Chapter 3

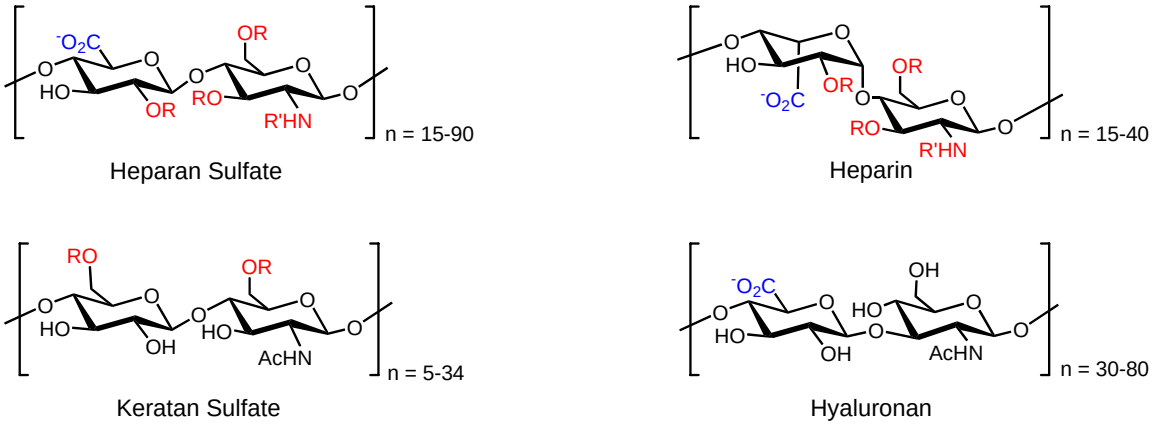
Synthesis of a Differentially-Protected Chondroitin Sulfate Disaccharide[31]

Introduction

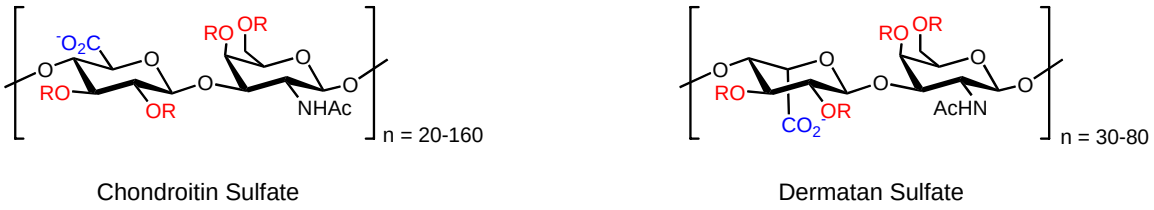
Described in the previous chapters is a method for quickly accessing differentially-protected monosaccharides.[1] This methodology could accelerate the synthesis of biologically active polysaccharides. To examine this theory, we decided to employ this method to synthesize a chondroitin sulfate tetrasaccharide.

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]

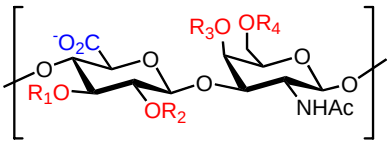
Glucosaminoglycan Class



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]

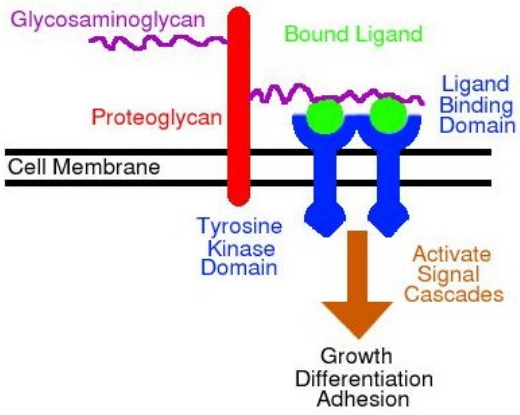


Figure 3.2: Glycosaminoglycans can be found at the cell surface or in the extracellular matrix.

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).

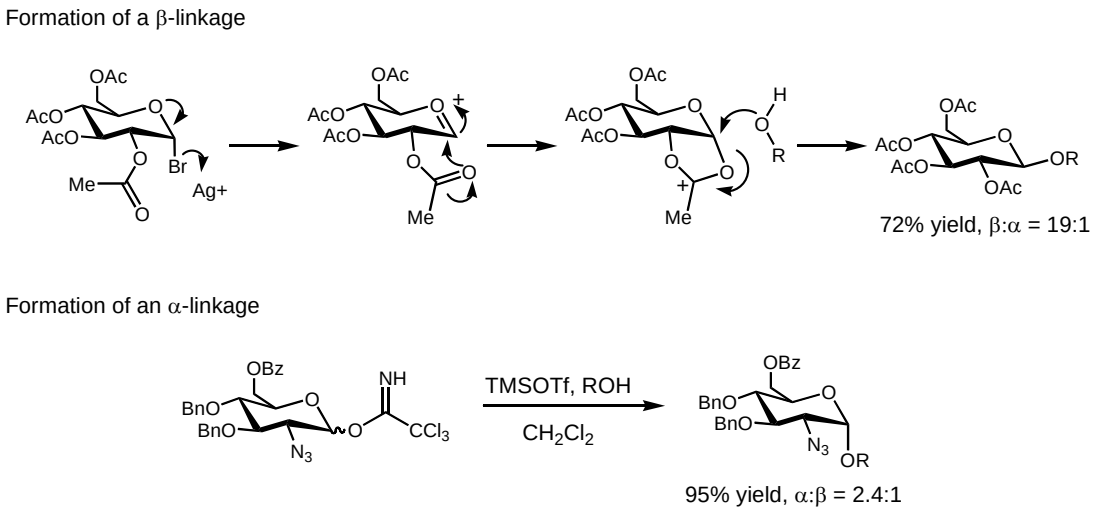
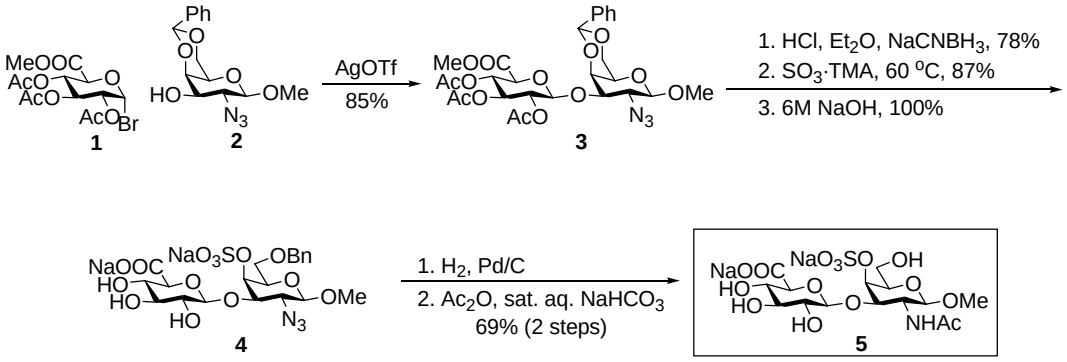


Figure 3.3: Participating groups refer to protecting groups that can block one face of the donor, forcing the acceptor to attack the unhindered face. Non-participating groups refer to protecting groups that do not perform in this manner.

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]



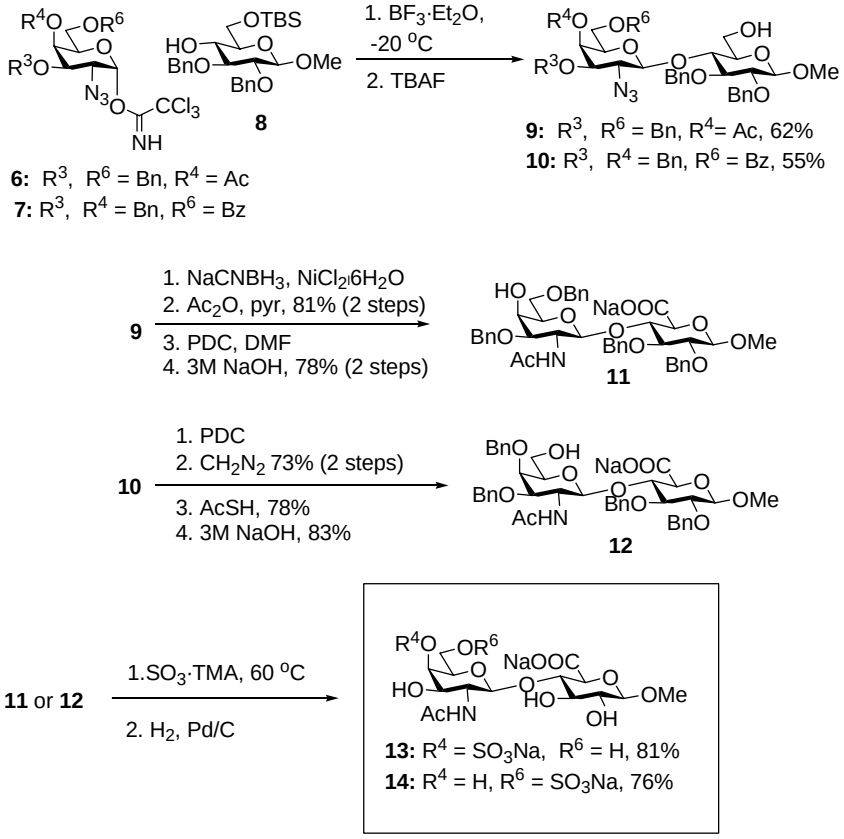
Scheme 3.1: The synthesis of a CS-A disaccharide reported by Sinay and coworkers

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]

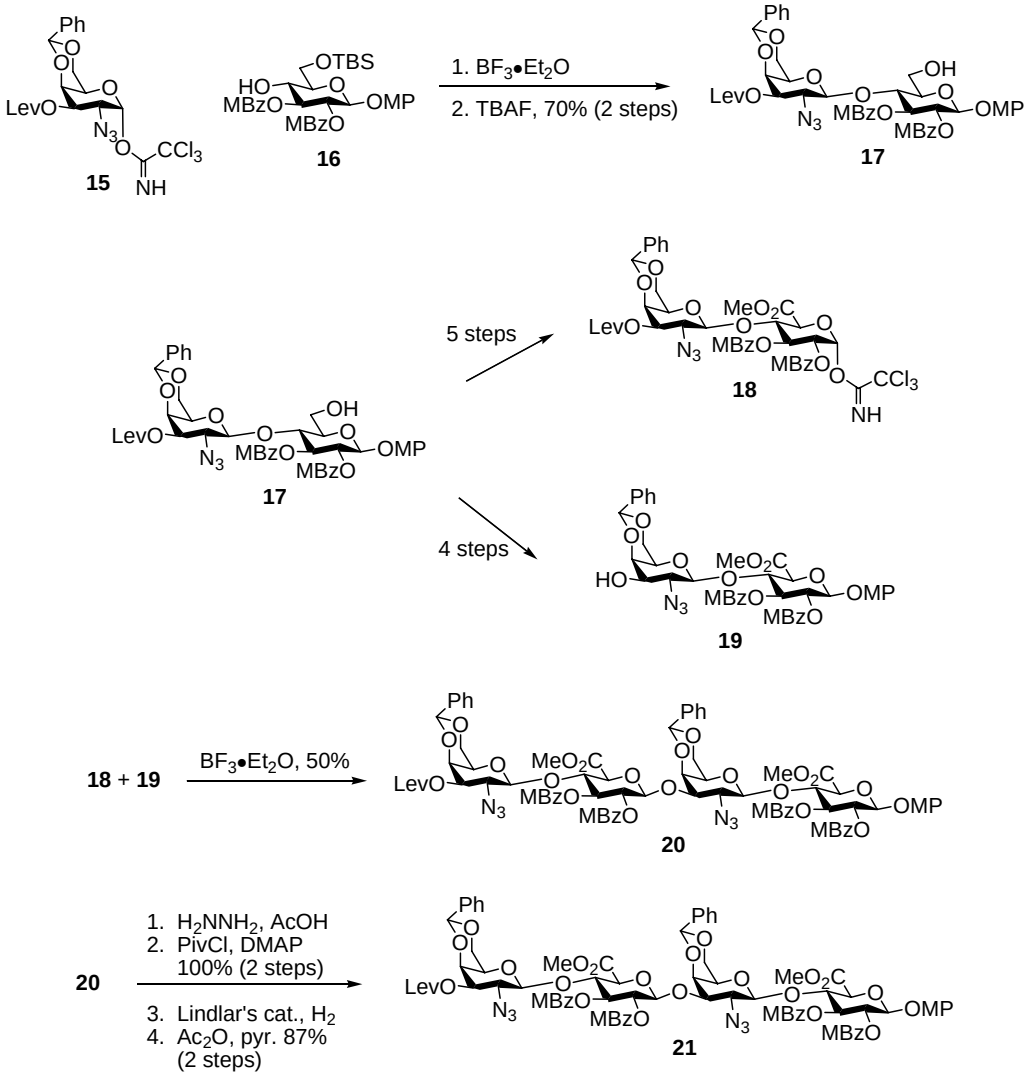
To conclude the synthesis, disaccharides **9** and **10** were elaborated to the C-4 and C-6 deprotected saccharides **11** and **12**, respectively. These disaccharides were sulfated before simultaneous hydrogenolysis of the benzyl groups and reduction of the azides. The resulting amines were acetylated 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

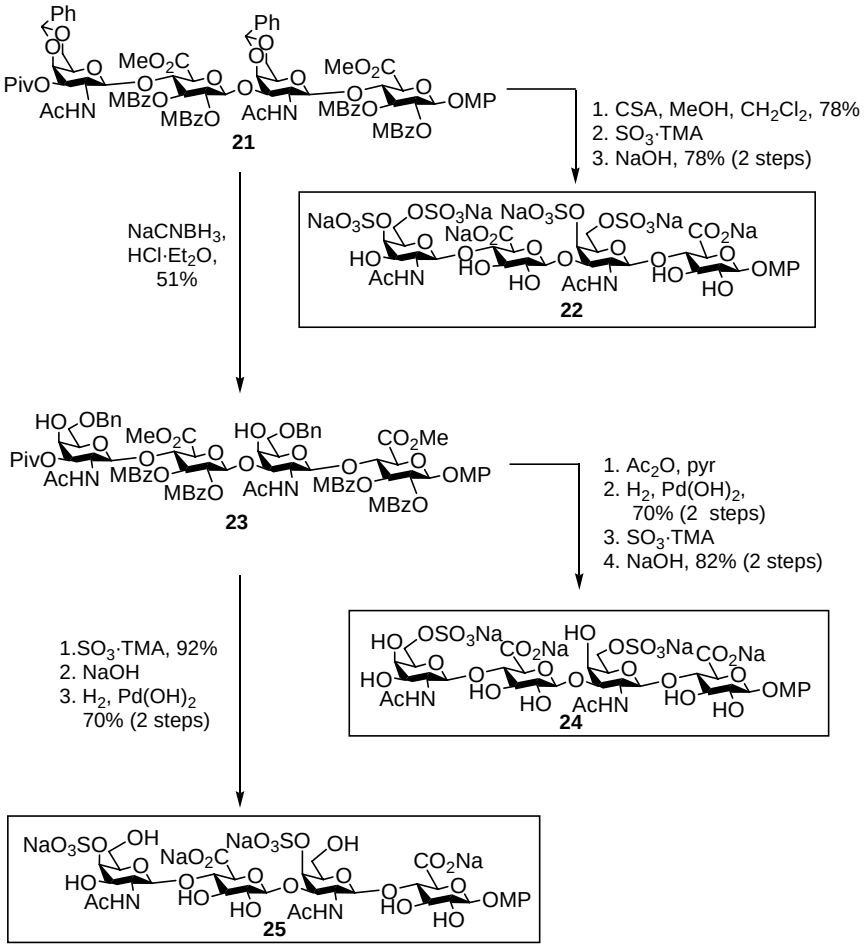
The Ogawa laboratory has completed the synthesis of CS-A, CS-C, and CS-E tetrasaccharides. As in the Jacquinet synthesis, monosaccharides **15** and **16** were coupled using Schmidt's imidate methodology and then desilated to produce disaccharide **17**. This disaccharide was then elaborated to both 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

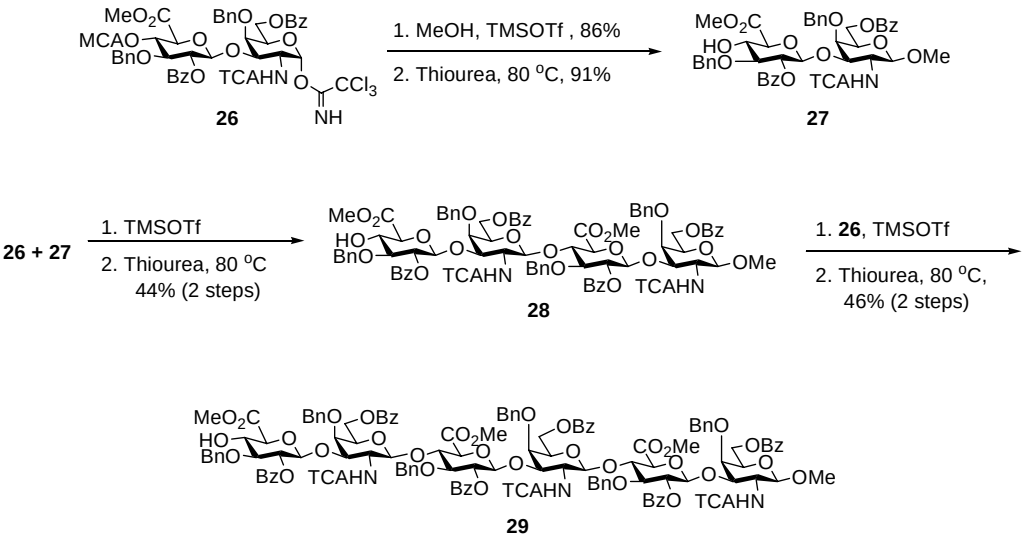
This synthesis was noteworthy for a number of reasons: This synthesis employs a divergent strategy, allowing access to 3 tetrasaccharides from a core disaccharide. Furthermore, it reaffirms the usefulness of trichloroacetimidate donors for coupling both mono- and disaccharides. In addition, this synthesis 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

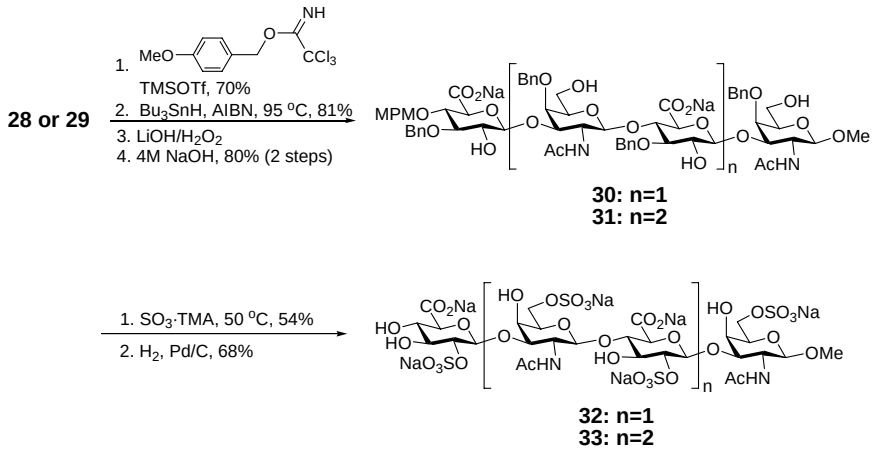
coupling conditions with donor **26** and, after chloroacetate deprotection with thiourea, afforded hexasaccharide **29** (Scheme 3.5)**.**



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

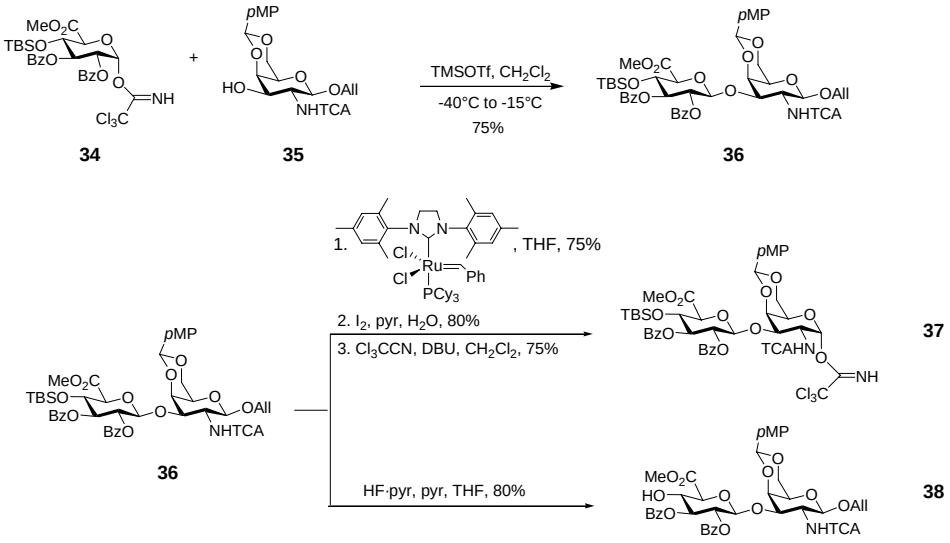
difficulty of sulfating the glucosyl C-2 hydroxyl allowed us to be aware of strong sulfation conditions that would be needed to affect this transformation.



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

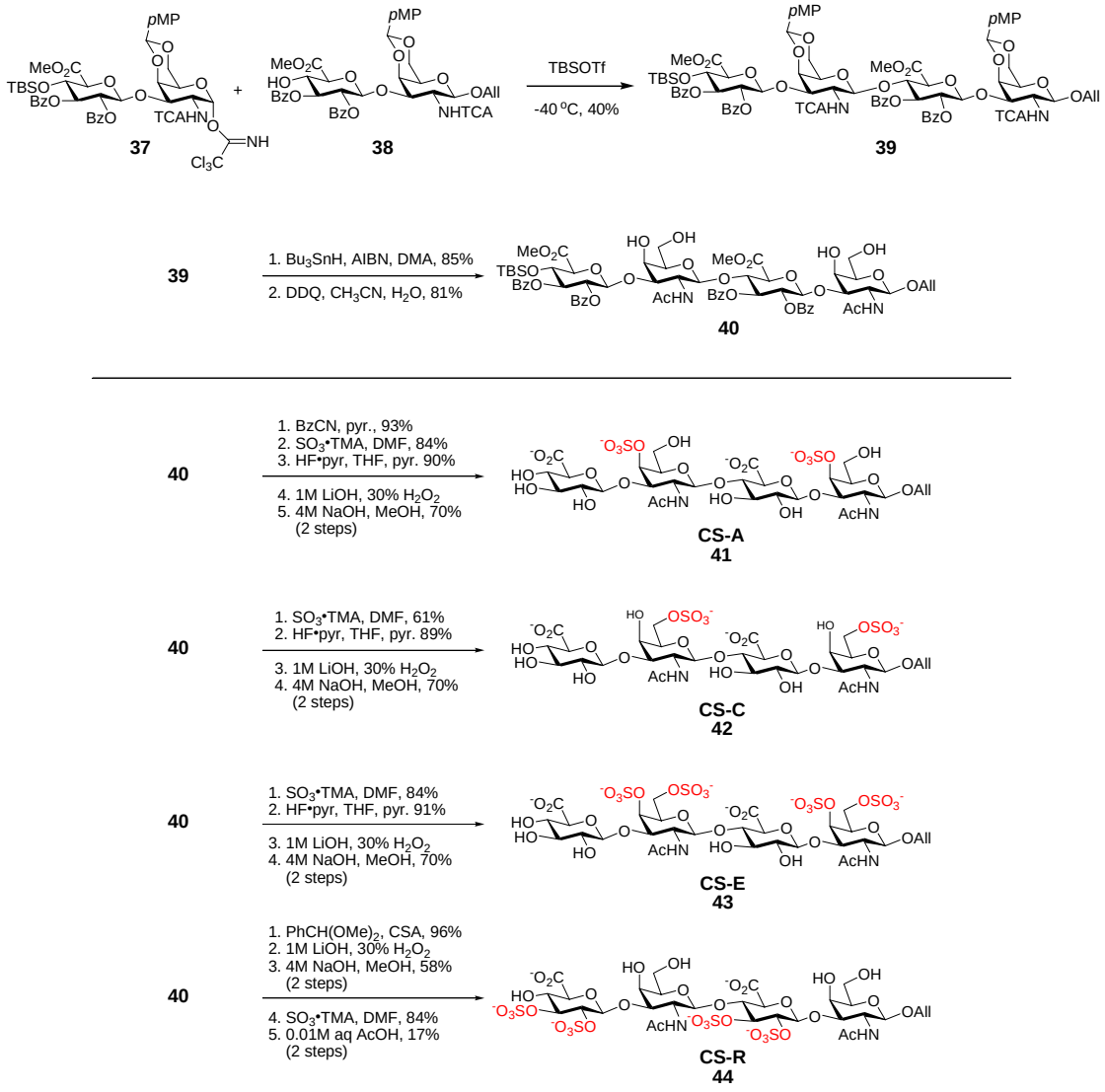
The greatest source of information came from our laboratory's experience synthesizing a library of CS-A, CS-C, CS-E, and CS-R tetrasaccharides. This synthesis converges at a core disaccharide and tetrasaccharide from which all four sulfation patterns are accessed. A TMSOTf-mediated coupling of imidate 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]

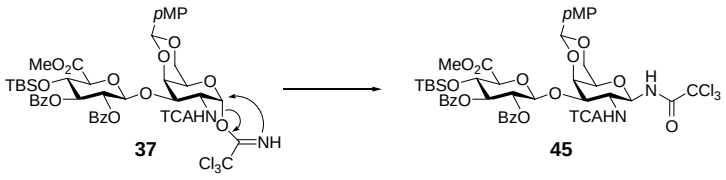


Figure 3.4: Donor **37** can undergo rearrangement to amide **45**.

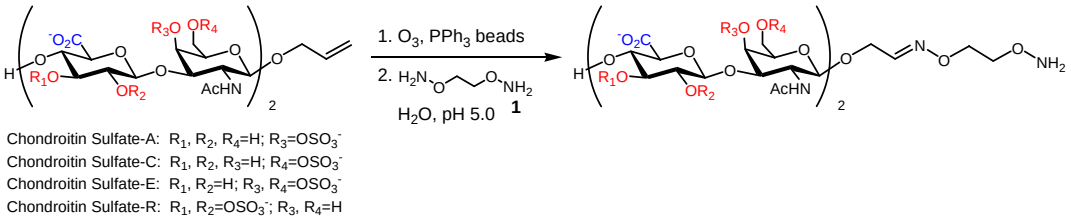
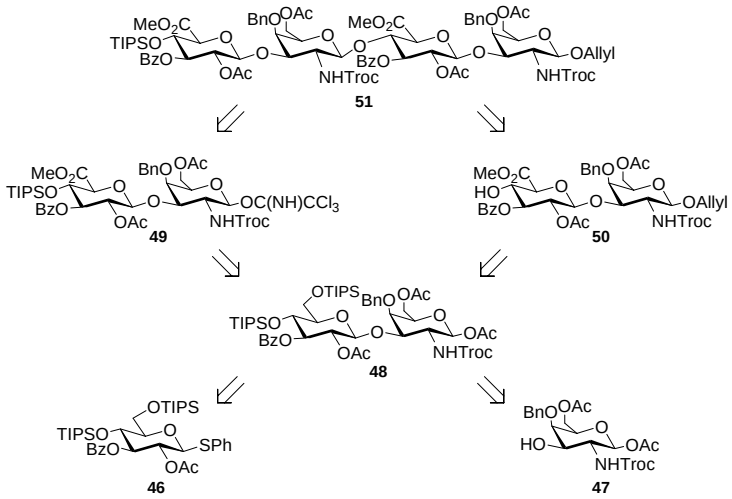


Figure 3.5: The allyl group can be used as a chemical handle for attaching tetrasaccharides to surfaces or other molecules.

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).

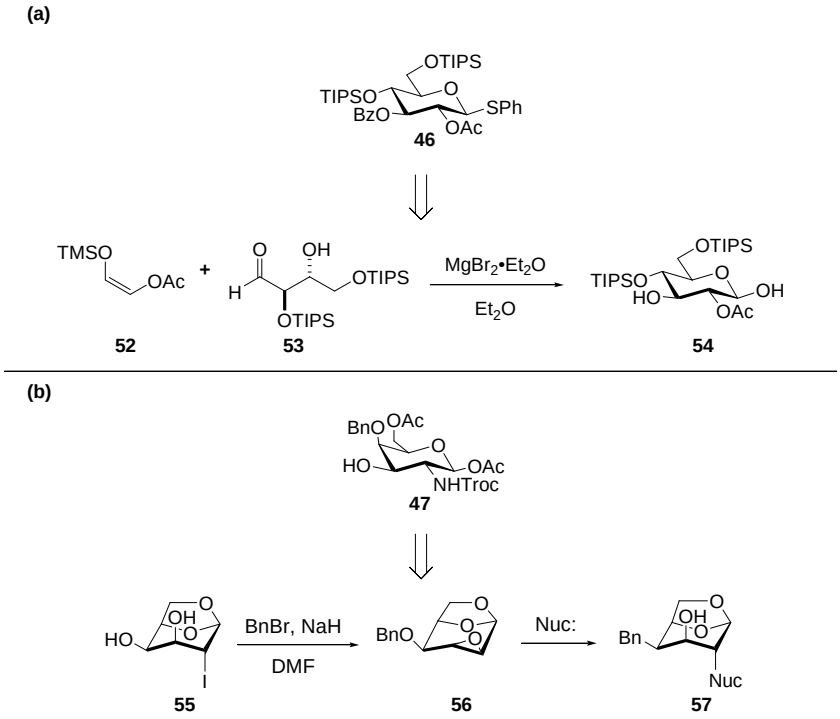
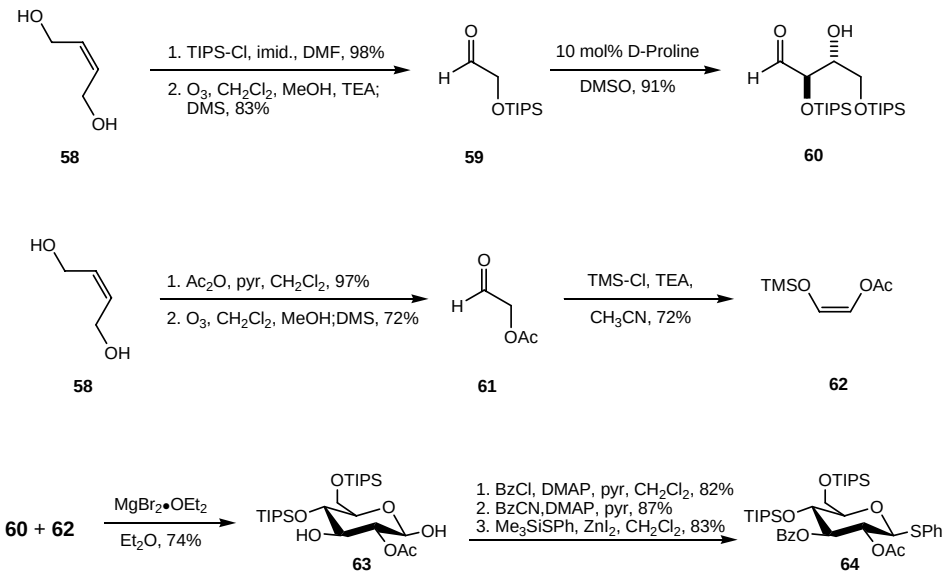


Figure 3.6: (a) Glucosyl monomer **46** can be accessed using an aldol methodology. (b) Galactosamine monomer **47** can be accessed through a Cerny epoxide.

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.

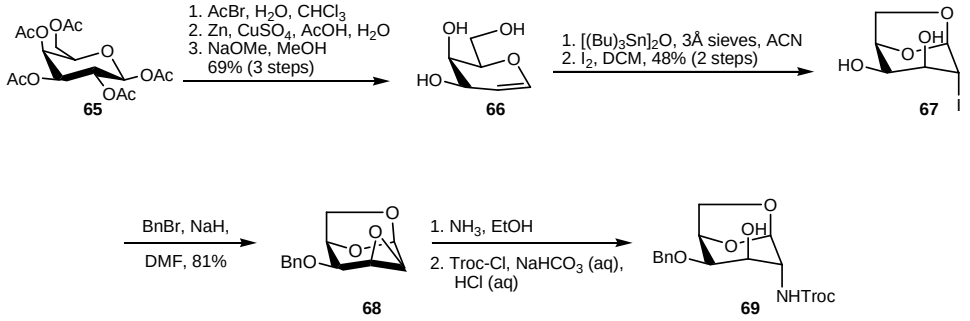
Synthesis of the CS Disaccharide

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 $MgBr_2 \bullet OEt_2$-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).



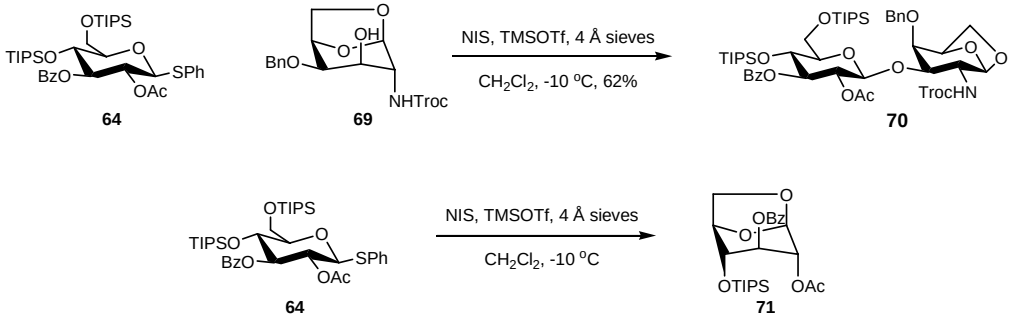
Scheme 3.10: Synthesis of glucosyl donor **64**

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).



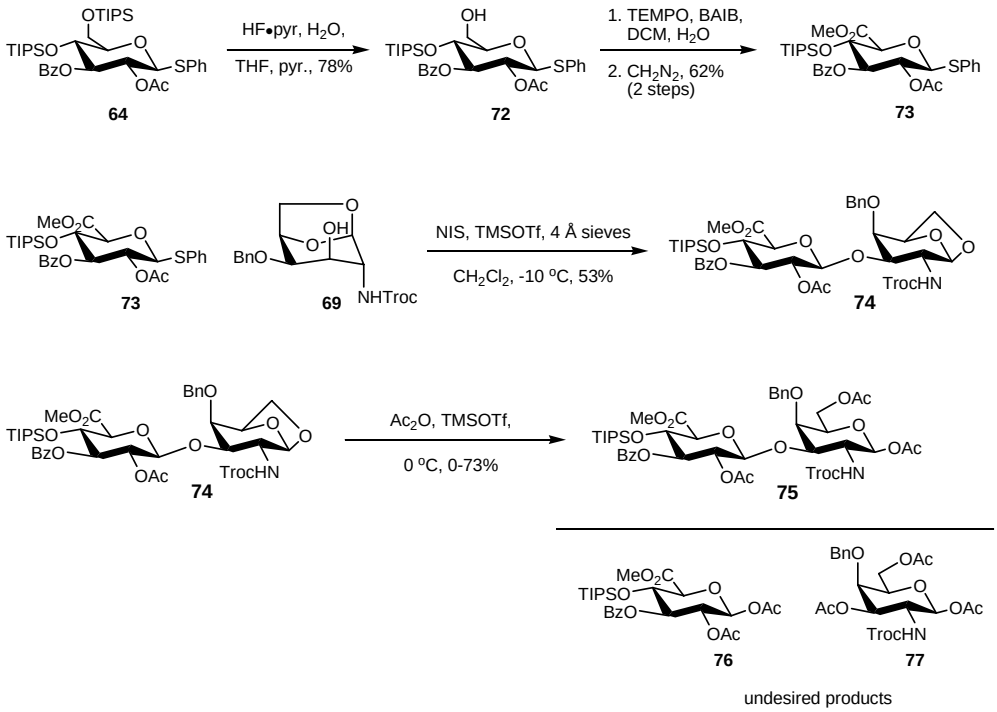
Scheme 3.11: Synthesis of galactosamine acceptor **69**

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).



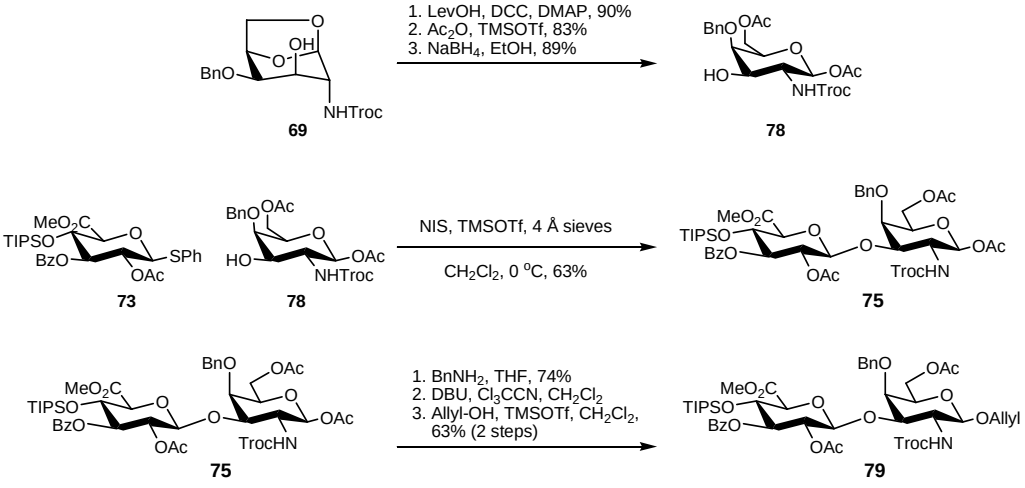
Scheme 3.12: Formation of disaccharide **70**

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

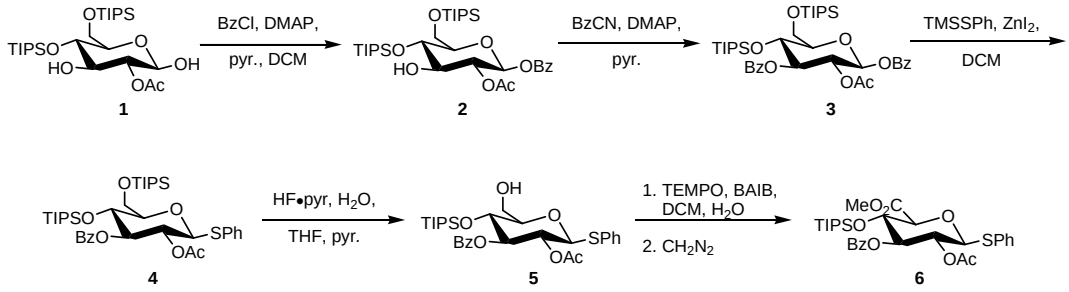
1100 Series chromatographs using a Chiralcel OD-H column (25 cm) and OD-H guard (5 cm) as noted. Liquid chromatography mass spectrometry analysis was performed on an Aglient 1100 series LCMS with an acetonitrile/water mobile phase treated with 0.1% acetic acid with a quadrapole detector. Thin-layer chromatography (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, $CHCl_3$).

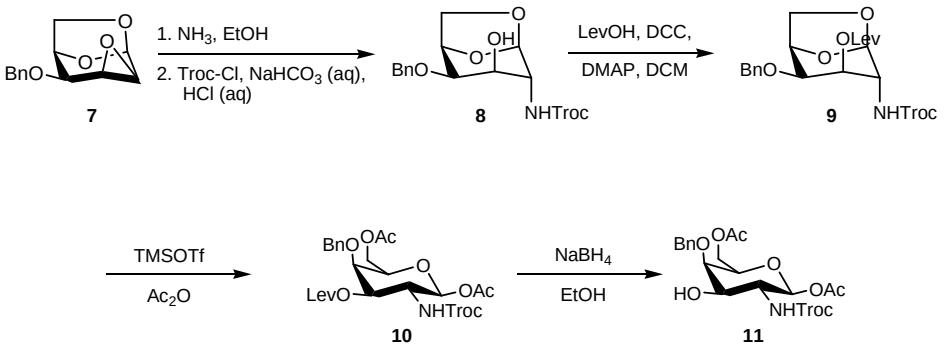
(3) In an oven-dried flask, compound **2** (1.19g, 1.86 mmol) was dissolved in pyridine (25 mL) and cooled to 0 °C. Dimethylaminopyridine (16 mg, 0.1 mmol) and benzoyl cyanide (1.32 g, 10.1 mmol) were added and the reaction was allowed to warm to room temperature. The reaction was stirred overnight. 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) $CO_2C\mathbf{H}_3$, 1.96 (s, 3H) $C(O)C\mathbf{H}_3$, 0.96 (m, 21H) $C\mathbf{H}(C\mathbf{H}_3)_2$. ^{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 **16** (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,

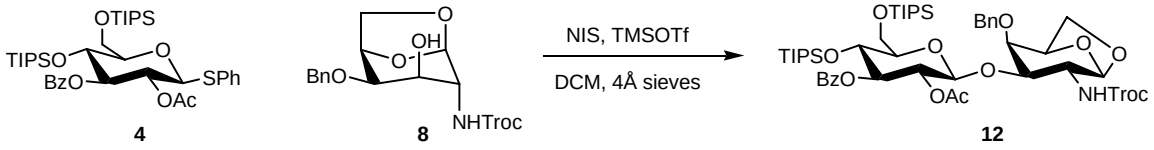
J = 20.7 Hz, C**H**$_2$Ph, 4.61 (d, 1H, J = 11.7 Hz) C**H**$_2$CCl$_3$, 4.43 (d, 1H J = 11.7 Hz) C**H**$_2$CCl$_3$, 4.41 (m, 2H) **H**-4, **H**-5, 3.94 (m, 1H) **H**-6, 3.74 (m, 2H) **H**-2, **H**-6; 2.79-2.64 (m, 4H) C**H**$_2$C**H**$_2$COCH$_3$, 2.16 (s, 3H) CH$_2$CH$_2$COC**H**$_3$. ^{13}C NMR (75 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$_8$NCl$_3$) 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, $CHCl_3$).

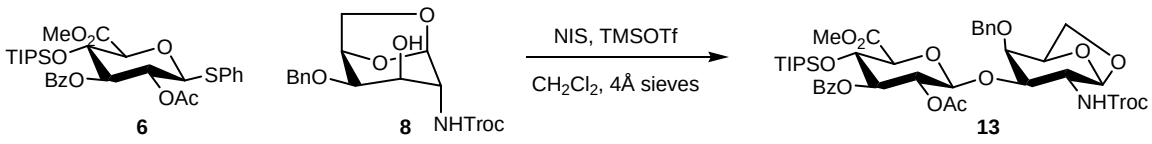
(11) To a solution of **10** (30 mg, 0.048 mmol) in ethanol (0.5 mL) was added sodium borohydride (2 mg, 0.06 mmol). The reaction was stirred for 30 minutes and then partitioned between ethyl acetate (3 mL) and water (1 mL). The organic layer was washed with brine (1 mL), dried over sodium sulfate, and 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



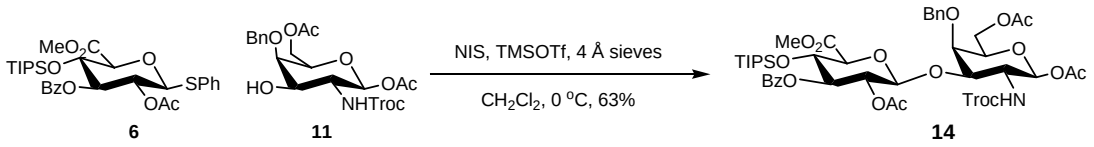
(12) Donor **4** (300 mg, 0.41 mmol) and acceptor **8** (175 mg, 0.41 mmol) were azeotroped three times with toluene placed under vacuum for 8 hours. They were then added as a solution in dichloromethane (2 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves and dichloromethane (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

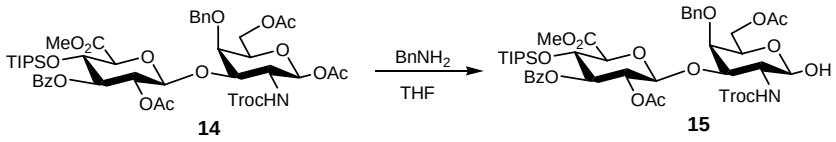
$Na_2S_2O_3$ (aq.) and 0.25 mL pyridine) and agitated until the red color changed to yellow. The reaction was partitioned between ethyl acetate (5 mL) and saturated aqueous sodium thiosulfate (2 mL). The aqueous layers were extracted with ethyl acetate (2x5 mL), and the organics were combined and washed 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)C**H**$_3$, 1.07 (m, 21H) C**H**$(CH_3)_2$. ^{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

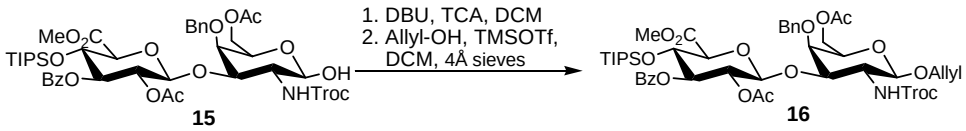
allowed to stir for 30 minutes before N-iodosuccinimide (52 mg, 0.23 mmol) was added under argon, and the reaction was allowed to stir for an additional 15 minutes. The reaction was then cooled to -40 °C. A solution of trimethylsilyl triflate in dichloromethane (0.6 mL, 0.05 M) was cooled to -78 °C and 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.



14 **15**

(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) $COC\mathbf{H}_3$, 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* 1040.3, found *m/z* 1042.1.

References

1. (a) Northrup, A. B.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 6798. (b) Northrup, A. B.; Mangion, I. K.; Hettche, F.; MacMillan, D. W. C. *Angew. Chem. Int. Ed.* **2004**, *43*, 2152. (c) Northrup, A. B.; MacMillan, D. W. C. *Science* 2004, *305*, 1753.
2. (a) Gama, C. I. and Hsieh-Wilson, L. C. *Curr. Op. Chem. Biol.* **2005**, *9*, 609. (b) Ernst, B.; Hart, G. W.; Sinay, P.; Eds. *Carbohydrates in Chemistry and Biology*, Wiley-VCH: New York, 2000.
3. (a) Sugahara, K. and Kitagawa, H. *Curr. Opin. Struct. Biol.* **2000**, *10*, 518. (b) Kursche-Gullberg, M. and Kjellen, L. *Curr. Opin. Struct. Biol.* **2003** *5*, 605.
4. (a) Rauch, U.; Feng, K.; Zhou, X.-H. *Cell. Mol. Life. Sci.* **2001**, *58*, 1842. (b) Kitagawa, H.; Tsutsumi, K.; Tone, Y.; Sugahara, K. *J. Biol. Chem.* **1997**, *272*, 50, 31377.
5. (a) Herman, T.; Hartwieg, E.; Horvitz, H. R. *Proc. Natl Acad. Sci. USA* **1999**, 96, 968. (b) Hwang, H.-Y.; Horvitz, H. R. *Proc. Natl Acad. Sci. USA* **2002**, 99, 14218. (c) Hwang, H.-Y.; Olson, S. K.; Esko, J. D.; Horvitz, H. R. *Nature*, **2003**, *423*, 439.
6. (a) Kluppel, M.; Wight, T.N.; Chan, C.; Hinek, A.; Wrana, J.L. *Development* **2005**, *132*, 3989. (b) Thiele, H.; Sakano, M.; Kitagawa, H.; Sugahara, K.; Rajab, A.; Hohne, W.; Ritter, H.; Leschik, G.; Nurnberg, P.; Mundlos, S. *Proc. Natl. Acad. Sci. USA,* **2004**, *101,* 10155.

7. (a) Yang, J.; Price, M.A.; Neudauer, C.L.; Wilson, C.; Ferrone, S.; Xia, H.; Iida, J.; Simpson, M.A.; McCarthy, J.B. *J. Cell Biol.* **2004**, *165*, 881. (b) Heredia, A.; Villena, J.; Romaris, M.; Molist, A.; Bassols, A. *Arch. Biochem. Biophys.* **1996**, *333*, 198. (c) Lu, H.; Yu, G.; Sun, L.; Zhang. Z.; Zhao, X.; Chai, W. *Oncology* **2007**, *72*, 347. (d) Pothacharoen, P.; Ong-Chai, S.; Supaphun, J.; Kumia, P.; Wanaphirak, C.; Sugahara, K.; Hardingham, T.; Kongtawelert, P. *J. Biochem. (Tokyo)* **2006**, *140*, 517. (e) Theocharis, A. D.; Tsara, M. E.; Papageorgacopoulou, N.; Karavias, D. D.; Theocharis, D. A. *Biochim. Biophys. Acta* **2000**, *1502*, 2, 201. (f) Theocharis, A. D.; Vynios, D. H.; Papageorgakopoulou, N.; Skandalis, S. S.; Theocharis, D. A. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 3, 376.

8. Nadanaka, S.; Clement, A.; Masayama, K.; Faissner, A.; Sugahara, K. *J. Biol. Chem.* **1998**, *278*, 6, 3296.

9. Bradbury, E.J.; Moon, L.D.; Popat, R.J.; King, V.R.; Bennett, G.S.; Patel, P.N.; Fawcett, J.W.; McMahon, S.B. *Nature*, **2002**, *416*, 636.

10. (a) Tully, S. E.; Mabon, R.; Gama, C. I.; Tsai, S. M.; Liu, X.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2004**, *126*, 7736. (b) Gama, C. I.; Tully, S. E.; Sotogaku, N.; Clark, P. M.; Rawat, M.; Vaidehi, N.; Goddard, W. A.; Nishi, A.; Hsieh-Wilson, L. C. *Nature Chem. Biol.* **2006**, *2*, 9, 467.

11. Marra, A.; Dong, X.; Petitou, M.; Sinay, P. *Carbohydr. Res.* **1989**, *195*, 39.

12. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.

13. Jacquinet, J. C. *Carbohydr. Res.* **1990**, *199*, 153.

14. (a) Tamura, J.; Neumann, K. W.; Kurono, S.; Ogawa, T. *Carbohydr. Res.* **1997**, *305*, 43. (b) Tamura, J.; Neumann, K. W.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1351.

15. Karst, N.; Jacquinet, J. C. *Eur. J. Org. Chem.* **2002**, 815.

16. Tully, S. E. *Synthesis and Biological Activity of Chondroitin Sulfate Biopolymers*, **2007**, Ph. D. Thesis, California Institute of Technology.

17. (a) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 2, 251. (b) Xue, J.; Khaja, S. D.; Locke, R. D.; Matta, K. L. *Synlett* **2004**, *5*, 861. (c) Yan, F.; Mehta, S.; Eichler, E.; Wakarchuk, W. W.; Gilbert, M.; Schur, M. J.; Whitfield, D. W. *J. Org. Chem.* **2003**, *68*, 2752.

18. Tully, S. E.; Rawat, M.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2006**, *128*, 24, 7740.

19. (a) Rucker, C. *Chem. Rev.*, **1995**, *95*, 1009. (b) Eisneberg, C.; Knochel, P. *J. Org. Chem.* **1985**, *50*, 4021. (c) Knight, D. W; Shaw, D.; Fenton, G. *Synlett*, **1994**, 295.

20. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, 3rd Ed.* Wiley: New York, 1999.

21. Egusa, K.; Kusumoto, S.; Fukase, K. *Eur. J. Org. Chem.* **2003**, *17*, 3435.

22. (a) Takatani, M.; Matsuo, I.; Yukishige, I. *Carbohydr. Res.* **2003**, *10*, 1, 1073. (b) Fukase, K.; Matsumoto, T.; Ito, N.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 10, 2643. (c) Brard, M.; Richter, W.; Benvegnu, T.; Plusquellec, D. *J. Am. Chem. Soc.* **2004**, *126*, 10003. (d) Stepowska, H.; Zamojski, A. *Pol. J. Chem.* **1994**, *68*, 11, 2229.

23. Arndt, S.; Hsieh-Wilson, L. C. *Org. Lett.* **2003**, *5*, 4197.

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.

25. (a) Kozikowski, A. P.; Lee, J. *J. Org. Chem.* **1990**, *55*, 3, 863. (b) Koto, S.; Asami, K.; Hirooka, M.; Nagura, K.; Takizawa, M.; Yamamoto, S.; Okamoto, N.; Mitsuko, S.; Tajima, H.; Yoshida, T.; Nonaka, N.; Sato, T.; Zen, S.; Yago, K.; Tomonaga, F. *Bull. Chem. Soc. J.* **1999**, *72*, 4, 765.

26. Leteux, C.; Veyrieres, A. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2647.

27. Cerny, M.; Julakova, O.; Pacak, J. *Coll. Czech. Chem. Comm.* **1974**, *39*, 5. 1391.

28. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.

29. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals;* 3rd ed. Pergamon Press, Oxford, 1988

30. W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978,** *43*, 2923.

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.

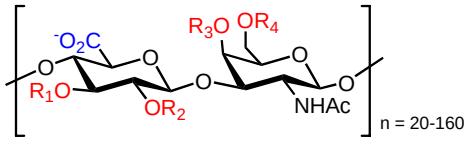
32. Figures in this section were modified from Tully, S. E. *Synthesis and Biological Activity of Chondroitin Sulfate Biopolymers*, **2007**, Ph. D. Thesis, California Institute of Technology.

Chapter 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).

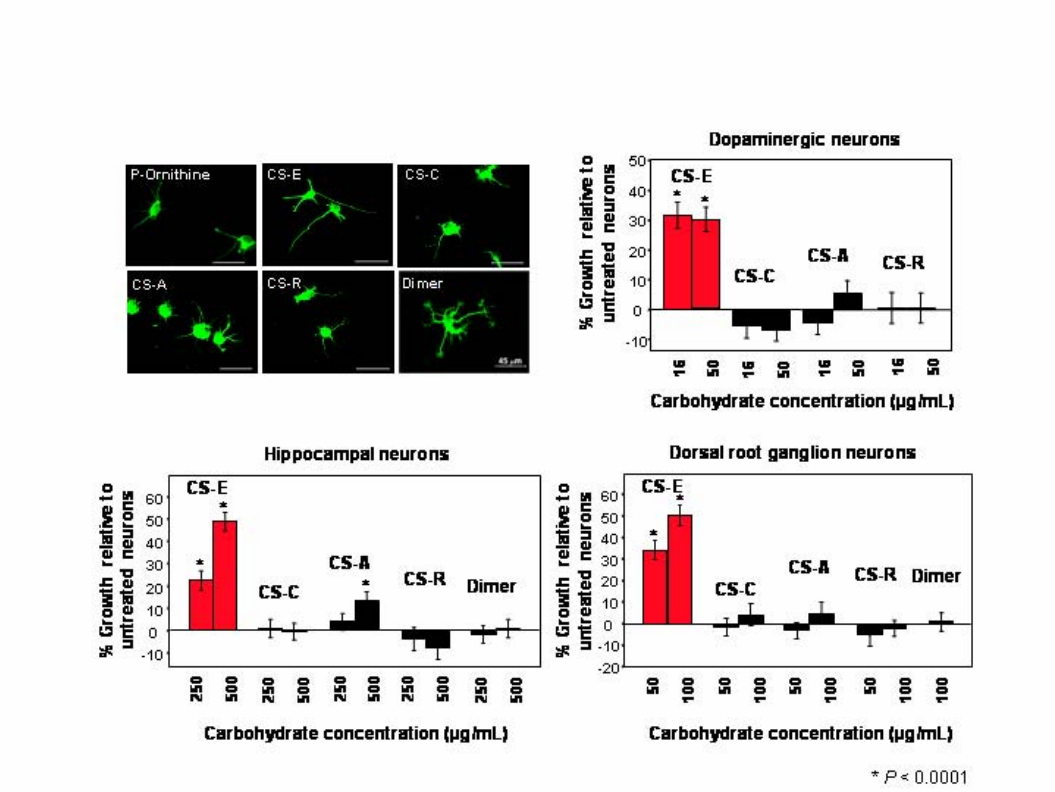


Figure 4.2: CS-E stimulated neurite outgrowth in dopaminergic, hippocampal, and dorsal root ganglion neurons.

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]

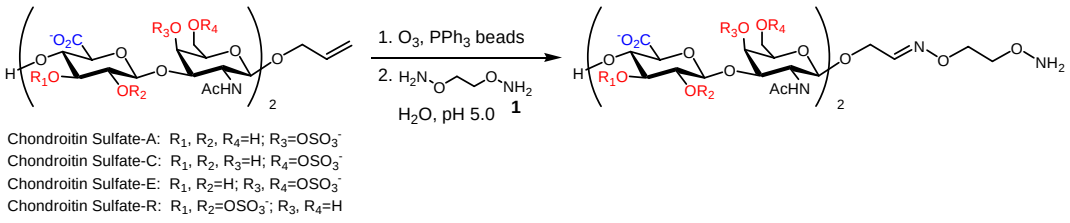


Figure 4.3: The allyl group can be used as a chemical handle for attaching tetrasaccharides to surfaces or other molecules.

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.

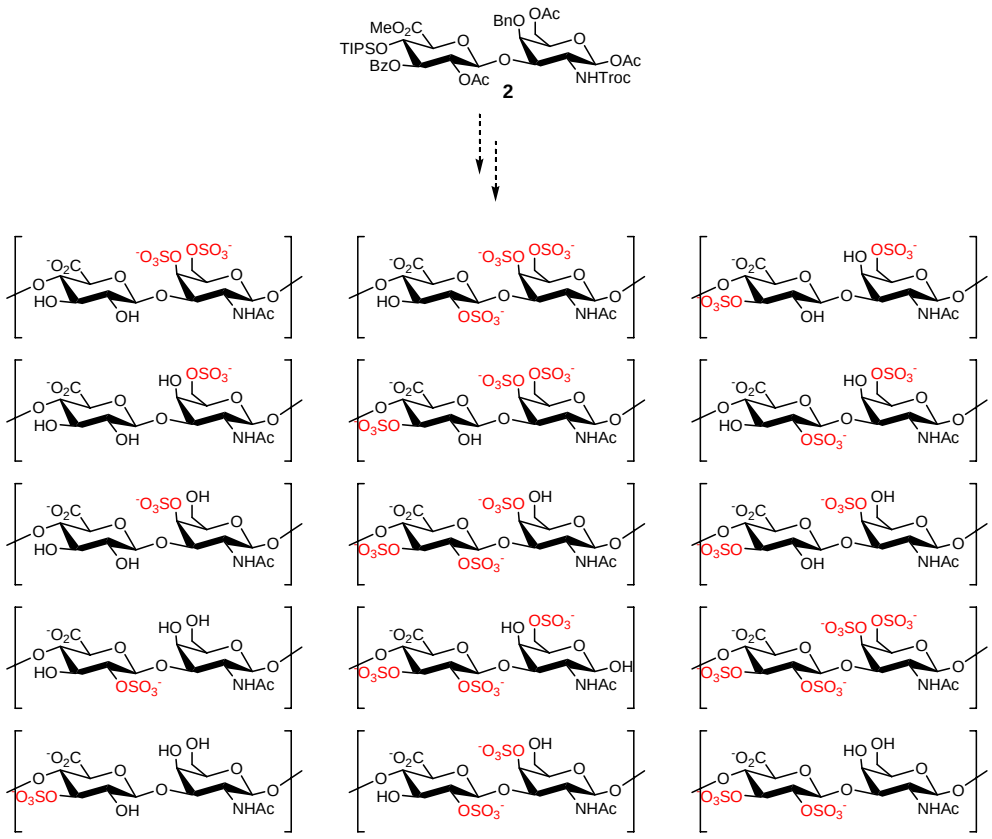


Figure 4.4: It was envisioned that a core disaccharide such as **2** could allow access to all possible sulfation patterns.

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]

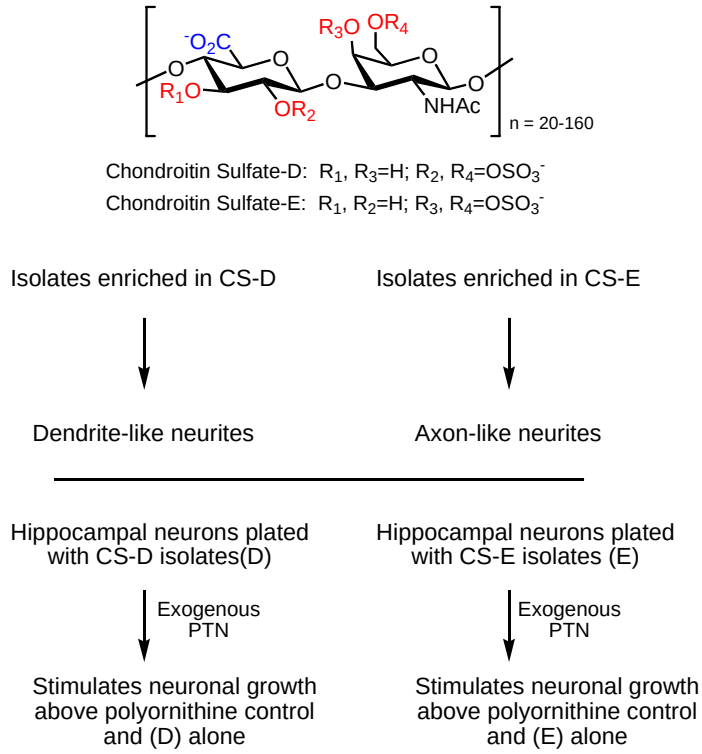


Figure 4.5: CS-D is believed to stimulate the growth of dendrites through the binding of pleiotrophin (PTN).

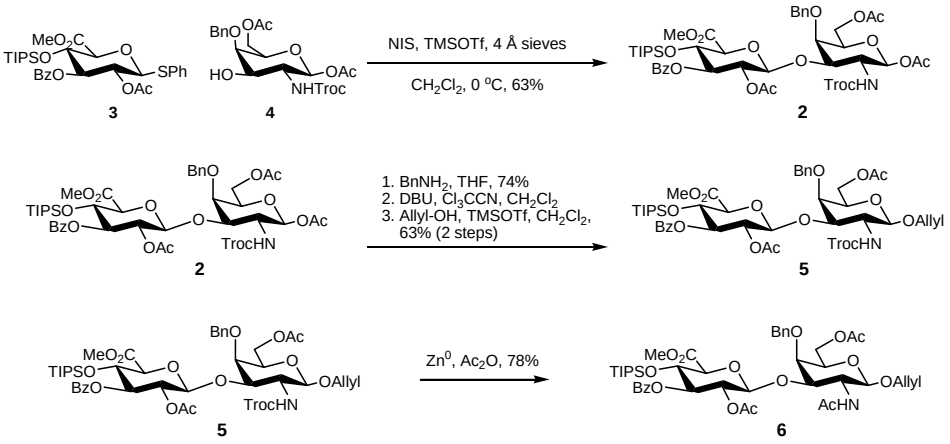
Studies with enriched CS-K isolates suggest it blocks retinoic acid receptor (RAR) signaling required for neuritogenic differentiation. Specifically, it was determined that as the production of CS-K increases, neurite outgrowth in LA-N-5 cells (a neuroblastoma cell line) decreases. In addition, there 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.

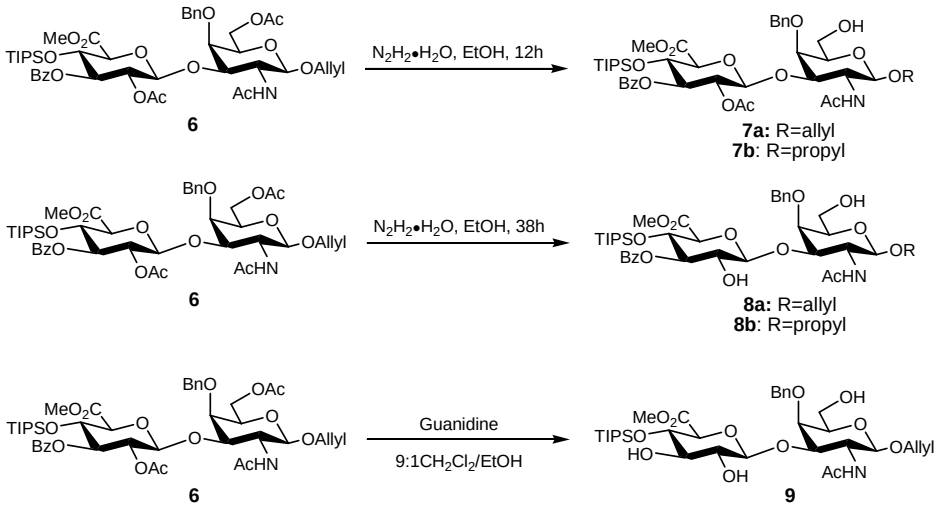
Testing the Disaccharide Protecting Group Strategy

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]



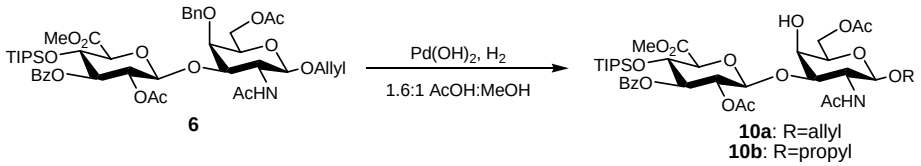
Scheme 4.1: Core disaccharide **2** was elaborated to form *N*-acetyl disaccharide **6**.

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.



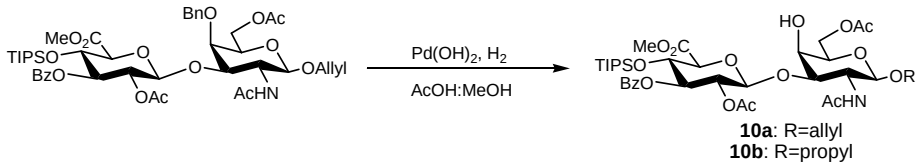
Scheme 4.2: Removal of the galactosamine C-6, glucosyl C-2, and glucosyl C-3 esters

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.

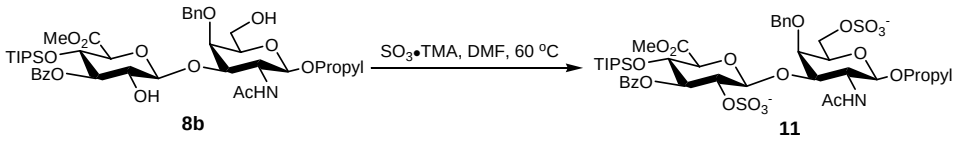
The final group to be evaluated was the C-4 benzyl ether. While we were confident we could remove the benzyl group in the presence of the ester and silyl protecting groups, we were hopeful that we could remove it without reducing the allyl group. There have been reports in the literature where benzyl 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.

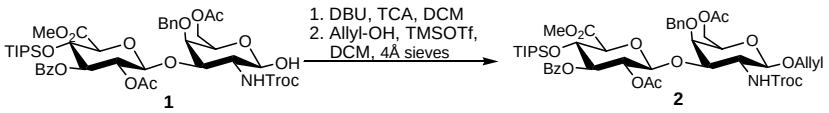
Supporting Information

General Methods

Unless stated otherwise, reactions were performed in oven-dried glassware that had been cooled in a desiccator over Drierite. 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).

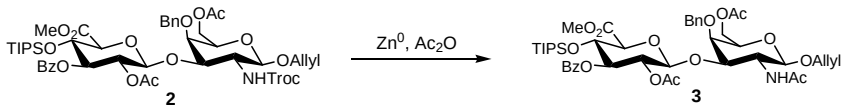
Synthesis of the Disaccharides



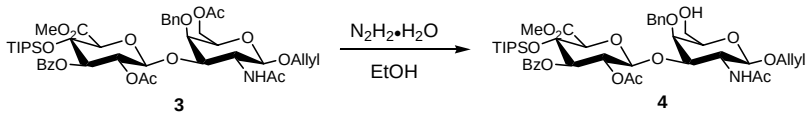
(2) Compound **1** (194 mg, 0.2 mmol) was azeotroped three times with toluene and placed under vacuum for 8 hours before being added to a flame-dried flask with flame-activated 4Å molecular sieves as a solution in dichloromethane (3.5 mL). The reaction mixture was allowed to stir for 15 minutes before 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 **1** (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.

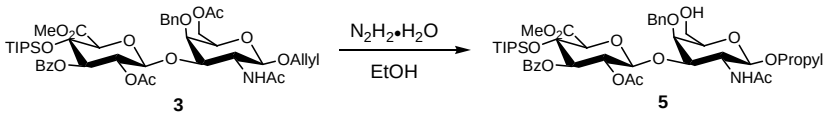


(3) Compound **2** (52 mg, 0.05 mmol) was stirred with activated zinc dust (13.4 mg, 20.4 mmol) in acetic anhydride (1 mL) for 12 hours, and the reaction was diluted with ethyl acetate (5 mL), filtered over celite, and the mother liquor was washed with saturated aqueous sodium bicarbonate (3x3 mL) and 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) $CH_2CHC\mathbf{H}_2O$, 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.



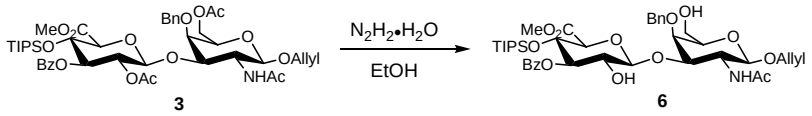
3 **4**

(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.



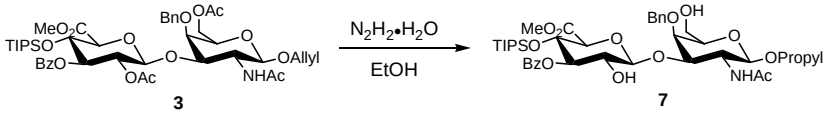
3 5

(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.



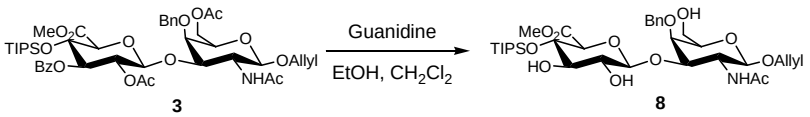
3 **6**

(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.



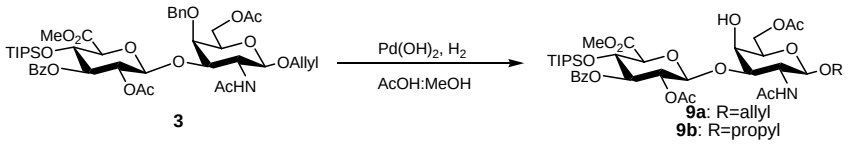
3 **7**

(28) Compound **27** (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 **28** (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**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.



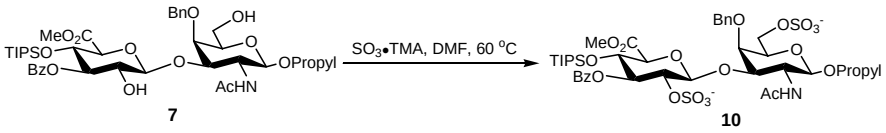
3 8

(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

(7) Compound **10** (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.

References

1. (a) Gama, C. I. and Hsieh-Wilson, L. C. *Curr. Op. Chem. Biol.* **2005**, *9*, 609. (b) Ernst, B.; Hart, G. W.; Sinay, P.; Eds. *Carbohydrates in Chemistry and Biology*, Wiley-VCH: New York, 2000.
2. For examples of these studies, see (a) Nadanaka, S.; Clement, A.; Masayama, K.; Faissner, A.; Sugahara, K. *J. Biol. Chem.* **1998**, *278*, 6, 3296. (b) Bao, X.; Mikami, T.; Yamada, S.; Faissner, A.; Muramatsu, T.; Sugahara, K. *J. Biol. Chem.* **2005**, *280*, 10, 9180.
3. (a) Tully, S. E.; Mabon, R.; Gama, C. I.; Tsai, S. M.; Liu, X.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2004**, *126*, 7736. (b) Gama, C. I.; Tully, S. E.; Sotogaku, N.; Clark, P. M.; Rawat, M.; Vaidehi, N.; Goddard, W. A.; Nishi, A.; Hsieh-Wilson, L. C. *Nature Chem. Biol.* **2006**, *2*, 9, 467.
4. Figure modified from Tully, S. E. *Synthesis and Biological Activity of Chondroitin Sulfate Biopolymers*, **2007**, Ph. D. Thesis, California Institute of Technology.
5. (a) Bao, X.; Mikami, T.; Yamada, S.; Faissner, A.; Muramatsu, T.; Sugahara, K. *J. Biol. Chem.* **2005**, *280*, 10, 9180. (b) Faissner, A.; Clement, A.; Lochter, A.; Streit, A.; Mandl, C.; Schachner, M. *J. Cell Biol.* **1994**, *126*, 783. (c) Hikino, M.; Mikami, T.; Faissner, A.; Vilela-Silva, A. C.; Pavao, M. S.; Sugahara, K. *J. Cell Biol.* **2003**,

278, 43744. (d) Maeda, N.; Fukazawa, N.; Nata, T. *J. Biol. Chem.* **2006**, *281*, 8, 4894.

6. (a) Dey, N.; De, P. K.; Wang, M.; Zhang, H.; Dobrota, E. A.; Robertson, K. A.; Durden, D. L. *Mol. Cell. Biol.* **2007**, *27*, 11, 4179. (b) Fongmoon, D.; Shetty, A. K.; Basappa, Yamada, S.; Sugiura, M.; Kongtawelert, P.; Sugahara, K. *J. Biol. Chem.* **2007**, *282*, 51, 36895.
7. (a) Kinoshita, A.; Yamada, S.; Haslam, S. M.; Morris, H. R.; Dell, A.; Sugahara, K. *Biochemistry*, **2001**, *40*, 12654. (b) Ueoka, C.; Kaneda, N.; Okazaki, I.; Nadanaka, S.; Muramatsu, T.; Sugahara, K. *J. Biol. Chem.* **2000**, *275*, 48, 37407.
8. (a) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135. (b) Saha, U.K., Schmidt, R.R. *J. Chem. Soc., Perkin Trans 1* **1997**, 1855.
9. Furst, A.; Berlo, R. C.; Hooton, S. *Chem. Rev.* **1965**, *65*, 1, 51.
10. For examples, see (a) Li, J.; Wang, Y. *Syn. Comm.* **2004**, *34*, 2, 211. (b) Compostella, F.; Ronchi, S.; Panza, L.; Mariotti, S.; Mori, L.; De Libero, G.; Ronchetti, F. *Chem. Eur. J.* **2006**, *12*, 21, 5587. (c) Xia, C.; Zhou, D.; Liu, C.; Lou, Y.; Yao, Q.; Zhang, W.; Wang, P. G. *Org. Lett.* **2006**, *8*, 24, 5493.
11. (a) Kunesch, N.; Meit, C.; Poisson, J. *Tetrahedron Lett.* **1987**, *28*, 3569. (b) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, 3rd Ed.* Wiley: New York, 1999.
12. 22. (a) Takatani, M.; Matsuo, I.; Yukishige, I. *Carbohydr. Res.* **2003**, *10*, 1, 1073. (b) Fukase, K.; Matsumoto, T.; Ito, N.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 10, 2643. (c) Brard, M.; Richter, W.; Benvegnu,

T.; Plusquellec, D. *J. Am. Chem. Soc.* **2004**, *126*, 10003. (d) Stepowska, H.; Zamojski, A. *Pol. J. Chem.* **1994**, *68*, 11, 2229.

13. Noti, C.; de Paz, J. L.; Polito, L.; Seeberger, P. H. *Chem. Eur. J.* **2006**, *12*, 8664.
14. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.
15. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals;* 3rd ed. Pergamon Press; Oxford, 1988
16. Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978,** *43*, 2923.

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.

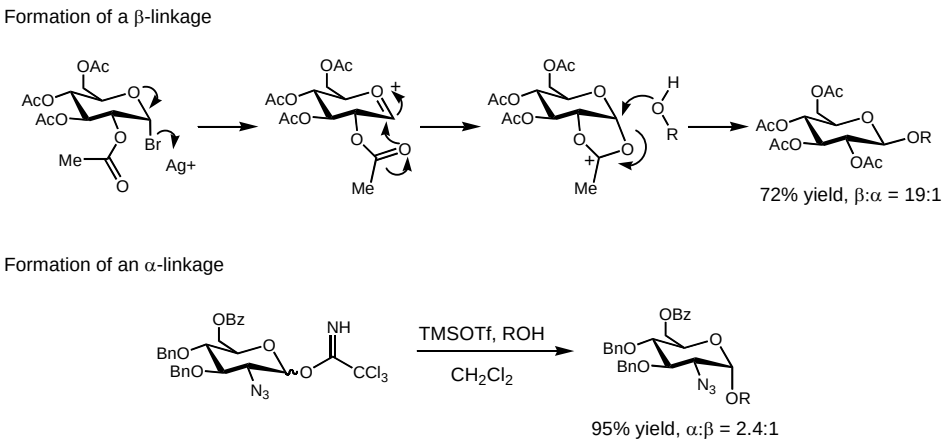


Figure 5.1: Participating groups can be used to direct the α:β ratio of coupling products.

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]

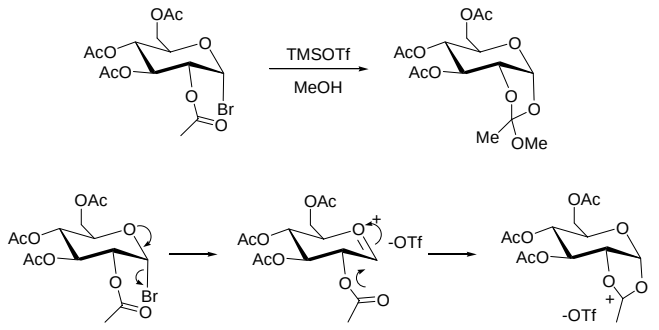


Figure 5.2: Orthoesters are formed when the nucleophile attacks the participating group instead of the anomeric position.

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]

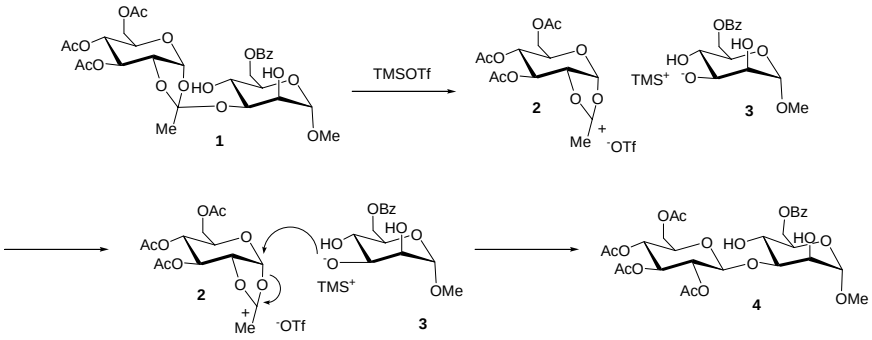


Figure 5.3: Proposed mechanism for orthoester rearrangement

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

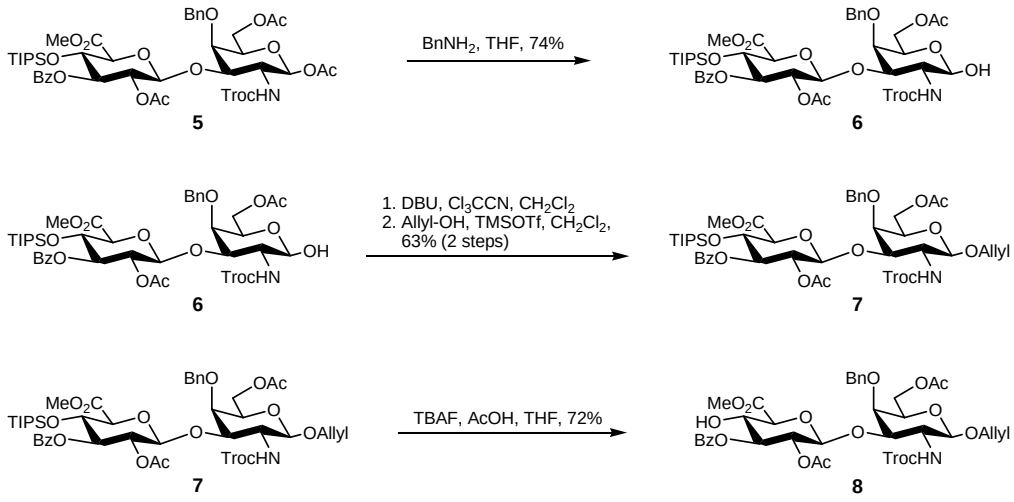
most reactive hydroxyl available on the acceptor.[5] The primary hydroxyl (6-OH) is considered the most reactive position on the acceptor, and it usually follows that the 3-OH is more reactive than the 2-OH, which is more reactive than the 4-OH.

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.

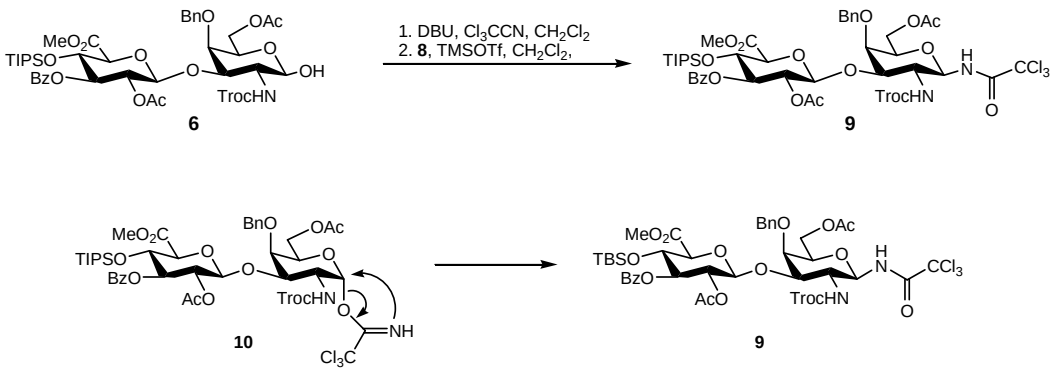
The classic glycosylation method is the Koenigs-Knorr reaction[7], which refers to a substitution reaction of a glycosyl halide with the acceptor alcohol to produce the new saccharide (Figure 5.1). Many other leaving groups have been introduced, such as the use of thioglycosides[8] and phosphates.[9] 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).

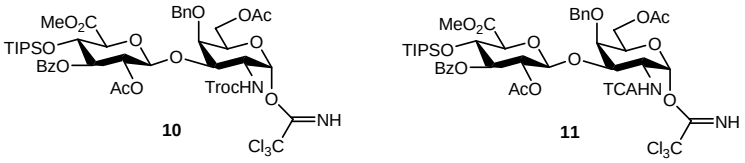
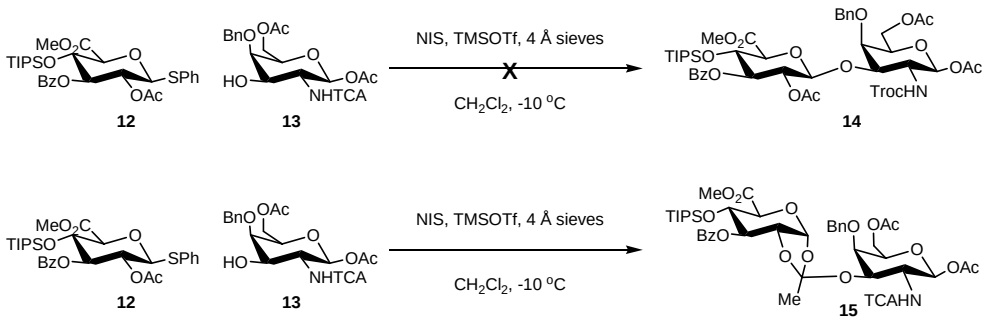


Figure 5.4: Model studies suggest that exchanging the Troc protecting group for a TCA protecting group minimizes rearrangement of the imidate.

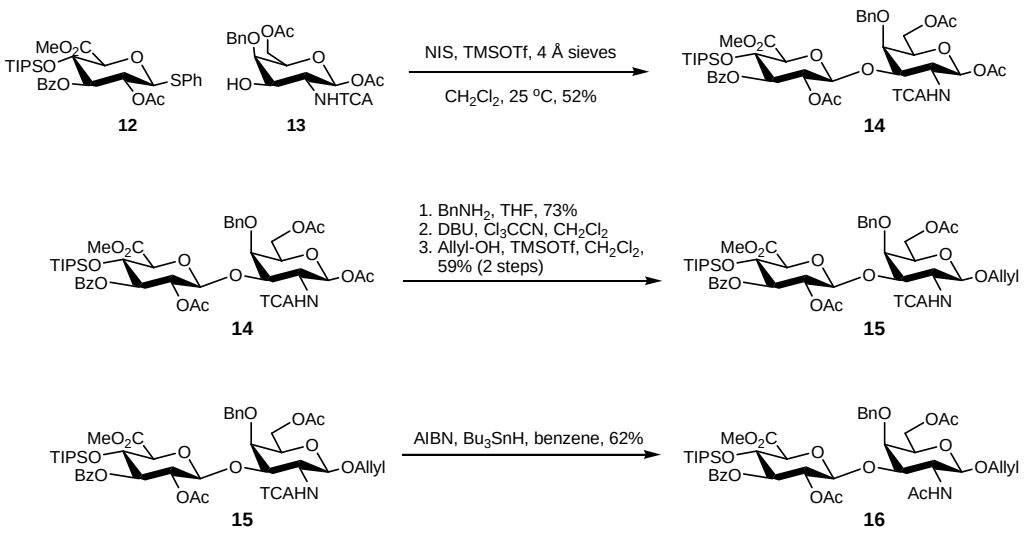
Because of these results, we decided to change the amine protecting group from a Troc group to a TCA group. To make core disaccharide **14**, we synthesized acceptor **13**[12]

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 ^{o}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.

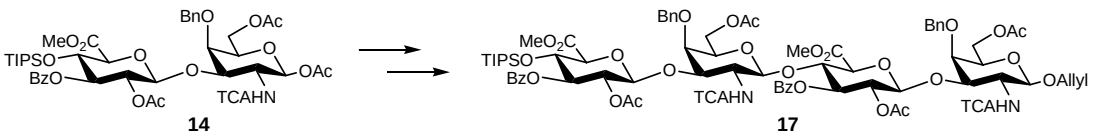


Figure 5.5: Core disaccharide **14** has been elaborated to tetrasaccharide **17**.

Conclusions

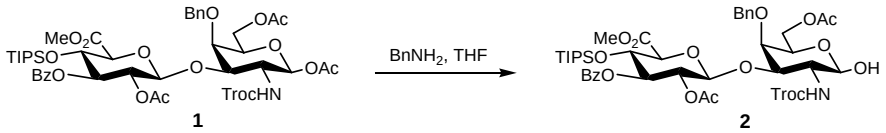
The choice of protecting groups can have a profound effect on glycosylation reactions. When attempting to couple donor **8** and acceptor **10**, a deleterious rearrangement of the donor occurred. To minimize this rearrangement, the galactosamine C-2 amine protecting group was changed from a Troc protecting 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

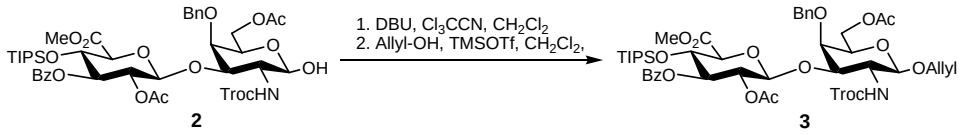
Unless stated otherwise, reactions were performed in oven-dried glassware that had been cooled in a desiccator over Dririte. Reactions were performed under an argon environment unless otherwise stated. All solvents were purified using the method of Grubbs.[13] Unless stated otherwise, all commercially 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).



1 2

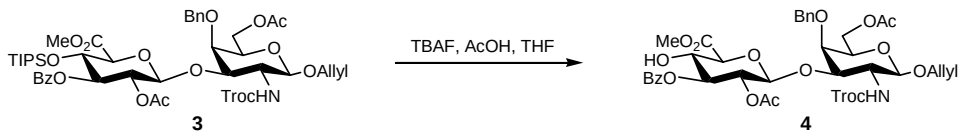
(1) To a solution of compound **2** (33 mg, 0.03 mmol) in THF (0.3 mL) was added benzylamine (0.01 mL, 0.1 mmol). The solution was stirred for 7 hours, and the reaction was quenched by the addition of ethyl acetate (5 mL) and poured into an extraction funnel. The organic layer was washed with water (2x1 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_2CH_3, 2.62 (bs, 1H) O**H**, 1.92 (s, 3H) C(O)C**H**3, 1.85 (s, 3H) C(O)CH_3, 0.89-0.84 (m, 21H) C**H**(CH_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.)

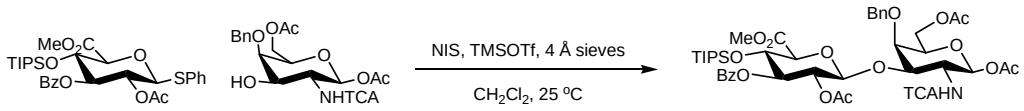
The imidate was added as a solution in dichloromethane (5 mL) to a flame-dried flask containing flame-activated 4Å molecular sieves. Freshly distilled allyl alcohol (0.12 mL, 1.75 mmol) was added, and the reaction mixture was stirred at room temperature for 15 minutes before being cooled to -78 °C for 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) $C\mathbf{H}_2CCl_3$, 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) $CH_2CHC\mathbf{H}_2O$, 4.12-4.05 (m, 3H) Glu **H**-1, $CH_2CHC\mathbf{H}_2O$, 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.



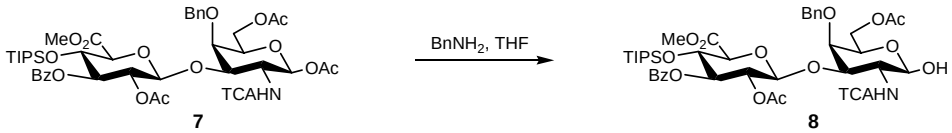
3 **4**

(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

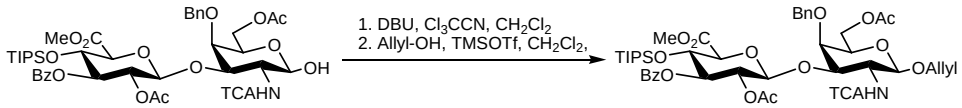
allowed to stir for 5 hours, at which time more TMSOTf (1 M solution in dichloromethane, 0.3 mL) was added. The reaction mixture was allowed to stir for an additional 17 hours. Upon completion, the reaction was diluted with ethyl acetate (30 mL) and washed with saturated sodium bicarbonate (2 x 10 mL), 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}, ^1H 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_2CH_3, 2.23 (s, 3H) C(O)C**H**$_3$, 2.06 (s, 3H) C(O)C**H**$_3$, 1.99 (s, 3H) C(O)C**H**$_3$, 1.02-0.90 (m, 21H) C**H**(C**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.



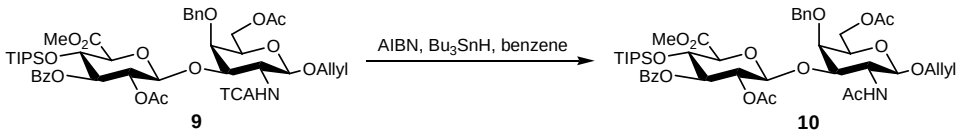
8 **9**

(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 ^oC 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. ^1H 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) $C\mathbf{H_2}CHCH_2O$, 5.17 (m, 1H) $C\mathbf{H_2}CHCH_2O$, 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) $C\mathbf{H_2}Ph$, 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**H**$_2$Ph, 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_2CHCH_2O, 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) $Si(CH(CH_3)_2)$. ^{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.

References

1. Ernst, B.; Hart, G. W.; Sinay, P.; Eds. *Carbohydrates in Chemistry and Biology*, Wiley-VCH: New York, 2000.
2. For a review of orthoester chemistry, see Kong, F. *Carbohydr. Res.* **2007**, *342*, 345.
3. (a) Ning, J.; Zhang, W.; Yi, Y.; Yang, G.; Wu, Z.; Yi, J.; Kong, F. *Bioorg. Med. Chem.* **2003**, *11*, 2193. (b) Zeng, Y.; Ning, J.; Kong, F. *Tetrahedron Lett.* **2002,** 3729**.**
4. Wang, W.; Kong, F. *Angew. Chem., Int. Ed.* **1999**, *38*,1247.
5. (a) Kanie, O.; Hindsgaul, O. *Curr. Opin. Struct. Biol.* **1992**, *2*, 674. (b) Becker, B.; Furneaux, R. H.; Reck, F.; Zubkov, O. A. *Carbohydr. Res.* **1999**, 315, 148. (c) Kaur, K. J.; Hindsgaul, O. *Glycoconjugate J.* **1991**, *8*, 90. (d) Figueroa-Perez, S.; Verez-Bencomo, V. J. *Carbohydr. Chem.* **1998**, *17*, 851. (e) Wang, W.; Kong, F. *J. Org. Chem.* **1998**, *63*, 5744 (f) Wang, W.; Kong, F. *Tetrahedron Lett.* **1998**,1937.
6. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, 3rd Ed.* Wiley: New York, 1999.
7. Koenigs, W.; Knorr, E. *Ber. Disch. Chem. Ges.* **1901**, *34*, 957.
8. Codee, J. D.C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Soc. Rev.* **2005**, *34*, 769.
9. Vankayalapati, H.; Jiang, S.; Singh, G. *Synlett*, **2002**, 16-25.
10. Schmidt, R. R. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.
11. (a) Jacquinet, J. C. *Carbohydr. Res.* **1990**, *199*, 153. (b) Tamura, J.; Neumann, K. W.; Kurono, S.; Ogawa, T. *Carbohydr. Res.* **1997**, *305*, 43. (c) Tamura, J.;

Neumann, K. W.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1351. (d) Karst, N.; Jacquinet, J. C. *Eur. J. Org. Chem.* **2002**, 815. (e) Tully, S. E.; Mabon, R.; Gama, C. I.; Tsai, S. M.; Liu, X.; Hsieh-Wilson, L. C. *J. Am. Chem. Soc.* **2004**, *126*, 7736.

12. Lee. S.-G.; Hsieh-Wilson, L. C. unpublished results.
13. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.
14. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals;* 3rd ed. Pergamon Press; Oxford, 1988
15. Still, W. C.; Kahn, M.; Mitra, A. J. *J. Org. Chem.* **1978,** *43*, 2923.
16. This work was performed in the Hsieh-Wilson laboratory with Dr. Song-Gil Lee and Ms. Maria Chiriac.