January 2019

Influence Of Side Chain Conformation On The Mechanism(s) Of Glycosylation Reactions; Investigation Of Sialidation Reaction Mechanism(s) By Kinetic Studies

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INFLUENCE OF SIDE CHAIN CONFORMATION ON THE MECHANISM(S) OF GLYCOSYLATION REACTIONS; INVESTIGATION OF SIALIDATION REACTION MECHANISM(S) BY KINETIC STUDIES

by

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DISSERTATION

Submitted to the Graduate School

of Wayne State University

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2019

MAJOR: CHEMISTRY (Organic)

Approved By:

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Advisor Date

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DEDICATION

I dedicate my PhD work to my parents, Mr. Aruna Amarasekara and Mrs. Jayanthi Sivurupitiya, my wife, Mrs. Udumbara Rathnayake, my daughter, Anuki Amarasekara, my sister, Miss. Sathyani Amarasekara, and my friends for their endless love, support, and guidance.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my advisor Prof. David Crich for his continuous support and motivation of my Ph.D. during the past six and a half years. I am honored to be one of his many Ph.D. students. He is one of the most influential personalities and role models that I have ever met in my life. He is the one who taught me how quality science can be done. Without his guidance, motivation, patience, immense knowledge, and insightful thoughts, I would not have been able to finish this dissertation.

I would like to extend my gratitude to the other members of my thesis committee, Professors Jeremy Kodanko, Vladimir Chernyak, and Peter Andreana, for their invaluable suggestions, insightful comments, and critical questions. Their dedication was indispensable for the completion of this dissertation.

I am also grateful to Professors Zhongwu Guo, Jeremy Kodanko, Jennifer L. Stockdill, Jin K Cha, Parastoo Hashemi, and Young-Hoon Ahn who taught me various courses during my first year at Wayne State University. The knowledge I acquired from them was a foundation for my research and studies during the following years.

I am grateful to Dr. Suresh Dharuman and Dr. Takayuki Kato for their invaluable contribution to some of the projects discussed in my dissertation. My appreciation also goes to the present and past Crich group members. Dr. Takayuki Kato, Dr. Takayuki Furukawa, Dr. Pavan K. Kancharla, and Dr. Appi Mandhapati helped me to acquire the basic laboratory techniques and familiarize myself with the laboratory during my first year. I have enjoyed working with Dr. Anura Wickramasinghe, Dr. Vikram Sarpe, Dr. Parasuraman Rajasekaran, Dr. Govind Pratap Singh, Dr. Sujit Mondal, Dr.

Chandrasekhar Navuluri, Dr. Salla Rajender, Dr. Szymon Buda, Dr. Oskar Popik, Dr. Myriame Moumé-Pymbock, Dr. Peng Wen, Dr. Amr Sonousi, Dr. Girish Sati, Dr. Philip Adero, Dr. Sandeep Dhanju, Dr. Bibek Dhakal, Dr. Xiaoxiao Liao, Dr. Guanyu Yang, Dr. Kapil Upadhyaya, Weiwei Chen, Michael G. Pirrone, Philemon Ngoje, Mohammed Hawsawi, Nuwan Kondasinghe, Timothy McMillan, Jonathan Quirke, Sameera Jayanath, Courtney Kondor, Rukshana Mohamad Ramshan, Emmanuel Onobun, Amirreza Samarbakhsh, Dean Jarois and Shuay Farhad, Jonathan Shaw, Brendan Blazejewski. Their help made my work and life much easier and enjoyable. I am also grateful to Melissa Rochon and all the staff members in the chemistry department for their kind support.

Lastly, I would like to thank my family: my parents Aruna Amarasekara and Jayanthi Sivurupitiya, my wife Udumbara Rathnayake, my daughter Anuki Amarasekara, my sister Sathyani Amarasekara, and my parents in law Premakumara Rathnayake and Kusuma Munasinghe for their endless love and spiritual support throughout my life.

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LIST OF ABBREVIATIONS

Ac	Acetyl
ACN	Acetonitrile
AIBN	Azobisisobutyronitrile
Ar	Aryl
ATP	Adenosine triphosphate
Boc	*tert*-Butyloxycarbonyl
Bn	Benzyl
BSP	1-Benzenesulfinyl piperidine
Bu	Butyl
Bz	Benzoyl
°C	Degree Celsius
CAM	Ceric ammonium molybdate
mCPBA	*m-Chloroperbenzoic acid*
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIAD	Diisopropyl azodicarboxylate
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMTST	Dimethyl(methylthio) sulfonium trifluoromethan
ESI	Electrospray ionization

Et	Ethyl
Gal	Galactose
Glc	Glucose
h	Hour(s)
HMBC	Heteronuclear multiple bond correlation
HRMS	High resolution mass spectrometry
Hz	Hertz
IgM	Immunoglobulin M
M	Molar concentration
Man	Mannose
Me	Methyl
MeOTf	Methyl trifluoromethanesulfonate
mmol	Millimole
MS	Molecular sieves
Ms	Methanesulfonyl
MW	Microwaves
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance
Ph	Phenyl
PMB	*p*-methoxybenzyl
ppm	Parts per million
pTSA	4-Toluenesulfonic acid

Pyr	Pyridine
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Ts	Tosyl
TTBP	2,4,6-Tri-*tert*-butylpyrimidine

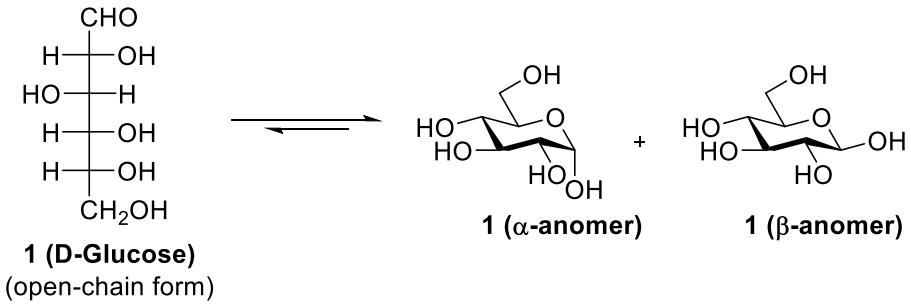
PART A. INFLUENCE OF SIDE CHAIN CONFORMATION ON THE MECHANISM(S) OF GLYCOSYLATION REACTIONS

CHAPTER 1. INTRODUCTION

Portions of the text and figures in this chapter were reproduced or adapted from: Adero, P. O.; Amarasekara, H.; Wen, P.; Bohé, L.; Crich, D., The Experimental Evidence in Support of Glycosylation Mechanisms at the S_N1–S_N2 Interface. *Chem Rev* **2018**, *118*, 8242–8284.

1.1 Background and Significance

Carbohydrates and saccharides are closely related types of molecules that mainly consist of the elements carbon (C), hydrogen (H), and oxygen (O) and have the empirical formula $C_x(H_2O)_y$. The prefix glyco- is used to denote carbohydrate-related fields, such as glycoscience, glycobiology, glycans, etc. Chemically, carbohydrates can be considered as polyhydroxy aldehydes and ketones that are often decorated with other functional groups, such as amines, thiol groups, carboxylic acids, etc. Open chain monosaccharides, the smallest building blocks of carbohydrates, undergo intramolecular reactions of an alcohol with the aldehyde or ketone, typically forming a 5- or 6-membered cyclic hemiacetal or hemiketal. The stereogenic center newly formed during the cyclization reaction is called the anomeric center, and the two stereoisomers are denoted as α- and β-anomers (**Scheme 1**).



Scheme 1. Equilibration of Acyclic and Cyclic Forms of Aldohexoses Illustrated for D-Glucopyranose.

Amplifying structural diversity and complexity, the monomer units combine to form dimeric, oligomeric, and polymeric structures, in which the monomers are linked to each other via an anomeric carbon and a hydroxyl or a heteroatomic group of another monomer unit (**Figure 1a**).[1]

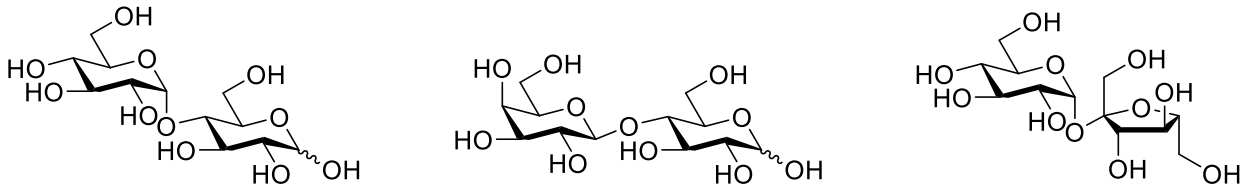


Figure 1. Examples of Regio- and Stereoisomeric Disaccharides.

Characteristically, carbohydrates contain multiple types of functional groups, even in their monomeric form, spread over around the carbohydrate ring. These spatially proximal functional groups can mutually interact and influence each other, thereby complicating the ability to predict reactivity. With their inherent structural complexity, carbohydrates have unique physical and biological properties and widely occur in biological systems. Therefore, carbohydrates are the most abundant class of biomolecules among the three other major classes of macromolecules, known as nucleic acids, proteins, and lipids.

1.2 Origin and Basic Roles of Carbohydrates

Initializing the biosynthesis process, plants and cyanobacteria use atmospheric carbon dioxide and energy from sunlight for the photosynthesis of carbohydrates and to generate molecular oxygen. In this process, during photosynthetic light reactions, radiation energy is conserved in the form of chemical energy as adenosine triphosphate (ATP) and of reducing power as adenine dinucleotide phosphate (NADPH) (**Figure 2**). In

the CO_2 fixing process known as the Calvin cycle, carbohydrates are generated from the reduction of CO_2 and H_2O. These carbohydrates serve both photosynthesizing and non-photosynthesizing organisms as an energy storage source to provide cellular energy and as a key component of the cell wall, to which they provide structural support.[2]

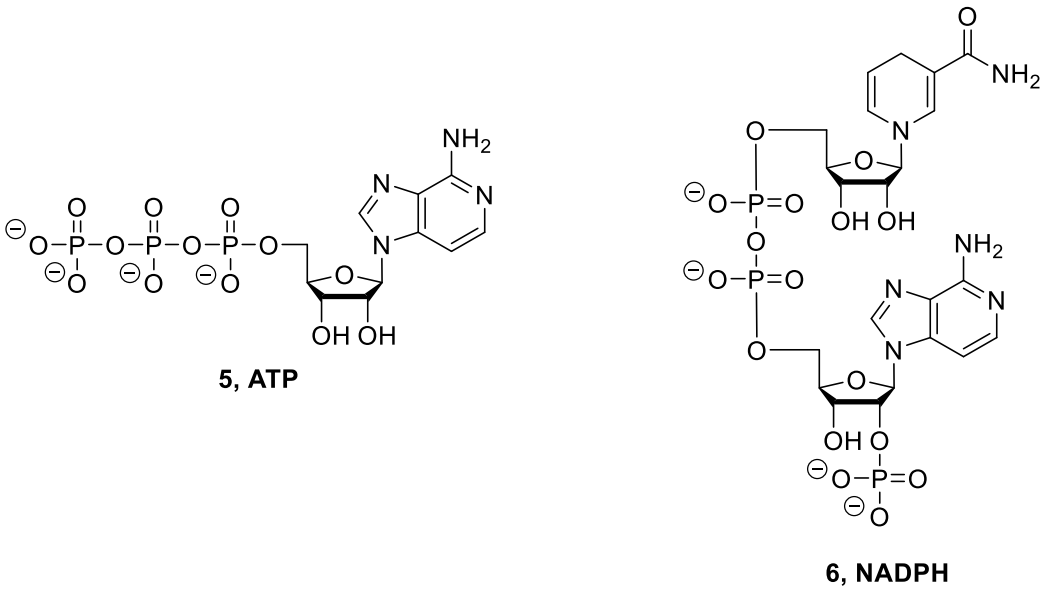


Figure 2. Structures of Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH).

1.2.1 Function as Energy Source and Structural Components

Recognition of the significance of carbohydrates as energy storage materials and as structural components dates back to the nineteenth century when the contributions of Emil Fischer in the determination of the basic concepts related to carbohydrate chemistry, i.e., structure and relative configuration of carbohydrates, was instrumental.[3-4] The process of energy production via oxidative carbohydrate metabolism is essentially the reverse process of photosynthesis. However, in general, carbohydrate metabolism is a highly complex process in which glucose-6-phosphate is considered as the key intermediate to several metabolic pathways (**Figure 3**).[1]

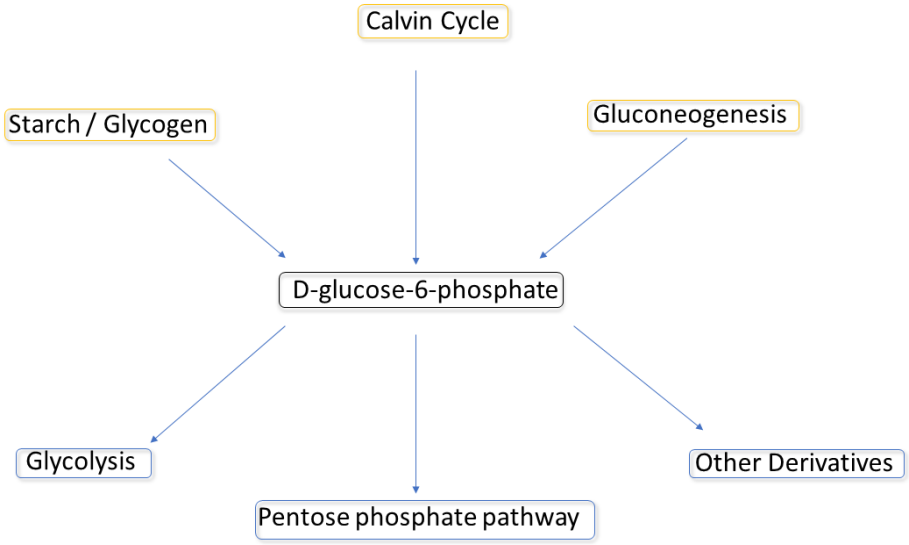


Figure 3. Carbohydrate Metabolism via Glucose-6-Phosphate as the Key Intermediate.

In plants, potential energy is stored as starch, a biopolymer of glucose with 1,4-α-glycosidic linkages. Further, ubiquitous structural components of plants are made of cellulose, which is also a biopolymer of glucose with 1,4-β-glycosidic linkages. Higher level animals and other organisms such as fungi, bacteria, etc. consume these plant materials and produce energy, mainly in the form of high energy molecules like ATP, via a series of processes called cellular respiration. Further, this process generates glucose as an intermediate, which can undergo further metabolism to generate ATP and other important biomolecules via glycolysis. Like in plants, excess glucose is stored in the form of glycogen, a multi-branched bio-polymer of glucose, through a process called glycogenesis. On the other hand, structural components such as the exoskeletons of arthropods and cell walls of fungi are made of chitin, a biopolymer of *N*-acetylglucosamine, which is also a derivative of glucose.[1]

1.3 Biological and Medicinal Importance of Carbohydrates

Despite the recognition of the basic functions of carbohydrates, interest in carbohydrate-related research was sparse until the 1960s. Recognition of the wide-spread occurrence and biological significance of complex carbohydrates in glycoconjugates that exist in soluble form or as basic constituents of all cell membranes triggered the rapid growth of carbohydrate-related sciences.[2, 5] In particularly, the important roles of glycoconjugates in cellular communication processes have been key to the increased interest in the carbohydrate field as now the study of the biology related to complex carbohydrate moieties of glycoconjugates has become a burgeoning field in cell-biology, named as glycobiology.

1.3.1 Significance of Glycoconjugates in Biology

Although carbohydrates can exist without being attached to any other type of biomolecule, they often tend to form more complex and important glycoconjugates by linking with non-carbohydrate natural products. These glycoconjugates consist of a vast range of sizes from small molecules to large polymeric structures. Smaller size glycoconjugates have significant importance in the pharmaceutical industry as they often exhibit therapeutic properties. In addition, pharmaceuticals, such as cardiac glycosides, galectin therapeutics, macrolides, drugs for diabetes mellitus, and anthracyclines, are some important classes of smaller sized glycoconjugates used in medicine.[6] Structurally, these molecules are glycosylated to different extents, embedding different physical, chemical, and biological properties. Glycans, the glycosyl parts of these conjugates, affect the properties of these glycoconjugates and can impart better pharmacodynamic and pharmacokinetic properties.[1, 7-10]

On the other hand, larger sized glycoconjugates are made by the combination of complex glycans with proteins and lipids, forming glycoproteins and glycolipids, respectively.[1-2] Glycoproteins and glycolipids are the two main components of the outer surface of the mammalian cells, where glycoproteins are involved in a range of functions, including several important biological processes, such as cell-cell adhesion, cell growth, viral replication, parasitic infections, etc.[2] Glycolipids are one of the key components of cellular energy production and the cellular recognition process as markers.

1.3.1.1 Glycoproteins, Glycolipids, and Glycocalyx

Before moving on to the details of the biological significance of carbohydrates, it is worth discussing the structure and occurance of glycoconjugates. Based on function and location, polysaccharide moieties of glycoconjugates consist of up to 20 monosaccharide units. For example, intracellular glycoproteins contain up to mono- or disaccharides, while the glycoproteins of eukaryotic cell membranes contain larger oligosaccharides.[1] These glycoconjugates are embedded into the lipid bilayer of the cell membrane in a way that these moieties are exposed to the extracellular side of the cell membrane (**Figure 4**).

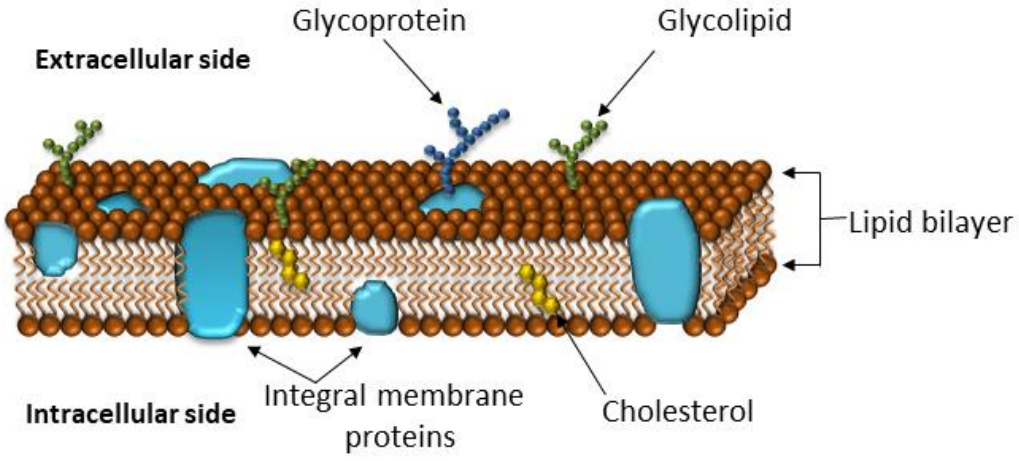


Figure 4. Glycoproteins and Glycolipids on the Lipid Bi-Layer of the Cell Membrane.

Further, glycoproteins can accommodate from one to several oligosaccharide units.[11] The linkages between these oligosaccharides and proteins have three major types, the *N*-glycosidic bonds, the *O*-glycosidic bonds, and the ethanolamine phosphate linkages (**Figure 5**). Moreover, these oligosaccharides are decorated with additional glycostructures, forming a carbohydrate coating called the glycocalyx.[12] This polysaccharide layer can extend to more than 100 nm.[1]

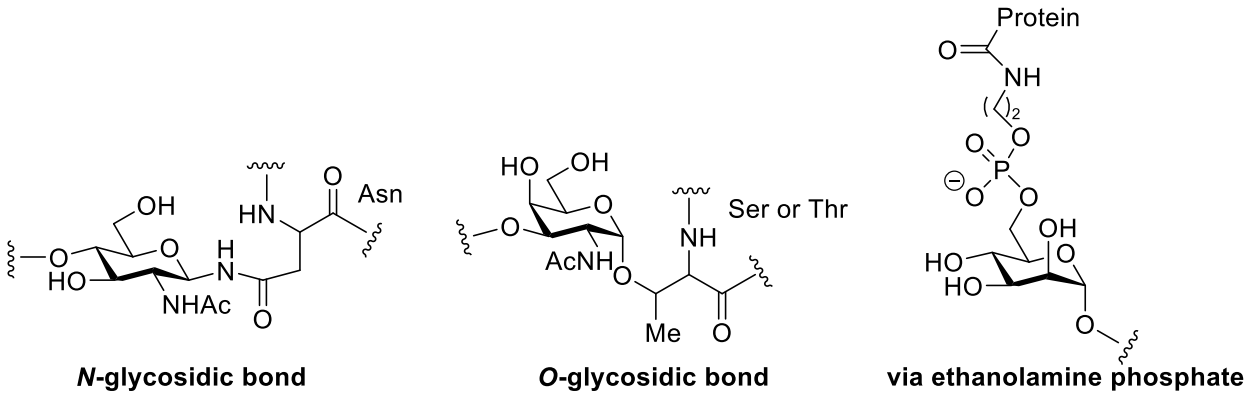
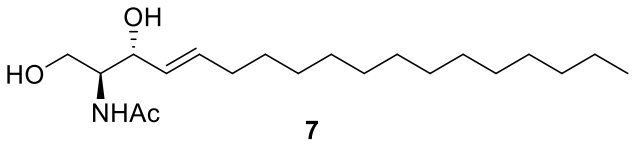


Figure 5. Major Types of Linkages in Glycoproteins.

On the other hand, glycolipids are made by the combination of a lipid part and a carbohydrate moiety, and these complex lipids are amphiphilic in nature. Generally, the lipophilic part is built from either *N*-acylsphingosine or 1,2-di-*O*-diacylglycerol, whereas the hydrophilic part consists of phosphate groups in the cases of glycerophospholipids or sphingophospholipids and of carbohydrates in the cases of glycoglycerolipids and glycosphingolipids.[13-14] Glycolipids are found in both plants and mammals; for example, glycosphingolipids are found in eukaryotic cell membranes and the lipophilic elements of these glycolipids are *N*-acetylsphingosine (**Figure 6**), called ceramide.[15]



7

Figure 6. Structure of Ceramide.

Despite the role of individual glycoconjugates, the nano-dimensional glycocalyx as a whole plays a critical role in cellular communication processes; hence, glycocalyx has great significance in glycobiology. The glycocalyx of a particular cell consists of a characteristic and unique glycosylated pattern governing the type of the cell, the developmental status of the cell, and the disease condition of the cell or organ.[1, 16-18] Moreover, such structural patterns change with dynamic processes, such as cell differentiation, cell degeneration, and other pathological modifications.[1, 17]

Carbohydrate-rich cell surfaces mediate the docking of foreign species, such as cells, molecules, or even pathogens. During these adhesion processes, carbohydrate-specific proteins, known as lectins,[19-21] interact with the glycan epitopes of a foreign species via carbohydrate recognition domains (CRD),[22] and these interactions are typically of a non-covalent nature.[23] These lectins are often integral membrane proteins with their CRDs facing the exterior of cells or the luminal space of organelles.[1, 22] In addition to the roles of lectins, carbohydrates present in the glycocalyx function as multivalent ligands. The three-dimensional structure, the stereochemistry of functional groups, and the supramolecular environment of these polysaccharide arrays of the glycocalyx are critical factors controling the efficient recognition, receptor binding, and biological signals induced by protein-carbohydrate signaling.[1, 24-25] However, the details of the molecular interactions of cellular communication processes are not yet fully understood.

1.3.2 Importance of Carbohydrates in Cellular Signaling Processes

Cellular signaling is an important part of cell communication, which governs almost all the intercellular and extracellular communication processes.[26] It allows millions of cells to work together in order to maintain the critical biological functions of living organisms. Efficient signal receipt and response are key attributes of cellular signaling, which govern vital functions, such as development, immunity, and tissue repair mechanisms.[27-28] Further, errors in cellular signaling and signal processing can result in serious disease conditions, such as cancer, autoimmunity, and diabetes, etc.[29-31] Therefore, it is crucial to understand cellular signaling processes for the diagnosis and treatment of such diseases.

1.3.2.1 Why Carbohydrates for Cellular Signaling ?

As discussed earlier, compared to the other classes of biomolecules, carbohydrates encompass an enormous structural diversity and complexity, providing a larger data storage capacity. Presumably, this unique structural feature is the major factor that governs the participation of carbohydrates in cellular communication.[1] However, as noted above, the mechanism of cellular communication via glycoconjugates is not completely resolved. In that aspect, some researchers are in search of a ‘glycocode’ similar to the genetic code,[32] while others are in search for novel functional principles to explain the unique roles of glycans in cellular communication. Overall, a deeper understanding of the three-dimensional structure of polysaccharides and their molecular dynamics under physiological conditions is essential to gain a better knowledge of the cellular communication mechanisms.[1]

1.3.2.2 Blood Group Classification and Other Cellular Communications

Blood group classification is a classic example of the significance of carbohydrate determinants in cell-recognition processes. Based on the structures of carbohydrate determinants, there are four types of blood groups present in humans (**Figure 7**). These structural determinants are small oligosaccharide units present in the glycoproteins, glycolipids, and soluble oligosaccharides, and these molecular structures are called blood-group antigens. Further, there are two types of structural modifications known as A- and B-type antigens, leading to blood groups A and B, respectively. When, both A- and B-antigens are present, the blood is AB type. In the absence of these structural modifications, A and B, the blood group is called phenotype O or H. Despite small differences in the structure, differences in the carbohydrate chains of blood cells have a significant impact on the properties of the blood. Therefore, the ABO blood group classification plays a vital role in safe blood transfusion, preventing the accidental mixing of different blood types, which can cause severe or even fatal complications.

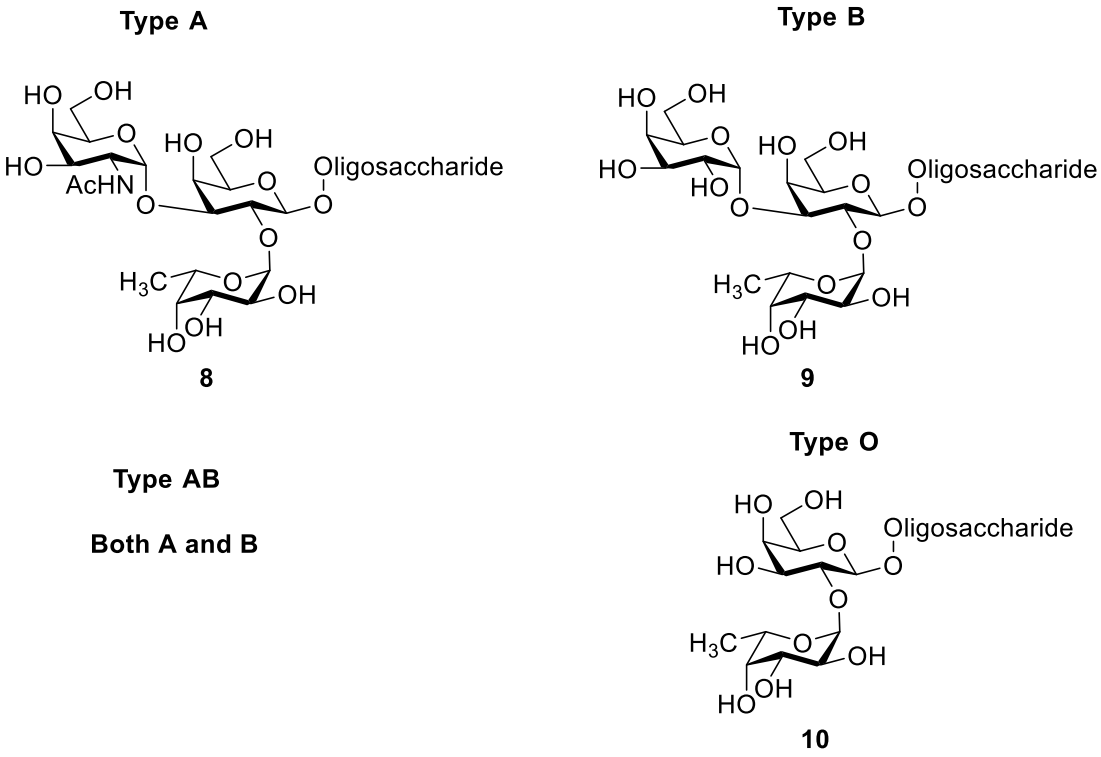


Figure 7. Minimal Determinant Saccharides of ABO Blood Group Classification.

Beyond the application of glycoconjugates in normal cells, some glycoconjugates are frequently found in the nervous system and are involved in cell-cell recognition, myelination, and neurotransmission.[33] Polysialic acids and sulfated glucuronyl glycoconjugates are characteristic nervous systems glycans.[34] Further, the anionic form of sulfated glucuronyl glycoconjugates is found in the myelin-associated glycoprotein and protein zero (P0), the most abundant glycostructure of myelin.[1, 35]

Overall, carbohydrates are involved in myriad important biological functions that are essential for the survival of living organisms. Therefore, the knowledge of the structure and biology related to these molecules is essential in understanding the related physiological functions and thus, for the prevention, diagnosis, and the treatment of diseases.

1.3.3 The Significance of Carbohydrates in Therapeutics

Glycotheraputics is an emerging field of medicinal chemistry, focusing on the development of carbohydrate-based drugs.[1] Anti-adhesion drugs, influenza drugs, and carbohydrate-based vaccines are a few important classes of the therapeutics that are used in modern medicine.

1.3.3.1 Anti Adhesion Drugs

The interactions between cell surface carbohydrates and proteins such as lectins of the pathogen surface are involved in the adhesions of toxins, viruses, bacteria, or parasites onto a healthy host cell surface.[36-38] In most infectious diseases, the adhesion process is a prerequisite for initiating the infection by docking the pathogen onto the host cell surface.[39] Molecules involving adhesion and related processes are the virulence factors of a pathogenic organism. Therefore, the blocking or inhibition of these factors, such as the blocking of bacterial lectins by the administration of natural or synthetic carhohydrate derivatives to compete with carbohydrate adhesion receptors of host cells,[40] are the basis of the anti-adhesion drugs. These types of therapeutics are mild and, therefore, safer compared to the stronger antibiotics used in bacterial infections. Moreover, the development of drug resistivity is minimal in these type of medicines because they do not kill or retard the growth of the pathogenic organisms. Therefore, the chances of evolving new drug-resistant strains of pathogenic organisms is reduced.

1.3.3.2 Influenza Drugs

Another important class of therapeutics based on carbohydrate derivatives is influenza drugs, belonging to the antiviral drug category. These drugs are neuramindase inhibitors which can inhibit the neuramindaze enzymes in the virus, preventing the

replication of the virus. These drugs are constructed based on the structure of natural 5-*N*-acetyl neuraminic acid derivatives. 4-amino-Neu5Ac2en **11**[41] and 4-guanidinium-Neu5Ac2en **12**[42] are two common examples of neuraminidase-inhibitory influenza drugs in the market (**Figure 8**).

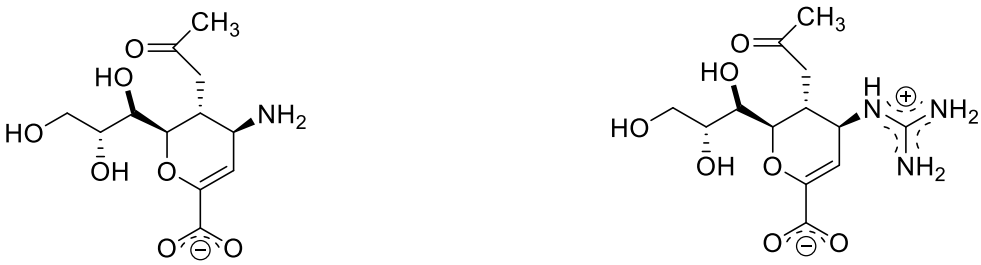


Figure 8. Neuraminic acid glycals as influenza drugs.

1.3.3.3 Carbohydrate Vaccines

Carbohydrate vaccines came into the picture as the surface of many pathogenic organisms are decorated with a thick coating of oligo- or polysaccharide layers in the forms of glycoconjugates or other types of polysaccharides. Depending on the type of pathogen, these saccharides have unique structural and chemical features to function as biomarkers in recognition events.[1] Therefore, these antigenic carbohydrates are critical in the development of disease-specific vaccines. Further, they are highly efficient as therapeutics in the prevention of many disease conditions and, therefore, have gained a serious attention from the scientific community.[43-44]

Initially, bacterial vaccines were designed only on the grounds of cell-surface polysaccharide arrays. However, such vaccines mostly induce the production of immunoglobulin M (IgM) and have low efficiency. On the other hand, smaller sized oligosaccharide-based vaccines with more specific glycan units have also been tested.

Unfortunately, due to their smaller sizes, these vaccines lack strong antigenic properties. To overcome these problems, smaller oligosaccharides and proteins have been linked together for use as therapeutics. These structural modifications have resulted in a remarkable increase in immunogenicity and specificity against many diseases.[1] During the last two decades, many conjugated vaccines came into the market to battle against several bacterial infections. Notably, *Haemophilus influenza* type b has a success rate of more than a 95 %. Moreover, conjugate vaccines were successfully used in several other bacterial diseases, such as *Salmonella typhi , Neisseria meningiditis,* and *Streptococcus pneumoniae*.[45]

1.4 Synthesis of Oligosaccharides

Obtaining pure samples of complex carbohydrates is essential to the study of their biology and beyond. Extraction from natural sources, such as pathogens, is one of the classical methods, but it has several practical difficulties, such as those associated with organism culturing and purification of complex carbohydrate mixtures.[46-47] Laboratory synthesis is the best solution to avoid those problems, and thus, the development of efficient synthetic routes, whether chemical or enzymatic, is in substantial demand.

The efficient synthesis of oligosaccharides requires a precise control of stereo- and regioselectivity over a sequence of glycosylation reactions. In this aspect, enzymatic synthesis of oligosaccharides from small-unprotected carbohydrates is very attractive. Mild reaction conditions and the lack of protecting group manipulation are the key advantages of highly regio- and stereoselective enzyme based glycosylations.[48] In addition, enzymatic methods can be utilized in multi-step oligosaccharide synthesis using a set of different enzymes in one pot. However, the high cost of reagents, the lack of

glycosyltransferases for many carbohydrate analogues, and the feedback inhibition of the enzymes by the products retard the rapid use of this method in oligosaccharide synthesis.[48-49] A combination of chemical and enzymatic glycosylation is another useful approach in oligosaccharide synthesis. This combinatorial approach promises great control of oligosaccharide synthesis because two methods can be utilized in distinct stages of the synthesis based on the requirements and the limitations of enzymatic and chemical methods.[50]

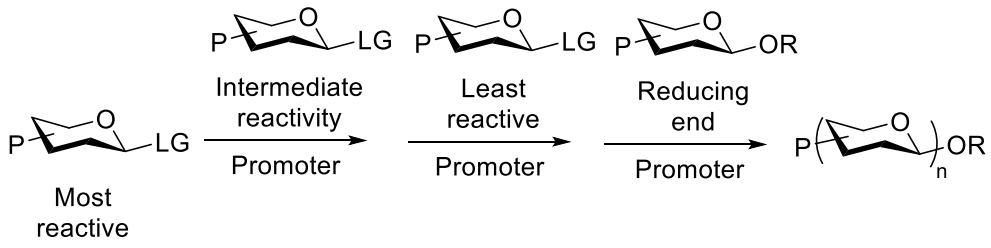
1.4.1 Chemical Approach to the Synthesis of Oligosaccharides

The glycosylation reaction is central to the synthesis of oligosaccharides and glycoconjugates. During the last few decades, an enormous number of different chemical glycosylation methods have been developed. These methods include the use of distinctly protected glycosyl donors with a variety of leaving groups, various types of promoters, a range of different solvents, and different additives, such as electrophile scavengers, bases, and acids. Therefore, the synthesis of oligosaccharides and glycoconjugates is enriched with a range of different glycosylation methods.[1, 51] Although there are many different glycosylation methods for a particular type of glycosidic bond formation, it is often hard to determine if one particular method has an advantage over another.[52] Among the several approaches of regio- and stereoselective glycosidic bond formation, modulating reactivity by protecting group manipulation is one of the widely used methods. This method is based on the varying reactivities of different glycosyl donors. Thus, depending on the protecting group system, different glycosyl donors have different reactivities and thus different relative reactivity values (RRV). In oligosaccharide synthesis, Wong's work is instrumental as his group has assembled a large pool of different glycosyl donors and

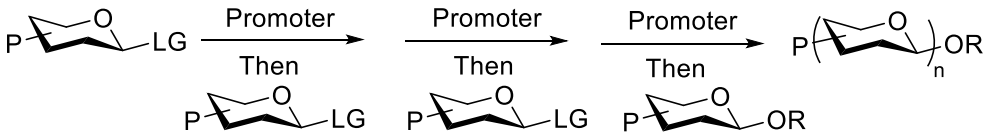
categorized them based on their RRV values. Further, based on these RRV values, Wong's group developed a software to choose the sequence of glycosyl donors for carrying out oligosaccharide synthesis. In addition to the selection of donors, based on the RRV values, the software facilitates the selection of acceptors with free hydroxyl groups. This strategy allows for the sequential activation of a set of glycosyl donors with varying reactivity (RRV values) in one pot. This method is called programmable one-pot oligosaccharide synthesis (**Scheme 2-a**).[53] The main advantage of this method is that it allows for the activation of a more-reactive donor in the presence of a less-reactive donor. However, intensive protecting group manipulation is a major drawback of the one-pot programmable synthesis.[47]

The pre-activation one-pot oligosaccharide synthesis method is a significant improvement to overcome intensive protecting group manipulations associated with the programmable one-pot method. In the preactivation method, the starting glycosyl donor is irreversibly activated prior to the introduction of the acceptor. After obtaining the first glycosylated product, it is then used in another preactivation-glycosylation step to obtain the second glycosylated product, and this sequence of reactions is carried forward until the desired oligosaccharide is obtained (**Scheme 2-b**).[54] This strategy is independent of complex protecting group strategies because the whole glycosylation sequence is controlled by the preactivation step of every glycosylation reaction. This preactivation strategy is successfully automated in smaller-sized oligosaccharide synthesis, yet this solution phase chemistry is difficult to apply in an automated system for the synthesis of larger-sized oligosaccharides.[55] Further, this method can lead to complex oligosaccharide mixtures, resulting in difficulties in purification.[47]

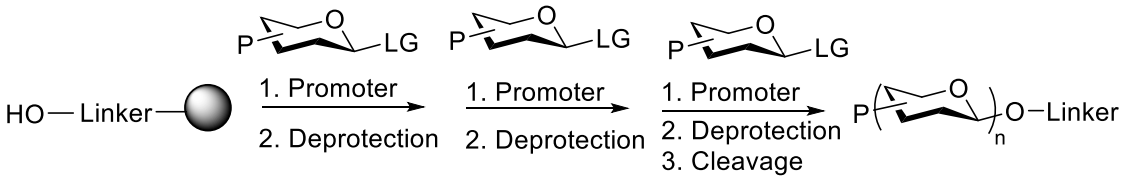
Inspired by the oligonucleotide and peptide synthesis, the Seeberger group introduced an automated solid phase synthesis to build oligosaccharides. In this strategy, a monosaccharide unit is first attached to a polymer support via a linker, and then this whole group is subjected to a sequence of successive glycosylation reactions to obtain the desired oligosaccharide bond to the polymer support. Finally, the polymer support and the protecting groups are cleaved to obtain the desired oligosaccharide (**Scheme 2-c**).[56-57]



a. Programmable one-pot oligosaccharide synthesis



b. Pre-activation one-pot oligosaccharide synthesis



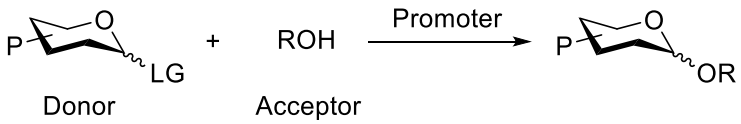
c. Automated solid-phase synthesis

Scheme 2. Oligosaccharide Synthesis Strategies **a**. Programmable One-Pot, **b**. Preactivation One-Pot, **c**. Automated Solid-Phase.

1.5 Glycosylation Reactions

Regardless of the different strategies used in oligosaccharide assembly, control of the glycosylation reaction is central to achieving better regio- and stereoselectivities of oligosaccharide synthesis. Accordingly, the higher demand of efficient and stereoselective glycosylation reactions is one of the key challenges in glycoscience.[58]

Glycosylation is a process in which the anomeric sp^3 carbon of a glycosyl donor is substituted by an incoming acceptor nucleophile, forming a new carbon-heteroatom bond known as a glycosidic bond (**Scheme 3**).The systematic development of such reactions critically depends on the knowledge of their mechanisms and the conformational dynamics of the glycosyl donors and acceptors, as well as other variables, such as temperature, solvent, additives, activators, catalysts, etc.[59-62] Insight into the glycosylation reactions can lead to the robust advancement of current glycosylation methods and to the introduction of efficient and greener reaction methodologies. Moreover, the criticality of the mechanistic aspects drives the increased interest towards developing novel methods for probing glycosylation mechanism(s) and extending the investigations towards understanding the factors affecting glycosylation reactions.



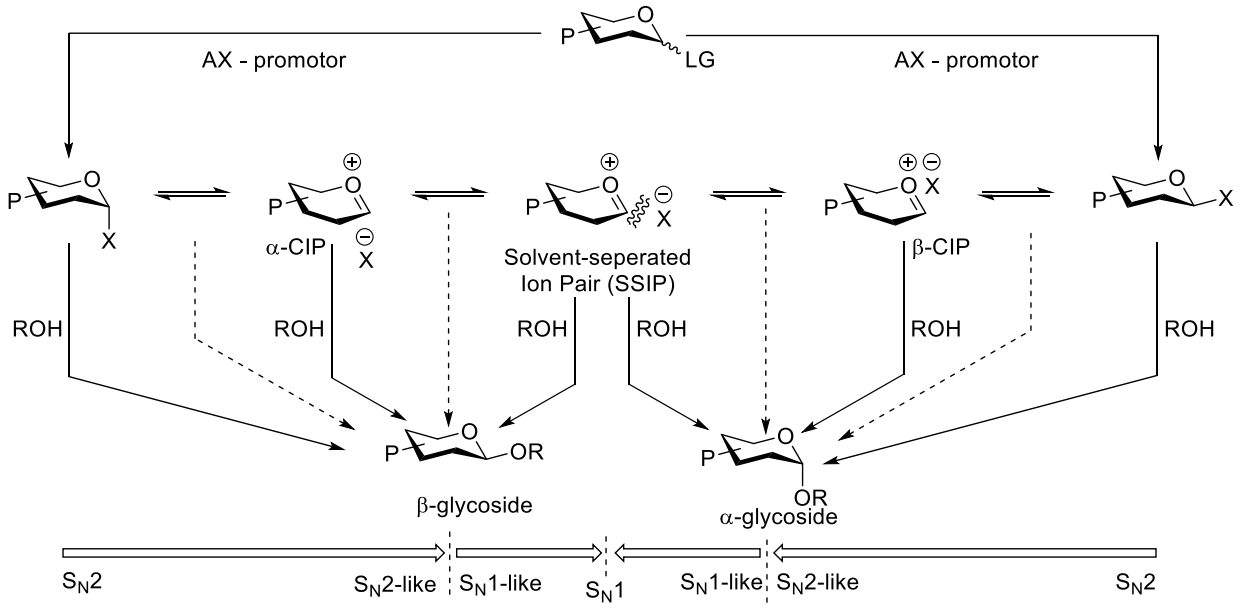
Scheme 3. General Glycosylation Reaction.

Before moving on to the details of complex glycosylation reactions, a brief consideration of the mechanism(s) of substitution reactions is important. In general, substitution reactions can be described by two extreme reaction mechanisms, known as dissociative (unimolecular-S_N1) and associative (bimolecular-S_N2) reaction pathways. In

the former, the leaving group departs to form an electrophilic intermediate, which then undergoes a nucleophilic substitution reaction with an appropriate nucleophile to form the substitution products. In the latter, the incoming nucleophile associates with the electrophilic center to form a penta-coordinated transition state followed by the departure of the leaving group in a concerted fashion, leading to the formation of the substituted products. Importantly, the two extremes are spanned by loosely or tightly associated ion pair mechanisms. However, mechanistic evidence for locating a particular reaction on the mechanistic spectrum is limited; hence, the demand for conducting physical organic type studies of such reactions is substantially higher.

1.5.1 Mechanism of Glycosylation Reactions

Glycosylation reactions can be considered as a subset of nucleophilic substitution reactions taking place at sp^3 carbon atom (anomeric center), which is bonded to the ethereal ring oxygen of, usually, 5-membered (furanosyl) or 6-membered (pyranosyl) rings. The glycosyl donor, which usually carries an electrophilic reaction center, can exist either in one or both of the two possible anomers. Activation of the glycosyl donor leads to the formation of a putative intermediate, glycosyl oxocarbenium ion located at the center of the S_N1-S_N2 continuum between the α- and β- ion pairs, whether they are tightly associated ion pairs (contact ion pair - CIP) or loosely associated ion pairs (solvent-separated ion pair - SSIP) (**Scheme 4**). The concept of the ion pair mechanism for the chemical glycosylation reactions was first introduced by Rhind-Tutt and Vernon.[63] Later, Lemieux and coworkers introduced a more graphical form of this ion pair mechanism.[64]



Scheme 4. S_N1-S_N2 Continuum for the Ion Pair Mechanism of Glycosylation Reactions.

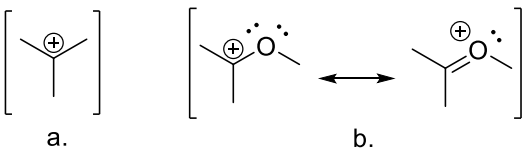
1.5.1.1 Approach to Probing the Glycosylation Mechanisms

According to Horenstein,[65] until recent years, most mechanistic studies of glycosylation reactions were based on studying the hydrolysis reactions of the glycosidic bond. For the mechanisms of glycosidic bond formation, with a few previous exceptions,[63, 66-68] scientists mostly relied on stereochemical evidence and favored oxocarbenium ion centric dissociative pathways. However, the uncritical use of stereochemical evidence alone for describing the mechanisms is not a reliable practice. Thus, later researchers expanded their studies of glycosidic bond formation mechanisms to include the isolation and characterization of intermediates and kinetic studies as well as those of substituent effects, preferentially, in terms of linear free energy relationships.[69] Such studies are, however, limited in number; thus, there remains a significant need for mechanistic studies.

1.5.2 Carbenium Ions and Oxocarbenium Ions in Glycosylation

The glycosyl oxocarbenium ion is the most widely invoked intermediate in glycosylation mechanisms. However, the evidence supporting such intermediates is limited, and therefore, those rationales are frequently suspect, particularly in cases where alternative explanations are available for explaining the stereochemical outcomes of glycosylation reactions. Therefore, it is critical to evaluate the existence, stability, and roles of oxocarbenium ions in glycosylation reactions, particularly from a physical organic chemistry perspective. Thus, before searching for physical evidence to support the existence of stable glycosyl oxocarbenium ions, the viability of the formation of oxocarbenium ions and the influence of substituents on the stability of oxocarbenium ions are analyzed under glycosylation reaction conditions.

In 1937, the Meerwein group first isolated and characterized oxocarbenium ions, which were formed by the reaction of aldehydes and ketones with triethyloxonium tetrafluoroborate.[70-71] Later, Rakhmankulov *et al.* reviewed non-carbohydrate oxocarbenium ions,[72] typically considered as resonance stabilized carbenium ions from α-oxygen (**Scheme 5**). In carbohydrate chemistry, apart from the α-oxygen, there are multiple C-O bonds at the β- and more remote positions of the carbohydrate framework; however, the influence of those substituents on the stability of glycosyl oxocarbenium ion is little considered. Therefore, evaluation of the oxocarbenium ion stabilities in the presence of different substituents on both the α- and β- positions is very important.[69]



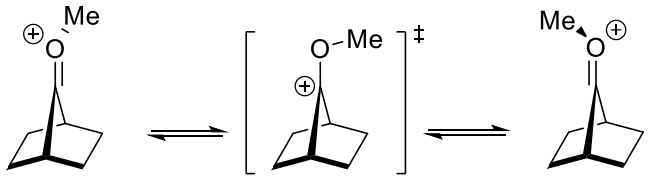
Scheme 5. **a**. Carbenium Ion and **b**. Resonance Stabilized Oxocarbenium Ion.

Computed hydride ion affinities (HIA) in gas phase reported by Keeffe and More O'Ferrall can be considered to estimate the relative stabilities and reactivities of carbenium ions (**Table 1**). As shown in **Table 1**, for both methoxymethyl and ethyl cations, the addition of extra methyl groups increases the stability in a comparable manner. Further, in the absence of β- and more remote C-O bonds, simple oxocarbenium ions have stability comparable with allylic and benzylic cations. Motivated from the conclusions of hydride ion affinity study, the Bennet and Withers groups compared the spontaneous hydrolysis of substituted cyclohexenyl mono- and dinitrophenyl ethers with correspondingly substituted mono- and dinitrophenyl glycosides. They observed that the glycosides and corresponding allylic ethers showed analogous kinetic parameters in their spontaneous hydrolysis experiments. Moreover, these allylic ethers undergo hydrolysis by certain glycosidase enzymes, indicating the analogous nature of the intermediates involved in these hydrolysis reactions.

Table 1. Computed Relative Hydride Ion Affinities (HIA) with Respect to tert-Butyl Cation (kcal.mol-1).

Carbocation	ΔHIA (g)
$MeCH_2^+$	33.8
Me_2CH^+	14.7
Me_3C^+	0
$MeOCH_2^+$	2.1
$MeOC(Me)H^+$	-15.7
$MeOCMe_2^+$	-26.7
$PhCH_2^+$	5.0

A measure of a barrier to rotation around the C-O bond is an alternative method to estimate the stabilities of oxocarbenium ions. The Winstein group determined a barrier of 18.4 kcal mol^{-1} for the *syn-anti* equilibration of 7-methoxy-7-norbonyl cation **13** using variable-temperature (VT) NMR spectroscopy in fluorosulfonic acid (**Scheme 6**), and, further, they estimated a 19.5 kcal mol^{-1} barrier for that of 2-methoxy-2-norbonyl cation.[73] In addition, Olah *et al.* estimated 60% π-character in oxocarbenium ions derived by alkylation and or protonation of aldehydes and ketones using ^{17}O, ^{1}H, and ^{13}C chemical shift values.[74-75] Overall, it is evident that, in the absence of remote electron-withdrawing groups, the electron delocalization from α-oxygen is prominent and has a significant impact on the stability of carbenium ions.



13

$\Delta G^{‡}$ = 18.4 kcal.mol^{-1} in FSO_3H

Scheme 6. Barrier to Rotation About the C-O π Bond in the 7-Methoxy-7-norbornyl Carbenium Ion.

However, when the electron delocalization from α-oxygen is structurally prohibited, adjacent carbenium ion is destabilized by the negative inductive effect of the α-oxygen atom. This destabilization can be investigated using the relative rates of hydrolysis experiments, proceeding via carbenium ions or oxocarbenium ion intermediates. Therefore, Meyer's and Martin's study on the relative rates of solvolysis of series of tosylates in 80% EtOH at 25 ˚C is instructive. According to their study, the relative rates of solvolysis decrease in the order of 1-adamantanyl tosylate **14**, 1-adamantanyl-2-oxa

tosylate **15**, and its 3-cyano derivative **16** (**Figure 9**).[76] These relative rates of hydrolysis can be attributed to the relative stabilities of the intermediate oxocarbenium ions. The formation of the 1-adamantanyl carbenium ion is relatively favored compared to its 2-oxa derivative because 1-adamantanoyl-2-oxa oxocarbenium ion (double bond on a bridgehead) is not favored as such oxocarbenium ions have substituents, which are distorted from the plane of the π-bond (C=O bond), violating Bredt's rule. Therefore, in the absence of resonance stabilization by α-oxygen, the carbenium ion is destabilized by the negative inductive effect of that oxygen atom. Further, the 3-cyano derivative has the lowest relative rate among three compounds, indicating that the cyano group boosts the negative inductive effect of α-oxygen by withdrawing electrons from the oxygen atom towards the C-N bond.

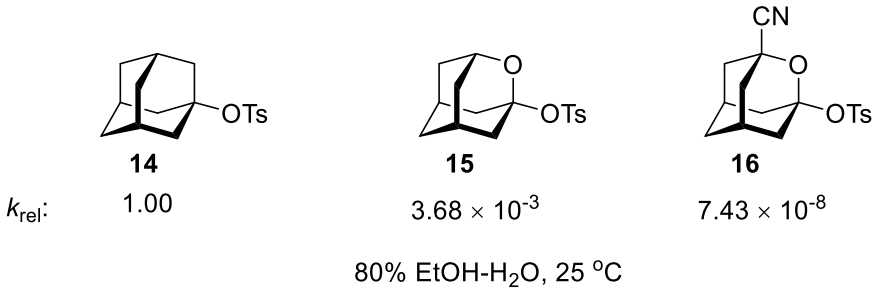


Figure 9. Relative Rate Constants of Solvolysis of 1-Adamantanyl Tosylate **14**, 2-Oxa-1-adamantanyl Tosylate **15**, and 3-Cyano-2-oxa-1-adamantanyl Tosylate **16**.

In addition, the thermal stabilities and reactivities of these compounds during column purification also follow the similar reactivity patterns observed in hydrolysis experiments. Overall, when the resonance stabilization of nascent carbenium ions is less feasible due to either steric reasons or/and the negative inductive effects of adjacent electron-withdrawing groups, the formation of carbenium ions is highly unfavorable. This critical discussion of the stability of simple oxocarbenium ions is very important as

analogous analysis can be applied to carbohydrate systems to predict the possible intermediates of glycosylation reactions and, therefore, to deduce glycosylation mechanisms.

1.5.2.1 Influence of C-O Bonds on the Stability of Glycosyl Oxocarbenium Ions

In contrast to simple aliphatic oxocarbenium ions, carbohydrate-based oxocarbenium ions have β- and multiple remote C-O bonds. Further, the hydroxyl groups of these C-O bonds can be protected by different types of protecting groups with various properties, such as electron-withdrawing, electron-donating, sterically hindered, and hydrogen bonding. In general, due to the electron-withdrawing nature of these C-O bonds, they exert a significant influence on the stability of the nascent carbenium ion; hence, the ease of formation of carbenium ions is critically dependent on these C-O bonds.

In order to estimate the stability of oxocarbenium ions, the relative rates of solvolysis reactions proceeding via carbenium ion intermediates can be investigated. Starting with non-carbohydrate analogues, a study of the solvolysis of secondary 2-nobornyl tosylates by Lambert *et al.* is instructive. It was observed that the relative rate of solvolysis of 2-endo-norbornyl tosylate **17** at 25 °C is 3000-fold faster than that of the 3-endo-acetoxy-2-endo-norbornyl tosylate **18** (**Figure 10**).[77] Later, in a similar type of study by the Kirmse group, it was demonstrated that the rate of solvolysis of 2-endo-norbornyl brosylate **19** at 25 °C was 10^5-fold faster in comparison to that of the brosylate **20** where the 6-methylene group is substituted with a ring oxygen atom (**Figure 10**).[78] In both comparisons, the retardation of the reaction rates can be attributed to the destabilization of nascent oxocarbenium ion intermediates due to the electron density removal by

adjacent electron-withdrawing atoms. Overall, when the neighboring group participation from β-C-O bonds is not plausible, there is a significant negative impact on the stabilities of carbenium ions due to the electron-withdrawing nature of such β-C-O bond. Similar effects are applicable in carbohydrate systems.

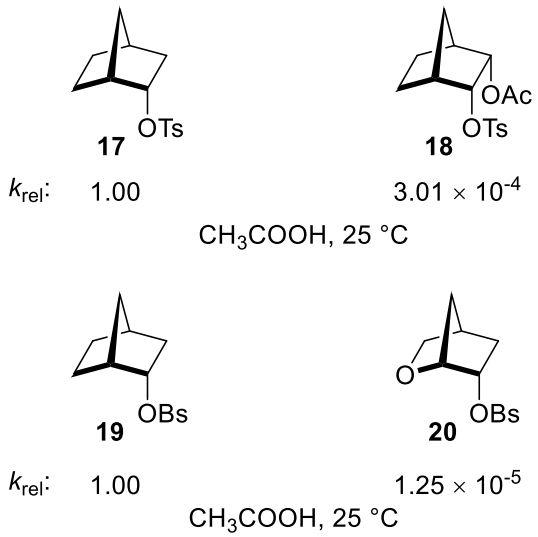


Figure 10. Influence of β-C-O Bonds on the Relative Rates of Solvolysis.

The influence of β- and more remote C-O bonds on the stability of glycosyl oxocarbenium ions has been estimated using different approaches. Employing the relative rates of glycoside hydrolysis as a measure of the oxocarbenium ion stability, Overend *et al*. studied the kinetics of acid-catalyzed hydrolysis of a series of methyl glycosides (**Table 2**).[79] Similarly, but in a more detailed approach, the Withers group studied the spontaneous hydrolysis of 2,4-dinitrophenyl glycosides (**Table 3**).[80] According to these studies, whether it is spontaneous hydrolysis of nitrophenyl glycosides or acid-catalyzed hydrolysis of methyl glycosides, 2-deoxy analogues are the most reactive glycosides. In the Withers study, due to the extreme reactivity of 2-deoxy 2,4-dinitrophenyl glycoside, it was observed that this glycoside was unstable even upon the attempted

column purification. Overall, the reactivity orders of the hydrolysis experiments for these two series of compounds follow the trend 2-deoxy >> 4-deoxy > 3-deoxy ~ 6-deoxy.[69]

Table 2. Relative Rates of Acid-Catalyzed Hydrolysis of Methyl Glycosides in 2 *N* HCl at 58 °C.

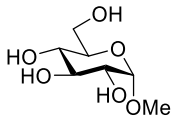
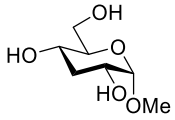
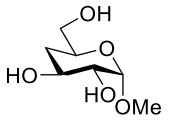
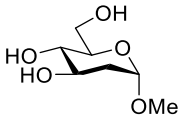
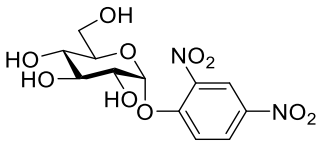
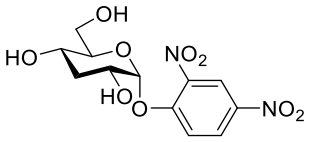
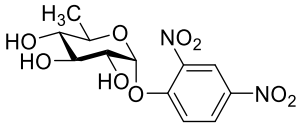
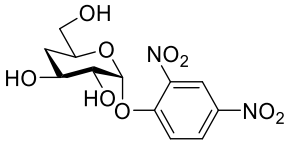
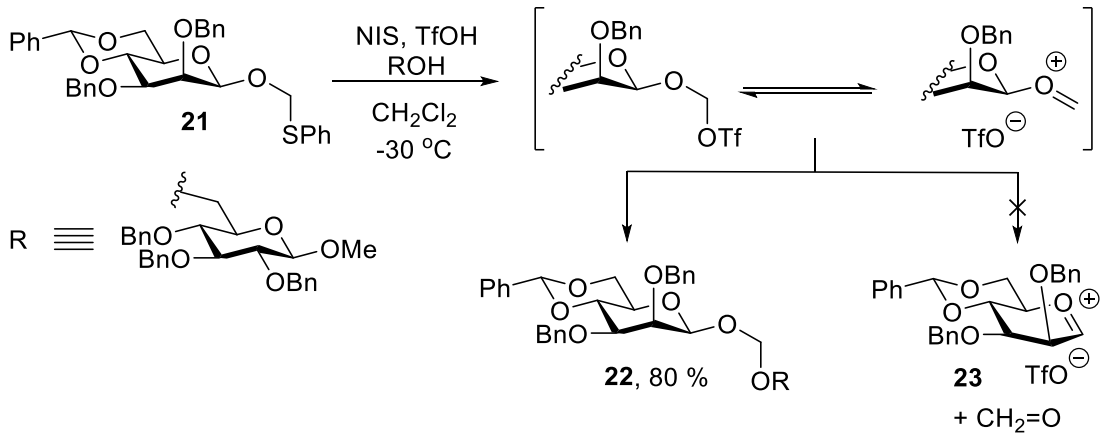
Glycoside	k_{rel}
	1
	20
	40
	2090 (In 0.1 *N* HCl)

Table 3. Relative Rates of Spontaneous Hydrolysis of 2,4-Dinitrophenyl Glycosides in 25 mM sodium phosphate buffer (pH 6.5) and 0.40 M KCl at 37 °C.

Glycoside	k_{rel}
	1.0
	4.0
	4.7
	22.4

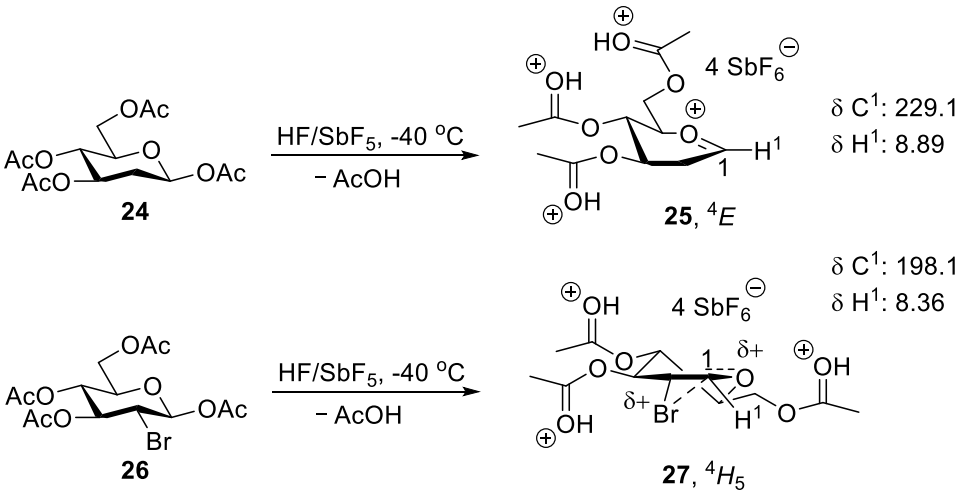
In another important study, Crich *et al.* conducted a series of glycosylation reactions of phenyl thiomethyl glycosyl donors in the presence of external acceptors and *N*-iodosuccinimide and triflic acid as the promotor in dichloromethane at -30 °C, and observed the formation of alkoxymethyl glycosides in good yield (**Scheme 7**).[81] These products were probably formed by a nucleophilic substitution at the external oxocarbenium ion intermediate, suggesting that the external oxocarbenium ion does not fragment to form a glycosyl oxocarbenium ion **23**. The destabilization of glycosyl oxocarbenium ion by β- and remote C-O bonds could be the prime reason for this observation.



Scheme 7. Substitution of a Phenylthiomethyl Glycoside without Fragmentation.

NMR-spectroscopic characterization of simple oxocarbenium ions was reported by several laboratories,[82-83] yet the detection and proper characterization of glycosyl oxocarbenium ions were not achieved until 2016. The enormous effort to characterize oxocarbenium ions using NMR spectroscopy reveals the difficulties associated with the observation of transient oxocarbenium ions, particularly glycosyl oxocarbenium ions with β-C-O bonds. Therefore, even if they exist, due to their highly reactive nature, they may have extremely short lifetimes compared to the NMR time scale. These observations

further explain the significance of β- and more remote C-O bonds on the stability of oxocarbenium ions. However, despite all these difficulties, in 2016 the Blériot group was able to successfully characterize glycosyl oxocarbenium ions using ^{13}C and ^{1}H NMR spectroscopy in HF/SbF_5 media, i.e., a super acid medium. It is important to note that all the carbonyl oxygen atoms of per-acetylated 2-deoxy-glucopyranoside and 2-bromo-2-deoxy glucopyranoside oxocarbenium ions were protonated, and they occupied ^{4}E and $^{4}H_5$ conformations, respectively (**Scheme 8**).[84-85]



Scheme 8. Generation and Characterization of Glycosyl Oxocarbenium Ions in Super-Acidic Media.

Though it is not a direct formation of oxocarbenium ions from a glycoside, in an important attempt to detect glycosyl oxocarbenium ions spectroscopically, Woerpel and coworkers generated simple monobenzyloxy tetrahydropyranosyl dioxacarbenium ions via the alkylation of corresponding lactones. NMR studies revealed that these oxocarbenium ions occupy half-chair conformations (**Figure 11**). Importantly, they observed that the electron-withdrawing 4-benzyloxy analogue prefers the pseudo axial benzyloxy group due to stereo-electronic reasons, while 4-methyl analogous prefers

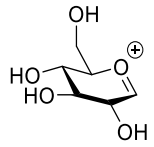
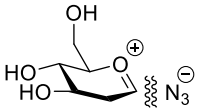
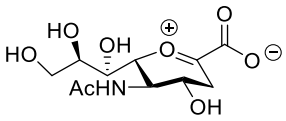
pseudo equatorial methyl groups due to their steric factors. These observations consistently reveal the significance of β- and remote substituents and their relative configuration on the stability of oxocarbenium ions.[86]

Figure 11. Conformational Changes of 1-Alkoxyoxocarbenium Ions as a Function of the Nature of the Substituents.

The inability of NMR spectroscopy to detect glycosyl oxocarbenium ions suggests that the lifetimes of glycosyl oxocarbenium ions are short on the NMR timescale. However, in the late 1980s, Jencks and Amyes were able to determine the lifetimes of the glucosyl oxocarbenium ion in water at 25 °C by diffusion-controlled trapping of glycosyl oxocarbenium ions using azides as external nucleophiles in a series of solvolysis experiments of α-azido ethers.[87] According to Jencks and co-authors, the lifetime of the glucopyranosyl oxocarbenium ion is 1 X 10^{-12} s (**Table 4**). Later, Bennet and co-workers showed that the same oxocarbenium ion has the lifetime 2.5 X 10^{-12} s.[88] These lifetimes are so short that the diffusional equilibration is unlikely. Further, the Bennet group was able to determine the lifetimes of α- and β- ion pairs of 2-deoxyglycopyranosyl oxocarbenium ions as 1.4 X 10^{-12} s and 2.7 X 10^{-12} s, respectively (**Table 4**).[89-90] Further, they concluded that the 2-deoxyglycopyranosyl oxocarbenium ion is not completely solvent equilibrated in an aqueous medium. Following a similar type of azide clock reaction, Horenstein and Brunner determined the lifetimes of sialyl oxocarbenium ions as ≥ 3 X 10^{-11} s (**Table 4**).[91] Overall, the extremely short lifetimes of these glycosyl oxocarbenium ions in water convey that the existence of such intermediates in an

aqueous medium is highly unlikely. Extrapolating these conclusions, survival of glycosyl oxocarbenium ions in non-polar organic solvents is extremely unlikely, and the use of such intermediates in corresponding mechanistic rationales is debatable. However, if the evidence supports the existence of glycosyl oxocarbenium ions, it is important to consider the other factors influencing the glycosylation reactions in order to rationalize such species as the intermediates of glycosylation reactions.

Table 4. Oxocarbenium Ion Lifetimes in Water.

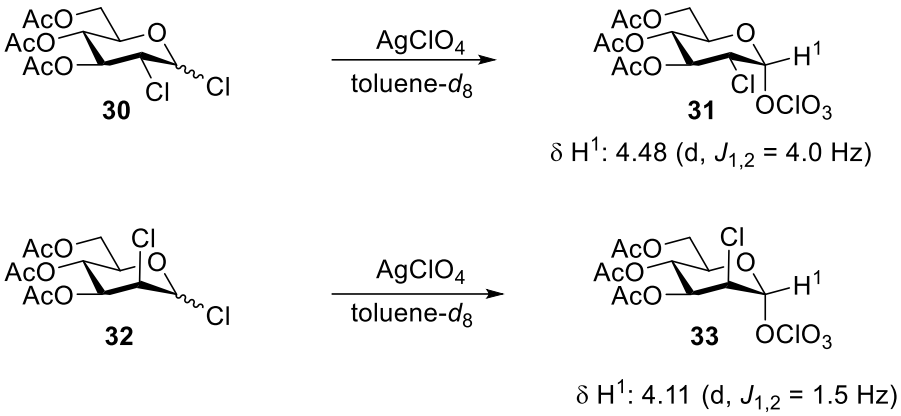
Oxocarbenium ions	Estimated lifetime (s)
	1×10^{-12} (Jencks), 2.5×10^{-12} (Bennet)
	1.4×10^{-11} (α)[a] and 2.7×10^{-11} (β)[a]
	$\geq 3 \times 10^{-11}$

[a] First formed intermediate is SSIP:M complex where M = 4-bromoisoquinoline

All in all, it is evident that the use of naked oxocarbenium ions, without valid evidence in the interpretation of the stereochemical outcomes of glycosylation reactions, is not a reliable approach in practicing the state of the art determination of the glycosylation reaction mechanisms. Therefore, it is important to explore evidence to predict the other possible intermediates in glycosylation reactions. In the search for other intermediates, the scientific community focuses their attention on possibilities of the existence of covalently bonded glycosyl intermediates, and several multidisciplinary laboratories have worked on the identification, isolation, and characterization of such important species.

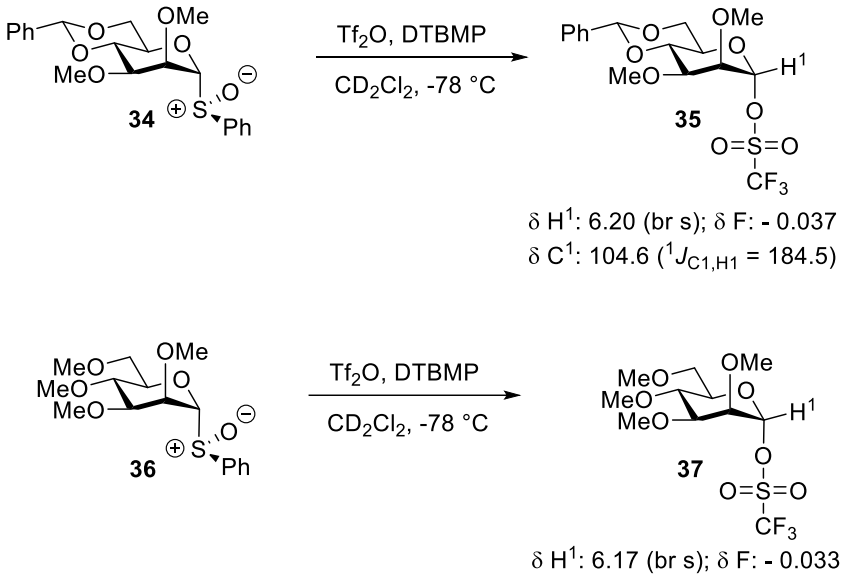
1.5.3 Other Intermediates or the Related Transition States in Glycosylations

The simplest form of glycosylation reactions proceeds via the direct displacement of the leaving group by the acceptor nucleophile. However, such glycosylation reactions[92] are extremely rare, and more often glycosylation reactions proceed via complex reaction pathways. Typically, the glycosyl donor is activated by a promoter, increasing the nucleofugacity of the leaving group. With the departure of the activated leaving group, another similar species, which is derived from the promoter or is an external additive, attacks the electrophilic center, forming a relatively unstable intermediate. Then, this transient intermediate undergoes nucleophilic attack from the acceptor nucleophile to form the final glycoside (**Scheme 4**). In fact, there is ample evidence to support the formation of covalently bonded intermediates rather than glycosyl oxocarbenium ions. Isolation of this kind of intermediate was first reported by the Igarashi group in 1970 when they were able to isolate α-gluco- and α-mannosyl perchlorate generated by the reaction of α- or β- configured gluco- and mannopyranosyl chlorides **30** and **32**, respectively (**Scheme 9**).[93] In another important work, Schuerch and co-workers activated per-*O*-benzylated α-glucopyranosyl bromide to give the corresponding sulfonates, which were characterized using NMR spectroscopy.[94-98]



Scheme 9. Formation of 2-Chloro-2-deoxy-gluco- and Mannopyranosyl Perchlorates.

In many common glycosylation reactions, the promoters are derived from triflates containing electrophiles, or triflates are being used as additives. In such reaction conditions, depending on the nature and the protecting group strategy of the glycosyl donor, the formation of glycosyl triflate intermediates is highly plausible. In an important series of low-temperature NMR studies, the Crich group was able to identify and characterize mannosyl triflates as the first ever spectroscopic evidence to support the formation of covalently bonded glycosyl triflates as glycosylation reaction intermediates. Further, they explained the role of that intermediate in controlling the stereoselectivities of mannosylation reactions (**Scheme 10**).[69, 99]



Scheme 10. Formation and Characterization of Mannopyranosyl Triflates.

In addition, based on glycosylation reaction conditions, the formation of several other glycosyl intermediates has been detected and characterized using spectroscopic methods. Among those intermediates, bromides,[64, 100-101] fluorides,[102] sulfonium ions,[103] phosphonium ions,[104] and isouronium ions[105] are worth mentioning (**Figure 12**).[69]

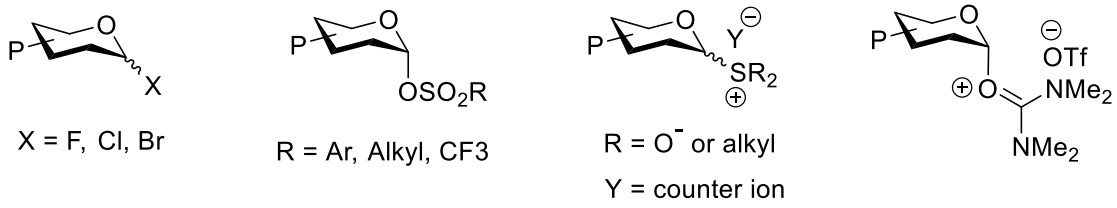


Figure 12. Covalently Bonded Activated Glycosyl Donors.

Manipulation of the solvent system is a common practice in regio- and stereoselective glycosylation reactions. In particular, acetonitrile-based solvent systems can increase the stereoselectivities of many glycosylations. These increased stereoselectivities are rationalized in terms of the formation of axial nitrilium ion intermediates (**Figure 13**). These intermediates then undergo S_N2 or S_N2-like reaction pathways to form glycosides with higher equatorial selectivity. The existence of such

intermediates is supported by the evidence from trapping experiments and NMR spectroscopy.[69, 106-108]

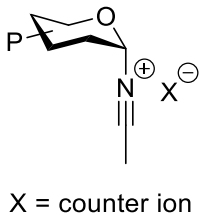


Figure 13. Nitrilium ions as Intermediates of Glycosylation Reactions.

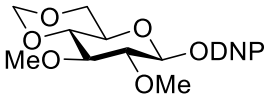
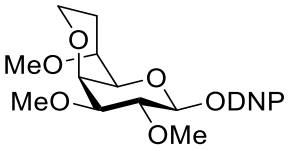
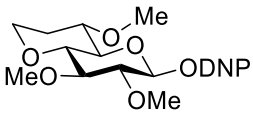
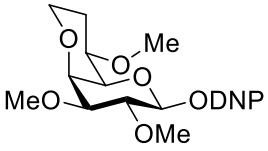
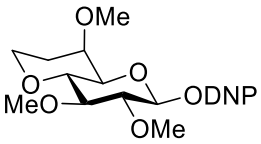
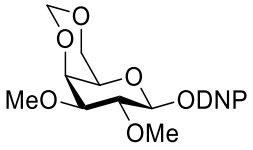
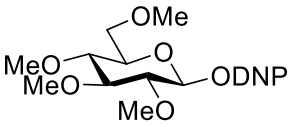
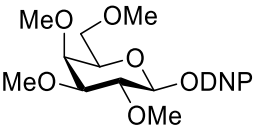
During the discussion of glycosylation reaction mechanisms, the formation and stability of putative glycosyl oxocarbenium ions were considered. Further, the discussion was extended to describe the covalently bonded intermediates in glycosylation reactions. However, each glycosylation reaction is such a unique sequence of reaction steps that the formation of intermediates, whether oxocarbenium ions or covalently-bonded intermediates, is governed by multiple factors, such as the type and configuration of substituents, side chain conformation, reaction temperature, reaction solvents, etc. Therefore, from a mechanistic perspective, it is very important to consider the influence of such factors in controlling glycosylation reactions.

1.6 Influence of Type and the Configuration of Substituents and Ring Conformation

In the above discussion, the influence of C-O bonds on the stabilities of oxocarbenium ions is surveyed as a function of the substituted position of C-O bonds in the carbohydrate ring. Similar but more specific studies have been conducted by the Bols and Crich groups, and specifically, their studies on the significance of side-chain conformation on the stability of oxocarbenium ions have been instrumental. They studied the kinetics of the spontaneous hydrolysis of bicyclic dinitrophenyl glycosides, where they

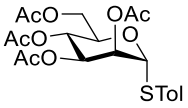
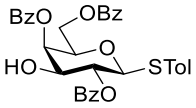
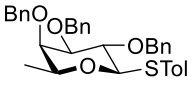
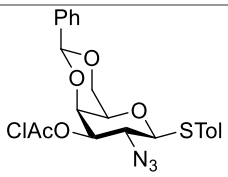
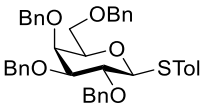
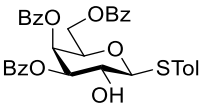
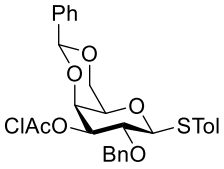
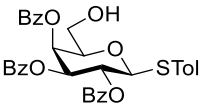
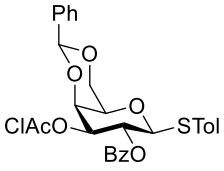
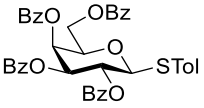
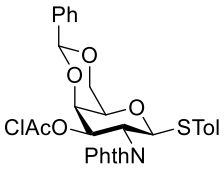
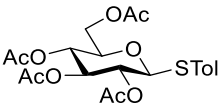
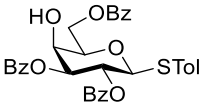
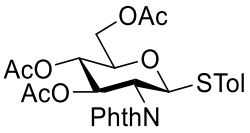
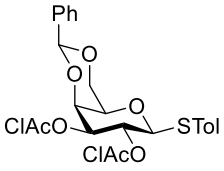
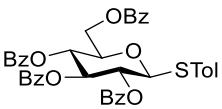
have imposed a selected configuration to the pyranoside side chain by introducing a rigid bicyclic system. From these studies, they were able to find the relative reactivities of different side chain conformations. Importantly, enforcement of the *trans-gauche (tg)* conformation to the side chain of the pyranoside leads to the lowest relative rate of hydrolysis compared to the other staggered conformers, *gauche-gauche (gg)* and *gauche-trans (gt)*. These observations imply that the electron-withdrawing nature of the C5-O6 bond of the *tg* conformer makes it difficult to hydrolyze the 2,4-dinitrophenyl glycosides (**Table 5**).[109-110] Later, the Bols group attributed the decrease in the rate of hydrolysis to the torsional effects because they observed that the replacement of O6 with a methylene group can lead to moderate β-selectivities between armed thioglycoside donors and disarmed 4,6-*O*-benzylidine protected donors in the corresponding glycosylation reactions. They suggested that this selective β-glycoside formation is caused by the preferential formation of axial triflate intermediates due to the destabilization of oxocarbenium ions by the torsional effect of benzylidene protection.[111] However, the Crich group later showed that this model, in which O6 is replaced by a methylene group, is not a good model to evaluate the 4,6-*O*-benzylidene effect as it completely disregards the extra torsional interactions that arise due to the methylene group, leading to the overestimation of the torsional effects. Further, they confirmed that there is a torsional component, yet the stereoelectronic effect of the structurally imposed *tg* conformation has a significant impact on the rate and the selectivities of glycosylation reactions.[110]

Table 5. Relative Rates of Spontaneous Hydrolysis of Conformationally locked Dinitrophenyl Glycosides at pH 6.5 at 37 °C.[109-110]

Substrate	Config	k_{rel} (glu)	Substrate	Config	k_{rel} (gal)
	*tg*	1		*tg*	1
	*gt*	2.1		*gt*	1.4
	*gg*	3.6		*gg*	2.5
	-	11.1		-	5.9

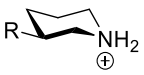
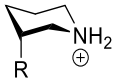
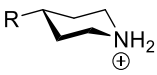
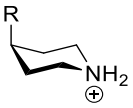
Further, from the hydrolysis experiments, it is clear that galactopyranosides are relatively more reactive than the corresponding gluco-analogues, implying that the axial substituents stabilize the formation positive charge (**Table 5**). As an alternative to the study of the hydrolysis of glycosides, studying the glycosylation of thioglycoside donors is also significant and informative. In that aspect, the Wong and Ley groups contributed extensively by providing relative reactivity values (RRVs) of the glycosylation reactions of a broad range of thioglycosides with different substituents and configurations.[53, 112] (**Table 6**) Further, electron-withdrawing protecting groups such as esters decrease the relative reactivity values in comparison to the corresponding ether protecting groups (**Table 6**). These observations draw parallels with the observations and conclusions of hydrolysis experiments of glycosides.

Table 6. Selected Examples of Wong Relative Reactivity Values (RRVs) for the Activation of Thioglycosides by NIS-TfOH in Dichloromethane at Room Temperature.

Substrate	RRV	Substrate	RRV
	1		28.9
	7.2×10^4		24.1
	1.7×10^4		17.6
	185.4		13.1
	118.7		5.7
	102.0		2.7
	67.1		1.7
	31.4		1.3

Through-space-electrostatic stabilization of oxocarbenium ions of pseudo-axial substituents is one of the key rationales used to explain the increased anomeric reactivity of carbohydrates with axial substituents over their equatorial counterparts. Due to the transient nature of oxocarbenium ions, direct measurements of the influence of protecting groups on oxocarbenium ion stability are difficult to obtain. Alternatively, piperidinium ions can be used as model compounds for oxocarbenium ions to study the stability of oxocarbenium ions. The Bols group employed the changes of pK_a values in a range of mono- and bicyclic piperidinium ions to estimate the stability of substituted piperidinium ions as a function of substituents, the orientation of the substituents, and the conformation of the cyclic piperidinium ions.[113] They observed that the unsubstituted piperidinium ions have the highest pK_a while the introduction of electron-withdrawing groups leads to a decrease in the pK_a values. Further, the axial electron-withdrawing substituents have a higher pK_a value compared to their equatorial counterparts. A set of substituent constants were derived in pH units by calculating the difference between the pK_a of unsubstituted piperidinium ion and that of the substituted ammonium ions. Since the piperidinium ions with axial hydroxyl groups have higher pK_a values compared to those of the equatorially substituted ones, the derived substituent constants of the former have smaller magnitudes while the latter have relatively higher values (**Table 7**).

Table 7. Axial and Equatorial Substituent Effects on the Base Strength (σ_s) of Piperidinium Derivatives in Water at 25 °C[a].

R group				
OH	1.3	0.5	0.6	0.2
F	2.3	1.5	1.0	-
COOMe	1.2	0.2	-	-
$CONH_2$	1.5	1.3	-	-
COO^-	0.5	-0.2	0.2	-
CN	2.8	3.0	-	-
CH_2OH	0.4	0.5	-	-

[a]σ_s values in pH units and they were calculated from the equation, $\sum\sigma_s$ = pKa (unsubstituted amine) - pKa (substituted amine).

These observations clearly demonstrate that the axial groups have a higher stabilization of developing positive charge compared to equatorial substituents. This stabilization is possibly a cumulative effect of the through-space electrostatic stabilization of positive charge by the axial hydroxyl groups and of the fact that the axial OH groups are not in a preferential configuration to withdraw the electron density from the nascent oxocarbenium ions to destabilize the positive charge. Further, in a series of hydrolysis experiments, a directly proportional relationship was observed between the sum of substituent constants for a given 2,4-dinitrophenyl glycoside versus the negative logarithm of the rate constants of their spontaneous hydrolysis (**Figure 14**).[113-114]

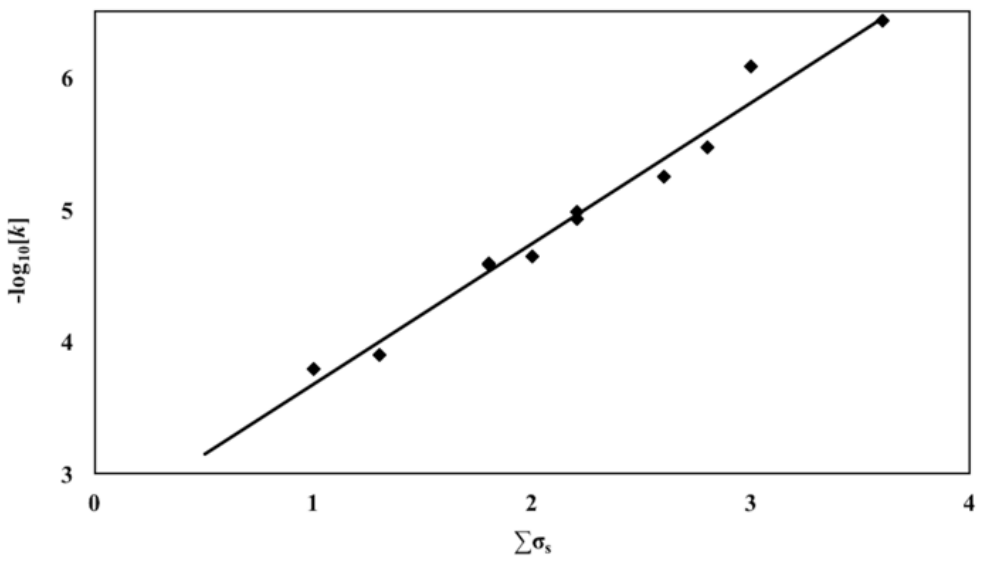


Figure 14. Plot of Negative Logarithm of the Rate Constant for Spontaneous Hydrolysis of 2,4-Dinitrophenyl Glycosides versus the Sum of their Stereoelectronic Constants for the Substrate.[113]

Application of this concept in studying the influence of protecting groups on the stability of glycosyl oxocarbenium ions further demonstrates that the electron-donating group and axial C-O bond of the glycosyl donor stabilizes the formation of oxocarbenium ions compared to their counterparts.[115] Thus, the conclusions of the study of pKa values using piperidinium models show parallel results to the reactivity pattern of glycosylations of the corresponding thioglycosides (**Figure 15**).[53, 69] Overall, the knowledge of factors influencing the glycosylation reactions, particularly the type of protecting groups, the configuration of ring C-O bonds, and the side-chain conformation, are of critical importance in mechanistic studies in carbohydrate chemistry.

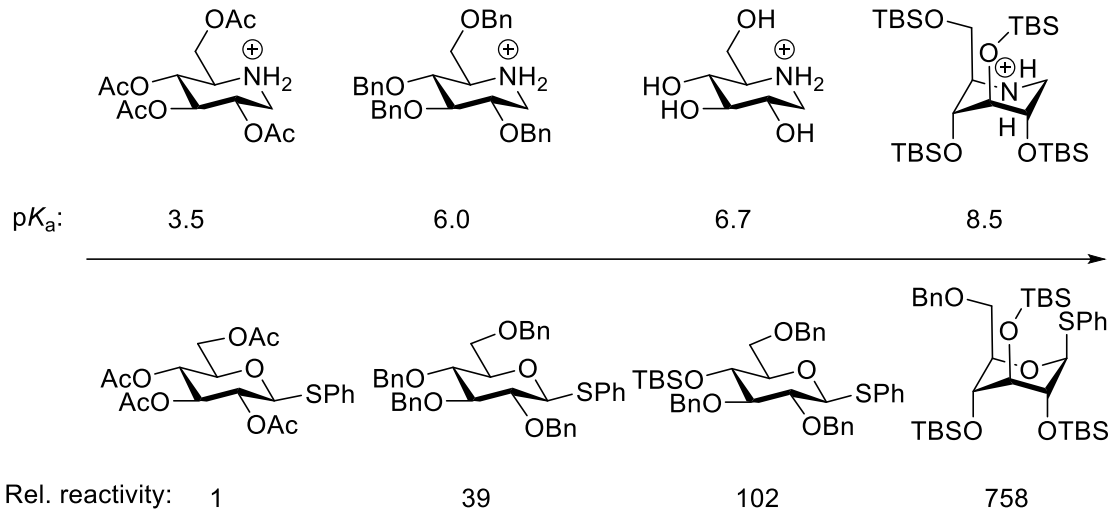
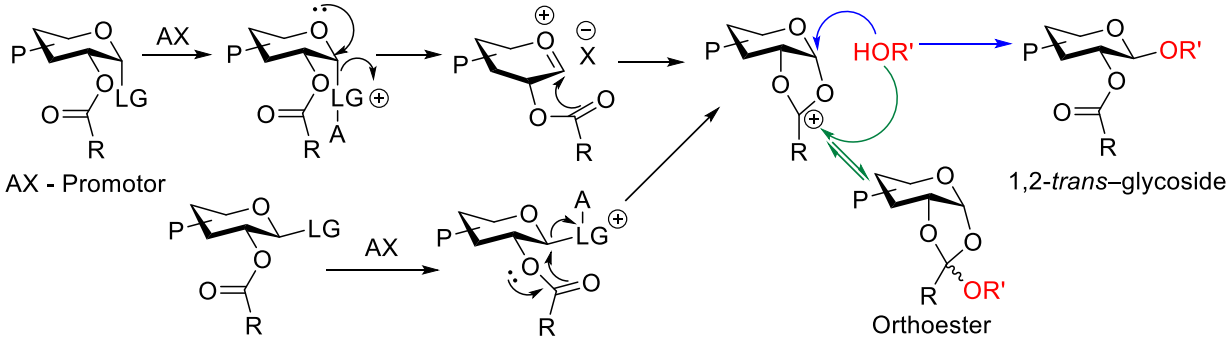


Figure 15. Comparison of the Relative Reactivity of Glycosyl Donors with the pK_a of the Corresponding Piperidinium Ions

1.7 Neighboring Group Participation

When considering the factors affecting glycosylation reactions, neighboring group participation[116] is one of the key concepts which is regularly invoked in explaining stereo-controlled glycosylation reactions. This concept has been invoked in a range of contexts.[117-118] In carbohydrate chemistry, this concept can be explained in terms of the nucleophilic participation by the oxygen atom of acyloxy group adjacent to the anomeric center (usually, 2-*O*-position), by forming a dioxalenium ion to stabilize the glycosyl carbenium ions.[119-120] The intermediate dioxalenium ion can undergo a nucleophilic substitution reaction with a nucleophile in S_N2 fashion to form 1,2-*trans* glycosides, or it can be trapped as an orthoester, which can eventually rearrange in the presence of an acid or a Lewis acid,[121] forming the 1,2-*trans* glycoside (**Scheme 11**). When the reaction rate is increased by the participation of the neighboring group, the phenomenon is called anchimeric assistant.[122] The intermediate dioxalenium ions have been characterized using NMR spectroscopy[119] as well as X-ray crystallography,[123] providing strong evidence

to support the concept of neighboring group participation. The formation of β-galacto- and glucopyranosides and α-mannopyranosides are few common examples for the stereoselective neighboring group participation.[124-125] In addition to the neighboring group participation by the protecting groups at the 2-position, the functional groups located at more distal positions to the anomeric center, such as 3-, 4-, and even 6-positions, have been invoked to explain the stereochemical outcomes of glycosylation reactions as 'stereodirecting participation by remote groups'. However, this latter rationale is not well supported by experimental evidence, rather the opposite, i.e. the unlikely nature of long range participation, is supported by mechanistic studies.[126] Overall, when the participating groups are present at the 2-position, neighboring group participation is a well-established explanation, whereas, stereodirecting participation by remote groups is a controversial concept.[126-131]



Scheme 11. Neighboring Group participation by 2-O-position Acyl Group.

1.8 Overall goals

The advancement of the glycobiology and glycochemistry fields depends critically on the introduction of efficient glycosylation methods and the optimization of current glycosylation reactions. Thus, insight into glycosylation reaction mechanisms is key to the success in carbohydrate related sciences.

Therefore, this thesis focuses on the mechanisms of glycosylation reactions and the factors influencing those reactions. In describing the glycosylation mechanisms, exploration of the evidence locating glycosylation reactions on the S_N1-S_N2 continuum of the ion pair mechanisms is crucial. The conformation and configuration of substituents have a critical roles in regulating glycosylation mechanisms. Therefore, evaluation of the influence of side-chain conformation on the anomeric reactivity is the first goal of this thesis. The accurate determination of side-chain conformation is highly dependent on the accuracy of experimental limiting coupling constants; hence, derivation of an accurate set of limiting coupling constants using model compounds was considered as the first goal. Then the application of the limiting coupling constants for the calculation of the side chain conformation of pyranosides and subsequence correlation of the side chain conformation with anomeric reactivity and selectivity is considered in the second project. A further goal of this thesis is the elucidation of sialidation mechanisms via kinetic studies.

CHAPTER 2. SYNTHESIS OF CONFORMATIONALLY LOCKED GALACTO- AND GLUCO-CONFIGURED *CIS*- AND *TRANS*-BICYCLIC DECANE DERIVATIVES AS MODEL COMPOUNDS FOR THE DETERMINATION OF LIMITING $^{3}J_{H,H}$ COUPLING CONSTANTS

Portions of the text and figures in this chapter were reproduced or adapted from: Amarasekara, H.; Dharuman, S.; Kato, T.; Crich, D., Synthesis of Conformationally-Locked *cis*- and *trans*-Bicyclo[4.4.0] Mono-, Di-, and Trioxadecane Modifications of Galacto- and Glucopyranose; Experimental Limiting $^{3}J_{H,H}$ Coupling Constants for the Estimation of Carbohydrate Side Chain Populations and Beyond. *J. Org. Chem.*, **2018**, *83*, 881–897. Copyright © 2018, American Chemical Society.

2.1 Introduction

The conformational dynamics of glycosyl donors, whether of the pyranoside or furanoside ring itself or of the side chain, have a significant influence on the reactivity and selectivity of glycosylation reactions.[109-110, 132-139] The conformationally mobile side chain is usually considered as a mixture of three staggered conformers (**Figure 16**). These conformers are described as *gauche-gauche* (*gg*), *gauche-trans* (*gt*), and *trans-gauche* (*tg*), in which the first and second descriptors denote the spatial relationship of O^6 with respect to the ring oxygen O^5 and C^4, respectively. The mole fractions (f_{gg}, f_{gt}, and f_{tg}) of these conformers can be calculated using the experimental $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants, given the availability of the accurate limiting coupling constants of the three ideal staggered conformers.[140-141]

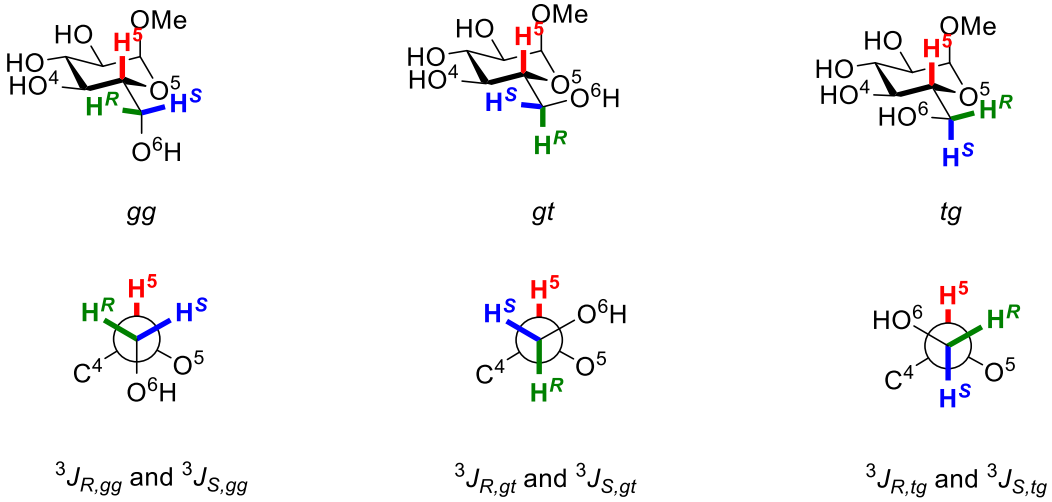


Figure 16. The Ideal Staggered Conformations of the Side Chain of Hexopyranoses (Methyl α-D-Glucopyranoside), and the Limiting Coupling Constants Describing Them.

The accuracy of the limiting coupling constants, whose values critically depend on the electronegativity and the configuration of the substituents attached to the coupled system,[142-143] is the most vital factor in determining accurate side chain conformational populations for a given system. Thus, the selection of the accurate limiting coupling constants has a significant influence on the correlation between structure and reactivity. Initially, the limiting coupling constants ($^3J_{R,gg}$ - $^3J_{S,tg}$) were derived by the parameterization of Karplus[144] and Haasnoot-Altona[145] equations. However, as summarized by Bock and Duus,[60] the accuracy of the parameterized limiting coupling constants depends on the underlying models. Later, those limiting coupling constants were revisited by several other authors.[146-148] Derivation of the experimental limiting coupling constants from model compounds is another approach. Adopting that approach, Bock and Duus used the C1-C2 bond of the 1,5-anhydroalditol **38** (*gg*), **39** (*tg*) and **40** (*tg*) as models of the C5-C6 bond to experimentally determine the limiting coupling constants (**Figure 17**).[60] Here, it is important to note that compounds **39** and **40** have an

enantiomeric relationship to the actual *tg* conformation of D-pyranoses, and therefore, despite the actual *pro*-6*R* and *pro*-6*S* relationships, H^{6S} and H^{6R} were assigned as shown in **Figure 17** for the ease of comparison. Further, Serianni and coworkers used pyruvate acetals **41** (*gg*), **43** (*tg*), and *trans*-2,5-(bishydroxymethyl)-1,4-dioxane **42** (*gt*) derivatives as models in deriving the limiting coupling constants (**Figure 17**).[147] In addition, earlier models[149] **44** (*gg*) and **45** (*gt*), with more conformationally mobile bicyclic frameworks, show significantly different limiting coupling constants from those of the more recent model compounds (**Figure 17**). Further, their conformational purity is ambiguous due to the mobile nature of the 9-membered ring containing the C5-C6 and C6-O6 bonds. Therefore, these early models **44** and **45** were discarded. Consideration of the *gg* and *tg* conformations of more recent models indicate that there are considerable differences between the limiting coupling constants reported by different authors (**Figure 17**). Further, in the absence of model **45**, there is only one model for the *gt* conformation. Overall, there are many inconsistencies in the limiting coupling constants reported by different authors, and the computed conformational populations from them include negative conformational populations for one of the three conformers.

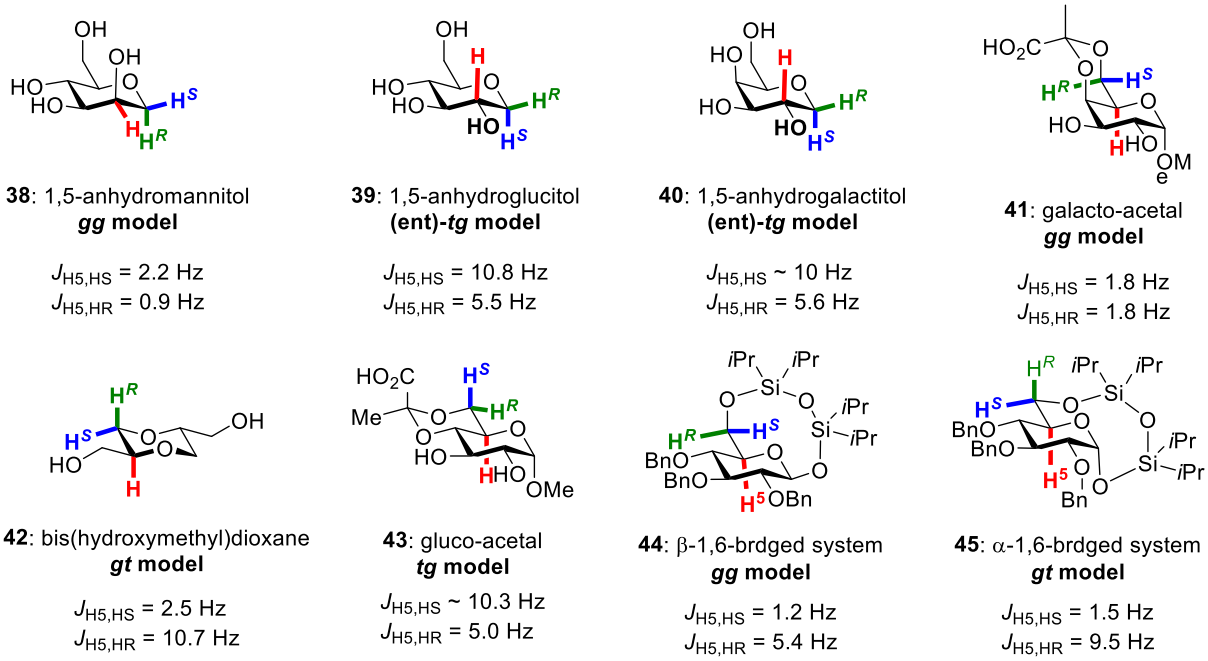


Figure 17. Literature Models for the *gg*, *gt*, and *tg* Conformers.

The accurate determination of the side chain conformational population is important in several ongoing projects in the Crich laboratory,[150-151] necessitating re-evaluation of the viability of using the literature limiting coupling constants. As discussed above, the discrepancies in the limiting coupling constants introduced by different authors and the negative populations calculated from those limiting coupling constants are indicative of possible errors in the data set. To address these problems, the synthesis and analysis of a series of conformationally rigid oxabicyclo[4.4.0]decane model compounds **46-57** were carried out to provide reliable limiting coupling constants (**Figure 18**). This series of compounds consists of both galacto- and gluco-configured systems such that, ideally, it can also be used to compare the effect of the orientation of the substituents, which are not directly attached to the coupled system (**Figure 18**).

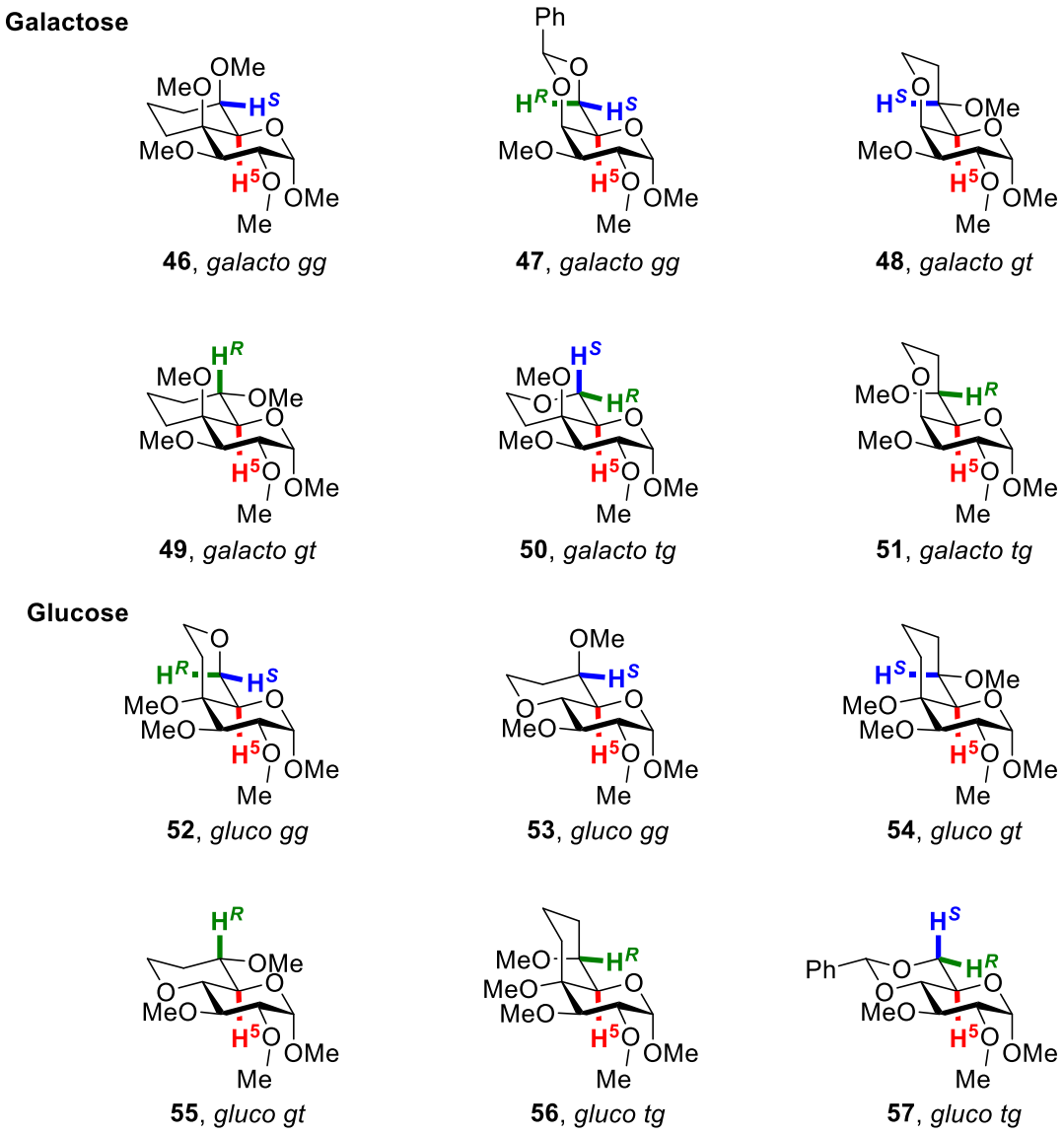


Figure 18. Oxa-, Dioxa-, and Trioxadecalins for the Determination of Limiting $^{3}J_{H,H}$ Coupling Constants About the C5-C6 bond in Galacto- and Glucopyranosides.

However, several potential problems are associated with these bicyclic model compounds, such as i) the conformational purity of the *cis*-fused model compounds and of the compounds with 1,3-diaxial interactions; ii) the derivation of coupling constants from the tetrasubstituted models for use in modeling the trisubstituted side chains in hexopyranoses; iii) a combination of i) and ii). Therefore, another series of compounds

58-63 were prepared for comparison purposes (**Figure 19**). The verification of the literature data was anticipated by the comparison of literature models **38** and **39** with the models **61** and **58**, respectively. Further, the comparative analysis of these four compounds is useful in the evaluation of the influence of the ether-type protecting groups on the coupling constants. It is important to note that similar to compounds **39** and **40**, the C2-C1 bond of compounds **58**-**60** have a pseudoenantiomeric relationship with the side chain (C5-C6) of D-sugars, and therefore, regardless of the absolute denotation of *pro*-6*R* and *pro*-6*S*, H^{6R} and H^{6S} are denoted as depicted in the **Figure 19** for ease of comparison.

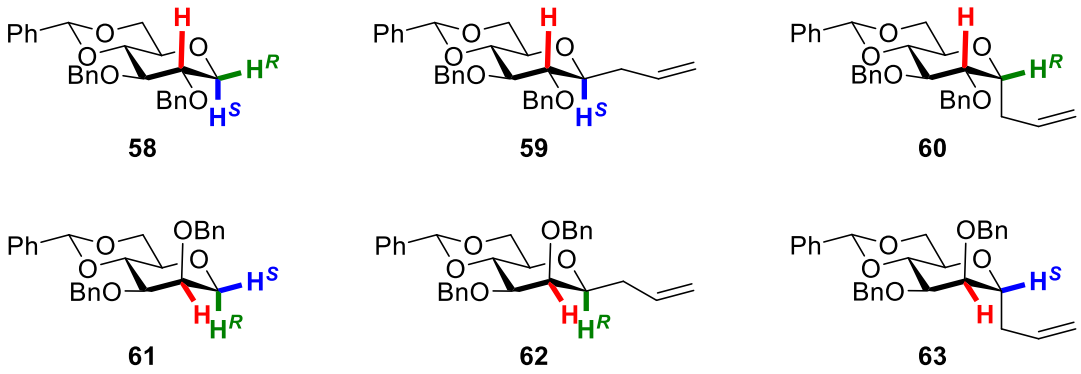


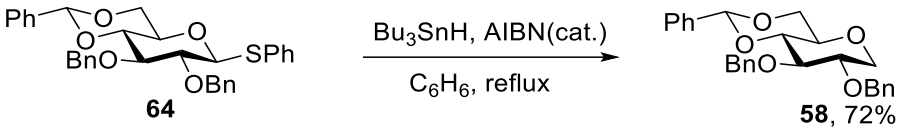
Figure 19. Bicyclic Model Systems for Comparison of Coupling Constants Between Tri- and Tetrasubstituted Systems.

2.2 Results and Discussion

2.2.1 Chemistry and Synthesis

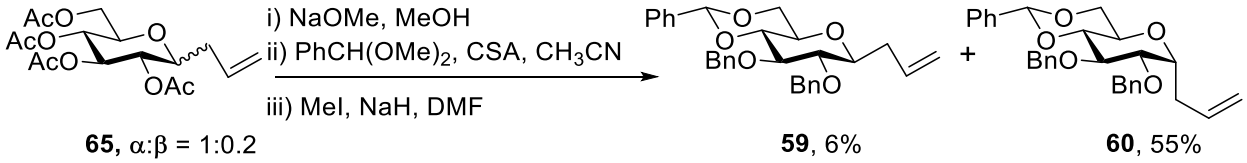
Compounds **47**,[152] **48**,[110] **51**,[110] **53**,[109] **55**,[109] **57**[153], **61**[154], **62**,[155] and **63**[155] are literature compounds that were synthesized as described in the literature. Dr. Suresh Dharuman and Dr. Takayuki Kato, whome I thank, carried out the synthesis of some literature models as well as some new model compounds. Literature compounds **58**-**60**[156-157] were prepared using alternative protocols as outlined in the **Scheme 12** and **13**. Thus,

the treatment of *S*-Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thia-β-D-glucopyranoside **64**[158] with tributylstannane in the presence of AIBN in benzene gave 1,5-anhydroglucitol **58** in 72% yield (**Scheme 12**).



Scheme 12. Synthesis of Compound **58**.

The synthesis of compounds **59** and **60** was started with the Zemplen deacetylation of an anomer mixture of 2, 3, 4, 6-tetra-*O*-acetyl-1-*C*-allyl-D-glucopyranose **65**[159] (α:β = 1:0.2) with sodium methoxide in methanol followed by benzylidine protection using benzaldehyde dimethyl acetal in the presence of camphor sulfonic acid in acetonitrile and finally, benzyl protection with benzyl bromide and sodium hydride in DMF to give **59** and **60** in 6% and 55% yield, respectively, over three steps (**Scheme 13**).



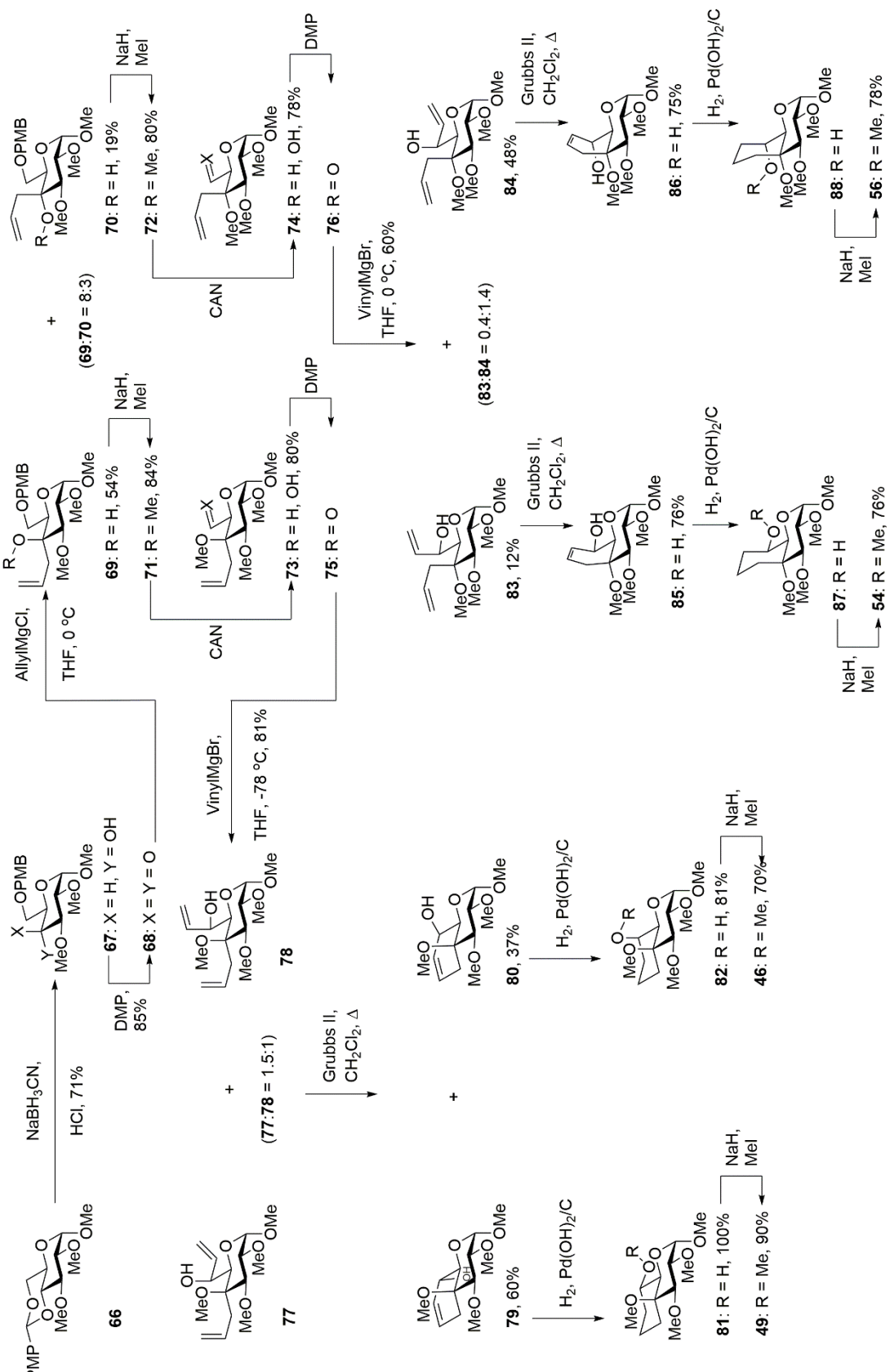
Scheme 13. Synthesis of Compounds **59**-**60**.

Synthesis of Compounds **46**, **49**, **54**, and **56** carried out as shown in **Scheme 14**. Thus, methyl 2,3-*O*-methyl,4,6-*O*-*p*-methoxybenzylidene-α-D-glucopyranoside **66**[160] was treated with sodium cyanoborohydride in the presence of HCl[161-162] to give 71% of the 6-*O*-*p*-methoxybenzyl ether **67,** followed by the oxidation using the Dess-Martin protocol[163] to give ketone **68** in 85% yield. Grignard reaction with allylmagnesium chloride in THF at 0 °C then gave the 4-*C*-allyl galacto- and glucopyranosides **69** and **70** in 54 and 19% yield, respectively. The galacto- and gluco isomers were separately methylated by

treating them with sodium hydride and methyl iodide to give compounds **71** and **72**. The *p*-methoxybenzyl groups were cleaved with CAN to give alcohols **73** and **74**. Dess Martin oxidation of the galacto-isomer **73** gave an aldehyde **75**, which was then treated with vinylmagnesium bromide in THF at -78 °C to give an 81% overall yield of an inseparable mixture (1.5:1) of the allylic alcohols **77** and **78**. The mixture of two isomers, **77** and **78**, was subjected to a sequence of reactions including ring-closing metathesis with the second generation Grubbs catalyst[164] to give **79** and **80** in 60% and 37%, respectively. The isomers were individually subjected to hydrogenation to give **81** and **82**, followed by methylation with sodium hydride and methyl iodide to give **49** and **46**, respectively. The configuration at the 6-position of the bicyclic compounds **49** and **46** was assigned with the help of ^{1}H-NMR spectral analysis. Thus, a comparative ^{1}H-NMR analysis between compound **46** and **49** over three solvents reveals that two compounds have average $^{3}J_{H5,H6}$ coupling constants of 3.2 Hz and 9.2 Hz, respectively. Based on the magnitude of the coupling constants, the smaller $^{3}J_{H5,H6}$ coupling constant of compound **46** asigned to the axial-equatorial (a,e) coupling between equatorial H^{6} and H^{5} proton, while the larger $^{3}J_{H5,H6}$ coupling constant of compound **49** is assigned to the axial-axial (a,a) coupling between axial H^{6} and H^{5} proton. Therefore, equatorial H^{6} of compound **46** and axial H^{6} of compound **49** are then assigned as H^{6S} and H^{6R}, respectively. The strong nOe correlation between methoxy groups at 4- and 6-positions of compound **46**, indicating 1,3-diaxial type interractions, also reveals the configuration of the methoxy group at 6-position (**Figure 22**).

Analogous to the synthesis of compounds **46** and **49**, the gluco-isomer **74** was used to synthesize the stereoisomers, **54** and **56**. Thus, oxidation of alcohol **74** gave an

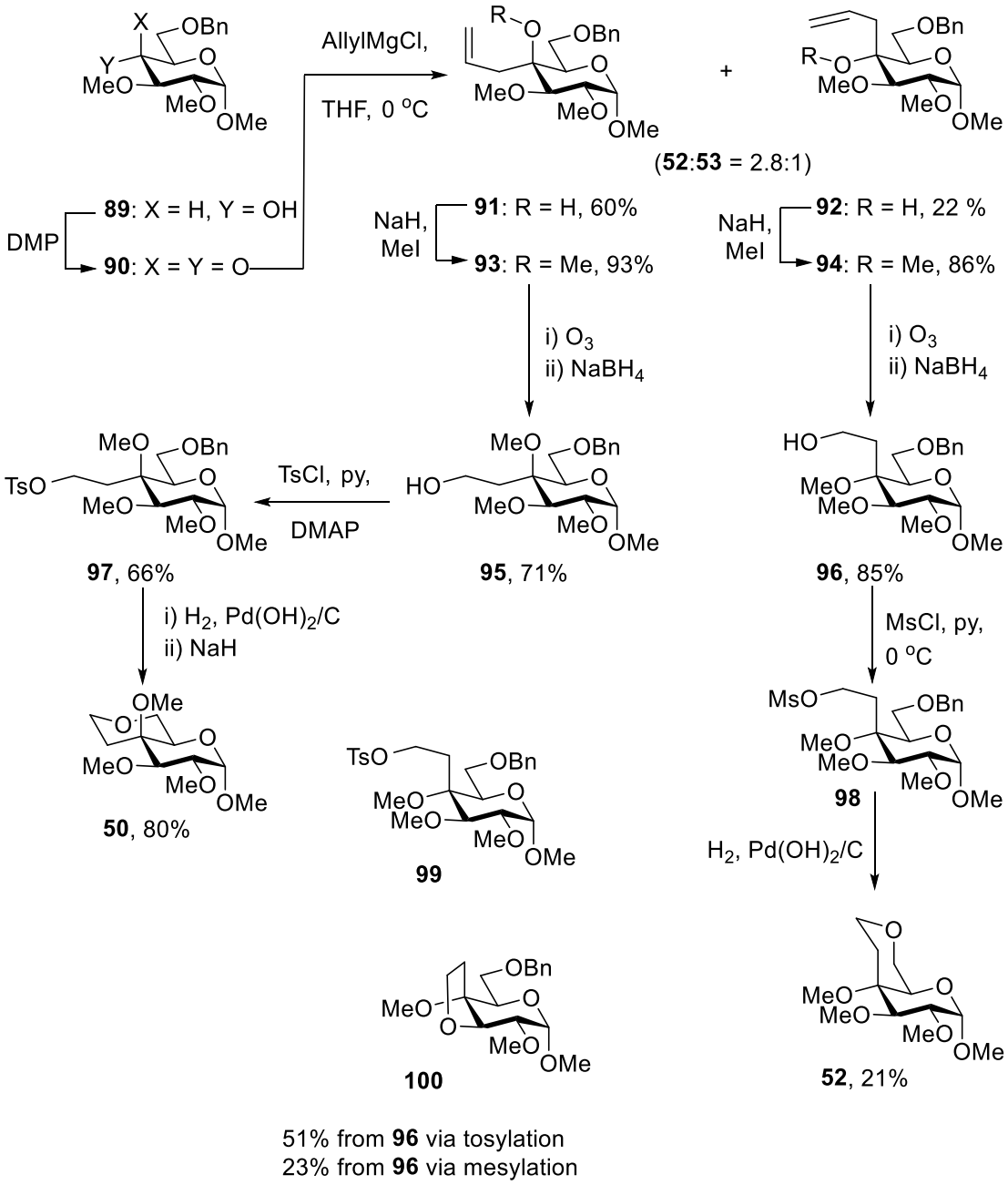
aldehyde **76**, followed by the treatment with vinyl magnesium bromide gave diastereoisomers **83** and **84** in 12 and 48% yields, respectively. Compounds **83** and **84** were then individually subjected to a sequence of ring-closing metathesis reactions to obtain **85** and **86**, respectively. The hydrogenation of individual isomers **85** and **86** gave alcohols **87** and **88**, respectively, and finally, the methylation of **87** and **88** gave **54** and **56**, respectively. Following a similar type of ^{1}H-NMR analysis as for bicyclic models **46** and **49**, the relative configurations at the 6-position of compounds **54** and **56** were assigned.



Scheme 14. Synthesis of the Bicyclic Pyranosides, **46**, **49**, **54**, and **56**.

Compounds **50** and **52** were synthesized as described in **Scheme 15**. Thus, Dess Martin oxidation of methyl 6-*O*-benzyl-2,3-di-*O*-methyl-α-D-glucopyranoside **89**[165] gave ketone **90**, which was then subjected to Grignard addition with allylmagnesium chloride in THF at 0 °C to obtain the 4-*C*-allyl galacto and 4-*C*-allyl-glucopyranosides **91** and **92** in 60% and 22% yield, respectively. The individual isomers were separately methylated using sodium hydride and methyl iodide to give the methyl ethers **93** and **94**. The compounds **93** and **94** were separately subjected to ozonolysis followed by reductive work up to give the 4-*C*-(2-hydroxyethyl) derivatives **95** and **96**, respectively, in good yields. The galacto-isomer **95**was treated with toluenesulfonyl chloride in pyridine to give the tosylate **97** in 66% yield. Tosylate **97** was then subjected to hydrogenolysis, and subsequent treatment with sodium hydride gave **50** in 80% yield. Following a similar sequence of reactions via mesylate **98**, the gluco-isomer **96** was transformed to the bicyclic compound **52** (**Scheme 15**). Attempted tosylation of **96** using different reaction conditions gave unstable tosylate **99** that decomposed on silica gel column chromatography and on standing to give *cis*-fused dioxyabicyclo-[4.3.0]-nonane derivative **100** in varying yields (**Scheme 15**). Compound **100** was also observed during the mesylation-hydrogenation-ring closing sequence of compound **96**, as a single diastereoisomer. The structure of **100** was deduced based on the HMBC and multiple nOe measurements. Thus, 2D-HMBC-NMR analysis of compound **100** showed correlations between H3 and H7 protons and H3 and H8 protons, indicating that C8 is attached to the oxygen at the 3-position, given that C4 is linked to the C7 position (**Figure 20**). In addition, the nOe analysis of the compound **100** revealed the correlations of H7

with H2 and H^{6R} protons and H7' with H^{6R} and H^{6S} protons and the methyl group at O5, supporting the proposed structure of compound **100** (**Figure 21**).



Scheme 15. Synthesis of the Bicyclic Pyranosides **50** and **52**.

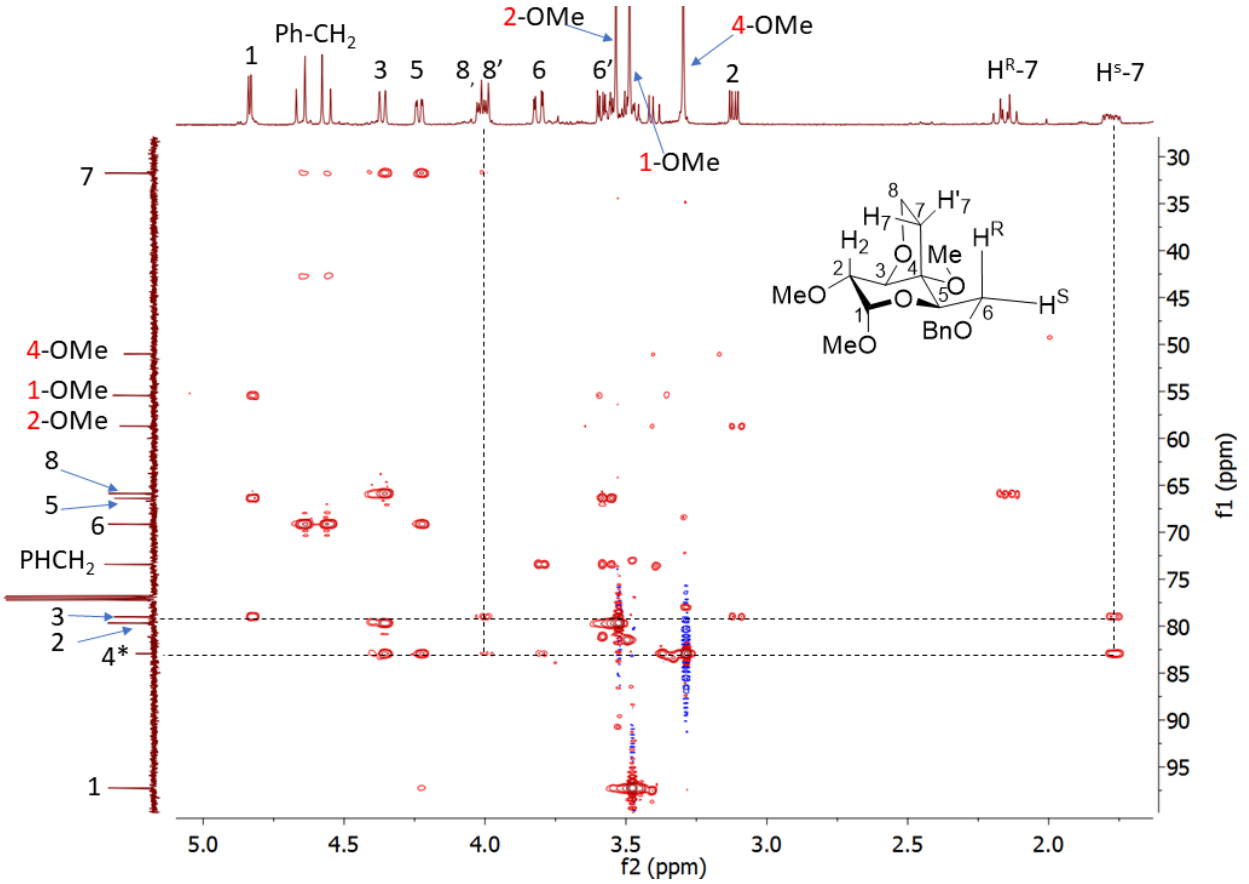


Figure 20. HMBC Correlations of Compound **100**.

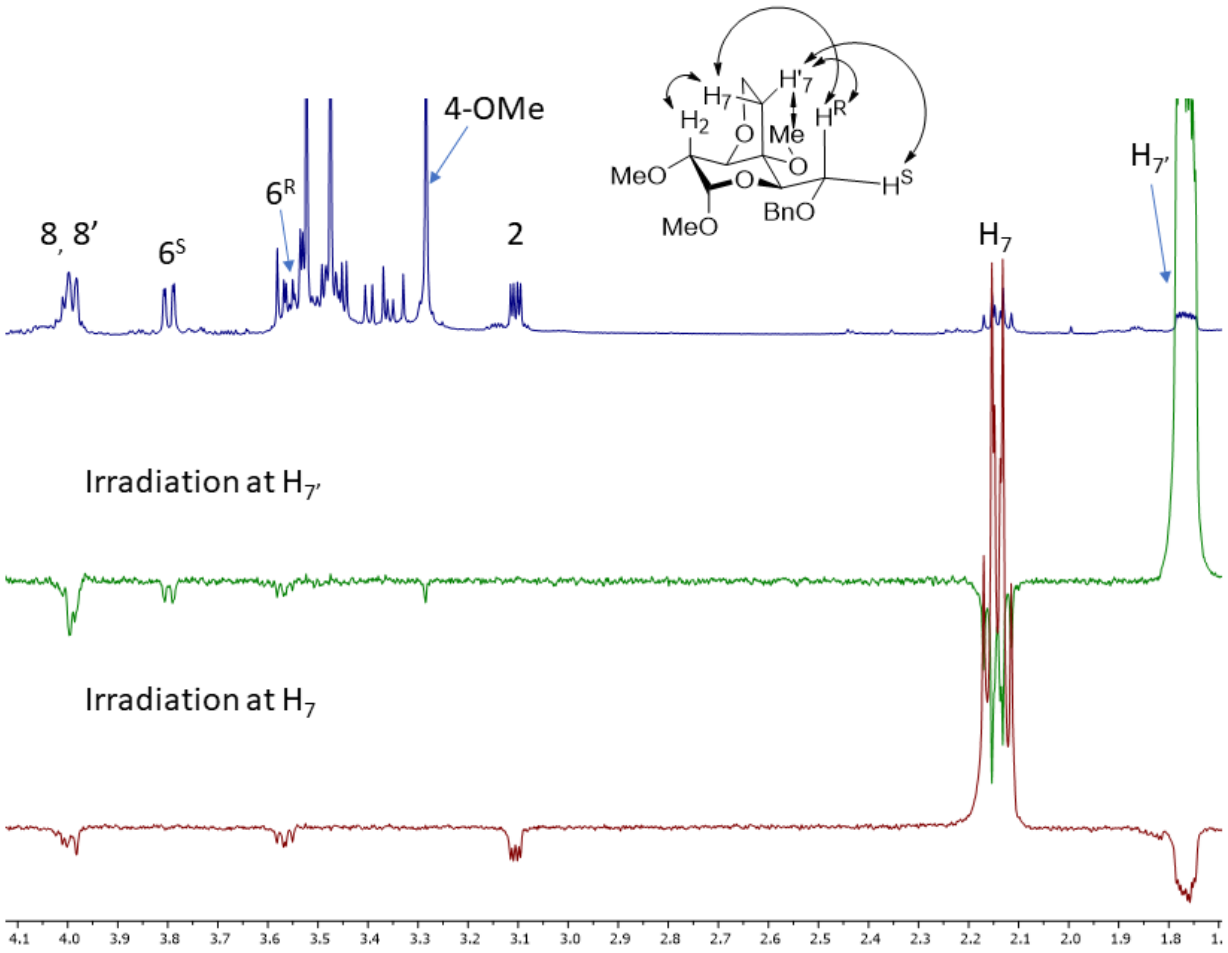


Figure 21. nOe Correlations of Compound **100**.

2.2.2 Determination of Coupling Constants and Influence of Solvent and Protecting Groups on the Coupling Constants

The ^{1}H-NMR spectra of model compounds **46**-**58** were recorded in C_6D_6, $CDCl_3$, and CD_3OD. All the coupling constants were extracted from the experimental spectra, except for those compounds with second-order spectra complicated by the presence of virtual couplings. In such cases, first order coupling constants were extracted from simulated ^{1}H-NMR spectra with the help of the spectral simulation tool, DAISY in the Bruker Topspin 3.5 software package. Analysis of the total set of coupling constants for compounds **46**-**58** shows minimal to no effect on the coupling constants across the three

solvents. Therefore, ^{1}H-NMR spectra of compounds **59**-**63** were only recorded in $CDCl_3$. In addition, $^{3}J_{H1,H2}$ coupling constants obtained in $CDCl_3$ for the benzylidine and 2,3-*O*-benzyl protected compounds **58** and **61** do not differ significantly from the $^{3}J_{H1,H2}$ coupling constants reported by Bock and Duus for the parent compounds, **39** and **38**, in D_2O. These observations suggest that the inclusion of a benzylidine ring or 2,3-*O*-benzyl protection or different NMR solvents does not have a significant influence on $^{3}J_{H1,H2}$ coupling constants of **38**, **39**, **58**, and **61** (**Table 8** and **9**).

The complete set of coupling constants for compounds **46**-**57** and **58-63** are tabulated in **Tables 8** and **9**, respectively. Further, $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants of the compounds **46**-**58** and **61**, and literature compounds **38**-**45**, are categorized into three **Tables 10**, **11**, and **12**, wherein each table consists of two sub sections: a first section for the $^{3}J_{H5,H6R}$ coupling constants and a second section for the $^{3}J_{H5,H6S}$ coupling constants. Moreover, in **Tables 10-12**, the coupling constants of the galacto-configured compounds precede those of the gluco-compounds.

Table 8. Coupling Constants ($^3J_{H,H}$ and $^2J_{H,H}$) Recorded in C_6D_6, $CDCl_3$ and CD_3OD for **46**-**57**.

Entry	Cmpd	Solvent	Coupling Constant $^3J_{H,H}$ and $^2J_{H,H}$																				
			1,2	2,3	3,4	4,5	5,6R	5,6S	6R, 7ax	6R, 7eq	6S, 7ax	6S, 7eq	6-vis	7a, 8ax	7ax, 8eq	7eq, 8ax	7eq, 8eq	7-vis	8ax, 9ax	8ax, 9eq	8eq, 9ax	8eq, 9eq	8-vis	9-vis
1	**46**	C_6D_6	3.7	10.0	-	-	-	3.2	-	-	3.1	3.1	-	13.5	3.2	2.9	2.9	14.3	13.5	3.4	3.2	3.2	13.4	14.6
		$CDCl_3$	3.8	10.0	-	-	-	3.0	-	-	3.1	3.1	-	14.1	3.3	2.8	2.8	14.3	13.7	3.4	3.3	3.3	13.5	14.7
		CD_3OD	3.8	10.1	-	-	-	3.2	-	-	3.3	3.3	-	14.0	3.0	3.3	3.3	14.4	13.3	3.1	2.8	2.8	13.3	14.7
2	**47**	C_6D_6	3.5	10.1	3.6	1.1	1.9	1.6	-	-	-	-	12.2	-	-	-	-	-	-	-	-	-	-	-
		$CDCl_3$	3.5	10.1	3.5	1.3	1.8	1.8	-	-	-	-	12.2	-	-	-	-	-	-	-	-	-	-	-
		CD_3OD	2.4	N/D	2.2	1.1	1.7	1.6	-	-	-	-	12.5	-	-	-	-	-	-	-	-	-	-	-
3	**48**	C_6D_6	3.7	10.0	3.2	~0.8	-	3.2	-	-	11.8	4.5	-	12.7	4.8	2.2	1.8	12.5	-	-	-	-	11.6	-
		$CDCl_3$	3.7	10.0	3.2	~0.8	-	3.2	-	-	11.9	4.5	-	12.6	4.8	2.2	1.7	12.4	-	-	-	-	11.7	-
		CD_3OD	3.7	10.1	3.2	~0.7	-	3.1	-	-	12.0	4.6	-	12.5	4.9	2.0	1.7	12.4	-	-	-	-	11.7	-
4	**49**	C_6D_6	3.7	10.0	-	-	9.2	-	10.9	4.8	-	-	-	13.5	4.5	3.7	2.9	12.6	13.5	3.4	4.4	2.8	13.5	14.6
		$CDCl_3$	3.8	10.0	-	-	9.2	-	10.8	4.9	-	-	-	13.6	4.4	3.6	2.9	12.9	13.7	3.3	4.0	2.6	13.7	14.6
		CD_3OD	3.8	10.1	-	-	9.2	-	10.8	4.8	-	-	-	13.7	4.4	3.8	2.9	12.8	13.7	3.3	4.1	2.6	13.7	14.7
5	**50**	C_6D_6	3.7	10.0	-	-	4.8	10.5	-	-	-	-	10.0	12.6	2.1	5.0	1.4	11.4	-	-	-	-	14.6	-
		$CDCl_3$	3.6[a]	9.9[a]	-	-	4.4[a]	10.1[a]	-	-	-	-	9.7[a]	12.7	2.1	5.0	1.5	11.5	-	-	-	-	14.7	-
		CD_3OD	3.7[a]	9.9[a]	-	-	4.7[a]	10.3[a]	-	-	-	-	9.8[a]	12.7	2.1	5.0	1.5	11.5	-	-	-	-	14.9	-
6	**51**	C_6D_6	3.6	10.1	3.4	1.1	3.0	-	2.8	2.9	-	-	-	13.0	7.6	2.8	1.1	14.1	-	-	-	-	N/D	-
		$CDCl_3$	3.6	10.1	3.4	1.1	3.1	-	2.8	3.7	-	-	-	13.1	5.3	2.6	1.3	14.3	-	-	-	-	11.4	-
		CD_3OD	3.6	10.1	3.4	1.1	3.1	-	2.9	3.0	-	-	-	13.0	5.4	2.1	1.2	14.3	-	-	-	-	11.3	-
7	**52**	C_6D_6	3.7[a]	9.5[a]	-	-	1.7[a]	1.6[a]	-	-	-	-	N/D	N/D	N/D	N/D	N/D	11.0	-	-	-	-	N/D	-
		$CDCl_3$	3.8	9.5	-	-	1.7	1.6	-	-	-	-	12.4	N/D	N/D	N/D	N/D	11.2	-	-	-	-	N/D	-
		CD_3OD	3.8	9.5	-	-	1.7	1.6	-	-	-	-	12.4	N/D	N/D	N/D	N/D	11.3	-	-	-	-	N/D	-
8	**53**	C_6D_6	3.6	9.4	9.2	10.1	-	2.6	-	-	2.5	3.6	-	12.7	5.4	2.5	1.5	14.1	-	-	-	-	11.4	-
		$CDCl_3$	3.6	9.4	8.9	10.1	-	2.6	-	-	2.2	3.6	-	12.7	5.5	2.2	1.5	14.5	-	-	-	-	11.6	-
		CD_3OD	3.7	9.5	9.0	10.1	-	2.6	-	-	2.4	3.6	-	12.8	5.6	2.2	1.5	14.5	-	-	-	-	11.5	-
9	**54**	C_6D_6	4.0	9.4	-	-	-	3.0	-	-	11.0	~ 5	-	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
		$CDCl_3$	3.9	9.5	-	-	-	3.1	-	-	11.7	4.4[b]	-	12.8	3.6	3.0	3.4	11.9	13.1	3.3	3.9	3.4	12.3	13.7
		CD_3OD	4.0	9.5	-	-	-	3.1	-	-	11.6	4.5	-	12.7	3.4	3.1	N/D	11.9	12.6	3.1	3.4	N/D	12.3	14.1
10	**55**	C_6D_6	3.7	9.2	9.2	9.7	9.2	-	11.0	5.1	-	-	-	12.7	5.2	2.4	1.6	13.1	-	-	-	-	11.8	-
		$CDCl_3$	3.7	9.3	9.3	9.6	9.0	-	11.0	5.1	-	-	-	12.9	5.3	2.3	1.5	13.2	-	-	-	-	11.9	-
		CD_3OD	3.8	9.4	9.3	9.4	9.2	-	10.8	4.8	-	-	-	12.8	5.2	2.3	1.6	13.2	-	-	-	-	11.9	-
11	**56**	C_6D_6	3.8	9.6	-	-	2.8	-	2.8	2.8	-	-	-	13.9	3.7	3.1	3.2	13.9	13.3	3.3	3.6	3.4	13.2	14.1
		$CDCl_3$	3.8	9.6	-	-	2.8	-	2.8	2.8	-	-	-	13.5	3.6	3.3	3.3	14.0	13.3	3.3	3.7	3.2	13.0	14.1
		CD_3OD	3.8	9.6	-	-	2.7	-	2.7	2.7	-	-	-	13.6	3.8	3.0	3.0	14.0	13.1	3.3	3.8	3.3	13.3	14.1
12	**57**	C_6D_6	3.7	9.2	9.2	9.6	4.9	10.3	-	-	-	-	10.2	-	-	-	-	-	-	-	-	-	-	-
		$CDCl_3$	3.7	9.2	9.3	9.6	4.7	10.1	-	-	-	-	10.2	-	-	-	-	-	-	-	-	-	-	-
		CD_3OD	3.6[a]	8.5[a]	8.2[a]	9.2[a]	4.7[a]	10.2[a]	-	-	-	-	9.5[a]	-	-	-	-	-	-	-	-	-	-	-

[a]simulated coupling constants using DAISY 1D spectra simulation tool in TopSpin 3.5 pl 7 software package; [b]coupling constant recorded with the help of TOCSY-1D experiment.

Table 9. Coupling Constants ($^3J_{H,H}$ and $^2J_{H,H}$) Recorded in C_6D_6, $CDCl_3$ and CD_3OD for **58**-**63.**

Entry	Cmpd	Solvent	Coupling Constant $^3J_{H,H}$ and $^2J_{H,H}$								
			1_{ax}, 2	1_{eq}, 2	1-vis	2,3	3,4	4,5	5,6R	5,6S	6-vis
1	**58**	C_6D_6	5.7	10.6	11.2	8.7	9.1	9.3	5.0	10.2	10.3
		$CDCl_3$	5.7	10.5	11.3	8.9	9.1	9.3	5.0	10.2	10.4
		CD_3OD	5.6	10.5	11.2	8.7	9.1	9.3	5.0	10.2	10.3
2	**59**	$CDCl_3$	9.6	-	-	8.4	9.3	9.3	5.0	10.3	10.4
3	**60**	$CDCl_3$	5.8	-	8.6	8.6	N/D	N/D	N/D	N/D	N/D
4	**61**	$CDCl_3$	1.1	2.2	12.5	3.3	9.9	9.5	4.8	9.9	10.3
5	**62**	$CDCl_3$	1.1	-	-	2.9	9.9	9.5	4.8	9.9	10.3
6	**63**	$CDCl_3$	-	1.5	-	3.3	9.9	9.5	4.8	9.9	10.3

Table 10. $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ Coupling Constants for *gg* Conformers.

Entry	Cmpd (bond)[a]	Config	Ring fusion	C6 Subst	Correction (Hz)[b]	Experimental and (corrected) $^{3}J_{H5,H6}$ values (Hz)			Comment[c]
						C_6D_6	$CDCl_3$	CD_3OD	
					H^5,H^{6R}				
1	**47**	galacto	cis	CH_2	na	1.9	1.8	1.7	exclude
2	**52**	gluco	cis	CH_2	na	1.7	1.7	1.7	exclude
3	**61**(C1,C2)	-	-	CH_2	na	nd	1.1	nd	include
4	**38**(C1,C2)	-	-	CH_2	na	0.9[d]			include
5	**41**	galacto	cis	CH_2	na	1.8[d]			exclude
6	**44**	gluco	-	CH_2	na	5.4			exclude
					H^5,H^{6S}				
7	**46**	galacto	trans	CH	-0.5	3.2 (2.7)	3.0 (2.5)	3.2 (2.7)	exclude
8	**47**	galacto	cis	CH_2	na	1.6	1.8	1.6	exclude
9	**53**	gluco	trans	CH	-0.5	2.6 (2.1)	2.6 (2.1)	2.6 (2.1)	include
10	**52**	gluco	cis	CH_2	na	1.6	1.6	1.6	exclude
11	**61**	-	-	CH_2	na	nd	2.2	nd	include
12	**38**(C1,C2)	-	-	CH_2	na	2.2[d]			include
13	**41**	-	cis	CH_2	na	1.8[d]			exclude
14	**44**	gluco	-	CH_2	na	1.2			exclude

a) Unless otherwise stated, the C5-C6 bond is considered. b) Deduced correction factor for the presence of the additional C-C bond. c) Validity of compounds for further consideration as discussed in the text. d) Measured in D_2O.

Table 11. $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ Coupling Constants for *gt* Conformers.

Entry	Cmpd (bond)[a]	Config.	Ring fusion	C6 Subst	Correction (Hz)[b]	Experimental and (corrected) $^{3}J_{H5,H6}$ values (Hz)			Comment[c]
						C_6D_6	$CDCl_3$	CD_3OD	
H^5,H^{6R}									
1	**49**	galacto	trans	CH	+2.0	9.2 (11.2)	9.2 (11.2)	9.2 (11.2)	include
2	**55**	gluco	trans	CH	+2.0	9.2 (11.2)	9.0 (11.0)	9.2 (11.2)	include
3	**42**(C1,C2)	-	-	CH_2	na	10.7[d]			include
4	**45**	gluco	-	CH_2		9.5			exclude
H^5,H^{6S}									
5	**48**	galacto	cis	CH	-0.5	3.2 (2.7)	3.2 (2.7)	3.1 (2.6)	include
6	**54**	gluco	cis	CH	-0.5	3.0 (2.5)	3.1 (2.6)	3.1 (2.6)	include
7	**42**(C1,C2)	-	-	CH_2	na	2.5[d]			include
8	**45**	gluco		CH_2	na	1.5			exclude

a) Unless otherwise stated, the C5-C6 bond is considered. b) Deduced correction factor for the presence of the additional C-C bond. c) Validity of compounds for further consideration as discussed in the text. d) Measured in D_2O.

Table 12. $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ Coupling Constants for *tg* Conformers.

Entry	Cmpd (bond)[a]	Config.	Ring fusion	C6 Subst	Correction (Hz)[b]	Experimental and (corrected) $^{3}J_{H5,H6}$ values (Hz)			Comment[c]
						C_6D_6	$CDCl_3$	CD_3OD	
H^5,H^{6R}									
1	**50**	galacto	trans	CH_2	na	4.8	4.4	4.7	include
2	**51**	galacto	cis	CH	0	3.0	3.1	3.1	exclude
3	**56**	gluco	cis	CH	0	2.8	2.8	2.7	exclude
4	**57**	gluco	trans	CH_2	na	4.9	4.7	4.7	include
5	**58** (C1,C2)	-	trans	CH_2	na	5.7	5.7	5.6	exclude
6	**39**	-	-	CH_2	na	5.5[d]			exclude
7	**40**	-	-	CH_2	na	5.6[d]			exclude
8	**43**	gluco	trans	CH_2	na	5.0[d]			include
9	**58** (C5,C6)	gluco	trans	CH_2	na	5.0	5.0	5.0	include
H^5,H^{6S}									
10	**50**	galacto	trans	CH_2	na	10.5	10.1	10.3	include
11	**57**	gluco	trans	CH_2	na	10.3	10.1	10.2	include
12	**58** (C1,C2)	-	trans	CH_2	na	10.6	10.5	10.5	exclude
13	**39**	-	-	CH_2	na	10.8[d]			exclude
14	**40**	-	-	CH_2	na	~10[d]			exclude
15	**43**	gluco	trans	CH_2	na	~10.3[d]			include
16	**58** (C5,C6)	gluco	trans	CH_2	na	10.2	10.2	10.2	include

a) Unless otherwise stated, the C5-C6 bond is considered. b) Deduced correction factor for the presence of the additional C-C bond. c) Validity of compounds for further consideration as discussed in the text. d) Measured in D_2O.

2.2.3 Analysis of Bicyclic Models 9-20.

The validity of these bicyclic model compounds, representing the three ideal staggered conformations of the pyranose side chain, critically depends on the conformational purity of their bicyclic framework (pure chair-chair conformation) as shown in **Figure 18**. Out of twelve compounds (**Figure 18**), six have the *trans*-decalin configuration. Since there are no significant variations in the coupling constant pattern (**Table 8**), compounds **49**, **50**, **53**, **55**, and **57** are considered as pure staggered conformers. Further, X-ray crystal structures of literature compounds, (e.g., CCDC: HEGREQ) which have < 2° deviation from the dihedral angle of the ideal *tg* conformation (about C5-C6 bond), further reveals that compound **57** is a suitable model for the ideal *tg* conformation. Comparison of the coupling constant pattern, particularly, axial-equatorial (a,e) coupling constants between H7 and H8, and H8 and H9, of the compound **49** with its diastereoisomer **46** (**Table 8**), reveals that there is a considerable discrepancy between those coupling constants. These differences may arise due to the plausible steric interactions of 1,3-diaxial methoxy groups present at C4 and C6 and/or the unfavorable dipolar interactions of those two methoxy groups present in compound **46**. Despite the deviations in coupling constants, nOe relationships indicate that compound **46** has a chair-like conformation (**Figure 22**). However, the considerable differences in coupling constants between compounds **46** and **49** provide enough evidence to conclude that compound **46** is not a suitable model for the ideal *gg* conformation, as the 1,3-diaxial interactions are sufficient to distort the bicyclic ring of compound **46**. Extrapolating from these observations, it can be predicted that the 4,6-di-*O*-methylated galactopyranoside and its derivatives have a very low or zero population of *gg* conformation.

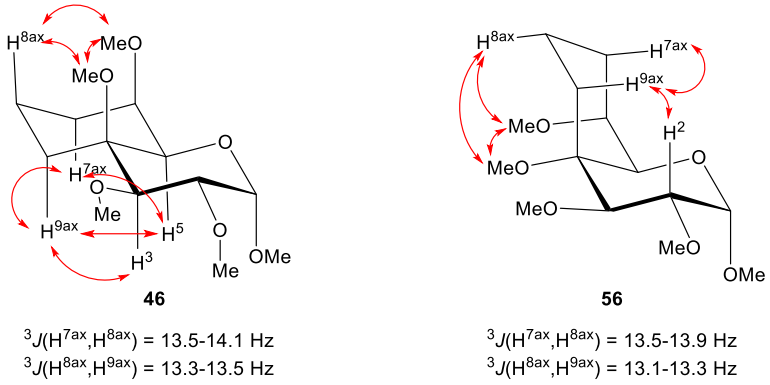


Figure 22. nOe Interactions and Coupling Constants (Mean Values Across the Three Solvents) for **46** and **56**.

On the other hand, *cis*-decalins **47**, **48**, **51**, **52**, **54**, and **56** are considered as more conformationally mobile systems due to the *cis*-fused ring junction of the two rings, and therefore, need careful examination. Despite the fact that the deviations in the coupling constants of the *cis*-decalin models are minimal (**Table 9**), previous crystalographic data from the two 4,6-*O*-benzylidine protected galactopyranoside[166] reveal the torsion angles of 67° and 68° for O5-C5-C6-O6 and of 52° and 52° for C4-C5-C6-O6, are enough evidence for the exclusion of benzylidine acetals **47** and the literature model **41** from further consideration as the ideal *gg* conformations. Since the $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants of compound **52** are almost identical with those of compound **47** (**Table 10**), compound **52** is also considered as a non-reliable model of the ideal *gg* conformation. According to the full set of coupling constant analysis of compounds **54** and **56** (**Table 8**), there are only minor differences in their conformations. Further, the nOe analysis of compound **56** does not indicate any evidence for the distortion of the structure of **56** from the ideal *cis*-bicyclic framework (**Figure 22**). However, the $^{3}J_{H5,H6R}$ coupling constant of **56** has a significant deviation from the corresponding coupling constants measured from *trans*-bicyclic models, **50** and **57**, leading to the exclusion of compound

56 from consideration. Moreover, compound **51** has a $^{3}J_{H5,H6R}$ coupling constant comparable to that of compound **56**, even after applying the suitable correction factors for the presence of an additional C-C bond (the derivation of correction factors is discussed below). Therefore, compound **51** is also excluded from further consideration. Importantly, *gt* models **48** and **54** have similar $^{3}J_{H5,H6S}$ coupling constants, and also those values are comparable to the corresponding coupling constants of the literature compound **42**, leading to the inclusion of *cis*-decalin derivatives **48** and **54** in the final data set. Overall, out of six *cis*-fused bicyclic models, compounds **47**, **51**, **52**, and **56** were excluded from further consideration, leaving compounds **48** and **54** for further analysis.

Another interesting observation from analysis of the coupling constants is that regardless of the *cis*- or *trans*-fused nature of the ring junction, the $^{3}J_{H2,H3}$ coupling constants of galacto compounds **46**-**51** are 10.0 ± 0.1 Hz, while gluco-configured compounds **52**-**57** show only 9.4 ± 0.2 Hz for the corresponding coupling constants (**Table 8**). The ~0.5 Hz increase in the galacto-series can be explained by the β-effect noted by Altona and Haasnoot, who noted that when one of the two hydrogens of a pair of antiperiplanar hydrogens is also antiperiplanar to an electron-withdrawing C-X bond in the β-position of antiperiplanar spin system, the coupling constant between two antiperiplanar hydrogens increases (**Figure 23**).[167]

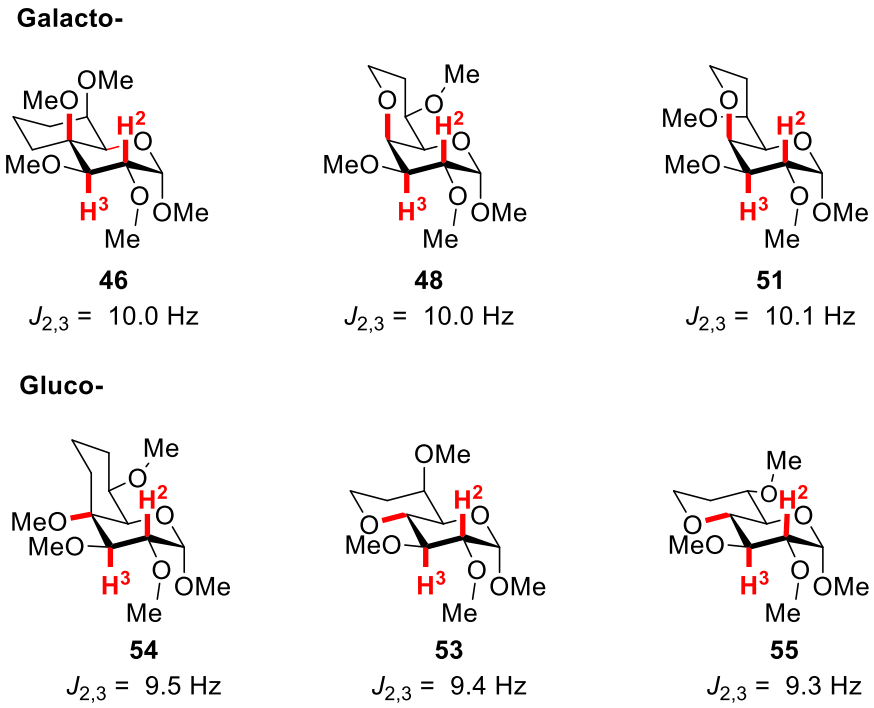
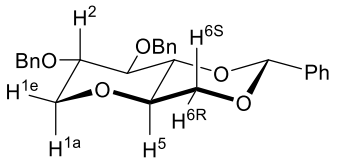


Figure 23. Comparison of $^{3}J_{2,3}$ Vicinal Coupling Constants between Selected Galacto-Models (with the Altona-Haasnoot β-Effect) and Gluco-Models (without the β-Effect).

2.2.3.1 Comparison of the 1,5-Anhydrohexitols and the 4,6-*O*-Acetals as Models for the *tg* Conformation

Two different sets of limiting coupling constants are available for the *tg* conformation. One can be extracted from the H1,H2 spin system and the other from the H^5,H^6 spin system. Comparison of the *tg* models **39** and **40** with the 4,6-*O*-alkylidene model **43** indicates that there are considerable differences between the two sets of coupling constants. Further, the internal comparison of those two sets of coupling constants within compound **58** also reveals clear differences in the coupling constants between the two types of spin systems (**Figure 24**).



$^3J_{H1e,H2}$ = 5.7 Hz, $^3J_{H1a,H2}$ = 10.5 Hz
$^3J_{H5,H6R}$ = 5.0 Hz, $^3J_{H5,H6S}$ = 10.2 Hz

Figure 24. Internal Comparison of $^3J_{H,H}$ Coupling Constants in the 4,6-*O*-Benzylidene Protected 1,5-Anhydrohexitol **58**.

A decision must, therefore, be made as to which of the two is the most reliable model. Based on the close analogy of the H^5,H^6 spin systems between compounds **43**, **57**, **58**, and **61** and the ideal *tg* conformation of the pyranose side chain, and the similarity of the $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ coupling constants between these models and the bicyclic model **50**, the H^5,H^6 spin system of models **43**, **57**, **58**, **61**, and **50** were selected over the H1,H2 spin system of compounds **39**, **40**, and **58**. In addition, the similarity of the $^3J_{H5,H6}$ coupling constants between the acetal-containing models and compound **50** reveals that the influence of the acetal functionality on the $^3J_{H5,H6}$ coupling constants is minimal.

2.2.4 Systematic Classification and Derivation of Correction Factors for the Use of Tetrasubstituted Stereodiads as Models for the Trisubstituted Side Chain of Pyranosides.

The exocyclic C6-O6 bond of the hexopyranose side chain is trisubstituted whereas the bicyclic model compounds **46**, **48**, **49**, **51**, **53**-**56** are tetrasubstituted. Therefore, the influence of the extra C-C bond of the tetrasubstituted models should be considered. According to Altona and Haasnoot, extra substituents do not require a correction for (a,e) and (e,e) coupling constants. However, in one of their two alternative interpretations of their data set, they proposed a + 0.4 Hz correction factor for the (a,a) coupling constant.[167] In the coupling constant analysis of compounds **46**-**57**, considerable

differences are observed between the tri- and tetrasubstituted spin systems with the same side chain conformation, necessitating careful attention to the influence of the additional C-C bond.

In order to derive correction factors with minimal systematic errors, a closer examination of the selected structures of the tri- and tetrasubstituted models is required. Thus, a systematic classification of these model compounds was considered. All the configurations of the bicyclic model compounds, whether *erythro* or *threo*, can be classified into three different Newman projections, leading to the total of six different conformations, of which II and III are pseudoenantiomeric (**Figure 25**). In addition, it is important to note that the bicyclic model compounds cannot be included in two of the configurations (I and V) shown in **Figure 25**, as those classes of configurations consist of antiperiplanar R and R' groups. However, for the completion of the data classification, those two classes are also included in this study.

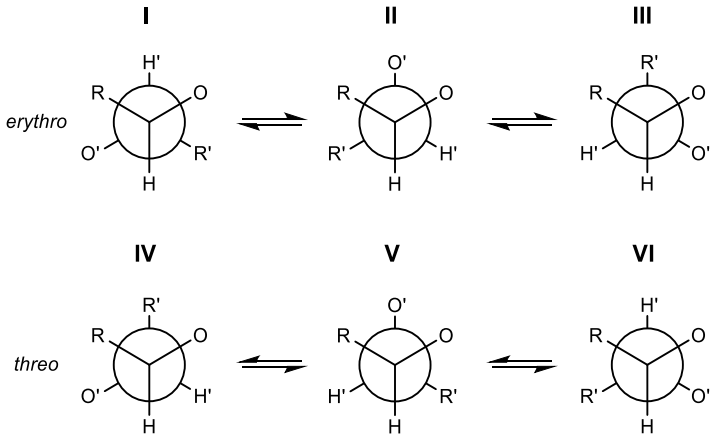


Figure 25. Staggered Conformations about the C5-C6 Bonds in the Bicyclic Models **46**-**57** and other 4,6-*O*-Acetals, the C2-C1 Bonds in the 1,5-Anhydrohexitols, and the C1-C2 Bonds in the 1,4-Dioxanes **42**, **101** and **102**. In the Tetrasubstituted Stereodiads R′ is an Alkyl Group, while in the Trisubstituted Systems R' is a Hydrogen Atom.

All staggered conformations about the C5-C6 bond of the tetrasubstituted models **46**, **48**, **49**, **51**, and **53**-**56** are categorized into Newman projections from I to VI (**Table**

13). Similarly, staggered conformations about the C5-C6 bond of trisubstituted compounds, **47**, **50**, **52**, and, **57** and about the C2-C1 bond of compounds **38**-**40**, **58**, and **61** can be categorized into Newman projections I to VI (**Table 13**). In the trisubstituted systems, R' is a hydrogen atom; therefore, they consist of only one stereogenic center. Consequently, for trisubstituted systems, projections I and IV are identical as are II and V, and III and VI. In addition, for comparison purposes, two other literature compounds, *cis*-2,3-dimethyl-1,4-dioxane **101** and *trans*-2,3-dimethyl-1,4-dioxane **102**, whose Newman projection about C1-C2 bonds belong to projections III and VI, respectively, were considered (**Figure 26**).[168-169] It is important to note that the vicinal coupling constants between chemically identical protons of these two compounds were obtained by the analysis of ^{13}C side bands at low temperature.[168-169]

101: *J*a,e' = 3.2 Hz

102: *J*a,a' = 8.5 Hz

Figure 26. *cis*- and *trans*-2,3-Dimethyl-1,4-dioxanes **101** and **102**.

Table 13. Assignment of Compounds According to Newman projections I-VI.

Entry	cmpd	config	projection	bond	O	R	H[a]	O'	R'	H',[a]
1	**43**, **50**, **57**, **58**, **61**	-	I (and IV)	5-6	O5	C4	H5(a)	O6	H6(e)	H6(a)
2	*ent*-**39**, *ent*-**40**, *ent*-**58**		I (and IV)	2-1	O2	C3	H2(a)	O5	H1(e)/(a)	H1(a)/(e)
3	*ent*-**59**	*erythro*	I	2-1	O2	C3	H2(a)	O5	allyl	H1(a)
4	**41**, **47**, **52**	-	II (and V)	5-6	O5	C4	H5(e)	O6	H6(a)	H6(e)
5	**46**, **53**	*erythro*	II	5-6	O5	C4	H5(a)	O6	C7	H6(e)
6	**38**, **61**	-	II (and V)	2-1	O2	C3	H2(e)	O5	H1(a)/(e)	H1(e)/(a)
7	**63**	*erythro*	II	2-1	O2	C3	H2(e)	O5	allyl	H1(e)
8	**42**	-	III (and VI)	1-2	O1	CH_2OH	H1(a)	O2	H2(a)/(e)	H2(e)/(a)
9	*ent*-**46**, *ent*-**53**	*erythro*	III	6-5	O6	C7	H6(e)	O5	C4	H5(a)
10	**48**, **54**	*erythro*	III	5-6	O5	C4	H5(e)	O6	C7	H6(a)
11	**101**	*erythro*	III	1-2	O1	Me	H1(a)	O2	Me	H2(e)
12	**51**, **53**	*threo*	IV	5-6	O5	C4	H5(a)	O6	C7	H6(e)
13	*ent*-**60**	*threo*	IV	2-1	O2	C3	H2(a)	O5	allyl	H1(e)
14	**62**	*threo*	V	2-1	O2	C3	H2(e)	O5	allyl	H1(a)
15	**49**, **55**	*threo*	VI	5-6	O5	C4	H5(a)	O6	C7	H6(a)
16	**102**	*threo*	VI	1-2	O1	Me	H1(a)	O2	Me	H2(a)

[a](a) and (e) refer to the axial or equatorial position of H and H' about the six-membered ring which includes the central C-C bond of the Newman projection.

Comparison of the models from **Table 13** entries 2 and 3, affords the correction factor for (a,a) coupling constant about the C1-C2 bonds of tri- and tetrasubstituted compounds of category I. Similarly, comparison of the models from **Table 13** entries 6 and 7, entries 8 and 11, entries 1 and 13, entries 6 and 14, and entries 8 and 16 leads to the correction factors for the classes II to VI (**Table 14**). In these comparisons, systematic errors are minimized as the correction factors are derived within the same class of compounds. The correction factors range from -0.5 Hz to +2.0 Hz. The largest value belongs to (a,a) couplings and is consistent with the suggestions of Altona and Haasnoot.[167] Finally, these derived correction factors are applied to the coupling constants measured from the model compounds (**Table 10-12**).

Table 14. Derivation of Correction Factors for Use with Tetrasubstituted Models.

Config	Project	Type	Correction Derivation		Correction (Hz)[a]	Relevant Models
			Trisubs cmpd, *J* (Hz)	Tetrasubs cmpd, *J* (Hz)		
erythro	I	(a,a)	**58**, 10.5	**59**, 9.6	+1.0	-
erythro	II	(e,e)	**61**, 2.2	**63**, 1.5	+0.5	-
erythro	III	(a,e)	**42**, 2.5	**101**, 3.2	-0.5	**46**, **48**, **53**, **54**
Threo	IV	(a,e)	**58**, 5.7	**60**, 5.8	0	**51**, **56**
Threo	V	(a,e)	**61**, 1.1	**62**, 1.1	0	-
Threo	VI	(a,a)	**42**, 10.7	**102**, 8.5	+2.0	**49**, **55**

[a]Correction factors are rounded to the nearest 0.5 Hz.

2.2.5 Selection of *gg*, *gt*, and *tg* Model Compounds and Limiting Coupling Constants

2.2.5.1 *gg* Conformation

According to the discussion in **Section 2.2.3**, literature compound **41** and the bicyclic models **46**, **47**, and **52** are considered as non-ideal models of the pure *gg* conformation and, therefore, are excluded from further consideration. Further, literature compound **44** is excluded as it has a significantly different $^{3}J_{H5,H6R}$ coupling constant from

all the others in **Table 10**, probably due to the distortions in the bicyclic structure of compound **7** as noted in **Section 2.1.** After the application of appropriate correction factors, gluco-configured *trans*-bicyclic model **53** and literature model **38** and it's benzylidene derivative **61** were selected as the appropriate models to represent the pure *gg* conformation. Thus, coupling constants 1.0 and 2.2 Hz are suggested as the limiting $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants, respectively (**Table 15, entry 1**).

2.2.5.2 *gt* Conformation

As per **Section 2.1**, the conformational purity of compound **45** is ambiguous, and the coupling constants derived from this compound are significantly different from all other models of the *gt* conformation (**Table 11**). Therefore, it is concluded that compound **45** is not a suitable model to represent the pure *gt* conformation. After the application of suitable correction factors, bicyclic compounds **49** and **55** together with the monocyclic model **42** were chosen as the best models to describe the pure *gt* conformation, and thus the derived limiting $^{3}J_{H5,H6R}$ coupling constant is 11.0 Hz (**Table 15, entry 1**). After application of the correction factors, compounds **48** and **54** are in greater agreement with the $^{3}J_{H5,H6S}$ coupling constant read from the monocyclic model **42,** and hence, the limiting $^{3}J_{H5,H6S}$ coupling constant is determined to be 2.5 Hz (**Table 15, entry 1**).

2.2.5.3 *tg* Conformation

As per the discussion in **Section 2.2.3**, compounds **51** and **56** are considered as inadequate models of the pure *tg* conformation and, therefore, are excluded from further consideration. Further, it is concluded that the H1, H2 spin system of the compounds **39**, **40**, and **58** are also inadequate models. On the other hand, the *tg* conformation about C5-C6 bonds of compounds **50, 57, 58,** and literature model **43** were considered as

suitable models to represent the *tg* conformation. Therefore, the limiting $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants of the *tg* conformation are assigned values of 4.8 and 10.2 Hz, respectively (**Table 15, entry 1**).

Table 15. Literature and Recommended Limiting Coupling Constants.

Entry	Source	$^{3}J_{H5,H6R}$			$^{3}J_{H5,H6S}$		
		gg	***gt***	***tg***	***gg***	***gt***	***tg***
1	This work	1.0	11.0	4.8	2.2	2.5	10.2
2	Haasnoot–Altona[145]	0.9	10.7	5.0	2.8	3.1	10.7
3	Bock-Duus[60]	0.9	10.7	5.5	2.2	2.5	10.7
4	Serianni (expt)[147]	1.8	10.7	5.0	1.8	2.5	10.3
5	Serianni (calc)[147]	0.8	9.9	4.5	1.3	1.5	10.8
6	Nishida[170]	1.7	10.8	4.1	2.2	2.4	11.1

2.2.6 Evaluation of Limiting Coupling Constants for the Application of Side Chain Population Calculations

2.2.6.1 Comparison of Newly Derived Limiting Coupling Constant with the Literature Values

As discussed above, several earlier set of limiting coupling constants are available for the *gg, gt*, and *tg* conformations. Those literature coupling constants were derived from the Hassnoot-Altona equation,[145] experimentally using models **38**-**40**[60] and **41**-**43**[147], and a similar set of benzylidene acetals,[170] and computationally using **41**-**43**[147] and are presented in **Table 15**. The limiting data obtained from this work is presented in **Table 15**, **entry 1**. Considering the data for $^{3}J_{H5,H6R}$, the newly introduced data for *gg*, *gt*, and *tg* conformations (**Table 15, entry 1**) closely resemble the data obtained from the Haasnoot-Altona equations (**Table 15, entry 2**), whereas the experimentally derived $^{3}J_{H5,H6R}$ coupling constant for the *gg* conformation from compound **41** and the same coupling constant computed for *gt* conformation from compound **42** deviate the most from data derived here (**Table 15, entry 4 and 5**). With regards to the $^{3}J_{H5,H6S}$ limiting coupling

constants, the data derived for compounds **38**-**40** (**Table 15, entry 3**) have the closest agreement with the data from this study.

2.2.6.1 Application of the Recommended Limiting Coupling Values in the Calculation of Hexopyranoside Side Chain Populations.

In order to evaluate the reliability of the new limiting coupling constants, the experimental $^3J_{H5,H6R}$ and $J_{H5,H6S}$ coupling constants measured by Bock and Duus[60] for methyl α-D-glucopyranoside (5.5 and 2.4 Hz), methyl β-D-glucopyranoside (6.1 and 2.5 Hz), and methyl β-D-galactopyranoside (7.5 and 4.8 Hz), respectively, were applied together with the limiting coupling constants from **Table 15** to solve equations 1-3.

$$^3J_{H5,H6R} = {}^3J_{R,gg}f_{gg} + {}^3J_{R,gt}f_{gt} + {}^3J_{R,tg}f_{tg} \quad \text{(eq. 1)}$$

$$^3J_{H5,H6S} = {}^3J_{S,gg}f_{gg} + {}^3J_{S,gt}f_{gt} + {}^3J_{S,tg}f_{tg} \quad \text{(eq. 2)}$$

$$1 = f_{gg} + f_{gt} + f_{tg} \quad \text{(eq. 3)}$$

Solution of the three equations gave the side chain populations of the test substrates. A comparison of the side chain populations calculated using new limiting coupling constants (**Table 16, entry 1**) and those which were calculated using literature limiting coupling constants (**Table 16, entries 2-6**) are presented in **Table 16**.

Table 16. Calculated Side Chain Populations Using Different Sets of Limiting Coupling Constants.

Entry	Source	Me α-D-Glc[a]			Me β-D-Glc[a]			Me β-D-Gal[a]		
		gg	*gt*	*tg*	*gg*	*gt*	*tg*	*gg*	*gt*	*tg*
1	This work	54	45	1	48	50	2	16	53	31
2	Haasnoot – Altona	57	50	-7	50	56	-6	19	58	23
3	Bock-Duus	53	47	1	46	52	2	17	54	29
4	Serianni (experimental)	56	40	4	49	47	4	16	53	31
5	Serianni (calculated)	42	47	11	35	54	12	5	59	36
6	Nishida	57	41	1	50	48	2	16	56	28

[a]Coupling constants of Bock and Duus:[60] Me α-D-Glc, $^3J_{H5,H6R}$ = 5.5 Hz, $^3J_{H5,H6S}$ = 2.4 Hz; Me β-D-Glc, $^3J_{H5,H6R}$ = 6.1 Hz, $^3J_{H5,H6S}$ = 2.5 Hz; Me β-D-Gal, $^3J_{H5,H6R}$ = 7.5 Hz, $^3J_{H5,H6S}$ = 4.8 Hz, were used to calculate the populations.

The absence of negative populations is an indication that the new set of limiting coupling constants is reliable for use in the determination of side chain conformational populations of hexopyranose systems. As described above, negative populations are usually caused by either i) the application of inaccurate limiting coupling constants,[60] or ii) the existence of the non-ideal conformers.[146-148] Therefore, the absence of negative populations with the new data set suggests that the equilibration between the three ideal staggered conformations of the side chain is an adequate model to describe the hexopyranoside side chains.

2.2.7 Application to Higher Carbon Sugars and Beyond.

Beyond the application of the new data set in the calculation of pyranose side chain populations, it can be applied to any terminal vicinal diol systems and corresponding ethers (i.e., HO-CH_2-CHOH-R' and R'O-CH_2-CHOHR'' groups, respectively). Further, the limiting coupling constant derived from compounds **48**, **49**, **53**, **54**, and **55** can be directly applied to internal vicinal diols (i.e., RCHOH-CHOH-R' group) without using correction factors. Such moieties are often found in higher carbon sugars, such as bacterial

heptopyranosides,[171-174] octulosonic, and non-ulosonic acids,[175-176] and acyclic polyols.[177-178] In addition, the coupling constants derived from the trisubstituted systems **50** and **57** can be applied to internal vicinal diols after the application of suitable correction factors.

2.3 Conclusions

A series of *cis*- or *trans*-fused bicyclo-[4.4.0]-octane type bicyclic compounds were synthesized as the models for ideal *gg*, *gt*, and *tg* conformations. After a critical analysis of the complete set of data, some compounds were excluded based on their deviation from the ideal chair-chair conformation. Consideration of another series of compounds along with some literature compounds facilitated the derivation of correction factors in the application of tetrasubstituted models, permitting them to be used as the models for trisubstituted systems. Ultimately, a new set of limiting coupling constants was introduced for the calculation of pyranoside side chain populations, and the reliability of the new data set was evaluated by applying it to literature compounds. The absence of negative populations calculated from the new data set, in comparison to the previously reported limiting coupling constants, provides strong evidence that the new data set is suitable for use as the limiting coupling constants. More importantly, the derivation of a reliable set of limiting coupling constants sets the background to an accurate determination of side chain conformational populations of hexopyranosides, which will eventually help to understand the relationship between conformation and mechanistic aspects of carbohydrate chemistry. In addition, applications of these limiting coupling constants can be extended to analyze the side chain conformations of the higher carbon sugars and even acyclic vicinal diols by applying an appropriate set of correction factors. Finally, the new set of

limiting data should serve the field of computational chemistry by providing reliable and advisory information for the development of new methods and tools for predicting the side chain population of carbohydrates.[179]

CHAPTER 3. INFLUENCE OF THE PROTECTING GROUPS ON THE SIDE CHAIN POPULATIONS AND ON THE GLYCOSYLATION REACTIONS

Portions of the text and figures in this chapter were reproduced or adapted from: Dharuman, S.; Amarasekara, H.; Crich, D., Interplay of Protecting Groups and Side Chain Conformation in Glycopyranosides. Modulation of the Influence of Remote Substituents on Glycosylation?. *J. Org. Chem.*, **2018**, *83*, 10334–10351. (Direct link: https://pubsdc3.acs.org/doi/full/10.1021/acs.joc.8b01459)

3.1 Introduction

The protecting group array of glycosyl donors has a significant influence on the reactivity and stereoselectivity of the corresponding glycosylation reactions.[180-187] Particularly, the configuration of protecting groups has a critical impact on anomeric reactivity (**Sections 1.4.2.1 and 1.5**). Ring conformation is another important factor that influences the anomeric reactivity. Thus, cyclic conformations with the maximum number of axial substituents (or pseudo-axial groups) have a higher anomeric reactivity compared to those with fewer axial substituents. This reactivity difference is clearly shown in the acid-catalyzed hydrolysis of methyl glycosides (**Figure 27**).[188-191] Thus, methyl glucopyranoside **103** with all equatorial hydroxyl groups has a significantly smaller relative rate compared to that of bicyclic glucopyranoside **104** with all axial hydroxyl groups.

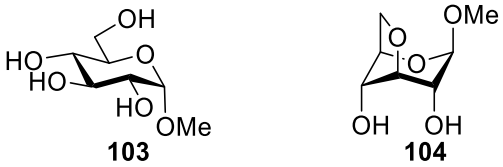


Figure 27. Effect of C-O Bond Orientation on the acid-catalyzed hydrolysis of methyl glycosides in 2 M HCl at 60 °C.[191-192]

The side chain conformation, that can be described as three staggered conformations (*gg, gt*, and *tg*), also has a significant influence on anomeric reactivity due

to the differing electron-withdrawing nature of the three conformations. Out of the three conformations, the *tg* conformation has the lowest reactivity as it has a highly electron-withdrawing antiperiplanar relationship between C5-O5 and C6-O6 bonds.[109-110, 176] Work by Vasquez and coworkers using NMR and CD methods showed that the anomeric configuration and the nature of the aglycone can influence the side chain populations,[193-197] and this study further demonstrates the significance of interplay between glycosidic bond and the side chain conformation.

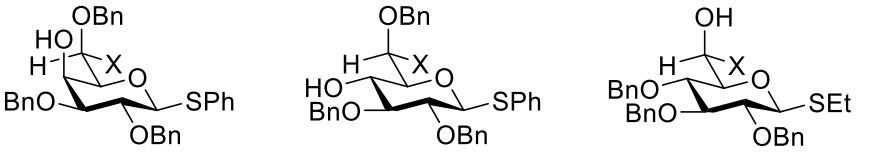
Apart from the well-known influence of the electron-withdrawing or donating nature of the protecting groups on the anomeric reactivity and selectivity,[69, 92, 198-201] protecting groups can modulate the side chain conformation, exerting an additional impact on the anomeric reactivity and selectivity. However, this interplay between protecting group array and the side chain conformation has not yet been studied in a systematic manner. Therefore, in this project, the influence of protecting groups on the modulation of side chain conformation and the consequent impact on the anomeric reactivity is studied. Due to the spatial and through bond proximity of the 4- and 6- protecting groups, the interactions between those groups are possibly the strongest forces, which influence the side chain conformation of pyranosides. Thus, two series of galacto- and gluco-thiopyranosides with different 4-*O*- protecting groups were prepared (**Figure 28**). To facilitate the unambiguous spectral assignment, *6S*-deuterio analogues of these two series were also prepared (**Figure 28**). The ^{1}H-NMR spectra of these compounds were recorded in both C_6D_6 and $CDCl_3$ to examine the influence of the solvent on the side chain conformation. Further, 6-*O*-derivatives of gluco- thiopyranosides and their *6S*-deuterio isotopomers were prepared to investigate the interplay between 6-position protecting

groups and the side chain conformation. This series also facilitates the comparative analysis of the influence of protecting groups on the side chain conformations between 4- and 6-derived glucopyranosides.

3.2 Results and Discussion

3.2.1 Experimental Design and Synthesis

Assuming that interactions between O4 and O6 protecting groups can be investigated by changing the O4 protecting group while keeping a constant protecting group at O6 of galacto- and gluco- pyranosides, or the inverse, 2,3,6-tri-*O*-benzyl-β-D-thiogalactopyranoside **105** (**Figure 28**)[202] and 2,3,4-tri-*O*-benzyl-β-D-thioglucopyranoside **107** (**Figure 28**)[203] were synthesized using literature protocols as the starting precursors of series 1 and 3 (**Figure 29**).



105, X = H
6*S*-D-105, X = D

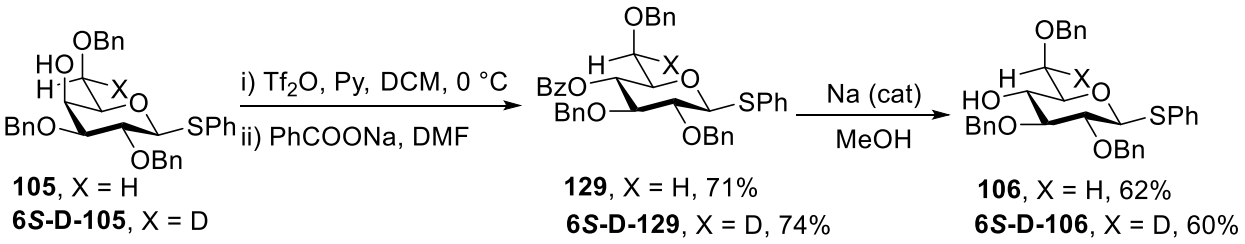
106, X = H
6*S*-D-106, X = D

107, X = H
6*S*-D-107, X = D

Figure 28. Structure of Compounds **105**, **106**, and **107** and their 6S-Deuterio Analogues.

For the convenience of the synthesis, literature compound 2,3,6-tri-*O*-benzyl-β-D-thioglucopyranoside **106** (**Figure 28**)[204] was prepared by the inversion of the 4-hydroxy group of its galacto-isomer **105**. Thus, treatment of compound **105** with trifluoromethanesulfonic anhydride (Tf_2O) and pyridine at 0 °C in DCM, followed by S_N2 displacement of 4-*O*-triflate with sodium benzoate gave benzoate ester **129** in 71% yield in two steps. The reaction of benzoate **129** with sodium metal (cat.) in methanol then gave alcohol **106** in good yield (**Scheme 16**). Compound **107** was prepared as an ethyl

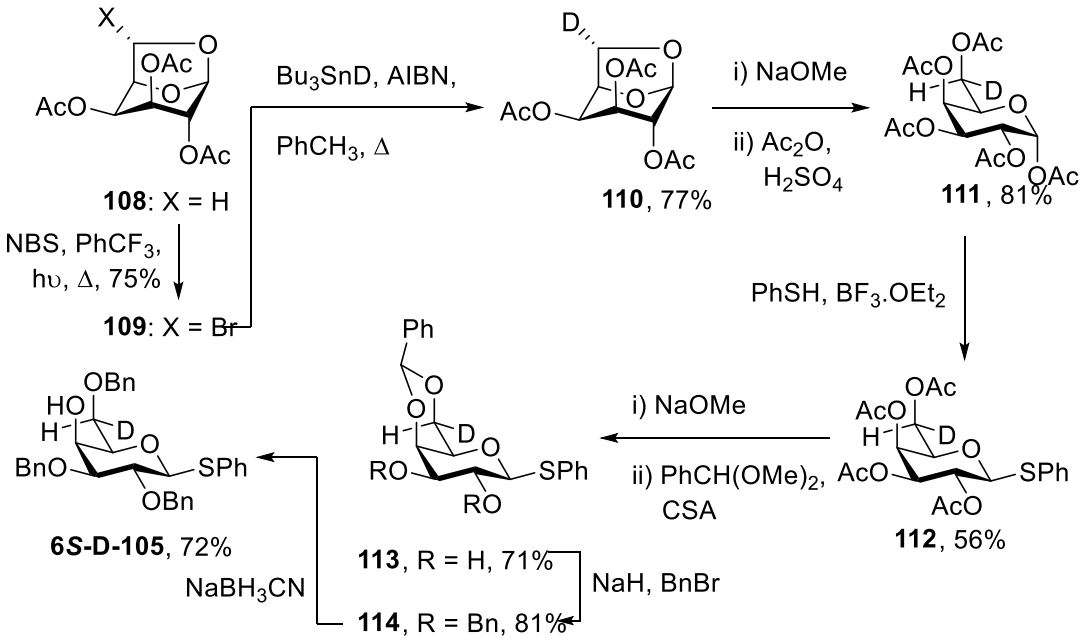
thioglycoside while the other two compounds, **105** and **106**, were obtained as phenyl thioglycosides. This is primarily due to the convenience of the synthesis, and, as implied by the final analysis of population data, no corrections were required for using different types of thioglycosides.



Scheme 16. Synthesis of Phenyl 2,3,6-tri-*O*-benzyl-β-D-thioglucopyranoside **106**.

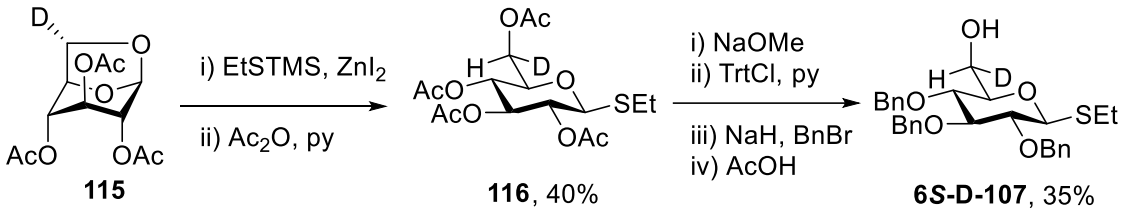
Accurate determination of side chain population is critical in correlating side chain conformation with the anomeric reactivity. Therefore, rigorous assignment of the *pro*-6*S* and *pro*-6*R* diastereotopic hydrogens is necessary and can be achieved by the synthesis of *6S*-deutereio isotopomers of compounds **105-107** (**6*S*-D-105** to **6*S*-D-107**). The synthesis of **6*S*-D-105** and **6*S*-D-107** was carried out as outlined in **Scheme 17** and **18**, while 6*S*-deuterio glucopyranoside **6*S*-D-106** was synthesized analogously to the synthesis of **106** using its C4-epimer, 6*S*-deuterio galactopyranoside **6*S*-D-105** (**Scheme 16**). Unambiguous synthetic routes involving the exoselective quenching of the radicals at the 6-position of 1,6-anhydropyranoses[205-209] were utilized in preference to shorter routes involving asymmetric reduction of 6-aldehydo pyranoses because of their unambiguous nature.[210-212] Thus, 2,3,4-tri-*O*-acetyl-1,6-anhydro-D-galactose **108** was converted to bromide **109** in 75% yield, following the literature protocol.[207] This reaction was carried out in the environmentally friendly solvent, *α,α,α*-trifluromethylbenzene,[213] replacing CCl_4; this modification was previously employed in the synthesis of the corresponding gluco-derivative **115**.[150] Radical debromination of **109** using tributyltin

deuteride, which was prepared according to Neumann *et al.*,[214] then gave 6*S*-deuterio anhydrogalactose **110** in good yield. Zemplen deacetylation of **110** then gave a triol, which on reaction with acetic anhydride in the presence of sulfuric acid gave peracetylated *6S*-dueterio galactopyranoside **111** in 81% yield over two steps. The reaction of compound **111** with thiophenol in the presence of boron trifluoride-diethyl etherate complex in DCM then gave thioglycoside **112** in a moderate yield. Zemplen deacetylation and subsequent benzylidene protection afforded diol **113** in 71% yield in two steps. Di-benzylation of free hydroxy groups with sodium hydride and benzyl bromide afforded compound **114** in 81% yield. Finally, regioselective ring opening of benzylidine acetal **113** using sodium cyanoborohydride gave 4-hydroxy galacto thiopyranoside **6*S*-D-105** in 72% yield (**Scheme 17**).



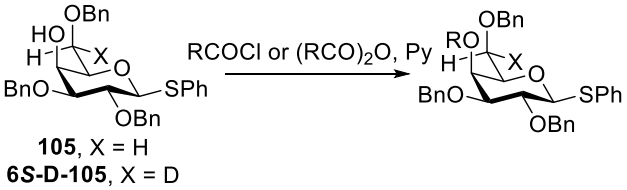
Scheme 17. Synthesis of Phenyl 2,3,6-tri-*O*-benzyl-6*S*-deuterio-β-D-thiogalactopyranoside **6*S*-D-105**.

The synthesis of deuterio analogue **6*S*-D-107** began with 6*S*-deuterio 1,6-anydroglucose **115**.[150, 205-206] Thus, compound **115** was treated with trimethylsilyl ethanethiol and zinc iodide[215] followed by the acetylation to give peracetylated-6*S*-deuterio thioglucopyranoside **116** in moderate yield. A series of standard transformations, Zemplen deacetylation, tritylation, benzylation, and acid-catalyzed deprotection of trityl group in the presence of acetic acid, were carried out to obtain compound **6*S*-D-107** (**Scheme 18**).



Scheme 18. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-6*S*-deuterio-β-D-thioglucopyranoside **6*S*-D-107**.

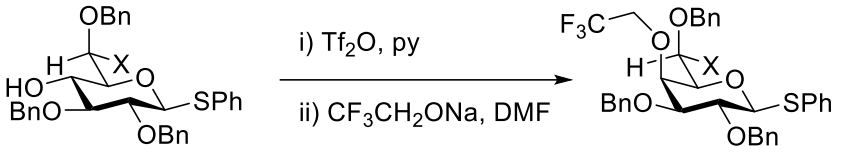
Dr. Suresh Dharuman, whom I thank, carried out the synthesis of 4-*O*-derived galactopyranosides to study the influence of substituents at the 4-position on the side chain conformation of galactopyranoside. Thus, compound **105** and its 6*S*-deuterio isotopomer **6*S*-D-105** were converted to a series of 4-*O*-esters **117**-**124** (**Figure 29, Series 1**) via standard esterification protocols using the relevant acid anhydride or acid chloride in the presence of pyridine (**Scheme 19**).



X = H	X = D
118, R = $COCMe_3$, 78%	**6*S*-D-117**, R = Ac, 81%
119, R = $COCF_3$, 65%	**6*S*-D-118**, R = $COCMe_3$, 90%
120, R = $COCCl_3$, 92%	**6*S*-D-119**, R = $COCF_3$, 87%
122, R = Bz-4-Me, 95%	**6*S*-D-120**, R = $COCCl_3$, 90%
123, R = Bz-4-OMe, 80%	**6*S*-D-121**, R = Bz, 84%
124, R = Bz-4-NO_2, 84%	**6*S*-D-122**, R = Bz-4-Me, 65%
	6*S*-D-123, R = Bz-4-OMe, 71%
	6*S*-D-124, R = Bz-4-NO_2, 83%

Scheme 19. Synthesis of 4-O-Esters of **105** and **6S-D-105**.

Benzyl ether **125**[216] (**Figure 29, Series 1**) and its deuterio isotopomer **6*S*-D-125** were prepared from the corresponding alcohols **105** and **6S-D-105** using literature protocols.[216] The 4-*O*-(2,2,2-trifluoroethyl) ether **126** and its 6*S*-deuterio isotopomer **6*S*-D-126** were obtained by the triflation of **106** and **6*S*-D-106** with triflic anhydride and pyridine followed by the S_N2 displacement of the triflates with sodium trifluoroethoxide in DMF (**Scheme 20**).

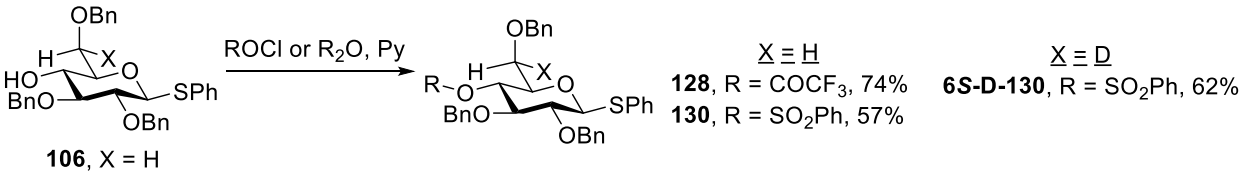


106, X = H
6*S*-D-106, X = D

126, X = H, 61%
6*S*-D-126, X = D, 30%

Scheme 20. Synthesis of Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,2,2-trifluoroethyl)-β-D-thiogalactopyranoside **126** and the Corresponding 6*S*-Deuterio Analog **6*S*-D-126**.

Similar to the galacto- series, glucose 4-*O*-derivatives were synthesized to probe the influence of 4-*O*- protecting groups on the side chain conformation of glucopyranosides (**Figure 29, Series 2**). Thus, compound **106** and its 6*S*-deuterio isotopomer **6*S*-D-106** were converted to a series of ester **127**, **128** and **130** using standard reaction protocols as described for the galacto- 4-*O*-series (**Scheme 21**).



106, X = H
6*S*-D-106, X = D

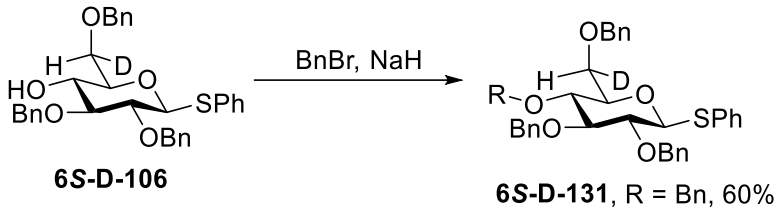
X = H
128, R = $COCF_3$, 74%
130, R = SO_2Ph, 57%

X = D
6*S*-D-130, R = SO_2Ph, 62%

Scheme 21. Synthesis of 4-*O*-esters of **106** and **6S-D-106**.

Benzyl ether **131**[217] was prepared using the literature protocol while its 6*S*-deuterio analogues **6S-D-131** was prepared by the benzylation of the corresponding alcohol **6S-D-106** in the presence of benzylbromide and sodium hydride in DMF (**Scheme 22**).

Benzoate esters **129** and **6*S*-D-129** were obtained as intermediate products during the synthesis of alcohols **106** and **6S-D-106** (**Scheme 16**), respectively.

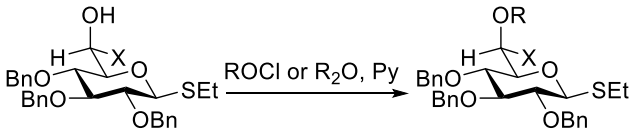


6*S*-D-106

6*S*-D-131, R = Bn, 60%

Scheme 22. Synthesis of 4-*O*-Benzyl Ether **6S-D-106**.

Finally, the synthesis of 6-*O*-derived thioglucopyranosides was carried out to investigate the influence of 6-*O*-substituents on the side chain conformation of glucopyranosides (**Figure 29, Series 3**). Thus, 6-hydroxy thioglucopyranoside **107** and its deuterio analogue **6*S*-D-107** were converted to a series of 6-*O*-esters **132**-**138** and their deuterio analogues **6*S*-D-132** - **6*S*-D-138** as described in the synthesis of the series 1 derivatives (**Scheme 23**).

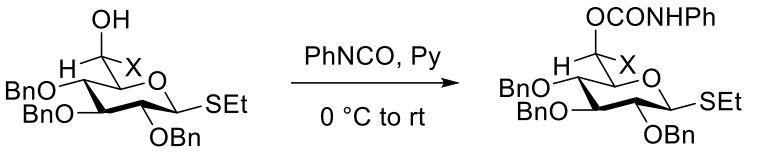


107, X = H
6*S*-D-107, X = D

X = H	X = D
133, R = $COCMe_3$, 92%	**6*S*-D-135**, R = Bz, 60%
134, R = $COCF_3$, 73%	**6*S*-D-136**, R = Bz-4-Me, 76%
136, R = Bz-4-Me, 95%	**6*S*-D-137**, R = Bz-4-OMe, 60%
137, R = Bz-4-OMe, 77%	**6*S*-D-138**, R = Bz-4-NO_2, quant.
138, R = Bz-4-NO_2, 96%	

Scheme 23. Synthesis of 6-*O*-esters of **107** and **6*S*-D-107**.

Benzyl ether **140**[217] and its deuterio isotopomer **6*S*-D-140** was prepared from the relevant alcohols **107** and **6*S*-D-107** using literature protocols.[217] In the synthesis of 6-*O*-carbamate analogues, treatment of alcohols **107** and ***6S*-D-107** with phenyl isocyanate and pyridine afforded corresponding carbamate **139** and ***6S*-D-139**, respectively, in good yields (**Scheme 24**).



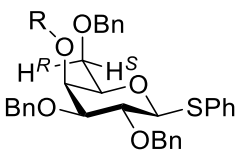
107, X = H
6*S*-D-107, X = D

139, X = H, 87%
6*S*-D-139, X = D, quant.

Scheme 24. Synthesis of Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-β-D-thioglucopyranoside **139** and the Corresponding 6*S*-Deuterio Analogue **6*S*-D-139**.

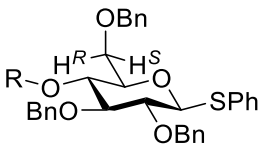
3.2.2 Measurement of Coupling Constants and Influence of Solvent

The 1D- and 2D-NMR spectra of alcohols **105**-**107** and their derivatives **117**-**140** along with the corresponding 6*S*-deuterio analogues were recorded in perdeuteriobenzene and $CDCl_3$. The comparison of ^{1}H-NMR spectra between non-deuterio and the corresponding deuterio analogues enabled rigorous assignment of *pro*-6*S* and *pro*-6*R* protons; here after they are denoted as H^{6R} and H^{6S}, respectively. All first-order coupling constants were extracted from the ^{1}H-NMR spectra, while second-order spectra complicated by the virtual coupling were simulated with the help of the spectral simulation tool in the MestRenove 9.0 suite of programs, and the coupling constants were determined based on the simulated spectra.



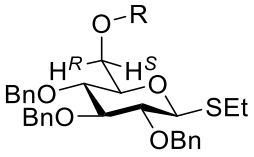
Series 1

105, R = H
117, R = Ac
118, R = $COCMe_3$
119, R = $COCF_3$
120, R = $COCCl_3$
121, R = Bz
122, R = Bz-4-Me
123, R = Bz-4-OMe
124, R = Bz-4-NO_2
125, R = Bn
126, R = CH_2CF_3



Series 2

106, R = H
127, R = Ac
128, R = $COCF_3$
129, R = Bz
130, R = SO_2Ph
131, R = Bn



Series 3

107, R = H
132, R = Ac
133, R = $COCMe_3$
134, R = $COCF_3$
135, R = Bz
136, R = Bz-4-Me
137, R = Bz-4-OMe
138, R = Bz-4-NO_2
139, R = CONHPh
140, R = Bn

Figure 29. 4- and 6-Hydroxy Galacto- and Gluco- Thiopyranosides and their Derivatives of Series 1-3.

The ^{1}H-NMR spectral data of compounds **105-107** and **117**-**140** (**Figure 29**) are classified into three tables (**Table 17, 18, and 19**). **Table 17** represents the chemical shifts and the corresponding coupling constants of the H^5, H^{6R}, and H^{6S} spin system of the 4-*O*-substituted galactopyranoside series. Similarly, the corresponding chemical shifts and the coupling constants of 4-*O*- and 6-*O*- derived glucopyranoside series are presented in **Table 18** and **19**, respectively. For ease of comparison, depending on the trends in the side chain conformation, alcohols are categorized first followed by the ethers and finally the esters.

Table 17. ^{1}H Chemical Shifts, $^{3}J_{H,H}$ Coupling Constants and Side Chain Populations for Compounds **105** and **117**-**126** in $CDCl_3$ and C_6D_6.

	4-*O*-Subs	**Chem Shift, ppm[a]**		**$^{3}J_{5,6}$, Hz[a]**		**Population, %[a]**		
		H^{6R}	H^{6S}	$J_{5,6R}$	$J_{5,6S}$	*gg*	*gt*	*tg*
105	H	3.78 (3.72)	3.81 (3.75)	5.8 (6.1)	5.7 (5.8)	25.6 (21.9)	31.8 (34.4)	42.6 (43.7)
125	$PhCH_2$	3.65 (3.57)	3.67 (3.66)	Nd (5.7)	nd (7.4)	nd (13.2)	nd (22.6)	nd (64.2)
126	CF_3CH_2	3.69 (3.55)	3.74 (3.68)	5.5 (5.7)	7.7 (7.6)	12.8 (11.7)	19.1 (21.7)	68.0 (66.7)
117	Ac	3.64 (3.47)	3.55 (3.42)	6.0 (6.1)	6.7 (6.5)	15.8 (16.4)	29.0 (31.0)	55.2 (52.6)
118	Me_3CCO	3.62 (3.49)	3.49 (3.42)	6.1 (6.0)	6.7 (6.8)	14.8 (15.0)	30.1 (28.6)	55.1 (56.4)
121	PhCO	3.69 (3.53)	3.59 (3.49)	5.8 (6.1)	6.8 (6.6)	17.0 (15.6)	26.5 (30.5)	56.5 (53.9)
122	*p*-MeC_6H_4CO	3.68 (3.56)	3.57 (3.52)	5.9 (6.2)	6.8 (6.5)	16.0 (15.4)	27.5 (32.0)	56.5 (52.5)
123	*p*-$MeOC_6H_4CO$	3.69 (3.57)	3.59 (3.54)	6.1 (6.2)	6.5 (6.4)	16.4 (16.2)	31.0 (32.5)	52.6 (51.3)
124	*p*-$O_2NC_6H_4CO$	3.69 (3.47)	3.56 (3.42)	5.7 (5.6)	7.2 (7.0)	14.8 (17.3)	23.6 (23.5)	61.6 (59.1)
119	CF_3CO	3.67 (3.39)	3.49 (3.36)	5.6 (5.7)	8.3 (8.1)	7.1 (7.7)	17.3 (19.2)	75.6 (73.0)
120	Cl_3CCO	3.71 (3.43)	3.59 (3.46)	5.7 nd	8.2 nd	6.9 nd	18.8 nd	74.3 nd

[a]Chemical shift, coupling constants, and populations in $CDCl_3$ (Chemical shift, coupling constants, and populations in C_6D_6).

Table 18. ^{1}H Chemical Shifts, $^{3}J_{H,H}$ Coupling Constants and Side Chain Populations for Compounds **106** and **127**-**131** in $CDCl_3$ and C_6D_6.

	4-*O*-Subs	**Chem Shift, ppm[a]**		**$^{3}J_{5,6}$, Hz[a]**		**Population, %[a]**		
		H^{6R}	H^{6S}	$J_{5,6R}$	$J_{5,6S}$	*gg*	*gt*	*tg*
106	H	3.76 (3.60)	3.80 (3.64)	5.3 (5.3)	4.1 (3.7)	43.1 (46.2)	34.5 (36.4)	22.4 (17.4)
131	$PhCH_2$	3.73 (3.63)	3.80 (3.63)	4.8 (nd)	1.9 (nd)	65.2 (nd)	40.0 (nd)	-5.3 (nd)
127	Ac	3.58 (3.54)	3.58 (3.58)	nd (5.8)	nd (3.2)	nd (45.3)	nd (43.9)	nd (10.8)
129	PhCO	3.65 (3.58)	3.65 (3.62)	nd (6.0)	nd (2.9)	nd (45.7)	nd (47.3)	nd (7.0)
128	CF_3CO	3.58 (3.30)	3.61 (3.37)	4.7 (4.3)	3.6 (3.5)	52.9 (57.6)	30.8 (27.2)	16.3 (15.2)
130	$PhSO_2$	3.51 (3.69)	3.71 (3.81)	5.6 (5.3)	2.1 (2.0)	55.9 (59.6)	47.1 (44.6)	-3.0 (-4.2)

[a]Chemical shift, coupling constants, and populations in $CDCl_3$ (Chemical shift, coupling constants, and populations in C_6D_6).

Table 19. ^{1}H Chemical Shifts, $^{3}J_{H,H}$ Coupling Constants and Side Chain Populations for Compounds **107** and **132**-**140** in $CDCl_3$ and C_6D_6.

	6-*O*-Subs	Chem Shift, ppm[a]		$^{3}J_{5,6}$, Hz[a]		population, %[a]		
		H^{6R}	H^{6S}	$J_{5,6R}$	$J_{5,6S}$	*gg*	*gt*	*tg*
107	H	3.71 (3.58)	3.87 (3.69)	4.8 (nd)	2.7 (nd)	59.0 (nd)	36.1 (nd)	4.9 (nd)
140	$PhCH_2$	3.69 (3.61)	3.76 (3.61)	5.0 (nd)	1.9 (nd)	63.3 (nd)	42.0 (nd)	-5.3 (nd)
132	Ac	4.20 (4.26)	4.33 (4.43)	5.4 (5.4)	2.4 (2.2)	55.5 (57.0)	43.7 (44.6)	0.9 (-1.7)
133	Me_3CCO	4.12 (4.23)	4.44 (4.55)	5.6 (5.7)	1.8 (2.1)	58.2 (54.9)	48.6 (48.2)	-6.8 (-3.0)
135	PhCO	4.46 (4.51)	4.65 (4.65)	nd (5.6)	nd (2.3)	nd (54.3)	nd (46.2)	nd (-0.5)
136	*p*-MeC_6H_4CO	4.40 (4.68)	4.59 (4.54)	5.5 (5.7)	2.2 (2.2)	56.1 (54.1)	45.6 (47.7)	-1.7 (-1.8)
137	*p*-$MeOC_6H_4CO$	4.58 (4.56)	4.41 (4.69)	5.5 (5.6)	2.3 (2.2)	55.3 (55.1)	45.2 (46.7)	-0.4 (-1.7)
138	*p*-$O_2NC_6H_4CO$	4.44 (4.40)	4.62 (4.56)	5.4 (5.6)	2.2 (2.3)	57.0 (54.3)	44.6 (46.2)	-1.7 (-0.5)
134	CF_3CO	4.32 (4.05)	4.54 (4.24)	6.3 (6.3)	2.1 (2.2)	49.0 (48.2)	54.2 (53.8)	-3.3 (-2.0)
139	PhNHCO	4.37 (4.46)	4.40 (4.43)	nd (4.6)	nd (2.5)	nd (62.5)	nd (35.1)	nd (2.4)

[a]Chemical shift, coupling constants, and populations in $CDCl_3$ (Chemical shift, coupling constants, and populations in C_6D_6).

Comparison of the coupling constants recorded in the two solvent systems (C_6D_6 and $CDCl_3$) (**Table 17-19**) reveals that there are no systematic changes in the coupling constants upon changing the solvent. This observation is consistent with the previous study of the bicyclic model systems (**Chapter 2**),[179] suggesting that the type of the solvent has a minimum effect on the side chain conformation. Therefore, coupling constants measured in one solvent can be applied in the other solvent, for the cases wherein the 1H-NMR spectra recorded in one of the two solvents is not adequately resolved to determine the coupling constants. However, careful analysis of 18 compounds whose ^{1}H-NMR were measured in both the solvents, thus of 36 different sets of coupling constants, indicate that there are unsystematic differences in the coupling constants over the two

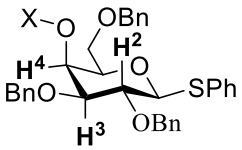
solvent systems. Consideration of those small differences in coupling constants allows an error limit of 0.4 Hz to be derived, which is in agreement with the digital resolution of the ^{1}H-NMR spectra of 0.38 Hz. Comparison of $^{3}J_{H5,\ H6R}$ and $^{3}J_{H5,\ H6S}$ coupling constants between 2,3,4,6-tetra-*O*-benzyl-protected thiophenyl glucoside **131** and 2,3,4,6-tetra-*O*-benzyl-protected thioethyl glucoside **140** reveals that the differences in the coupling constants are less than the error limit, 0.4 Hz; leading to the conclusion that the nature of the thioglycoside moiety has no influence on the coupling constant in question.

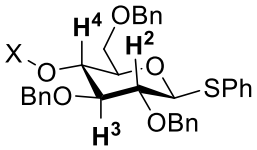
3.2.3 Calculation of Side Chain Populations and Associated Errors

The side chain populations of all compounds were calculated using experimental $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants obtained from compounds **105**-**107** and **117**-**140** with the help of equations 1-3 (**Chapter 2, Section 2.2.6.2**), given the experimental limiting $^{3}J_{R,gg}$ - $^{3}J_{S,tg}$ coupling constants of the pure *gg*, *gt*, and *tg* conformations derived using a series of conformationally rigid bicyclic models (**Chapter 2**).[179] After a comparative analysis of the coupling constants with the corresponding populations, the 0.4 Hz error limit in coupling constants is estimated to correspond to a 5% error in the side chain populations, and that error limit is applicable to all the populations presented in **Tables 17-19**. Further, **Tables 18** and **19** contain small negative populations of the *tg* conformation; however, as they are within the 5% error limit, these negative populations are considered as either zero or very close to it. Therefore, these negative values do not represent any physical meaning, and thus, further explanations are not required.

3.2.4 Estimation of Correction Factors for Coupling Constants Induced by the Electronegativity of Protecting Groups

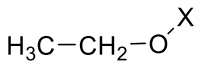
As the primary goal of this project is to study the interplay between protecting groups and the side chain conformation and the consequent influence on the anomeric reactivity, the vicinal $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants used in the calculation of side chain populations should not be affected by the electron-withdrawing/donating nature of the substituent across the series. However, it is widely accepted that vicinal coupling constants can be decreased by the presence of electronegative groups in the coupled system.[142-143, 145, 218] Nevertheless, the change in the coupling constant due to the replacement of an ether group by an ester group is not significant.[142, 219-220] Interestingly, in the H3,H4 spin system, replacing one of the two ether functionalities at the 3- and 4-positions with an ester group, leads to a 0.5 Hz increase in the $^{3}J_{H3,H4}$ coupling constant in both the galacto- and gluco- analogues with fixed *gauche* or *trans* relationships between the coupled protons (**Figure 30**). Further, this increase in the coupling constants is independent of the solvent, C_6D_6 or $CDCl_3$. However, in freely rotating spin systems, which have closer homology to the H^5,H^6 spin systems of compounds **107** and **132**-**140**, the difference in the coupling constant due to the replacement of an ether by an ester is as small as 0.1 Hz (**Figure 30**).[219] Since this difference is within the error limit (0.4 Hz), no corrections were made for the coupling data in **Table 19**.





X:	Bn	Ac	$COCF_3$
$J_{3,4}$ $CDCl_3$:	2.8	nd	3.2
$J_{3,4}$ C_6D_6:	2.8	3.3	3.2
$J_{2,3}$ $CDCl_3$:	9.2	nd	9.1
$J_{2,3}$ C_6D_6:	9.2	9.2	9.1

X:	Bn	Ac	$COCF_3$
$J_{3,4}$ $CDCl_3$:	8.9	9.4	9.6
$J_{3,4}$ C_6D_6:	9.0	9.2	9.5
$J_{2,3}$ $CDCl_3$:	8.8	8.8	8.8
$J_{2,3}$ C_6D_6:	8.7	8.7	8.7



X = Et, $J_{1,2}$ = 7.02
X = Bz, $J_{1,2}$ = 7.14

Figure 30. Comparison Between the Influence of Ether and Ester Groups on Vicinal Coupling Constants

Usually, the influence on coupling constant caused by changing an ether to an ester at the β-position of a coupled system is insignificant; as is evident from the unchanged nature of experimental $^3J_{H2,H3}$ coupling constant upon changing the 4-*O*-substituents of both galacto- and gluco- pyranosides (**Figure 30**). Thus, compounds **106** and **127-131** (**Series 2**), and **107** and **132**-**140** (**Series 3**) do not require any corrections for the influence of the nature of the 4-*O*-substituents. Further, comparison of the $^3J_{H2,H3}$ coupling constants between the galacto- and gluco- series reveals that the $^3J_{H2,H3}$ coupling constants of the galacto- series are ~0.4 Hz higher than those of the gluco- series (**Figure 30**). This difference in the coupling constants can be attributed to the β-effect noted by Haasnoot and Altona as described in **Chapter 2**, **Section 2.2.3**.[167] Considering the H^5,H^6 spin system of the galactopyranosides, a comparable relationship can be found between H^5, H^{6R} and O4 atoms of the *gt* conformation and the H^5, H^{6S}, and O4 atoms of the *tg* conformations (**Figure 31**). However, this kind of configurational relationship is not found

in the glucopyranoside systems. Considering the previous study on rigid bicyclic models, the comparison of $^{3}J_{H5,H6}$ coupling constants in the galactopyranosides with the corresponding coupling constants of the glucopyranosides reveals that there is no significant difference in the coupling constants between these two systems (**Chapter 2, Table 8**).[179] Therefore, it is concluded that the β-effect has no significant influence on the $^{3}J_{5H,H6R}$ coupling constant of the *gt* conformation and the $^{3}J_{H5,H6S}$ coupling constant of *tg* conformations of the galacto- series. Nevertheless, any possible minor influence from the Haasnoot and Altona β-effect on the galactopyranosides may cause overestimation of the *gt* and *tg* populations at the cost of *gg* populations (**Table 17**), compared to the corresponding glucopyranosides (**Table 18**). Overall, any residual effects will influence all the compounds systematically, and in the final analysis, no corrections of the coupling constants originating from the changes in the electronegativity of the substituents or by the Haasnoot and Altona β-effect were deemed necessary.

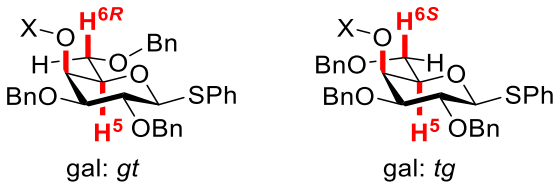


Figure 31. Configurations Possibly Subject to the Altona-Haasnoot β-Effect

3.2.5 Influence of Substituents on the Side Chain Population of Pyranoses

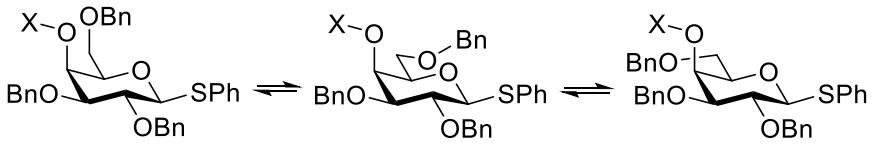
3.2.5.1 4-*O*-Substituents of Galactopyranosides (Series 1)

Here, the side chain populations of 4-*O*-H derivative **105** and its ethers and esters are considered. Thus, the side chain population of alcohol **105** is calculated to be *gg* (~25%), *gt* (~32%), and *tg* (~43%) (**Table 17** and **Figure 32**). Considering the 5% error in the side chain population, benzyl ether **125** and trifluoroethyl ether **126** have the almost

same side chain populations, *gg* (~13%), *gt* (~23%), and *tg* (~66%). Comparison of population data between alcohol **105** and ethers **125** and **126** reveals that the conversion of a 4-alcohol to a 4-*O*-ether has a significant impact on the side chain conformation (**Table 17** and **Figure 32**). Particularly, the 4-hydroxy derivative **105** has relatively higher populations of the *gg* and *gt* conformations, in comparison to those of the 4-*O*-ethers (**125 and 126**), whereas, the *tg* population of alcohol **105** is markedly lower compared to the corresponding ethers. These differences between the two types of compounds are possibly due to the unfavorable steric interactions between 4-*O*-benzyl/trifluoroethyl group and the 6-*O*-benzyl group of the ethers **125** and **126** or are the results of the favorable hydrogen bonding interactions between 4-hydroxy group and O6 atom of alcohol **105**. Since typical glycosylation reactions do not involve glycosyl donors with unprotected hydroxyl groups and the differences of the side chain population of alcohol and the ethers are significantly higher, from here on benzyl ether **125** is considered as the reference for comparing the population data of 4-*O*-derivatives of the galacto- series (**Series 1**).

Esterification of alcohol **105** gave the alkanoyl esters **117** and **118** and aroyl esters **121**-**124**. Comparison of the side chain of these esters with the ether **125** reveals that they are considerably different from each other; particularly, the *gg* populations of the ether **125** and the esters, **117**,**118**, and **121**-**124** are similar, but as a whole, the *tg* population has ~10% decrease in favor of the *gt* population on changing ether to ester transformation (**Table 17** and **Figure 32**). The trifluoroacetyl and trichloroacetyal protection of the 4-hydroxy group of alcohol **105** provided a separate set of esters **119** and **120**, respectively. The population analysis of those two esters shows a significant

decrease in the *gg* conformation in favor of *tg* conformations (**Table 17** and **Figure 32**). Further, internal comparison among the esters reveals a significant increase in the *tg* population (~73%) of highly electron-withdrawing esters **119** and **120**, while a reduction of both the *gt* and *gg* conformations with respect to the other esters **117**, **118**, and **121-124** (**Table 17** and **Figure 32**). Although the magnitude is relatively smaller, the 4-*O*-*p*-nitrobenzoate **124** follows the same population trend as of the more electron-withdrawing trichloroacetate **119** and trifluoroacetate **120**, suggesting that the increase in the *tg* population is a result of an increase in the electron-withdrawing nature of the 4-*O*-substituents.



X	*gg*	*gt*	*tg*
H	25	32	43
Alkyl	13	23	64
Acyl	16	30	54
CF_3CO	8	19	73

Figure 32. Summary of Modulations in Side Chain Population as a Function of 4-*O*-Protecting Groups of Galactopyranosides.

3.2.5.2 4-*O*-Substituents of Glucopyranosides (Series 2)

Similar to the 4-*O*- galactopyranoside series, derivatization of 4-*O*-H glucopyranoside **106**, forming 4-*O*- benzyl ether **131** results in a significant change in the side chain population. Particularly, the *tg* population decreases in favor of the *gg* and of the *gt* conformations to a relatively smaller extent (**Table 18** and **Figure 34**). Considering the spatial relationship of the O4 and O6 atoms, this pattern in the conformational change

is analogous to that of the glactopyranoside series. Specifically, in the galactopyranoside series, *gg* conformation has *syn*-pentane type interactions between O4 and O6, while in the glucopyranoside series the *tg* conformation has such a type of interactions between the same atoms. In both the cases, conversion of an alcohol to an ether results in a decrease in the population of the conformer with *syn*-pentane type configuration (*gg* of galacto- series and *tg* of gluco- series). As explained in the galacto- series, these conformational changes are caused by the increased steric interaction of *syn*-pentane type configuration during the conversion of alcohol to ether and/or the favorable hydrogen bonding between the 4-hydroxyl group and the O6 atom of alcohols **105** and **106** (**Figure 33**).

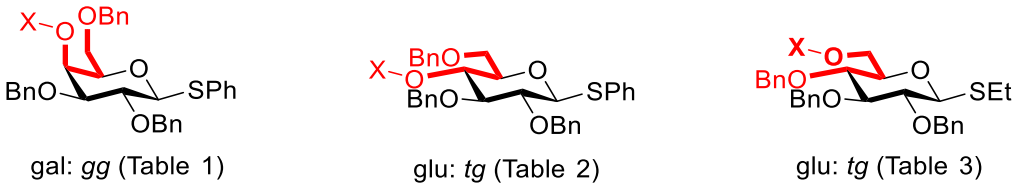
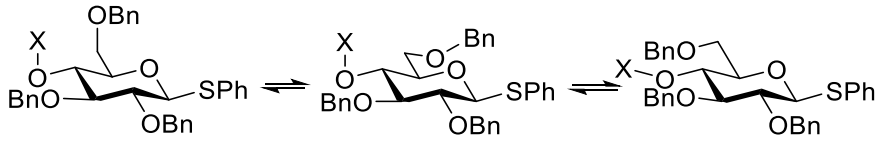


Figure 33. *syn*-Pentane Conformations of the Galacto- and Glucopyranoses.

Similar to the galacto- series, in the gluco- series, 4-*O*-benzyl ether **131** was used as the reference for comparing the influence of 4-*O*- protecting groups on the side chain conformation. The esters, 4-*O*-acetate **127** and 4-*O*-benzoate **129** have similar side chain populations, adopting smaller *gg* population and a relatively higher *tg* population compared to benzyl ether **131**. When the electron-withdrawing nature of 4-*O*-protecting group increases, for example in the case of trifluoroacetate **128**, the *tg* population increases, while the *gg* and *tg* populations are decreased with respect to alcohol **131**. Considering the side chain population of benzene sulfonate **130**, it has completely different side chain population compared to all other esters. Particularly, unlike to the

other esters, sulfonate ester **130** does not occupy the *tg* conformation (**Table 18** and **Figure 34**), possibly due to the strongly unfavorable *syn*-pentane interactions arise by the steric clash between the bulky 4-*O*-sulfonate group and the 6-*O*-benzyl group of the *tg* conformation. This decrease in the *tg* population results in an increase of *gg* population of 4-*O*-sulfonate **130** compared to acetate **127** and benzoate **129** (**Table 18** and **Figure 34**).



X	*gg*	*gt*	*tg*
H	43	35	22
Alkyl	57	43	0
Acyl	46	47	7
CF_3CO	58	27	15
$PhSO_2$	55	45	0

Figure 34. Summary of Modulations in Side Chain Population as a Function of 4-*O*-Protecting Groups of Glucopyranosides.

3.2.5.3 6-*O*-Substituents of Glucopyranosides (Series 3)

Unlike to the side chain modulations observed in galacto- and gluco- derivatives with respect to the 4-*O*-protecting groups, derivatization at the 6-position of 2,3,4-tri-*O*-benzyl glucopyranoside does not exert a significant influence on the side chain of glucopyranosides **132**-**140** (**Table 19**). Yet, some interesting changes in the side chain populations are worth mentioning. Specifically, 6-hydroxy glucopyranoside **107** and corresponding 6-carbamate derivative **139** have a small positive *tg* population, in spite of the sterically unfavorable *syn*-pentane type interactions of the *tg* conformation. These

small positive populations may be results of the hydrogen bonding interactions of the 4-OH group and 4-carbamate functionality with the O6 atom of the alcohol **107** and carbamate **140**, respectively. In addition, installation of an electron-withdrawing trifluoroacetyl group at the 6-position results in an increase of the *gt* population at the cost of the *gg* population. Overall, this series does not indicate any significant changes in the side chain population, suggesting that a parallel study on the 6-*O*-galactopyranosides was not required.

3.2.6 Influence of the Side Chain Population on Anomeric Reactivity

The changes in the side chain population with respect to the 4-*O*-protecting groups of both the galacto- and gluco- pyranosides are summarized in **Figures 32 and 34**. As the population changes are small, the energy differences related to those conformational changes are estimated a less than 1 kcal mol^{-1}. The relatively small differences in side chain populations on changes of protecting group are such that any interpretation of their origin would be questionable. Moreover, these population changes are difficult to calculate using computational software packages.[221-224] Despite the small magnitude of the protecting group-induced changes in the conformation, some general conclusions can be drawn.

Regardless of the type of the pyranoside, whether galacto- or gluco-, the replacement of an arming[199] protecting group at the 4-*O*-position by a disarming ester group results in a decrease in anomeric reactivity. This reduced reactivity is visible in typical glycosylation reactions involving the activation of thioglycoside,[53] or in the S_N2-type substitution reactions of an anomeric chloride/bromide[92] by an acceptor nucleophile. These diminished reactivities of ether-ester transformations are rationalized in the view

of the destabilization of nascent positive charge or the partial positive charge at the anomeric reaction center (**Chapter 1, Section 1.4.1, Scheme 4**).[53, 69, 80, 113, 199, 225] According to the population data (**Table 17** and **Figure 32**), in the galactopyranoside series, the disarming effect caused by the introduction of the ester groups could be moderated by changes in the side chain population. This is because, the introduction of an alkanoyl or an aroyl ester in the place of benzyl ether increases the population of the moderately reactive *gt* conformer at the expense of least reactive *tg* conformer and so partially compensates the electron-withdrawing destabilization effects introduced by the ester functionality. Analysis of the data in **Table 18** and **Figure 34** reveals that, in the 4-*O*-glucopyranoside series, the replacement of the benzyl ether by an alkanoyl or aroyl ester increases the population of the least reactive *tg* conformer while decreasing that of the most reactive *gg* conformer. Therefore, in contrast to the galactopyranoside series, substitution of an ether by an ester causes modulation of the side chain population in a way that the change in side chain conformation supports the added electron-withdrawing effect from the ester functionality, decreasing the anomeric reactivity of the 4-*O*-ester derivatives present in the gluco- series.

For both galacto- (**Table 17** and **Figure 32**) and gluco- (**Table 18** and **Figure 34**) series, the replacement of 4-*O*-ether by a strongly electron-withdrawing trifluoroacetate ester (trichloroacetate group in the galactopyranoside series), increases the population of the most electron-withdrawing (least-reactive) *tg* conformation, enhancing the electron-withdrawing effect introduced by the trifluoroacetyl and trichloroacetyl esters. In addition a 4-*O*-sulfonyl group, which has been utilized in numerous glycosylation reactions as a sterodirecting protecting group at the 4-position of 2,6-dideoxyglycopyranosyl donors,[226]

and of the 3-,4-, and 6-positions of the several other pyranosyl donors,[227-228] does not significantly change the side chain conformation of glucopyranosyl donors when compared to the corresponding 4-*O*-benzyl ethers (**Table 18** and **Figure 34**). This suggests that the reduced anomeric reactivity and related stereoselectivities seen on 4-*O*-sulfonylation are solely caused by the electron-withdrawing effect of this protecting group.

Overall, the modulations of the side chain population as a function of 4-*O*-alkanoyl and aroyl ester groups are not adequate to explain the anomeric reactivities and selectivities observed in compounds **121**-**124**[229] and the related analogues,[230] which were previously rationalized using the controversial concept of remote group participation.[126-131] Similar to 4-*O*- derivatives, the unchanged nature of the side chain conformation with respect to 6-*O*-protecting groups of the 2,3,4-tri-*O*-benzyl glucopyranosides suggests that the side chain conformation does not play any significant role in controlling the anomeric reactivity and selectivities observed during the corresponding glycosylation reactions.[231-233]

3.3 Conclusions

In the 2,3,6-tri-*O*-benzyl galactopyranoside series, replacing a benzyl ether at the 4-position with an alkanoyl or aroyl ester results in small but consistent changes in the population of the three staggered conformations; particularly, the moderately reactive *gt* conformation is increased at the expense of the least reactive *tg* conformation. This suggests that the reduced reactivity of the galactopyranoside, due to the electron-withdrawing effects enforced by the ether-ester transformation, is moderated by the conformational changes in the galactopyranoside side chain. In contrast to the

galactopyranoside series, in the corresponding glucopyranoside series, the ether-ester transformation results in an increase in the least reactive *tg* conformation, suggesting that such transformations complement the increased electron-withdrawing effects introduced by the ester group. With the substitution of strongly electron-withdrawing trifluoroacetyl groups at the 4-position of both the galacto- and gluco- series, the least reactive *tg* population is increased, suggesting that the trifluoroacetyl groups at the 4-position support the electron-withdrawing nature of the ester by modulating the side chain population. Overall, the changes in the side chain populations are small, but they have systematic patterns. It is important to note that these changes in the side chain conformation are studied in the glycosyl donors; the likelihood is that these changes can be significant at the stage of the transition state, in which a partial positive charge is developed at the ring oxygen. Therefore, this study opens gates to investigate the glycosylation mechanism at the level of the transition state with a novel viewpoint; the interplay between protecting groups and the side chain population, and the corresponding influence on the anomeric reactivity and selectivity. Such studies are currently underway in the Crich group.

PART B. INVESTIGATION OF SIALIDATION REACTION MECHANISM(S) BY KINETIC STUDIES

CHAPTER 4. INTRODUCTION

4.1 Background and Significance

Sialic acids are a group of nonulosonic acids composed of nine carbon keto aldonic acids and are considered as the most structurally diverse class among the large pool of carbohydrates.[234] Naturally occurring sialic acids are in different forms and different sialyl linkages, by which they are linked to various glycans.[235] A few examples of important sialic acid glycans are shown in **Figure 35a**. Out of about 50 sialic acid derivatives found in nature,[236-237] *N*-acetylneuraminic acid (Neu5Ac) is the most common member (**Figure 35b**). Considering the type of glycosidic bonds, equatorial (2→3) and (2→6) bonds with galactopyranose and equatorial (2→8) and (2→9) bonds with other sialic acid derivatives are widely observed in natural Neu5Ac glycans. After Neu5Ac, *N*-glycolyl neuraminic acid (Neu5Gc) is the most studied sialic acid derivative; it is found in extremely low quantities in human cells (**Figure 35b**). However, Neu5Gc derivatives are abnormally expressed in human cancer cells, and it is believed that Neu5Gc has key biological roles in mammalian evolution.[1, 238-239] In addition, the 5-deamino 5-hydroxy sialic acid, KDN,[240] is also found in various types of human cells in minor quantities (**Figure 35b**), and has similar types of glycosidic bonds, as of those found in other derivatives of sialic acid.[241] legionaminic acid (Leg) and pseudaminic acid (Pse) are also important derivatives of sialic acid (**Figure 35b**), which are found in microorganisms[242-244] responsible for several disease conditions in humans.[245-248]

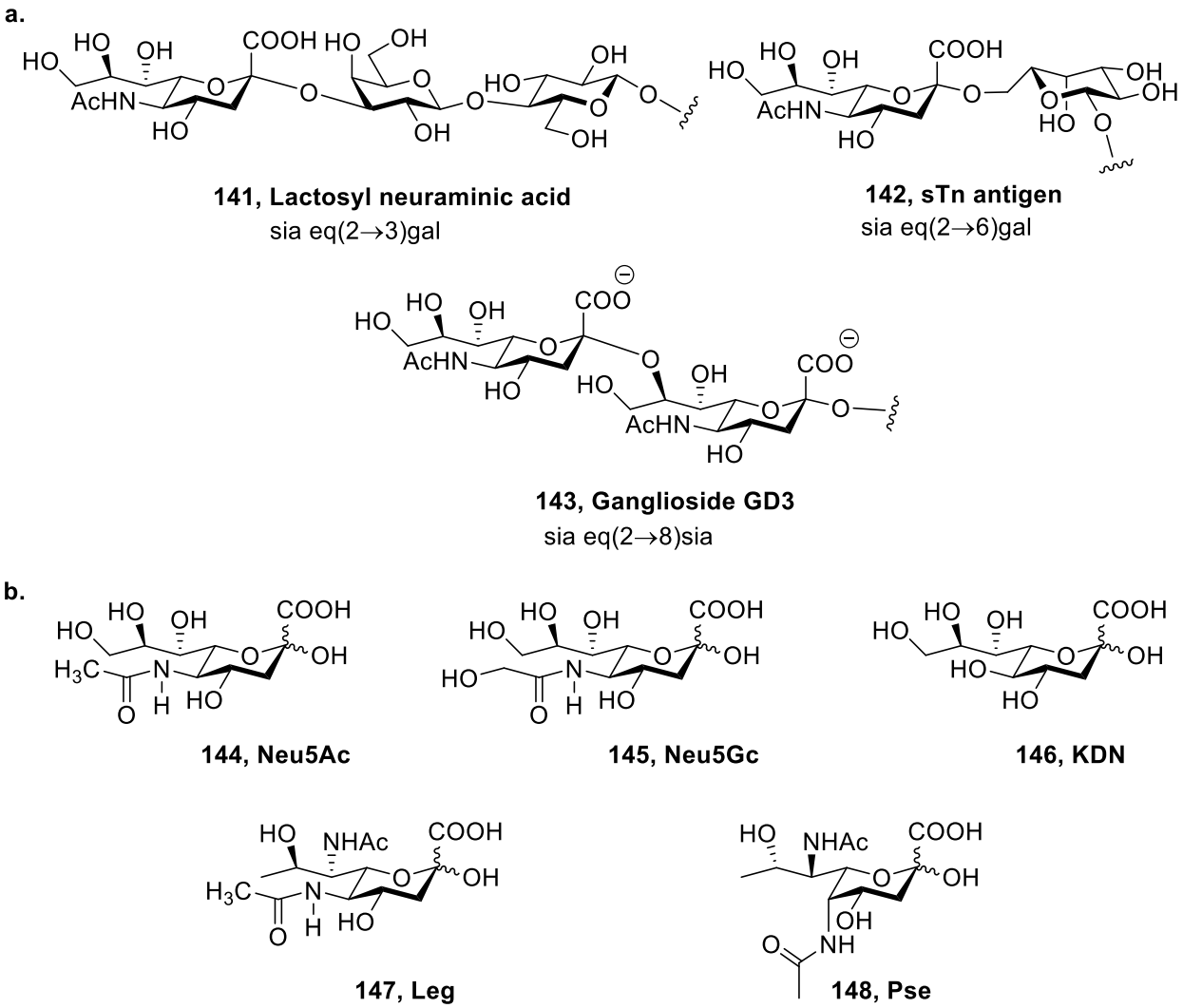


Figure 35. **a**. Examples of Naturally Occurring Sialic Acid Glycosides; **b**. Natural Variants of Sialic Acid: Neu5Ac, Neu5Gc, KDN, Leg, and Pse.

4.1.1 Biological and Medicinal Significance of Sialic Acid Derivatives

Sialic acid derivatives play significant roles in various types of normal and pathological processes in vertebrates and higher invertebrates. They are often found at the terminal, non-reducing end of glycoconjugate chains connected to the surface of the cells and soluble proteins.[2, 5, 237] The sialic acid glycosides are involved in the normal developmental stage of animals and also mediate binding of the pathogens and toxins to

host cells.[5, 249-253] During pathogenesis, the sialic acid binding proteins of pathogen bind to the sialic acid glycans of the host. These sialyl glycans have been carried with the host over a longer period of time, albeit they are detrimental to the host. Therefore, the presence of another set of interactions is important to facilitate the adhesion of the pathogen to the host. A recent study reveals a critical function of sialic acid glycans, known as molecular mimicry, by which the pathogen itself is decorated with sialic acid glycosides, mimicking the host cell surface. These glycans on the pathogen promote the interaction between the pathogen and the host,[250-254] by binding with intrinsic receptors, such as selectins and siglecs in vertebrate cells.[39, 251-253, 255] The following paragraph summarizes a few examples of this kind of pathogen-host interactions mediated by sialic acid glycosides.

Malaria is one of the most widespread infectious diseases in the tropical and sub-tropical regions in the world. During pathogenesis, merozoites of *Plasmodium falciparum* bind to red blood cells via sialic acid glycans. However, humans are not vulnerable to *Plasmodium reichnowii*, which is another type of malaria parasite that usually infects chimpanzees. The difference between the glycan chains of humans and chimpanzees leads to selective interactions from the binding proteins of the two types of pathogens, *P. falciparum* and *P. reichnowii,* respectively.[256] Sialic acids also play pivotal roles in several bacterial pathogens, which express them as capsular lipopolysaccharides (O-antigens) and polysaccharides (K-antigens). For example, *Neisseria meningitides* group B and C and *Escherichia coli* strain K1 carries sialic acid oligosaccharides and is involved in meningitis infection.[257] Apart from those pathogens, Campylobacter jejuni (Food-borne gastroenteritis), Helicobacter pylori (peptic ulcers),[258-259] and Vibrio cholera (cholera

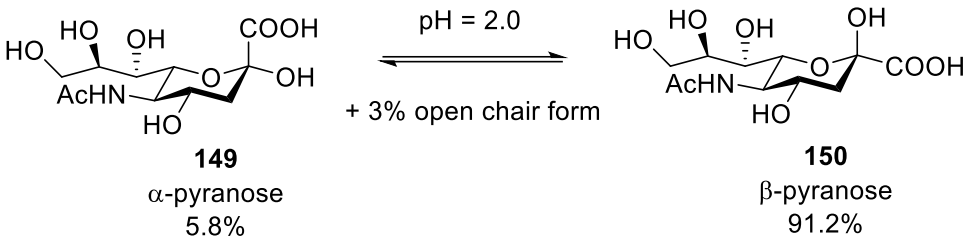
disease) also use sialic acid containing glycans in invading the host. Overall, sialic acid derived oligosaccharides are involved in a range of critical biological phenomena accountable for many different health conditions. Therefore, the structure elucidation and synthesis of these sialic acid containing glycans are of critical importance in biology and chemistry.

4.2 Sialidation Reactions and Recent Advances in Sialic Acid Chemistry

The higher demand and the difficulties associated with the isolation from natural sources necessitate the development of efficient and stereoselective synthetic methods to obtain sialic acid containing oligosaccharides and glycoconjugates. In the Neu5Ac series, naturally occurring equatorial glycosides are called α-glycosides, while the axial ones are β-glycosides. The α- or β- representation is based on the Rosanoff nomenclature which refers to the bottom-most stereogenic center of sugars in their Fischer projections.[260] Traditionally, the natural α-glycosides are considered as one of the most challenging types of glycosides to form, and accordingly, this area has been widely investigated and reviewed.[261-269]

4.2.1 Approaches to Selective Sialidation

Under thermodynamic conditions, over a wide range of pH, *N*-acetylneuraminic acid (Neu5Ac) equilibrates in water, to form >90:10 (β:α) mixture of anomers (**Scheme 18**).[270-271] Similarly, the methyl glycosides of Neu5Ac equilibrate under acidic conditions, preferring the axial or β-sialoside.[272-273] Consequently, kinetic conditions must be employed to obtain the equatorial or α-sialosides.

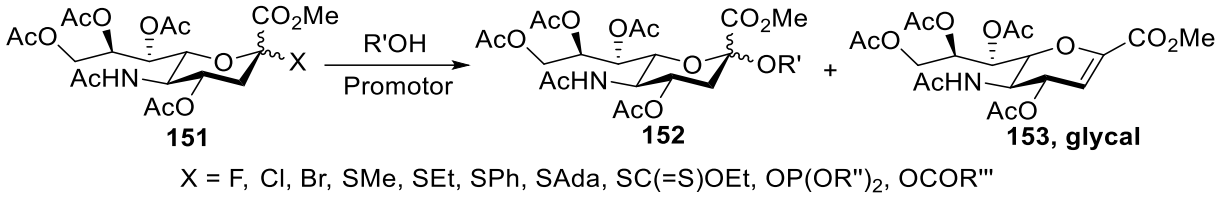


Scheme 25. Anomer Equilibration of Neu5Ac in Aqueous Medium.

Among the multiple factors influencing sialidation reactions, steric hindrance at the fully substituted anomeric carbon, the highly electron-withdrawing capacity of the carboxylic group, and the unsubstituted carbon atom at the 3-position favoring the formation of the eliminated byproduct (glycal), are the key factors which retard the efficient and stereoselective formation of α-sialosides. In addition, the unsubstituted carbon at the 3-position constrains the use of neighboring group participation. Overall, the presence of the carboxylate group and the unsubstituted nature of the C-3 position lead to competitive elimination, forming glycal (**Scheme 26**) and/or to poor stereoselectivity, forming undesired α-anomer.

Mainly, two synthetic approaches have been utilized to overcome the difficulties associated with α-*O*-sialidation;[263] indirect sialydation,[274] and direct sialydation.[275-276] In the case of the former, a pre-introduced participating group, mostly at the 3-position, is manipulated to achieve the desired α-sialosides. The subsequent removal of the auxiliary together with its introduction steps, mostly via glycal intermediates,[277] make the indirect method less efficient,[261-268] and therefore the direct method has gained the priority in sialoside synthesis. Therefore, this thesis is focused on direct sialydation reactions (**Scheme 26**). In the direct sialidation approach, traditionally, 2-chloro derivatives of *N*-acetyl sialic acid donors have commonly been used. In 1965, Meindl *et al.* were first reported sialidation reactions of 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-β-D-

neuraminic acid donor using Koenigs-Knorr reaction conditions in the presence of Ag_2CO_3 with moderate yields (30-50%).[278] The use of the free carboxylic acid can lead to several problems, thus, Kuhn *et al.* used methyl ester protected sialyl donors, and those donors were later employed in many synthesizes.[263] Methyl ester derived 2-chloro neuraminate derivatives have been activated using the classical Koenigs-Knorr method as well as the Helferich modification, where $Hg(CN)_2/HgBr_2$ were used as the promoter.[263, 278] Moreover, 2-bromo sialyl donors were used in the presence of $Hg(CN)_2/HgBr_2$ as the activator.[279] Many types of promotors, such as $ZnBr_2$, $ZnCl_2$, $Zn(OTf)_2$, $SnCl_2$, $Sn(OTf)_2$,[280] phase transfer catalysts,[281-282] AgOTf,[283-284] and silver zeolite,[285] etc. were investigated in the glycosylation of 2-halo sialyl donors, with varying yields and selectivities.



Scheme 26. Sialidation Reactions of Peracetylated Neu5Ac.

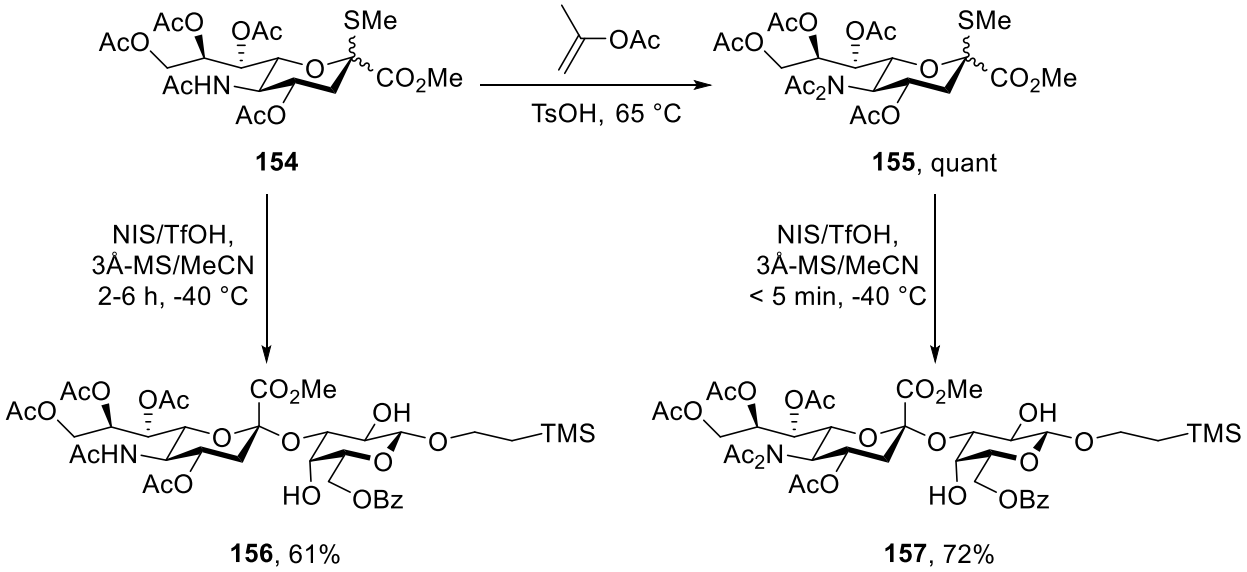
Among many different types of glycosyl donors employed in sialidation reactions, the most widely employed are thio alkyl (ethyl, methyl, and more recently adamantanyl) and thio aryl (phenyl and substituted phenyl) glycosides.[263] Excellent chemical stability together with the mild activation conditions (using soft thiophilic promotors)[286] make these thioglycosyl donors more versatile and the most employed precursors in oligosaccharide synthesis. Another advantage of using thiosialosides is that they can easily be transformed into several other leaving groups, such as sulfoxides and phosphates. Due to the wide spread applications of thioglycosides, this field has been reviewed on

numerous occasions.[286-294] Among the many different promotors (activators) employed in glycosylation reactions, *N*-iodosuccinimide/trifluoromethanesulfonic acid (NIS/TfOH), methyl trifluoromethanesulfonate (MeOTf), trimethylsilyl trifluoromethanesulfonate (TMSOTf), and dimethyl(methylthio) sulfonium trifluoromethanesulfonate (DMTST) have been widely used in the activation of thiosialosides.[263] Sialyl phosphates[263, 295-296] and xanthates[263, 297-298] are two other types of donors, which have been widely employed in glycosylation reactions with varying yields and selectivities.[266] More recently, glycosylation reactions of sialyl imidates and sialyl sulfoxides[299] were investigated. The Yu group introduced *N*-phenyltrifluoroacetamidates as a potent class of sialyl donors,[300] yet the synthesis and isolation of a stable trichloroacetamidates is a challenging task. Moreover, *S*-benzoxazolyl thioglycosides were recently added to the toolbox of sialidation reactions.[301-302] Overall, during the last few decades, the sialic acid related glycochemistry field underwent a remarkable development and has been reviewed several times.[263, 266, 303]

4.2.1.1 Influence of N5 Protecting Group on Reactivity and Selectivity

In 1998, Boons and Demchenko discovered that the N5 protecting group has a significant influence on both the reactivity of sialyl donors and their anomeric selectivities. This investigation is considered as on of the most influential findings in the sialic acid field during the last 15 years.[304] Consequently, it has been reviewed several times,[305-306] yet a brief discussion of their results is valuable. According to Boons and Demchenko, the addition of an extra acetyl group to the N5 of the peracetylated Neu5Ac donors (*N,N*-diacetyl donors) increases the anomeric reactivity compared to that of the parent *N*-

monoacetylated sialyl donors upon activation with NIS/TfOH in acetonitrile at -40 °C (**Scheme 27**).



Scheme 27. Comparison of Sialidation of *N*-Acetyl and *N,N*-Diacetyl Thiosialyl Donors.

Originally, a precise rationale for the increased reactivity of *N,N*-diacetyl sialyl donor was not available, but Boons *et al.* suggested that the decreased nucleophilicity of the imide group could be the reason for the higher reactivity of *N,N*-diacetyl donor compared to the *N*-acetyl donor.[307] Another explanation was advanced by the Kononov group, who proposed that the changes in the supramolecular aggregation induced by the alterations in the hydrogen bonding network with respect to the N5 protecting system may be the prime reason for this reactivity difference.[308]

Later, several other 5-amido and 5-imido protected sialyl donors, such as *N*-Boc acetamide sialosides[309] and *N*-benzoyl-*N*-acetyl sialyl donors[310] were employed in glycosylation reactions. Further, the Sugimoto group used phthalimide-protected sialyl donors in the synthesis of de-acetyl GM2 ganglioside analogues,[311] and later, the Fukase group took advantage of the phthalimide protecting group in conjunction with the *N*-

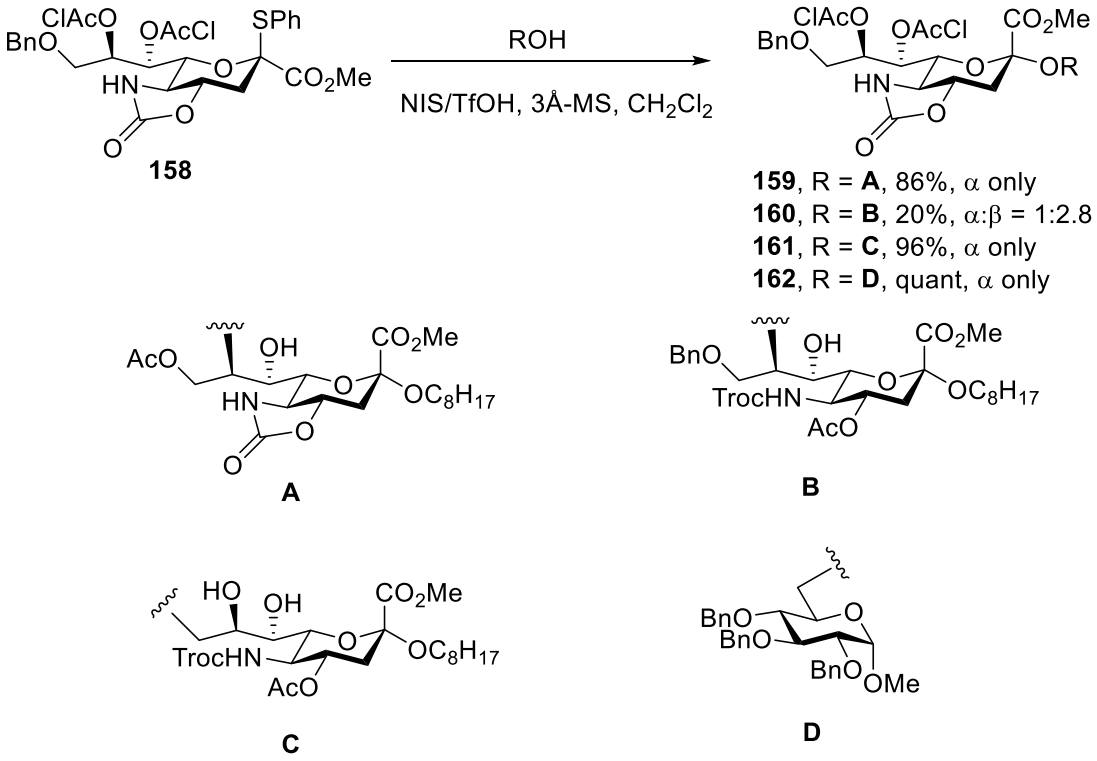
phenyltrifluoroacetimidate sialyl donors to obtain higher α-selectivity.[312] Other than these N5-variants, the use of *N*-trifluoroacetamide[313-317] and *N*-trichloroethylcarbonate (Troc)[312, 318-320] sialyl donors can be considered as significant improvements in comparison to the native 5-acetamido donors. Moreover, several other carbamate and amide protecting groups, such as the allyloxycarbonyl,[321] *tert*-butoxycarbonyl,[322] trichloroacetamide,[321] benzyloxycarbonyl,[321, 323] and 9-fluorenylmethylcarbonyl were explored in sialidation reactions, but did not deliver the anticipated results.

Following an initial report by the Unverzagt group,[324] the reactivity and anomeric selectivity of the glycosylation of 5-azido protected sialyl donors were investigated by several other groups.[325-328] Despite the reduced reactivity and selectivity of 5-azido sialyl donors in their glycosylations with secondary and hindered alcohols,[328] excellent reactivity and good yields were obtained from the glycosylation of 5-azido sialyl donors with 9-hydroxy sialic acid acceptors.[326] The increased selectivity is attributed to the low steric bulk of the azido group and its ability to stabilize the nitrilium ion intermediates, facilitating S_N2-like reactions pathways.

4.2.1.2 Influence of Cyclic Protecting Group on Reactivity and Selectivity

Other than the simple N5 protecting groups employed in sialidation reactions, several different cyclic protecting groups were explored.[304] Among those cyclic protecting groups, 4-*O*,5-*N*-oxazolidinone protected sialyl donors introduced by the Takahashi[329] and De Meo[330] groups and corresponding *N*-acetyl derivative introduced by the Crich group[331-332] are considered as the most significant improvements in sialidation reactions in recent years. According to the Takahashi group, silidation reactions of the 4-*O*,5-*N*-oxazolidinone protected donors with primary alcohols and 8-OH free oxazolidinone

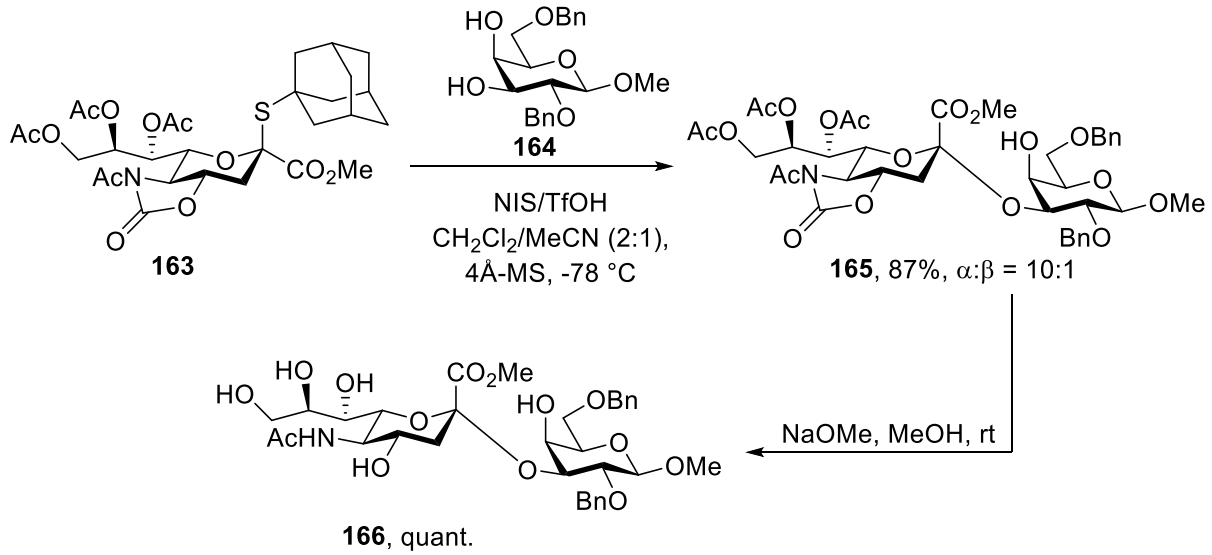
protected sialyl acceptors in dichloromethane at -78 °C afforded higher yields and α-selectivities compared to the corresponding *N*-acetyl and *N,N*-diacetyl sialyl donors (**Scheme 28**).[329] Another important feature of the silidation reactions of these bicyclic donors is that they do not afford 2,3-glycal biproducts, thereby increasing the yields of the desired sialosides. With these promising results, the Takahashi group employed this protecting system in oligosaccharide synthesis.[329, 333-335] Shortly after the report by Takahashi and co-workers, the De MeO group also reported α-selective and glycal-free sialidation reactions of oxazolidinone protected donors in the presence of NIS/TfOH promotor-combination in acetonitrile at -40 °C.[330]



Scheme 28. Glycosylation of 4-*O*,5-*N*-Oxazolidinone Protected Sialyl Donor.

For the *N*-acetyl version of oxazolidinone donors introduced by the Crich group, a combination of NIS/TfOH was used to activate those phenylthioglycoside sialyl donors in a mixture of dichloromethane and at -40 °C.[331] However, later, Crich and co-workers were

able to obtain better results by using 1-adamantanyl thioglycoside donors in the presence of same activating system, but at a lower temperature, -78 °C.[336] Apart from the excellent α-selectivity and glycal free nature of these sialidation reactions, the significant feature of this variant compared to the other oxazolidinone protected donors is that the *N*-acetyl oxazolidinone group can easily be deprotected under milder reaction conditions (i.e. sodium methoxide in methanol at room temperature) to obtain the natural *N*-acetyl neuraminic acid derivatives (**Scheme 29**).[331] Other than the oxazolidinone protecting group, 4,5-*O*-carbonate protecting groups in KDN donors,[337] 5-*N*,7-*O*-oxazinone-protected neuraminic acid donors,[338] and tricyclic neuraminic acid donors were studied in glycosylation reactions.[339]



Scheme 29. Glycosylation Reaction of *N*-acetyl 4-*O*,5-*N*-oxazolidinone Protected Sialyl Donor and The Deprotection of Oxazolidinone Group.

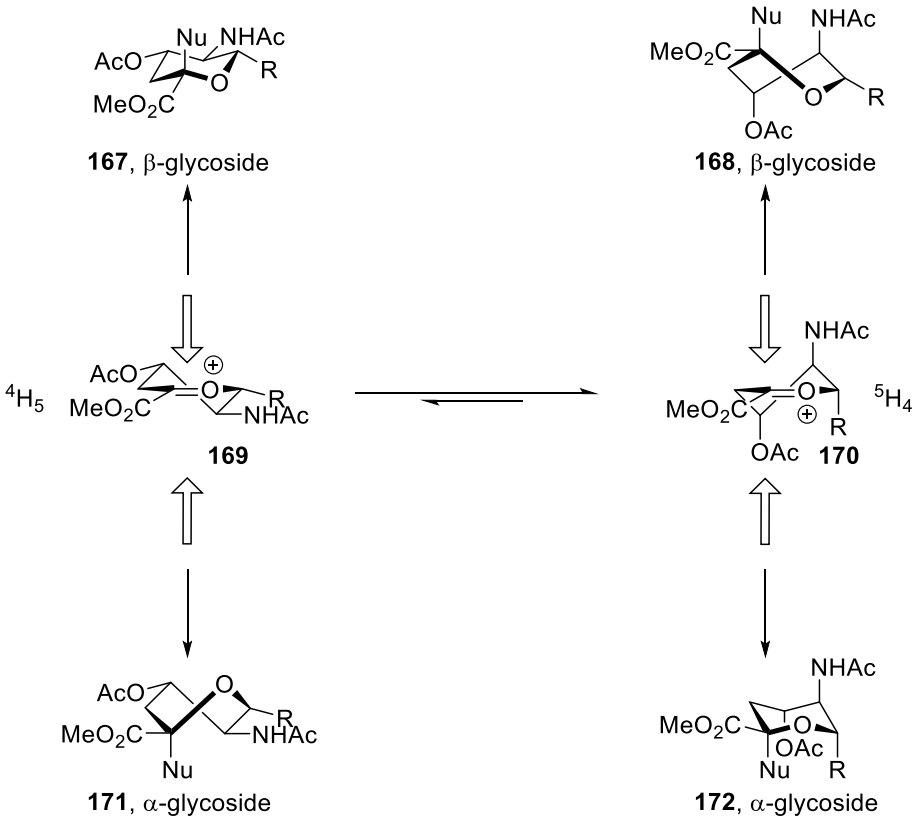
4.3 Mechanistic Aspects of Sialidation Reactions

Despite the significant improvements in the sialic acid field, the demand for even efficient sialidation conditions is high due to the increasing complexity of newly advancing

biologically important oligosaccharides. Yet, the lack of insight into sialidation mechanisms is one of the biggest challenges that retards the designing of optimal sialidation methods. Therefore, knowledge of sialidation mechanisms and the factors affecting sialidation reactions is of critical importance.

4.3.1 Evaluation of Sialyl-Oxocarbenium Ions Participating in Sialylation Reactions

As discussed in **Chapter 1** (**Section 1.4.2**), the most frequently invoked intermediates in glycosylation reactions are glycosyl oxocarbenium ions. However, the possibility that the stereoselective sialidation reactions proceed via oxocarbenium ion intermediates is very low (**Scheme 30**). Because, according to the **Scheme 30**, there is no preferential steric factor controlling the stereoselective nucleophilic attack on either face of the two interconverting half-chair conformations - more stable $^{5}H_{4}$-all axial half-chair conformation or the less stable $^{4}H_{5}$- all equatorial conformation.[139] These stability differences between the two half-chair conformations are attributed to stereoelectronic effects as discussed in **Chapter 1** (**Section 1.5**). Moreover, analysis of the boat conformations also leads to similar conclusions (**Scheme 30**). In addition, as noted in **Table 4** (**Section 1.4.2.1**), the lifetime of sialyl oxocarbenium ions in water is very short ($\geq 3 \times 10^{-11}$), suggesting that such intermediates may not be stabilized in non-polar organic solvents like dichloromethane. Overall, invoking sialyl oxocarbenium ion intermediates to explain sialidation reaction mechanisms without reasonable evidence is not a reliable practice, and therefore, the analysis of other factors is crucial.



Scheme 30. Stereoelectronic and Steric Considerations for the reactivity of Interconverting Sialyl oxocarbenium Ion.

In an important series of experiments, the Crich group studied the stability of sialyl oxocarbenium ions as a function of their protecting groups; particularly, N5 protecting groups, using mass spectral methods (electrospray ionization).[139] They reported that the minimum cone voltage of 78 V was required for the fragmentation of simple *N*-acetyl sialyl phosphate **173**, forming sialyl oxocarbenium ion. On the other hand, cyclic protecting groups, such as oxazolidinone- and *N*-acetyloxazolidinone-protected sialyl phosphates required minimum cone voltages of 93 and 83 V, respectively. These experiments clearly demonstrated that the introduction of cyclic protecting groups increases the minimum cone voltages required for the fragmentation of sialyl phosphates (**Figure 36**). Accordingly, the formation of oxazolidinone protected oxocarbenium ion **125** is less

favored compared to that of simple *N*-acetyl oxocarbenium ion **124**, leading to the conclusion that the oxazolidinone protected sialyl donors favor bimolecular S_N2 or S_N2-like reactions via covalent or contact ion pair type intermediates. Simple *N*-acetyl sialyl donors on the other hand favor S_N1 or S_N1-like reaction pathways, possibly via sialyl oxocarbenium ion type intermediates.

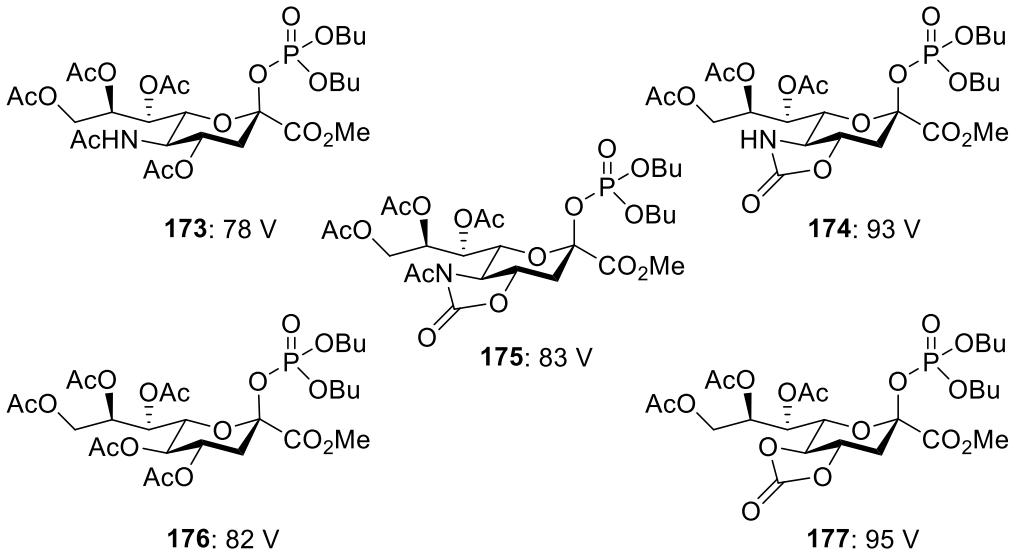


Figure 36. Cone Voltages for Fragmentation of Sialyl Phosphates.

The decreased stability of oxazolidinone-protected oxocarbenium ions is best explained by the higher electron-withdrawing nature of oxazolidinone group due to the alignment of its C4-O4 and C5-N5 bond dipoles in the mean plane of the pyranose ring, reinforcing the intrinsic electron-withdrawing nature of those two bond dipoles (**Figure 37**).

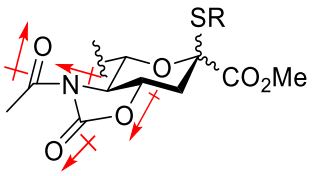


Figure 37. Orientations of key dipole moments of 4-*O*,5-*N*-oxazolidinone protecting group in thiosialosides (side chain is omitted for clarity).

This explanation is further supported by comparative analysis of the dipole moments between cyclic and acyclic protecting groups, where the cyclic and acyclic protecting groups have larger and smaller dipole moments, respectively (**Figure 38**).[139, 340-342] The influence of the 4-*O*,5-*O*-carbonate group on the reactivity the KDN series can also be rationalized in a similar manner. However, an alternative explanation has been advanced by the Kononov group for the varying stereoselectivities as a function of protecting groups (**Section 4.2.2.1**).[308]

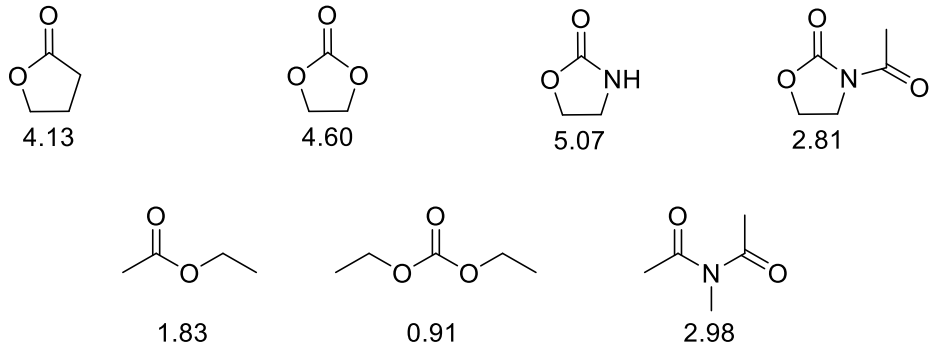


Figure 38. Dipole moment of Cyclic and Acyclic Protecting Groups in Debye units (μ).[139, 340-342]

4.3.2 Solvent Effects and Participation of Solvents in Sialylation Reactions

In the 1980s, the Hasegawa group introduced acetonitrile or acetonitrile-based mixtures of solvents, after which such solvents have been very widely used in sialidation reactions.[343-344] In the presence of acetonitrile, α-sialidation is favored and this outcome

is rationalized in terms of the participation of acetonitrile, forming a β-glycosyl-nitrilium ion, (**Figure 37, 129**) and subsequent nucleophilic attack from the glycosyl acceptor in an S_N2 fashion, forming α-*O*-sialoside. Nevertheless, for many years, no evidence for nitrilium ions was available, albeit the formation of a similar type of intermediate, the diaryl sulfoxide adduct of sialyl oxocarbenium ion, was reported (**Figure 39, 130**).[345] Nevertheless, in a recent study by the Crich group, evidence for the formation of nitrilium ion intermediates was revealed as described in this thesis (**Chapter 6**).[346] Apart from acetonitrile, the De Meo group has explored alternative participating solvents in sialidation reactions.[347]

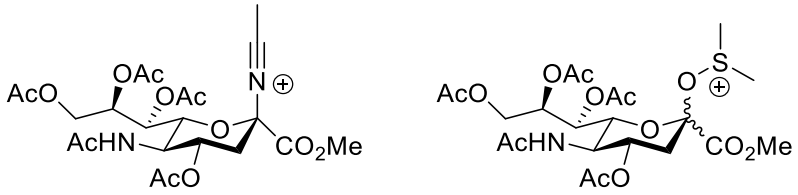


Figure 39. Sialyl Nitrilium and Sulfoxnium Ions.

4.4 Overall Goals.

The introduction of novel and efficient sialidation methodologies requires an in-depth understanding of their mechanisms, and thus, mechanistic investigations have drawn serious attention from physical organic chemists. In particular, locating sialidation reactions on the S_N1-S_N2 continuum is one of the main goals of these mechanistic studies.

Conducting kinetic experiments is one of the most reliable approaches of deriving rate parameters and thus locating reactions on S_N1-S_N2 continuum. Therefore, direct kinetic experiments of sialidation reactions were considered as the first goal of this study, wherein the focus was on probing the influence of the acceptor and acetonitrile

concentrations. As an alternative to direct kinetic experiments, competition kinetic experiments of sialyl donors were considered as the second goal with the emphasis on the investigation of the influence of the acceptor and acetonitrile concentrations and of protecting groups. The identification, isolation, and characterization of intermediates involve in sialidation reactions are also considered under this goal.

CHAPTER 5. INVESTIGATION OF THE SIALIDATION REACTION MECHANISM(S) VIA LOW-TEMPERATURE KINETIC EXPERIMENTS

5.1 Introduction

As summarized in **Chapter 4**, sialic acids are an extremely important class of carbohydrates and their synthesis requires a detailed description of sialidation mechanisms. Therefore, pseudo-first-order kinetic experiments were undertaken to elucidate the sialidation mechanism(s). To investigate kinetic parameters, conducting pseudo kinetic experiments and measuring initial rates of those experiments is considered as one of the most reliable and practical methods.[158, 348-350] In pseudo-first-order kinetic experiments, reaction conditions of a second order reaction are adjusted in such a way that the reaction appears to be first order with respect to one of the two reaction components while the order with respect to the other component appears to be zero (**Equation 4-6**).[351] This method is a common experimental design to determine the kinetics of second order reactions.[349]

Consider a bimolecular reaction with reactants, A and B that forms the product P:

$$A+B \longrightarrow P,$$

When the initial concentration of B is significantly higher than that of A, the rate law can be written as,

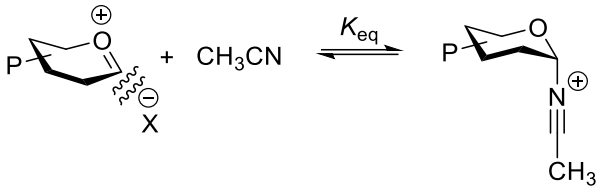
$dP/dt = k'[A]$, where k' = k[B] = pseudo-first-order rate constant (eq. 4)

By the application of equation 4 to sialidation reactions in the presence of a large excess of the donor, the rate equation can be represented as,

$d[\text{Glycoside}]/dt = k'[\text{Acceptor}]$, Where, k' = k[Donor] (eq. 5)

In addition, when acetonitrile is used as a solvent in glycosylation reactions, the participation of acetonitrile in the form of nitrilium ions is frequently postulated (**Chapter**

4, Section 4.3.2). To incorporate acetonitrile in the rate equation, an equilibrium between the oxocarbenium ion and glycosyl nitrilium ion (**Scheme 31**) is considered.



Scheme 31. Equilibrium Between Glycosyl Oxocarbenium Ion and Glycosyl Nitrilium Ion.

Thus, the rate equation 4 can be modified as,

$\mathrm{dP/dt} = k''[\mathrm{Acetonitrile}]$, where $k'' = kK_{eq}$ [Oxocarbenium ion][Acceptor] (eq. 6)

The error associated with the initial rate measurements is minimal because those rates are measured at the beginning of the reaction, where the reaction rate is maximum. Further, change in the concentration of the limiting reagent is minimal, and thus, the influence of concentration changes on the rate constant is negligible. The higher accuracy of initial rates is another advantage as such rates are derived from the most linear part (initial part of the curve) of the exponential curve from the concentration versus time relationship.[348]

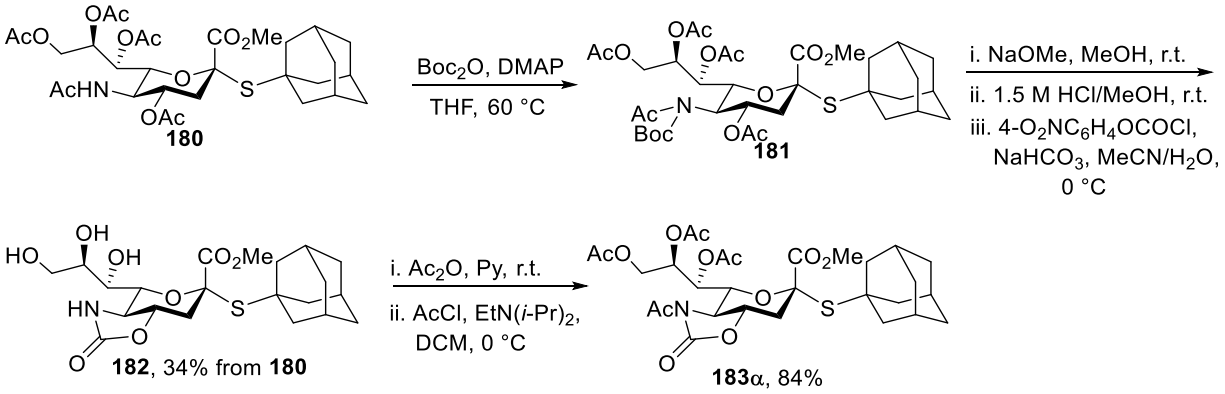
Two series of pseudo-first-order kinetic experiments with respect to the acceptor were conducted at a constant concentration of *N*-acetyl oxazolidinone protected sialyl donor (both α- and β- donors are used in separate series of reactions). These experiments provide the information required to determine the molecularity of the acceptor in sialidation reactions, and thus, to elucidate the sialidation mechanism(s). The influence of acetonitrile was also examined using two other series of kinetic experiments of both α- and β- sialyl donors, in which the percentage of acetonitrile in dichloromethane was varied

and the corresponding rate parameters were determined at constant donor and acceptor concentrations. These experiments provide the information necessary to derive the sialidation mechanism(s) in the presence of acetonitrile as an additive.

5.2 Results and Discussion

5.2.1 Hypothesis and Experimental Design

N-Acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl β-thiosialoside **183β** was synthesized following literature protocol.[336] Likewise, *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl α-thiosialoside **183α** was prepared from 1-adamantanyl thiosialoside **180** (**Scheme 32**).[352] Thus, the treatment of compound **180** with Boc_2O gave *N*-Boc sialoside **181**, which upon deacetylation with NaOMe in MeOH followed by the cleavage of Boc group in the presence of HCl in methanol and the introduction of 5-*N*,4-*O*-oxazolidinone group by treating with 4-nitrophenyl chloroformate in the presence of $NaHCO_3$ in H_2O/MeCN (1:1) gave compound **182** in 34% yield in four steps. The treatment of **182** with Ac_2O and pyridine followed by the *N*-acetylation using AcCl and $EtN(i\text{-}Pr)_2$ finally gave *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl α-thiosialoside **183α** in 84%.



Scheme 32. Synthesis of *N*-Acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl α-Thiosialoside.

Sialidation reactions are multicomponent reactions, which typically involve sialyl donor, acceptor, a combination of promotors (NIS/TfOH), solvent (DCM) or a mixture of solvents (DCM/MeCN), and acid-washed molecular sieves (AW-300), an in situ drying agent (**Scheme 33** and **34**). Therefore, these reactions are heterogeneous in nature, and thus, the sampling of known volumes from the reaction mixtures is not practically possible. To get around this problem, an internal standard (ISD), methyl 2-phthalimido-2-deoxy-β-D-glucopyranoside **184** (**Figure 40**),[353] which is inert under sialidation reaction conditions and has a UHPLC-MS trace independent from those of all other reaction components, was employed in situ, such that, the relative concentration of the products with respect to the internal standard can be determined from an unknown volume of the reaction mixture.

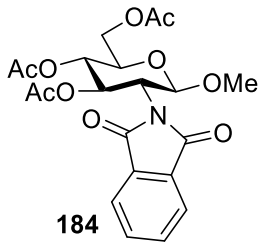


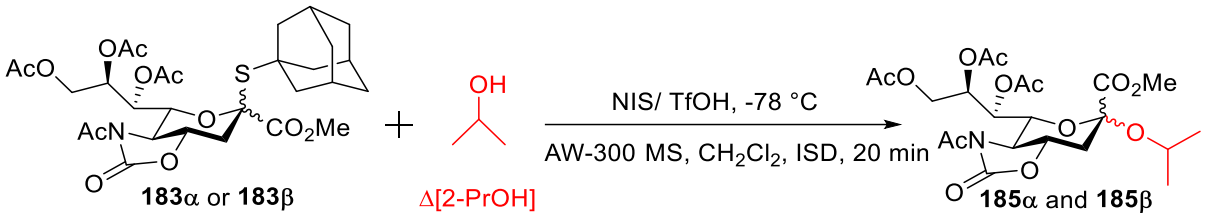
Figure 40. Structure of Methyl 2-phthalimido-2-deoxy-β-D-glucopyranoside.

5.2.2 Pseudo Kinetic Experiments of Sialidation Reactions

5.2.2.1 Influence of Acceptor Concentration

A series of pseudo-first-order kinetic experiments with respect to the glycosyl acceptor, 2-propanol, was conducted on *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl β-thiosialoside **183β** donor in dichloromethane at -78 °C (**Scheme 33**). Thus, the acceptor concentration was varied as 0.022, 0.028, 0.034, 0.039 and 0.045 mol dm^{-3}, while maintaining a constant concentration of glycosyl donor (0.056 mol dm^{-3}).

Then, the initial rate of product formation was determined by sampling the reactions over 20 minutes (starting from t = 0 min), while the conversion of glycosyl donor to product remained <10% that this conversion limit was not exceeded, was determined by the NMR analysis of the crude reaction mixture obtained on quenching the reaction with triethylamine at the end of the sampling (t = 20.5 min) and the subsequent work-up as described in the experimental section.



Scheme 33. Pseudo-First-Order Kinetic Experiment of α- and β-Thiosialosyl Donors.

All aliquots were analyzed using an ultra-high-performance liquid chromatography (UHPLC) coupled mass spectrometer (ESI-MS) equipped with a tunable ultra-violet (TUV) detector (**Figure 41**). The samples were monitored at the wavelength (λ) of 205 nm as it is highly sensitive (λ_{max}) to acetate and amide functional groups present in the reaction components. According to the Beer-Lambert law, absorbance is directly proportional to concentration; hence, individual peak areas of the TUV-trace are measurements of the concentration of the corresponding reaction components.

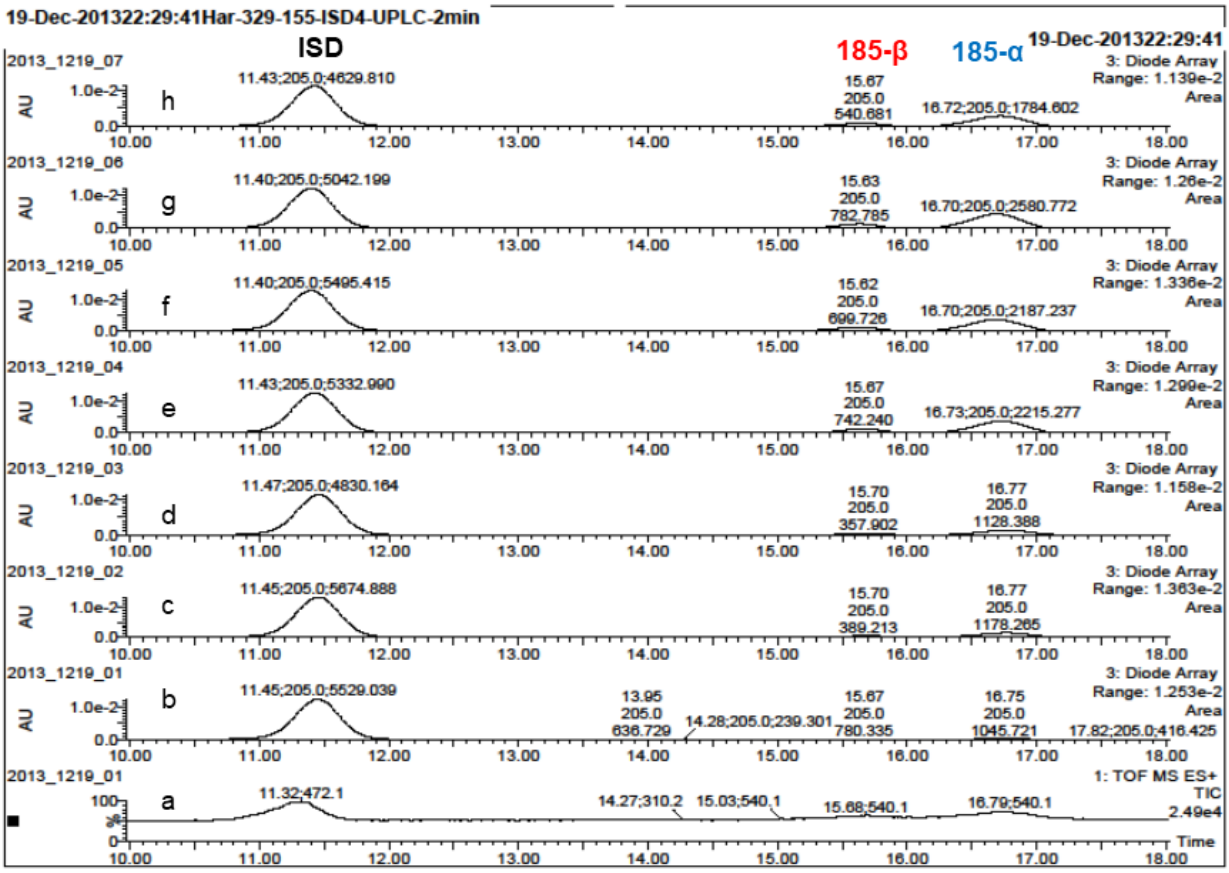


Figure 41. ESI-MS Coupled UPLC Analysis of Kinetic Experiments as Illustrated for the Pseudo-first-order Kinetic Experiment with Respect to the Donor (0.056 mol dm^{-3}) and Acceptor (0.034 mol dm^{-3}); [a]ESI-MS trace at t = 2 min; [b-h]TUV traces at different time points.

The integrated absorbance signal of the α-*O*-sialoside to that of the internal standard gave the relative concentration of the α-*O*-sialoside with respect to the internal standard at a given time point (**Table 20**). The relative concentrations of α-*O*-sialoside at different time points were plotted, and the relative rate of the sialidation reaction at that particular acceptor concentration was determined from the gradient of the graph (**Figure 42a**). Using this protocol, the relative rates of sialidation reactions at different acceptor concentrations were determined (**Table 21**), and the plots of these rates against acceptor concentrations were obtained (**Figure 42b**). It was found that, when the concentration of the accepter increased, the relative rate of α-*O*-sialoside formation increased in a linear

manner. The formation of β-*O*-sialoside on the other hand was much slower as it was not detected by TUV detector in most reaction samples. Consequently, only the kinetics of α-*O*-sialidation were considered in this study. This low rate of formation of β-anomer was expected as *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected thiosialyl donors are well known for their excellent α-selectivity (**Chapter 4**, **Section 4.2.1.2**).[329-331, 336]

Table 20. Change in the Relative Concentration of α-*O*-Sialoside as a Function of Time at the Wave Length of 205 nm [a].

Time/min	TUV Response of α-*O*-Sialoside/TUV Response of ISD (λ = 205 nm)
2	0.0000
5	0.0144
8	0.0148
11	0.0400
14	0.0500
20	0.0744

[a]Illustrated for glycosylation of β-thiosialyl donor (0.056 mol $dm^{-}3$) with the acceptor, 2-proponol (0.028 mol dm^{-3}).

Table 21. Relative Rate of α-*O*-Sialoside Formation as a Function of Acceptor Concentration for the Glycosylation of β-Thiosialyl Donor **183β**.

Concentration/mol dm^{-3}	Relative Rate of Reaction/min^{-1}
0.022	0.0004
0.028	0.0042
0.034	0.0055
0.039	0.0066
0.045	0.0072

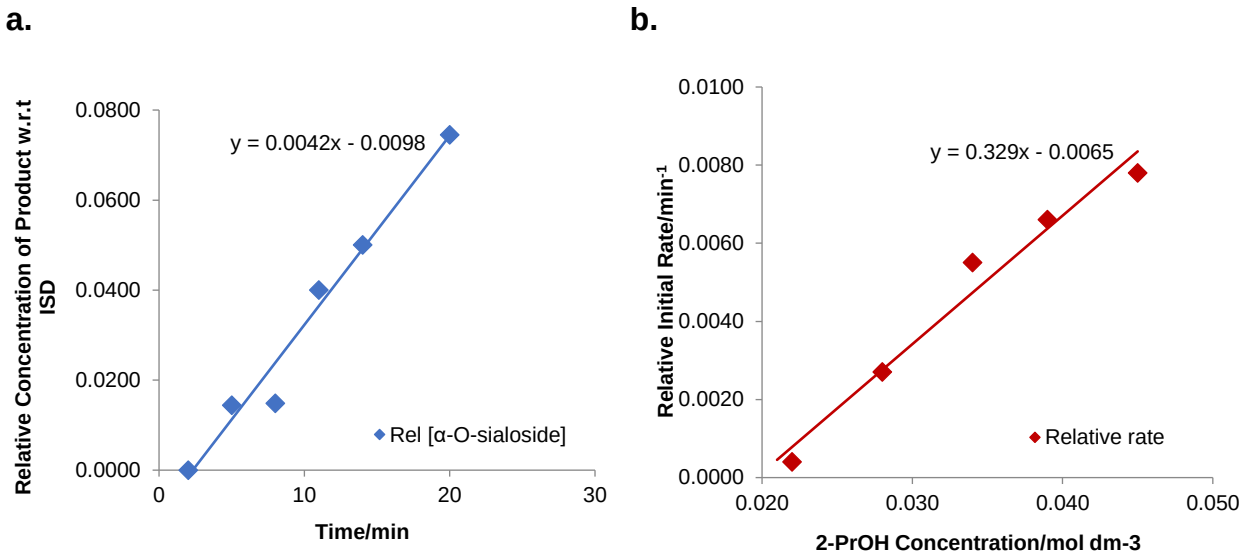


Figure 42. The Influence of Acceptor Concentration on α-*O*-Sialidation of β-Thiosialyl Donor **183β**; **a**. Relative Concentration of Product w.r.t. Internal Standard (ISD) vs time ([2-PrOH] = 0.028 M) and **b**. Relative Rate vs Concentration of 2-PrOH.

Analogously to the β-thiosialyl donor, kinetic experiments were also conducted with the *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected adamantanyl α-thiosialoside **183α**, for which relative rates with respect to different acceptor concentrations are presented in **Table 22** and plotted in **Figure 43**. Similar to the β-thiosialyl donor, the rate of α-*O*-glycosylation of α-thiosialyl donor was increased with the increase of acceptor concentration, in a linear manner, demonstrating a linear relationship between acceptor concentration and the rate of α-*O*-glycosylation reactions. As with the β-thiosialyl donor, the formation of β-*O*-sialoside was again insignificant in many reaction samples. Therefore, in this series also, only the kinetics of α-*O*-sialidation were considered.

Table 22. Relative Rate of α-*O*-Sialoside Formation as a Function of Acceptor Concentration for the Glycosylation of α-Thiosialyl Donor **183α**.

Concentration/mol dm^{-3}	Rate of Reaction/min^{-1}
0.022	0.0020
0.028	0.0030
0.034	0.0040
0.039	0.0120
0.045	0.0180

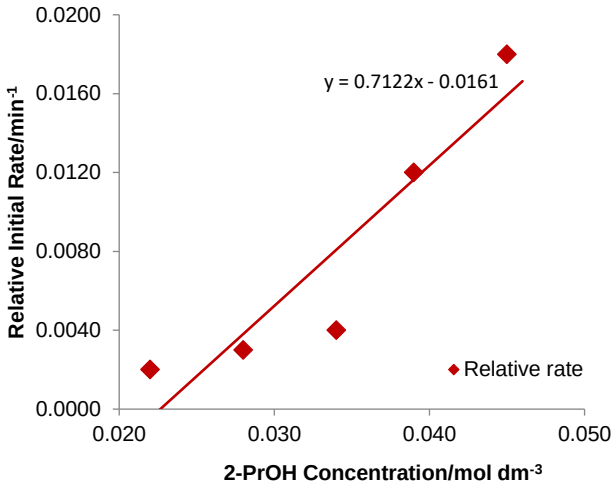
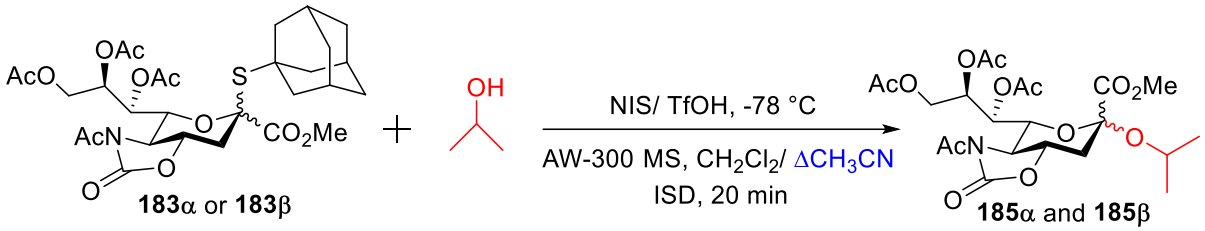


Figure 43. The Influence of Acceptor Concentration on α-*O*-Sialoside Formation from the α-Thiosialyl Donor.

5.2.2.2 Influence of Acetonitrile Percentage

The influence of acetonitrile on the kinetics of both β- and α-sialyl donors was investigated by varying the acetonitrile percentage from 0 to 40% in dichloromethane while choosing constant acceptor and donor concentrations of 0.028 mol dm^{-3} and 0.056 mol dm^{-3}, respectively (**Scheme 34**).



Scheme 34. Sialidation Reactions of Thiosialyl Donor with 2-Propanol at Different Percentages of Acetonitrile in Dichloromethane.

Except for the changes in the reaction solvent, these experiments were conducted analogously to the kinetic experiments discussed in the **Section 5.2.1.1**. In these two series also β-*O*-sialoside formation was not detectable by TUV-detector, and therefore only the relative rates of α-*O*-sialidation were taken into account in the final analysis. The change in the relative concentration of the α-*O*-sialoside product as a function of time is illustrated for the glycosylation of β-sialyl donor **183β** in 10% acetonitrile in dichloromethane. The data are tabulated (**Table 23**) and also represented in graphical format (**Figure 44a**). Analogously to the previous experiments (**Section 5.2.1.1**), the gradient of the graph gave the relative rate of α-*O*-sialidation at 10% acetonitrile in dichloromethane (**Figure 44a**). Similarly, the relative rates of α-*O*-sialidation of donor **183β** at different acetonitrile percentages were calculated (**Table 24**), and the corresponding graphical representation is shown in the **Figure 44b**. To investigate the influence of acetonitrile percentage on sialidation reactions as a function of anomeric configuration of the starting thiosialyl donor, another series of kinetic experiments was conducted using α-thiosialyl donor **183α,** and the kinetic data is presented in **Table 25** and plotted in **Figure 45**. Analogously to the β-thiosialyl donor, this series also demonstrated a linear relationship between acetonitrile percentage and the rate of α-*O*-sialidation.

Table 23. Change in the Relative Concentration of α-*O*-Sialoside as a Function of Time[a].

Time/min	TUV Response of α-*O*-sialoside/TUV Response of ISD
2	0.0000
5	0.0000
8	0.0336
11	0.0859
14	0.0837
17	0.1143
20	0.1301

[a]Illustrated for glycosylation of β-thiosialyl donor (0.056 mol $dm^{-}3$) with the acceptor 2-propanol (0.028 mol dm^{-3}) in 10% CH_3CN in dichloromethane.

Table 24. Relative Rate of α-*O*-Sialoside Formation as a Function of Acetonitrile Percentage in Dichloromethane.

Percentage of Acetonitrile	Rate of Reaction/min^{-1}
0.0	0.004
10.0	0.008
20.0	0.014
30.0	0.031
40.0	0.044

a. **b.**

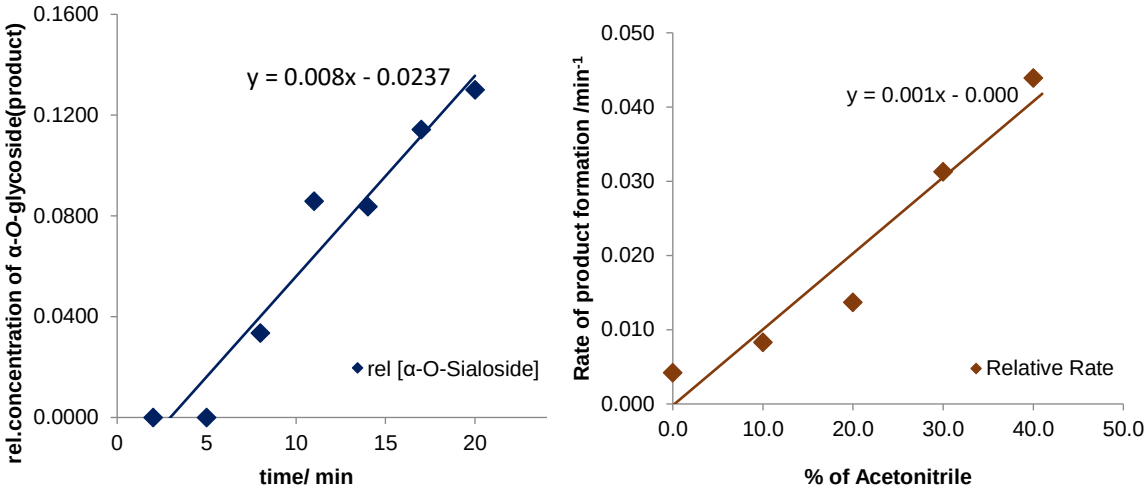


Figure 44. The Influence of Acetonitrile Percentage on the Formation of α-*O*-Sialoside in the Glycosylation of β-Thiosialyl Donor **183β**; **a**. Relative Concentration of Product w.r.t. Internal Standard (ISD) vs time (10% Acetonitrile in DCM) and **b**. Relative Rate vs Percentage of Acetonitrile in Dichloromethane.

Table 25. Relative Rate of α-*O*-Sialoside Formation as a Function of Acetonitrile Percentage in the Glycosylation of α-Thiosialyl Donor **183α**.

Percentage of Acetonitrile	Rate of Reaction/min^{-1}
0.0	0.002
10.0	0.013
20.0	0.023
30.0	0.040
40.0	0.038

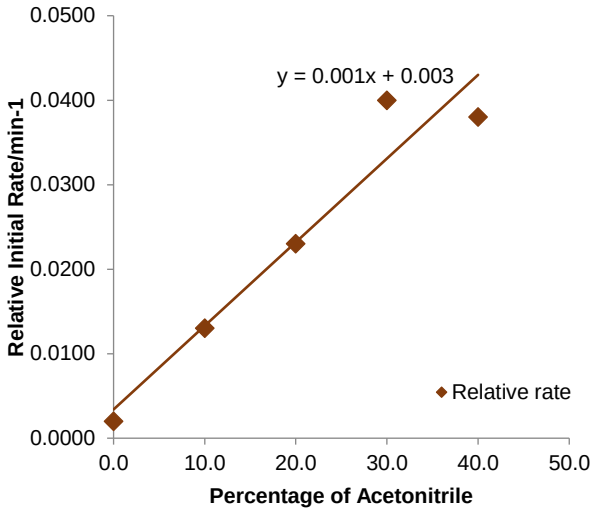


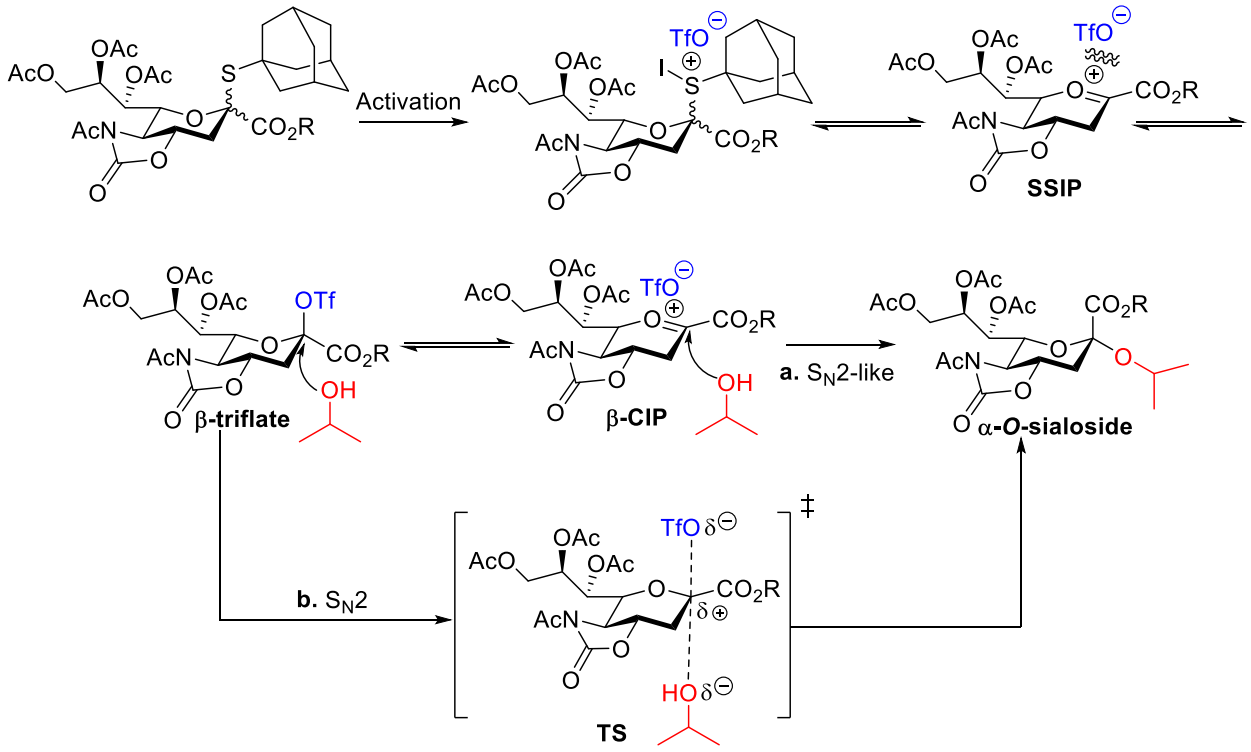
Figure 45. The Influence of Acetonitrile Percentage on the Formation of α-*O*-Sialoside in the Glycosylation of α-Thiosialyl Donor (**183α**).

5.2.3 Discussion

5.2.3.1 Influence of Acceptor Concentration on Glycosylation of β- and α-Thiosialyl Donors

For the reaction of thiosialoside **183β**, the linear increase in the relative rate of α-*O*-sialidation with respect to the increase of the acceptor concentration (**Figure 42b**) reveals that the rate of α-*O*-sialidation is dependent on the concentration of acceptor; thus, the order with respect to the acceptor is one. Therefore, the formation of α-*O*-sialoside from β-thiosialyl donor **183β** can be considered as a bimolecular reaction that follows the S_N2 or S_N2-like reaction mechanism. Similarly, the kinetics of the reaction of α-thiosiayl donor **183α** with 2-proponol also demonstrated the rate of α-*O*-sialidation to linearly increase with the increase of the acceptor concentration (**Table 3** and **Figure 43**), revealing the bimolecular nature of such reactions and their S_N2 or S_N2-like reaction mechanisms. As both the sialyl donors **183α** and **183β** of opposite anomeric configuration, lead to the same stereochemical outcome by bimolecular reaction

pathways, the rapid formation of a common intermediate is indicated. The most likely intermediate is the sialyl triflate when the reaction is conducted in dichloromethane alone as solvent (**Scheme 35**). Due to the anomeric effect, the axial triflate (β-sialyl triflate) is more stable than its equatorial counterpart (α-sialyl triflate), and therefore, the major nucleophilic substitution should take place on the β-sialyl triflate in an S_N2 or S_N2-like fashion forming the α-*O*-sialoside (**Scheme 35**). The formation of the α-sialyl triflate is not shown in **Scheme 35** as such intermediates are not required by the evidence presented here.

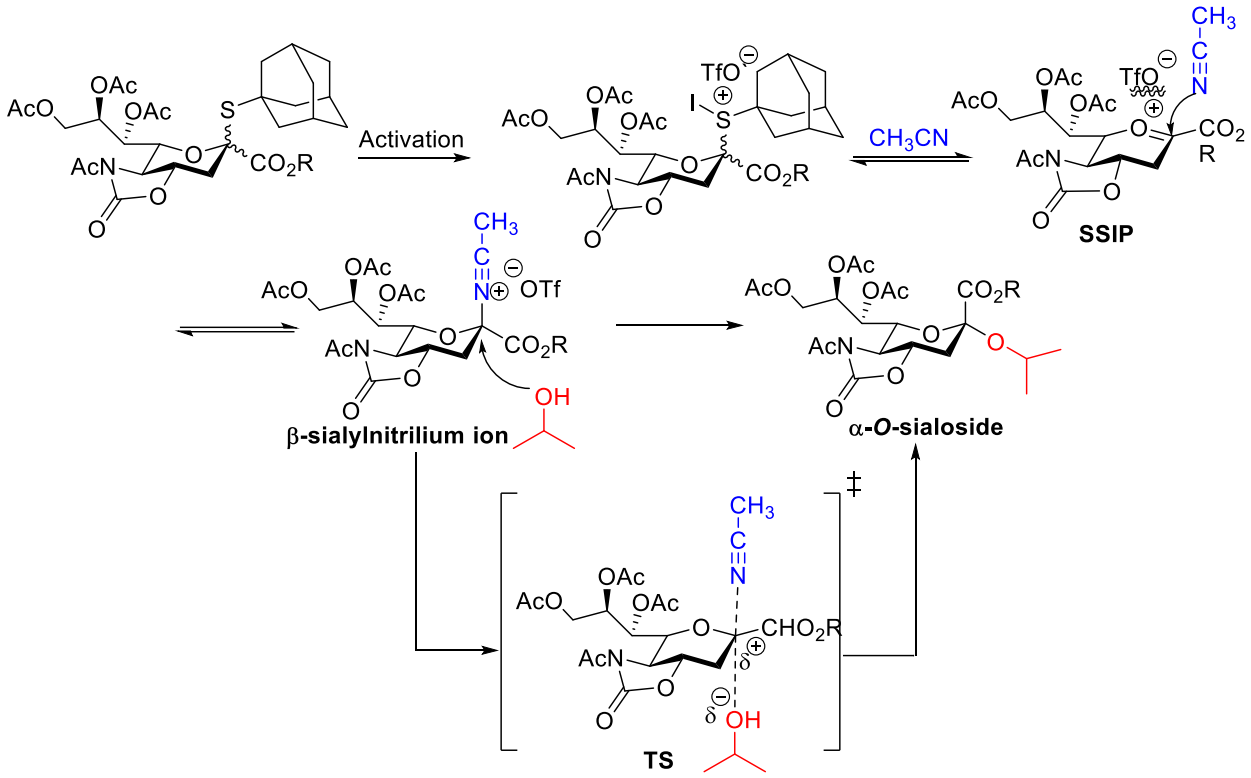


Scheme 35. Proposed Mechanism of α-*O*-Sialidation Reactions.

5.2.3.2 Influence of Acetonitrile Concentration on Glycosylation of β- and α-Thiosialyl Donors

Kinetic experiments with both the β- and α-thiosialyl donors (**183β** and **183α**) reveal that the concentration of acetonitrile in dichloromethane affects the rate of

formation of the α-*O*-sialoside **185α**. In particular, increasing the percentage of acetonitrile from 0 to 40 % in dichloromethane resulted in a linear increase in the relative rate of the α-*O*-sialidation reaction (**Table 5** and **6**, and **Figure 44b** and **45**). When the percentage of acetonitrile is doubled within the range of 10 to 30%, the rate of α-*O*-silidation was approximately increased by a factor of two (**Table 5** and **6**). The linear increase in the rate can be explained using equation 6, in which the rate and the concentration of acetonitrile have a directly proportional relationship. Specifically, with the increase in the acetonitrile percentage, the concentration of the sialyl nitrilium ion increases due to the shift of the equilibrium between sialyl oxocarbenium ions and nitrilium ion intermediates (**Scheme 31**). As explained for the sialyl triflate intermediates, the axial/β-sialyl nitrilium ion (**Scheme 36**) is more stable than its equatorial counterpart due to the anomeric effect. Thus, the β-sialyl nitrilium ions should undergo nucleophilic substitution reactions in an S_N2 or S_N2-like fashion, forming α-*O*-sialosides (**Scheme 36**). Overall, these observations further demonstrate the significance of additives in glycosylation reactions, where those additives participate in the formation of more reactive and stereoselective glycosyl intermediates.



Scheme 36. Mechanism of α-*O*-sialidation in the Presence of Acetonitrile as a Co-Solvent.

5.3 Error Analysis and Limitations

In some experiments, the relative concentrations obtained as a function of time have a considerable number of outliers, or they reach a plateau. Hence in those situations, the most linear plots were generated using a fewer number of data points that were in the best agreement to obtain the most linear average-trend line (R^2-value ~ 0.9) (**Figure 46**). This may decrease the accuracy of the data set.

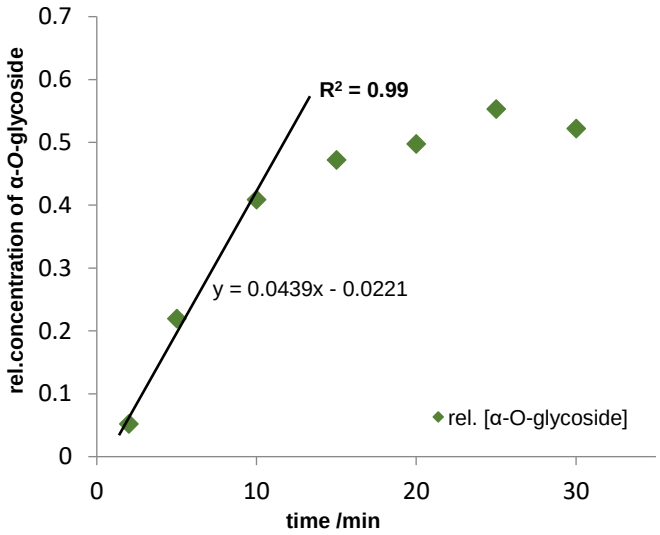


Figure 46. Relative Concentration of Product w.r.t. Internal Standard (ISD) vs time (β-thiosialyl donor - 40% Acetonitrile in DCM[a]); [a]Gradient of the graph is determined using the most linear part of the curve.

The errors in the data set may related to the sampling of these extremely moisture and temperature sensitive reactions. The multicomponent nature of these sialidation reactions also has a significant influence on the sampling. In particular, molecular sieves, which was used in situ as the drying agent of the reaction mixture, can interfer with the withdrawing of reaction aliquots as well as the releasing of the samples into the quenching vials. Atmospheric moisture is another factor, which can alter the outcome of the glycosylation reactions as a trace amount of moisture can robustly undergo nucleophilic reactions with the activated glycosyl donor, resulting in a complex reaction mixture. Overall, the prime reason for the errors associated with these kinetic experiments can be summarized as the offline monitoring of the kinetic experiments, i.e., individual reactions were sampled and quenched before their analysis; and therefore, unlike the real-time analysis, the data obtained may consist of a considerable degree of errors.

5.4 Conclusions

The sialidation mechanism was investigated using several series of pseudo-first-order kinetic experiments of both the α- and β- anomers of the 5-*N*,4-*O*-oxazolidinone protected sialyl donor. Irrespective of the anomeric configuration of the donor, the relative rate of the α-*O*-sialidation was increased with the increase of the acceptor concentration, suggesting that the sialidation reactions are strongly concentration dependent with regard to the acceptor. Therefore, it is concluded that the formation of the α-*O*-glycoside follows S_N2 or S_N2-like kinetics, where the reaction proceeds via covalently bonded sialyl triflate or contact ion pair type intermediates, respectively. In another two series of kinetic experiments with both the β- and α- sialyl donors, the rate of sialidation reactions was linearly increased with the increase of the percentage of acetonitrile in dichloromethane. This is a strong evidence for the participation of acetonitrile during the sialidation reactions via β-sialyl nitrilium ions (**Chapter 6, Section 6.1.1**),[346] on which an S_N2 or S_N2-like nucleophilic attack takes place. Overall, this study reveals that the α-*O*-sialidation reactions are concentration dependent on the acceptor as well as the additive, acetonitrile; thus, mechanistically, these reactions follow S_N2 or S_N2-like reaction pathways via sialyl triflates or sialylnitrilium ions, respectively. Therefore, to obtain better α-*O*-selectivity in sialidation reactions, maintaining the highest possible concentration with respect to both acceptor and sialyl donor and also utilizing participating solvents such as acetonitrile are highly recommended.

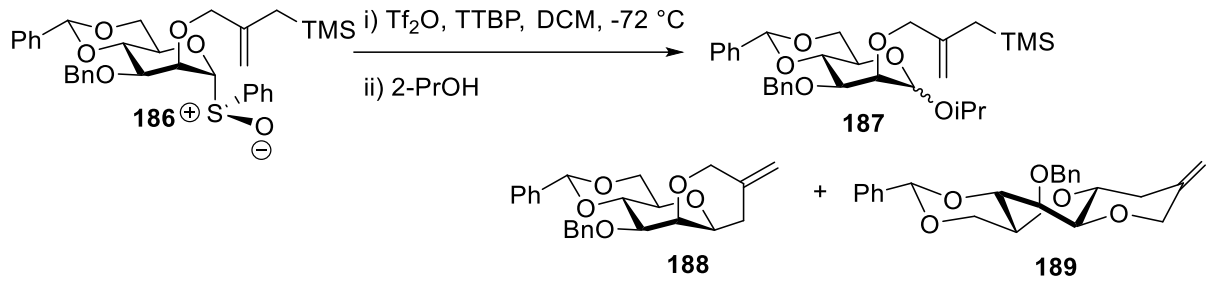
CHAPTER 6. PROBING THE MECHANISM(S) OF SIALIDATION REACTIONS WITH CATION CLOCKS

Portions of the text and figures in this chapter were reproduced or adapted from: Amarasekara, H.; Crich, D., Synthesis and intramolecular glycosylation of sialyl mono-esters of o-xylylene glycol. The importance of donor configuration and nitrogen protecting groups on cyclization yield and selectivity; isolation and characterization of a N-sialyl acetamide indicative of participation by acetonitrile. *Carbohydr Res.,* **2016**, *83*, 113–120.

6.1 Introduction

Competition kinetic experiments, which are commonly used in organic chemistry,[354] and can be a reliable method of studying reaction mechanisms. In glycoscience, the Jencks group introduced the azide clock reaction to estimate the lifetime of glycosyl oxocarbenium ions in aqueous media (**Chapter 1**, **Section 1.4.2.1**).[87] However, this azide clock reaction is not applicable to glycosylation reactions conducted in organic solvents at low temperatures. In another approach, by analogy to well-established radical clock reactions,[355] the Crich group developed cyclization of 2-*O*-(2-trimethylsilylmethallyl) glycosyl donors as clock reaction and utilized it in the investigation of the kinetics (cation clock kinetics) of manno- and glucopyranosyl systems.[356-357] In their study, the ratio of each glycoside, whether α- or β-, to the combined amount of cyclization products were determined as a function of acceptor concentration. For example, this experiment revealed that the β-*O*-mannosylation of 4,6-*O*-benzylidene protected mannopyranosyl donors is highly dependent on acceptor concentration; hence, such reactions follow S_N2 or S_N2-like reaction pathways. The α-*O*-mannosylation, one the other hand, did not indicate any significant dependence on the acceptor concentration, revealing S_N1 or S_N1-like reaction mechanisms (**Scheme 37**). The consistency of results obtained with the cation clock experiments and a series of kinetic isotope experiments indicates the reliability of the cation clock method.[358] Taking inspiration from the cation

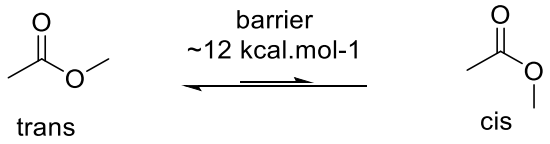
clock studies on the manno- and gluco- systems,[356-357] this chapter decribed the development of a cation clock reaction to investigate the mechanism of sialidation reactions.



Scheme 37. Cation Clock Kinetics of Mannosylation Reactions.

6.2 Experimental Design, Chemistry, and Discussion

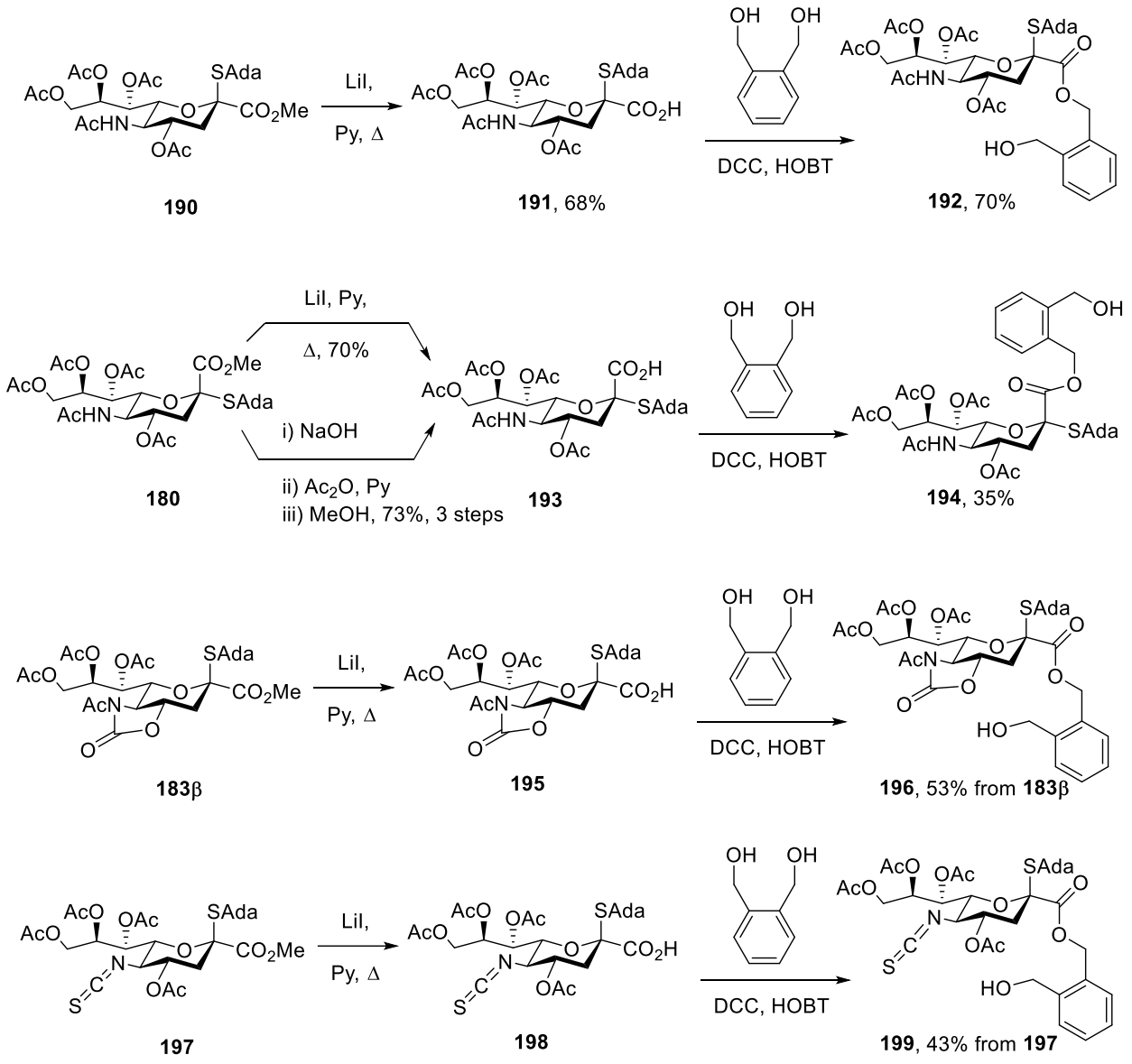
The intramolecular Sakurai reaction employed in the manno and glucopyranosyl systems[21,22] is not readily extended to the sialic acid series because of i) the lack of available alcohol for appendage of the allylsilane, and ii) the incompatibility of the allylsilane with typical sialidation conditions. Attention was therefore focused on the introduction of intramolecular alcohols linked to the carboxylate group as the intramolecular nucleophile. Ester groups favor the *trans*-configuration[359-360] and the barrier to *cis*-*trans* isomerization is estimated as 12 kcal mol^{-1} (**Scheme 38**).[361] Therefore, a 4-hydroxybutyl ester, which forms an eight-membered cyclic lactone[362-363] on cyclization directly from the ground state of the ester, was considered as the shortest alcohol-based clock precursor. To accelerate the cyclization, unsaturation was introduced into the butyl chain in the form of an aromatic ring, giving rise to the 2-hydroxymethylbenzyl esters selected for study.



Scheme 38. The Barrier to Rotation Between *cis*- and *trans*-Esters.

6.2.1 Synthesis

Compound **190**[336] was treated with lithium iodide in pyridine at reflux[311, 364-365] to deprotect the methyl ester and obtain carboxylic acid **191** in 68% yield, which was then coupled with benzene-1,2-dimethanol in the presence of dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole, to afford ester **192**, in 70% yield (**Scheme 39**). Then anomer **180**[352] of **190** was similarly converted to the carboxylic acid **193** in 73% yield by the treatment with lithium iodide in pyridine at refluxing condition. As an alternative route for the synthesis of **193**, compound **180** was saponified followed by re-installation of the acetyl groups in 73% overall yield. This saponification followed by reacetylation route was not successful in the case of compound **190** as it gave lactones as the byproducts during the acetylation step. Following the coupling protocol described for anomer **191**, compound **193** gave xylylene ester **194** in 35% yield. The reduced yield from the esterification of the axial carboxylic acid **193** compared to that of equatorial carboxylic acid **191** indicates the lower reactivity of axial acid owing to its higher steric hindrance. Similar to the demethylation and de-esterification protocols employed in compounds **190** and **180**, the *N*-acetyl-oxazolidinone and isothiocyanate derivatives **183β**[336] and **197**[366] were demethylated and coupled to form the esters **196** and **199**, via the intermediates **195** and **198**, in 53 and 43% overall yields, respectively (**Scheme 39**). Considering relative inaccessibility of **180**,[352] no attempt was made to study the α-anomers of compounds **196** and **199**.

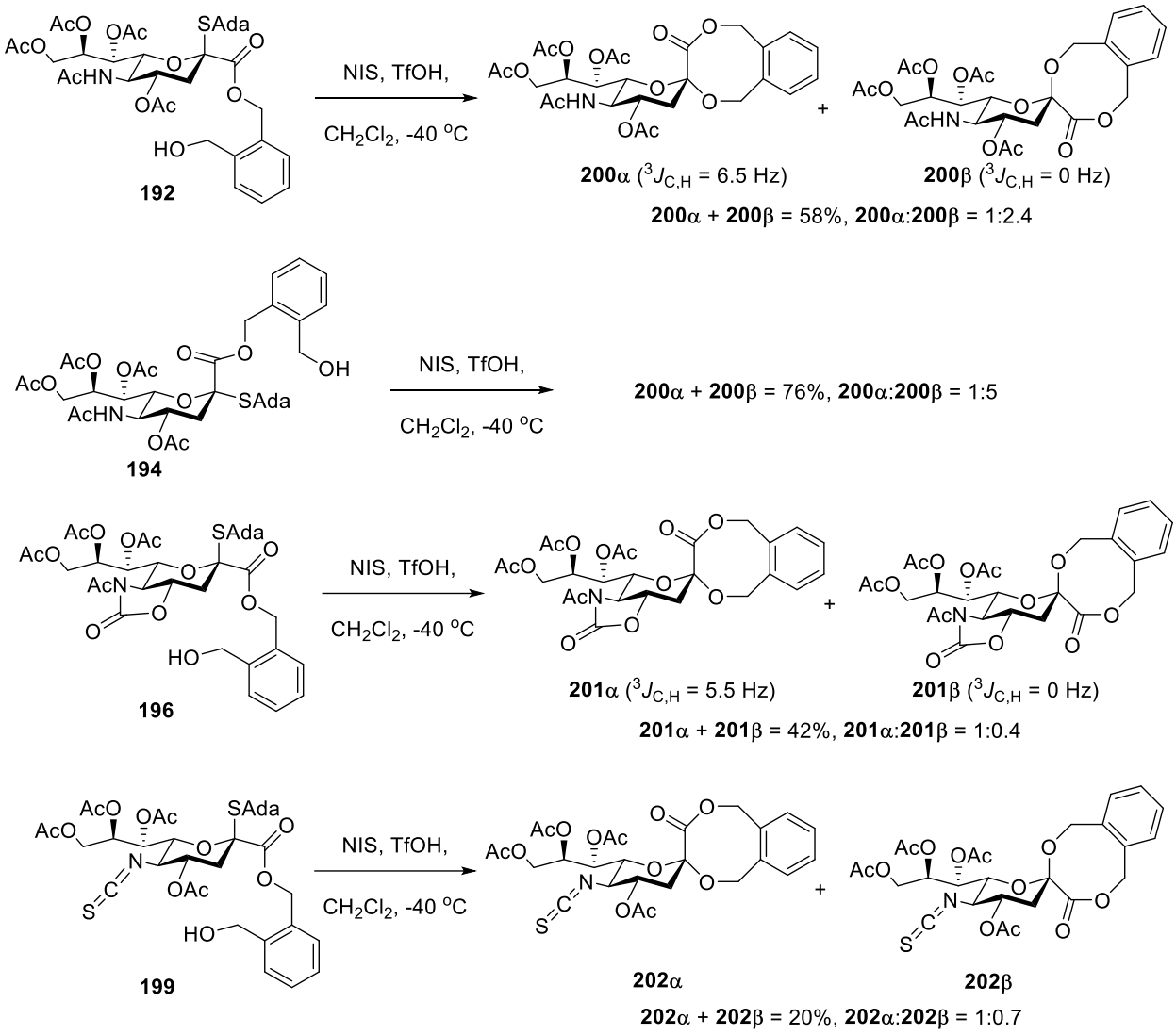


Scheme 39. Synthesis of Sialic Acid Esters for Cyclization.

6.2.2 Intramolecular Glycosylation Reactions

With regards to the cyclization reactions, first, the *N*-acetyl-β-thioglycoside **192** was activated using *N*-iodosuccinimide and triflic acid in dichloromethane at -40 °C in the presence of acid washed molecular sieves to give the diolides **200α** and **200β** in 58% combined yield and 1:2.4 (**200α**:**200β**) ratio (**Scheme 40**). The anomer of **192**, compound **194**, was activated using a similar procedure to afford diolides **200α** and **200β** in 76%

combined yield and 1:5 (**200α**:**200β**) ratio (**Scheme 40**). The 5-*N*-acetyl-5-*N*,4-*O*-oxazolidinone protected thiosialoside **196** was similarly activated using *N*-iodosuccinimide and triflic acid to obtain diolides **201α** and **201β** in 42% combined yield and 1:0.4 (**201α**:**201β**) ratio (**Scheme 40**). Finally, similar activation of the isothiocyanate **199** gave corresponding diolides **202α** and **202β** in 20 % yield and 1:0.7 (**202α**:**202β**) ratio (**Scheme 40**). Competing intermolecular glycosylation reactions were observed by mass spectrometry during all cyclization reactions, although the dimeric species could not be isolated pure and characterized due to the complexity of the multiple stereoisomers. These intermolecular self-condensations were the major pathway for the *N*-acetyloxazolidinone **196** and especially the isothiocyanate **199** as opposed to the acetamides **192** and **194**. The lower yields of diolides observed with donors **196** and **199** are reproducible.



Scheme 40. Intramolecular Glycosylation Reactions.

The anomeric configuration of **200α** and **200β** was assigned on the basis of the $^{3}J_{C,H}$ heteronuclear coupling constants as typically applied in sialic acid chemistry.[367-370] Accordingly, **200α** displays a $^{3}J_{C,H}$ coupling of 6.5 Hz between the axial hydrogen at C3 and the carbonyl carbon at C1, while the **200β** exhibits a $^{3}J_{C,H}$ coupling of 0 Hz (**Scheme 40**).

The configuration of compounds **201α** and **201β** was assigned by $^{3}J_{C,H}$ coupling constant, and the structure of **201α** was further confirmed by x-ray crystallographic

analysis (**Figure 47** - CCDC 149904). The *cis*-conformation of lactone **201α** was revealed from the crystal structure and is consistent with that of medium-size lactones as originally discussed by the Huisgen group.[363] The configuration of anomers **202α** and **202β** was established by converting them to the corresponding acetamides **200α** and **200β** by reacting with thioacetic acid,[366, 371-376] followed by comparison with the above standards by UHPLC analysis.

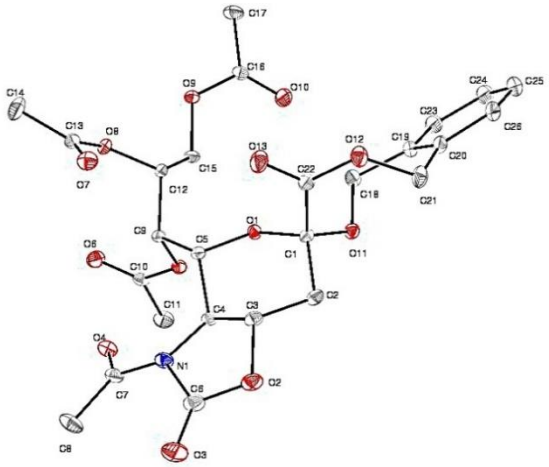
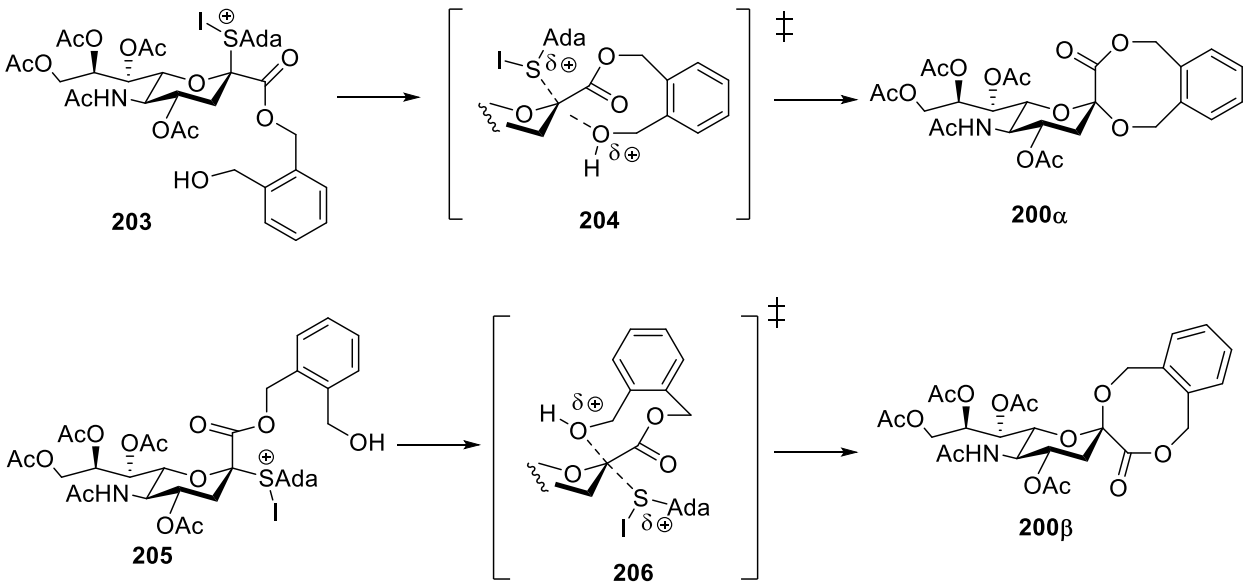


Figure 47. X-ray Crystal Structure of **201α**.

The anomeric configuration of the donors **192** and **194,** and the change of the protecting group system from acetyl through oxazolidinone to isothiocyanate (**Scheme 40**, donors **192**, **196**, and **199**, respectively) have a significant effect on the anomeric selectivity and yields of the cyclization reactions. Donors **192** and **194** have a higher preference to form the axial glycoside **200β**. However, the preference for the axial glycoside **200β** is higher, in the cyclization of **194** than of the equatorial thioglycosyl donor **192**, indicating that the major part of the intramolecular sialidation reactions does not involve a common oxocarbenium ion type intermediate. A significant fraction of inverted product may form via intramolecular displacement of the activated thioglycoside in S_N2

fashion. Accordingly, the activation of sialyl donor **192** with NIS/TfOH system[377] is anticipated to form iodosulfonium ion intermediate **203**, followed by an intramolecular nucleophilic attack from the alcohol in an S_N2 fashion, via a transition state **204**, which ultimately gives diolide **200α** (**Scheme 41**). In similar fashion, the activation of donor **194** forms predicted intermediate **205**, which then undergo intramolecular nucleophilic attack by the alcohol providing **200β** via transition state **206** (**Scheme 41**). It is important to note that transition states **205** and **206** do not imply synchronicity of bond cleavage (C-S) and bond formation (C-O), nor the extent to which the transition states are "exploded". The ester groups are drawn with the preferred *trans*-configuration assuming that relaxation to the *cis*-configuration on the formation of diolides, crystallographically revealed in **201α**, occurs after cyclization. The increased axial selectivity during the cyclization of donor **194** compared to donor **192** can be attributed to the extra stabilization of transition state **206** in comparison to **204** by the anomeric effect.



Scheme 41. Proposed Reaction Pathways for the Formation of Diolides **200α** and **200β** through S_N2-Like Mechanisms.

In the formation of **200α** and **200β**, the less than perfect stereoselectivity indicates the complex nature of the cyclization reactions of donors **192** and **194**. The competing reaction pathways involve the dissociation of activated thioglycosides **203** and **205**, forming sialyl oxocarbenium type intermediates that often are stabilized in the form of glycosyl triflates,[82, 99, 378] and of disulfonium ions as suggested by the Ellervik group.[379]

The cyclization reactions of axial thiosialyl donors **192**, **196**, and **199**, oxazolidinone **196** and isothiocyanate **199** show greater equatorial selectivity than the acetamide protected donor **192** (**Scheme 40**). This is consistent with earlier work on *N*-acetyloxazolidinone[331, 336] and iso-thiocyanate[366] protected sialyl donors. These protecting group-dependent selectivities can be described in terms of the sialidation mechanism, according to which the more electron-withdrawing oxazolidinone and isothiocyanate groups destabilize any oxocarbenium ions and lead to a greater proportion of the concerted path.

6.2.3 Cation Clock Kinetic Experiments

After investigating the intramolecular glycosylation reactions, the clock precursors were used in competition kinetics experiments with external acceptor 2-propanol. Accordingly, the thiosialyl donors **192**, **194**, **196**, and **199** were individually activated using TfOH/NIS system in dichloromethane at -40 ˚C in the presence of a known quantity of 2-propanol as the external acceptor and stirred for 2 h at -40 ˚C. The reactions were quenched at the same temperature, worked up, and concentrated to obtain the crude reaction mixtures. The crude reaction mixtures were analyzed using UHPLC coupled ESI-MS at 205 nm. This wavelength was selected because it is sensitive to the ester and amide functional groups present in the reaction components and the internal standard.

Following the same protocol, a series of kinetic experiments were carried out on each sialyl donor by changing the acceptor concentration. All the kinetic experiments were conducted at significantly low donor concentration (0.008 mol dm^{-3}) to minimize the intermolecular self-condensation among donor molecules. The crude reaction mixtures from multiple kinetic experiments with each sialyl donor were combined and purified by silica gel chromatography to obtain pure samples of the *O*-sialosides **207α** to **209β** (**Figure 48**), whose configuration was assigned based on the $^{3}J_{C,H}$ coupling constants.

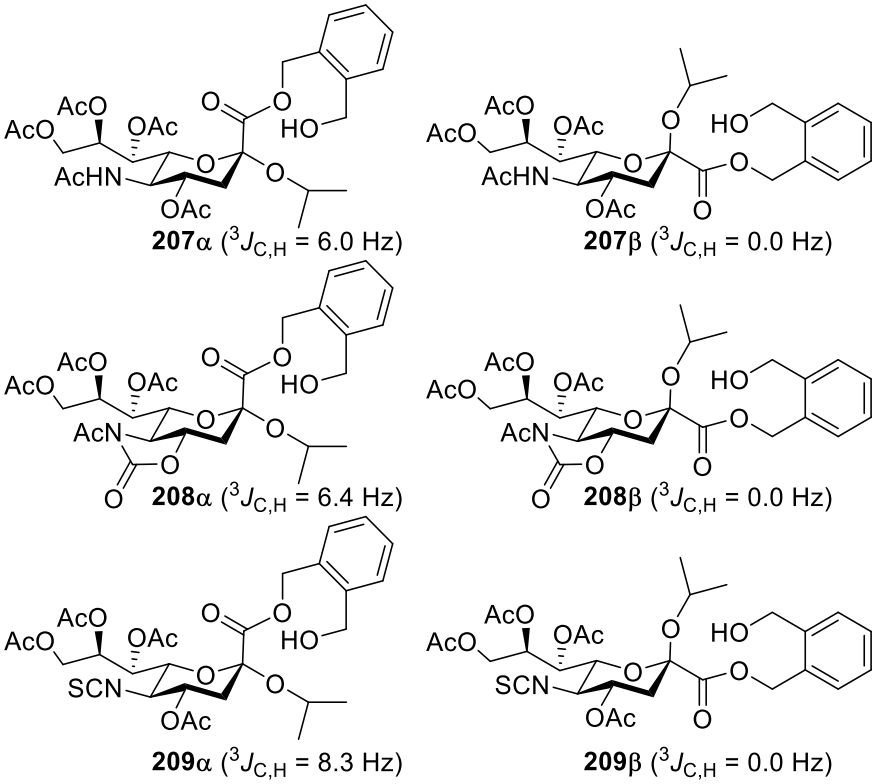


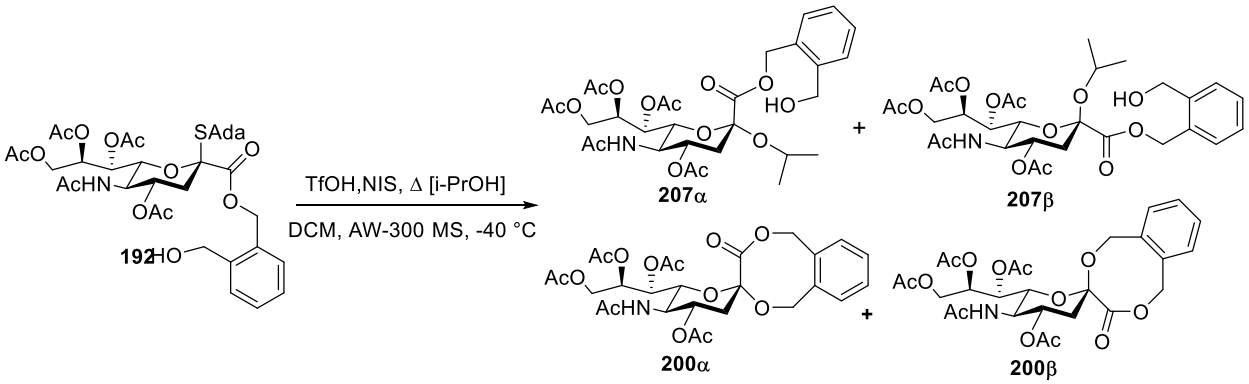
Figure 48. *O*-Sialosides Isolated from Competition Kinetic Experiments.

6.2.3.1 *N*-Acetyl β-Sialyl Donor (202β) at -40 °C

Activation of β-thiosialyl donor **192** by the general procedure for cation clock experiments (**Section 6.1.4**) led to the formation of α- and β-*O*-sialosides **207α** and **207β**, respectively, along with two cyclized products **200α** and **200β** (**Table 26**). The ratio of

each *O*-sialoside to the total amount of cyclized products **200α** and **200β** was determined as a function of the acceptor (2-propanol) concentration, resulting in the data tabulated in **Table 26** and plotted in **Figure 49**.

Table 26. *O*-Sialidation in Competition with Cyclization (peracetylated-β-thioglycoside donor).



Entry	2-PrOH[b]	α-gly/cycl	β-gly/cycl
		207α/(200α+200β)[c]	207β/(200α+200β)[c]
1	1.5(0.012)	0.085	0.053
2	2(0.016)	0.120	0.067
3	2.5(0.020)	0.149	0.081
4	3(0.024)	0.216	0.108
5	3.5(0.028)	0.238	0.118
6	4(0.032)	0.249	0.123
7	5(0.040)	0.322	0.179
8	8(0.064)	0.399	0.186
9	10(0.80)	0.438	0.184
10	15(0.120)	0.512	0.182
11	20(0.160)	0.547	0.162
12	30(0.240)	0.466	0.160

[a]Experimental conditions: NIS (2.4 equiv), TfOH (1 equiv.) at -40 °C; molecular sieves AW-300 MS; [b]Number of equivalents (concentration in mol dm^{-3}) [c]Molar ratios were determined by UHPLC/UV/MS

.

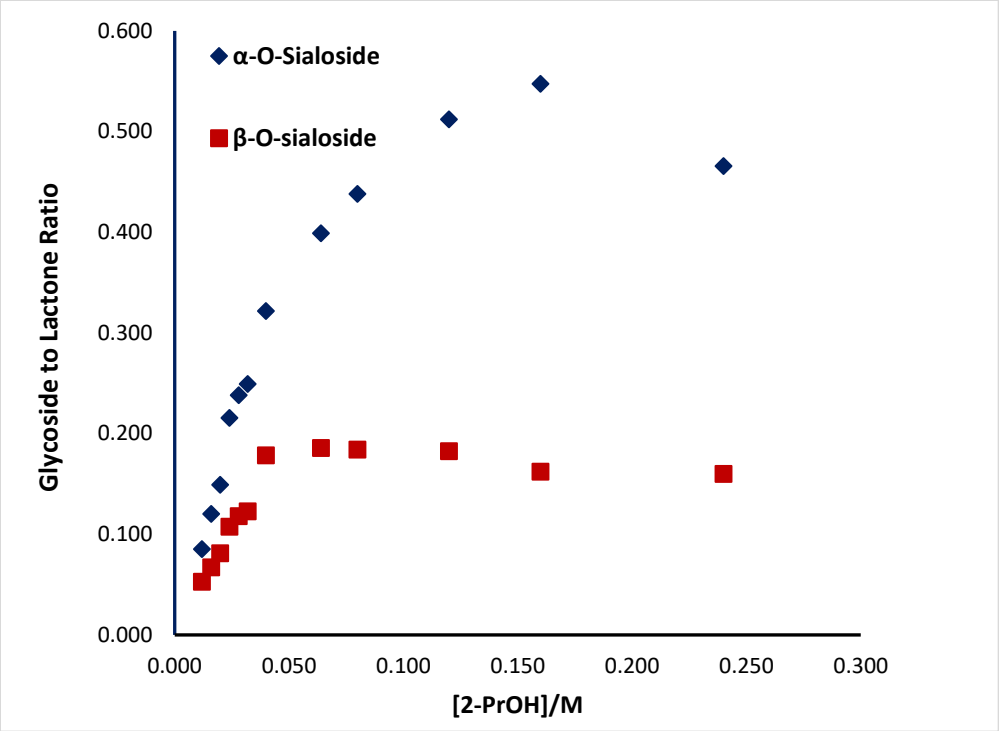


Figure 49. Ratios of α- and β-*O*-Sialosides to Cyclized Products as a Function of Acceptor Concentration at -40 ^{0}C (β-donor).

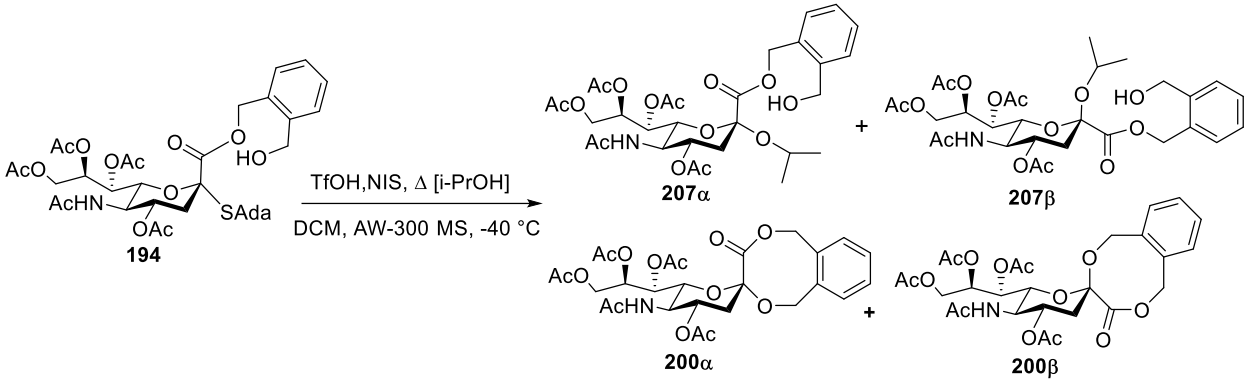
According to **Figure 49**, the relative rates of both the α- and β-*O*-sialidation reactions increase with the increase of acceptor concentration. After the addition of 5 equivalents (0.040 mol dm^{-3}) of the acceptor, the relative rate of β-*O*-sialidation reached a plateau, whereas that of α-*O*-sialidation continued to increase until the acceptor concentration reached 20 equivalents (0.240 mol dm^{-3}). Both the α- and β-*O*-sialidation reactions appear to be dependent on the acceptor concentration. However, the α-*O*-sialidation reaction demonstrates a higher concentration dependence with respect to the acceptor. Since the main focus of this study is to probe the α-*O*-sialidation mechanism, here on the priority is given to α-*O*-sialidation reaction. The nearly linear relationship between the acceptor concentration and relative rate of α-*O*-sialidation indicates first-order kinetics with regards to the acceptor concentration; hence, the α-*O*-sialidation

reaction should follow S_N2 or S_N2-like pathways via covalent sialyl triflate or contact ion pair type intermediates. These results are consistent with the direct kinetic experiments on sialidation reactions discussed in **Chapter 5**.

6.2.3.2 *N*-Acetyl α-Sialyl Donor at -40 °C

Similarly, the anomer of compound **192**, α-sialyl donor **194** was employed in a series of competition kinetic experiments using the general protocol for cation clock experiments (**Section 6.2.3**). Since, typical glycosylation reactions do not involve more than 10 equivalents of the acceptor, in this series, the amount of acceptor was varied from 1.5 to only 10 equivalents (0.800 M). The kinetic data are presented in **Table 27** and plotted in **Figure 50**.

Table 27. *O*-Sialidation in Competition with Cyclization (peracetylated-α-thioglycoside donor).



Entry [a]	iPrOH[b]	α-gly/cycl	β-gly/cycl
		207α/(200α+200β)[c]	207β/(200α+200β)[c]
1	1.5(0.012)	0.126	0.034
2	2(0.016)	0.162	0.055
3	2.5(0.020)	0.207	0.059
4	3(0.024)	0.201	0.062
5	3.5(0.028)	0.305	0.095
6	4(0.032)	0.301	0.095
7	5(0.040)	0.386	0.139
8	8(0.064)	0.517	0.240
9	10(0.80)	0.656	0.322

[a]Experimental conditions: NIS (2.4 equiv), TfOH (1 equiv.) at -40 °C; molecular sieves AW-300 MS; [b]Number of equivalents (concentration in mol dm^{-3}) [c]Molar ratios were determined by UHPLC/UV/MS

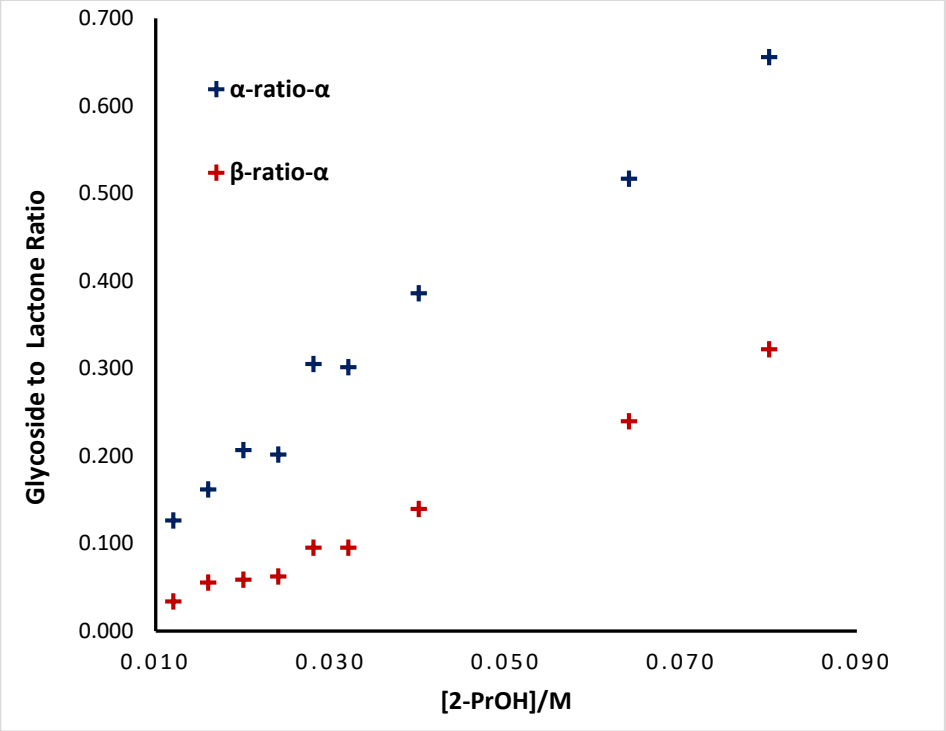


Figure 50. Ratios of α- and β-*O*-Sialosides to Cyclized Products as a Function of Acceptor Concentration at -40 ^{0}C (α-donor).

To compare the kinetics between thiosialyl donors **192** and **194**, the data from the two series were plotted on the same graph (**Figure 51**). No significant changes in the relative rates of sialidation reactions, whether α-*O*- or of β-*O*-sialidation, were observed. The analogy between the kinetics of two sialyl donors reveals that their mechanism is independent from the starting anomeric configuration, and both donors follow similar reaction pathways. In particular, α-*O*-sialidation of both donors **192** and **194** follow S_N2 or S_N2-like mechanisms most likely via axial sialyl triflate intermediate. This similarity between the two sialyl donors is consistent with the previous study of direct kinetic experiments on sialidation reactions (**Chapter 5**).

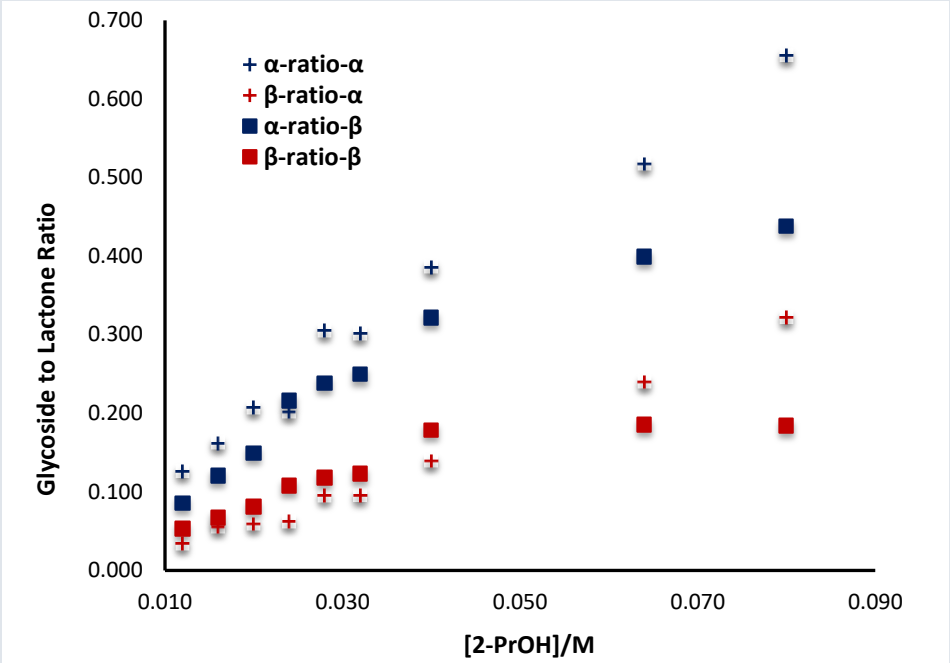
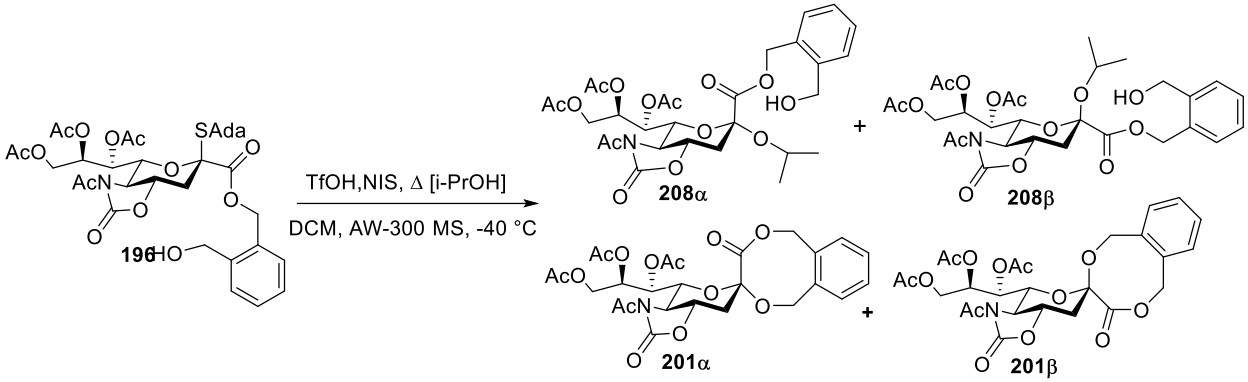


Figure 51. Comparison of Relative Rates Between α- and β-Thiosialyl Donors as a Function of Acceptor Concentration at -40 ^{0}C.

6.2.3.3 *N*-Acetyl 5-*N*,4-*O*-Oxazolidinone β- Sialyl Donor at -40 °C

Application of the general cation clock protocol (**Section 6.2.3**) to the *N*-acetyl-5-*N*,4-*O*-oxazolidinone protected β-thiosialyl donor resulted in *O*-sialosides **208α** and **208β**, along with cyclized products **201α** and **201β**. The ratio of each *O*-glycoside **208α** or **208β** to the combined amount of cyclization products **201α** and **201β** was determined as a function of 2-propanol concentration, resulting in the data presented in **Table 28** and tabulated in **Figure 52**.

Table 28. *O*-Sialidation in Competition with Cyclization (*N*-Acetyl-oxazolidinone-β-Thioglycoside Donor).



Entry[a]	*i*-PrOH[b]	α-gly/cycl	β-gly/cycl
		208α/(201α+201β)[c]	208β/(201α+201β)[c]
1	1.5(0.012)	0.183	0.000
2	2(0.016)	0.357	0.048
3	2.5(0.020)	0.454	0.103
4	3(0.024)	0.571	0.105
5	3.5(0.028)	0.574	0.301
6	4(0.032)	0.613	0.232
7	5(0.040)	0.768	0.348
8	6(0.048)	0.787	0.436
9	8(0.064)	0.892	0.697
10	9(0.072)	0.907	0.672
11	10(0.80)	0.957	0.611
12	15(0.120)	1.165	0.781
13	20(0.160)	1.462	0.828
14	25(0.200)	1.907	0.919

[a]Experimental conditions: NIS (2.4 equiv), TfOH (1 equiv.) at -40 °C; molecular sieves AW-300 MS; [b]Number of equivalents (concentration in mol dm^{-3}) [c]Molar ratios were determined by UHPLC/UV/MS.

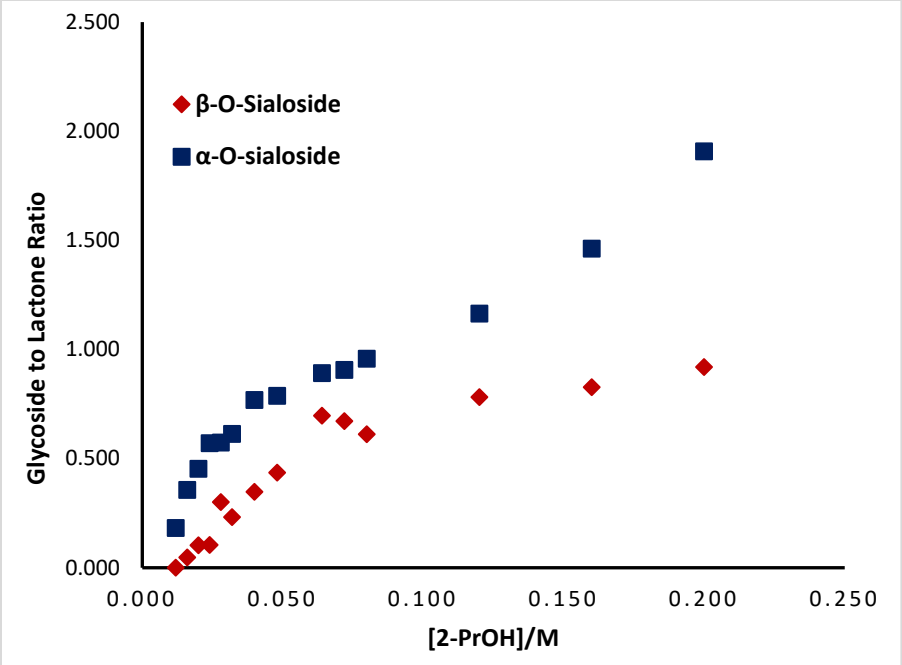


Figure 52. Ratios of α- and β-*O*-Sialosides to Cyclized Products as a Function of Acceptor Concentration at -40 ^{0}C (*N*-Acetyl-oxazolidinone-protected-β-donor).

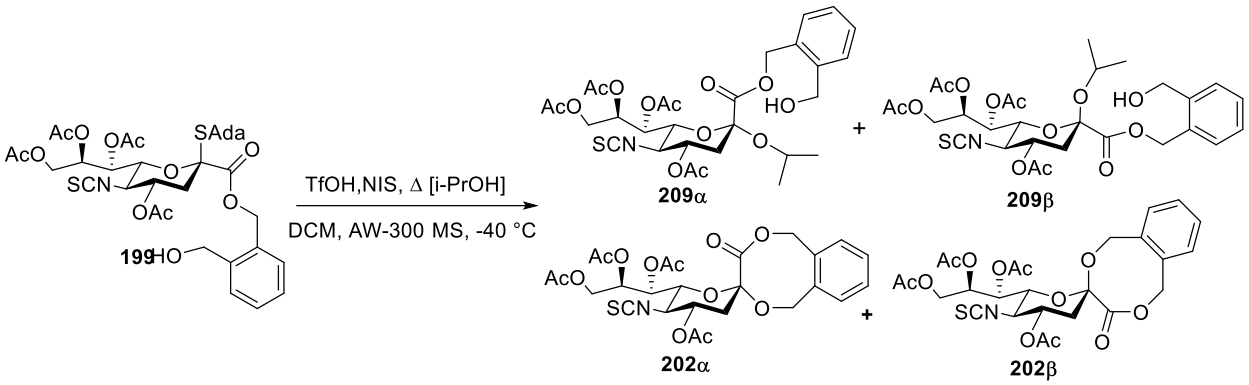
According to **Figure 52**, the rates of both the α- and β-*O*-sialidation reactions are dependent on the acceptor concentration; the α-*O*-sialidation has higher selectivity compared to that of the β-*O*-sialidation. The relative rate of β-O-sialidation is almost unchanged beyond ~8.0 eq of the acceptor; however, the kinetics at such extreme molar ratios of the acceptor are not important as such amounts are not utilized in typical glycosylation reactions. Also, the very high concentrations of 2-propanol in the latter experiments means that the polarity of the reaction solvent is considerably changed, and therefore the reaction mechanism can also be altered.

6.2.3.4 5-Isothiocyanate-protected β-Thiosialyl Donor at -40 °C

The competition kinetics of **199** with the highly electron-withdrawing isothiocyanate group resulted in *O*-sialosides **209α** and **209β** together with cyclization products **202α** and **202β**. The ratio of individual glycoside **209α** or **209β** to the total amount of cyclization

products **202α** and **202β** were calculated as a function of 2-propanol concentration, resulting in the data tabulated in **Table 29** and plotted in **Figure 53**.

Table 29. *O*-Sialidation in Competition with Cyclization (Isothiocyanato-β-thioglycoside donor).



Entry[a]	*i*-PrOH (M conc)[b]	α-gly/cycl	β-gly/cycl
		209α/(202α+202β)[c]	209β/(202α+202β)[c]
1	1.5(0.012)	0.418	0.000
2	2(0.016)	0.591	0.077
3	2.5(0.020)	0.976	0.208
4	3(0.024)	1.133	0.564
5	3.5(0.028)	1.217	0.546
6	4(0.032)	1.352	0.838
7	5(0.040)	1.463	0.907
8	6(0.048)	1.520	1.266
9	8(0.064)	1.623	1.761
10	10(0.800)	1.448	2.019
11	15(0.120)	1.820	2.645

[a]Experimental conditions: NIS (2.4 equiv), TfOH (1 equiv.) at -40 °C; molecular sieves AW-300 MS; [b]Number of equivalents (concentration in mol dm^{-3}) [c]Molar ratios were determined by UHPLC/UV/MS.

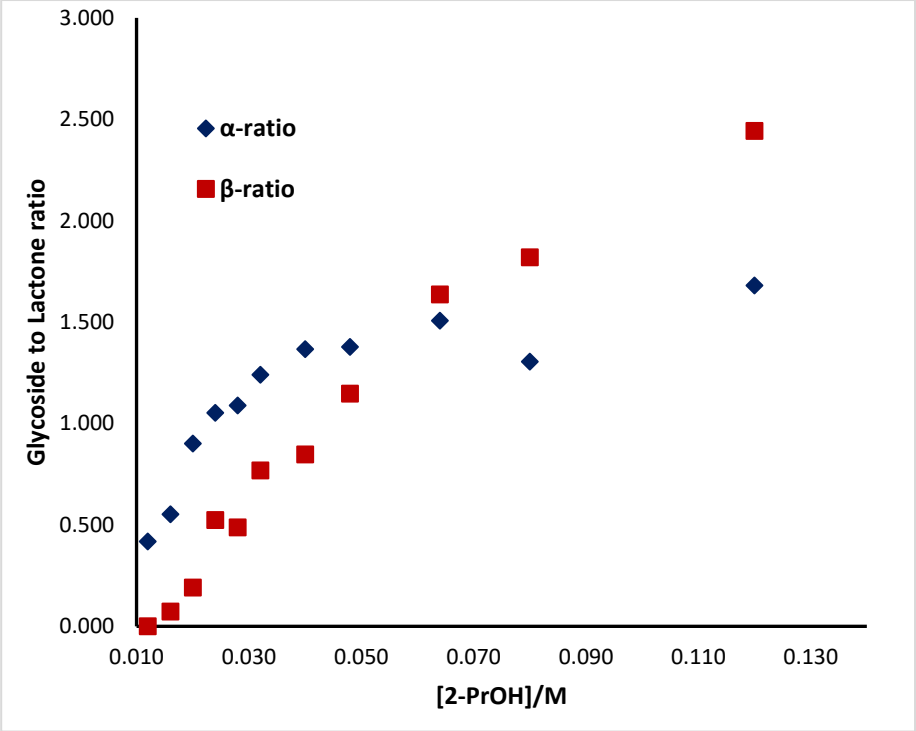


Figure 53. Ratios of α- and β-*O*-glycosides to cyclized products as a function of nucleophile concentration at -40 ^{0}C (Isothiocyanato-protected-β-donor).

As depicted in **Figure 53**, similar to the *N*-acetyl-oxazolidinone-protected β-thiosialyl donor, the rates of both the α- and β-*O*-sialidation reactions are dependent on the acceptor concentration, and the α-*O*-sialidation has higher preference compared to that of the β-*O*-sialidation. However, after the addition of 8 equivalents of 2-propanol, the selectivity of the sialidation reaction was changed, preferring the formation of the β-*O*-sialoside over α-*O*-sialoside. Such changes in the reactivity may be due to changes in the reaction mechanism with the introduction of extreme amounts of 2-propanol to the reaction medium.

Since the main focus is on the kinetics of α-*O*-sialidation reactions, the relative rates of α-*O*-sialoside formation from donors **3**, **9**, and **199** were tabulated in **Table 30** and plotted on one graph (**Figure 54**) for ease of comparison.

Table 30. Comparison of *O*-Sialidation of *N*-Acetyl, *N*-Acetyl-oxazolidinone and Isothiocyanato Protected Donors (β-donors) in Competition with Cyclization.

Entry [a]	iPrOH (M conc)[b]	α-gly/cycl	α-gly/cycl	α-gly/cycl
		207α/(200α+200β)[c]	**208α/(201α+201β)[c]**	**209α/(202α+202β)[c]**
1	1.5(0.012)	0.085	0.182	0.418
2	2(0.016)	0.120	0.357	0.591
3	2.5(0.020)	0.149	0.454	0.976
4	3(0.024)	0.216	0.571	1.133
5	3.5(0.028)	0.238	0.574	1.217
6	4(0.032)	0.249	0.613	1.352
7	5(0.040)	0.322	0.768	1.463
8	6(0.048)	-	0.787	1.520
9	8(0.064)	0.399	0.892	1.623
10	10(0.800)	0.438	0.907	1.448
11	15(0.120)	0.512	0.957	1.820

[a]Experimental conditions: NIS (2.4 equiv), TfOH (1 equiv.) at -40 °C; molecular sieves AW-300 MS; [b]Number of equivalents (concentration in mol dm^{-3}) [c]Molar ratios were determined by UHPLC/UV/MS.

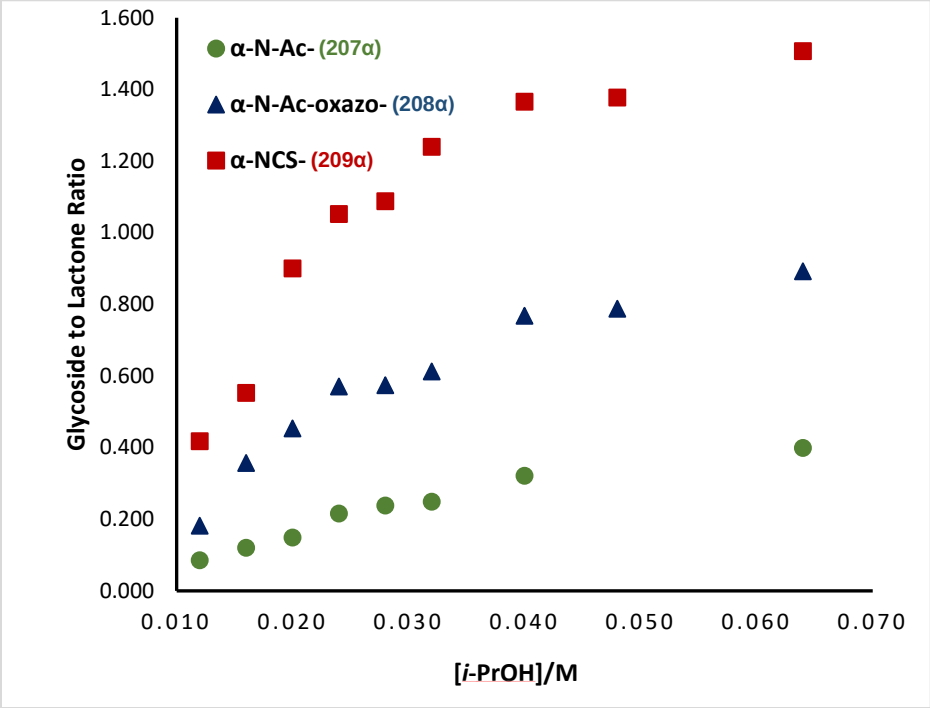
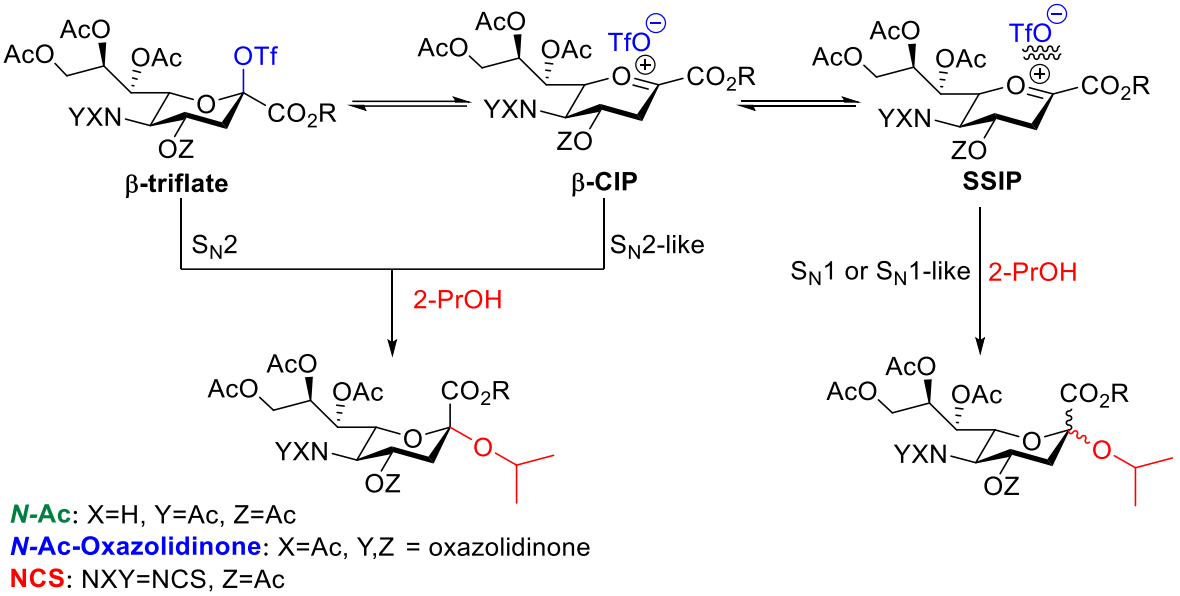


Figure 54. Comparison of Kinetics of Different Sialyl Donors at -40 ^{0}C.

As depicted in **Figure 54**, the influence of acceptor concentration on the rate of α-*O*-sialidation reactions increases from the *N*-acetyl to *N*-acetyl-oxazolidinone to isothiocyanate protected donors. This trend reveals that the increase of the electron-withdrawing nature of the protecting group enhances the dependence on acceptor concentration, consistent with increased S_N2 or S_N2-like reaction pathways via covalently bonded axial sialyl triflates or β-contact ion pair-type intermediates (**Scheme 42**). In other words, when the electron-withdrawing nature of the protecting group increases, the formation of oxocarbenium type intermediates is disfavored; thus, the equilibrium shifts towards the covalent or contact ion-pair type intermediates that favor S_N2 of S_N2-like mechanisms.



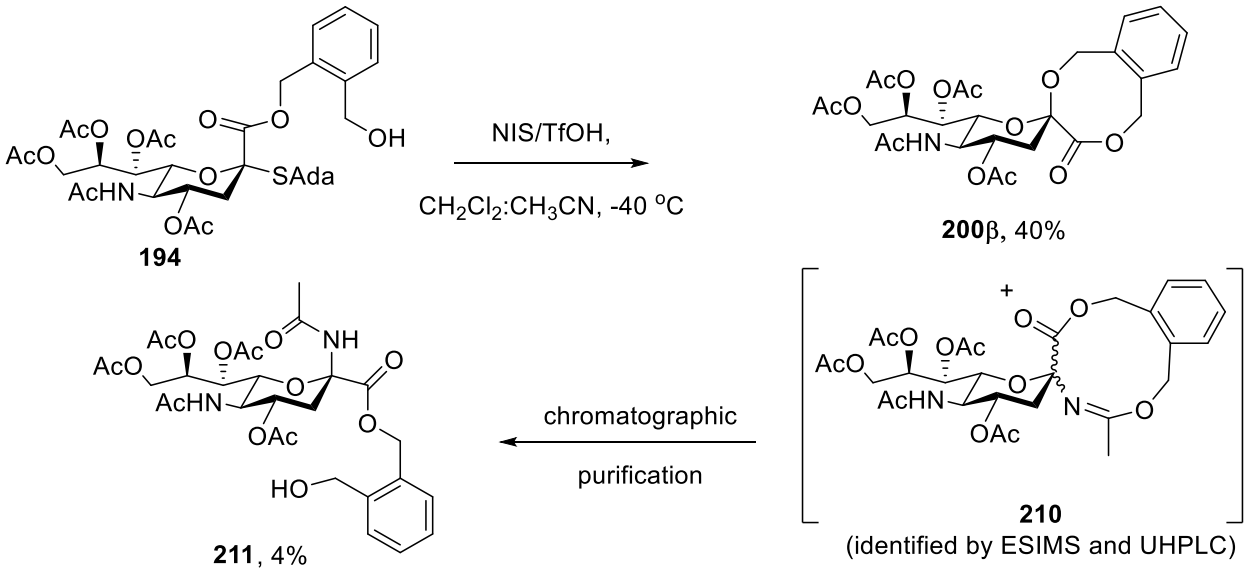
Scheme 42. Equilibration of a β-Sialyl Triflate with a β-Contact Ion Pair and Solvent-Separated Ion Pair.

6.2.4 Influence of Nitrile Solvents on Sialidation Reactions

Acetonitrile is one of the most widely used solvents in sialidation reactions[263, 269] typically resulting in better α-selectivities. The rationale for the enhanced stereoselectivity involves the formation of β-sialyl nitrilium ions followed by S_N2-like nucleophilic attack (**Chapter 4**, **Section 4.3.2**). However, no direct evidence for the formation of the nitrilium adduct has been reported.

Treatment of sialyl donor **194** with *N*-iodosuccinimide and triflic acid in a mixture of dichloromethane and acetonitrile (7:3) at – 40 °C, resulted in the formation of the **200β** and two new compounds in the UHPLC and ESI-MS (m/z 643) analysis ultimately assigned as diastereomers of structure **210** (**Scheme 43**). Chromatographic purification of the crude reaction mixture gave 40% of **200β** and the impure Ritter product **210** in ~6% yield. Attempted silica gel chromatographic purification of **210** resulted in isolation of the hydrolyzed product **211** in 4% yield (**Scheme 43**). The anomeric configuration of amide

211 was identified from the $J_{C1,H3ax}$ coupling constant of 0 Hz. The formation of **200β** as a single isomer can be explained by direct displacement of the activated thioglycoside **205** by an intramolecular nucleophile, consistent with the cyclization reactions in dichloromethane (**Scheme 40** and **41**). Overall, the isolation of the axial Ritter product **211** is stong evidence in support of the formation of β-sialyl nitrilium ions, which then undergo displacement reactions with nucleophiles in an S_N2-like pathway to form the equatorial glycosides (**Chapter 5**, **Scheme 36**).[346]



Scheme 43. Formation and Isolation of a Ritter Type Amide from the Sialidation in the Presence of Acetonitrile.

6.3 Conclusions

The yields and stereoselectivities of the cyclization reactions of donors **192**, **194**, **196**, and **199** are informative. Comparison of the stereoselectivities of the donors **192** and **194** suggests that the intramolecular glycosylation of these donors take place mainly via an S_N2-like pathway. Further, the protecting group system of sialyl donors has a significant influence on the yields and selectivities of cyclization reactions. The rate of α-

selective cyclization increases in a more pronounced manner with the introduction of more electron-withdrawing protecting groups, revealing the formation of β-sialyl triflate and subsequent S_N2 or S_N2-like cyclization reactions. In terms of yields of cyclization reactions, the *N*-acetyl-oxazolidinone and isothiocyanate protected systems gave low yields in their cyclization reactions due to the competing intermolecular glycosylations between two donor molecules.

Finally, in an important experiment, cyclization reaction of the *N*-acetyl donor **194** in the presence of acetonitrile as a co-solvent in dichloromethane resulted in the formation of a Ritter-type amide strongly supporting the formation of a sialyl nitrilium ion, which has been postulated as a key intermediate during sialidation reactions in the presence of acetonitrile.[343, 380]

Overall, this study reveals that the α-*O*-sialidation reaction proceeds via S_N2 or S_N2-like mechanisms, involving β-sialyl triflate or nitrilium ion intermediates. With the introduction of more electron-withdrawing protecting groups, corresponding sialidation reactions move more to the S_N2 or S_N2-like end of the mechanistic spectra.

CHAPTER 7. CONCLUSIONS

A series of oxabicyclo[4.4.0]decane derivatives were synthesized as models to represent the ideal *gg*, *gt*, and *tg* conformations of the pyranoside side chain. Analysis of the complete data set leads to the exclusion of several compounds on the grounds of non-ideal conformations. The remaining models were employed to derive a set of limiting coupling constants. These limiting coupling constants were used to determine the side chain populations of several literature compounds. The absence of negative populations in the analysis supports the viability of the new limiting coupling constants. The new limiting coupling constants also can be applied to calculate the side chain conformation of higher carbon sugars and even vicinal diols with the application of appropriate correction factors. The information from this study may also serve the computational chemistry field by providing reliable data with which to develop computational methods for the calculation of side chain conformations.

Application of the new limiting data set to three series of 4-*O*-derivatives of galacto- and glucopyranosides and 6-*O*-analogues of glucopyranosides gave their side chain populations as a function of different protecting groups. Despite the fact that the changes in the side populations are small, they can modulate the major effects exerted by electron-withdrawing or donating groups. In particular, the electron-withdrawing effects of 4-*O*-esters of galactopyranosides are moderated by a change in the side chain conformation to the more reactive *gg* and *tg* conformers. The side chain conformation of 4-*O*-esters of glucopyranosides, on the other hand, reinforces the electron-withdrawing effects of the esters. For both the galacto- and glucopyranose 4-*O*-series, the introduction of highly electron-withdrawing esters, such as trifluoro or trichloro esters, changes the side chain

population to support the electron-withdrawing effect of the ester. No significant modulatory effects were observed in the glucopyranose 6-*O*- series with regards to the change in protecting groups.

Pseudo-first-order kinetic experiments with respect to the glycosyl acceptor were conducted with both α- and β- anomers of an *N*-acetyl-4-*O*,5-*N*-oxazolidinone protected sialyl donor. Both donors demonstrated a strong dependence on the acceptor concetration for the formation of the α-*O*-sialoside, revealing first order kinetics with respect to the acceptor. Thus, an overall S_N2 or S_N2-like reaction mechanism via a β-sialyl triflate intermediate is proposed. Kinetic experiments at constant donor and acceptor concentration with varied amounts of acetonitrile as a co-solvent in dichloromethane revealed that the concertation of acetonitrile has a significant influence on the rate of α-*O*-sialidation reactions. This is a clear indication of the participation of acetonitrile, probably in the form of a β-sialyl nitrilium ion, favoring S_N2 or S_N2-like reactions in the formation of α-*O*-sialosides. Overall, this study recommends using the highest possible concentrations of glycosyl donor and acceptor and employing acetonitrile as an additive in sialidation reactions to obtain better α-selectivity.

A cation clock reaction was developed to probe the sialidation reaction mechanism(s). Thus, a series of sialyl donors (cation clock precursors) having different protecting groups at 5-*N*- or 4-*O*,5-*N*-positions were prepared, and their cyclization reactions were investigated. For the cyclization of α- and β- *N*-acetyl thiosialyl donors, an S_N2 type reaction mechanism was proposed on the basis of the results. Cyclization yields from *N*-acetyl-oxazolidinone and isothiocyanate precursors were only moderate, rather favoring dimer formation. A series of competition kinetic experiments of these cation clock

precursors demonstrated that the rate of α-*O*-sialidation is strongly dependent on the acceptor concentration and that this influence is amplified with the increasing electron-withdrawing effect of the protecting groups (*N*-acetyl to *N*-acetyl-oxazolidinone to isothiocyanate, respectively). Thus, an S_N2 or S_N2-like mechanism is proposed for the formation of α-*O*-sialosides especially in the presence of more electron-withdrawing protecting groups. Attempted kinetic experiments in the presence of acetonitrile revealed the formation of cyclic imidates, which upon purification by silica gel chromatography gave an axial anomeric acetamide; strong evidence for the formation of β-nitrilium ions in the presence of acetonitrile. Thus, an S_N2-like reaction pathway via a β-sialyl nitrilium ion is proposed for the formation of α-*O*-sialosides under these conditions. Overall, the results are consistent with the direct kinetic experiments; thus, employing *N*-acetyl-oxazolidinone or isothiocyanate protected sialyl donors with a maximum possible overall concentration in acetonitrile containing solvent mixture is recommended to secure a better α-selectivity.

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Title: The Experimental Evidence in Support of Glycosylation Mechanisms at the SN1–SN2 Interface

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Publication: Chemical Reviews

Publisher: American Chemical Society

Date: Sep 1, 2018

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CHAPTER 8. EXPERIMENTAL SECTION

General Experimental.

Commercial reagents were used without further purification unless otherwise stated. Commercially available anhydrous solvents were used for moisture sensitive reactions unless otherwise stated. Molecular sieves were activated using Buchi glass oven B-585 kugelrohr at 254 °C overnight. Anhydrous CH_2Cl_2 and CH_3CN were dried over 4 Å and 3 Å molecular sieves respectively for use in glycosylation reactions. All extracts were dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure at ~ 35 °C. Flash column chromatographic purifications were carried out over silica gel unless otherwise stated. HPLC purifications were performed on Varian prep star HPLC using an Agilent DYNAMAX SI-column (5 µm, 21.4 x 250 mm) at ambient temperature with a flow rate of 21.5 mL/min. UHPLC analysis was performed with a BEH C18 1.7 µm (2.1 × 100 mm) column equipped with LC-MS and TUV (205 nm) detector. NMR spectra were recorded in CD_3OD, $CDCl_3$, or C_6D_6 with 400, 500, or 600 MHz instruments unless otherwise stated. The DAISY 1D-NMR spectra simulation tool in the TopSpin 3.5 pl 7 or MestReNova 9.0 suite of programs was used to simulate ^{1}H-NMR spectra. ESI-HRMS were recorded using a Waters LCT Premier Xe time of flight mass spectrometer. Specific rotations were recorded on an automatic polarimeter with a path length of 100 mm in the solvent specified.

Methyl 4,6-*O*-Benzylidene-2,3-di-*O*-methyl-α-D-galactopyranoside (47).

Compound **47** was synthesized following literature protocol.[152] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 – 750 (m, 2H), 7.37 – 7.29 (m, 3H), 5.54 (s, 1H), 4.98 (d, *J* = 3.5 Hz, 1H), 4.34 (dd, *J* = 3.5, 1.3 Hz, 1H), 4.26 (dd, *J* = 12.5, 1.8 Hz, 1H), 4.07 (dd, *J* =

12.5, 1.8 Hz, 1H), 3.78 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.67 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.63 (td, *J* = 1.8, 1.3 Hz, 1H), 3.52 (s, 3H), 3.50 (s, 3H), 3.44 (s, 3H).

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 7.71 – 7.70 (m, 2H), 7.15 – 7.03 (m, 3H), 5.27 (s, 1H), 4.75 (d, *J* = 3.5 Hz, 1H), 4.06 (dd, *J* = 12.2, 1.6 Hz, 1H), 3.80 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.77 (dd, *J* = 3.6, 1.1 Hz, 1H), 3.62 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.44 (dd, *J* = 12.2, 1.9 Hz, 1H), 3.28 (s, 3H), 3.18 (s, 3H), 3.10 (s, 3H), 3.05 (ddd, *J* = 1.9, 1.6, 1.1 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 7.49 – 7.47 (m, 2H), 7.36 – 7.30 (m, 3H), 5.59 (s, 1H), 4.95 (d, *J* = 2.4 Hz, 1H), 4.45 (dd, *J* = 2.2, 1.1 Hz, 1H), 4.15 (dd, *J* = 12.5, 1.6 Hz, 1H), 4.12 (dd, *J* = 12.5, 1.7 Hz, 1H), 3.66 – 3.65 (m, 2H), 3.64 (ddd, *J* = 1.7, 1.6, 1.1 Hz, 1H), 3.46 (s, 3H), 3.44 (s, 3H), 3.41 (s, 3H).

Methyl 4,8-Anhydro-7-deoxy-2,3,6-tri-*O*-methyl-α-D-*glycero*-D-*galacto*-octopyranoside (48).

Compound **48** was synthesized following literature protocol.[110] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.95 (d, *J* = 3.7 Hz, 1H), 4.13 (ddd, *J* = 11.9, 4.8, 1.7 Hz, 1H), 4.00 (dd, *J* = 3.2, 0.8 Hz, 1H), 3.68 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.68 (dd, *J* = 3.2, 0.8, 1H), 3.55 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.52 (s, 3H), 3.49 (s, 3H), 3.47 (s, 3H), 3.41(s, 3H), 3.40 (ddd, *J* = 12.6, 11.7, 2.2, 1H), 3.34 (ddd, *J* = 11.9, 4.5, 3.2 Hz, 1H), 1.98 (dddd, *J* = 12.6, 12.4, 11.9, 4.8 Hz, 1H), 1.74 (dddd, *J* = 12.4, 4.5, 2.2, 1.7 Hz, 1H).

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.81 (d, *J* = 3.7 Hz, 1H), 3.88 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.80 (ddd, *J* = 11.6, 4.8, 1.8 Hz, 1H), 3.71 (d, *J* = 3.2 Hz, 1H), 3.57 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.29 (s, 3H), 3.27 (s, 3H), 3.22 (dd, *J* = 3.2, 0.8 Hz, 1H), 3.22 (s, 3H), 3.10 (s, 3H), 2.92 (ddd, *J* = 12.7, 11.6, 2.2 Hz, 1H), 2.80 (ddd, *J* = 11.8, 4.5, 3.2 Hz, 1H), 2.10 (dddd, *J* = 12.7, 12.5, 11.8, 4.8 Hz, 1H), 1.31 (dddd, *J* = 12.5, 4.5, 2.2, 1.8 Hz, 1H).

In CD_3OD: ^1H NMR (600 MHz, CD_3OD) δ 4.90 (d, *J* = 3.7 Hz, 1H), 4.01 (dd, *J* = 3.1, 0.7 Hz, 1H), 4.00 (ddd, *J* = 11.7, 4.9, 1.7 Hz, 1H), 3.77 (dd, *J* = 3.2, 0.7 Hz, 1H), 3.58 (dd, *J* = 10.1, 3.7 Hz, 1H), 3.52 (dd, *J* = 10.1, 3.2 Hz, 1H), 3.45 (ddd, *J* = 12.0, 4.6, 3.1 Hz, 1H), 3.44 (s, 3H), 3.42 (ddd, *J* = 12.5, 11.7, 2.0 Hz, 1H), 3.41 (s. 6H), 3.39 (s, 3H), 1.86 (dddd, *J* = 12.5, 12.4, 12.0, 4.9 Hz, 1H), 1.71 (dddd, *J* = 12.4, 4.6, 2.0, 1.7 Hz, 1H).

Methyl 4,8-Anhydro-7-deoxy-2,3,6-tri-*O*-methyl-β-L-*glycero*-D-*galacto*-octopyranoside (51).

Compound **51** was prepared using literature protocol.[110] **In $CHCl_3$:** ^1H NMR (600 MHz, $CDCl_3$) δ 4.86 (d, *J* = 3.6 Hz, 1H), 4.00 (dd, *J* = 3.4, 1.1 Hz, 1H), 3.84 (ddd, *J* = 11.4, 5.3, 1.3 Hz, 1H), 3.71 (ddd, *J* = 13.1, 11.4, 2.6 Hz, 1H), 3.67 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.61 (dd, *J* = 3.1, 1.1 Hz, 1H), 3.52 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.52 (s, 3H), 3.49 – 3.46 (m, 4H), 3.42 (s, 3H), 3.39 (s, 3H), 1.97 (dddd, *J* = 14.3, 13.1, 5.2, 2.8 Hz, 1H), 1.65 (dddd, *J* = 14.3, 3.7, 2.6, 1.3 Hz, 1H).

In C_6D_6: ^1H NMR (600 MHz, C_6D_6) δ 4.84 (d, *J* = 3.6 Hz, 1H), 4.15 (dd, *J* = 3.4, 1.1 Hz, 1H), 4.00 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.76 – 3.69 (m, 3H), 3.40 (ddd, *J* = 3.0, 2.9, 2.8 Hz, 1H), 3.37 (s, 3H), 3.27 (s, 3H), 3.19 (s, 3H), 3.00 (s, 3H), 2.05 (dddd, *J* = 14.1, 13.0, 7.6, 2.8 Hz, 1H), 1.36 (dddd, *J* = 14.1, 2.9, 2.8, 1.1 Hz, 1H).

In CD_3OD: ^1H NMR (600 MHz, CD_3OD) δ 4.89 (d, *J* = 3.6 Hz, 1H), 4.01 (dd, *J* = 3.4, 1.1 Hz, 1H), 3.73 (ddd, *J* = 11.3, 5.4, 1.2 Hz, 1H), 3.68 (ddd, *J* = 13.2, 11.4, 2.1 Hz, 1H), 3.62 (dd, *J* = 3.1, 1.1 Hz, 1H), 3.60 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.50 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.47 (ddd, *J* = 3.1, 3.0, 2.9 Hz, 1H), 3.46 (s, 3H), 3.41 (s, 3H), 3.40 (s, 3H), 3.40 (s, 3H), 1.95 (dddd, *J* = 14.3, 13.0, 5.4, 2.9 Hz, 1H), 1.67 (dddd, *J* = 14.3, 3.0, 2.8, 1.2 Hz, 1H).

Methyl 4,8-Anhydro-7-deoxy-2,3,6-tri-*O*-methyl-α-D-*glycero*-D-*gluco*-octopyranoside (53).

Compound **53** was synthesized using the literature protocol.[381] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.89 (d, *J* = 3.6 Hz, 1H), 3.75 (ddd, *J* = 11.6, 5.5, 1.5 Hz, 1H). 3.72 (ddd, *J* = 3.6, 2.6, 2.2 Hz, 1H), 3.69 (ddd, *J* = 12.7, 11.6, 2.2 Hz, 1H), 3.60 (s, 3H), 3.56 (dd, 10.1, 8.9 Hz, 1H), 3.54 (dd, 10.1, 2.6 Hz, 1H), 3.50 (s, 3H), 3.46 (dd, *J* = 9.4, 8.9 Hz, 1H), 3.43 (s, 3H), 3.42 (s, 3H), 3.28 (dd, *J* = 9.4, 3.6 Hz, 1H), 1.98 (dddd, *J* = 14.5, 3.6, 2.2, 1.5 Hz, 1H), 1.71 (dddd, *J* = 14.5, 12.7, 5.5, 2.2 Hz, 1H).

In C_6D_6: H NMR (600 MHz, C_6D_6) δ 4.71 (d, *J* = 3.6 Hz, 1H), 3.96 (dd, *J* = 10.1, 9.2 Hz, 1H), 3.83 (dd, *J* = 9.4, 9.2 Hz, 1H), 3.73 (ddd, *J* = 12.7, 11.4, 2.5 Hz, 1H), 3.68 (s, 3H), 3.63 (dd, *J* = 10.1, 2.6 Hz, 1H), 3.55 (ddd, *J* = 11.4, 5.4, 1.5 Hz, 1H), 3.40 (ddd, *J* = 3.6, 2.6, 2.5 Hz, 1H), 3.27 (s, 3H), 3.26 (s, 3H), 3.24 (dd, *J* = 9.4, 3.6 Hz, 1H), 3.16 (s, 3H), 1.45 (dddd, *J* = 14.1, 3.6, 2.5, 1.5 Hz, 1H), 1.38 (dddd, *J* = 14.1, 12.7, 5.4, 2.5 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.88 (d, *J* = 3.7 Hz, 1H), 3.70 (ddd, *J* = 11.5, 5.6, 1.5 Hz, 1H), 3.70 (ddd, *J* = 3.6, 2.6, 2.4 Hz, 1H), 3.64 (ddd, *J* = 12.8, 11.5, 2.2 Hz, 1H), 3.53 (s, 3H), 3.52 (dd, *J* = 10.1, 2.6 Hz, 1H), 3.48 (dd, *J* = 10.1, 9.0 Hz, 1H), 3.47 (s, 3H), 3.43 (s, 3H), 3.40 (s, 3H), 3.36 (dd, *J* = 9.5, 9.0 Hz, 1H), 3.19 (dd, *J* = 9.5, 3.7 Hz, 1H), 1.95 (dddd, *J* = 14.5, 3.6, 2.2, 1.5 Hz, 1H), 1.74 (dddd, *J* = 14.5, 12.8, 5.6, 2.4 Hz,1H).

Methyl 4,8-Anhydro-7-deoxy-2,3,6-tri-*O*-methyl-β-L-*glycero*-D-*gluco*-octopyranoside (55).

Compound **55** was synthesized following literature protocol.[381] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.87 (d, *J* = 3.7 Hz, 1H), 4.00 (ddd, *J* = 11.9, 5.3, 1.5 Hz, 1H), 3.61

(s, 3H), 3.52 (s, 3H), 3.50 (t, *J* = 9.3 Hz, 1H), 3.47 (s, 3H), 3.45 (s, 3H), 3.42 (dd, *J* = 9.6, 9.0 Hz, 1H), 3.42 (ddd, *J* = 12.9, 11.9, 2.3 Hz, 1H), 3.30 (ddd, *J* = 11.0, 9.0, 5.1 Hz, 1H), 3.24 (dd, *J* = 9.3, 3.7 Hz, 1H), 2.98 (dd, *J* = 9.3, 9.6 Hz, 1H), 2.04 (dddd, *J* = 13.2, 5.1, 2.3, 1.5 Hz, 1H), 1.55 (dddd, *J* = 13.2, 12.9, 11.0, 5.3 Hz, 1H).

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.65 (d, *J* = 3.7 Hz, 1H), 3.74 (t, *J* = 9.2 Hz, 1H), 3.62 – 3.60 (ddd, *J* = 11.8, 5.2, 1.6 Hz, 1H), 3.59 (s, 3H), 3.57 (dd, *J* = 9.7, 9.4 Hz, 1H), 3.27 (s, 3H), 3.22 (s, 3H), 3.19 (s, 3H), 3.17 (dd, *J* = 9.2, 3.7 Hz, 1H), 2.99 (dd, *J* = 9.7, 9.2 Hz, 1H), 2.99 (ddd, *J* = 11.0, 9.0, 5.1 Hz, 1H), 2.98 (ddd, *J* = 12.7, 11.8, 2.4 Hz, 1H), 1.51 (dddd, *J* = 13.1, 5.1, 2.4, 1.6 Hz, 1H), 1.40 (dddd, *J* = 13.1, 12.7, 11.0, 5.2 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.85 (d, *J* = 3.8 Hz, 1H), 3.94 (ddd, *J* = 11.8, 5.2, 1.6 Hz, 1H), 3.51 (s, 6H), 3.45 (s, 3H), 3.42 (ddd, *J* = 12.8, 11.9, 2.3 Hz, 1H), 3.39 (s, 3H), 3.37 (dd, *J* = 9.4, 9.3 Hz, 1H), 3.33 (ddd, *J* = 10.8, 9.2, 4.8 Hz, 1H), 3.31 (t, *J* = 9.2 Hz, 1H), 3.20 (dd, *J* = 9.4, 3.8 Hz, 1H), 2.96 (dd, *J* = 9.4, 9.3 Hz, 1H), 2.06 (dddd, *J* = 13.2, 4.8, 2.3, 1.6 Hz, 1H), 1.48 (dddd, *J* = 13.2, 12.8, 10.8, 5.2, 1H).

Methyl 4,6-*O*-Benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside (57).

Compound **57** was synthesized by following the literature protocol.[153] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 – 7.48 (m, 2H), 7.39 – 7.33 (m, 3H), 5.54 (s, 1H), 4.86 (d, *J* = 3.7 Hz, 1H), 4.28 (dd, *J* = 10.2, 4.7 Hz, 1H), 3.81 (ddd, *J* = 10.1, 9.6, 4.7 Hz, 1H), 3.73 (dd, *J* = 10.2, 10.1 Hz, 1H), 3.69 (dd, *J* = 9.3, 9.2 Hz, 1H), 3.64 (s, 3H), 3.55 (s, 3H), 3.53 (dd, *J* = 9.6, 9.3 Hz, 1H), 3.45 (s, 3H), 3.30 (dd, *J* = 9.2, 3.7 Hz, 1H).

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 7.65 – 7.61 (m, 2H), 7.21 – 7.18 (m, 2H), 7.15 – 7.11 (m, 1H), 5.37 (s, 1H), 4.63 (d, *J* = 3.7 Hz, 1H), 4.16 (dd, *J* = 10.2, 4.9 Hz, 1H), 3.90

(dd, *J* = 10.3, 9.6, 4.9 Hz, 1H), 3.88 (t, *J* = 9.2 Hz, 1H), 3.56 (s, 3H), 3.52 (dd, *J* = 10.3, 10.2 Hz, 1H), 3.50 (dd, *J* = 9.6, 9.2 Hz, 1H), 3.28 (s, 3H), 3.21 (dd, *J* = 9.2, 3.7 Hz, 1H), 3.06 (s, 3H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 7.48 – 7.45 (m, 2H), 7.38 – 7.32 (m, 3H), 5.57 (s, 1H), 4.91 (d, *J* = 3.6 Hz, 1H), 4.21 (dd, *J* = 9.3, 4.0 Hz, 1H), 3.75 (t, *J* = 10.3 Hz, 1H), 3.73 – 3.68 (m, 1H), 3.58 – 3.53 (m, 5H, H-4, OCH_3), 3.49 (s, 3H), 3.43 (s, 3H), 3.32 (dd, *J* = 9.5, 3.6, 1H).

1,5-Anhydro-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucitol (58).

A mixture of *S*-phenyl 2,3-di-*O*-benzyl-4,6-O-benzylidene-1-deoxy-1-thia-β-D-glucopyranoside **64**[382] (0.025 g, 0.046 mmol), tributylstannane (18.7 µL, 0.069 mmol), and azobisisobutyronitrile (catalytic) in anhydrous benzene (0.5 mL) was sparged with Ar and heated to reflux overnight. Then the reaction mixture was concentrated and partitioned between hexane and acetonitrile, and the acetonitrile layer was concentrated under vacuum. The crude residue was purified by silica gel chromatography, eluting with hexane/ ethylacetate (19:1 to 9:1), to afford **58** (14.5 mg, 72%) with spectral date consistent with the literature.[383] **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) 7.51 – 7.48 (m, 2H), 7.41 – 7.27 (m, 13H), 5.56 (s, 1H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.81 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.32 (dd, *J* = 10.4, 5.0 Hz, 1H), 4.01 (dd, *J* = 11.3, 5.7 Hz, 1H), 3.75 (dd, *J* = 9.1, 8.9 Hz, 1H), 3.68 (dd, *J* = 10.2, 10.3 Hz, 1H), 3.66 (ddd, *J* = 10.5, 8.9, 5.7 Hz, 1H), 3.62 (dd, *J* = 9.1, 9.3 Hz, 1H), 3.37 (ddd, *J* = 10.2, 9.3, 5.0 Hz, 1H), 3.33 (dd, *J* = 11.3, 10.5 Hz, 1H).

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 7.63 (dd, *J* = 8.1, 0.9 Hz, 2H), 7.42 (dd, *J* = 7.9, 0.9 Hz, 2H), 7.27 – 7.17 (m, 7H), 7.15 – 7.07 (m, 4H), 5.31 (s, 1H), 5.04 (d, *J* = 11.8

Hz, 1H), 4.85 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.43 (d, *J* = 11.9 Hz, 1H), 4.17 (dd, *J* = 10.2, 5.0 Hz, 1H), 3.87 (dd, *J* = 11.2, 5.7 Hz, 1H), 3.68 (dd, *J* = 9.1, 8.7 Hz, 1H), 3.56 (ddd, *J* = 10.6, 8.7, 5.7 Hz, 1H), 3.47 (dd, *J* = 9.1, 9.3 Hz, 1H), 3.44 (t, *J* = 10.2 Hz, 1H), 3.14 (ddd, *J* = 10.1, 9.3, 5.0 Hz, 1H), 3.09 (dd, *J* = 11.2, 10.6 Hz, 1H).

In CD_3OD: ^1H NMR (600 MHz, CD_3OD) δ 7.48 – 7.45 (m, 2H), 7.37 – 7.22 (m, 13H), 5.58 (s, 1H), 4.88 (d, *J* = 11.5 Hz, 1H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.23 (dd, *J* = 10.3, 5.0 Hz, 1H), 4.04 (dd, *J* = 11.2, 5.6 Hz, 1H), 3.70 (dd, *J* = 9.1, 8.7 Hz, 1H), 3.68 (dd, *J* = 10.2, 10.3 Hz, 1H), 3.63 (ddd, *J* = 10.5, 8.7, 5.6 Hz, 1H), 3.60 (dd, *J* = 9.1, 9.3 Hz, 1H), 3.35 (ddd, *J* = 10.2, 9.3, 5.0 Hz, 1H), 3.31 (dd, *J* = 11.2, 10.5 Hz, 1H).

1-Allyl-2,3-di-*O*-benzyl-4,*6*-*O*-benzylidene-1-deoxy-β-D-glucopyranose (59) and 1-Allyl-2,3-di-*O*-benzyl-4,*6*-*O*-benzylidene-1-deoxy-α-D-glucopyranose (60).

A mixture of 2,3,4,6-tetra-*O*-acetyl-1-*C*-allyl-D-glucopyranose **65**[384] (α:β = 1:0.2, 0.40 g, 1.1 mmol) and NaOMe (cat.) in anhydrous methanol (5.0 mL) was stirred at room temperature under argon for 5 h before quenching with Amberlyst-15 (pH~5). Then the reaction mixture was filtered and concentrated to dryness. The residue was dissolved in anhydrous acetonitrile (7.8 mL) followed by addition of benzaldehyde dimethyl acetal (0.24 mL, 1.6 mmol) and campher sulfonic acid (0.012 g, 0.05 mmol). The reaction mixture was stirred at room temperature overnight under argon, before it was quenched by adding triethylamine (0.35 mL) and concentrated to dryness. The crude residue (0.238 g, 0.81 mmol) was dissolved in anhydrous DMF (4.1 mL) and cooled to 0 ˚C before 60% NaH in mineral oil (0.100 g, 2.44 mmol) was added followed by benzyl bromide (288 µL, 2.44 mmol). The reaction mixture was stirred at room temperature overnight under argon,

then cooled to 0 ˚C before quenching with water. The reaction mixture was partitioned between ethyl acetate and H_2O, the organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The residue was purified using silica gel column chromatography, eluting with hexanes/ethyl acetate (20:1 to 10:1), to afford **59** (0.022 g, 6%) and **60** (0.210 g, 55%), both with spectral data consistent with the literature.

(**59**).[157] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 – 7.49 (m, 2H), 7.41 – 7.27 (m, 13H), 5.91 – 5.83 (m, 1H), 5.59 (s, 1H), 5.12 – 5.07 (m, 2H), 5.01 (d, *J* = 11.2 Hz, 1H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 10.8 Hz, 1H), 4.35 (dd, *J* = 10.4, 5.0 Hz, 1H), 3.85 (dd, *J* = 9.3, 8.4 Hz, 1H), 3.72 (t, *J* = 10.3 Hz, 1H), 3.67 (t, *J* = 9.3 Hz, 1H), 3.49 (ddd, *J* = 9.6, 7.2, 3.3 Hz, 1H), 3.42 (ddd, *J* = 10.3, 9.3, 5.0 Hz, 1H), 3.38 (dd, *J* = 9.6, 8.4 Hz, 1H), 2.61 (dddt, *J* = 14.8, 6.4, 3.3, 1.6 Hz, 1H), 2.30 (dtt, *J* = 14.8, 7.4, 1.1 Hz, 1H).

(60).[155, 157] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.54 – 7.52 (m, 2H), 7.43 – 7.29 (m, 13H), 5.80 (ddt, *J* = 17.0, 10.2, 6.9 Hz, 1H), 5.60 (s, 1H), 5.18 – 5.11 (m, 2H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.85 (d, *J* = 11.4 Hz, 1H), 4.80 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.31 – 4.24 (m, 1H), 4.11 (ddd, *J* = 8.3, 7.2, 5.8 Hz, 1H), 3.94 – 3.88 (m, 1H), 3.79 (dd, *J* = 8.6, 5.8 Hz, 1H), 3.73 – 3.65 (m, 3H), 2.57 (br t, *J* = 7.1 Hz, 2H).

1,5-Anhydro-4,6-*O*-benzylidene-2,3-di-*O*-benzyl-D-mannitol (61).

Compound **61** was synthesized using literature protocol.[154] ^{1}H NMR (600 MHz, $CDCl_3$) 7.60-7.20 (m, 15H); 5.56 (s, 1H); 4.85-4.65 (d x4, *J* = 12.5, 12.1 Hz, 4H); 4.29 (dd, 4.8, 10.3 Hz, 1H); 4.26 (dd, 9.9 Hz, 9.5 Hz, 1H); 4.07 (dd, 12.5, 2.2 Hz, 1H); 3.86 (t, 9.9,

10.3 Hz, 1H); 3.28 (m, 1.1, 2.2, 3.3 Hz, 1H); 3.68 (dd, 3.3, 9.9 Hz, 1H); 3.44 (dd, 12.5, 1.1 Hz, 1H); 3.34 (dt, 9.9, 4.8 Hz, 1H).

1-Allyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-β-D-mannopyranose (62).

Compound **62** was synthesized as described in literature.[155] ^{1}H NMR (600 MHz, $CDCl_3$) d 7.20-7.60 (m, 15H); 5.69 (m, 1H); 5.66 (s, 1H); 5.10, 4.71 (d x2, *J* = 12.5 Hz, 2H); 5.08-5.02 (m, 2H); 4.95, 4.78 (d x2, *J* = 12.5 Hz, 2H); 4.30 (dd, *J* = 4.8, 10.3 Hz, 1H); 4.27 (dd, *J* = 9.9, 9.5 Hz, 1H); 3.87 (t, *J* = 9.9,10.3 Hz, 1H); 3.82 (br.d, *J* = 1.1, 2.9 Hz, 1H); 3.75 (dd, *J* = 2.9, 9.9 Hz, 1H); 3.64 (t, *J* = 1.1, 7.0 Hz, 1H); 3.40 (dt, *J* = 9.5, 9.9, 4.8 Hz, 1H, H5); 2.48 (m, 1H); 2.28 (m, 1H).

1-Allyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-α-D-mannopyranose (63).

Compound **26** was synthesized as described in literature.[155] ^{1}H NMR (600 MHz, $CDCl_3$) d 7.10-7.60 (m, 15H); 5.65 (s, 1H); 5.60 (m, 1H); 5.02 (dd, *J* = 9.2, 1.1 Hz, 1H); 4.95 (dd, *J* = 16.9, 1.1 Hz, 1H); 4.81 (d, *J* = 12.5 Hz, 1H); 4.74 (s, 2H); 4.65 (d, *J* = 12.5 Hz, 1H); 4.27 (dd, *J* = 9.9, 9.5 Hz, 1H); 4.21 (dd, *J* = 4.8, 10.3 Hz, 1H); 4.04 (dt, *J* = 1.5, 7.7 Hz, 1H); 3.84 (dd, *J* = 3.3, 9.9 Hz, 1H); 3.83 (t, *J* = 9.9, 10.3 Hz, 1H); 3.69 (dd, *J* = 1.5, 3.3 Hz, 1H); 3.60 (dt, *J* = 9.5, 9.9, 4.8 Hz, 1H); 2.42 (m, 1H); 2.17 (m, 1H).

Methyl 6-*O*-(*p*-Methoxybenyl)-2,3-di-*O*-methyl-α-D-glucopyranoside (67).

To a stirred solution of compound **66**[160] (6.5 g, 18.9 mmol) and $NaCNBH_3$ (5.97 g, 94.9 mmol) in dry THF (100 mL) was added 2 M HCl in diethyl ether at 0 °C. After 0.5 h at 0 °C, the reaction mixture was poured into an ice-cold solution of saturated aqueous $NaHCO_3$. The aqueous phase was extracted with EtOAc. The combined extracts were washed with saturated aqueous 1 N HCl , dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by silica gel

column chromatography, eluting with hexanes/ethyl acetate 1:1, to yield **67** (4.6g, 71%) as a colorless oil. $[\alpha]^{22}$ = +82.3 (*c* 1.05, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.6 Hz, 2H), 6.76 (d, *J* = 8.6 Hz, 2H), 4.76 (d, *J* = 3.5 Hz, 1H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.40 (d, *J* = 11.8 Hz, 1H), 3.67 (s, 3H), 3.64 – 3.55 (m, 3H), 3.53 (s, 3H), 3.46 – 3.41 (m, 1H), 3.38 (s, 3H), 3.37 (t, *J* = 9.4 Hz, 1H), 3.33 (s, 3H), 3.26 (br s, 1H), 3.14 (dd, *J* = 9.3, 3.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 130.0, 129.2, 113.7, 113.7, 97.3, 83.0, 81.6, 73.1, 70.5, 70.0, 69.1, 61.0, 58.4, 55.1, 55.1. HRMS (ESI) m/z calcd for $C_{17}H_{26}O_7Na$ $[M+Na]^+$, 365.1576; found, 365.1580.

Methyl 6-*O*-(*p*-Methoxybenzyl)-2,3-di-*O*-methyl-α-D-*xylo*-hexopyranosid-4-ulose (68).

Dess-Martin periodinane (4.48 g, 10.7 mmol) was added to a mixture of alcohol **67** (2.80 g, 8.2 mmol) in anhydrous CH_2Cl_2 (94 mL) at room temperature. The reaction mixture was stirred overnight before addition of CH_2Cl_2 (80 mL) and a mixture of saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$ and water (1:1:1, 120 mL). The reaction mixture was then stirred for another 0.5 h before the organic layer was separated. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified over silica gel, eluting with hexanes/ethyl acetate (2:1 to 1:2), to obtain the ketone **68** (2.4 g, 85%). $[\alpha]^{22}{}_D$ = +71.2 (*c* 2.00, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.26 – 7.22 (m, 2H), 6.87 – 6.83 (m, 2H), 5.02 (d, *J* = 3.5 Hz, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.24 (dd, *J* = 6.2, 3.5 Hz, 1H), 4.09 (d, *J* = 10.2 Hz, 1H), 3.85 (dd, *J* = 10.9, 3.5 Hz, 1H), 3.78 (s, 3H), 3.64 (dd, *J* = 11.0, 6.2 Hz, 1H), 3.57 (s, 3H), 3.53 (s, 3H), 3.52 – 3.51 (m, 1H), 3.51 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ

201.8, 159.2, 129.9, 129.3, 113.7, 97.6, 84.5, 82.4, 73.3, 72.6, 67.2, 60.2, 59.7, 56.1, 55.2; HRMS (ESI) m/z calcd for $C_{17}H_{24}O_7Na$ $[M+Na]^+$, 363.1420; found, 363.1415.

Methyl 4-*C*-Allyl-6-*O*-(*p*-methoxybenzyl)-2,3-di-*O*-methyl-α-D-galactopyranoside (69) and Methyl 4-*C*-Allyl-6-*O*-(*p*-methoxybenzyl)-2,3-di-*O*-methyl-α-D-glucopyranoside (70).

A freshly prepared solution of allyl magnesium chloride in THF (1 M, 30 mL, 30.0 mmol) was added to a solution of ketone **68** (2.50 g, 7.3 mmol) in anhydrous THF (36 mL) at 0 °C. Then the reaction mixture was stirred at 0 °C until the completion as indicated by TLC (50% ethyl acetate in hexane). The reaction was quenched by addition of saturated aqueous NH_4Cl and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The resultant residue (30:31 = 8:3) was purified by silica gel chromatography, eluting with hexanes/ethyl acetate (7:3 to 1:1), to give **69** (1.52 g, 54 %) and **70** (0.53 g, 19 %) as colorless oils.

(69). $[\alpha]^{22}{}_D$ = +89.6 (*c* 1.50, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.24 – 7.22 (m, 2H), 6.86 – 6.83 (m, 2H), 5.70 (ddt, *J* = 17.5, 10.2, 7.5 Hz, 1H), 5.04 (dd, *J* = 10.2, 1.5 Hz, 1H), 4.99 (dd, *J* = 17.0, 1.6 Hz, 1H), 4.87 (d, *J* = 3.7 Hz, 1H), 4.54 – 4.49 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.7 Hz, 1H), 3.77 (s, 3H), 3.76 – 3.69 (m, 3H), 3.66 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.58 (s, 3H), 3.47 (s, 3H), 3.39 (s, 3H), 3.38 (d, *J* = 9.5 Hz, 1H), 3.16 (br s, 1H), 2.61 (dd, *J* = 14.2, 7.4 Hz, 1H), 2.14 (dd, *J* = 14.2, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3, 132.6, 129.6, 129.5, 118.8, 113.8, 97.6, 80.1, 79.0, 76.1, 73.3, 69.6, 68.8, 61.3, 58.8, 55.3, 55.2, 39.9; HRMS (ESI) m/z calcd for $C_{20}H_{30}O_7Na$ $[M+Na]^+$, 405.1889; found, 405.1888.

(70). $[\alpha]^{22}_{D}$ = +67.9 (*c* 2.20, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.22 (m, 2H), 6.86 – 6.84 (m, 2H), 6.02 – 5.86 (m, 1H), 5.13 – 5.06 (m, 2H), 4.82 (d, *J* = 3.7 Hz, 1H), 4.46 (d, *J* = 11.2 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 3.91 (dd, *J* = 7.1, 5.2 Hz, 1H), 3.78 (s, 3H), 3.72 (dd, *J* = 9.7, 5.3 Hz, 1H), 3.60 – 3.54 (m, 1H′), 3.58 (s, 3H), 3.47 (s, 3H), 3.46 (d, *J* = 10.1 Hz, 1H), 3.42 (s, 3H), 3.25 (dd, *J* = 10.0, 3.9 Hz, 1H), 2.90 (br s, 1H), 2.58 (dd, *J* = 14.4, 6.2 Hz, 1H), 2.31 (dd, *J* = 14.5, 8.5 Hz, 1H′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 134.5, 129.8, 129.2, 118.0, 113.7, 97.2, 85.7, 80.0, 74.9, 73.0, 71.8, 68.7, 61.8, 58.8, 55.2, 55.0, 34.1; HRMS (ESI) m/z calcd for $C_{20}H_{30}O_7Na$ $[M+Na]^+$, 405.1889; found, 405.1882.

Methyl 4-*C*-Allyl-6-*O*-(*p*-methoxybenzyl)-2,3,4-tri-*O*-methyl-α-D-galactopyranoside (71).

To a stirred solution of alcohol **69** (1.5 g, 3.92 mmol) in dry DMF (10 mL) was added NaH (60%, 313 mg, 7.84 mmol) followed by MeI (0.49 mL, 7.84 mmol) dropwise at 0 °C (ice-water bath). The reaction mixture was stirred at 0 °C for 0.5 h before TLC (60 % ethyl acetate in hexane) showed completion. The reaction mixture was quenched with water, extracted with EtOAc, and washed with brine solution. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. Column chromatography on silica gel (eluent: 40% ethyl acetate in hexane) afforded **71** (1.3 g, 84%) as a colorless oil. $[\alpha]^{22}$ = +86.9 (*c* 1.40, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.22 (m, 2H), 6.86 – 6.84 (m, 2H), 5.80 – 5.67 (m, 1H), 5.14 – 5.04 (m, 2H), 4.89 (d, *J* = 3.6 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.40 (d, *J* = 11.7 Hz, 1H), 3.82 (dd, *J* = 7.1, 2.8 Hz, 1H), 3.80 – 3.75 (m, 1H), 3.77 (s, 3H), 3.68 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.56 (s, 3H), 3.54 – 3.47 (m, 2H), 3.50 (s, 3H), 3.42 (s, 6H), 2.97 (dd, *J* = 13.6, 7.8 Hz, 1H), 2.15 (dd,

J = 13.6, 7.1 Hz, 1H); ^{13}C NMR (101 MHz, $CDCL_3$) δ 170.5, 159.1, 132.2, 130.3, 129.1, 119.3, 113.7, 97.4, 80.9, 80.2, 79.3, 73.0, 72.1, 68.5, 61.8, 58.6, 55.2, 52.8, 33.7; HRMS (ESI) m/z calcd for $C_{21}H_{32}O_7Na$ $[M+Na]^+$, 419.2046; found, 419.2039.

Methyl 4-*C*-Allyl-6-*O*-(*p*-methoxybenzyl)-2,3,4-tri-*O*-methyl-α-D-glucopyranoside (72).

NaH (60%, 0.08 g, 1.9 mmol) followed by MeI (0.12 ml, 1.9 mmol) were added to a stirred solution of **70** (0.37 g, 1.0 mmol) in anhydrous DMF (3.4 ml) at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h. The reaction was quenched with H_2O after cooling to 0 °C, and extracted with ethyl acetate, and the organic layers were washed with brine and dried over anhydrous Na_2SO_4 before evaporation to dryness. The resultant residue was purified by silica gel chromatography, eluting with hexane/ethyl acetate (9:1 to 7:3) to afford **72** (0.30 g, 80%) as a colorless oil. $[\alpha]^{22}{}_D$ = +61.4 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.21 (m, 2H), 6.89 – 6.83 (m, 2H), 5.94 (dddd, *J* = 17.1, 10.1, 8.8, 5.2 Hz, 1H), 5.07 (dd, *J* = 17.2, 1.0 Hz, 1H), 5.02 – 4.95 (m, 1H), 4.83 (d, *J* = 4.0 Hz, 1H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.6 Hz, 1H), 4.04 (d, *J* = 8.0 Hz, 1H), 3.79 (s, *J* = 3.8 Hz, 3H), 3.76 – 3.65 (m, 2H), 3.63 – 3.56 (m, 4H), 3.51 – 3.48 (m, 3H), 3.46 (s, 3H), 3.38 – 3.32 (m, 4H 1H), 2.38 (dd, *J* = 15.2, 8.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.17, 135.00, 130.68, 129.08, 116.79, 113.82, 96.98, 81.44, 80.89, 78.57, 73.06, 71.31, 68.93, 60.70, 58.92, 55.38, 55.07, 50.81, 32.86; HRMS (ESI) m/z calcd for $C_{21}H_{32}O_7Na$ $[M+Na]^+$, 419.2046; found, 419.2045.

Methyl 4-*C*-Allyl-2,3,4-tri-*O*-methyl-α-D-galactopyranoside (73).

To a stirred solution of **71** (1.2 g, 3.03 mmol) in CH_3CN (120 mL) was added a solution of CAN (8.3g, 15.13 mmol) in water (35 mL) at 0 ^{o}C. The reaction mixture was

stirred at room temperature for 15 min, then diluted with CH_2Cl_2 and washed with water. The combined organic phases were dried, concentrated, and purified by silica gel column chromatography (eluent: 60% EtOAc in hexane) to give **73** (0.9 g, 80%) as a colorless oil. $[\alpha]^{22}_D$ = +84.0 (*c* 1.85, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.73 (ddt, *J* = 17.3, 10.1, 7.4 Hz, 1H, -C*H*=CH_2), 5.18 (dd, *J* = 17.0, 1.4 Hz, 1H, -CH=C*H*$_2$), 5.15 (d, *J* = 102, 1.2 Hz, 1H, -CH=C*H*$_2$), 4.91 (d, *J* = 3.7 Hz, 1H), 3.90 (dd, *J* = 11.9, 6.3 Hz, 1H), 3.74 – 3.71 (m, 1H′), 3.69 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.63 (dd, *J* = 6.3, 3.0 Hz, 1H), 3.55 (s, 3H), 3.50 (s, 3H), 3.49 (d, *J* = 9.8 Hz, 1H), 3.46 (s, 3H), 3.39 (s, 3H), 3.03 (dd, *J* = 13.9, 7.3 Hz, 1H, -C*H*$_2$-CH=CH_2), 2.65 (d, *J* = 7.3 Hz, 1H), 2.19 (dd, *J* = 13.9, 7.6 Hz, 1H, -C*H*$_2$-CH=CH_2); ^{13}C NMR (150 MHz, $CDCl_3$) δ 131.9, 119.6, 97.8, 81.0, 80.2, 80.1, 71.7, 61.9, 61.0, 58.8, 55.3, 52.8, 33.6; HRMS (ESI) m/z calcd for $C_{13}H_{24}O_6Na$ $[M+Na]^+$, 299.1471; found, 299.1476.

Methyl 4-*C*-Allyl-2,3,4-tri-*O*-methyl-α-D-glucopyranoside (74).

A solution of CAN (2.05 g, 3.74 mmol) in H_2O (8.9 mL) was added to a mixture of **72** (0.30 g, 0.75 mmol) in CH_3CN (31.3 mL) at 0 °C, and the resulting mixture was stirred for 15 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with water, before it was dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was purified by column chromatography over silica gel, eluting with hexane/ethyl acetate (1:1 to 1:4), to obtain alcohol **74** (0.16 g, 78%) as a colorless oil. $[\alpha]^{22}_D$ = +112.3 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.96 (dddd, *J* = 17.2, 10.1, 8.5, 5.3 Hz, 1H), 5.11 (dd, *J* = 17.2, 1.1 Hz, 1H), 5.03 (dd, *J* = 10.1, 0.7 Hz, 1H), 4.80 (d, *J* = 4.0 Hz, 1H), 3.89 (dd, *J* = 7.8, 4.1 Hz, 1H), 3.82 – 3.72 (m, 2H), 3.69 (d, *J* = 9.8 Hz, 1H), 3.59 (s, 3H), 3.49 (s, 3H), 3.43 (s, 3H), 3.40 (s, 3H), 3.33 (dd, *J* = 9.7, 4.0 Hz, 1H), 2.73

– 2.67 (m, 1H), 2.42 (dd, *J* = 15.3, 8.5 Hz, 1H), 2.06 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 134.77, 117.04, 97.20, 81.53, 81.45, 78.85, 72.00, 61.52, 60.60, 58.96, 55.26, 51.30, 32.15; HRMS (ESI) m/z calcd for $C_{13}H_{24}O_6Na$ $[M+Na]^+$, 299.1471; found, 299.1475.

Methyl 4-*C*-Allyl-7,8-dideoxy-2,3,4-tri-*O*-methyl-α-D-*glycero*-D-*galacto*-7-en-octopyranoside and Methyl 4-*C*-Allyl-7,8-dideoxy-2,3,4-tri-*O*-methyl-β-L-*glycero*-D-*galacto*-7-en-octopyranoside (77 and 78).

To a stirred solution of alcohol **73** (300 mg, 1.08 mmol) in CH_2Cl_2 (11.0 mL) was added Dess–Martin periodinane (690 mg, 1.63 mmol) at room temperature. After 2 h, the excess of oxidant was quenched by addition of saturated aqueous $Na_2S_2O_3$ (15 mL) and saturated aqueous $NaHCO_3$ (15 mL). After 15 min vigorous stirring, the mixture was diluted with CH_2Cl_2, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. A solution of crude aldehyde (300 mg, 1.09 mmol) in dry THF (7 mL) was treated with vinylmagnesium bromide (2.7 mL, 1 M in THF) at −78 °C and stirred for 5 h before the temperature was raised to 0 °C and the reaction was quenched with saturated aqueous NH_4Cl (10 mL). The reaction mixture was extracted with EtOAc, and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified on silica gel column chromatography (eluent: 60% EtOAc in hexane), to give **77** and **78** (265 mg, 81%) as a mixture of diastereomers (1.5:1) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.08 – 5.96 (m, 1H, minor isomer), 5.81 – 5.71 (m, 2.5H, both isomers), 5.64 – 5.57 (m, 1.5H , major isomer), 5.20 – 4.99 (m, 10H, both isomers), 4.79 (d, *J* = 3.6 Hz, 1.5H, major isomer), 4.73 (d, *J* = 3.5 Hz, 1H, minor isomer), 4.45 (d, *J* = 5.3 Hz, 1.5 H, major isomer), 4.29 (br s, 2.5H, both

isomers), 3.60 – 3.51 (m, 3.5H, 2H minor isomer, 1H major isomer), 3.44 (s, 4.5H, major isomer), 3.43 (s, 3H, minor isomer), 3.41 (s, 4.5H, major isomer), 3.37 – 3.35 (m, 2.5H, both isomers), 3.37 (s, 4.5H, major isomer), 3.36 (s, 3H, minor isomer), 3.34 (s, 3H, minor isomer), 3.25 (s, 3H, minor isomer), 3.22 (s, 4.5H, major isomer), 3.05 (dd, *J* = 13.8, 6.9 Hz, 1.5H, major isomer), 2.89 (dd, *J* = 13.3, 8.2 Hz, 1H, minor isomer), 2.63 (br s, 1H, minor isomer), 2.37 (dd, *J* = 13.4, 6.2 Hz, 1H, minor isomer), 2.26 (dd, *J* = 13.8, 7.9 Hz, 1.5H, major isomer); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.8, 138.1, 133.0, 131.6, 119.7, 119.1, 115.3, 114.3, 97.6, 97.5, 81.9, 81.0, 80.7, 80.1, 79.9, 79.8, 73.7, 72.0, 71.4, 71.2, 61.8, 61.5, 58.6, 58.5, 55.2, 52.6, 52.4, 33.7, 33.3; HRMS (ESI) m/z calcd for $C_{15}H_{26}O_6Na$ $[M+Na]^+$, 325.1627; found, 325.1635.

Methyl 2,3,4-Tri-*O*-methyl-4,6-(prop-2-en-1,3-diyl)-β-L-*glycero*-α-D-*galacto*-nonopyranoside (79) and Methyl 2,3,4-Tri-*O*-methyl-4,6-(prop-2-en-1,3-diyl)-α-D-*glycero*-α-D-*galacto*-nonopyranoside (80).

A solution of **77** and **78** (210 mg, 0.69 mmol) in anhydrous CH_2Cl_2 (42 mL) was treated with Grubbs 2nd generation catalyst (11 mg, 5% weight) and heated to reflux for 1 h. The reaction mixture was concentrated to give the crude product, which was purified by silica gel chromatography (eluent: CH_2Cl_2/EtOAc, 1:1) to give **79** (113 mg, 60%) and **80** (70 mg, 37%) as colorless oils.

(79). $[\alpha]^{22}_D$ = + 160.9 (*c* 0.55, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.67 – 5.63 (m, 1H), 5.61 – 5.58 (m, 1H), 4.92 (d, *J* = 3.6 Hz, 1H), 4.39 (d, *J* = 6.2 Hz, 1H), 3.69 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.57 (d, *J* = 8.0 Hz, 1H), 3.51 (s, 3H), 3.48 (d, *J* = 5.4 Hz, 3H), 3.39 (s, 3H), 3.37 (s, 3H), 3.34 (d, *J* = 10.0 Hz, 1H), 2.94 – 2.90 (m, 1H, H-9), 2.31 (d, *J* = 3.1 Hz, 1H), 2.01 – 1.96 (m, 1H, H-9′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 129.2, 123.7, 97.9, 85.2,

79.7, 77.1, 75.7, 68.6, 62.1, 58.8, 55.2, 53.7, 28.9; HRMS (ESI) m/z calcd for $C_{13}H_{22}O_6Na$ $[M+Na]^+$, 297.1314; found, 297.1309.

(80). $[\alpha]^{22}_D$ = +35.6 (*c* 1.30, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 5.94 (ddd, *J* = 9.7, 4.8, 3.1 Hz, 1H), 5.71 – 5.61 (m, 1H), 5.03 (d, *J* = 3.5 Hz, 1H), 4.08 – 4.02 (m, 1H), 3.75 – 3.70 (m, 2H, O-*H*), 3.59 (d, *J* = 10.5 Hz, 1H), 3.51 (s, 3H), 3.49 (s, 3H), 3.41 (s, 3H), 3.39 (s, 3H), 3.36 (d, *J* = 10.2 Hz, 1H), 3.06 – 3.03 (m, 1H, H-9), 1.93 – 1.89 (m, 1H, H-9′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 130.0, 123.2, 98.3, 85.1, 79.3, 78.1, 70.2, 65.6, 62.2, 58.9, 55.5, 53.3, 29.1; HRMS (ESI) m/z calcd for $C_{13}H_{22}O_6Na$ $[M+Na]^+$, 297.1314; found, 297.1316.

Methyl 2,3,4-Tri-*O*-methyl-4,6-(propan-1,3-diyl)-β-L-*glycero*-D-*galacto*-nonopyranoside (81).

A stirred solution of **79** (30 mg, 0.11 mmol) in CH_3OH (2.0 mL) was treated with 20% $Pd(OH)_2/C$ (3.0 mg) at room temperature and stirred under 1 atm of H_2 (balloon) for 0.5 h. The reaction mixture was filtered through Celite and the combined filtrates were evaporated under vacuum. The crude product was purified using silica gel column chromatography (eluent: 40% EtOAC in hexane) to give **81** (30 mg, quantitative) as a colorless oil. $[\alpha]^{22}_D$ = +120.0 (*c* 1.50, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 4.94 (d, *J* = 3.7 Hz, 1H), 3.87 (td, *J* = 10.7, 4.9 Hz, 1H), 3.67 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.50 (s, 6H), 3.43 (s, 3H), 3.41 (s, 3H), 3.27 (d, *J* = 10.1 Hz, 1H), 3.25 (d, *J* = 9.3 Hz, 1H), 2.47 (dd, *J* = 14.6, 1.7 Hz, 1H, H-9), 2.27 (br s, 1H), 2.01 (d, *J* = 9.5 Hz, 1H), 1.58 (dd, *J* = 8.9, 4.8 Hz, 1H), 1.49 – 1.34 (m, 1H′), 1.32 – 1.18 (m, 1H′), 1.08 (td, *J* = 14.3, 4.1 Hz, 1H, H-9′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 98.1, 85.5, 79.8, 77.8, 77.5, 68.2, 62.1, 59.0, 55.2, 52.0,

31.5, 26.7, 18.6; HRMS (ESI) m/z calcd for $C_{13}H_{24}O_6Na$ $[M+Na]^+$, 299.1471; found, 299.1465.

Methyl 2,3,4-Tri-*O*-methyl-4,6-(propan-1,3-diyl)-α-D-*glycero*-D-*galacto*-nonopyranoside (82).

Compound **82** (25 mg, 81%) was prepared by hydrogenation of **80** (30 mg) analogously to **81**. $[\alpha]^{22}$ = +102.5 (*c* 0.95, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.98 (d, *J* = 3.7 Hz, 1H), 4.40 (d, *J* = 9.1 Hz, 1H), 3.97 (d, *J* = 4.9 Hz, 1H), 3.70 (dd, *J* = 10.2, 3.7 Hz, 1H), 3.50 (s, 3H), 3.49 (s, 3H), 3.47 (s, 3H), 3.42 (d, *J* = 3.3 Hz, 1H), 3.41 (s, 3H), 3.22 (d, *J* = 10.1 Hz, 1H), 2.57 (dd, *J* = 14.8, 2.5 Hz, 1H, H-9), 1.97 (d, *J* = 14.4 Hz, 1H), 1.70 – 1.58 (m, 1H), 1.48 – 1.42 (m, 2H′, H-8′), 1.08 (td, *J* = 14.0, 3.3 Hz, 1H, H-9′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 98.4, 85.1, 79.9, 79.6, 71.2, 68.9, 62.2, 59.0, 55.4, 52.1, 32.5, 26.7, 14.4; HRMS (ESI) m/z calcd for $C_{13}H_{24}O_6Na$ $[M+Na]^+$, 299.1471; found, 299.1472.

Methyl 2,3,4,6-Tetra-*O*-methyl-4,6-(propan-1,3-diyl)-β-L-*glycero*-D-*galacto*-nonopyranoside (49).

To a stirred solution of alcohol **42** (30 mg, 0.11 mmol) in dry DMF (1.0 mL) was added NaH (60%, 9 mg, 0.22 mmol) followed by MeI (14 µL, 0.22 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h before TLC (40 % ethyl acetate in hexane) showed completion. The reaction mixture was quenched with water, extracted with EtOAc, and washed with brine. The combined extracts were dried over Na_2SO_4 and concentrated under vacuum. Column chromatography on silica gel (eluent: 30% ethyl acetate in hexane) afforded **49** (28 mg, 90%) as a colorless oil. $[\alpha]^{22}{}_D$ = +111.2 (*c* 1.20, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.95 (d, *J* = 3.8 Hz, 1H), 3.68 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.50 (s, 3H), 3.49 (s, 3H), 3.46 (s, 3H), 3.42 (s, 6H), 3.40 (ddd, *J* =

10.8, 9.2, 4.9 Hz, 1H), 3.36 (d, *J* = 9.2 Hz, 1H), 3.25 (d, *J* = 10.0 Hz, 1H), 2.46 (dddd, *J* = 14.6, 3.3, 2.6, 1.9 Hz, 1H, H-9eq), 2.07 (ddddd, *J* = 12.9, 4.9, 3.6, 2.9, 1.9 Hz, 1H), 1.55 (ddddd, *J* = 13.7, 4.4, 4.0, 2.9, 2.6 Hz, 1H), 1.38 ((tddd, *J* = 13.7, 13.6, 3.6, 3.3 Hz, 1H), 1.15 (dddd, *J* = 13.6, 12.9, 10.8, 4.4 Hz, 1H), 1.02 (ddd, *J* = 14.6, 13.7, 4.0 Hz, 1H, H-9ax); ^{13}C NMR (150 MHz, $CDCl_3$) δ 97.7, 85.6, 79.8, 78.4, 77.5, 77.2, 62.1, 58.9, 58.7, 54.9, 51.8, 30.6, 26.5, 18.5; HRMS (ESI) m/z calcd for $C_{14}H_{26}O_6Na$ $[M+Na]^+$, 313.1627; found, 313.1614.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.87 (d, *J* = 3.7 Hz, 1H), 3.78 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.60 (ddd, *J* = 10.9, 9.2, 4.8 Hz, 1H), 3.44 (d, *J* = 9.2 Hz, 1H), 3.43 (s, 3H), 3.38 (s, 3H), 3.36 (s, 3H), 3.32 (d, *J* = 10.0 Hz, 1H), 3.31 (s, 3H), 3.18 (s, 3H), 2.19 (dddd, *J* = 14.6, 3.3, 2.6, 2.1, 1H), 1.95 (ddddd, *J* = 12.6, 4.8, 3.7, 2.9, 2.1 Hz, 1H), 1.23 (qdd, *J* = 13.5, 3.7, 3.4 Hz, 1H), 1.20 (ddddd, *J* = 13.5, 4.5, 4.4, 2.9, 2.8 Hz, 1H), 1.12 (dddd, *J* = 13.5, 12.6, 10.9, 4.5 Hz, 1H), 0.55 (ddd, *J* = 14.6, 13.5, 4.4 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.89 (d, *J* = 3.8 Hz, 1H), 3.58 (dd, *J* = 10.1, 3.8 Hz, 1H), 3.48 (s, 6H), 3.41 (s, 3H), 3.40 (s, 3H), 3.39 (ddd, *J* = 10.8, 9.2, 4.8 Hz, 1H), 3.38 (s, 3H), 3.35 (d, *J* = 9.2 Hz, 1H), 3.24 (d, *J* = 10.1 Hz, 1H), 2.46 (dddd, *J* = 14.7, 3.3, 2.6, 2.1 Hz, 1H), 2.07 (ddddd, *J* = 12.8, 4.8, 3.8, 2.9, 2.1 Hz, 1H), 1.57 (ddddd, *J* = 13.7, 4.4, 4.1, 2.9, 2.6 Hz, 1H), 1.38 (qdd, *J* = 13.7, 3.8, 3.3 Hz, 1H), 1.15 (dddd, *J* = 13.7, 12.8, 10.8, 4.4 Hz, 1H), 1.11 (ddd, *J* = 14.7, 13.7, 4.1 Hz, 1H).

Methyl 2,3,4,6-Tetra-*O*-methyl-4,6-(propan-1,3-diyl)-α-D-*glycero*-D-*galacto*-nonopyranoside (46).

Compound **9** (14 mg, 70%) was synthesized from **82** (20 mg) analogously to **49**. $[\alpha]^{22}$ = +10.4 (*c* 0.30, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.95 (d, *J* = 3.8

Hz, 1H), 3.85 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.51 (td, *J* = 3.1, 3.0, 1H), 3.50 (s, 3H), 3.48 (s, 3H), 3.44 (s, 3H), 3.41 (s, 3H), 3.40 (d, *J*= 3.1 Hz, 1H), 3.33 (s, 3H), 3.19 (d, *J* = 10.0 Hz, 1H), 2.57 (ddd, *J* = 14.7, 3.4, 3.3 Hz, 1H, H-9eq), 2.09 (ddt, *J* = 14.3, 3.1, 2.8 Hz, 1H), 1.64 (ddddd, *J* = 14.1, 13.7, 13.5, 3.4, 2.8 Hz, 1H), 1.35 (dqd, *J* = 13.5, 3.3, 2.8 Hz, 1H), 1.26 (dddd, *J* = 14.3, 14.1, 3.3, 3.1 Hz, 1H-7ax), 1.02 (ddd, *J* = 14.7, 13.7, 3.3 Hz, 1H, H-9ax); ^{13}C NMR (150 MHz, $CDCl_3$) δ 98.5, 86.7, 79.7, 77.3, 72.5, 62.0, 58.9, 57.9, 55.4, 51.8, 27.6, 27.3, 15.1; HRMS (ESI) m/z calcd for $C_{14}H_{26}O_6Na$ $[M+Na]^+$, 313.1627; found, 313.1617.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.85 (d, *J* = 3.7 Hz, 1H), 3.88 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.53 (s, 3H), 3.49 (s, 3H), 3.48 (dt, *J* = 3.2, 3.1, 1H), 3.39 (s, 3H), 3.35 (d, *J* = 3.2 Hz, 1H), 3.25 (d, *J* = 10.0 Hz, 1H), 3.19 (s, 3H), 3.16 (s, 3H), 2.41 (ddd, *J* = 14.6, 3.4, 3.2 Hz, 1H, H-9), 1.89 (ddt, *J* = 14.3, 3.1, 2.9 Hz, 1H), 1.85 (dddt, *J* = 13.5, 13.4, 3.4, 2.9 Hz, 1H), 1.10 (dqd, *J* = 13.4, 3.2, 2.9 Hz, 1H), 1.03 (dddd, *J* = 14.3, 13.5, 3.2, 3.1 Hz, 1H), 0.63 (ddd, *J* = 14.6, 13.5, 3.2 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.91 (d, *J* = 3.8 Hz, 1H), 3.64 (dd, *J* = 10.1, 3.8 Hz, 1H. H-2), 3.51 (dt, *J* = 3.3, 3.2 Hz, 1H), 3.48 (d, *J* = 3.2 Hz, 1H), 3.48 (s, 3H), 3.47 (s, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 3.30 (s, 3H), 3.20 (d, *J* = 10.1 Hz, 1H), 2.55 (ddd, *J* = 14.7, 3.1, 2.8 Hz, 1H), 2.06 (dq, *J* = 14.4, 3.3 Hz, 1H), 1.58 (dtdd, *J* = 14.0, 13.3, 3.3, 3.1 Hz, 1H), 1.35 (dddt, *J* = 13.3, 3.3, 3.0, 2.8 Hz, 1H), 1.36 (dddd, *J* = 14.4, 14.0, 3.3, 3.0 Hz, 1H), 1.13 (ddd, 14.7, 13.3, 2.8 Hz, 1H).

Methyl 4-*C*-Allyl-7,8-dideoxy-2,3,4-tri-*O*-methyl-α-D-*glycero*-D-*gluco*-7-en-octopyranoside (83) and Methyl 4-*C*-Allyl-7,8-dideoxy-2,3,4-tri-*O*-methyl-β-L-*glycero*-D-*gluco*-7-en-octopyranoside (84).

A mixture of alcohol **74** (0.15 g, 0.56 mmol) and Dess-Martin Periodinane (0.36 g, 0.84 mmol) in anhydrous CH_2Cl_2 (5.7 mL) was stirred for 2.5 h before addition of CH_2Cl_2 (5.0 mL) and a mixture of saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and H_2O (1:1:1, 7.5 mL). The reaction mixture was stirred for another 0.5 h before the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with brine before it was dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was dissolved in anhydrous THF (3.9 mL), cooled to 0 ˚C, and treated with vinyl magnesium bromide in THF (1 M, 1.4 mL, 1.4 mmol). The reaction mixture was stirred for 25 min before it was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue (**83:84** = 0.4:1.0) was then purified by column chromatography over silica gel, eluting with hexane/ethyl acetate (7:3 to 1:1), to give **83** (0.02 g, 12%) and **84** (0.08 g, 48%) as colorless oils.

(83). $[\alpha]^{22}_{D}$ = +103.6 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.16 – 6.08 (m, 1H), 5.98 – 5.90 (m, 1H), 5.39 (d, *J* = 17.2 Hz, 1H), 5.26 – 5.17 (m, 2H), 5.12 (d, *J* = 10.2 Hz, 1H), 4.74 (d, *J* = 4.0 Hz, 1H), 4.43 (dd, *J* = 9.3, 5.6 Hz, 1H), 3.63 (d, *J* = 9.8 Hz, 1H), 3.59 (s, 3H), 3.51 – 3.44 (m, 7H), 3.37 (s, 3H), 3.31 (dd, *J* = 9.8, 4.0 Hz, 1H), 2.87 (dd, *J* = 15.3, 5.9 Hz, 1H), 2.64 (dd, *J* = 15.3, 8.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.47, 133.91, 118.07, 116.04, 97.01, 82.84, 81.45, 81.02, 71.55, 71.47, 60.51, 58.81, 55.53,

52.42, 31.59; HRMS (ESI) m/z calcd for $C_{15}H_{26}O_6Na$ $[M+Na]^+$, 325.1627; found, 325.1630.

(84). $[\alpha]^{22}_D$ = +80.5 (*c* 0.6, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 6.20 – 6.10 (m, 1H), 5.99 (ddd, *J* = 17.1, 10.5, 5.3 Hz, 1H), 5.36 (dt, *J* = 17.2, 1.5 Hz, 1H), 5.20 – 5.13 (m, 2H), 5.06 (dd, *J* = 10.2, 0.9 Hz, 1H), 4.83 (d, *J* = 4.0 Hz, 1H), 4.52 – 4.46 (m, 1H), 3.66 (d, *J* = 9.9 Hz, 1H), 3.61 (d, *J* = 3.4 Hz, 1H), 3.59 (s, 3H), 3.48 (s, *J* = 10.7 Hz, 3H), 3.40 (s, *J* = 4.7 Hz, 3H), 3.38 (dd, *J* = 9.9, 4.0 Hz, 1H), 3.35 (s, 3H), 2.79 – 2.72 (m, 1H), 2.59 (dd, *J* = 15.2, 8.1 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 134.77, 117.04, 97.20, 81.53, 81.45, 78.85, 72.00, 61.52, 60.60, 58.96, 55.26, 51.30, 32.15; HRMS (ESI) m/z calcd for $C_{15}H_{26}O_6Na$ $[M+Na]^+$, 325.1627; found, 325.1630.

Methyl 2,3,4-Tri-*O*-methyl-4,6-(prop-2-en-1,3-diyl)-α-D-*glycero*-α-D-*gluco*-nonopyranoside (85).

A mixture of **83** (9 mg, 0.03 mmol) and Grubbs 2nd generation catalyst (1 mg, 10% weight) in anhydrous CH_2Cl_2 (1.2 mL) was heated to reflux for 1 h before concentration. The crude residue was purified by silica gel chromatography, eluting with hexane/ethyl acetate (7:3 to 1:1), to give **85** (0.006 g, 76%) as a colorless oil. $[\alpha]^{22}_D$ = +80.0 (*c* 0.4, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 5.73 – 5.64 (m, 2H), 4.86 (d, *J* = 4.1 Hz, 1H), 4.41 – 4.35 (m, 1H), 4.00 (d, *J* = 4.7 Hz, 1H), 3.70 (d, *J* = 9.1 Hz, 1H), 3.60 (s, 3H), 3.50 (s, 3H), 3.44 (s, 3H), 3.37 (s, 3H), 3.36 (dd, *J* = 9.2, 4.2 Hz, 1H), 2.35 – 2.25 (m, 2H), 2.20 – 2.14 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 128.28, 125.09, 97.39, 82.31, 77.45, 66.12, 65.05, 60.83, 58.98, 55.33, 51.08, 26.12; HRMS (ESI) m/z calcd for $C_{13}H_{22}O_6Na$ $[M+Na]^+$, 297.1314; found, 297.1318.

Methyl 2,3,4-Tri-*O*-methyl-4,6-(prop-2-en-1,3-diyl)-β-L-*glycero*-α-D-*gluco*-nonopyranoside (86).

Compound **86** was synthesized, by the same procedure as described for the compound **85** from diene **84** (9 mg, 0.03 mmol) and Grubbs 2nd generation catalyst (1 mg, 10% weight) in anhydrous CH_2Cl_2 (1.2 mL). After purification by silica gel column chromatography, eluting with hexane/ethyl acetate (1:1 to 2:8), **86** (0.006 g, 75%) was obtained as a colorless oil. $[\alpha]^{22}{}_D$ = +213.3 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.93 – 5.88 (m, 1H), 5.80 – 5.75 (m, 1H), 4.79 (d, *J* = 4.1 Hz, 1H), 3.91 – 3.84 (m, 2H), 3.64 (d, *J* = 9.3 Hz, 1H), 3.59 (s, 3H), 3.48 (s, 3H), 3.45 (s, 3H), 3.39 (s, 3H), 3.27 (dd, *J* = 9.3, 4.1 Hz, 1H), 2.68 (d, *J* = 11.3 Hz, 1H), 2.58 (dd, *J* = 19.2, 5.5 Hz, 1H), 2.15 – 2.08 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 127.28, 124.49, 97.21, 84.85, 82.50, 76.00, 68.84, 67.08, 60.70, 58.72, 55.44, 52.12, 23.31. HRMS (ESI) m/z calcd for $C_{13}H_{22}O_6Na$ $[M+Na]^+$, 297.1314; found, 297.1321.

Methyl 2,3,4,6-Tetra-*O*-methyl-4,6-(propan-1,3-diyl)-α-D-*glycero*-D-*gluco*-nonopyranoside (54).

A mixture of **85** (6 mg, 0.02 mmol) and 20% $Pd(OH)_2$ (3 mg) in CH_3OH (0.4 mL) was stirred under H_2 atmosphere (1 atm) over night at room temperature, then filtered through Celite before concentration to dryness. The resultant crude compound **87** was dissolved in anhydrous DMF (0.2 mL) and cooled to 0 ˚C before addition of 60% NaH (0.002 g, 0.04 mmol) followed by MeI (2.6 µL, 0.04 mmol). The reaction mixture was stirred under an argon atmosphere for 2 h and cooled to 0 ˚C before quenching with H_2O. The reaction mixture was partitioned between ethyl acetate and H_2O, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined

organic layers were dried over anhydrous Na_2SO_4 before concentration to dryness. The residue was purified by column chromatography over silica gel, eluting with hexane/ethyl acetate (7:3 to 1:1), to afford **54** (4.7 mg, 76%) as a colorless oil. $[\alpha]^{22}{}_D$ = +150.0 (*c* 0.3, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.87 (d, *J* = 3.9 Hz, 1H), 3.96 (d, *J* = 3.1 Hz, 1H. H-5), 3.63 (d, *J* = 9.5 Hz, 1H), 3.58 (s, 3H), 3.49 (s, 3H), 3.46 (ddd, *J* = 11.7, 4.4, 3.1 Hz, 1H), 3.46(s, 3H), 3.39 (s, 3H), 3.38 (s, *3*H), 3.30 (dd, *J* = 9.5, 3.9 Hz, 1H), 1.94 (ddd, *J* = 13.7, 3.4, 3.4 Hz, 1H, H-9_{eq}), 1.82 (dddd, *J* = 11.9, 4.4, 3.4, 3.0, 1H), 1.62 (dddt, *J* = 12.3, 3.9, 3.6, 3.4, 1H), 1.52 (dddd, *J* = 12.8, 11.9, 11.7, 3.6 Hz, 1H), 1.46 (ddddd, *J* = 13.1, 12.8, 12.3, 3.3, 3.0 Hz, 1H), 1.39 (dddd, *J* = 13.7, 13.1, 3.9 Hz, 1H, H-9_{ax}); ^{13}C NMR (150 MHz, $CDCl_3$) δ 97.48, 82.48, 82.05, 78.31, 76.60, 66.11, 60.72, 58.86, 56.63, 55.23, 50.46, 25.08, 23.23, 19.01; HRMS (ESI) m/z calcd for $C_{14}H_{26}O_6Na$ $[M+Na]^+$, 313.1627; found, 313.1630.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.72 (d, *J* = 4.0 Hz, 1H), 4.17 (d, *J* = 3.0 Hz, 1H), 3.81 (d, *J* = 9.4 Hz, 1H), 3.59 (ddd, *J* = 11.0, 5.0, 3.0, 1H), 3.50 (s, 3H), 3.30 (s, 3H), 3.26 (dd, *J* = 9.4, 4.0 Hz, 1H), 3.23 (s, 6H), 3.17 (s, 3H), 2.20 – 2.14 (m, 1H), 1.88 – 1.80 (m, 2H), 1.54 – 1.44 (m, 3H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.86 (d, *J* = 4.0 Hz, 1H), 3.94 (d, *J* = 3.1 Hz, 1H), 3.54 (d, *J* = 9.5 Hz, 1H), 3.53 (s, 3H), 3.48 (ddd, *J* = 11.6, 4.5, 3.1 Hz, 1H), 3.44 (s, 3H), 3.41 (s, 3H), 3.374 (s, 3H), 3.369 (s, 3H), 3.33 (dd, *J* = 9.5, 4.0 Hz, 1H), 1.93 (br d, *J* = 14.1 Hz, 1H), 1.76 (br d, *J* = 11.9 Hz, 1H), 1.60 (br d, *J* = 12.3 Hz, 1H), 1.52 (dddd, *J* = 12.7, 11.9, 4.5, 3.4 Hz, 1H), 1.46 (dddt, *J* = 12.7, 12.6, 12.3, 3.1 Hz, 1H), 1.39 (ddd, *J* = 14.1, 12.6, 3.4 Hz, 1H).

Methyl 2,3,4,6-Tetra-*O*-methyl-4,6-(propan-1,3-diyl)-β-L-*glycero*-D-*gluco*-nonopyranoside (**56**)**.**

A solution of **86** (0.006 g, 0.02 mmol) and 20% $Pd(OH)_2$ (0.003 g) in CH_3OH (0.4 mL) was stirred under H_2 atmosphere (1 atm) overnight followed by filtration through Celite and concentration to dryness. The resultant crude alcohol **88** was dissolved in anhydrous DMF (0.2 mL) and cooled to 0 ˚C before addition of 60% NaH (0.002 g, 0.04 mmol) followed by MeI (2.6 µL, 0.04 mmol). The reaction mixture was then stirred under an argon atmosphere for 3 h and cooled to 0 ˚C before quenching with H_2O. The crude compound **56** was obtained by following the same workup as described for compound **54**. The residue was purified by column chromatography over silica gel, eluting with hexane/ethyl acetate (7:3 to 1:1), to afford **56** (5.2 mg, 78 %) as a colorless oil. $[\alpha]^{22}{}_D$ = +99.4 (*c* 0.3, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.75 (d, *J* = 3.8 Hz, 1H), 3.87 (br s, 1H. H-5), 3.71 (d, *J* = 9.6 Hz, 1H), 3.58 (s, 3H), 3.49 (s,3H), 3.41 (s, 3H), 3.39 – 3.33 (m, 6H), 3.35 (q, *J* = 2.8 Hz, 1H, H-6_{eq}), 3.25 (dd, *J* = 9.6, 3.8 Hz, 1H), 1.95 (br d, *J* = 14.1 Hz, 1H), 1.82 (dtd, *J* = 14.0, 3.3, 2.8 Hz, 1H), 1.76 (dddt, *J* = 13.5, 13.3, 13.0, 3.3 Hz, 1H), 1.50 (dddd, *J* = 14.0, 13.5, 3.6, 2.8 Hz, 1H), 1.39 (ddd, *J* = 14.1, 13.3, 3.7 Hz, 1H, H-9_{ax}), 1.33 (ddddd, *J* = 13.0, 3.7, 3.6, 3.3, 3.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 97.38, 81.45, 80.89, 78.79, 76.43, 64.65, 60.89, 58.96, 57.42, 55.33, 50.58, 25.13, 24.96, 15.37; HRMS (ESI) m/z calcd for $C_{14}H_{26}O_6Na$ $[M+Na]^+$, 313.1627; found, 313.1635.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.63 (d, *J* = 3.8 Hz, 1H), 4.15 (br s, 1H), 3.99 (d, *J* = 9.6 Hz, 1H), 3.58 (s, 3H), 3.48 (q, *J* = 2.8 Hz, 1H), 3.39 (s, 3H), 3.27 (s, 3H), 3.26 (dd, *J* = 9.6, 2.8 Hz, 1H), 3.20 (s, 3H), 3.17 (s, 3H), 2.33 (ddd, *J* = 14.1, 3.4, 3.3 Hz, 1H),

2.24 (ddddd, *J* = 13.9, 13.3, 13.2, 3.3, 3.1 Hz, 1H), 1.89 (dddd, *J* = 13.9, 3.2, 3.1, 2.8 Hz, 1H), 1.70 (tdd, *J* = 13.9, 3.7, 2.8 Hz, H-7_{ax}), 1.58 (ddd, *J* = 14.1, 13.3, 3.6 Hz, 1H), 1.38 (ddddd, *J* = 13.2, 3.7, 3.6, 3.4, 3.2 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.80 (d, *J* = 3.8 Hz, 1H), 3.86 (br s, 1H), 3.64 (d, *J* = 9.6 Hz, 1H), 3.53 (s, 3H), 3.45 (s, 3H), 3.39 (s, 3H), 3.36 (q, *J* = 2.7 Hz, 1H), 3.34 (s, 3H), 3.34 (s, 3H) 3.27 (dd, *J* = 9.6, 3.8 Hz, 1H. H-2), 1.90 (dt, *J* = 14.1, 3.3 Hz, 1H), 1.78 (dtd, *J* = 14.0, 3.0, 2.7 Hz, 1H), 1.74 (ddddd, *J* = 13.6, 13.3, 13.1, 3.3, 3.0 Hz, 1H), 1.55 (dddd, *J* = 14.0, 13.6, 3.8, 2.7 Hz, 1H), 1.44 (ddd, *J* = 14.1, 13.1, 3.8 Hz, 1H), 1.31 (dtdd, *J* = 13.3, 3.8, 3.3, 3.0 Hz, 1H).

Methyl 4-*C*-Allyl-6-*O*-benzyl-2,3-di-*O*-methyl-α-D-galactopyranoside (91) and Methyl 4-*C*-Allyl-6-*O*-benzyl-2,3-di-*O*-methyl-α-D-glucopyranoside (92).

A mixture of alcohol **89**[165, 385] (0.53 g, 1.70 mmol) and Dess-Martin Periodinane (0.94 g, 2.21 mmol) in anhydrous CH_2Cl_2 (23.0 mL) was stirred overnight under an argon atmosphere before dilution with CH_2Cl_2 and addition of a mixture of saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, and H_2O (1:1:1, 60 mL). The reaction mixture was then stirred for another 0.5 h before the organic layer was separated. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. The resultant crude ketone **90** was dissolved in anhydrous THF (8.0 mL) and cooled to 0 ˚C before a freshly prepared solution of allyl magnesium chloride in THF (1 M, 12.0 mL, 12.00 mmol) was added. The reaction mixture was stirred for 15 min at 0 ˚C before it was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate. The combined organic layers were washed with brine, and the organic layer was dried over anhydrous Na_2SO_4 before concentration. The residue (**91:92** = 2.8:1) was purified by column chromatography over

silica gel, eluting with hexane/ethyl acetate (7:3 to 1:1), to obtain **91** (0.36 g, 60 %) and **92** (0.13 g, 22 %) as colorless oils.

(91). $[\alpha]^{22}_{D}$ = +101.3 (*c* 1.30, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 5H), 5.72 (ddt, *J* = 17.5, 10.1, 7.5 Hz, 1H), 5.05 (dd, *J* = 10.1, 1.1 Hz, 1H, H-9), 5.00 (dd, *J* = 17.0, 1.3 Hz, 1H, H-9′), 4.89 (d, *J* = 3.7 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.54 (d, *J* = 12.0 Hz, 1H), 3.81 (dd, *J* = 10.6, 2.6 Hz, 1H), 3.77 (dd, *J* = 10.6, 4.9 Hz, 1H′), 3.76 – 3.73 (m, 1H), 3.66 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.59 (s, 3H), 3.49 (s, 3H), 3.41 (s, 3H), 3.40 (d, *J* = 9.2 Hz, 1H), 3.09 (s, 1H), 2.62 (dd, *J* = 14.3, 7.4 Hz, 1H), 2.17 (dd, *J* = 14.3, 7.6 Hz, 1H′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 132.5, 128.4, 127.8, 118.8, 97.6, 80.1, 78.9, 76.0, 73.7, 69.7, 69.1, 61.3, 58.7, 55.3, 39.9; HRMS (ESI) m/z calcd for $C_{19}H_{28}O_6Na$ $[M+Na]^+$, 375.1784; found, 375.1786.

(92). $[\alpha]^{22}_{D}$ = +85.1 (*c* 0.85, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.26 (m, 5H), 6.01 – 5.90 (m, 1H), 5.16 – 5.06 (m, 2H), 4.84 (d, *J* = 3.9 Hz, 1H), 4.53 (br m, 2H), 3.99 – 3.92 (m, 1H), 3.77 (dd, *J* = 9.9, 4.8 Hz, 1H), 3.62 (dd, *J* = 9.9, 2.5 Hz, 1H), 3.60 (s, 3H), 3.49 (s, 3H), 3.48 – 3.47 (m, 1H), 3.44 (s, 3H), 3.27 (dd, *J* = 10.1, 3.9 Hz, 1H), 2.84 (br s, 1H), 2.60 (dd, *J* = 14.4, 6.2 Hz, 1H), 2.33 (dd, *J* = 14.5, 8.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 134.4, 128.7, 128.4, 128.0, 127.7, 127.5, 118.2, 97.2, 85.7, 80.1, 74.8, 73.3, 71.9, 69.1, 61.9, 58.8, 55.0, 34.1; HRMS (ESI) m/z calcd for $C_{19}H_{28}O_6Na$ $[M+Na]^+$, 375.1784; found, 375.1777.

Methyl 4-*C*-Allyl-6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-galactopyranoside (93).

To a stirred solution of alcohol **91** (0.2 g, 0.56 mmol) in dry DMF (5 mL) was added NaH (60%, 0.045 g, 1.13 mmol) followed by MeI (0.24 mL, 3.84 mmol) dropwise at 0 °C (ice-water bath). The reaction mixture was stirred at 0 °C for 0.5 h before it was quenched

with water (5 mL), extracted with EtOAc, and washed with brine. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. Column chromatography on silica gel (eluent: 20% ethyl acetate in hexane) afforded **93** (0.19 g, 93%) as a colorless oil. $[\alpha]^{22}_D$ = +84.4 (*c* 2.50, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 5H), 5.76 (ddt, *J* = 17.4, 10.2, 7.5 Hz, 1H), 5.15 – 5.05 (m, 2H), 4.90 (d, *J* = 3.7 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.48 (d, *J* = 12.1 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.81 (dd, *J* = 10.6, 2.9 Hz, 1H), 3.69 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.58 – 3.56 (m, 1H), 3.57 (s, 3H), 3.53 (d, *J* = 10.1 Hz, 1H), 3.51 (s, 3H), 3.44 (s, 3H), 3.43 (s, 3H), 2.98 (dd, *J* = 13.7, 7.8 Hz, 1H), 2.16 (dd, *J* = 13.6, 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 132.3, 128.3, 127.5, 119.3, 97.4, 80.9, 80.2, 79.3, 73.4, 72.2, 68.8, 61.8, 58.7, 55.2, 52.8, 33.7; HRMS (ESI) m/z calcd for $C_{20}H_{30}O_6Na$ $[M+Na]^+$, 389.1940; found, 389.1933.

Methyl 4-*C*-Allyl-6-*O*-benzyl-2,3,4-tri-*O*-methyl-α-D-glucopyranoside (94).

A solution of alcohol **92** (0.076 g, 0.22 mmol) in anhydrous DMF (0.8 mL) was cooled to 0 ˚C before addition of 60% NaH (0.017 g, 0.43 mmol) followed by MeI (27.0 µL, 0.43 mmol). The reaction mixture was stirred under an argon atmosphere for 0.5 h at room temperature. The reaction mixture was cooled to 0 ˚C before it was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude residue was purified by column chromatography over silica gel (eluting with hexane/ethyl acetate 9:1 to 1:1) to obtain **94** (0.068 g, 86 %) as a sticky gum. $[\alpha]^{22}_D$ = +71.9 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 5H), 5.94 (dddd, *J* = 17.2, 10.1, 8.8, 5.2 Hz, 1H), 5.07 (d, *J* = 17.2 Hz, 1H), 4.99 (d, *J* = 10.1 Hz, 1H), 4.84 (d, *J* = 4.0 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 4.07 (d, *J* = 7.9 Hz, 1H), 3.75 – 3.69 (m, 2H), 3.67 – 3.61

(m, 1H), 3.60 (s, 3H), 3.50 (s, 3H), 3.47 (s, 3H), 3.38 – 3.33 (m, 4H), 2.70 – 2.60 (m, 1H), 2.39 (dd, *J* = 15.2, 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.61, 134.97, 128.43, 127.57, 127.43, 116.83, 97.00, 81.47, 80.95, 78.56, 73.37, 71.35, 69.24, 60.70, 58.93, 55.07, 50.84, 32.83; HRMS (ESI) m/z calcd for $C_{20}H_{30}O_6Na$ $[M+Na]^+$, 389.1940; found, 389.1935.

Methyl 6-*O*-Benzyl-4-*C*-(2-hydroxyethyl)-2,3,4-tri-*O*-methyl-α-D-galactopyranoside (95).

Ozone was bubbled through a solution of olefin **93** (0.18 g, 0.49 mmol) in CH_2Cl_2/MeOH (30 mL/20 mL) at -78 ^oC until the solution turned to a persistent blue color. Ar gas was bubbled through the solution for 5 min to remove excess ozone before $NaBH_4$ (0.18 g, 4.90 mmol) was added at −78 ^oC. The reaction mixture was slowly brought to room temperature, stirred for 10 h, and concentrated under reduced pressure. The crude reaction mixture was dissolved in ethyl acetate and washed with aqueous hydrochloric acid (1 M), brine, dried over Na_2SO_4, and concentrated to dryness. Silica gel column chromatography (eluent: hexane/ethyl acetate = 3:2) afforded **95** (129 mg, 71%) as a colorless oil. $[\alpha]^{22}_D$ = +55.8 (*c* 2.75, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.25 (m, 5H), 4.88 (d, *J* = 3.7 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.50 (d, *J* = 11.9 Hz, 1H), 3.93 (t, *J* = 5.0 Hz, 1H), 3.85 (dd, *J* = 10.2, 4.7 Hz, 1H), 3.76 – 3.66 (m, 3H), 3.57 (d, *J* = 10.6 Hz, 1H), 3.55 (s, 3H), 3.54 – 3.51 (m, 1H), 3.50 (s, 3H), 3.43 (s, 6H), 2.47 (dt, *J* = 13.8, 6.1 Hz, 1H), 2.17 (br s, 1H), 1.71 (dt, *J* = 14.3, 7.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.8, 128.42 127.75 127.7, 97.5, 81.4, 80.0, 78.7, 73.5, 72.1, 69.3, 61.9, 58.7, 58.4, 55.3, 52.8, 31.4; HRMS (ESI) m/z calcd for $C_{19}H_{30}O_7Na$ $[M+Na]^+$, 393.1889; found, 393.1888.

Methyl 6-*O*-benzyl-4-*C*-(2-hydroxyethyl)-2,3,4-tri-*O*-methyl-α-D-glucopyranoside (96).

A solution of alkene **94** (0.051 g, 0.14 mmol) in a 2:1 mixture of CH_2Cl_2/CH_3OH (14.2 mL) was cooled to -78 ˚C before being sparged with ozone until a persistent blue was color observed. The solution was then purged with argon and treated with $NaBH_4$ (0.053 g, 1.39 mmol) at -78 ˚C. The reaction mixture was then stirred at room temperature for 4 h before concentration. The crude residue was dissolved in ethyl acetate and washed with 1 N HCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude was purified using silica gel column chromatography, eluting with hexane/ethyl acetate (1:1 to 1:4), to afford **96** (0.043g, 85 %) as a colorless oil; $[\alpha]^{22}_D$ = +86.2 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 5H), 4.83 (d, *J* = 3.9 Hz, 1H), 4.63 (d, *J* = 12.2 Hz, 1H), 4.50 (d, *J* = 12.1 Hz, 1H), 4.13 (dd, *J* = 8.1, 1.1 Hz, 1H), 3.89 (d, *J* = 9.8 Hz, 1H), 3.84 – 3.76 (m, 1H), 3.73 (dd, *J* = 10.6, 1.4 Hz, 1H), 3.67 – 3.59 (m, 4H), 3.53 – 3.46 (m, 8H), 3.31 (s, 3H), 2.09 – 1.99 (m, 1H), 1.62 – 1.53 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.29, 128.53, 127.75, 127.50, 97.16, 81.26, 78.89, 78.77, 73.55, 70.30, 68.33, 61.21, 59.17, 58.19, 55.21, 49.82, 33.86; HRMS (ESI) m/z calcd for $C_{19}H_{30}O_7Na$ $[M+Na]^+$, 393.1889; found, 393.1895.

Methyl 6-*O*-Benzyl-2,3,4-tri-*O*-methyl-4-*C*-(*p*-tolunesulfonyloxyethyl)-α-D-galactopyranoside (97).

To a stirred solution of alcohol **95** (0.07 g, 0.18 mmol) in pyridine (1 mL) was added tosyl chloride (0.043 g, 0.22 mmol) and DMAP (2.5 mg, 0.018 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h before TLC (40% ethyl acetate in hexane) showed completion. The reaction mixture was concentrated to

dryness, and the residue was dissolved in ethyl acetate, washed with 1 N HCl, washed with brine, and dried over anhydrous Na_2SO_4. The organic layer was concentrated under vacuum and the residue was purified by silica gel column chromatography, eluting with 40 % EtOAc in hexane, to afford **97** (65 mg, 66%) as a colorless oil. $[\alpha]^{22}_D$ = +55.5 (*c* 1.70, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 – 7.75 (m, 2H), 7.37 – 7.24 (m, 7H), 4.84 (d, *J* = 3.6 Hz, 1H), 4.50 (d, *J* = 11.9 Hz, 1H), 4.44 (d, *J* = 11.9 Hz, 1H), 4.16 (td, *J* = 9.4, 6.5 Hz, 1H), 4.06 (td, *J* = 9.5, 5.7 Hz, 1H), 3.72 (dd, *J* = 10.2, 4.4 Hz, 1H), 3.66 – 3.60 (m, 2H), 3.52 – 3.47 (m, 1H), 3.48 (s, 3H), 3.45 (s, 3H), 3.39 (s, 3H), 3.38 (s, 3H), 3.35 (d, *J* = 9.8 Hz, 1H), 2.59 – 2.50 (m, 1H), 2.41 (s, 3H), 1.95 – 1.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 137.9, 132.8, 129.9, 128.4, 127.9, 127.6, 127.5, 97.4, 81.6, 79.8, 77.9, 73.4, 71.9, 69.0, 66.4, 61.8, 58.8, 55.4, 52.9, 28.6, 21.6; HRMS (ESI) m/z calcd for $C_{26}H_{36}O_9SNa$ $[M+Na]^+$, 547.1978; found, 547.1953.

Methyl 4-*C*,6-*O*-(Ethan-1,2-diyl)-2,3,4-tri-*O*-methyl-α-D-galacto-octopyranoside (13).

A solution of tosylate **97** (0.060 g, 0.011 mmol) in CH_3OH:EtOAc (2 mL, 1:1) was treated with $Pd(OH)_2$/C (20 mg, 20 wt%) and stirred under 1 atm of H_2 (balloon) for 1 h, then filtered through Celite. The combined filtrates were evaporated under vacuum to give a crude alcohol (0.05g, 0.011 mmol), which was taken up in dry DMF (1.0 mL), treated with NaH (14 mg, 0.034 mmol, 60% in oil) at 0 °C and stirred for 1 h at room temperature. The reaction mixture was poured into iced water and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (eluent: ethyl acetate/hexane 5% to 20%) to afford **50** (24 mg, 80%) as a colorless oil. $[\alpha]^{22}_D$ = +138.9 (*c* 1.10, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.88 (d, *J* = 3.8 Hz, 1H), 3.73

(ddd, *J* = 11.5, 5.0, 1.5 Hz, 1H), 3.70 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.66 (dd, *J* = 9.7, 5.8 Hz, 1H), 3.62 – 3.61 (m, 1H), 3.61 – 3.60 (m, 1H), 3.52 (s, 3H), 3.50 (s, 6H), 3.49 (ddd, *J* = 12.7, 11.5, 2.1 Hz, 1H), 3.39 (s, 3H), 3.31 (d, *J* = 9.9 Hz, 1H), 2.41 (ddd, *J* = 14.8, 2.1, 1.5 Hz, 1H), 1.45 (ddd, *J* = 14.8, 12.7, 5.0 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 98.4, 85.2, 79.9, 74.2, 68.9, 64.2, 62.9, 62.1, 58.9, 55.3, 52.2, 27.9; HRMS (ESI) m/z calcd for $C_{12}H_{22}O_6Na$ $[M+Na]^+$, 285.1314; found, 285.1323.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.75 (d, *J* = 3.7 Hz, 1H), 3.93 (dd, *J* = 10.5, 10.0 Hz, 1H), 3.80 (dd, *J* = 10.0, 4.8 Hz, 1H), 3.77 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.73 (dd, *J* = 10.5, 4.8 Hz, 1H), 3.52 (ddd, *J* = 11.4, 5.0, 1.4 Hz, 1H), 3.38 (ddd, *J* = 12.6, 11.4, 2.1 Hz, 1H), 3.37 (s, 3H), 3.33 (d, *J* = 10.0 Hz, 1H), 3.32 (s, 3H), 3.14 (s, 3H), 3.08 (s, 3H), 2.01 (ddd, *J* = 14.7, 2.1, 1.4 Hz, 1H), 1.07 (ddd, *J* = 14.7, 12.6, 5.0 Hz, 1H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.86 (d, *J* = 3.8 Hz, 1H), 3.71 (ddd, *J* = 11.5, 5.0, 1.5 Hz, 1H, 7eq), 3.66-3.62(m, Hz, 1H), 3.62 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.55 – 3.53 (m, 2H), 3.51 (s, 3H), 3.47 (ddd, *J* = 12.7, 11.5, 2.1 Hz, 1H), 3.49 (s, 3H), 3.47 (s, 3H), 3.37 (s, 3H), 3.31 (d, *J* = 10.0 Hz, 1H), 2.44 (ddd, *J* = 14.9, 2.1, 1.5 Hz, 1H), 1.49 (ddd, *J* = 14.9, 12.7, 5.0 Hz, 1H).

Methyl 4-*C*,6-*O*-(Ethan-1,2-diyl)-2,3,4-tri-*O*-methyl-α-D-gluco-octopyranoside (52) and Methyl 6-*O*-Benzyl-3-*O*,4-*C*-(ethan-1,2-diyl)-2,4-di-*O*-methyl-α-D-gluco-octopyranoside (100).

Compound **96** (7.2 mg, 0.019 mmol) was dissolved in anhydrous pyridine (0.2 mL) and cooled to 0 ˚C before addition of MsCl (1.9 µL, 0.024 mmol). The reaction mixture was stirred for 1.5 h at 0 ˚C before it was diluted with ethyl acetate and washed with H_2O. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to

dryness. The resultant residue and 20% $Pd(OH)_2$ (3 mg) were stirred in a 1:1 mixture of CH_2Cl_2/CH_3OH (0.3 mL) under 1 atm of H_2 atmosphere for 6 h. The reaction mixture was filtered through Celite and concentrated to dryness. The residue was purified by column chromatography, eluting with hexane/ethyl acetate (7:3 to 1:1), to obtain **52** (0.0011 g, 21%) and **100** (0.0012g, 23%) as colorless oils.

(52). $[\alpha]^{22}{}_D$ = +72.0 (*c* 0.2, CH_2Cl_2); **In $CHCl_3$:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.87 (d, *J* = 3.8 Hz, 1H), 3.83 (dd, *J* = 12.4, 1.7 Hz, 1H), 3.80 (dt, *J* = 11.2, 3.2 Hz, 1H), 3.76 (dd, *J* = 12.4, 1.6 Hz, 1H), 3.68 (d, *J* = 9.5 Hz, 1H), 3.66 – 3.62 (m, 1H), 3.59 (s, 3H), 3.52 (br s, 1H), 3.50 (s, 3H), 3.42 (s, 3H), 3.40 (s, 3H), 3.34 (dd, *J* = 9.5, 3.8 Hz, 1H), 1.89 – 1.86 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 97.72, 81.86, 81.31, 73.75, 66.16, 65.17, 63.28, 60.63, 58.88, 55.44, 50.58, 25.27; HRMS (ESI) m/z calcd for $C_{12}H_{22}O_6Na$ $[M+Na]^+$, 285.1314; found, 285.1325.

In C_6D_6: ^{1}H NMR (600 MHz, C_6D_6) δ 4.68 (d, *J* = 3.7 Hz, 1H), 3.90 – 3.89 (m, 2H), 3.81 (d, *J* = 9.5 Hz, 1H), 3.80 (dt, *J* = 11.0, 3.5 Hz, 1H), 3.72 – 3.66 (m, 1H), 3.50 (br s, 1H), 3.47 (s, 3H), 3.25 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.15 (s, 6H), 3.08 (s, 3H), 1.98 – 1.95 (m, 2H).

In CD_3OD: ^{1}H NMR (600 MHz, CD_3OD) δ 4.86 (d, *J* = 3.8, 1H), δ 3.80 (dd, *J* = 12.4, 1.7 Hz, 1H), 3.71 (dt, *J* =11.3, 3.6 Hz, 1H), 3.67 (dd, *J* = 12.4, 1.6, 1H), 3.65 – 3.63 (m, 1H), 3.61 (d, *J* = 9.5 Hz, 1H), 3.54 (s, 3H), 3.52 (br s, 1H), 3.46 (s, 3H), 3.39 (s, 3H), 3.38 (s, 3H), 3.33 (dd, *J* = 9.5, 3.8 Hz, 1H), 1.85 – 1.82 (m, 2H).

(100). $[\alpha]_D^{22}$ = +74.0 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 4.84 (d, *J* = 3.7 Hz, 1H), 4.66 (d, *J* = 12.1 Hz, 1H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.36 (d, *J* = 8.1 Hz, 1H), 4.23 (dd, *J* = 7.8, 1.8 Hz, 1H), 4.04 – 3.98 (m, 2H), 3.81 (dd, *J* = 10.7,

2.0 Hz, 1H), 3.60 – 3.57 (m, 1H), 3.54 (s, 3H), 3.49 (s, 3H), 3.30 (s, 3H), 3.12 (dd, *J* = 8.2, 3.7 Hz, 1H), 2.16 (dt, *J* = 13.1, 9.9 Hz, 1H), 1.78 (ddd, *J* = 13.3, 5.8, 3.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.35, 129.73, 128.58, 128.51, 127.73, 127.60, 97.41, 83.10, 79.84, 79.18, 73.59, 69.31, 66.56, 66.05, 58.87, 55.60, 51.18, 31.92; HRMS (ESI) m/z calcd for $C_{18}H_{26}O_6Na$ $[M+Na]^+$, 361.1627; found, 361.1627.

Phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (105).

Compound **1** was synthesized as described in the literature.[202] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.57 (m, 2H), 7.42 – 7.41 (m, 2H), 7.36 – 7.21 (m, 13H), 7.25 – 7.23 (m, 3H), 4.83 (d, *J* = 10.3 Hz, 1H), 4.75 (d, *J* = 10.3 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.64 (d, *J* = 9.9 Hz, 1H), 4.57 (br s, 2H), 4.11 (d, *J* = 3.2 Hz, 1H), 3.81 (dd, *J* = 5.7, 10.0 Hz, 1H), 3.78 (dd, *J* = 5.8, 10.0 Hz, 1H), 3.76 (t, *J* = 9.5 Hz, 1H), 3.61 – 3.60 (m, 1H), 3.58 (dd, *J* = 3.3, 9.5 Hz, 1H), 2.55 (s, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.68 (dd, *J* = 8.2, 1.0 Hz, 2H), 7.40 (d, *J* = 7.6 Hz, 1H, 2H), 7.23 (d, *J* = 7.6 Hz, 1H, 2H), 7.17 – 6.96 (m, 13H), 6.91 (t, *J* = 7.4 Hz, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.57 (d, *J* = 9.8 Hz, 1H), 4.34 – 4.26 (m, 4H), 3.83 (t, *J* = 9.3 Hz, 1H), 3.79 (d, *J* = 2.7 Hz, 1H), 3.75 (dd, *J* = 9.8, 5.8 Hz, 1H), 3.72 (dd, *J* = 9.8, 6.1 Hz, 1H), 3.25 (t, *J* = 5.9 Hz, 1H), 3.20 (dd, *J* = 8.9, 3.2 Hz, 1H), 2.39 (s, 1H). IR (neat) *v* 3480 cm^{-1} (O-H).

Phenyl (6*S*)-[6-$^2H_1$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-105).

Compound **6*S*-D-105 (**0.40 g, 72%) was synthesized from compound **114** analogously to **105**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.57 (m, 2H), 7.42 – 7.40 (m, 2H), 7.36 – 7.21 (m, 13H), 7.25 – 7.23 (m, 3H), 4.83 (d, *J* = 10.3 Hz, 1H), 4.75 (d, *J* = 10.3 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.64 (d, *J* = 9.9 Hz,

1H), 4.56 (br s, 2H), 4.10 (d, *J* = 3.1 Hz, 1H), 3.76 (d, *J* = 2.9 Hz, 1H), 3.75 (t, *J* = 9.5 Hz, 1H), 3.60 – 3.59 (m, 1H), 3.58 (dd, *J* = 3.2, 9.3 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.69 – 7.68 (m, 2H), 7.41 – 7.40 (m, 2H), 7.23 – 6.89 (m, 16H), 4.84 (d, *J* = 10.8 Hz, 1H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.57 (d, *J* = 9.8 Hz, 1H), 4.31 (d, *J* = 11.9 Hz, 1H), 4.29 – 4.27 (m, 3H), 3.82 (t, *J* = 9.2 Hz, 1H), 3.78 (d, *J* = 3.1 Hz, 1H), 3.70 (d, *J* = 6.2 Hz, 1H), 3.23 – 3.22 (m, 1H), 3.19 (dd, *J* = 8.9, 3.2 Hz, 1H). HRMS (ESI) m/z calcd for $C_{33}H_{33}DO_5SNa$ $[M+Na]^+$, 566.2087; found, 566.2079.

Phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (106).

Compound **129** (0.01 g, 0.015 mmol) was dissolved in anhydrous methanol (0.5 mL) and cooled to 0 °C before it was treated with Na (catalytic amount). The reaction mixture was stirred under an argon atmosphere at room temperature overnight, quenched with Amberlyst-15 (pH ~ 4), filtered, and concentrated to dryness. The crude residue was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (19:1 to 4:1) to afford compound **106** (0.005 g, 62%) with spectral data consistent with the literature.[386] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.55 (m, 2H), 7.43 – 7.24 (m, 18H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.79 (d, *J* = 11.4 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H,), 4.70 (d, *J* = 9.6 Hz, 1H), 4.60 (d, *J* = 11.9 Hz, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 3.80 (dd, *J* = 10.4, 4.1 Hz, 1H), 3.76 (dd, *J* = 10.4, 5.3 Hz, 1H), 3.66 (dd, *J* = 9.6, 8.9 Hz, 1H), 3.54 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.50 (ddd, *J* = 9.6, 5.3, 4.1 Hz, 1H), 3.49 (dd, *J* = 9.6, 8.8 Hz, 1H), 2.54 (br s, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.69 – 7.65 (m, 2H), 7.45 – 7.42 (m, 2H), 7.31 – 6.94 (m, 16H), 4.94 (d, *J* = 10.6 Hz, 1H), 4.87 (d, *J* = 11.7 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 9.7 Hz, 1H), 4.33 (d, *J* = 11.9 Hz, 1H), 4.30 (d, *J* = 11.9 Hz, 1H), 3.66 (dd, *J* = 9.8, 8.9 Hz, 1H), 3.64 (dd, *J* = 10.4,

3.7 Hz, 1H), 3.60 (dd, *J* = 10.4, 5.3 Hz, 1H), 3.51 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.43 (dd, *J* = 8.9, 8.7 Hz, 1H), 3.22 (ddd, *J* = 9.8, 5.3, 3.7 Hz, 1H), 2.23 (s, 1H). IR (neat) *v* 3441 cm^{-1} (O-H).

Phenyl (6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-106).

Compound **6*S*-D-106** (0.011 g, 60%) was synthesized from **6*S*-D-129** (21.7 mg, 0.034 mmol) analogously to **106**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.53 (m, 1H), 7.44 – 7.22 (m, 18H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.91 (d, *J* = 10.4 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.74 (d, *J* = 10.4 Hz, 1H), 4.69 (d, *J* = 9.6 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 3.74 (d, *J* = 5.4 Hz, 1H), 3.66 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.54 (t, *J* = 8.8 Hz, 1H), 3.48 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.48 (dd, *J* = 9.6, 5.4 Hz, 1H), 2.53 (br s, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.71 – 7.64 (m, 2H), 7.46 – 7.41 (m, 2H), 7.30 – 6.93 (m, 16H), 4.94 (d, *J* = 10.6 Hz, 1H), 4.87 (d, *J* = 11.7 Hz, 1H), 4.75 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 9.7 Hz, 1H), 4.32 (d, *J* = 12.2 Hz, 1H), 4.30 (d, *J* = 12.2 Hz, 1H), 3.65 (ddd, *J* = 9.7, 8.8, 2.9 Hz, 1H), 3.57 (d, *J* = 5.3 Hz, 1H), 3.51 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.42 (t, *J* = 8.8 Hz, 1H), 3.22 (dd, *J* = 9.7, 5.3 Hz, 1H), 2.19 (d, *J* = 2.9 Hz, 1H). HRMS (ESI) m/z calcd for $C_{33}H_{33}DO_5SNa$ $[M+Na]^+$, 566.2087; found, 566.2083.

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (107).

Compound **107** was synthesized as described in the literature.[203] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 15H), 4.93 (d, *J* = 11.0Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.87 (d, *J* = 11.0 Hz, 1H), 4.86 (d, *J* = 10.9 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.66 (d, *J* = 10.9 Hz, 1H), 4.51 (d, *J* = 9.9 Hz, 1H), 3.87 (dd, *J* = 12.2, 2.7 Hz, 1H), 3.71 (dd, *J* = 9.1, 8.8 Hz, 1H), 3.70 (dd, *J* = 12.2, 4.8 Hz, 1H), 3.58 (dd, *J* = 9.6, 9.1 Hz, 1H), 3.41 (dd, *J* = 9.9, 8.8 Hz, 1H), 3.38 (ddd, *J* = 9.6, 4.8, 2.7 Hz, 1H) 2.81 – 2.70 (m, 2H), 1.82 (br s, 1H),

1.33 (t, *J* = 7.4 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.45 – 7.42 (m, 2H), 7.35 – 7.31 (m, 2H), 7.26 – 7.23 (m, 2H), 7.19 – 7.05 (m, 9H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.85 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.74 (d, *J* = 10.6 Hz, 1H), 4.61 (d, *J* = 11.2 Hz, 1H), 4.36 (d, *J* = 9.7 Hz, 1H), 3.77 – 3.71 (m, 1H), 3.66 – 3.56 (m, 3H), 3.43 (dd, *J* = 9.7, 8.4 Hz, 1H), 3.13 – 3.09 (m, 1H), 1.60 (br s, 1H) 2.56 – 2.42 (m, 2H), 1.11 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 3484 cm^{-1} (O-H).

Ethyl (6*S*)-[6-^{2}*H*$_1$]-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-107).

A solution of compound **116** (0.105 g, 0.27 mmol) in anhydrous methanol (1.0 mL) was cooled to 0 °C and treated with Na (cat) and stirred under an argon atmosphere at room temperature for 1.5 h. Then the reaction was quenched with Amberlyst-15 resin (pH ~7) and filtered through cotton before it was concentrated to dryness. The crude residue was dissolved in anhydrous pyridine (0.8 mL) and treated with tritylchloride (84 µL, 0.32 mmol) at room temperature. The reaction mixture was stirred in dark at room temperature for 3 days. The reaction was then quenched with methanol (~ 70 µL), stirred for 1 h, and concentrated to dryness. The crude residue was dissolved in chloroform, washed with cold aqueous saturated $NaHCO_3$, and cold brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was dissolved in anhydrous DMF (0.9 mL) and cooled to 0 °C before it was treated with 60% NaH in mineral oil (48 mg, 1.2 mmol) followed by benzyl bromide (141 µL, 1.2 mmol). The reaction mixture was stirred under an argon atmosphere at room temperature for 6 h. The reaction mixture was cooled to 0 °C before it was quenched with water. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was

passed through a short pad of silica, eluting with hexane/ethyl acetate (7:3) and concentrated. The residue was dissolved in a mixture of glacial acetic acid and water (4:1, 1.3 mL) and heated at 80 °C for 2.5 h. The reaction mixture was concentrated to dryness and purified using silica gel column chromatography, eluting with hexane/ethyl acetate (4:1 to 7:2), obtain compound **6*S*-D-107** (0.045 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 15H), 4.94 (d, *J* = 10.9 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.66 (d, *J* = 10.9 Hz, 1H), 4.51 (d, *J* = 9.8 Hz, 1H), 3.72 (t, *J* = 9.0 Hz, 1H), 3.68 (d, *J* = 4.9 Hz, 1H), 3.58 (dd, *J* = 9.7, 9.0 Hz, 1H), 3.41 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.38 (dd, *J* = 9.7, 4.9 Hz, 1H), 2.84 – 2.69 (m, 2H), 1.33 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{29}H_{33}DO_5SNa$ $[M+Na]^+$, 518.2087; found, 518.2066.

(6*S*)-l,6-Anhydro-2,3,4-tri-*O*-acetyl-6-bromo-β-D-galactopyranose (109).

A mixture of l,6-anhydro-2,3,4-tri-O-acetyl-β-D-galactopyranose **108**[387] (0.77 g, 2.67 mmol) and *N*-bromosuccinimide (1.9 g, 10.6 mmol)) in trifluorotoluene (40 mL) was refluxed over a 300-W heat lamp for 8 h. After 8h, the solvent was evaporated under reduced pressure and the crude product dissolved in EtOAc (50 mL). The solution was successively washed with aqueous saturated $Na_2S_2O_3$, aqueous saturated $NaHCO_3$, and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave a crude product which was purified through silica-gel column chromatography (eluent: 20% EtOAc in hexane) to give **109** (0.73g, 75%) as a yellowish oil. $[\alpha]^{22}{}_D$ = −63.6 (*c* 1.50, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (s, 1H), 5.81 (s, 1H), 5.29 – 5.20 (m, 2H), 4.74 (dd, *J* = 4.0, 1.3 Hz, 1H), 4.71 (br s, 1H), 2.14 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 169.1, 168.9, 101.7, 82.6, 79.6, 69.3, 66.9, 64.4, 20.7 (2), 20.5;

HRMS (ESI) m/z calcd for $C_{12}H_{15}{}^{79}BrO_8Na$ $[M+Na]^+$, 388.9848; found, 388.9849; calcd for $C_{12}H_{15}{}^{81}BrO_8Na$ $[M+Na]^+$, 390.9828; found, 390.9828.

(6*S*)-[6-$^2H_1$]-l,6-Anhydro-2,3,4-tri-*O*-acetyl-β-D-galactopyranose (110).

To a solution of **109** (2.3 g, 6.26 mmol) and azobisisobutyronitrile (0.1 g, 0.63 mmol) in toluene (170 mL) was added freshly prepared tri-*n*-butyltin deuteride[214] (5.5 g, 18.79 mmol) and the reaction mixture was refluxed for 0.5 h. The cooled reaction mixture was evaporated in *vacuo* to give a crude product which was purified on silica-gel column chromatography (eluent: 40% EtOAc in hexane) to give **110** (1.4 g, 77%) as a colorless oil. $[\alpha]^{22}{}_D = -10.0$ (*c* 1.15, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (s, 1H), 5.29 – 5.20 (m, 2H), 4.75 (s, 1H), 4.45 (d, *J* = 2.7 Hz, 1H), 4.31 (s, 1H), 2.12 (s, 6H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 169.3, 169.2, 98.9, 72.0, 71.0, 67.4, 64.2 (t, *J* = 23.5 Hz), 20.8, 20.7, 20.5; HRMS (ESI) m/z calcd for $C_{12}H_{15}DO_8Na$ $[M+Na]^+$, 312.0806; found, 312.0818.

(6*S*)-[6-$^2H_1$]-1,2,3,4,6-Penta-*O*-acetyl-α-D-galactopyranose (111).

To a solution of compound **110** (1.4 g, 4.84 mmol) in MeOH (20 mL) was added Na (catalytic amount) slowly and the reaction mixture was stirred for 7 h, then neutralized with Amberlite® IR120, filtered, and concentrated under reduced pressure to give (6*S*)-[6-$^2H_1$]-1,6-anhydrogalactose. A solution of (6*S*)-[6-$^2H_1$]-1,6-anhydrogalactose (0.73 g, 4.47 mmol) in Ac_2O (20 mL) was treated with conc.H_2SO_4 (0.35 mL) at 0 °C and stirred for 3 h. The reaction mixture was poured into a saturated aqueous NaOAc solution (50 mL) and extracted with $CHCl_3$ (3 × 20 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated under high vacuum to give the crude product. Column chromatography (eluent: 30% EtOAc in hexane) on silica-gel yielded **111** (1.5 g, 81% over 2 steps) as a

colorless syrup with spectral data consistent with the literature.[207] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (d, *J* = 2.2 Hz, 1H), 5.42 (s, 1H), 5.29 – 5.19 (m, 2H), 4.27 (d, *J* = 6.4 Hz, 1H), 3.99 (d, *J* = 6.5 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 1.96 (d, *J* = 2.5 Hz, 3H), 1.95 (d, *J* = 3.9 Hz, 3H), 1.93 (s, 3H).

Phenyl (6*S*)-[6-^{2}*H*$_1$]-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (112).

To a stirred solution of (6*S*)-[6-^{2}*H*$_1$]-1,2,3,4,6-penta-*O*-acetyl-α-D-galactopyranose **111** (1.5 g, 3.83 mmol) and thiophenol (047 mL, 4.59 mmol) in CH_2Cl_2 was added BF3·Et2O (1.9 mL, 7.66 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 22 h, then quenched with aqueous saturated $NaHCO_3$ solution (50 mL) and extracted with CH2Cl2 (3 × 25 mL), dried over Na_2SO4, and concentrated under high vacuum. The crude product was purified on silica-gel column (eluent: 20% EtOAc in hexane) chromatography to give **112**[388] (900 mg, 56%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.48 (m, 2H), 7.30 – 7.28 (m, 3H), 5.40 (d, *J* = 2.8 Hz, 1H), 5.22 (t, *J* = 10.0 Hz, 1H), 5.03 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 1H), 4.17 (d, *J* = 6.5 Hz, 1H), 3.92 (d, *J* = 6.8 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.2, 170.0, 169.4, 132.5, 132.4, 128.9, 128.1, 86.6, 74.3, 72.0, 67.2, 61.3 (t, *J* = 22.2 Hz), 20.8, 20.6, 20.6, 20.6; HRMS (ESI) m/z calcd for $C_{20}H_{23}DO_9SNa$ $[M+Na]^+$, 464.1101; found, 464.1114.

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-(6*S*)-[6-^{2}*H*$_1$]-1-thio-β-D-galactopyranoside (114).

To a stirred solution of **112** (0.9 g, 2.04 mmol) in MeOH (10.0 mL) was added Na metal (catalytic amount) slowly. The reaction mixture was stirred for 2 h, then neutralized with Amberlite® IR120, filtered, and concentrated under reduced pressure to give a crude

thiogalactoside. The crude residue was dissolved in dry CH_3CN (30 mL) and treated with benzaldehyde dimethylacetal (0.41 mL, 2.75 mmol) followed by camphorsulfonic acid (4.2 mg, 0.18 mmol) at room temperature. The reaction mixture was stirred for 2.5 h, then neutralized with triethylamine (0.5 mL), and concentrated under reduced pressure to give a crude compound **113**. After filtration through a short silica-gel column, compound **113** (0.52 g) was dissolved in dry DMF (10 mL) then treated with NaH (0.17 g, 4.15 mmol) and BnBr (2.27 g, 1.6 mL, 13.3 mmol) at 0 ^oC. The resulting solution was warmed to room temperature and stirred for 0.5 h. Upon completion of the reaction (TLC), the excess NaH was quenched using sat. NH_4Cl solution. The product was extracted with EtOAc (3 × 25 mL), the combined organic layer was washed with brine (10 mL), dried over Na_2SO_4, and concentrated under high vacuum. The crude product was purified on silica-gel column chromatography (30% EtOAc in hexane) to give **114** (0.63 g, 81%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.71 (m, 2H), 7.55 – 7.53 (m, 2H), 7.43 – 7.18 (m, 16H), 5.50 (s, 1H), 4.74 – 4.68 (m, 4H), 4.62 (d, *J* = 9.5 Hz, 1H), 4.16 (d, *J* = 3.2 Hz, 1H), 3.98 (br s, 1H), 3.91 (t, *J* = 9.4 Hz, 1H), 3.63 (dd, *J* = 9.2, 3.3 Hz, 1H), 3.42 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.6, 138.2, 138.0, 132.9, 132.8, 129.2, 129.0, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.6, 126.8, 101.5, 86.7, 8.5, 75.6, 75.5, 73.8, 72.0, 69.9, 69.3 (t, *J* = 21.7 Hz). HRMS (ESI) m/z calcd for $C_{33}H_{31}DO_5SNa$ $[M+Na]^+$, 564.1931; found, 564.1927.

Ethyl (6*S*)-[6-$^2H_1$]-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (116).

Compound **115**[150] (0.1 g, 0.346 mmol) was dissolved in anhydrous dichloroethane (10.5 mL) and treated with (ethylthio)trimethylsilane (167 µL, 1.03 mmol) followed by ZnI_2 (332 mg, 1.03 mmol). The reaction mixture was stirred under an argon atmosphere at

room temperature for 3 h before it was diluted with ethyl acetate and filtered through Celite. The organic layer was washed with aqueous saturated $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude residue was dissolved in a mixture of THF:H_2O (1:1, 20 mL), and treated with K_2CO_3 (200 mg, 1.44 mmol) and stirred for 15 min. The reaction mixture was then diluted with ethyl acetate and washed with water, brine, and treated with anhydrous Na_2SO_4 before it was concentrated to dryness. Then the crude residue was passed through a short pad of silica gel, eluting with hexane/ethyl acetate (1:1), and the eluent was concentrated to dryness. The residue (60 mg) was dissolved in pyridine (1.4 mL), treated with acetic anhydride (0.7 mL), and stirred overnight under an argon atmosphere at room temperature. The reaction mixture was then concentrated to dryness and the crude reaction mixture was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (7:3), to obtain **116** (0.051 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.22 (t, *J* = 9.4 Hz, 1H), 5.08 (dd, *J* = 10.0, 9.4 Hz, 1H), 5.03 (dd, *J* = 10.0, 9.4 Hz, 1H), 4.49 (d, *J* = 10.0 Hz, 1H), 4.22 (d, *J* = 5.0 Hz, 1H), 3.70 (dd, *J* = 10.0, 5.0 Hz, 1H), 2.78 – 2.63 (m, 2H), 2.07 (s, *J* = 2.8 Hz, 3H), 2.06 (s, 3H), 2.02 (s, *J* = 3.5 Hz, 3H), 2.00 (s, *J* = 3.1 Hz, 3H), 1.27 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.2, 169.4, 83.5, 75.9, 73.9, 69.8, 68.3, 61.9 (t, *J* = 22.8 Hz), 24.2, 20.7, 20.6, 20.6, 14.8. HRMS (ESI) m/z calcd for $C_{16}H_{23}DO_9SNa$ $[M+Na]^+$, 416.1102; found, 416.1098.

Phenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (117).[229]

Compound **13** was synthesized as described in the literature.[228] NMR (600 MHz, $CDCl_3$) δ 7.60 – 7.58 (m, 2H), 7.42 – 7.40 (m, 2H), 7.37 – 7.25 (m, 16H), 5.65 (br s, 1H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.77 (d, *J* = 10.3 Hz, 1H), 4.75 (d, *J* = 10.0 Hz, 1H), 4.71 – 4.67

(m, 1H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.50 (d, *J* = 11.0 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 3.77 – 3.76 (m, 1H), 3.67 – 3.66 (m, 2H), 3.64 (dd, *J* = 9.5, 6.0 Hz, 1H), 3.55 (dd, *J* = 9.5, 6.7 Hz, 1H), 2.09 (s, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.65 – 7.61 (m, 2H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.31 (d, *J* = 7.3 Hz, 2H), 7.25 (d, *J* = 7.4 Hz, 2H), 7.17 – 7.01 (m, 10H), 6.99 – 6.91 (m, 2H), 5.58 (dd, *J* = 3.3, 1.1 Hz, 1H), 4.80 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 10.7 Hz, 1H), 4.66 (d, *J* = 11.2 Hz, 1H), 4.56 (d, *J* = 9.7 Hz, 1H), 4.28 (d, *J* = 11.6 Hz, 2H), 4.17 (d, *J* = 11.9 Hz, 1H), 3.80 (t, *J* = 9.5 Hz, 1H), 3.47 (dd, *J* = 9.4, 6.1 Hz, 1H), 3.42 (dd, *J* = 9.4, 6.5 Hz, 1H), 3.33 (dd, *J* = 9.2, 3.3 Hz, 1H), 3.32 (ddd, *J* = 6.5, 6.1, 1.1 Hz, 1H), 1.65 (s, 3H). IR (neat) *v* 1742 cm^{-1} (C=O).

Phenyl 4-*O*-acetyl-(6*S*)-[6-^{2}*H*$_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-117).

Compound **6S-D-117** (0.021 g, 81%) was synthesized from **6S-D-105** analogously to **13**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 – 7.57 (m, 2H), 7.41 – 7.23 (m, 18H), 5.63 (d, *J* = 1.6 Hz, 1H), 4.79 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.72 – 4.68 (m, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.0 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 3.76 (d, *J* = 6.1 Hz, 1H), 3.68 – 3.66 (m, 2H), 3.61 (d, *J* = 6.0 Hz, 1H), 2.10 (s, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.65 – 7.62 (m, 2H), 7.40 (d, *J* = 7.6 Hz, 2H), 7.31 (d, *J* = 7.5 Hz, 2H), 7.24 (t, *J* = 10.8 Hz, 2H), 7.18 – 6.91 (m, 12H), 5.58 (d, *J* = 3.2 Hz, 1H), 4.80 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 10.7 Hz, 1H), 4.66 (d, *J* = 11.2 Hz, 1H), 4.56 (d, *J* = 9.7 Hz, 1H), 4.28 (d, *J* = 11.4 Hz, 2H), 4.17 (d, *J* = 11.9 Hz, 1H), 3.79 (t, *J* = 9.4 Hz, 1H), 3.47 – 3.45 (m, 1H), 3.35 – 3.26 (m, 1H), 1.65 (s, 3H).

HRMS (ESI) m/z calcd for $C_{35}H_{35}DO_6SNa$ $[M+Na]^+$, 608.2193; found, 608.2191.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-pivolyl-1-thio-β-D-galactopyranoside (118).[229]

A solution of **105** (0.050 g, 2.77 mmol) in dry pyridine (0.5 mL) was treated with pivaloyl chloride (0.25 mL) at room temperature and stirred at 80 °C for 1 h. The solvents were evaporated under high vacuum to give a crude residue. The crude product was purified by silica gel column chromatography (eluent: 20 % ethyl acetate in hexane) to afford **118** (0.045 g, 78%) as a colorless oil. $[\alpha]_D^{22}$ = +6.6 (*c* 0.50, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.57 (m, 2H), 7.37 – 7.24 (m, 18H), 5.63 (d, *J* = 2.9 Hz, 1H), 4.73 (d, *J* = 10.6 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.67 (d, *J* = 10.3 Hz, 1H), 4.63 (d, *J* = 9.5 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.45 (d, *J* = 11.0 Hz, 1H), 3.81 (t, *J* = 6.3 Hz, 1H), 3.65 (dd, *J* = 9.2, 3.3 Hz, 1H), 3.62 (dd, *J* = 9.5, 6.1 Hz, 1H), 3.57 (t, *J* = 9.2 Hz, 1H), 3.49 (dd, *J* = 9.5, 6.7 Hz, 1H), 1.14 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.4, 138.1, 137.8, 137.6, 133.2, 132.3, 128.8, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.4, 87.0, 81.4, 76.3, 76.1, 75.6, 73.7, 71.6, 68.2, 66.3, 39.0, 27.1. ^{1}H NMR (600 MHz, C_6D_6) δ 7.66 – 7.63 (m, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.30 – 7.24 (m, 4H), 7.18 – 6.91 (m, 12H), 5.56 (d, *J* = 3.2 Hz, 1H), 4.78 (d, *J* = 10.8 Hz, 1H), 4.76 (d, *J* = 10.9 Hz, 1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.49 (d, *J* = 9.9 Hz, 1H), 4.30 (d, *J* = 11.9 Hz, 1H), 4.22 (d, *J* = 11.9 Hz, 1H), 4.20 (d, *J* = 11.8 Hz, 1H), 3.69 (t, *J* = 9.4 Hz, 1H), 3.49 (dd, *J* = 9.2, 6.0 Hz, 1H), 3.42 (dd, *J* = 9.2, 6.8 Hz), 3.37 – 3.35 (m, 1H), 3.27 (dd, *J* = 9.0, 3.2 Hz, 1H), 1.08 (s, 9H). IR (neat) *v* 1732 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{38}H_{42}O_6SNa$ $[M+Na]^+$, 649.2600; found, 649.2584.

Phenyl 4-*O*-pivaloyl-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-118).

Compound **6S-D-118** (0.011 g, 90%) was synthesized from **6S-D-105** analogously to **118**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.57 (m, 2H), 7.37 – 7.23 (m, 18H), 5.63 (d, *J* = 3.1 Hz, 1H), 4.73 (d, *J* = 10.4 Hz, 1H), 4.70 (d, *J* = 10.4 Hz, 1H), 4.66 (d, *J* = 10.3 Hz, 1H), 4.64 (d, *J* = 9.7 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.48 (d, *J* = 11.8, 1H), 4.44 (d, *J* = 11.0 Hz, 1H), 3.80 (d, *J* = 6.1 Hz, 1H), 3.64 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.60 (d, *J* = 6.1 Hz, 1H), 3.56 (t, *J* = 9.3 Hz, 1H), 1.14 (s, 9H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.66 – 7.6 (m, 2H), 7.40 (d, *J* = 7.1 Hz, 2H), 7.30 – 7.25 (m, 4H), 7.17 – 6.91 (m, 12H), 5.56 (d, *J* = 3.2 Hz, 1H), 4.79 (d, *J* = 10.9 Hz, 1H), 4.76 (d, *J* = 10.9 Hz, 1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.49 (d, *J* = 9.7 Hz, 1H), 4.30 (d, *J* = 11.9 Hz, 1H), 4.21 (d, *J* = 11.5 Hz, 1H), 4.20 (d, *J* = 11.5 Hz, 1H), 3.70 (t, *J* = 9.4 Hz, 1H), 3.47 (d, *J* = 6.0 Hz, 1H), 3.36 (d, *J* = 5.9 Hz, 1H), 3.27 (dd, *J* = 9.0, 3.3 Hz, 1H), 1.08 (s, 9H). HRMS (ESI) m/z calcd for $C_{38}H_{41}DO_6SNa$ $[M+Na]^+$, 650.2663; found, 650.2662.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-trifluoroacetyl-1-thio-β-D-galactopyranoside (119).[229]

To a stirred solution of **105** (0.05 g, 0.092 mmol) in dry CH_2Cl_2 (1.0 mL) were added pyridine (0.030 mL, 0.18 mmol), trifluoroacetic anhydride (0.026 mL, 0.18 mmol), and DMAP (1.0 mg, 0.009 mmol) at 0 ^{o}C. The reaction mixture stirred was stirred for 0.5 h before it was quenched with water (5 mL) and extracted with CH_2Cl_2 (3 X 10 mL). The combined extracts were washed with 1 N HCl (5.0 mL) solution, dried over Na_2SO_4, and concentrated under high vacuum. Silica gel column chromatography (eluent: 10% ethyl acetate in hexane) afforded **119** (0.038 g, 65%) as a colorless oil. $[\alpha]_D^{22}$ = −6.9 (*c* 0.80, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7.52 (m, 2H), 7.37 – 7.23 (m, 18H), 5.74

(dd, *J* = 3.2, 0.9 Hz, 1H), 4.75 (d, *J* = 11.2 Hz, 1H), 4.68 (d, *J* = 10.2 Hz, 1H), 4.65 (d, *J* = 9.5 Hz, 1H), 4.64 (d, *J* = 9.9 Hz, 1H), 4.52 (d, *J* = 11.3 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 3.82 (ddd, *J* = 8.2, 5.6, 1.0 Hz, 1H), 3.70 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.67 (dd, *J* = 9.3, 5.6 Hz, 1H), 3.61 (t, *J* = 9.5 Hz, 1H), 3.49 (dd, *J* = 9.3, 8.3 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.8 (q, *J* = 42.7 Hz, *C*OCF3), 137.9, 137.1, 137.1, 132.8, 132.1, 128.9, 128.5, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 114.5 (q, *J* = 285.8 Hz, *C*F_3CO), 87.4, 80.5, 76.0, 75.7, 74.7, 73.8, 72.3, 71.3, 67.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −74.7 (COC*F*$_3$). ^{1}H NMR (600 MHz, C_6D_6) δ 7.59 (d, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 7.4 Hz, 2H), 7.19 – 6.91 (m, 16H), 5.57 (d, *J* = 3.1 Hz, 1H), 4.63 (d, *J* = 10.7 Hz, 1H), 4.53 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.37 (d, *J* = 9.5 Hz, 1H), 4.19 (d, *J* = 11.4 Hz, 1H), 4.15 (d, *J* = 11.7 Hz, 1H), 4.06 (d, *J* = 11.7 Hz, 1H), 3.71 (t, *J* = 9.4 Hz, 1H), 3.34 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.31 (dd, *J* = 9.1, 8.1 Hz, 1H), 3.24 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.19 – 3.15 (m, 1H). IR (neat) *v* 1790 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{35}H_{33}O_6F_3SNa$ $[M+Na]^+$, 661.1848; found, 661.1844.

Phenyl 4-*O*-trifluoroacetyl-(6*S*)-[6-^{2}*H*$_1$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-119).

Compound **6S-D-119** (0.021 g, 87%) was synthesized from **6S-D-105** analogously to **119**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7. 52 (m, 2H), 7.37 – 7.24 (m, 18H), 5.74 (d, *J* = 3.0 Hz, 1H), 4.75 (d, *J* = 11.2 Hz, 1H), 4.68 (d, *J* = 10.2 Hz, 1H), 4.65 (d, *J* = 9.5 Hz, 1H), 4.64 (d, *J* = 9.9 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.50 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 11.6 Hz, 1H), 3.81 (d, *J* = 5.6 Hz, 1H), 3.70 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.65 (d, *J* = 5.6 Hz, 1H), 3.61 (t, *J* = 9.4 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.61 – 7.58 (m, 2H), 7.33 (d, *J* = 7.2 Hz, 2H), 7.21 – 6.92 (m, 16H), 5.58 (d, *J* = 3.0 Hz, 1H), 4.63 (d, *J* = 10.8 Hz,

1H), 4.53 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 11.4 Hz, 1H), 4.38 (d, *J* = 9.7 Hz, 1H), 4.19 (d, *J* = 11.4 Hz, 1H), 4.16 (d, *J* = 11.8 Hz, 1H), 4.06 (d, *J* = 11.8 Hz, 1H), 3.71 (t, *J* = 9.4 Hz, 1H), 3.32 (d, *J* = 5.6 Hz, 1H), 3.25 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.17 (d, *J* = 5.7 Hz, 1H). HRMS (ESI) m/z calcd for $C_{35}H_{32}F_3DO_6SNa$ $[M+Na]^+$, 662.1910; found, 662.1905.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-trichloroacetyl-1-thio-β-D-galactopyranoside (120).[229]

Compound **120** (0.058 g, 92%) was synthesized from **105** analogously to **119**, as a colorless oil. $[\alpha]^{22}{}_D$ = −8.0 (*c* 1.80, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.52 (m, 2H), 7.39 – 7.21 (m, 18H), 5.71 (d, *J* = 2.9 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.65 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 9.9 Hz, 1H), 4.62 (d, *J* = 10.3 Hz, 1H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.51 (d, *J* = 11.4 Hz, 1H), 4.49 (d, *J* = 11.4 Hz, 1H), 3.87 (dd, *J* = 7.9, 6.0 Hz, 1H), 3.74 – 3.72 (m, 1H), 3.71 (dd, *J* = 9.2, 5.7 Hz, 1H), 3.68 (t, *J* = 9.3 Hz, 1H), 3.59 (dd, *J* = 9.2, 8.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 137.9, 137.3, 137.3, 132.6, 132.3, 129.0, 128.6, 128.3, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 89.9, 87.1, 80.9, 75.6, 75.6, 75.2, 73.9, 72.2, 72.1, 67.3. ^{1}H NMR (600 MHz, C_6D_6) δ 7.62 – 7.60 (m, 2H), 7.33 (d, *J* = 7.4 Hz, 2H), 7.24 – 7.19 (m, 4H), 7.16 – 7.07 (m, 11H), 6.95 – 6.91 (m, 1H), 5.53 (d, *J* = 3.0 Hz, 1H), 4.69 (d, *J* = 10.8 Hz, 1H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.38 (d, *J* = 9.9 Hz, 1H), 4.23 (d, *J* = 11.7 Hz, 1H), 4.19 (d, *J* = 11.4 Hz, 1H), 4.18 (d, *J* = 11.7 Hz, 1H), 3.79 (t, *J* = 9.4 Hz, 1H), 3.46 (m, 1H), 3.43 (m, 1H), 3.29 – 3.28 (m, 1H), 3.28 – 3.25 (m, 1H). IR (neat) *v* 1769 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{35}H_{33}O_6Cl_3SNa$ $[M+Na]^+$, 709.0961; found, 709.0955.

Phenyl 4-*O*-trichloroacetyl-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-120).

Compound **6S-D-120** (0.029 g, 90%) was synthesized from **6S-D-105** analogously to **105**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.52 (m, 2H), 7.39 – 7.21 (m, 18H), 5.70 (d, *J* = 2.9 Hz, 1H), 4.79 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 10.9 Hz, 1H), 4.63 (d, *J* = 9.9 Hz, 1H), 4.62 (d, *J* = 10.6 Hz, 1H), 4.53 (t, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.49 (d, *J* = 11.4 Hz, 1H), 3.86 (d, *J* = 5.7 Hz, 1H), 3.72 (dd, *J* = 9.1, 3.0 Hz, 1H), 3.69 (d, *J* = 5.7, 1H, H), 3.68 (t, *J* = 9.3 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.63 – 7.59 (m, 2H), 7.33 (d, *J* = 7.4 Hz, 2H), 7.23 – 7.20 (m, 4H), 7.17 – 6.90 (m, 12H), 5.53 (d, *J* = 3.0 Hz, 1H), 4.69 (d, *J* = 10.8 Hz, 1H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.52 (d, *J* = 11.3 Hz, 1H), 4.39 (d, *J* = 9.7 Hz, 1H H-1), 4.22 (d, *J* = 11.7 Hz, 1H), 4.19 (d, *J* = 11.4 Hz, 1H), 4.17 (d, *J* = 11.8 Hz, 1H), 3.79 (t, *J* = 9.4 Hz, 1H), 3.43 (d, *J* = 5.7 Hz, 1H, H), 3.29 – 3.26 (m, 2H). HRMS (ESI) m/z calcd for $C_{35}H_{32}Cl_3DO_6SNa$ $[M+Na]^+$, 710.1024; found, 710.1016.

Phenyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (121).[229]

Compound **121** was synthesized as described in the literature.[228] ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 – 7.93 (m, 2H), 7.66 – 7.65 (m, 2H), 7.61 – 7.59 (m, 1H), 7.47 – 7.45 (m, 2H), 7.40 – 7.39 (m, 2H), 7.35 – 7.22 (m, 16H), 5.91 (d, *J* = 3.0 Hz 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.74 (br s, 2H), 4.70 (d, *J* = 9.5 Hz, 1H), 4.54 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 10.6 Hz, 1H), 4.46 (d, *J* = 10.6 Hz, 1H), 3.92 – 3.89 (m, 1H), 3.77 (d, *J* = 9.2, 3.3 Hz, 1H), 3.72 (t, *J* = 9.4 Hz, 1H), 3.69 (dd, *J* = 9.6, 5.8 Hz, 1H), 3.59 (dd, *J* = 9.6, 6.8 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.11 (d, *J* = 8.1 Hz, 2H), 7.70 (dd, *J* = 12.2, 5.2 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.20 (d, *J* = 7.9 Hz, 2H), 7.17 – 6.95 (m, 15H), 5.87 (d, *J* = 3.1 Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.67

(d, *J* = 10.9 Hz, 1H), 4.54 (d, *J* = 9.7 Hz, 1H), 4.31 (d, *J* = 11.4 Hz, 1H), 4.19 (d, *J* = 11.8 Hz, 1H), 4.12 (d, *J* = 11.9 Hz, 1H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.53 (dd, *J* = 9.3, 6.1 Hz, 1H), 3.49 (dd, *J* = 9.3, 6.6 Hz, 1H), 3.42 (t, *J* = 4.8 Hz, 1H), 3.42 – 3.40 (m, 1H). IR (neat) *v* 1722 cm^{-1} (C=O).

Phenyl 4-*O*-benzoyl-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-121).

Compound **6S-D-121** (0.021 g, 84%) was synthesized analogously as **121** from **6S-D-105**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 – 7.99 (m, 2H), 7.67 – 7.64 (m, 2H), 7.61 – 7.59 (m, 1H), 7.47 – 7.45 (m, 2H), 7.40 – 7.39 (m, 2H), 7.37 – 7.21 (m, 16H), 5.91 (d, *J* = 3.1 Hz, 1H), 4.87 (d, *J* = 11.2 Hz, 1H), 4.74 (br s, 2H), 4.71 (d, *J* = 9.5 Hz, 1H), 4.54 (d, *J* = 11.2 Hz, 1H), 4.52 (d, *J* = 11.8 Hz, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 3.90 (d, *J* = 6.0 Hz, 1H), 3.77 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.72 (t, *J* = 9.3 Hz, 1H), 3.69 (d, *J* = 6.0 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.11 (d, *J* = 7.7 Hz, 2H), 7.70 (d, *J* = 7.3 Hz, 2H), 7.39 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 7.4 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 2H), 7.14 (t, *J* = 7.6 Hz, 2H), 7.09 – 6.94 (m, 13H), 5.87 (d, *J* = 3.1 Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.67 (d, *J* = 10.9 Hz, 1H), 4.54 (d, *J* = 9.7 Hz, 1H), 4.31 (d, *J* = 11.4 Hz, 1H), 4.19 (d, *J* = 11.9 Hz, 1H), 4.12 (d, *J* = 11.9 Hz, 1H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.51 (d, *J* = 6.0 Hz, 1H), 3.42 – 3.40 (m, 2H). HRMS (ESI) m/z calcd for $C_{40}H_{37}DO_6SNa$ $[M+Na]^+$, 670.2350; found, 670.2344.

Phenyl 4-*O*-(p-methylbenzoyl)-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (122).[229]

Compound **122** (0.057 g, 95%) was synthesized from **105** analogously to **123**, as a colorless oil. $[\alpha]_D^{22}$ = +20.9 (*c* 0.55, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 – 7.88

(m, 2H), 7.64 – 7.63 (m, 2H), 7.38 – 7.21 (m, 20H), 5.87 (d, *J* = 3.0 Hz, 1H), 4.84 (d, *J* = 11.2 Hz, 1H), 4.71 (br s, 2H), 4.69 (d, *J* = 9.5 Hz, 1H), 4.53 (d, *J* = 11.0 Hz, 1H), 4.51 (d, *J* = 11.7 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 3.89 – 3.87 (m, 1H), 3.74 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.69 (t, *J* = 9.2 Hz, 1H), 3.68 (dd, *J* = 9.5, 5.9 Hz, 1H), 3.57 (dd, *J* = 9.5, 6.8 Hz, 1H), 2.44 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.7, 143.9, 138.3, 137.6, 132.9, 132.8, 130.0, 129.1, 128.8, 128.4, 128.3, 128.3, 128.2, 128.2, 127.9, 127.7, 127.7, 127.6, 127.0, 87.1, 81.4, 76.5, 76.4, 75.7, 73.7, 71.7, 68.5, 67.1, 21.7. ^{1}H NMR (600 MHz, C_6D_6) δ 8.09 (d, *J* = 8.0 Hz, 2H), 7.74 – 7.70 (m, 2H), 7.39 (d, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 7.6 Hz, 2H), 7.21 (t, *J* = 8.6 Hz, 2H), 7.15 (dd, *J* = 15.7, 8.0 Hz, 2H), 7.12 – 6.95 (m, 10H), 6.84 (d, *J* = 8.0 Hz, 2H), 5.88 (d, *J* = 3.2 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.67 (d, *J* = 10.9 Hz, 1H), 4.55 (d, *J* = 9.7 Hz, 1H), 4.32 (d, *J* = 11.8 Hz, 1H), 4.20 (d, *J* = 11.8 Hz, 1H), 4.14 (d, *J* = 10.8 Hz, 1H), 3.86 (t, *J* = 9.4 Hz, 1H), 3.56 (dd, *J* = 9.4, 6.2, Hz, 1H), 3.52 (dd, *J* = 9.4, 6.5 Hz, 1H), 3.45 – 3.43 (m, 1H), 3.42 – 3.41 (m, 1H) 1.90 (s, 3H). IR (neat) *v* 1720 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{41}H_{40}O_6SNa$ $[M+Na]^+$, 683.2443; found, 683.2446.

Phenyl 4-*O*-(p-methylbenzoyl)-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-122).

Compound **6S-D-122** (0.016 g, 65%) was synthesized from **6S-D-105** analogously to **122**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.1 Hz, 2H), 7.64 – 7.63 (m, 2H), 7.39 – 7.19 (m, 20H), 5.86 (d, *J* = 3.0 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.70 (br s, 2H), 4.68 (d, *J* = 9.5 Hz, 1H), 4.50 (d, *J* = 11.1 Hz, 1H), 4.49 (d, *J* = 11.8 Hz, 1H), 4.43 (d, *J* = 11.8 Hz, 1H), 3.87 (d, *J* = 5.9 Hz, 1H), 3.74 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.68 (t, *J* = 9.3 Hz, 1H), 3.66 (d, *J* = 6.0 Hz, 1H), 2.44 (s, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.09 (d, *J* = 8.2 Hz, 2H),

7.75 – 7.67 (m, 2H), 7.39 (d, *J* = 7.2 Hz, 2H), 7.28 (d, *J* = 7.2 Hz, 2H), 7.22 (d, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.6 Hz, 2H), 7.11 – 6.95 (m, 10H), 6.84 (d, *J* = 8.1 Hz, 2H), 5.88 (d, *J* = 3.1 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.67 (d, *J* = 10.8 Hz, 1H), 4.55 (d, *J* = 9.7 Hz, 1H), 4.32 (d, *J* = 11.4 Hz, 1H), 4.19 (d, *J* = 11.9 Hz, 1H), 4.14 (d, J = 11.9 Hz, 1H), 3.87 (t, *J* = 9.4 Hz, 1H), 3.54 (d, *J* = 6.2 Hz, 1H), 3.44 – 3.41 (m, 2H), 1.90 (s, 3H). HRMS (ESI) m/z calcd for $C_{41}H_{39}DO_6SNa$ $[M+Na]^+$, 684.2506; found, 684.2499.

Phenyl 4-*O*-(p-methoxybenzoyl)-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (123).[229]

To a stirred solution of **105** (0.050 g, 0.092 mmol) in dry pyridine (1.0 mL) was added 4-methoxy benzoyl chloride (0.025 mL, 0.184 mmol) and DMAP (1.0 mg, 0.009 mmol) at room temperature. The reaction mixture was stirred for 8 h before the solvents were evaporated under high vacuum to give a crude product, which was dissolved in CH_2Cl_2 (10 mL) and washed with 1 N HCl (5 mL) solution, followed by sat. $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated under high vacuum. Silica gel column chromatography (eluent: 20 % ethyl acetate in hexane) afforded **123**[127] (0.050 g, 80%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 – 7.95 (m, 2H), 7.66 – 7.62 (m, 2H), 7.40 – 7.39 (m, 2H), 7.36 – 7.20 (m, 16H), 6.94 – 6.92 (m, 2H), 5.87 (d, *J* = 3.0 Hz, 1H), 4.86 (d, *J* = 11.4 Hz, 1H), 4.74 (br s, 2H), 4.71 (d, *J* = 9.2 Hz, 1H), 4.53 (d, *J* = 11.0, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 3.89 (s, 3H), 3.89 – 3.87 (m, 1H), 3.75 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.71 (t, *J* = 9.2 Hz, 1H), 3.69 (dd, *J* = 9.6, 6.1 Hz, 1H), 3.59 (dd, *J* = 9.6, 6.5 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.11 (d, *J* = 8.7 Hz, 2H), 7.71 (d, *J* = 7.4 Hz, 2H), 7.40 (d, *J* = 7.4 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 7.23 (d, *J* = 7.3 Hz, 2H),

7.17 – 6.93 (m, 12H), 6.56 (d, *J* = 8.8 Hz, 2H), 5.88 (d, *J* = 3.0 Hz, 1H), 4.79 (d, *J* = 11.4 Hz, 1 H), 4.76 (d, *J* = 10.8 Hz, 1H), 4.71 (d, *J* = 10.8 Hz, 1H), 4.57 (d, *J* = 9.7 Hz, 1H), 4.33 (d, *J* = 11.4 Hz, 1H), 4.21 (d, *J* = 11.9 Hz, 1H), 4.15 (d, *J* = 11.8 Hz, 1H), 3.87 (t, *J* = 9.4 Hz, 1H), 3.57 (dd, *J* = 9.4, 6.2 Hz, 1H), 3.54 (dd, *J* = 9.4, 6.4 Hz, 1H), 3.47 – 3.41 (m, 2H), 3.08 (s, 3H). IR (neat) *v* 1728 cm^{-1} (C=O).

Phenyl 4-*O*-(p-methoxybenzoyl)-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-123).

Compound **6S-D-123** (0.009 g, 71%) was synthesized from **6S-D-105** analogously to **123**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 – 7.91 (m, 2H), 7.69 – 7.58 (m, 2H), 7.39 – 7.18 (m, 18H), 6.91 (d, *J* = 8.9 Hz, 2H), 5.85 (d, *J* = 3.1 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.72 (br s, 2H), 4.68 (d, *J* = 9.5 Hz, 1H), 4.51 (d, *J* = (dd, J = 11.6 Hz OCH_2Ph, 4.49 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.7 Hz, 1H), 3.89 (s, 3H), 3.87 – 3.85 (m, 1H), 3.73 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.69 (t, *J* = 9.3 Hz, 1H), 3.66 (d, *J* = 6.2 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.11 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 7.3 Hz, 2H), 7.40 (d, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 7.23 (d, *J* = 7.3 Hz, 2H), 7.17 – 6.93 (m, 12H), 6.55 (d, *J* = 8.8 Hz, 2H), 5.88 (d, *J* = 3.1 Hz, 1H), 4.79 (d, *J* = 11.4 Hz, 1H), 4.76 (d, *J* = 10.8 Hz, 1H), 4.71 (d, *J* = 10.8 Hz, 1H), 4.57 (d, *J* = 9.7 Hz, 1H), 4.33 (d, *J* = 11.4 Hz, 1H), 4.21 (d, *J* = 11.9 Hz, 1H), 4.15 (d, *J* = 11.9 Hz, 1H), 3.87 (t, *J* = 9.4 Hz, 1H), 3.55 (d, *J* = 6.1 Hz, 1H), 3.45 – 3.41 (m, 2H), 3.08 (s, 3H). HRMS (ESI) m/z calcd for $C_{41}H_{39}DO_7SNa$ $[M+Na]^+$, 700.2455; found, 700.2449.

Phenyl 4-*O*-(p-nitrobenzoyl)-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (124).[229]

Compound **124** (0.053 g, 84%) was synthesized from **105** analogously to **123**, as a colorless oil. $[\alpha]^{22}_{D}$ = +19.5 (*c* 1.95, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 – 8.24 (m, 2H), 8.07 – 8.06 (m, 2H), 7.65 – 7.63 (m, 2H), 7.39 – 7.19 (m, 18H), 5.90 (d, *J* = 2.3 Hz, 1H), 4.83 (d, *J* = 10.6 Hz, 1H), 4.78 (d, *J* = 10.3 Hz, 1H), 4.73 (d, *J* = 10.3 Hz, 1H), 4.70 (d, *J* = 9.5 Hz, 1H), 4.54 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 3.91 (t, *J* = 6.1 Hz, 1H), 3.78 (dd, *J* = 9.2, 3.3 Hz, 1H), 3.69 (dd, *J* = 9.5, 5.7 Hz, 1H), 3.64 (t, *J* = 9.4 Hz, 1H), 3.56 (dd, *J* = 9.5, 7.2 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.8, 150.6, 138.1, 137.3, 137.3, 135.1, 133.3, 132.5, 130.9, 128.8, 128.4, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 123.5, 87.1, 81.3, 76.5, 75.8, 75.7, 73.7, 72.1, 68.5, 67.9. ^{1}H NMR (600 MHz, C_6D_6) δ 7.70 – 7.65 (m, 4H), 7.59 – 7.55 (m, 2H), 7.41 (d, *J* = 7.3 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 2H), 7.15 (dd, *J* = 16.1, 7.8 Hz, 4H), 7.08 – 6.91 (m, 10H), 5.79 (d, *J* = 3.1 Hz, 1H), 4.85 (d, *J* = 10.7 Hz, 1H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.67 (d, *J* = 11.1 Hz, 1H), 4.52 (d, *J* = 9.7 Hz, 1H), 4.28 (d, *J* = 11.0 Hz, 1H), 4.24 (d, *J* = 11.0 Hz, 1H), 4.13 (d, *J* = 11.9 Hz, 1H), 3.73 (t, *J* = 9.3 Hz, 1H), 3.47 (dd, *J* = 8.9, 5.6 Hz, 1H), 3.42 (dd, *J* = 8.9, 7.0 Hz, 1H), 3.41 – 3.39 (m, 1H), 3.38 – 3.37 (m, 1H). IR (neat) *v* 1729 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{40}H_{37}NO_8SNa$ $[M+Na]^+$, 714.2138; found, 714.2120.

Phenyl 4-*O*-(p-nitrobenzoyl)-(6*S*)-[6-^{2}*H*$_1$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-124).

Compound **6S-D-124** (0.010 g, 83%) was synthesized from **6S-D-105** analogously to **124**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 – 8.24 (m, 2H), 8.07 – 8.06 (m, 2H), 7.65 –

7.64 (m, 2H), 7.44 – 7.18 (m, 18H), 5.90 (d, *J* = 3.1 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.78 (d, *J* = 10.8 Hz, 1H), 4.73 (d, *J* = 10.3 Hz, 1H), 4.70 (d, *J* = 9.7 Hz, 1H), 4.54 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.43 (d, *J* = 11.8 Hz, 1H), 3.91 (d, *J* = 5.7 Hz, 1H), 3.78 (dd, *J* = 9.1, 3.1 Hz, 1H), 3.67 (d, *J* = 5.7 Hz, 1H), 3.64 (t, *J* = 9.4 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.69 – 7.65 (m, 4H), 7.59 – 7.55 (m, 2H), 7.41 (d, *J* = 7.3 Hz, 2H), 7.25 (d, *J* = 7.3 Hz, 2H), 7.18 – 6.90 (m, 14H), 5.78 (d, *J* = 3.1 Hz, 1H), 4.85 (d, *J* = 10.7 Hz, 1H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.67 (d, *J* = 11.1 Hz, 1H), 4.51 (d, *J* = 9.7 Hz, 1H), 4.28 (d, *J* = 11.1 Hz, 1H), 4.24 (d, *J* = 12.0 Hz, 1H), 4.12 (d, *J* = 11.9 Hz, 1H), 3.72 (t, *J* = 9.4 Hz, 1H), 3.45 (d, *J* = 5.7 Hz, 1H, H), 3.40 – 3.35 (m, 2H). HRMS (ESI) m/z calcd for $C_{40}H_{36}NDO_8SNa$ $[M+Na]^+$, 715.2200; found, 715.2206.

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (125).

Compound **125** was synthesized as described in the literature.[216] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.56 (m, 2H), 7.39 – 7.27 (m, 21H), 7.20 – 7.18 (m, 2H), 4.98 (d, *J* = 11.7 Hz, 1H), 4.79 (d, *J* = 10.3 Hz, 1H), 4.75 (d, *J* = 11.7 Hz, 1H), 4.74 – 4.73 (m, 1H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.65 (d, *J* = 9.9 Hz, 1H), 4.61 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 3.99 (dd, *J* = 2.8, 1.0 Hz, 1H), 3.95 (t, *J* = 9.5 Hz, 1H), 3.67 (m, 1H), 3.65 (m, 1H), 3.63 – 3.62 (m, 1H), 3.61 (dd, *J* = 2.8, 9.2, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.70 – 7.67 (m, 2H), 7.37 (d, *J* = 7.2 Hz, 2H), 7.30 (d, *J* = 7.2 Hz, 2H), 7.21 (d, *J* = 7.2 Hz, 2H), 7.18 (d, *J* = 7.1 Hz, 2H), 7.14 – 7.09 (m, 8H), 7.05 (dt, *J* = 14.7, 7.4 Hz, 4H), 6.99 – 6.89 (m, 3H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.79 (d, *J* = 10.7 Hz, 1H), 4.64 (d, *J* = 10.8 Hz, 1H), 4.59 (d, *J* = 9.6 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.42 (d, *J* = 11.9 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.22 (d, *J* = 11.8 Hz, 1H), 4.16 (d, *J* = 11.8 Hz, 1H), 4.11 (t, *J* = 9.4 Hz, 1H), 3.78 (dd, *J* = 2.9, 1.1 Hz, 1H), 3.66 (dd, *J* = 9.1, 7.4 Hz, 1H), 3.57

(dd, *J* = 9.1, 5.7 Hz, 1H), 3.32 (ddd, *J* = 7.3, 5.7, 1.0 Hz, 1H), 3.29 (dd, *J* = 9.2, 2.8 Hz, 1H).

Phenyl (6*S*)-[6-$^{2}H_{1}$]-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (6*S*-D-125).

Compound **6S-D-125** (0.012 g, 70%) was synthesized from **6S-D-105** analogously to **125**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 – 7.56 (m, 2H), 7.40 – 7.26 (m, 21H), 7.21 – 7.16 (m, 2H), 4.97 (d, *J* = 11.5 Hz, 1H), 4.79 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 11.7 Hz, 1H), 4.74 – 4.73 (m, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.65 (d, *J* = 9.7 Hz, 1H), 4.61 (d, *J* = 11.5 Hz, 1H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.42 (d, *J* = 11.7 Hz, 1H), 3.99 (d, *J* = 2.6 Hz, 1H), 3.94 (t, *J* = 9.5 Hz, 1H), 3.65 (d, *J* = 5.8 Hz, 1H, H), 3.62 – 3.61 (m, 2H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.70 – 7.67 (m, 2H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 7.5 Hz, 2H), 7.21 – 7.16 (m, 4H), 7.15 – 7.02 (m, 12H), 6.99 – 6.89 (m, 3H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.79 (d, *J* = 10.7 Hz, 1H), 4.64 (d, *J* = 10.8 Hz, 1H), 4.59 (d, *J* = 9.6 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.42 (d, *J* = 11.9 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.22 (d, *J* = 11.9 Hz, 1H), 4.16 (d, *J* = 11.8 Hz, 1H), 4.11 (t, *J* = 9.4 Hz, 1H), 3.78 (d, *J* = 2.6 Hz, 1H), 3.55 (d, *J* = 5.6 Hz, 1H), 3.33 – 3.26 (m, 2H). HRMS (ESI) m/z calcd for $C_{40}H_{39}DO_5SNa$ $[M+Na]^+$, 656.2557; found, 656.2548.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(1′,1′,1′-trifluoroethyl)-1-thio-β-D-galactopyranoside (126).[229]

To a solution of phenyl 2,3,6-tri-*O*-benzyl-1-thio-D-glucopyranoside **106** (0.05 g, 0.092 mmol) in dry CH_2Cl_2 was added pyridine (0.03 mL, 0.37 mmol) and trifluoromethanesulfonic anhydride (0.02 mL, 0.12 mmol) at 0 °C. The reaction mixture was stirred for 1 h before it was quenched with water. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with brine (5.0 mL), dried over Na_2SO_4, and concentrated

under high vacuum. Without further purification, the triflate was dissolved in dry DMF (1.0 mL) and freshly prepared sodium trifluoroethoxide (0.03 g, 0.27 mmol) was added at room temperature. The reaction mixture was stirred for 4 h at room temperature before TLC (10% ethyl acetate in hexane) showed completion. The reaction mixture was quenched with saturated aqueous NH_4Cl solution, extracted with ethyl acetate (2 X 10 mL), dried over Na_2SO_4, and concentrated. Silica gel column chromatography (eluent: 20 % EtOAc in hexane) afforded **126** (0.035 g, 61% over 2 steps) as a semi-solid. $[\alpha]^{D}_{22}$ = −11.0 (*c* 0.55, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7.55 (m, 2H), 7.37 – 7.21 (m, 18H), 4.79 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 11.7 Hz, 1H), 4.68 – 4.67 (m, 1H), 4.66 (d, *J* = 10.3 Hz, 1H), 4.61 (d, *J* = 9.5 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.7 Hz, 1H), 4.28 (dq, *J* = 12.1, 8.9 Hz, 1H), 3.93 (d, *J* = 2.2 Hz, 1H), 3.92 – 3.88 (m, 1H), 3.85 (t, *J* = 9.5 Hz, 1H), 3.74 (d, *J* = 9.2, 7.7 Hz, 1H), 3.69 (dd, *J* = 9.2, 5.5 Hz, 1H), 3.61 – 3.59 (m, 1H), 3.57 (dd, *J* = 9.2, 2.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.1, 137.8, 137.7, 133.8, 131.5, 129.7, 128.9, 128.8, 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.9, 127.8, 127.7, 127.5, 127.2, 124.7, 122.8, 87.7, 83.7, 77.1, 76.6, 76.0, 75.6, 73.6, 73.4, 69.4 (q, *J* = 34.1 Hz, CF_3CH_2), 68.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −74.7 (t, *J* = 8.7 Hz, CF_3CH_2O). ^{1}H NMR (600 MHz, C_6D_6) δ 7.66 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 7.6 Hz, 2H), 7.19 (d, *J* = 7.6 Hz, 2H), 7.16 – 7.00 (m, 13H), 6.93 (dd, *J* = 10.6, 4.2 Hz, 1H), 4.80 (d, *J* = 10.7 Hz, 1H), 4.54 (d, *J* = 10.7 Hz, 1H), 4.48 (d, *J* = 9.9 Hz, 1H), 4.38 (d, *J* = 11.1 Hz, 1H), 4.23 – 4.19 (m, 3H), 4.14 (dt, *J* = 17.8, 8.9 Hz, 1H), 3.95 (t, *J* = 9.4 Hz, 1H), 3.68 (dd, *J* = 9.1, 7.6 Hz, 1H), 3.66 – 3.60 (m, 1H), 3.57 (d, *J* = 1.7 Hz, 1H), 3.55 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.22 – 3.18 (m, 1H), 3.15 (dd, *J* = 9.2, 2.6 Hz, 1H). HRMS (ESI) m/z calcd for $C_{35}H_{35}O_5F_3SNa$ $[M+Na]^+$, 647.2055; found, 647.2067.

Phenyl (6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-4-*O*-(1′,1′,1′-trifluoroethyl)-1-thio-β-D-galactopyranoside (6*S*-D-126).

Compound **6S-D-126** (0.005 g, 30% over 2 steps) was synthesized from **6S-D-106** analogously to **126**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 – 7.55 (m, 2H), 7.37 – 7.21 (m, 18H), 4.78 (d, *J* = 10.1 Hz, 1H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 11.8 Hz, 1H), 4.65 (d, *J* = 10.2 Hz, 1H), 4.60 (d, *J* = 9.7 Hz, 1H), 4.51 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.27 (dq, *J* = 12.0, 9.0 Hz, 1H), 3.93 (d, *J* = 2.3 Hz, 1H), 3.92 – 3.87 (m, 1H), 3.84 (t, *J* = 9.4 Hz, 1H), 3.66 (d, *J* = 5.5 Hz, 1H, H), 3.60 – 3.59 (m, 1H), 3.57 (dd, *J* = 9.5, 2.6 Hz, 1H). ^{1}H NMR (600 MHz, C_6D6) δ 7.67 (d, *J* = 7.4 Hz, 2H), 7.35 (d, *J* = 7.4 Hz, 2H), 7.21 -7.19 (m, 2H), 7.16 – 6.90 (m, 14H), 4.80 (d, *J* = 10.7 Hz, 1H), 4.54 (d, *J* = 10.6 Hz, 1H), 4.48 (d, *J* = 9.6 Hz, 1H), 4.37 (d, *J* = 11.8 Hz, 1H), 4.22 – 4.19 (m, 3H), 4.18 – 4.10 (m, 1H), 3.95 (t, *J* = 9.4 Hz, 1H), 3.66 – 3.60 (m, 1H), 3.57 (d, *J* = 2.3 Hz, 1H), 3.53 (d, *J* = 5.6 Hz, 1H, H), 3.19 (d, *J* = 6.0 Hz, 1H), 3.15 (dd, *J* = 9.2, 2.7 Hz, 1H). HRMS (ESI) m/z calcd for $C_{35}H_{34}DF_3O_5SNa$ $[M+Na]^+$, 648.2118; found, 648.2102.

Phenyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (127).

Compound **127** was synthesized as described in the literature.[389] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.55 (m, 2H), 7.41 – 7.21 (m, 18H), 5.01 (dd, *J* = 9.4, 8.7 Hz, 1H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.70 (d, *J* = 10.3 Hz, 1H), 4.69 (d, *J* = 9.8 Hz, 1H), 4.64 (d, *J* = 11.4 Hz, 1H), 4.51 (s, 2H), 3.67 (dd, *J* = 9.4, 8.8 Hz, 1H), 3.61 – 3.57 (m, 3H), 3.55 (dd, *J* = 9.8, 8.8 Hz, 1H), 1.85 (s, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.69 – 7.64 (m, 2H), 7.43 – 7.39 (m, 2H), 7.33 – 7.29 (m, 2H), 7.28 – 7.24 (m, 2H), 7.21 – 6.94 (m, 12H), 5.33 (dd, *J* = 10.6, 9.2 Hz, 1H), 4.86 (d, *J* = 10.9 Hz, 1H), 4.76 (d, *J* = 11.8 Hz, 1H), 4.64 (d, *J* = 9.6 Hz, 1H), 4.59 – 4.57 (m, 2H), 4.31 (s, 2H), 3.58 (dd, *J* =

10.6, 3.2 Hz, 1H), 3.54 (dd, *J* = 10.6, 5.8 Hz, 1H), 3.52 (dd, *J* = 9.2, 8.7 Hz, 1H), 3.48 (dd, *J* = 9.6, 8.7 Hz, 1H), 3.36 (ddd, *J* = 10.1, 5.8, 3.2 Hz, 1H), 1.54 (s, 3H). IR (neat) *v* 1745 cm^{-1} (C=O).

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-trifluoroacetyl-1-thio-β-D-glucopyranoside (128).

Compound **106** (0.009 g, 0.016 mmol) was dissolved in anhydrous pyridine (0.05 ml) and cooled to 0 °C before the addition of trifluoroacetic anhydride (4.5 µl, 0.032 mmol). The reaction mixture was then stirred for 4 h under an argon atmosphere and concentrated to dryness. The crude residue was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (7:3), to obtain **128** (0.008 g, 74%) as a colorless oil. $[\alpha]^{D}_{22}$ = +37.0 (*c* 0.33, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 – 7.54 (m, 2H), 7.41 – 7.17 (m, 18H), 5.24 (dd, *J* = 9.8, 9.6 Hz, 1H), 4.91 (d, *J* = 10.2 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.70 (d, *J* = 10.2 Hz, 1H), 4.69 (d, *J* = 9.8 Hz, 1H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.54 (d, *J* = 11.6 Hz, 1H), 4.47 (d, *J* = 11.6 Hz, 1H), 3.77 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.69 (ddd, *J* = 9.8, 4.7, 3.6 Hz, 1H), 3.62 (dd, *J* = 10.7, 3.6 Hz, 1H), 3.58 (dd, *J* = 10.7, 4.7 Hz, 1H), 3.58 (dd, *J* = 9.8, 8.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.5 (d, *J* = 42.5 Hz), 137.8, 137.6, 137.5, 133.2, 132.4, 129.2, 128.6, 128.5, 128.3, 128.2, 128.1, 128.01, 127.95, 127.9, 114.6 (d, *J* = 285.8 Hz), 87.9, 83.2, 80.8, 76.5, 75.9, 75.7, 74.7, 73.9, 69.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ -75.0 ($OCOCF_3$). ^{1}H NMR (600 MHz, C_6D_6) δ 7.61 – 7.55 (m, 2H), 7.40 – 7.36 (m, 2H), 7.27 – 6.94 (m, 16H), 5.40 (dd, *J* = 10.0, 9.5 Hz, 1H), 4.83 (d, *J* = 10.6 Hz, 1H), 4.71 (d, *J* = 11.3 Hz, 1H), 4.55 (d, *J* = 11.3 Hz, 1H), 4.50 (d, *J* = 10.6 Hz, 1H), 4.46 (d, *J* = 9.7 Hz, 1H), 4.26 (d, *J* = 12.0 Hz, 1H), 4.18 (d, *J* = 12.0 Hz, 1H), 3.43 (dd, *J* = 9.5, 8.7 Hz, 1H), 3.37 (dd, *J* = 10.6, 3.5 Hz, 1H), 3.34 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.30 (dd, *J* = 10.6, 4.3 Hz, 1H), 3.00 (ddd, *J* = 10.0, 4.3, 3.5 Hz, 1H).

IR (neat) *v* 1793 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{35}H_{33}O_6SF_3Na$ $[M+Na]^+$, 661.1848; found, 661.1843.

Phenyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (129).

Compound **105** (0.010 g, 0.018 mmol) was dissolved in anhydrous dichloromethane (0.5 mL) and cooled to 0 °C. Anhydrous pyridine (15 µL, 0.18 mmol) was added followed by Tf_2O (10 µL, 0.060 mmol) and the reaction mixture stirred for 1.5 h before it was quenched with water at 0 °C. The reaction mixture was diluted with water and extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was then dissolved in anhydrous DMF (0.15 mL), and sodium benzoate (7.9 mg, 0.055 mmol) was added. The reaction mixture was stirred under an argon atmosphere at room temperature for 3 h then diluted with water and extracted with ethyl acetate. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude residue was purified by column chromatography over silica gel, eluting with hexane/ethyl acetate (8:2) to obtain **129** (0.008 g, 71%) with spectral data consistent with the literature.[228] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 – 7.93 (m, 2H), 7.64 – 7.53 (m, 3H), 7.44 – 7.04 (m, 20H), 5.29 (dd, *J* = 10.1, 9.4 Hz, 1H), 4.90 (d, *J* = 10.2 Hz, 1H), 4.77 (d, *J* = 9.8 Hz, 1H), 4.75 (d, *J* = 11.0 Hz, 1H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.62 (d, *J* = 11.0 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 11.6 Hz, 1H), 3.83 (dd, *J* = 9.4, 8.7 Hz, 1H), 3.79 – 3.75 (m, 1H), 3.66 – 3.64 (m, 2H), 3.62 (dd, *J* = 9.8, 8.7 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.07 – 8.04 (m, 2H), 7.73 – 7.68 (m, 2H), 7.45 – 7.42 (m, 2H), 7.27 – 6.94 (m, 19H), 5.65 (dd, *J* = 10.0, 9.4 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.72 (d, *J* = 9.8 Hz, 1H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.62 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 10.8 Hz, 1H), 4.26

– 4.20 (m, 2H), 3.68 (dd, *J* = 9.4, 8.6 Hz, 1H), 3.62 (dd, *J* = 10.7, 2.9 Hz, 1H), 3.58 (dd, *J* = 10.7, 6.0 Hz, 1H), 3.54 (dd, *J* = 9.8, 8.6 Hz, 1H), 3.48 (ddd, *J* = 10.0, 6.0, 2.9 Hz, 1H). IR (neat) *v* 1726 cm^{-1} (C=O).

Phenyl 4-*O*-benzoyl-(6*S*)-[6-$^{2}H_{1}$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-129).

Compound **6*S*-D-129** (0.030 g, 74%) was synthesized from **6*S*-D-106** analogously to **129**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 – 7.93 (m, 2H), 7.62 – 7.54 (m, 3H), 7.45 – 7.04 (m, 20H), 5.29 (dd, *J* = 10.0, 9.4 Hz, 1H), 4.90 (d, *J* = 10.2 Hz, 1H), 4.77 (d, *J* = 9.8 Hz, 1H), 4.77 (d, *J* = 10.9 Hz, 1H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.62 (d, *J* = 10.9 Hz, 1H), 4.49 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 3.83 (dd, *J* = 9.4, 8.8 Hz, 1H), 3.76 (dd, *J* = 10.0, 6.4 Hz, 1H), 3.65 – 3.60 (m, 2H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.07 – 8.03 (m, 2H), 7.73 – 7.68 (m, 2H), 7.46 – 7.41 (m, 2H), 7.27 – 6.92 (m, 19H), 5.64 (dd, *J* = 10.0, 9.4 Hz, 1H), 4.89 (d, *J* = 10.7 Hz, 1H), 4.72 (d, *J* = 9.8 Hz, 1H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.62 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 10.7 Hz, 1H), 4.25 (d, *J* = 11.8 Hz, 1H), 4.22 (d, *J* = 11.8 Hz, 1H), 3.68 (dd, *J* = 9.4, 8.7 Hz, 1H), 3.56 (d, *J* = 6.1 Hz, 1H), 3.54 (dd, *J* = 9.8, 8.7 Hz, 1H), 3.47 (dd, *J* = 10.0, 6.1 Hz, 1H). HRMS (ESI) m/z calcd for $C_{40}H_{37}DO_6SNa$ $[M+Na]^+$, 670.2350; found, 6703.2354.

Phenyl 4-*O*-benzenesulfonyl-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (130).

Compound **106** (0.007 g, 0.014 mmol) and 4-dimethylaminopyridine (catalytic amount) were dissolved in anhydrous pyridine (0.1 mL) and cooled to 0 °C. Benzenesulfonyl chloride (5 µL, 0.039 mmol) was added at 0 °C and the reaction mixture was stirred overnight under an argon atmosphere at room temperature. The reaction mixture was concentrated to dryness and the crude residue was dissolve in ethyl acetate

and filtered through a cotton plug before the organic layer was concentrated to dryness. The crude residue was then purified by silica gel column chromatography, eluting with hexane/ethyl acetate (9:1 to 4:1) to obtain **130** (0.005 g, 57%) as a colorless oil. $[\alpha]^{D}_{22}$ = +13.3 (*c* 0.15, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 – 7.81 (m, 2H), 7.59 – 7.16 (m, 23H), 4.82 (dd, *J* = 9.9, 9.2 Hz, 1H), 4.81 (d, *J* = 10.2 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.63 (d, *J* = 9.8 Hz, 1H), 4.60 (d, *J* = 10.2 Hz, 1H), 4.55 (d, *J* = 11.2 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 11.6 Hz, 1H), 3.71 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.66 (dd, *J* = 9.2, 8.7 Hz, 1H), 3.62 (ddd, *J* = 9.9, 5.6, 2.1 Hz, 1H), 3.54 (dd, *J* = 9.8, 8.7 Hz, 1H), 3.51 (dd, *J* = 10.8, 5.6 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 133.7, 132.2, 129.1, 129.1, 128.5, 128.5, 128.3, 128.1, 127.8, 127.7, 127.6, 127.5, 87.5, 83.7, 81.0, 78.0, 77.8, 75.6, 75.4, 73.5, 68.9. ^{1}H NMR (600 MHz, C_6D_6) δ 7.83 – 7.77 (m, 2H), 7.67 – 7.64 (m, 2H), 7.43 – 6.60 (m, 23H), 5.08 (dd, *J* = 9.9, 9.1 Hz, 1H), 4.80 (d, *J* = 10.4 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H) , 4.49 (d, *J* = 9.4 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 10.4 Hz, 1H), 4.38 (d, *J* = 11.4 Hz, 1H), 4.36 (d, *J* = 11.4 Hz, 1H), 3.81 (dd, *J* = 10.8, 2.0 Hz, 1H), 3.69 (dd, *J* = 10.8, 5.3 Hz, 1H), 3.45 – 3.35 (m, 2H), 3.24 (ddd, *J* = 9.9, 5.3, 2.0 Hz, 1H). IR (neat) *v* 1360 cm^{-1} (S=O), 1188 cm^{-1} (S=O). HRMS (ESI) m/z calcd for $C_{39}H_{38}O_7S_2Na$ $[M+Na]^+$, 705.1957; found, 705.1954.

Phenyl 4-*O*-benzenesulfonyl-(6*S*)-[6-$^{2}H_1$]-2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-130).

Compound **6*S*-D-130** (0.005 mg, 62 %) was synthesized from **6*S*-D-106** analogously to **130**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 – 7.81 (m, 2H), 7.60 – 7.17 (m, 23H), 4.81 (dd, *J* = 9.9, 9.2 Hz, 1H), 4.81 (d, *J* = 10.1 Hz, 1H), 4.70 (d, *J* = 10.8 Hz, 1H), 4.64 (d, *J* = 9.7 Hz, 1H), 4.60 (d, *J* = 10.1 Hz, 1H), 4.55 (d, *J* = 10.8 Hz, 1H), 4.49 (d, *J* =

11.8 Hz, 1H), 4.45 (d, *J* = 11.8 Hz, 1H), 3.66 (dd, *J* = 9.2, 8.7 Hz, 1H), 3.61 (dd, *J* = 9.9, 5.7 Hz, 1H), 3.54 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.50 (d, *J* = 5.7 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.82 – 7.79 (m, 2H), 7.67 – 7.64 (m, 2H), 7.43 – 6.62 (m, 21H), 5.07 (dd, *J* = 9.9, 9.0 Hz, 1H), 4.80 (d, *J* = 10.6 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.49 (d, *J* = 9.5 Hz, 1H), 4.47 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 10.6 Hz, 1H), 4.39 (d, *J* = 11.5 Hz, 1H), 4.36 (d, *J* = 11.5 Hz, 1H), 3.67 (d, *J* = 5.3 Hz, 1H), 3.44 – 3.36 (m, 2H), 3.24 (dd, *J* = 9.9, 5.3 Hz, 1H). HRMS (ESI) m/z calcd for $C_{39}H_{37}DO_7S_2Na$ $[M+Na]^+$, 706.2019; found, 706.2014.

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (131).

Compound **131** was synthesized as described in the literature.[217] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 – 7.58 (m, 2H), 7.41 – 7.18 (m, 23H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.89 (d, *J* = 10.1 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.83 (d, *J* = 10.9 Hz, 1H), 4.73 (d, *J* = 10.1 Hz, 1H), 4.67 (d, *J* = 9.8 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 10.9 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 3.80 (dd, *J* = 10.8, 1.9 Hz, 1H), 3.73 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.71 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.66 (dd, *J* = 9.6, 8.9 Hz, 1H), 3.52 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.51 (ddd, *J* = 9.6, 4.8, 1.9 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.73 – 7.70 (m, 2H), 7.46 – 7.42 (m, 2H), 7.35 – 7.27 (m, 4H), 7.23 – 6.95 (m, 17H), 4.95 (d, *J* = 10.7 Hz, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.83 – 4.79 (m, 2H), 4.72 (d, *J* = 10.7 Hz, 1H), 4.65 (d, *J* = 9.6 Hz, 1H), 4.56 (d, *J* = 11.3 Hz, 1H), 4.44 (d, *J* = 12.0 Hz, 1H), 4.35 (d, *J* = 12.0 Hz, 1H), 3.70 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.64 – 3.62 (m, 2H), 3.61 (dd, *J* = 9.0, 8.7 Hz, 1H), 3.56 (dd, *J* = 9.6, 8.7 Hz, 1H), 3.27 – 3.23 (m, 1H).

Phenyl (6*S*)-[6-^{2}*H*$_1$]-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-131).

A solution of compound **6*S*-D-106** (0005 g, 0.008 mmol) in anhydrous DMF (0.2 mL) was cooled to 0 °C and treated with NaH (60%, 1 mg, 0.025 mmol) and benzyl

bromide (5.1 µl, 0.025 mmol), respectively. The reaction mixture was then stirred for 3.5 h under an argon atmosphere at room temperature and quenched with water at 0 °C. The aqueous layer was extracted with ethyl acetate, and the organic layer was washed with brine, treated with anhydrous Na_2SO_4 and concentrated to dryness. The crude residue was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (19:1 to 4:1) to obtain **6*S*-D-131** (0.004 g, 80%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 – 7.56 (m, 2H), 7.41 – 7.17 (m, 23H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.89 (d, *J* = 10.3 Hz, 1H), 4.85 (d, *J* = 11.0 Hz, 1H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.73 (d, *J* = 10.3 Hz, 1H), 4.67 (d, *J* = 9.8 Hz, 1H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.59 (d, *J* = 10.8 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 3.73 – 3.69 (m, 2H), 3.67 – 3.61 (m, 1H), 3.51 (dd, *J* = 9.8, 8.7 Hz, 1H), 3.50 (dd, *J* = 9.7, 4.9 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.75 – 7.67 (m, 2H), 7.46 – 7.41 (m, 2H), 7.35 – 6.93 (m, 21H), 4.96 (d, *J* = 10.5 Hz, 1H), 4.89 (d, *J* = 11.4 Hz, 1H), 4.83 – 4.78 (m, 2H), 4.72 (d, *J* = 10.7 Hz, 1H), 4.65 (d, *J* = 9.6 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.36 (d, *J* = 12.0 Hz, 1H), 3.70 (dd, *J* = 9.7, 9.2 Hz, 1H), 3.63 – 3.59 (m, 2H), 3.56 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.25 (dd, *J* = 9.7, 4.5 Hz, 1H). HRMS (ESI) m/z calcd for $C_{40}H_{39}DO_5SNa$ $[M+Na]^+$, 656.2557; found, 656.2559.

Ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (132).

Compound **132** was synthesized as described in the literature.[203] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.24 (m, 15H), 4.95 (d, *J* = 10.9 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 4.47 (d, *J* = 9.8 Hz, 1H), 4.33 (dd, *J* = 11.9, 1.9 Hz, 1H), 4.20 (dd, *J* = 11.9, 5.1 Hz, 1H), 3.71 (dd, *J* = 8.8, 8.7 Hz, 1H), 3.54 (dd, *J* = 9.8, 8.7 Hz, 1H), 3.51 (ddd, *J* = 9.8, 5.1, 1.9 Hz, 1H), 3.44 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.81 – 2.70 (m, 2H), 2.04 (s, 3H),

1.33 (t, *J* = 7.4 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.43 – 7.40 (m, 2H), 7.34 – 7.32 (m, 2H), 7.27 – 7.25 (m, 2H), 7.20 – 7.05 (m, 9H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.95 (d, *J* = 11.2 Hz, 1H), 4.81 (d, *J* = 11.2 Hz, 1H), 4.81 (d, *J* = 11.2 Hz, 1H), 4.70 (d, *J* = 10.6 Hz, 1H), 4.49 (d, *J* = 11.2 Hz, 1H), 4.43 (dd, *J* = 11.9, 2.2 Hz, 1H), 4.35 (d, *J* = 9.7 Hz, 1H), 4.26 (dd, *J* = 11.9, 5.4 Hz, 1H), 3.61 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.48 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.47 (dd, *J* = 9.7, 8.9 Hz, 1H), 3.26 (ddd, *J* = 9.8, 5.4, 2.2 Hz, 1H), 2.59 (dq, *J* = 12.6, 7.4 Hz, 1H), 2.49 (dq, *J* = 12.6, 7.4 Hz, 1H), 1.64 (s, 3H1.13 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 1742 cm^{-1} (C=O).

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-pivolyl-1-thio-β-D-glucopyranoside (133).

Compound **107**[203] (0.008 g, 0.016 mmol) was dissolved in anhydrous pyridine (0.05 mL) and treated with pivolyl chloride (2.5 µl, 0.020 mmol). The reaction mixture was then stirred under an argon atmosphere at room temperature overnight and concentrated to dryness. The crude residue was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (4:1 to 7:3) to obtain **133** (0.007 g, 92%) as a colorless oil. $[\alpha]^{D}_{22}$ = +14.0 (*c* 0.57, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 15H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 10.7 Hz, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.58 (d, *J* = 10.7 Hz, 1H), 4.48 (d, *J* = 9.8 Hz, 1H), 4.44 (dd, *J* = 11.9, 1.8 Hz, 1H), 4.12 (dd, *J* = 11.9, 5.6 Hz, 1H), 3.71 (dd, *J* = 8.8, 8.7 Hz, 1H), 3.53 (ddd, *J* = 9.9, 5.6, 1.8 Hz, 1H), 3.50 (dd, *J* = 9.9, 8.7 Hz, 1H), 3.44 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.78 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.70 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.32 (t, *J* = 7.4 Hz, 3H), 1.21 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.2, 138.4, 138.0, 137.8, 128.7, 128.6, 128.6, 128.4, 128.2, 128.0, 128.0, 127.9, 86.7, 84.8, 81.9, 78.2, 76.0, 75.7, 75.4, 63.5, 27.4, 24.9, 15.4. ^{1}H NMR (600 MHz, C_6D_6) δ 7.45 – 7.41 (m, 2H), 7.35 – 7.31 (m,

2H), 7.27 (dd, *J* = 8.5, 1.7 Hz, 2H), 7.19 – 7.05 (m, 9H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.95 (d, *J* = 11.2 Hz, 1H), 4.85 (d, *J* = 11.1 Hz, 1H), 4.82 (d, *J* = 11.2 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.55 (dd, *J* = 11.9, 2.1 Hz, 1H), 4.50 (d, *J* = 11.1 Hz, 1H), 4.34 (d, *J* = 9.7 Hz, 1H), 4.23 (dd, *J* = 11.9, 5.7 Hz, 1H), 3.60 (dd, *J* = 9.0, 8.8 Hz, 1H), 3.48 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.46 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.26 (ddd, *J* = 9.8, 5.7, 2.1 Hz, 1H), 2.63 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.49 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.18 (s, 9H), 1.16 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 1731 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{34}H_{42}O_6SNa$ $[M+Na]^+$, 601.2600; found, 601.2602.

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoroacetyl-1-thio-β-D-glucopyranoside (134).

Compound **134** was synthesized using the same procedure as described for the synthesis of the compound **128** from compound **107** (0.007 g, 0.014 mmol) and trifluoroacetic anhydride (3.8 µL, 0.028 mmol) in anhydrous pyridine (0.05 mL). After purification by silica gel column chromatography, eluting with hexane/ethyl acetate (7:3) **134** (6.0 mg, 72 %) was obtained as a colorless oil. $[\alpha]^{D}_{22}$ = +16.6 (*c* 0.31, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 15H), 4.96 (d, *J* = 10.9 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.74 (d, *J* = 10.2 Hz, 1H), 4.56 (d, *J* = 11.1 Hz, 1H), 4.54 (dd, *J* = 11.6, 2.1 Hz, 1H), 4.48 (d, *J* = 9.8 Hz, 1H), 4.32 (dd, *J* = 11.6, 6.3 Hz, 1H), 3.72 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.58 (ddd, *J* = 9.9, 6.3, 2.1 Hz, 1H), 3.49 (dd, *J* = 9.9, 8.9 Hz, 1H), 3.45 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.75 (dq, *J* = 12.8, 7.4 Hz, 1H), 2.68 (dq, *J* = 12.8, 7.4 Hz, 1H), 1.30 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.27 (q, *J* = 42.5 Hz), 138.3, 137.9, 137.5, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.98, 127.95, 114.6 (q, *J* = 285.4 Hz), 86.7, 85.2, 81.8, 76.3, 76.0, 75.7, 75.3, 66.7, 25.1, 15.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ -74.9($COCF_3$). ^1H NMR (600 MHz,

C_6D_6) δ 7.42 – 7.39 (m, 2H), 7.32 – 7.28 (m, 2H), 7.20 – 7.06 (m, 11H), 4.95 (d, *J* = 10.6 Hz, 1H), 4.92 (d, *J* = 11.2 Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.75 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 10.6 Hz, 1H), 4.34 (d, *J* = 11.4 Hz, 1H), 4.24 (dd, *J* = 11.3, 2.4 Hz, 1H), 4.23 (d, *J* = 9.7 Hz, 1H), 4.05 (dd, *J* = 11.5, 6.3 Hz, 1H), 3.51 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.37 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.26 (dd, *J* = 9.9, 8.9 Hz, 1H), 3.07 (ddd, *J* = 9.9, 6.3, 2.2 Hz, 1H), 2.55 (dq, *J* = 12.8, 7.4 Hz, 1H), 2.43 (dq, *J* = 12.8, 7.4 Hz, 1H), 1.12 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 1790 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{31}H_{33}O_6SF_3Na$ $[M+Na]^+$, 613.1848; found, 613.1850.

Ethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (135).

Compound **135** was synthesized as described in the literature.[390] ^1H NMR (600 MHz, $CDCl_3$) δ 8.07 – 8.04 (m, 2H), 7.60 – 7.56 (m, 1H), 7.49 – 7.40 (m, 4H), 7.39 – 7.24 (m, 13H), 4.99 (d, *J* = 10.8 Hz, 1H), 4.97 (d, *J* = 10.2 Hz, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.79 (d, *J* = 10.2 Hz, 1H), 4.66 – 4.63 (m, 1H), 4.64 (d, *J* = 10.8 Hz, 1H), 4.55 (d, *J* = 9.8 Hz, 1H), 4.48 – 4.44 (m, 1H), 3.78 (t, *J* = 8.8 Hz, 1H), 3.69 – 3.67 (m, 2H), 3.52 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.79 (dq, *J* = 12.7, 7.5 Hz, 1H), 2.73 (dq, *J* = 12.7, 7.5 Hz, 1H), 1.32 (d, *J* = 7.5 Hz, 3H). ^1H NMR (600 MHz, C_6D_6) δ 8.18 – 8.16 (m, 2H), 7.43 – 7.40 (m, 2H), 7.34 – 7.31 (m, 2H), 7.28 – 7.25 (m, 2H), 7.20 – 7.01 (m, 12H), 4.98 (d, *J* = 10.6 Hz, 1H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.65 (dd, *J* = 11.8, 2.3 Hz, 1H), 4.52 (d, *J* = 11.1 Hz, 1H), 4.51 (dd, *J* = 11.8, 5.6 Hz, 1H), 4.38 (d, *J* = 9.7 Hz, 1H), 3.64 (dd, *J* = 9.0, 8.8 Hz, 1H), 3.56 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.51 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.36 (ddd, *J* = 9.8, 5.6, 2.3 Hz, 1H), 2.59 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.46 (dq, *J* = 12.7, 7.5 Hz, 1H), 1.11 (d, *J* = 7.5 Hz, 3H). IR (neat) *v* 1721 cm^{-1} (C=O)

Ethyl (6*S*)-[6-$^{2}H_{1}$]-6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-135).

Compound **6*S*-D-135** (0.007 g, 60%) was synthesized from **6*S*-D-107** analogously to **135**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 – 8.01 (m, 2H), 7.58 – 7.54 (m, 1H), 7.46 – 7.24 (m, 17H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.89 (d, *J* = 10.7 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 2H), 4.76 (d, *J* = 10.2 Hz, 1H), 4.62 (d, *J* = 10.7 Hz, 1H), 4.53 (d, *J* = 9.8 Hz, 1H), 4.42 – 4.40 (m, 1H, H), 3.76 (dd, *J* = 8.8, 8.7 Hz, 1H), 3.66 – 3.64 (m, 2H), 3.49 (dd, *J* = 9.7, 8.8 Hz, 1H), 2.77 (dq, *J* = 12.7, 7.5 Hz, 1H), 2.70 (dq, *J* = 12.7, 7.5 Hz, 1H), 1.29 (t, *J* = 7.5 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.19 – 8.16 (m, 2H), 7.44 – 7.41 (m, 2H), 7.35 – 7.32 (m, 2H), 7.28 – 7.25 (m, 2H), 7.20 – 7.01 (m, 12H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.83 (d, *J* = 11.3 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.52 (d, *J* = 11.1 Hz, 1H), 4.49 (d, *J* = 5.6 Hz, 1H), 4.37 (d, *J* = 9.7 Hz, 1H), 3.64 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.55 (dd, *J* = 9.8, 8.9 Hz, 1H), 3.51 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.35 (dd, *J* = 9.8, 5.6 Hz, 1H), 2.59 (dq, *J* = 12.8, 7.5 Hz, 1H), 2.46 (dq, *J* = 12.8, 7.5 Hz, 1H), 1.11 (t, *J* = 7.5 Hz, 3H). HRMS (ESI) m/z calcd for $C_{36}H_{37}DO_6SNa$ $[M+Na]^+$, 622.2350; found, 622.2358.

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-p-methylbenzoyl-1-thio-β-D-glucopyranoside (136).

Compound **107** (0.015 g, 0.030 mmol) was dissolved anhydrous pyridine (0.1 mL) and cooled to 0 °C before the addition of *p*-methylbenzoyl chloride (5.6 µL, 0.061 mmol). The reaction mixture was then warmed up to room temperature and stirred overnight under an argon atmosphere. The reaction mixture was then diluted with ethyl acetate and washed with aqueous hydrochloric acid (1 M), saturated aqueous $NaHCO_3$, and brine. The organic layer was separated and dried over anhydrous Na_2SO_4 and concentrated to

dryness. The resultant crude residue was purified using silica gel column chromatography, eluting with hexane/ethyl acetate (6:1 to 4:1), to afford the compound **136** (0.018 g, 95%) as a white semi solid. $[\alpha]^{D}_{22}$ = +20.5 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 – 7.90 (m, 2H), 7.39 – 7.37 (m, 2H), 7.35 – 7.21 (m, 17H), 4.95 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.87 (d, *J* = 10.7 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.60 (d, *J* = 10.7 Hz, 1H), 4.59 (dd, *J* = 12.0, 1.6 Hz, 1H), 4.51 (d, *J* = 9.8 Hz, 1H), 4.40 (dd, *J* = 12.0, 5.2 Hz, 1H), 3.74 (t, *J* = 8.8 Hz, 1H), 3.67 – 3.63 (m, 2H), 3.48 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.76 (dq, *J* = 12.8, 7.4 Hz, 1H), 2.69 (dq, *J* = 12.8, 7.4 Hz, 1H), 2.40 (s, 3H), 1.28 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.4, 143.9, 138.4, 138.0, 137.7, 129.8, 129.2, 128.7, 128.6, 128.4, 128.2, 128.2, 128.1, 128.0, 128.0, 127.4, 86.8, 85.2, 82.0, 78.1, 77.3, 76.1, 75.7, 75.4, 63.8, 25.2, 21.8, 15.3. ^{1}H NMR (600 MHz, C_6D_6) δ 8.17 – 8.14 (m, 2H), 7.44 – 7.41 (m, 2H), 7.35 – 7.32 (m, 2H), 7.29 – 7.27 (m, 2H), 7.19 – 7.03 (m, 9H), 6.89 – 6.86 (m, 2H), 4.99 (d, *J* = 10.6 Hz, 1H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.68 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.54 (dd, *J* = 11.8, 5.7 Hz, 1H), 4.53 (d, *J* = 11.0 Hz, 1H), 4.38 (d, *J* = 9.7 Hz, 1H), 3.64 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.56 (dd, *J* = 9.8, 8.9 Hz, 1H), 3.51 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.37 (ddd, *J* = 9.8, 5.7, 2.2 Hz, 1H), 2.61 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.48 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.95 (s, 3H), 1.13 (t, *J* = 7.4 Hz, 1H). IR (neat) *v* 1718 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{37}H_{40}O_6SNa$ $[M+Na]^+$, 635.2443; found, 635.2474.

Ethyl (6*S*)-[6-$^{2}H_{1}$]-6-*O*-p-methylbenzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-136).

Compound **6*S*-D-136** (0.003 g, 76%) was synthesized from **6*S*-D-107** analogously to **136**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 – 7.85 (m, 2H), 7.42 – 7.20 (m, 19H), 4.99 – 4.92 (m, 2H), 4.90 – 4.84 (m, 2H), 4.76 (d, *J* = 10.1 Hz, 1H), 4.61 (d, *J* = 10.7 Hz, 1H), 4.52 (d, *J* = 9.7 Hz, 1H), 4.39 (d, *J* = 5.0 Hz, 1H), 3.75 (t, *J* = 8.8 Hz, 1H), 3.68 – 3.61 (m, 2H), 3.49 (dd, *J* = 9.7, 8.8 Hz, 1H), 2.82 – 2.65 (m, 2H), 2.41 (s, 3H), 1.29 (t, *J* = 7.4 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.18 – 8.14 (m, 2H), 7.44 – 7.41 (m, 2H), 7.35 – 7.26 (m, 4H), 7.20 – 7.02 (m, 9H), 6.89 – 6.86 (m, 2H), 4.99 (d, *J* = 10.5 Hz, 1H), 4.96 (d, *J* = 11.0 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.82 (d, *J* = 11.1 Hz, 1H), 4.71 (d, *J* = 10.5 Hz, 1H), 4.53 (d, *J* = 11.1 Hz, 1H), 4.51 (d, *J* = 5.8 Hz, 1H, H), 4.38 (d, *J* = 9.7 Hz, 1H), 3.64 (t, *J* = 8.8 Hz, 1H), 3.56 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.51 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.37 (dd, *J* = 9.8, 5.8 Hz, 1H), 2.65 – 2.57 (m, 1H), 2.51 – 2.45 (m, 1H), 1.95 (s, 3H), 1.13 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{37}H_{39}DO_6SNa$ $[M+Na]^+$, 636.2506; found, 636.2508.

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-p-methoxybenzoyl-1-thio-β-D-glucopyranoside (137).

Compound **137** was synthesized by the same procedure as described for the compound **136** from compound **107** (0.015 g, 0.030 mmol) and *p*-methoxybenzoyl chloride (0.010 g, 0.061 mmol) in anhydrous pyridine (0.1 mL). After purification by silica gel column chromatography, eluting with hexane/ethyl acetate (6:1 to 4:1), compound **137** (0.015 g, 77%) was obtained as a white semi solid. $[\alpha]^{D}_{22}$ = +23.4 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 – 7.97 (m, 2H), 7.41 – 7.37 (m, 2H), 7.36 – 7.23 (m, 13H), 6.93 – 6.90 (m, 2H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 10.8

Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.76 (d, *J* = 10.2 Hz, 1H), 4.61 (d, *J* = 10.8 Hz, 1H), 4.58 (dd, *J* = 11.8, 1.7 Hz, 1H), 4.52 (d, *J* = 9.8 Hz, 1H), 4.41 (dd, *J* = 11.8, 5.2 Hz, 1H), 3.87 (s, 3H), 3.75 (dd, *J* = 8.9, 8.8 Hz, 1H), 3.67 – 3.63 (m, 2H), 3.49 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.80 – 2.67 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 163.6, 138.4, 138.0, 137.7, 131.7, 128.6, 128.6, 128.4, 128.3, 128.1, 128.1, 128.0, 128.0, 122.5, 113.7, 86.8, 85.2, 82.0, 78.1, 77.3, 76.1, 75.7, 75.4, 63.7, 55.6, 25.2, 15.3. ^{1}H NMR (600 MHz, C_6D_6) δ 8.21 – 8.18 (m, 2H), 7.44 – 7.41 (m, 2H), 7.35 – 7.32 (m, 2H), 7.31 – 7.28 (m, 2H), 7.20 – 7.02 (m, 9H), 6.65 – 6.60 (m, 2H), 5.00 (d, *J* = 10.6 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.73 (d, *J* = 10.6 Hz, 1H), 4.69 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.56 (dd, *J* = 11.8, 5.6 Hz, 1H), 4.55 (d, *J* = 11.0 Hz, 1H), 4.39 (d, *J* = 9.7 Hz, 1H), 3.65 (dd, *J* = 8.9, 8.7 Hz, 1H), 3.59 (dd, *J* = 9.8, 8.9 Hz, 1H), 3.54 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.38 (ddd, *J* = 9.8, 5.6, 2.2 Hz, 1H), 3.13 (s, 3H), 2.62 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.49 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.13 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 1714 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{37}H_{40}O_7SNa$ $[M+Na]^+$, 651.2392; found, 651.2399.

Ethyl (6*S*)-[6-$^{2}H_1$]-6-*O*-p-methoxybenzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-137).

Compound **6*S*-D-137** (0.004 g, 60%) was synthesized from **6*S*-D-107** analogously to **137**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 – 7.96 (m, 2H), 7.44 – 7.22 (m, 15H), 6.94 – 6.88 (m, 2H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.76 (d, *J* = 10.2 Hz, 1H), 4.61 (d, *J* = 10.8 Hz, 1H), 4.52 (d, *J* = 9.8 Hz, 1H), 4.39 (d, *J* = 5.0 Hz, 1H, H6), 3.87 (s, 3H), 3.75 (t, *J* = 8.8 Hz, 1H), 3.68 – 3.61 (m, 2H), 3.49 (dd, *J* = 9.7, 8.8 Hz, 1H), 2.77 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.70

(dq, *J* = 12.7, 7.4 Hz, 1H), 1.30 (t, *J* = 7.4 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 8.22 – 8.17 (m, 2H), 7.45 – 7.40 (m, 2H), 7.35 – 7.32 (m, 2H), 7.30 – 7.28 (m, 2H), 7.20 – 7.02 (m, 9H), 6.64 – 6.60 (m, 2H), 5.00 (d, *J* = 10.8 Hz, 1H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.84 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 10.8 Hz, 1H), 4.53 (d, *J* = 5.6 Hz, 1H, H6), 4.39 (d, *J* = 9.7 Hz, 1H), 3.65 (dd, *J* = 8.9, 8.7 Hz, 1H), 3.58 (dd, *J* = 9.7, 8.9 Hz, 1H), 3.53 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.38 (dd, *J* = 9.7, 5.7 Hz, 1H), 3.13 (s, 3H,), 2.62 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.49 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.13 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{37}H_{39}DO_7SNa$ $[M+Na]^+$, 652.2455; found, 652.2468.

Ethyl 6-*O*-p-nitrobenzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (138).

Compound **138** was synthesized using the same procedure as described for the synthesis of the compound **136** from compound **107** (0.015 g, 0.030 mmol) and *p*-nitrobenzoyl chloride (0.011 g, 0.061 mmol) in anhydrous pyridine (0.1 mL). After chromatographic purification using silica gel (hexane/ethyl acetate 6:1 to 4:1), compound **138** (0.019 g, 96 %) was obtained as a white semi solid. $[\alpha]^{D}_{22}$ = +29.7 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.29 – 8.26 (m, 2H), 8.17 – 8.14 (m, 2H), 7.41 – 7.20 (m, 15H), 4.98 (d, *J* = 10.8 Hz, 1H), 4.95 (d, *J* = 10.2 Hz, 1H), 4.90 (d, *J* = 11.0 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.77 (d, *J* = 10.2 Hz, 1H), 4.62 (dd, *J* = 11.8, 2.2 Hz, 1H), 4.62 (d, *J* = 11.0 Hz, 1H), 4.53 (d, *J* = 9.8 Hz, 1H), 4.44 (dd, *J* = 11.8, 5.4 Hz, 1H), 3.77 (t, *J* = 8.8 Hz, 1H), 3.66 (ddd, *J* = 9.8, 5.4, 2.2 Hz, 1H), 3.61 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.49 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.76 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.70 (dq, *J* = 12.7, 7.4 Hz, 1H, 1H), 1.29 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.4, 150.7, 138.3, 137.9, 137.6, 135.4, 130.9,128.7, 128.6, 128.4, 128.2, 128.2, 128.1, 128.0, 123.6, 86.8, 85.4, 81.9, 77.6, 76.9,

76.1, 75.7, 75.2, 64.8, 25.3, 15.3. ^{1}H NMR (600 MHz, C_6D_6) δ 7.76 – 7.73 (m, 2H), 7.66 – 7.63 (m, 2H), 7.45 – 7.41 (m, 2H), 7.36 – 7.33 (m, 2H), 7.26 – 7.23 (m, 2H), 7.20 – 6.97 (m, 9H), 5.02 (d, *J* = 10.6 Hz, 1H), 5.00 (d, *J* = 11.1 Hz, 1H), 4.85 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.74 (d, *J* = 10.6 Hz, 1H), 4.56 (dd, *J* = 11.8, 2.3 Hz, 1H), 4.51 (d, *J* = 11.2 Hz, 1H), 4.40 (dd, *J* = 11.8, 5.6 Hz, 1H), 4.37 (d, *J* = 9.7 Hz, 1H), 3.66 (t, *J* = 8.8 Hz, 1H), 3.53 (dd, *J* = 9.7, 8.8 Hz, 1H), 3.50 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.31 (ddd, *J* = 9.8, 5.6, 2.3 Hz, 1H), 2.57 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.46 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.09 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 1726 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{36}H_{37}NO_8SNa$ $[M+Na]^+$, 666.2138; found, 666.2148.

Ethyl (6*S*)-[6-^{2}*H*$_1$]-6-*O*-p-nitrobenzoyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-138).

Compound **6*S*-D-138** (0.004 g, 100%) was synthesized from **6*S*-D-107** analogously to **138**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 – 8.25 (m, 2H), 8.18 – 8.12 (m, 2H), 7.44 – 7.19 (m, 15H), 4.98 (d, *J* = 10.8 Hz, 1H), 4.95 (d, *J* = 10.2 Hz, 1H), 4.90 (d, *J* = 11.0 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.76 (d, *J* = 10.2 Hz, 1H), 4.61 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 9.8 Hz, 1H), 4.42 (d, *J* = 5.4 Hz, 1H), 3.76 (t, *J* = 8.8 Hz, 1H), 3.65 (dd, *J* = 9.8, 5.4 Hz, 1H), 3.60 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.48 (dd, *J* = 9.7, 8.8 Hz, 1H), 2.76 (dq, *J* = 12.7, 7.4 Hz, 1H), 2.70 (dq, *J* = 12.7, 7.4 Hz, 1H), 1.29 (t, *J* = 7.4 Hz, 1H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.76 – 7.72 (m, 2H), 7.66 – 7.63 (m, 2H), 7.45 – 7.41 (m, 2H), 7.36 – 7.33 (m, 2H), 7.26 – 7.22 (m, 2H), 7.20 – 6.97 (m, 9H), 5.02 (d, *J* = 10.7 Hz, 1H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.85 (d, *J* = 11.3 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.74 (d, *J* = 10.7 Hz, 1H), 4.51 (d, *J* = 11.3 Hz, 1H), 4.38 (d, *J* = 5.5 Hz, 1H), 4.37 (d, *J* = 9.6 Hz, 1H), 3.66 (t, *J* = 8.8 Hz, 1H), 3.53 (dd, *J* = 9.6, 8.8 Hz, 1H), 3.50 (dd, *J* = 9.8, 8.8 Hz, 1H),

3.30 (dd, *J* = 9.8, 5.5 Hz, 1H), 2.57 (dq, *J* = 12.8, 7.4 Hz, 1H), 2.46 (dq, *J* = 12.8, 7.4 Hz, 1H), 1.09 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{36}H_{36}DNO_8SNa$ $[M+Na]^+$, 667.2200; found, 667.2196

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-(N-phenylcarbamoyl)-1-thio-β-D-glucopyranoside (139).

Compound **107** (0.010 g, 0.020 mmol) was dissolved in anhydrous pyridine (0.4 mL) and cooled to 0 °C before the addition of phenyl isothiocyanate (13.3 µL, 0.120 mmol). The reaction mixture was then stirred for 48 h under an argon atmosphere and concentrated to dryness. The crude reaction mixture was dissolved in ethyl acetate and filtered through cotton before it was concentrated to dryness. The crude residue was purified over silica gel column chromatography, eluting with hexane/ethyl acetate (9:1 to 4:1), to afford the compound **139** (0.011 g, 87%) as a white solid with m.p 119-121 0 °C. $[\alpha]^{D}_{22}$ = +3.5 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 19H), 7.09 – 7.06 (m, 1H), 6.57 (br s, 1H), 4.96 (d, *J* = 10.9 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.86 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.63 (d, *J* = 10.7 Hz, 1H), 4.49 (d, *J* = 9.8 Hz, 1H), 4.41 – 4.35 (m, 2H), 3.73 (t, *J* = 8.8 Hz, 1H), 3.58 – 3.52 (m, 2H), 3.45 (dd, *J* = 9.8, 8.8 Hz, 1H), 2.81 – 2.70 (m, 2H), 1.31 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.1, 138.5, 138.0, 137.8, 137.7, 129.2, 128.7, 128.63, 128.6, 128.5, 128.2, 128.1, 127.9, 123.7, 118.7, 86.8, 85.4, 81.9, 77.2, 75.9, 75.7, 75.2, 63.9, 29.9, 25.3, 15.2. ^{1}H NMR (600 MHz, C_6D_6) δ 7.47 – 7.40 (m, 2H), 7.39 – 7.30 (m, 4H), 7.29 – 7.01 (m, 13H), 6.86 – 6.79 (m, 1H), 6.06 (br s, 1H), 5.01 (d, *J* = 10.6 Hz, 1H), 4.98 (d, *J* = 11.4 Hz, 1H), 4.86 (d, *J* = 11.4 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.73 (d, *J* = 10.6 Hz, 1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.46 (dd, *J* = 11.7, 4.6 Hz, 1H), 4.43 (dd, *J* = 11.8, 2.5

Hz, 1H), 4.39 (d, *J* = 9.7 Hz, 1H), 3.66 (dd, *J* = 9.0, 8.8 Hz, 1H), 3.57 – 3.52 (m, 2H), 3.29 (ddd, *J* = 9.8, 4.6, 2.5 Hz, 1H), 2.62 (dq, *J* = 12.6, 7.4 Hz, 1H), 2.52 (dq, *J* = 12.6, 7.4 Hz, 1H), 1.13 (t, *J* = 7.4 Hz, 3H). IR (neat) *v* 3329 cm^{-1} (N-H), 1716 cm^{-1} (C=O). HRMS (ESI) m/z calcd for $C_{36}H_{39}NO_6SNa$ $[M+Na]^+$, 636.2396; found, 636.2374.

Ethyl 2,3,4-tri-*O*-benzyl-(6*S*)-[6-$^{2}H_{1}$]-6-*O*-(N-phenylcarbamoyl)-1-thio-β-D-glucopyranoside (6*S*-D-139).

Compound **6*S*-D-139** (0.004 g, 100%) was synthesized from **6*S*-D-107** analogously to **139**. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (m, 19H), 7.10 – 7.05 (m, 1H), 6.57 (br s, 1H), 4.96 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 10.0 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.86 (d, *J* = 10.6 Hz, 1H), 4.75 (d, *J* = 10.0 Hz, 1H), 4.62 (d, *J* = 10.6 Hz, 1H), 4.49 (d, *J* = 9.8 Hz, 1H), 4.35 (d, *J* = 4.5 Hz, 1H, H), 3.72 (t, *J* = 8.8 Hz, 1H), 3.59 – 3.50 (m, 2H), 3.44 (dd, *J* = 9.8, 9.0 Hz, 1H), 2.87 – 2.66 (m, 2H), 1.31 (t, *J* = 7.4 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.45 – 7.41 (m, 2H), 7.37 – 7.32 (m, 4H), 7.30 – 7.02 (m, 13H), 6.84 – 6.79 (m, 1H), 6.04 (br s, 1H), 5.01 (d, *J* = 10.5 Hz, 1H), 4.98 (d, *J* = 11.2 Hz, 1H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.83 (d, *J* = 11.0 Hz, 1H), 4.73 (d, *J* = 10.5 Hz, 1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.44 (d, *J* = 4.9 Hz, 1H), 4.38 (d, *J* = 9.6 Hz, 1H), 3.66 (t, *J* = 8.7 Hz, 1H), 3.56 – 3.52 (m, 2H), 3.29 (dd, *J* = 9.8, 4.9 Hz, 1H), 2.62 (dq, *J* = 12.6, 7.4 Hz, 1H), 2.52 (dd, *J* = 12.6, 7.4 Hz, 1H), 1.13 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{36}H_{38}DNO_6SNa$ $[M+Na]^+$, 637.2459; found, 637.2466.

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (140).

Compound **140** was synthesized as described in the literature.[217] ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 18H), 7.19 – 7.16 (m, 2H), 4.93 (d, *J* = 10.9 Hz, 1H), 4.92 (d, *J* = 10.1 Hz, 1H), 4.86 (d, *J* = 10.9 Hz, 1H), 4.82 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 10.1

Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 10.7 Hz, 1H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.47 (d, *J* = 9.8 Hz, 1H), 3.76 (dd, *J* = 10.8, 1.9 Hz, 1H), 3.69 (dd, *J* = 9.0, 8.9 Hz, 1H), 3.69 (dd, *J* = 10.8, 5.0 Hz, 1H), 3.62 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.48 (ddd, *J* = 9.8, 5.0, 1.9 Hz, 1H), 3.45 (dd, *J* = 9.8, 8.9 Hz, 1H), 2.81 (dd, *J* = 12.7, 7.5 Hz, 1H), 2.75 (dd, *J* = 12.7, 7.5 Hz, 1H), 1.34 (d, *J* = 7.5 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.40 – 7.00 (m, 20H), 4.94 (d, *J* = 10.7 Hz, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.81 (d, *J* = 11.2 Hz, 1H), 4.79 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 10.7 Hz, 1H), 4.55 (d, *J* = 11.2 Hz, 1H), 4.41 (d, *J* = 12.2 Hz, 1H), 4.36 (d, *J* = 9.7 Hz, 1H), 4.34 (d, *J* = 12.2 Hz, 1H), 3.67 (dd, *J* = 9.7, 9.1 Hz, 1H), 3.61 – 3.60 (m, 2H), 3.60 (dd, *J* = 9.1, 8.7 Hz, 1H), 3.46 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.28 – 3.24 (m, 1H), 2.59 (dq, *J* = 12.6, 7.4 Hz, 1H), 2.48 (dq, *J* = 12.6, 7.4 Hz, 1H), 1.11 (t, *J* = 7.4 Hz, 3H).

Ethyl (6*S*)-[6-^{2}*H*$_1$]-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (6*S*-D-140).

Compound **6*S*-D-140** was synthesized, by the same procedure as described for **6*S*-D-131** from **6*S*-D-107** (0.010 g, 0.020 mmol), NaH (1.0 mg, 0.025 mmol) and benzyl bromide (2.8 µl, 0.024 mmol) in anhydrous DMF (0.1 ml). After purification by silica gel column chromatography, eluting with hexane/ethyl acetate (7:1 to 7:3), gave the title compound (0.011 g, 94%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 18H), 7.19 – 7.15 (m, 2H), 4.93 (d, *J* = 11.0 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.85 (d, *J* = 10.9 Hz, 1H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.75 (d, *J* = 10.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 4.56 (d, *J* = 12.2 Hz, 1H), 4.47 (d, *J* = 9.8 Hz, 1H), 3.69 (dd, *J* = 9.0, 8.9 Hz, 1H), 3.66 (d, *J* = 5.0 Hz, 1H), 3.61 (dd, *J* = 9.8, 9.0 Hz, 1H), 3.47 (dd, *J* = 9.8, 5.0 Hz, 1H), 3.45 (dd, *J* = 9.8, 8.9 Hz, 1H), 2.80 (dd, *J* = 12.7, 7.5 Hz, 1H), 2.75 (dd, *J* = 12.7, 7.5 Hz, 1H), 1.34 (d, *J* = 7.5 Hz, 3H). ^{1}H NMR (600 MHz, C_6D_6) δ 7.39 – 7.00 (m, 20H),

4.94 (d, *J* = 10.7 Hz, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.81 (d, *J* = 11.3 Hz, 1H), 4.79 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 10.7 Hz, 1H), 4.55 (d, *J* = 11.3 Hz, 1H), 4.41 (d, *J* = 12.2 Hz, 1H), 4.36 (d, *J* = 9.7 Hz, 1H), 4.35 (d, *J* = 12.2 Hz, 1H), 3.67 (dd, *J* = 9.7, 9.1 Hz, 1H), 3.60 (dd, *J* = 9.1, 8.7 Hz, 1H), 3.59 (d, *J* = 4.6 Hz, 1H), 3.46 (dd, *J* = 9.7, 8.7 Hz, 1H), 3.26 (dd, *J* = 9.7, 4.6 Hz, 1H), 2.59 (dq, *J* = 12.6, 7.4 Hz, 1H), 2.48 (dq, *J* = 12.6, 7.4 Hz, 1H), 1.11 (t, *J* = 7.4 Hz, 3H). HRMS (ESI) m/z calcd for $C_{36}H_{39}DO_5SNa$ $[M+Na]^+$, 608.2557; found, 608.2551.

Methyl (1-Adamantanyl 5-*N*,4-*O*-carbonyl-3,5-dideoxy-2-thio-D-glycero-*α*-D-galacto-non-2-ulopyranoside)onate (182).

A solution of compound **180** (1.9 g, 3.0 mmol) in anhydrous THF (12.7 ml) was treated with di-*tert*butyl dicarbonate (6.81 g, 31.2 mmol) and DMAP (153 mg, 1.25 mmol) and stirred overnight at 60 °C before it was concentrated to dryness. The crude reaction mixture was passed through a short pad of silica to obtain **181** (~1.4 g), which was then treated with NaOMe (cat.) in anhydrous methanol (9 ml) and stirred for 1.5 h. The reaction mixture was quenched with Amberlyst-15 (pH = ~4) and filtered through Celite and concentrated to dryness. The crude residue was then treated with 1.5 M HCl in methanol (22 ml, 33 mmol) for 2.5 h and concentrated to dryness. The crude residue and $NaHCO_3$ (0.8 g, 9.5 mmol) were dissolved in MeCN (8 mL) and H_2O (16 mL) and cooled to 0 °C. Then, a solution of 4-nitrophenyl chloroformate (0.94 g, 4.7 mmol) in MeCN (8 ml) was added to a vigorously stirred reaction mixture through a dropping funnel. Then the reaction mixture was stirred for 3 h at 0 °C before it was extracted with ethyl acetate (3 times). Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude residue was purified using silica gel

chromatography, eluting with hexane/ethyl acetate (10:1 to 6:1), to obtain **182** (470 mg, 34% from **180**). $[\alpha]^{RT}_{D}$ = +30.4 (*c* 1.7, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 4.04 (dd, *J* = 9.8, 2.0 Hz, 1H), 3.85 (s, 3H), 3.86 – 3.79 (m, 2H), 3.69 – 3.64 (m, 1H), 3.57 (dd, *J* = 11.2, 9.8 Hz, 1H), 3.52 (dd, *J* = 8.8, 2.0 Hz, 1H), 2.97 (dd, *J* = 11.7, 3.7 Hz, 1H), 2.09 (dd, *J* = 12.7, 11.8 Hz, 1H), 2.04 – 1.92 (br m, 9H), 1.73 – 1.65 (br s, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 171.4, 160.8, 85.5, 77.8, 71.4, 70.0, 63.2, 57.0, 52.4, 50.4, 43.4, 38.8, 35.7, 29.9. ESIHRMS Calcd for $C_{21}H_{31}NO_8SNa$ $[M + Na]^+$, 480.1668; found, 480.1668.

Methyl (1-Adamantanyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (183α).

A solution of **182** (400 mg, 0.87 mmol) in pyrindene (8 ml) was treated with acetic anhydride (9 ml) and stirred overnight at room temperature before it was concentrated to dryness. The crude reaction mixture was dissolved in anhydrous CH_2Cl_2, treated with EtN-(*i*-Pr)$_2$ (1.5 ml, 8.6 mmol) and cooled to 0 °C before it was treated with acetyl chloride (0.51 ml, 7.1 mmol). Reaction mixture was warmed to room temperature (~ 20 min) and poured into a saturated aqueous $NaHCO_3$ solution, organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 times). Combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude residue was purified by silica gel column chromatography, eluting with hexane/ethyl acetate (3:1 to 1:1), to obtain **183α** (0.54 g, 84% in two steps). $[\alpha]^{RT}_{D}$ = +37.2 (*c* 4.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.58 (d, *J* = 8.0, 1H), 5.41 (ddd, *J* = 8.4, 6.0, 2.8 Hz, 1H), 4.51 (d, *J* = 9.6, 1H), 4.36 (dd, *J* = 12.3, 2.9 Hz, 1H), 3.86 – 3.76 (m, 1H), 3.79 (s, 3H), 3.68 – 3.58 (m, 1H), 2.97 (dd, *J* = 12.0, 3.6 Hz, 1H), 2.46 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.13 – 2.08 (m, 1H), 2.01 (s, 3H), 1.99 – 1.84 (m, 9H), 1.72 – 1.61 (m, 6H);

^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8, 170.6, 170.3, 170.0, 169.9, 153.4, 84.9, 76.4, 75.5, 71.9, 69.8, 62.5, 59.5, 53.0, 51.3, 43.6, 38.6, 35.9, 29.9, 24.7, 21.1, 21.0, 20.7. ESIHRMS Calcd for $C_{29}H_{39}NO_{12}SNa$ $[M + Na]^+$, 648.2073; found, 648.2091.

General Protocol for Pseudo Kinetic Experiment in Dichloromethane.

N-acetyl-5-*N*,4-*O*-oxozolidinone protected adamantanyl thiosialoside **183α** or **183β**[336] (50.0 mg, 0.08 mmol), internal standard, acetylated-methyl-2-phthalimido-2-deoxy-β-D-glucopyranoside **184**[353] (3.3 mg, 0.007 mmol) ~10 mol %, and sialidation acceptor 2-PrOH in anhydrous CH_2Cl_2 (1.45 ml) were stirred with pre-activated, acid washed 4 Å molecular sieves (157 mg) at room temperature for 0.5 h followed by another 0.5 h at -78 ˚C. Then the reaction was activated with *N*-iodosuccinimide followed by trifluoromethanesulfonic acid at -78 ˚C (time = 0 min). The sampling was started after two minutes (time = 2 min). During every three minutes time intervals (t = 2, 5, 8, 11, 14, 17, and 20 min), aliquots of reaction mixture were taken out using 20 µL Kimble microcapillary pipettes (sampling volume ~ 5 µL) and quenched with 9 mM Et_3N in acetonitrile solution (1 ml). The samples were filtered through Millipore LG-0.20 µm syringe filters. All the aliquots were analyzed using an ultra-high-performance liquid chromatography (UHPLC) coupled mass spectrometer (ESI-MS) equipped with a tunable ultra-violet (TUV) detector at the wave length (λ) of 205 nm. After 20.5 min, reaction mixture was quenched with Et_3N (16 µl, 0.12 mmol). Then, reaction mixture was diluted with dichloromethane and washed with aqueous saturated $Na_2S_2O_3$, aqueous saturated $NaHCO_3$, brine, and dried over anhydrous Na_2SO_4 before it was concentrated to dryness. ^{1}H-NMR spectrum of the crude reaction mixture was recorded to ensure <10% conversion of the donor.

Pseudo-first-order Kinetic Experiments with Respect to the Acceptor, 2-Proponol.

A series of pseudo first-order kinetic experiments with respect to the glycosyl acceptor, 2-propanol, was conducted separately on sialyl donors **183β** and **183α** in dichloromethane at -78 °C using general protocol. Thus, the acceptor, 2-propanol concentrations was varied as 0.022, 0.028, 0.034, 0.039 and 0.045 mol dm^{-3}, while keeping a constant concentration of glycosyl donor at 0.056 mol dm^{-3}.

Kinetic Experiments by Changing the Acetonitrile Percentage in Dichloromethane.

Series of kinetic experiments were conducted separately on sialyl donors **183β** and **183α** with 2-proponol as the acceptor. Here, the general protocol was modified to keep constant donor (0.056 mol dm^{-3}) and acceptor (0.028 mol dm^{-3}) concentrations, while the acetonitrile percentage was varied from 0 to 40% in dichloromethane at -78 °C.

The crude reaction mixtures from multiple kinetic runs were combined and purified using silica gel chromatography, eluting with hexanes/ethyl acetate 3:1 to 1:1, to obtain a mixture of *O*-sialosides **185α** and **185β**.

Methyl (2-propyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (185α).

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.59 (dd, *J* = 8.2, 1.6 Hz, 1H), 5.43 (ddd, *J* = 8.2, 6.7, 2.9 Hz, 1H), 4.57 (dd, *J* = 9.4, 1.6 Hz, 1H), 4.37 (dd, *J* = 12.3, 2.9 Hz, 1H), 4.05 (dd, *J* = 12.3, 6.7 Hz, 1H), 4.00 – 3.95 (m, 1H), 3.94 (ddd, *J* = 14.0, 11.2, 3.5 Hz, 1H), 3.79 (s, 3H), 3.66 (dd, *J* = 11.1, 9.4 Hz, 1H), 2.83 (dd, *J* = 12.1, 3.6 Hz, 1H), 2.48 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H) 2.02 (t, *J* = 12.8 Hz, 1H), 1.25 (d, *J* = 6.1 Hz, 3H), 1.08 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.0, 170.6, 170.1, 170.0, 169.3 ($^{3}J_{C1,H3ax}$ = 6.1 Hz), 153.7, 99.2, 75.2, 75.1, 71.7, 69.8, 69.1, 67.5, 63.0, 59.2, 52.7, 37.0, 24.7,

24.6, 22.9, 21.1, 20.9, 20.7. ESIHRMS Calcd for $C_{29}H_{40}NO_{12}S$ [M + Na]$^+$, 540.1693; found, 540.1699.

Methyl (2-propyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranoside)onate (185β).

^{1}H NMR (600 MHz, $CDCl_3$ δ 5.54 (m, 1H), 5.28 – 5.18 (m, 1H), 4.70 (dd, *J* = 12.2, 2.4 Hz, 1H), 4.52 (td, *J* = 12.0, 3.7 Hz, 1H), 4.37 (dd, *J* = 9.1, 1.6 Hz, 1H), 4.22 – 4.07 (m, 2H), 3.80 (s, 3H), 3.66 (t, *J* = 10.3 Hz, 1H), 2.71 (dd, *J* = 12.1, 3.6 Hz, 1H), 2.44 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 1.97 (t, *J* = 12.4 Hz, 1H), 1.23 (d, *J* = 6.3 Hz, 3H), 1.04 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 171.1, 170.5, 169.8, 167.4 ($^3J_{C1,H3ax}$ = 2.5 Hz), 153.8, 97.3, 75.0, 74.9, 73.5, 72.4, 67.6, 59.4, 52.7, 43.5, 36.9, 35.9, 29.8, 29.6, 24.1, 22.5, 21.1, 20.7, 20.7. ESIHRMS Calcd for $C_{29}H_{40}NO_{12}S$ [M + Na]$^+$, 540.1693; found, 540.1699.

1-Adamantanyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid (191).

A mixture of **190**[332] (1.00 g, 1.56 mmol) and anhydrous pyridine (14.2 mL) was added to anhydrous LiI (0.75 g, 5.58 mmol) under an argon atmosphere. The reaction mixture was heated to reflux for 3.5 h before it was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with 1N HCl and brine, dired and evaporated to dryness. The crude residue was purified by chromatography over neutral alumina, eluting with ethyl acetate/glacial acetic acid 20:1 to 10:1, to obtain the carboxylic acid **191** (0.51 g, 68%). $[\alpha]^{RT}_D$ = -51.6 (*c* 2.7, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.55 (d, *J* = 10.3 Hz, 1H), 5.42 – 5.39 (m, 1H), 5.36 (dt, *J* = 11.4, 4.4 Hz, 1H), 5.25 – 5.21 (m, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.49 (dd, *J* = 10.6, 2.7 Hz, 1H), 4.13 (dd, *J* = 12.5, 8.1 Hz, 1H), 4.10 (q, *J* = 10.6 Hz,

1H), 2.60 (dd, *J* = 13.7, 4.5 Hz, 1H), 2.12 – 2.07 (m, 7H), 2.02 (s, 3H), 2.02 (s, 3H), 2.01 – 1.94 (m, 9H), 1.88 (s, 3H), 1.65 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.7, 171.2, 171.1, 170.8, 170.7, 170.3, 85.5, 72.9, 72.2, 69.3, 68.8, 63.4, 51.1, 49.6, 43.7, 39.6, 36.0, 30.0, 23.2, 21.5, 21.0, 20.9, 20.8. ESIHRMS Calcd for $C_{29}H_{40}NO_{12}S$ $[M - H]^-$, 626.2271; found, 626.2279.

2-Hydroxymethylbenzyl (1-adamantanyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (192).

A solution of *N,N′*-dicyclohexylcarbodiimide (0.10 g, 0.47 mmol) in anhydrous CH_2Cl_2 (0.3 mL) was added to a mixture of **191** (0.18 g, 0.29 mmol), 1,2-benzenedimethanol (0.16 g, 1.17 mmol) and hydroxybenzotriazole (0.08 g, 0.59 mmol) in anhydrous CH_2Cl_2 (0.4 mL) at 0 ^{0}C. The reaction mixture was stirred for 5 min then warmed to room temperature and stirred for 48 h under an argon atmosphere. The reaction mixture was then filtered through Celite, and the organic layer was diluted with CH_2Cl_2 before washing with sat. $NaHCO_3$, water, and brine. The organic layer was evaporated to dryness and purified by silica gel chromatography, eluting with hexane/ethyl acetate 3:1 to 3:2, to give **192** (0.15 g, 70 %). $[\alpha]^{RT}_D$ = - 51.0 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.3 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.38 – 7.35 (m, 1H), 7.31 – 7.28 (m, 1H), 5.45 – 5.43 (m, 1H), 5.43 – 5.34 (m, 3H), 5.29 (dt, *J* = 10.5, 4.5 Hz, 1H), 5.18 – 5.15 (m, 1H), 4.87 (dd, *J* = 12.3, 1.6 Hz, 1H), 4.80 (s, 2H), 4.50 (dd, *J* = 10.5, 2.6 Hz, 1H), 4.28 (dd, *J* = 12.3, 8.7 Hz, 1H), 4.05 (q, *J* = 10.4 Hz, 1H), 2.56 (br s, 1H), 2.49 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.12 (s, 3H), 2.06 (s, 3H), 1.99 (s, 7H), 1.90 (s, 3H), 1.85 (s, 2H), 1.84 – 1.74 (m, 7H), 1.63 – 1.53 (m, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 171.0, 170.7, 170.4, 170.3, 169.2, 140.2, 132.8, 130.8, 129.4, 129.3, 128.1, 86.4,

73.7, 73.0, 69.4, 69.0, 65.9, 63.2, 62.9, 51.0, 49.8, 43.6, 39.9, 35.9, 29.9, 23.3, 21.2, 21.0, 20.9, 20.8; ESIHRMS Calcd for $C_{37}H_{49}NO_{13}SNa$ $[M + Na]^+$, 770.2822; found, 770.2830.

1-Adamantanyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid (193).

A mixture of **180**[17,21] (0.10 g, 0.16 mmol) and anhydrous pyridine (1.4 mL) was added to anhydrous LiI (0.07 g, 0.56 mmol) under an argon atmosphere. The reaction mixture was heated to reflux for 4 h under an argon atmosphere before it was cooled to room temperature and concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, and the organic layer was washed with 1N HCl, brine, and anhydrous Na_2SO_4 before drying over Na_2SO_4 and evaporation to dryness. The crude residue was then purified by chromatography over neutral alumina (eluting with ethyl acetate/glacial acetic acid 20:1 to 10:1) to give the carboxylic acid **193** (0.07 g, 70%).

A solution of **180**[332, 352] (0.98 g, 1.52 mmol), methanol (9.8 mL) and 1N NaOH (19.5 mL) was stirred for 24 h, then co-evaporated with ethanol (×2) and toluene (×3). The crude residue was dissolved in pyridine (8.9 mL), treated with acetic anhydride (10.3 mL) at 0 ^{0}C, and stirred overnight at room temperature. The reaction mixture was then treated with methanol (15.0 mL) at 0 ^{0}C and stirred at room temperature for 30 min. After concentration, the crude residue was purified by chromatography over using neutral alumina, eluting with ethyl acetate/acetic acid 20:1 to 10:1, to afford **193** (0.94 g, 73%). $[\alpha]^{RT}_D$ = + 20.3 (*c* 2.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.36 (s, 1H), 5.31 (s, 2H), 4.86 (dt, *J* = 11.1, 4.4 Hz, 1H), 4.38 (d, *J* = 12.0 Hz, 1H), 4.21 – 4.16 (m, 2H), 4.05 (q, *J* = 10.4 Hz, 1H), 2.71 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.15 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H), 2.02 – 1.96 (m, 13H), 1.88 (s, 3H), 1.65 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.8,

171.7, 171.3, 170.71, 170.67, 170.4, 84.4, 74.4, 70.6, 70.1, 68.2, 62.1, 51.1, 48.7, 43.5, 39.8, 35.9, 29.8, 22.8, 21.1, 21.0, 20.82, 20.76; ESIHRMS Calcd for $C_{29}H_{41}NO_{12}SNa$ [M + Na]$^+$, 650.2247; found, 650.2247.

2-Hydroxymethylbenzyl (1-adamantanyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (194).

Compound **194** was prepared by the same procedure as compound **192** from acid **193** (0.50 g, 0.80 mmol), 1,2-benzenedimethanol (0.44 g, 3.19 mmol), hydroxybenzotriazole (0.22 g, 1.6 mmol) and *N,N′*-dicyclohexylcarbodiimide (0.26 g, 1.27 mmol) in anhydrous CH_2Cl_2 (2.0 mL). After chromatographic purification (gradient elution with hexanes/acetone 3:1 to 3:2), **194** (0.21 g, 35 %) was obtained. $[\alpha]^{RT}_D$ = - 4.3 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.4 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.30 (t, *J* = 7.3 Hz, 1H), 5.42 (d, *J* = 11.9 Hz, 2H), 5.28 (dd, *J* = 8.6, 1.6 Hz, 1H), 5.26 – 5.22 (m, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.16 (d, *J* = 9.9 Hz, 1H), 4.79 (d, *J* = 12.8 Hz, 1H), 4.75 (d, *J* = 12.8 Hz, 1H), 4.73 (dt, *J* = 11.4, 4.4 Hz, 1H), 4.32 (dd, *J* = 12.5, 2.4 Hz, 1H), 4.12 (dd, *J* = 12.5, 4.5 Hz, 1H), 4.06 (dd, *J* = 10.8, 1.5 Hz, 1H), 3.94 (q, *J* = 10.3 Hz, 1H), 2.67 (dd, *J* = 12.8, 4.6 Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.99-1.91 (m, 7H), 1.86 (s, 3H), 1.89 – 1.79 (m, 3H), 1.62 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 170.8, 170.3, 170.2, 170.1, 169.3, 140.3, 132.6, 131.3, 129.6, 129.2, 128.2, 84.8, 74.1, 69.8, 69.5, 67.5, 66.0, 63.1, 62.0, 51.6, 49.4, 43.7, 40.1, 36.1, 30.1, 23.4, 21.3, 21.1, 21.0, 20.9; ESIHRMS Calcd for $C_{37}H_{49}NO_{13}SNa$ [M + Na]$^+$, 770.2822; found, 770.2792.

2-Hydroxymethylbenzyl (1-adamantanyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (194).

A mixture of **183β**[332] (0.60 g, 0.96 mmol) and anhydrous pyridine (8.7 mL) was added to LiI (0.46 g, 3.43 mmol) under an argon atmosphere, and the mixture heated to reflux at 135 ^{0}C for 3.5 h under argon. After cooling to room temperature and drying under reduced pressure, the residue was dissolved in CH_2Cl_2, and the organic layer was washed with 1N HCl and brine before evaporation to dryness. A solution of *N,N'*-dicyclohexylcarbodiimide (0.32 g, 1.53 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added to a stirred solution of the resulting residue, 1,2-benzenedimethanol (0.53 g, 3.83 mmol) and hydroxybenzotriazole (0.26 g, 1.92 mmol) in anhydrous CH_2Cl_2 (1.4 mL) at 0 ^{0}C. The mixture was stirred for 5 min before warming to room temperature and stirring for another 36 h under an argon atmosphere. The crude product was obtained by following the same workup as described for compound **192**, and was purified by silica gel chromatography, eluting with hexane/ethyl acetate 3:1 to 1:1, to afford **196** (0.37 g, 53%). $[\alpha]^{RT}_D$ = - 39.6 (*c* 3.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.1 Hz, 1H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.40 – 7.35 (m, 1H), 7.33 – 7.27 (m, 1H), 5.71 – 5.68 (m, *J* = 3.5 Hz, 1H), 5.43 (d, *J* = 12.1 Hz, 1H), 5.34 (d, *J* = 12.1 Hz, 1H), 5.27 – 5.21 (m, 1H), 4.79 (s, 2H), 4.75 – 4.66 (m, 2H), 4.60 (dd, *J* = 12.2, 2.3 Hz, 1H), 4.12 (dd, *J* = 12.2, 7.6 Hz, 1H), 3.67 (dd, *J* = 11.3, 9.3 Hz, 1H), 2.77 (dd, *J* = 12.9, 3.6 Hz, 1H), 2.62 (br s, 1H), 2.48 (s, 3H), 2.22 (t, *J* = 12.9 Hz, 1H), 2.10 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.92 (br s, 3H), 1.87 – 1.72 (m, 6H), 1.65 – 1.53 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 170.8, 170.7, 169.7, 168.8, 153.7, 140.2, 132.5, 130.8, 129.4, 129.2, 127.9, 85.8, 75.1, 74.3, 72.7, 71.7, 66.0, 63.0,

62.8, 60.3, 51.4, 43.6, 38.2, 35.8, 29.8, 24.7, 21.2, 20.7, 20.6. ESIHRMS Calcd for $C_{36}H_{45}NO_{13}SNa$ $[M + Na]^+$, 754.2509; found, 754.2491.

2-Hydroxymethylbenzyl (1-adamantanyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-2-thio-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (199).

A solution of **197**[366, 391] (0.24g, 0.38 mmol) in anhydrous pyridine (3.4 mL) was added to anhydrous LiI (0.18 g, 1.36 mmol) under an inert atmosphere, and the mixture heated to reflux for 2 h before cooling to room temperature and concentration to dryness. The resultant residue was dissolved in CH_2Cl_2, and the organic layer was washed with 1N HCl and brine before evaporation to dryness. A stirred solution of the residue, 1,2-benzenedimethanol (0.21 g, 1.51 mmol) and hydroxybenzotriazole (0.10 g, 0.76 mmol) in anhydrous CH_2Cl_2 (0.6 mL) was treated with a solution of *N,N'*-dicyclohexylcarbodiimide (0.12 g, 0.61 mmol) in anhydrous CH_2Cl_2 (0.4 mL) at 0 ^{0}C and stirred for 5 min before warming up to room temperature and stirring for 48 h. The reaction mixture was worked up as described for compound **192**, and the resultant residue was purified by silica gel chromatography, eluting with toluene/acetone 5:1, and then by preparative HPLC, eluting by hexanes/ethyl acetate 9:1 to 4:6 over 50 min, to give **199** (0.12 g, 43%). $[\alpha]^{RT}_D$ = - 90.5 (*c* 1.01, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.3 Hz, 1H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.36 (dt, *J* = 7.5, 3.7 Hz, 1H), 7.30 (dt, *J* = 7.5, 3.8 Hz, 1H), 5.46 (dd, *J* = 4.7, 2.4 Hz, 1H), 5.43 – 5.31 (m, 3H), 5.28 – 5.23 (m, 1H), 4.77 (s, 2H), 4.60 (dd, *J* = 12.5, 2.2 Hz, 1H), 4.48 (dd, *J* = 10.3, 2.4 Hz, 1H), 4.27 (dd, *J* = 12.5, 6.8 Hz, 1H), 3.65 (t, *J* = 10.1 Hz, 1H), 2.60 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.45 (br s, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.90 (s, 4H), 1.86 – 1.70 (m, 6H), 1.63– 1.51 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.4, 169.7, 169.5, 169.0, 140.3, 139.6, 132.6, 130.8, 129.5,

129.3, 128.1, 86.1, 71.3, 71.1, 69.8, 69.6, 66.0, 63.0, 62.4, 57.4, 51.2, 43.5, 39.3, 35.9, 29.9, 21.2, 21.1, 20.9, 20.7; ESIHRMS Calcd for $C_{36}H_{45}NO_{12}S_2Na$ $[M + Na]^+$, 770.2281; found, 770.2263.

General Procedure for cyclization reactions.

A solution of substrate (0.008 M) in anhydrous CH_2Cl_2 was stirred under argon at room temperature with freshly activated AW-300 moleular sieves (100 mg/mL) for 30 min and then at – 40 ^{0}C for another 30 min. *N*-Iodosuccinimide (2.4 equiv) and TfOH (1 equiv) were then added respectively and the reaction mixture stirred for 2 h before Et_3N (~1.5 equiv) was added. The reaction mixture was diluted with CH_2Cl_2, filtered through Celite, washed with saturated aqueous $NaHCO_3$, saturated aqueous $Na_2S_2O_3$, brine, and finally concentrated to dryness. The crude residue was purified by silica gel column chromatography to obtain mixtures of cyclized products from which the pure anomers were obtained by preparative HPLC.

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (200α) and 2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (200β).

***From* 192**. According to the general procedure for cyclization, compounds **200α** and **200β** were prepared using peracetylated thioglycoside donor **192** (0.051 g, 0.068 mmol), freshly activated AW-300 moleular sieves (0.880 g), anhydrous CH_2Cl_2 (8.4 mL), *N*-iodosuccinimide (0.036 g, 0.162 mmol) and TfOH (5.9 µL, 0.066 mmol). The crude residue was purified using silica gel column chromatography (gradient elution with

toluene/2-propanol 14:1 to 6:1) to give **200α** and **200β** (0.022 g, 58%) as a mixture (1:2.4, α:β) of anomers.

From 194. The cyclization reaction was carried using the general procedure for cyclization using donor **194** (0.013 g, 0.017 mmol), AW-300 molecular sieves (0.221 g), anhydrous CH_2Cl_2 (2.1 mL), *N*-idodosuccinimide (0.009 g, 0.040 mmol) and TfOH (1.5 μL, 0.017 mmol) to afford **200α** and **200β** (0.008 g, 76%) as a mixture (1:5, α:β) of anomers.

Purification. The combined mixtures of products from the cyclization of **192** and **194** was separated by preparative HPLC, gradient elution with hexanes/ethyl acetate 1:1 to 1:4 over 30 min, to obtain pure **200α** and **200β**. UHPLC was used to analyze the pure samples of **200α** and **200β** (UHPLC method, flow rate: 0.35 mL/min; Initial: CH_3CN 30% in H_2O; 10.5 min: CH_3CN 30% in H_2O; 15.0 min: CH_3CN 100%; t_R (min): **200β** = 6.08, **200α** = 7.37).

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (200α).

$[\alpha]^{RT}_D$ = + 39.4 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34 (t, *J* = 7.4 Hz, 1H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.22 (d, *J* = 7.4 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 1H), 6.12 (d, *J* = 10.0 Hz, 1H), 6.04 (d, *J* = 13.0 Hz, 1H), 5.51 (ddd, *J* = 8.4, 5.5, 2.6 Hz, 1H), 5.34 (dd, *J* = 9.2, 2.0 Hz, 1H), 5.23 (d, *J* = 15.1 Hz, 1H), 5.11 (td, *J* = 11.8, 4.7 Hz, 1H), 4.98 (d, *J* = 13.1 Hz, 1H), 4.86 (d, *J* = 15.2 Hz, 1H), 4.62 (d, *J* = 10.5 Hz, 1H), 4.26 (dd, *J* = 12.5, 2.5 Hz, 1H), 4.21 (q, *J* =10.4 Hz, 1H), 4.09 (dd, *J* = 12.5, 5.6 Hz, 1H), 2.70 (dd, *J* = 13.0, 4.7 Hz, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.08 (t, *J* = 12.8 Hz, 1H), 2.02 (s, 6H), 1.87 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2 ($^3J_{C1,H3ax}$ = 6.5 Hz), 170.8, 170.7, 170.5, 170.2, 169.9,

134.8, 133.9, 129.5, 129.3, 128.8, 128.4, 99.3, 73.7, 71.9, 69.0, 68.8, 68.2, 67.1, 62.6, 49.2, 37.2, 23.1, 21.2, 21.04, 21.02, 20.9; ESIHRMS Calcd for $C_{27}H_{33}NO_{13}Na$ $[M + Na]^+$, 602.1850; found, 602.1824.

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (200β).

$[\alpha]^{RT}_D$ = - 32.2 (*c* 1.5, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 7.30 – 7.25 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 1H), 7.12 (d, *J* = 6.6 Hz, 1H), 5.66 (d, *J* = 13.5 Hz, 1H), 5.60 (d, *J* = 9.3 Hz, 1H), 5.43 (d, *J* = 4.7 Hz, 1H), 5.40 (d, *J* = 13.4 Hz, 1H), 5.34 – 5.24 (m, 2H), 4.81 (dd, *J* = 12.4, 2.3 Hz, 1H), 4.46 (d, *J* = 13.4 Hz, 1H), 4.16 – 4.05 (m, 3H), 2.35 (dd, *J* = 13.3, 4.9 Hz, 1H), 2.14 (s, 3H), 2.04 – 2.02 (m, 4H), 2.00 (s, 3H), 1.99 (s, 3H), 1.86 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 170.70, 170.67, 170.4, 170.3, 168.1($^3J_{C1,H3ax}$ = 0.0 Hz), 135.9, 133.0, 132.1, 128.9, 128.8, 128.1, 97.7, 71.6, 69.4, 69.0, 68.2, 67.1, 62.2, 49.6, 36.0, 23.3, 21.1, 21.0, 20.9; ESIHRMS Calcd for $C_{27}H_{33}NO_{13}Na$ $[M + Na]^+$, 602.1850; found, 602.1846.

2-Hydroxymethylbenzyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (201α) and 2-Hydroxymethylbenzyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (201β).

The general procedure for cyclization was used to synthesize a mixture of **201α** and **201β** from **196** (0.044 g, 0.060 mmol), AW-300 molecular sieves (0.781 g), anhydrous CH_2Cl_2 (7.5 mL), *N*-idodosuccinimide (0.032 g, 0.141 mmol) and TfOH (5.2 μL, 0.059 mmol). After silica gel chromatography using hexanes/ethyl acetate as eluent, products **201α** and **201β** (0.014 g, 42%) were obtained as a mixture (1:0.4, α:β) of anomers.

2-Hydroxymethylbenzyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (201α).

After preparative HPLC of the mixture of cyclized anomers (eluting with hexanes/ethyl acetate 7:3 to 1:1 over 40 min), an analytical sample of **201α** was obtained. Recrystallization from CH_2Cl_2/Hexanes (3:7) gave crystals of **201α** suitable for X-ray analysis. Mp 208 ^{0}C; $[\alpha]^{RT}_D$ = + 63.4 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, 2H), 7.23 (d, *J* = 7.2 Hz, 1H), 7.17 (d, *J* = 6.9 Hz, 1H), 5.96 (d, *J* = 13.0 Hz, 1H), 5.61 (dd, *J* = 8.6, 1.6 Hz, 1H), 5.53 – 5.48 (m, 1H), 5.32 (d, *J* = 14.8 Hz, 1H), 5.14 (d, *J* = 13.0 Hz, 1H), 4.84 (d, *J* = 14.8 Hz, 1H), 4.78 (dd, *J* = 9.4, 1.6 Hz, 1H), 4.42 (dd, *J* = 12.3, 2.9 Hz, 1H), 4.29 (ddd, *J* = 13.4, 11.3, 3.7 Hz, 1H), 4.08 (dd, *J* = 12.3, 6.8 Hz, 1H), 3.80 (t, *J* = 10.3 Hz, 1H), 2.89 (dd, *J* = 12.3, 3.8 Hz, 1H), 2.51 (s, 3H), 2.19 – 2.17 (m, 4H), 2.09 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 170.8, 170.4 ($^3J_{C1,H3ax}$ = 5.5 Hz), 170.2, 170.1, 153.7, 134.1, 134.1, 130.1, 129.4, 128.9, 128.6, 99.1, 76.3, 74.4, 71.6, 71.4, 69.4, 68.9, 63.3, 59.0, 35.4, 24.8, 21.17, 21.15, 20.9; ESIHRMS Calcd for $C_{26}H_{29}NO_{13}Na$ $[M + Na]^+$, 586.1537; found, 586.1535.

2-Hydroxymethylbenzyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (201β).

After preparative HPLC of the mixture of cyclized anomers (eluting with hexanes/ethyl acetate 7:3 to 1:1 over 40 min), an analytical sample of **201β** was obtained. $[\alpha]^{RT}_D$ = - 5.9 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.29 (m, 2H), 7.27 – 7.24 (m, 1H), 7.17 (d, *J* = 7.1 Hz, 1H), 5.69 – 5.64 (m, 2H), 5.52 (d, *J* = 13.0 Hz, 1H), 5.40 (ddd, *J* = 7.4, 4.5, 2.8 Hz, 1H), 5.36 (d, *J* = 13.6 Hz, 1H), 4.69 (dd, *J* = 12.3, 2.6 Hz, 1H), 4.59 (td, *J* = 12.4, 3.7 Hz, 1H), 4.53 (dd, *J* = 9.4, 1.4 Hz, 1H), 4.49 (d, *J* = 13.0 Hz, 1H),

4.11 (dd, *J* = 12.2, 7.6 Hz, 1H), 3.72 (dd, *J* = 11.1, 9.6 Hz, 1H), 2.59 (dd, *J* = 12.5, 3.8 Hz, 1H), 2.51 (s, 3H), 2.24 (t, *J* = 12.6 Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 171.0, 170.7, 170.1, 167.9 ($^3J_{C1,H3ax}$ = 0.0 Hz), 153.9, 136.3, 133.2, 132.5, 129.3, 128.8, 127.8, 97.0, 75.2, 74.5, 72.9, 71.7, 69.2, 67.6, 62.8, 59.6, 34.9, 24.8, 21.2, 21.0, 20.9; ESIHRMS Calcd for $C_{26}H_{29}NO_{13}Na$ $[M + Na]^+$, 586.1537; found, 586.1530.

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (202α) and 2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (202β).

Cyclization of compound **199** was conducted by the general procedure for cyclization using sialyl donor **199** (0.031 g, 0.041 mmol), AW-300 molecular sieves (0.540 g), anhydrous CH_2Cl_2 (5.2 mL), *N*-idodosuccinimide (0.022 g, 0.097 mmol) and TfOH (3.6 μL, 0.041 mmol), and afforded a mixture of **202α** and **202β** (0.04 g, 20 %, 1:0.4, α:β).

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosidonic acid spirolactone (202α).

Preparative HPLC of the mixture of cyclized anomers (eluting with hexanes/ethyl acetate 9:1 to 7:3 over 50 min) afforded an analytical sample of **202α**. $[\alpha]^{RT}_D$ = +13.3 (*c* 0.06, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 – 7.29 (m, 2H), 7.22 (dd, *J* = 5.4, 3.5 Hz, 1H), 7.15 (dd, *J* = 5.3, 3.5 Hz, 1H), 5.77 (d, *J* = 13.2 Hz, 1H), 5.53 (dd, *J* = 9.5, 1.4 Hz, 1H), 5.48 (ddd, *J* = 9.5, 4.2, 2.3 Hz, 1H), 5.37 (d, *J* = 14.2 Hz, 1H), 5.21 (d, *J* = 13.1 Hz, 1H), 5.15 (ddd, *J* = 11.9, 10.0, 4.9 Hz, 1H), 4.69 (d, *J* = 14.2 Hz, 1H), 4.34 (dd, *J* = 12.6, 2.3 Hz, 1H), 4.21 (dd, *J* = 12.6, 4.2 Hz, 1H), 4.15 (dd, *J* = 10.5, 1.4 Hz, 1H), 3.64 (t,

J = 10.2 Hz, 1H), 2.68 (dd, *J* = 13.1, 4.9 Hz, 1H), 2.21 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 1.77 (t, *J* = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 170.0, 170.0, 169.5, 140.7, 135.1, 133.4, 131.5, 129.1, 128.9, 128.4, 98.3, 72.2, 70.4, 69.8, 68.8, 67.9, 67.6, 61.8, 56.6, 36.2, 21.2, 21.1, 20.9, 20.9; ESIHRMS Calcd for $C_{26}H_{29}NO_{12}SNa$ [M + Na]$^+$, 602.1308; found, 602.1306.

2-Hydroxymethylbenzyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosidonic acid spirolactone (202β).

Preparative HPLC of the mixture of cyclized anomers (eluting with hexanes/ethyl acetate 9:1 to 7:3 over 50 min) afforded an analytical sample of **202β**. $[\alpha]^{RT}_D$ = -40.0 (*c* 0.03, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 5.6, 3.3 Hz, 1H), 7.23 – 7.21 (m, 1H), 7.16 – 7.14 (m, 1H), 5.65 (d, *J* = 13.7 Hz, 1H), 5.56 (d, *J* = 7.3 Hz, 1H), 5.41 – 5.34 (m, 3H), 5.30 (d, *J* = 13.4 Hz, 1H), 4.58 (dd, *J* = 12.7, 2.4 Hz, 1H), 4.47 (d, *J* = 13.5 Hz, 1H), 4.17 (dd, *J* = 12.7, 5.3 Hz, 1H), 4.08 (dd, *J* = 10.5, 1.4 Hz, 1H), 3.65 (t, *J* = 10.2 Hz, 1H), 2.48 (dd, *J* = 13.5, 5.0 Hz, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 1.89 (t, *J* = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 170.2, 169.9, 169.7, 167.8, 140.6, 135.6, 132.9, 132.0, 129.1, 129.0, 128.2, 97.8, 70.4, 70.4, 69.9, 69.8, 69.7, 68.2, 68.2, 68.0, 67.4, 61.7, 56.8, 35.5, 21.13, 21.11, 20.94, 20.87. ESIHRMS Calcd for $C_{26}H_{29}NO_{12}SNa$ [M + Na]$^+$, 602.1308; found, 602.1307.

Conversion of 202α to 200α.

To a stirred solution of lactone **202α** (0.002 g, 0.003 mmol), 18-crown-6 (0.0001 g, 0.0003 mmol) and thioacetic acid (0.0003 g, 0.004 mmol) in anhydrous THF (0.03 mL), potassium *tert*-butoxide (0.0001 g, 0.001 mmol) was added under an argon atmosphere. The reaction mixture was stirred at 60 ^{0}C for 4 h, then cooled to room temperature, diluted

with ethyl acetate, washed with 1N HCl, saturated aqueous $NaHCO_3$, brine, and concentrated to dryness. UHPLC analysis revealed the product to be identical to **200α.**

Conversion of 202β to 200β.

To a stirred solution of lactone **202β** (0.002 g, 0.003 mmol), 18-crown-6 (0.0001 g, 0.0003 mmol) and thioacetic acid (0.0003 g, 0.004 mmol) in anhydrous THF (0.03 mL), potassium *tert*-butoxide (0.0001 g, 0.001 mmol) was added under an argon atmosphere. The reaction mixture was stirred at 60 ^{0}C for 4 h, then cooled to room temperature, diluted with ethyl acetate, washed with 1N HCl, saturated aqueous $NaHCO_3$, brine, and concentrated to dryness. UHPLC analysis revealed the product to be identical to **200β**.

General Procedure for Competition kinetic experiments.

Thiosialyl donors (cation clock precursor) **192**, **194**, **196**, and **199** were separately used in series of competition kinetic experiments. First, thiosialyl donor (0.013 mmol, 0.008 M) was co-evaporated with toluene (×3) and kept in vacuum overnight. AW-300 molecular sieves (174 mg, 102 mg/mL) anhydrous CH_2Cl_2 (1.7 mL), and 2-propanol (range specified for each donor - **Tables 26**-**29**) were added to the reaction flask under argon atmosphere and stirred at room temperature for 0.5 h before it was cooled to -40 °C. Then the reaction mixture was stirred for another 0.5 h at -40 °C under argon atmosphere. The reaction mixture was then activated by adding *N*-Iodosuccinimide (7 mg, 0.031 mmol) followed by the addition of trifluoromethanesulfonic acid (TfOH) (1.2 µl, 0.013 mmol), and the reaction mixture was stirred under argon at -40 ^{0}C for 2 h until the completion of the reaction. The reaction mixture was quenched with triethylamine (3.0 µl), diluted with CH_2Cl_2 (2 mL), and filtered through a pad of Celite. Organic layer was diluted again with CH_2Cl_2 (8.0 mL), washed with saturated aqueous $NaHCO_3$, saturated aqueous

$Na_2S_2O_3$, brine solution, and concentrated to dryness. The crude residue was dissolved in CH_2Cl_2 (0.5 mL), and an aliquot (20 µl) was diluted with acetonitrile (1.0 mL) and used in UHPLC/UV/MS analysis at the wavelength (λ) of 205 nm.

Competition Kinetics with Peracetylated β-Thiosialyl Cation Clock Precursor (193) at -40 °C.

Following the general procedure for competition kinetic experiments, β-thioglycoside **192** (0.010 g, 0.013 mmol) was used in series of experiments by changing the concentration of 2-propanol (1.5-30 equiv. – **Table 26**). All the kinetic runs were analyzed using the UHPLC method, flow rate: 0.35 mL/min; Initial: CH_3CN 30% in H_2O; 10.5 min: CH_3CN 30% in H_2O; 15.0 min: CH_3CN 100%; t_R (min): **200β** = 6.08, **207β** = 6.53, **200α** = 7.37, **207α** = 9.37. Relative ratios of *O*-sialosides with respect to the cyclized products were determined using the integrated peaks of the TUV trace (Table **26**).

Competition Kinetics with Peracetylated α-Thiosialyl Cation Clock Precursor (194) at -40 °C.

Analogously to the competition kinetics of donor **192**, a series of competition kinetic experiments was conducted using α-thioglycoside **194** (0.010 g, 0.013 mmol) and 2-propanol (1.5-10 equiv.). The crude reaction mixtures were analyzed using identical UHPLC method as for the donor **192** (Table **27**).

2-Hydroxymethylbenzyl (2-propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (207α) and 2-Hydroxymethylbenzyl (2-propyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (207β).

Pooling of crude reaction mixtures of kinetic runs from donor **3** and **194** followed by chromatographic purification using silica gel (eluting with hexanes/ethyl acetate 1:1 to 1:9) and further purification using preparative HPLC (eluting with hexanes/ethyl acetate 9:1 to 4:6 over 50 min) gave an analytical sample of pure **207α** and **207β**.

(**207α**). $[\alpha]^{RT}_D$ = - 5.5 (*c* 0.8, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (m, 1H), 7.38 (m, 2H), 7.32 (m, 1H), 5.45 (d, *J* = 11.9 Hz, 1H), 5.29 (s, 2H), 5.25 (d, *J* = 9.0 Hz, 1H), 5.15 (d, *J* = 11.9 Hz, 1H), 4.82 – 4.78 (m, 1H), 4.77 (s, 2H), 4.32 (d, *J* = 11.5 Hz, 1H), 4.14 – 3.98 (m, 4H), 2.80 (br s, 1H), 2.60 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.87 (m, 4H), 1.21 (d, *J* = 6.1 Hz, 3H), 0.99 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 170.8, 170.4, 170.4, 170.3, 168.2 ($^3J_{C1,H3ax}$ =6.0 Hz), 140.1, 132.7, 131.1, 129.6, 129.1, 128.3, 99.0, 72.6, 69.6, 69.4, 68.8, 67.6, 65.7, 63.1, 62.5, 49.2, 38.5, 24.7, 23.4, 23.2, 21.3, 21.1, 21.0, 20.9. ESIHRMS Calcd for $C_{30}H_{41}N_3O_{16}Na$ $[M + Na]^+$, 662.2425; found, 662.2413.

(**207β**). $[\alpha]^{RT}_D$ = - 1.3 (*c* 0.3, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (m, 1H), 7.40 – 7.35 (m, 2H), 7.30 (m, 1H), 5.40 – 5.26 (m, 4H), 5.26 – 5.21 (m, 1H), 5.20 – 5.17 (m, 1H), 4.77 (d, *J* = 3.9 Hz, 1H), 4.73 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.08 – 4.00 (m, 4H), 2.49 (dd, *J* = 13.1, 4.8 Hz, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.87 (s, 3H), 1.82 (t, *J* = 12.1 Hz, 1H), 1.16 (d, *J* = 6.1 Hz, 3H), 0.97 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 171.0, 171.0, 170.4, 170.3, 167.7 ($^3J_{C1,H3ax}$ = 0.0 Hz),

140.1, 132.7, 130.5, 129.4, 128.8, 128.1, 98.2, 72.1, 72.1, 69.0, 68.8, 67.56, 65.57, 62.9, 62.6, 49.8, 38.2, 24.3, 23.3, 22.8, 21.2, 21.1, 21.0, 20.9. ESIHRMS Calcd for $C_{30}H_{41}N_3O_{16}Na$ $[M + Na]^+$, 662.2425; found, 662.2407.

Competition Kinetics with 5-*N*,4-O-oxazolidinone protected β-Thiosialyl Cation Clock Precursor (196) at -40 ^{0}C.

The general procedure for competition kinetic experiments was applied in a series of kinetic experiments using sialyl donor **196** (0.010 g, 0.013 mmol) and 2-propanol (1.5-25.0 equiv). The crude reaction mixtures from kinetic runs were analyzed using UHPLC (flow rate: 0.35 mL/min; Initial: CH_3CN 35% in H_2O; 12.0 min: CH_3CN 35% in H_2O; 15.0 min: CH_3CN 100%; t_R (min): **201β** = 8.68, **201α** = 9.10, **208β** = 9.72, **208α** = 11.27). Relative ratios of *O*-glycosides with respect to cyclized products were determined by integrating the corresponding peaks of UV spectra (Table **28**).

2-Hydroxymethylbenzyl (2-propyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (208α) and 2-Hydroxymethylbenzyl (2-propyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (208β).

. The crude reaction mixtures from multiple kinetic runs were combined and purified using silica gel chromatography (eluting with hexanes/ethyl acetate 3:1 to 1:1) to afford a mixture of **208α** and **208β**, which was further purified by HPLC (eluting with hexanes/ethyl acetate 9:1 to 1:1 for 50 min) to afford analytical samples of pure **208α** and **208β**.

(**208α**). $[\alpha]^{RT}_D$ = + 1.5 (*c* 1.0, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.28 (m, 4H), 5.54 (dd, *J* = 7.7, 1.5 Hz, 1H), 5.38 – 5.29 (m, 3H), 4.74 (s, 2H), 4.54 (dd, *J* = 9.4, 1.5 Hz, 1H), 4.35 (dd, *J* = 12.3, 2.8 Hz, 1H), 4.02 (dd, *J* = 12.3, 7.0 Hz, 1H), 4.00 – 3.90

(m, 2H), 3.67 (dd, *J* = 11.2, 9.4 Hz, 1H), 2.85 (dd, *J* = 12.0, 3.5 Hz, 1H), 2.47 (s, 3H), 2.33 (br s, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.03 – 2.00 (m, 4H), 1.21 (d, *J* = 6.0 Hz, 3H), 0.95 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.3, 170.8, 170.6, 170.2, 168.9 ($^3J_{C1,H3ax}$ = 6.4 Hz), 153.9, 139.7, 132.7, 130.6, 129.6, 129.4, 128.5, 99.4, 75.6, 75.1, 72.0, 70.0, 69.7, 65.6, 63.2, 63.2, 59.3, 37.1, 24.9, 24.8, 23.1, 21.3, 21.0, 20.9. ESIHRMS Calcd for $C_{26}H_{29}NO_{13}Na$ $[M + Na]^+$, 646.2112; found, 646.2110.

(**208β**). $[\alpha]^{RT}_D$ = - 20.0 (*c* 0.1, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.28 (m, 4H), 5.60 (dd, *J* = 4.0, 2.1 Hz, 1H), 5.36 (s, 2H), 5.25 – 5.22 (m, 1H), 4.78 (s, 2H), 4.57 (dd, *J* = 12.2, 2.5 Hz, 1H), 4.51 (ddd, *J* = 12.5, 11.3, 3.7 Hz, 1H), 4.37 (dd, *J* = 9.4, 2.0 Hz, 1H), 4.07 (hept, *J* = 6.0 Hz, 1H), 4.01 (dd, *J* = 12.2, 7.7 Hz, 1H), 3.65 (dd, *J* = 11.2, 9.5 Hz, 1H), 2.73 (dd, *J* = 12.1, 3.7 Hz, 1H), 2.49 (s, 3H), 2.09 (s, 3H), 2.07 (s, 1H), 2.04 – 1.99 (m, 4H), 1.16 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.5, 171.1, 171.0, 170.0, 167.2 ($^3J_{C1,H3ax}$ = 0.0 Hz), 153.9, 140.1, 132.7, 130.6, 129.6, 129.0, 128.2, 98.0, 75.1, 74.8, 73.2, 71.8, 67.9, 65.9, 63.0, 62.9, 59.6, 37.2, 24.9, 24.4, 22.8, 21.3, 20.9, 20.8. ESIHRMS Calcd for $C_{26}H_{29}NO_{13}Na$ $[M + Na]^+$, 646.2112; found, 646.2103.

Competition Kinetics with 5-Isothiocyanate Protected β-Thioglycoside Cation Clock Precursor (199) at -40 °C.

Thioglycoside donor (**199**) (0.010 g, 0.013 mmol) and the acceptor 2-propanol (1.5-15 equiv) were used in series of kinetic runs following the general procedure for competition kinetic experiments. The crude reaction mixtures from kinetic runs were analyzed using UHPLC (flow rate: 0.35 mL/min; Initial: CH_3CN 39% in H_2O; 10.0 min: CH_3CN 39% in H_2O; 12.0 min: CH_3CN 41% in H_2O; 22.0 min: CH_3CN 41% in H_2O; 24.0

min: CH_3CN 100%; t_R (min): **202α/202β** = 17.13, **209β** = 17.87, **209α** = 19.53). Relative ratios of *O*-glycosides with respect to cyclized products were determined by integrating the corresponding peaks of UV spectra (Table **29**).

2-Hydroxymethylbenzyl (2-propyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-D-glycero-α-D-galacto-non-2-ulopyranosid)onate (209α).

Pooling of crude reaction mixtures of the kinetic runs of donor **199** followed by chromatographic purification using silica gel (eluting with hexanes/ethyl acetate 2:1) gave a mixture of **209α** and **209β**, which was further purified using preparative HPLC (eluting by hexanes/ethyl acetate 8:2 to 6:4 over 50 min) to afford pure sample of **209α**. $[\alpha]^{RT}_D$ = - 18.8 (*c* 0.2, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.26 (m, 1H), 5.51 – 5.44 (m, 1H), 5.44 (d, *J* = 11.5 Hz, 1H), 5.36 (ddd, *J* = 9.1, 4.3, 2.4 Hz, 1H), 5.14 (d, *J* = 11.9 Hz, 1H), 4.82 (d, *J* = 4.2 Hz, 1H), 4.80 – 4.75 (m, 1H), 4.30 (ddd, *J* = 12.7, 10.2, 2.5 Hz, 1H), 4.19 (dd, *J* = 12.6, 4.4 Hz, 1H), 4.03 (dd, *J* = 10.5, 1.6 Hz, 1H), 3.95 (hept, *J* = 6.0 Hz, 1H), 3.55 (t, *J* = 10.2 Hz, 1H), 2.71 (dd, *J* = 12.9, 4.7 Hz, 1H), 2.21 (s, 3H), 2.16 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 1.67 (t, *J* = 12.6 Hz, 1H), 1.18 (d, *J* = 6.0 Hz, 3H), 0.95 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 170.2, 170.1, 169.5, 167.5 ($^3J_{C1,H3ax}$ = 8.3 Hz), 140.1, 139.8, 132.2, 130.8, 129.5, 128.7, 128.1, 98.6, 71.6, 70.2, 69.3, 68.0, 67.6, 65.8, 62.9, 61.8, 56.4, 37.9, 24.5, 22.9, 21.1, 21.0, 20.8, 20.7. ESIHRMS Calcd for $C_{29}H_{37}NO_{13}Na$ $[M + Na]^+$, 662.1883; found, 662.1873.

2-Hydroxymethylbenzyl (2-propyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (209β).

Pooling of crude reaction mixtures of the kinetic runs of donor **199** followed by chromatographic purification using silica gel (eluting with hexanes/ethyl acetate 2:1) gave

a mixture of **209α** and **209β**, which was further purified using preparative HPLC (eluting by hexanes/ethyl acetate 20:7 to 6:4 over 50 min) to afford an analytical sample of **209β**. $[\alpha]^{RT}_{D}$ = - 37.9 (*c* 0.6, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.26 (m, 1H), 5.46 (dd, *J* = 6.4, 1.8 Hz, 1H), 5.34 – 5.25 (m, 4H), 4.75 (d, *J* = 1.3 Hz, 2H), 4.54 (dd, *J* = 12.6, 2.4 Hz, 1H), 4.12 (dd, *J* = 12.6, 6.0 Hz, 1H), 3.96 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.90 (h, *J* = 6.1 Hz, 1H), 3.61 (t, *J* = 10.1 Hz, 1H), 2.58 (dd, *J* = 13.2, 4.9 Hz, 1H), 2.14 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.62 (dd, *J* = 13.1, 11.6 Hz, 1H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 170.1, 169.5, 169.5, 167.1 ($^3J_{C1,H3ax}$ = 0.0 Hz), 140.0, 140.0, 132.4, 130.3, 129.3, 128.8, 128.0, 97.9, 70.4, 70.1, 69.5, 68.7, 67.9, 65.6, 62.7, 61.9, 56.8, 37.3, 24.0, 22.5, 20.99, 20.97, 20.7, 20.6. ESIHRMS Calcd for $C_{29}H_{37}NO_{13}Na$ $[M + Na]^+$, 662.1883; found, 662.1865.

2-Hydroxymethylbenzyl (Acetyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-amino-D-glycero-β-D-galacto-non-2-ulopyranosid)onate (211).

A solution of **194** (0.057 g, 0.076 mmol) in anhydrous CH_3CN/CH_2Cl_2 (3:7, 9.8 mL) was stirred at room temperature with AW300 molecular sieves (1.022 g) for 30 min before being cooled to -40 ^{0}C and stirred for another 30 min. *N*-Iodosuccinimide (0.048 g, 0.180 mmol) and TfOH (6.7 µL, 0.075 mmol) were added and reaction mixture was stirred at -40 ^{0}C under argon for 4.5 h before triethylamine (17.0 µL) was added. The reaction mixture was diluted with CH_2Cl_2, filtered through Celite, washed with saturated aqueous $NaHCO_3$, saturated $Na_2S_2O_3$, brine, and concentrated to dryness. The residue was purified by silica gel chromatography, eluting with hexanes/ethyl acetate 1:1 to 1:8 followed by toluene/ethanol 6:1 containing 1 % triethylanime, to afford **200β** (0.015 g, 40 %) and **211** (0.002 g, 4%). $[\alpha]^{RT}_{D}$ = - 12.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ

7.47 (d, *J* = 7.2 Hz, 1H), 7.39 – 7.35 (m, 2H), 7.32 – 7.28 (m, 1H), 5.75 (d, *J* = 9.0 Hz, 1H), 5.45 (td, *J* = 10.9, 5.1 Hz, 1H), 5.36 (d, *J* = 12.2 Hz, 1H), 5.29 (d, *J* = 12.3 Hz, 1H), 5.27– 5.24 (m, 2H), 5.16 – 5.12 (m, 1H), 4.75 (s, 2H), 4.53 (dd, *J* = 12.4, 2.3 Hz, 1H), 4.14 – 4.07 (m, 2H), 3.76 (q, *J* = 9.9 Hz, 1H), 2.71 (dd, *J* = 13.5, 5.0 Hz, 1H), 2.10 (s, 3H), 2.07– 2.03 (m, 4H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 171.14, 171.08, 171.0, 170.8, 170.7, 168.1 ($^3J_{C1,H3ax}$ = 0.0 Hz), 140.4, 132.9, 130.4, 129.4, 129.4, 128.0, 84.2, 71.8, 70.9, 68.9, 68.5, 66.7, 62.8, 62.6, 50.7, 35.8, 23.4, 23.3, 21.2, 21.0, 20.9; ESIHRMS Calcd for $C_{29}H_{38}N_2O_{14}Na$ $[M + Na]^+$, 661.2221; found, 661.2209

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ABSTRACT

**INFLUENCE OF SIDE CHAIN CONFORMATION ON THE MECHANISM(S) OF GLYCOSYLATION REACTIONS;
INVESTIGATION OF SIALIDATION REACTION MECHANISM(S) BY KINETIC STUDIES**

by

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May 2019

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Major: Chemistry (Organic)

Degree: Doctor of Philosophy

Glycosylation, being the key reaction of glycobiology and carbohydrate chemistry, demands robust and stereoselective glycosylation methods. The rational development of such methods necessitates knowledge of the glycosylation reaction mechanism and the factors influencing glycosylation reactions. The work discussed in this dissertation is focused on studying glycosylation reaction mechanisms and exploring the key factors influencing them.

Chapter one reviews the background to the research, starting with a brief introduction of the significance of carbohydrates as structural and energy-storage materials. The significance of cell surface carbohydrates and their roles in communication and related processes are discussed along with examples of their applications in biology and therapeutics. Approaches to the synthesis of carbohydrates, glycosylation reactions, and their mechanisms are then discussed. The stability of glycosyl oxocarbenium ions and the influence of protecting groups on oxocarbenium ions are also discussed. This chapter ends with a short description of neighboring group participation.

Chapter two describes the synthesis of a series of oxabicyclo[4.4.0]decane type compounds as models representing the ideal *gg*, *gt*, and *tg* conformations of the pyranoside side chain, and the derivation of experimental limiting coupling constants from them. The preparation of the model compounds, NMR analysis, triage of some compounds on the grounds of lack of conformational purity, derivation of correction factors, and finally, the application of limiting coupling constants in the calculation of side chain population are described. The absence of negative populations on the application of the limiting coupling constants to a series of literature compounds reveals the reliability of the new limiting data set.

Chapter three discusses the influence of protecting groups on the modulation of side chain conformation and the subsequent impact on the glycosylation reactions. The synthesis of a series of 4-*O*-derivatives of galacto- and glucopyranosides and 6-*O*-derivatives of glucopyranosides, their NMR analysis, and calculation of their side chain populations, and their correlation with the anomeric reactivity are discussed. Both aroyl and alkanoyl esters modulate the side chain population and moderate the electron-withdrawing effects of the ester, whereas, the 4-*O*-esters of glucopyranosides change the side chain conformation to support the electron-withdrawing effect of the ester. In both the galacto- and glucopyranose 4-*O*-series, highly electron-withdrawing esters, such as trifluoroacetyl and trichloroacetyl esters, modulate the side chain conformation to reinforce the electron-withdrawing effect of the esters. The glucopyranose 6-*O*-series did not show any significant modulatory effects as a function of protecting groups. Overall, this study reveals small but consistent changes in the side chain population, with corresponding small influences on glycosylation reactions.

Chapter four starts with a brief introduction to the biological importance of sialic acid glycosides, their occurrence, and synthesis. Approaches to stereoselective sialidation reactions are discussed, and recent advances in sialic acid chemistry along with manipulation of protecting groups in obtaining better α-selectivity are presented. Sialidation reaction mechanisms and the influence of protecting groups and additives, such as acetonitrile, are also surveyed. This chapter ends with a critical note on the significance of kinetic studies and the identification of intermediates in describing sialidation reaction mechanisms.

Chapter five describes a series of direct kinetic experiments on *N*-acetyl-oxazolidinone protected α- and β- thiosialyl donors designed to probe the influence of acceptor concentration and acetonitrile concentration in sialidation reactions. The synthesis of the thiosialyl donors, pseudo-first order kinetic experiments with respect to the acceptor, and kinetics experiments varying the acetonitrile percentage at constant donor and acceptor concentrations are reported. Both the α- and β-*O*-sialidation reactions strongly dependent on the acceptor concentration, revealing S_N2 or S_N2-like reaction pathways via β-sialyl triflate intermediate. The rate of the α-*O*-siliadation reaction also depends on the concentration of acetonitrile, indicating the likely participation of acetonitrile in the form of a β-sialyl nitrilium ion, which then undergoes S_N2 type reactions giving α-*O*-sialosides. Overall, this study recommends using the highest possible concentrations of the glycosyl acceptor and donor and employing acetonitrile as a cosolvent in the reaction medium to obtain better α-selectivities.

Chapter six describes the development of a cation clock reaction to probe the kinetics of sialidation reactions as an alternative to direct kinetic experiments. The

synthesis of a series of cation clock donors is described. Study of the intramolecular glycosylation (cyclization) reactions reveals an S_N2 type mechanism for the cyclization. This chapter also describes a series of competition kinetic experiments conducted by changing the acceptor concentration, revealing a considerable dependence on acceptor concentration in the formation of the α-*O*-sialosides. Hence, α-*O*-sialidation reactions follow S_N2 type reaction pathways, and increasing the electron-withdrawing effect of the protecting groups favors the S_N2 end of the reaction to a greater extent. The use of acetonitrile in sialidation reactions led to the isolation and characterization of a β-acetamide, which is strong evidence in support of the participation of acetonitrile in the form of β-sialyl nitrilium ions. Overall, the evidence presented in this study is consistent with the direct kinetic experiments.

The dissertation ends with a conclusion chapter and a complete set of experimental data for the studies discussed.

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Publications

- **Harsha Amarasekara**, Szymon Buda, Appi R. Mandhapati, and David Crich. Protecting Group Strategies for Sialic Acid Derivatives. In *Protecting Groups: Strategies and Applications in Carbohydrate Chemistry*, Wiley-VCH, **2019**; 283 – 306.
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- **Harsha Amarasekara**, David Crich. Probing the Mechanism of Sialidation Reactions via Kinetic studies. 16th Annual Chemistry Graduate Symposium, Department of Chemistry, Wayne State University, Detroit, MI, **2014**