Methodologies for the Rapid Synthesis of Hexoses and Their Application Towards a Differentially-Protected Chondroitin Sulfate Tetrasaccharide

Thesis by

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ACKNOWLEDGEMENTS

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When I went to Georgia Tech, I was so lucky to be allowed into the laboratory of Prof. David Collard my freshman year. Thankfully, Daniel Connor, my graduate student mentor, didn't run in fear when he noticed that my first notebook entries were drawn with CH_2-CH_2 instead of line-bond drawings. It was truly learning organic chemistry by immersion, and I loved every minute of it. Rob Kriegel, Suzi Moore, and Sahar Javanmard also mentored me as I made my way through the Collard laboratory. I am so grateful that they were patient enough both to step in and teach me when I needed help and to not step in too early so I could work things out myself.

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In addition, I need to thank Heather Wiencko and Ger Norton for being such wonderful friends. There are too many adventures to go into, but suffice it to say that they are both great characters. I also have to thank Angela Tooker who was my first year room mate, bridesmaid, Starbuck's buddy, and voice of reason throughout graduate school. Finally, I would also like to thank Panna Felsen for her encouragement and friendship.

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Finally, I would like to thank my husband, Warren, for so many reasons: for coming into lab with me late at night so I would have someone to talk to, for washing my glassware when I was so busy I didn't have time, for understanding when I had to stay behind instead of moving with him to New York, for being my best friend. For these reasons and so many more, I dedicate this thesis in his honor.

ABSTRACT

Carbohydrates play many roles in biology, but their study has been hindered by the paucity of methods available to rapidly access hexoses. In 2004, the MacMillan laboratory published a two-step aldol methodology that allows access to the erythrohexoses allose, glucose, and mannose. Described herein is the development of two methodologies to access hexoses. First, the two-step aldol methodology for accessing the erythrohexoses was expanded to allow access to a differentially-protected mannosamine and gulose. Also described is the discovery of a one-step aldol methodology for accessing hexoses, which has allowed access to a protected allose and gulose.

This methodology was applied to the synthesis of a differentially-protected chondroitin sulfate di- and tetrasaccharide. Chondroitin sulfate is a complex linear polysaccharide composed of alternating glucuronic acid and galactosamine residues that are heterogeneously sulfated. Combining the aldol methodology with a Cerny epoxide methodology developed in the Hsieh-Wilson laboratory, a core disaccharide was accessed. Model studies confirmed each position could be accessed selectively. Elaboration of this disaccharide to the protected tetrasaccharide was hindered by an unfavorable rearrangement during the tetrasaccharide coupling, so a second core disaccharide was synthesized. This core disaccharide was elaborated to a common intermediate to confirm that it should still allow selective access to each position, and then the disaccharide was elaborated towards the desired protected tetrasaccharide.

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LIST OF ABBREVIATIONS

$[\alpha]_D$	specific rotation at wavelength of sodium D line
A	CS-A tetrasaccharide
Å	Angstrom
A_{280}	absorbance at 280 nm
Ac	acetyl, acetate
All	Allyl
Aq	Aqueous
BAIB	Bisacetoxyiodobenzene
BDNF	Brain-derived neurotrophic factor
Bn	Benzyl
Boc	*Tert*-butyl carbamyl
Bu	Butyl
Bz	Benzoyl
C	CS-C tetrasaccharide
º C	degrees Celsius
Calcd	Calculated
Cat	Catalyst
CAN	ceric ammonium nitrate
CNAcOH	cyanoacetic acid
COD	*Cis*,*cis*-1,5-cyclooctadiene
CS	chondroitin sulfate
CSA	(±)-DL-camphor-10-sulfonic acid
Cy	Cyclohexyl
D	Doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DCM	Dichloromethane
Dd	doubly distilled or doublet of doublets
DDQ	2,3-dichloro-5,6-dicyano-*p*-benzoquinone
DIBAL-H	Diisobutylaluminum hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMSO	dimethyl sulfoxide
DMF	*N*,*N*-dimethylformamide

DNA	deoxyribonucleic acid
DNBA	2,4-dinitrobenzoic acid
Dr	diastereomeric ratio
DRG	dorsal root ganglion
DS	dermatan sulfate
E	CS-E tetrasaccharide
Ee	enantiomeric excess
Eq	Equation
Eq	Equivalent
Equiv	Equivalent
ESI	electrospray ionization
Et	Ethyl
FAB	fast atom bombardment
FGF-1	Fibroblast growth factor-1
G	gram(s)
GAG	Glycosaminoglycan
Gal	Galactose
GalNAc	*N*-acetylgalactosamine
Gem	Geminal
GlcA	glucuronic acid
GlcN	Glucosamine
GlcNAc	*N*-acetyl galactosamine
Glu	Glucose
H	hour(s)
HOMO	highest occupied molecular orbital
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HS	heparin sulfate
Hsepi	Heparan sulfate C-5 epimerase
Hz	hertz
I	*iso*
IdoA	iduronic acid
IR	infrared spectroscopy
J	coupling constant
Kcal	Kilocalorie
Λ	Wavelength
L	Liter
LA	Lewis acid
LA-N-5	Neuroblastoma cell line

LC	liquid chromatography
Lev	Levulinoyl
LUMO	lowest unoccupied molecular orbital
M	multiplet, milli or messenger
m/*z*	mass to charge ratio
M	micro
M	metal
MCA	monochloroacetyl
Me	methyl
MHz	megahertz
Min	minute(s)
MM	MacroModel
Mol	mole(s)
MS	mass spectrometry
MS	molecular sieves
mRNA	Messenger RNA
N	Nano
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
OH	Alcohol
P	Para
Pd/C	Palladium on Carbon
PDC	pyridinium dichromate
PG	Proteoglycan, protecting group
Ph	Phenyl
pH	hydrogen concentration in aqueous solution
PhMe	Tolyl
Phth	Phthaloyl
Piv	Pivolate
PMB	*para*-methoxybenzyl
*p*MP	*para*-methoxyphenyl
Ppm	parts per million
Pr	Propyl
PTN	Pleiotrophin
p-TSA	*para*-toluenesulfonic acid
Pyr	Pyridine
Q	Quartet
R	alkyl group
R	CS-R tetrasaccharide

R_f	retention factor
RAR	Retinoic acid receptor
RNA	ribonucleic acid
Rt	room temperature
SOMO	Singly Occupied Molecular Orbital
S	Singlet
S	Secondary
Sqv	squashed vulva
T	Triplet
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBHP	*Tert*-butyl hydrogen peroxide
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetyl
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl
TES	Triethylsilyl
Tert	Tertiary
Tetra	tetrasaccharide
Tf	trifluoromethanesulfonate
TFA	Trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin-layer chromatography
TMA	trimethylamine
TMS	Trimethysilyl
Troc	trichloroethoxycarbamyl
Ts	*p*-toluenesulfonyl
UV	Ultraviolet
X	Halogen

C h a p t e r 1

Investigations into a Two-Step Method to Access Aminosugars[34]

An Introduction to Organocatalytic Aldol Reactions

For the past 40 years, asymmetric synthesis has been a key focus in organic chemistry. This movement was christened by independent reports from Knowles and Noyori where chiral catalysts were used to produce chiral products from achiral substrates.[1] Since these reports, it has evolved into a multifaceted field of research involving the use of many different modes of catalysis.[2] Acknowledging the importance of asymmetric catalysis, the Nobel committee awarded the 2001 Nobel Prize to Knowles, Noyori, and Sharpless for their work in this field.[3]

Lewis acid catalysis has been extensively used in asymmetric synthesis to affect a range of organic transformations including oxidations, reductions, cycloadditions, conjugate additions, and π-bond activation reactions. A Lewis acid is considered to be an electron pair acceptor. Usually, a Lewis acid catalyst reversibly coordinates to the electrophilic substrate and lowers the energy of the lowest unoccupied molecular orbital (LUMO) of the electrophilic substrate to make it more susceptible to attack by the highest occupied molecular orbital (HOMO) of the nucleophile. Conversely, Lewis acids have also been shown to raise the HOMO of a nucleophilic substrate, which makes it more capable of attacking the LUMO of an electrophile (Figure 1.1). While these reactions are a staple in the chemist's toolbox, they are not without their drawbacks. Lewis acids can be

sensitive to air and water, so they require the use of anhydrous conditions and special handling. Also, the use of metals in the synthesis of human consumables such as pharmaceuticals has to be done judiciously to avoid metal contamination of the final products. In addition, many of these catalysts are costly to use on a large scale.[2]

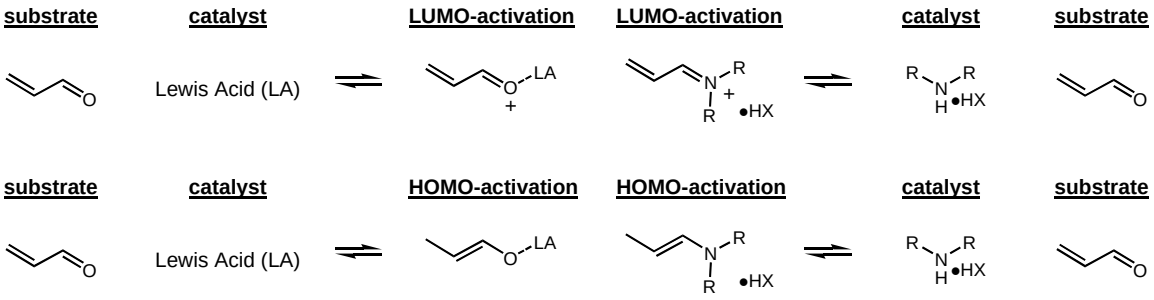
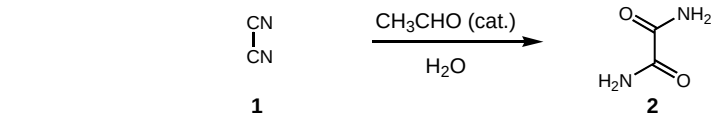


Figure 1.1: Secondary amines can behave like Lewis acids by performing both HOMO- and LUMO-activation.

Organocatalysts (that is, catalysts composed only of organic molecules) have been able to duplicate the reactivity of many Lewis acids. Furthermore, these catalysts do not possess many of the detrimental qualities of Lewis acids. Organic catalysts are typically air and water stable and are more cost-effective than their Lewis acid counterparts. [4]

The beginnings of the field of organocatalysis consist of a few unrelated reactions that peppered the early literature. The earliest reports of an organocatalytic reaction come in 1859 from Liebig who described the synthesis of oxamide from dicyan and water using acetaldehyde as a catalyst (Equation 1.1).[5] In 1912, Bredig and Friske described an alkaloid-catalyzed cyanohydrin methodology that afforded moderate enantioselectivities

(Equation 1.2).[6] In one example, using quinine as a catalyst, hydrogen cyanide was added to benzaldehyde to produce chiral cyanohydrin **4**. This methodology allowed access to both stereoisomers. While the enantioselectivities are low in both cases, these reactions represent the first enantioselective organocatalytic reactions described in the literature. The next report comes nearly half a century later when Pracejus describes an organocatalytic ketene methanolysis reaction to produce ester **6** (Equation 1.3).[7] In this reaction, the methanolysis of the ketene is catalyzed by strychnine (**7**). In the 1970's, two groups led by Hajos and Weichert described a proline-catalyzed Robinson annulation that now bears their name (the Hajos-Parrish-Eder-Sauer-Weichert reaction, Equation 1.4).[8] These results again lay dormant until the past decade when organocatalysis has become a defined field in organic chemistry. Since its awakening, organocatalysis has come to encompass a plethora of catalysts and activation methods that afford a variety of structural motifs.[4]



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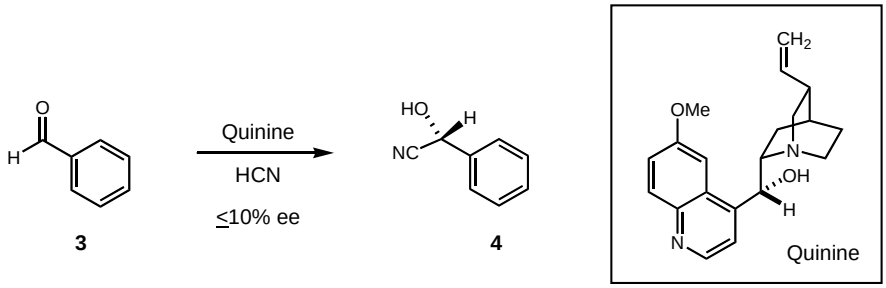

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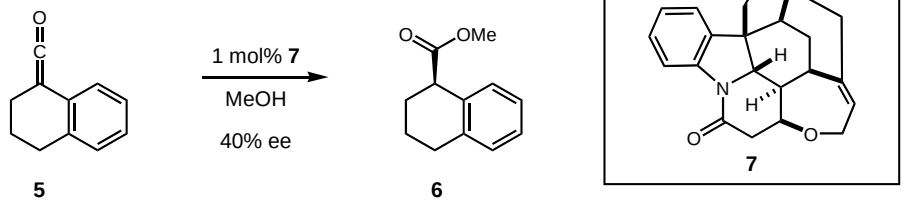

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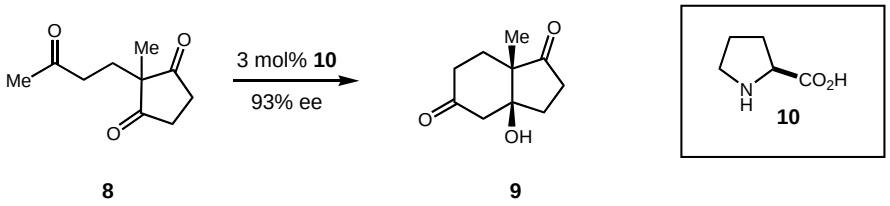


(1.4)

One of the first synthetic organocatalysts was the MacMillan imidazolidinone catalyst. This catalyst is capable of activating aldehydes and ketones in a fashion similar to Lewis acids and is both air and water stable (Figure 1.1). Its ability to impart chirality comes from the pendant groups on either side of the nitrogen that, when the substrate is bound to the catalyst, shield one face of the substrate while opening the other to attack (Figure 2.1).[4]

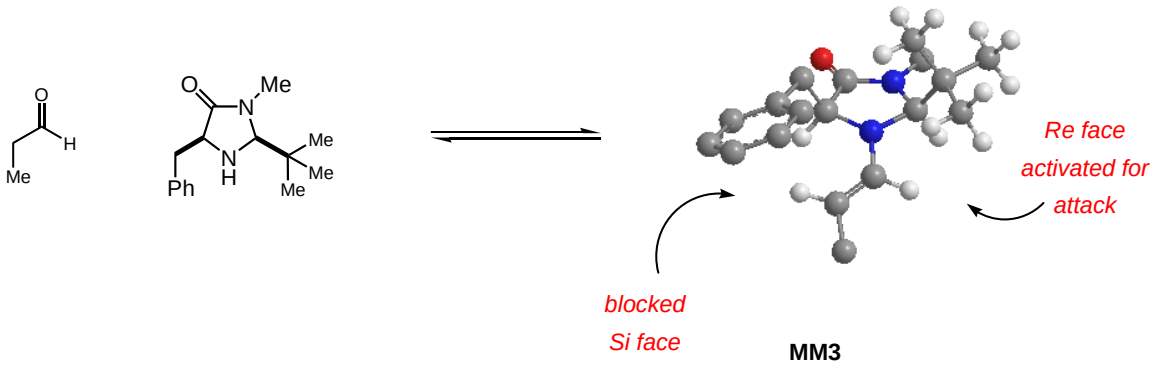


Figure 1.2: Chiral imidazolidinone catalysts allow enantiofacial discrimination.

This catalyst type has been used to affect iminium,[9] enamine,[10] and SOMO-activated[11] reactions (Figure 1.3), and both this catalyst and proline have been used by the MacMillan group to affect transformations such as the aldol reaction.[10b, 12]

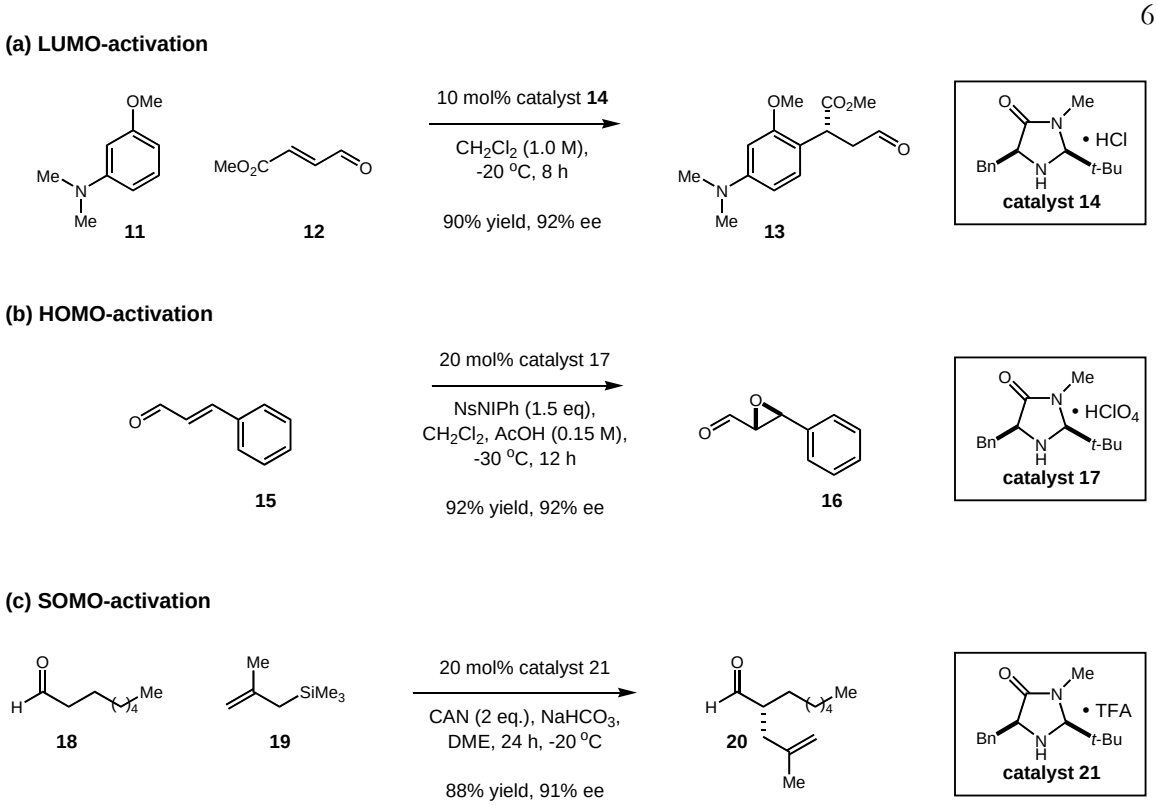


Figure 1.3: (a) Imidazolidinone **14** catalyzes the enantioselective 1,4-addition of substituted benzene **11** to α,β-unsaturated aldehyde **12**. (b) Imidazolidinone **17** catalyzes the epoxidation of α,β-unsaturated aldehyde **15** to epoxide **16**. (c) Single electron oxidation of the enamine formed between catalyst **21** and aldehyde **13** allows formation of aldehyde **20** through a radical pathway.

The aldol reaction is the general name for a reaction where an enolate nucleophile attacks an electrophilic carbonyl to form a new C-C bond (Figure 1.4). It was independently described by both Charles-Adolphe Wurtz and Alexander Borodin in 1872.[13] Since the discovery of the aldol reaction, numerous variants have been developed and it has

been employed in countless syntheses.[14] One challenge of the aldol reaction is controlling which reactant acts as the donor and which reactant acts as the acceptor (Figure 1.4).

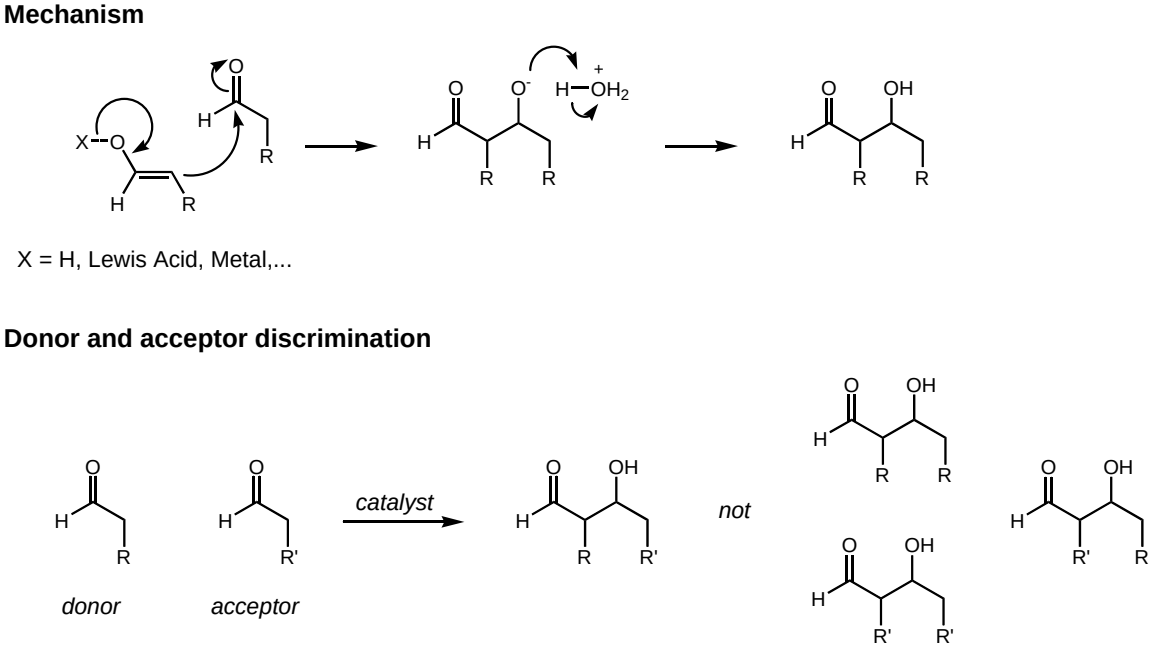
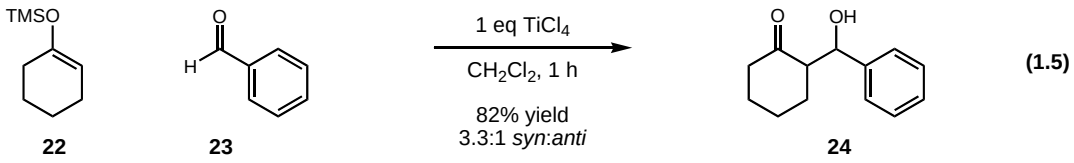


Figure 1.4: Mechanism and mechanistic challenges associated with the aldol reaction

One variant, known as the Mukaiyama aldol reaction, uses a preformed silyl enolate to help assign the role of donor and acceptor. Specifically, this reaction describes the

Lewis acid promoted or catalyzed addition of nucleophilic silyl enol ethers to aldehydes or ketones. The first description of this reaction was published by Mukaiyama in 1973 and described the titanium tetrachloride promoted addition of the silyl enol ether of cyclohexanone (**22**) into benzaldehyde (**23**) to produce the aldol product with an approximately 3:1 syn:anti relationship (**24**, Equation 1.5).[15]



22 **23** **24** **(1.5)**

Another variant on the aldol reaction that produces enantioenriched or enantiopure materials uses the Evans oxazolidinone, a chiral auxiliary that is appended to the aldehyde prior to enolization. This creates a chiral enolate for the purpose of the aldol reaction, and then the auxiliary can be cleaved to restore the aldehyde or provide other functional groups such as an acid or amide (Figure 1.5).[16] The Evans oxazolidinone has long been regarded as a major breakthrough in aldol technology, and countless natural products and pharmaceuticals owe their completion to this method. A drawback of this system is that the attachment and cleavage of the chiral auxiliary adds steps to the process of performing the aldol reaction, which ultimately decreases the overall yield.

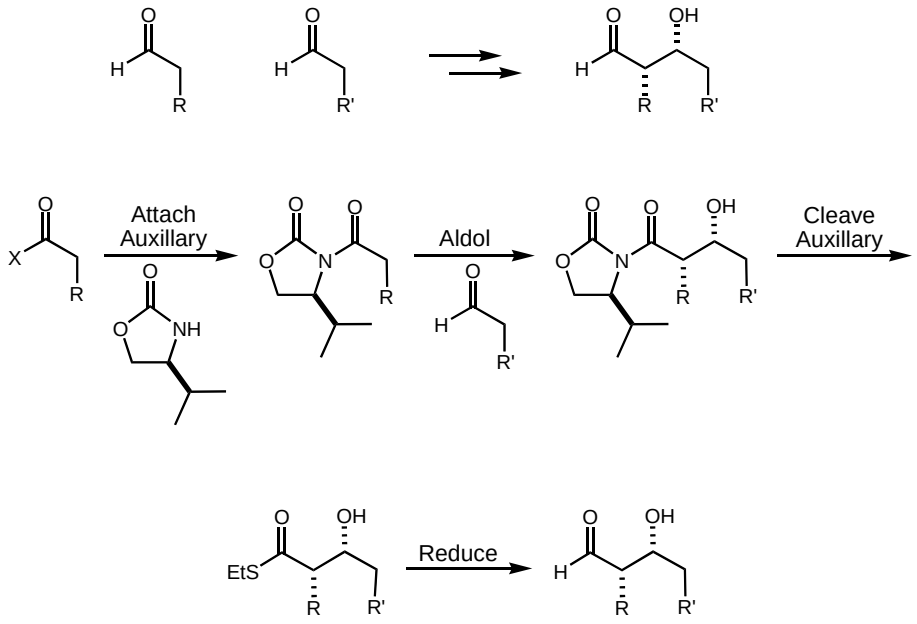


Figure 1.5: The Evans oxazolidinone methodology proceeds through the following steps: (a) attachment of the chiral auxiliary to the aldehyde, (b) aldol addition, (c) cleavage of the auxiliary, and (d) reduction of the cleaved product to the aldehyde. To add a second aldehyde to the aldol product, these steps would be repeated.

Therefore, the publication by Northrup and MacMillan in 2002 of a proline-catalyzed cross reaction of aldehydes produced considerable interest. This publication was followed by others detailing proline-catalyzed aldol reactions between α-oxyaldehydes and imidazolidinone-catalyzed aldol reactions to produce both syn- and anti-aldol products (Figure 1.6).[12] The catalytic cycles for the imidazolidinone and proline catalysts are believed to be similar. They both begin with the formation of an iminium ion that converts to the reactive enamine, and the aldol reaction takes place. However, the product of the imidazolidinone-catalyzed aldol reacts with another aldehyde in an acid-catalyzed acetal formation, while the proline-catalyzed reaction simply releases the product which does not

react further in the reaction media (Figure 1.7). The proline-catalyzed aldol reaction of α-oxyaldehydes provided a framework for the two-step synthesis of hexoses. Because both the imidazolidinone- and proline-catalyzed reactions failed to undergo multiple iterations, it was clear that a second aldol technology would have to be employed to produce hexoses.

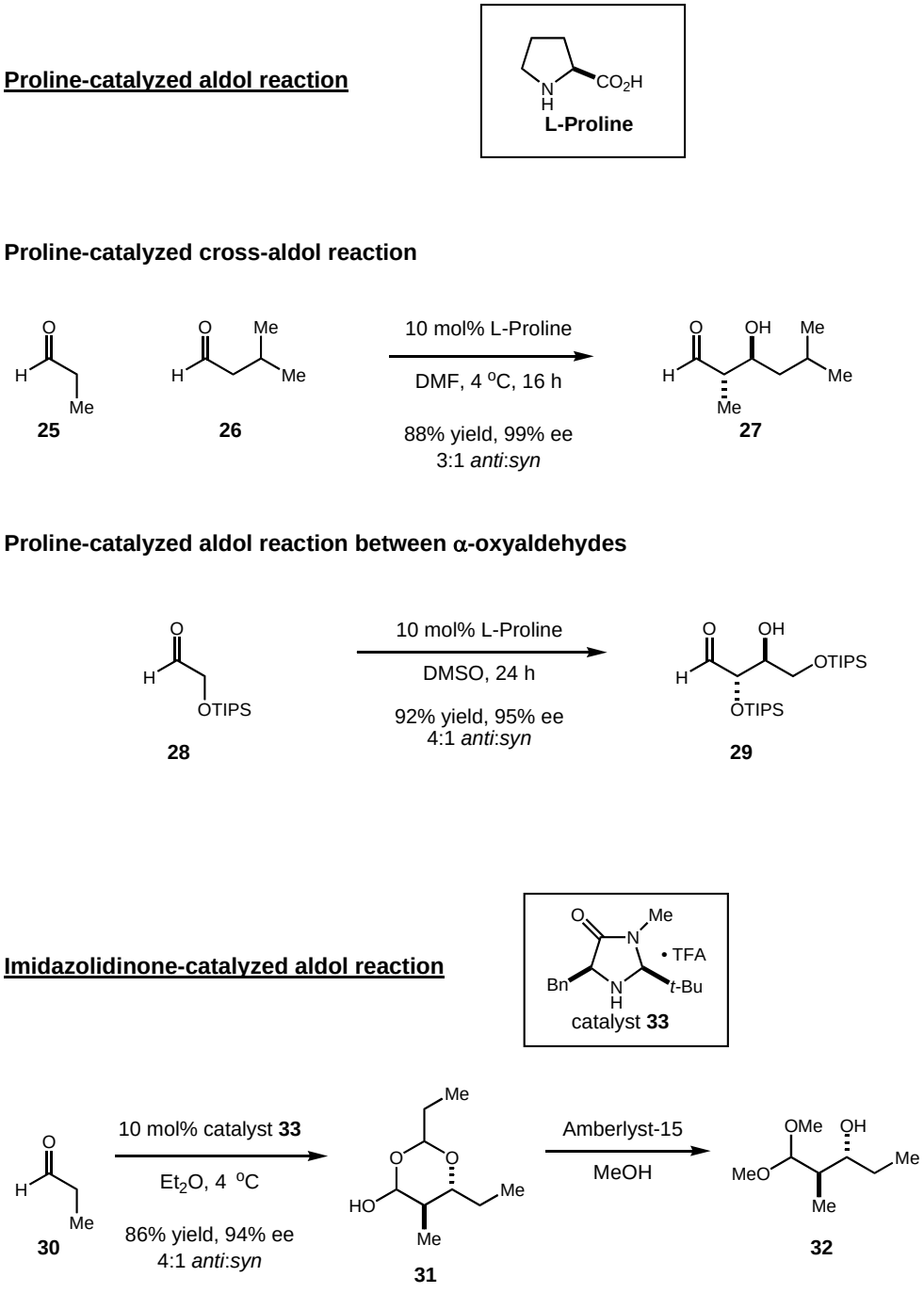


Figure 1.6: Both proline and imidazolidinone catalyst **35** can catalyze aldol additions.

A two-step synthesis of hexoses represented a great step forward for carbohydrate synthesis. Historically, the syntheses of hexose monomers have followed similar paths. One would begin with the natural hexose and elaborate it through a series of protections and deprotections to a usable saccharide for coupling. Protection motifs similar to the ones produced by the MacMillan methodology routinely took more than 15 steps to access.[17] This methodology also allowed the production of rare sugars: allose, L-sugars, and ^{13}C-labeled sugars. The most recent technology in selective glycoside protection was published by Wang, et al. and described a similar protection motif for glucose in four steps using a novel one-pot protection method.[18]

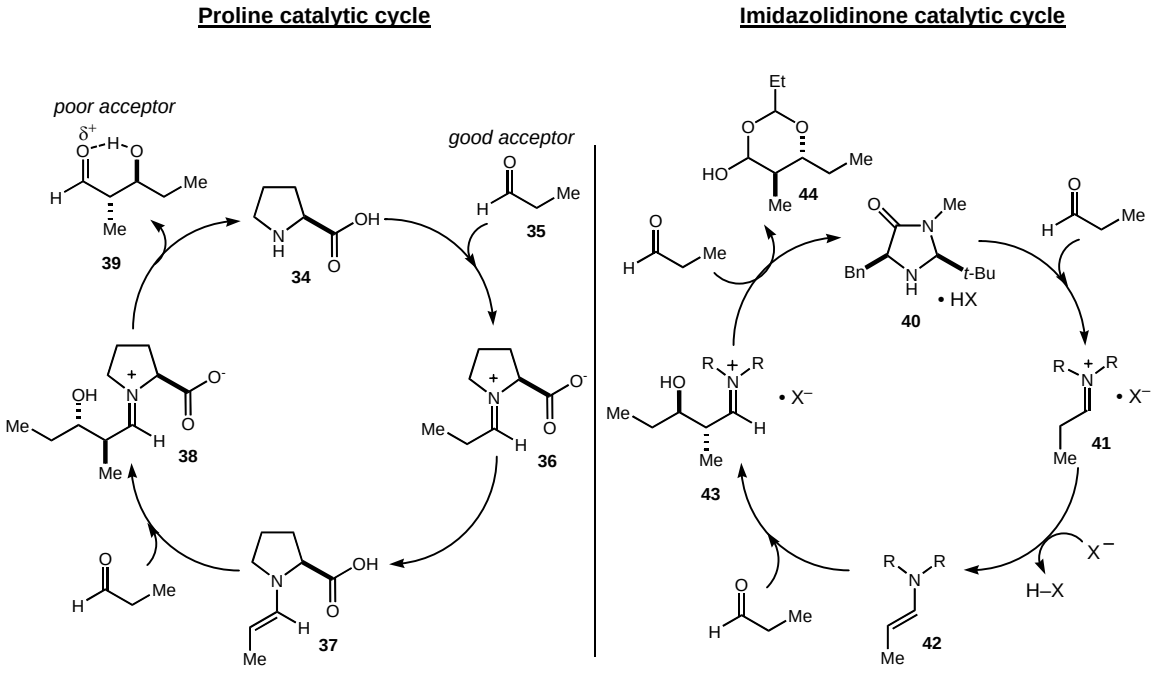
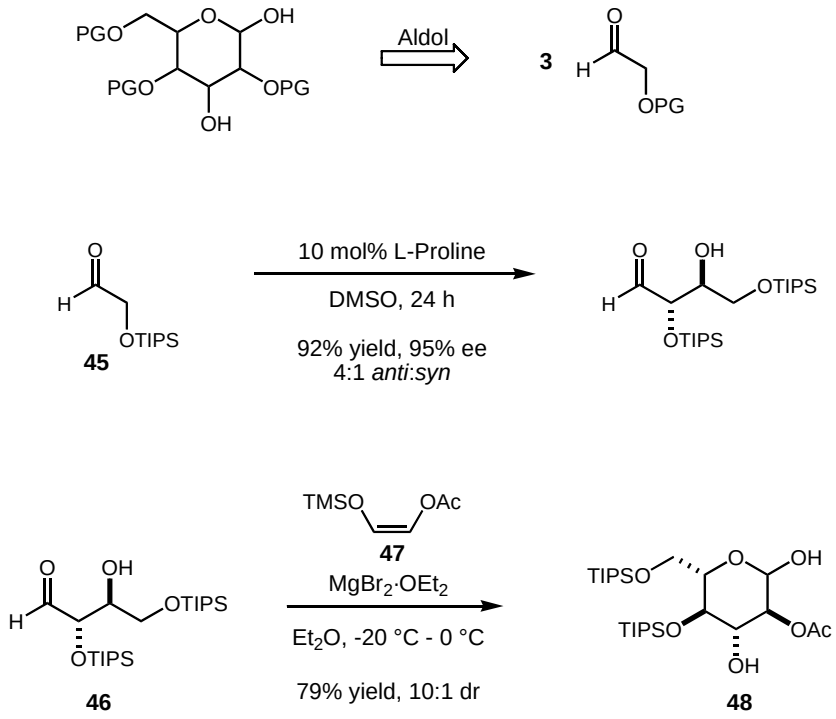


Figure 1.7: In both the proline and imidazolidinone catalytic cycles, the secondary amine condenses with the aldehyde to form an iminium ion which is converted to an enamine. This species adds into the second aldehyde to form the aldol product.

The concept behind the two-step synthesis is simple. A hexose can be thought of as the product of two aldol reactions combining three aldehydes. This two-step synthesis is the realization of this concept and relies on the combination of a proline-catalyzed aldol reaction between two protected α-oxyaldehydes, followed by a Mukaiyama aldol reaction between the resulting α,β,γ-oxyerythrose and a silyl enol ether. The aldol product cyclizes to form the hexose (Scheme 1.1).[20] This result was exciting because it was thought this reaction might produce a polymer instead of cyclizing (Figure 1.8).



Scheme 1.1: In the retrosynthetic sense, a hexose can be considered the product of two aldol reactions between three aldehydes. This has been achieved in the forward sense through a two-step procedure. First, TIPS-aldehyde **45** is dimerized through a proline-catalyzed aldol reaction to produce erythrose **46**. Then erythrose **46** and enolate **47** combine in a second aldol reaction to form protected glucose **48**.

The reagents used in this methodology were carefully chosen. The aldehyde used in the Mukiayama aldol reaction is the triisopropylsilyloxy-protected erythrose (TIPS-erythrose, **46**) produced by the proline-catalyzed reaction (Scheme 1.1). This aldehyde was selected for three reasons. First, the triisopropylsilyl (TIPS) protecting group is considered to be a very acid- and base-stable silyl protecting group and is useful in saccharide synthesis because it allows for other protecting groups to be installed and removed without

fear of removing the silyl group. Second, there are many ways reported in the literature to selectively remove a primary TIPS group in the presence of a secondary TIPS group.[20] Finally, the TIPS-erythrose could be prepared in good yield and diastereoselectivity (92% yield, 4:1 *anti*:*syn,* Scheme 1.1), and the diastereomers could be separated via column chromatography. The enolates were chosen to place a protecting group at the 2-position of the resulting hexose that would be compatible with the conditions used to remove the TIPS groups (hydrogen fluoride in pyridine or tetrabutylammonium fluoride (TBAF) can be used to remove a TIPS group).[20] In addition, the protecting groups on the enolate were chosen to allow the installation of either a participating or nonparticipating group (Figure 1.11). The choice of solvent and Lewis acid greatly affected the stereochemical outcome of the reaction. For example, when the TIPS dimer was combined with acetoxyenolate **47** in dichloromethane with titanium (VI) tetrachloride, the resulting saccharide was protected allose **49**. When the Lewis acid was changed to a magnesium bromide diethyl etherate complex, protected mannose **50** was produced. When the solvent of this reaction was changed to ether, the resulting saccharide was glucose **51** (Scheme 1.2).

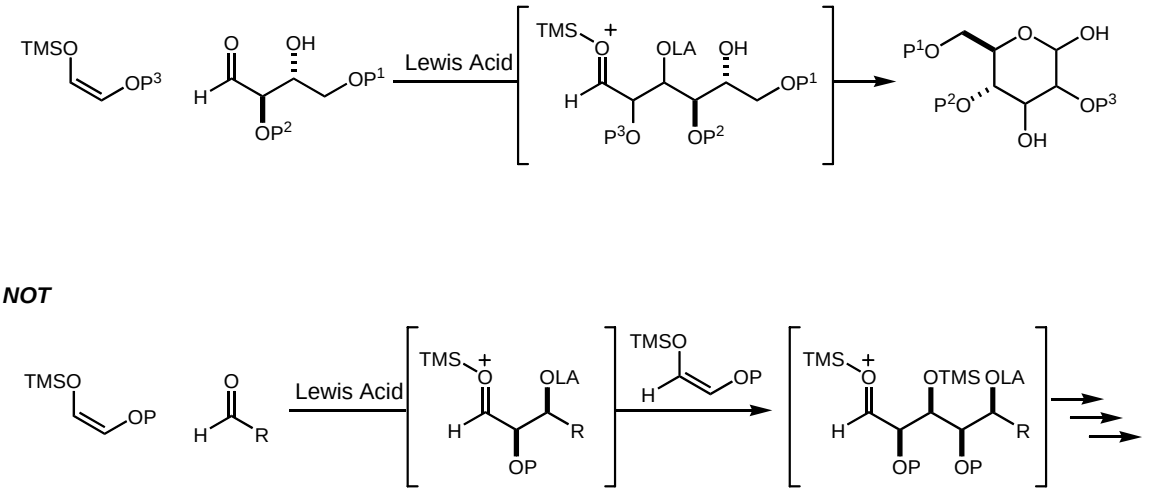
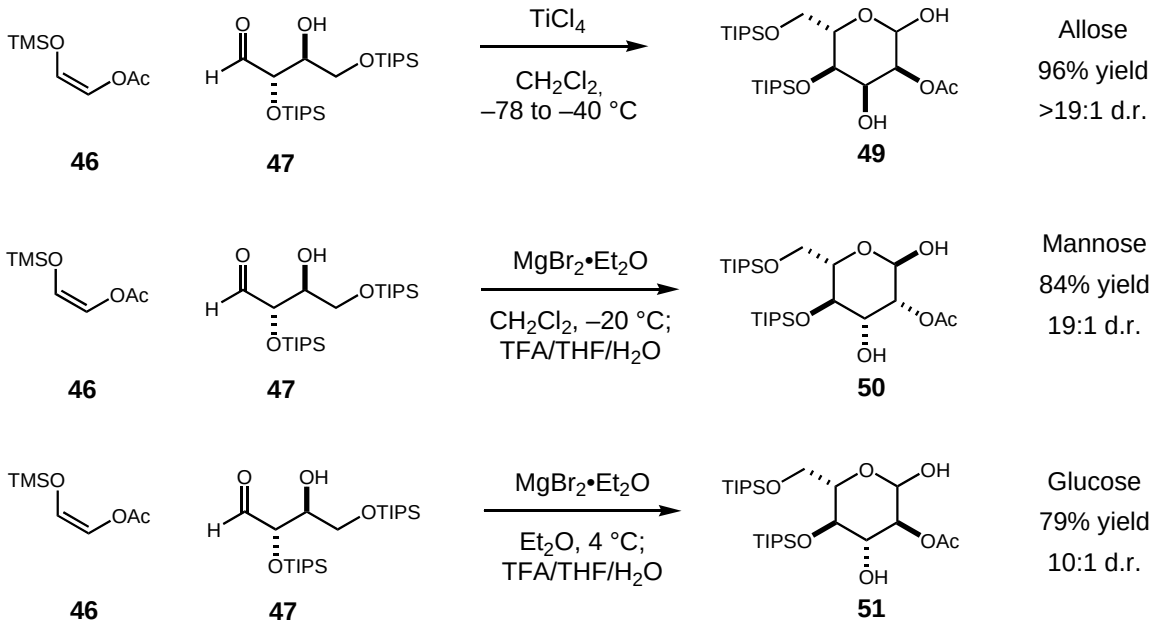


Figure 1.8: The δ-hydroxyaldehyde cyclizes to form a protected hexose, preventing subsequent aldol reaction which would produce a polymer.

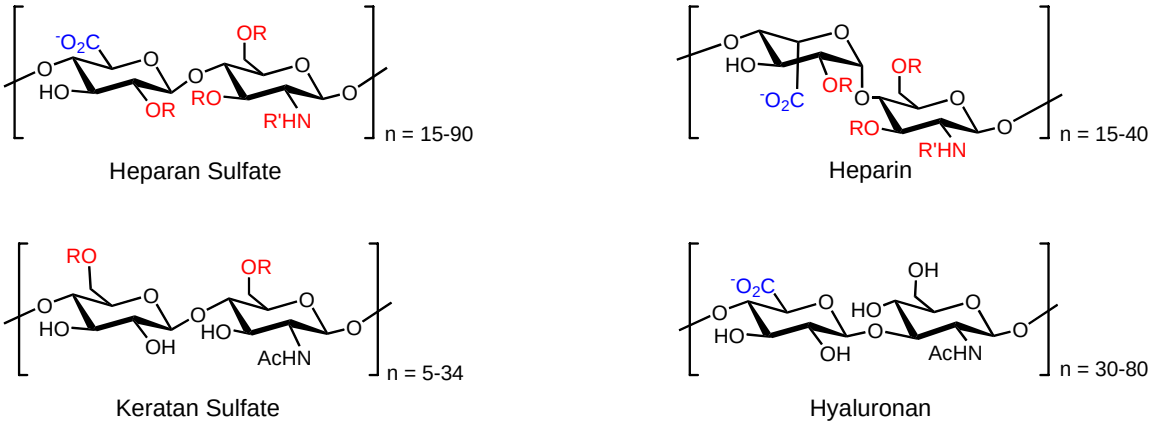
This methodology has been lauded for allowing chemists to efficiently access both natural and non-natural sugars. As the biological importance of glycosylation has become more apparent, the ability to rapidly synthesize saccharides becomes more and more necessary. For many biologically active polysaccharides, the ability to synthesize the saccharide of interest or non-natural saccharide probes has allowed scientists to better explore the biological roles that these sugars play.



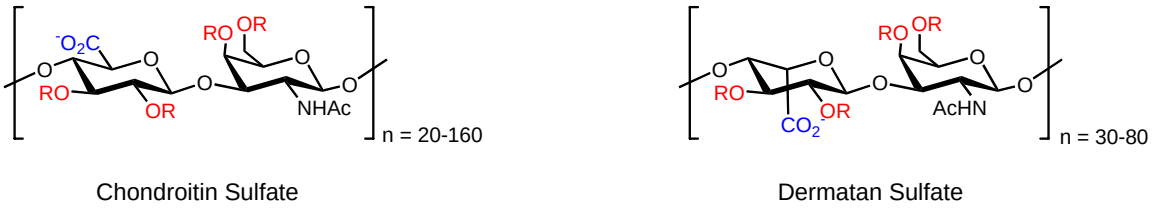
Scheme 1.2: By varying Lewis acid and solvent, acetoxyenolate **46** and TIPS-erythrose **47** can be combined to form a differentially-protected allose, mannose, and glucose.

For example, those studying glycosaminoglycans (GAGs) have relied heavily on synthesis to allow them to analyze the many biological roles these molecules play. Made up of alternating uronic acid and aminosugar residues, GAGs are polymeric and can consist of 2~200 disaccharide units. This broad class of molecules is commonly thought to have two subclasses (the glucosaminoglycan class and the galactosaminoglycan class). The glucosaminoglycan class is made up of four types: heparan sulfate, heparin, keratan sulfate, and hyaluronan. The galactosaminoglycan class consists of chondroitin sulfate and dermatan sulfate. These molecules can be sulfated at various positions around the ring, and they are given a letter designation to refer to each sulfation pattern (Figure 1.9).[21]

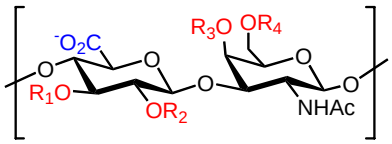
Glucosaminoglycan Class



Galactosaminoglycan Class



R = H or OSO_3^-
R' = Ac or OSO_3^-
n = average lengths



Chondroitin Sulfate-A: R_1, R_2, R_4=H; R_3=OSO_3^-
Chondroitin Sulfate-C: R_1, R_2, R_3=H; R_4=OSO_3^-
Chondroitin Sulfate-D: R_1, R_3=H; R_2, R_4=OSO_3^-
Chondroitin Sulfate-E: R_1, R_2=H; R_3, R_4=OSO_3^-

Figure 1.9: Glycosaminoglycans can be divided into two classes, glucosaminoglycans and galactosaminoglycans, based on the aminosugar residue present in the polysaccharide chain. These classes are further divided based on the uronic acid or glycosyl residue present in the polysaccharide backbone and the sulfation pattern displayed along the chain.

Glycosaminoglycans exist as heterogeneous polymeric chains that can consist of a mixture of glycosaminoglycan subtypes or one subtype, and the chain can display a variety of sulfation patterns. They usually reside on the cell surface and in the extracellular matrix where they are involved in numerous biological functions, ranging from cell growth to protein activity regulation. It is believed that the glycosaminoglycan conformation and sulfation pattern determines the biological activity. Synthetic glycosaminoglycans have been used to help determine the specific motif responsible for a biological event.[21]

For example, heparin is known to act as an anticoagulant, though the mechanism for this behavior was unclear. In the 1980s, the Sinay and Choay groups collaborated to synthesize a heparin pentasaccharide, and they used this synthetic pentasaccharide to determine how heparin inhibits coagulation. It was determined that a pentasaccharide expressing a specific sulfation pattern binds to antithrombin III, which induces a conformational change. This new complex binds an assortment of proteins and proteases involved in coagulation and inhibits clot formation (Figure 1.10).[21a]

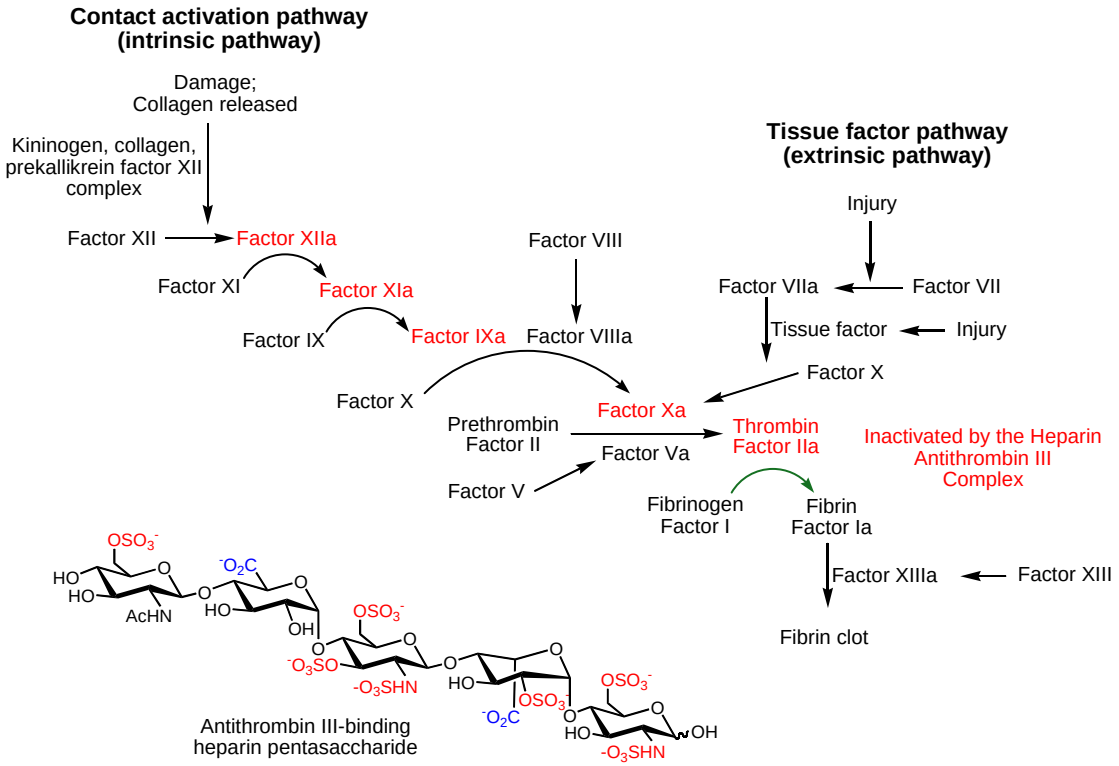


Figure 1.10: Heparin inhibits coagulation by binding and activating antithrombin III, which inhibits many factors along the coagulation pathway.

The synthesis of the heparin pentasaccharide by Sinay and Choay took 62 steps (33 in the longest linear sequence) and at least two years to complete.[21a] A more modern approach by Seeberger took 67 steps (with 28 in the longest linear sequence) to access a sulfated heparin tetrasaccharide.[22] The ability to access these molecules more quickly would be a great asset to glycobiologists.

The Synthesis of 2-Aminosugars Using a Two-Step Approach

The MacMillan sugar methodology allowed chemists to make many hexoses, but there were still sugars that needed to be accessed. For example, 2-aminosugars (sugars like glucosamine and galactosamine) are present in a plethora of glycoconjugates. It was believed that this technology should also allow an amine to be placed at the 2-position. To apply the same aldol technologies to 2-aminosugars, we first needed to find a way to make enolates of aminoaldehydes.

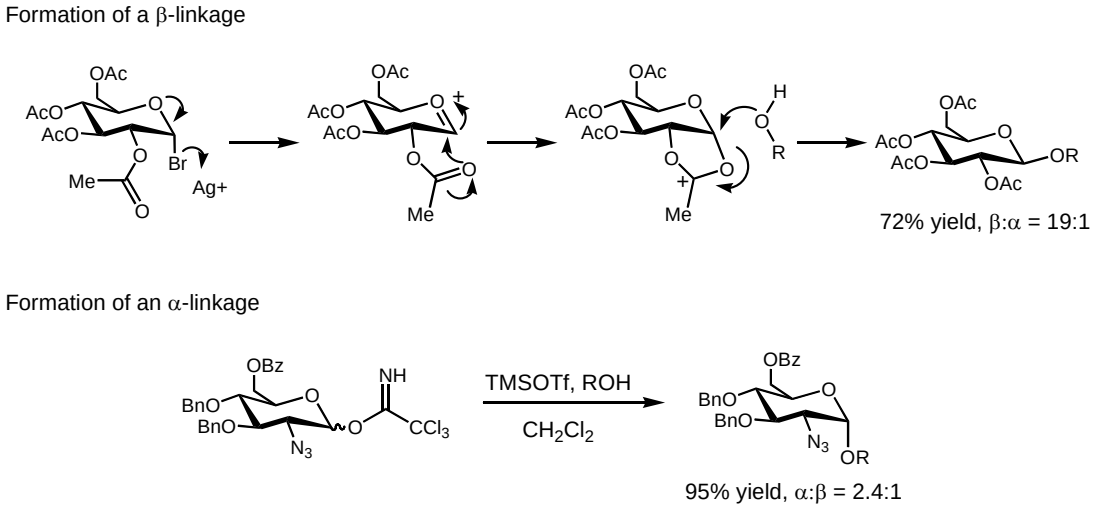
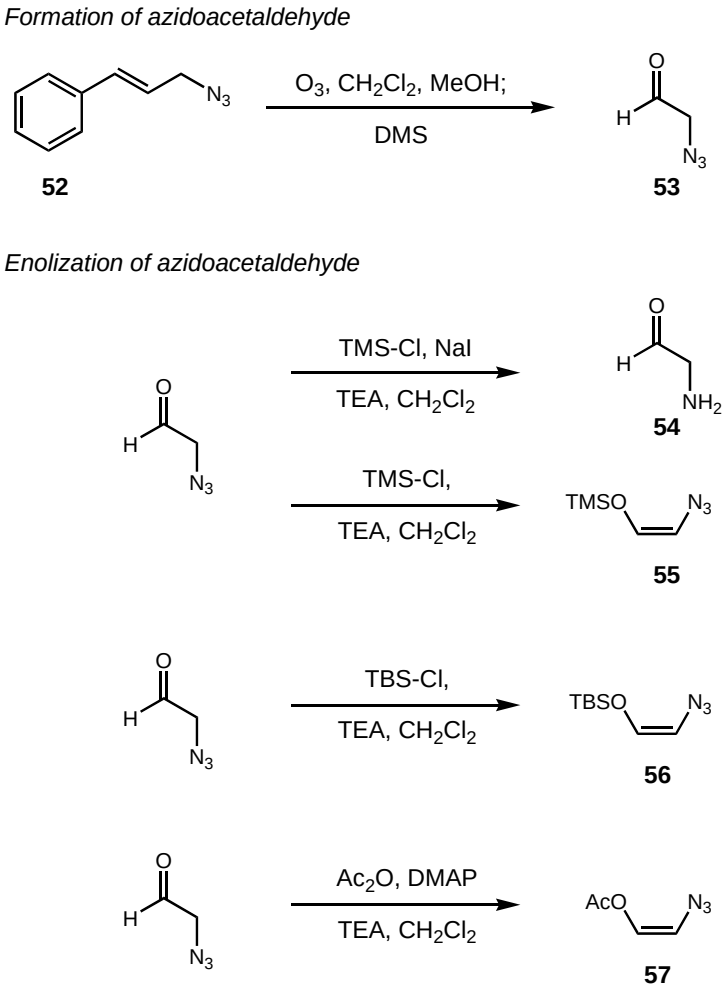


Figure 1.11: Participating groups refer to protecting groups that can block one face of the donor, forcing the acceptor to attack the unhindered face. Non-participating groups refer to protecting groups that do not perform in this manner.

Because any protecting group at the 2-position of a pyranose can affect the α:β ratio during coupling through the participating group effect (Figure 1.11), it was important to choose both protecting groups that participate in the coupling reaction and ones that do not participate. The first aldehyde chosen for enolization was azidoacetaldehyde (**53**, Scheme 1.3). The azide group does not participate in coupling reactions and can be converted to an amine via hydrogenation.[23]

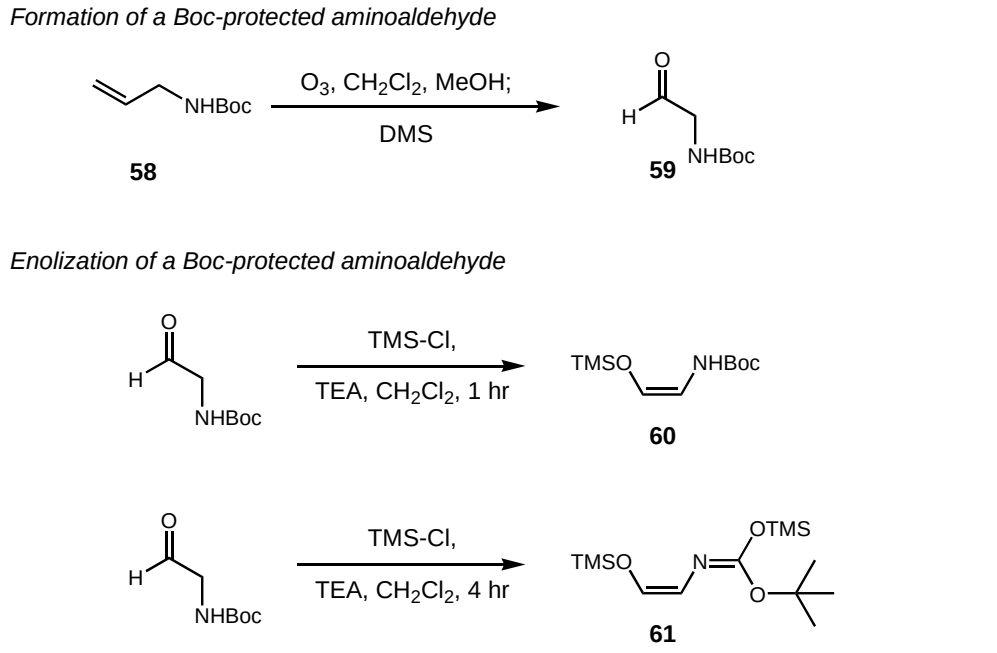
Forming the enolate of azidoacetaldehyde posed a few challenges. Aldehyde **53** was synthesized via the ozonolysis of cinnamylazide (**52**, Scheme 1.3).[32] Azidoacetaldehyde was not bench stable, and a trimer byproduct would begin to form when the azidoacetaldehyde was in concentrations greater than 0.5 M. This meant that isolation of the aldehyde and the conditions for its subsequent enolization had to be modified to allow for the purification and enolization to be performed on a 0.5 M solution of the aldehyde.



Scheme 1.3: Azidoacetaldehyde (**53**) was formed via ozonolysis of cinnamylazide (**52**) and enolized to form the TMS-, TBS-, and Ac-enolates.

Also, the aldehyde is typically distilled prior to enolization. However, there was a concern that the azidoacetaldehyde may be explosive when distilled. In addition, on the two occasions that the aldehyde was distilled, distillation was determined to be a low-yielding method for purification. Fortunately, experiments showed that the aldehyde could be enolized without being distilled.

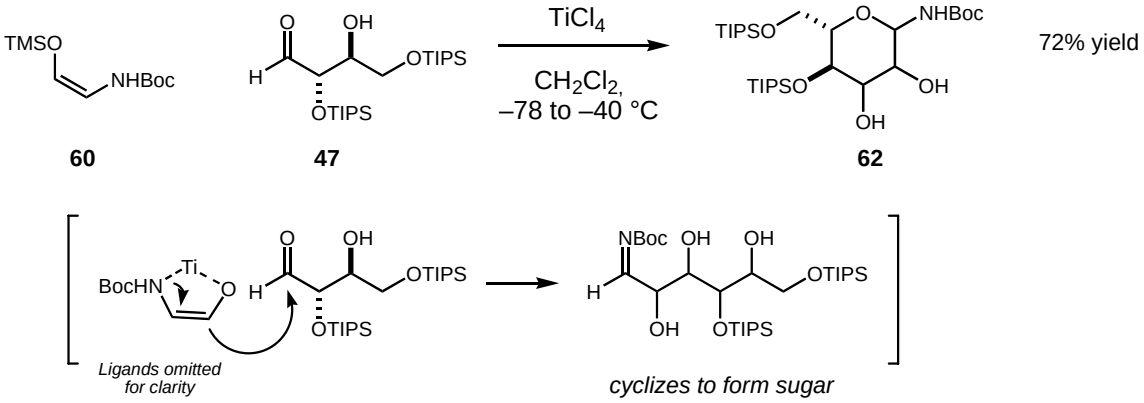
Next, the original conditions used for enolization were incompatible with the azide functionality. When the azidoacetaldehyde was exposed to the original enolization conditions (triethylamine, trimethylsilyl chloride, and NaI in acetonitrile), the azide was reduced to the amine. It was believed that the iodotrimethylsilane generated *in situ* was reducing the azide.[24] Accordingly, when the sodium iodide was omitted, the enolization reaction proceeded as desired (Scheme 1.3).



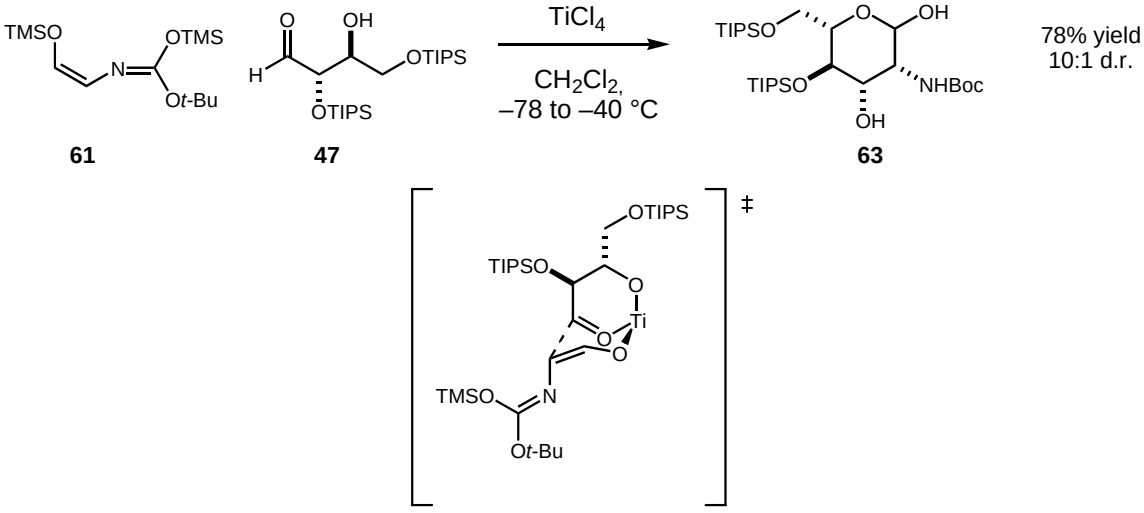
Scheme 1.4: Boc-protected aminoaldehyde **59** was synthesized via ozonolysis of Boc-protected allylamine **58.** Aldehyde **59** was elaborated to aminoenolate **60** and iminoenolate **61**.

Once formed, azidoenolate **55** was difficult to purify. Normally, to purify an enolate, the reaction is condensed and the residue is washed with dry ethyl ether. The ethereal extracts are then condensed and distilled. The enolate was unstable to distillation; ^{1}H NMR analysis of the distillate showed only the aldehyde. Column chromatography over triethylamine-treated silica also hydrolyzed the enolate. Consequently, the ethereal extracts were concentrated and used without distillation. The tributyldimethylsilyl-enolate (**56**) and the acetoxy-enolate (**57**) of azidoacetaldehyde were also synthesized with the hopes of creating a more stable enolate for purification, but both enolates proved unstable to distillation and column chromatography (Scheme 1.3).

Formation of a 1-aminosugar



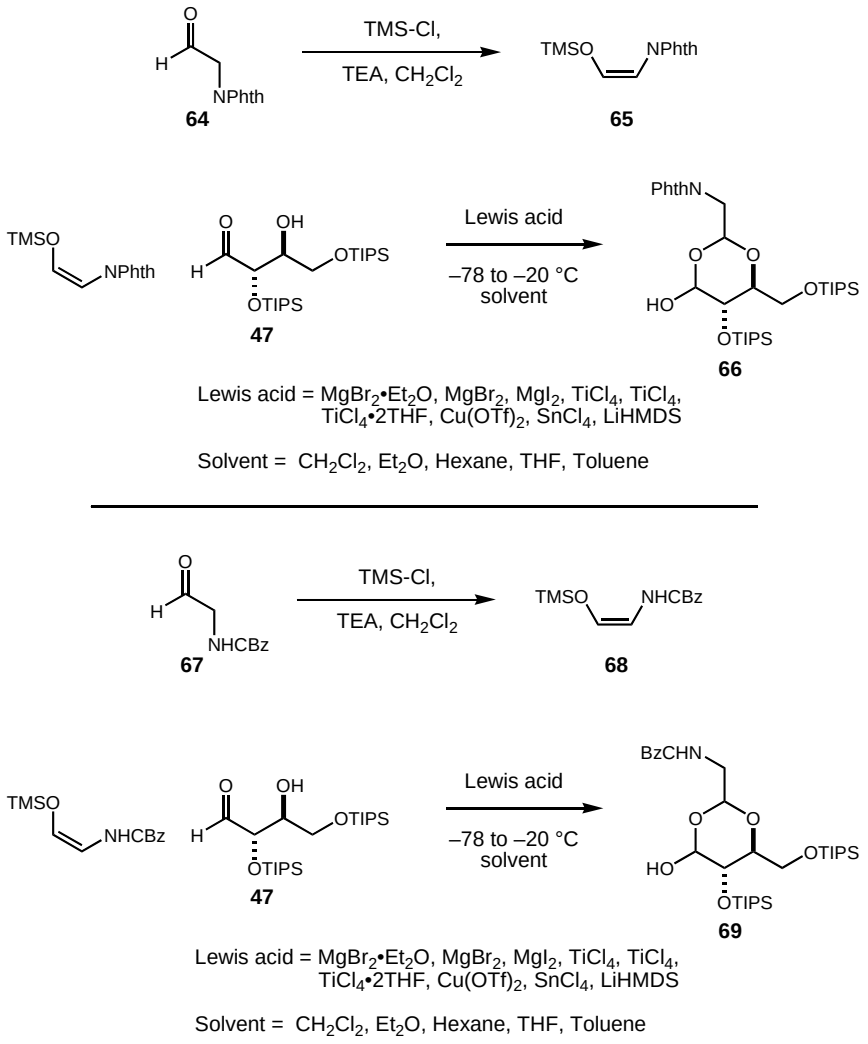
Formation of a 2-aminosugar



Anti-Felkin, *anti*-aldol

Figure 1.12: Aminoenolate **60** and TIPS-erythrose **47** combine to form 1-aminosugar **62**, while iminoenolate **61** and TIPS-erythrose **47** combine to form 2-aminosugar **63**.

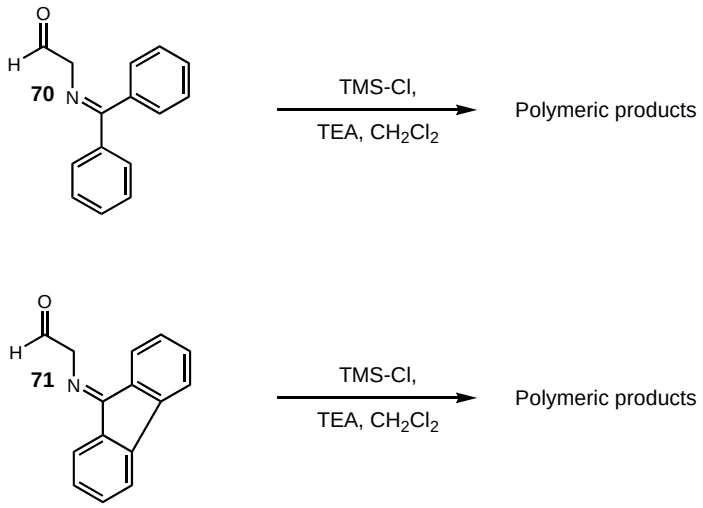
All three enolates were subjected to the aldol conditions with a variety of Lewis acids, though none afforded a 2-aminosugar product. Subjecting the azidoenolates to either tin (VI) tetrachloride or titanium (VI) tetrachloride reduced the azide to the amine, as shown by the isolation of aminoacetaldehyde from these reactions. Because of these complications, a different protecting group was chosen for installing an amine at the 2-position.



Scheme 1.5: Aminoenoates **64** and **67** did not participate in an aldol reaction with TIPS-erythrose **47.**

The *tert*-butylcarbamyl (Boc) protecting group is used frequently in carbohydrate chemistry because it functions as a good participating group in couplings and is stable to base and mild acids.[25] Though Boc-protected aminoaldehyde **59** is commercially available, it can also be easily prepared via an ozonolysis.[32] When the Boc-protected aminoaldehyde

was exposed to the enolization conditions for only an hour, the product was Boc-protected aminoenolate **60**. Surprisingly, exposing the Boc-protected aminoaldehyde to the enolization conditions for longer (3~5 hours) produced iminoenolate **61** (Scheme 1.4). Both enolates were tried in an aldol reaction with the TIPS-erythrose (using titanium (IV) tetrachloride and dichloromethane) with interesting results. The Boc-protected aminoenolate produced a 1-aminosugar (**62**). This implied that the nitrogen lone pair was donating into the π*-orbital of the enolate and initiating the reaction (Figure 1.12). We suspected that the iminoenolate would not react though this pathway because the nitrogen lone pair should be less active. Gratifyingly, iminoenolate **61** reacted as we expected, producing protected mannosamine **63** in 78% yield. We theorized that this originates from a closed, anti-Felkin transition state to give the *anti*-aldol product (Figure 1.12). Having established that an amine could be placed at the 2-position, the iminoenolate was tested with a variety of Lewis acids and solvents with the hope of accessing other stereochemistries, but none of these conditions produced usable yields of a 2-aminosugar.



Scheme 1.6: Exposure of aldehydes 70 and 71 to enolization conditions initiated polymerization of each aldehyde.

A variety of other aldehyde aminoenolates and iminoenolates were considered to access other 2-aminosugars. Aminoenolates **65** and **68** were made from their corresponding aldehydes. Interestingly, the CBz-protected aldehyde did not produce an iminoenolate despite varying the equivalents of TMS-Cl and reaction time. Use of aminoaldehyde enolates **65** and **68** did not provide access to the desired 2-aminosugar products. Instead, they both afforded a trimer-like product (Scheme 1.5). Iminoaldehydes **70** and **71**[33] were unable to be enolized, instead converting mostly to a polymeric material (Scheme 1.6). Also synthesized by a colleague, Dr. Akio Kayano, was thioester **72**, which can be converted to an enolate *in situ* via soft enolization techniques. This thioester provided access to a protected allosamine (**73**) when combined with the TIPS-erythrose in dichloromethane with titanium (VI) tetrachloride and Hunig's base (Figure 1.13). Having

determined that a stereochemistry other than mannose was accessible through this aldol technology, thioester **72** and the TIPS-erythrose were combined with a variety of Lewis acids, but none produced another stereochemistry or performed as well as titanium (VI) tetrachloride for the synthesis of the protected allosamine.

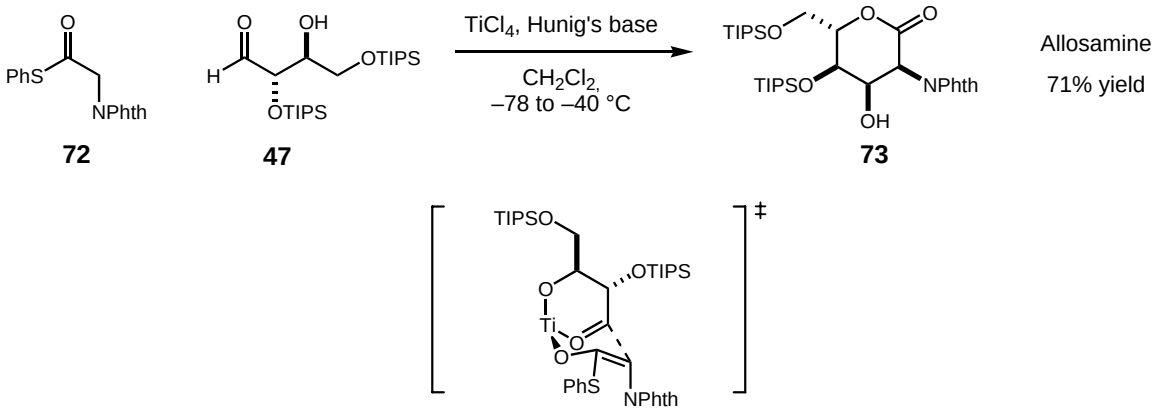


Figure 1.13: Titanium-mediated soft enolization of thioester **72** produced allosamine **73**.

The synthesis of protected mannosamine and allosamine were both exciting. Non-natural derivatives of mannosamine have been shown to have anti-tumor activity against T-cell lymphoma. Mannosamine has also implicated in the inhibition of proteoglycan breakdown, and derivatives of mannosamine are being investigated as anti-arthritics.[26] Allosamine, a rare aminosugar, is a key component of allosamidin, a chitinase inhibitor. Both asthma and allergies have been related to higher chitinase expression levels, and allosamidin and derivatives of allosamidin are being explored as a possible treatment

option for both conditions.[27] The ability to rapidly synthesize differentially-protected mannosamine and allosamine should accelerate the exploration of these molecules as treatment options.

Conclusions

Described above is the extension of the MacMillan sugar methodology to 2-aminosugars. Many enolates were not competent in this reaction, and a Boc-protected aminoenolate produced a protected 1-aminosugar. This methodology was successful at synthesizing a protected mannosamine using an iminoenolate. A protected allosamine was synthesized with a thioester using soft enolization conditions. Both products can serve as synthetic precursors for saccharides that are implicated as therapeutics for cancer, asthma, and arthritis.

Supporting Information

General Information. Commercial reagents were purified prior to use following the guidelines of Perrin and Armarego.[28] All solvents were purified according to the method of Grubbs.[29] Non-aqueous reagents were transferred under nitrogen via syringe or cannula. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using an ice-water bath for volatile samples. Chromatographic purification of products was accomplished using forced-flow chromatography on ICN 60 32-64 mesh silica gel 63 according to the method of Still.[30] Thin-layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica gel 60-F plates. Visualization of the developed chromatogram was performed by fluorescence quenching or by anisaldehyde, ceric ammonium molybdate, or $KMnO_4$ stain.

^{1}H and ^{13}C NMR spectra were recorded on a Mercury 300 (300 MHz and 75 MHz) or an Inova 500 (500 MHz and 125 MHz) as noted, and are internally referenced to residual protio solvent signals. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constant (Hz) and assignment. Data for ^{13}C NMR are reported in terms of chemical shift (δ ppm). IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra were obtained from the California Institute of Technology Mass Spectral Facility. Gas liquid chromatography (GLC) was performed on Hewlett-Packard 6850 and 6890 Series gas chromatographs equipped with a split-mode capillary injection system and flame

ionization detectors using a J&W Scientific DB-1701 (30 m x 0.25 mm) column as noted. High performance liquid chromatography (HPLC) was performed on Hewlett-Packard 1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted.

Preparation of Aldehyde Enolsilanes

Azidoacetaldehyde. (53) Previously prepared by Whitesides et al.,[31] ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.67 (s, 1H, CHO); 4.03 (d, 2H, J = 1.2 Hz, CH_2N_3).

(*Z*)-Azido 2-(trimethylsilanyloxy)-vinyl ester. (55) Azidoacetaldehyde (0.3 g, 3.6 mmol) in dichloromethane (10 ml) was slowly added in a single portion to a 0° C solution of chlorotrimethylsilane (1.3 ml, 7.1 mmol), triethylamine (2.0 ml, 14.3 mmol), and dicloromethane (5 ml) in a flame-dried flask under an argon atmosphere. Within five minutes, the solution became a yellow suspension that continued to darken over time. The solution was allowed to warm to room temperature. Volatiles were removed *in vacuo* and the residue was extracted with three portions of anhydrous diethyl ether. The ether was removed *in vacuo* to afford the title compound (19:1 *Z*:*E*) in 70% yield as a translucent, red liquid. ^{1}H NMR (300 MHz, $CDCl_3$) *Z* isomer: δ 5.95 (d, 1H, J = 4.2 Hz, CHOTMS); 5.01

(d, 1H, J = 4.2 Hz, CHN_3); 0.16 (s, 9H, $Si(CH_3)_3$); *E* isomer: δ 6.41 (d, 1H, J = 10.8 Hz, CHOTMS); 5.94 (d, 1H, J = 10.8 Hz, CHN_3); 0.19 (s, 9H, $Si(CH_3)_3$).

(*Z*)-Azido 2-(tert-butyldimethylsilanyloxy)-vinyl ester. (56) Azidoacetaldehyde (0.3 g, 6.6 mmol) in dichloromethane (10 ml) was slowly added in a single portion to a 0 °C solution of *tert*-butyl dimethyl silyl chloride (1.1g, 7.2 mmol), triethylamine (2.0 ml, 14.3 mmol), and dicloromethane (5 ml) in a flame-dried flask under an argon atmosphere. Within five minutes, the solution became a yellow suspension that continued to darken over time. The solution was allowed to warm to room temperature. Volatiles were removed *in vacuo* and the residue was extracted with three portions of anhydrous diethyl ether. The ether was removed *in vacuo* to afford the title compound (16:1 *Z*:*E*) in 72% yield as a translucent, orange liquid. IR (film) 2955, 2930, 2858, 2360, 2382, 2108 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *Z* isomer: δ 5.64 (d, 1H, J = 3.6 Hz, CHOTMS); 4.42 (d, 1H, J = 3.6 Hz, CHN_3); 0.93 (s, 9H, $CH_3)_3C$), 0.17 (s, 6H, $Si(CH_3)_2$); *E* isomer: δ 6.35 (d, 1H, J = 11.1 Hz, CHOTMS); 5.59 (d, 1H, J = 11.1 Hz, CHN_3); 0.87 (s, 9H, $CH_3)_3C$), 0.17 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) *Z* isomer: δ 139.1, 112.9, 51.5, 31.7, 23.9, 17.2; HRMS (Cl+) exact mass calcd for $[M + H]^+$ ($C_8H_{18}N_3OSi$) requires *m/z* 200.1219, found *m/z* 200.1212.

(*Z*)-Azido 2-(acetoxy)-vinyl ester. (57) Azidoacetaldehyde (0.61 g, 7.1 mmol) in dichloromethane (15 ml) was added dropwise over 15 minutes to a 0 °C solution of acetic anhydride (4.0 ml, 42.8 mmol), triethylamine (4.0 ml, 28.5 mmol), and 4-

dimethylaminopyridine (0.17 g, 1.4 mmol) in a flame-dried flask under an argon atmosphere. The solution was allowed to warm to room temperature. Volatiles were removed *in vacuo,* and the residue was extracted with three portions of anhydrous diethyl ether. The ether was removed *in vacuo* to afford the title compound (>19:1 *Z*:*E*) in ^{1}H NMR (300 MHz, $CDCl_3$) *Z* isomer: δ 6.83 (d, 1H, *J* = 4.8 Hz, CHOAc); 5.51 (d, 1H, *J* = 4.8 Hz, CHN_3); 2.13(s, 3H, $COCH_3$).

((*Z*)-[2-(Trimethylsilanyloxy)-vinyl]-carbamate. (60) (2-Oxo-ethyl)-carbamic acid *tert*-butyl ester (0.2 g, 1.2 mmol) was added in a single portion as a solution in 1 mL of acetonitrile to a room temperature solution of chlorotrimethylsilane (0.3 mL, 2.5 mmol), triethylamine (0.7 mL, 5.0 mmol), and acetonitrile (2 mL). In less than five minutes, the solution became a hot white suspension that turned into a rust-colored suspension within fifteen minutes. After stirring for 30 minutes, volatiles were removed *in vacuo* and the residue was extracted with three portions of anhydrous diethyl ether. Distillation of the ethereal extracts afforded the title compound (0.19 g, 0.82 mmol, b.p. 53-56 °C, 0.1 mmHg, 5:1 *Z*:*E*) in 68% yield as a clear, colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.36 (bs, 1H, NH); 5.95 (d, 1H, *J* = 4.8 Hz, CHOTMS); 5.24 (d, 1H, *J* = 4.8 Hz, CHN); 1.46 (s, 9H, $C(CH_3)_3$); 0.21 (s, 9H, $Si(CH_3)_3$).

((*Z*)-[2-(Trimethylsilanyloxy)-vinyl]-carbamic acid *tert*-butyl ester)-trimethylsilyl-imidate. (61) (2-Oxo-ethyl)-carbamic acid *tert*-butyl ester (3.0 g, 18.8

mmol) was added in a single portion as a solution in 10 mL of acetonitrile to a room temperature solution of chlorotrimethylsilane (4.78 mL, 37.7 mmol), triethylamine (10.51 mL, 75.4 mmol), and acetonitrile (30 mL). In less than five minutes, the solution became a hot white suspension that turned into a rust-colored suspension within fifteen minutes. After stirring for 3 hours, volatiles were removed *in vacuo* and the residue was washed with anhydrous diethyl ether (3 x 20 mL). Distillation of the ethereal extracts afforded the title compound (3.67 g, 12.1 mmol, b.p. 66-68 °C, 0.25 mmHg, 13:1 *Z*:*E*) in 64% yield as a clear, colorless liquid. IR (film) 2977, 1709, 1689, 1482, 1392, 1367, 1313, 1251, 1170, 1086, 847.7, 784.3, 755.6 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.97 (d, 1H, *J* = 2.7 Hz, CHOTMS); 5.25 (d, 1H, *J* = 2.7 Hz, CHN); 1.49 (s, 9H, $C(CH_3)_3$); 0.24 (s, 9H, $Si(CH_3)_3$); 0.20 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.1, 134.7, 111.4, 80.1, 28.6, 0.74, –0.25; HRMS (FAB+) exact mass calcd for $[M + H]^+$ ($C_{13}H_{29}NO_3Si_2$) requires *m/z* 303.1686, found *m/z* 303.1695. The product ratios were determined by ^1H NMR integration of the crude reaction mixture.

(*Z*)-Phthalimido-2-(trimethylsilanyloxy)-vinyl ester. (65)

Phthalimidoacetaldehyde (0.50 g, 2.65 mmol) in acetonitrile (8 mL) was slowly added in a single portion to a 0° C solution of chlorotrimethylsilane (0.67 ml, 5.3 mmol), triethylamine (1.5 ml, 10.6 mmol), and acetonitrile (8 ml) in a flame-dried flask under an argon atmosphere. Within five minutes, the solution became a yellow suspension that continued to darken over time. The solution was allowed to warm to room temperature. Volatiles were removed *in vacuo* and the residue was extracted with three portions of

anhydrous diethyl ether. The ether was removed *in vacuo* to afford the title compound (1.5:1 *Z*:*E*) in 86% yield as a translucent, red liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ *Z* isomer: 7.91-7.78 (m, 2H) ArH, 7.72-7.65 (m, 2H) ArH, 6.47 (d, 1H, *J* = 4.8 Hz, C**H**OTMS); 5.43 (d, 1H, *J* = 4.8 Hz, CHN_3); 0.12 (s, 9H, $Si(CH_3)_3$); *E* isomer: δ 7.91-7.78 (m, 2H) ArH, 7.72-7.65 (m, 2H) ArH, 7.50 (d, 1H, *J* = 11.4 Hz, C**H**OTMS); 6.42 (d, 1H, *J* = 11.4 Hz, CHN_3); 0.11 (s, 9H, $Si(CH_3)_3$).

Preparation of Sugars

(2*S*, 3*S*)-3-Hydroxy-2,3-bis-triisopropylsilanoxy-propionaldehyde. (46) A suspension of triisopropylsilanoxy-acetaldehyde (5.00 g, 23.0 mmol) and L-proline (133 mg, 1.15 mmol) in methyl sulfoxide (50 mL) was stirred for 24 h at room temperature. The resulting solution was diluted with ethyl ether (150 mL) and washed with water (3x50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Crude ^{1}H NMR analysis indicated complete conversion to a mixture of 4:1 *anti*:*syn* diastereomers. Flash chromatography (3% ether in pentane) afforded the title compound as a clear, colorless oil that froze upon storage at –20 °C (1.86 g, 4.3 mmol, 37%) as well as a faster-eluting fraction of a mixture of *syn*- and *anti*-diastereomers (2.74 g, 6.3 mmol, 55%) in 92% combined yield, 95% ee (*anti*-diastereomer). IR (film) 3483, 2945, 2892, 2868, 1734, 1464, 1385, 1117, 1069, 883, 683 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.68 (d, 1H, *J* = 2.1 Hz, CHO); 4.25 (dd, 1H, *J* = 3.9, 2.1 Hz, C**H**CHO); 4.10-3.94 (m, 1H, C**H**OH); 3.84

(dd, 1H, J = 9.9, 6.6 Hz, CH_2OR); 3.79 (dd, 1H, J = 9.6, 6.3 Hz, CH_2OR); 2.40 (d, 1H, J = 5.4 Hz, OH); 1.16-1.00 (m, 42H, 6 $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.1, 78.9, 74.3, 62.7, 18.0 (12C), 12.4 (3C), 11.9 (3C); HRMS (CI+) exact mass calcd for $[M+H]^+$ ($C_{22}H_{49}O_4Si_2$) requires *m/z* 433.3169, found *m/z* 433.3176; $[\alpha]_D$ = –3.6 (c = 4.0, $CHCl_3$).

1-*tert*-Butylcarbamato-1-deoxy-4,6-bis-*O*-triisopropylsilyl-α,β-L-pyranose. (62) Titanium (IV) tetrachloride (38 μL, 0.35 mmol) was added dropwise to a stirring solution of (2*S,* 3*S*)-3-hydroxy-2,3-bis-triisopropylsilanoxy-propionaldehyde (50 mg, 0.12 mmol), ((*Z*)-[2-(Trimethylsilanyloxy)-vinyl]-carbamate (107 mg, 0.46 mmol) and dichloromethane (1.2 mL) at –78 °C. The solution turned dark red upon addition of $TiCl_4$ and was stirred at –78 °C for 5 hours. It was then allowed to warm gradually over 5 hours to –40 °C. After stirring for an additional 24 hours at –40 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl, extracted three times with ethyl acetate (4 mL), washed with 10% $NaHCO_3$ (2 x 5 mL)$_3$, brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Flash chromatography (2:5 ether:hexanes) afforded the title compound as a clear, colorless oil (49 mg, 0.08 mmol, R_f = 0.3, stains teal blue in anisaldehyde) in 72% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (bs, 1H, NH); 4.91 (bd, 1H, J = 9.0 Hz, H1); 4.01 (dd, 1H, ,J = 2.4, 11.1 Hz, H6); 3.87 (dd, 1H, J = 2.4, 11.1 Hz, H6); 3.77 (dd, 1H, J = 9.0, 9.0 Hz, H2); 3.54 (dd, 1H, J = 9.0, 8.8 Hz, H3); 3.34 (m, 1H, H5); 3.21 (dd, 1H, J = 9.0, 8.8 Hz, H4); 2.57 (bs, 1H, OH); 2.54 (bs, 1H, OH); 1.16-1.00 (m, 42H, 6 $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 77.4, 74.9, 73.6, 71.5, 71.2, 68.4, 65.3, 29.2, 18.6, 18.6,

13.5, 12.2,; 300 MHz COSY spectra support the above ^{1}H NMR assignments; HRMS (EI+) exact mass calcd for $[M+H]^{+}$ ($C_{29}H_{62}NO_7Si_2$) requires *m/z* 592.4065, found *m/z* 592.4066.

2-*tert*-Butylcarbamato-2-deoxy-4,6-bis-*O*-triisopropylsilyl-α,β-L-mannopyranose. (63) Titanium (IV) chloride (38 μL, 0.35 mmol) was added dropwise to a stirring –78 °C solution of (2*S*, 3*S*)-3-hydroxy-2,3-bis-triisopropylsilanoxy-propionaldehyde (50 mg, 0.12 mmol), ((*Z*)-[2-(trimethylsilanyloxy)-vinyl]-carbamic acid *tert*-butyl ester)-trimethylsilyl-imidate (175 mg, 0.58 mmol) and dichloromethane (2.3 mL). The resulting red solution was stirred at –78 °C for 5 hours, then allowed to warm gradually over 5 hours to –40 °C. After stirring for an additional 48 hours at –40 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl, extracted three times with ethyl acetate (5 mL), washed with 10% $NaHCO_3$ (2 x 7 mL), brine (7 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Crude ^{1}H and ^{13}C NMR analysis indicated complete conversion to a 10:1 mixture of mannose:allose-derived diastereomers as well as some minor acetal side-products. Flash chromatography (1:3 ether:hexanes, R_f = 0.4, stains red in anisaldehyde) afforded the title compound as a clear, colorless oil (51 mg, 0.09 mmol, 2:1 α:β, 74%). IR (film) 3436, 2943, 2893, 2867, 1699, 1510, 1464, 1368, 1248, 1151, 1122, 1066, 883.0, 763.3, 680.9 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (d, 1H, *J* = 9.0 Hz, OH); 7.59 (bs, 1H, NH); 5.13 (d, 1H, *J* = 3.0 Hz, H1); 4.95 (m, 1H, H3); 3.95 (m, 1H, H2); 4.10 (m, 1H, H4); 3.85 (m, 1H, H5); 3.96 (m, 2H, H6); 1.47 (d, 1H, *J* = 3.0 Hz, C3 OH); 1.45 (s, 9H, $C(CH_3)_3$); 1.22-1.06 (m, 42H, 6 $CH(CH_3)_2$); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 163.1, 94.0, 80.5, 75.5, 71.2, 70.0 63.5, 54.5, 28.5, 18.5, 18.5, 18.2, 18.1, 13.0, 12.2; 500 MHz COSY spectra support the above ^1H NMR assignments; HRMS (EI+) exact mass calcd for $[M+H]^+$ ($C_{29}H_{62}NO_7Si_2$) requires *m/z* 592.4065, found *m/z* 592.4064; $[\alpha]_D$ = –27.1 (c = 2.00, $CHCl_3$, 2:1 α:β mixture). Stereochemistry was confirmed by comparison to an authentic 2-acetamido-2-dexoy-1,3,4,6-tetra-*O*-acetyl-α-mannopyranose.

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34. This work was performed in the MacMillan laboratory with the assistance of Drs. Alan Northrup and Akio Kayano.

C h a p t e r 2

A Novel Method to Access Hexoses[23]

Introduction

The methodologies described in the previous chapter explored the reactivity of the TIPS-erythrose (**1**) and allowed scientists to access a variety of hexoses (Figure 2.1), but the reactivity of the TIPS-threose (**7**) still remained to be investigated. It was thought that the threose may allow access to the syn-sugars: gulose, galactose, talose, and idose (Figure 2.2).

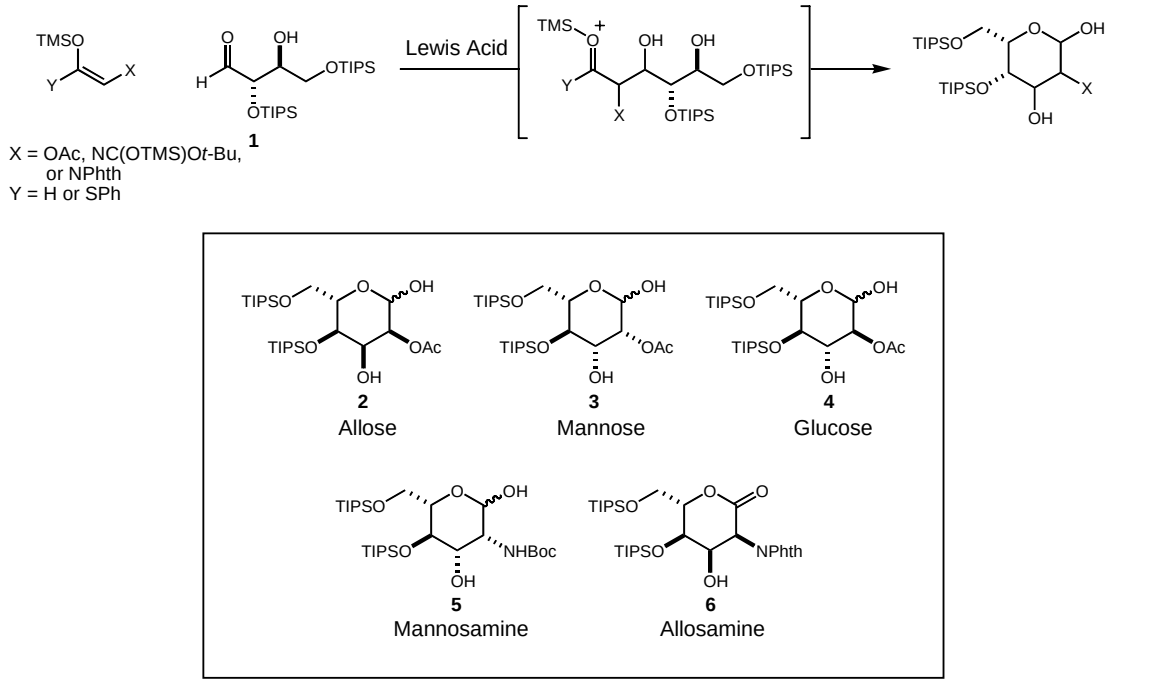


Figure 2.1: The TIPS-erythrose (**1**) has been elaborated to a differentially-protected allose, mannose, glucose, allosamine, and mannosamine.

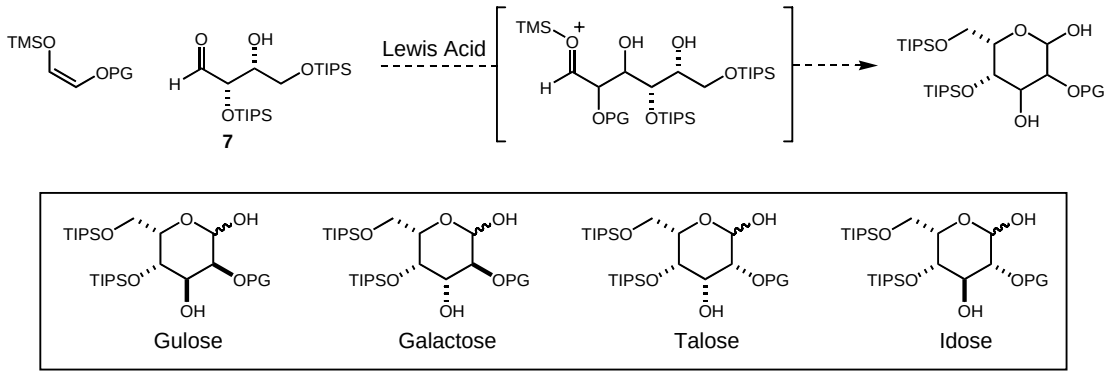


Figure 2.2: The TIPS-threose (**7**) may allow access to the syn-sugars, gulose, galactose, talose, and idose.

Many sugars are available in large quantities from natural sources. Syntheses often employ these sugars as starting materials because they can be easily obtained. In contrast, many of the syn-sugars are rare and are usually found as a component of a larger natural product.[2] In addition, many of these sugars often exist in modified forms, such as the uronic acid or a deoxygenated sugar. For example, free idose has not been isolated from nature even though iduronic acid is a key component of both heparin and dermatan sulfate (Figure 2.3). It is formed when heparan sulfate glucuronyl C5-epimerase (Hsepi) isomerizes D-glucuronic acid in heparan sulfate to L-iduronic acid.[3] Because of the impracticality or impossibility of isolating many of the syn-sugars from natural sources, methods for their synthesis have been explored.[2, 4]

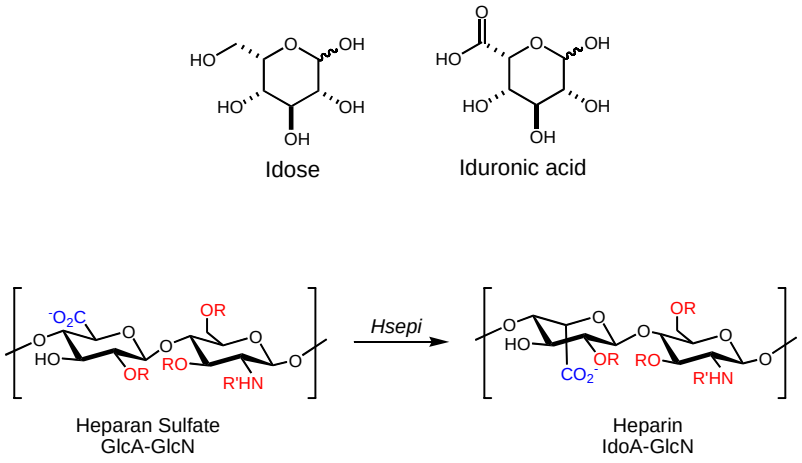
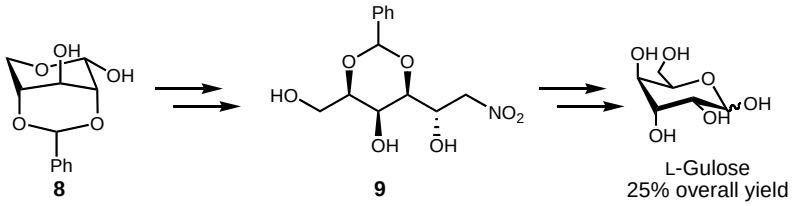


Figure 2.3: Glucuronic acid is epimerized to iduronic acid by heparan sulfate glucuronyl C5-epimerase (Hsepi).

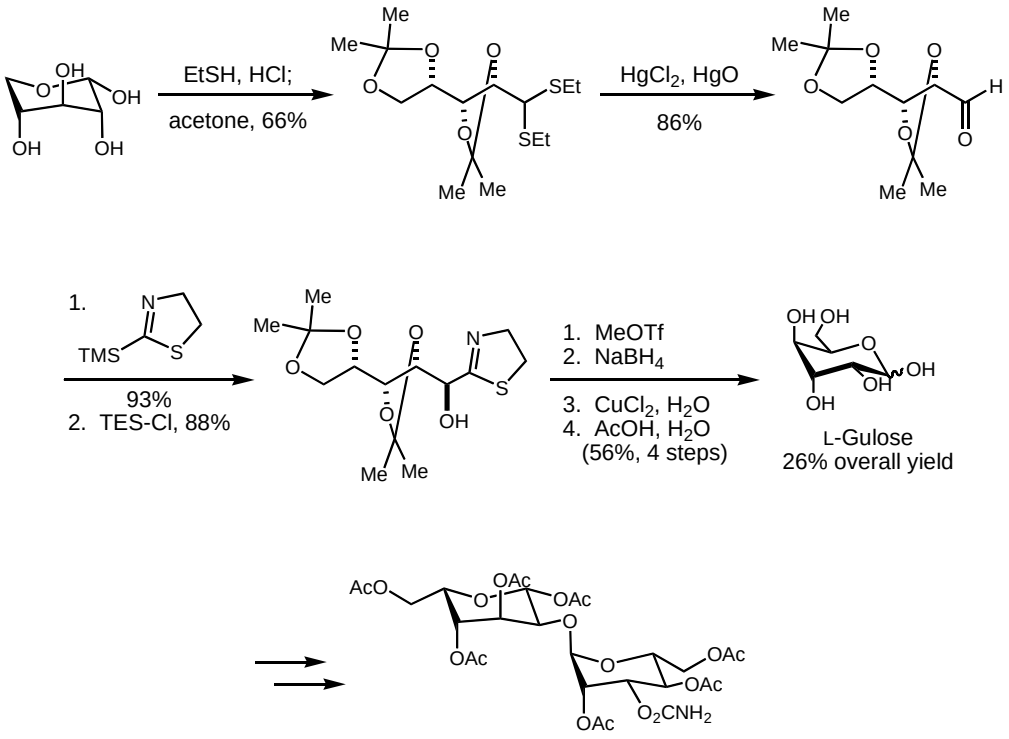
Despite the ease with which nature prepares hexoses, they have posed a synthetic challenge for researchers. One method for synthesizing many rare sugars is to start with a commonly occurring sugar and then elaborate it to the desired syn-sugar.[4] For example, L-gulose has classically been synthesized from L-xylose. Sowden and Fischer used benzylidene L-xylose **8** to synthesize L-gulose in 25% overall yield (Scheme 2.1).[4a, b] While this method has seen extensive use, even the authors note that it is not a feasible method for large-scale synthesis.[4b] A more recent synthesis starts with commercially available L-xylose and synthesizes L-gulose in 8 steps and 26% overall yield.[4c] After converting xylose to gulose, Dondoni and coworkers used this saccharide to produce the gulose-mannose disaccharide moiety of Bleomycin A_2 (Scheme 2.2).



Scheme 2.1: Sowden and Fischer's method for accessing gulose

Though choosing starting materials from the chiral pool has long been a practice in synthesis, there are drawbacks to this method. Often effort must be spent modifying the chosen starting material before progress can begin towards the desired product. Because of the drawbacks of this method, researchers have looked for other ways to access hexoses. Even so, there are few methods for accessing hexoses from achiral starting materials. The

methodologies that have been developed can be divided into four categories: asymmetric oxidation, allylation/oxidation, hetero-Diels-Alder, and aldol.



Scheme 2.2: Dondoni and Massi's method for accessing gulose

Asymmetric olefin oxidation technologies have been widely used to access carbohydrates due to their generality and the high enantioselectivities afforded by the chiral catalysts employed to set the vicinal oxygen stereocenters (Figure 2.4).[5]

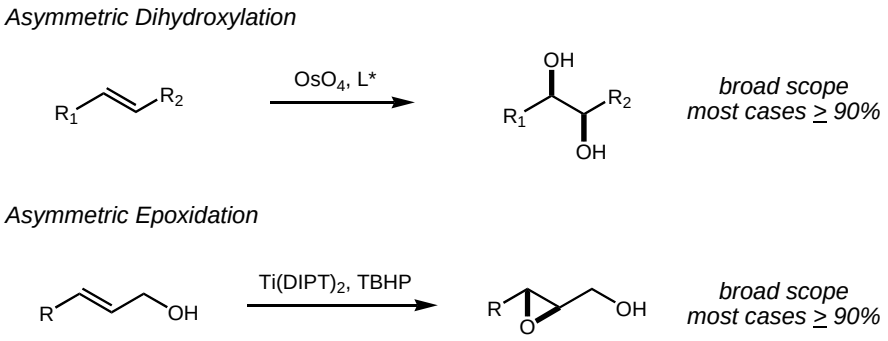


Figure 2.4: Asymmetric dihydroxylation and epoxidation strategies

Using these methodologies, Sharpless and coworkers were able to access all 8 of the L-hexoses. Through an iterative approach, they prepared each hexose in 20 steps (Figure 2.5).[6] Unfortunately, due to the requirement of acetonides as protecting groups for stereochemical purposes, this strategy cannot produce differentially-protected hexoses, and so further elaboration would be required for polysaccharide synthesis.

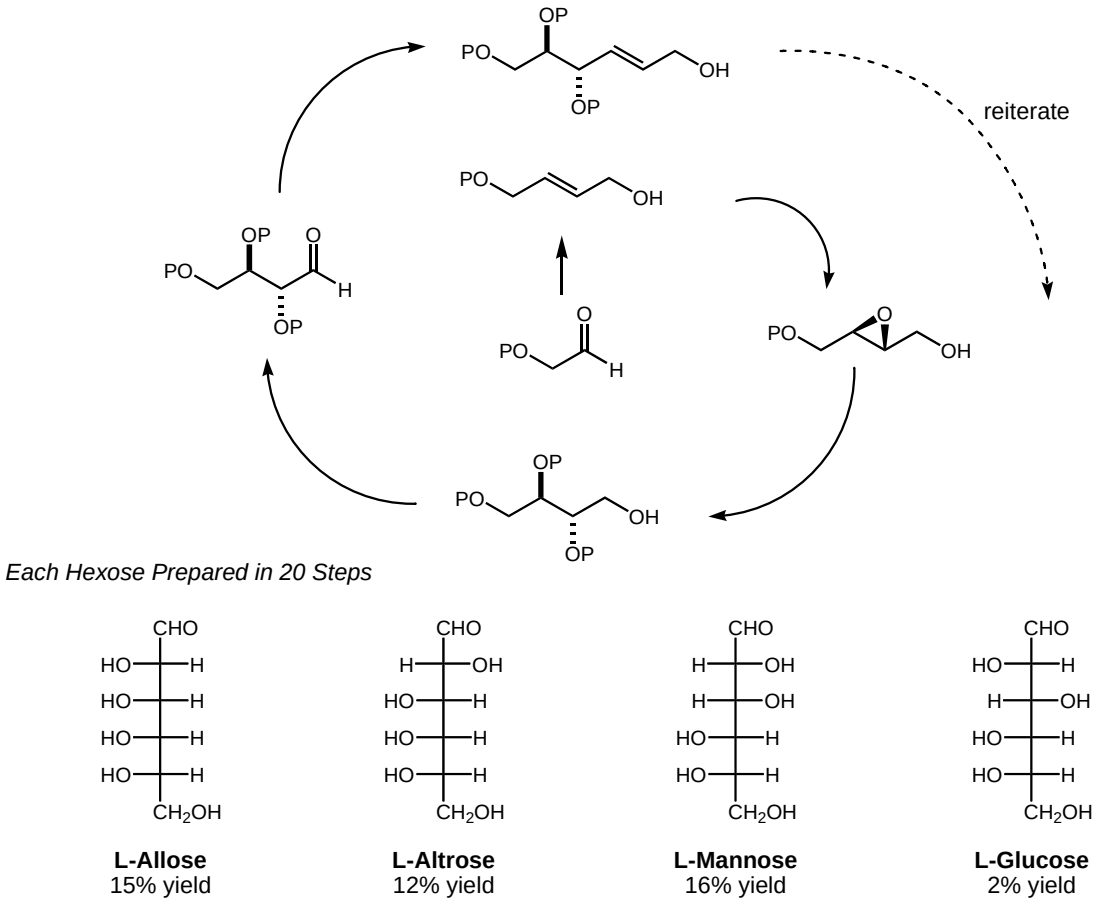


Figure 2.5: Asymmetric epoxidation has been used to synthesize the L-hexoses. It was noted that L-altrose was obtained as the 1,6-anhydro-β-L-altropyranose.

The allylation of aldehydes with chiral-metal reagents is another approach used for the construction of carbohydrates (Figure 2.6).[7] While this approach has been successfully used for natural and non-natural polysaccharides, the lengthy syntheses required to produce the chiral metal reagents, in addition to their toxicity and short shelf-lives, are drawbacks. In addition, this method again uses an iterative approach that lengthens the synthesis.

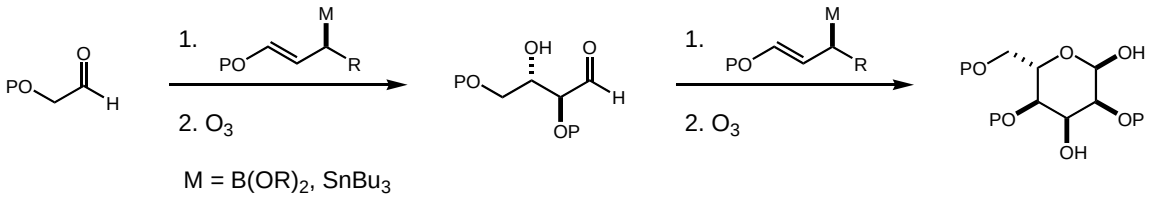


Figure 2.6: Allylmetal reagents have been used to synthesize carbohydrates.

Diels-Alder-based strategies have been employed for the synthesis of hexoses. For example, Danishefsky and coworkers have synthesized numerous natural and non-natural monosaccharides through the use of Danishefsky's diene (**10).** A Diels-Alder reaction between diene **10** and aldehyde **11** produces pyran **12**, which can be elaborated to form saccharides in the mannose, glucose, galactose, and talose families (Figure 2.7).[8] While this mode of synthesis is highly convergent, it cannot efficiently access all hexose stereochemistries since a large majority of Diels-Alder reactions favor the *endo* product.

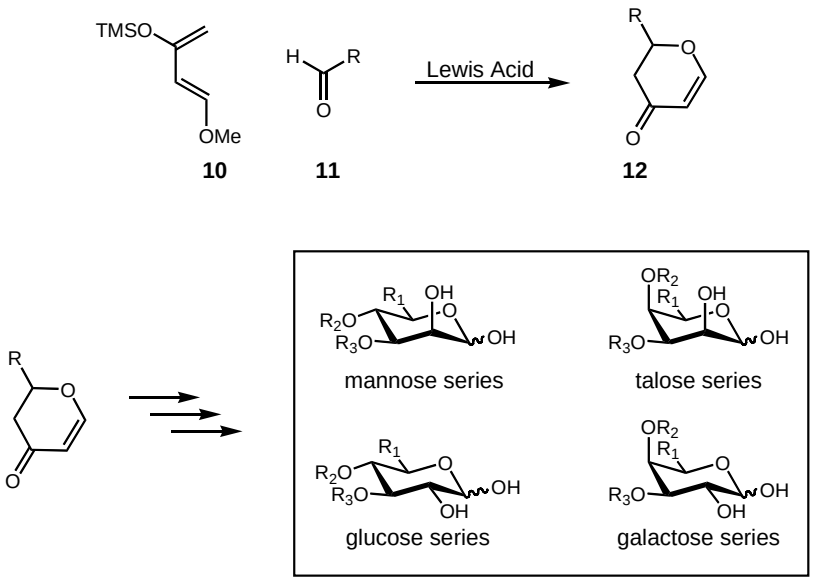


Figure 2.7: Diels-Alder chemistry has been used to access a variety of hexoses.

Another methodology applied to the synthesis of hexoses has been the aldol reaction. As described in Chapter 1, the aldol reaction is an important carbon-carbon bond-forming reaction that has been applied to the synthesis of many monosaccharides. One approach has involved the use of enzymes (such as kinase, aldolase, phosphatase, and isomerase enzymes) to form saccharides (Figure 2.8).[9] In addition to providing access to many useful natural monosaccharides, enzymatic aldol reactions have also allowed access to some non-natural monosaccharides. Unfortunately, the use of enzymes limits the substrate scope and limits or prevents the use of protecting groups. To circumvent this problem, other groups have used standard metal-catalyzed aldol technology to synthesize carbohydrates (Figure 2.9).[10] However, the use of standard

aldol conditions required lengthy syntheses to access hexoses. Because of this, they have seen limited use in synthesis.

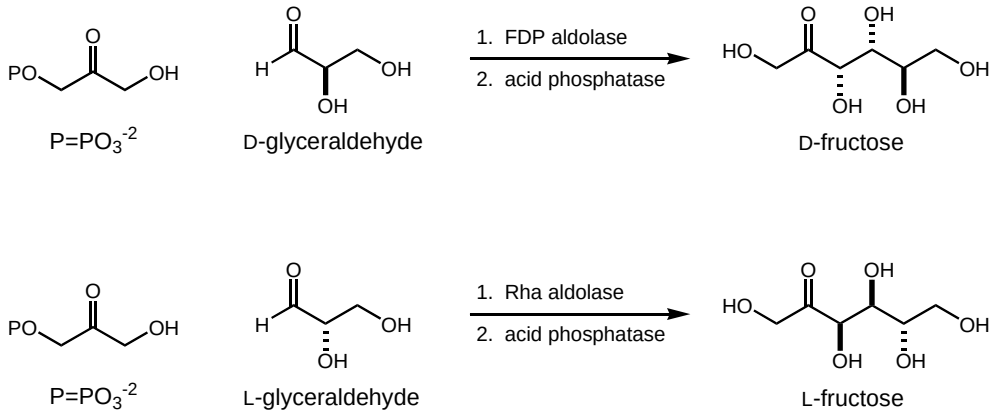


Figure 2.8: Aldolase enzymes have been applied to the synthesis of sugars.

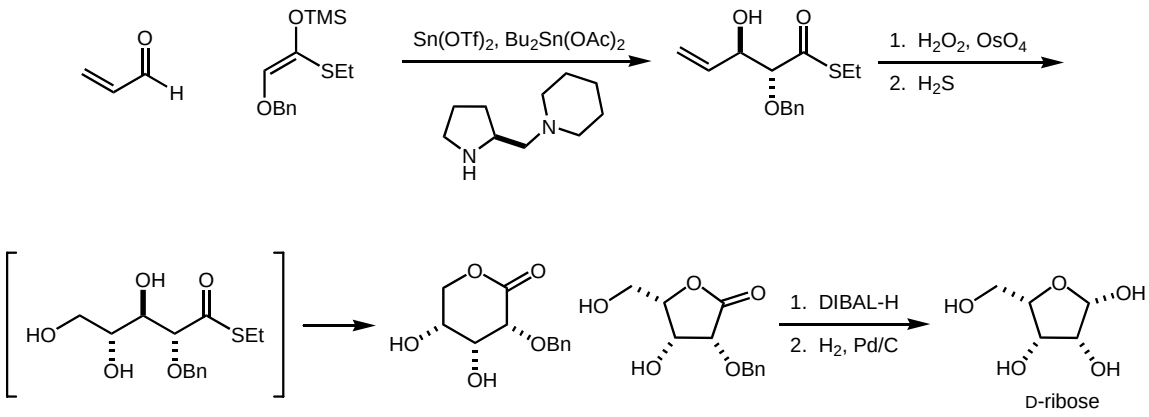
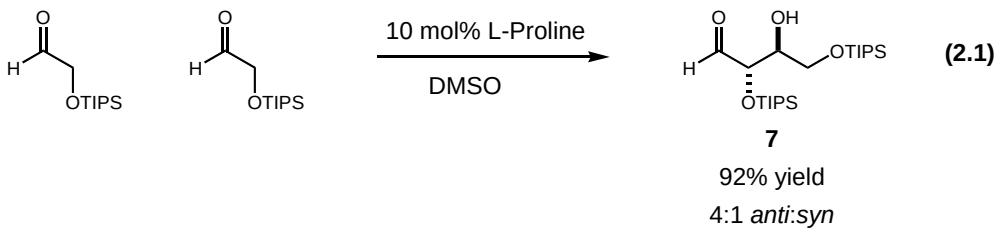


Figure 2.9: Enantioselective aldol chemistry has been applied to carbohydrate synthesis.

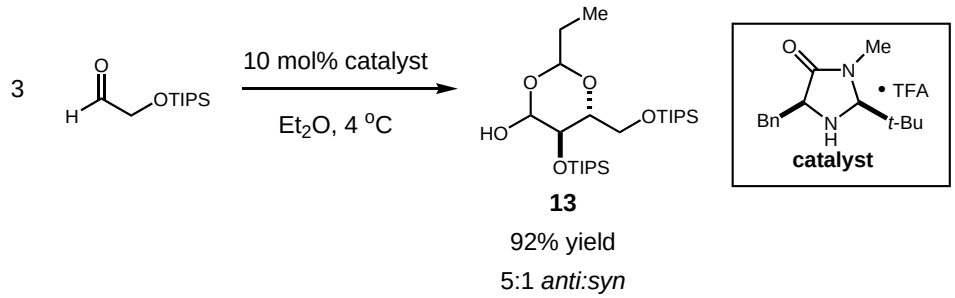
As described in Chapter 1, the MacMillan group has developed a new aldol methodology to access differentially-protected allose, mannose, and glucose from TIPS-erythrose **1** (Figure 2.1).[1] To extend this technology to the TIPS-threose (**7**), two reactions needed to be developed. Since the TIPS-threose is the minor product of the proline-catalyzed aldol reaction (Equation 2.1), a method needed to be developed to access it in larger quantities. Once the TIPS-threose could be readily accessed, conditions would have to be determined to promote the formation of the syn-hexoses.



Preparation of the TIPS-Threose

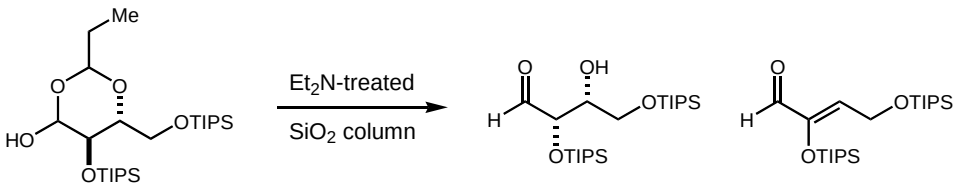
The TIPS-threose (**7**) is the minor product of a proline-catalyzed aldol reaction described in Chapter 1 (Equation 1).[1b] Because it constitutes only 20 percent of the product, a method was needed to access threose **7** more efficiently. Work in the MacMillan laboratory revealed that an imidazolidinone-catalyzed aldol reaction could produce TIPS-protected hemiacetal **13** (Equation 2.2). Furthermore, purification on a diethylamine-

treated column produced the TIPS-threose from hemiacetal **13** in good yield.[11] However, this hydrolysis reaction was troublesome to reproduce. The diastereomeric ratio and overall yields would vary from column to column.



(2.2)

The first aim was to standardize the column conditions used to generate the threose. Half-gram samples of hemiacetal **13** were purified over 100, 150, 200, and 300 grams of silica gel treated initially with 800 mL of a 15% solution of diethylamine in pentane, washed with 500 mL of pentane, and eluted with a 5% ether in pentane solution. While the 200 and 300 g columns produced the syn product, they also produced a β-elimination product **15**. The 100 g column did not completely hydrolyze the hemiacetal. The 150 g column effectively hydrolyzed the hemiacetal with minimal β-elimination product production (Table 2.1).

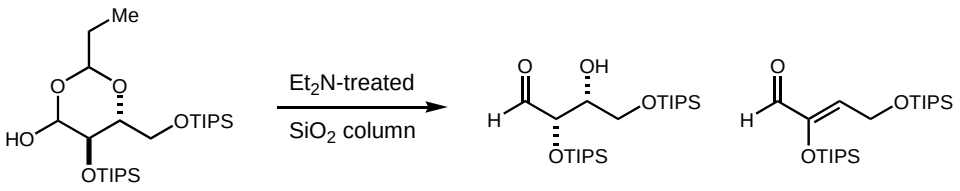


13 **14** **15**

entry	Amt. SiO_2 (g)	%**13**	%**14**	%**15**
1	100	32	41	0
2	150	0	52	14
3	200	0	39	23
4	300	0	37	27

Table 2.1: Column length affects the product ratio. Longer columns produced a larger amount of the β-elimination product (**15**) while a shorter column did not provide sufficient time for hydrolysis and separation.

It was suspected that excess diethylamine could be causing the β-elimination product to form, so the amount of diethylamine was evaluated next. Columns of 150 grams of silica were washed with 800 mL of a 5%, 10%, or 15% solution of diethylamine in pentane. Each column was washed with 500 mL of pentane and then a one gram sample of hemiacetal **13** was loaded onto each column. The 15% diethylamine/pentane treatment produced only 17% yield of the desired threose and while producing a 57% yield of the β-elimination product. The 10 % diethylamine/pentane treatment produced 57% yield of the threose and only a 12% yield of the β-elimination product, and the 5% diethylamine/pentane treatment failed to completely open the hemiacetal product (Table 2.2).

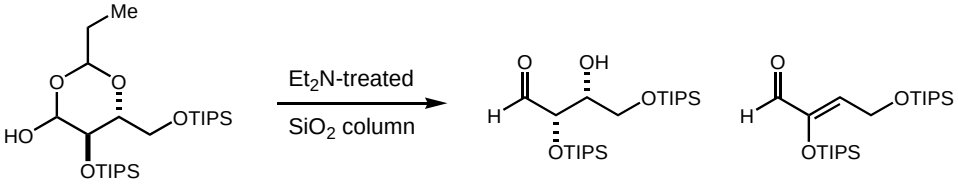


13 **14** **15**

entry	% Et_2N	%**13**	%**14**	%**15**
1	5	19	43	2
2	10	0	57	12
3	15	0	17	57

Table 2.2: The amount of β-elimination product formed correlates to the amount of Et_2N used to pretreat the column.

It was noticed that the amount of the β-elimination product formed seemed to correspond to the flow rate used for the column, so this was the next parameter examined. One-gram samples of hemiacetal **13** were chromatographed over 150 grams of silica gel treated with a 10% solution of diethylamine in pentane and washed with 500 mL of pentane. The samples were eluted in a 3% solution of diethyl ether in pentane with a flow rate of 52, 115, 196, or 273 mL/min. It was determined that a faster flow rate produced less of the β-elimination product (Table 2.3). When the flow rate was pushed to 273 mL/min, the TIPS dimer was obtained in 92% yield and in 4:1 diastereomeric ratio with only a trace of the β-elimination product.



13 **14** **15**

entry	flow rate (mL/min)	%**13**	%**14**	%**15**
1	52	0	16	59
2	115	0	23	48
3	196	0	58	22
4	273	0	92	2

Table 2.3: Affect of flow rate on the yield of TIPS-threose. The flow rate was determined by collecting the void volume into a graduate cylinder for 30 seconds.

These results are consistent with the theory that the hemiacetal hydrolysis is equilibrium process that is driven by the chromatographic separation of the resulting aldehyde products. This idea is also supported by the observation that silica gel and diethylamine do not cause hydrolysis in solution. While there is no direct precedent for this chemistry, the Rychnovsky laboratory has shown that the formation of β-hydroxy aldehydes from hemiacetals is facile in the presence of an amine base.[12]

The Reactivity of the TIPS-Threose

After the method for obtaining the TIPS-threose was determined, the reactivity of the threose could be explored. The TIPS-threose was first evaluated with benzyloxy- and

acetoxyacetaldehyde enolates[1c] (**16** and **17**, Figure 2.10). Despite efforts to produce a reaction by varying Lewis acid, solvent, temperature, and concentration, the TIPS-threose was not competent in the aldol reaction. One theory for this observation is that the TIPS-threose may be less active due to unfavorable steric interactions between the enolate nucleophile and the axial TIPS group (Figure 2.11). It was determined in previous studies that the TIPS dimer assumes a chair conformation. While the TIPS groups on the erythrose are equatorial, the threose geometry places a TIPS group axial, hindering nucleophilic attack.

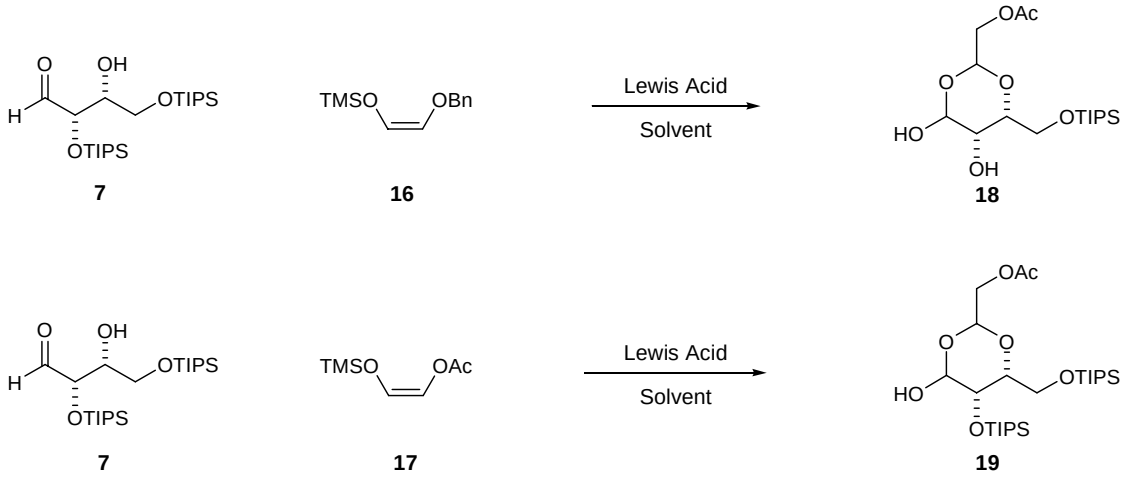
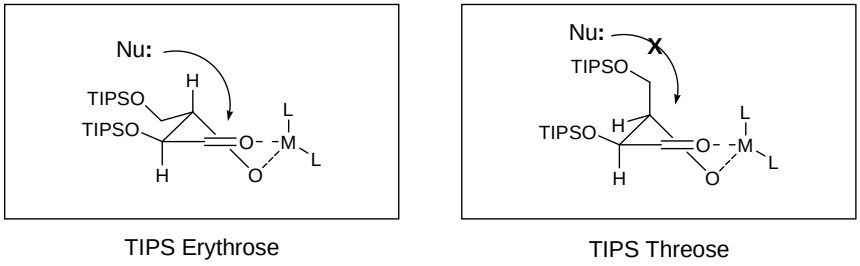


Figure 2.10: The TIPS-erythrose (**7**) did not produce the desired hexoses despite variations in Lewis acid, solvent, concentration, and temperature.



TIPS Erythrose TIPS Threose

Figure 2.11: The TIPS-threose may be less reactive due to an unfavorable steric interaction.

Further evidence that the TIPS-threose is less reactive than the erythrose was found when the threose was subjected to thioester soft-enolization reaction conditions. Thioester enolates are known to be more reactive than aldehyde enolates, so it was hoped these enolates would be better partners for the TIPS-threose.[13] Excitingly, when the TIPS-threose (**7**) was combined with benzyloxy thioester **20**, titanium tetrachloride, and Hunig's base in dichloromethane, it produced gulolactone **22**. However, it took 36 hours at 4 ^{o}C for this reaction to proceed. By comparison, when the TIPS-erythrose (**1**) was combined with benzyloxy thioester **20**, titanium tetrachloride, and Hunig's base in dichloromethane, it reacted in four hours at -40 ^{o}C to produce allolactone **21** (Figure 2.12).[20]

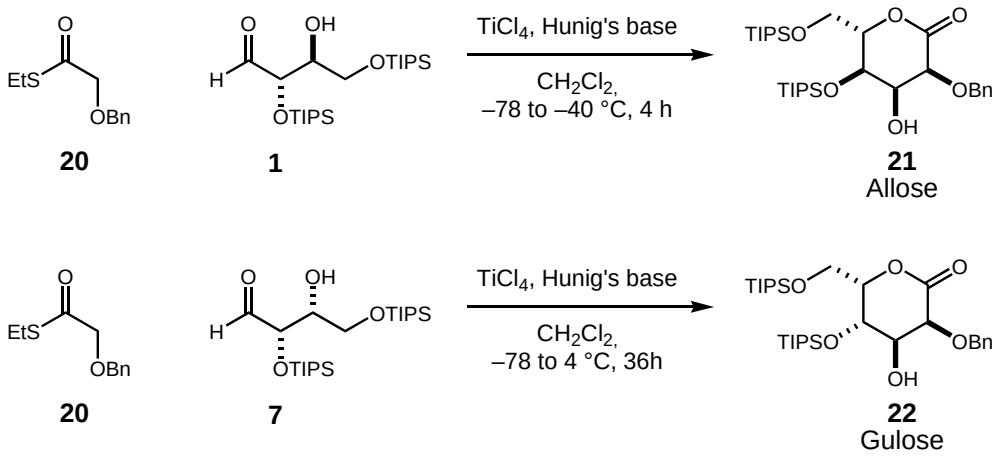


Figure 2.12: The TIPS-threose is less reactive than the TIPS-threose.

Efforts were then concentrated on accessing other stereochemistries through the use of benzyloxy thioester **20**, but the only hexose formed by these reactions was gulolactone **22.** Furthermore, none of the Lewis acids tried were as high yielding as $TiCl_4$, which produced gulolactone **22** in 75% yield. The production of gulolactone **22** represented a five-step synthesis (4 steps in the longest linear sequence) that produced a differentially-protected gulolactone in 48% overall yield from *cis*-butene diol. This was exciting because L-gulose has been found to be the key saccharide moiety of the pendant disaccharide on many anti-cancer therapeutics such as bleomycin A_2, bleomycin B_2, and phleomycin D_1. These drugs perform oxidative strand scission on double-stranded DNA. It is believed that the gulose moiety could be responsible for both entry into the cell and for stabilizing the iron (III) oxygen complex responsible for strand scission.[14] Furthermore, the presence of gulose in bacterial glycoproteins and glycolipids has been linked to higher virulence of the bacterial strain.[15] Because this sugar is rare, it can be costly to obtain (anywhere from

$500~$3000 for 1g of either D- or L-gulose).[16] Furthermore, once purchased, the free sugar still has to be modified before it can be used in polysaccharide synthesis. Therefore, it was pleasing to find a more efficient way to produce this saccharide.

Development of a One-Step Method to Access Hexoses

Because the thioester chemistry was unable to produce the other syn-hexose stereochemistries, different methods were sought to achieve this goal. It was considered that if an enolate was able to add to the TIPS dimer and cyclize to produce a hexose, then it should be possible to add two equivalents of an enolate to an aldehyde and it cyclize to form a hexose (Figure 2.13). To quickly evaluate this theory, acetoxyenolate **17** and TIPS-aldehyde **26** were combined with an assortment of Lewis acids and allowed to react until examination by thin layer chromatography (TLC) showed full consumption of the TIPS-aldehyde. The reaction was then extracted and the organic layer was filtered over silica. The organic layer was concentrated, and the residue was exposed to a 1:1 solution of TBAF and acetic acid in THF. After 1 hour, dichloromethane, triethylamine, and acetic anhydride were added, and the reactions were stirred for 8 hours. Previous work in the laboratory has shown that this sequence converts similar TIPS-protected hexoses to their pentaacetates.[1] Upon completion of this sequence, the reactions were extracted and purified. The isolated pentaacetates were then compared to known compounds to determine the stereochemical product of the aldol reaction. While we were able to isolate pentaacetates from a number of the reactions, only a few conditions selectively produced one hexose over another.

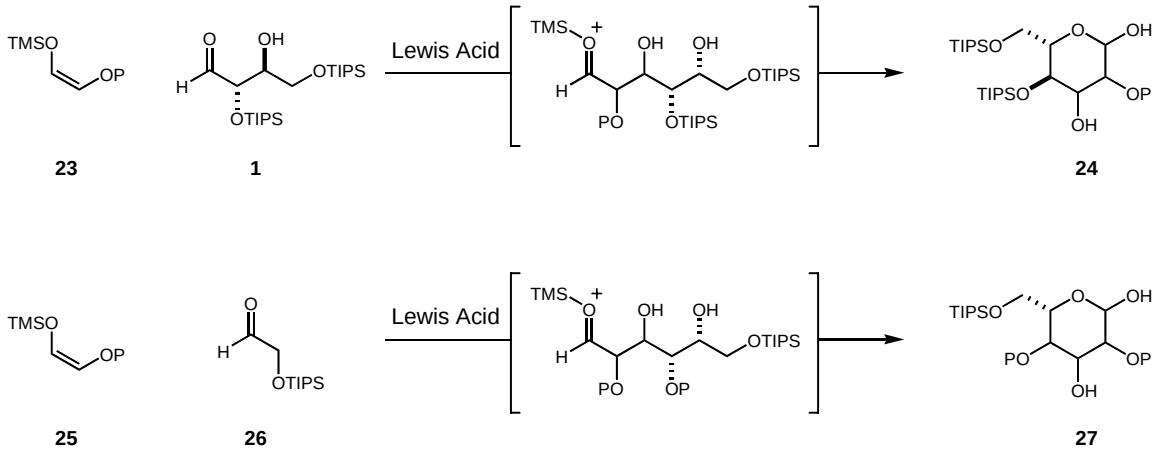


Figure 2.13: If the TIPS-erythrose (**1**) and enolate **23** are able to form a hexose instead of polymerizing, then it should be possible to form a hexose from TIPS-aldehyde **26**.

Specifically, both allose pentaacetate **28** and gulose pentaacetate **29** have been accessed through this chemistry. Using $TiCl_4 \cdot 2THF$ as the Lewis acid, we were able to isolate allose pentaacetate at the end of the reaction sequence. Similarly, when we used $MgBr_2 \cdot OEt_2$, we were able to access gulose pentaacetate. These results represented the first example of a one-step assembly of hexoses from achiral starting materials. Work is in progress to expand this technology to other stereochemistries. Studies are also underway to develop an asymmetric variant of this reaction.

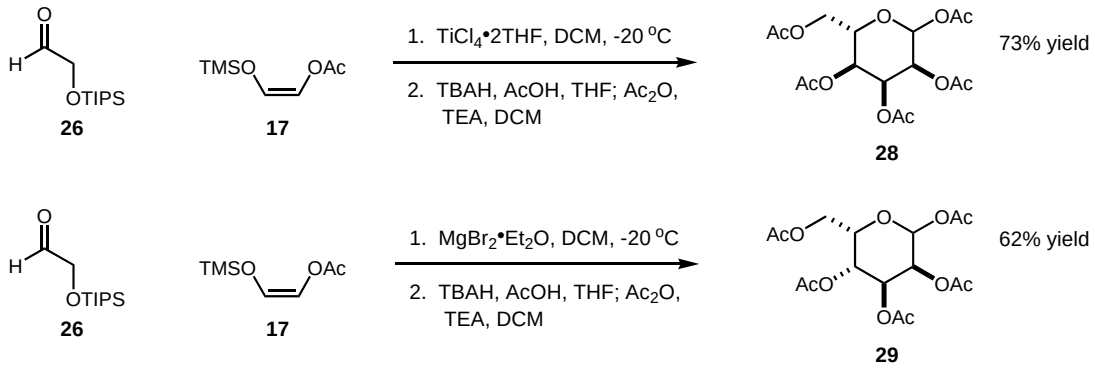


Figure 2.14: Both threo- and erytho-hexoses have been accessed through a one-step aldol reaction.

Conclusions

Described above are the efforts taken to access the syn hexoses. First developed was a method to efficiently access the TIPS-threose. This threose was tested in the two-step sugar methodology, and a protected gulolactone was formed via a thioester soft-enolization aldol reaction. Finally, a one-step method for synthesizing hexoses was developed and has produced a protected allose and gulose.

Supporting Information

General Information. Commercial reagents were purified prior to use following the guidelines of Perrin and Armarego.[17] All solvents were purified according to the method of Grubbs.[18] Non-aqueous reagents were transferred under nitrogen via syringe or cannula. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using an ice-water bath for volatile samples. Chromatographic purification of products was accomplished using forced-flow chromatography on ICN 60 32-64 mesh silica gel 63 according to the method of Still.[19] Thin-layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica gel 60-F plates. Visualization of the developed chromatogram was performed by fluorescence quenching or by anisaldehyde, ceric ammonium molybdate, or $KMnO_4$ stain.

^{1}H and ^{13}C NMR spectra were recorded on a Mercury 300 (300 MHz and 75 MHz) or an Inova 500 (500 MHz and 125 MHz) as noted, and are internally referenced to residual protio solvent signals. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constant (Hz) and assignment. Data for ^{13}C NMR are reported in terms of chemical shift (δ ppm). IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra were obtained from the California Institute of Technology Mass Spectral Facility. Gas liquid chromatography (GLC) was performed on Hewlett-Packard 6850 and 6890 Series

gas chromatographs equipped with a split-mode capillary injection system and flame ionization detectors using a J&W Scientific DB-1701 (30 m x 0.25 mm) column as noted. High-performance liquid chromatography (HPLC) was performed on Hewlett-Packard 1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted.

Preparation of the TIPS-threose

(2*S*, 3*R*)-3-Hydroxy-2,3-bis-triisopropylsilanoxy-propionaldehyde. (14) A suspension of triisopropylsilanoxy-acetaldehyde (1.0 g, 4.6 mmol) and catalyst **1** (111 mg, 0.31 mmol) in diethyl ether (1.5 mL) was stirred for 40 h at 4° C. The resulting solution was diluted with ethyl acetate (10 mL) and washed successively with water (2 x 5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in pentane (5 ml) and flashed on a diethylamine treated silica column (3.0" diameter column was filled with 150 g silica that has been stirred with a 10% diethylamine: pentane solution, washed with 800 mLof pentane, loaded with a 5 mLsolution of 0.5 g of trimer in pentane, and eluted with a 3% ether in pentane solution at a flow rate of 273 mL/min) to afford the title compound as a clear, colorless liquid (306 mg, 0.71 mmol, 92% yield) as a mixture of *syn*- and *anti*-diastereomers (4:1, determined by ^{1}H NMR) IR (film) 3483, 2945, 2892, 2868, 1734, 1464, 1385, 1117, 1069, 883, 683 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) *syn*-isomer: δ 9.74 (d, 1H, *J* = 1.5 Hz, CHO); 4.28 (dd, 1H, *J*

= 4.9, 1.5 Hz, C**H**CHO); 3.97 (dd, 1H, *J* = 9.9, 2.7 Hz, CH_2OR); 3.89 (m, 1H, C**H**OH); 3.77 (dd, 1H, *J* = 9.9, 4.5 Hz, CH_2OR); 2.73 (d, 1H, *J* = 9.9 Hz, OH); 1.16-1.00 (m, 42H, 6 $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) *syn*-isomer: δ 203.8, 74.4, 62.2, 18.0 (12C), 12.3 (3C), 11.9 (3C), one signal obscured by solvent. HRMS (CI+) exact mass calcd for $[M+H]^+$ ($C_{22}H_{49}O_4Si_2$) requires *m/z* 433.3169, found *m/z* 433.3176.

Preparation of the Hexoses

2-*O*-Benzyl-4,6-bis-*O*-triisopropylsilyl-L-gulolactone. (22) To a solution of benzyloxyacetyl ethyl thioester (73.0 mg, 0.35 mmol)[21] in dichloromethane (1 ml) at –78 °C was added neat titanium (IV) chloride (23.0 μl, 0.35 mmol). The yellow solution was allowed to stir for 20 minutes before the addition of Hunig's Base (60.0 μl, 0.35 mmol). The resulting red solution was stirred for another hour before a solution of (2*S*, 3*R*)-3-hydroxy-2,3-bis-triisopropylsilanoxy-propionaldehyde (50.0 mg, 0.12 mmol)[1] in dichloromethane (0.7 ml) was added. The solution was stirred at –78 °C for an additional hour before being moved to 4 °C for an additional 36 hours. The reaction was quenched with saturated aqueous NH_4Cl and extracted twice with dichloromethane. The organic layers were combined and washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica to afford the title compound as a pale yellow oil (50.5 mg, 0.09 mmol, 10 % ethyl acetate in hexane, stains pale blue in anisaldehyde) in 75% yield. IR (film) 2922, 2340, 1756, 1732, 1682,

1456, 1372, 1223, 1101 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$) δ 7.28-7.40 (m, 5H, ArH); 5.13 (d, *J* = 11.7 Hz, 1H, $ArCH_2$); 4.83 (d, 1H, *J*= 11.7 Hz, $ArCH_2$) 4.62-4.67 (m, 1H); 4.78 (dd, *J*= 1.7, 4.6, 1H); 4.12-4.20 (m, 2H); 3.84-4.02 (m, 2H); 2.82 (bs, 1H, OH); 0.97-1.15 (m, 42H, 6 $CH(CH_3)_2$);^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 137.0, 128.6, 128.5, 128.3, 79.4, 74.0, 72.5, 71.2, 68.4, 61.0, 18.1, 17.9, 12.7, 12.0. HRMS calcd for $[M+H]^+$ requires *m/z* 581.3615, found 581.3616 *m/z*. $[\alpha]_D$ = +28.98. Stereochemistry was confirmed by comparison to authentic gulose pentaacetate.

β-Allose pentaacetate. (28) To a stirring solution of $TiCl_4$-2THF (618 mg, 1.85 mmol) in dichloromethane (1.25 mL) at -78 ^oC was added triisopropylsilanoxy-acetaldehyde[1] (100 mg, 0.462 mmol) in dichloromethane (1 mL) and (*Z*)-acetic acid 2-(trimethyl-silanyloxy)-vinyl ester[1] (242 mg, 1.39 mmol). The solution was warmed to -20 ^oC and allowed to stir for 24 hours. The reaction was quenched with saturated aqueous NH_4Cl (1 mL) and extracted twice with dichloromethane (2 mL). The organic layers were combined and washed with saturated $NaHCO_3$ (2 x 3 mL) and brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in THF (1 mL), and to this was added a 2 M solution of TBAF and AcOH in THF (1 mL). The reaction was allowed to stir for 1 hour. Then dichloromethane (3 mL), triethylamine (1 mL, 7.2 mmol), and acetic anhydride (0.75 mL, 7.94 mmol) were added to the reaction mixture, and the reaction was allowed to stir for 8 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with saturated aqueous NH_4Cl (7 mL), saturated $NaHCO_3$ (4 x 7 mL), and brine (7 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica to afford the title

compound as a clear syrup that became a white solid upon standing (131 mg, 0.336 mmol, 50% ethyl acetate in hexane, stains green in anisaldehyde, R_f=0.47) in 73% yield. Identity of the product was determined by comparison with known β-allose pentaacetate.[22] ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.99 (d, *J* = 9.0 Hz, 1H, H-1); 5.68 (dd, 1H, *J*= 3.3, 3.0 Hz, H-3) 5.01-4.95 (m, 2H) H-4, H-2; 4.27-4.12 (m, 3H) H-5, H-6, H-6; 2.15 (s, 3H) Ac; 2.10 (s, 3H) Ac, 2.06 (s, 3H) Ac, 2.00 (s, 3H) Ac, 1.99 (s, 3H) Ac. ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 169.5, 169.4, 169.2, 169.2, 90.2, 71.3, 68.5, 68.3, 65.9, 62.1, 21.3, 21.1, 21.1, 21.1, 20.9.

β-Gulose pentaacetate. (29) To a stirring solution of $MgBr_2$-OEt_2 (448 mg, 1.85 mmol) in dichloromethane (1.25 mL) at -78 ^{o}C was added triisopropylsilanoxy-acetaldehyde[1] (100 mg, 0.462 mmol) in dichloromethane (1 mL) and (*Z*)-acetic acid 2-(trimethyl-silanyloxy)-vinyl ester[1] (242 mg, 1.39 mmol). The solution was warmed to -20 ^{o}C and allowed to stir for 24 hours. The reaction was quenched with saturated aqueous NH_4Cl (1 mL) and extracted twice with dichloromethane (2 mL). The organic layers were combined and washed with saturated $NaHCO_3$ (2 x 3 mL) and brine (3 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in THF (1 mL), and to this was added a 2 M solution of TBAF and AcOH in THF (1 mL). The reaction was allowed to stir for 1 hour. Then dichloromethane (3 mL), triethylamine (1 mL, 7.2 mmol), and acetic anhydride (0.75 mL, 7.94 mmol) were added to the reaction mixture, and the reaction was allowed to stir for 8 hours. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with saturated aqueous NH_4Cl (7 mL), saturated $NaHCO_3$ (4 x 7 mL), and brine (7 mL). The organic layer was dried over anhydrous Na_2SO_4 and

concentrated *in vacuo*. The residue was chromatographed on silica to afford the title compound as a clear, colorless syrup (112 mg, 0.287 mmol, 50% ethyl acetate in hexane, stains dark green in anisaldehyde, R_f=0.47) in 62% yield and 4:1 d.r. Identity of the major product was determined by comparison with known β-gulose pentaacetate.[22] ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98 (d, 1H, *J* = 8.7 Hz) H-1); 5.42 (dd, 1H, *J*= 3.3, 3.6 Hz) H-3, 5.12-5.08 (m, 1H) H-2, 4.99-4.97 (m, 1H) H-4, 4.38-4.33 (m, 1H) H-5, 4.20-4.05 (m, 2H) H-6, H-6; 2.16 (s, 3H) Ac; 2.15 (s, 3H) Ac, 2.13 (s, 3H) Ac, 2.05 (s, 3H) Ac, 2.00 (s, 3H) Ac. ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 169.6, 169.5, 169.3, 169.1, 90.1, 71.6, 67.8, 67.6, 67.5, 61.8, 21.3, 21.1, 21.1, 21.1, 21.0. The minor product was unable to be separated sufficiently from the major product for comparison to known pentaacetates.

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23. This work was performed in the MacMillan laboratory with the assistance of Drs. Alan Northrup, Akio Kayano, and Ian Mangion.

C h a p t e r 3

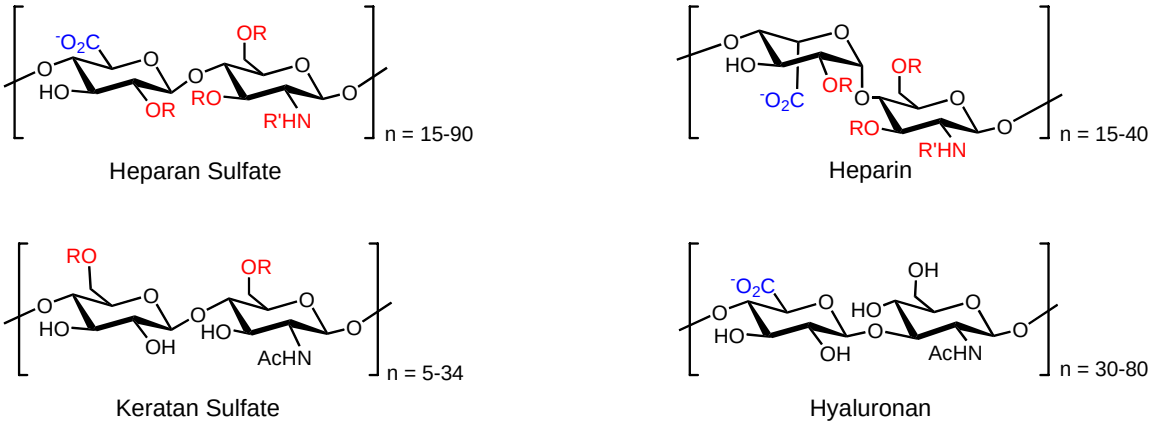
Synthesis of a Differentially-Protected Chondroitin Sulfate Disaccharide[31]

Introduction

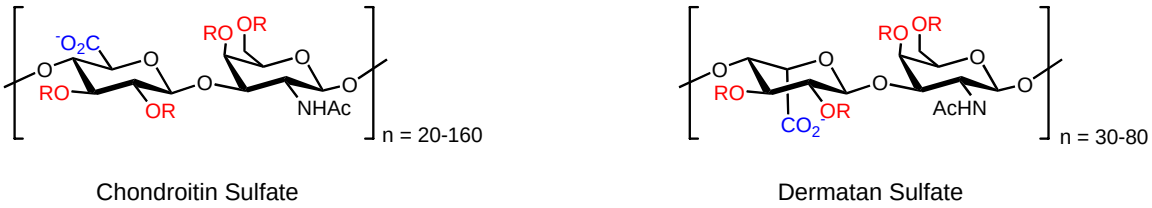
Described in the previous chapters is a method for quickly accessing differentially-protected monosaccharides.[1] This methodology could accelerate the synthesis of biologically active polysaccharides. To examine this theory, we decided to employ this method to synthesize a chondroitin sulfate tetrasaccharide.

Chondroitin sulfate (CS) is a member of a class of linear, sulfated polysaccharides known as glycosaminoglycans (GAGs). Glycosaminoglycans (GAGs) are comprised of repeating disaccharide subunits which alternate in uronic acid and aminosugar residues. These polysaccharides vary in stereochemistry, length, and patterns of sulfation. GAGs are classified into two broad categories depending on the amino sugar present in the polymer. The glucosaminoglycans (hyaluronan, keratan sulfate, heparin, heparan sulfate) are based on D-glucosamine, while the galactosaminoglycans (chondroitin sulfate and dermatan sulfate) are based on D-galactosamine. The uronic acid residue present in a CS disaccharide is glucuronic acid (GlcA), while either GlcA or iduronic acid (IdoA) can be present in heparan sulfate (HS), heparin, and dermatan sulfate (Figure 3.1).[2]

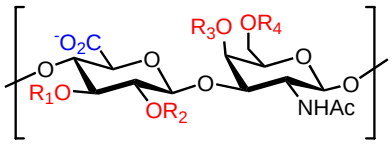
Glucosaminoglycan Class



Galactosaminoglycan Class



R = H or OSO_3^-
R' = Ac or OSO_3^-
n = average lengths



Chondroitin Sulfate-A: R_1, R_2, R_4=H; R_3=OSO_3^-
Chondroitin Sulfate-C: R_1, R_2, R_3=H; R_4=OSO_3^-
Chondroitin Sulfate-D: R_1, R_3=H; R_2, R_4=OSO_3^-
Chondroitin Sulfate-E: R_1, R_2=H; R_3, R_4=OSO_3^-

Figure 3.1: Glycosaminoglycans can be divided into two classes, glucosaminoglycans and galactosaminoglycans, based on the aminosugar residue present in the polysaccharide chain. These classes are further divided based on the uronic acid or glycosyl residue present in the polysaccharide backbone and the sulfation pattern displayed along the chain.

CS is a heterogeneous, linear polysaccharide that can be found on the cell surface or in the extracellular matrix (Figure 3.2). Its biosynthesis begins in the Golgi apparatus when a tetrasaccharide linker is attached to specific serine residues on certain proteins. Alternating units of GalNAc and GlcA are added, and then sulfotransferases sulfate specific hydroxyl groups along the oligosaccharide chain. CS can be sulfated at any or all of the free hydroxyl groups, representing 256 different possible sulfation motifs for a simple tetrasaccharide. Each sulfation pattern is denoted by a letter, such as CS-A (Figure 3.1).[2-3]

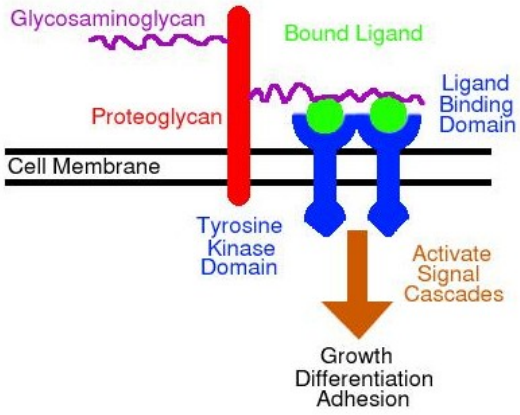


Figure 3.2: Glycosaminoglycans can be found at the cell surface or in the extracellular matrix.

For years scientists have been interested in understanding the many roles that CS proteoglycans play in biology. Monitoring mRNA levels of CS proteoglycans from both

embryonic rat[4a] and chick[4b] brains at various stages of development has shown that CS proteoglycan expression is highly regulated in the developing brain. Phenotypic studies have also highlighted the importance of CS. Mutations in 8 squashed vulva (sqv) genes have been shown to affect the biosynthesis of CS and heparan sulfate (HS) glycosaminoglycans in *Caenorhabditis elegans*, and these mutations are known to cause defects in cytokinesis and in vulval morphogenesis. Further studies in *Caenorhabditis elegans* have confirmed that it the disruption of CS synthesis can lead to the observed phenotype. [5] Mutation of the chondroitin-4-sulfotransferase 1 gene in mice resulted in severe chondrodysplasia and a disorganized cartilage growth plate, and deletion of a chondroitin-6-sulfotransferase gene in humans causes severe skeletal defects.[6] Furthermore, altered expression of CS proteoglycans is common with a number of cancers including melanoma, pancreatic, ovarian, and gastric cancers and believed to stimulate cancer cell proliferation, migration, and invasion.[7]

CS has also been shown to regulate the development of the nervous system and has been assigned both stimulatory and inhibitory roles in neuronal growth and development. CS has been shown to stimulate neurite outgrowth in rat hippocampal cultures,[8] but CS proteoglycans have also been shown to repel migrating neurons.[9] While suggesting the importance of sulfation in CS activity, these studies were unable to conclusively establish which sulfation pattern produces each biological response observed.

Many studies have attempted to explore this relationship with CSs isolated from natural sources. Unfortunately, natural isolates, while enriched in a particular motif, still possess other sulfation motifs, so no clear relationships can be drawn between structure and function. The use of synthetic CS tetrasaccharides with defined sulfation patterns has

allowed examination of the role the sulfation pattern. Studies performed in the Hsieh-Wilson laboratory using synthetic chondroitin sulfate tetrasaccharides have confirmed that the sulfation pattern determines the biological activity. Four tetrasaccharides (CS-A, CS-C, CS-E, and CS-R) were synthesized and tested with hippocampal, dopaminergic, and dorsal root ganglion (DRG) neurons, and it was shown that only the CS-E tetrasaccharide stimulated neuronal growth. These studies also established the tetrasaccharide as the minimum structure needed to produce a biological effect.[10]

Syntheses of Chondroitin Sulfate[32]

With these exciting results, it was desirable to expand the library of CS tetrasaccharides available to our laboratory. We set out to design a single, differentially-protected tetrasaccharide that would access all the desired sulfation patterns. With this goal, we began to plan our synthetic strategy, examining carefully the knowledge gained from previous syntheses of CS.

I. Sinay's Synthesis of a CS-A Disaccharide

Sinay and coworkers reported the synthesis of a CS-A disaccharide in 1989. In their synthesis, glucosyl donor **1** and galactosamine acceptor **2** were joined via a silver triflate-mediated coupling to produce the β-(1,3)-linkage that forms CS disaccharide **3**.

The acetate protecting group present on the glucosyl 2-position served as a participating group to promote the formation of a β-(1,3)-linkage over the α-(1,3) linkage (Figure 3.3).

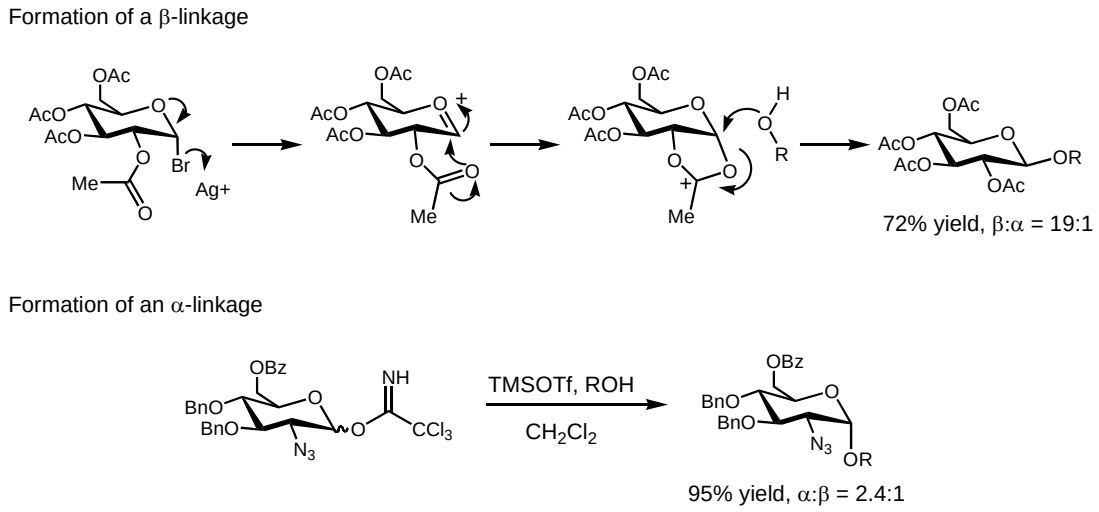
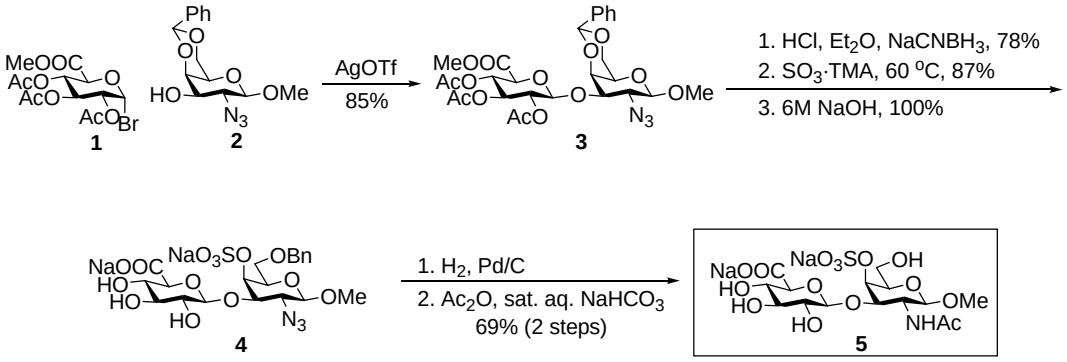


Figure 3.3: Participating groups refer to protecting groups that can block one face of the donor, forcing the acceptor to attack the unhindered face. Non-participating groups refer to protecting groups that do not perform in this manner.

Next, the benzylidene ring was selectively opened to produce the C-6 benzyl group, and the exposed C-4 position was sulfated. Removal of the acetates and simultaneous saponification of the methyl ester produced disaccharide **4**. Simultaneous hydrogenolysis of the benzyl protecting group and reduction of the azide followed by acetate protection of the resulting amine produced the CS-A disaccharide (**5**, Scheme 3.1). This synthesis established many reaction conditions that have become staples in CS synthesis.[11]



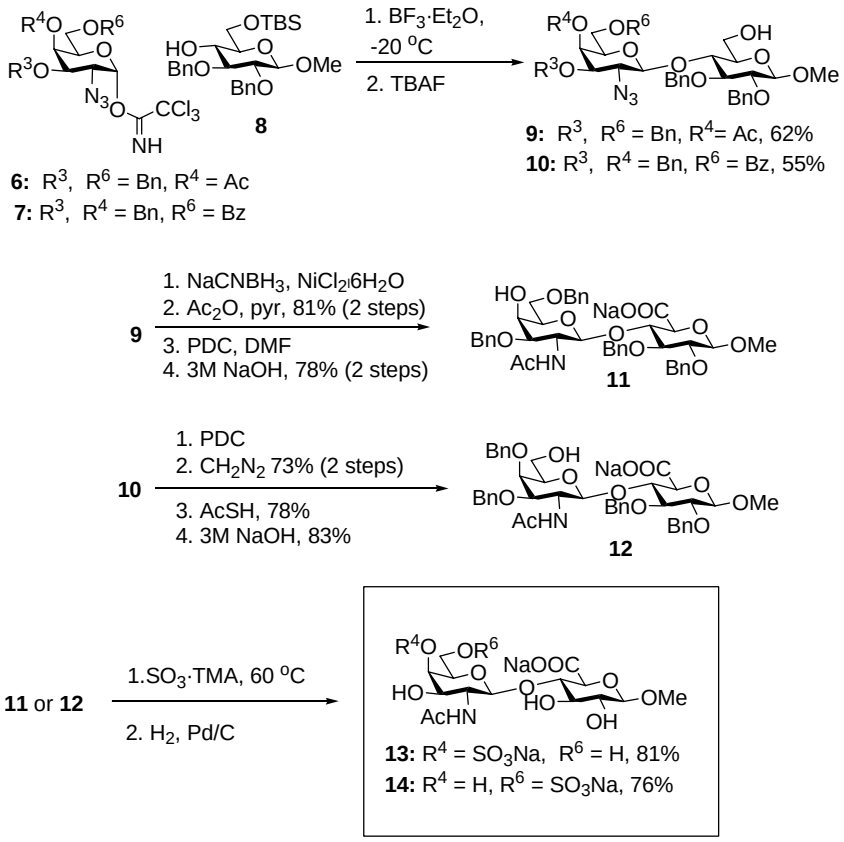
Scheme 3.1: The synthesis of a CS-A disaccharide reported by Sinay and coworkers

II. Jacquinet's Synthesis of CS-A and CS-C Disaccharides

A year later, Jacquinet and coworkers reported the synthesis of two CS-A and CS-C disaccharides. They produced two disaccharides, one CS-A and the other CS-C, that contained the β-(1,3)-linkage and two that contained the β-(1,4)-linkage. The formation of the β-(1,4)-linkage is notable for two reasons. First, the C-4 position of the acceptor is known to be the least reactive position around the ring, and formation of the β-(1,4)-linkage can be complicated by this decrease in reactivity. Second, the β-linkage was formed without the use of a participating group To form CS-A and CS-C, galactosamine donors **6** and **7** were generated from galactal and coupled to glucosyl acceptor **8.** The resulting disaccharides were subjected to TBAF to remove the primary TBS group to produce disaccharides **9** and **10.** The boron-mediated coupling was inspired by work from Schmidt and coworkers, who showed that α-trichloroacetimidates undergo inversion during low-temperature, boron-mediated coupling reactions to afford β-(1,6)-, β-(1,4)-, and β-(1,3)-

linkages with high levels of selectivity (β:α ratios from 4:1 ~ >19:1) without the use of a C-2 participating group.[12]

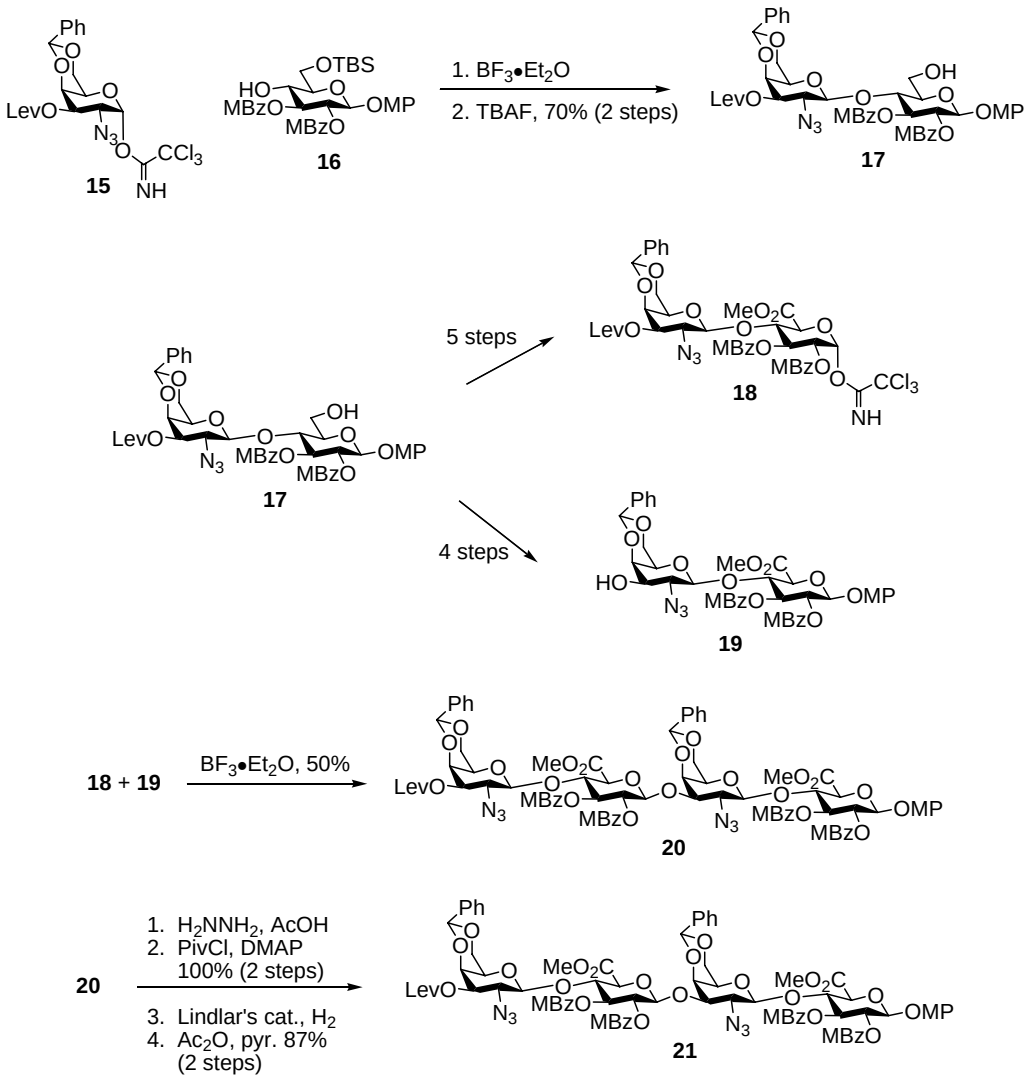
To conclude the synthesis, disaccharides **9** and **10** were elaborated to the C-4 and C-6 deprotected saccharides **11** and **12**, respectively. These disaccharides were sulfated before simultaneous hydrogenolysis of the benzyl groups and reduction of the azides. The resulting amines were acetylated to produce CS-A and CS-C disaccharides **13** and **14** (Scheme 3.2).[13]



Scheme 3.2: Jacquinet's synthesis of CS-A and CS-C disaccharides

III. Ogawa's Synthesis of CS-A, CS-C, and CS-E Tetrasaccharides

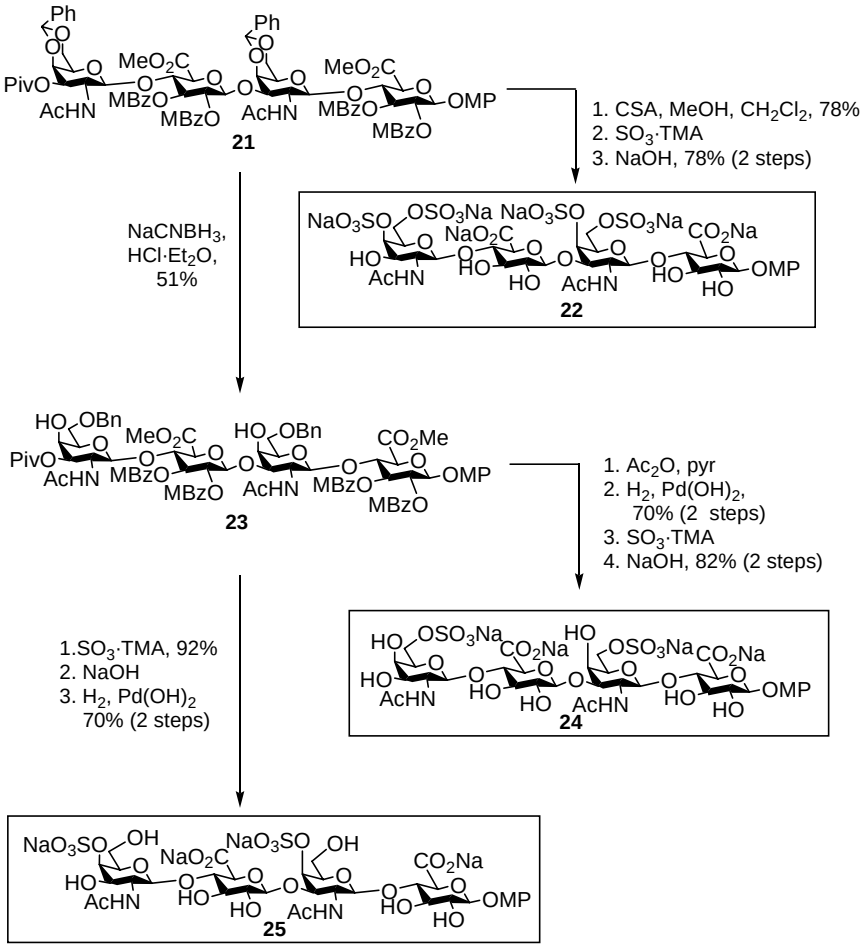
The Ogawa laboratory has completed the synthesis of CS-A, CS-C, and CS-E tetrasaccharides. As in the Jacquinet synthesis, monosaccharides **15** and **16** were coupled using Schmidt's imidate methodology and then desilated to produce disaccharide **17**. This disaccharide was then elaborated to both donor **18** and acceptor **19**, which were joined together via a second boron-mediated coupling to produce protected tetrasaccharide **20**. The levulinoyl group on tetrasaccharide **20** was replaced with a more stable pivolate ester before the azide was reduced to the amine and acetylated to form tetrasaccharide **21** (Scheme 3.3).



Scheme 3.3: Ogawa's synthesis of a core CS tetrasaccharide.

Tetrasaccharide **21** was elaborated to CS-A, CS-C, and CS-E through the following transformations: To access the CS-E tetrasaccharide (**22**), the 4,6-benzylidene was removed, and the revealed positions were sulfated. Finally, the remaining protecting groups were removed to afford the desired tetrasaccharide. To access CS-A and CS-C, the benzylidene was selectively opened to reveal the C-4 position. To produce the CS-A

tetrasaccharide (**25**), the C-4 hydroxyl was sulfated and then the remaining protecting groups were removed. To access the CS-C tetrasaccharide, the C-4 hydroxyl of tetrasaccharide **23** was acetylated before subjecting it to hydrogenation to remove the C-6 benzyl group. The exposed position was sulfated, and the remaining protecting groups were removed to afford the CS-C tetrasaccharide (**24**, Scheme 3.4).[14]



Scheme 3.4: Elaboration to CS-A, CS-C, and CS-E tetrasaccharides

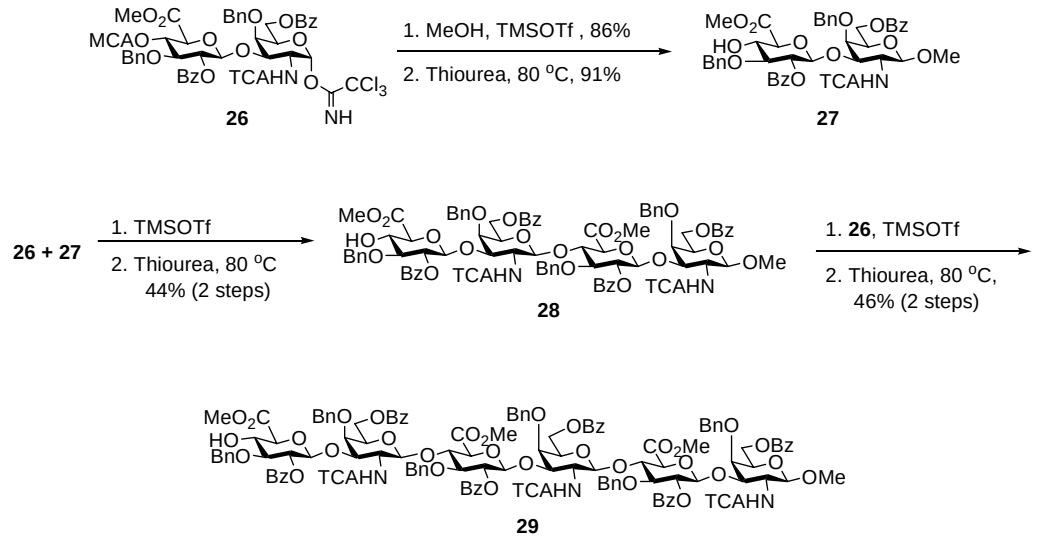
This synthesis was noteworthy for a number of reasons: This synthesis employs a divergent strategy, allowing access to 3 tetrasaccharides from a core disaccharide. Furthermore, it reaffirms the usefulness of trichloroacetimidate donors for coupling both mono- and disaccharides. In addition, this synthesis proves that it is feasible to perform late-stage protecting group manipulation on a tetrasaccharide to access other sulfation patterns.

However, this synthesis also revealed issues to consider as we approached our synthetic design. First, it illustrated the importance of choosing protecting groups that can withstand all reaction conditions between its application and removal. Second, the Ogawa group reported difficulties separating some of the products in the synthesis, suggesting the need to explore a range of purification methods for our synthesis. Finally, the ability of tetrasaccharide **21** to access other sulfation patterns was limited in part by the lack of differentiation between the glucosyl C-2 and C-3 position, highlighting an issue to focus on during the design of our synthesis.

IV. Jacquinet's Synthesis of CS-D Tetra- and Hexasaccharides

The Jacquinet laboratory has published the synthesis of both a CS-D tetra- and hexasaccharide. Acceptor **27** was prepared from disaccharide **26** via TMSOTf-mediated methanolysis of the imidate. The C-4 ester was selectively removed with thiourea to afford disaccharide **27.** This disaccharide was then joined with donor **26** via a TMSOTf-promoted coupling, and the C-4 chloroacetate of the resulting tetrasaccharide was removed with thiourea to afford tetrasaccharide **28**. This tetrasaccharide was subjected to similar

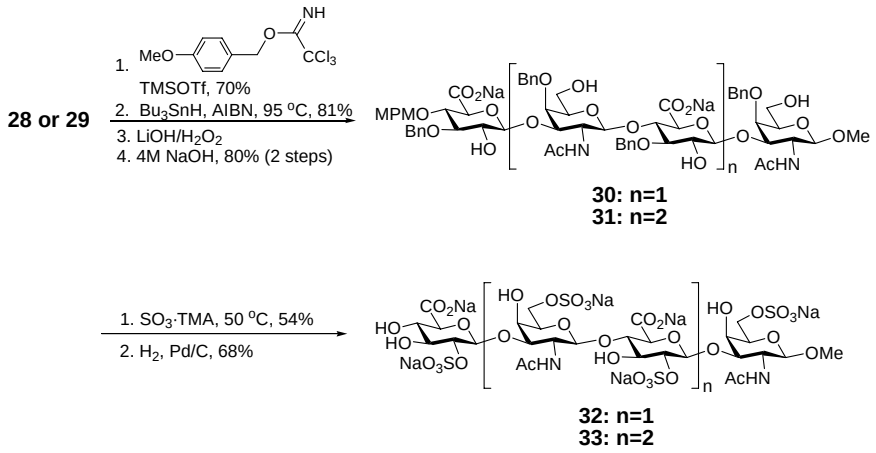
coupling conditions with donor **26** and, after chloroacetate deprotection with thiourea, afforded hexasaccharide **29** (Scheme 3.5)**.**



Scheme 3.5: Jacquinet's synthesis of the core tetra- and hexasaccharides

Both tetrasaccharide **28** and hexasaccharide **29** were elaborated through the following chemistry. First, the C-4 hydroxyl was protected using *p*-methoxybenzyl (MPM) trichloroacetimidate. Next, the *N*-trichloroacetyl group was converted to the natural *N*-acetyl group through exposure to AIBN and tributyltin hydride. Saponification of the C-5 methyl ester and removal of the benzoyl groups afforded tetra- and hexasaccharides **30** and **31**. Sulfation of the revealed hydroxyls followed by hydrogenolysis of the benzyl and MPM groups produced CS-D tetra- and hexasaccharides **32** and **33** (Scheme 3.6).[15] This synthesis was most notable for differentiating between the glucosyl C-2 and C-3 positions. Furthermore, Jacquinet's observations concerning the

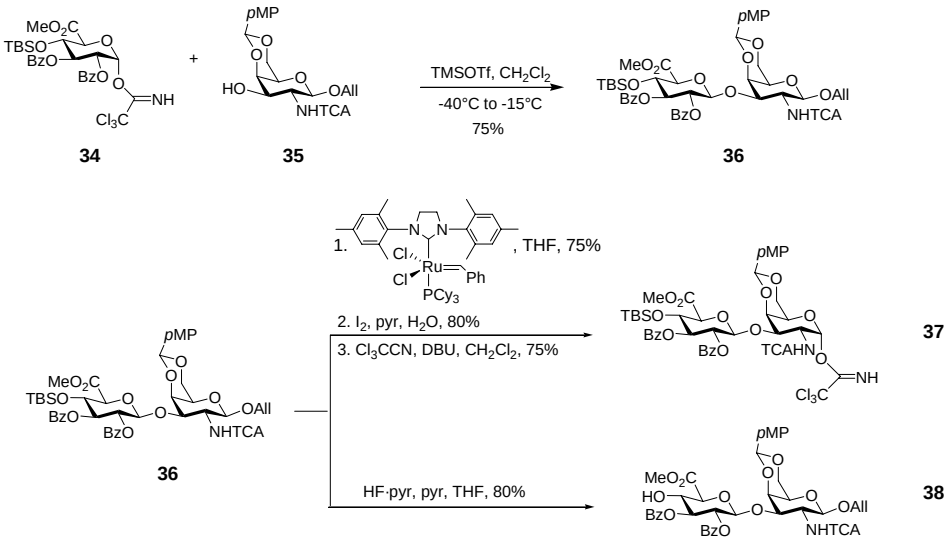
difficulty of sulfating the glucosyl C-2 hydroxyl allowed us to be aware of strong sulfation conditions that would be needed to affect this transformation.



Scheme 3.6: Elaboration to the CS-D tetra- and hexasaccharides

V. Hsieh-Wilson's synthesis of CS-A, CS-C, CS-E, and CS-R Tetrasaccharides

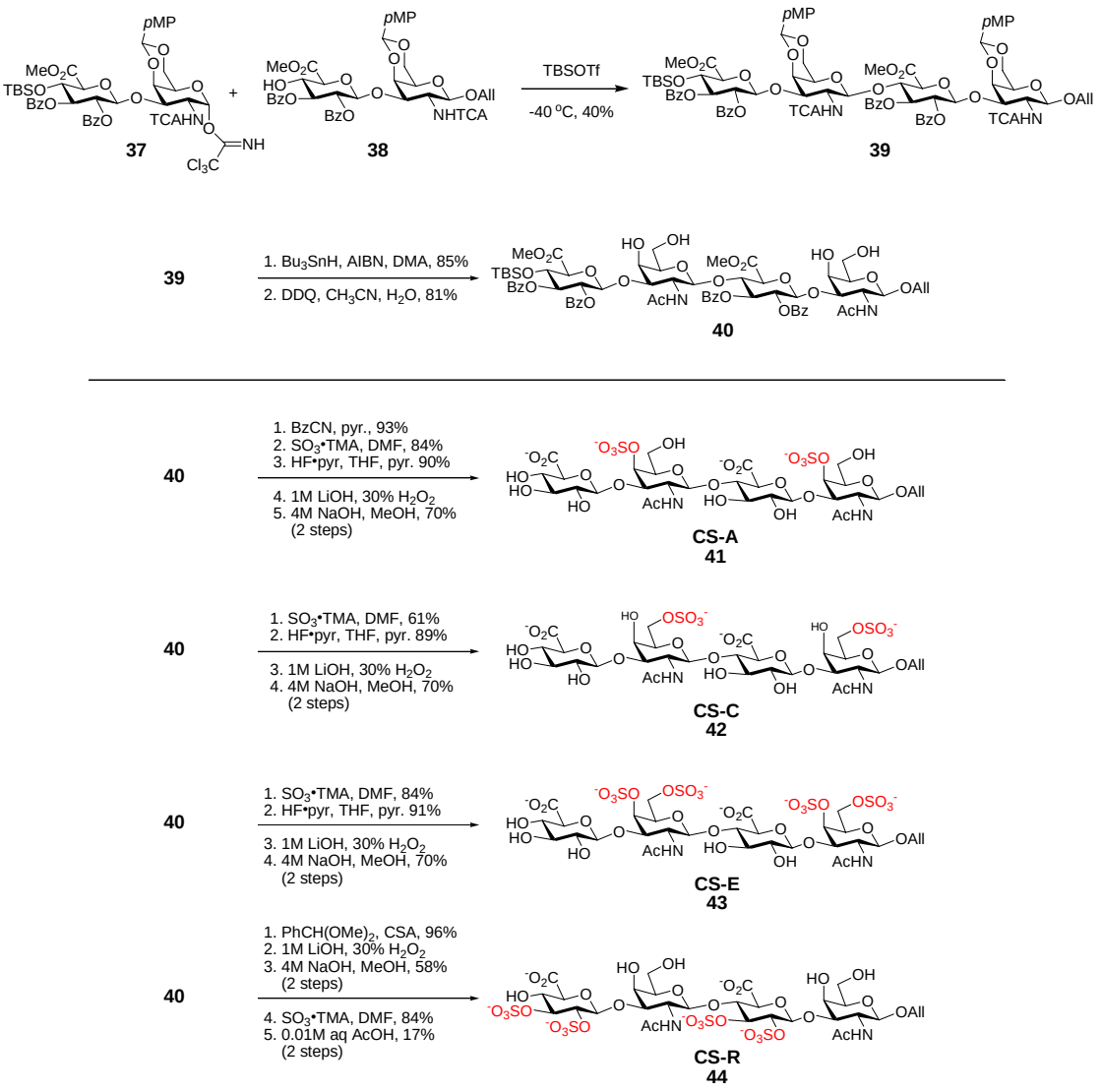
The greatest source of information came from our laboratory's experience synthesizing a library of CS-A, CS-C, CS-E, and CS-R tetrasaccharides. This synthesis converges at a core disaccharide and tetrasaccharide from which all four sulfation patterns are accessed. A TMSOTf-mediated coupling of imidate donor **34** and galactosamine acceptor **35** afforded disaccharide **36**. Exposure of disaccharide **36** to the Grubbs second-generation catalyst followed by hydrolysis of the resulting enol ether removed the anomeric allyl group. The resulting disaccharide was elaborated to form trichloroacetimidate donor **37**. Disaccharide **36** was elaborated to acceptor **38** through removal of the TBS ether (Scheme 3.7).



Scheme 3.7: Synthesis of the donor and acceptor disaccharides

Donor **37** and acceptor **38** were joined via a TBSOTf-mediated coupling reaction to form tetrasaccharide **39**. This tetrasaccharide was elaborated to core tetrasaccharide **40** through conversion of the *N*-trichloroacetyl groups to *N*-acetyl groups through the use of tributyltin hydride and AIBN, followed by removal of the *p*-methoxybenzylidene rings. To access CS-A, the galactosamine C-6 hydroxyl positions were selectively benzoylated, and the C-4 hydroxyls were sulfated through exposure to SO_3-TMA. Removal of the TBS ether, saponification of the methyl esters, and cleavage of the ester protecting groups afforded CS-A tetrasaccharide **41**. To form CS-C tetrasaccharide **42**, the galactosamine C-6 hydroxyl positions were selectively sulfated, and the remaining protecting groups were removed as before. Accessing the CS-E tetrasaccharide proceeded through a similar pathway. Sulfation of the galactosamine C-4 and C-6 hydroxyls followed by removal of

the remaining protecting groups produced CS-E tetrasaccharide **43**. To access the CS-R tetrasaccharide, two benzylidene rings were installed to protect the C-4 and C-6 hydroxyls. Next, saponification of the methyl esters followed by removal of the benzoyl protecting groups revealed the glucosyl C-2 and C-3 hydroxyls for sulfation. After sulfation, simultaneous removal of the TBS ether and the benzylidene rings afforded CS-R tetrasaccharide **44**.[10]



Scheme 3.8: The Hsieh-Wilson synthesis of CS-A, CS-C, CS-E, and CS-R tetrasaccharides.

Certain details of this synthesis were noted when we designed the second-generation tetrasaccharide. This synthesis reaffirmed the use of a core disaccharide to access multiple tetrasaccharides. It was also noted that the sulfation conditions used to produce CS-R tetrasaccharide **44** were found to be acidic enough to remove the *p*-

methoxybenzylidene protecting groups, so acid-sensitive protecting groups should be used judiciously. In addition, this synthesis provided conditions to selectively protect or sulfate the galactosamine C-6 position over the neighboring C-4 position. In addition, a rearrangement was observed during the tetrasaccharide coupling (Figure 3.4), and it was hoped that substitution of the *N*-trichloroacetyl group for a more activating protecting group would minimize this rearrangement and improve coupling yields.[16] The trichloroethyl carbamate had been shown to be a more active group in disaccharide couplings with no reported rearranged product, so this group was chosen as the amine protecting group for our synthesis.[17] Finally, later work with these tetrasaccharides revealed that the allyl group can serve as a useful chemical handle, so it was decided that this functional group would remain but be installed later in the synthesis to avoid the two-step deprotection necessary to remove it (Figure 3.5).[10b, 16, 18]

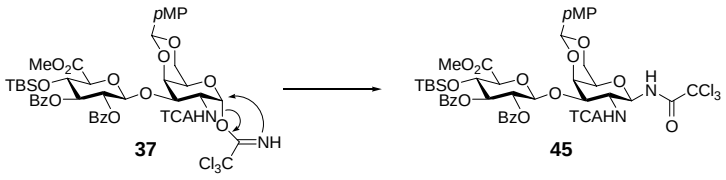


Figure 3.4: Donor **37** can undergo rearrangement to amide **45**.

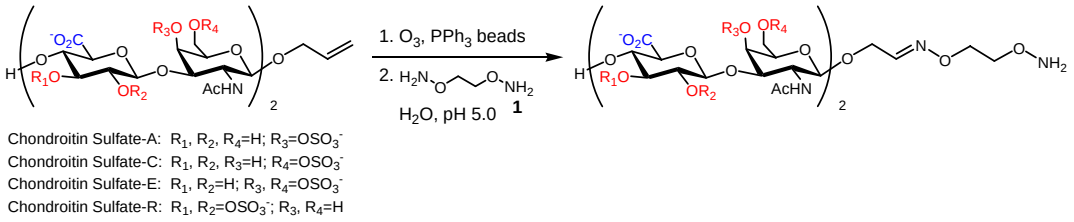
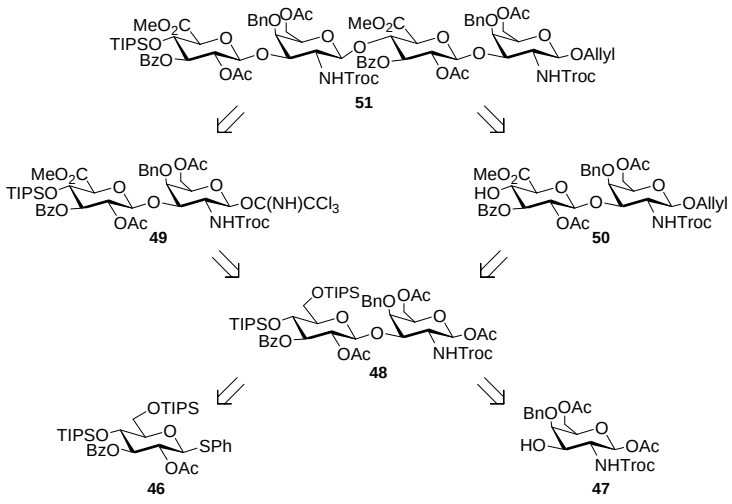


Figure 3.5: The allyl group can be used as a chemical handle for attaching tetrasaccharides to surfaces or other molecules.

Retrosynthesis of the CS Disaccharide

The retrosynthetic analysis of our tetrasaccharide (**51**) begins by cleaving the β-(1,4) linkage to produce disaccharides **49** and **50** that can both be accessed from a core disaccharide (**48**, Scheme 3.9). A TIPS protecting group was chosen for the glucosyl C-4 position on disaccharide **48** because this silyl ether can be selective cleaved using HF·pyridine or TBAF in the presence of many other protecting groups. Furthermore, the TIPS group has been described as the most stable of the silyl protecting groups, making it likely that it will be able to withstand coupling and sulfation conditions.[19] As mentioned before, it was hoped that the MacMillan sugar methodology (described in Chapters 1 and 2) could be used to accelerate this synthesis. This methodology would allow access to a glucose monosaccharide that possessed TIPS groups at the C-6 and C-4 hydroxyl groups. The primary TIPS group can be selectively removed for oxidation of the C-5 position.[1c] This would occur before removal of the C-4 TIPS ether was necessary, so this protecting group scheme was acceptable. A number of groups were considered for the C-2 and C-3 glucosyl positions. For the C-2 hydroxyl group, it was important to choose a participating

group (Figure 3.3) which would allow access to the β-(1,3)-linkage found in CS. Initially, a levulinoyl (Lev) group was chosen for the C-2 position because it can be selectively removed with sodium borohydride,[20] but this was abandoned when evidence of Lev-group migration was found. It was then decided that an acetate group may be a better choice for the C-2 hydroxyl. Benzoyl esters were chosen to protect the glucosyl C-1 and C-3 hydroxyls for two reasons. First, the acetyl protecting groups can be selectively removed in the presence of benzoyl group through the use of hydrazine or guanidine, allowing differentiation between the C-2 and C-3 position.[2b, 20] Second, an anomeric benzoyl group can be directly activated to form a thioglycoside.[21]



Scheme 3.9: Retrosynthetic analysis of the core disaccharide

Next, the protecting groups for the galactosamine residue were determined. An anomeric acetate group was chosen for the disaccharide because it is highly precedented in carbohydrate chemistry as a group that is stable to coupling reactions and can be removed selectively for coupling.[2b, 20] A trichloroethylcarbamate (Troc) protecting group was selected to protect the amine for reasons described above.[17] A benzyl group was chosen for the galactosamine C-4 hydroxyl group because it can be selectively removed in the presence of the ester and silyl protecting groups.[20] Furthermore, evidence suggested that it could be removed without reduction of the allyl group at the galactosamine anomeric position.[22] The allyl group at the anomeric position was chosen because it can be converted via ozonolysis to the aldehyde, which can serve as a chemical handle for the attachment to surfaces.[10b, 16, 18] Finally, an acetate group was selected for the galactosamine C-6 position because the removal of a primary acetate group in the presence of a secondary acetate is precedented in carbohydrate chemistry.[2b]

This disaccharide could come from the coupling of monosaccharides **46** and **47** (Scheme 3.9). To quickly access the desired monosaccharides, two methodologies would be employed—the two-step sugar methodology developed by the MacMillan laboratory[1] and a Cerny epoxide methodology developed in the Hsieh-Wilson laboratory.[23] Briefly, the MacMillan methodology allows differentiation of the C-2 and C-3 hydroxyl groups by assembling the monosaccharide through two aldol reactions of three protected α-oxyaldehydes. The Hsieh-Wilson methodology provides an efficient way to access aminosugars through the use of Cerny epoxides. Cerny epoxides are known to isomerize under basic conditions, but through simultaneous epoxide formation and C-4 hydroxyl

protection, the Hsieh-Wilson methodology traps the desired kinetic epoxide. This epoxide can be opened to provide a 3-hydroxyhexose ready for coupling (Figure 3.6).

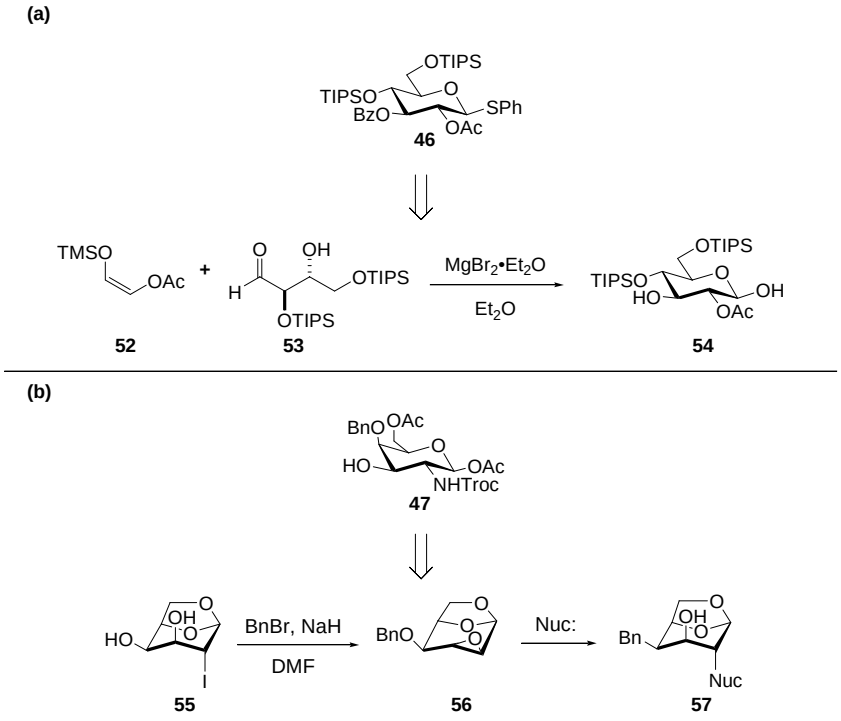
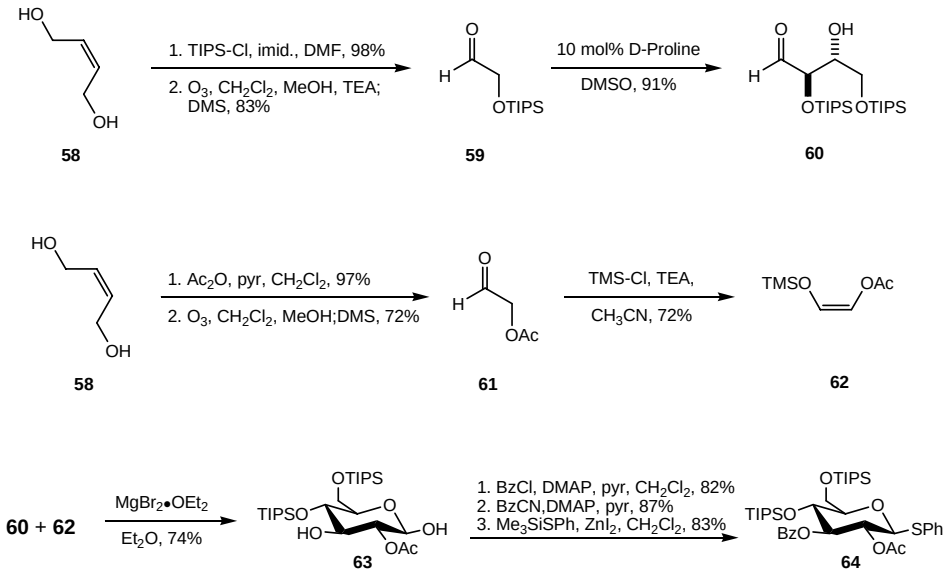


Figure 3.6: (a) Glucosyl monomer **46** can be accessed using an aldol methodology. (b) Galactosamine monomer **47** can be accessed through a Cerny epoxide.

Monosaccharides **46** and **47** were chosen as the coupling partners. It was initially decided that the glucosyl C-5 methyl ester would be formed after synthesizing core disaccharide **48** because C-5 esters have been shown to deactivate the donor in coupling reactions.[2b] Opening the 1,6-anhydro ring on the galactosamine monomer after the coupling would eliminate two steps (protection and deprotection of the galactosamine C-3 hydroxyl group) from the synthesis, so this pathway was chosen.

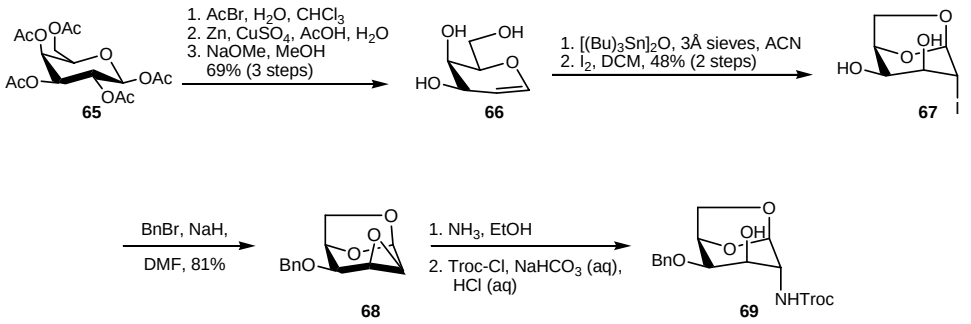
Synthesis of the CS Disaccharide

In the forward sense, we began by synthesizing the glucose and galactosamine monomers. The synthesis of the glucose monomer proceeded as follows: First, *cis*-butene diol **58** was protected with TIPS-Cl and ozonolyzed to create the TIPS-aldehyde (**59**). This aldehyde was dimerized via a proline-catalyzed aldol reaction to produce TIPS-erythrose **60**. Acetoxyenolate **62** was also synthesized from butane diol. Diol **58** was acetate protected and ozonlyzed to produce acetoxyaldehyde **61**.[24] This aldehyde was enolized with TMS-Cl and triethylamine to afford enolate **62**. The TIPS-erythrose and enolate **62** were combined in a $MgBr_2$•OEt_2-mediated aldol reaction to produce glucose **63**.[1] Efforts were made to protect the anomeric and C-3 hydroxyls in one step, but migration of the C-2 acetate to the anomeric position occurred in conditions basic enough to protect the C-3 position. Reducing the basicity to avoid migration prevented protection of the C-3 hydroxyl group. In the end, a two-step method was used where the anomeric position was protected using mildly basic conditions, and then more basic reaction conditions were used to protect the C-3 hydroxyl group. Finally, the anomeric position was activated with ZnI_2 to afford thioglycoside **64** (Scheme 3.10).



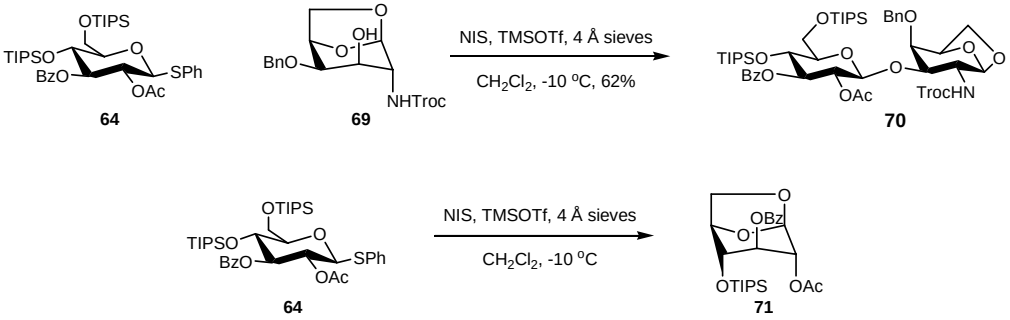
Scheme 3.10: Synthesis of glucosyl donor **64**

The galactosamine monomer synthesis begins with peracetylated galactose **65**. Bromination of the anomeric position followed by a zinc-mediated elimination produced tri-*O*-acetyl galactal, which was deprotected with sodium methoxide in methanol to produce galactal (**66**).[25] Galactal was then subjected to a two-step iodo-cyclization reaction to provide iodogalactal **67**.[26] The iodogalactal was simultaneously benzyl-protected at the C-4 position and epoxidized to form epoxide **68**.[23] The epoxide was opened with ammonia[27] and the resulting amine was Troc-protected to afford galactosamine **69** (Scheme 3.11).



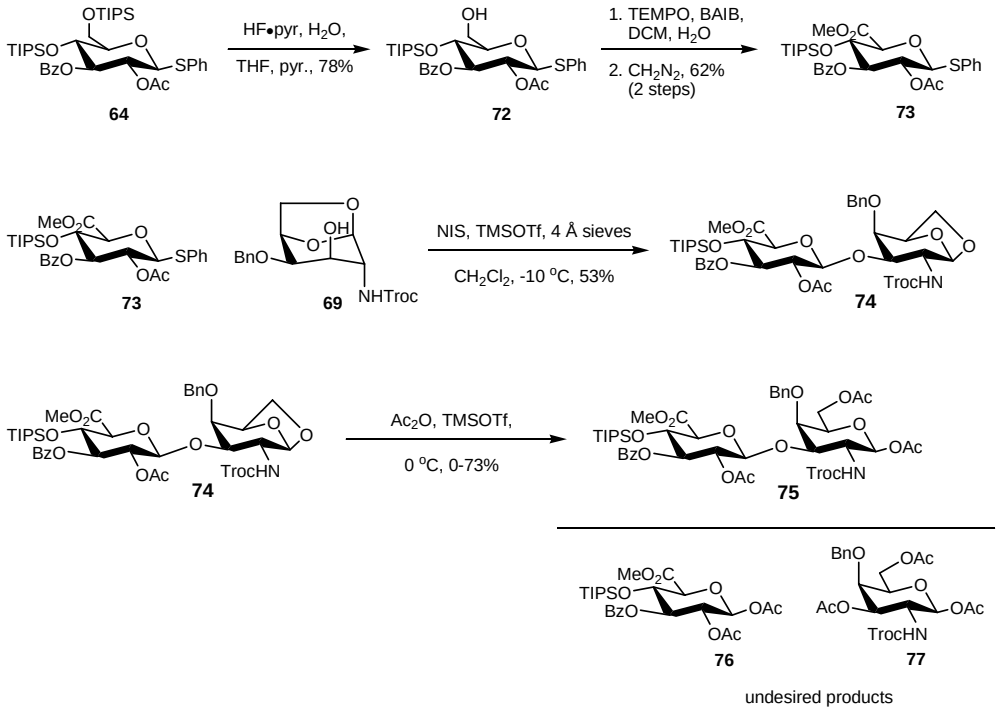
Scheme 3.11: Synthesis of galactosamine acceptor **69**

Glucosyl donor **64** and galactosamine acceptor **67** were combined via a TMSOTf-mediated coupling to provide disaccharide **70** in 62% yield. Investigations of this reaction revealed that the primary TIPS group was unstable to the coupling conditions, permitting an intramolecular cyclization to produce 1,6-anhydro glucose **71**. It was then decided to install the C-5 methyl ester before coupling, because this would prevent the formation of the 1,6-anhydro glucose byproduct (Scheme 3.12).



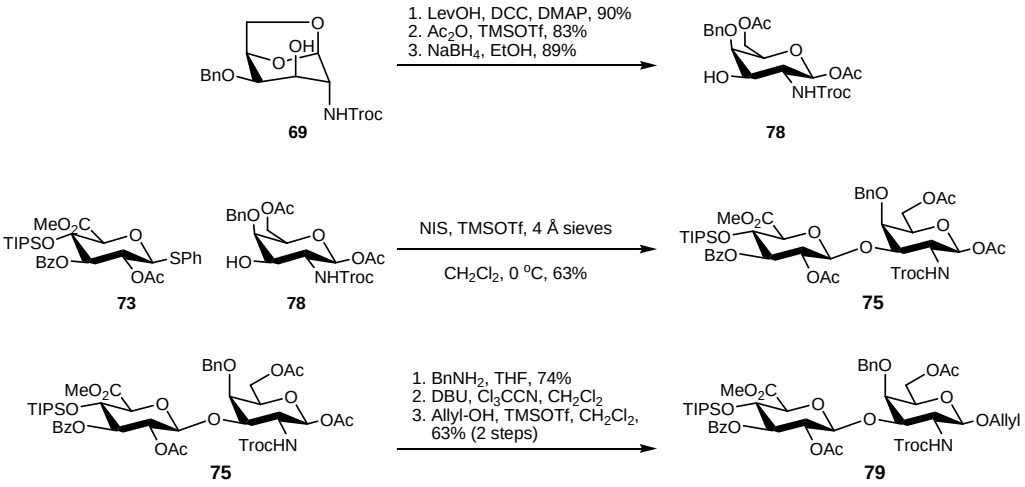
Scheme 3.12: Formation of disaccharide **70**

To that end, the primary TIPS group was removed on glucosyl donor **64** with HF·pyridine to produce glucose **72**. The C-6 hydroxyl was subjected to a TEMPO-oxidation to form the acid, which was converted to the methyl ester through treatment with diazomethane. This new glucosyl donor (**73**) was subjected to similar coupling conditions with galactosamine **69** to afford disaccharide **74** in 53% yield. Attempts to open the 1,6-anhydro ring of disaccharide **74** with acetic anhydride and TMSOTf produced widely variable yields due to cleavage of the glycosidic bond to produce monosaccharides **76** and **77**. Because of this, it was decided that the 1,6-anhydro ring would be opened prior to coupling (Scheme 3.13).



Scheme 3.13: Synthesis of glucosyl donor **73** allowed formation of disaccharide **74**, but attempts to open the 1,6-anhydro ring produced the desired disaccharide **75** variable yields.

To open the 1,6-anhydro ring, galactosamine **69** was first protected at the C-3 hydroxyl group with a levulinoyl ester. Exposure to acetic anhydride and catalytic TMSOTf opened the 1,6-anhydro ring, and then the Lev group was removed with sodium borohydride. This new galactosamine acceptor (**78**) was combined with glucosyl donor **73** in a TMSOTf-mediated coupling reaction to produce disaccharide **75**. The anomeric acetate group was removed with benzylamine in THF, and the anomeric position was activated to the imidate. The imidate was then coupled to allyl alcohol to produce allyl disaccharide **79**.



Scheme 3.14: Synthesis of core disaccharide **79**

Conclusions

Synthesis of CS polysaccharides with defined length and pattern of sulfation has been achieved by many groups, and the use of synthetic saccharides provides a great opportunity to probe the role of sulfation in CS biological activity. Described above is the design and synthesis of a core CS disaccharide that can access a wide range of CS sulfation motifs. Glucosyl donor **73** has been accessed in 13 steps through the use of the MacMillan sugar methodology. A methodology developed in the Hsieh-Wilson laboratory was used to access galactosamine acceptor **76** in 11 steps. These monosaccharides were coupled to produce core disaccharide **79**.

Supporting Information

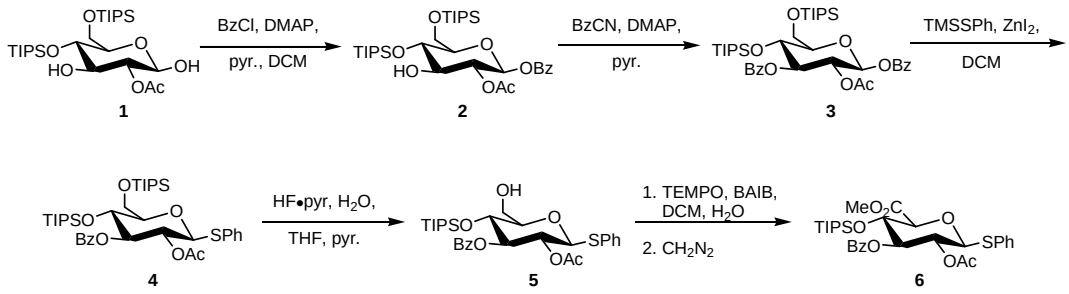
General Methods

Unless stated otherwise, reactions were performed in oven-dried glassware that had been cooled in a desiccator over Dririte. Reactions were performed under an argon environment unless otherwise stated. All solvents were purified using the method of Grubbs.[28] Unless stated otherwise, all commercially obtained reagents were used as received. Chemicals that were purified after purchase were purified according to the guidelines provided by Perrin and Armarego.[29] Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using an ice-water bath for volatile samples. High-performance liquid chromatography (HPLC) was performed on Hewlett-Packard 1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted. Liquid chromatography mass spectrometry analysis was performed on an Aglient 1100 series LCMS with an acetonitrile/water mobile phase treated with 0.1% acetic acid with a quadrapole detector. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by fluorescence quenching, cerium ammonium molybdate stain, *p*-anisaldehyde, potassium permanganate stain, or ninhydrin stain as necessary. Flash chromatography was performed on ICN silica gel (particle size 0.032 - 0.063 mm) using the method of Still.[30]

^{1}H NMR and proton decoupling spectra were recorded on a Varian Mercury 300 (300 MHz) or a Varian Inova 500 (500 MHz) and the ^{1}H NMR spectra are reported in parts per million (δ) relative to the residual solvent peak. Data for ^{1}H are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) in Hz, and integration. ^{13}C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) spectrometer and 500 (125 MHz) spectrometer and are reported in parts per million (δ) relative to the residual solvent peak. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). A JASCO P-1010 instrument was used to measure optical rotation. Mass spectra obtained from the Protein/Peptide MicroAnalytical Laboratory were generated on a Perkin Elmer/Sciex API 365 triple quadrapole mass spectrometer with nano spray ion source. Samples were dissolved in 50/50 MeOH/CH_2Cl_2, and infused at 0.2 microliter per minute. High-resolution mass spectrometry was performed at the Mass Spectrometry Facility at the California Institute of Technology on either a JEOL JMS-600H High Resolution Mass Spectrometer or a UPLC-LCT Premier XT TOF Mass Spectrometer using Leu-Enkephalin as lock mass in order to obtain exact mass. The UPLC-LCT Premier XT was purchased in 2006 with a grant from the National Science Foundation Chemistry Research Instrumentation and Facilities Program (CHE-0541745).

Synthesis of the Monosaccharides

Glucose Donor



(2) Compound **1**[1] (1.0 g, 1.9 mmol) was dissolved in dichloromethane (20 mL) in an oven-dried round bottom flask and cooled to 0 °C. To this solution was added freshly distilled benzoyl chloride (1.1 mL, 9.4 mmol), pyridine (0.3 mL, 1.0 mmol), and dimethylaminopyridine (45 mg, 0.4 mmol), and the reaction was allowed to warm to room temperature. The reaction was stirred for 16 hours when it was quenched by the addition of dichloromethane (50 mL) and poured into an extraction funnel. The organic layer was washed with saturated aqueous ammonium chloride (25 mL), saturated aqueous sodium bicarbonate (25 mL), and brine (25 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The residue was purified by flash chromatography (20% EtOAc/Hexane) to afford compound **2**, as a waxy solid in 82% yield. (R_f = 0.4, 1:1 Et_2O/Hexane) IR (film, $CDCl_3$) 3533, 2944, 2892, 2866, 2866, 1790, 1740, 1600, 1464, 1452, 1269, 1091, 1065 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.05 (m, 2H) Ar**H**, 7.65-7.40 (m, 3H) Ar**H**, 5.93 (d, 1H, J = 8.4 Hz) **H**-1, 5.13 (dd, 1H, J = 8.7, 8.1 Hz)

H-2, 4.08-3.92 (m, 4H), 3.78 (dd, 1H, J = 8.7, 8.4 Hz) **H**-4, 3.54 (ddd, 1H, J = 8.7, 3.3, 3.3) **H**-5, 2.07 (s, 3H) C(O)C**H**$_3$, 1.15-0.84 (m, 42H) C**H**(C**H**$_3$)$_2$. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8, 164.9,133.5, 130.0, 129.4, 129.1, 92.4, 78.6, 76.3, 73.4, 71.4, 62.1, 20.9, 18.3, 18.3, 17.8, 17.7, 13.0, 12.0. HRMS (ES+) exact mass calcd for [M+Na] requires *m/z* 661.3568, found *m/z* 661.3567. $[\alpha]_D$ = +5.0, (c = 1.00, $CHCl_3$).

(3) In an oven-dried flask, compound **2** (1.19g, 1.86 mmol) was dissolved in pyridine (25 mL) and cooled to 0 °C. Dimethylaminopyridine (16 mg, 0.1 mmol) and benzoyl cyanide (1.32 g, 10.1 mmol) were added and the reaction was allowed to warm to room temperature. The reaction was stirred overnight. The reaction was concentrated via rotary evaporation and the residue was purified via flash chromatography (10% EtOAc/Hexane) to afford compound **3** as a waxy solid (1.20 g, 1.62 mmol, R_f = 0.5 in 1:1 Et_2O/Hexane) in 87% yield. IR (film, $CDCl_3$) 2944, 2892, 2867, 1739, 1464, 1452, 1262, 1091, 1069 ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05 (m, 4H) Ar**H**, 7.60-7.43 (m, 6H) Ar**H**, 6.08 (d, 1H, J = 7.5 Hz) **H**-1, 5.50 (dd, 1H, J = 9.0, 9.0 Hz) **H**-3, 5.31 (dd, 1H, J = 8.0, 7.5 Hz), **H**-2, 4.35 (dd, 1H, J = 8.5, 8.5 Hz) **H**-4, 4.02 (m, 2H) C**H**$_2$, 3.68 (m, 1H) **H**-5, 1.88 (s, 3H) C(O)C**H**$_3$, 1.17-0.96 C**H**(C**H**$_3$)$_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.7, 165.7, 164.7, 133.6, 133.3, 132.9, 130.2, 129.7, 129.5, 129.0, 128.3, 92.1, 78.5, 76.4, 71.3, 68.6, 61.8, 20.6, 18.1, 18.1, 17.9, 17.7, 13.2, 12.0. HRMS (FAB) exact mass calcd. for $[M+Na]^+$ ($C_{40}H_{62}O_9Si_2Na$) requires *m/z* 765.3825, found *m/z* 765.3825. $[\alpha]_D$ = +6.3, (c = 2.00, $CHCl_3$).

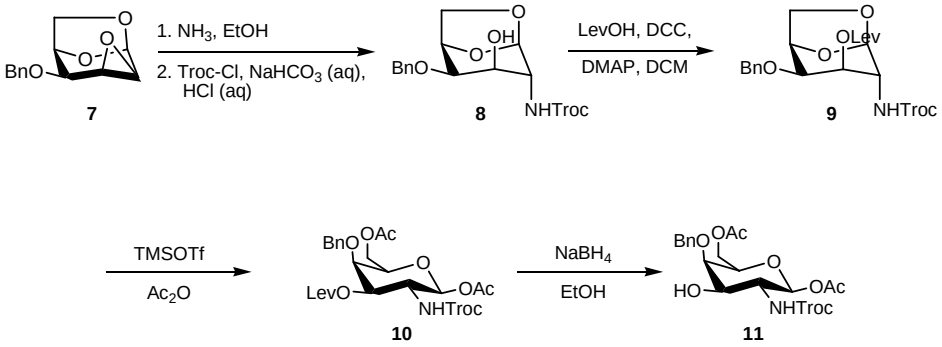
(4) To a flame-dried flask at 0 °C charged with zinc iodide (1.42 g, 4.4 mmol) and dichloromethane (50 mL) was added compound **3** (6.3 g, 8.5 mmol) as a solution in dichloromethane (20 mL). Trimethylsilyl thiophenol (2.4 mL, 12.7 mmol) was added, and the reaction was allowed to stir for 10 hours. The reaction was diluted with dichloromethane (70 mL) and poured into an extraction funnel. The organic layer was washed with saturated aqueous ammonium chloride (70 mL), a saturated aqueous bicarbonate solution (70 mL), and brine (70 mL), and the organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The residue was purified via flash chromatography (column 1: 10-20% EtOAc/Hexane, column 2: 30-60% Toluene/Hexane) to afford compound **4** as a waxy solid (5.2 g, 7.1 mmol, R_f = 0.55 in 1:1 Et_2O/Hexane) in 83% yield. IR (film, $CDCl_3$) 3062, 2944, 2891, 2867, 1753, 1730, 1464, 1452, 1440, 1268, 1224, 1155, 1111, 1087, 1068, 1050, 1026, 883 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (m, 2H) Ar-**H**, 7.61-7.24 (m, 8H) Ar-**H**, 5.41 (dd, 1H, J = 8.7, 8.7 Hz) **H**-3, 5.05 (dd, 1H, J = 9.9, 9.3 Hz) **H**-2, 4.92 (d, 1H, J = 10.2 Hz) **H**-1, 4.10 (m, 2H) C**H**$_2$, 3.92 (dd, 1H, J = 10.8, 5.4 Hz) **H**-4, 3.52 (m, 1H) **H**-5, 1.92 (s, 3H) C(O)C**H**$_3$, 1.09 (m, 21H) C**H**(C**H**$_3$)$_2$, 0.95 (m, 21H) C**H**(C**H**$_3$)$_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.0, 166.0, 134.3, 134.5, 131.5, 130.0, 129.8, 129.1, 128.6, 127.6, 86.4, 82.4, 77.9, 71.3, 69.9, 63.2, 21.0, 18.3, 18.3, 18.2, 18.2, 13.6, 12.2. HRMS (FAB+) exact mass calcd. for [(M+H)-H_2] ($C_{39}H_{61}O_7Si_2$) requires *m/z* 729.3677, found *m/z* 729.3698. $[\alpha]_D$ = +21.2, (c = 1.00, $CHCl_3$).

(5) In a polypropylene flask equipped with a stir bar, compound **4** (3.5 g, 4.8 mmol) in THF (108 mL) was cooled to 0 °C. Pyridine (78 mL) and water (25 mL) were added to the reaction solution. In a separate polypropylene flask, pyridine (30 mL) was cooled to 0 °C, and a solution of hydrogen fluoride in pyridine (70% HF in 30% pyridine, 30 mL) was slowly and carefully added. Dissolving the hydrogen fluoride into the pyridine produced an exotherm that was allowed to cool before proceeding. Once the hydrogen fluoride solution cooled, it was added dropwise over 10 minutes to flask containing compound **4**. The reaction was sealed and allowed to stir at 0 °C for 19 hours. The reaction was carefully poured into an Erlenmeyer flask containing saturated aqueous sodium bicarbonate (250 mL) and ethyl acetate (100 mL) at 0 °C to quench the hydrogen fluoride before pouring the solution into an extraction funnel. The organic layer was washed with saturated aqueous sodium bicarbonate (3x100 mL) and brine (100 mL) before being dried over sodium sulfate, filtered, and concentrated via rotary evaporation. The residue was purified via flash chromatography (20% EtOAc/Hexane, R_f = 0.6 in 30% EtOAc/Hexane) to afford compound **5** (2.2 g, 3.8 mmol) in 78% yield as clear, colorless oil. (R_f = 0.4 in 1:1 Et_2O/Hexane). IR (film, $CDCl_3$) 3505, 3061, 2994, 2891, 2867, 1749, 1730, 1269, 1223, 1109, 1090, 1069, 1050, 1026, 883 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (m, 2H) Ar-**H**, 7.61-7.24 (m, 8H) Ar-**H**, 5.42 (dd, 1H) **H**-3, 5.04 (dd, 1H) **H**-2, 4.91 (d, 1H) **H**-1, 4.10 (dd, 1H) **H**-4, 3.96 (ddd, 1H) **H**-5, 3.79 (m, 1H) **H**-6, 3.54 (m, 1H) **H**-6, 1.96 (dd, 1H) O**H**, 1.93 (s, 3H) C(O)C**H**$_3$, 0.98-0.86 (m, 21H) C**H**(C**H**$_3$)$_2$. HRMS (FAB+) exact mass calcd. for $[M+H]^+$ ($C_{30}H_{43}O_7SSi$) requires *m/z* 575.2499, found *m/z* 597.2496. $[\alpha]_D$ = +6.8, (c = 1.00, $CHCl_3$).

(6) Compound **5** (3.5 g, 6.0 mmol) was dissolved in dichloromethane (40 mL), and to this solution was added water (20 mL), TEMPO (191 mg, 1.2 mmol), and iodobenzene diacetate (4.9 g, 15.2 mmol). The reaction was stirred for 3 hours before being quenched by the addition of dichloromethane (40 mL). The reaction mixture was poured into an extraction funnel containing saturated aqueous sodium thiosulfate (40 mL). The aqueous layer was extracted with dichloromethane (2x15 mL) and the combined organics were dried over sodium sulfate, filtered, and cooled to 0 °C. Diazomethane in diethyl ether was added dropwise to the dichloromethane solution until TLC showed that all the acid (R_f = 0.2 in 60% Et_2O/Hexane) was consumed. The reaction was stirred for 30 minutes before being allowed to warm to room temperature over an hour. The reaction was quenched by the addition of acetic acid in toluene (0.5 mL in 10 mL, dropwise until bubbling ceases), and the solvent was removed via rotary evaporation. The residue was purified by flash chromatography (10% EtOAc/Hexane) to afford compound **6** (2.2 g, 3.7 mmol) in 62% yield as a hard, slightly beige solid (R_f = 0.5 in 60% Et_2O/Hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98-7.30 (m, 10H) Ar-**H**, Ar-**H**, 5.41 (dd, 1H, J = 8.7, 8.1 Hz) **H**-2, 5.08 (dd, 5.1, 3.6 Hz) **H**-3, 4.95 (d, 1H, J = 10.2 Hz) **H**-1, 4.44 (dd, 1H, J = 8.4, 8.4 Hz) **H**-4, 4.09 (d, 1H, J = 8.4 Hz) **H**-5, 3.72 (s, 3H) CO_2CH_3, 1.96 (s, 3H) $C(O)CH_3$, 0.96 (m, 21H) $CH(CH_3)_2$. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 168.1, 165.6, 133.3, 132.4, 129.7, 129.4, 129.0, 128.4, 128.1, 86.3, 80.1, 76.4, 70.6, 70.4, 29.7, 20.7, 17.9, 17.8, 13.0. HRMS

(ES+) exact mass calcd. for $[M+Na]^+$ ($C_{31}H_{42}O_8SSiNa$) requires *m/z* 625.2267, found *m/z* 625.2265 $[\alpha]_D = +7.3$, (c = 1.00, $CHCl_3$).

Galactosamine Acceptor



(8) Compound **7**[23] (650 mg, 2.78 mmol) was dissolved in ethanol (13 mL) in a Schlenk flask and cooled to 0 °C. Ammonia was bubbled through the ethanolic solution for 30 minutes. The flask was immediately sealed, placed behind a blast shield, and heated to 110 °C while stirring for 24 hours. The reaction was cooled to room temperature and concentrated via rotary evaporation and a conversion of 69% was obtained by determining the molar ^{1}H NMR ratio of two peaks, δ 5.48 (s) to δ 5.63 (d). This method of determining yield assumes only two products to be present, the free amine and the starting epoxide. The crude mixture containing the free amine (352 mg, 1.40 mmol) and the starting epoxide were dissolved in THF (1.0 mL) and cooled to 0 °C. In a vial, a 1N aqueous sodium bicarbonate solution (2.4 mL) and a 1N aqueous hydrochloric acid solution (1.0 mL) were combined

and added to the reaction mixture. Then 2,2,2-trichloroethylchloroformate (208 μL, 1.54 mmol) was added slowly, and the reaction was allowed to warm to room temperature. The reaction stirred for 4 hours, and was quenched by the addition of dichloromethane (10 mL). The reaction was poured into an extraction funnel and the aqueous layer was extracted with dichloromethane (3x10 mL). The organics were combined and washed with brine (10 mL), dried over sodium sulfate, filtered, and the solvent was removed via rotary evaporation. The residue was purified via flash chromatography (30 % EtOAc/Hexane) to afford compound **8** (508 mg, 1.2 mmol) in 85% yield as a white solid. (R_f = 0.35 in 50% EtOAc/Hexane). IR (film, $CDCl_3$) 3430, 3307, 3033, 2954, 2899, 2360, 1736, 1531, 1235, 1135, 1095, 1032, 932 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (m, 5H) Ar**H**, 5.38 (s, 1H) N**H**, 5.16 (d, 1H, J = 9.3 Hz) **H**-1, 4.77-4.61 (m, 4H) C**H**$_2$Ph, C**H**$_2$CCl_3, 4.41 (m, 2H) **H**-4, **H**-5, 4.04 (m, 2H) **H**-3, **H**-6, 3.69 (m, 2H) **H**-2, **H**-6, 2.70 (broad s, 1H) O**H**. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.6, 137.1, 128.8, 128.4, 127.8, 100.6, 95.3, 74.7, 72.6, 71.6, 71.6, 68.8, 64.8, 54.7. HRMS (FAB+) exact mass calcd. for $[M+H]^+$ ($(C_{16}H_{19}O_6NCl_3)$ requires *m/z* 426.0278, found *m/z* 426.0290. $[\alpha]_D$ = -24.0, (c = 2.00, $CHCl_3$).

(9) To a solution of **8** (258 mg, 0.6 mmol) in dichloromethane (7 mL) at 0 °C was added levulinic acid (0.13 mL, 1.3 mmol), dimethylaminopyridine (10 mg, 0.8 mmol), and dicyclohexylcarbodiimide (261 mg, 1.3 mmol). The reaction was allowed to warm to room temperature and stir for 6 hours. The reaction was quenched by dilution with dichloromethane (20 mL), and the organic layer was washed with water (5 mL) and brine

(5 mL). The organic layer was dried over sodium sulfate, filtered, and the solvent was removed via rotary evaporation. The residue was purified via flash chromatography (50% EtOAc/Hexane) to afford compound **9** (286 mg, 0.54 mmol) in 90% yield as a white solid. (R_f = 0.3 in 50% EtOAc/Hexane). IR (film, $CDCl_3$) 3325, 2960, 1741, 1720, 1529, 1234, 1154, 1139, 1030, 929 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39-7.26 (m, 5H) Ar**H**, 5.40 (m, 2H) **H**-1, **H**-3; 5.30 (d, 1H, J = 9.2 Hz) N**H**, 4.74 (d, 1H, J = 20.7 Hz) C**H**$_2$Ph, 4.70 (d, 1H, J = 20.7 Hz, C**H**$_2$Ph, 4.61 (d, 1H, J = 11.7 Hz) C**H**$_2$$CCl_3$, 4.43 (d, 1H J = 11.7 Hz) C**H**$_2$$CCl_3$, 4.41 (m, 2H) **H**-4, **H**-5, 3.94 (m, 1H) **H**-6, 3.74 (m, 2H) **H**-2, **H**-6; 2.79-2.64 (m, 4H) C**H$_2$**C**H$_2$**$COCH_3$, 2.16 (s, 3H) CH_2CH_2COC**H$_3$**. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 206.1, 171.6, 153.4, 137.3, 128.5, 128.1, 127.8, 100.3, 95.2, 74.8, 73.3, 71.5, 70.1, 68.2, 64.9, 53.9, 37.9, 29.8, 28.1. HRMS (FAB+) exact mass calcd. for $[M+H]^+$ (($C_{21}H_{25}O_8NCl_3$) requires *m/z* 524.0646, found *m/z* 524.0628. $[\alpha]_D$ = -60.1, (c = 1.00, $CHCl_3$).

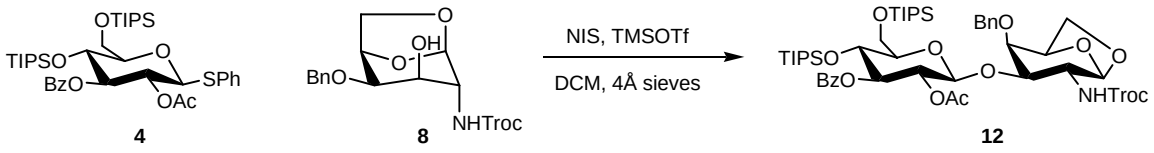
(10) To a solution of compound **9** (51 mg, 0.1 mmol) at 0 °C in freshly distilled acetic acid (0.15 mL) was added trimethylsilyl triflate (0.04 mL of a 0.05 M solution in acetic acid). The reaction was stirred at 0 °C for 5 hours and then quenched by dilution with ethyl acetate (2 mL). The solution was poured over saturated aqueous sodium bicarbonate (1 mL), and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate (3x1 mL) and brine (1 mL), dried over sodium sulfate and filtered. The solvent was removed via rotary evaporation, and the residue was purified by flash chromatography (30-50% EtOAc/Hexane) to afford compound **10** (52 mg) in 83% yield as

a white solid. (R_f = 0.4 in 50% EtOAc/Hexane). IR (film, $CDCl_3$) 3334, 3032, 1955, 1742, 1537, 1371, 1229, 1155, 1047, 933 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37-7.30 (m, 5H) Ar**H**, 6.25 (d, 1H, J = 3.6 Hz) **H**-1, 5.22 (dd, 1H, J = 3.0, 11.4 Hz) **H**-3, 5.01 (d, 1H, J = 9.9 Hz) N**H**, 4.87 (d, 1H, J = 11.4 Hz) C**H**$_2$CCl$_3$, 4.81 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.68 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.64 (m, 1H) **H**-2, 4.61 (d, 1H J = 11.4 Hz) C**H**$_2$CCl$_3$, 4.19 (m, 1H) **H**-6, 4.05 (m, 2H) **H**-5, **H**-6; 3.96 (1H, m) **H**-4, 2.84-2.48 (m, 4H) C**H$_2$**C**H$_2$**COCH$_3$, 2.18 (s, 3H) COC**H$_3$**, 2.15 (s, 3H) COC**H$_3$**, 1.98 (s, 3H) COC**H$_3$**. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 206.5, 173.3, 170.6, 169.2, 154.3, 137.6, 128.8, 128.5, 128.3, 91.6, 77.5, 75.3, 74.9, 73.6, 71.3, 70.5, 62.3, 49.6, 38.0, 30.0, 28.2, 21.1, 21.0. HRMS (FAB+) exact mass calcd. for $[M+Na]^+$ ($C_{25}H_{30}O_{11}NCl_3Na$) requires *m/z* 648.0787, found *m/z* 648.0783. $[\alpha]_D$ = +59.7, (c = 2.00, $CHCl_3$).

(11) To a solution of **10** (30 mg, 0.048 mmol) in ethanol (0.5 mL) was added sodium borohydride (2 mg, 0.06 mmol). The reaction was stirred for 30 minutes and then partitioned between ethyl acetate (3 mL) and water (1 mL). The organic layer was washed with brine (1 mL), dried over sodium sulfate, and filtered. The solvent was removed via rotary evaporation, and the residue was purified via flash chromatography (20-50% EtOAc/Hexane) to afford compound **11** (23 mg, 0.043) in 89% yield as a white solid. (R_f = 0.15 in 50% EtOAc/Hexanes). IR (film, $CDCl_3$) 3583, 3437, 3339, 3065, 2954, 2922, 1742, 1535, 1373, 1229, 1111, 1092, 1045, 1011, 932 ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38-7.30 (m, 5H) Ar**H**, 6.23 (d, 1H, J = 3.6 Hz) **H**-1, 5.02 (d, 1H, J = 8.7 Hz) N**H**, 4.76 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.75 (s, 2H) C**H$_2$**CCl$_3$, 4.70 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.23 (m,

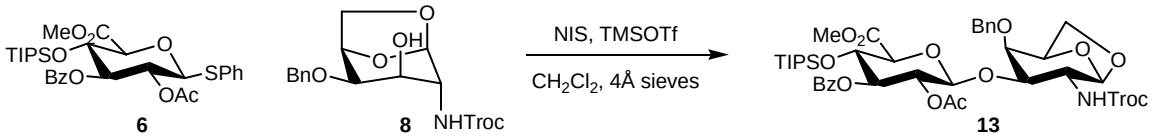
2H) **H**-2, **H**-3; 4.13 (m, 1H) **H**-6, 4.04 (m, 1H) **H**-6, 3.90 (m, 1H) **H**-4, 3.83 (1H, m) **H**-5, 2.27 (d, 1H, J = 9.6 Hz) O**H**, 2.14 (s, 3H) COC**H**$_3$, 2.04 (s, 3H) COC**H**$_3$. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 169.3, 155.3, 137.6, 129.0, 128.6, 128.3, 91.6, 76.2, 76.0, 75.1, 70.6, 70.1, 62.7, 52.0, 21.3, 21.1. HRMS (FAB+) exact mass calcd. for $[M+H]^+$ ($C_{20}H_{25}O_9NCl_3$) requires *m/z* 528.0595, found *m/z* 528.0604. $[\alpha]_D$ = +23.5, (c = 1.00, $CHCl_3$).

Synthesis of the Disaccharides



(12) Donor **4** (300 mg, 0.41 mmol) and acceptor **8** (175 mg, 0.41 mmol) were azeotroped three times with toluene placed under vacuum for 8 hours. They were then added as a solution in dichloromethane (2 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves and dichloromethane (6 mL). The reaction mixture was allowed to stir for 30 minutes before N-iodosuccinimide (111 mg, 0.493 mmol) was added under argon, and the reaction was allowed to stir for an additional 15 minutes. The reaction was then cooled to -40 °C. A solution of trimethylsilyl triflate in dichloromethane (1.64 mL, 0.05 M) was cooled to -78 °C and added to the reaction. The reaction was warmed to -10 °C and held at that temperature for 72 hours. The reaction was quenched by adding saturated aqueous

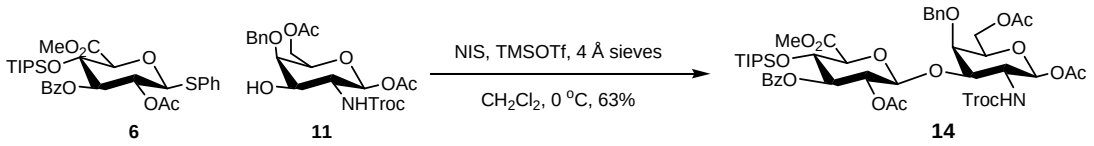
sodium thiosulfate with pyridine (5 mL $Na_2S_2O_3$ (aq.) with 0.25 mL pyridine) and agitated until the red color changed to yellow. The reaction was partitioned between ethyl acetate (100 mL) and saturated aqueous sodium thiosulfate (25 mL). The aqueous layers were extracted with ethyl acetate (2x25 mL), and the organics were combined and washed with brine (25 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed via rotary evacuation. The residue was purified via flash chromatography (10-35% EtOAc/Hexane) to provide compound **12** (266 mg) in 62% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98-7.24 (m, 10H) Ar-**H**, 5.46 (dd, 1H) **H**-3 GlcA, 5.16 (apparent s, 1H) **H**-1 GalN, 5.03 (d, 1H, J = 1.2 Hz) **H**-1 GlcA, 4.95 (d, 1H, J = 9.0 Hz) N**H**, 4.69 (dd, 1H) **H**-2 GlcA, 4.58-4.42 (m, 5H) C**H**$_2$CCl$_3$, C**H**$_2$OBn, **H**-4 GalN, 4.21 (m, 1H) **H**-5 GalN, 4.06 (dd, 1H) **H**-4 GlcA, 3.93 (apparent d, 1H) **H**-2 GalN, 3.85 (apparent s, 2H) **H**-6 GlcA, 3.75-3.69 (m, 2H) **H**-6 GalN, **H**-5 GlcA, 3.56-3.48 (m, 2H) **H**-3 GalN, **H**-6 GalN, 2.00 (s, 3H) C(O)C**H**$_3$, 1.10 (m, 21H) **TIPS**, 0.98 (m, 21H, **TIPS**). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.7, 165.7, 153.2, 137.5, 132.9, 130.3, 129.4, 128.6, 128.2, 128.0, 127.5, 100.2, 99.4, 95.4, 77.2, 74.9, 74.6, 73.7, 72.9, 72.2, 71.2, 70.0, 65.7, 65.0, 61.7, 54.7, 20.7, 18.1, 18.0, 18.0, 17.9, 12.9, 12.2. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{49}H_{74}Cl_3NNaO_{13}Si_2$) requires *m/z* 1068.3, found *m/z* 1068.0.



(13) Donor **6** (19 mg, 0.03 mmol) and acceptor **8** (16 mg, 0.04 mmol) were azeotroped three times with toluene placed under vacuum for 8 hours. They were then added as a

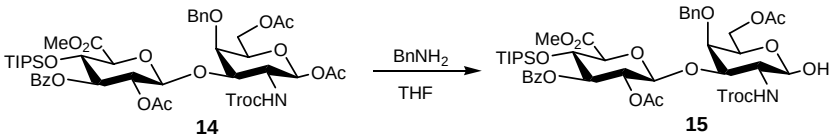
solution in dichloromethane (0.1 mL) to a flame dried flask containing flame-activated 4Å molecular sieves and dichloromethane (0.2 mL). The reaction mixture was allowed to stir for 30 minutes before N-iodosuccinimide (14 mg, 0.06 mmol) was added under argon, and the reaction was allowed to stir for an additional 15 minutes. The reaction was then cooled to -40 °C. A solution of trimethylsilyl triflate in dichloromethane (0.44 mL, 0.05 M) was cooled to -78 °C and added to the reaction. The reaction was warmed to -10 °C and held at that temperature for 17 hours. The reaction was quenched by adding saturated aqueous sodium thiosulfate with pyridine (0.5 mL of a solution containing 5 mL $Na_2S_2O_3$ (aq.) and 0.25 mL pyridine) and agitated until the red color changed to yellow. The reaction was partitioned between ethyl acetate (5 mL) and saturated aqueous sodium thiosulfate (2 mL). The aqueous layers were extracted with ethyl acetate (2x5 mL), and the organics were combined and washed with brine (10 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed via rotary evacuation. The residue was purified via flash chromatography (10-35% EtOAc/Hexane) to provide compound **13** (15 mg) in 53% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.99–7.17 (m, 10H) Ar-**H**, 6.09 (d, 1H, J = 3.9 Hz) **H**-1 GlcA, 5.49 (dd, 1H, J = 2.4, 2.1 Hz) **H**-3 GlcA, 5.34 (apparent s, 1H) **H**-1 GalN, 5.15 (d, 1H, J = 9.3 Hz) N**H**, 4.71 (d, 1H, J = 11.7 Hz), 4.59-4.35 (m, 7H), 4.23-4.13 (m, 3H), 3.72-3.64 (m, 2H), 3.59 (s, 3H) CO_2CH_3, 1.88 (s, 3H) $C(O)CH_3$, 1.07 (m, 21H) $CH(CH_3)_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.5, 170.3, 164.9, 153.6, 137.7, 133.9, 130.2, 128.8, 128.7, 128.2, 127.8, 124.3, 100.6, 95.4, 74.9, 74.8, 74.7, 73.5, 71.1, 70.6, 70.1, 69.1, 68.1, 65.2, 60.6, 54.6, 52.4, 24.5, 18.1, 18.1, 12.5. LRMS

(Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{41}H_{54}Cl_3NNaO_{14}Si$) requires *m/z* 940.2, found *m/z* 940.0.



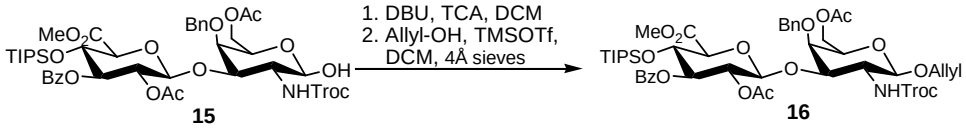
(14) Donor **6** (100 mg, 0.17 mmol) and acceptor **11** (92 mg, 0.18 mmol) were azeotroped three times with toluene placed under vacuum for 8 hours. They were then added as a solution in dichloromethane (1 mL) to a flame-dried flask containing flame- activated 4Å molecular sieves and dichloromethane (2.4 mL). The reaction mixture was allowed to stir for 30 minutes before N-iodosuccinimide (52 mg, 0.23 mmol) was added under argon, and the reaction was allowed to stir for an additional 15 minutes. The reaction was then cooled to -40 °C. A solution of trimethylsilyl triflate in dichloromethane (0.6 mL, 0.05 M) was cooled to -78 °C and added to the reaction. The reaction was warmed to 0 °C and held at that temperature for 10 hours. The reaction was quenched by adding saturated aqueous sodium thiosulfate with pyridine (2 mL of a solution containing 5 mL $Na_2S_2O_3$ (aq.) and 0.25 mL pyridine) and agitated until the red color changed to yellow. The reaction was partitioned between ethyl acetate (15 mL) and saturated aqueous sodium thiosulfate (10 mL). The aqueous layers were extracted with ethyl acetate (2x5 mL), and the organics were combined and washed with brine (10 mL). The organic layer was dried over sodium sulfate and filtered, and the solvent was removed via rotary evacuation. The residue was purified via flash chromatography (10-40% EtOAc/Hexane) to provide compound **14** (109

mg) in 63% yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.91 (d, 2H, J = 7.5 Hz) ArH, 7.51 (t, 1H, J = 7.5 Hz) ArH, 7.37-7.22 (m, 7H) Ar-**H**, 6.19 (d, 1H, J = 3.5 Hz) Gal H-1, 5.30 (dd, 1H, J = 8.5, 9.0 Hz) Glu H-3, 5.05 (dd, 1H, J = 8.0, 9.0 Hz) Glu H-4, 4.97-4.89 (m, 4H) CH_2CCl3, Glu H-5, NH; 4.70 (d, 1H, J = 12.0 Hz) CH_2Ph, 4.60 (d, 1H, J = 12 Hz) CH_2Ph, 4.50 (m, 1H), 4.44 (m, 1H), 4.40 (dd, 1H, J = 8.5, 8.5 Hz), 4.07 (d, 1H, J = 8.5 Hz) Glu H-1, 4.04 (m, 1H), 3.96 (m, 8H), 3.73 (s, 3H) $CO_2C\mathbf{H}_3$, 3.69 (m, 1H), 2.10 (s, 3H) $C(O)C\mathbf{H}_3$, 1.91 (s, 3H) C(O)C**H**3, 1.86 (s, 3H) $C(O)C\mathbf{H}_3$, 0.89-0.84 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ =170.8, 170.1, 169.1, 168.5, 165.8, 154.7, 138.0, 133.7,130.1, 129.6, 128.8, 128.5, 128.1, 128.0, 94.3, 91.9, 91.6, 76.7, 76.6, 75.1, 74.9, 74.7, 72.0, 71.1, 70.7, 63.1, 62.1, 52.5, 49.8, 21.4, 21.2, 20.9, 18.1, 12.4. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{45}H_{60}Cl_3NNaO_{17}Si$) requires *m/z* 1042.3, found *m/z* 1042.0.



(15) To a solution of compound **14** (33 mg, 0.03 mmol) in THF (0.3 mL) was added benzylamine (0.01 mL, 0.1 mmol). The solution was stirred for 7 hours, and the reaction was quenched by the addition of ethyl acetate (5 mL) and poured into an extraction funnel. The organic layer was washed with water (2x1 mL) and brine (1 mL) and dried over sodium sulfate. The organic layer was filtered, and the solvent was removed via rotary evaporation. The residue was purified via flash chromatography to afford compound **15**

(22 mg) in 74% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97 (d, 2H, J = 6.9 Hz) ArH, 7.60 (t, 1H, J = 7.2 Hz) ArH, 7.49-7.33 (m, 7H) Ar-**H**, 6.36 (bs, 1H), 5.53 (bs, 1H), 5.45 (m, 1H), 5.01-4.61 (m, 6H), 4.37 (bs, 1H), 4.28 (m, 1H), 4.20 (bs, 1H), 4.15-3.81 (m, 5H), 3.48 (s, 3H) $CO_2C\mathbf{H}_3$, 2.62 (bs, 1H) O**H**, 1.92 (s, 3H) C(O)C**H**3, 1.85 (s, 3H) $C(O)C\mathbf{H}_3$, 0.89-0.84 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 170.1, 164.6, 154.9, 138.1, 134.2, 130.2, 128.9, 128.9, 128.6, 128.1, 125.8, 94.2, 93.3, 92.4, 76.5, 75.4, 74.9, 74.8, 74.7, 70.1, 69.1, 68.8, 68.6, 66.9, 63.9, 52.5, 21.4, 21.0, 18.1, 12.3. LRMS (Ion Spray) exact mass calcd. for $[M+Cl']^-$ ($C_{43}H_{58}Cl_4NO_{16}Si$) requires *m/z* 1012.2, found *m/z* 1012.1.



(16) Compound **15** (194 mg, 0.2 mmol) was azeotroped three times with toluene and placed under vacuum for 8 hours before being added to a flame-dried flask with flame-activated 4Å molecular sieves as a solution in dichloromethane (3.5 mL). The reaction mixture was allowed to stir for 15 minutes before being cooled to 0 °C for the addition of freshly distilled 1,8-diazabicyclo[5.4.0]undec-7-ene (6 μL, 0.04 mmol) and freshly distilled trichloroacetonitrile (0.2 mL, 2 mmol). The reaction was warmed to room temperature to stir for 6 hours. Toluene (0.2 mL) was added to the reaction mixture, which was concentrated before loading on a silica column pretreated with a mixture of hexane:toluene:ethyl acetate:triethylamine (50:20:20:1). The column was eluted with a mixture of hexane:toluene:ethyl acetate (5:2:2) with triethylamine (0.1 mL), and the

fractions containing the imidate were concentrated and kept under vacuum for 4 hours before being used in the next reaction. (R_f = 0.5 in hexane:toluene:ethyl acetate (5:2:2) with 0.1 mL triethylamine. NOTE: this compound is easily hydrolyzed so TLC plates must be pre-eluted with the hexane:toluene:ethyl acetate:triethylamine solution (50:20:20:1) before being spotted.)

The imidate was added as a solution in dichloromethane (5 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves. Freshly distilled allyl alcohol (0.12 mL, 1.75 mmol) was added, and the reaction mixture was stirred at room temperature for 15 minutes before being cooled to -78 °C for the addition of trimethylsilyl triflate as a solution in dichloromethane (0.23 mL, 0.05 M). The reaction was warmed to 0 °C and stirred for 6 hours before being quenched by the addition of solution of pyridine in ethyl acetate (0.5 mL pyridine in 20 mL ethyl acetate). The organic layer was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL), and the organic layer was dried over sodium sulfate and filtered. The solvent was removed via rotary evaporation, and the residue was purified via flash chromatography (hexane:toluene:ethyl acetate, 5:2:2) to afford compound **16** (122 mg) in 63% yield as a white solid. (500 MHz, $CDCl_3$): δ = 8.01 (dd, 2 H J = 1.5, 8.5 Hz) Ar**H**, 7.96 (m, 1H) Ar**H**, 7.46-7.29 (m, 7H) Ar**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.38 (dd), 1H, J = 10.0, 10.0 Hz) Glu **H**-3, 5.28-5.24 (m, 2H) $C\mathbf{H}_2CHCH_2O$, N**H**; 5.18 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.10 (dd, 1H, J = 8.0, 8.0 Hz) Glu **H**-4, 4.94 (m, 1H) Glu **H**-5, 4.89 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.85 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.80 (m, 1H) Gal **H**-1, 4.71-4.64 (m, 2H) $C\mathbf{H_2}CCl_3$, 4.53 (m, 1H) Gal **H**-3, 4.49 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-2, 4.36-4.31 (m, 2H) $CH_2CHC\mathbf{H_2}O$, 4.12-4.05 (m, 3H) Glu **H**-1, $CH_2CHC\mathbf{H_2}O$, Gal **H**-6; 4.04 (m, 2H) Gal **H**-4, Gal **H**-5, 3.81 (s, 3H) $CO_2C\mathbf{H}_3$, 3.66 (m,

1H) Gal **H**-6, 3.53 (m, 1H) Gal **H**-2, 1.94 (s, 3H) COC**H**$_3$, 1.93 (s, 3H) COC**H**$_3$, 0.97-0.85 (m, 21H) Si(CH(C**H**$_3$)$_2$)$_3$ ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4, 169.4, 160.5, 154.2, 137.9, 137.2, 133.7, 133.5, 129.6, 129.4, 128.6, 128.5, 128.4, 128.3, 128.2, 125.3, 117.8, 95.6, 75.1, 74.6, 73.2, 73.0, 72.0, 70.9, 70.9, 70.0, 62.3, 52.5, 50.0, 20.7, 21.5, 20.9, 20.9, 20.7, 17.9, 12.9. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{46}H_{62}Cl_3NNaO_{16}Si$) requires *m/z* 1040.3, found *m/z* 1042.1.

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24. It is important to note that while the acetoxyaldehyde proved safe to distill many times, an explosion did occur during one distillation despite the liquid to be distilled showing no signs of the ozonide either when tested with KI paper or when analyzed by ^{1}H NMR.

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31. This research was carried out in the Hsieh-Wilson laboratory with the assistance of Drs. Song-Gil Lee and Sarah Tully, Ms. Maria Chiriac, and Mr. Robinson Moncure.

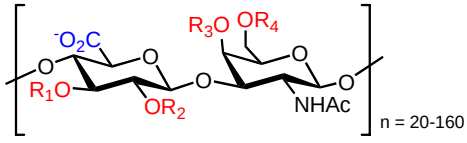
32. Figures in this section were modified from Tully, S. E. *Synthesis and Biological Activity of Chondroitin Sulfate Biopolymers*, **2007**, Ph. D. Thesis, California Institute of Technology.

C h a p t e r 4

Development of Selective Deprotection Conditions to Elaborate the Core Chondroitin Sulfate Disaccharide [17]

Introduction

Chondroitin sulfate (CS) is a linear polysaccharide composed of repeating glucuronic acid (GlcA) and galactosamine (GalNAc) residues. This polymeric scaffold can be sulfated at any of the free hydroxyls along the chain to produce a range of sulfation patterns (which are denoted by letter, such as CS-A, CS-C, CS-D, and CS-E (Figure 4.1)).[1]



Chondroitin Sulfate-A: R_1, R_2, R_4=H; R_3=OSO_3^-
Chondroitin Sulfate-C: R_1, R_2, R_3=H; R_4=OSO_3^-
Chondroitin Sulfate-D: R_1, R_3=H; R_2, R_4=OSO_3^-
Chondroitin Sulfate-E: R_1, R_2=H; R_3, R_4=OSO_3^-

Figure 4.1: Chondroitin sulfate is a linear polysaccharide composed of repeating GlcA and GalNAc residues. It can be sulfated along the backbone to produce a heterogeneous polymer composed of a range of sulfation patterns. Each pattern is denoted by a letter.

Many studies have been conducted to investigate the effects of sulfation on the biological activity of CS, but until recently, these studies were performed on CS natural isolates that, while enriched in a certain sulfation pattern, still contain other CS motifs.[2] Recent work in the Hsieh-Wilson laboratory has established that the bioactivity of CS lies in its sulfation pattern.[3] Using synthetic CS tetrasaccharides with defined sulfation motifs, they were able to show that one sulfation pattern, CS-E, stimulated axonal growth in hippocampal, dopaminergic, and dorsal root ganglion (DRG) neurons. Specifically, the synthetic tetrasaccharides were coated with polyornithine onto glass coverslips. Neurons were cultured on these coverslips for 48 hours, after which they were fixed and immunostained with an antibody to tubulin. They were examined by confocal fluorescence microscopy to quantify neurite length. CS-E stimulated neurite outgrowth up to 50% when compared to the polyornithine control in all three types of neurons. CS-C and CS-R did not significantly stimulate neurite outgrowth. CS-A did display some stimulatory activity at high concentrations (500 go/mL) with hippocampal neurons, but did not display significant stimulatory effects with the dopaminergic or DRG neurons (Figure 4.2).

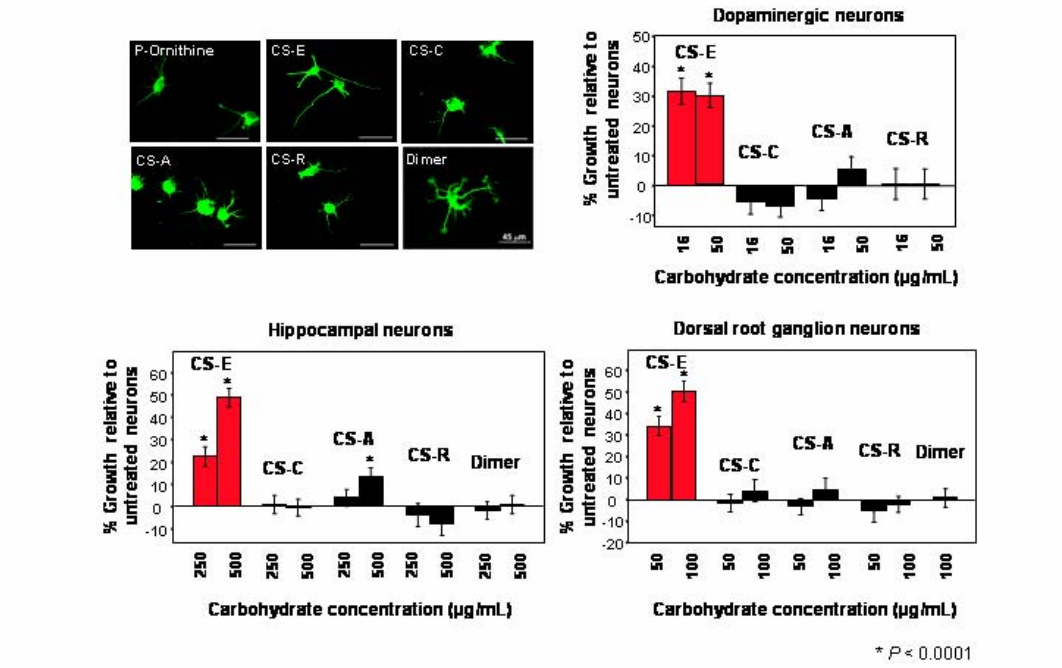
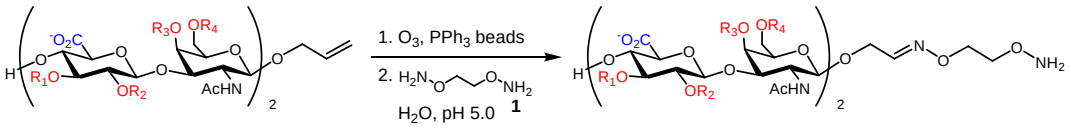


Figure 4.2: CS-E stimulated neurite outgrowth in dopaminergic, hippocampal, and dorsal root ganglion neurons.

Further studies determined that CS-E stimulates neuronal growth by binding both midkine and BDNF (brain-derived neurotropic factor). Microarray technology developed in our laboratory showed that CS-E interacts with the growth factors midkine and BDNF. Using a high-precision contact-printing robot, CS-A, CS-C, CS-E, and CS-R tetrasaccharides were spotted on aldehyde-coated slides. These tetrasaccharides were synthesized from the original allyl-tetrasaccharides through a two-step procedure: First the allyl group on each tetrasaccharide was ozonolyzed to the aldehyde, and this aldehyde was

reacted with oxime linker **1** to produce the activated tetrasaccharides (Figure 4.3). The oxime linker allowed these tetrasaccharides to covalently attach to the aldehyde plates. After washing the plates and quenching any unreacted aldehyde moieties on the suface, the micorarrays were probed with midkine and BDNF. Both growth factors were found to selectively bind to CS-E at 5 μM concentration.

To confirm the involvement of these growth factors, antibodies against midkine, BDNF, and fibroblast growth factor-1 (FGF-1) were added separately to plates containing hippocampal neurons cultured either on a substratum of polyornithine or polyornithine plus CS-E tetrasaccharide. As expected, CS-E stimulated neuronal growth almost 50% above control in the plates without added antibodies. However in plates with antibodies to midkine and BDNF, the ability of CS-E to stimulate neuronal growth was blocked. An antibody to FGF-1 had no affect on the ability of CS-E to stimulate neuronal growth, which was expected since CS-E is not known to operate through FGF-1.[3]



Chondroitin Sulfate-A: R_1, R_2, R_4=H; R_3=OSO_3^-
Chondroitin Sulfate-C: R_1, R_2, R_3=H; R_4=OSO_3^-
Chondroitin Sulfate-E: R_1, R_2=H; R_3, R_4=OSO_3^-
Chondroitin Sulfate-R: R_1, R_2=OSO_3^-; R_3, R_4=H

Figure 4.3: The allyl group can be used as a chemical handle for attaching tetrasaccharides to surfaces or other molecules.

These studies demonstrated the value of synthetic CS saccharides for probing the roles that sulfation plays in neuronal growth and development. With this in mind, we set out to design a core disaccharide **2** from which any desired sulfation pattern could be accessed (Figure 4.4). The design and synthesis of this disaccharide is detailed in Chapter 3. One goal with this disaccharide is to differentiate each position so that it will allow access to oversulfated chondroitin sulfates, such as CS-D, that are suggested to have important biological activities.

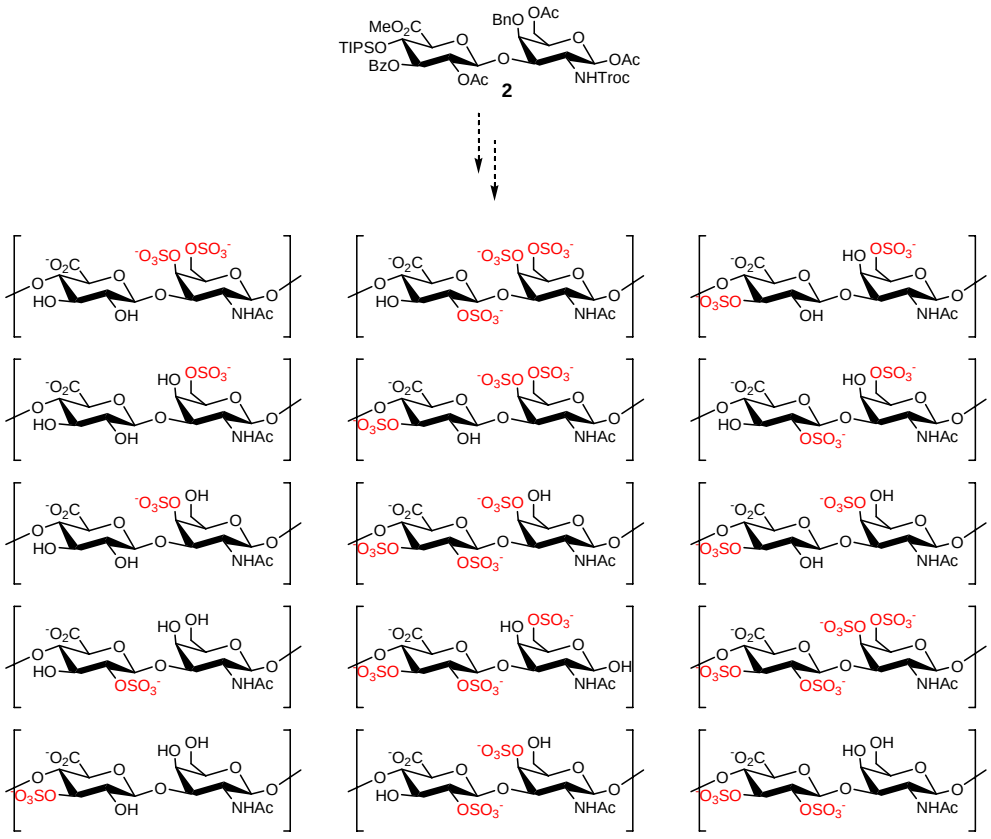


Figure 4.4: It was envisioned that a core disaccharide such as **2** could allow access to all possible sulfation patterns.

Studies with natural isolates suggest that the oversulfated CS saccharides may possess interesting biological functions. For example, there is evidence to suggest CS-D may affect neuronal growth through the binding of pleiotrophin. Even more interesting is the fact that CS-D may stimulate the extension of dendrites. In work by Sugahara and coworkers, hippocampal neurons were grown on coverslips coated with either polyornithine or polyornithine plus CS isolates enriched in certain sulfation motifs. It was found that CS-D was able to stimulate the growth of hippocampal neurons almost 60% above control. In further work by the same laboratory, they digested and fractionated CS-E polysaccharides (via elution over a pleiotrophin-immobilized affinity column) into two pools—low-affinity CS polysaccharides (LACS) and high-affinity CS polysaccharides (HACS). It was found that LACS (which was shown to have CS-D through binding of monoclonal antibody 473HD) promoted the outgrowth of dendrites while HACS promotes the outgrowth of axon-like neurons. Further studies have also shown that hippocampal neurons plated with CS-D isolates and exogenous pleiotrophin stimulated the growth of neurons over polyornithine control and over polyornithine and CS-D without exogenous pleiotrophin. In contrast, when similar studies were performed with CS-E isolates, pleiotrophin had no effect on neurite outgrowth, suggesting that CS-D and not CS-E operates through the binding of pleiotrophin. These results suggest each sulfation motif triggers distinct pathways (Figure 4.5).[5]

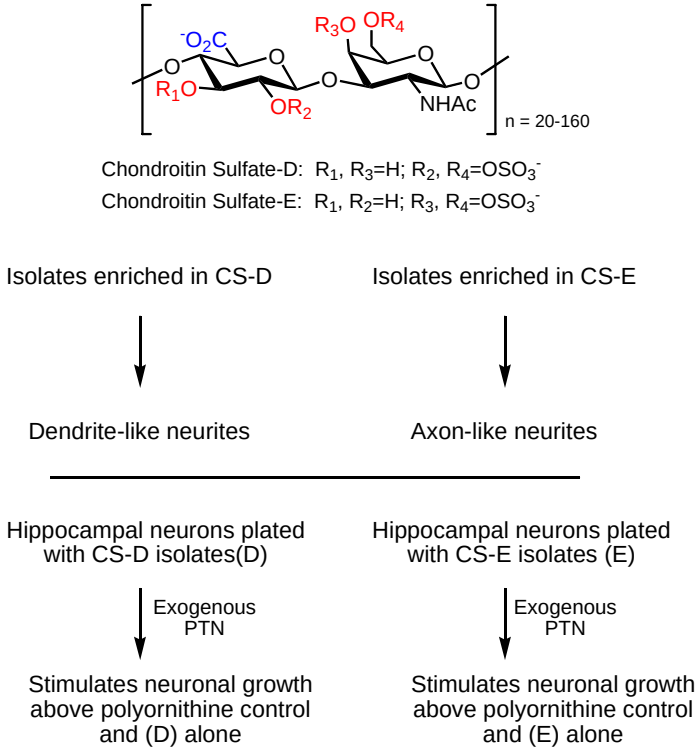


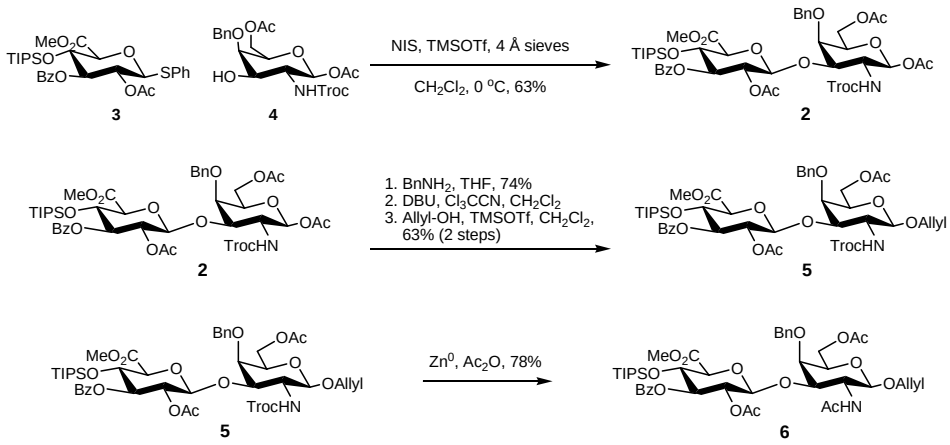
Figure 4.5: CS-D is believed to stimulate the growth of dendrites through the binding of pleiotrophin (PTN).

Studies with enriched CS-K isolates suggest it blocks retinoic acid receptor (RAR) signaling required for neuritogenic differentiation. Specifically, it was determined that as the production of CS-K increases, neurite outgrowth in LA-N-5 cells (a neuroblastoma cell line) decreases. In addition, there are distinct morphological changes between LA-N-5 cells grown with normal or overstimulated CS-K production.[6] Other studies have suggested that other oversulfated CSs such as CS-L and CS-M may also effect neuronal growth and differentiation.[7]

Our laboratory hopes to evaluate the biological activity of these sulfation patterns using synthetic saccharides. Upon accessing core disaccharide **2** it was necessary to confirm that we could differentiate between the positions before proceeding. Issues specifically examined are as follows: the ability to convert the Troc group to the desired *N*-acetyl group; differentiation of the galactosamine C-6, glucosyl C-2, and glucosyl C-3 esters; and removal of the galactosamine C-4 benzyl group.

Testing the Disaccharide Protecting Group Strategy

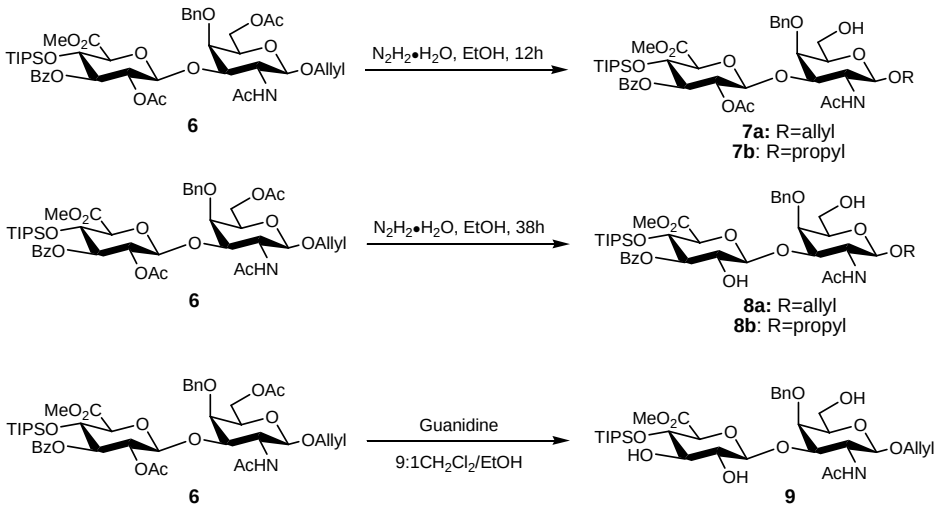
As shown in Scheme 4.1, core disaccharide **2** was obtained via a TMSOTf-mediated coupling of glucosyl donor **3** and galactosamine acceptor **4**. Removal of the anomeric acetate, activation to the imidate, and a TMSOTf-mediated coupling to allyl alcohol produced disaccharide **5** (as described in Chapter 3). Initial efforts focused on removing the Troc group in two steps, but this route was low yielding. Happily, a one-step procedure to simultaneously remove the Troc group and reprotect the resulting amine using zinc and acetic anhydride produced disaccharide **6** in good yield.[8]



Scheme 4.1: Core disaccharide **2** was elaborated to form *N*-acetyl disaccharide **6**.

We next examined if the ester protecting groups could be removed sequentially as we planned. We hoped to be able to deprotect the primary acetate in the presence of the secondary acetate and to deprotect both acetates in the presence of the benzoyl protecting group. It was found that 12-hour exposure of disaccharide **6** to hydrazine hydrate in ethanol removed the primary acetate, producing a deprotected saccharide that corresponds to CS-C. Extended exposure of disaccharide **6** to hydrazine hydrate removed both acetates without removing the glucosyl C-3 benzoyl ester. This deprotection pattern corresponds to CS-D. Interestingly-when removing the acetyl groups-hydrazine, hydrazine hydrate, and hydrazine acetate most often produced the propyl disaccharides **7b** and **8b** instead of allyl disaccharides **7a** and **8a**. While hydrazine has been used as a hydrogen source to reduce olefins,[9] this result was surprising since there are numerous reports of the use of hydrazine in the presence of an allyl group with no incident of reduction.[10] Even more troublesome is

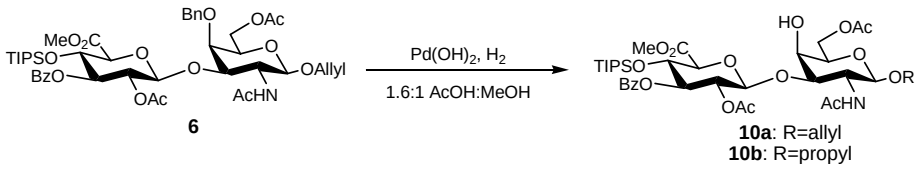
that these conditions have on occasion produced allyl disaccharides **7a** and **8a** (Scheme 4.2). Despite this unusual observation, it was concluded that hydrazine hydrate allowed differentiation between the primary and secondary acetate esters.



Scheme 4.2: Removal of the galactosamine C-6, glucosyl C-2, and glucosyl C-3 esters

Guanidine has been noted as another method for removing acetate esters in the presence of benzoyl esters.[11] The free base of guanidine was prepared as described by Kunesch and coworkers[11a] and was combined with disaccharide **6** . Instead of selectively removing the acetates, the reaction quickly proceeded to remove all ester groups to produce

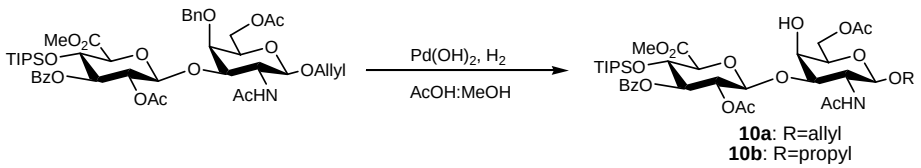
disaccharide **9**. While it was unfortunate that this method did not allow differentiation of the acetates, these conditions did provide us with a way to remove the benzoyl ester without affecting the silyl and benzyl ethers (Scheme 4.2).



Scheme 4.3: The galactosamine C-4 benzyl group can be removed with palladium hydroxide, though these conditions also lead to reduction of the allylic olefin.

The final group to be evaluated was the C-4 benzyl ether. While we were confident we could remove the benzyl group in the presence of the ester and silyl protecting groups, we were hopeful that we could remove it without reducing the allyl group. There have been reports in the literature where benzyl protecting groups have been removed without reducing allyl protecting groups on the same molecule.[12] Our first attempts involved exposing disaccharide **6** to palladium hydroxide in acetic acid and methanol under an atmosphere of hydrogen (Scheme 4.5). These conditions produced both disaccharides **10a** and **10b**. In addition, this reaction also produced several monosaccharide byproducts

which are believed to be the result of the acidity of these reaction conditions. Reduction in the amount of acetic acid prevented hydrolysis of the glycosidic bond, but this also favored formation of disaccharide **10b** (Table 4.1). Work is ongoing to determine selective reaction conditions. If methods cannot be developed to preserve the allyl group, other functional handles are available that are compatible with hydrogenation conditions.[13]

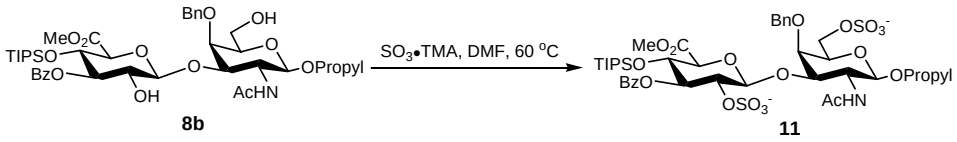


entry	AcOH:MeOH	**10b:10a**
1	1.6:1	2.3:1
2	1:1	5.6:1
3	0.5:1	**10b**
4	0:1	**10b**

Table 4.1: Hydrogenolysis of the galactosamine C-4 benzyl group. The ratio of **10a** to **10b** was determined by LCMS analysis of the crude reaction mixture.

Another concern was that the remaining protecting groups would not be stable to the acidity of the sulfation reaction. To test the stability of the disaccharide to these conditions, disaccharide **8b** was exposed to 30 equivalents of SO_3-TMA in dimethylformamide. Unfortunately, instead of producing the 2,6-sulfated disaccharide, the

products of this reaction were monosaccharides. The pH of this reaction was monitored, and it was determined that it rapidly became strongly acidic (pH 3~4). To counter this, triethylamine was added to maintain a pH of 6~8, but this reaction did not proceed to a sulfated disaccharide and the starting material began to decompose. It was thought that the large excess of SO_3-TMA may be the cause of this decomposition pathway, so 3 equivalents of SO_3-TMA were used. This resulted in progression to a monosulfated disaccharide (determined by MS of the reaction), and the addition of 20 more equivalents of SO_3-TMA permitted the formation of the disulfated product (**11**, Scheme 4.4).



Scheme 4.4: Sulfation of disaccharide **8b**

Conclusions

The sulfation patterns expressed by CS are responsible for the biological activity it displays. To determine the activities associated with each sulfation pattern, it is necessary to create a disaccharide that allows differentiation between the glucosyl 2- and 3-hydroxyl

groups and the galactosamine 4- and 6-hydroxyl groups. It was determined that the protecting group scheme chosen for the core disaccharide will allow differentiation around the disaccharide. Unfortunately, it is not clear that the allyl group can easily be preserved, so other chemical handles may need to be explored.

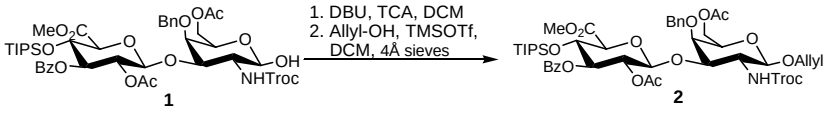
Supporting Information

General Methods

Unless stated otherwise, reactions were performed in oven-dried glassware that had been cooled in a desiccator over Dririte. Reactions were performed under an argon environment unless otherwise stated. All solvents were purified using the method of Grubbs.[28] Unless stated otherwise, all commercially obtained reagents were used as received. Chemicals that were purified after purchase were purified according to the guidelines provided by Perrin and Armarego.[29] Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using an ice-water bath for volatile samples. High-performance liquid chromatography (HPLC) was performed on Hewlett-Packard 1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted. Liquid chromatography mass spectrometry analysis was performed on an Aglient 1100 series LCMS with an acetonitrile/water mobile phase treated with 0.1% acetic acid with a quadrapole detector. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by fluorescence quenching, cerium ammonium molybdate stain, *p*-anisaldehyde, potassium permanganate stain, or ninhydrin stain, as necessary. Flash chromatography was performed on ICN silica gel (particle size 0.032 - 0.063 mm) using the method of Still.[30]

^{1}H NMR and proton decoupling spectra were recorded on a Varian Mercury 300 (300 MHz) or a Varian Inova 500 (500 MHz) and the ^{1}H NMR spectra are reported in parts per million (δ) relative to the residual solvent peak. Data for ^{1}H are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) in Hz, and integration. ^{13}C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) spectrometer and 500 (125 MHz) spectrometer and are reported in parts per million (δ) relative to the residual solvent peak. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). A JASCO P-1010 instrument was used to measure optical rotation. Mass spectra obtained from the Protein/Peptide MicroAnalytical Laboratory were generated on a Perkin Elmer/Sciex API 365 triple quadrapole mass spectrometer with nano spray ion source. Samples were dissolved in 50/50 MeOH/CH_2Cl_2, and infused at 0.2 microliter per minute. High-resolution mass spectrometry was performed at the Mass Spectrometry Facility at the California Institute of Technology on either a JEOL JMS-600H High Resolution Mass Spectrometer or a UPLC-LCT Premier XT TOF Mass Spectrometer using Leu-Enkephalin as lock mass in order to obtain exact mass. The UPLC-LCT Premier XT was purchased in 2006 with a grant from the National Science Foundation Chemistry Research Instrumentation and Facilities Program (CHE-0541745).

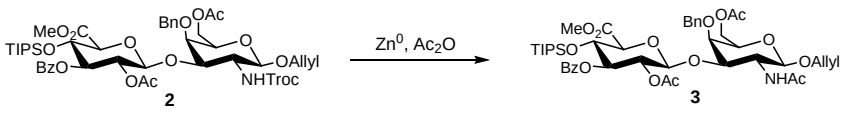
Synthesis of the Disaccharides



(2) Compound **1** (194 mg, 0.2 mmol) was azeotroped three times with toluene and placed under vacuum for 8 hours before being added to a flame-dried flask with flame-activated 4Å molecular sieves as a solution in dichloromethane (3.5 mL). The reaction mixture was allowed to stir for 15 minutes before being cooled to 0 °C for the addition of freshly distilled 1,8-diazabicyclo[5.4.0]undec-7-ene (6 μL, 0.04 mmol) and freshly distilled trichloroacetonitrile (0.2 mL, 2 mmol). The reaction was warmed to room temperature to stir for 6 hours. Toluene (0.2 mL) was added to the reaction mixture, which was concentrated before loading on a silica column pretreated with a mixture of hexane:toluene:ethyl acetate:triethylamine (50:20:20:1). The column was eluted with a mixture of hexane:toluene:ethyl acetate (5:2:2) with triethylamine (0.1 mL), and the fractions containing the imidate were concentrated and kept under vacuum for 4 hours before being used in the next reaction. (R_f = 0.5 in hexane:toluene:ethyl acetate (5:2:2) with 0.1 mL triethylamine. NOTE: this compound is easily hydrolyzed so TLC plates must be pre-eluted with the hexane:toluene:ethyl acetate:triethylamine solution (50:20:20:1) before being spotted.)

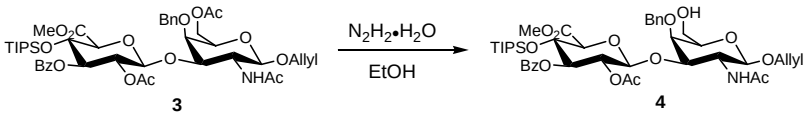
The imidate was added as a solution in dichloromethane (5 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves. Freshly distilled allyl alcohol (0.12 mL, 1.75 mmol) was added, and the reaction mixture was stirred at room temperature for 15 minutes before being cooled to -78 °C for the addition of trimethylsilyl triflate as a

solution in dichloromethane (0.23 mL, 0.05 M). The reaction was warmed to 0 °C and stirred for 6 hours before being quenched by the addition of solution of pyridine in ethyl acetate (0.5 mL pyridine in 20 mL ethyl acetate). The organic layer was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL), and the organic layer was dried over sodium sulfate and filtered. The solvent was removed via rotary evaporation, and the residue was purified via flash chromatography (hexane:toluene:ethyl acetate, 5:2:2) to afford compound **2** (122 mg) in 63% yield as a white solid. (500 MHz, $CDCl_3$): δ = 8.01 (dd, 2 H J = 1.5, 8.5 Hz) Ar**H**, 7.96 (m, 1H) Ar**H**, 7.46-7.29 (m, 7H) Ar**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.38 (dd), 1H, J = 10.0, 10.0 Hz) Glu **H**-3, 5.28-5.24 (m, 2H) $C\mathbf{H}_2CHCH_2O$, N**H**; 5.18 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.10 (dd, 1H, J = 8.0, 8.0 Hz) Glu **H**-4, 4.94 (m, 1H) Glu **H**-5, 4.89 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.85 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.80 (m, 1H) Gal **H**-1, 4.71-4.64 (m, 2H) $C\mathbf{H_2}CCl_3$, 4.53 (m, 1H) Gal **H**-3, 4.49 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-2, 4.36-4.31 (m, 2H) $CH_2CHC\mathbf{H_2}O$, 4.12-4.05 (m, 3H) Glu **H**-1, $CH_2CHC\mathbf{H_2}O$, Gal **H**-6; 4.04 (m, 2H) Gal **H**-4, Gal **H**-5, 3.81 (s, 3H) $CO_2C\mathbf{H}_3$, 3.66 (m, 1H) Gal **H**-6, 3.53 (m, 1H) Gal **H**-2, 1.94 (s, 3H) $COC\mathbf{H}_3$, 1.93 (s, 3H) $COC\mathbf{H}_3$, 0.97-0.85 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)_3$ ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4, 169.4, 160.5, 154.2, 137.9, 137.2, 133.7, 133.5, 129.6, 129.4, 128.6, 128.5, 128.4, 128.3, 128.2, 125.3, 117.8, 95.6, 75.1, 74.6, 73.2, 73.0, 72.0, 70.9, 70.9, 70.0, 62.3, 52.5, 50.0, 20.7, 21.5, 20.9, 20.9, 20.7, 17.9, 12.9. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{46}H_{62}Cl_3NNaO_{16}Si$) requires *m/z* 1042.3, found *m/z* 1042.1.



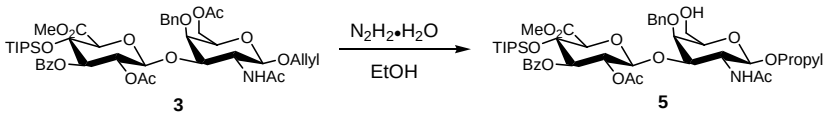
2 3

(3) Compound **2** (52 mg, 0.05 mmol) was stirred with activated zinc dust (13.4 mg, 20.4 mmol) in acetic anhydride (1 mL) for 12 hours, and the reaction was diluted with ethyl acetate (5 mL), filtered over celite, and the mother liquor was washed with saturated aqueous sodium bicarbonate (3x3 mL) and brine (3 mL). The organic layer was concentrated via rotary evaporation, and the residue was purified via flash chromatography (40-70% EtOAc/Hexane) to afford compound **3** (35 mg) as a white solid in 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, 2H, J = 7.0 Hz) Ar-**H**, 7.57 (t, 1H, J = 7.0 Hz) Ar-**H**, 7.44-7.28 (m, 7H) Ar-**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.63 (d, 1H, J = 7.0 Hz) N**H**, 5.38 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-3, 5.24 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.17 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.09 (dd, 1H, J = 8.0, 8.5 Hz) Glu **H**-4, 4.98 (d, 1H, J = 8.5 Hz) Gal H-1, 4.90 (d, 1H, J = 12.5 Hz) $C\mathbf{H}_2Ph$, 4.88 (d, 1H, J = 8.5 Hz) Glu H-5, 4.74 (m, 1H) Gal H-3, 4.66 (d, 1H, J = 11.5 Hz) $C\mathbf{H}_2Ph$, 4.47 (dd, 1H, J = 8.5, 8.5 Hz) Glu H-2, 4.14 (m, 1H) Gal **H**-6, 4.10 (d, 1H, **J** = 11.5 Hz) Glu H-1, 4.05 (m, 2H) $CH_2CHC\mathbf{H_2}O$, 3.91 (m, 1H) Gal **H**-5, 3.89 (m, 1H) Gal H-4, 3.77 (s, 3H) CO_2CH_3**,** 3.66 (m, 1H) Gal **H**-6, 3.39 (m, 1H) Gal **H**-2, 2.00 (s, 3H) $COCH_3$, 1.93 (s, 6H) $COCH_3$, 0.95-0.89 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.3, 168.6, 165.8, 138.4, 134.1, 133.6, 129.9, 129.6, 129.5, 128.7, 128.5, 127.9, 118.1, 101.8, 98.2, 78.2, 76.8, 75.7, 72.7, 72.0, 71.1, 70.3, 63.3, 55.7, 52.7, 28.1, 27.1, 24.1, 20.9, 18.7, 18.2, 17.8, 13.9, 13.2. HRMS (FAB+) exact mass calcd. for $[M+H]^+$ ($C_{45}H_{64}NO_{15}Si$) requires *m/z* 886.4045, found *m/z* 886.4028.



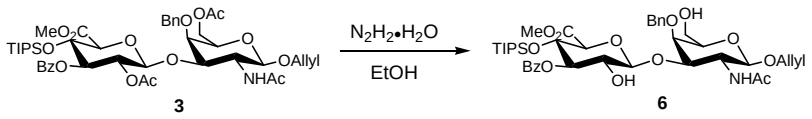
(4) To a solution of compound **3** (10 mg, 0.01 mmol) in ethanol (0.2 mL) was added hydrazine hydrate (50-60% solution, 0.12 mL), and the reaction was allowed to stir for 13 hours. The reaction was diluted with ethyl acetate (2 mL) and then washed with water (1 mL) and brine (1 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo.* The residue was purified via flash chromatography (30-80% EtOAc/Hexane) to afford compound **4** (6.9 mg, 82% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, 2H, J = 7.5 Hz) Ar-**H**, 7.57 (t, 1H, J = 7.5 Hz) Ar-**H**, 7.44-7.29 (m, 7H) Ar-**H**, 5.86 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.64 (d, 1H, J = 7.0 Hz) N**H**, 5.39 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-3, 5.26-5.23 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.18-5.16 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.10 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-4, 4.98 (d, 1H, J = 8.0 Hz) Gal **H**-1, 4.90-4.88 (m, 2H) $C\mathbf{H}_2$Ph, Glu **H**-5; 4.74-4.72 (m, 1H) Gal **H**-3, 4.69 (d, 1H, J = 12.0 Hz) $C\mathbf{H}_2$Ph, 4.48 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-2, 4.32-4.28 (m, 1H) Gal **H**-6, 4.11 (d, 1H, **J** = 8.5 Hz) Glu **H**-1, 4.10-4.05 (m, 1H) $CH_2CHC\mathbf{H_2}O$, 3.90 (m, 1H) $CH_2CHC\mathbf{H_2}O$, 3.80 (m, 1H) Gal **H**-4, 3.77 (s, 3H) CO_2CH_3, 3.67-3.62 (m, 1H) Gal **H**-2, 3.49 (m, 1H) Gal **H**-5, 3.43-3.36 (m, 1H) Gal **H**-6, 2.97 (bs, 1H) O**H**, 2.01 (s, 3H) $COCH_3$, 1.94 (s, 3H) $COCH_3$, 0.95-0.89 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.2, 169.5, 168.6, 165.8, 138.4, 134.2, 133.6, 130.0, 129.9, 129.7, 128.7, 128.6, 128.3, 118.0, 101.9, 98.6, 78.5, 77.4, 76.8, 75.8, 74.5, 74.3, 72.7, 71.1, 70.4, 62.1, 55.8, 52.7, 24.0. 21.0, 18.1, 13.2. LRMS (Ion

Spray) exact mass calcd. for $[M+Cl]^-$ ($C_{43}H_{61}ClNO_{14}Si)^-$ requires *m/z* 878.4, found *m/z* 878.5.



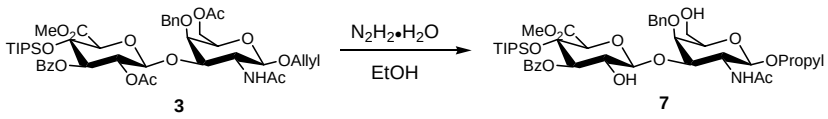
(5) To a solution of compound **3** (23 mg, 0.03) in ethanol (0.6 mL) was added hydrazine hydrate (50-60% solution, 0.5 mL), and the reaction was allowed to stir for 11 hours. The reaction was diluted with ethyl acetate (4 mL) and then washed with water (1 mL) and brine (1 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo.* The residue was purified via flash chromatography (30-80% EtOAc/Hexane) to afford compound **5** (19.3 mg, 76% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.06 (d, 2H, J = 7.0 Hz) Ar-**H**, 7.59 (t, 1H, J = 7.5 Hz) Ar-**H**, 7.45-7.29 (m, 7H) Ar-**H**, 5.72 (d, 1H, J = 6.5 Hz) N**H**, 5.28 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-3, 4.90-4.85 (m, 3H) Gal **H**-1, Glu **H**-4, C**H**$_2$Ph, 4.68 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.63 (d, 1H, J = 7.5 Hz) Glu **H**-5, 4.52 (m, 1H) Gal **H**-3, 4.41 (dd, 1H, J = 8.0, 8.5 Hz) Glu **H**-2, 4.15-4.11 (m, 1H) Gal **H**-6, 4.05 (d, 1H, **J** = 9.0 Hz) Glu **H**-1, 3.91 (m, 1H) Gal **H**-6, 3.88 (m, 1H) Gal **H**-4, 3.80 (s, 3H) CO_2CH_3, 3.78 (m, 2H), propyl CH_2, 3.66-3.60 (m, 3H) propyl CH_2, Gal **H**-5, 3.43-3.41 (m, 1H) Gal **H**-2, 2.94 (bs, 1H) OH, 1.98 (s, 3H) $COCH_3$, 1.92 (s, 3H) $COCH_3$, 0.95-0.89 (m, 24H) Si(CH(C**H**$_3$)$_2$), propyl CH_3. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.1, 170.7, 168.8, 167.0, 138.5, 133.6, 130.0, 129.9, 129.3, 128.6, 128.5, 127.9, 105.2, 99.7, 80.2, 78.1, 76.8, 74.3, 73.5, 72.1, 71.5, 71.0, 66.1, 63.3, 55.1, 52.6, 24.0, 22.9, 21.0, 18.2, 18.1,

18.1,13.2, 10.6. LRMS (Ion Spray) exact mass calcd. for $[M+Cl]^-$ $(C_{43}H_{63}ClNO_{14}Si)^-$ requires *m/z* 880.4, found *m/z* 881.5.

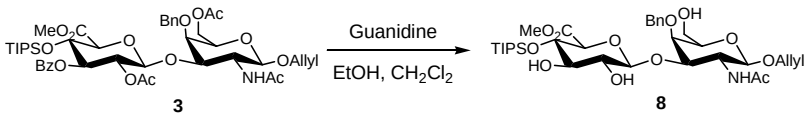


3 6

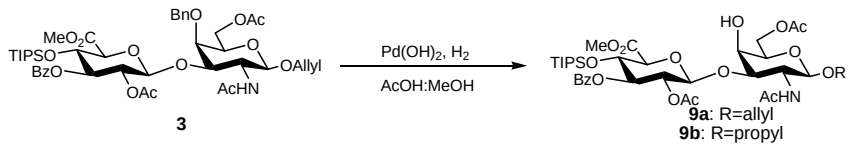
(6) To a solution of compound **3** (13 mg, 0.015 mmol) in ethanol (0.3 mL) was added hydrazine hydrate (50-60% solution, 0.2 mL), and the reaction was allowed to stir for 42 hours. The reaction was diluted with ethyl acetate (3 mL) and then washed with water (1 mL) and brine (1 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo.* The residue was purified via flash chromatography (40-80% EtOAc/Hexane) to afford compound **6** (8.1 mg, 67% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.98 (d, 2H, J = 7.5 Hz) Ar-**H**, 7.56-7.30 (m, 8H) Ar-**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.62 (bs, 1H) N**H**, 5.40-5.25 (m, 2H) $C\mathbf{H}_2CHCH_2O$, Glu **H**-3, 5.20-5.15 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.02-4.69 (m, 6H), 4.51-4.45 (m, 2H), 4.07-3.90 (m, 4H), 3.87-3.72 (m, 2H), 3.78 (s, 3H) CO_2CH_3, 3.44 (m, 1H) Gal **H**-2, 2.93 (bs, 1H) O**H**, 2.89 (bs, 1H) O**H**, 2.01 (s, 3H) $COCH_3$, 0.95-0.89 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. LRMS (Ion Spray) exact mass calcd. for $[M-H]^-$ $(C_{41}H_{58}NO_{13}Si)^-$ requires *m/z* 800.4, found *m/z* 800.6.



(7) Compound **3** (23 mg, 0.03 mmol) was dissolved in ethanol (1 mL) and to this was added hydrazine hydrate (16 μL). The reaction was stirred for 38 hours before being quenched by the addition of ethyl acetate (5 mL). The organic layer was washed with water (2x1 mL) and brine (1 mL) and dried over sodium sulfate before being filtered and concentrated via rotary evaporation. The residue was purified via flash chromatography (50-70% EtOAc/Hexane) to afford compound **7** (21 mg) in a 93% yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05 (m, 2H) Ar**H**, 7.59-7.30 (m, 8H) Ar-**H**, 5.70 (d, 1H, J = 7.0 Hz) N**H**, 5.29 (dd, 1H, J = 9.0, 9.0 Hz) Glu **H**-3, 4.88 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.85 (d, 1H, J = 8.5) Gal **H**-1, 4.72 (m, 1H) Glu H-**4**, 4.71 (d, 1H, J = 12.0 Hz) C**H**$_2$Ph, 4.63 (d, 1H, J = 7.5) Glu **H**-5, 4.47 (dd, 1H, J = 3.0, 11.0 Hz) Gal **H**-3, 4.42 (dd, 1H, 9.0, 9.0 Hz) Glu **H**-2, 4.05 (d, 1H, J = 9.0 Hz) Glu **H**-1, 3.87 (m, 1H) Gal **H**-4, 3.80 (s, 3H) $CO_2C\mathbf{H}_3$**,** 3.77 (m, 2H) propyl C**H**$_2$**,** 3.66 (m, 4H) propyl C**H**$_2$, Gal-**H**-5, Gal **H**-6) **,** 3.47 (m, 1H) Gal **H**-6, 3.43 (m, 1H) Gal **H**-2. 2.96 (bs, 1H) O**H**, 2.91 (bs, 1H) O**H**, 1.98 (s, 3H) $COCH_3$, 0.97-0.88 (m, 24H) Si(CH(C**H**$_3$)$_2$, propyl C**H**$_3$ ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.1, 170.3, 167.0, 153.6, 138.3, 133.6, 129.9, 129.6, 128.7, 128.6, 105.3, 99.8, 80.5, 76.8, 74.5, 73.6, 71.0, 61.0, 52.7, 24.0, 23.0, 18.2. 18.1, 18.0, 13.2, 10.6. LRMS (Ion Spray) exact mass calcd. for $[M-H]^-(C_{41}H_{60}NO_{13}Si)^-$ requires *m/z* 802.4, found *m/z* 802.2.

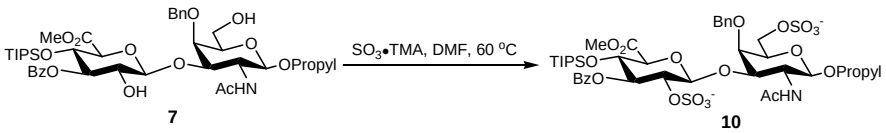


(8) Guanidine (0.7 M solution in ethanol, 10 μL) was added to a solution of compound **3** (6 mg, 0.01 mmol) at 0 ^{o}C in ethanol (80 μL) and dichloromethane (10 μL). This reaction was stirred for 2 hours and then diluted with ethyl acetate (2 mL). The organic layer was washed with water (0.5 mL) and brine (0.5 mL), and the organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo.* The residue was purified via column chromatography (60-100% EtOAc/Hexane) to afford compound **8** (3.5 mg, 73 % yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41-7.30 (m, 5H) Ar-**H**, 5.88 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.64 (d, 1H, J = 7.5 Hz) N**H**, 5.30 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.18 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 4.86 (d, 1H, J = 12.0 Hz) $C\mathbf{H}_2Ph$, 4.77-4.67 (m, 2H), 4.42 (d, 1H, J = 7.5 Hz), 4.33-4.26 (m, 2H), 4.10-4.06 (m, 2H), 3.88-3.77 (m, 3H), 3.80 (s, 3H) CO_2CH_3, 3.62-3.54 (m, 2H), 3.44-3.40 (m, 2H), 3.34-3.29 (m, 1H), 3.07 (bs, 2H) O**H**, 2.54 (bs, 1H) O**H**, 2.00 (s, 3H) $COCH_3$, 0.95-0.89 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. LRMS (Ion Spray) exact mass calcd. for $[M-H]^-$ $(C_{34}H_{54}NO_{12}Si)^-$ requires *m/z* 696.3, found *m/z* 696.9.



(9) Compound **3** (5 mg, .005 mmol) was dissolved in methanol (0.127 mL) and to this was added acetic acid (1 μL). Hydrogen was bubbled through the solvent for 1 minute and then

palladium hydroxide (2 mg) was added. The reaction was placed under 1 atmosphere of hydrogen and allowed to stir for 2 hours. The reaction was then filtered over celite, diluted with ethyl acetate (5 mL) and washed once with a saturated aqueous sodium bicarbonate solution (1 mL) and brine (1 mL). The organic layer was dried with sodium sulfate, filtered, and concentrated via rotary evaporation. The residue was purified via column chromatography (10-60% EtOAc/Hexane) to provide a 2.3:1 mixture of **9a** and **9b**. ^{1}H NMR (300 MHz, $CDCl_3$): **9a:** δ = 7.99-7.94 (m, 2H) Ar-**H**, 7.52-7.30 (m, 3H) Ar-**H**, 5.86 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.57 (bs, 1H), 5.37-5.12 (m, 3H), 5.09-4.91 (m, 2H), 4.86-4.70 (m, 2H), 4.47-3.82 (m, 7H), 3.76 (s, 3H) CO_2CH_3, 3.72 (m, 1H), 3.39-3.30 (m, 2H), 2.01 (s, 3H) $COCH_3$, 1.95 (s, 3H) 1.93 (s, 3H) $COCH_3$, 0.95-0.64 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. ^{1}H NMR (300 MHz, $CDCl_3$): **9b:** δ = 7.99-7.94 (m, 2H) Ar-**H**, 7.52-7.30 (m, 3H) Ar-**H**, 5.60 (bs, 1H), 5.36 (m, 1H) 5.09-4.91 (m, 2H), 4.86-4.70 (m, 2H), 4.47-3.82 (m, 6H), 3.78 (s, 3H) CO_2CH_3, 3.72 (m, 1H) 3.39-3.30 (m, 3H), 2.01 (s, 3H) $COCH_3$, 1.95 (s, 3H) $COCH_3$, 1.93 (s, 3H) COCH3, 1.54-1.48 (m, 2H), 1.10-0.89 (m, 24H) $Si(CH(C\mathbf{H}_3)_2)$. **9a:** LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{38}H_{57}NO_{15}SiNa$) requires *m/z* 818.3, found *m/z* 818.4. **9b:** LRMS (Ion Spray) exact mass calcd. for [M+Na] ($C_{38}H_{59}NO_{15}SiNa$) requires *m/z* 820.4, found *m/z* 820.5.



7 10

(10) Compound **7** (9 mg, 0.01 mmol) was dissolved in dimethylformamide (0.4 mL), and sulfur trioxide trimethylamine complex (4.1 mg, 0.03 mmol) was added. The reaction was heated to 60 °C and stirred for 7 hours. Then, more sulfur trioxide trimethylamine complex was added (46 mg, 0.3 mmol), and the reaction was allowed to stir for an additional 22 hours. The reaction was cooled to room temperature. Methanol (0.5 mL) was added, and the reaction was stirred for 1 hour. The reaction was concentrated via rotary evaporation and the residue loaded onto a Sephadex LH-20 column and eluted in 1:1 dichloromethane/methanol to afford compound **10**. This compound was unable to be separated from residual sulfur trioxide trimethylamine salts. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.95 (d, 2H, J = 7.0 Hz) Ar-**H**, 7.46-7.24 (m, 8H) Ar-**H**, 5.30 (dd, 1H, J = 9.0, 9.0 Hz) Glu **H**-3, 4.90 (d, 1H, J = 7.5 Hz) Glu **H**-5, 4.80 (d, 1H, J = 10.5 Hz) C**H**$_2$Ph, 4.63-461 (m, 2H) C**H**$_2$Ph, Gal **H**-1, 4.46 (dd, 1H, J = 9.0, 8.0 Hz), Glu **H**-4, 4.31 (dd, 1H, J = 9.0, 8.5 Hz) Glu **H**-2, 4.23-4.16 (m, 4H), 4.12 (bs, 1H) Gal **H**-4, 4.06 (d, 1H, J = 9 Hz) Glu **H**-1, 3.71 (s, 3H) CO_2CH_3, 2.02 (s, 1H)$COCH_3$, 0.95-0.89 (m, 21H) Si(CH(C**H**$_3$)$_2$). Other peaks were masked by SO_3TMA salts. LRMS (Ion Spray) exact mass calcd. for $[M-H]^-$ ($C_{41}H_{60}NO_{19}S_2Si$) requires *m/z* 962.3, found *m/z* 962.5.

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17. This research was carried out in the Hsieh-Wilson laboratory with the assistance of Drs. Song-Gil Lee and Sarah Tully, Ms. Maria Chiriac, and Mr. Robinson Moncure.

Chapter 5

Synthesis of a Second Differentially-Protected Chondroitin Sulfate Disaccharide and Its Elaboration to a Differentially-Protected Chondroitin Sulfate Tetrasaccharide

Introduction

The construction of CS requires the stereospecific formation of β-(1,3)- and β-(1,4)-glycosidic linkages. These coupling reactions can be affected by many variables, including the protecting group scheme and the method of activation. The protecting group scheme can have two influences on the reaction. First, the presence of a participating group or non-participating group at the 2-position can affect the α:β ratio of the coupled products. Second, the protecting groups can deactivate the ring. To that end, a variety of activation methods have been developed to improve the reactivity of the donor and to bias the coupled products towards a higher α or β ratio.[1]

The use of participating and non-participating protecting groups is the primary method employed by carbohydrate chemists to direct coupling reactions. Participating groups are known to set a β-linkage. They typically possess a carbonyl that adds into the anomeric position, forcing the acceptor to attack from the opposite face (Figure 5.1). One drawback of using participating groups is that doing so can lead to the formation of undesired orthoester products.

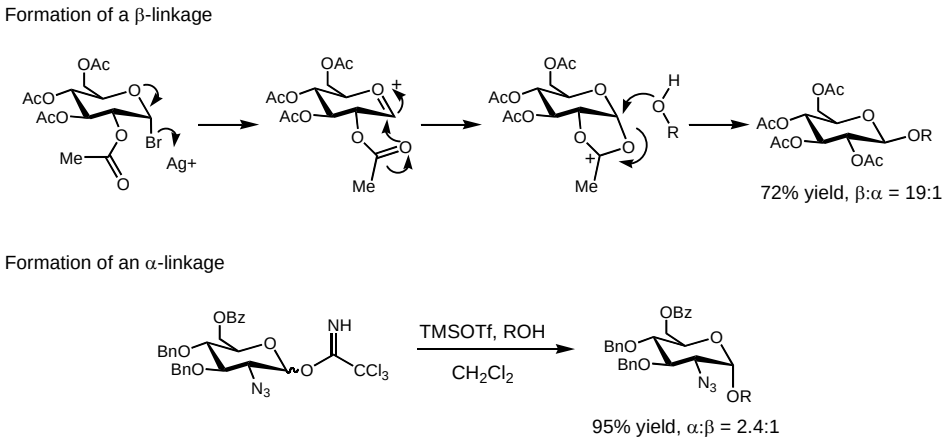


Figure 5.1: Participating groups can be used to direct the α:β ratio of coupling products.

Orthoesters are side products that result from the acceptor hydroxyl attacking the electrophilic carbon of the participating group instead of the anomeric position (Figure 5.2). While they are usually accessed inadvertently, stable orthoesters can also be formed intentionally and used to direct coupling reactions. An orthoester will usually revert to a coupled saccharide when exposed to a Lewis acid. In addition an orthoester will typically impart a stereochemical outcome on the coupled product.[2]

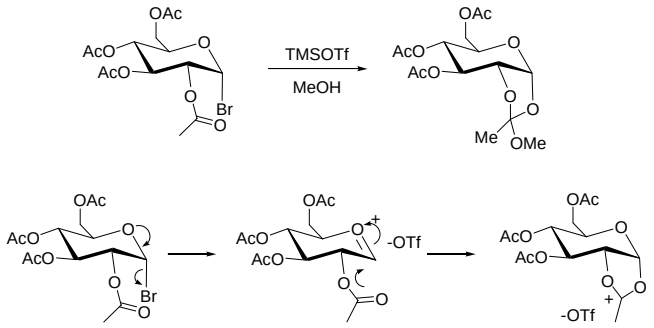


Figure 5.2: Orthoesters are formed when the nucleophile attacks the participating group instead of the anomeric position.

While there are reported instances of glucosyl orthoesters leading to α-linked saccharides[2], these orthoesters are usually considered to produce β-linkages. Mechanistic studies suggest that orthoesters undergo a rearrangement that begins with cleavage of the C-O bond between the orthoester carbon and the eventual acceptor. Then the released acceptor attacks the anomeric position of the donor to produce the desired disaccharide. For example, it is believed that disaccharide **4** is formed from orthoester **1** through the following pathway. First, the orthoester C-O bond of saccharide **1** is cleaved to produce monosaccharides **2** and **3**. Acceptor **3** then attacks the anomeric position of donor **2** to produce the β-(1,3)-linkage of disaccharide **4** (Figure 5.3).[1,3]

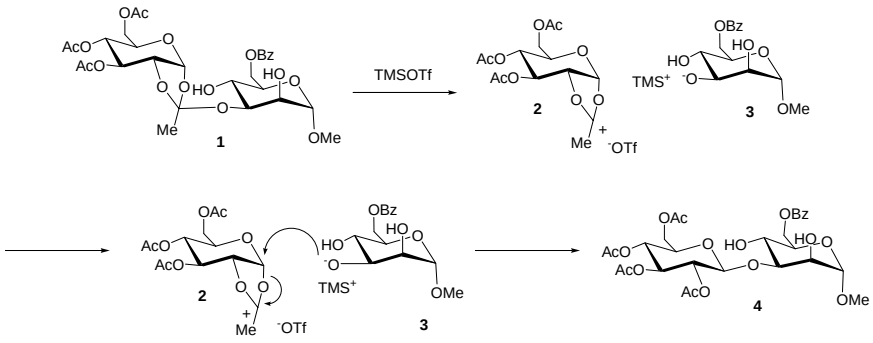


Figure 5.3: Proposed mechanism for orthoester rearrangement

It should also be noted that this reaction proceeds to selectively form the β-(1,3)-linked disaccharide in 82% yield, despite the fact that the acceptor C-2 and C-4 hydroxyls are also available nucleophiles. Many orthoester rearrangements can be carried out on partially-deprotected substrates to provide the desired linkage if the desired hydroxyl is the most reactive hydroxyl available on the acceptor.[5] The primary hydroxyl (6-OH) is considered the most reactive position on the acceptor, and it usually follows that the 3-OH is more reactive than the 2-OH, which is more reactive than the 4-OH.

In addition to protecting groups participating in reactions to direct the α:β ratio of coupling reactions, protecting groups can also affect the reactivity of coupling partners.[1,8] For example, ester protecting groups are known to deactivate the ring.[1] Even so, ester protecting groups are widely used in carbohydrate chemistry because they are stable in acidic conditions, able to be easily removed, and can serve as participating groups.[1, 6] In addition, a C-5 methyl ester (such as the one on glucuronic acid) can also deactivate the

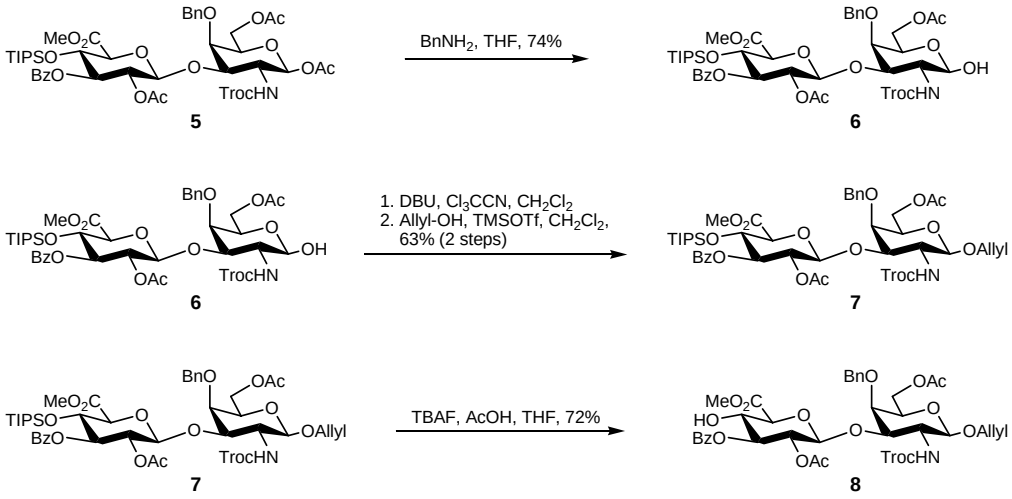
ring. To overcome the lowered reactivity, many methods have been developed to couple saccharides.

The classic glycosylation method is the Koenigs-Knorr reaction[7], which refers to a substitution reaction of a glycosyl halide with the acceptor alcohol to produce the new saccharide (Figure 5.1). Many other leaving groups have been introduced, such as the use of thioglycosides[8] and phosphates.[9] One of the most active leaving groups is the trichloroacetimidate. Developed by Schmidt and coworkers,[10] trichloroacetamides have been widely used in carbohydrate chemistry. In CS chemistry, the trichloroacetimidate donor has been particularly helpful for setting the β-(1,4)-linkage between the galactosamine (GalNAc) and glucuronic acid (GlcA) moieties.[11]

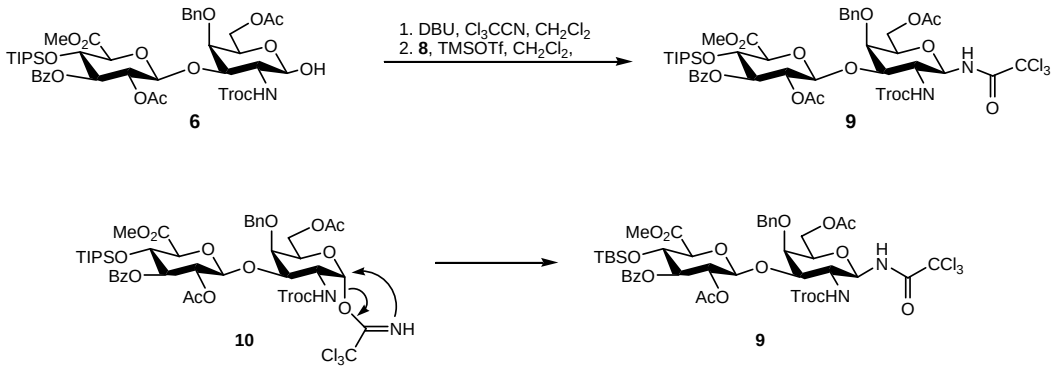
Progress Towards the Tetrasaccharide

To produce the donor and acceptor for the tetrasaccharide coupling, core disaccharide **5** was elaborated through the following steps. We first removed the anomeric acetate with benzylamine in tetrahydrofuran and formed the imidate (as described in Chapter 3). A portion of the imidate disaccharide was coupled to allyl alcohol to form disaccharide **7**, and the C-4 TIPS group was removed with HF·pyridine (Scheme 5.1). The resulting disaccharide (**8**) was combined with imidate donor **10** and TMSOTf, but instead of producing the desired tetrasaccharide, rearranged donor product **9** was formed, preventing the coupling of disaccharides **8** and **10** (Scheme 5.2). This product most likely

forms when the nucleophilic amine of the displaced trichloroacetimidate attacks the electrophilic anomeric position.



Scheme 5.1: Elaboration of core disaccharide **5** to produce the disaccharide donor and acceptor



Scheme 5.2: Attempts to couple disaccharides **8** and **10** resulted in a rearrangement to form disaccharide **9**.

During the course of our laboratory's synthesis of CS-E, a similar rearrangement product was observed. In fact, it was thought that replacement of the trichloroacetyl (TCA) protecting group with a Troc protecting group would minimize this rearrangement because a more active donor would react faster with the acceptor. However, studies performed by Dr. Song Gil Lee of both disaccharide **10** and **11** revealed that the TCA-protected donor **11** was a superior coupling partner to disaccharide **11** (Figure 5.4).

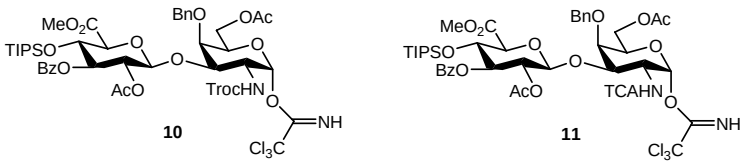
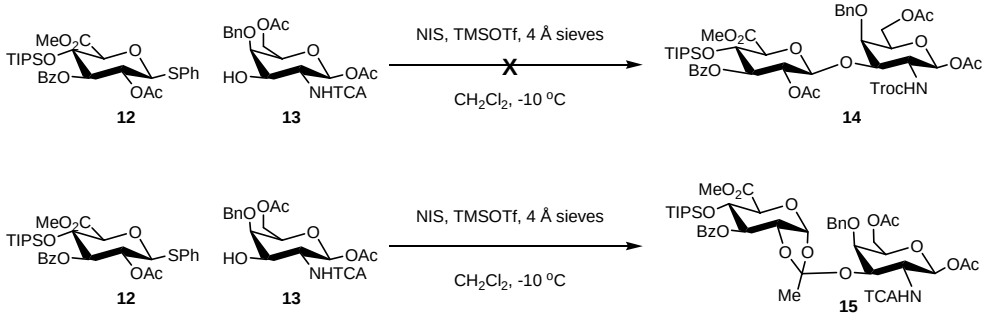


Figure 5.4: Model studies suggest that exchanging the Troc protecting group for a TCA protecting group minimizes rearrangement of the imidate.

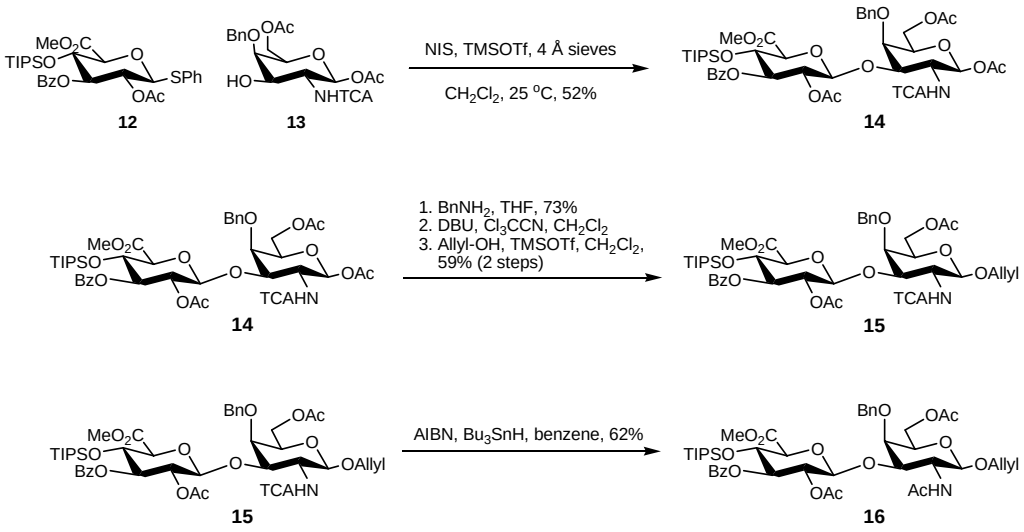
Because of these results, we decided to change the amine protecting group from a Troc group to a TCA group. To make core disaccharide **14**, we synthesized acceptor **13**[12] and combined it with donor **12** in a TMSOTf-mediated coupling reaction at -10 ^{o}C. However, instead of producing disaccharide **14**, orthoester **15** was produced as the main product (Scheme 5.3).



Scheme 5.3: Donor **12** and acceptor **13** produce orthoester **15**.

Knowing that orthoesters should be able to rearrange to form the desired disaccharide, we set out to find conditions that would permit this reaction. First, we increased the amount of TMSOTf added to the reaction from 0.2 equivalents to 0.7 equivalents and allowed the reaction to warm to room temperature. This change increased the production of disaccharide **14**. Next we divided the TMSOTF into two additions, adding 0.4 equivalents of TMSOTf in the beginning and an additional 0.3 equivalents of

TMSOTf after 5 hours. Finally, we tried warming the reaction to 25 °C. When these factors were combined, the desired disaccharide (**14**) was accessed in 52% yield.



Scheme 5.4: Elaboration of core disaccharide **14** to common intermediate **16**.

With this new core disaccharide, we needed to determine two things. First, we needed to confirm that this disaccharide could still access the desired sulfation patterns. To do so, disaccharide **14** was converted to common intermediate **16** through the following steps. First, the anomeric acetate was removed with benzylamine in tetrahydrofuran. Next, the resulting disaccharide was activated to the trichloroacetimidate donor and coupled to allyl alcohol to form disaccharide **15**. Finally, a radical conversion of the TCA group to the *N*-acetyl group produced disaccharide **16** (Scheme 5.4). Second, we needed to confirm that it would also allow access to the desired tetrasaccharide. Work by Dr. Song-Gil Lee

has shown that disaccharide **14** can be elaborated to form tetrasaccharide **17** (Figure 5.5).[12] With these results, the syntheses of the core differentially-protected disaccharide and tetrasaccharide were complete.

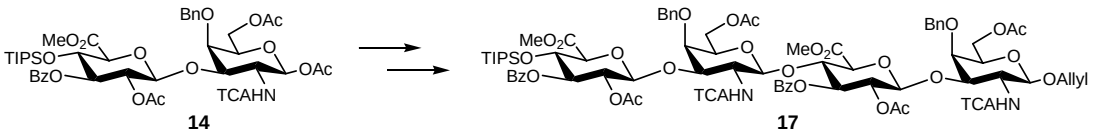


Figure 5.5: Core disaccharide **14** has been elaborated to tetrasaccharide **17**.

Conclusions

The choice of protecting groups can have a profound effect on glycosylation reactions. When attempting to couple donor **8** and acceptor **10**, a deleterious rearrangement of the donor occurred. To minimize this rearrangement, the galactosamine C-2 amine protecting group was changed from a Troc protecting group to a TCA protecting group. A new core disaccharide (**14**) was synthesized and elaborated to form disaccharide **16** which intersects the previous synthesis and has been used to form tetrasaccharide **17**.

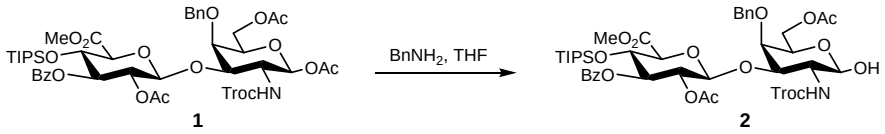
Supporting Information

General Methods

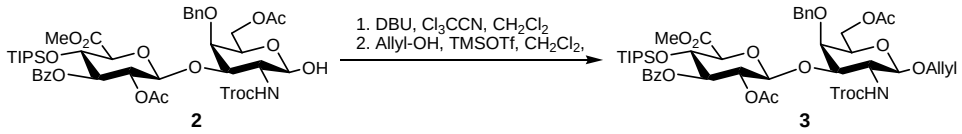
Unless stated otherwise, reactions were performed in oven-dried glassware that had been cooled in a desiccator over Dririte. Reactions were performed under an argon environment unless otherwise stated. All solvents were purified using the method of Grubbs.[13] Unless stated otherwise, all commercially obtained reagents were used as received. Chemicals that were purified after purchase were purified according to the guidelines provided by Perrin and Armarego.[14] Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator using an ice-water bath for volatile samples. High-performance liquid chromatography (HPLC) was performed on Hewlett-Packard 1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted. Liquid chromatography mass spectrometry analysis was performed on an Aglient 1100 series LCMS using an acetonitrile/water mobile phase treated with 0.1% acetic acid with a quadrapole detector. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by fluorescence quenching, cerium ammonium molybdate stain, *p*-anisaldehyde stain, potassium permanganate stain, or ninhydrin stain, as

necessary. Flash chromatography was performed on ICN silica gel (particle size 0.032 - 0.063 mm) using the method of Still.[15]

^{1}H NMR and proton decoupling spectra were recorded on a Varian Mercury 300 (300 MHz), a Varian Inova 500 (500 MHz), and Varian Mercury 600 (600 MHz) spectrometers and the ^{1}H NMR spectra are reported in parts per million (δ) relative to the residual solvent peak. Data for ^{1}H are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) in Hz, and integration. ^{13}C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) spectrometer and 500 (125 MHz) and are reported in parts per million (δ) relative to the residual solvent peak. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). A JASCO P-1010 was used to measure optical rotation. Mass spectra obtained from the Protein/Peptide MicroAnalytical Laboratory were generated on a Perkin Elmer/Sciex API 365 triple quadrapole mass spectrometer with nano spray ion source. Samples were dissolved in 50/50 MeOH/CH2Cl2, and infused at 0.2 microliter per minute. High-resolution mass spectrometry was performed at the Mass Spectrometry Facility at the California Institute of Technology on either a JEOL JMS-600H High Resolution Mass Spectrometer or a UPLC-LCT Premier XT TOF Mass Spectrometer using Leu-Enkephalin as lock mass in order to obtain exact mass. The UPLC-LCT Premier XT was purchased in 2006 with a grant from the National Science Foundation Chemistry Research Instrumentation and Facilities Program (CHE-0541745).



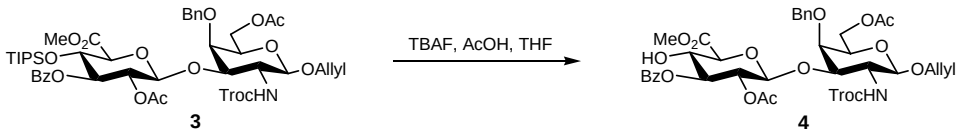
(2) To a solution of compound **1** (33 mg, 0.03 mmol) in THF (0.3 mL) was added benzylamine (0.01 mL, 0.1 mmol). The solution was stirred for 7 hours, and the reaction was quenched by the addition of ethyl acetate (5 mL) and poured into an extraction funnel. The organic layer was washed with water (2x1 mL) and brine (1 mL) and dried over sodium sulfate. The organic layer was filtered, and the solvent was removed via rotary evaporation. The residue was purified via flash chromatography to afford compound **2** (22 mg) in 74% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97 (d, 2H, J = 6.9 Hz) ArH, 7.60 (t, 1H, J = 7.2 Hz) ArH, 7.49-7.33 (m, 7H) Ar-**H**, 6.36 (bs, 1H), 5.53 (bs, 1H), 5.45 (m, 1H), 5.01-4.61 (m, 6H), 4.37 (bs, 1H), 4.28 (m, 1H), 4.20 (bs, 1H), 4.15-3.81 (m, 5H), 3.48 (s, 3H) $CO_2C\mathbf{H}_3$, 2.62 (bs, 1H) O**H**, 1.92 (s, 3H) C(O)C**H**3, 1.85 (s, 3H) $C(O)C\mathbf{H}_3$, 0.89-0.84 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9, 170.1, 164.6, 154.9, 138.1, 134.2, 130.2, 128.9, 128.9, 128.6, 128.1, 125.8, 94.2, 93.3, 92.4, 76.5, 75.4, 74.9, 74.8, 74.7, 70.1, 69.1, 68.8, 68.6, 66.9, 63.9, 52.5, 21.4, 21.0, 18.1, 12.3. LRMS (Ion Spray) exact mass calcd. for $[M+Cl']^-$ ($C_{43}H_{58}Cl_4NO_{16}Si$) requires *m/z* 1012.2, found *m/z* 1012.1.



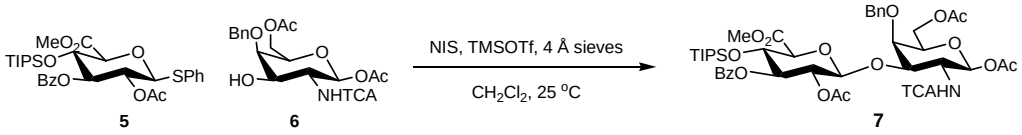
(3) Compound **2** (194 mg, 0.2 mmol) was azeotroped three times with toluene and placed under vacuum for 8 hours before being added to a flame-dried flask with flame-activated 4Å molecular sieves as a solution in dichloromethane (3.5 mL). The reaction mixture was allowed to stir for 15 minutes before being cooled to 0 °C for the addition of freshly distilled 1,8-diazabicyclo[5.4.0]undec-7-ene (6 μL, 0.04 mmol) and freshly distilled trichloroacetonitrile (0.2 mL, 2 mmol). The reaction was warmed to room temperature to stir for 6 hours. Toluene (0.2 mL) was added to the reaction mixture which was concentrated before loading on a silica column pretreated with a mixture of hexane:toluene:ethyl acetate:triethylamine (50:20:20:1). The column was eluted with a mixture of hexane:toluene:ethyl acetate (5:2:2) with triethylamine (0.1 mL), and the fractions containing the imidate were concentrated and kept under vacuum for 4 hours before being used in the next reaction. (R_f = 0.5 in hexane:toluene:ethyl acetate (5:2:2) with 0.1 mL triethylamine, NOTE: this compound is easily hydrolyzed so TLC plates must be pre-eluted with the hexane:toluene:ethyl acetate:triethylamine solution (50:20:20:1) before being spotted.)

The imidate was added as a solution in dichloromethane (5 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves. Freshly distilled allyl alcohol (0.12 mL, 1.75 mmol) was added, and the reaction mixture was stirred at room temperature for

15 minutes before being cooled to -78 °C for the addition of trimethylsilyl triflate as a solution in dichloromethane (0.23 mL, 0.05 M). The reaction was warmed to 0 °C and stirred for 6 hours before being quenched by the addition of solution of pyridine in ethyl acetate (0.5 mL pyridine in 20 mL ethyl acetate). The organic layer was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL), and the organic layer was dried over sodium sulfate and filtered. The solvent was removed via rotary evaporation, and the residue was purified via flash chromatography (hexane:toluene:ethyl acetate, 5:2:2) to afford compound **3** (122 mg) in 63% yield as a white solid. (500 MHz, $CDCl_3$): δ = 8.01 (dd, 2 H J = 1.5, 8.5 Hz) Ar**H**, 7.96 (m, 1H) Ar**H**, 7.46-7.29 (m, 7H) Ar**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.38 (dd), 1H, J = 10.0, 10.0 Hz) Glu **H**-3, 5.28-5.24 (m, 2H) $C\mathbf{H}_2CHCH_2O$, N**H**; 5.18 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.10 (dd, 1H, J = 8.0, 8.0 Hz) Glu **H**-4, 4.94 (m, 1H) Glu **H**-5, 4.89 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.85 (d, 1H, J = 29.0) $C\mathbf{H}_2Ph$, 4.80 (m, 1H) Gal **H**-1, 4.71-4.64 (m, 2H) $C\mathbf{H_2}CCl_3$, 4.53 (m, 1H) Gal **H**-3, 4.49 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-2, 4.36-4.31 (m, 2H) $CH_2CHC\mathbf{H_2}O$, 4.12-4.05 (m, 3H) Glu **H**-1, $CH_2CHC\mathbf{H_2}O$, Gal **H**-6; 4.04 (m, 2H) Gal **H**-4, Gal **H**-5, 3.81 (s, 3H) $CO_2C\mathbf{H}_3$, 3.66 (m, 1H) Gal **H**-6, 3.53 (m, 1H) Gal **H**-2, 1.94 (s, 3H) $COC\mathbf{H}_3$, 1.93 (s, 3H) $COC\mathbf{H}_3$, 0.97-0.85 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)_3$ ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.4, 169.4, 160.5, 154.2, 137.9, 137.2, 133.7, 133.5, 129.6, 129.4, 128.6, 128.5, 128.4, 128.3, 128.2, 125.3, 117.8, 95.6, 75.1, 74.6, 73.2, 73.0, 72.0, 70.9, 70.9, 70.0, 62.3, 52.5, 50.0, 20.7, 21.5, 20.9, 20.9, 20.7, 17.9, 12.9. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{45}H_{60}Cl_3NNaO_{17}Si$) requires *m/z* 1042.3, found *m/z* 1042.0.

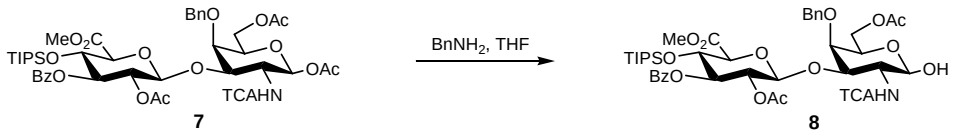


(4) Compound **3** (17 mg, 0.02 mmol) was dissolved in tetrahydrofuran (0.1 mL) and cooled to 0 ^{o}C. To this solution was added 1.0 M TBAF in THF (25 µL) and acetic acid (2 µL), and the reaction was allowed to stir for 3 hours. This reaction was diluted with ethyl acetate (2 mL), washed with saturated sodium bicarbonate (2 x 0.5 mL), water (0.5 mL), and brine (0.5 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via flash chromatography (30-70% EtOAc/Hexane) to afford compound **4** (6.8 mg, 0.01 mmol) in 47% yield. (300 MHz, $CDCl_3$): δ = 7.99 (m, 2 H) Ar**H**, 7.60-7.24 (m, 8H) Ar**H**, 6.05 (m, 1H), 5.82 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.48-5.45 (m, 1H), 5.33-5.10 (m, 4H), 4.91-4.74 (m, 4H), 4.67-4.61 (m, 2H), 4.46 (d, 1H, J = 9.5 Hz), 4.37-4.25 (m, 2H), 4.21-4.12 (m, 2H), 4.09-3.97 (m, 3H), 3.63 (s, 3H) $CO_2C\mathbf{H}_3$, 3.15-3.10 (m, 1H), 2.30 (bs, 1H) O**H**, 1.94 (s, 3H) $COC\mathbf{H}_3$, 1.90 (s, 3H) $COC\mathbf{H}_3$. LRMS (Ion Spray) exact mass calcd. for $[M-H]^-$ ($C_{37}H_{41}Cl_3NO_{16}$) requires *m/z* 860.1, found *m/z* 860.0.



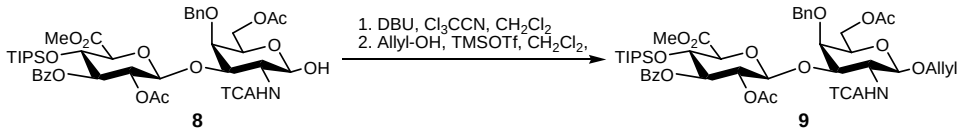
(7) Donor **5** (500 mg, 0.83 mmol) and acceptor **6** (434 mg, 0.87 mmol) were azeotroped three times with toluene and placed under vacuum overnight. They were then dissolved in dichloromethane (2 mL) and added into a flame-dried vial containing dichloromethane (6 mL) and activated 4 Å molecular sieves. This mixture was allowed to stir for 30 minutes.

NIS (464 mg, 1.16 mmol) was added, and the reaction mixture was allowed to stir for an additional 15 minutes. The reaction mixture was cooled to 0 ^{o}C and TMSOTf (1 M in dichloromethane, 0.4 mL) was added. This reaction was quickly warmed to 25 ^{o}C and allowed to stir for 5 hours, at which time more TMSOTf (1 M solution in dichloromethane, 0.3 mL) was added. The reaction mixture was allowed to stir for an additional 17 hours. Upon completion, the reaction was diluted with ethyl acetate (30 mL) and washed with saturated sodium bicarbonate (2 x 10 mL), water (10 mL), and brine (10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via column chromatography (20-80% EtOAc/Hexane) to provide compound **7** (428 mg, 0.43 mmol) in 52% yield. IR (film, $CDCl_3$) 2946, 2867, 1751, 1731, 1524, 1452, 1372, 1266, 1221, 1146, 1112, 1091, 1068, 1027, 1013 cm^{-1}, ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.04-8.02 (m, 2H) Ar**H**, 7.70-7.35 (m, 8H) Ar**H**, 6.75 (d, 1H, J = 8.5 Hz) N**H**, 6.34 (d, 1H, J = 3.5 Hz) Gal **H**-1, 5.39 (dd, 1H, J = 8.0, 8.0 Hz) Glu **H**-3, 5.26 (dd, 1H, J = 8.0, 9.0 Hz) Glu **H**-4, 5.09 (d, 1H, J = 8 Hz) Glu **H**-5, 5.03 (d, 1H, J = 11.0 Hz) CH_2Ph, 4.77-4.72 (m, 1H) Gal H-2, 4.61 (d, 1H, J = 11.0 Hz) CH_2Ph, 4.53 (dd, 1H, J = 8.0, 8.0 Hz) Glu **H**-2, 4.31-4.28 (m, 1H) Gal H-3, 4.32 (d, 1H, J = 8.0 Hz) Glu **H**-1, 4.21-4.10 (m, 3H) Gal H-5, Gal H-6, Gal H-6, 4.02 (bs, 1H) Gal H-4, 3.84 (s, 3H) $CO_2C\mathbf{H}_3$, 2.23 (s, 3H) $C(O)C\mathbf{H}_3$, 2.06 (s, 3H) $C(O)C\mathbf{H}_3$, 1.99 (s, 3H) $C(O)C\mathbf{H}_3$, 1.02-0.90 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{13}C NMR (500 MHz, $CDCl_3$): δ = 176.7, 170.9, 170.1, 169.0, 168.5, 165.6, 161.8, 137.9, 133.8, 132.7, 130.2, 129.9, 129.6, 128.7, 128.2, 100.3, 92.7, 91.2, 77.2, 75.4, 75.2, 75.0, 74.8, 71.3, 71.1, 70.7, 62.8, 52.9, 50.2, 21.1, 21.0, 20.9, 18.2, 18.1, 18.1, 13.2.



7 8

(8) Compound **7** (67 mg, 0.07 mmol) was dissolved in tetrahydrofuran (0.4 mL), and to this was added benzylamine (40 μL). This solution was allowed to stir for 7 hours. The reaction was then diluted with ethyl acetate (3 mL) and washed with water (1 mL) and brine (1 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via column chromatography to provide disaccharide **8** (46 mg, 0.05 mmol) in 73 % yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.97-7.95 (m, 2H) Ar**H**, 7.58-7.30 (m, 8H) Ar**H**, 6.83 (d, 1H, J = 9.5 Hz) N**H,** 5.34-5.30 (m, 2H) Gal H-1, Glu H-3, 5.16 (dd, 1H, J = 9.0, 9.0 Hz) Glu **H**-4, 5.01 (d, 1H, J = 11.0 Hz) CH_2Ph, 4.89 (d, 1H, J = 8 Hz) Glu **H**-5, 4.64-4.60 (m, 2H) CH_2Ph, Gal **H**-2, 4.45 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-2, 4.20-4.04 (m, 5H) Gal **H**-3, Glu **H**-1, Gal **H**-5, Gal **H**-6, Gal **H**-6, 3.94 (bs, 1H) Gal **H**-4, 3.80 (s, 3H) $CO_2C\mathbf{H}_3$, 3.06, (bs, 1H) O**H**, 2.02 (s, 3H) C(O)C$\mathbf{H}_3$, 1.95 (s, 3H) C(O)C$\mathbf{H}_3$, 0.96-0.92 (m, 21H) C**H**(C$\mathbf{H}_3$)$_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.2, 170.8, 167.0, 164.6, 157.7, 135.4, 134.1, 125.9, 125.6, 125.2, 124.7, 124.7, 124.2, 97.5, 88.3, 88.0, 84.1, 71.6, 71.3, 71.2, 67.6, 67.3, 65.1, 59.7, 48.9, 47.2, 26.6, 16.9, 14.17, 9.3.



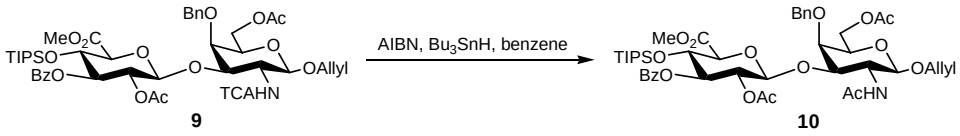
8 9

(9) Compound **8** (222 mg, 0.23 mmol) was azeotroped three times with toluene and placed under vacuum for 8 hours before being added to a flame-dried flask with flame-activated

4Å molecular sieves as a solution in dichloromethane (5 mL). The reaction mixture was allowed to stir for 15 minutes before being cooled to 0 °C for the addition of freshly distilled 1,8-diazabicyclo[5.4.0]undec-7-ene (7 μL, 0.05 mmol) and freshly distilled trichloroacetonitrile (0.23 mL, 2.3 mmol). The reaction was warmed to room temperature to stir for 6 hours. Toluene (0.2 mL) was added to the reaction mixture, which was concentrated before loading on a silica column pretreated with a mixture of hexane:toluene:ethyl acetate:triethylamine (50:20:20:1). The column was eluted with a mixture of hexane:toluene:ethyl acetate (5:2:2) with triethylamine (0.1 mL), and the fractions containing the imidate were concentrated and kept under vacuum for 4 hours before being used in the next reaction. (R_f = 0.5 in hexane:toluene:ethyl acetate (5:2:2) with 0.1 mL triethylamine. NOTE: this compound is easily hydrolyzed so TLC plates must be pre-eluted with the hexane:toluene:ethyl acetate:triethylamine solution (50:20:20:1) before being spotted.)

The imidate was added as a solution in dichloromethane (3 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves. Freshly distilled allyl alcohol (0.15 mL, 2.22 mmol) was added, and the reaction mixture was stirred at room temperature for 15 minutes before being cooled to -78 °C for the addition of trimethylsilyl triflate as a solution in dichloromethane (0.6 mL, 0.05 M). The reaction was warmed to 0 °C and stirred for 6 hours before being quenched by the addition of solution of pyridine in ethyl acetate (30 mL, 3% pyridine in ethyl acetate). The organic layer was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL), and the organic layer was dried over sodium sulfate and filtered. The solvent was removed via rotary evaporation, and the residue was purified via flash chromatography (hexane:toluene:ethyl

acetate, 5:2:2) to afford compound **9** (136 mg, 0.14 mmol) in 59% yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.96 (d, 2H, J = 7.5 Hz) Ar**H**, 7.56-7.28 (m, 8H) Ar**H**, 7.07 (d, 1H, J = 7.0 Hz) N**H,** 5.86 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.32 (dd, 1H, J = 8.5, 8.5 Hz) Glu H-3, 5.26-5.23 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.19-5.17 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.12 (dd, 1H, J = 8.0, 8.5 Hz) Glu H-4, 4.97-4.90 (m, 3H) CH_2Ph, Glu **H**-5, Gal H-1, 4.67 (d, 1H, J = 11.5 Hz) CH_2Ph, 4.65-4.62 (m, 1H) Gal H-3, 4.47 (dd, 1H, J = 8.5, 8.5 Hz) Glu H-2, 4.34-4.30 (m, 1H) $CH_2CHC\mathbf{H_2}O$, 4.19-4.04 (m, 3H) Gal H-6, Glu H-1, $CH_2CHC\mathbf{H_2}O$, 3.98-3.96 (m, 2H) Gal H-4, Gal H-6, 3.76 (s, 3H) $CO_2C\mathbf{H}_3$, 3.77-3.72 (m, 1H) Gal H-2, 3.70-3.67 (m, 1H) Gal H-5, 2.06(s, 3H) $C(O)C\mathbf{H}_3$, 1.97(s, 3H) $C(O)C\mathbf{H}_3$, 0.96-0.85 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.8, 169.9, 168.6, 165.7, 162.5, 133.6, 129.9, 129.4, 128.7, 128.6, 128.1, 118.6, 101.4, 97.7, 88.3, 77.5, 75.4, 74.9, 74.8, 72.2, 71.0, 70.9, 70.5, 63.1, 63.1, 52.7, 52.6, 21.1, 21.0, 18.1, 18.1, 13.1. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{45}H_{60}Cl_3NNaO_{15}Si$) requires *m/z* 1010.3, found *m/z* 1010.2



9 **10**

(10) Disaccharide **9** (138 mg, 0.14 mmol) was dissolved in benzene (4.6 mL), and to this solution was added tributyltin hydride (0.22 mL, 0.83 mmol) and AIBN (44 mg). The reaction was stirred at 80 ^{o}C under an argon atmosphere for 2 hours. It was then cooled to room temperature, the solvent was removed via rotary evaporation, and the residue was purified via column chromatography (40-70% EtOAc/Hexane) to afford disaccharide **10**

(76 mg, 0.09 mmol) in 62% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, 2H, J = 7.0 Hz) Ar-**H**, 7.57 (t, 1H, J = 7.0 Hz) Ar-**H**, 7.44-7.28 (m, 7H) Ar-**H**, 5.87 (m, 1H) $CH_2C\mathbf{H}CH_2O$, 5.63 (d, 1H, J = 7.0 Hz) N**H**, 5.38 (dd, 1H, J = 8.5, 8.5 Hz) Glu **H**-3, 5.24 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.17 (m, 1H) $C\mathbf{H}_2CHCH_2O$, 5.09 (dd, 1H, J = 8.0, 8.5 Hz) Glu **H**-4, 4.98 (d, 1H, J = 8.5 Hz) Gal H-1, 4.90 (d, 1H, J = 12.5 Hz) $C\mathbf{H}_2Ph$, 4.88 (d, 1H, J = 8.5 Hz) Glu H-5, 4.74 (m, 1H) Gal H-3, 4.66 (d, 1H, J = 11.5 Hz) $C\mathbf{H}_2Ph$, 4.47 (dd, 1H, J = 8.5, 8.5 Hz) Glu H-2, 4.14 (m, 1H) Gal **H**-6, 4.10 (d, 1H, **J** = 11.5 Hz) Glu H-1, 4.05 (m, 2H) $CH_2CHC\mathbf{H_2}O$, 3.91 (m, 1H) Gal **H**-5, 3.89 (m, 1H) Gal H-4, 3.77 (s, 3H) CO_2CH_3**,** 3.66 (m, 1H) Gal **H**-6, 3.39 (m, 1H) Gal **H**-2, 2.00 (s, 3H) $COCH_3$, 1.93 (s, 6H) $COCH_3$, 0.95-0.89 (m, 21H) $Si(CH(C\mathbf{H}_3)_2)$. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.3, 168.6, 165.8, 138.4, 134.1, 133.6, 129.9, 129.6, 129.5, 128.7, 128.5, 127.9, 118.1, 101.8, 98.2, 78.2, 76.8, 75.7, 72.7, 72.0, 71.1, 70.3, 63.3, 55.7, 52.7, 28.1, 27.1, 24.1, 20.9, 18.7, 18.2, 17.8, 13.9, 13.2. LRMS (Ion Spray) exact mass calcd. for $[M+Na]^+$ ($C_{45}H_{63}NNaO_{15}Si$) requires *m/z* 908.4, found *m/z* 908.5.

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16. This work was performed in the Hsieh-Wilson laboratory with Dr. Song-Gil Lee and Ms. Maria Chiriac.