CARB 1

Glycans at the stem cell surface

Laura L Kiessling, Kiessling@chem.wisc.edu. Departments of Chemistry and Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, United States

Human pluripotent stem cells (human embryonic stem cells and induced pluripotent stem cells) have the ability to self-renew indefinitely and differentiate into specialized types. Cell surface glycans are often used as markers of the pluripotent state but their functional roles are largely unexplored. The value of glycan binding was apparent when we devised and implemented a cell surface array screen: The surfaces displaying glycosaminoglycan (GAG)-binding peptides were the most effective at promoting human pluripotent stem (hPS) cell propagation. Matrigel—an undefined substratum derived from a murine tumor that consists of a mixture of >1800 proteins and other components—is a popular substratum for hPS cell culture, but the GAG-binding surfaces have superior properties. In this seminar, we will describe how chemically defined surfaces can not only serve as excellent substrata but also can reveal unexpected roles for GAGs in hPS cell propagation and differentiation.

CARB 2

Glycan arrays for analysis of influenza virus specificity

James C. Paulson, jpaulson@scripps.edu, Ryan McBride, Wenjie Peng, Robert P de Vries, Corwin M Nycholat, Department of Cell and Molecular Biology, Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California 92037, United States

The receptor specificity of influenza viruses for sialic acid containing receptors on host cells plays an important role as a barrier for transmission of animal viruses to humans. It is well documented that avian viruses primarily recognize a2-3 sialic acid receptors (avian-type) and human viruses recognize a2-6 receptors (human-type), and that potentially new human pandemic viruses originating from avian viruses must acquire mutations that confer specificity for human-type receptors. Moreover, recent studies suggest that binding to a2-6 sialosides is a prerequisite for respiratory droplet transmission in ferret models, and is a risk factor for transmission to and between humans. A major gap in knowledge is what glycans are actually recognized by influenza on the human airway. Glycan microarrays are now widely used as tools for rapidly assessing the receptor specificities of influenza viruses by laboratories around the world. To help understand the specificity of influenza viruses for glycans involved in infection, we are developing custom sialoside arrays comprising intact N- and O-linked glycans that are present on the human airway epithelium. Emphasis has been placed on chemo-enzymatic synthesis of an array of glycans spanning from trisaccharides to large branched N- and O-linked glycans with poly-lactosamine extensions, of over 35 monosaccharides in size. The resulting array of sialosides is revealing novel and important details about the adaptation of avian viruses to the human host. (Supported by NIH grants AI50143 and AI99141, NWO Rubicon Fellowship (RPdV))

CARB 3

Chemoenzymatic synthesis of glycoproteins for deciphering functions

Lai-Xi Wang, lwang@som.umaryland.edu. Institute of Human Virology and Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD
Protein glycosylation is one of the most prevalent and complex posttranslational modifications that can profoundly affect a protein's folding, stability, immunogenicity, and other important biological functions. However, a detailed understanding of the functions of glycosylation is often hampered by the difficulties to obtain homogeneous glycoproteins carrying structurally well-defined glycans. This lecture presents an efficient chemoenzymatic method for the synthesis of complex N-linked glycopeptides and for glycosylation remodeling of glycoproteins. The method explores the transglycosylation activity of a class of endoglycosidases for synthetic purpose. Recent development in generation of novel glycosynthases that lack product hydrolytic activity but can still take the highly activated glycan oxazoline as substrate for glycosylation of GlcNAc- or Glc-containing polypeptides will be discussed. The usefulness of this chemoenzymatic method for deciphering glycoprotein functions will be demonstrated with three examples: 1) synthesis of homogeneous HIV-1 glycopeptides for characterizing the glycan specificity of HIV-neutralizing antibodies; 2) synthesis of monoglucosylated glycoprotein glycoforms for probing lectin-mediated protein fold mechanism; and 3) Fc glycosylation engineering of monoclonal antibodies for enhancing their therapeutic efficacy.

CARB 4

Split personality of O-GlcNAc transferase

Suzanne Walker, suzanne_walker@hms.harvard.edu. Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts 02115, United States

O-GlcNAc Transferase (OGT) is an essential glycosyltransferase that catalyzes the attachment of N-acetylgalactosamine (GlcNAc) to serines and threonines of numerous nuclear and cytoplasmic proteins. Global O-GlcNAcylation levels increase with increased flux through the hexosamine biosynthetic pathway, which produces OGT's donor substrate, UDP-GlcNAc. Increased O-GlcNAcylation is associated with widespread changes in gene expression, but the molecular mechanisms underlying these changes remain poorly understood. It was recently reported that OGT catalyzes another post-translational modification: the cleavage of host cell factor 1 (HCF-1), a ubiquitous transcriptional coregulator found in several chromatin remodeling complexes. We will describe studies confirming that OGT has proteolytic activity and showing that HCF-1 cleavage occurs in the same active site as glycosylation. Based on structural work and biochemistry, a novel mechanism for proteolysis is proposed.

CARB 5

Developing an enzymatic approach to synthesize ultra-low and low molecular weight heparins

Jian Liu, jian_liu@unc.edu. Division of Chemical Biology and Medicinal Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599, United States

Heparan sulfate is a sulfated glycan that exhibits essential physiological functions, and heparin is a clinically used anticoagulant drug. Interrogation of the specificity of heparan sulfate-mediated activities demands a library of structurally defined oligosaccharides. Synthesis of heparan sulfate using enzymes provides a promising approach because of the high regioselectivity of heparan sulfate biosynthetic enzymes. The synthesis of heparan sulfate involves 15 different enzymes, including sulfotransferases, an epimerase and glycosyltransferases. Up to now, a number of
oligosaccharides with different sulfation patterns and sizes were synthesized. These oligosaccharides are now used to probe the biosynthetic mechanism of heparan sulfate and heparin, improving the synthesis of heparin drugs, and understanding the interaction of heparan sulfate with proteins. The availability of structurally defined heparan sulfate oligosaccharides will significantly advance the ability to understand the biology of heparan sulfate.

CARB 6

Quantifying the energetics of protein–carbohydrate interactions in the context of enhanced aromatic sequons

Wentao Chen¹,³, Joshua L Price¹,³, Elizabeth K. Culyba¹,³, Sarah R. Hanson¹,³, Apratim Dhar⁴, Chi-Huey Wong¹,³, Martin Gruebele⁴, Amelia A Fuller¹,³, Yanwen Fu¹,³, Evan T. Powers¹,²,³, Jeffery W Kelly¹,²,³, jkelly@scripps.edu. (1) Department of Chemistry, The Scripps Research Institute, United States (2) Department of Molecular and Experimental Medicine, The Scripps Research Institute, The Scripps Research Institute, United States (3) The Scripps Research Institute, The Skaggs Institute of Chemical Biology, United States (4) University of Illinois, Center for Biophysics and Computational Biology and Departments of Chemistry and Physics, United States

The 1/3 of the human proteome that traverses the cellular secretory pathway is generally co-translationally or post-translationally N-glycosylated at Asn within the Asn-Xxx-Thr/Ser sequon in the lumen of the endoplasmic reticulum. We have shown that placing an aromatic residue two or three positions prior to a N-glycosylated Asn in specific reverse turn types enables significant stabilizing hydrophobic interactions between the aromatic side chain, the first N-acetylglucosamine (GlcNAc) of the glycan and the Thr side chain. Glycosylation of a specific “enhanced aromatic sequon” in the complementary reverse turn type substantially stabilizes the native state of several different glycosylation-naïve proteins. However, the structure–energy relationships underpinning this native state stabilization were unclear. The structures and folding energetics of chemically synthesized glycoproteins will be discussed to provide insight into the contributions of the hydrophobic effect and CH–π interactions to carbohydrate–protein side chain packing interactions. We find that the hydrophobic effect contributes significantly to protein–carbohydrate interactions. Interactions between carbohydrates and aromatic amino acid side chains, however, are supplemented by CH–π interactions. The strengths of experimentally determined carbohydrate–π interactions do not correlate with the electrostatic properties of the involved aromatic residues, suggesting that the electrostatic component of CH–π interactions in aqueous solution is small. Thus, tight binding of carbohydrates and aromatic residues is driven by the hydrophobic effect and CH–π interactions featuring a dominating dispersive component.

References:


CARB 7
Chemical neurobiology is rapidly evolving and providing insights into the molecules and interactions involved in neuronal development, sensory perception and memory storage. We will describe the synergistic application of organic chemistry and neurobiology to understand how specific carbohydrate molecules contribute to the wiring of the brain during development, as well as the ability of axons to regenerate after injury. Chondroitin sulfate polysaccharides have traditionally been viewed as passive, “barrier” molecules that impede neuronal growth. By combining synthetic organic and polymer chemistry, computational chemistry and in vivo biology, we now show that these molecules actively participate in signaling processes that underlie the formation of neural circuits and neuroregeneration.

CARB 8

One-pot strategies for carbohydrate synthesis

Shang-Cheng Hung, schung@gate.sinica.edu.tw.Genomics Research Center, Academia Sinica, Taipei, Taiwan Republic of China

Glycans are exceptionally diverse and complex that deciphering the functions embedded within the glycome is a substantial challenge. The multiple regio- and stereochemical permutations for linking several monosaccharide units and the modifications that may follow chain assembly allowed these complex sugars to hold structural information densities that surpass DNA or proteins. With biosynthetic pathways that are regulated rather than template-driven, the sugars are usually expressed as an array of related structures that may possess subtle differences in activity. Several biological processes involve glycans, yet understanding their ligand specificities is impeded by their inherent diversity and difficult acquisition. Generating synthetic sugar libraries for bioevaluations forms the core of chemoglycomics approaches to unravel glycan structural information. To tackle this problem, a combination of “regioselective one-pot protection” and "stereoselective one-pot glycosylation” strategies have been developed to prepare a variety of cell-surface carbohydrates, including influenza virus-binding saccharides and heparan sulfate oligosaccharides. Affinity screening and further X-ray co-crystal analysis of these synthesized sugars with proteins provide key insights at the molecular level.

CARB 9

Hepatic delivery of drugs: A case of primaquine

Yuan Chuan Lee, yclee@jhu.edu.Department of Biology, Johns Hopkins University, Baltimore, MD 21218, United States

Plasmodium is responsible for malaria infection. Its liver phase is the longest in its life cycle (2 weeks). Targeting delivery of primaquine (PQ), an anti-malaria drug, can not only enhance the efficiency of PQ, but is a necessity for certain type of patients who cannot tolerate high dose of PQ. We attached a trivalent GalNAc ligand (triGalNAc), a powerful hepatic receptor ligand we had developed, to polyglutamic acid which carries multiple molecules of PQ. This polyplex was fluorescence-labeled for tracking. Highly efficient delivery of PQ to the liver was attained by the
above conjugate, but PQ-modified PGA without triGalNAc is quickly excreted to bladder.

**CARB 10**

Carbohydrate-based vaccines: Challenges and opportunities

**Chung-yi Wu, cyiwu@gate.sinica.edu.tw. The Genomics Research Center, Academia Sinica, Taipei, Taiwan Republic of China**

Advances in the synthesis of oligo- or polysaccharides and new technologies developed in glycobiology studies have opened a new avenue in carbohydrate vaccine design. In principle, various types of cell surface epitopes, characteristic for the invading organism or related to aberrant growth of cells, can be applied to develop vaccines. For example, carbohydrate epitopes on virus or cancer cells represent attractive targets for development of carbohydrate-based vaccines. Understanding the presentation of carbohydrate epitopes on cell surface allows us to more closely mimic the natural setting in the context for vaccine design. Numerous promising carbohydrate-based vaccine candidates have been prepared in recent years. This presentation briefly presents our recent advances involving carbohydrate based vaccines, including anti-bacteria and anti-cancers.

References:


**CARB 11**

Imaging glycans using a chemoenzymatic approach

**Peng Wu, peng.wu@einstein.yu.edu. Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461, United States**

In this talk, I will discuss our progress on using chemoenzymatic methods that combine the specificity of glycan modification enzymes and the fidelity of bioorthogonal click chemistry to imaging glycans in living organisms.

**CARB 12**

Directed evolution of glycopeptides and glycoDNA to design HIV vaccine candidates
We will describe the development of methods to optimize multivalent carbohydrate clustering via directed evolution. Although synthetic chemists often construct multivalent clusters of carbohydrates to improve on the otherwise weak interactions of single glycans with their target receptor, the maximum potential binding enhancement is rarely achieved. To systematically optimize glycocluster clustering for maximum binding enhancement, we have developed directed evolution methods in which a glycan can be clustered on \(10^{13}\) different peptide or DNA scaffolds, each of which results in a different geometric presentation of the glycans. Glycoclusters with high affinity are selected from the library by binding to immobilized target, then amplified to produce a new library which is superior to the first one, and then the process is repeated until excellent binding is achieved. We have applied this strategy to the development of oligomannose clusters which are strongly recognized by broadly-neutralizing anti-HIV antibody 2G12, obtaining some glycan clusters with picomolar affinity for 2G12. These structures should be excellent candidates for in vivo immunogenicity studies to re-elicit 2G12-like antibodies which may protect against HIV infection.

CARB 13

Chemical approaches to understanding O-GlcNAc modifications in human disease

Matthew R Pratt, matthew.pratt@usc.edu. Department of Chemistry, University of Southern California, Los Angeles, CA 90089, United States

The modification of proteins in the cytosol, nucleus, and mitochondria by the monosaccharide N-acetyl-glucosamine (O-GlcNAc) can have dramatic effects on their cellular function. Notably, the levels of O-GlcNAcylation are dramatically altered in several human diseases including cancer and neurodegeneration. Therefore, investigating the specific consequences of O-GlcNAcylation will
uncover new therapeutic opportunities. Here, I will present the use of a combination of bioorthogonal chemistries and synthetic protein chemistry to (1) visualize and identify important O-GlcNAcylated proteins and (2) understanding the molecular consequences of specific O-GlcNAcylation events on disease associated proteins.

CARB 14

Endowing glycan labeling and visualization with specificity and versatility

**Xing Chen**, xingchen@pku.edu.cn. College of Chemistry and Molecular Engineering, Peking University, Beijing, China

Strategies for labeling and visualizing glycans in live cells and living animals can facilitate elucidating their important functional roles in mediating molecular recognition, development, and cell signaling. Our research group works on developing chemical tools to metabolically probe and manipulate protein glycosylation. Here, we first present a cell-selective metabolic glycan labeling strategy based on ligand-targeted liposomes, which enables studying glycans in a specific cell type of interest within a complex biological system. Second, we design and synthesize bifunctional unnatural sugars that introduce simultaneously two functional groups into cellular glycans. The bifunctional sugar analogs are valuable tools for profiling glycan-protein interactions. Finally, we are interested in developing new imaging modalities for glycan visualization. A recently developed bioorthogonal Raman imaging technique will be discussed.

CARB 15

Understanding and manipulating protein glycosylation: Application in biofuel production

**Zhongping Tan**¹², zhongping.tan@colorado.edu, **Liqun Chen**¹², **Eric R Greene**², **Patrick K Chaffey**¹², **Matthew R Drake**¹². (¹ Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80303, United States ² BioFrontiers Institute, University of Colorado, Boulder, CO 80303, United States

Protein glycosylation is an important post-translational modification. It enhances the functional diversity of proteins and alters their conformation, stability and biological activity. However, because of the complexity of glycoproteins and glycan structures, establishing connections between glycan structures and their functions is difficult. Recently, we demonstrate that chemical synthesis can be employed as a useful tool to determine the effects of protein glycosylation on the stability and function of cellulases. The more precise understanding of protein glycosylation would aid in the development of cellulases with improved performance in biomass conversion.

CARB 16

Recent advances in the chemistry of the sialic acids

**David Crich**, dcrich@chem.wayne.edu. Department of Chemistry, Wayne State University, Detroit, MI 48202, United States

Recent work in the Crich laboratory on the stereoselective synthesis of sialic acid glycosides, and on their subsequent elaborations will be described.

CARB 17
Stereoselective synthesis of S-linked 2-deoxy sugars for biological studies

Jianglong Zhu, Jianglong.Zhu@utoledo.edu. Department of Chemistry, University of Toledo, Toledo, Ohio 43560, United States

2-Deoxy sugars are present in many bioactive natural products and clinical agents and significantly influence their biological activity as well as stability and solubility. Despite their critical role, the glycosidic linkage of 2-deoxy glycosides has been found to be susceptible to hydrolysis in acid media or by glycosyl hydrolases, which has made it difficult to probe the biological role of these 2-deoxy sugars, has resulted in toxicity and reduced activity of the parent molecules, and has limited their use as clinical agents. Thioglycosides (S-linked glycosides), in which the glycosidic oxygen atom is replaced by a sulfur atom, are resistant towards enzymatic cleavage as well as chemical degradation. In addition, thioglycosides maintain the biological activity of their parent O-linked glycosides and are tolerated by most biological systems. Therefore, they can be employed as an important tool for structural biology and attractive therapeutic agents. In this presentation, I will discuss the development of new glycosylation methods for stereoselective synthesis of S-linked 2-deoxy sugars for comparisons of their physical, chemical and biological properties with natural O-linked counterparts.

CARB 18

Approaches to 1,2-cis-2-amino glycosides via transition metal catalysis and application to the synthesis of heparin oligosaccharides

Hien M Nguyen, hien-nguyen@uiowa.edu. Department of Chemistry, University of Iowa, Iowa City, IA 52242, United States

We have developed new approach for the effective synthesis of 1,2-cis-2-aminosugars via nickel-catalyzed alpha-selective glycosylation with C(2)-N-substituted benzylideneamino trihaloacetimidate donors. These aminosugars make up one of the most important classes of oligosaccharides and glycoconjugates. In general, obtaining an adequate supply of these sugars from natural sources is exceedingly challenging. In many cases, high purity 1,2-cis-2-aminosugars can only be obtained by chemical synthesis. Even though there have been remarkable advances in the synthesis of 1,2-cis-2-amino glycosides, the disadvantages of current methods include the use of excess activating agents and unpredictable/poor anomeric selectivity.

Our approach to glycosylation forming aminosugar molecules is innovative in that we apply modern organometallic methodology to solve issues of yield and selectivity that cannot be achieved under the current state-of-the-art methods. Our approach relies on the nickel-ligand complex and the nature of metal-bound functional groups at the C(1)- and C(2)-position on glycosyl donors to control the alpha-selectivity. Our methods are broadly applicable and provide highly-yielding and selective products. The efficacy of our transition-metal catalyzed glycosylation method is being applied to the synthesis of heparin oligosaccharides. The development of the nickel-catalyzed selective glycosylation method and the synthesis and biological studies of heparin oligosaccharides will be presented at this conference.

CARB 19

Organoboron catalysts and promoters for selective activation of glycosyl acceptors

Mark S. Taylor, mtaylor@chem.utoronto.ca. Department of Chemistry, University of Toronto,
Catalyst-controlled, regioselective glycosylation represents a promising approach for streamlining the synthesis of oligosaccharides and probing structure-activity relationships in glycosylated natural products. Our efforts to develop synthetic catalysts for glycosyl donor activation take advantage of the strong and selective interactions between organoboron compounds and diols. The discovery of borinic acid-catalyzed methods for regioselective glycosylation will be discussed, along with mechanistic studies and the development of novel variants of this activation strategy. Applications to the construction of challenging classes of glycosidic linkages, the synthesis of oligosaccharides and the selective glycosylation of polyol natural products will be presented.

CARB 20

Reagent control in diastereoselective chemical glycosylation reactions

Clay S Bennett, clay.bennett@tufts.edu. Department of Chemistry, Tufts University, Medford, MA 02155, United States

Chemical glycosylation reactions frequently rely on the intrinsic stereochemical information in the coupling partners to control selectivity (substrate control). A consequence of this is that extensive modification of the coupling partners, or optimization of reaction conditions, is often necessary to obtain stereoselective reactions, especially with so-called difficult linkages. To address this we have developed glycosylation reactions where the promoter exerts absolute control over the stereochemical outcome of the reaction (reagent control). Using this approach in the 2-deoxy series we have found that it is possible to obtain either anomer of a particular glycosidic linkage from the same coupling partners simply by changing the promoter. The scope of this approach with different classes of donors, and mechanistic considerations will be discussed.

CARB 21

Genetically-encoded fragment-based discovery of glycomimetic ligands

Ratmir Derda, ratmir@ualberta.ca, Simon Ng. Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

Genetically encoded libraries, in which each ligand is attached to a DNA tag, are increasingly used for ligand discovery. Many therapeutic antibodies in clinical trials originate from phage display technology, in which a protein is encoded in bacteriophage genome and displayed as fusion with coat protein. Natural translation of DNA could yield only libraries made of natural 20 amino acids; chemical post-translational modification is a powerful strategy for diversification of these libraries [1]. We designed a quantitative chemical strategy for incorporation of glycans into phage libraries to yield glycopeptides with constant carbohydrate and variable peptide portions [2]. These libraries allow rapid fragment-based discovery of glycomimetic ligands that inhibit interaction of lectins with natural glycans. The glycan anchors the ligand at the desired binding site whereas peptide strengthens the binding. Selection of mannose-decorated libraries assisted by deep-sequencing [3] can discover ligands that have significant improvement over natural ligands for proteins like ConA and DC-SIGN. Our glycopeptide libraries can be used for identification of amino acids that mimic the majority of a complex oligosaccharide. Such “glyco-replaced” ligands, in turn, can be used as a simple synthetically-accessible starting point for the design of therapeutic compounds, diagnostic probes and tools for glycobiology research.
Photoredox catalytic O-glycosylation with selenoglycoside donors

Justin R. Ragains, jragains@lsu.edu, Mark Spell, Xiaoping Wang, Amir E. Wahba, Elizabeth Conner, Ranelka G. Fernando, George G. Stanley. Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, United States

Photoredox catalysis is a rapidly growing area of study in organic synthesis that harnesses the power of single-electron transfer processes under mild, user-friendly conditions. Photoredox catalytic O-glycosylation with chalcogenoglycoside donors is an emerging area of research in chemical glycosylation. These glycosylations are marked by mildness in addition to the avoidance of strong electrophiles and multistage procedures. Herein, we report on a visible light photoredox catalytic O-glycosylation using selenoglycoside donors. The initial development of both Ru-catalyzed and diphenyldiselenide-catalyzed variants will be discussed in addition to more recent lines of investigation to address the mechanism, yield, efficiency and stereoselectivity of this reaction.

Towards a practical redox cascade system for template-directed stereoselective glycosylation

Xinyu Liu, xinyuliu@pitt.edu. Department of Chemistry, University of Pittsburgh, Pittsburgh,
Template-directed synthesis is a broadly employed strategy by Mother Nature in the assembly of biomolecules in a living system. In this presentation, we report our effort to design a practical redox cascade system to enable the template-directed stereoselective generation of 1,2-cis glycosidic linkage in the chemical synthesis of complex oligosaccharides. This work stems from our early works on the development of 1) a sequential redox process to enable a one-pot single step intramolecular aglycan delivery reaction for the selective preparations of β-mannosides and β-rhamnosides;¹ 2) and a photoredox process for programmable catalytic glycosylations with seleno/thio-glycosides.² Discussions will be focused on the practical applicability of the newly developed system and the preparation of oligosaccharides containing continuous 1,2-cis glycosidic linkages in both solution and solid phases in the context of their roles in modulating innate immune response.


CARB 24

Direct and stereoselective synthesis of biologically significant 2-deoxy sugars

**Jianglong Zhu**, Jianglong.Zhu@Utoledo.edu.Department of Chemistry, University of Toledo, Toledo, Ohio 43560, United States

2-Deoxy sugars, especially 2,6-dideoxy and 2,3,6-trideoxy sugars, are an important class of carbohydrates which exist in numerous biologically active natural products and clinical agents. These sugars have been found to play critical roles in their biological activity as well as stability and solubility. Although considerable synthetic studies have been reported, stereoselective synthesis of 2-deoxy glycosides, especially 2-deoxy-β-glycosides, remains notoriously challenging due to the absence of directing group at C2. In this presentation, I will discuss our progress towards the development of new glycosylation methods for direct and stereoselective synthesis of 2-deoxy sugars.

CARB 25

Facile glycosylation reactions using air- and water-stable iodonium salt promoters

**Clay S Bennett**, clay.bennett@tufts.edu.Department of Chemistry, Tufts University, Medford, MA 02155, United States

Chemical glycosylation reactions typically require air- and water- sensitive reagents, extremely strong acids, toxic heavy metal salts, and/or ultra low temperatures to run. As a result, the chemical synthesis of carbohydrates requires extensive synthetic training to successfully execute. Here we present new conditions for room temperature chemical glycosylation reactions using air- and water-stable iodonium salt promoters. The reaction setup is trivial, and extensive drying of reagents and solvents is not necessary. We envision that this approach will one day allow chemical biologists to routinely prepare their own carbohydrate libraries, as they do with peptides today. The scope of the reaction and its use in diastereoselective glycosylation reactions will be discussed.
General approach for the synthesis of libraries of symmetrical and asymmetrical N-glycans

Geert-Jan Boons, gjboons@ccrc.uga.edu.Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30606, United States

Despite their biological importance, there are no methods available to systematically and efficiently produce asymmetrically branched N-glycans needed to populate diverse glycan libraries and investigate the specificities and biology of glycan binding proteins. We report here the chemical synthesis of a pentasaccharide that is common to all eukaryotic N-linked glycans and is modified at positions where branching points can occur with the protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonate (Fmoc), allyloxycarbonate (Alloc), and 2-naphthylmethyl (Nap). A library of complex branched bi-, tri-, and tetra-antennary structures has been generated by sequential removal of the protecting groups followed by chemical glycosylations using a diverse set of glycosyl donors. Furthermore, the use of acetylated or benzylated LacNAc and GlcNAc donors gave precursor glycans that at each antenna could be selectively extended by a panel of glycosyltransferases to rapidly give large numbers of highly complex asymmetrically substituted N-glycans. A similar strategy was employed to prepare a library of core-fucose modified N-linked glycans. The glycans were printed as microarrays and screened for binding to lectins and influenza-virus hemagglutinins, which demonstrated that recognition is modulated by presentation of minimal epitopes in the context of complex N-glycans.

De novo approaches to oligosaccharides

George A O’Doherty, g.odoherty@neu.edu.Chemistry, Northeastern University, Boston, MA 02115, United States

The O’Doherty research group has been working in two related areas of organic synthesis: carbohydrate synthesis and natural product synthesis. The unifying theme that connects our research in these two areas is our method of synthesis (asymmetric catalysis), target selection (mono-, di-, tri-, oligo-saccharide containing natural products) and biological activity (anti-cancer/anti-microbial agents). A recurring theme in the group’s synthetic approaches to the oligosaccharide natural products is our reliance on asymmetric catalysis for the control of asymmetry. Fundamental to our approach is the development of highly efficient routes that transform, via catalysis, inexpensive achiral starting materials into enantiopure products, which are poised for the conversion into complex molecules with biologically relevant properties (i.e. enantioselective synthesis of a new “chiral pool” via asymmetric catalysis). Recently, we have found that these approaches have matured to the point where we have developed enantioselective routes to these complex molecules in sufficient quantities that are amenable for biomedical investigations. Our lastest results along these lines will be discussed.

New glycosylation promoters for automated glycan synthesis with simplified building blocks

Nicola L. B. Pohl, npohl@indiana.edu.Department of Chemistry, Indiana University, Bloomington, IN 47405, United States
Many advances in glycobiology are stalled by the lack of diverse and chemically well-defined glycan structures. For automation to play as important a role in the synthesis of oligosaccharides as it does currently in peptide and nucleic acid production, the major bottleneck of building block access must be overcome. Here, progress toward addressing this building block issue, in part by the development of simpler thioglycoside activation protocols, will be discussed.

**CARB 29**

**Electrochemical assembly for oligosaccharide synthesis**

*Toshiki Nokami, tnokami@chem.tottori-u.ac.jp. Department of Chemistry and Biotechnology, Tottori University, Tottori, Tottori 680-8552, Japan*

The iterative assembly of small building blocks by the integration of reactions in a one-pot sequential manner serves as a powerful method for constructing oligosaccharides. We developed a new method for automated solution-phase synthesis of oligosaccharides based on electrochemical activation of thioglycosides to generate glycosyl triflates in the absence of a building block. The method enables us to make up oligosaccharides in the different direction of assembly from Seeberger method based on the acceptor-bound solid-support approach. If the present method would be a solid-support approach, it would be the donor-bound version. We synthesized partial structures of poly-β-D-(1-6)-N-acetylglucosamine (PNAG) by assembling six thioglycosides in a one-pot sequential manner using an automated electrochemical synthesizer developed for the method.

**CARB 30**

**From chemical glycosylation to expeditious oligosaccharide synthesis**

*Alexei V Demchenko, demchenkoa@umsl.edu. Department of Chemistry and Biochemistry, University of Missouri - St. Louis, St. Louis, Missouri 63121, United States*

From the building blocks of nature to disease-battling therapeutics and vaccines, carbohydrates have had a profound impact on evolution, society, economy, and human health. Numerous applications of these essential biomolecules in many areas of science and technology exist, foremost of which can be found in the area of development of therapeutic agents and diagnostic
platforms. Although carbohydrates are so desirable for biological and medical community, chemically these molecules are still very challenging targets. Carbohydrate chemists need to address the need for chemical functionalization, perform protecting and leaving group manipulations, control anomeric stereoselectivity, etc. Advances in chemistry and biochemistry have certainly simplified the synthesis of some classes of carbohydrates. However, the development of practical and general methods for chemical glycosylation and expeditious oligosaccharide synthesis remain demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical glycosylation and expeditious oligosaccharide assembly. These innovative tools for oligosaccharide synthesis will be discussed in light of recent results. The effectiveness of methods developed will be illustrated by the synthesis of medicinally relevant oligosaccharides and conjugates thereof. This work has been generously supported by awards from the NIGMS, NSF, Pfizer, and Mizutani Foundation for Glycoscience.

References


CARB 31

Drug discovery in undergraduate research: Defining an appropriate target in the struggle against lipopolysaccharides

Robert Woodward, woodwarl@mountunion.edu, Alyssa Greenwell, Emily Loosli, Lauren Gosser.Department of Chemistry & Biochemistry, The University of Mount Union, Alliance, OH 44601, United States
The emergence of antibiotic-resistant infections has led to an increased need for the development of novel therapeutic agents. While the introduction of such antibiotics has lagged considerably over the past decades, lipopolysaccharide biosynthesis, more specifically the enzyme LpxC, has emerged as an attractive target. Nevertheless, the large-scale screening efforts frequently employed to discover novel therapeutics for such targets are not feasible at most predominantly undergraduate institutions. We therefore focused on an alternative strategy to aid in the discovery process by recognizing that an adequate high-throughput screen for LpxC inhibitors has not been developed. This deficiency has primarily arisen due to the cost-prohibitive nature of the LpxC substrate. Accordingly, two novel total syntheses of the LpxC substrate are in progress, one which is purely chemical and a second which is chemoenzymatic.

The chemical total synthesis relies on a silane-based protecting group strategy which acts to prevent the problematic acyl migration noted in previous syntheses. In contrast, the chemoenzymatic synthesis seeks to exploit the activity of a lipase, phosphotransferase and uridylyltransferase to assemble the target substrate. Above all, these projects provide an example of how one can overcome equipment/material limitations and still offer undergraduate researchers the opportunity to become involved in the drug discovery field.

**CARB 32**

**Synthesis and anti-oxidant activity of phenylpropanoid glycosides**

*Jennifer Koviach-Côté, jkoviach@bates.edu, Jennifer Brown, Joanna Mangar, Zena Sabbath, Rebecca Otley. Department of Chemistry, Bates College, Lewiston, ME 04240, United States*

Phenylpropanoid glycosides, isolated from plants, show significant activity as radical scavengers and antioxidants. As part of a study to better understand the mechanism by which these compounds scavenge free radicals, we have synthesized a library of compounds containing 1-4 phenylpropanoid groups on a glucose core. We then measured the radical scavenging activity of each compound in both a protic and aprotic solvent using a well established assay. We found that anti-oxidant activity is most dependent on the number of phenol groups present in the compound, but preliminary data suggests that proximity of two groups may also play a role.

**CARB 33**

**Virus nanoparticles that perturb the heparin-coagulation mechanism**
Heparin use in clinical settings is plagued by the polymer's heterogeneity, leading to challenges associated with production, purification, dosage, and antagonism when immediate reversal is needed. To address these issues we have employed bacteriophage Qß nanoparticles, whose multi-valent nature makes it an attractive candidate for perturbing the coagulation mechanism that heparin polymers participate in. First, we have developed antagonists that safely reverse the anti-coagulant activity of heparin. To interact with the heparin polyanion, polycationic particles were generated both chemically and by mutation; in particular, mutant T18R, which mutates a single site on each of the 180 coat proteins that make up the capsid, showed full reversal of heparin activity. Chromatography using a heparin-sepharose column confirmed a strong interaction between heparin and the T18R particle. Binding studies using fluorescein-labeled heparin (HepFL) resulted in a concentration-dependent change in fluorescence intensity, which could be perturbed by the addition of unlabeled heparin. Analysis of the fluorescence data yielded a dissociation constant of approximately 1 nM and a 1:1 binding stoichiometry for HepFL:VLP. Studies using dynamic light scattering (DLS) suggest that T18R forms discrete complexes with heparin when the VLP:heparin molar ratios are equivalent. In vitro clotting assays confirm the 1:1 binding stoichiometry as full antagonism of heparin is achieved when the particle to heparin molar ratio approaches unity. These studies validate T18R for potential clinical use as an effective, non-toxic heparin antagonist.

We have also conducted studies towards the opposite approach: generating specifically sulfated phage that may elicit heparin-like activity. Surface lysine residues on Qß have been positioned to allow for maximum solvent accessibility; these positions are then modified with either singly or triply sulfated molecules. Factor Xa and activated partial thrombin time assays are used to determine the efficacy of the sulfated nanoparticles in prolonging coagulation.

**CARB 34**

**Preparation of C-glycosides as potential antihyperglycemic agents at Saginaw Valley State University, a primarily undergraduate institution**

*Jennifer L. Chaytor, jchaytor@svsu.edu, Amanda Paris, Jacqueline Spearman, Craig Tucker. Department of Chemistry, Saginaw Valley State University, University Center, Michigan 48710, United States*

Type II diabetes mellitus affects millions of people worldwide, and there is an urgent need for novel anti-hyperglycemic drugs to combat this disease. In this project, aryl-C-glycosides have been synthesized via standard cross coupling and carbohydrate chemistry. Their structures were designed based upon known anti-hyperglycemic agents. The target compounds have a carbohydrate moiety linked to an aromatic portion via a short linker, and both the carbohydrate and aromatic portions can be varied to provide a small library of compounds. These compounds will subsequently be evaluated in enzymatic assays as potential anti-hyperglycemic agents for the treatment of Type II diabetes. The results of our experiments to date will be presented. In addition, the challenges and successes of conducting research at a primarily undergraduate institution will be discussed.

**CARB 35**

**Chemical tools to discover and target Helicobacter pylori's glycoproteins**
Virulence of the gastric pathogen *Helicobacter pylori* (*Hp*) appears to be directly linked to the bacteria’s ability to glycosylate proteins. Although *Hp* synthesizes a vast array of glycoproteins, it is not clear which of these species are involved in host-pathogen interactions, what machinery is responsible for their synthesis, and how glycans can be harnessed to treat chronic *Hp* infection. My lab has employed a chemical technique, metabolic glycan labeling, to study *Hp* glycoproteins and to covalently deliver therapeutic probes to surface glycans on *Hp*. Here we will present our most-recent findings, including the targeted identification of 125 glycosylated proteins in *Hp* and recruitment of the host's immune system to target *Hp* based on its surface glycans. Broadly, this work validates metabolic labeling as an effective and efficient approach to discover and target bacterial glycoproteins.

**CARB 36**

**Metal-mediated synthesis of carbohydrate porphyrin (CPCs) and carbohydrate bacteriochlorin conjugates (CBCs)**

*Joshua V Ruppel*, jruppel@uscupstate.edu. Division of Natural Sciences and Engineering, University of South Carolina Upstate, Spartanburg, SC 29303, United States

Carbohydrate porphyrin (CPCs) and carbohydrate bacteriochlorin conjugates (CBCs) have gained attention for use as theranostic agents in photodynamic therapy (PDT) to address several limitations of the current photosensitizers including solubility in biological fluids, selectivity, and weak absorbance at clinically useful excitation wavelengths (NIR). The development of a concise route for the synthesis of brominated porphyrins and bacteriochlorins has allowed for the development of a modular metal-mediate synthetic approach for the synthesis of CPCs and CBCs. As part of efforts to generate CPCs and CBCs, herein we report an accessible approach for the synthesis of CPCs and CBCs using Buchwald-Hartwig and Cu(I)-mediated 1,3-dipolar cycloaddition type reactions. The advantages and challenges of performing collaborative research at PUIs involving undergraduate researchers will also be discussed.

**CARB 37**

**Investigations into the mutagenic potential of the prominent DNA lesion, 8-oxo-2'-deoxyguanosine, using nucleotide analogs**

*Michele Hamm*, mhamm@richmond.edu. Department of Chemistry, University of Richmond, Richmond, VA 23059, United States

8-oxo-2'-deoxyguanosine (OdG) is a prominent DNA lesion produced from the reaction of dG with reactive oxygen species. Because OdG can form stable base pairs to both dC and dA, it can direct the insertion of dATP during replication, leading to dG → T transversions. The potential for OdG to cause mutation is dependent on the preference for dCTP or dATP insertion opposite OdG, as well as the ability to extend past the resulting base pairs. The C2-amine and C8-oxygen could play major roles during these reactions since both can lie outside the Watson-Crick cognate base pairs shape and both can form strong interactions, like hydrogen bonds. In order to gain a more general understanding how the C2-amine and C8-oxygen of OdG affect its mutagenic potential, the incorporation opposite and extension past seven analogues of dG/OdG that vary at C2 and/or C8
were characterized for several DNA polymerases.

**CARB 38**

**New thio-click and domino approach to glycomimetics**

**Zbigniew J. Witczak, zbigniew.witczak@wilkes.edu. Pharmaceutical Sciences, Wilkes University, Nesbitt School of Pharmacy, Wilkes-Barre, PA 18766, United States**

The recent development in thio-click chemistry became a well-accepted part of the click reaction toolbox and is rapidly developing in many strategic areas of thio-conjugation. This strategy requires specifically modified chiral building blocks with sugar framework in the locked conformation of the pyranose ring. One of the ideal synthon fitting this category is 4-deoxy-1, 2-O-isopropylidene-β-L-threo-4-enopyranose 1, conveniently prepared from L-arabinose. Regioselective functionalization of 1, via thio-click/domino cyclization produced an adduct 3 in high yield. Subsequent thio-click functionalization of 3 with 1-thiosugar 4 proceeds in stereoselective manner with the formation of thiosaccharide 5 in excellent 90% yield.

The tentative mechanism of formation of thio-click/domino reaction products, with alternative reverse sequence of the reaction will be discussed in details.

![Diagram of the reaction process](image)

**CARB 39**

**Hydrolysis of carbohydrates into platform chemicals**

**Xincheng Wang, wxcnathan@gmail.com, Yanlei Song, Chongpin Huang, wxcnathan@gmail.com, Biaohua Chen, chenbh@mail.buct.edu.cn. Department of Chemical Engineering, Beijing University of Chemical Technology, Beijing, Beijing 100029, China**

Because of the adjustable functionality of ionic liquids, we have been involved in the use of ionic liquids(ILs) as a probe catalyst, rather than as a solvent, in the conversion of carbohydrates into 5-HMF. Different kinds of acid-base ILs were prepared via modification of cationic or anionic parts of ILs.

Brønsted acidic ILs, 1-(3-sulfonicacid)propyl-3-methyl imidazolium phosphotungstate ([MIMPS]_3PW_{12}O_{40}). gave a maximum yield of 99.1% with fructose as substrate. The introduction of alkaline ILs didn't improve the yield from fructose but did promote the dehydration of glucose, which almost doubled in 5-HMF yield(15%) catalyzed by acidic ILs. However, in both cases, the absolute yield from glucose was low.
For weaker alkaline or even neutral ILs, the hydroxyl ILs, 1-hydroxyethyl-3-methylimidazolium tetrafluoroborate ([C$_2$OHMIM]BF$_4$), producing the highest yields of 5-HMF for both fructose and glucose (95.7% and 35.7%), was a more efficient and environmentally friendly catalyst.

Furthermore, poly(imidazole-epichlorohydrin)BF$_4^-$ was also synthesized, which showed much better performance towards the conversion of glucose (66.1% 5-HMF yield).

**CARB 40**

Synthesis of degradable sugar poly(ortho esters)

*Wenjun Du, du1w@cmich.edu, Lingyao Li, Ian James Milligan, Jun Wang, Emily Anne*
The synthesis of novel sugar poly(ortho esters) were achieved through tetrabutylammonium iodide (TBAI)-catalyzed polymerization of di-functional AB monomers--- 2,3,4-tri-O-acetyl-a-D-glucopyranosyl bromide and 2,3,4-tri-O-acetyl-a-D-galactopyranosyl bromide. Having a 1-Br and 6-OH group on the same molecule, these monomers are stable at ambient conditions, but could be activated by TBAI to give sugar poly(ortho esters) with molecular weights up to 7.0 kDa in over 70% yield. The synthesized sugar poly(ortho esters) exclusively contain orthoester linkages between each sugar unit, indicating the polymerization is highly chemoselective. These sugar poly(ortho esters), containing highly acid-labile orthoester linkages, were used to construct biocompatible and pH-responsive nanoparticles.

CARB 41

2D oligosaccharide syntheses: A comprehensive approach

XINYU LIU, xinyuliu@pitt.edu. Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Chemical synthesis of complex carbohydrates constitutes a leading method of choice for providing homogeneous glycans for their functional studies at a glycomics age. To emulate the efficiency of oligonucleotide and oligopeptide synthesis, continuing innovation remains needed on the development of fundamental chemistries that are related to the chemical assembly of an oligosaccharide, namely novel glycosylating agent and protecting group chemistry and strategy. In this presentation, I will summarize our laboratory's work in the past three years that represents a comprehensive effort on designing new chemical strategy and tactics to transform a multidimensional challenge associated with an oligosaccharide synthesis, to a two dimensional problem that is reminiscent of the scenario for peptide and nucleic acid syntheses. Specifically, I will discuss the development of 1) a modular designer thioglycoside-based glycosylating system, 2) a set of tunable acid-sensitive hydroxyl protecting groups, 3) a panel of designer linker systems, which collectively improve the efficiency of complex oligosaccharide syntheses and allow for their preparations on unconventional solid supports and surfaces for biomedical and material applications.


CARB 42

Complementary one-pot multi-enzyme systems for heparosan oligosaccharide synthesis

Musleh M Muthana, mmu@ucdavis.edu, Jinqe Qu, Timofey Klyuchnik, Alex Siu, Mengyang Xue, Yanhong Li, Lei Zhang, Xi Chen*. Department of Chemistry, University of California Davis, Davis, CA 95616, United States

Heparan sulfate (HS) is a highly sulfated polysaccharide that is widely expressed in mammalian
cell surface and extracellular matrix. HS is important in many biological processes such as regulating cell growth, blood coagulation, inflammation, viral and bacterial infection, signal transduction, lipid metabolism, and cell differentiation. Heparin produced by mast cells can be considered as a special form of heparan sulfate. It is commonly used as an anticoagulant therapeutic. After the 2007 heparin contamination incident, concerns have been raised about the safety of heparin extracted from animals. This further supports the need for cost effective synthetic methods for heparin. Most chemoenzymatic methods for synthetic heparin rely on commercially expensive sugar nucleotides. In this work, a complementary one-pot multienzyme (OPME) system has been developed for heparosan synthesis. UDP-sugars are generated in situ and used by glycosyltransferases for elongating heparosan. This strategy can be applied to other glycosaminoglycans (GAGs).

CARB 43

Synthesis of biologically active N- and O-linked glycans with multi-sialylated poly-N-acetyllactosamine extensions using P. damsela α2-6 sialyltransferase

Wenjie Peng¹, wenjiep@scripps.edu, Corwin M. Nycholat¹, Ryan Mcbride¹, Robert P. de Vries¹, Aristotelis Antonopoulos², Anne Dell², Stuart M. Haslam², James C. Paulson¹. (¹) Departments of Cell and Molecular Biology, and Chemical Physiology, The Scripps Research Institute, La Jolla, CA 92037, United States (²) Department of Life Sciences, Imperial College London, London, United Kingdom

N- and O-linked glycans are biologically important post-translational modifications of glycoproteins on the cell surface, which play a fundamental role in many processes, including cell adhesion, signal transduction, and immune response. Extended poly-N-acetyllactosamine (pLN) glycans found on glycoproteins as well as glycolipids of some cells, are able to be further modified by sialylation, fucosylation and O-sulfation. Extended sialosides have shown to influence receptor-binding specificity in different biological studies. Though sialic acids are typically found α2-3 or α2-6-linked to a terminal non-reducing end galactose, poly-LacNAc extended core-3 O-linked glycans isolated from rat salivary glands and human colonic mucins have been reported to contain multiple internal Neu5Aca2-6Gal epitopes. Due to the complexity of natural glycans, the availability of synthetic structures for biological studies has been limiting. Here, we have developed an efficient approach for the synthesis of a library of N- and O-linked glycans with multi-sialylated poly-LacNAc extensions, including naturally occurring multi-sialylated core-3 O-linked glycans. We have found that a recombinant α2-6 sialyltransferase from Photobacterium damsela (Pd2,6ST) exhibits unique regioselectivity and is able to sialylate internal galactose residues in poly-LacNAc extended glycans which was confirmed by MS/MS analysis. Using a glycan microarray displaying this library we found that Neu5Aca2-6Gal specific influenza virus hemagglutinins, siglec and plant lectins are largely unaffected by adjacent internal sialylation, and in several cases the internal sialic acids are recognized as ligands. (Funded by NIH Grant AI099274).

CARB 44

Synthesis of C-vinyl sugars via a Pd(0)-catalyzed cyclization of Octenitols

Vivian C Ezeh, vezeh@colgate.edu, Matthew J Feeney, Ernest G Nolen. Department of Chemistry, Colgate University, Hamilton, New York 13346, United States

C-Linked glycoconjugates can be used as stable mimics to O-linked glycoconjugates in glycobiology studies. In this study, the stereoselective synthesis of vinyl-Glc, Gal and Man is
presented. These sugars will act as effective precursors to the synthesis of C-linked glycoconjugates. The synthetic steps involved making allylic acetates from the target monosaccharides, which were named octenitols. Cyclization of the octenitols catalyzed by Pd(0), afforded a mixture of α- and β-anomers of C-vinyl sugars. Attempts to expand the scope to other glycosides and to enhance this nascent selectivity using asymmetric ligands will be reported.

CARB 45

Examining furanoside conformational preference and reactivity: Computation, synthesis, and NMR

Jonathan S Rhoad, jrphoad1@missouriwestern.edu, David Freeman, Brett Cagg, Brian Dow, Chris Ruark, Adam Hunt, Mike Quaney, Torin McKinley, Melanie Edlin, Nathan Harms. Department of Chemistry, Missouri Western State University, Saint Joseph, MO 64506, United States

The conformational preferences of furanosides are better understood than they once were, but there is much we still don't know. We are examining permethylated furanosides, minimally substituted tetrahydrofurans and perfluorinated analogues using ring potential energy surface scanning to better describe their conformational preferences. The compounds are then synthesized so that the $^{3}J_{H,H}$ coupling constants can be compared to those predicted by theoretical methods. Initial measured coupling constants are in good agreement with calculated values.

CARB 46

Structure affects the interactions of cell-penetrating compounds with cell-surface glycosaminoglycans and lipid vesicle model membranes

Kristin J Braden, Amber R Schoenecker, Nicolas C Benish, Lisa E Prevette, prev0050@stthomas.edu. Chemistry, University of St. Thomas, St. Paul, MN 55105, United States

Cell-penetrating compounds (CPCs) are commonly used to enhance drug internalization, but their cell uptake mechanism is poorly understood. We hypothesize that glycosaminoglycans (GAGs) play an integral role in the recognition and subsequent internalization of CPCs and their attached cargo. Here, the interactions of TAT peptide, G5 polyamidoamine dendrimer and polyethyleneimine with four GAGs were studied through microcalorimetry to determine binding thermodynamics. Significant differences in affinity and stoichiometry were seen, likely reflecting charge density and hydroxyl stereochemistry variations in GAG structure, which have been characterized by colorimetric assay and NMR. Competitive displacement assays were used to quantify the concentration of each GAG needed to disrupt DNA complexes of the CPCs, to model premature release of cargo at the cell surface. CPCs showed lower affinity to lipid vesicle model cell membranes than to GAGs, revealing the potential importance of their GAG interaction for cell surface recognition. These results lead to understanding the role these GAGs play in the cellular internalization of biomaterials, which will contribute to the design of systems with enhanced uptake ability and have implications in targeted delivery to a variety of cell types with different expression levels of these GAG receptors.

CARB 47

Towards a better understanding of sialylations: Effects of substituents, solvent, and isotopic labeling
Sialic acids constitute a diverse family of more than 50 naturally occurring 2-keto-3-deoxy-nononic acids, amongst which N-acetylneuraminic acid (Neu5Ac) is the most ubiquitous. As the terminal component of oligosaccharide chains in glycoconjugates, Neu5Ac is typically attached to galactose, galactosamine or another sialic acid unit. Sialic acid-containing glycoconjugates are involved in a wide variety of biological phenomena ranging from cell-cell adhesion, cell growth regulation, immune response, to oncogenesis and recognition by viruses and bacteria. The chemical and enzymatic syntheses of glycoconjugates-containing sialic acid residues offer an essential tool for a better understanding of their biological proprieties, involvement in pathogenesis of various diseases, and for the design of sialic acid-based mimetics, therapeutics, and vaccines. Unfortunately, the chemical synthesis of sialosides is still a very challenging task in carbohydrate chemistry, due to the concomitant elimination reaction and lack of full stereocontrol. Herein, recent developments in the chemistry of sialylations which make an important contribution to the understanding of the chemistry of sialic acids will be presented. The main focus will be placed on stereoselective sialylations and expeditious syntheses of complex sialo-oligosaccharides.

**CARB 48**

**Carbohydrate-conjugated cinnamates via Huisgen cycloaddition reaction**

*Mo Hunsen, hunsenm@kenyon.edu.Chemistry Department, Kenyon College, Gambier, Ohio 43022, United States*

Carbohydrates and glycoconjugates are recognized by the pharmaceutical industry as an important class of molecules. Cinnamic acid and its derivatives are also attractive natural products due to their interesting anti-cancer, anti-microbial and anti-inflammatory properties. We report here the preparation of carbohydrate-conjugated cinnamates by a Huisgen cycloaddition reaction. Cinnamic acid derivatives with an alkynyl functionality were first prepared and then coupled with glycosyl azides using a Cu catalyst to afford the corresponding carbohydrate-tethered cinnamate triazoles. We also report a mild one-pot protocol for the preparation of glycosyl azides directly from free sugars. Our approach involves preparation of the glycosyl bromide intermediates using in situ generated HBr followed by a reaction with sodium azide under mild conditions. The carbohydrate-conjugated cinnamates are expected to possess improved bioavailability.

**CARB 49**

**Lessons learned from the total synthesis of the repeating pentasaccharide unit of pneumococcal serotype 31**

*Nicole L Snyder, nisnyder@davidson.edu.Department of Chemistry, Davidson College, Davidson, NC 28035, United States*

Invasive pneumococcal disease (IPD) is one of the leading causes of illness in adults and children worldwide. IPD is caused by the common gram positive bacterium *Streptococcus pneumoniae*, of which over 90 different serotypes are known to exist. In this symposium we will report on the lessons we have learned from our total synthesis of the repeating pentasaccharide unit of pneumococcal serotype 31 (PS31), a highly invasive strain of *Streptococcus pneumoniae* responsible for rising mortality rates in adult patients in Europe.
Controlling gene expression with nucleic acids

David Corey, david.corey@utsouthwestern.edu. Pharmacology, UT Southwestern, Dallas, TX 75390, United States

Nucleic acids are versatile tools for controlling gene expression. My laboratory has examined a wide variety of synthetic oligonucleotides for activity against targets ranging from chromosomal DNA, noncoding RNA, and trinucleotide repeat mRNA. This presentation will present our most recent advances in understanding the properties of nucleic acids inside cells and their control of gene expression.

Targeting microRNAs with small molecules: A novel therapeutic approach for cancer treatment

George A. Calin, gcalin@mdanderson.org, Paloma del C. Monroig, Maitri Shah, Nazila Nouraei. Department of Experimental Therapeutics, University of Texas MD Anderson Cancer Center, Houston, Texas 77054, United States

MicroRNAs (miRNAs) are a type of noncoding RNA (RNA that do not codify for proteins but have regulatory functions) that has been shown to be differentially expressed in numerous tissues, key cellular processes and multiple diseases. Medical communities have significantly underestimated the spectrum of consequences that their altered expression may cause in patients. In cancer, miRNAs act by targeting crucial tumor suppressor proteins in a post-transcriptional manner. During the past years, they have been proven to be involved in the processes of tumor initiation, progression and metastases; moreover, their signatures have been associated with diagnosis, staging, progression, prognosis and response to treatment. Since therapeutic strategies based on the modulation of miRNA activity have not met the needed requirements, we are in the urgent need of exploiting new treatment options. We recently embarked on the development of small molecules that target oncogenic miRNAs (rather than proteins) for cancer therapy. We named these agents SMIRs (small molecule inhibitors targeting miRNAs), and are focusing on using these small molecules to target overexpressed miRNAs as a new therapeutic approach. By performing high-throughput screening cellular-based assays, we are aiming to achieve a transformative concept that can be extended to multiple oncogenic miRNAs as well as different cancer types, bringing these molecules to the clinical practice.

Oligonucleotide conjugates

Marc M Lemaitre, marc.m.lemaitre@gmail.com, Md. Rowshon M Alam. Nitto Avecia Inc., Cincinnati, OH 45215, United States

Delivery of oligonucleotides as well as accessibility to the "site of action" has been a problem that researchers have been addressing for more than 25 years. Many different approaches have been proposed and tested. One of the most interesting consists in conjugating oligonucleotides to small molecules to either use the ability of the adduct to bind specific receptors or to improve the inclusion of oligonucleotides in delivery particles.
In the talk we provide an overview of recent developments regarding conjugates with peptides, carbohydrates and small molecules as ligands. We will also discuss our own experience with this approach from research-appropriate small scale to clinical large scale synthesis.

**CARB 53**

**Short interfering RNA guide strand modifiers from computational screening**

*Peter Beal*, pabeal@ucdavis.edu. Chemistry, University of California, Davis, Davis, CA 95616, United States

Short interfering RNAs (siRNAs) are promising drug candidates for a wide range of targets including those previously considered “undruggable”. However, properties associated with the native RNA structure limit drug development and chemical modifications are necessary. Here we describe the structure-guided discovery of functional modifications for the guide strand 5' end using computational screening with the high resolution structure of human Ago2, the key nuclease on the RNA interference pathway. Our results indicate the guide strand 5'-end nucleotide need not engage in Watson-Crick (W/C) H-bonding but must fit the general shape of the 5'-end binding site in MID/PIWI domains of hAgo2 for efficient knockdown. 1,2,3-Triazol-4-yl bases formed from the CuAAC reaction of azides and 1-ethynylribose, which is readily incorporated into RNA via the phosphoramidite, perform well at the guide strand 5'-end. In contrast, purine derivatives with modified Hoogsteen faces or N2 substituents are poor choices for 5'-end modifications. Finally, we identified a 1,2,3-triazol-4-yl base incapable of W/C H-bonding that performs well at guide strand position 12, where base pairing to target was expected to be important. This work expands the repertoire of functional nucleotide analogs for siRNAs.

**CARB 54**

**Synthesis and properties of amide-modified RNA for applications in RNA interference**

*Eriks Rozners*, erozners@binghamton.edu, Chelliah Selvam, Paul Tanui, Scott D Kennedy, Daniel Mutisya, Benjamin D Lunstad, Pradeep Pallan, Amanda Haas, Devin Leake, Martin Egli. (1) Department of Chemistry, Binghamton University, Binghamton, NY 13904, United States (2) Department of Biochemistry and Biophysics, University of Rochester School of Medicine, Rochester, NY 14642, United States (3) Global Research and Development in Molecular Biology, Thermo Fisher Scientific Bioscience Division, Lafayette, CO 80026, United States (4) Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, TN 37232, United States

Discovery of RNA interference has reinvigorated interest in chemical modifications of RNA for in vivo applications. Our current work is focused on the development of novel nonionic analogues of RNA that have the phosphodiesters replaced by amide linkages.
We hypothesize that the reduced negative charge and the hydrophobic nature of such modifications will not only increase the enzymatic stability but also have the potential to optimize potency, cellular uptake, and pharmacokinetics of small interfering RNAs. A further advantage of amide modifications in RNA is that they can be prepared using relatively straightforward peptide type couplings. This presentation will discuss synthesis and biophysical properties of amide-modified RNA analogues. The results obtained using osmotic stress, NMR spectroscopy and x-ray crystallography suggest interesting insights into how the formally hydrophobic modifications interact with hydration of RNA (1). Preliminary results on biological activity show that amides are well tolerated in both strands of modified siRNAs. The implications of these studies for practical applications of modified RNA analogues in RNA interference will be discussed.


CARB 55

Effect of co-administration of a nonsense oligonucleotide on the potency of cEt BNA containing Gapmer antisense oligonucleotide in mouse liver

Thazha P Prakash¹, tprakash@isisph.com, Ed Wancewicz³, Alfred E Chappell², Hans Gaus², Eric E. Swayze¹, (1) Medicinal Chemistry, Isis Pharmaceuticals, Carlsbad, California 92010, United States  (2) Structural Biology, Isis Pharmaceuticals, Carlsbad, California 92010, United States  (3) Neuro Drug Discovery, Isis Pharmaceuticals, Carlsbad, California 92010, United States

Abstract:

Approaches that improve upon the pharmacokinetic and pharmacodynamic properties of single-strand antisense oligonucleotides are an active research topic. Antisense oligonucleotide phosphorothioates (ASOs) are highly protein bound. Therefore, it is likely that they traffic into and through cells via a protein to protein shuttling mechanism driven by binding affinity gradients.¹ The ASO binding events are likely required for transport and uptake into cells, and it should be a saturable event. Nonlinear and saturable uptake has been reported in numerous in vivo pharmacokinetic studies in the literature.¹ Additionally, it is clear from the literature reviewed that there are multiple ASO uptake mechanisms. It has been reported that co-administration of nonsense oligonucleotide improved potency of a 2′-O-(2-methoxyethyl) Gapmer ASO.¹ We investigated the effect of co-administration of nonsense oligonucleotide on the activity of cEt BNA ASO in mouse and rat. Co-administration of nonsense oligonucleotide significantly improved the potency of cEt BNA ASO in both mouse and rat. The tissue level of active drug was much lower while pharmacology was improved. This data suggests that a nonsense oligonucleotide can compete off the active ASO from the non-productive compartment and improve active ASO uptake into the productive compartment. Onset and duration of effect of active drug with and without nonsense oligonucleotide was similar.


CARB 56

Structural characterization of unknown di-phosphorylated bovine submaxillary mucin O-
We have examined bovine submaxillary mucin (bsm) oligosaccharides by HPAEC-PAD, and MS. We have also done MS/MS of m/z 219 anion. We use a novel method for isolating O-linked oligosaccharide derivatives from bsm glycoprotein. This method is compatible for isolating derivatives of phosphoryl, sulfuryl and sialyl substituted oligosaccharides.

- We have discovered that there are two components, one major and one minor, of these oligosaccharides, by chromatography, when isolated in the manner above.
- The major component comes from either di-sulfated or di-phosphorylated analogue. By a novel method for discerning phosphate vs. sulfate substituted carbohydrate esters we have found that this molecule is di-phosphorylated.

We make a case for the phosphorylation sites as O-1 and O-3 or O-4, of hexosamine, leaving the O-6 position for substitution by sialic acid.

- We characterize the major product as 1, 4-di-mono-dehydrido-phosphoryl-6-N-acetyl neuraminyl N-acetyl hexosamine.
- This is the first report of the isolation of BSM O-linked oligosaccharide in the unreduced hex NAc form as this derivative. It is also the first report of di-phosphorylated BSM O-linked oligosaccharide. Except for 1-phosphorylated mucin O-linked oligosaccharides isolated by Warachuck et al., and report of 1-phosphorylated sialyl Tn antigen this is the first report of BSM O-linked oligosaccharide phosphorylated in the 1- position.

The isolated derivative is crucial to the determination of its structure.
Glycosidic linkage and the solution conformational entropy of gluco- and mannobioses

Andre M. Striegel\textsuperscript{1}, andre.striegel@nist.gov, Mallory J. Morris\textsuperscript{1,2}. (1) Chemical Sciences Division, National Institute of Standards & Technology (NIST), Gaithersburg, MD 20899, United States (2) Department of Chemistry, Florida State University, Tallahassee, FL 32306, United States

The flexibility differences imparted carbohydrates via their various glycosidic linkages directly impact inter- and intracellular recognition processes as well as protein-carbohydrate interactions. Size-exclusion chromatography (SEC), an entropically-controlled separation technique, serves here to determine the solution conformational entropy (-\(\Delta S\)) of (1\(\rightarrow\)2)-, (1\(\rightarrow\)3)-, (1\(\rightarrow\)4)-, and (1\(\rightarrow\)6)-linked gluco- and mannobioses with an \(\alpha\) anomeric configuration, at quasi-physiological conditions of solvent, temperature, and pH. The experiments allowed for comparison both among and between each series of disaccharides. Results included quantitative information on how the additional degrees of freedom of the (1\(\rightarrow\)6) linkage influence -\(\Delta S\), as well as on the influence on solution conformational entropy of a single axial hydroxyl (OH) group and of the relative positioning of the glycosidic linkage and the anomeric hydroxyl group. We also employed SEC to contrast the \(\alpha\)-(1\(\rightarrow\)4)-linked gluco- and mannobioses to their counterparts with a \(\beta\) anomeric configuration. Comparison between \(\beta\)-(1\(\rightarrow\)4)-linked glucobiose (cellobiose) and \(\beta\)-(1\(\rightarrow\)4)-linked mannobiose showed that the restrictive effect on solution flexibility of the axial OH in the latter disaccharide is offset by the combined effect of hydroxyl group orientation and anomeric configuration on intramolecular hydrogen bonding.

Sequence selective recognition of double-stranded RNA using nucleobase-modified peptide nucleic acids

Eriks Rozners, erozners@binghamton.edu, Thomas Zengeya, Ming Li, Pankaj Gupta. Department of Chemistry, Binghamton University, Binghamton, NY 13902, United States

The importance of non-coding RNAs in cell biology makes them attractive targets for molecular recognition. However, designing of small molecules that selectively recognize RNA has been a challenging and involved process because RNA helix presents little opportunity for shape-selective recognition. We discovered (1) that nucleobase-modified peptide nucleic acids (PNA), as short as six nucleobases, bind with low nanomolar affinity and high sequence selectively to double-stranded RNA via triple helix formation under physiological conditions.
Interestingly, little to no binding is observed to dsDNA of the same sequence, which suggests that the modified PNAs have unique selectivity for dsRNA. We also demonstrated that this binding could be used for highly specific recognition of biologically important double stranded RNA, such as ribosomal RNA and microRNAs. PNAs carrying M and Lys modifications were efficiently taken up by HEK-293 cells, while the unmodified PNA showed little uptake (2). This presentation will discuss our most recent results on sequence selective recognition of biologically relevant double-stranded RNAs using chemically modified PNA analogues under physiological conditions.


**CARB 59**

**Discovery of novel glycan-mediated binding partners for cholera toxin**

*Amberlyn Wands*, amberlyn.wands@UTSouthwestern.edu, Akiko Fujita, Janet McCombs, Andrea Rodriguez, Jennifer Kohler. Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390-9028, United States

Cholera is a severe disease that is epidemic and endemic in many parts of the world. The causative agent is the bacterium *Vibrio cholerae* and its secreted virulence factor cholera toxin (CT). Cholera toxin utilizes the thick glycan coating on the plasma membrane of the intestinal epithelium to invade host tissue, and its retrograde transport to the cytosol results in the massive loss of fluids and electrolytes associated with severe dehydration. It is widely accepted that the mono-sialoganglioside GM1 is cholera toxin's sole receptor to initiate this process. However, there have been increasing reports over the last 40 years suggesting that additional cell-surface binding partners may exist. As glycan-protein interactions are notoriously low affinity interactions, our lab has developed a photocrosslinking strategy utilizing a diazirine-containing sialic acid analog, SiaDAz, to covalently capture/detect complexes formed between cholera toxin subunit B (CTB) and its sialylated binding partners. With this method, we have shown via immunoblot that photocrosslinking of CTB to SiaDAz-producing intestinal epithelial cell lines results in the appearance of a new toxin-containing higher molecular weight species (∼200 kDa), consistent with formation of a covalent adduct between CTB and a glycoprotein binding partner. We have since utilized small-molecule inhibitors to interfere with various glycosyltransferases within the secretory pathway to gain knowledge about the class of glycoprotein that is mediating this interaction. Our current research focus is on the identification of this glycoprotein receptor by mass spectrometry, and deciphering its functional implications for toxin internalization and intoxication. These studies would enable the characterization of cholera toxin's distinct endocytic trafficking pathways and lead to the development of novel treatments for cholera infection.

**CARB 60**

**Physicochemical and sensory characteristics of novel sweetener turanose biosynthesized using amylosucrase from Neisseria polysaccharea**
Turanose (3-O-α-D-glucopyranosyl-D-fructose), a structural isomer of sucrose, is a low-calorigenic and non-cariogenic disaccharide. It has a potential to be a high-quality functional sweetener, but there has been no competitive way to produce turanose as well as no available information related to its physicochemical and sensory properties. Recently, an efficient way of turanose production was developed by applying a recombinant Neisseria amylosucrase. In this study, physicochemical and sensory properties of turanose were investigated and compared to those of sucrose tagatose, erythritol, and palatinose, to examine the feasibility of turanose as a novel sucrose substitute. Turanose remained stable at 30-90 °C under pHs 7 and 3 while sucrose was easily degraded at 90°C under acidic pH. Solubility (223.5 g) of turanose at 25°C was almost identical to sucrose (224.4 g) in 100 g water, while erythritol, palatinose, and tagatose were much less soluble. Aqueous solution of turanose drastically developed brown color in the presence of glycine when heated to 120°C on pH 5.5, but sucrose solution did not show any significant change in color. Turanose displayed similar viscosity and moisture sorption-desorption curve with sucrose as determined by rheometer and by dynamic vapor sorption, respectively, within the tested temperature and concentration ranges. Relative sweetness of turanose was 0.39 as determined by 54 screened panels using 2-alternative forced choice method when compared to 5% (w/v) sucrose solution. Turanose demonstrated no significant difference in sensory bitterness, artificial/chemical flavor, astringency, irritating sensation, viscosity, and sweetness-lasting compared to sucrose, whereas erythritol and stevioside displayed greater bitterness, astringency, irritating sensation, and sweetness-lasting.

CARB 61

Water/air-stable iodonium salt as a powerful thiophilic promoter

An-Hsiang Adam Chu, an-hsiang.chu@tufts.edu, Andrei Minciunescu, Clay S Bennett. Department of Chemistry, Tufts University, Medford, MA 02155, United States

The various different roles of carbohydrates in biology stem from its inherent structural complexity. Consequently, chemical glycosylation has been the subject of intense research for its application in constructing complex oligosaccharides with therapeutic potentials. Most typical glycosylation procedures, however, involve using highly toxic and extremely unstable reagents to generate reactive intermediates, thus creating a significant barrier for laboratories with limited synthetic settings to routinely execute. Here we report the use of a novel water/air stable iodonium reagent to effectively activate both armed and disarmed thioglycoside donors for subsequent glycosylation in excellent yields (up to 97 %) under room temperature. Reaction is robust, and reaction times range from 10 mins to 3 hrs. Synthesis of the iodonium reagent as well as the substrate scope will be discussed.

CARB 62

Chemoenzymatic synthesis of a high-mannose type N-glycan library for functional studies

Christian Toonstra, ctoonstra@ihv.umm.edu, Joseph V. Lomino, Lai-Xi Wang. Department of Biochemistry and Molecular Biology, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD 21201, United States
High-mannose (HM) type N-glycans, ranging from Man$_5$GlcNAc$_2$ to Man$_9$GlcNAc$_2$, are a major glycan component of HIV-1 envelope glycoprotein gp120. They play an active role in dendritic cell-mediated viral transmission by serving as the ligand for the dendritic cell-associated lectin (DC-SIGN). These high-mannose N-glycans are also targets for several broadly neutralizing antibodies including 2G12, PG9, PG16, and PGT128. Thus facile preparation of a library of structurally well-defined high-mannose N-glycans will facilitate functional evaluation and vaccine design. While pure chemical synthesis provides a way to make high-mannose N-glycans, it usually takes multiple tedious steps. We report here a top-down approach that consists of preparation of the full-size HM N-glycan, Man$_9$GlcNAc$_2$Asn, and its successive enzymatic degradation and chromatographic separation of the resulting distinct HM glycans. The starting material, Man$_9$GlcNAc$_2$Asn, was successfully obtained from soybean flour through isolation of crude soybean agglutinin (SBA) and thorough pronase digestion. It was then labeled at the free asparagine group with fluorenylmethyloxycarbonyl (Fmoc) group. Controlled digestion of the Fmoc-labeled Man$_9$GlcNAc$_2$Asn with an α-1, 2-mannosidase (cloned from *bacteroides thetaiotaomicron*) yielded a mixture of Man$_9$-Man$_5$GlcNAc$_2$Asn-Fmoc. The distinct HM-glycoforms were successfully separated by normal phase high-performance liquid chromatography (NP-HPLC) and characterized by MALDI-TOF MS and HPAEC-PAD (high performance anion-exchange chromatography with pulsed amperometric detection) methods. This approach provides a facile means to obtain distinct high-mannose type oligosaccharides and isomers with sufficient quantities for various applications, including the synthesis of HIV glycopeptide antigens for epitope characterization and the preparation of HM-glycan microarrays for functional glycomics studies.

CARB 63

Metabolic production of photocrosslinking O-GlcNAc: Method improvement and application

*Andrea C Rodriguez*, andrea.rodriguez@utsouthwestern.edu, Seok-Ho Yu, Bin Li, Jennifer J Kohler. Department of Biochemistry, UT Southwestern Medical Center, Dallas, Texas 75390, United States

O-linked N-acetyl-D-glucosamine (O-GlcNAc) is an abundant and highly dynamic single sugar post-translational modification found on hundreds of mammalian proteins. Two enzymes regulate O-GlcNAc-ylation: O-GlcNAc transferase (OGT) modifies proteins with O-GlcNAc at serine/threonine residues, while O-GlcNAcase (OGA) removes the modification. Altered O-GlcNAc-ylation is associated with human diseases, such as cancer and Alzheimer’s. While more than 1000 O-GlcNAc-modified proteins have been identified, much less is known about how O-GlcNAc-ylation alters protein function. To gain insight into the functional consequences of O-GlcNAc-ylation, the Kohler lab reported a method to metabolically incorporate the diazirine photocrosslinking group onto O-GlcNAc residues in cellular proteins. Photocrosslinking O-GlcNAc, which we call O-GlcNDAz, can be activated by UV irradiation, yielding covalent crosslinks between O-GlcNAcylated proteins and their binding partners. Further analysis of the crosslinked complexes can reveal protein-protein interactions that are promoted by O-GlcNAc-ylation. However, the metabolic labeling technology comes with limitations: OGT prefers to produce O-GlcNAc, rather than O-GlcNDAz, and OGA can’t remove the O-GlcNDAz modification. I will describe how we mutated OGT to improve O-GlcNDAz production and report on efforts to mutate OGA to allow O-GlcNDAz hydrolysis. In addition, I will describe the application of O-GlcNDAz crosslinking to discover binding partners of NUP98 fusion proteins produced due to chromosomal translocation in leukemia. NUP98 fusion proteins encode the phenylalanine-glycine (FG) repeat domain of nucleoporin NUP98 fused to homeodomain transcription factors or other proteins that interact with nucleic acids. FG domains of NUPs are natively unfolded and highly modified by O-GlcNAc. Cell-based O-GlcNDAz
crosslinking experiments demonstrate that NUP98 fusions proteins (NUP98-HOXA9 and NUP98-DDX10) each interact with discrete sets of proteins in cell lines derived from both leukemia and cervical carcinoma. Ongoing work includes efforts to identify the binding partners of NUP98 fusions and determine the role of O-GlcNAc modification of NUP98 fusions in leukemogenesis.

**CARB 64**

**Synthesis of homogeneous HIV-1 V3 glycopeptides for characterizing the glycan specificity of glycan-dependent HIV-neutralizing antibodies**

**Jared Orwenyo**, jorwenyo@ihv.umaryland.edu, **Mohammed Amin**, **Joseph V Lomino**, **Lai-Xi Wang**. Institute of Human Virology and Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland 21201, United States

The discovery of glycan-dependent broadly neutralizing antibodies (bnAbs) targeting regions of the envelope glycoprotein gp120 has driven the need to elucidate their epitope structures for vaccine development. Several bnAbs, including PGT128, PGT121, and 10-1074, have been reported to target glycan-dependent epitopes on the V3 region of gp120, but their glycan specificity remains to be fully characterized. We sought to clarify this by using the chemoenzymatic approach to synthesize homogeneous glycopeptides modeled on the V3 region of gp120 and with glycosylation at the conserved Asn332 residue, which was shown essential for antibody recognition. We utilized solid phase peptide synthesis (SPPS) to synthesize the GlcNAc-peptide precursor in which a GlcNAc-Asn was inserted at the Asn-332 site. Then various high-mannose and complex type N-glycans were attached at the pre-determined site via chemoenzymatic glycosylation using glycosynthase as the enzyme and glycan oxazoline as the donor substrate. Our SPR binding studies indicated that the PGT121 antibody recognized the V3 peptide epitope with a complex-type glycan at the Asn332 site while the PGT128 and antibody 10-1074 could efficient bind to the synthetic V3 glycopeptides having a high mannose Man$_9$ glycan at the Asn332 site. Interestingly, free N-glycans or non-glycosylated V3 peptides showed no or only marginal binding to those antibodies under the same conditions, showing the requirement of both the appropriate glycan and the peptide portion for high-affinity interactions. Further studies are ongoing to probe the effect of other glycosylation sites in the V3 domain on the recognition by these HIV-neutralizing antibodies.

**CARB 65**

**Synthesis of high molecular weight sugar poly(ortho esters) through DMAP-promoted polycondensation**

**Lingyao Li**, li4l@cmich.edu. Department of Chemistry, Central Michigan University, Mount Pleasant, Michigan 48858, United States

A 4-(dimethylamino)pyridine (DMAP)-promoted polycondensation was developed as an efficient polymerization method to synthesize high molecular weight poly(ortho esters). A di-functional AB-monomer, 2,3,4-tri-O-acetyl-a-D-glucopyranosyl bromide was synthesized and subjected to DMAP-promoted polycondensation, which produced poly(ortho esters) with molecular weights up to 8.0 kDa. The NMR analyses indicate that the polymerization is highly chemoselective with exclusive formation of orthoester linkages between each sugar units. The chain-end functionalization was achieved using alkynyl groups. Finally, poly(ethylene glycol) (PEG) was added to yield di-block polymer through Click chemistry.

**CARB 66**
In(III)-catalyzed amino glycosidation of 1,2-anhydrosugar with 3-amino azetidinone: A novel method for optical resolution

Ram N. Yadav, Armando Paniagua, apaniagua@broncs.utpa.edu, Sunena Chandra, Bimal K. Banik. Department of Chemistry, The University of Texas-Pan American, Edinburg, TX 78539, United States

The β-Lactam is the central core unit of various antibiotics and is also an important precursor of various biological systems. The synthesis of the optically active stereodefined β-lactam is one of the major challenges to the organic chemists. Herein we present the highly stereoselective In(III)-induced facile ring opening of the 1,2-anhydrosugar with 3-amino azetidinone.

CARB 67

Structural characterization and cell proliferation effect on HepG2 of a novel acid polysaccharide from the viscera of Haliotis discus hannai

Guoyun Li1,2,3, liguoyun88@gmail.com, Yuming Wang1, Xiaofang Liu1, Fengjuan Wu1, Lingyun Li2,3, Changhu Xue1, Robert J Linhardt1,2,3. (1) College of food science and Engineering, Ocean university of China, Qingdao, Shandong 266003, China (2) Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, United States (3) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, New York 12180, United States

Abalone, Haliotis discus hannai Ino, is a large algivorous marine gastropod, and one of the most commercially important species of gastropods in aquaculture for Asia. During the processing of abalone, large amounts of viscera, consisting chiefly of gonad and digestive tract, are discharged as by-products. A novel sulfated polysaccharide (AVAP) was isolated and purified from the viscera Haliotis discus hannai, and the structure of the AVAP was elucidated by negative-ion electrospray tandem mass spectrometry and NMR. The main backbone units of the AVAP were →3)-β-D-GlcpA-(1→4)-α-L-Rhap-(3SO4)-(1→ and →3)-β-D-GlcpA-(1→4)- α-L-Rhap-(2SO4)-(1→ with a ratio of 2:1, besides α-L-Rhap and α-L-Rhap-(4SO4)-(1→3)- β-D-Galp were branched at the sulfated rhamnose of the two backbone structural units respectively. The proliferation activity on HepG2 cell was assessed, AVAP was found to promote HepG2 cell proliferation by regulating the genes expression and accelerating the cell cycle process. Our results offer a comprehensive method for utilizing the abalone viscera which is usually considered wastes.

CARB 68

Catalytic effects of indium salt on O- and S-glycosylation of bromo sugar: A one pot approach for the synthesis of a chiral acid

Ram N. Yadav, Sunena Chandra, schandra@broncs.utpa.edu, Armando Paniagua, Bimal K. Banik. Department of Chemistry, The University of Texas-Pan American, Edinburg, TX 78539, United States

Glycosylation is a very important reaction which involves the coupling between a glycosyl donor and an alcohol as a glycosyl acceptor to construct the alpha and beta glycosyl linkages at the anomeric position of the sugar unit. In connection with our study on the indium salt-induced O and S-glycosylation of acetobromo alpha-D-glucose, we describe herein a versatile method for
glycosylation of various benzyl ester alcohols. We have discovered unprecedented hydrolysis of a benzyl ester group during the course of the glycosylation reaction.

CARB 69

**Effect of O-substituents in sialylation reactions**

Scott Geringer, cdemeo@siue.edu, Seyed Aalaei, Cristina De Meo. Chemistry, Southern Illinois University Edwardsville, Edwardsville, Illinois 62025, United States

Sialic acid-containing glycoconjugates are natural products and, for their biological functions, very important synthetic targets. Efficient synthesis (yield and stereoselectivity) of the glycosidic linkages with sialic acid is still quite challenging. The study of O-protecting groups in the mainstream of carbohydrate chemistry occupies a very important niche and represents the major venue to control reactivities and stereoselectivities of glycosyl donors. However, O-substitution of sialic acids, beyond traditional acetates, and the effect that O-substituents may have on the outcome of sialylation remains practically unknown. Recently, we showed that sialic acids bearing TBDMS and benzoyl substituents at O-4 and/or O-7 positions possess very interesting and rather unpredictable sialyl donor properties. As part of a broader investigation on the effect of O-protecting groups in sialic acid chemistry, herein we present the expansion of these studies.

CARB 70

**Synthesis of 2,3-dehydro derivatives of neuraminic acid from the isotopically labeled precursors**

Clare Wallace, cdemeo@siue.edu, Rachael Starner, Cristina De Meo. Southern Illinois University Edwardsville, Edwardsville, Illinois 62025, United States

The chemical synthesis of sialic acid-containing glycoconjugates requires efficient methods for sialylation. It is well-known that sialylation is commonly accompanied by concomitant elimination reactions, yielding 2,3-dehydro derivatives (glycals) as undesired side products. On the other hand, glycals have found application in the developments of medicinally and biologically relevant compounds (e.g. neuraminidase inhibitors). Therefore, their targeted synthesis represents a valuable venue for research. Since a majority of studies are dedicated to suppressing the elimination reaction, little is known about the mechanism of their formation. As a part of a program centered on a better understanding of the mechanism of sialylations, and therefore elimination reactions in competition, herein we describe the synthesis of 2,3-dehydro derivatives from isotopically labeled thiophenyl sialyl donor.

CARB 71

**Purification and characterization of chemoenzymatically synthesized heparin oligosaccharides**

Chao Cai, caichao1076@gmail.com, Demitria Dickinson, Victor Schultz, Kathryn Linkens, Jian Liu, Robert J. Linhardt. (1) Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, United States (2) Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, United States
Ultralow molecular weight (ULMW) heparins are sulfated glycans that are clinically used to treat thrombotic disorders. Currently, we are focusing on the chemoenzymatic construction of heparin/HS oligosaccharides with defined sulfate groups. Diverse tags including reverse phase, fluorous and magnetic particles are designed to simplify the purification procedure, and the substrates synthesized during each enzymatic step are characterized through 1D, 2D NMR, LC-MS and final heparin/HS oligosaccharides are investigated on their biological activity. O-Sulfotransferases, N-sulfotransferase and C5-epimerase are used to partially or completely modify these oligosaccharides to afford heparin/HS libraries.

CARB 72

New ester protecting group and its application in oligosaccharide synthesis

Feng Cai, feng.cai@live.com, Zhongwu Guo. National Glycoengineering Research Center, Shandong University, Jinan, Shandong 250013, China

Neighboring group participation of a 2-O-acyl functionality, beyond all doubt, is the most powerful protocol with which to achieve high stereoselectivity in glycosylation. A new ester protecting group is developed for the purpose gave excellent diastereoselectivity and high yields in coupling reactions. This ester group can be cleaved globally with other benzyl type protecting groups by hydrogenolysis.

CARB 73

Effects of sialic acid biosynthesis on the hexosamine biosynthetic pathway and N-linked glycans

Nam D Pham, nam.pham@utsouthwestern.edu, Jennifer J Kohler. Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390, United States

Sialic acid is an essential biomolecule and critical for mammalian life – homozygous inactivation of the enzyme that catalyzes the first committed step of sialic acid biosynthesis results in early embryonic lethality in mice. By serving as the terminal sugar on many glycan chains, sialic acid regulates diverse cellular processes and disruptions in sialic acid metabolism lead to a variety of human diseases ranging from muscle conditions to neurologic disease to autoimmune disorders. Additionally, cancer cells are known to display unusual sialylated epitopes that are thought to aid in immune system evasion and cancer cell survival. Given the sensitivity of human health to sialic acid content, I hypothesize that sialic acid levels are tightly regulated and alterations in sialic acid metabolism lead to changes in signaling and metabolism.

I designed experiments to reveal how changes in sialic acid production affect cellular homeostasis.
Microarray analysis and metabolomics studies suggest a defect with sialic acid biosynthesis causes only minimal changes in global gene expression and metabolism. However, a deficiency in sialic acid production does perturb the hexosamine biosynthetic pathway. Sialic acid is produced from UDP-GlcNAc, and cells that make sialic acid appear to have reduced levels of UDP-GlcNAc. UDP-GlcNAc serves as the precursor to both the critical O-GlcNAc modification and to N-linked glycan branching. Immunoblot analysis suggests that global levels of O-GlcNAc are unaffected by sialic acid production, but lectin binding experiments demonstrate that reduced sialic acid production alters N-linked glycan branching. Furthermore, the change in N-glycan branching leads to altered galectin-1 binding. Thus, sialic acid synthesis alters the N-linked glycome of membrane proteins and consequently affects interactions that occur with N-linked glycans.

CARB 74

Fast determination of lactose and lactulose in dairy products using a 4 µm particle column and high-performance anion-exchange chromatography with pulsed amperometric detection

Carl A Fisher, carl.fisher@thermoscientific.com, Terri Christison, Monika Verma, Hua Yang, Linda Lopez. Ion Chromatography and Sample Preparation, Thermo Fisher Scientific, Sunnyvale, CA 94085, United States

The primary carbohydrate found in milk is lactose. Dairy products that are lactose-free cater to those individuals who have a lactase deficiency, which can lead to considerable digestive discomfort if un-modified dairy products are consumed. Dairy products labeled “Lactose-free” should not have any lactose present, while “lactose-reduced” should have significantly reduced levels. Lactulose is formed as a result of heating milk to pasteurize or sterilize it, delaying the onset of spoilage. Determining the levels of lactulose in milk and dairy products can be used to verify the extent of heating during milk processing. The method presented here has the advantage of high-efficient, fast separation by high-performance anion-exchange chromatography with baseline resolution and elution of lactose and lactulose from a variety of dairy products within six minutes. A high-pressure ion chromatography system with a high-efficiency 4 µm particle CarboPac SA10-4µm column was used to achieve direct and sensitive quantification with pulsed amperometric detection (PAD).

CARB 75

Synthesis of cyclic compounds from D-glucal using ionic liquids

Sumiea ElTayeb, sumiea.eltayeb@student.shu.edu, Cecilia H Marzabadi. Department of Chemistry & Biochemistry, Seton Hall University, South Orange, New Jersey 07079, United States

Reactions of carbohydrates in organic solvents typically require protection of the hydroxyl groups as non-polar-moieties to enhance their solubility. Later, a subsequent deprotection step must be carried out. We are interested in the cycloaddition reactions of glycals.
We report our results of acid catalyzed reactions of unprotected D-glucal in ionic liquids in the presence of different dienes. These reactions lead to various cyclic sugar adducts in modest yields as well as, furan diol as a by-product.

CARB 76
WITHDRAWN

CARB 77
Synthesis of the disaccharide αGal-1,3-αGal and its conjugation to a carrier protein

Matthew S Anderson, mand340@mail.midland.edu, Katja Michael, Nathaniel S Schocker. Department of Chemistry, University of Texas at El Paso, El Paso, Texas 79902, United States

The surface of the protozoan parasite Trypanosoma cruzi (T. cruzi), the causative agent of Chagas disease, is heavily coated by glycoproteins containing highly immunogenic O-glycans. My research group has previously synthesized eleven T. cruzi related saccharides with either non-reducing terminal α-galactosyl or α-rhamnosyl moieties related to these O-glycans and conjugated them to bovine serum albumin (BSA) for the generation of a glycoarray in a microplate format. The recognition of the saccharides by antibodies was studied by chemiluminescent enzyme-linked immunosorbent assay (CL-ELISA) using pooled sera from patients with chronic Chagas disease. The study revealed the disaccharide Galα(1,3)Galβ as the immunodominant glycotope, however, the study had not included the disaccharide Galα(1,3)Galα, and therefore the role played by the anomeric configuration of the reducing end sugar is not known. Here we present the synthesis of the disaccharide Galα(1,3)Galα and its conjugation to BSA for CL-ELISA studies. This disaccharide has been synthesized using known protecting group strategies, and was fully characterized using NMR spectroscopy and mass spectrometry. The disaccharide was then covalently linked to an Fmoc-protected amino acid for incorporation into a peptide, which will later be studied as a potential B-cell and T-cell epitope. This work has implications for the development of a fully synthetic carbohydrate-based vaccine, and diagnostic and chemotherapy follow-up biomarkers for Chagas disease.

CARB 78
Extraction of carbohydrates from hydrolysis reaction solutions

Taylor H Goodie, taylor.goodie@gmail.com, William M Reichert. Chemistry, University of South Alabama, Mobile, AL 36688, United States

Ionic liquids (ILs) provide the unique opportunity to provide a solvent for the dissolution of biomass, a catalyst for the hydrolysis, and an extractant for the separation of the products. This study will investigate the extraction of carbohydrates from ionic liquid reaction solutions through the use of boronic acid functionalized ionic liquids such as 3-(8-boronoctyl)-1-methyl-1H-imidazol-3-ium bis(trifluoromethylsulfonyl)amide. This study will investigate cation structure on hydrophobicity and extraction properties of boronic acid functionalized ionic liquids. Test solutions will be made from ionic liquid/glucose solutions and reaction mixtures from the hydrolysis of cellulose in ionic liquids. The carbohydrate products will then be analyzed through DART Mass Spectroscopy (DART) and
Facile, selective one-pot synthesis of 4-O-benzylated glycals

Izabela Fokt, ifokt@mdanderson.org, Marta Krawczyk, Marcin Cybulski, Szymon Kosinski, Stanislaw Skora, Waldemar Priebe. Experimental Therapeutics, University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030, United States

The importance of glycals as useful organic substrates and intermediates in carbohydrate chemistry is well recognized and they are extensively used as glycosylating agents, especially in the synthesis of 2-deoxy sugars and oligosaccharides and as precursors of C-glycosyl compounds. They are also widely recognized as versatile chiral building blocks in multistep organic syntheses. These applications often require availability of selectively protected glycals. Benzyl ethers are widely used as a protective group in sugar chemistry because of their stability in both acidic and basic conditions and the ability to remove them in neutral conditions simply by catalytic hydrogenation using Pd/C, even in the presence of many other protective groups. Up to this point, various methods have been used to obtain selective benzylated carbohydrates—all of them multistep processes. We propose a novel approach that selectively and efficiently produces 4-substituted derivatives directly from peracetylated glycals. The usefulness of this easily scalable, high-yield, one-pot synthetic process was demonstrated by the synthesis of 4-O-benzylated glycals directly from per-O-acetylated-D-glucal, -D-galactal, -L-fucal and -L-rhamnal. We will discuss the synthetic procedures and mechanistic origins of the observed selectivity.

Synthesis and study of covalent inhibitors of Mycobacterium tuberculosis GlgE

Sandeep Thanna, sandeep.thanna@gmail.com, Vishwanath Gaitonde, Jared J. Lindenberger, Donald R. Ronning, Steven J. Sucheck. Department of Chemistry, The University of Toledo, Toledo, Ohio 43606, United States

Tuberculosis is a contagious disease caused by Mycobacterium tuberculosis (Mtb). Maltosyltransferase GlgE has been identified as an essential enzyme in mycobacterium tuberculosis, contributing to the synthesis of a cytoplasmic α-glucan. GlgE is responsible for transferring a maltosyl units to an elongating α-1,4-glucan chain. Inhibiting GlgE results in accumulation of maltose-1-phosphate (M1P) triggering a lethal cell response anticipated to kill the Mtb cell as a result of self-poisoning. The compounds 2,2-dideoxy-2,2-difluoro-α-maltosylfluoride; 2,2-dideoxy-2,2-difluoro-β-maltosylfluoride and deoxy-5-fluoro-α-L-iodofluoride have been synthesized as mechanism based inhibitors for GlgE, in order to characterize the enzyme mechanism and inform further design of GlgE inhibitors. Synthesis of 2,2-dideoxy-2,2-difluoro-α-maltosylfluoride and 2,2-dideoxy-2,2-difluoro-β-maltosylfluoride involves addition of a fluorine
electrophile on the glycal of maltose followed conversion to a fluoro-glycal. The later was used in a second electrophilic fluorination using Selectfluor®. The α and β compounds were characterized by $2\text{J}_{\text{H1-F\alpha}}$, $3\text{J}_{\text{H1-F\alpha}}$ coupling constants (50.98, 2.93 Hz for α and 50.25, 12.84 Hz for β). Deoxy-5-fluoro-α-L-iodofluoride was synthesized utilizing a free radical bromination at C-5 position on an α-glycosylfluoride followed by $\text{SN}_2$ substitution using silver fluoride. These fluoro compounds were deprotected by purging them NH$_3$ gas in methanol. Studies for identifying the nucleophile in the enzymatic reaction using mass spectral analysis instrument are in progress. The inhibitory activity studies for these substances will be evaluated using a malachite green assay that measures the free inorganic phosphate produced during GlgE-catalyzed α-glucan biosynthesis.

**CARB 81**

**Structural effect of phosphorous-nitrogen containing flame retardant derivatives on thermal behaviors of treated cotton**

SeChin Chang, sechin.chang@ars.usda.gov, Thach-Mien Nguyen, Brian Condon. United States Department of Agriculture, Southern Regional Research Center, New Orleans, LA 70124, United States

The present research is aimed at studying the structural effect of two phosphoramidate derivatives such as *Diethyl 3-hydroxypropylphosphoramidate* and *Dimethyl 3-hydroxypropylphosphoramidate* as flame retardants (FRs) for cotton. These FRs were obtained in very high yield and purity by one step procedures. Cotton twill fabrics treated with the two compounds at different add-ons (5 - 20 wt%) were characterized. Vertical flammability, limiting oxygen index (LOI), thermogravimetric (TGA), and micro-scale combustion calorimeter (MCC) analyses were performed. A study of the functional groups which appeared on the treated fabrics by attenuated total reflection infrared (ATR-IR) spectroscopy revealed different binding mechanisms between each compound and cotton cellulose. Analysis of the released gas products by thermogravimetric analysis-fourier transform infrared (TGA-FTIR) spectroscopy showed some distinctive details in the degradation of the treated fabrics during the burning process.

**CARB 82**

**Chemo-enzymatic synthesis of inner core oligosaccharides of *E. coli***

Peng George Wang, pwang11@gsu.edu. Department of Chemistry, George State University, Atlanta, GA 30303, United States

Lipopolysaccharides (LPS) are major virulence determinant in Gram-negative bacteria, which are responsible for many pathophysiological responses to bacterial infections and can elicit strong immune responses in animals. LPS typically consist of three main parts: an endotoxic lipid A component with a bisphosphorylated and acylated β-(1→6)-inter-linked glucosamine disaccharide backbone, a nonrepeating core oligosaccharide, and a distal polysaccharide named as O-antigen with O-antigenic component specific to each bacterial species. The research on LPS has attracted much interest for the development of vaccine candidates, diagnostic tools and therapeutics. Many lipid A and its analogs have been found as a potent non-toxic vaccine adjuvant. In order to better understand the role of LPS in host-pathogen interactions and elucidate the antigenic and immunogenic properties of LPS inner core region. Based on biosynthetic pathway of LPS, chemo-enzymatic synthesis of inner core oligosaccharides of *E. coli* was performed in a sequential enzymatic reactions using chemically synthetic lipid A as substrate. Synthesis of these well-defined
oligosaccharides can also provide various substructures for establishing structure-activity relationships for determining minimal epitope which can elicit protective immune response. Therefore, chemo-enzymatically constructed pure *E. coli* inner core-lipid A conjugates would represent as a novel set of wide-spectrum and stand-alone vaccine candidates.

CARB 83

On the mechanism of tumor cell killing by bleomycin

*Basab Roy*¹, *Chenhong Tang*¹, *Rupesh Nanjunda*², *Trevor C. Bozeman*¹, *W. David Wilson*², *Sidney M. Hecht*¹, sid.hecht@asu.edu.  (1) Center for BioEnergetics, Biodesign Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287, United States  (2) Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, United States

The bleomycins are a family of antitumor antibiotics used clinically for the treatment of tumors of soft tissues, including squamous cell carcinomas and malignant lymphomas. Bleomycin has been shown to mediate the oxidative cleavage of DNA; it seems reasonable to think that this cleavage contributes to the killing of tumor cells by bleomycin. However, the nature of the lethal event has not been described. Because the clinical dose of bleomycin is quite low, chromosomal DNA must be in excess relative to BLM in a clinical setting, implying that bleomycin may bind preferentially to that subset of chromosomal DNA which exhibits the best affinity for the drug. To model this situation, we have selected a library of hairpin DNAs which bind avidly to bleomycin. These DNAs exhibit unusual patterns of cleavage by Fe-bleomycin. We have also studied the dynamics of interaction of representative strongly bound DNAs with Fe-bleomycins and defined the affinity constants, as well as off- and on-rates under a variety of conditions. For those DNAs studied, we observed a single strong bleomycin binding site, along with one or more weaker binding sites. In contrast, each of the DNAs was cleaved at multiple sites, more than one of which was cleaved efficiently. Further study of the nature of the cleavage of these DNAs has provided insights into the events that may lead to tumor cell killing.

CARB 84

RNA targeting antibiotics

*Yitzhak Tor*, ytor@ucsd.edu. Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, United States

The emergence of virulent, drug-resistant bacterial strains coupled to a minimal output of new pharmaceutical agents to combat them makes this a critical time for antibacterial research. Interestingly, a large fraction of clinically useful antibiotics target the bacterial ribosome, including the aminoglycosides and the macrolides. Aminoglycosides are a well-studied, highly potent class of naturally occurring antibiotics with scaffolds amenable to modification, thus providing an excellent starting point for the development of semi-synthetic, next-generation agents. The lecture will describe our efforts to explore the potential of this approach and our insight into mechanisms of action, as well as the development of fluorescence-based assays for the discovery of new ribosome-targeting antibiotics. Additionally, although believed to operate via totally distinct mechanisms, the lecture will also highlight the similarity between aminoglycosides and polymyxins, another family of highly charged and potent antibiotics. The lecture will demonstrate that the latter can also bind to A-site RNA constructs, and interfere with eukaryotic translation in vitro but not with bacterial translation.
CARB 85

DNA G-quadruplexes as potential anticancer drug targets

Danzhou Yang, yang@pharmacy.arizona.edu. Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, United States
Dept of Chemistry, University of Arizona, Tucson, AZ 85721, United States
BIO5 Institute, University of Arizona, Tucson, AZ 85721, United States
Arizona Cancer Center, Tucson, AZ 85721, United States

G-quadruplex DNA secondary structures, formed in specific G-rich sequences, have recently emerged as a new class of molecular targets for cancer therapeutics. The DNA G-quadruplex secondary structures have been demonstrated as potential regulatory elements in regions of biological significance, such as in the promoters regions of a number of oncogenes and in human telomeres. Significantly, DNA quadruplexes can readily form in solution under physiological conditions. In my presentation, I will discuss our recent progress on structural studies of the biological relevant G-quadruplexes, as well as progress on G-quadruplex-targeted small molecules.

CARB 86

Small molecule nucleic acid hybrids (SMNH) as antiviral agent

Rajendra K Pandey, rpandey@springbankpharm.com, Radhakrishnan P Iyer. Spring Bank Pharmaceuticals, Milford, Massachusetts 01757, United States

Small Molecule Nucleic acid Hybrids (SMNH) as Antiviral Agents

Rajendra K Pandey and Radhakrishnan P Iyer

Spring Bank Pharmaceuticals, 113 Cedar St. Milford, 01757 MA, United States

Abstract:

Oligonucleotides and analogs are in great demand for their use in aptamer, ribozyme, antisense and siRNA-based diagnostic and therapeutic applications. Small molecule nucleic acid hybrids (SMNH), which are short chain oligonucleotide units can be rationally designed to target nucleic acid-protein interactions. A series of SMNH compounds were synthesized on polystyrene-based beads using a focused combinatorial approach and screened against fluorescently labeled proteins. Special emphasis on the immobilization of SMNH compounds on support materials and interaction studies of support-bound SMNH compounds with target proteins will be described. This screening strategy provides a novel approach to high-throughput screening and lead optimization of SMNH compounds against a target protein.

CARB 87

Strand exchange reactions at the frontier: Modular reaction networks

Andrew D Ellington, EllingtonAdmin@gmail.com. Department of Molecular BioSciences, University of Texas at Austin, Austin, Texas 78712, United States

There has been a quiet revolution in the ability to carry out programmed reactions based on strand exchange. A variety of non-enzymatic DNA circuits have now been built that can carry out complex computations, including taking square roots and acting as neural networks. We have explored a
reaction class known as catalytic hairpin assembly (CHA), and shown that it can be used for amplification and signal transduction in a variety of analytical applications. In particular, it has proven to be extremely useful as a means of monitoring enzyme-based isothermal amplification reactions. By rationally modifying the basic design and chemistry of the CHA reaction, we have been able to increase signal, suppress noise, and modulate interactions with a variety of amplifiers and amplicons at a variety of temperatures.

CARB 88

Expansion of the genetic alphabet

**Floyd Romesberg**, floyd@scripps.edu. Department of Chemistry, The Scripps Research Institute, La Jolla, California 92037, United States

Expansion of the genetic alphabet to include a third base pair would be a fundamental accomplishment that would not only have immediate utility for a number of biotechnology applications, such as site-specific labeling of DNA and RNA, but would also lay the foundation for a semi-synthetic organism with increased potential for information storage and retrieval. We have developed a class of unnatural base pairs, d5SICS-dNaM and dTPT3-dNaM that form based on packing and hydrophobic interactions rather than complementary H-bonding, but are nonetheless replicated and transcribed with efficiencies and fidelities approaching those of a natural base pair. Structural studies, as well as several paractical applications of the unnatural base pairs will be discussed, as will our recent progress towards replicating DNA containing an unnatural base pair within a living cell.

CARB 89

Selenium nucleic acid chemistry and biology

**Zhen Huang**, huang@gsu.edu, Wen Zhang, Huiyan Sun, Jia Sheng, Jozef Salon. Department of Chemistry, Georgia State University, Atlanta, GA 30303, United States

The elucidation of functions and crystal structures of nucleic acids by atom-specific modification and X-ray crystallography contributes to the understanding of molecular mechanisms of DNAs, RNAs and their protein complexes. 3D structure studies of nucleic acids and their protein complexes provide novel insights into them. Crystallography is a powerful tool for structure determination of DNAs, RNAs and their protein complexes with high resolution. However, crystallization and phase determination, two major bottle-neck problems, have largely slowed down structural determination of DNAs, RNAs and their ligand complexes. Our laboratory has pioneered and developed atom-specific substitution of nucleic acid oxygen with selenium (Ref. 1-3) that can be used as an atomic probe for structure and function studies of nucleic acids. As oxygen and selenium are in the same elemental family, the atom-specific substitution by replacing nucleotide oxygen with selenium or tellurium has revealed novel chemistry, structure, function and mechanism of nucleic acids and their protein complexes. Our selenium-nucleic acid (SeNA) strategy has demonstrated great potentials as a general methodology for structure and function studies of nucleic acids as well as their protein complexes. Moreover, we find that the Se-derivatized nucleic acids have virtually identical structures to the corresponding natives. Furthermore, we found that the Se-derivatization can facilitate crystallization, phase determination, and high-resolution structure determination. Our Se derivatization strategy via the atom-specific substitution will significantly facilitate functional and structural studies of nucleic acids as well as their protein complexes. Excitingly, we have recently determined the first RNA/DNA-protein complex via the
Invaders: Recognition of double-stranded DNA using oligonucleotide duplexes with interstrand zippers of intercalator-functionalized nucleotides

Sanne Andersen, Brooke A Anderson, Benjamin Denn, Dale C Guenther, Saswata Karmakar, Rie L Rathje, Sujay P Sau, Patrick J Hrdlicka, hrdlicka@uidaho.edu. Department of Chemistry, University of Idaho, Moscow, ID 83844-2343, United States

Development of compounds that recognize double-stranded DNA (dsDNA) in a sequence-specific manner is a research area, which is motivated by the prospect for molecular tools that can detect, regulate and modify genes. Significant advances have been made with triplex-forming oligonucleotides, peptide nucleic acids (PNAs), polyamides, pseudo-complementary PNA, gamma PNA and engineered proteins, among other approaches. However, given the restrictions of the current approaches, development of alternative strategies for specific mixed-sequence recognition of dsDNA at physiological conditions remains a highly desirable goal.

Our laboratory is exploring Invaders, i.e., double-stranded oligonucleotide probes that are activated for dsDNA-recognition through modification with interstrand zipper arrangements of intercalator-functionalized monomers based on 2’-amino-alpha-L-LNA, 2’-N-methyl-2’-amino-DNA or RNA scaffolds. We have recently demonstrated that the stability difference between Invader probes and probe-target complexes can be used to drive mixed-sequence recognition of linear dsDNA targets, ii) DNA hairpins and iii) chromosomal DNA.1-3

In this presentation, I will outline the Invader concept, discuss structure-property relationships and disclose results from biological dsDNA-recognition experiments. The results suggest that previously inaccessible DNA targets may become available to exogenous control, which has exciting implications for karyotyping, in vivo imaging, and gene regulation.

References

Glycoside-based inhibitors of *Mycobacterium tuberculosis* GlgE

Sri Kumar Veleti, Jared J Lindenburger, Donald R Ronning, **Steven J Sucheck**, steve.sucheck@utoledo.edu. Department of Chemistry, The University of Toledo, Toledo, OH 43606, United States

*M. tuberculosis* (*Mt*) GlgE is a recently identified (1→4)-α-D-glucan:phosphate-α-D-maltosyltransferase involved in α-glucan biosynthesis in bacteria and a genetically validated anti-tuberculosis drug target. The enzyme uses maltose-1-phosphate (M1P) as a substrate and exhibits an α-retaining catalytic mechanism during glucan polymerization. We have embarked on studies to identify substrate and mechanism-based inhibitors targeting the enzyme. We have synthesized the following putative GlgE inhibitors: 2,2-dideoxy-2,2-difluoro-α-maltosyl fluoride, 2,2-dideoxy-2,2-difluoro-α-maltosyl fluoride, and a 5-deoxy-5-fluoro-β-L-idosyl fluoride. In addition to these inhibitors, we have prepared a novel maltosyl-C1-phosphonate which is isosteric with M1P the natural substrate for GlgE. The C-glycoside was accessed via a Wittig olefination as a key step starting from maltose. To evaluate the inhibitors we have synthesized M1P the substrate for GlgE, through an efficient sequence of chemical transformations. We are currently in the process of evaluating the inhibitory activity of these materials against *Mt* GlgE.

**CARB 92**

Synthesis and biological evaluation of the tumor associative alpha-aminooxy disaccharide of the TF antigen conjugated to a polysaccharide immune stimulant

**Peter R Andreana**, peter.andreana@utoledo.edu. Chemistry, The University of Toledo, Toledo, Ohio 43606-3390, United States

Vaccines are powerful tools for disease prevention and therapy. Aberrant carbohydrates on cancer cell surfaces are important targets for the possible development of effective cancer vaccines. However, as single entities, void of lipids/peptides, carbohydrates have long been known to only invoke the B-cell mediated humoral arm of the immune system in a T-cell-independent manner, eliciting low-affinity IgM antibodies (Abs). For effective immunity, a strong and long-term response MUST be generated through both cellular and humoral arms of the immune system, involving class II major histocompatibility complex (MHCII) proteins, CD4+ T-cells and B-cells in a T-cell-dependent cascade. In recent years, a new class of bacterial polysaccharides, characterized by an alternating zwitterionic charge motif on adjacent monosaccharides, has been shown to stimulate T- and B-cell immune responses effectively. Motivated by this paradigm-shifting discovery and its potential application to the development of carbohydrate cancer vaccines, we hypothesize that chemically conjugating tumor-associated carbohydrate antigens (TACAs) to zwitterionic polysaccharides (ZPSs), such as PS A1, will lead to carbohydrate vaccines that can stimulate strong immunity.

This talk will therefore focus on a semi-synthetic vaccine construct, TF-PS A1, and will describe, in detail, its preparation. As our current understanding of the Thomsen-nouveaux (Tn)-PS A1 immunogen expands in scope, and applicability, a new construct consisting of the tumor associated carbohydrate antigen Thomsen-Friedenreich (TF) antigen conjugated to PS A1 draws to completion. The amino-oxy TF synthesis is assembled using a key Schmidt glycosylation strategy. Donor was prepared in three steps while the glycosyl acceptor was obtained in four. The key glycosylation was mediated by TMSOTf to give the disaccharide. The intermediate will then be converted into the hydroxylamine derivative, which after deprotection provides the amino-oxy TF antigen. With the TF antigen/hapten in hand, we propose to link this TACA to selectively oxidized PS A1 to obtain the vaccine candidate. Biological evaluation data will be revealed commencing with
murine immunizations followed by a series of in-vitro assays determining viability and function.

CARB 93

Development of neutralizable homogeneous biotinylated heparin as a novel anticoagulant

Peng George Wang, pwang11@gsu.edu. Department of Chemistry, George State University, Atlanta, GA 30303, United States

Current heparin-based treatment for thromboembolic disorder mainly relies on the heterogeneous unfractionated (UF) or low molecular weight (LMW) heparins that are either extracted from natural animal source or degraded forms of such extractions. UF and LMW heparins therapy faces the challenges in product quality control owing to the non-uniformity of the animal sources. In contrast, the synthetic homogenous ultralow molecular weight (ULMW) heparins bear multiple advantages over the aforementioned two forms in terms of pharmacokinetics (such as, longer circulation half-life and low IC50 value) and product quality control. One of the examples, among others, is the currently marketed Arixtra®, a densely sulfated pentasaccharide developed on the basis of natural antithrombin-binding activating domain of UF heparin. However, one prominent issue associated with Arixtra is that there is still no antidote currently available to efficiently neutralize their anticoagulant activity in the case of overdose or after surgical procedures.

Herein, we demonstrate a chemoenzymatic synthetic technique to prepare the biotinylated ULMW heparin (i.e. hexasaccharide) using chemically synthesized biotinylated glucuronic acid as initiating point of polymerization, followed by actions of a series of recombinant heparin sulfate biosynthetic enzymes. Such enzymatic approach, which is more environmental friendly and did not use organic solvents during the synthesis, dramatically shortens the overall synthetic steps (i.e. total 10 steps) as compared with the chemical synthesis (around 50 steps) that is currently used for preparation of Arixtra. The in vitro bioassay study has shown that final constructed biotinylated ULMW heparin has comparable anticoagulant activity to Arixtra, more significantly, it could be irreversibly neutralized by non-toxic avidin protein while Arixtra could not. Therefore, the biotinylated heparin we prepared could potentially substitute the ULMW heparin as a novel neutralizable anticoagulant agent.

CARB 94

Structure of an unusual oxidation product of cellulose

Alfred D French1, al.french@ars.usda.gov, Kurt Mereiter2, Thomas Rosenau3. (1) Southern Regional Research Center, U.S. Department of Agriculture, New Orleans, Louisiana 70124, United States (2) Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria (3) Department of Chemistry, University of Natural Resources and Life Sciences Vienna, Tulln, Austria

A dimer of 1,4-dimethyl 3-keto glucoside was unexpectedly found as the major product of this glucoside treated under conditions of Lyocell processing (molten NMMO monohydrate, 100°C). The molecule, essentially a 2:4′:2′:4′ dianhydride, is formed with the loss of two methanol molecules with concomitant linkage of the two ring structures. Translated to the cellulose case, taking the starting compound as a model for an oxidized anhydroglucose unit - this means chain cleavage and simultaneous crosslinking. And indeed, the same compound was isolated by methanolysis of oxidized and aged pulps in small amounts. Its crystal structure shows that the molecule comprises three six-membered rings and two eight-membered rings, all enveloped by a 10-membered ring.
One of the two rings from the original glucose rings has the $^1C_4$ conformation and the other has the normal $^4C_1$ shape. Short distances across the eight-membered rings between the carbonyl carbon atoms and the ring oxygens are identified as weak bonds by Atoms-in-Molecules electron density analysis. Similar bonds were found by Jack Dunitz in the 1970s. The dimer has subsequently been identified in cellulose. With an eye towards understanding the development of color in aging cellulotic materials, it is interesting that the central six-membered ring has a chemical structure similar to 2,5-dihydroxy-1,4-benzoquinone, a potent key chromophore.

CARB 95

Comprehensive comparison between woody and tunicate cellulosics

**Yadong Zhao**, yadong@kth.se, **Jiebing Li**, jbing@kth.se. Department of Fiber and Polymer Technology, Royal Institute of Technology, KTH, Stockholm, Stockholm 10044, Sweden

Cellulose is the most abundant natural and renewable polymer and an important raw material for energy e.g. bioethanol, chemicals after e.g. various derivatisations, and materials e.g. of different films or composites. Trees and tunicates are common sources for cellulose and the practical application technique will be largely dependent on the cellulose structure obtained. In this work, woody cellulose (WC) obtained from typical softwood (spruce) and hardwood (eucalyptus) and tunicate cellulose (TC), obtained from 5 different tunicate species, *Ciona intestinalis*, *Styela atlantica*, *Halocynthia roretzi*, *Styela plicata* and *Bothrylloides violaceous*, have been comprehensively compared by Size Exclusion Chromatography for molecular weight distribution, X-Ray Diffraction for crystallinity, Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) for enzymatic digestibility, Thermogravimetric Analysis and Differential Scanning Calorimetry for thermal properties, and Scanning Electronic Microscopy (SEM) for morphological structures. The comparison indicated that generally TC was of higher molecular weights than WC. The TC was composed of the almost-pure cellulose I$_\beta$ allomorph while the cellulose I$_\alpha$ was dominant in the WC. The higher degree of polymerization and crystallinity index were found in the TC which also made TC less accessible to enzymatic treatment than the WC. Reasonably, the TC with higher molecular weights showed better thermal stability than the WC though no very significant difference was found. Compared with relatively thin cellulose microfibril found in the WC, the longer and wider cellulose microfibril was characteristic for the TC which might make TC a more suitable candidate for composite reinforcement. From this study, it could therefore be concluded that different cellulosics have different physical and chemical properties depending on the sources and the TC and WC would have great potentials to be used in producing biomaterials, chemicals and fuels. There are different advantages and disadvantages of these two cellulosics, which will be decisive for different applications.

CARB 96

Generation and characterization of the pyranosyl and furanosyl oxocarbenium ions from 2-deoxyaldoses

**Geoffrey R Akien**, g942a423@ku.edu, Bala Subramaniam. Center for Environmentally Beneficial Catalysis, Lawrence, Kansas 66047, United States

Previously, we were able to generate and spectroscopically characterize the (tertiary) oxocarbenium ions from various protected ketofuranoses and ketopyranoses, in superacidic solutions at low temperature. For such complex species, it was perhaps surprising that they were
stable at all in superacidic media.

We have now extended this methodology to the even more reactive secondary oxocarbenium ions obtainable from various protected 2-deoxyaldoses. Through the measurement of J-couplings, this has allowed for the first determination of the conformation of these important intermediates in solution.

![Chemical structure](image)

CARB 97

Glycan microarrays prepared via a beam pen lithography induced thiol-acrylate photopolymerization

Adam B Braunschweig, a.braunschweig@miami.edu, Shudan Bian. Department of Chemistry, University of Miami, Coral Gables, FL 33146, United States

Multivalent glycan microarrays comprised of methacrylate brush polymers side-chain functionalized with α-glucose have been prepared by a Beam Pen Lithography (BPL) induced thiol-acrylate polymerization. Polymer length was controlled with UV irradiation time, resulting in a 3D lithographic method that achieves sub-micrometer control over feature position, feature diameter, and feature height. The binding of these brush polymers towards Concanavalin A (ConA) was compared to glycan arrays composed of monolayers of α-mannosides and α-glucosides prepared by the thiol-ene photochemical reaction or the copper-catalyzed azide-alkyne cycloaddition. At high ConA concentration the fluorescence signal of the brush polymer was nearly 20 times greater than the glycan monolayers, and the brush polymer arrays had a detection limit nearly two orders of magnitude better than their monolayer counterparts. Because of the ability of this method to control precisely the polymer length, the relationship between detection sensitivity and multivalency could be explored, and it was found that the longer polymers (136 nm) are an order of magnitude more sensitive toward ConA binding than the shorter polymers (8 nm), and that binding sensitivity affinity decreased systematically with length. These glycan arrays are a new tool to study the role of multivalency on carbohydrate recognition, and the photopolymerization route towards forming multivalent glycan scaffolds described herein is a promising alternative to the complex and multistep synthesis otherwise used to create the multivalent carbohydrates that are common to glycan arrays.
New reactivity of carbohydrates in ionic liquids and application for biomass conversion to 5-HMF

Valentine P. Ananikov, val@ioc.ac.ru. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russian Federation

Task-specific design of chemical process is a leading trend in modern research and technology in order to develop a new generation of sustainable chemical procedures. Outstanding progress in this field was stimulated in recent decades by discovery and application of several breakthrough technologies [1].

In the area of carbohydrate chemistry successful example of task-specific optimization has been achieved using ionic liquids, whose anionic and cationic properties can be efficiently optimized in order to meet the desired target reactivity. We have found that dissolution of carbohydrates in imidazolium ionic liquids initiates new reactivity as compared to regular solutions in water and organic solvents [2]. Ionic liquids were originally designed as non-flammable, non-volatile and non-explosive reaction media with high thermal stability. Ionic liquids have shown a promising potential to make a valuable environmental contribution as a replacement for volatile and flammable standard organic solvents.

Mechanistic studies of carbohydrates chemistry in ionic liquids using NMR spectroscopy [3], mass-spectrometry [4] and computational procedures [5] have revealed the possibility of carbohydrates transformation to 5-(hydroxymethyl)furfural (5-HMF) – a valuable platform chemical in the biomass conversion technology.

New directions of carbohydrate chemistry in ionic liquids, results of mechanistic findings and efficient catalytic systems for carbohydrates conversion to 5-HMF will be presented and discussed.

References

CARB 99

New oversulfated polysaccharide impurity in heparin and determination by radical depolymerization and liquid chromatography-mass spectrometry

Guoyun Li\textsuperscript{1,2,3}, liguoyun88@gmail.com, Chao Cai\textsuperscript{1,2}, Lingyun Li\textsuperscript{1,2}, Li Fu\textsuperscript{1,2}, Yuqing Chang\textsuperscript{1,2}, Changhu Xue\textsuperscript{3}, Fuming Zhang\textsuperscript{1,2}, Robert J Linhardt\textsuperscript{1,2}. (1) Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, United States (2) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, New York 12180, United States (3) College of food science and Engineering, Ocean university of China, Qingdao, Shandong 266003, China

Heparin is a critically important anticoagulant drug that was contaminated with a persulfonated polysaccharide in 2008 resulting in a number of adverse reactions some leading to death. Some controversy remains as to the precise composition of the 2008 contaminant and new information suggests that heparin may now be subject to adulteration with a new, difficult to detect, contaminant, \textit{N}-sulfo oversulfated chondroitin sulfate. This study describes the use of radical depolymerization followed by liquid chromatography-mass spectrometry to confirm the structure of the 2008 contaminant to be oversulfated chondroitin sulfate and to detect \textit{N}-sulfo oversulfated chondroitin sulfate.

CARB 100

WITHDRAWN

CARB 101

Modeling inhibition of ribonucleotide reductase by 2-substituted hexofuranoses

Mukesh M Mudgal\textsuperscript{1}, mmudg001@fiu.edu, Stanislaw F Wnuk\textsuperscript{1}, Morris J Robins\textsuperscript{2}. (1) Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33172, United States (2) Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, United States

Ribonucleotide Reductases (RNRs) are crucial enzymes that catalyze reduction of ribonucleotides to deoxyribonucleotides, which are required for the biosynthesis of DNA. Biomimetic reactions that are intended to mimic the radical-initiated reactions postulated to occur at active sites of RNR with 2-substituted hexofuranoses \textit{1} were studied. The 6-\textit{O}-nitro homoriboses \textit{1} with chlorine, bromine or tosylate substituents at the C2 position were prepared from diacetone glucose via multistep synthetic routes, which includes selective deoxygenation at C1 and C5, inversion of configuration at C3, and halogenation or tosylation at C2. Treatment of \textit{1} (X = Cl, Br, OTs) with Bu\textsubscript{3}SnH/AIBN in toluene at 95°C effected departure of the substituent from C2 to yield 1,2,5-trideoxy-D-glycero-hexofuranose-3-ulos\textit{e 2} in equilibrium with cyclic hemiacetal \textit{3} (Z = H; 40-90%). Benzoylation of this mixture (BzCl/pyridine) gave 6-\textit{O}-benzoyl-3-ulos\textit{e 4} (80%) as a single product. Analogous treatment of \textit{1} with Bu\textsubscript{3}SnD yielded a mixture of \textit{2} and \textit{3} as 2-deuterio epimers (Z = D). Mechanistic and kinetic aspects will be discussed.
Maillard reaction products (MRPs) between xylan and chitosan or its derivatives was used to develop a series of antioxidants, antibacterial agents and absorbents on heavy metal ions, including xylan-chitosan, xylan-chitooligomer, xylan-glucosamine hydrochloride, xylan-taurine, xylan-chitooligomer-zinc complex (XCGZC) and highly porous chitosan-xylan-nanoTiO₂ hybrid (CXTH). Their structure, UV absorbance, browning intensity, fluorescence changes, antioxidant activity and antimicrobial assessment were investigated to evaluate their promising applications. The results indicated that the properties of MRPs were closely related to molecular weight of model systems. The MRPs samples exhibited high radical scavenging activity. The XCGZC samples displayed the inhibition against *E.coli* and *S. aureus* as well as excellent antibacterial activity against *Bacillus subtilis*, *salmonella typhimurium*, *bacillus megaterium*. In addition, the water absorption of CXTH reached 1507 wt% in distilled water and 821 wt% in 0.9 wt% NaCl solution, showing smart salt-sensitivity behavior, and CXTH could adsorb efficiently the heavy metal ions such as Cu²⁺, Cr⁶⁺, Ni²⁺, Cd²⁺ and Hg²⁺.