, we have revealed several hot spots on Mincle surface for the lipid recognition. Based on the results of the characteristic amino acid residues of Mincle surface for hydrophobic moiety recognition site, we also synthesized fluorescence-labeled molecular probes and utilized them for the observation of the intracellular behavior of Mincle and the ligand.



The model of Mincle-

Trehalose ester containing a polar functional group-modified lipid moiety

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Labeled Xylosides As Tools For Glycosaminoglycan Investigation

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Proteoglycans (PGs) consist of a core protein and long negatively charged carbohydrate chains called glycosaminoglycans (GAGs). The PGs are located mainly in the extracellular matrix of mammalian cells and are involved in a variety of important processes including cell signalling, proliferation, and anticoagulation. A key molecule for GAG biosynthesis is the carbohydrate xylose, which is the anchor point between the core protein and the GAG chains. This carbohydrate acts as a substrate for the enzyme β4GalT7 and it is previously known that xylose with certain aglycons can produce soluble GAGs. The biosynthesis of GAGs is quite elusive and therefore it is of interest to fluorescently label xylose or already biosynthesized GAGs in order to use them as tools for investigation.

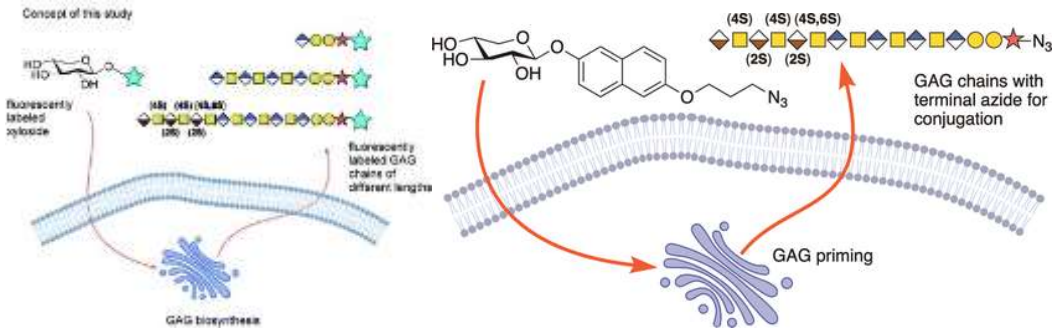

***Figure 1**: Concept of the biosynthesis of fluorescent or azide labeled GAGs.*

To achieve this, several fluorescent xylosides[1] and an azide labeled xyloside[2] were synthesized. The fluorescent xylosides could then be studied with confocal microscopy to visualise GAG biosynthesis, co-localisation, and transport in the cell.

The azide labeled naphthoxylosides primed full-length soluble GAGs which could thereafter be isolated. Using copper-free click chemistry the GAGs could then be conjugated with a multitude of appropriate alkyne containing molecules and be used for further GAG investigation.

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Epitope Mapping Of The Pa Surface Polysaccharide Pel

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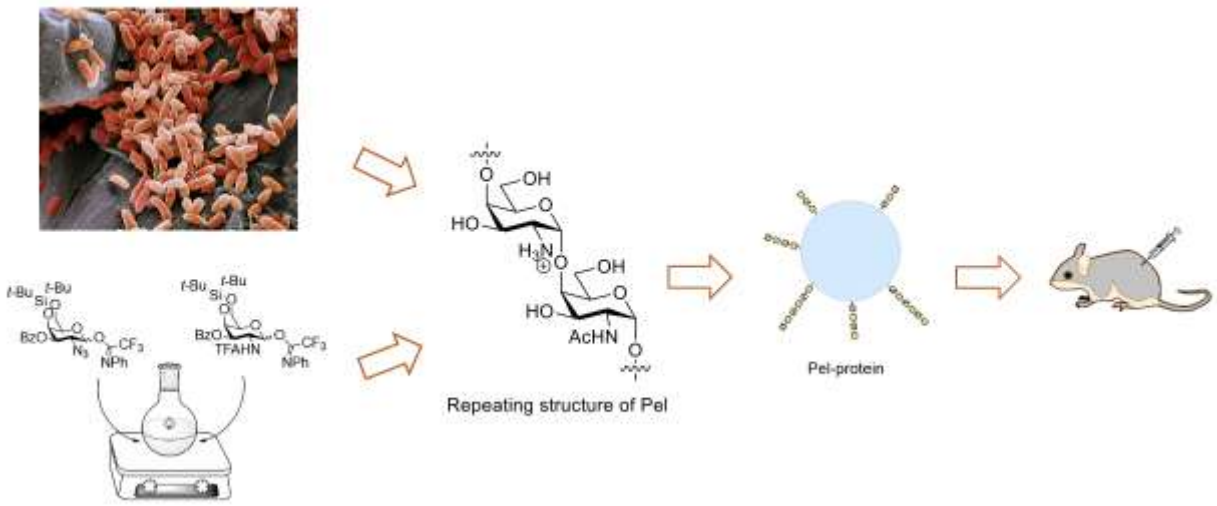
Pseudomonas aeruginosa (PA) is an antimicrobial resistant pathogen responsible for serious infections and it belongs to the ESKAPE pathogens, which are the main cause of nosocomial infections and a growing concern for antibiotic resistance. Developing a vaccine against *P. aeruginosa* is therefore a promising approach. Three exopolysaccharides, which are considered potential targets for bacterial vaccine development, have been identified in the *P. aeruginosa* biofilm: alginate, Psl, and Pel.

The structure of exopolysaccharide Pel has recently been elucidated and is thought to be composed of 1,4-alpha-linked galactosamine and N-acetyl galactosamine[1-2]. Well-defined Pel fragments have been synthesized and will be used as standards for structure elucidation studies, as well as being conjugated to CRM_{197} and BSA for use in immunogenicity studies. Meanwhile, natural Pel will be purified from the *P. aeruginosa* strain for vaccine generation and structural analysis experiments.



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Exploiting Cyclic Sulfates And Sulfamidates For Targeting Glycosidases

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Glycosyl hydrolases (GHs) play a crucial role in breaking down glycosidic bonds within complex carbohydrates, making them essential enzymes in both health and disease. Targeting specific GHs with precision is of paramount importance in biomedical research, as it underscores the development of precise therapeutic strategies for disorders associated with carbohydrate metabolism. These enzymes fall into two main categories based on their reaction mechanisms: retaining GHs which follow a two-step mechanism involving the formation of a covalent intermediate, and inverting GHs, which employ a single-step S_N2 type reaction. In the process, they undergo various conformational states to adapt to the steric and electronic properties of their carbohydrate substrates. Mimicking these conformational transitions has proven highly effective in designing selective inhibitors for various glycosidases. For instance, covalent nanomolar inhibitors for α-glucosidases have been developed using 1,6-*cis*-cyclic sulfate electrophiles, mimicking the $^4C_1$ initial Michaelis complex conformation (Figure 1).[1] Similarly, introducting a nitrogen atom linked to the pseudo-anomeric position has yielded 1,6-*cis*-cyclic sulfamidates functioning as selective competitive inhibitors for α-galactosidases[2] and α-glucosidases[3], depending on their respective configurations. More recently, we have developed 1,6-*trans*-cyclic sulfates and sulfamidates exhibiting a flipped $^1C_4$ chair conformation. Since inverting GH47-α-mannosidases follow a unusual $^3S_1$ (Michaelis complex) → $^3H_4$ (transition state) → $^1C_4$ (product) conformational itinerary, a mannose-configured 1,6-*trans*-cyclic sulfamidate mimics the $^1C_4$ chair conformation of the product state, targeting GH47-α-mannosidases. This rational design has ultimately led to the development of new selective inhibitors for GH47-α-Mannosidases through a “bump and hole” strategy.[4]

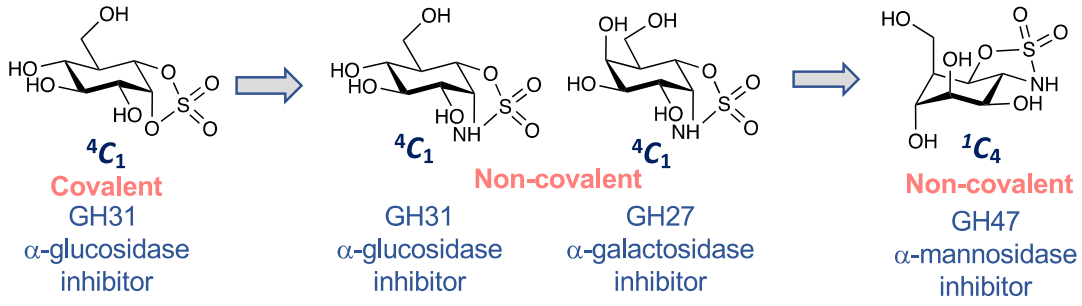

Figure 1. Cyclosulfate and cyclosulfamidate-based cyclitols as selective glycosidase inhibitors.

This communication will delve into the conformational itineraries of glucosidases, galactosidases, and mannosidases, as well as the rational design principles underlying conformational and mechanism-based cyclic sulfates and sulfamidates: an new chemical modality for targeting glycosidases, and potentially in the future glycosyltransferases.

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Squaryl Group-Modified Lysophosphatidyl-β-D-Glucoside Analogs As GPR55 Regulators

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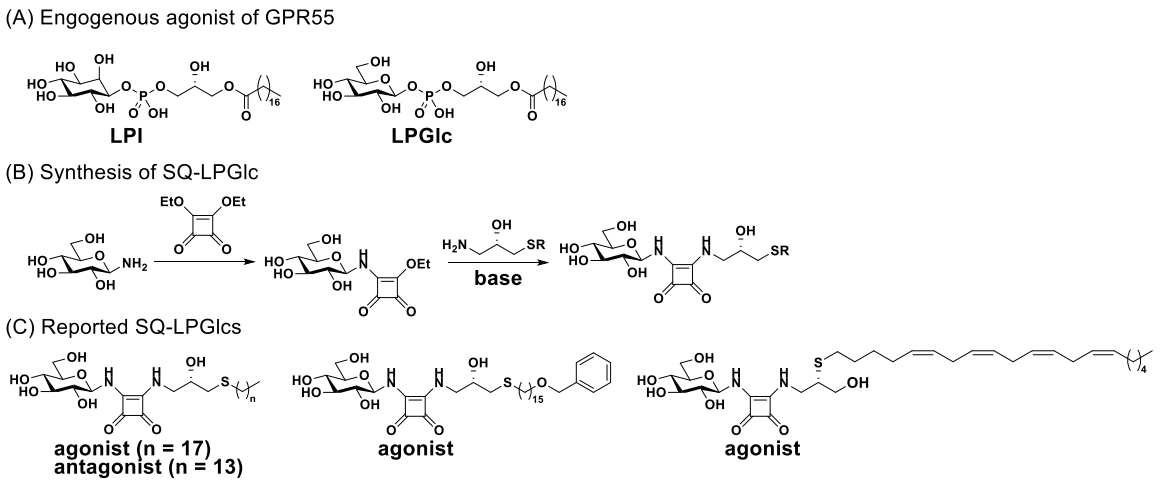
GPR55 is one of G protein-coupled receptors (GPCRs), which is related to diverse biological phenomena.[1] It was initially deorphanized as a cannabinoid receptor, but lysophosphatidylinositol (LPI) was reported to work as the endogenous agonist.[2] More recent study identified lysophosphatidyl-β-D-glucoside (LPGlc) as another endogenous agonist of GPR55.[3] Our study aimed to explore the structure activity relationship (SAR) of LPGlc to GPR55. However, the synthesis of LPGlc derivatives, such as derivatives possessing different alkyl chain length, requires multi-step transformations and tedious purification steps, inevitably making the SAR study labor-intensive. Therefore, we exploited the unique four-membered ring squaramide which is known as a surrogate of phosphodiester.[4] Squaramide containing analogs can be assembled in a technically simple manner by sequential reactions of nucleophiles to diethylsquarate without protecting and deprotecting steps, drastically facilitating the SAR study. In fact, our preceding studies demonstrated the relevance of the squaryl group-modified analogs of LPGlc (SQ-LPGlc) as agonists[5] or antagonists.[6] The synthesis was carried out by using diethylsquarate and β-D-glucosylamine, followed by the nucleophilic replacement with alkylamine. Various SQ-LPGlc can be obtained by changing alkylamine which is secondary nucleophile. Besides, we discovered SQ-LPGlc possessing a leaving group in its structure exhibited the irreversible inhibition, presumably through covalent binding to GPR55.[7] The synthesis and biological activity of the SQ-LPGlcs will be discussed in detail.



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Development Of Saponin-Based Glycoconjugates As Self-Adjuvanted Vaccine Candidates

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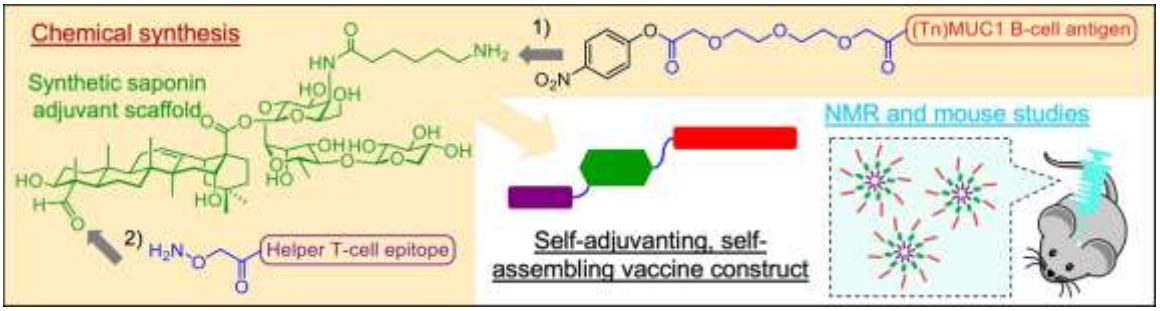
Subunit vaccines based on carbohydrate and (glyco)peptide antigens are weakly immunogenic and require immunopotentiating approaches to generate optimal immune responses. An emerging but underutilized strategy involves the design of unimolecular self-adjuvanted vaccine constructs that do not need to be conjugated to carrier proteins or coadministered with external adjuvants. Here, we report the design, synthesis, immunological evaluation in mice, and NMR structural studies of a novel self-adjuvanting and self-assembling tricomponent vaccine platform based on tumor-associated (TA) glycopeptide antigens (e.g. MUC1) chemically conjugated to a QS-21-derived saponin adjuvant. These candidates were synthesized from unprotected building blocks in high yields using a modular chemo-selective approach that harnessed two distal attachment points on the adjuvant platform to attach the corresponding (glyco)peptide elements via orthogonal ligations. In mouse immunizations, the tricomponent constructs induced significant IgG antibodies that recognized the native antigen on MCF7 cancer cells, unlike dicomponent or unconjugated combinations of the individual components. By NMR, these immunogenic candidates were shown to self-assemble into stably organized, particulate aggregates that displayed the more hydrophilic TA-MUC1 moiety on the surface and were not disrupted upon dilution. The observed correlation between higher molecular organization, structural stability, and increased immunogenicity suggests a longer half-life and enhanced antigen multivalent presentation of these conjugates in physiological media. This, together with the modularity and streamlined nature of the synthetic strategy, make these self-adjuvanting tricomponent vaccines promising candidates for further development.



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Creation Of Mannosylerythritol Lipid Analogues Exhibiting Selective Cytotoxicity Against Cancer Cells And Recovery Effects On Damaged Skin Cells

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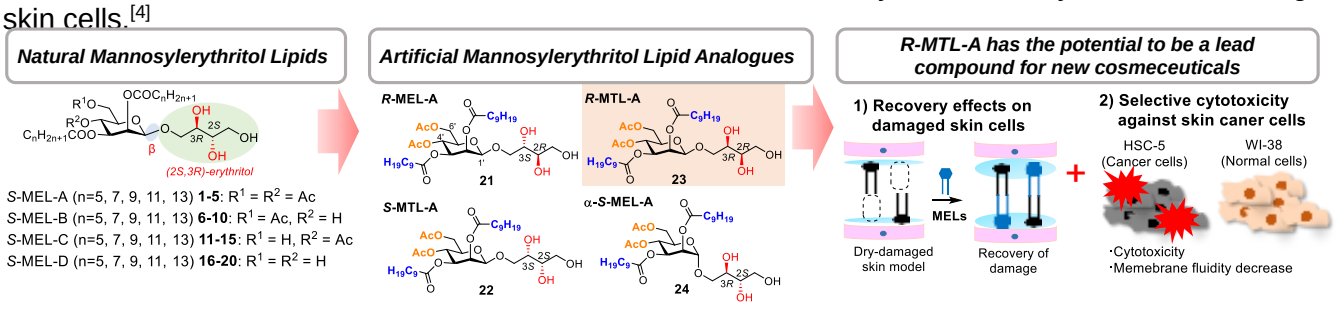
Mannosylerythritol lipids (MELs) are a class of amphipathic molecules with a hydrophilic 4-*O*-β-D-mannosyl-D-erythritol skeleton and two hydrophobic fatty acyl chains. These structural features give MELs unique physicochemical properties and biological activities. So far, our lab had total synthesized 20 members of *S*-MELs **1**-**20** using boron-mediated aglycon delivery (BMAD) method.[1] It was revealed that *S*-MEL-A-D (n=9) **3**, **8**, **13**, and **18** have high skin permeability and show remarkable recovery effects on damaged skin cells.[2] Also, it has been reported that natural *S*-MEL-A (C_8-C_{14}) shows cytotoxicity against cancer cells.[3] Therefore, we hypothesized that **3**, **8**, **13**, **18** and their analogues might exhibit both high recovery effects on damaged skin cells and high selective cytotoxicity against skin cancer cells. Compounds demonstrating these two properties are currently attracting attention as next-generation cosmeceuticals. Herein, we report on the creation of highly functional MEL analogues exhibiting both the recovery effects on damaged skin cells and selective cytotoxicity against cancer cells.

Firstly, we evaluated the selective cytotoxicity of **3**, **8**, **13**, and **18** against human skin cancer HSC-5 cells, and results showed that **3** exhibited the greatest cancer cell-selective manner. Next, we designed and synthesized four kinds of **3** analogues *R*-MEL-A **21**, *S*-mannosylthreitol lipid (*S*-MTL)-A **22**, *R*-MTL-A **23**, and *α*-*S*-MEL-A **24**, and functionally evaluated their selective cytotoxicity against HSC-5 cells. As a result, we found that **23** exhibits better selective cytotoxicity than does **3**. This demonstrated for the first time that the anomeric configuration and slight differences in the steric configuration of the erythritol moiety in MEL can affect the selective cytotoxicity of the compound. Next, a mechanistic analysis of cancer cell death induced by **3** and **23** suggested that **3** and **23** induce necrosis-like cell death in a cancer cell-selective manner, and that **3** and **23** selectively decrease cancer cell membrane fluidity. These results suggested that the change in cell membrane fluidity is important for the selective cytotoxicity of MEL and its analogues. Finally, we evaluated the recovery effect of **3** and **23** on damaged skin cells. Synthetic analog **23** exhibited higher activity than natural type MEL **3**, indicating that **23** is a promising candidate for cosmeceuticals, with both cancer cell-selective toxicity and recovery effects on damaged skin cells.[4]



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To be Determined…

One-pot glycans assembly based on glycosyl PVB: from oligosaccharides to polysaccharides

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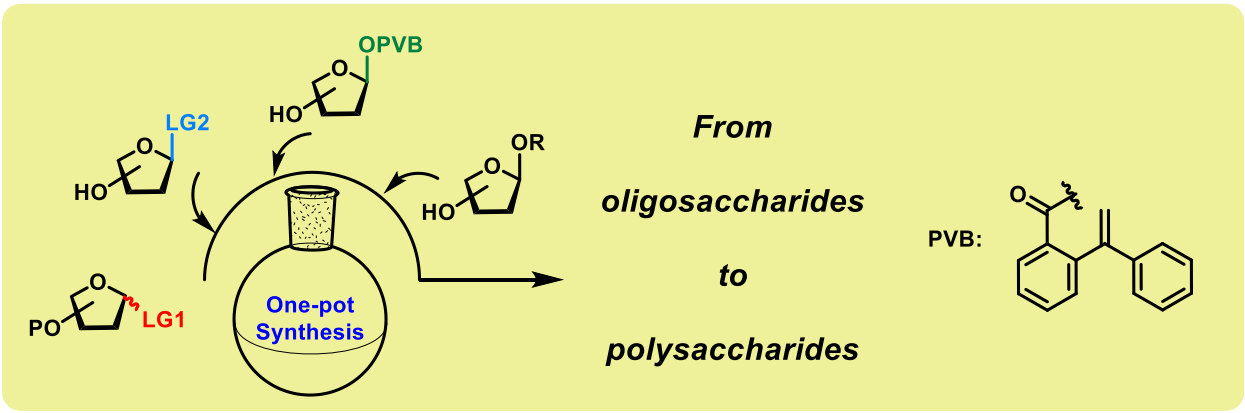
Carbohydrates are essential bio-molecules, which play important roles in numerous biological processes, such as bacterial and viral infections, cell growth and proliferation, and immune responses. Thus, carbohydrates-based molecules have been studied for a long time to decipher their functions and develop carbohydrates-based drugs. However, the synthetic accessibility to pure and well-defined glycans remains the bottleneck for the advances of carbohydrates sciences. To address these issues, in 2020, we introduced a new glycosylation method for efficient synthesis of both *O*-glycosides and nucleosides, using glycosyl *ortho*-(1-phenylvinyl)benzoates (PVB) as donors.[1] In 2021, we further developed one-pot glycans assembly strategy based on glycosyl PVB, which avoided the potential issues such as aglycone transfer, undesired interferences of departing species and unpleasant odor of thiol inherent to orthogonal one-pot glycosylation based on thioglycosides.[2] We applied this new one-pot glycosyaltion strategy on the basis of PVB glycosylation to efficient synthesis of a variety of complex carbohydrates, including capuramycin, TMG-chitotriomycin, 14-mer from *Lentinus giganteus* glycans,[3] rhynchosporosides, 13-mer from *Cordyceps militaris* glycans, nonasaccahride from *Phellinus ribis* glycans, 13-mer from *Bacteroides vulgatus* lipopolysaccharides,[4] 20-mer from *Cordyceps sinensis* EPS-1A glycan, 14-mer from *Ganoderma lucidum* GLSWA-1, *Acinetobacter baumannii* capsular polysaccharides K43, K47 and K88 repeating units, mucin-related tumor associated carbohydrates antigens,[5] 19-mer from *Ganoderma sinense* polysaccharides[6] and mannose capped lipoarabinomannan up to 101-mer[7] from *Mycobacterium tuberculosis* cell wall.[8]



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Chemical Glycobiology Tools For The Study Of Pseudaminic Acid

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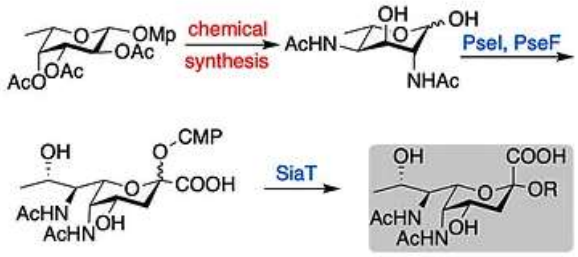
Sugars are the most abundant biomolecules on earth and play essential roles in a myriad of biological processes. The ubiquitous nonulosonic acid sugar Neu5Ac is present on the surface of all human cells and is therefore well studied, but its ‘evil twin’ pseudaminic acid (Pse), which is present on a range of pathogenic bacteria, is poorly understood in comparison. In this talk I will outline our progress[1-4] towards establishing a chemical glycobiology toolkit for dissection of the bacterial ‘Pseome', using a combination of enzymatic and synthetic carbohydrate chemistry to access Pse enzyme substrates, probes and inhibitors which afford molecular level insight into nonulosonic acid processing.



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2-Naphthylmethyl (NAP) Protecting Group Assisted Heparin Synthesis

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The high cost and lengthy process of heparin synthesis impede the clinical application of synthetic heparin oligosaccharides. To address this issue, we have reported the modular synthesis of heparin oligosaccharides using benzyl (Bn) as the permanent protecting group[1–3]. However, the intermediates are unsuitable for slurry separation and filtration. Also, the last Bn removal step required excessive hydrogenation. In contrast, the structurally similar 2-naphthylmethyl (NAP) protecting group can be removed under mild conditions, and its larger planar structure promotes intermolecular stacking, enabling slurry separation and filtration. Utilizing NAP as the permanent protecting group, we successfully achieved the total synthesis of a heparin pentasaccharide. This strategy offers an effective and efficient approach for synthesizing heparin oligosaccharides, providing a valuable tool for advancing heparin chemistry and its applications.

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Benzylidene-Directed Glycosylations – Mechanistic Insights From Cryogenic Infrared Spectroscopy

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The stereoselective formation of 1,2-cis glycosidic linkages is challenging. A promising method to preferentially obtain 1,2-*cis* glycosides are 4,6-*O*-benzylidene directed glycosylations, which were initially introduced by Crich[1] and further refined by many groups.[2,3] The stereoselectivity of this reaction is thought to be driven by a covalent intermediate, which reacts via an S_N2 mechanism.[4] However, the role of cationic S_N1-type intermediates in this reaction is unclear. Here, we elucidate the structure of glycosyl cations carrying 4,6-O-benzylidene groups using cryogenic infrared ion spectroscopy and computational methods. The data reveal that the intermediates unexpectedly form anhydro cations, which correlates well with the stereoselective outcome of S_N1-type glycosylations.[5] The study highlights how cryogenic infrared spectroscopy can unravel novel intermediates in sugar chemistry and how this structural data can be linked to reactions in solution.[6-8]

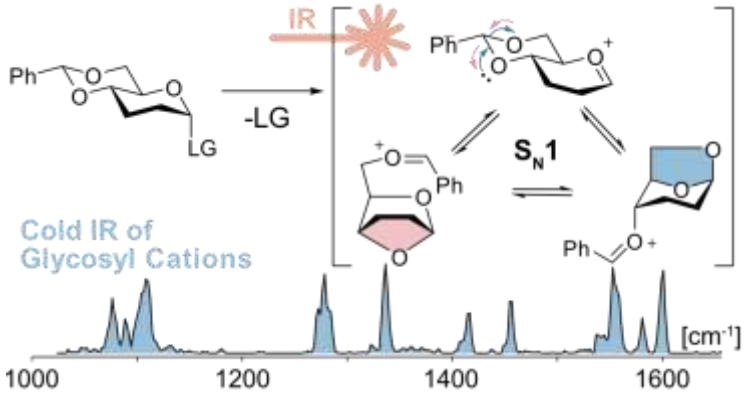

Figure 1: Postulated mechanism for benzylidene-mediated glycosylation reactions. We present here the first direct structural characterization of glycosyl cation using cryogenic IR. The predicted stereoselectivity for anhydro-type intermediates is correlated with the stereochemical outcome in S_N1-mechanism.

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Divergent Synthesis Of Linkage-Editing A-Galactosylceramides: A Strategy To Create Pseudo-Glycans With Altered Biological Activities

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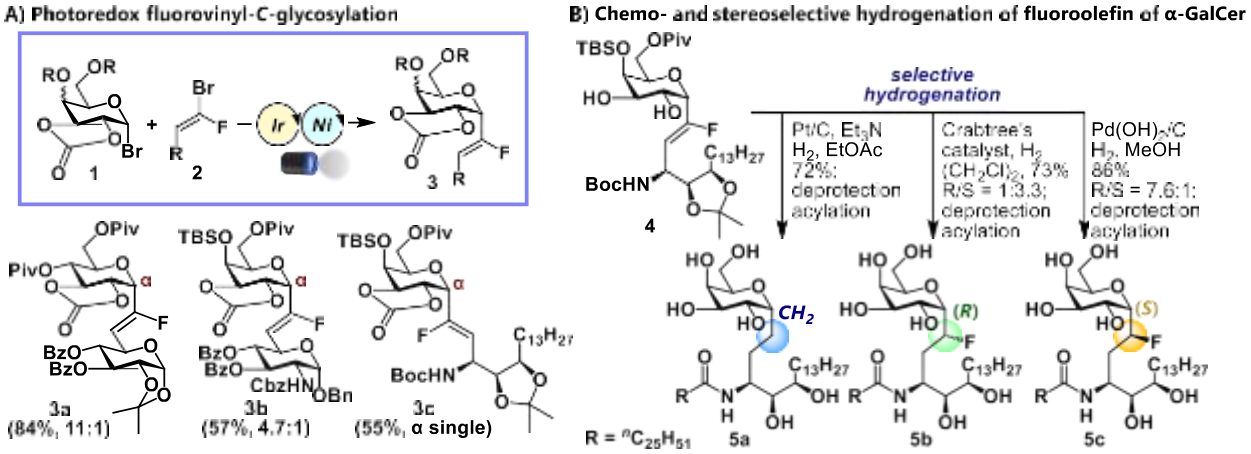
The glycans consist of saccharide and aglycone moieties, which are interconnected through glycosidic linkage. While combination of readily available saccharides and aglycones provides a wide range of chemical space for glycans, the diversity of linkage structure is limited. Instead of native *O*-glycoside, we have developed three different *C*-linked analogs, CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycosides, that are not cleaved by intracellular glycoside hydrolases.[1,2] While *O*-glycosides and CHF-glycosides occupy distinct conformations regulated by the exo-anomeric effect or the fluorine gauche effect, respectively, the conformation of CH_2-glycosides is not regulated by stereoelectronic effect, resulting in a more flexible conformation. These different conformational properties could give rise to new pseudo-glycans with different biological activities. To demonstrate the altered biological function of these linkage-edited glycan analogs, we synthesized various C-linked analogs of bioactive glycans, such as isomaltose,[2] α-galactosylceramide (α-GalCer),[2,3] melibiosamine,[4] and evaluated their biological activities.

In this presentation, we present the synthesis and biological evaluation of C-linked α-GalCer analogs. The first crucial step in the divergent synthesis is metallaphotoredox-catalyzed coupling reaction of glycosyl bromide **1** and bromofluoroolefin (BFO, **2**). This method proved effective in producing a variety of fluorovinyl-*C*-glycosides including isomaltose **3a**, melibiosamine **3b**, and α-galactosylceramide **3c**, with good yield and α-selectivity. Upon removing the carbonate group, fluoroolefin **4** underwent a catalyst-controlled chemo- and stereoselective hydrogenation, enabling the divergent synthesis of CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycosides **5a-c**. We evaluated adjuvant activities, cytokine production, and iNKT cell activation activities of C-linked α-GalCer analogs **5a-c** and native *O*-linked α-GalCer, revealing that (*R*)-CHF-glycoside **5b** acts as an antagonist of iNKT cell activation.



Keywords: C-glycoside, pseudo-glycan, glycolipid, α-GalCer, photoredox

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A Glycan Foldamer That Harvests Carbohydrate-Aromatic Interactions To Perform Catalysis

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In nature, the ability to catalyze reactions is a prerogative of proteins[1-2]. Inspired by these systems, peptide-based catalysts were designed to accelerate chemical reactions[3-4] and/or ensure regio- and stereo-selective transformations[5-6]. We wondered if other biomolecules (i.e. glycans) could be designed to perform catalytic functions, expanding the portfolio of synthetic functional oligomers. Herein, we report a glycan foldamer that acts as catalyst in a chemical reaction. This glycan-based catalyst benefits from structural rigidity and modular adaptability, incorporating a substrate-recognition motif alongside a catalytic active site. Leveraging the inherent ability of carbohydrates to engage in CH/π interactions with aromatic rings, we demonstrate the recruitment and functionalization of an aromatic substrate. Our modular glycan catalyst accelerates the reaction kinetics, enabling the modification of peptides in aqueous environments. Our findings not only pave the way for the development of glycan-based catalysts, but also suggest the possibility of catalytic capabilities of glycans in biological contexts.

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Targeting An Emerging Therapeutic Biomarker, Heparanase

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Heparan sulfate proteoglycans (HSPGs), major components in the extracellular matrix (ECM) of all tissue types, maintain ECM structural integrity and regulate cellular signaling via binding with ECM components and protein ligands such as growth factors and chemokines.[1] Heparanase, the only known enzyme that can cleave the heparan sulfate (HS) side chains of HSPGs, regulates many cellular processes including ECM remodeling and homeostasis of cell-associated HS, and controls the bioavailability and activity of molecules attached to HS.[2] Increased heparanase activity is mostly linked with cancer progression, angiogenesis, metastasis, various types of inflammation, fibrosis and kidney diseases. Our research focuses on the development of molecular tools that can target and visualize the spatiotemporal activities of heparanase in both cells and living organisms.[3] We apply our molecular probes of heparanase in both diagnostics and drug discovery.[4]-[9]

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Total Synthesis Of Trisaccharide Repeating Unit Of *Staphylococcus Aureus* Strain M

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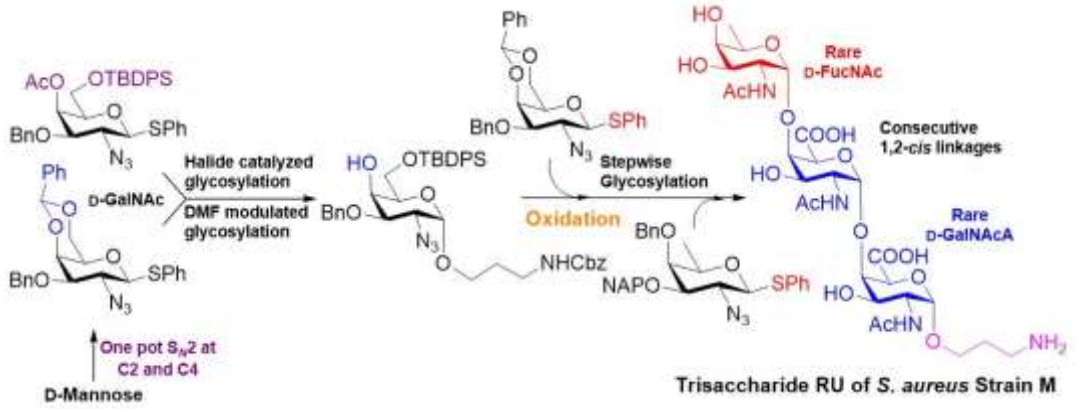
Multidrug resistance in bacteria has become a global concern. As a result, there is an urgent and unprecedented need for the development of novel antibiotics and prophylaxis strategies. Structurally unique glycans present on the bacterial cell surfaces are looked upon as excellent candidates for therapeutics and vaccine development. The ESKAPE pathogen has become a severe threat among all the MDR bacteria. *Staphylococcus aureus* strain M is a capsular polysaccharide (CPS). This opportunistic Gram-positive bacterium, which is responsible for the infection of skin, lungs, and joints and can cause life-threatening conditions such as endocarditis or toxic shock syndrome.[1] Considering this pathogen's biological importance, we have designed an efficient route for synthesizing a conjugation-ready trisaccharide repeating unit of *Staphylococcus aureus* strain M. The main challenges involved in this synthesis are the procurement of rare sugars, which is achieved by using in situ S_N2 bistriflate displacement[2] (D-FucNAc and D-GalNAcA). The challenge of stereoselective 1,2-*cis* glycosylation with the linker acceptor was achieved with easily accessible benzylidene protected D-galactosamine thioglycoside by employing a DMF modulated preactivation glycosylation method.[3] The consecutive 1,2-*cis* linkages were installed with the help of solvent participation. The carboxylic acid functionality was introduced via postglycosylation oxidation on the disaccharide moiety. The total synthesis of the trisaccharide repeating unit was accomplished with the longest linear sequence of 24 steps in a 4.5% overall yield.[4]



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Polymer Chemistry-Inspired Approaches to Native and Pseudo-Polysaccharides

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Polysaccharides are a major class of biomacromolecules that play important roles in both biology and material sciences. A long challenge for the characterization and utilization of polysaccharides is the heterogeneity of highly diverse natural polysaccharides. The efforts for chemically synthesizing complex polysaccharides have undergone rapid development in the past decade, but methods for the scalable preparation of polysaccharides remain elusive. In this talk, I will present our efforts toward the chemical synthesis of native and pseudo-polysaccharides for biological and material applications by taking inspiration from polymer chemistry. This talk will cover the synthesis of both native polysaccharides with glycosidic bonds and pseudo-polysaccharides with non-glycosidic linkages via chemical polymerization. Taken together, these developments provide promising glycobiomaterials with improved precision tailored for fundamental and applied biological investigations.

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Glucose-Based Imidazolium Salts And Their Various Applications

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Our research group has recently found a simple, straightforward and efficient approach for the synthesis of imidazolium salts bearing glucose functions (Figure 1). The synthesis of these molecules, that bear structural resemblance to commonly used imidazolium based ionic liquids, is highly flexible and allows for many variations, like different glycosidic groups, free hydroxy groups or protected hydroxy groups, varied side-chains on the imidazole or anion exchanges. Furthermore, these glucose-based imidazolium salts have been proven to exhibit a considerably higher biocompatibility than common imidazolium ionic liquids.[1]

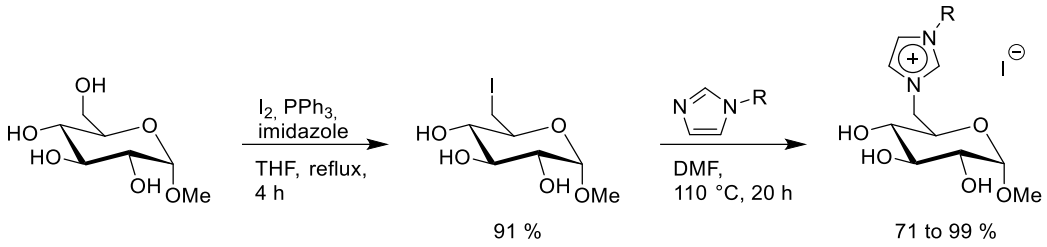

Figure 1: Synthesis of glucose-based imidazolium salts

Carbohydrate-based ionic liquids and salts (in short CHILS) are highly interesting molecules that serve as natural feedstock derived green alternatives to commercial ionic liquids. Like ILs, CHILS are applied in many fields beyond serving as solvents, like as starting materials for hydrogels,[2] as supports for biocatalysts[3] or as ligands for metal complexes[4] (Figure 2).

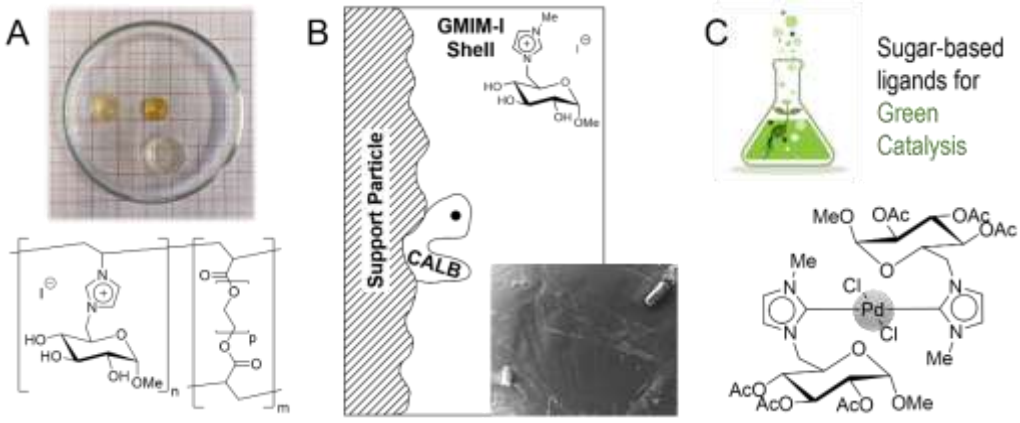

Figure 2: A) Scheme and photo of PEGDA-crosslinked hydrogels from glucosyl vinyl imidazolium iodide, B) Scheme and SEM of glucosyl methyl imidazolium iodide as support on Novozym 435, C) Pd-NHC complexes from acetylated glucosyl imidazolium salts

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Characterization Of The Intact N-Glycopeptide Of *Haemonchus Contortus* H-Gal-Gp Antigen

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Haemonchus contortus is a parasitic nematode of ruminants, seriously endangering the health of cattle and sheep and causing major economic losses to the global breeding industry. Currently, the drug resistance situation of this parasite is becoming increasingly severe, therefore, there is an urgent need on the research and development of vaccines. The native H-gal-GP is one of the most promising natural antigens for vaccine development, which is rich in N-glycosylation and plays a key role in the process of nematodes digesting the host blood to obtain nutrients for itself. However, the immune protection of recombinant proteins is greatly reduced because the recombinant proteins cannot obtain the correct protein glycosylation as the native proteins. Here, we first integrated the glycoprotein sequence and N-glycan structure of *H. contortus* to construct a complete glycoprotein database based on our previous work, and then used the complete glycoproteomics technology combined with GPSeeker (searching for N-glycans with $Man_3GlcNAc_2$ core structure) and pGlyco3.0 (supplemental search for truncated core structure), and identified a total of two aspartyl protease molecules, four zinc metalloproteinase molecules and one cysteine protease molecule, including 16 glycosylation sites and 110 corresponding intact N-glycopeptides, which is rich in many glycan motifs with known immunogenicity, including the Gal-Fuc motif, the di-fucose and tri-fucose motif, as well as the terminal LDNF motif. On the basis of the reported structural information of H-gal-GP by cryo-EM, the site-specific glycan was added to the corresponding N-glycosylation site by 3D visualization technology, and the complete structure and N-glycosylation characteristics of the native H-gal-GP antigen were obtained. Taken together, it provides a theoretical basis for the precise development of anti-parasitic glycoprotein synthesis vaccines.

Highly Regio-/Stereoselective Synthesis Of Carbohydrates With Unsaturated Glycosyl Donors Under Mild Conditions

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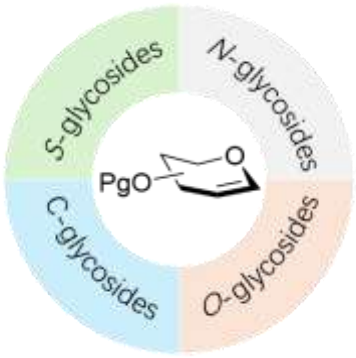
Carbohydrates and their conjugates play important roles in life activities and drug development. Our group was committed to the general and effective glycosylation methods and their application in chemical biology using unsaturated glycosyl donors by transition metal catalysis. In the past five years, we have reported some synthetic strategies with high stereoselectivity and milder conditions compared with conventional reported methods. In particular, high chemo-/regio- and stereoselective *O*-glycosylation, *C*-glycosylation and *S*-glycosylation could be achieved via palladium catalysis under open-air conditions at room temperature. In this work, we introduced our research progress in constructing four types of glycosides: *O*-, *C*-, *N*- and *S*-glycosides.



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To be Determined…

Development Of Research Strategy To Understand The Biological Significance Of Protein Glycosylation

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In nature, numerous proteins, such as lectins, antibodies, cytokines, and various enzymes, commonly undergo posttranslational glycosylation modifications. However, to date, our ability to predict the biological role of glycosylation and understand the mechanisms by which glycosylation affects the properties and functions of proteins remains limited. In order to delve deeper into this issue, we have long utilized the Family 1 Carbohydrate-Binding Module (*Tr*CBM1) as a model molecule to study glycosylation. Building upon this, we have systematically explored and developed a comprehensive research strategy for studying protein glycosylation. By applying this strategy, we have gained the ability to predict the role of protein glycosylation and develop a deeper understanding of its profound significance. Overall, through our study, we offer new strategies, capabilities, perspectives, and research directions for unraveling and predicting the role of protein glycosylation, as well as developing detailed mechanisms and molecular bases for protein glycosylation. These strategies and findings are expected to significantly advance the field of glycobiology, injecting new vitality into its continued development and deeper exploration.

OGT And m^{6}A: A Trilogy

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N6-methyladenosine (m^{6}A) is the most prevalent RNA modification, and its regulators include writers, readers, and erasers. m^{6}A is under stringent control and takes part in many biological events, but it is not known whether there is an interplay between m^{6}A and glycosylation. In our current work, we investigated the role of O-GlcNAcylation of three m^{6}A readers: YTHDC1, YTHDF1 and YTHDF2. YTHDC1 has been shown to be recruited to the DNA-RNA hybrid at DNA damage sites and regulate homologous recombination (HR) during DNA damage repair. We found that YTHDC1 is subject to O-linked β-N-acetylglucosamine (O-GlcNAc) modification at Ser396 upon DNA damage. And YTHDC1 O-GlcNAcylation promotes HR-mediated DNA damage repair and cell survival, probably through recruitment of Rad51 to the damage sites. In the second part of our work, we investigated YTHDF1 O-GlcNAcylation and revealed its role in tumorigenesis. In the third part, we show that YTHDF2 O-GlcNAcylations antagonizes ERK-dependent phosphorylation. Our work thus unveiled a new link between O-GlcNAcylation and m^{6}A mRNA regulation.

Key words: O-GlcNAcylation, m^{6}A mRNA, YTHDF1, YTHDF2, YTHDC1

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Precise Regulation Of Protein Glycosylation And Its Application In Live Cells

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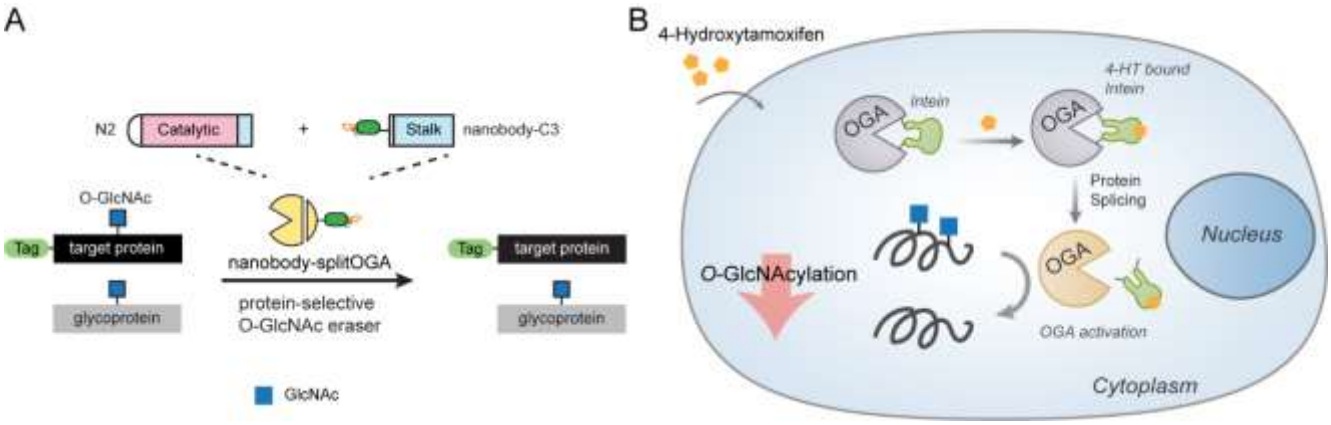
Glycosylation is the most abundant and diverse post-translational modification of proteins, encompassing N-glycosylation, O-GlcNAcylation, and other types. Notably, the nutrient sensor O-GlcNAc is dynamically and reversibly regulated by OGT and OGA to fine-tune protein functions. Current approaches for modulating O-GlcNAc in live cells have limited specificity and spatiotemporal resolution. To address this, we designed a nanobody-directed O-GlcNAc eraser to dissect distinct O-GlcNAc functions on specific substrates through protein engineering. The modularity and specificity of this tool are well demonstrated at the proteome level. Additionally, we utilized a small-molecule responsive intein for controllable activation of OGA in a dose-dependent and spatiotemporally resolved manner, showcasing its potential for tracking O-GlcNAc homeostasis and combinatory cancer therapies. We envision that these tools will achieve precise regulation of O-GlcNAc in live cells, enhancing our understanding of O-GlcNAc functions. Furthermore, we have engineered N-glycosylation on proteins, particularly nanobodies, to confer additional desired functions. We equipped an anti-CD47 nanobody with high-mannose-type N-glycosylation and loaded it onto cancer cell-derived vesicles. In two mouse tumor models, this tripartite ensemble of high-mannose glycan, anti-CD47 nanobody, and cellular vesicles has been validated to significantly activate antitumor immunity and suppress tumor growth. In summary, chemical biology strategies for glycosylation regulation enable us to study and leverage glycan functions in biological processes.



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Allosteric Modulation Of Dc-Sign

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DC-SIGN is a C-type lectin receptor essential for bridging innate and adaptive immunity. Expressed on antigen-presenting cells, it facilitates cellular adhesion, antigen presentation, and immune cell activation. In particular, its role in entry and dissemination of pathogens, such as HIV-1 and SARS-CoV-2, has fueled interest in developing small molecules that selectively modulate this receptor. Nevertheless, owing to its shallow and polar Ca^{2+}-dependent carbohydrate binding site, the number of selective chemical probes targeting DC-SIGN remains limited. Previous studies have reported the existence of remote secondary sites, one of which allosterically modulates the carbohydrate binding site of DC-SIGN[1,2]. Targeting this allosteric site offers the opportunity to overcome low druggability and promiscuity of the carbohydrate binding site, while modulating receptor activity.

This presentation will outline our current efforts to elucidate the structural determinants and functional effects of binding to the allosteric site in DC-SIGN. I will highlight how we have used molecular dynamics simulations for the rational design of DC-SIGN mutants, which in turn allowed us to experimentally validate the ligand-induced local flexibility of the allosteric site as well as long-range effects on the primary carbohydrate binding site. Moreover, I will provide insights into our developed Python-based workflow for predicting allosteric secondary sites to translate the findings for DC-SIGN to other human C-type lectins. Overall, our observations indicate that allosteric pockets represent a promising avenue for targeting DC-SIGN and have the potential to stimulate drug discovery campaigns that target other pharmaceutically relevant members of the C-type lectin family.

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Accurate Carbohydrate-Binding Site Prediction

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As the most abundant organic substances in nature, carbohydrates are essential for life. Understanding how carbohydrates regulate proteins in the physiological and pathological processes presents opportunities to address crucial biological problems and develop new therapeutics. However, the diversity and complexity of carbohydrates pose a challenge in experimentally identifying the sites where carbohydrates bind to and act on proteins. Here, we introduce a deep learning model, DeepGlycanSite, capable of accurately predicting carbohydrate-binding sites on a given protein structure. Incorporating geometric and evolutionary features of proteins into a deep equivariant graph neural network with the transformer architecture, DeepGlycanSite remarkably outperforms previous state-of-the-art methods (Table 1) and effectively predicts binding sites for diverse carbohydrates[1]. Integrating with a mutagenesis study, DeepGlycanSite has been employed to reveal the nucleotide-sugar-recognition site of several important G-protein coupled receptors[1,2]. These findings demonstrate DeepGlycanSite is invaluable for carbohydrate-binding site prediction and could provide insights into molecular mechanisms underlying carbohydrateregulation of therapeutically important proteins.

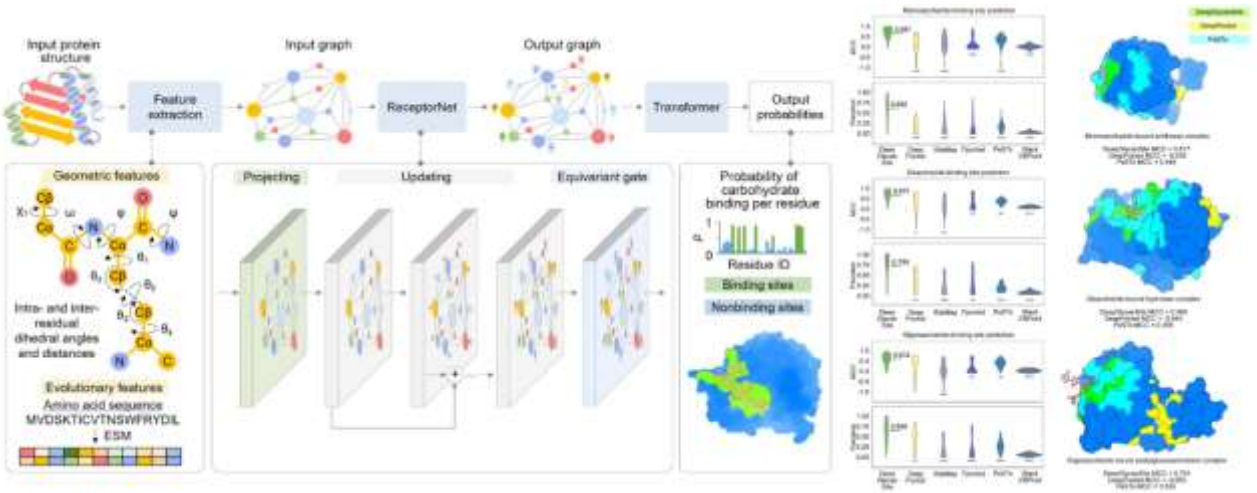

Figure 1 Overview of DeepGlycanSite and its performance

Table 1. Comparing DeepGlycanSite with previous binding site predictors on the independent dataset.

Method	MCC	Precision	Balanced accuracy
StackCBPred	0.018	0.052	0.525
Fpocket	0.191	0.194	0.617
SiteMap	0.227	0.201	0.717
DeepPocket	0.288	0.292	0.760
PeSTo	0.336	0.235	0.815
DeepGlycanSite (ours)	0.625	0.631	0.829

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O-Glcnacylation Regulates Melanoma Metastasis Via Akt-NfκB Signaling Pathway

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Enhancement of O-GlcNAcylation was associated with cancer development and progression. In this study, we have demonstrated the involvement of O-GlcNAcylation in melanoma metastasis. Based on the data from GEO database (GSE46517), O-GlcNAcylation and its related enzymes (GFAT, OGT, and OGA) were elevated in metastatic melanoma compared with primary tumors and normal tissues. Functional analyses in MNT-1, SK-MEL-28, and A-375 melanoma cell lines showed that knockdown of siOGT significantly suppressed O-GlcNAcylation and migration/invasion abilities of the cells. Moreover, phosphorylation of Akt (S473) and NFκB (S536) was also drastically suppressed in siOGT treated cells, suggesting the possibility of O-GlcNAcylation in regulating melanoma metastasis through Akt-NFκB signaling pathway. In addition, we found that the NFκB target gene–MCT-1 was significantly upregulated and correlated with O-GlcNAcyaltion level in metastatic tumors. This information suggested the role of O-GlcNAcyaltion in controlling melanoma metastasis via upregulating MCT-1 through activation of Akt-NFκB signaling pathway.

Keywords: glycosylation; O-GlcNAc; melanoma; metastasis, Akt, NFκB

O-Glcnacylation Of Enolase 1 Serves As A Dual Regulator Of Aerobic Glycolysis And Immune Evasion In Colorectal Cancer

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Aerobic glycolysis and immune evasion are two key hallmarks of cancer [1]. However, how these two features are mechanistically linked to promote tumor growth is largely unknown. Here, we show that the glycolytic enzyme enolase-1 (ENO1) is dynamically modified with an O-linked β-N-acetylglucosamine (O-GlcNAcylation), and simultaneously regulates aerobic glycolysis and immune evasion via differential glycosylation. Glycosylation of threonine 19 (T19) on ENO1 promotes its glycolytic activity via the formation of active dimers. On the other hand, glycosylation of serine 249 (S249) on ENO1 inhibits its interaction with PD-L1, represses the association of PD-L1 with E3 ligase STUB1, resulting in decreased proteasomal degradation of PD-L1. Consequently, blockade of T19 glycosylation on ENO1 inhibits glycolysis, and decreases cell proliferation and tumor growth. Blockade of S249 glycosylation on ENO1 reduces PD-L1 expression and enhances T cell–mediated immunity against tumor cells (Figure 1). Notably, elimination of glycosylation at both sites synergizes with PD-L1 monoclonal antibody therapy to promote antitumor immune response. Clinically, ENO1 glycosylation levels are upregulated and show a positive correlation with PD-L1 levels in human colorectal cancer. Thus, our findings provide a mechanistic understanding of how O-GlcNAcylation bridges aerobic glycolysis and immune evasion to promote tumor growth, suggesting new therapeutic opportunities.

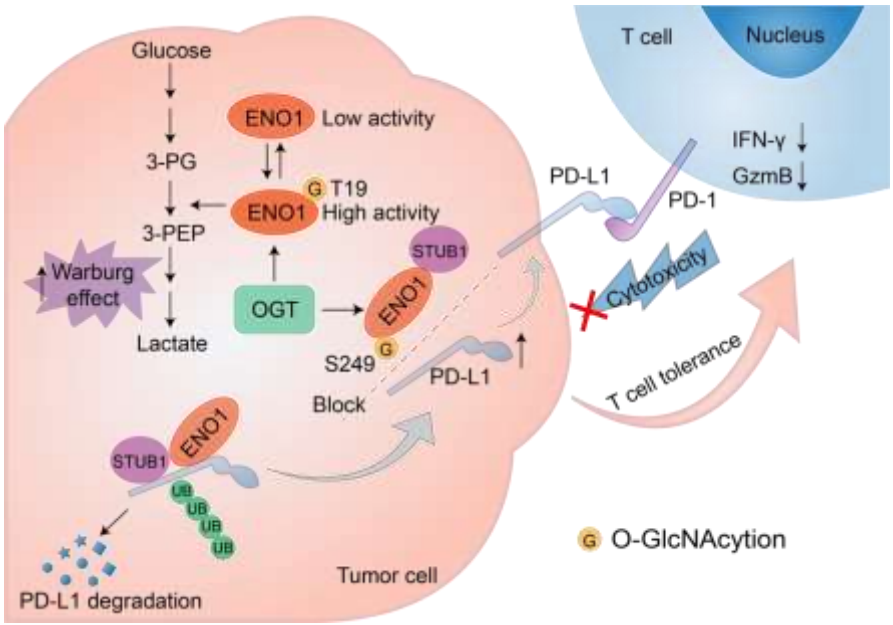

Figure 1. A graphical model of ENO1 O-GlcNAcylation in regulating aerobic glycolysis and tumor immune evasion.

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Inverting The Anomeric Configuration Of A Glycomimetic Ligand For Human Langerin Leads To Enhanced Targeted Delivery To Murine Langerin Expressing Cells

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Langerhans cells (LCs) play a crucial role in immune regulation in the epidermis and are vital targets for transdermal applications of vaccines and other therapies. Langerin (CD207) is a C-type lectin expressed on LCs and recognizes carbohydrates as its natural ligands. We previously developed a glycomimetic ligand for efficient targeting of LCs through human langerin[1] and showed that liposomes conjugated with the targeting ligand can deliver antigens effectively.[2,3] However, sensitive biophysical assays suggest this ligand does not bind to murine langerin receptor . To understand the key structural differences between the two homolog receptors that lead to the binding difference, we carefully examined a model of murine langerin with targeting ligand. This study revealed a clash between R302 in murine langerin and the linker extending from the anomeric position. In contrast, human langerin does not encounter this problem having a smaller amino acid (K299) in this position. To gain further insights, we first evaluated the fluorinated mannoside reporter that was originally designed and used in a ^{19}F reporter displacement assay. This mannoside showed decreased affinity for murine langerin compared with the human homologue, but maintained a similar affinity for murine langerin mutant R302K, which has a similar topography in the carbohydrate binding site with human langerin. The model of the mannoside with murine langerin highlighted the clash between its axial C2-*N*HAc and R302. Therefore, glucosides with equatorial configuration of C2-*N*HAc were tested via ^{19}F RDA, showing enhanced affinities for murine langerin. These findings suggested that the clash between the linker and R302 in murine langerin is the underlying cause of the issue. Consequently, the α-O-linked ethylamino linker of the ligand was altered into a beta linkage, directing the linker away from Arg302. The alpha anomer displayed enhanced affinity towards murine langerin while maintaining a similar affinity towards human langerin in isothermal titration calorimetry (ITC). Notably, the alpha anomer modified liposome binds to both murine and human langerin$^+$ model cells. Ongoing efforts focus on exploring the internalization of liposomes modified with these two targeting ligands by Langerhans cells to ascertain whether the enhanced affinity observed in biophysical assays leads to improved biological performance.

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Mgat1 Facilitates Liver Metastasis Of Colorectal Cancer Via Activation Of C-Met

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Colorectal cancer is among the most common and lethal malignant tumors worldwide, and liver metastasis is the main cause of death for patients with colorectal cancer. As an important type of post-translational modification, the dysregulation of protein glycosylation has been frequently observed in various kinds of tumors. However, whether abnormal protein glycosylation is involved in liver metastasis of colorectal cancer remains unclear. By employing the CRISPR library targeting glycosylation-related genes to screen liver metastasis-associated genes, we identified glycosyltransferase MGAT1 as an important regulator for the liver metastasis of colorectal cancer. Depletion of MGAT1 attenuated the invasion, anoikis resistance, and hepatic colonization of colorectal cancer cells. Through glycoproteomic analysis, it was found that MGAT1 affected the glycosylation maturation of receptor tyrosine kinase c-MET, and MGAT1 facilitated the membrane expression and activation of c-MET via N-linked glycosylation. Impairing the N-glycan branching therapeutically prevented the liver metastasis of colorectal cancer. Our study may provide new evidence for understanding the mechanism of liver metastasis of colorectal cancer from the perspective of protein glycosylation, and provide new targets and potential strategies for clinical treatment of CRC liver metastasis.

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Unveil The Accurate Site-Specific *N*- And *O*-Glycosylation Of A Novel Hypersialylated Erythropoietin Drug Candidate By An Integrated Approach

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Glycoprotein function is heavily influenced by its glycosylation site and glycan structure. Hyperglycosylation represents a crucial strategy in the development of long-acting protein drugs. Hyperglycosylated erythropoietin (EPO) drug, Aranesp® (darbepoetin alfa), demonstrates enhanced potency and a longer half-life than the recombinant human EPO. Despite the potential advantages of customizing glycosylation in EPO drug development, the site-specific glycosylation of Aranesp remains unknown, given its glycoform complexity and proximity of N-glycosylation sites, which pose significant challenges in analysis. Our collaborator has recently developed a novel hyperglycosylated EPO drug candidate, XL-EPO, exhibiting superior *in vivo* activities and a longer half-life. However, the site-specific glycosylation difference between the two hyperglycosylated EPO (hyper-EPO) drugs is unclear.

To obtain the accurate site-specific glycosylation of hyperEPO, we initially investigated a variety of enzymatic systems to generate peptides of a single glycosylation site across all the glycosylation sites, particularly at the adjacent sites (N24, N30, and N38; N83 and N88). The optimized digestion conditions, along with our customized glycopeptide enrichment-separation method, allow for the simultaneous detection of *N*- and *O*-glycopeptides. Furthermore, to address ambiguities in identification raising from large glycan structures and diverse peptide sequences, in addition to combining two complementary tandem mass spectrometry techniques, the data were processed through a released glycan library-based search and a novel library-free filter-based search, respectively. Because XL-EPO is characterized by heavily sialylated tetra-antennary glycans, this cross-validation approach efficiently excludes outliers, thereby elevating the assignment accuracy. Moreover, we conducted a comprehensive glycosylation comparison between the novel hyperglycosylated EPO drug candidate and Aranesp® at the intact protein level, glycopeptide level, and released glycan level. Overall, both hyperEPO proteins exhibit high site occupancy, large tetra-sialylated glycans, and similar types of sialic acid linkages, but they demonstrate markedly different site-specific glycoform distributions. Collectively, our study reveals the site-specific *N*- and *O*- glycosylation of a novel hyper-glycosylated EPO drug candidate and provides a state-of-the-art strategy for the accurate site-specific glycosylation characterization of hyperglycosylated proteins.

Protein Engineering-Based Precise Glycan Profiling And Modulation

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Affinity labeling of biomacromolecules is vital for bioimaging and functional studies. However, affinity probes recognizing glycans with high specificity remain scarce. We report the design and development of glycan recombinant affinity binders (GRABs) based on mutant glycosidases from bacteria, which are enzymatically inactive and preserve stringent specificity for recognizing the glycan substrates. By mutating a key catalytic residue of a pan-specific sialidase and an α2,3 sialidase, we develop GRAB-Sia and GRAB-Sia3 recognizing total sialoglycans and α2,3-linked sialosides, respectively. GRAB-Sia and GRAB-Sia3 exhibit stringent substrate and linkage specificity. The GRABs are further tetramerized with streptavidin, which greatly enhances the avidity of sialoglycan binding. The GRABs and tetrameric GRABs (tetra-GRABs) are compatible for probing sialoglycans in the settings of immunoblotting, flow cytometry, immunoprecipitation, and fluorescence imaging. Furthermore, the tetra-GRABs can be used simultaneously to enable multiplexed analysis of different sialoglycan subtypes. Finally, the spatially distinct sialoglycans in the mouse organs including the heart, kidney, lung, and intestine are visualized by tetra-GRABs.[1]

Bacterial glycosyltransferases are essential for developing chemoenzymatic tools for the precise synthesis and engineering of glycoproteins in a site-specific manner. Unlike N-glycosylation, protein O-linked glycosylation is not conserved between bacteria and eukaryotes. In search of bacterial cytoplasmic protein O-glycosyltransferases that uses nucleotide sugar donors and are able to modify eukaryotic proteins, we turned our attentions to glycosyltransferase effectors from pathogenic bacteria. Mechanistically, glycosyltransferase effectors use host nucleotide sugars to modify host proteins, making them promising tools for synthesis of eukaryotic O-glycoproteins. One of the major impediments is that most of the discovered glycosyltransferase effectors have specific protein targets. We report a powerful tool for site-specifically O-glucosylating a broad range of eukaryotic proteins, recognizing the strikingly short two-residue motifs (G-T/S and S-G) which ubiquitously exist in eukaryotic proteins and can be easily introduced. Additionally, the developed tool accepts 6-azido analog of UDP-glucose and enables site-specific bioorthogonal conjugation of proteins with various functional probes.[2]

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Unveiling The Complex Glycoproteome Of Resting And Activated Platelets Using Glycomics-Assisted Glycoproteomics

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Platelets play central roles in the vascular and immune systems, including in haemostasis, thrombosis, inflammation and carcinogenesis. Tissue injury promptly activates resting platelets, triggering profound morphological changes and granule exocytosis, resulting in the release of granular proteins (releasate) that mediate injury-related response processes. Despite the documented importance of protein glycosylation in platelet biology, the platelet glycoproteome remains poorly defined. This study employs complementary glycomics and glycoproteomics approaches to comprehensively map the *N*-glycoproteome of the lysate and releasate of resting and thrombin-activated platelets. Primary platelets were isolated in their resting condition from blood donors. Platelets were left unstimulated (n=10) or were either partially (n=6) or fully (n=5) activated with α-thrombin, a potent platelet agonist. Thrombin-mediated platelet activation was confirmed with flow cytometry for PAC-1 binding and CD62P positivity. The releasate fractions were then collected and separated from platelet cellular fractions (lysate). The *N*-glycome of platelet lysate and releasate fractions was quantitatively profiled using PGC-LC-MS/MS-based glycomics. Platelet lysates and releasates displayed profound *N*-glycome diversity rich in sialylated and core fucosylated complex-type *N*-glycans across both resting and activated conditions. Interestingly, a thrombin dose-dependent elevation of sialylated and fucosylated complex-type *N*-glycans displaying a higher degree of branching and a higher global protein occupancy with a concomitant reduction in bisecting GlcNAcylation were observed in the releasate under activated conditions. The *N*-glycoproteomics data recapitulated and expanded on the glycomics findings by uncovering a total of 339 unique *N*-glycopeptides from 92 different source *N*-glycoproteins, the highest glycoproteome coverage of platelets to date. Our data also suggested platelets exhibit subcellular-specific *N*-glycosylation featuring prominent sialofucosylation in the α-granules, paucimannosylation in lysosomes and, surprisingly, oligomannosylation on the platelet surface. In summary, this study provides the hitherto most detailed view into the resting and activated platelet *N*-glycoproteome, forming a valuable resource to further explore the fascinating platelet glycobiology in human health and disease.

Fucoidan attenuates ethanol-induced injury in Kupffer cells and *in vivo* via PON1/HDL/NF-κB axis

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Main body: In our previous study, the active fucoidan JHCF4 was successfully isolated from *Hizikia fusiformis* and its therapeutic activity against progressive alcoholic liver injury was established. In this study, we verify a hypothesis that paraoxonase 1(PON1)/high-density lipoprotein (HDL)/NF-κB axis plays a crucial role in alcohol-related liver disease (ALD) therapy and demonstrate how JHCF4 serves as a strong liver protective agent via the axis. *In vitro* study, silenced PON1 gene Kupffer cells were used to investigate the levels of HDL and the expression of NF-κB pathway-related proteins. We confirmed that the antioxidant activity of HDL is closely related to the expression of PON1. Further, JHCF4 was found to promote the transportation of Sterol Regulatory Element Binding Protein-2 (SREBP2) to the nucleus, thereby increasing the content of PON1 and subsequently inhibiting the NF-κB pathway. *In vivo*, ethanol was stimulated within the silenced PON1 gene C57BL/6J mice liver injury, while simultaneously, the concentrations of JHCF4 against the ethanol-induced damage were reduced through limiting *r*eactive oxygen species (ROS) production, NF-κB signals, and promoting PON1 and HDL contents. The cumulative results of our study demonstrate that the PON1/HDL/NF-κB axis has a significant role in the regulation of Kupffer cells and the promotion of liver function. JHCF4 increased the expression of PON1 and HDL, decreased ROS production, and inhibited the NF-κB proteins, thus providing a new potential therapeutic ingredient for the treatment of ALD.

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Conformational Space Of Complex Glycosyl Cations

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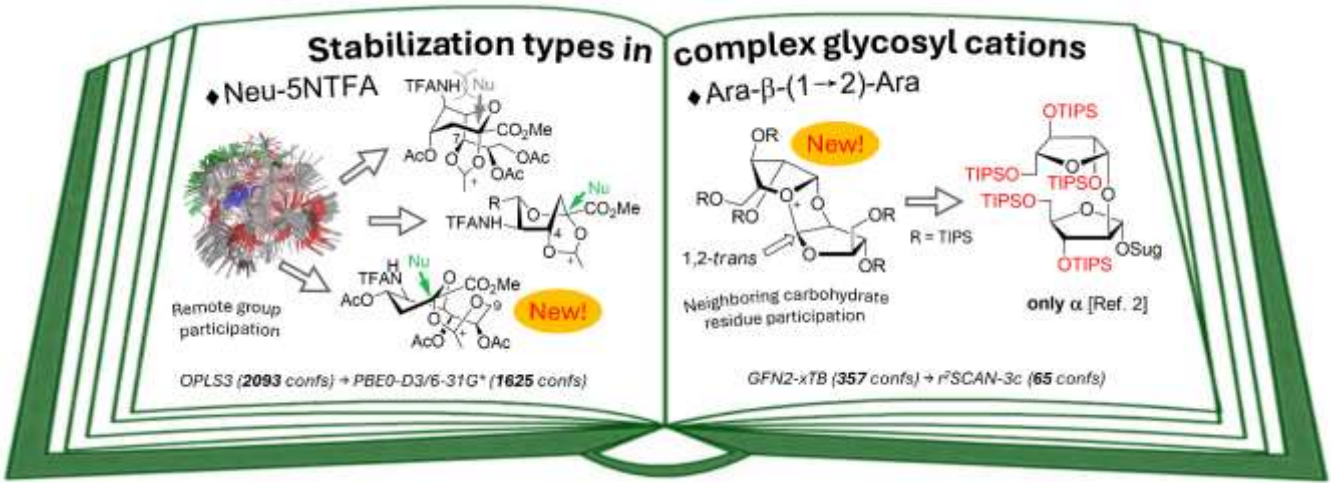
Quantum chemistry plays the central role in elucidating the structures of glycosylation reaction intermediates (in particular, glycosyl cations), since it is difficult to detect such short-living species experimentally under glycosylation conditions. Expectably, starting geometries significantly affect the results of glycosyl cations optimization during quantum-chemical calculations, so conformational searches are extremely needful to obtain lowest-energy geometries. This is especially critical for the cases of complex glycosyl cations, such as sialyl cation with a flexible side chain as well as disaccharide glycosyl cations with the additional degree of freedom coming from the glycosidic bond.

We show how systematic analysis of glycosyl-cation conformations – both low- and high-energy ones – allowed us to shed light on possibilities of glucosyl cations stabilization and its potential influence on glycosylation. Analysis of unique conformers obtained after conformational searches using either molecular mechanics (for the 4,7,8,9-OAc-5-NTFA-substituted sialyl cation[1]) or semi-empirics (for Ara-β-(1→2)-Ara glycosyl cations) with subsequent optimization at DFT level of theory revealed the possibility of remote groups participation (4-, 7-, and 9-OAc) in the sialyl cation, as well as the stabilization of disaccharide glycosyl cation by the neighboring carbohydrate ring. The found participation of a neighboring carbohydrate ring in fully TIPS-substituted Ara-β-(1→2)-Ara glycosyl cation made it possible to explain the exceptional α-selectivity observed[2] during glycosylation with the corresponding fully TIPS-substituted glycosyl donor. The proposed method of conformational search and clustering of the obtained conformers can be expanded for glycosyl cations of any complexity.



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Biantennary Guerbet-Type Glycolipids For Vesicular Drug Delivery: Synthetic Challenges Overview Through A Green Approach

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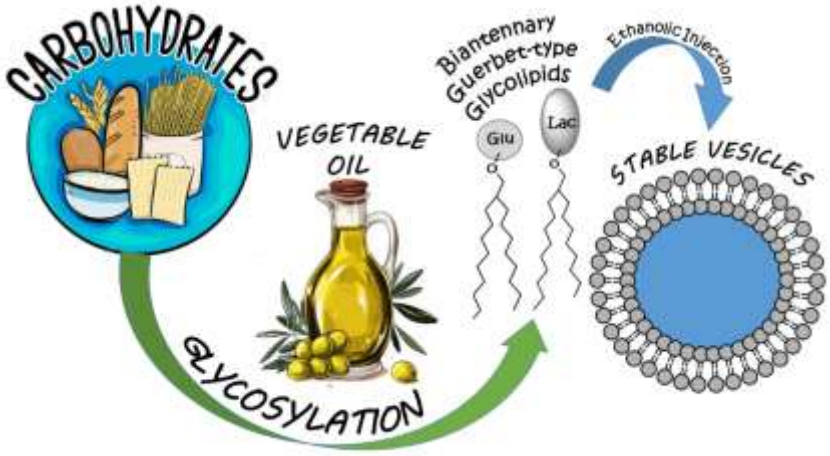
The adverse side effects of medical treatments have generated a need for drug delivery systems. A widely acknowledged approach aims at the entrapment of therapeutic agents in lipid assemblies. Due to their high stability, vesicles are of particular interest.[1] While fatty acid-based liposomes are well-known for their advantages in vesicular delivery systems, their stability is compromised under various conditions due to their ionic nature. Non-ionic amphiphilic structures can ensure greater stability of the assemblies.[2] Glycolipids are ideal alternatives for this purpose owing to their good amphiphilic properties and high biocompatibility. The complex design of biological membranes and separation challenges disfavors the application of natural glycolipids. Instead, synthetic compounds are typically suitable alternatives. Owing to the sensitive chemical linkage of ester bonds, synthetic glycolipids usually replace natural glyco-glycero-lipids. The biantennary Guerbet-type glycolipids are interesting replicates, which reflect glycosides with a biantennary hydrocarbon chain. Typically, this type of glycolipids can be synthesized via glycosylation of carbohydrates and Guerbet alcohols. Previous studies have confirmed that glycosides based on medium- and long-chain Guerbet alcohols effectively form vesicular aggregates in an aqueous medium.[3] However, medium and specifically long-chain Guerbet alcohols are not easily accessible due to their industrial synthetic constraints such as high purification cost and side reactions. To this end, different synthetic approaches were developed and investigated, leading to a novel, renewable feedstock-based approach for synthesizing biantennary Guerbet-type glycolipids from vegetable oil.[4] This article particularly discusses all synthetic challenges, limitations, and potential applications of the synthesized products in vesicle formulations.



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Expeditious Synthesis Of Glycoamphiphiles As Adsorbents/Ligands In Liquid Crystal-Based Biosensor For Pathogens Detection

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Nosocomial infections, also called healthcare-associated infections (HAIs), are caused by Pathogenic micro-organisms such as *Pseudomonas aeruginosa (P. aeruginosa).* This opportunistic bacterium is responsible for severe Broncho-pulmonary infections, poses a huge threat to cystic fibrosis patients, as well as those suffering from immunodeficiency and utilizes specific protein receptors in adhesion to host tissues [1]. Those specific proteins, so-called Lectins, are sugar-binding proteins recognizing specific structures of carbohydrates present on membrane cell surfaces and play an important role during the initiation of the infection process [2]. Traditional diagnostic tools used for pathogen detection are excessively time-consuming and complex to use. Due to their selectivity and sensitivity, the liquid-crystal (LC) based biosensors provide an alternative approach to rapid, simple and efficient bio-molecular detection, as well as an amplified output signal that can be directly observed by the naked eye [3].

We hereby innovate access to Glyco-Amphiphiles (GAs) associated with LC biosensor technology implemented here for the first time to detect pathogenic lectins, involved in HAIs. In this context, a new microwave-assisted strategy of GAs synthesis by *N*-aryl-glycosylation of unprotected carbohydrates was developed. Their stability to chemical hydrolysis was assessed using high-performance liquid chromatography (HPLC). The nano-structures of the GAs self-assembly have been investigated by Transmission Electron Microscopy (TEM) and the recognition between the carbohydrate moieties of our GAs and their specific Lectins has been proved using isothermal titration calorimetry (ITC) and quartz crystal microbalance with dissipation monitoring (QCM-D). All these results are validated pre-requisite for applying our synthesized GAs on the LC biosensors.

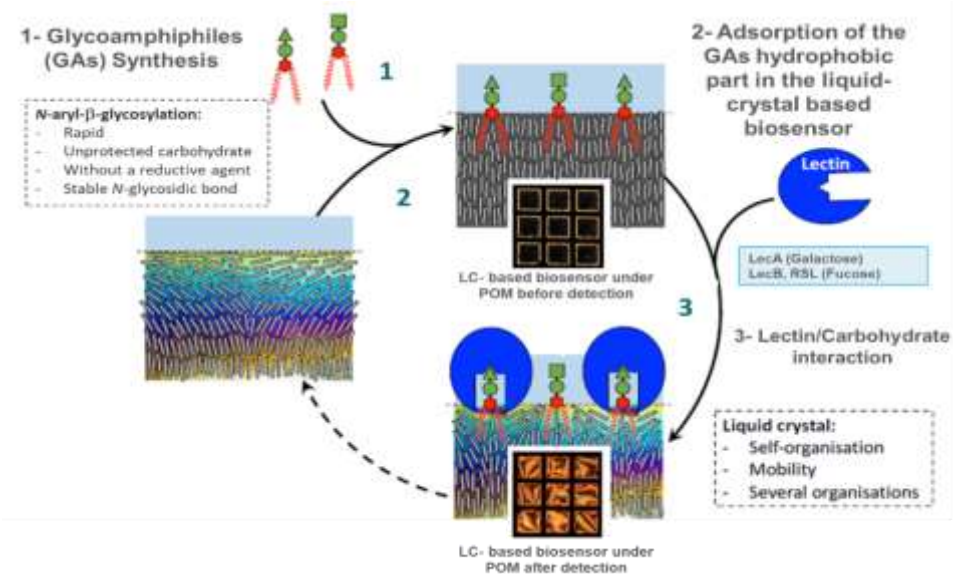

Figure 2: Schematic illustration of the LC sensing strategy for detecting lectins from pathgens

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Peptide-Scaffolded Detergent Assembly For Membrane Protein Studies

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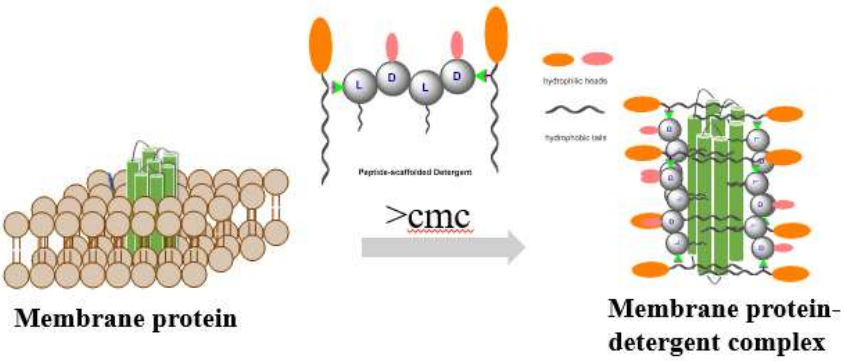
Abstract: Membrane proteins (MPs) undertake a broad range of physiological functions, and therefore are very important drug targets. Structural and functional studies of MPs require suitable amphiphilic system that mimic the natural lipid environment. Detergent is a class of amphiphilic molecules which have been widely used in the solubilization, purification and structural analysis of MPs. Different types of MPs show different preferences for detergents, but the commercially available types of detergents are limited. It is necessary to further the diversity of detergent. In the past decades, new detergents have mostly emerged in enlarged molecular sizes, featuring with multiple heads and multiple tails as assembled from traditional monomeric detergents. In our previous works, we have designed a type of β-strand peptides which enabled clear visualization of flexible conformations of MsbA, a bacterial ABC transporter. We also successfully introduced two classes of pre-assembled detergents via Click chemistry and Ugi reaction, respectively. This strategy enabled the two-dimensional expansion of detergent diversity for the first time. In this work, we report the synthesis of peptide-scaffolded detergents by pre-assembly of detergent monomers (OG and DDM) on peptides in various length (dipeptides and tetrapeptides) through Click reaction. We characterized the physical properties of these detergents and evaluated the thermal stability of $A_{2A}AR$ in these detergents, which belongs to the family of Class A G protein-coupled receptor. We found that one of the detergent C2B2 outperformed in the screening, indicating the great potential in the future MP study.



Keywords: Click reaction; Detergent; Membrane protein; Peptide detergent

Chiroptical Switching Of Photoswitchable Glycomacrocycles

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Macrocyclic compounds are of particular interest because of their unique structural and physicochemical properties as well as their potential applications in chemistry, material sciences, biology, drug discovery and drug delivery.[1] These properties are highly linked to the conformation and the cavity of macrocyclic structures which can be modulated through external stimuli such as pH, chemicals, heat and light.[2] Light can be easily and precisely controlled in time, location, wavelength and intensity, thus enabling the precise activation and deactivation of chemical and biological function. Photomodulation offers therefore a unique pathway to control over the structure, conformation and properties by using photoisomerization reaction of molecular photoswitches like azobenzene.[3] A variety of photoswitchable macrocycles have been investigated for the development of nanomaterials, smart polymers, molecular containers, and sensors.[4] Development of carbohydrates-based photoswitchable macrocycles is appealing since it allows to obtain enantiomerically pure macrocyclic compounds which could enlarge applications.

As a continuing interest in the development of photoswitchable glycomacrocycles,[5] we have designed and synthesized triazole-linked cyclic glycoazobenzenes through intramolecular glycosylation approach with interesting β-stereoselectivity for the mannosides. These photoswitchable glycomacrocycles displayed chiroptical properties which can be switched by light, solvent, acid and metal ions. All these original results will be presented.

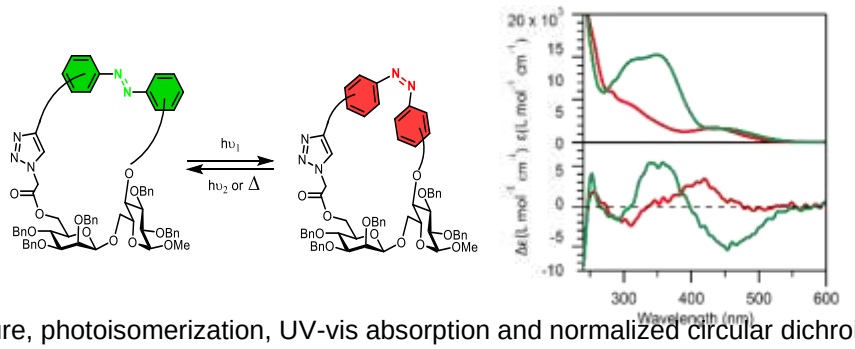

Figure: Structure, photoisomerization, UV-vis absorption and normalized circular dichroism spectra of cyclic glycoazobenzene.

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Synthetic And Analytical Study On Endo-Acting Enzymatic Degradation Of Plant α-L-Arabinofuranosidic Linkages

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For the functional analysis of arabinogalactan-degrading GH39 3-*O*-α-D-galactopyranosyl-α-L-arabinofuranosidase (GA*f*ase) and GH43 α-L-arabinofuranosidase (α-L-Ara*f*ase) from *Bifidobacterium longum*, the structural analysis of the products by enzymatic hydrolysis have been carried out by combined chemical methods and found that the limited degradative oligosaccharide structure, α-L-rhamnopyranosyl-(1→4)-β-D-glucuronyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactose in the AGP fermentation of *B. longum*.[1]

The GH39 homologue from *B. catenulatum* was treated with Seyal-type gum arabic, and the resulting hydrolyzed glycans were analyzed by combination of chemical synthesis and modifications, and NMR and MS analysis. The degradation products was found to contain [β-L-arabinofuranosyl-(1→2)]$_n$-β-L-arabinopyranosy-(1→3)-α-L-arabinofuranoside (n = 0–3) in this study, although the presence of the disaccharide moiety β-L-arabinopyranosy-(1→3)-α-L-arabinofuranoside has been reported in Senegal-type gum arabic[2] and the modification of β-L-arabinofuranosides is in the hydrophilic moiety of extensin as β-arabino-oligosaccharides, respectively.[3] Therefore, it was clarified that this GH39 enzyme cleaves up to pentasaccharide at α-L-arabinofuranoside of the inner residue of branched glycan on Seyal-type gum arabic. It was revealed that the branched glycan on galactan backbone of the Seyal-type gum arabic contain the terminal pentaarabinoside structure.[4]

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Multi-Reducing End Polysaccharides And Its Derived Hydrogels

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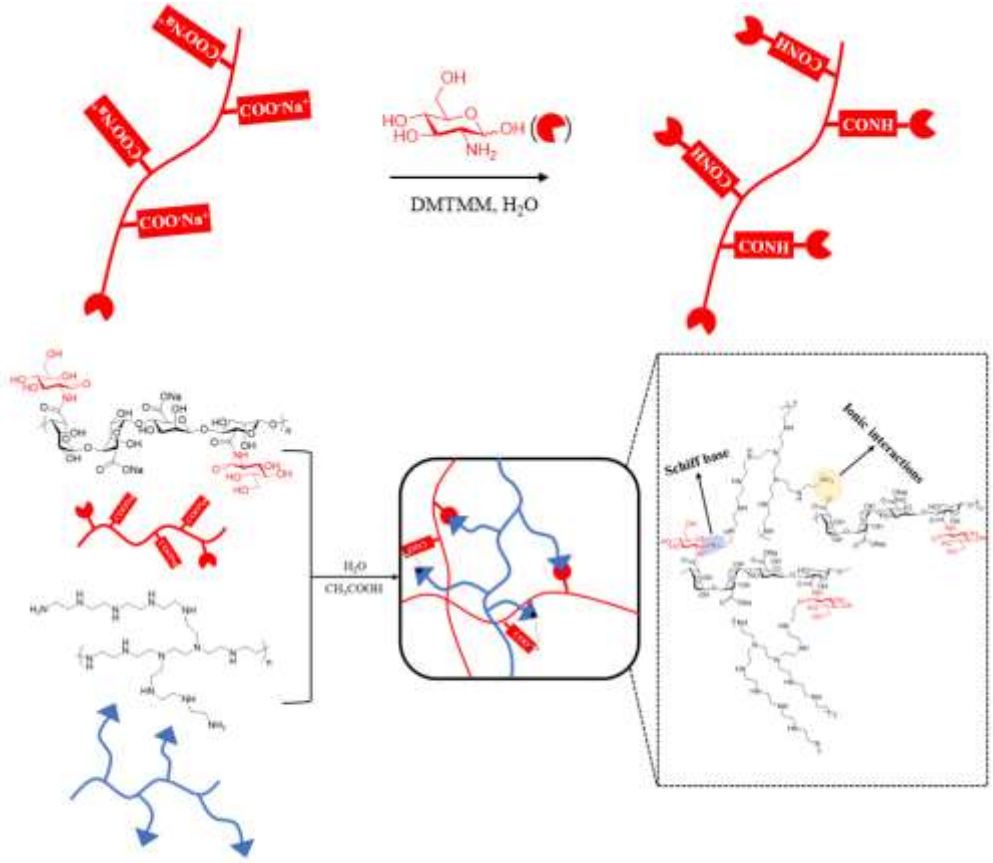
We developed a new approach to introduce multiple reducing ends to each polysaccharide molecule through amide formation[1]. Amine groups on monosaccharides such as glucosamine or galactosamine can react with carboxyl groups of polysaccharides, whether natural uronic acids like alginates, or derivatives with carboxyl-containing substituents such as carboxymethyl cellulose (CMC) or carboxymethyl dextran (CMD). Amide formation is assisted using the coupling agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). By linking the C2 amines of monosaccharides to polysaccharides in this way, a new class of polysaccharide derivatives possessing many reducing ends can be obtained. We refer to this class of derivatives as multi-reducing end polysaccharides (MREPs). The application of the multi-reducing end polysaccharides has been demonstrated by making hydrogels using multi-reducing end alginate with polyethyleneimine (PEI). The two polymer solutions can form a gel at room temperature after 24 hours. Acetic acid can be added to accelerate the gelation process, which 5 ul acetic acid can induce fast gelation within seconds[2].



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Deciphering The Complexity Of Microbiota

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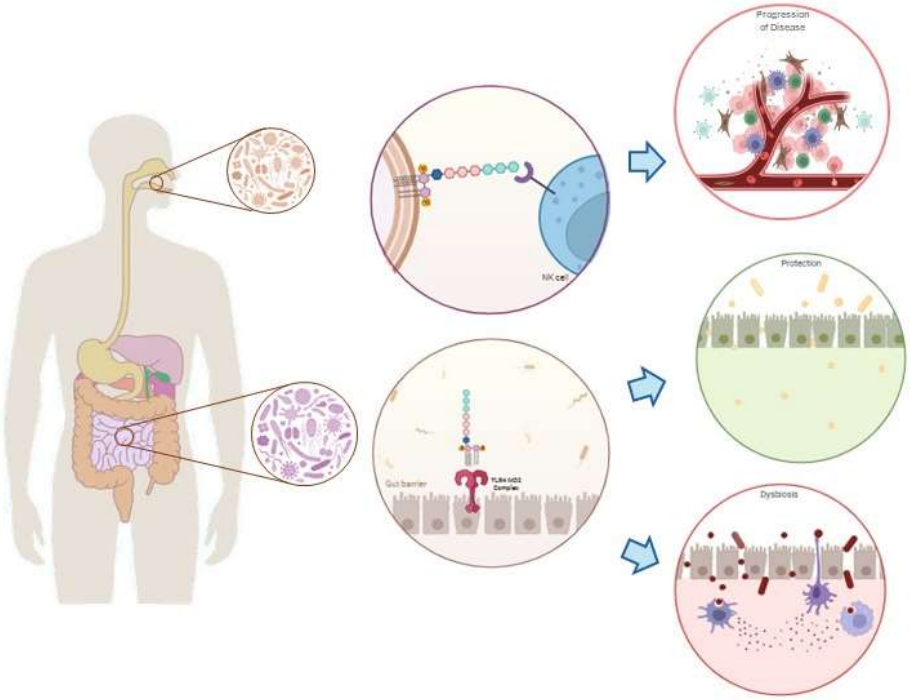
In recent years our understanding of the crucial role played by the gut microbiota in human health has increased. Lipopolysaccharide (LPS) derived from gut bacteria exhibit a nuanced functionality and are involved in countless critical pathways linked to their precise structural composition. They can trigger systemic pro-inflammatory responses in the presence of pathogens but can also evoke immunomodulatory responses in commensal organisms. [1] These intricate processes are integral to determining outcomes related to disease, commensalism, and the establishment of mutualistic relationships within the gut ecosystem. In addition, the elucidation of bacterial LPSs is challenged by their structural complexity and by bacterial diversity of the gut. Nevertheless, not only the gut microbiota is involved in such important processes, in the rest the body also plays essential roles, for example in the oral microbiota *Fusobacterium nucleatum*, Gram-negative obligate anaerobe, has beed identified as a key player in dysbiosis and colorectal cancer progression,[2] has further highlighted the intricate relationship between humans and bacteria. In this context, we report on the characterization of LPS from different human-associated bacteria achieved through the combined use of chemical, spectroscopic, spectrometric, computational techniques, and preliminary immunological assays.



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Chemoproteomic Profiling Of Siganling Metabolite Fructose-1,6-Bisphosphate Interactome In Cancer Cells

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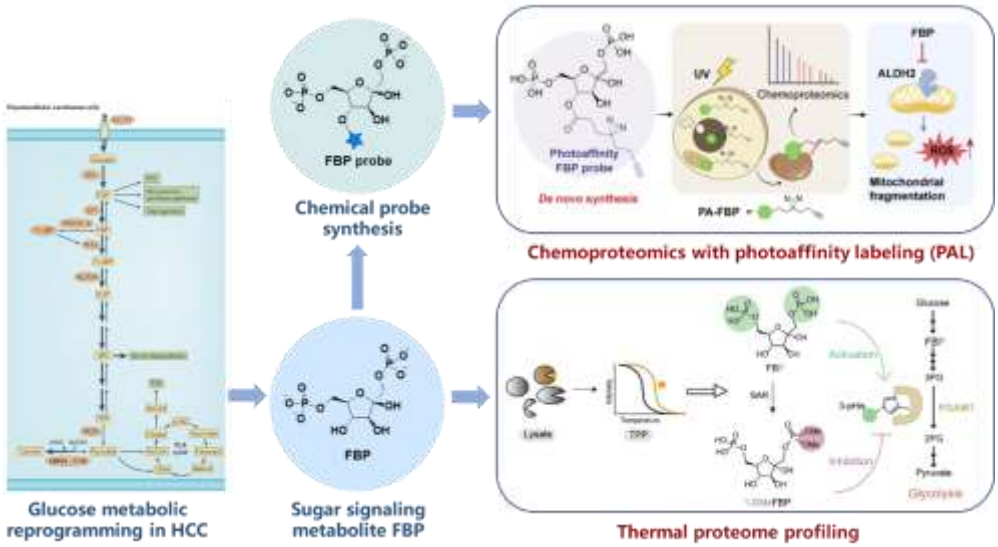
Sugar metabolism serves as the resources of energy and biomass for life while its homeostasis guarantees cellular resilience to stress from internal and external environment. Sugar metabolic reprogramming as well as the accumulation of certain sugar metabolites have been considered as hallmarks of metabolic diseases including cancer. Emerging evidences have shown that sugar metabolites act as signaling molecules to regulate various cellular events through interacting with important proteins. A deep understanding of sugar metabolite-protein interactions should provide valuable implications in human physiopathology. Although tremendous efforts have been made for determining individual sugar metabolite-protein interaction through cellular molecular biological or genetic approaches, global profiling of such interactome remains challenging.

Here we describe the global target identification and functional validation of glycolytic signaling metabolite fructose-1,6-bisphosphate (FBP), based on two different quantitative chemoproteomic approaches. Firstly, de novo synthesis of a photoaffinity FBP probe (PA-FBP) enabled comprehensive chemical proteomic mapping of FBP-interacting proteins based on photoaffinity labeling (PAL) directly in living cancer cells and identified mitochondrial metabolic enzyme aldehyde dehydrogenase 2 (ALDH2) as a new target of FBP. FBP was found to enter into the mitochondria and inhibit ALDH2 activity, resulting in the increase of cellular reactive oxygen species (ROS) level along with mitochondrial fragmentation. This might act as a new mode of glucose sensing and signaling mediated by FBP-ALDH2-ROS axis.[1] Likewise, thermal proteome profiling uncovered a full spectrum of FBP interactome as well as a unique covalent signaling function of FBP that activates intrapathway enzyme PGAM1 *via* histidine phosphorylation, therefore feedforwards Warburg effect to support cell proliferation in cancer cells.[2]



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Structural Insights Into The Interaction Between A Gonococcal Mimitope Vaccine Candidate And Its Cognate Monoclonal Antibody

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The global spread of multidrug-resistant strains of Neisseria gonorrhoeae (Ng) presents a global health emergency; thus, the development of a safe and effective vaccine against gonorrhea is urgently needed. It is known that the monoclonal antibody (mAb) 2C7 recognizes a glycan epitope on Ng lipooligosaccharide (LOS) that is expressed by almost every clinical isolate (Figure 1) and elicits complement-dependent bactericidal activity making LOS a good vaccine candidate. [1]
Previously, we identified a peptide that mimics the gonococcal 2C7 LOS epitope, which when cyclized and formulated as a multi-antigen peptide vaccine, attenuates vaginal colonization of mice by gonococci.[2] In the present study, we combined X-ray crystallography, NMR spectroscopy, and other biophysical techniques to perform structural and conformational analyses of 2C7 elucidating the three-dimensional complex of 2C7 and the cyclized peptide (CP2). The crystal structure of the Fab2C7-CP2 complex showed that CP2 formed a beta-hairpin bound to the Fragment antigen-binding (Fab) primarily through hydrophobic interactions. NMR spectroscopy and molecular dynamics simulations mapped the 2C7 epitope and identified the bioactive conformation of CP2 (Figure 1). Isothermal titration calorimetry and native mass spectrometry provided further information on the energetics and assembly state of the complex. Collectively, our multidisciplinary studiy suggests strategies for humanizing mAb 2C7 as a therapeutic against gonococcal infection and for optimizing peptide CP2 as a vaccine antigen.

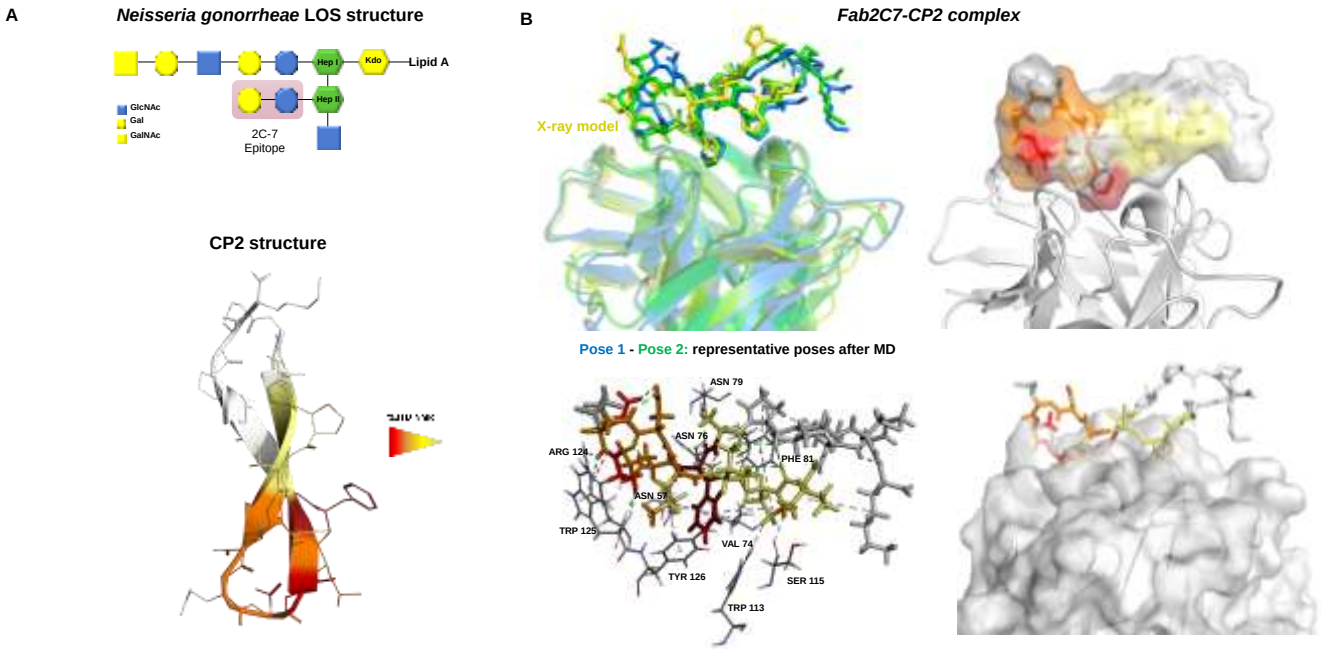

Figure 1. A: Representations of Ng LOS and CP2 structures. **B:** Different 3D views of Fab2C7-CP2 complex.

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Probing The Exopolysaccharide Interactome Of Staphylococcus Epidermidis Biofilms Through Live Cell Proximity Labelling

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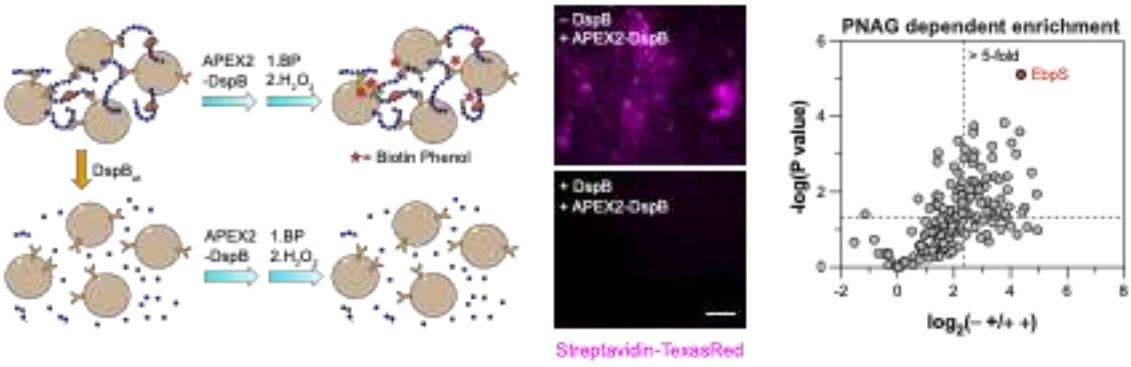
Bacterial biofilms are composed of surface attached bacterial cells embedded within an extracellular polymeric substance (EPS) composed of exported polysaccharides, proteins and extracellular DNA, which facilitates both cell–cell and cell–surface adhesion and serves as a protective barrier. Exopolysaccharides, like β-(1→6)-poly-*N*-acetylglucosamine (PNAG), are a significant structural component of the biofilm EPS of both Gram-positive and Gram-negative human pathogens, including *Staphylococcus epidermidis* and *Staphylococcus aureus*, but there is little known about how they mediate cell–cell interactions. Infections by biofilm forming bacteria are estimated to contribute to between 60-80% of all human infections, and the bacteria within the biofilm are up to one thousand-fold more resistant to antibiotic treatments. Thus, there is an interest in identifying new targets for anti-biofilm therapeutics that could be used to disrupt biofilms and potentiate the effectiveness of existing antibiotics. These efforts, however, require a detailed understanding of the intermolecular interactions that contribute to the integrity of live biofilms. Here, we have developed a live cell proximity labelling approach combined with quantitative mass spectrometry-based proteomics to map the PNAG interactome of live *S. epidermidis* biofilms.[1] Using this approach, we identified that the lysin motif domain found at the C-terminus of the *S. epidermidis* membrane protein EbpS functions as a PNAG binding protein, contributing to cell–matrix binding interactions in *S. epidermidis* biofilms. This live cell proximity labelling approach provides a rapid method to characterize exopolysaccharide interacting proteins of diverse bacterial biofilms and will lead to the identification of new proteins that contribute to biofilm integrity.



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Supramolecular Glycosyl-Nanoproteins Based On Host-Guest Chemistry For Targeted Co-Delivery In Vivo

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Supramolecular nanoproteins based on host-guest interaction can integrate different therapeutic functions into a single nano-delivery system to effectively overcome the limitations of chemotherapy. In this work, the biohybrid supramolecular glycosyl-nanoproteins (Bio-WP5G-Avi) were constructed via self-assembly of biotinylated glycosyl-supramolecule complex (Bio-WP5G) with streptavidin (Avi), where Bio-WP5G is formed through host-guest interaction between biotinylated pillar[5]arene (Bio-WP5) and galactosamine derivative (G), which displays excellent GSH responsiveness, enhanced hepatoma-targetability, and efficient co-delivery. The host molecule Bio-WP5 contains disulfide bonds that respond to high concentrations of GSH in the tumor microenvironment after entering hepatoma cells, while the guest molecule G contains ammonium cations for loading of polyanionic siRNA by electrostatic interaction and galactose residues for targeting hepatoma cells by carbohydrate-lectin specific interactions. In in vitro experiments, Bio-WP5G-Avi loaded with DOX/siRNA not only significantly improved the transfection efficiency of siRNA and exhibited effective gene silencing ability, but also enhanced the cytotoxicity of drugs to HepG2/ADR cells. Furthermore, in vivo experiments with Bio-WP5G-Avi@DOX/siRNA using HepG2/ADR subcutaneous tumor-bearing nude mice model confirmed good biosafety, enrichment at tumors and enhanced therapeutic efficacy. This work provides a convenient strategy to construct biohybrid glycosyl-supramolecular nanoproteins for targeted co-delivery of drug/siRNA, which can be used to overcome multidrug resistance tumors with a minimal risk of adverse outcomes.

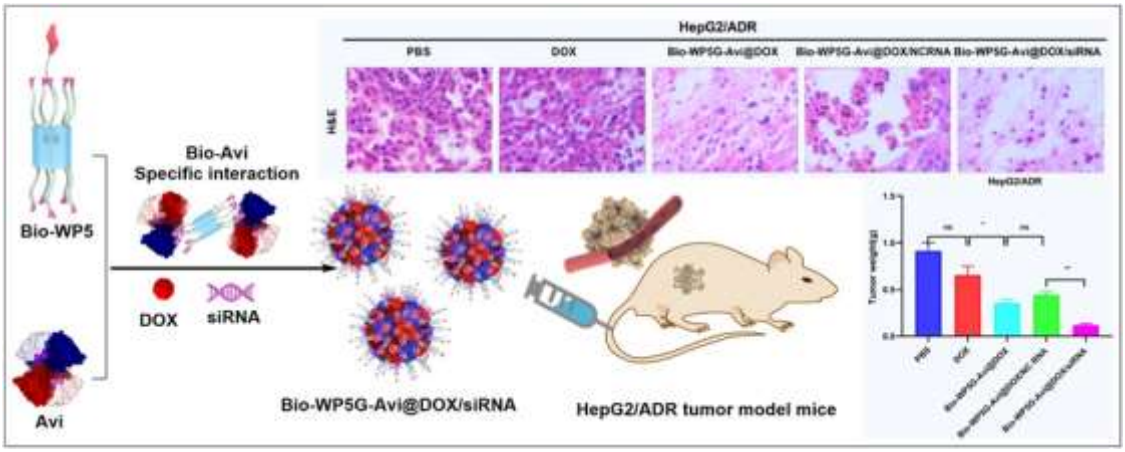

Figure 1. Illustration of supramolecular glycosyl-nanoproteins and their targeted co-delivery.

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Synthesis Of Difluoromethylene Bisphosphonates As Pyrophosphate Mimetics To Probe Bacterial Capsular Polysaccharide Biosynthesis Machinery

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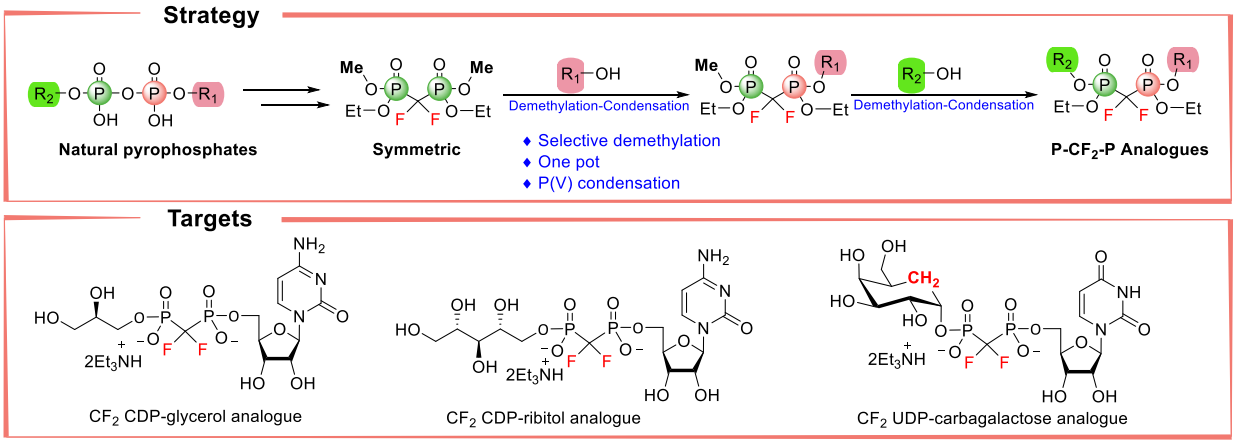
Naturally occurring pyrophosphates play important roles as building blocks in numerous vital biological processes, such as metabolism and the biosynthesis of bacterial capsular polysaccharides and teichoic acids.[1] The chemical modification of natural pyrophosphates has delivered critically important tools to study the mechanism of pyrophosphate processing enzymes and the use of these mimics in studying the function of a wide variety of enzymes is well documented. The sensitivity of the P-O-P bond towards both enzymatic and chemical hydrolysis offers a unique opportunity for the design and synthesis of stable non-hydrolysable inhibitors. In particular, difluoromethylene bisphosphonate-linked (P-CF_2-P) analogues are highly sought after as these analogues closely resemble the natural pyrophosphate, in terms of pKa-value (as the electron-withdrawing effect of fluorine atoms increase the acidity of the phosphonates), as well as the bond angles and lengths.[2]

We will present a synthetic method to generate difluoromethylene bisphosphonate analogues of various natural products using a desymmetrization strategy of diethyl- (dimethyl)difluoromethylene bisphosphonate which can be orthogonally deprotected to set the stage for one or two consecutive condensation reactions through P(V) condensation chemistry.[3]

These analogues will be used to probe and inhibit their cognate enzymes, to advance our understanding of the biosynthesis of components of bacterial cell wall and contribute to the development of novel therapeutic agents, and antimicrobial strategies.



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Chemical Glycobiology Studies On Bacterial Pseudaminic Acid

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Pseudaminic acid (Pse), first discovered in 1984, has been identified in a variety of pathogenic bacteria as important surface glycans. Belonging to the nonulosonic acid carbohydrate family, Pse is structurally related to its well-known congener, sialic acid. Pse exists with both α and β configuration in native glycoconjugates with variable substitution patterns at *N*5 and *N*7. Such structural diversity makes the structure-function relationship of Pse-containing glycans both intriguing but also challenging to study. The biological and evolutional significances of bacterial pseudaminic acid and its glycoconjugates remain largely unexplored, mainly due to the lack of synthetic access to pseudaminic acid and the structurally defined pseudaminylated glycoconjuates. Over the past years, we have been working on the chemical synthesis, biochemistry, biosynthesis, biology studies, and vaccine development on the bacterial pseudaminic acid. In this presentation, I will present our updated progress.

Keywords: pseudaminic acid, chemical glycobiology, chemical synthesis, vaccine development

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Synthesis Of Well-Defined Glycopolymers Towards Biomedical Applications

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Carbohydrates are not only the storage units that constitute the support and energy of the organism but also the information transfer players in many important physiological processes.[1,2,3] Synthetic glycopolymers serve a crucial role as biomimetics of natural polysaccharides in various biological applications by mimicking the function of cell-surface saccharides and effectively interacting with proteins, cells, and pathogens through the multivalent effects. Glycopolymers have also been demonstrated as a promising substrate due to their significant advantage in well-designed structural properties, which can be utilized in the form of nanoparticles, nanogels, and other advanced structures. To investigate the multivalent effects, the well-defined synthesis and pharmaceutical development of distinct glycopolymers have been preliminarily studied in our group. A series of glycopolymers were fabricated *via* ring-opening metathesis polymerisation (ROMP) catalyzed by hoveyda-grubbs II in phase transfer catalysis (PTC).[4] These synthetic glycopolymers, functionalized with specific sulfate patterns, have exhibited promising antitumor properties and broad-spectrum antiviral activities.[5,6] In addition, the glycopolymers derived from the hydrophilic skeleton possess a more flexible structure than the assembled polymers, which is more inclined to the non-assembly morphology as the natural polysaccharide. The approaches developed herein offer distinct and well-defined glycopolymers for deciphering the biological roles of natural bioactive polysaccharides.

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Synthesis of C-Oligosaccharides via Ni-Catalyzed Reductive Hydroglycosylation

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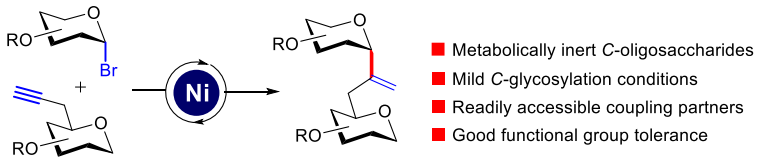
Glycans play crucial roles in a myriad of biological processes, such as cell adhesion, the immune response, inflammation, cancer metastasis, and viral and bacterial infections. Compared to the native glycans that are assembled mostly via *O*-glycosidic linkages, *C*-linked glycans (*C*- oligosaccharides) could retain the biological and pharmaco- logical properties while being metabolically stable, making them artificial surrogates and/or mimics of the native glycans with therapeutic potential. In recent decades, a tremendous amount of effort has been devoted to the development of methods for the synthesis of C-glycosidic linkages, employing glycosyl electrophilic/cationic species, anionic species, free radical species, or transition-metal complexes as glycosylation intermediates. However, methods that can be applied to the synthesis of C-oligosaccharides remain limited. In 2021, we disclosed an effective method for the synthesis of vinyl C-glycosyl amino acids/peptides via Ni-catalyzed reductive coupling of alkyne derivatives of amino acids/peptides with glycosyl bromides. Given the broad functional group tolerance of this hydroglycosylation reactions and the ready accessibility of alkynyl sugar derivatives, we set out to examine the applicability of this protocol to the synthesis of the challenging C-oligosaccharides. We reported a straightforward approach to the synthesis of vinyl C-linked oligosaccharides via the Ni-catalyzed reductive hydroglycosylation of alkynyl glycosides with glycosyl bromides.



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Chemoenzymatic Synthesis Of *N*-Glycans Of *S. Mansoni* To Investigate Anti-Parasite Immune Responses

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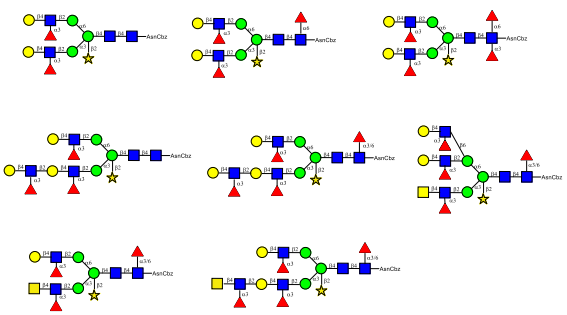
Schistosomiasis poses a public health challenge due to its high infectiousness and chronic persistence. Schistosoma species express a variety of glycan antigens on their cell surface that play pivotal roles in host immunity and escape strategies. In particular, *Schistosoma mansoni* displays a remarkable structural diverse set of *N*-glycans that have various core modifications and terminal epitopes. Modifications such as *α*-1,3/1,6 fucose (CF3 and CF6) and *β*-1,2 xylose (CX) are typical of schistosome glycans and recognized as common allergens[1]. While core xylosylation does not inhibit terminal epitope recognition, combination of core xylose and *α*-1,3 fucose constitute cross-reactive carbohydrate determinants. Terminal antigens, including motifs such as Lewis X (Le^x), Lac-di-NAc (LDN), and fucosyl Lac-di-NAc (LDN-F) are present on surface *N*-glycans. They can engage with the DC-SIGN on dendritic cells, facilitating pathogen internalization and antigen processing making DC-SIGN a critical target for therapeutic interventions. Multi-antennary *N*-glycans can have higher binding avidity for DC-SIGN than mono-valent epitopes, emphasizing the importance of *N*-glycan architecture on immune recognition and response [2]. However, such modulation of binding is not well-understood due to a lack of panels of well-defined *Schistosoma* glycans.



We are to exploring the full potential of schistosome *N*-glycans to modulate immune responses and develop innovative therapeutics by synthesizing large panels of di- and tri- antennary *N*-glycan having different patterns of core fucosylation (CF3/CF6), core xyloside and terminal epitopes such as Le^x, LDN, LDN-F. The synthetic approach is based on newly identified glycosyl transferases and chemoenzymatic strategies that make it possible to modify each antenna with a unique epitope. The compounds have been printed as a glycan microarray to examine interactions with glycan binding proteins and serum antibodies. This research is providing an understanding of *N*-glycan-mediated immune dynamics and supports the utilization of these structures in vaccine development.

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Synthetic MUC1 Glycopeptide Vaccines And Strategies To Enhance The Immune Responses

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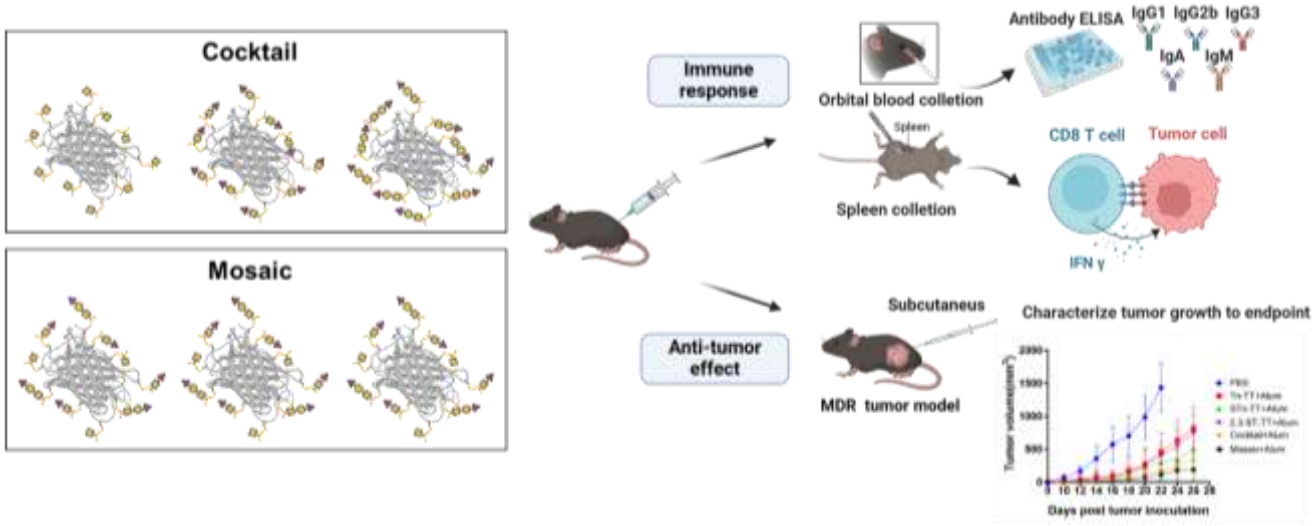

Mucin 1 (MUC1) is overexpressed on tumor cells with aberrant glycosylation on its side chain, which makes it an important tumor-associated antigen. Aberrant oligosaccharides on tumor MUC1 include Tn and T antigen, as well as their modifications with sialic acid. Changes in the structure of the glycan chain may affect the immunogenicity and efficacy of anti-tumor glycopeptide vaccines. Here, we synthesized MUC1 glycopeptides with different oligosaccharides by solid-phase peptide synthesis and chemoenzymatic methods, and covalently coupled the glycopeptide to protein carriers to obtain multivalent carrier-based glycopeptide vaccines. To further develop effective anti-tumor glycopeptide vaccines, Mosaic and Cocktail strategies were developed to display glycopeptide antigens with different oligosaccharides multivalently. All the vaccines could stimulate the immune system to a certain extent and induce relatively high antibody titer. The Mosaic and Cocktail multivalent glycopeptide vaccine could induce the highest expression of antibody, and the antibody showed strongest binding to B16MUC1 cells compared with others. Through fluorescence-activated cell sorting (FACS), we demonstrated that Mosaic and Cocktail multivalent glycopeptide vaccine could effectively stimulate and activate CD8+T cells and promote the proliferation of CD8+T cells, resulting in enhanced efficacy to prevent tumor growth. Our findings show that constructing glycopeptide vaccine by Mosaic method and Cocktail method is an effective approach to develop anti-tumor vaccine. Additionally, compared with Tn glycopeptide vaccine, plasma antibodies produced by mice immunized with salic acid-modified Tn (STn) glycopeptide vaccine showed stronger binding to B16MUC1 cells, and STn glycopeptide vaccine showed a stronger anti-tumor response, implying that targeting tumor sialylation may be a promising therapeutic strategy.

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Chemical Synthesis and Functions of Symbiotic Bacterial Lipid A for Safe Vaccine Adjuvant Development

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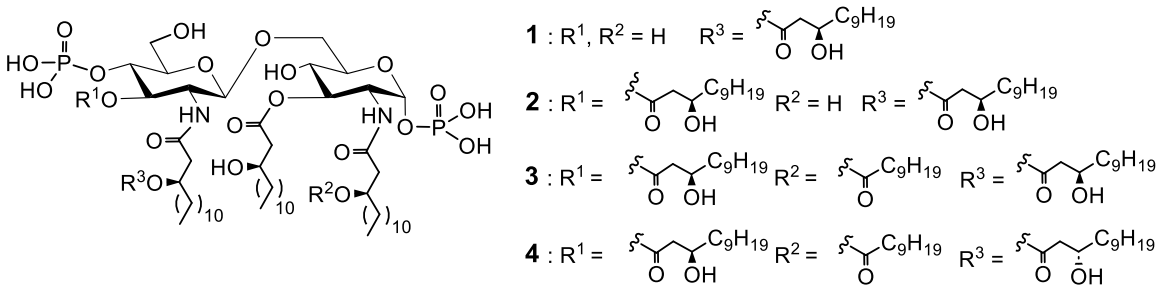
Lipopolysaccharide (LPS) is a major glycoconjugate in outer membrane of Gram-negative bacteria and canonical *Escherichia coli* LPS activate innate immunity to induce lethal strong inflammation. The terminal glycolipid lipid A is the active principle of LPS. Low inflammatory lipid A have been expected as vaccine adjuvants.

We hypothesized that co-evolved parasitic and symbiotic bacterial components should modulate host immunity moderately with low toxicity. We synthesized parasitic [1] and symbiotic [2] bacterial lipid A and elucidated the molecular basis of immunoregulation, and developed safe and useful adjuvants. In this presentation, we introduce the structure determination, chemical synthesis, and structure-activity relationship studies of lipid A from *Alcaligenes faecalis* inhabiting gut-associated lymphoid-tissue (GALT) that is responsible for the mucosal immunity regulation.

We synthesized *A. faecalis* lipids A **1**-**3** with diverse acyl group patterns and identified the active center as hexa-acylated **3**[2]. Lipid A **3** was confirmed to exhibit non-toxic but useful adjuvant function (enhancing antigen-specific IgA and IgG production) [3-7], and that vaccine model using **3** was found to be significantly protective against bacterial infection [4]. Since IgA is responsible for mucosal immune homeostasis, by focusing on GALT symbiotic bacteria, we found promising adjuvant that can safely regulate mucosal immunity. Furthermore, lipid A **4**, which reversed the stereochemistry of the acyl side chain hydroxy group, was found to be more active than **3**[8], and the molecular basis of the adjuvant function is also becoming clear.



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Arabinofuranose Functionalized Polystyrene Microbeads Selectively Target *Staphylococcus Aureus*

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Staphylococcus aureus is a Gram-positive bacterium, classified as one of the "ESKAPE" pathogens that pose a significant risk to human health through emergence of multi-drug resistant strains.[1] Current diagnostic techniques for *S. aureus* include cell culture, DNA amplification, and antibody-based detection assays. These are often slow and/or require expensive equipment, highlighting the pressing need for rapid, operationally simple, and cost-effective methods for *S. aureus* detection at the point of care.[2]

D-arabinofuranose is a rare sugar, found as a key component of the mycobacterial cell wall as well as in the pili of several *Pseudomonas* species.[3a,b] Additionally, it has been shown that analogues of D-arabinose are metabolised by *Mycobacterium smegmatis* and are uptaken by *Escherichia coli* [3a,c]. Therefore, D-arabinose was of interest to our group as a selective targeting motif in bacterial diagnostics.

Consequently, the Galan group and FluoretiQ Ltd. investigated the use of D-arabinose in a rapid bacterial detection assay based on carbohydrate-functionalized polystyrene microspheres (**Fig. 1**).[4] A library of arabinose derivatives conjugated to microbeads was synthesized, varying in functionalization site and linker length, and subsequently used in agglutination assays. These experiments reveal selective binding of C-2 linked arabinose moieties to *S. aureus*, over a panel of common bacterial pathogens.[5] The interaction is observed *via* clustering of microbeads after incubation with bacteria, due to multivalent binding between the surface arabinose and *S. aureus*. The clustering effect can be quantified using image processing software developed by FluoretiQ and allows subtle differences in D-arabinose-bacteria interactions to be studied in detail.

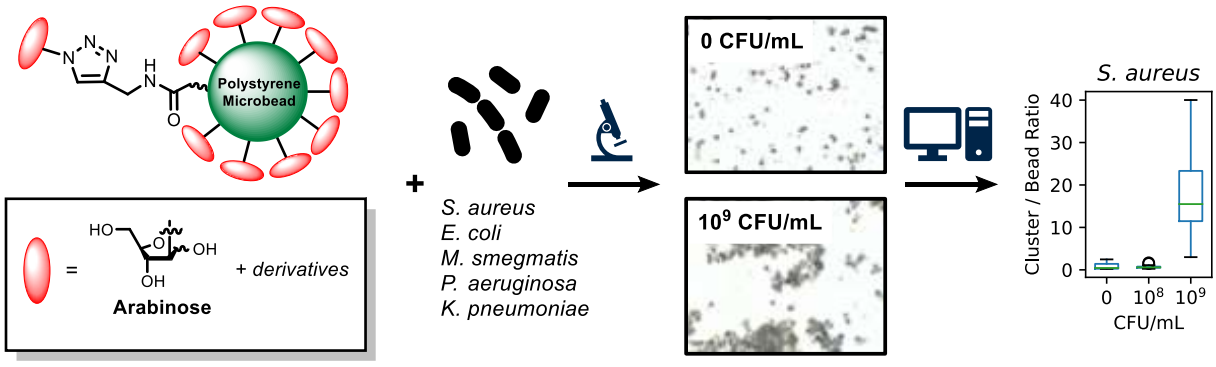

Figure 1: Arabinose functionalized microbead agglutination assay allowing detection of *S. aureus*.

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A Sensor Array To Discriminate *Pseudomonas Aeruginosa* Pathoadaptation

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Microbial infections collectively accounted for over 13 million deaths in 2019, 559,000 of which were due to *Pseudomonas aeruginosa* (*Pa.*).[1] Bacterial pathogens readily adapt to the environment within their host, often altering virulence factors associated with adhesion and infection. Potential biomarkers for identifying these pathoadaptations include carbohydrate binding proteins, or lectins, that are associated with a variety of pathogens such as bacteria, viruses and fungi. Lectins bind to glycans displayed on almost all cell surfaces and play a key role in cellular recognition during pathogenesis.

Inspired by the mammalian glycocalyx, the design of a sensor array for the detection of bacterial lectins and associated pathoadaptations is reported. Ten fluorescent glycopolymers, differing only by carbohydrate moiety were synthesised. Upon addition of bacteria to our array, a unique 'fingerprint' of fluorescence responses were observed. The array was tested against a library of Pa. transposon mutants and clinical isolates, linear discriminant analysis conducted on the resulting dataset resulted in discrimination of each of mutant. Leave-one-out cross-validation suggested this label-free approach enabled the effective discrimination of genetically engineered *Pa.* transposon mutants, and clinical samples.

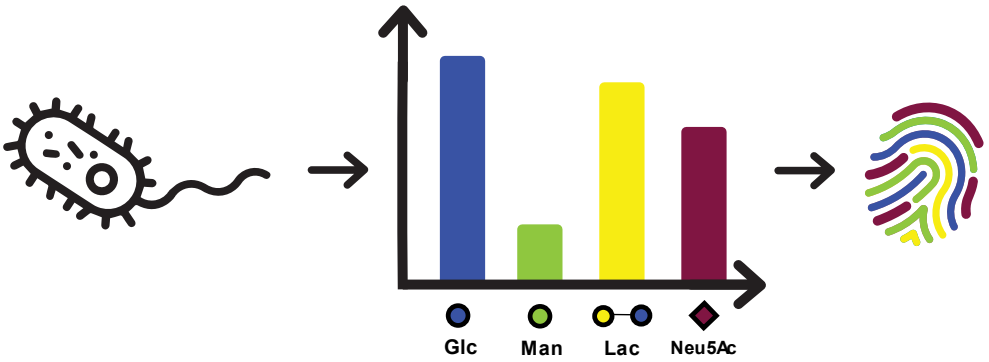

Fig. The principle of our array with each glycopolymer generating a response to a bacterium that are combined to generate a 'fingerprint'.

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Preparation And Immunogenicity Study Of Paucimannose Glycoconjugate

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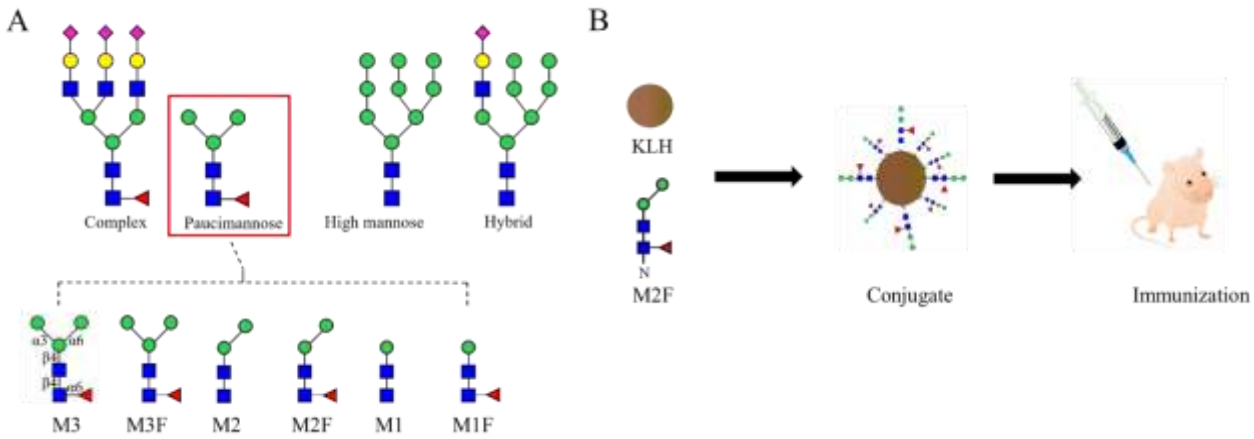
Paucimannose is a kind of oligomannose type *N*-glycosylation structure with important biological activity, which exists in a large number of plants and invertebrates, while its content in human body is relatively low. In recent years, many studies have proved that this unusual *N*-glycan structure has a certain relationship with human immune system, infection process, cell development and even tumor formation.[1] Human paucimannose contains a total of six glycoforms, among which two glycoforms, namely M2F and M3F, have the largest characteristics. Currently, there is little research on M2F and M3F, and no report on their *in vitro* preparation. On the other hand, there is only one antibody on the market that can recognize the paucimannose, but some studies have reported that its recognition ability is poor, and only has good specificity for two of the six glycoforms.[2] Therefore, it is essential to prepare M2F and M3F glycoforms *in vitro*, and to study their biological properties and further develop the specific antibodies.

M2F and M3F glycoforms are abundantly expressed in some pathological tissues and tumor cells, and regarded as the biomarkers of some cancers.[1] In this study, M2F and M3F analogues were successfully synthesized and characterized *in vitro*. The two glycoforms consist of two N-acetylglucosamine (GlcNAc), one fucose (Fuc), two mannoses (Man), or three Man, respectively. Glycoprotein conjugates were prepared by coupling oligosaccharides with proteins (Disuccinimidyl glutarate, DSG) using Keyhole Limpet Hempet Hemocyanin (KLH) as carrier protein. Immunological experiments were conducted using the two glyco-KLH conjugates prepared, and two immune adjuvants (aluminum hydroxide and Frehde adjuvant) were used. The result showed that both conjugates can stimulate the production of antibodies with high titers and strong antigen specificity, indicating that the antibodies have potential clinical application value.



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Heparan Sulfate As A Scaffold For Stable Presentation Of Cell-Surface Rna

Zeshi Li[§], Bhagyashree S. Joshi[§], Ruud Wijdeven, Irene Santos Barriopedro, Milad Shademan, Erick Bos, Geert-Jan Boons, Marvin Tanenbaum, Thom Sharp, Vered Raz, Sjaak Neefjes, Michiel Vermeulen, Chirlmin Joo

[§] equal contribution

Recent discoveries have shown RNA's localization on the cell surface, defying the traditional view that RNA functions only intracellularly, However, the mechanistic understanding of how cell-surface RNA (csRNA) is stably presented on the plasma membrane is lacking. Here, we exploit the RNA-sensing ability of TLR7 and established it could be used as a probe for csRNA. Using the TLR7 probe, we identified heparan sulfate (HS) as a crucial factor for RNA presentation on cell surface by a CRISPR-Cas9-mediated genome-wide knockout screening. By performing proximity labeling and proteomic analysis, we further revealed HS-RNA association required RNA-binding proteins. We then demonstrated these heparan sulfate-associated csRNA could modulate the interaction between poliovirus receptor (PVR) and killer cell immunoglobulin-like receptor 2DL5 (KIR2DL5) by acting as a co-binder to enrich the latter protein on the cell surface, unveiling a new layer of complexity in the regulation of cell surface receptor-ligand interactions.

Development Of Mannose-Presenting Colicin Bioconjugates To Exploit Native Bacteria Adhesin Pathways

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Numerous bacterial species form biofilms[1] which are often integral to pathogenesis.[2] As antibiotic resistant bacteria become more prevalent, there is an increasing need to develop new non-bactericidal agents against adhesive bacteria. Both adherent invasive *E. coli* (AICE) (which is implicated in inflammatory bowel disease in Crohns disease), and Uropathogenic *E. coli* (which is responsible for 80% of uncomplicated urinary tract infections) adhere to terminal mannose units on epithelial glycoproteins using the FimH adhesin, located at the tip of the type 1 pilus.[7] A number of monovalent α-D-mannopyranoside-based binders have previously been developed to prevent the binding of adherent bacteria to epithelial cells, in this talk we will present a novel strategy to simultaneously target both FimH and the bacterial cell surface receptor BtuB, in order to develop new agents capable of aggregating adhesive bacteria.

We report the preparation of colicin E9 bioconjugates that bind to the BtuB receptor on bacterial cell surfaces,[11] but also present functional motifs, such as biotin, fluorescein and α-D-mannopyranosides, and show that these bioconjugates are capable of redecorating the surface of *E. coli*. Furthermore, we demonstrate that the incubation of *E. coli* strain BW25113 with α-D-mannopyranoside-presenting colicin E9 bioconjugates results in *E. coli* aggregation, likely due to the formation of intercellular crosslinks between FimH units and BtuB receptors located on the bacterial surface (Fig. 1).

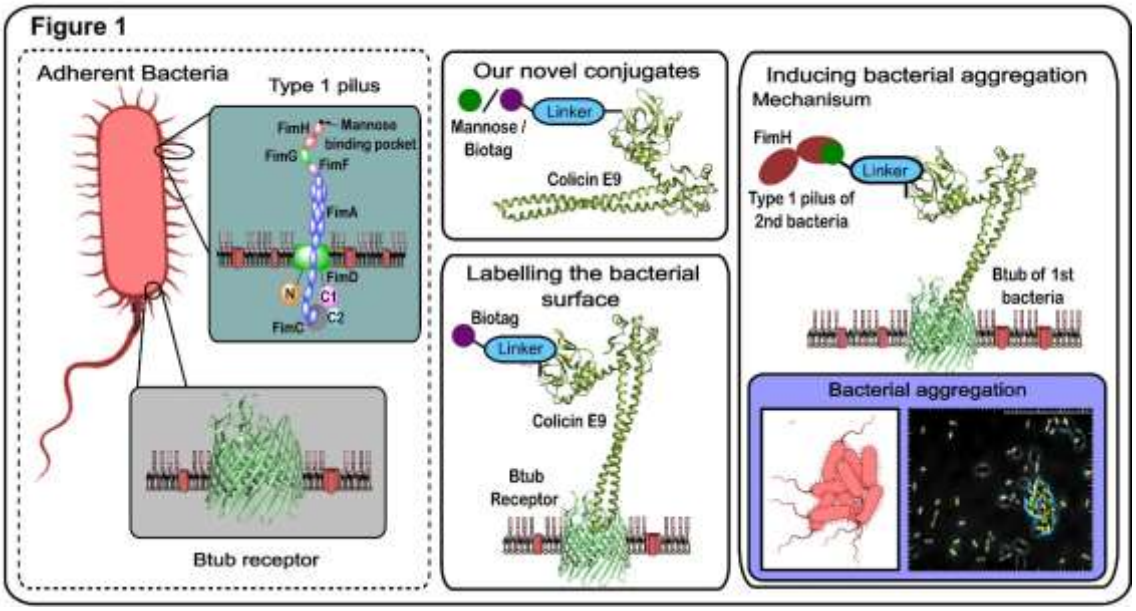

Figure 1. A novel approach to anti-adhesion therapy using bacterial aggregation-inducing Mannose-Colicin bioconjugates.

Merging total synthesis and NMR technology for deciphering the realistic structure of natural 2,6-dideoxyglycosides

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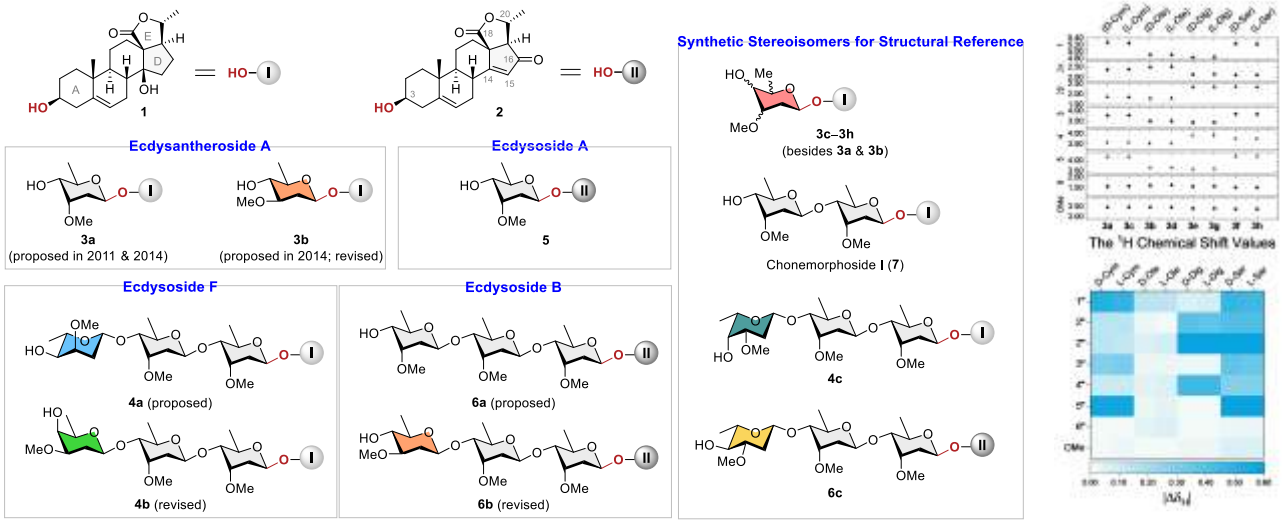
The structural identification and efficient synthesis of bioactive 2,6-dideoxyglycosides is a daunting challenge. Here we report the total synthesis and structural revision of a series of 2,6-dideoxyglycosides from folk medicinal plants *Ecdysanthera rosea* and *Chonemorpha megacalyx*, which feature pregnane steroidal aglycones bearing a 18,20-lactone and glycans consisting of 2,6-di-deoxy-3-*O*-methyl-β-pyranose residues, including ecdysosides A, B, F, and ecdysantheroside A. All the eight possible 2,6-di-deoxy-3-*O*-methyl-β-pyranoside stereoisomers (of the proposed ecdysantheroside A) have been synthesized, that testifies the effective gold(I)-catalyzed glycosylation methods for the synthesis of various 2-deoxy-β-pyranosidic linkages and lays a foundation via NMR data mapping to identify these sugar units which occur promiscuously in the present and other natural glycosides. Moreover, some synthetic natural compounds and their isomers have shown promising anticancer, immunosuppressive, anti-inflammatory, and anti-ZIKV activities.



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Stereocontrolled 1,2-Trans-Arabinofuranosylation In The Absence Of 2-O-Acyl Group In Glycosyl Donor

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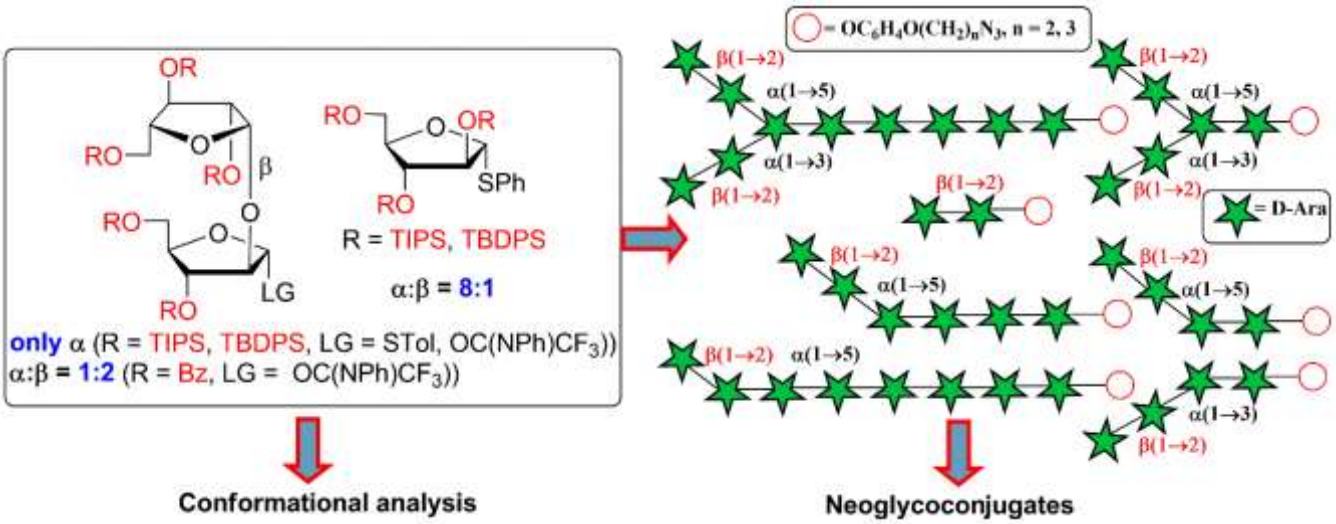
Oligoarabinofuranosides related to the polysaccharides of the *M. tuberculosis* are an attractive synthetic target due to the necessity of the development of drugs, vaccines, and diagnostics. [1]. We investigated the influence of the nature of the protective and leaving groups in stereoselective 1,2-*trans*-α-arabinofuranosylation with mono- and disaccharide glycosyl donors bearing acyclic silyl protective groups. The main challenge is associated with the absence of stereocontrolling 2-*O*-acyl group in glycosyl donor. Complete stereoselectivity of 1,2-*trans*-α-arabinofuranosylation was achieved for Ara-β-(1→2)-Ara glycosyl donors containing five TIPS [2] or TBDPS groups. In contrast, in the case of polysilylated (TIPS, TBDPS) monosaccharide thioglycosides, the stereoselectivity is lower (α:β = 8:1). It is important to note that the glycosylation with Ara- β-(1→2)-Ara containing only benzoyl protection led to loss of stereocontrol (α:β = 1:2) [2]. Silylated Ara-β-(1→2)-Ara glycosyl donors were successfully used in the synthesis of the library of oligoarabinofuranosides with 4-(ω-azidoalkoxy)phenyl aglycones and neoglycoconjugates thereof related to the arabinan domain of the polysaccharides of the *M. tuberculosis*.
This work was financially supported by the Russian Science Foundation (Project No. 21-73-20164).



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Dehydroxylative Radical N-Glycosylation Of Heterocycles Enabled By Copper Metallaphotoredox Catalysis

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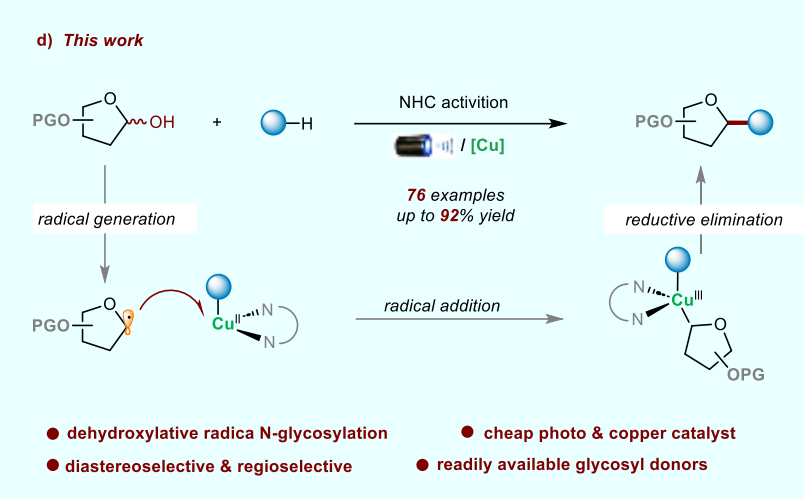

N-Glycosylated heterocycles play important roles in biological systems and drug development. The synthesis of these compounds heavily relies on ionic N-glycosylation, which is usually constrained by factors such as labile glycosyl donors, precious metal catalysts, and stringent conditions. Herein, we report a dehydroxylative radical method for synthesizing *N*-glycosides by leveraging copper metallaphotoredox catalysis, in which stable and readily available 1-hydroxy carbohydrates are activated for direct N-glycosylation. Our method employs inexpensive photo- and copper- catalysts and can tolerate some extent of water. The reaction exhibits a broad substrate scope, encompassing 76 examples, and demonstrates high stereoselectivity, favoring 1,2-*trans* selectivity for furanoses and α-selectivity for pyranoses. It also exhibits high site-selectivity for substrates containing multiple N-atoms. The synthetic utility is showcased through the late-stage functionalization of bioactive compounds and pharmaceuticals like Olaparib, Axitinib, and Metaxalone. Mechanistic studies prove the presence of glycosyl radicals and the importance of copper metallaphotoredox catalysis.

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Indium(III)-Catalyzed Gold-Free Glycosylation Using Alkyne-Based Glycosyl Donors

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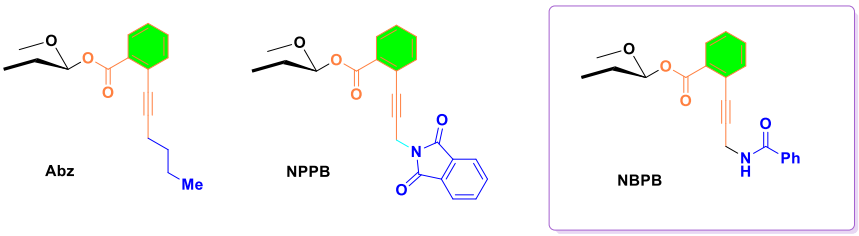
Abstract: Carbohydrates play an important role in the development of new drugs for many diseases, such as tumor, immune regulation, anti-oxidation, anti-virus and so on. The pharmaceutical prospects have greatly promoted the research of carbohydrate chemical synthesis to which the construction of glycosidic bonds is often the key. Since the first chemical synthesis of glycosides in 1879, many glycosylation methods have been developed and used in the synthesis of complex sugar-containing natural products. Among many glycosyl donors, alkynyl-containing donors are an attractive category due to their convenient preparation, stability, and mild orthogonal activation. One representative is glycosyl *ortho*-alkynylbenzoate (Abz), thus far known as Yu's donor, featuring the neutral Au(I) catalysis. This mild catalysis allows the efficient glycosylation of acid labile acceptor and thus the accomplishment of many natural products. However, considering the high cost, it is necessary to develop a safe and economic catalytic system. Recently, a new glycosylation donor, NPPB, has been reported, which can be catalyzed by Cu(II). We have discovered a novel glycosyl donor, based on the installment of N-Propargyl groups, which can be efficiently activated by cheap and readily available $In(OTf)_3$. This promotion condition is orthogonal to that for the previously reported Yu's donor and its recently modified NPPB donors. The mechanistic studies, as well as the applications of new donors in the construction of glycan and glycosyl conjugates are currently in the progress.

Key Words: Glycosylation, In(III) catalysis, novel glycosyl donor



	Au(I)	Cu(II)	In(III)
Abz	✓	✗	✗
NPPB	○	✓	✗
NBPB	○	○	✓

- Inexpensive and readily available catalyst
- Mild catalytic condition
- Shelf-stable donors
- Broad substrate scope

Unexpected Formation Of Furanose Form During Deacetylation Of Pyranose Gluco-Oxazoline

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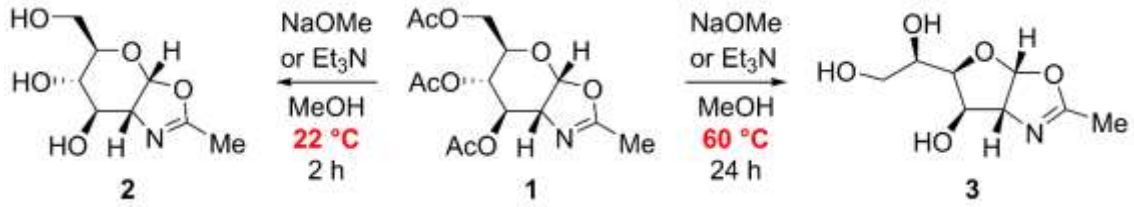
Since *N*-acetyl-D-glucosamine in the pyranose form is one of the most frequently encountered hexosamines in biologically important glycoconjugates a variety of methods for chemical synthesis of its glycosides have been developed.[1] Recently, there has been an increasing interest in glyco-oxazolines functioning as glycosyl donors in glycosylation reactions.[2, 3]

We have found that during conventional deacetylation of well-known (3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**1**)[4] with MeONa in MeOH at ambient temperature a previously unknown furanose isomer of gluco-oxazoline triol **3** is formed along with the expected pyranose form of gluco-oxazoline triol **2** (Scheme 1). Similarly, treatment of **1** with methanolic Et_3N leads to formation of both **2** and **3** in variable amounts. Importantly, prolonged reaction times or higher temperatures increase the share of the furanose form **3** in the reaction mixture, suggesting that this isomer is more thermodynamically stable. The reaction can be selectively directed to pyranose **2** or furanose **3** by appropriate choice of reaction conditions. Thus, after 2 h at 22 °C pyranose form of gluco-oxazoline triol **2** is formed exclusively, while prolonged heating at 60 °C (24 h) cleanly gives furanose form of gluco-oxazoline triol **3**. Both isomers of gluco-oxazoline triol **2** and **3** were isolated in quantitative yields without admixture of the other isomer and their structures established by 2D NMR spectroscopy and high-resolution mass spectrometry. The structure of pyranose isomer of gluco-oxazoline triol **2** was additionally confirmed by its transformation to the starting acetate **1** under acetylation conditions (Ac_2O, Py).

A possibility of preparation of unprotected furanose form of gluco-oxazoline **3** opens a new pathway to poorly studied (see[5-10] and references cited therein) furanose forms of glyco-oxazolines with various *O*-protective groups. Investigation of the prospects of the use of derivatives of furanose oxazoline **3** in glycosylation reactions is currently underway in our laboratory.



Scheme 1. Deacetylation of oxazoline **1** to give the gluco-oxazoline triol in pyranose (**2**) or furanose (**3**) forms.

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Promoter-Controlled Synthesis And Conformational Analysis Of Cyclic Mannosides Up To A 32-Mer

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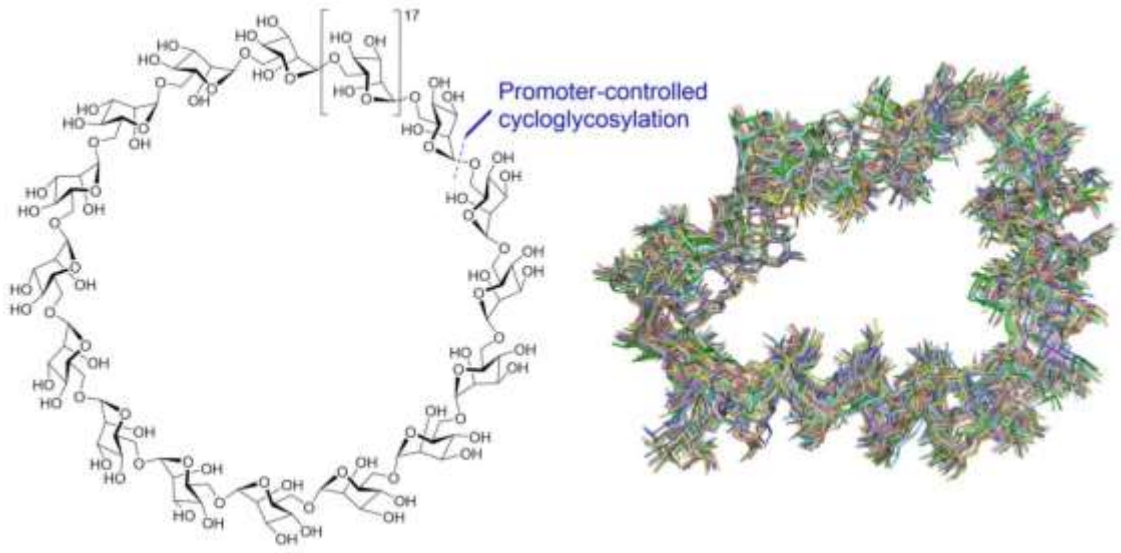
Cyclodextrins are widely used as carriers of small molecules for drug delivery due to their remarkable host properties and excellent biocompatibility. However, cyclic oligosaccharides with different sizes and shapes are limited. Cycloglycosylation of ultra-large bifunctional saccharide precursors is challenging due to the constrained conformational spaces. Here we present a promoter-controlled cycloglycosylation approach for the synthesis of cyclic α-(1→6)-linked mannosides up to a 32-mer. Cycloglycosylation of the bifunctional thioglycosides and ynenoates was found to be highly dependent on the promoters. In particular, sufficient amount of gold(I) complex played a key role in the proper preorganization of the ultra-large cyclic transition state, providing a cyclic 32-mer polymannoside, which represents the largest synthetic cyclic polysaccharide to date. NMR experiments and computational study revealed that the cyclic 2-mer, 4-mer, 8-mer, 16-mer, and 32-mer mannosides adopted different conformational states and shapes.



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Green And Efficient Selective Protection Of Carbohydrates---From Organotins To Stannous Chloride

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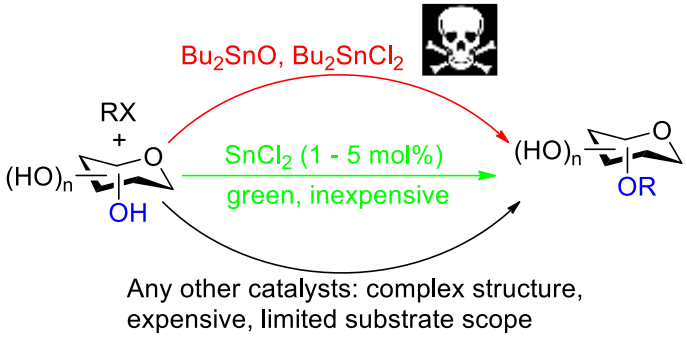

Organotin-mediated selective protection of carbohydrates had been widely used for decades in carbohydrate synthesis.[1] For example, by using stoichiometric amounts of dibutyltin oxide, a wide range of substrates, including 1,2- and 1,3-diols, as well as glycosides containing *cis*-adjacent diol or *trans*-adjacent diol, can be selectively acylated and alkylated with good selectivity. Unfortunately, the application of organotins has been severely limited due to their highly toxicity. Therefore, researchers have been trying to find low or non-toxic reagents to replace organotins in carbohydrate protection strategies for decades. However, these developed reagents often have complex molecular structures resulting in much higher costs than organotins, and are not as effective as organotins in one or more aspects such as selectivity, substrate scope, or reaction efficiency. These may be the reasons why toxic organotins are still often employed by many researchers to date. Our group has long been committed to the development of green selective carbohydrate protection strategies. Especially in recent years, we have successfully applied iron (III)-catalysts instead of organotins in the protection strategies.[2-4] In the most recent, our group has found that stannous chloride ($SnCl_2$) is the most ideal catalyst for the selective carbohydrate protection,[5,6] which is not only safe and non-toxic, but also comparable to organotins in terms of cost, selectivity, substrate scope, reaction efficiency, and ease of handling. Our mechanistic studies continue to support that the catalytic activity and selectivity derive from the fact that cyclic five-membered and six-membered dioxolane/dioxane-type intermediates are generated between the diol and the Sn species under alkaline conditions.

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Janus Aglycones: Synthesis And Functionalization Of *P*-Hydroxyphenyl Glycosides

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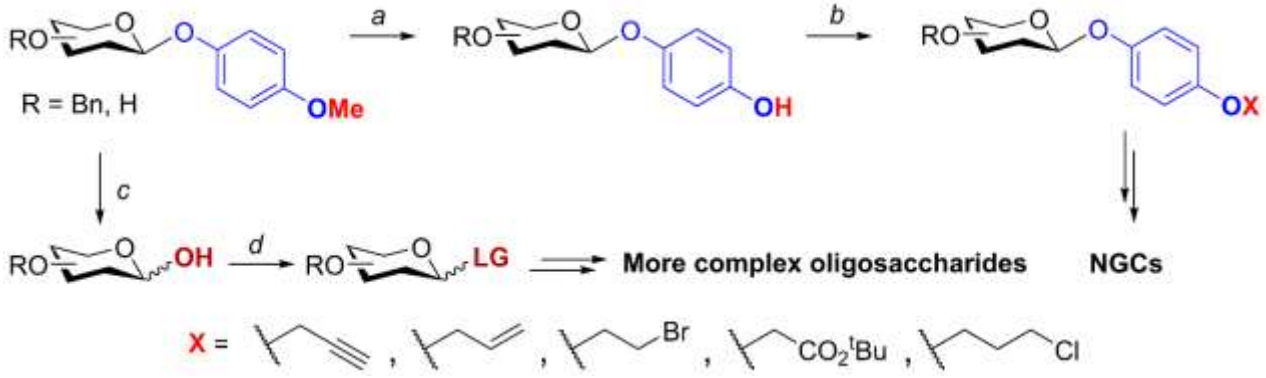
Janus glycosides are featured by the presence of a cleavable pre-spacer aglycone (Janus aglycone),[1] which allows these glycosides to be used both for the preparation of neoglycoconjugates (NGCs) and, after cleavage of the aglycone and introduction of a leaving group, as building blocks in the synthesis of more complex oligosaccharides (Scheme 1). We have recently introduced 4-(ω-chloroalkoxy)phenyl aglycones as potent Janus aglycones and successfully used them for the block synthesis of oligosaccharides and NGCs thereof (see[2] and references cited therein).

As part of the development of this concept, *p*-methoxyphenyl (MP) aglycone was studied as a candidate for inclusion to the family of Janus aglycones. Since MP aglycone is widely used as an anomeric protective group, the key remaining issue was to design an approach for functionalization of MP aglycon. If a straightforward method for demethylation of MP glycosides could be devised, then selective functionalization of the more acidic phenolic hydroxy group in the resulting *p*-hydroxyphenyl glycoside would not constitute any problem.

After comparison of several feasible demethylation approaches and considerable experimentation (more details will be shown in the poster) we elaborated a robust and efficient demethylation protocol that included treatment of an (un)protected MP glycoside with EtSH and NaOH in *N*-methyl-2-pyrrolidone (NMP) at 130 °C. Importantly, intersaccharidic glycosidic linkages in oligosaccharide substrates were stable under these conditions. The alkylation of the phenolic hydroxy group in protected and unprotected *p*-hydroxyphenyl glycosides, obtained in almost quantitative yields, cleanly gave the corresponding pre-spacer glycosides with a functional group in aglycon (or its precursor), which would allow a straightforward conjugation to a carrier, also in nearly quantitative yields (Scheme 1).

This work was financially supported by the Russian Science Foundation (Project No. 21-73-20164).



Scheme 1. MP aglycone as an example of a Janus aglycone. *Reagents and conditions: a.* EtSH, NaOH, NMP, 130 °C. *b.* Alkylating agent (XBr or XOTs), K_2CO_3, polar aprotic solvent, *c.* $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O. *d.* Introduction of a leaving group (LG).

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Hypervalent Selenium Catalysis For The Activation And Stereoselective Glycosylation Of 2-Deoxy Hemiacetals

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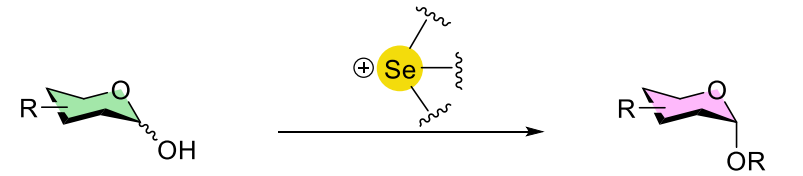
The development of stereoselective glycosylation methods is vital for enabling the synthesis of biologically relevant carbohydrate-based molecules.[1] Often glycosylation reactions result in a mixture of anomers, leading to purification problems and lower enantiomeric yields. Additionally, achieving stereoselectivity is more challenging when using 2-deoxy sugars which lack substituents at C-2 that can direct nucleophilic attack. Our group is interested in the development of novel catalytic methods for the synthesis of deoxyglycosides to address this challenge, through the activation of both glycals and hemiacetals.[2,3]

In this work, we demonstrate our latest effort to apply group 16 elements in transition-metal free catalysis for carbohydrate synthesis. For the first time the application of hypervalent chalcogenonium catalysis to synthesise both 2-deoxy and fully-oxygenated glycosides via the activation of hemiacetal donors has been demonstrated. In this work, we disclose a practical and direct α-stereoselective glycosylation strategy applied to a range of orthogonally protected hemiacetal substrates in fair to excellent yields, with both primary and secondary OH nucleophiles, and high stereocontrol. Moreover, ^{1}H-NMR and kinetic isotope studies provided insights towards the reaction mechanism pathways and unravel the key steps in the activation process.[4]



2-deoxy hemiacetal

α- selective
>25 examples
14-93 %

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Unprotected Glycosyl Radical Functionalizations

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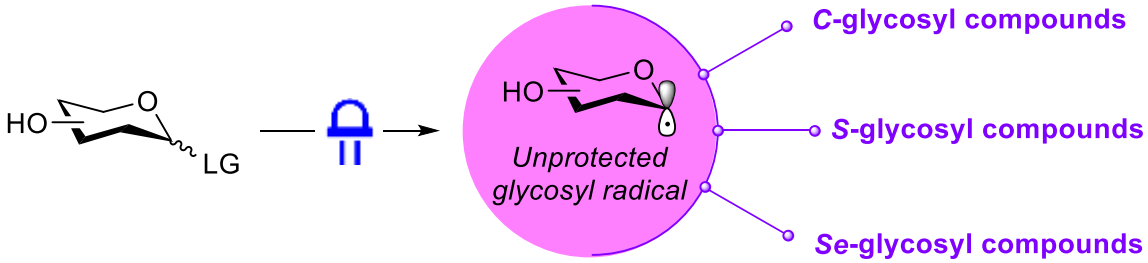
Development of new strategies for constructing densely functionalized saccharides is a central objective in carbohydrate chemistry. In the past four years, we have harnessed the power of nonprecious base metal catalysis and/or photochemistry to afford reactive glycosyl radical intermediates[1-3], which can be trapped with appropriate reagents to access various classes of medicinally relevant glycosyl compounds with good control of site and stereoselectivity[4-10]. This approach potentially overcomes undesired reactions such as elimination and epimerization that are common in transformations involving glycosyl ionic species[1]. Recent progress in my group focused on leveraging fully unprotected glycosyl radicals, which are generated from suitable donors under photoinduced conditions, in homolytic (one-electron) chemistry to furnish the desired sugar products[8, 11]. In this presentation, we will highlight our contributions to this growing area.



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A Protection-free procedure for the Synthesis of 1,2-cis C-aryl Furanosides

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C-glycosides are widely present in nature and exhibit significant biological activities.[1] The superior stability of the C−C glycosidic bond linkages against hydrolysis renders C-aryl glycosides better bioavailability, and makes them excellent surrogates of classical O- and N-glycosides in medical chemistry. The synthesis of C-glycosides have attracted considerable attentions in the field of synthetic chemistry.[2] By leveraging the chemistry of transition metal catalyzed C−H glycosylation, glycosylidene carbene mediated C−B insertion, Petasis reaction & halogenation enabled deaminative cyclization, we developed a series of methods for the synthesis of C-aryl, -vinyl and -alkyl glycosides.[3-6] These methods use readily available glycosyl donors, demonstrate broad compatibility and enable the synthesis natural products Cam-HrTH-I, neopuerarin A and antidiabetic agent tofogliflozin.

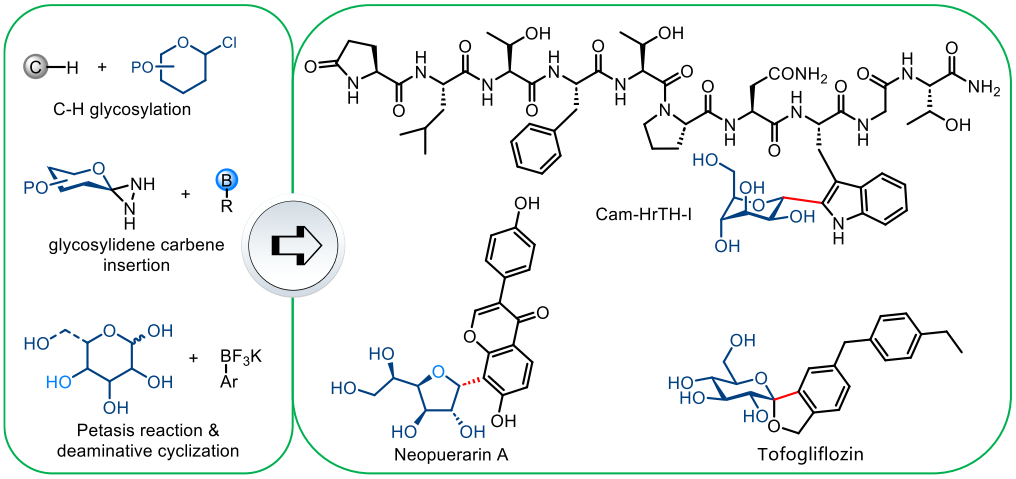

Figure 1. Synthesis of *C*-Glycosides

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Synthesis Of Sialylated Human Milk Oligosaccharides By Automated Glycan Assembly

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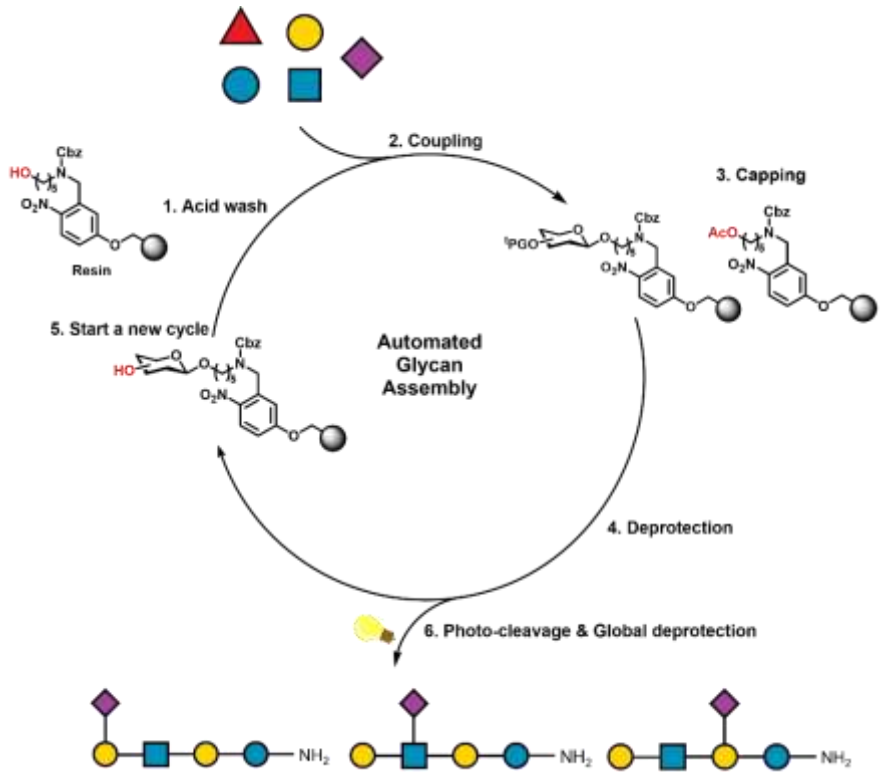
Human milk oligosaccharides (HMOs), diverse unconjugated glycans, are third largest component of breast milk. Numerous studies show human infants rely on HMOs for development of their immune system and modulation of their gut microbial environment[1]. However, accessing to homogeneous HMOs remains the major challenge for elucidating their biological functions. Automated Glycan Assembly (AGA) has seen major developments as a fast and reliable method to synthesize oligosaccharides such as mammalian and bacterial glycans[2]. At present, there is no suitable automated method for sialylation. The challenges of chemical sialylation are efficiency and stereoselectivity because of its quaternary anomeric center with an adjacent electron-withdrawing group and the lack of participating group on C-3. It is necessary to develop a universal solution for construction of oligosaccharides containing sialic acid by AGA. In our project, we used automated glycan assembly (AGA) to systematically construct a library of sialylated HMOs. With approved building blocks[3] and optimized conditions, we could efficiently obtain a collection of sialylated HMOs.



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Development Of Carbohydrate-Based Anticancer Vaccines Using Bacteriophage Q Virus-Like Particles

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Vaccination can provide long-term protection to the host with few side effects.[1,2] Tumor-associated carbohydrate antigens (TACAs) are important targets for developing anticancer vaccines. MUC1 (mucin-1) is an essential antigenic target. To develop a MUC1-based vaccine, we identified SAPDT*RPAP (* is the glycosylation site) as a protective epitope of MUC1 for the first time.[3,4] This epitope was conjugated to bacteriophage Qβ virus-like particles (VLPs) to construct a Qβ-MUC1-Tn conjugate vaccine, which induced more than 1 million IgG titers in MUC1.Tg mice, which was the highest MUC1-Tn IgG titer in Tg mice.[4] Inspired by this, we went on to synthesize and evaluate Qβ-MUC1-Tf, Qβ-MUC1-STn, and Qβ-MUC1-βTf conjugate vaccines.[5,6]

In addition, 9-O-acetyl-GD2 (9OAc-GD2) is a highly specific ganglioside tumor target antigen. However, the 9-O-acetyl group in 9OAc-GD2 is unstable and difficult to synthesize. Therefore, we designed an unnatural ganglioside 9NHAc-GD2 that carries N-acetamide (NHAc) in non-reducing terminal neuraminic acid as a suitable alternative to 9OAc-GD2. 9NHAc-GD2 was synthesized by the chemoenzymatic method. The Qβ-9NHAc-GD2 conjugate vaccine was constructed by conjugating 9NHAc-GD2 to Qβ VLP. Strikingly, Qβ-9NHAc-GD2 induced 70-fold higher antibody titers than KLH-GD2 in mice, demonstrating its high clinical translation potential.[7]

The wild-type (Wt) Qβ was used to develop the abovementioned vaccines. However, the WtQβ-TACA vaccine induces potent TACA-specific antibodies and high Qβ-specific antibodies. Removal of Qβ B cell epitopes can reduce anti-Qβ titers and increase anti-TACA titers. In order to address this issue, multiple mutant (m) Qβ VLPs were constructed. It was found that mQβ(A38K/A40C/D102C) was a superior carrier to WtQβ. As such, 9NHAc-GD2 antigen was conjugated to mQβ(A38K/A40C/D102C) to produce mQβ-9NHAc-GD2, which induced higher levels of 9NHAc-GD2-specific IgG compared to WtQβ-9NHAc-GD2.[8] Furthermore, we also synthesized and evaluated an mQβ-Globo-H conjugate vaccine, which caused a more robust Globo-H-specific antibody than KLH-Globo-H.[9]

In summary, we developed a variety of carbohydrate-based anticancer vaccines, including WtQβ-MUC1-Tn, WtQβ-MUC1-Tf, WtQβ-9NHAc-GD2, mQβ-9NHAc-GD2, mQβ-Globo-H, which have great clinical translational potential.

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Hepatic Glucuronyl C5-Epimerase Combats Obesity By Stabilising GDF15[1]

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Background & Aims: Disturbed hepatic metabolism frequently results in excessive lipid accumulation in the adipose tissue. However, the specific role of the liver–adipose axis in maintaining lipid homeostasis, as well as the underlying mechanism, has not yet been fully elucidated. In this study, we investigated the role of hepatic glucuronyl C5-epimerase (*Glce*) in the progression of obesity.

Methods: We determined the association between the expression of hepatic *Glce* and body mass index (BMI) in obese patients. Obesity models were established in hepatic *Glce*-knockout and wild-type mice fed a high-fat diet (HFD) to understand the effect of *Glce* on obesity development. The role of *Glce* in the progression of disrupted hepatokine secretion was examined via secretome analysis.

Results: Hepatic *Glce* expression was inversely correlated with BMI in obese patients. Moreover, *Glce* level was found to be decreased in the liver of a HFD murine model. Hepatic *Glce* deficiency led to impaired thermogenesis in adipose tissue and exacerbated HFD-induced obesity. Interestingly, decreased level of growth differentiation factor 15 (GDF15) was observed in the culture medium of *Glce*-knockout mouse hepatocytes. Treatment with recombinant GDF15 obstructed obesity progression derived from the absence of hepatic *Glce*, similar to the effect of *Glce* or its inactive mutant overexpressed both *in vitro* and *in vivo*. Furthermore, liver *Glce* deficiency led to diminished production and increased degradation of mature GDF15, resulting in reduced hepatic GDF15 secretion.

Conclusions: Hepatic *Glce* deficiency facilitated obesity development, and decreased *Glce* expression further reduced hepatic secretion of GDF15, thereby perturbing lipid homeostasis *in vivo*. Therefore, the novel *Glce*–GDF15 axis plays an important role in maintaining energy balance and may act as a potential target for combating obesity.

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Glycosylation Quality Control By Golgi Structure Formation

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The Golgi apparatus plays a central role in protein trafficking, sorting, and glycosylation within the secretory pathway. Its unique stacked structure of flattened cisternae provides a platform for Golgi enzymes to carry out their functions. Through a multidisciplinary approach, we have investigated the molecular mechanisms of Golgi structure formation and its importance in protein glycosylation [1,2]. Our findings demonstrate that Golgi cisternal stacking is crucial for accurate N-glycosylation as well as glycosaminoglycan synthesis, sulfation, and secretion [3,4]. This structural organization of Golgi stacks ensures the orderly distribution of glycosylation machineries and facilitates the fidelity and quality of sugar chain processing [5].

Additionally, the Golgi is responsible for synthesizing sphingolipids and glycolipids. Defects in Golgi structure can cause mis-sorting and secretion of lysosomal enzymes, such as HEXA and HEXB, resulting in a substantial elevation in the levels of monosialotetrahexosylganglioside (GM1) with concomitant reductions in the levels of globotriaosylceramide (Gb3) [6], critical molecules involved in myelination and neuronal function, leading to rare neurodegenerative disorders.

Importantly, in Alzheimer's disease, Golgi fragmentation accelerates amyloid cleavage of the amyloid beta precursor protein, and restoring Golgi structure reduces Aβ production [7]. Moreover, our investigations into how the Golgi responds to different cellular stresses have revealed that the Golgi stacking protein GRASP55 functions as an energy sensor via O-GlcNAcylation to facilitate autophagosome maturation [8,9]. This function regulates the unconventional secretion and aggregation of proteopathic proteins, such as tau, TDP43, α-synuclein, and mutant huntingtin [10]. These findings highlight the Golgi as a potential drug target for disease treatment.

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Polyguluronate Sulfate Could Mitigate Hyperlipidemia By Inhibiting Pcsk9-Mediated Degradation Of Ldlr

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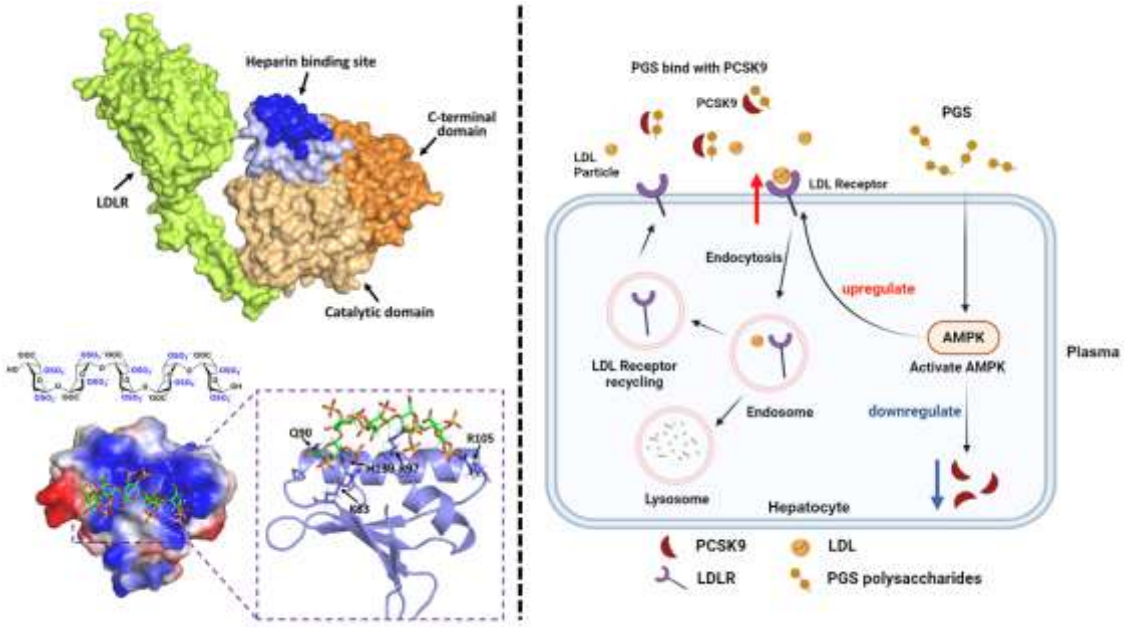
Hyperlipidemia has become one of the leading causes of mortality worldwide. Circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) protein in plasma induce lysosomal degradation of hepatic LDL receptors (LDLR), thereby reducing the clearance of low-density lipoprotein (LDL). In this study, we identified that polyguluronate sulfate (PGS), a derivative of the marine sulfated polysaccharide lipid-lowering drug PSS, is capable of binding to the positively charged domain of PCSK9, thereby inhibiting the PCSK9-mediated degradation of LDLR. Moreover, we further demonstrated that the lipid-lowering activity of PGS is exerted through modulation the PCSK9/LDLR pathway and activating the AMP-activated protein kinase (AMPK) pathway. In summary, this research provides theoretical support for the development of PGS as a novel lipid-lowering drug candidate.



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Understanding Siglec-15 Ligand Selectivity For The Efficient Design Of High-Affinity Sugar Mimetics

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Siglecs (Sialic acid-binding immunoglobulin-type lectins) constitute a large family of membrane receptors present on most of white blood cells. They allow immune cells to distinguish between self and non-self antigens by interacting with the sialic acid moieties that commonly decorate complex glycans.

Over the last decade, siglecs have become important therapeutic targets for treatment of several diseases, especially cancer, asthma, allergies and autoimmune diseases. In particular, Siglec-15 upregulation has been described in TAMs (Tumor Associated Macrophages)[1] and it might have a signalling role in osteoclastogenesis as well[2]. So far, research efforts have been focused on antibody-based therapies and on the development of high affinity ligands. Both approaches aim to disrupt the sialic acid-siglec axis and modulate the immune outcome. However, while the sialic acid-lectin interaction has been well characterized at a molecular level for other human and murine siglecs, the selectivity of siglec-15 for sialic-acid containing glycans still remain elusive. Indeed, the few data reported so far are not fully consistent. Some of us have recently described the X-Ray crystallographic 3D structure of Siglec-15 bound to an antibody and disclose the basic recognition features required for its binding to natural sialoglycans[3]. Herein, we have employed a ligand-based NMR approach to establish a first molecular basis that allows understanding glycan selectivity and opening new avenues to find improved, high affinity sialic acid mimetics.

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The Claim Of Primacy Of Human Gut *Bacteroides Ovatus* In Dietary Cellobiose Degradation

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Although cellulose is widespread in human diets as components of the cell walls of plants, it cannot be digested by human. However, a demonstration of cellulose degrading bacterium from human gut changed our view that human cannot utilize the cellulose due to the β1,4-glycosidic bond rendered cellulose inaccessible to the limited repertoire of digestive enzymes encoded by the human genome. But in fact, the distribution of this species is not clear till now. On top of that, how cellulose is degraded into glucose remains unknown. To address, the smallest unit of cellulose utilized by a gut key member *Bacteroides ovatus* (BO) in the human gut was selected for uncovering the underlying molecular mechanism. As shown in Figure 1, we found a core polysaccharide utilization locus (PUL), which contained two cell surface cellulase belonging to GH5 family with similar biological structure with Cel5A and Cel5R, two cellulase from soil bacteria, was responsible for the degradation of cellobiose. The catalytic sites were highly conservative with two glutamate residues. *In vivo* test, we observed that cellobiose reshaped the composition of gut microbiota, and specifically increased the abundance of BO and *Lactobacillus reuteri* (LR), and probably modified the metabolic function of bacteria especially for the enrichment of β-N-acetylhexosaminidase and KEGG pathway and Histidine. Briefly, our work sheds light on the molecular mechanism that BO has evolved to utilize the cellobiose in the highly competitive environment of the human gut microbiota. The findings that microbes in the human gut possess the capacity to utilize the cellobiose generated from ubiquitous cereal highlights the relevance of potential therapeutic interventions based on the gut microbiota. Our work also sets the stage for future work to understand the significance of cellobiose to human wellness.

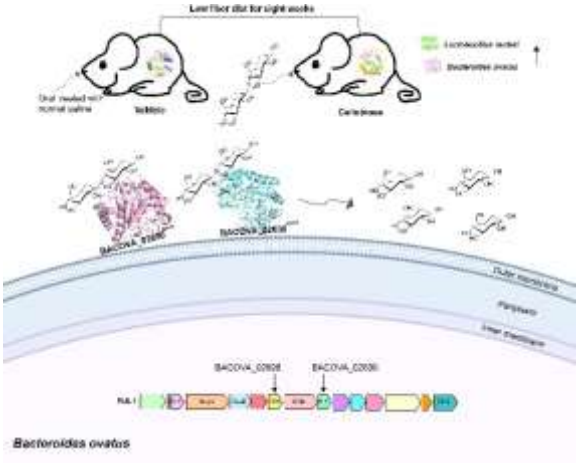

Figure 1. Graphical abstract. Model of degradation of cellobiose by *Bacteroides ovatus* (BO). Two new cellulases on the cell surface conferred the degradation of cellobiose into glucose were determined. *In vivo* test, we observed that cellobiose reshaped the composition of gut microbiota, and the abundance of LR and BO were enriched significantly after eight weeks administration by gavage.

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Structural characterization and biological activities of polysaccharides from Trametes sanguinea

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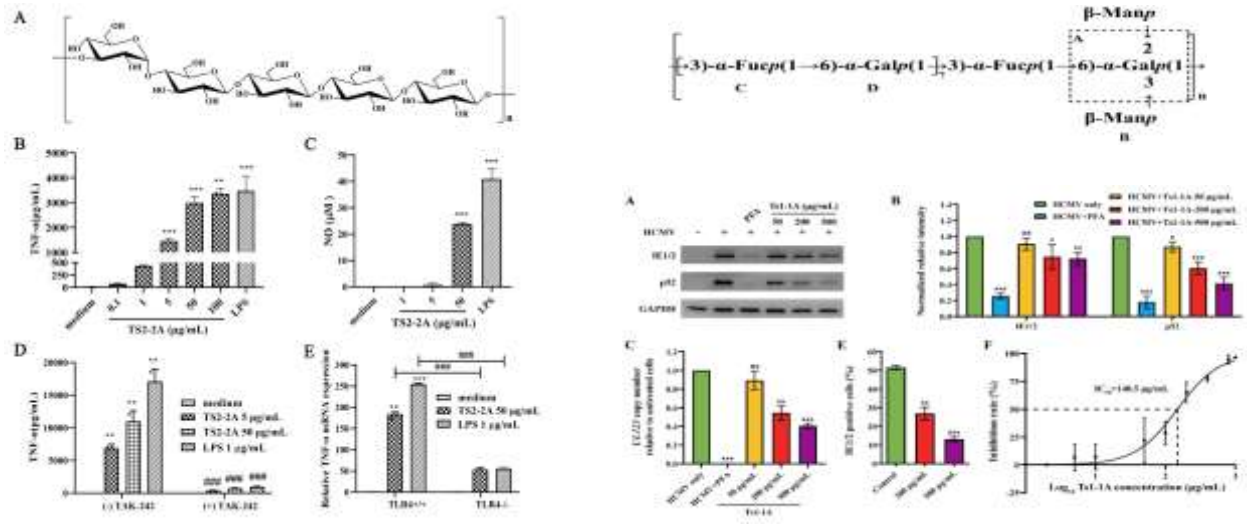
Hepatoma is the second leading cause of tumor death in China. Traditional Chinese medicine with multi-component synergistic regulation advantage has great potential in the research of anti-hepatoma drugs with high efficiency and low toxicity. As a medicinal fungus with Qingrejiedu, The total polysaccharide TsLTP, extracted from *Trametes sanguinea*, possed significant anti-angiogenesis and immune-enhancement activities, and showed effective inhibition of tumor growth in H22 tumor-bearing mice. Our team intends to prepare and clarify the anti-hepatoma effective components of *Trametes sanguinea* polysaccharides, and study its anti-hepatoma mechanism from the point of view of inducing tumor vascular normalization and reprogramming tumor immune microenvironment.



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Glycogen Structural Abnormality In Rat Liver With Diethylnitrosamine-Induced Carcinogenic Injury

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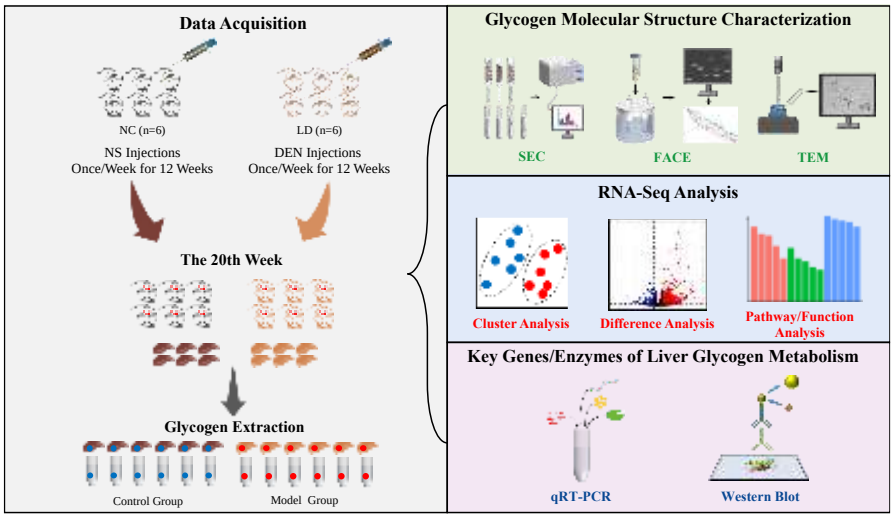
Abstract Growing evidence confirms associations between glycogen metabolic re-wiring and the development of liver cancer. Previous studies showed that glycogen structure changes abnormally in liver diseases such as cystic fibrosis, diabetes, etc. However, few studies focus on glycogen molecular structural characteristics during liver cancer development, which is worthy of further exploration. In this study, a rat model with carcinogenic liver injury induced by diethylnitrosamine (DEN) was successfully constructed, and hepatic glycogen structure was characterized. Compared with glycogen structure in the healthy rat liver, glycogen chain length distribution (CLD) shifts towards a short region. In contrast, glycogen particles were mainly present in small-sized β particles in DEN-damaged carcinogenic rat liver. Comparative transcriptomic analysis revealed significant expression changes of genes and pathways involved in carcinogenic liver injury. A combination of transcriptomic analysis, RT-qPCR, and western blot showed that the two genes, *Gsy1* encoding glycogen synthase and *Gbe1* encoding glycogen branching enzyme, were significantly altered and might be responsible for the structural abnormality of hepatic glycogen in carcinogenic liver injury. Taken together, this study confirmed that carcinogenic liver injury led to structural abnormality of hepatic glycogen, which provided clues to the future development of novel drug targets for potential therapeutics of carcinogenic liver injury.



Graphical Abstract

Blood Group Positive Microbes Stimulate The Development Of Anti-Blood Group Antibody Formation

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Anti-ABO(H) antibodies, which form spontaneously within the first few months of life, represent the most common immunological barrier to transfusion and transplantation. These antibodies can vary widely between individuals, with significant differences in anti-ABO(H) antibody levels and specificity that have direct clinical consequences on ABO(H) incompatible outcomes. However, the factors that influence this variability remain incompletely understood. As ABO(H) blood group antigens (hereafter referred to as BG) are carbohydrate structures largely confined to humans as polymorphic structures, preclinical models capable of defining factors that influence anti-BG antibody formation have not been available. To overcome this limitation, we generated a novel preclinical model that recapitulates key features of naturally occurring anti-BG antibody formation. By knocking out the enzyme required for the synthesis of the murine blood group B-like antigen (murine B or B^m), we generated blood group O-like (murine O or O^m) mice that spontaneously develop varying levels of anti-B^m antibodies. Similar to what occurs clinically, transfusion of B^m RBCs into O^m recipients results in varying degrees of hemolytic transfusion reactions (HTRs), where the magnitude of B^m RBC hemolysis correlates with pre-existing anti-B^m antibody levels. To define factors that contribute to varying anti-B^m antibody levels, O^m recipients were separately housed over several generations based on their levels of anti-B^m antibodies. Using this approach, distinct colonies of O^m mice were selected for that generate either high or no detectable anti-B^m antibodies despite having similar total IgM. Consistent with recent data suggesting that the microbiota does not impact naturally occurring antibody formation, analysis of the total microbiota at the genus level failed to detect key differences between O^m recipients with distinct anti-B^m antibody levels. Microbiota at the genus level failed to detect key differences between O^m recipients with distinct anti-B^m antibody levels. However, anti-B^m antibodies eluted from B^m RBCs did recognize distinct microbiota. Sorting and culturing of anti-B^m antibody reactive microbiota identified a strain of *Klebsiella pneumoniae* that specifically expresses the B^m antigen. Exposure of O^m recipients with undetectable anti-B^m antibodies to B^{m+} *K. pneumoniae* induced anti-B^m antibodies independent of CD4 T cells that were capable of causing HTRs. These results suggest that anti-BG antibody formation reflects exposure to BG+ microbes, providing critical insight into key factors that drive the most common immunological barrier to transfusion and transplantation.

The Role Of Branched Sugar On Capsular Polysaccharide Of *Streptococcus Pneumoniae*

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Streptococcus pneumoniae causes severe invasive diseases such as pneumonia, septicemia and meningitis[1]. The pathogen is surrounded by capsular polysaccharides (CPS), which is the key virulence factor and important antigen[2,3]. More than 100 serotypes have been found in *S. pneumoniae* and 63 CPSs exhibit branched structures[4]. This bacterium generates novel serotype simply by altering branched structures. However, the functions of CPS branches remain less known. Serotype 14 is one of the most common pneumococcal serotypes that cause invasive pneumococcal diseases worldwide[5]. CPS14 is composed of oligosaccharide repeating units, which consists of four monosaccharides including a branched β1-4 galactose[6]. In this study, we deleted the galactose transferase gene *wchM* and the initiating glycosyltransferase gene *wchA* to obtain Δ*wchM* strain and Δ*wchA* strain. Dot-blotting assay and dextran size exclusion assay showed that Δ*wchA* lost capsule while Δ*wchM* produced significantly reduced capsule with high permeability. In consistent with this result, Δ*wchM* displayed a sparse capsule layer watching by transmission electron microscopy. The purified CPS of Δ*wchM* had a weak reaction with specific antibodies against serotype 14 by immunodiffusion assay. These results suggested that the branched galactose is a key antigenic determinant of serotype 14 capsule.

Surprisingly, we observed the suppression mutation in *wchA* gene accompanied with a knockout of *wchM* gene. Through immunoblotting of polysaccharides, we found that the wild type strain produced more high-molecular-weight polysaccharides but Δ*wchM* produced fewer low-molecular-weight polysaccharides, suggesting that the structurally incomplete oligosaccharides were not effectively recognized by polymerase Wzy thus produce short polysaccharide chains. Furthermore, the free lipid carrier Und-P was significantly reduced in Δ*wchM* by UPLC-MS analysis, indicating that Und-P is sequestered by these oligosaccharides and thus prevented the production of capsule. This study reveals the crucial role of branched sugar in biosynthesis and antigenicity of capsular polysaccharide.

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A Novel *Klebsiella Pneumoniae* Serotype O13 Encoded By The Ol101 Locus

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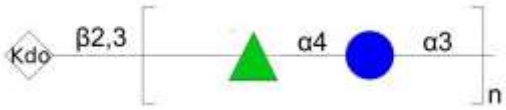
Klebsiella pneumoniae is a nosocomial pathogen and one of the priority species, pointed out by the World Health Organization as critical regarding highly limited options of treatment of infections. Lipopolysaccharide (LPS, O antigen) and capsular polysaccharide (CPS, K antigen) are major surface and virulence factors of the species. *K. pneumoniae* has been perceived as of limited variety of O antigens (dozen of O serotypes and subtypes identified to date) [1]. That trait makes O antigen an attractive target for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics.

Since structural analyses of O antigens are effectively supported by bioinformatics (for example Kaptive tool)[2], novel O loci encoding O-specific polysaccharides of LPS may be identified and linked with exact chemical structures of the O antigens. The OL101 has been one of the novel *K. pneumoniae* O locus for which the antigen structure has not been elucidated so far. In this study, four clinical isolates (ABC152, BC738, BC13-986, 3936/19) predicted as OL101 were characterized and found to have the following O antigen structure numbered as O13 serotype:



Identification of the β-Kdo*p* terminus was based on the analysis of the native LPS molecule by the ^{1}H, ^{13}C HR-MAS NMR spectroscopy.

The MLST analysis revealed that all of the isolates were genetically diverse, each representing another sequence type (ST), namely ST11 (ABC122), ST485 (BC738), ST1427 (3936/19) and ST3658 (BC13-986). ABC122 strain revealed the same structure of the *rfb* cluster and high match confidence between its all nine *rfb* genes and those of the OL101 reference strain. The bioinformatic analysis of 71,377 *K. pneumoniae* genomes from public databases (July 2023) revealed a notable OL101 prevalence of 6.55 % [3].

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In situ survey and structural identification of glycoRNAs

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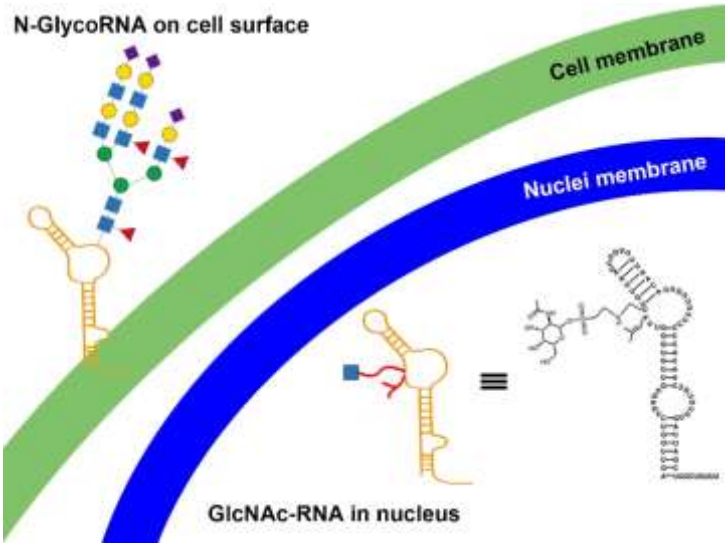
GlycoRNA is a newly discovered type of glycoconjugate, which has been reported to be presented in cell surface and modified with N-glycans.[1] Several methods have been developed to image the glycoRNAs on cell surfaces with hybridization-mediated proximity ligation assay[2] and hierarchical coding strategy.[3] Unfortunately, the accurate structure and glycan-modification site still remains unknown. Here, we supplemented this discovery with evidence that intracellular RNAs were also glycosylated but with single GlcNAc monosaccharide. Using the metabolic technology and the customized fluorescent DNA probes, the GlcNAc modified Y5 RNA in nucleus was firstly imaged by fluorescence resonance energy transfer, which was further collected by the specially designed gathering procedure. The structure of GlcNAc conjugated ribonucleotide was accurately identified by specially designed gel electrophoresis and MALDI-TOF analysis, and the glycan-modification site on the RNA sequence was also inferred. We find that the intracellular Y5 RNA is modified with GlcNAc monosaccharide and attached on pi6Aptm5s2U, which locates at the first AU site from the 5' end. Collectively, these findings extremely supplement the distribution and detailed structure information of glycoRNAs, which greatly promotes the functional research of glycoRNAs.



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The Structure And Immunological Properties Of *Bacteroides Stercoris* Lipopolysaccharide

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The human body is a complex symbiotic system of diverse microorganisms comprising bacteria, yeasts, and viruses.[1] This massive and heterogenous assemblage of microbes, known as the microbiota, is key in physiological and pathological processes occurring in our body, which also include protection against pathogens and immune system development.[2] In this frame, a vast and dynamic community of microbes inhabits the human gut, i.e. the gut microbiota, and comprises commensals and beneficial species for human health. Nevertheless, most of these bacteria are Gram-negative and therefore they possess lipopolysaccharides (LPS) on their outer membranes.[3] LPS are glycoconjugates traditionally associated to potent immune inflammatory reactions in Mammals which occur in a manner that is strongly dependent on the LPS chemical structure. Nevertheless, many harmless Gram-negative colonize the gut without producing any dangerous immune response while promoting the well-being of the host by modulating the immune system itself. [1,3] Therefore, is LPS harmful or beneficial? Deciphering the chemistry responsible for the delicate balance between “beneficial” LPS and “harmful” LPS is pivotal to answer this question. In this communication I will show unreported data related to the characterization of the structure and immunological properties of the LPS from one of the predominant Gram-negative residing the human gut and considered a beneficial bacterium [3], i.e. *Bacteroides stercoris*. A novel chemical structure and uncommon immunological behaviour will be presented, providing insights into the chemistry that might be responsible for establishing harmless relationship between *B. stercoris* and the human host.

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Intramolecular crankshaft isomerization of a quinazolinone-based glycoconjugate

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UV or visible light-induced structural changes have been known for decades and play a fundamental role in many biological processes [1]. The structural change is often accompanied by a rotation around bonds, resulting in a conformational change from a more energetically favourable (*anti*-form) to a less energetically favourable conformation (*syn*-form). This process, called photoisomerisation, is very common in conjugated systems containing heteroatoms, mainly oxygen and nitrogen. A significant part of this group is represented by heterocyclic compounds, many of which can contain highly conjugated systems with aromatic substituents attached to the aliphatic chain [2,3].

Here we present the photoisomerisation of a new glycoconjugate consisting of two *β*-glucopyranoses attached to a quinazolinone-like structure. NMR spectroscopy and DFT calculations showed that simultaneous rotation around the −N−N= and =CH−C− bonds of the −N−N=CH−C− linkage (so-called crankshaft rotation) occurs upon exposure to UV light [4]. The unique structure of the investigated compound allowed a detailed analysis in three different solvents (water, dimethylsulfoxide and methanol). The experimental data demonstrated the formation of the *syn*-forms and also showed that the attachment of the carbohydrate residues to the photoactive compounds can significantly alter the isomerisation process, while the overall molecular structure remains virtually unchanged.

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The Discovery And Mechanism Study Of Polysaccharides From Traditional Chinese Herb Medicine Against Alzheimer'S Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease, which leads to cognitive dysfunction and neuronal death. The prevalence of AD induces great pressure on the social and health-care systems of aging population's society. Diverse evidences suggested that amyloid-β (Aβ) peptides have a causal role in the pathogenesis of AD. The drug development targeting Aβ is one of the most effective strategies towards AD. Recent studies reported the neuroprotective effects of some edible traditional Chinese herb medicine. However, the active polysaccharide substances of these herb medicines against AD were largely unknown.

In the present study, we firstly isolated and purified some homogeneous polysaccharides with different structures from *Lonicera japonica* Thunb., *Lycium barbarum* L., Mulberry Fruit, flowers of *Panax notoginseng* and *Semiaquilegia adoxoides* using traditional water extraction as well as enzyme extraction. Then, the inhibitory effects of homogeneous polysaccharide on aggregation, neurotoxicity and secretion of $A\beta_{42}$ were evaluated. Until now, we have found polysaccharides named LJW0F2[1], LBP1A1-1[2], LBP1C-2[3] and FMP-6-S4[4] could suspend $A\beta_{42}$ aggregation using the Thioflavine T (ThT) fluorescence assay. Subsequently, we evaluated the inhibitory effects of polysaccharides on $A\beta_{42}$ production in amyloid precursor protein (APP) and β-site APP cleaving enzyme 1(BACE1) double stably transfected Chinese hamster ovary (CHO) cells (CHO/APPBACE1 cells) and APP Swedish mutant transfected HEK293 cells (HEK293-APPsw) by ELISA assay, the results revealed that polysaccharides LBP1A1-1[2], LBP1C-2[3], FMP-6-S4[4], RN1, SA05B and SA02C[5] could suppress $A\beta_{42}$ secretion in a concentration dependent manner. In addition, the MTT and CCK-8 assay revealed that these polysaccharides showed no obvious effects on the tested cell growth. Further studies indicated that some of the aforementioned glycans could regulate the processing of APP, and targeting autophagy to promote $A\beta_{42}$ phagocytosis.

Based on the above results, our study demonstrated that as a major bioactive molecule in herb medicine, polysaccharide showed potential use in the treatment of AD through multiple effects. Our project will provide several leading compounds to the anti-AD drug discovery based on polysaccharide.

Key Words: Alzheimer's disease, polysaccharide, Aβ.

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Polyesters Based On Carbohydrate Derived Diglyoxylic Acid Xylose As Soil-Release Polymers For Synthetic Fabric Surfae Modification

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Due to the increasing presence of synthetic fibers in fabrics, soil release polymers (SRPs) have become a highly desired polymer additive found in fabric care formulations to enhance cleaning efficiency. Conventional SRPs contain a poly(ethylene terephthalate) subunit capped by a hydrophilic methoxy-poly(ethylene glycol) unit which aids in the dispersal of the polymer in solution whilst preventing the redeposition of the soil onto the fabric surface. To improve the sustainability profile of these additives, a novel class of SRPs have been synthesised using the carbohydrate derived monomer diglyoxylic acid xylose (DGAX), which can be isolated from lignocellulosic biomass (**Figure 1**). This sugar-derived monomer provides an attractive alternative to the petrochemically derived terephthalate, which is sourced from the oxidation of p-xylene, due to its high abundance and inherent degradable nature. The potential viability of the poly(propylene diglyoxylic acid xylose) based SRPs as potential replacements to current commercialised SRPs were assessed through soil release and anti-redeposition performance tests to determine the stain removal index and whiteness maintenance of the fabric sample. Further insight into the differences in behavior presented by the increasing percentage of the carbohydrate derived monomer incorporated into a terephthalate system was further investigated with dynamic light scattering, contact angle measurements and scanning electron microscopy.

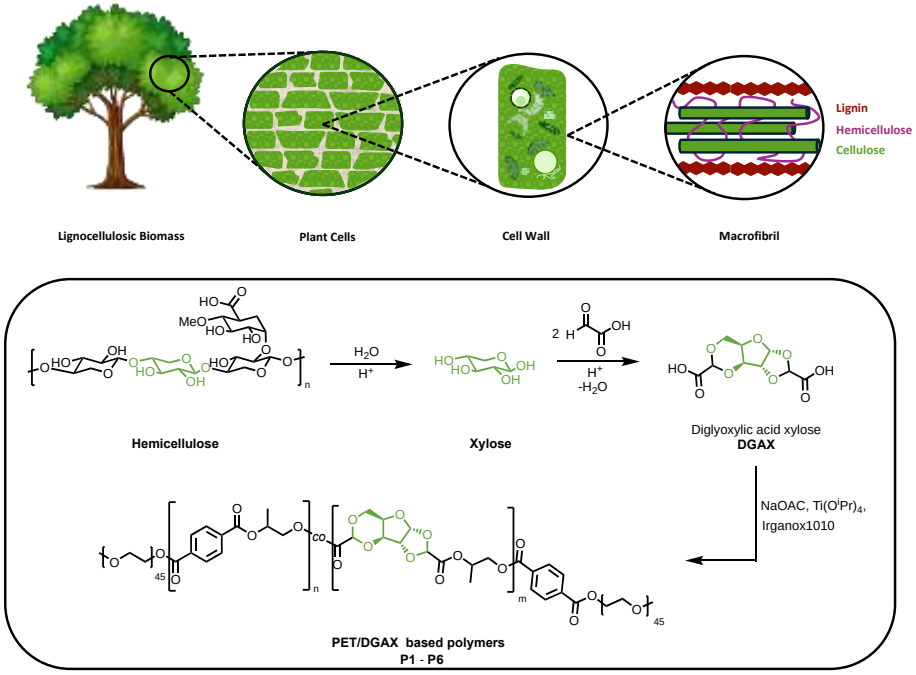

Figure 1: A schematic showing the extraction of the carbohydrate-based monomer, diglyoxylic acid xylose, from hemicellulose1 for the synthesis of a series of polymers.

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Cyclodextrin-Based Giant Bola-Amphiphiles: Synthesis, Self-Assembly And Gene Delivery Capabilities

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Nucleic acid therapeutics face challenges including poor bioavailability and susceptibility to chemical and enzymatic degradation, necessitating effective delivery systems. Among nonviral vectors, lipid and polymer carriers have been the most extensively studied, with lipid vectors notably utilized in the successful development of the first mRNA vaccines for COVID-19. Alternatively, molecular vectors leveraging macrocyclic platforms like cyclodextrins (CDs) offer a promising avenue for tailored vector design, owing to their well-defined structure and cooperative multivalency. In this context, Janus-type amphiphilic derivatives that capitalize simultaneously on the unique structural properties of CDs and the delivery characteristics of lipid and polymer vectors have been extensively explored. We sought to investigate whether altering the arrangement of cationic and lipid domains would influence the self-assembly and nucleic acid delivery capabilities. Specifically, in this study, we designed, synthesized, and characterized giant bola-amphiphilic βCD vectors with varied cationic heads and hydrophobic connectors evenly distributed at opposing O6 and O2 positions (Figure 1), emulating bola-amphiphilic dendrimers A comprehensive examination of these novel systems, focusing on their supramolecular properties and nucleic acid binding, will be presented.

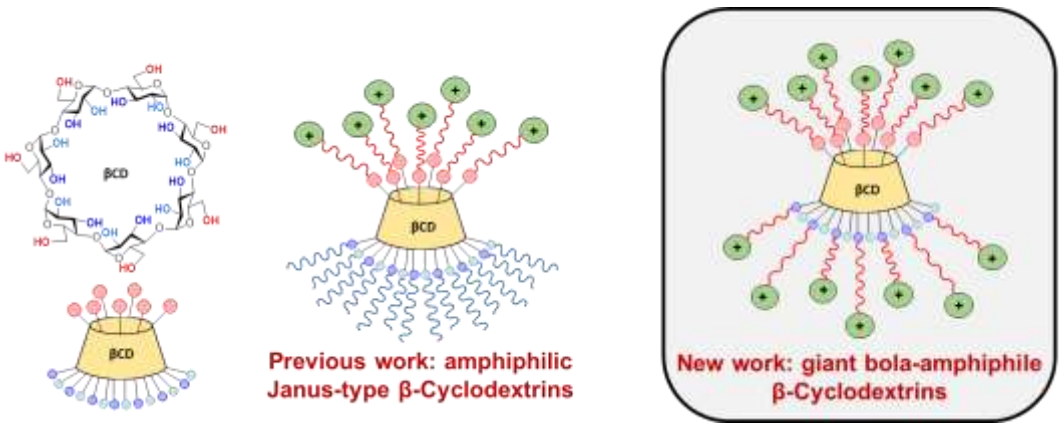

Figure 1. Schematic representation of CD-based Janus-type and giant bola-amphiphiles.

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Sweeten Bioprocesses: Inputs To The Study And Analysis Of Polysaccharides From Terrestrial Plants And Marine Biomass

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Abstract: France has published its roadmap for achieving sustainable development goals by 2030. This action framework for climate and energy over the next decade also outlines strategies at the European Union level regarding (i) reducing greenhouse gas emissions, (ii) enhancing carbon sequestration, (iii) increasing the use of renewable energies, and (iv) promoting "green" processes inspired by nature, which emphasize the use of natural molecules and byproducts. Clermont Auvergne University and Clermont Auvergne INP, currently undergoing restructuring, are carrying an I-SITE CAP 20-25 project which focuses on these new models of life, including also sustainable production. Despite this favorable environmental and societal background, it is crucial to rethink and optimize the use of natural resources. The development of new macromolecules and biobased materials, such as polysaccharides, is central to the research and studies concerning natural resource utilization, commonly referred to as "biomass." Polysaccharides have a key role to play in the ecological and societal transition over the next two decades, regarding the availability of fossil resources, mobility needs and health risks arising from complex and delusional environmental conditions. Through several examples, our projects (4Bio Team) aim to identify and characterize the (i) structural features; (ii) biological activities, (anti-inflammatory, anti-complement, anti-coagulant, anti-bacterial, antioxidant); (iii) physico-chemical behaviors in solution (thickener, gelling, stabilizing, film forming, hydro/aerogel design) and (iv) agronomic properties (biostimulants of natural plant defenses) of various natural poly- and oligosaccharides extracted and/or designed by our own chemo-enzymatic multiscale approaches.

Keywords: Polysaccharides; Bioprocess; Chromatography; Enzymes; Physico-chemical and biological properties

Strategies To Recover Polysaccharides And Oligosaccharides With Prebiotic Activity From Agri-Food Byproducts

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There are a high number of unexploited sources of prebiotic molecules due the lack of strategies for its recovery from insoluble matrices, such as agri-food byproducts. These by-products are rich in polysaccharides that compose the dietary fiber, although insoluble. Soluble polysaccharides and oligosaccharides are prebiotic compounds that promote the growth of beneficial gut bacteria and contribute to improved health. Industrial juice production results in juices with moderate amounts of soluble dietary fiber because most polysaccharides that constitute the dietary fiber are retained in the pomace. Incorporation of glucanases and pectinases into the juice production process can therefore enrich the juice with soluble fibre and possible prebiotic effects. Pear and apple cloudy juice was only composed of 5 % and 4 % of fibre (dry basis), respectively, mainly pectic polysaccharides. Pear and apple polysaccharides (mostly insoluble fibre) are retained in pomace (41-37%) after the industrial juice processing. Pear pomace is richer in xyloglucans and apple pomace richer in cellulose and pectic polysaccharides. Hydrolysis of apple pomace with endo- and exo-cellulase released a high amount of material (63.9 %), composed of monosaccharides and a variety of cellulose and xyloglucan oligosaccharides. Hydrolysis with pectin lyase, polygalacturonase, and pectin methylesterase hydrolysis release 57.7% material composed of pectic polysaccharides and derived oligosaccharides.
In lignified by-product materials, more drastic strategies need to be used to recover polysaccharides and oligosaccharides with prebiotic activity. Pine nut skin, a by-product obtained during pine nut processing, is a source of insoluble dietary fiber[1]. Subcritical water extraction allowed to obtain an extract rich in phenolic compounds and mono- and oligosaccharides, and an extract rich in pectic polysaccharides and xyloglucans[2]. The fermentation of mono- and oligosaccharides and phenolic compounds did not cause a significant microbial shift upon fermentation. Nonetheless, it stimulated significantly ($p< 0.05$) the growth of the prebiotic *Bifidobacterium adolescentis* and the short-chain fatty acids production, specifically acetate and propionate. Pectic polysaccharides and xyloglucans had an impact on the microbiota composition similar ($p< 0.05$) to fructooligosaccharides used as positive control, also increasing the abundance of *B. adolescentis*. The core microbiome upon these polysaccharides' fermentation was composed of multiple butyrate producers, which lead to an increase ($p< 0.05$) of butyrate concentration. Besides, it was observed a microbial utilization selectivity towards xyloglucans in detriment to rhamnose-containing pectic polysaccharides and type II arabinogalactans.

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Molecular Assembled Crystalline Nanoxylan Synthesized From Hot Water Extracted Wood Xylan

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Nanostructured polysaccharides are certain to be a mainstay of the forthcoming green materials revolution in the transformation of societal sustainability [1]. Xylan is the third most abundant polysaccharide in nature after cellulose and chitin, accounting for approximately one-third of all renewable organic carbon on Earth [2]. In this work, as a contribution to expanding accessibility in the territory of bio-based nanomaterials, we adopt a bottom-up strategy to convert water-soluble and amorphous xylan obtained in a pressurized hot-water extraction (PHWE) biorefinery to crystalline nanoxylan (CNX) as stable hydrocolloids. Amorphous PHWE-xylan is water-soluble with branching side-sugar and acetyl, which is different from the currently existing preparations of CNX using the alkali-extracted, water-insoluble xylan that possesses highly ordered orientation but is poorly controllable towards a complete debranching efficiency.

The unique innovation is the strategy to reform the end-group by borohydride reduction to a primary alcohol, which effectively prevents the main-chain peeling when the substitutions of side-sugar units and acetyl groups are cleaved at an elevated temperature under alkaline conditions. Multi-instrumental analysis (composition analysis and NMR) confirmed the almost complete removal of side substitutions. This eventually tailor makes the branched PHWE-xylans as almost linear biopolymer composed of more than 90 wt% xylose units. Nanoprecipitation by a gradual pH decrease resulted in a stable hydrocolloid dispersion in the form of worm-like nanoclusters assembled with primary crystallites in uniform diameter of 10 – 15 nm, owing to the self-assembly of debranched xylan driven by strong intra-/inter-chain H-bonds and van der Waals attraction. With evaporation-induced self-assembly, we can further construct the hydrocolloids as dry submicron spheroids of CNX with a high average elastic modulus of 47–83 GPa. Taking the advantage that the chain length and homogeneity of PHWE-xylan can be tailored, we refined the xylan fractions to access well-defined macromolecular characteristics and strategically to increase the structural order in the as-prepared CNX. Importantly, rigid clusterization that is constructed within the nanoxylan of high structural order and crystallinity has contributed to the enhanced phosphorescent emission of this crystalline biopolymer. Hydrocolloids of self-assembled CNX increase the economic feasibility in PHWE-biorefinery and broaden the landscape of bio-based and functional nanomaterials.

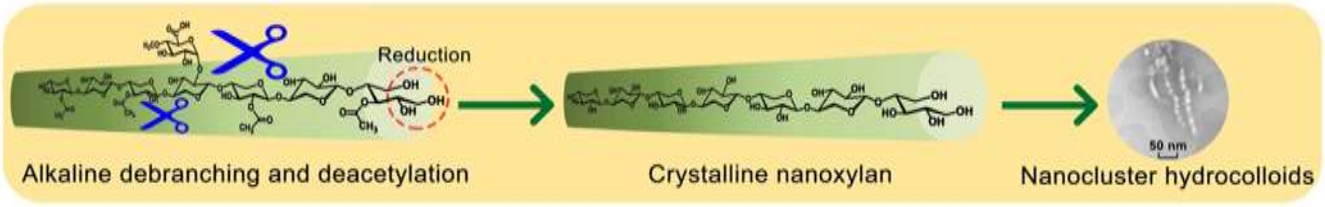

Figure 1. Schematic illustration of the preparation of CNX and TEM image of CNXs assembled head to toe in nanosuspension.

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Gram-Scale Production And Chemical Modification Of A Novel Exopolysaccharide From *L. Pentosus*

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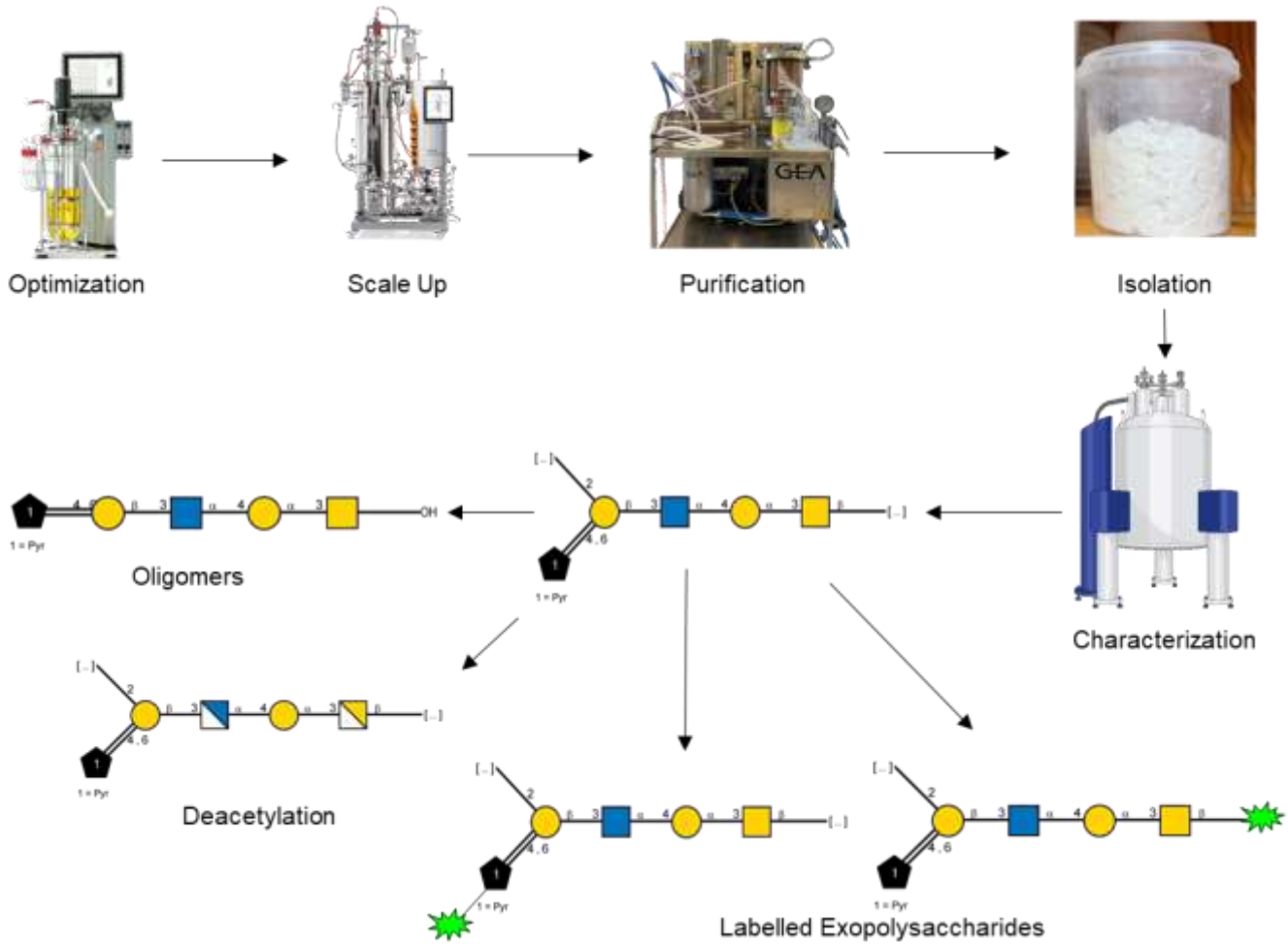
Bacterial exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) have gained attention due to their structural diversity in nature, physicochemical properties applicable across various industrial domains, and health benefits in functional foods.[1] In the present study, a novel EPS was purified from the LAB strain *Lactiplantibacillus pentosus* KW1. In brief, growth conditions were initially optimized to maximise the EPS yield, employing a 100-liter fermentation process in a semi-defined medium. Subsequent EPS extraction and purification gave 15 g of EPS in isolated yield. The polysaccharide structure was elucidated by NMR, revealing a novel structure similar to a glycosylaminoglycan (GAG), owing its negative charge to pyruvic acid acetals. The EPS was finally modified by chemical methods to gain acces to deacetylated, labeled or oligomerized EPS for intented use in applications ranging from enzymology to microbiology.



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Advanced Glycosyl Nanomaterials Based On Supramolecular Chemistry For Targeted Cancer Therapy

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Supramolecular assembly based on supramolecular chemistry provides great advantages in fabrication of complex multifunctional nanomaterials via rational design of the molecules or selection of the building blocks, which have received wide interests in the fields of material science and biology, especially in drug delivery and cancer therapy. Considering there are overexpressed asialoglycoprotein receptors on the cancer cell membrane which can be specifically recognized by carbohydrates such as galactose or lactose, we rationally designed and synthesized several lactose derivatives to fabricate advanced glycosyl nanomaterials with different nanostructures from nanovesicles to metal organic frameworks. These advanced nanomaterials not only show good targetability to hepatocellular carcinoma, but also offer enhanced delivery efficiency and efficacy in targeted cancer therapy.

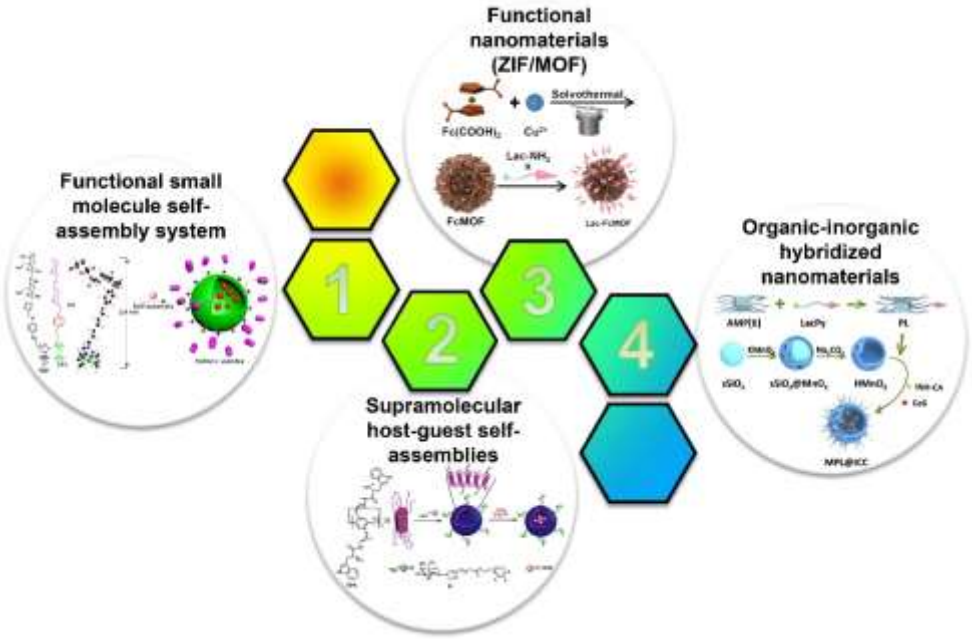

Figure 1. Advanced glycosyl nanomaterials obtained via supramolecular assembly.

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Unlocking Cell- And Organ-Specific Transfection With Cyclodextrins: Geometrically Frustrated Amphiphiles

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In this research, we introduce an innovative β-cyclodextrin (βCD)-prototype for delivering nucleic acids, which we term "geometrically frustrated amphiphiles (GFAs)." These GFAs are designed with cationic centers evenly distributed across the primary O6 and secondary O2 positions of the βCD scaffold, while hydrophobic tails are anchored at the seven O3 positions. Such unique arrangement of functional elements breaks away from the conventional sharp division between cationic and lipophilic domains typically observed in Janus architectures, endowing the system with an exceptional capacity for encoding topological data. Our objective in this communication is to outline the design, synthesis, and supramolecular properties of βCD-based GFAs, including their self-assembly behavior and interaction with DNA upon co-assembly. Additionally, through *in vitro* and *in vivo* transfection experiments, we highlight their potential for effectively formulating cell- and organ-specific nucleic acid therapeutics (Figure 1).

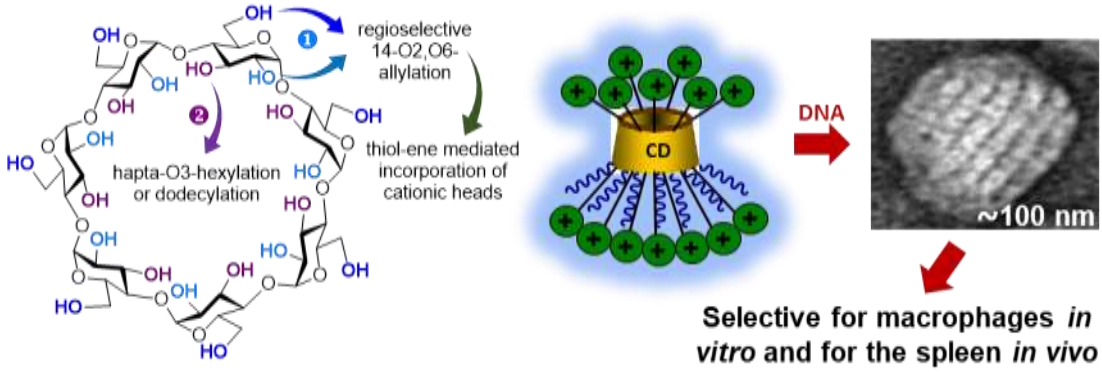

Figure 1. Schematic representation of the βCD-based geometrically frustrated amphiphiles reported in this work and their self-assembly with DNA to afford transfectious nanoparticles

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Innovative Oxidized Microcellulose Based 3D-Bioink For Tissue Engineering

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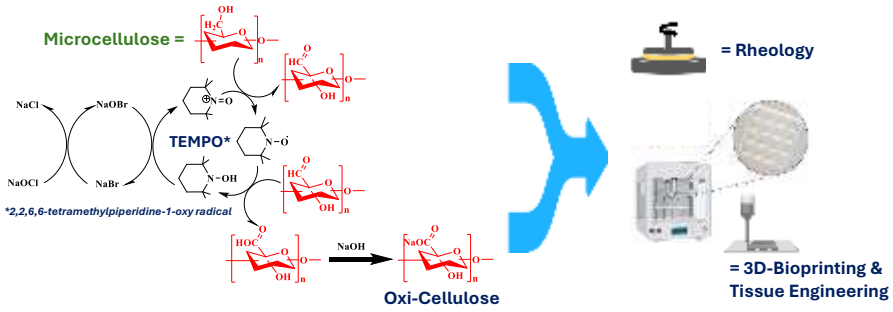
Cellulose is a polysaccharide composed of repeated *β*-D-glucose sugar units linked by *β*-1,4-glycosidic bonds and characterized by numerous intramolecular/intermolecular hydrogen bonds, resulting in a plethora of hydroxyl groups along the cellulose chain. These hydrogen bonds confer unique stability, hydrophilicity, and abundant sites for chemical modification with various functional groups[1]. Notably, both the original and chemically modified forms of cellulose find extensive application in tissue engineering due to their notable advantages. These include high specific mechanical properties, non-immunogenicity, non-toxicity, abundance in sources, and cost-effectiveness of production[2].The oxidation process mediated by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) selectively targets the primary hydroxyl group of cellulose, leading to the conversion of the surface C6 hydroxyl group into a carboxyl group. The oxidized cellulose undergoes mechanical treatment to yield oxidized nanocellulose with a reduced diameter. These oxidized nanocellulose particles exhibit high viscosity owing to their excellent water solubility and possess the capability to cross-link with divalent metals, such as calcium ions, to form hydrogels[3]. Cellulose nanofibers (CNFs) have demonstrated suitability for facilitating cell diffusion and maintaining phenotypic morphology, thus holding promise for application in bone tissue engineering[4].To simplify and reduce costs process, we developed a very innovative TEMPO/NaBr/NaOCl system to oxidize microcellulose and generate high yield gram scale oxi-cellulose. The physico-chemical properties of this anionic cellulose were studied (FTIR, carboxyl%, Transmittance, gelation time using $CaCl_2$/nano-hydroxyapatite, rheology, injectability/printability, cell viability, bone cell adhesion) to propose new generation of 3D-bioink for tissue engineering scaffolds without additional polysaccharides.



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The Retaining Pse5Ac7Ac Pseudaminyltransferase Kpss1 Defines A Previously Unreported Glycosyltransferase Family (GT118)

Abigail J. Walklett, Emily K. P. Flack, Harriet S. Chidwick, Natasha E. Hatton, Tessa Keenan, Darshita Budhadev, Julia Walton, Gavin. H Thomas and Martin A. Fascione

Cell surface sugar 5,7-diacetyl pseudaminic acid (Pse5Ac7Ac) is a bacterial analogue of the ubiquitous sialic acid, Neu5Ac, and contributes to the virulence of several multidrug resistant bacteria, including ESKAPE pathogens *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Despite its discovery in the surface glycans of bacteria over thirty years ago, to date no glycosyltransferase enzymes (GTs) dedicated to the synthesis of a pseudaminic acid glycosidic linkage have been unequivocally characterised in vitro. In our research, we demonstrate that *A. baumannii* KpsS1 is a dedicated pseudaminyltransferase enzyme (PseT) which constructs a Pse5Ac7Ac-α(2,6)-Glc*p* linkage (Figure 1), and proceeds with retention of anomeric configuration. We utilise this PseT activity in tandem with the biosynthetic enzymes required for CMP-Pse5Ac7Ac assembly, in a two-pot, seven enzyme synthesis of an α-linked Pse5Ac7Ac glycoside. Due to its unique activity and protein sequence, we also assign KpsS1 as the prototypical member of a previously unreported GT family (GT118).[1]

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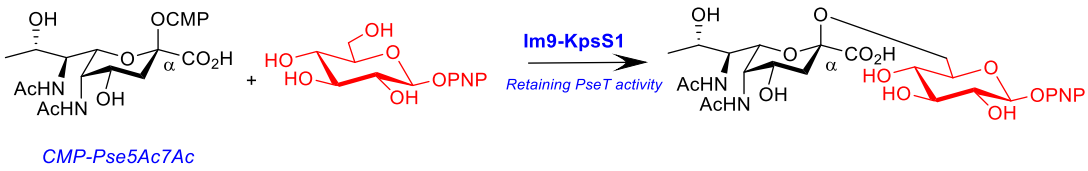

Figure 1: Im9-KpsS1 reacting CMP-Pse5Ac7Ac (donor substrate) with a PNP-β-D-Glc*p* (acceptor substrate) to produce a Pse5Ac7Ac-α(2,6)-Glc*p* linkage.

Chemistry And Biology Of Immunomodulatory Lps Mimetics

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Pro-inflammatory signaling mediated by pattern recognition receptors (PRRs) plays a key role in immediate protection against infection and in supporting immune homeostasis.[1, 2] Activation of these innate immune receptors also enhances adaptive immunity, which is being exploited in the development of vaccine adjuvants through the use of minimally toxic PRRs activating ligands. The development of novel synthetic lipopolysaccharide (LPS/lipid A) mimetics with simplified structure targeting key PRRs such as caspase-4/11 and/or Toll-like receptor 4 (TLR4) opens up promising opportunities to harness the benefits of TLR4- or caspase-4-mediated immunity for therapeutic applications such as vaccine adjuvant development,[3, 4] management of sepsis[5] and chronic inflammation[6] or immunotherapy of solid tumors.[7, 8]

Using crystal structure-based design, we have developed LPS/lipid A mimetics in which the inherently flexible β(1→6)-linked diglucosamine backbone of lipid A is replaced by a synthetic conformationally restricted (1↔1)-linked disaccharide scaffold. We show that the molecular shape of the non-reducing disaccharides as scaffolds for immunomodulatory glycolipids, determined by the anomeric configuration around the 1,1´-glycosidic linkage, is critical for the expression of TLR4-specific activity. Thus, LPS-mimetics derived from β,α-1,1-linked diglucosamine express anti-inflammatory activity, whereas glycolipids based on a synthetic α,α-1,1-linked disaccharide scaffold are potent activators of cellular responses.[9-12]

The atomic Cryo-EM structures of heterodimeric TLR4/MD-2/LPS-mimetic complexes (obtained at 2.2 – 2.9 Å resolution) have allowed the elucidation of the structural basis of the recognition of synthetic LPS-mimetics by the TLR4 complex and provided a structural background for the correlation with biological activity. While the 3D molecular shape of the non-reducing disaccharide backbone determines the biological activity (TLR4 agonist or antagonist), the attachment sites of the phosphate groups and the branched lipid chains to the sugar backbone control the mode of ligand binding to the receptor complex and correlate with the potency of the immune response.

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Glycoconjugates As Immunostimulants And Vaccines

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Immunostimulants have the properties of activating nonspecific immunity and enhancing immune response. Rational use of immune agonists as adjuvants for subunit vaccines can induce long-term and efficient specific immune responses, or change the types of immune responses, improve the body's protective ability, and at the same time reduce the dosage of immune substances and the production cost of vaccines.

Subunit vaccine has the advantages of good safety, easy production, storage and transportation, and is one of the most effective means to prevent many infectious pathogens. However, due to the poor immunogenicity of antigen, the effectiveness of subunit vaccine is limited, and it is urgent to develop various technologies to solve this problem.

In order to develop safe and effective subunit vaccines, we developed glycoconjugates as adjuvants to address the issue of poor immunogenicity:

1. Through rational design, a series of highly effective immune agonists of glycolipids were synthesized, such as NKT cell agonist αGalCer analogs and TLR4 agonist MPLA analogs, which can efficiently induce immune cells to elicit cytokines and enhance immune responses;

2. Preparation of "adjuvant-protein" conjugates by site-specific coupling. The lipophilic glyco-adjuvant is conjugated to the N-terminal of the protein. The protein with built-in adjuvant is then prepared as liposomal vaccine, which induces strong activation of immune cells and significantly enhanced humoral and cellular reactions in vivo. The results show that "adjuvant-protein" conjugate is a new strategy for developing potent vaccine.

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Chondroitin Sulfates Synthesized Via A Microbiology-Chemistry Strategy And Their Multiple Effects In Modulating The Neurite Outgrowth

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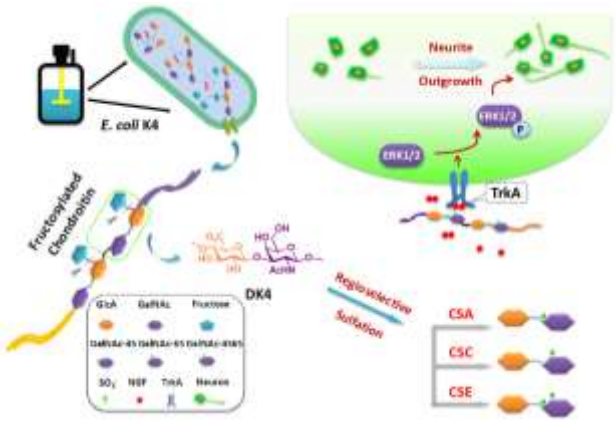
Neurite outgrowth is a critical but challenging function for the regeneration of impaired neurons. Chondroitin sulfate (CS) as a ubiquitous and heterogeneous component in brain plays key roles in regulating cellular behaviors of neurons, which still attracted less attention on how CS modulates their outgrowth[1]. Presently, this work aims at controlling the sulfate pattern and molecular weight of CS subtypes based on the hybrid of microbiology-chemistry strategy[2,3]. Both of the native CS precursor and its degraded fraction with low molecular weight were subjected to the regioselective sulfation[3]. The chemical structures of three synthesized CS subtypes were characterized by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, disaccharide analysis, and elementary analysis to determine their regioselective sulfation and degree of sulfation. The isolated hippocampal neuron was exposed to three CS products to study how sulfation pattern of CS affects the outgrowth of neurons. We found that CS with dual sulfates promoted the outgrowth and survival of neurons, whereas CS with mono sulfate displayed an inhibitory effect. Finally, the extracellular signal-regulated kinase (ERK) signaling pathway was proved to be the regulating mechanism by CS in outgrowth of hippocampal neurons[4]. This work provided a sustainable strategy in producing sulfated glycosaminoglycans, which would benefit the efforts in discovering their novel functions and therapeutic application.



Scheme 1. The route of semi-synthesis of CS subtypes by using the fructosylated chondroitin from E. coli K4 as precursor, and the potential mechanism of neurite outgrowth regulated by CS.

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Acknowlegements

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Assessment Of All Configurational Isomers Of The 3,4,5-Trihydroxypiperidine With Their *N*-Alkylated Derivatives As Gcs, Gba And Gba2 Inhibitors

Qiang Ma, Maria J. Ferraz, Richard J. B. H. N. van den Berg, Johannes M. F. G. Aerts and Hermen S. Overkleeft

1-Deoxynojirimycin (DNJ) is a biologically active natural compound, from which many isomers and derivatives have been studied for their biological potential,[1] including inhibition of the three glucosylceramide processing enzymes, glucosylceramide synthase (GCS), lysosomal glucosylceramidase (GBA) and non-lysosomal glucosylceramidase (GBA2). Recently, there has been reported that the hydroxymethyl group of DNJ is crucial to neither potency nor selectivity of β-glucosidase.[2] Additionally, GBA inhibition is improved after removing this sidechain of DNJ.[3] With the aim of developing more potent and selective inhibitors of these three glucosylceramide processing enzymes, this project described the synthesis and biological evaluation of in total 4 configurational isomers of 3,4,5-trihydroxylpiperidines, as well as their *N*-alkylated derivatives. Screening the library against the three enzymes indicates that the hydroxymethyl group is not crucial to their inhibition potency but affects the selectivity toward GCS/GBA2 over GBA. In addition, this inhibition profile confirms that appropriately substituted DL-*glu*/DL-*ido* configured 3,4,5-trihydroxylpiperidines are still effective as dual GCS/GBA2 inhibitors, which have been studied as putative therapeutics for the treatment of the lysosomal storage disorder, Gaucher disease. We believe that our finding here could contribute to the design of next-generation inhibitors of glucosylceramide processing enzymes.

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N-Glyfind^{tm} – A High-Specificity Affinity Reagent For Detection And Enrichment Of *N*-Glycosylated Proteins

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N-glycans play crucial roles in nearly every aspect of biological processes, and their distinct properties make them appealing as disease biomarkers and therapeutics targets. However, due to their highly branched and variably linked nature, *N*-glycans remain a challenge to detect, purify, and analyze structurally. Despite current advances in analytical techniques and instrumentation, there is still a great need for high-affinity reagents with well-defined epitope specificity that can be used to interrogate and enrich biological samples. Lectenz Bio has been engineering glycan-processing enzymes and glycan-binding proteins into high-affinity glycan-binding reagents with tunable specificities. Here, we report the development of *N*-GlyFind^{TM}, an asparagine-linked glycan (*N*-glycan) specific reagent engineered via directed evolution from a mouse F-box only protein 2 (FBXO2 or Fbs1). Our approach harnesses molecular dynamics (MD) simulations to explore the dynamic nature of protein-glycan interactions, enabling the identification of specific amino acid residues for construction and screening of a combinatorial yeast display library. The resulting *N*-glycan core-specific candidates were further validated by a panel of assays such as Glycan Microarray, Western Blot, Bio-Layer Interferometry, ELISA, and Affinity Chromatography. The lead candidate, called *N*-GlyFind^{TM}, has been identified as an *N*-glycan affinity reagent exhibiting high pan-specificity towards *N*-glycosylated peptides and proteins. (Supported by United States National Institutes of Health grant OD035390.)

Fucosylated Chondroitin Sulfate Oligosaccharides Against Parkinson's Disease Through Inhibiting Endocytosis And Amplification Of α-Syn Aggregates

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Parkinson’s disease (PD) is a neurologic disorder marked by abnormal aggregation of α-Synulcein (α-Syn) protein in the substantia nigra. Identification of substances that stop or reverse the aggregation process could therefore be a promising treatment approach for PD. Here, we demonstrated that in a cellular model of PD caused by α-Syn preformed fibrils (PFFs) α-Syn pathology was decreased by oligosaccharides (3-18mer) derived from the depolymerization of a sea cucumber fucosylated chondroitin sulfate (fCS). First, the results of biophysical and biochemical tests indicate that the fCS oligosaccharides exhibited a strong ability to suppress α-Syn fibrillation in a chain length-dependent manner via interacting with α-Syn. Second, the fCS oligosaccharides have the ability to prevent the clathrin-mediated endocytosis of α-Syn aggregates. Finally, it was discovered that fCS oligosaccharides could cross the blood-brain barrier, and their inhibition on α-Syn aggregation was confirmed in a α-Syn PFFs-injected PD mouse model. Overall, this study demonstrates that the fCS oligosaccharides may be an effective treatment option for PD.

Keywords: Sea cucumber fucosylated chondroitin sulfate, Parkinson’s disease, α-Syn aggregation

Synthesis, biofunctional evaluation and computational analysis of Th2-selective glycolipid antigens

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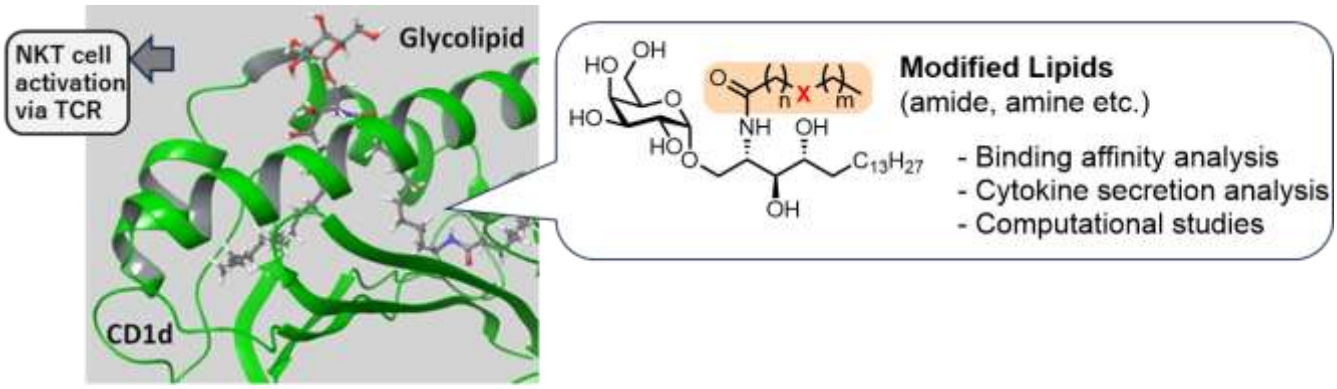
CD1d is present on antigen presenting cells and recognizes glycolipid antigens to form complexes. NKT cells recognize these complexes which leads to its activation, resulting in secretion of various cytokines for modulation of the immune system. Through exploration of lipid-modified glycolipid antigen ligands of CD1d, we have shown that the binding affinity, cytokine induction activity and selectivity can be adjusted by polar-functional group modification of the acyl group of the glycolipids.[1] For further exploration of the functions of CD1d and its ligands, we developed Th2-biased glycolipids, performed its synthesis and biofunctional evaluation, along with computational analysis to visualize the dynamics of the CD1d-ligand complex.

En route to the synthesis of the novel α-GalCer derivatives with modified acyl groups, we developed a new synthetic method to introduce functional groups to the acyl groups, and achieved a highly efficient synthesis. The synthesized fatty acids were installed to a glycolipid intermediate, prepared from D-galactose and phytosphingosine as previously reported,[1] at the final stages to achieve the synthesis of the target α-GalCer derivatives. The biological activities of the synthesized α-GalCer derivatives were evaluated by competitive binding affinity assays with the CD1d protein and cytokine induction assays with mouse splenocytes, and compared with the data of the previously reported amide and amine modified Th2-biased α-GalCer derivatives. Results indicated that the novel α-GalCer derivatives exhibits increased binding affinity towards CD1d, and possesses a significantly high Th2-selective cytokine induction profile, compared with the previously reported derivatives.

MD simulations of the α-GalCer derivatives were also performed to analyze the dynamic motion of the ligand within the CD1d-ligand complex. The distance of CD1d-sugar moiety was analyzed to evaluate the movement of the ligand within the complex throughout the simulation. Details of the experimental and computational results will be further discussed in the poster presentation.



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From A Unique Tetrasaccharide Scaffold To Vaccine Candidates Targeting Diverse *Shigella* Serotypes

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Shigella flexneri are Gram-negative enterobacteria and the main causative agent of endemic shigellosis, a major diarrheal disease especially in children under five from low- and middle-income countries. Disease burden calls for a *Shigella* vaccine that would induce broad serotype protection in the population most at risk. Protective immunity is believed to be achieved to a large extent by antibodies specific for the O-antigen (O-Ag) component of the *Shigella* lipopolysaccharide, making the O-Ag a prime target for vaccine development. Most *S. flexneri* serotypes exhibit closely related O-Ags whose common backbone features a unique repeating unit (RU). Structural diversity reflecting serotype specificity derives from diverse site-selective substitutions on this tetrasaccharide core (Figure 1).[1]

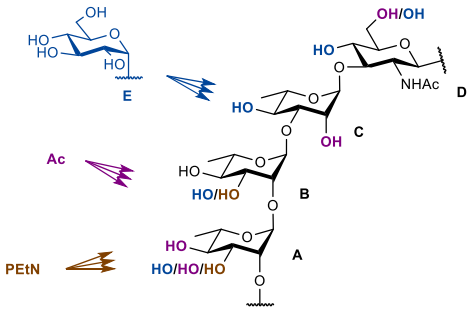

Figure 1. Backbone repeating unit of most *S. flexneri* O-Ags and type-specific substitutions thereof.

A multidisciplinary strategy toward vaccine candidates encompassing synthetic glycans mimicking the putative protective determinants carried by the O-Ag of selected *Shigella* serotypes was undertaken. A semi-synthetic glycoconjugate was designed to help protect against *S. flexneri* 2. Promising data in healthy adults in a first-in-human clinical trial trial[2] support the development of novel strategies enabling serotype broadening to answer the need in the field.[3]

This presentation will first highlight the concept of synthetic O-Ag functional mimics and recent achievements in the context of *S. flexneri 2a*. Next, focus will be on the design of oligosaccharides acting as functional mimics of the O-Ags characterizing the most predominant *S. flexneri* serotypes. Going beyond original achievement,[3] we will report a concept whereby key RU building blocks featuring serotype-specific substitutions are built from a single orthogonally protected scaffold. A three-step strategy was implemented: 1) synthesis of a fine-tuned scaffold featuring suitable orthogonal protecting groups at relevant substitution sites; 2) selective unmasking, on demand, of selected hydroxyl groups to provide ready-for-modification well-designated acceptors; 3) controlled 1,2-*cis* glucosylation of those acceptors to provide fully protected intermediates representative of the RUs of the selected *S. flexneri* O-Ags. Lastly, we will discuss chain elongation at either end of the glucosylated bricks to deliver the required panel of linker-equipped serotype-specific oligosaccharides and conversion of the later into potent immunogens. The proof-of-concept for a broad coverage synthetic glycan-based *Shigella* conjugate vaccine will be illustrated.

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Design, Synthesis And Sar Study Of Iminosugar-Type Glycosidase Inhibitors

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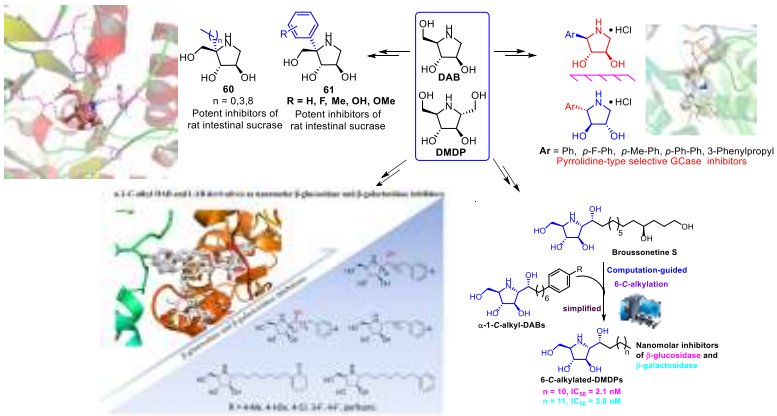
Iminosugars have shown important applications in the development of drugs for treatment of many diseases, such as diabetes, viral infection and cancer etc[1]. Development of series of iminosugar derivatives will promote the discovery of lead compounds with potent and selective glycosidase inhibitory activities and favorable pharmaceutical properties, and will also devote to systematic study of biological activities and establishment of reliable structure-activity relationship (SAR). In order to obtain iminosugars with optimal glycosidase inhibition potency and selectivity, we selected some iminosugars with important biological activities as parent compounds to construct a molecular-diversed iminosugar derivatives library with *C*-branch, *N*-alkylation, fluorination and glycosylation as the main structure modification strategies.

Herein, we present our recent research in *C*-branched DAB and DMDP derivatives. DAB (1,4-dideoxy-1,4-imino-D-arabinitol)[2] is a powerful α-glucosidase inhibitor, whereas DMDP (2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine) [3] is a potent inhibitor of bovine liver β-glucosidase and β-galactosidase. Based on the SAR of the two iminosugars and related naturally occurring broussonetines and radicamines, C-1 and C-4 alkylated/arylated DAB derivatives[4-6] and 6-*C*-alkyl-DMDP derivatives have been designed and assayed as glycosidase inhibitors. Up to now, a series of potent and selective rat intestinal sucrase inhibitors, specific GCase (Human lysosomal acid β-glucosidase) inhibitors and nanomolar bovine liver β-galactosidase and β-glucosidase inhibitors have been obtained. Subsequent SAR study in the aid of molecular docking calculations helped to provide guidance for design and development of more pyrrolidine pharmacological chaperones for lysosomal storage diseases, and potential lead compounds for treatment of viral defection, type II diabetes etc.



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Structure Characteristics Of A Novel Pectic Polysaccharide From Fructus Corni And Its Protective Effect On Alcoholic Fatty Liver

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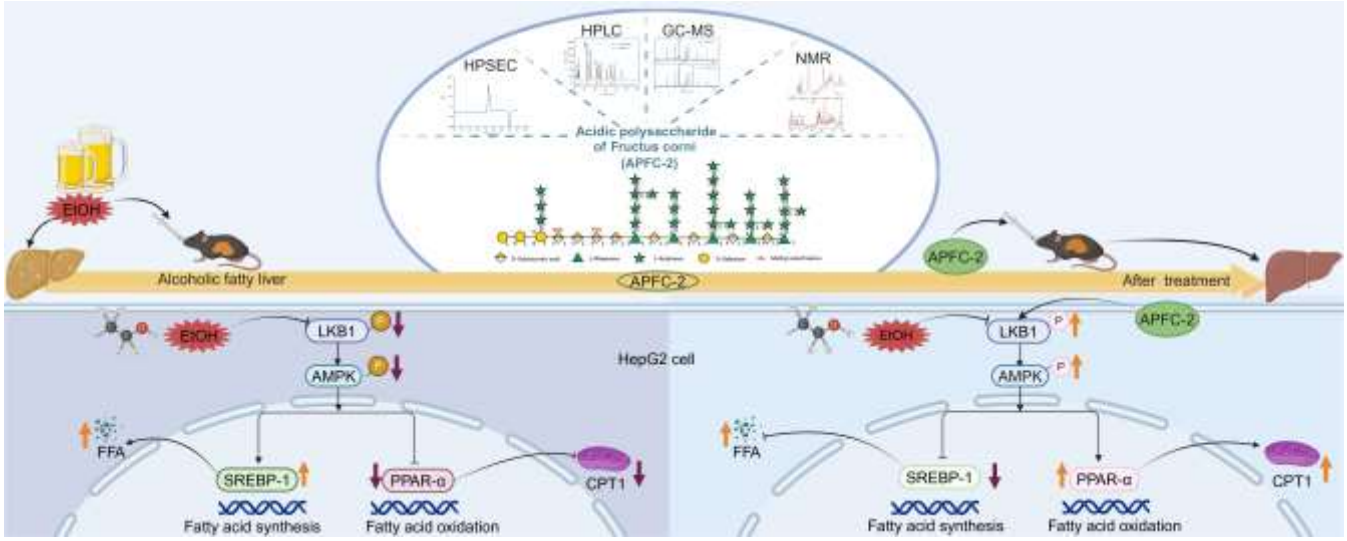
Alcoholic fatty liver disease (AFLD) is characterized by hepatic lipid accumulation and still no effective treatment [1-2]. Previous studies suggest that polysaccharide is a very promising natural product for AFLD prevention and treatment [3]. In this study, a novel homogeneous polysaccharide, APFC-2, was isolated from the dried pulps of Fructus corni, which demonstrated a markedly protective effect against AFLD. APFC-2 is a 63.0 kDa pectic polysaccharide, whose backbone consists of T-α-Gal*p*-(1→6)-α-Gal*p*-(1→3,6)-α-Gal*p*-(1→[4)-α-Gal*p*A-OMe-(1→4)-α-Gal*p*A(1→]$_m$→[2,4)-α-Rha*p*-(1→4)-α-Gal*p*A(1→]$_n$, and whose branches contain T-Ara*f*-(1→, →3)-α-Ara*f*-(1→, →3,5)-α-Ara*f*-(1→ and →5)-α-Ara*f*-(1→. APFC-2 significantly reduced hepatic steatosis, fasting triglyceride (TG) and cholesterol (CHO) levels in alcohol-induced AFLD mice. The lipid-decreasing effect of APFC-2 in vitro was evaluated by oil red O staining and consumption assays, and the results showed that APFC-2 concentration-dependently enhanced lipid metabolism and significantly improved cell viability in alcohol-induced HepG2 liver cells. Mechanistically, APFC-2 markedly inhibited the formation of lipid both *in vitro* and *in vivo* by activating liver kinase B1 (LKB1) and then regulating adenosine 5'-monophosphate-activated protein kinase (AMPK)/sterol-regulatory element binding protein-1 and AMPK/peroxisome proliferator-activated receptor-α pathways. This research provides a potential therapeutic polysaccharide as a specific inhibitor of LKB1 for treating AFLD.



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Protein-Bound Beta-Glucan From Edible Mushroom Coriolus Versicolor Alleviates Obesity Via Ra-Iga-Intestinal Akkermansia Muciniphila

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Obesity has become a major disease burden worldwide and seriously threatens public health. There is an urgent need to develop strategies for the prevention and treatment of obesity. *Coriolus versicolor* is a food source mushroom that also has been used in traditional Chinese medicine for thousands of years. An extremely broad range of physiological activities have been linked to the use of *C. versicolor*, including immunomodulatory, anticancer, antioxidant and hepatoprotective effects. We separated the polysaccharides from the dried fruiting bodies of *C. versicolor* by water extraction and ethanol precipitation. The chemical structure was well elucidated with overall consideration of monosaccharide composition, methylation analysis and 1D/2D-NMR spectra data. With molecular weight of 29.7 kDa, the isolated polysaccharide was composed of (1→4)-β-/(1→3)-β-D-glucopyranosyl group as backbone with branches attached at O-6 site. Further, the anti-obesity activities of this polysaccharide (named as PBG) were evaluated. Our results indicated that PBG can reduce obesity and metabolic inflammation in mice fed with a high-fat diet (HFD). Gut microbiota analysis reveals that PBG markedly increases the abundance of *Akkermansia muciniphila*, although it does not rescue HFD-induced change in the Firmicutes to Bacteroidetes ratio. It appears that PBG alters host physiology and creates an intestinal microenvironment favorable for *A. muciniphila* colonization. Fecal transplants from PBG-treated animals in part reduce obesity in recipient HFD-fed mice. Further, PBG is shown to upregulate expression of a set of genes related to host metabolism in microbiota-depleted mice. Since the intestinal microbiota could utilize immunoglobulin A (IgA) for mucosal colonization, thus, we deduced that PBG could promote *A. muciniphila* colonization via IgA secretion. In order to verify the key role of IgA in the anti-obesity effect of PBG, we examined the IgA content in different intestinal parts. Compared with the HFD group, the IgA contentin plasma and stools of PBG treated mice showed significantly increased, indicating that PBG could significantly promote IgA secretion. The IgA content was positively correlated to the *A. muciniphila* content. Moreover, the $IgA^{-/-}$ C57BL/6 mice were adopted, and PBG treatment could significantly increase the abundance of intestinal *A. muciniphila* abundance of $IgA^{-/-}$ mice. The in-depth multi-omics analysis showed that PBG profoundly affected the retinoic acid metabolism pathway with ALDH1A1, ALDH1A2, retinol dehydrogenase 1 (RDH1) and RDH9 genes highly expression. The PCR verification analysis was performed on the target genes of retinoic acid metabolic pathway. All these data highlighted that PBG might exert its ant-obesity effects by affecting RA metabolism pathway, promoting intestinal IgA secretion thus facilitating intestinal *A. muciniphila* colonization. Our study revealed the molecular mechanism of PBG from *C. versicolor* in obesity improvement and provided a reference for intestinal immunity of polysaccharide prebiotics in anti-obesity research, which is of great significance for the prevention and treatment of obesity.

Keywords: *Coriolus versicolor*; beta-glucan; obesity; *Akkermansia muciniphila*; polysaccharide;

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Biogenesis Of Outer Membrane Vesicles In *Bacteroides Thetaiotaomicron*: Is Lps Biosynthesis Altered?

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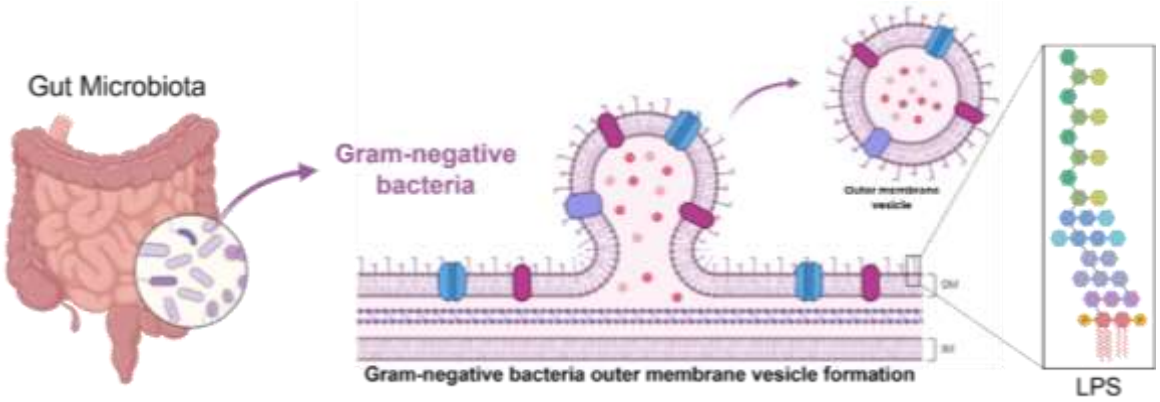
Human microbiota encompasses a large population of microorganisms inhabiting specific body districts, such as the oral and intestinal regions, where it plays pivotal roles in maintaining overall well-being, influencing a multitude of body functions, which also include immune system development and maturation.[1] Seminal studies have identified outer membrane vesicles (OMVs), spherical structures produced by blebbing of the Gram-negative outer membrane, as key mediators of these functions exhibiting anti-inflammatory and immunomodulatory properties.[2,3] In particular, it has been shown that the mutualistic gut microbe *Bacteroides thetaiotaomicron* produces large quantities of OMVs as the result of an active and regulated process. Indeed, Feldman *et al.* have recently identified a unique feature of Bacteroidota, i.e. a novel family of Dual Membrane-spanning Anti-sigma factors (Dma), which regulate OMV biogenesis in *B. thetaiotaomicron*.[2] In fact, deletion of Dma1, the founding member of the Dma family, resulted in hypervesiculation in this bacterium. Moreover, they demonstrated that NigD1, which belongs to a family of uncharacterized lipoproteins found exclusively in Bacteroidota, is required for the induction of vesiculation in the absence of Dma1, upregulating the number of lipopolysaccharides (LPS) present in OMVs.[2] More knowledge about regulation of LPS biosynthesis in *Bacteroides* is required to fully appreciate how Dma and NigD1 control OMV biogenesis.
In this communication I will show the results of a detailed structural characterization of LPS isolated from the wild-type B. *thetaiotaomicron* strain VPI-5482 and from a hypovesiculating ΔDma strain, with the aim to give a first glimpse of whether Dma and NigD1 impact on LPS biosynthesis while controlling OMV biogenesis.



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Studies In Deoxy Sugar And Other Rare Sugar Synthesis

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The oligosaccharides found on many bioactive natural products and the O-antigens of Gram-negative bacteria are often composed of so-called rare sugars. Assembling these rare sugars into oligosaccharides presents a challenge as they often do not contain functionality at the C2 and C6 positions used to control the stereochemical outcome of glycosylation reactions.[1] Furthermore, because these monosacchardes are not commercially available, their construction is often a bottleneck in the synthesis of oligosaccharides for glycoconjugate vaccine synthesis or the use of natural product glycodiversification for therapeutic development. As such there remains a need for methods that permit the efficient production of these molecules on rapid time scales, as well as methods for the stereoselective assembly of rare sugars into oligosaccharides.

This talk will discuss methods developed in our lab to facilitate deoxy sugar and other rare oligosaccharide synthesis. In the case of deoxy sugar oligosaccharide synthesis we have found that activating hemiacetals with sulfonyl chlorides leads to the formation of species that undergo β-specific glycosylation reactions.[2] Mechanistic studies that show that this chemistry proceeds through an S_N2-like manifold as well as applications to deoxysugar oligosaccharide synthesis will be discussed.[3-5] To address the need to obtain suitably functionalized monosaccharides, we have developed a continuous flow-based platform for deoxy sugar monosaccharide production. This platform reduces the production time for unusual sugars form a period of days to just a few hours.[6] In addition, we have demonstrated that 2,4-diamino-2,3,6-trideoxyhexoses (bacillosamines) associated with many pathogenic bacteria can be obtained in good yield and 6-7 steps from threonine via Ir-mediated allylation chemistry.[7] The scope of both methods will be presented.

Keywords: Glycosylation, deoxy sugar, de novo synthesis, automated synthesis, rare sugars

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Advancing Glycoconjugate Vaccine Design For Enhanced Antibody Response

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Introduction: Glycoconjugate vaccines offer significant global health benefits, yet their effectiveness in high-risk populations remains a challenge. Recent insights highlight the importance of carbohydrate presentation to T cells by antigen-presenting cells, suggesting that peptides could serve as alternative carriers for glycoconjugate vaccines, replacing whole proteins[1-2]. In this study, we present a platform for optimizing glycopeptide vaccine design, including determining the ideal polysaccharide chain length, peptide composition, and conjugation site. Animal studies were conducted to compare the immune response induced by these optimized glycopeptide vaccines with conventional glycoprotein conjugate vaccines.

Materials and Methods: Female Balb/c mice or rabbits received intraperitoneal immunizations with Group B Streptococcus type III (GBSIII) and Francisella tularensis (Ft) glycoprotein or glycopeptide conjugate vaccines, administered three times at two-week intervals using Alum or Complete Freund Adjuvant (CFA). Antigen-specific antibody responses were monitored during the experiment by ELISA. In the GBSIII model, the functional activity of vaccine-induced antibodies was evaluated using opsonophagocytic killing assays, as well as by transferring immunized rabbit sera to mice and assessing protection against lethal bacterial challenge. Additionally, challenge studies were conducted in both the GBSIII and Ft models to assess the level of protection conferred by the different glycoconjugate vaccines against lethal bacterial challenges.

Results: The peptide linker and conjugation site significantly impacts conjugation efficiency and immune response. Notably, in mouse models of GBS and Ft infection, glycopeptide conjugate vaccines outperform standard glycoprotein conjugate vaccines in protecting against bacterial challenges, despite inducing lower levels of anti-carbohydrate specific IgG antibodies. Furthermore, using a full-length polysaccharide optimally enhances immunogenicity for glycopeptide conjugate vaccines compared to smaller-sized polysaccharides.

Discussion and Conclusions: Our observation challenges the conventional belief that the amount IgG induced by glycoconjugate vaccines directly correlates with conferred protection. Ongoing research aims to comprehensively evaluate the humoral and cellular response elicited by both glycopeptide and glycoprotein conjugate vaccines[3-4]. The knowledge gained from this evaluation will be pivotal in developing glycoconjugate vaccines that provide enhanced efficacy and longer-lasting immunity.

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Stereoselective 1,2-*Cis*-Glucosylations

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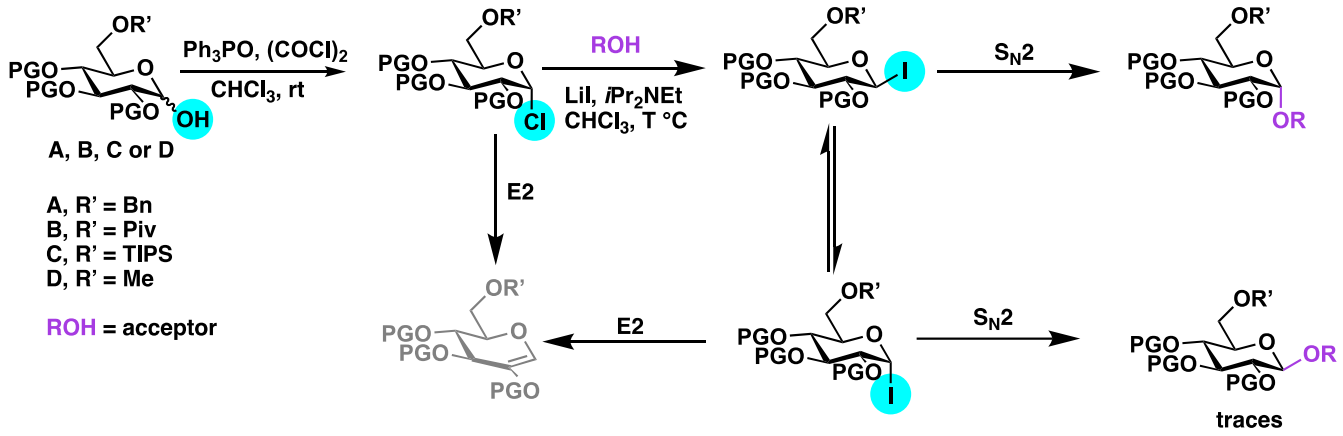
Oligosaccharide synthesis, consisting of multiple glycosylation steps, poses many difficulties with respect to regio- and stereoselectivity[1]. Depending on the reaction conditions, 1,2-*cis*- or 1,2-*trans*-glycosides can be obtained, of which the former are usually more difficult to synthesize. Previously, the McGarrigle group reported access to 1,2-*cis*-glycosides, by treatment of the glycosyl hemiacetal donor with Denton's catalytic Appel conditions[2,3], followed by reaction with LiI, *i*Pr$_2$NEt and the acceptor[4]. This procedure was successfully applied to the stereoselective synthesis of β-mannosides and β-rhamnosides.

In contrast to β-mannosides and β-rhamnosides, we will describe how glucosyl hemiacetal donors give α-glucosides. Glucosyl hemiacetal donors and a range of acceptors have been tested (**Scheme 1**). Optimization studies were required to prevent unwanted elimination of the glycosyl iodide intermediate to form the corresponding glucal side product (**Scheme 1**, grey). Changing the rate of addition of base *i*Pr$_2$NEt was found to limit the formation of the side product, affording an increase in the acceptor conversion, and still with an excellent *α/β* selectivity. To demonstrate the usefulness of the method, a target pentasaccharide was also synthesized using these conditions[5].



Scheme 1. General scheme for the stereoselective synthesis of α-glucosides.

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Glycosyl N-Phenyl Pentafluorobenzimidates: A New Generation Of Imidate Donors For Glycosylation

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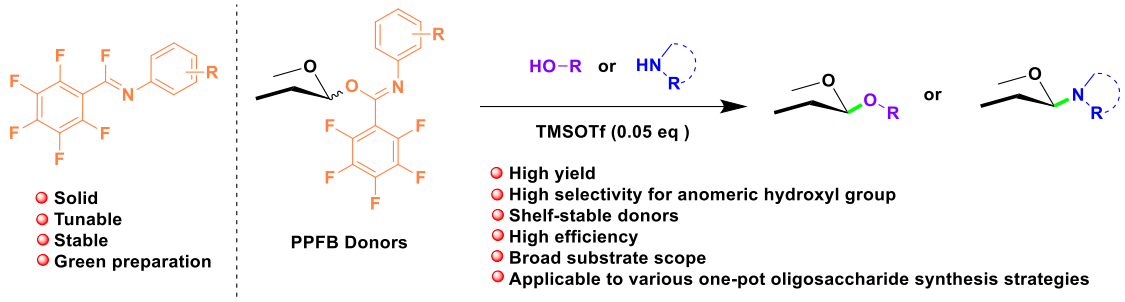
Abstract: Glycosyl imidate are among the most popular donors in the synthesis of glycans and glycoconjugates. Since the first report, a great number of imidates have been disclosed. Representative imidates include glycosyl trichloroacetimidates (TCAs) and N-phenyl trifluoroacetimidates (PTFA). The continuous efforts have brought new values on imidates, like the easy accessibility, stability and tunability, by the introduction of new structural motifs. Here we report N-phenyl pentafluorobenzimidoyl (PPFB) glycoside as a new generation of imidate donors. PPFBs can be synthesized by nucleophilic substitution on the corresponding imidoyl fluorides, which showed a higher chemoselectivity on the anomeric hydroxyl group over the others. The PPFB glycosylation features broad substrate scope, ranging from primary to tertial alcohols, electron rich and deficient phenols, glycosyl acceptors, amino acid derivative and nucleobases. These glycosylations are often in good to excellent yields, except purines. In addition, we found that PPFBs were generally more stable than PTFAs when evaluated by ^{19}F NMR over an extended period. Consistently, PPFBs showed less activity than TCAs and PTFAs in the glycosylation, which facilitates the chemoselective one-pot synthesis of glycans that is currently on the progress.



Keywords: Imidate donor; Glycosylation; N-phenyl pentafluorobenzimidoyl glycoside

Facile Synthesis Of 1,2-Cis-α-1-Thiosugars And Trehalose Type α-α-Thiodisaccharides Via Photoclick Chemistry

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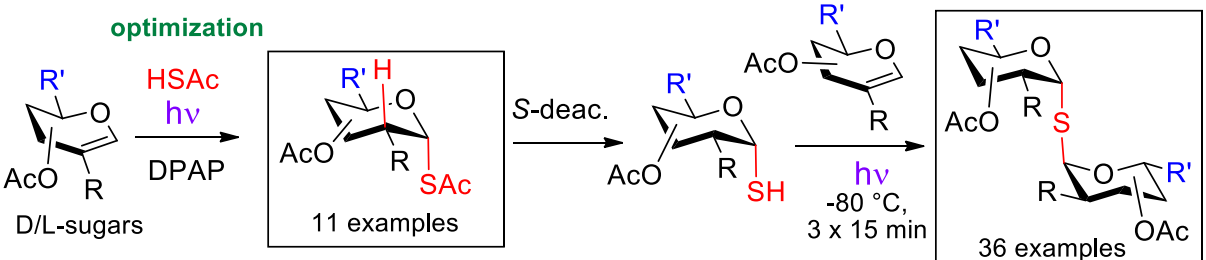
Glycomimetics, in which a sulfur atom replaces the interglycosidic oxygen, are resistant to glycosidase enzymes and are therefore of great interest as drug candidates and useful tools in glycobiological studies. The radical mediated, photoinitiated thiol-ene addition reaction (thio click reaction) has emerged as an efficient method for the stereoselective synthesis of stable thioglycomimetics, including α,β-linked trehalose-type thiodisaccharides. [1-3] At the same time, there is no generally applicable, stereoselective synthesis method for α,α-linked trehalose-type thiodisaccharides, even though these structures have an exceptionally high biological potential.

Here we present a fully stereoselective method for the synthesis of α,α-thiotrehalose and their analogues based on a double thiol-ene coupling. In the first photoinitiated thiol-ene addition step, thioacetic acid is introduced into the anomeric position of the 2-substituted glycal with full 1,2-cis-α-selectivity, and after selective S-deacetylation the resulting α-1-thiosugar is coupled to another 2-substituted glycal, in a second thiol-ene reaction, to obtain various homo- and hetero-thiodisaccharides with complete α-selectivity. Thioacetic acid initially showed low reactivity in the radical addition reaction, therefore a thorough optimization study was performed to efficiently obtain α-thioacetates. The α-thiols were also were also utilized for the sznthesis of symmetric α,α-diglycosyl disulfides. All thioglycosides and diglycosyl disulfides were deprotected to make them suitable for biological assays.



R = OAc / NHAc; DPAP: 2,2-dimethoxy-2 phenylacetophenone
R' = CH_2OAc, CH_2OBz, $CH_2OTBDSP$, CH_3, COOMe
Optimized conditions: photosensitizer, -80 °C, 3 x 1 h irradiation in AcOH; 60-95% yield

In the interaction study between *Pseudomonas aeruginosa* LecA and LecB lectins and selected thiodisaccharides, α-L-Fucp-α-L-Fucp and α-D-Galp-α-D-Galp showed excellent affinity against one of the lectins, while α-L-Fucp-α-D-Galp proved to be an excellent bispecific a ligand.

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Palladium-Catalyzed Stereospecific Glycosylation With 3,4-Carbonate Glycal Donors: Access To Diverse Glycoside Scaffolds

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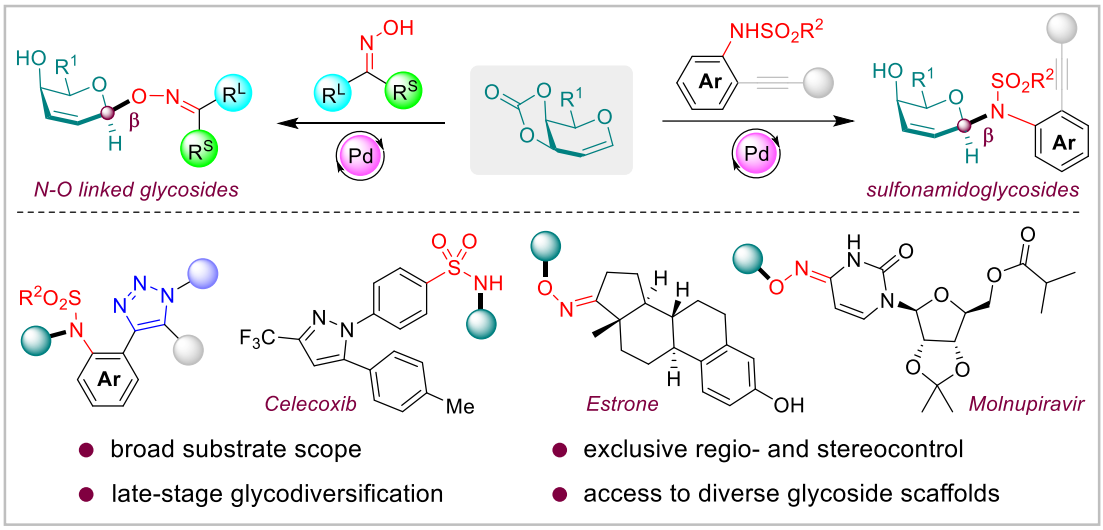
Carbohydrates play pivotal roles in numerous physiological/pathological processes and have important applications in modern drug research and development. Therefore, we are dedicated to developing novel stereoselective glycosylation methods and strategies and applying them to the efficient synthesis of complex oligosaccharides and glycoconjugates. In recent years, transition-metal catalyzed glycosylation has emerged as a powerful tool for constructing glycosidic bonds due to its versatility and robustness.[1,2] Herein, we present a highly efficient, palladium-catalyzed stereospecific glycosylation between 3,4-O-carbonate glycals and oximes/sulfonamides.[3,4] This approach features broad substrate scope, high functional group tolerance, and easy scalability, delivering a wide range of glycosyl oximes/sulfonamides in excellent yields with exclusive β-selectivity. The power of this method is demonstrated by a set of site-selective transformations of glycosylation products and late-stage functionalization of pharmaceutically relevant molecules. Overall, our strategy provides an efficient toolkit for facile access to diverse N−O-linked glycosides and N-glycosylated sulfonamides, providing excellent opportunities for their in-depth biological evaluations.



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Synthesis Of Circulating Anodic Antigen (Caa) Oligosaccharides For Schistosomiasis Diagnostics

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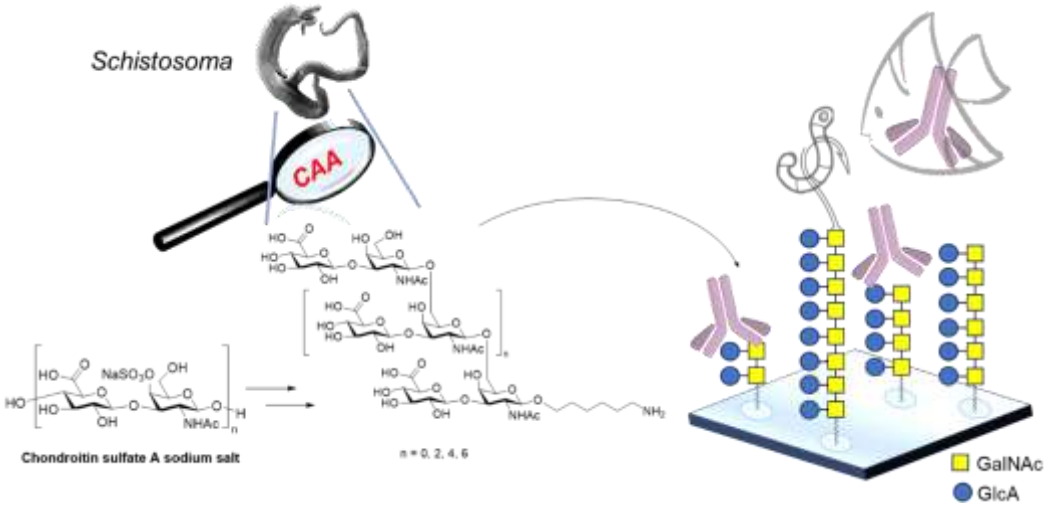
Schistosomiasis, caused by parasites of the genus *Schistosoma*, affects over 250 million people worldwide. Developing improved diagnostic methods is crucial for effective disease control and elimination. Schistosoma parasites express a complex array of glycans that are prime targets for antibodies generated during infection. Among these, the circulating anodic antigen (CAA), a polymer composed of repeating units of *N*-acetyl galactosamine and glucuronic acid ([-6-(GlcA-β1-3)-GalNAc-β1-]$_n$), has emerged as a promising target.[1]

We realized that the CAA polysaccharide shares the same repeating unit with chondroitin sulfate, although the connection of the dimer repeats in both polysaccharides differs. We therefore here report the development of an effective semi-synthesis[2] route towards CAA oligosaccharides, using disaccharides sourced from the cheap and readily available chondroitin sulfate. Depolymerisation and installation of the appropriate protecting groups delivered the required disaccharide repeating unit building blocks that were used for to generate oligosaccharides of various lengths. These oligosaccharides underwent two-step deprotection, resulting in well-defined CAA fragments including a dimer, tetramer, octamer, dodecamer and hexadecamer, each containing an amine-functionalized spacer for conjugation purposes. Preliminary microarray-based evaluation[3] demonstrated the remarkable specificity and sensitivity of CAA, indicating its potential as a diagnostic tool for primary infection. Concurrently, structural studies of CAA fragments are ongoing with the aim to establish a correlation between the glycan structure and the elicited immunological response. In all, our studies may advance the development of novel diagnostic methods for Schistosomiasis.



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Facile Generation Of Glycosyl Radicals And Their Application For The Synthesis Of *C*-Glycosides Using 1,4-Bis(Diphenylamino)Benzene

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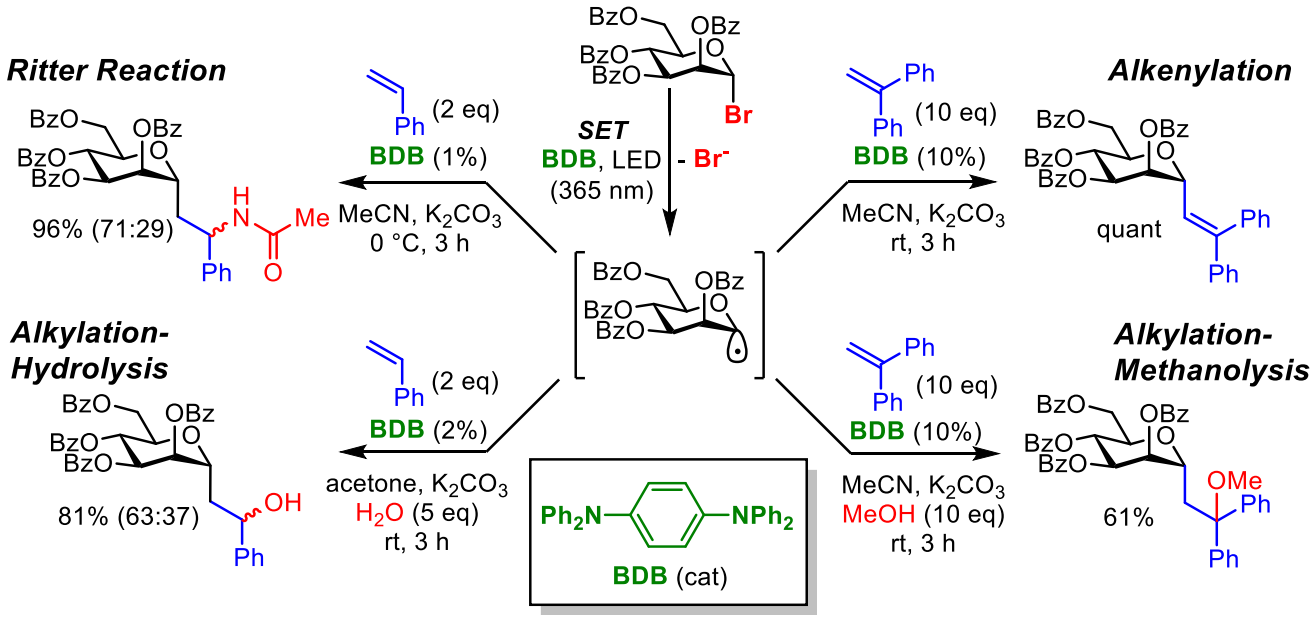
Aromatic compounds equipped with diarylamino substituents have already been utilized as highly reducing organic photocatalysts by Koike and co-workers in their pioneering works.[1] Here we report a practical method to generate glycosyl radicals from glycosyl bromides using 1,4-bis(diphenylamino)benzene (BDB) as an organic photocatalyst. Glycosyl bromides served as glycosyl radical precursors by LED light (365 nm) irradiation in the presence of BDB and K_2CO_3. The generated glycosyl radicals were coupled with alkenes to afford alkenylation and radical-polar crossover (RPCO)-type products in moderate to quantitative yields. This method is applicable to glycosyl bromides of pyranohexoses to prepare various *C*-glycosides which are important analogues of glycosides.[2]



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The Exploration And Practice On Research And Development Of Natural Polysaccharide Innovative Drugs

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With unique structure, bioactivity and mechanism, polysaccharide drug has become an important part of the research and development of international innovative drugs. Polysaccharide, as a biological macromolecule, has highly complex structure due to their large molecular weight, diverse monosaccharide composition, substituents and glycosidic bond linkages. Moreover, their properties of mixtures and microscopic heterogeneity cause great challenges in the druggability study and new drug development.

Our group has long been dedicated to the research of natural polysaccharides, including isolation and purification, structural identification, structure-activity relationship, function and mechanism, and the discovery of innovative drug [1~3]. As core members, we discovered a natural polysaccharide-derived "first-in-class" drug, which has been approved by the US FDA for clinical trials (IND153593), as an antithrombotic polysaccharide innovative drug with low bleeding risk. Internationally, it is the first potent and selective inhibitor of intrinsic coagulation factor Xase.

In recent years, our group has conducted systematic and in-depth studies on a series of natural polysaccharides. We have reported different polysaccharides with novel structural and potent activity from dozens of animal and plant species. For instance, the fructan and galactan from *Polygonatum cyrtonema* can significantly promote the proliferation of intestinal probiotics [4]. A glucomannan from *Bletilla formosana* showed good anti-inflammatory activity [5]. A glycosaminoglycan the pleopod of *Achatina fulica*, with heparan sulfate-like regular structure, can significantly promote the healing of chronic wounds in diabetes [6]. Moreover, a natural polysaccharide-based bioadhesive from the mucus of *Achatina fulica*, possesses excellent hemostatic property and pro-healing effects for both acute and chronic wounds [1].

In summary, despite some progresses were achieved, it's still challenging in the research and development of polysaccharide-derived innovative drugs. Particularly, structural identification of irregular polysaccharides is lack of efficient method, and the pharmacological mechanism of some polysaccharides with exact pharmaceutical effect have not been thoroughly studied. Therefore, further exploration is needed. We hope to take root in the southwest China, dedicate to innovative drug discovery that are based on polysaccharides from the abundant medicinal resources, which would be benefit to public health.

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Glycosylation With Sialyl Chlorides: S_N1–S_N2 Dichotomy

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The synthesis of sialic acid glycosides with the natural α-configuration is difficult due to the lack of stereocontrolling groups. Most stereoselective sialylation reactions proceed via the S_N2-like route.[1] During our studies of the possibility of using sialyl chlorides in unpromoted glycosylation of phenols under phase-transfer catalysis[2] conditions (in flask and microfluidic conditions), we discovered unusual sensitivity of the yield of aryl sialosides (10–66%) and stereoselectivity of the reaction (α:β = 0.9:1–32 :1) not only to the nature of the electrophile/nucleophile, but also to the concentration of the reagents (5–200 mmol/L), and additionally, in microfluidic conditions, to the flow rate (2–1000 μL/min) and the type of mixer used.[3,4] To rationally explain the entire set of facts, we were forced to assume that this reaction proceeds along the S_N1-like route (via the glycosyl cation), and the stereochemical outcome is determined exclusively by the presentation[4] of sialyl chloride molecules on the surface of the supramers (see the reviews[5,6]), which determines the spatial accessibility different faces of the glycosyl cation formed from sialyl chloride (Fig. 1), and is modulated by the concentration and mode of mixing of the reagents, as well as (in microfluidic conditions) by the flow regime (see a review[7]).

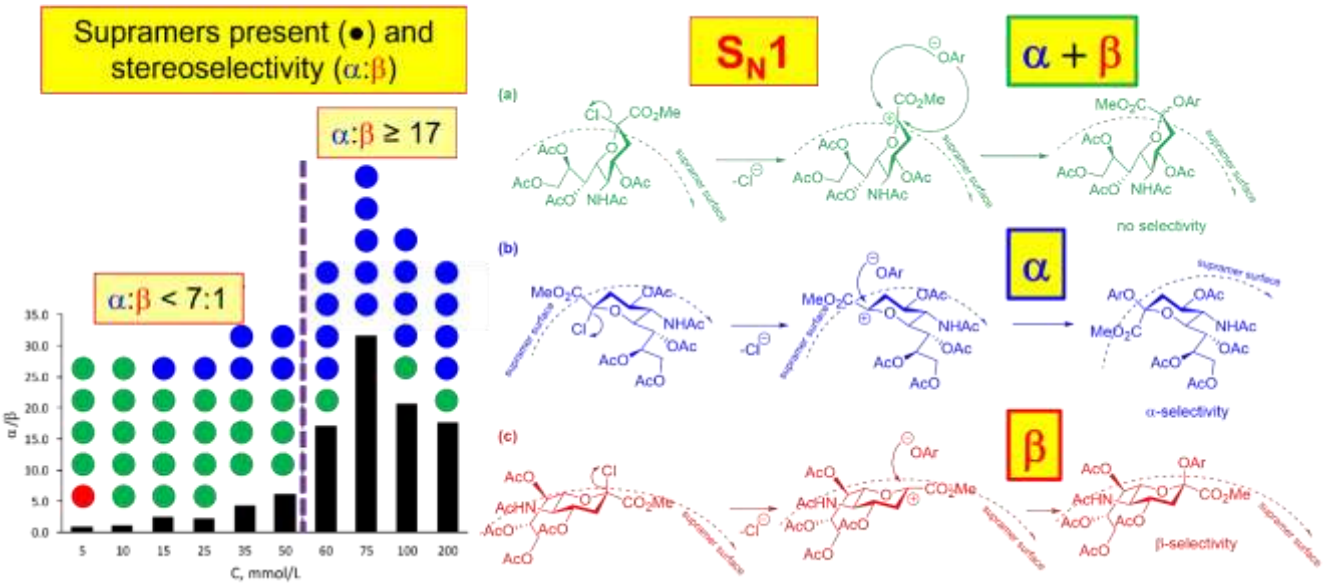

Figure 1. Concentration dependence of stereoselectivity of sialylation of 4-(3-chloropropoxy)phenol with $Ac_4Neu5AcCl$ (left) and the presentation of the glycosyl cation on supramer surface (right): (a) both faces of the glycosyl cation are available for attack by a nucleophile, which leads to a non-selective reaction; (b) and (c) – only one face of the glycosyl cation is available for attack, resulting in the formation of only one anomer (α or β).

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Towards Automated Synthesis Of Monosaccharide Building Blocks And Applications In Oligosaccharide Synthesis

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Developing effective techniques for synthesising carbohydrates with complex structural organisation is crucial to the discipline of glycoscience. Despite significant progress in the synthesis of oligosaccharides, the synthesis of targets featuring complex glycosidic linkages of monosaccharide building blocks remains a challenge. These compounds are present in a wide range of biologically relevant compounds.

While much of the emphasis in the development of automated platforms for carbohydrate synthesis has been on the construction of oligosaccharides, manual syntheses of monosaccharide building blocks can represent up to 90% of the synthetic effort and thus constrain throughput[1]. This is often laborious and time-consuming. Furthermore, excess amounts of glycosyl donor building blocks are frequently used in glycosylations, presenting a pressing need to develop methods for streamlining the acquisition of monosaccharides.

The aim of this work is to improve the purification of monosaccharides, which is often a bottleneck in the preparation of important carbohydrates. By using a purification tag, TIDA,[2] the process of purifying monosaccharides can be made simpler and more efficient. One of the key findings of this research is that the silica binary affinity properties of the TIDA tag can be extended to monosaccharides bearing a variety of protecting groups. This characteristic proved beneficial during the synthesis of the tagged molecules, as it simplified purification and eliminated the need for arduous column chromatography. As a result, this process is potentially amenable to automation. Additionally, the tagged building blocks have been used to synthesise a trisaccharide in high yield, indicating that the TIDA tag is appropriate for the synthesis of oligosaccharides.

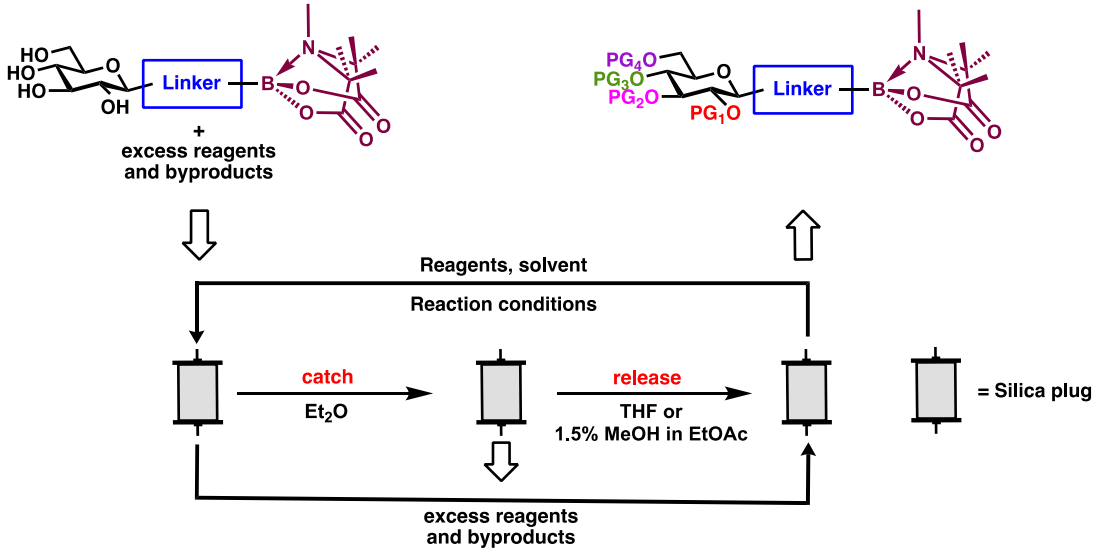

Figure 1: Catch and release purification

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Regioselective C-4 Functionalization Of Unprotected Sugars Through Photoredox

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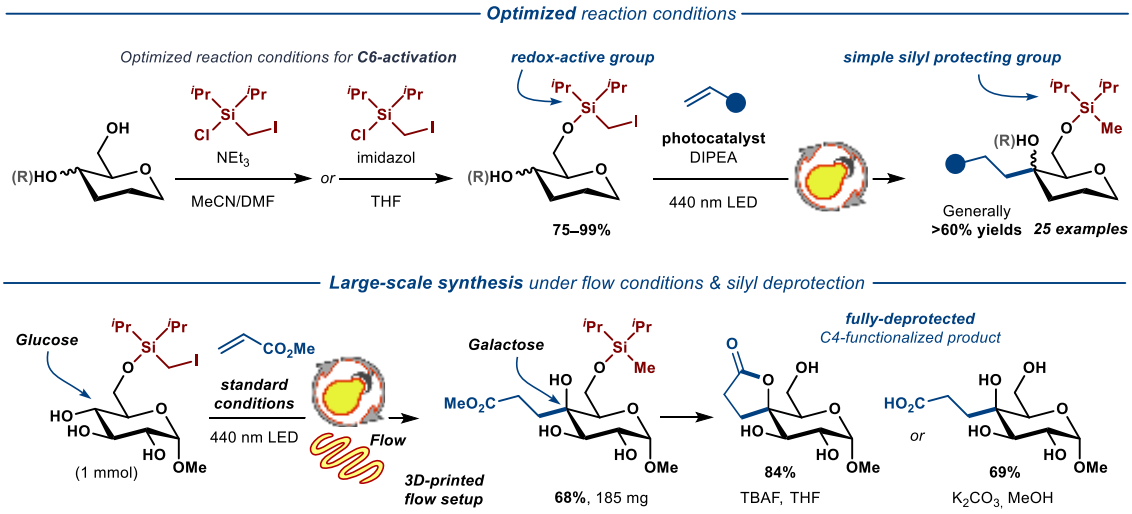
Unconventional C-functionalized carbohydrates are common bacterial metabolites, which constitute an elusive synthetic target and have long been accessible only through elaborate synthetic sequences. In recent years, access to such carbohydrate derivatives was greatly simplified by the emerging photoredox-mediated methods for C–H bond activation in both protected and unprotected carbohydrates.[1] The major drawback of these methods stems from the limited control over site- and stereoselectivity of the C-functionalization reactions. In this work, we address these limitations by employing a “traceless” redox-active tethering group, which allows fully-regioselective C4-functionalization of unprotected pyranosides through a sequence of inter- and intramolecular halogen- and hydrogen-atom transfer reactions, while stereoselectivity of the C–C bond-forming step if firmly defined by the anomeric configuration of the substrate. The selected silyl-based tethering group[2] is easily attached to the primary hydroxy functionality of the substrate in one step and is activated under benign photoredox catalytic conditions. In course of the reaction, this group is transformed into a simple trialkyl silyl protecting group, which can be readily removed under mild conditions to deliver the desired fully-deprotected C4-functionalized carbohydrate target.



The reaction was amenable to upscale under continuous flow conditions, demonstrating the same yield and selectivity as under batch conditions.

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Biosynthesis Of Fungal Cell Wall Polysaccharides: Molecular Basis, Drug Action And Drug Resistance Mechanism

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Invasive fungal infections cause over 3 million deaths annually, posing a serious threat to public health [1]. The current antifungal therapies suffer from daunting challenges such as limited classes of drugs and emerging drug-resistant strains [2]. The synthesis of β-1,3-glucan and chitin, the core components of fungal cell wall, are appealing targets for antifungal drugs such as echinocandin and nikkomycin Z [3]. However, the lack of their mechanistic insights has hampered further drug development.

Here we present multiple evidences to firmly demonstrate for the first time that FKS1 is the specific β-1,3-glucan synthase [4]. Further structure-guided functional characterizations identify the active site for glucan polymerization and a glucan translocation path across the membrane. Drug-resistant mutations are clustered at a region near TM5–6 and TM8 of FKS1, depicting a possible drug binding site. The structure of FKS1 S643P reveals altered lipid arrangements in this region, suggesting a novel drug-resistant mechanism.

As for the chitin biosynthesis, we report the cryo-EM structures of fungal chitin synthase Chs1 in apo- and NikZ (nikkomycin Z)-bound forms [5]. Chs1 forms a unique dimeric functional form. These structures and functional analysis reveal crucial catalytic residues for chitin polymerization and a membrane tunnel for chitin translocation. NikZ specifically binds in the active site and act through a competitive mode.

Our work on FKS1 and Chs1 reveals the desired molecular basis underlying fungal cell wall biosynthesis. It will serve as a framework for developing broad-spectrum drugs against invasive fungal infections.

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Mechanism and inhibition of the *Mtb* cell wall assembly

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Mycobacterium tuberculosis (*Mtb*), the causative agent of global tuberculosis (TB), is one of the leading causes of human death globally. The complex *Mtb* cell wall comprised of glycan and lipids plays a pivotal role in *Mtb* viability and pathogenicity. The enzymes involved in *Mtb* cell wall biosynthesis are well-defined anti-TB drug targets, such as the arabinosyl-transferases EmbA/B/C, the targets of front-line anti-TB drug ethambutol. However, the function of these cell wall enzymes remains poorly understood on the molecular level. We have revealed that ethambutol binds the EmbA-EmbB and $EmbC_2$ complexes (enzymes in AG and LAM biosynthesis, respectively) and inhibits their enzymatic activities by competing with the donor DPA and acceptor substrates as a competitive inhibitor (Science, 2020). Based on this discovery, we continued our research in essential enzymes involved in the cell wall arabinan pathway. The arabinose donor DPA is essential in both AG and LAM pathway. We found a unique membrane-bound phosphoribosyl transferase (PRTase) is key to DPA biosynthesis and therefore is theoretically a promising new drug target. We have determined the cryo-EM structures of this PRTase and revealed the structural basis of the cell envelop precursor synthesis (Nature Microbiology, 2024). In another work, we have determined the cryo-EM structure of the arabinan priming enzyme AftA (the first enzyme that utilizes DPA for AG biosynthesis) and suggested the basis for priming selectivity (PNAS, 2023). Targeting these enzymes will be of great interest in anti-TB drug development. In this talk, I will describe our findings in these critical enzymes involved in *Mtb* cell wall arabinan pathway, primarily from a structural point of view.

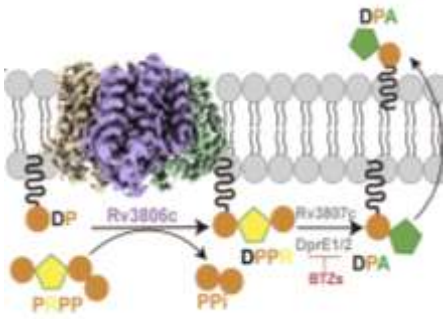

Fig.1 The front-line anti-TB drug ethambutol binds to the arabinosyl-transferases (left) EmbA-EmbB and

(right) EmbC-EmbC.

Fig.2 Molecular mechanism of a mycobacterial membrane-bound phophoribosyl-transferase

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Discovery And Structural Insights Of Lacto-*N*-Biosidases In The Cazy Family GH20

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Carbohydrate-active enzymes (CAZymes) represent powerful tools for glycoengineering and carbohydrate structure elucidation thanks to their high specificity and regioselectivity [1]. Among these, enzymes within the Glycoside Hydrolase family 20 (GH20) play crucial roles in glycan degradation including human milk oligosaccharides and host glycans. Family GH20, predominantly composed of N-acetylhexosaminidases that catalyze the release of terminal N-acetylglycosamine moieties in oligosaccharides, also includes lacto-*N*-biosidases [2]. These enzymes catalyze the release of the disaccharide lacto-*N*-biose (β-D-Gal-(1→3)-D-GlcNAc) [3]. However, despite the vast sequence diversity within this family, only two lacto-*N*-biosidases have been identified among the 133 enzymes characterized thus far.

In this presentation, we will detail our investigation of the family GH20, to uncover its potential and diversity. We first clustered GH20 enzymes according to their sequence similarities [4], and then employed an approach combining sequence and 3D model analysis, to identify additional lacto-*N*-biosidases. By exploring different enzyme cluster groups, we identified nine novel lacto-*N*-biosidases, two of which were successfully crystallized. Our findings not only expand the repertoire of known lacto-*N*-biosidases with diverse catalytic and biochemical properties but also provide deeper insights into the structure-function relationships of those enzymes. This study underscores the importance of exploring sequence space to uncover novel enzymatic activities, ultimately contributing to advancements in the fields of glycoscience and biotechnology.

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Molecular Dissection Of *Fusobacterium Nucleatum* Interaction With Siglec-7

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Fusobacterium nucleatum (*Fn*) is a prominent Gram negative pathobiont in the human oral cavity and rarely found in the lower gastrointestinal tract of healthy individuals.[1, 2] Nevertheless, *Fn* is capable of systemic dissemination and is associated with disease states characterized by a disturbed microbial balance, or dysbiosis, becoming interesting for its role in various systemic diseases including colorectal cancer (CRC). Thus, *Fn*'s ability to evade the immune system and contribute to oncogenic processes makes it a critical subject of study in cancer research.

The interaction between *Fn* and immune cells, particularly through Siglec-7, a sialic acid-binding lectin predominantly expressed on natural killer (NK) cells, is of significant interest. Siglec-7 is known for its inhibitory role in immune regulation, which it performs by recognizing sialylated structures on cell surfaces, leading to the suppression of immune activation.[3, 4] By engaging with Siglec-7, *Fn* may modulate immune responses, promoting its own survival and facilitating disease progression. Studying this interaction is crucial, not only for understanding *Fn*'s role in immune evasion but also for exploring potential therapeutic targets that could disrupt this interaction to enhance immune response against tumors.

Here we tackle the importance of dissecting the molecular basis of *Fn*-Siglec-7 interaction, performed following a multidisciplinary approach (Figure 1) by combining wet lab, NMR spectroscopy (protein and ligand-based methods), computational modeling (Docking, MD), and biophysical assays (Fluorescence, SPR). Our data revealed that the core region of *Fn* ATCC 10953 LPS did not interacted with Siglec-7; indeed, similar association constants were measured for the full O-antigen portion and for the oligosacchairdes containing various number of repeating units. In addition, a single repeating unit, including a reducing sialic acid was not interacting with the protein, suggesting the necessity of a longer glycan chain to induce recognition process. Additionally, our results highlighted the essential role of the internal sialic acid residue, in forming stable complexes through a key salt bridge with Arg124. These findings not only advance our understanding of the intricate mechanisms governing immune cell recognition and bacterial evasion but also underscore the potential of targeting Siglec-7 for therapeutic strategies against colorectal cancer and other related diseases.

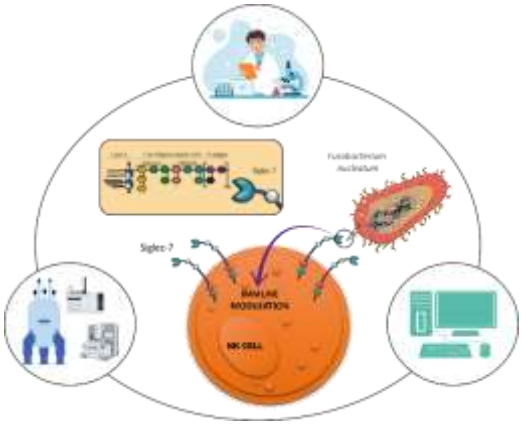

Figure 1: Schematic representation of the recognition of *Fusobacterium nucleatum* ATCC 10953 LPS by Siglec-7 as investigated using NMR, other biophysical techniques, and computational approaches.

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Prediction And Detection Of Glycan Epitoes On Cells

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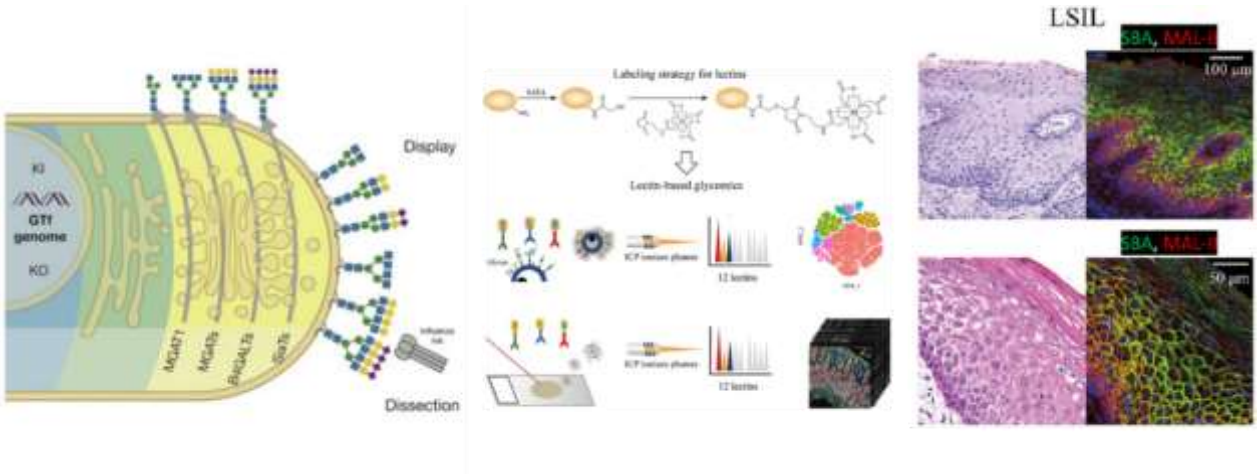
The sequence variation and structural complexity of glycans on cells are closely associated with various pathophysiology processes. Deciphering the "glycan code" on cells presents a challenging task. Cellular heterogeneity poses a significant challenge in decoding the "glycan code" expressed by individual cells, thus becoming the next frontier in glycobiology research. In recent years, single-cell sequencing has elucidated the issue of cellular heterogeneity in life processes, offering potential avenues for deciphering the sequence and structure of glycans expressed by individual cells. Clausen *et al.* research suggests that analyzing the transcriptome levels of glycosyltransferases (200 types) can predict the glycan structures [1]. Similar to this approahc, we have examed the expression levels of more than 400 glycosyltransferases, glycosidases and other carbohydrate related enzymes for a variety of cells in different pathophysiology condistions, predicted the dominant sugar epitopes on those cells. we have discovered during COVID-19 infection that the upregulation of hyaluronan synthase 2 and mucin promotes the secretion of hyaluronan by lung fibroblasts, leading to intractable pulmonary inflammation.

Validating the predicted structure and sequence of glycans expressed by cells based on transcriptomics requires robust experimental verification. Here, we have developed LectoScape, a method for imaging tissue glycoproteins at a resolution of 1 μm using imaging mass cytometry[2]. This method utilizes 12 different lectins that recognize various glycans, enabling multidimensional detection of multiple glycans. Using LectoScape, we successfully delineated unique polysaccharide structures in various cell types, enhancing our understanding of the distribution of glycans in human tissues. Our method has identified specific glycan markers, such as α2,3-sialylated Galβ1, 3GalNAc in O-glycan, and terminal GalNAc, as diagnostic indicators for cervical intraepithelial neoplasia. LectoScape holds tremendous potential in cancer diagnosis by detecting abnormal glycosylation.



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In Vivo Antibody Glycoengineering By Mrna-Encoded Glycosyltransferases And Its Applications

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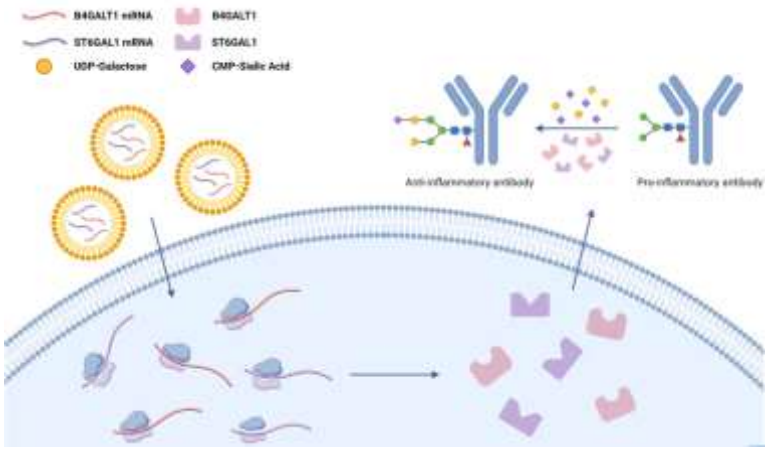
Aberrant antibody glycosylation patterns have been implicated in various diseases including autoimmune diseases and metabolic disorders. Studies have demonstrated changes in the glycosylation profiles of IgG antibodies in rheumatoid arthritis and obese individuals, characterized by increased levels of pro-inflammatory glycans, such as agalactosylated and asialylated glycans, and decreased levels of anti-inflammatory glycans. In vivo glycoengineering of antibodies is an emerging field with promising potential for therapeutic applications. Recent advances in mRNA technology and therapies enable efficient production of glycan-modifying enzymes and precise modulation of glycan structures in vivo. Our study targets two crucial enzymes, galactosyltransferase B4GALT1 and sialyltransferase ST6GAL1, involved in antibody glycan synthesis, aiming to develop mRNA drugs that enhance anti-inflammatory antibody glycosylation. In vitro and in vivo experiments confirmed the activity of the enzymes encoded by mRNAs encapsulated into lipid nanoparticles (LNPs). The mRNA-LNP treatment significantly reduced the incidence and clinical scores of arthritis in collagen-induced arthritis mouse models and mitigated weight loss and intestinal damage in ulcerative colitis models. The elevation of IgG galactosylation and sialylation levels were confirmed by antibody N-glycan analysis. These findings suggest that mRNA-LNP can serve as a novel therapeutic strategy for autoimmune diseases by regulating antibody functions through glycosylation. Given the emerging link between inflammation, immune regulation and obesity, we explored the efficacy of glycosyltransferase mRNA-LNP in high-fat diet mice. The treated mice exhibited a significant decrease in body weight and fat mass compared to the control group. The glucose tolerance and insulin sensitivity were improved in the treatment group, suggesting an enhancement in metabolic function. The research suggests that glycosyltransferase-encoding mRNA-LNP could be a promising therapeutic approach for aberrant antibody glycosylation-related disorders.



Sulfated Glycans In Asfv Virus Infection

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African swine fever is a deadly porcine disease that has become a serious threat to the global pig industry and pork production. Breeding the ASFV disease resistance pig is an effective strategy for ASFV prevention and control. However, the key host resistance genes are absent to hinder the development of disease-resistant breeding, due to the complexity of the pathogenic mechanism and structure of ASFV. The glycan covered with the cell surface of mammalian plays an important role in viral infection as a direct or indirect receptor. Thus, screening the glycoprotein related to ASFV entry is one of the important ways to identify ASFV resistance genes. Here, we discovered that HP5, a heparan sulfate proteoglycan, bound the ASFV by combining the glycan microarray screening, genome-wide CRISPR knockout libraries screening, and RNA-seq analysis. Moreover, cell biology experiments analysis showed the inhibition of HP5 gene expression level in PAM cells, the host cell of ASFV, decreased the ASFV infection, whereas HP5 overexpression in non-susceptible cells 3D4 increased the ASFV infection. In addition, we found HP5 involved in ASFV endocytosis by interacting with the envelope protein of ASFV. Our results indicate HP5 is a candidate ASFV receptor, which provides the key information to implement ASFV disease resistance breeding.
Keywords: heparan sulfate proteoglycan, African swine fever virus, endocytosis

Chemoenzymatic Glycoengineering Approach For The Formation Of Single Glycoform Monoclonal Antibodies

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Monoclonal antibodies (mAbs) are the most widely used protein therapeutics in the treatment of cancers, arthritis, and other human disorders worldwide. Currently the production of mAbs by mammalian (e.g. CHO cells) or alternative cell culture platforms gives rise to heterogenous mAb structures [1]. Contrastingly, homogenous mAbs demonstrate higher specificity and selectivity to their ligands (FcRs) [2]. The value of this is that the patient dose can be decreased for the same clinical outcome and consequently the value of the antibody can be enhanced. *N*-glycans have the ability to fine tune immunological responses such as antibody-dependent cellular cytotoxicity (ADCC) or anti-inflammatory properties. The formation of these highly useful single glycoforms still remains a challenge. Two current glycoengineering methods include a) cell line engineering and b) media supplementation and process parameter alterations [1]. Here we will present a chemoenzymatic workflow (**Figure 1**) for the design and characterisation of a single glycoform mAbs using a previously developed *N*-glycoanalytical technology [3,4]. In brief, the workflow sequentially removes key glycan motifs such as sialic acids, galactose, *N*-acetylglucosamine and fucose residues from the mAb originator molecules followed by addition of selective glycan epitopes utilizing glycosyltransferase enzymes and sugar donors to generate a family of glycoengineered mAbs for improved pharmacokinetics and enhanced effector functions.

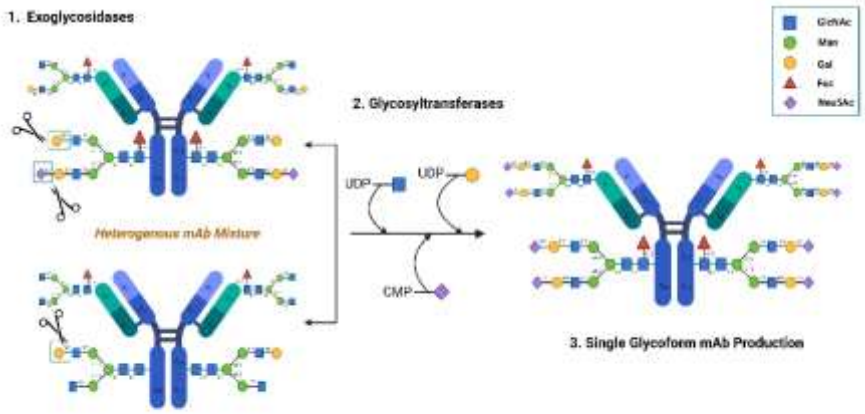

Figure 1. General scheme for chemoenzymatic synthesise of single glycoform mAbs. UDP, uridine diphosphate; CMP; cytidine monophosphate, Glc; glucose, Gal; galactose, GlcNAc; *N*-acetylglucosamine, Fuc; fucose, and Neu5Ac; *N*-acetylneuraminic acid.

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Bisecting GlcNAc Modification Reverses The Chemoresistance Via Attenuating The Function Of P-gp

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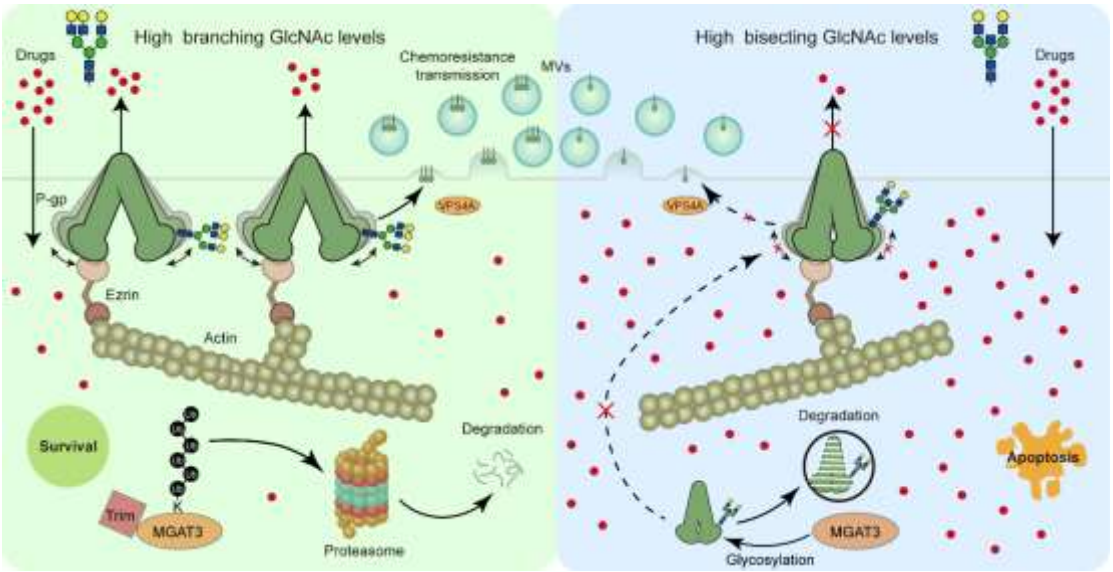
Chemoresistance is a key factor contributing to the failure of anti-breast cancer chemotherapy. Although abnormal levels of glycosylation, e.g. bisecting GlcNAc, are closely correlated with breast cancer progression and metastasis, the function of glycoconjugates in chemoresistance remains poorly understood. Here, we observed significantly reduced levels of bisecting GlcNAc and its glycosyltransferase MGAT3, accompanied by enhanced expression of P-glycoprotein (P-gp) in the chemoresistant breast cancer cell. Elevating bisecting GlcNAc levels effectively reversed chemoresistance by reducing P-gp expression in the chemoresistant cells. Our mechanical study revealed that bisecting GlcNAc modification impaired the association between Ezrin and P-gp, leading to decreased P-gp expression on the cell membrane. Bisecting GlcNAc also suppressed VPS4A-mediated P-gp recruitment into microvesicles, resulting in a decrease in chemoresistance transmission. Structural dynamics analysis further suggested that bisecting GlcNAc at Asn494 could introduce structural constraints that rigidified the conformation and suppressed the activity of P-gp. Together, our findings highlight the crucial role of bisecting GlcNAc in chemoresistance and suggest the possibility of reversing chemoresistance by modulating the specific glycosylation in breast cancer therapy



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Eliminating Tumor'S Protective Umbrella By Antibody-Glycosidase Fusion: An Approach And Perspectives

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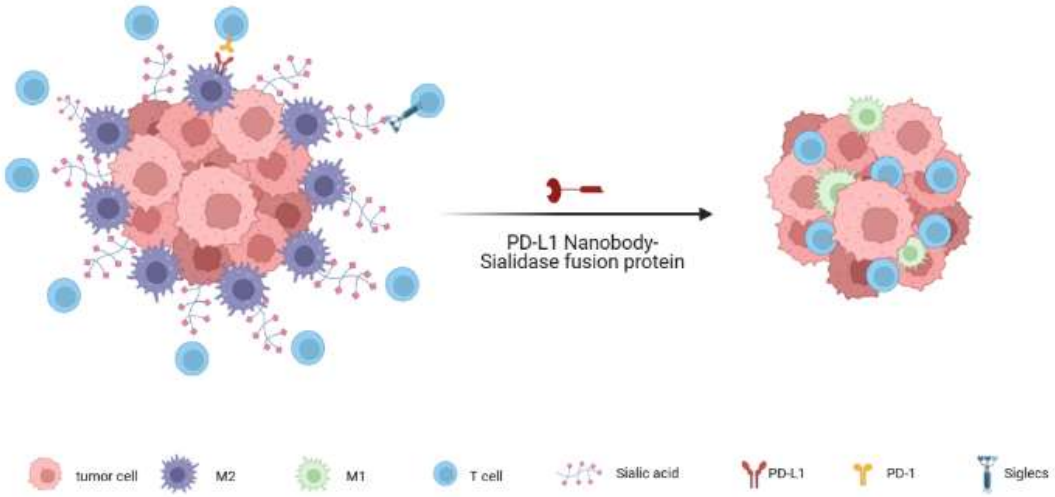
An anti-PDL1 and sialidase fusion was made and subjected to in vitro and in vivo tests. In colorectal cancer models, it demonstrated a significant anti-tumor activity and a unique way of action illustrated by pathological analysis. The results will be shared and a glycosidase-based strategy aiming at tumor's protective umbrella will be discussed. The following illustration provides a schematic diagram of the mechanism of action of the antibody-glycosidase fusion (AGF) protein developed in our study.



Specific GPI-attachment Signal Peptides Upregulate GPI Biosynthesis With ARV1

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Glycosylphosphatidylinositol (GPI) anchoring of proteins is a conserved post-translational modification carried out in the endoplasmic reticulum (ER). GPI are synthesized in the ER and transferred by GPI transamidase to many proteins. In GPI transamidase defective cells, all GPI exist as non-protein-linked GPI (free GPI). Previously, we found that deficiency of ER-associated degradation (ERAD) pathway in GPI transamidase defective cells, free GPI expression is enhanced nearly 10-fold. Based on a genome-wide CRISPR–Cas9 screen, we found that a widely expressed GPI-anchored protein CD55 precursor and ER-resident ARV1 are involved in upregulation of GPI biosynthesis under ERAD-deficient conditions. In cells defective in GPI transamidase, GPI-anchored protein precursors fail to obtain GPI, remaining the uncleaved GPI-attachment signal at the C-termini. Accumulation of the CD55 precursor in ERAD deficient cells, which in turn upregulates GPI biosynthesis, where the GPI-attachment signal peptide is the active element. ARV1 is prerequisite for the GPI upregulation by CD55 precursor. In this presentation, we would like to discuss the regulation of GPI biosynthesis through ARV1 and precursors of specific GPI-anchored proteins.

Keywords:
glycosylphosphatidylinositol; endoplasmic reticulum-associated degradation; CD55, ARV1

Biobased Polysaccharidic Biostimulant For Root Application: Enhancement Of Wheat Plants Tolerance To Drought Stress

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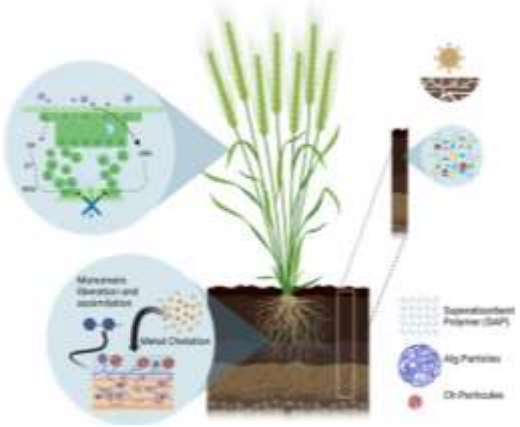
Biostimulants from polysaccharides are known to improve plant resilience against abiotic stresses by modulating cellular and molecular mechanisms, such as for example, abscisic acid hormonal pathway and the Reactive Oxygen/Nitrogen Species (ROS and NOS) regulation[1,2]. Also, the oligomeric form has been used for bio elicitation and biostimulation, for example, oligoalginates and oligochitosans tested on wheat in response to drought might induce the stomatal closure and the decrease damages generated by ROS-NOS species, increasing the antioxidant system protection[3,4].
In order to reduce inputs in the soil, root application appears to be a more suitable solution to establish synergic effects between plants, microorganisms and biostimulants. The application of polysaccharides based biostimulants might increase the plant performance at different levels of the plant physiology under abiotic stresses. Furthermore, the evaluation of biostimulant efficiency is important at different plant developmental stages, from the seed germination to maturation. In this study, wheat plants were treated with an innovative 100% biobased polysaccharide biostimulant in the soil under 2 water regimes (well-watered and drought) in order to study its effect on wheat performance and the water reserves in wheat tissues.



Our results **(Figure 1**) suggest that the root application of the developed biostimulants may help the plants to manage water in tissues, decreasing the gas exchanges by partial stomatal closure. Moreover, the treatments have shown to protect the external tissues of the plants against cellular damages, caused by a severe drought episode, probably linked to the ROS/NOS regulation. Further investigations are under process to determine the effects of the treatment on the soil microorganisms' diversity to decipher the interactions between biodegraded biostimulants and the rhizosphere.

Figure 1: Applying SAP close to the root system, the Chitosan (Ch) and alginate (Alg) particles may enhance the closure of stomata by production of ROS and ABA signaling dependent Pathway. SAP could chelate some micronutrients that cannot be absorbed by the plant under normal conditions. Also, interactions between microbiome-SAP-roots may lead to the biodegradation of the polymer. After its breakdown, subunits could be assimilated by the microbiome and lead to a slow release of chitosan and alginate particles. In combination with SAP the water holding capacity (WHC) of the soil may be improved when applying the biomolecules in the soil and plant tolerance under drought could be increased.

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Bi-component Nanoparticles As Bacterial Antigen Displaying Systems

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Nanoparticle (NP) technology was proven to be a potent tool to improve viral protein immunogenicity, thanks to antigen multi-display and an optimal dimension for antigen uptake. Recently, bi-component NPs have been computationally designed aiming to co-display different antigens on the same scaffold for boosting and promoting cross-protective antibody production.[1] I53 bi-component NP has an icosahedral geometric structure composed by 20 trimeric and 12 pentameric subunits. Protein antigens of interest are genetically fused to I53 trimers and NPs are easily produced *in-vitro* by mixing with I53 pentamers. I53-50 bi-component NPs have been recently used for SARS-COV-2 vaccine (licensed in South Korea and UK), while a tetravalent Pan-Sarbecovirus vaccine is under development.[2] Here, for the first time, I53-50 NP has been tested as a scaffold for bacterial saccharide antigens using model bacterial antigens.[3] Different approaches were tested with the aim to understand the optimal way to efficiently produce resulting conjugates.[4, 5] Animal studies are ongoing to evaluate the immune response elicited by these new constructs in comparison with traditional glycoconjugates (**Figure 1**).

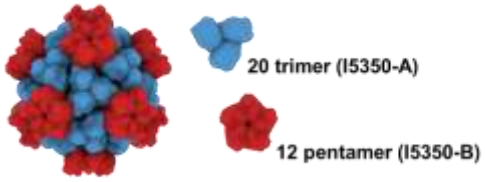

Figure 1: I53-50 bi-component NP structure.

In conclusion, the innovative use of I53-50 bi-component nanoparticles applied to bacterial antigens shows the value of integrating computational protein design and bacterial expertise. This work will provide valuable insights into the potential of this technology as a versatile platform for the development of more effective vaccines for global health.

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Characterization Of Testicular O-Glycoproteome In Mice Using Lectin-Based Mass Spectrometry Analysis

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Among human organs, the testis is relatively unique and mysterious, possessing the largest number of tissue-specific proteins of unknown function [1]. While O-glycosylation is a post-translational modification that plays important roles in regulating cell differentiation and tissue development, our previous findings showed that testis is armed with highly O-glycan [2], indicating O-glycan is critical for sperm formation and male fertility. However, the spatial distribution and diversity of the O-glycoproteome in testis and temporal dynamics during spermatogenesis remain unmapped. Closing this knowledge gap is important because it can provide targets and resource for studying the the mechanism of O-glycosylation in sterility, and more importantly, it can provide new insights for investigating the function of testicular proteins whose functions are currently unknown.

In this study, we performed the first qualitative and quantitative analysis of site-specific O-glycosylation in mouse testis using a lectin affinity chromatography coupled with LC-MS/MS approach. We established the hitherto largest O-glycoproteome map with a total of 349 O-glycoproteins and 799 unambiguous O-glycosite from testes of 24 days and 12 weeks mice, of which 85 proteins are found to be O-glycosylated for the first time. Moreover, we comprehensively investigated the distribution properties of O-glycosylation in testis and found dynamic changes in O-glycosylation with upregulation of Tn-glycopeptides and downregulation of T-glycopeptides in 12-week-old testes, which may contribute to elongated spermatid maturation and fertilization. Notably, we preliminarily explored the function of O-glycan catalyzed by ppGalNAc-T3 on acrosomal proteins, which provide potential targets to explain male sterility induced by ppGalNAc-T3 knockout. Collectively, these data illustrate the global properties of O-glycosylation in testicular germ cells and lay the foundation for functional study of site-specific O-glycosylation in male infertility.

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Advancing Nucleic Acid Therapeutics: Trehalose-Sucrose Macrocycles As Non-Viral Vectors

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The remarkable success of COVID-19 mRNA vaccines has highlighted lipid nanoparticles (LNPs) as exemplary nonviral vectors for nucleic acid therapeutics. However, LNPs encounter stability and immunogenicity issues, stemming from their heterogeneous composition comprising ionizable and neutral lipids, stabilizers, polyethylene glycol, cholesterol, etc. Simplifying vector design to one-component formulations based on molecularly defined carriers presents an attractive alternative. Most efforts have focused on macrocyclic molecular nanoparticle platforms, notably cyclomaltooligosaccharides (cyclodextrins; CDs; Figure 1A) and α,α'-trehalose-based macrocycles (cyclotrehalans; CTs; Figure 1B), which have demonstrated significant potential. Introducing a novel prototype within this category, here we present cyclosucrotrehalans (CSTs), distinguished from CTs by the inclusion of a sucrose module in the backbone. CSTs offer a modular synthesis approach, facilitating the selective incorporation of cationic and lipid domains with precise spatial arrangements (Figure 1C), enhancing nucleic acid nanocomplexation efficiency. Moreover, the sucrose segment is expected to enhanced flexibility and increased pH-sensitivity, facilitating endosomal escape. This communication outlines the synthesis, supramolecular properties, formulation characteristics, and transfection capabilities of CSTs, illustrating their potential as versatile nucleic acid carriers.

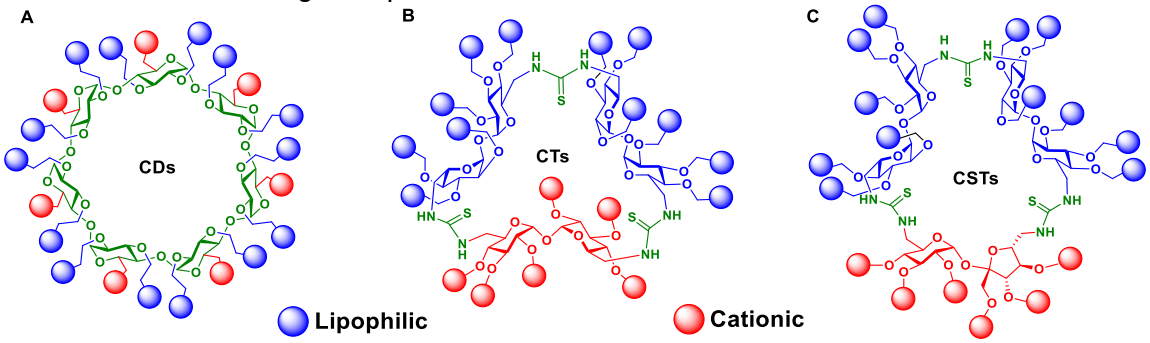

Figure 1. Schematic representation of the previously reported polycationic amphiphilic CDs and CTs, and of the new CSTs synthesized in this work.

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An Integrated One-Tube *N*-Glycan Preparation Method For Mass Spectrometry-Based Glycome Profiling

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Sialic acids attached to nonreducing ends of *N*-glycans play diverse biological roles. In particular, different linkage isomers (α-2,3- or α-2,6-linkage) of sialylated *N*-glycans are involved in specific interactions and physiological events. Accurately characterizing sialylated glycans and distinguishing linkage isomers pose an inherent challenge due to the instability of sialic acids and their structural similarity. Moreover, highly sensitive and reproducible profiling of the glycome from a limited amount of biological sample is often hindered by sample loss during preparation and inferior ionization efficiency of glycans in mass spectrometry (MS). Esterification and amidation are commonly used derivatization strategies that provide enhanced structural stability, reduced identification ambiguities, and the improved MS signal of both sialylated and non-sialylated glycans in positive ion mode.

Here, we have established a rapid and robust sample preparation method for MS-based sialylated *N*-glycomics analysis, integrating sample lysis, protein reductive alkylation, *N*-glycan release, non-linkage-specific or linkage-specific sialic acid derivatization, reducing end labeling, glycan enrichment and desalting in a single microcentrifuge tube. The output glycans are ready for matrix-assisted laser desorption/ionization (MALDI)-MS analysis, showing improved signal. The entire one-tube process can be completed within 10 hours without sample transferring, enabling high throughput and highly sensitive *N*-glycan analysis. In our method, linkage-specific salic acid derivatization consists of two sequential steps, resulting in the ethyl esterification of α-2,6-linked sialic acids and subsequently amidation with ammonia of α-2,3-linked sialic acids, and all the reaction conditions are mild, allowing unstable modifications on sialic acids such as *O*-acetylations to be preserved. Additional MS signal boosting through all glycans was obtained by reductive amination. The one-tube method not only facilitates the MALDI-MS glycan profiling of glycoprotein at the nanogram level, hundreds of cells, and other complex biological samples in minute amounts but also provides linkage information of sialic acid isomers with high accuracy. Overall, the integrated one-tube method offers a simplified and robust strategy for comprehensive *N*-glycome profiling of precious biological samples.

Mass Spectrometry-Based Techniques To Elucidate The Structure Of Glycans

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Cells encode information in the sequence of biopolymers, such as nucleic acids, proteins, and glycans. Although glycans are essential to all living organisms, surprisingly little is known about their biological role. The reason glycobiology lags behind its counterparts dealing with nucleic acids and proteins lies in the complexity of carbohydrate structures, which renders their analysis extremely challenging. Building blocks that may differ only in the configuration of a single stereocenter, combined with the vast possibilities to connect monosaccharide units, lead to an immense variety of isomers, which poses a formidable challenge to conventional mass spectrometry.

In recent years, a combination of innovative ion activation methods, commercialization of ion mobility–mass spectrometry, progress in gas-phase ion spectroscopy, and advances in computational chemistry have led to a revolution in mass spectrometry-based glycan analysis.[1] Here we showcase for a couple of examples how complex oligosaccharide structures can be unambiguously identified using ion mobility mass spectrometry[2] and cryogenic ion spectroscopy.[3] Particular focus will be put on particularly challenging glycoconjugates such as mucin-type O-glycans and glycosaminoglycans. Further, we discuss the potential of mass spectrometry-based techniques combined with theory to elucidate the structure of reactive intermediates from glycosylation reactions such as glycosyl cations.[4-6]

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Detecting Glycan Changes By A Combined Approach Using Lectin-Based Microarrays And Mass Spectrometry

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Protein glycosylation and its changes are among the key aspects of cellular communication affecting countless biological functions. Changes in glycan structures of glycoproteins and other glycoconjugates are in relationship with, for example, pathogen-host interactions, immune system, stem cells, fertilization, etc. as well as with various diseases such as cancer, inflammatory diseases, neurological diseases, psychiatric diseases and many others. Microarrays have become an elegant choice to discern and evaluate aberrant glycosylation in a high-throughput manner. Precise recognition of target glycan ligands among the plethora of other different ones is central for any glycan-targeting reagent being tested by microarray analyses. Ever since the introduction of microarray as an elemental tool for high throughput glycotyping numerous distinct array platforms possessing different customizations and assemblies for the applications in glycomic have been developed. We have designed and prepared lectin-based glycoprotein microarray biochips in the reverse-phase format, the technique defined as a high-throughput approach enabling simultaneous and rapid analysis of a large cohort of samples by a set of lectins without the need for the separation of glycans from proteins. The microarray biochip is prepared by printing tens to hundreds of samples on the microarray substrate which is then allowed to interact with a panel of biotinylated lectins, and the detection of interaction is carried out using a fluorescent tag conjugated with streptavidin. This method provides effective glycotyping of samples and screening of glycan biomarkers with attractive applications in biomedicine, biotechnology and biology. Although interaction with lectins does not allow accurate identification of glycan structures, this method provides effective glycotyping of samples and screening of glycan biomarkers with attractive applications in biomedicine, biotechnology and biology. To identify glycan structures, we combine microarray analysis with analysis by MS methods enabling us to validate glycosylation changes detected by microarray. We have applied our combined analytical approach in various cases[1-3] such as cancer, kidney diseases, COVID-19, and therapeutic proteins, and herein we present its application for the screening of aberrant glycosylation in congenital disorders of glycosylation (CDG) and for studying glycan changes in attention-deficit hyperactivity disorder (ADHD). Acknowledgement: This work was supported by the grants VEGA 2/0120/22, APVV-20-0243, APVV-21-0108 and COST CA20117. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00049.

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Large-Scale Pattern Analysis Of N- And O-Glycoproteomics Using Ion-Mobility Assisted Mass Spectrometry

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Glycosylation plays crucial roles in biological processes such as cell signaling, immune response, protein folding, cell adhesion, and recognition[1]. Aberrant glycosylation can significantly affect immune activation, antigen recognition, antigen-antibody binding affinity and malignant cellular immune escape, thus leading to immune disorders and promoting the pathogenesis of diseases[2,3]. Here, we employed trapped ion mobility tandem time-of-flight mass spectrometry (timsTOF MS) to investigate the patterns and associations of protein N- and O-glycosylation. To begin with, we assessed the feasibility of tandem-TIMS platform with optimal PASEF mode utilizing three purified glycoproteins including fetuin, recombinant erythropoietin (rEPO), and Apolipoprotein E (ApoE). Subsequently, we performed a large-scale characterization of N- and O-glycosylation for more complex biological samples, including human lung cancer cells, serum, and exosomes. Next, we delved into serum antibody glycosylation and systematically compared the aberrant glycosylation patterns of serum antibodies in eight immune disorders, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, amyloid light-chain amyloidosis, mild IgA nephropathy, severe IgA nephropathy, acute myeloid leukemia, and acute lymphoblastic leukemia.

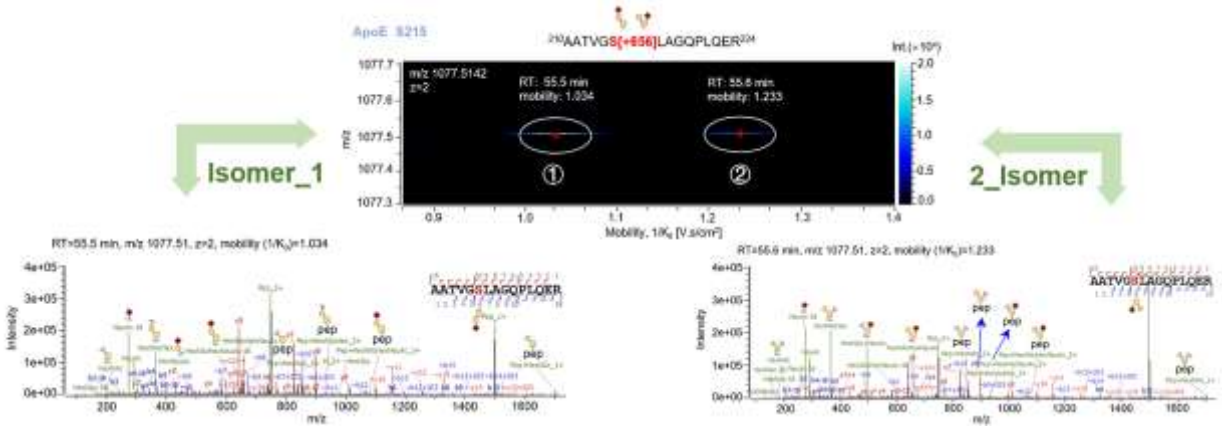

Fig.1 TIMS-TOF MS enhances physical separation of isomeric glycopeptides

We demonstrated that this platform could generate high-quality mass spectra and produce abundant four-dimensional information for intact glycopeptides identification. The results displayed the presence of numerous glycoproteins exhibiting significant biological functions in cells, cell exosomes, serum, and serum exosomes. We observed unique glycoprotein profiles in each of these four types of samples, showing specific patterns and variations in glycosylation in different biological regions. We validated that N-glycans conservatively occupied the Fc domain which was consistent with pioneer studies, while O-glycans were more flexible and ubiquitous, and distributed across the entire functional regions, suggesting that N- and O-glycosylation have distinct roles in antibody function. Our informative patterns of N- and O-glycosylation facilitated a better understanding of the pathogenesis of immune-mediated disorders.

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Serum Antibody Fc Glycosylation Profiling Of Igg, Iga1 and Igm By Light Chain Affinity Capturing Coupled With Nano-Lc-Ms Analysis

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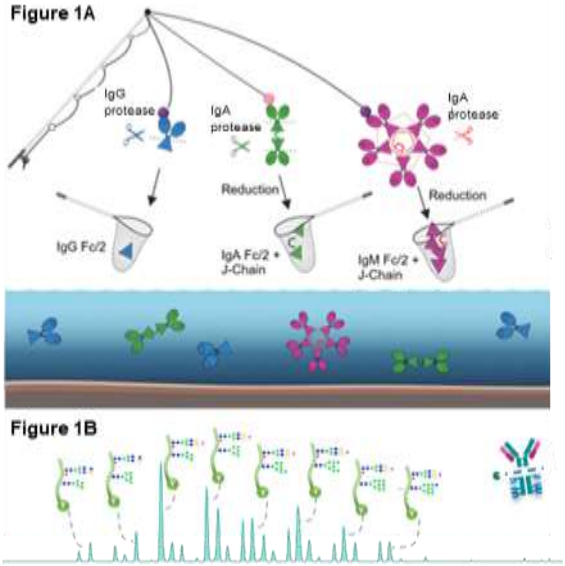

The constant domain (Fc) of antibodies is responsible for binding to effector cells and activate component mediators therefore triggering downstream immune responses. Differences in the amino acid sequence of the Fc define the antibody isotype (with the most abundant being IgG, IgA and IgM) all sharing regions of the light chain with two potential variations (kappa and lambda light chain). Furthermore, antibodies exhibit post-translational modifications, with glycosylation as one of the most important, which can modulate their biological functions. Characterization of antibody Fc structure contribute to understand (altered) immune responses. Up to now, endogenous antibodies are characterized using bottom-up approaches resulting in a loss of combinational information of multiple PTMs such as multiple N-glycans of IgA and IgM.

With new developments of mass spectrometry, intact or middle-up protein analysis are increasingly applied for glycoform characterization study, since it provides comprehensive structural information compared to peptide analysis. In middle-up analysis, antibodies are cleaved via specific proteases into two subunits, constant domain (Fc) and variable domain (Fab). So far, only IgG has been studied using middle-up approaches for Fc characterization. For endogenous IgGs, normally the antibodies are captured by FcXL beads which bind to the Fc of IgG, followed by a hinge-region cleavage by IdeS and elution of the Fc subunits under acidic conditions. For IgA or IgM there is no analytical platform for middle-up Fc profiling yet. In this project, we have developed a middle-up analysis platform for sequential Fc profiling of IgG, IgA and IgM.

To allow capturing of all antibody isotypes a light chain affinity capturing, using a mixture of kappa and lambda light chain beads, was established (Figure 1A). After capturing, the Fc/2 subunits of IgG, IgM and IgA1 were sequentially released by specific IgG, IgM and IgA proteases providing directly the Fc portions and eliminating the elution step required in Fc affinity strategies. The Fc/2 subunits of each isotype were individually analyzed by nanoRPLC-MS. The IgG Fc/2 profiles showed no bias between the developed light chain capturing method and the classical FcXL capturing. For IgM Fc/2 subunits, very complex glycosylation profiles containing 2 and 3 N-glycosylation sites were observed (Figure 1B). Next to glycosylation, other modifications such a c-terminal tyrosine truncation were detected for IgA and IgM. The mass spectra of IgA and IgM Fc/2 glycoforms was annotated by integrating the intact subunit and the site-specific bottom-up information. In addition to Fc/2 subunits, the corresponding joining chains from IgA and IgM were detected and annotated with different glycoforms. The proposed method was applied to the characterization of the Fc/2 subunits of three independent donors resulting in different profiles, therefore illustrating the potential of the approach to study antibody Fc/2 changes.

Human Kidney Glycome Array To Identify Damage-Associated Glycan Patterns Recognized By Collectin 11 In Acute Kidney Injury

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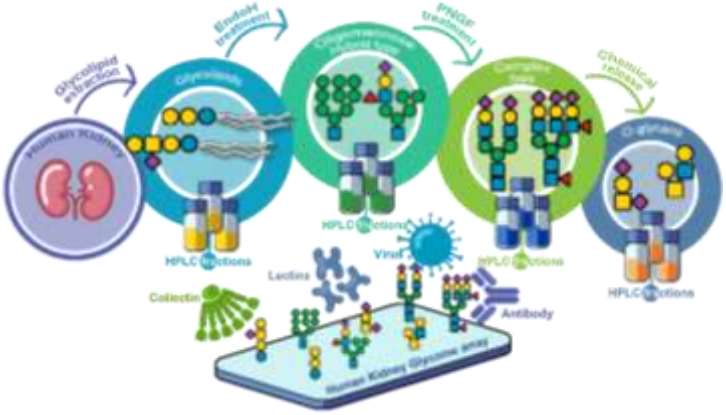
Pathogen- or damage-associated molecular patterns interacting with human collectin 11 (CL-11) and leading to complement activation are known to cause tissue necrosis during acute kidney injury (AKI) and organ transplantation reactions. Previous studies have shown that CL-11 recognizes both mannose- and fucose-containing glycans[1,2], but the specific damage-associated glycan ligands in ischaemic human kidney tissue remain unknown. The identification of such glycan ligands for CL-11 is crucial for better understanding of the roles of CL-11 in AKI pathobiology and developing new therapeutic approaches, particularly the design of antagonistic drugs.

In this communication, we describe an integrated tissue glycome array strategy that combines mass spectrometry (MS)-based glycomic analysis with immunohistochemical (IHC) staining to elucidate the tissue-specific CL-11 glycan ligands. As outlined in the scheme below, a workflow for sequential isolation of glycolipids, N- and O-linked glycans was applied to construct a glycome array from human kidney cortex tissue. The technological highlights include i) harvesting oligomannose and hybrid-type N-glycans before releasing complex-type N-glycans to resolve the various mannose- and fucose-containing N-glycan ligands for CL-11, and ii) using a novel trifunctional linker to facilitate the glycome array preparation and quality control[3].



With preparative-scale glycan release, combined with glycan array, MS analyses, and IHC, we were able to conduct in-depth studies of the structure, abundance, location and binding properties of various types of glycans present in human kidney, achieving a much greater level of detail compared to earlier tissue glycomics studies[4,5]. Despite the high abundance of fucosylated glycans in the tissue glycome, predominantly Lewis^x-related sequences, our data revealed that mannose-terminating N-glycans are the preferred CL-11 ligands, largely localized in the cytoplasm of the proximal tubules but, in addition, some basolateral staining . This human kidney tissue glycome array is also being applied for recognition studies of other binding systems related to human kidney, including kidney-associated pathogens. The integrated tissue glycome array strategy can be applied to other tissues, to address the knowledge gap regarding natural glycan ligands for diverse glycan recognition systems.

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De Novo Glycan Sequencing by An Electronic Excitation Dissociation Based Ms^2-Guided Ms^3 Approach

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Abstract

Comprehensive de novo glycan sequencing remains an elusive goal due to the structural diversity and complexity of glycans. Present strategies employing collision-induced dissociation (CID) and higher energy collisional dissociation (HCD)-based multi-stage tandem mass spectrometry (MS^n) or MS/MS combined with sequential exoglycosidase digestions are inherently low-throughput and difficult to automate. Compared to CID and HCD, electron transfer dissociation (ETD) and electron capture dissociation (ECD) each generate more cross-ring cleavages informative about linkage positions, but electronic excitation dissociation (EED) exceeds the information content of all other methods and is also applicable to analysis of singly charged precursors. Although EED can provide extensive glycan structural information in a single stage of MS/MS, its performance has largely been limited to FTICR MS, and thus it has not been widely adopted by the glycoscience research community. Here, the effective performance of EED MS/MS was demonstrated on a hybrid Orbitrap-Omnitrap QE-HF instrument, with high sensitivity, fragmentation efficiency, and analysis speed. In addition, a novel EED MS^2-guided MS^3 approach was developed for detailed glycan structural analysis. Automated topology reconstruction from MS^2 and MS^3 spectra could be achieved with a modified GlycoDeNovo software. We showed that the topology and linkage configurations of the $Man_9GlcNAc_2$ glycan can be accurately determined from first principles based on one EED MS^2 and two CID-EED MS^3 analyses, without reliance on biological knowledge, a structure database or a spectral library. The presented approach holds great promise for autonomous, comprehensive and de novo glycan sequencing.

Structure-Guided Discovery Of Protein And Glycan Components In Native Mastigonemes

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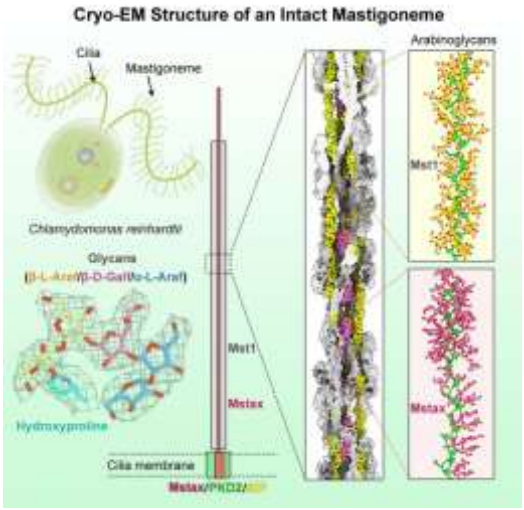
Sugar is essential for life: Carbohydrate molecules represented by glucose are one of the main energy sources for cell metabolism. The cell wall in plants is composed of cellulose. Glycosylation is also one of the major forms of Posttranslational modifications (PTM), which can significantly expand the functional diversity of proteins. However, the intrinsic stereochemical complexity of glycan molecules has hindered the systematic study of them, and the lack of information on the sugar-containing structure has severely limited human understanding of the functions of this important group of biomolecules. In this study, we used a combination of biophysics, cell biology, and bioinformatics methods to elucidate the molecular mechanism of glycans in building biomacromolecules. Plants and algae are known to possess a unique form of Hyp O-glycosylation in which the glycan module consists mainly of arabinose with a small amount of galactose. This Hyp O-glycosylation dependent form is fundamental for plants and algae to exercise normal life activities. Previously, no structural information about glycan-mediated assembly of bio-architectures was available. In this study, a density of more than 1000 sugar molecules was clearly observed, which is the largest complex structure containing sugar molecules. By analyzing the interaction between polysaccharides and proteins, this study revealed the key role of arabinoglycans in the assembly of biological structures, providing important clues to understand the role of structural glycans in life processes, and reflecting the transformation of modern structural biology from a tool for structural confirmation to a tool for de nove discovery.



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Highly Acetylated Mannoglucan From Cremastra Appendiculata (Orchidaceae) Ameliorates Non-Alcoholic Fatty Liver Disease By Modulating Gut Microbiota And Regulating Fatty Acid Metabolic Pathways

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Globally, the high-incidence and concurrent status of nonalcoholic fatty liver disease (NAFLD) heavily threatens human health, and no curative therapeutic agents appear on the market[1]. Functional polysaccharides promotes beneficial gut microbiota expansion by providing energy and accommodating the gut microenvironment [2]. *Cremastra appendiculata* (Orchidaceae) is a popular Chinese medicine for heat clearing and detoxifying, reducing phlegm and resolving masses especially the sticky sputum. It is commonly prescribed as the major drug for lipid elimination in folk for a long time. After extraction and purification, we have obtained a homogenous polysaccharide (named as CAP) with Mw 557.5 kDa from *C. appendiculata* (Orchidaceae). As it was well characterized, CAP was a 1,4-β-mannoglucan with 20.8% acetylation substitution, which was a hexasaccharide repeating unit composed of 1,4-β-mannose and 1,4-β-glucose. We further investigated the role of the high acetylated mannoglucan CAP on anti-NAFLD. Our results indicated that CAP could significantly reverse the body weight increase induced by HFD, decrease the hepatic TG level and alleviate the liver pathological state of HFD-induced mice. According to the 16S rRNA microbiome results, HFD-induced mice showed gut microbiota disorder, the diversity decrease, the composition changes in the phylum and genus levels, and the abundance of harmful bacteria increase. However, all the indicators of gut microbiota were reversed after CAP treatment. Specially, the abundance of beneficial Lachnospiraceae were greatly up-regulated and it was negatively correlated to the liver TG level. Combined with the RNA-Seq anq qPCR results, it was clearly indicated that CAP significantly affect the gene expression levels related to the fatty acid metabolic process. The gene expression levels of lipolysis gene ATGL were significantly increased ($P<0.001$), and the gene expression levels of fatty acid synthesis related genes including FASN and SCD1 were significantly inhibited. The corresponding ATGL protein level was significantly elevated and the TG level decreased, the DG level increased. Importantly the fatty acid esters of hydroxy fatty acids (FAHFAs) were profoundly increased according to the lipidomics analysis.These results indicated that CAP might ameliorate NAFLD through a) reshaping the gut microbiota and sequentially increasing the beneficial bacteria especially *Lachnospiraceae* and *Bifidobacterium*; b) promoting ATGL lipolysis expression to reduce hepatic TG accumulation and produce FAHFAs benifical lipids [3]. Our in-depth study elucidates the anti-NAFLD mechanism of mannoglucan from *C. appendiculata* (Orchidaceae) and provides solid data information for the prevention and treatment of NAFLD.

Keywords: mannoglucan; gut microbiota, nonalcoholic fatty liver disease, Cremastra appendiculata (Orchidaceae) polysaccharide

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Development Of A Novel High-Precision Glycosaminoglycan Oligosaccharide Microarray For Probing Microbe-Host Interactions

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Glycosaminoglycans (GAGs) play important roles in a plethora of biological activities through interactions of a diverse range of proteins.[1] Due to their structural heterogeneities, defining the specificity of GAG-protein interactions remains a great challenge. The value of GAG microarrays, a much sought-after high-throughput tool for probing GAG-mediated interactions, has been highlighted in recent studies with the neoglycolipid-base GAG oligosaccharide microarrays[2, 3] and microarrays of synthetic heparan sulphate oligosaccharides.[4, 5] However, a limitation of all the GAG array systems is the lack of accurate methods to quantify GAG probes of different sizes and charges retained on the microarrays after deposition; this is important for obtaining quantitative data.

In this communication, we describe the development of a new generation of GAG oligosaccharide probes based on a tri-functional Fmoc-protected-Amino Azido-Aminoxyl linker (FAAO) These FAAO-GAG probes, designed based on the recently described FAA-glycan probes[6], achieve higher yields for derivatizing different classes of GAG oligosaccharides with differing chain length, benefiting from the high efficiency of glycan conjugation via oxime-ligation.[7] The Fmoc group in the linker facilitates monitoring glycan probe conjugation and purification. Upon Fmoc deprotection the amino group allows arraying of derivatized glycans onto functionalized glass slides using amide-coupling reaction. The azido functionality allows 'on-array' visualization and semi-quantitative measurement by microarray scanner of the FAAO-GAG probes attached to the array surface. Moreover, the azido group adds versatility, allowing the FAAO-GAG probes to be converted into other functional probes, e.g. lipid-linked, biotinylated, fluorescent probes for diverse glycan-recognition studies.

A new GAG oligosaccharide array has been constructed with over 50 FAAO-GAG oligosaccharide probes, including those derived from oligosaccharide fractions of hyaluronic acid, chondroitin sulphates A, C and D, dermatan sulphate, and heparins obtained from different biological sources which have distinct sulphation features together with their variously desulphated variants. Promising results have been obtained in microarray analyses of a wide range of viruses, including human papillomavirus, human cytomegalovirus, different serotypes of human adenoviruses and a number of bacterial adhesins. The application of this array for analysing whole bacterial cells is currently underway. Thus, this new GAG array platform holds significant promise for investigating GAG interactions with various microbes, not only to enrich our understanding of the GAG Interactome at the host-microbe interface, but also to leading to novel therapeutic opportunities.

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Novel Fluorescent Probes For *N*-Glycan Labelling And Therapeutic Glycoprotein Detection

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Over 50% of human proteins are glycosylated and over 80% of biopharmaceuticals contain *N*-glycosylation [1]. Sugars or glycans are often altered when our immune system is dysregulated and glycosylation can also influence the safety and efficacy of therapeutics. As such tools to enhance detection of *N*-glycans, particularly for low-abundant proteins are desirable to the Glycoscience and the Biopharmaceutical community alike. Our innovative design will be based on fully automated methods developed in the O'Flaherty lab utilising current gold standards, 2-aminobenzamide (2-AB) and aminoquinoline carbamate (AQC) [2]. These methods will also allow direct comparison of the novel tags to the present state of the art. Novel tags synthesised as part of this project build on expertise within the Elmes Group at MU [3]. Coupling reactions are based on squaric acid intermediates, such as diethyl-, dimethyl, ditertbutyl and dichloro squarate, where these derivatives have found increased utility as a useful method of bioconjugation because of facile reaction protocols, high reaction yields in aqueous solution, high functional-group tolerance and usually very high conversions [3,4]. These protocols have been combined with various fluorophore derivatives and optimised to for efficient *N*-glycan labelling during my research. The novel tags have been labelled to *N*-glycans released from human IgG using established approaches[5,6]. The structure of human IgG presented can have up to 6 *N*-glycosylation sites (2 in Fc and 4 in Fab region) which serves as a suitable starting point as there is much research on its *N*-glycosylation profile and is the basis for a large number of biopharmaceutical drugs eg. monoclonal antibodies. Experimentally, the protein is denatured, reduced, alkylated and the *N*-glycans are enzymatically released. Following this step, labelling can occur via traditional chemistries, ie reductive amination using 2AB [6], nucleophilic substitution via AQC [2] or through the novel squaramide chemistries which utilises nucleophilic substitution presented in this work.

Figure 1: Proof of Principle Fluorophore design

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Structural Elucidation Of An Active Polysaccharide From *Radix Puerariae Lobatae* And Its Protection Against Acute Alcoholic Liver Disease

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Abstract: *Radix Pueraria lobata* can be used as medicine and food, whose polysaccharide is one of the main bioactive ingredients. To explore the effect and mechanism of *Pueraria lobata* polysaccharide, a homogeneous and novel water-soluble polysaccharide (PLP1) was successfully isolated and purified from *P. lobata* by column chromatography in the current study. Structure analysis revealed that PLP1 (M_w = 10.43 kDa) was constituted of the residues including (1→4)-α-D-glucose and (1→4, 6)-α-D-glucose, which were linked together at a ratio of 5:1 and represented the main glycosidic units. *In vitro* experiments indicated that PLP1 exhibited a better free radical-scavenging ability than amylose and amylopectin, meanwhile *in vivo* experiments indicated that PLP1 effectively protected against liver injury in mice with acute ALD through significantly inhibiting oxidative stress to regulate lipid metabolism, increasing short-chain fatty acid production, and maintaining intestinal homeostasis by regulating intestinal flora. Taken together, our results illustrate that PLP1 can regulate intestinal microecology as a feasible therapeutic agent for protecting against ALD on the ground of the gut-liver axis, thus laying a theoretical foundation for the rational exploitation and utilization of *P. lobata* resources in the clinic.

Keywords: *Pueraria lobata polysaccharide*, Structural characterization, Acute ALD, Oxidative stress, Intestinal flora

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Chemoenzymatic Synthesis Of Sialosides Containing 4-*N*-Derivatives Of Sialic Acid As Probes For Influenza A Virus Neuraminidases

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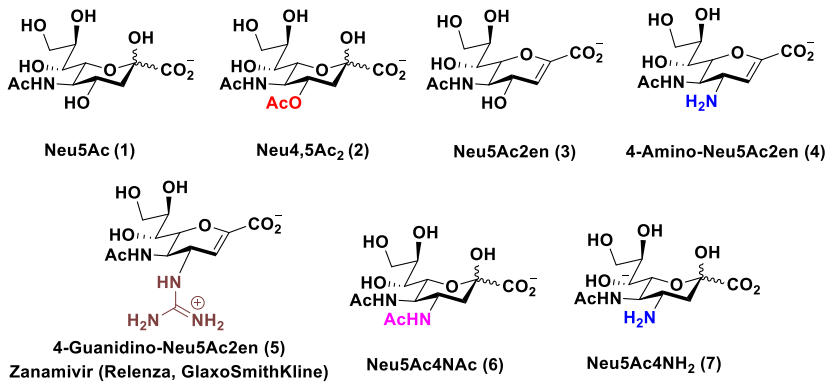
Sialic acids are acidic monosaccharides that participate in many molecular recognition events. They are commonly found at the terminal positions of the carbohydrate components on cell surface, and can be recognized by pathogens including influenza viruses. *N*-Acetylneuraminic acid (Neu5Ac, **1**) is the most abundant sialic acid form in nature. 4-*O*-Acetylated Neu5Ac ($Neu4,5Ac_2$, **2**) has been found on glycoconjugates from some species, but the impact of this modification has been difficult to study due to its susceptibility to base-catalyzed cleavage.

On the other hand, 2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en, **3**) is a transition state analog inhibitor against neuraminidases that catalyze the hydrolysis of sialosides. Its 4-amino- (**4**) and 4-guanidino- (Zanamivir, **5**) derivatives have been shown to be highly efficient and selective against neuraminidases from influenza A viruses (IAVs). However, it is unclear whether the hemagglutinins from IAVs can bind to sialoside ligands containing 4-amino- or 4-guanidino derivatives of Neu5Ac.

To help answer these questions and to overcome the instability issues of $Neu4,5Ac_2$ (**2**) without changing its structure significantly, I have designed and chemically synthesized its 4-*N*-acetyl analog Neu5Ac4NAc (**6**) and its chemoenzymatic synthon 4-amino-analog $Neu5Ac4NH_2$ (**7**). $Neu5Ac4NH_2$ (**7**) has been further used in a highly efficient stepwise one-pot multienzyme (StOPME) system to construct a comprehensive library of sialosides containing $Neu5Ac4NH_2$ (**7**) and its derivatives.

These sialosides are being used as probes in high-throughput substrate specificity and binding assays for neuraminidases and hemagglutinins from different IAVs. The study can lead to the development of new diagnostic tools and potential therapeutics against IAVs.

Graphic:



Chemoenzymatic Approaches To Glycans, Glycoproteins And Proteoglycans

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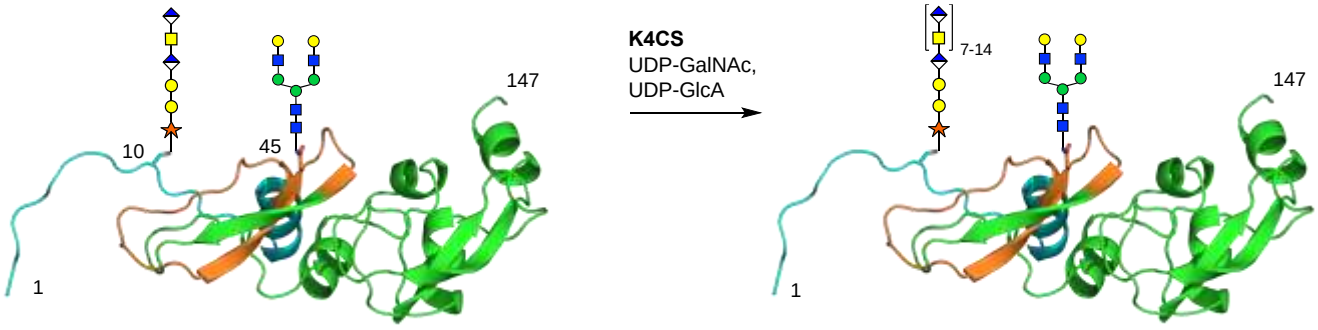
Nature is utilizing carbohydrates not only as an energy source but also as a versatile set of universal building blocks for the modification of biomolecules. These modifications by carbohydrates are typically carried out by enzymes. Thus, the use of enzymes is straightforward whenever natural glycoconjugates are of interest. We focus on the synthesis and the biological evaluation of *N*-glycoproteins. In these entities the carbohydrate part is modulating the biological properties. Of high relevance are therapeutic *N*-glycoproteins (e.g. recombinant EPO and antibodies), which are by default composed of a multitude of individual glycoforms. In this context we have developed chemoenzymatic approaches for the synthesis of *N*-glycans, glycopeptides[1] and homogenous glycoproteins.[2] Chemoenzymatic synthesis has in our hands facilitated the systematic synthesis of libraries of *N*-glycans, which serve as ligands for lectin crystals,[3] NMR-studies,[4] and glycan arrays. Systematic libraries of asymmetric *N*-glycans provide new standards for precision glycomic analyses. Along this line libraries of synthetic glycoproteins[5] can be obtained not only by modifying the intermediate glycopeptide segments but also the final glycoproteins.[6] We have recently developed a strategy to utilize recombinant glycosyltransferases in the synthesis of proteoglycans exemplified by semisynthetic *N*,*O*-glycosylated human bikunin bearing a chondroitin chain with up to about 30 sugar units.



bikunin
(AF-P02760-F1-model_v4 AMBP Bikunin)

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Design And Synthesis Of Covalent Inhibitors For Inverting A-Glucosidases

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Inverting glycosidases are an important and widespread class of enzymes (Fig. 1 A). As for retaining glycosidases, these enzymes can be promising targets in biomedicine and biotechnology. Inverting α-glucosidases are of particular interest as they play an important role in cellular functions, such as the correct folding of proteins in the endoplasmic reticulum. However, selective inverting α-glucosidase inhibitors are scarce, and chemical probes that selectively report on their activity in biological samples do not exist.

To overcome the absence of inhibitors we sought to apply activity-based protein profiling (ABPP) to this class of enzymes. This powerful method depends heavily on the availability of mechanism-based enzyme inhibitors to develop affinity-based probes (ABPs, Fig. 1B). Herein we present the rational design and synthesis of potential covalently binding inhibitors targeting inverting α-glucosidases. The designs are based on the use of carbaglucose scaffold, carrying an epoxide, mimicking the $^4H_3$ conformation of the natural substrate in the transition state of the hydrolysis reaction. To enable the formation of a covalent bond between the inhibitor and the enzyme, a suitable electrophile is introduced that can take up the space normally occupied by the water molecule involved in the hydrolysis reaction (Fig. 1C). In addition, a specially developed assay was implemented and used for biological evaluation.

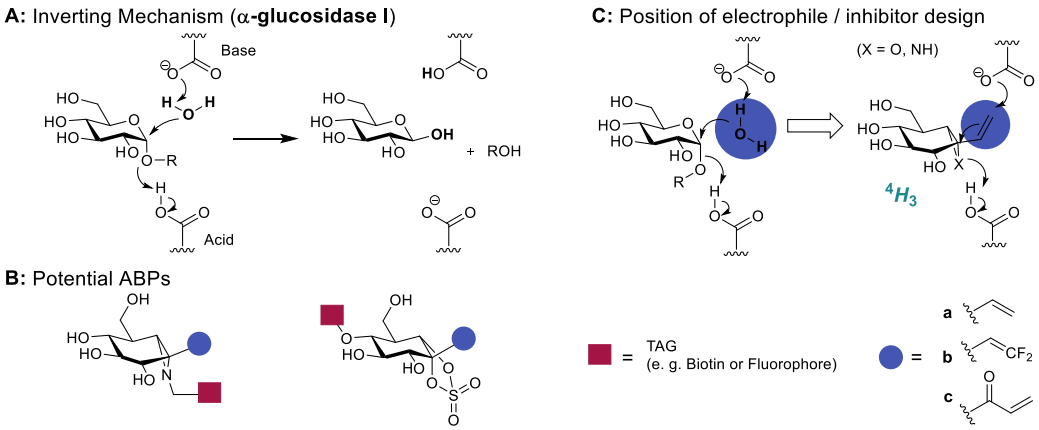

Figure 1: Design approach for covalently binding inverting α-glucosidase inhibitors for ABPP.

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Chemical Synthesis Of A-Dystroglycan With Defined O-Mannosylation Pattern

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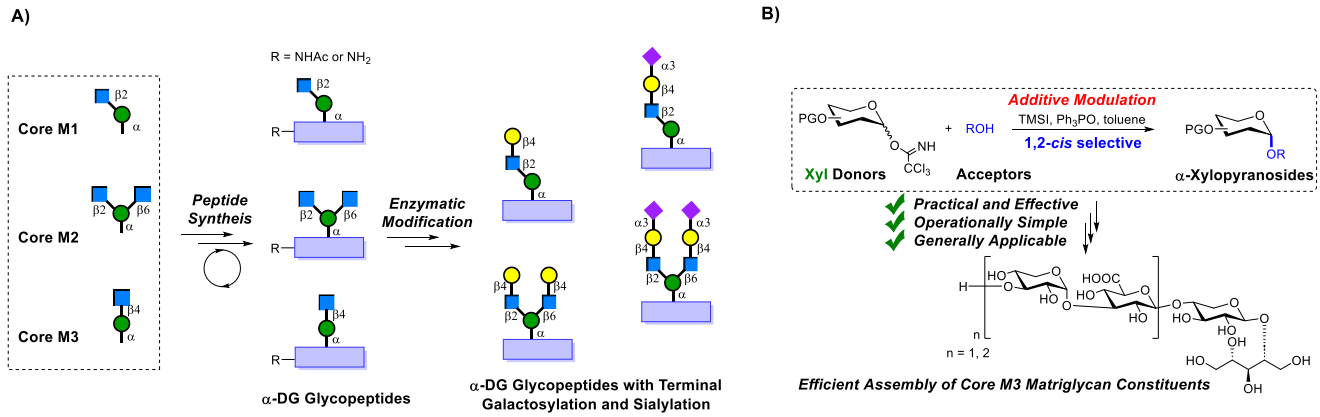
O-Mannosylation plays a vital role in the regulation of a variety range of biological processes, for instance, brain and muscle development. However, the precise function remains largely unknown due to its innate heterogeneity. In this regard, it is still welcome to develop efficient methods to access diverse structurally defined O-mannosyl glycopeptides. In this study, a diversity-oriented assembly of O-mannosyl α-dystroglycan (α-DG) glycopeptides has been achieved via a chemoenzymatic strategy. This strategy features (i) gram scale divergent synthesis of core M1, core M2 and core M3 mannosylated amino acids from judiciously designed protecting group strategies and chemical glycosidation; (ii) efficient glycopeptide assembly via the optimized microwave assisted solid phase peptide synthesis (SPPS); and (iii) enzymatic elaboration of the core glycan structures to install galactosyl and sialyl galactosyl moieties. The efficiency and flexibility of this chemoenzymatic approach was demonstrated with the construction of 12 glycopeptides with different core M1, core M2 and core M3 mannosyl glycans, including a core M2 glycopeptide bearing a hepta-saccharide for the first time.

As a major constituent of extending core M3 glycan, the matriglycan remains a synthetic challenge. To address the long-standing technical hurdle in 1,2-*cis*-xylopyranoside bond formation, a highly stereoselective strategy was established via a preactivation-based, additive-modulated trichloroacetimidate glycosidation strategy. The current protocol is mild, practical, and successful with various xylopyranosyl donors and glycosyl acceptors, including acceptors that are reported to be less reactive due to steric hindrance. The utility of this method was demonstrated with the facile assembly of matriglycan constituent tetra- and hexasaccharides.



Extended N-Glycans Are Preferred Receptors Of H3N2 Influenza Viruses On Human Ciliated Epithelial Cells

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Influenza virus pandemics have resulted from the introduction of an avian/swine virus progenitor. Transmission in the human population requires that the hemagglutinin (HA) specificity shifts from recognition of α2,3-linked sialoside receptors (‘avian type’) to α2,6-linked sialoside receptors (‘human-type’) found on human airway epithelial cells. Since the introduction of H3N2 viruses in the 1968 flu pandemic, the H3 HA has retained human-type receptor preference but has acquired a restricted specificity for the poly-LacNAc extended α2,6-sialosides. This change has impacted binding to cells used for laboratory viral assays and membranes of eggs commonly used for vaccine production due to the lack of adequate extended glycan receptors. To understand the basis of this change in receptor specificity, we analyzed the glycome of human airway epithelial cells, and confirmed the presence of extended glycan receptors. An N-linked glycan library representing the structural diversity of H3N2 virus candidate receptors in the airway glycome was constructed by chemo-enzymatic synthesis and tested for the specificity of H3N2 HAs from 1968-2020, revealing that the strict specificity for extended receptors was retained in current strains. Histochemical staining of human airway biopsy samples shows recent H3N2 HAs preferentially bind to ciliated cells not the entire apical surface, which we believe is a result of preferential expression of extended glycans on these cells. (Funded in part by NIH grant R01AI114730, and CEIRR contract 75N93021C00015).

Keywords: H3N2 influenza virus, receptor specificity, chemo-enzymatic synthesis

Chemoenzymatic Synthesis Of Fucosylated And/Or Sialylated Linear Human Milk Oligosaccharides (Hmos) With A Hexaose Core

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Human milk oligosaccharides (HMOs) constitute a major component of human milk. There is an increasing attention on the contribution of HMOs to the health of breast-fed infants. They are not digested by infants but instead found in their guts, urine and plasma, which can serve as prebiotics to suppress the growth of some pathogenic bacteria, anti-adhesive decoy receptors for some pathogenic microbes, antibiofilm antimicrobials, brain-gut axis modulators, immune modulators, infant colon epithelial cell response modulators, and cell maturation stimulators. The detailed functions of specific HMOs, especially those with more complex structures, are not clear. Exploring the applications of HMOs as infant formula additives, nutraceuticals, and/or therapeutics has begun but has been slow due to the limited access to structurally defined HMOs in sufficient amounts. I have contributed to the development of a highly efficient user-friendly glycosyltransferase-based synthetic platform to access target HMOs in a systematic manner. I have been working on developing efficient routes for systematic chemoenzymatic synthesis of HMOs containing a hexose core (pLNnH, pLNH) using various approaches including Enzyme Assembly Synthetic Maps (EASyMaps), Stepwise One-Pot Multienzyme (StOPMe) with *in situ*-generation of sugar nucleotides, glycosyltransferase substrate engineering strategy, and glycosyltransferase engineering, etc. (Figure 1) The products are essential probes and reagents for elucidating the roles of HMOs and exploring their applications which is studied in the HMO-protein binding assays. The chemoenzymatic synthetic process is readily scalable for large-scale production of HMOs in the future.

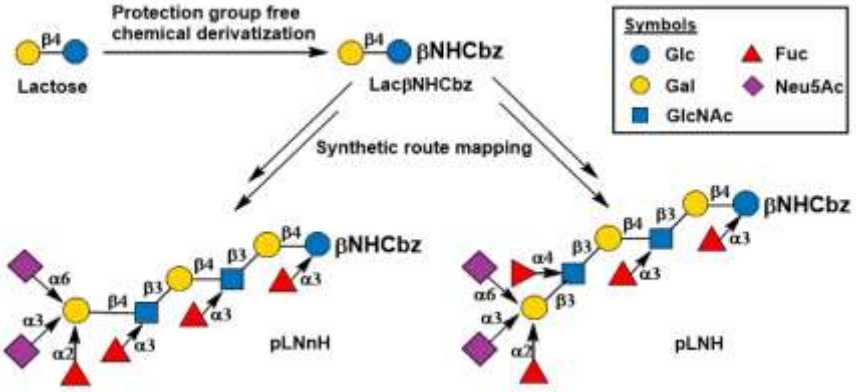

Figure 1. Strategy of chemoenzymatic route mapping and target-orientated construction of pLNnH and pLNH derivatives.

Expanding The Scope Of Solid-Phase Glucan Synthesis

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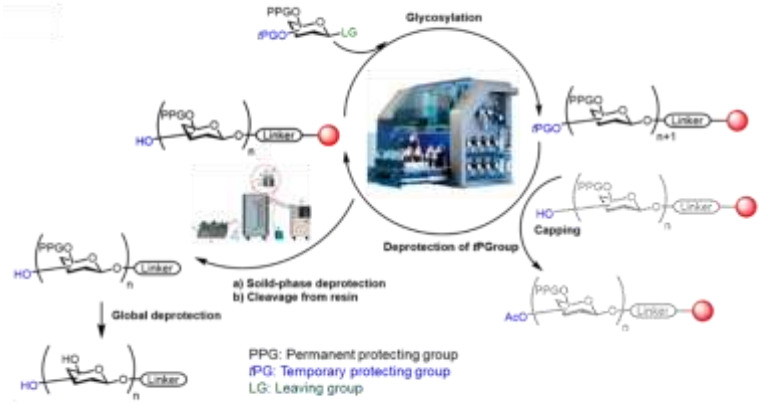
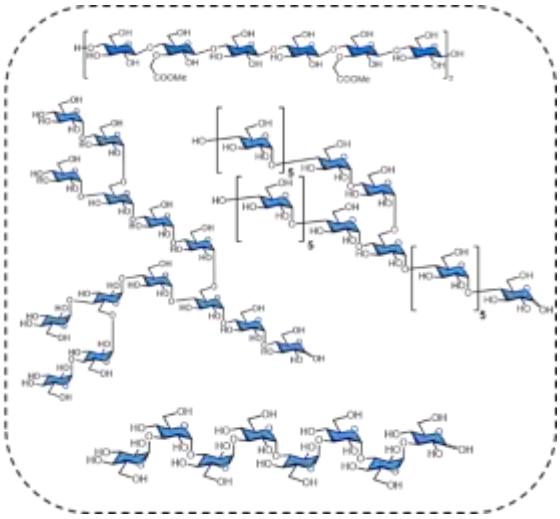
Polysaccharides are Nature´s most abundant biomaterials essential for cell wall construction and energy storage. Seemingly minor structural differences result in entirely different functions: cellulose, a β (1-4) linked glucose polymer, forms fibrils that can support large trees, while amylose, an α (1-4) linked glucose polymer forms soft hollow fibers used for energy storage. A detailed understanding of polysaccharide structures requires pure materials that cannot be isolated from natural sources. Solid-Phase Glycan Synthesis (SPGS) is a powerful method for the quick production of well-defined natural and unnatural oligosaccharides. Here we reported some recent progress on the synthesis of ionic β (1-4) glucan (analogues of cellulose), also the stereoselective installation of multiple *cis*-glycosidic linkages present in α (1-4) glucan (amylose) and α (1-3) glucan (fungal cell wall glycan). Using thioglycoside building blocks with optimized conditions, we prepared cellulose analogs with a well-defined charge pattern. We also achieved excellent stereoselectivity during the synthesis of linear and branched α-glucan polymers with up to 20 *cis*-glycosidic linkages. The molecules prepared in this study will serve as probes to understand the biosynthesis and the structure of glucans. The α (1-3) glucan with well-defined structure can be used to develop novel therapy tools against pathogenic fungi infection.





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Immobilized Enzymes Monolithic Reactor Designed For Exploitation Of Funcrional Poly- And Oligosaccharides

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Abstract: The exploitation of functional poly- and oligosaccharides has attracted extensive interest due to their diverse applications in cosmetics, health, nutraceuticals, food, etc. [1,2] Over the past decade, immobilized enzymatic catalysis, as a green-chemistry technique, has been utilized to modify polysaccharide molecules and exploit new active oligosaccharides. [1,2,3] In this study, an immobilized enzyme reactor (IMER) system comprising two primary enzymatic compartments was constructed. The first compartment, consisting of a monolithic Convective Interaction Media® (CIM®) carboxy imidazole (CDI) disk with immobilized laccases (EC 1.10.3.2) from *Trametes versicolor*, was designed to add phenol groups onto polysaccharides. Dextran T40, chosen as a model polysaccharide, was selected to assess the phenolization process. The second compartment was intended to deconstruct polysaccharides, thereby producing oligosaccharides. A CIM® CDI disk with immobilized glucuronan lyases (EC 4.2.2.14) from *Rhizobium rosettiformans* was used to deconstruct glucuronan. In both compartments, the kinetic parameters of free and immobilized enzymes were quantified, including the maximum rate (Vmax) and Michaelis constant (Km). The design of experiments (DOE) and respond surface methodology (RSM) were used to explore the operating effects, i.e., substrate concentration, flow rate, and reaction time, on enzymatic catalysis for finally optimizing the operating conditions. The stability of immobilized enzymes reactor was also monitored to evaluate its life span. Finally, we aim to integrate these two compartments with a membrane filtration system to create a multistep enzymatic reactor system for the development of poly- and oligosaccharides.

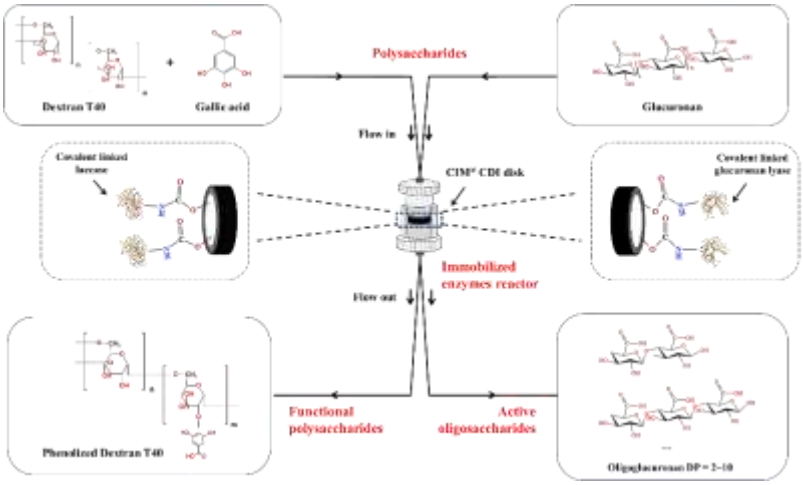

Fig. 1. Schematic diagram of CIM® CDI monolithic reactors immobilized with laccases or glucuronan lyases for producing functional polysaccharides or active oligosaccharides, respectively.

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Automated Solid Phase Innovation For Glycan Synthesis

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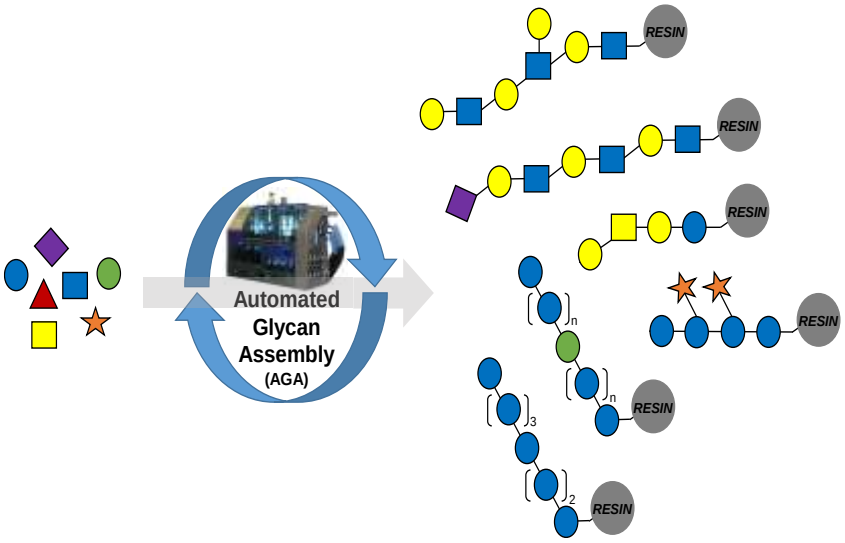
Oligosaccharides are involved in important roles in biological processes. Despite tremendous advances in glycan synthesis in the last years, there is still the need to explore different avenues to deal with the issues connected with the intrinsic chemical complexity of glycans and the difficulties for generating global and holistic enzymatic/synthetic procedures able to generate any glycan at will. [1] Automated Glycan Assembly (AGA) has smoothed the acquisition of synthetic glycans, to be exploited in diagnostics, vaccine development, enzyme characterization and structure-function relationship studies. [2] We are exploring the state-of-the-art of AGA by employing the *Glyconeer* synthesizer to be able to prepare a set of original oligosaccharides (both natural and labelled with stable isotopes) for molecular recognition and biomedical studies. Herein, we present some examples using the first Glyconeer 2.1 based in Spain, focused on polyLacNAc structures and analogues as Galectin and Siglec ligands as well as in the preparation of diverse glycans to be used in the prion propagation area.



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Chemoenzymatic Synthesis Of O- And N-glycans

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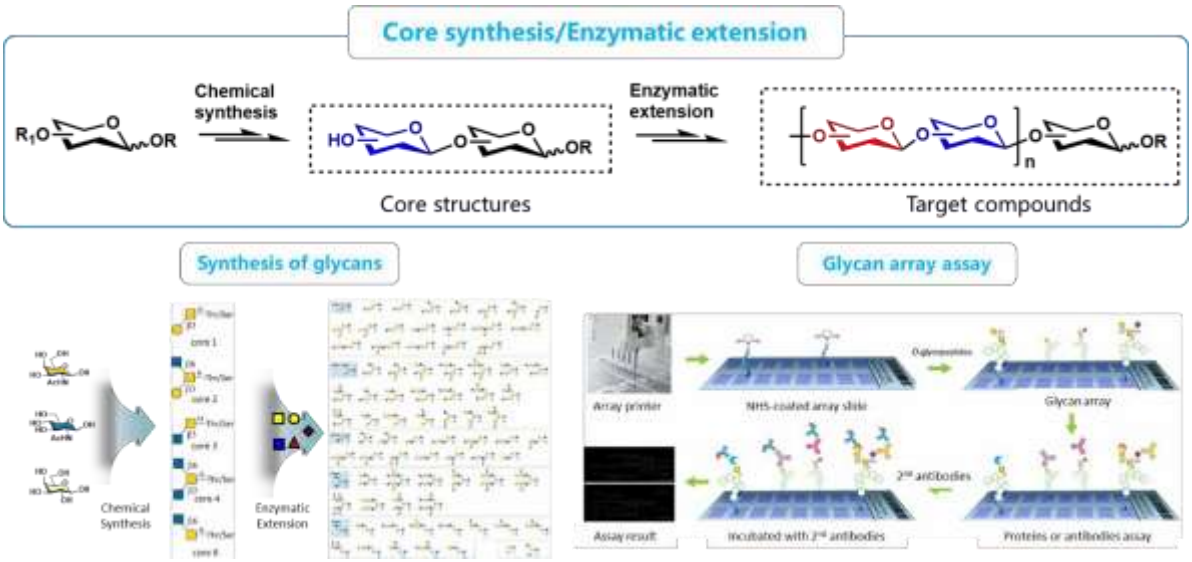
Glycans, recognized as the 'third chain of life' beyond nucleic acids and proteins, play pivotal roles in physiological processes. The efficient synthesis of glycans stands as a fundamental challenge in the field of glycoscience, demanding innovative solutions. We developed different chemoenzymatic methods for the synthesis of O- and N-glycans. Firstly, we developed a 'core synthesis, enzymatic extension' strategy for the assembly of O-glycans. This strategy enables the chemical synthesis of simple core structures and enzymatic elongation of complex glycan epitopes, combining the advantages of chemical and enzymatic methods. Using this approach, over 150 O-glycans (O-Man and O-GalNAc glycans) and 40 N-glycans were assembled. The function of these glycans was further elucidated using a glycan microarray. Secondly, we expanded the application of glycosyltransferases in the glycan analysis field, furnishing robust tools for decrypting glycomic data and probing the functional and regulatory paradigms of glycans in essential biological processes. Over 30 N-glycopeptides, with or without stable isotope-labeled fucose, were synthesized and applied for the absolute quantification of N-glycans in serum antibodies



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Reaction Rate And Stereoselectivity Enhancement In Glycosidations Due To Catalysis By A Lewis Acid–Nitrile Cooperative Effect

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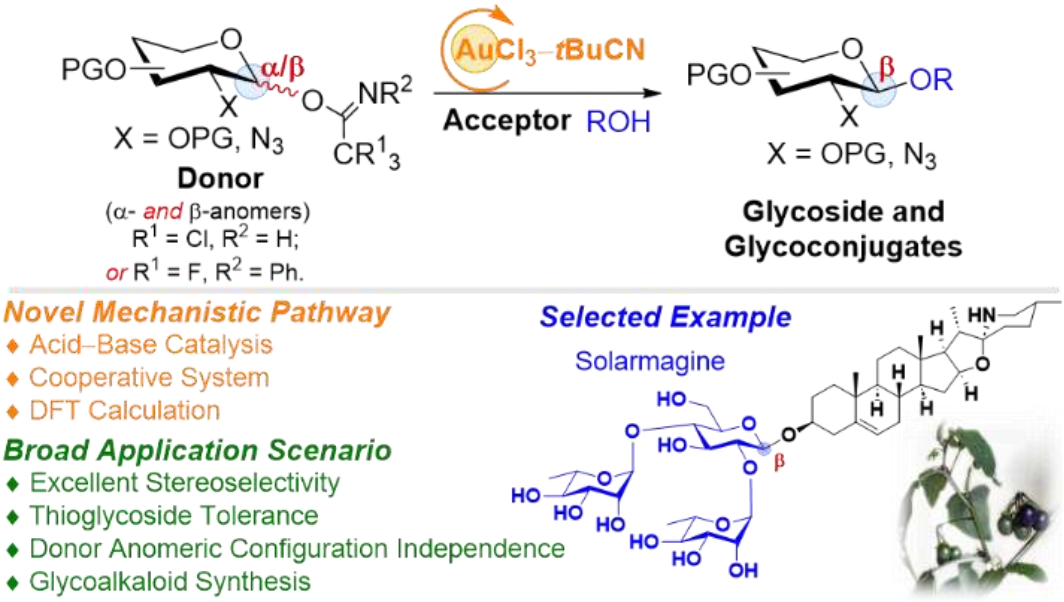
Activation of O-glycosyl trihaloacetimidate glycosyl donors with $AuCl_3$ as a catalyst and pivalonitrile (*t*BuCN) as a ligand led to excellent glycosidation results in terms of yield and anomeric selectivity. This way, various β-D-gluco- and β-D-galactopyranosides were obtained conveniently and efficiently. Experimental studies and density functional theory (DFT) calculations, in order to elucidate the reaction course, support formation of the tBuCN−$AuCl_2$−OR(H)$^+$ $AuCl_4$ complex as decisive intermediate in the glycosidation event. Proton transfer from this acceptor complex to the imidate nitrogen leads to donor activation. This way, guided by the C-2 configuration of the glycosyl donor, the alignment of the acceptor complex enforces the stereoselective β-glycoside formation in an intramolecular fashion, thus promoting also a fast reaction course. The high stereocontrol of this novel 'Lewis acid−nitrile cooperative effect' is independent of the glycosyl donor anomeric configuration and without the support of neighboring group or remote group participation. The power of the methodology is shown by a successful glycoalkaloid solamargine synthesis.



Regio- And Stereoselective Organocatalysed Relay Glycosylations To Synthesize 2-Amino-2-Deoxy-1,3-Dithioglycosides

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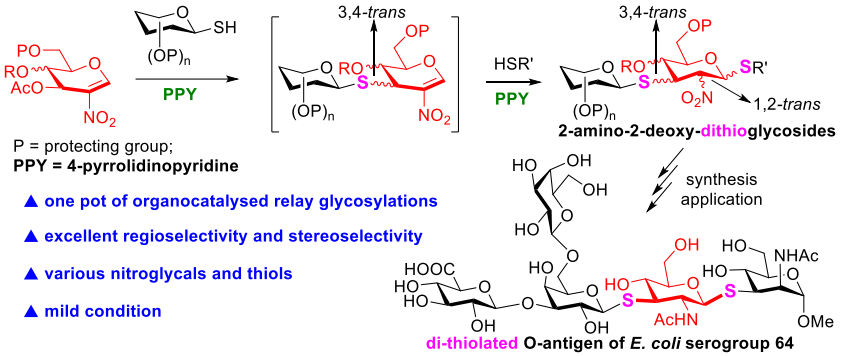
Thioglycosides are carbohydrate mimetics and are resistant to acid-mediated or enzymatic hydrolysis, with applications in glycobiology and drug development. We here describe novel methodology for the regio- and stereoselective convergent synthesis of 2-amino-2-deoxy-dithioglycosides via the one-pot 4-pyrrolidinopyridine-mediated relay glycosylation of 3-*O*-acetyl-2-nitroglycal donors. This unique organo-catalysis relay glycosylation features excellent site- and stereoselectivity, good to excellent yields, mild reaction conditions, and a broad substrate scope. 2-Amino-2-deoxy-glucosides/mannosides bearing 1,3-dithio-linkages were efficiently obtained from 3-*O*-acetyl-2-nitroglucal donors in both stepwise and one-pot glycosylation protocols, while 2-amino-2-deoxy-idosides bearing 1,3-dithio-linkages were produced from 3-*O*-acetyl-2-nitrogalactal donors. The di-thiolated O-antigen of *E. coli* serogroup 64 was successfully synthesized using this newly developed method.



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The reducing toxicity effect of acetyl D-glucosamine on the organic small molecules anticancer activity

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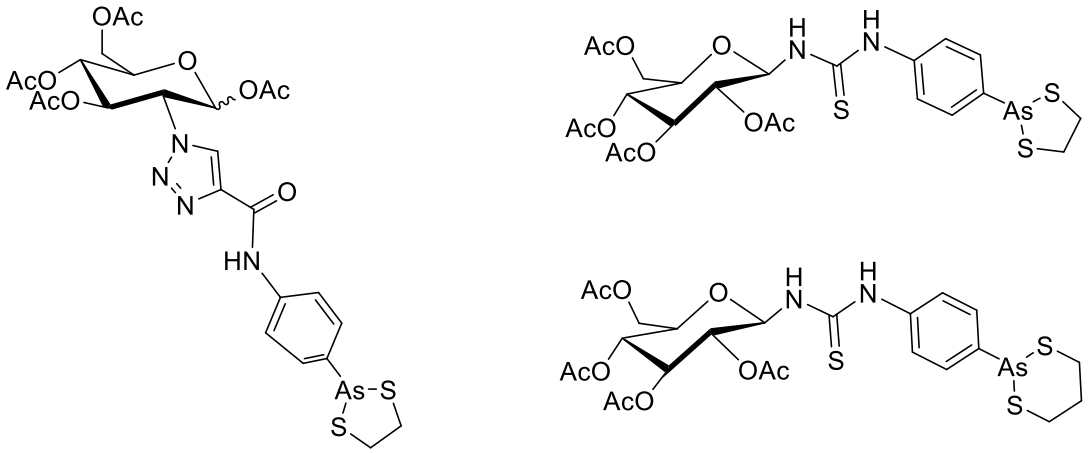
Abstract: Acetyl D-glucosamine has been reported that it has the reducing effect toxicity of acetyl D-glucosamine on the anticancer activity of organic arsenic by our group. The different strategies were used to synthesize the carbohydrate-conjugated organic arsenic to study their anticancer activities and selectivity via click reaction and nucleophilic substitution. The biological test in vitro results showed that 2-position modified carbohydrate-conjugated organic arsenics have better selectivity to HCT-116 cells than commercial avalible 5-FU. Simliarly, the derivatives of carbohydrate-conjugated organic small moleclues were reported by our group.



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Engineering and Photoelectric Driving Application Of Lytic Polysaccharide Monooxygenases From *Myceliophthora Thermophila*

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Lytic polysaccharide monooxygenases (LPMOs) are a newly discovered class of metalloenzymes that show great potential in degrading recalcitrant polysaccharides such as cellulose. The copper active site of LPMOs is activated by reductants and use O_2 or H_2O_2 as co-substrates to oxidatively cleave glycosidic bonds. We systematically explored strategies to enhance the performance of LPMOs through enzyme engineering modifications and the development of innovative reaction systems.

We present the first comprehensive analysis of N- and O-glycosylation within the catalytic domain of LPMO, delving into their complex action mechanisms. The experimental results revealed the N- and O-glycosylation sites and glycan structures within the LPMO catalytic domain, showing significant heterogeneity in glycosylation and a widespread presence of O-glycosylation. By constructing 38 glycosylation mutants, we deeply explored the effects of N- and O-glycosylation on the catalytic activity, structural stability, and overall performance of LPMO. The research findings suggest that glycosylation modifications can significantly affect the catalytic efficiency by modulating the interaction interface between LPMO and the substrate, as well as the interaction between the copper active site and the substrate.

In modular engineering modifications, we explore the impact of CBM on the performance of LPMO. By constructing CBM-truncated variants and CBM fusion mutant, it was found that CBM significantly influenced substrate binding affinity, enzymatic activity, and H_2O_2 tolerance. These results show that CBM can significantly enhance LPMO catalytic activity and reduce auto-oxidative damage to the copper active site by enhancing substrate binding affinity and optimizing the utilization of H_2O_2. Furthermore, through MD simulations, we confirmed that CBM fusion increases the binding sites between LPMO and its substrate. CBM can adjust the spatial proximity between the copper active site and the substrate, facilitating localized cleavage and influencing the interaction between the copper active site and H_2O_2[1].

In terms of innovation in the reaction system, we successfully developed a photoelectrical driven LPMO reaction system. Compared to the conventional ascorbic acid reductant, our method increased the enzyme activity by more than 2.3 times. By controlling the lighting conditions, the system can regulate the generation of reductants, optimizing the stability and efficiency of the LPMO catalytic process, and achieving control over the catalytic reaction with light switches. Additionally, the experimental results confirmed that a continuous supply of reductants is essential to maintain high catalytic activity in O_2-driven LPMO catalysis. In contrast, in H_2O_2-driven LPMO catalysis, only the initial addition of a reductant is required to initiate the reaction [2].

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Dissecting GAG Biosynthesis – From *In Vitro* To *In Situ*

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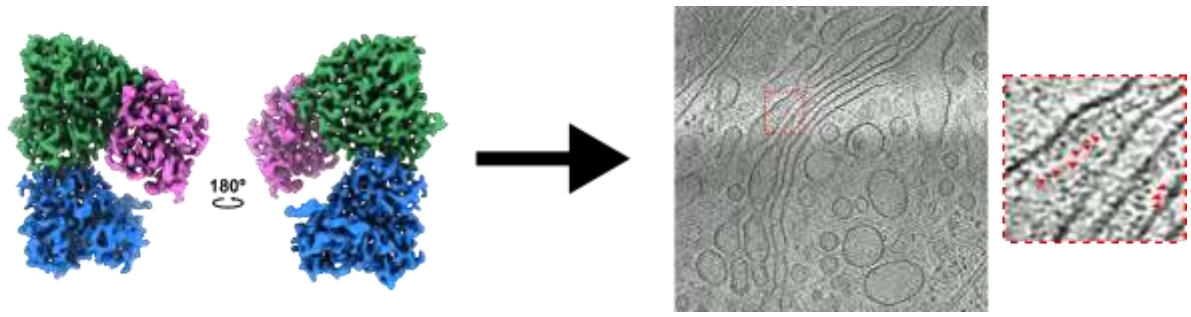
Mammalian glycosaminoglycan (GAG) biosynthesis is a complex process, mediated by multiple enzymes within the Golgi complex, which add and modify carbohydrates on core proteins as they transit from ribosomes to the cell surface. Non-templated GAG biosynthesis leads to the creation of diverse heterogenous polysaccharides, which play essential roles in biological processes including cell adhesion, cytokine signalling and host-pathogen binding[1]. Understanding GAG biosynthesis is complicated by the continual interplay between multiple enzymes and substrates within the pathway. Structural methods can help to deconvolute such complexities, by resolving individual enzymes and their interactions with each other.

Here, we present recently solved structures of heparan sulfate N-deacetylase-N-sulfotransferase (NDST)1, the first enzyme that acts on nascent HS after its polymerization[2]. NDST1 shows an unusual back-to-back arrangement of its catalytic domains, which imposes strong steric constraints on functional cooperativity. Aided by novel activity modulating nanobodies, we carried out biochemical and biophysical analysis of NDST1, which suggest that non-catalytic binding must operate alongside catalytic turnover to mediate cooperativity.

We are now working to place *in vitro* structures of NDST1 and other GAG biosynthesis enzymes into a broader *in situ* framework. A pipeline for high-resolution cryo-electron tomography imaging of Golgi complexes has been optimized, enabling visualization of biosynthesis events directly within cells. Current challenges to annotate these information rich datasets will be discussed, including efforts to develop new tools for labelling. We envision that combining classical *in vitro* structural biology with newer *in situ* methods will help to dissect the complexities of mammalian GAG biosynthesis.



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Biosynthesis Of Mollusc Glycosylation

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Molluscs are vital components of many different ecosystems. They inhabit terrestrial, marine and fresh water habitats. Molluscs are filter feeders, improving the quality of water, they are decomposers and serve as food source for many other species, including humans. On the other hand, herbivorous gastropods are a serious threat to agriculture, and many molluscs often serve as intermediate hosts for the developmental stages of human or livestock parasites. Presumably to enable parasite invasion and development, molluscs and parasites share some structural features of their N- and O-glycans. Glycosylation patterns play an important role in many recognition processes and seem to have a high impact in host-parasite interactions. Therefore, analysing the glycosylation pathway of molluscs is essential for a better understanding of parasite-host interactions.

Recently we were able to clone, express and characterise a number of glycosyltransferases[1-4] and glycosidases[5-6] from mollusc origin, which are involved in the biosynthesis of N- and O-glycans. Compared to the well investigated corresponding vertebrate versions, the mollusc enzymes exhibit some novel features in their structure, their substrate specificity as well as in their biochemical characteristics which seem to be unique to this phylum. For example, a T-synthase from *Pomacea canaliculata* lacks a consensus sequence (CCSD) which was previously considered indispensable for this kind of enzymes[3], or in contrast to most vertebrate enzymes, some mollusc glycosyltransferases do not require divalent cations for their activity, but can transfer a monosaccharide even in the presence of EDTA.

Here, we provide an overview of the mollusc enzymes involved in glycan biosynthesis with a focus on the newly discovered structural and biochemical parameters. The description of these enzymes is not only an important step for the understanding of mollusc glycosylation, but perhaps in the future these enzymes may also be used for the production of highly specific glycans.

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Multi-Path Optimization For Efficient Production Of 2'-Fucosyllactose In An Engineered *Escherichia Coli*

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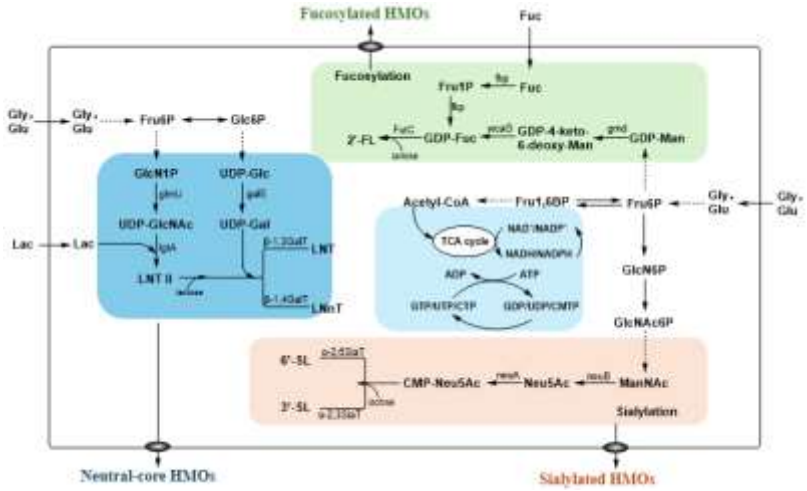
Human milk is unique in terms of complex oligosaccharides content, known as human milk oligosaccharides (HMOs), which is the third most abundant solid components in human milk. Their role in the development of intestinal flora blocking the attachment of pathogens and modulating the immune system of the infant are well-recognized. Compared to chemical and enzymatic synthesis, microbial production has obvious advantages in terms of production efficiency, reduced cost and environmental friendly.



In order to obtain high performance strains, the activity and selectivity of α-1,2-fucosyltransferase were enhanced significantly by directed evolution firstly. Subsequently, utilizing the strategy of "Design-Build-Test-Learn" to balance and finely regulate the 2'-FL metabolic network, including 1) strengthening the key precursor GDP-fucose metabolic pathway; 2) Weakening of degradation pathways of intermediate products; 3) Improvement of lactose and carbon utilization efficiency; 4) Balance between cofactors and energy; 5) Screening and expression of pump proteins. Ultimately, we obtained a high-yield 2'-FL producing strain. Furthermore, advanced precision fermentation technology was adopted for Scale up step by step, 2'-FL yield enhanced up to 200g/L, and with a lactose conversion rate of 95%.

Through the construction of the 2'-FL producing strain, we have also developed a series of high-yield strains of HMOs, including 3'-FL, 3'-SL, 6'-SL, LNnT, LNT, which laying the foundation for the production of high-end infant formula milk powder.

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Ogt-Mediated O-Glcnacylation Inhibits Activation And Pyroptosis Of Microglia By Targeting Atf2 During Sae

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Sepsis-associated encephalopathy (SAE) is a diffused brain dysfunction caused by systemic immune dysregulation after sepsis. Microglia pyroptosis-mediated neuroinflammation is thought to be an important pathological basis for SAE. β-N-acetylglucosamine transferase (OGT)-mediated O-GlcNAcylation affects multiple physiological and pathophysiolocal functions. Increased O-GlcNAc stimulation has been reported to protect against sepsis associated mortality. However, its specific functional mechanism in SAE progression remain undetermined. In the present study, we observed a typical U-shape curve of the global protein O-GlcNAcylation level in the brain of SAE mice and the microglial inflammatory activation model following LPS administration. Inhibition of O-GlcNAcylation in microglia by knocking down Ogt gene significantly exaggerated the LPS-induced production of pro-inflammatory cytokine IL-6, IL-1β, which implied a potential regulatory role of O-GlcNAcylation modification in the LPS induced acute microglial activation. Inhibition of O-GlcNAcylation induces pyroptosis of microglia in vivo and in vitro, and impairs cognitive function of mice. Mechanistically, we found that OGT interacts with activation transcription factor 2 (ATF2) and regulates the balance of O-GlcNAcylation and phosphorylation of ATF2 in microglia. OGT defciency induces the activation of NLRP3 inflammasome by regulating the phosphorylation modification and nuclear translocation of ATF2, and causes microglial pyroptosis and excessive immune response. In addition, knocking down OGT in microglia leads to neuroinflammation and neuronal loss, and exacerbates the progression of SAE. Furthermore, we identified the O-GlcNAcylation of ATF2 on serine 44, and found that mutation of ATF2 S44 to alanine exacerbates NLRP3 inflammasome activation and promotes LPS stimulated production of inflammatory cytokines (IL-18, IL-1β) in microglia. Taken together, our study reveals the protective function of OGT-mediated O-GlcNAcylation of ATF2 in microglia, which might provide a new target for the treatment of SAE.

Binding Specificities Of Disialylated Gangliosides By Siglec-7

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Gangliosides are glycosphingolipids composed of an extra-cellular carbohydrate moiety that is linked to ceramide, a hydrophobic lipid portion embedded in the cellular membrane. They are widely distributed in human cells and tissues and play crucial roles in cellular processes, such as neurotransmission, interaction with regulatory proteins of the nervous system, cell-cell recognition and modulation of signal transduction pathways.[1] Notably, gangliosides containing sialic acids have been found to pronounce effects in cancers, influencing cell behaviors such as proliferation, migration, invasion, adhesion, and angiogenesis, but also mediating immunosuppression of tumors.[2] In recent years, the study of gangliosides has made significant strides, particularly with respect to their relevance to pathology and their interactions with sialic acid binding receptors.[3] Among these, Siglecs, sialic acid-binding immunoglobulin-like receptors, are I-type lectins found on most white blood cells of the immune system and have in common an N-terminal Ig domain that recognizes sialic acid–containing glycans. Evidence indicates that certain Siglecs are engaged by endogenous gangliosides to trigger important physiological and pathophysiological signaling events.[4] The presence of ligands for Siglec-7 and Siglec-9 has been found to be high in various cancer types, such as pancreatic cancer and melanoma.[5] For example, sialoglycans expressed on cancer cells surface can engage Siglec-7 on natural killer (NK) cells, leading to the inhibition of immune responses.[6] We here present a comprehensive analysis of the structure, conformation, and interactions of α2,8-linked gangliosides, including GD3 and its derivatives DSGb3α3 and DSGb3α6, as well as DSGB5 that contains both α2-3- and α2-6-linked sialic acids and densely populated on renal cell carcinoma (RCC).[7,8] Understanding the dynamics of these interactions holds great promise for providing insights into disease mechanisms and potentially opening the door to the development of diagnostic and therapeutic strategies.[9] To this aim we combined structural biology methodologies, NMR, techniques, chemical-physical studies, and computational approaches to provide information on binding affinities and 3D models of the complexes.[10,11] This can have significant implications in the field of immunotherapy, where targeting these interactions may lead to novel strategies for conditions such as cancer, which often exploits sialic acid-containing molecules in immune evasion mechanisms.

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Heparan Sulfate Differentially Regulates Cell Binding By Extracellular Vesicles And Human Cytomegalovirus

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Introduction: Extracellular vesicles (EVs) are lipid-enclosed biological nanoparticles produced by all cells as a mode of intercellular communication. During viral infection, cells produce EVs whose cargo can be modulated in response to infection. This may have functional implications for the EVs, including binding to recipient cells to deliver their cargo. While human cytomegalovirus (HCMV) and other viruses are known to utilize heparan sulfate (HS) and/or other glycosaminoglycans (GAGs) for cell tethering, less is known about the role of GAGs in cellular binding by EVs. Therefore, we have studied GAG- and cell-binding properties of EVs produced by uninfected and HCMV-infected cells to identify GAGs that mediate binding of these particles to recipient cells, and to understand whether HCMV infection modulates this function.

Methods: Conditioned media containing EVs was prepared from uninfected fibroblast cells. Conditioned media containing HCMV virions and EVs was prepared from HCMV-infected fibroblast cells and subjected to high-speed centrifugation to separate EVs from HCMV virions. EVs from uninfected and infected cells were further purified by ultrafiltration and size exclusion chromatography and labelled using NHS-ester-AF647. Labelled EVs or HCMV virions were applied to GAG oligosaccharide microarrays and fibroblast cells to investigate GAG and cell binding, respectively. Cell binding was assessed by confocal microscopy (for labelled EVs/HCMV) and qPCR (for HCMV). Enzymes were used to remove HS or CS from cell or EV surfaces to understand their roles in interactions between EVs and cells.

Results: Glycan microarrays showed that EVs from both uninfected and HCMV-infected cells bound heparin, a highly sulfated form of HS, and, to a lesser extent, CS and keratan sulfates. Removal of HS from fibroblasts or EVs promoted binding by EVs produced by uninfected, but not HCMV-infected, cells. Conversely, HCMV virions required HS for cell binding, as evidenced by a drastic reduction in virion binding following HS removal from fibroblasts. Removal of CS from either fibroblasts or EVs had no obvious effect on the interactions between EVs and cells.

Conclusion: Our results demonstrate that HCMV and co-produced EVs depend on distinct and non-overlapping cell surface components for binding to cells. In contrast to HCMV, EVs, while interacting with GAGs on microarrays, did not appear to depend on these for cellular binding. In fact, removal of HS from either the cell surface or the EV surface promoted cell binding by EVs produced by uninfected cells, potentially via exposure of additional EV binding ligands. Therefore, HS serves opposing roles in cellular binding by EVs and HCMV virions.

Molecular Insights Into *Neisseria Meningitidis* Serogroup Y CPS Interaction With Siglec-7

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Siglecs (sialic acid immunoglobulin type lectins) are essential receptors on immune cells, modulating immune responses through specific interactions with sialic acid residues.[1] Exploited by pathogens and tumor cells for immune evasion, they play a significant role in infectious diseases and cancer. Siglec-7, expressed primarily on Natural Killer cells, acts as an inhibitory receptor, regulating immune responses. [3] We here show that serogroup Y (Men-Y) capsular polysaccharide (CPS), crucial for meningococcal meningitidis, interacts with Siglec-7 (Figure 1), a critical immunomodulatory receptor, contributing to immune evasion mechanisms.
Therefore, this presentation delved into the molecular aspects of Men-Y CPS recognition by Siglec-7, employing ELISA, fluorescence studies, NMR spectroscopy, and computational techniques.[4] The partial depolymerization of Men-Y CPS facilitated the isolation of oligosaccharides for detailed analysis. Molecular dynamics simulations provided insights into the conformational behavior of Men-Y CPS when bound to Siglec-7. In addition, by the employment of different experiments, it was confirmed Siglec-7 preferential recognition of Men-Y ([-4)-α-Neu5Ac-(2,6)-α-Glc-(1]$_n$ over Men-W ([-4)-α-Neu5Ac-(2,6)-α-Gal-(1]$_n$), despite their structural similarities. So, we here aim at shedding light on immune evasion mechanisms employed by *Neisseria meningitidis* evaluating the interaction between Men-Y CPS and Siglec-7. Understanding these interactions could inform the development of targeted therapies against meningococcal infections.

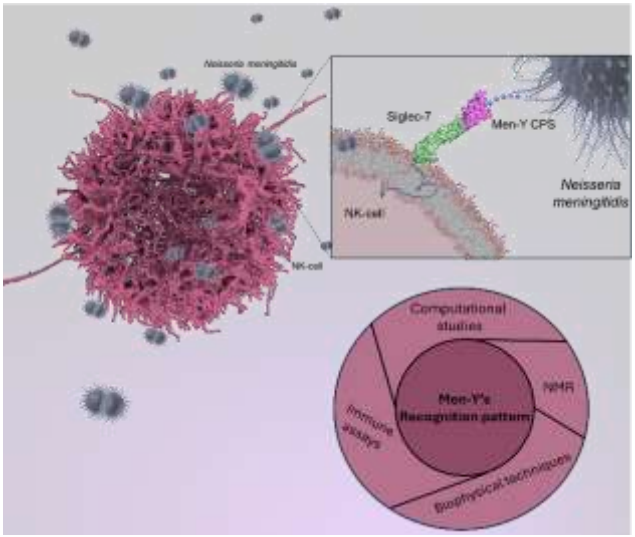

Figure 1. Schematic representation of the interaction between meningococcal CPS and Siglec-7.

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Nanopore Glycomics

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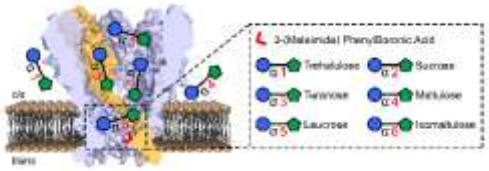
Nanopores are emerging single molecule sensor techniques. Existing nanopore sequencing techniques, as pioneered by Oxford Nanopore Technologies, can already sequence DNA or RNAs with an exceptionally long read length. However, to date, nanopore based glycan sequencing has not yet been demonstrated, largely due to the structural complexity of polysaccharides. By engineering a hetero-octameric *Mycobacterium smegmatis* porin A (MspA) nanopore to contain a phenylboronic acid (PBA) adapter, we have demonstrated the first nanopore sensor which can simultaneous resolve all major monosaccharides[1] and glycoside linkages[2]. This nanopore is also suitable for the analysis of alditol, RNA nucleoside monophosphates (NMP), nucleotide drugs, nucleotide sugars. Assisted by custom machine learning algorithms, all single molecule results can be automatically analyzed with a high accuracy. By the introduction of an NTA ligand to MspA, the pore can further chelate a nickel ion (Ni^{2+}) to generate a metal embedded biological nanopore, named MspA-NTA-Ni. The MspA-NTA-Ni unambiguously and simultaneously identifies all 20 proteinogenic amino acids and their post translational modifications, suggesting its potential use in the analysis of glycosylation sites on proteins[3].



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A Chemoenzymatic Approach for Precise Synthesis of Heparan Sulfate Oligosaccharides

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Heparan sulfates (HS) are highly sulfated linear polysaccharides present on the cell surface and in the extracellular matrix of all mammalian cells, playing crucial roles in various biological processes through protein interactions.[1] The biosynthesis of HS is a highly regulated multi-step process involving various enzymes within the Glogi apparatus. [2] It initiates with the assembly of a polysaccharide chain composed of alternating α-1,4-GlcNAc and β-1,4-GlcA. The resulting polymer undergoes several enzymatic reactions involving *N*-deacetylation/*N*-sulfation, C5 epimerization and *O*-sulfation at various positions, mediated by *N*-deacetylase/*N*-sulfotransferases (NdAc/NST), C5 epimerases (C5-epi), 2-*O*-sulfotransferase (2-OST), 6-OST and 3-OST. Incomplete enzymatic modifications lead to the structural diversity of HS. Several laboratories have reported elegant chemical[3] or chemoenzymatic methodologies[4] for HS oligosaccharides. However, chemical synthetic methods involve many steps. Enzyme-mediated methods require fewer steps, but the promiscuity of the biosynthetic enzymes makes it challenging to precisely control the modification sites. Previous studies have shown that 6-*O*-metylation modification of GlcNS can not only block sulfation of C-6 hydroxyl group, but also prevent epimerization of the upstream GlcA moiety.[5] However, C6 methylation could not be removed without destroying HS oligosaccharides after enzymatic modifications.

In this work, we describe a novel chemoenzymatic approach that employs a removable group, benzyl group modified at C6 hydroxyl of GlcNS moiety to address deficiencies of enzymatic modifications and provide numbers of well-defined HS oligosaccharides. A modular synthetic approach has been developed in which a C6 benzyl ether modification of GlcNS serves as an HS primer. Evaluations of this primer with HS synthetic enzymes such as NST, PmHS2, C5-epi, 2-OST, 6-OST indicate that C6 benzyl modified GlcNS can tolerate NST and PmHS2. Additionally, observations indicate that introducing the C6 benzyl group on GlcNS can self-blocking 6-*O*-suflation without impacting the sulfation of adjacent GlcNS units. Furthermore, we have found that benzyl group not only allows for controlled epimerization of a single adjacent GlcA residue but also can be removed under mild condition. This discovery paves the way for the development of chemoenzymatic synthesis of HS oligosaccharides with precise control. This modified group can be removed at different stages of the enzymatic modification, providing access to differently sulfated derivatives from a single HS precursor.

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Analytical Approaches To The Study Of Glycans Using Liquid Chromatography Mass Spectrometry

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Glycans are released/digested enzymatically or chemically using reductive beta elimination and analyzed as reduced glycans or modified with 2-aminobenzamide.

Glycans and/or glycopeptides are characterized with liquid chromatography-mass spectrometry. We routinely use porous graphitized carbon columns and C18 columns connected to ion trap or orbitrap mass spectrometers. The obtained MS^2 or MS^3 spectra are interpreted manually and compared to reference spectra compiled in-house. Glycans are semiquantified using Progenesis software, allowing us high throughput quantitative analyses of a large number of samples. Below are shown the sample preparation workflow and results from analyses of selected projects.

Novel Technologies And Challenges Of Sequencing Pectic Polysaccharides

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***Ab*stract**

Pectic polysaccharides, as essential dietary fiber components, have functions such as regulating gut microbiota, controlling obesity, and reducing the risk of chronic diseases. Hence, the elucidation of pectic polysaccharide structures is fundamental for exploring their functions. This research focuses on the elucidation extensive and delicate structures of pectic polysaccharides. (1) This research uses LC/QqQ-MS to dynamically monitor the characteristic ion pairs of 3-phenyl-5-methyl-5-pyrazolone (PMP) derivatized monosaccharides, allowing for the analysis of 23 common monosaccharides within 10 minutes with detection limits ranging from picomoles to micromolar. The investigation of polysaccharides extracted from different plants through EDTA-NaOH two-step extraction. (2) Through a process involving methylation in a 96-well plate, acid hydrolysis, and PMP derivatization, combined with LC/QqQ-MS analysis of monosaccharides, oligosaccharides, and polysaccharide standards, a library containing 94 glycosidic linkages has been constructed, enabling the separation and resolution of different neutral and acidic glycosidic residue within 30 minutes. (3) Based on the HILIC-MS/MS analysis of 332 plant oligosaccharides, a reference library containing over 500 mass spectrometry data was established for the first time. Construction of an automatic analysis scheme for pectic polysaccharide structure using an oligosaccharide MS reference library combined with bioinformatics software. Through an investigation encompassing the dimensions of monosaccharide composition, linkage and oligosaccharide constituents, this study endeavors to formulate an automated analysis framework by amalgamating an oligosaccharide liquid chromatography mass spectrometry reference library with bioinformatics software.

A Glycoengineering Strategy For Labelling Lipooligosaccharides And Detecting Native Sialyltransferase Activity In Live Gram-Negative Bacteria With Neuraminic Acids Analogs

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Lipooligosaccharides (LOS) are the most abundant cell surface glycoconjugates on the outer membrane of Gram-negative bacteria. They play important roles in host-microbe interactions. Some pathogenic bacteria cap their LOS with *N*-acetylneuraminic acid (Neu5Ac) to mimic host glycans, a way of protecting themselves from recognition by the hosts immune system. The process of molecular mimicry is not fully understood and remains under investigated for many Gram-negative bacteria. To investigate the functional role of sialic acid-capped lipooligosaccharides (LOS) at the molecular level, it is important to have tools readily available for the detection and manipulation of both Neu5Ac on glycoconjugates and the involved sialyltransferases, preferably in live bacteria. We and others have shown that the native sialyltransferases of some Gram-negative bacteria can incorporate extracellular unnatural sialic acid nucleotides onto their LOS. We here report on glycoengineering strategy for the use of native bacterial sialyltransferases to incorporate Neu5Ac analogues with a reporter group into the LOS of a variety of relevant bacteria Gram-negative bacteria to human health and disease. We show that this approach offers a quick method to screen clinically relevant pathogenic bacteria for the expression of functional sialyltransferases and the ability to use exogenous CMP-sialic acids to decorate their glycoconjugates, and provides means to modify, label and visualize bacterial LOS.

Profiling Of N-Glycans In Different Types Of Diseases By Orthogonal Methods

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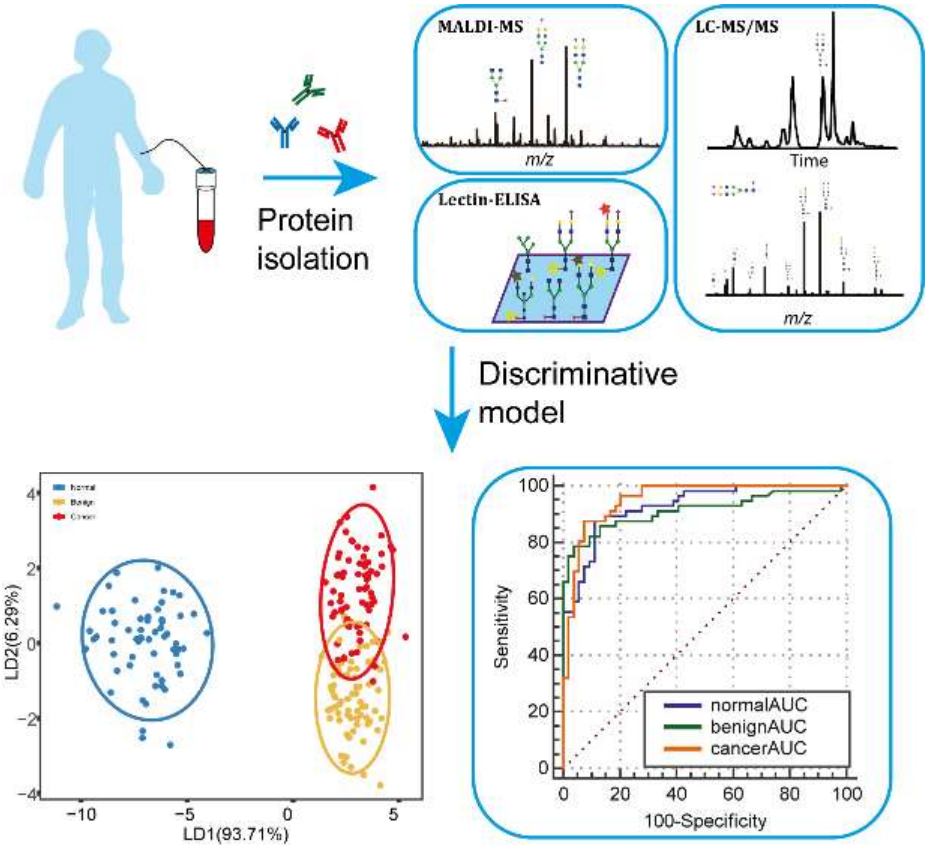
Numerous studies have shown significant changes in protein *N*-glycosylation during inflammatory activity and disease development, and specific *N*-glycans are potential biomarkers [1]. With the high-throughput orthogonal mass spectrometry analytical methods, we have comprehensively and systematically analyzed the *N*-glycome profiles in solid tumors, multiple myeloma and representative rare disease (Haff disease). Taking gastrointestinal tumors as an example, serum *N*-glycome or subclass-specific IgG *N*-glycome profile can effectively distinguish colorectal cancer, esophageal cancer, and gastric cancer. Using transfer learning algorithms, classification and diagnosis models for different tumors can be established, and possible tumor-specific *N*-glycan molecular profiles can be mined [2]. It is worth mentioning that N-glycomics can be used as a key to preliminarily revealing the pathogenic mechanism of rare disease (Haff disease) with unknown etiology [3]. Based on the antibody glycome profile, the utility of glycoengineering methods can achieve more efficient treatment of the disease.



For TOC

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Oligosaccharide Mapping Analysis By Hilic-Esi-Hcd-Ms/Ms For Structural Elucidation Of Complex Sulfated Polysaccharides

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The elucidation of precise structure of sulphated fucan and rhamnosan is essential for understanding their structure-function relationship. For the complexity of the sulphated polysaccharides, the strategy of combination of thermal degradation and HILIC-ESI-HCD-MS/MS analysis with PRM model was used to elucidate the oligosaccharide mapping for structural elucidation of sulfated polysaccharides from *Holothuria floridana* and *Chlorella pyrenoidosa.*

The sulphated rhamnosan was degraded at 110°C, pH 4.0 for 30 min to obtain the various oligosaccharides from large chains, with pH lower than sulphated fucan (pH 5.0).The sequence and sulfation position of all fucoidan oligosaccharide dp2-dp12 and their alditols by $NaBD_4$ reduction was confirmed by HILIC-ESI-HCD-MS/MS, by selection of fully-depronized precursor ions during spray ionization, HCD and PRM during MSMS analysis. Abundant fragment ions of deprotonated molecular ion m/z 331.91 (z=7) of $Fuc_{12}S_7$ (Fig.1) and its alditol confirmed the sequences of 4 symmetrical isomers [1].

The sequence and oligosaccharides mapping of dp2-dp9 from *Chlorella pyrenoidosa* and their alditols were eluciated by HILIC-HCD MS/MS. Remarkably, some new sulfated hexa-rhamnosan oligosaccharides with sulfation (S), methylation (Me) and acetylation (Ac) were identified such as $Rha_6S_3+Ac_3$, $Hex_3Rha_3S_2+Ac_2$, $Hex_4Rha_4S_3+Me_2$, $Hex_3Rha_5S_3+Ac_1+Me_4$, $Hex_3Rha_4Pent_1S_3+Me_2$, $Hex_3S_2+Ac_2+Me_1$ and $Hex_2Rha_2Pent_1S_2+Ac_2$, with sulfation on C2 of rhamnose (Rha) and C4 of hexose (Hex), methylation on C2 or C4 of Rha, acetylation on C2 of Rha. Fig.2 shows the HCD MSMS of the deprotonated molecular ion of $Hex_3Rha_3Ac_2S_3$ (m/z=421.0766, z=3) and its alditol confirmed its sequence as Rha2S-Hex2Ac-Rha2S-Hex2Ac-Rha-Hex4S.

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Fig.1 $Fuc_{12}S_7$

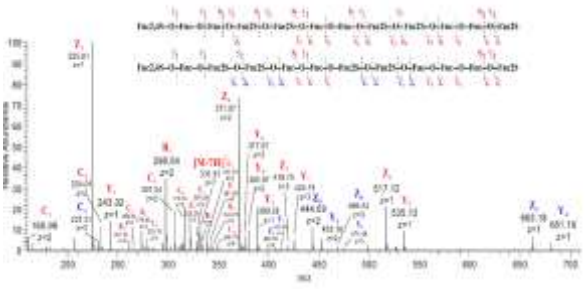
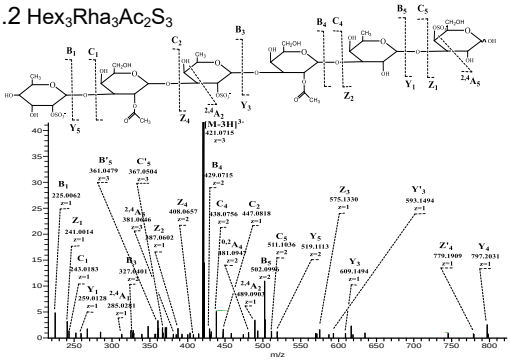


Fig.2 $Hex_3Rha_3Ac_2S_3$



O-Glycomics: Ion Mobility Vs Liquid Chromatography

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O-glycosylation is a common post-translational modification that is essential for the defensive properties of mucus barriers. Incomplete and altered *O*-glycosylation is often linked to severe diseases, such as cancer, cystic fibrosis, and chronic obstructive pulmonary disease. *O*-glycans are often present as complex mixtures containing multiple isomers. Therefore, the analysis of complex *O*-glycans usually requires hyphenation of orthogonal techniques such as liquid chromatography (LC), ion mobility spectrometry, and mass spectrometry (MS).

We developed a mucin-type *O*-glycomics workflow that utilizes hydrophilic interaction liquid chromatography for separation, and fluorescence labeling for detection and quantification.[1] In combination with MS, detailed analyses on the relative abundance of specific mucin-type *O*-glycan compositions are performed.

Detailed LC-MS workflows are laborious and time-consuming which hinders high-throughput implementation of *O*-glycomics in a clinical setting. To address this issue, we present a rapid alternative for separating and identifying *O*-glycans released from mucins based on trapped ion mobility mass spectrometry.[2] Compared to LC-MS, the acquisition time is reduced from an hour to two minutes. To test the validity, the developed workflow was applied to sputum samples from cystic fibrosis patients to map *O*-glycosylation features associated with disease.

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Poster Presentions

Poster Presentations Part-A

July, 14-15

Synthetic And Analytical Study On Endo-Acting Enzymatic Degradation Of Plant α-L-Arabinofuranosidic Linkages

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For the functional analysis of arabinogalactan-degrading GH39 3-*O*-α-D-galactopyranosyl-α-L-arabinofuranosidase (GA*f*ase) and GH43 α-L-arabinofuranosidase (α-L-Ara*f*ase) from *Bifidobacterium longum*, the structural analysis of the products by enzymatic hydrolysis have been carried out by combined chemical methods and found that the limited degradative oligosaccharide structure, α-L-rhamnopyranosyl-(1→4)-β-D-glucuronyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactose in the AGP fermentation of *B. longum*.[1]

The GH39 homologue from *B. catenulatum* was treated with Seyal-type gum arabic, and the resulting hydrolyzed glycans were analyzed by combination of chemical synthesis and modifications, and NMR and MS analysis. The degradation products was found to contain [β-L-arabinofuranosyl-(1→2)]$_n$-β-L-arabinopyranosy-(1→3)-α-L-arabinofuranoside (n = 0–3) in this study, although the presence of the disaccharide moiety β-L-arabinopyranosy-(1→3)-α-L-arabinofuranoside has been reported in Senegal-type gum arabic[2] and the modification of β-L-arabinofuranosides is in the hydrophilic moiety of extensin as β-arabino-oligosaccharides, respectively.[3] Therefore, it was clarified that this GH39 enzyme cleaves up to pentasaccharide at α-L-arabinofuranoside of the inner residue of branched glycan on Seyal-type gum arabic. It was revealed that the branched glycan on galactan backbone of the Seyal-type gum arabic contain the terminal pentaarabinoside structure.[4]

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N-Modification Influences Inhibition Behaviour of Isofagomine: Synthetic and Biological Insights

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Isofagomine (**1**) is widely recognized as a potent β-glucosidase inhibitor. [1] Analogues of isofagomine have been explored for their diverse biological activities. Highly effective pharmacological chaperones as well as inhibitors have been prepared by modifying the carbon backbone of **1**. [2] The N-modified analogues, however, have not garnered as much attention, except for a few examples. [3] In this study, we present an alternative [4] synthetic strategy for the preparation of isofagomine (**1**). Our synthesis relies on a stereoselective Henry reaction, resulting in nitro compound **2**, from which **1** is easily accessible in a few simple synthetic steps. Further on, **1** can chemoselectively be modified with polar and apolar spacer arms carrying varying terminal moieties. Interestingly, the N-modification entails a change in potency and α/β-preference of inhibitory activity against tested glycoside hydrolases from different sources when compared to the parent compound **1**. Concretely, significant diminished activities towards GH1 family β-glucosidases from three different sources have been observed. In contrast the conducted N-modifications improved inhibition potency against α-glucosidase from *Saccharomyces cerevisiae* (GH13). Moreover, and contrary to isofagomine, deactivations of β-galactosidase from *Escherichia coli* (GH2) as well as α-mannosidase from *Canavalia ensiformis* (GH38) have not been verified for this series of compounds. Synthetic details and biological results will be presented.

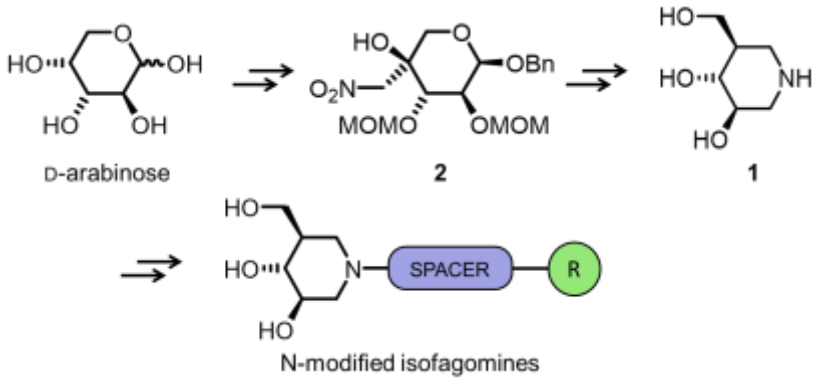

Figure: Key structures in the presented synthetic route.

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Chemical Synthesis and Functions of Symbiotic Bacterial Lipid A for Safe Vaccine Adjuvant Development

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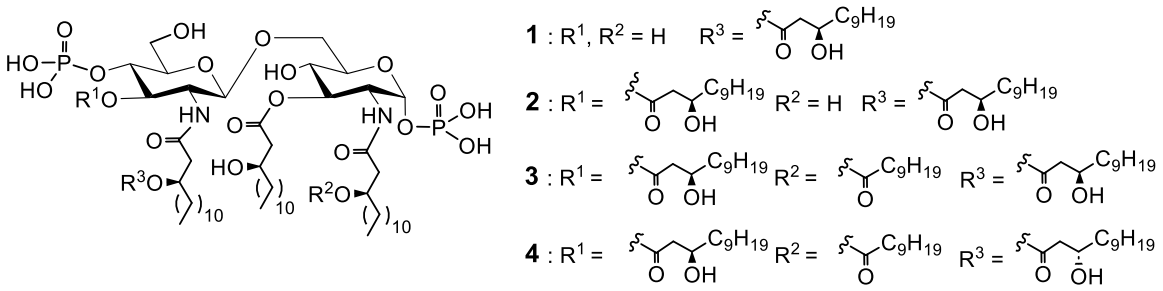
Lipopolysaccharide (LPS) is a major glycoconjugate in outer membrane of Gram-negative bacteria and canonical *Escherichia coli* LPS activate innate immunity to induce lethal strong inflammation. The terminal glycolipid lipid A is the active principle of LPS. Low inflammatory lipid A have been expected as vaccine adjuvants.

We hypothesized that co-evolved parasitic and symbiotic bacterial components should modulate host immunity moderately with low toxicity. We synthesized parasitic [1] and symbiotic [2] bacterial lipid A and elucidated the molecular basis of immunoregulation, and developed safe and useful adjuvants. In this presentation, we introduce the structure determination, chemical synthesis, and structure-activity relationship studies of lipid A from *Alcaligenes faecalis* inhabiting gut-associated lymphoid-tissue (GALT) that is responsible for the mucosal immunity regulation.

We synthesized *A. faecalis* lipids A **1**-**3** with diverse acyl group patterns and identified the active center as hexa-acylated **3**[2]. Lipid A **3** was confirmed to exhibit non-toxic but useful adjuvant function (enhancing antigen-specific IgA and IgG production) [3-7], and that vaccine model using **3** was found to be significantly protective against bacterial infection [4]. Since IgA is responsible for mucosal immune homeostasis, by focusing on GALT symbiotic bacteria, we found promising adjuvant that can safely regulate mucosal immunity. Furthermore, lipid A **4**, which reversed the stereochemistry of the acyl side chain hydroxy group, was found to be more active than **3**[8], and the molecular basis of the adjuvant function is also becoming clear.



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Unveilig The Potential Of Light-Sensitive Glycosidase Inhibition With Sp^2-Iminosugar Azobenzene Glycosides

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We have leveraged the inherent chemical mimicry of sp^2-iminosugars to create photoswitchable *p*- and *o*-azobenzene α-glycosides, using the gluco-configured ONJ representative as a model. Notably, we achieved exceptionally high switching factors for glycosidase inhibition, favoring either the *E*- or *Z*-isomer depending on the aglycone structure. Our findings suggest a link between the isomeric state of the azobenzene module and the selectivity towards α- and β-glucosidase isoenzymes. For instance, compound in Figure 1 exhibited a $2.5 \cdot 10^3$-fold increase in inhibitory potency towards human β-glucocerebrosidase (Gcase) in its *Z* isomeric form compared to the *E* form. This distinct variation aligns with the potential for ex-vivo drug activation and controlled self-deactivation under physiological conditions. Moreover, our results demonstrate that through chemical tailoring, we can engineer photocommutators capable of reversibly switching between inhibiting different glycosidases, thereby broadening the versatility and potential therapeutic applications of this strategy.

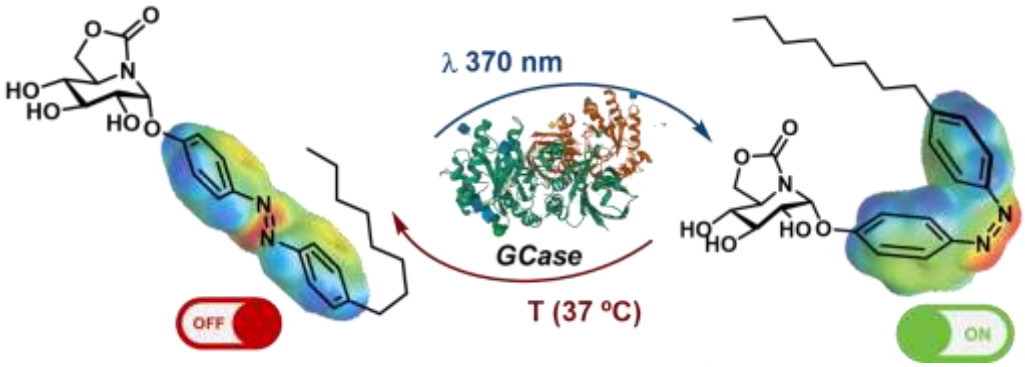

Figure 1. Structure of a photoswitchable D-*gluco* configured sp^2-iminosugar azobenzene conjugate with high (>2500-fold) switching factor against GCase, in favor of the *Z*-isomer.

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Development Of A Mucin O-Glycan Library For Investigating Pathogen Virulence

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The mucosal layer lining the epithelial surfaces of the respiratory, gastrointestinal, and urogenital tracts is widely recognized for its significant influence on the microbiome and serves as an initial defense against infections. Several recent studies have reported on the ability of mucin glycoproteins and their associated O-glycans to downregulate the expression of virulence-associated genes in diverse cross-kingdom pathogens. However, native mucin glycans are structurally diverse and the isolation of pure, defined individual structures in suitable amounts has proven extremely challenging due to their similar physical and chemical characteristics. This has prevented the study of the virulence attenuating properties of individual glycan structures. Therefore, to elucidate which specific glycans are responsible, we have been developing a convergent and scalable approach to obtain a comprehensive library of structurally defined mucin O-glycans in sufficient quantity and quality (>30 mg of target glycan). An initial library of core 1 & core 2-type methyl glycosides was first established[1] and has been successfully used to identify discrete glycan structures responsible for virulence attenuation in fungal pathogen *Candida albicans*[2,3] and prominent Gram-negative bacterial pathogen *Vibrio cholerae*.[4]

In subsequent work, we have recently expanded the mucin glycan library to include core 3 & core 4-type structures.[5] These compounds were synthesized as methyl α-glycosides retaining the stereochemistry of the natural GalNAc-Ser/Thr linkage in mucins. The strategy of the glycan assembly was based on building blocks developed in our previous work, aiming to maximize convergence in our process. Our focus primarily centered on core 3, core 3-Gal (type I), core 3-Gal (type II) and core 4 methyl glycoside derivatives. These structures provide a further expansion of our mucin glycan library to assess the virulence attenuating capabilities of individual glycans.

As the molecular mechanisms through which mucin O-glycans regulate pathogen virulence remain poorly understood, the development of synthetic methods to generate a comprehensive library of mucin O-glycans in sufficient quantity and purity will enable exploration of these processes and subsequently the development of novel therapeutic strategies.

Acknowledgements: Innovation project supported by Innosuisse (104.462 IP-LS), the University of Basel, and the Swiss National Science Foundation (CRSK-3_196773).

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Structural Elucidation Of An Active Polysaccharide From *Radix Puerariae Lobatae* And Its Protection Against Acute Alcoholic Liver Disease

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Abstract: *Radix Pueraria lobata* can be used as medicine and food, whose polysaccharide is one of the main bioactive ingredients. To explore the effect and mechanism of *Pueraria lobata* polysaccharide, a homogeneous and novel water-soluble polysaccharide (PLP1) was successfully isolated and purified from *P. lobata* by column chromatography in the current study. Structure analysis revealed that PLP1 (M_w = 10.43 kDa) was constituted of the residues including (1→4)-α-D-glucose and (1→4, 6)-α-D-glucose, which were linked together at a ratio of 5:1 and represented the main glycosidic units. *In vitro* experiments indicated that PLP1 exhibited a better free radical-scavenging ability than amylose and amylopectin, meanwhile *in vivo* experiments indicated that PLP1 effectively protected against liver injury in mice with acute ALD through significantly inhibiting oxidative stress to regulate lipid metabolism, increasing short-chain fatty acid production, and maintaining intestinal homeostasis by regulating intestinal flora. Taken together, our results illustrate that PLP1 can regulate intestinal microecology as a feasible therapeutic agent for protecting against ALD on the ground of the gut-liver axis, thus laying a theoretical foundation for the rational exploitation and utilization of *P. lobata* resources in the clinic.

Keywords: *Pueraria lobata polysaccharide*, Structural characterization, Acute ALD, Oxidative stress, Intestinal flora

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Synthesis of C-Oligosaccharides via Ni-Catalyzed Reductive Hydroglycosylation

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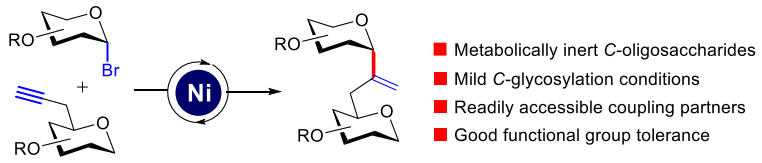
Glycans play crucial roles in a myriad of biological processes, such as cell adhesion, the immune response, inflammation, cancer metastasis, and viral and bacterial infections. Compared to the native glycans that are assembled mostly via *O*-glycosidic linkages, *C*-linked glycans (*C*- oligosaccharides) could retain the biological and pharmaco- logical properties while being metabolically stable, making them artificial surrogates and/or mimics of the native glycans with therapeutic potential. In recent decades, a tremendous amount of effort has been devoted to the development of methods for the synthesis of C-glycosidic linkages, employing glycosyl electrophilic/cationic species, anionic species, free radical species, or transition-metal complexes as glycosylation intermediates. However, methods that can be applied to the synthesis of C-oligosaccharides remain limited. In 2021, we disclosed an effective method for the synthesis of vinyl C-glycosyl amino acids/peptides via Ni-catalyzed reductive coupling of alkyne derivatives of amino acids/peptides with glycosyl bromides. Given the broad functional group tolerance of this hydroglycosylation reactions and the ready accessibility of alkynyl sugar derivatives, we set out to examine the applicability of this protocol to the synthesis of the challenging C-oligosaccharides. We reported a straightforward approach to the synthesis of vinyl C-linked oligosaccharides via the Ni-catalyzed reductive hydroglycosylation of alkynyl glycosides with glycosyl bromides.



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Design And Synthesis Of Gpr119 Agonist

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In our preliminary studies, we identified a molecule that demonstrated promising activity against the GPR119 target[1]. Building on this finding, we employed the RdKit library[2] in Python to screen various molecular fragments. Subsequent to this screening, we conducted docking studies, applied five filtering rules, and carried out molecular dynamics simulations, followed by coding reactions. Concurrently, we investigated different synthetic pathways and selected the most promising candidates for further biological testing.

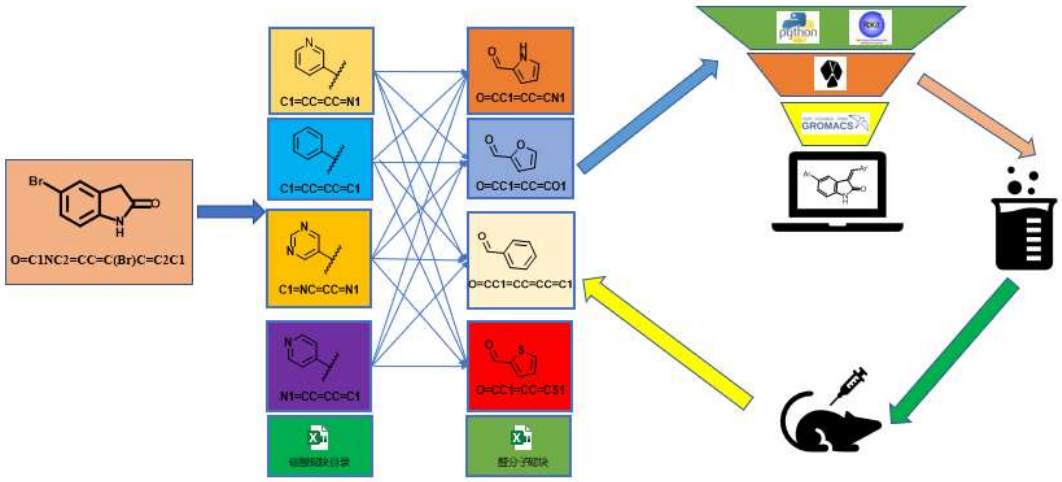

Fig2. The workflow for screening GPR-119 agonists

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Development Of Glycomimetic Ligands For C-Type Lectin Receptors

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Carbohydrate-protein interactions are widely found to play a crucial role in numerous aspects of biological processes. For example, cellular interactions take place between receptors on cell membrane and a highly glycosylated environment, with dense glycans, glycolipids, glycoproteins on the eukaryotic cell plasma membrane, and glycosaminoglycans, mucins in extracellular milieu.[1,2] C-type lectins (CTLs) are a superfamily of carbohydrate binding proteins and are widely found in diverse organisms where they cover multiple functions, including immune response, cell-cell adhesion, and apoptosis.[3] Intriguingly, CTLs are expressed by several subsets of dendritic cells (DCs), immune cells that are specialized in the internalization of pathogens and cross-presentation of antigens.[4] Thus, CTLs represents an attractive target receptors for vaccination applications.

To target CTLs, carbohydrates are natural ligands. However, meanwhile the shallow and polar nature of the recognition binding site makes CTLs undruggable. Glycomimetics mimic the structure and function of native carbohydrates, and have been thoroughly studied to enhance affinities, selectivity, and bioavailabilities. One path to the development of a glycomimetic is to engage in secondary interactions near the primary carbohydrate binding site, which can improve selectivity and affinity. [5] To address this issue, we designed and synthesized a glycomimetic library from shared monosaccharide scaffolds. A parallel synthesis strategy was applied to efficiently generate divergent glycomimetics basing on bioisosteres design.[6] We conjugated our glycomimetic library to lipids then formulated liposomes as a potential targeting delivery system and screened for binding and uptake efficiency testing against a larger panel of CTLs expressed on model cell lines. This assay is an important first step to evaluate specific delivery to natural DCs and our liposomes with preferred glycomimetic ligands showed highly selective affinity to specific CTLs expressed by DCs.[7] As a result, our liposome delivery platform would provide novel modalities to target DCs for immune- and chemotherapy purposes.

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Antineoplastic Effect Accompanied By Alleviating Anti-Myelosuppression Using Mannose-Modified Azocalix[4]Azene As A Delivering Host

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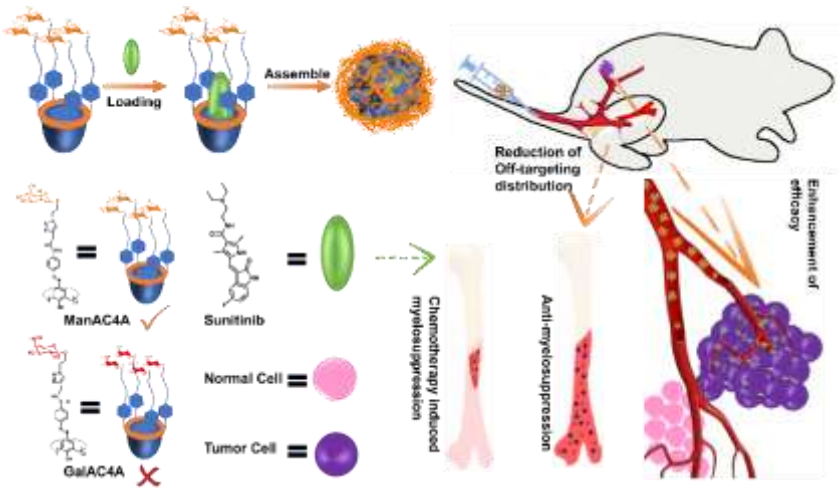
The abnormal blood vessels within tumor tissue, combined with the widespread dispersion of chemotherapeutic agents, often result in numerous unintended adverse effects during antineoplastic therapy. These include hematotoxicity[1, 2] and myelosuppression[3, 4], resulting in poor prognosis. To address the issues, we report a supramolecular integration of multifunctional nanomaterials based on mannose-modified azocalix[4]arene (ManAC4A) and sunitinib. ManAC4A possesses hypoxia-responsiveness, together with active targeting and biosafety. Collectively, the Sunitinib @ManAC4A assembly simply prepared by two components is integrated with multifunction, including triple targeting (EPR targeting, active targeting, and hypoxia-targeted release) and effective anti-tumor therapy. Sunitinib@ManAC4A showed earlier antiproliferative effects, as well as enhanced apoptosis and inhibition of angiogenesis, than free sunitinib on MCF-7 bearing mouse. Meanwhile, Sunitinib@ManAC4A alleviates thrombocytopenia, neutropenia, and myelosuppression compared with Sunitinib@GalAC4A (galactose-modified azocalix[4]arene, GalAC4A, is selected as the control group). Importantly, this design principle offers the novel supramolecular material for reducing off-target effects and attenuating side effects of chemotherapy drugs.



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A-Galcer Sp²-Iminoglycolipid Analogs As Cd1d-Dependent Inkt Modulators: Adjuvancy And Immunotherapeutic Potential

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Invariant natural killer T (iNKT) cells exhibit characteristics that blend those of conventional T cells and classical NK cells, playing critical roles in both innate and adaptive immune responses. α-Galactosylceramide (α-GalCer), also known as KRN7000, serves as the prototype antigen for iNKT cells. However, its efficacy in clinical studies has been suboptimal due to the concurrent secretion of Th1 and Th2 cytokines. To address this limitation, alternative glycolipids have been explored. Nevertheless, challenges persist in the stereoselective synthesis of α-glycosides and in the susceptibility of these compounds to degradation by α-glycosidases, hindering clinical progress. Our strategy aims to overcome these challenges by replacing the carbohydrate moiety in α-GalCer-type glycolipids with a sp²-iminosugar surrogate. These sp²-iminosugars, characterized by a carbamoyl-type nitrogen instead of the endocyclic oxygen atom found in monosaccharides, offer chemical mimicry properties and enable precise control over α-selectivity in glycosylation reactions via an intensified anomeric effect. Evaluation of the resulting α-GalCer sp²-iminoglycolipid (sp²-IGL) immunomodulatory properties has revealed them to be a novel class of CD1d ligands capable of either antagonizing or agonizing iNKT activation. Computational studies provide structural insight into these experimental observations. Additionally, our exploration of the potential applications of selected α-GalCer-related sp²-IGLs in asthma and autoimmune hepatitis immunotherapy, as well as their use as adjuvants, in murine models, has identified promising candidates for drug development.

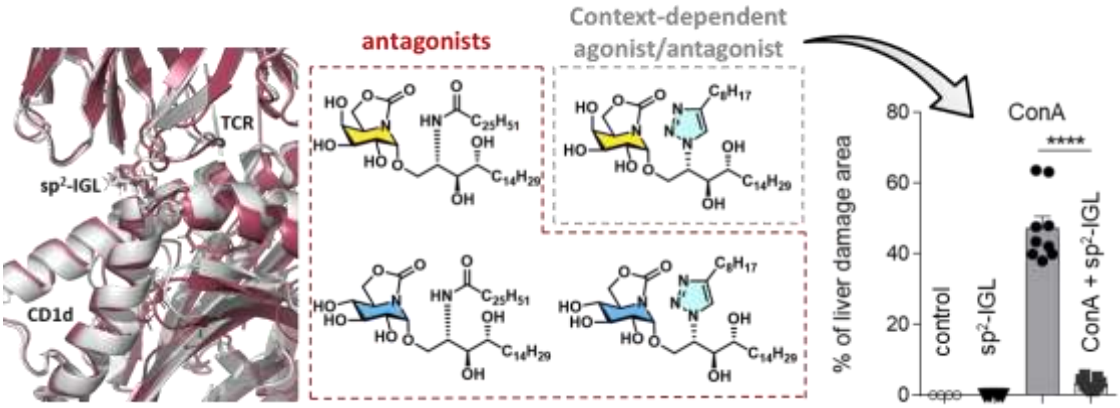

Figure 1. Structures of the new αGalCer-mimicking sp²-IGLs (middle), superposition of the ternary CD1d-(sp²-IGL)-TCR complexes for agonist and antagonist representatives (D-*galacto* configuration; left) and reduction of liver damage in the ConA-induced liver injury model (mouse; right).

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Serum Antibody Fc Glycosylation Profiling Of Igg, Iga1 and Igm By Light Chain Affinity Capturing Coupled With Nano-Lc-Ms Analysis

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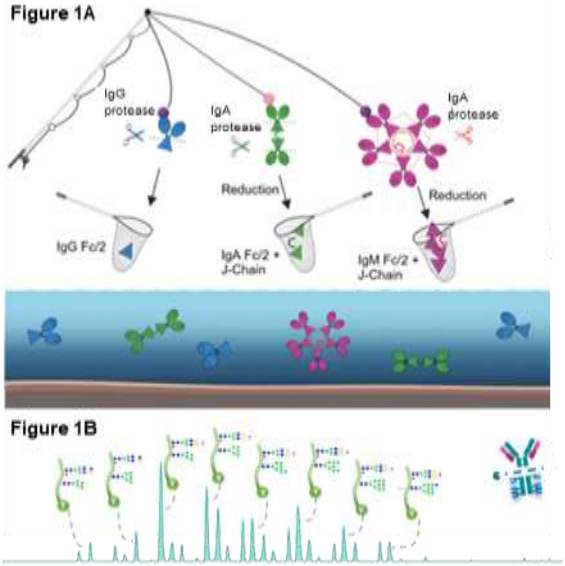

The constant domain (Fc) of antibodies is responsible for binding to effector cells and activate component mediators therefore triggering downstream immune responses. Differences in the amino acid sequence of the Fc define the antibody isotype (with the most abundant being IgG, IgA and IgM) all sharing regions of the light chain with two potential variations (kappa and lambda light chain). Furthermore, antibodies exhibit post-translational modifications, with glycosylation as one of the most important, which can modulate their biological functions. Characterization of antibody Fc structure contribute to understand (altered) immune responses. Up to now, endogenous antibodies are characterized using bottom-up approaches resulting in a loss of combinational information of multiple PTMs such as multiple N-glycans of IgA and IgM.

With new developments of mass spectrometry, intact or middle-up protein analysis are increasingly applied for glycoform characterization study, since it provides comprehensive structural information compared to peptide analysis. In middle-up analysis, antibodies are cleaved via specific proteases into two subunits, constant domain (Fc) and variable domain (Fab). So far, only IgG has been studied using middle-up approaches for Fc characterization. For endogenous IgGs, normally the antibodies are captured by FcXL beads which bind to the Fc of IgG, followed by a hinge-region cleavage by IdeS and elution of the Fc subunits under acidic conditions. For IgA or IgM there is no analytical platform for middle-up Fc profiling yet. In this project, we have developed a middle-up analysis platform for sequential Fc profiling of IgG, IgA and IgM.

To allow capturing of all antibody isotypes a light chain affinity capturing, using a mixture of kappa and lambda light chain beads, was established (Figure 1A). After capturing, the Fc/2 subunits of IgG, IgM and IgA1 were sequentially released by specific IgG, IgM and IgA proteases providing directly the Fc portions and eliminating the elution step required in Fc affinity strategies. The Fc/2 subunits of each isotype were individually analyzed by nanoRPLC-MS. The IgG Fc/2 profiles showed no bias between the developed light chain capturing method and the classical FcXL capturing. For IgM Fc/2 subunits, very complex glycosylation profiles containing 2 and 3 N-glycosylation sites were observed (Figure 1B). Next to glycosylation, other modifications such a c-terminal tyrosine truncation were detected for IgA and IgM. The mass spectra of IgA and IgM Fc/2 glycoforms was annotated by integrating the intact subunit and the site-specific bottom-up information. In addition to Fc/2 subunits, the corresponding joining chains from IgA and IgM were detected and annotated with different glycoforms. The proposed method was applied to the characterization of the Fc/2 subunits of three independent donors resulting in different profiles, therefore illustrating the potential of the approach to study antibody Fc/2 changes.

Biosynthesis Of Mollusc Glycosylation

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Molluscs are vital components of many different ecosystems. They inhabit terrestrial, marine and fresh water habitats. Molluscs are filter feeders, improving the quality of water, they are decomposers and serve as food source for many other species, including humans. On the other hand, herbivorous gastropods are a serious threat to agriculture, and many molluscs often serve as intermediate hosts for the developmental stages of human or livestock parasites. Presumably to enable parasite invasion and development, molluscs and parasites share some structural features of their N- and O-glycans. Glycosylation patterns play an important role in many recognition processes and seem to have a high impact in host-parasite interactions. Therefore, analysing the glycosylation pathway of molluscs is essential for a better understanding of parasite-host interactions.

Recently we were able to clone, express and characterise a number of glycosyltransferases[1-4] and glycosidases[5-6] from mollusc origin, which are involved in the biosynthesis of N- and O-glycans. Compared to the well investigated corresponding vertebrate versions, the mollusc enzymes exhibit some novel features in their structure, their substrate specificity as well as in their biochemical characteristics which seem to be unique to this phylum. For example, a T-synthase from *Pomacea canaliculata* lacks a consensus sequence (CCSD) which was previously considered indispensable for this kind of enzymes[3], or in contrast to most vertebrate enzymes, some mollusc glycosyltransferases do not require divalent cations for their activity, but can transfer a monosaccharide even in the presence of EDTA.

Here, we provide an overview of the mollusc enzymes involved in glycan biosynthesis with a focus on the newly discovered structural and biochemical parameters. The description of these enzymes is not only an important step for the understanding of mollusc glycosylation, but perhaps in the future these enzymes may also be used for the production of highly specific glycans.

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Design And Synthesis Of Covalent Inhibitors For Inverting A-Glucosidases

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Inverting glycosidases are an important and widespread class of enzymes (Fig. 1 A). As for retaining glycosidases, these enzymes can be promising targets in biomedicine and biotechnology. Inverting α-glucosidases are of particular interest as they play an important role in cellular functions, such as the correct folding of proteins in the endoplasmic reticulum. However, selective inverting α-glucosidase inhibitors are scarce, and chemical probes that selectively report on their activity in biological samples do not exist.

To overcome the absence of inhibitors we sought to apply activity-based protein profiling (ABPP) to this class of enzymes. This powerful method depends heavily on the availability of mechanism-based enzyme inhibitors to develop affinity-based probes (ABPs, Fig. 1B). Herein we present the rational design and synthesis of potential covalently binding inhibitors targeting inverting α-glucosidases. The designs are based on the use of carbaglucose scaffold, carrying an epoxide, mimicking the $^4H_3$ conformation of the natural substrate in the transition state of the hydrolysis reaction. To enable the formation of a covalent bond between the inhibitor and the enzyme, a suitable electrophile is introduced that can take up the space normally occupied by the water molecule involved in the hydrolysis reaction (Fig. 1C). In addition, a specially developed assay was implemented and used for biological evaluation.

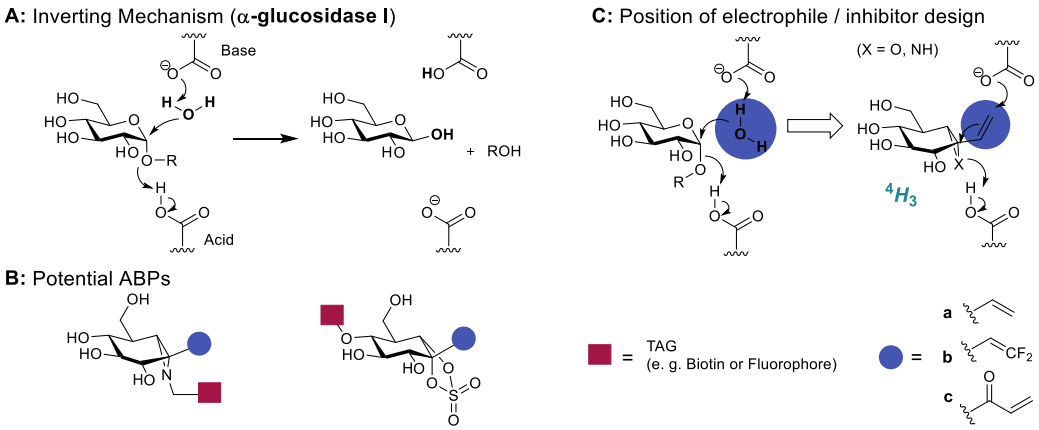

Figure 1: Design approach for covalently binding inverting α-glucosidase inhibitors for ABPP.

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Enzymatic Synthesis Of The *S*-Diastereoisomer Of Pro-Xylane

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Glycosaminoglycans (GAGs) are long, linear polysaccharides comprised of repeating disaccharide units with pleiotropic biological functions. GAGs are a well-recognized skincare target due to their high endogenous expression in skin, pleiotropic biological action and attenuated expression/activity within aged skin.[1] Previous studies show that hydroxypropyl tetrahydropyrantriol (Pro-Xylane) can effectively promote the production of GAGs, thereby increasing the firmness and moisture of skin.[2] The chemical synthesis of Pro-Xylane requires the use of sodium borohydride for stereoselective reduction, which results in borate impurities in the final products.[3] Here, we show an enzymatic method to produce *S*-configuration Pro-Xylane with better eco-footprint. A natural sorbitol dehydrogenase was employed and engineered using directed evolution, to catalyze the ketone reduction. The obtained *S*-configuration Pro-Xylane showed better effect in stimulating GAG synthesis, as well as the type IV collagen in human fibroblasts. In addition, *S*-configuration Pro-Xylane penetrate across the skin, making it a promising ingredient to restore skin elasticity and firmness against ageing.

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Molecular Dissection Of *Fusobacterium Nucleatum* Interaction With Siglec-7

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Fusobacterium nucleatum (*Fn*) is a prominent Gram negative pathobiont in the human oral cavity and rarely found in the lower gastrointestinal tract of healthy individuals.[1, 2] Nevertheless, *Fn* is capable of systemic dissemination and is associated with disease states characterized by a disturbed microbial balance, or dysbiosis, becoming interesting for its role in various systemic diseases including colorectal cancer (CRC). Thus, *Fn*'s ability to evade the immune system and contribute to oncogenic processes makes it a critical subject of study in cancer research.

The interaction between *Fn* and immune cells, particularly through Siglec-7, a sialic acid-binding lectin predominantly expressed on natural killer (NK) cells, is of significant interest. Siglec-7 is known for its inhibitory role in immune regulation, which it performs by recognizing sialylated structures on cell surfaces, leading to the suppression of immune activation.[3, 4] By engaging with Siglec-7, *Fn* may modulate immune responses, promoting its own survival and facilitating disease progression. Studying this interaction is crucial, not only for understanding *Fn*'s role in immune evasion but also for exploring potential therapeutic targets that could disrupt this interaction to enhance immune response against tumors.

Here we tackle the importance of dissecting the molecular basis of *Fn*-Siglec-7 interaction, performed following a multidisciplinary approach (Figure 1) by combining wet lab, NMR spectroscopy (protein and ligand-based methods), computational modeling (Docking, MD), and biophysical assays (Fluorescence, SPR). Our data revealed that the core region of *Fn* ATCC 10953 LPS did not interacted with Siglec-7; indeed, similar association constants were measured for the full O-antigen portion and for the oligosacchairdes containing various number of repeating units. In addition, a single repeating unit, including a reducing sialic acid was not interacting with the protein, suggesting the necessity of a longer glycan chain to induce recognition process. Additionally, our results highlighted the essential role of the internal sialic acid residue, in forming stable complexes through a key salt bridge with Arg124. These findings not only advance our understanding of the intricate mechanisms governing immune cell recognition and bacterial evasion but also underscore the potential of targeting Siglec-7 for therapeutic strategies against colorectal cancer and other related diseases.

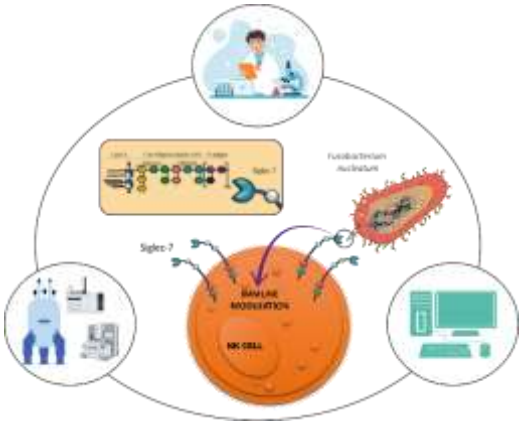

Figure 1: Schematic representation of the recognition of *Fusobacterium nucleatum* ATCC 10953 LPS by Siglec-7 as investigated using NMR, other biophysical techniques, and computational approaches.

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Investigation Of Galectin-9n Selectivity For B-Mannoside Triazolyl Analogues Of Galactoside Galectin Inhibitors

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Lund University

Investigating galectin-9N selectivity for β-mannoside triazolyl analogues of galactoside galectin inhibitors Mannoside β-C-1 amidotriazoles have previously been reported as having selectivity towards galectin-9N inhibitors over corresponding galactoside C-3 amidotriazoles, which were more selective for galectin-3. This study further investigated this by synthesis of mannoside analogues to high-affinity galectin-3 inhibitors. Following synthesis, affinity measurements using competititve fluorescence polarization assays were performed which indicated low affinity and selectivity. From conformational calculations it was implied that the mannoside analogues were not able to find the binding pose of the parent galactoside compounds.

Structure-Activity Relationship Studies Of The Glycolipid Mpiase Essential For Membrane Protein Integration In *E. Coli*

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MPIase was discovered in the inner membrane of *E. coli* as an essential component for the integration of membrane proteins into the membrane and for membrane translocation of secretory proteins[1,2]. Despite its protein-like name, MPIase is a glycolipid composed of 9 to 11 repeating trisaccharide units consisting of *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine uronic acid, and 4-*N*-acetyl-D-fucosamine, with diacylglycerol linked via a pyrophosphate (Fig. 1). To overcome the scarcity and heterogeneity of natural MPIase and to elucidate the mechanism of action of MPIase, we have systematically synthesized MPIase analogs[3,4]. Structure-activity relationship studies using homogeneous synthetic analogs revealed the contribution of distinctive functional groups and the effect of the MPIase glycan length on membrane protein integration activity. In addition, both the synergistic effects of these analogs with the membrane chaperone YidC, and the chaperone-like activity of the phosphorylated glycan were observed[5]. These results demonstrated the translocon-independent membrane protein integration mechanism in the inner membrane of *E. coli*, in which MPIase captures the highly hydrophobic nascent proteins via its characteristic functional groups, prevents protein aggregation, attracts the proteins to the membrane surface, and delivers them to YidC in order to regenerate its own integration activity.

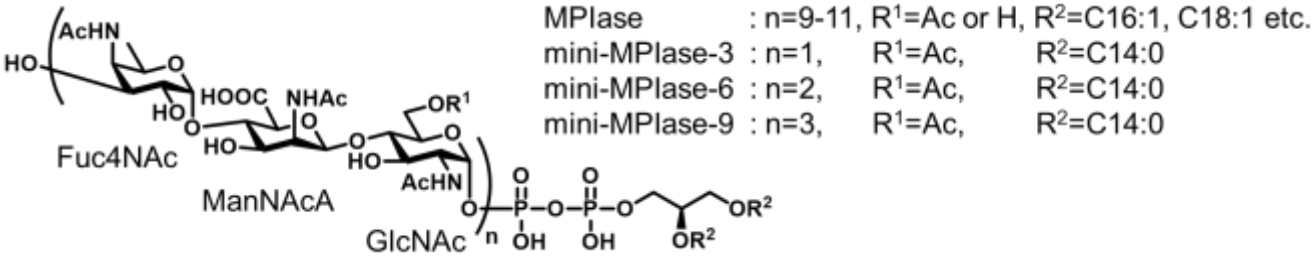

Figure 1. Structure of MPIase and its synthetic analogs

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Design And Synthesis Of C-6 Fluorinated (-)-Swainsonine And (+)-Swainsonine Derivatives: High Selective And Potent Inhibitors Of A-Mannosidase And A-L-Rhamnosidase

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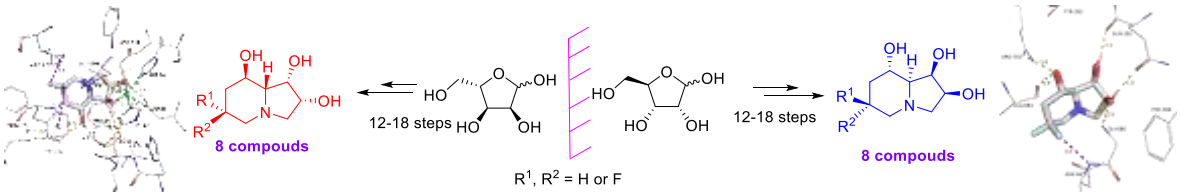
Swainsonine is a potent inhibitor of Golgi mannosidase Ⅱ,[1] and therefore endows it important potential in treatment of cancers.[2] However, a phase Ⅱ clinical trial with renal carcinoma was discouraging due to severe side effects such as fatigue, anorexia, nausea and diarrhea.[3] Structure modification has been proved an effective strategy to improve glycosidase inhibitory activities and adjust inhibitory spectrum. In this work, six C-6 fluorinated (-)-swainsonine derivatives and their enantiomers were designed and synthesized based on initial docking calculations. These compounds were evaluated as potential inhibitors of a wide panel of glycosidases with (-)-swainsonine and its enantiomer as contrast, and part of them were found to be highly selective and potent inhibitors of α-mannosidase and α-L-rhamnosidase. The work presented herein is an important part of our research in fluorinated iminosugars,[4-8] and has laid good foundation for development of more potent α-mannosidase inhibitors as well as structure-activity relationship (SAR) study.



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Synthesis Of Circulating Anodic Antigen (Caa) Oligosaccharides For Schistosomiasis Diagnostics

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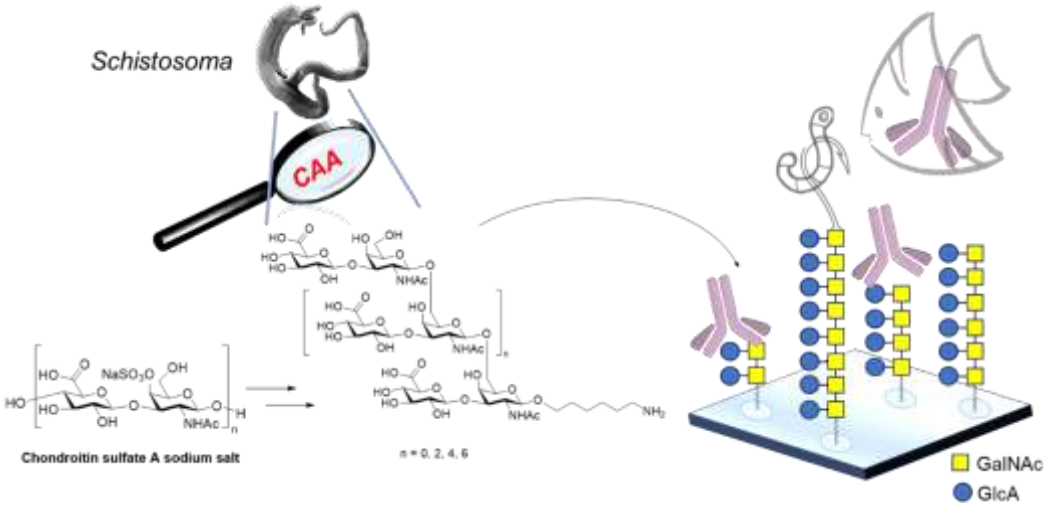
Schistosomiasis, caused by parasites of the genus *Schistosoma*, affects over 250 million people worldwide. Developing improved diagnostic methods is crucial for effective disease control and elimination. Schistosoma parasites express a complex array of glycans that are prime targets for antibodies generated during infection. Among these, the circulating anodic antigen (CAA), a polymer composed of repeating units of *N*-acetyl galactosamine and glucuronic acid ([-6-(GlcA-β1-3)-GalNAc-β1-]$_n$), has emerged as a promising target.[1]

We realized that the CAA polysaccharide shares the same repeating unit with chondroitin sulfate, although the connection of the dimer repeats in both polysaccharides differs. We therefore here report the development of an effective semi-synthesis[2] route towards CAA oligosaccharides, using disaccharides sourced from the cheap and readily available chondroitin sulfate. Depolymerisation and installation of the appropriate protecting groups delivered the required disaccharide repeating unit building blocks that were used for to generate oligosaccharides of various lengths. These oligosaccharides underwent two-step deprotection, resulting in well-defined CAA fragments including a dimer, tetramer, octamer, dodecamer and hexadecamer, each containing an amine-functionalized spacer for conjugation purposes. Preliminary microarray-based evaluation[3] demonstrated the remarkable specificity and sensitivity of CAA, indicating its potential as a diagnostic tool for primary infection. Concurrently, structural studies of CAA fragments are ongoing with the aim to establish a correlation between the glycan structure and the elicited immunological response. In all, our studies may advance the development of novel diagnostic methods for Schistosomiasis.



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Heparan Sulfate Proteoglycans In ASFV Virus Infection

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African swine fever is a deadly porcine disease that has become a serious threat to the global pig industry and pork production. Breeding the ASFV disease resistance pig is an effective strategy for ASFV prevention and control. However, the key host resistance genes are absent to hinder the development of disease-resistant breeding, due to the complexity of the pathogenic mechanism and structure of ASFV. The glycan covered with the cell surface of mammalian plays an important role in viral infection as a direct or indirect receptor. Thus, screening the glycoprotein related to ASFV entry is one of the important ways to identify ASFV resistance genes. Here, we discovered that HP5, a heparan sulfate proteoglycan, bound the ASFV by combining the glycan microarray screening, genome-wide CRISPR knockout libraries screening, and RNA-seq analysis. Moreover, cell biology experiments analysis showed the inhibition of HP5 gene expression level in PAM cells, the host cell of ASFV, decreased the ASFV infection, whereas HP5 overexpression in non-susceptible cells 3D4 increased the ASFV infection. In addition, we found HP5 involved in ASFV endocytosis by interacting with the envelope protein of ASFV. Our results indicate HP5 is a candidate ASFV receptor, which provides the key information to implement ASFV disease resistance breeding.
Keywords: heparan sulfate proteoglycan, African swine fever virus, endocytosis

Semisynthesis And Biological Evaluation Of Chitin-Binding Protein Cbp21 And Its Analogs

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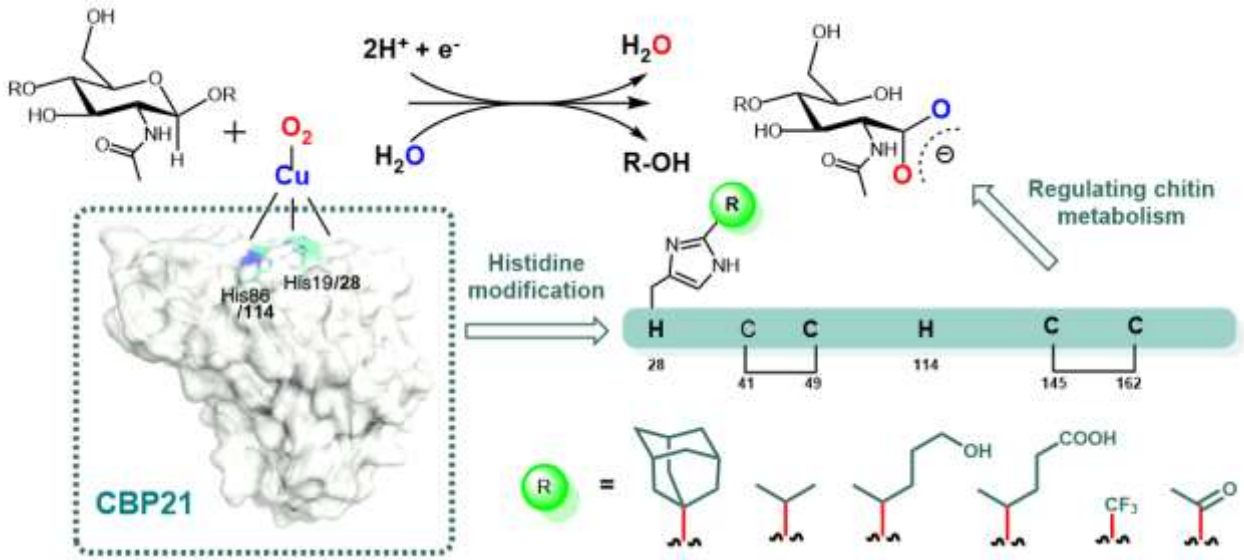
During the past decades, the effects of incorporating unnatural amino acids into proteins with potential applications in biochemistry and chemistry have been increasingly explored, and offer unprecedented opportunities to expand their utility beyond the twenty proteinogenic amino acids. However, these methods have been limited in specific scaffold of unnatural amino acids, and an extensive screening of t-RNA and aminoacyl-tRNA synthetases are required. For example, histidine plays unique roles in protein structures and functions due to its imidazole ring. It is discouraging that no effect method has been developed to replace histidine residues with its analogs, which are essential in enzyme catalysis. CBP21 is an enzyme derived from family of lytic polysaccharide monooxygenases, which can catalyze cleavage of glycosidic bond in chitin, histidine of CBP21 is crucial for its activity. [1] Here we will synthesize a library of CBP21 with various histidine modifications via a combination of express protein ligation and histidine-specific modification method. [2] We foresee the engineering of histidine residue will contribute to a deep understanding of the function of imidozle ring and offer a practical way to modify histidine residue in protein.



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Glycogen Structural Abnormality In Rat Liver With Diethylnitrosamine-Induced Carcinogenic Injury

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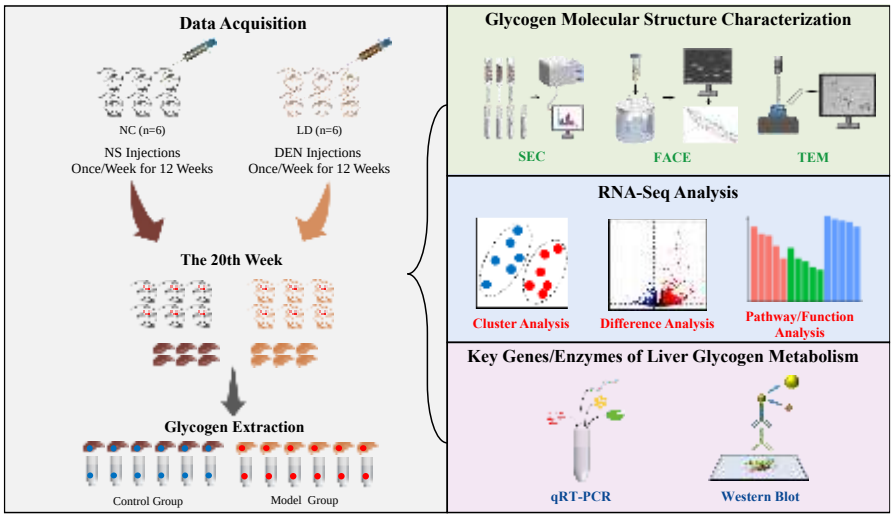
Abstract Growing evidence confirms associations between glycogen metabolic re-wiring and the development of liver cancer. Previous studies showed that glycogen structure changes abnormally in liver diseases such as cystic fibrosis, diabetes, etc. However, few studies focus on glycogen molecular structural characteristics during liver cancer development, which is worthy of further exploration. In this study, a rat model with carcinogenic liver injury induced by diethylnitrosamine (DEN) was successfully constructed, and hepatic glycogen structure was characterized. Compared with glycogen structure in the healthy rat liver, glycogen chain length distribution (CLD) shifts towards a short region. In contrast, glycogen particles were mainly present in small-sized β particles in DEN-damaged carcinogenic rat liver. Comparative transcriptomic analysis revealed significant expression changes of genes and pathways involved in carcinogenic liver injury. A combination of transcriptomic analysis, RT-qPCR, and western blot showed that the two genes, *Gsy1* encoding glycogen synthase and *Gbe1* encoding glycogen branching enzyme, were significantly altered and might be responsible for the structural abnormality of hepatic glycogen in carcinogenic liver injury. Taken together, this study confirmed that carcinogenic liver injury led to structural abnormality of hepatic glycogen, which provided clues to the future development of novel drug targets for potential therapeutics of carcinogenic liver injury.



Graphical Abstract

Glycomimetics In Ligand Directed Protein Profiling For Selective Labeling Of Glycoside Hydrolases

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Glycoside hydrolases together with glycosyl transferases represent the two largest families of so-called carbohydrate-active enzymes (CAZymes) [1,2]. Their combined activities lead to a sensitive homeostasis of formation and degradation of carbohydrate presenting metabolites. Due to the essential role of glycoside hydrolases in cells, including metabolism, antibacterial defense, and pathogenesis, profiling of the location and amount of active glycosidases is essentially important. Due to their delicate structure, often presenting a pocket-shaped active site leaving little space for large probes and tags, probe design targeting glycosidases is challenging [1]. Ligand-directed chemistry (LDC) [3,4] as a variant of well-known activity-based protein profiling (ABPP) [5,6] enables covalent, chemical modification of a protein of interest (POI). Essential for the LDC probe design is the availability of a ligand (Fig. 1, A) for the target protein. The linker region, connecting the ligand (A) and the terminal reporter tag (C), is equipped with a cleavable electrophilic reactive group (B), which is used for covalent bond formation. This is enabled by the presence of a nucleophilic amino acid residue located in close proximity to the ligand binding site of the POI [3]. As a consequence of the mechanism of LDC and the use of reversible inhibitors as ligands, the labeled protein remains its activity after covalent tagging [3,4]. Here we present the design, synthesis and biological evaluation of glycomimetic based probes for selective profiling of glycosidases, applying the ligand-directed chemistry approach. Experimental details and results of biological activity will be presented.

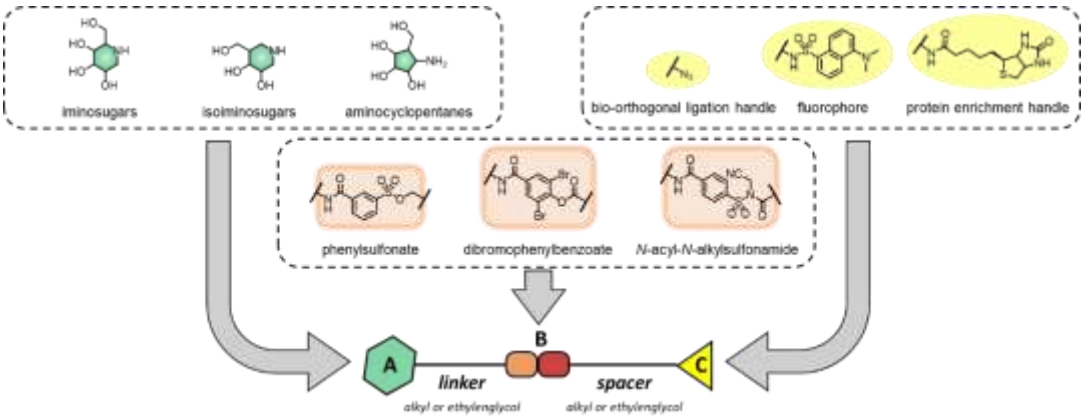

Figure 3: Building block concept for ligand directed chemistry (LDC) probes targeting glycoside hydrolases. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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Development Of Tpe-Based Fluorescent Molecular Rotors For Real-Time Biosensing Of Specific Receptors

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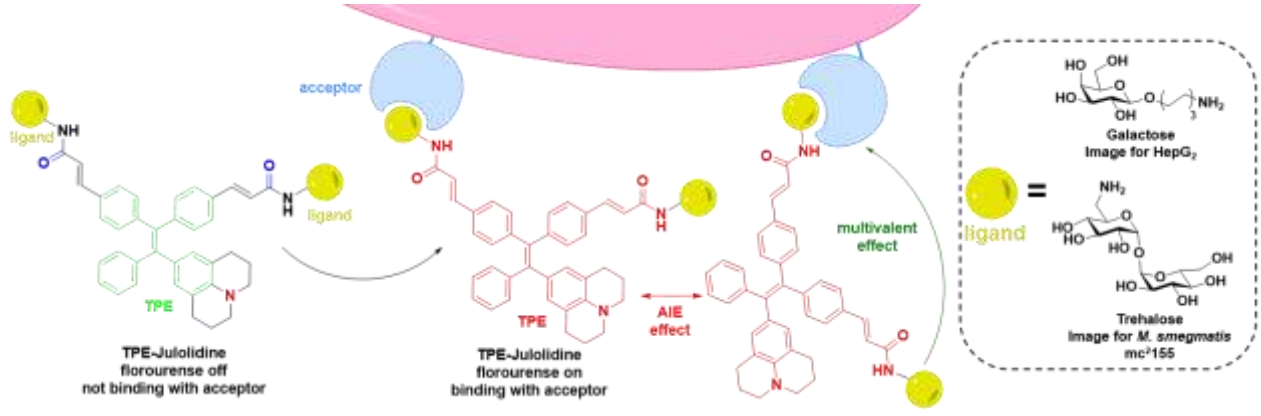
Tetraphenylethylene (TPE) compounds have gained significant interest as aggregation-induced emission (AIE) fluorophores due to their simple structure, ease of synthesis, and modification in recent years. Hence, we have designed a fluorogenic probe combined with tetraphenylethylene (TPE) and julolidine moieties. According to these two structures, we anticipate that the probe will exhibit aggregation-induced emission (AIE) and twisted intramolecular charge transfer (TICT), and it will show a redshift in the absorption and emission spectra compared with traditional TPE. Moreover, TPE has been demonstrated to exhibit its AIE effect on cell membrane imaging[1] by altering membrane fluidity to changing viscosity. We aim to linked two ligands, galactose and trehalose, with the probe. Because of galactose-binding receptors are present on cancer cell membranes and trehalose plays a vital role in the synthesis of the cell wall in *M. tuberculosis.* Additionally, interactions between glycans and glycan-binding proteins (GBPs) are usually weak and irreversible[2]. Thus, we have synthesized TPE-Julolidine linked two galactoses for the further multivalent effects[3]. Futhermore, We develope a new method to have an efficient *O*-sialyation at room temperature by using 2-Fluorosialyl donor.[4] In the future, we will use this method to synthsize sia-α linkage which is common discovered on cancer cell. We aim to linked it with TPE-Julolidine to image cancer cells.



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Biosynthesis Of Fungal Cell Wall Polysaccharides: Molecular Basis, Drug Action And Drug Resistance Mechanism

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Invasive fungal infections cause over 3 million deaths annually, posing a serious threat to public health [1]. The current antifungal therapies suffer from daunting challenges such as limited classes of drugs and emerging drug-resistant strains [2]. The synthesis of β-1,3-glucan and chitin, the core components of fungal cell wall, are appealing targets for antifungal drugs such as echinocandin and nikkomycin Z [3]. However, the lack of their mechanistic insights has hampered further drug development.

Here we present multiple evidences to firmly demonstrate for the first time that FKS1 is the specific β-1,3-glucan synthase [4]. Further structure-guided functional characterizations identify the active site for glucan polymerization and a glucan translocation path across the membrane. Drug-resistant mutations are clustered at a region near TM5–6 and TM8 of FKS1, depicting a possible drug binding site. The structure of FKS1 S643P reveals altered lipid arrangements in this region, suggesting a novel drug-resistant mechanism.

As for the chitin biosynthesis, we report the cryo-EM structures of fungal chitin synthase Chs1 in apo- and NikZ (nikkomycin Z)-bound forms [5]. Chs1 forms a unique dimeric functional form. These structures and functional analysis reveal crucial catalytic residues for chitin polymerization and a membrane tunnel for chitin translocation. NikZ specifically binds in the active site and act through a competitive mode.

Our work on FKS1 and Chs1 reveals the desired molecular basis underlying fungal cell wall biosynthesis. It will serve as a framework for developing broad-spectrum drugs against invasive fungal infections.

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Development And Application Of High-Affinity Ligands For Siglecs In Tumor Immunotherapy

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Sialic acid binding immunoglobulin-like lectins (Siglecs) are expressed on the majority of immune cells, which play key roles in antigen endocytosis and immune cell signaling through interaction with sialoglycan ligands [1]. Because of their involvement in the formation of immune tolerance and tumor immune escape, Siglecs have been recognized as novel glyco-immune checkpoints and become potential therapeutic targets for human autoimmune diseases and tumor immunotherapies. To target the Siglecs, high-affinity ligands have been developed by chemical modifications of sialic acid [2] which were applied to suppress the activity of immune cells and deliver toxins to tumor cells. However, due to the low affinity, poor specificity and synthetic issues, current Siglec high-affinity ligands have limit applications in the fundamental research and therapeutic fields. Here, by rationally engineering the NmCSS (CMP-sialic acid synthetase from *Neisseria meningitidis*), a key enzyme to activate the Sia, we successfully broaden its substrate scope by screening a library of sialic acid derivatives, which were prepared by click chemistry. One-pot Two Enzymes (OPTE, combination of NmCSS mutant and sialyltransferase) approach allows us to enzymatically transfer sialic acid derivatives to the acceptors on cell surface in one step. Moreover, developed OPTE strategy was utilized to glyco-engineer the immune cells, facilitating to target tumor cells, therefore increasing the antitumor activity and specificity.

Key words: Siglecs, protein engineering, cell surface glycan editing, high affinity ligands

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Structure-Guided Discovery Of Protein And Glycan Components In Native Mastigonemes

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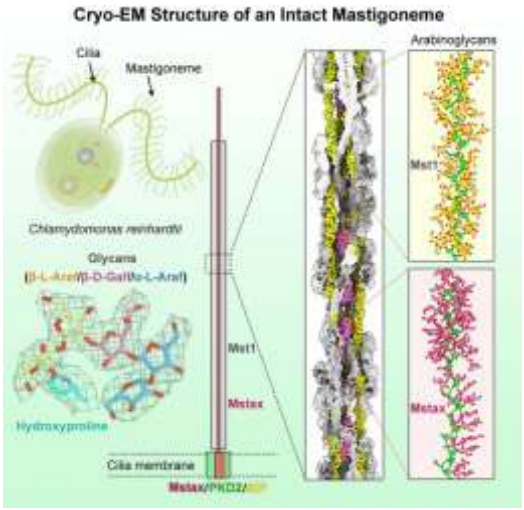
Sugar is essential for life: Carbohydrate molecules represented by glucose are one of the main energy sources for cell metabolism. The cell wall in plants is composed of cellulose. Glycosylation is also one of the major forms of Posttranslational modifications (PTM), which can significantly expand the functional diversity of proteins. However, the intrinsic stereochemical complexity of glycan molecules has hindered the systematic study of them, and the lack of information on the sugar-containing structure has severely limited human understanding of the functions of this important group of biomolecules. In this study, we used a combination of biophysics, cell biology, and bioinformatics methods to elucidate the molecular mechanism of glycans in building biomacromolecules. Plants and algae are known to possess a unique form of Hyp O-glycosylation in which the glycan module consists mainly of arabinose with a small amount of galactose. This Hyp O-glycosylation dependent form is fundamental for plants and algae to exercise normal life activities. Previously, no structural information about glycan-mediated assembly of bio-architectures was available. In this study, a density of more than 1000 sugar molecules was clearly observed, which is the largest complex structure containing sugar molecules. By analyzing the interaction between polysaccharides and proteins, this study revealed the key role of arabinoglycans in the assembly of biological structures, providing important clues to understand the role of structural glycans in life processes, and reflecting the transformation of modern structural biology from a tool for structural confirmation to a tool for de nove discovery.



Huang, Junhao (黄隽豪) et al. “Structure-guided discovery of protein and glycan components in native mastigonemes.” Cell vol. 187,7 (2024): 1733-1744.e12. doi:10.1016/j.cell.2024.02.037

Convergent Synthesis And Anti-Pancreatic Cancer Cell Growth Activity Of A Highly Branched Heptadecasaccharide From *Carthamus Tinctorius*

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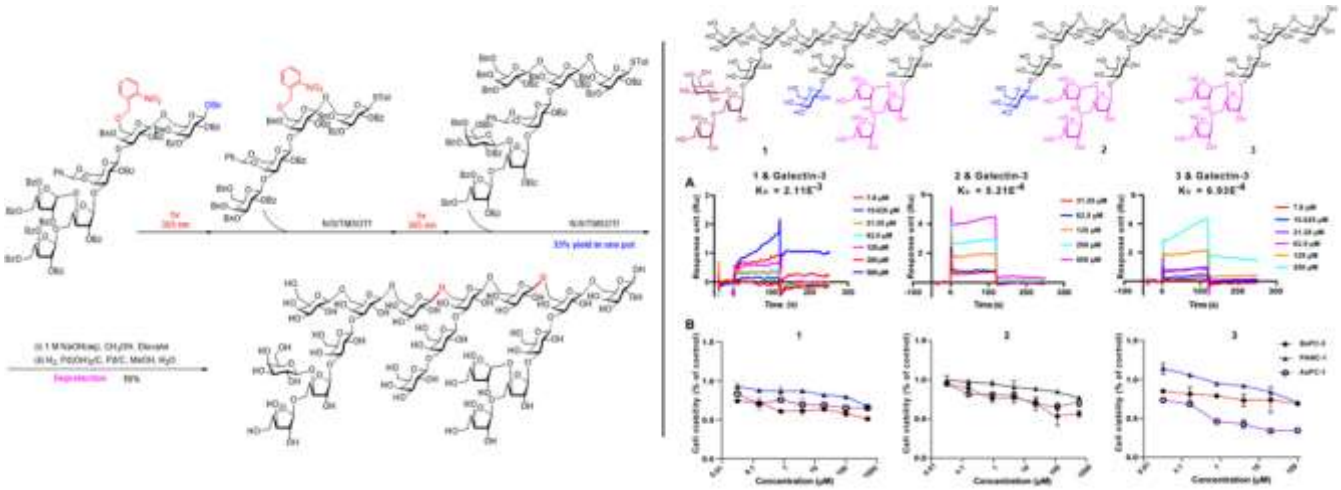
Bioactive polysaccharides from natural resources target various biological processes and are increasingly used as potential target molecules for drug development. However, the accessibility of branched and long complex polysaccharide active domains with well-defined structures remains a major challenge. Herein we describe an efficient first total synthesis of a highly branched heptadecasaccharide moiety of the native bioactive galectin-3-targeting polysaccharide from Carthamus tinctorius L. as well as shorter fragments of the heptadecasaccharide. The key feature of the approach is that a photo-assisted convergent [6+4+7] one-pot coupling strategy enables rapid assembly of the heptadecasaccharide, whereby a photoremovable o-nitrobenzyl protecting group is used to generate the corresponding acceptor for glycosylation in situ upon ultraviolet radiation, during which the aglycon transfer was eliminated because the sequence of assembly is from reducing end to non-reducing end of the oligosaccharide. Biological activity tests suggest that the heptadecasaccharide can target galectin-3 and inhibit pancreatic cancer cell growth. This work demonstrates a representative example to understand the active domains of the polysaccharide, which could be synthesized for structure-activity relationship studies, allowing for further structure modification and potential drug candidate development.



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Total Synthesis Of Curcluigoside A – Arylglucoside From Medicinal Plant Curculigo Orchioides

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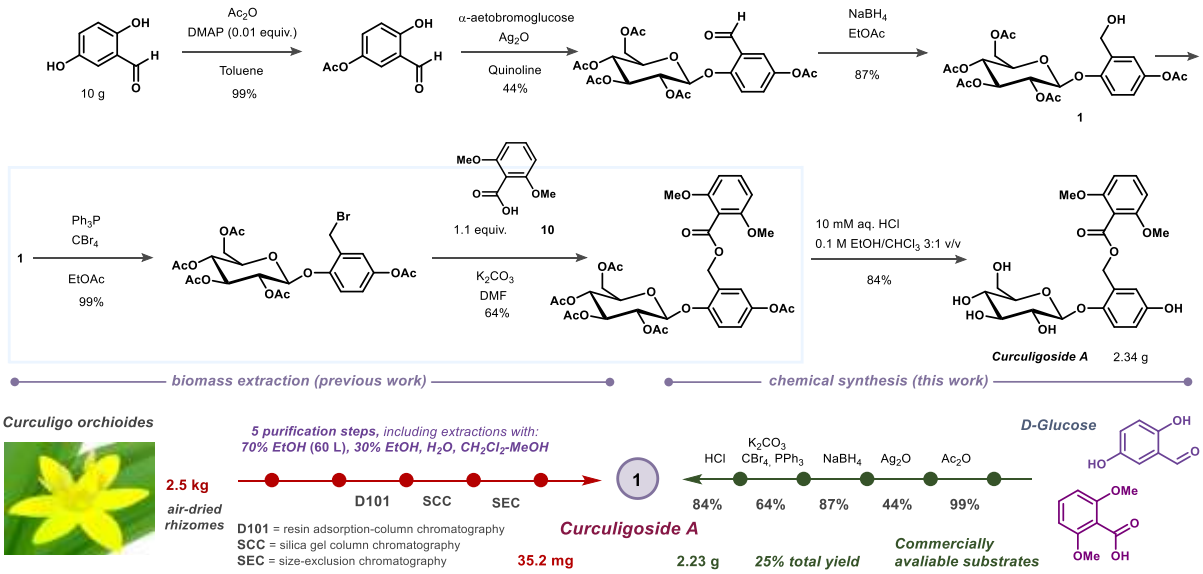
Curculigo orchioides Gaertn. (*Amaryllidaceae*) is abundantly found in subtropical regions of Asia, particularly southern China and India. It is esteemed in traditional medicine and is utilized for treating osteoporosis, menorrhagia, [1] and is believed to possess aphrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer and antidiabetic properties. [2] The dried rhizome of *C. orchioides* is notably rich in phenolic glycosides, curculigoside A being predominant component in itd ethanolic extract. [3] Research suggest its potential as a neurovascular recovery agent for stroke and brain injury, [4] a therapeutic agent for osteoporosis, [5] and its relevance in studying the mechanism of Parkinson's disease and its prevention. [6]



Traditional methods of obtaining the valuable substance through extraction are inefficient and demand significant energy and resources. For example, from 2.5 kg of dry plant material, 35.2 mg of pure product was obtained. [5] Our proposed synthesis yields 2.32 g of curculigoside A, from 10 g of the starting 2-hydroxysalicylic aldehyde. Total organic synthesis offers the potential to producea chemically pure substance in substantially larger quantities, and the developed synthesis is versatile, enabling the production of several types of arylglycosides of the curculigoside family. The Ministry of Education and Science of the Russian Federation (Program No. 075-03-2024-118/1) is gratefully acknowledged.

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Glycosidic Linkage Analysis Of Arabino-Mannan Oligosaccharides In Carbohydrate-Protein Interactions

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Arabinomannan oligosaccharides are putative glycoconjugates in the bacterial cell wall structures. Whereas their bacterial cell wall functions are studied intensely, synthetic congeners of arabinomannans aid the in-depth analysis of their protein binding behaviour. A study was undertaken to identify the lectin binding behaviour of a series of arabinomannan oligosaccharides (**figure 1**), in order to evaluate the linkage dependence on the lectin binding. Carbohydrate-protein interactions gain good binding affinities through multivalency, either intra- (cis) or inter- (trans) molecular binding possibilities. The synthetic derivatives provide a route to assess these binding properties. Synthetic di-, tri- and pentasaccharide arabinomannans, possessing α-D-mannopyranoside at the non-residing end and an α-D-arabinofuranoside at the core, are undertaken for the study. The synthetic ligands are subjected to studies with lectin Con A, through isothermal titration calorimetry, in addition to dynamic light scattering and atomic force microscopy. Among the ligands, one trisaccharide and the pentasaccharide exhibit lectin binding, adhering to the bivalent structural and functional valencies. Whereas the remaining oligosaccharides display only a functional monovalency, even when the ligands possess bivalent structural valency. The trisaccharide with (1→2)(1→3) glycosidic bond connectivity, fulfilling both structural and functional valencies, stands out as the smallest bivalent ligand, engaging in lectin interaction through a trans-mode.

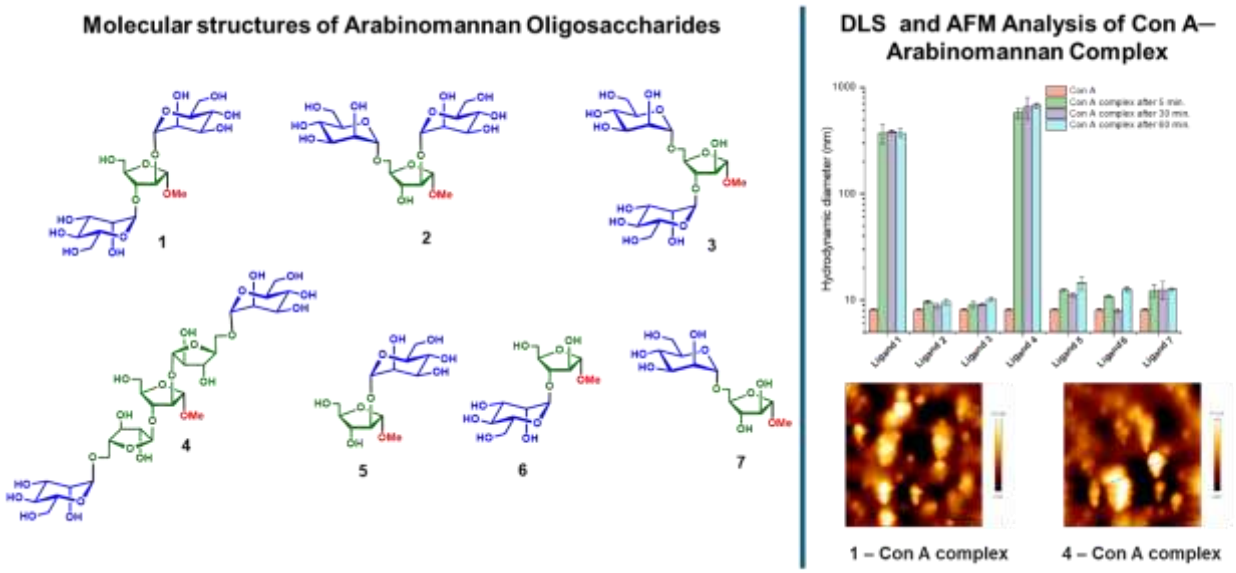

Figure 1. Molecular structures of Arabinomannan ligands and Plot of the changes in the hydrodynamic diameters of Con A upon complexation with ligand **1**-**7**.

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Synthesis Of Difluoromethylene Bisphosphonates As Pyrophosphate Mimetics To Probe Bacterial Capsular Polysaccharide Biosynthesis Machinery

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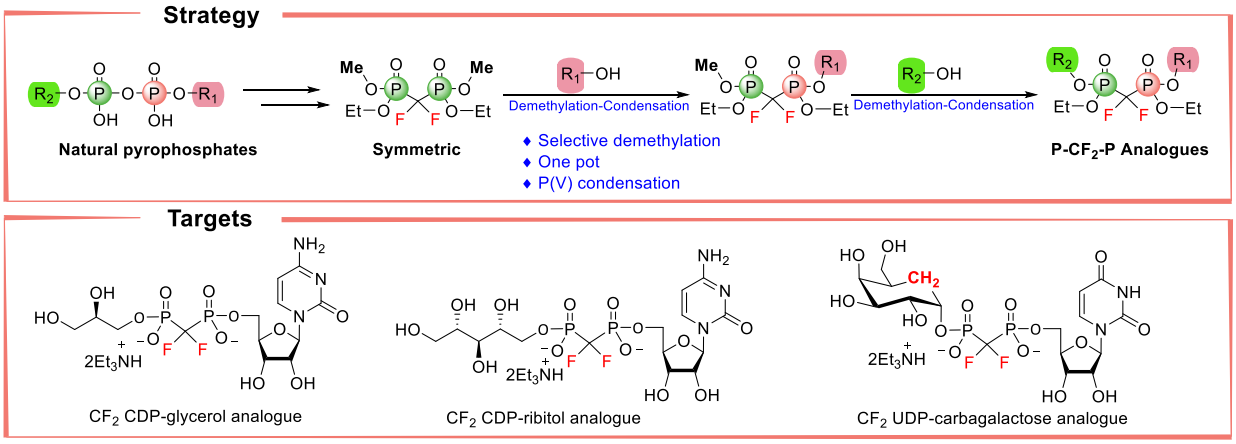
Naturally occurring pyrophosphates play important roles as building blocks in numerous vital biological processes, such as metabolism and the biosynthesis of bacterial capsular polysaccharides and teichoic acids.[1] The chemical modification of natural pyrophosphates has delivered critically important tools to study the mechanism of pyrophosphate processing enzymes and the use of these mimics in studying the function of a wide variety of enzymes is well documented. The sensitivity of the P-O-P bond towards both enzymatic and chemical hydrolysis offers a unique opportunity for the design and synthesis of stable non-hydrolysable inhibitors. In particular, difluoromethylene bisphosphonate-linked (P-CF_2-P) analogues are highly sought after as these analogues closely resemble the natural pyrophosphate, in terms of pKa-value (as the electron-withdrawing effect of fluorine atoms increase the acidity of the phosphonates), as well as the bond angles and lengths.[2]

We will present a synthetic method to generate difluoromethylene bisphosphonate analogues of various natural products using a desymmetrization strategy of diethyl- (dimethyl)difluoromethylene bisphosphonate which can be orthogonally deprotected to set the stage for one or two consecutive condensation reactions through P(V) condensation chemistry.[3]

These analogues will be used to probe and inhibit their cognate enzymes, to advance our understanding of the biosynthesis of components of bacterial cell wall and contribute to the development of novel therapeutic agents, and antimicrobial strategies.



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Affinity-Based Covalent Sialyltransferase Probes

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Sialyltransferases (ST) are enzymes that catalyses sialylation, which is the transfer of sialic acids onto growing glycan chains. Sialylation plays many important roles in healthy human physiology, but it has also been implicated as a key cancer and bacteria pathogenicity marker.[1–5] Various sialic acid mimics have been developed as reversible inhibitors and fluorescent probes to aid in the study of ST[6–8], but their use is limited by the lack of a stable covalent linkage to the enzyme.

While activity-based covalent probes have been successfully developed and proved invaluable in profiling and studying many glycosidases[9,10], such probes have not been reported for ST or glycosyltransferases in general. This effort is in part hindered by the single displacement S_N2 mechanism by which all ST and many glycosyltransferases operate[11–13], where no covalent intermediate with the enzyme is formed and therefore a stable covalent adduct cannot be trapped by stabilising such an intermediate.

Here we report a strategy to develop covalent probes for ST using ligand-directed chemistry, resulting in so-called affinity-based probes. Such an approach has been widely applied in protein labelling and profiling.[14] However, its application in chemical glycobiology research is scarce, with only one example reported on isolated glycosidases.[15]

We here show that these affinity-based probes are highly specific for the sialyltransferases selected in our study. We further demonstrate the utility of these probes by *in vitro* and *in vivo* labelling of sialyltransferases in bacteria.

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Switchable and Stereoselective Glycosylation Enabled by Electroactivation of Glycosyl Imidates

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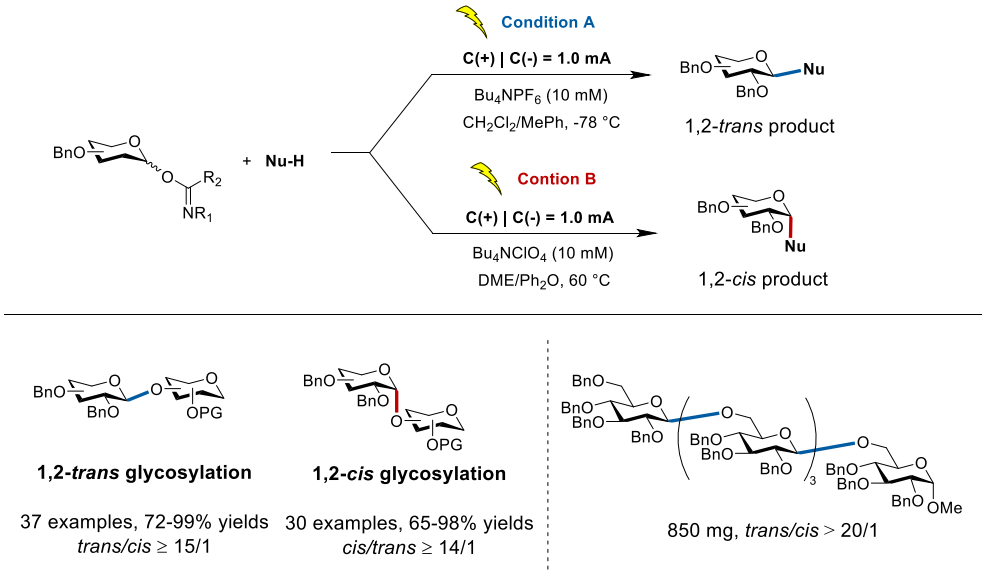
Glycosyl imidate donors promoted by Lewis-acid have been widely used in glycosylation reactions[1][2], but the activation of glycosyl imidates under neutral condion remains an unsolved challenge. We developed a neutral electro-glycosylation from *N*-phenyl-trifluoroacetimidates, trichloroacetimidates and other glycosyl imidates. Highly 1,2-*trans*- and 1,2-*cis*-stereoselective glycosylations have been achieved under corresponding electrolysis conditions. This method was suitable for a wide array of substrates and displayed good reaction yields and stereoselectivity. The electro-glycosylation proceeded well in application to acid-sensitive substrates. The value of this method was further demonstrated by a gram-scale synthesis of β-(1→6)-glucose pentasaccharide. Mechanistic studies indicated that this reaction involved a radical intermediate and anodic oxidation of imidate was key to the reaction.



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Glycosylation Of 2, 4-Oh Mannosides And Galactoside Acceptors And Their Regioselective Control

Yang-Fan Guo, Jian-Cheng Yu and Hai Dong*

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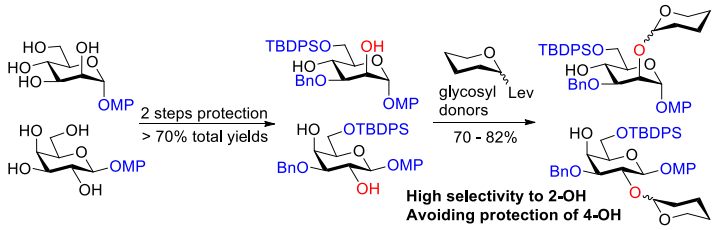

In the synthesis of oligosaccharides, due to multiple hydroxyl groups on the glycosyl acceptor being potential sites for glycosylation coupling, it is unavoidable to encounter the trouble of how to control the acceptor coupling site. One solution is to use acceptors with a minimal number of protecting groups (minimizing the need for protection and deprotection operations in the synthesis of the acceptors) in regio/site-selective glycosylation strategies.[1] In such strategies, the hydroxyl group involved in the glycosylation reaction must exhibit much higher reactivity than the other hydroxyl groups since the acceptor contains at least two hydroxyl groups. It is possible to reduce or even avoid the protection/deprotection operations in the preparation of glycosyl acceptors, and regio/site-selective glycosylation strategies are therefore of great importance for the efficient synthesis of oligosaccharides. Based on our previous studies, regioselective protection of 3- and 6-OHs of mannoside and galactoside is readily achieved.[2,3,4] Therefore, we systematically investigated the regioselective glycosylation of 2, 4-OH mannoside and galactoside acceptors by altering the protecting groups of their 1,3,6-positions. The results showed that their 2, 4-OHs exhibited different regioselectivity during glycosylation under the influence of different protecting groups and regioselective glycosylation of their 2-OHs was successfully achieved. By using the *p*-methoxyphenyl 3-OBn, 6-OTBDPS, α-mannoside and β-galactoside acceptors, their glycosylation with various glycosyl donors led to 1,2-linked products in 70-82% yields.

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Synthesis Of Deoxy Pyranosides Via Fe(III) Catalyzed Regioselective Thiocarbonylation Of Partially Protected Carbohydrates

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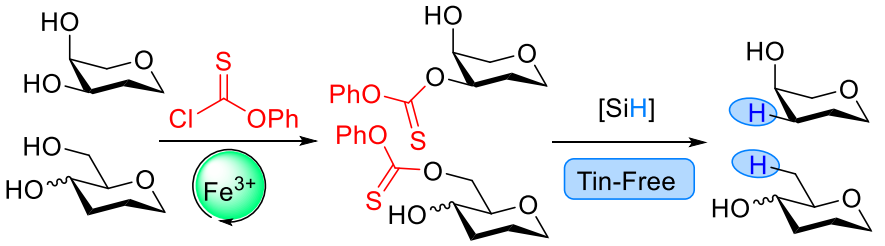
Rapid and efficient synthesis of targeted deoxygenated glycosides is highly desired. Herein, we disclose a two-step procedure for the synthesis of deoxysugars from partially protected pyranosides via site-selective thiocarbonylation followed by tin-free Barton–McCombie deoxygenation. The selectivity can be smoothly achieved through $FeCl_3$ as the catalyst assisted by low-priced benzoyl trifluoroacetonate (Hbtfa) ligand in the presence of K_2CO_3 as base. Further deoxygenation can be carried out using greener silicon reagent, avoiding highly toxic organotin reagents in traditional procedures. Conspicuous features of this method include mild conditions, easy operation, high selectivity and non or low toxic reagents, making it a more attractive strategy.



● *Excellent selectivity* ● *Straightforward operation* ● Tin-free conditions

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Regiodivergent Functionalization Of Protected And Unprotected Carbohydrates Via An Adaptive Activating Strategy

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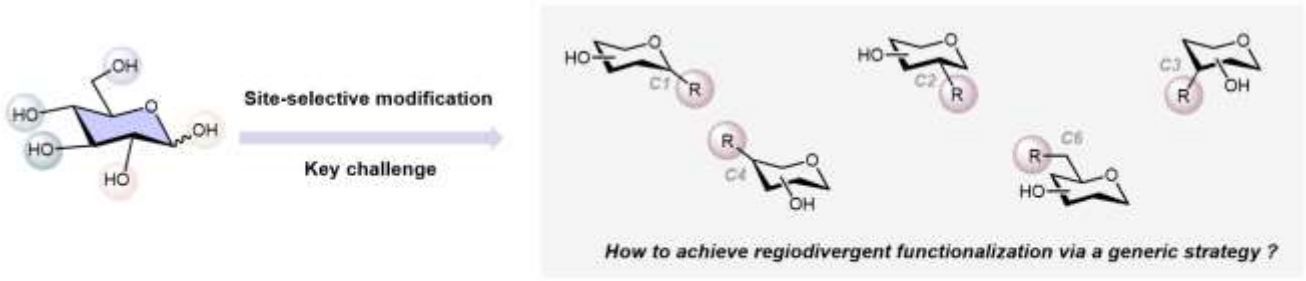
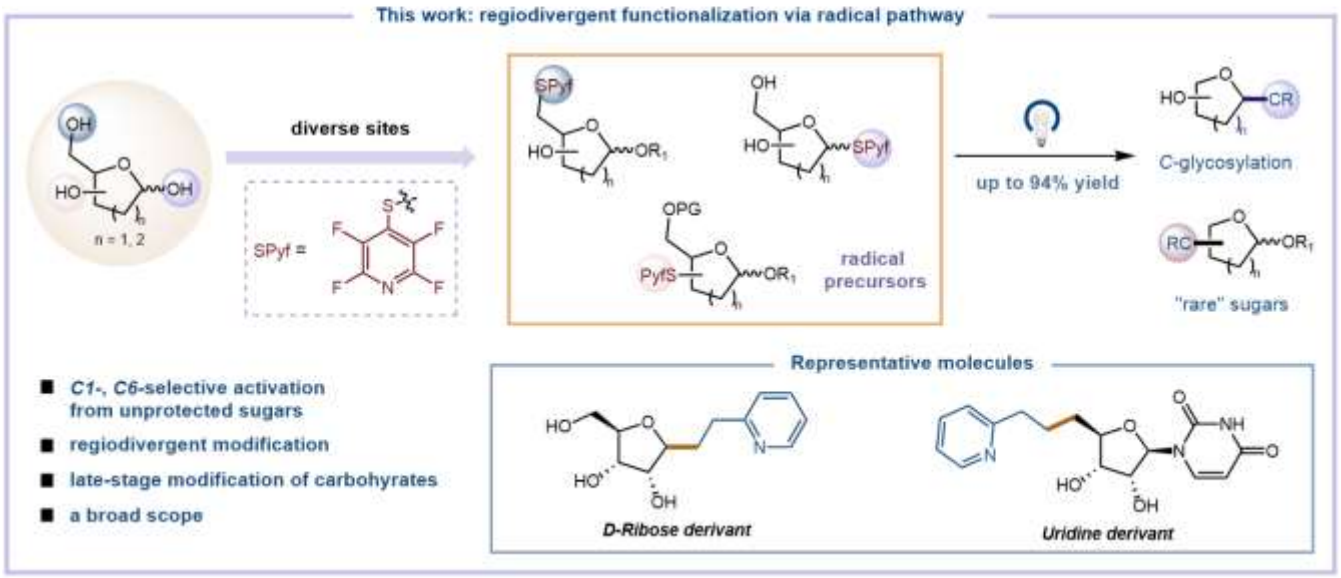
Hangzhou Institute for Advanced Study,UCAS

The selective functionalization of carbohydrates holds a central position in synthetic carbohydrate chemistry, driving the ongoing quest for ideal approaches to manipulate these compounds. In this study, we introduce a general strategy that enables the regiodivergent functionalization of saccharides. The use of electron-deficient photoactive 4-tetrafluoropyridinylthio (SPyf) fragment as an adaptable activating group, facilitated efficient functionalization across all saccharide sites. More importantly, this activating group can be directly installed at the C1, C5 and C6 positions of biomass-derived carbohydrates in a single step and in a site-selective manner, allowing for the efficient and precision-oriented modification of unprotected saccharides and glycans**.**





Photosensitizer-Free Visible-Light-Promoted Glycosylation Enabled By 2-Glycosyloxy Tropolone Donors

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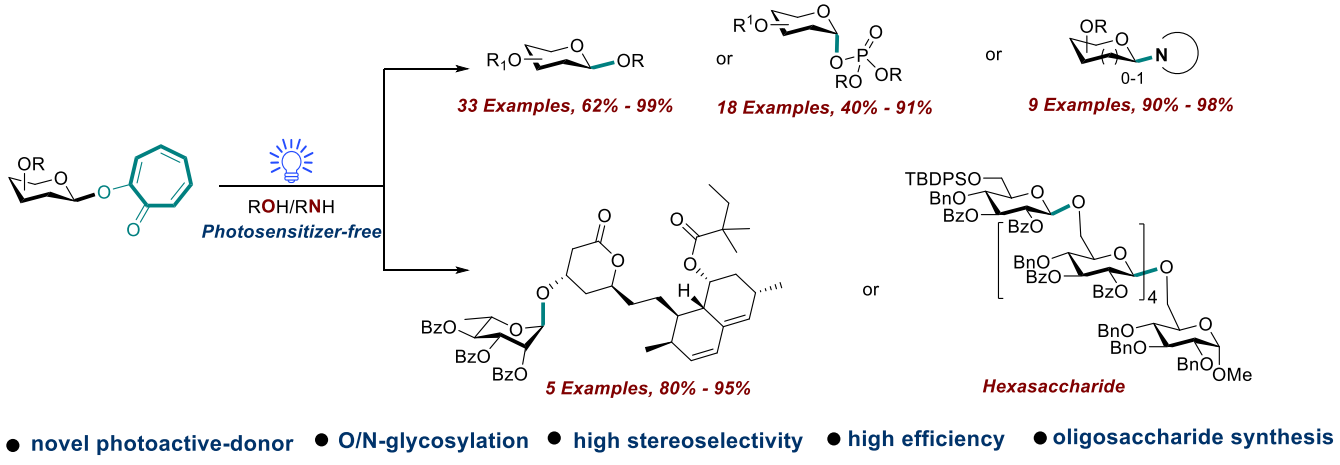
Abstract: Photochemistry, as a key technology in organic synthesis, has attracted widespread attention in recent years for its application in photochemical glycosylation[1-3]. However, to the best of our knowledge, visible-light-promoted glycosylation via photoactive glycosyl donor has not been reported. In the study, we reported report a novel photosensitizer free visible-light-mediated glycosylation approach using a photoactive 2-glycosyloxy tropone as the donor. This glycosylation reaction proceeds at ambient temperature to give a wide range of *O*-glycosides or oligosaccharides with yields up to 99%. This glycosylation method is further applied in the stereoselective preparation of various functional glycosyl phosphates/phosphosaccharides. Perbenzylated 2-glycosyloxy tropolone donors could react with phosphate acceptors to obtain α-glycosylphosphate/glycosyl phosphosaccharides with high yields and selectivity, which α/β ratio up to 20/1. 2-Glycosyloxy tropolones can also be used for visible-mediated *N*-glycosylation reactions to obtain corresponding pyranosyl-/furanosyl-N-glycosides in 90% to 98% yields. Glycosylation of natural products and pharmaceuticals is evaluated on a gram-scale by using the above glycosylation method. The mono/di-glycosylation of 2-glycosyloxy tropolone with epiandrosterone, estradiol benzoate, β-estradiol, cholesterol and simvastatin generated the corresponding glycosides (1.20-1.70 g) with 80-95% yields. Furthermore, a hexasaccharide is prepared via iteratively visible-light-promoted glycosylation and desilylation in a satisfactory isolated yield. The mechanism of the glycosylation reaction was preliminarily investigated. The protocol featured features uncomplicated conditions, operational simplicity, wide substrate scope (58 examples), excellent compatibility with functional groups, scalability of products (7 examples), and high yields. It provides an efficient glycosylation method for accessing *O*/*N*-glycosides and glycans.



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Stereoselective Synthesis Of O-antigen Of *A. baumannii* ATCC 17961

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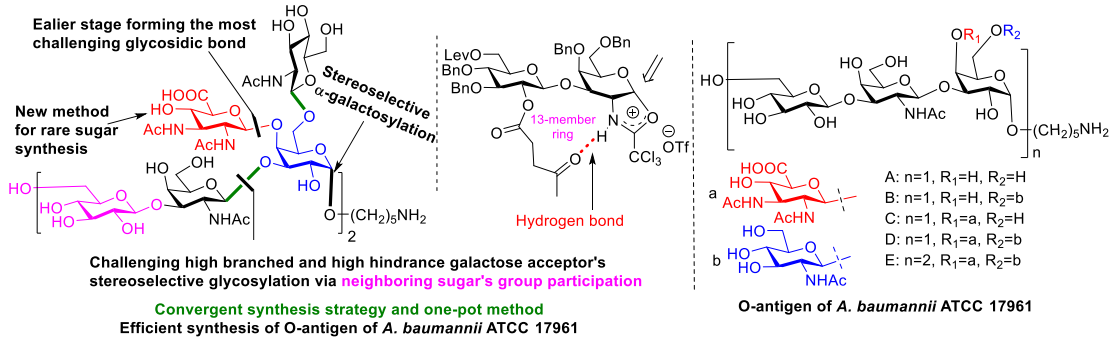
Abstract: *Acinetobacter baumannii* is currently posing a serious threat to global health. Lipopolysaccharide (LPS) is a potent virulence factor of pathogenic Gram-negative bacteria. To explore the antigenic properties of *A. baumannii* LPS, *A. baumannii* strain ATCC 17961 O-antigen were synthesized. The oligosaccharide molecules include trisaccharide **A**, tetrasaccharide **B** and tetrasaccharide **C**, pentasaccharide **D** with single repeating unit and decasaccharide **E** with two repeating units. The efficient synthesis of the rare sugar 2,3-diacetamido-glucuronate was achieved using our recently introduced organocatalytic glycosylation method. Next, it is the first time to find that neighboring sugar's levulinoyl group participation via a hydrogen bond in glycosylations can result in a significantly improved β-selectivity, solving the stereoselectivity problem of high branched galactose acceptors. The proposed mechanism was supported by control experiments and DFT computations. Benefiting from the long-range levulinoyl group participation strategy, pentasaccharide were obtained via an efficient [2 + 1 + 2] one-pot glycosylation method.



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Synthesis Of Polyporus Polysaccharide Fragments And Oligosaccharide Conjugates For Structure-Activity Relationship Study

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Polyporus umbellatus (Pers.) Fries is a fungus belonging to the family Polyporaceae. The sclerotium is its main medicinal part and has been widely used in Asia for treatments of edema, scanty urine, vaginal discharge, as well as jaundice and diarrhea. Polyporus polysaccharides (PPS) have been reported as one of the major ingredients responsible for its activities such as diuretic, anti-tumor, anti-oxidation, immune regulation and DNA repair and the activities of PPS are closely related to their chain conformation and structure. However, the structure of PPS isolated from natural source is of micro-heterogenity, which deters the study of structure-activity relationship(SAR) of PPS. In this study, PPS fragments with defined structure were synthesized by chemical method, and polyporus oligosaccharide conjugates were prepared efficiently. This will lay a foundation for PPS SAR study and provide a new insight into the function of carbohydrates.

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Sterereoselective Synthesis Of *O*-Glycosides With Borate Acceptors

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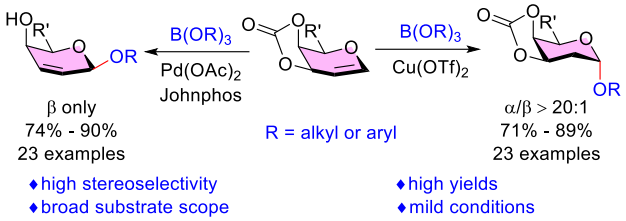
Borate esters have been applied widely as coupling partners in organic synthesis. However, the direct utilization of borate acceptors in *O*-glycosylation with glycal donors remains underexplored. Herein we describe a novel *O*-glycosylation resulting in the formation of 2,3-unsaturated *O*-glycosides and 2-deoxy *O*-glycosides mediated by palladium and copper catalysis respectively. This *O*-glycosylation method tolerated a broad scope of trialkyl/triaryl borates and various glycals with exclusive stereoselectivities in high yields. All the desired aliphatic/aromatic *O*-glycosides and 2-deoxy *O*-glycosides were generated successfully, without the hemiacetal by-products and O→C rearrangement because of the nature of borate esters. The utility of this strategy was demonstrated by functionalizing the 2,3-unsaturated glycoside products to form saturated β-O-gulosides, 2,3-deoxy *O*-glycosides and 2,3-epoxy *O*-glycosides.



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SODS/Tf_2O: A Potent Combo For *S*-Glycosides Activation

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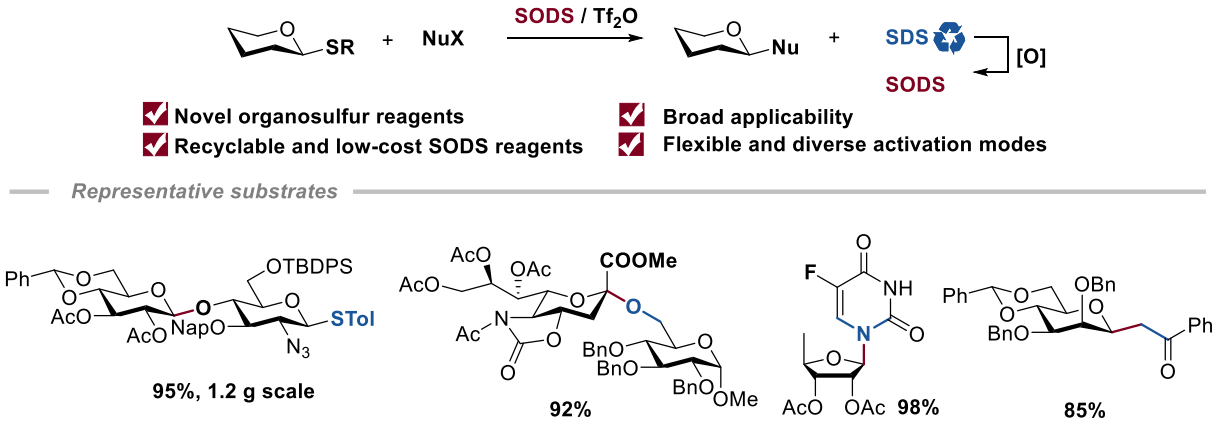
S-glycosides are commonly utilized as glycosyl donors in oligosaccharide synthesis due to their easy preparation, good stability, and excellent compatibility with different conditions. The development of powerful and easy-handling promoters for the activation of S-glycosides is an ongoing area of research. In this study, we present the stable and low-cost SODS reagents in combination with triflic anhydride (Tf_2O) as a powerful thiophilic promoter system. This combo offers several advantages: 1) flexible and diverse activation modes such as stoichiometric and substoichiometric activation, one-shot activation, pre-activation, and progressive activation; 2) efficient and stereoselective formation of various glycosidic bonds (O-, C-, N-); 3) the ability to regenerate SODS reagents through simple oxidation.



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Synthesis Of 2-Methyl-6-Methoxy-4-Quinolinecarboxylic Acid N-Hydroxysuccinimide Ester (MMQC-Osu) For Streamlined And Effective HPLC-Based Fluorescence Detection Of Aliphatic Amines In Environmental Samples

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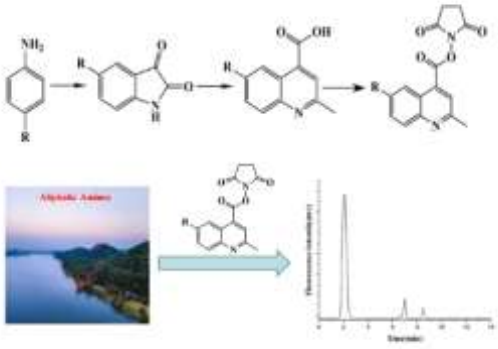
Aliphatic amines are widely distributed in the environment and food, posing a risk to human health through skin and mucosal irritation. The quantification of aliphatic amines can reflect the quality of the local environment, making their detection crucial for environmental assessment. We established an innovative synthetic route that promotes the synthesis of succinimidyl esters with an array of desirable substituents. This advancement enabled the creation of efficient and highly sensitive reagents for the detection of aliphatic amines. Among them, 2-methyl-6-methoxy-4-quinolinecarboxylic acid N-hydroxysuccinimide ester (MMQC-OSu) exhibited the best detection performance. MMQC-OSu not only exhibits high detection sensitivity, but also operates at a low reaction temperature, with a short reaction time and simple operation. This innovative synthesis method allows for the study of substituent electronic effects on the fluorescence properties of succinimidyl esters, as well as providing valuable insights into the effects of substituent polarization and spatial localization on its fluorescence properties



Keywords: MMQC-OSu, Aliphatic amines, HPLC, Succinimidyl esters,

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Glycosyl N-Phenyl Pentafluorobenzimidates: A New Generation Of Imidate Donors For Glycosylation

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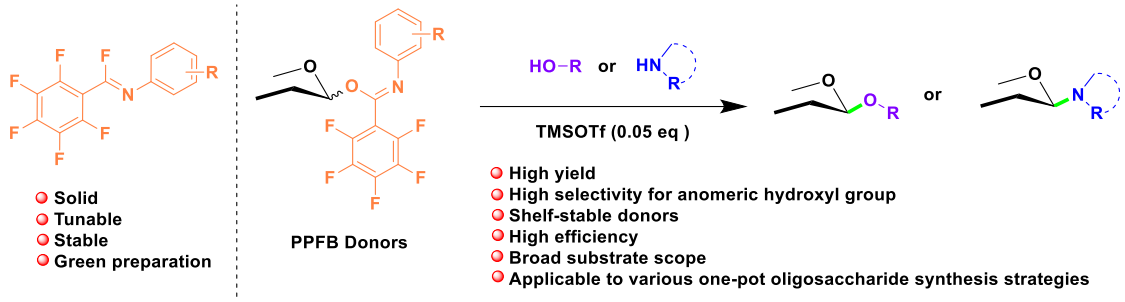
Abstract: Glycosyl imidate are among the most popular donors in the synthesis of glycans and glycoconjugates. Since the first report, a great number of imidates have been disclosed. Representative imidates include glycosyl trichloroacetimidates (TCAs) and N-phenyl trifluoroacetimidates (PTFA). The continuous efforts have brought new values on imidates, like the easy accessibility, stability and tunability, by the introduction of new structural motifs. Here we report N-phenyl pentafluorobenzimidoyl (PPFB) glycoside as a new generation of imidate donors. PPFBs can be synthesized by nucleophilic substitution on the corresponding imidoyl fluorides, which showed a higher chemoselectivity on the anomeric hydroxyl group over the others. The PPFB glycosylation features broad substrate scope, ranging from primary to tertial alcohols, electron rich and deficient phenols, glycosyl acceptors, amino acid derivative and nucleobases. These glycosylations are often in good to excellent yields, except purines. In addition, we found that PPFBs were generally more stable than PTFAs when evaluated by ^{19}F NMR over an extended period. Consistently, PPFBs showed less activity than TCAs and PTFAs in the glycosylation, which facilitates the chemoselective one-pot synthesis of glycans that is currently on the progress.



Keywords: Imidate donor; Glycosylation; N-phenyl pentafluorobenzimidoyl glycoside

Stereoselective *O*-Glycosylation Of Glycals With Arylboronic Acids Using Air As The Oxygen Source

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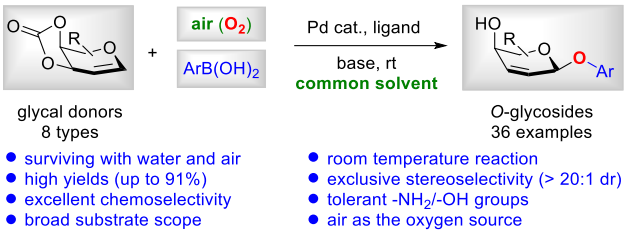

An open-air palladium-catalyzed *O*-glycosylation was developed using glycals and arylboronic acids with base additives at ambient conditions. The novel approach enabled facile access to various *O*-glycosides in high yields, with exclusive 1,4-cis-stereoselectivity tolerating reactive hydroxyl/amino groups. Mechanistic studies indicated that chemo-/stereoselectivity arose from the coordination between palladium and phenols generated in situ by oxidizing arylboronic acids, followed by an intramolecular attack. Isotope-labeling experiments revealed that the oxygen of *O*-glycosidic bonds came from O_2.

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1,3-Trans Glycosylation Of 3-Aminosugars And The Application Thereof

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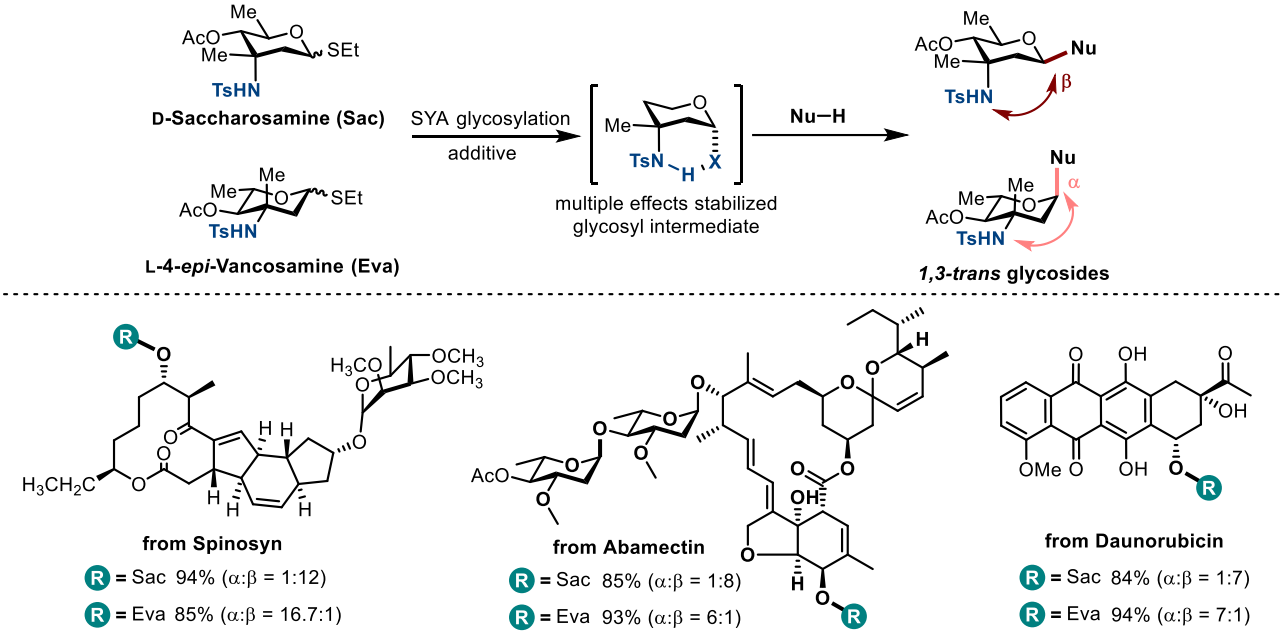
1,3-Trans-glycosidic linkages involving 3-amino glycosides, such as saccharosamine β-glycosides and 4-*epi*-vancosamine α-glycosides, play a crucial role as structural elements in various biologically active compounds and pharmaceuticals. The precise and selective formation of these acid-labile glycosidic bonds, especially in the absence of neighboring participating groups at C-2, has posed a significant synthetic challenge in the production of such 1,3-trans glycosides. In this study, a direct method for establishing 1,3-trans-glycosidic connections using thioglycosides as donors under mild reaction conditions is presented. A diverse array of substrates was investigated, leading to high levels of stereoselectivity (β-stereoselectivity for saccharosamine and α-stereoselectivity for 4-*epi*-vancosamine) facilitated by multiple effects. The efficacy of this methodology is illustrated through the structural modification of natural products and pharmaceuticals containing 3-aminosugars, as well as the synthesis of fragments of saccharomicin A and B.



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New Look At Acidic Ethanolysis Of Acetyl Groups In Carbohydrates

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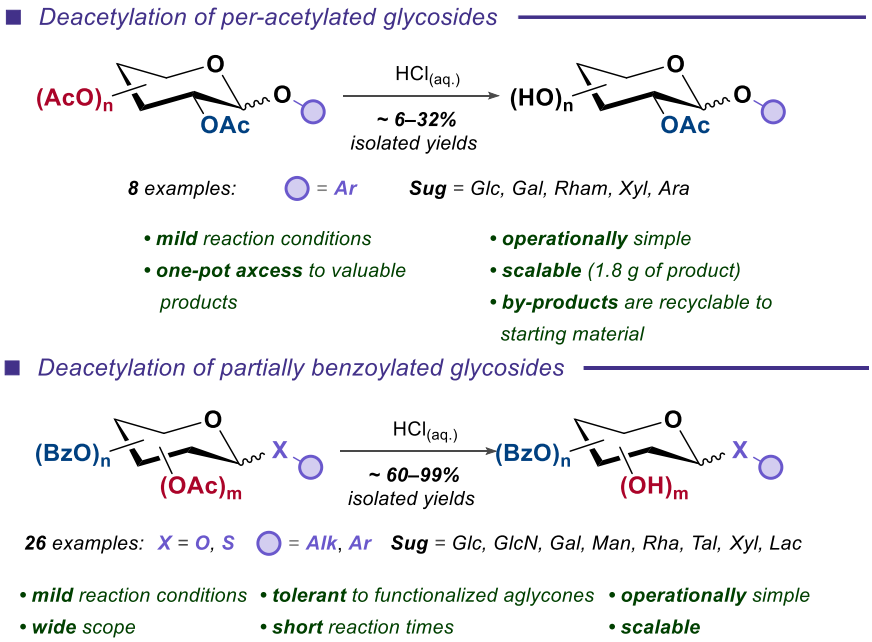
In the present work, we disclose a protocol for the regioselective deacetylation of *per*-acetylated and partially benzoylated aryl glycosides under mild acidic conditions (HCl in EtOH and $CHCl_3$). [1]This approach enables the direct synthesis of 2-O-acetylated aryl glycosides in a single step, utilizing readily available reagents and a straightforward procedure. Importantly, this methodology offers a significant reduction in synthetic steps compared to traditional multi-step strategies involving protecting groups. We have successfully applied the deacetylation protocol for the preparation 2-*O*-acetylated glycosides with moderate yields (6-23%). Further, we propose a simple method for regenerating byproducts to convert them back to the starting material, thereby enhancing the overall yield of the desired 2-O-acetate (68% of 2-*O*AcGal*p* after six cycles). Furthermore, the outlined acid-catalyzed protocol allows for the removal of acetyl groups in presence of more inert benzoyl groups using mild acidic conditions (HCl in EtOH and $CHCl_3$, 70 °C). Thus, we synthesized 26 partially benzoylated compounds with high yields (66-99%) featuring various functionalized aglycons and configurations of carbohydrate moiety. The Ministry of Education and Science of the Russian Federation (Program No. 075-03-2024-118/1) is gratefully acknowledged.



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Synthesis Of 2-Amino Imidazolium Glycosides As Novel Precursors Of *N*-Heterocyclic Carbenes

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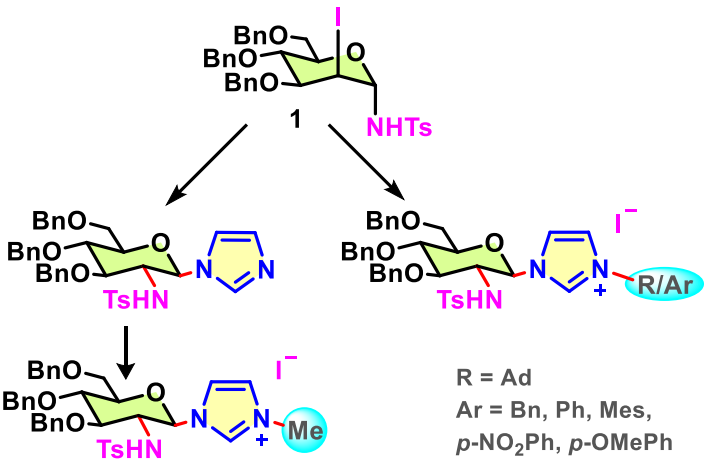
N-heterocyclic carbene (NHC) synthesis and applications have been thoroughly investigated in the field of organic asymmetric transformations during the past few decades. However, development of NHCs tethered to carbohydrates are very limited despite their inherent chirality. In 2014, Galan and co-workers[1] reported the synthesis of β-glucosamine-based NHC.HCl salts and mannosamine-based NHC.HCl salts. In 2020, the same group[2] also developed the synthesis of carbohydrate-based imidazolium salts having sterically bulky gorups at C1 and C3 of carbohydrates respectively. Maruoka and co-workers[3] synthesized C_2-symmetric chiral imidazolium salts from readily accessible chiral amines. Tomioka and co-workers[4] synthesized dihydroimidazolium tetrafluoroborate salt with the mesityl substituents on both the nitrogen atoms. Herein, we report the development of carbohydrate-based novel NHC-precursor possessing an amino group at the C2 position of the carbohydrate skeleton in two steps from readily available tri-*O*-benzyl-D-glucal proceeding thorugh the intermedaicy of iodo-sulfonamide **1**. The synthesis proceeds via the formation of the unstable aziridine ring followed by attack of substituted imidazoles at the C1 position. The synthesis of the starting material **1**[5] has already been reported from our group. The purification of these NHC-precursors involves either one time or multiple times precipitation.

- Novel NHC-precursors
- One-step synthesis
- Excellent yield
- Highly scalable
- Having a C2-amino group
- Simple purification technique



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Study On Structural Characteristics, Bioactivity And Targeting Molecule Of *Campsis Grandiflora* Polysaccharide

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The *Campsis grandiflora* (Thunb.) Schum. flower possesses significant medicinal value due to its effects of clearing heat and cooling blood, resolving blood stasis and dispersing nodules, as well as dispelling wind and alleviating itching [1-2]. However, there are few reports on the structure and target molecules of the active macromolecular polysaccharides in *Campsis grandiflora*. Here, the purified homogeneous polysaccharide LXH2 from *C. grandiflora* was subjected to structural analysis, investigation of its anti-colitis activity, and related target molecules discovery. Specifically, molecular weight determination revealed that the molecular weight of LXH2 was 19.44 kDa. Additionally, monosaccharide composition analysis indicated that LXH2 contained mannose, rhamnose, glucuronic acid, galacturonic acid, galactose, and arabinose in a molar ratio of 0.8: 1.2: 39: 1: 1. It is a straight-chain structure consisting of 1,4-α-GalA ,with a T-α-HexA attached at the C-3 position of the 1,4-α-GalA and T-β-GalA linkage at C-4 position. Furthermore, molecular interaction studies demonstrated that LXH2 might weakly bind to Galectin-4 with an equilibrium dissociation constant (KD) of 2.3×10^{-5}. Biological activity studies showed that the polysaccharide inhibited the inflammatory factor TNF-α by more than 74% in the LPS-induced intestinal epithelial cell inflammation model. Overall, these findings lay a theoretical foundation for the anti-colitis properties of *C. grandiflora* polysaccharides.

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Structure Of The O-Specific Polysaccharide Of *Asaia Bogorensis* Atcc Baa-21 Lipopolysaccharide

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asaia bogorensis is a gram-negative, rod-shaped and peritrichously flagellated, aerobic bacterium belonging to *acetobacteraceae* family. *a. bogorensis* is isolated from flowers and fruits found in tropical climate[1], gut, and the *reproductive system* including the ovaries and salivary glands of mosquitoes[2], and rarely isolated from immunocompromised patients[3]. in europe, *a. bogorensis* is responsible for the contamination of flavored mineral waters with the addition of natural juices or natural flavors. lipopolysaccharide is an important virulence factor triggering an immune response. it consists of three basic regions such as lipid a anchoring the entire macromolecule in the outer membrane, core oligosaccharide, and o-specific polysaccharide (o-antigen), which is the outermost component of the lps composed of repeating oligosaccharide units. the polysaccharide part is the most variable region, determining serological specificity, used to identify and distinguish serotypes of gram-negative bacteria. to date, no data on the structural analysis of *a. bogorensis* lps have been published. therefore, for the first time, here we present the results of a structural analysis of the o-specific polysaccharide of *a. bogorensis* atcc baa-21 lps. this strain was isolated from an orchid tree flower in indonesia. to the best of our knowledge, the o-ps structure is the first report on lipopolysaccharide of *asaia bogorensis* and the entire *asaia* genus. chemical and nmr analysis of the o-ps of *a. bogorensis* atcc baa-21 lps revealed a branched trisaccharide repeating unit →6)-α-D-glc*p*-(1→2)-[β-D-glc*p*-(1→3)]-α-L-rha*p*-(1→.

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Large-Scale Pattern Analysis Of N- And O-Glycoproteomics Using Ion-Mobility Assisted Mass Spectrometry

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Glycosylation plays crucial roles in biological processes such as cell signaling, immune response, protein folding, cell adhesion, and recognition[1]. Aberrant glycosylation can significantly affect immune activation, antigen recognition, antigen-antibody binding affinity and malignant cellular immune escape, thus leading to immune disorders and promoting the pathogenesis of diseases[2,3]. Here, we employed trapped ion mobility tandem time-of-flight mass spectrometry (timsTOF MS) to investigate the patterns and associations of protein N- and O-glycosylation. To begin with, we assessed the feasibility of tandem-TIMS platform with optimal PASEF mode utilizing three purified glycoproteins including fetuin, recombinant erythropoietin (rEPO), and Apolipoprotein E (ApoE). Subsequently, we performed a large-scale characterization of N- and O-glycosylation for more complex biological samples, including human lung cancer cells, serum, and exosomes. Next, we delved into serum antibody glycosylation and systematically compared the aberrant glycosylation patterns of serum antibodies in eight immune disorders, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, amyloid light-chain amyloidosis, mild IgA nephropathy, severe IgA nephropathy, acute myeloid leukemia, and acute lymphoblastic leukemia.

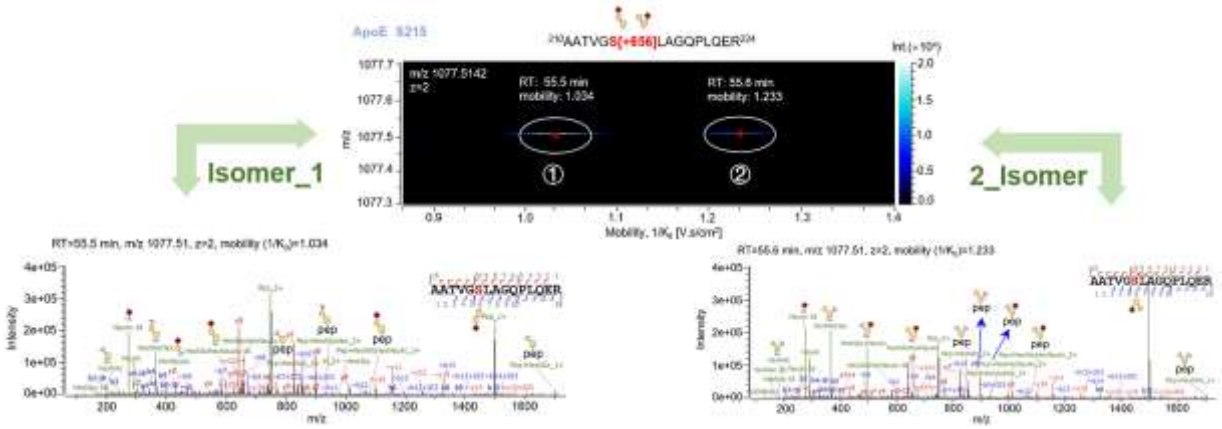

Fig.1 TIMS-TOF MS enhances physical separation of isomeric glycopeptides

We demonstrated that this platform could generate high-quality mass spectra and produce abundant four-dimensional information for intact glycopeptides identification. The results displayed the presence of numerous glycoproteins exhibiting significant biological functions in cells, cell exosomes, serum, and serum exosomes. We observed unique glycoprotein profiles in each of these four types of samples, showing specific patterns and variations in glycosylation in different biological regions. We validated that N-glycans conservatively occupied the Fc domain which was consistent with pioneer studies, while O-glycans were more flexible and ubiquitous, and distributed across the entire functional regions, suggesting that N- and O-glycosylation have distinct roles in antibody function. Our informative patterns of N- and O-glycosylation facilitated a better understanding of the pathogenesis of immune-mediated disorders.

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Dissecting the Conformational Stability of a Glycan Hairpin

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Dissecting the conformational stability of a glycan hairpin Systematic structural studies of model oligo-peptides revealed important aspects of protein folding and offered design principles to access non-natural materials. Similarly, synthetic oligosaccharides could be valuable substrates to dissect the rules that regulates glycan folding, but their analysis is often limited due to synthetic and analytical complexity. Taking a glycan capable of spontaneously folding into a hairpin conformation as model system, we analysed the factors that contribute to its conformational stability in water solution. Systematic chemical modifications of the glycan sequence, including the introduction of NMR labels and staples, revealed that conformational proclivity and multiple glycan-glycan interactions are the major determinants of folding stabilization. Nuclear magnetic resonance assisted by molecular dynamics simulations revealed that minor modifications in the glycan primary sequence can be used to tune the rigidity of structural motifs remote to the modification sites. These results could inspire the design of other glycan architectures with implications is glycobiology and material sciences.

Yadav, N., et al., Dissecting the Conformational Stability of a Glycan Hairpin. J Am Chem Soc, 2024. 146(9): p. 6369-6376.

Total Synthesis Of Trisaccharide Repeating Unit Of *Staphylococcus Aureus* Strain M

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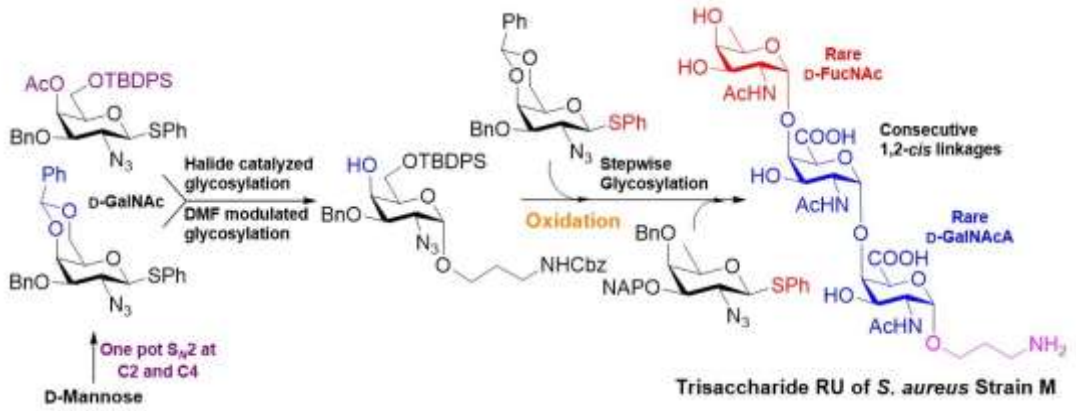
Multidrug resistance in bacteria has become a global concern. As a result, there is an urgent and unprecedented need for the development of novel antibiotics and prophylaxis strategies. Structurally unique glycans present on the bacterial cell surfaces are looked upon as excellent candidates for therapeutics and vaccine development. The ESKAPE pathogen has become a severe threat among all the MDR bacteria. *Staphylococcus aureus* strain M is a capsular polysaccharide (CPS). This opportunistic Gram-positive bacterium, which is responsible for the infection of skin, lungs, and joints and can cause life-threatening conditions such as endocarditis or toxic shock syndrome.[1] Considering this pathogen's biological importance, we have designed an efficient route for synthesizing a conjugation-ready trisaccharide repeating unit of *Staphylococcus aureus* strain M. The main challenges involved in this synthesis are the procurement of rare sugars, which is achieved by using in situ S_N2 bistriflate displacement[2] (D-FucNAc and D-GalNAcA). The challenge of stereoselective 1,2-*cis* glycosylation with the linker acceptor was achieved with easily accessible benzylidene protected D-galactosamine thioglycoside by employing a DMF modulated preactivation glycosylation method.[3] The consecutive 1,2-*cis* linkages were installed with the help of solvent participation. The carboxylic acid functionality was introduced via postglycosylation oxidation on the disaccharide moiety. The total synthesis of the trisaccharide repeating unit was accomplished with the longest linear sequence of 24 steps in a 4.5% overall yield.[4]



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Towards Automated Synthesis Of Monosaccharide Building Blocks And Applications In Oligosaccharide Synthesis

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Developing effective techniques for synthesising carbohydrates with complex structural organisation is crucial to the discipline of glycoscience. Despite significant progress in the synthesis of oligosaccharides, the synthesis of targets featuring complex glycosidic linkages of monosaccharide building blocks remains a challenge. These compounds are present in a wide range of biologically relevant compounds.

While much of the emphasis in the development of automated platforms for carbohydrate synthesis has been on the construction of oligosaccharides, manual syntheses of monosaccharide building blocks can represent up to 90% of the synthetic effort and thus constrain throughput[1]. This is often laborious and time-consuming. Furthermore, excess amounts of glycosyl donor building blocks are frequently used in glycosylations, presenting a pressing need to develop methods for streamlining the acquisition of monosaccharides.

The aim of this work is to improve the purification of monosaccharides, which is often a bottleneck in the preparation of important carbohydrates. By using a purification tag, TIDA,[2] the process of purifying monosaccharides can be made simpler and more efficient. One of the key findings of this research is that the silica binary affinity properties of the TIDA tag can be extended to monosaccharides bearing a variety of protecting groups. This characteristic proved beneficial during the synthesis of the tagged molecules, as it simplified purification and eliminated the need for arduous column chromatography. As a result, this process is potentially amenable to automation. Additionally, the tagged building blocks have been used to synthesise a trisaccharide in high yield, indicating that the TIDA tag is appropriate for the synthesis of oligosaccharides.

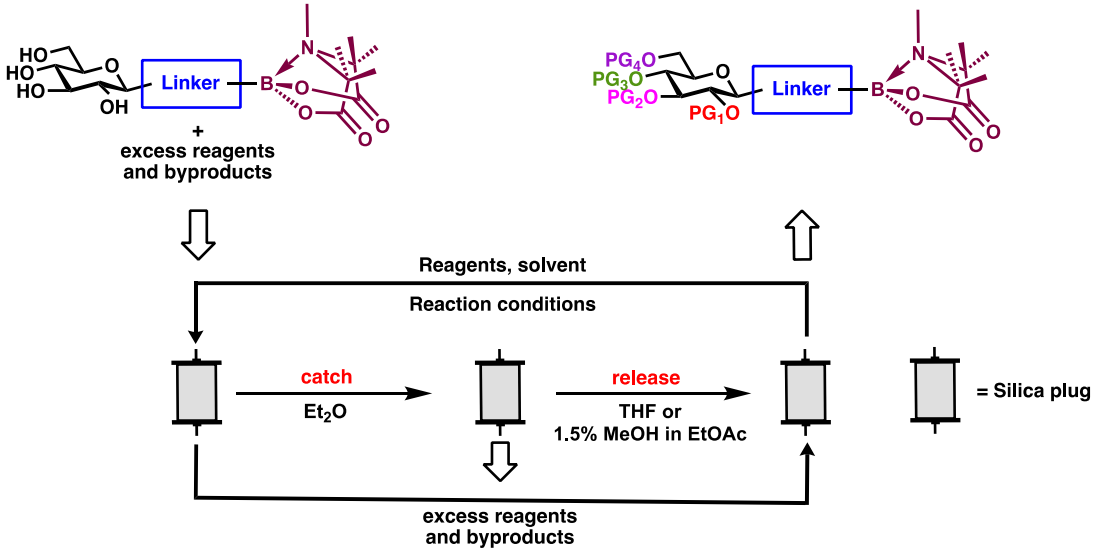

Figure 1: Catch and release purification

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Janus Aglycones: Synthesis And Functionalization Of *P*-Hydroxyphenyl Glycosides

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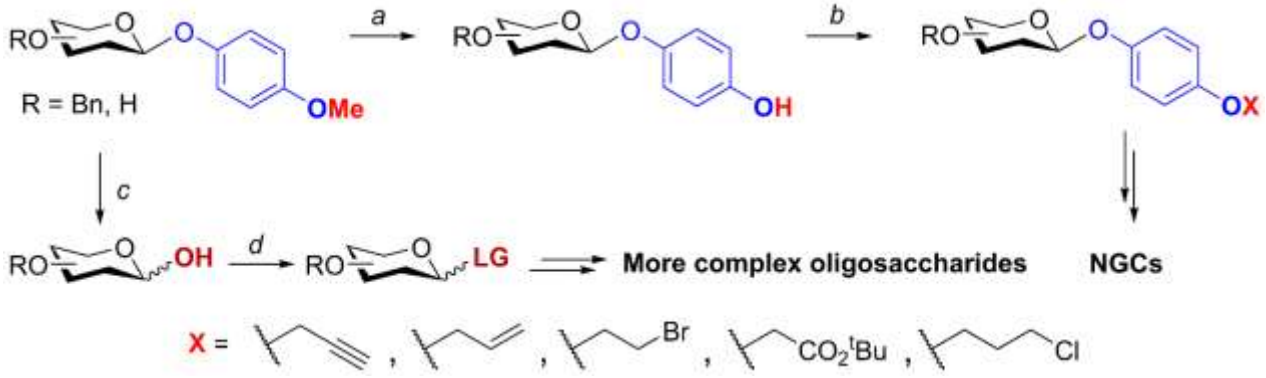
Janus glycosides are featured by the presence of a cleavable pre-spacer aglycone (Janus aglycone),[1] which allows these glycosides to be used both for the preparation of neoglycoconjugates (NGCs) and, after cleavage of the aglycone and introduction of a leaving group, as building blocks in the synthesis of more complex oligosaccharides (Scheme 1). We have recently introduced 4-(ω-chloroalkoxy)phenyl aglycones as potent Janus aglycones and successfully used them for the block synthesis of oligosaccharides and NGCs thereof (see[2] and references cited therein).

As part of the development of this concept, *p*-methoxyphenyl (MP) aglycone was studied as a candidate for inclusion to the family of Janus aglycones. Since MP aglycone is widely used as an anomeric protective group, the key remaining issue was to design an approach for functionalization of MP aglycon. If a straightforward method for demethylation of MP glycosides could be devised, then selective functionalization of the more acidic phenolic hydroxy group in the resulting *p*-hydroxyphenyl glycoside would not constitute any problem.

After comparison of several feasible demethylation approaches and considerable experimentation (more details will be shown in the poster) we elaborated a robust and efficient demethylation protocol that included treatment of an (un)protected MP glycoside with EtSH and NaOH in *N*-methyl-2-pyrrolidone (NMP) at 130 °C. Importantly, intersaccharidic glycosidic linkages in oligosaccharide substrates were stable under these conditions. The alkylation of the phenolic hydroxy group in protected and unprotected *p*-hydroxyphenyl glycosides, obtained in almost quantitative yields, cleanly gave the corresponding pre-spacer glycosides with a functional group in aglycon (or its precursor), which would allow a straightforward conjugation to a carrier, also in nearly quantitative yields (Scheme 1).

This work was financially supported by the Russian Science Foundation (Project No. 21-73-20164).



Scheme 1. MP aglycone as an example of a Janus aglycone. *Reagents and conditions: a.* EtSH, NaOH, NMP, 130 °C. *b.* Alkylating agent (XBr or XOTs), K_2CO_3, polar aprotic solvent, *c.* $(NH_4)_2Ce(NO_3)_6$, MeCN–H_2O. *d.* Introduction of a leaving group (LG).

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Synthesis And Biological Profiling Of Seven D-Glucuronic Acid Containing Heparinoid Trisaccharides

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Heparin and heparan sulfate (HS) are linear anionic glycosaminoglycan polysaccharides. Both are composed of alternating α-D-glucosamine and hexuronic acid units, but differ in L-iduronic acid content and degree of sulfation.[1] Heparin is used in medical practice as an anticoagulant, but heparinoids also have growth factor-inhibitory, anti-inflammatory and cell growth-inhibitory effects.[2] Heparin and its derivatives are also being investigated for the treatment of many disorders, including cancer.[3] Our research group has been working on the synthesis of heparin-analog oligosaccharides for a long time,[4] and recently we produced three trisaccharides containing D-glucuronate (GlcA), which showed a significant and selective inhibitory effect on the growth of tumor cells.[5]

Based on the above results, we designed an efficient synthesis route of six Glc-GlcA-Glc and one GlcN-GlcA-Glc sequenced heparin-related trisaccharides with various sulfation, and acetylation patterns. The *in vitro* anti-proliferative and anti-inflammatory activity and cytotoxicity of our compounds have been tested. In this poster, we present the synthesis of trisaccharides and the results of their biological tests.

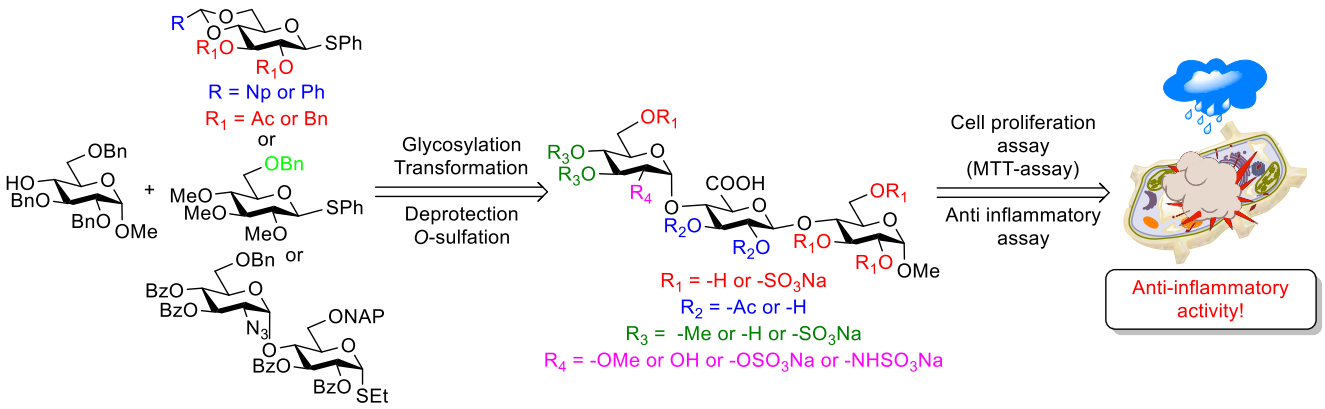

Figure 1. Synthesis of the heparin analogue trisaccharides

The authors gratefully acknowledge financial support for this research from the National Research, Development and Innovation Office of Hungary (NKFIH, FK 137924) and by ÚNKP-23-4 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund

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Stereoselective 2-Deoxy-Glycosylation Enabled By Nitroarenes Catalysis

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The stereoselective synthetically acquisition of 2-deoxy-glycosides keeps a long-standing challenge, due to the absence of directing groups at the C2 position, albeit kinds of strategies have been developed in direct or indirect manner so far[1,2]. Nitroarene compounds are increasingly applied in organic catalysis due to its distinct electrical properties[3,4]. We hypothesized the potential nitroarenes may activate glycals via π interaction, and recruit acceptors at the same time to form the activated intermediate, resulting to enzyme-like catalytic glycosylation. We begin our investigation by screening the nitroarenes as catalysts to promote the 2-deoxy-glycosylation of glycal and acceptor, and methyl 4-nitrobenzoate (**Cat1**) was selected as the most effective molecule. After systematic reaction condition screening, 40 examples were presented in excellent yields and α stereoselectivity. Such catalytic glycosylation (*Cat-glycosylation*) was universal for diverse glycals and acceptors (alcohols, carbohydrates and natural products). Subsequent gram scale synthesis and iterative synthesis of decasaccharide further manifested the superiority of this method. Moreover, we have successfully achieved the synthesis of pregnane glycoside **P57** (an appetite-suppressant isolated from a South African cactus-like *Hoodia gordonii*) involving two-step *Cat-glycosylation*. Mechanism studies and DFT calculation suggested the -NO_2 of **Cat1** can simultaneously interact with both donor and acceptor leading to a ternary complex to start the catalytic process. In conclusion, our work have presented a new method and perspective for stereoselective 2-deoxy-glycosylation and enzyme-mimetic catalysis of organocatalysts.

Keywords: 2-deoxy-glycosylation; Nitroarenes; Organocatalysis

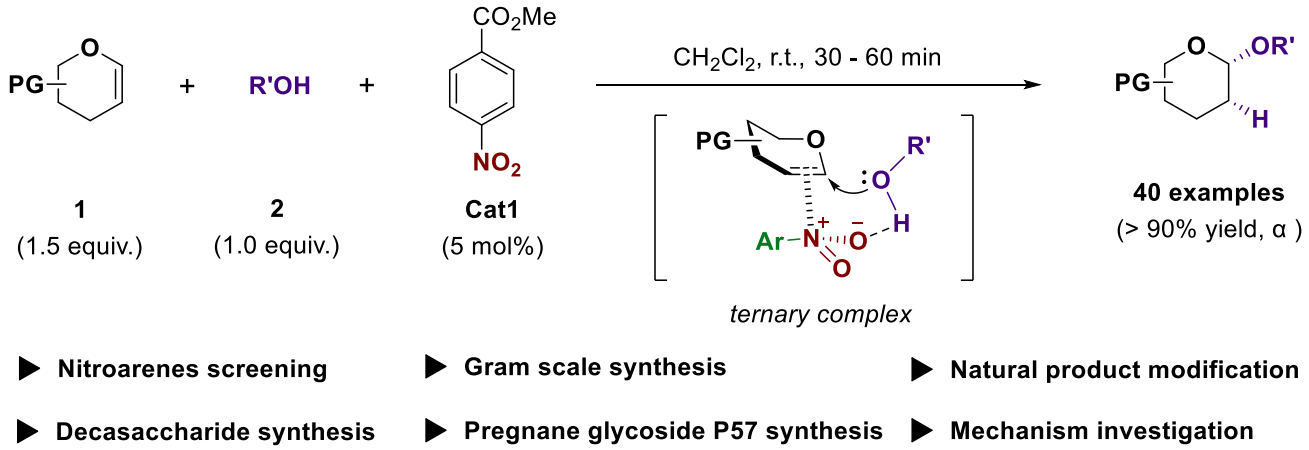

Fig. 1 The 2-deoxy-glycosylation of glycals catalyzed by nitroarenes

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Benzylidene-Directed Glycosylations – Mechanistic Insights From Cryogenic Infrared Spectroscopy

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The stereoselective formation of 1,2-cis glycosidic linkages is challenging. A promising method to preferentially obtain 1,2-*cis* glycosides are 4,6-*O*-benzylidene directed glycosylations, which were initially introduced by Crich[1] and further refined by many groups.[2,3] The stereoselectivity of this reaction is thought to be driven by a covalent intermediate, which reacts via an S_N2 mechanism.[4] However, the role of cationic S_N1-type intermediates in this reaction is unclear. Here, we elucidate the structure of glycosyl cations carrying 4,6-O-benzylidene groups using cryogenic infrared ion spectroscopy and computational methods. The data reveal that the intermediates unexpectedly form anhydro cations, which correlates well with the stereoselective outcome of S_N1-type glycosylations.[5] The study highlights how cryogenic infrared spectroscopy can unravel novel intermediates in sugar chemistry and how this structural data can be linked to reactions in solution.[6-8]

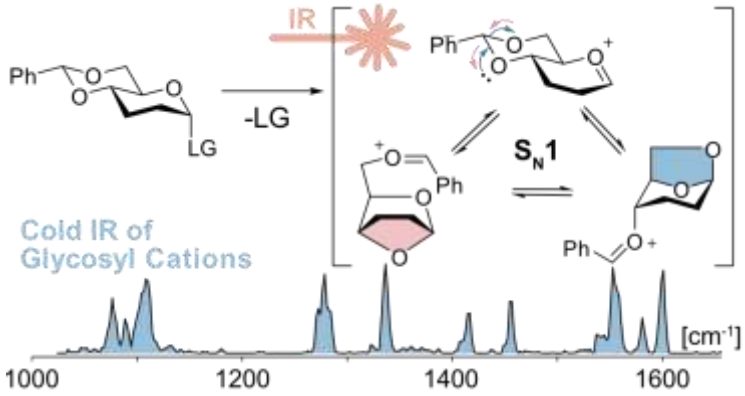

Figure 1: Postulated mechanism for benzylidene-mediated glycosylation reactions. We present here the first direct structural characterization of glycosyl cation using cryogenic IR. The predicted stereoselectivity for anhydro-type intermediates is correlated with the stereochemical outcome in S_N1-mechanism.

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Adjacent Hydroxyl Groups Are Key To Enhance The Hydrolysis Of Amine-Protecting Groups In Glycans Via Water—Mediated Intramolecular Hydrogen Bonds

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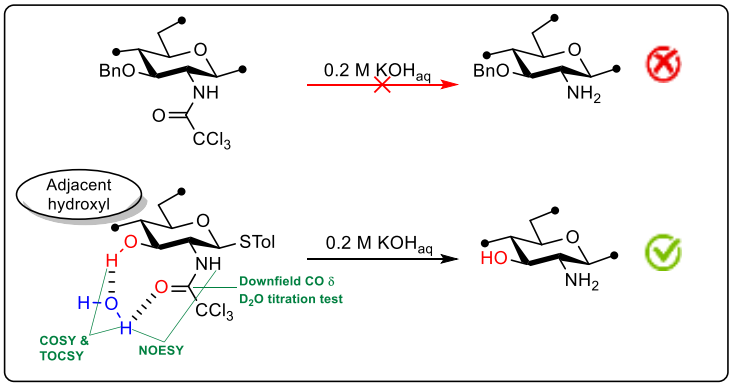
Various *N*-protecting groups were developed in carbohydrate chemistry, especially for 2-amino-2-deoxy glucosides. However, their deprotection procedures usually require harsh conditions such as high temperature, long reaction time or hydrogenolysis, where may not be compatible for other protecting groups. Herein, we report the findings that adjacent hydroxyl group(s) represent a key to remove an amide-protecting group under a mild condition. A plausible mechanism is also proposed with supporting evidence. A series of *N*-trichloroacetylated (TCA) mono-*ol*-, di-*ol*-, and tri-*ol*-containing glucosides were initially synthesized and subjected to the hydrolysis in a KOH solution at room temperature. The result indicated that adjacent hydroxyl group(s) are important to speed up the reaction with higher yields. Mechanistic studies, including D_2O titration test and NMR spectroscopic methods (2D-NOESY), were also carried out to support the interaction of 3-OH with the carbonyl of TCA in the presence of water molecules. To further expand the range of applications, we also surveyed several other *N*-protecting groups (e.g., *N*-2,2,2-trichloroethoxycarbamate and *N*-phthaloyl group) and different glycosides (e.g., sialosides).



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Zinc(II)-Mediated Stereoselective Construction Of 1,2-*Cis* 2-Azido-2-Deoxy Glycosidic Linkage

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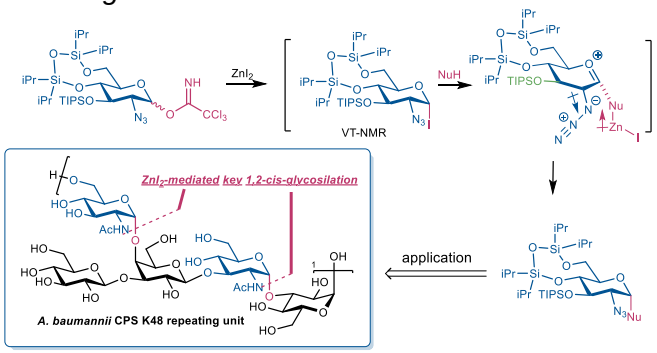
The capsular polysaccharide (CPS) of the pathogenic *Acinetobacter baumannii* is a major virulence factor and a promising target for vaccine development. However, the synthesis of 1,2-*cis*-2-amino-2-deoxyglycoside core of CPS remains challenging. Here we develop a highly α-selective ZnI_2-mediated 1,2-*cis* 2-azido-2-deoxy glycosylation strategy using 2-azido-2-deoxy glycose donors with various 4,6-*O*-tethered groups. Among them the tetraisopropyldisiloxane (TIPDS)-protected 2-azido-2-deoxy-D-glucosyl donor afforded predominantly α-isomer (α:β > 20:1) in the highest yield. We demonstrated the versatility and effectiveness of this novel approach by applying it to a wide substrate scope, including various aliphatic alcohols, sugar alcohols, and natural products, and succeeded in synthesizing *A. baumannii* K48 capsular pentasaccharide repeating fragments by employing this strategy in the key step of constructing the 1,2-*cis* 2-azido-2-deoxy glycosidic linkage. The reaction mechanism was explored with combined experimental variable-temperature NMR (VT-NMR) studies and mass spectroscopy (MS) analysis, and theoretical density functional calculations, which suggested the formation of covalent α-$C1^{GlcN}$-iodide intermediate in equilibrium with separated oxocarbenium–counter ion pair, followed by an S_N1-like α-nucleophilic attack most likely from separated ion pairs by the ZnI_2-activated acceptor complex under the influence of the 2-azido *gauche* effect.



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Application Of Catalytic Strain-Release Of Donor-Acceptor Cyclopropane In Efficient Carbohydrate Synthesis

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As a foundational organic transformation, the catalytic nucleophilic ring-opening of strained molecules has found extensive applications in diverse fields such as organic synthesis, materials science, and bioconjugation. Inspired by our previous facile and customizable thioglycoside activation strain-release transformation utilizing donor-acceptor cyclopropane (DAC) as the thiophilic reagent,[1] we have developed two innovative types of glycosyl donors: glycosyl *ortho*-2,2-dimethoxycarbonylcyclopropylbenzoates (CCBz) and *ortho*-2,2-dimethoxycarbonylcyclopropylbenzyl (CCPB) thioglycosides. These stable yet reactive glycosyl donors have enabled highly efficient and versatile glycosylation reactions, including *C*-, *N*-, *S*-, and *O*-glycosylation, yielding satisfactory outcomes.[2,3] The utility of the leaving group CCBz in efficient carbohydrate synthesis extends to steroidation reactions, facilitating the conjugation of carbohydrates with steroids to yield a novel class of pseudo-steroidal glycosides exhibiting excellent anti-inflammatory activities, employing a steroidal CCBz donor.[4] Building upon the strain-release strategy, we have developed CCBz-doped benzyl ester and anhydride reagents, enabling catalytic etherification and esterification under weak acidic conditions, yielding good-to-excellent yields.[5] These advancements highlight the versatility and synthetic potential of the strain-release approach, as showcased by the synthesis of a variety of biologically and medicinally relevant oligosaccharides, including TMG-chitotriomycin, lipid IV, and TD139.

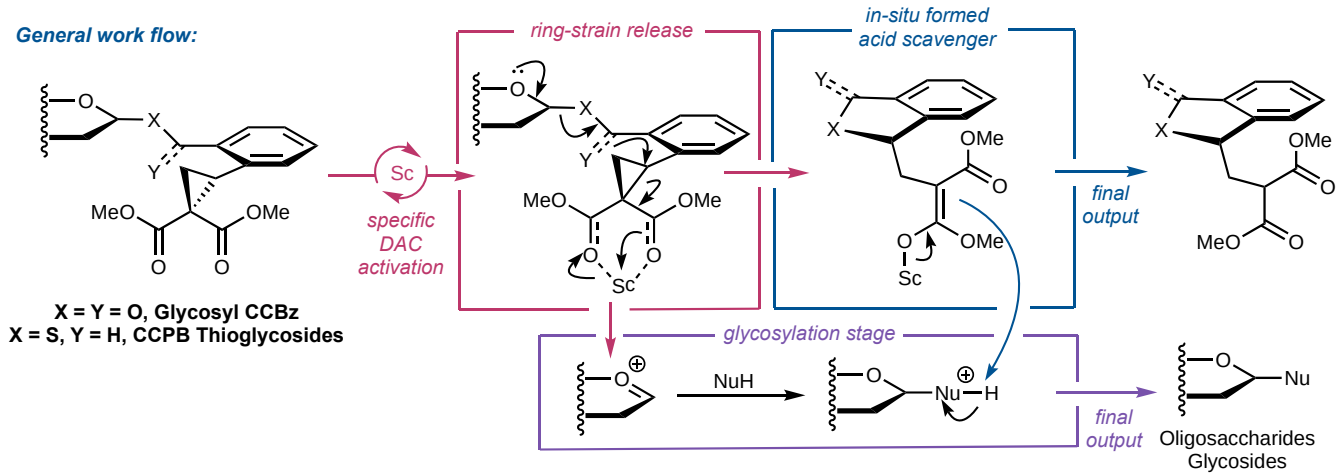

Figure 1 General Working Flow of Strain-Release Glycosylation

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Regioselective C-4 Functionalization Of Unprotected Sugars Through Photoredox

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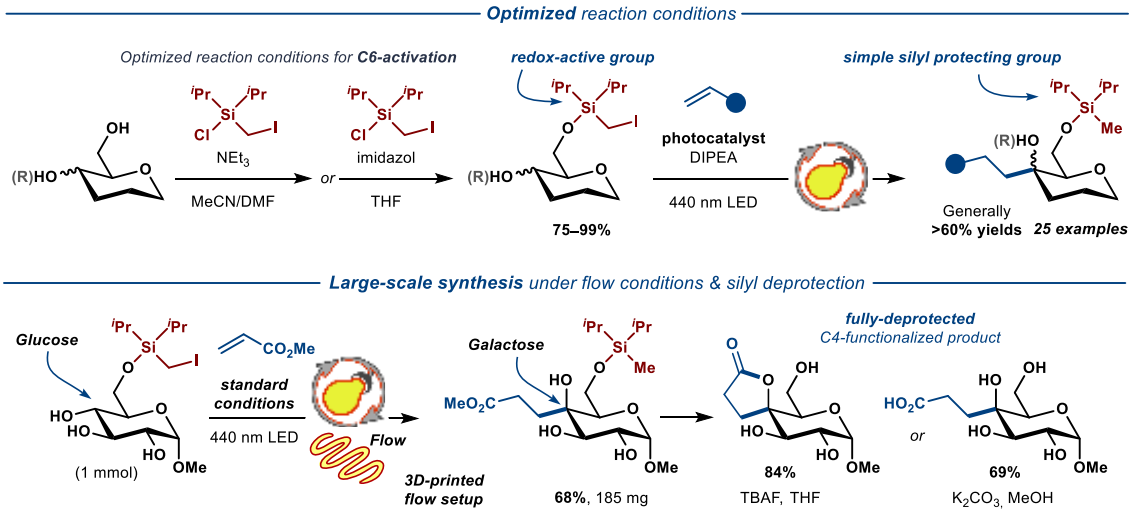
Unconventional C-functionalized carbohydrates are common bacterial metabolites, which constitute an elusive synthetic target and have long been accessible only through elaborate synthetic sequences. In recent years, access to such carbohydrate derivatives was greatly simplified by the emerging photoredox-mediated methods for C–H bond activation in both protected and unprotected carbohydrates.[1] The major drawback of these methods stems from the limited control over site- and stereoselectivity of the C-functionalization reactions. In this work, we address these limitations by employing a “traceless” redox-active tethering group, which allows fully-regioselective C4-functionalization of unprotected pyranosides through a sequence of inter- and intramolecular halogen- and hydrogen-atom transfer reactions, while stereoselectivity of the C–C bond-forming step if firmly defined by the anomeric configuration of the substrate. The selected silyl-based tethering group[2] is easily attached to the primary hydroxy functionality of the substrate in one step and is activated under benign photoredox catalytic conditions. In course of the reaction, this group is transformed into a simple trialkyl silyl protecting group, which can be readily removed under mild conditions to deliver the desired fully-deprotected C4-functionalized carbohydrate target.



The reaction was amenable to upscale under continuous flow conditions, demonstrating the same yield and selectivity as under batch conditions.

The Ministry of Education and Science of the Russian Federation (Program No. 075-03-2024-118/1) is gratefully acknowledged.

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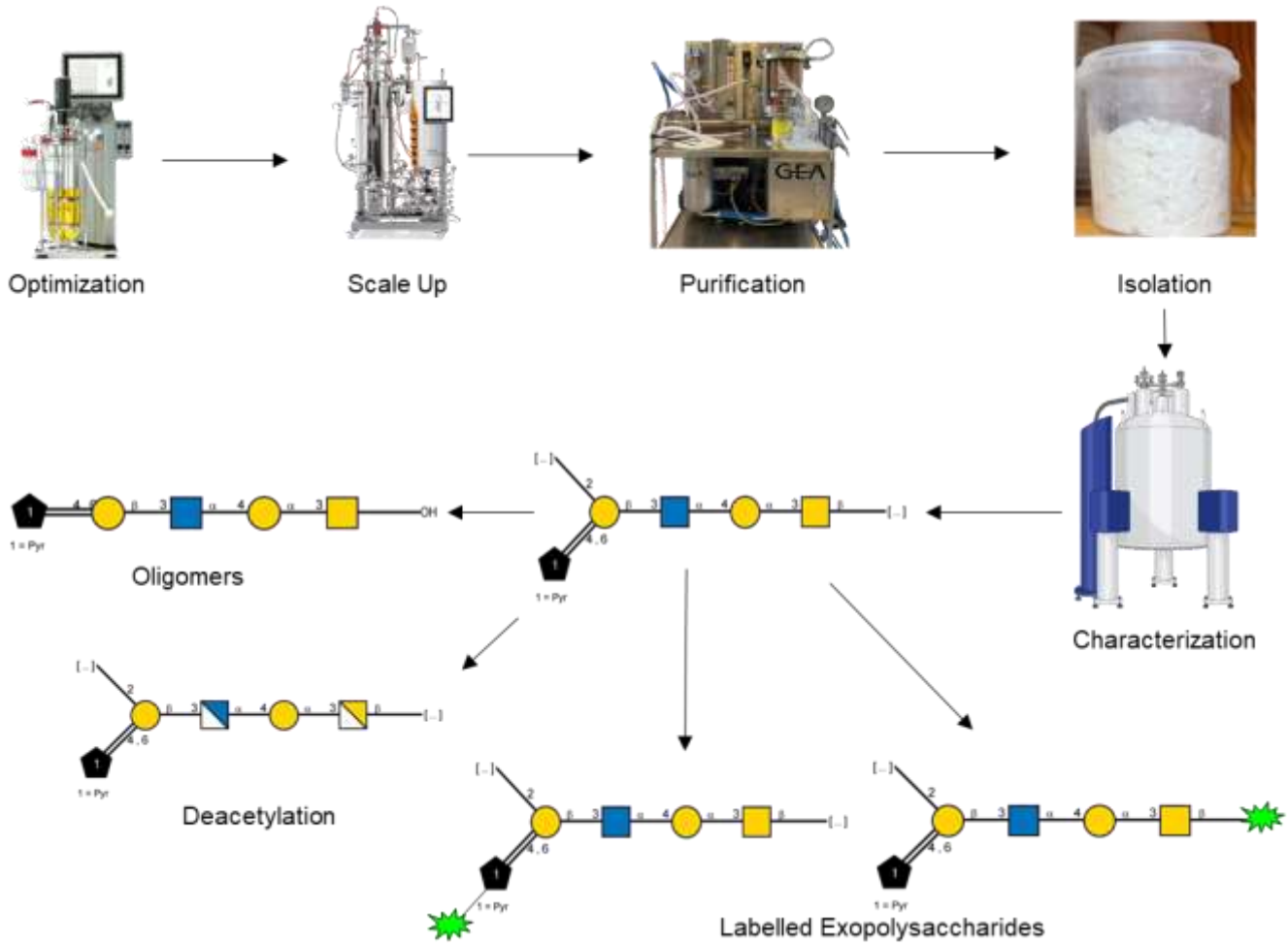
Gram-Scale Production And Chemical Modification Of A Novel Exopolysaccharide From *L. Pentosus*

Gordon Jacob Boehlich, Ahmad Tsjokajev, Pascal Michael Mrozek, Victor Daisuke Kietzmann, Philipp Garbers, Gustav Vaaje-Kolstad and Bjørge Westereng

Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Ås, Norway

Bacterial exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) have gained attention due to their structural diversity in nature, physicochemical properties applicable across various industrial domains, and health benefits in functional foods.[1] In the present study, a novel EPS was purified from the LAB strain *Lactiplantibacillus pentosus* KW1. In brief, growth conditions were initially optimized to maximise the EPS yield, employing a 100-liter fermentation process in a semi-defined medium. Subsequent EPS extraction and purification gave 15 g of EPS in isolated yield. The polysaccharide structure was elucidated by NMR, revealing a novel structure similar to a glycosylaminoglycan (GAG), owing its negative charge to pyruvic acid acetals. The EPS was finally modified by chemical methods to gain acces to deacetylated, labeled or oligomerized EPS for intented use in applications ranging from enzymology to microbiology.



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Synthetic Anhydro-Murnac Moiety As Unusual Glycosyl Acceptor Terminates Bacterial Peptidoglycan Elongation

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Bacterial transglycosylases (TGases) catalyze the polymerization of glycan strands in peptidoglycan (PG) biosynthesis, marking their essential role as attractive targets for antibiotic development.[1] Despite of current TGase inhibitors, like moenomycin A, that always bind the TGase donor sites,[2] alternative strategies to disrupt natural elongation of PG have not been widely exploited. Anhydromuropeptides are naturally generated from PG recycling by lytic transglycosylases (LTs) in bacteria.[3] Inconsideration of their similarity with the natural substrates of TGases, Lipid II, we hypothesized that anhydromuropeptides could effectively incorporate into the growing PG as glycosyl acceptors, leading to the termination of successive PG elongation due to the absence of the reducing end in their 1,6-anhydro-MurNAc termini. Hence, we synthesized 4-O-(N-acetyl-β-D-glucosaminyl)-1,6-anhydro-N-acetyl-β-D-muramyl-L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala, **1**, within 15 steps, and the *in vitro* assays showed that this anhydromuropeptide and its analogue without the pentapeptide, **1-deAA**, were both readily utilized by TGase as non-canonical anhydro glycosyl acceptors. The Incorporation of anhydro-muramyl moiety into the growing glycan chains of PG by TGases led to the effective termination of glycan strands elongation. The subsequent *in vitro* studies of **1-deAA** against *Staphylococcus aureus* demonstrated that **1-deAA** served as a potential antimicrobial adjunct of vancomycin.[4] These findings imply that small molecule mimics of anhydromuropeptides may act as novel PG-terminating inhibitors to interfere with the PG elongation, paving the way for new strategies in antibiotic development.

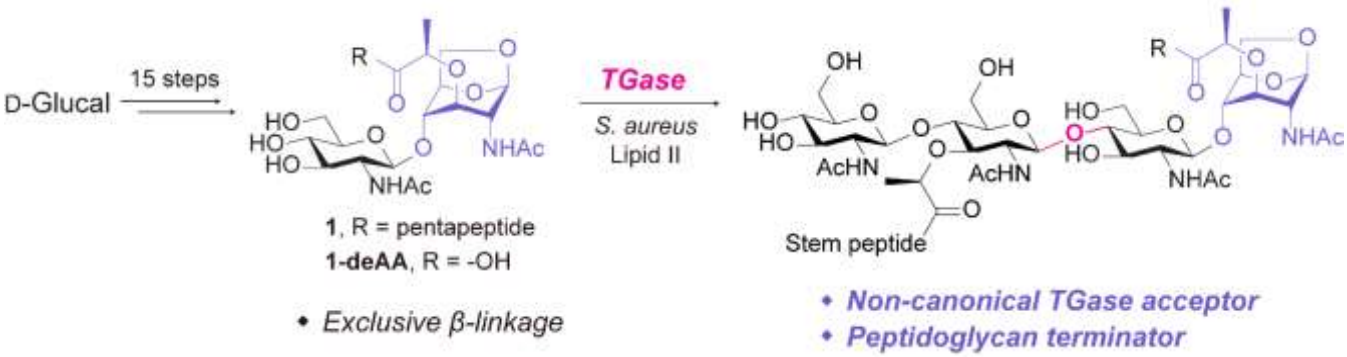

Figure 1. Synthetic **1** and **1-deAA** incorporate into the growing PG as glycosyl acceptors that covalently terminators the bacterial PG elongation.

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Photo-Induced Glycosylation Using The Edible Polyphenol Curcumin

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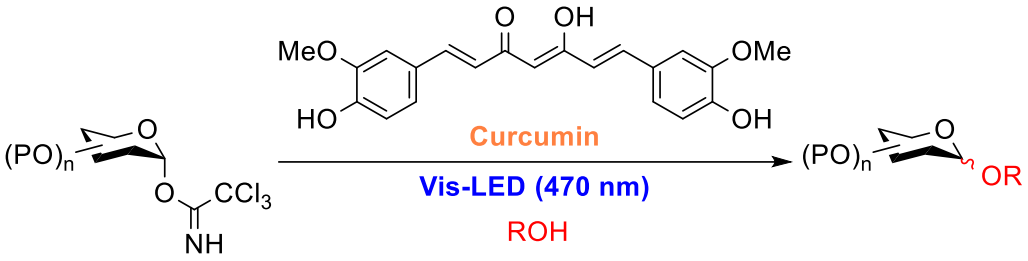
Many glycosides, including glycoconjugates and oligosaccharides, are commonly found in biologically active natural products, pharmaceuticals and high-functional molecules. Elucidating the precise biological and functional roles and structure-activity relationships of the glycosides requires homogeneous and structurally well-defined glycosides. In this context, glycosylation, a crucial synthetic reaction for binding sugars to other sugar moieties or other molecules (aglycon), is increasingly important in chemistry, biology, and materials science. Great effort has been made to develop efficient glycosylation reactions, with various efficient glycosylation methods reported to date. However, most conventional glycosylation methods still use environmentally harmful catalysts such as strong acids and toxic metal reagents, making environmentally friendly glycosylation methods increasingly important as environmental problems accumulate and Sustainable Development Goals (SDGs) gain importance. Photo-induced glycosylation using reusable photocatalysts is an attractive approach to green and sustainable chemistry.

We recently reported the photo-induced glycosylation of trichloroacetimidate donors and several alcohols using organo Brønsted photoacid (BPA) catalysts (2-naphthol,[1] 5,8-dicyano-2-naphthol[1] and 1,3-bis[3,5-bis(trifluoromethyl)-phenyl]thiourea[2]) or organo Lewis photoacid (LPA) catalysts (bis(2-naphthyl)disulfide[3] and 3,11-dimethoxydinaphthothiophen[4]) under long-wavelength UV light irradiation.[5]

Herein, we report that the edible polyphenol curcumin can act as an activator to effectively promote photo-induced glycosylation under mild reaction conditions. To our knowledge, this is the first demonstrated example of a chemical glycosylation reaction using an edible chemical as an activator.[6]



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Photoredox-Catalyzed α-Ketonylation Of Glycosyls: Glycosyl Tetrafluoropyridinyl Sulfides As Radical Precursors

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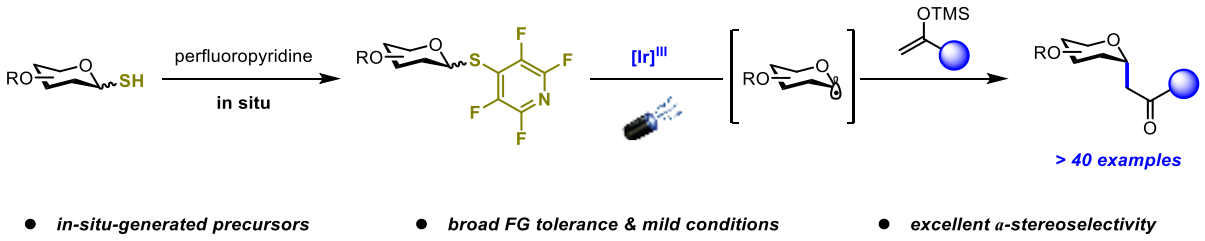
The hydrolytic stability of *C*-glycosyl compounds in vivo makes them particularly advantageous in developing artificial mimetics for natural glycosyl compounds, especially a large number of *O*-glycosides with enzymatically susceptible C–O acetal linkages.[1-2] Although β-ketonyl glycosyl compounds have been stereoselectively synthesized by a number of strategies,[3-9] the selective approaches to α-isomers are limited.[10-13] We herein devise a glycosyl radical-based approach to facilely access α-ketonyl glycosyl compounds via an Ir-photoredox-catalyzed desulfurative addition to silyl enol ethers, using in-situ-generated glycosyl tetrafluoropyridinyl sulfides from glycosyl 1-thiols as novel precursors. This protocol features readily prepared starting materials, mild conditions, excellent functional group tolerance, satisfactory scale-up, and notable amenability to late-stage modification of pharmaceutically relevant complex molecules, which constitutes general access to a diverse array of α-*C*-glycosyl compounds. Experimental studies provide insight into the reaction mechanism, which indicates a radical addition pathway and a non-chain radical mechanism.



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Glycan Recognition Mode Of Osteopontin, Insights From An Nmr Perspective

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Osteopontin (OPN) is a highly phosphorylated and glycosylated protein found in the extracellular matrix of cells. It contributes to cell spreading and adhesion; being involved in a myriad of processes like bone remodeling, wound healing, inflammation and tumor growth and progression.[1]

The glycosylation pattern of OPN modulates these cellular processes by interacting with glycan binding proteins.[2] However, the precise mechanism through which *O*-glycosylation of OPN influences these processes remains not fully understood to date.

Here, the OPN protein was expressed in human HEK293F cells with uniformly ^{13}C-labelled glycans.[3] By using state-of-the-art NMR experiments, such as ^{1}H-^{13}C-HSQC, ^{1}H-STD and ^{1}H-STD-^{1}H-^{13}C-HSQC NMR, we elucidate OPN's glycans interactions with diverse glycan-binding proteins, such as Siglecs and Galectins. Altogether, these results shed light on the structural basis of the elusive glycan recognition mode of OPN, aiming to provide a better understanding of the role of OPN post-translational modifications in regulating biochemical processes.

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Targeted Protein O-GlcNAcylation Using Bifunctional Small Molecules

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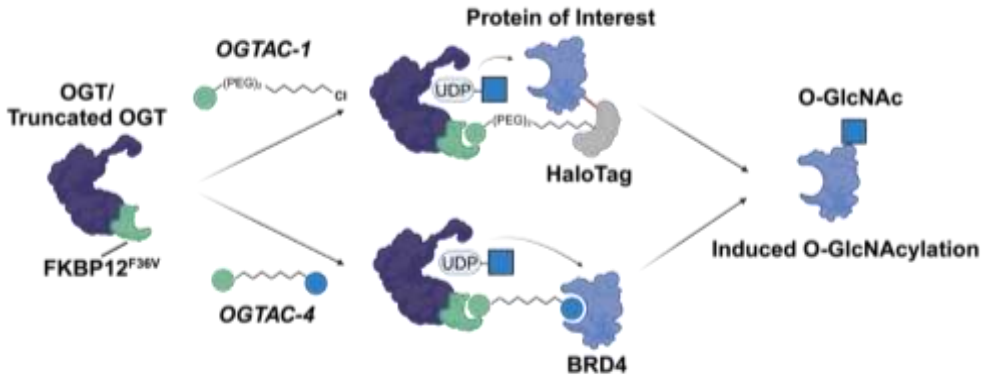
Protein O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) plays a significant role in the regulation of transcription, metabolism, cell signaling, protein stability, and nucleocytoplasmic trafficking.[1] Abnormal O-GlcNAcylation was reported to reprogram cellular metabolism to favor cancer proliferation.[2] Of note, both hyper- and hypo-O-GlcNAcylation are associated with tumorigenesis and resistance to anti-cancer therapy, suggesting that O-GlcNAc homeostasis is one of the underappreciated hallmarks of cancers.[3,4]

However, the lack of precise approaches for the manipulation of protein-specific O-GlcNAcylation greatly hindered the comprehensive dissection of its functions in human diseases. To address this, we have developed heterobifunctional small molecules, named O-GlcNAcylation TArgeting Chimeras (OGTACs), which enable protein-specific O-GlcNAcylation in living cells.[5] OGTACs promote O-GlcNAcylation of proteins such as BRD4, CK2α, and EZH2 *in cellulo* by recruiting FKBP12^{F36V}-fused O-GlcNAc transferase (OGT), with temporal, magnitude, and reversible control. Overall, the OGTACs represent a promising approach for inducing protein-specific O-GlcNAcylation, thus enabling functional dissection and offering new directions for O-GlcNAc-targeting therapeutic development.



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Structural Characterization of Two Polysaccharides from *Rosa Multiflora* and Their Liver Protection Bioactivity

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*Rosa multiflora (*RM*)*, is the flower of the deciduous small shrub Rosa of the family Rosaceae. In the concept of traditional Chinese medicine, Rose multiflora belongs to the class of medicinal materials that invigorate blood circulation and dispel blood stasis, Such herbs often have a positive effect on the treatment of heart and liver diseases. Meanwhile, Polysaccharides are an crucial macromolecular substance that constitutes the active ingredients of traditional Chinese medicine, and a large number of studies have shown that polysaccharides have abundant biological activity such as Hepatic Fibrosis [1], Anti-inflammatory [2]and Angiogenesis Inhibition [3], etc. Therefore, we purified the two active polysaccharides RM 132 and RM 234 by anion exchange column (DEAE-sepharose fast flow column) and gel chromatography column (sephacryl HR column). Structure analyses suggested that RM132 was a novel arabinogalactan with the molecular weight (Mw) of 6.1 kDa.The backbone was consist of T-linked Arabinose, 1,5-linked Arabinose, 1,3,6-linked Arabinose, T-linked Galactose, 1,3-linked Galactose, 1,4 linked Galactose, 1,6-linked Galactose and 1,3,6-linked Galactose. RM 234 was a novel galacturonan with the molecular weight (Mw) of 4.2 kDa, the backbone was mainly consist by 1,4-linked Galacturonic acid . Bioactivity test showed that RM132 has a certain inhibitory effect on the degree of fibrosis induced by TGF-β in human hepatocytes and it has no obvious toxic side effects on normal human liver cells. In addition, in the drug screening experiments for promoting liver regeneration using the Ki67 genetic labeling system induced by 4-OHT, it was found that polysaccharide RM234 has an effect on promoting the proliferation of hepatocytes at a concentration of 40 μM. These results suggested that polysaccharide RM 132 and RM 234 could be a potential liver protection compound for functional food and new drug development.Reference

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Chemoproteomics Reveals Fructose-1,6-Bisphosphate Signaling Mediated By Mitocondrial Aldh2 In Living Cells

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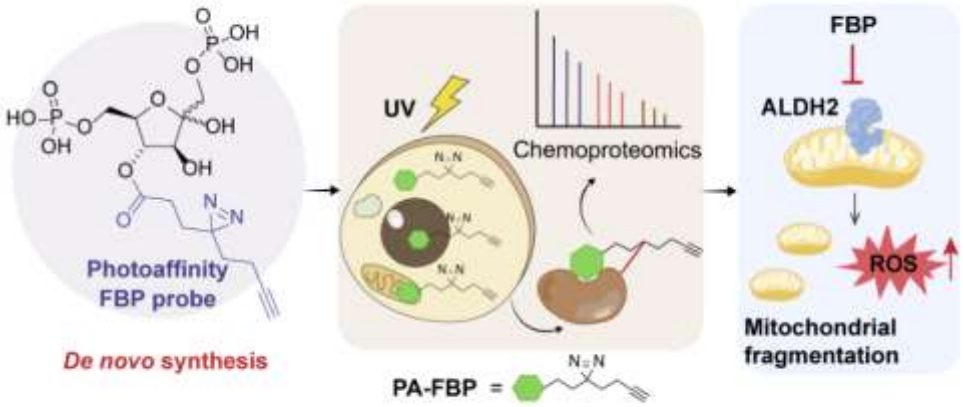

Abstract: Fructose-1,6-bisphosphate (FBP), a cellular endogenous sugar metabolite in the glycolytic pathway, has recently been reported to act as signaling molecule to regulate various cellular events through engagement of important proteins.[1-2] Though tremendous progress has been made in identifying specific FBP-protein interactions, the comprehensive identification of FBP-interacting proteins and their regulatory mechanisms remain largely unexplored. Here we describe a concise synthetic approach for the scalable preparation of a photoaffinity FBP probe, that enables the quantitative chemoproteomic profiling of FBP-protein interactions based on photoaffinity labeling (PAL) directly in living cells. Using such protocol, we identified over 50 potential FBP-interacting proteins with high confidence that include the known targets of FBP such as PKM2 and MDH2. Furthermore, among unknown FBP-interacting proteins, we identified a mitochondrial metabolic enzyme aldehyde dehydrogenase 2 (ALDH2) and found out that FBP is able to enter into the mitochondria and inhibit ALDH2 activity, resulting in the increase of cellular reactive oxygen species (ROS) level along with mitochondrial fragmentation. These results suggest that sugar metabolite FBP transmits the signal of accelerated glucose uptake and glycolytic flux to ROS signals and mitochondrial morphological changes through mitochondrial signal transduction. This might work as a new mode of glucose sensing and signaling event and it expands our knowledge of sugar metabolite signaling in mammalian biology.[3]

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Kinetic And Structural Aspects Of Glycosaminoglycan–Monkeypox Virus Protein A29 Interactions Using Surface Plasmon Resona

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Monkeypox virus (MPXV), a member of the Orthopoxvirus genus, has begun to spread into many countries worldwide. While the prevalence of monkeypox in Central and Western Africa is well-known, the recent rise in the number of cases spread through intimate personal contact, particularly in the United States, poses a grave international threat. Previous studies have shown that cell-surface heparan sulfate (HS) is important for vaccinia virus (VACV) infection, particularly the binding of VACV A27, which appears to mediate the binding of virus to cellular HS. Some other glycosaminoglycans (GAGs) also bind to proteins on Orthopoxviruses. In this study, by using surface plasmon resonance, we demonstrated that MPXV A29 protein (a homolog of VACV A27) binds to GAGs including heparin and chondroitin sulfate/dermatan sulfate. The negative charges on GAGs are important for GAG–MPXV A29 interaction. GAG analogs, pentosan polysulfate and mucopolysaccharide polysulfate, show strong inhibition of MPXV A29–heparin interaction. A detailed understanding on the molecular interactions involved in this disease should accelerate the development of therapeutics and drugs for the treatment of MPXV.

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Improving Impact Of Heparan Sulfate On The Endothelial Glycocalyx Abnormalities In Atherosclerosis As Revealed By Glycan-Protein Interactome

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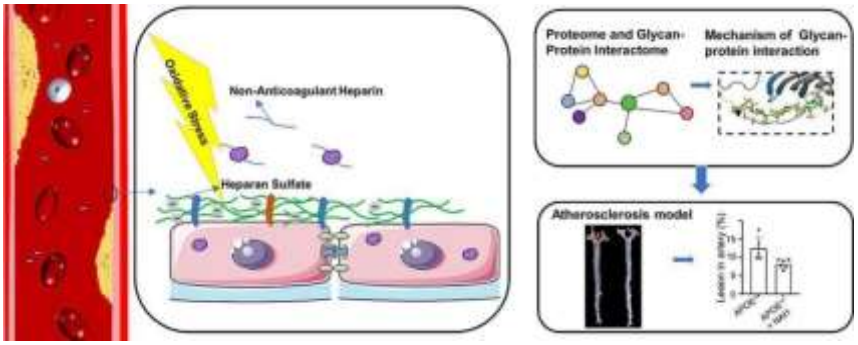
Endothelial dysfunction induced by oxidative stress is an early predictor of atherosclerosis, which can cause various cardiovascular diseases. The glycocalyx layer on the endothelial cell surface acts as a barrier to maintain endothelial biological function, and it can be impaired by oxidative stress. However, the mechanism of glycocalyx damage during the development of atherosclerosis remains largely unclear. Herein, we established a novel strategy to address these issues from the glycomic perspective that has long been neglected. Using countercharged fluorescence protein staining and quantitative mass spectrometry, we found that heparan sulfate, a major component of the glycocalyx, was structurally altered by oxidative stress. Comparative proteomics and protein microarray analysis revealed several new heparan sulfate-binding proteins, among which alpha-2Heremans-Schmid glycoprotein (AHSG) was identified as a critical protein. The molecular mechanism of AHSG with heparin was characterized through several methods. A heparan analog could relieve atherosclerosis by protecting heparan sulfate from degradation during oxidative stress and by reducing the accumulation of AHSG at lesion sites. In the present study, the molecular mechanism of anti-atherosclerotic effect of heparin through interaction with AHSG was revealed. These findings provide new insights into understanding of gly cocalyx damage in atherosclerosis and lead to the development of corresponding therapeutics.



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Chitosan-Mediated Modulation Of The Ovarian Microenvironment And Gut Microbiota To Alleviate Diminished Ovarian Reserve (Dor)

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Abstract

Background: Age-related changes in the ovarian microenvironment are linked to impaired fertility in women. Macrophages play important roles in ovarian tissue homeostasis and immune surveillance. However, the impact of aging on ovarian macrophage function and ovarian homeostasis remains poorly understood.

Methods: Senescence-associated beta-galactosidase staining, immunohistochemistry, and TUNEL staining were used to assess senescence and apoptosis, respectively. Flow cytometry was employed to evaluate mitochondrial membrane potential (MMP) and apoptosis in granulosa cells lines (KGN), and macrophages phagocytosis. After a 2-month treatment with low molecular weight Chitosan (LMWC), ovarian tissues from mice were collected for comprehensive analysis.

Results: Compared with the liver and uterus, the ovary displayed accelerated aging in an age-dependent manner, which was accompanied by elevated levels of inflammatory factors and apoptotic cells, and impaired macrophage phagocytic activity. The aged KGN cells exhibited elevated reactive oxygen species (ROS) and apoptotic levels alongside decreased MMP. H_2O_2-induced aging macrophages showed reduced phagocytosis function. Moreover, there were excessive aging macrophages with impaired phagocytosis in the follicular fluid of patients with diminished ovarian reserve (DOR). Notably, LMWC administration alleviated ovarian aging by enhancing macrophage phagocytosis and promoting tissue homeostasis.

Conclusions: Aging ovarian is characterized by an accumulation of aging and apoptotic granulosa cells, an inflammatory response and macrophage phagocytosis dysfunction. In turn, impaired phagocytosis of macrophage contributes to insufficient clearance of aging and apoptotic granulosa cells and the increased risk of DOR. Additionally, LMWC emerges as a potential therapeutic strategy for age-related ovarian dysfunction.

Keywords: Ovary; Macrophages; Aging; Phagocytosis; Chitosan; Diminished ovarian reserve

Down Regulation Of *Engase* In *Caenorhabditis Elegans* May Improve Its Stresses Adaptivity

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Abstract

Endo-beta-N-acetylglucosaminidase (ENGASE) is one of the key enzymes involved in the regulation of structure and function of glycoproteins. It is conserved from prokaryotic to eukaryotic cells. Although their activities *in vitro* and applications have been well studied, the biological function of ENGASE remains to be illustrated. In this study, we analyzed the molecular and physiological function of *Engase* from *Caenorhabditis elegans* homolog *eng-1*(*CeEngase*). We found that *CeEngase* knockout or knockdown increased the environmental stresses adaptability, such as heat stress and osmotic stress. Preliminary glycomics analysis showed that the basement membrane proteins of extracellular matrix may be the main targets of CeENGASE. In addition, CeENGASE may selectively prefer to N2H7 glycans on glycoproteins. In conclusion, our data illustrated that the defection and/or down regulation of *CeEngase* may provide a beneficially adaptation for stresses.

A Nanobody-Enzyme Fusion Protein Targeting Pd-L1 And Sialic Acid Exerts Anti-Tumor Effects By Affecting Tumor Associated Macrophages

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Abstract

Cancer cells employ various mechanisms to evade immune surveillance. Their surface features, including a protective "sugar coat" and immune checkpoints like PD-L1 (programmed death ligand 1), can impede immune cell recognition. Sialic acids, which carry negative charges, may hinder cell contact through electrostatic repulsion, while PD-L1 transmits immunosuppressive signals to T cells. Furthermore, cancer cells manipulate macrophages within the tumor microenvironment to facilitate immune escape. Prior research has demonstrated the effectiveness of separately blocking the PD-L1 and sialic acid pathways in eliciting anti-tumor effects. In this study, we investigated the relationship between PD-L1 expression and genes associated with sialic acid in clinical databases. Subsequently, we developed a novel nanobody enzyme fusion protein termed Nb16-Sia to simultaneously target both PD-L1 and sialic acid pathways. In vivo experiments confirmed the anti-tumor activity of Nb16-Sia and highlighted its dependence on macrophages. Further investigations revealed that Nb16-Sia could polarize macrophages towards the M1 phenotype through the C-type lectin pathway in vitro and eliminate tumor-associated macrophages in vivo. In conclusion, our findings demonstrate that the fusion of PD-L1 nanobody with sialidase effectively targets tumor-associated macrophages, resulting in significant anti-tumor effects. This approach holds promise for drug development aimed at enhancing immune responses against cancer.

Evaluating the Immunogenicity of Heparin and Heparin Derivatives by Measuring Their Binding to Platelet Factor 4 Using Biolayer Interferometry

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Heparin (HP) is a polysaccharide that is widely used in the clinic as an anticoagulant. A major side effect associated with HP is the heparin-induced thrombocytopenia (HIT), which is initiated by the immune response to complex formed by HP and platelet factor 4 (PF4). Low molecular weight heparins (LMWHs) are the depolymerized version of HP, which have reduced risks of inducing HIT. However, it is still necessary to evaluate the immunogenicity of LMWHs to ensure their drug safety. Since HIT involves very complicated processes, the evaluation of HP and LMWH immunogenicity requires experiments from multiple aspects, of which the binding affinity between HP and PF4 is a key property to be monitored. Herein, we developed a novel competitive biolayer interferometry (BLI) method to investigate the binding affinity between HP and PF4. The influence of different domains in HP on its immunogenicity was compared for better understanding of the molecular mechanism of HP immunogenicity. Furthermore, the half maximal inhibitory concentration (IC50) of HP and LMWH can be measured by competitive combination, which is important for the quality control during the developing and manufacturing of HP and LMWH drugs.

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Characterization Of The Intact N-Glycopeptide Of *Haemonchus Contortus* H-Gal-Gp Antigen

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Haemonchus contortus is a parasitic nematode of ruminants, seriously endangering the health of cattle and sheep and causing major economic losses to the global breeding industry. Currently, the drug resistance situation of this parasite is becoming increasingly severe, therefore, there is an urgent need on the research and development of vaccines. The native H-gal-GP is one of the most promising natural antigens for vaccine development, which is rich in N-glycosylation and plays a key role in the process of nematodes digesting the host blood to obtain nutrients for itself. However, the immune protection of recombinant proteins is greatly reduced because the recombinant proteins cannot obtain the correct protein glycosylation as the native proteins. Here, we first integrated the glycoprotein sequence and N-glycan structure of *H. contortus* to construct a complete glycoprotein database based on our previous work, and then used the complete glycoproteomics technology combined with GPSeeker (searching for N-glycans with $Man_3GlcNAc_2$ core structure) and pGlyco3.0 (supplemental search for truncated core structure), and identified a total of two aspartyl protease molecules, four zinc metalloproteinase molecules and one cysteine protease molecule, including 16 glycosylation sites and 110 corresponding intact N-glycopeptides, which is rich in many glycan motifs with known immunogenicity, including the Gal-Fuc motif, the di-fucose and tri-fucose motif, as well as the terminal LDNF motif. On the basis of the reported structural information of H-gal-GP by cryo-EM, the site-specific glycan was added to the corresponding N-glycosylation site by 3D visualization technology, and the complete structure and N-glycosylation characteristics of the native H-gal-GP antigen were obtained. Taken together, it provides a theoretical basis for the precise development of anti-parasitic glycoprotein synthesis vaccines.

Identification And Characterization Of An A-1,3 Mannosidase From *Elizabethkingia Meningoseptica* And Its Potential Attenuation Impact On Allergy Associated With Cross-Reactive Carbohydrate Determinant

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Abstract

Core α-1,3 mannose is structurally near the core xylose and core fucose on core pentasaccharide from plant and insect glycoproteins. Mannosidase is a useful tool for characterization the role of core α-1,3 mannose in the composition of glycan related epitope, especially for those epitopes in which core xylose and core fucose are involved. Through functional genomic analysis, we identified a glycoprotein α-1,3 mannosidase and named it MA3. We used MA3 to treat allergen horseradish peroxidase (HRP) and phospholipase A2 (PLA2) separately. The results showed that after MA3 removed α-1,3 mannose on HRP, the reactivity of HRP with anti-core xylose polyclonal antibody almost disappeared. And the reactivity of MA3-treated PLA2 with anti-core fucose polyclonal antibody decreased partially. In addition, when PLA2 was conducted enzyme digestion by MA3, the reactivity between PLA2 and allergic patients' sera diminished. These results demonstrated that α-1,3 mannose was a critical component of glycan related epitope.

Insights Into Galectin-4 Binding To Abh Antigen Containing Molecules

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Galectin-4 (Gal-4) is a member of the galactoside-binding protein family Galectins, which are multifaceted lectins performing different functions both intra and extracellularly and are in fact involved in multiple biological events.[1] As a tandem-repeat Galectin, Gal-4 has a dimeric architecture, with two different Carbohydrate Recognition Domains (CRD) covalently attached through a flexible peptide fragment. Although the dimeric architecture of tandem-repeat type galectins modulates their biological activities,[2] the underlying molecular mechanisms have remained elusive.

Among their multiple roles, emerging evidence show that tandem-repeat galectins are important players in the innate immunity by recognizing carbohydrate antigens present on the surface of certain pathogens, which very often mimic the structures of the human self-glycan antigens. Herein, the binding preferences of the isolated CRD domains of Gal-4 towards the ABH-carbohydrate antigens with different core presentations have been analyzed and their recognition features have been rationalized by employing a combination of experimental approaches.[3,4] The behavior of the full-length tandem-repeat form (Gal-4FL) has been additionally scrutinized, to show that both domains within Gal-4FL bind to ABH oligosaccharides independently of each other. In this context, the heterodimeric architecture does not play any major role, apart from the complementary A and B-antigen binding preferences. However, upon binding to a bacterial lipopolysaccharide (LPS) containing a multivalent version of an H-antigen mimetic as O-antigen, the significance of the galectin architecture was revealed. Indeed, our data point to the linker peptide domain and the F-face of the C-domain as key elements that provide Gal-4 with the ability to cross link multivalent ligands, beyond the glycan binding capacity of the dimer.

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The Retaining Pse5Ac7Ac Pseudaminyltransferase Kpss1 Defines A Previously Unreported Glycosyltransferase Family (GT118)

Abigail J. Walklett, Emily K. P. Flack, Harriet S. Chidwick, Natasha E. Hatton, Tessa Keenan, Darshita Budhadev, Julia Walton, Gavin. H Thomas and Martin A. Fascione

Cell surface sugar 5,7-diacetyl pseudaminic acid (Pse5Ac7Ac) is a bacterial analogue of the ubiquitous sialic acid, Neu5Ac, and contributes to the virulence of several multidrug resistant bacteria, including ESKAPE pathogens *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Despite its discovery in the surface glycans of bacteria over thirty years ago, to date no glycosyltransferase enzymes (GTs) dedicated to the synthesis of a pseudaminic acid glycosidic linkage have been unequivocally characterised in vitro. In our research, we demonstrate that *A. baumannii* KpsS1 is a dedicated pseudaminyltransferase enzyme (PseT) which constructs a Pse5Ac7Ac-α(2,6)-Glc*p* linkage (Figure 1), and proceeds with retention of anomeric configuration. We utilise this PseT activity in tandem with the biosynthetic enzymes required for CMP-Pse5Ac7Ac assembly, in a two-pot, seven enzyme synthesis of an α-linked Pse5Ac7Ac glycoside. Due to its unique activity and protein sequence, we also assign KpsS1 as the prototypical member of a previously unreported GT family (GT118).[1]

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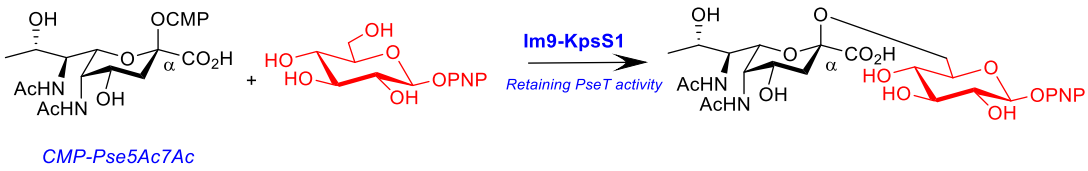

Figure 1: Im9-KpsS1 reacting CMP-Pse5Ac7Ac (donor substrate) with a PNP-β-D-Glc*p* (acceptor substrate) to produce a Pse5Ac7Ac-α(2,6)-Glc*p* linkage.

Identification of A Novel Histone Modification Marker That Is Derived from Isobutyryl-Coa

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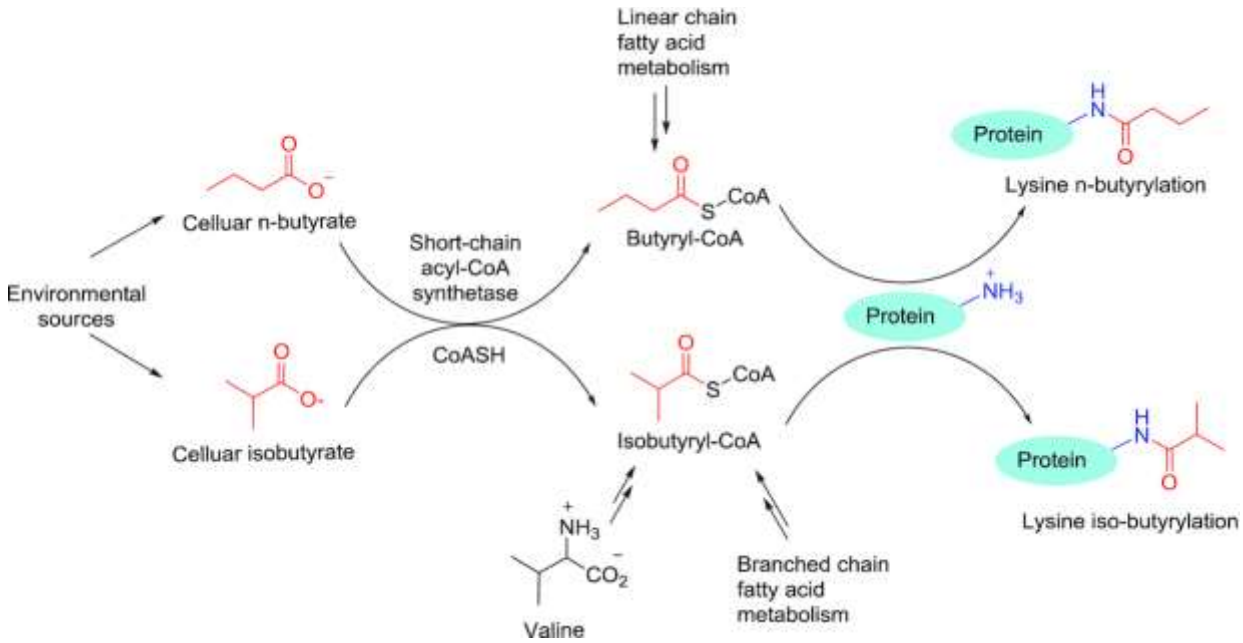
Short-chain acylations of lysine residues in eukaryotic proteins are acknowledged as crucial posttranslational modifications (PTMs) that play a pivotal role in regulating various cellular processes, including transcription, cell cycle, metabolism, and signal transduction. Lysine butyrylation was initially identified as a straight chain acylation known as normal butyrylation (Knbu). In this report, we present the discovery of its structural isomer, branched chain butyrylation, specifically lysine isobutyrylation (Kibu), which represents a novel PTM occurring on nuclear histones. Notably, isobutyryl-CoA exhibits a unique derivation from valine catabolism and branched-chain fatty acid oxidation, setting it apart from the metabolic pathway of n-butyryl-CoA. In vitro studies have indicated that several histone acetyltransferases, particularly p300 and HAT1, possess lysine isobutyryltransferase activity. Further transfection and western blot experiments have demonstrated the regulatory role of p300 in modulating histone isobutyrylation levels within cells. Additionally, X-ray crystal structures of HAT1 in complex with isobutyryl-CoA have provided valuable atomic-level insights into HAT-catalyzed isobutyrylation. Moreover, RNA-Seq profiling unveiled that isobutyrate exerted a significant impact on the expression of genes linked to various pivotal biological pathways. Collectively, our findings establish Kibu as a novel chemical modification marker in histones, highlighting its extensive role in regulating epigenetics and cellular physiology.



Structurally defined heparin octasaccharide domain for binding to SARS-CoV-2 Omicron BA.4/BA.5/BA.5.2 spike protein RBD

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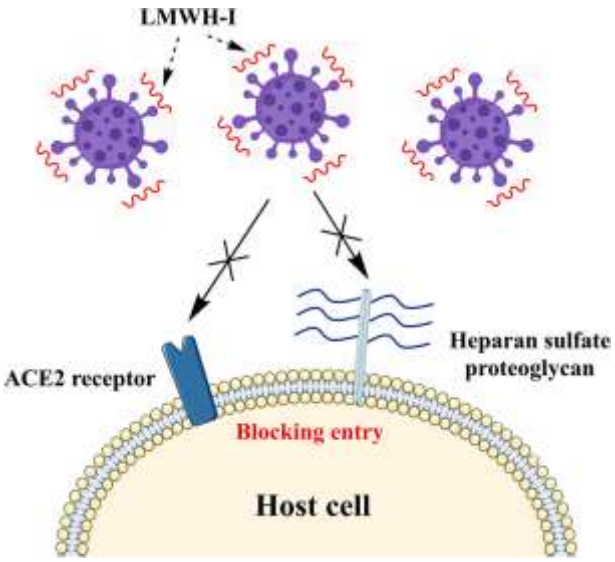

Heparin, a bio-molecule with the highest negative charge density, is pharmaceutically important to prevent SARS-CoV-2 infection due to its strong competitive binding to spike protein compared with cellular heparan sulfate, which was confirmed as a co-receptor for virus-host cell interaction. Hence, the refined structural characterization of heparin targeting viral protein-HS interaction was significant for developing antiviral pharmaceuticals. In our study, heparin oligomers (dp ≥ 4) were prepared using heparinase I. The affinity oligosaccharides binding to Omicron spike protein RBD were separated by affinity chromatography and size exclusion chromatography. HILIC-ESI-FTMS was used for chain mapping analysis. The basic building blocks were analyzed and the binding domain sequence was produced by Seq-GAG software and further measured by SAX chromatography. As results, heparin octasaccharide was found with significantly higher binding ability than hexasaccharide and tetrasaccharide, and the octasaccharide [ΔUA-GlcNS6S-GlcA-GlcNS6S-IdoA2S-GlcNS6S-IdoA2SGlcNS6S] with 12 sulfate groups showed high binding to RBD. The mechanism of this structurally well-defined octasaccharide binding to RBD was further investigated by molecular docking. The affinity energy of optimal pose was − 6.8 kcal/mol and the basic amino acid residues in RBD sequence (Arg403, Arg452, Arg493 and His505) were identified as the major contribution factor to interacting with sulfate/carboxyl groups on saccharide chain. Our study demonstrated that heparin oligosaccharide with well-defined structure could be potentially developed as anti-SARS-CoV-2 drugs.

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Chemical Synthesis Of The Oligosaccharides Related To *Fusobacterium Nucleatum* Atcc 23726 O-Antigen

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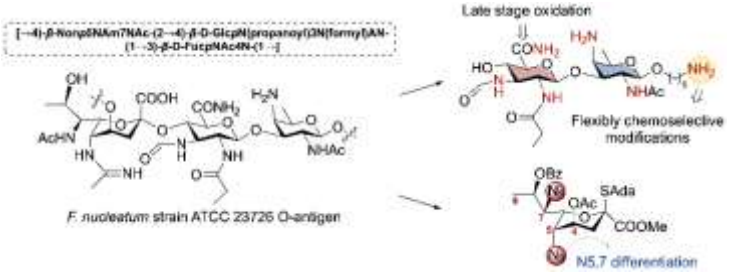
Colorectal cancer (CRC) is the third most prevalent malignant tumor globally. The existence of oncomicrobial risk factors for CRC has long been suspected. The most well-known oncomicrobe associated with CRC is *Fusobacterium nucleatum*, which has been considered as a potential target for CRC diagnosis and treatment. To date, there is no vaccine against *F. nucleatum* on the market. Developing carbohydrate-based vaccines against *F. nucleatum* have been hampered by challenges in identifying promising carbohydrate antigens. The chemical synthesis of homogeneous and well-defined oligosaccharides is an attractive means. The trisaccharide repeating unit of *F. nucleatum* ATCC 23726 O-antigen has been characterized as [→4)-*β*-Non*p*5NAm7NAc-(2→4)-*β*-D-Glc*p*N(propanoyl)3N(formyl)AN-(1→3)-*β*-D-Fuc*p*NAc4N-(1→]. The trisaccharide can be divided into an aminodisaccharide [→4)-*β*-D-Glc*p*N(propanoyl)3N(formyl)AN-(1→3)-*β*-D-Fuc*p*NAc4N-(1→] and a novel bacterial nonulosonic acid (NulO) 7-acetamido-5-acetimidoylamino-3,5,7,9-tetradeoxy-D-glycero-L-gluco-2-nonulosonic acid. Focusing on the aminodisaccharide fragment[1], there are five amino groups with five different modifications including carboxamidation, free amine, acetylation, formylation, and propanoylation. Rare 2,3-diamido-D-glucuronic acid amide and 2-acetamido-4-amino-D-fucose were synthesized from D-glucosamine through configuration inversion, nucleophilic substitution, C6 oxidation, and C6 deoxygenation. A judicious choice of protecting groups and reaction conditions enabled the selective installation of different *N*-substitutions. A reactivity comparison between the amino groups on the linker and the AAT sugar ring indicated that an amine linker installed at the reducing end can be used for further biological study. The 5-amino-7-azido-3,5,7,9-tetradeoxy-D-glycero-L-gluco-2-nonulosonic acid was designed as a glycosyl donor or acceptor.[2] The novel NulO was synthesized from *N*-acetylneuraminic acid with sequential performance of C5,7 azidation, C9 deoxygenation, C4 epimerization, and N5,7 differentiation. The C5 azido group in the obtained 5,7-diazido-NulO can be regioselectively reduced to differentiate the two amino groups. These studies provide an opportunity for further synthesizing the trisaccharide repeating unit of *F. nucleatum* ATCC 23726 O-antigen.



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A 3d-Printable Polysaccharides-Based Hydrogel Scaffold To Enable Porcine Muscle Stem Cells Expansion And Differentiation For Cultured Meat Development

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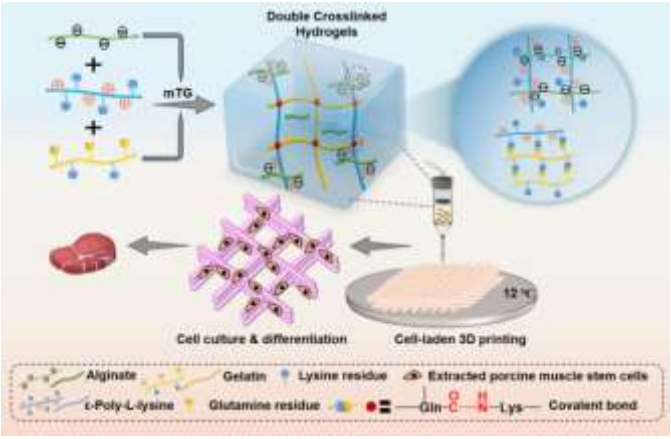
Global trends in increasing meat consumption and excessive environmental pollution underline the growing need to supplement animal-based meat production with alternative sources of dietary protein. Cultured meat, a promising future alternative for meat food, is produced directly from cells. The mass proliferation of seed cells and imitation of meat structures remain challenging for cell-cultured meat production. Tissue engineering aims to use appropriate cell types, scaffolding materials, and manufacturing techniques to mimic complex natural tissue. With excellent biocompatibility, high water content and porosity, hydrogels are frequently-studied materials for anchorage-dependent cell scaffolds in biotechnology applications. Herein, a scaffold based on gelatin/alginate/ε-Poly-L-lysine (GAL) hydrogel is developed for skeletal muscle cells. ε-Poly-L-lysine with the wealthy amino groups can form stable crosslinking with alginate by electrostatic interaction. Meanwhile, the lysine residues of gelatin or of ε-PL can be covalently bonded with the glutamine residues of gelatin to form strong intra- or intermolecular covalent bridges as cross-linking points. Thus, the gelatin/alginate/ε-Poly-L-lysine (GAL) hydrogels were formed via both covalently and ionically cross-linking. In this work, the hydrogel GAL-4:1, composed of gelatin (5%, *w/v*), alginate (5%, *w/v*) and ε-Poly-L-lysine (molar ratio vs. alginate: 4:1) is selected as cell scaffold based on Young's modulus of 11.29 ± 1.94 kPa, satisfactory shear-thinning property and suitable porous organized structure. The commercially available C2C12 mouse skeletal myoblasts and porcine muscle stem cells (PMuSCs), are cultured in the 3D-printed scaffold. The cells show strong ability of attachment, proliferation and differentiation after induction, presenting high biocompatibility. Furthermore, the cellular bioprinting is performed with GAL-4:1 hydrogel and freshly extracted PMuSCs. The extracted PMuSCs exhibit high viability and display early myogenesis (desmin) on the 3D scaffold, suggesting the great potential of GAL hydrogel as 3D cellular constructs scaffolds. Overall, we develop a novel GAL hydrogel as a 3D-printed bioactive platform for cultured meat research.



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Mechanism and inhibition of the *Mtb* cell wall assembly

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Mycobacterium tuberculosis (*Mtb*), the causative agent of global tuberculosis (TB), is one of the leading causes of human death globally. The complex *Mtb* cell wall comprised of glycan and lipids plays a pivotal role in *Mtb* viability and pathogenicity. The enzymes involved in *Mtb* cell wall biosynthesis are well-defined anti-TB drug targets, such as the arabinosyl-transferases EmbA/B/C, the targets of front-line anti-TB drug ethambutol. However, the function of these cell wall enzymes remains poorly understood on the molecular level. We have revealed that ethambutol binds the EmbA-EmbB and $EmbC_2$ complexes (enzymes in AG and LAM biosynthesis, respectively) and inhibits their enzymatic activities by competing with the donor DPA and acceptor substrates as a competitive inhibitor (Science, 2020). Based on this discovery, we continued our research in essential enzymes involved in the cell wall arabinan pathway. The arabinose donor DPA is essential in both AG and LAM pathway. We found a unique membrane-bound phosphoribosyl transferase (PRTase) is key to DPA biosynthesis and therefore is theoretically a promising new drug target. We have determined the cryo-EM structures of this PRTase and revealed the structural basis of the cell envelop precursor synthesis (Nature Microbiology, 2024). In another work, we have determined the cryo-EM structure of the arabinan priming enzyme AftA (the first enzyme that utilizes DPA for AG biosynthesis) and suggested the basis for priming selectivity (PNAS, 2023). Targeting these enzymes will be of great interest in anti-TB drug development. In this talk, I will describe our findings in these critical enzymes involved in *Mtb* cell wall arabinan pathway, primarily from a structural point of view.

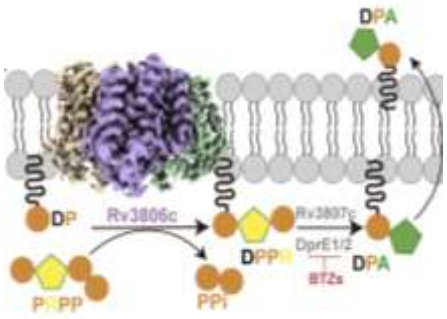

Fig.1 The front-line anti-TB drug ethambutol binds to the arabinosyl-transferases (left) EmbA-EmbB and

(right) EmbC-EmbC.

Fig.2 Molecular mechanism of a mycobacterial membrane-bound phophoribosyl-transferase

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Thermal Proteome Profiling Reveals Fructose-1,6-Bisphosphate As A Phosphate Donor To Activate Phosphoglycerate Mutase 1

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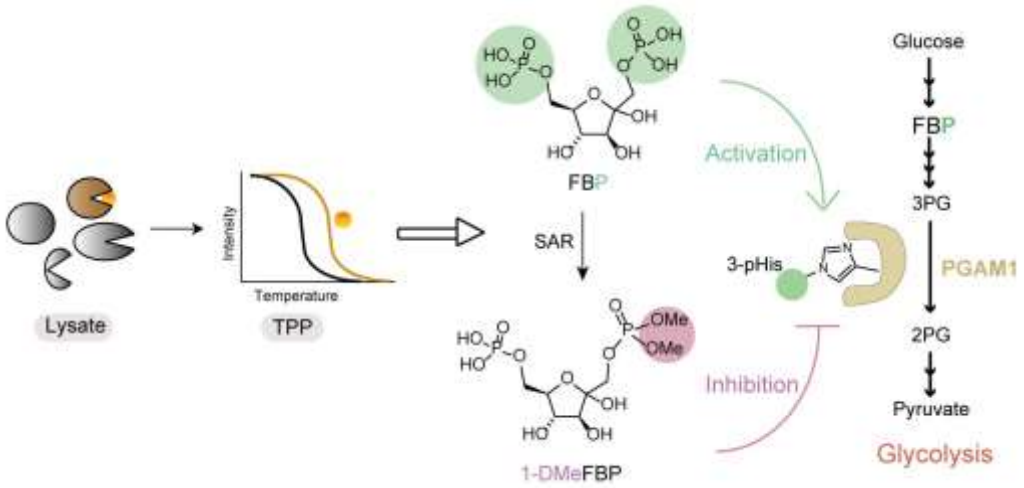
Emerging evidences have shown that disregulated sugar metabolites from sugar metabolic reprogramming process act as signaling molecules to regulate various cellular events through interacting with specific proteins.[1-4] A deep understanding of sugar metabolite-protein interactions should provide valuable implications in human physiopathology. Although tremendous efforts have been made for determining individual sugar metabolite-protein interaction, global profiling of such interactome remains challenging. In this study, we describe a thermal proteome profiling strategy for comprehensive mapping of central glycolytic metabolite fructose-1,6-bisphosphate (FBP)-interacting proteins. Our results reveal a chemical signaling role of FBP which acts as a phosphate donor to activate phosphoglycerate mutase 1 (PGAM1) and contribute an intrapathway positive feedback for glycolysis and cancer cell proliferation. At the molecular level, FBP donates its C1/C6-*O*-phosphate to the catalytic histidine of PGAM1 to form 3-phosphate histidine (3-pHis) modification. Importantly, structure-activity relationship studies of such interaction facilitate the discovery of PGAM1 orthostatic inhibitors which can potentially restrain cancer cell proliferation. Collectively we have profiled a spectrum of FBP interactome, and discovered a unique covalent signaling function of FBP that supports Warburg effect *via* histidine phosphorylation which inspires the development of pharmacological tools targeting metabolism.[5]



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Chemoenzymatic Glycoengineering Approach For The Formation Of Single Glycoform Monoclonal Antibodies

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Monoclonal antibodies (mAbs) are the most widely used protein therapeutics in the treatment of cancers, arthritis, and other human disorders worldwide. Currently the production of mAbs by mammalian (e.g. CHO cells) or alternative cell culture platforms gives rise to heterogenous mAb structures [1]. Contrastingly, homogenous mAbs demonstrate higher specificity and selectivity to their ligands (FcRs) [2]. The value of this is that the patient dose can be decreased for the same clinical outcome and consequently the value of the antibody can be enhanced. *N*-glycans have the ability to fine tune immunological responses such as antibody-dependent cellular cytotoxicity (ADCC) or anti-inflammatory properties. The formation of these highly useful single glycoforms still remains a challenge. Two current glycoengineering methods include a) cell line engineering and b) media supplementation and process parameter alterations [1]. Here we will present a chemoenzymatic workflow (**Figure 1**) for the design and characterisation of a single glycoform mAbs using a previously developed *N*-glycoanalytical technology [3,4]. In brief, the workflow sequentially removes key glycan motifs such as sialic acids, galactose, *N*-acetylglucosamine and fucose residues from the mAb originator molecules followed by addition of selective glycan epitopes utilizing glycosyltransferase enzymes and sugar donors to generate a family of glycoengineered mAbs for improved pharmacokinetics and enhanced effector functions.

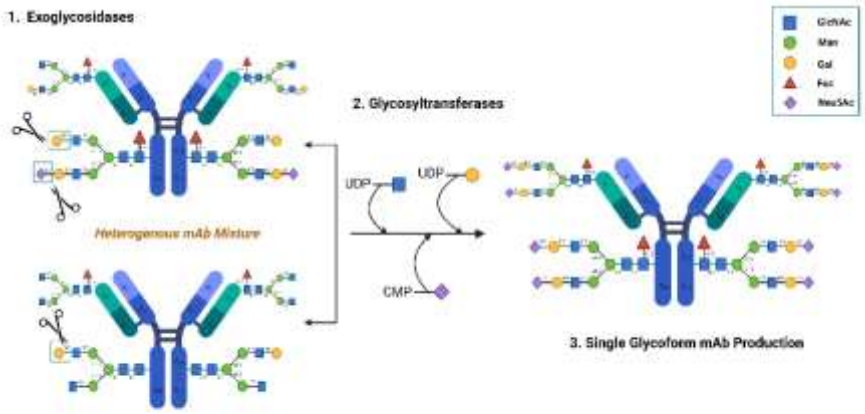

Figure 1. General scheme for chemoenzymatic synthesise of single glycoform mAbs. UDP, uridine diphosphate: CMP; cytidine monophosphate, Glc; glucose, Gal; galactose, GlcNAc; *N*-acetylglucosamine, Fuc; fucose, and Neu5Ac; *N*-acetylneuraminic acid.

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Binding Specificities Of Disialylated Gangliosides By Siglec-7

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Gangliosides are glycosphingolipids composed of an extra-cellular carbohydrate moiety that is linked to ceramide, a hydrophobic lipid portion embedded in the cellular membrane. They are widely distributed in human cells and tissues and play crucial roles in cellular processes, such as neurotransmission, interaction with regulatory proteins of the nervous system, cell-cell recognition and modulation of signal transduction pathways.[1] Notably, gangliosides containing sialic acids have been found to pronounce effects in cancers, influencing cell behaviors such as proliferation, migration, invasion, adhesion, and angiogenesis, but also mediating immunosuppression of tumors.[2] In recent years, the study of gangliosides has made significant strides, particularly with respect to their relevance to pathology and their interactions with sialic acid binding receptors.[3] Among these, Siglecs, sialic acid-binding immunoglobulin-like receptors, are I-type lectins found on most white blood cells of the immune system and have in common an N-terminal Ig domain that recognizes sialic acid–containing glycans. Evidence indicates that certain Siglecs are engaged by endogenous gangliosides to trigger important physiological and pathophysiological signaling events.[4] The presence of ligands for Siglec-7 and Siglec-9 has been found to be high in various cancer types, such as pancreatic cancer and melanoma.[5] For example, sialoglycans expressed on cancer cells surface can engage Siglec-7 on natural killer (NK) cells, leading to the inhibition of immune responses.[6] We here present a comprehensive analysis of the structure, conformation, and interactions of α2,8-linked gangliosides, including GD3 and its derivatives DSGb3α3 and DSGb3α6, as well as DSGB5 that contains both α2-3- and α2-6-linked sialic acids and densely populated on renal cell carcinoma (RCC).[7,8] Understanding the dynamics of these interactions holds great promise for providing insights into disease mechanisms and potentially opening the door to the development of diagnostic and therapeutic strategies.[9] To this aim we combined structural biology methodologies, NMR, techniques, chemical-physical studies, and computational approaches to provide information on binding affinities and 3D models of the complexes.[10,11] This can have significant implications in the field of immunotherapy, where targeting these interactions may lead to novel strategies for conditions such as cancer, which often exploits sialic acid-containing molecules in immune evasion mechanisms.

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Isolation and structure characterization of a polysaccharide from *Edgeworthia chrysantha* Lindl. and its hepatocyte proliferation promoting bioactivity.

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Edgeworthia chrysantha Lindl. (ECL) is a traditional Chinese medicine. And it is also a Chinese herbal medicine of channel tropism of live. Polysaccharide is a biological macromolecule with various bioactivities, such as anti-inflammatory, inhibiting angiogenesis[1], shaping the gut microbiota[2], inducing apoptosis and against autophagy[3] and so on. Here, an acidic polysaccharide ECL222 with an average molecular weight of 93 kDa, was extracted by boiled water. It was purified from dried herb *Edgeworthia chrysantha* Lindl. by DEAE Sepharose™ Fast Flow and Sephacry S-300 HR columns. Monosaccharide composition results showed that the polysaccharide of ECL222 was composed of rhamnose, arabinose,galactose and galacturonic acid in a molar ratio of 25.09: 10.83: 31.54: 32.54.The backbone of ECL222 was consisted of 1,2-linked rhamnose, 1,2,4-linked rhamnose, 1,4-linked galacturonic acid. And the branch chain of ECL 222 contained the 1,3-linked galcatose, 1,4-linked galactose, terminal galcatose, 1,5-linked arabinose, terminal-linked arabinose, 1,3,5-linked arabinose and 1,3,6-linked galactose, according to the results of partial acid hydrolysis analysis, methylation analysis and NMR spectra. Bioactivity screen results showed that this polysaccharide may promote hepatocyte proliferation in a concentration-dependent manner.

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Oriental Covalent Immobilization Of N-Glycan Binding Protein Via N-Terminal Selective Modification

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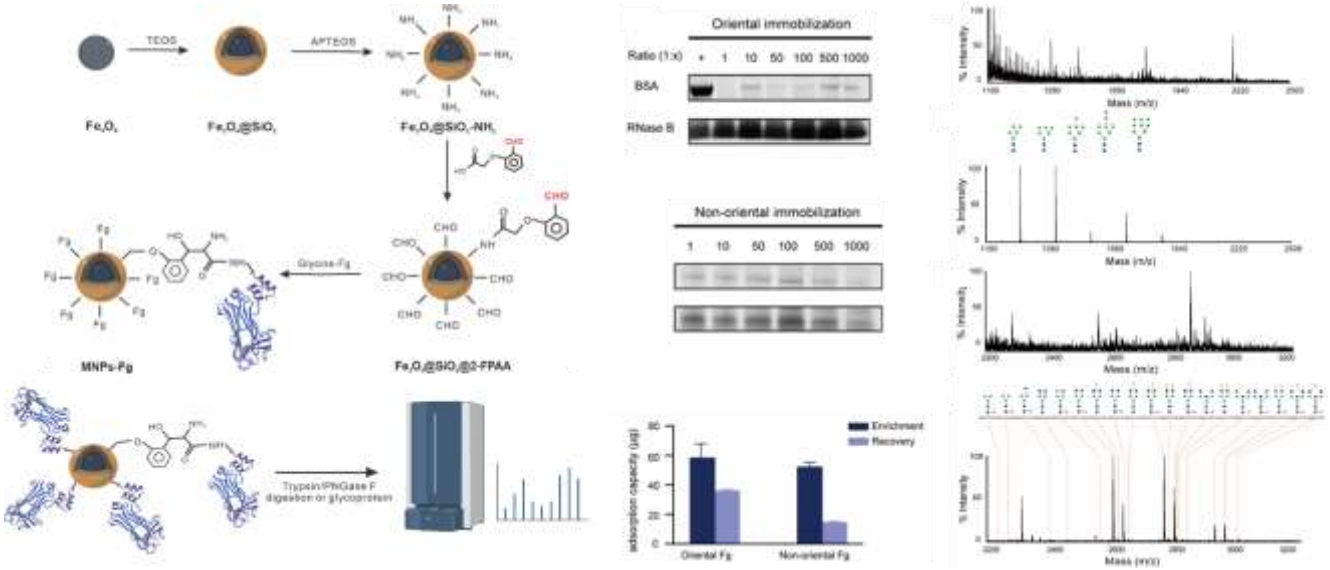
Lectin affinity chromatography is one of powerful tools for the study of protein glycosylation. Different lectin proteins can recognize different structures of monosaccharides or oligosaccharide units, allowing for the selective separation of glycopeptides or glycoproteins containing different polysaccharide structures. However, the N-glycans were only partially captured by most of common lectins, reducing the coverage rate of identifying N-glycoconjugates. Recently, it has been reported that the engineering variant of glycan binding protein Fbs1 has a high affinity for innermost Man3GlcNAc2 structure and is able to bind diverse types of N-glycans, which can be suitable for the analysis of protein N-glycosylation. However, efficient immobilization of protein to separation matrix is particularly challenging as it requires the functionality and integrity of the protein to be preserved. Herein, we describe a simple and robust strategy for oriental covalent immobilization of proteins on magnetic nanoparticles by N-terminal selective labeling techniques. We inserted the enterokinase cleavage site to produce the specific N-terminal glycine of protein. Under physiological conditions, the protein was immobilized on the surface of the MNPs by this glycine tag, and the enrichment process could be completed within 30 minutes. A whole enrichment and purification of glycan and glycopeptides were completed and analyzed by MALDI TOF-MS. The functional materials achieved stable enrichment of sugar structure in different enzyme digestion systems or complex samples, showing excellent anti-interference and applicability.



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Maltooligosaccharide@Gadolinium Contrast Agent On Targeted Diagnosis Of Bacterial Infections

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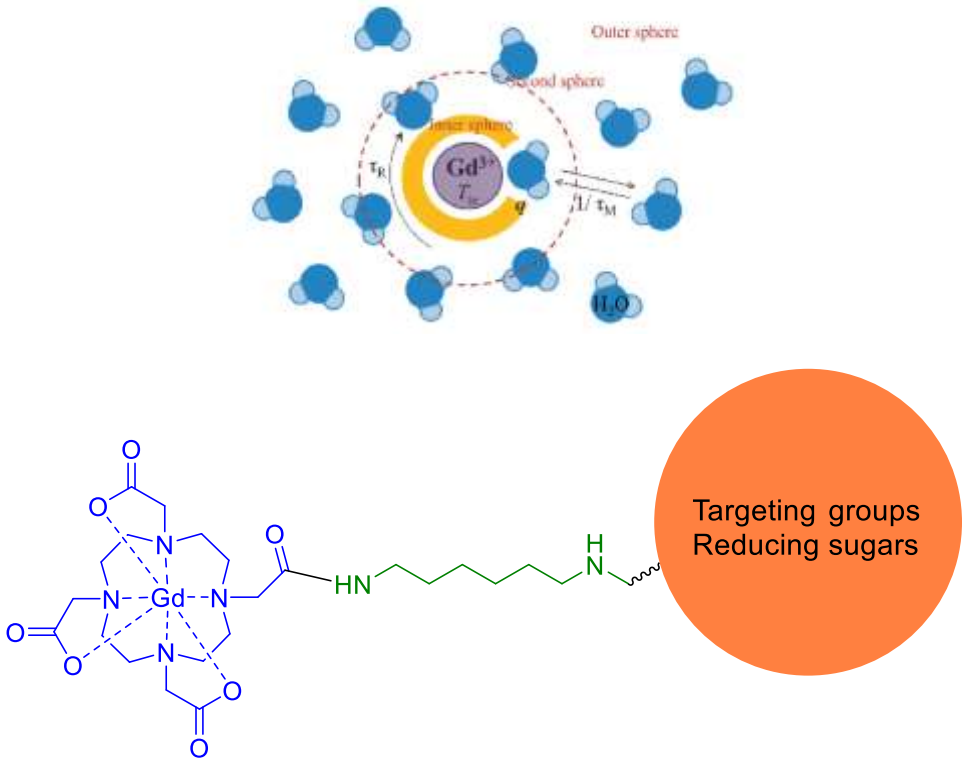
Magnetic resonance imaging (MRI) is a noninvasive imaging technique with many advantages such as high resolution and no ionizing radiation, which is widely used in clinical medicine. Contrast agents are a class of auxiliary diagnostic agents that can effectively increase the contrast between normal and diseased tissues, and is currently used in close to 50% of MRI tests in clinical practice. Gadolinium-based contrast agents (GBCA) are the most widely used contrast agents[2], and there are 8 GBCAs on the market, occupying almost the entire contrast agent market. Comparatively the r1 values of these GBCAs are still unsatisfactory, with unsatisfactory imaging effects, poor targeting of tissues and cells, and the risk of releasing free[3] Gd^{3+}. In this regard, we propose to design a class of maltooligosaccharide@gadolinium contrast agents (Gd-Mal), which are specifically targeted to bacteria through the ABC transport mechanism of maltooligosaccharide. By studying the targeting imaging ability of gadolinium contrast agents with different structures and dosages on bacterial infected lesions, it is possible to provide a new method for the bacterial infection detection and subsequent therapeutic observation.



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A Glycoengineering Strategy For Labelling Lipooligosaccharides And Detecting Native Sialyltransferase Activity In Live Gram-Negative Bacteria With Neuraminic Acids Analogs

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Lipooligosaccharides (LOS) are the most abundant cell surface glycoconjugates on the outer membrane of Gram-negative bacteria. They play important roles in host-microbe interactions. Some pathogenic bacteria cap their LOS with *N*-acetylneuraminic acid (Neu5Ac) to mimic host glycans, a way of protecting themselves from recognition by the hosts immune system. The process of molecular mimicry is not fully understood and remains under investigated for many Gram-negative bacteria. To investigate the functional role of sialic acid-capped lipooligosaccharides (LOS) at the molecular level, it is important to have tools readily available for the detection and manipulation of both Neu5Ac on glycoconjugates and the involved sialyltransferases, preferably in live bacteria. We and others have shown that the native sialyltransferases of some Gram-negative bacteria can incorporate extracellular unnatural sialic acid nucleotides onto their LOS. We here report on glycoengineering strategy for the use of native bacterial sialyltransferases to incorporate Neu5Ac analogues with a reporter group into the LOS of a variety of relevant bacteria Gram-negative bacteria to human health and disease. We show that this approach offers a quick method to screen clinically relevant pathogenic bacteria for the expression of functional sialyltransferases and the ability to use exogenous CMP-sialic acids to decorate their glycoconjugates, and provides means to modify, label and visualize bacterial LOS.

Novel Fluorescent Probes For *N*-Glycan Labelling And Therapeutic Glycoprotein Detection

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Over 50% of human proteins are glycosylated and over 80% of biopharmaceuticals contain *N*-glycosylation [1]. Sugars or glycans are often altered when our immune system is dysregulated and glycosylation can also influence the safety and efficacy of therapeutics. As such tools to enhance detection of *N*-glycans, particularly for low-abundant proteins are desirable to the Glycoscience and the Biopharmaceutical community alike. Our innovative design will be based on fully automated methods developed in the O'Flaherty lab utilising current gold standards, 2-aminobenzamide (2-AB) and aminoquinoline carbamate (AQC) [2]. These methods will also allow direct comparison of the novel tags to the present state of the art. Novel tags synthesised as part of this project build on expertise within the Elmes Group at MU [3]. Coupling reactions are based on squaric acid intermediates, such as diethyl-, dimethyl, ditertbutyl and dichloro squarate, where these derivatives have found increased utility as a useful method of bioconjugation because of facile reaction protocols, high reaction yields in aqueous solution, high functional-group tolerance and usually very high conversions [3,4]. These protocols have been combined with various fluorophore derivatives and optimised to for efficient *N*-glycan labelling during my research. The novel tags have been labelled to *N*-glycans released from human IgG using established approaches[5,6]. The structure of human IgG presented can have up to 6 *N*-glycosylation sites (2 in Fc and 4 in Fab region) which serves as a suitable starting point as there is much research on its *N*-glycosylation profile and is the basis for a large number of biopharmaceutical drugs eg. monoclonal antibodies. Experimentally, the protein is denatured, reduced, alkylated and the *N*-glycans are enzymatically released. Following this step, labelling can occur via traditional chemistries, ie reductive amination using 2AB [6], nucleophilic substitution via AQC [2] or through the novel squaramide chemistries which utilises nucleophilic substitution presented in this work.

Figure 1: Proof of Principle Fluorophore design

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CD44 Targeting Nanodrug Based On Chondroitin Sulfate For Melanoma Therapy By Inducing Mitochondrial Apoptosis Pathways

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Neovascularization is crucial to the occurrence and progression of tumors, and the development of anti-angiogenic drugs has essential theoretical value and clinical significance. However, antiangiogenesis therapy alone cannot meet the needs of tumor therapy. Meanwhile, polysaccharides are ideal drug carriers with prom ising applications in drug modification and delivery. In this research, we developed a novel redox and acid sensitive nanodrug (CDDP-CS-Cys-EA, CCEA) composed of chondroitin sulfate (CS), antiangiogenic peptide (endostatin2-alft1, EA) and chemotherapeutic drug (cisplatin, CDDP). CCEA exhibited redox and acid respon siveness, better blood hemocompatibility (hemolysis rate.

Keywords: chondroitin sulfate, antiangiogenic peptide, cisplatin, antitumor, tumor microenvironment

Reconstituting The Immune Killing Functions And Improving The Pharmacokinetics Of Nanobody By Rhamnolipid Conjugation

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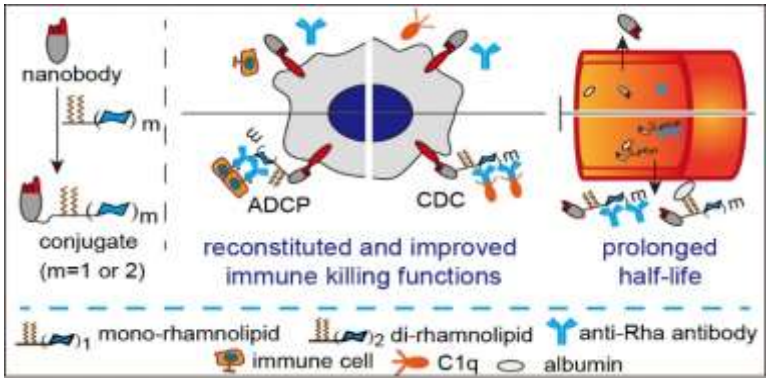
Abstracts: Nanobodies (Nbs) hold great promise as next-generation therapeutics in cancer immunotherapy,[1-2] but their efficacy is hindered by poor pharmacokinetics and the inability to trigger Fc-mediated immune killing functions.[3] In this study, we address these limitations by designing and synthesizing rhamnolipid-modified Nbs and evaluating their biological profiles *in vitro* and *in vivo*. 7D12, an EGFR-targeting Nb, was selected and site-specifically conjugated with a series of rhamnolipid derivatives. Structure-activity relationship results revealed that the number of rhamnose (Rha) units and the spacer length in the conjugates affected anti-tumor activities. Conjugate **R5**, which contained two Rha units and a PEG2 spacer, exhibited the most potent antibody-dependent cell-mediated phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) activities. Furthermore, **R5** gained a significantly improved half-life through the engagement of albumin and endogenous anti-Rha antibodies. Ultimately, the optimized conjugate **R5** demonstrated potent *in vivo* antitumor activity in xenograft mice. Our findings highlight the potential of rhamnolipidation as a strategy to enhance cancer immunotherapy and provide a cost-effective platform for improving the therapeutic efficiency of Nbs.



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Characterization Of Sialylated Oligosaccharides Based On Core Of Sialyllacto-N-Tetraose A By Derivation And Electrospray Tandem Mass

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Disialyllacto-N-tetraose (DSLNT) has been proved to be the main active ingredient in human milk oligosaccharides (HMOs) for the prevention of necrotizing colitis (NEC). At present, the real structure of DSLNT can only be synthesized by human *N*-acetylgalactosaminide α2,6-sialyltransferase (ST6GalNAc) when using sialyllacto-*N*-tetraose a (LSTa) as the substrate at the lab scale [1]. Bacteria α2,6-sialyltransferase have been used to synthesize the analogues of DSLNT when lacto-*N*-tetraose (LNT) was used as a substrate [2]. Herein, four recombinant bacterial α2,6-sialyltransferase were expressed in *Escherichia coli* to screen for synthesizing DSLNT using sialyllacto-*N*-tetraose a (LSTa) as the acceptor substrate. High-sensitivity online HPLC coupled with electrospray tandem mass spectrometry was used to characterize the derivatized DSLNT and other byproducts. Among the identified structures, three recombinant α2,6-sialyltransferases can synthesize DSLNT accompanied by structurally different by-products, and one DSLNT analogue mono-sialylated LSTa (MSLSTa) and two tri-sialylated LNT (TSLNT I and TSLNT II) were identified for the first time. By controlling the proportion of substrates, the highest DSLNT yield can be effectively achieved. This study revealed different reaction mechanisms for four α2,6-sialyltransferases and would be expected to provide an effective method for the large-scale synthesis of DSLNT.

Table 1 Oligosaccharides identified in this study by LC-ESI-MS/MS

RT[a]	$[M+H]^+$	Composition[b]	Structure[c]	Name
12.9	$[M+H]^+$ *m/z* 1119.3	H3N1S1	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	LSTa
22.0	$[M+H]^+$ *m/z* 1410.5	H3N1S2	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc	DSLNT
23.8	$[M+H]^+$ *m/z* 1410.5	H3N1S2	Neu5Acα2-3Galβ1-3GlcNAcβ1-3(Neu5Acα2-6)Galβ1-4Glc	MSLSTa
37.7	$[M+2H]^{2+}$ *m/z* 851.3	H3N1S3	Neu5Acα2-3(Neu5Acα2-6)Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc	TSLNT I
			Neu5Acα2-3(Neu5Acα2-6)Galβ1-3GlcNAcβ1-3(Neu5Acα2-6)Galβ1-4Glc	TSLNT II

[a] Retention time (in min) of 2AB derivatives; [b] H, Hex; N, HexNAc; S, Neu5NAc; [c] Proposed structure based on MS/MS and comparison with literature data.

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Comprehensive Profiling Of Extracellular Vesicles N-Glycome In Epilepsy

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N-glycosylation on the surface of extracellular vesicles is involved in biological processes such as vesicle biogenesis, intercellular communication, interneuronal communication, immune regulation, and disease progression. Perturbations in the N-glycosylation patterns of EV surface proteins, which include key membrane constituents such as ion channels and receptors, may precipitate increased neuronal excitability, potentially culminating in the induction of epileptic seizures. Given the permeability of EVs across the blood-brain barrier (BBB), EVs originating from cerebral neurons and glial cells can also be detected in peripheral circulation. Accurate and detailed profiling of N-linked glycosylation on EVs derived from the serum of epilepsy patients not only facilitates a deeper understanding of EV-mediated biological mechanisms but also bolsters the clinical translation of EV-centric diagnostic and therapeutic strategies for epilepsy. This study initially focused on assessing the impact of various advanced separation techniques on the glycosylation profiles of EVs, subsequently selecting the most efficacious method for deploying in the glycomic analysis of epilepsy-associated exosomes. Employing a high-throughput glycomic technique, we characterized the N-glycome signatures of serum-derived EVs from both healthy control subjects and individuals with epilepsy, aiming to identify potential biomarkers for pediatric epilepsy detection and monitoring. This investigation encompassed the analysis of serum and serum-derived EVs from a cohort of 235 participants, comprising 108 patients diagnosed with epilepsy and 127 age- and gender-matched healthy controls. The findings reveal distinct EV glycan alterations that correlate significantly with both the type and status of the disease in patients. EV-based biomarkers demonstrated superior diagnostic accuracy over traditional plasma biomarkers, effectively differentiating between epilepsy patients and healthy individuals. In conclusion, this research marks a substantial advancement in the field of neuropsychiatric disorders, underscoring the potential of EV-based biomarkers in facilitating the personalized diagnosis and management of epilepsy, thereby reinforcing their value in clinical applications.

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New Insights Into The Binding Of Pf4 To Long Heparin Oligosaccharides In Ultralarge Complexes Using Mass Spectrometry

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Heparin-induced thrombocytopenia (HIT) is a serious complication caused by heparin drugs. The ultralarge complexes formed by platelet factor 4 (PF4) with heparin or low molecular weight heparins (LMWHs) are important participants in inducing the immune response and HIT. In this work, the characteristics of PF4–enoxaparin complexes after incubation in different molar ratios and concentrations were analyzed by multiple analytical methods, especially liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry with multiple reaction monitoring were developed to qualitatively and quantitatively monitor heparin oligosaccharides and PF4 in HIT-inducing complexes. The results showed that the largest proportion of ultralarge complexes formed by PF4 and enoxaparin was at a specific molar ratio, ie, a PF4/enoxaparin ratio of 2:1, while the ultralarge complexes contained PF4 tetramer and enoxaparin at a molar ratio of approximately 2:1. A binding model of PF4 and enoxaparin in ultralarge complexes is proposed with one heparin oligosaccharide chain (~ dp18) bound to 2 PF4 tetramers in different morphologies to form ultralarge complexes, while PF4 tetramer is surrounded by multiple heparin chains in smaller complexes. Our study provides new insights into the structural mechanism of PF4–LMWH interaction, which help to further understand the mechanism of LMWH immunogenicity and develop safer heparin products.

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New Solutions For The Food Industry: Reducing Sugar Content By Enhancing Prebiotic Functionality

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Fruit preparations are rich in vitamins and nutrients but often contain high levels of intrinsic caloric sugars, contributing to the rise of diseases like obesity and cancer. The World Health Organization (WHO) recommends reducing sugar consumption to below 25 grams per day. Consumers now seek foods with good nutritional content and health-promoting properties, prompting the food industry to innovate. Fructo-oligosaccharides (FOS), valued for their prebiotic benefits, have been incorporated into various foods. FOS are selectively fermented by gut-healthy probiotic bacteria, improving both the metabolic and immune systems, thereby reducing the risk of developing a wide range of diseases, including obesity, diabetes, cardiometabolic diseases, intestinal inflammation, cancer, as well as cognition, and neuropsychological disorders. This study aimed to develop a low-sugar functional fruit preparation, for incorporation into dairy products, by converting sucrose into prebiotic FOS (*in situ*) using fructosyltransferase enzymes [1,2].

Four commercial enzymatic complexes were studied: Pectinex® Ultra SP-L, Viscozyme® L, Novozym® 960, and Catazyme® 25L. Reaction conditions, including temperature, pH, and enzyme/preparation ratio, were optimized to maximize the yield in FOS. The most effective was further applied in a commercial strawberry preparation. The prebiotic preparation was digested using the INFOGEST 2.0 protocol. Subsequently, the samples underwent a sensory analysis with a panel of 100 participants and 13 trained technical tasters applying the Temporal Check-All-That-Apply (TCATA) method. Under optimized conditions, the highest FOS yield ($g_{FOS}/g_{initial\ sucrose}$) was achieved with Novozym (0.629±0.002); Viscozyme (0.624±0.003) and Pectinex (0.619±0.003) showed similar production yields, followed by Catazyme (0.444±0.001). Thus, Novozym was chosen for further application in the strawberry preparation. An enzyme/preparation ratio of 1/100 (v/v) was found to be optimal. In a lab-scale setup, Novozym synthesized 293.2±0.7 g/L of FOS after a 1.25 h reaction, with a production yield of 0.630±0.003 $g_{FOS}/g_{initial\ sucrose}$. Similar outcomes were obtained in a pilot-plant study (290.2±0.8 g/L FOS). More than 90% of FOS remained unhydrolyzed after digestion showing prebiotic functionality. The preparation was well accepted by consumers, with a 93% approval rate.

In conclusion, the resultant strawberry preparation contained 56% (w/w) FOS, with an 85% reduction in initial sucrose content and an 18% reduction in caloric value. The *in-situ* approach demonstrated high efficiency and suitability for industrial applications.

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Characterization Of A Crystalline Unsaturated Hyaluronic Acid Disaccharide

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Unsaturated hyaluronic acid disaccharide (ΔDiHA) is a bioactive compound derived from the enzymatic breakdown of hyaluronic acid, with unsaturation occurring at the 4,5-glycosidic linkage of glucuronic acid. Previous studies have shown its excellent antioxidant properties and capacity to promote the proliferation of human vein endothelial cells and corneal epithelial cells, indicating its substantial therapeutic potential in the medical field. Moreover, high-purity ΔDiHA serves as a standard for assessing the content and purity of hyaluronic acid and related products. However, the usual preprepared ΔDiHA exists as amorphous powder, and shows strong hygroscopicity with 10% higher moisture content. To solve this problem, high-purity amorphous product of ΔDiHA was obtained via enzyme hydrolysis, tangential flow filtration and desalination. Subsequently, a systematic polymorph screening was conducted using various techniques including room temperature slurring, high-temperature slurring, anti-solvent, solvent evaporation, slow cooling, and vapor diffusion etc. Eventually, a special crystalline ΔDiHA was abstained using an anti-solvent crystallization strategy. Characterization with X-ray powder diffraction (XRPD) and polarized light microscopy (PLM) confirmed its crystalline form. Further thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) indicate its 5% lower moisture content. Ultimately, dynamic vapor sorption (DVS) shows a significant improvement in hygroscopicity. This stable crystal form of ΔDiHA has significant implications for its physicochemical properties, thus presenting broad prospects for its application in the medical field.

Keywords:
Unsaturated hyaluronic acid disaccharide; crystalline; hygroscopicity; dynamic vapor sorption

Novel Technologies And Challenges Of Sequencing Pectic Polysaccharides

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***Ab*stract**
Pectic polysaccharides, as essential dietary fiber components, have functions such as regulating gut microbiota, controlling obesity, and reducing the risk of chronic diseases. Hence, the elucidation of pectic polysaccharide structures is fundamental for exploring their functions. This research focuses on the elucidation extensive and delicate structures of pectic polysaccharides. (1) This research uses LC/QqQ-MS to dynamically monitor the characteristic ion pairs of 3-phenyl-5-methyl-5-pyrazolone (PMP) derivatized monosaccharides, allowing for the analysis of 23 common monosaccharides within 10 minutes with detection limits ranging from picomoles to micromolar. The investigation of polysaccharides extracted from different plants through EDTA-NaOH two-step extraction. (2) Through a process involving methylation in a 96-well plate, acid hydrolysis, and PMP derivatization, combined with LC/QqQ-MS analysis of monosaccharides, oligosaccharides, and polysaccharide standards, a library containing 94 glycosidic linkages has been constructed, enabling the separation and resolution of different neutral and acidic glycosidic residue within 30 minutes. (3) Based on the HILIC-MS/MS analysis of 332 plant oligosaccharides, a reference library containing over 500 mass spectrometry data was established for the first time. Construction of an automatic analysis scheme for pectic polysaccharide structure using an oligosaccharide MS reference library combined with bioinformatics software. Through an investigation encompassing the dimensions of monosaccharide composition, linkage and oligosaccharide constituents, this study endeavors to formulate an automated analysis framework by amalgamating an oligosaccharide liquid chromatography mass spectrometry reference library with bioinformatics software.

Biobased Polysaccharidic Biostimulant For Root Application: Enhancement Of Wheat Plants Tolerance To Drought Stress

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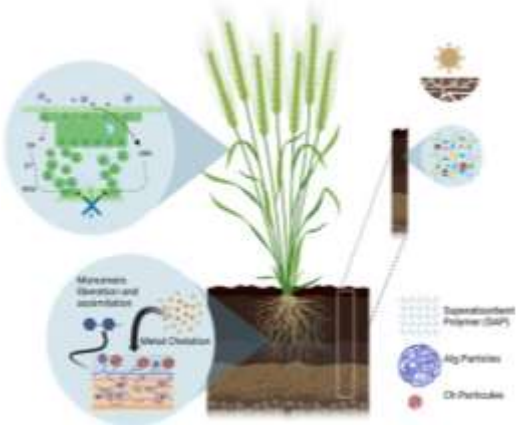
Biostimulants from polysaccharides are known to improve plant resilience against abiotic stresses by modulating cellular and molecular mechanisms, such as for example, abscisic acid hormonal pathway and the Reactive Oxygen/Nitrogen Species (ROS and NOS) regulation[1,2]. Also, the oligomeric form has been used for bio elicitation and biostimulation, for example, oligoalginates and oligochitosans tested on wheat in response to drought might induce the stomatal closure and the decrease damages generated by ROS-NOS species, increasing the antioxidant system protection[3,4].
In order to reduce inputs in the soil, root application appears to be a more suitable solution to establish synergic effects between plants, microorganisms and biostimulants. The application of polysaccharides based biostimulants might increase the plant performance at different levels of the plant physiology under abiotic stresses. Furthermore, the evaluation of biostimulant efficiency is important at different plant developmental stages, from the seed germination to maturation. In this study, wheat plants were treated with an innovative 100% biobased polysaccharide biostimulant in the soil under 2 water regimes (well-watered and drought) in order to study its effect on wheat performance and the water reserves in wheat tissues.



Our results **(Figure 1**) suggest that the root application of the developed biostimulants may help the plants to manage water in tissues, decreasing the gas exchanges by partial stomatal closure. Moreover, the treatments have shown to protect the external tissues of the plants against cellular damages, caused by a severe drought episode, probably linked to the ROS/NOS regulation. Further investigations are under process to determine the effects of the treatment on the soil microorganisms' diversity to decipher the interactions between biodegraded biostimulants and the rhizosphere.

Figure 1: Applying SAP close to the root system, the Chitosan (Ch) and alginate (Alg) particles may enhance the closure of stomata by production of ROS and ABA signaling dependent Pathway. SAP could chelate some micronutrients that cannot be absorbed by the plant under normal conditions. Also, interactions between microbiome-SAP-roots may lead to the biodegradation of the polymer. After its breakdown, subunits could be assimilated by the microbiome and lead to a slow release of chitosan and alginate particles. In combination with SAP the water holding capacity (WHC) of the soil may be improved when applying the biomolecules in the soil and plant tolerance under drought could be increased.

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A Sensor Array To Discriminate *Pseudomonas Aeruginosa* Pathoadaptation

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Microbial infections collectively accounted for over 13 million deaths in 2019, 559,000 of which were due to *Pseudomonas aeruginosa* (*Pa.*).[1] Bacterial pathogens readily adapt to the environment within their host, often altering virulence factors associated with adhesion and infection. Potential biomarkers for identifying these pathoadaptations include carbohydrate binding proteins, or lectins, that are associated with a variety of pathogens such as bacteria, viruses and fungi. Lectins bind to glycans displayed on almost all cell surfaces and play a key role in cellular recognition during pathogenesis.

Inspired by the mammalian glycocalyx, the design of a sensor array for the detection of bacterial lectins and associated pathoadaptations is reported. Ten fluorescent glycopolymers, differing only by carbohydrate moiety were synthesised. Upon addition of bacteria to our array, a unique 'fingerprint' of fluorescence responses were observed. The array was tested against a library of Pa. transposon mutants and clinical isolates, linear discriminant analysis conducted on the resulting dataset resulted in discrimination of each of mutant. Leave-one-out cross-validation suggested this label-free approach enabled the effective discrimination of genetically engineered *Pa.* transposon mutants, and clinical samples.

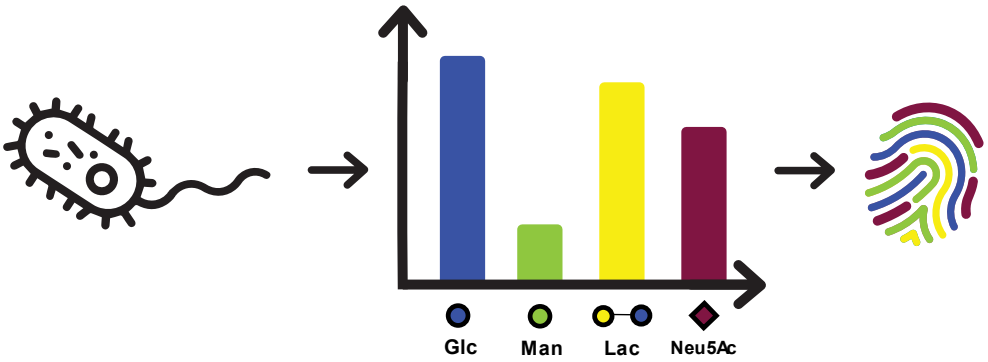

Fig. The principle of our array with each glycopolymer generating a response to a bacterium that are combined to generate a 'fingerprint'.

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Analytical Approaches To The Study Of Glycans Using Liquid Chromatography Mass Spectrometry

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Glycans are released/digested enzymatically or chemically using reductive beta elimination and analyzed as reduced glycans or modified with 2-aminobenzamide.

Glycans and/or glycopeptides are characterized with liquid chromatography-mass spectrometry. We routinely use porous graphitized carbon columns and C18 columns connected to ion trap or orbitrap mass spectrometers. The obtained MS^2 or MS^3 spectra are interpreted manually and compared to reference spectra compiled in-house. Glycans are semiquantified using Progenesis software, allowing us high throughput quantitative analyses of a large number of samples. Below are shown the sample preparation workflow and results from analyses of selected projects.

Molecular Assembled Crystalline Nanoxylan Synthesized From Hot Water Extracted Wood Xylan

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Nanostructured polysaccharides are certain to be a mainstay of the forthcoming green materials revolution in the transformation of societal sustainability [1]. Xylan is the third most abundant polysaccharide in nature after cellulose and chitin, accounting for approximately one-third of all renewable organic carbon on Earth [2]. In this work, as a contribution to expanding accessibility in the territory of bio-based nanomaterials, we adopt a bottom-up strategy to convert water-soluble and amorphous xylan obtained in a pressurized hot-water extraction (PHWE) biorefinery to crystalline nanoxylan (CNX) as stable hydrocolloids. Amorphous PHWE-xylan is water-soluble with branching side-sugar and acetyl, which is different from the currently existing preparations of CNX using the alkali-extracted, water-insoluble xylan that possesses highly ordered orientation but is poorly controllable towards a complete debranching efficiency.

The unique innovation is the strategy to reform the end-group by borohydride reduction to a primary alcohol, which effectively prevents the main-chain peeling when the substitutions of side-sugar units and acetyl groups are cleaved at an elevated temperature under alkaline conditions. Multi-instrumental analysis (composition analysis and NMR) confirmed the almost complete removal of side substitutions. This eventually tailor makes the branched PHWE-xylans as almost linear biopolymer composed of more than 90 wt% xylose units. Nanoprecipitation by a gradual pH decrease resulted in a stable hydrocolloid dispersion in the form of worm-like nanoclusters assembled with primary crystallites in uniform diameter of 10 – 15 nm, owing to the self-assembly of debranched xylan driven by strong intra-/inter-chain H-bonds and van der Waals attraction. With evaporation-induced self-assembly, we can further construct the hydrocolloids as dry submicron spheroids of CNX with a high average elastic modulus of 47–83 GPa. Taking the advantage that the chain length and homogeneity of PHWE-xylan can be tailored, we refined the xylan fractions to access well-defined macromolecular characteristics and strategically to increase the structural order in the as-prepared CNX. Importantly, rigid clusterization that is constructed within the nanoxylan of high structural order and crystallinity has contributed to the enhanced phosphorescent emission of this crystalline biopolymer. Hydrocolloids of self-assembled CNX increase the economic feasibility in PHWE-biorefinery and broaden the landscape of bio-based and functional nanomaterials.

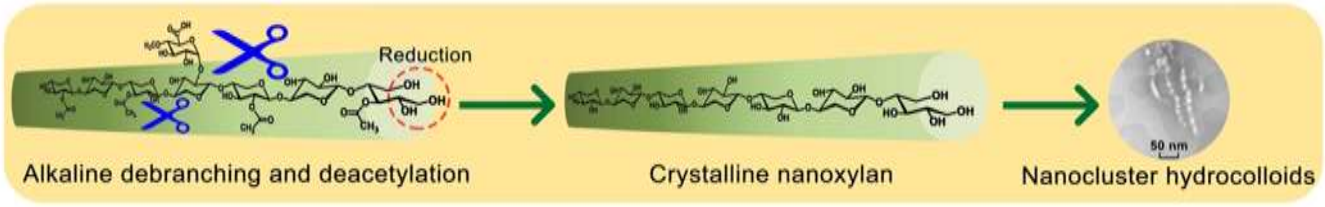

Figure 1. Schematic illustration of the preparation of CNX and TEM image of CNXs assembled head to toe in nanosuspension.

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Innovative Oxidized Microcellulose Based 3D-Bioink For Tissue Engineering

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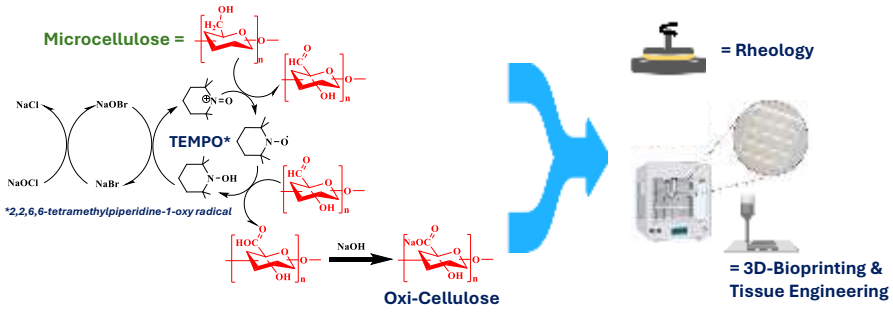
Cellulose is a polysaccharide composed of repeated *β*-D-glucose sugar units linked by *β*-1,4-glycosidic bonds and characterized by numerous intramolecular/intermolecular hydrogen bonds, resulting in a plethora of hydroxyl groups along the cellulose chain. These hydrogen bonds confer unique stability, hydrophilicity, and abundant sites for chemical modification with various functional groups[1]. Notably, both the original and chemically modified forms of cellulose find extensive application in tissue engineering due to their notable advantages. These include high specific mechanical properties, non-immunogenicity, non-toxicity, abundance in sources, and cost-effectiveness of production[2].The oxidation process mediated by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) selectively targets the primary hydroxyl group of cellulose, leading to the conversion of the surface C6 hydroxyl group into a carboxyl group. The oxidized cellulose undergoes mechanical treatment to yield oxidized nanocellulose with a reduced diameter. These oxidized nanocellulose particles exhibit high viscosity owing to their excellent water solubility and possess the capability to cross-link with divalent metals, such as calcium ions, to form hydrogels[3]. Cellulose nanofibers (CNFs) have demonstrated suitability for facilitating cell diffusion and maintaining phenotypic morphology, thus holding promise for application in bone tissue engineering[4].To simplify and reduce costs process, we developed a very innovative TEMPO/NaBr/NaOCl system to oxidize microcellulose and generate high yield gram scale oxi-cellulose. The physico-chemical properties of this anionic cellulose were studied (FTIR, carboxyl%, Transmittance, gelation time using $CaCl_2$/nano-hydroxyapatite, rheology, injectability/printability, cell viability, bone cell adhesion) to propose new generation of 3D-bioink for tissue engineering scaffolds without additional polysaccharides.



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Glycol-Split Heparin-Linked Prodrug Nanoparticles Target The Mitochondrion Apparatus For Cancer Metastasis Treatment

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[1] These authors contributed equally to this work.

The progression and metastasis of solid tumors rely strongly on neovascularization. However, angiogenesis inhibitors alone cannot meet the needs of tumor therapy. This study prepared a new drug conjugate (PTX-GSHP-CYS-ES2, PGCE) by combining polysaccharides (heparin without anticoagulant activity, GSHP), chemotherapeutic drugs (paclitaxel, PTX), and antiangiogenic drugs (ES2). Furthermore, a tumor-targeted prodrug nanoparticle delivery system was established. The nanoparticles appear to accumulate in the mitochondrial of tumor cells and achieve ES2 and PTX release under high glutathione and acidic environment. It has been confirmed that PGCE inhibited the expression of multiple metastasis-related proteins by targeting the tumor cell mitochondrial apparatus and disrupting their structure. Furthermore, PGCE nanoparticles inhibit migration, invasion, and angiogenesis in B16F10 tumor-bearing mice and suppress tumor growth and metastasis *in vitro*. Further *in vitro* and *in vivo* experiments show that PGCE has strong antitumor growth and metastatic effects and exhibits efficient anti-angiogenesis properties. This multi-targeted nanoparticle system potentially enhances the antitumor and anti-metastatic effects of combination chemotherapy and antiangiogenic drugs.

Keywords: Heparin; Anti-metastatic; Anti-angiogenesis; Anti-tumor; Prodrug nanoparticles

Integrated Glycomics Analysis Reveals Predominant Serum IgG Glycans Associated With Aging Across Multiple Time Points And Interventions

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NHC Key Laboratory of Glycoconjugates Research, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Fudan University

Abstract

IgG N-glycans have been identified as associated with aging; however, previous studies predominantly quantified changes based on the relative percentages of each glycan within the total glycan pool, neglecting the absolute concentration changes of individual glycans. This approach can be misleading, as variations in relative percentages do not necessarily reflect actual changes in glycan levels and fail to account for individual differences, thus affecting the efficacy of anti-aging interventions. For a more comprehensive and accurate understanding of glycan alterations during aging, it is essential to consider both absolute and relative changes in glycan levels. In this study，Serum IgG N-glycans in C57BL/6 mice from aging (n=70, 7 time points) and caloric restriction (n=116, 7 time points) studies were analyzed using integrated glycomics to assess both relative and absolute changes. Our comprehensive glycomics analysis revealed key features: downregulation of a bisected glycan and upregulation of a digalactosylated glycan. These glycan changes showed significant fold changes from an early stage. The GlycoAge index suggested a younger state under caloric restriction, with an average age reduction of 15.7-21.4 weeks. This analysis enhances our understanding of glycan alterations, accounts for individual variability, and aids in designing effective anti-aging strategies. These findings highlight the crucial roles of the bisected glycan and digalactosylated glycan as potential biomarkers for aging and health.

Key words：IgG N-glycome, aging, calorie restriction, biomarker

Critical Role Of Presentation Of Molecules On The Surface Of Supramers For The Outcome Of Glycosylation Reaction

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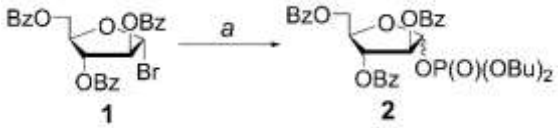
We have recently discovered[1] a dramatic increase of α-stereoselectivity of glycosylation of $(BuO)_2P(O)OH$ with glycosyl bromide **1** (reaction time t = 2 h) upon dilution. Here we studied the influence of concentration of **1** (C = 5–100 mM) on the outcome of this reaction for different reaction times (t = 1–60 min) both under batch and microfluidic (Comet X-01 micromixer) conditions (Scheme 1). Anomerization of neither glycosyl bromide **1** nor glycosyl phosphate **2** does not contribute to the observed changes in stereoselectivity. Stereoselectivity of microfluidic glycosylation (Fig. 1, *a*) follows a trend similar to that observed under batch conditions. Higher stereoselectivity in the low concentration range (C ≤20 mM) can be rationalized using the supramer approach,[2] which suggests that submicron-sized supramers of reagents are present in macroscopically homogeneous reaction solutions and are the real reacting species. The supramer analysis of reaction solutions by polarimetry (Fig. 1, *b*) revealed formation of different suparmers of glycosyl donor **1** in the low and high concentration ranges separated by a critical concentration (C = 20 mM).[3] The observed differences in stereoselectivity below and above 20 mM are apparently related to the changes in the presentation[4] of molecules of **1** on the surface of different supramers, which exist in different concentration ranges, featured by different accessibility of the anomeric center for the attack of a nucleophile (Fig. 1, *c*). Thus, α-selectivity dramatically increases in the low concentration range, where supramers of $\{\mathbf{1}\}^{III}$-type dominate.



Scheme 1. Glycosylation with glycosyl bromide **1** to give glycosyl phosphate **2**. *Reagents and conditions: a*. 1) $(BuO)_2P(O)OH$ (4 equiv.), Pr^i_2NEt (4 equiv.), MeCN, 20 °C; 2) quenching with satd aq $NaHCO_3$.

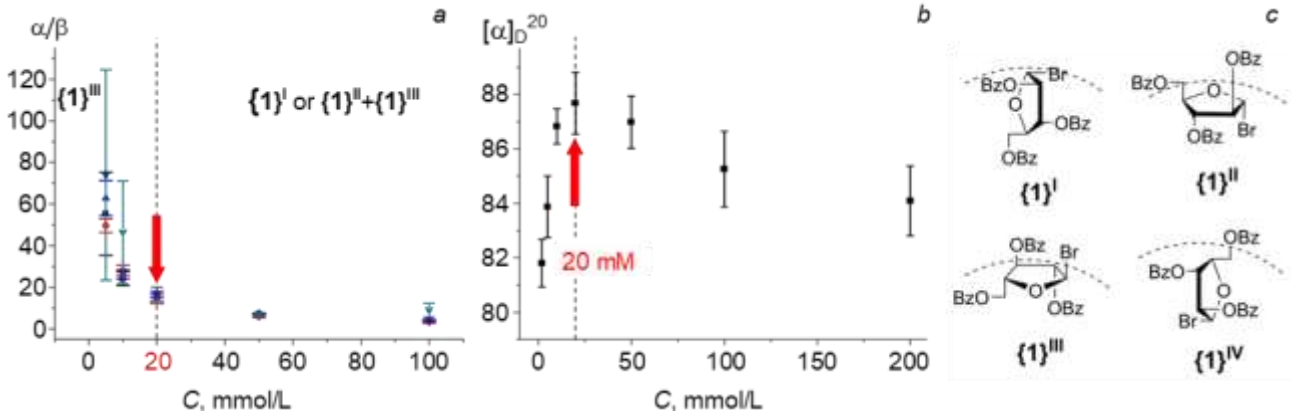


Figure 1. (*a*) Dependence of stereoselectivity on concentration of glycosyl bromide **1** for microfluidic glycosylation (n = 5). Residence time: ■ – 1 min, ● – 15 min, ▲ – 30 min, ▼ – 60 min. (*b*) Specific rotation of **1** *vs* concentration in MeCN.[2] (*c*) Types of supramers with different presentation of glycosyl bromide **1** on their surface.

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Multi-Reducing End Polysaccharides And Its Derived Hydrogels

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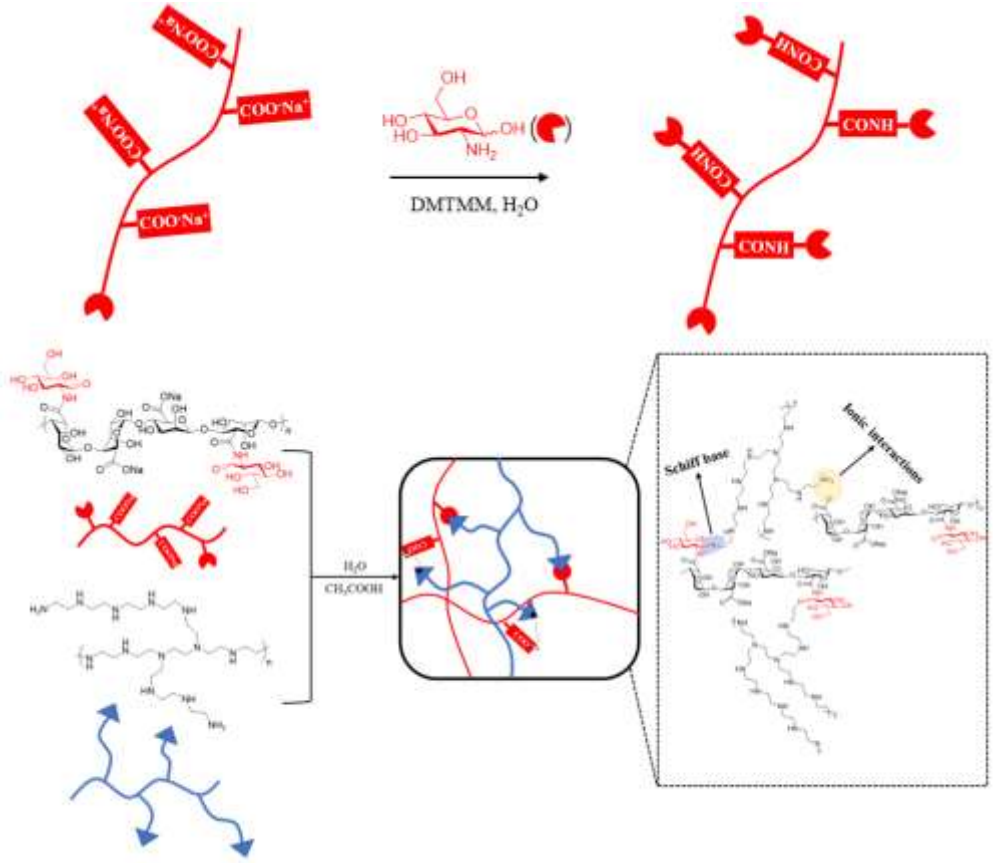
We developed a new approach to introduce multiple reducing ends to each polysaccharide molecule through amide formation[1]. Amine groups on monosaccharides such as glucosamine or galactosamine can react with carboxyl groups of polysaccharides, whether natural uronic acids like alginates, or derivatives with carboxyl-containing substituents such as carboxymethyl cellulose (CMC) or carboxymethyl dextran (CMD). Amide formation is assisted using the coupling agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). By linking the C2 amines of monosaccharides to polysaccharides in this way, a new class of polysaccharide derivatives possessing many reducing ends can be obtained. We refer to this class of derivatives as multi-reducing end polysaccharides (MREPs). The application of the multi-reducing end polysaccharides has been demonstrated by making hydrogels using multi-reducing end alginate with polyethyleneimine (PEI). The two polymer solutions can form a gel at room temperature after 24 hours. Acetic acid can be added to accelerate the gelation process, which 5 ul acetic acid can induce fast gelation within seconds[2].



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Polyesters Based On Carbohydrate Derived Diglyoxylic Acid Xylose As Soil-Release Polymers For Synthetic Fabric Surfae Modification

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Due to the increasing presence of synthetic fibers in fabrics, soil release polymers (SRPs) have become a highly desired polymer additive found in fabric care formulations to enhance cleaning efficiency. Conventional SRPs contain a poly(ethylene terephthalate) subunit capped by a hydrophilic methoxy-poly(ethylene glycol) unit which aids in the dispersal of the polymer in solution whilst preventing the redeposition of the soil onto the fabric surface. To improve the sustainability profile of these additives, a novel class of SRPs have been synthesised using the carbohydrate derived monomer diglyoxylic acid xylose (DGAX), which can be isolated from lignocellulosic biomass (**Figure 1**). This sugar-derived monomer provides an attractive alternative to the petrochemically derived terephthalate, which is sourced from the oxidation of p-xylene, due to its high abundance and inherent degradable nature. The potential viability of the poly(propylene diglyoxylic acid xylose) based SRPs as potential replacements to current commercialised SRPs were assessed through soil release and anti-redeposition performance tests to determine the stain removal index and whiteness maintenance of the fabric sample. Further insight into the differences in behavior presented by the increasing percentage of the carbohydrate derived monomer incorporated into a terephthalate system was further investigated with dynamic light scattering, contact angle measurements and scanning electron microscopy.

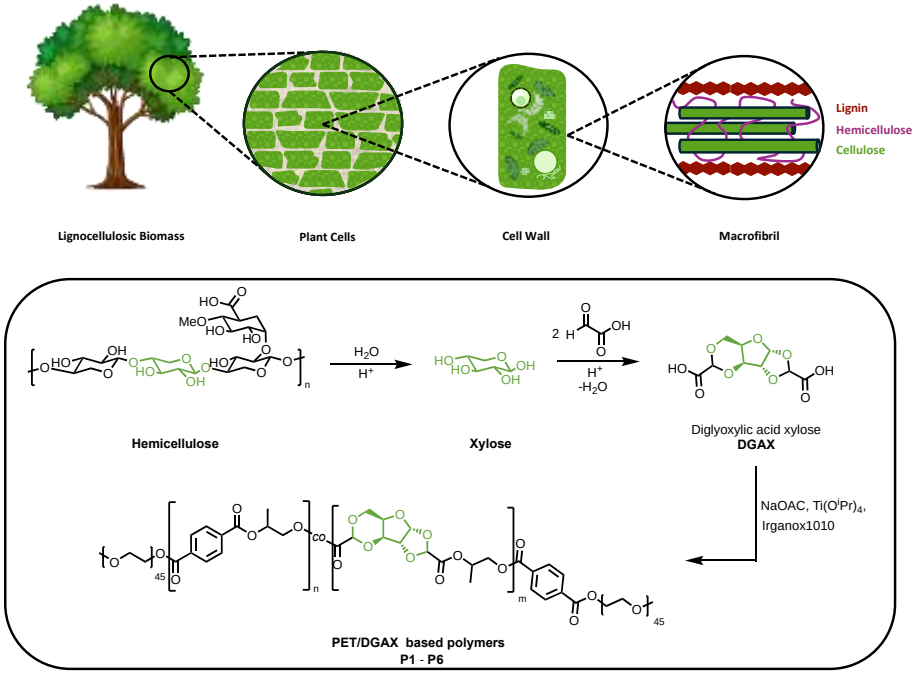

Figure 1: A schematic showing the extraction of the carbohydrate-based monomer, diglyoxylic acid xylose, from hemicellulose1 for the synthesis of a series of polymers.

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Variations In Metabolic Enzymes Cause Differential Changes Of Heparan Sulfate And Hyaluronan In High Glucose Treated Cells On Chip

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Glycocalyx dysfunction is believed as the first step in diabetic vascular disease. However, few studies have systematically investigated the influence of high glucose (HG) on the glycocalyx as a whole and its major constituent glycans towards one type of cell. Furthermore, most studies utilized traditional two-dimensional (2D) cultures in vitro, which can't provide the necessary fluid environment for glycocalyx. Here, we utilized vascular glycocalyx on chips to evaluate the changes of glycocalyx and its constituent glycans in HG induced HUVECs. Fluorescence microscopy showed up-regulation of hyaluronan (HA) but down-regulation of heparan sulfate (HS). By analyzing the metabolic enzymes of both glycans, a decrease in the ratio of synthetic/degradative enzymes for HA and an increase in that for HS were demonstrated. Two substrates (UDP-GlcNAc, UDP-GlcA) for the synthesis of both glycans were increased according to omics analysis. Since they were firstly pumped into Golgi apparatus to synthesize HS, less substrates may be left for HA synthesis. Furthermore, the differential changes of HA and HS were confirmed in vessel slides from db/db mice. This study would deepen our understanding of impact of HG on glycocalyx formation and diabetic vascular disease.

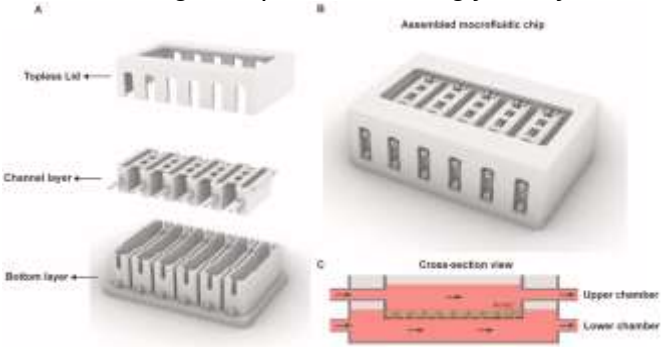

Fig. 1. Design and fabrication of the microfluidic chip.

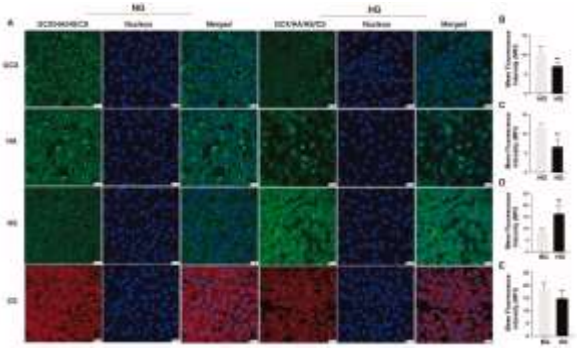

Fig. 2. Effects of HG on whole GCX, HA, HS and CS in HUVEC cells.

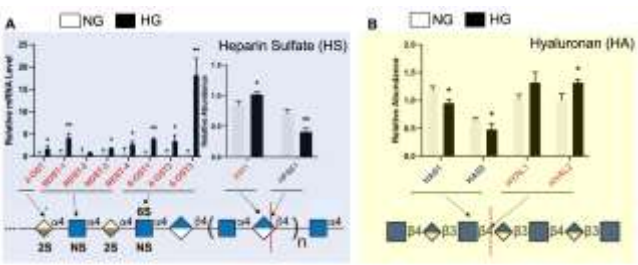

Fig. 3. Differential changes of metabolic enzymes of HA and HS in HG treated HUVECs.

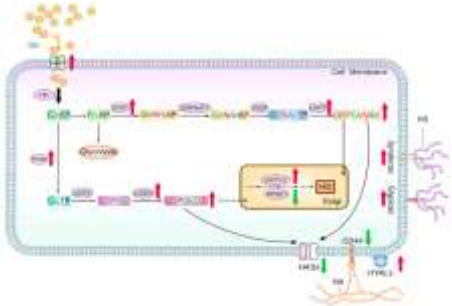

Fig. 4. Schematic diagram showing the possible mechanism from glucose imported into cells to differential synthesis of HA and HS in HG treated HUVECs

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5-Hydroxymethylfurfural (5-Hmf) And 5-Glucosyloxymethylfurfural (Gmf) As Biobased Platforms Towards Fine Chemicals

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5-Hydroxymethylfurfural (HMF) is directly derived from sugars and is considered one of the most promising renewable building blocks due to its rich chemical composition.[1] It is a remarkable example of biobased platform molecule in the context of the use of biomass as a renewable resource for chemistry.[2] Its glucosylated analogue, 5-glucosyloxymethylfurfural (GMF), obtained by dehydrating the commercial disaccharide isomaltulose,[3] also offers an aldehyde group capable of yielding a variety of novel structures which incorporate the intact glucose moiety. Thus, such carbohydrate-based furan aldehydes offer a foundational structure for designing novel chemicals, yet it also necessitates mild and selective methods that can accommodate their specific chemical sensitivities.
In this communication, we show the variety of possible directions offered by HMF or GMF for the design of novel chemicals, including the Morita-Baylis-Hillman (MBH) or *aza*-MBH reactions,[4] the Biginelli reaction,[5] Kabachnik-Fields reaction,[6] nitrone dipolar cycloaddition.[7] A focus will be made on the latest applications to the design of carbohydrate-derived amphiphiles exploiting the highly polar nature of the carbohydrate moiety of GMF,[8] and nitrogen-containing heterocyclic compounds by [4+2+1] cycloaddition[9] and Hantzsch dihydropyridine synthesis reaction.[10]

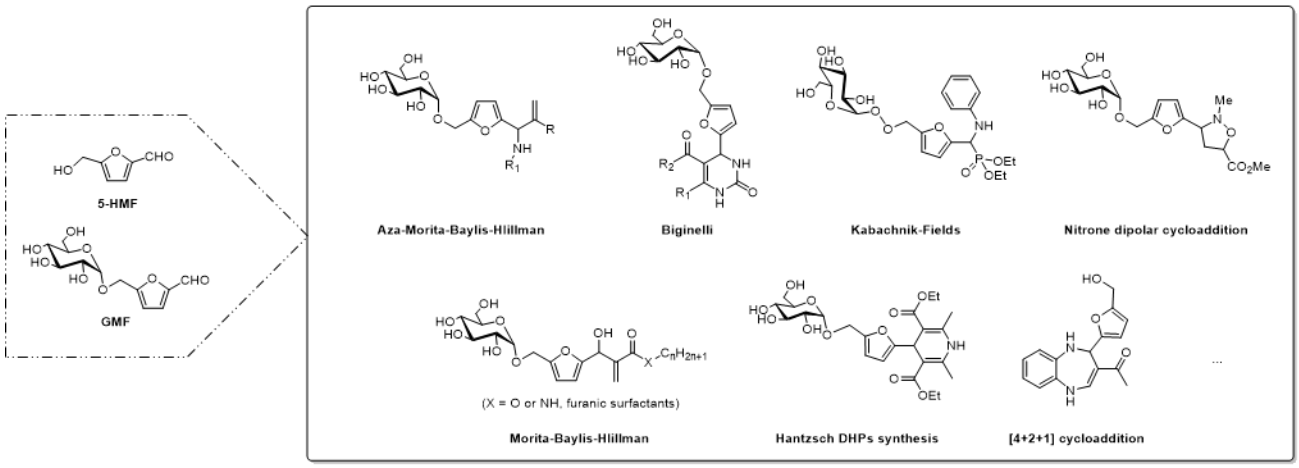

Fig. 1: Examples of fine chemicals obtained from HMF and GMF

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Prediction And Detection Of Glycan Epitoes On Cells

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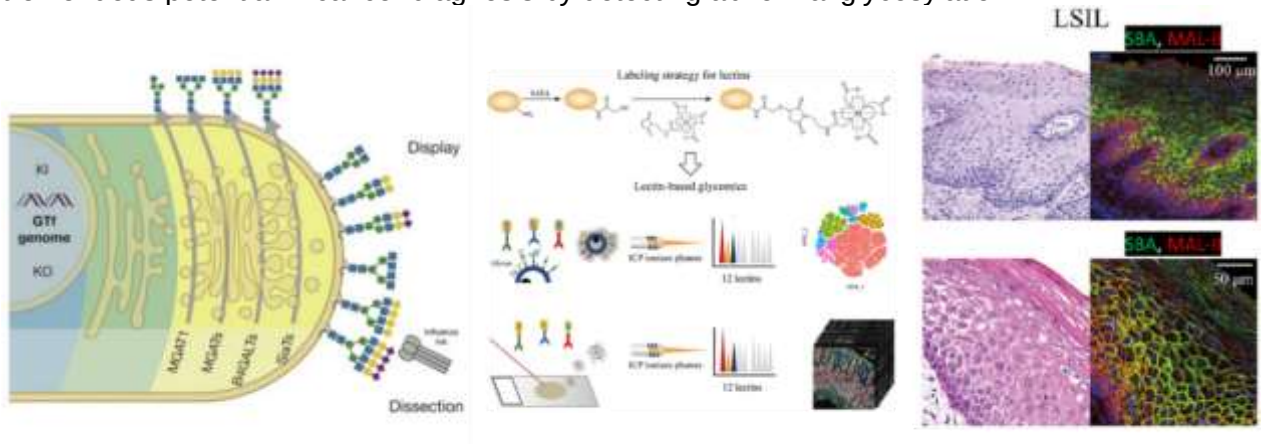
The sequence variation and structural complexity of glycans on cells are closely associated with various pathophysiology processes. Deciphering the "glycan code" on cells presents a challenging task. Cellular heterogeneity poses a significant challenge in decoding the "glycan code" expressed by individual cells, thus becoming the next frontier in glycobiology research. In recent years, single-cell sequencing has elucidated the issue of cellular heterogeneity in life processes, offering potential avenues for deciphering the sequence and structure of glycans expressed by individual cells. Clausen *et al.* research suggests that analyzing the transcriptome levels of glycosyltransferases (200 types) can predict the glycan structures [1]. Similar to this approahc, we have examed the expression levels of more than 400 glycosyltransferases, glycosidases and other carbohydrate related enzymes for a variety of cells in different pathophysiology condistions, predicted the dominant sugar epitopes on those cells. we have discovered during COVID-19 infection that the upregulation of hyaluronan synthase 2 and mucin promotes the secretion of hyaluronan by lung fibroblasts, leading to intractable pulmonary inflammation.

Validating the predicted structure and sequence of glycans expressed by cells based on transcriptomics requires robust experimental verification. Here, we have developed LectoScape, a method for imaging tissue glycoproteins at a resolution of 1 μm using imaging mass cytometry[2]. This method utilizes 12 different lectins that recognize various glycans, enabling multidimensional detection of multiple glycans. Using LectoScape, we successfully delineated unique polysaccharide structures in various cell types, enhancing our understanding of the distribution of glycans in human tissues. Our method has identified specific glycan markers, such as α2,3-sialylated Galβ1, 3GalNAc in O-glycan, and terminal GalNAc, as diagnostic indicators for cervical intraepithelial neoplasia. LectoScape holds tremendous potential in cancer diagnosis by detecting abnormal glycosylation.



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Immobilized Enzymes Monolithic Reactor Designed For Exploitation Of Funcrional Poly- And Oligosaccharides

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Abstract: The exploitation of functional poly- and oligosaccharides has attracted extensive interest due to their diverse applications in cosmetics, health, nutraceuticals, food, etc. [1,2] Over the past decade, immobilized enzymatic catalysis, as a green-chemistry technique, has been utilized to modify polysaccharide molecules and exploit new active oligosaccharides. [1,2,3] In this study, an immobilized enzyme reactor (IMER) system comprising two primary enzymatic compartments was constructed. The first compartment, consisting of a monolithic Convective Interaction Media® (CIM®) carboxy imidazole (CDI) disk with immobilized laccases (EC 1.10.3.2) from *Trametes versicolor*, was designed to add phenol groups onto polysaccharides. Dextran T40, chosen as a model polysaccharide, was selected to assess the phenolization process. The second compartment was intended to deconstruct polysaccharides, thereby producing oligosaccharides. A CIM® CDI disk with immobilized glucuronan lyases (EC 4.2.2.14) from *Rhizobium rosettiformans* was used to deconstruct glucuronan. In both compartments, the kinetic parameters of free and immobilized enzymes were quantified, including the maximum rate (Vmax) and Michaelis constant (Km). The design of experiments (DOE) and respond surface methodology (RSM) were used to explore the operating effects, i.e., substrate concentration, flow rate, and reaction time, on enzymatic catalysis for finally optimizing the operating conditions. The stability of immobilized enzymes reactor was also monitored to evaluate its life span. Finally, we aim to integrate these two compartments with a membrane filtration system to create a multistep enzymatic reactor system for the development of poly- and oligosaccharides.

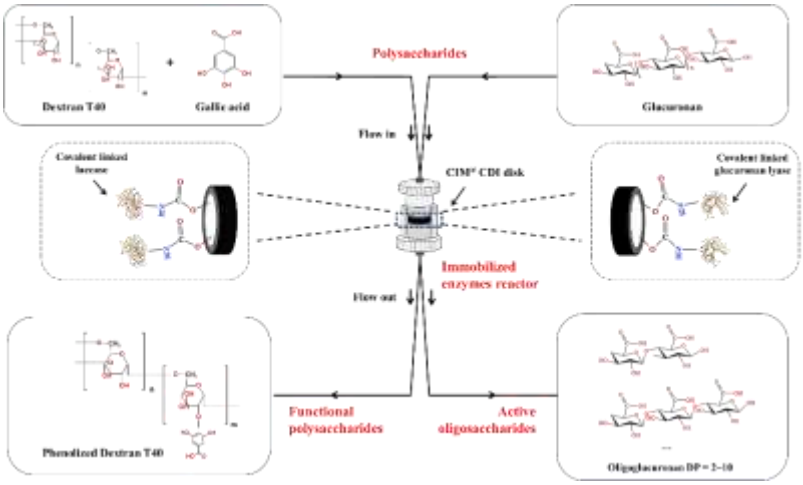

Fig. 1. Schematic diagram of CIM® CDI monolithic reactors immobilized with laccases or glucuronan lyases for producing functional polysaccharides or active oligosaccharides, respectively.

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Photolabile Ortho-Nitro-Benzyl Carbonate As A Permanent Protecting Group For Stereocontrolled Synthesis Of Diverse Glycosides

Jintao Shang, Jibin Zheng, Hongyu Chen, Lvfeng Zhang, Youling Liang, Dongsheng Chang, and You Yang*

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Traditional protecting groups are often removed under harsh conditions with potentially hazardous reagents, thereby impeding the convenient synthesis of oligosaccharides and glycosides. Herein we present to utilize photolabile ortho-nitro-benzyl carbonate (*o*NBC) as a permanent protecting group for stereocontrolled synthesis of glucosides, galactosides, and 2-deoxy-2-amino glycosides. The Ph_3PO-modulated glycosylation with strongly disarmed per-*o*NBC-protected glycosyl ynenoates preferred to afford glycosides with excellent α-selectivities via the β-phosphonium transition state, whereas the gold(I)-catalyzed glycosylation with *o*NBC-protected 2-deoxy-2-N-phthaloyl (Phth)-, 2-deoxy-2-N-trichloroacetyl (TCA)-, and 2-deoxy-2-N-*o*NBC-glycosyl ynenoates proceeded smoothly to provide 2-deoxy-2-amino glycosides with exclusive β-selectivities via the neighboring group participation (NGP) effect. The multiple O- and N-*o*NBC protecting groups installed on the glycosides could be readily removed under irradiation at 365 nm with aminomethyl polystyrene as the carbonyl scavenger. Based on the *o*NBC-mediated galactosylation, synthesis of the glycolipid digalactosyl diacylglycerol (DGDG) containing six double bonds and two esters was achieved in a straightforward manner.

Keywords: carbohydrates, protecting group, glycosylation, glycolipids, glycosides

A Semisynthetic Oligomannuronic Acid-Based Glycoconjugate Vaccine Against *Pseudomonas Aeruginosa*

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Pseudomonas aeruginosa is one of the leading causes of nosocomial infections and becomes increasingly resistant to multiple antibiotics. However, development of novel classes of antibacterial agents against multidrug resistant *P. aeruginosa* is extremely difficult. Herein we develop a semisynthetic oligomannuronic acid-based glycoconjugate vaccine that confers broad protection against infections of both mucoid and nonmucoid strains of *P. aeruginosa*. The well-defined glycoconjugate vaccine employing a highly conserved antigen elicited a strong and specific immune response and protected mice against both mucoid and nonmucoid strain of *P. aeruginosa*. The resulting antibodies recognized different strains of *P. aeruginosa* and mediated the opsonic killing of the bacteria in varied levels depending on the amount of alginate expressed on the surface of the strains. Vaccination with the glycoconjugate vaccine significantly promoted the pulmonary and blood clearance of the mucoid PAC1 strain of *P. aeruginosa*, and considerably improved the survival rates of mice against the nonmucoid PAO1 strain of *P. aeruginosa*. Thus, the semisynthetic glycoconjugate is a promising vaccine that may provide broad protection against both types of *P. aeruginosa*.

Peptide-Scaffolded Detergent Assembly For Membrane Protein Studies

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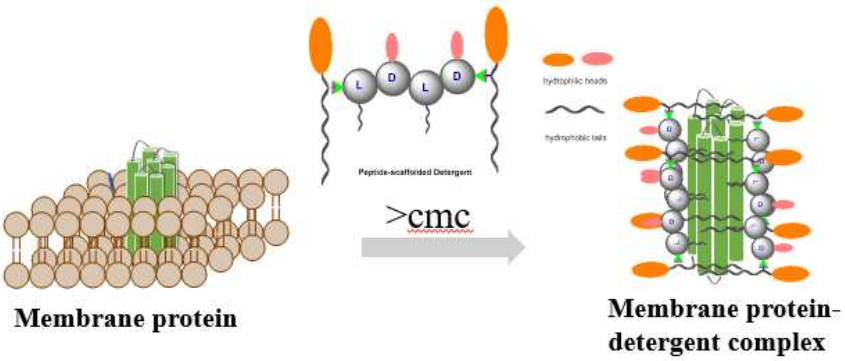
Abstract: Membrane proteins (MPs) undertake a broad range of physiological functions, and therefore are very important drug targets. Structural and functional studies of MPs require suitable amphiphilic system that mimic the natural lipid environment. Detergent is a class of amphiphilic molecules which have been widely used in the solubilization, purification and structural analysis of MPs. Different types of MPs show different preferences for detergents, but the commercially available types of detergents are limited. It is necessary to further the diversity of detergent. In the past decades, new detergents have mostly emerged in enlarged molecular sizes, featuring with multiple heads and multiple tails as assembled from traditional monomeric detergents. In our previous works, we have designed a type of β-strand peptides which enabled clear visualization of flexible conformations of MsbA, a bacterial ABC transporter. We also successfully introduced two classes of pre-assembled detergents via Click chemistry and Ugi reaction, respectively. This strategy enabled the two-dimensional expansion of detergent diversity for the first time. In this work, we report the synthesis of peptide-scaffolded detergents by pre-assembly of detergent monomers (OG and DDM) on peptides in various length (dipeptides and tetrapeptides) through Click reaction. We characterized the physical properties of these detergents and evaluated the thermal stability of $A_{2A}AR$ in these detergents, which belongs to the family of Class A G protein-coupled receptor. We found that one of the detergent C2B2 outperformed in the screening, indicating the great potential in the future MP study.



Keywords: Click reaction; Detergent; Membrane protein; Peptide detergent

Oligosaccharide Mapping Analysis By Hilic-Esi-Hcd-Ms/Ms For Structural Elucidation Of Complex Sulfated Polysaccharides

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The elucidation of precise structure of sulphated fucan and rhamnosan is essential for understanding their structure-function relationship. For the complexity of the sulphated polysaccharides, the strategy of combination of thermal degradation and HILIC-ESI-HCD-MS/MS analysis with PRM model was used to elucidate the oligosaccharide mapping for structural elucidation of sulfated polysaccharides from *Holothuria floridana* and *Chlorella pyrenoidosa.*

The sulphated rhamnosan was degraded at 110°C, pH 4.0 for 30 min to obtain the various oligosaccharides from large chains, with pH lower than sulphated fucan (pH 5.0).The sequence and sulfation position of all fucoidan oligosaccharide dp2-dp12 and their alditols by $NaBD_4$ reduction was confirmed by HILIC-ESI-HCD-MS/MS, by selection of fully-depronized precursor ions during spray ionization, HCD and PRM during MSMS analysis. Abundant fragment ions of deprotonated molecular ion m/z 331.91 (z=7) of $Fuc_{12}S_7$ (Fig.1) and its alditol confirmed the sequences of 4 symmetrical isomers [1].

The sequence and oligosaccharides mapping of dp2-dp9 from *Chlorella pyrenoidosa* and their alditols were eluciated by HILIC-HCD MS/MS. Remarkably, some new sulfated hexa-rhamnosan oligosaccharides with sulfation (S), methylation (Me) and acetylation (Ac) were identified such as $Rha_6S_3+Ac_3$, $Hex_3Rha_3S_2+Ac_2$, $Hex_4Rha_4S_3+Me_2$, $Hex_3Rha_5S_3+Ac_1+Me_4$, $Hex_3Rha_4Pent_1S_3+Me_2$, $Hex_3S_2+Ac_2+Me_1$ and $Hex_2Rha_2Pent_1S_2+Ac_2$, with sulfation on C2 of rhamnose (Rha) and C4 of hexose (Hex), methylation on C2 or C4 of Rha, acetylation on C2 of Rha. Fig.2 shows the HCD MSMS of the deprotonated molecular ion of $Hex_3Rha_3Ac_2S_3$ (m/z=421.0766, z=3) and its alditol confirmed its sequence as Rha2S-Hex2Ac-Rha2S-Hex2Ac-Rha-Hex4S.

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Fig.1 $Fuc_{12}S_7$

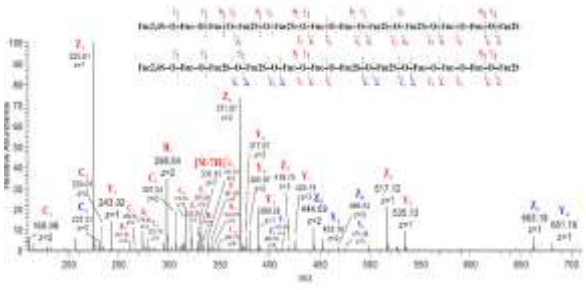
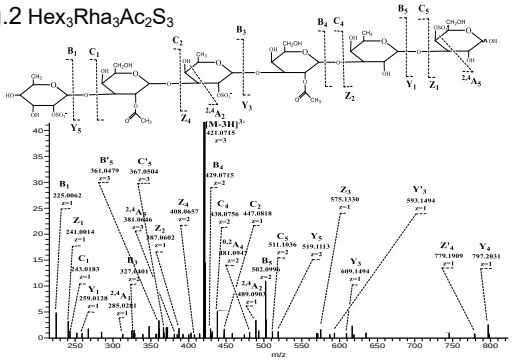


Fig.2 $Hex_3Rha_3Ac_2S_3$



Characterization Of Testicular O-Glycoproteome In Mice Using Lectin-Based Mass Spectrometry Analysis

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Among human organs, the testis is relatively unique and mysterious, possessing the largest number of tissue-specific proteins of unknown function [1]. While O-glycosylation is a post-translational modification that plays important roles in regulating cell differentiation and tissue development, our previous findings showed that testis is armed with highly O-glycan [2], indicating O-glycan is critical for sperm formation and male fertility. However, the spatial distribution and diversity of the O-glycoproteome in testis and temporal dynamics during spermatogenesis remain unmapped. Closing this knowledge gap is important because it can provide targets and resource for studying the the mechanism of O-glycosylation in sterility, and more importantly, it can provide new insights for investigating the function of testicular proteins whose functions are currently unknown.

In this study, we performed the first qualitative and quantitative analysis of site-specific O-glycosylation in mouse testis using a lectin affinity chromatography coupled with LC-MS/MS approach. We established the hitherto largest O-glycoproteome map with a total of 349 O-glycoproteins and 799 unambiguous O-glycosite from testes of 24 days and 12 weeks mice, of which 85 proteins are found to be O-glycosylated for the first time. Moreover, we comprehensively investigated the distribution properties of O-glycosylation in testis and found dynamic changes in O-glycosylation with upregulation of Tn-glycopeptides and downregulation of T-glycopeptides in 12-week-old testes, which may contribute to elongated spermatid maturation and fertilization. Notably, we preliminarily explored the function of O-glycan catalyzed by ppGalNAc-T3 on acrosomal proteins, which provide potential targets to explain male sterility induced by ppGalNAc-T3 knockout. Collectively, these data illustrate the global properties of O-glycosylation in testicular germ cells and lay the foundation for functional study of site-specific O-glycosylation in male infertility.

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Study on Structure Characterization, Target Molecules Discovery and Structure-Activity Relationship of Active Polysaccharides from *Malva Verticillate*

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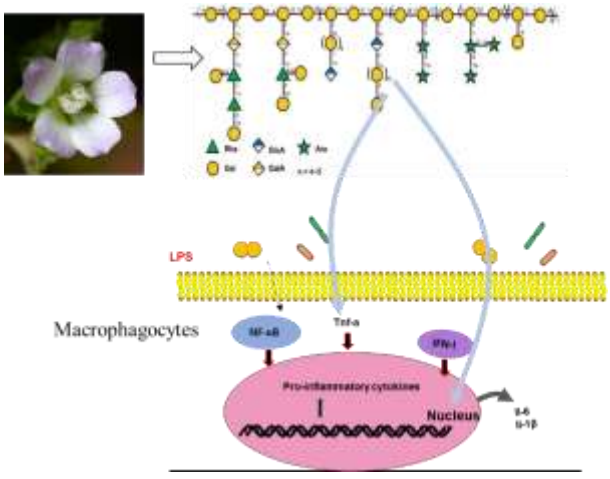
Chinese medicinal polysaccharides have complex and diverse biological functions and show great potential for immune regulation [1]. *Malva verticillata* L.is used as medicine, while relatively few studies have been conducted on the structural analysis and immunomodulatory activity of polysaccharides from it. Herein, the structure of one soluble polysaccharides from *Malva verticillata* L (DF111) was elucidated. The main chain of DF111 consists of alternating 1, 3, 6-β-Gal and 1, 6-β-Gal in a 1:1 ratio, forming multiple side chains. The first side chain is attached to the main chain 1, 3, 6-β-Gal at the O-3 position by 1, 4-α-GalA; the O-4 position of 1,4-α-GalA is linked to the C-1 position of 1, 2, 4-α-Rha sugar residue. Additionally, 1,2-α-Rha and T-β-Gal are connected to the O-2 and O-4 positions of 1, 2, 4-α-Rha, respectively, with T-β-Gal attached to the O-2 position of 1, 2-α-Rha. The second side chain also contains 1, 4-α-GalA, 1, 2, 4-α-Rha, and T-β-Gal. The third side chain consists of 1, 3-β-Gal and T-α-GlcA sugar residues in an 8:1 ratio. The fourth side chain is composed of 1, 4-β-GlcA, 1, 4-β-Gal, and T-β-Gal in a 1:12:1 ratio. Furthermore, two classic arabogalactan side chains are present, with one consisting of 1, 5-α-Ara and T-α-Ara in a 1:1 ratio, and another composed of 1, 3, 5-α-Ara and T-α-Ara also in a 1:2 ratio. Screening revealed that Malva verticillata polysaccharide DF111 may have immunomodulatory activity, inhibiting the release of the inflammatory factor IL-6. Based on the results, we speculate that DF111 from *Malva verticillata* is a structurally novel arabinogalactan to exert immunomodulatory effects.



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Accurate Carbohydrate-Binding Site Prediction

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As the most abundant organic substances in nature, carbohydrates are essential for life. Understanding how carbohydrates regulate proteins in the physiological and pathological processes presents opportunities to address crucial biological problems and develop new therapeutics. However, the diversity and complexity of carbohydrates pose a challenge in experimentally identifying the sites where carbohydrates bind to and act on proteins. Here, we introduce a deep learning model, DeepGlycanSite, capable of accurately predicting carbohydrate-binding sites on a given protein structure. Incorporating geometric and evolutionary features of proteins into a deep equivariant graph neural network with the transformer architecture, DeepGlycanSite remarkably outperforms previous state-of-the-art methods (Table 1) and effectively predicts binding sites for diverse carbohydrates[1]. Integrating with a mutagenesis study, DeepGlycanSite has been employed to reveal the nucleotide-sugar-recognition site of several important G-protein coupled receptors[1,2]. These findings demonstrate DeepGlycanSite is invaluable for carbohydrate-binding site prediction and could provide insights into molecular mechanisms underlying carbohydrateregulation of therapeutically important proteins.

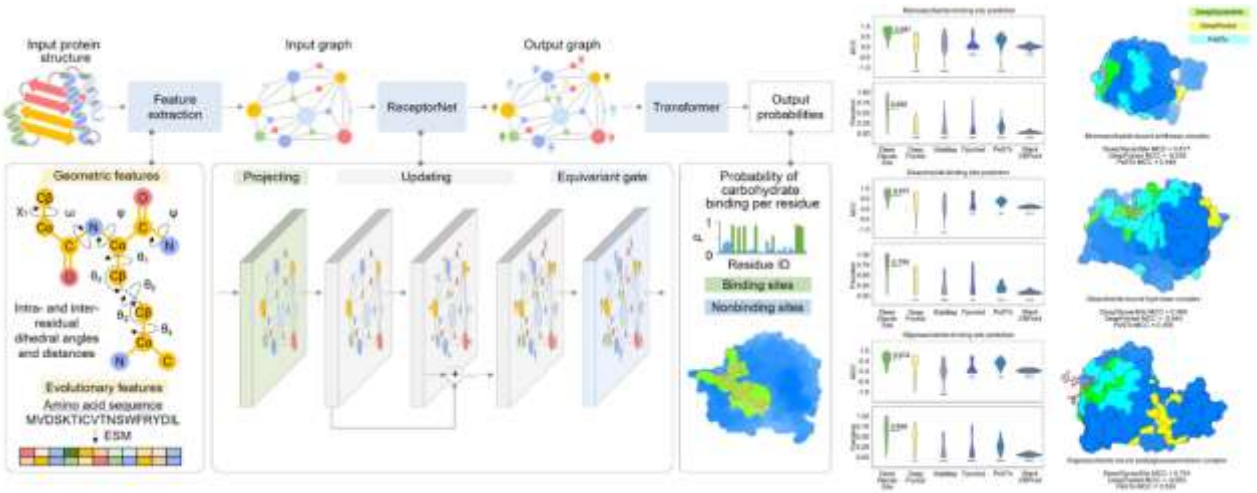

Figure 1 Overview of DeepGlycanSite and its performance

Table 1. Comparing DeepGlycanSite with previous binding site predictors on the independent dataset.

Method	MCC	Precision	Balanced accuracy
StackCBPred	0.018	0.052	0.525
Fpocket	0.191	0.194	0.617
SiteMap	0.227	0.201	0.717
DeepPocket	0.288	0.292	0.760
PeSTo	0.336	0.235	0.815
DeepGlycanSite (ours)	0.625	0.631	0.829

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Direct Identification Of Complex Glycans Via A Highly Sensitive Engineered Nanopore

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Main body: The crucial roles glycans played in biological systems are determined by their structures. However, the analysis of glycan structures still has numerous bottlenecks due to their inherent complexities. The development of nanopore technology has emerged as a powerful sensor for the DNA sequencing and peptides detection. This has a significant impact on the development of the related research area. Currently, nanopores are beginning to be applied for the detection of simple glycans, but the analysis of complex glycans by this technology is still challenging. Here, we designed an engineered α-hemolysin nanopore M113R/T115A to achieve the sensing of complex glycans at micromolar concentration and label-free conditions. By extracting characteristic features to depict a 3D scatter plot, glycans with different numbers of functional groups, various chain lengths ranging from disaccharide to decasaccharide, and distinct glycosidic linkages could be distinguished. Molecular dynamics simulations (MD) show different behaviors of glycans with 1,3 or 1,4 glycosidic bonds in nanopores. More importantly, the designed nanopore system permitted the discrimination of each glycan iso-mer with different lengths in a mixture with a separation ratio of over 0.9. This work represents a proof-of-concept demonstration that complex glycans can be analyzed by using nanopore sequencing technology.

Keywords: Engineered α Hemolysin nanopore, Glycan, Glycosidic bond, Isomer, 3D fingerprints

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Expeditious Synthesis of Gwanakoside A and the Chloronaphthol Glycoside Congeners

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The synthesis of gwanakoside A, a chlorinated naphthol bis-glycoside, and its analogues was achieved through stepwise chlorination and donor-equivalent controlled regioselective phenol glycosylation with glycosyl N-phenyltrifluoroacetimidates as donors. Gwanakoside A displayed considerable inhibitory effects against various cancer cells and Staphylococcus aureus strains.

Referance:

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Poster Presentions Part-B

July, 16-17

Deciphering Affinities In Tandem-Repeat Galectins: A Leap Towards Glycomimetic Inhibitors

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Galectins, a group of animal proteins, play a critical role in human metabolism, aiding cellular communication, migration, adhesion, and the regulation of apoptosis. Gal-1 and Gal-3 are particularly noteworthy in cancer research due to their immunomodulatory effects on tumor progression [1]. Tandem-repeat galectins [2], such as Gal-4, Gal-8, Gal-9, and Gal-12, feature two carbohydrate recognition domains and show promise in diagnostics and therapy due to their distinct binding affinities. This research underlines the importance of developing specific inhibitors of these galectins to enhance our understanding of their function and therapeutic potential.
In our study, we focused on Gal-8 and Gal-9, and developed novel aryl-modified carbohydrate molecules that bind more strongly to these proteins than their unmodified counterparts. In addition to a competitive immunoassay (ELISA), these symmetric 3,3'-*O*- disubstituted TDG-based glycomimetics showed high efficiency in inhibiting these galectins in an *in vitro* competitive binding assay on the surface of A549 lung carcinoma cells. These results were correlated with molecular modelling, giving an even deeper insight into the binding mechanism of the glycomimetics. These findings highlight the potential of small-molecule glycomimetics to disrupt harmful protein interactions in cancer cells, paving the way for innovative therapeutic strategies in targeting tandem-repeat galectins. This research marks an important step towards understanding and potentially controlling the role of tandem-repeat galectins in cancer.

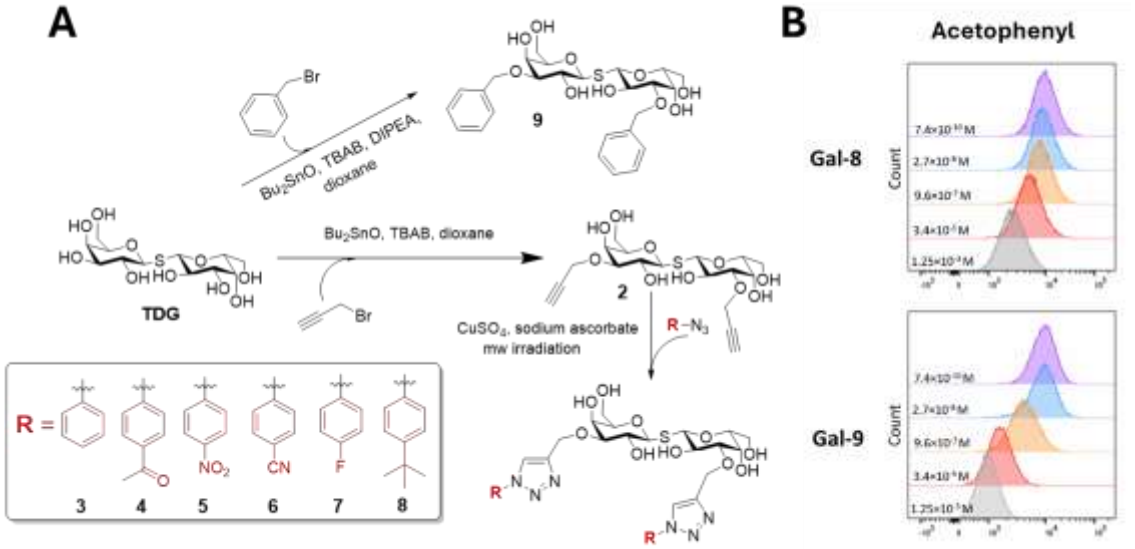

Figure1.**1A** - Synthesis of 3,3'-*O*-disubstituted TDG-based glycomimetics; **1B -** flow cytometry histograms of competitive inhibition of binding of Gal-8 or Gal-9 to the surface of A549 lung cancer cells

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Mannose-Functionalised Micelles Encapsulating Iridium Complexes For Anti-Cancer Therapy

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Cancer, the second leading cause of death worldwide, leads to around 10 millions death every year. Chemotherapy using small molecule anti-cancer drugs faces challenges, including drug resistance and off-target effects. Photodynamic therapy is a non-invasive cancer treatment, which requires a photosensitizer to induce cell apoptosis, by producing radical oxygen species under light irradiation. Iridium complexes showed high singlet oxygen-photosensitisation efficiency as a promising anti-cancer photosensitiser.[1] Without light activation, the iridium complexes remain non-toxic towards healthy cells, reducing undesired cell death.

Macromolecular drug delivery system can target towards cancer tissues, improve cell permeability and minimize drug's toxicity with normal cells. Moreover, mannose receptor (MR) is overexpressed in tumours. It has been used as a stable marker and target on cancer cells for targeted drug delivery platform.[2] In this work, iridium complex was encapsulated in pH-sensitive micelles, which could respond to acidic environment in tumour. Mannose-modified micelles surface could overcome the problem of poor cell selectivity, as mannose has stronger binding to tumour cells compared to healthy cells. The selective accumulation in tumours can lead to efficient internalisation and uptake of iridium complexes into the cells, enable radical oxygen species interact and destroy organelles, causing cell death.

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Study Of Fucoidan Derivatives As The Galectin-4 Inhibitor For The Suppression Of Peritoneal Metastasis Of Gastric Cancer

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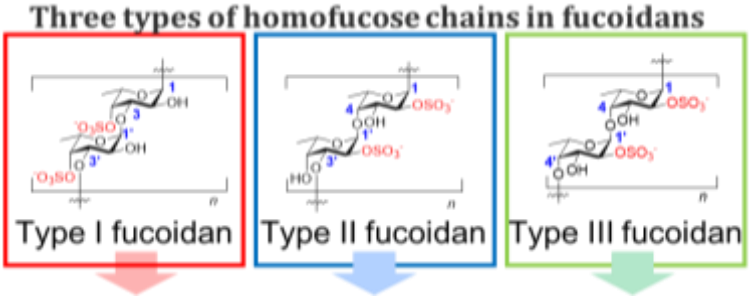
Peritoneal dissemination is an inoperable condition for which effective treatment is lacking. We clarified the role of galectin-4 in regulating the proliferation and peritoneal metastasis of malignant gastric cancer cells by downregulating the expression of activated c-MET and CD44 [1]. Based on these results, we hypothesized that galectin-4 inhibitors might inhibit peritoneal metastasis in gastric cancer. Fucoidans are sulfated polysaccharides derived from seaweeds that have been reported to have a wide range of biological activities, including anticancer effects. We previously found that fucoidan from *Fucus vesiculosus* showed high inhibitory activity against galectin-4 [2]. However, structural heterogeneity and non-uniformity make it difficult to conduct further studies. Fucoidans can be classified into three groups, as shown on the right. Recently, three types of fucoidan derivatives with different sulfation patterns were synthesized. We proceeded with our study using chemically synthesized fucoidan derivatives with well-defined structures[3].



Since we found that the suppression of galectin-4 expression inhibited the proliferation of gastric cancer cells, we next studied whether the addition of fucoidan derivatives also affected proliferation. Initially, the inhibitory activity against the proliferation of MKN45 gastric cancer cells was assessed using an ATP assay to screen the 14 fucoidan derivatives. Five fucoidan derivatives that significantly reduced cell proliferation were selected. The fucoidan derivative conjugated with cholestanol showed the strongest growth-inhibitory activity. Furthermore, to further explore whether inhibition by fucoidan derivatives was linked to the suppression of galectin-4, we assessed the inhibitory activities against galectin-4 knockout (KO) NUGC4 cells. Fucoidan derivatives exhibited reduced growth inhibitory activity against galectin-4 KO cells compared to wild-type NUGC4 cells, indicating the involvement of galectin-4 in the growth inhibition induced by fucoidan derivatives. The interaction between fucoidan derivatives and galectin-4 was assessed using surface plasmon resonance (SPR) with galectin-4 immobilized on a sensor chip. Interestingly, the cholestanol-conjugated derivative exhibited a significantly increased response compared to other derivatives. The inhibitory activities of the derivatives were evaluated in a dose-dependent manner using ELISA. Molecular docking was employed to map potential binding sites of fucoidan derivatives on the surface of galectin-4 with support from a mutagenesis study using SPR. The relationship between structure and inhibitory activity will be discussed.

In this study, a fucoidan derivative with a strong affinity for galectin-4 was shown to be a candidate inhibitor for the suppression of peritoneal metastasis of galectin-4-positive gastric cancer cells.

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Squaryl Group-Modified Lysophosphatidyl-β-D-Glucoside Analogs As GPR55 Regulators

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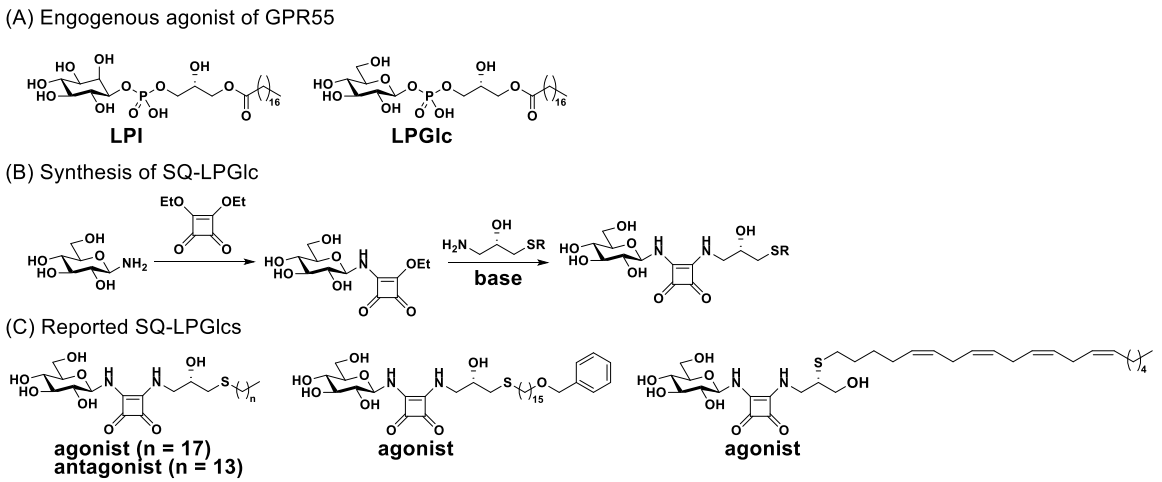
GPR55 is one of G protein-coupled receptors (GPCRs), which is related to diverse biological phenomena.[1] It was initially deorphanized as a cannabinoid receptor, but lysophosphatidylinositol (LPI) was reported to work as the endogenous agonist.[2] More recent study identified lysophosphatidyl-β-D-glucoside (LPGlc) as another endogenous agonist of GPR55.[3] Our study aimed to explore the structure activity relationship (SAR) of LPGlc to GPR55. However, the synthesis of LPGlc derivatives, such as derivatives possessing different alkyl chain length, requires multi-step transformations and tedious purification steps, inevitably making the SAR study labor-intensive. Therefore, we exploited the unique four-membered ring squaramide which is known as a surrogate of phosphodiester.[4] Squaramide containing analogs can be assembled in a technically simple manner by sequential reactions of nucleophiles to diethylsquarate without protecting and deprotecting steps, drastically facilitating the SAR study. In fact, our preceding studies demonstrated the relevance of the squaryl group-modified analogs of LPGlc (SQ-LPGlc) as agonists[5] or antagonists.[6] The synthesis was carried out by using diethylsquarate and β-D-glucosylamine, followed by the nucleophilic replacement with alkylamine. Various SQ-LPGlc can be obtained by changing alkylamine which is secondary nucleophile. Besides, we discovered SQ-LPGlc possessing a leaving group in its structure exhibited the irreversible inhibition, presumably through covalent binding to GPR55.[7] The synthesis and biological activity of the SQ-LPGlcs will be discussed in detail.



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Synthesis, biofunctional evaluation and computational analysis of Th2-selective glycolipid antigens

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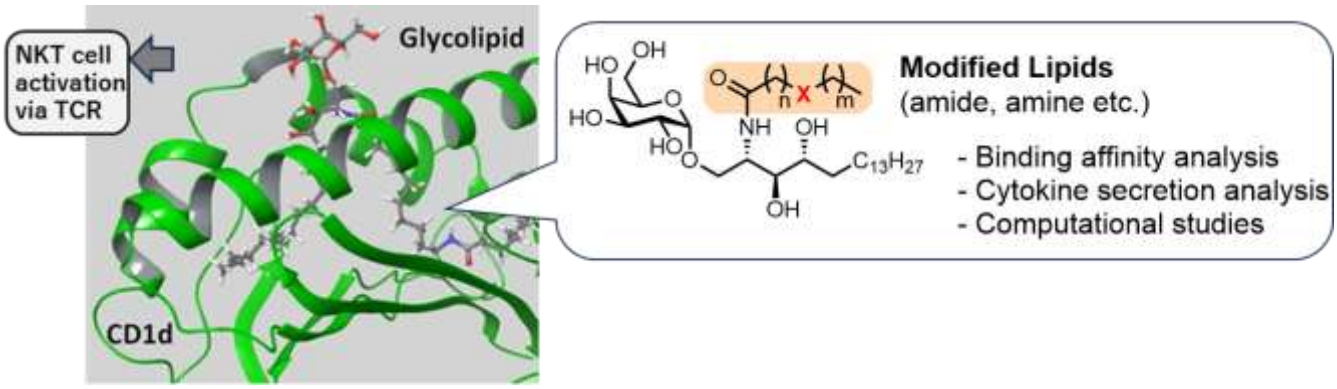
CD1d is present on antigen presenting cells and recognizes glycolipid antigens to form complexes. NKT cells recognize these complexes which leads to its activation, resulting in secretion of various cytokines for modulation of the immune system. Through exploration of lipid-modified glycolipid antigen ligands of CD1d, we have shown that the binding affinity, cytokine induction activity and selectivity can be adjusted by polar-functional group modification of the acyl group of the glycolipids.[1] For further exploration of the functions of CD1d and its ligands, we developed Th2-biased glycolipids, performed its synthesis and biofunctional evaluation, along with computational analysis to visualize the dynamics of the CD1d-ligand complex.

En route to the synthesis of the novel α-GalCer derivatives with modified acyl groups, we developed a new synthetic method to introduce functional groups to the acyl groups, and achieved a highly efficient synthesis. The synthesized fatty acids were installed to a glycolipid intermediate, prepared from D-galactose and phytosphingosine as previously reported,[1] at the final stages to achieve the synthesis of the target α-GalCer derivatives. The biological activities of the synthesized α-GalCer derivatives were evaluated by competitive binding affinity assays with the CD1d protein and cytokine induction assays with mouse splenocytes, and compared with the data of the previously reported amide and amine modified Th2-biased α-GalCer derivatives. Results indicated that the novel α-GalCer derivatives exhibits increased binding affinity towards CD1d, and possesses a significantly high Th2-selective cytokine induction profile, compared with the previously reported derivatives.

MD simulations of the α-GalCer derivatives were also performed to analyze the dynamic motion of the ligand within the CD1d-ligand complex. The distance of CD1d-sugar moiety was analyzed to evaluate the movement of the ligand within the complex throughout the simulation. Details of the experimental and computational results will be further discussed in the poster presentation.



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Enzyme-Sialylation-Controlled Chemical Sulfation of Glycan Epitopes for Decoding Tte Binding of Siglec Ligands

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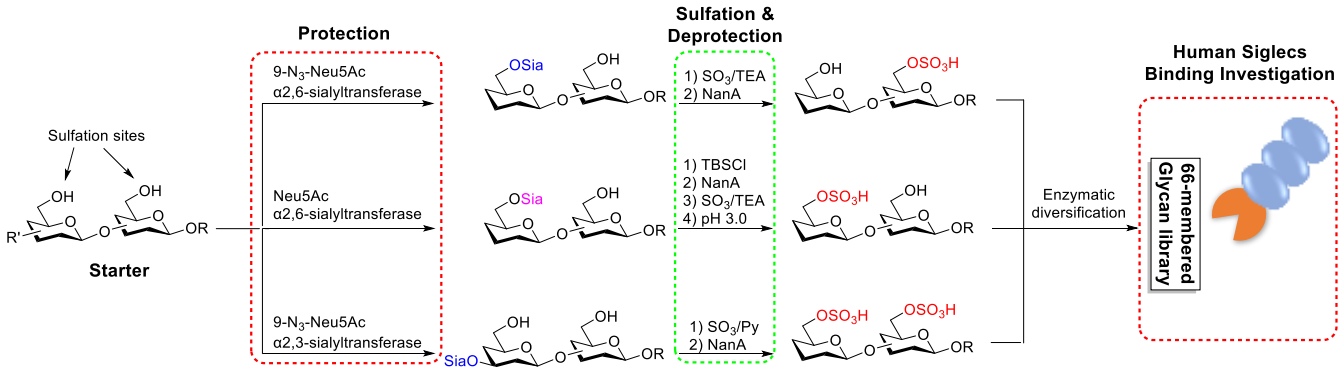
†These authors contributed equally to this work.

Widely distributed in nature, sulfated glycan epitopes play important roles in diverse pathophysiological processes.[1,2] However, due to their structural complexity, the preparation of glycan epitopes with structurally defined sulfation patterns is challenging, which significantly hampers the detailed elucidation of their biological functions at the molecular level. Here, we introduce a strategy for site-specific chemical sulfation of glycan epitopes, leveraging enzymatic sialylation and de-sialylation processes to precisely control the regio-specificity of glycan sulfation. Using this method, a sulfated glycan library covering the most common sulfated glycan epitopes was prepared with high yield and efficiency. By screening a microarray prepared with this glycan library, we systematically probed their binding specificity with human Siglecs (sialic acid-binding immunoglobulin-type lectins), many of which function as glyco-immune checkpoints to suppress the immune system activation.[3] Our investigation revealed that sulfation and sialylation patterns serve as important determinants of Siglec binding affinity and specificity. Thus, these findings offer new insights for the development of research tools and potential therapeutic agents targeting glyco-immune checkpoints by modulating the Siglec signaling pathway.



Starter = Galβ1,4GlcNAc-proN$_3$; Galβ1,4Glc-proN$_3$; Galβ1,3GlcNAc-proN$_3$; Galβ1,3GalNAc-proN$_3$

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Solution Structure Of Heparin Tetrasaccharide And Its Complex With Tau Peptide ^{218}Pro-^{230}Arg

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The manifold properties of sulphated saccharides are surprisingly large and originate from the structural arrangement of these saccharide derivatives. The methods of quantum chemistry, combined with NMR spectroscopy, allow a detailed description of the molecular properties of these molecules. The present study deals with the 3D molecular structure and NMR parameters of heparin, dermatan sulphate and chondroitin sulphate oligosaccharides, taking into account explicit solvent molecules. Analysis of the data showed that the formation of a complex hydrogen bonding network and strong ionic interactions influence the first hydration shell and play an important role in shaping the 3D saccharide molecules. We also investigated the complexation between heparin tetrasaccharide and tau peptide corresponding to the sequence ^{218}Pro-^{230}Arg (numbering according to the longest full length CNS tau isoform 2N4R, uniport ID P10636-8), which has been shown to interact with heparin within the full-length tau molecule[1] and has been crystallised in the complex with an antibody (PDB ID 4TQE,[2]). First, the DFT analysis, using the MN15/6-31+G(d) approach, allowed the description of the energy minima in solution of both the tetrasaccharide and the tau peptide ^{218}Pro-^{230}Arg. The interaction between the heparin tetrasaccharide and the tau sequence was then investigated by DFT with implicit and explicit solvent models. Theoretical data showed that complex formation is accompanied by significant changes in both the heparin fragment and the peptide with respect to the solution structure.

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Antibody-Carbohydrate Conjugates: Versatile Platforms For Ligand Discovery And Immunotherapy

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Lectins, or professional glycan-binding proteins, play a critical role in orchestrating a diverse range of immune responses, highlighting their potential as targets for immunotherapy. However, individual carbohydrate-lectin interactions are weak and thus *require* multivalency on both sides, complicating the design and study of targeting ligands. One such example is CD206, a C-type lectin overexpressed on tumor-associated macrophages (TAMs). Notably, TAMs are mis regulated immune cells that aid cancer cells in metastasis, angiogenesis, and proliferation via diverse immunosuppressive mechanisms.[1,2] We first demonstrated how antibody-carbohydrate conjugates can be utilized as a platform to rapidly screen multivalent ligands for CD206 directly on living cells. This approach resulting in the discovery of novel small molecule binders: tris-fucose (**1**) and tris 3-SO_4 galactose (**2**) (**Fig. 1A**).

Next, we developed Tumor-Immune Cell Targeting Chimeras (TICTACs), which are antibody- carbohydrate conjugates that are capable of selectively depleting inhibitory immune checkpoint proteins (ICPs) on TAMs. These bifunctional chimeras comprise of a CD206-binding small molecule that is chemically conjugated to a non-blocking antibody that binds but does not inhibit the ICP. By simultaneously engaging CD206, which constitutively recycles between the plasma membrane and early endosomes, these conjugates facilitate robust removal of ICPs such as signal regulatory protein alpha (SIRPa) from the surface of $CD206^{high}$ TAMs, while having no effect on healthy $CD206^{low}$ macrophages (**Fig. 1B** and **C**). By decoupling antibody selectivity from its blocking function, we present a new class of tumor-specific immune therapies with reduced risk of systemic immune activation and associated toxiciti

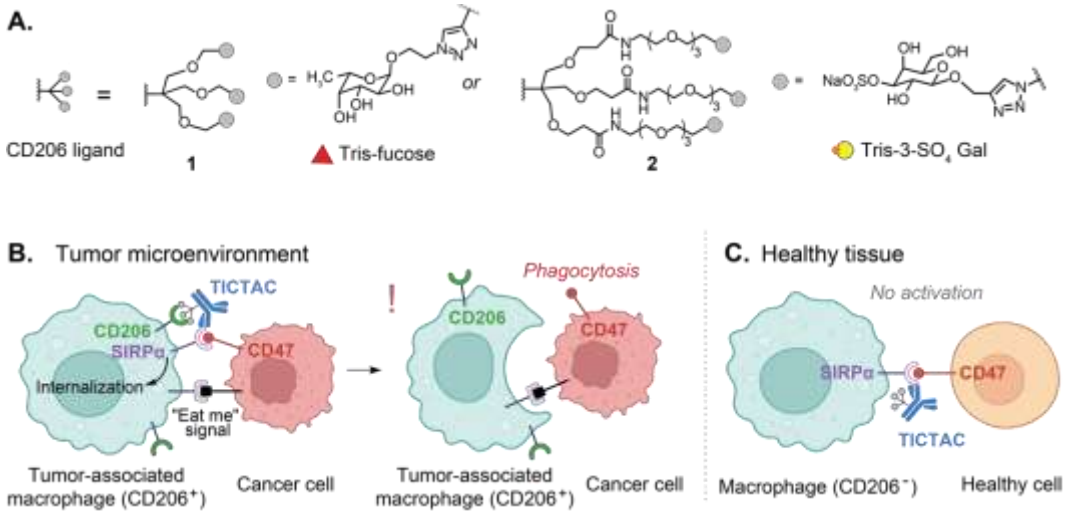

Figure 1. A. Structures of CD206-binding molecules; **B.** TICTACs enable robust depletion of immune checkpoints such as SIRPa on TAMs, leading to immune activation; **C.** TICTACs have no effect on healthy tissue, where macrophages have low expression of CD206.

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Conformationally Locked Iminosugars As Potential Glycosidase Inhibitors

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Glycosidases (or glycoside hydrolases) play a crucial role in breaking down glyosidic bonds, making them essential for the catabolism of carbohydrates in all living organisms. Given their fundamental biological significance and the therapeutic advantages associated with targeting these enzymes pharmacologically, there is a considerable motivation for the development of new inhibitor classes. Compounds capable of selectively inhibiting these enzymes are the conformationally locked sugar analogues cyclophellitol (**1**), cyclophellitol aziridine (**2**), α-cyclosulfate (**3**), β-cyclosulfate (**4**) [1] or basic sugar analogues such as the iminosugar 1-deoxynojirimycin (**5**) and its numerous derivatives [2, 3]. Motivated by the compelling biological prospects offered by these two substance classes, this presentation will showcase the design, synthesis, and potential biological applications of new conformationally locked iminosugars, exemplified by compounds **6** and **7**.

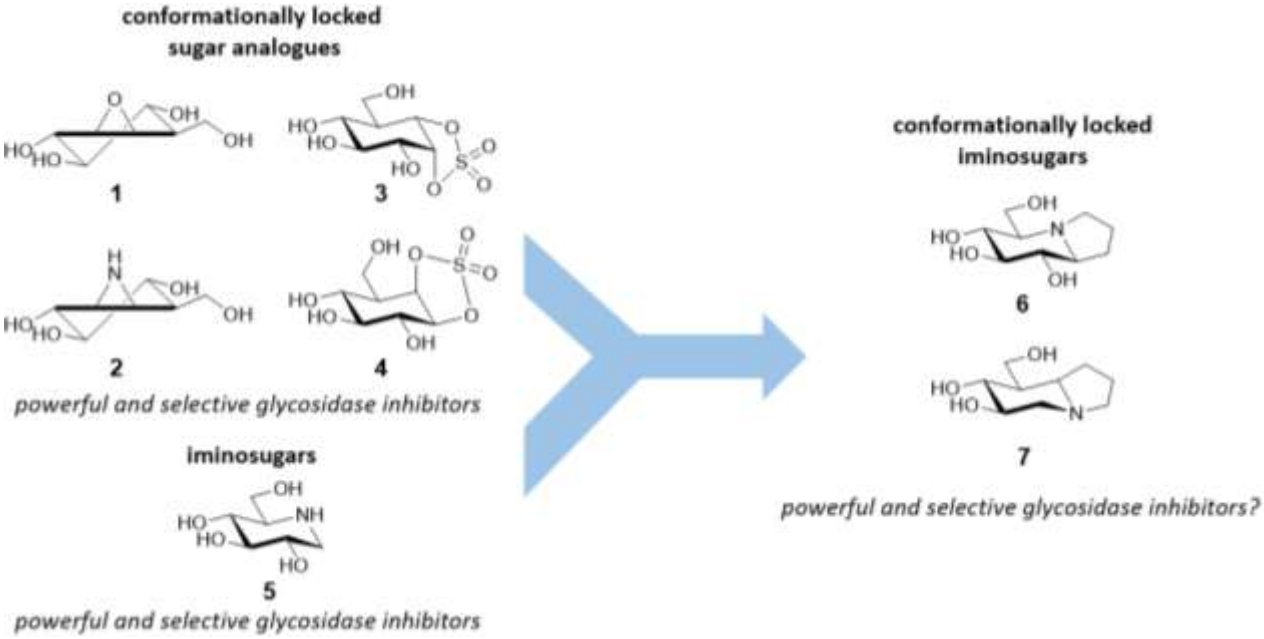

Figure: Conformationally locked iminosugars as potential glycosidase inhibitors.

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A Sialomimetic Glycan Array for The Identification of Viral Entry Inhibitors

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Zoonotic viruses can rapidly emerge from animals and transmit to humans, causing pandemics that lead to millions of deaths and a massive economic burden for humanity. Some widely recognized respiratory viruses such as the Spanish flu, Swine flu, MERS-CoV, and SARS-CoV-2, have resulted in significant human casualties due to the lack of effective drugs and vaccines.[1] Those pandemic influenza and corona viruses exploit animal and human sialic acid receptors for transmission and infection.

Sialic acids are a family of monosaccharides composed of a nine-carbon backbone. They are typically located at the terminal end of carbohydrate chains, attached to several glycoproteins and glycolipids on the cell membrane and thus serve as primary receptors by many viruses for establishing infection.[2] These receptors therefore constitute an important target for drug research against these viruses.[3]

Our research aims to develop molecules that perfectly lock the virus sialic acid binding proteins and thereby block viral infection. To this end, we systematically varied the sialic acid motif through chemical synthesis, ensuring ongoing enrichment of a sialomimetic library. These synthetic sialomimetics will be immobilized onto glass slides in an array format.[4] These arrays will then be exposed to recombinant sialic acid binding lectins and ultimately intact viruses to evaluate receptor-specific binding. The information obtained will guide the development of potent drugs capable of scavenging viruses. Within the context of pandemic preparedness, this pipeline prepares for potential future outbreaks of similar unknown viruses.

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Structural Insights Into The Interaction Between A Gonococcal Mimitope Vaccine Candidate And Its Cognate Monoclonal Antibody

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The global spread of multidrug-resistant strains of Neisseria gonorrhoeae (Ng) presents a global health emergency; thus, the development of a safe and effective vaccine against gonorrhea is urgently needed. It is known that the monoclonal antibody (mAb) 2C7 recognizes a glycan epitope on Ng lipooligosaccharide (LOS) that is expressed by almost every clinical isolate (Figure 1) and elicits complement-dependent bactericidal activity making LOS a good vaccine candidate. [1]
Previously, we identified a peptide that mimics the gonococcal 2C7 LOS epitope, which when cyclized and formulated as a multi-antigen peptide vaccine, attenuates vaginal colonization of mice by gonococci.[2] In the present study, we combined X-ray crystallography, NMR spectroscopy, and other biophysical techniques to perform structural and conformational analyses of 2C7 elucidating the three-dimensional complex of 2C7 and the cyclized peptide (CP2). The crystal structure of the Fab2C7-CP2 complex showed that CP2 formed a beta-hairpin bound to the Fragment antigen-binding (Fab) primarily through hydrophobic interactions. NMR spectroscopy and molecular dynamics simulations mapped the 2C7 epitope and identified the bioactive conformation of CP2 (Figure 1). Isothermal titration calorimetry and native mass spectrometry provided further information on the energetics and assembly state of the complex. Collectively, our multidisciplinary studiy suggests strategies for humanizing mAb 2C7 as a therapeutic against gonococcal infection and for optimizing peptide CP2 as a vaccine antigen.

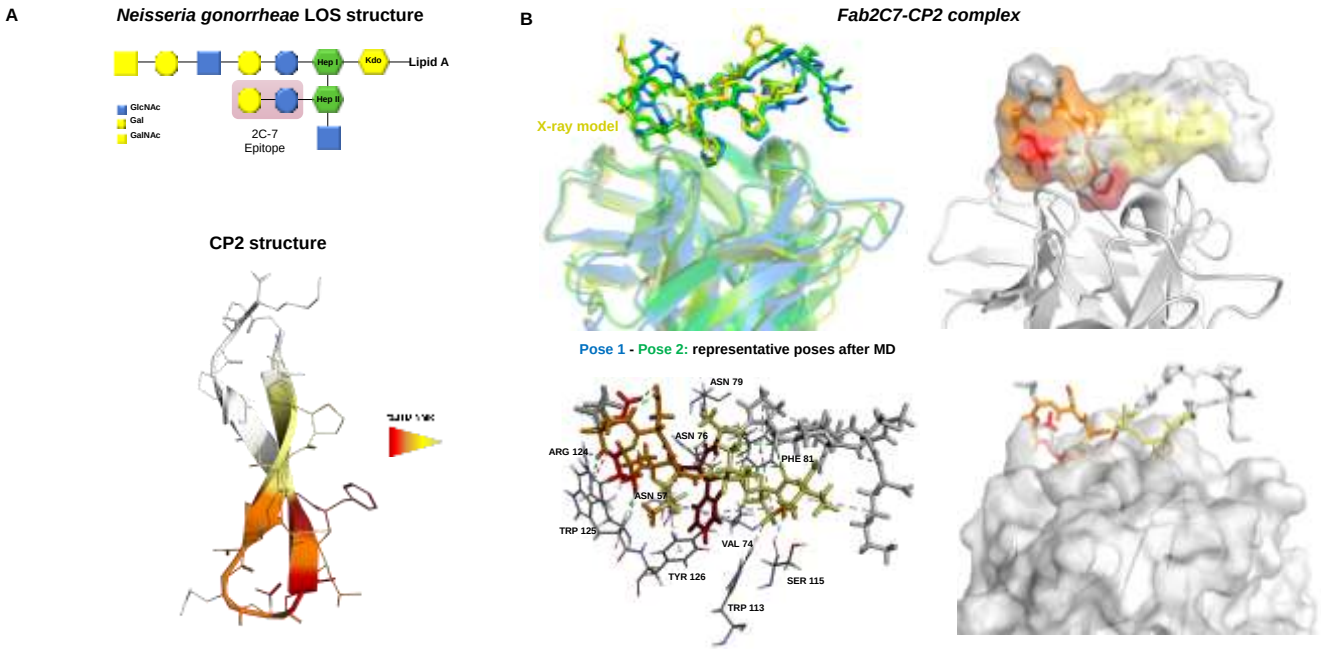

Figure 1. A: Representations of Ng LOS and CP2 structures. **B:** Different 3D views of Fab2C7-CP2 complex.

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Labeled Xylosides As Tools For Glycosaminoglycan Investigation

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Proteoglycans (PGs) consist of a core protein and long negatively charged carbohydrate chains called glycosaminoglycans (GAGs). The PGs are located mainly in the extracellular matrix of mammalian cells and are involved in a variety of important processes including cell signalling, proliferation, and anticoagulation. A key molecule for GAG biosynthesis is the carbohydrate xylose, which is the anchor point between the core protein and the GAG chains. This carbohydrate acts as a substrate for the enzyme β4GalT7 and it is previously known that xylose with certain aglycons can produce soluble GAGs. The biosynthesis of GAGs is quite elusive and therefore it is of interest to fluorescently label xylose or already biosynthesized GAGs in order to use them as tools for investigation.

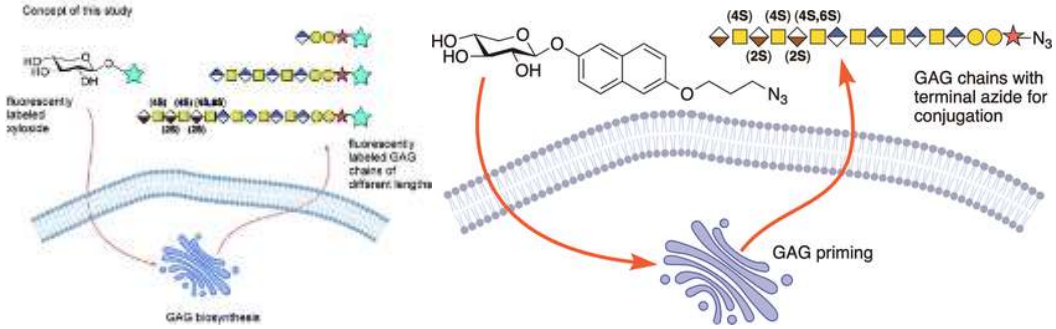

***Figure 1**: Concept of the biosynthesis of fluorescent or azide labeled GAGs.*

To achieve this, several fluorescent xylosides[1] and an azide labeled xyloside[2] were synthesized. The fluorescent xylosides could then be studied with confocal microscopy to visualise GAG biosynthesis, co-localisation, and transport in the cell.

The azide labeled naphthoxylosides primed full-length soluble GAGs which could thereafter be isolated. Using copper-free click chemistry the GAGs could then be conjugated with a multitude of appropriate alkyne containing molecules and be used for further GAG investigation.

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An Integrated Chemoenzymatic Approach To Streamline The Assembly Of Complex Glycopeptides In The Liquid Phase

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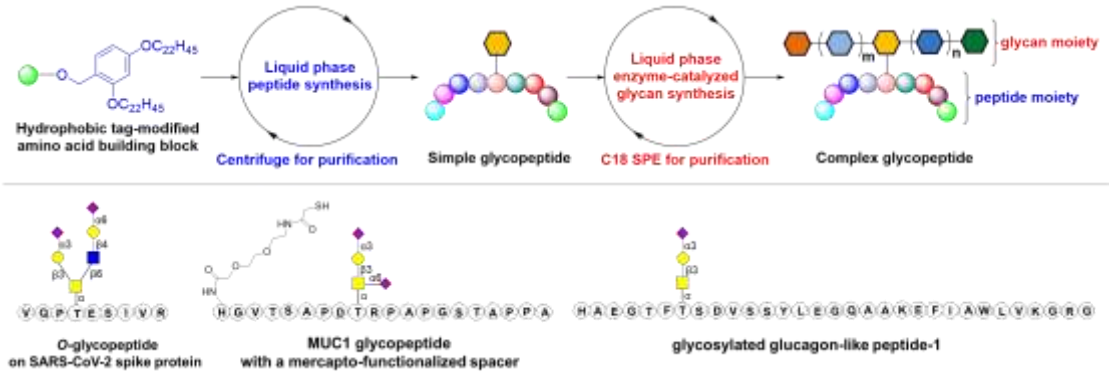
Protein glycosylation is a ubiquitous and complex post-translational modification. Despite the significant progress in glycoproteomics, the precise functions of glycoproteins remain ambiguous due to the difficulty in obtaining homogeneous glycopeptides or glycoproteins. Here we report an efficient chemoenzymatic approach to prepare complex glycopeptides by integrating hydrophobic tag-supported chemical synthesis and enzymatic glycosylations. The hydrophobic tag is utilized to facilitate peptide chain growing in liquid phase and expeditious product separation. After removal of the tag, a series of glycans is installed on the peptides via efficient glycosyltransferase-catalyzed reactions. The broad scope of this method is demonstrated by the streamlined synthesis of 16 structurally well-defined O-glycopeptides on the SARS-CoV-2 spike protein, 4 complex MUC1 glycopeptides, and a 31-mer glycosylated glucagon-like peptide-1. Based on the general applicability and robustness of this streamlined approach, it is possible to implement complex glycopeptide synthesis in many laboratories thereby greatly facilitating progress in glycoscience.



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Chemical Synthesis And Biological Study Of ADP-Ribosylated Nucleic Acids

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ADP-ribosylation is an important post-translational modification of proteins within cells, mediating numerous physiological and pathological processes. Recent reports have unveiled a surprising extension of this modification to nucleic acids, where ADP-ribose (ADPr) becomes covalently linked to either the 5’ phosphate[1] (ADPr-5’ P-nucleotide, as depicted in Fig. 1) or the nucleic base[2], [3] (ADPr-Thymidine) of DNA or RNA. Importantly, some of these modifications can be reversed by ADP-ribose hydrolases such as PARG, TARG1, and DARG. Despite being poorly understood, this newly discovered glycosylation of nucleic acids actively participates in DNA damage repair, bacterial growth, and various physiological processes[1], [2], [3]. However, the lack of synthetic methods and molecular tools for studying ADP-ribosylated nucleic acids presents a significant hurdle in biological research. In response, our recent efforts have centered on the chemical synthesis of ADP-ribosylated nucleic acids, including ADPr-T-Biotin and ADPr-5’P-nucleotide. We successfully tackled the challenges associated with synthesizing the 1,2-cis N-glycosidic bond, labile pyrophosphate linkage, and α-phosphate glycosidic bond. These synthetic products could be employed in chemical biology studies, enabling a deeper investigation into the roles played by ADPr-modified nucleic acids in bacterial growth, cancer, and other cellular processes.

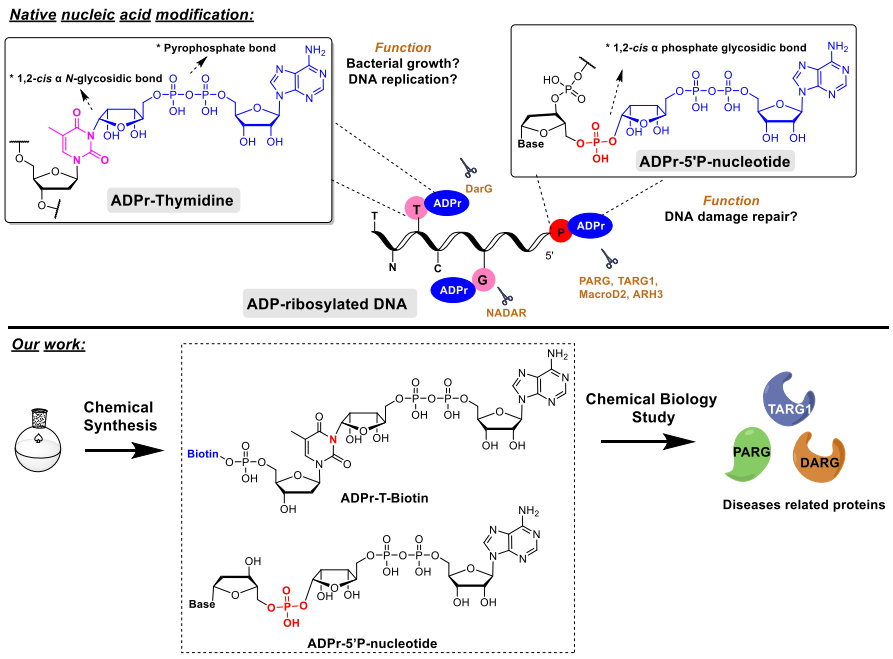

Figure.1 Native structure of ADP-ribosylated DNA, the related hydrolases, possible biological functions of ADPr-nucleic acid (upper) and our work (lower).

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Semi-Synthesis Of Homogeneous Glycosylated Tumor Necrosis Factor-Alpha

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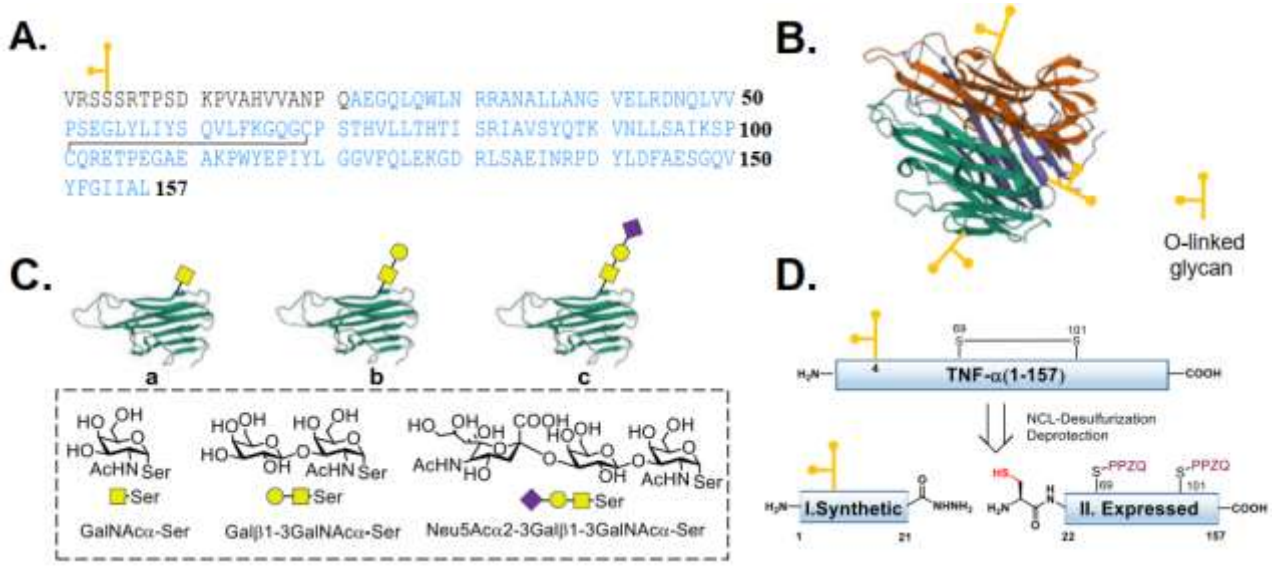
Tumor Necrosis Factor-alpha (TNF-α) is an important cytokine[1]. To study it's structure-activity relationship between glycan and protein, we synthesized three glycosylated TNF-α, which bearing defined structure glycoform at Ser4. Combining with Solid Phase Peptide Synthesis (SPPS), Native Chemical Ligation (NCL), and Expressed Protein Ligation (EPL), the full sequence of TNF-α with various O-glycans have been successfully assembled. In conclusion, we successfully synthesized TNF-α containing a homogeneous carbohydrate for the first time. Furthermore, we conducted a comprehensive study on the immune effects of different carbohydrates on mice[2]. This research played a crucial role in elucidating the structureactivity relationship between carbohydrates and TNF-α.



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The *N*-glycan structural characteristics and significance of CD133 protein in tumor-initiating cells of intrahepatic cholangiocarcinoma

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Tumor-initiating cells (TICs) is a small group of cancer cells with self-renewal and unlimited proliferation potential, which are closely related to tumor formation and growth and plays an important role in the treatment of malignant tumors. To date, the identification of TICs has mainly relied on the recognition of surface markers, in which CD133 is widely used as a biomarker in TICs-related research. During the differentiation of TICs, the *N*-glycan structure of CD133 was changed. However, the exact *N*-glycan structure of CD133 in TICs is unknown. The relationship between the *N*-glycan structure of CD133 and stem cell characteristics is also unclear, which greatly limits the application of CD133 in tumor stem cell sorting and targeted therapy. In order to overcome these limitations, our study found that the level of α-1,2-mannosylated CD133 was associated with the level of stemness genes in intrahepatic cholangiocarcinoma (iCCA) tissues. α-1,2-mannosylated CD133 cells possessed the characteristics of tumor-initiating cells. The loss of the Golgi α-mannosidase I coding gene MAN1C1 resulted in the formation of α-1,2-mannosylated CD133 in iCCA-initiating cells. Analysis of iCCA samples showed that the level of α-1,2-Man and cytoplasmic CD133 was associated with poor iCCA prognosis. In summary, α-1,2-mannosylation of CD133 is a functional marker of iCCA-initiating cells, our finding provides the relationship between the *N*-glycan structure of CD133 and the characteristics of stem cells and provides a new strategy to eliminate the iCCA-initiating cells. This work was supported by the Shanghai Natural Science Foundation (23ZR1413900), National Natural Scientific Foundation of China (32071273, 8207327), the Shanghai Biomedical Technology Support Special Project (23S11900100).

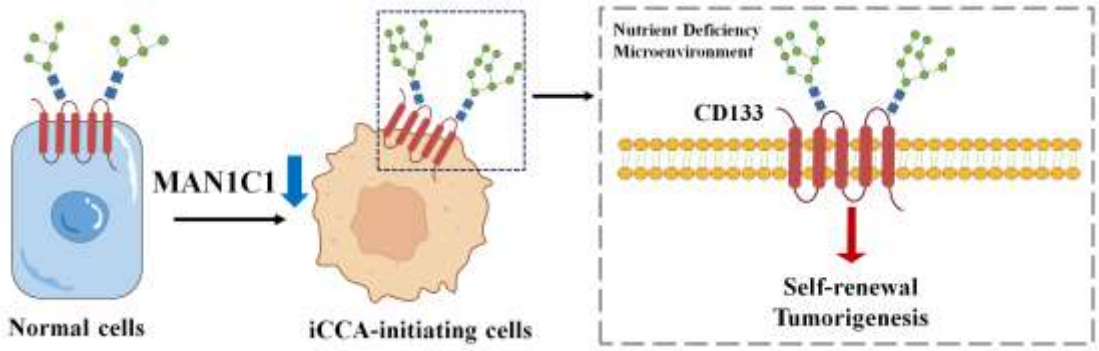

Figure 4 α-1,2-mannosylation of CD133 is a functional marker of iCCA-initiating cells

Keywords: α-1,2-mannosylation, tumor-initiating cell, high mannose glycan, CD133, *N*-glycan

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Structure Characteristics Of A Novel Pectic Polysaccharide From Fructus Corni And Its Protective Effect On Alcoholic Fatty Liver

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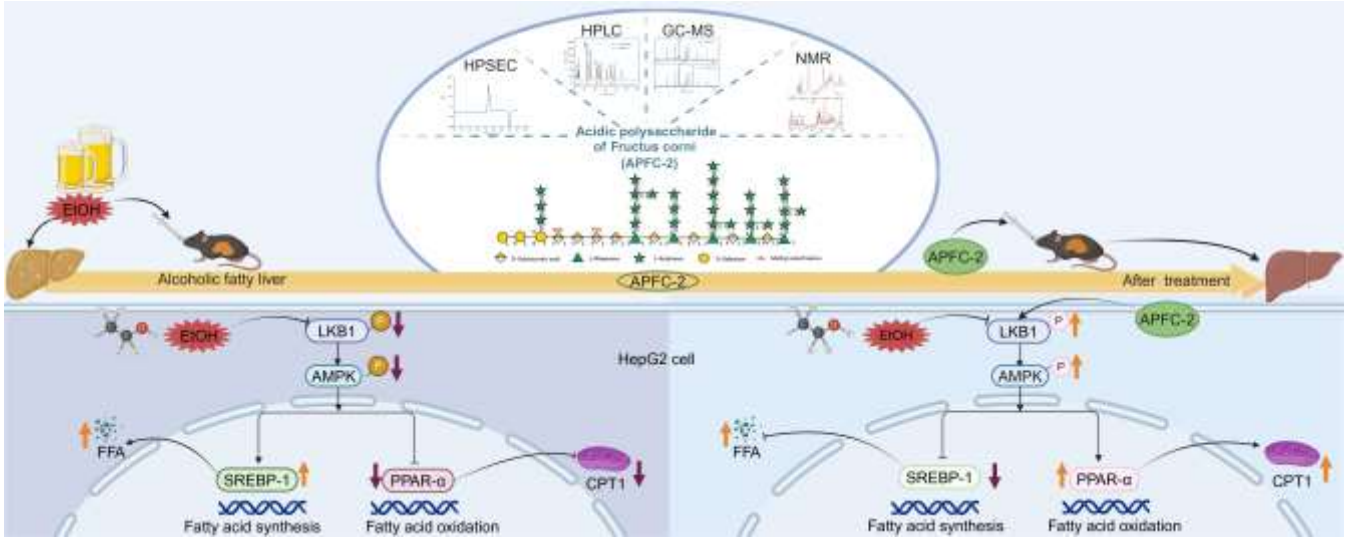
Alcoholic fatty liver disease (AFLD) is characterized by hepatic lipid accumulation and still no effective treatment [1-2]. Previous studies suggest that polysaccharide is a very promising natural product for AFLD prevention and treatment [3]. In this study, a novel homogeneous polysaccharide, APFC-2, was isolated from the dried pulps of Fructus corni, which demonstrated a markedly protective effect against AFLD. APFC-2 is a 63.0 kDa pectic polysaccharide, whose backbone consists of T-α-Gal*p*-(1→6)-α-Gal*p*-(1→3,6)-α-Gal*p*-(1→[4)-α-Gal*p*A-OMe-(1→4)-α-Gal*p*A(1→]$_m$→[2,4)-α-Rha*p*-(1→4)-α-Gal*p*A(1→]$_n$, and whose branches contain T-Ara*f*-(1→, →3)-α-Ara*f*-(1→, →3,5)-α-Ara*f*-(1→ and →5)-α-Ara*f*-(1→. APFC-2 significantly reduced hepatic steatosis, fasting triglyceride (TG) and cholesterol (CHO) levels in alcohol-induced AFLD mice. The lipid-decreasing effect of APFC-2 in vitro was evaluated by oil red O staining and consumption assays, and the results showed that APFC-2 concentration-dependently enhanced lipid metabolism and significantly improved cell viability in alcohol-induced HepG2 liver cells. Mechanistically, APFC-2 markedly inhibited the formation of lipid both *in vitro* and *in vivo* by activating liver kinase B1 (LKB1) and then regulating adenosine 5'-monophosphate-activated protein kinase (AMPK)/sterol-regulatory element binding protein-1 and AMPK/peroxisome proliferator-activated receptor-α pathways. This research provides a potential therapeutic polysaccharide as a specific inhibitor of LKB1 for treating AFLD.



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Synthesis Of Biological Probes By Chemoenzymatic Strategies At GlycoNet Integrated Services

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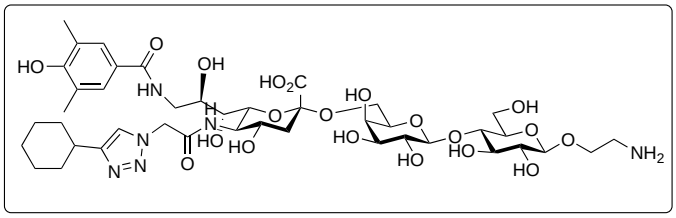
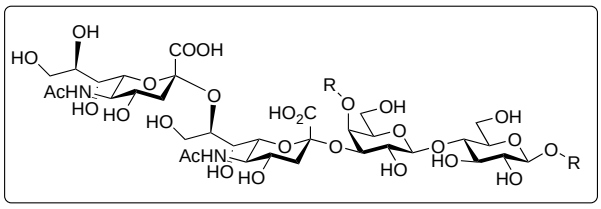
Canadian Glycomics Network (GlycoNet) is a network which comprises 190 research teams and 160 partner organizations from industry, academia, etc. As part of its mandate, GlycoNet funds and supports research projects, develops strategic partnerships, enables technology translation, commercialization, and company creation, and provides outstanding training to trainees.

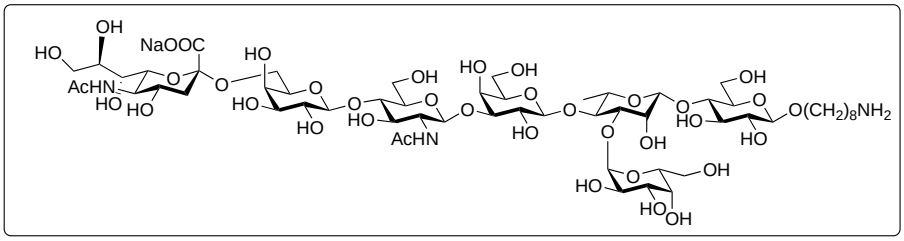
GlycoNet also leads GlycoNet Integrated Services (GIS), a distributed glycomics service facility across seven Canadian institutions including the carbohydrate synthesis. Our core service team at University of Alberta has the expertise on synthesis of complex glycans by chemical or chemoenzymatic approach and automated glycan assembly with the Glyconeer system. In this poster, I will describe our capability in synthesizing various biological probes to contribute the development of glycomic research.



CD33 ligand[1]



GD_3^2, R = H
GD_2^2, R = GalNAc



Streptococcus suis Antigen[3]

Figure 1: examples of glycans made in core service facility[1,2,3].

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The Structure And Immunological Properties Of *Bacteroides Stercoris* Lipopolysaccharide

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The human body is a complex symbiotic system of diverse microorganisms comprising bacteria, yeasts, and viruses.[1] This massive and heterogenous assemblage of microbes, known as the microbiota, is key in physiological and pathological processes occurring in our body, which also include protection against pathogens and immune system development.[2] In this frame, a vast and dynamic community of microbes inhabits the human gut, i.e. the gut microbiota, and comprises commensals and beneficial species for human health. Nevertheless, most of these bacteria are Gram-negative and therefore they possess lipopolysaccharides (LPS) on their outer membranes.[3] LPS are glycoconjugates traditionally associated to potent immune inflammatory reactions in Mammals which occur in a manner that is strongly dependent on the LPS chemical structure. Nevertheless, many harmless Gram-negative colonize the gut without producing any dangerous immune response while promoting the well-being of the host by modulating the immune system itself. [1,3] Therefore, is LPS harmful or beneficial? Deciphering the chemistry responsible for the delicate balance between “beneficial” LPS and “harmful” LPS is pivotal to answer this question. In this communication I will show unreported data related to the characterization of the structure and immunological properties of the LPS from one of the predominant Gram-negative residing the human gut and considered a beneficial bacterium [3], i.e. *Bacteroides stercoris*. A novel chemical structure and uncommon immunological behaviour will be presented, providing insights into the chemistry that might be responsible for establishing harmless relationship between *B. stercoris* and the human host.

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N-Glyfind^{tm} – A High-Specificity Affinity Reagent For Detection And Enrichment Of *N*-Glycosylated Proteins

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N-glycans play crucial roles in nearly every aspect of biological processes, and their distinct properties make them appealing as disease biomarkers and therapeutics targets. However, due to their highly branched and variably linked nature, *N*-glycans remain a challenge to detect, purify, and analyze structurally. Despite current advances in analytical techniques and instrumentation, there is still a great need for high-affinity reagents with well-defined epitope specificity that can be used to interrogate and enrich biological samples. Lectenz Bio has been engineering glycan-processing enzymes and glycan-binding proteins into high-affinity glycan-binding reagents with tunable specificities. Here, we report the development of *N*-GlyFind^{TM}, an asparagine-linked glycan (*N*-glycan) specific reagent engineered via directed evolution from a mouse F-box only protein 2 (FBXO2 or Fbs1). Our approach harnesses molecular dynamics (MD) simulations to explore the dynamic nature of protein-glycan interactions, enabling the identification of specific amino acid residues for construction and screening of a combinatorial yeast display library. The resulting *N*-glycan core-specific candidates were further validated by a panel of assays such as Glycan Microarray, Western Blot, Bio-Layer Interferometry, ELISA, and Affinity Chromatography. The lead candidate, called *N*-GlyFind^{TM}, has been identified as an *N*-glycan affinity reagent exhibiting high pan-specificity towards *N*-glycosylated peptides and proteins. (Supported by United States National Institutes of Health grant OD035390.)

Synthesis And Functional Evaluation Of 2-C-Substituted Glycolipids As A Mincle Ligand

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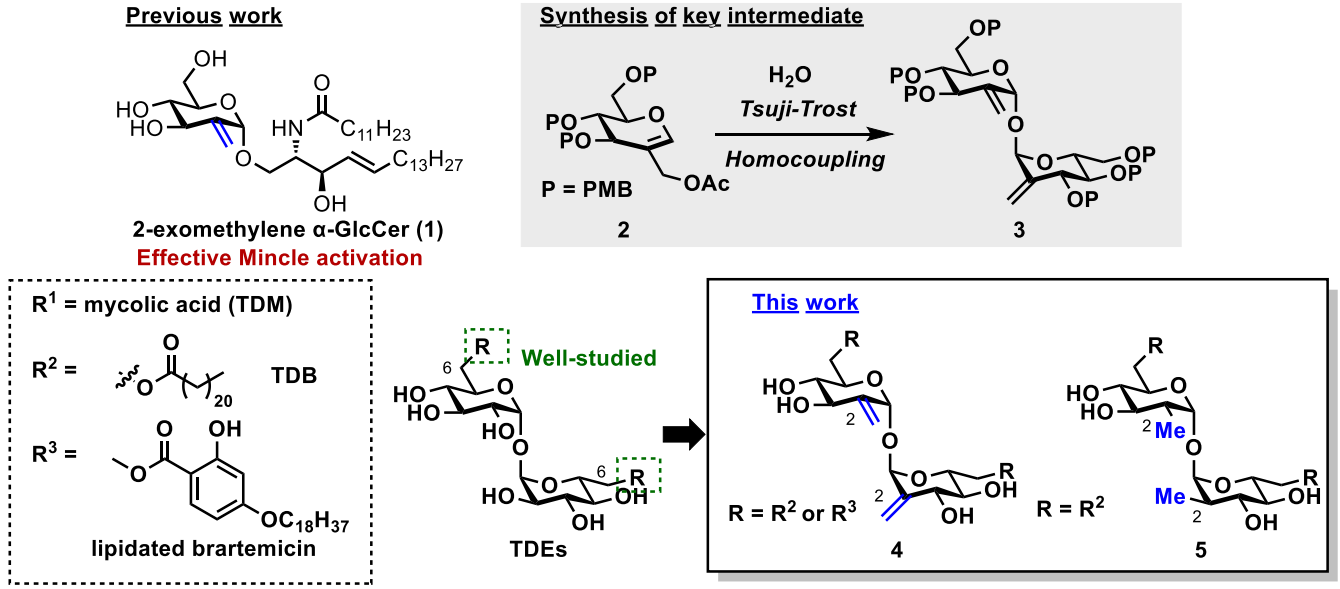
The Macrophage-inducible C-type lectin (Mincle) receptor recognizes glycolipids, including trehalose dimycolate (TDM) and trehalose dibehenate (TDB), thereby triggering immune responses.[1] Mincle has been recognized as a potential target molecule for vaccine adjuvants. Structure-activity relationships of the trehalose diesters (TDEs), especially for the lipid structures at C6, have been well studied, and highly active adjuvants such as the lipidated brartemicin derivatives have been developed.[2] In contrast, few Mincle ligands with modified hydroxyl groups at C2 have been reported. We recently developed ligand-controlled, stereoselective synthesis of both α- and β-glycosides containing a 2-exomethylene group.[3] Furthermore, we demonstrated that 2-exomethylene α-GlcCer analog **1** has exhibited superior Mincle activation activity compared to the corresponding α-GlcCer. The introduction of exomethylene group results in the loss of hydrogen bonding ability at C2, but the π-bond could cause different intermolecular interactions. However, the reasons for the enhanced activity upon introducing the exomethylene group at C2 remained unclear.

In this study, we verified whether introducing the exomethylene groups at C2 enhances the Mincle activity not only in α-GlcCer but also in TDEs. Additionally, to evaluate the influences of steric and electronic effects, we also designed a 2,2'-dimethyl TDE with the simple functional group. The key intermediate **3** was efficiently synthesized by stereoselective Tsuji-Trost reaction using glucal derivative **1** and water. We synthesized 2,2'-diexomethylene- and 2,2'-dimethyl-pseudo-trehalose glycolipids **4** and **5** from **3**. We will report the details of the synthesis and the discussion on molecular mechanisms of glycolipids with the 2-exomethylene group based on biological evaluation and docking simulations.



Keywords: pseudo-glycoconjugates, Tsuji-Trost reaction, Mincle

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Heparan Sulfate Differentially Regulates Cell Binding By Extracellular Vesicles And Human Cytomegalovirus

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Introduction: Extracellular vesicles (EVs) are lipid-enclosed biological nanoparticles produced by all cells as a mode of intercellular communication. During viral infection, cells produce EVs whose cargo can be modulated in response to infection. This may have functional implications for the EVs, including binding to recipient cells to deliver their cargo. While human cytomegalovirus (HCMV) and other viruses are known to utilize heparan sulfate (HS) and/or other glycosaminoglycans (GAGs) for cell tethering, less is known about the role of GAGs in cellular binding by EVs. Therefore, we have studied GAG- and cell-binding properties of EVs produced by uninfected and HCMV-infected cells to identify GAGs that mediate binding of these particles to recipient cells, and to understand whether HCMV infection modulates this function.

Methods: Conditioned media containing EVs was prepared from uninfected fibroblast cells. Conditioned media containing HCMV virions and EVs was prepared from HCMV-infected fibroblast cells and subjected to high-speed centrifugation to separate EVs from HCMV virions. EVs from uninfected and infected cells were further purified by ultrafiltration and size exclusion chromatography and labelled using NHS-ester-AF647. Labelled EVs or HCMV virions were applied to GAG oligosaccharide microarrays and fibroblast cells to investigate GAG and cell binding, respectively. Cell binding was assessed by confocal microscopy (for labelled EVs/HCMV) and qPCR (for HCMV). Enzymes were used to remove HS or CS from cell or EV surfaces to understand their roles in interactions between EVs and cells.

Results: Glycan microarrays showed that EVs from both uninfected and HCMV-infected cells bound heparin, a highly sulfated form of HS, and, to a lesser extent, CS and keratan sulfates. Removal of HS from fibroblasts or EVs promoted binding by EVs produced by uninfected, but not HCMV-infected, cells. Conversely, HCMV virions required HS for cell binding, as evidenced by a drastic reduction in virion binding following HS removal from fibroblasts. Removal of CS from either fibroblasts or EVs had no obvious effect on the interactions between EVs and cells.

Conclusion: Our results demonstrate that HCMV and co-produced EVs depend on distinct and non-overlapping cell surface components for binding to cells. In contrast to HCMV, EVs, while interacting with GAGs on microarrays, did not appear to depend on these for cellular binding. In fact, removal of HS from either the cell surface or the EV surface promoted cell binding by EVs produced by uninfected cells, potentially via exposure of additional EV binding ligands. Therefore, HS serves opposing roles in cellular binding by EVs and HCMV virions.

Concise Chemoenzymatic Synthesis Of *N*-Glycans

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Protein *N*-glycosylation, which refers to the attachment of N-acetylglucosamine (GlcNAc) to the nitrogen atom of an Asn side chain through a β *N*-linkage, is one of the most common protein post-translational modifications. [1] All naturally occurring N-glycans contain a conserved structure, named core pentasaccharide, to which a variable number of monosaccharides can be added or removed, resulting in extremely diverse *N*-glycan structures in living cells. [2,3] The attachment of N-glycans to proteins could affect many properties of glycoproteins, and thus, regulate many physiological and pathological processes. [4] It is well-established that aberrant *N*-glycosylation including changes in site occupancy and glycan structure, has a strong relationship with cancer and many other diseases. [5] Therefore, well-structured N-glycans have great potential for application in basic research and medicinal chemistry.

At present, the methods for obtaining well-defined N-glycans include extraction from natural sources, chemical synthesis and chemoenzymatic synthesis. However, the extraction from natural sources has the problem of low abundance, time-consuming and labor-intensive. Chemical and chemoenzymatic synthesis requires dozens to more than 100 steps to prepare high-grade intermediates, and the synthesis requirements are high. Therefore, the synthesis of a large number of N-glycans with complex structures has always been an obstacle to glycoscience.

In living cells, *de novo* biosynthesis of N-glycans starts from the dolichol phosphate at the cytosolic side of the endoplasmic reticulum membrane, and asparagine-linked glycosyltransferase (ALG) family is responsible for the beginning few steps. Based on the biosynthetic pathway, we designed a chemoenzymatic strategy for the total synthesis of N-glycans (Figure 1). The most challenging problem in the enzymatic synthesis of N-glycans is the preparation of core precursors. Starting with the disaccharide GlcNAcβ1,4 GlcNAc, we synthesized several substrates with different lipid tails ($GlcNAc_2$-PP20, $GlcNAc_2$-PP11, $GlcNAc_2$-PP6) for ALG1 and ALG2 activity tests. We found that a substrate containing an 11-carbon lipid tail ($GlcNAc_2$-PP11) can be completely reacted by ALG1 and ALG2. After the synthesis of Man_3-$GlcNAc_2$-PP11 in large quantities, the core pentasaccharides and core tetrasaccharides could be successfully prepared by acidolysis or Endo S enzymatic hydrolysis. After 9 simple chemical reactions and enzyme reactions, a large number of core pentaccharides and core tetrasaccharides can be obtained. These two N-glycan core precursors are extended by glycosyltransferases to produce a variety of symmetrical and asymmetric N-glycans. In particular, we developed a reversible enzymatic galactosylation as a protective strategy for the synthesis of asymmetric N-glycans, and successfully prepared 49 complex symmetric and asymmetric N-glycans. The described method allows for large-scale, efficient preparation of N-glycans without the need for cumbersome purification operations.

In summary, we have successfully developed a platform for the total chemoenzymatic synthesis of *N*-glycans. This work provides a simple and high-yield strategy for assembling core pentasaccharides and core tetrasaccharides from common starting materials. Using the precursors of core pentaccharides and core tetrasaccharides, a variety of symmetrical and asymmetric biantennary *N*-glycans were synthesized. We believe that this work will accelerate the synthesis and application of complex *N*-glycans and glyconjugates in glycoscience.

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Development Of A Method For Accurate Site-Specific Glycosylation Characterization Of Hyperglycosylated Proteins

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Hyperglycosylated proteins, characterized by multiple glycosylation sites, complex glycan structures, and high sialic acid content, present significant challenges in glycosylation research. Despite the importance of hyperglycosylation in protein drug development, obtaining accurate site-specific glycosylation of such proteins is often hindered by the proximity of glycosylation sites and labile sialic acids. Conventional analytical workflows could be inadequate due to several aspects. First, the dense glycosylation sites can result in a high yield of missed cleavages and glycopeptides with multiple glycosylation sites through traditional specific enzymatic digestion thereby complicating data interpretation. Second, the retention and separation of glycopeptides of large glycans are typically unfavourable in routine reverse phase liquid chromatography. Furthermore, larger glycan motif brings more ambiguities in glycopeptide identification[1, 2].

In our study, using novel erythropoiesis stimulating proteins (NESP) as examples, we developed a comprehensive method to address the aforementioned analytical challenges in the site-specific glycosylation analysis of hyperglycosylated proteins. NESP is a glycoengineered hypersialylated protein drug containing a substantial amount of sialic acid, with glycosylation accounting for half of its molecular weight. Five *N*-glycosylation sites and one *O*-glycosylation site (S120) have been reported from this protein; however, the close proximity of its *N*-glycosylation sites (N24, N30 and N38; N83 and N88) poses a significant challenge for accurate site-specific glycosylation characterization[3]. Instead of using specific enzymatic digestion, we selectively employ non-specific enzymes to uncover all the glycosylation sites by producing glycopeptides of a single glycosylation site. In addition to RPLC, HILIC and PGC based columns were studied to improve the analysis of glycopeptides derived from non-specific enzymatic digestion. Introducing complementary tandem mass spectrometry techniques and sialic acid derivatization could refine the accuracy in identification. Moreover, a custom-built program was developed to reanalyze the glycopeptides identified by commercial software tools, thus reducing the false-positive assignments.

This integrated and innovative method allows the successfully elucidation of site-specific glycosylation differences between a commercial NESP drug (darbepoetin alfa) and a novel NESP drug candidate. The advancement in accurate site-specific glycosylation analysis provides profound insights into hyperglycosylated proteins, potentially enhancing the characterization and development of glycoprotein drugs.

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Encouraging Solution To The Problem Of Semi-Synthesizing Of Glycoproteins

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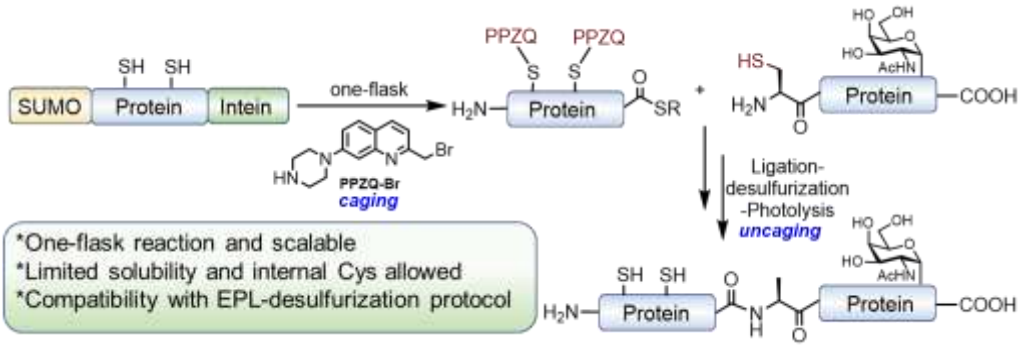
Glycosylation is the most common and complex post-translational modification (PTM) of proteins, and it plays a critical role in the immune response, infection and disease[1]. Expressed protein ligation (EPL) provides a powerful tool to access glycoproteins with precise structures. Existing methods for constructing the critical protein thioester for EPL have predominantly relied on the recombinant intein fusion expressed in Escherichia coli (*E. coli*). Despite its powerful applications, the expression of thioester derived from eukaryotic protein in *E. coli* inherently suffers from its limited solubility, the inactivity of intein, premature hydrolysis and low yields, which restricts the semi-synthesis of glycoproteins[2].

To overcome these obstacles, we present herein the facile one-flask synthesis of inaccessible protein α-thioester via a SUMO-protein-intein (SPI) sandwich model[3].The utility of SPI enhances the protein express yield and solubility, prevents premature hydrolysis and simplifies the purification process, and the inaccessible protein thioester can be readily produced, which is otherwise difficult to obtain using traditional methods.With the protein thioester in hand, we used EPL to efficiently achieve the semi-synthesis of corresponding complex glycoproteins such as Siglec-15, IL-15 and G-CSF. In conclusion, our strategy greatly expands the current toolbox for glycoprotein synthesis. We anticipate its widespread application in the synthesis of various complex glycoproteins.



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Electrochemical Detection Of Viruses Using Diamond Electrode Modified With Glycans Obtained By Saccharide Primer Method

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Infectious diseases caused by viruses remain a serious threat to public health. The recent pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) killed millions of people and severely affected economic activity worldwide. Beside, influenza virus (IFV) causes seasonal epidemic of disease almost every year, which are estimated to result in about 3-5 million serious illnesses annually around the world. Diagnosis at early phase of infection is important for preventing circulation of infectious diseases. Immunochromatography is the gold standard method for virus detection, but its sensitivity is low. Therefore, development of alternative detection method is required. Biosensors based on electrochemistry are rapid and sensitive detection. In this study, we developed boron-doped diamond (BDD) electrodes modified with glycans as receptors for viruses, and IFV and SARS-CoV-2 S1 protein were detected electrochemically. BDD electrode has many excellent properties, such as physicochemical stability, low background current and chemical inertness. Glycan mixture was obtained from cultured cells by administration of a saccharide primer, 12-azidododecyl β-lactoside (Lac-C12N3), which structurally mimic intermediates in the biosynthetic pathway of glycolipid (Fig.1). Lac-C12N3 were glycosylated in MDCK cells, and the glycosylated products were released into culture medium. The structures of glycosylated products were analyzed by liquid chromatography-mass spectrometry (LC-MS), and found to be enriched in sialylated and sulfated glycans. BDD electrode was prepared by chemical vapor deposition method, followed by modification with alkyne-terminated linker molecule electrochemically. The glycosylated products having azido group were reacted with the linker on the surface of the electrode by click reaction. After incubation the glycan-modified electrode, hemagglutinin (HA), H1N1 subtype IFV, and SARS-CoV-2 S1 protein were detected by electrochemical impedance spectroscopy.

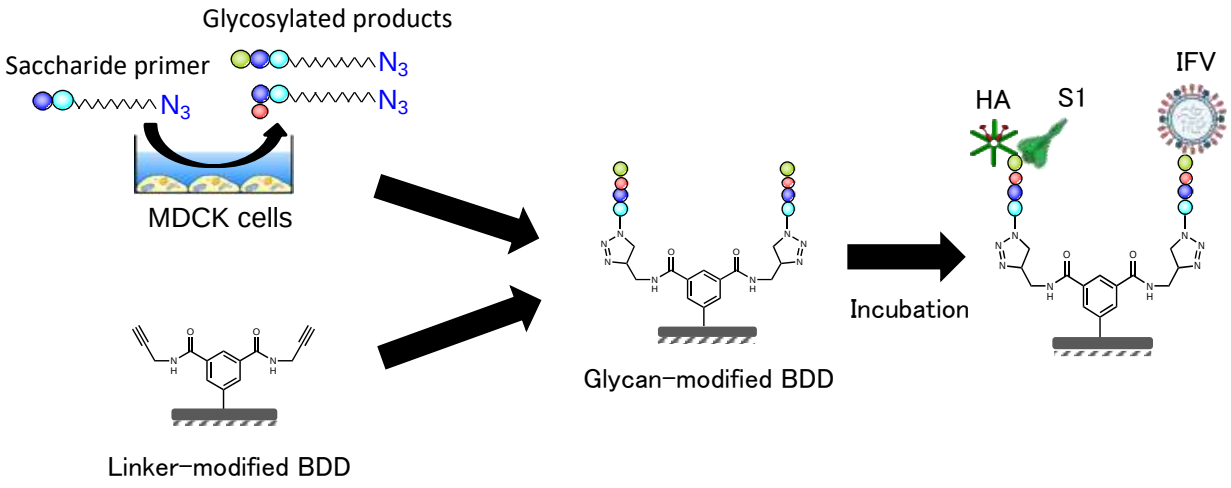

Fig.1 Preparation of glycan-modified BDD.

Synthesis Of Sialylated Human Milk Oligosaccharides By Automated Glycan Assembly

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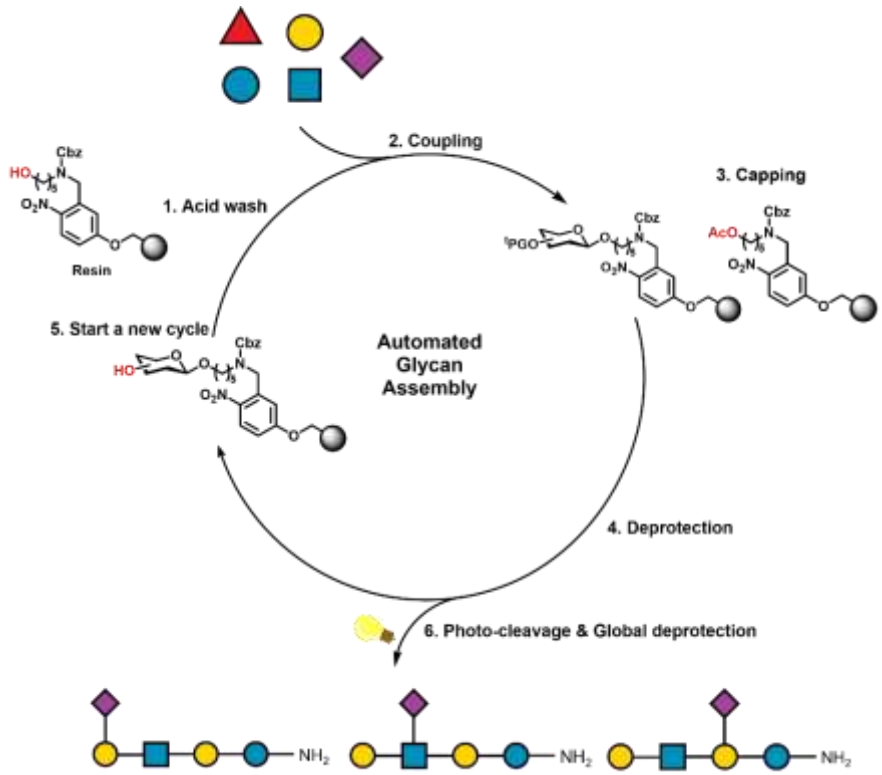
Human milk oligosaccharides (HMOs), diverse unconjugated glycans, are third largest component of breast milk. Numerous studies show human infants rely on HMOs for development of their immune system and modulation of their gut microbial environment[1]. However, accessing to homogeneous HMOs remains the major challenge for elucidating their biological functions. Automated Glycan Assembly (AGA) has seen major developments as a fast and reliable method to synthesize oligosaccharides such as mammalian and bacterial glycans[2]. At present, there is no suitable automated method for sialylation. The challenges of chemical sialylation are efficiency and stereoselectivity because of its quaternary anomeric center with an adjacent electron-withdrawing group and the lack of participating group on C-3. It is necessary to develop a universal solution for construction of oligosaccharides containing sialic acid by AGA. In our project, we used automated glycan assembly (AGA) to systematically construct a library of sialylated HMOs. With approved building blocks[3] and optimized conditions, we could efficiently obtain a collection of sialylated HMOs.



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Development Of A Novel High-Precision Glycosaminoglycan Oligosaccharide Microarray For Probing Microbe-Host Interactions

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Glycosaminoglycans (GAGs) play important roles in a plethora of biological activities through interactions of a diverse range of proteins.[1] Due to their structural heterogeneities, defining the specificity of GAG-protein interactions remains a great challenge. The value of GAG microarrays, a much sought-after high-throughput tool for probing GAG-mediated interactions, has been highlighted in recent studies with the neoglycolipid-base GAG oligosaccharide microarrays[2, 3] and microarrays of synthetic heparan sulphate oligosaccharides.[4, 5] However, a limitation of all the GAG array systems is the lack of accurate methods to quantify GAG probes of different sizes and charges retained on the microarrays after deposition; this is important for obtaining quantitative data.

In this communication, we describe the development of a new generation of GAG oligosaccharide probes based on a tri-functional Fmoc-protected-Amino Azido-Aminoxyl linker (FAAO) These FAAO-GAG probes, designed based on the recently described FAA-glycan probes[6], achieve higher yields for derivatizing different classes of GAG oligosaccharides with differing chain length, benefiting from the high efficiency of glycan conjugation via oxime-ligation.[7] The Fmoc group in the linker facilitates monitoring glycan probe conjugation and purification. Upon Fmoc deprotection the amino group allows arraying of derivatized glycans onto functionalized glass slides using amide-coupling reaction. The azido functionality allows 'on-array' visualization and semi-quantitative measurement by microarray scanner of the FAAO-GAG probes attached to the array surface. Moreover, the azido group adds versatility, allowing the FAAO-GAG probes to be converted into other functional probes, e.g. lipid-linked, biotinylated, fluorescent probes for diverse glycan-recognition studies.

A new GAG oligosaccharide array has been constructed with over 50 FAAO-GAG oligosaccharide probes, including those derived from oligosaccharide fractions of hyaluronic acid, chondroitin sulphates A, C and D, dermatan sulphate, and heparins obtained from different biological sources which have distinct sulphation features together with their variously desulphated variants. Promising results have been obtained in microarray analyses of a wide range of viruses, including human papillomavirus, human cytomegalovirus, different serotypes of human adenoviruses and a number of bacterial adhesins. The application of this array for analysing whole bacterial cells is currently underway. Thus, this new GAG array platform holds significant promise for investigating GAG interactions with various microbes, not only to enrich our understanding of the GAG Interactome at the host-microbe interface, but also to leading to novel therapeutic opportunities.

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Synthetic Homogeneous Glycoforms Of The Sars-Cov-2 Spike Receptor-Binding Domain For Profiling The Correlations Between Glycan Composition And Function

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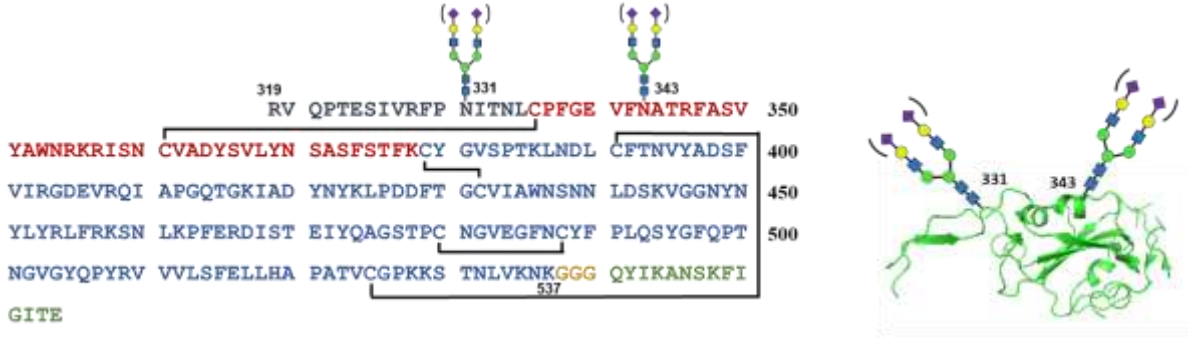
SARS-CoV-2 attaches to its host receptor, angiotensin-converting enzyme 2 (ACE2), via the receptor-binding domain (RBD) of the spike protein, a critical viral immunogen, is affected by the heterogeneity of its glycan structures and relatively low immunogenicity. Vaccines have been the primary remedy in the global fight against SARS-CoV-2. The RBD glycoprotein is a critical target for the development of neutralizing antibodies and vaccines against SARS-CoV-2. However, the high heterogeneity of RBD glycoforms may lead to an incomplete neutralization effect and impact the immunogenic integrity of RBD-based vaccines. Investigating the role of different carbohydrate domains is of paramount importance for vaccine developing.

Herein we described a highly efficient and scalable strategy for the preparation of homogeneously glycosylated RBDs bearing structure-defined glycoforms, facilitating the elucidation of carbohydrate structure-function relationships. Relative to natural HEK293-derived RBD, synthetic RBDs with biantennary N-glycan elicited a higher level of neutralising antibodies against SARS-CoV-2 in mice. Furthermore, RBDs containing T_{pep} elicited significant immune responses in transgenic mice expressing human ACE2. Our collective data suggested that trimming the N-glycans and T_{pep} conjugation of RBD could potentially serve as an effective strategy for developing subunit vaccines.



Key words: peptide ligation, glycosylation, glycoprotein

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Synthesis And Biological Activities Of Diacetylene Polyols Compounds

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Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences

Polyacetylene compounds are a class of natural product with two or more conjugated acetylenic and olenic bonds. Researches revealed that most of them exhibit antibacterial, antiviral, anti-inflammatory, anti-oxidant, antitumor and immune enhancing activities and have been treated as components in traditional Eastern medicine[1]. Due to their novel structure features, impressive broad-band biological activities, diacetylene polyols compounds caused our attention[2]. We have reported the total synthesis of the related diacetylene polyols petrosiols[3-4], and found that some of petrosiols could Induce the differentiation of neuronal progenitors and in protecting them against oxidative stress[5]. Herein, we would like to report the design and synthesis of a series of diacetylene polyols derivatives and their preliminary biological activities. Our results reveal that compounds LYF-1, LYF-2, LYF-8 presented better anti-inflammatory activities in comparison with natural product petrosiol E.

Chemoenzymatic Synthesis Of Sialosides Containing 4-*N*-Derivatives Of Sialic Acid As Probes For Influenza A Virus Neuraminidases

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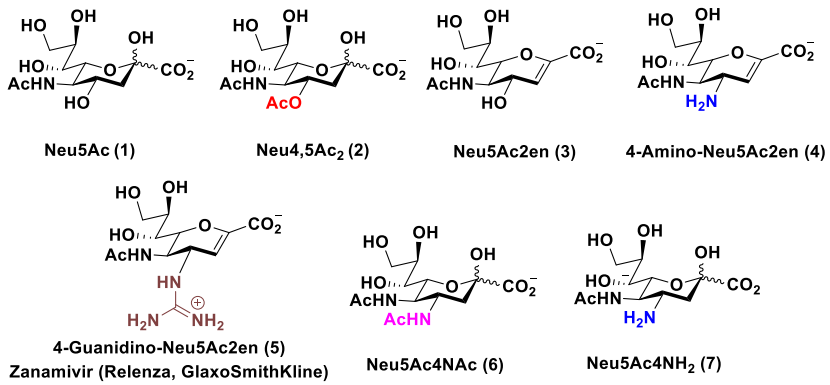
Sialic acids are acidic monosaccharides that participate in many molecular recognition events. They are commonly found at the terminal positions of the carbohydrate components on cell surface, and can be recognized by pathogens including influenza viruses. *N*-Acetylneuraminic acid (Neu5Ac, **1**) is the most abundant sialic acid form in nature. 4-*O*-Acetylated Neu5Ac ($Neu4,5Ac_2$, **2**) has been found on glycoconjugates from some species, but the impact of this modification has been difficult to study due to its susceptibility to base-catalyzed cleavage.

On the other hand, 2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en, **3**) is a transition state analog inhibitor against neuraminidases that catalyze the hydrolysis of sialosides. Its 4-amino- (**4**) and 4-guanidino- (Zanamivir, **5**) derivatives have been shown to be highly efficient and selective against neuraminidases from influenza A viruses (IAVs). However, it is unclear whether the hemagglutinins from IAVs can bind to sialoside ligands containing 4-amino- or 4-guanidino derivatives of Neu5Ac.

To help answer these questions and to overcome the instability issues of $Neu4,5Ac_2$ (**2**) without changing its structure significantly, I have designed and chemically synthesized its 4-*N*-acetyl analog Neu5Ac4NAc (**6**) and its chemoenzymatic synthon 4-amino-analog $Neu5Ac4NH_2$ (**7**). $Neu5Ac4NH_2$ (**7**) has been further used in a highly efficient stepwise one-pot multienzyme (StOPME) system to construct a comprehensive library of sialosides containing $Neu5Ac4NH_2$ (**7**) and its derivatives.

These sialosides are being used as probes in high-throughput substrate specificity and binding assays for neuraminidases and hemagglutinins from different IAVs. The study can lead to the development of new diagnostic tools and potential therapeutics against IAVs.

Graphic:



Bisecting GlcNAc Modification Reverses The Chemoresistance Via Attenuating The Function Of P-gp

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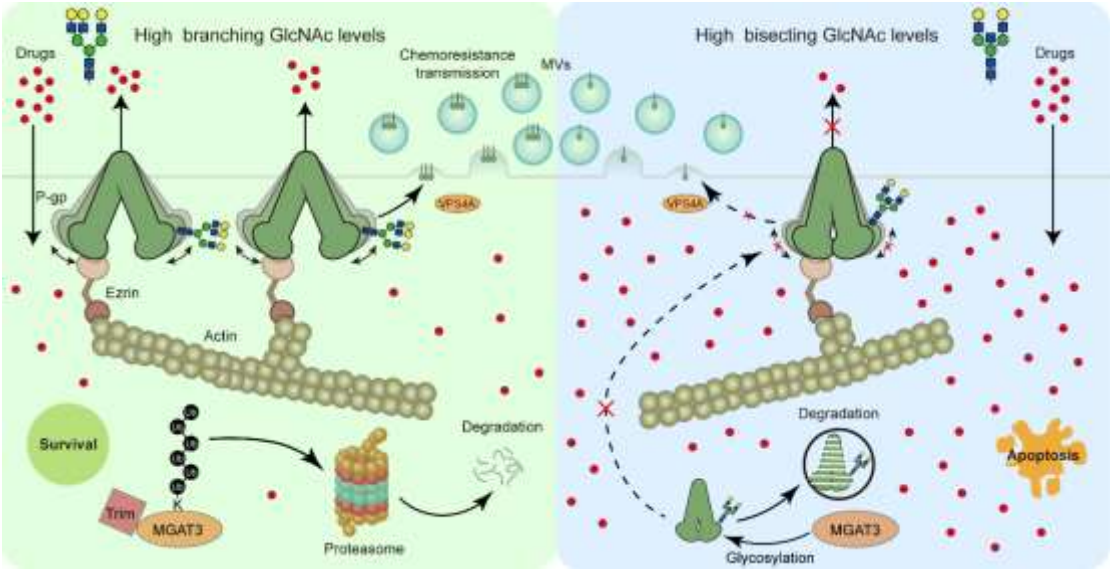
Chemoresistance is a key factor contributing to the failure of anti-breast cancer chemotherapy. Although abnormal levels of glycosylation, e.g. bisecting GlcNAc, are closely correlated with breast cancer progression and metastasis, the function of glycoconjugates in chemoresistance remains poorly understood. Here, we observed significantly reduced levels of bisecting GlcNAc and its glycosyltransferase MGAT3, accompanied by enhanced expression of P-glycoprotein (P-gp) in the chemoresistant breast cancer cell. Elevating bisecting GlcNAc levels effectively reversed chemoresistance by reducing P-gp expression in the chemoresistant cells. Our mechanical study revealed that bisecting GlcNAc modification impaired the association between Ezrin and P-gp, leading to decreased P-gp expression on the cell membrane. Bisecting GlcNAc also suppressed VPS4A-mediated P-gp recruitment into microvesicles, resulting in a decrease in chemoresistance transmission. Structural dynamics analysis further suggested that bisecting GlcNAc at Asn494 could introduce structural constraints that rigidified the conformation and suppressed the activity of P-gp. Together, our findings highlight the crucial role of bisecting GlcNAc in chemoresistance and suggest the possibility of reversing chemoresistance by modulating the specific glycosylation in breast cancer therapy



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Bacteria Lectin Recognition Towards Synthetic Mucin Tandem Repeat Glycopeptides

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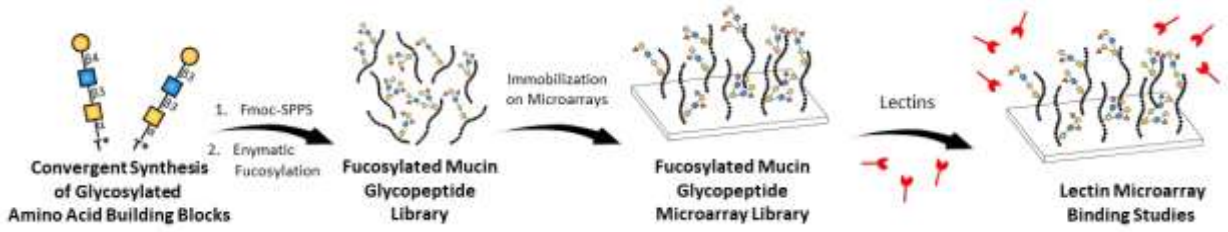
Mucins are densely *O*-glycosylated membrane-bound and secreted proteins ubiquitously found on the epithelial cell surface.[1] In the intestines the membrane-bound mucins are coated with an inner and outer mucus layer. The mucus consist to a large extend of secreted mucins and serve as a protective barrier, which keeps bacteria on distance from the epithelial tissue.[2] However, the microbiota have co-evolved with the human host and developed strategies to feed, degrade and penetrate the mucus barrier and thereby enable adhesion to carbohydrate ligands on the host cell-surface using lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria lectins and carbohydrate ligands presented on mucin peptide backbones. For instance binding specificities will be presented of fucose binding lectins from the toxin A of *Clostridium difficile* (TcdA), a bacterium that causes gastrointestinal disorders.[3] Selected synthetic mucin core and extended core tandem repeat glycopeptides were enzymatically modified with Lewis a, Lewis x, or H-type motifs as well as bi-fucosylated Lewis b and Lewis y structures. [4] Then the glycopeptides were immobilized on microarray slides and applied to evaluate TcdA binding preferences. Binding preferences of bacteria CBMs from mucin degrading enzymes may additionally be presented.



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Activity Screening And Application Of *Lycium Barbarum* Peptidoglycan Lbpw In Improving Liver Fibrosis

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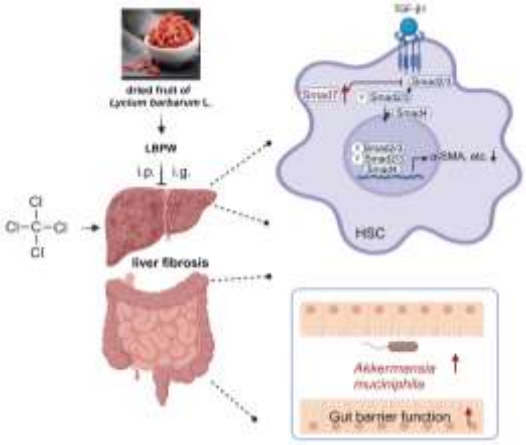
Liver fibrosis has fueled considerable morbidity and mortality in the world with an ascending trend. When liver tissue is repeatedly or continuously damaged, the liver parenchyma will undergo scarring, forming liver fibrosis. Evidence shows that the process of liver fibrosis may be reversible. However, the development of a safe and specific anti-fibrotic drug remains an unmet challenge, despite extensive research efforts. Lycii fructus is the mature fruit of Solanaceae *Lycium barbarum* L. (LB). *Shen Nong's Materia Medica* documents that it has the therapeutic function of nourishing the liver and kidneys. Polysaccharides, recognized as a principal constituent of *Lycium barbarum* L., have been previously documented to exert a role in the amelioration of liver fibrosis. For the further exploration of potential hepatoprotective effects in improving liver fibrosis through other biological macromolecules from LB, an O-linked peptidoglycan, denoted as LBPW, was isolated from LB. LBPW contains a sugar content of 58.6% and a protein content of 38%. The polysaccharide portion was composed of glucose (34.1%), arabinose (20.2%), galactose (19.0%), mannose (16.8%), xylose (8.4%), and rhamnose (1.6%); meanwhile, the protein content of this peptidoglycan is 40%, among which, serine (1.3 mg/g), alanine (1.1 mg/g), and glutamic acid (1.0 mg/g) were identified as the predominant constituents. Subsequent analysis aimed to delineate its hepatoprotective properties. Indeed, a remarkable prevention of liver damage and fibrosis induced by carbon tetrachloride (CCl_4) in mice was observed following the intraperitoneal injection and intragastric administration of LBPW. LBPW ameliorates liver fibrosis by impeding hepatic stellate cell (HSC) activation through upregulating Smad7 and subsequent suppression of the TGF-β/Smad cascade. Furthermore, LBPW betters the intestinal environment as evidenced by the boosted abundance of *Akkermansia muciniphila* (*A. muciniphila*) and fortified gut barrier. *A. muciniphila* might be responsible for the efficacy of LBPW since interfered with the abundance of this bacterium by antibiotic cocktail blocking the antifibrotic effect of LBPW. Taken together, LBPW is a novel type of molecule from fruit of LB, which exerts a hepatoprotective effect and is conducive to developing drug candidates or nutraceuticals for treating liver fibrosis.



Graphic abstract: LBPW might exert an anti-fibrogenesis effect on CCl_4-induced mice via rebalancing TGF-β/Smad7 signaling in HSC and propagating gut commensal *A. muciniphila*.

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Stereoselective Synthesis Of Pseudo-Glycoconjugates With 2-Exomethylene Group And Biological Activity Of Pseudo-Glucosylceramides

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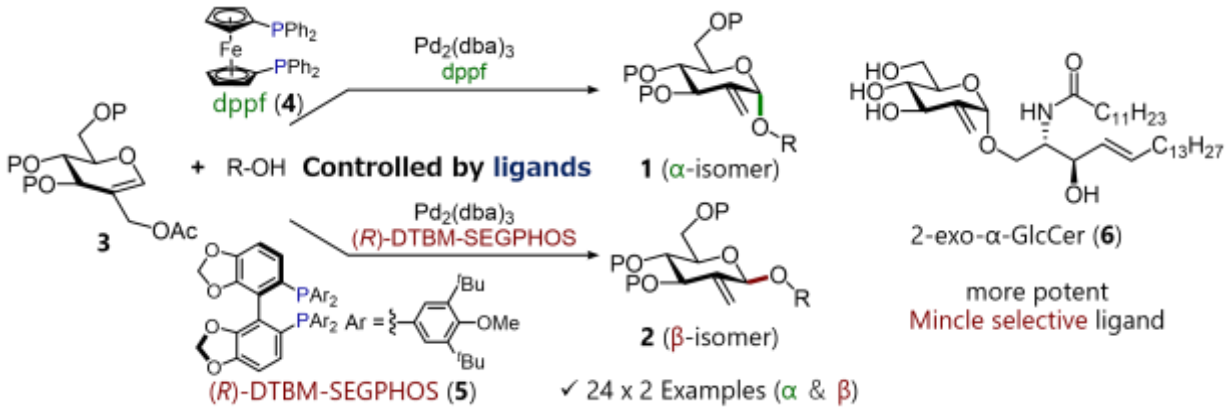
Our group is investigating the development of “pseudo-glycoconjugates”, in which the original activity or function are altered by slight structural modification of the original glycoconjugates. We recently developed novel pseudo-glycoconjugates by replacing the functional group at C2, which characterizes the function of glycans, to the exomethylene functionality. The introduction of an exomethylene group results in the loss of hydrogen bonding ability at functional group at C2, but the π-bond can cause different intermolecular interaction. In addition, the ring strain caused by the introduction of sp^2 carbon results in a unique conformational property from that of native glycoconjugates. We expected that these steric and electronic property of pseudo-glycoconjugates with 2-exomethylene group would lead to finding new biological-active glycan analogues.

In the first step, we challenged to develop a methodology for the efficient and stereoselective synthesis of various 2-exomethylene-type α-glucosides **1** and β-glucosides **2** in the first step. In this study, we investigated the Tsuji-Trost-type reaction of glucal derivative **3** as a common precursor. of **1** and **2**. We anticipated that the stereoselectivity could be controlled by a ligand of the Pd catalyst. Treatment of **3** and an acceptor and catalytic amount of $Pd_2(dba)_3$ and dppf (**4**) under heated conditions gave the α-glucoside **1** in high yield and in a stereoselective manner. Further examination of the ligand revealed that the use of (*R*)-DTBM-SEGPHOS (**5**) reversed the stereoselectivity and selectively gave β-glucoside **2**. These methods were applicable to 24 species of acceptors with primary alcohols or secondary alcohols [1].

We synthesized the pseudo-glucosylceramide (pseudo-GlcCer) **6** and evaluated its function as a ligand for immune receptor. Finally, we found that it has different property from that of native GlcCers as expected. We will report the development of synthetic methodology and the biological activities of pseudo-GlcCers in detail.



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Targeting The Biosynthesis Of Gags By Phosphonate-Xyloside Constructs

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β-1,4-galactosyltransferase 7 (β4GalT7) plays a crucial role in glycosaminoglycan (GAG) biosynthesis by initiating the transfer of the initial galactose unit onto xylose within the linker region. In our pursuit of novel inhibitors targeting GAG biosynthesis, we employed saturation transfer difference NMR spectroscopy to examine the binding interactions between β4GalT7 and various pentosides in the presence of UDP-donors. These investigations confirmed β4GalT7's specificity in glycosylation and highlighted the significant roles of the naphthalene and uridine components in the acceptor and donor binding, respectively, with the galactose moiety exhibiting less significance. Building upon these insights, we explored the synthesis of UDP-naphthoxyloside conjugates aiming to mimic transition states. These synthesized compounds, prepared via a streamlined procedure, were then evaluated for inhibitory activity in a β4GalT7 assay. Remarkably, one truncated analog, a bisphosphonate-xyloside construct, demonstrated noteworthy inhibition (IC50: 188 μM). These results pave the way for the development of a novel class of GAG biosynthesis inhibitors.

A Chemoenzymatic Approach for Precise Synthesis of Heparan Sulfate Oligosaccharides

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Heparan sulfates (HS) are highly sulfated linear polysaccharides present on the cell surface and in the extracellular matrix of all mammalian cells, playing crucial roles in various biological processes through protein interactions.[1] The biosynthesis of HS is a highly regulated multi-step process involving various enzymes within the Glogi apparatus. [2] It initiates with the assembly of a polysaccharide chain composed of alternating α-1,4-GlcNAc and β-1,4-GlcA. The resulting polymer undergoes several enzymatic reactions involving *N*-deacetylation/*N*-sulfation, C5 epimerization and *O*-sulfation at various positions, mediated by *N*-deacetylase/*N*-sulfotransferases (NdAc/NST), C5 epimerases (C5-epi), 2-*O*-sulfotransferase (2-OST), 6-OST and 3-OST. Incomplete enzymatic modifications lead to the structural diversity of HS. Several laboratories have reported elegant chemical[3] or chemoenzymatic methodologies[4] for HS oligosaccharides. However, chemical synthetic methods involve many steps. Enzyme-mediated methods require fewer steps, but the promiscuity of the biosynthetic enzymes makes it challenging to precisely control the modification sites. Previous studies have shown that 6-*O*-metylation modification of GlcNS can not only block sulfation of C-6 hydroxyl group, but also prevent epimerization of the upstream GlcA moiety.[5] However, C6 methylation could not be removed without destroying HS oligosaccharides after enzymatic modifications.

In this work, we describe a novel chemoenzymatic approach that employs a removable group, benzyl group modified at C6 hydroxyl of GlcNS moiety to address deficiencies of enzymatic modifications and provide numbers of well-defined HS oligosaccharides. A modular synthetic approach has been developed in which a C6 benzyl ether modification of GlcNS serves as an HS primer. Evaluations of this primer with HS synthetic enzymes such as NST, PmHS2, C5-epi, 2-OST, 6-OST indicate that C6 benzyl modified GlcNS can tolerate NST and PmHS2. Additionally, observations indicate that introducing the C6 benzyl group on GlcNS can self-blocking 6-*O*-suflation without impacting the sulfation of adjacent GlcNS units. Furthermore, we have found that benzyl group not only allows for controlled epimerization of a single adjacent GlcA residue but also can be removed under mild condition. This discovery paves the way for the development of chemoenzymatic synthesis of HS oligosaccharides with precise control. This modified group can be removed at different stages of the enzymatic modification, providing access to differently sulfated derivatives from a single HS precursor.

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Facile Synthesis Of Polyglutamic Acid And Scalable Preparation Of Glycopeptides Via One-Pot Strategy

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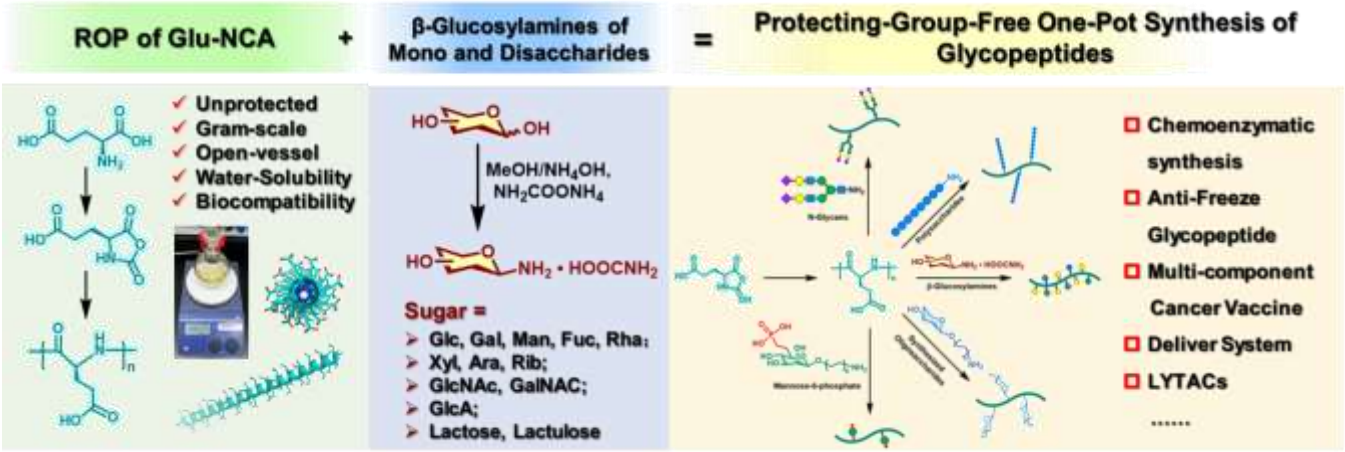
Glycoproteins play a significant role in numerous physiological processes, including cell signaling, cell differentiation, viral infection, fertilization and cancer metastasis. The distinct biological functions of glycoproteins are highly relevant to their structure, making it a significant challenge to obtain well-defined glycopeptides.[1] Therefore, glycoconjugates are carefully designed serving as the biomimetics of glycopeptides and glycoproteins. However, most of the glycopolymers are synthesized with the non-hydrophilic backbone. Synthetic polypeptide backbones are more biocompatible and water-soluble, which can be synthesized via the polymerization of α-amino acid N-carboxyanhydride (NCA).[2] Glycopeptides can be synthesized by the direct ring-opening polymerization with the glycosylated NCA monomers and post-modification. In this study, we have developed a robust and straightforward methodology for synthesizing the polyglutamic acid backbone from unprotected glutamic acid, followed by their subsequent post-glycosylation modification. The moisture-tolerant synthesis and polymerization of $_L$-glutamate NCA can be conducted in standard chemistry laboratories without the need for a glovebox (environmental humidity: 70% or higher) and can be easily executed on a decagram scale. This protecting-group-free one-pot synthesis of glycopeptides enables the acquisition of diverse multivalent well-defined glycoconjugates, thereby facilitating the synthesis of glycomimetic drugs and process development in medicinal chemistry.



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Chemical Synthesis Of A-Dystroglycan With Defined O-Mannosylation Pattern

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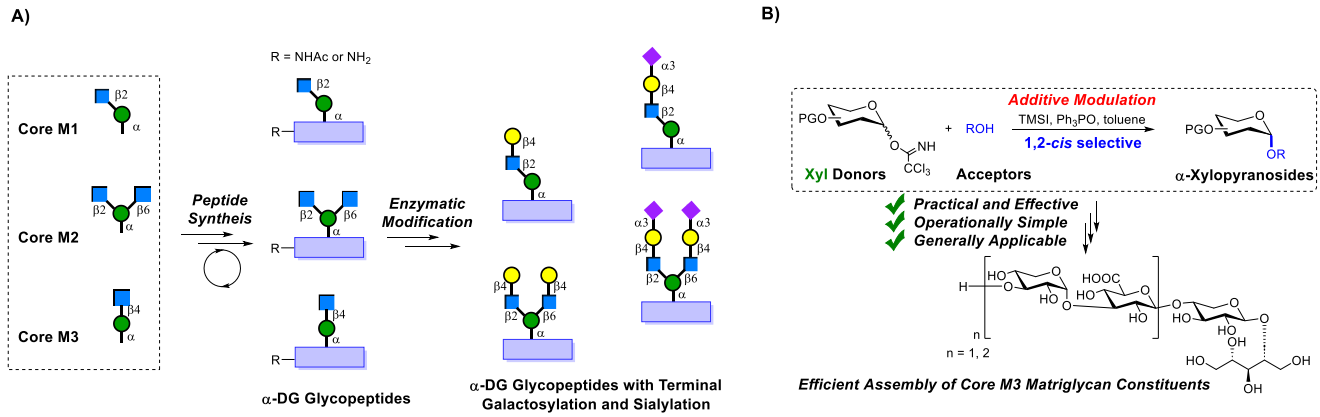
O-Mannosylation plays a vital role in the regulation of a variety range of biological processes, for instance, brain and muscle development. However, the precise function remains largely unknown due to its innate heterogeneity. In this regard, it is still welcome to develop efficient methods to access diverse structurally defined O-mannosyl glycopeptides. In this study, a diversity-oriented assembly of O-mannosyl α-dystroglycan (α-DG) glycopeptides has been achieved via a chemoenzymatic strategy. This strategy features (i) gram scale divergent synthesis of core M1, core M2 and core M3 mannosylated amino acids from judiciously designed protecting group strategies and chemical glycosidation; (ii) efficient glycopeptide assembly via the optimized microwave assisted solid phase peptide synthesis (SPPS); and (iii) enzymatic elaboration of the core glycan structures to install galactosyl and sialyl galactosyl moieties. The efficiency and flexibility of this chemoenzymatic approach was demonstrated with the construction of 12 glycopeptides with different core M1, core M2 and core M3 mannosyl glycans, including a core M2 glycopeptide bearing a hepta-saccharide for the first time.

As a major constituent of extending core M3 glycan, the matriglycan remains a synthetic challenge. To address the long-standing technical hurdle in 1,2-*cis*-xylopyranoside bond formation, a highly stereoselective strategy was established via a preactivation-based, additive-modulated trichloroacetimidate glycosidation strategy. The current protocol is mild, practical, and successful with various xylopyranosyl donors and glycosyl acceptors, including acceptors that are reported to be less reactive due to steric hindrance. The utility of this method was demonstrated with the facile assembly of matriglycan constituent tetra- and hexasaccharides.



The Synthesis Of *O-/C*-Aryl Glycosides With Stereodefined Quaternary Pseudoanomeric Centers

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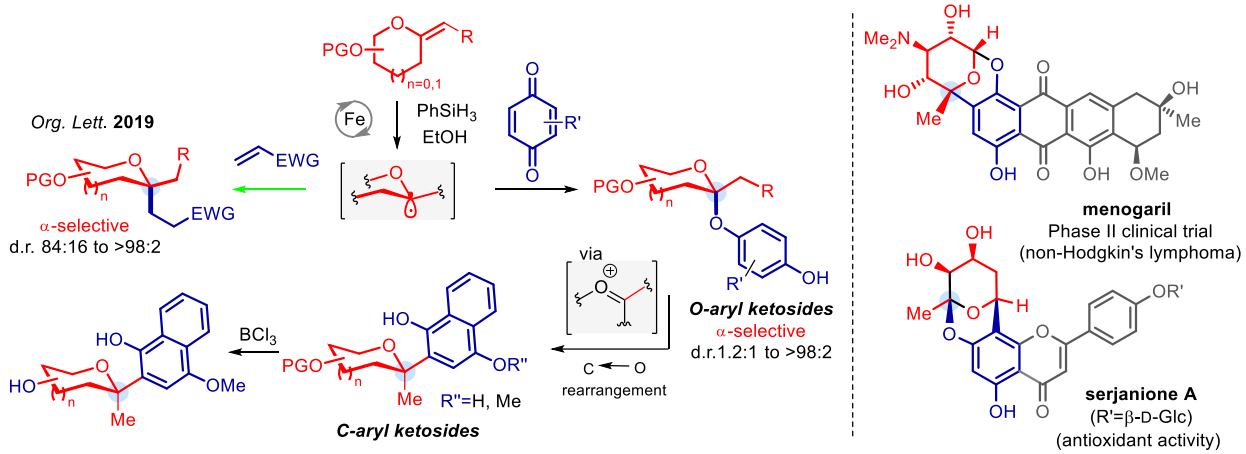
O- and *C*-aryl glycosides represent important classes of synthetic and natural compounds of therapeutic interest.[1] Among them, serjanione A[2a] and menogaril[2b], a clinically active antitumor drug derived from the natural product nogalamycin, are synthetically attractive. In these unique structures, the sugar residue is joined to the aromatic moiety *via* both glycosidic and C-C bonds to form a benzoxocin ring system. In our previous report on the synthesis of *C,C*-glycosides from *exo*-glycals by way of a Metal-mediated Hydrogen Atom Transfer (MHAT).[3] The capture of the transient tertiary pseudoanomeric radicals by a range of Michael acceptors enables the stereocontrolled *C*-quaternization of the anomeric center. With the objective of developing a convergent, step-economical access to the benzoxocin core found in *C,O*-fused glycosyl hetarenes such as serjanione A or nogalamycin, we envisioned the direct coupling of MHAT-generated glycosyl radicals with 1,4-quinones. The control in regio- chemo- and diastereo-selectivity in the generation of quaternary (pseudo)anomeric centers using such a demanding cross-coupling transformation involving an *exo*-glycal and a quinone was expected to be highly challenging. In our works, quinones can be successfully coupled with a variety of *exo*-glycals to produce phenolic *O*-ketosides, demonstrating their applicability in demanding cross-coupling transformations initiated by iron-catalyzed HAT.[4] Following this, the synthesis of *C*-aryl ketosides via unprecedented Lewis acid-catalyzed O → C glycoside rearrangement was also demonstrated, opening the way to a unified strategy for the construction of *C*-glycoside motifs characterized by a stereodefined quaternary pseudoanomeric center bearing an exocyclic *O*- or *C*-aryl substituent.[5] Finally, the availability of *C,C*-aryl glycosides with naked hydroxyl groups will probably contribute new horizons to the synthesis of drugs and natural products.



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A Novel Bio-Inspired Semi-Synthetic Strategy Toward 143 Glycosyl-Interleukin-6

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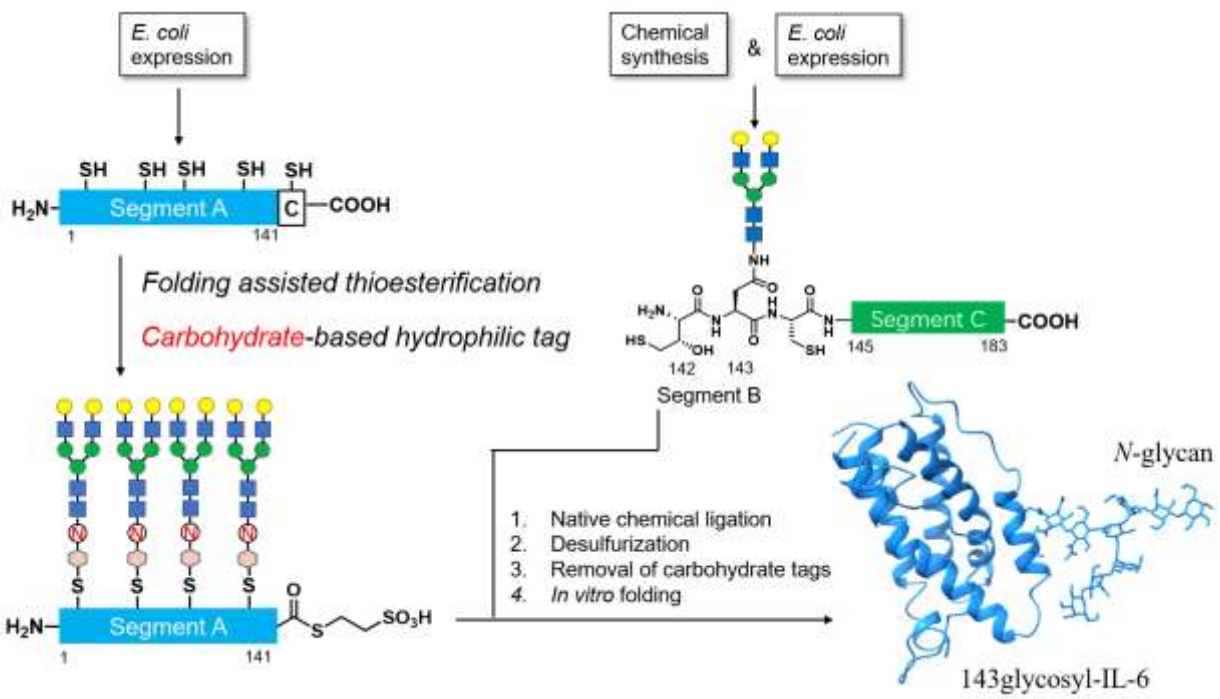
Although the chemical synthesis based on solid-phase peptide synthesis (SPPS) has been widely used to obtain homogeneous glycoproteins with structurally defined *N*-glycans, the SPPS-free semi-synthesis might open a new path toward the synthesis of challenging glycoprotein targets. Herein, we demonstrate a novel bio-inspired semi-synthetic strategy to synthesize a rare glycoform of human interleukin-6 (IL-6) bearing N-glycan at Asn143 without using SPPS. A regioselective activation method for expressed peptide thioesterification was developed based on the folding process of protein. The problem of hydrophobicity and aggregation of IL-6 was solved by using an unprecedent hydrophilic tag based on carbohydrate, which greatly facilitate the ligation reaction and in vitro folding to produce folded 143glycosyl-IL6. The bioassay and MD simulation results suggested that synthetic 143glycosyl-IL6's employed suitable bioactive-conformation, although the wrong conformation around 143 position of IL6 was reported owing to non-glycosylation. We believe that this novel semi-synthetic strategy combining a novel thioesterification method and hydrophilic tag would provide facile access toward homogeneous glycoproteins, which is essential for elucidation of glycoprotein-structure and glycan function.[1]



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Precision Glycosaminoglycans From Living Polymerization

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The polysaccharides with precise compositions, sequences and lengths have a significant impact on their biological and physical properties. However, obtaining high-molecular-weight and structurally well-defined polysaccharides through precise preparation remains a formidable challenge in the fields of polymer chemistry and glycoscience. It is difficult to thoroughly understand their structure-function relationship for quite a long time. This work introduced a strategy to realize the living polymerization of heteropolysaccharides preparation, including glycosaminoglycans and its proteoglycans, with high molecular weight and diverse architectural forms, through the designed dormant substates as polymerization initiators. We designed and synthesized a series of substrates or initiators in living polymerization. Our method afforded a facile and versatile pathway to prepare medium to ultra-high molecular weight (600-1000 kg mol^{-1}) glycosaminoglycans with narrow dispersity. Glycosaminoglycans with precisely distributed N_3 or *N*-TFA in up to nine-block were prepared, with the minimum block being at least 10 kg mol^{-1}. Meanwhile, proteoglycans could also be prepared from the core protein backbone by in situ living polymerization, and the highest molecular weight could be reached in the millions. This approach will facilitate the establishment of a structure-function relationship linking structural parameters, such as molecular weight, to their material properties and biological functionalities. The development of biomaterials based on structurally well-defined polysaccharides by living polymerization not only propels the field of polymer synthesis forward, but also enhances our comprehension of the biological functions and regulatory mechanisms underlying their sequences.

Keywords: glycosaminoglycans, precise synthesis, living polymerization

Divergent Synthesis Of Linkage-Editing A-Galactosylceramides: A Strategy To Create Pseudo-Glycans With Altered Biological Activities

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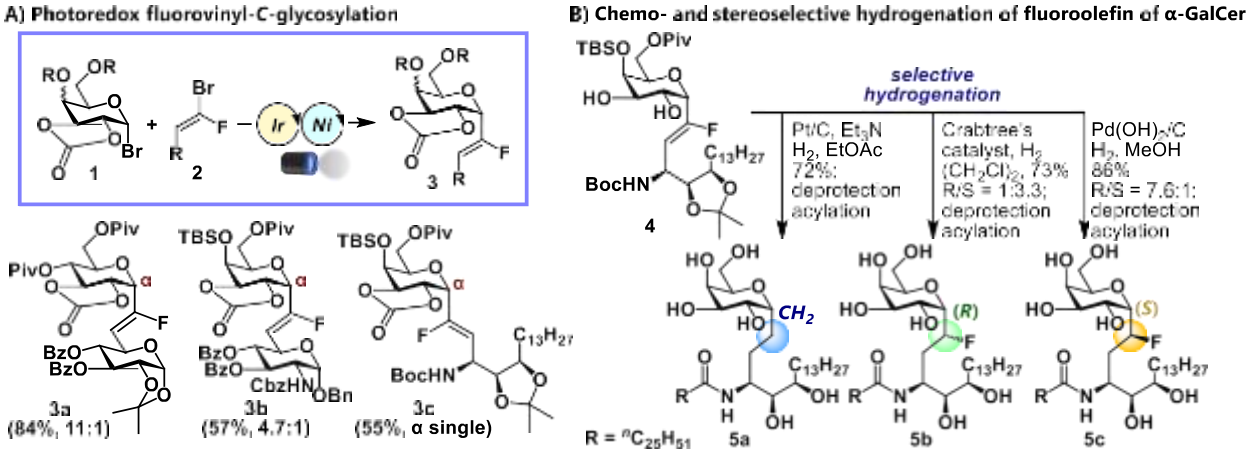
The glycans consist of saccharide and aglycone moieties, which are interconnected through glycosidic linkage. While combination of readily available saccharides and aglycones provides a wide range of chemical space for glycans, the diversity of linkage structure is limited. Instead of native *O*-glycoside, we have developed three different *C*-linked analogs, CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycosides, that are not cleaved by intracellular glycoside hydrolases.[1,2] While *O*-glycosides and CHF-glycosides occupy distinct conformations regulated by the exo-anomeric effect or the fluorine gauche effect, respectively, the conformation of CH_2-glycosides is not regulated by stereoelectronic effect, resulting in a more flexible conformation. These different conformational properties could give rise to new pseudo-glycans with different biological activities. To demonstrate the altered biological function of these linkage-edited glycan analogs, we synthesized various C-linked analogs of bioactive glycans, such as isomaltose,[2] α-galactosylceramide (α-GalCer),[2,3] melibiosamine,[4] and evaluated their biological activities.

In this presentation, we present the synthesis and biological evaluation of C-linked α-GalCer analogs. The first crucial step in the divergent synthesis is metallaphotoredox-catalyzed coupling reaction of glycosyl bromide **1** and bromofluoroolefin (BFO, **2**). This method proved effective in producing a variety of fluorovinyl-*C*-glycosides including isomaltose **3a**, melibiosamine **3b**, and α-galactosylceramide **3c**, with good yield and α-selectivity. Upon removing the carbonate group, fluoroolefin **4** underwent a catalyst-controlled chemo- and stereoselective hydrogenation, enabling the divergent synthesis of CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycosides **5a-c**. We evaluated adjuvant activities, cytokine production, and iNKT cell activation activities of C-linked α-GalCer analogs **5a-c** and native *O*-linked α-GalCer, revealing that (*R*)-CHF-glycoside **5b** acts as an antagonist of iNKT cell activation.



Keywords: C-glycoside, pseudo-glycan, glycolipid, α-GalCer, photoredox

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Unexpected Formation Of Furanose Form During Deacetylation Of Pyranose Gluco-Oxazoline

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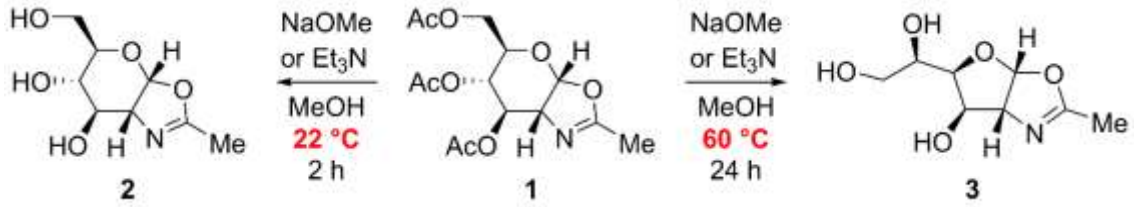
Since *N*-acetyl-D-glucosamine in the pyranose form is one of the most frequently encountered hexosamines in biologically important glycoconjugates a variety of methods for chemical synthesis of its glycosides have been developed.[1] Recently, there has been an increasing interest in glyco-oxazolines functioning as glycosyl donors in glycosylation reactions.[2, 3]

We have found that during conventional deacetylation of well-known (3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**1**)[4] with MeONa in MeOH at ambient temperature a previously unknown furanose isomer of gluco-oxazoline triol **3** is formed along with the expected pyranose form of gluco-oxazoline triol **2** (Scheme 1). Similarly, treatment of **1** with methanolic Et_3N leads to formation of both **2** and **3** in variable amounts. Importantly, prolonged reaction times or higher temperatures increase the share of the furanose form **3** in the reaction mixture, suggesting that this isomer is more thermodynamically stable. The reaction can be selectively directed to pyranose **2** or furanose **3** by appropriate choice of reaction conditions. Thus, after 2 h at 22 °C pyranose form of gluco-oxazoline triol **2** is formed exclusively, while prolonged heating at 60 °C (24 h) cleanly gives furanose form of gluco-oxazoline triol **3**. Both isomers of gluco-oxazoline triol **2** and **3** were isolated in quantitative yields without admixture of the other isomer and their structures established by 2D NMR spectroscopy and high-resolution mass spectrometry. The structure of pyranose isomer of gluco-oxazoline triol **2** was additionally confirmed by its transformation to the starting acetate **1** under acetylation conditions (Ac_2O, Py).

A possibility of preparation of unprotected furanose form of gluco-oxazoline **3** opens a new pathway to poorly studied (see[5-10] and references cited therein) furanose forms of glyco-oxazolines with various *O*-protective groups. Investigation of the prospects of the use of derivatives of furanose oxazoline **3** in glycosylation reactions is currently underway in our laboratory.



Scheme 1. Deacetylation of oxazoline **1** to give the gluco-oxazoline triol in pyranose (**2**) or furanose (**3**) forms.

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Stereoselective 1,2-*Cis*-Glucosylations

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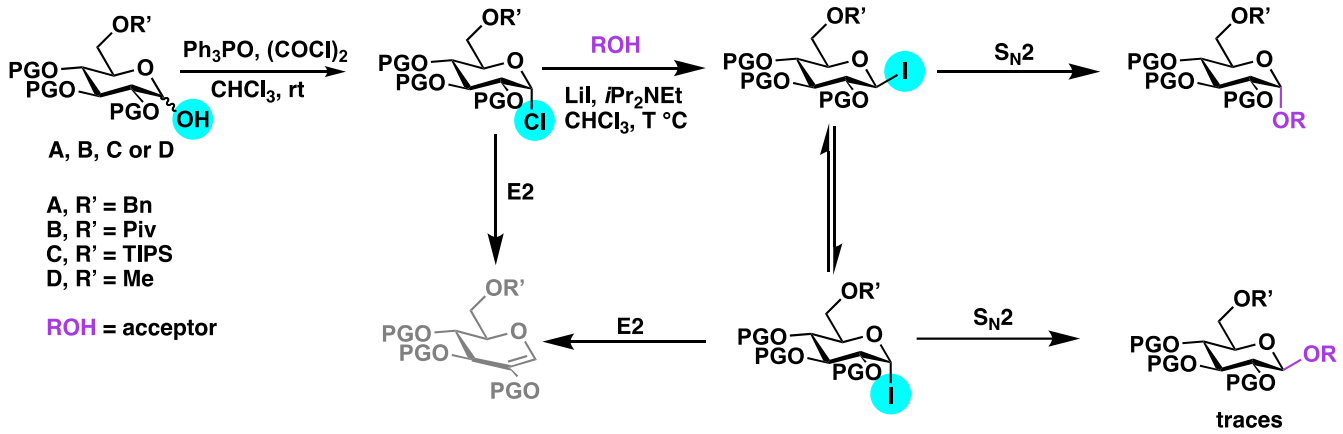
Oligosaccharide synthesis, consisting of multiple glycosylation steps, poses many difficulties with respect to regio- and stereoselectivity[1]. Depending on the reaction conditions, 1,2-*cis*- or 1,2-*trans*-glycosides can be obtained, of which the former are usually more difficult to synthesize. Previously, the McGarrigle group reported access to 1,2-*cis*-glycosides, by treatment of the glycosyl hemiacetal donor with Denton's catalytic Appel conditions[2,3], followed by reaction with LiI, *i*Pr$_2$NEt and the acceptor[4]. This procedure was successfully applied to the stereoselective synthesis of β-mannosides and β-rhamnosides.

In contrast to β-mannosides and β-rhamnosides, we will describe how glucosyl hemiacetal donors give α-glucosides. Glucosyl hemiacetal donors and a range of acceptors have been tested (**Scheme 1**). Optimization studies were required to prevent unwanted elimination of the glycosyl iodide intermediate to form the corresponding glucal side product (**Scheme 1**, grey). Changing the rate of addition of base *i*Pr$_2$NEt was found to limit the formation of the side product, affording an increase in the acceptor conversion, and still with an excellent *α/β* selectivity. To demonstrate the usefulness of the method, a target pentasaccharide was also synthesized using these conditions[5].



Scheme 1. General scheme for the stereoselective synthesis of α-glucosides.

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Chemical Synthesis Of The Trisaccharide Repeating Unit Of The O-Antigen Of *Fusobacterium Nucleatum* Atcc 51191

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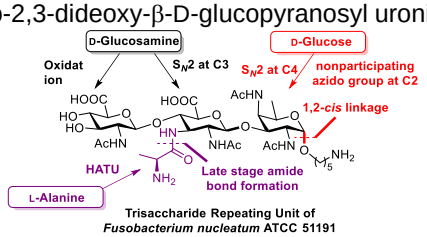
Fusobacterium nucleatum, a Gram-negative bacterium, is an opportunistic pathogen of the oral microbiota. Recent studies have shown that it was associated with various human diseases, including periodontitis, halitosis, chorioamnionitis, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease. In addition, increasing evidence points toward a key role of *F. nucleatum* in cancer, particularly colorectal cancer (CRC) and oral squamous cell carcinoma (OSCC). Studies have demonstrated that *F. nucleatum* can cause tumor cell proliferation and chemotherapy resistance, resulting in poor prognosis. It is a therapeutic target for CRC and is treated with antibiotics such as metronidazole. However, antibiotic resistance and the threat to normal beneficial flora in the gut make the development of vaccines particularly attractive. The outer membrane of *F. nucleatum* is mainly composed of lipopolysaccharide (LPS), which is a potent elicitor of innate immune responses and an important target for vaccine development. Hence, the trisaccharide repeating unit (RU) of the LPS present on the surface of *F. nucleatum* ssp. *animalis* ATCC 51191 is a potential vaccine candidate. The structure of the trisaccharide RU was established as [→4)-β-D-Glc*p*NAcA-(1→4)-β-D-Glc*p*NAc3NAlaA-(1→3)-α-D-Fuc*p*NAc4NR-(1→], R = Acetyl (60%) or H, and contains three special units: 2,4-dideoxy-diacetamino-fucose, 3-NAla-2-acetamido-2,3-dideoxy-β-D-glucopyranosyl uronic acid, and 2-acetamido glucuronic acid.



The target molecule could be obtained from fully protected trisaccharide via post-glycosylation modification (amidation, removal of temporary protective group, oxidation), and hydrogenolysis would convert all trichloroacetamido (TCA) groups to acetamido groups and remove all benzyl (Bn) and benzyloxycarbonyl (Cbz) groups. Bis(trichloroacetamido)-D-fucose linker-appended acceptor was synthesized from known benzylidene-protected 3-(2-naphthylmethyl)-2-azido-2-deoxyselenoglycoside after 11-steps reactions in 14% overall yield. The observed α-stereoselectivity in this case may be attributed to the presence of the bulky phthalimide group at the C4 position in the axial orientation, which may hinder β face attack, or involve in the remote participation in fucosylation. By using a [2+1] glycosylation strategy to improve acceptor reactivity, the target trisaccharide was synthesized in high yield. The L-Ala was efficiently coupled by using HATU as an activating reagent. In addition, the target molecule was installed with an aminopentyl linker at the reducing end to enable conjugation with a carrier protein for further immunological evaluation.

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Reaction Rate And Stereoselectivity Enhancement In Glycosidations Due To Catalysis By A Lewis Acid–Nitrile Cooperative Effect

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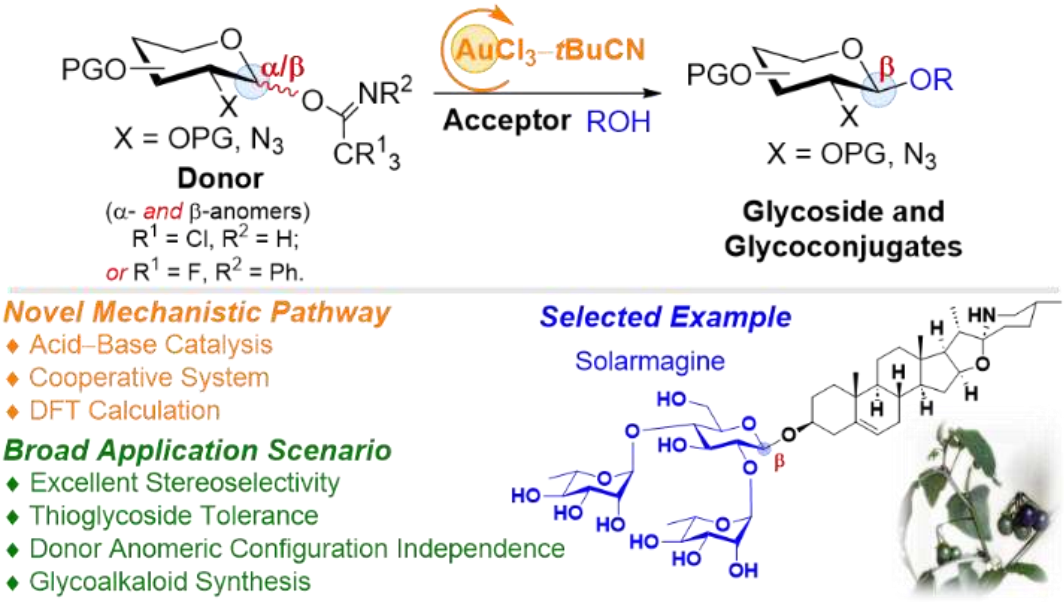
Activation of O-glycosyl trihaloacetimidate glycosyl donors with $AuCl_3$ as a catalyst and pivalonitrile (*t*BuCN) as a ligand led to excellent glycosidation results in terms of yield and anomeric selectivity. This way, various β-D-gluco- and β-D-galactopyranosides were obtained conveniently and efficiently. Experimental studies and density functional theory (DFT) calculations, in order to elucidate the reaction course, support formation of the tBuCN−$AuCl_2$−OR(H)$^+$ $AuCl_4$ complex as decisive intermediate in the glycosidation event. Proton transfer from this acceptor complex to the imidate nitrogen leads to donor activation. This way, guided by the C-2 configuration of the glycosyl donor, the alignment of the acceptor complex enforces the stereoselective β-glycoside formation in an intramolecular fashion, thus promoting also a fast reaction course. The high stereocontrol of this novel 'Lewis acid−nitrile cooperative effect' is independent of the glycosyl donor anomeric configuration and without the support of neighboring group or remote group participation. The power of the methodology is shown by a successful glycoalkaloid solamargine synthesis.



Assessment Of All Configurational Isomers Of The 3,4,5-Trihydroxypiperidine With Their *N*-Alkylated Derivatives As Gcs, Gba And Gba2 Inhibitors

Qiang Ma, Maria J. Ferraz, Richard J. B. H. N. van den Berg, Johannes M. F. G. Aerts and Hermen S. Overkleeft

1-Deoxynojirimycin (DNJ) is a biologically active natural compound, from which many isomers and derivatives have been studied for their biological potential,[1] including inhibition of the three glucosylceramide processing enzymes, glucosylceramide synthase (GCS), lysosomal glucosylceramidase (GBA) and non-lysosomal glucosylceramidase (GBA2). Recently, there has been reported that the hydroxymethyl group of DNJ is crucial to neither potency nor selectivity of β-glucosidase.[2] Additionally, GBA inhibition is improved after removing this sidechain of DNJ.[3] With the aim of developing more potent and selective inhibitors of these three glucosylceramide processing enzymes, this project described the synthesis and biological evaluation of in total 4 configurational isomers of 3,4,5-trihydroxylpiperidines, as well as their *N*-alkylated derivatives. Screening the library against the three enzymes indicates that the hydroxymethyl group is not crucial to their inhibition potency but affects the selectivity toward GCS/GBA2 over GBA. In addition, this inhibition profile confirms that appropriately substituted DL-*glu*/DL-*ido* configured 3,4,5-trihydroxylpiperidines are still effective as dual GCS/GBA2 inhibitors, which have been studied as putative therapeutics for the treatment of the lysosomal storage disorder, Gaucher disease. We believe that our finding here could contribute to the design of next-generation inhibitors of glucosylceramide processing enzymes.

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Stereocontrolled Synthesis Of *α*-3-Deoxy-D-manno-oct-2-ulosonic Acid (*α*-Kdo) Glycosides Using C3-*p*-Tolylthio-Substituted Kdo Donors

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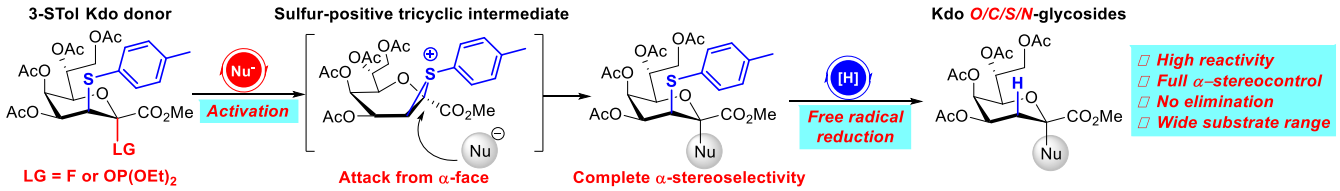
3-Deoxy-D-manno-2-octulosonic acid (Kdo) is an eight-carbon monosaccharide widely distributed in bacterial lipopolysaccharides and capsule polysaccharides, and has the potential to be used as new vaccines or immunization reagents. It has been demonstrated through crystal structure that Kdo-containing units in LPS inner core can bind to Toll-like receptors/MD-2 complex. However, Kdo glycosylation is one of the most challenging glycosylation reactions because of the low reactivity, the uncontrolled stereoselectivity and the bulk formation of 2,3-ene byproducts. The highly efficient and stereoselective synthesis of Kdo glycosides is still an unsolved problem in glycosylation methodology, which limits the biology studies of Kdo-containing oligosaccharides.

Recently, we developed an indirect methodology for the stereoselective synthesis of *α*-Kdo glycosides with C3-*p*-tolylthio-substituted Kdo donors (Scheme 1).[1, 2] The presence of the *p*-tolylthio group enhanced the reactivity, suppressed the formation of elimination by-products (2,3-enes), and provided complete *α*-stereocontrol. A variety of Kdo *α*-glycosides were synthesized by our method in excellent yields (up to 98%). After glycosylation, *p*-tolylthio group can be efficiently removed by the free radical reduction. Subsequently, the orthogonality of phosphite donor and thioglycoside donor was demonstrated by the one-pot synthesis of a trisaccharide in *Helicobacter pylori* and *Neisseria meningitidis* LPS. Moreover, an efficient total synthesis route for the challenging 4,5-branched Kdo trisaccharide in LPS from several *A. baumannii* strains was highlighted. To demonstrate the high reactivity of our approach further, the highly crowded 4,5,7,8-branched Kdo pentasaccharide was synthesized as a model molecule for the first time. Additionally, the reaction mechanism was investigated by the density functional theory (DFT) calculation. In this report, we present the latest findings from recent research.



Scheme 1. Stereocontrolled synthesis of *α*-3-deoxy-D-manno-oct-2-ulosonic acid (*α*-Kdo) glycosides using C3-*p*-tolylthio-substituted Kdo donors.

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Towards Efficient Electrochemical Synthesis Of Cyclic (1,3;1,6)-β-Glucan Dodecasaccharide

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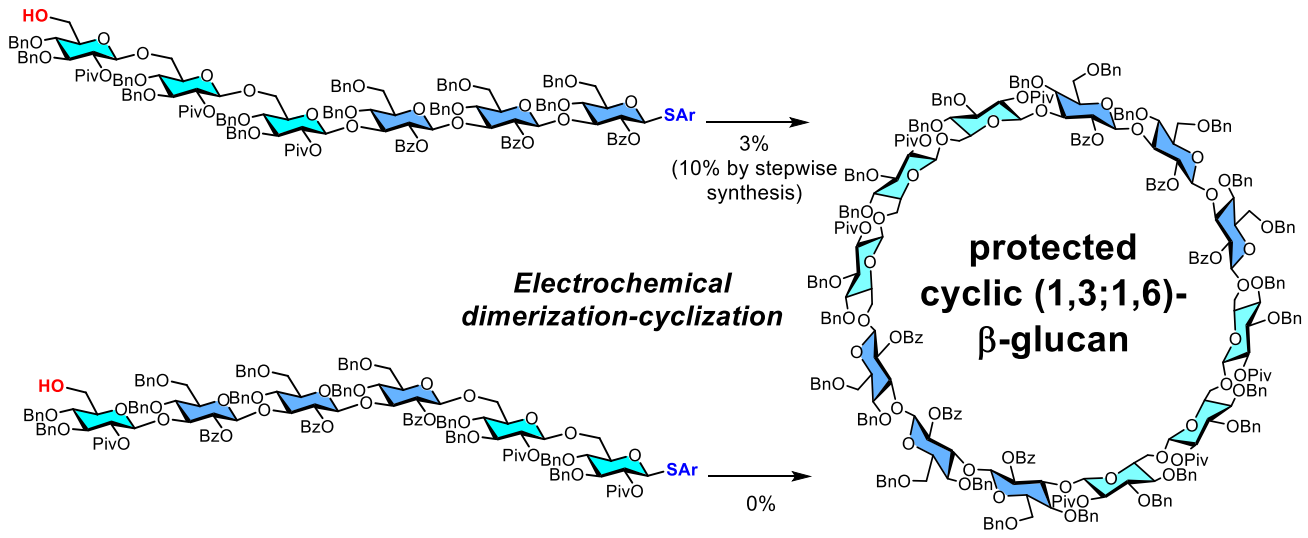
Electrochemical glycosylation[1] pioneered by Noyori is a powerful method to synthesize oligosaccharides including unnatural cyclic oligosaccharides.[2,3] We have also been interested in synthesizing natural cyclic oligosaccharides such as cyclic (1,3;1,6)-β-glucans[4] which have different glycosidic linkages; however, the potential precursor hexasaccharide was hard to make in large scale.[5] Recently, we have synthesized a hexasaccharide and provided for the one-pot synthesis under the electrochemical dimerization-cyclization condition.[6] Although the yield was only 3% (10% by stepwise synthesis), we obtained the corresponding protected cyclic dodecasaccharide. To our surprise hexasaccharides with a similar structure did not afford the cyclic oligosaccharide at all. Both hexasaccharides have protecting-group-free 6-OH and the same thioaryl leaving group (Ar = 4-chlorophenyl). Therefore, we have prepared model disaccharides for two hexasaccharides and compered their oxidation potentials[7] and relative reactivity values (RRVs)[8] to elucidate the origin of their reactivity difference.



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Chemoenzymatic Synthesis Of O- And N-glycans

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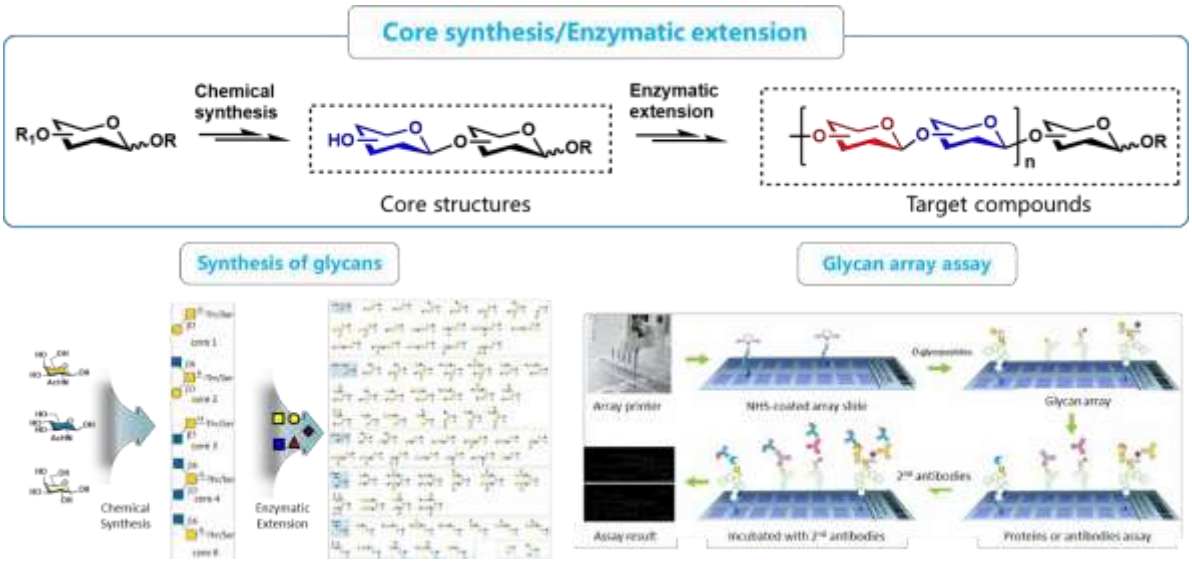
Glycans, recognized as the 'third chain of life' beyond nucleic acids and proteins, play pivotal roles in physiological processes. The efficient synthesis of glycans stands as a fundamental challenge in the field of glycoscience, demanding innovative solutions. We developed different chemoenzymatic methods for the synthesis of O- and N-glycans. Firstly, we developed a 'core synthesis, enzymatic extension' strategy for the assembly of O-glycans. This strategy enables the chemical synthesis of simple core structures and enzymatic elongation of complex glycan epitopes, combining the advantages of chemical and enzymatic methods. Using this approach, over 150 O-glycans (O-Man and O-GalNAc glycans) and 40 N-glycans were assembled. The function of these glycans was further elucidated using a glycan microarray. Secondly, we expanded the application of glycosyltransferases in the glycan analysis field, furnishing robust tools for decrypting glycomic data and probing the functional and regulatory paradigms of glycans in essential biological processes. Over 30 N-glycopeptides, with or without stable isotope-labeled fucose, were synthesized and applied for the absolute quantification of N-glycans in serum antibodies



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DE-Epimerization Of Monosaccharides By Dynamic Kinetic Resolution: Ligand-Controlled Synthesis Of *O*-Aryl-Glycosides Through Copper-Catalyzed Cross-Coupling

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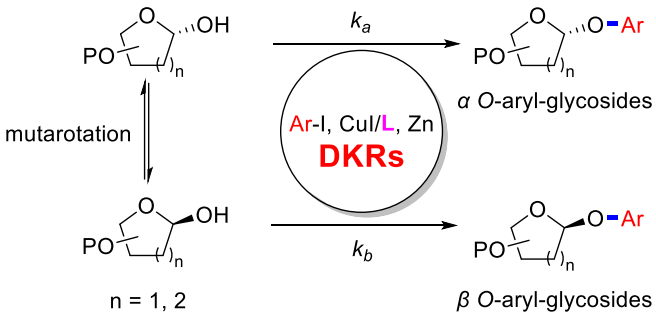
Glycosylation plays a crucial role in carbohydrate chemistry and pharmaceutical chemistry, which can modulate the physiological properties and functional modes of monosaccharide and glycoconjugates, with improved metabolic stability, membrane permeability and biodistribution.[1] Though many methods for the glycosylation have been developed, stereoselective glycosylation remains one of the most important topics in carbohydrate chemistry.

We reported a strategy for stereoselective *O*-aryl-glycoside synthesis by copper-catalyzed cross-coupling[2] of a variety of anomeric sugars and (hetero)aromatic iodides. Stereocontrol of the α/β selectivity can be successfully realized by slight structural modifications of the oxalic diamide ligands. Mechanistic studies indicated a dynamic kinetic resolution (DKR) reaction mechanism controlled by the ligand structures. This reaction could be performed on gram scale, and has also been applied to the synthesis of some natural products.[3]



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A Simple Synthetic Strategy: Concise Access To Isoiminosugars And Beyond

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Isoiminosugars (**A**) are glycomimetics in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group [1]. In general, these compounds are selective and highly potent inhibitors of glycoside hydrolases [2-4]. Moreover, *C*-5a-chain elongated derivatives of this compound class, i.e. *C*-5a-chain extended entities (**2**) of 4-*epi*-isofagomine (4-*epi*-IFG, **1**) have been proven as highly potent pharmacological chaperones for the treatment of GM1 gangliosidosis [2,3]. As a matter of fact, the indicated structural characteristics of isoiminosugars (**A**) remain synthetically challenging. However, valuable synthetic strategies towards this compound class have been reported [2-4]. In context with our interest in the design and synthesis of such structures, we have found an efficient and concise synthetic approach towards isoiminosugars (**A**). This strategy relies on a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated glycopyranoses (**I**) leading to corresponding C-2 carbon chain branched glycofuranosides (**II**) [5]. We applied this ring contraction for the synthesis of isoiminosugars (**A**). Employing different configurations of **I** and variations in the reaction sequence open the avenue to various modifications in the substitution pattern. Herein, synthetic and mechanistic details as well as the scope and limitations of this approach will be presented.

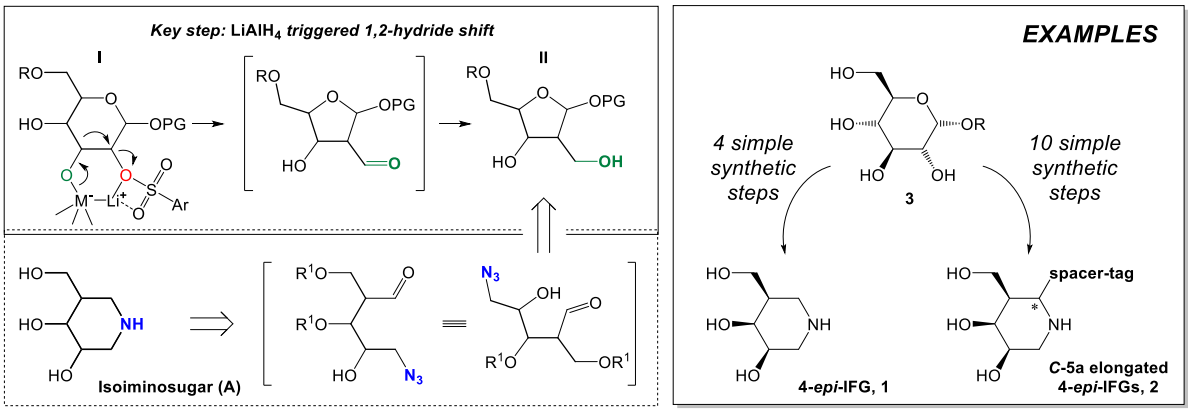

Figure: Schematic overview of a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated pyranosides (**I**) as key-step in our general (retro-) synthetic concept for isoiminosugars (**A**) (left), and its demonstration in a simple 4 step synthesis of 4-*epi*-isofagomine (**1**) and according conversions to *C*-5a elongated derivatives (**2**) starting from common α-D-glucopyranosides (**3**) respectively (right).

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Direct Site-Specific Detailed Glycan Characterization By Higher Energy Electron Activated Dissociation Tandem Mass Spectrometry

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Glycosylation is recognized as the most complex post-translational modification due to the presence of macro- and micro-heterogeneities, with its biological function influenced by the glycosylation site and the glycan fine structure[1]. Site-specific detailed glycan characterization remains a major challenge by existing tandem mass spectrometry (MS/MS) methods, where collision-based dissociation produces predominantly glycosidic cleavages and electron capture or transfer dissociation generates mostly peptide backbone cleavages[2]. Alternative approaches are therefore needed to achieve simultaneous site localization and glycan structural assignment at the intact glycopeptide level. Here, higher energy electron activated dissociation (heExD) MS/MS, in particular electronic excitation dissociation (EED), demonstrated extraordinary potential for in-depth N-glycopeptide sequencing by providing information on the glycosylation site, peptide sequence, glycan topology and linkages in one analysis[1,3]. Higher energy collision-induced dissociation (HCD) and electron-transfer/higher-energy collisional dissociation (EThcD) MS/MS spectra were acquired on an Orbitrap Lumos mass spectrometer (Thermo Scientific). All other ExD MS/MS experiments were performed on a Q Exactive HF instrument (Thermo Scientific) modified with an Omnitrap platform (Fasmatech). Manual data interpretation was achieved using the Glycoworkbench software and a home-built program. In this work, we have performed a systematic investigation of the effect of electron energy and charge carriers on the ExD fragmentation behaviors of N-linked glycopeptides bearing different glycan structures, including high-mannose glycans and sialylated glycans. Overall, we have found that, for site-specific glycopeptide analysis, EED outperforms all other fragmentation methods tested here, including HCD, sceHCD, ETD, EThcD, and low-energy ECD, as it can reveal the peptide sequence, determine the glycosylation site, and elucidate the glycan topology and the linkages, in a single MS/MS experiment. The exceptional EED efficiency achieved on the Omnitrap instrument, with a typical irradiation time of under two hundred milliseconds, makes EED MS/MS compatible with on-line LC separation, which could provide a powerful tool for structure-focused glycoproteomics studies.

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Application Of Photocatalytic Dehydrolanine Conjugate Addition Reaction In Glycopeptide Synthesis

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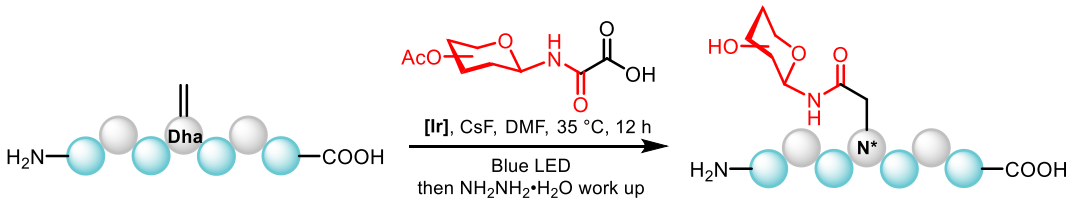
The glycosylation of proteins and peptides profoundly impacts their intrinsic properties and plays a crucial role in biological processes. [1] Chemical synthesis is an effective method for obtaining homogeneous glycopeptides. We report the first visible-light-driven radical conjugate addition of N-glycosyl oxamic acid to dehydroalanine derivatives, generating glycopeptides in good yields. N-glycosyl oxamic acids are readily accessible radical precursors that can be converted to carbamoyl radicals via photoredox reaction under mild conditions. Dehydroalanine, an unnatural amino acid with good biocompatibility, can be introduced into peptides and proteins. Due to its reactivity, dehydroalanine is often selected for chemical modification of proteins and peptides. [2] Utilizing these two substrates, we synthesized a series of glycosylated peptides in good yields, providing a novel strategy for glycopeptide synthesis. This methodology offers a mild, efficient, and potentially scalable approach using visible light as the driving force, aligning with green chemistry principles. The compatibility of dehydroalanine with biological systems enables exploring the effects of glycosylation on protein and peptide properties in vivo.



Peptides containing dehydroalanine

Peptides containing glycosylation

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Enhancing Versatile And Stereoselective N-Glycosylation: A Radical Activation Strategy

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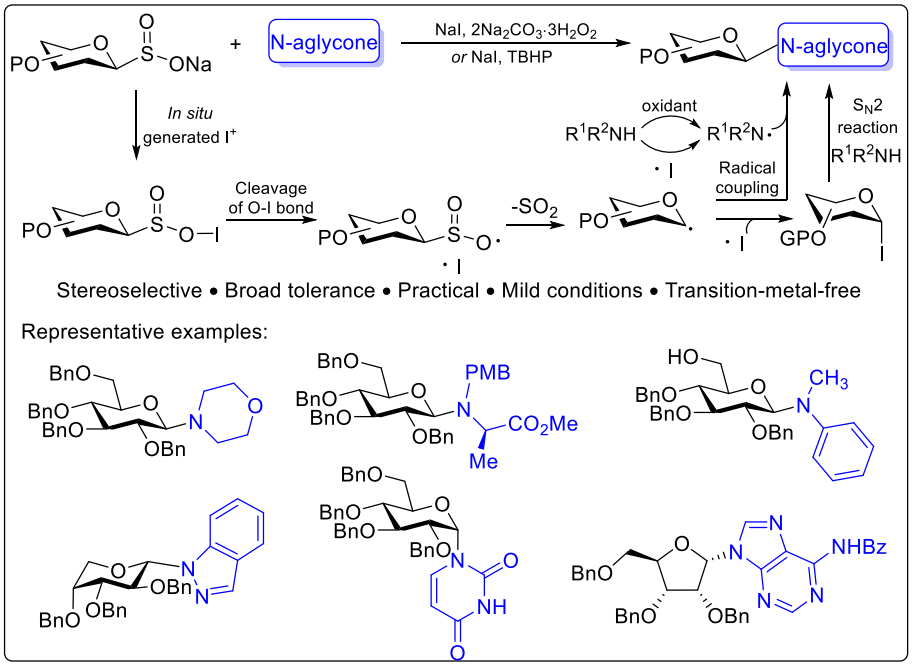
Abstract: Prior N-glycosylation predominately proceeded under acidic conditions, facing challenges of low stereoselectivity and limited scope. Herein, we introduce a radical activation strategy that enables versatile and stereoselective N-glycosylation using readily accessible glycosyl sulfinate as a donor under basic conditions, exhibiting exceptional tolerances towards various N-aglycons, encompassing alkyl, aryl, heteroaryl and nucleobase functionalities. Preliminary mechanistic studies indicate a pivotal role of iodide, orchestrating the formation of glycosyl radical from glycosyl sulfinate and subsequent generation of the key intermediate, configurationally well-defined glycosyl iodide, which was subsequently attacked by N-aglycones in a stereospecific SN2 manner to give the desired N-glycosides. Additionally, an alternative route involving the coupling of a glycosyl radical and a nitrogen-centered radical is also proposed, affording the exclusive 1,2-trans product. This novel approach promises to broaden the synthetic landscape of N-glycosides, offering a powerful tool for the construction of complex glycosidic structures under mild conditions.



Highly Regio-/Stereoselective Synthesis Of Carbohydrates With Unsaturated Glycosyl Donors Under Mild Conditions

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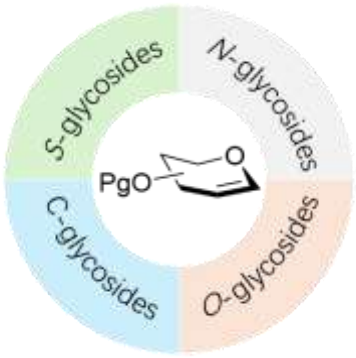
Carbohydrates and their conjugates play important roles in life activities and drug development. Our group was committed to the general and effective glycosylation methods and their application in chemical biology using unsaturated glycosyl donors by transition metal catalysis. In the past five years, we have reported some synthetic strategies with high stereoselectivity and milder conditions compared with conventional reported methods. In particular, high chemo-/regio- and stereoselective *O*-glycosylation, *C*-glycosylation and *S*-glycosylation could be achieved via palladium catalysis under open-air conditions at room temperature. In this work, we introduced our research progress in constructing four types of glycosides: *O*-, *C*-, *N*- and *S*-glycosides.



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Automated Solution-Phase Multiplicative Synthesis Of Complex Glycans Up To A 1080-Mer

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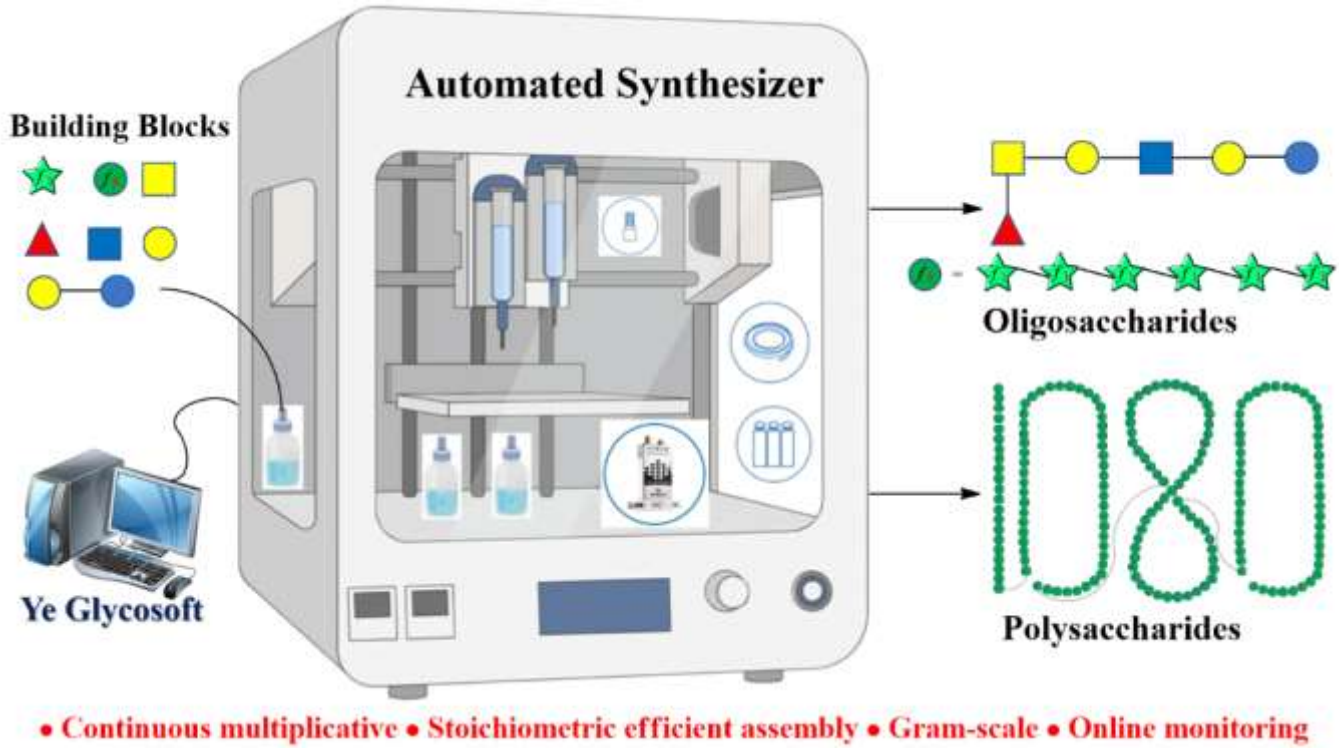
Carbohydrates play essential roles in nature, such as in cell-cell communication, cell growth and immunoresponse. However, the synthesis of structurally well-defined carbohydrates, especially large-sized glycans, is a challenging task. We herein report an automated solution-phase multiplicative synthesis of complex glycans enabled by preactivation-based, multi-component, one-pot glycosylation and continuous multiplying amplification. This was achieved by making a dual-mode automated solution-phase glycan synthesizer. Using this synthesizer, a library of oligosaccharides covering various glycoforms and glycosidic linkages was assembled rapidly, either in the general promoter-activation mode or in the light-induced activation mode. The automated synthesis of a fully-protected Fondaparinux pentasaccharide (an anticoagulant) was realized on gram-scale. Furthermore, automated ten-component tandem reactions were performed successfully. Finally, the automated multiplicative synthesis of complex polysaccharides up to a surprising 1080-mer was achieved, taking glycan synthesis to the new height far beyond the synthesis of nucleic acids (up to 200-mer) and proteins (up to 472-mer).[1] This automated platform will not only benefit non-specialists who will be able to assemble target glycans, but it can also be applied to the streamlined synthesis of other organic molecules of interest.[2-3]



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Palladium-Catalyzed Stereospecific Glycosylation With 3,4-Carbonate Glycal Donors: Access To Diverse Glycoside Scaffolds

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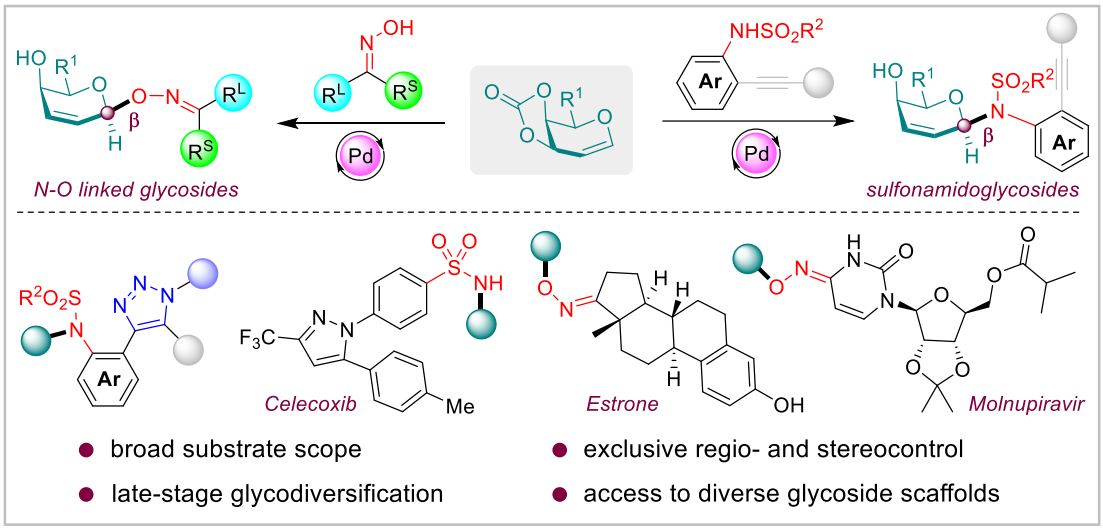
Carbohydrates play pivotal roles in numerous physiological/pathological processes and have important applications in modern drug research and development. Therefore, we are dedicated to developing novel stereoselective glycosylation methods and strategies and applying them to the efficient synthesis of complex oligosaccharides and glycoconjugates. In recent years, transition-metal catalyzed glycosylation has emerged as a powerful tool for constructing glycosidic bonds due to its versatility and robustness.[1,2] Herein, we present a highly efficient, palladium-catalyzed stereospecific glycosylation between 3,4-O-carbonate glycals and oximes/sulfonamides.[3,4] This approach features broad substrate scope, high functional group tolerance, and easy scalability, delivering a wide range of glycosyl oximes/sulfonamides in excellent yields with exclusive β-selectivity. The power of this method is demonstrated by a set of site-selective transformations of glycosylation products and late-stage functionalization of pharmaceutically relevant molecules. Overall, our strategy provides an efficient toolkit for facile access to diverse N−O-linked glycosides and N-glycosylated sulfonamides, providing excellent opportunities for their in-depth biological evaluations.



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Direct Radical Functionalization Of Native Sugars

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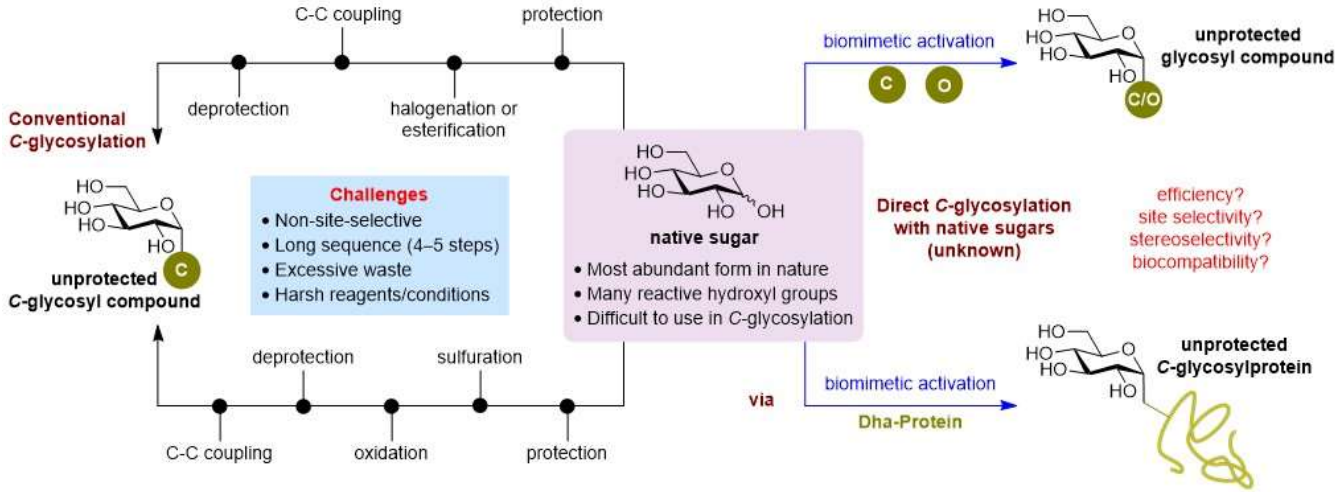
Native sugars encompass multiple nucleophilic and Brønsted acidic hydroxyl groups of similar reactivity in heterolytic (two-electron) chemistries[1,2]. Current methods therefore hinge on multi-step protecting-group manipulation strategies[3] to convert these natural feedstocks into applicable glycosylating reagents for further derivatizations, which are intrinsically inefficient in practice. Direct transformation of native sugars to complex glycosides as a straightforward and practical alternative yet remains an insurmountable challenge. Herein, we demonstrate a biomimetic activation approach to achieve site- and stereo-selective chemical glycosylation of fully unprotected natural sugar feedstocks, through homolytic (one-electron) chemistry. The transformation process mimics enzymatic glycosylation in nature[4] through chemoselective generation of a transient glycosyl donor, which could undergo diversified radical glycosylation upon photoactivation. Such a strategy eliminates laborious hydroxyl group masking and manipulation, as well as expands the repertoire of glycosylation reactions to access metabolically robust glycosyl compounds that are analogous to nature's diversification of natural products. Further biocompatible extension of this strategy to direct post-translational glycosylation of proteins underscores the potential far-reaching application of the established biomimetic activation strategy.

Key words: biomimetic activation, glycosyl radical, unprotected glycosides, glycoprotein, native sugars



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Reaction Of New Sialyl Halides With Meoh And *I*-Proh Without An Added Promoter

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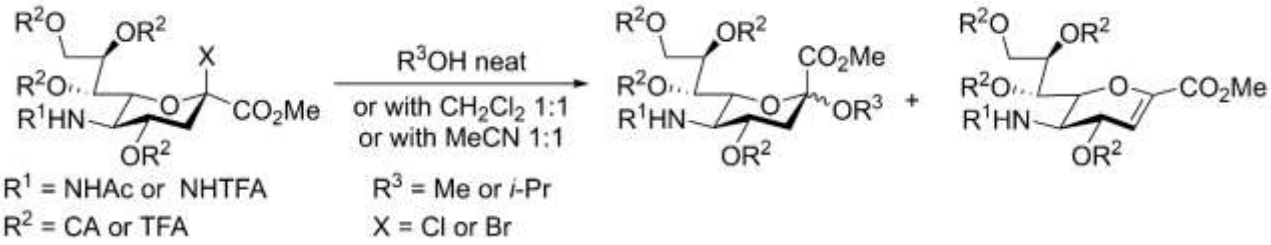
Glycoconjugates containing residues of α-bound sialic acids are omnipresent in various organisms where they are involved in a wide range of biological phenomena. Synthesis and biomedical research of glycoconjugates, oligosaccharides and their analogues containing sialic acid is an important area of research aimed at understanding their biological roles and determining their therapeutic significance.

The synthesis of sialic acid glycosides with the α-configuration is a difficult task. Although it has recently been shown that stereoselective α-sialylation is possible, proceeding through the formation of a glycosyl cation along an S_N1-like route,[1] approaches in which glycosylation proceeds along an S_N2-like route[2,3] are considered more promising.

Here we report the results of reactions of new *N*-acetyl- and *N*-trifluoroacetyl-sialyl chlorides and bromides with *O*-chloroacetyl (CA) and *O*-trifluoroacetyl (TFA) protective groups, recently synthesized by us,[4] with simple alcohols without addition[5,6] of a promoter to estimate their relative reactivities and selectivities (Scheme 1). The sialyl halides could be involved in reactions with MeOH (in case of chlorides and bromides) and *i*-PrOH (only in case of bromides).



Scheme 1. Reactions of sialyl donors with MeOH and *i*-PrOH.

Firstly, we found that bromides were significantly more reactive than chlorides. At the same time, the stereoselectivity of reactions in case of bromides was significantly lower than that of chlorides for similarly substituted derivatives. Secondly, *N*-TFA derivatives gave lower α:β ratios of glycosides than those obtained with *N*-Ac derivatives, but they also gave less glycal (by-product) and were generally less reactive. Thirdly, *O*-TFA groups accelerated the reaction with MeOH compared to *O*-CA groups, but made impossible a reaction with *i*-PrOH (instead of the glycosylation, decomposition of the donor occurred). The high stereoselectivities obtained in some reactions with MeOH (α:β up to 29:1) suggest that in these cases the sialylation seems to proceed along an S_N2-like route, while much lower stereoselectivities obtained in reactions with *i*-PrOH (α:β = 1.3:1–3.9:1) might indicate an increased contribution of an S_N1-like pathway in these cases.

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Indium(III)-Catalyzed Gold-Free Glycosylation Using Alkyne-Based Glycosyl Donors

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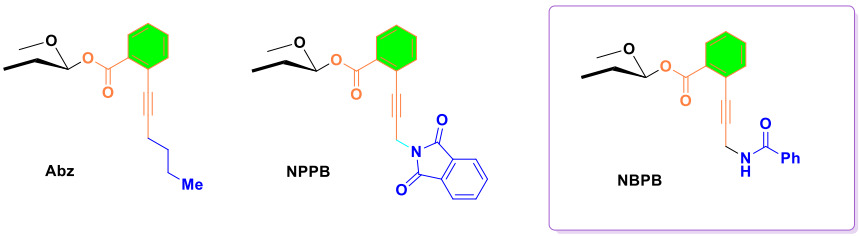
Abstract: Carbohydrates play an important role in the development of new drugs for many diseases, such as tumor, immune regulation, anti-oxidation, anti-virus and so on. The pharmaceutical prospects have greatly promoted the research of carbohydrate chemical synthesis to which the construction of glycosidic bonds is often the key. Since the first chemical synthesis of glycosides in 1879, many glycosylation methods have been developed and used in the synthesis of complex sugar-containing natural products. Among many glycosyl donors, alkynyl-containing donors are an attractive category due to their convenient preparation, stability, and mild orthogonal activation. One representative is glycosyl *ortho*-alkynylbenzoate (Abz), thus far known as Yu's donor, featuring the neutral Au(I) catalysis. This mild catalysis allows the efficient glycosylation of acid labile acceptor and thus the accomplishment of many natural products. However, considering the high cost, it is necessary to develop a safe and economic catalytic system. Recently, a new glycosylation donor, NPPB, has been reported, which can be catalyzed by Cu(II). We have discovered a novel glycosyl donor, based on the installment of N-Propargyl groups, which can be efficiently activated by cheap and readily available $In(OTf)_3$. This promotion condition is orthogonal to that for the previously reported Yu's donor and its recently modified NPPB donors. The mechanistic studies, as well as the applications of new donors in the construction of glycan and glycosyl conjugates are currently in the progress.

Key Words: Glycosylation, In(III) catalysis, novel glycosyl donor



	Au(I)	Cu(II)	In(III)
Abz	✓	✗	✗
NPPB	○	✓	✗
NBPB	○	○	✓

- Inexpensive and readily available catalyst
- Mild catalytic condition
- Shelf-stable donors
- Broad substrate scope

Epitope Mapping Of The Pa Surface Polysaccharide Pel

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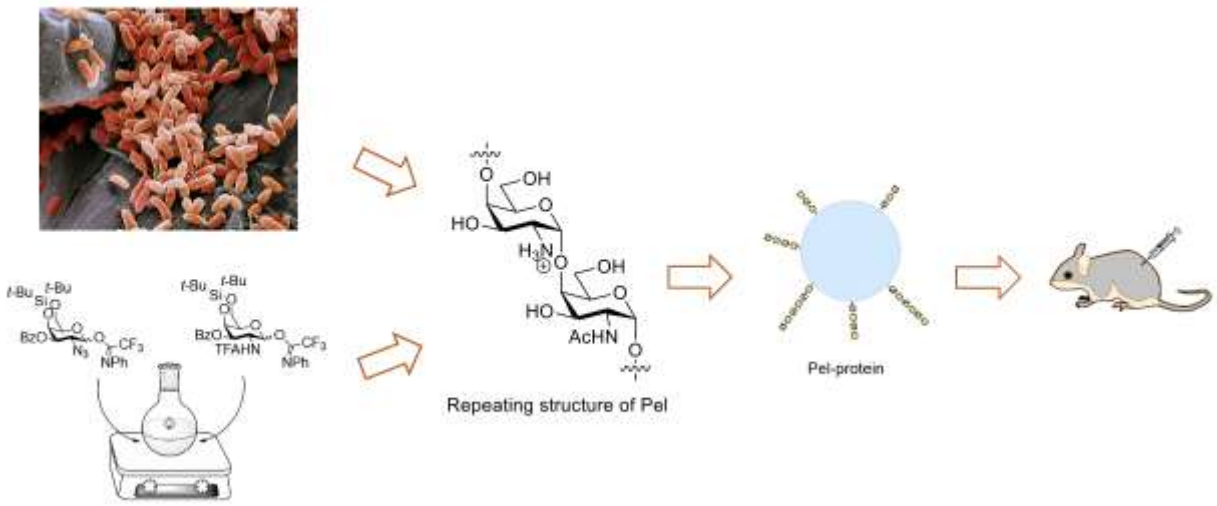
Pseudomonas aeruginosa (PA) is an antimicrobial resistant pathogen responsible for serious infections and it belongs to the ESKAPE pathogens, which are the main cause of nosocomial infections and a growing concern for antibiotic resistance. Developing a vaccine against *P. aeruginosa* is therefore a promising approach. Three exopolysaccharides, which are considered potential targets for bacterial vaccine development, have been identified in the *P. aeruginosa* biofilm: alginate, Psl, and Pel.

The structure of exopolysaccharide Pel has recently been elucidated and is thought to be composed of 1,4-alpha-linked galactosamine and N-acetyl galactosamine[1-2]. Well-defined Pel fragments have been synthesized and will be used as standards for structure elucidation studies, as well as being conjugated to CRM_{197} and BSA for use in immunogenicity studies. Meanwhile, natural Pel will be purified from the *P. aeruginosa* strain for vaccine generation and structural analysis experiments.



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Highly Reactive Glycosylation With 1-*O*-(Thiomethyl)Thiocarbonyl Glycosyides As Donors

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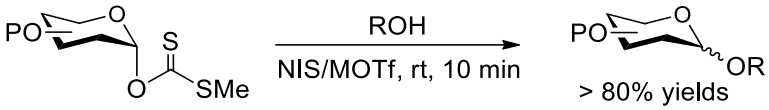

Oligosaccharides and glycoconjugates are widely found in all kinds of biological organisms and play significant roles in biological activities. Glycosylation is the most important and fundamental reaction for synthesizing oligosaccharides and glycoconjugates. Usually, the glycosyl donor defines the glycosylation strategy by which glycosidic bonds are constructed to form glycosylation products. So far, many different types of glycosyl donors have been developed, such as halide glycosyl donors, *O*-imidate glycosyl donors, thioglycosyl donors, alkynyl benzoate glycosyl donors, and phosphate ester glycosyl donors, etc.[1-3] Although they each have their own advantages, glycosyl tricholoroacetimidate are the preferred donors for glycochemists because of their simplicity of preparation, mild glycosylation reaction conditions and high reactivity. However, glycosyl tricholoroacetimidate donors are not shelf-stable and sometimes form stable trichloroacetamide by-products by rearrangements during glycosylation, being their major drawbacks.[2] Therefore, there is still a need to develop a new type of glycosyl donor that is simple to synthesize, highly active without by-products, and shelf-stable. Herein we have developed a glycosylation method using 1-*O*-(thiomethyl)thiocarbonyl glycosides as donors. Such donors were synthesized in a simple and structurally stable manner. This catalytic glycosylation system is easy to operate, conducted at room temperature, has a broad substrate scope, and provides the corresponding glycosylated products with good to excellent yields. We believed that such 1-*O*-glycosyl xanthate donor would be used as a complete substitute for the trichloroacetamide donor in oligosaccharide synthesis.

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Total Synthesis Of Dragocins A-D

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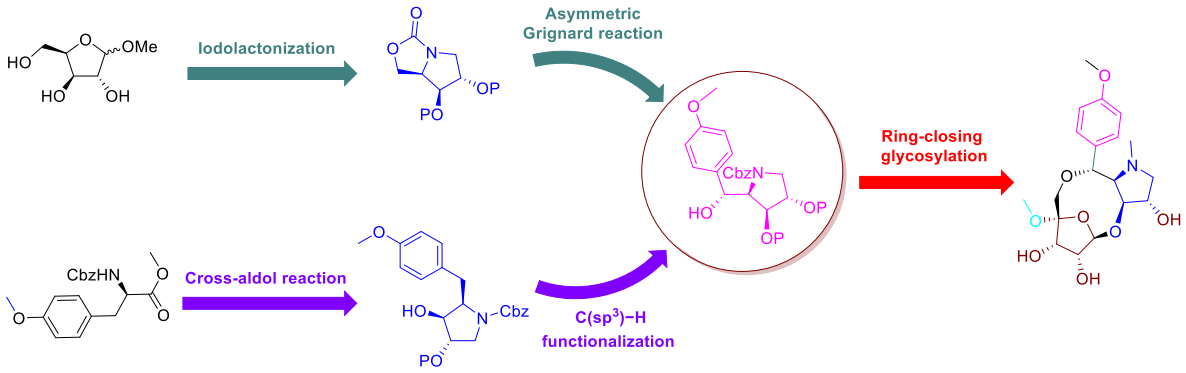
Dragocins A-D are iminosugar alkaloids separated from marine cyanobacterium,[1] and feature with a common nine-membered macrocyclic core and substitutions at the C-4′ position of a ribofuranose unit. [2]The dragocin derivatives have been proved with significant inhibitions of the proliferation of H-460 lung cancer cells, yet their anti-cancer mechanism has not been fully clarified. The main hindrance that restricted their mechanism study and further structure-activity relationship (SAR) study lies in the difficulty to obtain sufficient amount of dragocins efficiently. Recently, we launched the total synthesis of dragocins A-D from two completely different routes. The first de novo synthetic strategy towards dragocin A has been accomplished with 26 steps in the longest linear sequence, with iodolactonization, asymmetric Grignard reaction and ring-closing glycosylation as key steps. The currently undergoing biomimetic synthetic strategy has also fulfilled the construction the dragocins core successfully in a more concise way, utilizing direct organocatalytic asymmetric cross-aldol reaction of aldehydes[3]and asymmetric C(sp3)−H functionalization as key steps. The open synthesis strategies have laid solid foundation for the efficient construction of dragocin compound library and systematic SAR study.



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Expanding The Scope Of Solid-Phase Glucan Synthesis

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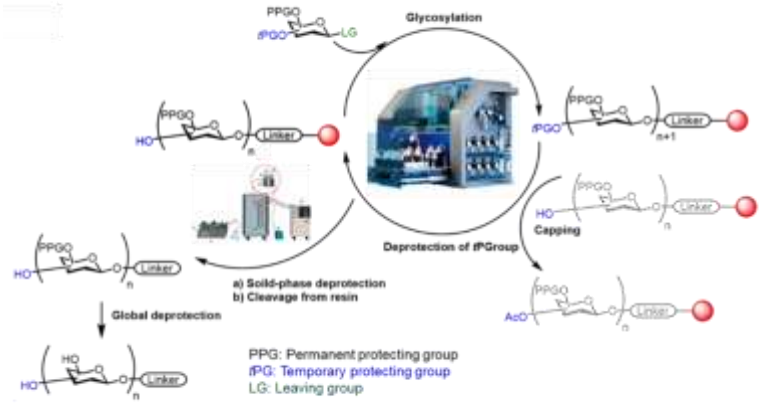
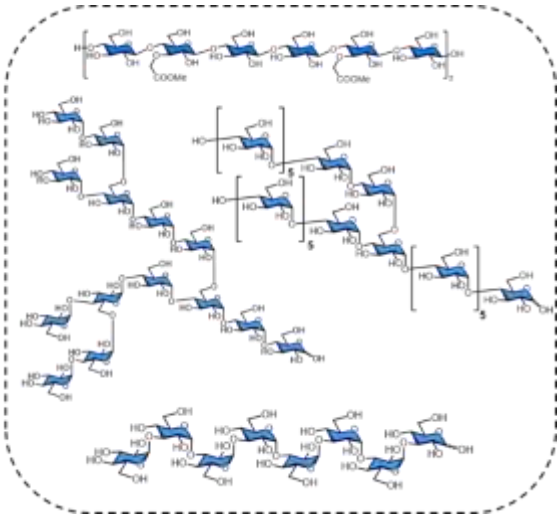
Polysaccharides are Nature´s most abundant biomaterials essential for cell wall construction and energy storage. Seemingly minor structural differences result in entirely different functions: cellulose, a β (1-4) linked glucose polymer, forms fibrils that can support large trees, while amylose, an α (1-4) linked glucose polymer forms soft hollow fibers used for energy storage. A detailed understanding of polysaccharide structures requires pure materials that cannot be isolated from natural sources. Solid-Phase Glycan Synthesis (SPGS) is a powerful method for the quick production of well-defined natural and unnatural oligosaccharides. Here we reported some recent progress on the synthesis of ionic β (1-4) glucan (analogues of cellulose), also the stereoselective installation of multiple *cis*-glycosidic linkages present in α (1-4) glucan (amylose) and α (1-3) glucan (fungal cell wall glycan). Using thioglycoside building blocks with optimized conditions, we prepared cellulose analogs with a well-defined charge pattern. We also achieved excellent stereoselectivity during the synthesis of linear and branched α-glucan polymers with up to 20 *cis*-glycosidic linkages. The molecules prepared in this study will serve as probes to understand the biosynthesis and the structure of glucans. The α (1-3) glucan with well-defined structure can be used to develop novel therapy tools against pathogenic fungi infection.





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Unexpectable Substrate Dependence In Ag_2CO_3-Promoted Glycosylations With Sialyl Chlorides

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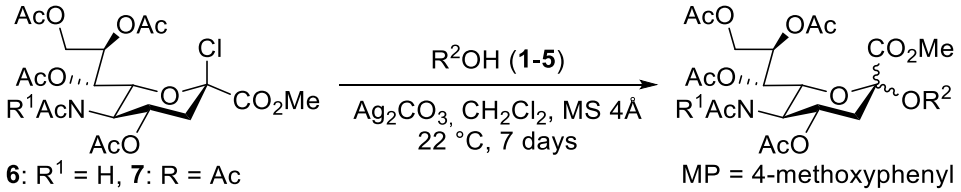
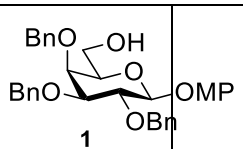
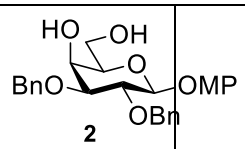
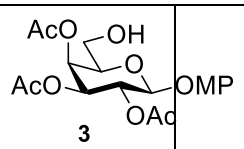
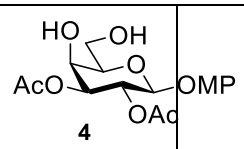
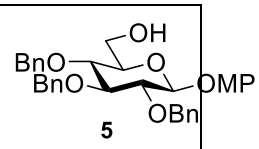
Due to the ease of preparation, acyl-protected sialyl halides[1] are attractive glycosyl donors in the synthesis of biologically significant sialoglycoconjugates. While their reactions with lower alcohols can proceed as a "simple" solvolysis,[2] more sterically demanding carbohydrate-based glycosyl acceptors (even those with a primary hydroxy group) require a metal ion (often Ag^+) as a promoter. Previously, Koenigs–Knorr conditions (Ag_2CO_3, chlorinated solvent, ambient temperature) were successfully used[3] for highly stereoselective α2→6 sialylation of a large series of sugar 4,6-diols (note that most of them had 3-(trifluoroacetamido)propyl aglycone and/or 3-*O*-Ac group) with *N*-acetylsialyl chloride **6**, suggesting a wide applicability of the method.

We examined glycosylation of partially protected 4-methoxyphenyl galactosides **1**–**4** and glucoside **5** with two acetylated sialyl chlorides (**6** and **7**) using the Koenigs–Knorr conditions (identical to those described[3]). To our surprise, *N*-acetylsialyl chloride **6** showed no reaction over 7 days with all but one glycosyl acceptor (**4**), glycosylation of which resulted in preferential formation of β-anomer of disaccharide in a low yield. Addition of AgOTf led to glycal formation. The more reactive *N*,*N*-diacetylsialyl chloride **7** formed glycosides with three of the five acceptors (one 4,6-diol and two 6-monohydroxy derivatives), albeit with poor stereoselectivity. Conversely, a seemingly similar glycosylation of two partially protected 3-(trifluoroacetamido)propyl glycosides (not shown here) with *N*-acetylsialyl chloride **6** under identical conditions expectably gave disaccharide products in high yield and almost complete α-stereoselectivity as described[3] previously for other glycosyl acceptors.



6: R^1 = H, **7**: R = Ac

MP = 4-methoxyphenyl

R^2OH	 **1**	 **2**	 **3**	 **4**	 **5**
6	no reaction	no reaction	no reaction	6%, α:β = 1:2	no reaction
7	60%, α:β = 1:1.6	no reaction	no reaction	58%, α:β = 1.6:1	15%, α:β = 1:2.1

These results cannot be explained by differences in nucleophilicity of 6-OH or by steric hindrance, and no obvious reactivity pattern can be deduced therefrom. Further investigation of the sialylation of glycosyl acceptors bearing various aglycones and protective groups is currently underway in our laboratory.

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Chemoenzymatic Synthesis Of Fucosylated And/Or Sialylated Linear Human Milk Oligosaccharides (Hmos) With A Hexaose Core

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Human milk oligosaccharides (HMOs) constitute a major component of human milk. There is an increasing attention on the contribution of HMOs to the health of breast-fed infants. They are not digested by infants but instead found in their guts, urine and plasma, which can serve as prebiotics to suppress the growth of some pathogenic bacteria, anti-adhesive decoy receptors for some pathogenic microbes, antibiofilm antimicrobials, brain-gut axis modulators, immune modulators, infant colon epithelial cell response modulators, and cell maturation stimulators. The detailed functions of specific HMOs, especially those with more complex structures, are not clear. Exploring the applications of HMOs as infant formula additives, nutraceuticals, and/or therapeutics has begun but has been slow due to the limited access to structurally defined HMOs in sufficient amounts. I have contributed to the development of a highly efficient user-friendly glycosyltransferase-based synthetic platform to access target HMOs in a systematic manner. I have been working on developing efficient routes for systematic chemoenzymatic synthesis of HMOs containing a hexose core (pLNnH, pLNH) using various approaches including Enzyme Assembly Synthetic Maps (EASyMaps), Stepwise One-Pot Multienzyme (StOPMe) with *in situ*-generation of sugar nucleotides, glycosyltransferase substrate engineering strategy, and glycosyltransferase engineering, etc. (Figure 1) The products are essential probes and reagents for elucidating the roles of HMOs and exploring their applications which is studied in the HMO-protein binding assays. The chemoenzymatic synthetic process is readily scalable for large-scale production of HMOs in the future.

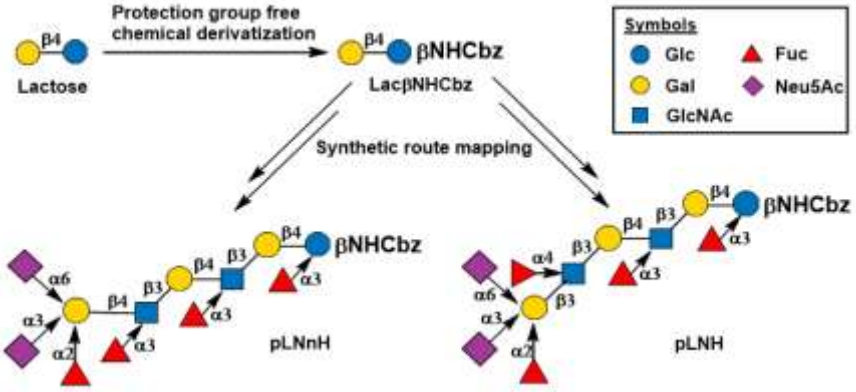

Figure 1. Strategy of chemoenzymatic route mapping and target-orientated construction of pLNnH and pLNH derivatives.

Nickel Catalysed Migita-Like Cross Coupling of Glycosyl Thiols in Aqueous Solution

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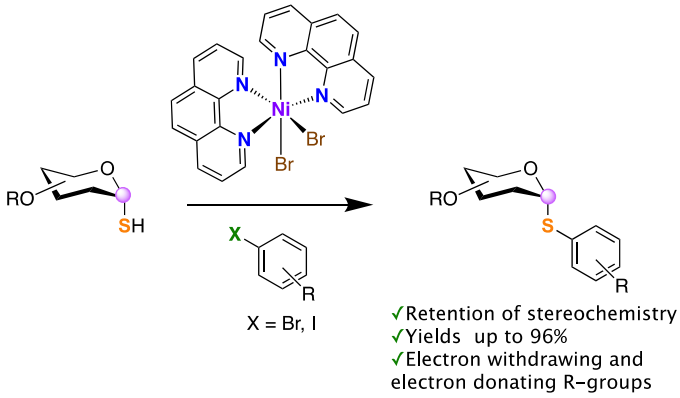
Thioglycosides have become universal in carbohydrate chemistry not only as oligosaccharide building blocks, but also as enzymatically stable glycomimetics.[1] In both cases, the identity of the aglycone and the anomeric configuration can greatly affect the properties of the glycoside.[2,3] Traditionally, thioglycosides have been synthesised by reaction of a glycosyl acetate with an alkyl or aryl thiol in the presence of excess Lewis acid, but this method can have drawbacks including necessity of foul-smelling thiol reagents, limitation of available thiols, and difficulty in obtaining 1,2-*cis* stereochemistry. Our group has already demonstrated the utility of using α-glycosyl thiols as a nucleophilic starting material which can be alkylated to form thioglycosides with 1,2-*cis* stereochemistry.[4] Similarly, glycosyl thiols can be arylated using transition metal catalysed cross coupling methods to form aryl thioglycosides with retention of anomeric stereochemistry.[5]

Recently, Lipschutz has reported a surfactant-mediated, nickel catalysed C-S cross coupling which can be carried out in aqueous solution.[6] Using the *bis*-phenanthrolino Ni(II) catalyst and surfactant TPGS-750-m employed by Lipschutz group, we have aimed to carry out a similar cross coupling reaction for glycosyl thiols, achieving arylation with inexpensive and abundant nickel, and avoiding the need for toxic or environmentally harsh organic solvents. We have shown this methodology to be effective in the coupling of multiple mono- and disaccharide 1-thiols with aryl halides bearing both electron-donating and electron-withdrawing substituents. The reaction proceeds under relatively mild conditions in yields of up to 96% with retention of native anomeric stereochemistry.



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Hepatic Glucuronyl C5-Epimerase Combats Obesity By Stabilising GDF15[1]

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Background & Aims: Disturbed hepatic metabolism frequently results in excessive lipid accumulation in the adipose tissue. However, the specific role of the liver–adipose axis in maintaining lipid homeostasis, as well as the underlying mechanism, has not yet been fully elucidated. In this study, we investigated the role of hepatic glucuronyl C5-epimerase (*Glce*) in the progression of obesity.

Methods: We determined the association between the expression of hepatic *Glce* and body mass index (BMI) in obese patients. Obesity models were established in hepatic *Glce*-knockout and wild-type mice fed a high-fat diet (HFD) to understand the effect of *Glce* on obesity development. The role of *Glce* in the progression of disrupted hepatokine secretion was examined via secretome analysis.

Results: Hepatic *Glce* expression was inversely correlated with BMI in obese patients. Moreover, *Glce* level was found to be decreased in the liver of a HFD murine model. Hepatic *Glce* deficiency led to impaired thermogenesis in adipose tissue and exacerbated HFD-induced obesity. Interestingly, decreased level of growth differentiation factor 15 (GDF15) was observed in the culture medium of *Glce*-knockout mouse hepatocytes. Treatment with recombinant GDF15 obstructed obesity progression derived from the absence of hepatic *Glce*, similar to the effect of *Glce* or its inactive mutant overexpressed both *in vitro* and *in vivo*. Furthermore, liver *Glce* deficiency led to diminished production and increased degradation of mature GDF15, resulting in reduced hepatic GDF15 secretion.

Conclusions: Hepatic *Glce* deficiency facilitated obesity development, and decreased *Glce* expression further reduced hepatic secretion of GDF15, thereby perturbing lipid homeostasis *in vivo*. Therefore, the novel *Glce*–GDF15 axis plays an important role in maintaining energy balance and may act as a potential target for combating obesity.

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The Regulation Of Peritoneal Metastasis Of Gastric Cancer Cells By Galectin-4 And Glycosphingolipids

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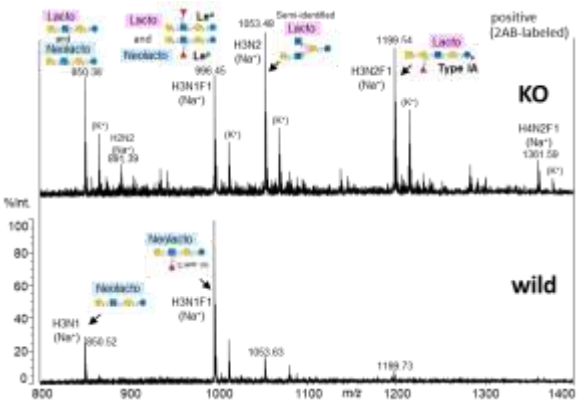
Peritoneal metastasis frequently accompanies metastatic and/or recurrent gastric cancer, leading to a dismal prognosis for patients due to the lack of effective treatment. Hence, there is a pressing need to enhance our comprehension of the mechanisms and molecules driving peritoneal metastasis. In a prior study, our focus was on galectin-4, highly expressed in poorly differentiated gastric cancer cells with potent metastatic abilities. Inhibition of galectin-4 notably impedes peritoneal metastasis in murine models. Our unveiled the involvement of galectin-4 participates in the peritoneal dissemination of malignant gastric cancer cells, primarily by fostering cell proliferation. Additionally, it interacts with various molecules, including c-MET and CD44[1], contributing to yet unidentified mechanisms.

To comprehend the intricate mechanisms underlying galectin-4-mediated regulation, particularly concerning glycosylation, we scrutinized the glycan profiles of cell surface proteins and glycosphingolipids (GSLs) in cells with varying tumorigenic potentials. Through detailed mass spectrometry analysis, we observed a noteworthy alteration in the neutral GSLs profile, while no significant change was observed in the N-glycan profile. Galectin-4 knockout (KO) cells exhibit increased expression of lacto-series GSLs with β1,3-linked galactose, including Le^a, Type I H, and Type I A, while showing no significant alterations in neolacto-series GSLs, including Le^x[2].



We conducted real-time PCR analysis to pinpoint the candidate glycosyltransferase (GT) responsible for synthesizing increased levels of GSLs in KO cells. Subsequently, we introduced the candidate GT gene into the cells and isolated clones that exhibited stable expression of the gene. The glycan profiles of GSLs from the established clones (H1 and H2) resembled those from the KO cells. Notably, the H1 and H2 clones exhibited diminished proliferative capacity, and there was a notable suppression in tumor formation within the peritoneal cavity of mice. Additionally, both H1 and H2 clones showed suppressed expression of galectin-4 and reduced activation of AKT.

Moreover, colocalization of galectin-4 with Flotillin-2 (a raft marker) decreased in candidate GT-expressing cells, implicating GSLs in galectin-4 localization to lipid rafts. Utilizing sucrose gradient sedimentation analysis and immunofluorescence staining of cells, we found that treatment with MβCD (cholesterol removal) or D-PDMP (GlcCer synthase inhibitor) affected galectin-4 localization in rafts, suggesting the involvement of GSL microdomains.

In this study, we discovered that GT plays a crucial role in regulating peritoneal metastasis of malignant gastric cancer cells by suppressing cell proliferation, and modulating lipid rafts and galectin-4 through unknown mechanisms. Given the significance of GSLs in lipid rafts, the GT responsible for synthesizing specific glycans of GSL, assumes a pivotal role. Our findings pave the way for novel therapeutic strategies targeting peritoneal metastases of gastric cancer.

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A Novel *Klebsiella Pneumoniae* Serotype O13 Encoded By The Ol101 Locus

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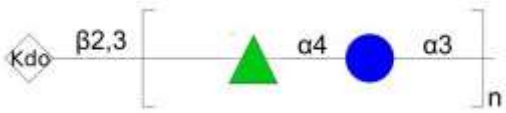
Klebsiella pneumoniae is a nosocomial pathogen and one of the priority species, pointed out by the World Health Organization as critical regarding highly limited options of treatment of infections. Lipopolysaccharide (LPS, O antigen) and capsular polysaccharide (CPS, K antigen) are major surface and virulence factors of the species. *K. pneumoniae* has been perceived as of limited variety of O antigens (dozen of O serotypes and subtypes identified to date) [1]. That trait makes O antigen an attractive target for antibody-based therapies (vaccines and passive immunization) as an alternative to antibiotics.

Since structural analyses of O antigens are effectively supported by bioinformatics (for example Kaptive tool)[2], novel O loci encoding O-specific polysaccharides of LPS may be identified and linked with exact chemical structures of the O antigens. The OL101 has been one of the novel *K. pneumoniae* O locus for which the antigen structure has not been elucidated so far. In this study, four clinical isolates (ABC152, BC738, BC13-986, 3936/19) predicted as OL101 were characterized and found to have the following O antigen structure numbered as O13 serotype:



Identification of the β-Kdo*p* terminus was based on the analysis of the native LPS molecule by the ^{1}H, ^{13}C HR-MAS NMR spectroscopy.

The MLST analysis revealed that all of the isolates were genetically diverse, each representing another sequence type (ST), namely ST11 (ABC122), ST485 (BC738), ST1427 (3936/19) and ST3658 (BC13-986). ABC122 strain revealed the same structure of the *rfb* cluster and high match confidence between its all nine *rfb* genes and those of the OL101 reference strain. The bioinformatic analysis of 71,377 *K. pneumoniae* genomes from public databases (July 2023) revealed a notable OL101 prevalence of 6.55 % [3].

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Albumin-Mediated Delivery Of Tn Antigen To Lymph Nodes Leads To Enhanced Immune Response

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The Tn antigen, a tumor-associated carbohydrate antigen, is a promising target for cancer vaccine. This study introduces a strategy of complexation of a designed Tn antigen with albumin for a target-driven delivery and enhance the immune response against cancer cells. Polyethylene glycol-lipid conjugate, namely, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine derivative (DSPE-PEG2000), modified with covalently bound Tn antigen, facilitates the complexation with circulating albumin and the antigen is delivered to lymph nodes. This delivery mechanism is critical for the subsequent production of antibodies against the antigen. Biophysical studies demonstrate the serum binding capability of the synthesized Tn antigen conjugate. The chemically synthesized Tn antigen glycoconjugate undergoes micellization at 500 nM in PBS buffer, which is disrupted upon incubation with BSA, leading to a 1:1 complex formation between BSA and Tn DSPE-PEG2000. Higher concentrations of Tn glycoconjugate result in the coexistence of the micelle and BSA-bound complex. In vitro studies demonstrate the preferential uptake of the Tn glycoconjugate by cells, increasing over time. Immunological assessments in mice models show that albumin complexation significantly enhances the delivery of the Tn glycoconjugate to antigen-presenting cells within lymph nodes, eliciting a robust humoral immune response. A superior binding affinity is assessed through ELISA titers, where a saturation dilution of 1:51200 is observed for the Tn glycoconjugate, as compared to the Tn-BSA covalent conjugate at a saturation dilution of 1:6400. Immunohistochemical staining confirms the targeted delivery of the Tn glycoconjugate to lymph nodes, particularly within the subcapsular sinus and interfollicular areas (**Fig. 1**). These results underscore the potential of Tn antigen derivative-albumin complexation as a targeted delivery strategy in cancer immunotherapy.

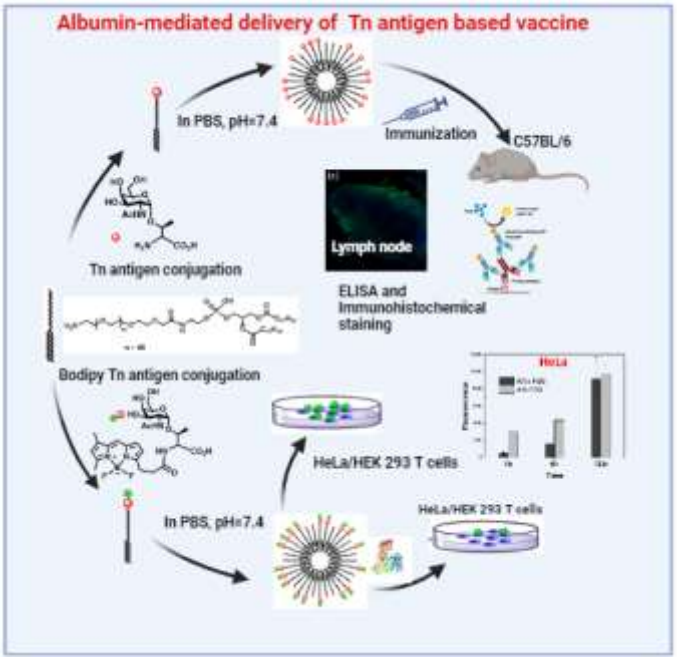

Figure 1. Albumin-mediated delivery of Tn antigen to lymph nodes.

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Polyguluronate Sulfate Could Mitigate Hyperlipidemia By Inhibiting Pcsk9-Mediated Degradation Of Ldlr

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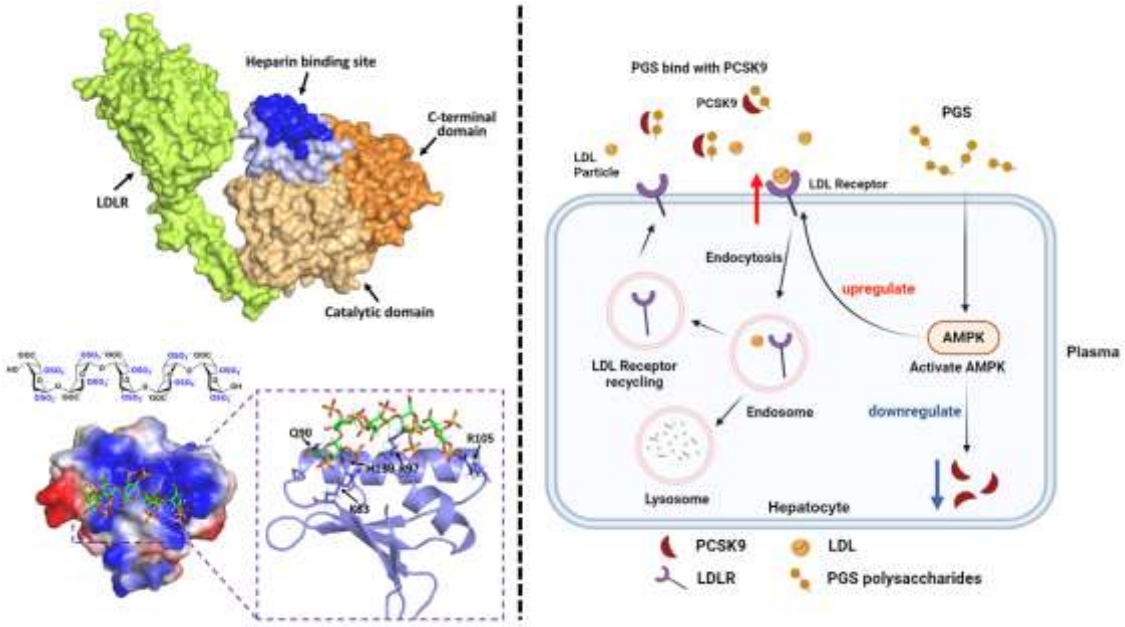
Hyperlipidemia has become one of the leading causes of mortality worldwide. Circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) protein in plasma induce lysosomal degradation of hepatic LDL receptors (LDLR), thereby reducing the clearance of low-density lipoprotein (LDL). In this study, we identified that polyguluronate sulfate (PGS), a derivative of the marine sulfated polysaccharide lipid-lowering drug PSS, is capable of binding to the positively charged domain of PCSK9, thereby inhibiting the PCSK9-mediated degradation of LDLR. Moreover, we further demonstrated that the lipid-lowering activity of PGS is exerted through modulation the PCSK9/LDLR pathway and activating the AMP-activated protein kinase (AMPK) pathway. In summary, this research provides theoretical support for the development of PGS as a novel lipid-lowering drug candidate.



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The Claim Of Primacy Of Human Gut *Bacteroides Ovatus* In Dietary Cellobiose Degradation

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Although cellulose is widespread in human diets as components of the cell walls of plants, it cannot be digested by human. However, a demonstration of cellulose degrading bacterium from human gut changed our view that human cannot utilize the cellulose due to the β1,4-glycosidic bond rendered cellulose inaccessible to the limited repertoire of digestive enzymes encoded by the human genome. But in fact, the distribution of this species is not clear till now. On top of that, how cellulose is degraded into glucose remains unknown. To address, the smallest unit of cellulose utilized by a gut key member *Bacteroides ovatus* (BO) in the human gut was selected for uncovering the underlying molecular mechanism. As shown in Figure 1, we found a core polysaccharide utilization locus (PUL), which contained two cell surface cellulase belonging to GH5 family with similar biological structure with Cel5A and Cel5R, two cellulase from soil bacteria, was responsible for the degradation of cellobiose. The catalytic sites were highly conservative with two glutamate residues. *In vivo* test, we observed that cellobiose reshaped the composition of gut microbiota, and specifically increased the abundance of BO and *Lactobacillus reuteri* (LR), and probably modified the metabolic function of bacteria especially for the enrichment of β-N-acetylhexosaminidase and KEGG pathway and Histidine. Briefly, our work sheds light on the molecular mechanism that BO has evolved to utilize the cellobiose in the highly competitive environment of the human gut microbiota. The findings that microbes in the human gut possess the capacity to utilize the cellobiose generated from ubiquitous cereal highlights the relevance of potential therapeutic interventions based on the gut microbiota. Our work also sets the stage for future work to understand the significance of cellobiose to human wellness.

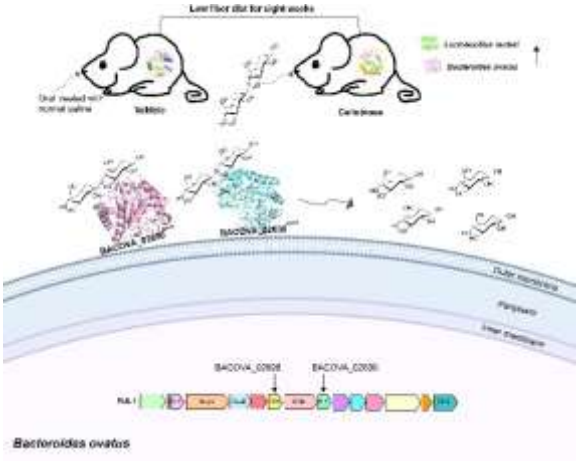

Figure 1. Graphical abstract. Model of degradation of cellobiose by *Bacteroides ovatus* (BO). Two new cellulases on the cell surface conferred the degradation of cellobiose into glucose were determined. *In vivo* test, we observed that cellobiose reshaped the composition of gut microbiota, and the abundance of LR and BO were enriched significantly after eight weeks administration by gavage.

Reference

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Anti-Cancer Immunotherapy Using β-glucan/antisense Oligonucleotide Complex

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Cancer is considered as a leading cause of death worldwide. Immunotherapy is promising in cancer treatment. However, targeted delivery of drugs to immune cells with minimal immunogenicity remains a great challenge. In this study, we present an immune cell targeted delivery system which consists of helix β-glucan and antisense oligonucleotide (ASO). Helix β-glucan is dissolved in alkali solution and dimethyl sulfoxide (DMSO) with the addition of ASO in water or acid solution, which forms the helix β-glucan. β-glucan can resist the endogenous enzymes degradation [1] and be selectively recognized by immune cells through pattern recognition receptors such as Dectin-1 [2]、Toll like receptors (TLRs) and complement receptor 3 (CR3) to deliver ASO to monocytes, silencing the important genes [3] and remodel tumor microenvironment. Reprogramed monocytes release cytokines and chemokines to achieve antitumor effects. The helix β-glucan/ASO delivery system shows good biocompatibility and desirable immunomodulatory activity, providing a promising strategy for cancer immunotherapy.

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Novel Carbohydrate Antigens Revealed For Immunoglobulin Receptors Of Chronic Lymphocytic Leukaemia Cells

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Chronic lymphocytic leukemia (CLL) is the most prevalent B cell neoplasm in the Western World. It is striking as a malignancy in which a cell proliferative state is maintained by signaling events that arise at the B-cell receptor (BCR). The VH gene usage among the BCR immunoglobulins (Igs) of CLL cells is not random but it is biased, indicating that the tumor cells originate from CD5 B cells were previously stimulated by specific antigens [1]. In support of this are the observations that the BCR Igs of around 40% of CLLs are encoded by IGHV-IG-HD-IGHJ genes that have remarkably similar HCDR3 regions referred to as 'stereotyped' often associated with antigen-binding features [2]. Prime examples of this are CLLs of the subset 4 of VH4-34. BCRs encoded by specific VDJ gene rearrangements giving rise to IGHV4-34/IGHD5-18/IGHJ6 and IGKV2-30/IGKJ2 combinations [3]. Several CLL BCRs with IGHV1-2, IGHV1-3 and IGVH1-69 show binding to cell surface-associated vimentin of stromal cells [4]; those with IGHV1-69 bind antigens on HIV-1 and hepatitis C virus as well as those of intestinal commensal bacteria [5]. The antigenic determinants remain to be elucidated, however. Although it has been proposed that microbial antigens and autoantigens induce BCR signaling in CLLs, epitopes of many of the antigens bound remain elusive. It remains unknown whether these antigens are glycans in nature. However, there are new opportunities to address these important questions with advanced glyco-technologies. Carbohydrate microarrays have emerged as powerful tools for unravelling glycan recognition systems involved in physiological processes in innate and acquired immunity and in diverse pathological processes, infection, inflammation, and malignancy. Here we have capitalized on glycan array technology and a recombinant lymphoma antibody expression method and have examined for glycan recognition by microarray analyses the BCRs of a cohort of 172 CLLs. We identify glycan antigens for around 15% of the cohort as either auto-antigens or microbial antigen-related and we demonstrate that the carbohydrate antigens can induce CLL cell signaling.

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Probing Altered Receptor Specificities Of Antigenically Drifting Human H3N2 Viruses By Chemoenzymatic Synthesis, NMR, And Modeling

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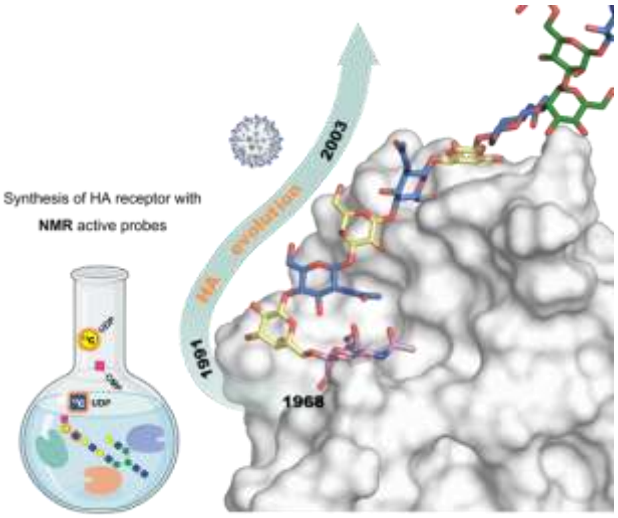

Prototypic receptors for human influenza viruses are N-glycans carrying α2,6-linked sialosides. Due to immune pressure, A/H3N2 influenza viruses have emerged with altered receptor specificities that bind α2,6-linked sialosides presented on extended N-acetyl-lactosamine (LacNAc) chains. The binding modes of such drifted hemagglutinin's (HAs) have been examined by chemoenzymatic synthesis of N-glycans having ^{13}C-labeled monosaccharides at strategic positions. The labeled glycans have been employed in 2D ^{1}H-STD-^{1}H,^{13}C-HSQC NMR experiments to pinpoint which monosaccharides of the extended LacNAc chain engage with evolutionarily distinct HAs. The NMR data in combination with computation and mutagenesis demonstrated that mutations distal to the receptor binding domain of recent HAs create an extended binding site that accommodates the extended LacNAc chain. Fluorine containing sialosides have been used as NMR probes to derive relative binding affinities.[1]

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Unveiling The Complex Glycoproteome Of Resting And Activated Platelets Using Glycomics-Assisted Glycoproteomics

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Platelets play central roles in the vascular and immune systems, including in haemostasis, thrombosis, inflammation and carcinogenesis. Tissue injury promptly activates resting platelets, triggering profound morphological changes and granule exocytosis, resulting in the release of granular proteins (releasate) that mediate injury-related response processes. Despite the documented importance of protein glycosylation in platelet biology, the platelet glycoproteome remains poorly defined. This study employs complementary glycomics and glycoproteomics approaches to comprehensively map the *N*-glycoproteome of the lysate and releasate of resting and thrombin-activated platelets. Primary platelets were isolated in their resting condition from blood donors. Platelets were left unstimulated (n=10) or were either partially (n=6) or fully (n=5) activated with α-thrombin, a potent platelet agonist. Thrombin-mediated platelet activation was confirmed with flow cytometry for PAC-1 binding and CD62P positivity. The releasate fractions were then collected and separated from platelet cellular fractions (lysate). The *N*-glycome of platelet lysate and releasate fractions was quantitatively profiled using PGC-LC-MS/MS-based glycomics. Platelet lysates and releasates displayed profound *N*-glycome diversity rich in sialylated and core fucosylated complex-type *N*-glycans across both resting and activated conditions. Interestingly, a thrombin dose-dependent elevation of sialylated and fucosylated complex-type *N*-glycans displaying a higher degree of branching and a higher global protein occupancy with a concomitant reduction in bisecting GlcNAcylation were observed in the releasate under activated conditions. The *N*-glycoproteomics data recapitulated and expanded on the glycomics findings by uncovering a total of 339 unique *N*-glycopeptides from 92 different source *N*-glycoproteins, the highest glycoproteome coverage of platelets to date. Our data also suggested platelets exhibit subcellular-specific *N*-glycosylation featuring prominent sialofucosylation in the α-granules, paucimannosylation in lysosomes and, surprisingly, oligomannosylation on the platelet surface. In summary, this study provides the hitherto most detailed view into the resting and activated platelet *N*-glycoproteome, forming a valuable resource to further explore the fascinating platelet glycobiology in human health and disease.

Biogenesis Of Outer Membrane Vesicles In *Bacteroides Thetaiotaomicron*: Is Lps Biosynthesis Altered?

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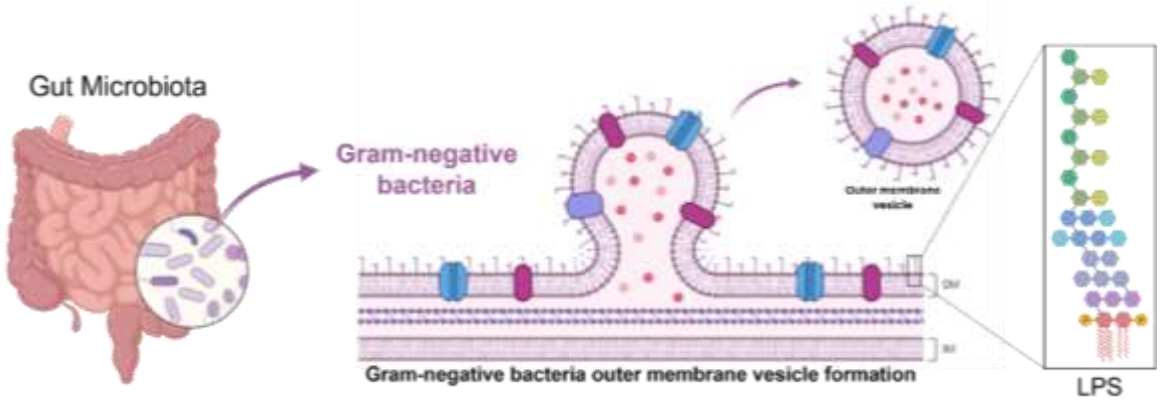
Human microbiota encompasses a large population of microorganisms inhabiting specific body districts, such as the oral and intestinal regions, where it plays pivotal roles in maintaining overall well-being, influencing a multitude of body functions, which also include immune system development and maturation.[1] Seminal studies have identified outer membrane vesicles (OMVs), spherical structures produced by blebbing of the Gram-negative outer membrane, as key mediators of these functions exhibiting anti-inflammatory and immunomodulatory properties.[2,3] In particular, it has been shown that the mutualistic gut microbe *Bacteroides thetaiotaomicron* produces large quantities of OMVs as the result of an active and regulated process. Indeed, Feldman *et al.* have recently identified a unique feature of Bacteroidota, i.e. a novel family of Dual Membrane-spanning Anti-sigma factors (Dma), which regulate OMV biogenesis in *B. thetaiotaomicron*.[2] In fact, deletion of Dma1, the founding member of the Dma family, resulted in hypervesiculation in this bacterium. Moreover, they demonstrated that NigD1, which belongs to a family of uncharacterized lipoproteins found exclusively in Bacteroidota, is required for the induction of vesiculation in the absence of Dma1, upregulating the number of lipopolysaccharides (LPS) present in OMVs.[2] More knowledge about regulation of LPS biosynthesis in *Bacteroides* is required to fully appreciate how Dma and NigD1 control OMV biogenesis.
In this communication I will show the results of a detailed structural characterization of LPS isolated from the wild-type B. *thetaiotaomicron* strain VPI-5482 and from a hypovesiculating ΔDma strain, with the aim to give a first glimpse of whether Dma and NigD1 impact on LPS biosynthesis while controlling OMV biogenesis.



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A New Generation Glycoconjugate Vaccine Against *Streptococcus Suis*

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Streptococcus suis causes significant economic losses to the swine industry and raises concerns about animal welfare. This organism is also an emerging zoonotic pathogen, mainly in Asian countries. In the absence of effective commercial vaccines, the incidence of disease in pigs is controlled by extensive antimicrobial prophylaxis. *S. suis* is covered by a capsular polysaccharide (CPS), which is the major virulence factor. Of the 29 described serotypes based on the CPS antigenicity, serotype 2 is the most clinically prevalent in both, pigs and humans. Studies have proven the protective capacity of CPS-specific antibodies. CPS is thus an attractive antigen but its poor immunogenicity limits its use as vaccine. However, when conjugated to protein carriers to produce glycoconjugate vaccines, carbohydrates acquire the required immunochemical ability, as shown by their successful application in human medicine. We established a proof-of-concept vaccine that protects pigs against *S. suis* serotype 2 challenge using native CPS conjugated to tetanus toxoid[1]. Unfortunately, glycoconjugate standard production methods are complex, resulting in high-cost vaccines. Recent advances in chemical synthesis and formulation design have spawned a new generation of carbohydrate-based vaccines. These developments overcome many of the limitations associated with traditional glycoconjugate vaccines.

In this study, we designed the first chemically-synthesized glycoconjugate vaccine against *S. suis* and provided proof-of-concept of its protective capacity. Eight fragments of the CPS from *S. suis* serotype 2, ranging in size from a monosaccharide to a heptasaccharide (1, 4, 7 and 10–14, **Fig. 1A**) were selected for synthesis. These compounds were prepared bearing an 8-azidooctyl (or 8-aminooctyl) linker to facilitate their conjugation to the carrier protein CRM197 (a non-toxic mutant of diphtheria toxin), using a maleimide–thiol coupling reaction.

Mouse immunization pre-trials were performed for a preliminary target selection based on the capacity of conjugated-CPS fragments to induce a high anti-CPS antibody response, a diversity of IgG sub-classes and functional ability of antibodies to eliminate *S. suis* by opsonophagocytosis. In the swine immunization and challenge model, the levels of antibodies induced by the different pre-selected conjugated-CPS fragments (#1, #4, #7, and #10) vary but the IgG isotype pattern was similar. However, not all conjugated-CPS fragments were efficient in inducing protection. Conjugated-CPS fragments #1 (**Fig. 1B**) and #10 showed strong to partial protection, respectively, and are thus promising targets for a swine vaccine. Our findings demonstrate the importance of not only chemically design the right epitope but also that clinical evaluation in pigs is required to predict the real value of a chemically-synthesized fragment as a vaccine candidate.

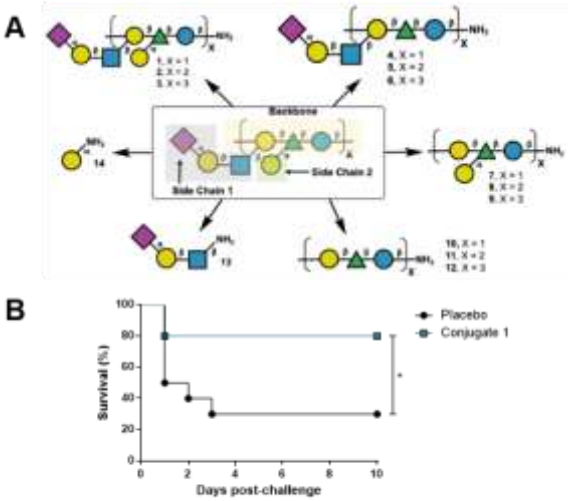

Figure 1. A) Schematic representation of CPS fragments (epitopes) targets. **B)** Survival rate of piglets vaccinated with glycoconjugate #1.

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Assignment Of The Function Of Genes Involved In *Lactobacillus Helveticus* Cnrz32 Exopolysaccharide Biosynthesis

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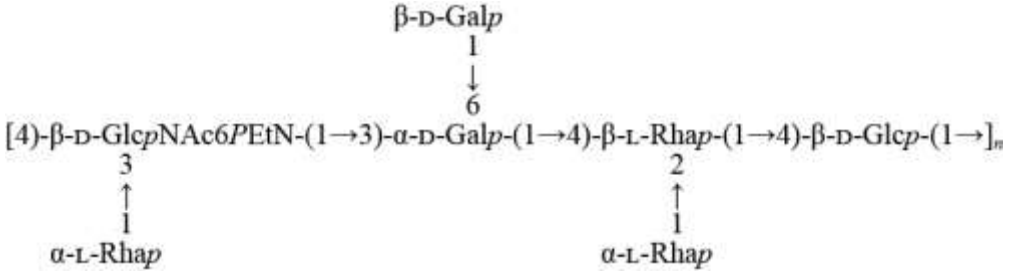
The repeating unit of the exopolysaccharide (EPS) produced by *Lactobacillus helveticus* strain CNRZ32 has been determined to have the following structure:



Seven glycosyltransferases, one phosphotransferase, and a polymerase are thus assumed to be involved in its assembly. The *L. helveticus* CNRZ32 partial *eps* operon and complete genome have been sequenced and annotated (GenBank accession numbers DQ826082, CP002081, and NC_021744).[1,2] The first four genes were involved in EPS synthesis and transport regulation, similarly to other *eps* operon organization. The following nine genes encoded the enzymes involved in the EPS subunit synthesis and polymerization.

In this study, we tentatively assigned functions of genes involved in EPS biosynthesis on the basis of the structure and sequence homologies of putative *eps* gene products.

Among the nine *eps* synthesis genes, the first enzyme (undecaprenylphosphate transferase) could be implied in the linkage of glucose to the lipid anchor. It was followed by the polymerization enzyme gene. The next two enzymes had strong homologies with β1,4- and α1,2-rhamnosyl transferases. No homology was found for the following enzyme, but the next one could be a β1,3-*N*-acetylglucosaminyl transferase. Most interestingly, from its homologies, the next enzyme could be involved in the formation of a phosphodiester linkage. The last two genes coded for proteins having strong homologies to α1,3-rhamnosyl and β1,6-galactosyl transferases. These results were in agreement with the EPS structure.

On these bases, we can propose the function of encoded enzymes from the *eps* operon of *L. helveticus* CNRZ32, which appeared to be organized in agreement with their sequential action during the synthesis.

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Investigating The Carbohydrate Processing Enzymes Encoded By A Key Polysaccharide Utilization Locus Of A Beneficial Gut Bacterium For Child Malnutrition Recovery

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In underdeveloped regions, where malnutrition persists as a pressing issue, microbiome-directed food intervention is emerging as a new promising strategy to facilitate the recovery of children suffering from malnutrition. Understanding the relationships between nutrition, the development of a thriving gut microbiome and the healthy growth of infants is key to this approach [1, 2].

In recent studies, a Polysaccharide Utilization Locus (PUL) was identified within a *Prevotella copri* strain sequenced from the microbiomes of children from Bangladesh suffering from malnutrition. This strain was positively associated with weight gain in malnourished children that received a microbiome-directed food treatment [1, 2]. To understand this finding, our focus is to elucidate the functional role of this PUL through detailed enzymatic analysis and comparative genomic studies. Comprising five carbohydrate-active enzymes [3], including four glycoside hydrolases and one carbohydrate esterase, this PUL is dedicated to the complete degradation of glucomannans, while also facilitating the initial breakdown of other complex glycans like mannan, galactomannan and xyloglucan.

By deciphering the intricate enzymatic mechanisms employed by beneficial gut bacteria to degrade complex carbohydrates found in food, this study aims to contribute to the development and optimization of microbiome-directed food formulations. These therapeutic foods can be designed to promote the growth of beneficial microbial communities, to develop healthy gut microbiomes and improve nutritional outcomes in vulnerable populations.

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Probing The Exopolysaccharide Interactome Of Staphylococcus Epidermidis Biofilms Through Live Cell Proximity Labelling

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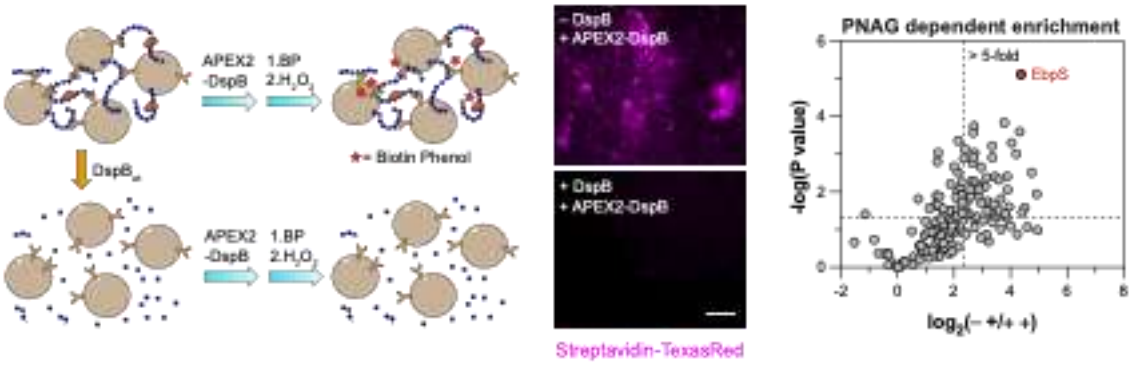
Bacterial biofilms are composed of surface attached bacterial cells embedded within an extracellular polymeric substance (EPS) composed of exported polysaccharides, proteins and extracellular DNA, which facilitates both cell–cell and cell–surface adhesion and serves as a protective barrier. Exopolysaccharides, like β-(1→6)-poly-*N*-acetylglucosamine (PNAG), are a significant structural component of the biofilm EPS of both Gram-positive and Gram-negative human pathogens, including *Staphylococcus epidermidis* and *Staphylococcus aureus*, but there is little known about how they mediate cell–cell interactions. Infections by biofilm forming bacteria are estimated to contribute to between 60-80% of all human infections, and the bacteria within the biofilm are up to one thousand-fold more resistant to antibiotic treatments. Thus, there is an interest in identifying new targets for anti-biofilm therapeutics that could be used to disrupt biofilms and potentiate the effectiveness of existing antibiotics. These efforts, however, require a detailed understanding of the intermolecular interactions that contribute to the integrity of live biofilms. Here, we have developed a live cell proximity labelling approach combined with quantitative mass spectrometry-based proteomics to map the PNAG interactome of live *S. epidermidis* biofilms.[1] Using this approach, we identified that the lysin motif domain found at the C-terminus of the *S. epidermidis* membrane protein EbpS functions as a PNAG binding protein, contributing to cell–matrix binding interactions in *S. epidermidis* biofilms. This live cell proximity labelling approach provides a rapid method to characterize exopolysaccharide interacting proteins of diverse bacterial biofilms and will lead to the identification of new proteins that contribute to biofilm integrity.



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Biopanning To Identify Peptides Recognizing Sars-Cov-2 Spike Proteins In Acoustically Levitated Droplets

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19, which escalated into a global pandemic in 2020. Even now, several years after the pandemic, there is still concern about the prevalence of variants. The spike (S) protein of SARS-CoV-2 decorates the viral surface and binds to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. The S protein plays a crucial role in infection and is an important target for the development of diagnosis, therapy, and vaccines. Artificially designed molecules that bind to the S1 subunit of the S protein are expected to be used as infection inhibitors and virus-detection sensor materials. In general, non-specific adsorption of phage leads to the reduction of affinity selection efficiency. To prevent the non-specific adsorption of phage, the phage display method was developed with acoustically levitated droplets. One of the aims of this study is to apply the phage display method in levitated droplets to obtain peptides effectively.

Results: To obtain SARS-CoV-2 S1-binding peptides, we used a phage display method having a 15-mer random peptide library against S1 protein. Phages were selected under three conditions, including two conventional and levitation methods (Fig. 1). The phages binding to S1 were eluted with acid buffer and glycoconjugates. Compared to the conventional two selections, phage recovery was improved much by the levitation method. Sequence analysis of 166 phage clones showed that a total of 12 peptide sequences were enriched. Enriched phage clones were evaluated for binding activity by phage ELISA. Phage ELISA results indicated that the enriched clones were bound to S1. Of the acquired sequences, we chemically synthesized four biotinylated peptides and determined the binding affinity of the peptides with the S1, by avidin-biotin peroxidase complex method. Several peptides have an affinity for the S1 protein, especially peptide AC4 can bind to S1 with a dissociation constant (K_d) of 21.5 μM. These S1-binding peptides have potential application as an inhibitor of SARS-CoV-2 infection.

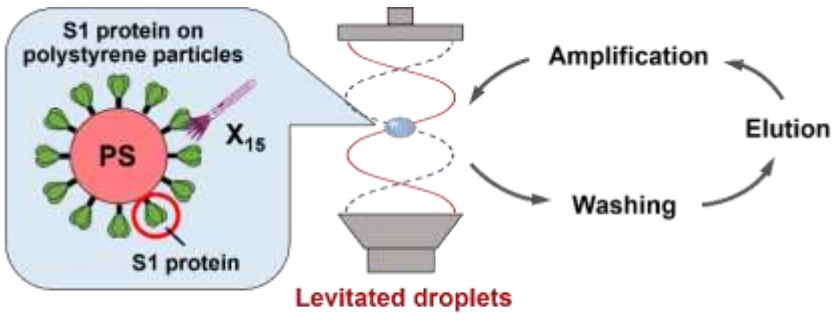

Fig. 1 Biopanning in acoustically levitated droplets.

In situ survey and structural identification of glycoRNAs

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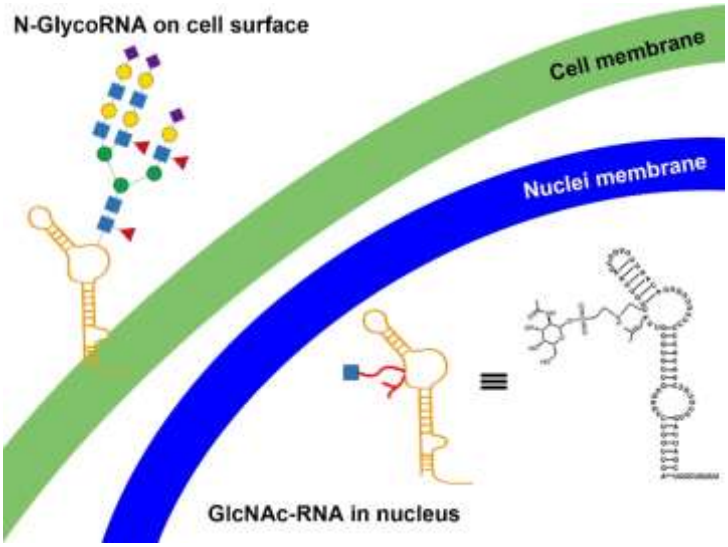
GlycoRNA is a newly discovered type of glycoconjugate, which has been reported to be presented in cell surface and modified with N-glycans.[1] Several methods have been developed to image the glycoRNAs on cell surfaces with hybridization-mediated proximity ligation assay[2] and hierarchical coding strategy.[3] Unfortunately, the accurate structure and glycan-modification site still remains unknown. Here, we supplemented this discovery with evidence that intracellular RNAs were also glycosylated but with single GlcNAc monosaccharide. Using the metabolic technology and the customized fluorescent DNA probes, the GlcNAc modified Y5 RNA in nucleus was firstly imaged by fluorescence resonance energy transfer, which was further collected by the specially designed gathering procedure. The structure of GlcNAc conjugated ribonucleotide was accurately identified by specially designed gel electrophoresis and MALDI-TOF analysis, and the glycan-modification site on the RNA sequence was also inferred. We find that the intracellular Y5 RNA is modified with GlcNAc monosaccharide and attached on pi6Aptm5s2U, which locates at the first AU site from the 5' end. Collectively, these findings extremely supplement the distribution and detailed structure information of glycoRNAs, which greatly promotes the functional research of glycoRNAs.



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Structural Elucidation Of An Active Arabinoglucan From *Gomphrena Globosa* And Its Protection Effect And Mechanism Against Metabolic Dysfunction-Associated Steatohepatitis.

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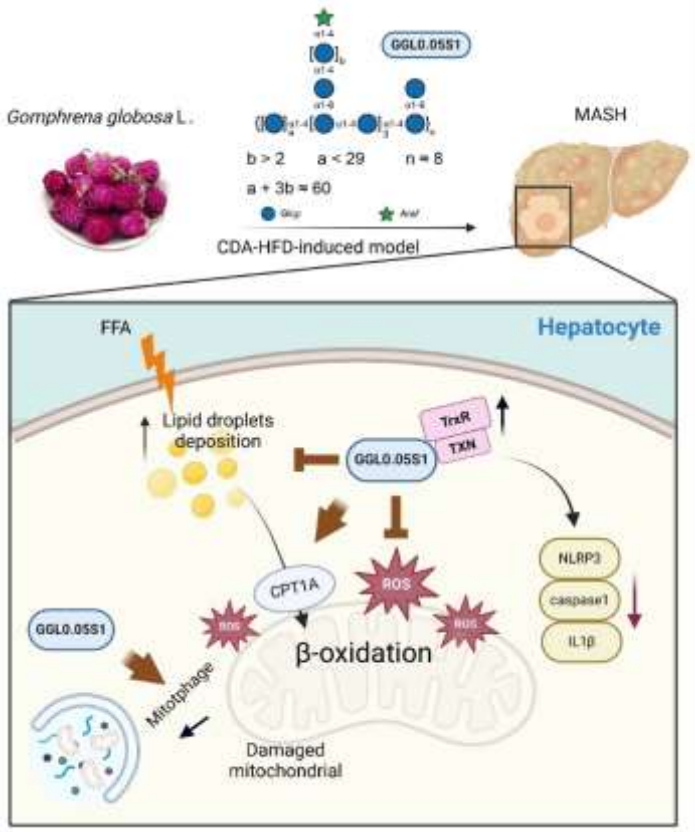
The flower of *Gomphrena globosa* (*G. globosa*.) is used as Chinese traditional medicine and functional food. Extractions from *G. globosa*. have been reported to exert hepatoprotective effects. We further hypothesized that the polysaccharide components, as a bioactive ingredient, might have anti-fatty and hepatitis function. Here, a novel homogeneous arabinoglucan GGL0.05S1 (*Mw* = 83.9 kDa) from this flower, was characterized by monosaccharide composition analysis, methylation analysis, and NMR. Structural analysis showed that the backbone of GGL0.05S1 consists of →4)-α-Glc*p*-(1→ and →4,6)-α-Glc*p*-(1→ residues, and the branched chain linked to the main chain via C-6 of →4,6)-α-Glc*p*-(1→ in the form α-Ara*f*-(1→[4)-α-Glc*p*-(1]$_b$→ (b > 2). *In vitro* experiments demonstrated that GGL0.05S1 could inhibit lipid deposition and ROS overload in free fatty acid-induced hepatocytes, meanwhile *in vivo* tests showed that GGL0.05S1 effectively protected against liver injury, steatohepatitis, and fibrosis in a CDA-HFD-fed MASH model. Mechanism study further uncovered that GGL0.05S1 augmented the expression and antioxidant ability of thioredoxin protein that ameliorated oxidative stress, promoted fatty acid β-oxidation and mitophagy, up to reducing lipotoxicity and alleviating inflammation via inhibiting NLRP3 signaling pathway. Overall, GGL0.05S1 might be a potential novel active compound with liver-protective effect from *G. globosa*., and which is informative for anti-MASH new drug development.



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Extended N-Glycans Are Preferred Receptors Of H3N2 Influenza Viruses On Human Ciliated Epithelial Cells

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Influenza virus pandemics have resulted from the introduction of an avian/swine virus progenitor. Transmission in the human population requires that the hemagglutinin (HA) specificity shifts from recognition of α2,3-linked sialoside receptors (‘avian type’) to α2,6-linked sialoside receptors (‘human-type’) found on human airway epithelial cells. Since the introduction of H3N2 viruses in the 1968 flu pandemic, the H3 HA has retained human-type receptor preference but has acquired a restricted specificity for the poly-LacNAc extended α2,6-sialosides. This change has impacted binding to cells used for laboratory viral assays and membranes of eggs commonly used for vaccine production due to the lack of adequate extended glycan receptors. To understand the basis of this change in receptor specificity, we analyzed the glycome of human airway epithelial cells, and confirmed the presence of extended glycan receptors. An N-linked glycan library representing the structural diversity of H3N2 virus candidate receptors in the airway glycome was constructed by chemo-enzymatic synthesis and tested for the specificity of H3N2 HAs from 1968-2020, revealing that the strict specificity for extended receptors was retained in current strains. Histochemical staining of human airway biopsy samples shows recent H3N2 HAs preferentially bind to ciliated cells not the entire apical surface, which we believe is a result of preferential expression of extended glycans on these cells. (Funded in part by NIH grant R01AI114730, and CEIRR contract 75N93021C00015).

Keywords: H3N2 influenza virus, receptor specificity, chemo-enzymatic synthesis

Blood Group Positive Microbes Stimulate The Development Of Anti-Blood Group Antibody Formation

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Anti-ABO(H) antibodies, which form spontaneously within the first few months of life, represent the most common immunological barrier to transfusion and transplantation. These antibodies can vary widely between individuals, with significant differences in anti-ABO(H) antibody levels and specificity that have direct clinical consequences on ABO(H) incompatible outcomes. However, the factors that influence this variability remain incompletely understood. As ABO(H) blood group antigens (hereafter referred to as BG) are carbohydrate structures largely confined to humans as polymorphic structures, preclinical models capable of defining factors that influence anti-BG antibody formation have not been available. To overcome this limitation, we generated a novel preclinical model that recapitulates key features of naturally occurring anti-BG antibody formation. By knocking out the enzyme required for the synthesis of the murine blood group B-like antigen (murine B or B^m), we generated blood group O-like (murine O or O^m) mice that spontaneously develop varying levels of anti-B^m antibodies. Similar to what occurs clinically, transfusion of B^m RBCs into O^m recipients results in varying degrees of hemolytic transfusion reactions (HTRs), where the magnitude of B^m RBC hemolysis correlates with pre-existing anti-B^m antibody levels. To define factors that contribute to varying anti-B^m antibody levels, O^m recipients were separately housed over several generations based on their levels of anti-B^m antibodies. Using this approach, distinct colonies of O^m mice were selected for that generate either high or no detectable anti-B^m antibodies despite having similar total IgM. Consistent with recent data suggesting that the microbiota does not impact naturally occurring antibody formation, analysis of the total microbiota at the genus level failed to detect key differences between O^m recipients with distinct anti-B^m antibody levels. Microbiota at the genus level failed to detect key differences between O^m recipients with distinct anti-B^m antibody levels. However, anti-B^m antibodies eluted from B^m RBCs did recognize distinct microbiota. Sorting and culturing of anti-B^m antibody reactive microbiota identified a strain of *Klebsiella pneumoniae* that specifically expresses the B^m antigen. Exposure of O^m recipients with undetectable anti-B^m antibodies to B^{m+} *K. pneumoniae* induced anti-B^m antibodies independent of CD4 T cells that were capable of causing HTRs. These results suggest that anti-BG antibody formation reflects exposure to BG+ microbes, providing critical insight into key factors that drive the most common immunological barrier to transfusion and transplantation.

Molecular Insights Into *Neisseria Meningitidis* Serogroup Y CPS Interaction With Siglec-7

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Siglecs (sialic acid immunoglobulin type lectins) are essential receptors on immune cells, modulating immune responses through specific interactions with sialic acid residues.[1] Exploited by pathogens and tumor cells for immune evasion, they play a significant role in infectious diseases and cancer. Siglec-7, expressed primarily on Natural Killer cells, acts as an inhibitory receptor, regulating immune responses. [3] We here show that serogroup Y (Men-Y) capsular polysaccharide (CPS), crucial for meningococcal meningitidis, interacts with Siglec-7 (Figure 1), a critical immunomodulatory receptor, contributing to immune evasion mechanisms.

Therefore, this presentation delved into the molecular aspects of Men-Y CPS recognition by Siglec-7, employing ELISA, fluorescence studies, NMR spectroscopy, and computational techniques.[4] The partial depolymerization of Men-Y CPS facilitated the isolation of oligosaccharides for detailed analysis. Molecular dynamics simulations provided insights into the conformational behavior of Men-Y CPS when bound to Siglec-7. In addition, by the employment of different experiments, it was confirmed Siglec-7 preferential recognition of Men-Y ([-4)-α-Neu5Ac-(2,6)-α-Glc-(1]$_n$ over Men-W ([-4)-α-Neu5Ac-(2,6)-α-Gal-(1]$_n$), despite their structural similarities. So, we here aim at shedding light on immune evasion mechanisms employed by *Neisseria meningitidis* evaluating the interaction between Men-Y CPS and Siglec-7. Understanding these interactions could inform the development of targeted therapies against meningococcal infections.

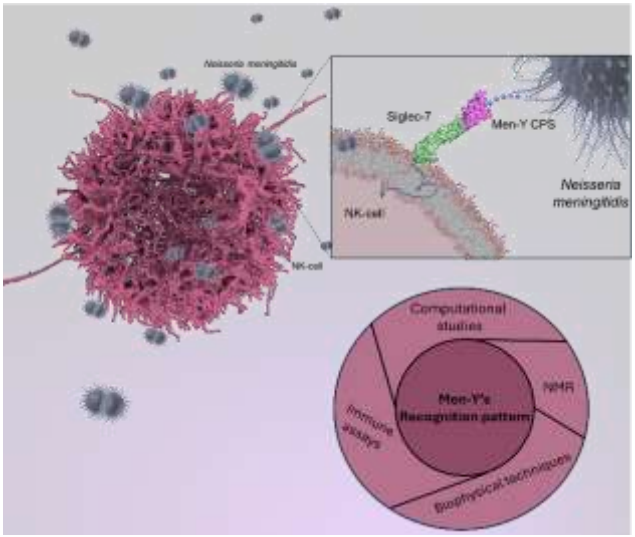

Figure 1. Schematic representation of the interaction between meningococcal CPS and Siglec-7.

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Anti-Hepatoma Activity And Mechanism Of Trametes Sanguinea Polysaccharides In H22 Tumor-Bearing Mice

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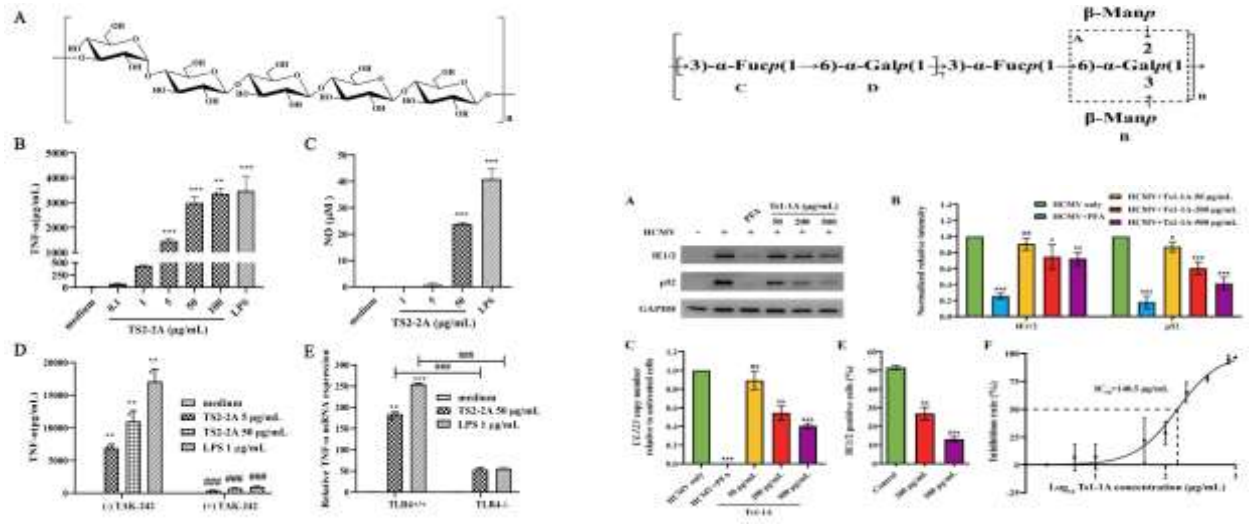
Hepatoma is the second leading cause of tumor death in China. Traditional Chinese medicine with multi-component synergistic regulation advantage has great potential in the research of anti-hepatoma drugs with high efficiency and low toxicity. As a medicinal fungus with Qingrejiedu, The total polysaccharide TsLTP, extracted from *Trametes sanguinea*, possed significant anti-angiogenesis and immune-enhancement activities, and showed effective inhibition of tumor growth in H22 tumor-bearing mice. Our team intends to prepare and clarify the anti-hepatoma effective components of *Trametes sanguinea* polysaccharides, and study its anti-hepatoma mechanism from the point of view of inducing tumor vascular normalization and reprogramming tumor immune microenvironment.



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The Commensal Gut Bacteria-Derived Peptidoglcyan Fragment, Glcnac-Murnac (Gm) Dissaccharide, Confers Immunomodulatory And Protective Effects Via Tlr4 Signaling

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Main body: Gut microbiota-derived peptidoglycan fragments (PGNs) are key signaling molecules that regulate multiple aspects of the host's health. Yet the exact structures of natural PGNs in hosts have not been fully elucidated. Herein, we developed an LC-HRMS/MS analytical platform for global quantification and profiling of natural PGN subtypes in host gut and sera, unexpectedly revealing the abundance of saccharide-only PGN motifs in hosts that do not resemble canonical ligands of classic mammalian NOD1/2 sensors. Focusing on the disaccharide GlcNAc-MurNAc (GM), a natural PGN that does not stimulate NOD receptors yet still exhibits robust immunostimulatory effects in immune cells, we unambiguously established GM as a TLR4 agonist, which adds to the growing understanding of NOD-independent sensing mechanisms of PGNs in hosts. Importantly, the administration of GM to mice effectively protects them against colonic inflammations in the colitis model, highlighting the in vivo significance of GM-induced TLR4 signaling for maintaining host intestinal homeostasis.

Keywords: gut microbiota, peptidoglycan fragments, immuno-modulatory, TLR4, intestinal inflammation

Heparan Sulfate As A Scaffold For Stable Presentation Of Cell-Surface Rna

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Recent discoveries have shown RNA's localization on the cell surface, defying the traditional view that RNA functions only intracellularly, However, the mechanistic understanding of how cell-surface RNA (csRNA) is stably presented on the plasma membrane is lacking. Here, we exploit the RNA-sensing ability of TLR7 and established it could be used as a probe for csRNA. Using the TLR7 probe, we identified heparan sulfate (HS) as a crucial factor for RNA presentation on cell surface by a CRISPR-Cas9-mediated genome-wide knockout screening. By performing proximity labeling and proteomic analysis, we further revealed HS-RNA association required RNA-binding proteins. We then demonstrated these heparan sulfate-associated csRNA could modulate the interaction between poliovirus receptor (PVR) and killer cell immunoglobulin-like receptor 2DL5 (KIR2DL5) by acting as a co-binder to enrich the latter protein on the cell surface, unveiling a new layer of complexity in the regulation of cell surface receptor-ligand interactions.

Ogt-Mediated O-Glcnacylation Inhibits Activation And Pyroptosis Of Microglia By Targeting Atf2 During Sae

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Sepsis-associated encephalopathy (SAE) is a diffused brain dysfunction caused by systemic immune dysregulation after sepsis. Microglia pyroptosis-mediated neuroinflammation is thought to be an important pathological basis for SAE. β-N-acetylglucosamine transferase (OGT)-mediated O-GlcNAcylation affects multiple physiological and pathophysiolocal functions. Increased O-GlcNAc stimulation has been reported to protect against sepsis associated mortality. However, its specific functional mechanism in SAE progression remain undetermined. In the present study, we observed a typical U-shape curve of the global protein O-GlcNAcylation level in the brain of SAE mice and the microglial inflammatory activation model following LPS administration. Inhibition of O-GlcNAcylation in microglia by knocking down Ogt gene significantly exaggerated the LPS-induced production of pro-inflammatory cytokine IL-6, IL-1β, which implied a potential regulatory role of O-GlcNAcylation modification in the LPS induced acute microglial activation. Inhibition of O-GlcNAcylation induces pyroptosis of microglia in vivo and in vitro, and impairs cognitive function of mice. Mechanistically, we found that OGT interacts with activation transcription factor 2 (ATF2) and regulates the balance of O-GlcNAcylation and phosphorylation of ATF2 in microglia. OGT defciency induces the activation of NLRP3 inflammasome by regulating the phosphorylation modification and nuclear translocation of ATF2, and causes microglial pyroptosis and excessive immune response. In addition, knocking down OGT in microglia leads to neuroinflammation and neuronal loss, and exacerbates the progression of SAE. Furthermore, we identified the O-GlcNAcylation of ATF2 on serine 44, and found that mutation of ATF2 S44 to alanine exacerbates NLRP3 inflammasome activation and promotes LPS stimulated production of inflammatory cytokines (IL-18, IL-1β) in microglia. Taken together, our study reveals the protective function of OGT-mediated O-GlcNAcylation of ATF2 in microglia, which might provide a new target for the treatment of SAE.

Deciphering Initial Site Preference Of The Polypeptide Galnac Transferases In Muc1 O-Glycosylation Process

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Mucin1 (MUC1) is an attractive target for anticancer vaccines, due to its overexpression and highly aberrant O-GalNAc glycosylation in many prevalent cancers. The varied O-GalNAc glycan types and sites on MUC1 are essential for its biological activity, which is controlled by 20 members of the polypeptide N-acetyl-α-galactosaminyltransferases (GalNAc-Ts) family in human cells. However, the site-specific O-glycosylation processes of MUC1 by each GalNAc-T isoform are still incompletely understood.

Here, we successfully obtained 14 members of human GalNAc-Ts family with high catalytic activity based on a simple bacterial expression system. Using chromatography and mass spectrometry technologies, we systematically examined the site-specific preference of these GalNAc-Ts against MUC1-derived peptides. Based on the initial acceptor sites, we grouped 11 active isoenzymes of GalNAc-Ts into two clusters: cluster1, which targets GVTS first, and cluster2, which initially modifies GSTA. Further molecular dynamics simulations and site-directed mutagenesis revealed the initial O-glycosite preference is controlled by two critical residues in the peptide-binding pocket of GalNAc-Ts. Moreover, our results demonstrated that the GSTA and GVTS motifs on MUC1 exhibit high occupancy, and the O-glycosylation preference of GalNAc-Ts for these two motifs is conserved across multiple MUC1-derived peptides. Overall, our work reveals the underlying enzymatic mechanisms of the biosynthesis of MUC1 O-glycosylation, which contribute to a deeper understanding of the correlation between the abnormal glycosylation of MUC1 and immune regulation in cancer, and facilitate the development of more effective MUC1 vaccines in the future.

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Nanoscale Sialyltransferase Inhibitor For Tumor Therapy

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Immune checkpoint blockade (ICB) has significantly improved the prognosis of patients with cancer, although the majority of such patients achieve low response rates; [1,2] consequently, new therapeutic approaches are urgently needed. The upregulation of sialic acid-containing glycans is a common characteristic of cancer-related glycosylation, which drives disease progression and immune escape via numerous pathways.[3] Herein, we report the development of self-assembled core-shell nanoscale coordination polymer nanoparticles carrying a high loading of sialyltransferase inhibitor, termed as NCP-STI which effectively stripped diverse sialoglycans from cancer cells, providing an antibody-independent pattern for disruption the emerging Siglec-sialic acid glyco-immune checkpoint. Furthermore, NCP-STI inhibited sialylation of the concentrated nucleoside transporter 1 (CNT1), promoted the intracellular accumulation of anticancer agent gemcitabine (Gem), and enhanced Gem-induced immunogenic cell death (ICD). As a result, the combination of NCP-STI and Gem (NCP-STI/Gem) evoked a robust antitumor immune response and exhibited superior efficacy in restraining the growth of multiple murine tumors and pulmonary metastasis (Figure 1).[4,5] Collectively, our findings demonstrate a novel form of small molecule-based chemo-immunotherapy approach which features sialic acids blockade that enables cooperative effects of cancer cell chemosensitivity and antitumor immune responses for cancer treatment.

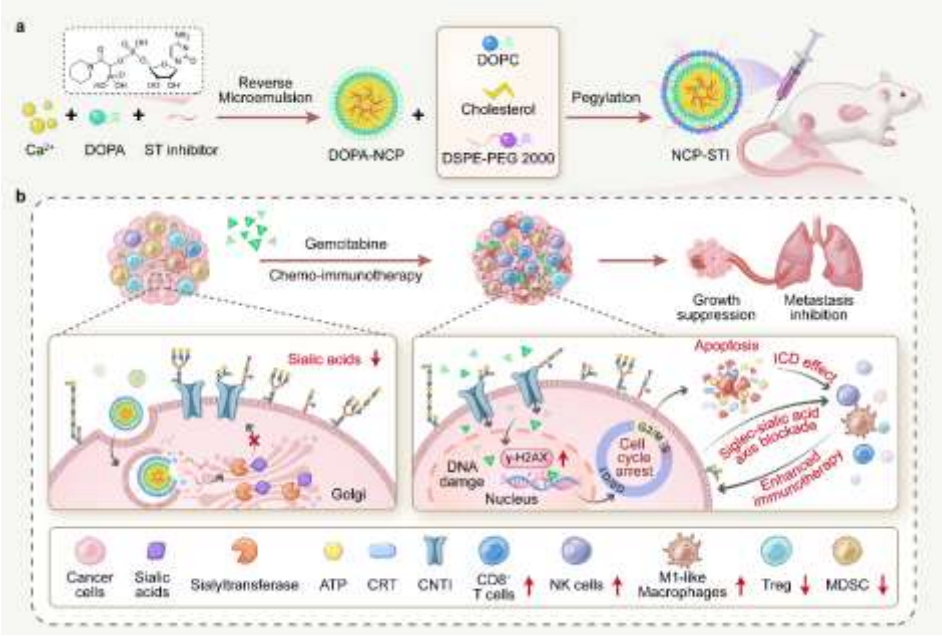

Figure 1. Schematic illustration of chemo-immunotherapy featuring sialic acids blockade for cancer treatment.

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DMBT1 Promotes SARS-Cov-2 Infection In Mucosal Compartment

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Abstract

DMBT1 is a large scavenger receptor cysteine rich (SRCR) B protein, which is densely O-glycosylated and expressed by mucosal compartment with highest abundance at airway submucosal glands. It has been proposed as a tumor suppressor gene and a co-receptor for HIV-1 infection. Here we found DMBT1 is a major mucosal protein bound to SARS-CoV-2. Overexpression of DMBT1 in 293T cells enhanced infection by coronaviruses in ACE2 dependent manner. Blocking experiments using overlapping peptide library of SRCR domain of DMBT1 showed that CQGRVEVLYRGSWGTV peptide, which was reported to inhibit bacteria binding, could inhibit SARS-CoV2 infection with IC50 at 40 micromolar range. These results indicate DMBT1 promotes coronavirus infection, and serve as a candidate target for antivirals development.

Keywords: DMBT1; Group B Scavenger Receptor Cysteine-Rich (SRCR) domain; SARS-CoV-2; Submucosal gland; Antivirals

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Developing Rhamnose-Modified Biomolecule For Cancer Immunotherapy By Recruiting Natural Antibody

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Abstract：Endogenous antibodies are naturally occurring antibodies present in the human circulatory system, targeting antigens such as the dinitrophenyl (DNP) group, galactose-α-(1,3)-galactose (αGal), and rhamnose (Rha).[1-3] Harnessing these antibodies for specific disease cell targeting has the potential to enable adaptive immunities for selective and effective therapy.[4-7] In this study, we designed and synthesized a series of multivalent rhamnose (Rha)-modified nanobody conjugates and investigated their antitumor activities, as well as their potential to overcome cetuximab resistance.

Structure-activity relationship studies revealed that the multivalent conjugate **D5**, which carried sixteen Rha haptens, was able to trigger the most potent Fc-mediated innate immunity to promote cancer cell death, highlighting its potential for cancer immunotherapy. Notably, **D5** exhibited complete resistance to enzymatic degradation by the serine protease (PRSS), which is commonly found in the tumor microenvironment of metastatic colorectal cancer (mCRC) patients and can degrade cetuximab. In contrast, cetuximab lacked the ability to induce complement-dependent cytotoxicity (CDC) due to the presence of complement regulatory proteins on tumor cells, while **D5** demonstrated robust in vitro CDC cytotoxicity. The remarkable antitumor activity of conjugate **D5** was demonstrated in a cetuximab-resistant tumor cell derived xenograft mice model as well.

These results highlight the potential of Rha-Nanobody (Nb) conjugates as a promising therapeutic strategy for treating cetuximab-resistant tumors. By enhancing innate Fc effector immunity and promoting the recruitment of endogenous antibodies to facilitate cancer cell clearance by innate immune cells, Rha-Nb conjugates offer a novel approach to address cetuximab resistance and improve therapeutic outcomes.[8]

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Fractional Extraction And Structural Characterization Of Glycogen Particles From The Whole Cultivated Caterpillar Fungus *Ophiocordyceps Sinensis*

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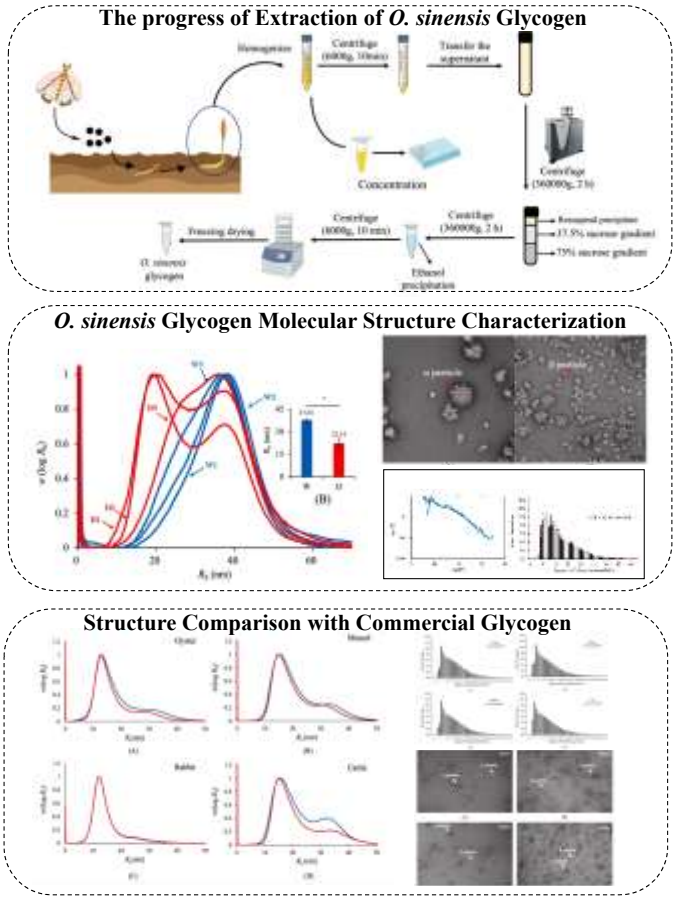
Abstract *Ophiocordyceps sinensis* (formerly known as *Cordyceps sinensis*) is a precious traditional Chinese medicine with many bioactive components, among which polysaccharide is one of the key constituents with important medical effects. Glycogen is a functional polysaccharide widely identified in eukaryotes including many fungal species. However, there is currently no definitive report of glycogen in O. sinensis, which is worth in-depth investigation. In this study, we fractionated glycogen-like polysaccharides from O. sinensis through the sucrose density gradient ultracentrifugation method, which was characterized for fine molecular structure via techniques such as size exclusion chromatography and fluorophore-assisted carbohydrate electrophoresis, etc. The results confirmed that the extracted polysaccharide was glycogen and provided a novel structural overview of O. sinensis glycogen. Taken together, glycogen was identified in O. sinensis for the first time, which provided insights into its physiological functions that shall be further explored.



Advancing Nucleic Acid Therapeutics: Trehalose-Sucrose Macrocycles As Non-Viral Vectors

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The remarkable success of COVID-19 mRNA vaccines has highlighted lipid nanoparticles (LNPs) as exemplary nonviral vectors for nucleic acid therapeutics. However, LNPs encounter stability and immunogenicity issues, stemming from their heterogeneous composition comprising ionizable and neutral lipids, stabilizers, polyethylene glycol, cholesterol, etc. Simplifying vector design to one-component formulations based on molecularly defined carriers presents an attractive alternative. Most efforts have focused on macrocyclic molecular nanoparticle platforms, notably cyclomaltooligosaccharides (cyclodextrins; CDs; Figure 1A) and α,α'-trehalose-based macrocycles (cyclotrehalans; CTs; Figure 1B), which have demonstrated significant potential. Introducing a novel prototype within this category, here we present cyclosucrotrehalans (CSTs), distinguished from CTs by the inclusion of a sucrose module in the backbone. CSTs offer a modular synthesis approach, facilitating the selective incorporation of cationic and lipid domains with precise spatial arrangements (Figure 1C), enhancing nucleic acid nanocomplexation efficiency. Moreover, the sucrose segment is expected to enhanced flexibility and increased pH-sensitivity, facilitating endosomal escape. This communication outlines the synthesis, supramolecular properties, formulation characteristics, and transfection capabilities of CSTs, illustrating their potential as versatile nucleic acid carriers.

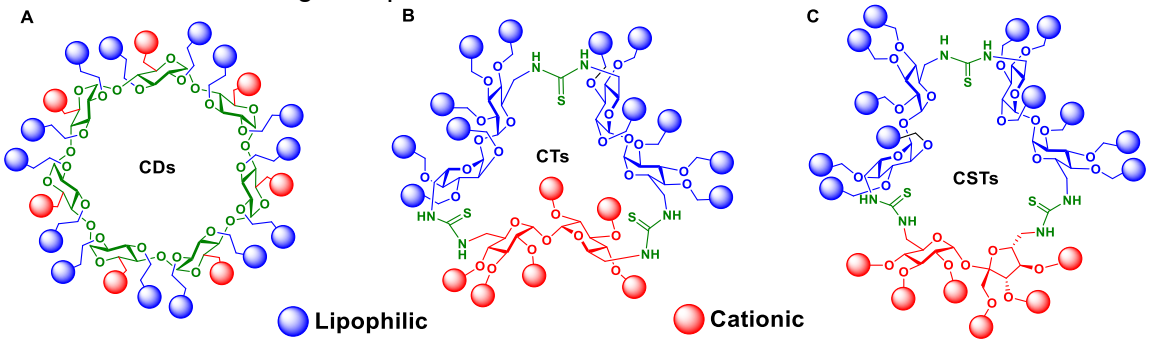

Figure 1. Schematic representation of the previously reported polycationic amphiphilic CDs and CTs, and of the new CSTs synthesized in this work.

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Chemoenzymatic Synthesis Of *N*-Glycans Of *S. Mansoni* To Investigate Anti-Parasite Immune Responses

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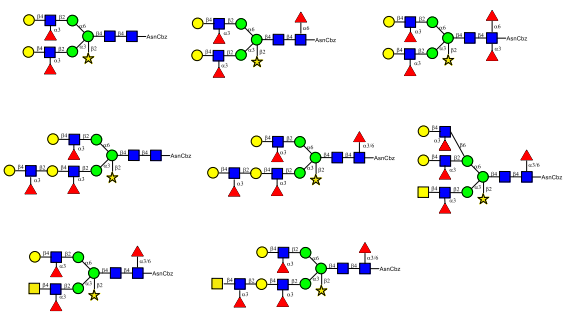
Schistosomiasis poses a public health challenge due to its high infectiousness and chronic persistence. Schistosoma species express a variety of glycan antigens on their cell surface that play pivotal roles in host immunity and escape strategies. In particular, *Schistosoma mansoni* displays a remarkable structural diverse set of *N*-glycans that have various core modifications and terminal epitopes. Modifications such as *α*-1,3/1,6 fucose (CF3 and CF6) and *β*-1,2 xylose (CX) are typical of schistosome glycans and recognized as common allergens[1]. While core xylosylation does not inhibit terminal epitope recognition, combination of core xylose and *α*-1,3 fucose constitute cross-reactive carbohydrate determinants. Terminal antigens, including motifs such as Lewis X (Le^x), Lac-di-NAc (LDN), and fucosyl Lac-di-NAc (LDN-F) are present on surface *N*-glycans. They can engage with the DC-SIGN on dendritic cells, facilitating pathogen internalization and antigen processing making DC-SIGN a critical target for therapeutic interventions. Multi-antennary *N*-glycans can have higher binding avidity for DC-SIGN than mono-valent epitopes, emphasizing the importance of *N*-glycan architecture on immune recognition and response [2]. However, such modulation of binding is not well-understood due to a lack of panels of well-defined *Schistosoma* glycans.



We are to exploring the full potential of schistosome *N*-glycans to modulate immune responses and develop innovative therapeutics by synthesizing large panels of di- and tri- antennary *N*-glycan having different patterns of core fucosylation (CF3/CF6), core xyloside and terminal epitopes such as Le^x, LDN, LDN-F. The synthetic approach is based on newly identified glycosyl transferases and chemoenzymatic strategies that make it possible to modify each antenna with a unique epitope. The compounds have been printed as a glycan microarray to examine interactions with glycan binding proteins and serum antibodies. This research is providing an understanding of *N*-glycan-mediated immune dynamics and supports the utilization of these structures in vaccine development.

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An Integrated One-Tube *N*-Glycan Preparation Method For Mass Spectrometry-Based Glycome Profiling

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Sialic acids attached to nonreducing ends of *N*-glycans play diverse biological roles. In particular, different linkage isomers (α-2,3- or α-2,6-linkage) of sialylated *N*-glycans are involved in specific interactions and physiological events. Accurately characterizing sialylated glycans and distinguishing linkage isomers pose an inherent challenge due to the instability of sialic acids and their structural similarity. Moreover, highly sensitive and reproducible profiling of the glycome from a limited amount of biological sample is often hindered by sample loss during preparation and inferior ionization efficiency of glycans in mass spectrometry (MS). Esterification and amidation are commonly used derivatization strategies that provide enhanced structural stability, reduced identification ambiguities, and the improved MS signal of both sialylated and non-sialylated glycans in positive ion mode.

Here, we have established a rapid and robust sample preparation method for MS-based sialylated *N*-glycomics analysis, integrating sample lysis, protein reductive alkylation, *N*-glycan release, non-linkage-specific or linkage-specific sialic acid derivatization, reducing end labeling, glycan enrichment and desalting in a single microcentrifuge tube. The output glycans are ready for matrix-assisted laser desorption/ionization (MALDI)-MS analysis, showing improved signal. The entire one-tube process can be completed within 10 hours without sample transferring, enabling high throughput and highly sensitive *N*-glycan analysis. In our method, linkage-specific salic acid derivatization consists of two sequential steps, resulting in the ethyl esterification of α-2,6-linked sialic acids and subsequently amidation with ammonia of α-2,3-linked sialic acids, and all the reaction conditions are mild, allowing unstable modifications on sialic acids such as *O*-acetylations to be preserved. Additional MS signal boosting through all glycans was obtained by reductive amination. The one-tube method not only facilitates the MALDI-MS glycan profiling of glycoprotein at the nanogram level, hundreds of cells, and other complex biological samples in minute amounts but also provides linkage information of sialic acid isomers with high accuracy. Overall, the integrated one-tube method offers a simplified and robust strategy for comprehensive *N*-glycome profiling of precious biological samples.

Structural Diversity Among *Aeromonas Salmonicida* O-Polysaccharides Isolated From Ill And Healthy Fish

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Aeromonas salmonicida is an important pathogen of fish, producing the systemic disease furunculosis. Since the annual worldwide losses of farmed fish due to diseases involve millions of dollars, this pathogen has been subjected to considerable investigation [1]. One of the principal virulence factors of this pathogen is an S-layer (named the A-layer) that consists principally of a 2-dimensional crystalline tetragonal protein (A-protein) array, which is tethered to the cell by lipopolysaccharide (LPS). The A-layer appears to cover most of the surface of virulent *A. salmonicida*, although some LPS may also be exposed. This structure has been shown to protect this bacterium from killing by serum in a manner that somehow requires both LPS and the A-layer. The LPS is one of the major structural and immunodominant molecules of the outer membrane. It consists of three domains: lipid A, core oligosaccharide, and O-specific polysaccharide (O-antigen).
By now, only one structure of *A. salmonicida* lipid A, one core oligosaccharide and three O-polysaccharide (structure A, B, C) have been identified and published [2-7]. The chemical structure of LPS molecule together with the A-layer can strongly determine the bacterial resistance against antibiotics, what has been known as a problem for many decades.
The 41 of *A. salmonicida* strains from ill and healthy fish have been isolated. The O-polysaccharides were compared by using ^{1}H,^{13}C high-resolution magic angle spinning (HR-MAS) NMR spectroscopy. The structure A has been identified in 31 strains, the structure B in 5, and the structure C in 3. The O-polysaccharide structure in strains 11/A/658 and 10/A/646 has been identified as a new one. This new structure was investigated by ^{1}H and ^{13}C NMR spectroscopy and matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The following structure of the linear hexasaccharide repeatig unit of *A. salmonicida* O-antigen has been established:

→3)-α-L-Rha*p*-(1→3)-α-D-Man*p*NAc-(1→2)-β-D-Glc*p*-(1→3)-α-L-Rha*p*2OAc4OAc-(1→3)-β-D-Man*p*NAc-(1→3)-α-D-Glc*p*-(1→

Identification of *A. salmonicida* O-polysaccharides (different LPS chemotypes) presented in ill fish could be very useful for veterinarian either to confirm the etiologic agent of disease and either in improving biosecurity of fish farms: by having a quick tool to detect the presence of pathogenic *A. salmonicida* O-polysaccharides before the level of the pathogen reach density and causes disease.

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Deciphering The Complexity Of Microbiota

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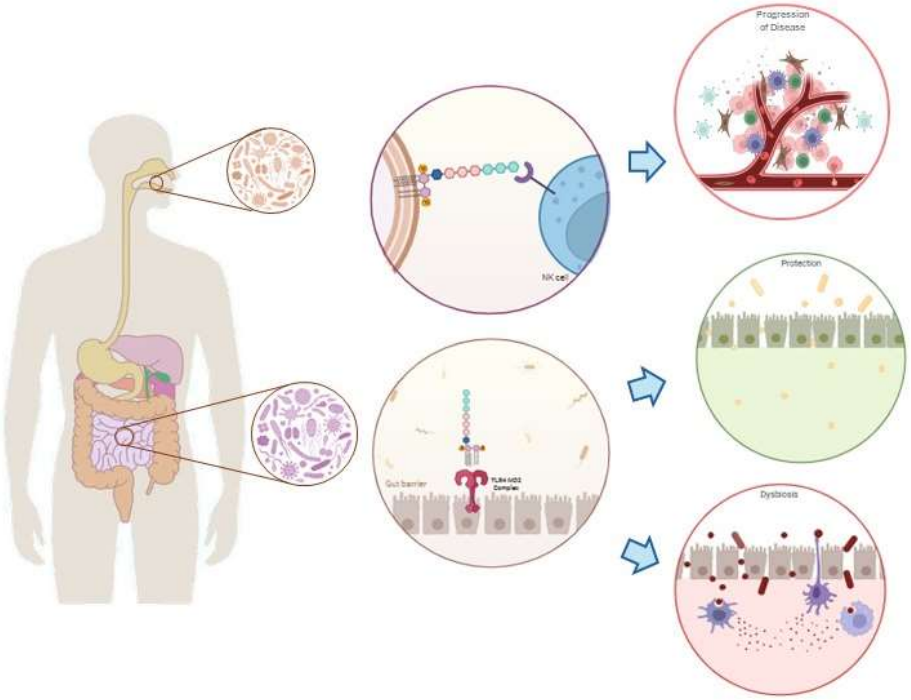
In recent years our understanding of the crucial role played by the gut microbiota in human health has increased. Lipopolysaccharide (LPS) derived from gut bacteria exhibit a nuanced functionality and are involved in countless critical pathways linked to their precise structural composition. They can trigger systemic pro-inflammatory responses in the presence of pathogens but can also evoke immunomodulatory responses in commensal organisms. [1] These intricate processes are integral to determining outcomes related to disease, commensalism, and the establishment of mutualistic relationships within the gut ecosystem. In addition, the elucidation of bacterial LPSs is challenged by their structural complexity and by bacterial diversity of the gut. Nevertheless, not only the gut microbiota is involved in such important processes, in the rest the body also plays essential roles, for example in the oral microbiota *Fusobacterium nucleatum*, Gram-negative obligate anaerobe, has beed identified as a key player in dysbiosis and colorectal cancer progression,[2] has further highlighted the intricate relationship between humans and bacteria. In this context, we report on the characterization of LPS from different human-associated bacteria achieved through the combined use of chemical, spectroscopic, spectrometric, computational techniques, and preliminary immunological assays.



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De Novo Glycan Sequencing by An Electronic Excitation Dissociation Based Ms^2-Guided Ms^3 Approach

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Abstract

Comprehensive de novo glycan sequencing remains an elusive goal due to the structural diversity and complexity of glycans. Present strategies employing collision-induced dissociation (CID) and higher energy collisional dissociation (HCD)-based multi-stage tandem mass spectrometry (MS^n) or MS/MS combined with sequential exoglycosidase digestions are inherently low-throughput and difficult to automate. Compared to CID and HCD, electron transfer dissociation (ETD) and electron capture dissociation (ECD) each generate more cross-ring cleavages informative about linkage positions, but electronic excitation dissociation (EED) exceeds the information content of all other methods and is also applicable to analysis of singly charged precursors. Although EED can provide extensive glycan structural information in a single stage of MS/MS, its performance has largely been limited to FTICR MS, and thus it has not been widely adopted by the glycoscience research community. Here, the effective performance of EED MS/MS was demonstrated on a hybrid Orbitrap-Omnitrap QE-HF instrument, with high sensitivity, fragmentation efficiency, and analysis speed. In addition, a novel EED MS^2-guided MS^3 approach was developed for detailed glycan structural analysis. Automated topology reconstruction from MS^2 and MS^3 spectra could be achieved with a modified GlycoDeNovo software. We showed that the topology and linkage configurations of the $Man_9GlcNAc_2$ glycan can be accurately determined from first principles based on one EED MS^2 and two CID-EED MS^3 analyses, without reliance on biological knowledge, a structure database or a spectral library. The presented approach holds great promise for autonomous, comprehensive and de novo glycan sequencing.

Cyclodextrin-Based Giant Bola-Amphiphiles: Synthesis, Self-Assembly And Gene Delivery Capabilities

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Nucleic acid therapeutics face challenges including poor bioavailability and susceptibility to chemical and enzymatic degradation, necessitating effective delivery systems. Among nonviral vectors, lipid and polymer carriers have been the most extensively studied, with lipid vectors notably utilized in the successful development of the first mRNA vaccines for COVID-19. Alternatively, molecular vectors leveraging macrocyclic platforms like cyclodextrins (CDs) offer a promising avenue for tailored vector design, owing to their well-defined structure and cooperative multivalency. In this context, Janus-type amphiphilic derivatives that capitalize simultaneously on the unique structural properties of CDs and the delivery characteristics of lipid and polymer vectors have been extensively explored. We sought to investigate whether altering the arrangement of cationic and lipid domains would influence the self-assembly and nucleic acid delivery capabilities. Specifically, in this study, we designed, synthesized, and characterized giant bola-amphiphilic βCD vectors with varied cationic heads and hydrophobic connectors evenly distributed at opposing O6 and O2 positions (Figure 1), emulating bola-amphiphilic dendrimers A comprehensive examination of these novel systems, focusing on their supramolecular properties and nucleic acid binding, will be presented.

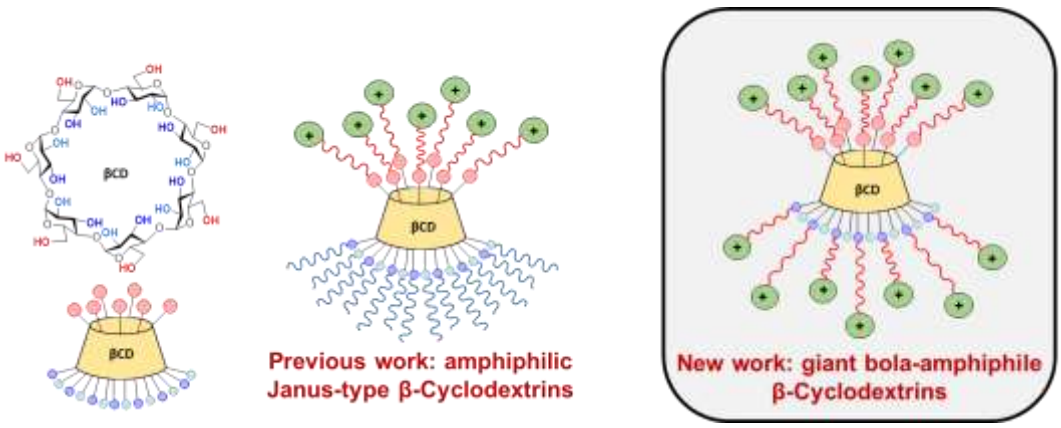

Figure 1. Schematic representation of CD-based Janus-type and giant bola-amphiphiles.

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Development Of Low-Molecular-Mass Organo-Gelators Derived From Starch That Can Gel Various Oils At 1 Wt%

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1. Introduction: Various industries, including cosmetics, food, and pharmaceuticals, require gel thickeners that can regulate oil flowability. Moreover, naturally derived functional ingredients have gained attention due to the recent SDGs. To cater to these industry needs, we have developed novel organo-gelators capable of gelling a variety of oils at only 1 wt%. We synthesized these novel compounds by esterifying 1,5-anhydro-D-glucitol (AG), derived from naturally abundant starch via fermentation technology, with various linear saturated fatty acids or linear saturated fatty acids containing amide groups in the chain[1][2].

2. Results and discussion:

2.1 Syntheses: We synthesized the C-AG series, where linear saturated fatty acids (10-18 and 22 carbons) of different lengths are introduced by reacting AG with various fatty acid chlorides, and the GABA-AG series, where linear saturated fatty acids (12, 14, 16 and 18 carbons) are attached to the amino group of 4-aminobutyric acid (GABA) via an amide bond and then esterified with a GABA carboxyl group and AG, using organic chemical methods (Figure 1).

2.2 Gelation Experiments: In the C-AG series, C16AG with 16 carbons exhibited the highest gelation ability[1], and in the GABA-AG series, it was C14GABA-AG with 14 carbons[2]. Gelation tests were conducted on various oils at 1 wt% of each gelator, and a variety of oils from low to high polarity were gelatinized. Interestingly, C16AG also gelled ethanol and various silicone oils (Figure 1)[1].

2.3 High temp cost-effective syntheses: A high-temperature, solvent-free synthesis method was established for the practical application of the C-AG series. The synthesis was successfully accomplished with more than 90% isolated yield by simply mixing powdered AG and powdered fatty acids and stirring at 230°C for 4 hours. This enabled very low-cost synthesis[3].

3. Conclusion: Currently, we are working on the social implementation of these gelling agents in fields such as cosmetics and civil engineering, etc.

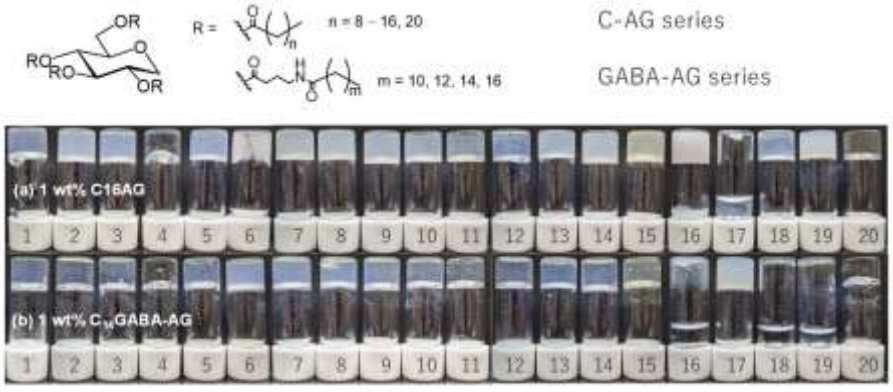

Figure 1: Actual photographs of the formed gels of each solvent. (a) 1 wt% gel of C16AG in various organic solvents. (b) 1 wt% gel of C14GABA-AG in various organic solvents. The solvents of each gel were as follows: (1) liquid paraffin #350, (2) liquid paraffin #70, (3) olive squalene, (4) hydrogenated polyisobutene (kinetic viscosity 300 mm^2/s), (5) pentaerythrityl tetraisostearate, (6) isopropyl myristate, (7) ethylhexyl palmitate, (8) triethylhexanoin, (9) jojoba oil, (10) canola oil, (11) castor oil, (12) diisostearyl malate, (13) polyglyceryl-2 triisostearate, (14) 2-octyl-1-dodecanol, (15) rice bran oil, (16) ethanol (99.5), (17) ethanol/H_2O v/v 8:2, (18) dimethicone (viscosity 10 mm^2/s), (19) cyclopentasiloxane, and (20) diphenylsiloxy phenyl trimethicone.

4. Acknowledgments: This work is supported by the Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) from the Japan Science and Technology Agency (JST), Grant Number JPMJTR20U3, and JSPS KAKENHI, Grant Number JP21K05418.

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Synthesis of 2-Indolyl C-Glycoside Neopetrosins A and C and Congeners via Ni-Catalyzed Photoreductive Cross-Coupling

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Abstract

Neopetrosins A (**1**) and C (**2**) (**Figure 1A**), two 2-indolyl *C*-*α*-d-mannopyranosides, were identified from the marine sponge *Neopetrosia chaliniformis* by Li and co-workers in 2022. (1) Interestingly, neopetrosin A (1), fused with a lactone, exerted a moderate hepatoprotective effect in a zebrafish model at the concentration of 20 *μ*M, whereas the uncyclized counterpart **2** showed no activity. (1) In fact, a few compounds containing the 2-indolyl C-glycosidic linkage have been found in nature. In 2011, He et al. reported cytotoxic 2-indolyl C-glycoside **3** from the medicinal plant Isatis indigotica; (2) however, the structure of the sugar unit in **3** remained uncertain. (3) More prominently, *C*-mannosyl tryptophan (4), which was initially found in RNase 2 from human urine and erythrocytes, (4) has been proved as a promising biomarker of renal function. (5) Herein we report a necessary modification of the Ni-catalyzed photoreductive cross-coupling reported by Goddard-Borger et al.(6) using indole C2-iodides as the coupling partners, and the enabling synthesis of 22 2-indolyl C-pyranosides, including neopetrosins A (**1**) and C (**2**) (**Figure 1B**).

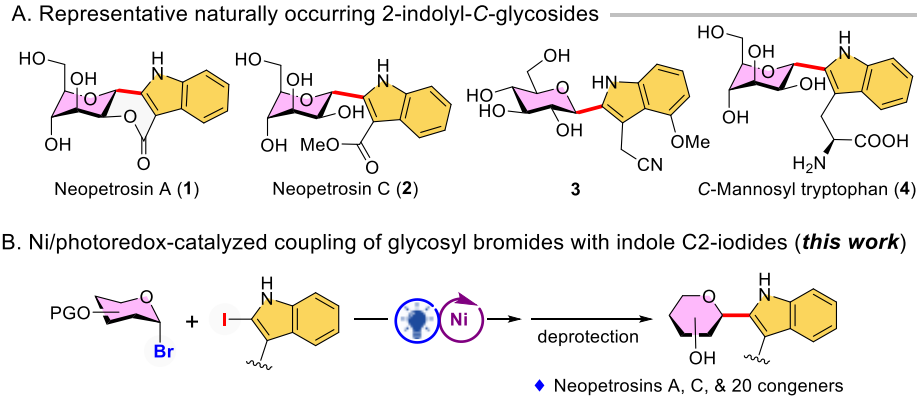

Figure 1. Representative naturally occurring 2-indolyl-C-glycosides, PG = protecting group.

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Genetic Basis Of Alpha-1,3 Galactose Epitopes In Animal Reservoirs Of Coronaviruses

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The Galα1-3Galβ1-4GlcNAc-R αGal epitope is found in all nonprimate mammals, lemurs, and New World monkeys but not in Old World monkeys, apes or humans[1]. Humans possess natural anti-αGal antibodies that have been hypothesized to prevent virus spillover through neutralization and complement-mediated inactivation of glycosylated viruses carried by animal hosts. In this study, we cloned the α-1,3-galactosyltransferase family from the animal reservoir of coronaviruses and investigated its ability to modify surface glycoproteins of the SARS-CoV-2 pseudovirus. The results showed that the bat and pangolin genomes contain functional α3GalT1 and α3GalT2 glycosyltransferase genes. The bat and pangolin α3GalT1 can catalyze the expression of the αGal epitope on SARS-CoV-2 envelope glycoproteins. αGal antibodies neutralize SARS-CoV-2 pseudoviruses packaged in glycoengineered 293T cells expressing the αGal epitope. Our findings revealed the RNA transcription of functional α3GalTs in animal reservoirs of coronaviruses. Glycoengineered human cells expressing the αGal epitope may serve as safer tools for studying coronaviruses. Ongoing studies are being focused on the role of anti-αGal natural antibodies in cross-species virus transmission, as well as the mechanism through which such antibodies are induced in humans. The minimum level of anti-αGal natural antibodies for protection in vivo against animal viruses remain to be determined in at-risk populations such as infants[2].

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GalNAc-based degradable glycopolymer for liver-targeted siRNA delivery

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RNA interference technology has made great progress in cancer therapy by silencing pathogenic gene and down-regulating pathogenic protein [1]. Survivin, a member of inhibitor of apoptosis proteins (IAPs) [2], which is overexpressed in various tumor cells, plays an important role in inhibiting cell apoptosis, promoting cell proliferation and regulating cell cycle, making it a potential cancer therapeutic target. Knocking down its expression by RNA interference will dramatically inhibit tumor proliferation. Among numerous nucleic acid delivery system, GalNAc-siRNA conjugates [3] exhibit superior liver-targeted properties due to their ability to specifically bind to asialoglycoprotein receptors (ASGPR), which are highly expressed on the surface of hepatocytes. Nevertheless, the use of conventional glycopolymer carriers is associated with significant toxicity due to their tissue accumulation, which restricts their utility in nucleic acid therapy. Numerous efforts have been undertaken to develop biodegradable polymers for gene delivery [4]. In this study, we design and construct several different GalNAc-based glycopolymers [5] for Survivin-siRNA delivery. Due to liver targeting property of GalNAc units, Survivin-siRNA is specifically delivered to liver cancer cells by covalent coupling or electrostatic adsorption with carriers. In view of the pH responsiveness of glycopolymers, siRNA is effectively released in the acidic lysosome, which is beneficial for its subsequent gene interference function. *In vitro* experiments show excellent gene silencing effect of these nucleic acid delivery system, providing a new strategy for liver cancer treatment.

Keywords: RNA interference; GalNAc-based glycopolymer; Asialoglycoprotein receptor; Survivin; Degradable polymer

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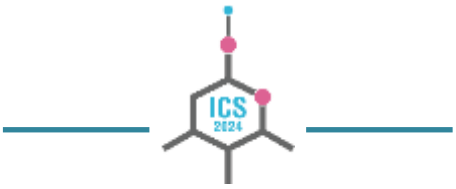

Other

Synthetic glycans bearing zwitterionic modifications For dipteran glycan array

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The interactions of lectins, pentraxins or antibodies with glycans from insect sources, especially from dipteran species, such as *Drosophila melanogaster*, *Glossina morsitans*, *Aedes aegypti* and *Anopheles gambiae*, which are the model organisms or pathogen vectors are poorly characterised. In particular, mosquitoes which are important vectors for a number of human and animal pathogens in the tropics, including the malaria parasite *Plasmodium,*[1] flaviviruses such as Dengue[2] or West Nile Virus deserve special attention, while tsetse flies (*G. morsitans*) transmit the African trypanosomes which cause sleeping sickness in humans and Nagana in cattle. Significantly, climate change is driving an increased geographical range of these insect-borne diseases, whose transmission depends on glycan-mediated interactions.

Many of the non-mammalian N-glycomes show modifications with anionic and /or zwitterionic moieties such as sulphate or phosphoethanolamine (PE) and phosphorylcholine (PC).[3-5] The best-known insect-transmitted pathogens are the flaviviruses, including Zika, West Nile and Dengue viruses specifically harbored by mosquitoes. The entry of flaviviruses into mammalian cells is also lectin-mediated and thus dependent on mosquito-type glycosylation of viral glycoproteins, whereby N-glycans may play a role in binding the mosquito cell-derived viral protein to DC-SIGN.[6] Pentraxins (serum amyloid P and C-reactive protein),[7, 8] which are rather ancient components of the mammalian innate immune system capable of activating the complement cascade, are also known to bind zwitterionic moieties.

In order to have a set of basic antennal glycan elements for the study of protein-glycan interactions, we have chemically synthesised a series of linker-equipped trisaccharides, focusing on zwitterionic modifications of the terminal GlcNAc moiety that mimic insect N-glycan antennae motifs. Using advanced P(III) chemistry, we developed a novel approach to introduce a phosphoethanolamine modification into synthetic glycans. A series of synthetic PE/PC-modified trisaccharides were used for glycan array binding studies.

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Direct Site-Specific Detailed Glycan Characterization By Higher Energy Electron Activated Dissociation Tandem Mass Spectrometry

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Glycosylation is recognized as the most complex post-translational modification due to the presence of macro- and micro-heterogeneities, with its biological function influenced by the glycosylation site and the glycan fine structure[1]. Site-specific detailed glycan characterization remains a major challenge by existing tandem mass spectrometry (MS/MS) methods, where collision-based dissociation produces predominantly glycosidic cleavages and electron capture or transfer dissociation generates mostly peptide backbone cleavages[2]. Alternative approaches are therefore needed to achieve simultaneous site localization and glycan structural assignment at the intact glycopeptide level. Here, higher energy electron activated dissociation (heExD) MS/MS, in particular electronic excitation dissociation (EED), demonstrated extraordinary potential for in-depth N-glycopeptide sequencing by providing information on the glycosylation site, peptide sequence, glycan topology and linkages in one analysis[1,3]. Higher energy collision-induced dissociation (HCD) and electron-transfer/higher-energy collisional dissociation (EThcD) MS/MS spectra were acquired on an Orbitrap Lumos mass spectrometer (Thermo Scientific). All other ExD MS/MS experiments were performed on a Q Exactive HF instrument (Thermo Scientific) modified with an Omnitrap platform (Fasmatech). Manual data interpretation was achieved using the Glycoworkbench software and a home-built program. In this work, we have performed a systematic investigation of the effect of electron energy and charge carriers on the ExD fragmentation behaviors of N-linked glycopeptides bearing different glycan structures, including high-mannose glycans and sialylated glycans. Overall, we have found that, for site-specific glycopeptide analysis, EED outperforms all other fragmentation methods tested here, including HCD, sceHCD, ETD, EThcD, and low-energy ECD, as it can reveal the peptide sequence, determine the glycosylation site, and elucidate the glycan topology and the linkages, in a single MS/MS experiment. The exceptional EED efficiency achieved on the Omnitrap instrument, with a typical irradiation time of under two hundred milliseconds, makes EED MS/MS compatible with on-line LC separation, which could provide a powerful tool for structure-focused glycoproteomics studies.

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Convenient Synthesis Dragocins A-C

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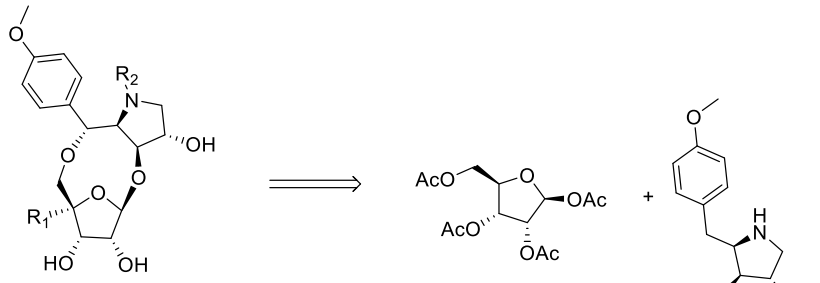
Dragocin A-C were isolated by Gerwick and colleagues from a marine cyanobacterium found near Boca del Drago on the Caribbean coast. Preliminary biological investigation revealed dragocins exhibit moderate cytotoxic activity against human lung cancer cells (H-460). In order to explore the rich activity of the tricyclic [6.2.1]-undecane core structure of dragocins A-C, we have developed an efficient and modular synthetic method for the synthesis of the dragocins skeleton using dehydrogenative cross-coupling strategy.



Dragocin A (**1**):R_1= OMe, R_2= CH_3
Dragocin B (**2**):R_1= Cl, R_2 = H
Dragocin C (**3**): R_1= Cl, R_2= CH_3

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Convergent Synthesis Of Oligosaccharide Derivatives Related To Galactomannan From *Antrodia Cinnamomea*

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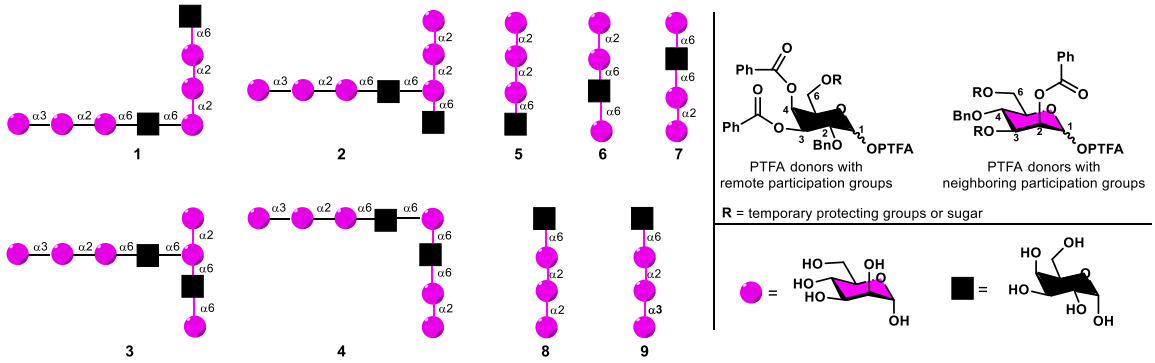
Galactomannan with an octasaccharide-repeating unit (ACP) was isolated from medicinal mushroom that significantly enhanced the phagocytosis and bactericidal activity of J774A.1 murine macrophages against *Escherichia coli*.[1] Now, we have completed the efficient synthesis of homogenous and structurally well-defined tetrasaccharides (**5**-**9**) and octasaccharides(**1**-**4**) *via* highly convergent [2+2] and [4+4] glycosylation strategy. In these syntheses, di- and tetrasaccharide trifluoroimidate (PTFA)[2] were proved to be more active glycosyl donors to efficiently accomplish the synthesis of target molecules. Also, all *α*-glycosidic bonds were stereoselectively controlled *via* the neighboring group participation effect and the remote participation effect[3] and successfully achieved in satisfying yields. The immunological evaluation of synthetic ACP oligosaccharides is being tested.



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Engineering and Photoelectric Driving Application Of Lytic Polysaccharide Monooxygenases From *Myceliophthora Thermophila*

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Lytic polysaccharide monooxygenases (LPMOs) are a newly discovered class of metalloenzymes that show great potential in degrading recalcitrant polysaccharides such as cellulose. The copper active site of LPMOs is activated by reductants and use O_2 or H_2O_2 as co-substrates to oxidatively cleave glycosidic bonds. We systematically explored strategies to enhance the performance of LPMOs through enzyme engineering modifications and the development of innovative reaction systems.

We present the first comprehensive analysis of N- and O-glycosylation within the catalytic domain of LPMO, delving into their complex action mechanisms. The experimental results revealed the N- and O-glycosylation sites and glycan structures within the LPMO catalytic domain, showing significant heterogeneity in glycosylation and a widespread presence of O-glycosylation. By constructing 38 glycosylation mutants, we deeply explored the effects of N- and O-glycosylation on the catalytic activity, structural stability, and overall performance of LPMO. The research findings suggest that glycosylation modifications can significantly affect the catalytic efficiency by modulating the interaction interface between LPMO and the substrate, as well as the interaction between the copper active site and the substrate.

In modular engineering modifications, we explore the impact of CBM on the performance of LPMO. By constructing CBM-truncated variants and CBM fusion mutant, it was found that CBM significantly influenced substrate binding affinity, enzymatic activity, and H_2O_2 tolerance. These results show that CBM can significantly enhance LPMO catalytic activity and reduce auto-oxidative damage to the copper active site by enhancing substrate binding affinity and optimizing the utilization of H_2O_2. Furthermore, through MD simulations, we confirmed that CBM fusion increases the binding sites between LPMO and its substrate. CBM can adjust the spatial proximity between the copper active site and the substrate, facilitating localized cleavage and influencing the interaction between the copper active site and H_2O_2[1].

In terms of innovation in the reaction system, we successfully developed a photoelectrical driven LPMO reaction system. Compared to the conventional ascorbic acid reductant, our method increased the enzyme activity by more than 2.3 times. By controlling the lighting conditions, the system can regulate the generation of reductants, optimizing the stability and efficiency of the LPMO catalytic process, and achieving control over the catalytic reaction with light switches. Additionally, the experimental results confirmed that a continuous supply of reductants is essential to maintain high catalytic activity in O_2-driven LPMO catalysis. In contrast, in H_2O_2-driven LPMO catalysis, only the initial addition of a reductant is required to initiate the reaction [2].

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Esswntial Roles Of Protein O-Glycosylation In Plant Growth And Defense Response

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Glycosylation, a crucial co- and post-translational modification of proteins, plays significant roles in numerous biological processes, including plant growth and defense responses. Emerging evidence suggests the importance of protein glycosylation, yet the roles of newer types, such as protein O-GlcNAcylation and O-fucosylation, require further exploration, which we address in this study.

The impact of protein O-GlcNAcylation on plant growth was partly revealed through studies related to *At*AGM. As an essential enzyme in the uridine diphosphate (UDP)-GlcNAc biosynthesis pathway, the significant role of N-acetylglucosamine phosphate mutase (AGM) remains unknown in plants. In the present study, a functional plant AGM (*At*AGM) was identified from *Arabidopsis thaliana*. *At*AGM catalyzes the isomerization of GlcNAc-1-P and GlcNAc-6-P, and has broad catalytic activity on different phosphohexoses. UDP-GlcNAc contents were significantly decreased in *At*AGM T-DNA insertional mutants, which caused temperature-dependent growth defects in seedlings and vigorous growth in adult plants. Further analysis revealed that protein O-GlcNAcylation but not N-glycosylation was dramatically impaired in *Atagm* mutants due to UDP-GlcNAc shortage. Combined with the results from O-GlcNAcylation or N-glycosylation deficient mutants, and O-GlcNAcase inhibitor all suggested that protein O-GlcNAcylation impairment mainly leads to the phenotypic variations of *Atagm* plants [1]. In conclusion, based on the essential role in UDP-GlcNAc biosynthesis, *At*AGM is important for plant growth mainly via protein O-GlcNAcylation-level regulation.

The role of protein O-glycosylation in plant defense was explored using glycosylation-deficient mutants in the interaction between *Arabidopsis* and *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*). Analysis across multiple levels, including phenotypic data, disease indices, bacterial growth on infected leaves, and the expression of *Pst* marker genes, indicated that protein O-GlcNAcylation-deficient mutants (*sec*-1, 2, and 5) exhibited more severe symptoms compared to wild type (WT), highlighting a positive role of protein O-GlcNAcylation in plant immunity. Conversely, the protein O-fucosylation mutant *Atpofut* displayed enhanced defense capabilities compared to WT, suggesting a negative role in plant defense. These contrasting results hint at potential antagonism between protein O-GlcNAcylation and O-fucosylation, possibly due to competitive modification at the same sites on key defense proteins, a hypothesis we plan to investigate further.

In summary, protein O-GlcNAcylation positively influences both plant growth and defense responses, whereas protein O-fucosylation appears to play a detrimental role in plant immunity.

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Protein-Bound Beta-Glucan From Edible Mushroom Coriolus Versicolor Alleviates Obesity Via Ra-Iga-Intestinal Akkermansia Muciniphila

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Obesity has become a major disease burden worldwide and seriously threatens public health. There is an urgent need to develop strategies for the prevention and treatment of obesity. *Coriolus versicolor* is a food source mushroom that also has been used in traditional Chinese medicine for thousands of years. An extremely broad range of physiological activities have been linked to the use of *C. versicolor*, including immunomodulatory, anticancer, antioxidant and hepatoprotective effects. We separated the polysaccharides from the dried fruiting bodies of *C. versicolor* by water extraction and ethanol precipitation. The chemical structure was well elucidated with overall consideration of monosaccharide composition, methylation analysis and 1D/2D-NMR spectra data. With molecular weight of 29.7 kDa, the isolated polysaccharide was composed of (1→4)-β-/(1→3)-β-D-glucopyranosyl group as backbone with branches attached at O-6 site. Further, the anti-obesity activities of this polysaccharide (named as PBG) were evaluated. Our results indicated that PBG can reduce obesity and metabolic inflammation in mice fed with a high-fat diet (HFD). Gut microbiota analysis reveals that PBG markedly increases the abundance of *Akkermansia muciniphila*, although it does not rescue HFD-induced change in the Firmicutes to Bacteroidetes ratio. It appears that PBG alters host physiology and creates an intestinal microenvironment favorable for *A. muciniphila* colonization. Fecal transplants from PBG-treated animals in part reduce obesity in recipient HFD-fed mice. Further, PBG is shown to upregulate expression of a set of genes related to host metabolism in microbiota-depleted mice. Since the intestinal microbiota could utilize immunoglobulin A (IgA) for mucosal colonization, thus, we deduced that PBG could promote *A. muciniphila* colonization via IgA secretion. In order to verify the key role of IgA in the anti-obesity effect of PBG, we examined the IgA content in different intestinal parts. Compared with the HFD group, the IgA contentin plasma and stools of PBG treated mice showed significantly increased, indicating that PBG could significantly promote IgA secretion. The IgA content was positively correlated to the *A. muciniphila* content. Moreover, the $IgA^{-/-}$ C57BL/6 mice were adopted, and PBG treatment could significantly increase the abundance of intestinal *A. muciniphila* abundance of $IgA^{-/-}$ mice. The in-depth multi-omics analysis showed that PBG profoundly affected the retinoic acid metabolism pathway with ALDH1A1, ALDH1A2, retinol dehydrogenase 1 (RDH1) and RDH9 genes highly expression. The PCR verification analysis was performed on the target genes of retinoic acid metabolic pathway. All these data highlighted that PBG might exert its ant-obesity effects by affecting RA metabolism pathway, promoting intestinal IgA secretion thus facilitating intestinal *A. muciniphila* colonization. Our study revealed the molecular mechanism of PBG from *C. versicolor* in obesity improvement and provided a reference for intestinal immunity of polysaccharide prebiotics in anti-obesity research, which is of great significance for the prevention and treatment of obesity.

Keywords: *Coriolus versicolor*; beta-glucan; obesity; *Akkermansia muciniphila*; polysaccharide;

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Ion Mobility Mass Spectrometry For Glycomics: Challenges And Opportunities When Met With Artificial Intelligence

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***Abs*tract**

Glycans possess complex and unique stereochemical features, endowing them with distinguishing functions. An urgent need to develop a glycan sequencing method to elucidate the determine stereo diversity of glycan precisely. A currently promising approach is ion mobility mass spectrometry (IM-MS), which offers an additional dimension separation and collision cross-section (CCS) value for the unambiguous identification of glycan isomers. This research highlights the evolution of IM-MS technologies to support comprehensive glycan analysis. Furthermore, and the CCS values of different food glycan are collected based on Fenton degradation and HILIC-IM-MS strategy. We attempt to decipher unknown food-derived polysaccharide structures through matching 4D data (Rt, *m/z*, MS/MS, CCS), rapidly uncovering more detailed structural information. Eventually, we utilized AI prediction software (CCSondemand) to predict the CCSs of food-derived glycans, achieving reliable predictions with an error range controlled within 5%, with the smallest error being only 0.3%. This fully demonstrates that by combining IM-MS technology and deep learning techniques, we can enhance the resolution strategy of food-derived glycobiology, increasing throughput and depth. This research not only aims to provide a deeper understanding of IM-MS on food-derived glycan research but also discuss the road toward AI era.

Keywords: Food glycan; Structure; Ion mobility spectrometry; Ion mobility-mass spectrometry; Collision cross section; Artificial intelligence

Integrated Analysis Of Natural Glycans Using A Versatile Pyrazolone-Type Heterobifunctional Tag Anpmp

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Glycans mediate various biological processes through carbohydrate-protein interactions, and glycan microarrays have become indispensable tools for understanding these mechanisms. However, advances in functional glycomics are hindered by the absence of convenient and universal methods for obtaining natural glycan libraries with diverse structures from glycoconjugates. To address this challenge, we have developed an integrative approach that enables one-pot release and simultaneously capture, separation, structural characterization, and functional analysis of *N/O*-glycans. Using this approach, glycoconjugates are incubated with a pyrazolone-type heterobifunctional tag-ANPMP to obtain glycan-2ANPMP conjugates, which are then converted to glycan-AEPMP conjugates. We prepared a tagged glycan library from porcine gastric mucin, soy protein, human milk oligosaccharides, etc. Following derivatization by *N*-acetylation and permethylation, glycans were subjected to detailed structural characterization by ESI-MS^n analysis, which revealed more than 83 highly pure glycan-AEPMPs containing various natural glycan epitopes. A shotgun microarray is constructed to study the fine details of glycan-bindings by proteins and antisera.

Keywords: Glycans; ANPMP; ESI-MS; 2D-HPLC; Natural glycan microarray

Revisiting The Structure Of Arabinogalactan From *Lycium Barbarum* And The Impact Of Its Side Chain On Anti-Ageing Activity

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Lycium barbarum, known as "longevity fruit", is a good product for prolonging life. Previously our group showed that the crude *Lycium barbarum* polysaccharide (LBP) fraction, mainly composed of arabinose and galactose, had significant anti-aging activity, but the effector components and the relationship between the structure and activity of were unclear. Studies showed that the backbone of *Lycium barbarum* arabinogalactan was composed of →3,6)-Galp-(1→. It needs to be further identified whether the backbone structure of *Lycium barbarum* arabinogalactan contained →6)-Galp-(1→ as backbone with partial substitutions at the C3 site, or →3)-Galp-(1→ as backbone with partial substitutions at the C6 site. In this study, we obtained all the homogeneous LBGP70 (arabinogalactan-protein complex), LBGP70-OL (sugar chain of LBGP70), and LBGP70-OL-Ⅰ (backbone part of LBGP70-OL) fraction from crude LBP by stepwise precipitation. The structural features of LBGP70-OL were investigated by a series of analytical techniques and chemical methods, which was identified as a highly branched polysaccharide with an average of 9 branches per 10 sugar backbone units. The backbone was only →6)-β-Gal*p*-(1→ residues, substituted at the C3 position. The side chains contained α-Ara*f*-(1→3)-β-Ara*f*-(1→3)-β-Ara*f*-(1→, α-Ara*f*-(1→3)-β-Ga*p*-(1→3)-β-Gal*p*-(1→, β-Gal*p*-(1→, α-Ara*f*-(1→, α-Ara*f*-(1→[5)-β-Ara*f*-(1]$_4$→3)-β-Gal*p*-(1→. LBGP70-OL had the highest anti-ageing activity, with its side chain and backbone exhibiting a synergistic effect. LBGP70-OL exerted the anti-ageing activity by attenuating SA-β-Gal activity, preventing cell cycle arrest, increasing antioxidant enzyme activity, protecting the cell membranes from oxidative damage, and regulating the senescence-related genes. These results lay the fundamental for further studies on the structure-function relationships of LBP.

Keywords: anti-ageing, arabinogalactan, *Lycium barbarum* polysaccharides, structure characterisation

A New Perspective On Structural Characterisation And Immunomodulatory Activity Of Arabinogalactan In Larix Kaempferi From Qinling Mountains

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Abstract

In this study, crude polysaccharide (LAG-C) and homogeneous arabinogalactan (LAG-W) were isolated from Qinling *Larix kaempferi* of Shaanxi Province. Bioactivity assays showed that LAG-W and LAG-C enhanced the phagocytic ability, NO secretion, acid phosphatase activity, and cytokine production (IL-6, IL-1β, and TNF-α) of RAW264.7 macrophages. Notably, LAG-W exhibited a significantly stronger immunomodulatory effect than LAG-C. The primary structure of LAG-W was characterised by chemical methods (monosaccharide composition, methylation analysis, and alkali treatment) and spectroscopic techniques (gas chromatography-mass spectrometry, high-performance liquid chromatography-mass spectrometry, and 1D/2D nuclear magnetic resonance). LAG-W was identified as a 22.08 kilodaltons (kDa) neutral polysaccharide composed of arabinose and galactose at a 1:7.5 molar ratio. Its backbone consisted of repeated →3)-β-Gal*p*-(1→ residues. Side chains, connected at the O-6 position, were mainly composed of T-β-Gal*p*-(1→ and T-β-Gal*p*-(1→6)-β-Gal*p*-(1→ residues. And it also contained small amounts of T-β-Ara*p*-(1→, T-α-Ara*f*-(1→6)-β-Gal*p*-(1→6)-β-Gal*p*-(1→, and T-α-Ara*f*-(1→3)-α-Ara*f*-(1→6)-β-Gal*p*-(1→ residues. By structurally and functionally characterising *L. kaempferi* polysaccharides, this study opens the way for the valorisation of this species.

Keywords: *Larix kaempferi*, Arabinogalactan, Structural characterisation, Immunomodulation

Sugar Amino Acid Mimetics Of Heparan Sulfate

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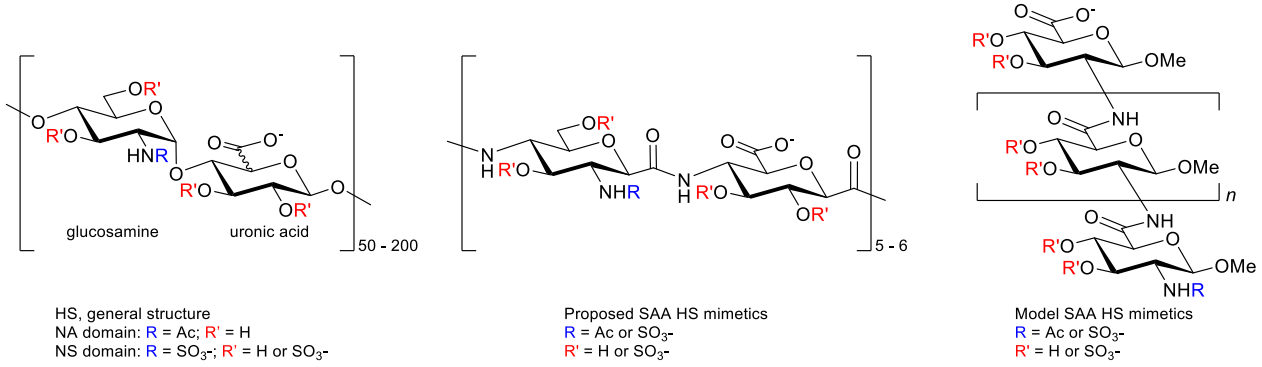
Heparan sulfate (HS) is a sulfated glycosaminoglycan (GAG) found on the surface of all animal cells. It is involved in a huge range of cell signaling and regulatory processes in which its protein interactions are attributable to its sulfation pattern.[1] Nature provides HS as a population of linear glycans with a heterogenous distribution of sulfates making exploration of relationships between sulfation sequence and biological activity challenging. It is scarcely available and obtained as a side product of porcine-derived heparin – a related GAG listed as a World Health Organisation essential medicine. Further, synthesis of HS is complex and resource intensive even to access relatively low molecular weight oligomers.[2] Synthetic sugar amino acid (SAA) mimetics of HS are proposed to overcome these obstacles.

This work explores the synthesis of HS SAA mimetics from sulfated SAA monomers. Use of sulfated SAA monomers enables specific sulfation sequences to be assembled as an oligomer exploiting the stablished amide-coupling reactions established in peptide synthesis. There are limited examples of sulfated species in amide-coupling reactions in the literature with sulfates typically installed following oligomerisation. Investigations into the assembly of these highly polar materials using both liquid and solid phase techniques will be discussed.



General structure of HS, the proposed mimetics, and model SAA mimetics.

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Chemical Synthesis And *Functional Study* Of Complex Glycoproteins

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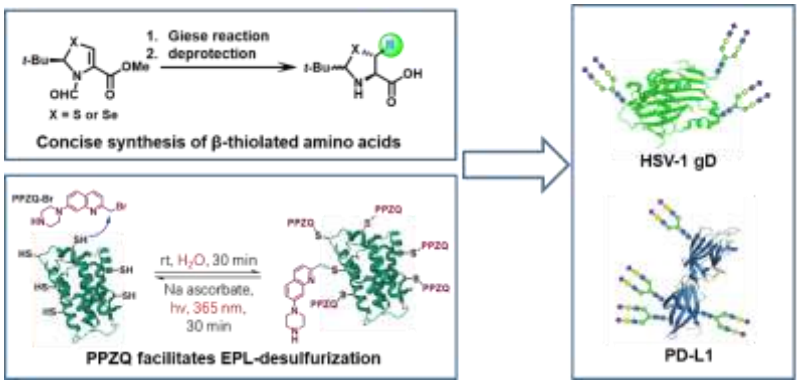
Protein glycosylation plays a key role in all aspects of life activities. Glycosylation is controlled by a variety of glycosyltransferases and glycosidases, resulting in its high heterogeneity. Chemical synthesis of glycoprotein can provide homogeneous glycoproteins. However, the process is complicated and usually provides a small amount of glycoprotein. First, we have developed a general method to prepare β-mercapto amino acids (including Leu, Glu, Gln, Lys, Phe, Arg, Trp, and Met) via a photoredox-catalyzed asymmetric Giese reaction, which can facilitate the crucial NCL-desulfurization for complex proteins. Second, 7-(piperazin-1-yl)-2-(methyl)quinolinyl (PPZQ)-assisted ligation that allows for the EPL-desulfurization approach to construct proteins bearing multiple native Cys residues in the expressed protein segment, facilitating the preparation of complex and large proteins. The two methods provide powerful solutions for the synthesis of glycoproteins such as glycoprotein d of herpes simplex virus-1 and PD-L1.



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Dietary Glycoproteins Modulate Gut Microbiota And Inhibit Pathogens: Sialylation Is A Key Factor

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Food-derived mucins are glycoproteins rich in sialic acid, but their digestive properties and potential health benefits for humans have been scarcely investigated. In first work, ovomucin (OVM, rich in Neu5Ac, ~3%), porcine small intestinal mucin (PSIM, rich in Neu5Gc, ~1%), the desialylated OVM (AOVM) and the desialylated PSIM (APSIM) were selected to examine their digestion and their impact on the gut microbiota of elderly individuals. The results shown that, the proportion of low-molecular-weight proteins increased after simulated digestion of these four mucins, with concomitant comparable antioxidant activity observed. Desialylation markedly increased the degradation and digestion rate of mucins. In vitro fecal fermentation was conducted with these mucins using fecal samples from individuals of different age groups: young, low-age and high-age elderly. Fecal fermentation with mucin digestive solution stimulated the production of organic acids in the group with fecal sample of the elderly individuals. Among them, the OVM group demonstrated the most favorable outcomes. The OVM and APSIM groups elevated the relative abundance of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, while diminishing the presence of pathogenic bacteria such as *Klebsiella*. Conversely, the probiotic effects of AOVM and PSIM were attenuated or even exhibited adverse effects. Hence, mucins originating from different sources and possessing distinct glycosylation patterns exhibit diverse biological functions.

In another work, sialoglycopeptide (SGP) was prepared by hydrolyzing edible bird's nest (EBN, a salivary mucin from Swiftlet) with pronase & trypsin. EBN SGP exhibit dose-dependent and significant inhibitory effects on *Candida albicans* and *Helicobacter pylori* within a certain concentration. MAL-II is used as a mimic of avian influenza virus to study the ability of EBN SGP to resist influenza virus. When the system does not contain EBN SGP, MAL-II can significantly bind to MDCK cells. When 0.5 mg/mL EBN SPG is added to the system, the binding of MAL-II to MDCK cells is significantly inhibited. At the same titer (10 mg/mL), compared to Neu5Ac, 3'-SL, and CGMP, EBN SGP shows stronger inhibitory effects. Our result indicates that glycopeptides and glycoproteins rich in sialic acid and with higher molecular weight have better ability to capture, bind, and inhibit influenza viruses, while glycopeptides containing N-glycans have stronger ability to inhibit pathogens such as *C. albicans* and *H. pylori*.

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Structural Elucidation Of The Capsular Polysaccharide From *Streptococcus Pneumoniae* Serotype 38

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Keywords: *Streptococcus pneumoniae*; capsular polysaccharides; carbohydrate antigens; glycoconjugates; bacterial vaccines

Streptococcus pneumoniae (SP) is one of the globally important encapsulated human pathogens and more than 100 different serotypes have been identified. Despite very extensive genetic and immune-serological studies, the structure of capsular polysaccharide repeating unit of serotype 38 has not been determined yet. Employing the state-of-art techniques including composition, linkage, NMR and mass spectroscopy analyses, we discovered that serotype 38 polysaccharide is composed of a penta-saccharide repeat unit →3)-[β-D-Gal*f*(1→2)]-β-D-Gal*p*A6(Ser)-(1→3)-α-D-Glc*p*NAc-(1→3)-α-Sug*p*-(1→4)-α-D-Gal*p*(2OAc)-(1→. The polysaccharide is O-acetylated at O-2 of the α-Gal residue on approximately 80% of the repeat units.

'Decorative groups' such as pyruvate and O-acetyl on polysaccharides have been identified in many SP serotypes. The presence of serine has been found in O-polysaccharide of *Providencia stuartii* O43 but this is the first case that an amino acid is found to be present as an integral component of a SP polysaccharide. This evidence opens questions on the possible glycobiology mechanism which can explain the presence of amino acids in SP CPS repeating units.

Synthesis Of Sialylmimetic Quinic Acid Derivatives And Their Evaluation Against Trypanosome *Cruzi* Parasite

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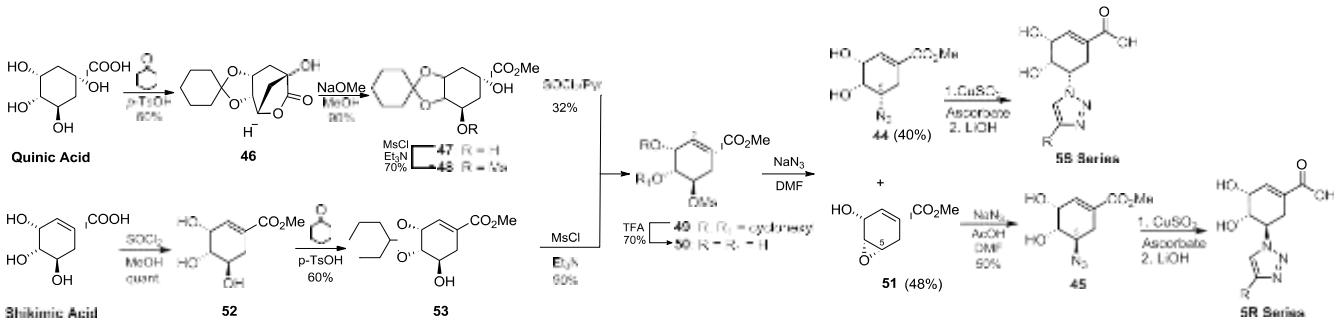
Sialidases are enzymes involved in the cleavage of glycans as they remove the terminal sialic acid for other glycans. Furthermore, terminal sialic acid-containing glycans on the cell membrane are essential in the cell recognition process and play essential roles in infectious diseases caused by viruses, bacteria and parasites. Among parasite sialidase, *trans*-sialidase from *Trypanosoma cruzi* (TcTS) plays an important role in the development of Chagas's disease which affects numerous people mainly in South and Central America. TcTS is a retaining glycosyl transferase responsible for transferring sialic acid residues from mammalian host glycoconjugates to terminal β-Gal residues of mucins on the parasite cell surface, an initial process for parasite invasion on host macrophage.[1] Taking into account that sialic acid-based TcTS inhibitors can occupy the donor binding site and hinder or block the sialic acid transfer reaction, we design and synthesized a series of novel sialylmimetics using Cu(I)-assisted 1,3-dipolar azide-alkyne cycloaddition (CuAAC) reaction. Based on the structural resemblance between quinic acid and shikimic acid with silalic acid and their high enantiomeric purity, we pursued the synthesis of eighteen analogs featuring different side chains at C-5' position, R goups, such as: (phenyl (**a**), *p*-methoxy-phenyl (**b**), *p*-nitro-phenyl (**c**), *t*-butyl-alcohol (**d**), pyridine (**e**), *p*-methyl-phenyl (**f**), benzyl (**g**). The coupling reaction was achieved from commercially available alkynes and quinic acid derivatives bearing the azide group, as depicted in Scheme. The key intermediate 50 was obtained through two alternative routes from natural precursors, quinic acid or shikimic acid, but it was evident the straightforward reaction and high yield obtained from the later, in spite of its high cost.



Scheme.

After treating intermediate 50 with sodium azide, we successfully synthesized the expected S-azido (44) with an inversion of configuration at C-5. Interestingly, this reaction also provided an unexpected epoxy product (51), which was further converted in azido (45) with R-configuration. Both isomers (44, 45) were coupled with a panel of alkynes to give the final 1,4-disubstituted 1,2,3-triazoles (Scheme), through CuAAC reactions. TcTS assays revealed that both isomers, 5R and 5S-p-nitro-phenyl (R groups) derivatives were the most active of the series, IC50 226 and 399 µM, respectively. The TcTS inhibitions were significant because donor mimetic substrates already described in the lit. have IC_{50} in a mM range. On the other hand, in vitro antitrypanosomal assays against strain Y showed 5S isomer containing the p-methoxy-phenyl, as the R group (Scheme), with moderate activity (IC_{50} 32 µM). The cytotoxicity (CC_{50}) on LLC-MK2 cell line was observed at concentrations above 500 µM.

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Regio- And Stereoselective Organocatalysed Relay Glycosylations To Synthesize 2-Amino-2-Deoxy-1,3-Dithioglycosides

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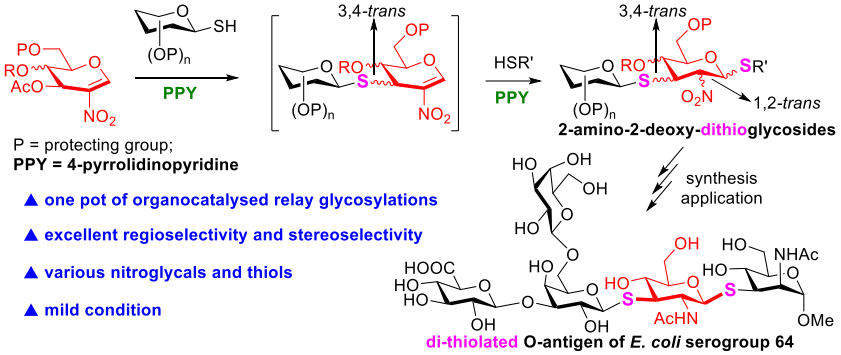
Thioglycosides are carbohydrate mimetics and are resistant to acid-mediated or enzymatic hydrolysis, with applications in glycobiology and drug development. We here describe novel methodology for the regio- and stereoselective convergent synthesis of 2-amino-2-deoxy-dithioglycosides via the one-pot 4-pyrrolidinopyridine-mediated relay glycosylation of 3-*O*-acetyl-2-nitroglycal donors. This unique organo-catalysis relay glycosylation features excellent site- and stereoselectivity, good to excellent yields, mild reaction conditions, and a broad substrate scope. 2-Amino-2-deoxy-glucosides/mannosides bearing 1,3-dithio-linkages were efficiently obtained from 3-*O*-acetyl-2-nitroglucal donors in both stepwise and one-pot glycosylation protocols, while 2-amino-2-deoxy-idosides bearing 1,3-dithio-linkages were produced from 3-*O*-acetyl-2-nitrogalactal donors. The di-thiolated O-antigen of *E. coli* serogroup 64 was successfully synthesized using this newly developed method.



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New Look At Acidic Ethanolysis Of Acetyl Groups In Carbohydrates

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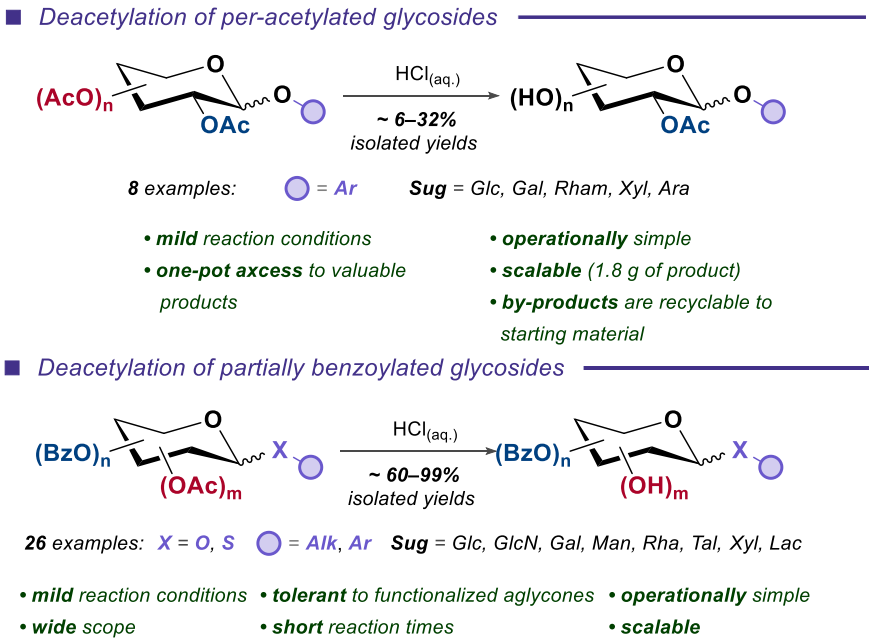
In the present work, we disclose a protocol for the regioselective deacetylation of *per*-acetylated and partially benzoylated aryl glycosides under mild acidic conditions (HCl in EtOH and $CHCl_3$). [1]This approach enables the direct synthesis of 2-O-acetylated aryl glycosides in a single step, utilizing readily available reagents and a straightforward procedure. Importantly, this methodology offers a significant reduction in synthetic steps compared to traditional multi-step strategies involving protecting groups. We have successfully applied the deacetylation protocol for the preparation 2-*O*-acetylated glycosides with moderate yields (6-23%). Further, we propose a simple method for regenerating byproducts to convert them back to the starting material, thereby enhancing the overall yield of the desired 2-O-acetate (68% of 2-*O*AcGal*p* after six cycles). Furthermore, the outlined acid-catalyzed protocol allows for the removal of acetyl groups in presence of more inert benzoyl groups using mild acidic conditions (HCl in EtOH and $CHCl_3$, 70 °C). Thus, we synthesized 26 partially benzoylated compounds with high yields (66-99%) featuring various functionalized aglycons and configurations of carbohydrate moiety. The Ministry of Education and Science of the Russian Federation (Program No. 075-03-2024-118/1) is gratefully acknowledged.



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Direct Identification Of Complex Glycans Via A Highly Sensitive Engineered Nanopore

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Main body: The crucial roles glycans played in biological systems are determined by their structures. However, the analysis of glycan structures still has numerous bottlenecks due to their inherent complexities. The development of nanopore technology has emerged as a powerful sensor for the DNA sequencing and peptides detection. This has a significant impact on the development of the related research area. Currently, nanopores are beginning to be applied for the detection of simple glycans, but the analysis of complex glycans by this technology is still challenging. Here, we designed an engineered α-hemolysin nanopore M113R/T115A to achieve the sensing of complex glycans at micromolar concentration and label-free conditions. By extracting characteristic features to depict a 3D scatter plot, glycans with different numbers of functional groups, various chain lengths ranging from disaccharide to decasaccharide, and distinct glycosidic linkages could be distinguished. Molecular dynamics simulations (MD) show different behaviors of glycans with 1,3 or 1,4 glycosidic bonds in nanopores. More importantly, the designed nanopore system permitted the discrimination of each glycan iso-mer with different lengths in a mixture with a separation ratio of over 0.9. This work represents a proof-of-concept demonstration that complex glycans can be analyzed by using nanopore sequencing technology.

Keywords: Engineered α Hemolysin nanopore, Glycan, Glycosidic bond, Isomer, 3D fingerprints

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Component Determination Of Polysaccharide Antigens By A Quantitative Nmr Method Using A Single Internal Standard

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Carbohydrate-based vaccines have provided tremendous health benefits in the world, and hold great promise for numerous diseases, such as pneumonia and meningitis caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib). Quality control of polysaccharide antigens is a crucial factor in the manufacture of carbohydrate-based vaccines. A significant portion of quality control involves component determination of polysaccharide antigens. Given that phosphate group exists in the capsular polysaccharides (CPSs) of Hib and *S. pneumoniae* types 6A, 6B, 7B, 18C, 19A, 19B, 19C, 19F, 23F, 32A, and 32F, it is necessary to determine the phosphorus content. In addition, residual C-polysaccharide (C-Ps) existing in the *S. pneumoniae* CPS is an important index for the quality control of pneumococcal vaccines. A quantitative ^{1}H- and ^{31}P-NMR method using a single internal standard hexamethylphosphoramide (HMPA) was developed for the simultaneous determination of ribose and phosphorus contents in Hib CPS.[1] The 2-H signal of ribose and the methyl signals of HMPA were chosen as characteristic signals for the quantitative ^{1}H-NMR determination of Hib CPS. For the determination of ribose and phosphorus contents, 15−20 mg·mL^{-1} was the optimal concentration range of Hib CPS in D_2O solution. The quantitative ^{1}H- and ^{31}P-NMR method was further applied to simultaneously determine CPS, C-Ps, and phosphorus contents of carbohydrate antigens from *S. pneumoniae*, ensuring quality control of pneumococcal vaccines.[2] Focusing on *S. pneumoniae* types 6A, 6B, 19A, 19F, and 23F CPS with low viscosity, the C6 methyl signal of rhamnose was chosen as the quantitative signal, and 5−25 mg·mL^{-1} was the optimal concentration range for determination. For *S. pneumoniae* type 18C CPS with high viscosity, the *O*-Ac methyl signal was selected as a quantitative signal and 3−15 mg·mL^{-1} was the optimal concentration range for determination. Moreover, for the determination of pneumococcal C-Ps, another more efficient and accurate quantitative method using ^{1}H- and ^{31}P-NMR was established with HMPA as the internal standard.[3] The optimal concentration ranges of *S. pneumoniae* serotype 4 and 7F CPS for determination are 3−15 mg·mL^{-1} and 15−25 mg·mL^{-1}, respectively. This quantitative ^{1}H- and ^{31}P-NMR method using a single internal standard shows good specificity, accuracy, and precision, providing a valuable approach for the quality control of carbohydrate-based vaccines.

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Screening Of Enzymatic Complexes For The *In-Situ* Production Of Prebiotics In Dairy Products

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Consumers' awareness of the negative impact of high-sugar consumption on health has increased. As a result, the food industry has been pressed to develop innovative products with reduced sugar. Herein, we aimed to develop a functional fruit preparation for further inclusion into dairy products, by converting its sucrose content into prebiotic fructo-oligosaccharides (FOS) [1,2].

Commercial enzymatic complexes with transfructosylation activity (Pectinex®Ultra SP-L, Viscozyme®L, Novozym®960, and Catazyme®25L) were studied in the catalysis of sucrose conversion into FOS using an *in-situ* approach. Reaction conditions were optimized to maximize the yield in FOS. The selected enzymatic complex was then applied in a commercial strawberry preparation.

At optimal conditions, maximal FOS yield in a sucrose model solution ($g_{FOS}/g_{ini.sucrose}$) was attained for Novozym– 0.629±0.002 (47.4 ℃, pH 5.4, *t*=0.5 h), followed by Viscozyme– 0.624±0.003 (60.1 ℃, pH 5.7, *t*=5.5 h), Pectinex– 0.619±0.003 (58.7℃, pH 5.7, *t*=6 h), and Catazyme– 0.444±0.001 (49.1℃, pH 5.7, 2 h). Thus, Novozym was chosen for further application in the strawberry preparation, in an enzyme:substrate ratio of 1:99 (v/v). Novozym produced 293.2±0.7 g/L of FOS, yielding 0.630±0.003 $g_{FOS}/g_{ini.sucrose}$ after 1.25 h reaction in a lab-scale setup. Similar results were obtained in a pilot-plant study (290.2±0.8 g/L FOS).

The *in-situ* approach proved to be efficient and suitable for industrial applications. The strawberry preparation prototype had 56% (w/w) FOS incorporated, less 85% of its initial sucrose amount, and a caloric value reduced by 27%. Results on *in-vitro* gastrointestinal digestion, gut microbiota modulation, and sensory experience with the new formulation will be presented at the conference.

Referance:

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Caveolin-1 Facilitates Cell Migration By Upregulating Nr4a2/Rxrα-Mediated St6gal-I Expression In Human Hepatocarcinoma Cells

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It has been reported that caveolin-1 (Cav-1) acts as a tumor promoter in hepatocellular carcinoma (HCC). Our previous studies showed that Cav-1 promoted mouse hepatocarcinoma cell adhesion to fibronectin by upregu- lating β-galactoside α2,6-sialyltransferase I (ST6Gal-I) expression. However, the detailed mechanism by which Cav-1 regulates ST6Gal-I is not fully understood. In this study, we found that the expression levels of Cav-1 and ST6Gal-I were increased in HCC tissues and correlated with poor prognosis. Cav-1 upregulated ST6Gal-I expression to promote the migration and invasion of HCC cells by inducing epithelial-to-mesenchymal transi- tion. Importantly, the binding of the transcription factor nuclear receptor 4A2/retinoid X receptor alpha (NR4A2/RXRα) to the -550/-200 region of the ST6GAL1 promoter was critical for Cav-1-induced ST6GAL1 gene expression. Furthermore, Cav-1 expression activated the phosphatidylinositol 3-kinase/protein kinase B/ mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway, followed by upregulation of NR4A2 expression and phosphorylation of RXRα, which facilitated the complex of NR4A2 and phosphorylated RXRα forming and binding to the ST6GAL1 promoter region to induce its transcription. Finally, in the diethylnitros-amine (DEN)-induced HCC murine model, the expression levels of NR4A2, p-RXRα, ST6Gal-I, and α2,6-linked sialic acid decreased in parallel in Cav-$1^{-/-}$ mice compared with Cav-$1^{+/+}$ mice, which was consistent with the above in vitro results. These findings provide insight into the mechanism of ST6GAL1 gene transcription mediated by Cav-1, which may lead to the development of novel therapeutic strategies targeting metastasis in HCC.

Inhibitors Of Dermatan Sulfate Epimerase 1 Decreased Accumulation Of Glycosaminoglycans In Mucopolysaccharidosis Type I Fibroblasts

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Genetic deficiency of alpha-L-iduronidase causes mucopolysaccharidosis type I (MPS-I) disease, due to accumulation of glycosaminoglycans (GAGs) including chondroitin/dermatan sulfate (CS/DS) and heparan sulfate (HS) in cells. Currently, patients are treated by infusion of recombinant iduronidase or by hematopoietic stem cell transplantation. An alternative approach is to reduce the L-iduronidase substrate, through limiting the biosynthesis of iduronic acid. Our earlier study demonstrated that ebselen attenuated GAGs accumulation in MPS-I cells, through inhibiting iduronic acid producing enzymes. However, ebselen has multiple pharmacological effects, which prevents its application for MPS-I. Thus, we continued the study by looking for novel inhibitors of dermatan sulfate epimerase 1 (DS-epi1), the main responsible enzyme for production of iduronic acid in CS/DS chains. Based on virtual screening of chemicals towards chondroitinase AC, we constructed a library with 1,064 compounds that were tested for DS-epi1 inhibition. Seventeen compounds were identified to be able to inhibit 27%–86% of DS-epi1 activity at 10 μM. Two compounds were selected for further investigation based on the structure properties. The results show that both inhibitors had a comparable level in inhibition of DS-epi1 while they had negligible effect on HS epimerase. The two inhibitors were able to reduce iduronic acid biosynthesis in CS/DS and GAG accumulation in WT and MPS-I fibroblasts. Docking of the inhibitors into DS-epi1 structure shows high affinity binding of both compounds to the active site. The collected data indicate that these hit compounds may be further elaborated to a potential lead drug used for attenuation of GAGs accumulation in MPS-I patients.

Keywords:
DS-epi1;inhibitors;MPS-I;substratereductiontherapy.

Mannose-Mediated Antibacterial Perylene Bisimide Derivativetitle

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Bacterial infection and the increasing resistance of bacteria to commercially available antibiotics have long become a threat to global human health[1]. To explore nonantibiotic antibacterials[2], we prepared a mannose-decorated spermine and perylene bisimide derivative (**PBI-spm-Man**) that formed a self-assembled glycocluster presenting multivalent mannose moieties and concentrated cations[3]. The introduction of mannose not only endows the molecule with targeting ability towards *Escherichia coli* (*E. coli*) through mannose-FimH interaction, but also greatly reduced the potential cytotoxicity. This is proved by a control molecule with an additional spermine moiety instead of mannose groups (**PBI-dispm**), which demonstrate potent antibacterial activity but very strong cytotoxicity (Figure 1).

Further investigation of the molecule evidenced that **PBI-spm-Man** could eliminate three clinical-isolated *E. coli strains* that are resistant to different antibiotics. *In vivo* full-thickness wound healing results showed that not **PBI-spm-Man** only reduced the residual bacteria at the wound site but also promoted wound healing. This work represents a universal design strategy for nonantibiotic antibacterial agents based on multivalent glycoconjugates.

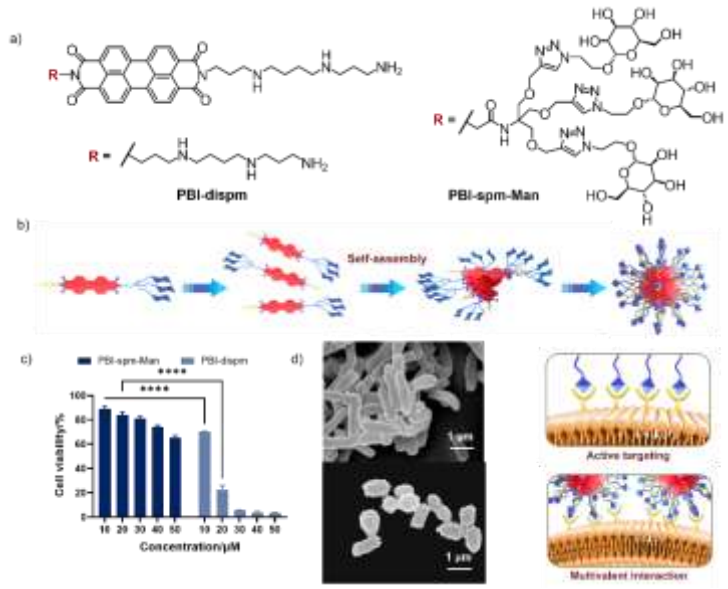

Figure 1. a) Structure of **PBI-spm-Man** and **PBI-dispm**; b) Self-assembly process of **PBI-spm-Man**; c) Reduction of cytotoxicity by incorporation of mannose; d) Morphology of E. coli before (up) and after (down) interaction with **PBI-spm-Man**.

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Chemical Synthesis Of A Key Precursor Relevant To The Tetrasaccharide Repeating Unit From *Treponema Medium* Atcc 700293

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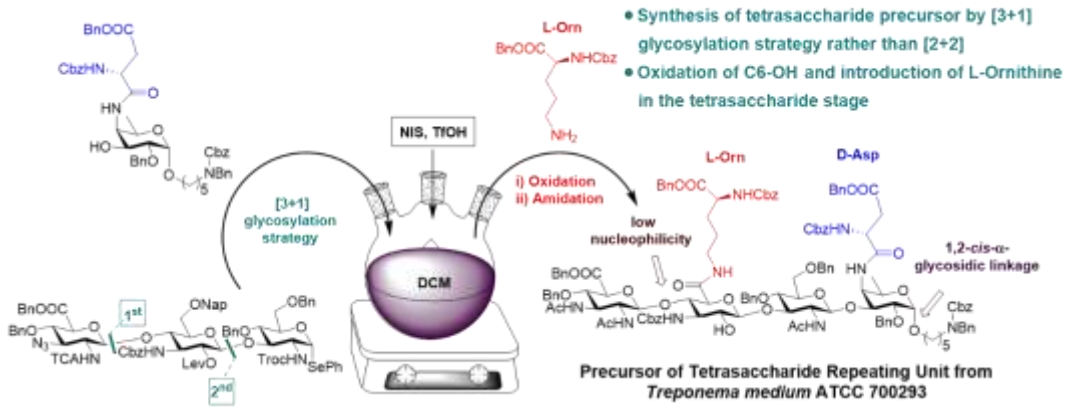
Treponema is a Gram-negative anaerobic bacterium, among which the pathogenic Treponema can cause various diseases, such as venereal syphilis (*Treponema pallidum*), yaws (*Treponema carateum*), and oral diseases (*Treponema denticola* and *Treponema medium*). Although different from conventional lipopolysac-charides, the extracellular glycoconjugate of Treponema may still be a potential antigen and provide a candidate for vaccine development. *Treponema medium* ATCC 700293 was isolated from subgingival plaque from an adult with periodontal disease. It is a Gram-negative, anaerobic, motile, helically coiled, and medium-sized treponeme. The structure of the polysaccharide repeating unit in the extracellular glycolipids of *T. medium* has been elucidated as the tetrasaccharide repeating unit, →4)-β-D-Glc*p*NAc3NAcA-(1→4)-β-D-Man*p*NAc3NAOrn-(1→3)-β-D-Glc*p*NAc(1→3)-α-D-Fuc*p*4NAsp(1→ containing a L-ornithine(L-Orn) and D-aspartic acid (D-Asp) residues.



Hence, we completed the first chemical synthesis of *Treponema medium* ATCC 700293 tetrasaccharide precursor containing L-ornithine (L-Orn) and D-aspartic acid (D-Asp) derivatives. The efficiency of non-reducing end disaccharide formation has been improved by optimizing the assembly of the protecting groups in the donors and acceptors. Our [3+1] glycosylation strategy attempted to reduce the length of the acceptor to increase the nucleophilicity of the hydroxyl group and, thus the efficiency of the target tetrasaccharide synthesis. The L-Orn derivative was introduced at the final stage due to its influence on the glycosylation stereospecificity and efficiency. Therefore, the successful introduction of two amino acid derivatives and the synthesis of a tetrasaccharide precursor with complex functional-group modifications have provided valuable insights for synthesizing other complex bacterial glycans.

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Dehydroxylative Radical N-Glycosylation Of Heterocycles Enabled By Copper Metallaphotoredox Catalysis

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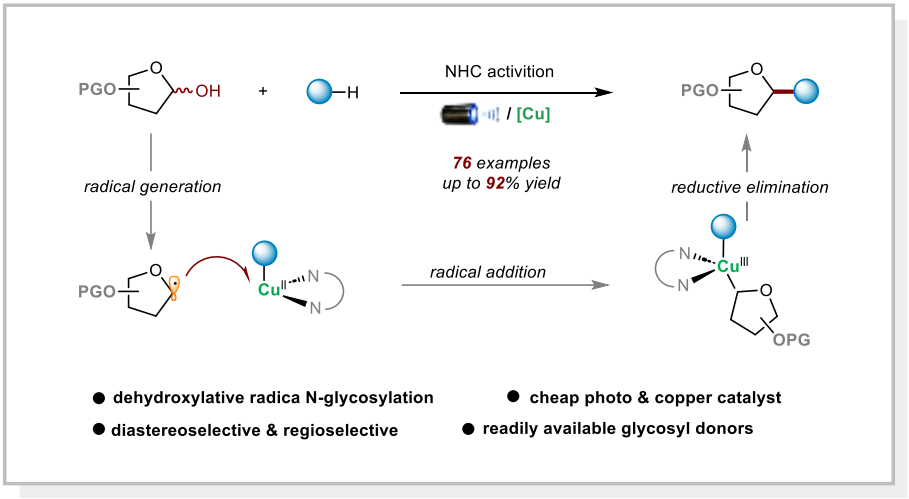

N-Glycosylated heterocycles play important roles in biological systems and drug development. The synthesis of these compounds heavily relies on ionic N-glycosylation, which is usually constrained by factors such as labile glycosyl donors, precious metal catalysts, and stringent conditions. In a continuation of our interest in glycosyl radical-based glycosylation,[1, 2] we report herein a dehydroxylative radical method for synthesizing *N*-glycosides by leveraging copper metallaphotoredox catalysis, in which stable and readily available 1-hydroxy carbohydrates are activated for direct N-glycosylation.[3] Our method employs inexpensive photo- and copper- catalysts and can tolerate some extent of water. The reaction exhibits a broad substrate scope, encompassing 76 examples, and demonstrates high stereoselectivity, favoring 1,2-*trans* selectivity for furanoses and α-selectivity for pyranoses. It also exhibits high site-selectivity for substrates containing multiple N-atoms. The synthetic utility is showcased through the late-stage functionalization of bioactive compounds and pharmaceuticals like Olaparib, Axitinib, and Metaxalone. Mechanistic studies prove the presence of glycosyl radicals and the importance of copper metallaphotoredox catalysis.

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The Influence Of Steaming Process On Polysaccharides And Their Anti-Colitis Activity In *Polygonatum Cyrtonema*

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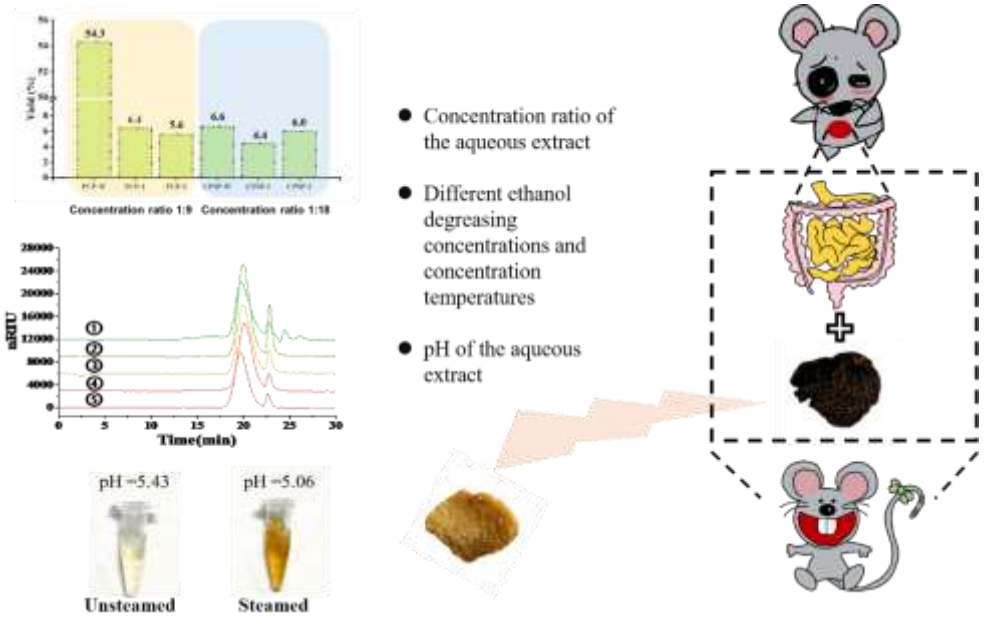
Polygonatum cyrtonema, commonly known as Huangjing, is a frequently used medicinal herb. It typically requires steaming and drying to enhance its therapeutic effects. Polysaccharides are an important active component in Huangjing and play a significant role in treating various diseases, such as antioxidant, anti-tumor, hypoglycaemic, and anti-colitis. However, there has been little research and reporting on how steaming affects the content, structure, and biological activity of the polysaccharides in Huangjing. To reveal the impact of the steaming process on polysaccharides, the concentration ratio of the aqueous extract was first examined. It was found that the higher the concentration ratio, the more significant the degradation of the polysaccharide yield. Subsequently, the concentration of ethanol for degreasing and the temperature for aqueous extraction were examined. It was found that 95% ethanol should be used for degreasing, and the concentration temperature should not be too high. Additionally, we tested the pH value of the aqueous extract and found that the pH decreases during the processing. Furthermore, anti-inflammatory effects were observed in mice with colitis, indicating that the steamed extract exhibited better anti-colitis activity. In summary, the differences in anti-inflammatory activity may be related to the changes in polysaccharide content and structure caused by steaming. These findings lay the foundation for exploring the processing mechanism of Huangjing.



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Convergent Synthesis And Anti-Pancreatic Cancer Cell Growth Activity Of A Highly Branched Heptadecasaccharide From *Carthamus Tinctorius*

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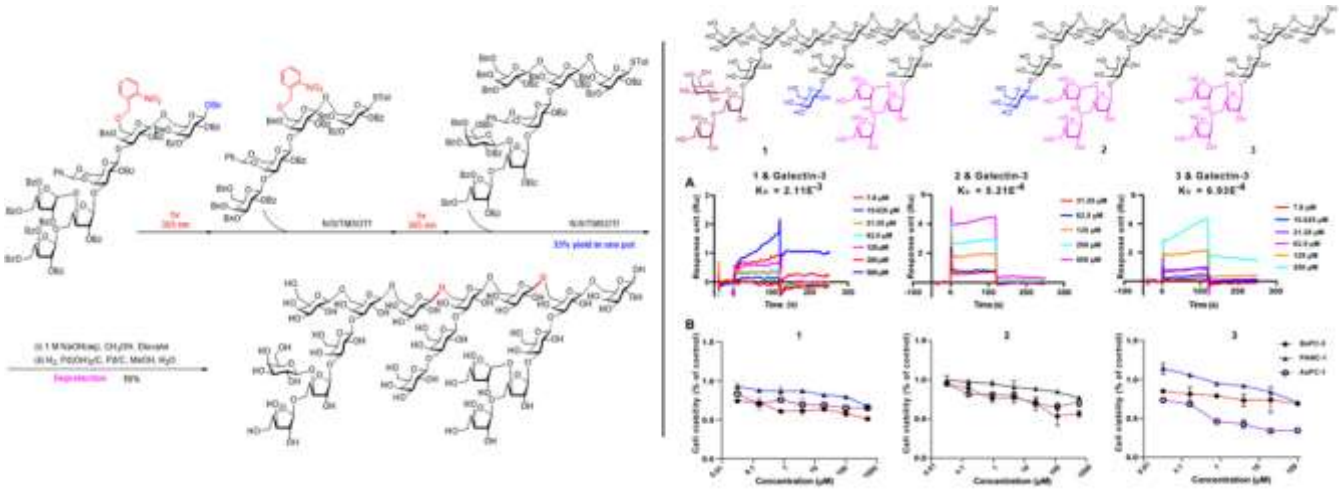
Bioactive polysaccharides from natural resources target various biological processes and are increasingly used as potential target molecules for drug development. However, the accessibility of branched and long complex polysaccharide active domains with well-defined structures remains a major challenge. Herein we describe an efficient first total synthesis of a highly branched heptadecasaccharide moiety of the native bioactive galectin-3-targeting polysaccharide from Carthamus tinctorius L. as well as shorter fragments of the heptadecasaccharide. The key feature of the approach is that a photo-assisted convergent [6+4+7] one-pot coupling strategy enables rapid assembly of the heptadecasaccharide, whereby a photoremovable o-nitrobenzyl protecting group is used to generate the corresponding acceptor for glycosylation in situ upon ultraviolet radiation, during which the aglycon transfer was eliminated because the sequence of assembly is from reducing end to non-reducing end of the oligosaccharide. Biological activity tests suggest that the heptadecasaccharide can target galectin-3 and inhibit pancreatic cancer cell growth. This work demonstrates a representative example to understand the active domains of the polysaccharide, which could be synthesized for structure-activity relationship studies, allowing for further structure modification and potential drug candidate development.



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Total Synthesis Of Curcluigoside A – Arylglucoside From Medicinal Plant Curculigo Orchioides

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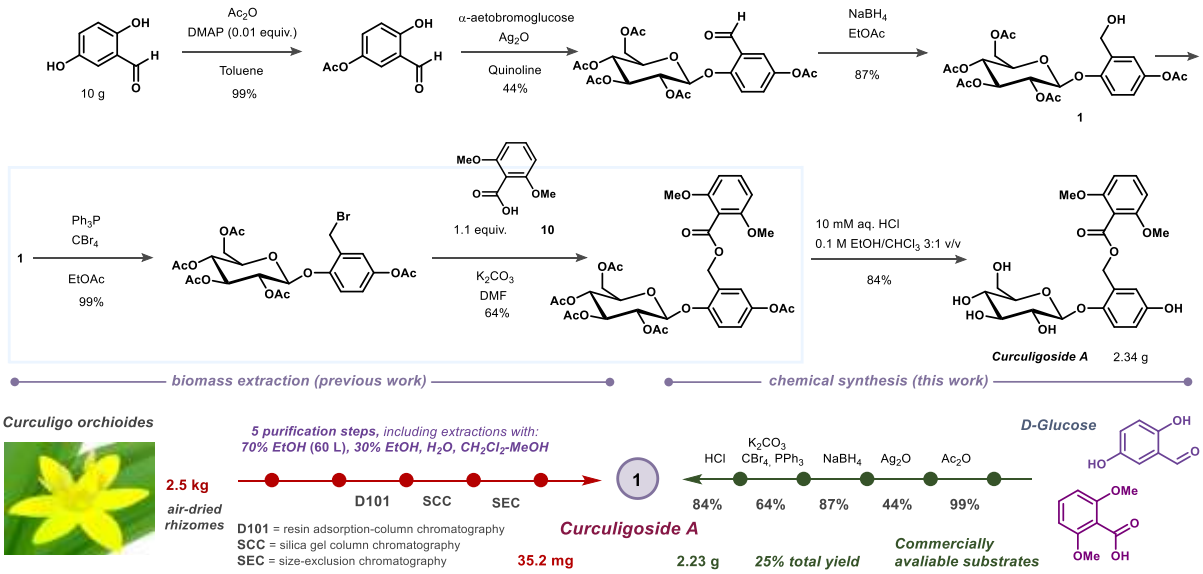
Curculigo orchioides Gaertn. (*Amaryllidaceae*) is abundantly found in subtropical regions of Asia, particularly southern China and India. It is esteemed in traditional medicine and is utilized for treating osteoporosis, menorrhagia, [1] and is believed to possess aphrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer and antidiabetic properties. [2] The dried rhizome of *C. orchioides* is notably rich in phenolic glycosides, curculigoside A being predominant component in itd ethanolic extract. [3] Research suggest its potential as a neurovascular recovery agent for stroke and brain injury, [4] a therapeutic agent for osteoporosis, [5] and its relevance in studying the mechanism of Parkinson's disease and its prevention. [6]



Traditional methods of obtaining the valuable substance through extraction are inefficient and demand significant energy and resources. For example, from 2.5 kg of dry plant material, 35.2 mg of pure product was obtained. [5] Our proposed synthesis yields 2.32 g of curculigoside A, from 10 g of the starting 2-hydroxysalicylic aldehyde. Total organic synthesis offers the potential to producea chemically pure substance in substantially larger quantities, and the developed synthesis is versatile, enabling the production of several types of arylglycosides of the curculigoside family. The Ministry of Education and Science of the Russian Federation (Program No. 075-03-2024-118/1) is gratefully acknowledged.

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Perylene Monoimide Mannose Derivatives For Photothermal Antibacterial And Wound Healing

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Bacterial infections pose a serious threat to public health and are a huge challenge facing the global public health sector[0]. Bacterial infections can lead to many diseases [0]. Therefore, the development of new antibacterial methods and pathways are a focus of attention. Recently, a large number of antibacterial studies based on the interaction between bacterial surface sugar receptors and sugar molecules have been carried out[0], and gratifying results have been achieved.

Based on the multivalent recognition between mannose and the lectin FimH on the surface of *Escherichia coli*, the mannose modified perylene monoimide derivative (PMI-3Man) was designed and synthesized (Figure 1). Furthermore, Under the concentration of 20 μM, 32 μM, 58 μM, 80 μM, 100 μM and 138 μM (Figure1), PMI-3Man exhibited the bacterial survival rate of 72.10%, 50.54%, 32.26%, 22.85%, 15.93% and 0.38% with laser irradiation. These results indicated that PMI-3Man showed good bactericidal effects on *Escherichia coli* and provided a beneficial attempt for sugar targeted self-assembly photothermal inhibition research.

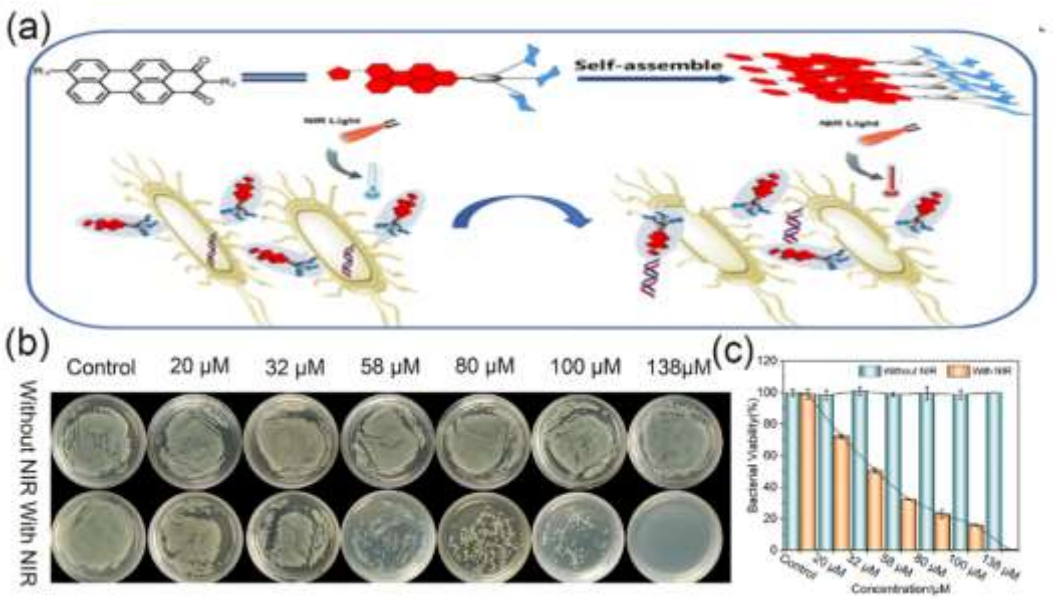

Figure 1. (a) Schematic illustration the structure of the PMI-3Man and the applications of antibiotic-free antibacterial; Photographs of bacterial colonies (b) and relevant quantitative analysis (c) after exposed to PMI-3Man with and without a laser irradiation.

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Hypervalent Selenium Catalysis For The Activation And Stereoselective Glycosylation Of 2-Deoxy Hemiacetals

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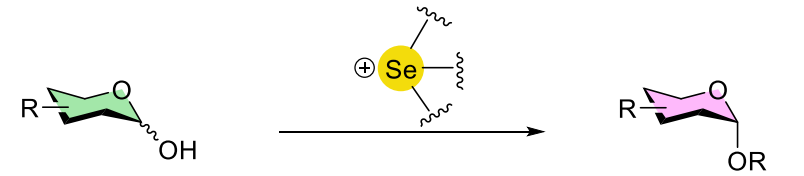
The development of stereoselective glycosylation methods is vital for enabling the synthesis of biologically relevant carbohydrate-based molecules.[1] Often glycosylation reactions result in a mixture of anomers, leading to purification problems and lower enantiomeric yields. Additionally, achieving stereoselectivity is more challenging when using 2-deoxy sugars which lack substituents at C-2 that can direct nucleophilic attack. Our group is interested in the development of novel catalytic methods for the synthesis of deoxyglycosides to address this challenge, through the activation of both glycals and hemiacetals.[2,3]

In this work, we demonstrate our latest effort to apply group 16 elements in transition-metal free catalysis for carbohydrate synthesis. For the first time the application of hypervalent chalcogenonium catalysis to synthesise both 2-deoxy and fully-oxygenated glycosides via the activation of hemiacetal donors has been demonstrated. In this work, we disclose a practical and direct α-stereoselective glycosylation strategy applied to a range of orthogonally protected hemiacetal substrates in fair to excellent yields, with both primary and secondary OH nucleophiles, and high stereocontrol. Moreover, ^{1}H-NMR and kinetic isotope studies provided insights towards the reaction mechanism pathways and unravel the key steps in the activation process.[4]



2-deoxy hemiacetal

α- selective
>25 examples
14-93 %

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Natural Glycan Target Molecule Discovery

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Glycan is the polymer which composes of monosaccharides through glycosidic bond. More and more evidence show that different structure of glycan (including oligosaccharide and polysaccharide) demonstrates distinct bioactive molecule with impact on physiological and pathological function linked to infectious disease, cancer, metabolic disease, neuropsychiatric disorders, immunopathy, and angiocardiopathy. However, whether glycan has the specific structure motif which codes corresponding bioactivity, and whether glycan has unambiguous functional target(s) are still equivocal. In addition, whether those bioactivities owing to the intact glycan or part of the glycan or even has the active domain is vague. In this lab, we are trying to understand impact of different monosaccharide composition, linkage type, glycan sequence, and the whole structure motif, etc. on glycan target discovery. Our previous study had shown that polysaccharides from different herbs might target one or multiple target molecules to impede tumor cells growth, suppress liver or lung fibrosis, and block angiogenesis, etc. Nevertheless, these targets are sensitive to the structure motifs differentiation.

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Visible Light Induced Palladium-Catalyzed Suzuki-Miyaura Cross-Coupling Of Glycosyl Chlorides To Form C-Aryl Glycosides

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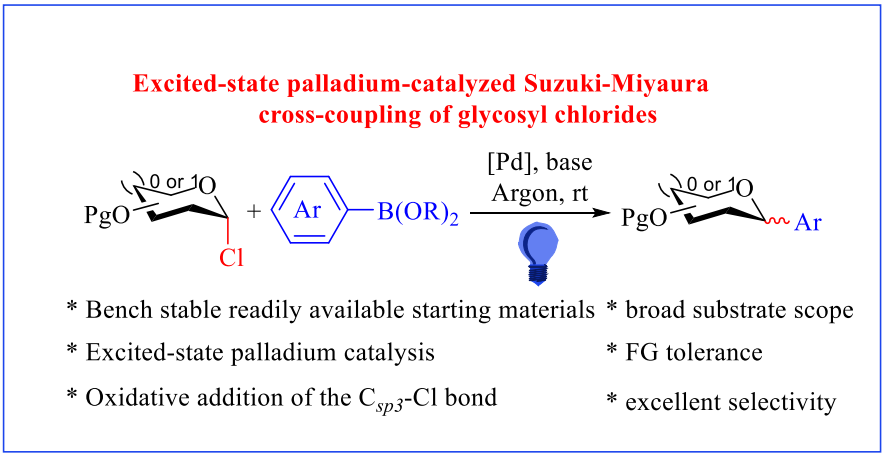
Both the natural and the mimetic *C*-aryl glycosides have diverse biological activity and play important role in drug design.[1] Even though various methods have been developed for synthesis of *C*-aryl glycosides[2], the Suzuki-Miyaura reaction of glycosyl chlorides to form anomeric *C*-aryl glycosides (Csp^3-Csp^2 coupling) was not systematic studied owing to the difficult oxidative addition of a transition metal to the C–Cl bond. Here we report the simple and powerful method applying excited-state palladium-catalyzed Suzuki-Miyaura cross-coupling (Csp^3-Csp^2) to synthesize *C*-aryl glycosides. The method can be applied to a wide range of aryl and heteroaryl boronic seter substrates and glycosyl chlorides. A variety of *C*-aryl pyranosides and furanosides can be straightforward synthesized. And it also offers a tool for late-stage modification of more complex drug molecules.



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Palladium catalysis enables cross-coupling–like S_N2-glycosylation of phenols

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Abstract: Despite their importance in life and material sciences, the efficient construction of stereo-defined glycosides remains a challenge. Studies of carbohydrate functions would be advanced if glycosylation methods were as reliable and modular as palladium (Pd)-catalyzed cross-coupling. However, Pd-catalysis excels in forming sp2-hybridized carbon centers whereas glycosylation mostly builds sp3-hybridized C–O linkages. We report a glycosylation platform through Pd-catalyzed S_N2 displacement from phenols toward bench-stable, aryl-iodide–containing glycosyl sulfides. The key Pd(II) oxidative addition intermediate diverges from an arylating agent (Csp2 electrophile) to a glycosylating agent (Csp3 electrophile). This method inherits many merits of cross-coupling reactions, including operational simplicity and functional group tolerance. It preserves the S_N2 mechanism for various substrates and is amenable to late-stage glycosylation of commercial drugs and natural products.

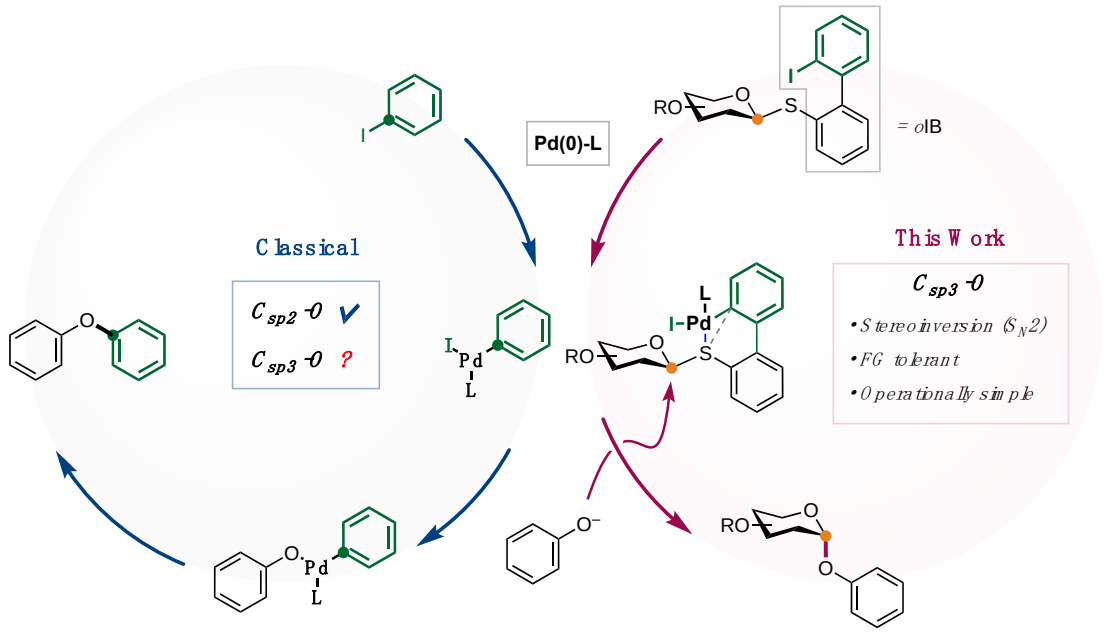

Figure 1. Classical Pd-catalyzed C–O cross-coupling and novel Pd-catalyzed S_N2 glycosylation

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Engineering Liquid-Liquid Phase Separation To Control Microstructure And Cell Behavior In Polysaccharide Hydrogels

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The compositional heterogeneity and structural diversity of native extracellular matrix are essential in regulating cell behavior and promoting tissue regeneration. Thermoresponsive polysaccharide-based materials with tunable transition temperatures and phase-separated microstructure offer substantial opportunities in tissue engineering, drug delivery, and wound healing applications. To develop novel synthetic thermoresponsive polysaccharides, we employed versatile chemical routes to attach intrinsically hydrophobic adducts to the backbone of hydrophilic dextran and developed protocols to form hydrogels with defined microstructures. Systematically conjugating methacrylate moieties to the dextran backbone yielded a continuous increase in macromolecular hydrophobicity that induced a reversible phase transition whose lower critical solution temperature can be systematically modulated *via* variations in polysaccharides concentration, molecular weight, degree of methacrylation, ionic strengths, and Hofmeister salts. Photo-initiated radical polymerization permits facile chemical crosslinking and kinetic capture of phase separation, enabling the formation of hydrogels with defined microdomains. The resulting heterogeneous hydrogels feature tunable microstructures and exhibited both microspheres and continuous phases that promoted enhanced cell adhesion in 2D and interfacial-driven cell migration in 3D. Engineering macromolecular hydrophobicity with temperature-triggered phase separation of conventional hydrophilic, non-phase separating polysaccharides to generate heterogeneous hydrogels with controlled microstructures will find potential applications in chronic wound healing.

O-GlcNAcylation Of FTO Determines The FTO Stability And Negatively Regulates Pyrin mA Demethylation And Inflammatory Response

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The fat mass and obesity-associated protein (FTO), as a key RNA N6-methyladenosine (m^6A) demethylase, has been implicated in modulating inflammatory response. Here we report a mechanism that O-GlcNAcylation modification controls the biological functions of the FTO protein. We uncover that FTO undergoes O-GlcNAcylation at the Ser95 site, and Lipopolysaccharide (LPS) or Glucosamine treatment can enhance FTO O-GlcNAcylation in macrophages. Surprisingly, O-GlcNAcylation of FTO determines the FTO stability and promotes its K48 ubiquitination degradation, which is orchestrated by the E3 ligase TRIM21. Decreased stability of FTO due to O-GlcNAcylation negatively regulates the m^6A demethylation of the target gene *Mefv* (encoding Pyrin) and consequently increases Pyrin expression. FTO O-GlcNAcylation also promotes Pyrin dephosphorylation and negatively regulates NLRP3 inflammasome assembly and inflammatory cytokines TNF-α/IL-1β/IL-6 expression of macrophages. FTO O-GlcNAcylation protects against LPS-induced inflammatory responses and septic shock. Our study reveals a mechanism that protein post-translational modification controls the biological functions of the FTO protein. Modulating FTO O-GlcNAcylation levels may offer a promising therapeutic strategy to prevent endotoxin-induced inflammatory sepsis.

Keywords: O-GlcNAcylation, FTO, Inflammation, NLRP3-inflammasome, Pyrin (*Mefv*), m^6A

The Potential Of Carob As A Prebiotic And Antioxidant Beverage

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Prebiotics are dietary substrates selectively utilized by gut probiotic microorganisms, conferring health benefits to humans. Modulating gut microbiota equilibrium through prebiotic foods has emerged as a promising approach for preventing and treating a wide range of diseases such as obesity, intestinal inflammation, cancer, and neuropsychological disorders [1]. Fructo-oligosaccharides (FOS) are well-established prebiotics, while polyphenols have recently demonstrated their potential as prebiotics [2]. FOS are produced enzymatically from sucrose by microbial enzymes with transfructosylation activity. Carob pulp, with its high sucrose content (65–75%) and high levels of polyphenols, shows great potential to be transformed into a prebiotic food [3]. Herein, we aimed to develop a prebiotic beverage using carob pulp by enzymatically converting its sucrose content into FOS (*in-situ* approach) [4].

Carob pulp extraction conditions were optimized to maximize sucrose extraction. Antioxidant, phenolic and flavonoids content of the carob pulp extract were analyzed. Four commercial enzymatic complexes were evaluated for FOS synthesis: Pectinex® Ultra SP-L, Viscozyme® L, Novozym® 960, and Catazyme® 25L. FOS resistance through digestion was evaluated by applying the standardized INFOGEST protocol [2]. Optimal carob pulp sucrose extraction conditions were determined to be a carob:water ratio of 1:3 (w/v), a temperature of 46.1 °C, and an extraction time 132 minutes. The obtained extract showed an antioxidant activity of 88.9 ± 0.2 µmol TEq/g DW by FRAP and 114.99 µg/mL by ABTS. The total phenolic and flavonoid contents were 8.35 ± 0.01 mg GAEq/g DW and 1.413 ± 0.007 mg CEq/g DW, respectively. Sucrose content was 86 ± 1 g/L. The complex Novozym® 960 produced the best results, yielding 0.42 ± 0.01 g of FOS per g of carob sucrose after 45 minutes of reaction, with a purity of 29.6 ± 0.3% (w/w). FOS showed resistance to gastrointestinal digestion, with only 9.9% of FOS hydrolysis.

In conclusion, a prebiotic beverage, including 30% (w/w) FOS, was successfully produced through enzymatic conversion of carob's inherent sucrose content using the Novozym® 960 enzymatic complex. Bioactive compounds, such as phenolic acids and flavonoids, were also identified in the carob beverage, suggesting its potential as antioxidant, anti-inflammatory, and anti-carcinogenic [2]. This substrate proved to be a promising alternative to pure sucrose in FOS production, offering a cost-effective process coupled with improved nutritional value and functionality.

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A Structure Defined Polysaccharide From *Dendrobium Officinale* Is Deconstructed By Human Gut Bacteroides Spp

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Arabinogalactan (AG) is an excellent prebiotic because it improves intestinal barrier function and partially restores intestinal microbiota [1], [2]. Hence, we hypothesize that more complex AG-like polysaccharides have better gut bacterial activity due to longer fermentation in the distal colon. Here, an AG-like polysaccharide TF112 from flowers of *Dendrobium officinale* could easily support the growth of *BT* and *BC* strains. Structure analysis showed that TF112, with *Mw* of 54.7 kDa, contained rhamnose, galacturonic acid, galactose, and arabinose in the molar ratio of 1.00: 1.01:4.00: 2.55. Its backbone included 1, 4-linked α-Gal*p*A, 1, 2, 4-linked α-Rha*p*, 1, 3, 6-linked β-Gal*p*, 1, 4-linked α-Gal*p* and 1, 3-linked β-Gal*p* with branches substituted at C-4 and C-3 position of rhamnose and galactose, respectively. The branches comprised terminal (T)- and 1, 6-linked β-Gal*p*, T- and 1, 5-linked α-Ara*f*. Studying the interaction between TF112 and intestinal bacteria revealed that TF112 promoted the growth of Bacteroides spp., leading to the production of acetic acid and propionic acid. The Bacteroides could also competitively utilize oligosaccharides with other strains. The AG-like polysaccharide TF112 appeared to be a better prebiotic, and dehydrogenate production suggested a new mode for intestinal Bacteroides to metabolize polysaccharides. These findings provide new insights into the metabolized mechanisms of prebiotics liked AG.

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sp²-Iminosugar-Based Trimannosyl Glycolipid Conjugate Mimics As Cell Membrane-Targeted Tlr4 Antagonists

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Trimannosyl glycolipid conjugates (MGCs), such as MGC-C25 (Figure 1), are composed of a branched core linked to three mannose units via triethylene glycol spacers and a saturated or unsaturated lipophilic chain. MGCs selectively block TLR4-mediated activation in human monocytes and dendritic cells (DCs) treated with LPS, but their action is not based on direct competition with LPS for binding to CD14 and/or MD-2, as is the case with most other TLR4 antagonists. Instead, compound MGC-C25 has a disruptive action on the glycosylphosphatidylinositol (GPI)-anchored proteins CD1a and CD14, located in lipid rafts, inducing their rapid cellular internalization. This result suggests that the plasma membrane may be the primary target through which the immunomodulatory effects of MGCs are mediated. In the presence of LPS, MGCs could prevent the colocalization of CD14 and TLR4, thereby acting as TLR4 antagonists. In this work, we explore the feasibility of replacing the α-D-mannopyranosyl substituents in MGCs with units of sp²-iminosugar motifs. Thus, conjugates that are chemically and enzymatically stable are obtained. On the other hand, the synthetic versatility of sp²-iminosugars is well suited to strategies aimed at molecular diversity, which has allowed us to address the preparation of a series of sp²-MGCs in which we have systematically modified both the configuration of the sp²-iminosugar and the type of glycosidic bond, as well as the nature of the spacer between them and the branched core or the lipid chain. Preliminary immunoactivity tests show that sp²-MGCs mirror the TLR4 antagonist capabilities of the parent MGCs.

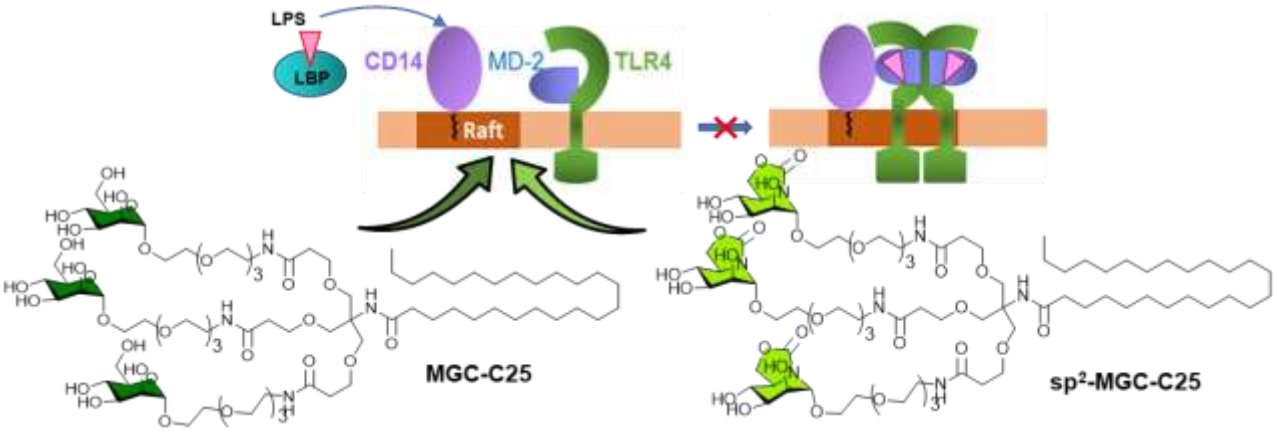

Figure 1. Structures of MGC-C25 and the corresponding sp²- MGC-C25, and illustration of the postulated membrane-targeted TLR4 antagonism mechanism.

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Preparation Of New *C*-Glycosyl-1,2,4,5-Tetrazines

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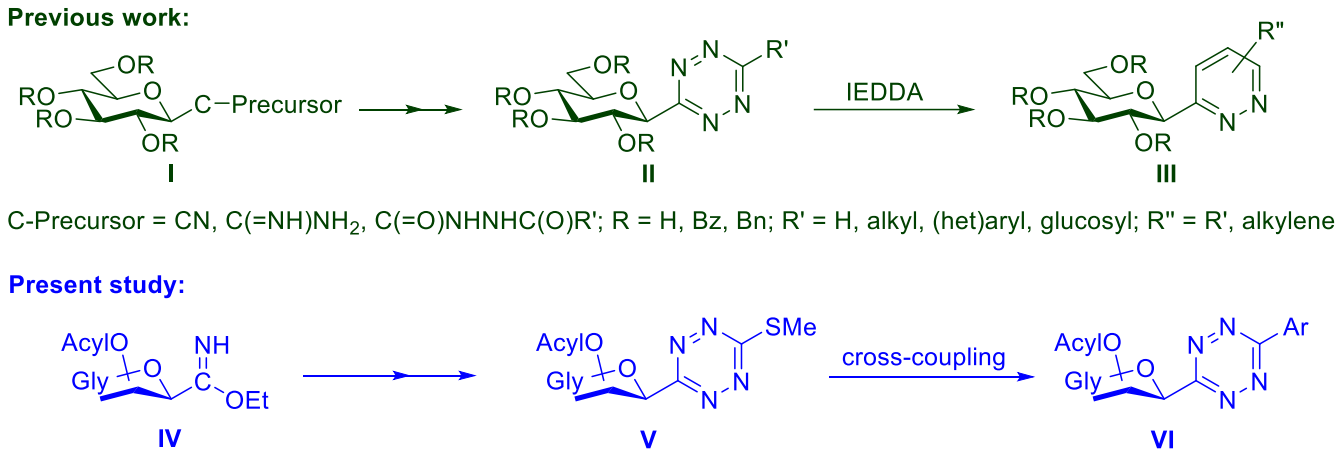
1,2,4,5-Tetrazines (*s*-tetrazines) are commonly used azadienes, whose [4+1] and [4+2] cycloadditions pave the way for constructing further N-heterocycles, such as pyrazoles, triazines and pyridazines [1]. Among these ring-transformations, the inverse electron-demand Diels-Alder reactions (IEDDA) promoted with strained cyclic dienophiles have become the most intensively studied ones in recent years. This type of transformations has emerged as one of the most powerful bioorthogonal reactions, providing possibilities for exploring physiological processes by labeling of biomolecules [2].
In a recent paper [3], we reported the first representatives of *C*-glycosyl-1,2,3,5-tetrazines, which were completely unknown in the literature prior to our work. A set of 3-β-D-glucopyranosyl-1,2,4,5-tetrazines (Scheme 1, **II**) were prepared by ring-closing reactions of different *C*-β-D-glucopyranosyl precursors (**I**). In addition, the synthetic applicability of these heterocyclic monosaccharides by their conversions into 3-β-D-glucopyranosyl pyridazines (**III**) was demonstrated [3].
As a continuation of this study, a new synthetic route to get additional *C*-glycosyl-1,2,4,5-tetrazines (**V**), suitable for functionalization (even with fluorescent moiety) by cross-couplings (**V**→**VI**), have been examined. Furthermore, the IEDDA reactions of tetrazines **II** and **VI** with strained cycloalkynes have also been under investigation to test their possible use as sugar-based bioorthogonal labeling agents.



Scheme 1.

In the presentation the details of route **IV**→**V**→**VI** as well as the first results of the strain-promoted IEDDA reactions of compounds **II** and **VI** will be reported.

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Bi-Component Nanoparticles As Bacterial Antigen Displaying Systems

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Nanoparticle (NP) technology was proven to be a potent tool to improve viral protein immunogenicity, thanks to antigen multi-display and an optimal dimension for antigen uptake. Recently, bi-component NPs have been computationally designed aiming to co-display different antigens on the same scaffold for boosting and promoting cross-protective antibody production.[1] I53 bi-component NP has an icosahedral geometric structure composed by 20 trimeric and 12 pentameric subunits. Protein antigens of interest are genetically fused to I53 trimers and NPs are easily produced *in-vitro* by mixing with I53 pentamers. I53-50 bi-component NPs have been recently used for SARS-COV-2 vaccine (licensed in South Korea and UK), while a tetravalent Pan-Sarbecovirus vaccine is under development.[2] Here, for the first time, I53-50 NP has been tested as a scaffold for bacterial saccharide antigens using model bacterial antigens.[3] Different approaches were tested with the aim to understand the optimal way to efficiently produce resulting conjugates.[4, 5] Animal studies are ongoing to evaluate the immune response elicited by these new constructs in comparison with traditional glycoconjugates (**Figure 1**).

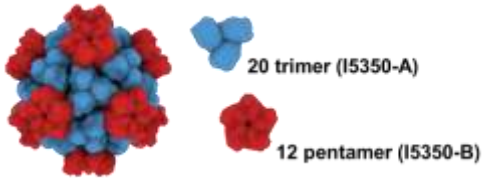

Figure 1: I53-50 bi-component NP structure.

In conclusion, the innovative use of I53-50 bi-component nanoparticles applied to bacterial antigens shows the value of integrating computational protein design and bacterial expertise. This work will provide valuable insights into the potential of this technology as a versatile platform for the development of more effective vaccines for global health.

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Discovery Of A Class Of Glycosaminoglycan Lyases With Ultra-Broad Substrate Spectrum And Evolutionary Transition Characteristics

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Glycosaminoglycan (GAG) lyases are often strictly substrate-specific, especially difficult to simultaneously degrade GAGs with different type of glycosidic bonds, such as chondroitin sulfate (CS) and heparan sulfate (HS). In this study, we found a class of novel GAG lyases (GAGases) from PL35 family that can efficiently degrade hyaluronan, CS, HS and even alginate. Specifically, GAGases prefer to act on the domains composed of non-/6-*O*-/*N*-sulfated hexosamines and D-glucoronic acids in GAGs or D-mannuronic acids in alginate but not domains with other sulfation patterns or epimerized hexuronic acids in GAGs/alginate. Crystal structure analysis showed that GAGases consist of a N-terminal $(\alpha/\alpha)_7$ toroid multi-domain and a C-terminal two-layered *β*-sheet domain with a Mn^{2+} involved in the catalysis of these enzymes. Notably, structural comparison combined with site-directed mutagenesis of key amino acids in the catalytic cavity demonstrated that although GAGases share the same Brønsted acid/base catalytic mechanism as various GAG/alginate lyases, they have a higher homology with alginate lyases than GAG lyases. Considering the view that alginate evolved earlier than GAGs, we can reasonably speculate that these GAGases with more similar folds to alginate lyases even alginate-degrading capacity should be transitional forms from alginate lyases evolving to GAG lyases. In addition, as a result of multiple structural alignments of GAGases with identified alginate lyases and crucial catalytic site-directed mutagenesis, we simultaneously identified key residues of GAGases associated with alginate degradation, which provides an important reference for the study of the catalytic mechanism of related enzymes. Overall, the discovery of GAGases not only provide important tool enzymes for structure-function studies of GAGs and preparation of related oligosaccharides, but also expands our understanding about the diversity of GAG lyases and provides a key clue to solve the puzzle regarding the divergent evolution of GAG lyases adapting substrates.

Glycomimetics In Ligand Directed Protein Profiling For Selective Labeling Of Glycoside Hydrolases

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Glycoside hydrolases together with glycosyl transferases represent the two largest families of so-called carbohydrate-active enzymes (CAZymes) [1,2]. Their combined activities lead to a sensitive homeostasis of formation and degradation of carbohydrate presenting metabolites. Due to the essential role of glycoside hydrolases in cells, including metabolism, antibacterial defense, and pathogenesis, profiling of the location and amount of active glycosidases is essentially important. Due to their delicate structure, often presenting a pocket-shaped active site leaving little space for large probes and tags, probe design targeting glycosidases is challenging [1]. Ligand-directed chemistry (LDC) [3,4] as a variant of well-known activity-based protein profiling (ABPP) [5,6] enables covalent, chemical modification of a protein of interest (POI). Essential for the LDC probe design is the availability of a ligand (Fig. 1, A) for the target protein. The linker region, connecting the ligand (A) and the terminal reporter tag (C), is equipped with a cleavable electrophilic reactive group (B), which is used for covalent bond formation. This is enabled by the presence of a nucleophilic amino acid residue located in close proximity to the ligand binding site of the POI [3]. As a consequence of the mechanism of LDC and the use of reversible inhibitors as ligands, the labeled protein remains its activity after covalent tagging [3,4]. Here we present the design, synthesis and biological evaluation of glycomimetic based probes for selective profiling of glycosidases, applying the ligand-directed chemistry approach. Experimental details and results of biological activity will be presented.

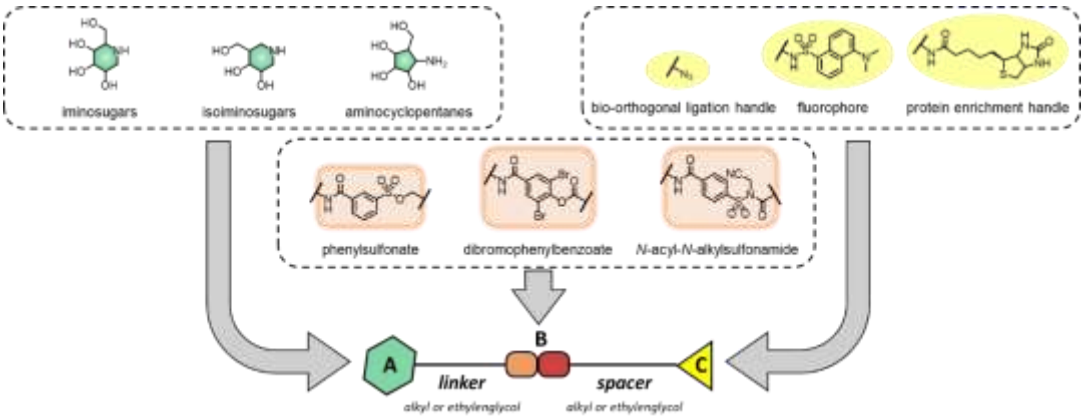

Figure 5: Building block concept for ligand directed chemistry (LDC) probes targeting glycoside hydrolases. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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The Methyl-Esters And Acetyl Influence The Intervention Of Homogalacturonan Obtained From The Fruits Of *Ficus Pumila* L. On Dss-Induced Colitis In Mice

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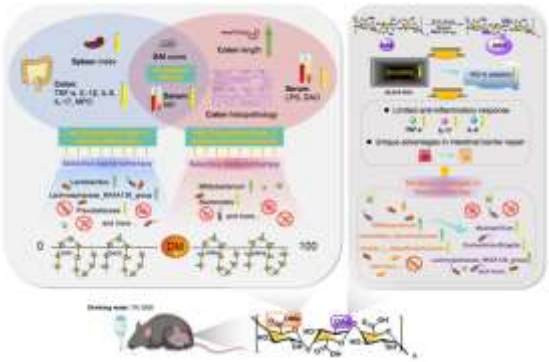
Currently, considering factors such as efficacy and safety, dietary fiber has attracted widespread attention in the prevention and treatment of inflammatory bowel disease (IBD). Adequate dietary fiber intake has been associated with a reduced risk of IBD development and favorable outcomes in disease management [1]. However, the differential effects of various dietary fiber types on IBD necessitate further investigation to elucidate the most efficacious options. Homogalacturonan (HG), the predominant component in pectin, is an edible dietary fiber found widely in nature. HG effectively improves symptoms of colitis [2], yet the impact of its structural parameters (methyl esterification and acetylation) is not completely understood.

Herein, typical reported HG from the fruits of *Ficus pumila* L. as the raw material was modified by chemical methods and the intervention effect of modified HG with different degrees of methyl-esterification (DM) and acetylation (DA) on dextran sulfate sodium (DSS)-induced colitis in mice was explored. Our results indicated that low-DM HG (DM3 and DM25) mainly alleviated colitis by reducing inflammation, such as inhibiting the expression of TNF-α, IL-1β, IL-17, and IL-6. Conversely, high-DM HG (DM54 and DM94) primarily alleviated colitis by repairing the intestinal barrier. These effects may be attributed to the preferential regulation of gut microbiome by HG with different DM. An increase in DA reduced the solubility of HG and didn't significantly promote the anti-inflammatory response, as observed in the inhibition of TNF-α, IL-6, and IL-17. However, it had unique advantages in intestinal barrier repair and microbiome regulation. Additionally, various structural parameters and substitution degrees showed no significant impact on HG's regulation of oxidative stress reactions. This study emphasizes the importance of structural parameters for the functional role of HG, providing a robust foundation for the design and development of functional dietary fiber supplements to prevent intestinal inflammation and other related conditions.



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α-Stereoselective Kdo *O*-glycosylation With *p*-toluenethioglycoside As Donor By $(p\text{-Tol})_2SO/Tf_2O$ Preactivation Strategy

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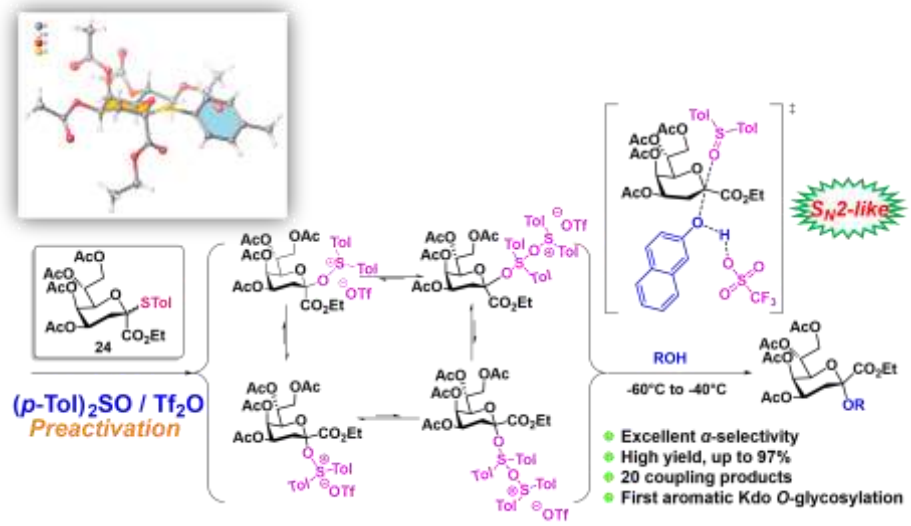
α-Kdo glycosides, as the key part of inner core of LPS of a variety of pathogenic bacteria show enormous values in immunology. However, synthesizing α-Kdo glycosides in high yields and with good stereoselectivity remains a great challenge due to the special structure of the anomeric carbon. Here, a convenient and efficient approach was developed to synthesize α-Kdo *O*-glycosides based on $Tf_2O/(p\text{-Tol})_2SO$ preactivation strategy using peracetylated Kdo thioglycoside as donor. The structure of donor **24β** was determined by X-ray single crystal diffraction, which showed a normal chair conformation and *tg* conformation. Under the optimized reaction conditions, several *O*-glycoside products, including α-(2→1)-, α-(2→2)-, α-(2→3)- and α-(2→6)-Kdo products were stereoselectively synthesized in high yields. Remarkably, utilizing $(p\text{-Tol})_2SO/Tf_2O$ preactivation strategy, a series of aromatic α-Kdo *O*-glycosides were first and successfully constructed in high yields. In addition, through this strategy, the shortcomings of thioglycosides on the aromatic *O*-glycosylation could be overcome. The free energy of possible intermediates and transition states were calculated and discussed. DFT calculations together with experimental results showed that the *O*-glycosylation of peracetylated Kdo thioglycoside would proceed via the *S_N2-like* mechanism to generate α-Kdo *O*-glycosides.



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Catalyst-Free Coupling Reactions Of Anhydro-Aldose Tosylhydrazones With 1,2,3-Triazoles

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In the past two decades *N*-tosylhydrazones **1** were generally used in metal-catalysed and metal-free C-C and C-heteroatom bond formation reactions as coupling agents [1, 2]. *N*-tosylhydrazones are readily available and stable molecules from which reactive carbenes can be generated *in situ* [1, 2]. Our simple method makes also anhydro-aldose tosylhydrazones **3** readily available [3]. We have systematically studied the coupling reactions of **3** with alcohols and phenols [4], thiols [5], aryl-bromides [6], benzyl-bromides [7], boronic acids [8], tetrazoles [9, 10] to access *C*-glycosylmethyl derivatives that can be useful glycomimetics. After having established a coupling methodology of **1** to give 2,4-disubstituted-1,2,3-triazoles **2**, in this work our goal was to extend the coupling reactions of **3** to form potentially biologically active 2-glycosylmethyl-4-substituted-1,2,3-triazole derivatives **4**.

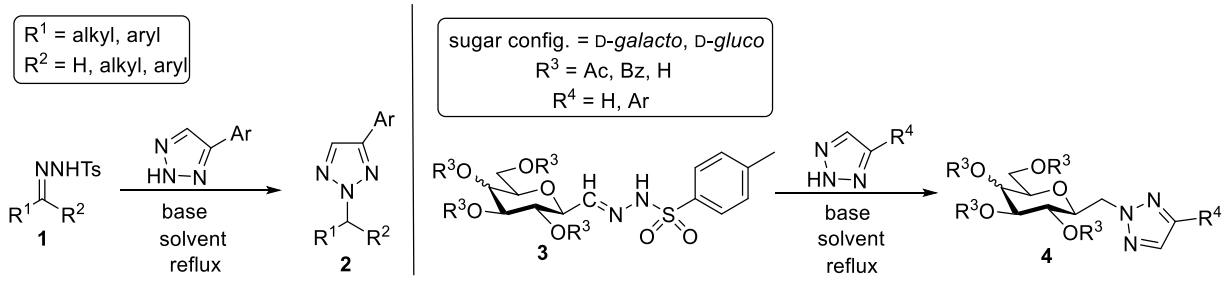

Figure 1. Synthesis of 2,4-disubstituted 1,2,3-triazoles

Acknowledgement: The research was supported by the National Research, Development and Innovation Office under the project FK128766 and K146147.

Keywords: coupling, *N*-tosylhydrazones, anhydro-aldose tosylhydrazones, 1,2,3-triazoles

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Half-Sandwich Type Platinum-Group Metal Complexes With Isoxazol(in)e Glycoconjugates As Ligands For Potential Biological Applications

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Platinum-based compounds such as cisplatin spearheads the anticancer chemotherapeutics, but their use is limited by resistance and toxicity. Based on this, extensive research is conducted to find drug candidates as effective or surpassing the benefits of cisplatin [1,2].

In our previuos studies half-sandwich type complexes of platinum-group metal ions (Ru(II), Os(II), Rh(III) and Ir(III)) with *O*-protected *C*- and *N*-glycosyl heterocyclic N,N-bidentate ligands (pyridin-2-yl or quinolin-2-yl substituted 1,2,4- and 1,3,4-oxadiazoles, 1,2,3-triazole) were developed and showed promising antitumor and antimicrobial effects [2,3]. Since the azole moiety of the above ligands proved to be decisive for the biological activity, in this work our aim is to investigate the effect of a new heterocycle, namely isoxazol(in)e coupled to various sugar forms (pyranose, furanose, open chain) on the biological efficiency (Figure 1).

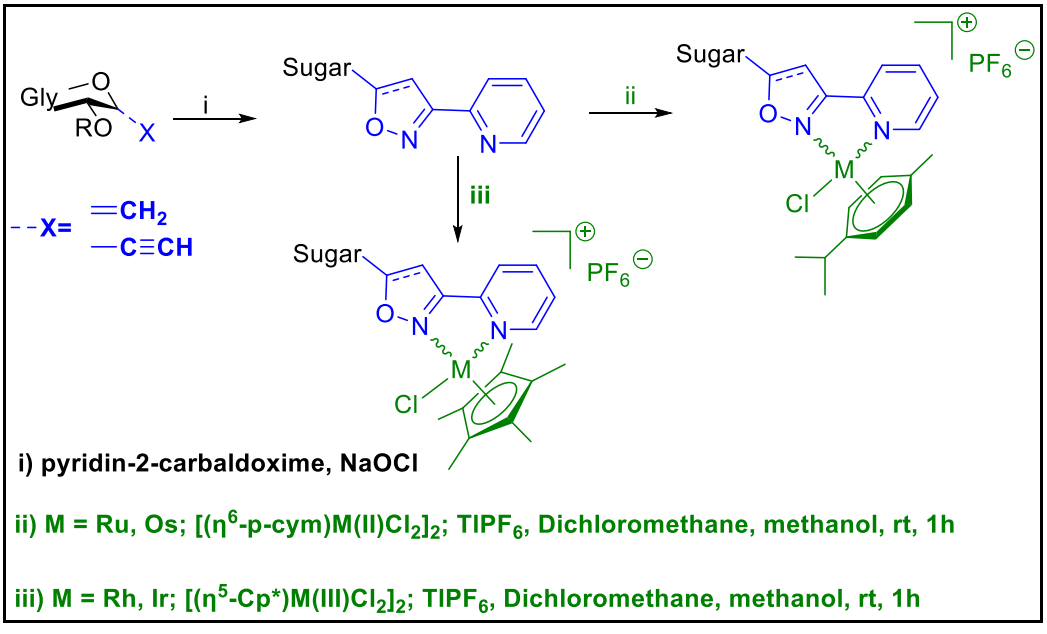

Figure 1.

In the presentation synthetic details for the preparation of the ligands as well as those of the outlined complexes will be highlighted.

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New Half-Sandwich Platinum-Group Metal Complexes With *N*- And *C*-Glucopyranosyl-1,2,3-Triazole Type N,N-Bidentate Ligands For Potential Biological Utilization

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The square planar platinum(II) complexes, the so-called platins (e.g. cisplatin, oxaliplatin, and carboplatin), represent one of the most commonly used types of chemotherapeutics [1]. Nevertheless, due to their inadequate selectivity towards tumor cells and their decreasing effectiveness in a long-term treatment, there is an ongoing research aimed at to find new metal complex-based drug alternatives with better anticancer potential [1,2]. In this regard, recent years have seen a significant amount of interest in the half-sandwich type complexes of other platinum-group metal ions, such as Ru(II), Os(II), Ir(III), and Rh(III) [1,2].

Recently, our research group synthesized a series of half-sandwich platinum-group metal complexes of the above metal ions with *N*- and *C*-glycopyranosyl azole type N,N-bidentate ligands (Figure 1, **I**). Several of them showed (sub)micromolar cytostatic activity against different cancer cell lines, and also exerted bacteriostatic effect on multiresistant Gram positive bacteria [3,4]. The most potent member of the set was a *p*-cymene containing Os(II) complex with *O*-perbenzoylated 1-*N*-(β-D-glucopyranosyl)-4-(2-quinolyl)-1,2,3-triazole ligand (**II**).

As a continuation of this study, the modification of the sugar derived ligand of **II** by changing the anomeric configuration from β to α (**III-A**) and the conjugation mode of the heterocycle to *C*-glycosylic derivatives (**III-B**) were envisaged. In the poster presentation the synthetic details of the glucopyranosyl heterocyclic ligands and their half-sandwich complexes will be reported.

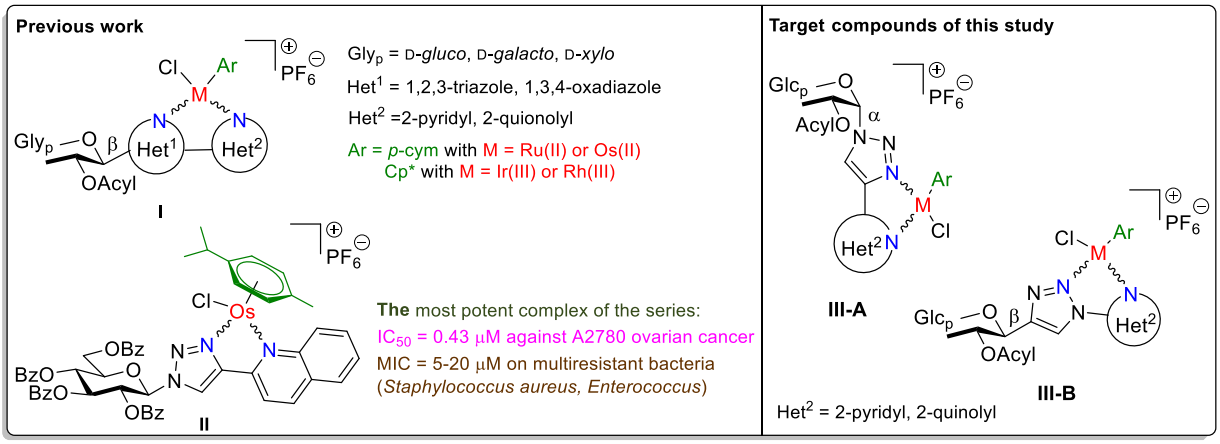

Figure 1.

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Glycosidic Linkage Analysis Of Arabino-Mannan Oligosaccharides In Carbohydrate-Protein Interactions

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Arabinomannan oligosaccharides are putative glycoconjugates in the bacterial cell wall structures. Whereas their bacterial cell wall functions are studied intensely, synthetic congeners of arabinomannans aid the in-depth analysis of their protein binding behaviour. A study was undertaken to identify the lectin binding behaviour of a series of arabinomannan oligosaccharides (**figure 1**), in order to evaluate the linkage dependence on the lectin binding. Carbohydrate-protein interactions gain good binding affinities through multivalency, either intra- (cis) or inter- (trans) molecular binding possibilities. The synthetic derivatives provide a route to assess these binding properties. Synthetic di-, tri- and pentasaccharide arabinomannans, possessing α-D-mannopyranoside at the non-residing end and an α-D-arabinofuranoside at the core, are undertaken for the study. The synthetic ligands are subjected to studies with lectin Con A, through isothermal titration calorimetry, in addition to dynamic light scattering and atomic force microscopy. Among the ligands, one trisaccharide and the pentasaccharide exhibit lectin binding, adhering to the bivalent structural and functional valencies. Whereas the remaining oligosaccharides display only a functional monovalency, even when the ligands possess bivalent structural valency. The trisaccharide with (1→2)(1→3) glycosidic bond connectivity, fulfilling both structural and functional valencies, stands out as the smallest bivalent ligand, engaging in lectin interaction through a trans-mode.

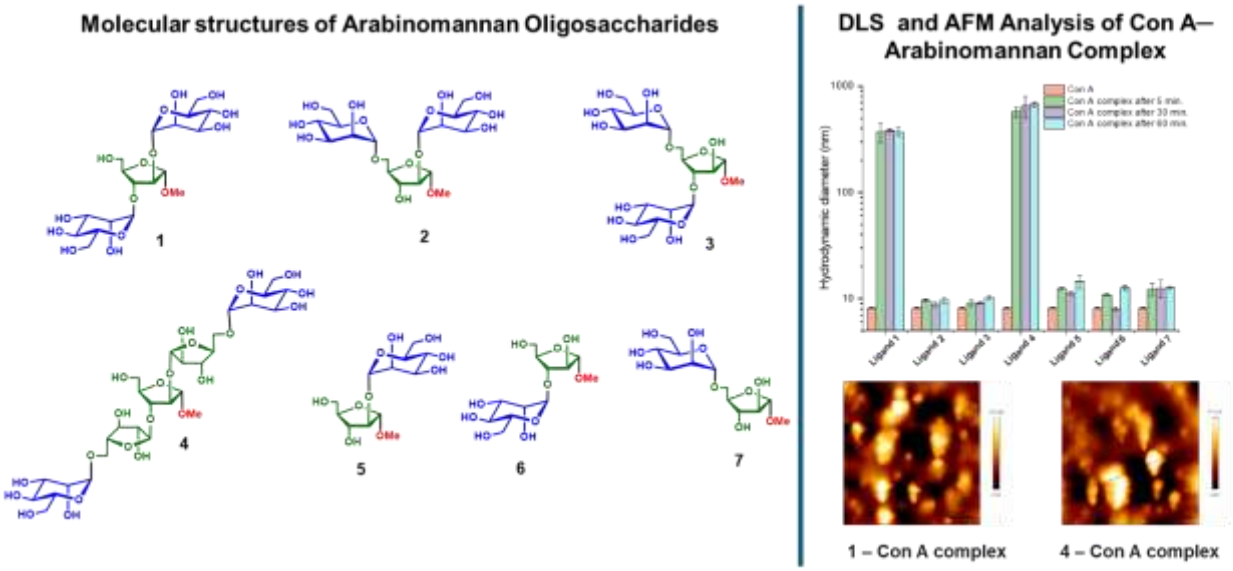

Figure 1. Molecular structures of Arabinomannan ligands and Plot of the changes in the hydrodynamic diameters of Con A upon complexation with ligand **1**-**7**.

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Heparan Sulfate: Towards A Human Therapeutic

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Our research has generated a potential human therapeutic based on heparan sulfate. This technology builds on this complex molecule's ability to moderate the bioactivity, selectively, of relevant growth factors.[1] As heparan sulfate is ubiquitous in the body it exhibits perfect biocompatibility; therefore, such a technology should direct cellular repair and speed tissue regeneration without any undesirable side-effects.

Our research focuses on the chemical composition of heparan sulfates (HS).[2] While the closely related anticoagulant Heparin, derived from porcine mucosa, is the most widely used natural product human therapeutic and commands an international market worth US$ ~10 B[3] there are no HS products on the market. Heparin, as the most negatively charged natural polymer[4] is also arguably the most information-rich biomolecule in nature. While only mast cells produce Heparin, and it is present in our body in very small amounts, *every* cell generates the closely related HS. However, commercial supply of HS is problematic and a non-trivial issue in developing a potential human therapeutic.

This presentation will detail our work towards generating bioactive fractions of heparan sulfate,[4] difficulties in the supply and characterization of this fascinating molecule, and its application in human clinical trials to facilitate rapid tissue repair. The characterization of this highly complex class of molecule, its application in wound repair technologies[5] and research towards synthetic mimetics will also be presented.

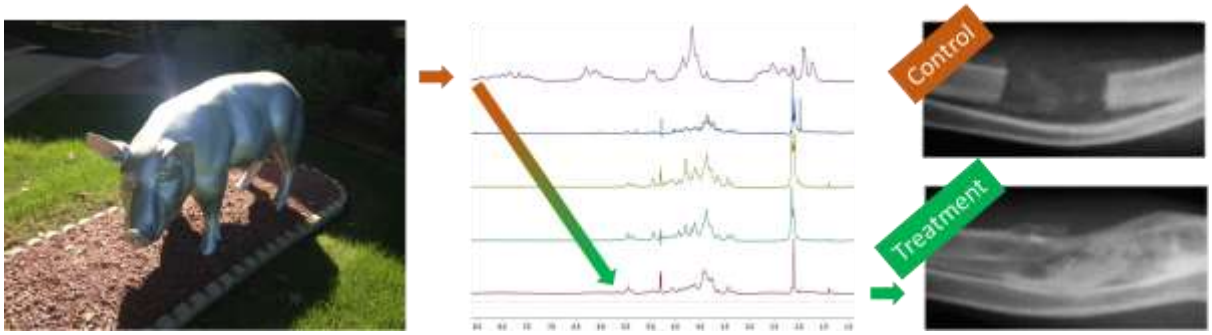

Figure 1. Porcine derived heparan sulfate after affinity purification promotes rapid bone repair.[5]

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Platform For The Cell-Free N-Glycosylation Of Polypeptides

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Cell-free protein expression enables the fast production of small quantities of peptides and proteins [1]. However, to date most commercial cell-free production systems are unable to generate glycosylated proteins. Here, we present a cell-free platform for the *N*-glycosylation of polypeptides complementing and expanding existing chemoenzymatic and chemical methods [2]. The platform is a synthetic copy of the eukaryotic *N*-glycosylation machinery of the Endoplasmic Reticuluum (ER) and Golgi apparatus. It consists of three parts. (1) An oligosaccharyltransferase (OST), STT3A from *T. brucei*, that inherently transfers glycans to the *N*-glycosylation consensus sequence (Asn-X-Ser/Thr) of polypeptides circumventing the limitations of bacterial OSTs that require extended amino acid sequences [3]. (2) An enzyme cascade of three ER mannosyltransferases for the generation of the lipid-linked core glycan $GlcNAc_2Man_3$ as substrates for glycan transfer reactions [4,5]. (3) A toolbox of human, trans-membrane deleted glycosyltransferase to glycoengineer the conjugated *N*-glycans on glycoprotein and glycopeptides [6]. For the latter, the expression and purification of a wide range of *E. coli*-produced glycosyltransferases for the generation of complex-type, multi-antennary *N*-glycans have been established.

In a case study, aglycosylated influenza A virus hemagglutinin peptides (HA1) (strain: A/Puerto Rico/8/1934) were in-vitro *N*-glycosylated using the platform. First, synthetic phytanol-linked $GlcNAc_2Man_3$ was produced in a multi-step one-pot reaction. The unpurified mix was then used as substrate for the transfer using recombinant OST STT3A and the peptides. The successful transfer was confirmed by LC-MS/MS and gel electrophoresis with laser induced fluorescence detection (CGE-LIF). Subsequently, transmembrane-deleted β-1,4-*N*-acetylglucosaminetransferases MGAT1 and MGAT2, β-1,4-galactosyltransferase and α-2,6-sialyltransferase were used in multi-step one-pot reaction to generate terminal α-2,6-sialylated, complex-type *N*-glycans on the influenza virus H1 peptides.

In summary, the platform is a powerful alternative to laborious chemical methods and limited chemoenzymatic methods for the *N*-glycosylation of polypeptides. Moreover, the toolbox of trans-membrane deleted glycosyltransferases alone can also be utilized to generate and study homogeneous glycoforms of (therapeutic) proteins including subunit vaccine candidates.

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Catalytic Epimerization Of α-C-Glycosyl Acetaldehyde: Synthesis Of 6-*O*-Methyl-D-*Glycero*-L-*Gluco*-Heptopyranosyl Fluoride Related To *Campylobacter Jejun*i NCTC 11168

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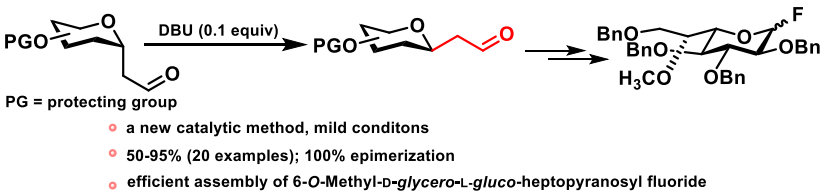

C-glycosides, similar to *O*-glycosides, are abundantly found in natural products and exhibit remarkable biological activities including anti-viral, antibacterial, and anticancer[1]. Additionally, due to the difficulty of C-glycosides have being hydrolyzed by the enzyme in the body, they have become artificial substitutes or analogues for natural *O*-glycosides. Definitely, the above content has greatly promoted the development of stereoselective strategies for constructing *C*-glycosides. Numerous methods have been reported in the past few years, leading to rapid advancements in this field. Compared to alkyl-α-*C*-glycosides, the synthesis of alkyl-β-*C*-glycosides is often more complex due to their conformational and electronic properties[2].In this study, we induced the epimerization of α-C-glycosylmethyl aldehydes with catalytic amounts of organic strong base to supplement the synthesis of β-anomers, thereby avoiding the tedious steps and reaction conditions involved in traditional synthesis methods.The method was also successfully applied to the preparation of 6-*O*-methyl-D-*glycero*-L-*gluco*-heptopyranosyl donor, providing a more convenient and efficient method for the preparation of this donor and providing the basis for the synthesis of *C. jejuni* NCTC11168 CPS tetrasaccharide repeating unit. Moreover, we are striving to implement this method for the synthesis of other L-heptoses.

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The Preventive And Therapeutic Effects Of Acute & Severe Inflammatory Disorders With Heparinoid

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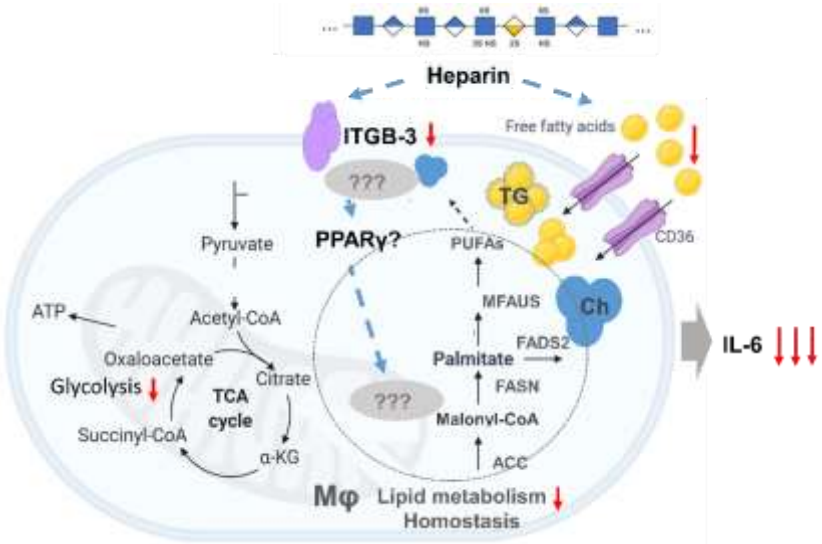
Sepsis is not a disease, but life-threatening syndromes of systemic inflammatory response developed from infection or other inducements. It is necessary to find drugs for preventing or interfering with the symptoms in the clinics. The anti-coagulant agent, heparin, effectively alleviated inflammatory reactions and prevented sepsis happen. But the efficacy is on issue, and the exact mechanism is not fully understood.

To confirm the therapeutic effects of heparin and investigate the mechanisms behind, we administrated heparin, 6-desulfulted heparin and N-acylated-heparin on the mouse models induced by “Two Hit” strategy with L-arginine. The results showed these compounds effectively suppressed the histological pancreatic damage, reduced the substantially enhanced cytokine concentration, and prevented multiple organ failures and death. Meanwhile, they significantly reduced macrophage infiltration in the pancreas, and lowered the extracellular HMGB-1 levels [1]. Further, they also inhibited the HMGB-1 secretion in RAW264.7 cell lines stimulated with the damaged pancreatic tissue. The emergence of the concept of immunometabolism as a major controller of immune response has raised a new hope for identifying new targets for immunomodulatory therapeutic approaches. By combined use of transcriptomics and metabonomics. Heparin was found keep lipid mentalism homostasis, inhibiting the changes the phenotype and function of macrophages (ITGB-3+) through CD36/PPARγ pathway, paving the way for new target deconvolution.



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Synthesis Of The Minimal Saponin Vaccine Adjuvant Based On QS-21

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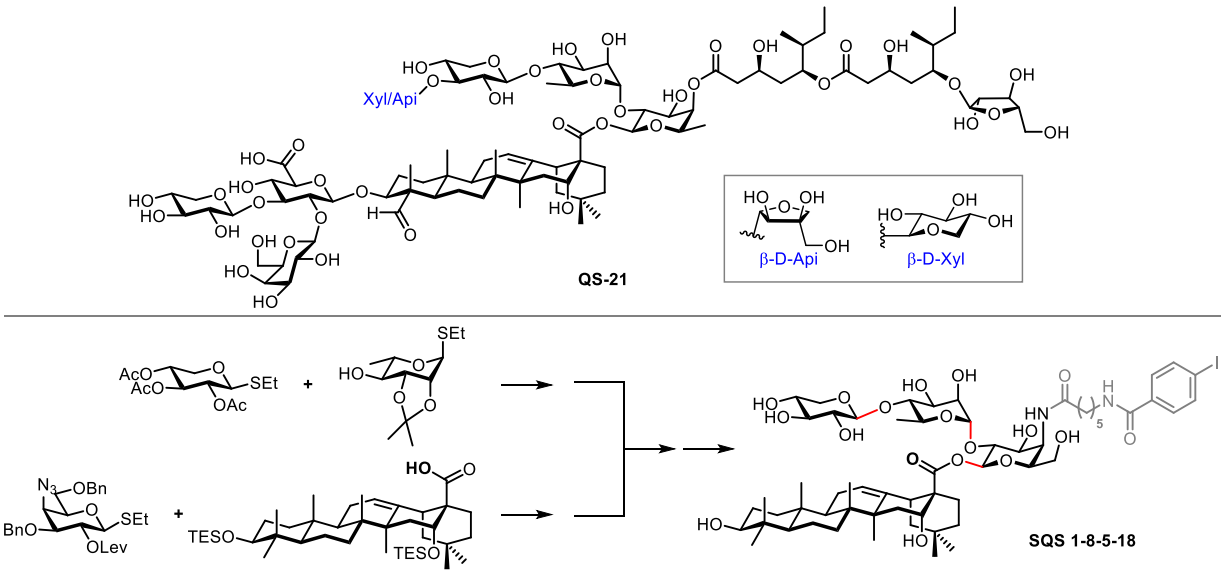
QS-21, a saponin natural product derived from the Quillaja saponaria tree, boasts exceptional immunoadjuvant activity. Its applications span across the treatment of various cancers and infectious diseases such as malaria, acquired immunodeficiency syndrome (AIDS), hepatitis, and tuberculosis. Despite its broad utility, QS-21 encounters certain limitations including restricted availability from natural sources, toxic side effects, and chemical instability due to the spontaneous hydrolysis of its acyl chain. In 2014, Gin and co-works identified SQS-1-8-5-18, which is the trisaccharide saponin component derivative of QS-21, as a minimal immunological adjuvant with reduced toxicity. The clinical potential of SQS-1-8-5-18 is promising, generating significant interest among researchers. Leveraging our recently developed SYA glycosylation method, we developed a practical syntehtic strategy toward SQS-1-8-5-18. By employing three SYA glycosylation, the key trisaccharide saponin intermediate of SQS-1-8-5-18 was obtained in excellent yield and on a decagrams scale.



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Glycopeptide From Mountain-Cultivated Ginseng Attenuates Oxidant-Induced Cardiomyocyte And Skeletal Myoblast Injury

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Abstract: In Asian traditional medicine, ginseng has been referred to as the “King of Herbs” because to its extensive therapeutic and pharmacologic characteristics, particularly in the treatment of type 2 diabetes mellitus and illnesses connected to diabetes. The 80% ethanol extracts of cultivated, red, and mountain-cultivated ginseng were liquid partitioned with hexane, chloroform, ethyl acetate, and n-butanol, respectively. The residues produced were processed with enzyme-assisted extraction by different enzymes. Cardiomyocytes, skeletal myoblasts, wild-type AB line zebrafish and *Tg* (kdrl:EGFP) zebrafish were used to screen and verify the protective effect of extracts. APMCG-1 is precipitated by alkaline protease-assisted extract from mountain-cultivated ginseng with 30% ethanol, which has a strong scavenging effect on hydroxyl radicals. In palmitic acid-induced H9c2 cells, APMCG-1 greatly enhanced cell viability while reducing reactive oxygen species generation and lactate dehydrogenase levels. Additionally, it reduced endoplasmic reticulum and mitochondrial dysfunction by increasing the Ca^{2+} level and membrane potential of mitochondria in H9c2(2-1) cells. APMCG-1 boosted glucose uptake while lowering creatine kinase levels in C2C12 cells. More significantly, 5 days after fertilization *Tg* (kdrl:EGFP) zebrafish and 1-month-old wild-type zebrafish with type 2 diabetic symptoms both had lower blood sugar and lipid levels attributed to APMCG-1. Further, APMCG-1 was identified as a glycopeptide containing O-linked glycopeptide bonds. As a PI3K/AKT activator, APMCG-1 protects the dysfunction of oxidant induced cardiomyocytes and skeletal myoblasts in type 2 diabetes, and is a potential therapeutic drug for diabetes.

Keywords: Mountain-cultivated ginseng; Glycopeptides; Type 2 diabetes mellitus Zebrafish; Cardiomyocytes Skeletal myoblasts

Catalytic Glycosylation For Minimally Protected Donors And Acceptors

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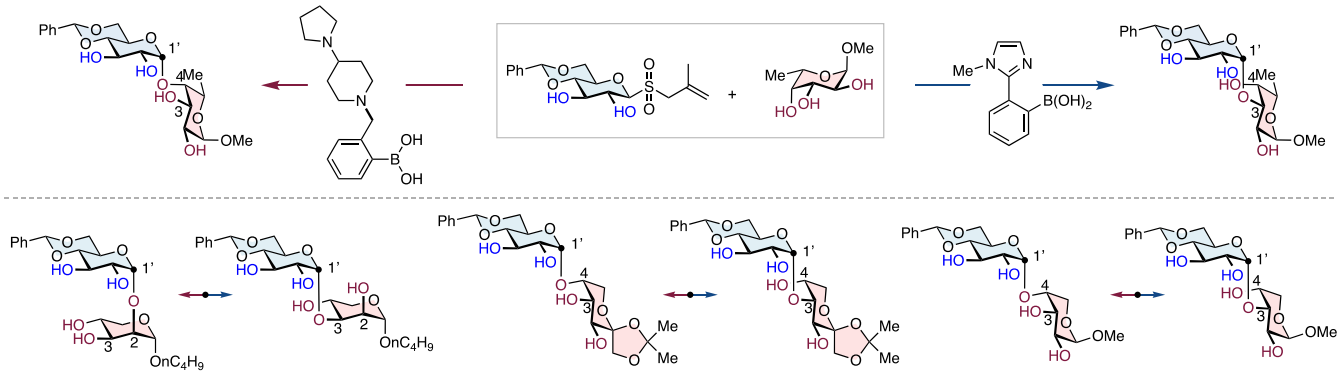
Abstract: Oligosaccharides, crucial participants in nearly all biological processes, represent among the most coveted yet structurally complex targets in synthesis. With a dense concentration of consecutive stereocenters and hydroxyl groups, their assembly through *O*-glycosylation requires simultaneous control of site-, stereo-, and chemoselectivities. To tackle this grand challenge, Nature has evolved a suite of enzymatic tools, while chemists have traditionally relied on protecting group manipulations. **Here we report a glycosylation platform that enables selective coupling between unprotected or minimally protected donors and acceptors, producing 1,2-cis-*O*-glycosides in a catalyst-controlled, switchable site-selective manner**. Our strategy initiates with a radical-based activation of allyl glycosyl sulfones to form glycosyl bromides. The key designed aminoboronic acid catalysts bring this reactive intermediate close to an acceptor through a network of noncovalent hydrogen bonding and reversible covalent B–O bonding interactions, allowing precise glycosyl transfer. The site of glycosylation can be switched by altering catalysts' structures and their interaction modes with substrates. The method accommodates a wide range of sugar types, amenable to preparing naturally occurring sugar chains and pentasaccharides containing 11 free hydroxyls. Experimental and computational studies provide insights into the origin of selectivity outcomes.



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Immunomodulation And Anti-Hepatocarcinoma Effects Of Oligosaccharide Derivatives-Related To *Antrodia Cinnamomea* Galactomannan

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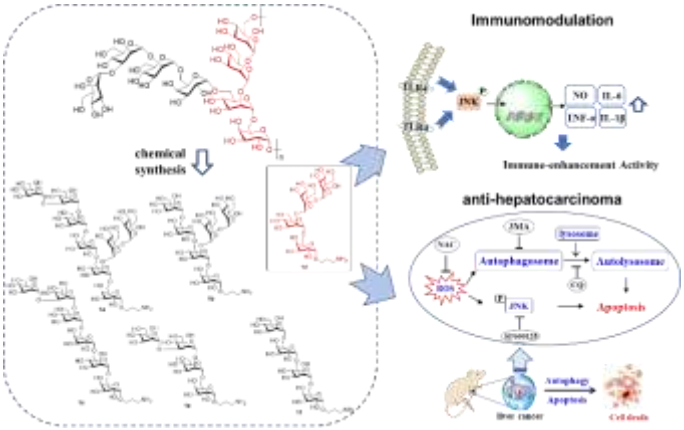
Antrodia cinnamomea (AC), a unique medicinal fungus grown in Taiwan Province of China, exhibits various pharmacological effects, such as immune regulation, anti-tumor, hepatoprotective, and antioxidation, etc[1]. Clinically, it has been widely used to treat liver disease, abdominal pain, diarrhea, drug poisoning, skin itching, hypertension, and cancer[2]. Polysaccharides in AC have been identified as one of the main pharmacologically active components[3]. The galactomannan (GM) is a neutral polysaccharide isolated from the cold-water extract of *A. cinnamomea*, and its repeat unit is an octasaccharide fragment composed of mannose and galactose, in which all the glycosidic bonds are connected by α-configuration. Moreover, this polysaccharide has been demonstrated to significantly enhance the phagocytic activity and bactericidal capacity of macrophages, showing great potential as an immunostimulant or adjuvant in immunotherapy and vaccine development[2].

Herein, we report the first chemical synthesis of the intact repeating octasaccharide unit, **1a**, of AC galactomannan, as well as its substructures, including two hexasaccharide fragments, **1b** and **1c**, and three tetrasaccharide fragments, **1d-1f**, for in-depth research on their immunological and anti-hepatocarcinoma activity[4]. Utilizing the structurally well-defined GM oligosaccharides **1a-1f** as the research subjects, a preliminary immunological study was performed to assess their impact on the proliferative activity, phagocytic capacity, and cytokine secretion of Raw264.7 cells in vitro. Ultimately, the tetrasaccharide backbone **1d** was identified as the active oligosaccharide epitope. Subsequently, the anti-hepatoma effects of **1d** were investigated in vitro and in vivo and its direct anti-tumor mechanism was also explored from the perspectives of autophagy and apoptosis. The results indicated that **1d** induced hepatoma cell death through ROS/JNK signaling pathway-mediated apoptosis and ROS-mediated autophagy-dependent apoptosis.



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Natural Polysaccharide Afg From Snail Mucus For Wound Repair

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Diabetic chronic ulcers are one of the most common and dangerous complications of diabetes, posing a significant threat to human life and health. Currently, clinical treatment methods are relatively limited, with a low cure rate, urgently necessitating the development of a new generation of drugs with clear mechanisms and definite therapeutic effects.

The authors found that the snail polysaccharide AFG can significantly promote acute/chronic wound healing, increase the regeneration of granulation tissue at the wound site, collagen deposition, and neovascularization. It also significantly downregulates the expression levels of pro-inflammatory cytokines such as TNF-α， IFN-γ， IL-6 in tissues, with effects superior to those of widely used clinical alginate dressings [1]. In-depth mechanistic studies revealed that AFG can upregulate the phosphorylation level of signal transducer and activator of transcription 3 (STAT3), promoting macrophages to polarize towards an anti-inflammatory M2 phenotype. Moreover, AFG can bind to inflammatory chemokines such as IP-10 and IL-8 with high affinity. Through these two pharmacological actions, AFG weakens the persistent inflammatory response in the microenvironment of chronic diabetic wounds, thereby repairing difficult-to-heal diabetic wound surfaces. Using a biomimetic strategy, a wound dressing based on AFG, AFG/GelMA hydrogel, was prepared. It can significantly promote wound healing in both type I diabetic rats and type II diabetic mice [2], making it a new wound dressing with clear efficacy, good biocompatibility, and ease of industrial preparation. These research findings provide potential new drugs and methods for common yet difficult-to-cure chronic wounds in clinical settings, with great application prospects.

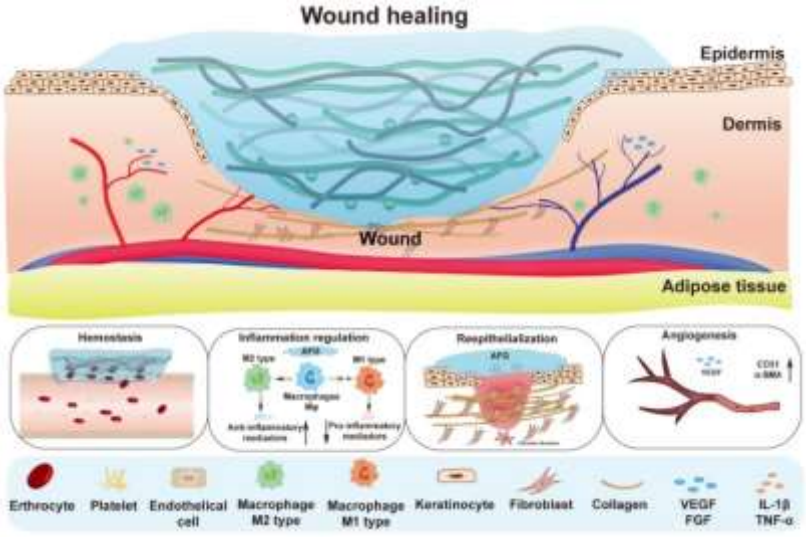

Figure: Schematic diagram of the mechanism of snail mucus gel healing chronic wounds

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Multi-Path Optimization For Efficient Production Of 2’ -Fucosyllactose In An Engineered *Escherichia Coli*

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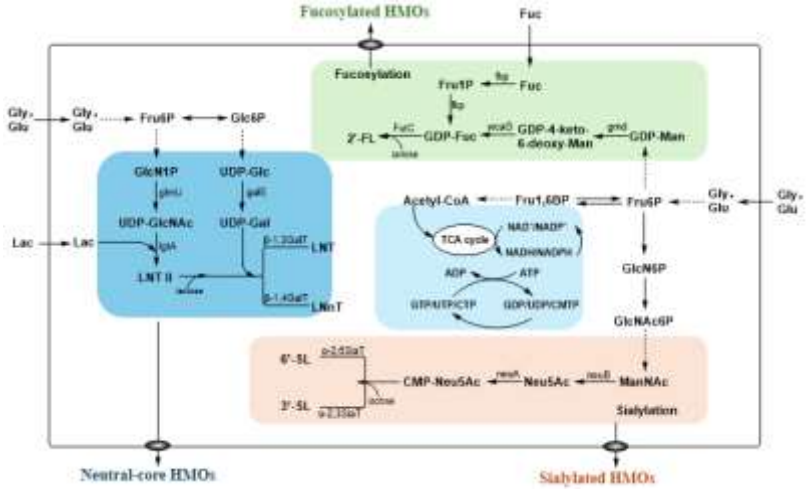
Human milk is unique in terms of complex oligosaccharides content, known as human milk oligosaccharides (HMOs), which is the third most abundant solid components in human milk. Their role in the development of intestinal flora blocking the attachment of pathogens and modulating the immune system of the infant are well-recognized. Compared to chemical and enzymatic synthesis, microbial production has obvious advantages in terms of production efficiency, reduced cost and environmental friendly.



In order to obtain high performance strains, the activity and selectivity of α-1,2-fucosyltransferase were enhanced significantly by directed evolution firstly. Subsequently, utilizing the strategy of “Design-Build-Test-Learn” to balance and finely regulate the 2'-FL metabolic network, including 1) strengthening the key precursor GDP-fucose metabolic pathway; 2) Weakening of degradation pathways of intermediate products; 3) Improvement of lactose and carbon utilization efficiency; 4) Balance between cofactors and energy; 5) Screening and expression of pump proteins. Ultimately, we obtained a high-yield 2'-FL producing strain. Furthermore, advanced precision fermentation technology was adopted for Scale up step by step, 2’-FL yield enhanced up to 200g/L, and with a lactose conversion rate of 95%.
Through the construction of the 2'-FL producing strain, we have also developed a series of high-yield strains of HMOs, including 3’-FL, 3’-SL, 6’-SL, LNnT, LNT, which laying the foundation for the production of high-end infant formula milk powder.

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A Simple Synthetic Strategy: Concise Access To Isoiminosugars And Beyond

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Isoiminosugars (**A**) are glycomimetics in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group [1]. In general, these compounds are selective and highly potent inhibitors of glycoside hydrolases [2-4]. Moreover, *C*-5a-chain elongated derivatives of this compound class, i.e. *C*-5a-chain extended entities (**2**) of 4-*epi*-isofagomine (4-*epi*-IFG, **1**) have been proven as highly potent pharmacological chaperones for the treatment of GM1 gangliosidosis [2,3]. As a matter of fact, the indicated structural characteristics of isoiminosugars (**A**) remain synthetically challenging. However, valuable synthetic strategies towards this compound class have been reported [2-4]. In context with our interest in the design and synthesis of such structures, we have found an efficient and concise synthetic approach towards isoiminosugars (**A**). This strategy relies on a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated glycopyranoses (**I**) leading to corresponding C-2 carbon chain branched glycofuranosides (**II**) [5]. We applied this ring contraction for the synthesis of isoiminosugars (**A**). Employing different configurations of **I** and variations in the reaction sequence open the avenue to various modifications in the substitution pattern. Herein, synthetic and mechanistic details as well as the scope and limitations of this approach will be presented.

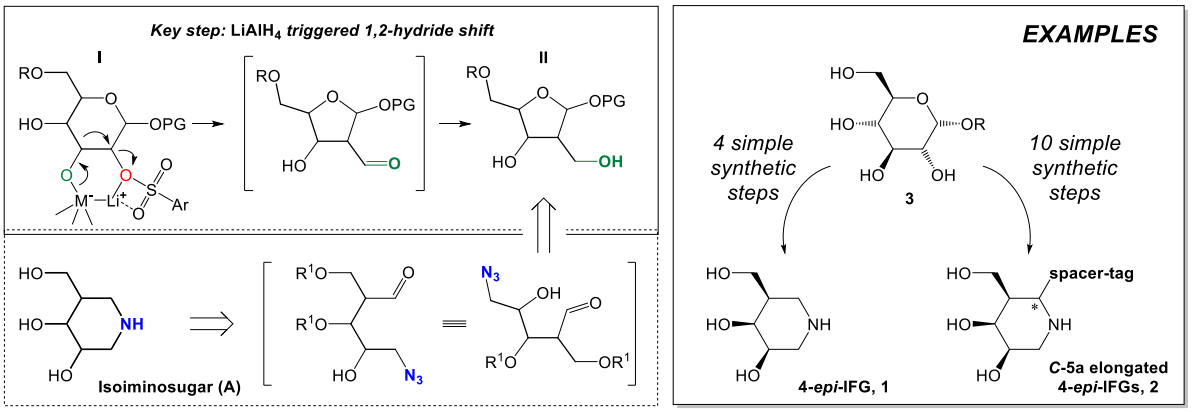

Figure: Schematic overview of a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated pyranosides (**I**) as key-step in our general (retro-) synthetic concept for isoiminosugars (**A**) (left), and its demonstration in a simple 4 step synthesis of 4-*epi*-isofagomine (**1**) and according conversions to *C*-5a elongated derivatives (**2**) starting from common α-D-glucopyranosides (**3**) respectively (right).

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Dehydroxylative Radical N-Glycosylation Of Heterocycles Enabled By Copper Metallaphotoredox Catalysis

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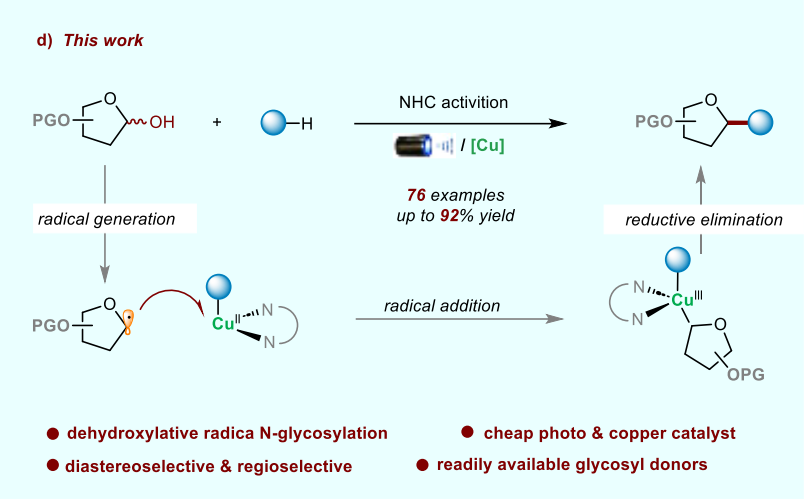

N-Glycosylated heterocycles play important roles in biological systems and drug development. The synthesis of these compounds heavily relies on ionic N-glycosylation, which is usually constrained by factors such as labile glycosyl donors, precious metal catalysts, and stringent conditions. Herein, we report a dehydroxylative radical method for synthesizing *N*-glycosides by leveraging copper metallaphotoredox catalysis, in which stable and readily available 1-hydroxy carbohydrates are activated for direct N-glycosylation. Our method employs inexpensive photo- and copper- catalysts and can tolerate some extent of water. The reaction exhibits a broad substrate scope, encompassing 76 examples, and demonstrates high stereoselectivity, favoring 1,2-*trans* selectivity for furanoses and α-selectivity for pyranoses. It also exhibits high site-selectivity for substrates containing multiple N-atoms. The synthetic utility is showcased through the late-stage functionalization of bioactive compounds and pharmaceuticals like Olaparib, Axitinib, and Metaxalone. Mechanistic studies prove the presence of glycosyl radicals and the importance of copper metallaphotoredox catalysis.

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Synthesis And Immunogenicity Evaluation Of Oligosaccharide Epitopes For The Development Of A Glycoconjugate Vaccine Against *Streptococcus Pneumoniae* Serotype 3

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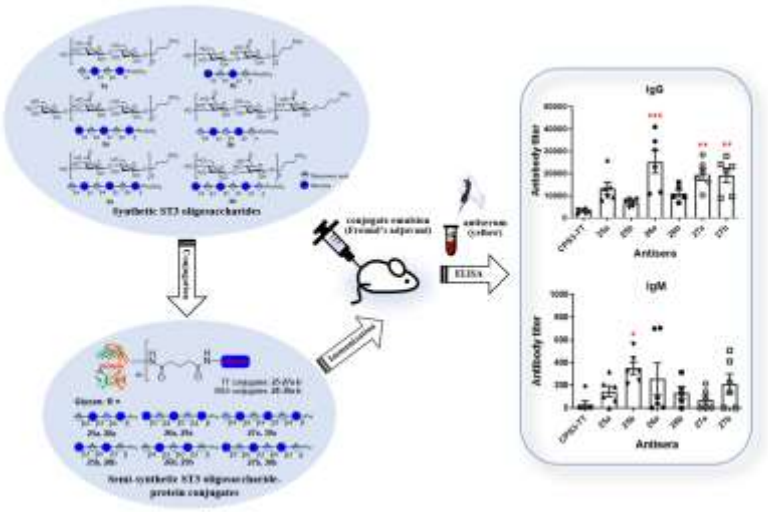
Streptococcus pneumoniae is a conditionally pathogenic bacterium that can cause a variety of invasive diseases[1]. It usually leads to high mortality rate. *S. pneumoniae* serotype 3 (ST3) is one of the most pathogenic bacteria, and vaccination against pneumonia has proved to be a relatively cost-effective measure for preventing and controlling transmission and morbidity from this organism in terms of safeguarding public health. However, the low immunogenicity of ST3 capsular polysaccharide (CPS) observed in currently used commercial conjugate vaccines has prompted the development of more effective anti-ST3 vaccines. Glycoconjugates based on ST3 CPS oligosaccharide fragments have been demonstrated to induce protective IgG antibodies against ST3 challenge and thus recognized as promising antigen candidates for developing novel ST3 vaccine[2-4]. Herein, a series of ST3 CPS oligosaccharide derivatives with different glycan chain lengths and sequences in the structure were efficiently constructed through a one-pot pre-activation glycosylation or convergent glycosylation, and the corresponding oligosaccharide-TT conjugates were then subsequently prepared using the bifunctional glutaryl linker[5]. Immunological studies in mice disclosed that all of the synthesized oligosaccharide conjugates induced T-cell dependent immunity that was comparable with or stronger than that of the polysaccharide conjugate CPS3-TT. The antisera induced by these oligosaccharide conjugates shown noteworthy cross-reactivity with the ST3 CPS polysaccharide, suggesting that the synthesized oligosaccharide, particularly hexasaccharide **3b**, has great potential as ideal antigenic epitopes for vaccine development.



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Zinc(II)-Mediated Stereoselective Construction Of 1,2-*Cis* 2-Azido-2-Deoxy Glycosidic Linkage

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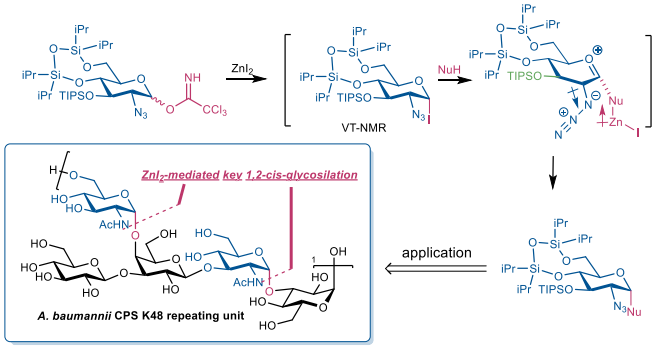
The capsular polysaccharide (CPS) of the pathogenic *Acinetobacter baumannii* is a major virulence factor and a promising target for vaccine development. However, the synthesis of 1,2-*cis*-2-amino-2-deoxyglycoside core of CPS remains challenging. Here we develop a highly α-selective ZnI_2-mediated 1,2-*cis* 2-azido-2-deoxy glycosylation strategy using 2-azido-2-deoxy glycose donors with various 4,6-*O*-tethered groups. Among them the tetraisopropyldisiloxane (TIPDS)-protected 2-azido-2-deoxy-D-glucosyl donor afforded predominantly α-isomer (α:β > 20:1) in the highest yield. We demonstrated the versatility and effectiveness of this novel approach by applying it to a wide substrate scope, including various aliphatic alcohols, sugar alcohols, and natural products, and succeeded in synthesizing *A. baumannii* K48 capsular pentasaccharide repeating fragments by employing this strategy in the key step of constructing the 1,2-*cis* 2-azido-2-deoxy glycosidic linkage. The reaction mechanism was explored with combined experimental variable-temperature NMR (VT-NMR) studies and mass spectroscopy (MS) analysis, and theoretical density functional calculations, which suggested the formation of covalent α-$C1^{GlcN}$-iodide intermediate in equilibrium with separated oxocarbenium–counter ion pair, followed by an S_N1-like α-nucleophilic attack most likely from separated ion pairs by the ZnI_2-activated acceptor complex under the influence of the 2-azido *gauche* effect.



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SYNTHESIS And Application Of Hydrazide Containing Fluorescent Probe

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Glycans play important biological functions in life activities such as cell recognition, signal transduction, and pathogen infection. Therefore, the study of the structure-activity relationship of glycans is crucial. However, the lack of chromogenic groups or reactive active groups such as amino groups in glycans molecules hinders the development of their structure-activity relationship research. Fluorescence imaging technology has the advantages of high detection sensitivity, fast response, and visualization, playing an important role in studying the tracing and localization of target molecules in the body. However, due to the complexity and specificity of glycans structures, the research on fluorescence labeling is relatively lagging behind that of biological macromolecules such as proteins and nucleic acids. Meanwhile, the types of fluorescent reagents that can be used for glycans labeling are relatively scarce and expensive. Based on this, we aimed to synthesize a fluorescent probe, fluorescein-5-thiosemicarbazide (FTSC), which can be used for labeling of glycans with reducing end or active aldehydes. In our study, nitrofluorescein was synthesized by condensation reaction of resorcinol and 4-nitrophthalic acid. Aminofluorescein was synthesized by a reduction reaction of nitrofluorescein. Then fluorescein isothiocyanate (FITC) was synthesized by an esterification reaction. Finally, FITC was converted into FTSC through an addition reaction. The synthesized FTSC was characterized by NMR and MS. The fluorescence spectrum of FTSC was very similar to that of popular fluorescence probe FITC; the fluorescence intensity of FTSC is 0.9 times over that of the FITC, showing potential in fluorescent imaging. A MTT assay showed that FTSC have slight effect on cell viability under 25-200 μM. Finally, we successfully applied FTSC to the fluorescent imaging detection of sialylated glycans on four cell lines, demonstrating the potential of FTSC in fluorescent imaging of glycans in *vivo*.

Down Regulation Of *Engase* In *Caenorhabditis Elegans* May Improve Its Stresses Adaptivity

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Abstract

Endo-beta-N-acetylglucosaminidase (ENGASE) is one of the key enzymes involved in the regulation of structure and function of glycoproteins. It is conserved from prokaryotic to eukaryotic cells. Although their activities *in vitro* and applications have been well studied, the biological function of ENGASE remains to be illustrated. In this study, we analyzed the molecular and physiological function of *Engase* from *Caenorhabditis elegans* homolog *eng-1*(*CeEngase*). We found that *CeEngase* knockout or knockdown increased the environmental stresses adaptability, such as heat stress and osmotic stress. Preliminary glycomics analysis showed that the basement membrane proteins of extracellular matrix may be the main targets of CeENGASE. In addition, CeENGASE may selectively prefer to N2H7 glycans on glycoproteins. In conclusion, our data illustrated that the defection and/or down regulation of *CeEngase* may provide a beneficially adaptation for stresses.

A Novel Bifunctional Agarase Of *Pseudoalteromona* sp. Q02

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Abstract: The backbone of agar is a linear molecule consisting of disaccharide units that are connected by repeated β-1,4 glycosidic bonds, and the disaccharide unit is composed of a β-D-galactose (G) and a 3,6-endoethergalactose (A) linked by an α-1,3 glycosidic bond. Recent studies have shown that a Glactosidase plays the same essential role in agar metabolism as an Agarase, while the mechanism remains unclear. *Pseudoalteromonas* sp. Q02 was a polysaccharide-degrading bacterium isolated from ocean. With genome sequencing and data mining, ORF0276 was obtained, which encoded a predicted product with a novel modular composition and organization type, and was classified as a disaccharide-yielding β-agarase within the GH50 family. The gene was initially cloned into the pET30a(+) plasmid and finally transformed into *E. coli* (DE3) cells, which were then stained with Lugol's iodine solution after induction, resulting in clear zones around the recombinant strains, suggesting that the gene encodes an agarase. The recombinant enzyme was then purified to a purity >95%, and determined by the DNS-reducing sugar method: the optimum was 50°C, pH 7. However, the enzyme was sensitive to metal ions and chemicals, and easily inactivated. TLC analysis showed that the main product was a neo-disaccharide (NA2) whereas with suspected dispersion of monosaccharides, which means a non-typical agarase. The enzyme-polysaccharide reacted mixtures were labeled with 2-aminobenzamide (2-AB) at the reducing end, then purified by HPLC, and finally subjected to mass spectrometry, resulting in a product dominated by 2-AB-G and 2-AB-GA signals,but few 2-AB-A or 2-AB-A-derived signals, suggesting that the enzyme can catalyze the *β*-1,4 glycosidic bonds as a β-agrase, as well as an α-glactosidase. In addition,TLC analysis also showed that the enzyme could incompletely degrade lactose (disaccharide) molecules, suggesting the cleavage of inner *β*-1,4 bonds. Therefore, the enzyme ORF0276 combines the activities of both a β-agarase and an galactosidase, which have rarely been reported in the same category, making it a novel enzyme tool and also beneficial to discover associated mechanisms.
Keywords: Agarase; *Pseudoalteromonas*; Genome; Glactosidase; Glycosidic bond;
Findings: The Natural Science Foundation of Science and Technology Department of Shandong Province (No. ZR2019MD038) and Shandong Department of Education Graduate Education Joint Cultivation Base Construction Project (No.SDYJD18001).

Effect Of A Novel Polysaccharide From *Lentinus Edodes* On Gut Microbiota

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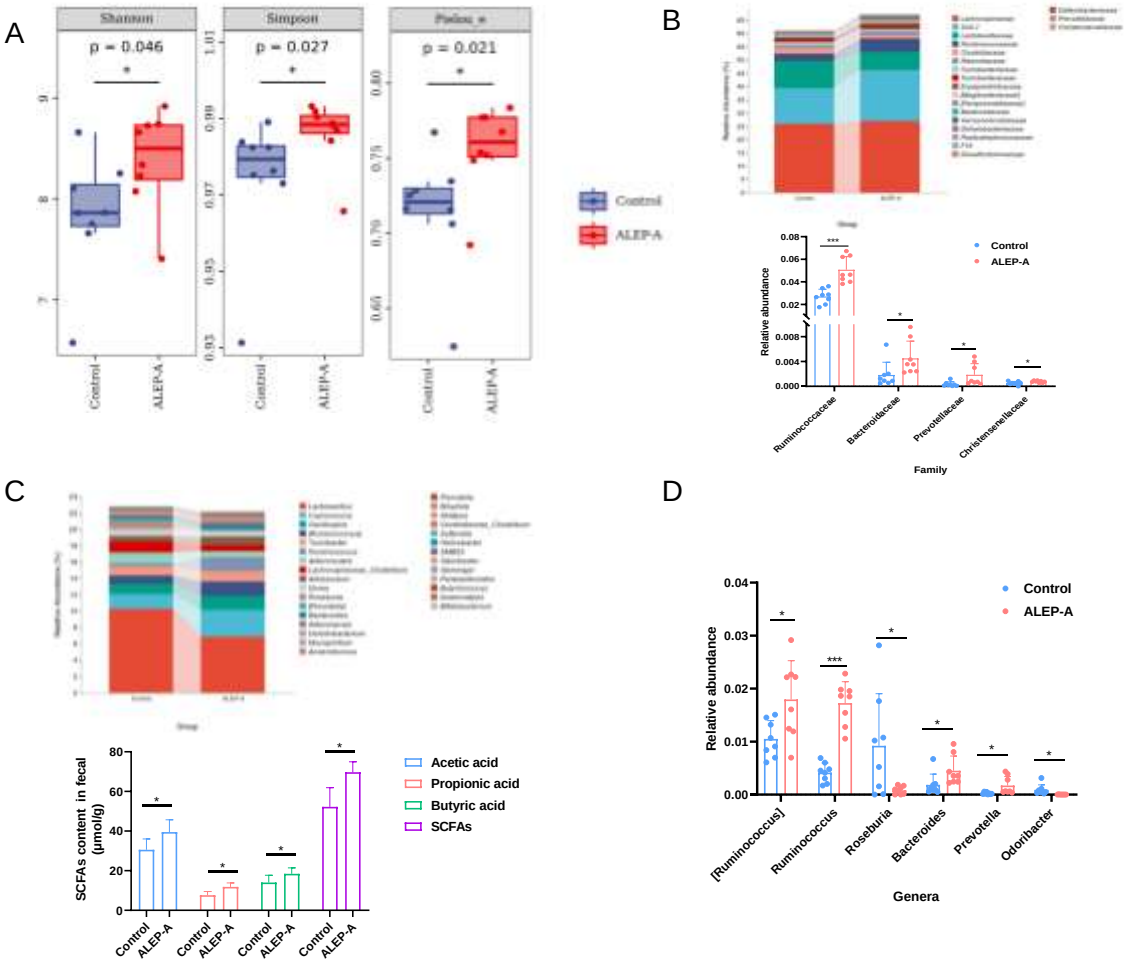

Polysaccharides from *Lentinus edodes* (*L. edode*s) have a variety of biological activities[1]. In the present study, a novel polysaccharide named ALEP-A was obtained from the water extraction residue of *L. edodes* fruiting bodies by hot alkali extraction method. 16S rRNA sequencing results indicated that ALEP-A significantly increased the α diversity of gut microbiota (Figure A) and upregulated the relative abundance of Ruminococcaceae, Bacteroidaceae, Prevotellaceae and Christensenellaceae at the family level (Figure B). At the genus level, ALEP-A promoted the relative abundance of *[Ruminococcus]*, *Ruminococcus*, *Bacteroides and Prevotella*, and decreased the abundance of *Roseburia* and *Odoribacter* (Figure C). In line with previous findings[2], ALEP-A significantly increased the concentrations of acetic acid, propionic acid and butyric acid in the cecal contents (Figure D). Collectively, our study suggested that ALEP-A could significantly regulate the composition and structure of gut microbiota, and may play an immunomodulatory role partly by promoting the production of short chain fatty acids (SCFAs).

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Structural Characterization And Antitumor Activity Of A Pectin Polysaccharide From *Euphorbia Humifusa* W.

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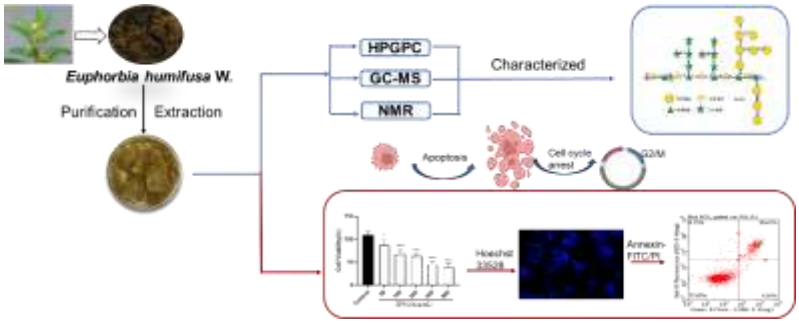
As one of the most malignant tumors in the world, Human hepatocellular carcinoma brings a huge burden to people Chemotherapy, as the most commonly used and effective modality for the treatment of liver cancer, has the drawbacks of high cost, non-specific cytotoxicity, and multiple adverse effects, especially toxicity to normal cells[1]. Therefore, it is important to explore natural antitumor drugs with strong cancer inhibition and low toxicity. A large number of studies have shown that natural plant polysaccharides have significant antitumor activity and obvious toxic side effects. *Euphorbia humifusa* W. is the dried whole grass of *Euphorbiaceae* family, which has the efficacy of clearing heat and detoxification. Recent studies indicated that *Euphorbia humifusa* W. had a variety of bioactive functions such as antitumor, anti-inflammatory, antioxidant and antiviral. However, its antitumor components are not clear, and polysaccharides, as the main components, may be the material basis for the antitumor activity.

In this study, a rhamnogalacturonan Ⅰ polysaccharide EPS-2A was separated on a DEAE Sepharose Fast Flow, purified on a Superdex™ 75 column, and eluted with 0.2 mol/L NaCl from *Euphorbia humifusa* W. Its molecular weight was estimated to be 55.6 kDa by high performance gel permeation chromatography (HPGPC). The structure characterization of EPS-2A was analyzed by the monosaccharide composition, partial acid hydrolysis, methylation analysis[2], and NMR analysis. Its backbone was predominantly composed of alternate 1,2-α-Rha*p* and 1,4-α-Gal*p*A, with branches of 1,6-β-Gal*p*, 1,3,6-β-Gal*p*, T- ,1,5-linked, 1,3-linked, 1,3,5-linked, 1,2,5-linked α-Ara*f*, which were attached to the *C*-4 of 1,2-α-Rha*p*. MTT assay showed that EPS-2A reduced the cell viability of HepG2 cells in a concentration-dependent manner. To assess whether the growth inhibitory effects of EPS-2A on HepG2 cells was associated with apoptosis, both control and EPS-2A-treated cells were stained with the fluorescent Hoechst 33258 nuclear dye and visualized by a fluorescent microscope. The result showed that the HepG2 cells treated with EPS-2A were observed cell shrinkage, chromatin compaction and nuclear fragmentation. To further quantify the apoptosis caused by EPS-2A on HepG2 cells, the percentages of apoptotic cells were analyzed using flow cytometry. The numbers of late apoptotic cells were significantly dose-dependently increased as compared to control group. These findings suggested the anti-tumor potential of EPS-2A in the treatment of human liver tumors.



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Copper-Catalyzed Stereoselective Synthesis Of A-2-Deoxygalactosides

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Deoxysugars exist in a variety of biologically active natural products and clinical formulations; and their chemical properties are of great research value in carbohydrate chemistry[1]. 2-Deoxyglycosides have unique chemical properties, that is, they lack a guiding group at C-2 to control its selectivity in glycosylation, and generally a mixture of α/β was present in the products. Therefore, it is very important to develop stereoselective synthetic methods of 2-deoxyglycoside linkage. In the past decade, many research groups have made outstanding contributions in this field. For example, Kashyap's group used CuI/$NaIO_4$ catalytic system to catalyze glycals into 2-deoxy-2-iodo glycosides, which requires a further deiodization for final product[2]. Liu's research group successfully synthesized 2-deoxydisaccharide using Yu's glycosylation[3]. Galan's group synthesized a series of α-2-deoxyglycosides successfully using a gold/silver system and perbenzyl-protected glycals as donor[4]. Our research group have been studying the synthesis of 2-deoxygalactoside, using fully acylated glycals[6]. In recent years, there have been many advances in this area, such as Yao 's group using Fe (OTf) $_3$[6], and Yu's synthesis of deoxyglycoside drug ecdysoside F[7]. Based on considerations of atomic economy, our group tried to synthesize 2-deoxyglycosides from D-Galactal with different promotors. We used glycals protected by 3, 4-carbonates as substrate, $Cu(OTf)_2$ as catalyst (0.1eq), alcohols and phenols as acceptors at 80° in a short time (<30mins). 2-Deoxyglycosides were synthesized with good yields (63-92%, ~20 examples) and a single α-selectivity (α/β>30:1). Besides, a deoxygalactosyltrisaccharide was synthesized to demonstrate the substrate applicability of our method.

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Development of novel vaccine candidates and passive immunotherapy against multidrug-resistant hypervirulent *Klebsiella pneumoniae*

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Klebsiella pneumoniae (KP), which was first identified in 1882 as a causative pathogen for pneumonia, was highlighted by WHO in 2017 as one of the most threatening multidrug-resistant pathogens urgently demanding development of new efficient therapies with highest priority.[1] The acquisition of multiple antibiotic resistances gradually increased the failure of traditional treatments. The emergence of hypervirulent Klebsiella pneumoniae (hvKP) even worsens the issue, and the convergence of multidrug-resistant and hypervirulent KP stains significantly increases mobility and mortality.[2] As encapsulated Gram-negative pathogen, KP was surrounded by the capsular polysaccharide (CPS) layer. CPS is critical for the survival of KP in harsh environment (like antibiotic treatment) and its infection capability. The CPS structure of KP shows great diversity, and up to 78 K-serotypes have been identified from KP strains till today, and K1 and K2 cover more than 70% clinical cases of hvKP infection. The passive immunotherapy offers prevention against KP by immunizing host with the KP capsular polysaccharide. Great efforts have been made by using native CPS as antigens. In 1985, Cryz et al. reported the safety and immunogenicity study of KP K1 CPS vaccine in human volunteers.[3] Single dose vaccination elicited IgG response specifically against K1 CPS, and the isolated human antisera showed good protecting effect in mice burn wound sepsis model. In further study, a 24-valent CPS-based KP vaccine was tested in Phase 1 clinical trial and showed promising result.[4] However, the production and heterogenicity of native CPS are problematic due to KP's virulence and purification process. As the alternative route towards CPS-based hvKP vaccine, the development of chemically synthesized oligosaccharide conjugate vaccines is quite appealing, due to the exact structural homogeneity of the sugar epitope and the possibility for fine optimization of the epitope structures, carrier proteins and ligation strategies. Recently, a synthetic tetrasaccharide-CRM197 conjugate vaccine based on K2 CPS of KP was reported by Wu et al., [5] and a more complicated hexasaccharide-CRM197 vaccine based on a clinically isolated carbapenem-resistant KP CPS (showing structural similarity to K19 and K34) was reported by Seeberger and Pereira.[6] These works demonstrated the feasibility of the development of synthetic oligosaccharide conjugate vaccine against multidrug resistant kvKP. Thus, we plan to tackle the challenge multidrug resistant hvKP by developing synthetic oligosaccharide conjugate vaccine based on K1/K2 derived epitopes and generating anti-K1/2 CPS monoclonal antibodies for potential diagnostic and therapeutic use.

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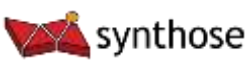
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