

## **CARB 1**

### **Chemical probes of glycan assembly in microbes**

**Laura L. Kiessling**, *kiesslin@mit.edu. Dept of Chemistry Biochemistry, University of Wisconsin Madison, Madison, Wisconsin, United States*

Surface glycans are an evolutionarily conserved feature of cells. Glycans participate in biological processes ranging from energy storage to cell structural integrity to pathogenesis. The characteristic cell envelope glycans of pathogens, such as *Mycobacterium tuberculosis*, can be critical for viability. We are exploring how the cell surface carbohydrate coat of *M. tuberculosis* and related pathogens is built, as an increased understanding could lead to new anti-infective agents. One of the integral components of this cell wall is the arabinan, an essential polysaccharide. The arabinan is a branched polysaccharide built by multiple transmembrane glycosyltransferases. Generating this target is challenging, as neither chemical synthesis nor in vitro enzyme-based synthesis provides the means to readily address fundamental molecular questions about arabinan function. To this end, we are developing NMR and fluorogenic probes that co-opt the biosynthetic machinery and can therefore be used to assess the roles of key components of the mycobacterial cell envelope, including the arabinan.

## **CARB 2**

### **Human milk oligosaccharides in antimicrobial chemotherapy**

**Steven D. Townsend**, *steven.d.townsend@vanderbilt.edu. Chemistry, Vanderbilt University, Nashville, Tennessee, United States*

The introduction of antimicrobial agents into broad clinical use is a landmark advance in contemporary medicine. Unfortunately, while antimicrobial chemotherapy is an indispensable resource to treating human infections, it has also contributed to the belief that infectious diseases are conquerable. In reality, the juxtaposition between extensive use and misuse of antibiotics, the current attrition of drug candidates, and the accelerated rate of antimicrobial resistance is a threat to human health. This talk will focus on our investigation of human milk oligosaccharides (HMOs) as broad-spectrum antimicrobial and antivirulence agents.

## **CARB 3**

### **Building oligosaccharides and building community**

**Nicola L. Pohl**, *npohl@indiana.edu. Dept of Chem 120A Simon Hall, Indiana University, Bloomington, Indiana, United States*

The automated assembly of monosaccharide building blocks is still far from a robust process, in part because of the challenges of obtaining suitable quantities of these

blocks to rapidly test hypotheses about coupling chemistries in complex systems and to build libraries of glycans. This talk will focus on the development of a solution-phase automated oligosaccharide synthesis platform that uses fluororous tags rather than a solid phase to purify intermediates and will present the challenges remaining to make automated glycan synthesis routine in the context of larger efforts to bring researchers in the area together to tackle problems collectively.

#### **CARB 4**

##### **Carbohydrate chemistry in the service of anti-infective drug discovery**

*David Crich, dcrich@chem.wayne.edu. Department of Chemistry, Wayne State University, Detroit, Michigan, United States*

This lecture will cover recent advances in the Crich group on the design and development of new and improved aminoglycoside antibiotics for the treatment of multidrug resistant infectious diseases.

#### **CARB 5**

##### **Synthesis as an enabling technology for understanding bacterial glycan biosynthesis and function**

*Todd L. Lowary, tlowary@ualberta.ca. Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada*

This seminar will focus on recent work from the group on preparing, through chemical synthesis, complex glycoconjugates as biological probes. Particular areas of emphasis will be the synthesis of glycosyl-phospholipids for use in understanding bacterial polysaccharide assembly and the generation of an array of glycans for elucidating carbohydrate-mediated mycobacteria–host interactions.

#### **CARB 6**

##### **Approaches to 1,2-*cis*-2-aminosugars and heparan sulfate mimicking glycopolymers**

*Hien M. Nguyen<sup>1,2</sup>, hien-nguyen@uiowa.edu. (1) Department of Chemistry, Wayne State University, Detroit, Michigan, United States (2) Department of Chemistry, University of Iowa, Iowa City, Iowa, United States*

In this symposium, I will present new approaches to novel carbohydrate coupling methods that utilize nickel catalyst to stereoselectively promote formation of high purity 1,2-*cis*-2-aminosugars, one of the most important classes of naturally occurring complex carbohydrates and glycoproteins. The hydrophobic/hydrophilic effects of the protecting groups on the selectivity at the newly-formed glycosidic bond and the mechanistic

studies of nickel-catalyzed glycosylation reaction will be discussed. The utility of the nickel-catalyzed coupling method in the design, synthesis, and evaluation of heparan sulfate mimicking glycopolymers to modulate heparanase activity will also be presented. Heparanase is an enzyme which cleaves heparan sulfate (HS) polysaccharides of the extracellular matrix. It is a regulator of aggressive tumor behavior, plays a key role in kidney related diseases and autoimmune diabetes. We will discuss the use of computational studies to extract the natural HS-heparanase interactions as a template for the design of HS mimicking glycopolymers. Upon evaluation, glycopolymer with twelve repeating units was determined to be the most potent heparanase inhibitor and to have tight-binding characteristics. In addition, this glycopolymer lacks anticoagulant activity and is hydrolytically stable towards heparanase.

## **CARB 7**

### **Expedient methods for the stereocontrolled synthesis of oligosaccharides**

**Clay Bennett**, *clay.bennett@tufts.edu*. Department of Chemistry, Tufts University, Medford, Massachusetts, United States

Controlling the stereochemical outcome glycosylation reactions remains a significant challenge in oligosaccharide synthesis. Traditional approaches to oligosaccharide synthesis, based on the use of protecting groups, can be time consuming, and do not always provide high levels of selectivity, especially in unusual systems. Our group has developed chemical glycosylation methods where the selectivity of the reaction is controlled by the chemical promoter. Through this approach we have found it is possible to get very high, to nearly perfect  $\beta$ -selectivity with both 2-deoxy-sugars, and more traditional substrates containing oxygenation at the C-2 position. Furthermore, we have found that we can change the stereochemical outcome of these reactions to selectively provide  $\alpha$ -linked products, simply by changing the promoter. Recent developments in this work, including applications to synthesis will be discussed.

## **CARB 8**

### **Allostery in C-type lectins**

*Jonas Hanske*<sup>1</sup>, *Stevan Aleksić*<sup>2</sup>, *Jonas Aretz*<sup>1</sup>, *Hannes Baukmann*<sup>1</sup>, *Robert Wawrzinek*<sup>1</sup>, *Jessica Schulze*<sup>1</sup>, *Bettina Keller*<sup>2</sup>, **Christoph Rademacher**<sup>1</sup>, *Christoph.Rademacher@mpikg.mpg.de*. (1) Department of Biomolecular Systems, Max Planck Institute of Colloids Interfaces, Berlin-Dahlem, Germany (2) Freie Universitaet Berlin, Berlin, Germany

Innate immune cells are major contributors to pathogen uptake and consequently, antigen uptake and processing. Thereby these cells initiate the immune response. Many members of the C-type lectin family are expressed by these innate immune cells and primarily recognize pathogens by their glycan structures. Some of these lectins are endocytic pattern recognition receptors that, upon glycan binding trigger internalization

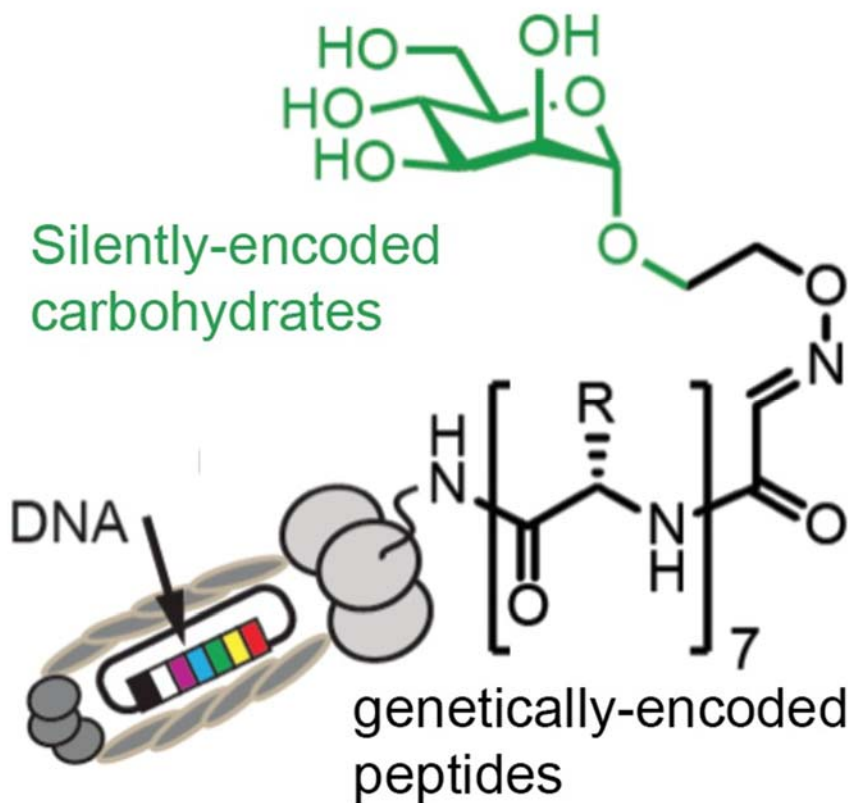
of their cargo upon ligand binding. Hence, pathogen recognition and uptake are tightly coupled to immune cell activation and antigen processing. C-type lectin receptors recognize their carbohydrate ligands utilizing a central calcium ion coordinated by conserved amino acids in the lectin binding site. The fate of the cargo is likely determined by the kinetics and endosomal routing of the C-type lectin receptor. Endosomal calcium channels open to reduce the effective calcium concentration and additional acidification concerted leads to cargo release. These mechanisms affect members of the C-type lectin family differently and also vary with cell type. We chose Langerin as an endocytic model receptor. This homotrimeric C-type lectin is highly expressed on epidermal Langerhans cells capturing invading pathogens such as HIV. We studied the molecular mechanisms involved in calcium binding using isothermal titration calorimetry and at atomic resolution applying protein NMR spectroscopy and molecular dynamics simulations. Biomolecular NMR provided intriguing insight into a network of amino acids involved in  $\text{Ca}^{2+}$  recognition, while Markov state models constructed from molecular dynamics simulations led to a detailed picture of the conformational dynamics of Langerin. Together these complementary techniques indicate a potential intradomain allosteric network, while overall no interdomain allostery of the trimeric protein could be observed. We discuss these results in light of the physiological role of Langerin.

## **CARB 9**

### **Genetically-encoded toolbox for discovery of ligands for glycan-binding proteins (GBPs)**

*Ratmir Derda, ratmir@ualberta.ca. Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada*

The power of genetic-encoding and large scale genetic libraries combined with the regioselective chemical modification of bacteriophage M13 gives rise to versatile approaches that can discover new ligands for glycan-binding proteins (GBP) and give rise to inhibitors of protein carbohydrate interactions. Silent encoding of diverse glycan modification within this framework further upgrades this system and permits unsupervised discovery of carbohydrate-binding preferences of GBP. In this talk, I will describe development and utility of several technologies that combine genetic encoding of peptide libraries and silent genetic encoding of glycosylation.



## CARB 10

### Building drug delivery systems: *In vitro* and *in vivo* studies of drug-hydroxybutenyl cyclodextrins complexes

**Charles M. Buchanan**, [cmbuchanan@btes.tv](mailto:cmbuchanan@btes.tv). Eastman Chemical Company, Kingsport, Tennessee, United States

In many respects, drug delivery is the perfect field for an organic chemist whose interest is in structure-property relationships; synthesis and characterization of a drug delivery system and subsequent introduction of this drug delivery vehicle into animals or humans is a complex and challenging structure-property problem. In this regard, we developed hydroxybutenyl cyclodextrin (HBenCD) for oral and intravenous delivery of pharmaceutical actives having poor water solubility and low bioavailability. In this presentation, we describe the synthesis and characterization of HBenCD, formation of drug-HBenCD complexes, *in vitro* solubility and dissolution studies of drug-HBenCD complexes, and pharmacokinetic studies in animals after oral and intravenous administration of drug-HBenCD complexes.

## CARB 11

## Cyclodextrins in the therapy of Niemann-Pick C disease

**Kostantin Dobrenis**, *kostantin.dobrenis@einstein.yu.edu*, **Cristin Davidson**, **Andrew Smith**, **Steven Walkley**. *Neuroscience, Albert Einstein College of Medicine, Bronx, New York, United States*

Niemann-Pick C (NPC) disease is a fatal neurodegenerative storage disorder involving accumulation of unesterified cholesterol (UC) and glycosphingolipids (GSLs) in the lysosomal system. NPC disease animal model studies by us and others have shown the cyclodextrin (CD), 2-hydroxypropyl- $\beta$ -CD (HP $\beta$ CD), to significantly: reduce neuronal UC and GSL accumulation; ameliorate behavioral deficits; delay cerebellar Purkinje neuron (PN) loss; and increase life span. Ongoing clinical trials are encouraging but efficacy limited by lower HP $\beta$ CD dosages required to avoid hearing loss noted in animal studies. Our current work aims to identify safer, efficacious alternatives and the mechanisms of CD therapeutic action. In long-term studies with disease mice, subQ injections of sulfobutylether- $\beta$ -CD (SBE $\beta$ CD) and sulfobutylether- $\gamma$ -CD (SBE $\gamma$ CD) produced little to no hearing loss after 9 weeks, yet still delayed motor deficits and extended life span. SBE $\gamma$ CD was more effective than SBE $\beta$ CD but less than HP $\beta$ CD. HP $\beta$ CD has been thought to exert its effect within the lysosome, directly interacting with stored UC to promote its egress, a process normally carried out by NPC proteins deficient in the disease. However prior collaborative [Dr. L. Szente (Cyclolab, Hungary)] short-term studies comparing 9 CDs, showed no correlation between relative effectiveness in reducing neuronal UC storage in vivo and relative aqueous solubilization of UC. Using disease neuronal cultures, relative UC reduction by different CDs was similar to that in vivo suggesting the disparity between efficacy and solubilization is not due to differential CD penetration of brain. Results of additional culture modeling studies argue against indirect depletion of neuronal UC storage. We also treated mice with HP $\beta$ CD and miglustat, a GSL synthesis inhibitor previously shown to rescue PNs. HP $\beta$ CD was more efficacious than miglustat, and combination therapy showed minor improvement over HP $\beta$ CD alone, primarily for cerebellum. Genome-wide expression studies on mouse neocortex found 34 of 77 gene expression levels altered by disease were returned to wild type levels by HP $\beta$ CD. Combination therapy did not correct additional genes. Altered expression was identified for several genes not previously implicated in NPC disease, including *Ttyh2*, *Sez6l2*, *Gpr37* and *Plexin B3*. Similar ongoing studies on microdissected PNs to identify changes in common for miglustat and HP $\beta$ CD could help pinpoint critical genes underlying rescue of PNs.

## CARB 12

### Cyclodextrins from toy to tool: Excipients and therapeutic agents

**Lajos Szente**, *szente@cyclolab.hu*. *Organic Synthesis, Cyclolab Ltd., Budapest, Hungary*

Cyclodextrins (CDs) have long been known and used as functional excipients in different pharmaceutical dosage forms. The last 40 years in CD technology has

witnessed the successful utility of CDs for stabilisation, solubilisation and bioavailability improvement of poorly soluble APIs. At present more than 40, CD-enabled human pharmaceutical products are on the market.

Recently, the therapeutic uses of “empty” non-occupied CD nano cavities have been emphasized. The first such application was published in 1983, where empty CDs were used to selectively remove toxic retinoids from the circulation in A vitamin intoxication. Based on this recognition, AKZO-Organon initiated a program aiming at selective removal of neuromuscular blocking agent during anesthesia using a tailor-made CD that binds target API. These efforts resulted in the approval of a uniquely modified gammaCD nano-cavity. The first cyclodextrin as a drug, a selective binding agent was marketed recently in USA, EU and Japan (Bridion® by Merck, Co.)

The safe and potent solubilizing excipient, 2-hydroxypropyl- $\beta$ -CD (HPBCD) listed in both the US and EU Pharmacopoeias, has an Orphan Drug designation, due to its selective cholesterol binding property. It has been established that HPBCD improves the clinical status of patients suffering from a deadly lysosomal storage disease, called Nieman-Pick-Type C (NPC). Soon, after clinical trials, in 2010, first US FDA, later EU granted Orphan drug designation for HPBCD to treat NPC. Currently more than 30 patients world-wide are treated using this cyclodextrin derivative with promising results. Similar lipid complexing properties were recently used to apply HPBCD as a lead drug candidate for two orphan kidney indications (Focal Segmental Glomerulosclerosis and Alport Syndrome) by Variant Pharmaceuticals. A number of further therapeutic applications of CDs themselves are expected to come.

A unique, diagnostic application of a CD has been developed and the method marketed by Oxford Nanopore Ltd for automatized DNA sequencing. A bacterial protein nanopore is combined with CD as analyte-recognizing adapter sensor. This enables quick, reliable and cheap DNA sequencing. The devices named MinON®, SmigdION® are currently in practical use.

## **CARB 13**

### **Sequence-based design of potent and selective small molecules targeting RNA**

*Matthew D. Disney, Disney@scripps.edu, Hafeez Haniff, Matthew Costales, Alicia angelbello, Sai Velagapudi, Suzanne Rzuczek. Department of Chemistry, The Scripps Research Institute, Jupiter, Florida, United States*

A challenge in molecular recognition has been identifying small molecules that can selectively recognize biological receptors. One of the major biomolecules that is considered to be a challenge to recognize with small molecules is RNA. In fact, many have viewed RNA, despite being involved in nearly every disease, as being “undruggable” with small molecules. We have taken a non-traditional approach to define small molecules targeting RNA that has proven to be broadly successful in defining lead small molecules that target RNA that precisely and selectively affects its biology in cells. Most chemical probe discovery efforts focus on a screen to identify lead compounds targeting a single biomolecule. Our approach is not focused on a single target but

focused on defining selective interactions between small molecules and RNA folds. An ultra-high throughput library-versus-library screening approach termed two-dimensional combinatorial screening (2DCS) studies of the binding of small molecules to libraries of RNA motifs. This results in an annotated library of druggable RNA motifs and small molecules to drug them. This information is mined against the human transcriptome to identify disease-causing RNAs that have RNA motifs that are bound by small molecules by using an approach termed Inforna. These studies have defined precise small molecules that target a host of RNAs involved in various incurable or difficult to treat diseases. Furthermore, a target validation approach has been developed to cross link small molecules to their RNA targets (termed Chem-CLIP) via a proximity-based reaction. By using Chem-CLIP broadly applicable rules have been established on how to drug non-coding RNAs in cells and to study target engagement in cells. These studies have helped to show that RNA – indeed – is druggable with small molecules. Furthermore, these studies also show that sequence-based design can be used to take a disease-causing biomolecule's sequence and quickly use it to enable the design of a lead medicine to target it.

## **CARB 14**

### **Design and synthesis of pi-extended nucleobases for sequence selective triple-helical recognition of RNA using peptide nucleic acids**

*James A. MacKay<sup>2</sup>, mackayj@etown.edu, Amanda K. Williams<sup>4</sup>, Holly A. Sofka<sup>3</sup>, Eriks Rozners<sup>1</sup>. (1) Department of Chemistry, Binghamton University, Binghamton, New York, United States (2) Chemistry and Biochemistry, Elizabethtown College, Elizabethtown, Pennsylvania, United States*

The selective recognition of RNA sequences and the ability to detect and inhibit function is a highly desirable goal for both fundamental research and practical applications in biotechnology that will likely uncover new discoveries of the functional importance of many RNA transcripts. Although anti-sense oligonucleotides target single-stranded RNA, many non-coding RNAs fold into double helical secondary structures that are challenging targets for molecular recognition. Recent research developments have shown that peptide nucleic acids (PNA) bind dsRNA through a triple helix in homopurine tracts under physiologically relevant conditions. Thymidine (T) is historically known to bind A-U, and more recently 2-aminopyridine (M) was demonstrated to bind selectively to G-C. Yet, binding of pyrimidines remains a challenge and there still exists no general method for the sequence selective recognition of any sequence of dsRNA.

Our research involves addressing this shortcoming through the rational design of nucleobases for Hoogsteen binding of PNA to dsRNA. Guided by computational analysis, we have determined and begun syntheses on several potential targets for binding studies that retain key structural features of known nucleobases (e.g. T and M) that bind purines, and extending them with a linker and additional heterocycle that can recognize the pyrimidine. In addition, our design aims for planar systems with the ability to pi-stack in the triple helix. Finally, an important feature of the design is use of the



same extended nucleobase for recognition of G-C and C-G base pairs and A-U and U-A base pairs. In doing so, we are in essence utilizing the strong binding of M and T bases to increase the binding affinity for the inverted base pair. This presentation will highlight both design and synthetic progress toward intended targets along with preliminary studies on PNA-RNA binding.

## **CARB 15**

### **Narrowing the gap between affinity and efficacy with RNA-targeted peptidomimetics**

**Benjamin L. Miller**, *benjamin\_miller@urmc.rochester.edu*, Viktoriya Anokhina, John D. McAnany, Oliver Swart, Thomas Hilimire, Jeffrey Chamberlain, Steve Dewhurst. *Univ of Rochester Medical Ctr, Rochester, New York, United States*

For “simple” RNA targets such as helices and stemloops, sequence-selective recognition has most frequently been achieved only with relatively large molecules. While effective in vitro, these compounds typically have bioavailability issues that limit their utility in cellular assays and in vivo. This issue manifests as a large discrepancy between the measured dissociation constant ( $K_D$ ) for RNA binding, and the effective concentration ( $EC_{50}$ ) in a cellular assay. This talk will discuss our efforts to develop general strategies for narrowing the gap between affinity and efficacy, and will focus on two primary targets: a stemloop responsible for regulating frameshifting in HIV, and pre-miRNAs associated with a range of cancers.

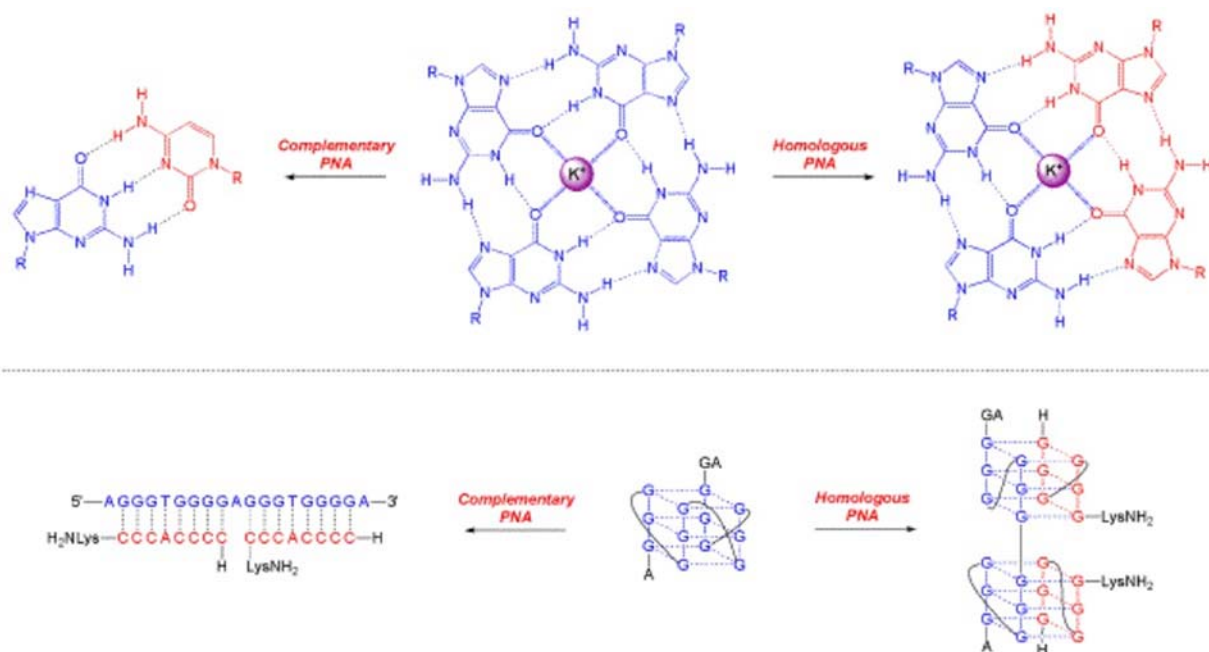
## **CARB 16**

### **Sequence-targeted invasion of DNA and RNA G quadruplexes by peptide nucleic acid**

**Bruce A. Armitage**, *army@andrew.cmu.edu*. *Chemistry, Carnegie Mellon University, Pittsburgh, Pennsylvania, United States*

G quadruplexes are secondary structures formed by guanine-rich DNA and RNA sequence motifs. Growing evidence implicates G quadruplexes in a wide variety of cellular processes, most notably in regulation of gene expression at numerous stages (e.g. transcription, splicing and translation). Given the biological importance of G quadruplexes, considerable effort is currently directed toward development of synthetic molecules that can bind and perturb the function of these structures. In contrast to the more common strategy of developing small molecule ligands that recognize quadruplexes based on shape, we are pursuing a sequence-targeted approach involving peptide nucleic acid oligomers. In one format, the PNA is complementary to the target. Upon invasion of the quadruplex structure, a PNA-DNA or PNA-RNA heteroduplex is formed. In a second format, the PNA is itself G-rich and invades quadruplex DNA or RNA to form heteroquadruplex structures. Biophysical and biochemical experiments will be presented showing low nanomolar  $K_D$  and  $IC_{50}$  values

for quadruplex-targeted PNAs. The benefits of using a second-generation PNA analogue, gammaPNA, will also be presented.



## CARB 17

### Structural plasticity in disease and pharmaceutical targeting of non-coding RNAs

**Gabriele Varani**, [varani@chem.washington.edu](mailto:varani@chem.washington.edu). Chemistry, University of Washington, Seattle, Washington, United States

Long and short non-coding RNAs regulate gene expression in healthy and diseased cellular states. Their mutation or abnormal expression is often observed in chronic diseases ranging from cancer to neurodegeneration. We have shown that a single nucleotide polymorphism within the promoter-associated transcript regulates expression of the tumor suppressor E-cadherin by causing an RNA structural switch which affects differential recruitment of epigenetic enzymes and results in differential prostate cancer progression. Metastable, switchable RNA structures within non coding RNAs provide attractive but unexploited targets for pharmaceutical intervention. We target non coding RNAs by using cyclic peptides and engineered proteins to inhibit expression of the oncogenic microRNA miR-21. This chemistry provides unprecedented affinity and specificity for stem-loop structures that constitute the dominant structural element within non-coding RNAs, penetrate cells readily and allow demonstration of cellular activity through RNA target engagement.

## CARB 18

## **NIH glycoscience program: Developing new facile methods for synthesis of biomedically relevant carbohydrates**

**Pamela Marino**, [marinop@nigms.nih.gov](mailto:marinop@nigms.nih.gov). PPBC, NIGMS, NIH, Bethesda, Maryland, United States

Carbohydrates are ubiquitous to all living organisms serving key biological functions in normal and disease processes. While nearly all aspects of mammalian biology are affected by glycan-mediated events, the complexity of carbohydrates, amplified by the presence of stereo-isomers, anomeric configurations, branched chains, and modifications has rendered study of their biological roles intractable to most researchers. Lack of approaches to produce affordable, well-characterized glycans and glycoconjugates spanning the chemical diversity of mammalian and microbial glycomes is a roadblock to understanding their functions. Chemical methods exist to synthesize only a small portion of biomedically relevant glycans. These methods are generally complex requiring sophisticated synthetic expertise and produce limited quantities of glycans/glycoconjugates at considerable cost. This has resulted in a paucity of glycan standards (including sets of isomers), and hampered the development of tools for decipher their biological function(s). Ideally, one would like to be able to efficiently and cost effectively synthesize all biomedically relevant mammalian and microbial glycomes at will, and to scale up synthesis when appropriate. To this end, the NIH Common Fund *Accelerating Translation of Glycoscience: Integration and Accessibility Program* is focused on creating accessible and affordable new methods, tools, and technologies for the study of carbohydrates such that biomedical researchers can significantly advance our understanding of the roles of these complex molecules in health and disease. The program supports three major initiatives: development of methods and technologies for synthesis of biomedically relevant carbohydrates; development of accessible tools for probing and analyzing carbohydrates and their interaction partners; and development of data integration and analysis tools. The synthetic initiative is focused on the development of new innovative methods (catalytic, chemical, and chemo-enzymatic), and technologies to facilitate the rapid, robust, and affordable synthesis, and/or functionalization of biomedically relevant glycans and glycoconjugates. To date 18 awards for synthesis have been made and awardees work as a team. A major focus of this team is the development of new robust, stereo-selective catalytic methods. Development of these methods are crucial for the program's success and will ultimately facilitate the automation of carbohydrate synthesis.

### **CARB 19**

#### **Cell-surface glyco-engineering using sialyl transferases and modified CMP-Neu5Ac derivatives**

**G Boons**, [gjboons@ccrc.uga.edu](mailto:gjboons@ccrc.uga.edu). Complex Carbohydrate Research Centre, University of Georgia, Athens, Georgia, United States

It has been difficult to explore the biological properties of specific glycans in the context of cells. We are addressing this deficiency by developing a cell-surface engineering strategy based on the use of a CMP-Neu5Ac derivative that is modified at C-5 by a bi-functional entity composed of a complex oligosaccharide and biotin. It was found that recombinant ST6GAL1 can readily transfer the modified sialic acid to glycoprotein acceptors of living cells resulting long-lived display. It made it possible to display a range of well-defined oligosaccharides, such heparan sulfate and matriglycan, on the surface of cells that do not present these oligosaccharides resulting in a gain of biological activity. The new approach makes it possible to establish structure-function relationships in the context of cells.

## **CARB 20**

### **Chemoselective amidoglycosylation of all four D-glycal 3-carbamate diastereomers: Synthesis of stereo-varied 2-amino sugar oxazolidinones**

*Christian M. Rojas, crojas@barnard.edu. Chemistry Department, Barnard College, New York, New York, United States*

Metal-complexed nitrene intermediates generated from glycal 3-carbamates provide one-pot, stereocontrolled C2–N bond formation as well as incorporation of a nucleophile at the anomeric center. All four D-glycal 3-carbamate diastereomers have been investigated, leading to a range of 2-amino sugar derivatives bearing a cis-2N,3O-oxazolidinone group. In developing this methodology, we discovered that the nitrene intermediate partitions between the desired alkene addition reaction (which leads to amidoglycosylation via a presumed glycosyl aziridine donor) and a competing C3–H oxidation that leads to a dihydropyranone byproduct. We have developed solutions to this chemoselectivity problem both through substrate control (varying the electronic, steric, and conformational properties of the glycals via the 4O,6O protecting groups) and catalyst control (use of Rh-, Cu-, Ag-, and Fe-complexed nitrenes). The reaction tolerates C6-azido functionality as well as a range of glycosyl acceptor alcohols. The resulting 2-amino sugar oxazolidinones can be further derivatized by N-arylation and also advanced to the fully deprotected free reducing sugars for dehydrative couplings with aglycons.

## **CARB 21**

### **Challenges in automating catalytic glycosylation methods**

*Nicola L. Pohl, npohl@indiana.edu. Dept of Chem 120A Simon Hall, Indiana University, Bloomington, Indiana, United States*

A major roadblock in the advance of glycobiology is ready access to chemically well-defined structures. Methods amenable to the automated assembly of monosaccharide building blocks promise such access, but the transfer of manual protocols to the automated liquid handling platforms needed as part of both solution-phase and solid-

phase-based synthesizers is nontrivial. With a focus on chemists interested in making their new methods broadly applicable to the future of glycan synthesis, this talk will discuss the requirements of chemical procedures for their ready adoption in automation platforms using catalytic and other glycosylation reactions as models.

## **CARB 22**

### **One-pot multienzyme (OPME) chemoenzymatic synthesis of carbohydrates and sialidase inhibitors**

***Xi Chen**, xiichen@ucdavis.edu. Dept of Chemistry, UC Davis, Davis, California, United States*

Carbohydrates are structurally diverse and functionally important biomolecules. Many naturally occurring carbohydrates contain modifications that are added after the formation of the glycosidic linkage, named as post-glycosylational modifications (PGMs). We have developed highly efficient glycosyltransferase-based one-pot multienzyme (OPME) systems for synthesizing structurally complex naturally occurring carbohydrates and their non-natural derivatives. In these systems, desired modifications are introduced at the monosaccharide building blocks by chemical synthesis. Substrate promiscuous enzymes are then used in one-pot for activation and transfer of the modified monosaccharides to suitable acceptors for glycosyltransferases. The OPME systems can be used in sequential for building up longer and complex structures. The straight-forward synthetic schemes can be combined with facile purification processes for efficient production of desired compounds. Sialidase inhibitors can also be synthesized by novel OPME systems from simple monosaccharides and derivatives. Our recent progress on the development of the OPME strategy will be presented.

## **CARB 23**

### **Regenerative glycosylation via nucleophilic catalysis**

***Alexei Demchenko**<sup>1</sup>, demchenkoa@umsl.edu, Yashopal Singh<sup>1</sup>, Keith J. Stine<sup>2</sup>. (1) Dept of Chem Biochem, UM - St. Louis, St. Louis, Missouri, United States (2) University of Missouri, Saint Louis, Missouri, United States*

From the building blocks of nature to disease-battling pharmaceuticals carbohydrates have had deep impact on many aspects of science and technology. Numerous applications of these biomolecules exist, most of which can be found in the areas of functional-food, therapeutic-agent, and diagnostic-platform development. Although carbohydrates are desirable for the biomedical community, these molecules are very challenging synthetic targets. The development of practical and general methods for chemical glycosylation and oligosaccharide synthesis represent important areas of research.

At the core of this presentation is the development of a new concept for chemical glycosylation introduced by our laboratory. Discussed herein are our recent results on defining the scope of the regenerative glycosylation method and its application to the oligosaccharide assembly via conventional stepwise approach and HPLC-assisted automated synthesis.

## **CARB 24**

### **Optical control of nucleic acid function in biological systems**

*Alexander Deiters, alexdeiters@gmail.com. Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, United States*

Synthetic oligonucleotides have been extensively used in the control of a wide range of biological processes such as gene expression, gene editing, DNA replication, and protein activity. Based on well-established sequence design rules that rely on Watson–Crick base pairing interactions, the function of these oligonucleotides can be targeted to any gene of interest. In order to provide conditional control over oligonucleotide function, we are installing light-cleavable chromophore onto nucleobases and in phosphodiester backbones. This allows us to both activate and deactivate gene expression photochemically at the transcriptional and translational level with spatial and temporal control in human cells and zebrafish embryos. Specifically, we have developed caged triplex-forming oligonucleotides, DNA decoys, and plasmids to regulate transcription, and we have controlled translation with light-activated antisense agents and splice-switching oligonucleotides, and I will present the overall approach and select examples.

## **CARB 25**

### **Molecular recognition of DNA: From discovery to oncology**

*Alexis Kurmis<sup>2</sup>, akurmis09@gmail.com, Peter B. Dervan<sup>1</sup>. (1) California Inst of Technology, Pasadena, California, United States (2) Chemistry, California Institute of Technology, Pasadena, California, United States*

Pyrrole-imidazole polyamides are synthetic molecules programmed to read the DNA double helix by a set of simple chemical principles. These small molecules achieve affinities comparable to DNA-binding proteins and are cell permeable. Research efforts are focused on the modulation of gene expression pathways by disruption of transcription factor-DNA interfaces. The oversupply or overactivity of one or more transcription factors may be required for the survival, growth, and metastatic behavior of all human cancers. The efficacy of Py-Im polyamide oligomers targeted against therapy resistant prostate cancer xenografts will be discussed.

## **CARB 26**

## **Modulating nucleic acid structure and function using shape-selective small molecules**

*David M. Chenoweth, dcheno@sas.upenn.edu. Dept of chemistry, University of Pennsylvania, Philadelphia, Pennsylvania, United States*

Nucleic acids play important roles in fundamental cellular processes and in the regulation of nearly all disease states. Recently, a dramatic number of human diseases are directly associated with RNA molecules. Most known RNA molecules have unknown structures beyond those of predicted secondary structure maps. Despite this lack of structural information, common themes have emerged through structure predictions. For example, three-way junctions are one of the most commonly observed RNA motifs, varying widely in terms of predicted structure at the junction interface. Many of these junctions are centrally located in the predicted RNA maps where structural perturbation could have a significant influence on function. Small molecule targeting of specific RNA structural elements, such as junctions, may allow for chemical control over many cellular processes and disease states. Our laboratory is developing new small molecule scaffolds for the recognition of RNA to control the structure and function of specific motifs.

### **CARB 27**

#### **Small molecule therapeutics targeting nucleic acids**

*Dev P. Arya, dparya@clermson.edu. Chemistry, Clemson University, Clemson, South Carolina, United States*

Nucleic Acids offer us exciting targets for molecular recognition and drug development. These targets include validated nucleic acid drug targets such as the bacterial ribosome and DNA, as well as new targets for therapeutic intervention, such as micro RNA. In this presentation, I will share our recent results describing the recognition of validated targets (pathogen ribosome) and new targets, such as oncogenic micro RNA. Tunable approaches with broad applicability in targeting various nucleic acid targets will be discussed.

### **CARB 28**

#### **Target-specific inhibition of transcription factors by designed, synthetic DNA ligands**

*W David Wilson, wdw@gsu.edu, Ananya Paul, Pu Guo, Tam D. Vo, Narinder Harika, Arvind Kumar, Abdelbasset A. Farahat, Beibei Liu, Gregory M. Poon, Markus W. Germann, Boykin W. David. Department of Chemistry, Georgia State University, Atlanta, Georgia, United States*

Small, synthetic molecules that strongly and selectively bind to DNA in biological systems and induce specific biological responses, such as changes in gene expression, are a central goal of biomolecular compound design and synthesis research as well as therapeutic development. Control of gene expression could be accomplished directly by either inhibiting or enhancing binding of transcription factors to their DNA promoter sites. Since transcription factors themselves are frequently classified as "undruggable", targeting the DNA component of DNA-protein complexes would facilitate progress and provide an important step forward in the area. Unfortunately, there has been very limited advancement in compound designs for recognizing mixed base pair (bp) DNA promoter sequences. Heterocyclic-cationic, minor-groove binders that can selectively interact with DNA have become a valuable resource in therapeutics. These successful agents have provided proof of concept that selective recognition of the DNA minor groove with a set of modules, combined in different ways for different sequences, is possible. The combinatorial design and preparation of modular minor-groove, sequence-specific compounds that can selectively recognize GC and AT bp in more complex DNA sequences than most current agents would be a major step forward in promoter site binding. Using this approach, compounds are being prepared with H-bond donors for AT bp binding and H-bond acceptors for GC bp interactions. Conformational matching of the synthetic agents to the shape and functionality of the minor groove is critical. We now have several compounds that can selectively recognize a single GC bp with flanking AT pairs, in contrast to the traditional AT-only binding of these type compounds. The critical question at this point, which we are now addressing, is how to increase selectivity and how to extend the initial compounds to recognize longer, more complex DNA sequences with additional GC bps. By combining modular GC recognition units, we expect to prepare agents that can selectively recognize two GC bps in DNA sequences such as (A/T)<sub>m</sub>-(G/C)-(A/T)<sub>n</sub>-(G/C)-(A/T)<sub>m</sub>. The compounds should have enough flexibility to match the shape and curvature of the DNA minor groove and an appropriate length with recognition modules spaced to bind to the DNA sequence and cover a full turn of the DNA helix. The design strategy and DNA interaction results will be described.

## **CARB 29**

### **De novo approaches to oligosaccharide assembly for stereochemical structure activity relationship studies (S-SAR)**

**George A. O'Doherty**, *g.odoherty@neu.edu*. Dept of Chemistry Chem Biology, Northeastern Univ, Boston, Massachusetts, United States

Over the years, the O'Doherty group has been working to develop practical catalytic asymmetric approaches to the synthesis and study of stereochemically complex structural motifs. The unifying theme that connects our research in these two areas is our method of synthesis (asymmetric catalysis) and target selection (stereochemically complexity and biological activity). A recurring theme in the group's synthetic approaches to any target is the reliance on asymmetric catalysis and synthetic design for the control of asymmetry. The application of these "atom economical" approaches



should allow simple conversion of readily available bulk chemicals (e.g., furans) to advanced chiral intermediates (e.g., pyranones) which in turn can be readily assembled into stereochemical complex molecules of interest (e.g., oligosaccharides). Fundamental to our approach is the development of highly efficient and stereoselective routes that transform, via catalysis, inexpensive and readily available building blocks into complex oligosaccharides and related stereoisomers for further biomedical investigations.

## **CARB 30**

### **Silane reagents for intramolecular glycosylation**

*John Montgomery, jmontg@umich.edu. Univ of Michigan, Ann Arbor, Michigan, United States*

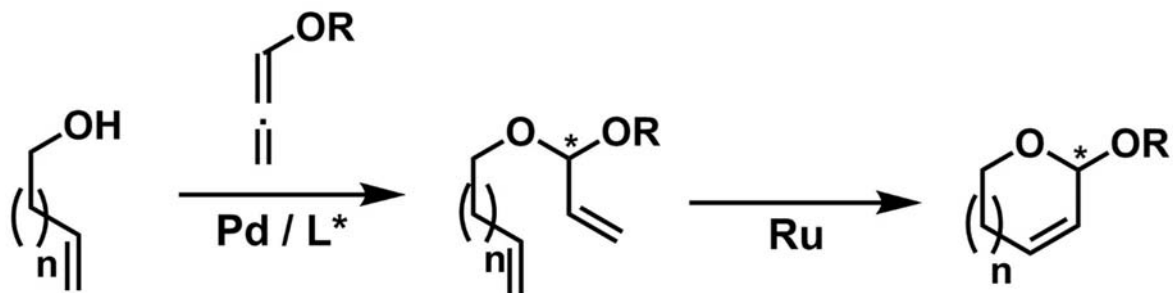
The assembly of carbohydrate-derived silane reagents (sugar silanes) and their utilization in glycosylation strategies will be described. The requisite sugar silane reagents may be prepared by simple condensations of sugar hydroxyls utilizing commercially available dialkylchlorosilane reagents. From these intermediates, an array of sugar acceptors including alcohols, ketones or aldehyde-alkyne reagent pairs can be installed through metal-catalyzed condensations or reductive coupling strategies. The resulting silicon-tethered assemblies can be utilized in versatile intramolecular glycosylations to prepare challenging frameworks including beta-mannosides, alpha-glucosides, or beta-2-deoxy-, 2-alkoxy-, and 2-azidoglycosides. Recent developments and mechanistic insights into this approach will be described.

## **CARB 31**

### **Pd-catalyzed asymmetric hydroalkoxylation of allene: A new synthetic method for carbohydrates**

*Young ho Rhee, yhrhee@postech.ac.kr, Mijin Kim, Juyeol Lee, Soyeong Kang. Chemistry, Pohang University of Science and Technology, Pohang, Kyungbook, Korea (the Republic of)*

In this presentation, we wish to report our recent studies on the Pd-catalyzed asymmetric intermolecular hydroalkoxylation of alkoxyallene. Upon combination with ring-closing-metathesis, this reaction proposes a conceptually new synthetic method for various mono- and oligosaccharides. A salient feature of this method is that the anomeric stereoselectivity is controlled by the chiral ligand. Mechanistic aspect of the reaction as well as some synthetic applications involving 2,3,6-trideoxyglycosides (such as amictose) and apiofuranoglycoside will be introduced.



## CARB 32

### Production of O-glycans and O-glycopeptides/glycoproteins by chemical and enzymatic catalysis production of O-glycans and O-glycopeptides/glycoproteins by chemical and enzymatic catalysis

**Peng G. Wang**, *pwang11@gsu.edu*. Department of Chemistry, Georgia State University, Atlanta, Georgia, United States

In mammalian glycomes, O-glycans and O-glycopeptides/glycoproteins compose an indispensable category. These O-glycans are linked to peptides/proteins via Ser/Thr. Although functional important, the synthesis of such O-glycans/glycopeptides/glycoproteins is still lagging behind, hindering further functional study.

By a core synthesis – enzymatic extension (CSEE) strategy, we achieved the complexity and diversity of O-glycans/glycopeptides/glycoproteins with most natural structural diversity. Starting with 8 chemically prepared O-GalNAc core structures and 2 O-Man cores, we diversified the glycans with specific glycosyltransferases to over 100 O-GalNAc glycans and over 40 O-Man glycans, which is by far the most complex O-glycan library. For O-glycopeptide/glycoprotein synthesis, the chemically prepared core glycans were incorporated into glycopeptide/glycoprotein by solid-phase peptide synthesis, and the glycans were further extended to provide fully glycosylated O-glycopeptides/glycoproteins. The O-glycans and the O-glycopeptides/glycoproteins prepared by this strategy should serve as standard for O-glycan structure characterization and are valuable for functional glycomic studies to better understand the functions and mechanisms of O-glycans as well as O-glycopeptides/glycoproteins.

## CARB 33

### New catalytic methods in carbohydrate synthesis

**Maciej A. Walczak**, *ffiord@gmail.com*. Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado, United States

Although carbohydrate synthesis is considered by many as a mature field, universal methods for chemical glycosylation resulting in high levels of anomeric control across a broad range of substrates are lacking. These limitations are inherent to nucleophilic displacement methods in which the glycosyl donor bears an electronegative element at the anomeric carbon. An alternative approach that focuses on the use of configurationally stable anomeric nucleophiles is a promising solution because of the predictable nature of the glycosylation reaction and direct stereochemical relationship between the substrate and the product. In this presentation, I will discuss recent developments from our group focused on the use of C1 nucleophiles in the synthesis of oligosaccharides of biomedical relevance.

## **CARB 34**

### **New catalytic methods for stereoselective glycosylation**

**Hien M. Nguyen**<sup>1,2</sup>, [hien-nguyen@uiowa.edu](mailto:hien-nguyen@uiowa.edu). (1) Department of Chemistry, Wayne State University, Detroit, Michigan, United States (2) Department of Chemistry, University of Iowa, Iowa City, Iowa, United States

The field of glycoscience has burgeoned in the last several decades, leading to the identification of many oligosaccharides and glycoconjugates which could serve critical roles in a range of biological processes. Meeting research demands require access to significant quantities of well-defined bioactive carbohydrates. Although numerous elegant strategies and methods have developed for the formation of glycosidic bonds,<sup>1-8</sup> stereoselective construction of alpha- and beta-glycosides remains challenging. Most of the current method relies on the nature of the substrate's protecting groups to control anomeric selectivity. In addition, glycosylation scenarios often require stoichiometric amounts of activating agents to sufficiently activate donors. In this symposium, I will present new two approaches to novel carbohydrate coupling methods, including nitrogen-containing heterocycle-catalyzed 1,2-*cis*-glycosylation and photoinduced copper-catalyzed stereoselective glycosylation.

## **CARB 35**

### **Synthesis and biological evaluations of mono- and poly-fluorogalactopyranosides: Preparation of selective galectin inhibitors**

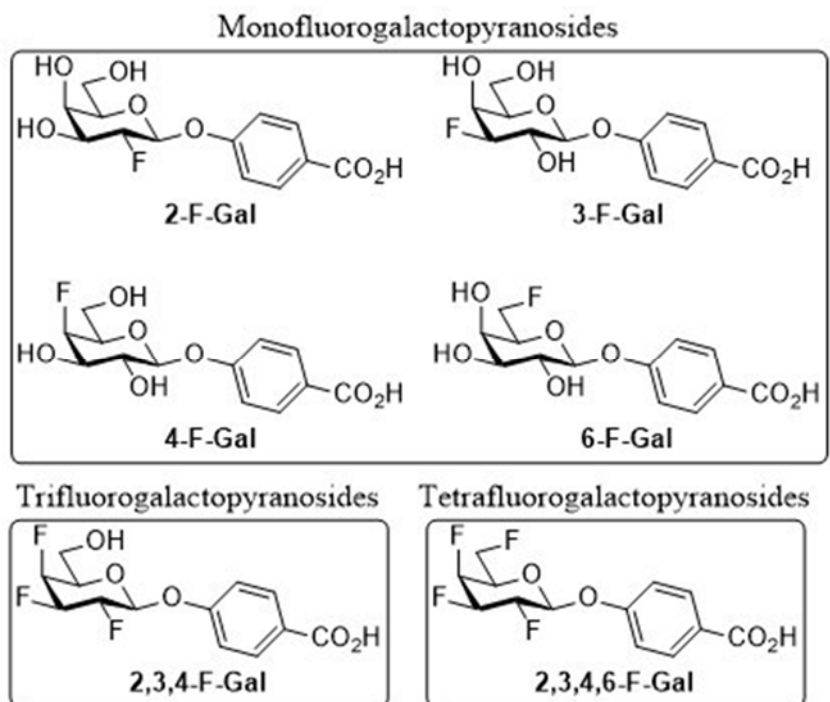
**Danny Laine**<sup>2</sup>, [danny.laine.1@ulaval.ca](mailto:danny.laine.1@ulaval.ca), Vincent Denavit<sup>3</sup>, Denis Giguere<sup>1</sup>. (1) Chemistry, Laval University, Quebec, Quebec, Canada (2) Chemistry, Université Laval, Quebec, Quebec, Canada (3) Chemistry, Université Laval, Quebec city, Quebec, Canada

Galectins are a family of galactose-binding proteins present in the cytoplasm with multiple biological activities. Galectin-3 is implicated in various cancers and galectin-1 increase HIV-1 attachment to target cells. The discovery of selective inhibitors of galectins could lead to new anti-cancer or antiviral agents.

The purpose of our research is the synthesis of selective galectin inhibitors with unique pharmacokinetic properties.

We propose that substituting the hydroxyl groups present on the galactopyranoside core will procure analogs with improved biological activities, superior metabolic stabilities and enhanced pharmacokinetic profile. The fluorinated carbohydrates represent a good strategy to improve protein-carbohydrate interactions via desolvation together with attractive dipolar interactions mediated by the polar C-F bonds. The incorporation of fluorine atoms in a pyranose sugar unit does not have a deleterious effect on the interactions with the binding site of the protein, despite the absence of any hydrogen bond donating capacity and the reduced aptitude to accept hydrogen bonds.

A Chiron approach was used to access a library of fluorinated galactosides: all the monofluorogalactosides, a trifluorogalactoside and a tetrafluorogalactoside. All the derivatives were evaluated over galectin-1 and galectin-3 using quantitative enzyme-linked immunosorbent assay (ELISA) tests. The results show that some fluorinated galactopyranosides have selectivity toward galectin-1. The stereochemistry of the fluorine groups were confirmed using X-ray crystallography and NMR spectroscopy.



## **New metabolic chemical reporter 6-azido-6-deoxy-glucose reveals an unexpected substrate promiscuity of O-GlcNAc transferase and the potential for protein modification by O-glucose**

**Narek Darabedian**, *darabedi@usc.edu*, Matthew Pratt. University of Southern California, Los Angeles, California, United States

Metabolic chemical reporters of glycosylation in combination with bioorthogonal reactions have been known for two decades and have been used by many different research labs for the identification and visualization of glycoconjugates. More recently, however, they have begun to see utility for the investigation of cellular metabolism and the tolerance of biosynthetic enzymes and glycosyltransferases to different sugars. Here, we take this concept one step further by using the metabolic chemical reporter 6-azido-6-deoxy-glucose (6AzGlc). We show that treatment of mammalian cells with the per-O-acetylated version of 6AzGlc results in robust labeling of a variety of proteins. Notably, the pattern of this labeling was consistent with O-GlcNAc modifications, suggesting that the enzyme O-GlcNAc transferase is quite promiscuous for its donor sugar substrates. To confirm this possibility, we show that 6AzGlc-treatment results in the labeling of known O-GlcNAcylated proteins, that the UDP-6AzGlc donor sugar is indeed produced in living cells, and that recombinant OGT will accept UDP-6AzGlc as a substrate *in vitro*. Finally, we use proteomics to directly identify 6AzGlc-modification in mammalian cells. These results support the possibility that OGT can endogenously modify proteins with both *N*-acetyl-glucosamine and glucose, raising the possibility that intracellular O-glucose modification will be found endogenously.

### **CARB 37**

#### **Direct coupling of amides and urea to glycosyl halides using silver triflate**

**Michael De Castro**, *michael.decastro@farmingdale.edu*. Chemistry, Farmingdale State College, Farmingdale, New York, United States

We herein report the direct addition of various amides and ureas to haloglycosides in the presence of silver triflate at room temperature. A 1:1 mixture of  $\alpha/\beta$  diastereomers was obtained when alkyl / heteroaryl amides and substituted ureas were added to the gluco and galacto haloglycoside. When the fully acetylated glucuronamide was employed in the reaction the  $\beta$  anomer of the corresponding pseudodissaccharide was obtained in good yields at room temperature. The newly synthesized compounds underwent viability studies using HeLa cancer cell. The results obtained are presented in this poster.

### **CARB 38**

#### **Improved HPAE-PAD method for glycoprotein monosaccharide determination**

**Sachin Patil**, [sachin.patil@thermofisher.com](mailto:sachin.patil@thermofisher.com), Jeffrey Rohrer. Thermo Fisher Scientific, Sunnyvale, California, United States

Determination of the monosaccharide composition of a glycoprotein pharmaceutical is a key quality control assay for glycoprotein-based therapeutics. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a well-established method for glycoprotein carbohydrate analysis. This work demonstrates an improved HPAE-PAD method using the new Thermo Scientific™ Dionex™ CarboPac™ PA20 column with 4 μm particle size to simultaneously quantify six major monosaccharides present in glycoprotein acid digests. The total run time is 20 minutes as compared to a 32-minute method with a 6.5 μm particle size column. The column's smaller particle size offers higher peak efficiencies leading to high resolution separations in significantly shorter run times.

Here, two different commercially available proteins, bovine fetuin and alpha1 acid glycoprotein were individually subjected to two different hydrolysis conditions using 1) HCl, which favors release of amino sugars like galactosamine and glucosamine and 2) TFA, which favors release of neutral sugars like mannose, glucose, and galactose. Figure 1 shows a representative chromatogram of a standard mix containing the six common glycoprotein carbohydrates. All peaks are well resolved and the separation is completed within 8 minutes. The method is linear at monosaccharide concentrations between 1.56 to 300 μM (Table 1). The monosaccharide retention time and peak area RSDs are less than 2.66%, indicating excellent method precision. The more efficient peaks also allow the injection of less sample for routine monosaccharide quantification. The method described here requires 0.5 μg injected protein compared to 2 μg for the method previously described. This assay was validated according to the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures. The method was shown to measure accurately the monosaccharide concentration in complex matrices like acid-hydrolyzed proteins. Moreover, the method is robust to experimental condition variations that occur during routine use.

Figure 1. Monosaccharide separation on CarboPac PA20-Fast-4μm, 2x100 mm column.

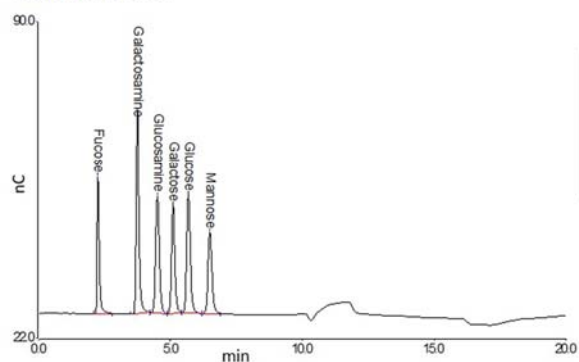


Table 1. Calibration (between 1.56 to 300 μM concentration of the monosaccharides) and precision data (10 μM concentration, n=3).

	Fucose	Galactosamine	Glucosamine	Galactose	Glucose	Mannose
Coefficient of Determination	0.995	1	1	0.995	0.993	0.994
Calibration Range (μM)	1.56-50	1.56-50	1.56-300	1.56-100	1.56-50	1.56-100
RT % RSD	0.43	0.39	0.32	0.38	0.43	0.34
Area %RSD	1.14	0.24	0.62	0.82	1.17	2.66

## **CARB 39**

### **Synthesis of the glycosylated amino acid bearing the Thomsen nouvelle antigen**

*Elizabeth R. Mercer, elizabethrosemercer@gmail.com, Donella Beckwith, Mare Cudic. Chemistry and Biochemistry, Florida Atlantic University, Ft Lauderdale, Florida, United States*

Metastatic breast cancer is the proliferation of cancerous cells from a primary breast tumor site. The spread of these cancerous cells increases the difficulty of detection and treatment, leading to an overall reduced survival rate. One of the key indicators of breast cancer is the overexpression and aberrant glycosylation of Mucin 1 (MUC1), a large transmembrane protein located on the apical surface of human epithelial cells. Aberrant carbohydrate expression of Tumor-Associated Carbohydrate Antigens (TACAs) in breast cancer cells has been shown to indicate tumor progression and metastasis. MUC1 consists of a variable number tandem repeat (VNTR) domains that generally favor serine, threonine, and proline residues, the presence of which promote O-glycosylation. As a protective and cell signaling protein, its structure can be defined as densely O-glycosylated with long, and branched sugars. The overexpression, irregular intercellular localization, and aberrant glycosylation of this protein are associated with breast cancer progression. Breast cancer cell lines express truncated O-glycans, such as the Thomsen Nouvelle Antigen (Tn) antigen. It is hypothesized that TACAs like the Tn antigen allow MUC1 to be more exposed and open to interactions with carbohydrate binding proteins (lectins) that are overexpressed in metastatic cancers. In particular, the macrophage galactose type C lectin (MGL) recognizes Tn and sialylated Tn antigen, and facilitates the uptake of the antigen and regulation of T-cell immune response. The objective of our research was to prepare gram quantities of the Tn- $\alpha$ -O-Ser/Thr building blocks suitable for use in the solid-phase peptide synthesis of glycopeptide models of tumor-associated MUC1, as a step towards understanding how they affect cancer biology. The synthesis of Tn was completed through four key reactions: per-acetylation of D-galactal, one-pot azidochlorination reaction, glycosylation of Ser/Thr residue, and reductive acetylation of an azide group to an N-acetyl group. The purity of the final Ser/Thr-Tn building blocks was confirmed using RP-HPLC, MALDI-TOF mass spectrometry,  $^1\text{H}$  and  $^{13}\text{C}$  NMR. The availability of this building block will allow us to proceed to the next step, the synthesis of cancer-associated MUC1 glycopeptide models, with the long-term goal of studying the role of altered glycosylation of MUC1 in tumor progression and metastasis.

## **CARB 40**

### **Comparison of furanoside conformational preferences between calculation and better resolved NMR measurement**

*Jonathan S. Rhoad, jrhoad1@missouriwestern.edu, Shawn Culver. Missouri Western State Univ, Saint Joseph, Missouri, United States*

In our continuing efforts to better understand conformational preferences of flexible furanoside rings, we have compared coupling constants based on updated Karplus relationships with Boltzmann distribution from energy scan calculations at the B3LYP/6-311(d,p) level of theory with three-bond coupling constants that have been revealed at higher resolution (>0.1 Hz) using the proton NMR 2D *J*-resolved coupling experiment.

## **CARB 41**

### **The influence of protecting group properties in the nickel-catalyzed formation of 1,2-*cis*-2-aminoglycosides**

**Alisa Fairweather**<sup>1</sup>, *Alisa-fairweather@uiowa.edu*, **Hien M. Nguyen**<sup>2</sup>. (1) Department of Chemistry, University of Iowa, Iowa City, Iowa, United States (2) Dept of Chemistry, University of Iowa, Iowa City, Iowa, United States

There has been success in the formation of 1,2-*cis*-amino glycosidic linkages through the utilization of commercially available nickel(II) triflate as an activator in the reagent-controlled glycosylation reaction. Activation of C(2)-*N-ortho*-(trifluoromethyl)benzylideneamino *N*-phenyltrifluoroacetimidate donors under these mild conditions has allowed high stereoselectivity in the construction of many traditionally difficult 1,2-*cis*-2-aminoglycosides. To use this current synthetic strategy with predictability, a systemic study was conducted on the protecting group influence on the stereoselective outcome of the glycosidic bond. A range of protecting groups and their location on several different C(2)-amino sugars were systematically varied on *N*-phenyltrifluoroacetimidate C(2)-amino donors. Counter to that of traditional rationale, the electronic properties (i.e. electron donating and electron withdrawing) of the protecting groups displayed no correlation in the  $\alpha:\beta$  selectivity of the glycosidic reaction. However, a near linear trend emerged when hydrophilicity of the protecting groups was utilized instead of their electronic factors. The trend showed that more hydrophilic (lower log *P* values) protecting groups are more  $\alpha$ -selective than the hydrophobic counterparts. For example, a C(4)-acetyl (log *P* of -0.24) yielded an  $\alpha:\beta$  selectivity of 15:1 whereas C(4)-benzyl (log *P* of 2.78) yielded just 2:1. Similar results were seen for a range of protecting groups with diverse hydrophilicities at C(3), C(4), and C(6) for glucosamine, galactosamine, as well as several L-aminosugars donors. As well, this systemic varying of the protecting groups has also divulged important data in determining the potential mechanism of the glycosylation reaction at hand.

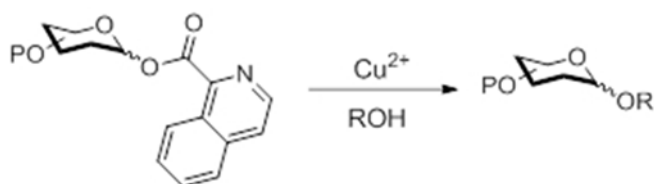
## **CARB 42**

### **Isoquinoline-1-carboxylate as a traceless leaving group for chelation-assisted glycosylations**

**Christopher Simmons**<sup>1</sup>, *csimmons3@wisc.edu*, **Haoyuan Wang**<sup>2</sup>, **Weiping Tang**<sup>3</sup>. (1) Chemistry, University of Wisconsin - Madison, Madison, Wisconsin, United States (2) University of Wisconsin-Madison, Madison, Wisconsin, United States (3) School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States



Oligosaccharides have been shown to have utility in medicinal chemistry, including anti-cancer therapeutics, antibiotics and vaccines. However, the synthesis of oligosaccharides can be challenging and often requires the formation of unstable glycosyl donors and harsh conditions for the formation of glycosidic bonds. We envisioned that having a coordinating ligand on the leaving group might provide a relatively stable glycosyl donor that can be activated under mild conditions. We discovered that an isoquinolinic ester could be selectively activated by copper (II) salts and acted as a traceless leaving group for glycosylation reactions. Although previous research had shown that picolinic esters could similarly be activated by copper (II), a competition experiment showed that the isoquinolinic esters were at least two orders of magnitude more reactive than picolinic esters in the presence of copper (II) salts. In addition, the isoquinolinic esters could also avoid the competitive transesterification side reactions observed for some picolinic substrates. Finally, the isoquinolinic ester was shown to be compatible with ortho-alkynyl esters that can be activated by gold (I) complexes, allowing for an iterative synthesis of a tetrasaccharide from monosaccharide units in only four steps.



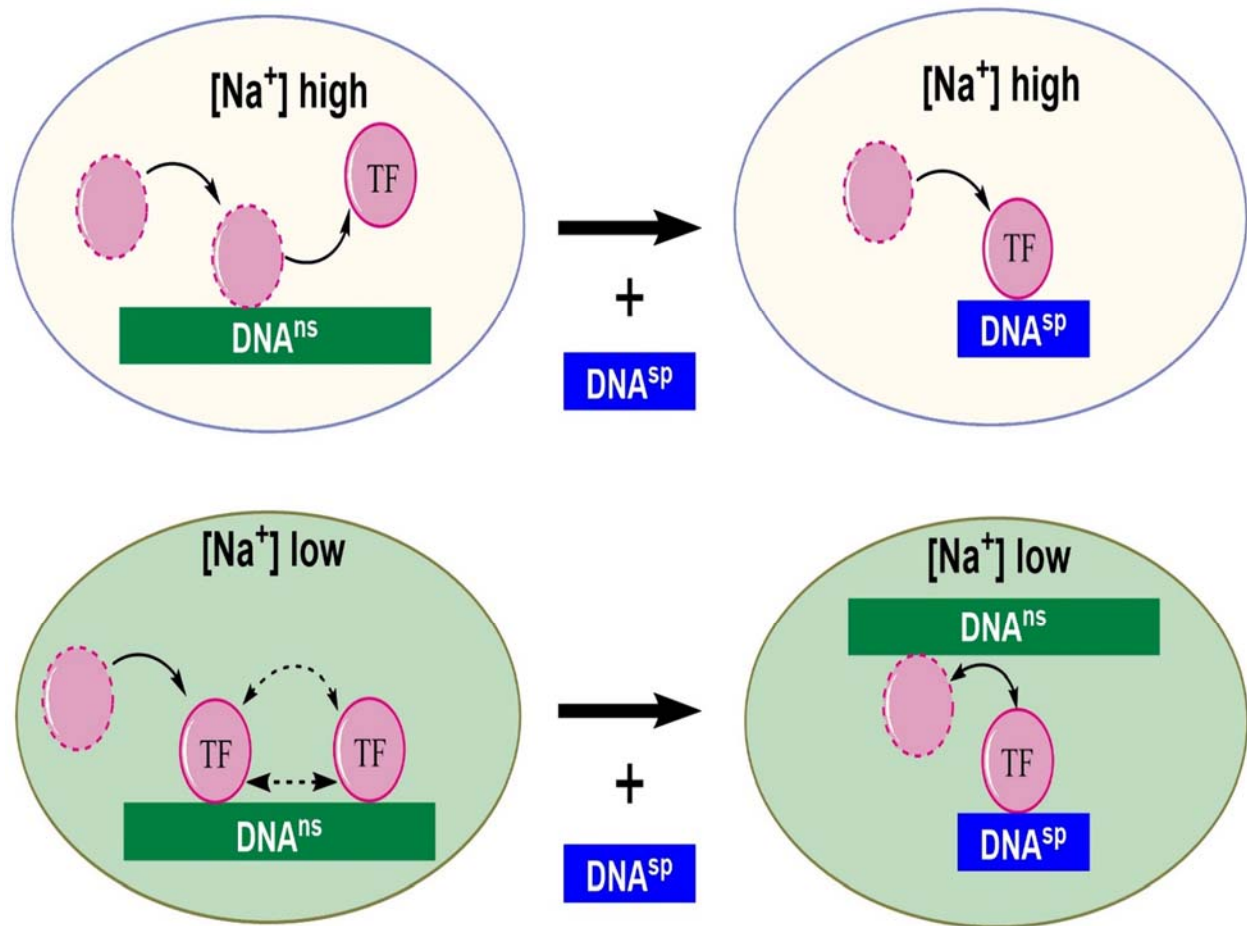
## CARB 43

### Electrostatic control of binding interactions between ETS transcription factors proteins and their cognate DNA

**Tam D. Vo**<sup>1</sup>, [tvo38@student.gsu.edu](mailto:tvo38@student.gsu.edu), Shuo Wang<sup>1</sup>, Gregory M. Poon<sup>1,2</sup>, W David Wilson<sup>1,2</sup>. (1) Chemistry, Georgia State University, Atlanta, Georgia, United States (2) Center of Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia, United States

The ETS family of transcription factors was found to be essential for many cellular functions and was involved in the development of diseases such as cancers. All the members of the ETS family recognize conserved DNA sequences with a helix-turn-helix motif at 5'-GGAA-3' in the major groove and contact the flanking DNA sequences in the minor groove via phosphate backbone. Despite the exponential increase in knowledge on the molecular biology of this family, there is limited information on the mechanism how these structural conserved transcription factors find their target DNA site. Transcription factors and other DNA binding proteins can efficiently search for their target DNA via facilitated diffusion such as sliding, hopping, and intersegmental transfer. The mechanisms of sliding and hopping have been scrutinized with different proteins

over the years and are well understood at the molecular level. Knowledge of the intersegmental transfer of proteins with a single DNA binding domain remains unveiled. We have developed a method using surface plasmon resonance (SPR) to examine the binding mechanism of ETS proteins to its DNA target site. In the previous study on ETV6, a member of ETS, we have shown that by changing salt concentration, we can directly observe the change in the mechanism that a protein initiates to find its target DNA. The results showed that target search by ETV6, which cannot contact the source and targeted DNA site spontaneously, still in fact governed by intersegmental transfer in a heterogeneous site environment. We have expanded the observation on four different ETS proteins that are evenly spaced on the evolutionary phylogenetic tree. The structures and interactions of many of the ETS transcription factors with their cognate DNA sequences are now understood in detail.

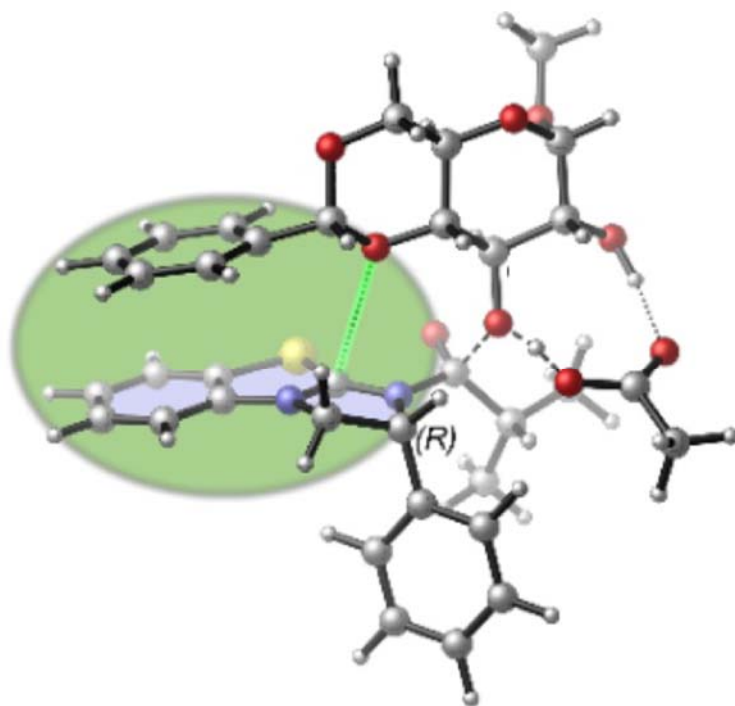


Schematic model of electrostatic toggle of target search by free diffusion at high salt and intersegmental jumping at low salt.

## Using chiral catalysts and cation- $n$ interactions to direct site-selective acylation of carbohydrates

Guozhi Xiao<sup>2</sup>, **Stephanie Blaszczyk**<sup>3</sup>, StephBlaszczyk8@gmail.com, Gabriel Cintron-Rosado<sup>1</sup>, Daniel A. Glazier<sup>3</sup>, Peng Liu<sup>1</sup>, Weiping Tang<sup>4,3</sup>. (1) Department of Chemistry, University of Pittsburgh, Los Angeles, California, United States (2) School of Pharmacy, University of Wisconsin, Madison, Wisconsin, United States (3) Chemistry, University of Wisconsin - Madison, South Beloit, Illinois, United States (4) School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States

Carbohydrates are ubiquitous in nature, yet general and efficient synthetic methods for their formation are rare. Furthermore, the site-selective manipulation of hydroxyl groups adds another level of difficulty to their longstanding synthetic issues. Using a pair of chiral catalysts, we can predictably differentiate many trans-1,2-diols in pyranoses. Our model is supported by DFT calculations, which indicate that site-selectivity hinges upon the presence or absence of a cation- $n$  interaction between the cation in the acylated catalyst and a suitable lone pair in the substrate. Initially using O-glycosides, but recently expanding to S-glycosides, we have further validated this model on a variety of substrates. The predictive power of this method makes great strides towards streamlining the chemical synthesis of carbohydrates and will be useful for the investigation of more complex targets in the future.



## **CARB 45**

### **Divergent stereoselective synthesis of rare amino-sugars**

**Daniel A. Glazier**<sup>1</sup>, [dglazier@wisc.edu](mailto:dglazier@wisc.edu), **Zhongpeng Zhu**<sup>1</sup>, **Weiping Tang**<sup>2</sup>. (1) *Chemistry, University of Wisconsin-Madison, Madison, Wisconsin, United States* (2) *School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States*

The desire to study the biological roles played by carbohydrates, such as cellular recognition and protein folding, has made the synthesis of rare and unnatural carbohydrates an important area of research for decades. De novo synthesis of carbohydrates from simple feedstocks has emerged as a promising method for the production of these rare mono- and oligosaccharides. Dihydropyrans, especially those obtained from the Achmatowicz rearrangement, have proven to be particularly useful intermediates for the de novo synthesis of carbohydrates. Aminosugars are an exceptionally interesting class of carbohydrates as they appear in an abundance of natural products, anti-biotics, and other medicinally interesting molecules. We have recently developed a novel stereoselective route to access all possible stereoisomers of 2,3,4,6-tetra-deoxy 4-amino sugars and their derivatives systematically from Achmatowicz rearrangement products. This strategy combines our dynamic kinetic diastereoselective acylation methodology, with a rhodium catalyzed asymmetric reduction and the Mitsunobu reaction to give these rare aminosugars in high yields and stereoselectivities.

## **CARB 46**

### **Development of a new class of carbohydrate-based adjuvants**

**Cecilia E. Marzabadi**<sup>2</sup>, [cecilia.marzabadi@shu.edu](mailto:cecilia.marzabadi@shu.edu), **Vikram Basava**<sup>1</sup>, **Constantine Bitsaktsis**<sup>1</sup>, **David Nichols**<sup>1</sup>. (1) *Seton Hall University, South Orange, New Jersey, United States* (2) *Chemistry Dept, Seton Hall University, South Orange, New Jersey, United States*

Recently we reported that a novel trisaccharide analog prepared in our lab was found to induce the production of TNF- $\alpha$  and IL-6 in macrophage cell lines.<sup>1</sup> Using TLR knockout mice, we determined that the carbohydrate small molecule bound to the TLR-4 receptor. Competition studies with lipopolysaccharide, suggested the two molecules bound to the receptor in an analogous fashion. We are currently investigating the mechanism of action by this novel molecule by evaluating its signaling pathways. The effects of structural modifications on the molecule on its activity (SAR) are also being studied. We will report on the data obtained thus far and hypothesize on how the molecule contributes to innate (and adaptive) signaling.

## **CARB 47**

### **A selective small-molecule inhibitor of O-GlcNAc transferase**

**Nichole Pedowitz**, *npedowit@usc.edu*. Chemistry, University of Southern California, Los Angeles, California, United States

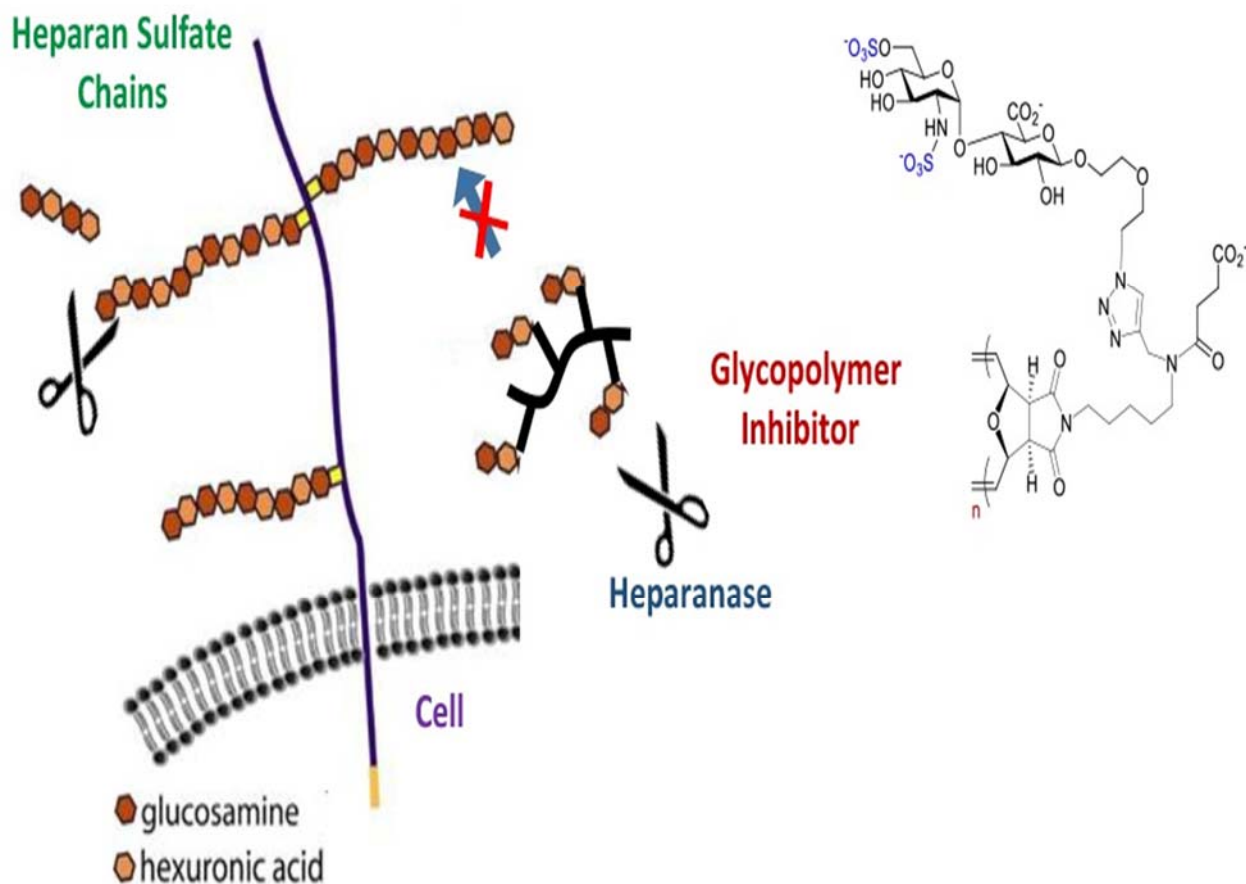
Past research demonstrates that the misregulation of posttranslational modifications (PTMs) is closely related to the occurrence of cellular malignancies. O-GlcNAcylation is one such PTM that refers to the addition of one monosaccharide to the side chain of serine and threonine residues. This modification has been found on ~1,000 proteins in mammals leading to a push to understand O-GlcNAc's function. One approach is to eliminate the sole enzyme responsible for facilitating the addition of O-GlcNAc on target residues, O-GlcNAc transferase (OGT). Though a handful of small molecule inhibitors have shown reduction in OGT activity, so far none have completely mitigated off-target effects making isolated study of OGT activity inaccessible. Even the best reported inhibitor to date, Ac45SGlcNAc, has been shown to inhibit other important glycosyltransferases that install cell surface glycans. Here, I present a new synthetic strategy aimed at developing more selective inhibitors of OGT allowing for previously impossible analysis of O-GlcNAc biochemistry to be studied in depth.

## **CARB 48**

### **Glycosidase inhibition by multivalent presentation of heparan sulfate saccharides on bottlebrush polymers**

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Glycosidases represent an important member of the enzyme family that largely remain underdeveloped as medicinal targets. We report the utilization of glycopolymers endowed with pendant heparan sulfate (HS) disaccharides for inhibition of the glycosidase, heparanase, which is a regulator in aggressive tumor behavior. To achieve inhibition the well-established glycotope of heparanase was manipulated *in silico* into an inhitope. The multivalent presentation of the attached inhitope allowed for an IC<sub>50</sub> value in the picomolar to low nanomolar range, a thousand-fold amplification over its monovalent counterpart. These studies concluded that (1) the glycopolymers are hydrolytic stable toward heparanase, (2) longer polymer length provides greater inhibition, and (3) increased local saccharide density is negligible due to the hindered active site of heparanase. Overall, the results of these studies show that the multivalent presentation of saccharides on bottlebrush polymers can serve as potent glycosidase inhibitors.



## CARB 49

### Application of indium bromide catalyst to the synthesis of disaccharides

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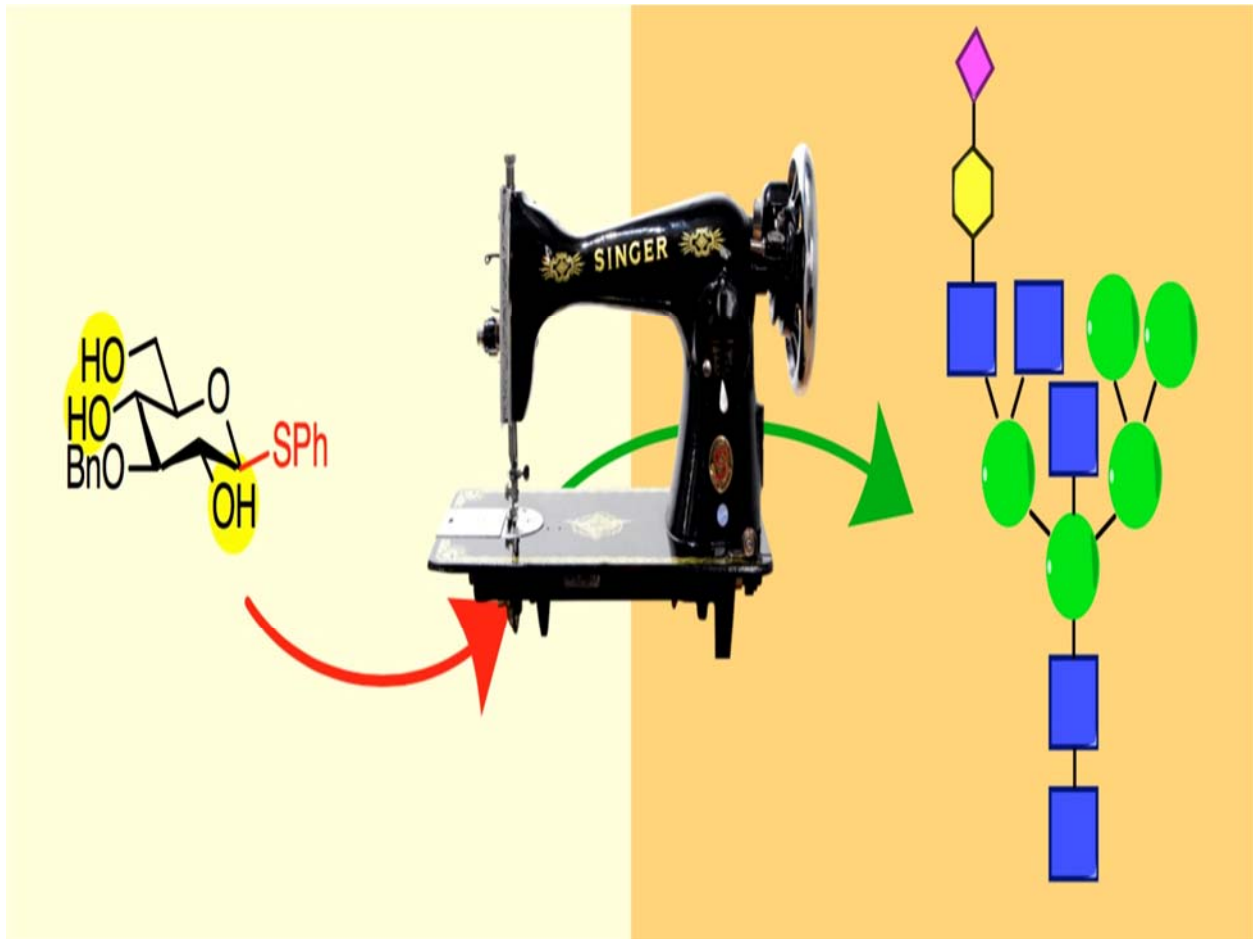
Recent synthetic efforts have demonstrated the ability of indium bromide to catalyze the stereoselective conjugation of beta-*N*-acetyl glucosamine to alcoholic amino acids in high yield. These glycosylated amino acids were used in solid-phase peptide synthesis for the preparation of site-specifically modified proteins. Given the simplicity and success of this reaction, we wondered if indium bromide could also catalyze the conjugation of beta-*N*-acetyl glucosamine to other sugars to form disaccharides. Formation of such disaccharides typically require complicated glucosamine donors and dry conditions. Herein, we report the application of indium bromide to the preparation of glucosamine disaccharides at various linkages without the need of complex glucosamine donor sugars and dry conditions.

## CARB 50

### Minimalist approach to assemble complex saccharides with unprotected donors

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Given the abundance of complex saccharides on the surface of the cell it is not surprising that these structures modulate and mediate cellular interactions, ranging from signalling to intercellular adhesion. Having such importance in biological systems, a handy chemical synthetic approach towards glycans is highly desired for further investigation. However, the specific stereo-configuration at each anomeric linkage and diversity in branching burdens the facile assembly of glycan chains. We herein demonstrate an approach for stereoselective oligosaccharide syntheses without the need for sequential protection/deprotection steps. Using dialkylboryl triflate masking reagent *in situ*, a wide array of glycosyl donors with one to three unprotected hydroxyl groups reacts with various glycosyl acceptors to furnish oligosaccharides with good regio- and stereoselectivity. This approach provides a straightforward access to important structural scaffolds for complex glycoconjugate synthesis.



## CARB 51

### Analysis of glycoprotein production in antibiotic resistant strains of *Helicobacter pylori*

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*Helicobacter pylori* (*Hp*) is a pathogenic bacterium that causes peptic ulcers and gastric cancer. The current antibiotic treatment is often ineffective because of growing numbers of antibiotic resistant strains and lasting harm inflicted on the host's microbiome. Therefore, research on alternative therapies for curing *Hp* infection is critical. Our laboratory has identified the sugar-coated proteins on the surface of *Hp* (glycoproteins) as promising targets for therapeutic intervention. My work aims to characterize two antibiotic resistant strains of *Hp* that are defective in glycoprotein biosynthesis. Metabolic oligosaccharide engineering with the azide-bearing monosaccharide *N*-azidoacetylglucosamine revealed glycoprotein biosynthesis defects within two antibiotic resistant isolates of *Hp*. Fitness assays revealed that these strains have altered motility



and biofilm formation relative to wildtype *Hp*. These results suggest that *Hp*'s glycoproteins are linked to pathogenesis and that the genes responsible for their biosynthesis are potential drug targets.

## **CARB 52**

### **Targeting of *Helicobacter pylori* using photodynamic therapy agents**

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The differential expression of glycans between bacteria and mammalian cells offers a means to design specific therapeutics that can eradicate pathogenic bacteria without damaging host cells. Metabolic oligosaccharide engineering (MOE) has been used in previous work to selectively label surface sugars present on target cells with non-toxic chemical handles capable of reacting with therapeutic agents. In this work, a two-component covalent therapeutic containing (1) a reactive cyclooctyne for delivery to azide-bearing sugars on cell surfaces and (2) the photosensitizer protoporphyrin IX (PpIX) capable of eliciting specific cell death in the presence of light was chemically synthesized, structurally characterized, and assessed for its ability to react with azide-bearing glycans on the surface of the gastric pathogen *Helicobacter pylori*. Western blot analysis indicates that the PpIX-containing cyclooctyne-based conjugate, Cyclo-PpIX, was efficiently delivered to azide-covered *H. pylori* with minimal delivery to azide-free cells. Currently, experiments are underway in our laboratory to further characterize this therapeutic conjugate and to explore its potential as a means to selectively eradicate *H. pylori*. Ultimately, the covalent targeting of bacterial pathogens through their unique glycans has the potential to provide clinicians with new and resourceful antibiotics.

## **CARB 53**

### **Mechanistic study of hydrodeoxygenation reaction on lignin beta5 model compounds using earth abundant metal catalyst**

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Increased atmospheric greenhouse gas concentrations, and other environmental issues resulting from fossil fuel combustion, have motivated the development of renewable energy sources. One such promising renewable energy source is lignocellulose, the main component of plant biomass, which is composed of carbohydrate polymers (cellulose, hemicellulose) and lignin. Lignin as the only nature source of aromatics composes up to 25% of the plants' total mass. Nevertheless, lignin is still of low utility, and is considered a waste product by biorefinery and paper pulp industry only for its heating value, due to its inherent complex structure and underdeveloped lignin valorization technologies. Lignin model compounds with simplified structures that represent lignin interunit linkage give us better understanding of the reaction mechanism during the hydrodeoxygenation process of native lignin. In this work, lignin

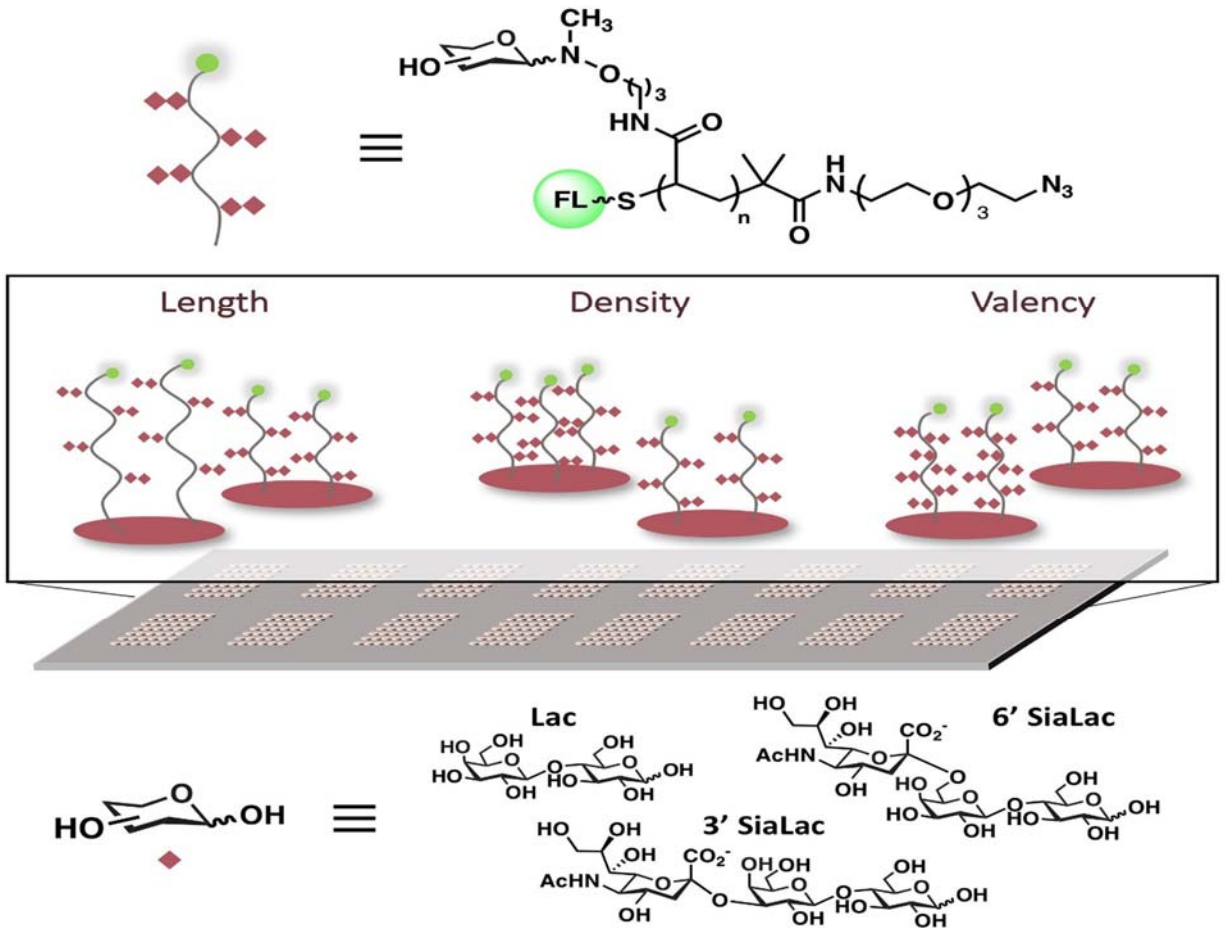
beta5 model compounds with different functional groups have been synthesized and studied. Hydrodeoxygenation reactions of lignin model compounds were performed using heterogeneous nickel catalyst supported on activated carbon, under mild conditions to study reaction mechanism. Benzylic ether CO linkage was observed to be selectively cleaved without aromatic ring being hydrogenated. Modification on the reaction conditions has been shown to have large effect on the distribution of hydrogenated products. Deuterium labeling reactions were performed and kinetic isotopic effect was studied to better understand the reaction mechanism. HPLC-UV, UPLC-MS, 1D and 2D NMR have been used for the qualitative and quantitative study.

## **CARB 54**

### **Using density variant microarrays to investigate the effect of glycan presentation on viral binding**

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Influenza A virus (IAV) is coated with multiple copies of two proteins, hemagglutinin (HA) and neuraminidase (NA), which bind sialylated glycans on host cells. Binding to host glycans by HA is essential for internalization of the virion which leads to its replication, whereas enzymatic cleavage of sialoglycans by NA is important for travel through host mucosal barriers, as well as release of budding virus. Glycan microarrays are commonly employed to uncover the affinity of glycan binding proteins (GBPs) to their partners and have been used to investigate viral binding specificity to underlying glycan substructures. The most prominent viral receptor may still require identification due to the vast heterogeneity of the human glycome. Much of this diversity can be attributed to glycan presentation, which has been shown to influence the binding of GBPs. In the case of IAV where the affinity of HA to a single receptor is low (~2 mM) and binding is increased through avidity, it is known that correct spacing of receptor decoys in multivalent inhibitors becomes important for strong binding. Our glycan microarray, comprised of polymers that mimic native mucin glycoprotein architectures, provides a platform to vary the presentation and density of glycan receptors. We print well-defined glycopolymers of different lengths bearing a sialyllactose (SiaLac) viral recognition motif at several valencies and a range of concentrations. Using a fluorescent tag, we can determine relative glycan density within each spot on the array. We determined the affinity of thoroughly studied GBPs to the diverse glycan presentations within the array. These experiments provide evidence that apparent surface dissociation constant ( $K_{d,surf}$ ) changes as glycan presentation is altered. Viral culture and characterization are currently underway, and we will be testing viral binding to the array at temperatures where NA is both active and inactive.



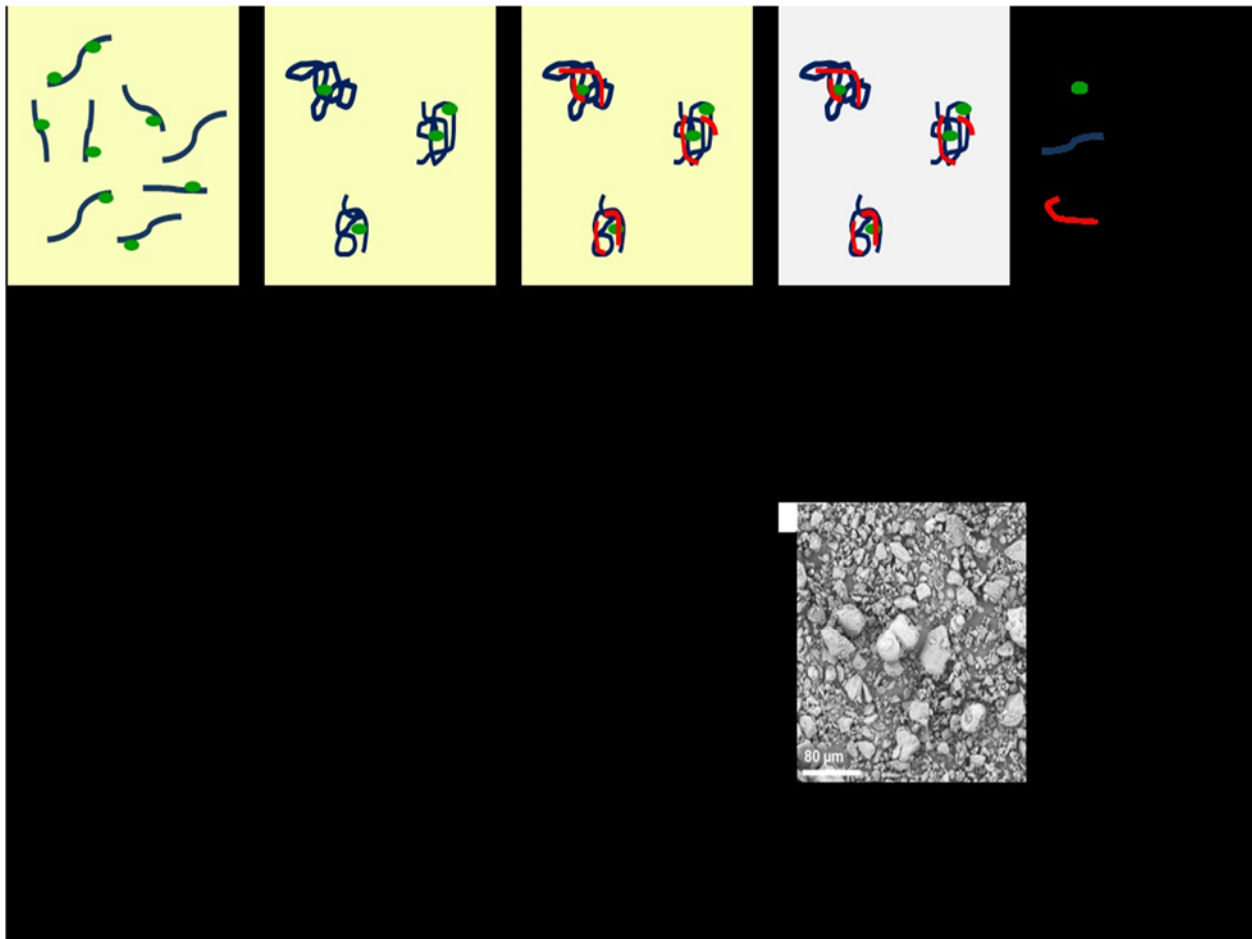
## CARB 55

### Characterizing nisin containing chitosan-alginate microparticles

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Nisin is a naturally occurring bacteriocin that has GRAS status for use in foods granted by the FDA. Encapsulation of nisin within microparticles can protect it from interactions with other food components, thereby increasing its stability and functionality. The effects of formulation parameters on the physico-chemical characteristics of nisin-loaded chitosan-alginate microparticles were studied. Chitosan-alginate microparticles were prepared using the ionotropic pre-gelation method, which involves inducing coiling of the alginate polymer by forming a calcium-alginate pre-gel state, and followed by chitosan complexation. Calcium-alginate pre-gel states were investigated using various calcium concentrations (0 – 5 mM) through viscosity measurements, and calcium

concentrations below 2 mM were found to promote the formation of a coiled alginate nucleus. Calcium concentration had a significant effect ( $p < 0.05$ ) on the size and zeta potential of microparticles. Nisin containing microparticles prepared using 2 mM calcium concentration had a particle size diameter of  $7.86 \pm 0.34 \mu\text{m}$ , and zeta potential value of  $-10.6 \pm 1.22$ . The average polydispersity index (PDI) of particles was found to be  $0.25 \pm 0.01$ , indicating a wide variation in particle sizes. SEM images revealed that the microparticles showed a tendency to aggregate, perhaps due to their low surface charge. Chitosan-alginate complex formation was confirmed using FTIR analysis, and the nisin peptide was found to be intact upon encapsulation, with no change in its secondary structure.



**CARB 56**

**Chemical reporter for dual cell-surface labeling of mycobacteria**

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(2) Department of Chemistry and Biochemistry, Central Michigan University, Mt Pleasant, Michigan, United States

Trehalose is non-reducing disaccharide that is essential to the biosynthesis of the mycobacterial cell envelope. It has recently been demonstrated that synthetic trehalose analogues are able to hijack cell envelope biosynthetic pathways to deliver chemical cargo specifically to the mycobacterial cell surface, permitting various applications. Here, we describe the design, synthesis, and evaluation of a new chemical reporter, which in a single step can simultaneously label the cell surface with azides and alkynes. Strain-promoted azide–alkyne cycloaddition (SPAAC) and in vivo-compatible Cu-catalyzed azide–alkyne cycloaddition (IVC-CuAAC) can be performed sequentially to enable dual surface modification of living mycobacterial cells without impacting cell viability. This tool may be useful for applications such as studying cell envelope dynamics, detecting mycobacteria with high specificity, or delivering cargo to modulate bacterial immunogenicity.

## CARB 57

### **Structural and functional analysis of the *N*-acetylglucosamine-6-phosphate deacetylase (NagA) from *Mycobacterium tuberculosis***

*Elizabeth Fullam*, e.fullam@warwick.ac.uk. University of Warwick, Coventry, United Kingdom

Tuberculosis (TB), is a major global pathogen caused by *Mycobacterium tuberculosis* (*Mtb*). TB is a major global pathogen: it is estimated that one-third of the world's population are latently infected with *Mtb*, and that over 1.8 million people die from TB each year, more than HIV and malaria. Despite the global threat of TB there are limited studies to investigate the nutrient requirements and subsequent metabolism of these nutrients for this pathogenic organism. Therefore, new targets and drugs are urgently needed.

Here we report the structures of the *Mtb* *N*-acetylglucosamine-6-phosphate deacetylase (NagA), an enzyme that is predicted to be essential in *Mtb* and responsible for the deacetylation of *N*-acetylglucosamine-6-phosphate (NAcGlcN6P) to glucosamine-6-phosphate (GlcN6P) - the key enzymatic step to generate essential amino-sugar precursors required for *Mtb* cell wall biosynthesis and influence recycling of *Mtb* cell wall peptidoglycan fragments. The structures reveal a two-subdomain architecture encompassing a  $(\beta/\alpha)_8$  barrel that encloses the active-site and a small  $\beta$ -barrel domain and  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  present in its active-site. The structure is dimeric, with the active-site formed at the dimer interface and the structure of the NagA complex has enabled the mode of NAcGlcN6P substrate binding to be determined. Kinetic studies reveal a distinct preference for NAcGlcN6P over epimeric sugar derivatives and site-directed mutagenesis indicate the importance of conserved residues in the active site which underpin selective substrate recognition and catalysis. Our studies establish the

mechanistic basis of substrate recognition for this important enzyme, providing a framework for exciting new opportunities for inhibitor design.

## **CARB 58**

### **Synthesis and evaluation of cell-permeable trehalose analogues for the protection of mammalian cells**

**Thomas E. Williamson**<sup>1</sup>, [williat3@union.edu](mailto:williat3@union.edu), **Margot G. Paulick**<sup>2</sup>. (1) Biology, Union College, Schenectady, New York, United States (2) Chemistry, Union College, Rexford, New York, United States

Mammalian cells are important for the success of many medical and research endeavors. Unfortunately, these cells are fragile; manipulation or long-term storage often leads to a significant decrease in cell viability. We have developed a novel strategy for protecting mammalian cells using the disaccharide trehalose. Trehalose is a natural cellular protectant that is biosynthesized by organisms such as fungi, insects, and bacteria when they are exposed to stressful conditions. A significant barrier to the introduction of trehalose into mammalian cells is the impermeability of mammalian cell membranes to hydrophilic sugars such as trehalose. We have synthesized cell-permeable analogues of trehalose that deliver high concentrations of free trehalose into mammalian cells. Critically, we have demonstrated that these analogs are able to protect mammalian cells from heat shock-induced apoptosis. In our current work, we have synthesized new cell-permeable trehalose analogues and are evaluating them for their protective effects. Our novel approach for the delivery of trehalose into mammalian cells has the ability to be widely applicable for the protection of mammalian cells exposed to a variety of conditions, ultimately providing a means to improve their longevity during storage, transport, and manipulation.

## **CARB 59**

### **A kinetic study of porcine liver esterase hydrolysis of cell-permeable analogues of trehalose**

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Live mammalian cells are used extensively in both the laboratory and the clinic. Unfortunately, these cells are often fragile and cannot easily be manipulated or stored for long periods of time without a significant decrease in cell viability. We have developed a method for protecting mammalian cells that exploits the disaccharide trehalose, a naturally-occurring cellular protectant. In this method, we have synthesized cell-permeable trehalose analogues in which the hydrophilic hydroxyl groups of trehalose are masked as esters. Once inside the cell, these ester groups are cleaved by endogenous esterases and release free trehalose. We have previously demonstrated that two of our analogues deliver high concentrations of trehalose into mammalian cells.

To better understand the esterase-catalyzed removal of esters from our trehalose analogues, we have developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based esterase assay using porcine liver esterase (PLE), a mammalian esterase, as a model enzyme. We are currently evaluating the kinetics of PLE-catalyzed hydrolysis of our trehalose analogues. Results from these experiments will provide us with a better understanding of the production of free trehalose inside mammalian cells when using esterified trehalose analogues and will aid us in the design of more effective trehalose analogues.

## **CARB 60**

### **Synthesis and evaluation of a fluorogenic probe for detecting mycobacteria**

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Tuberculosis (TB) is one of the top 10 causes of death worldwide. TB kills about 1.8 million people each year and it affects around 30% of the world's population worldwide. A key contributor to these statistics is the lack of rapid and accurate diagnostic tools for TB, which is particularly burdensome in low-resource areas where TB is prevalent. *Mycobacterium tuberculosis* (*Mtb*) is the bacterial causative agent of TB. Methods for the specific detection of *Mtb* in patient samples are critical to managing the disease. Sputum Smear Microscopy (SSM) is a widely used and inexpensive method of diagnosing *Mtb*, however the current reagents used by SSM have problems related to sensitivity, specificity, and/or convenience. New probes that sensitively and specifically detect *Mtb* are needed. Here, we report the synthesis and evaluation of a fluorescence resonance energy transfer (FRET)-based chemical probe—an analogue of the mycobacterial glycolipid trehalose dimycolate (TDM)—that is activated by the mycobacteria-specific enzyme trehalose dimycolate hydrolase (TDMH).

## **CARB 61**

### **Synthesis and evaluation of butyrylated trehalose analogues for the protection of mammalian cells**

**Rachel A. Riley**, [rachelriley55@gmail.com](mailto:rachelriley55@gmail.com), **Margot G. Paulick**. Chemistry, Union College, Schenectady, New York, United States

With the increasing use of mammalian cells in research and in the clinic, there is a critical need for effective methods to protect these cells during manipulation or long-term storage. We have developed a protection method that exploits trehalose, a naturally-occurring disaccharide. Trehalose serves as a cellular protectant for many types of organisms when they are subjected to stressful conditions like cold temperatures and dehydration. While trehalose cannot readily cross the mammalian cell

membrane, modified trehalose analogues, in which the hydroxyl groups have been converted into hydrophobic esters, can passively diffuse through the membrane. Once inside the cell, the ester groups are enzymatically cleaved to generate free trehalose. Previous work from our lab has demonstrated that esterified trehalose analogues successfully deliver free trehalose into mammalian cells and, most importantly, provide protection to these cells from heat shock. This research focuses on the use of butyrylated trehalose analogues for the protection of mammalian cells. We will discuss the synthesis of these analogues, as well as their ability to deliver free trehalose into mammalian cells and act as cellular protectants. Modified trehalose analogues, such as these butyrylated analogues, offer scientists a facile means to deliver high concentrations of trehalose into mammalian cells for use as a cellular protectant.

## **CARB 62**

### **Synthesis of fluorescence resonance energy transfer (FRET)-based fluorogenic probes for the investigation of arabinogalactan mycolate metabolism in mycobacteria**

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Mycobacteria possess an outer membrane rich in mycolic acids that are ester-linked to sugars such as trehalose and arabinogalactan, resulting in virulence-associated glycoconjugates such as trehalose monomycolate (TMM), trehalose dimycolate (TDM), and arabinogalactan mycolate (AGM). The mycoloyl esters in TMM and TDM can be transferred or hydrolyzed via known enzymes (e.g., Ag85 and trehalose dimycolate hydrolase) in processes associated with outer membrane biosynthesis or remodeling. While it is likely that AGM mycoloyl esters must also be broken down, the enzyme(s) that perform this task, as well as the implications for this process in mycobacterial physiology and pathogenesis, are unknown. This study aims to develop analogues of AGM bearing fluorescence resonance energy transfer (FRET) partners on the sugar and mycoloyl group, so that upon enzymatic separation of these two moieties in cells or lysate, fluorescence signal is generated. Here, we report progress toward the synthesis of fluorescence-quenched monosaccharide and disaccharide AGM FRET probes. Upon completion of these compounds, they will be used in whole-cell assays and native gel electrophoresis assays to identify and study the activity of enzymes that are involved in AGM metabolism.

## **CARB 63**

### **Possible aggregation effect in fluoros-tagged oligosaccharide synthesis**

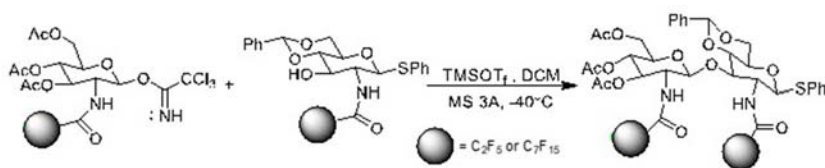
*Aleida G. Gonzalez<sup>1</sup>, aleida.gonzalez01@utrgv.edu, Idris H. Mahmud<sup>2</sup>, Zahraa Shahin<sup>2</sup>, Peter Goekjian<sup>2</sup>. (1) Chemistry, University of Texas Rio Grande Valley, Brownsville, Texas, United States (2) Lab. Chimie ORganique 2-Glycosciences, Universite de Lyon, Villeurbanne, Lyon, France*



Glycosidic coupling reactions between two fluorinated-tagged D-glucosamine moieties were investigated using several sizes of fluorinated tags. The glucosaminyl-glucosamine moiety is found in various bacterial oligosaccharides, although it is more unusual in mammalian systems. Glucosamine presents a certain number of challenges both as a glycosyl donor and as a glycosyl acceptor, including poor reactivity, poor solubility, and limitations in the choice of C2-participating nitrogen protecting groups.

D-Glucosamine derivatives bearing different length fluorinated amides at the C-2 position, C<sub>2</sub>F<sub>5</sub>, C<sub>3</sub>F<sub>7</sub>, and C<sub>7</sub>F<sub>15</sub>, were synthesized from D-Glucosamine hydrochloride via its 1,3,4,6-O-tetraacetate. Fluorinated-tagged glycosyl donors and acceptors were prepared. The trichloroacetimidate of the 3,4,6-O-acetyl-N-perfluoroalkanoyl-D-glucosamine was selected as the glycosyl donor. The glycosyl acceptor was 4,6-O-benzylidene-N-perfluoroalkanoyl-D-glucosamine phenyl thioglycoside, in which the free C-3 hydroxyl group is potentially deactivated by the hydrogen bond from the neighboring amide.

Issues of solubility, stereoselectivity, and reactivity were addressed by investigating different combinations of D-glucosamine donors and acceptors with different fluorinated tag lengths. Analysis of the coupling reactions was performed by using a 300 MHz NMR. We address whether, in addition to allowing efficient purification of the coupled products, the fluorinated tag can act as an efficient C2-participating group, modulate the solubility of the glucosamine precursors, and/or improve the reactivity in the glycosyl coupling reaction. A possible effect of aggregation on the reactivity of these compounds will be discussed.



## CARB 64

### Concise chemoenzymatic synthesis of trehalosamine, an aminoglycoside antibiotic and precursor to mycobacterial imaging probes

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Trehalosamine (2-amino-2-deoxy- $\alpha,\alpha$ -D-trehalose) is an aminoglycoside with antimicrobial activity against *Mycobacterium tuberculosis*, and it is also a versatile synthetic intermediate that has been used to access imaging probes for mycobacteria.

Published chemical syntheses of trehalosamine are generally lengthy and/or low-yielding. Here, we report an efficient 2-step chemoenzymatic synthesis of trehalosamine (63% overall yield) that features trehalose synthase (TreT)-catalyzed 1,1- $\alpha,\alpha$ -stereoselective glycosylation as the key transformation. We also demonstrate that chemoenzymatically synthesized trehalosamine can be readily elaborated to two complementary imaging probes, which label live mycobacterial cells via distinct metabolic pathways.

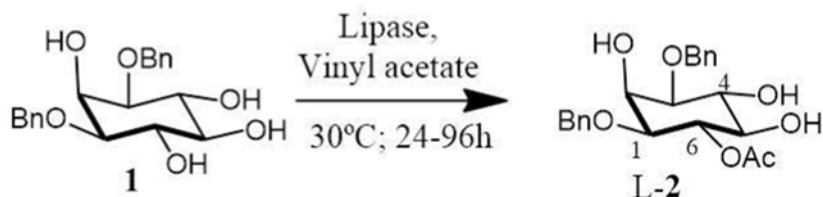
## CARB 65

### Chemoenzymatic desymmetrization of a potential synthetic precursor of bioactive *myo*-inositol phosphates

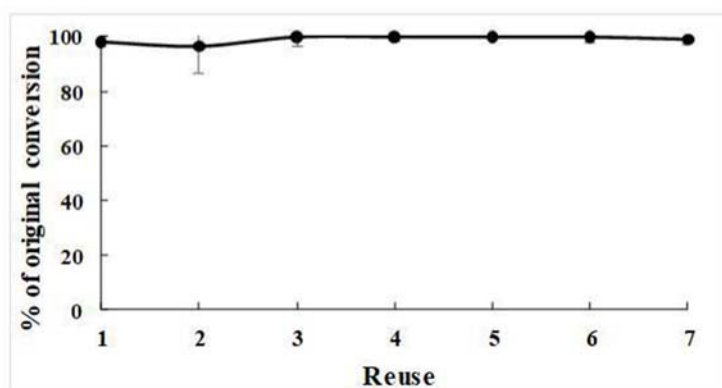
**Alessandro B. Simas**<sup>1</sup>, [abcsimas@yahoo.com](mailto:abcsimas@yahoo.com), **Barbara S. de Jesus**<sup>1</sup>, **Marcela F. Ribeiro**<sup>2</sup>, **Denise M. Freire**<sup>2</sup>, **Evelin A. Manoel**<sup>3</sup>. (1) IPPN, UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil (2) Departamento de Bioquímica, IQ, UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil (3) Faculdade de Farmácia, UFRJ, Rio de Janeiro, Rio de Janeiro, Brazil

Chiral *myo*-inositol derivatives play key roles in cell-signaling processes. Despite the relevance of these compounds, few syntheses rely on enantioselective catalytic reactions. Even fewer reports describe the use of desymmetrization of *myo*-inositol derivatives. In fact, most routes involve resolution by derivatization. We have previously found that TL-IM and RM-IM lipases are able to desymmetrize *myo*-inositol derivative **1**, a potential precursor of different bioactive inositol phosphates, leading to compound **2** in high ee (Scheme 1).

In this communication, we shall report on biocatalyst (TL-IM lipase) recycling under selected conditions (Figure 1), which could be carried out for 7 times without conversion decrease. A protocol for the determination of acetate **2** configuration, via conversion to reference compound D-2,5,6-tri-*O*-benzyl-*myo*-inositol, will also be disclosed.



**Scheme 1.** Lipase-catalyzed mono-*O*-acetylation of 1,3-di-*O*-*myo*-inositol (**1**)



**Figure 2.** Operational stability in the kinetic resolution of 1 by TL-IM (circles filled) under conditions: 5mg/mL of substrate; 200 U of enzyme, and 30°C of temperature in vinyl acetate/EtOAc.

## CARB 66

### Chemoenzymatic synthesis and evaluation of 5-deoxy-thio-D-trehalose as a trehalase-resistant trehalose surrogate

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Trehalose, a non-reducing disaccharide composed of two glucose units, is broadly used in the food, cosmetics, biotech, and pharmaceutical industries due to its exceptional biopreservation properties. In addition, due to its autophagic induction properties, trehalose has recently gained traction as a potential therapeutic due to its ability to mitigate disease burden in animal models of metabolic disease and neurodegeneration. However, trehalose can be efficiently broken down by the enzyme trehalase, which allows microorganisms to use trehalose as a food source and potentially contaminate trehalose-containing products. Furthermore, humans possess trehalase in the kidneys and intestinal tract, which could degrade and ultimately limit the effectiveness of trehalose when administered as a therapeutic. 5-Deoxy-5-thio sugars have previously been shown to be resistant to glycosidase-catalyzed hydrolysis, leading us to hypothesize that 5-deoxy-5-thio-D-trehalose may serve as a trehalase-resistant surrogate for trehalose that could be applied in situations where trehalose breakdown is a concern. Here, we report the one-step chemoenzymatic synthesis and conformational analysis of 5-deoxy-5-thio-D-trehalose, as well as its evaluation in trehalase degradation assays.

## CARB 67

## **Heterobifunctionalized poly(ethylene glycol) and poly(propylene glycol) polymers for bioconjugation applications**

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Poly(ethylene glycol) (PEG) is a biocompatible polymer often used as a spacer/linker for a wide variety of organic and biological compounds, or to enhance solubility of poorly water soluble compounds. In recent years, there have been several reports on the heterobifunctionalization of PEG. Poly(propylene glycol) (PPG) is a similar but more hydrophobic polymer that has received much less attention. To our knowledge, little has been studied on systematic schemes for the heterobifunctionalization of PPG. Once heterobifunctionalized PPG or PEG are prepared, those polymers may be used as linkers on bioconjugates. A robust and versatile synthetic method would provide a promising scheme for applications in other areas as well. Herein, we present our ongoing work on the synthesis of heterobifunctionalized PEG and PPG for preparation of carbohydrate-fluorophore conjugates utilizing “click chemistry”.

### **CARB 68**

#### **Glycan engineering for 3D embryonic stem cell bodies**

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Embryonic stem cell fate can be directed through cell surface growth factor recruitment by glycosaminoglycans (GAGs). Previous work has shown that GAG mimetics are able to rescue mouse embryonic stem cell differentiation in cells that lack the native GAG structures responsible for recruiting FGF2. These cells differentiate into neural rosettes through GAG mimetics facilitating the recruitment of FGF2 growth factor to the cell surface. While monolayer cell surface remodeling has been shown to work, stem cell differentiation in 3D embryoid bodies (EBs) is more representative of a developing embryo and allows access to each primary germ layer. We have shown that our GAG mimetics are able to influence differentiation into mesodermal cells from mouse stem cell EBs. Establishing a gradient of GAG mimetic incorporation would provide the tools to influence various layers of cells within the EBs to differentiate via growth factor recruitment. Creating a gradient in the EBs is performed by varying the structure of the GAG mimetics. Varying the length, attaching different sugars, and amount of overall charge influences GAG mimetic incorporation into EBs. The EBs are visualized via confocal microscopy after labeled GAG mimetic incorporation. We saw a dose dependent response for GAG mimetic penetration of EBs. Based on the length of the backbone and sugar charge of the GAG mimetic, we hope to establish a gradient of incorporation into EBs. This would allow for an investigation into FGF2 recruitment in gradients, and ultimately controlling stem cell fate using these gradients.

### **CARB 69**

## **Exploring the chemistry and the bonding in oligosaccharides derived from sugars and sugar acids**

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Chemical evolution explores the chemistry of complex organic molecule formation from simpler molecules through chemical reactions under prebiotic conditions- i.e. amino acids to polypeptides, nucleic acids to polynucleic acids, and monosaccharides to oligo- and polysaccharides. Employing mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, we report the chemistry and the nature of the bonding in the formation of oligosaccharides from simple sugars and sugar acids.

### **CARB 70**

#### **Effect of silyl groups at C-4 in sialylation reactions**

**Matt Lohman**, *malohma@siue.edu*, **Cristina De Meo**. *chemistry, Southern Illinois University Edwardsville, Edwardsville, Illinois, United States*

Sialic acids are a family of around 50 naturally occurring derivatives of neuraminic acid, with N-acetyl neuraminic acid as the most abundant member. The chemical synthesis of sialic acid containing derivatives is very challenging. Previously we reported that the introduction of tertbutyl dimethyl silyl ether at C-4 improves dramatically the stereoselectivity of some sialylation reactions. As a part of a research goal directed towards the development of new synthetic methodologies for stereoselective sialylation reactions, herein we describe the synthesis and testing of novel sialyl donors bearing different silyl functionalities at C-4.

### **CARB 71**

#### **Effects of side chain conformation in chemical sialylations of Neu5Ac derivatives via 8,9-O-substitution**

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Sialic acids are a family of carbohydrates regularly found in the terminal positions of glycoconjugates. The most common sialic acid derivative, N-acetylneuraminic acid, exists naturally in alpha glycosidic configuration. Obtaining modest yields with enhanced alpha stereoselectivity is especially difficult in chemical sialylation reactions. Recently, it was found that the conformation and configuration of the glycerol side chain influences both reactivity and stereoselectivity of chemical sialylations. As part of an effort to understand the effect of side chain conformation on chemical sialylations, several C-8,9-

isopropylidene and C-8,9-acetyl protected sialyl donors were synthesized. Herein we report the synthesis of these donors and the results of corresponding sialylations.

## **CARB 72**

### **Selective acetylation on sialic acid donors**

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N-Acetyl Neuraminic Acid is the most abundant member of sialic acids and is predominately found at the terminal ends of glycoconjugates in mammalian cells. These carbohydrates participate in cell-cell interactions in organisms and play a role in the development of infectious diseases such as influenza. The chemical synthesis of sialic acid building blocks and the methodologies in stereoselective sialylation reactions are still challenging. As a part of a program of understanding the effect of O-protecting groups in sialylations, herein we report a study of regioselective acetylation of sialic acid

## **CARB 73**

### **A non-woven fabric wound dressing containing layer-by-layer deposited hyaluronic acid and chitosan**

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Newly wound dressings composed of non-woven cotton (NWC) fabric and multilayer of hyaluronan (HA) and chitosan were built using layer-by-layer assembly technique. Factors affecting the building up of that dressings such as HA concentration, number of coating layers and nitrogen content of the NWC fabric quaternized form were studied. Meanwhile, some physico-chemical properties of such dressings were investigated. Moreover, to enhance the antibacterial properties of the aforementioned dressings, Silver nano-particles (Ag NPs) were prepared and incorporated as a functional additive in the final HA layer of such dressings. TEM image of the prepared Ag-NPs depicts that the particle size of that nano-particles was less than 13 nm. Furthermore, the prepared dressing surface was characterized via scanning electron microscope. The EDX of Ag NPs loaded dressings confirmed the presence of Ag NPs onto such dressings with Ag - content of 0.24% (w/w). The thermogravimetric analysis assured that the prepared dressings based on quaternized NWC fabric have higher thermal stability than the un-quaternized form.

## **CARB 74**

## **Inexpensive treatments for *M. lepramatous* could include milk trisacccharide, fetuin N-linked oigosaccharide and bovine submaxillary mucin O-linked oligosaccharide**

**Michael A. Madson**, *mmadson53@earthlink.net*, *Jesus Christus. Research & Development, BioLogistics LLC, Ames, Iowa, United States*

The TLR-2 'hook' on the exo-side of the cell membrane has sialyl groups known to bind sialyl group binding molecules like VCAM-1, ICAM-1 and VLA4 for signaling and to begin the process of translocation across the cell membrane in the inflammation process. Mycobacterium lepramatous (a form of leprosy) is known to compete with binding of these molecules, VCAM-1, ICAM-I and VLA-4, to the TLR-2 'hook.' We need a substitute for the current 'free' treatment given by the WHO, phthalidamide. Phthalidamide is known to cause horrible birth defects for progeny of leprous people. The isolatiion of the sialylated glycan di-phospho amino acid for bsm and fetuin, sialylated, might be used as treatments for leprosy. We give support for the prevalence of glycan di-phospho-amino acid molecules, here. Also available for possible *M. lepramatous* sialylated mimic, to inhibit *M. lepramatous* infection, would be the recently isolated bovine milk oligosaccharide, 5-acetamido-5-deoxy neuraminy (α 2-> 3') D-galactosyl (β1->4)-D-glucose (Madson, 2015)

We have evidence for protein carbohydrate linkages to protein in corn, both N and O-linkages; fetuin glycoprotein, bovine submaxillary mucin glycoprotein, and cellulose from switchgrass. These linkages, we propose, are through a di-phosphoryl group. In the case of O-linkages, it is through a di-phospho- ester serine linkage to protein. In the case of N-linkages it is through a di-phosphoryl amide linkage to protein. We have yield data in the case of corn glycans. And in the cases of fetuin, bovine submaxillary mucin and switchgrass cellulose, the evidence is from MS and MS<sup>2</sup> data. We have isolated the glycan di-phospho linkages through the treatment of the sample with, pH 11.4 NH<sub>4</sub>OH for all but corn. In the case of corn, after treatment with pH 11.4 NH<sub>4</sub>OH, we treat with 1% NaSO<sub>3</sub>. We propose mechanisms for the presence of these carbohydrate linkages in this presentation. The isolation of the sialylated glycan di-phospho amino acid for bsm and fetuin, sialylated, might be used as treatments for leprosy.

### **CARB 75**

#### **Investigation of accelerated hydrolysis of cellulose after chemical modification**

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Mechanism of accelerated hydrolysis of chemically modified cellulose is developed. It was found cellulose chemically modified by some compounds such as reactive dyes are more prone to acid hydrolysis. Since the discovery, the mechanism is not well investigated. Understanding the mechanism of accelerated hydrolysis of chemically

modified cellulose could not only improve mechanical strength cellulosic materials but also promote the conversion of cellulosic materials into various fine chemicals. With molar ratio of substitution on cellulose less than 1%, the overall hydrolysis rate of substituted cellulose is as 10 folds fast as that of unsubstituted cellulose. Through hydrolysis experiments and computer modeling, we believe the increased hydrolysis of cellulose is caused by lowered activation energy of hydrolysis, resulted from that negative charges on substituents stabilize intermediate of hydrolysis, oxocarbenium ions, through electrostatic interaction. Rate of hydrolysis was affected by both of distances between charged atoms or groups on substituents and oxocarbenium ions and amount of the negative charges on substituents. When the distance between charged groups and oxocarbenium ion is smaller than 5 Å, negative charges on substituents have great impact on hydrolysis rate of cellulose. When such distance is between 5 Å and 10 Å, impact of negative charges on rate of hydrolysis decreases. When this distance exceeds 10 Å, negative charges have little effect on rate of hydrolysis. Hydrolysis rates of cellulose could be tuned according to different purposes through slight modification of the constitutions of substituents.

## **CARB 76**

### **One-pot gram-scale synthesis of GlcNAcylated amino acids via indium bromide catalysis**

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O-GlcNAcylation is an important post-translational modification of intracellular proteins with implications in development, survival, and disease. It involves the dynamic addition of the single monosaccharide  $\beta$ -N-acetyl glucosamine to the side chains of serine and threonine residues of proteins. Synthetic proteins bearing site-specific post-translational modifications have revolutionized our understanding of their biological functions in vitro and in vivo. A current drawback to studying the biophysical and biochemical effects of O-GlcNAcylation is the synthetic preparation of anomerically pure GlcNAcylated amino acids for use in solid-phase peptide synthesis and unnatural amino acid mutagenesis. Herein, we describe the one-pot stereoselective synthesis of Fmoc-protected GlcNAc serine, threonine, and cysteine amino acids in high yield using catalytic amounts of indium bromide. Previous methods require the preparation of donor sugars, extensive protecting group chemistry, dry conditions, and often result in anomeric mixtures. However, our new method involves only inexpensive commercially available reagents that require no further modification or special handling. The reagents are simply mixed, dissolved, and heated under open air or inert atmosphere to afford the anomerically pure glycosylated amino acids in high yield. This operationally simple procedure should facilitate the study of O-GlcNAcylation without necessitating an expertise in synthetic carbohydrate chemistry.

## **CARB 77**



## **Efficient catalytic conversion sugar-rich microalgae into glycol, lactic acid and HMF in water**

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Large-scale cultivation of microalgae is an effective and sustainable measure to achieve multiple targets of carbon dioxide emission reduction and chemicals/fuels production. Recently, research on microalgae mainly focuses on the biofuel production. However, the efficient conversion of microalgae to value-added chemicals, such as alkanediols, HMF and lactic acid was rarely reported. Since the percentage of moisture in microalgae is very high, microalgae with its nutrient solution can be directly used as reaction system, avoiding the addition of other reaction solvents and complicated separation and purification processes compared with terrestrial raw biomass. Therefore, selective conversion of microalgae into high value-added polyols, especially alkanediols (e.g., EG and 1,2-PDO), HMF and lactic acid were of great interest. Herein, a nickel-based catalyst, HZSM-5 and Sn-Beta were used to convert microalgae (*Chlorococccum* sp.) into a series of chemicals in our group. The synthesized catalysts exhibited excellent tolerance of N-contained components and a 53.5%, 48.0% and 38.0% yield of polyols, HMF and lactic acid respectively could be achieved directly from microalgae under moderate reaction conditions. Meanwhile, the influences of reaction conditions were systematically investigated and the reaction pathways of microalgae conversion were proposed.

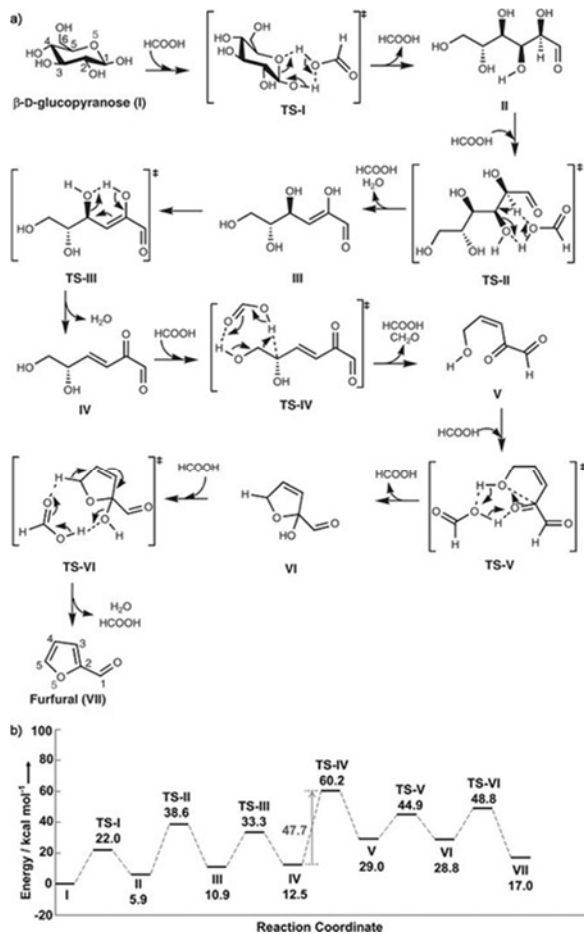
## **CARB 78**

### **Mechanistic studies on the catalytic transformation of glucose over acid or base catalysts**

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There is significant interest in using biomass as a renewable feedstock for chemical production, which has the additional benefit of being pre-functionalized. Among the biomass feedstocks, the hexose glucose is abundant in nature and can be readily obtained. However, the selective conversion of glucose to value-added chemicals such as the platform chemicals, furfural and lactic acid, usually require special energy forms such as microwave and/or acid-base catalysts in order for the process to be realized under relatively mild conditions. These along with the complex structure of the substrate make mechanistic understanding and process design a great challenge. To overcome these barriers, we performed extensive density functional theory calculations to reveal the underlying mechanisms for glucose selective conversion to furfural and lactic acid by closely collaborating with the experimental studies. We employed GC-MS and NMR techniques to analyze the microwave-assisted pyrolysis products of isotope-labeled glucose, and the mechanism revealed by the experimental analysis is consistent with

our first principles prediction. Our calculations further suggest the potential catalytic effect of acid products such as formic acid, which was also verified by our experiment. We also studied the formation of lactic acid from glucose over a Sn-Beta zeolite catalyst, and our experiment and theory both reveal the critical role of Lewis acid site due to the skeleton Sn in the zeolite framework. Our calculations further suggest the involvement of Brønsted acid site for the formation of lactic acid. Thus, we demonstrate the importance of combining experiment and theory in providing mechanistic understanding for biomass transformation.



Formation mechanism of furfural in microwave-assisted pyrolysis of glucose revealed by isotope-labeled experiment and first principles calculations with formic acid found to catalyze the reaction

## CARB 79

**BF<sub>3</sub>-N,N-dimethylformamide catalyst for synthesis minimally protected 1,2-*cis*-glycopyranosides in solution and solid-phase stereocontrolled synthesis**

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BF<sub>3</sub>-N,N-dimethylformamide is known as a mild Lewis acid and it is stable solid to air which represents practical and operational advantages. Methodologies are described for the stereoselective synthesis of 1,2-*cis* glycopyranosides in the *D*-galacto, *D*-gluco, and 2-azido-2-deoxy-*D*-glucopyranoside series utilizing minimally protected (3-bromo-2-pyridyloxy) β-*D*-glucopyranosyl donors in the presence of BF<sub>3</sub>-DMF as a catalyst and a variety of alcohol acceptors relying on the “remote activation concept”. Precursors to antifreeze glycopeptide components are synthesized in excellent yields and high α/β ratios. The method is adaptable to solid-supported synthesis giving access to diverse sets of minimally protected α-*D*-glucopyranosides as major products.

## **CARB 80**

### **Trying to identify and address grand challenges in carbohydrate science**

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Carbohydrates possess a near-unique molecular vantage point of tractable complexity in Biology that may be understood and manipulated through Chemistry. This lecture will give a perspective on possible 'Grand Challenges' that the group are exploring in areas ranging from pathogenic disease to food security.

## **CARB 81**

### **Chemical tools and strategies to decipher the role of glycans in human disease**

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Oligosaccharides, including human glycans, can engage in a myriad of recognition and signaling events that have potential therapeutic applications. Existing chemical methods to study saccharides are often applicable to selected problems and the key obstacles such as suboptimal selectivities in glycosylation reactions await broadly applicable solutions. In this presentation, I will describe our efforts focused on chemical synthesis of mammalian glycans, glycoconjugates, and small biologics and the use of these novel technologies for therapeutic benefits. Specifically, methods for stereoselective glycosylation, regioselective modifications of hydroxyl groups, and the synthesis of complex oligosaccharides of mammalian origin will be discussed.

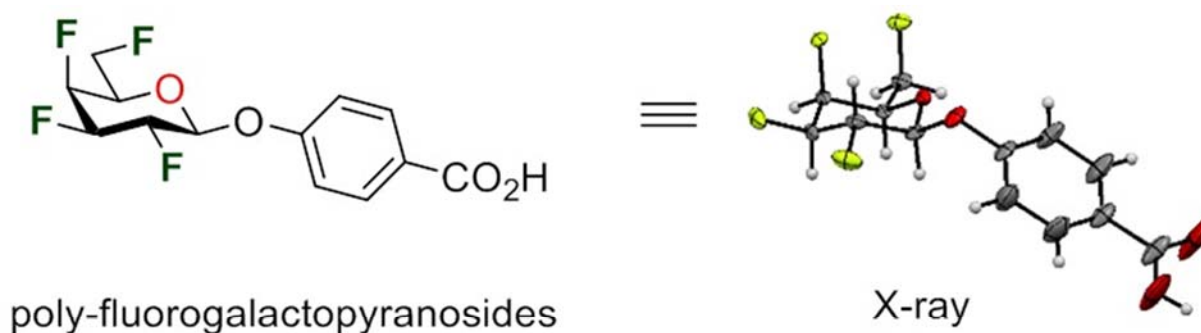
## **CARB 82**

## Mono- and poly-fluorinated carbohydrates: Synthetic challenges associated to a new class of bioactive molecules

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Fluorinated carbohydrates are invaluable tools to study various biochemical processes. In this seminar, recent work from the group on the synthesis of these molecules will be presented. The synthesis of all mono-fluorogalactopyranosides have been achieved and a particular emphasis will be on a new approach to 3-deoxy-3-fluoro and 4-deoxy-4-fluoro-galactopyranosides from levoglucosan. Moreover, the stereoselective preparation of a tri-fluorogalactopyranoside and a tetra-fluorogalactopyranoside have been completed. The biological evaluation of these fluorinated sugars was achieved over various human galectins.

Finally, the focus of this research is extended to the stereoselective synthesis of other heavily fluorinated hexopyranosides. This may open the path for the synthesis of complex fluorinated molecules containing contiguous chiral carbon centers.



## CARB 83

### Substrate recognition of reversible O-GlcNAcylation

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O-GlcNAcylation is the reversible modification of serine and threonine residues with *N*-acetylglucosamine (O-GlcNAc). This modification is attached by O-GlcNAc transferase (OGT) in the presence of sugar donor uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), and can be removed by O-GlcNAcase (OGA). O-GlcNAcylation dynamically modulates the functions of over 1,000 intracellular proteins in transcription, translation, and signal transduction, among others. Aberrant O-GlcNAcylation has been detected in many diseases such as cancer, diabetes, and Alzheimer's disease. Hence, there is a significant interest in understanding how OGT and OGA regulate this modification on a broad range of substrates that lack apparent sequence motifs. This talk presents our new chemical biology and structural approaches to elucidate the molecular basis underlying the substrate binding and recognition of OGT and OGA.

## CARB 84

### Exploiting the inhibitory function of CD22 on B-cells to prevent antibody responses

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Antibody responses protect us from a plethora of pathogens, but can also be pathogenic in allergic and autoimmune diseases. New approaches for selectively disarming these deleterious antibody responses would provide a breakthrough over broadly immunosuppressive therapies that can leave patients immunocompromised. Exploiting the natural function of inhibitory receptors on immune cells to drive immunological tolerance toward specific antigens is a promising strategy. One class of inhibitory receptors on immune cells is the sialic acid-binding immunoglobulin-type lectin (Siglec) family. This family couples recognition of their sialic acid-containing glycan ligands to regulation of immune cell function under the appropriate physiological

conditions. As B-cells are the ultimate source of antibodies, we have been investigating the natural function of Siglecs on these B-cells, most notably CD22 (Siglec-2). Through developing a multivalent platform to engage CD22, we have shown that it is possible to delete the 'disease-causing' B-cells while preserving the function of all other B-cells. This platform – called Siglec-engaging tolerances-inducing antigenic liposomes (STALs) – consists of liposomal nanoparticles that display both antigen and a high affinity, selective glycan ligand of CD22. STALs induce cross-linking of CD22 and the BCR and drive an apoptotic signal *only* in the antigenic B-cells. This modulator platform has allowed us to test different antigens, target both murine and human CD22 with different glycan ligands, as well as encapsulate an immunosuppressant drug to enhance tolerance. Progress towards using STALs for inducing tolerance to antigens in allergies (e.g. peanut allergies) and autoimmunity (e.g. rheumatoid arthritis) will be presented. (NIH grants AI050143, AI099141; DOD grant W81XWH-16-1-0303)

## **CARB 85**

### **Commandeering mycobacterial carbohydrate metabolism for applications in tuberculosis research**

**Benjamin M. Swarts**, *ben.swarts@cmich.edu*. Department of Chemistry and Biochemistry, Central Michigan University, Mt Pleasant, Michigan, United States

The causative agent of tuberculosis (TB), *Mycobacterium tuberculosis* (*Mtb*), possesses a complex cell envelope decorated with an array of unique glycoconjugates that provide protection to the bacterium and coordinate many of the host–pathogen interactions involved in *Mtb* pathogenesis. Many of these glycoconjugates, along with their associated metabolic pathways, are essential and mycobacteria-specific, making them highly attractive targets for TB drug, diagnostic, and vaccine development. However, progress in this area is impeded by the lack of tools for studying *Mtb* glycoconjugates, which ultimately prevents many TB researchers from pursuing fundamental studies and clinical applications related to these structures. Because the glycoconjugates of mycobacteria are structurally and functionally distinct from glycoconjugates in eukaryotes and even other bacteria, the vast majority of tools developed for these systems are not applicable to studying *Mtb*. The goal of our research program is to develop novel tools that facilitate the investigation of mycobacterial glycoconjugates, and to apply these tools in basic and applied TB research. We develop carbohydrate-based probes that exploit unique carbohydrate metabolic pathways in mycobacteria, which enables labeling and analysis of specific glycoconjugates in intact bacterial cells. This presentation focuses on the design, synthesis, and applications of trehalose-, trehalose monomycolate-, and trehalose dimycolate-based probes, which are valuable tools for studying virulence-associated glycoconjugates of the unique mycobacterial outer membrane, and which may have future applications as TB diagnostic tools.

## **CARB 86**

## **Towards catalytic site-selective alterations of glycopeptide antibiotics and other carbohydrates**

**Scott J. Miller**, *scott.miller@yale.edu*. Yale University, Dept of Chemistry, New Haven, Connecticut, United States

This lecture will describe our studies employing catalysts for the selective alteration of the complex glycopeptide antibiotics and related carbohydrates. In particular, catalysts that emerged from both combinatorial screening, as well as rational design will be presented. In the latter case, we will detail our efforts to co-opt molecular mechanisms of biological activity for the orthogonal goal of chemical derivatization of the structure. In addition to showing our results for various classes of bond forming reactions, we will present mechanistic studies that support some of the aspects of our original hypotheses. The biological activities of the new analogs we have synthesized will be presented in certain cases.

### **CARB 87**

#### **Coinage metal-catalyzed stereoselective glycosidic bond formation**

**Liming Zhang**, *zhang@chem.ucsb.edu*. Dept of Chemistry Biochemistry, University of California, Santa Barbara, California, United States

The development of generally applicable stereoselective glycosidic bond formation remains an on-going challenge in carbohydrate chemistry and organic synthesis in general. This presentation will discuss two of our recent endeavors in this area via coinage metal catalysis. The first study entails a silver-catalyzed activation of glycosyl esters en route to  $S_N2$ -type glycosidic bond formation. The second study employs thioglycosides as substrates. Their gold-catalyzed activation affords intermediates that are also capable of undergoing  $S_N2$ -type reactions with incoming acceptors. In this latter study, the tuning of the electronic characteristics of the HO group protecting groups improves the reaction stereoselectivity, and glucosides can be obtained with >20:1  $\alpha/\beta$  ratio.

### **CARB 88**

#### **Studies on regioselective glycosylation of natural polyols**

**Pavel Nagorny**<sup>3</sup>, *nagorny@umich.edu*, **Jia-Hui Tay**<sup>3</sup>, **Alonso J. Arguelles**<sup>3</sup>, **Matthew DeMars III**<sup>4</sup>, **Paul M. Zimmerman**<sup>2,3</sup>, **David H. Sherman**<sup>1</sup>. (1) Univ of Michigan, Ann Arbor, Michigan, United States (2) University of Michigan, Ann Arbor, Michigan, United States (3) Chemistry, University of Michigan, Ann Arbor, Michigan, United States (4) Chemistry, Life Science Institute, Ann Arbor, Michigan, United States

The ability to control the glycosylation patterns in oligosaccharides and glycoconjugates is of great significance to the field of drug discovery, as this provides one of the most

effective strategies for harnessing the physical and biological properties of various organic compounds. Many important molecular parameters might be greatly affected by the position and stereochemistry of the glycosidic linkages. However, despite the recent advances in the fields of chemical synthesis and catalysis, we are still very limited in our ability to introduce such alternations. The assembly and attachment of even moderately complex oligosaccharides may suffer from a large number of steps, which often renders such studies impractical for the drug discovery. Not surprisingly, a number of the recent efforts have been focused on developing catalytic methods for the regioselective and chemoselective synthesis of oligosaccharides and glycoconjugates. This presentation summarizes our efforts on achieving regioselective glycosylation of natural and unnatural polyols by employing chiral Bronsted acid-catalyzed reactions with carbohydrate-derived trichloroacetimidates. In particular, the use of chiral Bronsted acids to override the steric bias and introduce glycosylation at the less reactive sites will be discussed in the context of glycosylation of natural and unnatural 14-membered macrolactones. In addition, a complementary strategy based on a single pot traceless protection/glycosylation to achieve regioselective glycosylation of polyol-containing 14-membered macrolides and cardiotonic steroids will be presented.

## **CARB 89**

### **Organoboron catalysts and reagents for carbohydrate chemistry**

**Mark S. Taylor**, *mtaylor@chem.utoronto.ca*. Department of Chemistry, University of Toronto, Toronto, Ontario, Canada

It has been known for more than a century that boron compounds interact with carbohydrate derivatives. While organoboron compounds have been explored in depth as protective groups and synthetic receptors for sugars, opportunities exist to employ them in other ways to facilitate carbohydrate synthesis. My group has developed catalytic transformations that exploit the enhanced nucleophilicity of tetracoordinate carbohydrate–borinic acid adducts. Applications of these methods in the synthesis of complex, glycosylated natural products will be presented. The distinct behaviors of tricoordinate (protected) and tetracoordinate (activated) organoboron–diol complexes can be combined to achieve sequential, regioselective transformations of sugar derivatives. New types of reactivity enabled by organoboron–carbohydrate complexation will also be discussed.

## **CARB 90**

### **Chiral catalyst-directed site-selective functionalization of carbohydrates**

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Site-selective functionalization of hydroxyl groups in natural products especially carbohydrates is one of the long-standing challenges in chemistry. Using a pair of chiral catalysts, we now can differentiate hydroxyl groups systematically and predictably, including the most prevalent trans-1,2-diols in pyranoses, where both hydroxyl groups are in the equatorial positions. DFT calculations indicate that the key determining factor for the selectivity is the presence or absence of a cation- $\pi$  interaction between the cation in the acylated catalyst and an appropriate lone pair in the substrate. DFT calculations also provided a predictive model for site-selectivity and this model is validated by structurally diverse substrates, including trans-1,2-diols, cis-1,2-diols, 1,3-diols, diols in two different carbohydrate units, and even polyols in complex natural products. The cation- $\pi$  interaction offers a novel mode of interaction for the development of catalytic methods; and the site-selective method significantly simplifies chemical syntheses of carbohydrates and other natural products.

## **CARB 91**

### **Anti-pathogenic glycoconjugates**

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Since Fleming's discovery of penicillin, antibiotics have represented the only effective treatment for bacterial infections. Unfortunately, their efficacy has been compromised by over- and misuse. As antibiotic development stagnates, an orthogonal strategy is the development of adjuvant therapeutics that restore the efficacy of existing antibiotics, minimize the emergence of resistance, or lower required doses. Here we describe the synthesis and evaluation of understudied plant glycoconjugates as a defense against bacterial pathogens.

## **CARB 92**

### **Solving puzzles of glycoside hydrolase reaction mechanisms**

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Crystallography and activity studies provide invaluable information on how glycoside hydrolase (GH) enzymes break down glycosidic bonds on a subsecond timescale at ambient temperatures, despite these remarkably strong bonds having an estimated half-life on the order of millions of years. This experimental data has been the basis of hypothesized reaction mechanisms that have proven to be consistent for many, but not all, GH enzymes classified into families, as tracked on the CAZy website ([www.cazy.org](http://www.cazy.org)). To better understand these important carbohydrate-active enzymes, our group uses a range of computational techniques, including molecular dynamics (MM and QM/MM) simulations, enhanced sampling methods, and data science techniques to

reveal elusive mechanisms and protein structure-function relationships. This presentation will include insights into the elusive mechanism of a GH family 6 cellulase, and the role of a puzzlingly inactive GH family 9 enzyme. These studies help understand enzymatic activities that defy chemical intuition and improve our efforts toward rational enzyme design.

### **CARB 93**

#### **Mimetic sugar-nucleotides to probe a strategic bacterial dehydrogenase enzyme**

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The opportunistic human pathogen *Pseudomonas aeruginosa* (PA) causes chronic bacterial infections in cystic fibrosis patients, contributing to a reduction in lung function and increased mortality rates. The lung environment induces a switch of *P. aeruginosa* to its mucoid phenotype, which is characterised by an overproduction of the exopolysaccharide alginate. Composed of  $\beta$ -D-mannuronic acid and its C5 epimer  $\alpha$ -L-guluronic acid, alginate is a key component in the formation of a bacterial biofilm, which increases persistence of the bacteria in the airways and retards antimicrobial treatments. Studies of the alginate biosynthetic pathway reveal a central enzyme involved in its formation is GDP-mannose dehydrogenase (GMD), which catalyses an NAD<sup>+</sup>-dependent oxidation of GDP-D-Man to GDP-D-ManA: the alginate feedstock monosaccharide building block. The GMD crystal structure suggests that following initial oxidation of the 6-OH in GDP-Man to an aldehyde, the mechanism is further mediated by a key Cys residue to deliver GDP-ManA. The aim of this research is to design and synthesise structural GDP-ManA probes to interact with the GMD active site and, potentially Cys<sup>268</sup>, thus providing new mechanistic understanding for GMD and a view to rational inhibitor design to perturb exopolysaccharide alginate formation in mucoid PA.

### **CARB 94**

#### **Synthetic glycoproteins by polymerization of glycosylated NCAs**

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Glycopolymers have emerged as useful tools to probe glycan dependent biological interactions and as materials for diverse biomedical applications. Glycosylated polypeptides are particularly attractive polymers since they can display glycans in their native form, naturally take on complex secondary structures that drive self-assembly, and are biodegradable. A panel of carbohydrate functionalized polypeptides have been prepared via polymerization of N-carboxyanhydrides. Molecular weight and glycan density can be controlled by polymerization reaction stoichiometry to give glycopolypeptides of biologically-relevant length and composition. The

glycopolypeptides are dual end-functionalized with chemical handles that allow conjugation to a variety of substrates and fluorophores. These studies focused on preparation and study of synthetic mucin glycoproteins and synthetic pathogen-associated glycoproteins. Conformation and other physical properties were evaluated. Methods to display the synthetic glycoproteins were examined, such as covalent conjugation to live cell-surfaces or to beads. In particular, functionalization of fluorescent beads allowed study of the impact of carbohydrate composition and density on phagocytosis in a model cell line. We observed increased phagocytosis of glycopolypeptide-bead conjugates bearing canonical pattern-recognition motifs compared to controls. The polypeptides also elicited receptor-dependent cytokine expression in model cell lines. These materials provide a new platform for studying carbohydrate-dependent biological systems.

## **CARB 95**

### **Self-assembling glycopeptide hydrogels as selectively permeable mucus-like barriers**

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Mucus is a glycoprotein-rich gel that protects epithelial tissue surfaces from non-specific interactions with micro-organisms and macromolecules. Synthetic mucus-like barriers could enable new opportunities to confer implantable materials with non-fouling properties that are essential for biocompatibility and long-term efficacy. Unfortunately, mucus glycoproteins (i.e., “mucins”) derived from natural sources are highly variable and difficult to purify, while recombinant mucin production is impractical. To circumvent these challenges, here we report synthetic mucin analogs based on carbohydrate-modified peptides (i.e., “glycopeptides”) that self-assemble into beta-sheet nanofibers in water. The dense carbohydrate coating on the surface of glycopeptide nanofibers is similar to that of naturally derived mucins. Likewise, carbohydrates decorating the surface of glycopeptide nanofibers can be selectively recognized by their cognate lectins. Finally, the type of carbohydrate appended onto the nanofiber can be selectively and efficiently varied via glycosyltransferase enzymes. Using this approach, we synthesized nanofibers decorated with n-acetylglucosamine (GlcNAc) and n-acetyllactosamine (LacNAc) that selectively bind to wheat germ agglutinin (WGA) and galectins, respectively. At millimolar concentrations in water, glycopeptide nanofibers entangled into viscoelastic hydrated gels (i.e., “hydrogels”). GlcNAc-decorated glycopeptide hydrogels bound and retained WGA, but were permeable to various non-lectin proteins, including albumin and green fluorescent protein, as well as the mannose-binding lectin, concanavalin A. GlcNAc-decorated glycopeptide hydrogels coated onto a glass surface reduced *E. coli* adhesion by ~90%, whereas non-glycosylated peptide hydrogels only reduced bacterial adhesion by 50%. Additionally, glycopeptide hydrogels coated on glass completely inhibited NIH3T3 fibroblast adhesion in the presence of serum, whereas non-glycosylated peptide hydrogels were permissive to non-specific fibroblast adhesion. Together, these data demonstrate self-assembly of

glycopeptides into hydrogels with selective macromolecule permeability and resistance to non-specific cell interactions that is similar to natural mucinous gels. Moving forward, we anticipate that self-assembled glycopeptide hydrogels will be broadly useful as mucus-inspired surface coatings to improve the biocompatibility and efficacy of implantable devices, drug delivery vehicles, and biosensors.