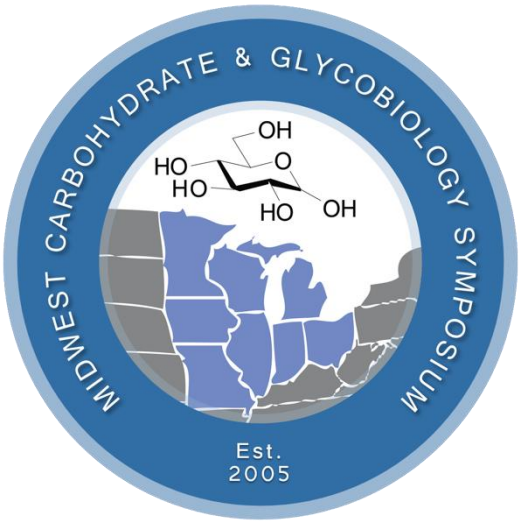
15th Annual Midwest Carbohydrate and Glycobiology Symposium (MCGS)

Jordan Hall of Science
Department of Chemistry and Biochemistry
University of Notre Dame
Notre Dame, Indiana



Meeting Website URL: http://sites.nd.edu/mcgs-nd2019/

September 20–21, 2019

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(Alphabetical Order)

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A Brief History of the MCGS

The Midwest Carbohydrate and Glycobiology Symposium (MCGS) was founded in 2005 by Xuefei Huang and Steven Sucheck, and was hosted for the first time by the University of Toledo in 2005. Since that time, the MCGS has convened annually at various academic institutions throughout the Midwest.

2005 (1st): University of Toledo – X. Huang and S. Sucheck, Organizers
2006 (2nd): Wayne State University – Z. Guo and P. Andreana, Organizers
2007 (3rd): The Ohio State University – P. G. Wang, Organizer
2008 (4th): Cleveland State University – X.-L. Sun and J. Hu, Organizers
2009 (5th): University of Cincinnati – S. Iyer, Organizer
2010 (6th): University of Toledo – S. Sucheck, Organizer
2011 (7th): Michigan State University – X. Huang, Organizer
2012 (8th): Wayne State University – P. Andreana, D. Crich and Z. Guo, Organizers
2013 (9th): University of Toledo – P. Andreana, S. Sucheck and J. Zhu, Organizers
2014 (10th): University of Michigan – P. Nagorny, Organizer
2015 (11th): Cleveland State University – X.-L. Sun, Organizer
2016 (12th): Central Michigan University – B. Swarts, Organizer
2017 (13th): University of Wisconsin – W. Tang, J. Jiang and L. Li, Organizers
2018 (14th): Michigan State University – X. Huang, Organizer
2019 (15th): University of Notre Dame – A. S. Serianni, Organizer

TECHNICAL PROGRAM

Friday, September 20, 2019

4:30 pm – 6:15 pm **Meeting Registration; Poster Setup**
Jordan Hall of Science – Galleria

SESSION I

Jordan Hall of Science – Room 105
Chair: Linda Hsieh-Wilson (CalTech)

6:25 – 6:30 pm **Introductory Remarks**
Anthony S. Serianni

6:30 – 7:00 pm Plenary 1
Dr. Richard D. Cummings
Professor, Department of Surgery, Harvard University
Recognizing Sugars – Chemical Biology and Physiological Roles

7:00 – 7:15 pm Nicholas Banahene
Student, Swarts Lab, Department of Chemistry and Biochemistry, Central Michigan University
O-Acylated Trehalose Analogues for Probing Cellular Processes and Components in Corynebacterineae

7:15 – 7:45 pm Invited 1
Dr. Joseph J. Barchi Jr.
Senior Scientist, Center for Cancer Research, National Cancer Institute
The Thomsen Friedenreich Tumor-Associated Carbohydrate Antigen in Anticancer Drug Discovery

7:45 – 8:15 pm **Break; Poster Session**
Jordan Hall of Science – Galleria

8:15 – 8:30 pm Shuyao Lang
Student, Huang Lab, Department of Chemistry and Biomedical Engineering, Michigan State University
Glyco-engineering of Natural Killer Cells with CD22 Ligands for Effective Anti-Cancer Immunotherapy

8:30 – 9:00 pm Invited 2
Dr. Roger A. Laine
Department of Biological Sciences, Department of Chemistry, Louisiana State University
Chief Executive Officer, TumorEnd, LLC.
Polysaccharides as Tumor Therapeutics, 1868-2019

9:00 pm **Reception; Poster Session**
Jordan Hall of Science – Galleria

Saturday, September 21, 2019

7:30 – 8:15 am **Continental Breakfast (Jordan Hall)**
Jordan Hall of Science – Galleria

SESSION II
Jordan Hall of Science – Room 105
Chair: Peter Andreana (University of Toledo)

8:15 – 8:30 am **Introductory Remarks**
Dr. Mary Prorok
Assistant Chair, Department of Chemistry and Biochemistry, University of Notre Dame
The Evolution of Laboratory Safety at Notre Dame

8:30 – 9:00 am Plenary 2
Dr. Linda Hsieh-Wilson
Professor, Division of Chemistry and Chemical Engineering, California Institute of Technology
Streamlined Methods for the Synthesis and Study of Heparin Sulfate Oligosaccharides

9:00 – 9:15 am Jicheng Zhang
Student, Huang Lab, Department of Chemistry, Michigan State University
Chemical Synthesis of Heparin Like Head to Tail Multimers

9:15 – 9:45 am Invited 3
Dr. Norman J. Dovichi
Professor, Department of Chemistry and Biochemistry, University of Notre Dame
Capillary Electrophoresis-Mass Spectrometry Reveals the N-Glycome During Vertebrate Embryogenesis

9:45 – 10:45 am **Break; Poster Session**
Jordan Hall of Science – Galleria

10:45 – 11:00 am Mithila D. Bandara
Student, Demchenko Lab, Department of Chemistry and Biochemistry, University of Missouri-St. Louis
Synthesis of Human Milk Oligosaccharides

11:00 – 11:30 am Invited 4
Dr. Jacquelyn Gervay-Hague
Professor, Department of Chemistry, University of California, Davis
Protecting Group Modulation of Glycosyl Iodide Reactivity to Yield Metabolic Probes of Interspecies Chemical Communication

11:30 – 11:45 am Sonia Tomar
Student, Sun Lab, Department of Chemistry, Cleveland State University
Investigation of Substrate Specificity of Sialidases with Membrane Mimetic Glycoconjugates

11:45 am – 1:45 pm **Lunch; Poster Session**
Jordan Hall of Science – Galleria

1:00 – 1:45 pm **PI Meeting**
Jordan Hall of Science – Reading Room

SESSION III
Jordan Hall of Science – Room 105
Chair: Jacquelyn Gervay-Hague (UC–Davis)

1:45 – 2:15 pm Plenary 3
Dr. Shahriar Mobashery
Professor, Department of Chemistry and Biochemistry, University of Notre Dame
Cell-Wall Recycling in Pseudomonas aeruginosa and the Nexus to Antibiotic Resistance

2:15 – 2:30 pm Jia Gao
Student, Huang Lab, Department of Chemistry, Michigan State University
Chemoenzymatic Synthesis of Heparan Sulfate Glycopeptide Mimetics and Evaluation of Its Biological Functions

2:30 – 3:00 pm Invited 5
Dr. Ming Tien
Professor, Department of Biochemistry and Molecular Biology, The Pennsylvania State University
Initiation, Elongation and Termination of Bacterial Cellulose Synthesis

3:00 – 3:30 pm **Break; Poster Session**
Jordan Hall of Science – Galleria

3:30 – 3:45 pm Dr. Jiaoyang Jiang
Associate Professor, Pharmaceutical Sciences Division, University of Wisconsin-Madison
Targeted Covalent Inhibition of O-GlcNAc Transferase in Cells

3:45 – 4:15 pm	Invited 6 Dr. Darón I. Freedberg Research Chemist, U.S. Food & Drug Administration *Hydrogen Bonding in the Conformations of Glycans*
4:15 – 4:30 pm	Matteo Panza Student, Demchenko Lab, Department of Chemistry and Biochemistry, University of Missouri-St. Louis *HPLC-Based Oligosaccharide Synthesis: Entirely Automated Glycan Synthesis*
4:30 – 5:00 pm	Invited 7 Dr. Robert J. Woods Professor, Department of Biochemistry and Molecular Biology, Department of Chemistry, Complex Carbohydrate Research Center, University of Georgia *Glycomimetics to Inhibit Influenza Infection*
5:00 – 5:30 pm	**Awards; Concluding Remarks**
5:30 – 6:15 pm	**Break; Poster Tear-down**
6:15 pm	**Dinner** Jordan Hall of Science – Galleria

ABSTRACTS
Oral Presentations

Plenary (1–3)

Recognizing Sugars – Chemical Biology and Physiological Roles

Richard D. Cummings[1]

[1]Beth Israel Deaconess Medical Center, Harvard Medical School, Harvard University, Cambridge, MA 02138 USA

Complex carbohydrates in humans are comprised of a dizzying array of amino acid derivatives of glycans linked to 9 of the 20 amino acids along with innumerable types of glycolipids varying in their glycan and lipid moieties. Chemical understanding of their structures, expression, and recognition requires novel technologies. We have developed new methods of derivatizing glycans and generating defined glycopeptides to study glycan recognition in context of aglycone components and other post-translational modifications. In addition, we have generated multiple types of glycan presentations on microarray formats and others to aid in studying glycan recognition and function. In addition, we have developed Smart Anti-Glycan Reagents using lamprey-derived antibodies that can recognize many human and mammalian glycans and provide new tools for exploring the temporal and spatial expression of glycans in development. Altogether, these new technologies have provided new insights in infectious diseases, as in influenza virus, relative to the human glycome, as well as other diseases such as tuberculosis, and autoimmune disorders. To further explore the physiological roles of glycans we have examined human disorders involving altered glycan expression and recognition, as well as murine models in which genetic alterations in glycan expression are targeted for modification. In observing the physiological outcomes in such animal models, we have uncovered novel functions of glycans in cell adhesion and signaling. There are tremendous translational opportunities in glycoscience from exploiting new observations in glycan structure and recognition.

Streamlined Methods for the Synthesis and Study of Heparan Sulfate Oligosaccharides

Linda Hsieh-Wilson

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125 USA

Heparan sulfate (HS) glycosaminoglycans (GAGs) are sulfated polysaccharides that mediate a wide range of important biological processes, including growth factor signaling, blood coagulation, viral infection, neural development, and cancer. The diverse biological functions of HS GAGs are thought to stem from their complex stereochemistry and sulfation patterns. However, understanding the structure-activity relationships of HS has been hampered by a lack of methods to synthesize large collections of oligosaccharides with defined sulfation sequences. A major obstacle is the preparation of suitably protected building blocks, whose synthesis typically requires 20-30 steps. We will describe a new approach to access all four of the core disaccharides required for HS assembly from natural heparin and heparosan polysaccharides. The use of disaccharides rather than monosaccharides as minimal synthons accelerates the synthesis of HS GAGs, providing strategically-protected building blocks and tetrasaccharides in about half the number of steps. Rapid access to key building blocks is greatly facilitating the generation of libraries of HS oligosaccharides for detailed investigations into the 'sulfation code.' If time permits, we will also describe the application of defined HS molecules to the discovery of a novel interaction between HS GAGs and the orphan receptor Tie1 and its implications for vascular development and homeostasis.

This work was supported by the National Institutes of Health (Common Fund Grant U01 GM116262-03 and R01 GM093627) and the National Science Foundation (DGE-1144469)

Cell-Wall Recycling in *Pseudomonas aeruginosa* and the Nexus to Antibiotic Resistance

Shahriar Mobashery

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46656 USA

Pseudomonas aeruginosa has the ability to sense damage inflicted to its cell wall by β-lactam antibiotics. The process involves chemical signaling, which will be a subject of my presentation. A primary mechanism for this sensing and signalling involves the events of cell-wall recycling. The cell wall is degraded for recycling and then it is resynthesized *de novo* for the repair function. The recycling events get initiated by the functions of a family of 11 lytic transglycosylases in *P. aeruginosa*, which generate the signalling factors that influence transcriptional events in the cytoplasm. The structures and mechanisms of these enzymes and those of the early cytoplasmic steps of recycling have been the subject of study in my lab, which I will disclose in my presentation.

Invited (1–7)

The Thomsen Friedenreich Tumor-Associated Carbohydrate Antigen in Anticancer Drug Discovery

Kevin R. Trabbic and Joseph J. Barchi Jr.

Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, NCI at Frederick, Frederick, MD 21702 USA

A hallmark of all cancers is the aberrant expression of cell-surface glycans; these aberrations cause distinct changes in cellular properties such as adhesion, signaling and metastasis. Many of these modified structures are known as Tumor-Associated Carbohydrate Antigens (TACAs) for their ability to elicit immune responses, and circulating antibodies to TACAs can be protective. One TACA disaccharide, the Thomsen-Friedenreich antigen (TF-ag), has been the target of a host of antitumor vaccine designs, while also being a motif that is directly linked to the metastatic spread of various solid tumor. We have developed gold nanoparticle platforms bearing the TF-ag or TF-ag-containing glycopeptides as both antimetastatic agents or immunotherapeutics. The design and development of these platforms along with their biological relevance will be discussed.

This work was supported by Federal funds from the Intramural Program of the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E

Polysaccharides as Tumor Therapeutics, 1868-2019

Roger A Laine

Department of Biological Sciences, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803 USA; TumorEnd, LLC., Suite 240, Parker Drive East, LSU Campus, Baton Rouge, LA 70803-0001 USA

Busch (1868) and Fehleisen (1880) showed sarcoma patients with nosocomial or induced **erysipelas** skin infections had tumor regression or elimination. Spronck (1891) showed that heat treated "*Streptococcus erysipelatos*" (now *S. pyogenes*) cultures, injected into sarcoma human or dog patients would likewise cause tumor regression. Heat stability was the first indication that polysaccharides might be the active pharmaceutical ingredient (API). Coley (1891, 1910) used a mixture of bacteria-free, heat treated culture medium of *S. erysipelatos* and *Bacillus prodigiosa* (*Serratia marsescens*) to successfully treat sarcoma patients. "Coley's Toxin" was produced by Parke-Davis for 20 years, however with inconsistent potency due to lack of knowledge of the API. Coley applied the toxins to sarcomas patients and included many case studies (Coley, 1910). Around 1920 the American Cancer Association recommended using radiation therapy instead of Coley's Toxin for cancer, because of the inconsistent potency of the Parke-Davis preparation and Coley's toxin fell into disuse, despite its potent effects. A Coley's Toxin review was published by Nauts, *et al*., 1980, a table reproduced in Novotny's review (1985) showing 5 year survival times of nearly 50% in 897 patients with 18 different cancers, treated with Coley's toxin. Shear continued work on the *Serratia* API component of Coley's Toxin, and in 1943, Hartwell and Shear reported the Serratia API was a polysaccharide, lacking peptide, containing lipid and phosphate, that caused tumor specific hemorrhage in mice. Their preparation was 1500 times as potent as the Parke-Davis Coley's Toxin. We have recently repeated Shear and Hartwell's work with modern chromatographic techniques and found the polysaccharide we call SP1 to be 250,000 in molecular weight by SEC and HPLC. Independently, in the 1980's Hellerqvist, Sundell, *et al.* at Vanderbilt, apparently without knowledge of Coley's Toxin or previous work, isolated an active polysaccharide toxin principle CM101 from Group B *Streptococcus*, (GBS toxin) which was responsible for lung capillary damage in Early Onset Disease, human neonates. They isolated a 270kDa polysaccharide from the GBS culture filtrate, with a lipid and phosphate attached. This GBS Toxin was later found by Hellerqvist to also cause *tumor specific capillary damage*, hemorrhage in tumors, and regression of tumors in rodent models. A successful Phase I clinical trial in volunteer stage 4 humans showed 33% effectivity (DeVore, *et al*. 1997). The capillary endothelial receptor was discovered to be Sialin by Hellerqvists group in 2002. Mechanism is proported to be complement activation, followed by inflammatory cytokine cascade and neutrophil destruction of capillaries. Voelz, et al., 2010, reported a nosocomial outbreak of Serratia in a neonate facility, where in the first 5 days, 9 neonates showed respiratory distress, febrility with a 50% death rate, similar to the GBS effects on newborns. Thus the receptor on the capillary endothelium for CM101 may be the same as for the Serratia SP1. We believe these two polysaccharides from Streptococcus and Serratia are the API's of Coley's Toxin.

Capillary Electrophoresis-Mass Spectrometry Reveals the *N*-Glycome During Vertebrate Embryogenesis

Yanyan Qu[1,3], Kyle M. Dubiak[1], Elizabeth H. Peuchen[1], Matthew M. Champion[1], Zhenbin Zhang[1], Alex S. Hebert[2], Sarah Wright[1], Joshua J. Coon[2], Paul W. Huber[1], Norman J. Dovichi[1]

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46656 USA; [2]Department of Biochemistry and Biomolecular Chemistry, University of Wisconsin, Madison WI 53706 USA; [3]Current affiliation: Astrazenca, Gaithersburg, MD 20878 USA

Glycans show differences in cell-surface expression, intracellular trafficking, and tissue distribution throughout vertebrate development. While studies have described the spatial distribution of specific monosaccharides in embryonic tissue, there is very little quantitative data on the evolution of glycan structure and abundances of glycans and glycoproteins during embryogenesis. We report the first quantitative study of the expression of both N-linked glycans and of N-glycoproteins during early development of Xenopus laevis. We employed aminoxyTMT isobaric labels and capillary electrophoresis-tandem mass spectrometry to quantify glycan expression at six development stages from the fertilized egg to the late tailbud stage. We quantified 110 N-glycans that spanned four orders of magnitude in abundance. Capillary electrophoresis was particularly useful in identifying charged glycans; over 40% of the observed glycan structures were sialylated. Glycan expression was relatively constant from fertilization through the midblastula transition, followed by a massive reprogramming, particularly for multi-sialylated glycans. We also identified 612 N-glycosylation sites on 350 glycoproteins in the stage 1 embryo and 1,682 N-glycosylation sites on 1,023 N-glycoproteins at stage 41. Serine protease inhibitor A6 (serpina6 or EP45), which dominates early stage glycoprotein abundance, is associated with oocyte maturation. In contrast, over two thirds of the N-glycoproteins identified at stage 41 are associated with neuron projection morphogenesis, suggesting a vital role of the N-gycome in neuronal development.

This work was supported by (Grant R01GM096767 – NJD and NIH P41 GM108538 - JJC).

Protecting Group Modulation of Glycosyl Iodide Reactivity to Yield Metabolic Probes of Interspecies Chemical Communication

Jacquelyn Gervay-Hague

Department of Chemistry, University of California, Davis, Davis, CA 95616 USA

Research in the Gervay-Hague lab is focused on developing experimental methods for the efficient synthesis of glycolipids. One-pot synthetic routes for large quantity production of alpha-galactosyl ceramides, phytosteryl glycosides and glycerol containing glycolipids using glycosyl iodide chemistry have been established. This synthetic chemistry platform exploits the unique reactivity of glycosyl iodides providing highly efficient routes to metabolic probes that can be pulled down from natural systems through chemical transformations. Methodologies for streamlining processes to minimize protecting group transformations and to increase yields through one-step processes have been invented. These advancements provide a reliable platform for making compounds that can used to interrogate interspecies chemical communication.

Initiation, Elongation and Termination of Bacterial Cellulose Synthesis

John B. McManus[1], Hui Yang[2], Liza Wilson[2], James D. Kubicki[3] and Ming Tien[1]

[1]Department of Biochemistry and Molecular Biology and [2]Department of Biology, The Pennsylvania State University, University Park, PA 16802 USA; [3]Department of Geological Sciences, University of Texas at El Paso, El Paso, TX 79968 USA

Cellulose is the major component of the plant cell wall and composed of β-linked glucose units. Use of cellulose is greatly impacted by its physical properties, which are dominated by the number of individual cellulose strand within each fiber and the average length of each strand. Our kinetic analysis of the bacterial cellulose synthase has provided a complete mechanism for cellulose synthase accounting for its processivity and mechanism of initiation. Using ionic liquids and gel permeation chromatography (GPC), we obtain kinetic constants for initiation, elongation and termination (release of the cellulose strand from the enzyme) for two bacterial cellulose synthases (*Gluconoaceto bacterhansenii* and *Rhodobacter sphaeroides*). Our results show that initiation of synthesis is primer-independent. After initiation, the enzyme undergoes multiple cycles of elongation until the strand is released. The rate of elongation is much faster than that of steady-state turnover. Elongation requires cyclic addition of glucose (from UDP-glucose) and then strand translocation by one glucose unit. Translocations greater than one glucose unit results in termination requiring re-initiation. The rate of the strand release, relative to the rate of elongation, determines the processivity of the enzyme. This mechanism and the measured rate constants were supported by kinetic simulation. With the experimentally-determined rate constants we are able to simulate steady-state kinetics and mimic the size distribution of the product. Thus, our results provide for the first time a mechanism for cellulose synthase that accounts for initiation, elongation and termination.

Hydrogen Bonding in the Conformations of Glycans

Marcos D. Battistel, Hugo F. Azurmendi and Darón I. Freedberg

U.S. Food & Drug Administration, 16071 Industrial Drive, HFZ-260, Gaithersburg, MD 20877 USA

Hydrogen bonds (Hbonds) are a recurrent structural feature found in biological molecules, such as proteins and nucleic acids. Hbonds help stabilize biological structural motifs in proteins, DNA and RNA, supported by a wealth of direct evidence in solution. However, direct evidence for Hbonds in glycans has been lacking until recently. The best tool for directly detecting Hbonds is solution NMR spectroscopy because it offers atomic resolution and the potential to directly establish Hbond donor and acceptor pairs via *J*-couplings. Thus, Hbond directionality can also be determined. Hbonds expected in glycans are different than those in proteins and nucleic acids, where the NH-based Hbonds are far more prominent and also dictates structure. Hbonding in glycans is thought to occur between multiple OH groups. Since OH in glycans are considered similar to those in H_2O, internal Hbonds are believed not to provide significant structural stabilization to yield long-lived conformations. Consequently, Hbonds are likely transient, therefore harder to detect than for other biomolecules. However, their detection may provide direct evidence of the presence of defined conformations, even while multiple conformations may exist simultaneously in equilibrium. In this context, our group considers detection of Hbonds a valuable tool for the characterization of a glycan's conformational space. In our recent work, we have detected NH→O, OH→OH, OH→O=C and CH→O Hbonds. In this talk, we report our progress towards detecting and characterizing Hbonds in glycans by direct NMR measurements.

Glycomimetics to Inhibit Influenza Infection

Robert J. Woods

Department of Biochemistry and Molecular Biology, Department of Chemistry, Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602 USA

Small-molecule anti-influenza agents, such as zanamivir and oseltamivir are neuraminidase (NA) inhibitors limit the release of progeny virions from infected cells through competitive binding to the viral NA. However, these drugs do not block infection. Vaccination is the most common approach for preventing influenza infection, but vaccine creation takes approximately 6 months and requires prior knowledge of the circulating virus strains. The impact of this limitation was evident in the 2009 swine flu pandemic, against which the seasonal vaccine was completely ineffective. The urgent need to develop complementary strategies to vaccination or NA inhibition is emphasized by the fact that between 2006 and 2009 seasonal flu developed almost complete resistance to oseltamivir, resulting from a single spontaneous mutation in the viral NA.

As a potential alternative to current flu antivirals, we are developing glycomimetics that block the interaction between the viral hemagglutinin (HA) and the host glycans that serve as flu-receptors. This interaction is essential for viral adhesion, and the universal receptor for flu A [15] and B [16] viruses is Sia-Gal (sialic acid, Sia, a.k.a. neuraminic acid, linked to galactose, Gal). HAs from either human (H1N1) or avian-infective (N5N1) flu strains bind to di- or trisaccharides terminating in Sia-Gal regardless of whether the Sia-Gal linkage is α2,6 or α2,3. This observation suggested that a small Sia-Gal glycomimetic might be developed that could inhibit a broad range of flu A strains, including flu B, and was critical in the design of our glycomimetic framework (FB127).

Here were report that FB127 shows dose-dependent inhibition of the infection of MDCK cells by a range of flu viruses from group 1 (H1N1, H5N1), group 2 (H3N2), and flu B, and that the influenza neutralizing ability of FB127 is within the same range as reported for glycodendrimers. Because FB127 is a synthetic small molecule, it can be rationally modified, and manufactured with a high degree of quality control and reproducibility. In this regard, FB127 is amenable to a number of formulations, including aerosol, oral, and intravenous routes that provide great flexibility in drug delivery.

SPS (1–7)

O-Acylated Trehalose Analogues for Probing Cellular Processes and Components in *Corynebacterineae*

Nicholas Banahene and Benjamin M. Swarts

Department of Chemistry and Biochemistry, Central Michigan University, Mount Pleasant, MI 48858 USA

Mycobacterium tuberculosis (*Mtb*), the pathogen that causes tuberculosis (TB), is a member of the *Corynebacterineae* suborder, which comprises hundreds of important species. TB is a global health crisis with about 10 million new cases and 1.5 million deaths in 2017. The mycobacterial cell envelope includes a unique outer membrane, also known as the mycomembrane, which is the major defense barrier that confers intrinsic drug tolerance to *Mtb*. The mycomembrane is typified by long-chain (up to 100 carbons), α-branched, β-hydroxy fatty acids called mycolic acids, which are esterified to various acceptors, including trehalose, forming trehalose mono- and dimycolate (TMM and TDM), as well as protein serine residues, forming *O*-mycoloylated proteins. These structures are generated by a process called mycoloylation, which refers to the Ag85-catalyzed transfer of mycolic acid from the donor molecule TMM to the acceptor molecule. We recently reported an *O*-acylated TMM analogue containing a terminal alkyne group on the acyl chain (O-AlkTMM-C7), which allowed for metabolic incorporation of the alkyne handle into mycomembrane components and enabled their visualization using click chemistry. Here, we describe an expanded toolbox of *O*-acylated TMM analogues bearing alkyne, azide, *trans*-cyclooctene, and fluorescent tags, which allowed us to test the substrate tolerance of Ag85 enzymes at the cellular level. In addition, these compounds provide significantly expanded experimental versatility including one- or two-step cell labeling, live cell labeling and rapid cell labeling via tetrazene ligation. Moreover, these compounds allow for the labeling and analysis of mycomembrane components, including *O*-mycoloylated proteins. Thus, these compounds have potential for the specific detection and surface engineering of live *Mtb*, as well as for the identification of novel mycomembrane components that may be attractive targets for drug development.

Glyco-engineering of Natural Killer Cells with CD22 Ligands for Effective Anti-Cancer Immunotherapy

Xianwu Wang[1], Shuyao Lang[2], Yunpeng Tian[3], Jianghong Zhang[3], Xu Yan[4], Zhihong Fang[5], Jian Weng[1], Na Lu,[6] Hongzhi Cao,[6] Zhu Li*,[3], Xuefei Huang*,[2]

[1]Department of Biomaterials, College of Materials, Xiamen University, 422 Siming Nan Road, Xiamen 361005, P. R. China; [2]Department of Chemistry and Biomedical Engineering, Institute for Quantitative Health Science and Engineering, Michigan State University, East Lansing, MI 48824 USA; [3]Xiamen Nuokangde Biological Technology Co., Ltd. Xiamen 361006, China;[4]School of Chemistry, Sun Yat-Sen University, Guangzhou 510275, China. [5]Department of Hematology, The First Affiliated Hospital of Xiamen University, Xiamen 361003, China; [6]National Glycoengineering Research Center, Shandong University, Qingdao, Shandong 266237, China 361003, China. Correspondence and requests for materials should be addressed to Z.L. (email: lizhu@nkdbio.com) or to X.H. (email: huangxu2@msu.edu).

Adoptive transfer of immune cells is being actively pursued for cancer treatment. Natural killer (NK) cells are a class of immune cells that can potentially kill tumor cells. Due to the lack of major histocompatibility complexes on cell surface, NK cells do not have inherent selectivities towards cancer. To bestow tumor targeting abilities and enhance anti-cancer efficacy, a new strategy is established to glyco-engineer NK cells. Carbohydrate based ligands for CD22, a marker for B cell lymphoma, can be introduced through either metabolic engineering or direct membrane insertion with an amphiphilic glyco-polymer onto NK cells. Such glyco-engineered NK cells exhibited much enhanced cytotoxicities towards $CD22^+$ lymphoma cells in a CD22 dependent manner. Importantly, both $CD22^+$ lymphoma cell lines and primary lymphoma cells from cancer patients can be effectively killed by the engineered NK cells. Furthermore, glyco-engineered NK cells provided significant protection to tumor bearing mice. Thus, NK cell glyco-engineering is an exciting new approach for cancer treatment without the need for genetic modification of the cells.

Chemical Synthesis of Heparin Like Head to Tail Multimers

Jicheng Zhang and Xuefei Huang

Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA

Heparin, a heterogeneously sulfated glycosaminoglycan (GAG), consists of α-1,4-linked glucosamine (GlcN) and uronic acid [either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA)] disaccharide repeating units. It has been used as an anticoagulant drug for over 80 years. In addition, it plays significant roles in various biological processes such as inflammation, growth factor regulation, bacterial and viral infection, cell adhesion, cell growth, tumor metastasis, lipid metabolism and diseases of the nervous system. However, the complex heterogeneity of natural heparin polysaccharides has hindered efforts to understand the relationship between their diverse structures and biological functions. While chemical and enzymatic syntheses of heparin oligosaccharides have seen tremendous advances in recent years, it is still challenging to prepare heparin analogs approaching the length of polysaccharides with distinct backbone structures and sulfation patterns. In this project, we are developing a new strategy, where (GlcN-IdoA) disaccharide modules with defined sulfation pattern are synthesized and conjugated through amide bond formation. Novel "head-to-tail" multimers will be accessed to mimic the linear connections in natural GAG polysaccharides.

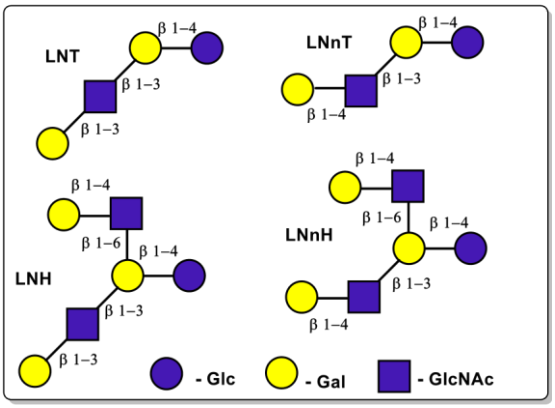
Synthesis of Human Milk Oligosaccharides

Mithila D. Bandara, Keith J. Stine, and Alexei V. Demchenko*

Department of Chemistry and Biochemistry, University of Missouri - St. Louis, One University Boulevard, St. Louis, MO 63121 USA; e-mail: wdbhhf@mail.umsl.edu

Human milk oligosaccharides (HMOs) are a family of structurally related glycans that are highly abundant in human milk. Oligosaccharide fraction is the third largest solid component in human milk after lactose and lipids.[1] There is an accumulating evidence that HMOs can provide significant benefits to the breast-fed infants.[2] However, understanding of the HMO functions is still incomplete due to the lack of individual HMOs in sufficient quantities. We believe that the availability of a library of pure individual HMOs will significantly enhance our ability to study these compounds.

We have been developing new methods for streamlined access to orthogonally protected monosaccharide building blocks and expeditious strategies for the oligosaccharide assembly. Reported herein is the synthesis of common core HMOs[3] including Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNnT), Lacto-N-hexaose (LNH) and Lacto-N-neohexaose (LNnH). Synthesis of HMOs was carried out in solution phase approach.



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Investigation of Substrate Specificity of Sialidases with Membrane Mimetic Glycoconjugates

Sonia Tomar and Xue-Long Sun*

Department of Chemistry, Chemical and Biomedical Engineering and Center for Gene Regulation in Health and Disease (GRHD), Cleveland State University, Cleveland, OH 44115 USA

Sialidases, or neuraminidases, are enzymes that cleave terminal sialic acids (SAs) from the glycans of both glycoproteins and glycolipids. Removal of SAs (desialylation) is an important mechanism in various physiological and pathological processes, such as receptor activation, cellular signaling, platelet clearance and others. To understand the biological significance of desialylation by sialidases, it is important to investigate their specificity with native substrates, either glycoproteins or glycolipids with sialic acids (SAs) in different linkages. Herein, we report the preparation of liposome ganglioside conjugates with different lipids to mimic the native membrane structure of glycolipids for evaluating substrate specificity of sialidases and the lipid effect on sialidase activity. Briefly, liposomes of phosphatidylcholine (PC) and cholesterol with ganglioside (GM3 or GM1) along with different percentage of phosphatidylserine (PS) or phosphatidylethanolamine (PE) were prepared and characterized. Their desialylation profiles with *Arthrobacter ureafaciens* (bacterial) sialidase and H1N1 (influenza viral) sialidase were quantified by HPLC method. A diversity of substrate preference was found for both bacterial and viral sialidase to the liposome ganglioside conjugate platform. This biomimetic substrate provides a better tool for unravelling the substrate specificity and the biological function of sialidases and for screening of functional sialidase inhibitors as well.

Chemoenzymatic Synthesis of Heparan Sulfate Glycopeptide Mimetics and Evaluation of its Biological Functions

Jia Gao[1], Yongmei Xu[2], Jian Liu[2] and Xuefei Huang[1]

[1]Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA
[2]Department of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA

In view of their multiple functionalities within numerous biological events, heparan sulfate proteoglycans (HSPGs) have been of great research interests for a few decades. However, isolation of substantial amounts of homogeneous HSPGs from natural sources for either research studies or clinical treatments has not been possible. This is largely due to the heterogeneous nature of this set of natural macromolecules. Considerable research efforts are being investigated into the chemical synthesis of important heparan sulfate constructs and related glycopeptides. Inspiringly, solid advances have been made in this field. On top of the groundbreaking total chemical synthesis of certain HSPG molecules, chemoenzymatic strategies of synthesis, integrating the advantages of both chemical and enzymatic synthesis, are unveiling their vast potentials, as reflected by improved yields, enhanced regio- and stereoselectivities, and compatibilities with scale-up. This project focuses on the application of chemoenzymatic methods towards the converged synthesis of HSPG mimetics that contains synstatin, a functional peptide derived from HSPG core protein, and investigations on potential synergistic effects from the glycan and synstatin on integrin bindings.

Targeted Covalent Inhibition of *O*-GlcNAc Transferase in Cells

Jiaoyang Jiang[1], Matthew Worth[2], Chia-Wei Hu[1], Hao Li[1], Dacheng Fan[1], Arielis Estevez[1], Dongsheng Zhu[1], and Ao Wang[1]

[1]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53705 USA; [2]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53705 USA

O-GlcNAc transferase (OGT) glycosylates numerous proteins and is implicated in many diseases. To date, most of OGT inhibitors lack either sufficient potency or characterized specificity in cells. We will present the first targeted covalent inhibitor that predominantly reacts with OGT but does not affect other functionally similar enzymes. This study provides a new strategy to interrogate cellular OGT functions and to investigate other glycosyltransferases.

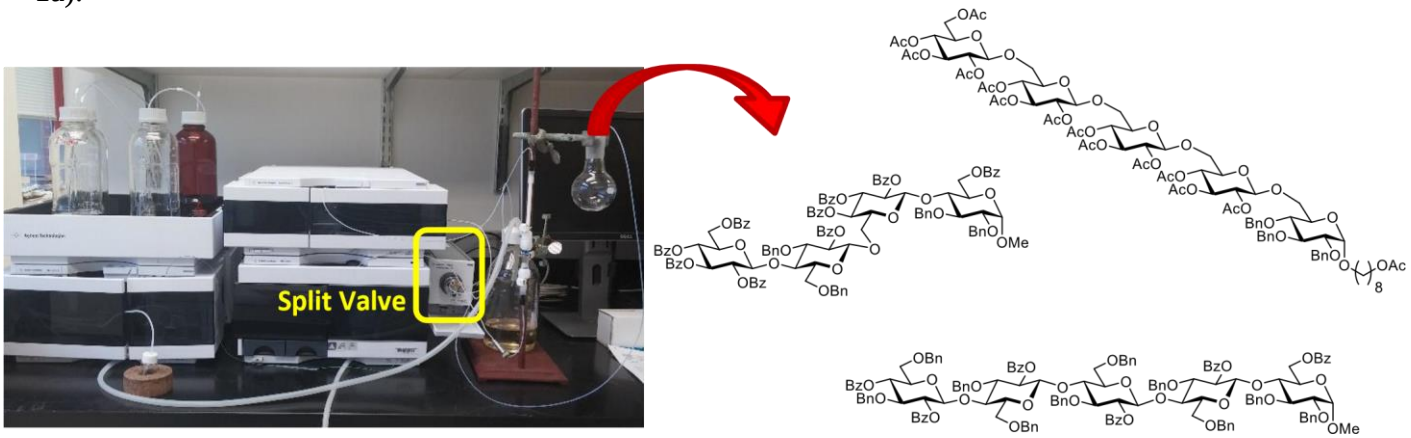
This work was supported by NIH R01 GM121718 and NIH Chemistry-Biology Interface Training Program T32 GM008505 (A. Estevez).

HPLC-Based Oligosaccharide Synthesis: Entirely Automated Glycan Assembly

Matteo Panza, Keith J. Stine, and Alexei V. Demchenko*

Department of Chemistry and Biochemistry, University of Missouri-St. Louis, St. Louis, MO 63121 USA; email: mp887@mail.umsl.edu

Since the introduction of solid supported synthesis of oligosaccharides in the last century [1], the field has seen a substantial progress with the development of automated devices [2]. The HPLC based system, developed by Demchenko and Stine [3], was upgraded with the implementation of an autosampler allowing for the automated delivery of the promoter for glycosylation reaction [4]. Despite that, the system still lacked the components for full automation. Presented herein is the introduction of a new computer operated split-valve module and a programmable autosampler in the design of fully automated synthetic sequences (Figure 1a).



a b

Figure 1. (a) HPLC-based synthesizer equipped with split valve and (b) target glycans

Simple programs have been written and combined into more sophisticated sequences to build target glycans (Figure 1b). All the reagents are delivered automatically through the autosampler while the quaternary pump delivers the solvents necessary for the reactions.

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1

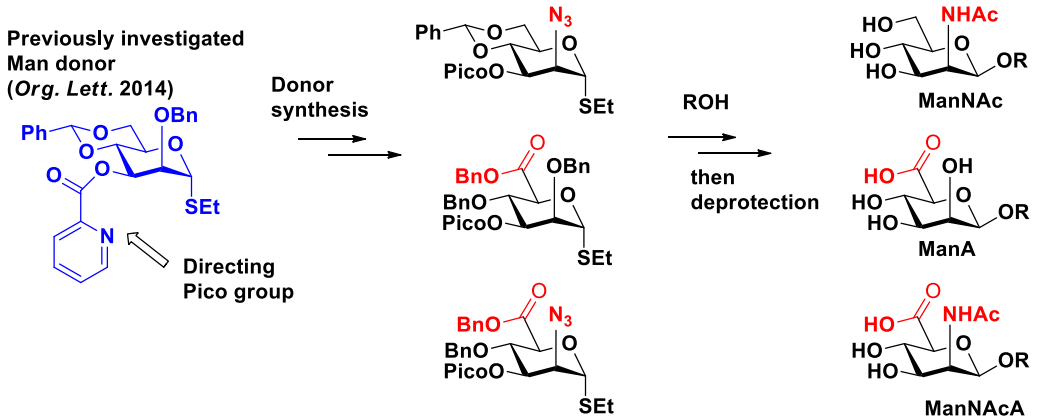
Hydrogen-Bond-Mediated Aglycone Delivery For Stereocontrolled β-Mannosylation

Catherine Alex, Satsawat Visansirikul, Alexei V. Demchenko*

Department of Chemistry and Biochemistry, University of Missouri - St. Louis, MO 63121 USA

Carbohydrates are the essential bio-molecules of life as they form the forefront of interactions with receptors, proteins, pathogens, or neighboring cells. On cell-surfaces, carbohydrates are mostly found as linear or branched glycoconjugates, and a majority of them are linked via either 1,2-*cis* or 1,2-*trans* *O*-glycosidic linkages. The synthesis of 1,2-*trans* glycosides can be reliably achieved via the neighboring group assistance. The synthesis of 1,2-*cis* glycosides is difficult because, beyond weak anomeric effects, there are no forces helping in directing the stereoselectivity.[1] The synthesis of β-mannosides is complicated further because the anomeric effect is working against it. Current methods for β-mannosylation require specialized donors and super-low temperatures.[2]

Hydrogen-bond-mediated aglycone delivery (HAD) method introduced by our lab makes use of remote picolinyl and picoloyl groups that are capable of providing a strong stereodirecting effect.[3] Among a variety of targets and substrates investigated, our lab determined that a highly stereoselective formation of β-mannosides can be achieved via the assistance of the remote 3-*O*-picoloyl group.[4] Presented herein is the extension of the HAD method to the stereoselective synthesis of oligosaccharides containing D-mannosamine (ManNAc), D-mannuronic acid (ManA), and D-mannosamine uronic acid (ManNAcA). These residues are quite abundant in the microbial glycans, wherein they are connected via β-(1,2-*cis*) linkages.



Scheme 1. Overview

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2

Characterizing Spatial and Metabolic Relationships Between the Gut Microbes *Bacteriodes thetaiotaomicron* and *Ruminococcus bromii* During Resistant Starch Degradation

Aathmaja Anandhi Rangarajan[1], Hannah Chia[1], Nicole M. Koropatkin[2] and Julie S. Biteen[1,*]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109 USA; [2]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109 USA

The human gut contains a highly diverse and complex set of microbial communities which greatly impact human health. This diverse microbial community results from an intricate synergy among several species which cross-feed on one another's metabolic byproducts. The factors involved in the maintenance and organization of mixed microbial communities are not well understood. Consumption of resistant starch has been shown to enhance gut microbial diversity, conferring health benefits to humans. Here, we focus on two prominent members of the human gut microbiota: *Ruminococcus bromii* (*Rb*) and *Bacteroides thetaiotaomicron* (*Bt*). *Rb* is a keystone species involved in resistant starch degradation. *Bt*, on the other hand, can metabolize a variety of mono- and oligosaccharides and possesses a well-characterized starch utilization system (Sus) for carbohydrate catabolism. It has been previously shown that *Bt* can utilize metabolic byproducts of resistant starch degradation by *Rb*. However, the spatial and metabolic relationships between *Bt* and *Rb* have not been not well studied.

Here, we aim to study the spatial relationship between *Bt* and *Rb* and characterize the intercellular cross-feeding with live-cell imaging and analytical chemistry. Initial experiments showed that *Bt* can utilize spent rum media isolated after the degradation of potato starch by *Rb*; *Bt* grows significantly less in rum media without *Rb*. Moreover, microscopy revealed that the phenotype of *Bt* is distinctly different under different nutrient conditions: the *Bt* cells had an elongated morphology (length = 2.3 μm) in rum media without *Rb* compared to the cells grown in spent rum isolated from *Rb* grown with resistant starch (length = 1.8 μm). Further analysis revealed that the longer phenotype of *Bt* is due to the presence of sodium carbonate in the absence of additional sugars. These results indicate that *Rb* may release sugars after resistant starch degradation; *Bt* grows to a conventional length of 1.8 μm in these sugars, whereas in the absence of sugars, *Bt* possesses a longer length of 2.3 μm. Finally, to visualize the bacterial cells in mixed communities, we established a two-color labeling method. The fatty-acid binding protein UnaG was chromosomally inserted to produce *Bt* that fluoresces in the blue channel in the presence of bilirubin, whereas *Rb* was labeled with an antibody against amylase-9 and with Alexa Fluor 594 secondary antibody to fluoresce in the yellow channel. Further analysis and modulation of the metabolic byproducts of the resistant starch degradation will enable us to measure and control spatial patterning between *Bt* and *Rb*.

This work was supported by Army Research Office Grant W911NF1810339.

3

Elucidating the Interactome of the Lytic Transglycosylase RlpA of *Pseudomonas aeruginosa*

Luis Felipe Avila, Stefania De Benedetti, Matthew Champion, Shahriar Mobashery

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogenic bacterium. Many existing antibiotics that treat *P. aeruginosa* infections target the cell wall of the organism. Inhibition of synthesis of the cell by β-lactam antibiotics results in bacterial cell lysis. However, drug-resistant *P. aeruginosa* strains have acquired the genes necessary to allow for the detection of β-lactams, which initiates a signaling pathway that culminates in the production of the AmpC β-lactamase, an antibiotic resistance enzyme, which hydrolytically degrades the β-lactam antibiotics. This pathway utilizes the cell-wall recycling process of the organism in its signaling. Lytic transglycosylases (LTs) are enzymes that facilitate cell-wall recycling by catalyzing the cleavage of peptidoglycan of the cell wall. Rare lipoprotein A (RlpA) is one of the 11 LTs of *P. aeruginosa* and little is known of its interactome, the complex assembly of other proteins that interact with it. In the present report, we have identified the putative binding partners of RlpA, using a pull-down approach, followed by mass spectrometry. We have cloned and purified the recombinant proteins detected in our mass spectrometry assay and have explored their interactions with RlpA using a modified dot-blot assay. Verified binding partners will be studied by surface-plasmon resonance and X-ray crystallography. Elucidating the interactome of the LTs, and specifically RlpA, is important in understanding the bacterial physiology of resistance and in aiding drug-discovery efforts.

4

Synthesis of a Comprehensive Heparan Sulfate Tetrasaccharide Library

Kedar N. Baryal[a], Sherif Ramadan[a], Changxin Huo[a], Yuetao Zhao[a], Jian Liu[b], Linda C. Hsieh-Wilson[c] and Xuefei Huang[a]*

[a]Departments of Chemistry and Biomedical Engineering, Michigan State University, East Lansing, MI 48823 USA; [b]Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599 USA; [c]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125 USA

Heparan sulfate (HS) is a member of glycosaminoglycan (GAG) family found on the cell surfaces and extracellular matrices as proteoglycan. It is a linear oligosaccharide with the repeating disaccharide units of glucosamine (GlcN)-α-1→4 linked to uronic acid, which can be either β-D-glucuronic acid or α-L-iduronic acid. The sulfation may occur at *O*-2 of uronic acid and *O*-3, *O-6* of GlcN, and GlcN amine can be sulfated, acetylated or unmodified. HS regulates a wide range of biological functions including blood coagulation, cell differentiation and viral infections.

It is challenging to obtain sufficient amounts of pure HS from natural sources for detailed study of structure-activity relationships (SAR) due to their highly heterogeneous nature. Chemical synthesis helps to access the target HS in pure and sufficient amounts. However, most of the approaches reported in literature till now, are designed for a few specific targets with limited structural variations. Hence, we will report our strategy towards the synthesis of a comprehensive library of HS tetrasaccharides with all the possible *O*-2, *O*-6 and *N*-sulfation motifs.

5

Cs_2CO_3-Mediated Anomeric *O*-Alkylation : Stereoselective Synthesis of *β*-Mannosamines and Synthesis of Representative Bacterial Capsular Polysaccharides

Bishwa Raj Bhetuwal, Fenglang Wu, Shuai Meng, Padam Prasad Acharya, Prakash Thapa, and Jianglong Zhu

Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, OH 43606 USA

2-Amino-2-deoxy-β-mannosides, i.e., β-mannosamines, are found extensively in the capsular polysaccharides (CPS) of various harmful bacteria strains. As a class of 1,2-cis-glycosidic linkages, β-mannosamines are difficult to construct by chemical synthesis due to the steric effect and the absence of anomeric effect. Although a number of strategies have been reported, there is lack of a direct and an efficient approach for the synthesis of 2-amino-2-deoxy-β-mannosides. Recently, our lab developed a direct and efficient method for the stereoselective synthesis of β-mannopyranosides via Cs_2CO_3-mediated anomeric *O*-alkylation. Based on this result, we used similar strategy to develop a feasible approach for stereoselective synthesis of 2-amino-2-deoxy-*β*-mannosides using cesium carbonate-mediated anomeric *O*-alkylation chemistry. In addition, application of this method for stereoselective syntheses of representative bacterial polysaccharides containing *β*-mannosamines will be discussed.

This work was supported by National Institutes of Health Common Fund Glycosciences Program (1U01GM125289) and The University of Toledo.

6

Chiral Benzotetramisole-Catalyzed Site-Selective Acylation of Carbohydrates Directed by Anomeric *S*-Adamantyl Group

Stephanie A. Blaszczyk[1,2], Peng Wen[1], Guozhi Xiao[1], Hua Hao[3], Jessica Wu[1], Bo Wang[1], Francisco Carattino[3], Ziyuan Li[1], Daniel A. Glazier[1,2], Bethany J. McCarty[1,2], Peng Liu[3,*], and Weiping Tang[1,2,*]

[1]School of Pharmacy, University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI 53705 USA; [2]Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, WI 53706 USA; [3]Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260 USA

The prevalent roles of carbohydrates in various physiological and pathological processes have entailed the vast investigation into their chemical synthesis. However, as one of the major classes of biopolymers, carbohydrates pose extra synthetic burdens compared to their proteins and nucleic acids counterparts. One of the major issues is that each carbohydrate building block represents a densely functionalized molecule bearing multiple hydroxyl groups with similar reactivities. An efficient synthesis of polysaccharides would require precise differentiation, and therefore site-selective functionalization of hydroxyl groups in each carbohydrate monomers. Pioneering works have exploited the subtle differences in steric hindrance and relative acidities of hydroxyl groups in particular chemical environments. More recent works rely on the use of chiral organo-catalysts or chelating reagents. On the basis of our previous works, we have discovered that an *S*-adamantyl group at the carbohydrate anomeric position is capable of directing the acylation of the hydroxyl group at *C*2 position in the presence of other free hydroxyl groups on the sugar ring. DFT calculation indicated that the dispersion interactions between the adamantyl C-H bonds and the π-system of the cationic acylated (*R*)-benzotetramisole ((*R*)-BTM) catalyst were the main contributor to the site-selectivity. Further study shows that this method is applicable to more challenging carbohydrate targets with no or minimal protecting groups.

7

Synthesis and Evaluation of Chain-End Functionalized *N*-Glycan Polymers as Biomimetic Macro-Glycoligands

Ka Keung Chan, Joshua Whited, Jinshan Tang, and Xue-Long Sun*

Department of Chemistry, Chemical and Biomedical Engineering and Center for Gene Regulation in Health and Disease (GRHD), Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115 USA

Carbohydrate recognitions are crucial events in many biological processes where glycoproteins, glycolipids, or proteoglycans exist as cell surface carbohydrates involved in cell−cell signaling, immune recognition events, pathogen/host interactions, tumor metastasis, tissue growth, repair, and many more. Glycoscience research specifically concerning the cell surface carbohydrates can provide an abundant opportunity to discover potential therapeutic targets or diagnostic mechanisms for various diseases. In the past decades, glycopolymer, polymers with glycan pendant groups, have been extensively explored as multivalent carbohydrate ligands for studying carbohydrate - protein interactions and for important biomedical applications because they can act as agonists or antagonists for understanding the molecular mechanisms of many biological processes, and also provide tremendous opportunities for therapeutic applications. Herein, we report a straightforward synthesis of chain-end functionalized *N*-glycan polymers from free glycans *via* glycosylamine intermediates followed by acrylation and polymerization *via* cyanoxyl-mediated free radical polymerization (CMFRP) in one-pot fashion. A typical synthetic procedure for *N*-glycan polymers from free monosaccharides, disaccharide, and trisaccharides was demonstrated. Overall, azide- and alkyne-chain end functionalized *N*-glucosyl polymer, *N*-galactosyl polymer, *N*-mannosyl polymer, *N*-GlcNAc polymer, *N*-lactosyl polymer, *N*-α2,6-sialolactosyl polymer, and *N*-α2,3-sialolactosyl polymer were synthesized. No protection and deprotection were used in either glycomonomer or glycopolymer synthesis. The polymer chain-end functionalization capabilities were investigated *via* click chemistry and photo cross-linking chemistry.

8

Exploring bacteriophage Qb virus like particle as a platform to design vaccines for various applications

Zahra Rashidi, Shivangi Chugh, Xuefei Huang*

Department of Chemistry, Michigan State University, East Lansing, MI 48823 USA

Vaccination has been used as a powerful strategy to combat various diseases. Our lab has been exploring the ability of bacteriophage Qb, a virus like particle (VLP) in eliciting immune responses against multiple types of antigens. Considering the VLPs as the nature-designed nanocarrier, we aim at exploring the encapsulation of different cargoes into VLP capsid. We have packaged IL-2, GM-CSF, two cytokines for immune cell activation, and different epitopes of human mucin-1 (MUC1) for T cell activation in separate Qb carriers. Since MUC1 epitopes are self-antigens, we are trying to optimize Qb-construct to overcome their low immunogenicity. In another project, we are constructing a vaccine against enzootic bovine leukosis using peptide-antigen derived from the envelope protein gp51 of Bovine Leukemia Virus. This peptide-antigen has been displayed in a highly organized manner through two different linker engineering strategies, homo-bifunctional nitrophenyl esters and isothiocyanates on the surface of Qb VLP. Our *in vivo* study confirmed the ability of this synthetic peptide vaccine to raise the anti-peptide and anti-gp51 IgG antibodies.

9

Synthesis of Entirely Carbohydrate Immunogen GD2-PS A1 and Production of Anti-GD2 mABs

Vinod Kumar Gattoji, Peter R. Andreana

Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606 USA

Aberrant glycosylation is the hallmark of malignant transformation of the normal healthy cells. These altered patterns of glycocalyx on the cancer cells are called Tumor Associated Carbohydrate Antigens (TACAs). These TACAs are poorly immunogenic i.e., they elicit T-cell independent immune responses and hence they are conjugated to carrier proteins like KLH, CRM197 to elicit T-cell dependent immune responses. Due to the carrier epitope suppression, capsular polysaccharides are conjugated instead of carrier proteins. PS A1, one of the capsular polysaccharide is a T-cell immune stimulating immunogen conjugated to TACAs synthesized in our lab. In part of our research, we are working on disialoganglioside GD2 which is a TACA for melanomas and neuroblastomas. Once synthesized, these TACAs are conjugated to PS A1 to make TACA-PS A1 conjugates. Previously we have reported TACA-PS A1 conjugates such as Tn-PS A1 and STn-PS A1, elicit robust MHC II dependent immune responses which leads to CD4+ T-cells proliferation and antibody production. GD2 is disialogangliside which is ranked 12 among 75 potential antigens based on therapeutic function, immunogenicity, role of antigen in oncogenicity, specificity, expression level. GD2 is oncofetal antigen which is expressed during fetal development and in mature neurons. Disialoganglioside GD2 is a tumor associated carbohydrate antigen (TACA) expressed on neuroblastomas (NB), a cancer which starts in early nerve cells i.e., neuroblasts of the sympathetic nervous system. Approximately 700 children under the age of five, are diagnosed with this cancer within the United States every year and it accounts for 6% of all childhood cancers. 99% of the NB cells have GD2 expressed on their cell surface and approximately 5-10 million molecules of GD2/cell. Over past two decades there has been immense demand for Anti-GD2 mAbs to high risk neuroblastomas. Unituxin®, an FDA approved monoclonal antibody (mAb) against neuroblastomas is the best proven immuno-therapeutic but has many limitations such as infusion reactions, bone marrow suppression, electrolyte abnormalities, etc. This therapeutic is also highly expensive. In an attempt to validate our entirely carbohydrate-based immunogen and seek to overcome some downfalls of Unituxin, we envisaged a strategy to chemically prepare a linker-free construct by synthesizing an aminooxy derivative of GD2 and then conjugating it to PS A1, isolated from B.fragilis ATCC 25285/NCTC 9343 to get GD2 specific mAbs.

10

Dynamics of Three Carbohydrate Utilization System Glycoside Hydrolases Probed by Single Molecule Fluorescence Measurements

Laurent Geffroy[1], Haley A. Brown[2], Nicole M. Koropatkin[2] and Julie S. Biteen[1]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109 USA; [2]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109 USA

The gut microbiota is made up of thousands of organisms whose composition is dictated by the available glycans. The well-studied starch utilization system (Sus) of *Bacteroides thetaiotaomicron* (*Bt*) is considered a prototype for glycan uptake by the bacteria. Interestingly, Sus includes five outer-membrane proteins (OMPs) which interact to bind and translocate starch into the periplasm. In particular, SusG is an α-amylase which breaks down the polymer into oligosaccharides. Although the molecular mechanisms of this system are well known, the dynamics and cooperativity of the Sus OMPs are still not fully characterized. In our previous studies, we used single-molecule and super-resolution fluorescence microscopy in single cells to show that fluorescently labeled SusG is mobile in the *Bt* outer membrane and diffuses slowly in the presence of starch.

Here, we compare the dynamics of three different glycoside hydrolases (GH): SusG, Bt4668, and Bt1760, which respectively target starch, galactan, and levan. Interestingly, these surface GH enzymes can also be co-expressed in a mixture of these carbon sources. First, we measured the mobility of each protein in its cognate glycan and observed that the hydrolase diffusion is more heterogeneous in the presence of the polymer than in glucose: some molecules move very slowly—perhaps due to substrate binding—whereas others diffuse very rapidly. Second, we tracked each protein in a mixture of carbohydrates to probe interactions between different carbohydrate utilization systems. Overall, these experiments provide dynamical data on three different carbohydrate utilization systems to both test and complete the paradigm for glycan uptake.

This work was supported by NIH Grant R01-GM118475-01.

11

Synthesis of Fluorogenic Trehalose and Trehalose Monomycolate Analogues for the Detection of Mycobacteria

Dana M. Gepford, Nicholas Banahene, Cameron J. Lee, Jessica M. Groenevelt, Yen-Pang Hsu, Michael S. VanNieuwenhze and Benjamin M. Swarts*

Department of Chemistry and Biochemistry, Central Michigan University, Mount Pleasant, MI 48858 USA

Mycobacterium tuberculosis (*Mtb*) manifests itself in the form of human tuberculosis, which claims 1.3 million lives each year and is one of the top ten leading causes of deaths in the world. Although early detection can significantly reduce mortality rates for tuberculosis, currently available diagnostics have limitations with respect to specificity, sensitivity, and/or cost effectiveness. This research is aimed at the synthesis of new trehalose-based probes, which are designed to label mycobacteria via specific pathways and thus may enable rapid and accurate detection of *Mtb* infections. Here, the syntheses of trehalose and trehalose monomycolate (TMM) analogues bearing molecular rotor-based fluorogenic moieties that operate through a twisted intramolecular charge transfer (TICT) mechanism are reported. It is hypothesized that upon incorporation into the densely packed mycobacterial outer membrane, probe rotation will be restricted, generating fluorescence. For comparison, a previously reported solvatochromism-based fluorogenic trehalose analogue and its TMM version were also synthesized.

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Pharmacological Targeting of O-GlcNAc Readers via Glycopeptide Mimetics

Jessica M. Groenevelt[†], Laura Hanselman[‡], Daniel Corey[†], Charlie Fehl *[,†]

[†]Department of Chemistry, Wayne State University, Detroit, MI 48202 USA;
[‡]Department of Chemistry, Kalamazoo College, Kalamazoo, MI 49006 USA

The transcription factor FOXO1 has been identified as a metabolic regulator subject to control via insulin dependent post translational modifications (PTMs) including O-GlcNAcylation. Given that sugar PTMs are dependent upon metabolic pathways, energy disorders such as diabetes can greatly alter the glycosylation levels and in turn glycoprotein signaling. In insulin resistant models increased O-GlcNAcylation on FOXO1 has shown an ability to activate transcription of gluconeogenic genes but the key PTM sites for glycoprotein signaling as well as potential readers for these sites have yet to be identified. Transcription factors are largely undruggable targets but readers of their PTMs could present a more accessible but equally potent target. Using solid phase peptide synthesis it is possible to design glycopeptide mimics of these PTM sites to drug these readers. By incorporating a cell-penetrating peptide and nuclear localization sequences, fluorophore, and a non-cleavable GlcNAc moiety we are designing glycopeptide mimics that can be delivered directly to the nucleus to probe the impact of O-GlcNAcylation on FOXO1 activation and regulation of glucose metabolism. Ultimately our glycopeptide mimics will allow for investigation into the role O-GlcNAc signaling plays in metabolic diseases.

This work is generously supported by WSU Chemistry Startup Fund and Wayne State University Research Grant.

13

Synthesis of Trisaccharide Oligomer of *Salmonella* E1 Capsular Polysaccharide *via* Anomeric *O*–Alkylation

Ishani Lakshika Hettiarachchi,[1] Xiaohua Li,[2] and Jianglong Zhu[1]

[1]Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, OH 43606 USA; [2]Department of Natural Sciences, University of Michigan-Dearborn, Dearborn, Michigan, 48128 USA

Salmonella sp has gained spotlight as an infectious agent due to the vast number of severe infections and lack of effective treatments. These circumstances have arisen because of the emergence of *Salmonella* sp that become antibiotic resistant, rendering infection by the strains very challenging to treat. It is a Gram negative foodborne pathogen, difficult to eliminate from its reservoir hosts, and food animals often serve as reservoirs of the pathogen. It causes highest number of illnesses, hospitalizations, and deaths allied with foodborne illness. *Salmonella* sp is a diverse species of bacteria consisting of more than 2500 different serotypes and most of them are multidrug- resistant. Therefore capsular polysaccharides (CPS) have been potential candidates for the synthesis of vaccines against *Salmonella* infections which is nontoxic and have the high immunogenic ability.

The capsular polysaccharide contains galactose, mannose and fucose monomers in its trisaccharide oligomer repeating units with α and β linkages. Construction of β mannopyranosidic linkage is a long-lasting challenge in glycobiology. Recently our group has reported an efficient method for the stereoselective construction of β mannopyranosidic linkages via anomeric *O*-alkylation. This method governs through the kinetic anomeric effect in conjunction with chelation control of cesium metal and has been used to synthesize the *Salmonella* E1 trisaccharide.

This work was supported by National Science Foundation (CHE-1464787), National Institutes of Health Common Fund Glycosciences Program (1U01GM125289), The University of Toledo, and University of Michigan–Dearborn.

14

Site-Specific Glyco-Engineering of Recombinant Thrombomodulin

Hailong Zhang, Joseph Keil, Xia Liu, and Xue-Long Sun*

Department of Chemistry and Chemical & Biomedical Engineering, Center of Gene Regulation of Health and Disease (GRHD), Cleveland State University, Cleveland OH 44115 USA

Thrombomodulin (TM), a membrane glycoprotein predominately expressed on endothelium, plays essential roles in keeping local haemostatic balance. Particularly, TM modulates the activity of thrombin from a procoagulant to an anticoagulant protease. When bound to TM, thrombin activates plasma protein C, which selectively inactivates coagulation factors Va and VIIIa. At the same time, TM-bound thrombin is unable to cleave fibrinogen or to activate platelets diminishing its procoagulant activity. To closely mimic the glycoprotein structural feature of native TM, we proposed a site-specific glyco-engineering of recombinant TM with glycopolymer and chondroitin sulfate. Briefly, recombinant TM containing the epidermal growth factor (EGF)-like domains 4, 5, and 6 (rTM_{456}) with a *C*-terminal azidohomoalanine or a C-terminal LPETG tag was expressed in *Escherichia coli* and was modified with a glycopolymer and chondroitin sulfate *via* copper-free click chemistry or sortase A-mediated ligation respectively. The glyco-modification of rTM_{456} was confirmed with SDS-PAGE, Western blot and protein C activation assay, respectively. The reported site-specific end-to-end protein glyco-conjugation approach facilitates uniform protein glycoconjugate formation *via* biocompatible chemistry and in high efficiency. The proposed biomimetic rTM_{456}-glycopolymer conjugate is expected to be a potential anticoagulant with enhanced pharmacokinetic properties.

15

Synthesis of Tetrahydrolistatin Congeners for Inhibition of *Mycobacterium tuberculosis* Protein Antigen85C

Saniya Shehbaz Khan

University of Toledo, Toledo OH 43606 USA

Mycobacterium tuberculosis (Mtb) is wrapped in a complex cell envelope which has inner and outer layers of membranes. The inner layer is primarily made of mycolyl arabinogalactan (mAG), whereas, the outer layer is made of glycolipids, phospholipids, sulpholipids, trehalose dimycolate (TDM) and trehalose monomycolate (TMM). This protective covering helps in survival of *Mtb*. Ag85C is essential for building of lipid rich cellular sheathing layers of *Mtb*. Ag85C facilitates the transfer of mycolic acid (mA) from TMM to arbanigalactan (AG), forming mAG during the process, while releasing free trehalose in pseudoperiplasmic space. Tetrahydrolipstatin (THL), a renowned covalent inhibitor of serine esterases and has a minimum inhibitory concentration (MIC) of 5µg/ml against the growth of *Mtb*. Serine^{124} of Ag85C attacks the carbonyl center of β-lactone ring of THL yielding a β-hydroxy ester. This results in covalent inhibition of Ag85C.

In our lab, we are substituting the hexanoyl chain of THL with different hydrophobic groups in order to increase the selectivity of β-lactones against Ag85C. This library will facilitate the structure-activity relationship (SAR) of these compounds against *Mtb.* Additional aspirations are to explore the activity–based protein profiling (ABPP) of potential cellular targets of THL by using click chemistry and a chemical proteomic approach. The results of SAR studies of the THL derivatives against *Mtb* might prove to be advantageous in improving the potency and specificity of the drug.

16

Design and Synthesis of a Bifunctional Inhibitor Against *Mycobacterium tuberculosis* Antigen 85 Complex

Alexander Landgraf[1], and Steven J. Sucheck[1]

[1]Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606 USA

The Antigen 85 complex (Ag85s) is composed of a family of three proteins Ag85A, Ag85B and Ag85C. This group of proteins is essential for the maintenance of the hydrophobic cell wall of *Mycobacterium tuberculosis* (*Mtb*), the organism responsible for tuberculosis (TB). Ag85s does this by catalyzing the transfer of mycolic acids forming trehalose dimycolate (TDM), trehalose monomycolate (TMM), and mycolyl-arabinogalactan (m-AG) which are all important components of the outer envelope of *Mtb*. It has been shown that inhibition of any member of the Ag85s leads to a decrease in the virulence and drug resistance of *Mtb*. Tetrahydrolipstatin, an FDA approved lipid esterase inhibitor, is shown to covalently inhibit Ag85C though interactions with the active site serine. It is also shown that allosteric inhibition of Ag85C can be achieved by covalent modification of cystine 209. The non-catalytic cysteine is only 6.7 Å away from the active site. Our lab is focusing on making a dual inhibitor of both the active site serine and the allosteric cystine. This will be achieved by modifying the amino acid side chain of THL to include a thiol reactive group that would be able to reach and react with cystine 209. We will use Micheal acceptors as our thiol reactive group. They will be appended to the back bone of THL which will be synthesized using a regioselective bimetallic $[\text{Lewis acid}]^{+}[Co(CO)_4]^{-}$ catalyzed carbonylation to generate the lactone ring. This dual-warhead design should increase selectivity and activity of THL and lead to a new class of inhibitor for Ag85s.

17

^{13}C-^{13}C Spin-Coupling Constants in Crystalline ^{13}C-Labeled Saccharides: Conformational Effects Interrogated by Solid-State ^{13}C NMR Spectroscopy

Wenhui Zhang,[1] Mi-Kyung Yoon,[1] Reagan J. Meredith,[1] Jieye Lin,[1] Jaroslav Zajicek,[1] Allen G. Oliver,[1] Matthew Hadad,[1] Michael H. Frey,[3] Ian Carmichael,[2] and Anthony S. Serianni[1]*

[1]Department of Chemistry and Biochemistry; [2]Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556-5670 USA; [3]JEOL USA, Inc., 11 Dearborn Rd., Peabody, MA 01960 USA

Solid-state ^{13}C NMR spectroscopy has been used in conjunction with selectively ^{13}C-labeled mono- and disaccharides to measure ^{13}C-^{13}C spin-couplings (J_{CC}) in crystalline samples. This experimental approach allows direct correlation of J_{CC} values with specific molecular conformations since, in crystalline samples, molecular conformation is essentially static and can be determined by x-ray crystallography. J_{CC} values measured in the solid-state in known molecular conformations can then be compared to corresponding J_{CC} values calculated in the same conformations using density functional theory (DFT). The latter comparisons provide important validation of DFT-calculated *J*-couplings, which is not easily obtained by other approaches and is fundamental to obtaining reliable experiment-based conformational models from redundant *J*-couplings by *MA'AT* analysis (Zhang *et al*., *J. Phys. Chem. B.* **2017**, *121*, 3042–3058). In this work, representative $^{1}J_{CC}$, $^{2}J_{CCC}$ and $^{3}J_{COCC}$ values were studied quantitatively as either intra-residue couplings in the aldohexopyranosyl rings of monosaccharides or inter-residue (trans-glycoside) couplings in disaccharides. The results demonstrate that (a) accurate J_{CC} values can be measured in crystalline saccharides that have been suitably labeled with ^{13}C, and (b) DFT-calculated J_{CC} values compare very favorably to those determined by solid-state ^{13}C NMR when molecular conformation is a constant in both determinations. [This work was supported by the National Science Foundation, CHE 1707660.]

18

Design, Synthesis and Screening of Carbohydrate Derivatives for Cancer Therapy

Mengxia Sun[1], Kunli Liu[1], Jian Hu[1,2], Xuefei Huang[1]

[1]Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA;
[2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48864 USA

Carbohydrate derivatives can be useful in modulating biological functions. Targeted cancer therapy is a treatment using certain substances blocking the growth and metastasis of cancer cells by interfering with specific molecules involved in carcinogenesis, tumor growth, and metastasis. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway is a signaling pathway that regulates several cellular processes including cell growth, survival, differentiation, and metabolism. The aberrant activation of PI3K/AKT/mTOR pathway has been detected in many different types of cancer, including prostate, breast and brain cancers. In the last decade, numerous studies have been carried out for the development of inhibitors of PI3K, AKT, and mTOR. In our lab, we are focusing on an upstream lipid kinase, phosphatidylinositol 4-phosphate 5-kinase (PIP5K), which predominantly produces phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]. PI(4,5)P2 is the substrate of PI3K and the generated PI(3,4,5)P3 recruits AKT to the cell membrane and initiates the signal cascades. Studies have shown that the growth of the tumor was inhibited by knocking down PIP5K in mice. The native substrate of PIP5K is PI4P, which consists of two hydrophobic fatty acid chains and a hydrophilic inositol head group with a phosphate group on the 4-position. In this study, we designed and synthesized a series of galactose based PI4P mimetic compounds, which are anticipated to bind to the substrate-binding pocket of PIP5K. Lipid kinase assays are being performed to test the inhibitory activities of these mimetics.

In the second part of the poster, we will present our efforts using carbohydrate derivatives to aid in drug delivery, which can potentially achieve higher efficiency and selectivity in delivering drugs to tumor sites. Cluster of differentiation 44 (CD44) is a cell adhesion protein overexpressed on cancer cell surface. The principle receptor of this protein is hyaluronic acid (HA), which is a polysaccharide consisting of repeating units of glucuronic acid and *N*-acetyl-D-glucosamine. HA is widely used as a targeting molecule for drug delivery to cancer cells. HA can not only bind to CD44, but also to other HA receptors, including HA receptor for endocytosis (HARE) and lymphatic vessel endothelial HA receptor-1 (LYVE-1). For drug delivery purposes, the binding of HA toward HARE and LYVE-1 is not desirable, since it would decrease the specificity and efficiency of drug delivery to CD44+ tumors. The goal of this project is to use the Ugi reaction to modify the carboxyl group on HA and to screen for HA derivatives that have stronger and more specific bindings toward CD44. Competitive enzyme-linked immunosorbent assay (ELISA) was used as the tool in screening. Compounds with stronger and more selective binding to CD44 were identified.

19

Mechanistic Investigations of β-Mannosylation via Cs_2CO_3-Mediated Anomeric *O*- Alkylation and Synthesis of Complex Biologically Significant Carbohydrate Molecules

Shuai Meng,[1] Bishwa Raj Bhetuwal,[1] Hai Nguyen,[1] Xiaotian Qi,[2] Cheng Fang,[2] Kevin Saybolt,[3] Xiaohua Li,[3] Peng Liu,[2] and Jianglong Zhu[1]

[1]Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo OH 43606 USA; [2]Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260 USA; [3]Department of Natural Sciences, University of Michigan-Dearborn, Dearborn, MI 48128 USA

β-Mannopyranoside is one of the key glycosidic linkages existing in numerous biologically significant targets, such as *N*-linked glycans, bacterial capsular polysaccharides, fungal metabolites, glycolipids, and etc. Stereoselective construction of β-mannopyranosides, however, remains as a long-standing challenge due to the steric effect of the axial C2- substituents as well as the absence of anomeric effect and anchimeric assistance, albeit remarkable success has been achieved. Recently, we developed a direct and efficient method for the stereoselective synthesis of β-mannopyranosides *via* Cs_2CO_3-mediated anomeric *O*-alkylation of mannose-derived lactols with suitable electrophiles. Based on the previous work, several structural features which are critical to this reaction have been revealed through extensive chemical experiments and computational studies. In this presentation, we will describe our mechanistic investigations of this β-mannosylation methodology. In addition, the application of this β-mannosylation to the syntheses of selected complex carbohydrate targets will also be discussed.

This work was supported by National Science Foundation (CHE-1464787), National Institutes of Health Common Fund Glycosciences Program (1U01GM125290 to P.L. and 1U01GM125289 to J.Z.), The University of Toledo, and University of Michigan–Dearborn.

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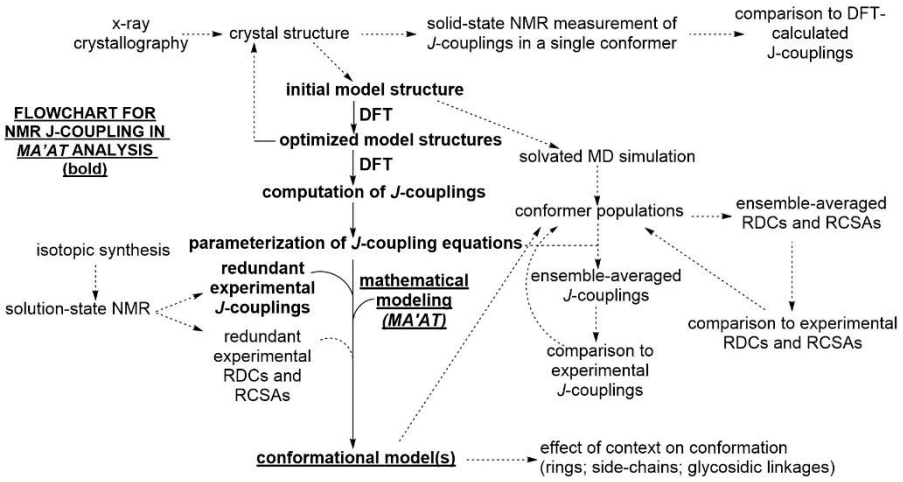
MA'AT Circular Statistics Software Development and Applications To Carbohydrate Structure Determination

Reagan J. Meredith,[1] Luke Sernau,[2] and Anthony S. Serianni[1]

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA; [2]Facebook Inc., Seattle, WA 74793 USA

Elucidating the structures of saccharides in solution remains challenging because they contain multiple, flexible, and often correlated conformational elements. This property has led to a heavy reliance on molecular dynamics (MD) simulations and related calculations to predict solution conformations and dynamics. Few experimental techniques are available to validate the results obtained from these simulations. We describe here the underlying mathematics and applications of a new technique called *MA'AT* analysis for the determination of saccharide conformation and dynamics in solution. *MA'AT* combines density functional theory (DFT) calculations and experimental NMR spin-couplings using circular statistics to generate single- and multi-state population distribution models (see chart). To date, the *MA'AT* method has been applied to different conformational elements/domains in saccharides, including *O*-glycosidic linkages (Zhang *et al*., *J. Phys. Chem. B.* **2017**, 121, 3042–3058; Zhang *et al*., *Biochem.* **2019**, 58, 546-560), furanose ring pseudorotation, exocyclic hydroxyl and hydroxymethyl group rotation, and *N*- and *O*-acetyl side-chain behavior (Turney *et al*., *J. Phys. Chem. B.* **2017,** 121, 66-77). For domains whose conformational properties are well characterized, the *MA'AT* method and MD simulations produce consonant continuous population models with small differences in mean molecular torsion angles and circular standard deviations. For domains whose conformational behaviors are less well understood, *MA'AT* and MD have yielded significantly different population models, presumably due to limitations in the force-fields used in the simulations. [This work was supported by the National Science Foundation, CHE 1707660.]

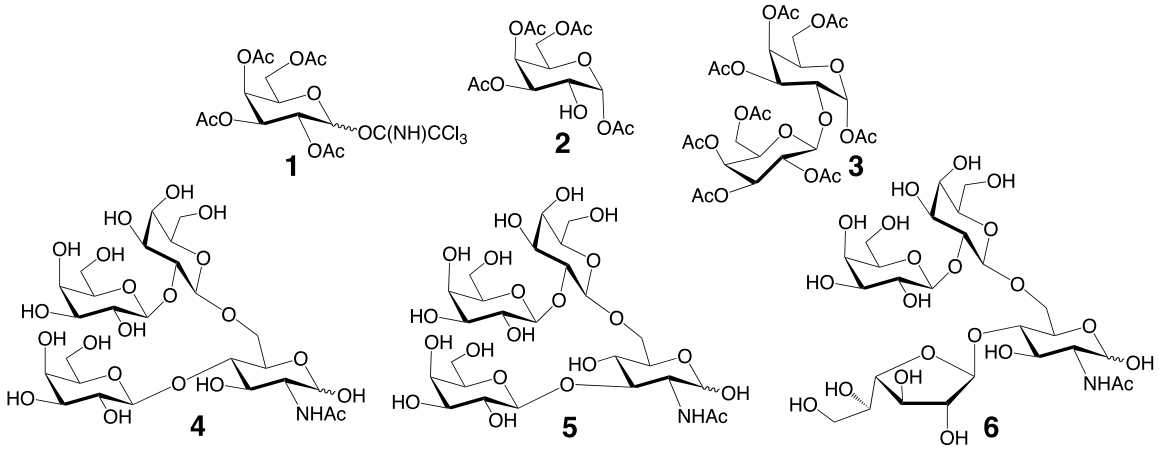


21

An Unusual Glycosylation Side-Reaction and Its Application in Glycan Synthesis

Qingfeng Pan and Anthony S. Serianni
Omicron Biochemicals, Inc., 115 South Hill Street, South Bend, IN 46617-2701 USA

An unusual and synthetically useful side-reaction occurs during chemical glycosylation involving a peracetylated D-galactohexopyranosyl trichloroacetimidate donor (**1**) and less-active glycosylation acceptors. The 2-OAc group on unreacted or excess **1** migrates to the anomeric center, displacing the trichloroacetimidate group to presumably give **2** as an intermediate, and the latter subsequently reacts with another molecule of **1** to give the peracetylated disaccharide, β-D-Gal*p*-(1→2)-D-α-Gal*p* (**3**) in 59% yield based on **1**, and whose structure was confirmed by MS and NMR analyses. This heretofore unreported side-reaction eliminates multi-step orthogonal syntheses of this common Gal-Gal motif observed in the glycan chains of *Trypanosoma cruzi* glycoproteins and in other biologically important glycans. Tetrasaccharides β-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→6)-[β-D-Gal*p*-(1→4)]-D-Glc*p*NAc (**4**), β-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→6)-[β-D-Gal*p*-(1→3)]-D-Glc*p*NAc (**5**) and β-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→6)-[β-D-Gal*f*-(1→4)]-D-Glc*p*NAc (**6**) were successfully synthesized from **3** after trichloroacetimidate activation. The β-linkages introduced using **3** as a trichloroacetimidate donor were obtained diastereoselectively due to the "nitrile effect" (Wulff, G.; Röhle, G. *Angew. Chem. Inter. Ed.*, **1974**, *13*, 157—170).

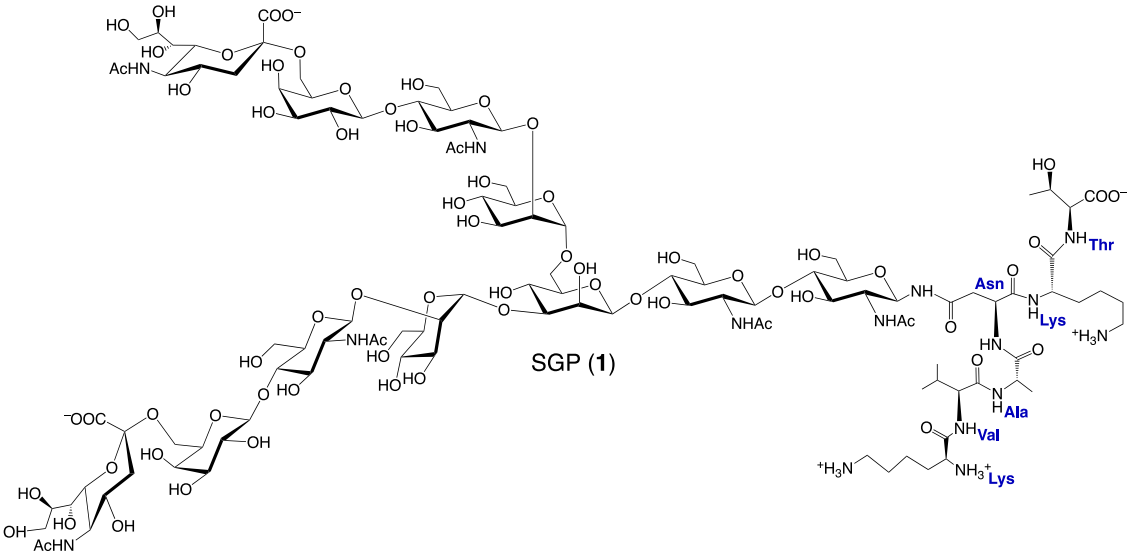


22

Isolation of Sialyl Glycopeptide from Commercial Hen Egg Yolk Powder

Rebecca Pan[1], Reagan Meredith[2] and Anthony S. Serianni[2]
[1]St. Joseph High School, 453 N. Notre Dame Ave., South Bend, IN 46617 USA;
[2]Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA

Sialylglycopeptide (SGP) (**1**) is an 11-residue *N*-glycan commonly found in hen egg yolk that contains two sialyl bi-antennary arms and a hexapeptide fragment, and has gained popularity as a starting material for the semi-synthesis of *N*-glycans. Two recently reported methods (J. Liu *et al.*, *Carbohydr. Res.* **2017**, *452*, 122–128; K. Alagesan and D. Kolarich, *MethodsX* **2019**, *6*, 773–778) to isolate **1** from commercially available hen egg yolk powder were revisited. Crude extracts were analyzed by SDS gel electrophoresis on a Tris-Tricine polyacrylamide gel. This electrophoretic assay provides convenient monitoring of the purification of **1** through final column chromatography purification steps. To mitigate the relatively slow flow rates through carbon/Celite chromatographic columns, the initial extract was passed through a 10-kD membrane filter prior to subsequent purification. LC-MS analysis of the membrane filtrate applied to an amide HILIC column (Waters XBridge BEH Amide) indicated the presence of **1** in amounts comparable to those reported previously (~0.8 mg per g of egg yolk). Ongoing purification of membrane filtrates by ion-exchange (DEAE Sephadex A-25) and/or size exclusion (Sephadex G-25) chromatography are expected to yield purities of **1** ranging from 95–98% as determined by high-resolution ^{1}H and $^{13}C\{^{1}H\}$ NMR spectroscopy. [This work was supported by the National Science Foundation, CHE 1707660.]



23

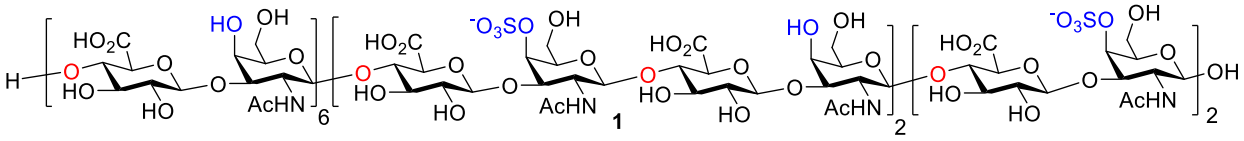
Synthesis of Chondroitin Sulfate (CS) Oligosaccharide Library and CS chain (24-mer) of Bikunin and its Anti-inflammatory Activity

Sherif Ramadan, Tianlu Li, Zeren Zhang, Xuefei Huang*
Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA

Chondroitin sulfates (CSs) and chondroitin sulfate proteoglycans (CSPG) belong to the family of glycosaminoglycans (GAGs). Those oligosaccharides are consisted of repeating disaccharide units of D-glucuronic acid (GlcA) β-1,3- linked to 2-acetamido-2-deoxy-D-galactosamine (GalN). The CS chains are linked to the carrier proteins through a serine residue forming chondroitin sulfate proteoglycans (CSPG). CSs and CSPGs interact with a large variety of biological molecules such as growth factors, chemokines, Aβ, and cell adhesion molecules. As a result, they play important roles in many biological events, including bacterial and viral infection, cancer metastasis, central nervous system development, regeneration after neural injury, and Alzheimer's disease.

The sulfation patterns of CS can be important determinants of their biological functions. As sulfation patterns in naturally existing CS are highly heterogeneous, it is challenging to decipher the detailed structure function relationship using CS isolated from nature. Hence, a divergent synthetic route towards a library of CS tetrasaccharides bearing all major sulfation patterns will be presented.

Bikunin, also known as urinary trypsin inhibitor, is a CSPG that is released in human plasma and urine. It is one of the main anti-inflammatory mediators that could inhibit many serine proteases such as trypsin, elastase, and thrombin. Clinically, it has been used as a drug to treat patients with acute inflammatory disorders such as pancreatitis, shock and disseminated intravascular coagulation. In this poster, we report the synthesis of the CS chain in bikunin for a better understanding of the roles of the GAG chain and the protein on the inflammatory response. For the first time, the longest CS chain to date (24-mer, glycan **1**) has been produced through chemical synthesis in an excellent yield on a mg scale. Furthermore, we demonstrate that both the glycan and the core protein moiety are important for anti-inflammatory activity of bikunin.



24

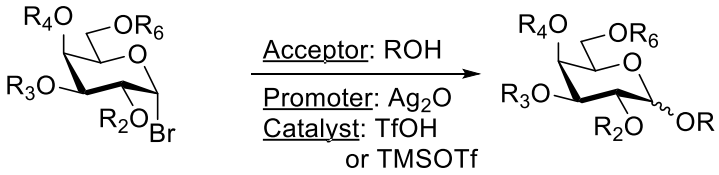
Stereocontrolled Synthesis of Galactosides using Acid-Catalyzed Koenigs-Knorr Reaction

Melanie Shadrick, Yashapal Singh, and Alexei Demchenko*

Department of Chemistry, University of Missouri-St. Louis, 1 University Blvd, St. Louis, MO 63121 USA

In spite of many methods developed for the synthesis of oligosaccharides,[1] glycosyl halide donors introduced by Arthur Michael[2] continue to find wide application.[3] Under classical Koenigs-Knorr reaction conditions,[4,5] a glycosyl bromide (or chloride) donor is coupled with a glycosyl acceptor (alcohol, ROH) in the presence of silver oxide (or carbonate). This reaction is slow, and even glycosidations of reactive, per-benzylated donors require many hours (or even days) to produce the respective glycoside products. This reaction is particularly sluggish with less reactive per-benzoylated bromides.

Recently, we reported that glycosidation of glycosyl bromides in the presence of silver(I) oxide can be dramatically accelerated in the presence of catalytic TMSOTf.[6] Our previous studies have been primarily focusing on glycosyl donors of the D-*gluco* and D-*manno* series. Presented herein is our first attempt to investigate this reaction in application to differentially protected glycosyl bromides of the D-*galacto* series. Excellent yields and stereoselectivity has been achieved for a broad range of substrates and targets.



Galactosyl donors:
R_2=R_3=R_4=R_6=Bn
R_2=R_3=R_4=R_6=Bz
R_2=R_3=R_4=Bn, R_6=Bz
R_2=R_3=R_6=Bn, R_4=Bz

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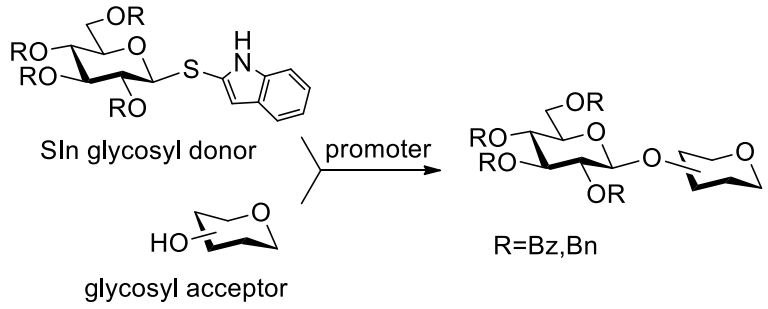
Indolyl Thioimidates as New Building Blocks for Chemical Glycosylation

Ganesh Shrestha, Matteo Panza, and Alexei V. Demchenko*

Department of Chemistry and Biochemistry, University of Missouri—St. Louis,
One University Boulevard, St. Louis, MO 63121, USA

Over the last decade, the thioimidate approach has evolved into a very robust methodology for chemical glycosylation.[1] Particularly, with the introduction of novel *S*-benzoxazolyl (SBox), *S*-thiazolinyl (STaz) and *S*-benzimidazolyl (SBiz) leaving groups,[2-6] it has become apparent that thioimidates can withstand many reaction conditions associated with protecting group manipulations. In addition, thioimidates are easily accessible from a variety of simple precursors. This class of glycosyl donors can typically be glycosidated under a range of relatively mild activation conditions. Superior stereoselectivity in comparison to other glycosylation methods is often achieved, and the thioimidoyl moiety can be selectively activated in the presence of other types of leaving groups. All of these important traits for both glycoside and oligosaccharide synthesis are rarely found in one class of a leaving group.

Capitalizing on our previous success with developing new methods for chemical glycosylation, presented herein are our first steps towards the development of a new class of glycosyl donors, indolyl thioimidates. The S-indolyl leaving group shows good stability towards protecting group manipulations, but it can be readily activated for glycosylation. Among a variety of promoter systems that are available for the activation of novel thioimidates, silver carbonate and trimethylsilyl trifluoromethanesulfonate (Ag_2CO_3/TMSOTf) provided the most promising results in terms of the reaction rates, stereoselectivities, and yields.



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26

Synthesis of Carbocyclic Maltose Derivatives Used as Potential Inhibitors of *Mycobacterium tuberculosis* GlgE

Anshupriya Si and Steven J. Sucheck*

The University of Toledo, Department of Chemistry and Biochemistry, Toledo, OH 43606 USA

Tuberculosis (TB) is a serious infectious disease and now it is global health threat. The persistence of TB globally is due to the hardiness of the bacterium *Mycobacterium tuberculosis* (*Mtb*), and the ability of *Mtb* to enter a dormant phase complicates the treatment. GlgE is a bacterial maltosyltransferase that catalyzes the elongation of a cytosolic, branched α-glucan. In *Mycobacterium tuberculosis* (*Mtb*), inactivation of GlgE (*Mtb* GlgE) results in the rapid death of the organism due to a toxic accumulation of the maltosyl donor, maltose-1-phosphate (M1P), which suggests that GlgE is a fascinating target for inhibitor design. GlgE is an α-maltose-1-phosphate:(1 → 4)-α-D-glucan-4-α-D-maltosyltransferase that catalyzes the addition of maltose to maltooligosaccharides. The essential biological roles played by glycosidic hydrolases and the great medicinal benefits of pharmacologically targeting these enzymes provides a premise for our work. Cyclophellitol epoxides, aziridines, 1,6-epi-cyclophellitol cyclosulfate and derivatives of valienamine are reported as α-glucosidase inhibitors. Inspired by this we proposed to synthesize carbocyclic mimics of maltose which include epoxide, aziridine and cyclosulfate moieties. Our synthesis started with a β-thio maltoside which was treated with *N*-bromosuccinimide to obtain a maltose hemiacetal. Then Wittig olefination was performed to obtain the vinyl glycoside. Oxidation of the secondary alcohol using Moffatt oxidation followed by stereoselective addition of vinyl magnesium bromide afforded the allyl alcohol. Intermolecular olefin metathesis using a first generation Grubbs catalyst gave the carbasaccharide. *Cis* dihydroxylation of the alkene will afford the α-cyclo sulfate which will be a potential inhibitor. On other hand the tertiary alcohol of the pseudodisaccharide will be converted to carbamate and subjected to an Ichikawa rearrangement to give protected maltosyl valienamine analog. We will also attempt the deoxygenation of the tertiary alcohol and epoxidation, aziridination of the alkene. Then subsequent deprotection of these derivatives will yield the desired inhibitors which will be tested for its inhibition activity against GlgE and various glycosidase enzymes.

27

Trehalose Analogues Block Utilization of Trehalose by Hypervirulent *Clostridium difficile*

Alicyn I. Stothard, Belinda K. Wilson, Qing Qing Dong, Noah D. Danielson, James Collins, Peter J. Woodruff, Robert A. Britton, and Benjamin M. Swarts*

Department of Chemistry and Biochemistry, Central Michigan University, Mount Pleasant, MI 48858 USA

Trehalose, a non-reducing disaccharide composed of two glucose units, is broadly used in various pharmaceutical and biotech applications and it is being investigated as a therapeutic for multiple diseases. However, its ability to be used as an energy source by microbes is problematic, as highlighted by the recent finding that low levels of trehalose can be efficiently metabolized by and enhance the virulence of epidemic strains of the intestinal pathogen *Clostridium difficile*. Here, we show that various trehalose analogues designed to resist enzymatic degradation, specifically the 5-deoxy-5-thio-D-trehalose and the manno- and lacto- epimers, are incapable of being used as carbon sources by *C. difficile*. Furthermore, these analogues block utilization of native trehalose by hypervirulent *C. difficile*. Thus, trehalase-resistant trehalose analogues are potentially valuable as surrogates for or co-additives with trehalose in applications where enzymatic breakdown is a concern.

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Engineering of Bacteriophage Qß for Use as a Conjugate Vaccine Platform

Hunter S. M[c]Fall-Boegeman[1], Zibin Tan[1], Xuefei Huang[1,2,3]

[1]Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA; [2]Department of Biomedical Engineering, Michigan State University, East Lansing, MI 48824 USA; [3]Institute for Quantitative Health Science and Engineering, Michigan State University, East Lansing, MI 48824 USA

Studies have shown the promise of the bacteriophage Qß as a carrier protein in conjugate vaccine design.[1] Qß is a virus-like particle (VLP) made up of 180 copies of a 14kDa coat protein and an RNA core. The highly repetitive surface structure allows for efficient decoration with carbohydrate-based antigens. This ordered display allows for efficient B cell activation with titers in excess of 1 million. The coat protein also contains a helper T cell (Th) epitope that drives the immune response towards a long-lasting memory response. Unfortunately, as a highly immunogenic protein, Qß can potentially hinder the desired immunity against the loaded antigen via a process called carrier induced epitope suppression (CIES). Another drawback is that of a lack of cytotoxic T cell (CTL) response against the desired antigen. Attempts to engineer Qß to solve the problems of CIES and CTL activation will be discussed.

In our previous vaccination studies[1,2] utilizing Tn (Ser/Thr α-O-GalNAc) as the model antigen, we observed super strong immunities generated against both the antigen and the carrier. Our previous results showed that 1,2,3-triazole ring presented in the vaccine induced unwanted immune response, inhibiting desired immunity against the antigen and avoiding such structure lead to better antibody quality and thus better protection. We hypothesized that by eliminating the immunity against the carrier, we can further improve the vaccination process and provide better protection against cancer. We utilized modified poly (oligo ethylene glycol methacrylate) grafted from the coat protein as a shield trying to reduce the recognition of B cell to the carrier. “Traceless” cleavable linker between the protein and the polymer was used to avoid disrupting the Th activation. Tn antigen was conjugated to the polymer to achieve surface display. Unfortunately, such modification resulted in lower immunity against Tn as tested by ELISA (enzyme-linked immunosorbent assay) and sera binding to cancer cells expressing Tn antigen. Possible reasons, including disruption of antigen transportation in the lymph system and randomization of previously patterned and ordered display will be discussed.

To elicit a comprehensive immune response, both B cells and CTLs should be activated as efficiently as possible. In order to maintain already excellent B cell responses, CTL epitope (CTLe) incorporation should occur on the interior of the capsid. The design process including conjugation site, effects of CTLe hydrophobicity, and linker structures will be discussed.

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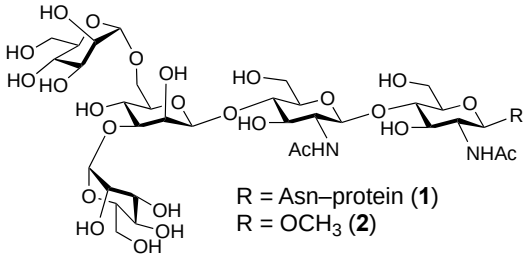
29

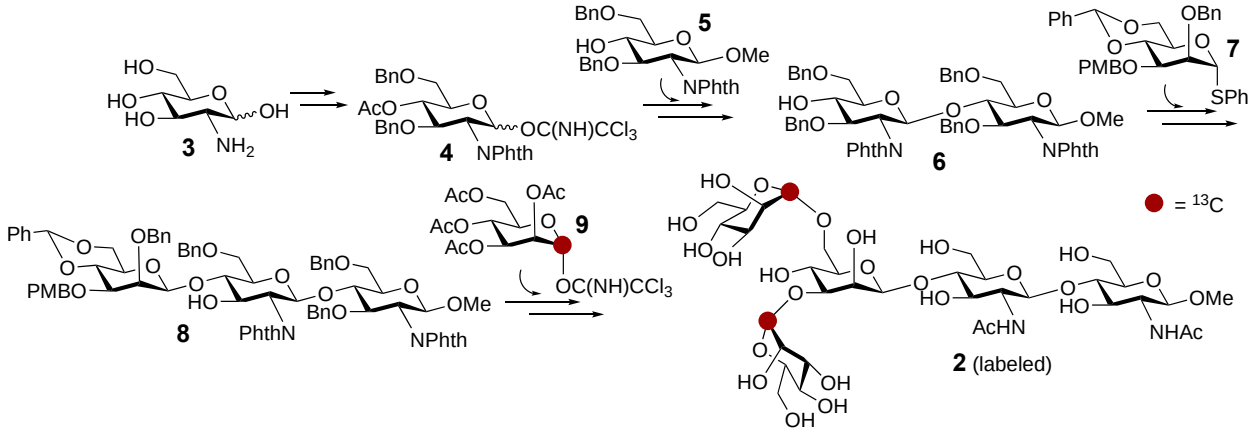
Synthesis and Conformational Studies of *O*-Glycosidic Linkages in ^{13}C-Labeled Complex-Type *N*-Glycans Constructed From a Labeled Man$_3$GlcNAc$_2$ Core

Timothy J. Tetrault,[1] Reagan Meredith,[1] Qingfeng Pan,[2] Wenhui Zhang,[1] and Anthony S. Serianni[1]*

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46656-5670 USA; [2]Omicron Biochemicals, Inc., 115 South Hill Street, South Bend, IN 46617-2701 USA

N-Glycans in eukaryotes share a common Man$_3$GlcNAc$_2$ core pentasaccharide (**1**) and an *N*-glycosidic β-linkage to a tripeptide consensus sequence, Asn-X-Ser/Thr, where X is any amino acid except Pro. Our aim is to develop a reliable, moderate scale, versatile (for isotopic labeling) and high-yield chemical route to **2** with or without selective ^{13}C-labeling for systematic elaboration by enzyme-mediated glycosylation to give higher-order complex-type *N*-glycans. The general elements of this route are shown below, starting from D-glucosamine (**3**), progressing to protected GlcNAc-GlcNAc disaccharide **6** and trisaccharide **8**, followed by installation of either one or both labeled αMan residues to give labeled **2**. Using the recently developed *MA'AT* method to model oligosaccharide conformation in solution (Zhang *et al*., *J. Phys. Chem. B*. **2017**, *121*, 3042–3058), we aim to





investigate systematically the effects of structural context on the conformations and dynamics of the αMan linkages in **2** and in larger oligosaccharides. Particular attention will be paid to the αMan(1→6) linkage in **2** and in elaborated structures, which has been implicated as a trigger of conformational change in glycoproteins bearing these *N*-glycans. *MA'AT*-determined conformational models of this linkage (and other linkages in targeted oligosaccharides) will be compared to those determined by aqueous MD simulation as a means of validating the MD-determined models and the underlying force-field used to derive them. [This work was supported by the National Science Foundation, CHE 1707660.]

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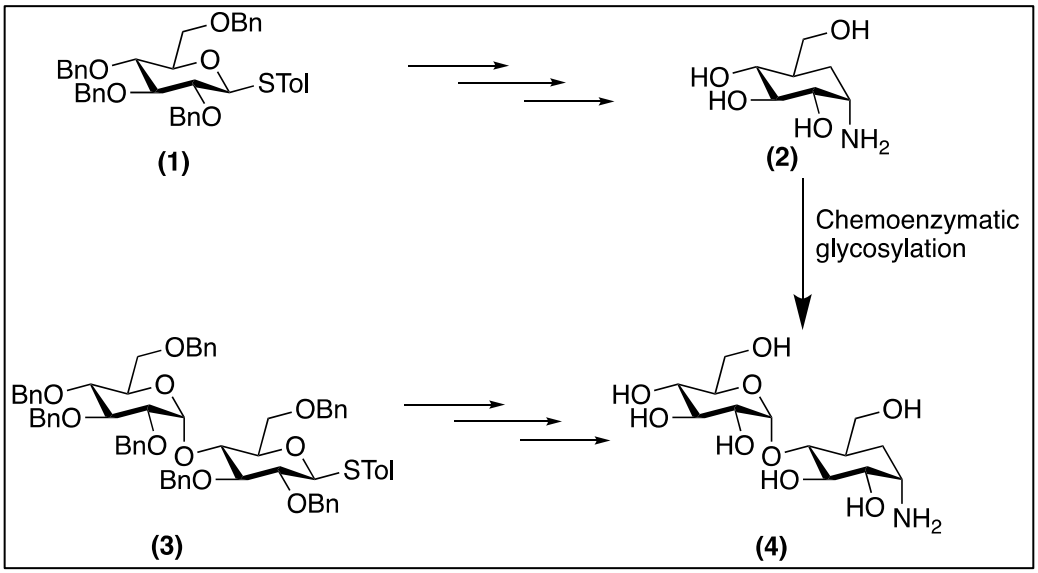
Synthesis of Potential Carbasugar-based Inhibitors of *Mycobacterium tuberculosis* GlgE

Radhika Thanvi

University of Toledo, Toledo, OH 43606 USA

Mycobacterium tuberculosis (Mtb) GlgE is an essential α-glycoside hydrolase like enzyme which is responsible for catalyzing the addition of maltose-1-phosphate (M1P) to maltooligosaccharides. Inhibiting the activity of this enzyme leads to the death of the Mtb cell due to the accumulation of M1P. Derivatives of valienamine (non-covalently binding) and cyclic-sulfates (co-valently binding) are known potent inhibitors of glycoside hydrolases. Bearing this in mind, we synthesized some targets starting from glucose and maltose derivatives. Compounds *p*-tolyl β -thioglucoside and *p*-tolyl β -thiomaltoside were subjected to *N*-bromosuccinimide to obtain a free reducing end on the sugar. Wittig olefination was performed using methyltriphenylphosphonium bromide, the resulting alcohol was subjected to Moffatt oxidation to yield a ketone. Addition into the carbonyl with vinyl magnesium bromide gave two separable diastereomers. Ring-closing metathesis using Grubb's catalyst afforded the desired carbasugars. The obtained tertiary alcohol will be converted to a carbamate using trichloroacetyl isocyanate and then rearranged to yield a protected valieneamine and its maltosyl analogue. Subsequent deprotection and chemoenzymatic glycosylation of the valienamine will yield the desired non-covalent carbasugar-based potential inhibitor. The synthesized molecule will be tested for its inhibition activity against GlgE and various glycoside hydrolase enzymes. Other potential target molecules in the series include epoxides, cyclic-sulfates and aziridines.



31

Stereoselective Synthesis of 2,3-Diamino-2,3-dideoxy-β-D-mannopyranosyl Uronates Existing in Various Bacterial Capsular Polysaccharides

Prakash Thapa,[1] Bishwa Raj Bhetuwal,[1] Alex Joyce,[1] Mira Chahine,[2] Xiaohua Li,[2] and Jianglong Zhu[1]

[1]Department of Chemistry and Biochemistry and School of Green Chemistry and Engineering, University of Toledo, Toledo, OH 43606 USA; [2]Department of Natural Sciences, University of Michigan-Dearborn, Dearborn, Michigan, 48128 USA

2,3-Diamino-2,3-dideoxy-β-mannuronic acids are the important constituents of various bacterial capsular polysaccharides. Stereoselective synthesis 2,3-diamino-2,3-dideoxy- β -mannuronic acids is a challenging task in the field of carbohydrate chemistry because of both anomeric effect and neighboring group participation (NGP) or steric effect of C2 axial substituent both favor the formation of α isomer. Several efforts were made in the past to develop a protocol for the stereoselective synthesis of this class of β-mannosamine and prominent success was achieved. However, an ideal protocol for the synthesis of β-mannosides and *β*-mannosamine yet to be developed. In the current research, we attempt to develop a robust methodology for the stereoselective synthesis of 2,3-diamino-2,3-dideoxy-β-mannosides via Cs_2CO_3-mediated anomeric *O*-alkylation. We synthesized 2,3-diazido-2,3-dideoxy-β-mannose donor, mannose-derived cyclic urea lactol donor and C3 *N*-benzyl protected lactol donor and their reactivity were studied with the different acceptor. In our preliminary results, C3 *N*-benzyl protected lactol donor is found to be effective over the remaining two other donors during glycosylation.

This work was supported by National Institutes of Health Common Fund Glycosciences Program (1U01GM125289), The University of Toledo, and University of Michigan–Dearborn.

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De Novo Synthesis of 3-Amino-2,3,6-Trideoxy Sugars

Peng Wen[1], Timothy C. Homan[1,2], Jessica Wu[1], and Weiping Tang[1,3*]

[1]School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53705 USA; [2] Department of Chemistry, Grove City College, Grove City, PA 16127 USA; [3]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706 USA

3-Amino-2,3,6-trideoxy sugars are an important class of rare sugars found in numerous pharmaceuticals (such as anthracycline antibiotics) and biologically active natural products (such as saccharomicin A and B). Their prevalence in these bioactive compounds has stimulated tremendous interests in their synthesis and diversification. Traditionally, 3-amino-2,3,6-trideoxy monosaccharides can be synthesized from both carbohydrate and non-carbohydrate precursors. However, stereo-controlled glycosylation for the synthesis of oligosaccharides or glycoconjugates containing 3-amino-2,3,6-trideoxy sugar remains to be challenging due to the lack of an appropriate directing group at *C*2-position. In an attempt to tackle this problem, we herein report a novel synthetic strategy which enables the highly stereoselective coupling of a glycosyl acceptor at an early stage and the subsequent derivatization of the key intermediate for the introduction of the *C*3-amino functionality. The key steps involve a chiral catalyst-directed dynamic kinetic diastereoselective acylation (DKDA) of Achmatowicz rearrangement products and a palladium-catalyzed stereospecific Tsuji-Trost reaction of the resulting allylic ester with an alcohol as glycosyl acceptor. Preliminary results have shown that an amino functionality can then be installed stereospecifically at the *C*3-position *via* a Rh-catalyzed intramolecular nitrene C-H insertion. The further diversification of the obtained bicyclic carbamate intermediate is currently under investigation.

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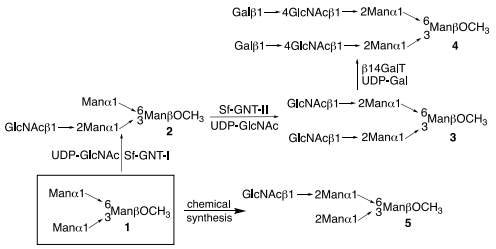
Chemo-enzymatic Synthesis and NMR Spin-Spin Coupling Constant Measurements on Complex-Type *N*-Glycans

Mi-Kyung Yoon,[1] Jieye Lin,[1] Qingfeng Pan,[2] Reagan Meredith,[1] Wenhui Zhang,[1] and Anthony S. Serianni [1,2]

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670 USA; [1]Omicron Biochemicals, Inc., South Bend, IN 46617-2701 USA

To investigate the solution conformations and dynamics of complex-type *N*-glycans using the newly developed *MA'AT* method (Zhang *et al*., *J. Phys. Chem. B.* **2017**, 121, 3042–3058; Zhang *et al*., *Biochem.* **2019**, 58, 546-560), six oligosaccharides (tri- to heptasaccharides) were prepared chemo-enzymatically, and NMR spin-couplings (J_{HH} , and J_{CH}) were measured in each compound. Trisaccharide αMan(1→3)-[αMan(1→6)]-βManOCH$_3$ (**1**) was synthesized via a chemical route and used as a substrate for α-1,3-mannosyl-glycoprotein 2-β-*N*-acetylglucosaminyltransferase from *Spodoptera frugiperda* (Sf-GNT-I), with UDP-GlcNAc serving as the sugar donor (see scheme). Tetrasaccharide βGlcNAc-(1→2)-αMan-(1→3)-[αMan-(1→6)]-βManOCH$_3$ (**2**) was produced in nearly quantitative yield (~30 mg), was isolated in >98% purity as determined by NMR and HRMS, and was used as a substrate for α-1,6-mannosyl-glycoprotein 2-β-*N*-acetylglucosaminyltransferase from *S. frugiperda* (Sf-GNT-II), again using UDP-GlcNAc as the sugar donor. Pentasaccharide βGlcNAc-(1→2)-αMan-(1→3)-[βGlcNAc-(1→2)-αMan(1→6)]-βManOCH$_3$ (**3**) was obtained in nearly quantitative yield (~25 mg) and was isolated in >98% purity as determined by NMR and HRMS. Sf-GNT-I and Sf-GNT-II were prepared from a baculovirus-insect cell expression system using plasmids obtained from Prof. Don Jarvis (University of Wyoming). Pentasaccharide **3** was treated with human β-1,4-galactosyltransferase I (β14GalT) and UDP-Gal to give heptasaccharide βGal-(1→4)-βGlcNAc-(1→2)-αMan-(1→3)-[βGal-(1→4)-βGlcNAc-(1→2)-αMan(1→6)]-βManOCH$_3$ (**4**) in nearly quantitative yield (~10 mg) and >98% purity after isolation (see scheme). Oligosaccharides **2**–**4** were purified by gel-filtration chromatography on Biogel P2. Tetrasaccharide αMan-(1→3)-[βGlcNAc-(1→2)-αMan-(1→6)]-βManOCH$_3$ (**5**) and pentasaccharide αMan-(1→3)-[αMan-(1→6)]-βMan-(1→4)-βGlcNAc-(1→4)-GlcNAcOH (**6**) were prepared by chemical synthesis (see scheme). NMR spin-couplings (J_{HH} and J_{CH}) were measured at natural abundance in **1**–6 using various 2D NMR methods, including ^{13}C, ^{1}H-HSQC, ^{13}C, ^{1}H-J-HMBC, and ^{13}C, ^{1}H-HSQC-HECADE. For the measurement of $^{1}J_{CH}$ values, ^{13}C-coupled ^{13}C, ^{1}H-HSQC spectra were used, whereas ^{13}C, ^{1}H-HSQC-HECADE spectra were used to measure intra-residue long-range $^{n}J_{CH}$ values. To obtain inter-residue (trans-glycosidic) $^{3}J_{COCH}$ values, 2D ^{13}C, ^{1}H-J-HMBC spectra were collected with different scaling factors. Potential second-order effects were taken into account in measurements of J_{HH} and J_{CH} values. Work is underway to prepare **2**–**5** with ^{13}C-labeling in select residues; for example, compounds containing ^{13}C-labeling at C1 of βGlcNAc residues is achieved using ^{13}C-labeled UDP-GlcNAc. The long-range goal of these studies is to determine quantitatively how structural context affects the conformation and dynamics of *N*-glycans in aqueous solution and to provide structural rationales for the observed behaviors. Related studies are underway using Man$_3$GlcNAc$_2$OCH$_3$ (core pentasaccharide prepared chemically) as a substitute for **1**, and in *N*-glycan oligosaccharides appended to peptides/proteins. [This work was supported by NSF CHE1402744 and Omicron Biochemicals, Inc.]



Preparation of oligosaccharides **2**–**4** chemo-enzymatically from **1** *via* Sf-GNT-I and Sf-GNT-II, and chemical synthesis of **5**.

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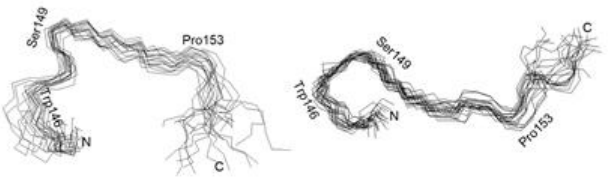
p53-Derived Peptides Are Stabilized Conformationally By *O*-GlcNAcylation

Mi-Kyung Yoon,[1] Reagan Meredith, [1] Jieye Lin[1], Wenhui Zhang[1], and Anthony S. Serianni[1,2]
[1]Department of Chemistry and Biochemistry, University of Notre Dame,
Notre Dame, IN 46556-5670 USA; [2]Omicron Biochemicals, Inc., 115 South Hill Street,
South Bend, IN 46617-2701 USA

Protein *O*-GlcNAcylation involves the addition of single *N*-acetyl-β-D-glucosamine (βGlcNAc) residues to either the Ser or Thr side-chains of nuclear and cytoplasmic proteins. *O*-GlcNAcylation has been shown to be involved in extensive crosstalk with phosphorylation, which regulates cellular processes such as cellular signaling, cell cycle, and transcription, and aberrant regulation of this crosstalk contributes to human diseases such as cancer. Major tumor suppressor proteins such as p53 are regulated by crosstalk between *O*-GlcNAcylation and phosphorylation. It is known that p53 is *O*-GlcNAcylated on Ser149, leading to decreased phosphorylation on Thr155 by the COP9 signalosome (CSN), and decreased ubiquitylation and degradation. Despite these known biological functions, however, the molecular mechanism underlying this crosstalk remains unclear. To initiate an investigation of this mechanism for p53, the structures of several p53-derived peptides, with and without *O*-GlcNAcylation, were determined by NMR. These structure and dynamics studies indicate that p53 peptides become more stable and rigid upon *O*-GlcNAcylation (see attached structures). The increased stability appears to be mediated by carbohydrate-π stacking interactions between the B-face of βGlcNAc and the indole ring of Trp, a non-covalent interaction known to contribute to protein stabilization by *N*-GlcNAcylation. Conformational studies of the βGlcNAc–Ser *O*-glycosidic linkage were conducted on an "isolated" βGlcNAc–O–Ser dimer and on *O*-GlcNAcylated peptides by NMR using the newly-developed *MA'AT* method. Related studies are underway to characterize linkage structure in the context of larger proteins. These structural studies, and others underway, are expected to provide deeper understandings of the role of crosstalk between post-translational modifications of p53 in the progression of cancer. [This work was supported in part by NSF grant CHE 1707660 to A.S., and by Omicron Biochemicals, Inc.]



NMR structures of unglycosylated (left) and glycosylated (right) p53 peptides

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Chemical Synthesis of Syndecan-4 Glycopeptide Bearing *O*-, *N*-sulfation and Multiple Aspartic Acids for Probing Impacts of the Glycan Chain and the Core Peptide on Biological Functions

Weizhun Yang, Xuefei Huang

Department of Chemistry, Michigan State University, East Lansing, MI 48824 USA
xuefei@chemistry.msu.edu

Proteoglycans are a family of complex glycoproteins with glycosaminoglycan chains attached to the core protein backbone. Due to the high structural heterogeneity of proteoglycans in nature, it is challenging to decipher the respective roles of the glycan chain and the core protein on biological functions. Synthesis of homogeneous proteoglycan glycopeptide faces many obstacles. Herein, we report the first synthesis of proteoglycan glycopeptides bearing multiple aspartic acids in core peptide and *O*- and *N*-sulfations in the glycan chain, as exemplified by the syndecan-4 glycopeptide. To overcome the high acid sensitivities of sulfates and base sensitivities of the glycopeptide, an effective synthetic strategy utilizing sulfate ester protecting groups has been developed to integrate sulfated glycan chain with a peptide sequence prone to formation of aspartimide side products. The availability of the structurally well-defined synthetic glycopeptide enabled the investigation of their biological functions including cytokine, growth factor binding and heparanase inhibition. Interestingly, the glycopeptide can have distinct activity trends compared to the peptide or glycan alone. This suggests that besides changes in sulfation patterns and backbone sequences, linking the glycosaminoglycan chain to core proteins as in proteoglycans may be an additional approach to modulate biological functions of glycosaminoglycans in nature.

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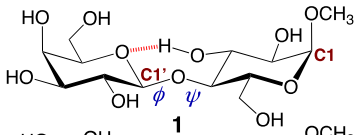
Inter-residue Hydrogen Bonding Does Not Determine the Conformation and Dynamics of β-(1→4) *O*-Glycosidic Linkages In Aqueous Solution

Wenhui Zhang,[1] Reagan Meredith,[1] Xiaocong Wang,[3] Robert J. Woods,[3]
Ian Carmichael,[2] and Anthony S. Serianni[1]*

[1]Department of Chemistry and Biochemistry, and [2]Radiation Laboratory,
University of Notre Dame, Notre Dame, IN 46556-5670 USA; [3]Complex Carbohydrate
Research Center, University of Georgia, Athens, GA 30602 USA

Inter-residue hydrogen bonding is believed to influence the *O*-glycosidic linkage conformations in some oligo- and polysaccharides, especially those containing β-(1→4) linkages like that found in methyl β-D-galactopyranosyl-(1→4)-α-D-glucopyranoside (**1**). To gain quantitative information on the influence of hydrogen bonding on linkage conformation in aqueous solution, *MA'AT* analysis (Zhang *et al*., *J. Phys. Chem. B*. **2017**, *121*, 3042–3058) was applied to evaluate the *phi* (ϕ) (defined as H1'–C1'–O1'–C4) and *psi* (ψ) (defined as C1'–O1'–C4–H4) torsion angles in ^{13}C-labeled isotopomers of **1** and the C3-deoxy analog **2** in which putative inter-residue hydrogen bonding between O3H (donor) and O5' (acceptor), which is observed in the crystal structure of **1** (Pan *et al*., *Acta Cryst*. **2005**, *C61*, o674–o677) and possibly in aqueous solutions of **1** and related disaccharides (Zhang *et al*., *Carbohydr. Res*. **2009**, *344*, 1582–1587; Zhao *et al*., *J. Org. Chem*. **2007**, *72*, 7071–7082), cannot occur. These analyses focused on experimentally determined mean values of both torsion angles, and equally important, on circular standard deviations (CSDs) of these mean values that report on librational motion. The *MA'AT* analyses indicate that the absence of O3 in **2** does not alter the mean values of ϕ and ψ appreciably (30° and 24° for ϕ, and –8° and –15° for ψ in **1** and **2**, respectively). *MA'AT*-determined CSDs are very similar for ϕ and ψ in **1** and **2** (26° for ϕ; 16–19° for ψ). These results compare favorably with those for the *dominant* linkage conformers of **1** (94%) and **2** (72%) determined by aqueous MD simulation (1-μs; GLYCAM06): 46° and 41° for ϕ in **1** and **2**, respectively, with CSDs of 12°; –1° and –6° for ψ in **1** and **2**, respectively, with CSDs of 18–23°. The *MA'AT* and MD models of the *O*-glycosidic linkages in **1** and **2** are in good agreement, with O3 deletion in **1** causing a small reduction in ϕ (less positive) and in ψ (more negative), although the *absolute* values of ϕ and ψ determined by MD are more positive than *MA'AT*-determined values by up to 17°. In addition, MD simulation indicates that 6% of **1** and 28% of **2** adopt linkage conformations that are not detected by *MA'AT* analysis. The *MA'AT* results show that librational motion about ϕ and ψ is not much affected by the loss of O3, a result consistent with the MD findings. These results suggest that, if inter-residue hydrogen bonding between O3H and O5' occurs in aqueous solutions of **1** (i.e., is persistent and relatively strong), the interaction plays little to no role in dictating the mean values of linkage torsion angles and does not affect the librational motions of these torsion angles appreciably. Inter-residue hydrogen bonding, if it occurs in **1**, appears to reinforce rather than dictate the intrinsic conformational properties of the linkage. Whether this conclusion applies to all such interactions in oligo- and polysaccharides remains an open question. [This work was supported by the National Science Foundation, CHE 1707660.]



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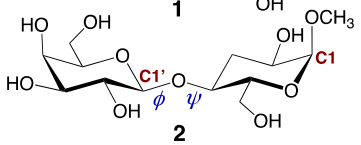

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Sialylation Status and Mechanical Properties of THP-1 Macrophages Upon LPS Stimulation

Yu Zhao, Gautam Mahajan, Chandrasekhar R. Kothapalli, Xue-Long Sun*

Department of Chemistry, Chemical & Biomedical Engineering and Center of Gene Regulation of Health and Disease (GRHD), Cleveland State University, Cleveland, OH 44115 USA

Cell surface receptors are the key contributors of macrophage function. Most macrophage cell surface receptors are glycoproteins with sialic acids at the terminal of their glycans. It is well recognized that biologic modulators such as lipopolysaccharide (LPS) induces cell surface sialylation changes that may in turn contribute to macrophage functions. In addition, cellular mechanics such as elasticity is also a major determinant of macrophage function, which in turn is modulated by LPS. In this report, we characterized the sialylation status of macrophages upon LPS stimulation and assessed the changes in its mechanical properties and function. Specifically, we confirmed that sialylation status is closely related to macrophage biomechanical characteristics (elastic modulus, tether force, tether radius, adhesion force, and membrane tension) and thus directly involved in macrophage function. Further, we modulated macrophage sialylation status by feeding the cell with exogenous free sialic acid (Neu5Ac, Neu5Gc) and sialidase inhibitors, and examined the resulting effects on cellular mechanics and function. A systematic recognition of sialylation status related to cellular mechanics of macrophages will contribute to defining their phenotypes and elucidate macrophage functional diversity. Further, it will provide novel mechanisms and approaches for diseases diagnosis and treatment.

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Synthesis of Next-Generation FRET Probes for Real-Time Monitoring of Glycolipid Processing in Mycobacteria

Nikolas H. Kambitsis, Nicholas Banahene, Nathan J. Holmes, B. Dillon Vannest, and Benjamin M. Swarts

Department of Chemistry and Biochemistry, Central Michigan University, Mount Pleasant, Michigan 48859, USA

The outer membrane of mycobacteria, referred to as the mycomembrane, is essential for the viability and virulence of *Mycobacterium tuberculosis*. The mycomembrane consists of several glycolipids, including the abundant species trehalose monomycolate (TMM) and trehalose mycolate (TDM). Mycobacterial acyltransferases and esterases utilize TMM and TDM as substrates to build or remodel the mycomembrane, respectively. In previous studies, it was shown that fluorescence resonance energy transfer (FRET)-equipped TMM and TDM analogues undergo fluorescence activation upon processing by these enzymes, potentially representing a tool to investigate their contributions to mycobacterial physiology and pathogenesis. However, these FRET-based probes lacked high specificity for mycobacterial enzymes. This project aims to address this issue by synthesizing novel fluorescence-quenched TMM and TDM analogues bearing mycolate chains that more closely resemble those found in mycomembranes. Native mycolates feature a branched acyl chain and a β-hydroxyl group, which were not present in the first generation of FRET probes. By adding these key structural features, we hypothesize that an increase in specificity for mycobacterial enzymes will be observed. Here, we report progress toward the synthesis of these next-generation FRET probes.

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Incorporating PDMAB to Monosaccharides as a Microwave Labile Protecting Group to Facilitate Neutral Glycosylation

A.J Liyanarachchi & P.R Andreana

Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606 USA

The use of neutral conditions for glycosylation benefits in making the process more effective and efficient. For instance, microwave-assisted glycosylation reactions are less time consuming compared to Lewis acid promoted glycosylation. The aim of this project is to develop methodologies that can carry out glycosylation in reagent-free environment by installing microwave labile group *p*-*N*,*N*-dimethylamino benzyl (PDMAB) on the C-6 position of monosaccharides. It is predicted that microwave irradiation will allow the PDMAB group to render a sugar alkoxide that can be utilized for other chemical transformations. In the presence of a suitable electrophile, the sugar alkoxide can attack an electrophile via substitution reactions, S_N2 or S_N2'. Varying conditions such as temperature, pressure, and power of the microwave reactor is enabling for product outcome analysis leading to optimized conditions while screening solvents. This approach will be used to facilitate orthogonality in carbohydrate chemistry and provide another method in the chemist's toolbox.

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Development of Neutral Glycosylation Protocol Using Novel Microwave Labile Functional Handle PDMAB/PDEAB Through *O*-Alkylation Strategy

Shanika Gamage, Peter R, Andreana

Department of Chemistry and Biochemistry, University of Toledo, Toledo, OH 43606 USA

Neutral glycosylation, under microwave irradiation, is a novel synthetic strategy in carbohydrate chemistry. Providing milder reaction conditions and decreased reaction times are the major advantages of microwave promoted reactions. Development of a neutral glycosylation protocol under microwave conditions is the main objective of this project. Installation of microwave labile functional groups to the anomeric position of monosaccharides will lead to *O*-glycosylation through two different pathways; formation of alkoxide ion and formation of oxocarbenium ion. The microwave labile group, *p*-*N*,*N*dimethyamino benzyl (PDMAB), which is installed at the anomeric position, can undergo glycosylation by forming an alkoxide ion via an aza-quinone methide intermediate in the presence of a suitable electrophile. Installation of activated leaving groups, in the form of esters, which are attached to the anomeric position of sugars, promotes the formation of oxocarbenium ions under microwave irradiation and glycosylation then pursues in the presence of a suitable acceptor.

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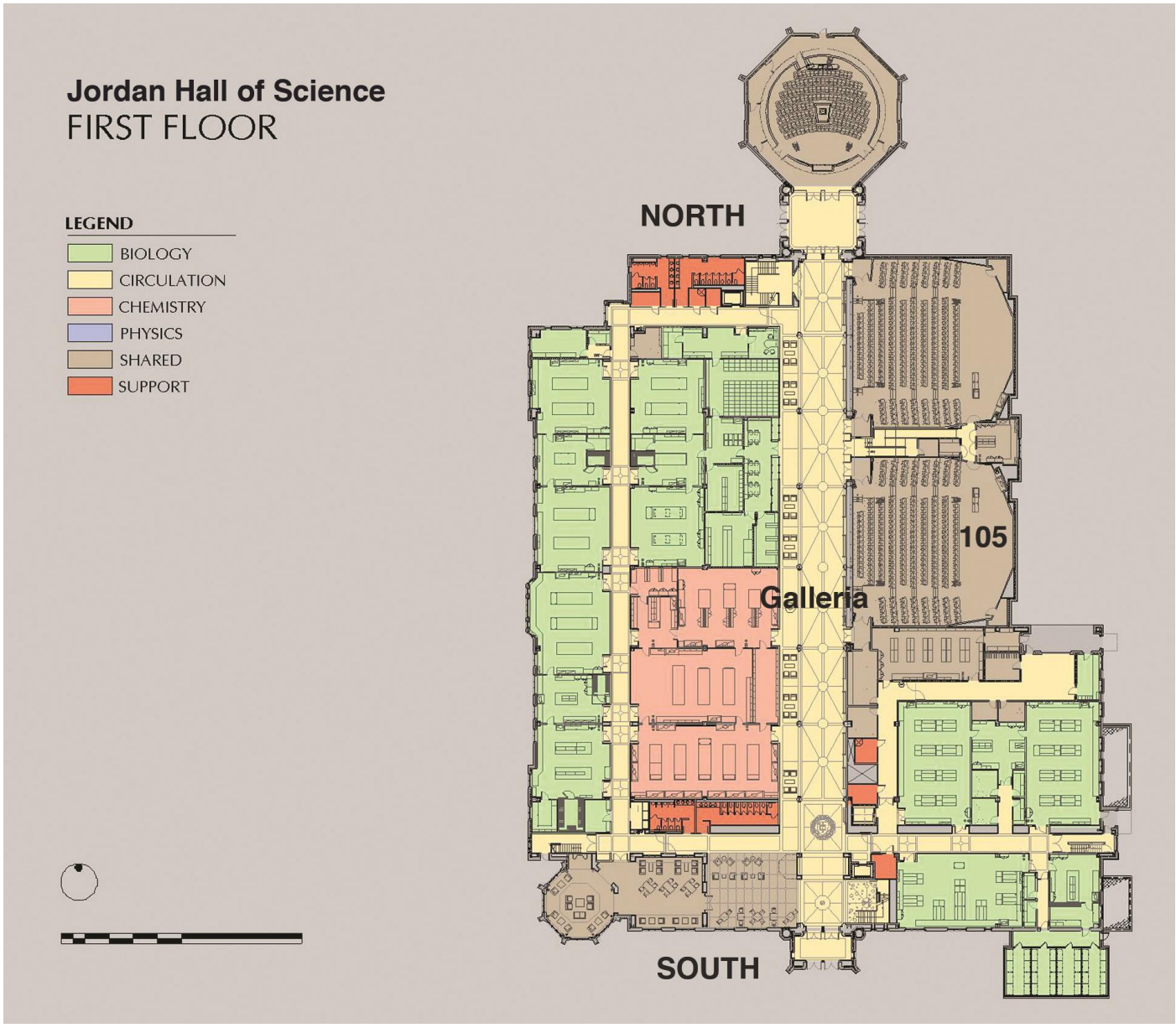


St. Joseph's Lake
St. Mary's Lake
Holy Cross Cemetery
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Basilica
Hesburgh Library
Snite Museum
Notre Dame Stadium
Joyce Center
Purcell Pavilion
Eck North Field
Eck South Field
Bookstore Basketball Courts
Hammes Bookstore
Eck Visitors Center
Main Gate
Cedar Grove Cemetery
DeBartolo Performing Arts Center
Irish Green
Sculpture Park
Compton Family Ice Arena
Main Entrance
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PARKING
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