ACS chemical biology

In Focus

pubs.acs.org/acschemicalbiology

Reports from the Award Symposia Hosted by the American Chemical Society, Division of Carbohydrate Chemistry at the 245th American Chemical Society National Meeting

Xuefei Huang*[,†] and David J. Vocadlo[‡]

[†]Department of Chemistry, Michigan State University, 578 S. Shaw Lane, East Lansing, Michigan 48864, United States

[‡]Departments of Chemistry, Molecular Biology, and Biochemistry, Simon Fraser University, 8888 University Dr., Burnaby, BC, V5A 1S6, Canada

The Division of Carbohydrate Chemistry of the American Chemical Society (ACS) offers several awards to glycoscientists. These include the Claude S. Hudson award, the Melville L. Wolfrom award, the Horace Isbell award, and the David Y. Gin New Investigator award, which honor both established and new investigators. At the spring ACS National Meeting, the Division holds award symposia to celebrate the accomplishments of the award winners. Each award symposium starts with presentations from several invited speakers, which are followed by a presentation from the award winner. In the following, we provide a report on the award symposia hosted by the Division of Carbohydrate Chemistry at the 245th ACS National Meeting in New Orleans on April 7 and 8, 2013.

Figure 1. The Ernest N. Morial Convention Center, New Orleans, venue for the 245th ACS National Meeting. Image courtesy of Anirban Mahapatra.

THE CLAUDE S. HUDSON AWARD SYMPOSIUM

The Hudson Award was established by the Division in 1946 and is presented biennially to "recognize outstanding contributions to carbohydrate chemistry in education, research, or applications". This is the most prestigious award given by the Division. Dr. Laura Kiessling (University of Wisconsin) is the 2013 Claude S. Hudson award winner.

Prof. Suzanne Walker (Harvard Medical School) kicked off the Hudson award symposium. Prof. Walker discussed the latest structural insights into the *O*-GlcNAc transferase (OGT). OGT is a glycosyl transferase catalyzing the attachment of *N*-acetylglucosamine (GlcNAc) to serines and threonines of intracellular proteins. *O*-GlcNAcylation is an important post-translational protein modification, which can affect diverse cellular processes such as transcription, signaling, and protein–protein interactions. To provide a better understanding of the molecular mechanism of OGT and facilitate tool development to study *O*-GlcNAcylation, the Walker group performed crystallography studies of OGT in the presence of a peptide substrate as well as an analogue of the UDP-GlcNAc glycosyl donor. Analysis of a set of the ternary complexes provided detailed structural views of the catalytic mechanism of glycosyl transfer. Key features regarding the mechanism and important factors driving catalysis gleaned from the crystal structures were discussed. Interestingly, besides catalyzing the transfer of GlcNAc, OGT has been found to possess another catalytic function. It was recently shown that OGT can facilitate the cleavage of host cell factor-1 (HCF-1), an important regulator in controlling cell cycle and transcription. Dr. Walker presented the structures of OGT complexed with the proteolytic repeat units of HCF-1 and discussed the mechanistic insights on how HCF-1 cleavage was mediated by OGT.

The second speaker of the Hudson award symposium was Prof. Jennifer Kohler (University of Texas, Southwestern Medical Center at Dallas). Carbohydrates mediate many cellular interactions. However, it is often difficult to study these interactions due to the low binding affinities and rapid rates of dissociation. To address this problem, the Kohler group has pioneered an approach taking advantage of photo-cross-linking.[1,2] Using metabolic engineering, they have introduced photoactivatable probes into glycans on the surface of living cells, in particular cell surface sialic acid containing glycans, which are commonly used by pathogens and toxins as receptors to gain entries into cells. Upon UV irradiation, stable covalent bonds are formed between the photoactive glycans and their binding proteins, allowing the purification and characterization of the glycan binding partners as well as the glycan bearing receptors. In her presentation, Dr. Kohler discussed their results on introducing diazirine moieties to *N*-acyl side chains of sialic acid containing glycans. One such glycan is the ganglioside GM1, which was thought to be the sole binding partner for cholera toxin subunit B (CTxB). Interestingly, the Kohler group discovered that independent of GM1, there were

Published: July 19, 2013

   dx.doi.org/10.1021/cb400398f | *ACS Chem. Biol.* 2013, 8, 1361–1365

multiple glycoproteins on cell surfaces functioning as CTxB binding partners. This photo-cross-linking approach, when coupled with metabolic engineering, presents a powerful method for the discovery of novel carbohydrate binding partners without requiring prior knowledge of the identity of either the glycoprotein receptor or the glycan binding protein.

The next presentation of the symposium was given by Prof. Lai-Xi Wang (University of Maryland), who addressed the challenges in carbohydrate-based anti-HIV vaccine design.[3] Broadly neutralizing antibodies are an important form of protective immunity against HIV-1 infection. Several such antibodies, which recognize epitopes present within the HIV-1 envelop glycoproteins, have been discovered from HIV-1 infected people. The surface of HIV-1 is heavily glycosylated with its envelope glycoprotein gp120 carrying more than 20 *N*-glycans. The heterogeneity of the glycoforms present on gp120 renders it extremely challenging to identify the specific epitopes responsible for the generation of neutralizing antibodies. Dr. Wang discussed the development of a novel chemoenzymatic approach utilizing engineered endoglycosidases to prepare glycopeptides bearing homogeneous *N*-glycans. A panel of gp120 glycopeptides containing various high mannose type or complex type *N*-glycans were efficiently synthesized and then subjected to binding studies with several known neutralizing antibodies. The glycan structures leading to strong antibody recognition were identified for each antibody. Interestingly, some of the peptide backbone sequences also played important roles in antibody binding. The identification of these novel glycopeptide epitopes may present a new direction in anti-HIV vaccine design, and these structures could be used to elicit an effective humoral immune response.

The last talk in the Hudson symposium was given by Dr. Laura Kiessling, the first female recipient of this prestigious award. In her presentation, Dr. Kiessling focused on her group's study of the biosynthesis of polysaccharides from bacteria, in particular the mycobacterial cell wall. Mycobacteria have a cell envelope rich in polysaccharides such as arabinogalactan and arabinomannan, which help to form an exceptionally strong barrier protecting these bacteria from environmental dangers. Despite the importance of these polysaccharides, the molecular mechanisms controlling their biosynthesis and structures are only now being uncovered. Important building blocks of these cell wall polysaccharides are various furanosides. Dr. Kiessling focused on their studies of GlfT2, an essential glycosyl transferase responsible for incorporating galactofuranosides into the growing polymer chain. Oligosaccharides bearing a lipid chain at the reducing end were prepared as primers for enzymatic elongation. The length of the lipid chain was found to influence the size the polymers synthesized. To shine light on how the growing polymer chain is processed by the enzyme, a novel mass spectrometry assay was developed where isotopically labeled substrate primers were used to compete with the growing chain. On the basis of the product isotope distribution patterns, it was concluded that GlfT2 goes through a processive rather than distributive mechanism by maintaining contact with the glycan substrate through successive monomer additions.[4,5] In addition to biosynthetic studies, Dr. Kiessling discussed the biological functions of the furanose-containing polysaccharides,[6] casting light on their roles through gene deletion studies as well as some remarkable chemical rescue experiments. Besides serving as armor for the microbe, the polysaccharides appear to have a potential role in controlling cell division. The more thorough understanding of the biosynthesis and functions of bacterial polysaccharides can facilitate the development of new antimicrobial agents.

THE MELVILLE L. WOLFROM AWARD SYMPOSIUM

The Melville L. Wolfrom Award "acknowledges outstanding service to the Division and to the field of carbohydrate chemistry". The Wolfrom award symposium, held in honor of the 2013 Wolfrom award recipient Prof. Todd Lowary (University of Alberta), provided a stimulating mix of chemistry and biology.

The symposium commenced with a more biology-focused talk by Dr. Chris Whitfield from the University of Guelph. Lipopolysaccharides (LPS), the set of complex glycolipids uniquely presented on the surface of Gram-negative bacteria, are crucial for bacterial viability and play a major role in diseases. Dr. Whitfield provided a summary of the biosynthetic pathways leading to the formation of LPS. He focused on the ABC transporter-dependent biosynthetic pathway responsible for incorporating the long repeating *O*-polysaccharide, which is the hypervariable antigenic component of LPS. Using an elegant combination of biochemistry, carbohydrate chemistry, and structural biology, his team identified the mannosyltransferases responsible for the synthesis of the polymannose chain,[7] as well as the enzyme defining the length of the polymannose chain by programmed termination through stepwise phosphorylation and methylation.[8] This methyl phosphate group caps the polymer, limiting its length and also serving as the mark enabling export of this polymer through the ABC-transporter. Once delivered to the periplasm, the *O*-polysaccharide is linked to the lipid A-core and then delivered to the surface of the bacterium. This elegant system underscores the remarkable beauty of biosynthetic pathways while also presenting a set of unique targets that could be exploited for antibiotics development.

The next speaker, Dr. Jeroen Codée (Leiden University), moved the session to a more synthetic focus with an overview of his team's ongoing effort to develop a robust synthesis of uronic acid oligomers that can be performed on solid-phase. Glucuronic acid monosaccharide building blocks are well-known to exhibit surprising reactivities. The mannuronic acids are much less studied, which present another level of complexity due to the challenge associated with generating the 1,2-*cis* glycosidic linkages. Using a variety of methods including competitive glycosylation experiments, Dr. Codée and co-workers highlighted the perplexing, but ultimately logical, reactivities of various uronic acid donors in glycosylations.[9] With the deliberate optimization of these donor and acceptor building blocks, the synthesis of several challenging uronic acid-containing glycans was accomplished.[10,11] The talk culminated in a discussion of cis-glycosidic linked oligomannuronic acids, which are found in alginate polysaccharides produced by the opportunistic Gram-negative pathogen *Pseudomonas aeruginosa*. Through careful study, the team developed a reliable automated route to the very rapid preparation of sizable fragments, up to twelve units, of these complex structures. The availability of these molecules and the potential to speedily generate derivatives should spur understanding of the biology of these complicated molecules, as well as potentially opening the way to vaccines that can protect against pathogens expressing such structures.

Wolfrom award winner Dr. Todd Lowary finished the session with an account of his team's efforts directed toward exploiting unusual oligomannosides produced by *Mycobacterium smegma-*

tis and some other mycobacterial species.[12,13] The oligosaccharides of interest are comprised of α-(1→4)-linked mannopyranose residues and are polymethylated at the 3-hydroxyl positions. The multiple methyl moieties confer on these molecules the ability to tightly bind long chain fatty acids such as palmitic acid. The ability to sequester hydrophobic compounds render the polysaccharides as potential agents to aid in bioremediation of oil contaminated water by helping to remove toxic contaminants such as the naphthenic acids. Several approaches to synthesize the targets were explored but failed to give the desired 3-*O*-methylated oligomannosides. The solution to the puzzle emerged after extensive optimization by using *n*-Bu_2SnCl_2-catalyzed tosylation at the 2-hydroxyl groups followed by standard methylation of the free 3-hydroxyl groups. Careful mass spectrometry analysis of the binding affinity of these synthetic methylmannose polysaccharides revealed they do not interact as tightly as previous reports suggested. Nevertheless, these polymethylated oligomannosides are able to sequester significant quantities of hydrocarbons from water, suggesting they might have potential in water treatment applications.

THE HORACE S. ISBELL AWARD SYMPOSIUM

The Horace S. Isbell Award acknowledges an investigator under the age of 45 with "excellence in and promise of continued quality of contribution to research in carbohydrate chemistry". The 2013 award winner is Prof. David Vocadlo. In the Isbell symposium, we had three speakers, Dr. Andrew Bennet, Dr. Mario Pinto, and Dr. David Vocadlo, all from Simon Fraser University.

Dr. Bennet's presentation was focused on understanding how sialidases catalyze the removal of sialic acid by using novel NMR methods. Sialic acids are often found at the nonreducing ends of oligosaccharides, which can serve as points of attachment for microbes during infection of mammalian cells. As mentioned above, cholera toxin gains entry into the cells mainly through binding with a sialic acid containing ganglioside GM1. *V. cholerae* sialidase is produced by the microbe to process higher order gangliosides to produce GM1. In this talk, Dr. Bennet presented his team's approach to using ^{13}C NMR spectroscopy to measure the relative cleavage rates of isotopically labeled substrates.[14] Based on a precisely measured set of competitive kinetic isotope effects for atoms close to the reaction center, the transition state structures of the enzyme catalyzed reactions were systemically probed. Using these isotope effects as constraints the transition state can be modeled, which supported a concerted and highly dissociative transition state.[15] Further results using new NMR methods, reveal this sialidase likely catalyzes the cleavage of sialic acid by first distorting the sialic acid into a skew boat conformation, thereby lowering the activation energy of the hydrolysis reaction. The results have important implications for the design of transition state analogues and development of such analogues as therapeutics for sialidases and neuraminidases.

Following Dr. Bennet's talk, Dr. Mario Pinto discussed how to use the deuterium isotope effect to better understand hyperconjugation. Hyperconjugation is of fundamental and practical importance in organic chemistry. In glycoscience, the hyperconjugation-related anomeric effect plays an essential role in determining the conformation of glycosides. Experimental evaluation of the hyperconjugative effect can be complicated by the difficulty in separating several contributing factors. In his talk, Dr. Pinto described their approach in utilizing deuterium substitution as a minimal perturbation to carefully probe the magnitude of hyperconjugation effects in 1,3-dioxane, -dithiane, and -diselenane systems. Using variable temperature dynamic NMR spectroscopy, the conformational deuterium isotope effects were established using Saunders' isotopic perturbation method showing a sequentially decreasing hyperconjugation effect from 1,3-dioxane to 1,3-dithiane and -diselenane. DFT calculations and natural bond orbital analysis were also presented, which supported the results obtained from the isotope studies.[16]

The next presentation was given by the 2013 Isbell award winner, Dr. David Vocadlo. Dr. Vocadlo presented his group's results on the understanding of chemical biology of *O*-GlcNAc. As reported above, *O*-GlcNAcylation plays roles in many essential biological processes. The levels of *O*-GlcNAc on proteins are regulated by two enzymes, OGT and *O*-GlcNAcase (OGA). Based on understanding of the control and catalytic mechanisms of OGT and OGA through kinetic[17,18] and structural studies, the Vocadlo group developed highly potent small-molecule inhibitors of these enzymes.[19,20] The availability of these inhibitors enabled them to modulate *O*-GlcNAc levels in cells as well as in animals, which is a powerful approach to probe the functional roles of *O*-GlcNAc. In the second half of his talk, Dr. Vocadlo shared their insights gained from using the inhibitors on how *O*-GlcNAcylation may impact neurodegenation and in particular Alzheimer's disease. The *O*-GlcNAcylation of tau protein was found to reduce tau aggregation *in vitro*. Using an Alzheimer's disease transgenic mouse model, the Vocadlo group discovered that upon treatment with the OGA inhibitor they developed, *O*-GlcNAcylation of tau was significantly increased and correlated with decreased neurofibrillary tangle formation and reduced neurodegeneration.[21] Interestingly, tau phosphorylation, a mechanism to induce tau aggregation, did not change even with increased *O*-GlcNAcylation. The knowledge gained from these results suggests OGA could be a potential target for disease-modifying therapeutics for Alzheimer's disease.

THE DAVID Y. GIN NEW INVESTIGATOR AWARD SYMPOSIUM

The David Y. Gin New Investigator Award "acknowledges and encourages outstanding contributions to research in carbohydrate chemistry by scientists in the first seven years of their independent career". The 2013 award winner is Prof. Peng Wu, Albert Einstein College of Medicine.

In the Gin New Investigator Award symposium, Dr. James C. Paulson from the Scripps Research Institute presented his team's latest results on targeting sialic acid-binding immunoglobulin-type lectins (siglecs), a class of sialic acid binding lectin that are found primarily on the surface of immune cells.[22−24] There are 15 members of the siglec family and some siglecs are highly restricted to specific cell types. For example, siglec-2 (CD22) is a B-cell inhibitory receptor, which regulates B-cell functions and prevents the overactivation of the immune system. Interestingly, although all siglecs recognize sialic acid, the glycans connected to the reducing end of the sialic acid, as well as modifications of the sialic acid structure can have a profound effect in determining the selectivity and affinity of binding to a specific type of siglec. Based on the co-crystal structures of a siglec with its ligand, the Paulson group previously developed siglec ligands with enhanced binding affinity. To improve the throughput of ligand discovery, Dr. Paulson discussed several novel screening methods where a

dx.doi.org/10.1021/cb400398f | *ACS Chem. Biol.* 2013, 8, 1361−1365

large number of sialic acid derivatives were generated, with some of the structures directly prepared on microarray slides. The availability of a library of diverse, yet well-defined compounds enabled them to discover novel ligands for several siglecs including CD22, sialoadhesin (siglec 1), siglec 7, and siglec-9. Some of the ligands developed have high nM binding affinity toward siglec receptors and show excellent selectivity (>50-fold) for their target over other siglecs. Such ligands have been incorporated into liposomes as a vehicle to deliver lipid antigens, immuogens and chemotherapeutic agents to cells expressing the specific siglec. In this way researchers can modulate cellular functions and target B-cells to treat diseases including cancer. The results presented here demonstrate the power of combining design and high-throughput screening for ligand discovery as well as the potential for using a carbohydrate recognition based approach to selectively target specific immune cell lineages of interest.

Following Dr. Paulson's presentation, Dr. Peng Wu discussed their results on developing novel catalysts to facilitate the copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction. The CuAAC reaction is one of the most prominent examples of click chemistry, which has been applied extensively in materials science and biological studies. However, for applications of the CuAAC reaction in living cells or organisms, the toxicity of the Cu(I) catalyst presents a serious challenge. Furthermore, the rates of CuAAC reaction using the traditional catalysts were found to be slow in aqueous solutions, which render it difficult to use this approach for real time monitoring of biological events. In order to address these challenges, Dr. Wu described their efforts to develop novel water-soluble triazole based ligands. The complexes formed between the new ligands and the Cu(I) ion were found to lack apparent toxicity in cellular systems at micromolar concentrations required to catalyze the reaction. Furthermore, the ligands led to rate enhancements of greater than 10-fold compared to traditional catalysts.[25,26] To demonstrate the utility of their approach, the Wu group incorporated alkynyl sugars into living cells through metabolic engineering. Incubation of the engineered cells with an azide containing reporter and the Cu/ligand complex led to rapid labeling of cell surface within minutes, allowing them to track the movement of cell-surface glycans (work done in collaboration with Dr. Ben Ovryn at Albert Einstein College of Medicine, unpublished results). In addition, this technology was applied to zebrafish embryos for multicolorimaging and monitoring of embryonic development, thus demonstrating the possibility for *in vivo* imaging as well as monitoring cell surface glycoprotein dynamics.

SUMMARY

We would like to congratulate all of the award winners for the well deserved honor. The award symposia provided a snapshot of some of the state-of-the-art research at the interface between chemistry and biology in the glycoscience field. The presentations serve as prime examples of the increasing integration of chemical and biological research in the area of glycoscience and how tools of chemistry can be applied to answer interesting, important, and fundamental biological questions. We look forward to many more years of exciting developments in the chemistry and chemical biology of glycoscience and anticipate improved tools and approaches will drive major advances while also spurring interests in the wider field.

AUTHOR INFORMATION

Corresponding Author

*E-mail: xuefei@chemistry.msu.edu.

REFERENCES

(1) Tanaka, Y., and Kohler, J. J. (2008) Photoactivatable crosslinking sugars for capturing glycoprotein interactions. *J. Am. Chem. Soc. 130*, 3278−3279.

(2) Bond, M. R., Whitman, C. M., and Kohler, J. J. (2010) Metabolically incorporated photocrosslinking sialic acid covalently captures a ganglioside-protein complex. *Mol. Biosys. 6*, 1796−1799.

(3) Amin, M. N., McLellan, J. S., Huang, W., Orwenyo, J., Burton, D. R., Koff, W. C., Kwong, P. D., and Wang, L. X. (2013) Synthetic glycopeptides reveal the glycan specificity of HIV-neutralizing antibodies. *Nat. Chem. Biol.,*.

(4) Levengood, M. R., Splain, R. A., and Kiessling, L. L. (2011) Monitoring processivity and length control of a carbohydrate polymerase. *J. Am. Chem. Soc. 133*, 12758−12766.

(5) May, J. F., Levengood, M. R., Splain, R. A., and Kiessling, L. L. (2012) Processive carbohydrate polymerases mediate bifunctional catalysis using a single active site. *Biochemistry 51*, 1148−1159.

(6) Kraft, M. B., Farias Martinez, M. A., and Kiessling, L. L. (2013) Synthesis of lipid-linked arabinofuranose donors for glycosyltransferases. *J. Org. Chem. 78*, 2128−2133.

(7) Greenfield, L. K., Richards, M. R., Li, J., Wakarchuk, W. W., Lowary, T. L., and Whitfield, C. (2012) Biosynthesis of the polymannose lipopolysaccharide O-antigens from Escherichia coli serotypes O8 and O9a requires a unique combination of single- and multiple-active site mannosyltransferases. *J. Biol. Chem. 287*, 35078−35091.

(8) Clarke, B. R., Richards, M. R., Greenfield, L. K., Hou, D., Lowary, T. L., and Whitfield, C. (2011) In vitro reconstruction of the chain termination reaction in biosynthesis of the Escherichia coli O9a O-polysaccharide: the chain-length regulator, WbdD, catalyzes the addition of methyl phosphate to the non-reducing terminus of the growing glycan. *J. Biol. Chem. 286*, 41391−41401.

(9) Walvoort, M. T., de Witte, W., van Dijk, J., Dinkelaar, J., Lodder, G., Overkleeft, H. S., Codée, J. D., and van der Marel, G. A . (2011) Mannopyranosyl uronic acid donor reactivity. *Org. Lett. 13*, 4360−4363.

(10) Walvoort, M. T. C., van den Elst, H., Plante, O. J., Krock, L., Seeberger, P. H., Overkleeft, H. S., van der Marel, G. A., and Codée, J. D. C. (2012) Automated solid-phase synthesis of beta-mannuronic acid alginates. *Angew. Chem., Int. Ed. 51*, 4393−4396.

(11) Walvoort, M. T. C., Volbeda, A. G., Reintjes, N., van den Elst, H., Plante, O. J., Overkleeft, H. S., van der Marel, G. A., and Codée, J. D. C. (2012) Automated solid phase synthesis of hyaluronan oligosaccharides. *Org. Lett. 14*, 3776−3779.

(12) Xia, L., and Lowary, T. L. (2013) Regioselective polymethylation of α-(1→4)-linked mannopyranose oligosaccharides. *J. Org. Chem. 78*, 2863−2880.

(13) Liu, L., Bai, Y., Sun, N., Xia, L., Lowary, T. L., and Klassen, J. S. (2012) carbohydrate−lipid interactions: Affinities of methylmannose polysaccharides for lipids in aqueous solution. *Chem.—Eur. J. 18*, 12059−12067.

(14) Chan, J., Lewis, A. R., Gilbert, M., Karwaski, M. F., and Bennet, A. J. (2010) A direct NMR method for the measurement of competitive kinetic isotope effects. *Nat. Chem. Biol. 6*, 405−407.

(15) Chan, J., Lewis, A. R., Indurugalla, D., Schur, M., Wakarchuk, W., and Bennet, A. J. (2012) Transition state analysis of Vibrio cholerae sialidase-catalyzed hydrolyses of natural substrate analogues. *J. Am. Chem. Soc. 134*, 3748−3757.

(16) Greenway, K. T., Bischoff, A. G., and Pinto, B. M. (2012) Probing hyperconjugation experimentally with the conformational deuterium isotope effect. *J. Org. Chem. 77*, 9221−9226.

(17) Shen, D. L., Gloster, T. M., Yuzwa, S. A., and Vocadlo, D. J. (2012) Insights into O-linked N-acetylglucosamine ([0−9]O-GlcNAc) processing and dynamics through kinetic analysis of O-

GlcNAc transferase and O-GlcNAcase activity on protein substrates. *J. Biol. Chem. 287*, 15395−15408.
(18) Greig, I. R., Macauley, M. .S, Williams, I. H., and Vocadlo, D. J. (2009) Probing synergy between two catalytic strategies in the glycoside hydrolase O-GlcNAcase using multiple linear free energy relationships. *J. Am. Chem. Soc. 131*, 13415−13422.
(19) Yuzwa, S. A., Macauley, M. S., Heinonen, J. E., Shan, X., Dennis, R. J., He, Y., Whitworth, G. E., Stubbs, K. A., McEachern, E. J., Davies, G. J., and Vocadlo, D. J. (2008) A potent mechanism-inspired O-GlcNAcase inhibitor that blocks phosphorylation of tau in vivo. *Nat. Chem. Biol. 4*, 483−490.
(20) Gloster, T. M., Zandberg, W. F., Heinonen, J. E., Shen, D. L., Deng, L., and Vocadlo, D. J. (2011) Hijacking a biosynthetic pathway yields a glycosyltransferase inhibitor within cells. *Nat. Chem. Biol. 7*, 174−181.
(21) Yuzwa, S. A., Shan, X., Macauley, M. S., Clark, T., Skorobogatko, Y., Vosseller, K., and Vocadlo, D. J. (2012) Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. *Nat. Chem. Biol. 8*, 393−399.
(22) Rillahan, C. D., Schwartz, E., Rademacher, C., McBride, R., Rangarajan, J., Fokin, V. V., and Paulson, J. C. (2013) On-Chip Synthesis and Screening of a Sialoside Library Yields a High Affinity Ligand for Siglec-7. *ACS Chem Biol.*, DOI: 10.1021/cb400125w.
(23) Rillahan, C. D., Schwartz, E., McBride, R., Fokin, V. V., and Paulson, J. C. (2012) Click and Pick: Identification of sialoside analogues for siglec-based cell targeting. *Angew. Chem., Int. Ed. 51*, 11014−11018.
(24) Nycholat, C. M., Rademacher, C., Kawasaki, N., and Paulson, J. C. (2012) In silico-aided design of a glycan ligand of sialoadhesin for in vivo targeting of macrophages. *J. Am. Chem. Soc. 134*, 15696−15699.
(25) Soriano del Amo, D., Wang, W., Jiang, H., Besanceney, C., Yan, A., Levy, M., Liu, Y., Marlow, F. L., and Wu, P. (2010) Biocompatible copper(I) catalysts for in vivo imaging of glycans. *J. Am. Chem. Soc. 132*, 16893−16899.
(26) Besanceney-Webler, C., Jiang, H., Zheng, T., Feng, L., Soriano Del Amo, D., Wang, W., Klivansky, L. M., Marlow, F. L., Liu, Y., and Wu, P. (2011) Increasing the efficacy of bioorthogonal click reactions for bioconjugation: A comparative study. *Angew. Chem., Int. Ed. 50*, 8051−8056.