Poster session 1

Monday 10th

15h30 - 17h30

Even numbers

Salle 151: P2 to P112

Salle 251: P114 to P224

Application of Synthetic Glycans to orchestrate animal gut health

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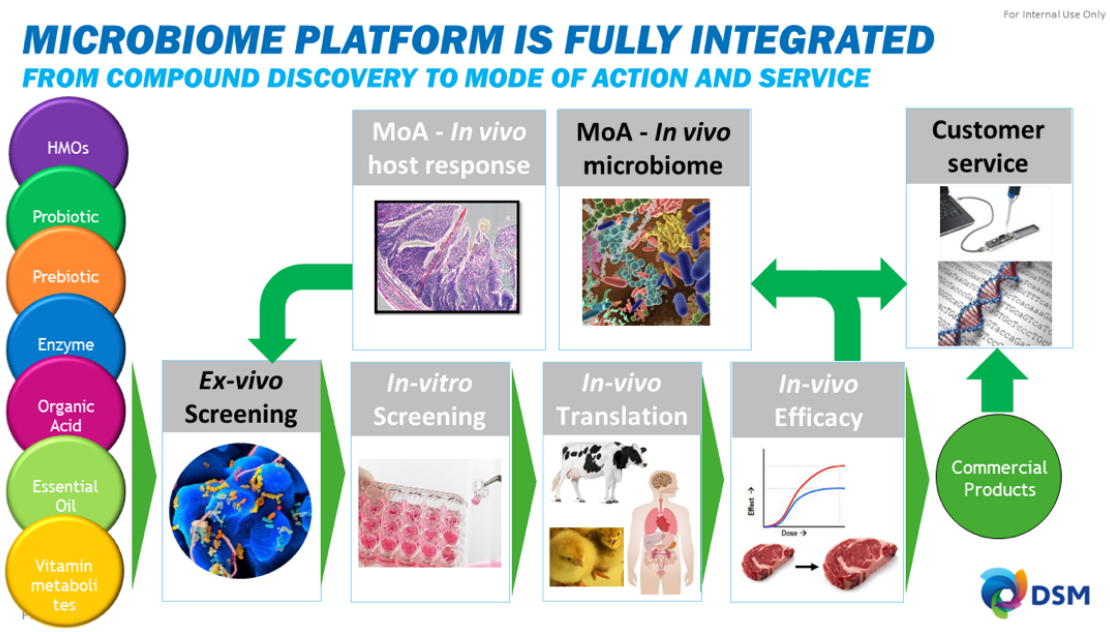
The role of glycans in modulating microbiome composition and promoting gut health is well-established. Specifically, glycans in the form of complex polysaccharides that resist human and animal digestion have been shown to have a significant positive impact on the gut microbiome by changing the relative abundances of bacterial species. In this study, we investigated the potential of using a diverse range of synthetic glycans to modulate the microbiome of poultry. Our results demonstrate that these glycans can promote cooperation amongst existing microbes, direct metabolite production by the microbiome, and optimize resilience to enteric stress, improve nutrient utilization, and reduce emissions. Importantly, these glycans also improved the welfare and productivity of the birds. In this presentation, we will present the results of our screening and characterization studies with these synthetic glycans, as well as the effects of optimized glycan mixtures on animal trials.



DSM-firmenich microbiome platform applied for complex glycans

Synthesis of potentially biologically active carbohydrates: transformation of anhydro-aldose oximes

Ágnes HOMOLYA [1.2], Tímea KASZÁS [1], Bence SZAKÁCS [1], Tekla BLÁGA [1], Kyle DOHERTY [3], Trinidad VELASCO-TORRIJOS [3], Hina SHAFIQUE [1], Zoárd ECSEDI [1], Éva JUHÁSZ-TÓTH [1], László JUHÁSZ [1], Ulf J. NILSSON [4], László SOMSÁK [1], Marietta TÓTH [1]

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Candida albicans is a yeast which can exist in a commensal state in the mucosae and gastrointestinal tract. *C. albicans* becomes pathogenic in immunocompromised patients under various conditions, like superficial and systemic infections. The high pathogenicity of *Candida* species may be related to their adherence to the host organism [1]. The cell surface glycans are important receptors for *C. albicans* and warrant the development of anti-adherence ligands that can mimic them, thus disrupting *C. albicans* - epithelial cell interactions. Mono- and disaccharideswere found to inhibit the adhesion of C. albicans,among these carbohydrate derivatives the bivalent galactoside derivativecontaining triazole units has been shown one of themost effective [2].

Our group have elaborated synthetic procedures for the preparation of anhydro-aldoximes by the transimination reaction of anhydro-aldose semicarbazones [3]. Oximes can be applied to *in situ* generation of nitrile oxides, these intermediates can be further transformed in various ways.

Based on these preliminaries, we aim systematically study the reactions of anhydro-aldose oximes derived nitrile oxides to synthesize glycopyranosylidene-spiro isoxazolines as galectin and glycogen phosphorylase inhibitors, and C-glycosyl-isoxazoles, -isoxazolines and 1,2,4-oxadiazoles, which may be potential anti-adhesion agents of C. albicans as these heterocycle units are bioisosteres of the triazole [2] which were prepared and tested in Maynooth University.

Acknowledgements

Research was supported by the N. R. D. I. Office under the project PD 142641, by the ÚNKP-22-3-I-DE-390, and by the COST under the project CA18132.

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Studies on anomeric phosphorylation under modified Mitsunobu reaction conditions

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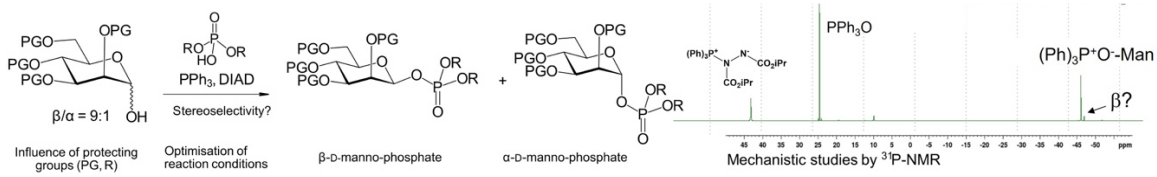
Glycosyl phosphates are important biomolecules involved in essential biological processes such as metabolism and oligosaccharide/phosphoglycan biosynthesis which requires the development of efficient approaches for the stereoselective synthesis of anomerically pure glycosyl phosphates as substrates or synthetic intermediates for NDP-sugars. The stereoselectivity of phosphorylation at the anomeric center generally reflects the anomeric ratio in the lactol precursors and, therefore, requires the preparation of anomerically enriched hemiacetals, which should then be directly converted to phosphates using P(III) or P(V) chemistry. For some pyranoses, however, the preferred anomeric configuration in lactols is the opposite of that required for the synthesis of biologically important glycosyl phosphates: D-Man and L-Ara4N are some of examples.[1] Also, D-manno-heptose, a precursor of ADP-(L/D)-glycero-β-D-manno-heptose and β-heptose 1,7-bisphosphate which serve as substrates for alpha-kinase 1, a recently discovered pattern recognition receptor that plays a key role in Gram-negative inflammation,[2] also shows a preferred α-configuration of the anomeric lactol. Here we investigated the feasibility of stereocontrolled synthesis of glycosyl phosphates by inversion of the anomeric configuration using modified Mitsunobu reaction conditions.[3,4] We performed mechanistic analysis by *in situ* ^{31}P-NMR spectroscopy using variably protected D-Man and examined the influence of protecting groups in both coupling partners on the efficiency and the stereoselectivity of the phosphorylation reaction.



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Synthesis of chemical inhibitors to monitor OGA activity

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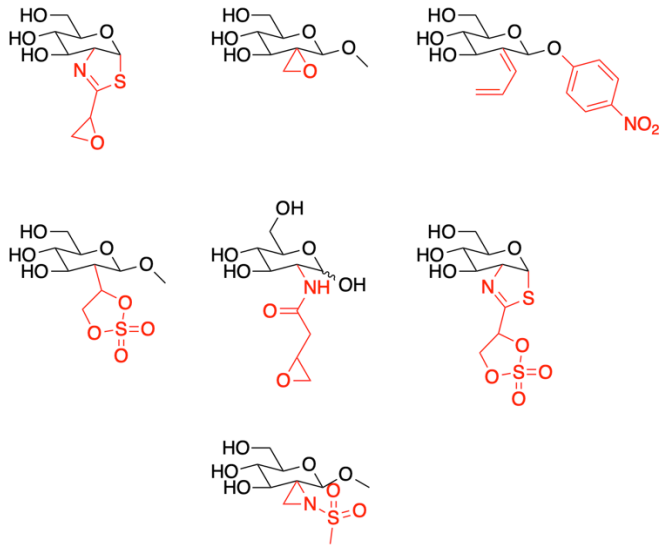
O-linked N-acetylglucosamine (O-GlcNAc) is a dynamic post-translational modification applied to serine and threonine residues on hundreds of intracellular proteins. O-GlcNAcylation is believed to play a role in several diseases, such as Alzheimer's disease (AD).[1] The modification is controlled by two enzymes: the O-GlcNAc transferase, OGT, and the O-GlcNAc hydrolase, OGA. OGA catalyses the breakdown of the GlcNAc-serine/threonine bond on a diverse number of substrates. Inhibiton of OGA has become of significant interest as target for therapeutic agents due to its role in disease, however the detection of enzyme activity and inhibition within a cellular context remains challenging. In this work, we aim to develop novel activity-based probes that allow us to monitor active enzyme levels in cells. Similarities between the active site of OGA and other hexosaminidases (HexA/HexB) alongside the lack of a covalent intermediate in the substrate assisted mechanism create difficulty in the generation of specific high affinity probes.[2, 3] We aim to use a variety of electrophilic warheads placed strategically to interact with the catalytic base of OGA (Asp174) to generate covalent enzyme inhibitors that will form the basis for the synthesis of activity-based probes. In addition to this we aim to use a natural pocket in the active site of OGA as a way to gain selectivity over HexA/HexB where this pocket is absent. We show the results of molecular dynamics studies on transition state mimics, and the



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Glyco-tagging of native proteins for developing improved therapeutics

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Proteins are increasingly used for drug development and in clinical practice,[1] and improving their physical, chemical, and pharmacological properties is an area of intensive research. In addition to mediating cell-cell and cell-matrix interactions, glycosylation exerts a major influence on protein folding, stability and pharmacokinetic properties.[2] Inspired by nature and aiming to recapitulate these important features, glycosylation is a strategy to enhance the therapeutic properties of peptide and protein drugs.[3] Here, we describe a novel chemoenzymatic methodology for the glyco-conversion of native proteins without introducing immunogenic modifications offering a streamlined approach to accelerate the discovery and improvement of biotherapeutics.[4]

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4. Manuscript in preparation.

Bacterial vesicles as new drug-delivery tool: the role of glycans

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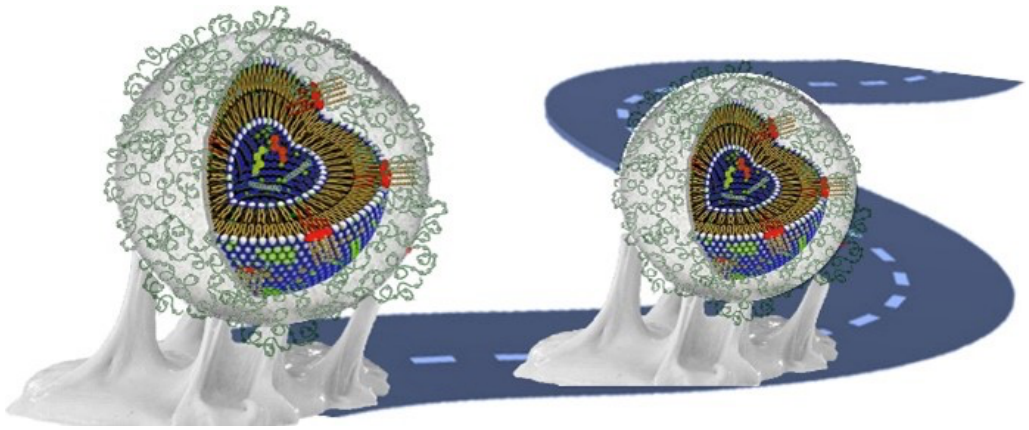
The treatment and the rapid detection of antimicrobial resistance diseases pose a formidable challenge in medicine. Nowadays, it is well established that antibiotics are not the only solution to treat these infections since microbes, especially bacteria, can adapt and develop resistance due to the selective pressure induced by the massive administration of prophylactic pharmaceuticals [1]. The translation from acute to chronic infections can cause the point of no return in their treatment [2]. The persistence of chronic infection is related to the bacteria's ability to form biofilms and to the lack of efficient detection techniques.

Biofilm is defined as communities of microorganisms embedded within a matrix, which consists of water, polysaccharides, extracellular vesicles, proteins, lipids, and nucleic acids [3]. A crucial role in promoting the emergence of multi-drug resistant (MDR) bacteria is also played by Extracellular Membrane Vesicles (EMVs), small vesicles (20–250 nm) formed by bacterial lipid-bilayer membranes comprising lipopolysaccharides (LPSs), proteins, peptidoglycans, DNA and RNA [4]. EMVs play different roles in the physiology and pathogenicity of bacteria: biofilm formation, delivery of toxins, antibiotic resistance, immunomodulation, stress response, horizontal gene transfer, and communication among cells and species [4]. By taking inspiration from the structural features of EMVs it is possible to design nanoparticles to be used for the prevention and diagnosis of biofilm infections. Recently, we described the polysaccharide "corona" from the EMVs of *Shewanella vesiculosa* HM13, demonstrating its role in the adhesion on polystyrene nanoparticles [5].

Here we described the structural characterization and determination of the physicochemical properties of glycans isolated from the Antarctic Gram-negative bacterium EMVs producer, as an innovative tool to deliver antibiofilm compound against *Staphylococcus epidermidis*.



Polysaccharide corona decorating the EMVs from Shewanella vesiculosa HM 13.

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9-Anthracenemethyl glycosides as chiral supramolecular synthons and bio-imaging agents

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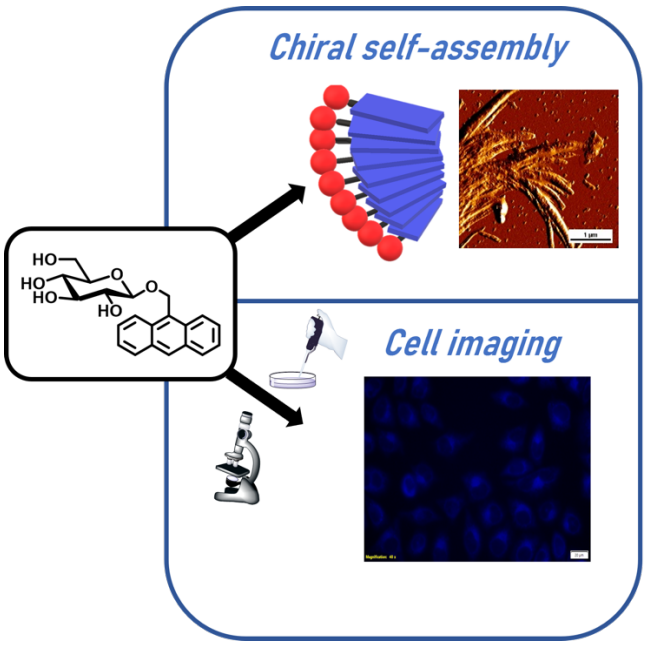
Self-assembly of amphiphiles, leading to the formation of supramolecular structures, occurs in biology and is studied in material chemistry. Chiral centers-rich carbohydrate moieties provide a rich source in the study of self-assembly, due to the feasibility of securing chiral supramolecular structures. Design of the monomers is critical in order to realize the chiral self-assembly of such synthons. 9-Anthracenemethyl glycosides were undertaken for the study covering (i) the formation of chiral supramolecular structures and (ii) the applicability of the photophysical properties of anthracene moiety at the biological interface. 9-Anthracenemethyl O-glycosides, installed with mono- and disaccharides, are studied for their self-assembly properties. Emerging chiral structures in aq. solutions follow the configuration of the attached sugar moiety. Monosaccharides D- and L-glucopyranosides alternate between left- and right-handed chiral structures, respectively. Whereas, a disaccharide-containing derivative does not exhibit chirality, even when the self-assembly occurred. A photochemical [4π+4π] cycloaddition occurs in the self-assembled structure in aq. solution, disrupting the chirality of the supramolecular structure. Anthracene as a probe to investigate bio-imaging properties of the newly-formed anthracenemethyl glycosides was studied. Cell viability assay using HeLa cells shows 80% cell viability, in the presence of 50 mM of 9-anthracenemethyl D-glucopyranoside. Significant uptake of this derivative occurred, whereas the corresponding D-galactopyranoside and L-glucopyranoside containing derivatives showed a relatively weaker cellular uptake. Bio-imaging was facilitated through fluorescence occurring at the perinuclear region of the cells, indicating an active transport of the glycosides, through the cell membrane. Results of the study will be presented.



We thank SERB-DST, New Delhi, for a financial support. AG acknowledges the CSIR, New Delhi, for a research fellowship.

A new synthetic pathway to 3-Amino-3-deoxyglycals: selective deprotections and glycosylation

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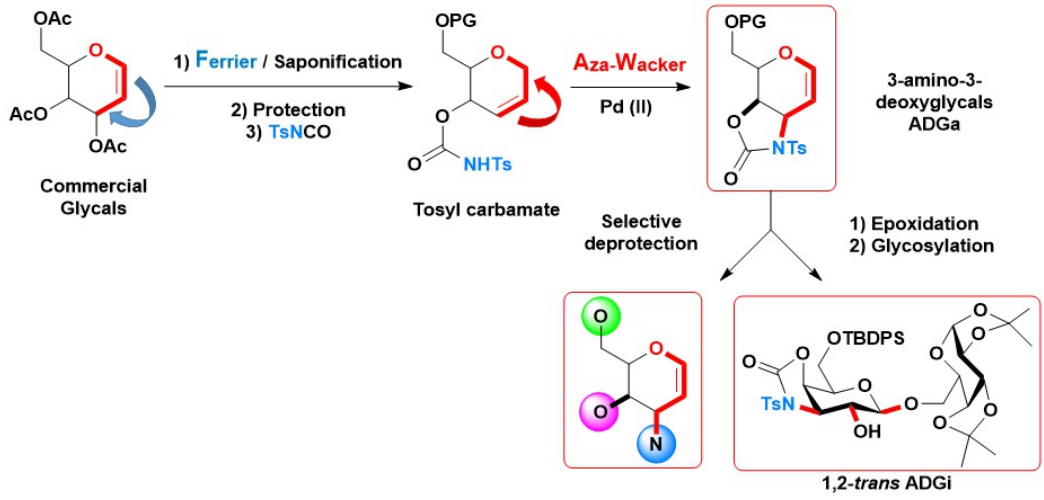
Among the most significant sugars in drug synthesis, 3-**A**mino-3-**D**eoxy**G**lycosides (**ADGi**) are bioactive compounds with applications in different domains.[1] They can either be found in oncology as anticancer agents or involved in fighting infectious diseases as antibiotics or antifungal medicines. The synthesis of efficient donors of these sugars is still a hot topic.

Fortunately, 3-**A**mino-3-**D**eoxy**G**lyc**a**ls (ADGa) could lead the way as key building blocks to access ADGi. In glycochemistry, glycals are well known for being efficient glycosyl donors (or precursors) for the synthesis of 2-deoxy and 1,2-*trans* glycosides.[2] The synthesis and reactivity of ADGa have been poorly studied. Most of the reported methods for their synthesis are either limited in terms of diversity or have an excessive number of steps, low yields or modest stereoselectivities.[3]

Here we describe a new sequence involving a type I Ferrier rearrangement and an aza-Wacker cyclization starting from commercially available glycals.[4] This new synthetic strategy allows rapid, gram-scale, and diastereoselective synthesis of orthogonally protected ADGa. These glycoplateforms can be selectively deprotected and engaged in an epoxidation / glycosylation sequence to obtain 1,2-*trans* ADGi.



Acknowledgements

ANR (JCJC, ANR-19-CE07-0011) is gratefully acknowledged for its financial support.

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Classification of protein-carbohydrate interfaces using unsupervised machine learning

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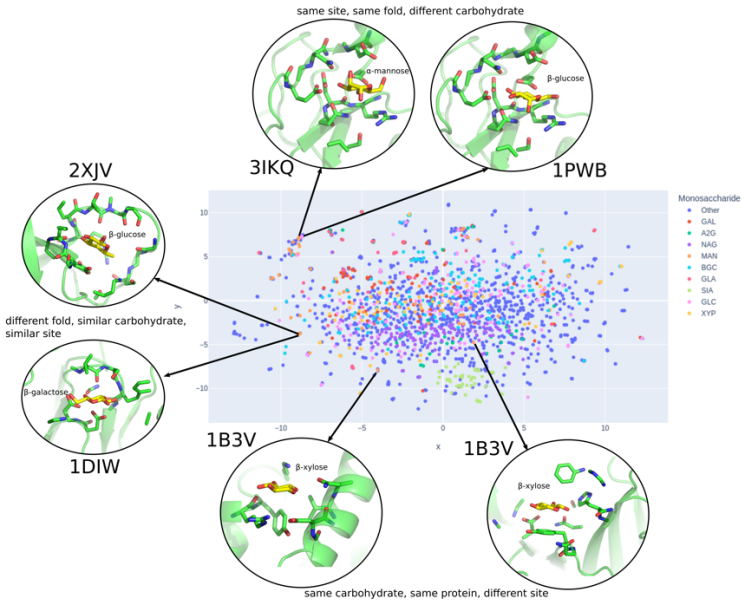
Numerous essential biochemical pathways are mediated by protein-carbohydrate (PC) interactions. However, their experimental characterization remains a technical challenge and the structural data on PC interfaces is scarce and underrepresented. Moreover, carbohydrate binding sites' (BS) diversity is very peculiar. Indeed, some similar protein regions can bind to different carbohydrates, while other BS targeting specific carbohydrates are found in proteins with very different folds (several examples are displayed in the figure attached). The goal of the current study was to provide a generalized view on different types of PC interfaces and perform their classification using unsupervised machine learning (ML) methods. We have extracted information on all the available BS present in Protein Data Bank (more than 20k) and performed a pairwise comparison of the most representative BS using a graph-based score evaluating local distance distortion between different atom types[1]. Then, we performed clustering for the obtained affinity score matrix and defined hierarchical classification of carbohydrate BS by their similarity. We show that most carbohydrate BS can be assigned to just a few categories: some of them being protein- or ligand-specific and others being more general, e.g. including proteins with very low homology relations. Our result paves the way to the development of the carbohydrate BS computational prediction tools, which have the potential to significantly impact understanding of fundamental biological processes and provide new drug design strategies.



t-SNE projection of representative carbohydrate binding sites present in the Protein Data Bank colored according to the carbohydrate name.

Bibliographic references:

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Design synthesis and biological evaluation of *C* and *S*-glycoside based galectins inhibitors

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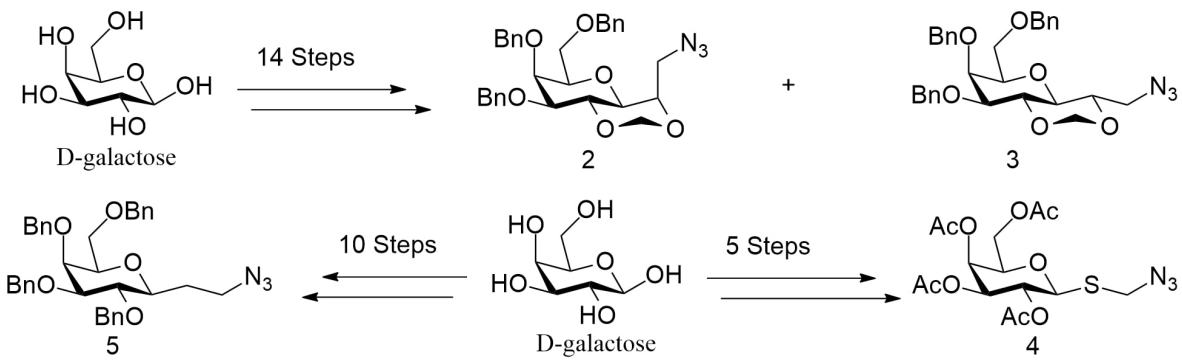
The galactoside binding specific subclass of lectins, galectins, are classified into three subfamilies based on the structure and number of carbohydrate recognition domains (CRD). Galectins involve in several cellular activities, certain cancers, infections, inflammations, fibrosis, HIV, and many other wide range of biological processes. The molecular basis for the selectivity of galectins is well documented and revolves around appropriate interactions of glycans with amino acid residues. The selectivity for galactose moiety stems largely from the hydrogen bonds (HBs) between histidine/arginine and the axial hydroxyl (-OH) at the 4-position. This axial hydroxyl is equatorial oriented in analogous O-linked β-glucosides. Mimetics of glycan ligands of galectins (glycomimetics) have been of interest.

We have synthesized "the clickable" intermediates (2, 3, 4 and 5) from β-D-galactose and current focus is given to its modification at the C1 and C3 position with the suitable pharmacophore to increase the affinity for galectins. For example, Nilsson et al[1] reported that galactose C3-modification via click chemistry stacked above the Arg144 sidechain, which in turn forms a water-mediated salt-bridge with Asp-148.

We will present the synthesis and biophysical evaluation of some b-C-glycosides derivatives, conformationally constrained C-glycosides, and an S-glycoside mimetic derived from 2-5. The compounds were tested as inhibitors of gal-1, 3, 8C, 8N, 9C, 9N and 4C. The synthesized compounds provided a basis of further modification and evaluation of their properties in search for better galectin inhibitors which is currently ongoing in the laboratory.



Synthesis Scheme of key intermediates (Fig: Synthesis of 2, 3, 4 and 5)

Bibliographic references:

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Synthesis of trifluoromethyl-substituted MEP analogues for the study of the non mevalonate pathway

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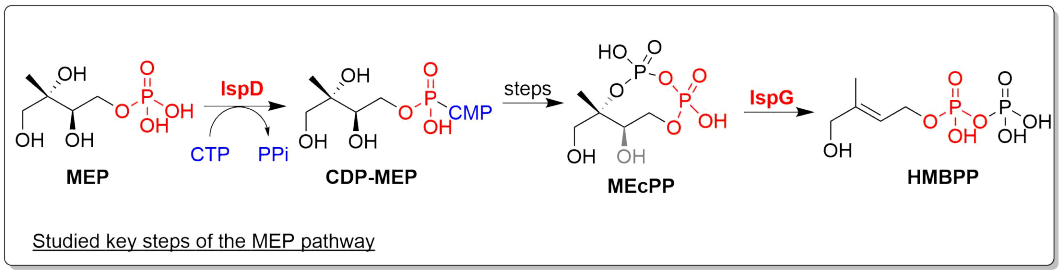
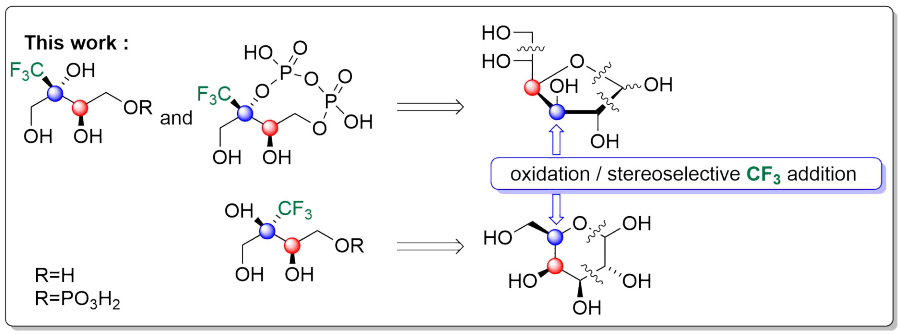
The 2-*C*-methyl-d-erythritol 4-phosphate (MEP) pathway is an essential biological process for numerous pathogenic bacteria species, while being absent from mammal metabolism (1). Its seven consequential enzymes have been reported as targets of interest for development of novel antibiotic drugs families (2). We focus on IspD (YgbP), a transferase catalyzing the transformation of 2-*C*-methyl-d-erythritol 4-phosphate (MEP) into 4-diphosphocytidyl-2-*C*-methyl-d-erythritol (CDP-MEP), and IspG (GcpE), a metalloenzyme catalyzing ring opening and reductive dehydration of 2-*C*-methyl-d-erythritol 2,4-cyclodiphosphate (MEcPP) into 4-hydroxy-3-methylbut-2-enyle 4-diphosphate (HMBPP).

Here, we present linear multistep stereoselective synthesis of 2-*C*-trifluoromethyl-d-erythritol in its carbohydrate, phosphate and cyclodiphosphate forms starting from d-glucose. We also present another strategy for the synthesis of 2-*C*-trifluoromethyl-d-threitol in its carbohydrate and phosphate forms. This structural modification is supposed to impair the enzymatic mechanism of MEP pathway enzymes by steric hindrance or destabilizing the activated complex, leading to inactivation or a low turnover (3). Some preliminary biological results will be presented.





Studied key steps of the MEP pathway and targets of the synthesis

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GlycoShape3D: a database and toolbox for structural glycomics

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To understand the architecture of glycan-mediated interactions, a detailed atomistic understanding of glycan structure is necessary. However, determining the 3D structure of glycans is difficult due to their intrinsic flexibility and micro/macro-heterogeneity. As a result, glycans represented within the Protein Data Bank (www.rscb.org) are often incomplete or presented in questionable and/or highly distorted conformations.

The release of the AlphaFold (AF) Protein Structure Database (alphafold.ebi.ac.uk) has dramatically increased the availability of protein structure data, yet this information is incomplete as it does not include co- and post-translational modifications, metals and cofactors that are often essential to protein structure and function. Some of us found that AF predicted glycoprotein regions where glycans should have been present are preserved in these models. [1] This facilitates the direct grafting of glycans onto these models, providing that the structure of the glycan is known [1].

Here we will present the design and principles of GlycoShape3D, a unique online open-access repository of structural data on free (unbound) glycans from equilibrium MD simulations [2-4]. GlycoShape3D will deliver complete and consistent structural information on glycan structure in a format that is accessible to glycobiology experts and non-experts alike, with a demonstration of some of the key potential applications and innovations of this novel repository.

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Investigating the role of carbohydrate sulfotransferases in the green seaweed ulva

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Polysaccharides are the major component in seaweed cell walls. Many of these polysaccharides are subject to extensive modifications, the two major modifications being acetylation and sulfation, carried out by carbohydrate acetyltransferases and sulfotransferases respectively. Interest in seaweed derived polysaccharides has grown in recent years and highly sulfated polysaccharides are of particular interest because their unique properties could benefit a variety of commercial and biomedical applications (1). Despite this, the physiological role of these polysaccharides and the enzymes that catalyse their modifications remain poorly understood.

My work looks at the Ulva genus of green seaweed whose species can be found in abundance along coastlines all across the globe (2). A large proportion of their cell wall is made up of ulvan, large heterogeneous polysaccharides that exhibit a high degree of sulfation. The genome for Ulva mutabalis/compressa was sequenced in 2018 and was the first green seaweed genome to be published, opening up new opportunities for research (3).

Using a homology-based approach, we have identified at least 19 putative carbohydrate sulfotransferase genes, suggesting a relatively recent gene expansion. However, based on previously collected RNAseq data, only four genes appear to be expressed under normal conditions. To investigate this further, I will be using FTIR to measure changes in levels of sulfation in response to different physiological stresses. This will be followed with qPCR to determine the link between expression, level of sulfation and stress.

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Exploring the synthetic application of *akkermansia muciniphila* α1,3-fucosyltransferase AkkFT

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Human milk oligosaccharides (HMOs), which are the third abundant solid component in breast milk, can be grouped into neutral, fucosylated, and sialylated glycans. Amongst, a majority of HMOs are fucosylated glycans. Studies showed that these glycans reduced the risk of infection in infants by acting as the soluble decoy receptors to the pathogens or viruses which potentially would bind to the surface of epithelial cells in the guts. Enzymatic catalysis is one of the powerful strategies to produce these valuable biomolecules. However, the majority of key enzymes for fucosylated glycans production, the fucosyltransferases (FucT), are recombinant enzymes from *Helicobacter pylori* and *Bacteroides fragilis*. Here we report a novel FucT named AkkFT which was isolated from *Akkermancia muciniphila* and demonstrated its synthetic application for human milk glycans production for the first time. The recombinant AkkFT was successfully expressed in *E. coli* BL21(DE3) with 3.68 mg/L yield. Biochemical characterization revealed that AkkFT exhibited a broad range of catalytic pH (from 6 to 8.5), with the highest activity observed at pH 7. AkkFT showed a 41% increase in FucT activity when magnesium ions were used as cofactors. Additionally, with the broad substrate tolerance, recombinant AkkFT was able to catalyze the α1,3-fucosylation on various human milk glycans including lactose, lacto-*N*-tetraose (LNT), and lacto-*N*-neotetraose (LNnT), to produce 3'fucosyllactose, Lacto-*N*-fucopentaose V (LNFP V) and Lacto-*N*-neofucopentaose (LNnFP V), respectively. The results indicated that AkkFT showed high regioselectivity on α1,3-fucosylating the reducing end of the lactose unit on a given glycans. These finding suggests that AkkFT would provide a promising new approach for human milk glycans synthesis.

Acknowledgements

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Heteromultivalent s

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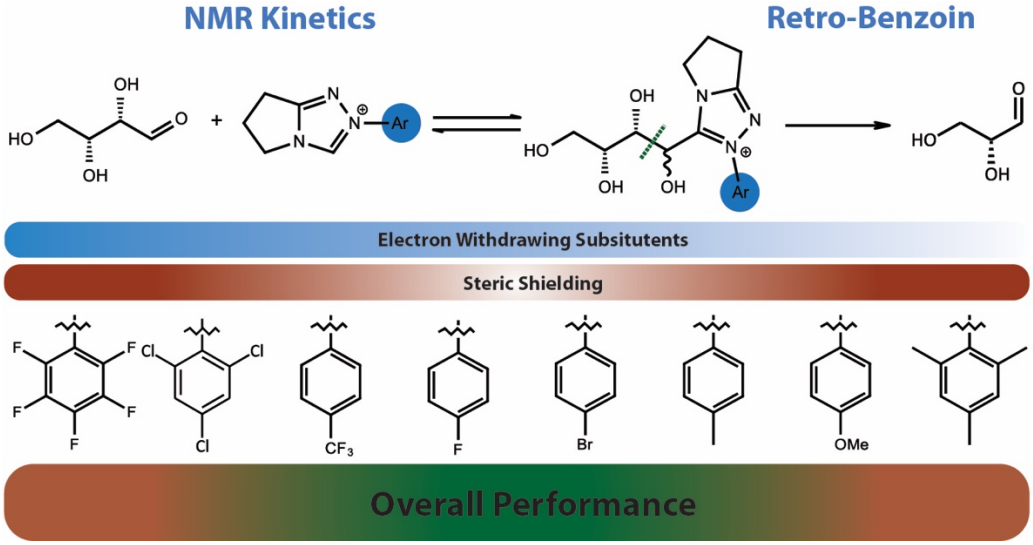
Classical carbohydrate chemistry often focuses on selectivity issues inherent to their polyhydroxy functions and the glycosidic linkage. Interestingly, their most reactive function – the aldehyde – receives comparably little attention. In this light, we study the interactions of aldoses' aldehyde moiety with aldehyde-selective *N*-heterocyclic carbene (NHC) catalysts, aiming to increase the use of carbohydrates as chiral pool materials in organic chemistry.

Recently, we demonstrated a selective dehomologation of selected carbohydrates using an NHC-catalysed retro-benzoin reaction in good yields. In this approach, a Stetter reaction with chalcone as substrate was used as the catalyst recycling step. However, applicability remains limited to particularly suitable substrates. Towards the ultimate goal of a general methodology, we are further elaborating the complex mechanism of this reaction from two points of view:

First, NMR-based kinetic measurements enabled us to understand the kinetics of the initial adduct formation between a specific NHC and an aldose, giving us new insights into catalyst requirements for their efficient carbohydrate activation. Second, we were indirectly probing for the successive retro-benzoin step, by monitoring the rate of formylation of chalcone, thereby recycling the catalyst.

Our investigations have hinted at a sweetspot between the efficiency of the retro-benzoin and Stetter reactions, which needs to be finely balanced.



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Total synthesis of *Campylobacter jejuni* NCTC11168 CPS assisted with iMAP 1,6-anhydrous strategy

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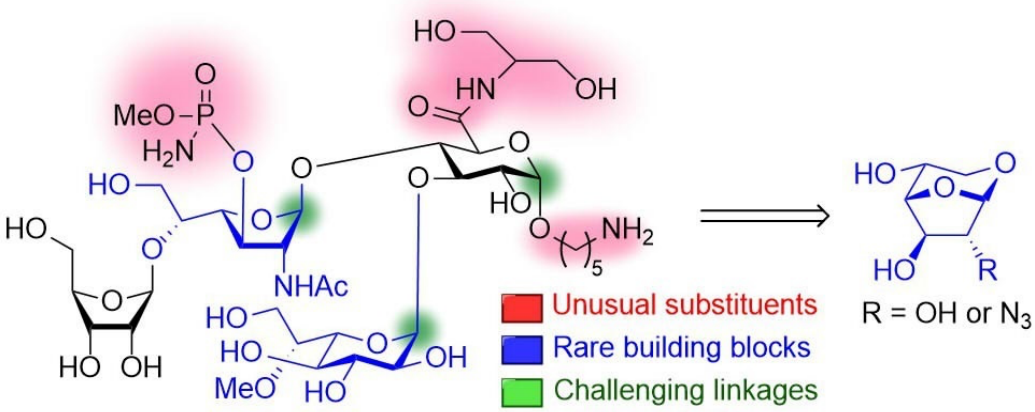
Campylobacter jejuni, a general gastrointestinal pathogen, causes severe diarrhea or Guillain-Balre´ Syndrome (GBS) and threatens human lives. W.H.O. has announced that *C. jejuni* ranks in high-priority of alternative therapy development due to the gradually increasing antibiotic resistance.[1] The pathogenicity of *C. jejuni* is highly related to capsular polysaccharide (CPS) with a regular structure. Therefore, these feature structures can provide a suitable antigen for vaccine development. Here, we report a total synthesis of *C. jejuni* NCTC11168 CPS repeating unit.

The synthesis utilized the strategy of intramolecular anomeric protection (iMAP), which generates a 1,6-anhydro-furanoside sugar residue in a one-pot manner from free-sugar, to concise the building block synthesis.[2] Accordingly, the synthesis of heptose building block from free-galactose in 6 steps, and the galactosamine building block from free-galactosamine in 2 steps. Consequently, merely 28 steps were used to synthesize product 1, including all building blocks synthesis from free-sugar. After structuring the tetrasaccharide skeleton with [2+1+1] glycosylation and completing the required modification, the desired product 1 shows a 13.46 ppm resonance on the ^{31}P NMR spectrum, similar to the native CPS chemical shift of 13.6 ppm.[3] In addition, this product 1 containing linker moiety on the glucose part can conjugate to carrier protein for further vaccination development and is undergoing.



Acknowledgements

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Length-controlled glycopolymers from a poly(norbornenyl azlactone) platform for lectin recognition

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Glycopolymers are synthetic backbones with pendant sugars that mimic natural polysaccharides. While the interaction between a single sugar molecule and the complementary carbohydrate-binding protein (lectin) is rather weak in a number of important biological processes, the simultaneous binding of numerous sugar groups of a multivalent polymer can result in a strong conjugation.[1] To prepare such structures, several synthetic strategies have been developed, and among them, click post-polymerization modification (PPM) of functionalized polymer platforms with appropriate sugar derivatives offers a rapid route to generate glycopolymer libraries with a wide range of carbohydrate structures, without requiring tedious protection-deprotection sequences. In this context, ring-opening polymerization metathesis (ROMP) has been widely used as a versatile and controlled method to synthesize functional polymers. In addition, the presence of carbon-carbon double bonds and cyclopentane along the polymer scaffold increases the rigidity of the structure, avoiding conformational entropy penalty and promoting target specificity.[2] The azlactone moiety has emerged as a powerful anchoring group able to react with amino-terminated functional moieties. Indeed, this group shows a high reactivity, full atom economy in a broad range of organic solvents as well as in aqueous solution at room temperature without generating by-products.[3] Moreover, the azlactone functionality is compatible with ruthenium-based catalysts, enabling the fast design of glycopolymers.[4] We report herein the ROMP of the *endo*-and *exo*-(norbornenyl azlactone) diastereoisomers with ruthenium-based third generation Grubbs catalyst in order to access a library of poly(norbornenyl azlactone) (PNBAzl) scaffolds with a wide range of number-average degree of polymerization (*DPn*) ranging from 10 to 1,000. The subsequent PPM with amine-functionalized mannoside and glucosyl ligands led to length-controlled glycopolymers. The mannose analogs were functionalized with a hydrophilic triethyleneglycol (TEG) spacer or hydrophobic heptyl chain. Finally, a TEG-glucosyl was also prepared as a mismatched sugar reference for lectin assays. The binding inhibition of the resulting glucose- and mannose-functionalized PNBAzl was evaluated against a set of models, and therapeutically relevant lectins, using a "lectin profiling" technology. We selected five mannose-binding lectins (ConA, FimH, Bc2L-A, DC-SIGN and langerin) for their biological relevance and marked sensitivity to multivalency.

Scheme 1. Strategy for the synthesis of glycopolymers from a poly(norbornenyl azlactone) platform

Acknowledgements

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Functional carbohydrates for health and nutrition

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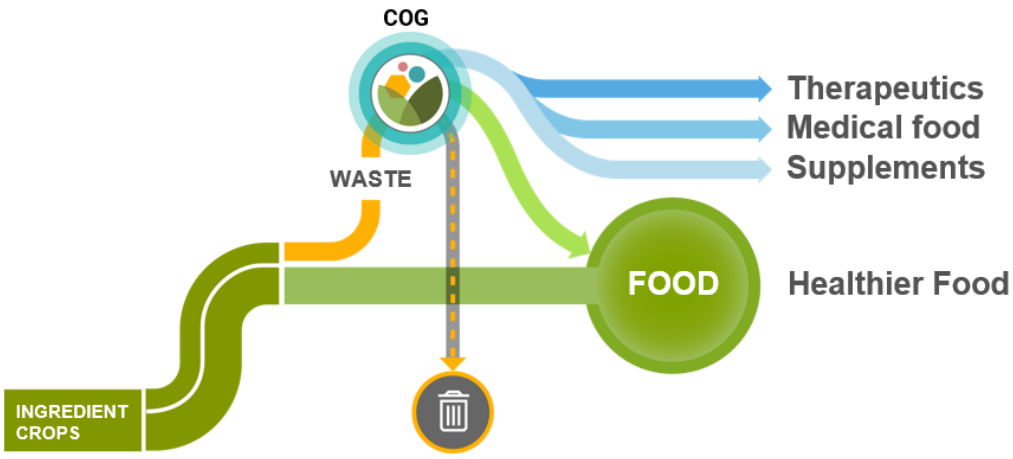
While gut microbiome research continues to uncover new associations with human health and disease, strategies for modulating the underlying bacterial communities have not kept up. Purely bacterial based interventions have received much attention but have not shown to be effective in community redesign. Alternatively, prebiotics have been shown to be more drastic drivers on gut microbiome modulation but are currently limited to just a few structurally diverse products with limited functionality. New strategies for creating novel and structurally diverse prebiotic oligosaccharides would enable new opportunities for impacting health. We have developed a Fenton oxidation-based approach for depolymerizing practically any polysaccharide-rich source material into pools of oligosaccharides with unique and diverse structures. To date, BCD Bioscience has employed this food-grade reaction on over fifty unique food processing waste streams, which has allowed us to generate the largest catalogue of structurally diverse oligosaccharides that can be mined for potential bioactivity. By combining extensive in-house LC/MS-based structural analysis and simulated gut microbiome fermentation assays, we have demonstrated microbial taxa modulation and have linked it to the defined carbohydrate structural features. By shaping the gut microbiome and thus microbial-derived metabolite production, we are exploiting fiber structure-function relationships to develop ingredients with bioactive potential for human health, as food ingredients, medical foods, and pharmaceutical products.



BCD Bioscience ingredient creation flowchart, through our patented COG (creative oligosaccharide generation) process

Stereo-controlled synthesis of 2-deoxy-C-glycosides with β-glycosyl boronate

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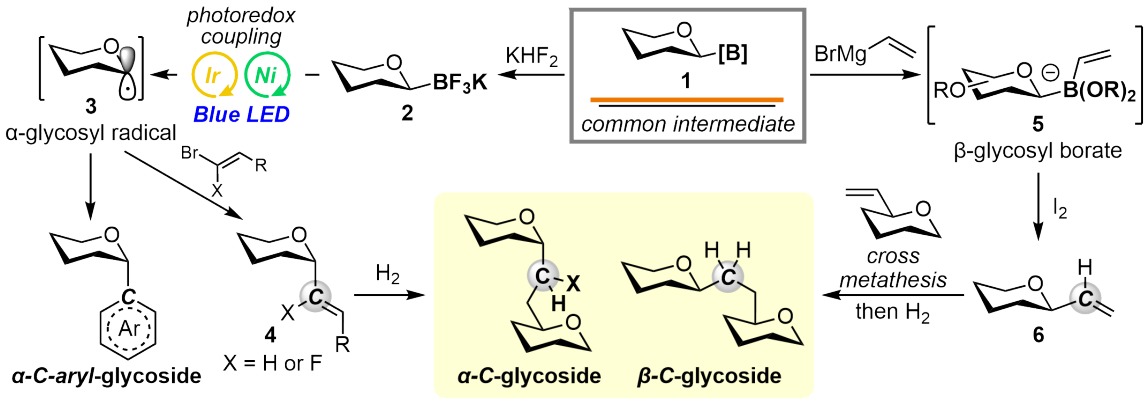
Native glycans are involved in a variety of biological phenomena as polysaccharides or glycoconjugates. Avoiding the enzymatic cleavage of cleavable *O*-glycoside bonds and maintaining the original glycan structures is one of the important issues to analyze their exact functions. *CH_2*-glycoside analogues, in which the oxygen atom of O-glycoside bond is replaced with the methylene group, are expected to be useful tools because of their stability against glycoside hydrolases. However, synthesis of glycan analogues (pseudo-glycans) with a *C*-glycoside linkage is generally complicated, which prevents their application to biological studies or investigation of their therapeutic agents. We envisioned β-glycosyl boronate **1** as the common intermediate for psudo-glycans with both α- and β-C-glycoside linkage [1, 2].

Single electron oxidation [3] of **2** generates α-glycosyl radical **3** stabilized by anomeric effect. Then, cross-coupling reaction with aryl or vinyl halide catalyzed by Ni-complex proceeded to give *C*-aryl-glycosides or α-*C*-vinyl-glycosides (**4**) in a high α-selectivity. Hydrogenation of **4** successfully gave the α-*CH_2*-linked 2'-deoxy-disaccharide analogues. On the other hand, nucleophilic addition to pinacol boronate **1** followed by oxidative 1,2-migration gave β-C-glycoside **6** stereospecifically [4]. Cross-metathesis reaction between **6** and olefins followed by hydrogenation enabled synthesis of β-*CH_2*-linked disaccharide analogues.



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Serum *N*-glycosylation RPLC-FD-MS assay to assess colorectal cancer surgical interventions

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A newly developed analytical strategy was applied to profile the total serum N-glycome of 65 colorectal cancer (CRC) patients before and after surgical intervention. In this cohort it was previously found that serum N-glycome alterations in CRC associated with patient survival. Here, fluorescent labeling of plasma N-glycans was applied using procainamide and followed by sialic acid derivatization specific for α2,6- and α2,3-linkage types via ethyl esterification and amidation, respectively. This strategy allowed efficient separation of specific positional isomers on reversed-phase liquid chromatography–fluorescence detection–mass spectrometry (RPLC-FD-MS) and complemented the previous glycomics data based on matrix-assisted laser desorption/ionization (MALDI)-MS that did not include such separations. The results from comparing pre-operative CRC to post-operative samples were in agreement with studies that identified a decrease of di-antennary structures with core fucosylation and an increase in sialylated tri- and tetra-antennary N-glycans in CRC patient sera. Pre-operative abundances of N-glycans showed good performance for the classification of adenocarcinoma and led to the revisit of the previous MALDI-MS dataset with regard to histological and clinical data.

This strategy has potential to monitor patient profiles before, during and after clinical events such as treatment, therapy or surgery and should also be further explored.

Acknowledgements

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Chemical synthesis of *A. faecalis* monophosphoryl Lipid A and a conjugate of Lipid A with Tn antigen

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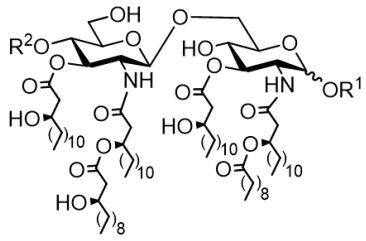
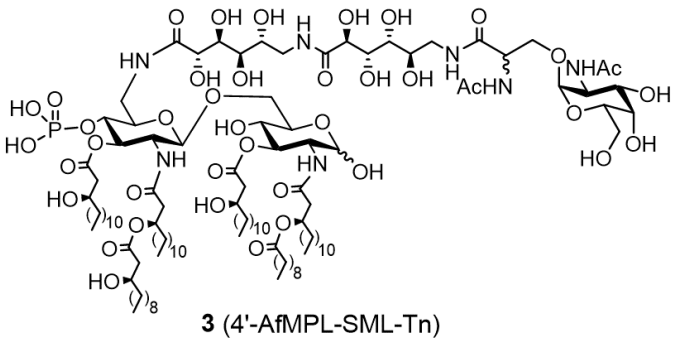
Lipopolysaccharide (LPS), a glycoconjugate constituting the outer membrane of Gram-negative bacteria, is known as a representative innate immunostimulant. LPS and its active principle lipid A have potential to function as adjuvants that enhance vaccine efficacy. While the canonical *E. coli* LPS is toxic, monophosphoryl lipid A (MPL), lacking one phosphate group was found to be an attenuated immunostimulant hence attractive for adjuvant applications. GlaxoSmithKline also developed an MPL analog, 3D-MPL[1], which was approved as a safe adjuvant for practical use in vaccines. Alternatively, our group revealed that symbiotic *Alcaligenes faecalis* lipid A (AfLA)[2], diphosphate type, showed useful adjuvant activity without toxicity[3]. Here we synthesized *A. faecalis* MPLs (AfMPLs) **1** and **2**. Both AfMPLs induced weaker IL-6 cytokine induction than AfLA. Their adjuvant activity is now under investigation. Meanwhile, self-adjuvanting vaccine strategies in which antigen and adjuvant are covalently linked have recently been well studied especially in the development of carbohydrate-based vaccines[4,5]. However, there are few reports of lipid A-based self-adjuvanting vaccines[5,6] because structural modification often inactivates lipid A. Here we successfully synthesized conjugate **3** consisting of 4'-AfMPL **2** covalently linked to a tumor-associated carbohydrate Tn antigen via a sugar mimic linker (SML). Conjugate **3** significantly induced IL-6 cytokine; about 10 times weaker than unmodified **2**, and now in *vivo* assay is underway.



1 (1-AfMPL)
R^1 = -P(=O)(OH)$_2$, (only α form), R^2 = H

2 (4'-AfMPL)
R^1 = H, R^2 = -P(=O)(OH)$_2$



3 (4'-AfMPL-SML-Tn)

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Development of novel D-glucuronamide-based nucleoside analogs containing 1,2,3-triazole units as potential anticancer agents

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The synthesis of D-glucuronamide-containing molecules has attracted increasing attention in the context of the search for new bioactive compounds, which is motivated by the biological profile reported for either natural or synthetic derivatives containing this glycosyl unit.[1]

Within our continuous interest in the synthesis of new potentially bioactive D-glucuronamide-based compounds, among them nucleoside analogs [2,3], in this communication we report on the synthesis of innovative nucleoside analogs constructed on D-glucuronamide scaffolds containing 1,2,3-triazole units. The synthesized molecules included [*N*-(glucuronamidyl)triazolyl]methyl phosphonates as potential sugar diphosphate mimetics, in which the (triazolyl)methyl phosphonate system was planned as a prospective neutral diphosphate mimetic, and glucuronamide-based (purinyl)methyl triazole nucleosides. For their access, D-glucofuranuronolactone was used as starting material and key synthetic steps included amidation, furanose to pyranose isomerization, anomeric azidation, azide-alkyne 1,3-dipolar cycloaddition or Arbuzov reaction. The biological evaluation demonstrated that some of the synthesized compounds have significant antiproliferative activities in cancer cells.

Acknowledgements

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A new solid-phase method for the chemoenzymatic synthesis of heparan sulfate and chondroitin sulfate

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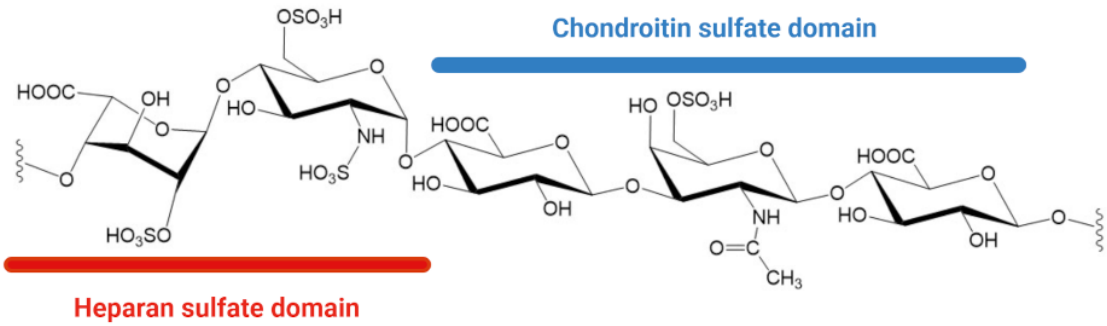
Heparan sulfate (HS) and chondroitin sulfate (CS) are two structurally distinct natural polysaccharides that play important roles in a variety of biological processes. Here, we report the development of a solid-phase enzymatic synthesis approach for the synthesis of HS and CS chimeras. Our approach utilizes specialized linkers that are covalently attached to a solid support. The linkers are then used to enzymatically synthesize HS or CS chains, which are subsequently released from the support by enzymatic digestion.

We also have successfully synthesized a library of seven structurally homogeneous HS and CS chimeric dodecasaccharides (12-mers). The chimeras contain a CS domain on the reducing end and a HS domain on the nonreducing end. The synthesized chimeras display anticoagulant activity as measured by both in vitro and ex vivo experiments. Furthermore, the anticoagulant activity of H/C 12-mer 5 is reversible by protamine. Our findings demonstrate the synthesis of unnatural HS-CS chimeric oligosaccharides using natural biosynthetic enzymes, offering a new class of glycan molecules for biological research.



Expression of α1,2-mannosidases impacts the protein *N*-glycan profiles in *Chlamydomonas reinhardtii*

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The green microalga *Chlamydomonas reinhardtii* is a model for studying cellular biological processes including synthesis of glycoproteins (**Mathieu-Rivet et al., 2020**). *N*-glycosylation represents a post-translational modification of major importance since it impacts the biological function of the expressed proteins. Structural analysis of glycans *N*-linked to *C. reinhardtii* proteins has demonstrated that mainly oligomannoside structures are synthesized, the main one being a non-canonical $Man_5GlcNAc_2$ (**Vanier et al., 2017**). In the context of bioproduction of recombinant proteins, the glyco-engineering of *C. reinhardtii* oligomannosides into humanized *N*-glycans requires the accumulation of the chassis $Man_3GlcNAc_2$. Indeed, this structure is a prerequisite since it allows the transfer of terminal *N*-acetylglucosamine and then decorations such as galactose or fucose to give human-like *N*-glycans. Thus, the accumulation of $Man_3GlcNAc_2$ in *C. reinhardtii*implies to remove the two α1,2-linked Man from $Man_5GlcNAc_2$. Towards this objective, we expressed in *C. reinhardtii,* the α1,2- mannosidases either from *Aspergillus saito* (AsMAN) or from *Arabidopsis thaliana* (AtMNS1). In the transformed microalgae expressing either AsMAN or AtMNS1, $Man_3GlcNAc_2$ was strongly increased to the detriment of $Man_5GlcNAc_2$. These results demonstrated that the glyco-engineering of the *C. reinhardtii N*-glycosylation pathway can be performed by expression of heterologous glycoenzymes and further confirm that the targeting mechanisms of glycoenzymes into the secretory system is conserved between microalga and plants.

Acknowledgements

The authors are thankful to the Region Normandie for funding this work through the SweetTrip RIN RECHERCHE 2021, and the SweetBioPharm RIN XLChem prog

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Glycoengineering lipooligosaccharides on bacterial pathogens

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Bacterial glycans play an important role in the interaction with their host. Lipooligosaccharides (LOS) are the most abundant cell surface glycoconjugates on the outer membrane of most Gram-negative bacteria. They contribute to the rigidity and impermeability of the cell wall and can stimulate the host immune system. Some bacteria display LOS capped with sialic acid as terminal epitopes to mimic host glycans. This phenomenon, known as molecular mimicry, can help bacteria hijack host biological mechanisms to facilitate infection and evade the host's immune system. To further investigate the functional role of sialic acid-capped LOS at the molecular level, it is important to have tools readily accessible for the detection and manipulation of Neu5Ac on glycoconjugates of live bacteria.

Here we report a novel strategy to incorporate Neu5Ac with a reporter group onto the lipooligosaccharides of a selection of Gram-negative bacteria. We show that the native sialyltransferases of several pathogens are able to accept extracellular unnatural sugar nucleotides, thereby introducing unnatural sialosides onto their LOS. This new technique, Labeling via Bacterial Native Sialyltransferases, is an efficient and rapid way to screen for bacteria that can decorate their glycoconjugates with exogenous sialic acid. This strategy complements other glycoengineering techniques, such as Metabolic Oligosaccharide Engineering (MOE) and Selective Exo-Enzymatic Labelling (SEEL), and can help to dissect host-bacterial glycan interactions.

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Optimization and chemical characterization of pectins from blackcurrant fruit pomace obtained by NADES extraction

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Nowadays, one of the main goals of the industry is to proceed efficient sustainable processes that result in high-quality products at low cost. For this reason, known procedures are modified to meet new requirements. Improvements are possible thanks to the optimization of technological processes, and thus the modifications of products or the course of individual processes. The changes result from the implementation of the principles of green chemistry and include environmentally friendly methods.

The fruit processing is an important part of the food industry in Poland, and juice production especially. Every year tons of fruit pomace are generated. These wastes are usually rich in polysaccharides, mainly pectin, which present a wide range of applications, i.e. as gelling agents, thickeners, emulsifiers, stabilizers, etc. [1,2]. Commercially, pectin isolation process is carried out using conventional methods based on mineral strong acid solutions and toxic organic solvents [3]. The synergy of the management of wastes from fruit processing for pectin extraction with clean separation methods is an attractive direction in the development of sustainable technologies. One of the most promising, green methods is the use of natural deep eutectic solvents (NADES).

The experimental model of pectins extraction from black currant pomace using NADES systems was developed. NADES were made from different molar ratios of choline chloride (HBA) and citric acid (HBD). The optimization was performed with response surface methodology (RSM). The products with the most favorable results were analyzed using spectroscopic and chromatographic methods.

Acknowledgements

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Rational design of gold(III)-glycoconjugates as antiviral agents against SARS-CoV-2

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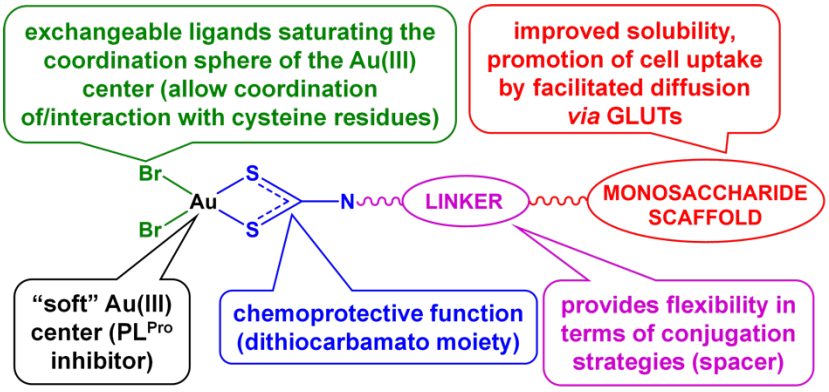
First identified in Wuhan (China) in December 2019, the Coronavirus Disease 2019 (COVID-19) pandemic has been causing a major global health (and, subsequently, socio-economic) emergency which undoubtedly exposed the fragility of the current globalized society. Remarkably, it was the third recent coronavirus-related outbreak after SARS-CoV (2002) and MERS-CoV (2012), thus highlighting the urgent need for dedicated antiviral therapeutics,[1] not an easy task given their extremely low approval rates.[2]

Notwithstanding the recent development of state-of-the-art vaccines has considerably reduced the spread of the virus and the associated risk of hospitalization and fatalities, infection figures still pose a significant threat to patients (especially unvaccinated and vulnerable), and are still putting the healthcare systems under major pressure.[3] Although substantial efforts in drug design and repurposing have been undertaken, to date there are only a few drugs (which were already marketed) have been officially approved for the treatment of COVID-19 infection, mostly recommended to treat patients at high risk.[4] In this context, metal derivatives are generally under-represented in the compound libraries used for screening in drug discovery campaigns, despite of the growing evidence of their role in medicinal chemistry. On account of the aforementioned considerations, based on solid encouraging preliminary results recently obtained in our group,[5] we here report on the design of monosaccharide-containing gold(III)-based derivatives as antiviral agents against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). The goal is to generate non-toxic metal-glycoconjugates to be taken up by coronavirus-infected cells and, once internalized, capable of acting as potent inhibitors of specific SARS-CoV-2 target proteins, such as the Papain-like Protease (PL^{Pro}), with a view to preventing the replication of the virus and its subsequent spread to other host cells.[6]



General designing strategy to the target metal-glycoconjugates against SARS-CoV-2.

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Promiscuity in glycosyltransferases: chitin nanofibrils formation by cellulose synthase

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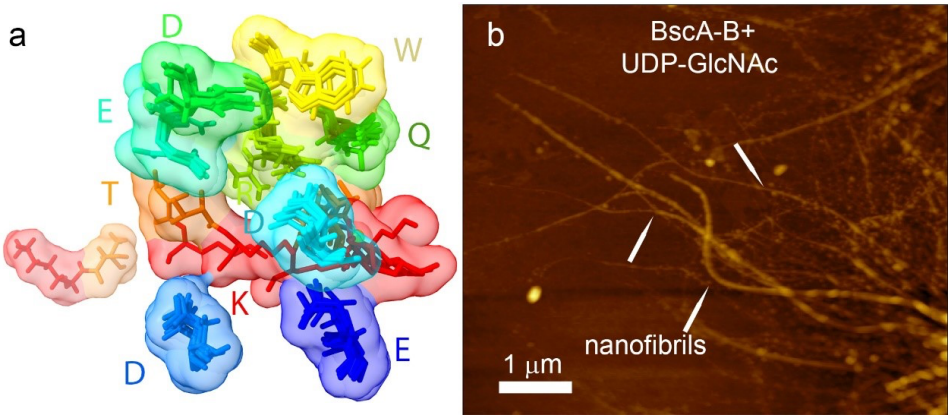
Glycosyltransferases (GTs) play essential roles in various biological processes and exhibit promiscuity in substrate specificity, allowing the synthesis of diverse glycoconjugates, crucial for the adaptation and survival of organisms. Cellulose and chitin synthases are examples of processive GTs involved in cellulose and chitin biosynthesis, respectively. Organisms that produced chitin do not produce cellulose and vice-versa. Recently, we have demonstrated that bacterial cellulose and chitin synthases share highly similar active site architecture despite low global amino acid sequence and 3D structure similarities [1], laying a theoretical framework for experimental validation. Here we demonstrate that recombinant bacterial cellulose synthase from *Rhodobacter sphaeroides*(BscA-BscB) catalyzes the cleavage of uridine diphosphate N-acetylglucosamine (UDP-α-D-GlcNAc), resulting in the formation of chitin nanofibrils in vitro glucose. BscA-BscB has a similar substrate affinity between UDP-α-D-GlcNAc and its natural substrate uridine diphosphate glucose (UDP-α-D-GlcN) but less efficient toward UDP-α-D-GlcNAc. *R. sphaeroides* grown in the presence of UDP-α-D-GlcNAc resulted in the formation of chitin oligomers.

This study highlights the catalytic promiscuity of bacterial cellulose synthase, providing insights into the formation of chitin nanofibrils and expanding our understanding of GTs' functional versatility. The findings contribute to the broader field of glycobiology and have implications for biotechnological applications involving glycoconjugate synthesis.



a, Conservation of bacterial cellulose and chitin active sites [1]. b, AFM of chitin fibrils

Acknowledgements

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Design and synthesis of covalent inhibitors for inverting α-glucosidases

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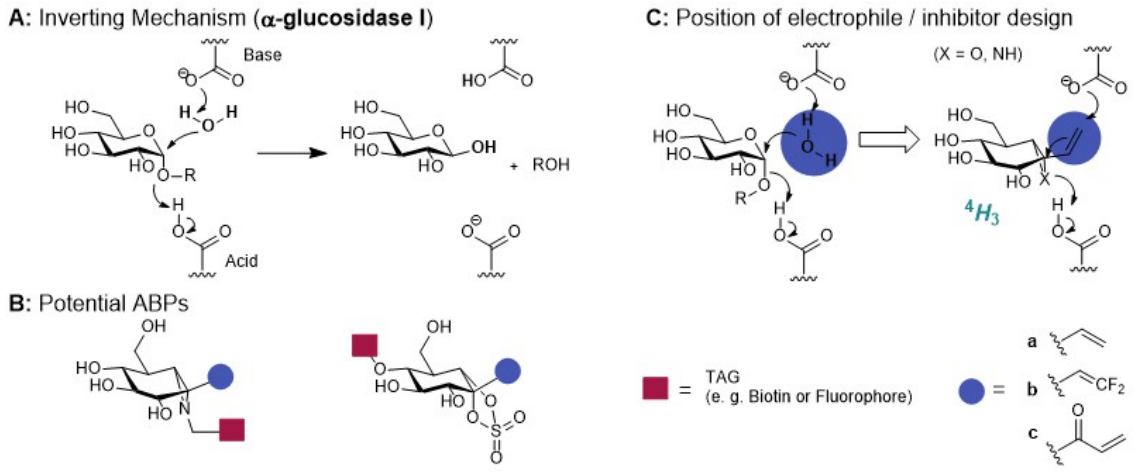
Inverting glycosidases are an important and widespread class of enzymes (Fig. 1 A). As for retaining glycosidases, these enzymes can be promising targets in biomedicine and biotechnology. Inverting α-glucosidases are of particular interest as they play an important role in cellular functions, such as 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.



Design approach for covalently binding inverting α-glucosidase inhibitors in ABPP.

Acknowledgements

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Design and synthesis of inhibitors for sialic acid esterases

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Human cells are coated with a dense array of glycoproteins and glycolipids called the glycocalyx [1]. Sialic acids are nine-carbon monosaccharides that are mostly found as the terminal residues of glycans forming the glycocalyx with N-acetyl-5-neuraminic acid (Neu5Ac) being the most common member of this family [2]. A common post translational modification of glycoproteins, is the O-acetylation of the hydroxyl at C-4, 7, 8, and/or 9 on Neu5Ac which is important in cell-cell signaling, autoimmunity and viral infection [3,4].

O-acetylated-Neu5Ac is a receptor for Influenza C and type 2a coronaviruses and toroviruses [5]. These viruses bind host cell surface O-acetylated-Neu5Ac-glycoproteins via hemagglutinin-esterases (HEs) or a spike protein followed by cleavage of the acetyl-group, leading to the destruction of the receptor. Viruses bearing non-functional HEs have reduced infectivity, highlighting their potential anti-viral targets [6].

The goal of this project is to design and synthesize covalent inhibitors targeting viral sialic acid esterases (SAEs). These inhibitors will be tested for their efficacy against recombinant 9-O-SAEs.

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Synthesis of nonglycosaminoglycan-type heparinoid trisaccharides with potential biological activity

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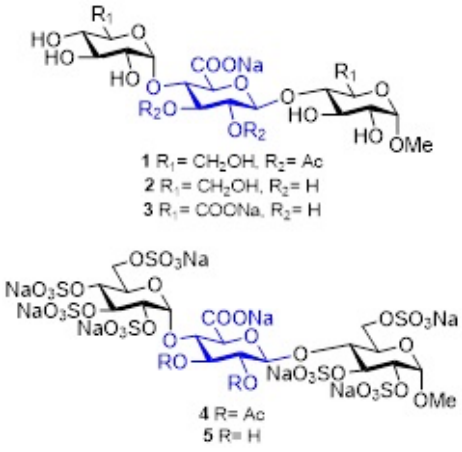
Heparin and heparan sulfate are linear anionic polysaccharides which are the members of glycosaminoglycans. Both are built up from alternating D-glucosamine and hexuronic acid units, but there are some structural differences between their saccharide sequences and sulfation degree.[1] Heparin has been employed in the medical practice as a blood-anticoagulant, but heparin and its analogues have many other biological effects such as growth factor inhibitiory, anti-inflammatory and cell growth inhibitory activity.[2] Heparin and its derivatives are also being investigated for the treatment of a number of disorders, including cancer.[3] Based on the aforementioned, non-glycosaminoglycan analogues of heparin might be important structures in the development of anticancer agents. Our research group has long been working on the synthesis of heparin-analogue oligosaccharides.[4] Moreover in our Department two D-glucuronate-containing trisaccharides were synthesized, which showed significant and selective inhibitory effects on the growth of tumor cells.[5] We supposed that, the newly synthesized trisaccharide fragments of heparin (Scheme 1., 1-5) might also display cell growth inhibitory activity. Based on the mentioned results we performed the efficient synthesis of four Glc-GlcA-Glc and a GlcA-GlcA-GlcA sequenced non-glycosaminoglycan, heparin-related trisaccharides with various sulfation, and acetylation patterns. The cell growth inhibitory effects of the compounds will be investigated against cancerous human cell lines and non-cancerous cell lines.



Scheme 1.: Structures of the planned trisaccharides (1-5)

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Glycosyltransferase engineering to dissect *N*-linked protein glycosylation

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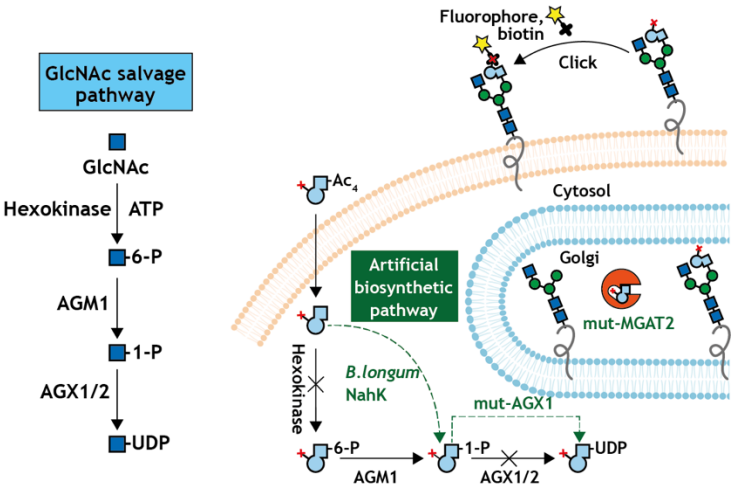
The pursuit of designing and synthesizing complex systems that closely resemble the highly glycosylated extracellular matrix (ECM) has gained significant interest in various fields: 3D cultures, drug delivery and tissue engineering [1]. Supramolecular hydrogels hold great potential for achieving this goal. However, the development of these hydrogels has been hindered by a lack of knowledge behind the fundamental parameters governing their hierarchical self-assembly. Furthermore, the limited examples of carbohydrate-based hydrogels in the literature predominantly involve homomultivalent presentation of a single carbohydrate, which falls short of replicating the complex heteromultivalent nature of the ECM [2].

To address these challenges, this project aims to synthesize biocompatible supramolecular hydrogels that emulate the highly and heterogeneously glycosylated ECM through a hierarchical supramolecular self-assembly approach, by employing rationally designed neoglycolipids. Various photopolymerizable neoglycolipids with distinct sugar headgroups, such as α-D-mannose, β-D-galactose, β-D-glucose, and β-lactose, have been synthesized. The hierarchical self-organization of these neoglycolipids into different hydrogel structures has been thoroughly characterized. Importantly, the resulting hydrogels exhibit multiple interactions with fluorescent lectins specific to the exposed sugars, highlighting their heteromultivalency.

In addition, hybrids of hydrogels and glyconanoring-coated carbon nanotubes have been developed to enhance the mechanical properties of the constructs.



Acknowledgements

We thank Richard Meek and Gideon Davies for providing MGAT5 enzymes. We thank Kevin Breummer and Carolyn Bertozz for sharing with us MGAT1 plasmids.

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Chemical synthesis of 4’-modified nucleoside analogues

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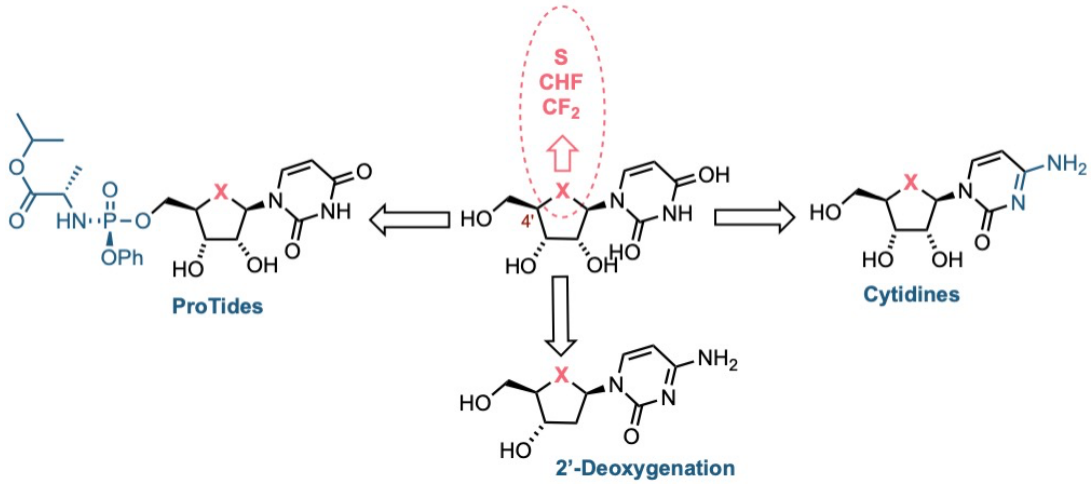
Nucleosides are the building blocks of DNA and RNA. Due to their involvement in a myriad of biological processes, nucleoside analogues represent an ideal starting point for the discovery of new drug candidates and have formed a cornerstone of treatment for viral infections and cancers. Notwithstanding their rich history in successful drug development, therapeutic intervention using nucleoside analogues is often limited by poor cellular uptake, low conversion to the active triphosphate metabolite, rapid degradation or clearance and development of resistance profiles in certain cell types. Consequently, research activity in this field continues to develop syntheses for next generations of biologically active nucleoside analogues that can overcome these limitations and provide new therapeutic options. Within this context, 4’-postion modification of the ribose ring represents an important structural motif for exploration. We have developed synthetic methods to chemcially synthesise 4’-thionucleosides, alongside replacing the furanosyl oxygen with CHF and CF_2 groups.

Exploring 4’-ring modification of pyrimidine nucleosides



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Predicting glycan structure from tandem mass spectrometry via deep learning

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Glycans constitute the most complicated post-translational modification, modulating protein activity in health and disease. However, structural annotation from tandem mass spectrometry data is a bottleneck in glycomics, preventing high-throughput endeavors and relegating glycomics to a few experts. Trained on a newly curated set of 300,000 annotated MS/MS spectra, we present CandyCrunch, a dilated residual neural network predicting glycan structure from raw LC-MS/MS data in seconds (Top1 Accuracy: 87.7%). We developed an open-access Python-based workflow of raw data conversion and prediction, followed by automated curation and fragment annotation, with predictions recapitulating and extending expert annotation. We demonstrate that this can be used for *de novo* annotation, diagnostic fragment identification, and high-throughput glycomics.

For maximum impact, this entire pipeline is tightly interlaced with our glycowork platform and can be easily tested at https://colab.research.google.com/github/BojarLab/CandyCrunch/blob/main/CandyCrunch.ipynb.

We envision CandyCrunch to democratize structural glycomics and the elucidation of biological roles of glycans.

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Preparation and surface properties of fluorinated nanocomposites containing gluconamide units

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Much attention has been focused on the synthetic sugar-containing polymers (glycopolymers), of whose polymers possess the pendent gluconic residues (pentaol units), owing to their role as biomimetic analogues and their applications in biomedical and technological fields such as paints and cosmetics [1]. These glycopolymers also have high potential use as polymeric surfactants [2]. Therefore, the studies on the preparation of the novel gluconamide units-containing polymers bearing longer fluoroalkyl groups are of particular interest, because these polymers would exhibit not only the unique characteristics related to the gluconamide units but also the surface active characteristic imparted by longer fluoroalkyl groups.

Here we report that two fluoroalkyl end-capped vinyltrimethoxysilane oligomer [3] can undergo the sol-gel reaction in the presence of *N*-(3-triethoxysilylpropyl)gluconamide under alkaline conditions to afford the corresponding fluorinated oligomeric silica nanocomposites containing gluconamide units. The modified surfaces treated with these obtained nanocomposites can provide the unique wettability such as superamphiphobic, highly oleophobic/superhydrophilic and superoleophilic/superhydrophobic characteristics. These results will be demonstrated in this conference.

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Synthesis of Man/Glc glycoclusters for systematic variation of glycoligand presentation in 3D space

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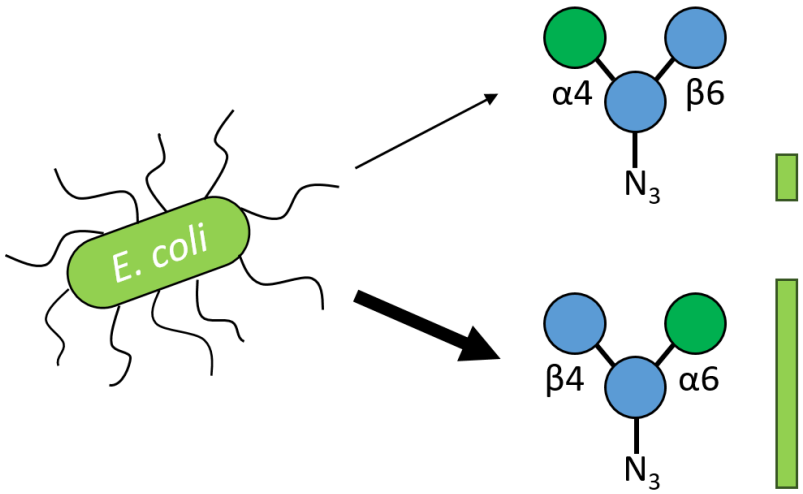
Carbohydrate recognition plays a fundamental role in biological processes like signal transduction, molecule transport, cell-cell recognition or cell adhesion.[1] These are complex processes comprising the interplay of different functional aspects such as, e.g., multivalency and heteromultivalency effects.[1] Furthermore, carbohydrate recognition is apparently regulated by the presentation mode of glycoligands in three-dimensional (3D) space. We have investigated the effect of 3D ligand presentation using synthetic glycomimetics allowing for the precise control of the spatial relation between a mannoside ligand (Man) and a glucoside moiety (Glc).[2,3]

Biological testing of these synthetic model systems in an adhesion inhibition assay[4] employing mannose-specific live *E. coli* bacteria revealed that different ligand presentation results in a significant difference in the inhibition of mannose-specific *E. coli* adhesion. Rationalisation of these results was obtained by computer-aided molecular docking of the bacterial lectin FimH and the synthetic regioisomeric glycoligands.

Future investigations will aim at a more complex testing system utilizing metabolic oligosaccharide engineering and human cells decorated with tailor-made synthetic glycoligands.



Regioisomeric trisaccharides demonstrate varying inhibition strengths against E. coli adhesion.

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Chemical synthesis of sugar nucleotide donors for the investigation of plant glycosyltransferases

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Knowledge of the structures and biosynthesis of plant cell wall polysaccharides is essential in the search for alternative, renewable resources for the production of fuels, chemicals and bio-based materials. Glycosyltransferases (GTs), the enzymes responsible for the construction of cell wall glycans, thereby play a key role, as they determine cell wall structure and properties. [1]

We report the chemical synthesis of sugar nucleotide donors related to the biosynthesis of plant cell wall glycans for the glycan array-based search for new biosynthetic GTs. [2] Oligosaccharide acceptors are immobilized as microarrays and incubated with putative GTs and the synthesized sugar nucleotide donors, which are chemically modified with an azido group, enabling detection of the products by click chemistry with a fluorescent dye.

UDP-N_3-galactose, *UDP-N_3-arabinofuranose*, *UDP-N_3-rhamnose* and *UDP-N_3-apiofuranose* have been chosen as the initial synthetic targets, as many unknown activities are to be discovered for GTs transferring these donors within AGP biosynthesis. We also report examples for the use of the synthesized sugar nucleotides in the aforementioned glycan array assay.

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Neu5Ac and Neu5,9Ac$_2$ in human plasma: potential biomarkers of cardiovascular disease

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CVD is characterized by high levels of inflammation which has been linked to a rise in the concentration of *N*-acetyl neuraminic acid (Neu5Ac) in blood. [1] Another sialic acid, 9-*O*-Acetyl-*N*-acetyl-neuraminic acid (Neu5,9Ac$_2$), has been of interest as a biomarker for diseases such as breast cancer but has not been studied in the case of CVD. Neu5Ac and Neu5,9Ac$_2$ concentrations were determined by quantitative analysis using liquid chromatography in plasma obtained from both patients with CVD and healthy controls. [2] Mean concentrations of Neu5Ac and Neu5,9Ac$_2$ were significantly elevated between the two sample groups (Neu5Ac: $P < 0.001$; Neu5,9Ac2: $P < 0.04$). Receiver operator curve analysis (ROC) further revealed the predictive power (AUC) of the two markers (Neu5Ac: 0.86; Neu5,9Ac2: 0.71).

A combined Neu5Ac/Neu5,9Ac$_2$ marker exhibited an AUC of 0.93. The sensitivity and specificity of each marker was then assessed, with the combined marker performing best overall. Neu5Ac appears to have good discriminatory power for CVD. Combining the two markers together may offer a better biomarker than either of the markers individually. Further analysis was undertaken to determine the *N*-glycan profile, percentage galactosylation of *N*-glycans and c-reactive protein concentration of each sample. ROC analysis was performed and AUC values were compared with the data for Neu5Ac and Neu5,9Ac$_2$. The samples were also analysed via nanoparticle prefractionation (*U.K. Patent Application Number 2117557.5*). [3] This allowed for the extraction of fibrinogen and analysis of fibrinogen derived glycans.

Acknowledgements

MRC (MR/P015786/1) and Ludger Ltd. for funding this research

Irish Research Council for supporting the study (Project EPSPG/2019/511)

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Stable electron-poor glycosylamides and their utilization

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Glycosylation has many roles in biochemical processes, cell-communication through lectin-carbohydrate interactions, and is a key factor in solubilization of lipophilic active compounds. The downside of glycosides often being the ease of cleavage of glycosidic bonds by glycosidases. Substitution of the glycosidic oxygen for other atoms or motifs can be a solution to this disadvantage.

Copying the natural *N*-glycosylation of peptide residues and building on 100 years old literature procedures, we have revived the methods for preparation of electron-poor glycosylamines and developed a better method of their acylation. This opened up a path to novel, hydrolytically stable scaffolds with potential use as lectin-ligands and drug-carriers.

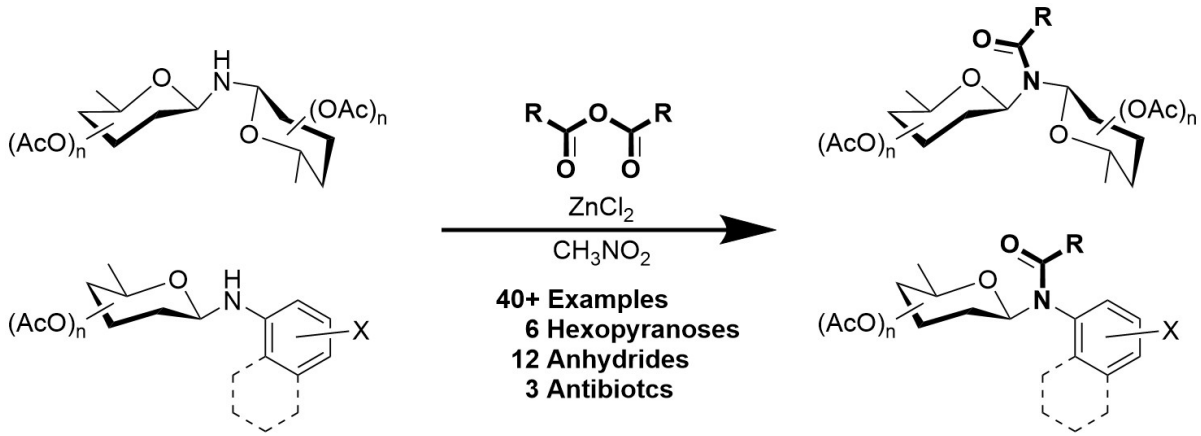

Figure 1: (A) HS repeating unit. (B) HPSE covalent inhibition. (C) Stabilized inhibitors.

This work was supported by the Czech Science Foundation (project 22-17586S) and grant of Specific University Research (grant No. A2_FPBT_2019_004).

Deciphering glycosaminoglycan structures using biological based nanopores

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Glycosaminoglycans (GAGs) are linear, highly complex, and heterogeneous polysaccharides mainly located in the extracellular matrix (ECM) and at the cell surface. Their polysaccharide backbones contain repeating disaccharide blocks composed of (a) one N-acetylhexosamine or hexosamine residue, linked to (b) one uronic acid or galactose residue. GAGs biosynthesis includes further modifications by sulfation, deacetylation, or epimerization, which lead to their vast structural diversity. Both their localization and diversity allow GAGs to interact with various extracellular proteins, and they are involved in different physiological and pathological events [1].

Despite of a great interest in studying GAGs structure-to-function relationships, structural characterization of GAGs is challenging due to their high complexity and polydispersity. Also, commonly employed analytical methods for GAG structural analysis are time-consuming and often fail to provide information on minor structural patterns [2]. On the other hand, recent developments in nanopore-based analysis demonstrated the potential of this method for in-depth structural characterization of GAGs and detection of their low abundant but crucial structural motifs. This method was shown to successfully determine the degrees of polymerization (DP), detecting differences in osidic bonds, sulfation patterns, and epimers of uronic acid residues of GAG oligosaccharides on a single molecule level [3]. Another advantage of biological nanopores is that they can be modified at the Å-level precision by protein-engineering techniques for improving fine structural discrimination of analytes. A replacement of a single amino acid residue in the case of aerolysin (AeL) nanopore showed promising results in analyzing structures of peptides [4].

The present study employs the wild-type AeL and its mutant R220S to determine the impact of this single-point mutation within the pore lumen on the nanopore structural analysis of GAG oligosaccharides. Ongoing work is as well focused on creating a specific signal database for known GAG structures. Preliminary results suggest that this approach can be used for fast and reliable fine structural characterization of different GAGs. Among our future goals is to employ this approach for analyzing fine structural differences between GAGs from pathological and non-pathological samples and further our knowledge on relations between GAG structures and function.

Acknowledgements

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Association of C3 glycosylation with the complement pathway proteins

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Complement is a central component of the innate immune system. It plays a key role in maintaining homeostasis and defense against pathogens. Activation of the complement system is possible through three different pathways: the classical, the alternative and the lectin pathways. Plasma proteins, mainly derived from the liver and membrane proteins expressed on the cell surface make up the complement system. The most abundant protein of the complement is C3. Like most plasma proteins, C3 is glycosylated. It has three potential N-glycosylation sites, but only two of them are occupied by glycans, exclusively the high-mannose type. In this study, we analyzed C3 glycosylation in a site-specific manner by nano LC-MS. C3 was enriched with concanavalin A lectin affinity matrix from plasma. This method was applied to plasma samples of four different groups. In addition, peptides of various complement proteins were measured in these samples. The C3a protein, released from C3 by C3-convertase and C5a, cleaved from C5 by C5-convertase were included in this study. Furthermore, components of the alternative pathway included in the analysis were Ba and Bb. C4a as a component of the classical and lectin pathways and SC5b-9 as a component of the terminal pathway were measured in each group. Preliminary statistical analysis showed a significant correlation between C3 glycopeptides and peptide levels of the complement system. The significant correlation points in the same direction in all four groups.

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Multivalent 9-*O*-Acetylated-sialic acidglycoclusters as potent inhibitors for SARS-CoV-2 infection

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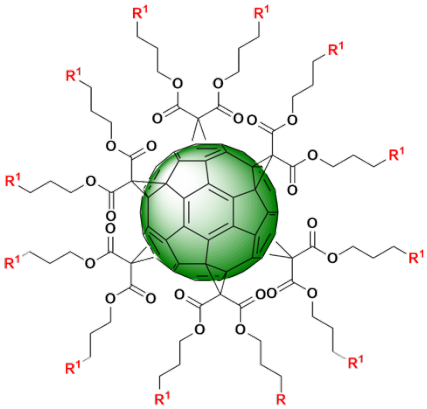
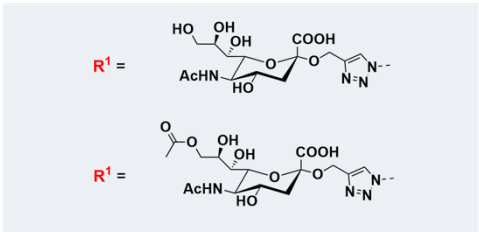
The recent emergence of highly transmissible SARS-CoV-2 variants illustrates the urgent need to better understand the molecular details of the virus binding to its host cell and to develop anti-viral strategies. While many studies focused on the role of the angiotensin-converting enzyme 2 receptor in the infection, others suggest the important role of cell attachment factors such as glycans. We first synthesized a series of glycoclusters based on various central scaffolds and a controlled number of sialic acids, and we use atomic force microscopy to study these early binding events with the focus on the role of sialic acids (SA). We show that SARS-CoV-2 binds specifically to 9-O-acetylated-SA with a moderate affinity, supporting its role as an attachment factor during virus landing to cell host surfaces.

For therapeutic purposes and based on this finding, we have designed novel blocking molecules with various topologies and carrying a 4 to 12 SA residues, enhancing affinity through a multivalent effect. Inhibition assays show that the AcSA-derived glycoclusters are potent inhibitors of cell binding and infectivity, offering new perspectives in the treatment of SARS-CoV-2 infection.





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Reductive Opening of 4,6-*O*-Benzylidene with $PhBCl_2/Et_3SiH$: how to prevent unreported side-reactions

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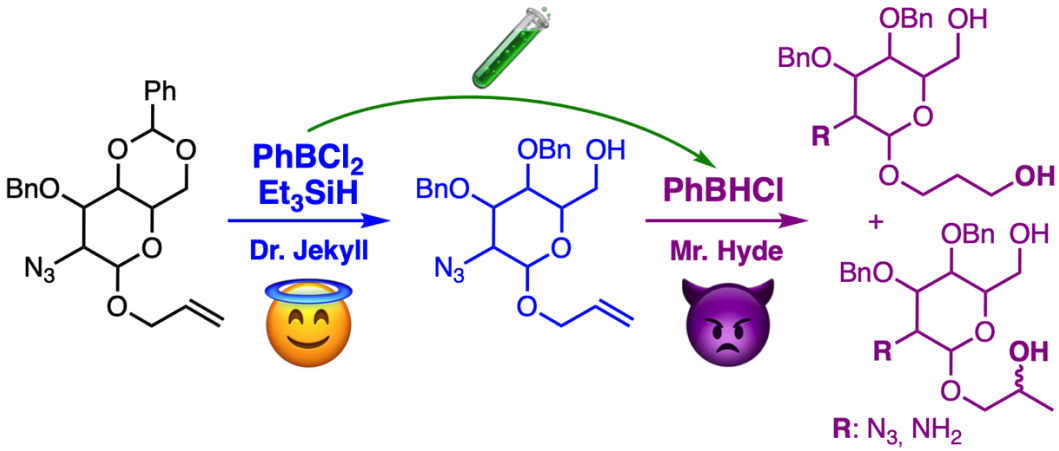
Arylidene acetals are widely used protecting groups, not only for the high regioselectivity of their introduction, but also for the possibility to perform further regioselective reductive opening in the presence of a hydride donor and acid catalyst. In this context, the $Et_3SiH/PhBCl_2$ system present several advantages: silanes are efficient, environmentally benign, and user-friendly hydride donors, while $PhBCl_2$ open the way to unique regioselectivity with regards to all other Brønsted or Lewis acids used with silanes. This system has been extensively used by several groups and we have demonstrated its high regioselectivity in the reductive opening of 4,6 and 2,4-*O*-*p*-methoxybenzylidene moieties in protected disaccharides.

Surprisingly, its use on the 4,6-*O*-benzylidene containing also *O*-allyl or azide moieties led to unreproducible yields due to the unexpected formation of several side products. Their characterizations allowed us to identify different pitfall potentially affecting the outcome of reductive opening of arylidenes with the $Et_3SiH/PhBCl_2$ reagent system: alkene hydroboration, azide reduction and/or Lewis acid promoted cleavage of the arylidene. With this knowledge, we optimized reproducible and high yielding reaction conditions that secure and extend the scope of the $Et_3SiH/PhBCl_2$ system as reagent for the regioselective opening of arylidenes in complex and multifunctional molecules.



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TheFrench MESR for P. QUELLIER's one.

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Structural analysis of *N*-glycans and their quantities in bovine submaxillary mucin by LC-MS/MS

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Bovine submaxillary mucin (BSM) is a heavily-glycosylated macromolecular (approximately 4MDa) protein that is used in various biomaterial applications in light of its high viscosity and biocompatibility, in addition to use as a biochemical substrate or inhibitor as a result of its abundant *O*-glycans. Although *N*-glycosylation has been reported to provides stability of human mucins, most BSM research has been focused on its *O*-glycans, while *N*-glycans have not been reported to date.

In this study, a common *N*-glycan core component was detected by monosaccharide analysis of BSM, and the structures and relative quantities of the *N*-glycans were determined by liquid chromatography–tandem mass spectrometry.

Seventeen *N*-glycans comprising ten complex-type [$Fucose_{0\sim2}Hexose_{3\sim4}$*N*-$acetylhexosamine_{1\sim6}Sulfate_{0\sim1}$; 61.1% (the sum of the relative quantities of each *N*-glycan out of the total *N*-glycans)], two high-mannose-type ($Hexose_{5\sim6}$*N*-$acetylhexosamine_2$; 12.0%), and five paucimannose type ($Fucose_{0\sim1}Hexose_{3\sim4}$*N*-$acetylhexosamine_{2\sim3}$; 26.9%) were identified, but no hybrid-type or sialylated *N*-glycans were found. Additionally, these are less-branched structures compared to human mucins. Of these, ten glycans (77.2%), including two sulfated glycans (8.0%), were core fucosylated, which confer unique biological functions to glycoproteins. These *N*-glycans are less-branched structures compared to human mucins. This is the first study to confirm *N*-glycosylation of BSM, and these results support further expansion of the biological function of non-human mucin.

Proposed structure[a]	LC-ESI-HCD-MS/MS				
	Mass (m/z)		Charge	Mass error (ppm)[b]	Relative quantity (%)[c]
	Theoretical $[M+H]^+$	Observed $[M+H]^+$			
	1583.6205	1583.6221	1	1.0	23.9
	1380.5411	1380.5435	1	1.7	12.2
	1786.6998 (893.8536)[e]	893.8536	2	0.0	10.8
	1177.4617	1177.4630	1	1.1	9.0
	1355.5095	1355.5111	1	1.2	7.1
	1745.6733 (873.3403)[e]	873.3414	2	1.3	5.3
	1866.6566 (933.8320)[e]	933.8330	2	1.1	5.2
	1517.5623	1517.5636	1	0.9	4.9
	1437.5625	1437.5631	1	0.4	3.6
	1989.7792 (995.3932)[e]	995.3940	2	0.7	3.2
	1234.4832	1234.4836	1	0.4	3.0
	2069.7360 (1035.3717)[e]	1035.3734	2	1.7	2.8
	1932.7577 (966.8825)[e]	966.8837	2	1.2	2.6
	1948.7527 (974.8800)[e]	974.8807	2	0.7	2.2
	1031.4038	1031.4049	1	1.1	1.8
	1843.7213 (922.3643)[e]	922.3638	2	0.6	1.5
	1396.5360	1396.5380	1	1.4	0.9

N-glycan analysis of BSM for determination of the structure and the relative quantity.

Identification and quantification of sialylated and core-fucosylated *N*-glycans in human transferrin

Jieun KIM [1], Mijung JIN [1], Jihye KIM [1], Chi Soo PARK [1], Chulmin MOON [1], Mirae KIM [1], Subin YANG [1], Leeseul JANG [1], Ji Yeon JANG [1], Chang MYEONG JEONG [1], Ha Hyung KIM [1]

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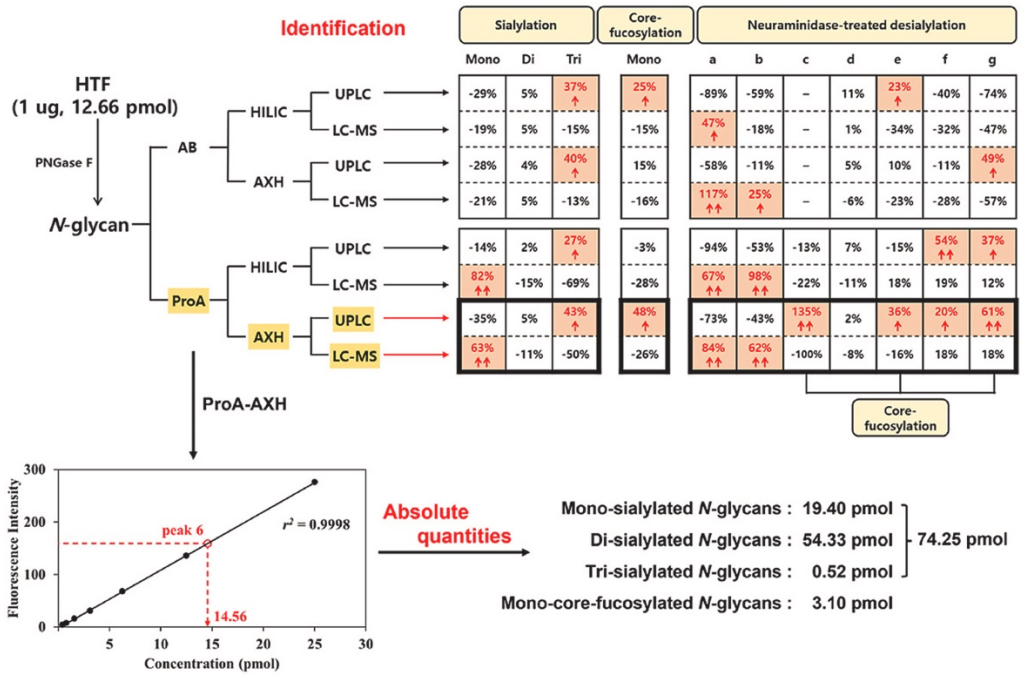
Sialylated and core-fucosylated *N*-glycans in human transferrin (HTF) are used as glycan biomarkers due to their increased or decreased characteristics in certain diseases. However, efficient identification and quantification of *N*-glycans in HTF remain unclear.

In this study, *N*-glycans of HTF were identified by UPLC and LC-MS/MS using combinations of fluorescence tags [2-aminobenzamide (AB) and procainamide (ProA)] and columns [HILIC and anion exchange chromatography-HILIC (AXH)].

The structures of 14 (including five core-fucosylated) *N*-glycans in total comprising two non-, six mono-, four di-, and two tri-sialylated *N*-glycans were identified. The quantities (%) of each *N*-glycan relative to the total *N*-glycans (100%) were obtained. HILIC and AXH were better for peak identification and separability except for desialylation, respectively. Specifically, sialylated (in ProA-HILIC and ProA-AXH by UPLC or LC-MS/MS) and core-fucosylated (in AB-HILIC and ProA-AXH by UPLC) *N*-glycans were efficiently identified. Seven neuraminidase-treated (including three core-fucosylated) *N*-glycans were efficiently identified in ProA-AXH, even with their poor separation. Additionally, ProA-AXH was more efficient for the estimation of the absolute quantities of *N*-glycans from the results of fluorescence intensity (by UPLC) and relative quantity (by LC-MS/MS).

These results first demonstrate that ProA is useful for identifying and quantifying sialylated, core-fucosylated, and neuraminidase-treated desialylated *N*-glycans in HTF using AXH by UPLC and LC/MS.



Efficient combination to identify and quantify N-glycans in HTF.

Acknowledgements

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Combating DC-SIGN-mediated SARS-CoV-2 Dissemination by Glycan-mimicking Polymers

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The C-type lectin receptor DC-SIGN is a promiscuous attachment factor for pathogenic viruses on innate immune cells. In the context of the recent SARS-CoV-2 pandemic, DC-SIGN-mediated virus dissemination has been identified as a mechanism of immune dysfunction that contributes to severe COVID-19.[1] Carbohydrate-based compounds can efficiently interfere in the interaction between viral surface glycoproteins and DC-SIGN and, thus, prevent virus binding to innate immune cells.[2-4] As a consequence, glycan-mimicking molecules represent a host-directed strategy to combat the spread of epidemic and pandemic viruses. A recent study identified preferred oligomannose fragments of DC-SIGN in high-mannose glycans.[5] Here, we aim to leverage this insight in order to enhance the activity of polyvalent DC-SIGN ligands. For this, poly-l-lysine polymers were functionalized with selected mono-, or oligosaccharide epitopes and evaluated for their ability to bind DC-SIGN. Hydrodynamic properties and multivalent interaction thermodynamics were characterized in biophysical assays and correlated with polymer activity in SARS-CoV-2 *trans*-infection studies.

Oligosaccharide epitopes containing a-d-Man-(1→2)-a-d-Man motifs displayed particularly high activity in the low nanomolar range. The optimized oligomannose glycopolymers identified here represent highly active and fully biocompatible lead candidates to enable a rapid host-directed response to known and newly emerging viral pathogens.

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Characterization of an *N*-Acetylglucosaminyltransferase I (GnT-I) from *Crassostrea gigas*

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The biosynthesis of hybrid and complex type N-glycans takes place in the medial-Golgi and requires the prior action of the α-1,3-mannosyl-glycoprotein 2-β-N-acetylglucosaminyltransferase I, GnT-I (encoded by *Mgat1*, EC 2.4.1.101). It catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine (UDP-GlcNAc) to the Man-alpha-1-3 arm of Man5GlcNAc2 (Man5), producing a suitable substrate for the further enzymes, such as alpha-mannosidase-II and subsequently GnT-II.

Although the enzyme has been cloned and characterized from several plants and animals, the GnT-I from invertebrates, especially from mollusks, remains rather undiscovered.

In this study, we present the expression of a ~50 kDa truncated GnT-I from the Pacific oyster, *C. gigas* (ΔR2-M24 GnT-I, NCBI Ref. Nr.: XP_034321804.1), in Sf9 insect cells and its characterization with 2-aminopyridin labelled Man5 N-glycan (Man5-PA) as the substrate.

The GnT-I shows highest activity at neutral environment, pH 7.0, and at a temperature of 30 °C. The addition of divalent cations such as Mn^{2+}, Co^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, and Ni^{2+} [24 mM] increases activity, with highest activity at 40 mM Mn^{2+}, while the addition of EDTA or Cu^{2+} abolishes the enzyme's activity completely. The activity is also negatively influenced by the addition of UMP, UDP, UTP or galactose [0.1 %]. Moreover, the GnT-I enzyme is sensitive towards storage in methanol (20 % v/v), acetonitrile (10 % v/v) or glycerol (10 % v/v).

Acknowledgements

This project is funded by the Austrian Science Fund (Förderung der wissenschaftlichen Forschung – FWF) grant agreement No. P-33239-B.

Chemoenzymatic synthesis of ^{13}C enriched sialic acids

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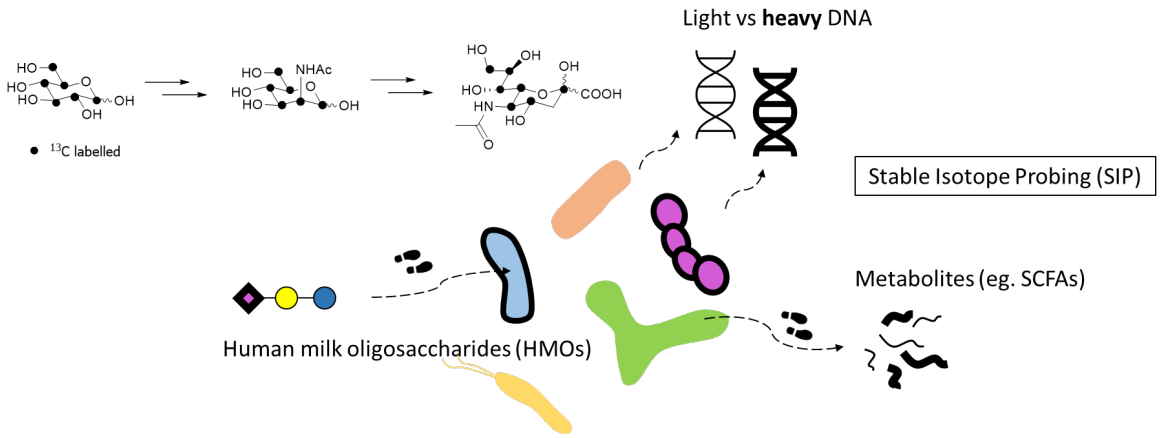
Sialic acid or N-acetylneuraminic acid (Neu5Ac) is a ubiquitous sugar residue found in the gut mucus layer. It is commonly found at the terminal location of sugar chains, and its prior removal is necessary for gut microbiota to gain access to the rich underlying sugar residues.

Metabolism of glycans by gut bacteria leading to the production of short chain fatty acids (SCFAs) is of great interest because of their known health benefits to human hosts. Through Stable Isotope Probing (SIP), 13C labelling of Neu5Ac can identify gut microbiota able to metabolise Neu5Ac and enable clear distinction of metabolites generated in the Neu5Ac metabolism pathway in the presence of other sugars.

To obtain these 13C enriched sugars, we have developed a facile chemoenzymatic synthesis route from the cheap and commercially available [U-13C]glucose. Chemical synthesis yielded the key intermediate, peracetylated N-acetyl mannosamine ([13C6]Ac4ManNAc) on a gram scale at 21% yield. Subsequently, enzymatic condensation of [13C6]ManNAc with [2-13C]pyruvate gave asymmetrically labelled [13C7]Neu5Ac, which can be used to prepare 3'-SL and 6'-SL through a facile one-pot enzymatic reaction using CMP-sialic acid synthetase and sialyltransferase. Synthesis of other forms of sialic acids are also possible as demonstrated by the synthesis of 2,7-anhydro Neu5Ac.



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Pyridoneimine-promoted aqueous anomeric functionalization of unprotected carbohydrates

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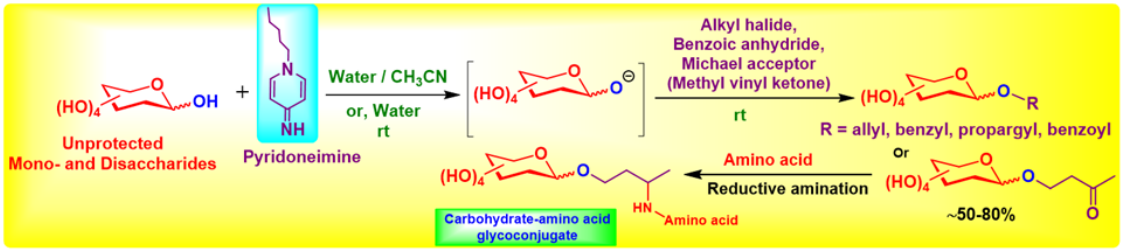
Anomeric functionalization of unprotected carbohydrates in water is ambitious, due to the presence of competing hydroxy moieties in a monosaccharide. As a result, the most often practiced route is to install required protecting groups that permit selective functionalization at the desired hydroxy functionality, including the anomeric lactol moiety. There is a reasonable basis to differentiate the anomeric lactol moiety from that of remaining hydroxy functionalities, factoring the pK_a differences. Difference in the p*K*a value of ~12.5 *vs* 16-18 for the anomeric and the remaining hydroxy groups, respectively, prompts that such a difference is available for the selective functionalization of anomeric lactol in carbohydrates.

With this basis, we have uncovered the potential of pyridoneimine as a suitable and selective base for deprotonation of the hemiacetal and subsequent reaction of the corresponding hemiacetal anion. Reactions of the resulting hemiacetal anion with alkyl halides, acid anhydrides and Michael acceptors lead to facile formation of the corresponding anomeric functionalized derivatives. Unprotected mono- and disaccharides of varied constitutions are subjected to the reactions and the selective functionalization at the anomeric carbon achieved, in aq. solutions. The anomeric oxa-Michael addition-derived keto-glycosides are resourceful for further glycoconjugations with amino acids, through reductive amination. The presentation will include the development of pyridoneimine as a base for selective anomeric functionalization of mono- and disaccharides, fulfilling the solubilities of carbohydrates in water, in a complete site-selective fashion in such a multi-functional synthons.



Hemiacetal deprotonation followed by further alkylation, acylation and oxa-Michael addition reaction

Acknowledgements

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2. CSIR for research fellowship

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Synthesis of a tetrasaccharide donor for the synthesis of rhamnogalacturonan II side chain A

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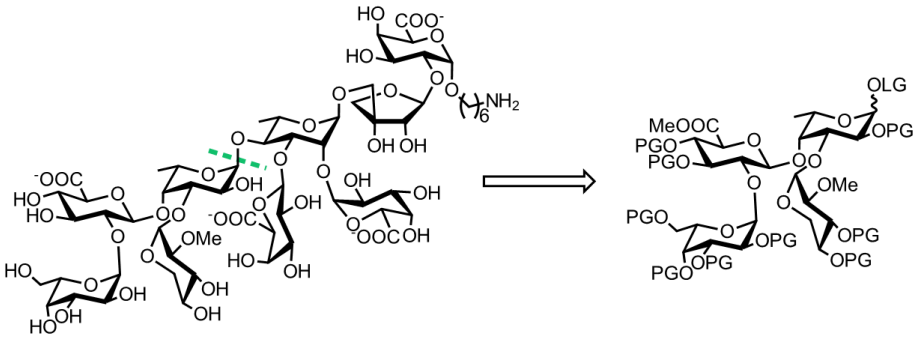
The plant cell wall contains three types of polysaccharides: cellulose, hemicellulose and pectin. Pectin is an acidic polysaccharide consisting of different domains with either homogalacturonan or rhamnogalacturonan backbone. The most complex part of pectin is rhamnogalacturonan-II (RG-II).[1] It mostly occurs in dimers, which are covalently linked by a borate diester which has been shown to be crucial for plant growth.[2]

For further investigations into the biosynthesis, structure and biological function of RG-II, oligosaccharide fragments of RG-II will be highly valuable. Here we present the synthesis of a tetrasaccharide donor (**2**) for the synthesis of the full side chain A nonasaccharide of RG-II (**1**) as well as different side A fragments. Glycosyl donor **2** is a branched tetrasaccharide, consisting of a fucose carrying an α-1,3-linked 2-*O*-methyl xylose a β-1,4-linked glucuronic acid, which in turn is substituted with an a-1,2-linked L-galactose. The glycosylation reactions for constructing tetrasaccharide **2** were mostly conducted using thioglycoside donors activated by *N*-iodosuccinimide and silver triflate. Importantly, a highly orthogonal set of protecting groups was chosen to achieve the branched structure of donor (**2**). Tetrasaccharide donor **2** will be reacted in a [4+5]-glycosylation reaction with a suitable pentasaccharide acceptor to provide full side chain A. Synthetic side chain A and fragments thereof generated using donor **2** will serve as substrates for glycosyltransferases, glycosyl hydrolases, and as ligands for carbohydrate receptors.[3]



Full Side Chain A (1) **Tetrasaccharide Donor (2)**

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Regioselective and Stereospecific β-Arabinofuranosylation Using a Boron-Mediated Aglycon Delivery

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β-Arabinofuranoside (β-Arb*f*)-containing glycans have garnered significant attention across numerous research fields due to their intriguing biological activities. However, achieving stereoselective construction of β-Arb*f* linkages has proven challenging due to the absence of neighboring-group participation, the unfavorable anomeric effect, and the steric hindrance of the substituent at the C2 position. Moreover, S_N1 pathway is preferential for furanoside formation compared to pyranoside formation owing to its structural and electronic properties, resulting in diminished stereoselectivity. With this in mind, we directed our attention towards our boron-mediated aglycon delivery (BMAD) method, which is capable of constructing 1,2-*cis* pyranosides with exceptional regio- and stereoselectivities and explored its applicability in β-arabinofuranosylation.

As a result of the investigation, it was found that the glycosylations of 1,2-anhydroarabinofuranoses and several sugar diols in the presence of boronic acid catalyst proceeded smoothly to provide corresponding β-Arb*f*s with complete β-stereoselectivities and high regioselectivities. In addition, a variety of diols, triols, and unprotected sugar acceptors could be employed for this present arabinofuranosylation, and the regioselectivity was completely reversed depending on the optical isomerism of the donor used and was predictable a priori using predictive models. Furthermore, the chemical synthesis of arabinogalactan fragments from Timothy grass demonstrated the usefulness of this glycosylation method.

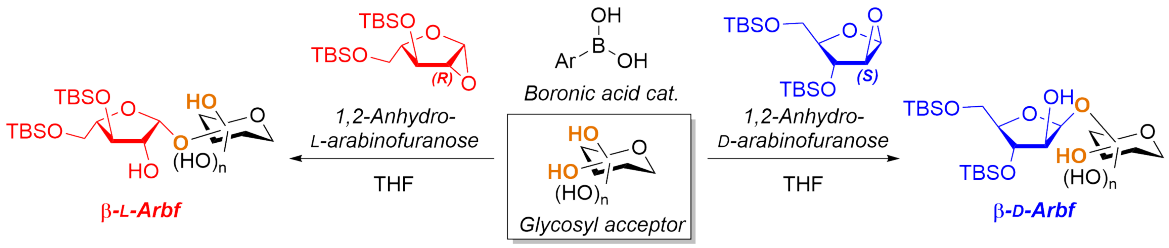

Figure 1. (a) Neoglycolipids. (b) Supramolecular characterization. (c) Heteromultivalency.

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Analysis of the interactions between ganglioside GM3 and insulin receptor transmembrane peptide

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Biochemical and cell biological experiments have shown that GM3, a cell membrane glycolipid, inhibits the function of insulin receptor (IR). However, due to the heterogeneity of the cell membrane, it has been difficult to analyze the interaction between GM3 and IR in detail. In this study, we synthesized a fluorescently labeled transmembrane peptide (NBD-IR-TM) and incorporated it into liposomes to construct a simplified model system for interaction analysis.

The localization of NBD-IR-TM in phase separated GUVs containing glycolipids was analyzed (Figure 1). The co-localization of NBD-IR-TM with GM3 was significantly increased compared to that of lactosylceramide (LacCer), a GM3 precursor without sialic acid. Furthermore, the fluidity of NBD-IR-TM in GUVs was significantly reduced by the addition of GM3; in MLVs, the association of NBD-IR-TM was inhibited by GM3, but not by LacCer. Finally, we analyzed the aggregation state of two model peptides with and without charge and found that only the association of the positively charged peptide was inhibited by GM3.

These results indicate that the basic amino acids and acidic sugars of each molecule are important for the interaction between transmembrane peptides and GM3, and that this electrostatic interaction also occurs between the insulin receptor and GM3.

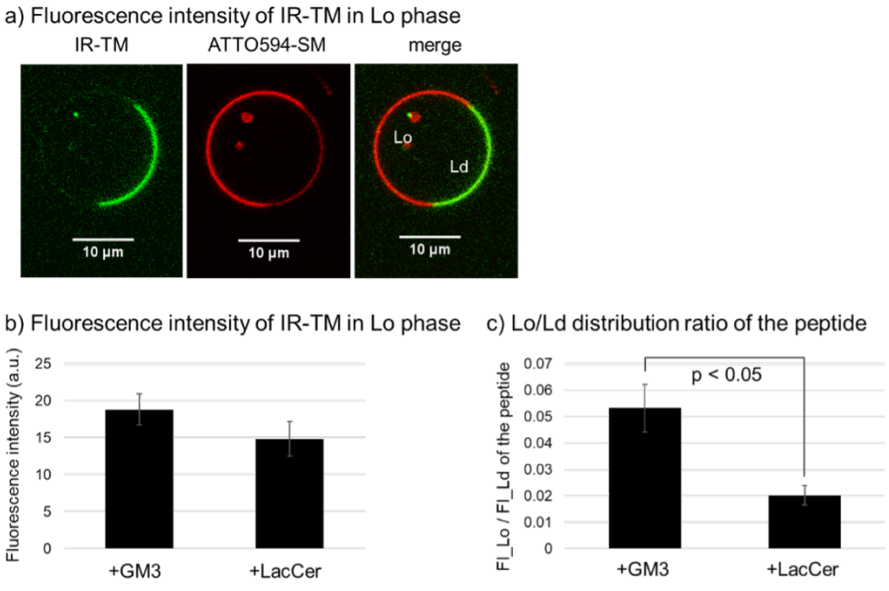

Figure 1 Localization analysis of IR-TM in phase-separated GUVs. GUVs were prepared with molar ratios of DOPC / sphingomyelin / GM3 or LacCer / cho

Glycosylation of Polyfluorinated Carbohydrates

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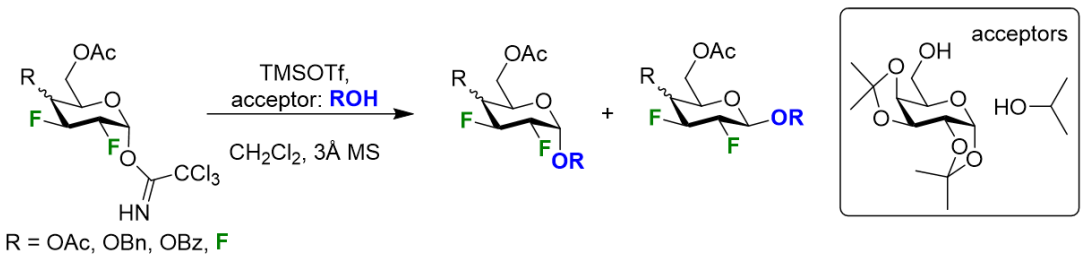
Glycosylation is a ubiquitous process in nature, where glycans are involved in metabolic pathways and recognition events. Thus, fluorinated glycans can act as probes in the investigation of protein-glycan interactions and epitome mapping studies. Currently, monofluorinated glycans can be made by enzymatic glycosylation of monofluorinated donors. There are no enzymatic glycosylations described with sugar donors having >1 fluorine atom in their ring. In carbohydrates, fluorination was shown to increase chemical stability and reduce their hydrophilicity.

Chemical glycosylation of deoxyfluorinated carbohydrates is challenging because the fluorine electron withdrawing effect destabilizes the transition states of anomeric C–O bond forming reactions. The effect is pronounced when the number of fluorine atoms increases and when they are located adjacent to the anomeric position. To date, there is limited precedence for the chemical glycosylation of polyfluorinated carbohydrates. Most of the glycosylation methods developed involve anomeric alkylation which results in inversion of a stereogenic centre on the acceptor. Most of the methods developed are glycosidation, i.e., involve non carbohydrate acceptors. This poster will describe our efforts to achieve conventional chemical glycosidation and glycosylation of 2,3-difluoro- and 2,3,4-trifluorinated glucose and galactose donors using trichloroacetimidate pre-activation. Two distinct goals were established: being able to reach full conversion and increasing the anomeric selectivity of the reaction.



Glycosylation of polyfluorinated carbohydrates through trichloroacetimidate pre-activation

Acknowledgements

K.H. and B.L. thank the University of Southampton for funding

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Design and synthesis of 5-thio-glucose based endo-glycosidase inhibitors

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Glycomimetics are molecules that are structurally similar to carbohydrates but differ in their architecture around the glycosidic linkage.[1] By altering this key feature, these molecules are able to mimic the behaviour of natural substrates while being customizable in reactivity and stability.[1] One way of increasing the stability of the glycosidic linkage is by substituting its endo-cyclic oxygen atom with a sulfur atom.[2] Known as thiosugars, the increased glycosidic stability of these compounds makes them useful tools in chemical biology, as thiosugar-linked substrates are known to act as inhibitors for endo-specific glycosidases.[3]

A prominent member of the endo-glycosidase family is heparanase (HPSE), an endo-β-D-glucuronidase that degrades the linear polysaccharide known as heparan sulfate (HS) (Fig. 1A).[4] Recently, the cyclophellitol derivative **1**, a mimic of the repeating disaccharide present in HS, was found to be a potent and covalent inhibitor of HPSE (Fig. 1B).[5] With this in mind, mimics featuring a thiosugar could potentially stabilize such an inhibitor by preventing exo-glycosidase cleavage of the glycosidic linkage, thereby increasing selectivity (Fig. 1C). Here, the development of such 5-thio-D-glucopyranoside linked inhibitors is presented.

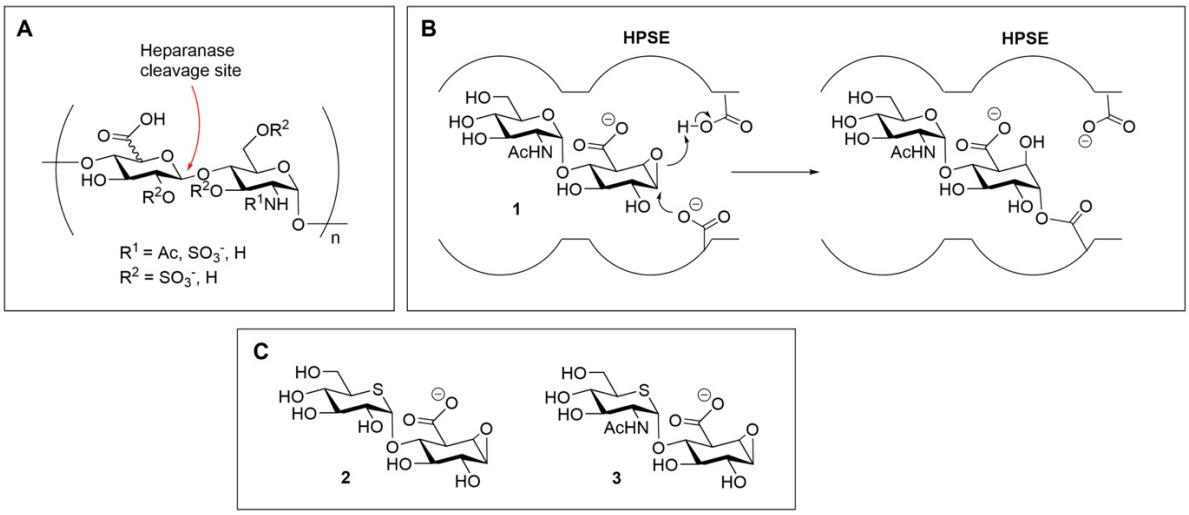

Figure 1: (A) HS repeating unit. (B) HPSE covalent inhibition. (C) Stabilized inhibitors.

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New supramolecular multivalent anti-adhesive agents against SARS-CoV-2

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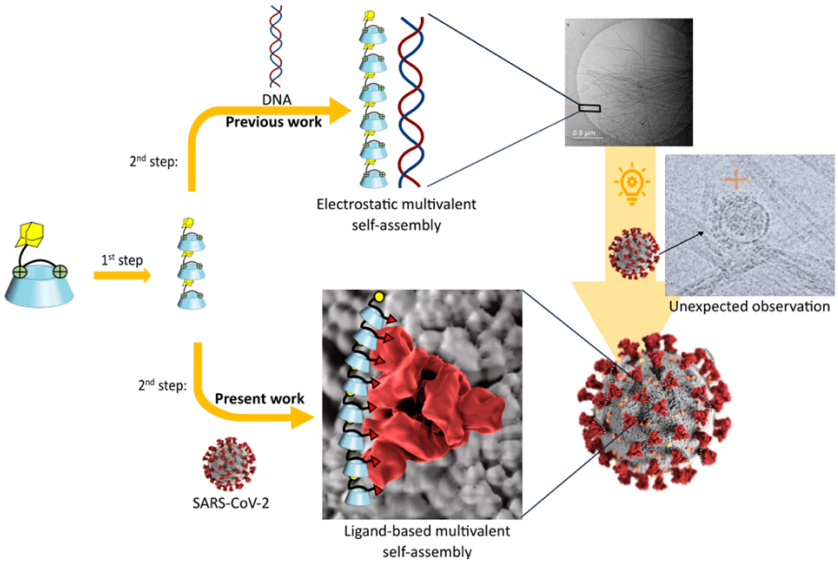
In 2020, during a CRYO-EM experiment, we unexpectedly observed a SARS-CoV-2 particle near self-assembled cyclodextrins fibers. We thus wondered if we could bring specific interactions between them and trigger this assembly on purpose. Indeed, our team previously showed that it was possible to form fibers from small oligomers of DNA and cyclodextrins functionalized by an adamantane and ammonium. The hydrophobic effect between cyclodextrins and adamantanes allows the formation of small supramolecular polymers by self-assembly (1st step). Then, through multivalent electrostatic interactions between the monomers and DNA, the co-assembly becomes a lot bigger (2nd step).[1]

The surprising observation encouraged us to explore the ability of cyclodextrins assemblies to cooperatively interact with SARS-CoV-2 particles and use them as anti-adhesive agents to potentially inhibit cell infection by this virus. We therefore changed non-specific electrostatic interactions into more specific ones, using sugar-based ligands allowing multivalent effect. For that, we functionalized cyclodextrins with an adamantane and the targeted ligand on the side. We are now studying their self-assemblies (1st step), their multivalent ability to interact with several receptors at the surface of SARS-CoV-2 and potentially observe cooperative assembly.



From CDs interactions with DNA to multivalent anti-adhesive agents against SARS-CoV-2

Bibliographic references:

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Synthesis of *Streptococcus pneumoniae* 6A/6C capsular polysaccharide fragments

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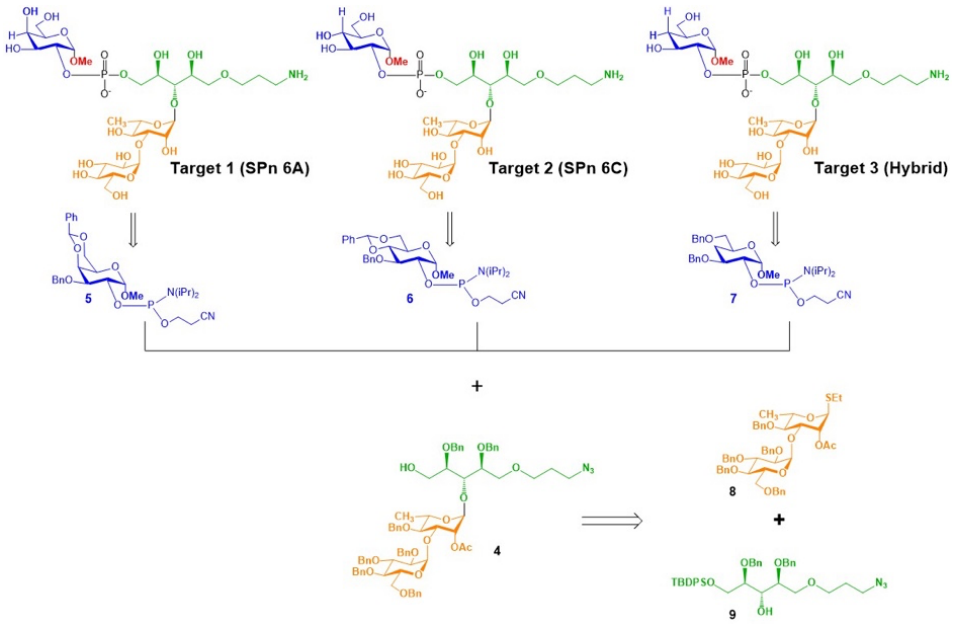
Streptococcus pneumoniae (SPn) is a significant cause of otitis media, pneumonia, and meningitis. Just 7 out of the approximately 100 serotypes were initially included in the pneumococcal polysaccharide conjugate vaccine in 2000, and this number has been increased in subsequent years. A significant rise of infections from non-vaccine serotypes SPn 6C was recorded following the widespread administration of pneumococcal vaccines containing serogroups 6A and 6B, a phenomenon usually referred to as serotype replacement1. Thus, the identification of novel antigens able to provide protection against more than one serotype is key to the future development of novel and more effective vaccines. The polysaccharide repeating units of SPn 6 serotypes have minimal structural differences: 6A and 6C have the same glycosidic linkages, but they differ in their monosaccharide composition, with glucose replacing galactose in 6C2. The tetrasaccharide fragments of SPn 6A (Target 1) and 6C (Target 2) serotypes are the primary synthetic goals of this project (Scheme 1). In addition, we designed the 4-deoxy derivative of both tetrasaccharides (Target 3), where the only structural difference between 6A and 6C CPS is removed. According to the retrosynthetic approach, compound **4** was synthesized from a suitable rhamnose derivative, which can be used both as an acceptor for the synthesis of disaccharide **8** and as a donor for the glycosylation of ribitol **9**. Finally, target tetrasaccharides **1**, **2** and **3** have been assembled using the phosphoramidite derivatives **5**, **6** and **7**. The synthetic tetrasaccharides will be eventually conjugated to a carrier protein, and the glycoconjugates obtained will be subjected to in-depth immunological studies. The results will help to identify protective epitopes to be employed for the development of an effective anti-SPn 6A/6C glycoconjugate vaccine.



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Development of a 2,3-difluorosialic acid based covalent neuraminidase probe

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Neuraminidase (NA), one of the major surface glycoproteins of influenza A virus, is an important diagnostic biomarker and antiviral therapeutic target. Probing NA provides important information on influenza virus biology, that can monitor the emergence of drug-resistant strains and guide the development of novel drugs and vaccines. However, there is still a lack of covalent NA probes with enhanced specificity and higher stability and this limits the in-depth exploration of NA.

Based on the covalent NA inactivators, 2,3-difluoro sialic acids (DFSA) synthesized by Wennekes and colleagues1, we modified DFSA with an azide mini-tag to converted it into a covalent probe. In this study, we chemically synthesized 5*N*-azidoacetyl-2,3- difluoro sialic acid as a covalent probe. The reactivation assay had shown the probe binding to NA without cleavage in 6 hours. The NA protein inhibited by the DFSA probe could be labeled with a biotin reporter via the CuAAC reaction, which also proved the covalent binding of the probe to the NA protein. Besides, the probe also kept an inhibition activity on NA with an IC50 value from 50 to 60 µM. With these properties, we expect the DFSA probes to be promising tools in labeling, visualizing, and mobilizing NA proteins and Influenza virus particles.

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Pharmacological chaperone therapy treat krabbe disease

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Krabbe disease (KD) is a rare and fatal neurodegenerative lysosomal storage disorder (LSD) caused by a deficiency of the lysosomal enzyme β-galactosylceramidase (GALC).[1] As a consequence the glycolipids galactosylceramides and psychosine accumulates (**Figure 1A**).[2] A deficiency of this enzyme leads to the build-up of galactosylceramide and then, through the action of acid ceramidase (AC), leads to the accumulation of psychosine, also a substrate of GALC. This psychosine metabolite is cytotoxic to neuronal cells, which give rise to KD.[2] Unfortunately, there is no cure for this neurodegenerative disease, ultimately leading to death within the first two years of life.

Pharmacological chaperone therapy (**Figure 1B**) is an innovative approach to treating protein misfolded diseases like KD. This therapy uses small molecules to act as molecular scaffolds to help stabilise and therefore rescue partially defective enzymes from premature degradation by the cell's quality control process.[3] Counterintuitively, competitive inhibitors are often utilised as pharmacological chaperones, to selectively bind to the active site of the mutated enzyme, facilitating its folding and proper trafficking.[1] Once at its site of utility, the pharmacological chaperone is displaced, and the mutated enzyme can break down the accumulated substrates. This presentation will cover the design and synthesis of new carbohydrate-based compounds that can act as pharmacological chaperone for the treatment of KD.

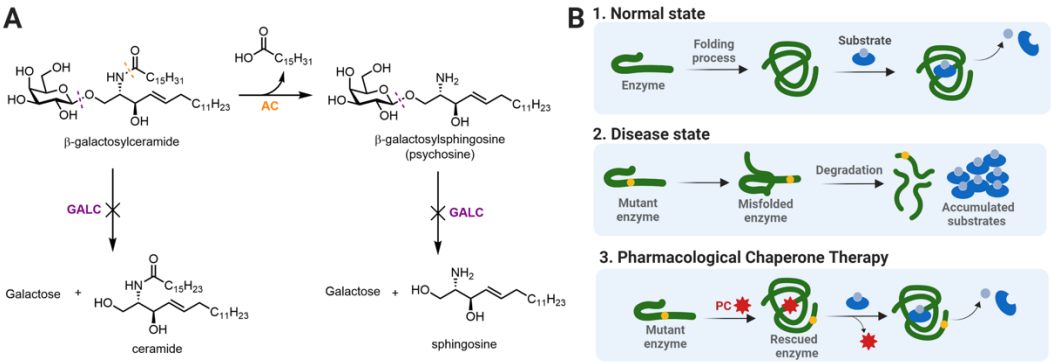

Figure 1: A) Enzymatic reaction of the hydrolysis of β-galactosylceramide by AC. B) Schematic representation of the proposed mechanism of PC action.

Acknowledgements

We would like to acknowledge the support of Research for Life for funding this travel and the Ministry of Business Innovation and employment for funding this research.

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New glycolipid synthase for alpha-galactosylceramide synthesis

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In an effort to achieve sustainable and efficient synthetic routes to glycoconjugates, glycobioengineering has focused to find out the biosynthetic catalysts present in nature, redesign and apply these enzymes in *in vitro* or/and *in vivo* biotechnological platforms.

Glycolipids are complex molecules that play important roles in cellular processes, and one such molecule, the glycosphingolipid alpha-galactosylceramide, has been found to have immunostimulatory properties, making it of interest in biomedicine [1,2]. Most glycolipids present beta glycosidic linkage but for clinical applications, the alpha configuration is especially important. To synthesize this molecule, we look for ceramide glycosyltransferase enzymes in natural bacterial producers [3,4].

Several GT4 enzymes from *Bacteroides fragilis* were evaluated, but only one, BF9343_3149, exhibited glycolipid synthase activity [5,6]. This non-processive glycosyltransferase prefers UDP-Gal as a donor substrate, and its maximum activity was observed at pH 7.3 and around 30-35°C [6]. Unlike other GT4 enzymes, it does not require metal cations for activity, but Zn^{2+} can inactivate it. The enzyme works best when the ceramide lipid acceptor is solubilized with BSA, but not in mixed micelles, and the presence of anionic lipids does not increase activity, as in other membrane-associated glycolipid synthases [6].

Acknowledgements

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Production of lacto-*N*-biose I using the extract of bifidobacterial cells

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Lacto-*N*-biose I (LNB) is the building block of the major type I oligosaccharides contained in human milk oligosaccharides. Bifidobacteria, particularly those present in the intestine of infant, have a unique metabolic system that selectively and efficiently utilizes type I oligosaccharides to increase the intestinal occupancy in breast-fed infants. Furthermore, the bifidobacteria-promoting effect of LNB has drawn attention as a prebiotic, hence, its use as a food ingredient has expected.

We succeeded in producing 200 g/L of LNB using sucrose and *N*-acetylglucosamine in a multi-enzyme reaction that takes advantage of the mechanism by which bifidobacteria utilize LNB to produce LNB. However, the commercialization of LNB production has not progressed due to the low consumer acceptance of recombinant enzymes prepared from genetically modified *E. coli*. Therefore, we developed a method to produce LNB using wild-type enzymes present in the bifidobacterial extract.

In addition to the four enzymes necessary for LNB production, the bifidobacteria extract contains other components. Among these, adenosine triphosphate, phosphoglucomutase, fructose 6-phosphate phosphoketolase, and glycogen phosphorylase are the inhibitors of LNB production. Therefore, we investigated methods such as membrane filtration, pancreatin treatment, and glucoamylase treatment to remove or suppress these inhibitors. Thereafter, all treated extracts were used for LNB production. The LNB concentration reached up to 288 mM in a 100 mL reaction, a 15-fold increase in LNB productivity compared with untreated extracts.

Acknowledgements

The authors appreciate Morinaga Milk Industry Co., Ltd. for the donation of the cells of Bifidobacterium species containing the enzymes.

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The Myrosinase-Glucosinolate reaction as a bioconjugational tool to prepare neoglycoproteins

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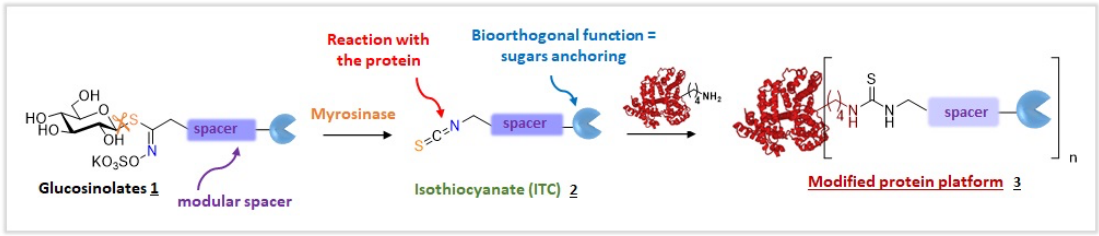
Multivalency is widely observed in Nature in glycan-proteins recognition to counterbalance the rather weak association constants of carbohydrate with their receptors (lectins for example). This strategy has been exploited by chemists to design and synthesize novel efficient carbohydrate-based ligands. Indeed, multivalent presentation of the ligands usually leads to significant enhancements in terms of affinity and selectivity and also induces specific supramolecular arrangement on the cell surface which may be critical for recognition.[1]

The central platform, from which multiple carbohydrate elements are displayed, influences the size and the shape of the multivalent ligand. Proteins, which have the advantages of being water-soluble, are commonly used as carriers for the multivalent presentation of glycans with valencies around 15 to 20. The resulting (semi)synthetic ligands are called *neo*glycoproteins, with respect to naturally occurring glycoproteins, and have been used for many years as probes for carbohydrate-proteins interactions.[2] We have recently developed a safe and biocompatible isothiocyanate-based conjugation process that relies on the *in situ* enzymatic generation of the reactive isothiocyanate species **2** from stable water-soluble synthetic glucosinolates precursors **1** by action of a highly specific β-thioglucoside hydrolase, namely myrosinase.[3]

The functionalized proteins **3** can then serve as platforms to anchor well-defined monosaccharides or synthetic oligosaccharides fragments to build high valency *neo*glycoproteins with a specific macromolecular architecture.[4] Thus, we would like to present here our recent results around the chemical modification of native proteins using an enzymatically triggered bioconjugation process.



Functionalization of native proteins using the myrosinase-glucosinolate reaction

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A building block tetrasaccharide as precursor to the RUs of prevalent *Shigella flexneri* serotypes

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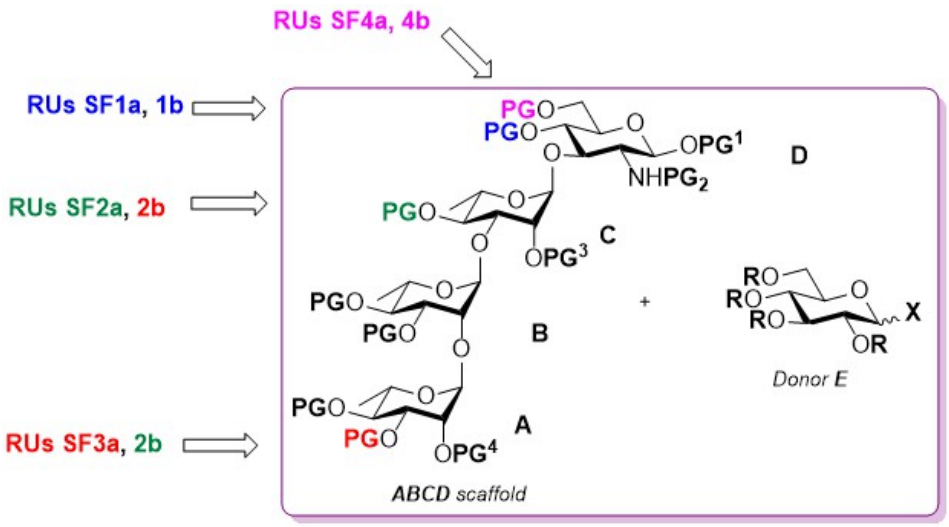
S. flexneri, a Gram-negative enterobacterium, is the main causative agent of the endemic form of shigellosis, a diarrheal disease of high prevalence in children under five in developing countries and a prime target for vaccine development. The O-antigen (O-Ag) part of the bacterial lipopolysaccharide (LPS) is considered to be the major target of the immune response against reinfection. Most *S. flexneri* serotypes exhibit closely related O-Ags. They share a common backbone featuring a unique repeating unit (RU) made of a *N*-acetyl-D-glucosamine and three L-rhamnose residues (**ABCD**) linked to one another by 1,2-*trans* glycosidic linkages. Structural diversity reflecting serotype specificity derives from the site-selective O-acetylation and α-D-glucosylation of the **ABCD** tetrasaccharide.

The identification of oligosaccharides acting as functional mimics of the O-Ags characterizing the most predominant *S. flexneri* serotypes (1a, 1b, 2a, 2b and 3a) is of interest as part of ongoing development toward a broad coverage synthetic carbohydrate-based *Shigella* vaccine. Going beyond original achievement, this communication reports an original concept whereby key pentasaccharide building blocks featuring serotype-specific substitutions are built from a single orthogonally protected tetrasaccharide. A three-step strategy was implemented : 1) synthesis of a fine-tuned core **ABCD** building block featuring suitable orthogonal protecting groups at all substitution sites ; 2) selective unmasking, on demand, of selected hydroxyl groups to provide ready-for-modification well-designated tetrasaccharide acceptors ; 3) controlled 1,2-*cis*chemical glucosylation of the resulting **ABCD** acceptors to provide fully protected pentasaccharides representative of the RUs of the selected *S. flexneri* O-Ags. Chemical chain elongation at either end post α-D-glucosylation will generate the required panel of serotype-specific oligosaccharides as potential haptens for vaccine design against shigellosis.



Effects of sialylation on human serum AAG-drug interactions assessed by ITC

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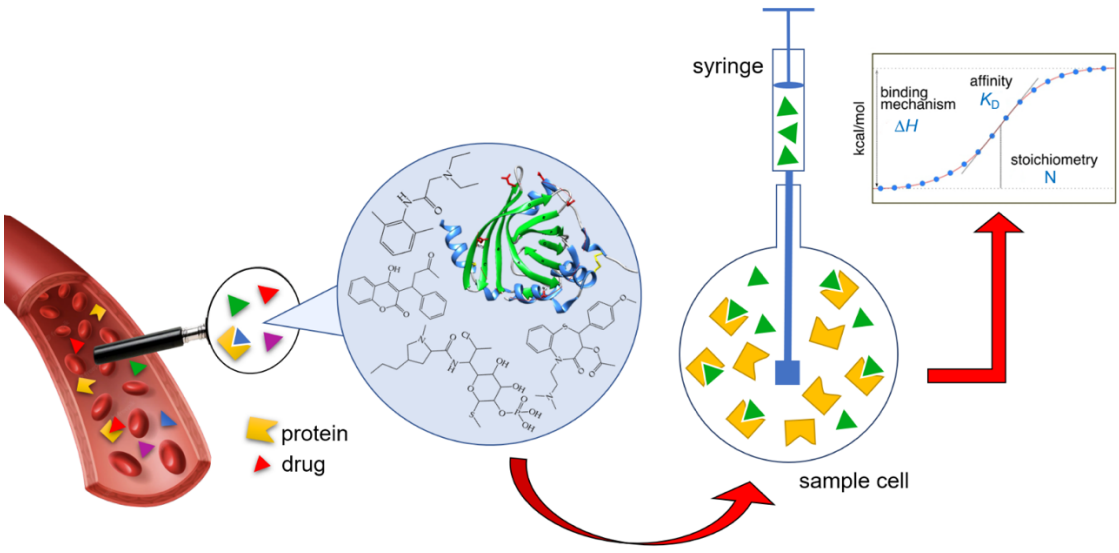
Human serum alpha-1 acid glycoprotein is an acute-phase plasma protein involved in the binding and transport of many drugs, especially basic and lipophilic substances. It has been reported that the sialic acid groups that terminate the N–glycan chains of alpha-1 acid glycoprotein change in response to certain health conditions and may have a major impact on drug binding to alpha-1 acid glycoprotein. The interaction between native or desialylated alpha-1 acid glycoprotein and four representative drugs-clindamycin, diltiazem, lidocaine, and warfarin-was quantitatively evaluated using isothermal titration calorimetry. The calorimetry assay used here is a convenient and widely used approach to directly measure the amount of heat released or absorbed during the association processes of biomolecules in solution and to quantitatively estimate the thermodynamics of the interaction.

The results showed that the binding of drugs with alpha-1 acid glycoprotein were enthalpy-driven exothermic interactions, and the binding affinity was in the range of 10^{-5} – 10^{-6} M. Desialylated alpha-1 acid glycoprotein showed significantly different binding with diltiazem, lidocaine, and warfarin compared with native AAG, whereas clindamycin showed no significant difference. Therefore, different degree of sialylation may result in different binding affinities, and the clinical significance of changes in sialylation or glycosylation of alpha-1 acid glycoprotein in general should not be neglected.



Potential clinical significance of protein sialylation on drug binding could contribute to the development of personalized medicine.

Linker, loading, reaction scale - influence on automated glycan assembly

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Automated glycan assembly (AGA) allows for fast synthesis of well-defined oligo- and polysaccharides.[1] Recently, the implementation of new synthetic strategies[2] as well as technological improvements[3] permitted access to highly complex carbohydrates.[4] Still, variations in yields are not always ascribable to the AGA process, with structures assembled in high purity, but isolated in relatively low yields. Herein we analyzed how parameters connected to the solid support (i.e. linker type, resin loading, reaction scale) affect the productivity of AGA (Fig.1). While loading and reaction scale did not significantly influence the AGA outcome, the chemical nature of the linker dramatically altered the isolated yields. This systematic study identified that the major determinants of AGA yields are cleavage from the solid support and post-AGA purification steps. Future efforts need to focus on the development of new linkers and the implementation of post-AGA manipulation steps on resin.

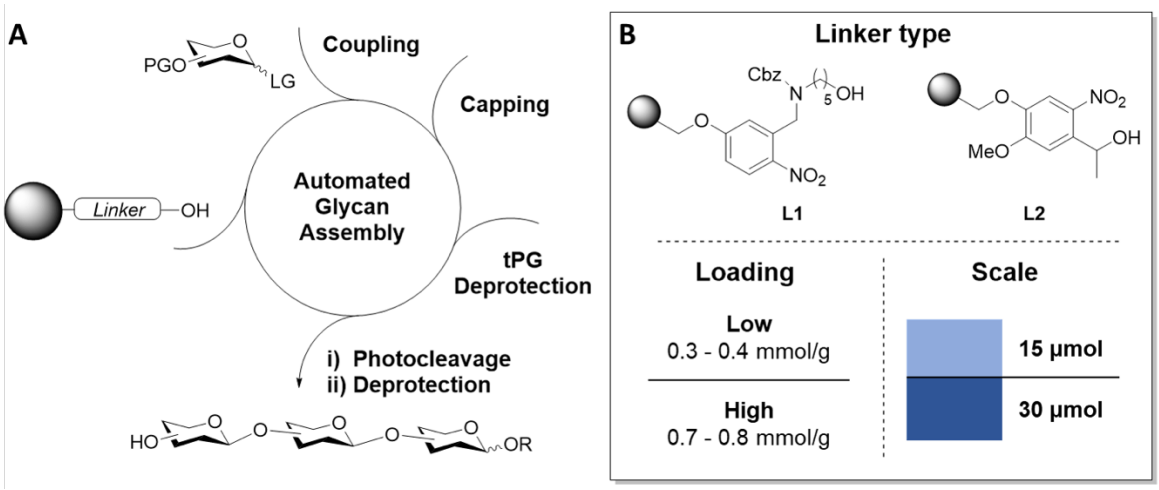

Figure 1. Schematic representation of the AGA process (A). Variables that can affect the AGA outcome

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A short synthetic approach towards isoiminosugars

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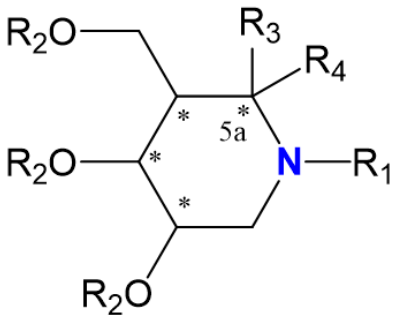
Isoiminosugars (**A**) are sugar analogues in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group. In general, these compounds are selective and highly potent inhibitors of their corresponding β-glycosidases. Moreover, selected *C*-5a-chain branched derivatives of this compound class, e.g. *C*-5a-chain extended derivatives of 4-*epi*-isofagomie (**1**) have been proven as highly potential pharmacological chaperones for the treatment of GM1 gangliosidosis [1,2], clearly rivalling any chemical chaperon published to date. As a matter of fact, the indicated structural characteristics remain to challenge the synthesis of isoiminosugars (**A**). However, valuable synthetic strategies towards this compound class have been reported [3-6].

In context with our interest in the advanced design and synthesis of such structures, we have found a novel, efficient and concise synthetic approach towards isoiminosugars (**A**). This method can be applied on different configurations and allows variations in the reaction sequence, opening the avenue to various modifications in the substitution pattern. Herein, synthetic details as well as the scope and limitations of this approach will be presented.

ISOIMINOSUGARS (**A**)



4-*epi*-isofagomine (**1**):
D-*galacto* configurated,
$R_1 = R_2 = R_3 = R_4 = H$

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From glycals to carbohydrate-tethered 2,5-disubstituted pyrazines as potential hypoglycemic agents

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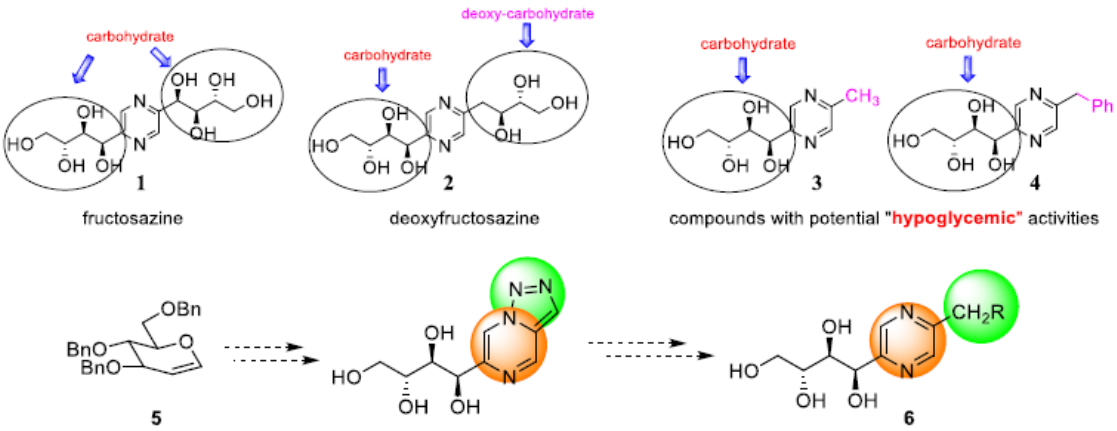
Fructosazine (FZ) **1** and deoxyfructosazine (DOF) **2** belong to the class of molecules containing a pyrazine structure tethered to a sugar in its open chain form and are very useful chemicals with varied applications, such as flavours in tobacco industry, treatment and prevention of diabetes (type II), resistance of cancers, treatment of immunological and inflammatory disease, reagents for DNA strand cleavage. Certain derivatives of pyrazines such as **3** and **4** have been identified as molecules with potential hypoglycemic activities. Though quite a few procedures are available for the synthesis of FZ and DOF, synthetic methods for making derivatives such as **3** and **4** are limited. To our knowledge, there is only one synthetic route available which involves the deoxygenation of the corresponding *N*-oxides. As of now, no synthetic strategy that provides access to a library of 2,5-disubstituted fructosazine containing a sugar moiety is available in literature.

Recently we have developed a practical access to the synthesis of a variety of carbohydrate-tethered 2,5-disubstututed pyrazines **6** through a sequence of novel organic transformations starting from readily available 3,4,6-tri-*O*-benzyl-D-glucal **5**. The details of the work will be presented.



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β-Galactosidase BgaD isoforms: comparison of the structure and functions

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β-Galactosidases catalyze the cleavage of lactose, but they can also use lactose as a glycosyl donor and acceptor and synthesize galactooligosaccharides. β-Galactosidase from *B. circulans* ATCC 31382 (BgaD) is unique due to its high synthetic potential. Four isoforms of this enzyme are formed by truncation of the C-terminal peptide by endogenous proteases [1]. The isoforms differ in size (A – 189 kDa, B- 155 kDa, C – 135 kDa, D – 92 kDa), structure, and transgalactosylation activity. In our previous study, recombinant β-galactosidase from *Bacillus circulans* isoform A (BgaD-A; EC 3.2.1.23) was produced and its synthetic potential was studied through mutagenesis [2]. To further explain the behavior, we crystallized the BgaD-A isoform. So far, only isoform D has been crystallized by Ishikawa et al [3]. Structural analysis of BgaD-A isoform by cryo-EM revealed new insights that are important for understanding the catalytic properties. The major difference between BgaD-A and BgaD-D is the flexible Big-4 domain, which is absent in BgaD-D. This domain is adjacent to the active site and hinders the binding of longer glycosyl substrates in BgaD-A. We confirmed this hypothesis by reactions with both isoforms under the same conditions, using lactose as donor and acceptor. BgaD-D produced higher amounts of more diverse mixtures of galactooligosacharides.

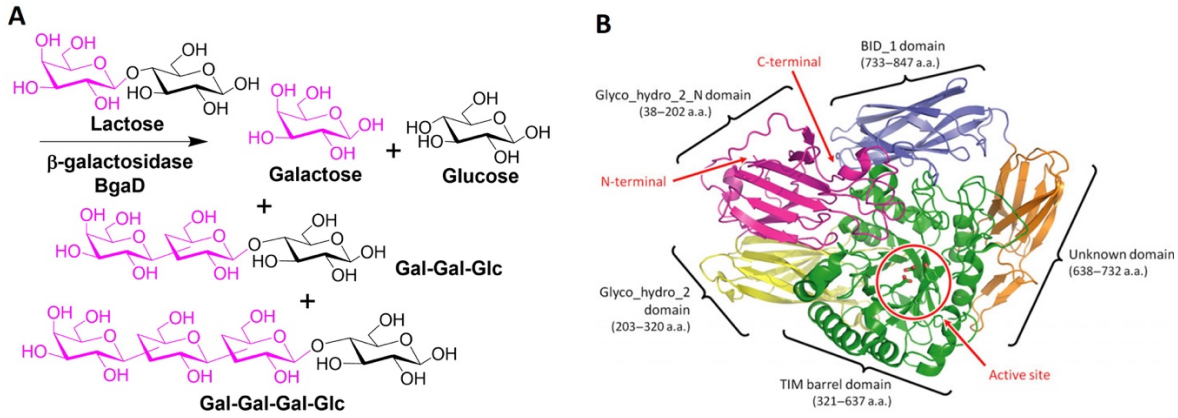

Figure. A. Synthesis of galactooligosacharides. B. Crystal structure of the β-galactosidase from B. circulans isoform D (BgaD-D), PDB code: 4YPJ [3].

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N-glycan profiles of acid alpha-glucosidases produced in transgenic rice cell suspension cultures

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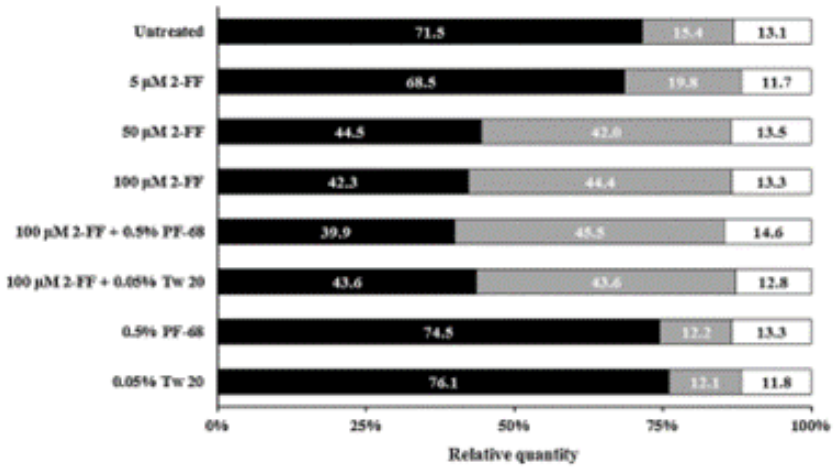
Recombinant human acid alpha-glucosidase (rhGAA) from Chinese hamster ovary cells is the only approved treatment for patients with Pompe disease.

In this study, rhGAAs were produced in transgenic rice cell suspension cultures under eight different conditions; untreated, 5 µM of 2-fluoro-L-fucose (2-FF), 50 µM of 2-FF, 100 µM of 2-FF, 100 µM of 2-FF + 0.5% Pluronic F-68 (PF-68), 100 µM of 2-FF + 0.05% Tween 20 (Tw 20), 0.5% PF-68, and 0.05% Tw 20. The *N*-glycans of eight rhGAAs were analyzed using liquid chromatography and tandem mass spectrometry. The relative quantity (%) of each glycan was obtained from the corresponding UPLC peak area per the sum (100%) of individual UPLC peak area. Fifteen *N*-glycans, comprising seven core-fucosylated glycans (71.5%, sum of each relative quantities) that have immunogenicity-inducing potential, three de-core-fucosylated glycans (15.4%), and five non-core-fucosylated glycans (13.1%), were characterized with high mass accuracy and glycan-generated fragment ions. The increases or decreases of relative quantities of each glycan from seven rhGAAs were compared with those of untreated control. These results indicate that the relative quantity of each glycan of rhGAA produced in rice cell suspension cultures is significantly affected by their culture condition.

This study performed the comparison of the *N*-glycan profiles of rice cell-derived rhGAA to identify the core-fucosylated glycans using tandem mass spectrometry and will provide useful insights for the development of plant-derived biotherapeutics focused on *N*-glycans.



The sum of relative quantities of the N-glycans of rhGAA.

Synthesis and biological evaluation of novel xylofuranosyl nucleoside phosphonates

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Nucleoside and nucleotide analogs are important group of molecules in medicinal chemistry owing to their ability to display a variety of biological activities. Their best-known therapeutic applications are in the field of anticancer and antiviral drug research, with several approved molecules approved as drugs acting as nucleic acid antimetabolites.[1,2] Their antimicrobial potential has also been reported in the literature, indicating that nucleos(t)ide-like structures may target various microbe cellular processes. and therefore be exploited towards new agents with unique mechanisms of action to circumvent antimicrobial resistance.[3]

Within our interest in the development of novel nucleos(t)ide analogs of potential therapeutic interest, [4,5] in this communication we report on the development of novel potentially bioactive nucleotide analogs based on an 3-*O*-dodecyl xylofuranyl unit and containing a phosphonate group. Motivation for their synthesis arose from the significant antiproliferative activities exhibited by previously reported related azido nucleosides from our group.[4] For their access, diacetone-D-glucose was used as starting material and key synthetic steps included sugar iodination, Arbuzov reaction or purine/pyrimidine N-glycosylation. The compounds were further studied for their antiproliferative effects on a panel of cancer cells and for their antibacterial activities on Gram-positive and negative bacterial pathogens.

Herein both the results of the synthetic work and those of the bioactivity screening will be disclosed.

Acknowledgements

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Cu(I) catalyzed stereoselective synthesis of deoxy glycosides on electron-poor glycal systems

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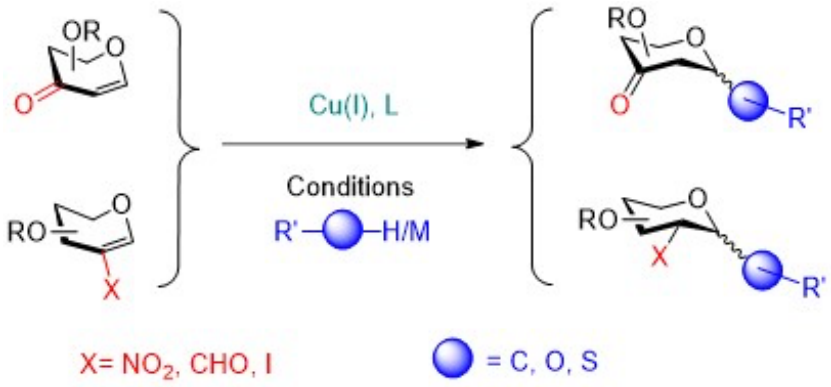
Deoxysugars play a crucial role in as key components of many natural products, such as glycoproteins, bacterial endotoxins, and secondary metabolites. Research into the functions of deoxysugars has revealed a fascinating array of diverse roles for these compounds.

Glycals have been identified as an effective starting material for synthesizing deoxyglycosides[1]., various direct glycosylation techniques have been developed that utilize glycals to produce 2-deoxy and 2-substituted-deoxy sugars. More recently modified glycals containing electron withdrawing groups, such as 2-deoxy nitro or 2-deoxy aldehyde or 2-deoxy sulfonyl or 3-keto moieties (scheme 1) have been identified as interesting scaffolds for the synthesis of glycoside analogues. To carry out glycosylation on these deactivated enol ether-containing systems, various methods have been explored, including lewis acid or base catalyzed glycosylation, transition metal catalysed or organocatalyzed glycosylations[2].

In this report, we describe a Cu(I)-catalyzed strategy that enables the use of modified electron-deficient glycal systems to generate C-, O-, and S-glycosylated products with moderate to high yields and high to complete stereoselectivity. The novel deoxy-analogues can be further modified upon removal of the electron withdrawing groups or their further functionalization to generate a range of naturally occurring glycans or glycomimetics that can be used to study biological pathways or even as potential drug candidates.



Graphical Abstract

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Neolectins, towards new tools for selective sugar targeting

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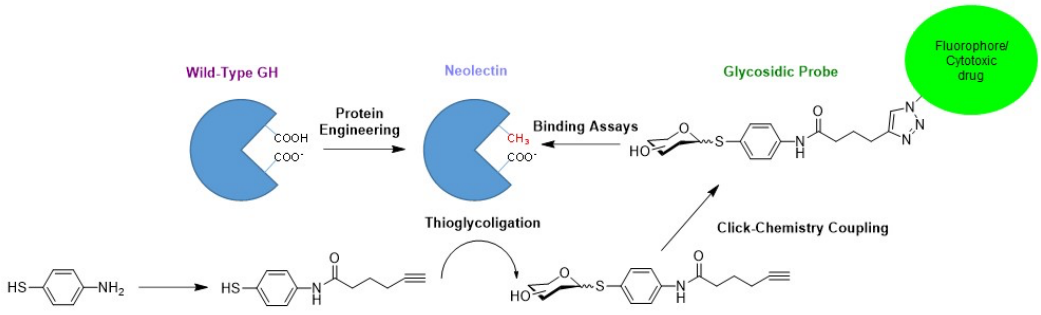
Lectins are carbohydrate-binding proteins specific for sugar molecules incorporated in glycosides or glycans at cell surface, playing a critical role in recognition processes at cellular and molecular levels. Engineering of lectins has been a challenge for many years. In our group, a synthetic lectin has recently been developed by engineering a galactofuranosidase, able to recognize and hydrolyse the galactofuranose entity, by removing its catalytic activity and retaining its sugar binding ability, resulting in a "neolectin".[1] Based on this result, we aim at developing a library of neolectins, by selecting glycoside hydrolase selective for targeted carbohydrate structures and turning them into corresponding "neolectin" by site-directed mutagenesis. However, to determine their binding affinity and sugar selectivity, a matching library of glycosides has to be synthesized to serve as chemical probes for *in-vitro* assays. In this context, an original biocatalytic approach has been chosen as the engineered neolectins are also thioglycoligase able to catalyze the formation of the corresponding *S*-glycosides.[2,3] Thus, the neolectins to be assayed as sugars receptors will also serve as biocatalysts to generate their own dedicated chemical probes. Several examples of chemo-enzymatic synthesis of such *S*-glycosides bearing either a fluorescent moiety or bioorthogonal functions for subsequent coupling will be presented.



Acknowledgements

The present work is supported by the grant of the Université of Orléans and MESRI (Ministère de l'Enseignement supérieur et de la Recherche).

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Exosomes as potential biomarkers for prostate cancer diagnosis

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Cancer is among leading causes of deaths worldwide. Prostate cancer (PCa) is one of the most common type of cancer diseases in men and one of the most frequently diagnosed cancer in more than half of the countries of the world [1]. Early diagnosis remains one of the most important factors of a successful treatment. Since PCa diagnostics lacks sensitivity and specificity, liquid biopsy is taking on an increasingly important role in early diagnosis research, detecting cancer-specific biomarkers in body fluids. Aberrant glycans attached to these markers are a frequent sign of tumour progression. Several predictive and prognostic biomarkers are already known today, and their serum levels can be analysed. The problem is elevated serum levels of these biomarkers lack sensitivity. Extracellular vesicles, especially exosomes, are considered as a potential biomarker for cancer diagnostics [2]. Exosomes, as naturally produced nanoparticles, are constantly released by the cells and tissues into various body fluids. They have the same topology as the parental cell and are enriched in different proteins, lipids, glycoconjugate and nucleic acids [3]. This work is focused on the analysis of exosomes isolated from benign prostate epithelial *RWPE1* cell line and cancerous prostate epithelial *22Rv1* cell line. The storage conditions, stability, production rate and mode of analysis of isolated exosomes are investigated in the experiments.

The authors wish to acknowledge the financial support received from the Slovak Research and Development Agency APVV 21-0329.

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2-Acetamido-2-deoxy-D-iminosugar *C*-glycosides: recent synthetic approaches and perspectives

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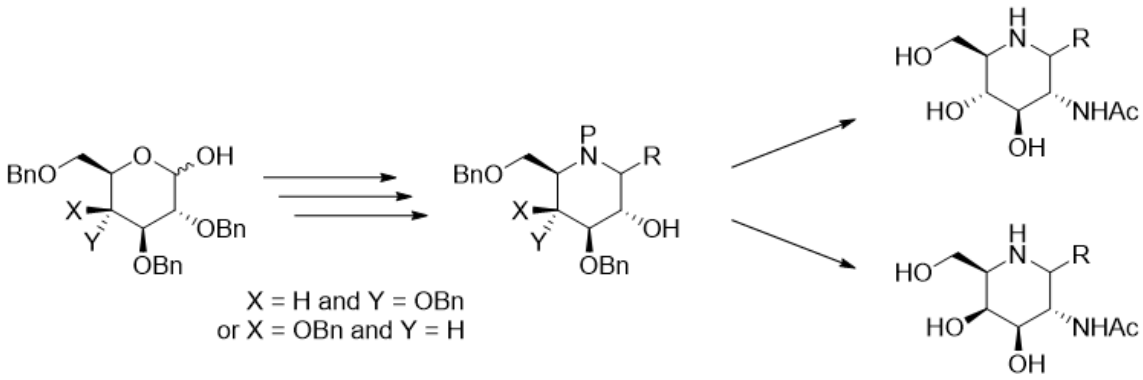
Whereas iminosugars are ranking among the most powerful glycosidase inhibitors *[1]*, their promising *C*-glycoside derivatives have not yet shown their full potential as glycosidase or glycosyltransferase inhibitors.

One of the main drawbacks associated with iminosugar-*C*-glycosides is their multistep sequence synthesis. This is even more obvious with 2-acetamido-2-deoxy-D-iminosugars, due to the presence of the sensitive NHAc moiety.

Because of the key biological role of GlcNAc and GalNAc and the valuable potential of relevant iminosugar analogues able to interfere with enzymes of high therapeutic interest *[2]*, our group is interested in developing new access to this class of compounds.

Since the first syntheses of GlcNAc and GalNAc homoiminosugar analogues *[3]*, we have shortened the synthetic sequences and enlarged molecular diversity of iminosugar-*C*-glycosides leading to L-derivatives *[4]*. These results open new perspectives in the mimicry of GlcNAc and GalNAc-derived glycoconjugates. Our last results in this field will be presented.



General strategy to access 2-acetamido-2-deoxy-D-iminosugar-C-glycosides

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Pneumococcal serogroup 10 CPS: conformational rationalization of immunological cross-reactivity

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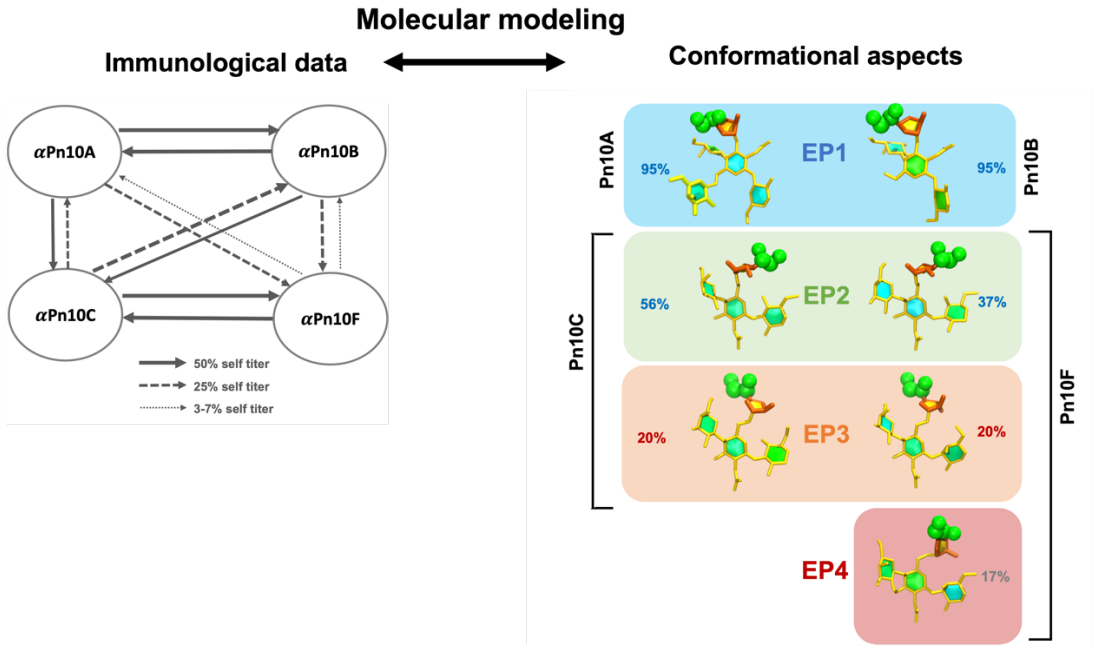
Molecular modeling can be used to correlate the conformational features of carbohydrate antigens with data on serotype cross-protection produced by immunological studies, revealing the important key epitopes for serotype cross-protection. *Streptococcus pneumoniae* is an encapsulated gram-negative bacterium and an important human pathogen responsible for significant disease and mortality in children under five. The streptococcal capsular polysaccharide (CPS) is essential for virulence and an important target antigen for vaccines. CPS structures of *S. pneumoniae* serogroup 10 are highly conserved, differing only in a 2- or 4-linked Rib-ol-5P backbone linkage and the presence/location of βDGal*f* and βDGal*p* side groups on a branching βDGalNAc residue. However, despite this similarity, serological data from immunological studies report complex, asymmetrical cross-reactivity between the four main constituents of the serogroup: Pn10A, Pn10B, Pn10C and Pn10F. Our conformational modelling of serogroup 10 identified four distinct conformational epitopes focused on an immunodominant βDGal*f* side group. These distinct epitopes provide a rationalization of the observed complex asymmetric cross-reactivity, and thus inform the design of the next generation of vaccine.



S. pneumoniae serogroup 10 rabbit antisera (α) cross-reactivity trends and representative conformational epitopes

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N-glycoproteomics at the cell surface

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Cell surface glycans are essential in establishing cell communication, adhesion, and migration. Interestingly, glycosylation changes in cancer cells, making carbohydrate antigens compelling targets for cancer diagnosis and therapy. However, because it remains challenging to obtain cell surface-specific information on glycoconjugate structures, it is unclear to what degree the observed cancer-associated glycan changes are related to cell surface glycans or rather biosynthetic intermediates present inside cells. Obtaining this information is essential for unraveling the functional role of glycans and for exploiting them as clinical targets.

To specifically analyze the N-glycoprotein forms expressed at the cell surface, we developed a mass spectrometry (MS)-based method for the sensitive analysis of cell surface enriched glycoproteins. Using human skin keratinocytes as a model system, we identified and quantified the site-specific N-glycosylation of hundreds of surface glycoproteins. This approach allowed us to study the glycoforms present at the functional relevant cell surface, omitting immaturely glycosylated proteins present in the secretory pathway. Furthermore, natural simplification of the sample was obtained by excluding non-glycosylated proteins from e.g., the nucleus and cytoplasm, allowing a deeper investigation of the glycoproteome.

With this approach, we also compared N-glycosylation sites of proteins expressed both on the cell surface and in a total cell lysate. The analysis showed profound differences in glycosylation between the two subcellular components.

Novel bioactive triazole- and guanidine-containing xylofuranosyl isonucleoside analogs

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Nucleoside and nucleotide analogs occupy a distinctive place in (bio)organic and in medicinal chemistry due to their propensity to interfere with nucleos(t)ide-dependent biological events that are crucial for life as well as for the progress of various diseases. Their therapeutic interest is demonstrated by the various examples of such compounds approved as anticancer and antiviral drugs [1], while their antimicrobial potential has been well reported [2].

Approaches for the design of nucleos(t)ide analogs include simple modifications at purine, pyrimidine or at ribose/2-deoxyribose moieties, the use of other nitrogenous heteroaromatic systems or other glycosyl units, the inclusion of phosphate group mimetic motifs or modification on the type or location of the bond connecting nucleobase and sugar.

In this communication the synthesis and biological evaluation of a variety of 5'-isonucleoside analogs constructed on xylofuranosyl templates and comprising a 1,2,3-triazole moiety and/or a guanidine group is reported. The triazole motif was envisaged as a surrogate of a nucleobase and was also connected to a phosphonate, phosphoramidate, or a phosphate moiety to establish new potential and rather stable neutral mimetics of the diphosphate system. The synthetic methodologies used azido xylofuranoses as precursors and employed key steps such as azide-alkyne 1,3-dipolar cycloaddition, phosphorylation, Arbuzov reaction, N-glycosylation, or guanidinylation.

From the molecules subjected to biological assays, some showed significant inhibition of acetylcholinesterase, potent antiproliferative activity in a breast cancer cell line or potent effects against the Gram-positive bacterial pathogen *Streptococcus pneumonia*, with activities comparable to those of reference drugs.

We thank FCT for funding: grant CEECIND/03881/2018, project EXPL/MED-QUI/1017/2021, projects UIDB/00100/2020, UIDP/00100/2020 and LA/P/0056/2020, UIDP/04378/2020, UIDB/04378/2020 and LA/P/0140/2020.

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Multivalent glycosidic vectors for the modulation of the immune system

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Among the different breast cancer types, the triple negative breast cancer (TNBC) is the most difficult to treat and to recover from [1]. Cancer immunotherapy is nowadays a consolidated strategy and Tumor-Associated Carbohydrate Antigens (TACAs) are used to develop therapeutic cancer vaccines (CVs). In designing a potential TACA-based CV it must be considered that saccharidic antigens suffer from a reduced metabolic stability *in vivo* and a poor T cells-dependent immunogenicity, which compromise a strong immune response, crucial for a promising CV. To overcome these issues TACA analogues can be designed to mimic the native antigens and to ensure a better stability and immunogenicity [2].

Over the past two decades, saccharidic structures mimicking the well-established MUC-1 TACAs, were successfully developed in our group.[3] To overcome an intrinsic low immunogenicity TACAs are generally covalently linked to immunogenic proteins; novel immunogenic vectors have recently been exploited in vaccine assembling to deliver and efficiently present TACAs. In this communication, we present the preliminary results obtained in the development of TNBC vaccine candidates which relies on a carrier-adjuvant conjugated with structurally immunogenic TACAs mimetics, specifically Tn and STn mimetics (Tn-mim and STn-mim in **Figure 1**).

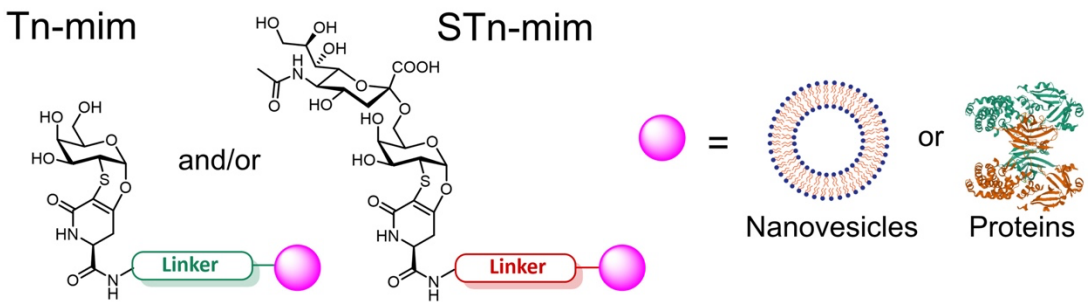

Figure 1: Carrier-adjuvant conjugated to Tn and STn mimetics.

Acknowledgements

The research leading to these results has received funding from AIRC under IG 2021 - ID. 25762 project – P.I. Nativi Cristina.

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New glycocalix[4]arenes for targeting galectins and their biodistribution

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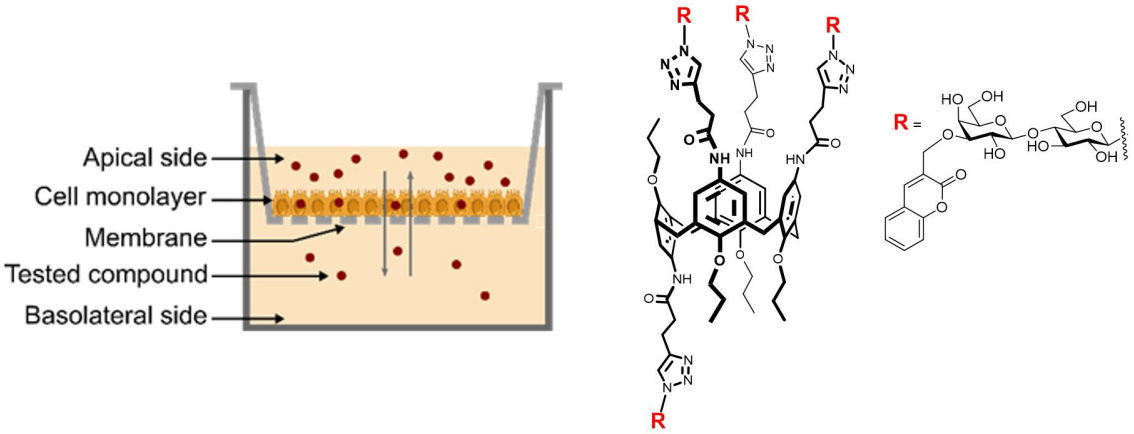
Galectins are carbohydrate-binding lectins that modulate important extra- and intracellular biological processes such as cell proliferation, adhesion, or migration. Elevated levels of galectins in serum and affected tissues are associated with various diseases, e.g., inflammation, carcinogenesis, fibrosis, and metabolic disorders [1]. Inhibition of galectins by high-affinity carbohydrate ligands, especially glycomimetics, opens new therapeutic routes. We show the synthesis of a library of glycocalix[4]arenes as ligands of prototype, chimeric and tandem-repeat galectins. The affinity of glycocalix[4]arenes to galectins was strongly steered by the linker and core structure. Moreover, they were able to induce supramolecular clustering of galectins [2]. A *partial cone* calix[4]arene carrying a novel glycomimetic, 3-*O*-coumaryllactose, proved to be promising for absorption into intestinal cells as shown in an established model of Caco-2 cell monolayer. Penetration into the intracellular compartment, otherwise hardly accessible for hydrophilic carbohydrates, may allow the interaction of glycocalix[4]arenes with intracellular targets such as galectin-3. Thus, the present glycocalix[4]arenes represent prospective tools for biomedical research.



Coumaryl-derived glycocalix[4]arene and its penetration through the monolayer of human colon epithelial cells Caco-2.

This study was supported by the project 20-00317S of the Czech Science Foundation.

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Selective involvement of UGGT2 in lipid glucosylation

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The ER is the primary site facilitating the biosynthesis of membrane-bound and secretory proteins. In addition, a large number of lipid classes are also biosynthesized at the ER. ER stress and UPR activation have been reported to be induced not only by accumulation of misfolding proteins but also by ER membrane lipid perturbation, with the latter playing a fundamental role in the pathogenesis of Alzheimer's disease, type 2 diabetes and cardiovascular disease. As a key component of the protein quality control system (PQC), Uridine diphosphate glucose:glycoprotein glucosyltransferase 1 (UGGT1) re-glucosylates misfolded glycoproteins to promote re-entry in the protein-folding cycle and curtails aggregation of misfolded glycoproteins [1,2]. The biological function of UGGT2, a UGGT1 paralogue, remains poorly understood [3,4], but has recently been proposed to be involved in the maturation of lysosomal proteins [5].

Our findings suggest that UGGT2 is largely unnecessary for efficient protein biogenesis and demonstrate that ER stress inducing phosphatidic acid derivatives with saturated fatty acyl chains are one of the physiological substrates of UGGT2, yielding lipid-raft resident phosphatidyl-β-D-glucoside. UGGT2 but not UGGT1 was protective during hypoxic stress by positively modulating autophagy and mitigating PERK-C/EBP homologous Protein (CHOP)-mediated apoptosis. Our findings are not limited to basic lipid biochemistry but provide a novel perspective on lipid induced ER stress and the up to now elusive "lipid quality control" (LQC) system.

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The first synthesis of autoinducer-2 prodrugs

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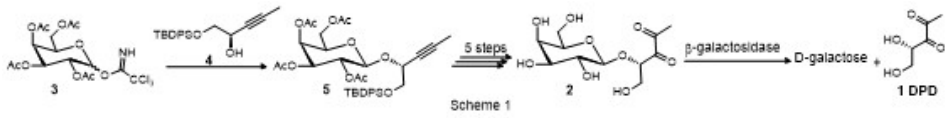
Bacteria are able to coordinate the behaviour of cell population by secreting and sensing small molecules called autoinducers.[1] This phenomenon is called quorum sensing (QS). Among the QS compounds, autoinducer-2 (AI-2) stands out, proposed to be a "universal" bacterial signalling molecule in inter-species communication. AI-2 plays an important role in controlling the colonisation and homeostasis of the gut microflora. There is an evidence that AI-2 can be used to ameliorate the effect caused by antibiotic-induced microbiota imbalances in the gut.[2] Thus, our premise is that synthetic AI-2 can help in the recovery of a healthy bacterial phyla ratio after antibiotic treatment.

To study the mechanisms involved in the response of the gut microbiota to the AI-2, it is necessary to synthesise a suitable chemical tool to deliver AI-2 unaltered to the gut. For this reason, we decided to follow the strategy of colon-specific drug delivery systems.[3] In this contribution we will discuss chemoenzymatic strategies towards linking **DPD 1** (the uncyclised precursor of AI-2) to a monosaccharide to create a prodrug **2** (Scheme 1). This prodrug will deliver **DPD** to intestine where it will be liberated by beta-D-galactosidases produced by the gut microbiota.

To verify the plausibility of this experimental approach, the development of an *in vitro* method for enzymatic hydrolysis of the glycosidic bond between the sugar and **DPD** using commercial beta-D-glycosidase will be presented together with subsequent quantification of AI-2 released using *Vibrio harveyi* bioluminescence assay.



Scheme 1

Acknowledgements

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Conformational space of glucose mono- and di-saccharides: comparison of GLYCAM06j and CHARMM36

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Carbohydrates are an important class of biomolecules, which are involved in a huge diversity of biological roles in living organisms. Typically, the pyranose ring adopts stable 4C1 or 1C4 conformations, but less predominant conformations also significantly influence the biological activity of carbohydrate-containing systems. The conformational analyses of carbohydrate molecules is of great importance to uncover the role in the related biological events.

Molecular dynamics is an important technique to study carbohydrates at atomic level. So far, the widely used and developed all-atom additive force fields for carbohydrates are GLYCAM06j and CHARMM36, with parameters available for most pyranoses and furanoses in eukaryotic. However, it is well-known that both of the two force fields over stabilize the chair conformation of pyranose molecules.

In this study, we use molecular dynamics with enhanced sampling at both molecular mechanics and quantum mechanics levels to address the force field issues. This study will be useful for future carbohydrates force fields development.

Chemical synthesis of oligosaccharides related to plant rhamnogalacturonan-I

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The plant cell wall is the outer fibre composite layer which plays an important role in plant growth as well as in plant–microbe interactions, such as the defence response against pathogens. It is a complex network to a large part composed of structural polysaccharides such as cellulose, hemicellulose and pectin. [1] Pectin comprises the domains homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II). The RG-I backbone is made up of alternating units of (1→2)-α-L-rhamnopyranose and (1→4)-α-D-galactopyranuronic acid, substituted with various L-arabinan, D-galactan, and arabinogalactan side chains. [2] Oligosaccharide fragments of RG-I represent important research tools for studying RG-I biosynthesis as well as its potential involvement in plant immune responses.

Towards the chemical synthesis of RG-I fragments, we employ a post-assembly-oxidation strategy using galactose building blocks that are later oxidized to the corresponding galacturonic acids. [3] The backbone is constructed using thioglycoside donors activated with NIS and AgOTf. Fluorenylmethoxycarbonyl (Fmoc) serves as a temporary protecting group for chain elongation, and benzoyl (Bz) protecting groups at C-6 of the galactose units provide remote participation to facilitate α-selective glycosylations before they are selectively removed and oxidized after backbone assembly. [4] The synthetic RG-I fragments will be used for glycan array-based studies with glycosyltransferases and plant immune receptors.

Acknowledgements

This work was supported by the Austrian Science Fund (FWF, grant P35404 to FP)

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Synthetic study of glycosyl-stem cell factor using β-mercapto norleucine

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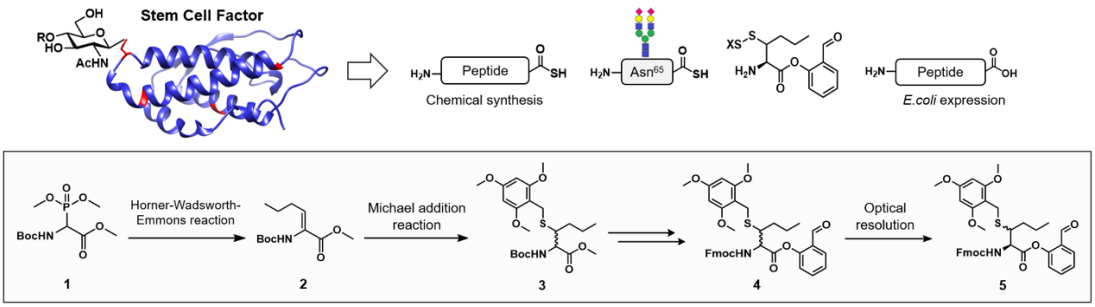
Stem Cell Factor (SCF) is a glycoprotein hormone and play important roles in hematopoiesis. SCF is known to dimerize and have asparagine-linked (N-) glycans near the dimeric interface. However, the function of these glycans has not yet been investigated. Therefore, we have synthesized homogeneous glycosyl SCF.

For the synthesis of glycosyl SCF by the convergent synthesis, we designed four building blocks: N-terminal peptide thioacid, Asn-(sialyloligosaccharide)-thioacid, β-mercaptonorleucine derivative, and C-terminal recombinant peptide. These compounds could be sequentially coupled by diacyl disulfide coupling [1], Ser/Thr ligation [2], and thioacid capture ligation [3].

First, we carried out the synthesis of β-mercaptonorleucine derivative, which can expand the choice of ligation sites. Glycylphosphonate was used for Horner-Wadsworth-Emmons reaction with butylaldehyde. The following Michael addition with a thiol derivative and protecting group manipulations were then performed. In addition, the optical resolution by chiral column successfully yielded the desired norleucine derivative having a thiol functionality at the β-position.

Furthermore, three peptide building blocks were successfully prepared by Boc solid phase peptide synthesis (SPPS) and E. coli expression system. Since all building blocks were already prepared, we examined the peptide coupling reactions to obtain the full-length glycosylated polypeptide. This presentation will describe these experiments in detail.



Strategy for the synthesis of β-mercaptonorleucine derivative

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Synthesis of β-(1→3)-glucans as a possible candidate for vaccines against *Cryptococcus neoformans*

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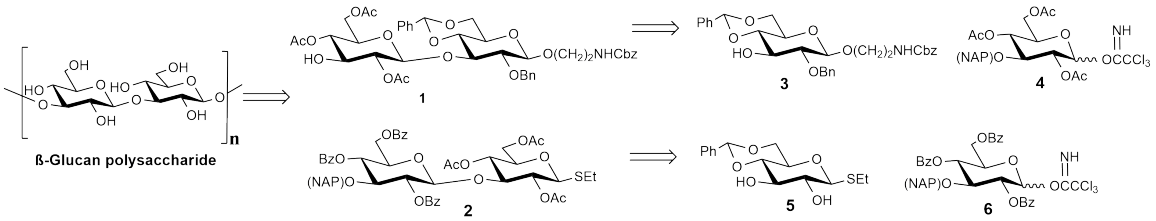
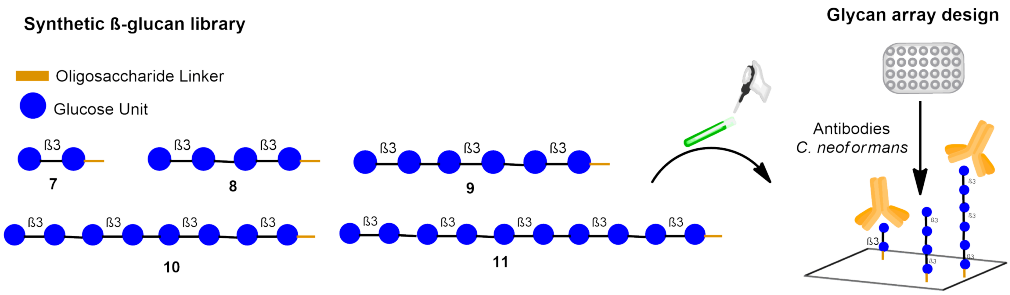
Cryptococcus neoformans, an opportunistic fungal pathogen that is ubiquitous in the environment, can cause systemic infection in the immunocompromised and it is estimated that 1 million infections occur annually [1]. *C. neoformans* is unique among pathogenic fungi in that its polysaccharide capsule is essential for virulence in mammals. The capsule and cell wall is composed of several constituents, including mannoproteins, β-glucans, galactoxylomannan (GalXM) and glucuronoxylomannan (GXM) [2]. In this context, β-glucan, which is functionally necessary for fungi and immunologically active, is an attractive target antigen [3].

That is why we proposed the synthesis of disaccharides acceptor **1** and donor **2** with the aim of obtaining different polysaccharides units of β-(1→3)-glucan, for immunological study. We synthesized disaccharide **1** derived with a spacer arm with an amino group, from precursors **3** and **4**. Donor disaccharide **2** was obtained with a similar strategy, using precursors **5** and **6**. Donors **4** and **6** were prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose.

The library of obtained β-glucan oligosaccharides of different length (**7**-**11**), will be printed to produce a glycan microarray that will be screened with sera from *Cryptococcus* infected patients to select for possible vaccine candidates.





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Towards the synthesis of defined carbohydrate-based ligands for the C-type lectin clec-2

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C-type lectin-like receptor 2 (CLEC-2) is a receptor expressed on human platelets. Platelets play an important role in several biological events such as hemostasis, inflammation, infection and immunity. CLEC-2 is activated by podoplanin, a mucin-type protein, causing platelet aggregation.[1] Another ligand that was reported to act as an agonist of CLEC-2 is fucoidan, a sulfated polysaccharide from the algae *Fucus vesiculosus*.[2]

We report on our research towards the chemical synthesis of podoplanin- and fucoidan-based ligands for studying CLEC-2 biology. The sialyl Tn (STn) disaccharide moiety of the glycoprotein ligand podoplanin interacts most closely with CLEC-2.[3] Therefore, aminoalkyl-linker-functionalized STn was prepared by enzymatic 2,6-selective sialylation of linker-functionalized *N*-acetyl-galactosamine with CMP-sialic acid. Podoplanin-based glycopeptide-ligands will be synthesized as well, starting from a galactosylated threonine building block. In this case, both the sugar and the peptide moiety will contribute to binding to the receptor.[4]

Fucoidan-based hexasaccharide ligands with alternating α1,3- and α1,4-linkages will be prepared through several rounds of glycosylation reactions using two different L-fucose thioglycoside building blocks followed by selective sulfation. The choice of the positions for sulfations are guided by the sulfation pattern of the natural fucoidan ligand for CLEC-2 from *Fucus vesiculosus*.[5] The binding between the ligands and CLEC-2 will be studied by biophysical techniques.

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One-pot biosynthesis of fluorescence lectins using a cell-free transcription-translation system

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Mushroom lectins harboring carbohydrate binding specificity are powerful tools to detect glycoconjugates with N-linked or O-linked glycans for diagnosis. However, the conventional preparation for these lectins with a chemical labeling are still a bottleneck. Here, we report one-pot biosynthesis of recombinant fluorescent lectins using a cell free transcription system with *Escherichia coli* extract. Codon-optimized genes encoding the eight different types of mushroom lectins were successfully expressed as green or red fluorescent protein-fused forms in a cell-free protein synthesis system. Although the production yields of the recombinant proteins were depended on their coding genes, the resulting recombinant fluorescent lectins are apparent homo-multicomplex proteins with different molecular sizes and pI values. In hemagglutination inhibition assay, the lectins demonstrated agglutination activities towards various glycoconjugates.

Moreover, the fluorescent lectins can be applicable to detect glycan binding specifies on using a glycan microarray. The glycan binding specificities of the florescent lectins are also useful to analyze glycan epitopes expressed in different cancer cell lines. Taken together, these results provide an efficient and optimized procedure for the high-throughput synthesis and screening of lectin coding gene products based on in vitro production of the recombinant fluorescent lectins.

Acknowledgements

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Biorefining of hemicellulosic beta-mannans and their utility as glycan donors in enzymatic synthesis

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beta-Mannans are abundant plant polysaccharides which today are underutilized. Pilot-scale recovery and purification of the main softwood hemicellulose galactoglucomannan (GGM) from a pulping stream were developed [1]. Lignin removal enhanced the yield of enzymatic GGM-conversion using Trichoderma reesei beta-mannanase TrMan5A. Several potential enzyme-generated products were demonstrated, e.g. monosugars, prebiotic oligomers [2] and activated beta-mannosides and alpha-galactosides, applicable in surface and material chemistry as monomers for synthesis of novel glyco-polymers [3-6]. We investigated the transglycosylation capacity of family GH5 beta-mannanases for the synthesis of surfactants (alkyl mannosides) and activated acrylate- or allyl- (galacto)mannosides using beta-mannans (galactomannan or GGM) as glycan donors and different alcohols or acrylates as glycan acceptors [3]. Product- and reaction-screening using mass-spectrometry, HPLC and NMR were set up. Bioinformatics and protein engineering approaches generated improved enzyme-variants [3, 4]. When using galactomannan as donor, enzyme synergy (combining TrMan5A with an alpha-galactosidase) gave improved yield [3] and demonstrated enzymatic synthesis of novel allyl- and propargyl-functionalised glycosides with applicability in further “click-chemistry” coupling [5]. Transglycosylation products were purified and analysed by NMR. Novel thermoresponsive mannoside-acrylamide co-polymers were generated from enzymatically synthesised mannoside-acrylates and acrylamide and their properties characterized [6].

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In silico virtual screening of small molecules targeting GlfT2

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Galactofuranosyltransferase 2 (GlfT 2) from *Mycobacterium tuberculosis* is a glycosyltransferase with homotetrameric structure catalyzing transfer of Gal*f* residue from UDP-Gal*f* donor to growing Gal*f* chain while creating β-(1→5) and β-(1→6) bonds between Gal*f* residues. Since GlfT2 is absent in human cells, it is an ideal candidate for development of novel tuberculosis drugs. The aim of our work was to find possible inhibitors of GlfT2 using methods of computational chemistry. Ligand structures were selected in cooperation with Dr. Sean Ekins (Collaborations Pharmaceuticals, Inc.) by virtual screening of Chembridge database. Selected 92 structures were prepared using LigPrep [1] tool and optimized by Jaguar [1,2] using DFT B3LYP-D3 method and 6-31G** basis set. Subsequently the optimized structures were docked using Glide [1,3] by Standard Precision protocol into model of GlfT2 without acceptor substrate as well as model with acceptor in position allowing transfer of galactofuranose to O5 oxygen of acceptor galactofuranose residue and second model with acceptor in position suitable for transfer targeting O6 oxygen. Best ligands were selected based on their binding affinity evaluated by means of docking score as well as other parameters including interaction with Mg^{2+} ion, catalytic Asp372 and other aspartates located in binding site.

Acknowledgements

This work was supported by the Slovak Grant Agency of Science (VEGA 2/0137/20) and the Slovak Research an Developmental Agency (APVV-20-0230).

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Lead generation for catechols as glycomimetic LecA inhibitors of *Pseudomonas aeruginosa*

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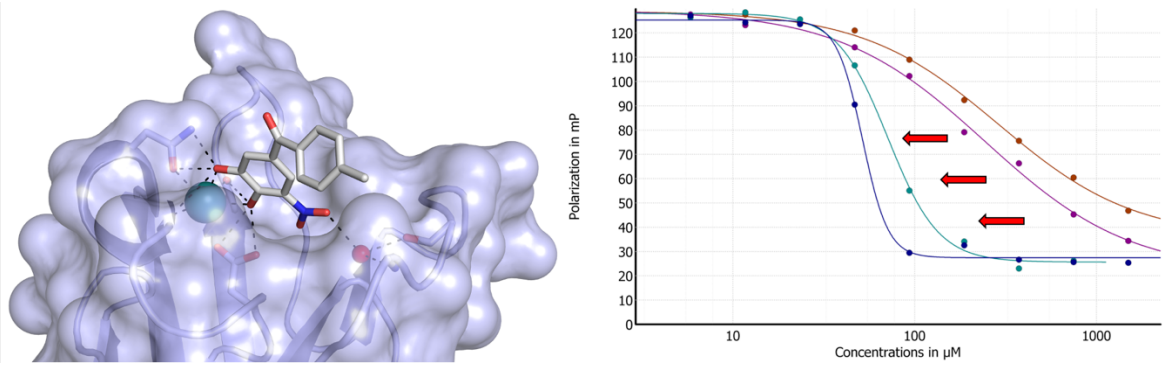
LecA is one of the lectins of *Pseudomonas aeruginosa* employed for host cell adhesion and invasion as well as biofilm formation.[1] Inhibitors of LecA therefore hold promise to synergize with antibiotics and aid in the treatment of pseudomonal infections.[1],[2]

A virtual screening followed by rigorous experimental hit conformation yielded catechols as novel glycomimetic binders of calcium-dependent lectins.[3] Here, we report on the on-going lead generation towards LecA.

Starting from the initial, fragment-like hit compounds, possessing excellent ligand efficiencies, a new frontrunner was identified (IC_{50} = 238 µM). The glycomimetic binding mode of this molecule – tolcapone, a licensed Parkinson drug marketed by Roche – was confirmed by x–ray crystallography. To gain deeper insights into the structure-activity relationship more than 3500 catechol and tolcapone derivatives of the Roche in-house library were tested experimentally at three concentrations against LecA in a competitive binding assay based on fluorescence polarization. Based on this data affinity cliffs were revealed and a set of 48 compounds was selected for further in-depth studies. The best compounds of this set reach low micromolar affinities, a 20-fold improvement compared to the initial screening hits.

This substantial increase in affinity provides a promising basis for further development of catechols as glycomimetic antivirulence drugs in light of the antibiotic resistance crisis.



Catechols are glycomimetic binders of LecA with drug-like properties

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Unreported sialylated *N*-glycans modified with *O*-acetylation of recombinant human acid α-glucosidase

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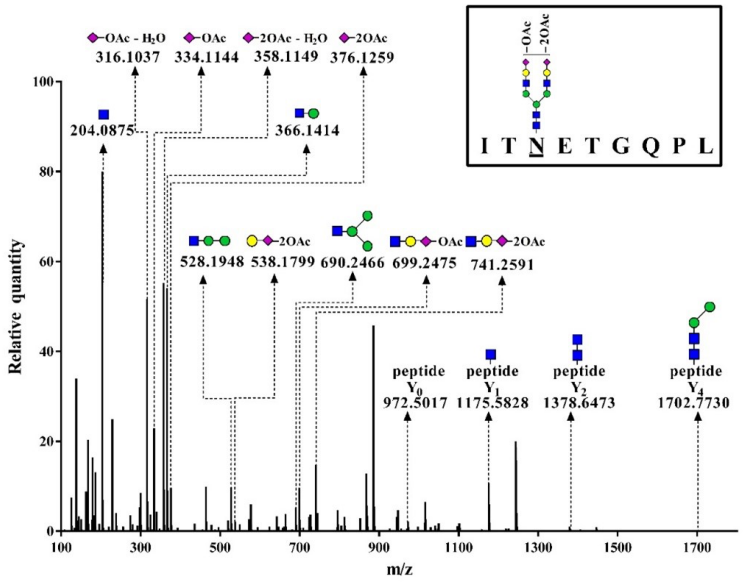
O-acetylated sialic acid (SA) attached to the *N*-glycans of therapeutic glycoproteins reportedly inhibit sialidase activity, increase protein half-life, decrease protein antigenicity, and stabilize protein conformation. Recombinant human acid α-glucosidase, Myozyme, is the only drug approved by the United States Food and Drug Administration for the treatment of Pompe disease.

In this study, structural analysis of *N*-glycans in Myozyme and the relative quantities of glycans with a focus on unreported sialylated *N*-glycans modified with *O*-acetylation were investigated using liquid chromatography (LC)-electrospray ionization (ESI)-high-energy collisional dissociation (HCD) tandem mass spectrometry (MS/MS).

The 17 *N*-glycans (6.4% of total glycans) containing mono-, di-, mono/di-, and di/di-*O*-acetylated *N*-acetylneuraminic acid (Neu5Ac) were identified. The analysis of peptides containing mono- and/or di-*O*-acetylated Neu5Ac ions sorted from all peptides using nano-LC-ESI-HCD-MS/MS confirmed six *O*-acetylation sites (Asn 140, Asn 233, Asn 390, Asn 470, Asn 652, and Asn 882), at least five of which (Asn 140, Asn 233, Asn 390, Asn 470, and Asn 652) could contribute to the drug efficacy or cellular uptake of Myozyme.

This is the first study to identify *N*-glycans containing *O*-acetylated Neu5Ac and *O*-acetylation sites in Myozyme. The results could be useful for the development of biobetter versions or glycoengineered α-glucosidase with enhanced drug efficacy or cellular uptake compared with that of the original Myozyme.



Nano-LC-ESI-HCD-MS/MS spectrum of O-acetylated glycopeptide obtained from human acid α-glucosidase.

Acknowledgements

This research was supported by the BK21 FOUR funded by the National Research Foundation (NRF) and the Ministry of Education of Korea.

Synthesis of various pseudo-glycans with a *C*-glycoside linkage by metallophotoredox cross-coupling

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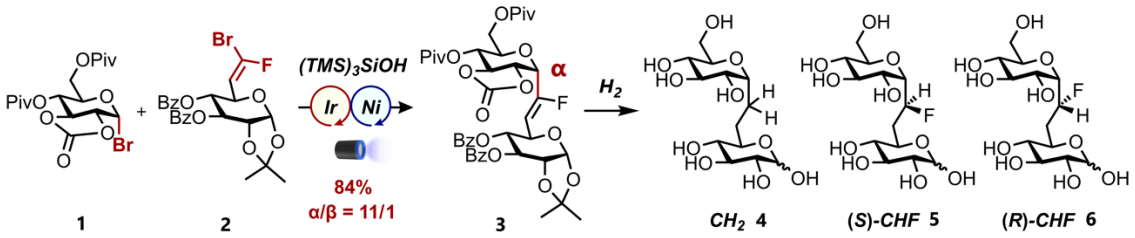
Glycan analogues with a C-glycoside linkage have resistance against glycoside hydrolases and different conformational properties from native glycans. Native O-glycoside linkage regulates glycan conformation by both steric and stereoelectronic effects (exo-anomeric effect). The simplest CH_2-linked analogues lose conformational control because of the lack of stereoelectronic effect, making their conformation more flexible than native glycans. In contrast, the conformation of CHF-linked analogues should be regulated to some extent by the gauche effect of a fluorine atom. Thus, the synthesis of three types of analogues with CH_2-, (*R*)-CHF-, and (*S*)-CHF-glycoside linkages would enable the creation of pseudo-glycans with different conformational properties as well as glycoside hydrolase-resistance, which we expected results in enhanced or altered biological activity to parent native glycans. Previously, we demonstrated that (*S*)-CHF-ganglioside GM3 analogue has exhibited superior biological activity to the native, CH_2-linked, or (*R*)-CHF-linked GM3[1]. However, the lack of a versatile synthetic method for CHF-glycosides made it difficult to apply this molecular design concept to other glycans or glycoconjugates.

In this study, we have developed a direct C-glycosylation reaction toward the efficient synthesis of CHF and CH_2-glycoside analogues. We employed the reductive cross-coupling of glycosyl bromide **1** and bromofluoroolefin **2** to furnish the disaccharide analogues **3** with fluoroalkene C-glycosides. As a result of several optimizations, we established the conditions to give **3** in high yield and stereoselectivity, by the metallophotoredox cross-coupling reaction developed by Macmillan and coworkers.[2] Furthermore, stereo- and chemoselective hydrogenation after the coupling reaction successfully produced CH_2, (*S*)-CHF, and (*R*)-CHF-linked disaccharide analogues (**4-6**). This methodology was found to be capable of synthesizing a variety of pseudo-disaccharides and pseudo-glycolipids.



✓ **α-selective C-glycosylation**
✓ **Applicable to disaccharides and glycolipids**
✓ **Stereo-and chemoselective hydrogenation**
✓ **Efficient synthesis of CH_2-, (S)-CHF- and (R)-CHF-glycosides**

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A glycochemical approach toward an active substance for fruit-body induction in *Pleurotus ostreatus*

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The fruit-body development of wood-rotting fungi is still unexplained. The hormones or external signal molecules, even though they are omnipresent in nature, have been not identified. In our trials to find chemical or natural substances that effectively stimulate the fruiting of *Pleurotus ostreatus* (an oyster mushroom) on agar medium [1-3], several effective compounds were found, including the sucrose ester of fatty acids (SE), synthetic triterpenoid glycoside (saponin), 3-*O*-alkyl-D-glucose, some glyceroglycolipid analogous compounds of GlcDAG, Glc_2DAG and ManDAG, and a commercially available glucosyl ceramide (GlcCer) of fungal origin, with methyl group at C-9 and the 4E double bond in the sphingoid base moiety.

With above findings as a background, we are now trying to isolate an endogenous active substance from *P. ostreatus*. We have successfully isolated the fungal type GlcCer from *P. ostreatus* by chromatographic fractionations of crude acetone extracts of the mycelium using silica-gel, suggested the possibility of inducing fruit-body formation in *P. ostreatus* at significantly low concentrations from the results of preliminary assay using paper disk. The GlcCer fractions were extracted from the three different development stages of *P. ostreatus*: the mycelium, primordia and matured fruit body, and most abundantly contained at the stage of primordia, just when fruit-body formation was started.

In this conference, we will discuss the potent biological role of GlcCer in terms of fruit-body formation in mushroom, referencing the first report on *Schizophyllum commune* [4].

Acknowledgements

This work was supported by JSPS KAKENHI Grant Numbers 18K19241 and 20H03051

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Naked-eye monitoring systems for carbohydrate-carbohydrate interactions

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Glycosphingolipids (GSLs) on cell surfaces aggregate laterally to form GSL-enriched microdomains. Since carbohydrate units of GSLs are prominently exposed on cell surfaces, GSL-enriched microdomains thus have densely packed carbohydrate clusters (glycoclusters) on their surfaces. It has been recognised that such glycoclusters on cell surfaces interact with other glycoclusters located at the neighbouring cell surfaces in intercellular fashions and induce various bioprocesses such as embryonic compactions and cancer metastases. We recently prepared bacterial cellulose hydrogels carrying Le^x trisaccharides, GM3 trisaccharides and Gg3 trisaccharides from commercially available *nata de coco* via $NaIO_4$ oxidation, reductive amination using propargylamine and Cu^+-catalyzed click coupling with the corresponding glycosyl azides.

Since these hydrogels have a visible scale, their homo- and hetero-assembly could be readily monitored in aqueous media without devices. This is the first example of the naked-eye monitoring system for Le^x-Le^x and GM3-Gg3 interactions, which are associated with embryonic compactions and cancer metastases. Reference experiments using similar bacterial cellulose hydrogels carrying *N*-acetyl-lactosaminides, *N*-acetyl-glucosaminides, lactosides or glucosides revealed the contributions of each carbohydrate subunit of Le^x, GM3 and Gg3 trisaccharides to their homo and hetero bindings.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP21K85298.

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Serum *N*-glycans analysis by LC-MS allows the prediction of patients' response to treatment for CD

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Crohn's disease (CD) is a type of inflammatory bowel disease that affects millions of people worldwide. As a lifelong condition, it is imperative for patients to have access to the right treatment as quickly as possible. Therefore, there is a need for reliable biomarkers to predict whether an individual patient will respond to a treatment. In this study, a comparison of serum *N*-glycan profiles between responders and non-responders to Vedolizumab (VDZ), one of the treatments for CD, was used to detect potential biomarkers for the response.

Serum samples from 58 CD patients were taken before (t1) and after (t2) initiation of VDZ treatment. After enzymatic release, the glycans were fluorescently labelled and analysed by HILIC-MS. From the relative abundances of each individual glycan, derived glycan traits were calculated by combining direct traits with shared structural similarities. Correcting for age and sex, the direct and derived traits were tested across treatment responses using logistic regression and cross-validation to determine which traits at t1 are predictive of response and traits at t2 are markers of response.

Two glycans, MAN6 (0.65 AUC) and FA2G2S2 (0.7 AUC) were higher in the responders prior to treatment and moderate predictors of response, with no improvement in a combined model. In accordance with previous studies, galactosylation levels were found to increase in responders after treatment. Overall, 17 direct traits and 4 derived traits were observed as markers of response after treatment, and collectively proved to be good predictors of response (0.80 AUC).

Mapping the miRNA Regulation of -1,2 Fucosylation

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MicroRNAs (miRNAs) are short segments of non-coding RNAs that regulate the translation of messenger RNA (mRNA) into protein. Glycogenes, i.e. the genes that control glycosylation, are highly regulated by miRNAs. The glycan motif *α*-1,2 fucose is a crucial component of Lewis^{b} and Lewis^{y} and is the defining feature of the blood group H antigen.

FUT1 and *FUT2* genes encode a Golgi stack enzyme α-1,2-fucosyltransferase that catalyzes the transfer of 1,2-linked fucose to galactose residue of glycans.

In this project, we aim to generate high throughput data that shows the regulation of *FUT1* by miRNA using miRFluR high-throughput assay. This assay utilized genetically encoded dual-colour fluorescence reporters to identify regulatory miRNAs thus generating a comprehensive map of the regulation of *FUT1* by approximately 2700 human miRNAs.

pMIR-3' UTR sensor of *FUT1* has been developed and by utilizing our high throughput assay we have identified 76 regulatory miRNAs for *FUT1*. We have validated the regulation of *FUT1* gene by these miRNAs at a protein and glycan level via western blot and lectin staining experiments, respectively in multiple cell lines. The dominant paradigm is that miRNA binding to mRNA represses protein expression. Nonetheless, this work and other ongoing and recently published work from our lab showed miRNA –mediated up-regulation of glycogenes via direct interaction. Our work overturns the current thinking of miRNA upon the regulation of glycogenes.

Variability of human alpha-1-acid glycoprotein *N*-glycome

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Alpha-1-acid glycoprotein (AGP) is a heavily glycosylated protein in human plasma and one of the most abundant acute phase proteins in humans. Glycosylation plays a crucial role in its biological functions, and alterations in AGP N-glycome have been associated with various diseases and inflammatory conditions. Still, large scale studies of AGP N-glycosylation in general population are lacking.

Using recently developed high-throughput glycoproteomic workflow for site-specific AGP N-glycosylation analysis, 803 individuals from Croatian island of Korcula were analysed and their AGP N-glycome data associated with biochemical and physiological traits.

Regression analysis revealed that 61 out of 79 AGP glycopeptides were significantly associated with sex, where the largest differences between males and females were observed in the levels of fucosylation and sialylation. While 38 out of 79 AGP glycopeptides were significantly associated with age, the strongest associations were not as strong as associations observed between AGP N-glycome and sex and the patterns of changes associated with age were not similar across different glycosylation sites.

Furthermore, regression analysis with age and sex included as covariates was performed on available biochemical and physiological traits. The results revealed strong associations between multiple AGP N-glycome traits and biochemical (triglycerides, uric acid, glucose, fibrinogen etc.) and clinical (height, weight, waist, and brachial circumference, etc.) parameters as well as smoking status.

Enzymatic glycosylation catalyzed by GH84 O-GlcNAcase using an oxazoline derivative

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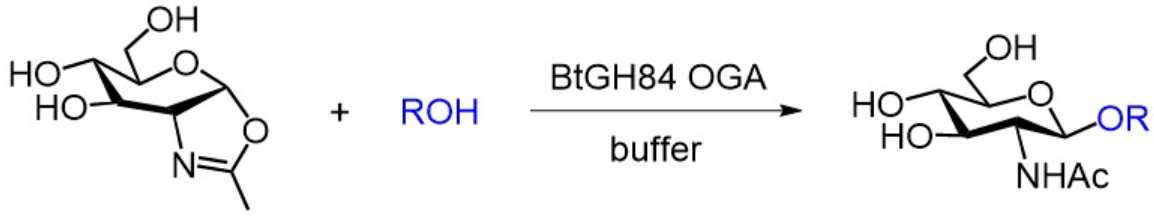
O-Glycosylated *N*-acetyl-β-D-glucosamine-selective *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.169) (OGA), belonging to glycoside hydrolase family 84 (GH84), has been found in various organisms ranging from humans and bacteria and catalyzes the elimination of *N*-acetyl-D-glucosamine (GlcNAc) from serine and threonine residues on proteins in humans. Bacterial OGA also belongs to GH84 and is a retaining glycosidase via a substrate-assisted hydrolysis mechanism through a sugar 1,2-oxazoline derivative as an intermediate. It is well known that retaining glycosidases have potential enzymatic transglycosylation. However, as far as the authors know, no transglycosylation catalyzed by GH84 OGA has been reported. In this study, we report the first enzymatic transglycosylation catalyzed by the GH84 OGA from *Bacteroides thetaiotaomicron* (BtGH84 OGA) [1].

The BtGH84 OGA-catalyzed transglycosylation using 1,2-oxazoline derivative of GlcNAc (GlcNAc-oxa) as a glycosyl donor substrate was examined. The transglycosylation product was observed in case of excess feeding of *N*-(2-hydroxyethyl)acrylamide as an acceptor substrate in a buffer pH 7.0. No glycosylation product was observed when pNP-GlcNAc was used as a glycosyl donor substrate under the same condition as that using GlcNAc-oxa. When the GlcNAc derivative with a triazole-linked acrylamide group were used as an acceptor substrate, b1,6-linked disaccharide of GlcNAc was obtained. Molecular docking simulation of the transglycosylation products for BtGH84 OGA suggests the substrate recognition around the catalytic site of the enzyme.



Scheme. Enzymatic transglycosylation catalyzed by BtGH84 OGA using GlcNAc-oxa.

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Elucidating mucinase and bacteria toxin binding specificities towards mucin *O*-glycopeptides

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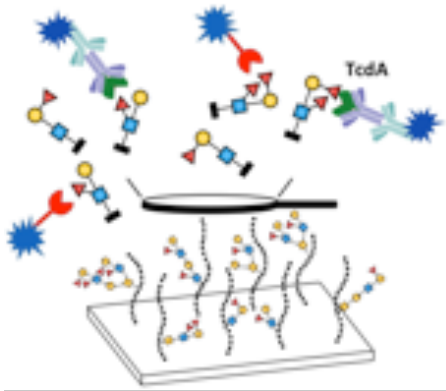
Mucins are densely *O*-glycosylated membrane-bound or secreted proteins ubiquitously found on the epithelial cell surface.[1] They are part of the innate immune system and play major roles as protective barriers to defend the host against invading pathogens.[2] However, bacteria and viruses have co-evolved with the human host and developed strategies to promote virulence for instance by adhering to carbohydrate ligands on the host cell-surface via pathogenic lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria adhesion proteins and *O*-glycans presented on mucin tandem repeat peptide backbones. Through the use of carbohydrate binding bacteria toxins such as Toxin A, interactions with glycans at the epithelial cell-surface, results in internalization of enzymes that promote virulence by modulation of the intracellular host GTPases. To enable penetration of the mucus layer and make the epithelial cell-surface accessible bacteria further secrete glycosidases and mucinases. In recent years we have through a chemoenzymatic approach prepared extensive libraries of mucin tandem repeat glycopeptides consisting of different O-glycan core structures modified with LacNAc, LacdiNAc, sialylation and fucosylation.[3-5] On Eurocarb we will present our latest results elucidating binding interactions between mucin *O*-glycopeptides and mucinase glycan binding domains as well as binding studies of toxin A originating from different pathogenic bacteria strains.



Elucidating Mucin O-glycopeptide binding partners.

This work was supported by grants from Kempe foundation, the Swedish Chemical Society, Deutsche Forschungsgemeinsch DFG (WE 4751/2-1) and Fonds der Chemischen Industrie.

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Towards catalytic aminoglycoside: probing the modification of kanamycin B at 3'- and 4'-positions

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The ongoing emergence of multidrug-resistant pathogens has become a severe clinical problem for the treatment of infectious diseases worldwide. To address this issue, we have recently set out to explore the concept of "catalytic antibiotics" as a new paradigm in antibiotics research.

Concerning the catalytic aminoglycoside, we synthesized a series of new derivatives of neomycin B in which the catalytic warhead, 1,2-diamine appendage was selectively attached at the 4'-position of ring I as a single arm to catalytically cleave the scissile phosphodiester bond of rRNA by general acid/general base catalysis. The new derivatives exhibited comparable antibacterial activity to the parent neomycin B against wild-type bacteria and were especially potent against resistant and pathogenic bacteria. However, all attempts to demonstrate cleavage of the scissile phosphodiester bond of rRNA have been unsuccessful. By using molecular dynamics simulations, we found that the designed single-arm ethylenediamine appendage was unable to efficiently cleave the scissile phosphodiester bond of rRNA.

To overcome this limitation, we decided to install the general base and general acid appendages on the aminoglycoside scaffold as two separate arms to allow efficient catalysis. Herein, we describe our efforts in probing potential sites of kanamycin B (KanB) scaffold to introduce the desired two catalytic arms. We initially selected the 4'- and 3'-positions of ring I as the modification sites and show the synthesis and evaluation of the new derivatives of KanB, compounds **2**-**5** (Fig. 1).

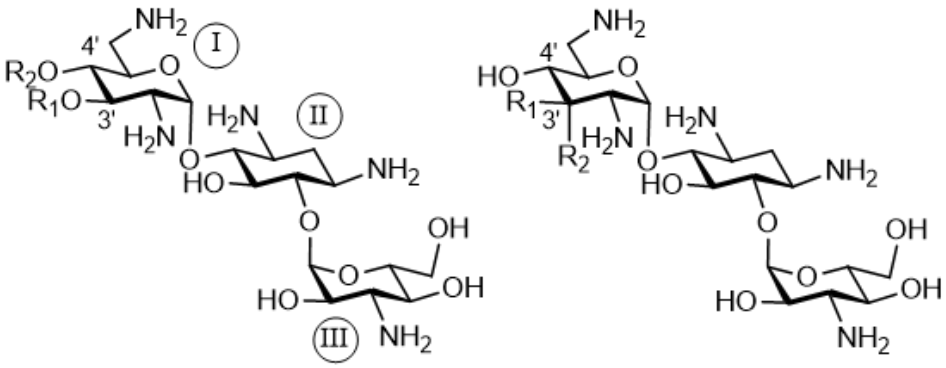

Figure 1. Kanamycin B and its new derivatives studied.

Genetic Engineering and Chemical Approaches for Enhancing Siglec-3 Binding in “HASLECs"

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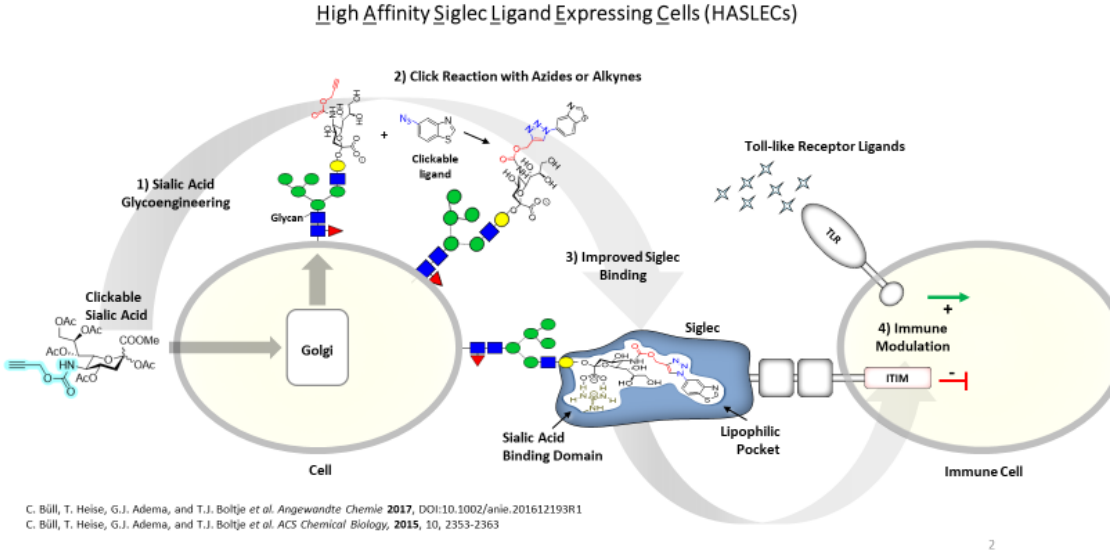
Sialic acids or N-acetylneuraminic acids (Neu5Ac) are a diverse family of 9-carbon monosaccharides synthesized in mammals and some prokaryotes, present at the outermost end of N-linked and O-linked carbohydrate chains and in lipid-associated glycoconjugates. Sialic acid sugars form the ligands for the sialic acid binding immunoglobulin-like lectin (Siglec) family, which are immunomodulatory receptors expressed by immune cells. Interactions between sialic acid and Siglecs regulate the immune system, and aberrations contribute to pathologies like autoimmunity and cancer. Sialic acid/Siglec interactions between living cells are difficult to study owing to a lack of specific tools.

We combined metabolic labeling using unnatural sialic acid derivatives in cells with combinatorial loss/gain of individual sialyltransferase/suflotransferase genes to optimize Siglec-3 binding. Using bioorthogonal chemistry, we introduced chemical modification of alkyne and azide containing sialic acid derivatives to afford High Affinity Siglec Ligand Expressing Cells (HASLECs). Combining this chemical editing of sialic acids with the genetic engineering of the sialyltransferases that underpins their biosynthesis, we identified very potent Siglec-3 binding HASLECs.



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beta-Stereoselective *C*-aryl glycosylation through intramolecular « aryl » delivery (IArD)

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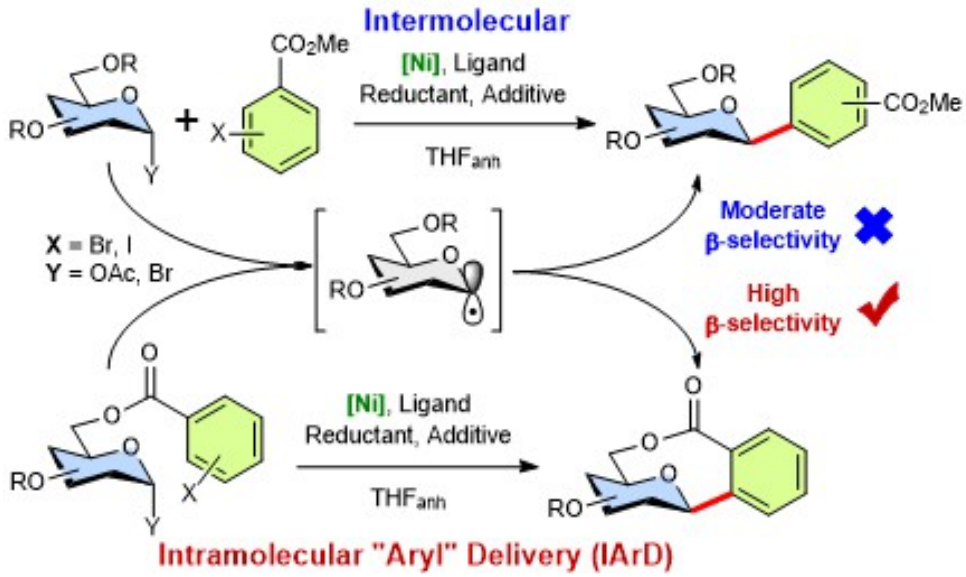
C-Aryl glycosides are an important class of natural products with various biological activities against cancer, bacteria or hypoglycemic agents in the context of type 2 diabetes. The control of glycaemia can be achieved by the inhibition of enzymes such as glycogen phosphorylase (GP) or the sodium/glucose co-transporter type 2 (SGLT2) for which the best ligands identified and marketed are *C*-aryl glycosides.[1] A particular attention must be paid to the α/β-stereoselectivity of the *C*-glycosylation since stereoelectronic effects favor the axial configuration of the anomeric radical thus leading to *C*-aryl αlpha-glycosides. Very few and recent methodologies propose alternatives with improved beta-selectivities in an intermolecular *C*-glycosylation under Ni(0) catalysis.[2] We investigate herein (1) the intermolecular approach towards such *C*-aryl b-glycosides using nickel catalysis, but also (2) the application of intramolecular "aryl" delivery[3] (**IArD**) for an exclusive beta-stereoselectivity in the *C*-glycosylation of hexopyranosides.

Conditions were initially tested under intermolecular coupling conditions using an aryl aglycon activated with an iodide (X = I) and glycosidic donors (Y = OAc, Br) in the presence of nickel ($Ni(acac)_2$), a terpyridine ligand, a reductant (Mn or Zn) and additive ($ZnCl_2$, NaI, $MgCl_2$). The results obtained showed an influence of several parameters such as additive and the reductant. The optimized conditions were then tested in the **IArD** strategy and provided, so far, limited yields of the macrocyclic *C*-aryl beta-glycoside. A discussion will be provided with additional data and a better understanding of the parameters governing both the yield of the *C*-glycosylation as well as its stereoselective outcome.

The *C*-aryl beta-glycosides will be synthesized in the glucose series towards GP or SGLT2 ligands and applications in the context of type 2 diabetes,[1] through functional diversification at the benzoic acid moiety. Furthermore, application into the galactose or fucose series will also be investigated to give access to potential ligands of lectins with application in glycobiology.[4]



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Rutinosidase and other diglycosidases: rising stars in biotechnology

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Diglycosidases are glycosidases catalyzing the cleavage of entire disaccharide moieties from the aglycone. Rutinosidases, main diglycosidase representatives, cleave rutinose (α-L-Rha-(1-6)-β-D-Glc) from rutin or other rutinosides (Fig. 1A). Some diglycosidases can be classified as monoglucosidases with extended substrate specificity. They also have distinct synthetic (transglycosylating) abilities. Rutinosidase from *A. niger*[1] and *A. oryzae* (GH5-23) can glycosylate various acceptors, including phenols, in a good yield using priceworthy rutin as a glycosyl donor. Surprisingly, they are able to glycosylate species such as inorganic azide to form β-rutinosyl azide [2] or carboxylic acids forming (anomeric) glycosyl esters [3], being a unique property of glycosidases. The variant of *A. niger* rutinosidase mutated at the catalytic nucleophile residue E319A is capable of generating α-rutinosyl azide [2]. It was found that rutinosidase is able to accept quercetin 3-β-glucopyranoside as a substrate and thus it is also able to transfer a β-glucosyl moiety [1]. This enzyme has a dual glycosylation activity, generating either rutinosides or glucopyranosides [4]. Its broad substrate specificity has also been demonstrated in the enzymatic cleavage of various 6"-acylated quercetin-3-*O*-β-glucopyranosides (Fig. 1B). Rhamnose-containing compounds (rutinose) are attracting attention due to their anti-cancer activity and as skin anti-aging agents [5]. Their easy availability through the action of rutinosidase opens a whole new avenue in cancer therapy, dermatology, and other fields.

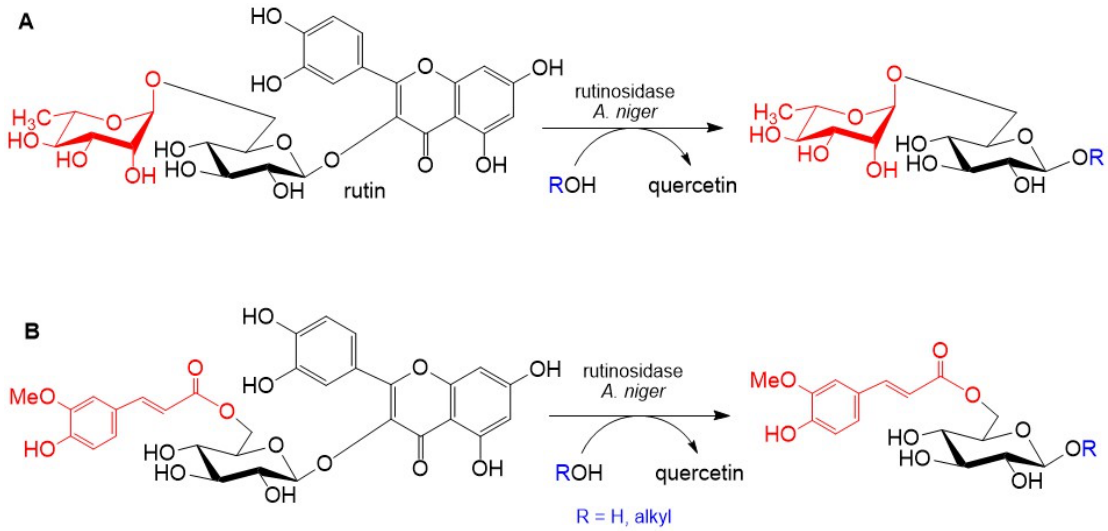

Fig.1 A Hydrolysis/transglycosylation by rutinosidase; B 6”-feruloyl quercetin 3-O-β-glucopyranoside

Acknowledgements

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Synthesis of advanced sialylated glycans libraries by automated glycan assembly

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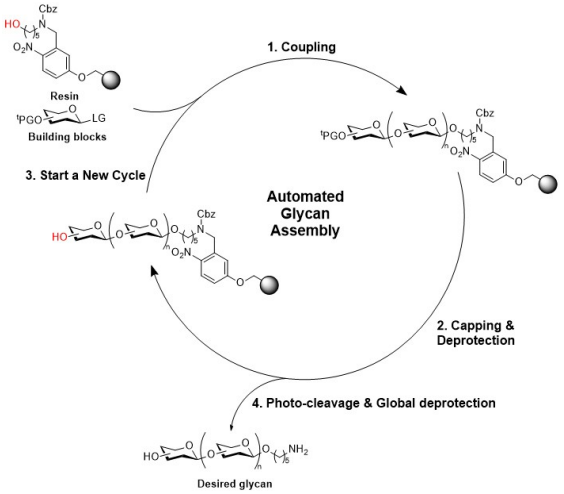
The pursuit of designing and synthesizing complex systems that closely resemble the highly glycosylated extracellular matrix (ECM) has gained significant interest in various fields: 3D cultures, drug delivery and tissue engineering [1]. Supramolecular hydrogels hold great potential for achieving this goal. However, the development of these hydrogels has been hindered by a lack of knowledge behind the fundamental parameters governing their hierarchical self-assembly. Furthermore, the limited examples of carbohydrate-based hydrogels in the literature predominantly involve homomultivalent presentation of a single carbohydrate, which falls short of replicating the complex heteromultivalent nature of the ECM [2].

To address these challenges, this project aims to synthesize biocompatible supramolecular hydrogels that emulate the highly and heterogeneously glycosylated ECM through a hierarchical supramolecular self-assembly approach, by employing rationally designed neoglycolipids. Various photopolymerizable neoglycolipids with distinct sugar headgroups, such as α-D-mannose, β-D-galactose, β-D-glucose, and β-lactose, have been synthesized. The hierarchical self-organization of these neoglycolipids into different hydrogel structures has been thoroughly characterized. Importantly, the resulting hydrogels exhibit multiple interactions with fluorescent lectins specific to the exposed sugars, highlighting their heteromultivalency.

In addition, hybrids of hydrogels and glyconanoring-coated carbon nanotubes have been developed to enhance the mechanical properties of the constructs.



Acknowledgements

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Deoxygenation as a useful tool for tuning the selectivity of glycosidase-targeted ABPs

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Glycosidases are enzymes abundant in nature that hydrolise glycosidic linkages. Inhibitors and activity-based probes (ABPs) of glycosidases are valuable tools to study these enzymes and enable therapeutic and biotechnological applications. [1]

One widespread class of glycosidase ABPs is the cyclophellitol aziridines. These usually are structural mimetics of natural sugars and bear an electrophilic aziridine warhead that facilitates irreversible binding with the enzyme. Because of the high degree of resemblance of "fully oxygenated" cyclophellitol-type inhibitors to their natural carbohydrate counterparts, high target selectivity can be achieved. However, the many hydroxyl groups present in these molecules prohibit broad-spectrum activity-based profiling studies and make these compounds less attractive from a medicinal chemistry point of view. Cyclophellitol aziridines with modified structures can ameliorate these issues.

Here we present the design and synthesis - using both chiral pool and *de novo* approaches – of a comprehensive library of deoxygenated cyclophellitol aziridines (24 compounds in total) that have been tested against therapeutically relevant GBA, GBA2 and GBA3 enzymes to provide insight into the contribution of each hydroxy group to binding affinity and deliver highly selective inhibitors. We demonstrate that deoxygenation of sugar-mimicking ABPs in certain positions can tune their reactivity and drastically change selectivity patterns among enzymes with similar natural substrates.

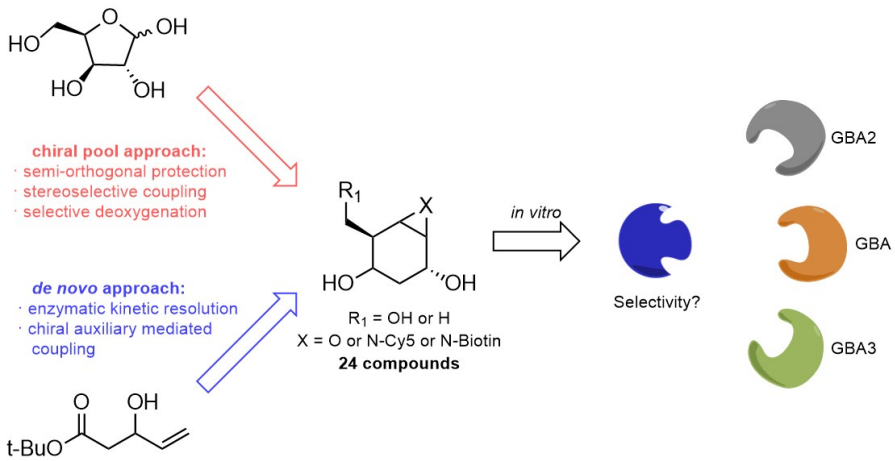

Figure 1: Structural concept of iminosugar based probes.

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Synthesis and study of photoswitchable glycosides for biological applications

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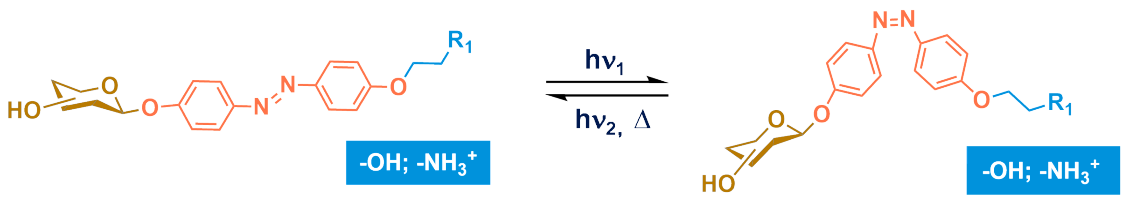
Photochromic molecules are bistable molecules featuring different structural and/or electronic properties which may be reversibly isomerized by light, with the possibility to cycle up to one million "round trips".[1] They offer numerous opportunities for reversibly photomodulating chemical, biological or pharmacological activities or proprieties.[2] Light is generally noninvasive and orthogonal toward most elements of living systems. It can be easily and precisely controlled in time, location, wavelength and intensity, thus enabling the precise activation and deactivation of biological function.

Carbohydrate-lectin interactions are crucially involved in the modulation of myriads of physiological and pathological events.[3] As a continuing interest in the development of photoswitchable glycosides,[4] we decided to prepare galactosyl and fucosyl azobenzene derivatives (Scheme 1) targeting Lectins LecA and LecB which are essential for the bacterial adhesion, biofilm formation and host cell invasion of *Pseudomonas aeruginosa*, a bacterium classified as a Priority 1 pathogen by the WHO.[5]

Photoisomerization of the azobenzene moiety could induce large conformational changes, which provides a clean, fast and easy way to control the geometry and spatial orientation of glycosides so as to reversibly modulate carbohydrate-lectin interaction by light. Synthesis of photoswitchable and their photoswitching properties will be presented.



Scheme 1: Structure and photoisomerisation property of azobenzene-functionalized glycosides.

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An activity-based probe, near-infrared (NIR) fluorogenic probe to detect *O*-GlcNAcase in mitochondria

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O-GlcNAcase (OGA), which promotes the cleavage of the β-O-GlcNAc monosaccharide from serine (Ser) and threonine (Thr) residues of proteins, is associated with the dynamic cycling of protein O-GlcNAcylation in conjunction with the action of O-GlcNAc transferase, which catalyzes the attachment of GlcNAc to Ser and Thr side chains. Dysregulation of protein O-GlcNAcylation is closely related to the pathogenesis of diverse human diseases.

Two isoforms (short and long) of OGA in the nucleus and cytosol have been characterized. Although OGA was found to be present in mitochondria, its function has not been well studied owing to the lack of suitable tools to detect this enzyme. We designed and synthesized an activity-based, near-infrared (NIR) fluorogenic probe that selectively responded to and captured mitochondrial OGA. The probe consisted of (1) a NIR fluorophore, (2) an ethyl carbamate group as a reactive group to covalently capture OGA, and (3) a triphenylphosphonium moiety as a mitochondria-targeting motif.

The intact probe displayed weak fluorescence. Addition of OGA to the probe in aqueous buffer led to label the protein concomitant with an increase in NIR fluorescence. The probe was utilized successfully for imaging of mitochondrial OGA and identify the protein. Details will be described in the presentation.

Monitoring the chemical sensitivity of carbohydrate-based furanic platforms: clean, mild and atom-ec

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In the field of the use of biomass as a renewable resource for chemistry, carbohydrate-based furanic platform molecules offer original building blocks for the design of novel chemical architectures, like original novel furanic surfactants or nitrogen-containing targets obtained by Morita-Baylis-Hillman, Biginelli, Kabachnik-Fields products, nitrone dipolar cycloaddition or oxazole C-H arylation reactions [1-4]. A key issue is to find the appropriate conditions which are compatible with 5-HMF specific reactivity and known sensitivity to harsh conditions.

We now report new atom-economical reactions forming nitrogen containing cyclic structures such as 1,5-benzodiazepines from 5-HMF, o-phenylenediamines and alkynones and 1,4-dihydropyridines, under remarkably mild and clean conditions. The study included careful optimization of the reaction conditions and their application to a wide scope of substrates, leading to a library of variously substituted products [5].

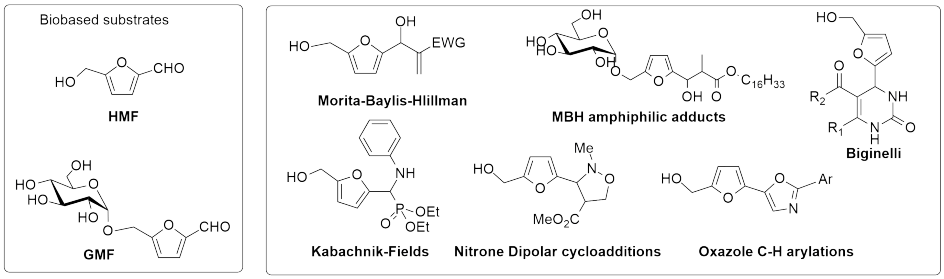
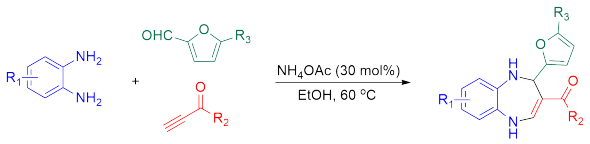


Fig. 1: Examples of fine chemicals derived from HMF and analogs



23 examples, up to 74% yield

√ ***One-pot MCR strategy, clean solvent, mild and new catalyst***
√ ***First access to 1,5-benzodiazepines involving 5-HMF***

Scheme 1: Multicomponent access to 5-HMF-containing 1,5-benzodiazepines

Acknowledgements

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One-pot synthesis of glycosyl azobenzenes and study of their photoswitching properties in water

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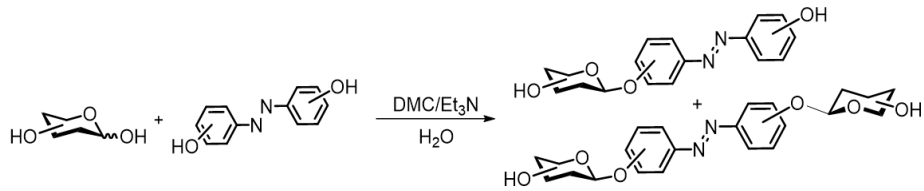
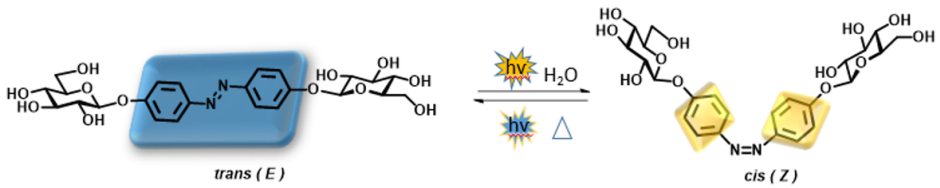
Molecular photoswitches with photoswitching ability in aqueous medium are highly demanded for biological applications and photopharmacology. *[1]* However, the common developed photoswitches like azobenzenes, diarylethenes are barely soluble in water. Linking carbohydrates to a photoswitching unit is an interesting approach to obtain water-soluble photochromic compounds.

Since several years, azobenzene-functionalized photoswitchable glycoconjugates have been developed for light-controlled carbohydrate-protein interactions, cell adhesion, enzyme inhibitors and glycolipid mimics, *[2]* and so on. Consequently, a rapid access to the target compounds with unprotected sugars in aqueous media is of interest. Recently, DMC (2-chloro-1,3-dimethylimidazolinium chloride) mediated glycosylation of unprotected sugars with phenols in aqueous medium has shown promising results. *[3]* As a continuing interest in the development of photoswitchable carbohydrates, *[4]* we are developing the DMC-mediated glycosylation of hydroxyazobenzene with unprotected sugars (Scheme **1**). After optimization of reaction conditions, we are able to prepare a series of water-soluble glycosyl azobenzenes. Furthermore, the synthesized glycosyl azobenzenes displayed remarkable photoswitching behavior in water (Scheme **2**). *[5]*These new results will be presented.



Scheme **1**: DMC-mediated glycosylation of hydroxy-azobenzene derivatives.



Scheme **2**: Isomerization of glycosyl azobenzene upon light and heat.

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Breaking down complex isomeric HMO structures with ^{1}H-^{15}N NMR m⍰ethods

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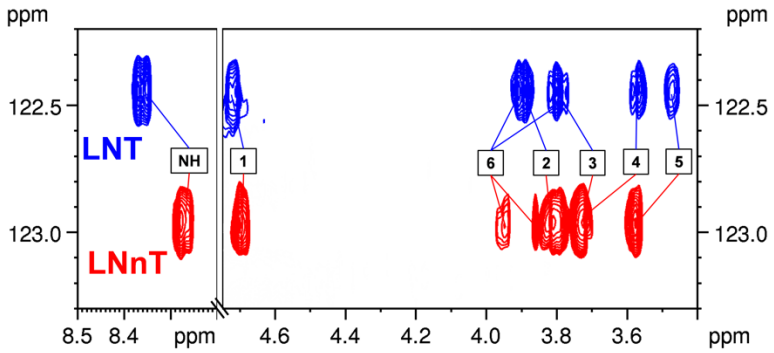
Human milk oligosaccharides (HMOs) are complex glycans that offer numerous health benefits to neonates. However, analytical methods for their routine investigation are still incomplete. NMR spectroscopy provides detailed structural information that can be used to indicate subtle structural differences, particularly for isomeric carbohydrates.

As most HMOs contain the NMR active nucleus ^{15}N incorporated into *N*-acetylglucosamine (GlcNAc) and/or *N*-acetylneuraminic acid (Neu5Ac) moieties, we aimed to introduce the ^{15}N and ^{1}H NMR chemical shifts of GlcNAc or Neu5Ac building blocks into the characterization of HMOs to facilitate the identification of isomeric structures: LNT/ LNnT, 3'-SL/6'-SL, LNFP II/LNFP III and LSTa/ LSTb.

Complete ^{1}H, ^{13}C, and ^{15}N NMR resonance assignments were achieved for the investigated HMOs. The isomeric HMO pairs showed remarkably different ^{15}N NMR resonances (obtained from the ^{1}H-^{15}N HSQC experiment). The highest chemical shift perturbation was observed between the tetrasaccharides LNT and LNnT bearing a GlcNAc unit, while the ^{15}N NMR chemical shifts of the Neu5Ac moiety in 3'SL and 6'SL trisaccharides were less responsive to the minor structural difference. When considering both ^{1}H and ^{15}N NMR chemical shifts of the GlcNAc and/or Neu5Ac moieties (obtained from the ^{1}H-^{15}N HSQC-TOCSY experiment), the distinction of the structural isomers was unambiguous.

This NMR-based method offers a straightforward approach for the identification of common HMOs, which may contribute to the analytical investigation of HMO standards, HMO products, or even human milk.



Overlaid 1H-15N HSQC-TOCSY spectra of LNT and LNnT with the 1H NMR assignment of their GlcNAc moiety

Acknowledgements

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Optimalization of lung tissue *N*-glycoprofile analysis

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Altered glycosylation is a common hallmark of a number of diseases, such as metabolic disorders, oncological diseases, and other pathological states. Therefore, a development of reliable method with the highest informative value about the N-glycan structures is an essential tool that can be utilized in biomarker discovery. In presented study, fresh lung resections were homogenized and proteins were extracted to be subjected to PNG-ase F treatment. Released glycans were isolated and analysed by MALDI TOF mass spectrometry in their unlabelled form, after permethylation and after linkage-specific derivatization of sialic acids. The appropriate composition of buffers used for homogenization and protein extraction, as well as the various introduced solid phase extractions with the focus on preserving the labile terminal sialic acid and increasing the obtained signal intensities were optimized.

By the established protocol, even larger tetra-sialylated or tetra-antennary N-glycan structures with *m/z* up to 4,938 (Hex9HexNAc8Fuc2NeuAc2) were observed in substantial relative intensities after the permethylation. Linkage-specific derivatization of sialic acids led to the characterization of signals with *m/z* up to 3,870 (Hex7HexNAc6Fuc2NeuAc4); however, larger structures were identified in low relative intensities. After the efficient glycan isolation from lung tissue and clean-up, both derivatization methods led to the successful detection of a plethora of hybrid, high-mannose and complex N-glycan structures.

Acknowledgements

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Synthesis and in vivo evaluation of MUC1-carbon dot conjugates as cancer vaccines

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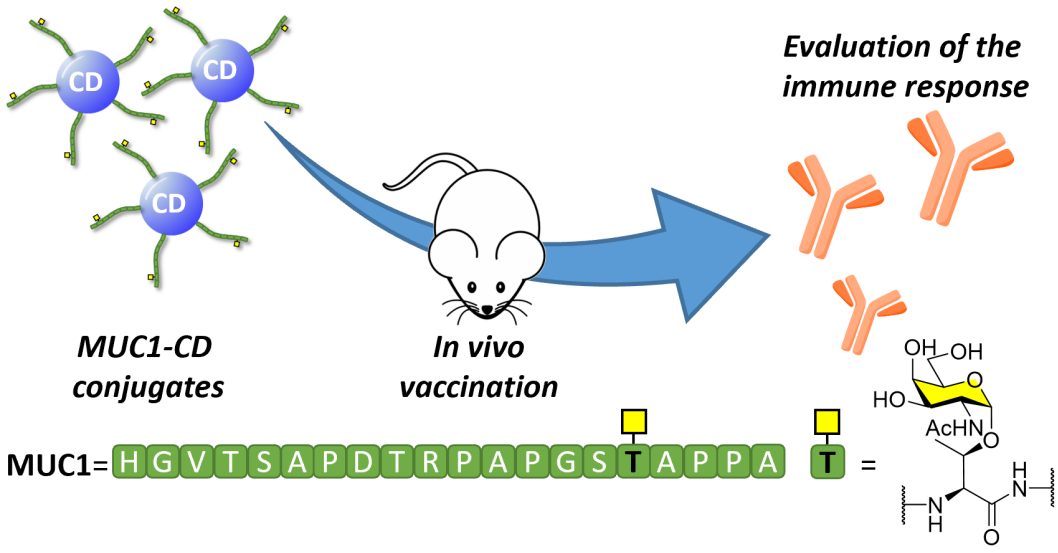
Carbon dots (CDs) are an emerging class of carbon-based nanoparticles which possess inherent immunostimulant properties [1]. Several types of CDs have been synthesized from different precursors, and conjugated with the MUC1 antigen. MUC1 is highly glycosylated glycoprotein expressed on the surface of epithelial cells. In cancer cells, it is overexpressed and presents truncated carbohydrate residues, such as the Tn antigen (α-*O*-GalNAc-Ser/Thr), which can be recognized by the immune system [2]. Unfortunately, MUC1 has low *in vivo* stability and low immunogenicity. Therefore, the conjugation of MUC1 on the surface of carrier proteins or nanoparticles such as CDs is essential to elicit a strong immune response [3].

Herein we report the preliminary results of a novel set of MUC1-CD conjugates. We show that the immunostimulant properties of CDs depend on the nature of the CD precursors, and that CD nanoparticles constitute promising scaffolds for the synthesis of novel self-adjuvant cancer vaccines.



Acknowledgements

This project has received funding from the EU H2020 Marie Skłodowska-Curie grant No 101034288, and Mizutani Foudation for Glycoscience grant No 220115

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Physico-chemical characteristics of pectin fractions from Flaxseed

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Flaxseed is a rich source of pectins with unique physico-chemical properties that make it a valuable component of various food products. Moreover, flaxseed pectins have potential health benefits due to their ability to form a gel-like matrix in the gastrointestinal tract, which can slow down nutrient absorption and promote satiety. These polysaccharides may also have prebiotic effects by promoting the growth of beneficial gut bacteria [1].

Natural deep eutectic solvents (NADES) are not only more environmentally safe, but also are a preferred medium for the extraction of polysaccharides. NADES have several advantages over strong inorganic acids, traditionally used in pectin separation, but aggressive and problematic. From the point of view of disposal NADES have several advantages over strong inorganic acids, i.e. show low toxicity and biodegradability [2,3].

The optimization for the proper combination of NADES components for flaxseed pectins extraction, using response surface methodology (RSM) and the I-optimal method, was applied. A mixture of choline chloride and citric acid in the appropriate combination, diluted in water to reduce viscosity, has been shown to be an extremely effective solvent in obtaining dietary fiber. Uronic acid-rich polysaccharides were obtained and subjected to GPC analysis to estimate their molecular weight. The degree of methylation of carboxyl residues was assessed by FT-IR, and the content of neutral sugars by GC-MS.

Acknowledgements

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