The Synthesis of Sulfated Carbohydrates Using a Sulfate Protecting Group Strategy

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Sulfated carbohydrates play key roles in a wide range of biological processes such as blood clotting, viral entry into cells, amyloidogenesis, neurite outgrowth, tumor growth and metastasis. However, their synthesis still remains a considerable challenge. A general approach to the synthesis of sulfated carbohydrates was examined in which sulfate group is incorporated at the beginning of the syntheses as a protected sulfodiester. Towards this end, a series of sulfuryl imidazolium salts (SIS), a new class of sulfating agents, was prepared and examined as reagents for incorporating 2,2,2-trichloroethyl-protected sulfate esters into monosaccharides. The SIS that contained a 1,2-dimethylimidazolium moiety proved to be a superior sulfating agent compared to SIS bearing no alkyl groups or bulkier alkyl groups on the imidazolium ring. Difficult *O*- and *N*- sulfations that required prolonged reaction times and a large excess of the SIS bearing a 1-methylimidazolium group were achieved in high yield and in less time when employing less than half the 1,2-dimethylimidazolium derivative. Efforts were then made to apply the sulfate protecting group strategy to the total synthesis of a class of chondroitin sulfate glycosaminoglycans. These studies revealed some of the limitations of the sulfate protecting group approach to the synthesis of sulfated oligosaccharides. Studies on the selective introduction and isomerization of the carbobenzyloxy protecting group into 2,3-diols of 4,6-O-benzylidene galactose derivatives are also reported.

Acknowledgements

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List of Abbreviations

Ac	acetyl
AIBN	2,2'-azobisisobutyronitrile
Ar	aryl
AT-III	antithrombin III
BAIB	bis-acetoxyiodobenzene (iodobenzene diacetate)
Bn	benzyl
BSP	1-benzenesulfinyl piperidine
Bz	benzoyl
CAN	cerium ammonium nitrate
Cbz	benzyloxycarbonyl
CS	chondroitin sulfate
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-*N*,*N*-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
eq	equivalents
ESI	electrospray ionization
Et	ethyl
GAG	glycosaminoglycan
Gal	galactose
GalNAc	*N*-Acetylgalactosamine
Glc	glucose
h	hour
HCC	human hepatocellular carcinoma
HRMS	high resolution mass spectrometry
Im	imidazole
m	multiplet
MCA	monochloroacetate
Me	methyl
MP	4-methoxyphenyl

NAP	2-napthylmethyl
NBS	N-bromosuccinimide
NIS	N-iodosuccinimide
NMR	nuclear magnetic resonance
PG	protecting group
Ph	phenyl
Phth	phthaloyl
Piv	pivaloyl
PMB	p-methoxybenzyl
py	pyridine
rt	room temperature
s	singlet
SAR	structure activity relationship
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBS	*tert*-butyldimethylsilyl
TCE	2,2,2-trichloroethyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFE	trifluoroethyl
THF	tetrahydrofuran
TMS	trimethylsilyl
Troc	2,2,2-trichloroethylcarbonyl
TTBP	tri-*tert*-butylpyrimidine

Chapter 1 - Introduction

1.1 Sulfated Carbohydrates

One of the unique features of carbohydrates is the limitless number of distinct structures that can be obtained from combining individual monosaccharide building blocks. Oligosaccharides are naturally assembled from monosaccharides in pyranose and furanose configurations, with different types of linkages, at varying positions in the carbohydrate ring, forming straight chain or branched polymers. Further modifications such as alkylation, phosphorylation, and sulfation provide additional structural complexity to the already diverse compounds.

The high degree of variability among biologically relevant sulfated carbohydrates is attributed to a number of factors. There is variability with respect to monosaccharides which are sulfated (*i.e.* glucose (Glc), galactose (Gal), the corresponding *N*-acetyl (NAc) amines GlcNAc, GalNAc, and mannose (Man) among others); the total number of sulfate moieties, and hydroxyl(s) to which the sulfate group(s) are linked (*i.e.* 2-*O*, 3-*O*, 4-*O*, 6-*O* sulfates, and any resulting combination from multiple sulfations); and also the varied structure of the underlying oligosaccharide moiety. An important consequence of this structural diversity is that each unique structure has the potential to be recognized by an individual receptor, making sulfated oligosaccharides ideal for carrying information in complex biological systems.

Sulfated carbohydrates are of extreme biological importance. The oligosaccharides play key roles in modulating bioactive proteins and peptides, and thus

are responsible for controlling many physiological events. Despite their widespread occurrence, a clear comprehension of the specific roles of these compounds is limited. Research to develop an understanding of the molecular-level function of many different oligosaccharides is hampered, mainly because these compounds are isolated as complex mixtures from natural sources, and the chemical synthesis of pure, well defined sulfated oligosaccharide fragments is extremely challenging.

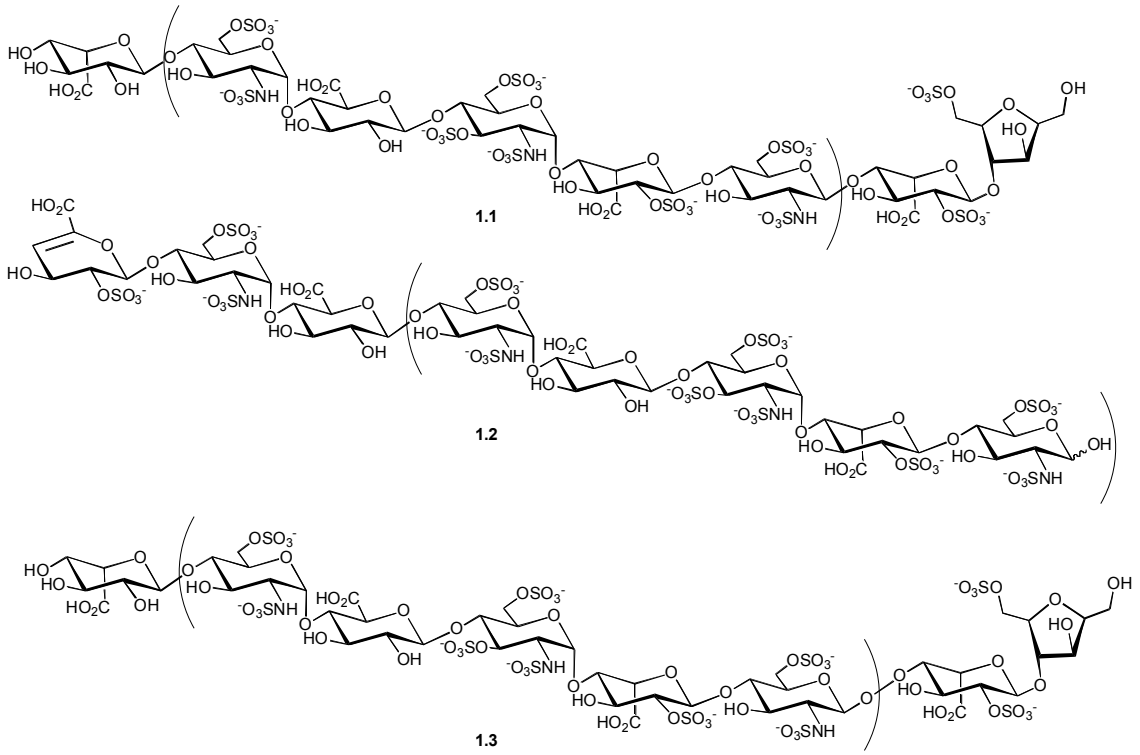


Figure 1.1. Typical fragments from heparin degradation.[1]

Some of the most well-known sulfated carbohydrates are heparin oligosaccharides (Figure 1.1).[1] Heparin is a highly sulfated glycosaminoglycan that has been clinically applied as an injected anticoagulant-antithrombotic agent since the early 1940's. It is one

of the oldest pharmaceutical drugs still currently in use, and is the only sulfated carbohydrate-based drug on the market.

The negatively charged, unbranched carbohydrate polymers were originally isolated from animal tissues as complex mixtures of sulfated oligosaccharides. Degradation of naturally occurring heparin yields a wide variety of oligosaccharides that consist predominantly of α-(1,4)-linked L-iduronic acid and N-, O-di-sulfated glucosamine residues (**1.1** – **1.3** Figure 1.1). Heparin binds and activates antithrombin III (AT-III), a serine protease inhibitor that blocks thrombin and factor Xa in the blood coagulation cascade.[2] In clinical use, natural heparin has several drawbacks, including a very short half life ($t_{1/2}$ = 1 h) and a lack of selectivity, which can lead to abnormal bleeding in patients.[3]

In the early 1980's, it was discovered that the interaction between heparin and AT-III was mediated by a unique pentasaccharide sequence (denoted in parenthesis in Figure 1.1, **1.4** in Figure 1.2), now called the antithrombin III binding domain (ABD).[4] The discovery and characterization of the active pentasaccharide **1.4** lead to a widespread drug development program lasting more than 20 years. The primary goals of this program were to establish a specific heparin structure-function relationship and create synthetic AT-III inhibitors with the heparin pentasaccharide **1.4** as a vantage point.[1]

Research efforts eventually lead to the synthesis of active heparin analogs such as pentasaccharide **1.5**, first synthesized in over 60 steps with low yield and purity, and its stabilized methyl glycoside **1.6** in over 55 synthetic steps. These pentasaccharides contain

an N-sulfate group in place of the N-acetyl group on the non-reducing end of the pentasaccharide.[5,6]

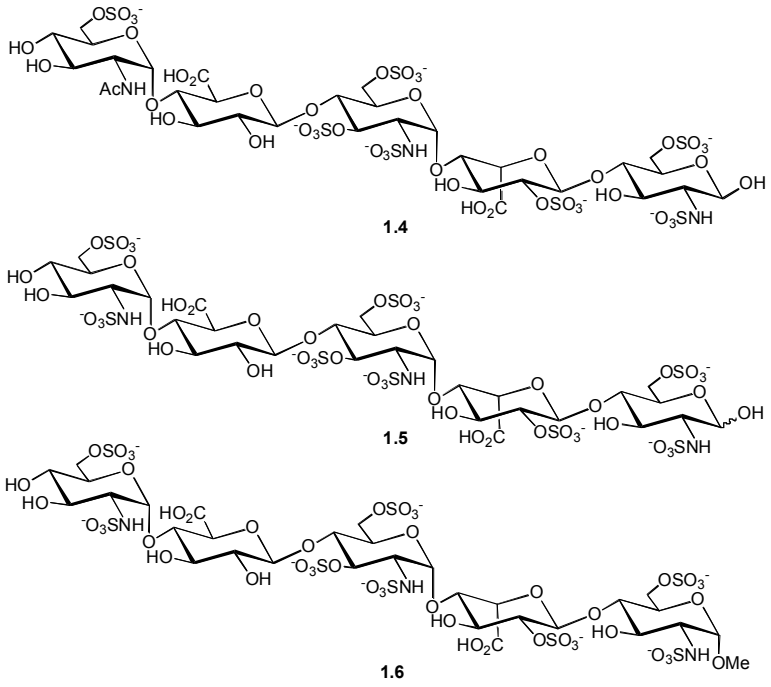
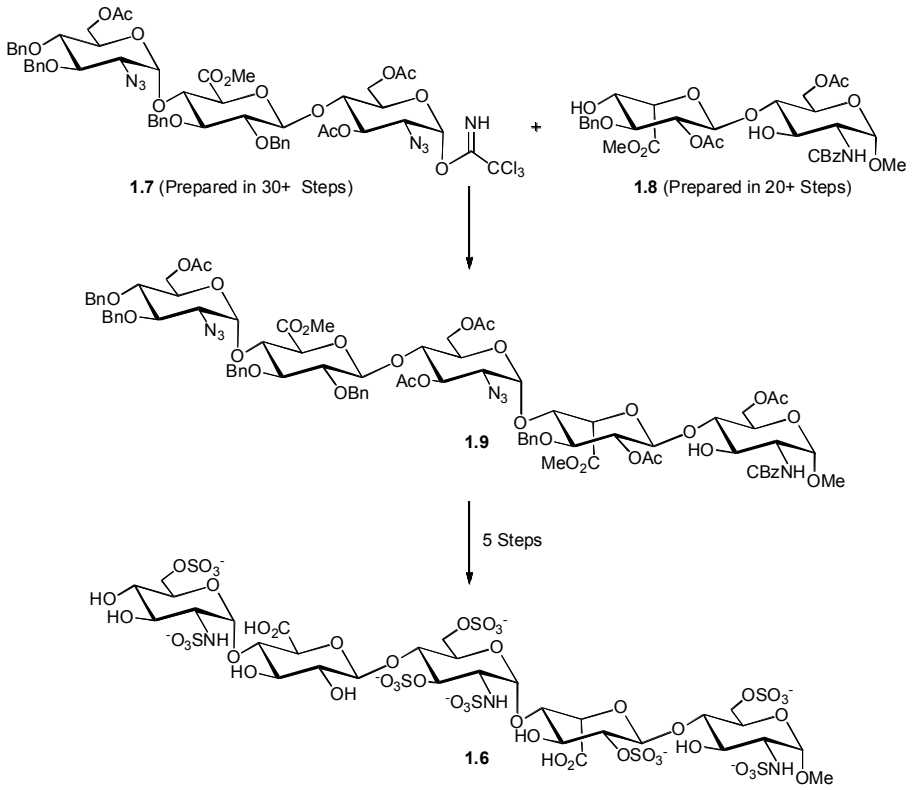


Figure 1.2. Heparin pentasaccharide and synthetic analogs.

In 1989 over 20 g of highly purified pentasaccharide **1.6** was prepared; and in 2001 after successes in toxicology testing and clinical studies, **1.6** was registered in the USA and Europe as a new antithrombotic drug under the name Arixtra (fondaparinux).[7] Even though it comprises almost 60 steps and a low overall yield, the multi-kg synthesis of highly pure **1.6** is still performed successfully in the pharmaceutical industry. Arixtra is superior to natural Heparin in that it requires a lower dosage, has a much longer half life ($t_{1/2}$ = 17 h) and is uncompromised by the adverse side effects.[7] As outlined in

Scheme 1.1, the key approach to the synthesis of the pentasaccharide is the application of an orthogonal protecting group strategy to differentiate the location of the sulfate group from other reactive positions in the oligosaccharide.[1]



Scheme 1.1. Synthesis of Arixtra

The fully protected pentasaccharide **1.9** is prepared by coupling trisaccharide donor **1.7** with disaccharide **1.8** (prepared in over 50 combined synthetic steps).[1,8] The positions that will carry sulfate groups are protected as acetate esters, whereas the hydroxyl groups in the product are masked as benzyl ether groups. The locations that bear the sulfates are deprotected and then *O*-sulfated. The remaining benzyl and azido groups are subjected to hydrogenolysis, and selective *N*-sulfation affords the desired pentasaccharide **1.6**. Through extensive structure-activity relationship (SAR) studies,

almost all of the sulfate groups (as indicated in Figure 1.3) on pentasaccharide **1.6** were determined to be essential groups, and therefore are required for biological activity.[1]

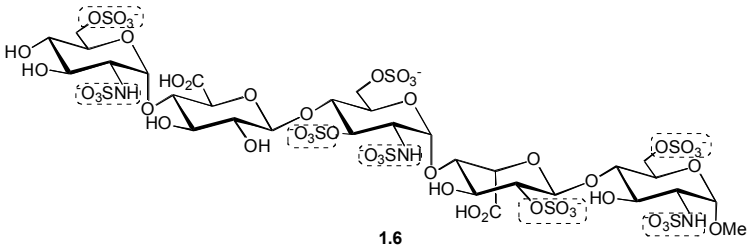


Figure 1.3. Essential sulfate groups in Arixtra

Further structure-activity studies of heparin-like pentasaccharides have lead to the development of simplified pentasaccharides such as Idraparinux **1.10**[1] and Idrabiotaparinux **1.11**[9] (Figure 1.4).

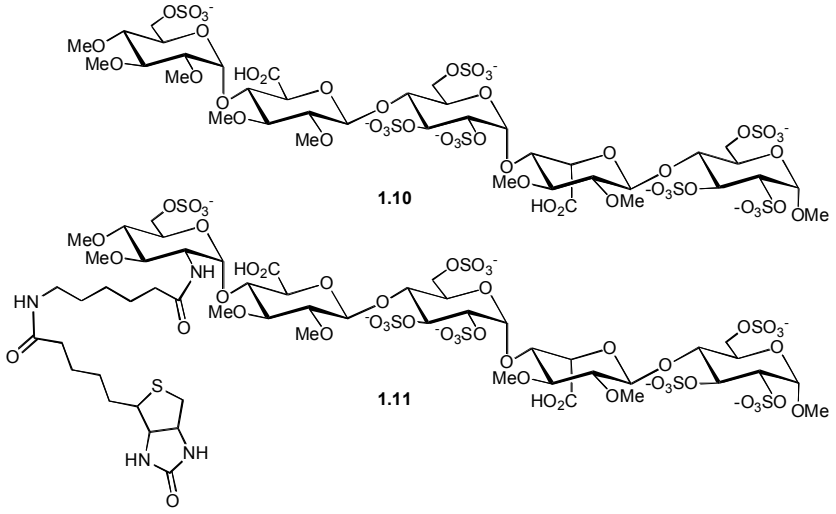
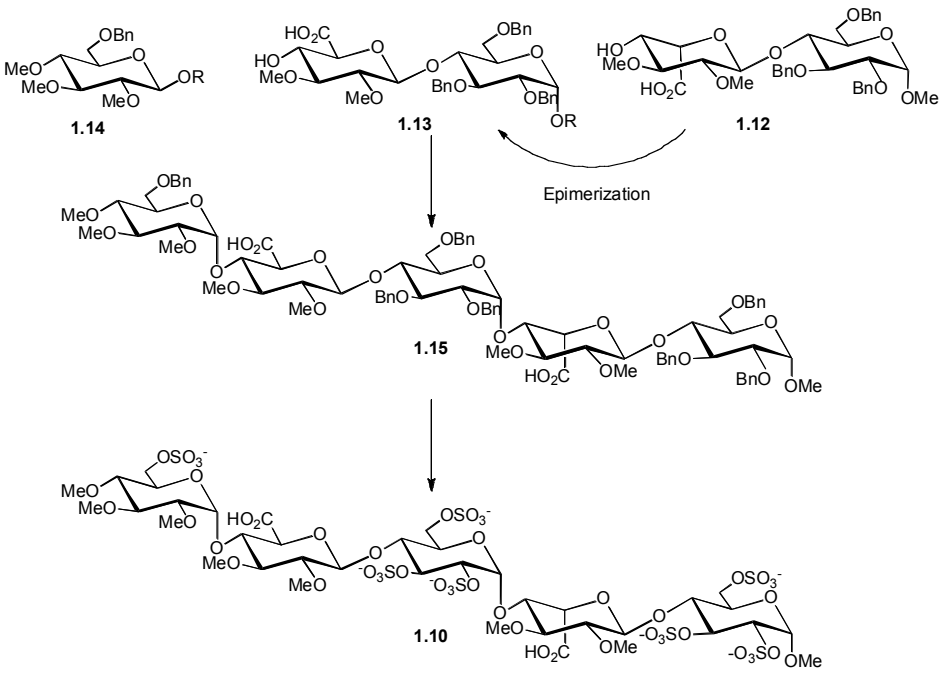


Figure 1.4. Idraparinux and Idrabiotaparinux

As a result of the simplified chemical structure of the pentasaccharide in **1.10** and **1.11**, the need for orthogonal protecting groups is eliminated, and the total synthesis of **1.10** is achieved in 'only' 25-30 steps (outlined in Scheme 1.2).[5,10,11] The synthesis utilizes disaccharide **1.12** which can be converted into, and then coupled to **1.13** to provide access to an intermediate tetrasaccharide. This tetrasaccharide is then coupled with donor **1.14** to give fully protected pentasaccharide **1.15**. Removal of the benzyl protecting groups followed by a global sulfation reaction provides Idraparinux **1.10**.



Scheme 1.2. Synthesis of Idraparinux

Idraparinux displayed higher activity and higher bio-availability than both heparin and Arixtra, ($t_{1/2}$ = 120 h), and by 2003 had reached phase III clinical trials. Unfortunately, the compound was found to be too active, and caused major bleeding

events in greater than 18% of the clinical trials.[9] To counteract this, a biotin moiety was tethered to the non-reducing end of **1.10** to give Idrabiotaparinux **1.11** (Figure 1.4). It is anticipated that the activity of Idrabiotaparinux can be controlled by the addition of Avidin, and this process is currently in phase III clinical trials.[9]

Similar to the heparin family of oligosaccharides, the chondroitin sulfate family of glycosaminoglycans is a very important class of naturally occurring sulfated oligosaccharides. In 2004, Hsieh-Wilson and coworkers reported that tetrasaccharide **1.16**, a fragment of a chondroitin sulfate glycoaminoglycan, stimulates neuronal growth.[12] The presence of the four sulfate residues was absolutely essential for the stimulatory activity.[12] Chondroitin sulfate glycosaminoglycans will be covered in more detail in chapter 3.

Other sulfated carbohydrates of interest include disaccharide **1.17** (Figure 1.5), known as sulfolipid-1 (SL-1), which is a virulence factor in strains of *Mycobacterium tuberculosis*.[13,14] SL-1 contains a trehalose core modified with four lipid chains, and a sulfate group at C2 of the first monomer. The sulfate group is critical for virulence of the bacterium.[13,14]

In 2004, Schroeder and co-workers isolated and identified a family of unusual sulfated nucleoside derivatives, such as the known compound **1.18** and previously unknown sulfated nucleosides such as **1.19**[15] and from the venom of the grass spider *Hololena curta*.[16] These neurotoxins are unique mono- or disulfated glyconucleosides that are found to have an uncommon ability to effectively block kainate receptors in

addition to weakly blocking calcium channels. Interestingly, these authors summarize their article by commenting on the fact that a literature search revealed very little synthetic information about these molecules, which results in very little evaluation of their biological properties. They concluded that the synthesis and subsequent biological activity studies remain important objectives for their work.[16]

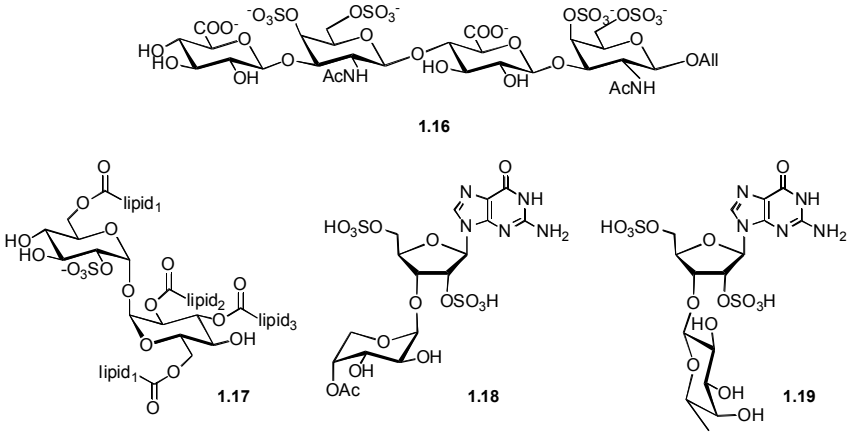
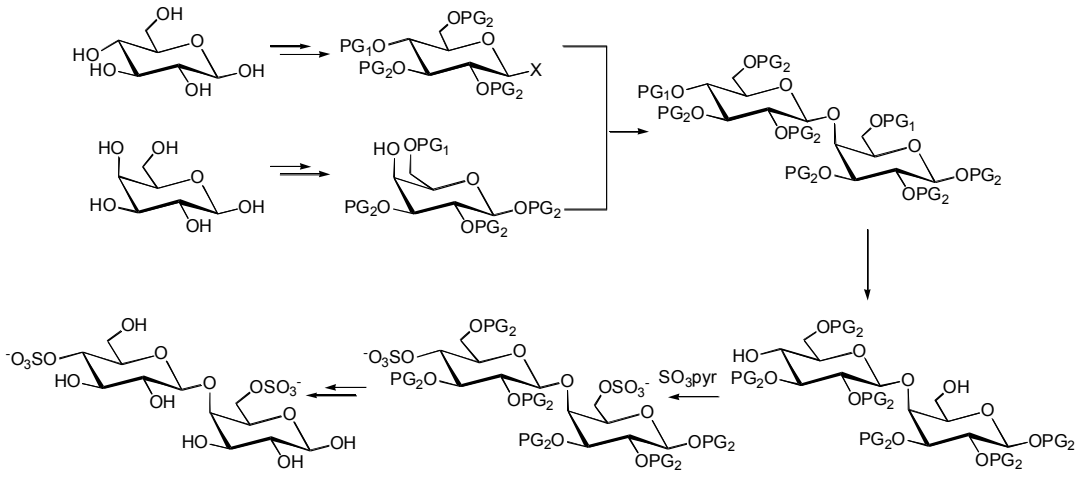


Figure 1.5. Biologically relevant sulfated carbohydrates

1.2 Synthesis of Sulfated Carbohydrates

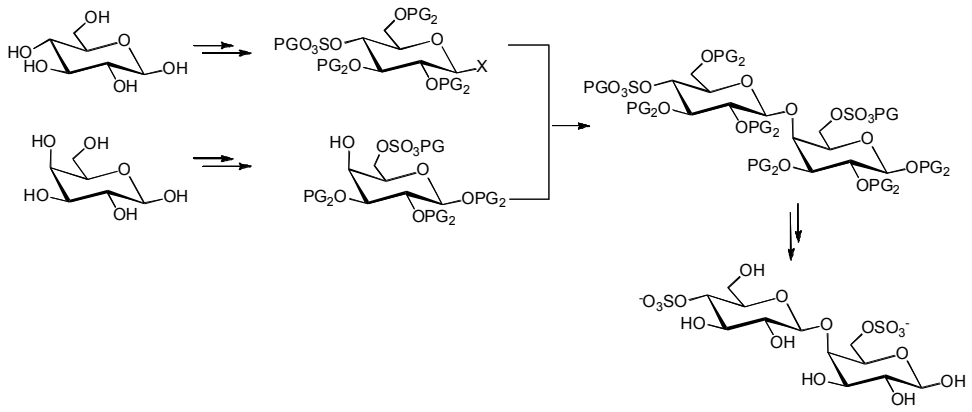
Although sulfated carbohydrates have been obtained synthetically for several decades, their synthesis still remains a considerable challenge. In the current approach, as outlined in Scheme 1.3, monosaccharide or disaccharide building blocks are fully protected, with the hydroxyls that will ultimately bear the sulfate groups protected in an orthogonal manner to those that will not be sulfated. After assembly of the oligosaccharide, the locations that are to be sulfated are selectively deprotected. The sulfate moiety is introduced using a conventional sulfation method such as a sulfur

trioxide-amine complex, and then all other protecting groups are removed to give the final product.



Scheme 1.3. Orthogonal protection strategy

An alternate approach to the conventional synthesis of sulfated carbohydrates is being advocated in this thesis. In this approach, the sulfate groups are introduced at the monosaccharide stage as protected sulfate diesters. This method reduces the required protecting group manipulations, and provides products which are less polar, and easier to manipulate. Once the fully protected oligosaccharide is assembled, the hydroxyl and sulfate protecting groups are removed, and the desired sulfopolysaccharide is obtained (Scheme 1.4).



Scheme 1.4. Incorporation of a protected sulfate moiety

1.3 Protecting Groups for the Sulfate Moiety

The idea of protecting the sulfate group during sulfocarbohydrate synthesis has been around for some time; however, applications of this approach have been very limited. The reason for this has to do with the number of sulfate protecting groups that are available as well as the ease by which they can be incorporated into the carbohydrate building blocks and then removed to provide the free sulfate. The limited number of protecting groups is a direct consequence of the chemical reactivity of sulfate mono- and diesters. Acid labile protecting groups cannot be used due to the well-known instability of sulfate monoesters to acid.[17] Furthermore, sulfate diesters are highly susceptible to nucleophilic attack, which can occur at either the sulfur atom or carbon atoms of the C-O-S bonds (Figure 1.6).[18]

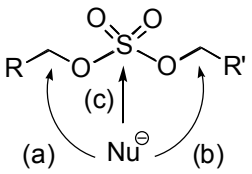


Figure 1.6. Nucleophilic attack on a sulfate ester – 3 possible routes

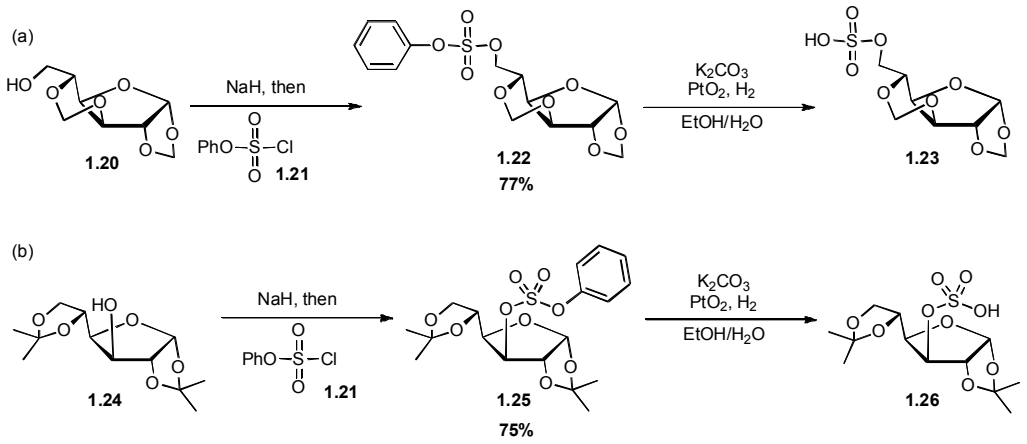
When R is a carbohydrate, substitution by path (a) is generally slow, especially so for the sulfate esters of secondary alcohols. The idea of protected carbohydrate sulfate diesters focuses on disfavouring attack by route (a) (Figure 1.6), with the strategic design of R' in a manner which favours attack by route (b) without rendering the protecting group ineffective, as it is expected to be stable to many different chemical manipulations.

The reactivity properties of sulfate diesters eliminates the possibility of using protecting groups that are removed by hydrogenolysis or photolysis, since these are usually benzylic moieties, and so are very susceptible to nucleophilic displacement. If base labile protecting groups are used, they would have to be designed such that they are stable enough to nucleophiles while being removed under conditions that are not too harsh. In light of these difficulties, it is not surprising that, until recently, only a few protecting groups for sulfate esters have ever been reported.

1.3.1 Phenyl Protection for Sulfate Monoesters

In 1981, Penney and Perlin were the first to propose and demonstrate the introduction of the sulfate group to a carbohydrate in the form of its protected organosulfate.[19] Employing monosaccharides as model systems, the authors showed that phenyl chlorosulfate **1.21** could be reacted with partially protected carbohydrates **1.20**

and **1.24** to afford the corresponding phenyl sulfocarbohydrates **1.22** in a 77 % yield, and **1.25** in a 75% yield (Scheme 1.5a,b).



Scheme 1.5. Incorporation and deprotection of phenyl sulfates

The authors studied the stability of the phenyl protected sulfate esters, and reported that **1.22** was stable to various conditions including NaOMe at room temperature; 2:1 NH_4OH in pyridine; CsF in acetonitrile or methanol; KF and 18-Crown-6; but formed unidentifiable products when reacted with TBAF in oxolane. Stability studies with **1.25** showed that the 5,6-isopropylidine group could be hydrolyzed in the presence of the protected sulfate using TFA in $CHCl_3$; the phenyl sulfate moiety was stable to cationic resin in water/oxolane at room temperature but not at elevated temperatures; and that the acetals could be removed using 1:1 Ac_2O/H_2SO_4 without affecting the phenyl sulfate.

Removal of the phenyl protecting group to provide the free sulfate was achieved by subjecting **1.22** and **1.25** to a solution of excess potassium carbonate and cat. platinum oxide / H_2 in ethanol-water. This resulted in the hydrogenation of the phenyl ring to a

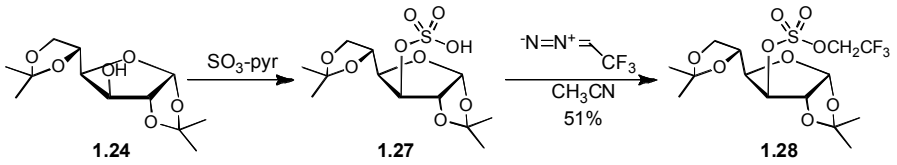
cyclohexyl group, which was removed to give the desired sulfated carbohydrates **1.23** and **1.26** (Scheme 1.5 a,b).[19] The authors did not comment on the yield of the deprotections, but did suggest that 10 % of desulfation had taken place during the reaction.

Perlin and co-workers further examined use of the phenyl protecting group in the synthesis of sulfated monosaccharides in the preparation of mono-and disulfated compounds;[20,21] however, the approach exhibited a number of limitations. Cleavage of the sulfate diester required conditions that are not compatible with protecting groups that are sensitive to base and hydrogenolysis. The deprotected products required tedious purifications involving anion exchange resins, and removing the phenyl protecting group was found to result in partial desulfation leading to poor yields of the desired products. Because of these limiting factors, Penney and Perlin's method was not widely applied to the synthesis of sulfated carbohydrates.

1.3.2 Trifluoroethyl Protection for Sulfate Monoesters

The idea of protected sulfates in carbohydrate chemistry did not resurface until sixteen years later, when Proud *et al.* offered an alternative to the phenyl group.[18] In 1997, the authors proposed the use of trihaloethyl sulfate esters, suggesting that these moieties would be stable to nucleophilic attack (as indicated in Figure 1.6) for both steric and electronic reasons, and could offer versatile protection in many aspects of carbohydrate chemistry. Their initial attempts focused on the 2,2,2-trichloroethyl (TCE) group, since it had previously been used for phosphate and carboxyl protection.[22] This

appeared to be an unusual first choice, since these authors anticipated that the TCE group could be successfully removed from the protected sulfocarbohydrate without the loss of the sulfate group using Zn/AcOH, which is unlikely due to the poor stability of the sulfate monoesters to acid. Nevertheless, they never got the opportunity to test their hypothesis, since they were unable to prepare the TCE-protected sulfocarbohydrates. The authors stated that the reaction of 2,2,2-trichloroethyl chlorosulfate with carbohydrate nucleophiles proceeded in yields that were too low to be of use, attributing this to steric reasons. They therefore decided to examine the trifluoroethyl (TFE) group instead. Again, reacting 2,2,2-trifluoroethyl chlorosulfate with carbohydrates proceeded in poor yields. They were, however, able to develop a two step approach in which conventional sulfation methods such as sulfur trioxide / amine complexes were first used to prepare a sulfated carbohydrate such as **1.27**, which was then treated with 2,2,2-trifluorodiazoethane to give the desired TFE-protected sulfocarbohydrate **1.28** (Scheme 1.6).[18]



Scheme 1.6. Preparation of TFE-protected carbohydrate sulfates

Proud *et al*. extended the chemistry to synthesize a number of sulfated monosaccharides commonly used in the synthesis of complex carbohydrates (Figure 1.7).

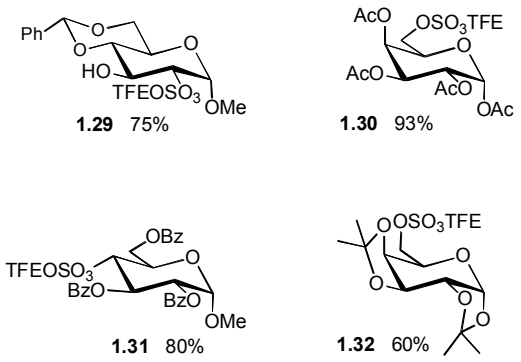
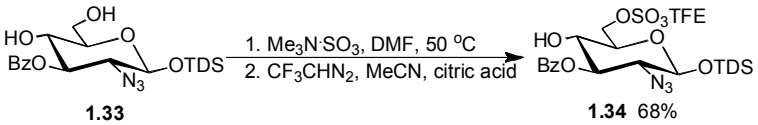


Figure 1.7. Formation of trifluoroethyl esters of carbohydrate sulfates

The TFE protecting group was reported to be stable to a variety of conditions such as TFA in EtOH, TBAF, hydrogenation, and NaOMe in MeOH at room temperature and reflux. This stability unfortunately, affected the ease with which the protecting group was removed; this transformation required refluxing the protected carbohydrate in potassium *t*-butoxide in *t*-butanol. The yields of deprotected products ranged from 82-96 %. In certain cases such as **1.29** in Figure 1.7, migration of the sulfate ester occurred during deprotection.[18]

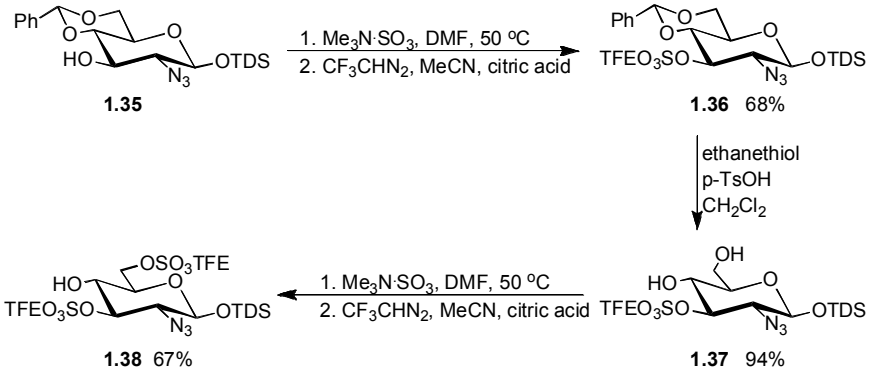
Despite the apparent potential that Proud *et al.*'s chemistry had for the synthesis of sulfated carbohydrates, it was another six years before it was examined in more detail. In 2003, Linhardt and coworkers published extensive studies on the use of the TFE group in the synthesis of fully differentiated hexosamine monosaccharides.[23] For example, selective 6-*O*-sulfation of carbohydrate **1.33** with sulfur trioxide-trimethyl amine followed by treatment with trifluorodiazoethane gave the TFE-protected sulfocarbohydrate **1.34** in 68% yield (Scheme 1.7).



1.33 **1.34** 68%

Scheme 1.7. Selective incorporation of a TFE sulfate

Linhardt's group also prepared disulfo-derivative **1.38** (Scheme 1.8) demonstrating that the TFE-sulfate moiety was compatible with the acidic conditions required for removing the benzylidene acetal in **1.36**, showing a greater versatility for the trifluoroethyl protecting group.



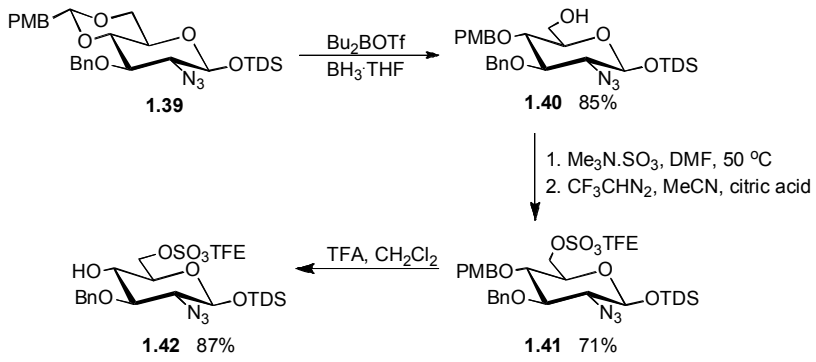
1.35 **1.36** 68%

1.37 94%

1.38 67%

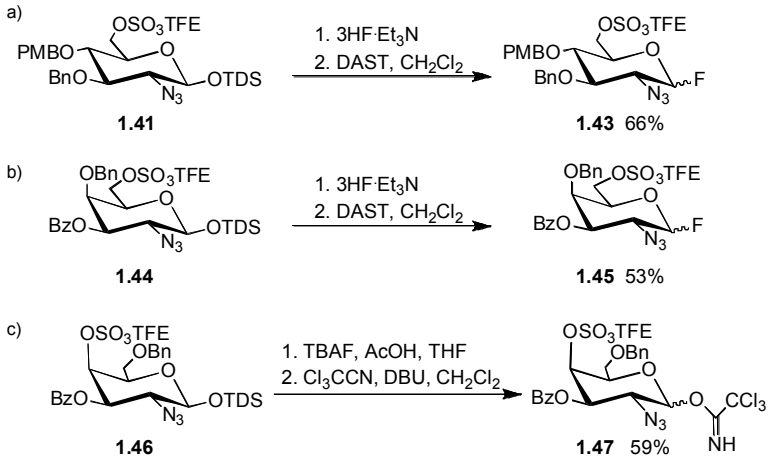
Scheme 1.8. Preparation of a TFE-protected disulfated acceptor

Monosaccharide building blocks employing *p*-methoxybenzylidene (PMB) protection at the 4,6-position such as glucosamine derivative **1.39** were subjected to regioselective opening of the benzylidene ring to unmask the 6-position for sulfation without affecting the TFE-protected sulfate. The PMB group was then selectively removed in acidic conditions to give glycosyl acceptor **1.42** (Scheme 1.7).[23]



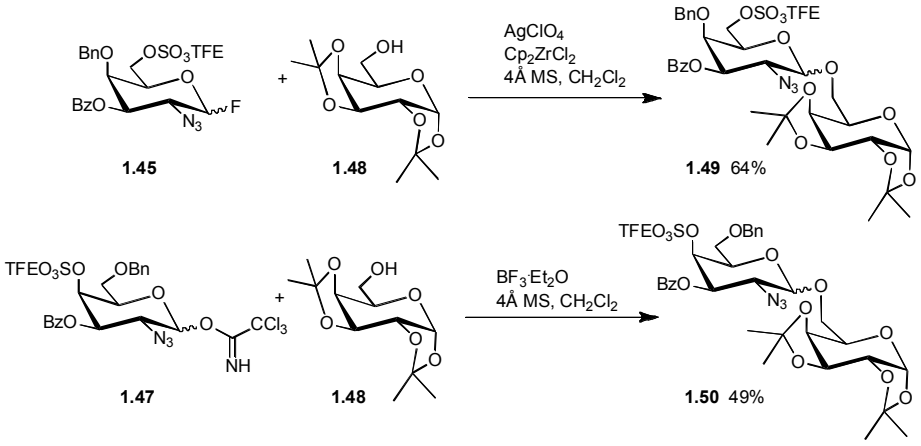
Scheme 1.9. PMB removal from TFE-protected carbohydrate sulfate

Linhardt and coworkers then studied the preparation of activatable carbohydrates bearing the TFE sulfate moiety. Both fluoride and trichloroacetimidate species were prepared (Scheme 1.10). It was reported that when present at the 6-position, the trifluoroethylsulfate moiety acted as a good leaving group when using only TBAF to remove the anomeric thexyldisilyl (TDS) group on carbohydrate **1.41**, as 1,6-anhydro sugars were recovered as side products for the reaction. This was corrected when excess acetic acid was added to TBAF, or by applying a milder reagent such as triethylamine trihydrofluoride (Scheme 1.10a,b). It was also noted that partial loss of the sulfate protecting group was observed under the basic conditions required to prepare trichloroacetimidate **1.47** (Scheme 1.10c).



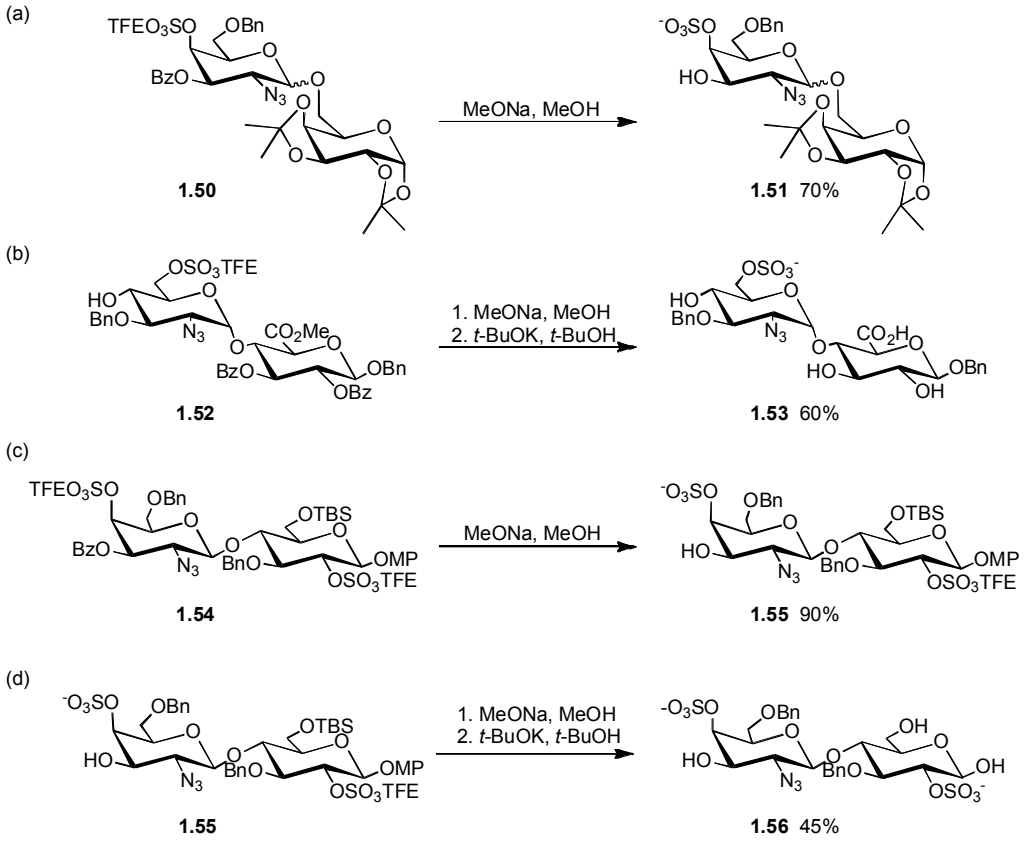
Scheme 1.10. Glycosyl donors bearing TFE sulfates

Finally, preliminary glycosylation attempts via the coupling of fluoride **1.45** and imidate **1.47** with the 6-OH acceptor of **1.48** were reported (Scheme 1.11).[23] Despite the electron-withdrawing character of the trifluoroethyl sulfonate, encouraging yields for the synthesis of **1.49** and **1.50** were reported in both cases.



Scheme 1.11. Glycosylation with sulfated donors

In July of 2004, a second publication from Linhardt and co-workers further described the synthesis of a variety of sulfo-protected monosaccharide donors and acceptors.[24] A wide variety of glycosylation reactions were studied, demonstrating the TFE sulfate group was compatible with a range of activation conditions commonly used with fluoride, imidate, and sulfoxide donors. Despite the extensive TFE-sulfate chemistry presented in the first publication, it was not until this second paper that Linhardt addressed the deprotection of the sulfate group (Scheme 1.12), demonstrating that removing the TFE protecting group was quite difficult.



Scheme 1.12. Deprotection of TFE-protected sulfates

The standard conditions of *t*-BuOK/*t*-BuOH first reported for TFE-sulfate deprotection[18] were found to be too harsh for disaccharide **1.50** resulting in substrate decomposition. Instead, it was demonstrated that the TFE group could be removed using NaOMe/MeOH, conditions which Proud *et al.* reported that the TFE group was stable to,[18] affording the deprotected product **1.51** in a 70 % yield (Scheme 1.12a).[24] Deprotection of the 6-*O*-sulfate **1.52** (Scheme 1.12b) presented an even greater challenge, as the *t*BuOK/*t*BuOH conditions lead to almost complete desulfation. It was found that the TFE protecting group could be removed under standard conditions only after the complete removal of the benzoyl esters in **1.52**, a method which resulted in minor loss of the sulfate group, compromising the final yield of **1.53**. Furthermore, for the 2,4-disulfate **1.54** (Scheme 1.12c) a stepwise deprotection was required. The 4-*O*-position sulfate was removed with NaOMe/MeOH to afford **1.55**, which was then subjected to *t*BuOK/*t*BuOH to deprotect the 2-*O*-position sulfate, providing **1.56** (Scheme 1.12d). Partial decomposition was also observed using this method, resulting in loss of the 6-OTBDMS and anomeric OMP protecting groups; therefore, the deprotected product **1.56** was obtained in only a 45% overall yield.[24]

The TFE protecting group has allowed for the synthesis of protected carbohydrate sulfate diesters, and has proved to be stable to a number of synthetic conditions for complex carbohydrates; however, this strategy has not been widely applied to the synthesis of these compounds as the approach exhibits some strong limitations. The method relies on conventional sulfation techniques that can be unreliable. Introducing the

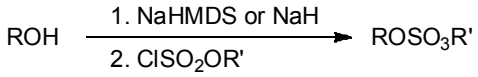
TFE group involves the use of trifluorodiazoethane, which is toxic and potentially explosive, and often proceeds in variable yields. Finally, and most significantly, removing the TFE group is difficult, and deprotection yields tend to be low when the substrate is more complex than a simple monosaccharide. While the approach is an excellent idea, alternative protecting groups and new methods for their introduction are required before this strategy becomes effective.

1.3.3 Neopentyl and Isobutyl Protection for Sulfate Monoesters

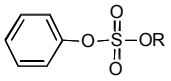
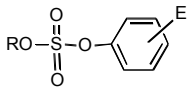
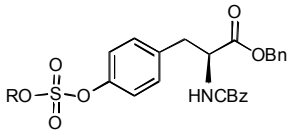
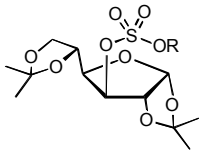
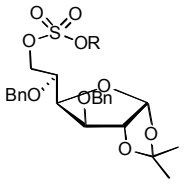
In 2006, Simpson and Widlanski published an article describing a comprehensive approach to the synthesis of sulfate esters.[25] Noting that there is no broadly useful method for the introduction of a protected sulfate monoester in the synthesis of complex molecules, the authors sought methodology to incorporate transient sulfate diesters that permit access to the target sulfates. In their approach, Simpson and Widlanski described the synthesis of sulfate esters employing neopentyl and isobutyl protecting groups for the protection of aromatic and aliphatic sulfate monoesters, basing their protecting group selection on the idea that the neopentyl and isobutyl groups are known to serve as good protecting groups for sulfonates.[25]

By treating an alkyl or aryl alcohol with a strong base, followed by the addition of neopentyl or isobutyl chlorosulfates **1.57** and **1.58,** respectively, a selection of protected sulfate diesters was prepared, with results summarized below in table 1.1.

Table 1.1. Preparation of Neopentyl and Isobutyl Sulfate Diesters



Where R' = nP **1.57** or R' = iBu **1.58**

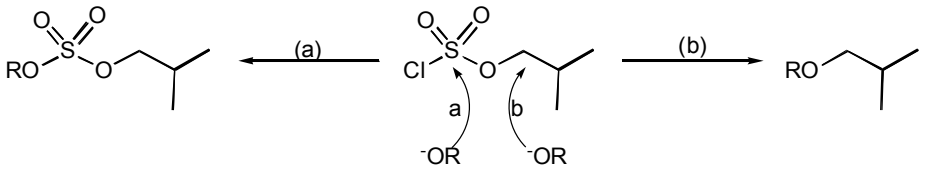
Entry	Product*	Protecting Group*	Yield (%)
1		**1.59** R = *n*P	95
		1.60 R = *i*Bu	80
2		**1.61** R = *n*P	98
		1.62 R = *i*Bu	82
3		**1.63** R = *n*P	99
4		**1.64** R = *n*P	95
		1.65 R = *i*Bu	95
5		**1.66** R = *i*Bu	86

* E = Estrone; *n*P = neopentyl; *i*Bu = isobutyl

The neopentyl protected sulfate monoesters were prepared by subjecting the corresponding alcohols and phenols to sodium hydride or sodium bis (trimethylsilyl)amide (NaHMDS) in THF (20% DMPU) at –75 ^{o}C, followed by treatment with a small excess of neopentyl chlorosulfate. Under these conditions, a variety of

neopentyl protected sulfated monoesters including phenolic derivative **1.59**, estrone sulfate **1.61**, protected tyrosine sulfate **1.63**, as well as the sulfocarbohydrate **1.64** were readily prepared from their hydroxyl substrates in excellent yields.

Simpson and Widlanski examined the isobutyl group as an alternative to the neopentyl group anticipating that it would be significantly more labile as the neopentyl protecting group is known to be extremely stable.[25] To prepare the isobutyl protected sulfate monoesters, the reaction conditions required slight adjustments to eliminate isobutyl ether side products which were assumed to arise form the attack of the sodium alkoxides or phenoxides at the methylene group in isobutyl chlorosulfate (Scheme 1.13b).

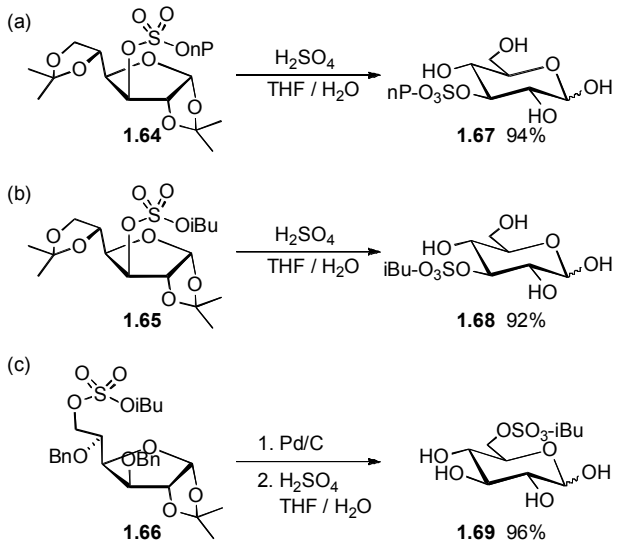


Scheme 1.13. Isobutyl ether side products

The modifications for the introduction of the isobutyl sulfate monoesters included discontinuing the use of DMPU, lowering the concentration, increasing the temperature, and using a larger excess of isobutyl chlorosulfate (5-10 eq.). Under the optimized conditions, the isobutyl protected sulfate esters of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **1.65** and 3,5-di-*O*-benzyl-1,2-di-*O*-isopropylidene-α-D-glucofuranose **1.66** were obtained in 95 % and 86 % yields, respectively.

The stability of the neopentyl and isobutyl protected sulfate esters were studied using NMR assays by subjecting protected phenyl sulfates **1.59** and **1.60** to different

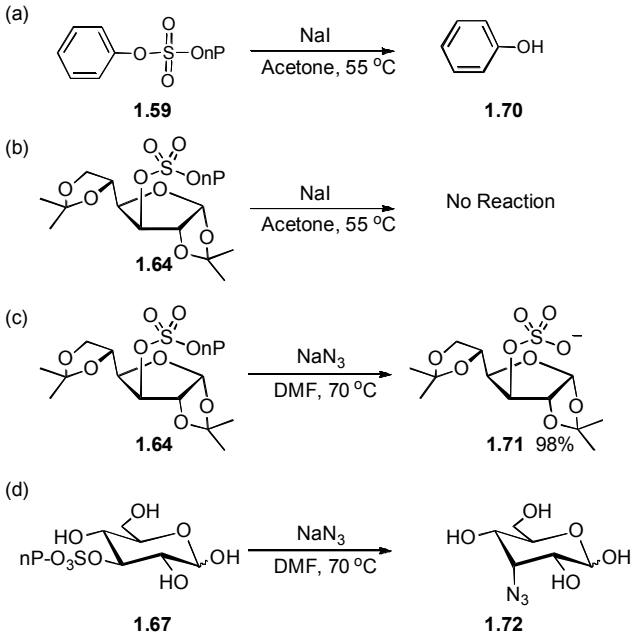
concentrations of TFA and piperidine. Both the isobutyl and neopentyl sulfate esters were found to be stable to 50% TFA, with less than 10% degradation observed after 48 h. The neopentyl sulfate ester was stable to 20% piperidine in chloroform, but the isobutyl sulfate ester was less tolerant, showing evidence of nucleophilic cleavage, even at 6% piperidine solution. From these studies, the authors concluded that both the neopentyl and isobutyl groups for sulfate esters offer viable protection in highly acidic reactions, with the isobutyl protecting group being much less effective under basic or nucleophilic conditions. The stability of the alkyl sulfates was not investigated under the TFA or piperidine conditions; however, carbohydrate sulfates **1.64**, **1.65**, and **1.66** were subjected to other transformations to demonstrate the versatility of the sulfate diesters (Scheme 1.14).



Scheme 1.14. Stability of alkyl protected carbohydrate sulfates

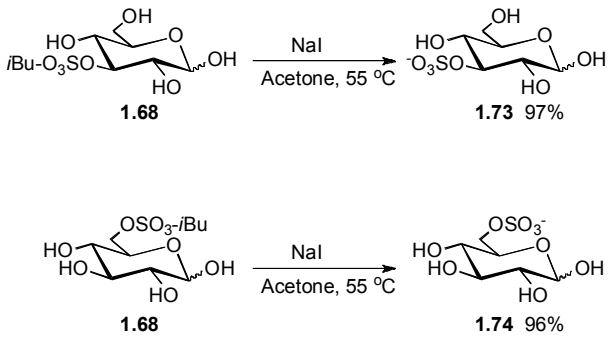
The stability of the neopentyl and isobutyl protecting groups towards hydrogenolysis and brief treatment with strong acids was demonstrated by removing the isopropylidene groups from protected carbohydrates **1.64**, and **1.65** using H_2SO_4 in THF /H_2O, and removal of the benzyl groups in **1.66** by hydrogenolysis with Pd/C and H_2, followed by treatment with aqueous H_2SO_4 in THF, with yields of the deprotected hexoses ranging from 92 – 96%.

To study the deprotection conditions required to cleave the neopentyl moiety, the protected sulfate esters were reacted with nucleophiles in polar solvents. The reaction of sodium iodide with phenyl neopentyl sulfate **1.59** (Scheme 1.15a) yielded the desulfated phenol rather than cleavage of the neopentyl group, and did not result in any reaction with the neopentyl protected glucose sulfate **1.64** (Scheme 1.15b). Other small nucleophiles such as azide and cyanide in DMF (60-70 ^{o}C) were reported to be effective for removing the neopentyl protecting group in protected aryl sulfates **1.59**, **1.61**, and **1.63**, as well as the protected sulfate of diacetone-D-glucose **1.65** (Scheme 1.15c) in near quantitative yields. Treating the unprotected glucopyranose neopentyl diester **1.67** with sodium azide in DMF, however, led to the displacement of the entire protected sulfate group providing 3-azido-3-deoxy D-allose **1.72** (Scheme 1.15d). This result strongly suggests that the neopentyl group is not useful for the protection for sulfates of primary or secondary alcohols, as the neopentyl group cannot be removed to afford the desired sulfate monoester products.



Scheme 1.15. Deprotection of neopentyl protected sulfate groups

Because nucleophilic substitution is significantly faster at isobutyl centers than at the more hindered neopentyl counterpart, a variety of nucleophiles were expected to cleave the isobutyl protecting group. The isobutyl protected sulfate esters of unprotected hexoses **1.68** and **1.69** were effectively cleaved with sodium iodide in acetone at 55 °C to provide the target sulfates **1.73** and **1.74** in excellent yields (Scheme 1.16). As was the case with the neopentyl aryl sulfates, iodide was able to displace phenoxides from the aryl alkyl sulfate esters; thus the isobutyl protecting group in sulfoestrone derivative **1.62** was cleaved using sodium thiocyanate and triethylamine in acetone at 55 °C to provide estrone sulfate in a 96 % yield.



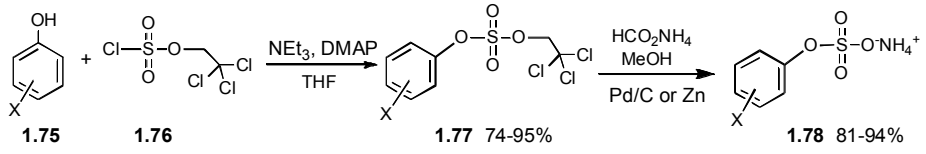
Scheme 1.16. Deprotection of isobutyl protected sulfate groups

The chemistry presented by Simpson and Widlanski does offer a unique approach for the synthesis of sulfated compounds; however, like other sulfation methods it is more successful for aryl sulfates. The neopentyl protected aryl sulfate monoesters could be highly effective for the synthesis of complex molecules but cleavage of the protecting group requires somewhat harsh conditions, to which other functionalities may not be tolerant. The isobutyl protected sulfate monoesters, designed to provide an alternative to the highly stable neopentyl counterparts, were much more sensitive to nucleophilic and basic conditions, which in turn limits their potential applications towards the synthesis of complex molecules. The authors also indicated that the isobutyl chlorosulfate approach would be effective for introducing sulfate monoesters at the last step in a synthesis. Although the method could be useful for increased yields of sulfation, it does not eliminate the unnecessary protecting group manipulations that are avoided by introducing the sulfate group at the beginning of a synthetic sequence, and therefore does not provide a significant alternative to conventional sulfation methods.

1.3.4 2,2,2-trichloroethyl Protection for Sulfate Monoesters

Although the 1997 paper by Proud *et al*. focused on the TFE moiety, this was not the starting point for their research.[18] As previously stated, initial attempts involved the use of the 2,2,2-trichloroethyl ester, a group that is used for phosphate and carboxyl protection.[18] Proud and coworkers had concluded that the TCE-sulfate moiety could not be incorporated into carbohydrates in good yields, and would not be useful for the synthesis of these compounds. The TCE group has an advantage over the TFE group in that it can be cleaved under very mild conditions (such as Zn/AcOH). In order for this group to be used, however, methodology would have to be developed for its introduction into sulfated carbohydrates in high yields without the use of toxic and explosive diazo derivatives, and a mild alternative for Zn/AcOH would have to be developed for it's removal.

In 2004, the Taylor group reported the use of the TCE moiety as the first group for the protection of aryl sulfates.[26] They demonstrated that 2,2,2-trichloroethyl chlorosulfate **1.76** can successfully be used to prepare protected aryl sulfates **1.77** in high yields (Scheme 1.17).



Scheme 1.17. Preparation of aryl sulfates

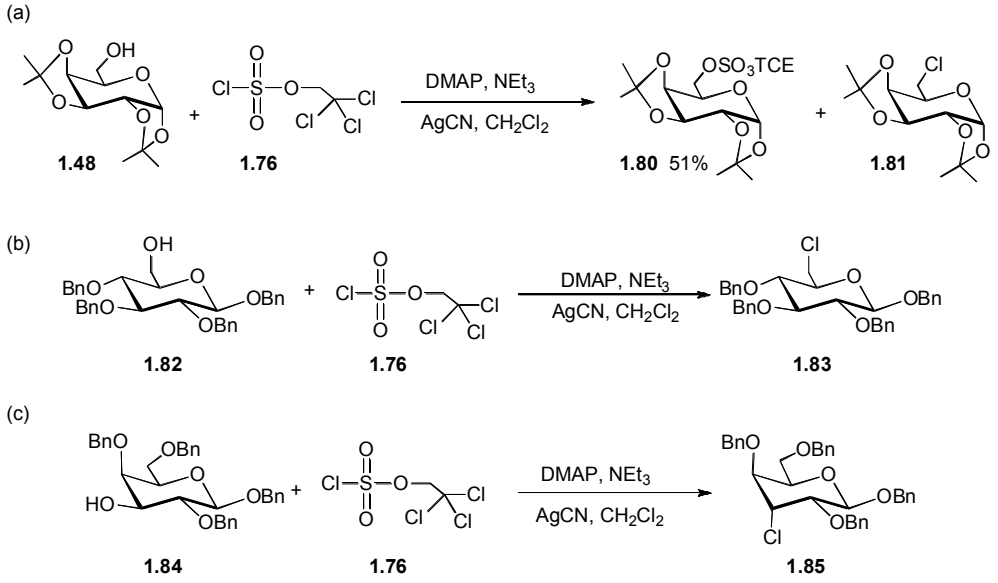
The TCE protecting group was easily removed in excellent yields under mild conditions by catalytic transfer hydrogenolysis using Pd/C and ammonium formate, or by using Zn and ammonium formate in methanol to obtain aryl sulfates **1.78**.[26] This success prompted further investigation into the use of the TCE group for protecting alkyl sulfates, specifically carbohydrate sulfates.

The methodology reported by the Taylor group was expanded to show that TCE chlorosulfate **1.76** can be reacted with diacetone glucose **1.24** to give the sulfated **1.79** in 82% yield, according to Scheme 1.18. This was an important development, since it contradicted Proud's previous conclusions that incorporation of the TCE group in high yields could not be achieved.[18]

Scheme 1.18. TCE protected sulfate moiety

Attempts to extend the chemistry to other carbohydrates were found to be more difficult than expected. For example, we found that reacting 1,2:3,4-di-*O*-isopropylidene galactose **1.48** with the TCE chlorosulfate **1.76** gave carbohydrate **1.80** in only a 51% yield (Scheme 1.19a). Analysis of the reaction products revealed that displacement of the TCE sulfate moiety by the liberated chloride ion to give chlorosugar **1.81** was a competing reaction. Attempts to repeat this chemistry to include benzyl-protected

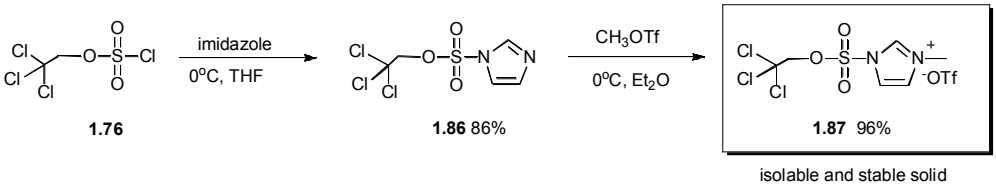
carbohydrates **1.81** and **1.83** (Scheme 1.19b,c) were less successful, as the reactions proceeded very slowly, and the chlorosugars **1.83** and **1.85** were the major products in both cases.



Scheme 1.19. Formation of chlorosugar by-products

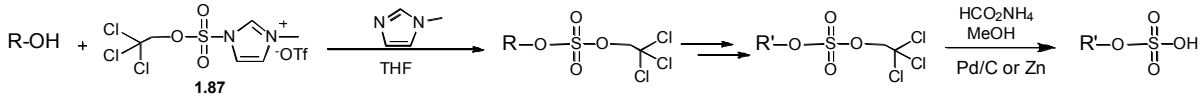
The unexpected side reactions described in Scheme 1.19 led to the investigation of alternative approaches. In January of 2006, we introduced the 2,2,2-trichloroethyl protecting group for sulfated carbohydrates.[27] To achieve this, an entirely new class of sulfating agents capable of introducing the TCE-protected sulfates to monosaccharide building blocks was reported. In search of a sulfating agent that did not liberate a nucleophilic species such as a chloride ion, we prepared the sulfuryl imidazolium triflate salt **1.87**, according to Scheme 1.20. Although sulfuryl imidazolium salts have never been reported, 2,2,2-trichloroethoxysulfuryl imidazole **1.86** was easily constructed (Scheme

1.20). 2,2,2-Trichloroethylsulfuryl chloride **1.76** was treated with an excess of imidazole to give TCE sulfuryl imidazole **1.86** in 86% yield. Treatment of **1.86** with methyl triflate (1.0 eq.) in dry diethyl ether provided the activated imidazolium triflate species **1.87**. As this reaction progressed, **1.87** was found to precipitate out of solution, and was isolated by simple filtration in near-quantitative yields, with no further purification required. Reagent **1.87** was found to be very stable and can be stored for months at room temperature or –20 ^{o}C with no detectable decomposition.



Scheme 1.20. TCE-Sulfuryl imidazolium triflate reagent

We then demonstrated that the TCE-sulfurylimidazolium triflate salt **1.87** could be reacted with a variety of monosaccharide building blocks (R in Scheme 1.21) in the presence of *N*-methylimidazole (NMI) in THF to provide the corresponding TCE-protected sulfocarbohydrates. The sulfated products could be transformed under other conditions to modified products, and at the end of the sequence, the TCE protecting group is easily removed to provide the free sulfate.[27]



Scheme 1.21. TCE Sulfate protection and deprotection

1.4 Summary and Thesis Outline

It is widely known that the chemical synthesis of complex carbohydrates is extremely challenging. The reluctance of the pharmaceutical industry to target molecules in this field is not surprising, as the industry depends on the ability to bring innovative products to the market in a quick and cost-effective matter, a difficult feat for oligosaccharide total synthesis. Research in the last 10 years, however, has demonstrated that carbohydrate-based compounds have immense therapeutic possibilities. Because of this, there are great efforts being conducted toward probing the functions and synthesis of oligosaccharides with interesting biological profiles.

Sulfated carbohydrates are included in the group of biologically relevant oligosaccharides, as they are implicated in a variety of biological processes; many of which depend solely on the 'sulfation code' of a particular oligosaccharide. There remains, however, no simple and efficient route to obtain pure, well-defined fragments of naturally occurring sulfated oligosaccharides

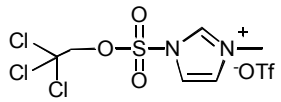
The remainder of this thesis focuses on my research contributions to the synthesis of sulfated carbohydrates. The ultimate goal was to devise an efficient and reliable method by which such compounds can be prepared, providing a viable alternative to the conventional methods currently in use. *Chapter 2* describes the development and applications of 2,2,2-trichloroethyl (TCE) sulfuryl imidazolium salts for the preparation of TCE-protected sulfated carbohydrates. This methodology, unlike those currently in use, allows for the incorporation of a protected sulfate diester at the monosaccharide

building block stage. Notable advantages of this approach include higher yields of sulfation, and products that are non-polar and easy to purify and handle for subsequent manipulations. *Chapter 3* describes the efforts towards the total synthesis of chondroitin-based sulfated oligosaccharides. These targets, which are known to be extremely difficult to prepare synthetically, are ideal targets to further evaluate the scope of our TCE-protecting group strategy. The successes and shortfalls in the synthesis of a selection of chondroitin sulfate oligosaccharides are discussed. *Chapter 4* covers the development of methodology that allows for selective 3-*O*-carbobenzoxylation of carbohydrate diols, and the formation of the 2-*O*-Cbz migrated products. This methodology will be applied towards the total synthesis of a disulfated SB_{1A} tetrasaccharide.

Chapter 2 - 2,2,2-trichloroethyl-protected Carbohydrate Sulfates

2.1 Background on the use of 2,2,2-Trichloroethoxysulfuryl Imidazolium Triflate in the Preparation of Sulfated Carbohydrates

As mentioned in Chapter 1, in 2006 we reported the synthesis of a 2,2,2-trichloroethoxysulfuryl-*N*-methylimidazolium triflate, (compound **1.87**, Figure 2.1) the first example of a sulfuryl imidazolium salt (SIS).[27] This reagent represented the first of a new class of potent sulfating agents, capable of reacting with nucleophilic substrates while liberating a non-nucleophilic leaving group.



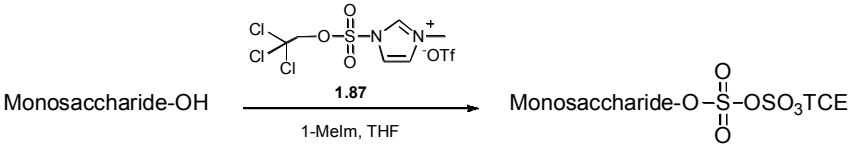
1.87

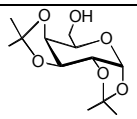
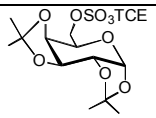
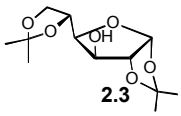
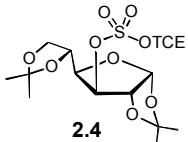
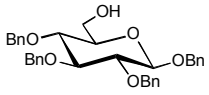
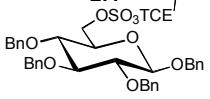
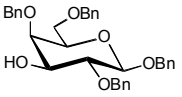
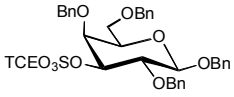
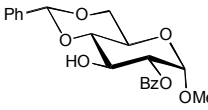
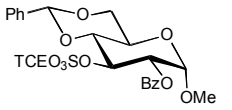
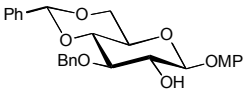
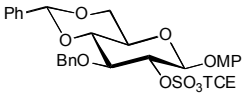
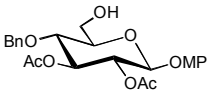
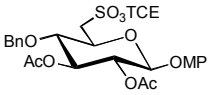
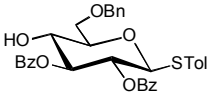
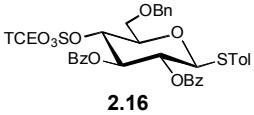
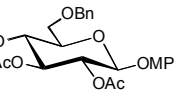
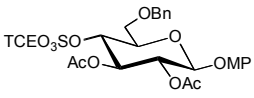
Figure 2.1. 2,2,2-Trichloroethoxysulfuryl-*N*-methylimidazolium triflate (**1.87**)

It was also mentioned in chapter 1 (Scheme 1.21) that carbohydrates containing TCE-protected sulfate groups could be prepared in good yields by subjecting partially protected carbohydrates containing a free hydroxyl group to **1.87** and 1-methylimidazole (1-MeIm).[27,28] A selection of the prepared sulfated carbohydrates is shown in Table 2.1. The target nucleophile substrates were designed with a number of factors in mind: (1) that the TCE sulfate group could be incorporated at the 2-*O,* 3-*O*, 4-*O*, and 6-*O* positions, (2) a variety of commonly used protecting groups are employed, including acetate and

benzoyl groups, benzyl ethers, as well as substituted acetals, and (3) the target TCE-protected sulfocarbohydrates should provide ready access to both glycosyl donors and acceptors. In most cases, primary and secondary hydroxyl groups were sulfated in good to excellent yields by subjecting them to **1.87** (2.0 – 5.0 eq.) and *N*-methylimidazole (1-MeIm) in THF (2.5 – 6.0 eq.) in THF for 16-46 hours. A select few sulfation reactions were found to be more difficult than others. In the case of **2.11**, 11.6 eq. of 1-MeIm 10.5 eq. of **1.87** and 72 hours were required to achieve a 90 % yield of the sulfated product **2.12**. Similarly, carbohydrate **2.19** was obtained in only a 76% yield, even after **2.17** was treated with 6 eq. of **1.87** over a 72 h period. For all reactions, the presence of 1-MeIm was essential, as other bases (NEt_3, Hunig's base, pyridine, 2,6-lutidine, and piperidine) were considerably less effective.

Table 2.1. Preparation of TCE-protected carbohydrate sulfates.[29]

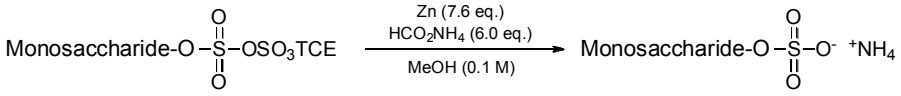


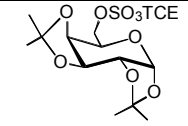
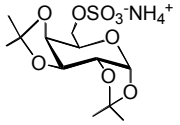
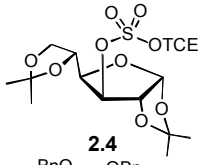
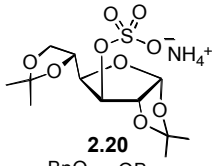
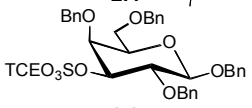
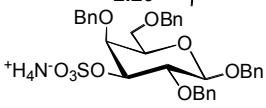
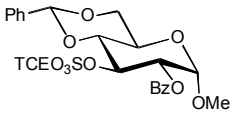
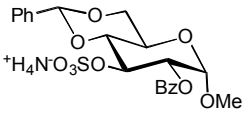
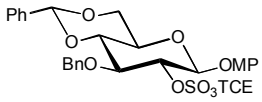
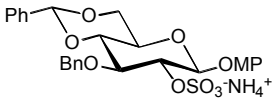
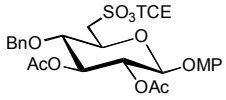
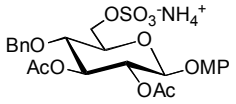
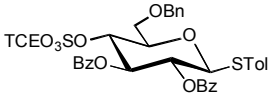
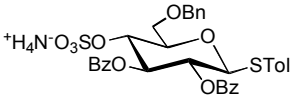
Entry	Monosaccharide	Sulfated Product	Yield (%)
1[a]	 **2.1**	 **2.2**	87
2[a]	 **2.3**	 **2.4**	90
3[a]	 **2.5**	 **2.6**	75
4[a]	 **2.7**	 **2.8**	94
5[b]	 **2.9**	 **2.10**	94
6[c]	 **2.11**	 **2.12**	90
7[a]	 **2.13**	 **2.14**	81
8[d]	 **2.15**	 **2.16**	91
9[e]	 **2.17**	 **2.18**	76

a) 2.5 eq. 1-MeIm, 2.0 eq. **1.87**; b) 5.3 eq. 1-MeIm, 4.7 eq. **1.87**; c) 11.6 eq. 1-MeIm, 10.5 eq. **1.87**; d) 5.2 eq. 1-MeIm, 4.3 eq. **1.87**; e) 4.0 eq. 1-MeIm, 6.0 eq. **1.87**

When a new protecting group is introduced, one of the most important factors to demonstrate is that the group can be removed without difficulty, and in respectable yields when required. The TCE group was easily removed in high yields from the sulfated carbohydrates by subjecting the compounds to zinc and ammonium formate in methanol (Table 2.2). Apart from the presence of $ZnCl_2$, $Zn(HCO_2)_2$ and NH_4Cl, the crude sulfates were essentially pure. These side products were easily removed by passing the carbohydrate through a short column of silica with CH_2Cl_2/MeOH/NH_4OH (20:4:1) as eluent. Deprotection studies were also performed with Pd/C–ammonium formate; however, the yields were slightly lower than those with Zn.[27]

Table 2.2. Removal of the TCE protecting group.[29]

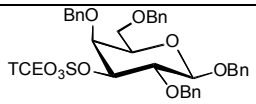
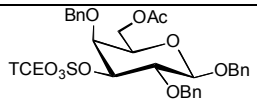
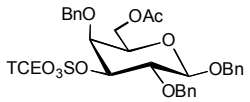
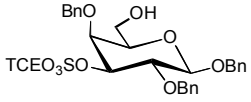
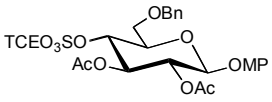
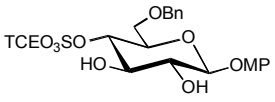
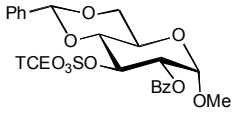
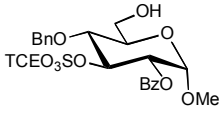
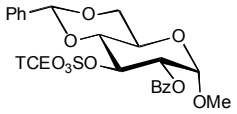
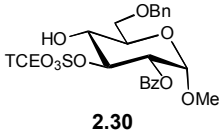
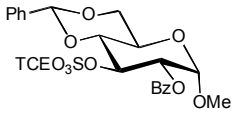
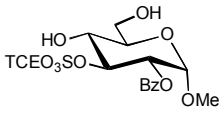
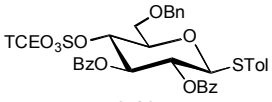
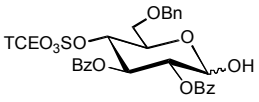
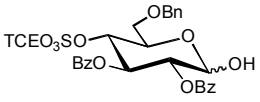
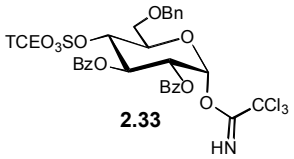
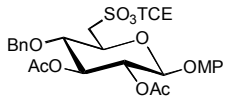
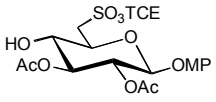


Entry	Sulfated Monosaccharide	Deprotected Product	Yield (%)
1	 **2.2**	 **2.19**	99
2	 **2.4**	 **2.20**	94
3	 **2.8**	 **2.21**	97
4	 **2.10**	 **2.22**	96
5	 **2.12**	 **2.23**	96
6	 **2.14**	 **2.24**	91
7	 **2.16**	 **2.25**	94

In order for the TCE-protected sulfate methodology to be applied to carbohydrate chemistry, it was essential to demonstrate that the TCE sulfates are stable to a wide variety of conditions commonly encountered in the synthesis of higher order

oligosaccharides. The $TCESO_3$ moiety should remain intact during routine protecting group manipulations, as this is one of the major components in the synthesis of complex carbohydrates. The protected sulfates must also tolerate conditions for anomeric activation; and survive conditions required for the coupling of monosaccharides, which often involve nucleophilic acceptors as well as strong promoters. Table 2.3 demonstrates that, for the most part, the TCE-protected sulfates are stable to many conditions commonly encountered in carbohydrate chemistry, and could offer a viable approach towards the synthesis of sulfated carbohydrates. Selective 6-*O*-debenzylation and acetylation of **2.8** with $ZnCl_2$/AcOH/Ac_2O gave **2.26**, which could be treated with catalytic NaOMe in MeOH to give the deacetylated product **2.27** in 85% yield.

Table 2.3. Manipulations of TCE-protected carbohydrate sulfates[29]

Entry	Substrate	Conditions	Product	Yield (%)
1	 **2.8**	$ZnCl_2$ (3.7 eq.), $AcOH/Ac_2O$, 3 h, rt	 **2.26**	95
2	 **2.26**	NaOMe (0.16 eq.) MeOH, 3 h, rt	 **2.27**	85
4	 **2.18**	CH_3COCl (3M in MeOH) 3 h, rt	 **2.28**	84
5	 **2.10**	$PhBCl_2$ (3.4 eq.), Et_3SiH (3.0 eq.), 4A MS, -78 °C, CH_2Cl_2, 1 h.	 **2.29**	96
6	 **2.10**	TfOH (3.4 eq.), Et_3SiH (3.0 eq), 4A MS, -78 °C, CH_2Cl_2, 1 h.	 **2.30**	87
7	 **2.10**	TsOH (0.1 eq.), CH_2Cl_2/MeOH, 45 °C, 16 h.	 **2.31**	94
8	 **2.16**	NBS (3.0 eq.), acetone, H_2O, 0 °C, 20 min	 **2.32**	74
9	 **2.32**	DBU (0.2 eq.), Cl_3CCN (16 eq.), -40 to -10 °C, CH_2Cl_2, 3 h.	 **2.33**	80
10	 **2.14**	NBS (3.5 eq.), $CaCO_3$ (5 eq.), CCl_4/H_2O, 250-W incandescent lamp	 **2.34**	91

Unfortunately, the ease with which the primary acetate in **2.26** was removed could not be extended successfully to other carbohydrates. Attempts to deacetylate **2.18**, which bears secondary acetate groups, under the same Zemplén conditions resulted in both de-acetylation and loss of the TCE-sulfate. This issue is easily overcome by stirring an acetylated carbohydrate (such as **2.18**) in acidic methanol to get the corresponding de-esterified compound (**2.28** for example) in good yields.

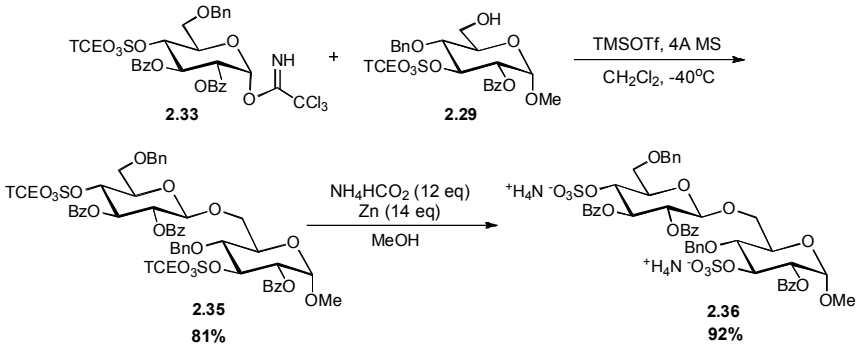
Benzylidene acetals are key protecting groups in carbohydrate chemistry, as they protect two hydroxyl groups with high regioselectivity. The $TCESO_3$ was found to be tolerant of the conditions required for the regioselective reductive opening of the benzylidene acetal. Sulfated carbohydrate **2.10** bearing a benzylidene acetal can be treated with $PhBCl_2$ or TfOH in the presence of Et_3SiH to access **2.29** with a free 6-OH, or **2.30** with a free 4-OH in good yields. The benzylidene acetal in **2.10** was also completely removed under acidic conditions to provide **2.31**, the corresponding 4,6-diol. No detectable loss of the $TCESO_3$ group was observed in any of these reactions.

Difficulties were initially encountered when attempting to selectively cleave a benzyl ether in the presence of the TCE-protected sulfate in **2.14** using hydrogenolysis, as the TCE protecting group is known to be removed under the standard hydrogenolysis (Pd, H_2) conditions. Photochemically initiated debenzylation was first documented by Binkley and Hehemann in 1990,[30] and was developed further by Riley and Grindley in 2001.[31] In this approach, sterically hindered benzyl ethers that could not be removed by hydrogenolysis, or those that progressed slowly, were readily removed by applying N-bromosuccinimide (NBS) and light in the presence of aqueous calcium carbonate.

Subjecting benzylated **2.14** to these conditions (table 2.3, entry 10) resulted in the selective 4-*O*-debenzylation to give easy access to the corresponding alcohol **2.34** in a 91% yield.

The stability of the TCE protecting group was further examined in the preparation of activatable carbohydrate donors and disaccharide formation. Thioglycoside **2.16**, bearing a TCE-protected sulfate group at C-4 was subjected to NBS in acetone/water to give the corresponding hemiacetal **2.32** in a 74 % yield. Reacting **2.32** with trichloroacetonitrile in the presence of a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at low temperature, provided the trichloroacetimidate **2.33** in 80 % yield (entry 9, table 2.3)

As mentioned in Chapter 1, Karst et al. removed the TFE moiety from sulfate groups in fully protected disaccharides in low to moderate yields.[24] It was anticipated that this would not be an issue with the TCE group, as it is removed under very mild conditions. To illustrate this, trichloroacetimidate donor **2.33** was coupled to glycosyl acceptor **2.29** in the presence of TMSOTf to give disaccharide **2.35** in 81 % yield (Scheme 2.1). Deprotection of **2.35** with zinc and ammonium formate gave the sulfo-deprotected product **2.36** in 92 % isolated yield (Scheme 2.1).



Scheme 2.1. Synthesis of a disulfated disaccharide

2.2 Objectives

One of the limitations of reagent **1.87** is that the sulfations need to be run in THF due to the low solubility of the triflate salt in other solvents, such as dichloromethane. This is a hindering factor for the reactions involving poorly nucleophilic substrates, as these reactions tend to proceed very slowly. It is suspected that for the slower reactions, reagent **1.87** begins to break down in THF before the sulfation occurs, thus the reaction requires additional aliquots over extended periods of time. For example, from table 2.1, the sulfation reaction with the 4-OH substrate **2.17** proceeded very slowly, and required up to 6 equivalents of **1.87** to reach completion over two days. Also, for the 2-*O*-sulfation of **2.11**, over 10 eq. of **1.87** were required over 72 hours. So although this new sulfation methodology gives the desired products in good yields, there is clearly room for improvement. The primary objective of the work described in this chapter is to develop an SIS with stability, solubility and sulfation properties that are superior to that of reagent **1.87**. Additional objectives are to examine the sulfate protecting group strategy as a means of preparing *N*-sulfated compounds including *N*-sulfated carbohydrates and to examine alternative means of removing the TCE groups from TCE-protected sulfate groups in carbohydrates.

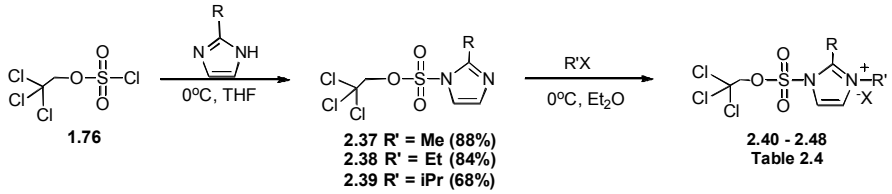
2.3 Results and Discussion

2.3.1 Derivatives of 2,2,2-Trichloroethoxysulfuryl Imidazolium Triflate (1.87)

*2.3.1.1 Synthesis of Derivatives of 2,2,2-Trichloroethoxysulfuryl Imidazolium Triflate (**1.87**)*

In conjunction with Dr. Ahmed M. Ali and Ahmed Desoky of the Taylor group, we set out to modify the imidazolium moiety in reagent **1.87** anticipating that the reactivity and/or stability of the sulfating agents could be tuned based on substitution on the imidazole ring. In doing so, we prepared a series of sulfuryl imidazolium triflates, all of which contained the TCE group, with different alkyl groups at the 2- and 3- positions of the imidazolium ring.[32] It was anticipated that modifying the imidazole moiety of reagent **1.87** with alkyl groups could potentially change the solubility enough such that the sulfation reactions could be performed in solvents that are less polar than THF such as dichloromethane. The tetrafluoroborate counterion was also examined. In most cases, the synthesis of these compounds was readily achieved by reacting 2,2,2-trichloroethylchlorosulfate **1.76** with an excess of the appropriate imidazole to give the corresponding TCE sulfuryl imidazole (compounds **2.37**-**2.39**). The sulfuryl imidazole compounds were then reacted with 1.0 eq. of methyl triflate, or trimethyl- or triethyloxonium tetrafluoroborate to give the sulfuryl imidazolium salts (table 2.4).

Table 2.4. Preparation of TCE Sulfuryl Imidazolium Salts.



Product	R	R'	X^-	Yield (%)
2.40	Me	Me	TfO^-	99
2.41	Et	Me	TfO^-	98
2.42	iPr	Me	TfO^-	85
2.43	Me	Me	BF_4^-	85
2.44	Et	Me	BF_4^-	79
2.45	iPr	Me	BF_4^-	ND
2.46	Me	Et	BF_4^-	81
2.47	Et	Et	BF_4^-	ND
2.48	iPr	Et	BF_4^-	ND

Upon formation, most of the modified imidazolium salts precipitated out of solution, and were easily isolated by filtration. It is likely that the precipitation of the product is required to drive the formation of the sulfuryl imidazolium salts. Compounds **2.45**, **2.47**, and **2.48** did not precipitate out of the reaction mixture irrespective of the solvent used (diethyl ether, THF, CH_2Cl_2), and the reaction did not go to completion even with extended reaction times. Attempts to selectively precipitate the products using non-polar solvents such as hexane or pentane were not successful, and semisolids consisting of both the starting material and product were obtained.

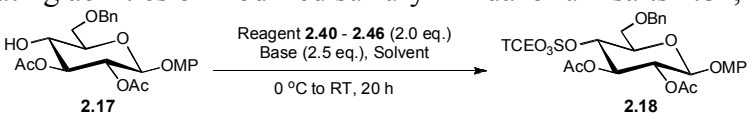
In general, sulfuryl imidazolium salts having the triflate counterion were obtained in higher yields than those having the tetrafluoroborate counterion. For example,

compound **2.42** was easily isolated in good yield, whereas the identical tetrafluoroborate salt **2.45** could not be isolated at all. In the preparation and isolation of derivatives **2.46**, **2.47**, and **2.48**, a few people encountered strong allergic reactions to the compounds, so these salts were not pursued any further. All of the isolated sulfuryl imidazolium salts were obtained in pure form, were white powders, and were stored at 4 °C for months without any detectable decomposition.

*2.3.1.2 Sulfating Abilities of TCE Sulfuryl Imidazolium Salts **2.40-2.46***

Carbohydrate **2.17** was chosen as a model compound to evaluate the sulfating abilities of each of the TCE sulfuryl imidazolium salts **2.40** to **2.46**, as it had previously been noted as being challenging to sulfate under the original optimized sulfating conditions (i.e. 2.5 eq. of **1.87** with 2.0 eq. 1-MeIm in THF, 0 °C). As previously mentioned in table 2.1, for the 4-*O*-sulfation of **2.17**, over 6 eq. of **1.87** was required over a 72 hour period to obtain sulfated **2.18** in a 76 % yield (table 2.1, entry 9). The yield of **2.18** was even lower when the reaction was performed in CH_2Cl_2, likely due to the very limited solubility of **1.87** in this solvent. To evaluate the new sulfuryl imidazolium salts, compound **2.17** was reacted with 2 eq. of the sulfating agents **2.40** to **2.46** in various solvents for 20 h. The results are shown in table 2.5. To have a direct comparison of each of the new reagents, the reaction was halted at 20 h, whether or not it had reached completion, and the isolated yield of **2.18** was determined after purification. This work was done in conjunction with Ahmed Desoky of the Taylor group.

Table 2.5. Sulfating abilities of modified sulfuryl imidazolium salts **1.87**, **2.40**-**2.44**, **2.46.**



Entry	Reagent	Base	Solvent	Yield (%)
1	**1.87**	1-MeIm	THF	70
2	**1.87**	1-MeIm	CH_2Cl_2	56
3	**1.87**	1-MeIm	DMF	35
4	**2.40**	1,2-DiMeIm	THF	65
5	**2.40**	1,2-DiMeIm	CH_2Cl_2	80
6	**2.40**	1-Me-2-iPrIM	CH_2Cl_2	53
7	**2.40**	1,2-DiMeIm	DMF	40
8	**2.41**	1-Me-2-EtIm	THF	18
9	**2.41**	1,2-DiMeIm	THF	58
10	**2.41**	1-Me-2-EtIm	CH_2Cl_2	21
11	**2.41**	1,2-DiMeIm	CH_2Cl_2	68
12	**2.42**	1-Me-2-iPrIm	THF	18
13	**2.42**	1,2-DiMeIm	THF	54
14	**2.42**	1-Me-2-iPrIm	CH_2Cl_2	30
15	**2.42**	1,2-DiMeIm	CH_2Cl_2	60
16	**2.43**	1,2-DiMeIm	CH_2Cl_2	79
17	**2.44**	1-Me-2-Et	CH_2Cl_2	38
18	**2.44**	1,2-DiMeIm	CH_2Cl_2	78
19	**2.46**	1-Et-2-MeIm	CH_2Cl_2	45
20	**2.46**	1,2-DiMeIm	CH_2Cl_2	75

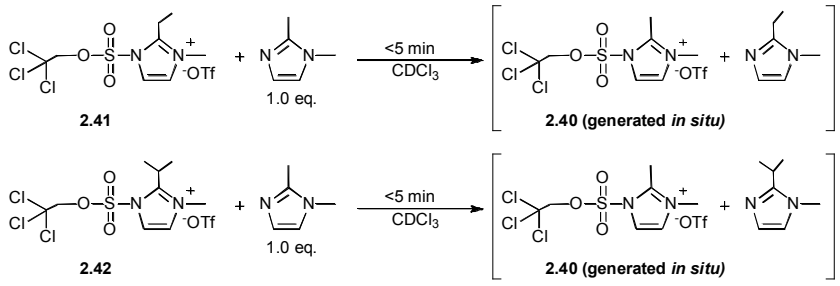
For sulfating agents **2.40** – **2.42**, CH_2Cl_2 proved to be a better reaction solvent than THF, as **2.18** was obtained in higher yields when the two solvents were compared under identical reaction conditions (for example, table 2.5 entry 1 vs. 2, and entry 4 vs.

5). Reagents **2.43** to **2.46** had very limited solubility in THF, thus were not evaluated in that reaction medium.

The use of sulfuryl imidazolium salts **2.40** and **2.43**, which bear a 1,2-dimethylimidazolium group, resulted in good yields when the sulfations were performed in CH_2Cl_2 using 1,2-dimethylimidazole (1,2-diMeIm) as the base (table 2.5, entries 5 and 16). The counterion (TfO^- or BF_4^-) had no effect on the isolated yield of **2.18**. Lower yields were obtained when the sulfating agents contained an ethyl or isopropyl group at the 2-position of the imidazole ring (**2.41**, **2.42**, **2.44**) if the base used was the same as the leaving group of the sulfating agent (1-methyl-2-ethylimidazole (1-Me-2-EtIm) for **2.41** and **2.44**; 1-methyl-2-isopropylimidazole (1-Me-2-*i*PrIm) for **2.42**, as shown by entries 10 and 17 in table 2.5. The yield of **2.18** increased considerably when the same sulfating reagents (**2.41** and **2.44**) were used in conjunction with 1,2-dimethylimidazole (entries 11 and 18, table 2.5) under the same reaction conditions.

To understand why 1,2-dimethylimidazole was a superior base to the others (i.e. 1-Me-2-EtIm, and 1-Me-2-*i*PrIm), nuclear magnetic resonance (NMR) investigations were performed on a selection of the sulfating agents and the corresponding bases. ^1H NMR studies in $CDCl_3$ revealed that just 1 eq. of 1,2-dimethylimidazole rapidly displaced the 1-methyl-2-ethylimidazole or the 1-methyl-2-isopropylimidazole from sulfating reagents **2.41** and **2.42** in less than five minutes, forming **2.40** in situ (Scheme 2.2), which has proven to be the better sulfating agent. Even after several hours, ^1H NMR provided no evidence that the reverse reaction to the original compound was occurring. This indicates that reagent **2.40** is more stable than **2.41** and **2.42**, possibly

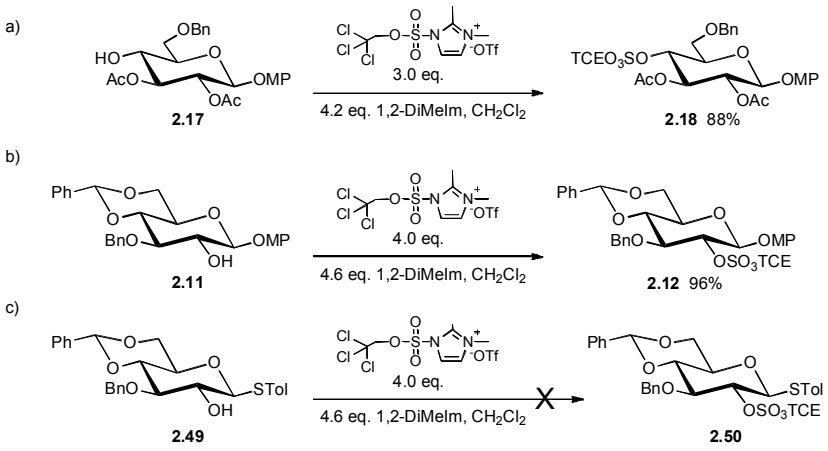
due to steric hindrance between the ethyl or isopropyl group at the 2-position of the imidazole ring, and the sulfate moiety and methyl group.



Scheme 2.2. In situ imidazolium exchange

Reduced yields of **2.18** were also observed using reagent **2.40** and 1-Me-2-iPrIm as a base in CH_2Cl_2 (table 2.5, entry 6). This suggests that the low yields encountered with sulfating agents **2.41**, **2.42**, and **2.44** could in part be due to the added imidazole itself, which is likely acting as a general base during the reaction. It is possible that there is greater steric crowding at the transition state of the reaction with reagents **2.41**, **2.42**, and **2.44** and the sterically hindered imidazole groups 1-Me-2-*i*PrIm and 1-Me-2-EtIm. Sulfating reagent **2.46**, which differs from **2.43** only by the presence of an ethyl instead of a methyl group on the alkylated nitrogen atom, gave lower yields than **2.43** when 1-ethyl-2-methylimidazole (1-Et-2-MeIm) was used a base (entry 16), but gave a similar yield when 1,2-DiMeIm was added (entries 19 and 20). These results again suggest that the 1-Et-2-MeIM group in **2.46** is exchanging in situ with 1,2-DiMeIm, forming a more reactive sulfating species.

Overall, sulfuryl imidazolium salt **2.40** was prepared in the highest yield, and provided the desired sulfated carbohydrate **2.18** in the highest yields. In light of this, reagent **2.40** was chosen for further study. Additional studies with reagent **2.40** and carbohydrate **2.17** revealed that **2.18** could be prepared in an 88 % yield in 24 h, using just 3 eq. of **2.40** and 4.3 eq. of 1,2-DiMeIm in CH_2Cl_2 (Scheme 2.3a). Furthermore, carbohydrate **2.12**, previously prepared in 90 % yield by subjecting **2.11** to 10.5 eq. of **1.87** in THF over 72 h (table 2.1) could be prepared using only 4 eq. of **2.40**, in a 96 % yield after just 24 h (Scheme 2.3b). Performing the reaction under the same conditions in THF gave only a 40 % of **2.12**, with unreacted starting material **2.11** still remaining after 24 h. This result demonstrates that these reactions can be subject to a significant solvent effect. Surprisingly, a complex mixture of products was obtained when **2.49**, the thioglycoside analogue of **2.11** was subjected to the optimized sulfating conditions (Scheme 2.3c). Despite numerous efforts, the target sulfated thioglycoside **2.50** could not be isolated. This was unexpected, as the sulfation of the 4-OH on thioglycoside **2.15** (table 2.1) progressed very smoothly to provide **2.16** in a 91 % yield. In the case of **2.49**, it is possible that upon 2-*O*-sulfation, an intramolecular displacement of the TCE sulfate by the sulfur atom occurs, resulting in the formation of a reactive episulfonium ion. Alternatively, it is possible that the thioglycoside is activated by the imidazolium triflate species, providing a reactive glycosyl donor that can decompose as the reaction proceeds.



Scheme 2.3. Improved Sulfation Reactions

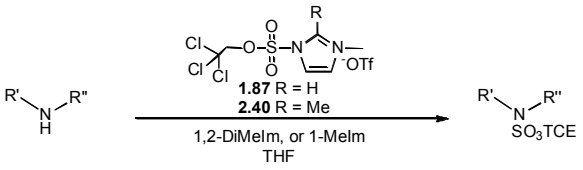
2.3.2 TCE-Protected *N*-Sulfate Moieties in Amines

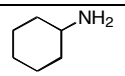
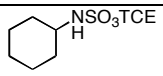
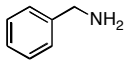
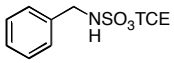
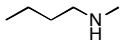
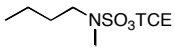
Glucosamine monomers are one of the most abundant sugars, found primarily in carbohydrate-based biomolecules. In naturally occurring polymers the residues can exist in the free amine form, but are most commonly found as the *N*-sulfated and *N*-acetylated derivatives. The scope and application of our TCE-protected sulfate chemistry was examined in the synthesis of both *N*- and *O*- sulfated glucosamine residues; and the compatibility of TCE protected sulfates with *N*-acetyl glucosamine donors was examined.

2.3.2.1 Sulfation of Simple Amines

The sulfation of simple amines was first examined as described in Table 2.6. Several primary and secondary commercially available amines were selected as substrates to examine sulfating abilities of reagents **1.87** and **2.40**. Table 2.6 summarizes the results of these studies.

Table 2.6. Sulfation of 1° and 2° aliphatic amines



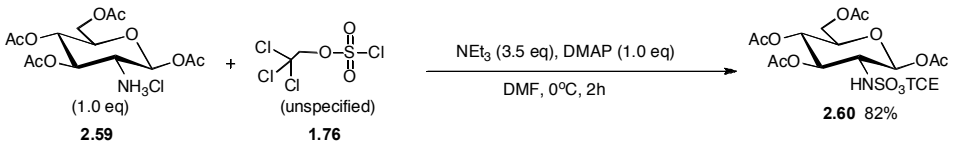
Amine	Product	Sulfation with 2.40[a]	Sulfation with 1.87[b]
		99%	13%
2.51	**2.52**		
		95%	14%
2.53	**2.54**		
		90%	ND
2.55	**2.56**		
		98%	ND
2.57	**2.58**		

[a] THF (0.23 M), 2.5 eq. 1,2-dimethylimidazole, 2.0 eq **1.87**, 8-14 h.
[b] THF (0.23 M), 2.5 eq. 1-methylimidazole, 2.0 eq. **2.40**, 8-14h

Sulfation of cyclohexylamine (**2.51**) and benzylamine (**2.53**) using reagent **2.40** gave the desired sulfated products **2.52** and **2.54** in excellent yields, whereas very poor yields were obtained using sulfating agent **1.87**.[32] *N*-Sulfation of secondary amines **2.55** and **2.57** with reagent **2.40** progressed smoothly affording the corresponding *N*-sulfates **2.56** and **2.58** in high yields. The sulfation of **2.55** and **2.56** with **1.87** was not examined. We do not have a clear explanation as to why SISs **1.87** and **2.40** behave so differently under identical conditions when amine nucleophiles are used, but demonstrate in the next section that this observation is not limited to simple aliphatic amines.

2.3.2.2 *Sulfation of 1,3,4,5-tetra-O-acetyl-β-D-glucosamine hydrochloride*

As our laboratory was investigating the sulfation of carbohydrate-based amines, Chen and Yu published the first literature example of *N*-sulfate protection with the TCE protecting group.[33] The authors demonstrated that readily available 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine hydrochloride (**2.59**) could be treated with our original sulfating agent, TCE-chlorosulfate **1.76,** in the presence of DMAP and NEt_3 to access the TCE-protected glucosamine-*N*-sulfate derivative **2.60** in an 82 % yield (Scheme 2.4). Unfortunately, very little experimental details (such as the required amount of chlorosulfate **1.76**) for the synthesis of **2.60** were given in the paper.



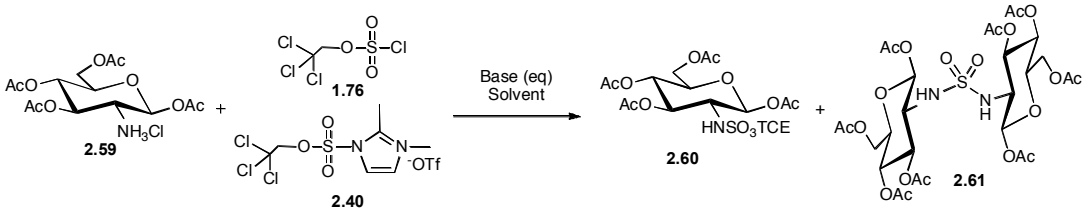
Scheme 2.4. TCE-protected *N*-Sulfate

Our initial attempts to repeat the literature sulfation of **2.59** published by Chen and Yu were unsuccessful, likely due to the lack of experimental details on the preparation of the compound. Yu was contacted to obtain the details of his synthesis, and it was learned that **2.60** was prepared by the addition of a solution of **2.59** and 3.0 eq. of NEt_3 in DMF over 1 h to a solution of **1.76** (6.0 eq) and DMAP (1.0 eq) in DMF. Under identical conditions, the best yield of **2.60** that could be obtained was 45 % (table 2.7, entry 1). The reaction produced two major products, one of which was the desired sulfated **2.60**, and second product was determined to be dimer **2.61**, a byproduct that was not mentioned in Chen and Yu's original report. This sulfation reaction was performed

numerous times under the literature conditions, and in each instance, **2.60** and **2.61** were formed in 40-45 % yields. Alternative reaction conditions were examined with hopes to increase the yield of **2.60**, while minimizing the formation of dimer **2.61** and the results are summarized in table 2.7. Adding 1.1 eq. reagent **1.76** to a solution of Et_3N and **2.59** in DMF at 0 ^oC resulted in only the formation of dimer **2.61** in low yield (entry 2). Performing the reaction under the same conditions described by Chen and Yu except CH_2Cl_2 was used in place of DMF (table 2.7, entry 3) gave exclusively dimer **2.61** in a 70 % yield.

Attempts to prepare compound **2.60** using reagent **2.40** also gave disappointing results with the best yield of **2.60** being only 29 % when the reaction was performed with 6.0 eq. of **2.40** in the presence of 3.5 eq. of 1,2-diMeIm (entry 7). In some instances we were able to isolate $(TCEO)_2SO_2$ (**2.62**). Performing the same reaction in DMF (entry 9, table 2.7) gave an even lower yield of **2.60** (18 %); however in neither case was dimer **2.61** formed. It was assumed that a stronger base such as NEt_3 is required for elimination of trichloroethanol from **2.60**, which would lead to dimer formation. Attempts to access **2.60** using 4.0 eq. of **2.40** and 1,2-diMeIm in the presence of NEt_3 or pyridine gave only trace amounts of the desired product (entries 10 and 11).

Table 2.7. Sulfation conditions for glucosamine **2.59.**



Entry	Reagent (eq)	Solvent	Base (Eq.)	2.60	2.61
1[a]	**1.76** (6.0 eq)	DMF	NEt_3 (3.5) DMAP (1.0)	40%	45%
2[b]	**1.76** (1.1 eq)	DMF	NEt_3 (3.5) DMAP (1.0)	0%	20%
3[a]	**1.76** (6.0 eq)	DCM	NEt_3 (3.5) DMAP (1.0)	0%	70%
4[b]	**2.40** (1.1 eq)	DMF	NEt_3 (3.5) DMAP (1.0)	0%	0%
5[b]	**2.40** (3.0 eq)	DCM	NEt_3 (3.5) DMAP (1.0)	0%	68%
6[b,c]	**2.40** (2.0 eq)	DCM	1,2-DiMeIm (4.0)	0%	0%
7[b,c]	**2.40** (6.0 eq)	DCM	1,2-DiMeIm (3.5)	29%	0%
8[a,c]	**2.40** (6.0 eq)	DCM	1,2-DiMeIm (3.5)	23%	0%
9[a]	**2.40** (6.0 eq)	DMF	1,2-DiMeIm (3.5)	18%	0%
10[a]	**2.40** (4.0 eq)	DCM	NEt_3 (1.0) 1,2-DiMeIm (4.0)	trace	Trace
11[a]	**2.40** (4.0 eq)	pyridine	NEt_3 (1.0)	trace	Trace

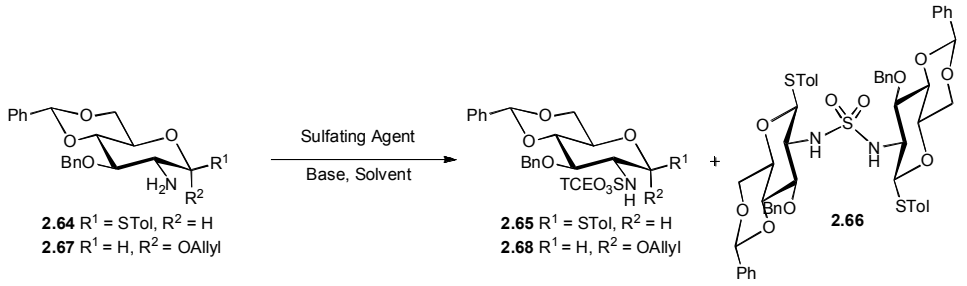
[a]**2.59** and base in solvent (0.1 M wrt **2.59**) added at 0 °C over 1 h to a solution of **1.76** or **2.40** and DMAP in solvent (2.0 M wrt **1.76** or **2.40**).
[b]**2.59** in solvent (0.05M) at 0 °C, add base then reagent **1.76** or **2.40**
[c] Isolated significant quantities of **2.62**

2.3.2.3 N-Sulfation of Glucosamine Derivatives as their Free bases

After encountering limitations when attempting to introduce the $TCESO_3$ moiety to per-acetylated glucosamine hydrochloride **2.61**, we turned our attention to glucosamine monomers bearing amino groups as their free bases. Two model substrates, **2.64** and **2.67**, were prepared as stable free amines, and were subjected to reagents **1.76**, **1.87** or **2.40** under a variety of conditions. The results are summarized in table 2.8.

Sulfation of **2.64** with reagent **1.76** using the conditions of Chen and Yu did not result in the formation of the *N*-sulfated product **2.65**; however dimer **2.66** was isolated in a 70 % yield (table 2.8, entry 1). Sulfation of **2.64** using reagent **1.76** in the presence of 1,2-DiMeIm gave **2.65** in a 60% yield (entry 2). Sulfation reactions using amine **2.64**, reagent **1.87** and 1-MeIm were unsuccessful or very low yielding as complex mixtures of products were formed (determined by TLC), and only trace quantities of the sulfated amine **2.65** were obtained (table 2.8, entries 3-5).

Table 2.8. *N*-Sulfations of glucosamine derivatives **2.64** and **2.67**

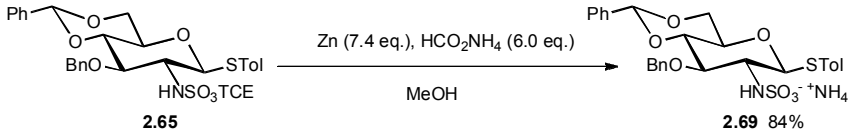


Entry	Substrate	Reagent (eq)	Solvent	Base (Eq.)	**2.65** or **2.68**	**2.66**
1	**2.64**	**1.76** (6.0)	DMF	NEt_3 (3.5), DMAP (1.0)	0%	70%
2	**2.67**	**1.76** (2.0)	THF	1,2-DiMeIm (4.0)	60%	N/A
3	**2.64**	**1.87** (2.0)	THF	1-MeIm (2.5)	Trace	0%
4	**2.64**	**1.87** (6.0)	THF	1-MeIm (4.0)	Trace	0%
5	**2.64**	**1.87** (6.0)	CH_2Cl_2	1-MeIm (4.0)	Trace	0%
6	**2.64**	**2.40** (6.0)	THF	1,2-DiMeIm (4.0)	94%	0%
7	**2.64**	**2.40** (6.0)	CH_2Cl_2	1,2-DiMeIm (4.0)	95%	0%
8	**2.67**	**2.40** (4.0)	THF	1,2-DiMeIm (3.5)	94%	N/A
9	**2.67**	**1.87** (6.0)	THF	1-MeIm (3.5)	9%	N/A

However, subjecting **2.64** to 6.0 eq. of **2.40** and 4.0 eq. of 1,2-DiMeIm in either THF or CH_2Cl_2 (entries 6 and 7) gave *N*-sulfated glucosamine **2.65** in 94-95% yield. Similarly, the sulfation of **2.67**, which bears an anomeric allyl protecting group in place of the thioglycoside, was readily achieved under the same conditions using reagent **2.40** and 1,2-DiMeIm to give *N*-sulfated product **2.68** in a 94 % yield (table 2.8, entry 8) while reagent **1.87** gave only a 9% yield of **2.68** (entry 9). Thus, the difference in reactivity between sulfating agents **1.87** and **2.40** that was observed during the sulfation of simple amines (table 2.6) was also observed in the sulfations of carbohydrate-based amines in

that little or none of the desired product was obtained with reagent **1.87**. We do not yet have a clear understanding as to why sulfating agents **1.87** and **2.40** behave so differently with amines. One possible explanation could be that a free amine may deprotonate the 2-position of the imidazole ring in **1.87**, forming a reactive imidazolium carbene species; a side reaction that could not occur with **2.40**. Alternatively, attack of the amine at the 2-position of the imidazolium ring of **1.87** may also occur. These hypotheses could not be investigated by NMR, however, as **1.87** is poorly soluble in $CDCl_3$.

The *N*-sulfated thioglycoside **2.65** was subjected to the standard zinc and ammonium formate deprotection conditions to provide the unmasked N-sulfamate **2.69** in an unoptimized 84% yield (Scheme 2.5).



Scheme 2.5. Deprotection of the TCE-protected *N*-sulfate

2.3.2.4 Attempted Synthesis of a Heparin Based Monosaccharide

While exploring the synthesis of *N*-sulfated monosaccharide residues, we turned our attention to heparin derivatives such as the disaccharide fragment shown in Figure 2.2.

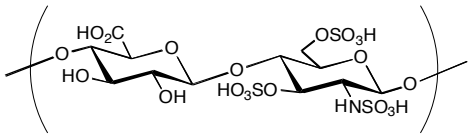
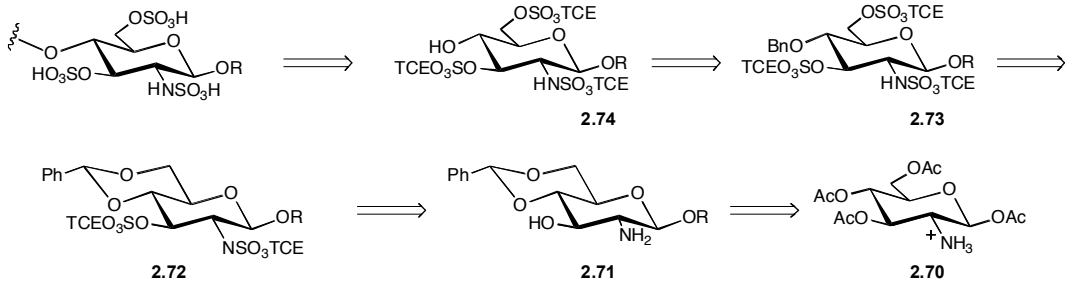


Figure 2.2. Heparin disaccharide fragment

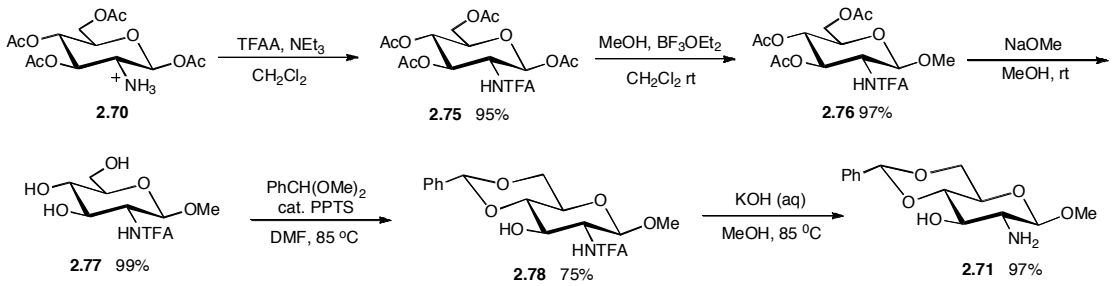
It was anticipated that the trisulfated glucosamine residue could easily be prepared using the TCE-protection chemistry, with the sulfate groups being introduced in a stepwise fashion as described in the retrosynthesis in Scheme 2.6.



Scheme 2.6. Retrosynthesis of a trisulfated heparin monosaccharide

The synthesis of the trisulfated heparin acceptor was designed such that the per-acetylated glucosamine hydrochloride **2.70** could be modified to obtain amino-alcohol **2.71**, which could be subjected to a simultaneous di-sulfation to obtain **2.72**. Regioselective reductive opening of the benzylidene acetal in **2.72** would lead to a free 6-OH, which can then be sulfated to provide the fully protected trisulfated monomer **2.73**.

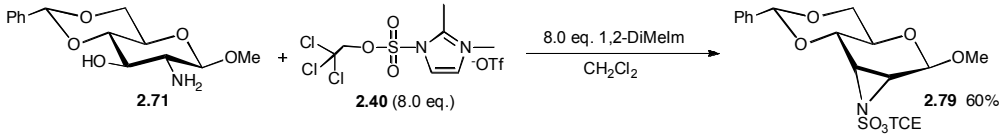
Scheme 2.7 describes the completed synthetic steps towards the synthesis of the trisulfated acceptor **2.74** (previously shown in scheme 2.6).[34]



Scheme 2.7. Synthesis of amino-alcohol **2.71**

Compound **2.71** was prepared from **2.70** using literature procedures.[34] Fully protected glucosamine residue **2.75**, was easily converted into methyl glycoside **2.76** with an anomeric protecting group exchange using methanol and the boron trifluoride diethyl etherate promoter. Selective removal of the acetate esters in **2.76** under Zemplén conditions yields triol **2.77**, which was subjected to 4,6-*O*-benzylidenation with dimethoxybenzaldehyde under acid catalyzed conditions to give partially protected **2.78** in moderate yields. Finally, alkaline hydrolysis of the trifluoroacetamide group provided the target amino-alcohol **2.71**.

Unfortunately, the *N*- and *O*- disulfation did not proceed as expected. The reaction progressed very slowly, and required a considerable excess of **2.40** to push the reaction to completion (as determined by consumption of starting material). More significantly, as shown in Scheme 2.8, none of the desired disulfated compound **2.72** was obtained. Instead, the only identifiable product was the sulfated aziridine **2.79**, isolated after column chromatography in a 60 % yield.



Scheme 2.8. Formation of *N*-sulfated aziridine product **2.79**

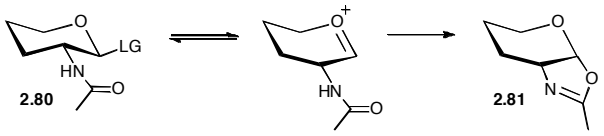
Despite subjecting **2.71** to a variety of conditions to introduce the TCE-protected sulfate groups, the formation of the *N*-sulfated aziridine product **2.79** could not be avoided.[35] This highlights one of the limitations of the TCE-protecting group chemistry,

as it cannot be used to introduce TCE-protected sulfates to a 1,2-*trans* amino-alcohol functionality.

2.3.3 Alternative Conditions for the Deprotection of TCE-protected sulfates

Naturally occurring glucosamine residues are most commonly found in the form of a 2-acetamido-2-deoxyglucopyranoside. These monosaccharides are constituents of biologically significant peptidoglycans, proteoglycans, glycosaminoglycans, glycoproteins including milk oligosaccharides, as well as the blood group glycolipids.[36] Because of this prevalence, chemical synthesis of naturally occurring oligosaccharides can often require the use of a 2-acetamido-2-deoxyglucopyranoside glycosyl donor.

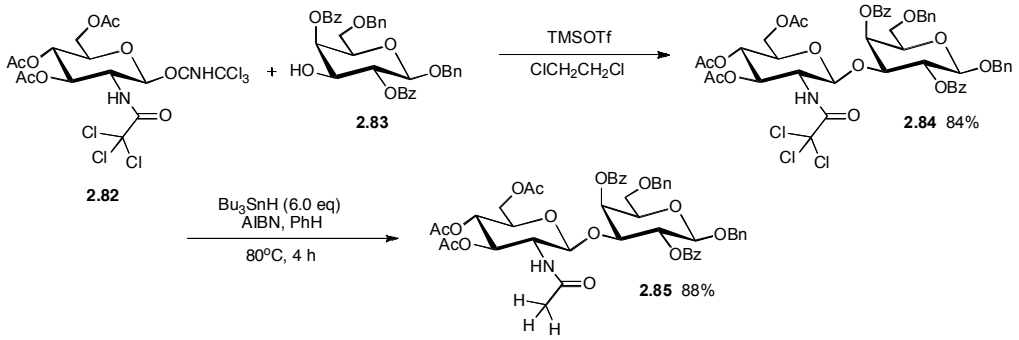
N-acetylated glucosamine donors (depicted by **2.80**) are not commonly used for difficult glycosylation reactions, as oxazoline compounds (such as **2.81**) can readily form upon donor activation (Scheme 2.9). In certain cases, these oxazoline compounds are very stable, and can prevent any glycosylation reactions from occurring.



Scheme 2.9. Stable oxazoline formation

In 1994, Blatter, Beau and Jacquinet provided a possible solution to this problem.[37] They proposed the use of a trichloroacetamide at the 2-position of the glycosyl donor, suggesting that upon activation the corresponding 2-(trichloromethyl)oxazolinium ion would form. This intramolecular trichloroacetimidate

species would prove to be a reactive glycosyl donor for the synthesis of 1,2-*trans*-2-aminodeoxyglycosides. Using *N*-trichloroacetylated amines such as **2.82** in Scheme 2.10, the authors demonstrated that the compounds could be effective donors for incorporating the 2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl units into disaccharides such as **2.84** (Scheme 2.10), in good yields and high 1,2-trans selectivity. The main advantage of their method over other 2-amino protection strategies is that the conversion of *N*-trichloroacetyl in **2.84** to the required *N*-acetyl counterpart **2.85** (Scheme 2.10) is achieved in a single step using tributylstannane and AIBN, affecting very few other protecting groups that are used in carbohydrate chemistry.

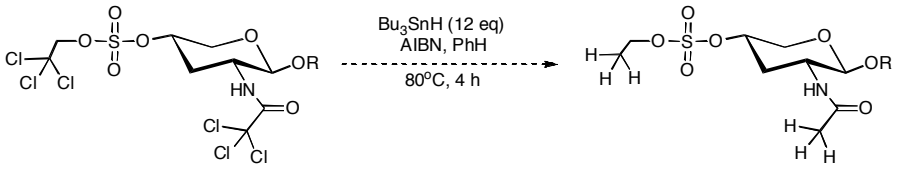


Scheme 2.10. 2-Trichloroacetamido-2-deoxyglucopyranosyl glycosyl donors[37]

2.3.3.1 Deprotection of TCE-protected sulfates using tributylstannane and AIBN

2-Trichloroacetamido-2-deoxyglucopyranosyl donors such as **2.82** presented a potential complication to our TCE-sulfate protecting strategy. Reduction of the trichloroacetamide using tributylstannane and AIBN could possibly result in some or complete reduction of the TCE sulfate moiety to a di- or monochloroethyl-protected sulfate or even to an ethyl-protected sulfate as shown in Scheme 2.11. This would not be

a problem as long as the sulfate group can eventually be completely deprotected at the end of the synthesis.

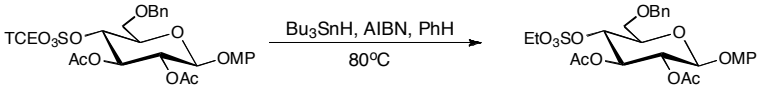


Scheme 2.11. TCE and TCA co-reduction using tributylstannane and AIBN

To determine how tributylstannane/AIBN would affect a TCE-protected sulfate group we subjected a model monosaccharide (**2.18**) to the literature reaction conditions (3 eq. tributylstannane, AIBN, benzene, 80 ^{o}C, 1 h) for the reduction of the *N*-trichloroacetamide. This resulted in a mixture containing mostly starting material, with traces of mono- and didechlorinated products (entry 1, table 2.9). Ideally, we wished to develop conditions that would result in the complete reduction of the 2,2,2-trichloroethyl group to the ethyl group. Increasing the amount of reducing agent as well as the reaction time lead to mixtures of the mono- di- and fully dechlorinated product, and in one instance (entry 4), the desired completely dechlorinated product **2.86** was isolated in a 91 % yield. Unfortunately, these reaction conditions could not be easily reproduced, and mixtures of partially and fully dechlorinated products were often obtained. The execution of these reductions required tedious work-ups because of the large excess of tin reagents; and the dechlorinated products were very difficult to purify, often requiring 3 to 4 purifications by flash column chromatography. It was then reasoned that it would take

at least 12 equivalents of tributylstannane to co-reduce one TCE group along with one trichloroacetamide, and the required work-up and purification would not be favourable for products more complicated than simple monosaccharides.

Table 2.9. Reduction of the 2,2,2-trichloroethyl group in compound **2.18** using tributylstannane, AIBN.

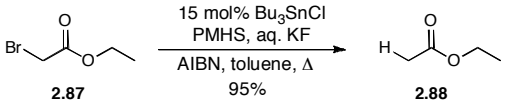


2.18 **2.86**

Entry	Bu_3SnH	Time	Work-Up / Purification	Result
1	3.0 eq.	1 h	H_2O Wash / 3 columns	Mostly **2.18**, trace mono, di-dechlorinated products
2	6.0 eq.	1 h	H_2O Wash / 4 columns	Mixture of mono-, di- and tri-dechlorinated product
3	6.0 eq.	2 h	CH_3CN-Hexanes / 2 columns	~95 % contaminated with trace didechlorinated
4	6.0 eq.	4 h	CH_3CN-Hexanes / 3 columns	91%

2.3.3.2 Deprotection of TCE-protected sulfates using polymethylhydrosiloxane, aqueous KF and catalytic Bu_3SnCl.

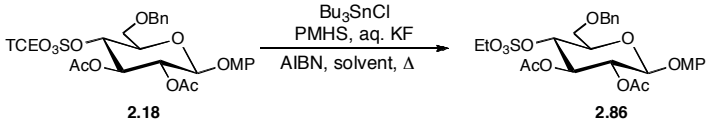
In 1990, Terstiege and Maleczka reported a radical dehalogenation pathway that was performed under catalytic tin conditions as described by Scheme 2.12.[38] In this reaction, the organotin hydride is generated *in situ*, using polymethylhydrosiloxane (PMHS) and aqueous KF.



Scheme 2.12. Catalytic tributyltin chloride reduction

The TCE-protected monosaccharide **2.18** was subjected to the catalytic reduction conditions developed by Terstiege and Maleczka with the intent to effectively convert the TCE moiety to the corresponding ethyl group. The results are summarized in table 2.10. Unfortunately, the desired compound was never obtained under the above conditions. Several variables were examined, including amount of tributyltin chloride, aliquots of PMHS with aqueous KF, solvent, and reaction time; but at best, only 2:1 mixtures of tri-di dechlorinated products were obtained (table 2.10, entries 5 and 6). Attempts to force the reaction to completion with excess KF and PMHS resulted in a complex mixture of products as determined by TLC. This approach was not pursued any further.

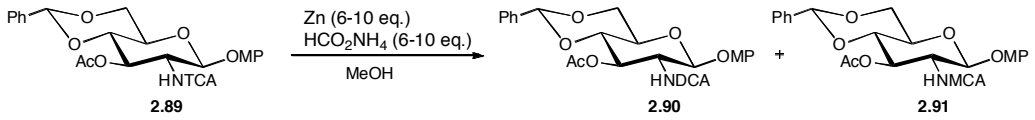
Table 2.10. Attempted catalytic reduction conditions



Entry	Bu_3SnCl	PMHS / aq. KF	Conditions	Result
1	0.1 eq	1.5 eq (x3, 2h)	$PhCH_3$, 8h	N.R.
2	0.1 eq	1.5 eq (x3, 2h)	PhH, 8h	Trace product
3	0.25 eq	1.5 eq (x3, 2h)	PhH, 8h	Trace product
4	0.5 eq	1.5 eq (x3, 2h)	PhH, 8h	Trace product
5	1.0 eq	3.0 eq (X2, 1h)	PhH, 2h	2:1 tri:di dechlorinated
6	1.0 eq	3.0 eq (x3, 1h)	PhH, 3h	2:1 tri:di dechlorinated
7	1.0 eq	3.0 eq (x6, 1h)	PhH, 6h	Complex mixture

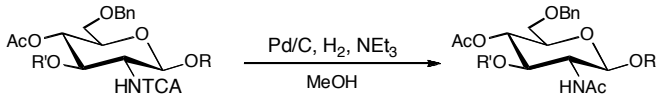
2.3.3.3 Deprotection of TCE-protected sulfates using Catalytic Hydrogenolysis in Basic Media

We then turned our attention to other dehalogenation conditions that, in theory, would be more mild for the carbohydrate starting material. Since the TCE group is easily removed using zinc and ammonium formate, it was reasoned that similar conditions might in fact reduce the trichloroacetamide moiety as well. As shown in Scheme 2.13, *N*-trichloroacetylated glucosamine derivative **2.87** was subjected to varying amounts of zinc and ammonium formate in methanol. Unfortunately, only mixtures of the mono- and di-dechlorinated products **2.90** and **2.91** were observed, with no evidence of the completely dechlorinated product being formed.



Scheme 2.13. Zinc and ammonium formate mediated dechlorination

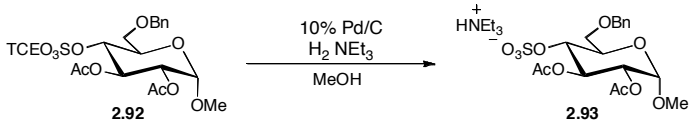
In 2000, Mulard and Ughetto-Monfin reported that during debenzylation of a protected pentasaccharide under catalytic hydrogenation conditions (Pd/H_2), the *N*-trichloroacetamide group was partially reduced to a monochloroacetate.[39] The authors took advantage of this and found that the *N*-trichloroacetate moiety could be efficiently transformed to the required *N*-acetamide group when the debenzylation was performed using Pd and H_2 under slightly basic media, as described in Scheme 2.14.



Scheme 2.14. Hydrogenation conditions for trichloroacetamide reduction

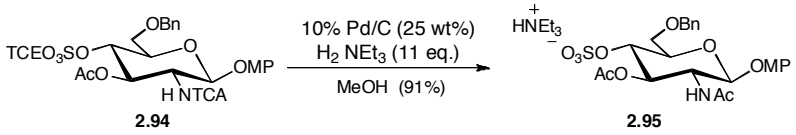
TCE-protected carbohydrate sulfate **2.92** was subjected to the basic catalytic hydrogenation conditions described by Scheme 2.13, to determine if the TCE protecting group could be removed without subsequent loss of the sulfate moiety (table 2.11). The literature procedure describes the use of "2 drops" of triethylamine; thus some optimization was required to obtain the reduced product in good yields. Initial attempts using the literature conditions resulted in desulfated product (table 2.11, entries 1-3). Presumably, HCl is formed as the deprotection progresses, and sulfate groups are known to be unstable in acidic media. Increasing the amount of triethylamine to 5 eq. allowed for the isolation of the desired sulfated carbohydrate **2.93** in an excellent yield (entry 4), and decreasing the amount of palladium catalyst from 50 wt % to 10 wt % increased the reaction time slightly, but had no effect on the yield of the desired product (entry 5).

Table 2.11. Pd/H_2 and NEt_3 conditions for TCE deprotection.



Entry	NEt_3 (eq.)	10 % Pd/C	Time	Result
1	5 drops	50 wt %	30 min	Desulfated product
2	1 eq	50 wt %	30 min	Desulfated product
3	2 eq	50 wt %	30 min	Desulfated product
4	5 eq	50 wt %	30 min	97 %
5	5 eq	10 wt %	90 min	98 %

To determine if the TCE and trichloroacetamide groups could be reduced simultaneously, TCE-protected sulfated monosaccharide **2.94** was subjected to the optimized conditions from table 2.9. Compound **2.95** was easily prepared in a 91% yield by subjecting **2.94** to catalytic hydrogenation conditions in the presence of triethylamine in methanol as described by Scheme 2.15.



Scheme 2.15. Co-reduction and deprotection of trichloroacetamide and TCE groups

2.4 Summary and Future Work

In 2004, we reported the first synthesis of a sulfuryl imidazolium salt. Compound **1.87** was effective for introducing the TCE-protected sulfates into simple monosaccharides in good yields. The resulting TCE-protected sulfocarbohydrates were found to be stable to various manipulations often encountered in carbohydrate synthesis;

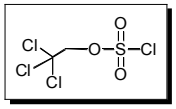
and yet the sulfate groups could easily be deprotected as required in excellent yields. However, reagent **1.87** was not without its limitations. To circumvent some of the limitations of **1.87** a series of modified sulfuryl imidazolium salts were prepared and studied. We demonstrated that by incorporating a methyl group at the 2-position of the imidazolium ring of **1.87** a more efficient sulfating agent, **2.40**, was obtained. *O*-Sulfations that required prolonged reaction times and a large excess of the original reagent **1.87**, were more readily achieved using reagent **2.40**. Attempts to incorporate a TCE-protected sulfate at the 2-position of a thioglycoside failed revealing a limitation of our sulfate protecting group approach. Furthermore, we expanded the scope of the chemistry to include the synthesis of selected *N*-sulfated glucosamine derivatives. Certain *N*-sulfated compounds that that were practically inaccessible using **1.87** could be obtained in excellent yield using SIS **2.40**. However, shortcomings were observed as a target 2-*N*, 3-*O*-disulfated product could not be prepared. The compatibility of the TCE-protected sulfate groups with the reduction of *N*-trichloroacetylated glucosamine donors was also resolved, as it was demonstrated that the *N*-trichloroacetamide and TCE-protected sulfate could be simultaneously reduced and deprotected under the same conditions. These successes suggest that the TCE-protection strategy could be considered a viable approach to the synthesis of complex sulfated oligosaccharides. Clearly the next step in this work is the application of our sulfate protecting group strategy to the total synthesis of a multisulfated oligosaccharide. Our efforts towards this goal are discussed in Chapters 3 and 4.

2.5 Experimental

2.5.1 General Information

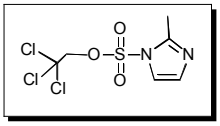
All reactions were carried out using freshly distilled solvents unless otherwise noted. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium metal in the presence of benzophenone under argon. CH_2Cl_2 was distilled from calcium hydride under nitrogen. Flash chromatography was performed using silica gel 60 Å (234-400 mesh). Chemical shifts (δ) for ^1H NMR spectra are reported in parts per million (ppm) relative to Me_4Si (0.0 ppm) or DMSO-d_6 (2.49 ppm) and are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened), integration, coupling constant in Hz, and assignment. Chemical shifts (δ) for ^{13}C spectra are reported in ppm relative to $CDCl_3$ (δ 77.0, central peak) or DMSO-d_6 (δ 39.5, central peak). Chemical shifts (δ) for ^{19}F spectra are reported in ppm relative to an external standard (δ 0.0, $CFCl_3$). All peak assignments were confirmed using 2D-NMR (COSY, HMQC) techniques. Optical rotations were measured at the sodium D line at ambient temperature in cells with 1 dm path length. All melting points are uncorrected. High resolution mass spectra were obtained at the University of Waterloo Mass Spectrometry Facility. HRMS data for chlorine containing compounds is reported for ^{35}Cl. Compounds **2.38**, **2.39**, and **2.41-2.48** were prepared by Dr. Ahmed Ali and Ahmed Desoky.

2.5.2 Experimental Syntheses

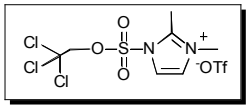


2,2,2-Trichloroethoxysulfuryl chloride (1.76). Procedure modified from the original synthesis.[40] Distilled sulfuryl chloride (20.0 mL, 0.250 mol) was added dropwise via syringe pump over 1 h to a solution of pyridine (20.5 mL, 0.250 mol) and 2,2,2-trichloroethanol (24.0 mL, 0.250 mol) in distilled Et_2O at –78 °C. The resulting slurry was stirred for an additional 1 h at –78 °C, then stirred for 3 h at room temperature. The precipitate was removed by suction filtration and the filtrate was concentrate to a crude oil. Purification by vacuum distillation afforded **1.76** as a clear colourless oil (54.4 g, 88%). Boiling point 71 °C/ 8 mm Hg. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90 (s, 2H, CH_2).

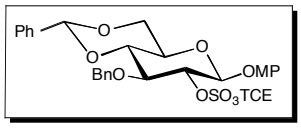
General procedure for the synthesis of 1-(2,2,2-Trichloroethoxysulfuryl) 2-substituted imidazoles (2.37, 2.38, 2.39). To a solution of appropriately 2-substituted imidazole (0.072 mol, 3.60 eq.) in dry THF (40 mL) at 0 ^{o}C was added dropwise a solution of 2,2,2-trichloroethoxysulfuryl chloride (**1.76**) (5.0 g, 0.02 mol, 1.00 eq.) in THF (50 mL). The reaction was stirred at 0 ^{o}C for 1 h, warmed to room temperature and stirred for an additional hour. The reaction mixture was filtered; residue washed with THF and the combined filtrate was concentrated under vacuum. The crude residue was purified by flash chromatography using (33:67 EtOAc/hexanes).



2,2,2-Trichloroethoxysulfuryl-(2-methyl)imidazole (2.37). To 2-methyl imidazole (0.493 g 6.0 mmol) in CH_2Cl_2 (1 mL) at 0 ^{0}C was added a solution of 2,2,2-trichloroethyl chlorosulfate **1.76** (0.5 g 2.0 mmol) in CH_2Cl_2 (1.5 mL). The reaction was gradually warmed to room temperature, and allowed to stir for 24 h. The crude material was concentrated, then purified by flash chromatography (33:67 EtOAc/hexanes) to provide **2.37** as a white solid (0.464 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (s, 3H, CH_3), 4.66 (s, 2H, CH_2), 6.96 (d, 1H, J = 1.5 Hz, ImH), 7.33 (d, 1H, J = 1.5 Hz, ImH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 79.9, 91.6, 120.0, 128.2, 146.4. Melting point 53-55 °C.

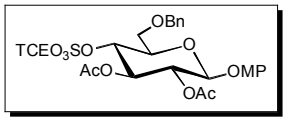


2,2,2-Trichloroethoxysulfuryl-(2-methyl)-*N*-methylimidazolium triflate (2.40). To a solution of 2,2,2-trichloroethoxysulfuryl-(2-methyl)imidazole **2.37** (5.0 g, 17.0 mmol) in Et_2O (65 mL) at 0 ^{o}C was added dropwise methyl triflate (2.1 mL, 18.7 mmol). The reaction was stirred for 3 h at 0 ^{o}C during which time a white precipitate formed. The desired product was isolated by suction filtration, in which the first filtrate was cooled to re-precipitate any remaining product (1x) and washed with cold Et_2O to afford **2.40** as a fluffy white solid (6.32 g, 81%). ^{1}H NMR (300 MHz, MeOD) δ 2.96 (s, 3H, CH_3), 3.96 (s, 3H, CH_3), 5.38, (s, 2H, CH_2), 7.77 (d, 1H, J = 1.6 Hz, ImH), 8.10 (d, 1H, J = 1.6 Hz, ImH); ^{13}C NMR (75 MHz, MeOD) δ 10.5, 35.3, 82.0, 91.6, 120.7, 123.5, 148.6. HRMS (+ESI) m/z = 306.9469, $C_7H_{10}Cl_3N_2O_5$ $(M\text{-}OTf)^+$ requires 306.9478.



4-Methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,2,2-trichloroethoxysulfo)-*β*-D-glucopyranoside (2.12): To carbohydrate **2.11** (0.200 g, 0.431 mmol) in freshly distilled dichloromethane (2.7 mL) at 0 °C was added 1,2-dimethylimidazole (0.100 g, 1.0 mmol) followed by sulfating agent **2.40** (0.400 g, 0.874 mmol). The reaction was gradually brought to room temperature by allowing the ice bath to melt, and then stirred for 16 hours. Analysis by TLC indicated remaining starting material, thus additional 1,2-dimethylimidazole (0.100 g, 1.0 mmol) and sulfating agent **2.40** (0.4 g, 0.874 mmol) were added at room temperature. The reaction was stirred for an additional 8 hours, at which point no starting material was detected by TLC. The reaction was diluted with dichloromethane (1.0 mL) and quenched with water (1.0 mL). The organic layer was separated and dried over $MgSO_4$, and concentrated to a brown crude oil. Purification by flash chromatography (25:75 EtOAc/hexanes) afforded **2.12** as an amorphous white solid (0.278 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (ddd, 1H, $J_{5,6ax}$ = 9.7, $J_{5,4}$ = 9.5, $J_{5,6eq}$ = 5.0 Hz, H5), 3.80 (s, 3H, CH_3), 3.86 ($t_{app,}$ 1H, $J_{6eq,6ax}$ = $J_{6ax,5}$ = 10.4 Hz, $H6_{ax}$), 3.88 ($t_{app,}$ 1H, $J_{4,5} = J_{4,3}$ = 9.2 Hz, H4), 3.98 (t_{app}, 1H, $J_{3,4} = J_{3,2}$ = 9.1 Hz, H3), 4.43 (dd, 1H, $J_{6eq,6ax}$ = 10.5, $J_{6eq,5}$ = 5.0 Hz, $H6_{eq}$), 4.68, 4.71 (AB, 2H, J = 11.1 Hz, CH_2CCl_3), 4.85 (m, 1H, H2), 4.87, 5.01 (AB, 2H, J = 11.2 Hz, CH_2Ph), 5.02 (d, 1H, $J_{1,2}$ = 7.8 Hz, H1), 5.61 (s, 1H, CHPh), 6.85-6.88 (m, 2H, ArH), 7.06-7.09 (m, 2H, ArH), 7.33-7.50 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 66.2, 66.3, 74.6, 77.9, 79.9, 81.2, 83.9, 92.6, 99.9, 101.4, 114.6, 118.8, 125.9, 127.9, 128.09, 128.11, 128.25, 128.27, 128.28, 128.4, 129.1, 136.7, 137.1, 150.2,

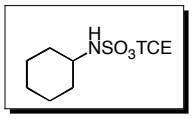
156.0; Melting point 105-107 °C; HRMS (EI) *m/z* = 674.0546, $C_{29}H_{29}Cl_3O_{10}S$ requires 674.0547.



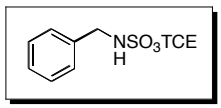
4-Methoxyphenyl 2,3-di-*O*-acetyl-6-*O*-benzyl-4-*O*-(2,2,2-trichloroethoxysulfo)- β-D-glucopyranoside (2.18):

To carbohydrate **2.17** (0.25 g, 0.54 mmol) in CH_2Cl_2 (2.2 mL, 0.25 M) at 0 °C was added 1,2-dimethylimidazole (0.130 g, 1.35 mmol) followed by the sulfating agent **2.40** (0.48 g, 0.104 mmol). The reaction was stirred at 0 °C, allowed to warm to room temperature by allowing the ice bath to melt. After 12 h, additional 1,2-dimethylimidazole (0.104 g, 1.08 mmol) and **2.40** (0.24 g, 0.524 mmoL) were added, and the reaction was stirred for an additional 12 h at rt. The solvent was removed under vacuum and the crude oil was immediately applied to a silica gel column. Flash chromatography (27:75 EtOAc/hexanes) yielded **2.18** as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.77-3.81 (m, 3H, H5, H6, H6'), 4.59, 4.62 (AB, 2H, J = 11.8 Hz, CH_2Ph), 4.68, 4.73 (AB, 2H, J = 11.1 Hz, CH_2CCl_3), 4.97 (d, 1H, $J_{1,2}$ = 7.9 Hz, H1), 5.00 (t_{app}, 1H, $J_{4,5} = J_{4,3}$ = 9.3 Hz, H4), 5.22 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{2,1}$ = 7.9 Hz, H2), 5.42 (t_{app}, 1H, $J_{3,4} = J_{3,2}$ = 9.3 Hz, H3), 6.78 – 6.81 (m, 2H, ArH), 6.94 – 6.97 (m, 2H, ArH), 7.26 – 7.34 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 20.7, 55.6, 67.9, 71.4, 73.2, 73.8, 79.3, 80.2, 92.5, 100.2, 114.6, 118.8, 127.8, 127.8, 128.4, 137.5, 150.8, 155.9, 169.3, 170.3; $[\alpha]_D^{25}$ =-40.7 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 688.0779 $(M+NH_4)^+$ $C_{26}H_{33}Cl_3NO_{12}S$ requires 688.0789.

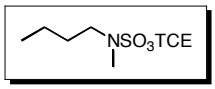
General procedure for preparation of sulfated amines 2.51-2.57. To the appropriate amine (1.0 eq) in THF (0.23 M) at 0 °C was added 1,2-dimethylimidazole or 1-methylimidazole (2.5 eq) followed by sulfating agent **2.40** or **1.87** (2.0 eq). The reactions were gradually brought to room temperature, and stirred for 8-14 hours. The crude material was applied directly to a silica gel column, and purified by flash chromatography (33:67 EtOAc/hexanes).



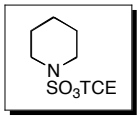
2,2,2-Trichloroethyl cyclohexylsulfamate (**2.52):** 99% as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22-1.41 (m, 5H, CH_2), 1.61-1.79 (m, 3H, CH_2), 2.06-2.11 (m, 2H, CH_2), 3.44-3.48 (m, 1H, CHN), 4.59 (d, 1H, J = 12.4 Hz, NH), 4.64 (s, 2H, CH_2CCl_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.59, 25.06, 33.59, 54.17, 78.15, 93.50; Melting point 80-82 °C; HRMS (EI) m/z = 308.9756, $C_8H_{14}Cl_3NO_3S$ requires 308.9760.



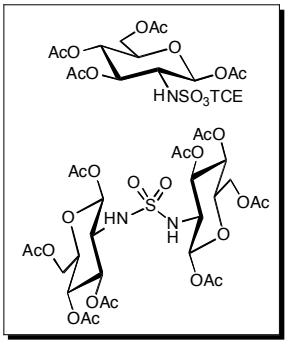
2,2,2-Trichloroethyl benzylsulfamate (2.54): 95% as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.38 (d, 2H, J = 5.8 Hz, CH_2Ph), 4.56 (s, 2H, CH_2CCl_3), 4.92 (br-s, 1H, NH), 7.32-7.35 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.12, 78.27, 93.40, 128.41, 128.50, 129.01, 135.52; Melting point 87-89 °C; HRMS (ESI) m/z = 334.9778, $C_9H_{14}Cl_3N_2O_3S$ ($M+NH_4$) requires 334.9791.



2,2,2-Trichloroethyl butyl(methyl)sulfamate (2.56): 90% as a clear colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3H, J = 7.3 Hz, $C\underline{H}_3CH_2CH_2CH_2N$), 1.34 (tq, 2H, $J_1 = J_2$ = 7.4 Hz, $CH_3C\underline{H}_2CH_2CH_2N$), 1.60 (tt, 2H, $J_1 = J_2$ =7.3 Hz, $CH_3CH_2C\underline{H}_2CH_2N$), 2.93 (s, 3H, CH_3), 3.27 (t, 2H, J = 7.4 Hz, $CH_3CH_2CH_2C\underline{H}_2N$), 4.57 (s, 2H, CH_2CCl_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.62, 19.59, 29.17, 35.49, 51.16, 77.67, 93.79; HRMS (EI) m/z = 296.9753, $C_7H_{14}Cl_3NO_3S$ requires 296.9760.

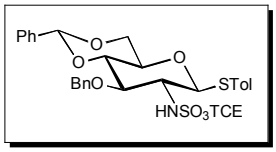


2,2,2-Trichloroethyl piperidine-1-sulfonate (2.58): 98% as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57-1.70 (m, 6H, CH_2), 3.35 (t, 4H, J = 5.3 Hz, CH_2N), 4.58 (s, 2H, CH_2CCl_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.38, 24.77, 47.86, 77.68, 93.79; Melting point 55-58 $^{\circ}$C; HRMS (EI) m/z = 294.9608, $C_7H_{12}Cl_3NO_3S$ requires 294.9603.

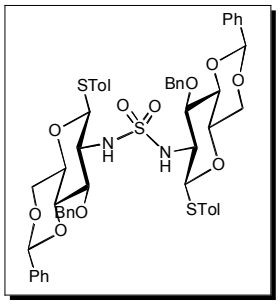


Acetyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-(2,2,2-trichloroethoxysulfamido)-β-D-glucopyranoside (2.60) and dimeric by-product (2.61): A solution containing carbohydrate **2.59** (0.200 g, 0.521 mmol) and NEt_3 (0.25 mL, 1.78 mmol) in dry DMF (10.0 mL) was added dropwise via syringe pump over 1.5 hours to a solution of reagent **1.76** (0.41 mL, 3.13 mmol) and DMAP (0.064 g, 0.521 mmol) in dry DMF (2.0 mL) at 0 °C. The reaction was stirred for

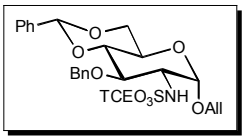
an additional 30 minutes at 0 °C, then diluted with dichloromethane (10 mL) and quenched with ice water (5 mL). The organic phase was separated, and the remaining aqueous phase was extracted with dichloromethane (2x5 mL). The combined organics were dried over $MgSO_4$ and concentrated to a crude yellow oil. Purification by flash chromatography (1:1 EtOAc/Hexanes) provided *N*-sulfated product **2.60** as a white foam (0.132 g, 45%) and the dimeric by product **2.61** as a yellow solid (0.088 g, 45%). **Compound 2.60**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 3.78 (m, 1H, H2), 3.87 (m, 1H, H5), 4.11 (dd, 1H, $J_{6,6'}$ = 12.5 Hz, $J_{6,5}$ = 1.8 Hz, H6), 4.28 (dd, 1H, $J_{6',6}$ = 12.5 Hz, $J_{6',5}$ = 4.4 Hz, H6'), 4.61 (s, 2H, CH_2), 5.08 (t_{app}, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.6 Hz, H4), 5.22 (t_{app}, 1H, $J_{3,4}$+$J_{3,2}$ = 19.8 Hz, H_3), 5.72 (d, 1H, $J_{1,2}$ = 8.6 Hz, H1), 5.99 (d, 1H, $J_{NH,2}$ = 8.0 Hz, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5, 20.7, 20.8, 21.0, 58.0, 61.5, 67.9, 72.4, 72.7, 78.8, 92.0, 93.2, 169.4, 169.7, 170.7, 171.8; HRMS (ESI) *m/z* = 575.0275, $C_{16}H_{26}N_2O_{12}SCl_3$ $(M+NH_4)^+$ requires 575.0272. **Dimer 2.61**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 3.50-3.59 (m, 1H, H_2), 3.84-3.86 (m, 1H, H5), 4.06 (br-d, 1H, $J_{6,6'}$ = 10.9 Hz, H6), 4.25 (dd, 1H, $J_{6',6}$ = 12.5 Hz, $J_{6',5}$ = 3.9 Hz, H6'), 4.98-5.06 (m, 2H, H4, H3), 5.42 (d, 1H, $J_{NH,2}$ = 9.8 Hz, NH), 5.56 (d, 1H, $J_{1,2}$ = 8.8 Hz, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.5, 20.6, 20.8, 21.0, 56.9, 61.3, 68.1, 72.4, 72.5, 92.1, 169.4, 170.0, 170.5, 171.2; HRMS (ESI) *m/z* = 774.2242, $C_{28}H_{44}N_3O_{20}S$ $(M+NH_4)^+$ requires 774.2239.



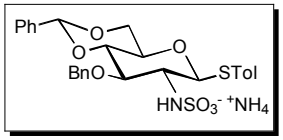
***4*-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroethoxysulfoxyamino)-ß-D-glucopyranoside (2.65):** To **2.64**[28] (0.50 g, 1.08 mmol) in THF (5.0 mL, 0.23 M) at 0 ^{o}C was added 1,2-dimethylimidazole (0.43 g, 2.7 mmol) followed by **2.40** (2.0 g, 4.32 mmol). The reaction was stirred at 0 ^{o}C, gradually warmed to room temperature by allowing the ice bath to melt, and then stirred overnight. After 14 h, additional **2.42** (1.0 g, 2.16 mmol) was added and after 10 h the system applied directly to silica gel column. Flash chromatography (33:67 EtOAc/hexanes) gave pure **2.65** as a white solid (0.68 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.37 (dt, 1H, $J_{2,3}$+ $J_{2,1}$ = 20.1, $J_{2,NH}$ = 7.1 Hz, H2), 3.52 (dt, 1H, $J_{5,6ax}$+$J_{5,4}$ = 19.4 Hz, $J_{5,6eq}$ = 5.0 Hz, H5), 3.70 (t 1H, $J_{4,5}$ = $J_{4,3}$ = 9.2 Hz, H4), 3.81 (t_{app}, 1H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.3 Hz, $H6_{ax}$), 3.99 (t_{app}, 1H, $J_{3,4}$ = $J_{3,2}$ = 9.3 Hz, H3), 4.41 (dd, 1H, $J_{6eq,6ax}$ = 10.5, $J_{6eq,5}$ = 5.0 Hz, $H6_{eq}$), 4.79, 4.83 (AB, 2H, J = 11.0 Hz, CH_2CCl_3), 4.83 (d, 1H, J = 11.0 Hz, 1/2 CH_2Ph), 4.90 (d, 1H, J = 10.4 Hz, H1), 4.95 (d, 1H, $J_{NH,2}$ = 7.1 Hz, NH), 5.00 (d, 1H, J = 11.0 Hz, 1/2 CH_2Ph), 5.55 (s, 1H, CHPh), 7.16 (d, 2H, J = 8.0 Hz, ArH), 7.35-7.46 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 59.3, 68.5, 70.1, 74.1, 78.5, 78.8, 82.3, 86.4, 93.4, 101.3, 126.0, 127.2, 128.1, 128.4, 128.56, 128.59, 129.2, 130.0, 133.7, 137.0, 137.6, 139.0; HRMS (EI) *m/z* = 673.0513, $C_{29}H_{30}Cl_3NO_4S_2$ requires 673.0529.



***p*-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-sulfamino-1-thio-ß-D-glucopyranoside dimer (2.66):** A solution containing carbohydrate **2.64** (0.135 g, 0.300 mmol) and NEt_3 (0.14 mL, 1.00 mmol) in dry DMF (3.0 mL) was added dropwise via syringe pump over 1.0 hour to a solution of reagent **1.76** (0.24 mL, 1.83 mmol) and DMAP (0.037 g, 0.300 mmol) in dry DMF (1.0 mL) at 0 °C. The reaction was stirred for an additional 30 minutes at 0 °C, then diluted with dichloromethane (10 mL) and quenched with ice water (5 mL). The organic phase was separated, and the remaining aqueous phase was extracted with dichloromethane (2x5 mL). The combined organics were dried over $MgSO_4$ and concentrated to a crude yellow oil. Purification by flash chromatography (1:1 EtOAc/Hexanes) provided **2.66** as a white solid (0.104 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.15 (ddd, 1H, $J_{5,6ax}$ = 10.3 Hz, $J_{5,4}$ = 9.2 Hz, $J_{5,6eq}$= 5.0 Hz, H5), 3.30 (t_{app}, 1H, $J_{3,4}$= $J_{3,2}$= 9.2 Hz, H3), 3.52 (dt, 1H, $J_{2,1}$ = 10.3 Hz, $J_{2,3}$= $J_{2,NH}$ = 9.2 Hz, H2), 3.62 (t_{app}, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.2 Hz, H4), 3.71 (d, 1H, $J_{1,2}$ = 10.3 Hz, H1), 3.74 (t_{app}, 1H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.3 Hz, $H6_{ax}$), 4.34 (dd, 1H, $J_{6eq,6ax}$ = 10.3, $J_{6eq,5}$ = 5.0 Hz, $H6_{eq}$), 4.57 (d, 1H, $J_{NH,2}$ = 9.2 Hz, NH), 4.59 (d, 1H, J = 11.0 Hz, 1/2 CH_2Ph), 5.17 (d, 1H, J = 11.0 Hz, 1/2 CH_2Ph), 5.52 (s, 1H, CHPh), 7.14-7.48 (m, 14H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 58.2, 68.6, 69.8, 73.9, 81.6, 87.3, 101.1, 126.0, 127.8, 128.3, 129.0, 129.1, 129.6, 133.6, 137.1, 138.0, 138.2; HRMS (ESI) *m/z* = 989.3168, $C_{54}H_{57}N_2O_{10}S_3$ $(M+H)^+$ requires 989.3175.

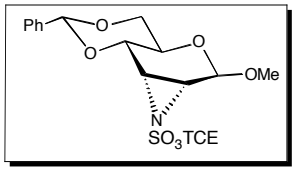


Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloro ethoxysulfoxyamino)-*ß*-D-glucopyranoside (2.68): To **2.67** (0.10 g, 0.252 mmol) in THF (1.1 mL, 0.23 M) at 0 ^{o}C was added 1,2-dimethylimidazole (0.06 g, 0.62 mmol) followed by **2.40** (0.460 g, 1.01 mmol). The reaction was stirred at 0 ^{o}C, gradually warmed to room temperature by allowing the ice bath to melt, and then stirred overnight. After 14 h no starting material was detected by TLC, and the system was applied directly to silica gel column. Flash chromatography (33:67 EtOAc/hexanes) gave pure **2.68** as an amorphous white solid (0.144 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (br-ddd, 1H, $J_{2,NH} = J_{2,3} = 8.8$ Hz, $J_{2,1} = 3.4$ Hz, H2), 3.73-3.94 (m, 4H, H3, H4, H5, $H6_{ax}$), 4.08 (dd, 1H, $J_{H,H} = 12.6$, 6.4 Hz, $OC\underline{H}_2CHCH_2$), 4.25 (dd, 1H, $J_{H,H} = 12.6$, 5.5 Hz, $OC\underline{H}_2CHCH_2$), 4.32 (dd, 1H, $J_{6eq,6ax} = 9.9$ Hz, $J_{6eq,5} = 4.3$ Hz, $H6_{eq}$), 4.58, 4.62 (AB, 2H, $J =$ 10.8 Hz, CH_2CCl_3), 4.76, 4.99 (AB, 2H, $J = 11.5$ Hz, CH_2Ph), 5.00 (br-d, $J_{NH,2} = 8.8$ Hz, NH), 5.15 (d, 1H, $J_{1,2} = 3.7$ Hz, H1), 5.27-5.37 (m, 2H, $OCH_2CHC\underline{H}_2$), 5.62 (s, 1H, CHPh), 5.88-6.01 (m, 1H, $OCH_2C\underline{H}CH_2$), 7.28-7.52 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 57.8, 62.7, 68.8, 69.0, 75.0, 75.5, 78.3, 82.8, 92.9, 96.9, 101.4, 118.8, 126.0, 128.0, 128.2, 128.3, 128.6, 129.1, 132.9, 137.1, 137.7; HRMS (ESI) m/z = 608.0665, $C_{25}H_{29}NO_8Cl_3S$ (M+H) requires 608.0679.



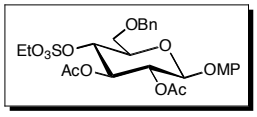
***p*-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-sulfoxyamino-1-thio-ß-D-glucopyranoside (2.69)**. To a suspension of ammonium formate (0.083 g, 1.3 mmol) in MeOH (1.3 mL, 1.0 M) was added glucosamine **2.65** (0.15 g, 0.22 mmol) followed by zinc dust

(0.1 g, 1.55 mmol). The reaction was stirred for 7 h at room temperature, at which point no starting material was detected using TLC. The reaction was filtered through celite, and concentrated to crude product. Flash chromatography (20:4:1 CH_2Cl_2/MeOH/NH_4OH) afforded a white solid, which was lyophilized (3x) from water to yield **2.69** as a light fluffy white product (0.105 g, 84%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.29 (s, 3H, CH_3), 3.01 (t_{app}, 1H, $J_{2,3}$+$J_{2,1}$ = 17.1 Hz, H2), 3.39-3.46 (m, 1H, H5), 3.65 (t_{app}, 1H, $J_{6ax,6eq}$ = $J_{6ax,5}$ = 10.2 Hz, $H6_{ax}$), 3.71 (t_{app}, $J_{4,5}$+$J_{4,3}$ = 18.3 Hz, H4), 4.20 (dd, 1H, $J_{6eq,6ax}$ = 10.2, $J_{6eq,5}$ = 5.1 Hz, $H6_{eq}$), 4.39 (t_{app}, $J_{3,4}$ = $J_{3,2}$ = 8.5 Hz, H3), 4.74 (d, 1H, J = 11.5 Hz, 1/2 CH_2Ph), 4.97 (d, 1H, J = 11.5 Hz, 1/2 CH_2Ph), 5.59 (d, 1H, J = 9.1 Hz, H1), 5.66 (s, 1H, CHPh), 5.76 (s, 1H, NH), 7.14-7.40 (m, 18 H, 14 ArH + NH_4); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 59.8, 68.5. 68.6, 74.0, 78.9, 81.7, 87.0, 100.6, 126.4, 126.5, 127.4, 128.2, 128.3, 128.5, 130.0, 131.2, 131.8, 138.3, 140.1; HRMS (ESI) *m/z* = 542.1306, $C_{27}H_{28}NO_7S_2$ requires 542.1307.



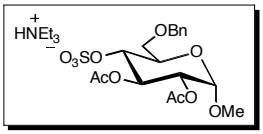
Methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-imino-*N*-(2,2,2-trichloroethoxysulfonyl)-β-D-glucopyranoside (2.79): To carbohydrate **2.71** (0.200 g, 0.716 mmol) in THF (4.0 mL, 0.18 M) at 0 ^{o}C was added 1,2-dMeIm (0.35 g, 3.64 mmol) followed by **2.40** (1.3 g, 2.86 mmol). The reaction was stirred at 0 ^{o}C, gradually warmed to room temperature by allowing the ice bath to melt. After 8 h, additional **2.40** (1.3 g, 2.86 mmol), and 1,2-dMeIm (0.35 g, 3.64 mmol) were added, and the reaction was allowed to stir for 16 h. The reaction was then applied directly to a silica gel column. Flash chromatography

(100% dichloromethane) gave pure **2.79** as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.42 (d, 1H, $J_{6ax,6eq}$ = 7.3 Hz, $H6_{ax}$), 3.49 (dd, 1H, $J_{6eq,6ax}$ = 7.3 Hz, $J_{6eq,5}$ = 2.2 Hz, $H6_{eq}$), 3.55 (s, 3H, CH_3), 3.62-3.79 (m, 2H, H3,H4), 4.08 (dd, 1H, $J_{4,5}$ = 8.7 Hz, $J_{4,3}$ = 2.4 Hz, H4), 4.30 (dd, 1H, $J_{5,4}$ = 8.8 Hz, $J_{5,6eq}$ = 2.9 Hz, H5), 4.86, 4.90 (AB, 2H, J = 10.6 Hz, CH_2CCl_3), 5.28 (s, 1H, H1), 5.59 (s, 1H, CHPh), 7.41-7.46 (m, 2H, ArH), 7.33-7.36 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 41.3, 44.3, 57.0, 61.8, 68.9, 74.3, 79.9, 92.5, 97.7, 102.6, 126.1, 128.3, 129.3, 136.6; $[\alpha]_D^{25}$ =-2.0 (c 0.5, $CHCl_3$); HRMS (ESI) m/z = 473.9960, $C_{16}H_{19}Cl_3NO_7S$ (M+H) requires 473.9948.



4-Methoxyphenyl 2,3-Di-*O*-acetyl-4-*O*-ethylsulfo-6-*O*-benzyl-β-D-glucopyranoside (2.86): To Carbohydrate **2.18** (0.201 g, 0.299 mmol) in benzene (6.5 mL, 0.05 M) was added AIBN (0.01 g, 0.061 mmol) followed by tributyltin hydride (0.48 mL, 1.78 mmol). The reaction mixture was stirred under argon for 1h, then heated at 80 °C for 3 h. Upon cooling, the solvent was removed *in vacuo*, and the remaining residue was stirred in a 1:1 mixture of CH_3CN:hexanes for 1h. The organic layers were separated, and the CH_3CN layer was concentrated to a clear colourless syrup. Flash chromatography (1:1 EtOAc/hexanes, performed twice) gave nearly pure **2.86** as a clear colourless glassy compound (0.154 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 7.1 Hz, CH_3), 2.05 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 3.63-3.79 (m, 5H, OCH_3, H6, H5), 3.86 (d, 1H, $J_{6,6'}$ = 10.6 Hz, H6'), 4.23-4.35 (m, 2H, CH_2), 4.55, 4.60 (AB, 2H, J = 11.8 Hz, CH_2Ph), 4.83 (t_{app}, 1H, $J_{4,3} = J_{4,5}$ = 9.3 Hz, H4), 4.94 (d, 1H, $J_{1,2}$ = 7.9 Hz, H1), 5.18 (t_{app}, 1H, $J_{2,1} + J_{2,3}$ = 17.4 Hz, H2), 5.37

(t_{app}, 1H, $J_{3,2} + J_{3,4}$ = 18.5 Hz, H3), 6.76 (d, 2H, J = 9.0 Hz, ArH), 6.95 (d, 2H, J = 9.0 Hz, ArH), 7.28-7.32 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 20.6, 20.7, 55.6, 68.1, 71.0, 71.4, 72.2, 73.5, 73.7, 76.5, 100.5, 114.5, 118.7, 127.8, 128.4, 137.7, 150.9, 155.8, 169.3, 170.3.



Methyl 2,3 Di-*O*-acetyl-6-*O*-benzyl-4-*O*-sulfo-α-D-glucopyranoside (2.93). A solution of **2.92** (0.200 g, 0.345 mmol) and triethylamine (0.24 mL, 1.72 mmol), and 10% Pd/C (0.02 g, 10% w/w) in methanol (5.0 mL) was stirred under H_2 (1 atm, balloon) for 90 min, until no starting material was detected using TLC. The reaction mixture was filtered through a pad of celite, and concentrated to a crude solid. Flash chromatography (20:4:1 CH_2Cl_2/MeOH/NH_4OH) provided a white solid, which was lyophilized (3x) from H_2O to yield **2.93** as a light white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, 9H, J = 7.3 Hz, 3xCH_3), 1.92 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 3.10 (q, 6H, J = 7.3 Hz, 3xCH_2), 3.32 (s, 3H, OCH_3), 3.52 (dd, 1H, $J_{6,6'}$ = 11.0 Hz, $J_{6,5}$ = 7.2 Hz, H6), 3.69 (t_{app}, 1H, $J_{5,6} + J_{5,4}$ = 16.6 Hz, H5), 3.96 (d, 1H, $J_{6',6}$ = 11.0 Hz, H6'), 4.05 (t_{app}, 1H, $J_{4,5} = J_{4,3}$ = 9.6 Hz, H4), 4.50 (s, 2H, CH_2Ph), 4.71 (dd, 1H, $J_{2,3}$ = 10.4, $J_{2,1}$ = 3.6 Hz, H2), 4.85 (d, 1H, $J_{1,2}$ = 3.6 Hz, H1), 5.23 (t_{app}, 1H, $J_{3,2} + J_{3,4}$ = 19.6 Hz, H3), 7.24-7.35 (m, 5H, ArH), 8.82 (br-s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 9.1, 20.9, 21.3, 46.5, 54.9, 69.7, 70.3, 70.5, 70.9, 72.7, 73.2, 96.2, 127.7, 127.8, 128.6, 139.2, 170.1, 170.2.

Chapter 3 - Target Based Synthesis of Sulfated Carbohydrates using a Sulfate Protecting Group Strategy

3.1 Introduction

In chapter 2 and in our previous reports[27,32] we demonstrated that 2,2,2-trichloroethyl-protected sulfates could be introduced into monosaccharides in good yield using sulfuryl imidazolium salts. We also demonstrated that the TCE group withstood many of the conditions that are commonly encountered in carbohydrate chemistry and a simple disaccharide containing TCE-protected sulfates could be prepared. Finally, we demonstrated that the TCE group can be removed under mild conditions usually in excellent yield. However the true test of our methodology will be in the synthesis of complex multisulfated oligosacharides. In this chapter we present our studies on the application of our sulfate protecting group strategy towards the synthesis of di- and tetrasaccharides derived from chondroitin sulfates C and D, which are two glycosamino glycans (GAG's) that have been implicated in a number of physiological processes.

3.1.1 Chondroitin Sulfate

Chondroitin sulfate (CS) glycosaminoglycans (GAGs) are unbranched polysaccharides of variable lengths that contain two alternating monosaccharide residues: D-glucuronic acid (GlcA) and *N*-Acetyl-D-galactosamine (GalNAc). There are several characterized CS compounds (Figure 3.1), each of which carries a different sulfation

code. CS-A and CS-C bear sulfate groups at the 4-*O*- and 6-*O*-positions, respectively; CS-D and CS-E are described as oversulfated chondroitin sulfates. CS-D contains a sulfate group at the 2-*O*-position of the GlcA monomer along with a 6-*O*-sulfate on the GalNAc residue. CS-E bears a 4,6-di-*O*-sulftaed GalNAc residue. CS-K and CS-L have a *3*-*O*-sulfate on the glucuronic acid residue, and differ by being 4-*O*-sulfated and 6-*O*-sulfated on the GalNAc monomer, respectively. CS-M is heavily sulfated, with groups at the 3-*O*-position on the GlcA monomer, as well as the 4,6-*O*-positions of the GalNAc ring.

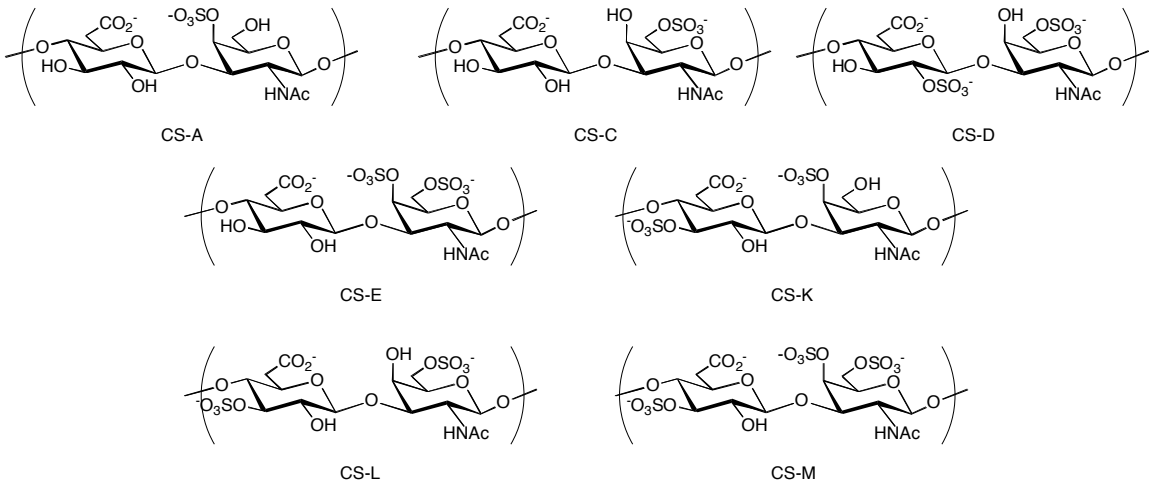


Figure 3.1. Chondroitin Sulfates A,C,D,E,K,L,M

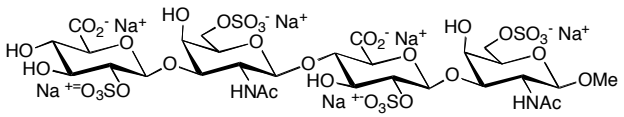
The specific biological functions of these compounds are a subject of intense investigation. It is now known that the roles of these oligosaccharides are intimately related to the distinctive sulfation pattern each GAG carries; and that these diverse patterns of sulfation are highly regulated in vivo. The CS GAGs are implicated in a

number of physiological processes including viral invasion.,[41] brain development and regeneration,[42] cell-cell recognition, [43] and spinal cord injuries, [44] as well as numerous other processes.

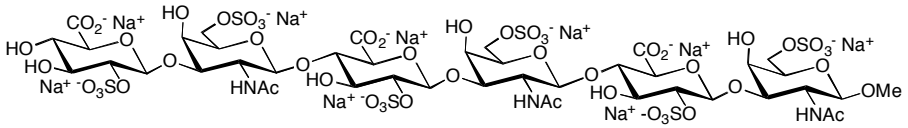
It is widely known that the synthesis of naturally occurring glycosaminoglycans is extremely challenging, and is even more so when the targets are modified with sulfation patterns. In order to better study the structure and activity of these compounds, pure oligosaccharide fragments of defined length and sulfation pattern are required. Isolation of well-defined CS fragments from natural sources is difficult due to the inherent microheterogeneity of the polymers, thus progress in this field must rely on chemical oligosaccharide synthesis. The groups of Jean-Claude Jacquinet and Linda Hsieh-Wilson are the leading researchers in the chemical synthesis of chondroitin sulfate oligosaccharides. The research efforts of each group are summarized below.

3.1.1.1 Research Efforts by the Jacquinet Group

Over 2000 to 2002, Karst and Jacquinet reported the first stereocontrolled total synthesis of CS-D based oligosaccharides.[45,46] In an admirable synthetic achievement, Karst and Jacquinet successfully prepared the CS-D related tetra- and hexasaccharides **3.1** and **3.2** described in Figure 3.2.



3.1

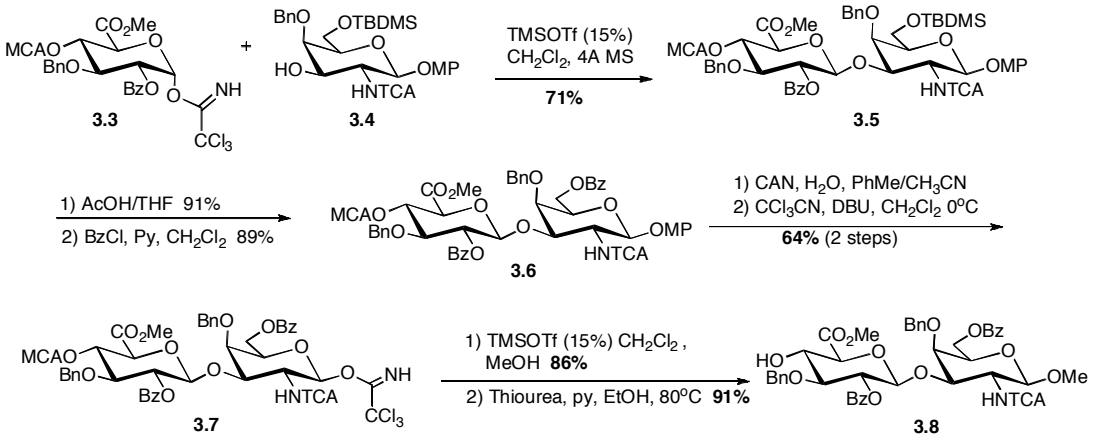


3.2

Figure 3.2. Synthetic CS-D Oligosaccharides **3.1** and **3.2**

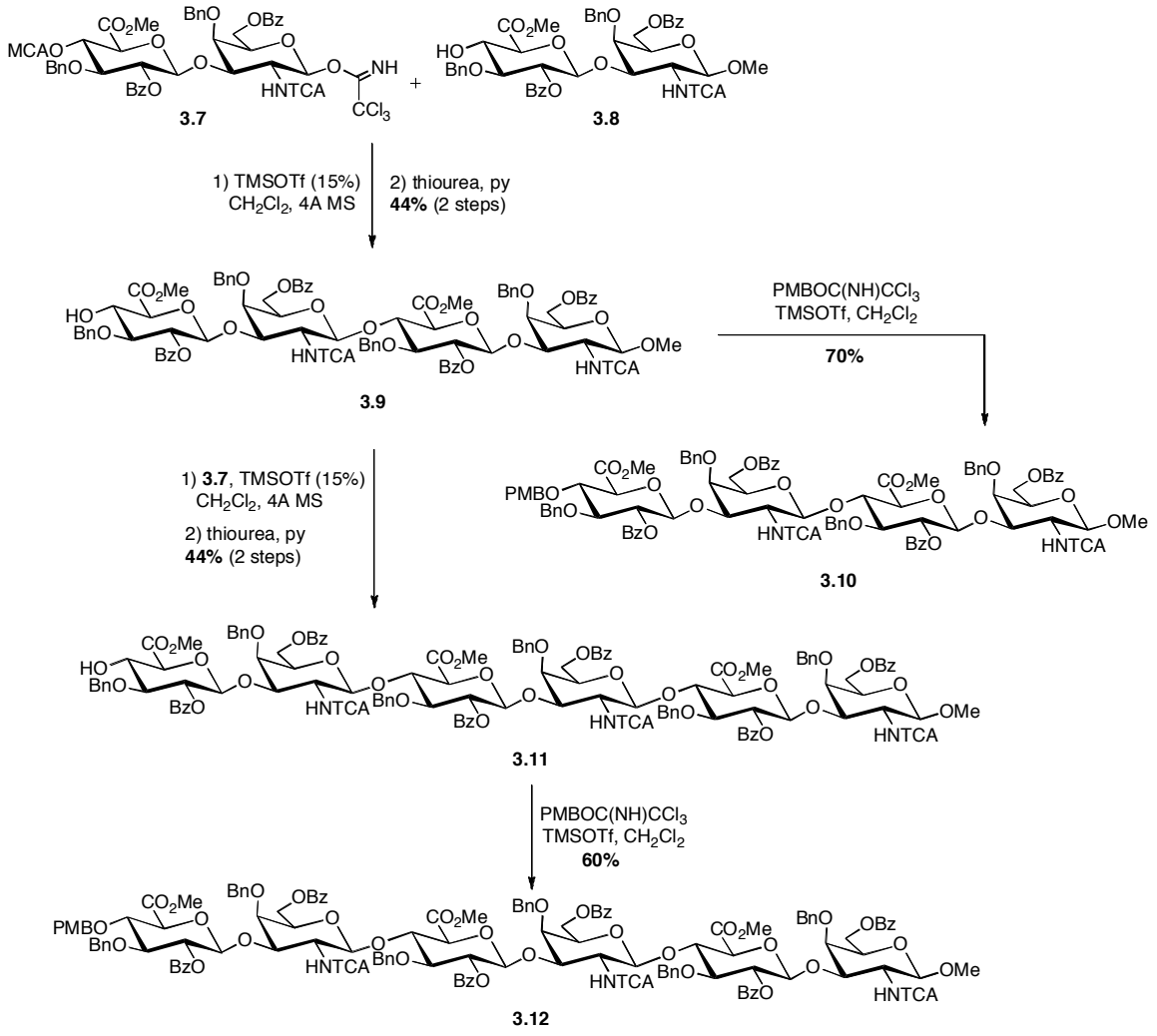
Their initial syntheses of CS-D fragments **3.1** and **3.2** are presented in Schemes 3.1 to 3.3. In the synthesis, benzyl ethers were selected as permanent protection for the hydroxyl groups to be free in the final product, and benzoate esters were used as temporary protecting groups for the hydroxyl groups to ultimately be sulfated. The synthesis of the targets focused on the preparation of disaccharide **3.7** (Scheme 3.1), which could be used in an iterative fashion to provide access to **3.1** and **3.2**.

To access the key disaccharide **3.7**, glycosyl donor **3.3** (prepared in 13 steps), and acceptor **3.4** (prepared in 15 steps) were coupled in the presence of TMSOTf to obtain disaccharide **3.5** in a 71 % yield. The primary silyl ether in **3.5** was exchanged for a benzoate ester, giving **3.6**. Oxidative hydrolysis of the anomeric OMP group in **3.6** followed by installation of the trichloroacetimidate moiety provided target disaccharide **3.7** as an α,β-mixture in 67% yield. Disaccharide **3.7** was then condensed with methanol in the presence of TMSOTf and then treated with thiourea to afford the reducing disaccharide acceptor **3.8** in 78 % yield over two steps (Scheme 3.1).



Scheme 3.1. Preparation of target disaccharides **3.7** and **3.8**[45]

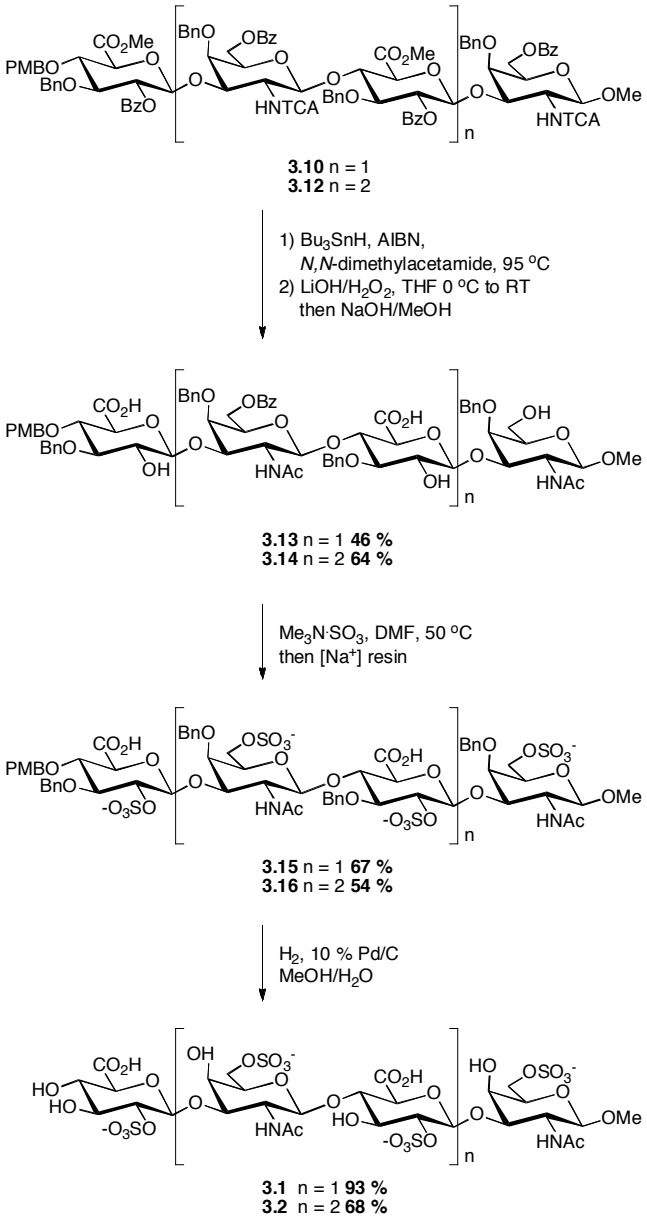
With disaccharide donor **3.7** and acceptor **3.8** in hand, the authors examined an iterative approach for the preparation of the target oligosaccharides. Coupling of imidate **3.7** with the 4-OH in acceptor **3.8** followed by *O*-dechloroacetylation provided tetrasaccharide **3.9** in a 44 % yield (Scheme 3.2). This tetrasaccharide bearing a free 4-OH at the non-reducing end served as the glycosyl acceptor when condensed a second time with key disaccharide donor **3.7**. The resulting free 4-OH positions of the glucuronic acid residues at the non-reducing ends of the tetra- and hexasaccharides **3.9** and **3.11** were then protected with hydrogenolyzable PMB (4-Methoxybenzyl) ethers using 4-methoxybenzyl trichloroacetimidate to give the fully protected oligosaccharides **3.10** and **3.12** (Scheme 3.2).



Scheme 3.2. Preparation of fully protected tetra- and hexasaccharides **3.10** and **3.12**[45]

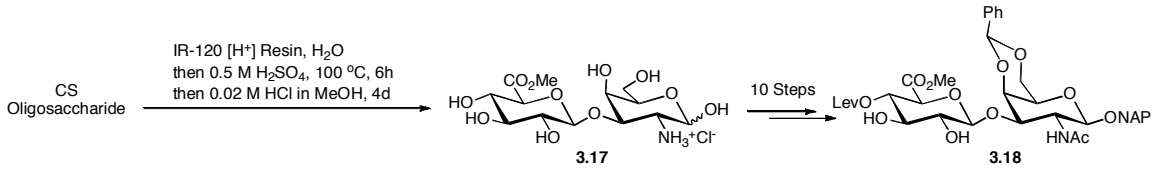
Before the sulfate esters could be introduced to the oligosaccharides, several modifications first had to be performed (Scheme 3.3). The *N*-trichloroacetate groups were transformed into *N*-acetyl groups using tributylstannane and AIBN; and the benzoate and methyl esters were saponified with treatment of lithium hydroperoxide followed by methanolic sodium hydroxide to give **3.13** and **3.14** in good yields. The partially protected **3.13** and **3.14** were then *O*-sulfated using the sulfur trioxide-trimethylamine

complex in DMF to give their sulfated counterparts **3.15** and **3.16** in 67% and 54% yields (Scheme 3.3). Final deprotection of **3.15** and **3.16** was achieved under catalytic hydrogenation conditions using 10% palladium on carbon to afford the target tetra- and hexasaccharides **3.1** and **3.2** in 93% and 68% yields, respectively.



Scheme 3.3. Access to CS-D tetra- and hexasaccharides **3.1** and **3.2**[45]

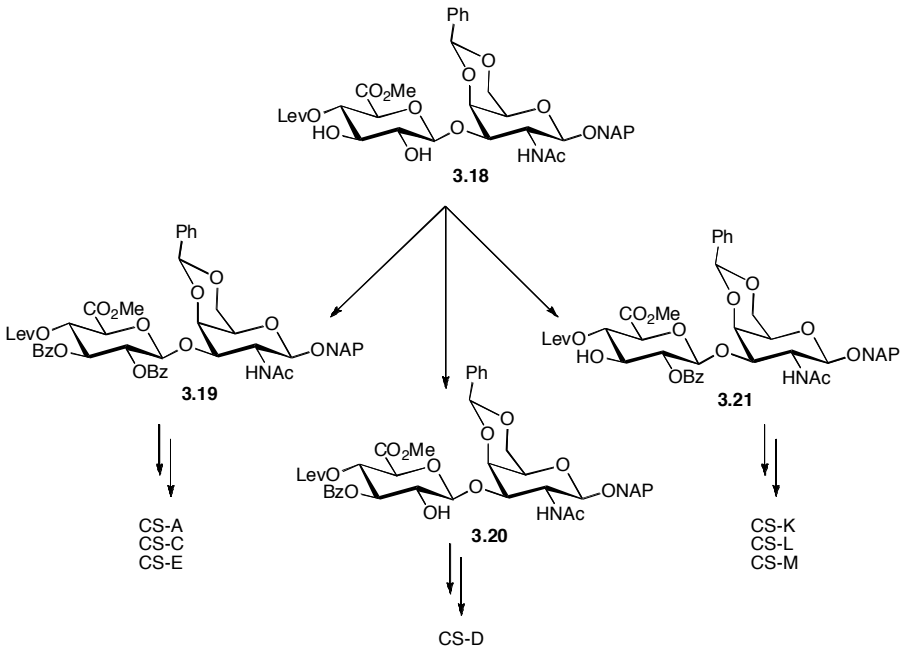
In 2006, a publication by Lopin and Jacquinet provided an alternate approach to the preparation of structurally defined CS polymers.[47] In this publication, the authors made note of the fact that for all of the previous preparations of chondroitin sulfate molecules, each synthesis started from chemically prepared monomeric units. Since each synthesis required a GalNAc monomer, a rare and expensive starting material, the derivatives were generally prepared via long synthetic routes. Taking advantage of the abundance of the naturally occurring GAGs, the authors sought to isolate and modify the basic CS disaccharide unit **3.17**, which is obtained from chemical hydrolysis of the CS oligosaccharides.[48,49] From pure **3.17** Lopin and Jacquinet prepared the functionalized disaccharide **3.18** and related derivatives in a series of protecting group manipulations over 10 steps and an overall 8.5 % yield from the CS-polymer.



Scheme 3.4. Modified CS fragments obtained from natural sources[47]

In 2009, a two-part publication by Jacquinet and co-workers further elaborated on the synthesis of structurally defined chondroitin sulfate polymers using starting materials obtained from chemical degradation of the natural polymer.[50,51] The authors designed a highly divergent chemical synthesis of all known variants (A, C, D, E, K, L, M) of chondroitin sulfate GAGs starting from the key disaccharide **3.18** (Scheme 3.5). The protecting group pattern in **3.18** allows for elongation at the reducing end through

oxidative removal of the anomeric NAP group; as well as modification at the non-reducing end through selective removal of the Lev protecting moiety. All hydroxyl groups in the synthesis requiring permanent protection were masked as benzyl ethers, and then easily regenerated at the end of the synthesis. The required CS variants were organized into two structural classes: those that have a sulfate moiety on the GalNAc monomer (CS-A, -C, -E), and those that are sulfated on both the GalNAc and GlcA monomers (CS-D, -K, -L, -M). To access all of the possible sulfation motifs, **3.18** was converted into **3.19** to ultimately produce CS-A, -C, and –E; **3.20** to provide access to CS-D, and **3.21** to yield CS-K, L, and -M as outlined in Scheme 3.5.

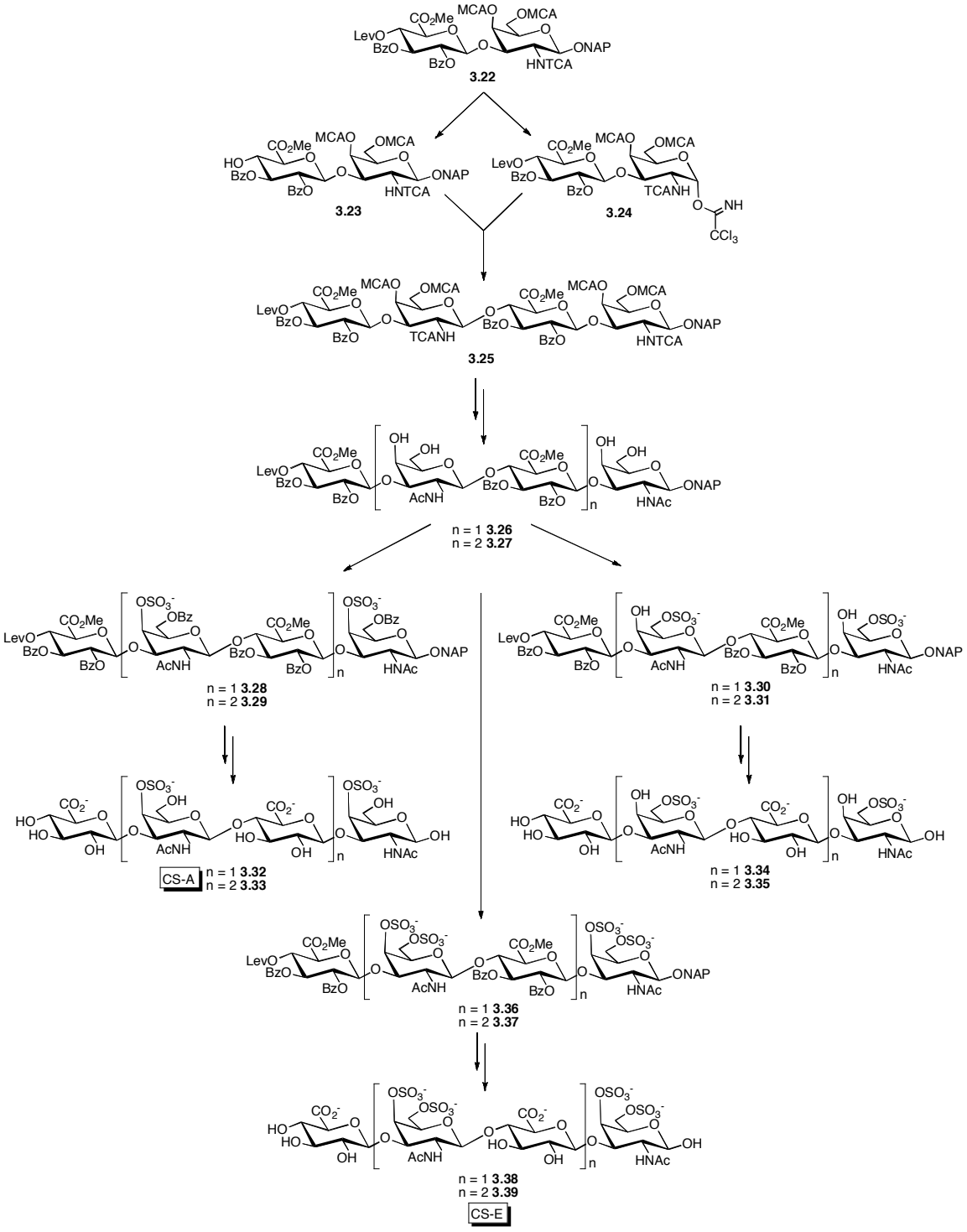


Scheme 3.5. Divergent approach to all CS variants[51]

CS-A, -C, and –E oligosaccharides were prepared from disaccharide **3.19** as described by Scheme 3.6. Disaccharide **3.22** was converted into acceptor **3.23** (89 %

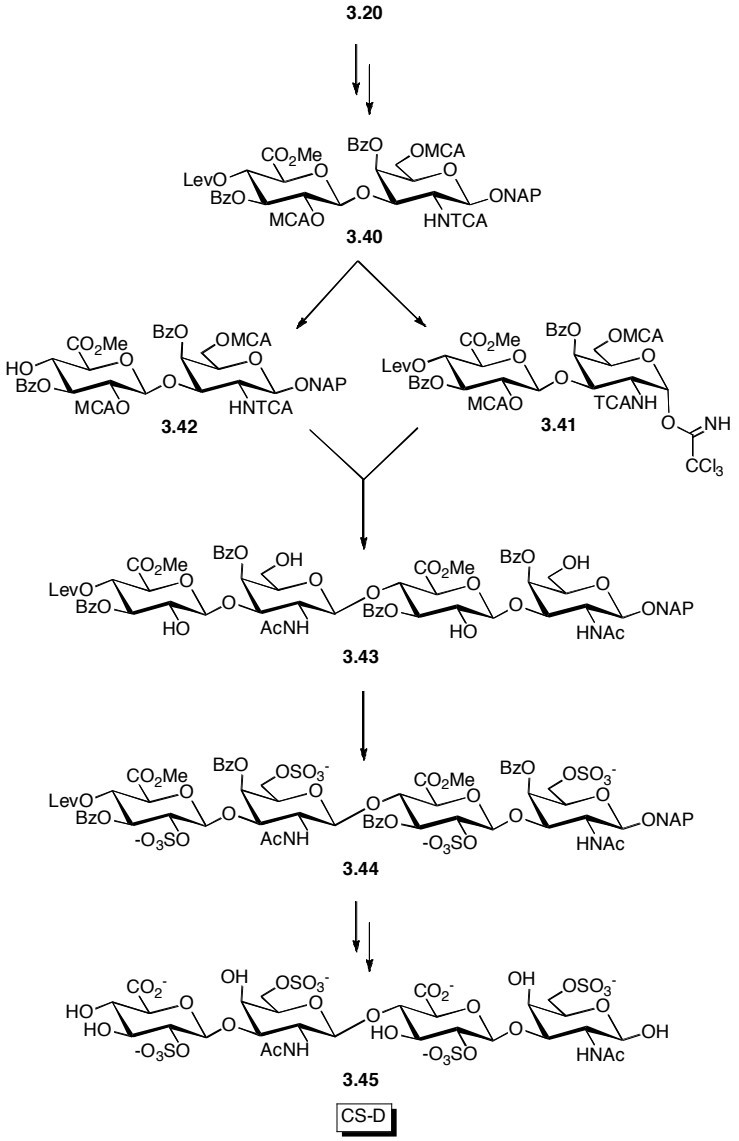
yield) and glycosyl donor **3.24** (61 %, 2 steps), which were then coupled to give tetrasaccharide **3.25** in 71 % yield. After removing the 4-*O*-Lev group at the non-reducing end, tetrasaccharide **3.25** was coupled to donor **3.24** in a second glycosylation reaction. Removal of the chloroacetate protecting groups with thiourea, followed by tributylstannane mediated reduction of the *N*-trichloroacetate moiety provided access to modified tetra- and hexasaccharides **3.26** and **3.27** (Scheme 3.6).

The fully protected tetra- and hexasaccharides **3.26** and **3.27** were then transformed into the target CS fragments (Scheme 3.6). Selective benzoylation with benzoyl cyanide gave the 6-*O*-Bz derivatives, which were sulfated using an excess of sulfur trioxide/trimethylamine complex in DMF to provide the 4-sulfated oligosaccharides **3.28** and **3.29** for the CS-A derivatives in 72% and 85% yields. Controlled regioselective sulfation of **3.26** and **3.27** followed by 4-*O*-acetylation gave the 6-sulfated derivatives **3.30** and **3.31** (83% and 81% yield) required for the CS-C fragments. Finally, total *O*-sulfation of **3.26** and **3.27** provided the 4,6-disulfated configuration in **3.36** and **3.37** (in 86% and 82%) required for CS-E. Complete deprotection of the oligosaccharides was achieved through a 2-step saponification with lithium hydroperoxide and sodium hydroxide, followed by catalytic hydrogenolysis to provide the six reducing oligosaccharides **3.32** – **3.35** and **3.38** – **3.39**.



Scheme 3.6. CS-A, -C, -E tetra- and hexasaccharides of defined lengths[51]

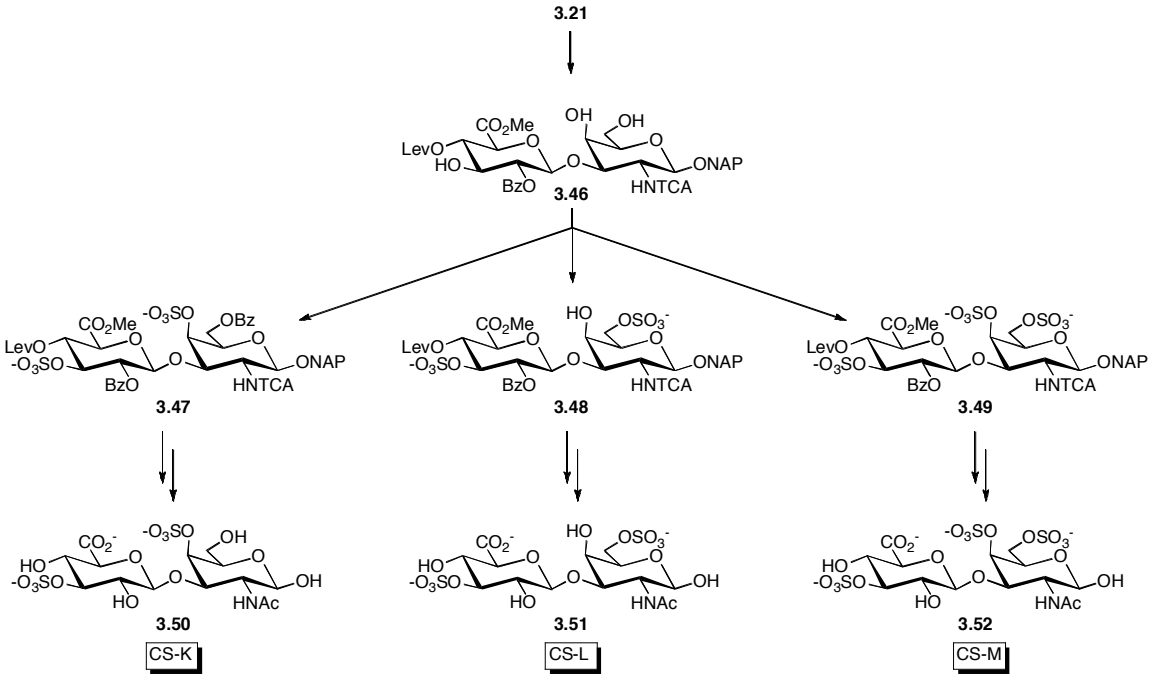
CS-D based di- and tetrasaccharides were prepared starting from disaccharide **3.20** as shown in Scheme 3.7. Fully protected **3.20** was subjected to standard protecting group manipulations to give differentiated derivative **3.40**. Oxidative cleavage of the anomeric NAP ether with installation of the imidate moiety; and removal of the 4-*O*-Lev group in **3.40** provided access to donor **3.41** and acceptor **3.42** which were coupled in the presence of TMSOTf. The resulting tetrasaccharide was then de-chloroacetylated and the *N*-trichloroacetate group was reduced to give partially protected **3.43**, which was then completely *O*-sulfated using sulfur trioxide/trimethylamine. Tetrasaccharide **3.44** was then saponified and hydrogenated under the previously described conditions to provide the CS-D tetrasaccharide **3.45** (Scheme 3.7).



Scheme 3.7. Synthesis of a CS-D reducing tetrasaccharide[51]

Synthesis of the less common CS-K, -L, and -M sulfoforms commenced with the synthesis of the basic reducing disaccharides starting from **3.21**. Radical reduction of the *N*-trichloroacetyl group followed by hydrolysis of the benzylidene acetal provided triol **3.46**, which served as a precursor to the required CS variants (Scheme 3.8). CS-K was

accessed through selective primary benzoylation followed by disulfation in 80% to give the fully protected **3.47**. Conversely, controlled selective sulfation of triol **3.46** gave the 6,3'-disulfated CS-L disaccharide **3.48** in 75 % yield (with approximately 10% of the trisulfated disaccharide). Finally, exhaustive *O*-sulfation of **3.46** gave the CS-M precursor **3.49**. The disaccharides were then subjected to saponification conditions followed by catalytic hydrogenolysis to provide the CS-K **3.50**, -L **3.51**, and -M **3.52** reducing disaccharide sulfoforms (Scheme 3.8).



Scheme 3.8. Synthesis of CS-K, -L, and –M disaccharides[51]

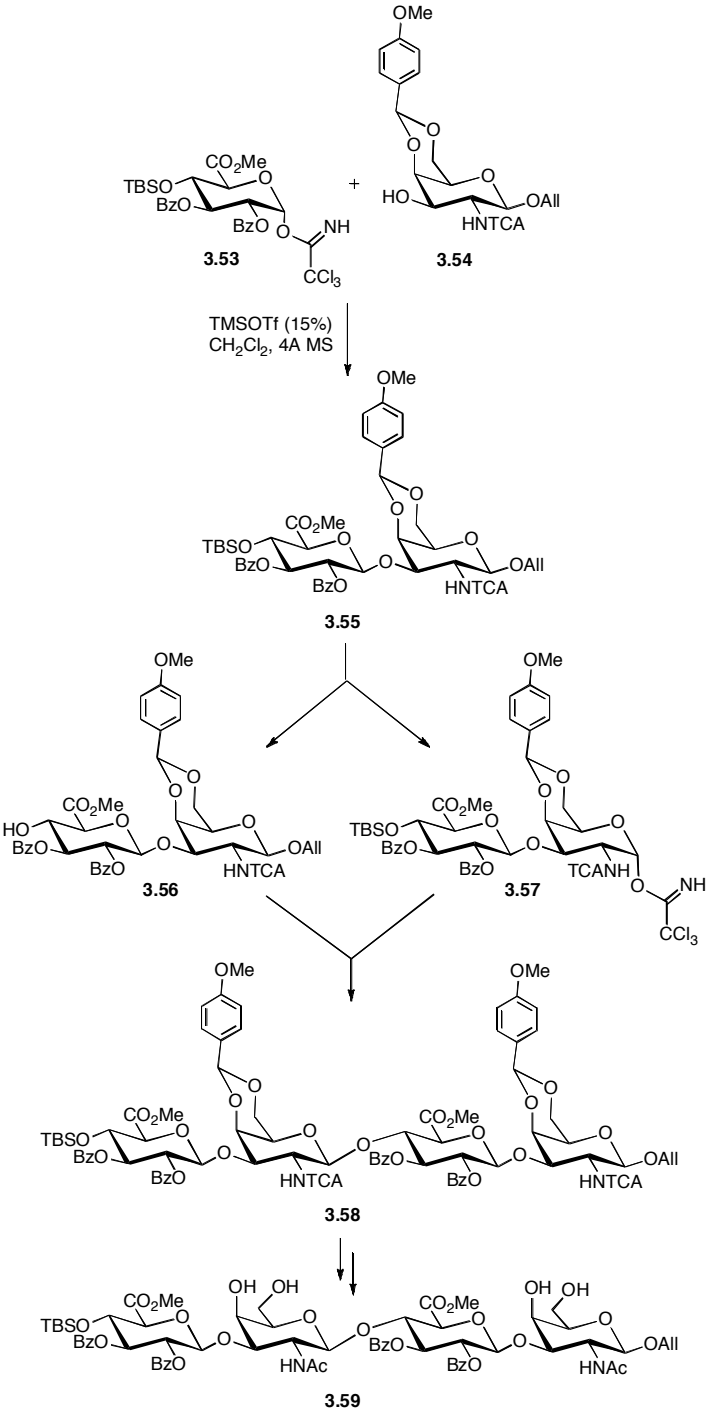
3.1.1.2 Synthesis of Chondroitin Sulfates by the Hsieh-Wilson Group

Over 2004 to 2006, the Hsieh-Wilson group also addressed the idea that chemical synthesis of these CS glycosaminoglycans could provide a powerful means to access well defined structures, and further enable systematic investigations into the biological roles of

specific sulfation patterns.[52,53] In two publications, the authors designed an efficient and modular approach to obtain target tetrasaccharides derived from CS-A, CS-C, CS-E, and the relatively rare CS-R sulfation sequences, while attempting to minimize the number of synthetic steps and maximize isolation quantities of the target oligosaccharides.

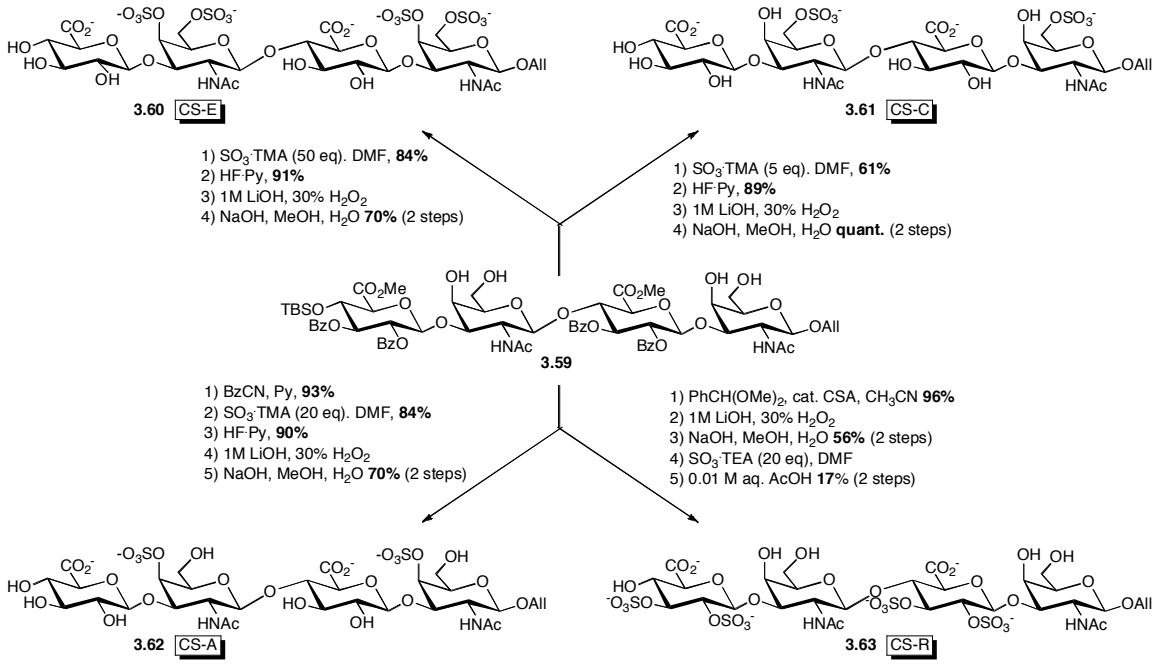
Similar to Jacquinet's approach (albeit from chemically prepared starting materials as opposed to those obtained from natural sources) the Hsieh-Wilson group employed *N*-trichloroacetamide or *O*-benzoyl groups to provide stereocontrol for the required β-glycoside synthesis; and an orthogonal protecting group strategy was developed to allow for the installation of specific sulfate sequences.

Starting from donor **3.53** (prepared in 11 steps) and acceptor **3.54** (prepared in 10 steps) disaccharide **3.55** was prepared in a 77% yield. This key disaccharide intermediate was readily converted into glycosyl acceptor **3.56** and trichloroacetimidate donor **3.57**, which were coupled in the presence of TMSOTf to give tetrasaccharide **3.58** in a 44 % yield with good stereoselectivity (Scheme 3.9). Conversion of the *N*-trichloroacetamide moiety to the corresponding *N*-acetamide followed by oxidative cleavage of the p-methoxybenzylidene (PMB) acetal afforded the tetra-ol intermediate tetrasaccharide **3.59** (Scheme 3.9).



Scheme 3.9. Preparation of key tetrasaccharide intermediate **3.59**[52,53]

With the functionalized tetrasaccharide **3.59** in hand, the CS structures with specific sulfation motifs were then assembled (Scheme 3.10). Vigorous sulfation of **3.59** provided the 4,6-*O*-tetrasulfated tetrasaccharide required to access CS-E. A selective primary di-*O*-sulfation of **3.59** provided the precursor to CS-C. To obtain the precursor to CS-A, the primary hydroxyl groups in **3.59** were selectively benzoylated with benzoyl cyanide, and the remaining free 4-OH positions were then sulfated. To obtain the CS-R sulfation code, a benzylidene acetal was introduced to tetrasaccharide **3.59**, the ester protecting groups were saponified and the resulting free hydroxyl groups were then sulfated. The target CS-E, CS-C, and CS-A tetrasaccharides **3.60**, **3.61**, and **3.62** were obtained after removal of the silyl- and benzoate-protecting groups. The unique CS-R tetrasaccharide **3.63** was isolated after acidic hydrolysis of the PMB acetal (Scheme 3.10.)



Scheme 3.10. Hsieh-Wilson's approach to defined CS variants[53]

The work completed by Jacquinet, Hsieh-Wilson and respective research groups is an outstanding synthetic achievement. For the first time, two highly divergent approaches to the preparation of all known CS-oligosaccharides have been developed and executed. These oligosaccharides are currently in high demand, and access to polymers of defined length and sulfation patterns will allow for in-depth study of these intriguing polysaccharides.

3.2 Objectives

The objectives of the work described in this chapter is to further evaluate the scope of our TCE-protecting group strategy by applying it to the synthesis of di- and tetrasaccharides derived from CS-C and CS-D (**3.64-3.66**, Figure 3.3). In the approaches of both Jacquinet and Hsieh-Wilson, the sulfate groups are incorporated late in the synthetic sequence, which necessitates the use of an orthogonal protecting group strategy. Because of this, almost all of the protecting group manipulations are performed on the di-, tetra- and hexasaccharide targets, often resulting in less than favourable yields, and products that are very difficult to characterize due to their complex nature. Furthermore, multiple sulfations on large targets can sometimes be low yielding (for example, 54% for the sulfation of **3.14** or 17% for the sulfation of **3.62**), and the sulfated products can be very difficult to isolate and purify. The yields of sulfate-incorporation into monosaccharides using the sulfuryl imidazolium salts described in chapter 2 are often higher than those performed on oligosaccharides with sulfur trioxide/amine complexes.

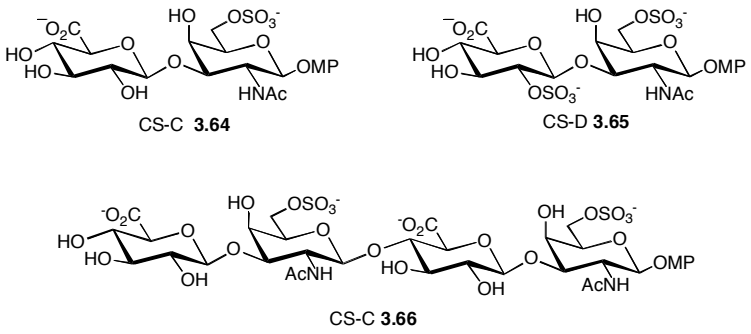


Figure 3.3. Target CS based molecules

Each target (**3.64**-**3.66**) presents unique synthetic challenges that will allow for further exploration of the scope and limitations of the protected sulfate approach. In each target we wished to demonstrate that a synthesis could be achieved whereby all of the protecting groups could be removed in a single step at the end of the syntheses by catalytic hydrogenolysis. The CS-C disaccharide **3.64**, the simplest of the three, provides a relatively simple model system to test this. The CS-D disaccharide **3.65** was chosen to investigate the reactivity of 2-*O*-sulfated glycosyl donors, as well as the effect of the TCE-protected sulfate moiety on the stereochemical outcome of the glycosylation reactions. The CS-C tetrasaccharide target **3.66** was chosen to demonstrate the TCE-protection strategy in the synthesis of higher order oligosaccharides. The synthesis of these CS-based targets is still very much a work in progress, and this section presents our preliminary developments in this area. Nevertheless, it is our hope that the chemistry developed in the preparation of these compounds will be applicable in the synthesis of higher order sulfated oligosaccharides.

3.3 Results and Discussion

3.3.1 Towards the Synthesis of CS-C Disaccharide 3.64

The retrosynthetic analysis of **3.64** is shown in Figure 3.4. We envisioned target **3.64** being obtained from its fully protected precursor **3.67** in a one-pot global deprotection, as all of the protecting groups in **3.67** can be removed and reduced under catalytic hydrogenolysis conditions. Disaccharide **3.67** was to be prepared from thioglycoside donor **3.68** and galactosamine acceptor **3.69**. The key features of this synthesis include the preparation of a GalNTCA monomer bearing a TCE-protected sulfate group at the 6-*O*-position, as well as the use of a carboxybenzyl (Cbz) group at the 2-*O*-position of the GlcA monomer. The Cbz group was an attractive choice for the synthesis of the CS-C target, as it has been shown that it can be readily introduced into carbohydrates, can provide the anchimeric assistance required to direct the β-glycosidic linkage and, unlike traditional esters, could be removed by catalytic hydrogenolysis.[54,55,56] The only potential problem in using the Cbz group is that under certain glycosidation conditions the benzyl group (derived from a Cbz group at the 2-position of the donor) can be transferred to the acceptor.[54] This issue will be discussed in more detail in a subsequent section in this chapter. Furthermore, the synthesis also employs a NAP ether at the 4-*O*-position of the glucuronic acid ring in **3.67**, and an OMP group at the reducing end of the molecule. This protecting group strategy can, based on literature precedence,[57] allow for chain elongation at the anomeric position of the reducing end by independent removal of the OMP protecting group; or elongation at the 4-*O*-position of the non-reducing GlcA monomer by removal of the NAP ether.

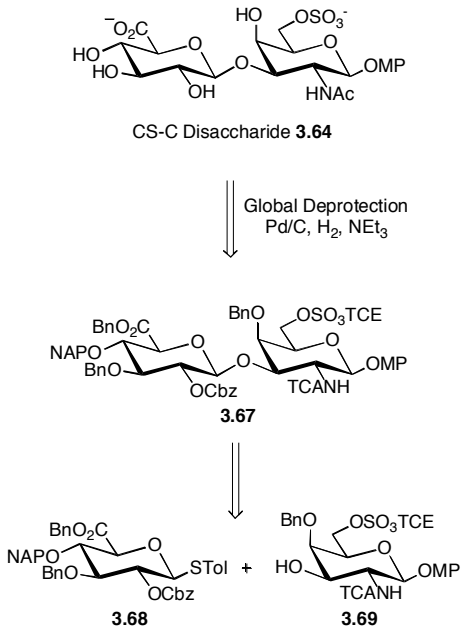
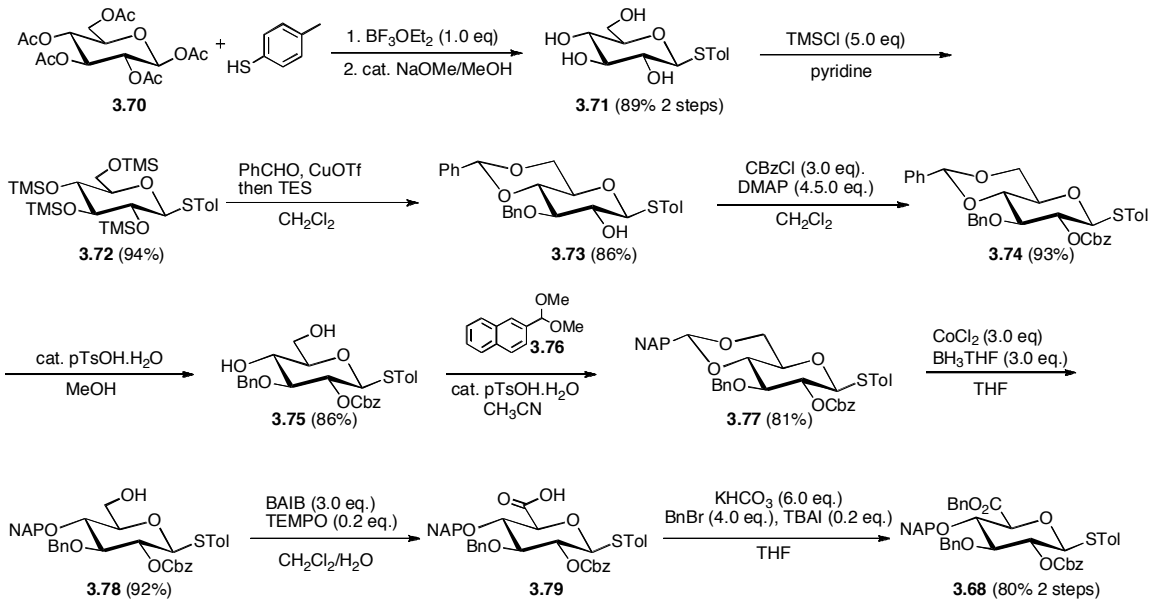


Figure 3.4. Retrosynthesis of CS-C disaccharide **3.64**

*3.3.1.1 Synthesis of the GlcA Thioglycoside Donor **3.68***

The challenge in the preparation of donor **3.68** was that by the end of the synthesis, each position in the carbohydrate ring had to be orthogonally protected from the others. The anomeric position required reliable protection, yet had to be easily activated for glycosylation when required. The 2-*O*-, and 3-*O*-positions required permanent protection; thus a benzyl protecting group was selected for the 3-*O*- position, and as mentioned above, a Cbz group was selected for the 2-position. Further manipulations of the target disaccharide would require selective access to a free 4-OH group, thus that position was protected with a NAP ether that can be removed in the presence of other benzyl groups.[57] Lastly, the 6-OH had to be oxidized to provide the corresponding glucuronic acid which required subsequent esterification. The synthesis of **3.68** is shown in Scheme 3.11.



Scheme 3.11. Synthesis of thioglycoside donor **3.68**

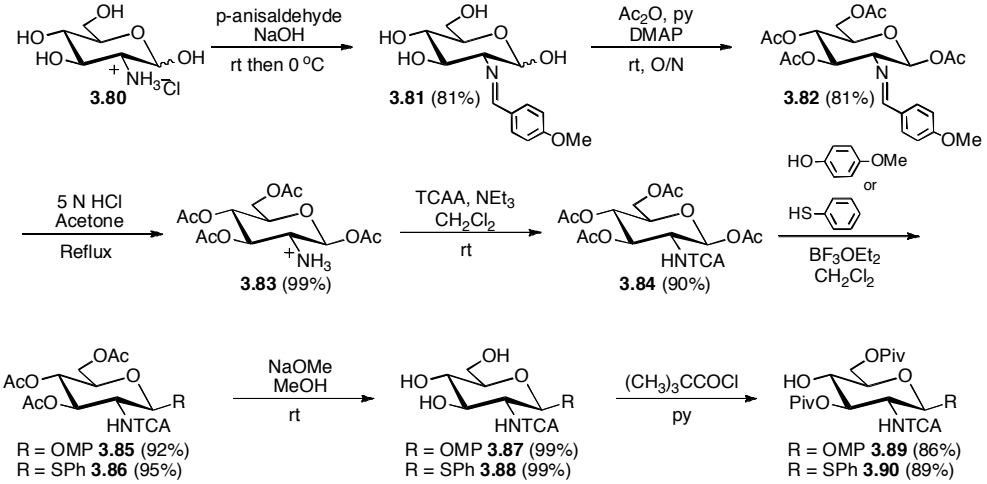
The synthesis of donor **3.68** began with the preparation of the tetrasilylated thioglycoside **3.72** in three steps from commercially available acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3.70**).[59] After an anomeric protecting group exchange and ester saponification under Zemplén conditions, tetraol **3.71** was silylated with chlorotrimethylsilane (TMSCl) in the presence of pyridine. Persilylated **3.72** served as the substrate for a regioselective one-pot protection reaction. The reaction concept was originally developed by Wang and co-workers,[58] however, we found that particular reaction protocol was very involved, and we were unable to repeat the literature yields. Instead, we applied a very elegant tandem catalysis reaction developed by Francais, Urban, and Beau.[59] In this reaction, **3.72** is treated with 1 mol% of CuOTf in the presence of benzaldehyde forming a di-benzylidenated intermediate (not shown). Addition of triethylsilane (TES) regioselectively opens the less stable 5-membered acetal to provide

the free 2-OH in **3.73** in high yields and excellent regioselectivity. The 2-*O*-position is then treated with CbzCl and DMAP to afford **3.74** in 82% yield. The presence of excess DMAP was essential for successful introduction of the Cbz protecting group, as attempts to do so with catalytic or equimolar amounts of DMAP and/or other organic bases (NEt_3, pyridine, Hunig's) were considerably less effective. Because the target required the 3-*O*-NAP protection, the benzylidene acetal was exchanged for a 2-naphthylidene acetal using **3.76**[60] to afford fully protected **3.77** in 81% yield. Regioselective reductive opening of the acetal using cobalt chloride and borane-THF[61] smoothly provided **3.78** in excellent yield. Lastly, a TEMPO/BAIB mediated primary oxidation provided glucuronic acid **3.79**, which was subsequently esterified using benzyl bromide to provide target donor **3.68** in 10 steps and an over 30% yield (89% average yield per step) from commercially available starting material.

*3.3.1.2 Synthesis of the GalNAc Acceptor **3.69***

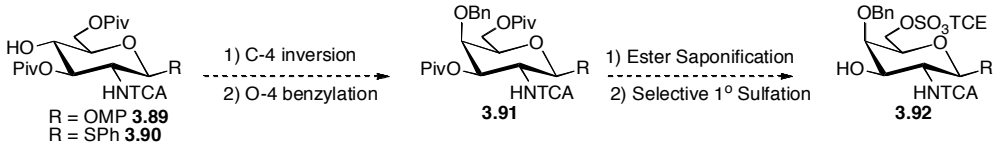
The synthesis of acceptor **3.69** was significantly more challenging and lengthy than that of donor **3.68**. The protecting group strategy was designed such that the anomeric group could easily be activated or left as a permanent protecting group at the end of a synthesis. The amine functionality was masked as a trichloracetamide that would be converted to the required *N*-acetatamide when needed. The free 3-OH position was required to form the desired glycosidic linkage, and the 4-OH was permanently protected with a benzyl group. Also, during the construction of **3.69**, the 6-OH required the introduction of the TCE-protected sulfate moiety.

The synthesis of acceptor **3.69** began with commercially available glucosamine hydrochloride (**3.80**). Using reliable literature procedures,[62,63] **3.80** was converted into tetraacetate **3.84** in four routine, high-yielding transformations (Scheme 3.12). At this point in the synthesis, it had not been determined whether the target would be prepared as a thioglycoside or an OMP-protected glycoside, thus both monomers were synthesized. Tetraacetate **3.84** was treated with BF_3OEt_2 in the presence of 4-methoxyphenol or benzenethiol to yield compounds **3.85** and **3.86,** respectively. Removal of the remaining acetate protecting groups gave carbohydrate triol compounds **3.87** and **3.88** in near quantitative yields. The 4-OH group in glucosamine derivatives is known to be notoriously unreactive.[64] Exploiting this well known fact, a slow addition of trimethylacetyl (pivaloyl, Piv) chloride to triol derivatives **3.87** and **3.88**[45] allowed for the selective 3,6-di-*O*-Piv protected carbohydrates **3.89** and **3.90** to be prepared and isolated in good yields (Scheme 3.12).



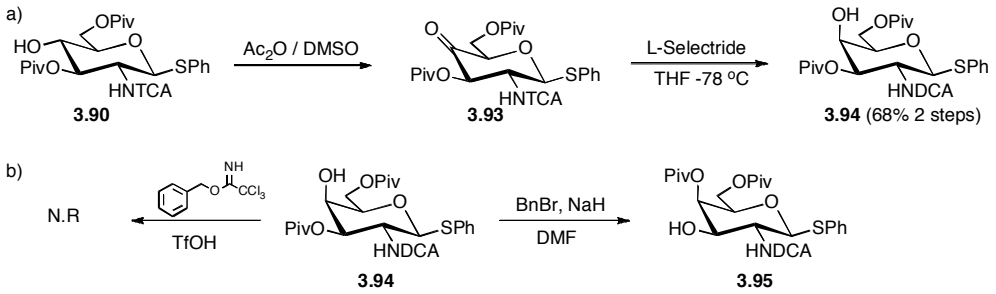
Scheme 3.12. Synthesis of 4,6-di-*O*-Piv monomers **3.89** and **3.90**

From this stage of the synthesis, we envisioned accessing the target acceptor as shown in Scheme 3.13. Because galactosamine based starting materials are rare and relatively expensive, C-4 inversion of configuration to transform glucosamine monomers **3.89** or **3.90** into their galactosamine counterparts is an alternative way to access the necessary carbohydrate configuration. The 4-OH of the newly formed galactosamine monomer would then be benzylated to give **3.91**, and the pivaloyl groups would be removed under Zemplén conditions to provide a partially protected monosaccharide bearing free hydroxyl groups at the 4- and 6-positions. Selective primary sulfation at the 6-position would then yield the target **3.92** acceptor in only 11 steps from glucosamine.



Scheme 3.13. Initial route to target acceptor

Unfortunately, the synthesis could not be executed as planned. In their synthesis of CS-D, Karst and Jacquinet had reported that upon triflate mediated inversion, the Piv protecting groups readily migrated to the 4-position of the galactosamine monomer. With this in mind, we chose to investigate an oxidation/reduction coupled inversion sequence[65] in which the equatorial 4-OH in **3.90** was oxidized to the corresponding ketone **3.93**, and then subsequently reduced with L-Selectride to provide the free axial 4-OH in **3.94** (Scheme 3.14a).

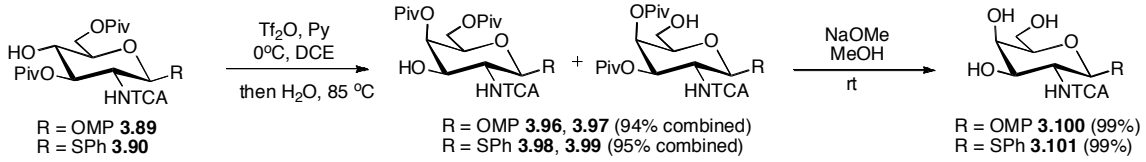


Scheme 3.14. Oxidation-reduction mediated C-4 inversion and unsuccessful benzyl protection

The 4-OH in **3.90** was oxidized to ketone **3.93** using DMSO and acetic anhydride. Subjecting **3.93** to L-Selectride at low temperatures resulted in the expected equatorial hydride attack to provide the free axial 4-OH in a 68 % yield over 2 steps (Scheme 3.14a). Unexpectedly, in this transformation, the *N*-trichloroacetamide was converted into a dichloroacetamide moiety, a side reaction which could not be avoided. This was not considered to be a problem as the trichloro- or dichloroacetamide would ultimately be reduced to the *N-acetamide* at the end of the synthesis. After work-up and purification of **3.94**, there was no evidence of Piv group migration, so attempts were made to benzyl-protect the axial 4-OH. Under standard benzylation conditions (NaH, BnBr, DMF), however, the only product that could be obtained was the 4,6-di-*O*-piv compound **3.95** resulting from a 3-*O*- to 4-*O*- ester migration. Attempts to install the benzyl protecting group under acidic conditions resulted in mostly starting material, with no indication that the target benzylated product was formed (Scheme 3.14b).

We then decided to return to the triflic anhydride/H_2O mediated C-4 inversion used by Karst and Jacquinet.[45] Carbohydrates **3.89** and **3.90** were each subjected to triflic anhydride then H_2O to effect the C-4 epimerization to achieve the required galactosamine

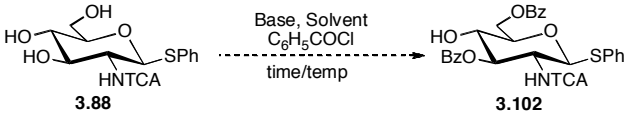
configuration (Scheme 3.15). Under these conditions, a mixture of the 4,6- and 3,4-di-*O*-Piv compounds **3.96** – **3.99** were obtained in good yields. These compounds can easily be separated by flash column chromatography; however it is not necessarily required as removal of the ester protecting groups using catalytic sodium methoxide in methanol provided the galactosamine triol **3.100** or **3.101** as a single isomer (Scheme 3.15).



Scheme 3.15. Glucosamine to galactosamine C-4 epimerization

The use of benzoyl group for the 3,6-*O*-protection of glucosamine triol **3.88** was also examined, to investigate whether the resulting benzoyl esters would be more or less prone to migration under the C-4 inversion conditions. In order to prepare the 3,6-di-*O*-benzoylated thioglycoside **3.102** several reaction conditions were examined (table 3.1).

Table 3.1. Preparation of 3,6-di-*O*-benzoylated **3.102**

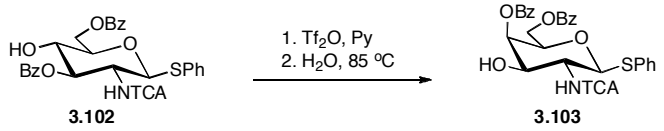


Entry	Base (eq.)	Solvent	BzCl (eq.)	Temp/Time	Result
1[a]	--	Py (0.03 M)	6.0	rt/8 h	3,4,6-tri-OBz
2	--	Py (0.03 M)	3.0	rt/8 h	3,4,6-tri-OBz
3	--	Py (0.03 M)	3.0	0 °C/5h	3,4,6-tri-OBz
4[b]	--	Py (0.03 M)	3.0	0 °C/5h	3,6- and 4,6-di-OBz (1:1)
5[b]	--	Py (0.03 M)	2.5	0 °C/5h	3,6- and 4,6-di-OBz (1:1)
6[b]	--	Py (0.03 M)	2.5	0 °C/5h	3,6- and 4,6-di-OBz (1:1)
7[b]	NEt_3 (5.0)	CH_2Cl_2 (0.1 M)	2.5	0 °C/5h	**3.102** 92%

a) literature conditions[66] b) BzCl added via syringe pump over 1h

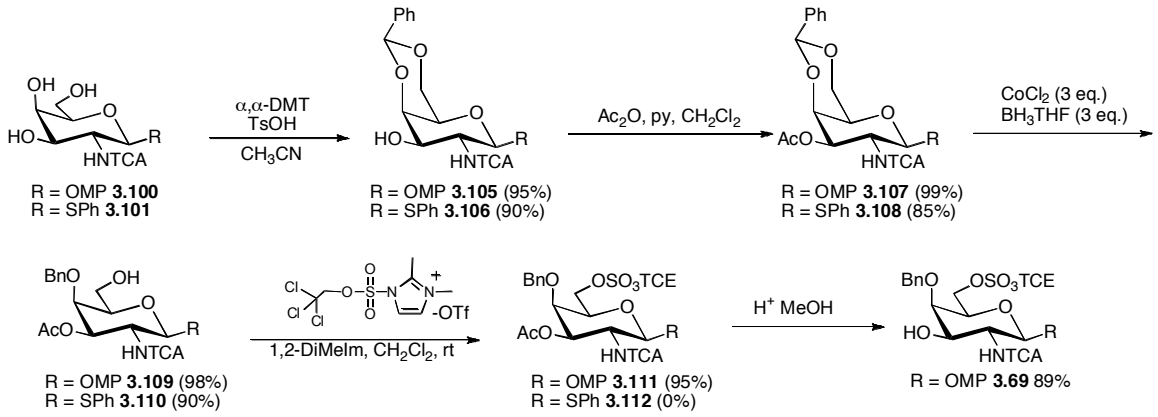
Applying the literature conditions[66] for the di-*O*-esterificaton to compound **3.88** with benzoyl chloride substituting for pivaloyl chloride (table 3.1, entry 1) resulted in complete benzoylation of the triol starting material. Decreasing the amounts of benzoyl chloride and cooling the reaction did not yield any selectivity in the introduction of the benzoyl groups (entries 2 and 3). Decreasing the addition rate by adding benzoyl chloride with a syringe pump at low temperatures resulted in a 1:1 mixture of the 3,4- and 4,6-di-*O*-benzoyl products (entries 4-6). Finally, changing the base from pyridine to triethylamine, and performing the reaction in dichloromethane provided the target di-benzoylated compound **3.102** in a 92% yield (entry 7).

Di-*O*-benzoylated **3.102** was then subjected to the C-4 inversion conditions as shown in Scheme 3.16. Not surprisingly, the benzoyl esters in **3.102** migrated as readily as the pivaloyl esters in **3.89**, and **3.103** was obtained. A year or so after this result had been observed, Rosuland and co-workers published an NMR and kinetic study on acyl group migrations in galactosamine monomers.[67] As was observed experimentally, the authors concluded that the rate of acyl group migration proceeds more slowly for Piv esters (rate OPiv<OBz<OAc), and that in galactopyranoside monomers, equatorial C-3 to axial C-4 acyl group migrations occur more rapidly than the reverse isomerization.



Scheme 3.16. Observed benzoyl migration after C-4 inversion

The synthesis of target acceptor **3.69** continued from triol **3.100**, and **3.101** (Scheme 3.17). Selective installation of the 4,6-*O*-benzylidene acetal was accomplished using benzaldehyde dimethyl acetal with catalytic p-toluenesulfonic acid ($TsOH \cdot H_2O$) to afford **3.105** and **3.106** in 95% and 90% yields respectively. Attempts to perform the regioselective opening of the benzylidene acetal in **3.106** were not successful (inseparable mixtures of the 4-OH and 6-OH isomers were obtained), thus the 3-OH was temporarily protected with an acetate ester. Fully protected **3.107** and **3.108** were then subjected to borane-THF and $CoCl_2$ to smoothly afford the desired 6-OH carbohydrates **3.109** and **3.110** in high yields.



Scheme 3.17. Completion of acceptor **3.69**

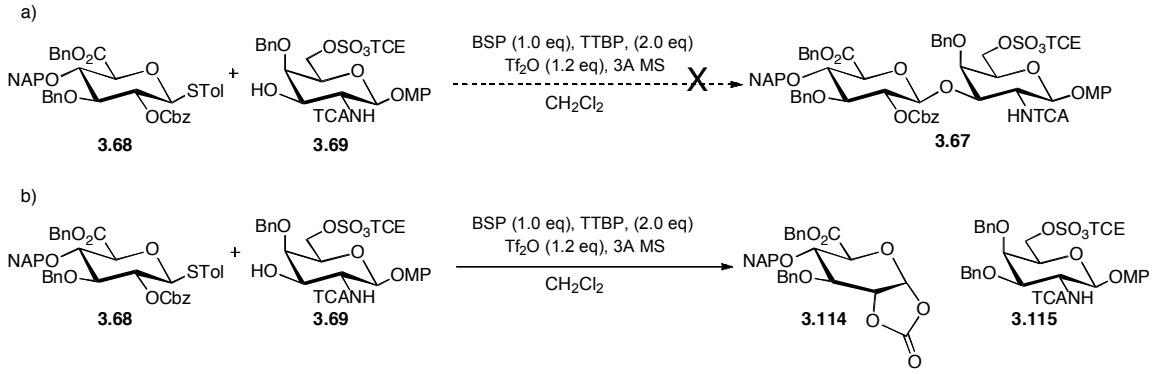
With the 1° 6-OH now available, **3.109** and **3.110** were subjected to our standard sulfation conditions with 2,2,2-trichloroethoxysulfuryl imidazolium triflate **2.40** in the presence of 1,2-dimethylimidazole, to access the TCE-protected sulfated monomers **3.111** and **3.112**. Surprisingly, the anomeric protecting group had a profound effect on the outcome of this reaction (Scheme 3.17). The sulfation reaction progressed very

smoothly with OMP protected **3.109**, providing the 6-*O*-TCE sulfate **3.111** in a 95% yield. Conversely, when thioglycoside **3.110** was subjected to identical conditions, a complex mixture of products resulted, and **3.112** could not be isolated or even detected in the reaction mixture. This result is not unlike that observed for the 2-*O*-sulfation of **2.51**, discussed in chapter 2. We do not have a clear understanding as to why certain thioglycoside compounds behave peculiarly under the developed sulfation conditions. This could be due to a reaction of the sulfur atom with the imidazolium salt or S_N2 attack on C-6 by the sulfur atom resulting in loss of the TCE sulfate and formation of a reactive sulfonium ion. From this point, the synthesis was carried through with OMP acceptor **3.69**, which was finally obtained after cleavage of the 3-*O*-acetate protecting group under acidic conditions. Target acceptor **3.69** was ultimately prepared in 14 steps and an overall 33% yield (92% average yield per step) from glucosamine hydrochloride.

*3.3.1.3 Glycosylation Conditions with Donor **3.68** and Acceptor **3.69***

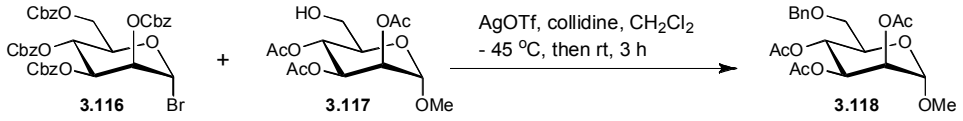
With the synthesis of thioglycoside donor **3.68** and acceptor **3.69** completed, we set out to prepare the fully protected disaccharide target **3.67** (Scheme 3.18a). There are a wide variety of glycosylation methods that can be used for a thioglycoside donors;[68] we chose to investigate the conditions developed by Crich and co-workers which utilizes the combination of 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) as a powerful means of activation for both armed and disarmed thioglycosides.[69,70] In this glycosylation reaction, a mixture of thioglycoside donor, BSP, and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in the presence of 3Å molecular sieves are activated at low temperatures with Tf_2O and then treated with the glycosyl acceptor

(Scheme 3.18a). Once again, however, the reaction did not proceed as planned, and target disaccharide **3.67** was not isolated. Instead, the major products isolated from the reaction were the cyclic carbonate **3.114** and benzylated acceptor **3.115** (Scheme 3.18b).



Scheme 3.18. Unsuccessful glycosylation using donor **3.68**

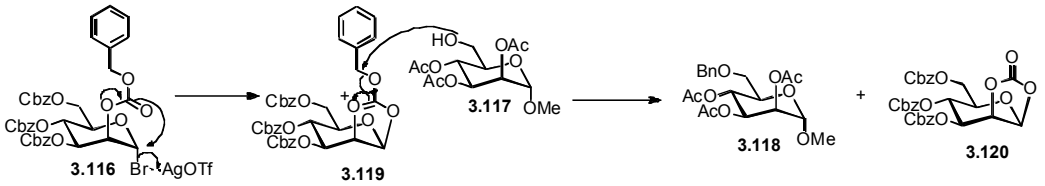
This type of benzyl group transfer reaction had been previously noted by Montero and coworkers and so was not entirely unexpected.[71] The authors have shown that the attempted coupling of donor **3.116** with acceptor **3.117** using AgOTf as activator resulted in the formation of monosaccharide **3.118** as the only isolated product (Scheme 3.19).



Scheme 3.19. Formation of **3.118** during the coupling of donor **3.116** to acceptor **3.117**[71]

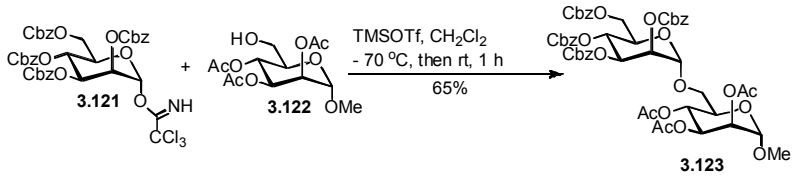
The authors suggested that upon activation, the 2-*O*-Cbz group of the donor participates (as expected) in the formation of the oxazolium ion **3.119**. The subsequent

nucleophilic attack by the acceptor however, did not occur at the anomeric position as required, but at the benzylic carbon in the Cbz protecting group, as shown in Scheme 3.20. The cyclic carbonate **3.120** was not isolated.



Scheme 3.20. Mechanism for cyclic carbonate formation as proposed by Montero and coworkers[71]

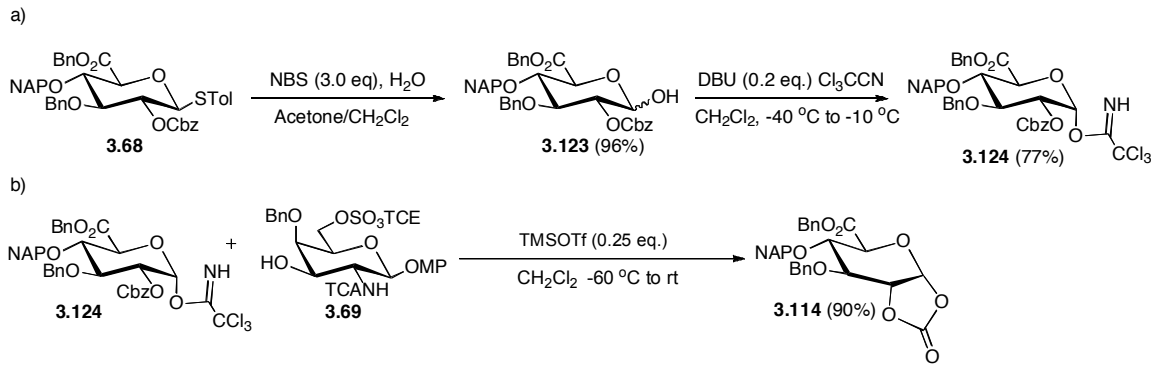
However, the authors also reported that the desired disaccharide **3.122** could be obtained in a 65 % yield by using trichloroacetimidate glycosyl donor **3.121**, as shown in Scheme 3.21. No product resulting from benzyl group transfer was isolated under these glycosylation conditions.



Scheme 3.21. Formation of the desired product **3.122** using imidate donor **3.121**

On the basis of these results we decided to examine whether trichloroacetimidate glycosyl donor **3.124** could be used in place of thioglycoside donor **3.68** to get the desired disaccharide (Scheme 3.22). Thus, thioglycoside **3.68** was smoothly converted to its corresponding hemiacetal using NBS/H_2O to afford **3.123** in a 96% yield. Subsequent

treatment of **3.123** with a catalytic amount of DBU in the presence of trichloroacetonitrile afforded imidate **3.124** in a 77% yield (Scheme 3.22a). The newly formed imidate **3.124** and glycosyl acceptor **3.69** were then combined and treated with TMSOTf at -60 °C in an attempt to produce the target disaccharide **3.67**. After work-up and purification, however, carbonate **3.114** was isolated in a 90% yield with only trace indication of any disaccharide formation (Scheme 3.22b). This undesired side reaction could possibly offer one explanation as to why 2-*O*-Cbz moieties are not widely used in carbohydrate synthesis. This result was somewhat of a disappointment, as changing the 2-*O*-protecting group would likely negate the possibility of the global one-pot deprotection at the end of the synthesis unless another protecting group can be found for this position that can be removed by hydrogenolysis. A more detailed discussion of the use of the Cbz group in carbohydrate chemistry will be presented in Chapter 4.



Scheme 3.22. Formation of carbonate **3.114** from trichloroacetimidate donor **3.124**

3.3.2 Investigations into the synthesis of CS-D based disaccharide 3.65

The following studies on the synthesis of the CS-D disaccharide **3.65** were concurrent with our studies on the synthesis of the CS-C disaccharide **3.64**. For what is

considered to be a more challenging synthesis, the retrosynthetic analysis of **3.65** is presented in Figure 3.5. The synthesis is once again designed to allow for a global one pot deprotection of disaccharide **3.125** as the protecting group strategy is designed to result in a disaccharide bearing only hydrogenolyzable protecting groups, along with the *N*-trichloroacetamide which is reduced under the catalytic hydrogenation conditions. The synthesis will make use of the previously prepared acceptor **3.69**, and will involve the preparation of the 2-*O*-sulfated glucuronic acid donor **3.125**. In the synthesis of this target, the effect of the 2-*O*-sulfate group on the outcome of a glycosylation reaction will be studied in order to determine if the 2-*O*-TCESO_3 moiety is sterically demanding enough to ensure the β-stereochemistry of the newly formed glycosidic linkage in **3.125**.

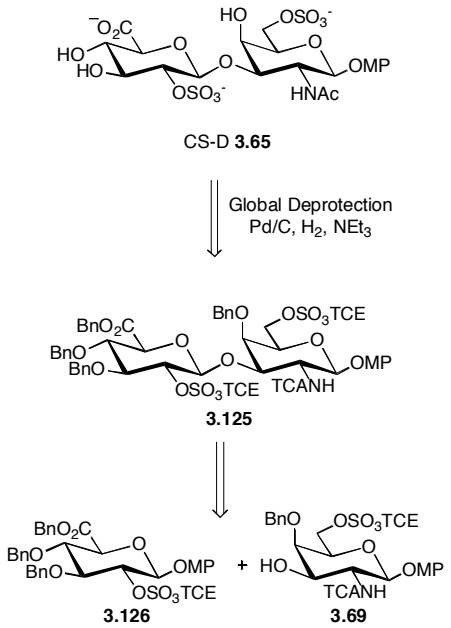
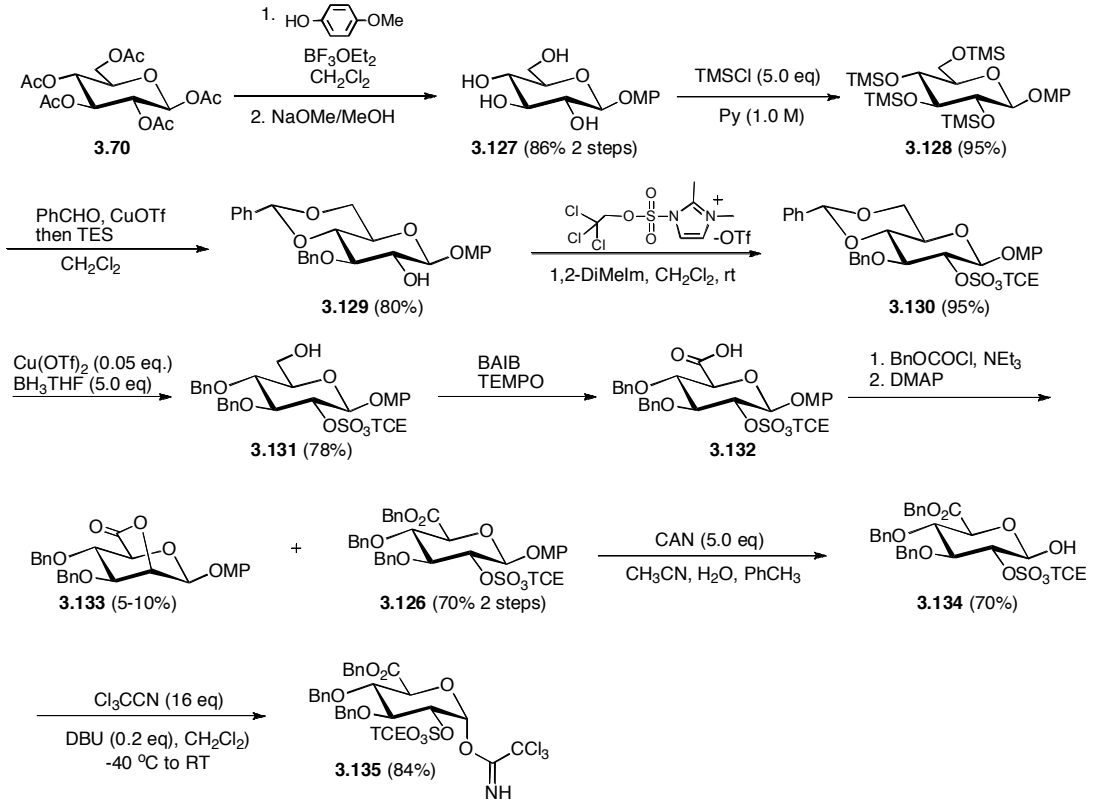


Figure 3.5. Retrosynthesis of CS-D disaccharide **3.65**

*3.3.2.1 Preparation of Sulfated Donor **3.135***

The synthesis of donor **3.135** is depicted in Scheme 3.23. The one pot regioselective protection chemistry previously shown in Scheme 3.11 was utilized once again in the preparation of carbohydrate **3.129**. From previous sulfation studies (ex. the 2-*O*-sulfation of **2.49**, chapter 2) it was known that 2-*O*-sulfation of a thioglycoside was not an option for the preparation of this target, thus **3.126** was designed to have an anomeric OMP moiety that could offer versatile protection yet be removed to provide access to the glycosyl donor. The literature substrates for the one-pot regioselective protection methodology contained either an anomeric α-methoxy, or β-thiol moiety;[59] but it was anticipated that the procedure would adapt to the β-OMP glycosides without complications. As was predicted, tetrasilylated **3.128** was easily prepared from OMP-protected tetraol **3.127**, and performed as an ideal substrate for the regioselective preparation of **3.129** in an 80% yield. 2-*O*-sulfation of **3.129** proceeds smoothly in a 95% yield using the 2,2,2-trichloroethoxysulfuryl imidazolium triflate sulfating agent **2.40** in the presence of 1,2-dimethylimidazole to provide fully protected **3.130** in a 95% isolated yield. Regioselective reductive opening of the benzylidene acetal using the standard $CoCl_2$ and BH_3•THF conditions progressed very slowly for substrate **3.130**, but substituting the $CoCl_2$ with CuOTf[72] provided **3.131,** which was subsequently oxidized to its corresponding glucuronic acid **3.132**. The TCE-sulfate moiety was found to be stable to both of these manipulations. Initial attempts to benzyl-protect the glucuronic acid **3.132** resulted solely in lactone **3.133**, as under the basic reaction conditions, the 2-*O*-$TCESO_3$ group was easily displaced by the carboxylate. The formation of this undesired

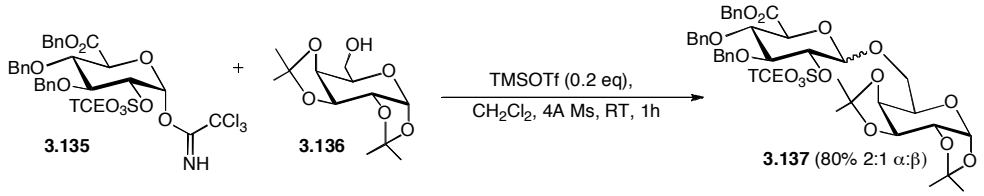
side product was, for the most part, circumvented by reversing the addition order of the reagents such that the NEt_3 is added to the reaction mixture after the addition of benzyl chloroformate. The formation of lactone **3.133** could not be avoided completely, but was often present in less than a 5-10% yield, and desired carbohydrate ester **3.126** could be isolated in a 70% yield after the oxidation and protection manipulations. To convert **3.126** into an activated glycosyl donor, oxidative hydrolysis of the anomeric OMP group using ammonium cerium nitrate (CAN) provided hemiacetal **3.134**, which was immediately used to prepare **3.135** in an 84% yield. Overall, trichloroacetimidate donor **3.135** was prepared in 20% (85% average per step) over a total of 10 steps (Scheme 3.23).



Scheme 3.23. Preparation of trichloroacetimidate donor **3.135.**

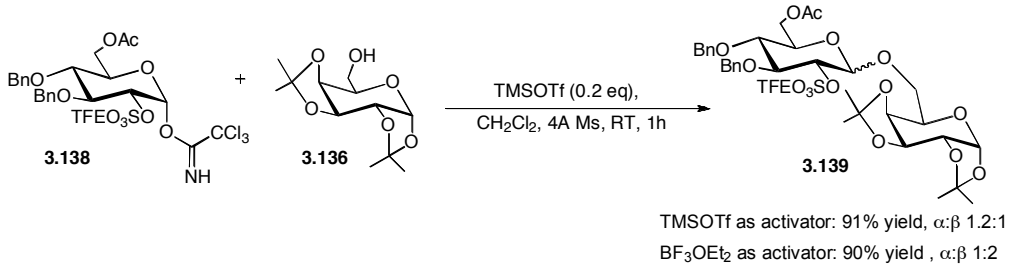
*3.3.2.2 Glycosylation with 2-O-Sulfated Donor **3.135***

The stereochemical outcome of a glycosylation reaction using donor **3.135** was first studied using 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**3.136**) as a simple nucleophilic acceptor, according to Scheme 3.24 The $TCEOSO_3$ moiety does not appear to participate in the formation of the oxazolium intermediate, and disaccharide **3.137** was isolated as a 2:1 α:β mixture in an overall 80% yield.



Scheme 3.24. Model glycosylation reaction with donor **3.135**

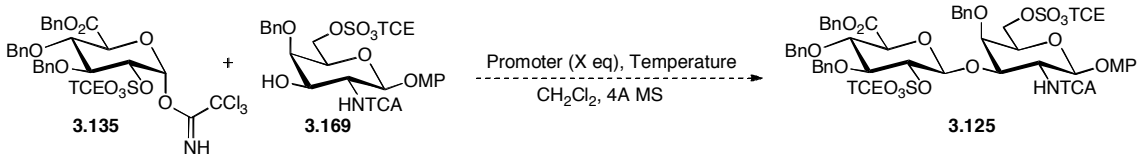
These results are similar to Linhardt and coworkers results during their studies on the use of trifluoroethyl-protected sulfates in the synthesis of sulfated carbohydrates.[24] For example, the authors have shown that the reaction of donor **3.138** and acceptor **3.136** resulted in the formation of disaccharide **3.139** in high yields but as α,β mixtures (Scheme 3.25).



Scheme 3.25. Linhardt and coworkers synthesis of disaccharide **3.139**

Despite obtaining an α/β mixture of **3.137**, the glycosylation reaction between donor **3.135** and acceptor **3.169** was attempted under various conditions (table 3.2). Under these circumstances, however, target disaccharide **3.125** could not be detected or isolated. For each reaction condition, the end result was a complex mixture of carbohydrate-based products that were very difficult to separate and identify. It appears that our sulfate protecting group strategy is not a very practical approach to preparing oligosaccharides having a sulfate group at the 2-position.

Table 3.2. Glycosylation conditions for donor **3.135** and acceptor **3.69**



Entry	Donor (eq)	Acceptor (eq.)	Temp.	Promoter (eq.)	Result
1	1.0	1.0	-40 °C to rt	TMSOTf (0.2)	Mixture
2	1.0	1.5	-40 °C to rt	TMSOTf (0.2)	Mixture
3	1.0	1.5	-40 °C to rt	BF_3OEt_2 (0.2)	Mixture
4	1.0	2.0	Rt	TMSOTf (0.5)	Mixture
5	1.0	1.5	Rt	BF_3OEt_2 (0.5)	Mixture
6	1.0	1.0	Rt	TMSOTf (1.0)	Mixture
7	2.0	1.0	Rt	BF_3OEt_2 (1.0)	Mixture

3.3.2.3 Preparation of the CS-D Target via Sulfation of a CS-C Disaccharide

While the negative results from the glycosylation attempts with 2-*O*-sulfated donor **3.135** were disappointing, they were not entirely unexpected. Because of this, a synthetic sequence was designed such that the target di-sulfated CS-D **3.65** would be obtained from the sulfation of a protected CS-C disaccharide **3.140**. The retrosynthesis

of both targets is outlined in Figure 3.6. In this approach, monosulfated CS-C disaccharide **3.141** would be assembled from thioglycoside donor **3.142** and previously prepared galactosamine acceptor **3.69** The advantage of the MCA protecting group is that it can be selectively cleaved in the presence of other acyl protecting groups under a variety of conditions including aqueous ammonia,[73] thiourea, [74] and DABCO, [75] among others. This is a key factor in the synthesis of CS-D, as the chloroacetate protecting group will need to be removed from **3.141** in the presence of a benzyl ester, as well as a TCE-protected sulfate moiety. Upon successful removal of the 2-O-MCA group, the second sulfate will be introduced to provide the fully protected CS-D disaccharide **3.125**. A one pot global deprotection reaction will then provide access to target **3.65**.

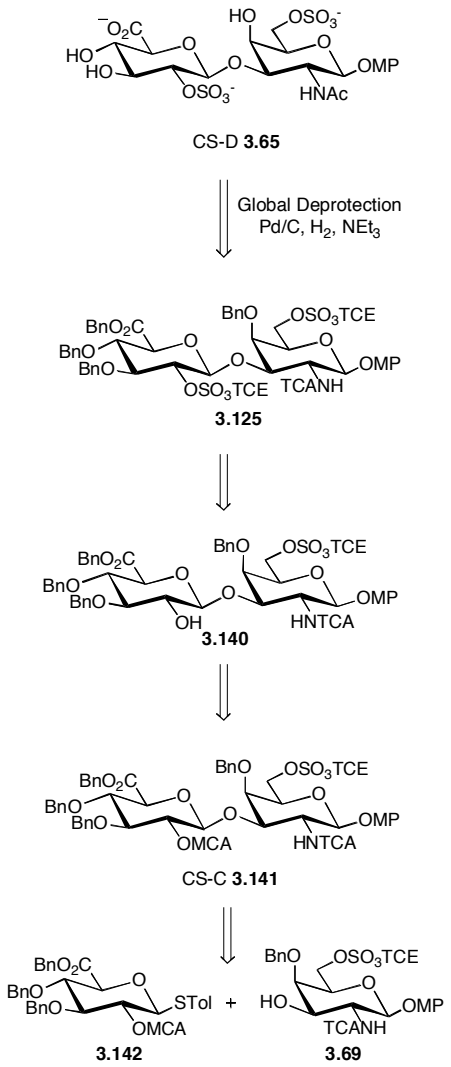
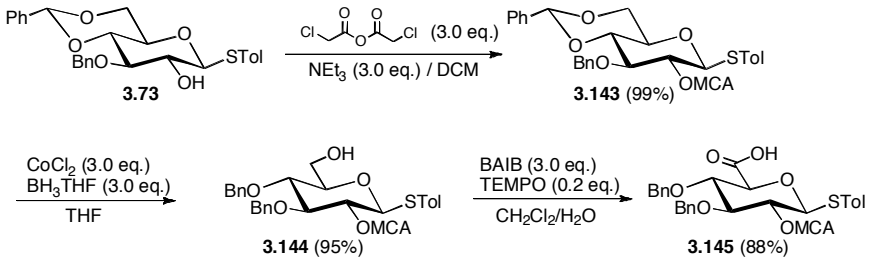


Figure 3.6. Retrosynthesis of CS-D and CS-C based disaccharides

The 2-*O*-MCA protecting group was introduced to thioglycoside **3.73** using chloroacetic anhydride and triethylamine to provide fully protected **3.143** in near quantitative yields (Scheme 3.26). At this point, we chose to forego the 4-*O*-NAP protection strategy in favor of a shorter synthesis; thus, **3.143** was treated with borane-THF to effect the regioselective opening of the benzylidene acetal affording the 6-OH

3.144 in 95% yield. Once again, the glucose monomer was converted to its corresponding glucuronic acid using a BAIB/TEMPO mediated oxidation to provide **3.145** in an 88 % yield (Scheme 3.26).

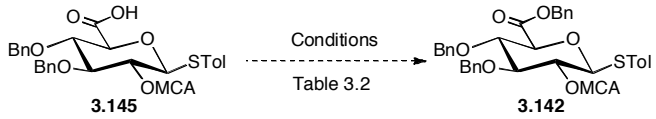


Scheme 3.26. Modifying the 2-*O*-protecting group

Initial attempts to benzyl protect the resulting carboxylic acid **3.145** under the conditions previously described in Scheme 3.11 (i.e. $KHCO_3$, BnBr, TBAI, DMF) were more complicated when **3.145** was the substrate. When previously performed on **3.79,** the 2-*O*-CBz derivative of **3.145**, the progression of the reaction was easily monitored by TLC. As the spot corresponding to the glucuronic acid substrate disappeared, a faster running product spot formed, until starting material no longer could no longer be detected. In the case of **3.79** isolation of the product spot resulted in pure **3.68** in good yields. When **3.145** was used as the substrate, the reaction progressed identically by TLC as expected; however, NMR analysis of the isolated product indicated that two very similar compounds were produced in the reaction as many of the signals in both the ^{1}H and ^{13}C spectra were complicated by what appeared to be shadow peaks. Under no circumstances could these products be separated by TLC, and because of this the absolute structure of the contaminating product could not be determined. Its possible that the

contaminating product results from an epimerization of the C5 position, but this theory could not be validated. A number of conditions were examined in attempts to minimize the occurrence of this unwanted by-product (table 3.3); however when strong bases were used for the protection reaction, pure **3.142** could not be isolated (entries 1-4). To prepare **3.142** under more mild esterification conditions, a decarboxylative benzylation procedure developed by Kim and Lee in 1985 was attempted.[76] In this reaction, the carboxylic acid **3.145** is treated with benzyl chloroformate in the presence of triethylamine to induce the formation of a mixed carboxylic-carbonic anhydride intermediate. Addition of a catalytic amount of DMAP then promotes the conversion of the mixed anhydride into the target benzyl ester **3.145** through a decarboxylative benzylation reaction. Fortunately, under these conditions, pure **3.145** could be isolated in a 73% yield (table 3.3, entry 7).

Table 3.3. Optimized benzyl esterification conditions.

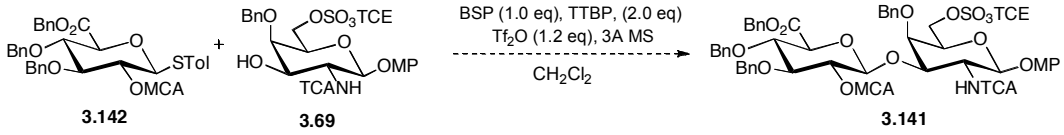


3.145 **3.142**

Entry	Solvent	Base (eq.)	Reagent (eq.)	Result
1	DMF	$KHCO_3$ (6.2 eq)	BnBr (4.0 eq), TBAI (0,2 eq)	Mixture
2	THF	$KHCO_3$ (6.2 eq)	BnBr (4.0 eq), TBAI (0,2 eq)	Mixture
3	THF	$KHCO_3$ (1.0 eq)	BnBr (4.0 eq), TBAI (0,2 eq)	Mixture
4	THF	NaH (1.5 eq)	BnBr (4.0 eq), TBAI (0,2 eq)	Mixture
6	CH_2Cl_2	NEt_3 (1.0 eq)	BnOCOCl (1.0 eq) then DMAP (0.2 eq)	64% **3.142**
7	CH_2Cl_2	NEt_3 (1.3 eq)	BnOCOCl (1.3 eq) then DMAP (0.2 eq)	73% **3.142**

With the preparation of a second derivative of the required donor completed, we returned to investigate the glycosylation conditions required for the preparation of the CS-based disaccharide **3.141**. Table 3.4 summarizes the glycosylation conditions examined for the preparation of disaccharide **3.141** Starting with a set of literature conditions[69] (entry 1) resulted in an encouraging 64% yield of target **3.141**. Decreasing the reaction temperature (entries 2, 3 and 4) had a negative effect on the isolated yield of **3.141**. Modifying the reaction so that the glycosyl donor **3.142** was present in excess of glycosyl acceptor **3.69** resulted in a significant increase in the yield of **3.141** to 84-86% (entries 7 and 8). Thus it appeared that we were able to obtain disaccharide **3.141** in a very good yield.

Table 3.4. Glycosylation conditions for the preparation of **3.141.**



Entry	Donor (eq)	Acceptor (eq.)	Temp.	Time	Result*
1	1.0	1.5	-60 °C to rt	3 h	64%
2	1.0	1.5	-78 to -40 °C	3 h	41%
3	1.0	1.5	-60 °C	3 h	50%
4	1.0	2.0	-60 °C to rt	5 h	68%
5	1.0	1.5	-60 °C to rt	16 h	54%
6	1.0	1.0	-60 °C to rt	3 h	55%
7	1.5	1.0	-60 °C to rt	3 h	84%
8	2.0	1.0	-60 °C to rt	3 h	86%

* Results for presumed product **3.141**. See structural reassignment section 3.3.2.5.

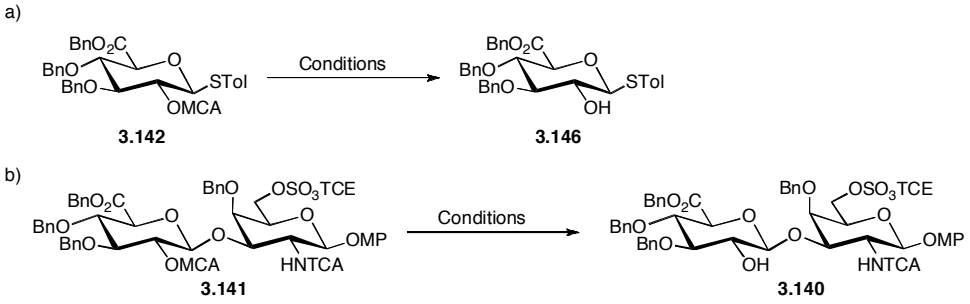
With the protected CS-C disaccharide fully prepared, we began to examine the conditions required for the removal of the MCA group. As previously described in

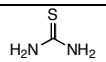
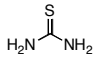
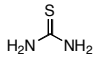
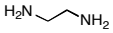
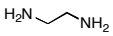
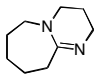
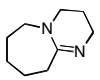
Figure 3.6, removal of the 2-*O*-MCA group from **3.141** would provide **3.160** with the free 2-*O*-position on the glucuronic acid monomer. Subjecting **3.140** to the standard sulfation conditions would provide target **3.125**, the fully protected precursor to the target CS-D disaccharide **3.65**. It was not known if the 1° SO_3TCE group or the benzyl ester would be tolerant of the basic and nucleophilic conditions for the removal of the MCA, thus a comparison study was performed with monosaccharide donor **3.142** and protected disaccharide **3.141**. For the deprotection to be successful on **3.142**, the benzyl ester on the GlcA monomer must remain intact; and to be successful on **3.141** the $TCESO_3$ group must not be affected. It would then be assumed that any conditions that successfully cleaved the MCA group from **3.142** but failed for **3.141**, did so due to complicating reactions with the 1° SO_3TCE moiety. The results from this study are presented in table 3.5.

Monosaccharide donor **3.142** was subjected to a variety of conditions (Table 3.5) to remove the MCA protecting group. Thiourea at 80 °C in ethanol and pyridine (entry 2), DABCO in ethanol (entry 4) and ethylenediamine in ethanol and pyridine (entry 10) all effectively removed the 2-*O*-MCA in **3.142** to afford **3.146** in 95-96% yields. Surprisingly, performing the same transformation on apparent disaccharide **3.141** was considerably more complex, and desired **3.140** could not be isolated. Instead, as the reaction progressed, the mixture got increasingly complicated (as analyzed by TLC), and subsequent work-up and purification did not produce any major identifiable carbohydrate based products. At this point, it was assumed that the 6-*O*-$TCESO_3$ group was intolerant

to the conditions required to remove the 2-*O*-MCA group, thus an alternate approach to the CS-D disaccharide was investigated.

Table 3.5. Nucleophilic and basic conditions for cleavage of the MCA group.



Entry	Carbohydrate	Reagent	Eq.	Solvent	Temp °C	Time	Result
1	**3.142**		4.0	EtOH/py	RT	24 h	NR
2	**3.142**		4.0	EtOH/py	80 °C	1 h	96 % **3.146**
3	**3.141**		4.0	EtOH/py	80 °C	1 h	Mixture
4	**3.142**		15.0	EtOH	55 °C	5 h	95% **3.146**
5	**3.141**		15.0	EtOH	55 °C	5 h	Mixture
6	**3.142**		1.5	EtOH/py	RT	24 h	NR
7	**3.142**		15.0	EtOH/py	RT	24 h	NR
8	**3.141**		1.5	EtOH/py	RT	24 h	NR
9	**3.141**		15.0	EtOH/py	RT	24 h	NR
10	**3.142**		1.9	EtOH/py	RT	24 h	95% **3.146**
11	**3.141**		1.9	EtOH/py	RT	9 h	Mixture
12	**3.142**		2	EtOH	RT	1h	90% **3.146**
13	**3.141**		2	EtOH	RT	1h	Mixture

3.3.2.4 CS-D via Disulfation of a Partially Protected Disaccharide

The approach to the CS-D target was then modified such that the protected disaccharide scaffold would be constructed, and then the two TCE-protected sulfate moieties would be introduced as described in Figure 3.7. The target disaccharide **3.125** can be accessed through a simultaneous disulfation of diol **3.147**, which is obtained from the fully protected **3.148**.

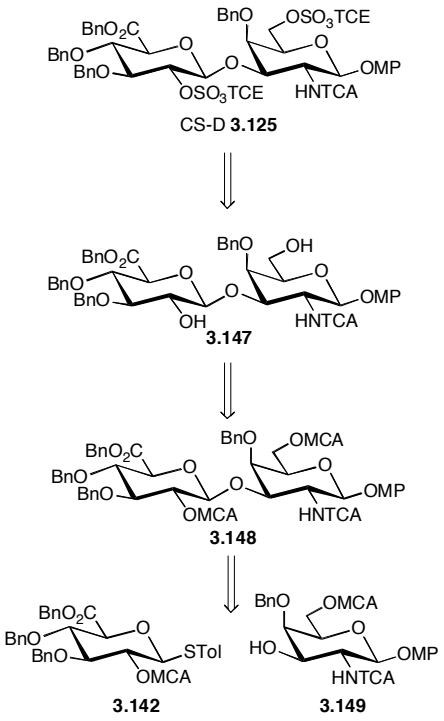
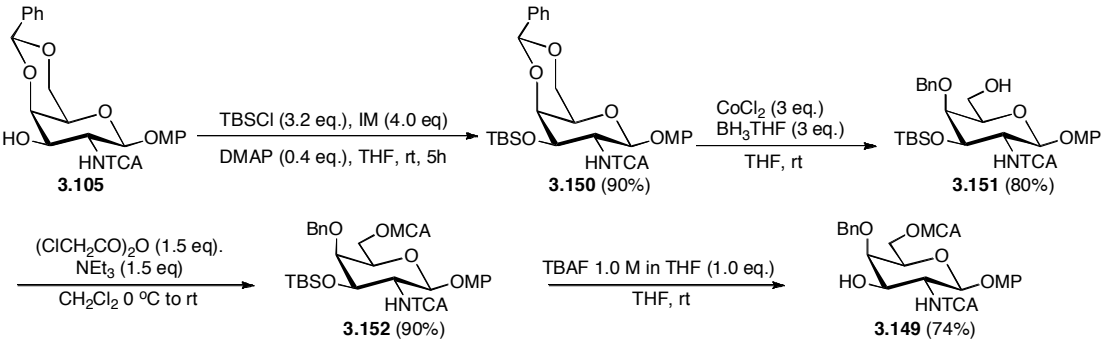


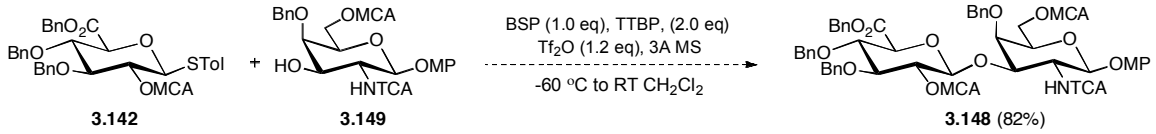
Figure 3.7. Alternative approach to CS-D disaccharide **3.125**

The previously prepared glucuronic acid donor **3.142** would serve to prepare disaccharide **3.148** without requiring any modifications. The galactosamine acceptor had to be redesigned with an alternative protecting group strategy so that the 3-OH group could be independently deprotected in the presence of the 6-*O*-MCA to provide glycosyl acceptor **3.149**. The synthesis of **3.149** is described in Scheme 3.27.



Scheme 3.27. Modified glycosyl acceptor **3.149**

Starting from intermediate **3.105** (from Scheme 3.17), a t-butyldimethylsilyl (TBS) group was introduced at the 3-*O*-position with TBDMSCl in the presence of imidazole and catalytic DMAP to give **3.150** in a 90 % yield. Cobalt chloride/borane THF directed opening of the 4,6-di-*O*-benzylidene acetal provided partially protected **3.151** in good yields. Subsequent introduction of the 6-*O*-MCA group followed by TBAF mediated removal of the 3-*O*-TBS group afforded glycosyl acceptor **3.149**.

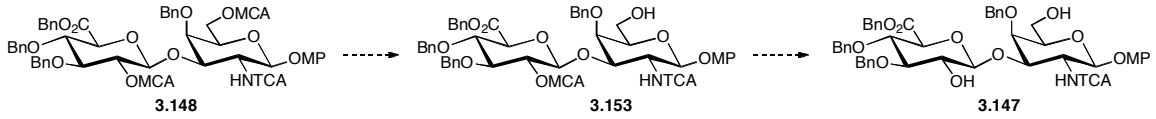


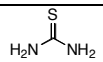
Scheme 3.28. Formation of protected disaccharide **3.149.** See structural resassignment in section 3.3.2.5.

The glycosylation reaction between thioglycoside donor **3.142** and 6-*O*-MCA acceptor **3.149** appeared to proceed smoothly to afford what was assumed to be fully protected disaccharide **3.148** in an 82% yield (The structure of **3.148** is reassigned in section 3.3.2.5). The next step in the synthesis was the simultaneous cleavage of the 2-*O*,

and 6'-*O*-MCA protecting groups to access diol **3.147**, the required target for the disulfation reaction. Presumably, the 6-*O*-MCA group is more labile than the 2-*O*-MCA protecting group, thus the di-deprotection reaction would likely proceed through monodeacetylated intermediate **3.153**. It was anticipated that this deprotection reaction would proceed smoothly, as we had previously demonstrated that the benzyl ester was tolerant of the conditions required for the dechloroacetylation (table 3.6); the $TCESO_3$ moiety was no longer present in the disaccharide structure; and the remaining benzyl ethers should not be affected by the basic reaction conditions. Unfortunately, this was not the case, as once again, the 2-*O*-MCA group appeared to be surprisingly difficult to remove. Table 3.6 summarizes the conditions attempted to remove the 2-*O*-MCA group from carbohydrates **3.148** or **3.153**.

Table 3.6. Di-Dechloroacetylation conditions for **3.148** and **3.153**



Entry	Carbohydrate	Reagent	Eq.	Solvent	Temp °C	Time	Result
1	**3.148**		4.0	EtOH/py	80 °C	5 h	**3.153** 71%
2	**3.153**		4.0	EtOH/py	80 °C	8 h	NR
3	**3.148**		30.0	EtOH	55 °C	5 h	**3.153** 65%
4	**3.153**		30.0	EtOH	55 °C	8 h	NR
5	**3.148**		2	EtOH	RT	10 min	**3.153** 65%
6	**3.153**		2	EtOH	RT	8 h	NR
7	**3.148**		8	EtOH	80 °C	5 h	**3.153**
8	**3.153**		15	EtOH	80 °C	12 h	Dec.

Subjecting disaccharide **3.148** to 2 eq. of DBU in EtOH for 10 minutes resulted in complete removal of the primary MCA protecting group giving disaccharide **3.153**, isolated in a 65% yield (entry 6). Treating the monodeprotected **3.153** with another 2 eq. of DBU did not result in the second deprotection, despite extended reaction times (entry 2). The 6-*O*-MCA group was also successfully removed using DABCO (entry 3) and

thiourea (entry 5) to provide the monodeprotected **3.153** in low to moderate yields. Di-deprotected **3.147** was not detected or isolated in any of the described reactions. Attempts to use more forcing conditions (entry 8) with a large excess of DBU in EtOH at elevated temperatures resulted in the formation and then decomposition of **3.153**.

From the previous deprotection study (table 3.5), removal of the 2-*O*-MCA protecting group from monosaccharide **3.142** was successful under a variety of nucleophilic and/or basic conditions. The same transformation on apparent disaccharides **3.141** and **3.148**, however, were not successful. Because of these results, it was assumed that the 6-*O*-$TCESO_3$ group was not surviving the dechloroacetylation reaction, and thus needed to be installed at the disaccharide stage. The results summarized in table 3.6, now suggest that this may not be the case.

*3.3.2.5 Structural Reassignment of Compounds **3.141** and **3.148***

The difficulties encountered in the removal of the 2-OMCA moiety lead us to analyze the structures of compounds **3.141** and **3.148** more closely. When **3.141** was first prepared, it was anticipated that the 2-OMCA group would provide the required anchimeric assistance to ensure the desired β-linkage. As the reaction progressed, there was no evidence of the formation of an α/β mixture, thus it was assumed that the 2-OMCA participated in the reaction, and the required β-linkage was formed. Upon analysis with ^{1}H NMR, the observed coupling constant for $J_{H1\text{-}H2}$ of the glucuronic acid moiety was calculated to be 3.5 Hz. While this is on the small side for a β-linked disaccharide, it was thought that the glucuronic acid ring might not be in the true $^4C_1$

conformation, and ring distortion could lead to distortion of the observed coupling constants.

During a discussion with Dr. Auzanneau (Dept. of Chemistry, University of Guelph), it was suggested that disaccharides **3.141** and **3.148** might in fact not be disaccharides, but could rather be orthoesters **3.154** and **3.155**. Orthoesters of this type have been reported by several groups.[77] Analysis of the ^{1}H NMR for carbohydrate **3.141** suggests that that theory is likely correct. Along with the skewed $J_{H1\text{-}H2}$ value for the glucuronic acid ring, a very notable change in the chemical shift of the H2 proton of the GlcA ring was observed. The H2 proton connected to the 2-OMCA moiety in monomer **3.142** occurs at 5.1 ppm. In what was assumed to be disaccharide **3.141**, the proton corresponding to H2 of the GlcA ring was found at 4.2 ppm, an unexpected upfield shift. The observed $J_{H2\text{-}H3}$ value for the same proton was also much smaller than expected ($J_{H2\text{-}H3}$ = 4.0 Hz)**.** The flattening of the carbohydrate ring through orthoester formation could account for the unusual values for the observed coupling constants. Furthermore, what had gone unnoticed was the appearance of an unusual quaternary carbon at 124 ppm in the aromatic region of the ^{13}C NMR. This could easily correlate to the quaternary carbon of the proposed orthoester. Unfortunately, the same characteristics were observed for disaccharide **3.148**. In light of these discoveries, the structures of **3.141** and **3.148** have been revised to be orthoesters **3.154** and **3.155** (Figure 3.8). In some instances orthoesters of the type shown in Figure 3.8 have been isomerized under acidic conditions to the desired β-linked disaccharides in good yield.[78] Studies to achieve the isomerization of **3.154** to **3.141** are currently in progress in the Taylor group.

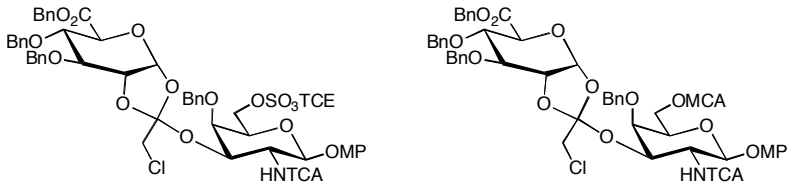
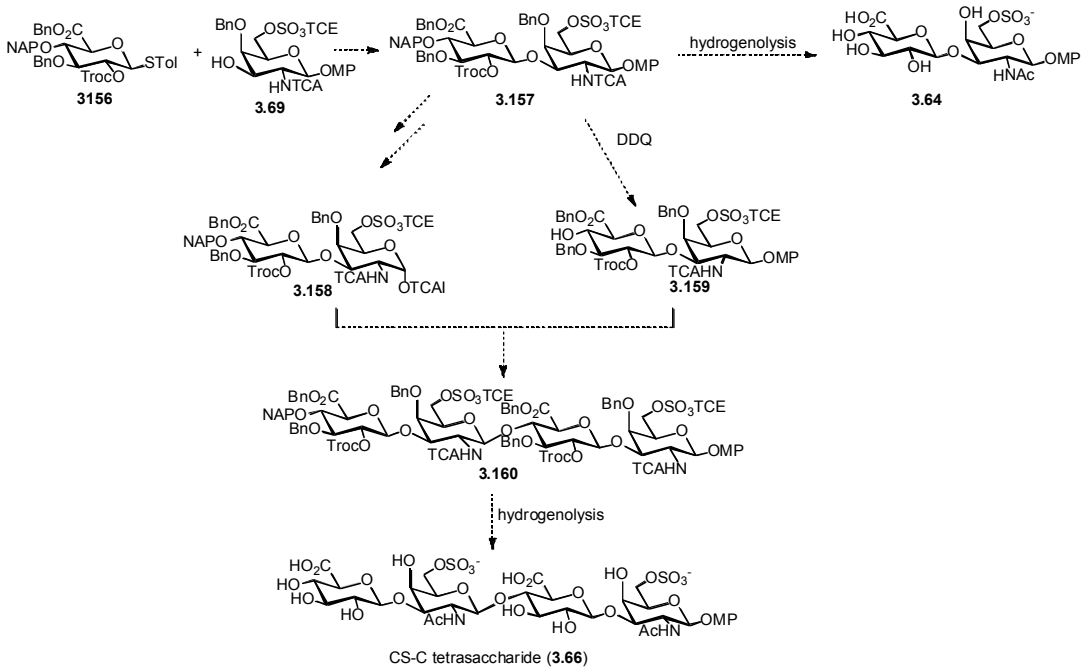


Figure 3.8. Revised structures of **3.141** and **3.148**

3.4 Summary and Future Work

Although the syntheses of the target compounds (**3.64-3.66**) remain unfinished, these studies have laid the groundwork for the completion of these three syntheses. For target **3.64**, it appears that having a Cbz group at the 2-position of the donor (i.e. compound **3.68**) is not going to work due to cyclic carbonate formation. It is very likely that we could readily prepare **3.64** by putting an acetyl, chloroacetyl or benzoyl group at the 2-position of the donor. However, we would still prefer to install a protecting group at this position that will provide the desired β-stereochemistry yet be removed by hydrogenolysis so that we could demonstrate that all of the protecting groups could be removed in a single step. Very few protecting groups meet these criteria. The 2,2,2-trichloroethylcarbonate (Troc) group is one possibility. This group has not been widely employed as a protecting group in carbohydrate chemistry. Nevertheless, Saulnier and coworkers have used this protecting group for installation of the carbohydrate portion of etoposide analogs.[79] Glucopyranose donors bearing the Troc group at positions 2 and 3 were employed (bis-Troc protected at positions 2 and 3) and the desired β-sterochemistry in the coupled products were obtained.[79] The Troc groups were removed by

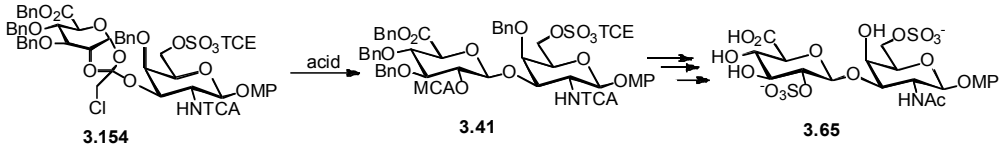
hydrogenolysis. Thus monomer **3.156** will be prepared with a Troc group at the 2-position and coupled to monomer **3.69** (Scheme 3.29). Hydrogenolysis will provide the CSC-disaccharide **3.64**. Disaccharide **3.157** will be used to prepare the CS-C tetrasaccharide **3.66** (Scheme 3.29).



Scheme 3.29. Proposed route to CS-C disaccharide **3.64**.

The studies described in this chapter also provided some insight as to the limitations of our sulfate protecting group approach to the synthesis of sulfated oligosaccharides. One limitation is that thioglycosides bearing a TCE-protected sulfate at the 6-position (i.e. compound **3.112**) appear to be unstable. Another, and perhaps the most significant limitation of our approach, is that it is not a very practical tactic for preparing oligosaccharides having a sulfate group at the 2-position as glycosidation

reactions employing donors bearing a TCE-protected sulfate at the 2-position yield coupled products as α/β mixtures. Consequently, the CS-D disaccharide **3.65** will be prepared by isomerizing **3.154** to **3.141** using acid, followed by removal of the MCA group to get **3.140**, sulfation using imidazolium salt **2.40** and then global deprotection using hydrogenolysis (Scheme 3.30). These studies are currently in progress in the Taylor group.



Scheme 3.30. Proposed route to CS-D disaccharide **3.65**.

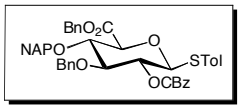
In spite of the above-mentioned limitations of our approach most of the difficulties encountered in the syntheses of the target compounds presented in this chapter were not a direct result of the presence of the TCE-protected sulfate group but instead stem from the inherently challenging synthesis of carbohydrates in general. While there still remains a great deal of research to be done, we are confident that the synthesis of sulfated carbohydrates using TCE-protected sulfate moieties will become a feasible approach in the future.

3.5 Experimental

3.5.1 General Considerations

For general information regarding solvents, NMR, MS, refer to section 2.5.1 in Chapter 2. All commercially available reagents in chapter 3 were purchased from either Sigma Aldrich or Alfa Aesar with the exception of BSP[80] and TTBP, [81] which were prepared exactly as reported in the literature.

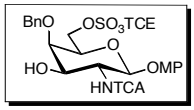
3.5.2 Experimental Syntheses and Characterization



Benzyl (4-Tolyl 3-*O*-benzyl-2-*O*-carbobenzyloxy-4-*O*-(2 naphthyl)methylene-1-thio-β-D-glucopyranoside) uronate (3.68).

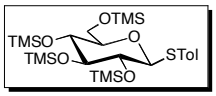
The crude acid **3.79** (0.624 g, crude) was dissolved in THF (9.0 mL). $KHCO_3$ (0.56 g, 5.5 mmol), TBAI (0.06 g, 0.17 mmol) and BnBr (0.6 mL, 5.0 mmol) were added sequentially. The reaction was stirred overnight for 16 hours, until no starting material remained by TLC. The reaction was quenched with MeOH, diluted with EtOAc, and washed with H_2O. The organic layer was separated, dried over Na_2SO_4 and concentrated to a crude solid. Purification by flash chromatography (100% CH_2Cl_2) afforded **3.68** as a white solid (0.554 g, 80% over 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.72 (t_{app}, 1H, $J_{3,2} = J_{3,4} = 8.8$ Hz, H3), 3.90 (t_{app}, 1H, $J_{4,3} + J_{4,5} = 18.3$ Hz, H4), 3.98 (d, 1H, $J_{5,4} = 9.7$ Hz, H5), 4.58-4.82 (m, 6H, H1, H2, CH_2Ph, CH_2NAP), 5.13 (s, 2H, CO_2CH_2Ph), 5.18, 5.22 (AB, 2H, $J = 12.2$ Hz OCO_2CH_2Ph), 7.04 (d, 2H, $J = 8.0$ Hz, ArH), 7.13-7.26 (m, 11H, ArH), 7.31-7.39 (m, 7H, ArH), 7.41-7.47 (m, 2H, ArH), 7.54

(s, 1H, ArH), 7.70-7.73 (m, 2H, ArH), 7.77-7.80 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.9, 67.4, 70.2, 75.5, 75.6, 78.2, 79.0, 83.5, 86.7, 125.8, 126.0, 126.1, 126.6, 127.6, 127.7, 127.78, 127.81, 127.9, 128.0, 128.36, 128.44, 128.5, 128.58, 128.61, 129.64, 133.0, 133.2, 133.9, 134.98, 135.03, 135.1, 137.7, 138.6, 154.3, 160.7, 168.1; HRMS (+ESI) m/z = 772.2932, $C_{46}H_{46}NO_8S$ $(M+NH_4)^+$ requires 772.2944.

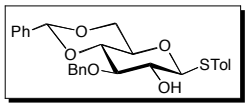


4-Methoxyphenyl 4-*O*-benzyl-2-deoxy-2-trichloroacetamido-6-*O*-(2,2,2-trichloroethoxysulfo)-β-D-galactopyranoside (3.69). A 1.5 M solution of acidic methanol was prepared by dissolving acetyl chloride (0.6 mL) in reagent grade methanol (6.0 mL) at 0 °C. This solution was then added to fully protected **3.111** (0.2 g, 0.258 mmol), and the reaction was stirred for 4 h at room temperature until no starting material was detected by TLC. The reaction was concentrated to approximately half volume (without heat), then diluted with CH_2Cl_2. The resulting solution was washed with H_2O, sat. aq. $NaHCO_3$ then H_2O and dried over $MgSO_4$. The reaction was then concentrated under reduced pressure (heat must be avoided in this process) to a white foam (0.158 g, 84%). This product decomposes *very* rapidly, thus is used immediately in the glycosylation reaction without any purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.82 (d, 1H, $J_{OH,3}$ = 7.9 Hz, OH), 3.77 (s, 3H, CH_3), 3.91 (d, 1H, $J_{4,3}$ = 2.4 Hz, H4), 3.94-3.96 (m, 1H, H3), 4.08-4.13 (m, 1H, H2), 4.24 (dd, 1H, $J_{6,6'}$ = 10.6 Hz, $J_{6,5}$ = 4.3 Hz, H6), 4.25-4.30 (m, 1H, H5), 4.54 (dd, 1H, $J_{6',6}$ = 10.6 Hz, $J_{6',5}$ = 7.7 Hz, H6'), 4.59, 4.62 (AB, 2H, J = 1.0.9 Hz, CH_2CCl_3), 4.73, 4.91 (AB, 2H, J = 11.6 Hz, CH_2Ph), 5.14 (d, 1H, $J_{1,2}$ = 8.3 Hz, H1), 6.79-6.83 (m, 2H, ArH), 6.93-6.98 (m, 2H,

ArH), 7.03 (d, 1H, $J_{NH,2}$ = 7.1 Hz, NH), 7.36-7.43 (m, 5H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.7, 56.7, 70.9, 72.0, 72.3, 74.9, 75.6, 79.6, 92.3, 92.4, 99.4, 114.7, 118.6, 128.4, 128.6, 128.8, 137.1, 150.7, 155.8, 163.0.

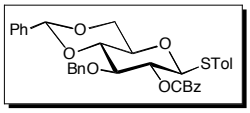


4-Tolyl 2,3,4,6-tetra-*O*-trimethylsilyl-1-thio-β-D-glucopyranoside (3.72). Prepared according to literature procedure. [59] To the tetraol **3.71**[28] (6.3 g, 22.0 mmol) in pyridine (22.0 mL) was added TMSCl (14.0 mL, 110.3 mmol) dropwise over 30 mins. The reaction quickly forms a thick white paste, and was stirred at rt for 3h. The slurry was diluted with Et_2O, washed with water, and concentrated to a crude oil. The crude product was taken up in toluene and re-concentrated (3x) under high vacuum to remove residual traces of pyridine; and left on the high vacuum pump until a white solid (**3.72**) formed (12.2 g, 96%). All spectra are in agreement with literature data for this compound.



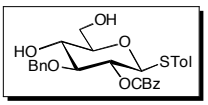
4-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (3.73). Prepared according to a modified literature procedure.[59] To a solution of the tetrasilylated **3.72** (5.0 g, 8.68 mmol) in CH_2Cl_2 at 0 °C was added benzaldehyde (2.5 mL, 23.9 mmol) followed by solution of freshly dried CuOTf in CH_3CN (0.0289 g in 1.0 mL , 0.080 mmol). The reaction was stirred for 2 h at 0 °C, and then triethylsilane (1.4 mL, 8.76 mmol) was added. After an additional 1 h at 0 °C, the reaction was concentrated with no heat to a crude slurry. The wet solid was taken up in CH_2Cl_2 washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and

concentrate to a white solid. The resulting crude solid was washed with EtOH, and filtered. Flash chromatography of the filtrate (100% CH_2Cl_2) and combination of the solids resulted in pure **3.73** (3.48 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.60 (br-s, 1H, OH), 3.48-3.55 (m, 2H, H2, H5), 3.63-3.74 (m, 2H, H3, H4), 3.81 (t_{app}, 1H, $J_{6,6'} = J_{6,5} = 10.2$ Hz, H6), 4.41 (dd, 1H, $J_{6',6} = 10.4$ Hz, $J_{6',6} = 4.8$ Hz, H6'), 4.60 (d, 1H, $J_{1,2} = 9.6$ Hz, H1), 4.82, 4.97 (AB, 2H, $J = 11.5$ Hz, CH_2Ph), 5.59 (s, 1H, CHPh), 7.16 (d, 1H, $J = 7.9$ Hz, ArH), 7.28 – 7.51 (m, 12H, ArH); All spectra are in agreement with literature data for this compound.[59]

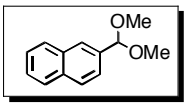


4-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-1-thio-*β*-D-glucopyranoside (3.74). To a solution of **3.73** (2.0 g, 4.31 mmol) and DMAP (1.9 g, 15.5 mmol) in CH_2Cl_2 (30 mL) at room temperature was added benzyl chloroformate (1.2 mL) dropwise over 30 min. The reaction was stirred for 12 h, and then a second portion of DMAP (0.5 g, 4.1 mmol) and benzyl chloroformate (0.6 mL, 4.30 mmol) were added. After an additional 8 h, no starting material remained by TLC. The reaction was quenched with cold MeOH, diluted with CH_2Cl_2 and washed with water. The organic layers were collected, dried over $MgSO_4$ and concentrated to a crude yellowish solid. Purification by flash chromatography (100% CH_2Cl_2) afforded **3.74** as a white solid (2.40 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.45 (ddd, 1H, $J_{5,6} = 9.6$ Hz, $J_{5,4} = 9.4$ Hz, $J_{5,6'} = 5.0$ Hz, H5), 3.70 (t_{app}, 1H, $J_{4,5} = J_{4,3} = 9.2$ Hz, H4), 3.74-3.81 (m, 2H, H3, H6ax), 4.37 (dd, 1H, $J_{6,6'} = 10.5$ Hz, $J_{6',5} = 5.0$ Hz, H6eq), 4.65 (d, 1H, $J_{1,2} = 9.9$ Hz, H1), 4.66, 4.83 (AB, 2H, $J = 11.9$ Hz, CH_2Ph), 4.81 (t_{app}, 1H, $J_{2,3} + J_{2,1}$

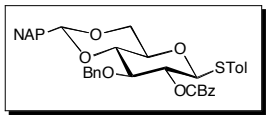
= 18.5 Hz, H2), 5.23 (s, 2H, CO_2CH_2Ph), 5.55 (s, 1H, CHPh), 7.09 (d, 2H, J = 8.0 Hz, ArH), 7.19-7.23 (m, 5H, ArH), 7.34-7.48 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 68.5, 70.1, 70.5, 74.6, 75.8, 79.9, 81.1, 86.8, 101.2, 126.0, 127.7, 127.8, 127.9, 128.3, 128.4, 128.61, 128.63, 129.1, 129.7, 133.8, 135.1, 137.1, 137.9. 138.7, 154.2; HRMS (+ESI) m/z = 599.2105 $(M+H)^+$ $C_{35}H_{35}O_7S$ requires 599.2104.



4-Tolyl 3-*O*-benzyl-2-*O*-carbobenzyloxy-1-thio-β-D-glucopyranoside (3.75). Carbohydrate **3.74** (3.0 g, 5.01 mmol) was dissolved in 2:1 MeOH: CH_2Cl_2 (150 mL). *p*-Toluenesulfonic acid monohydrate (0.6 g, 3.13 mmol) was added, and the reaction was heated at 45 °C for 5 h until no starting material remained by TLC. The reaction was neutralized with NEt_3, and, concentrated to approximately half-volume, and then diluted with CH_2Cl_2. The resulting solution was washed with H_2O, and sat. aq. $NaHCO_3$, and the organic layer was collected and dried over Na_2SO_4. Purification of the crude solid by flash chromatography (1:99 to 5:95 MeOH:CH_2Cl_2) afforded **3.75** as a white solid (2.20 g, 86%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.31 (s, 3H, CH_3), 2.31-2.33 (m, 1H, OH), 2.87 (d, 1H, $J_{OH,4}$ = 3.3 Hz, 4-OH), 3.29-3.35 (m, 1H, H5), 3.40 (t_{app}, 1H, $J_{3,2} + J_{3,4}$ = 17.6 Hz, H3), 3.61 (ddd, 1H, $J_{4,3}$ = 9.1 Hz, $J_{4,5}$ = 9.2 Hz, $J_{4,OH}$ = 3.1 Hz, H4), 3.69-3.76 (m, 1H, H6), 3.81-3.88 (m, 1H, H6'), 4.58-4.78 (m, 4H, H1, H2, CH_2Ph), 5.20, 5.21 (AB, 2H, J = 12.1 Hz, CO_2CH_2Ph), 7.08 (d, 2H, J = 8.0 Hz, ArH), 7.21-7.39 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 62.3, 70.05, 70.15, 74.9, 76.1, 79.3, 83.8, 86.4, 127.9, 128.0, 128.4, 128.5, 128.56, 128.62, 129.7, 133.2, 135.1, 137.9, 138.4, 154.2.

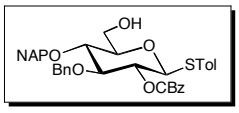


2-(dimethoxymethyl)naphthalene (3.76). Prepared according to the literature procedure.[60] Trimethylorthoformate (8.0 mL, 0.077 mol) and p-toluenesulfonic acid (0.025g) were added to a solution of 2-naphthaldehyde (8.0 g, 0.051 mol) in methanol (15.3 mL). The reaction was stirred overnight for 16 h, then diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4 and concentrated. The resulting orange-yellow viscous liquid (10.7 g) was used without any further purification. All spectra were in agreement with the literature data.[60]



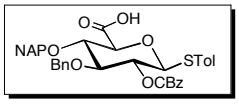
4 Tolyl 3-*O*-benzyl-2-*O*-carbobenzyloxy-4,6-*O*-(2-naphthyl)methylene-1-thio-*β*-D-glucopyranoside (3.77). Diol **3.75** (4.3 g, 8.42 mmol) was dissolved in CH_3CN (85 mL). 2-napthaldehyde dimethyl acetal **3.76** (2.04 g, 10.1 mmol) and p-toluenesulfonic acid (0.10 g, 0.53 mmol) were added. The reaction was stirred at room temperature for 30 min, until a clear gel-like precipitate formed and no starting material was detected by TLC. The resulting slurry was concentrated, taken up in CH_2Cl_2 washed with H_2O, sat. aq. $NaHCO_3$ and dried over Na_2SO_4. Flash chromatography of the crude solid provided **3.77** as a waxy solid (4.68 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 3.51 (ddd, 1H $J_{5,4} = J_{5,6ax} = 9.2$ Hz, $J_{5,6eq} = 4.9$ Hz, H5), 3.73-3.88 (m, 3H, H3, H4, H6ax), 4.42 (dd, 1H, $J_{6eq,6ax} = 10.5$ Hz, $J_{6eq,5} = 4.9$ Hz, H6eq), 4.66-4.70 (m, 3H, H1, 1/2 CH_2Ph), 4.82-4.88 (m, 2H, H2, 1/2 CH_2Ph), 5.26 (s, 2H, CO_2CH_2Ph), 5.70 (s, 1H, CHNAP), 7.12 (d, 2H, $J = 7.9$ Hz, ArH), 7.23 (s, 1H, 5H), 7.36-7.41 (m, 7H, ArH), 7.48-7.51 (m, 2H, ArH), 7.58 (d, 1H, $J = 8.4$ Hz, ArH), 7.83-7.88 (m, 3H, ArH), 7.95 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ

21.2, 68.6, 70.1, 70.5, 74.6, 75.8, 79.9, 81.2, 86.8, 101.4, 123.6, 125.5, 126.3, 126.5, 127.69, 127.73, 127.8, 127.9, 128.1, 128.3, 128.37, 128.4, 128.7, 129.8, 132.9, 133.7, 133.8, 134.5, 135.2, 137.0, 138.7, 154.2; HRMS (+ESI) m/z = 649.2263 $(M+H)^+$ $C_{39}H_{36}O_7S$ requires 649.2260.

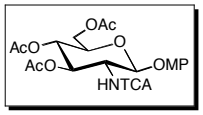


4-Tolyl 3-*O*-benzyl-2-*O*-carbobenzyloxy-4-*O*-(2-naphthyl)methylene-1-thio-*β*-D-glucopyranoside (3.78). To carbohydrate **3.77** (3.0 g, 4.62 mmol) in freshly distilled THF (15 mL) at rt was added $CoCl_2$ (1.8 g, 13.9 mmol) followed by BH_3THF (1.0 M soln. in THF, 14.4 mL, 1.4. mmol). When no starting material remains by TLC, (approximately 8 hours, subject to change drastically with quality of BH_3THF) the reaction was diluted with EtOAc, and the unused $CoCl_2$ is filtered off. The remaining filtrate was treated with aq. $NaBH_4$ (0.2 eq), and a second filtration was performed to remove the black precipitate. The resulting two phases were separated, the organic phase was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated to a crude solid. Flash chromatography (99:1 CH_2Cl_2:MeOH) provided **3.78** as a white waxy solid (2.76 g, 92%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.88 (br-s, 1H, OH), 2.32 (s, 3H, CH_3), 3.38-3.44 (m, 1H, H5), 3.61-3.78 (m, 3H, H3, H4, H6), 3.87-3.91 (m, 1H, H6'), 4.61 (d, 1H, $J_{1,2}$ = 9.8 Hz, H1), 4.67-4.84 (m, 4H, H2, CH_2Nap, 1/2 CH_2Ph), 4.95 (d, 1H, J =1 1.1 Hz, 1/2 CH_2Ph), 5.19, 5.23 (AB, 2H, J = 12.1 Hz, CO_2CH_2Ph), 7.07-7.10 (m, 2H, ArH), 7.18-7.26 (m, 5H, ArH), 7.31-7.40 (m, 8H, ArH), 7.44-7.47 (m, 2H, ArH), 7.67 (s, 1H, ArH), 7.75-7.82 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 62.0, 70.2, 75.2, 75.5, 76.4, 77.2, 79.5, 84.2, 86.1, 125.9, 126.0,

126.2, 126.8, 127.7, 127.77, 127.83, 128.0, 128.26, 128.33, 128.37, 128.40, 128.6, 129.8, 133.0, 133.2, 133.5, 135.10, 135.13, 137.9, 138.6, 154.3; HRMS (+ESI) m/z = 668.2663, $C_{39}H_{42}NO_7S$ $(M+NH_4)^+$ requires 668.2682.

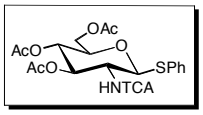


4-Tolyl 3-*O*-benzyl-2-*O*-carbobenzyloxy-4-*O*-(2-naphthylmethylene)-1-thio-β-D-glucopyranosyluronic acid (3.79). To carbohydrate **3.78** (0.60 g, 0.92 mmol) in 2:1 CH_2Cl_2:H_2O (4.5 mL) was added TEMPO (0.029 g, 0.18 mmol) followed by iodobenzene diacetate (BAIB, 0.89 g, 2.7 mmol). The biphasic reaction was vigorously stirred at rt for 45 min, then diluted with CH_2Cl_2 then quenched with 10 % $Na_2S_2O_3$ in H_2O (30 mL). The layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated to a crude yellow solid (0.624 g, crude). The crude material was directly applied in the synthesis of **3.69** with no purification or characterization.

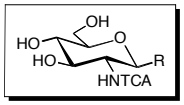


4-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (3.85). To tetraacetate **3.84**[62,63] (15.0 g, 30.4 mmol) in freshly distilled CH_2Cl_2 at 0 °C was added 4-methoxy phenol (15.0 g, 120.8 mmol) follwed by a dropwise addition of BF_3OEt_2 (15.0 mL, 118.0 mmol). The reaction was allowed to gradually warm to room temperature and stir overnight for 16 h. The solution was then cooled, diluted with CH_2Cl_2 and carefully quenched with sat. aq. $NaHCO_3$. The resulting layers were separated, and the organic layer was washed with cold 1 M NaOH, and H_2O, dried over $MgSO_4$ and concentrated to a yellow solid.

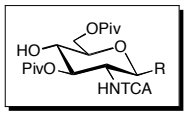
Recrystallization (EtOAc/Hexanes) afforded **3.85** as fine white needles (15.1 g, 92%). All spectra agree with literature data for this compound.[82]



Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy1-thio-2-trichloroacetamido-β-D-glucopyranoside (3.86). Synthesis performed exactly as described for 3.85. Tetraacetate 3.84 (15.0 g, 30.4 mmol), benzene thiol (15.0 mL, 136 mmol), BF_3OEt_2 (15.0 mL, 118 mmol). After work-up and recrystallization, 3.86 is isolated as a white solid (15.7 g, 95%). All spectra agree with literature data for this compound.[83]

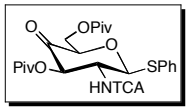


4-Methoxyphenyl 2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (3.87) or Phenyl 2-deoxy-1-thio-2-trichloroacetamido-β-D-glucopyranoside (3.88). Na° metal (0.3 eq.) was added to the appropriate starting material (**3.85** or **3.86**) in reagent grade MeOH (0.12 M solution). The reaction was stirred for 12 h, neutralized with Dowex H^+ resin, filtered and concentrated to a white solid. The resulting crude triols were used directly in the next step without any purification or characterization.

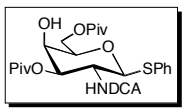


4-Methoxyphenyl 2-deoxy-3,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-glucopyranoside (3.89) or Phenyl 2-deoxy-3,6-di-*O*-pivaloyl-1-thio-2-trichloroacetamido-β-D-glucopyranoside (3.90). Prepared according to a modified literature procedure.[66] To the appropriate crude triol (3.86 or 3.87) in pyridine (1.2 M) was added trimethylacetyl chloride (6.0 eq) dropwise via syringe pump over 90 min. The

reaction was then removed from the ice bath, and stirred at room temperature for 5 h. The slurry was then cooled, quenched with MeOH and concentrated to dryness. The resulting solid was dissolved in CH_2Cl_2, washed with H_2O, 5% aqueous HCl, and sat. aq. $NaHCO_3$, dried over $MgSO_4$ and re-concentrated to dryness. Recrystalization of the crude solid and flash chromatography purification of the filtrate (EtOAc/Hexanes) provided pure **3.88** or **3.89**. All spectra for **3.89**[82] and **3.90**[83] were in agreement with literature data for these compounds.

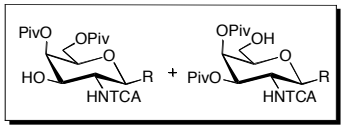


Phenyl 2-deoxy-3,6-di-*O*-pivaloyl-1-thio-2-trichloroacetamide-β-D-glucopyranosid-4-ulose (3.93). Acetic acid (11.3 mL) was added to a solution of carbohydrate **3.89** (2.0 g, 3.42 mmol) in dry DMSO (79 mL). The reaction flask was covered with aluminum foil, and stirred at room temperature for 16 h. The solution was then diluted with Et_2O and washed with H_2O. The aqueous layer was extracted 2x with Et_2O, and the combined organic phases were dried over $MgSO_4$ and concentrated to a crude oil. Purification by flash chromatography afforded impure **3.93** as a yellow solid (1.91 g, 96% incl. impurities). HRMS (+ESI) m/z = 582.0883, $C_{24}H_{31}Cl_3NO_7S$ $(M+H)^+$ requires 582.0887. The compound was used directly in the next reaction without any further characterization.



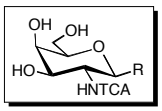
Phenyl 2-deoxy-2-dichloroacetamido-3,6-di-*O*-pivaloyl-1-thio-β-D-galactopyranoside (3.94). To a solution of **3.93** (1.91 g, 3.28 mmol) in THF (65 mL) at −78 °C was added L-Selectride (1.0 M in THF, 6.75 mL, 6.75 mmol)

dropwise via syringe pump over 45 min. The reaction was stirred at –78 °C for 4 h, at which point no starting material could be detected by TLC. The reaction was quenched with H_2O, diluted with Et_2O, and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with Et_2O, and the organic portions were collected, dried over $MgSO_4$ and concentrated to a crude oil. Purification by flash chromatography (25:75 EtOAc/Hexanes) afforded **3.94** as a slightly yellow foam (1.23 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 9H, 3xCH_3), 1.17 (s, 9H, 3xCH_3), 2.63 (br-s, 1H, OH), 3.83-3.83 (m, 1H, H5), 4.01 (br-s, 1H, H4), 4.27-4.42 (m, 3H, H2, H6, H6'), 4.90 (s, 1H, $J_{1,2}$ = 10.5 Hz, H1), 5.20 (dd, 1H, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 3.0 Hz, H3), 5.84 (s, 1H, $CHCl_2$), 6.94 (d, 1H, $J_{NH,2}$ = 9.4 Hz, NH), 7.22-7.26 (m, 3H, ArH), 7.46-7.49 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.06, 27.10, 30.7, 39.0, 49.9, 63.4, 66.2, 67.3, 72.7, 76.4, 87.9, 127.9, 128.9, 129.0, 132.0, 133.5, 164.4, 178.3, 178.4. HRMS (+ESI) m/z = 550.1420 $C_{24}H_{34}NO_7SCl_2$ $(M+H)^+$ requires 550.1433.

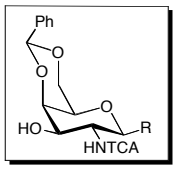


4-Methoxyphenyl 2-deoxy-4,6-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside (3.96) and 4-Methoxyphenyl 2-deoxy-3,4-di-*O*-pivaloyl-2-trichloroacetamido-β-D-galactopyranoside (3.97) *or* Phenyl 2-deoxy-4,6-di-*O*-pivaloyl-2-trichloroacetamido-1-thio-β-D-galactopyranoside (3.98) and Phenyl 2-deoxy-3,4-di-*O*-pivaloyl-2-trichloroacetamido-1-thio-β-D-galactopyranoside (3.99). Compounds prepared according to a modified literature procedure.[66] The appropriate glycoside (**3.89** or **3.90**), in 1,2-dichloroethane (0.16 M) at 0 °C was added pyridine (3.0 eq) followed by a

dropwise addition of triflic anhydride (1.2 eq.). The reaction was stirred for 1 h at 0 °C until no starting material is detected by TLC. H_2O (50 eq.) was then added, and the reaction was heated at 85 °C for 3 hours. The resulting solution was cooled, diluted with 1,2-dichloroethane, washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Purification by flash column chromatography (33:67 to 50:50 EtOAc/Hexanes) provided the appropriate separated 3,4- and 4,6-di-*O*-Piv compounds **3.96**/**3.97** (94% combined yield) or **3.98**/**3.99** (95 % combined yield). All spectra for these compounds are in agreement with literature characterization data.[82,83]

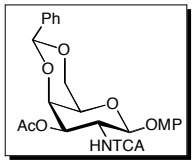


4-Methoxyphenyl 2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (3.100) or Phenyl 2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside (3.101). Na metal (0.3 eq.) was added to the appropriate starting material (3.85 or 3.86) in reagent grade MeOH (0.12 M solution). The reaction was stirred for 12 h, neutralized with dowex H^+ resin, filtered and concentrated to a white solid. The resulting crude triols **3.100** and **3.101** were used directly in the next step without any further purification or characterization.



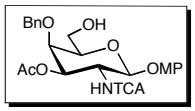
4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (3.105) *or* Phenyl 4.6-*O*-2-deoxy-1-thio-2-trichloroacetimido-β-D-galactopyranoside (3.106). A solution of **3.100** or **3.101** in benzaldehyde dimethyl acetal (3.5 eq.) and p-toluenesulfonic acid (0.05 eq.)

were stirred overnight (16 h) at room temperature. The solution was neutralized by addition of triethylamine, and concentrated to a crude yellow solid. For **3.105**: The resulting solid was washed with methanol, and the white precipitate was filtered and collected. Flash chromatography of the resulting filtrate (98:2 CH_2Cl_2/MeOH) afforded a white solid. **3.105** (96%). For **3.106**: The crude solid was purified by flash chromatography (1:1 EtOAc/Hexanes) to afford **3.106** (90%). All spectra for **3.105** and **3.106** were in agreement with literature data.[82]

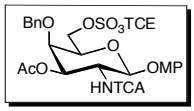


4-Methoxyphenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (3.107). To carbohydrate **3.105** (6.6 g, 12.7 mmol) in CH_2Cl_2 (70 mL) at 0 °C was added pyridine (5.3 mL, 61.6 mmol) followed by a dropwise addition of acetic anhydride (6.0 mL, 63.5 mmol). The reaction was removed from the ice bath and stirred until no starting material was detected by TLC (5 h). The solution was quenched with methanol and concentrated. The resulting residue was dissolved in CH_2Cl_2, washed with sat. aq. $NaHCO_3$ and H_2O, the organic layer was dried over $MgSO_4$ and concentrated to a crude foam. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **3.107** as a white solid (7.0 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H, CH_3), 3.62 (br-s, 1H, H5), 3.73 (s, 3H, OCH_3), 4.06 (dd, 1H, $J_{6,6'}$ = 12.4 Hz, $J_{6,5}$ = 1.2 Hz, H6), 4.31-4.42 (m, 3H,H6', H4, H2), 5.27 (d, 1H, $J_{1,2}$ = 8.3 Hz, H1), 5.47 (dd, 1H, $J_{3,2}$ = 11.3 Hz, $J_{3,4}$ = 3.4 Hz, H3), 5.51 (s, 1H, CHPh), 6.74-6.78 (m, 2H, ArH), 6.90 (d, 1H, $J_{NH,2}$ = 8.1 Hz, NH), 6.96-7.00 (m, 2H, ArH), 7.31-7.39 (m, 3H, ArH), 7.50-7.53 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ

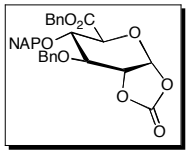
20.8, 53.0, 55.6, 66.5, 69.0, 69.5, 73.0, 92.4, 100.1, 100.9, 114.5, 119.4, 126.3, 128.2, 129.2, 137.4, 151.1, 155.7, 162.0, 170.8.



4-Methoxyphenyl 3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (3.109). To the fully protected **3.107** (4.3 g, 7.67 mmol) in freshly distilled THF (43 mL) at rt was added $CoCl_2$ (3.0 g, 23.1 mmol) followed by BH_3THF (1.0 M soln. in THF, 24.0 mL, 1.4. mmol). When no starting material remains as indicated by TLC analysis, (approximately 6 hours) the reaction is diluted with EtOAc, and the unused $CoCl_2$ is filtered off. The remaining filtrate is treated with aq. $NaBH_4$ (0.2 eq), and a second filtration is performed to remove the black precipitate. The resulting two phases are separated, the organic phase is washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated to a crude solid. Flash chromatography (99:1 CH_2Cl_2:MeOH) provides **3.109** as a white solid (4.19 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81 (br-s, 1H, OH), 2.03 (s, 3H, CH_3), 3.56-3.61 (m, 1H, H5), 3.64-3.68 (m, 1H, H6), 3.71 (s, 3H, OCH_3), 3.82 (dd, 1H, $J_{6',6}$ = 10.7 Hz, $J_{6',5}$ = 6.3 Hz, H6'), 3.90 (d, 1H, $J_{4,3}$ = 2.7 Hz, H4), 4.45-4.52 (m, 1H, H2), 4.56, 4.76 (AB, 2H, J = 11.7 Hz, CH_2Ph), 5.04 (d, 1H, $J_{1,2}$ = 8.3 Hz, H1), 5.29 (dd, 1H, $J_{3,2}$ = 11.3 Hz, $J_{3,4}$ = 2.9 Hz, H3), 6.71-6.76 (m, 2H, ArH), 6.84-6.94 (m, 3H, NH, ArH), 7.26-7.37 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 53.5, 55.6, 61.4, 72.3, 72.9, 74.9, 75.3, 92.4, 100.6, 114.6, 118.6, 128.2, 128.5, 128.6, 137.4, 151.2, 155.6, 162.1, 170.8.

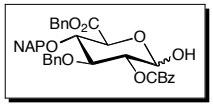


4-Methoxyphenyl 3-*O*-acetyl-4-*O*-benzyl-2-deoxy-2-trichloroacetamido-6-*O*-(2,2,2-trichloroethoxysulfo)-β-D-galactopyranoside (3.111). To **3.109** (2.853 g, 5.07 mmol) in CH_2Cl_2 (20 mL, 0.25 M) at 0 °C was added 1,2-DMI (1.25 g, 13.0 mmol) followed by **2.42** (4.75 g, 10.4 mmol). The reaction was stirred at 0 ^oC, gradually warmed to room temperature, and then stirred overnight. After 24 h, the system applied directly to a silica gel column, and **3.111** was obtained after flash chromatography (33:67 EtOAc/Hexanes) as a white solid (3.70 g, 95%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.13 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 4.01 (d, 1H, $J_{4,3}$ = 2.3 Hz, H4), 4.05 (br-dd, 1H, $J_{5,6'}$ = 6.9 Hz, $J_{5,6}$ = 4.9 Hz, H5), 4.28 (dd, $J_{6,6'}$ = 10.6 Hz, $J_{6,5}$ = 4.6 Hz, H6), 4.49-4.63 (m, 5H, H6', H2, 1/2 CH_2Ph, CH_2CCl_3), 4.90 (d, 1H, J = 11.4 Hz, 1/2 CH_2Ph), 5.19 (d, 1H, $J_{1,2}$ = 8.3 Hz, H1), 5.45 (dd, 1H, $J_{3,2}$ = 11.3 Hz, $J_{3,4}$ = 2.7 Hz, H3), 6.81 (d, 2H, J = 9.0 Hz, ArH), 6.88 (d, 1H, $J_{NH,2}$ = 8.6 Hz, NH), 6.96 (d, 2H, J = 9.0 Hz, ArH), 7.35-7.43 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.8, 53.3, 55.6, 71.6, 71.7, 72.1, 72.7, 75.1, 79.7, 92.31, 92.35, 100.0, 114.7, 118.6, 128.4, 128.5, 128.8, 136.8, 150.9, 155.8, 162.2, 170.6; HRMS (+ESI) m/z = 788.9805, $C_{26}H_{31}Cl_6N_2O_{11}S$ $(M+NH_4)^+$ requires 788.9780.

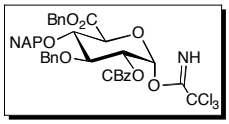


Carbonate (3.114). To a solution of **3.117** (0.285 g, 0.359 mmol), **3.69** (0.180 g, 0.246 mmol), and 4A molecular sieves (0.75 g) in CH_2Cl_2 at –40 °C was added TMSOTf (0.1 mL of a 1.0 M soln. in CH_2Cl_2, 0.1 mmol). The reaction was stirred for 30 min at –40 °C and then quenched with NEt_3 (0.1 mL). The reaction was diluted with CH_2Cl_2, filtered through celite and concentrated to a

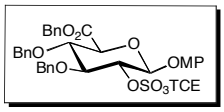
yellow foam. Purification by flash chromatography (33/67 EtOAc/Hexanes) afforded **3.114** as a white foam (0.186 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (t_{app}, 1H, $J_{3,4}$ + $J_{3,2}$ = 7.0 Hz, H3), 4.06 (t_{app}, 1H, $J_{4,5}$ + $J_{4.3}$ = 9.3 Hz, H4), 4.38 (s, 2H, CH_2Ar), 4.57 (d, 1H, $J_{5,4}$ = 5.1 Hz, H5), 4.61 (dd, 1H, $J_{2,1}$ = 6.2 Hz, $J_{2,3}$ = 2.8 Hz, H2), 4.68 (s, 2H, CH_2Ar), 5.07, 5.13 (AB, 2H, J = 12.2 Hz, CO_2CH_2Ph), 6.18 (d, 1H, $J_{1,2}$ = 6.3 Hz, H1), 7.07-7.35 (m, 2H, ArH), 7.23-7.34 (m, 9H, ArH), 7.47-7.51 (m, 2H, ArH), 7.63 (s, 1H, NAPH1), 7.76-7.84 (m, 3H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.6, 72.1, 72.6, 72.86, 72.93, 73.2, 73.8, 95.7, 125.8, 126.2, 126.3, 127.0, 127.7, 127.9, 128.0, 128.35, 128.37, 128.5, 128.6, 128.7, 133.2, 134.2, 134.8, 136.3, 152.2, 168.3; LRMS (+ESI) m/z = 558.2247, $C_{32}H_{32}NO_8$ $(M+NH_4)^+$ requires 558.1784.



Benzyl (3-*O*-benzyl-2-*O*-carbobenzyloxy-4-*O*-(2-naphthyl)methylene-D-glucopyranosyl)uronate (3.123). NBS (0.38 g, 2.12 mmol) was added to **3.68** (0.4 g, 0.530 mmol) in acetone/CH_2Cl_2/H_2O (5:5:1, 11 mL) at 0 °C. The reaction was stirred for 30 min at 0 °C then concentrated until turbidity developed. The remaining residue was dissolved in EtOAc, washed with H_2O, dried over $MgSO_4$ and concentrated to a yellow foam. Purification by flash chromatography (100% CH_2Cl_2) afforded **3.123** as a white foam (0.3129, 94%). HRMS (+ESI) m/z = 666.2679, $C_{39}H_{40}NO_9$ $(M+NH_4)^+$ requires 666.2703. **3.123** was used directly in the next reaction with no further characterization.

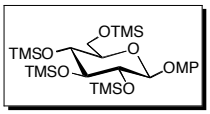


Benzyl (3-*O*-benzyl-2-*O*-carbobenzyloxy-4-*O*-(2-naphthyl)methylene-1-*O*-trichloroacetimidoyl-α-D-glucopyranosyl)uronate (3.124). To carbohydrate **3.123** (0.3291 g, 0.507 mmol) in CH_2Cl_2 (3.0 mL) at –40 ^oC was added trichloroacetonitrile (0.8 mL, 7.98 mmol), followed by DBU (0.10 mL of 1.0 M solution in CH_2Cl_2, 0.10 mmol). The reaction was stirred for approximately 4 hours then concentrated to a brown crude oil. Purification by flash chromatography (25:75 EtOAc/hexanes) afforded 3.123 as a white foam (0.3096, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (t_{app}, $J_{3,4} = J_{3,.2} = 9.7$ Hz, H3), 4.13 (t_{app}, $J_{4,5} + J_{4,3} = 19.0$ Hz, H4), 4.51 (d, 1H, $J_{5,4} = 9.9$ Hz, H5), 4.61, 4.86 (AB, 2H, $J = 10.9$ Hz, CH_2NAP), 4.80 (s, 2H, CO_2CH_2Ph), 5.00 (dd, 1H, $J_{2,3} = 9.8$ Hz, $J_{2,1} = 3.5$ Hz, H2), 5.08-5.19 (m, 4H, CH_2Ph), 6.66 (d, 1H, $J_{1,2} = 3.5$ Hz, H1), 7.21-7.26 (m, 12H, ArH), 7.34 (s, 5H, ArH), 7.45-7.48 (m, 2H, ArH), 7.55 (s, 1H, NapH1), 7.71-7.74 (m, 2H, ArH), 7.78-7.82 (m, 1H, ArH), 8.57 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.6, 70.1, 72.9, 75.5, 75.70, 75.74, 78.8, 78.9, 90.7, 93.4, 125.9, 126.0, 126.1, 126.8, 127.7, 127.8, 127.9, 128.0, 128.1, 128.36, 128.40, 128.48, 128.51, 128.58, 128.62, 128.7, 133.1, 133.2, 134.8, 135.0, 137.8, 154.3, 160.7, 168.1.

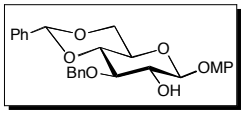


Benzyl (4-Methoxyphenyl 3,4-di-*O*-benzyl-2-*O*-trichoroethoxysulfo-β-D-glucopyranoside)uronate (3.126). To the glucuronic acid **3.132** (0.230 g, 0.332 mmol) in CH_2Cl_2 (1.2 mL) at 0 °C was added benzyl chloroformate (0.07 mL, 0.490 mmol) followed by a dropwise addition of NEt_3 (0.06 mL, 0.430 mmol). The reaction was stirred for 10 min, then DMAP (0.011 g, 0.090

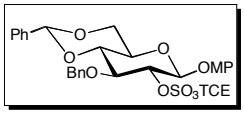
mmol) was added and the system was stirred for an additional 30 min at 0 °C. The reaction was concentrated to approximate half volume without the use of heat, and applied directly to a silica gel column. Flash chromatography (100% CH_2Cl_2) provided **3.126** as a white solid (0.188 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (s, 3H, CH_3), 3.85 (br-t, 1H, $J_{3,4} + J_{3,2}$ = 15.6 Hz, H3), 4.02-4.10 (m, 2H, H4, H5), 4.52 (d, 1H, J = 10.7 Hz, 1/2 CH_2Ph), 4.65-4.74 (m, 3H, CH_2CCl_3, 1/2 CH_2Ph), 4.80-4.92 (m, 3H, H2, CH_2Ph), 5.07 (d, 1H, $J_{1,2}$ = 7.3 Hz, H1), 5.14 (s, 2H, CO_2CH_2Ph), 6.80 (d, 2H, J = 8.9 Hz, ArH), 7.03 (d, 2H, J = 8.9 Hz, ArH), 7.13-7.35 (m, 14H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 67.6, 74.7, 75.0, 75.5, 78.8, 80.0, 80.8, 83.8, 92.6, 99.4, 114.7, 118.9, 127.8, 128.0, 128.1, 128.4, 128.49, 128.54, 128.6, 134.8, 137.0, 137.2, 150.1, 156.1, 167.3; HRMS (+ESI) m/z = 798.1285, $C_{36}H_{39}Cl_3NO_{11}S$ $(M+NH_4)^+$ requires 798.1309.



4-Methoxyphenyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucopyranoside (3.128). TMSCl (7.9 mL, 60.7 mmol) was added dropwise to a solution of carbohydrate **3.127** (3.4 g, 11.9 mmol) in pyridine (20 mL). The reaction was stirred for 2 h at room temperature, diluted with Et_2O, and washed H_2O. The resulting organic layer was dried over $MgSO_4$ and concentrated to dryness. The resulting oil was co-evaporated (2x) with toluene and left under vacuum overnight. Pure **3.128** was isolated as a white solid (6.48 g, 95%).

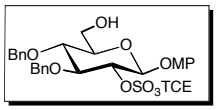


4-Methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-glucopyranoside (3.129). Tetrasilylated **3.128** (1.0 g, 1.74 mmol) was dissolved in CH_2Cl_2 (2.0 mL) at 0 °C. Benzaldehyde was added, and after 10 min a solution of freshly dried CuOTf in CH_3CN (0.023 M, 0.75 mL, 0.017 mmol) was added. After 1 h, triethylsilane (0.28 mL, 1.75 mmol), and the reaction was stirred for an additional 30 min at 0 °C. The reaction was then concentrated to a crude paste without the use of heat, redissolved in CH_2Cl_2 (45 mL) and was treated with a 1.0 M solution of TBAF in THF (3.0 mL, 3.0 mmol) at room temperature. The reaction was stirred for an additional hour and then concentrated to a crude solid. Purification by flash chromatography (100% CH_2Cl_2 to 98:2 CH_2Cl_2:MeOH for solubility reasons) afforded **3.129** as a white solid (0.6541, 80%). All spectra for this compound were in agreement with literature data.



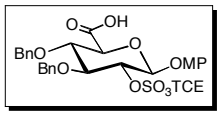
4-Methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,2,2-trichloroethoxysulfo)-*β*-D-glucopyranoside (3.130). To carbohydrate **3.129** (0.2 g, 0.431 mmol) in freshly distilled CH_2Cl_2 (2.7 mL) at 0 °C was added 1,2-DiMeIm (0.1 g, 1.0 mmol) follwed by **2.40** (0.4 g, 0.847 mmol). The reaction was stirred for 16 h, and then additional 1,2-DiMeIm (0.1 g, 1.0 mmol) and **2.40** (0.4 g, 0.847 mmol) were added. After an additional 8 h, the reaction was quenched with H_2O. The aqueous layer was extracted with CH_2Cl_2 and the organic layer was collected, dried over $MgSO_4$ and concentrated to a crude brown syrup. Purification by flash chromatography (25:75 EtOAc/Hexanes) afforded **3.130** (0.278 g, 95%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (ddd, 1H, $J_{5,6ax}$ = 9.7, $J_{5,4}$ = 9.5, $J_{5,6eq}$ = 5.0 Hz, H5), 3.80 (s, 3H, CH_3), 3.86 (t_{app}, 1H, $J_{6eq,6ax}$ = $J_{6ax,5}$ = 10.4 Hz, $H6_{ax}$), 3.88 (t_{app}, 1H, $J_{4,5}$ = $J_{4,3}$ = 9.2 Hz, H4), 3.98 (t_{app}, 1H, $J_{3,4}$ = $J_{3,2}$ = 9.1 Hz, H3), 4.43 (dd, 1H, $J_{6eq,6ax}$ = 10.5, $J_{6eq,5}$ = 5.0 Hz, $H6_{eq}$), 4.68, 4.71 (AB, 2H, J = 11.1 Hz, CH_2CCl_3), 4.85 (t_{app}, 1H, $J_{2,3}$ +$J_{2,1}$ =16.2 Hz, H2), 4.87, 5.01 (AB, 2H, J = 11.2 Hz, CH_2Ph), 5.02 (d, 1H, $J_{1,2}$ = 7.8 Hz, H1), 5.61 (s, 1H, CHPh), 6.85-6.88 (m, 2H, ArH), 7.06-7.09 (m, 2H, ArH), 7.33-7.50 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.54, 66.19, 66.30, 74.61, 77.87, 79.86, 81.21, 83.89, 92.57, 99.89, 101.36, 114.59, 118.77, 125.88, 127.92, 128.09, 128.11, 128.25, 128.27, 128.28, 128.38, 129.13, 136.63, 137.12, 150.16, 155.99; Melting point 105-107 ^{o}C; HRMS (EI) *m/z* = 674.0546, $C_{29}H_{29}Cl_3O_{10}S$ requires 674.0547.

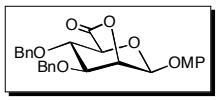


4-Methoxyphenyl 3,4-di-*O*-benzyl-2-*O*-(2,2,2-trichloroethoxysulfo)-*β*-D-glucopyranoside (3.131). Carbohydrate **3.130** (1.0 g, 1.48 mmol) was dissolved in 1.0 M BH_3·THF solution (7.4 mL, 7.4 mmol). CuOTf (0.027 g, 0.075 mmol) was added, and the reaction was stirred at room temperature for 10 h. Upon completion, the system was diluted with EtOAc, the black precipitate was filtered off, and the filtrate was washed with H_2O, sat. aq. $NaHCO_3$, dried over $MgSO_4$ an concentrated to a white foam. Purification by flash chromatography provided **3.131** (0.780 g, 78%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85 (br-s, 1H, OH), 3.45-3.51 (m, 1H, H5), 3.68-3.77 (m, 2H, H4,H6), 3.75 (s, 3H, CH_3), 3.82-3.88 (m, 2H, H3, H6'), 4.63-4.87 (m, 6H, H2, CH_2CCl_3, CH_2Ph, 1/2CH_2Ph), 4.98 (d, 1H, J = 8.5 Hz, 1/2 CH_2Ph), 5.03 (d, 1H, $J_{1,2}$ = 7.8 Hz, H1), 6.79-6.84 (m, 2H, ArH), 6.98-7.01

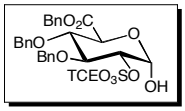
(m, 2H, ArH), 7.24-7.40 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 61.3, 75.2, 75.6, 75.8, 77.4, 80.0, 81.8, 84.2, 92.7, 98.9, 114.8, 118.3, 128.02, 128.05, 128.12, 128.2, 128.5, 128.6, 137.1, 137.3, 150.1, 155.9; HRMS (+ESI) m/z = 694.1028, $C_{29}H_{35}NO_{10}SCl_3$ $(M+NH_4)^+$ requires 594.1047.



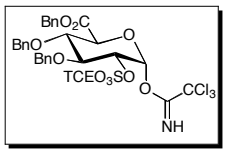
4-Methoxyphenyl 3,4-di-*O*-benzyl-2-*O*-(2,2,2-trichloroethoxysulfo)-*β*-D-glucopyranosyluronic acid (3.132). To carbohydrate **3.131** (0.3 g, 0.442 mmol) in 1:1 CH_2Cl_2:H_2O (2.5 mL) was added TEMPO (0.014 g, 0.090 mmol) followed by iodobenzene diacetate (BAIB, 0.310 g, 0.962 mmol). The biphasic reaction was vigorously stirred at rt for 3 h. The resulting layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated to a crude yellow solid. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) afforded **3.132** as a yellow foam (0.271 g, 88%). The crude acid was used directly in the next reaction with no further characterization.



Lactone (3.133). Isolated as a by-product in the preparation of **3.126**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74, (s, 4H, H3, CH_3), 3.84 (s, 1H, H5), 4.47-4.67 (m, 5H, H4, 2xCH_2Ph), 4.97 (s, 1H, H2), 5.48 (s, 1H, H1), 6.80 (d, 2H, J = 9.02 Hz, ArH), 6.97 (d, 2H, J = 9.02 Hz, ArH), 7.24-7.37 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 71.05, 71.14, 76.6, 77.4, 79.4, 94.7, 114.7, 118.2, 128.0, 128.1, 128.3, 128.4, 128.66, 128.69, 136.50, 136.52, 149.7, 155.5, 168.0; HRMS (+ESI) m/z =463.1765, $C_{22}H_{27}O_7$ $(M+H)^+$ requires 463.1757.

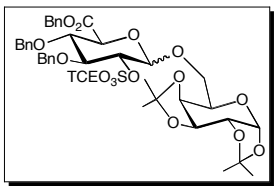


Benzyl (3,4-di-*O*-benzyl-2-*O*-(2,2,2-trichloroethoxysulfo)-D-Glucopyranosyl)uronate (3.134). Ceric ammonium nitrate (0.933 g, 0.96 mmol) wad added to carbohydrate **3.126** (0.250 g, 0.319 mmol) in $CH_3CN/H_2O/PhCH_3$ (4.2 mL:28 mL:0.3 mL). The reaction was stirred for 20 min, then poured into an ice/H_2O mixture. The resulting solution was extracted with EtOAc, and the resulting organic phase was collected and dried over $MgSO_4$. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **3.134** as a slightly yellow foam (0.158 g, 73%). For the α-anomer. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50 (br-s, 1H, OH), 3.86 (t_{app}, 1H, $J_{4,3} + J_{4.5}$ = 18.3 Hz, H4), 4.11 (t_{app}, 1H, $J_{3,4} + J_{3,2}$ = 18.3 Hz, H3), 4.47-4.63 (m, 5H, H2, H5, CH_2CCl_3, 1/2 CH_2Ph), 4.68-4.78 (m, 2H, 2x 1/2 CH_2Ph), 4.85 (d, 1H, J = 11.0 Hz, 1/2 CH_2Ph), 5.14, 5.16 (AB, 2H, J = 12.1 Hz, CO_2CH_2Ph), 5.68 (br-s, 1H, H1), 7.11-7.13 (m, 2H, ArH), 7.25-7.31 (m, 13H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 60.5, 67.7, 70.3, 75.1, 76.0, 77.8, 79.56, 79.64, 81.5, 90.2, 92.3, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 134.8, 137.2, 168.8; HRMS (+ESI) m/z =675.0637, $C_{29}H_{30}Cl_3O_{10}S$ $(M+H)^+$ requires 675.0625.



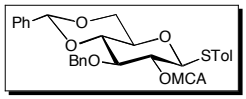
Benzyl (3,4-di-*O*-benzyl-1-*O*-trichloroacetimidoyl-2-*O*-(2,2,2-trichloroethoxysulfo)-α-D-glucopyranosyl)uronate (3.135). To carbohydrate **3.134** (0.172 g, 0.255 mmol) in CH_2Cl_2 (1.5 mL) at –40 ^{o}C was added trichloroacetonitrile (0.41 mL, 4.09 mmol), followed by DBU (0.05 mL of 1.0 M solution in CH_2Cl_2, 0.05 mmol). The reaction was stirred for approximately

4 hours as the temperature increased to rt, then concentrated to a brown crude oil. Purification by flash chromatography (25:75 EtOAc/hexanes) afforded **3.135** as a white foam (0.175, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.94 (t_{app}, 1H, $J_{4,5} = J_{4,3}$ =9.5 Hz, H4), 4.13 (t_{app}, 1H, $J_{3,2} = J_{3,4}$ = 9.5 Hz, H3), 4.41-4.48 (m, 2H, H5, 1/2 CH_2Ph), 4.55 (s, 2H, CH_2CCl_3), 4.69 (d, 1H, J = 10.6 Hz, 1/2 CH_2Ph), 4.90 (d, 1H, $J_{1,2}$ = 3.5 Hz, H1), 7.06-7.09 (m, 2H, ArH), 7.24-7.31 (m, 13H, ArH), 8.85 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.8, 72.6, 75.5, 76.1, 78.0, 79.4, 79.7, 80.1, 92.6, 128.04, 128.06, 128.14, 128.2, 128.5, 128.6, 128.7, 134.7, 136.87, 136.92, 160.3, 167.6; HRMS (+ESI) m/z = 817.9717, $C_{31}H_{30}Cl_6NO_{10}S$ $(M+H)^+$ requires 817.9722.



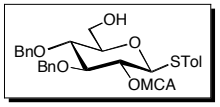
Benzyl 3,4-di-*O*-benzyl-2-*O*-(2,2,2-trichloroethoxysulfo)-α,β-D-glucopyronosyluronate-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (3.137). A mixture of imidate **3.135** (0.170 g, 0.207 mmol) and acceptor **3.136** (0.043 g, 0.165 mmol) and 4A molecular sieves were stirred in dry CH_2Cl_2 (2.0 mL) under rgon for 1 h at room temperature. A solution of 0.1 M TMSOTf (0.040 mL) was added, and the reaction was allowed to stir at rt for 30 min. The system was then treated with triethylamine (0.05 mL), filtered through celite and concentrated. Purification by flash chromatography (25:75 EtOAc/Hexanes) afforded in order of elution the α-anomer (0.085 g, 54%) and the β anomer (0.042 g, 27%). α anomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.76-3.91 (m, 3H, H4', H5, H6), 3.98-4.09 (m, 2H, H3', H6), 4.24-4.30 (m, 2H, H2, H3), 4.38 (d, 1H, $J_{5',4'}$ = 10.0 Hz, H5'), 4.47-4.61 (m,

5H, H2', H4', CH_2CCl_3, 1/2 CH_2Ph), 4.65-4.74 (m, 2H, 2x1/2CH_2Ph), 4.87 (d, 1H, *J*=11.0 Hz, 1/2 CH_2Ph), 5.17 (s, 2H, $COCH_2Ph$), 5.33 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H1'), 5.47 (d, 1H, $J_{1,2}$ =5.0 Hz, H1), 7.08-7.10 (m, 2H, ArH), 7.22-7.29 (m, 13H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 24.9, 25.9, 26.1, 65.9, 67.5, 70.4, 70.5, 70.6, 75.0, 75.9, 78.2, 79.6, 80.0, 81.1, 92.4, 96.2, 96.4, 108.6, 109.5, 127.78, 127.82, 127.9, 128.0, 128.4, 128.48, 128.51, 128.60, 128.64, 134.9, 137.26, 137.31, 168.8. β anomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 6H, 2xCH_3), 1.41 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.80-3.86 (m, 2H, H3', H6), 3.94-4.07 (m, 4H, H4, H4', H5, H6), 4.24 (d, 1H, $J_{5',4'}$ = 8.0 Hz, H5'), 4.31-4.33 (m, 1H, H2), 4.44, 4.68 (AB, 2H, *J*= 10.8 Hz, CH_2Ph), 4.55-4.65 (m, 2H, H2', H3), 4.74-4.81 (m, 2H, H1', 1/2 CH_2Ph), 4.86 (s, 2H, CH_2CCl_3), 4.93 (d, 1H, $J_{1,2}$ =5.0 Hz, H1), 7.07-7.09 (m, 2H, ArH), 7.24-7.31 (m, 13H, ArH); : ^{13}C NMR (75 MHz, $CDCl_3$-) δ 24.4, 24.9, 25.0, 67.46, 67.54, 68.9, 70.2, 70.7, 71.3, 74.9. 75.4, 79.3, 80.1, 81.0, 83.5, 93.2, 96.2, 99.7, 108.8, 109.4, 127.8, 127.9, 128.0, 128.2, 128.4, 128.5, 128.56, 128.60, 128.63, 134.8, 137.1, 137.2, 167.8; HRMS (+ESI) m/z = 917.1796, $C_{41}H_{48}Cl_3O_{15}S$ requires 917.1780.



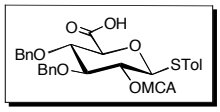
4-Tolyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-chloroacetyl-thio-*β*-D-glucopyranoside (3.143). To carbohydrate **3.73** (1.7 g, 3.66 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added NEt_3 (1.6 mL, 11.49 mmol) followed by chloroacetic anhydride (1.84 g, 10.76 mmol). The reaction was stirred for 1h at 0 °C then removed from the ice bath and allowed to stir at rt for 8 h. Methanol (20 mL) was slowly added, and the reaction was concentrated to a crude brown solid. Purification by flash

chromatography (99:1 CH_2Cl_2:MeOH) afforded **3.143** as a white solid (1.960 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.43-3.52 (m, 1H, H5), 3.66-3.81 (m, 3H, H3, H4, $H6_{ax}$), 3.83, 3.94 (AB, 2H, J = 14.9 Hz, CH_2Cl), 4.38 (dd, 1H, $J_{6az,6eq}$ = 10.8 Hz, $J_{6eq,5}$ = 4.9 Hz, $H6_{eq}$), 4.59-4.63 (m, 2H, H1, 1/2 CH_2Ph), 4.86 (d, 1H, J = 12.0 Hz, 1/2 CH_2Ph), 4.98 (d, 1H, $J_{2,1} = J_{2,3}$ = 9.0 Hz, H2), 5.56 (s, 1H, CHPh), 7.12 (d, 2H, J = 8.0 Hz, ArH), 7.23-7.48 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 40.7, 68.5, 70.5, 72.8, 74.4, 79.5, 81.2, 86.4, 101.2, 125.9, 127.5, 127.9, 128.1, 128.3, 128.4, 129.1, 129.7, 133.8, 137.0, 137.9, 138.8, 165.7.

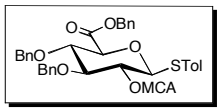


4 Tolyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-thio-β-D-glucopyranoside (3.144). To carbohydrate **3.143** (1.6 g, 2.96 mmol) in freshly distilled THF (16 mL) at rt was added $CoCl_2$ (1.15 g, 8.86 mmol) followed by BH_3THF (1.0 M soln. in THF, 8.80 mL, 8.8. mmol). The reaction was stirred at room temperature for 15 h, then was diluted with EtOAc, and the unused $CoCl_2$ was filtered off. The remaining filtrate was treated with aq. $NaBH_4$ (0.2 eq), and a second filtration was performed to remove the black precipitate. The resulting two phases were separated, the organic phase was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated to a crude solid. Flash chromatography (99:1 CH_2Cl_2:MeOH) provides **3.143** as a white solid (1.53 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.96 (br-s, 1H, OH), 2.32 (s, 3H, CH_3), 3.37-3.43 (m, 1H, H5), 3.61 (t_{app}, 1H, $J_{3,2} = J_{3,4}$ = 9.3 Hz, H3), 3.67-3.76 (m, 3H, H6, H4, 1/2 CH_2Cl), 3.87-3.93 (m, 2H, H6', 1/2 CH_2Cl), 4.56-4.69 (m, 3H, H1, 2x1/2 CH_2Ph), 4.81 (d, 1H, J = 10.9 Hz, 1/2 CH_2Ph), 4.82 (d, 1H, J = 11.6 Hz, 1/2 CH_2Ph),

4.94 (t_{app}, 1H, $J_{2,1}$ +$J_{2,3}$ = 18.9 Hz, H2), 7.09-7.12 (d, 2H, J = 9.0 Hz, ArH), 7.23-7.41 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 40.7, 61.8, 73.3, 75.2, 75.4, 77.6, 79.5, 83.8, 85.8, 127.9, 127.99, 128.10, 128.5, 128.6, 128.8, 133.3, 137.6, 138.0, 138.7, 165.9.

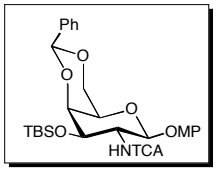


4-Tolyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-1-thio-*β*-D-glucopyranosyluronic acid (3.145). To carbohydrate **3.144** (1.0 g, 1.84 mmol) in 1:1 CH_2Cl_2:H_2O (10 mL) was added TEMPO (0.060 g, 0.38 mmol) followed by iodobenzene diacetate (BAIB, 1.31 g, 4.07 mmol). The biphasic reaction was vigorously stirred at rt for 1 h. The resulting layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated to a crude yellow solid. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) afforded **3.120** as a slightly yellow solid (0.902 g, 88%). The crude acid was used without any further characterization.



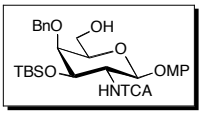
Benzyl (4-Tolyl-3,4-di-*O*-benzyl-2-*O*-chloroacetyl-1-thio-*β*-D-glucopyranoside)uronate (3.142). To the glucuronic acid **3.145** (3.30 g, 5.93 mmol) in CH_2Cl_2 (26 mL) at 0 °C was added NEt_3 (1.1 mL, 7.90 mmol) followed by a dropwise addition of benzyl chloroformate (1.1 mL, 7.71 mmol). The reaction was stirred for 10 min, then DMAP (0.21 g, 1.72 mmol) was added and the system was stirred for an additional 30 min at 0 °C. The reaction was concentrated to approximate half volume without the use of heat, and applied directly to a silica gel column. Flash chromatography (100% CH_2Cl_2) provided **3.142** as a white solid (2.90 g,

76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.72 (t_{app}, 1H, $J_{3,2} = J_{3,4}$ = 9.2 Hz, H3), 3.90, 3.93 (AB, 2H, J=14.8 Hz, CH_2Cl), 3.91 (t_{app}, 1H, $J_{4,3} + J_{4,5}$ = 18.7 Hz, H4), 4.00 (d, 1H, $J_{5,4}$ = 9.7 Hz, H5), 4,54, 4.72 (AB, 2H, J = 10.7 Hz, CH_2Ph), 4.59 (d, 1H, $J_{1,2}$ = 9.0 Hz, H1), 4.64, 4.83 (AB, 2H, J = 11.6 Hz, CH_2Ph), 5.00 (t_{app}, 1H, $J_{2,1} + J_{2,3}$ = 10.1 Hz, H2), 5.23 (s, 2H, CO_2CH_2Ph), 7.10 (d, 2H, J = 8.0 Hz, ArH), 7.17-7.19 (m, 2H, ArH), 7.25-7.40 (m, 16H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.2, 40.7, 67.5, 72.6, 75.1, 75.4, 78.2, 79.3, 81.2, 86.3, 127.4, 127.97, 127.99, 128.0, 128.1, 128.4, 128.5, 128.57, 128.59, 128.7, 129.7, 132.8, 137.5, 137.8, 138.9, 165.8, 167.5;



4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-*tert*-butyldimethylsilyl-2-trichloroacetamido-β-D-galactopyranoside (3.150). To 3.105 (0.500 g, 0.990 mmol) in dry THF (5.0 mL) was added TBSCl (0.49 g, 3.25 mmol) followed by imidazole (0.27 g, 3.97 mmol) and DMAP (0.05 g, 0.41 mmol). The reaction was stirred for 3 h at room temperature, then was quenched with sat. aq. $NaHCO_3$ and diluted with EtOAc. The aqueous layer was extracted three times with EtOAc, and the oranic layers were combined, dried over Na_2SO_4 and concentrated to a waxy crude compound. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) afforded 3.150 as a white waxy solid (0.560 g, 91%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.085 (s,3H, CH_3), 0.104 (s, 3H, CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 3.56 (br-s, 1H, H5), 3.73 (s, 3H, OCH_3), 3.84-3.93 (m, 1H, H2), 4.08 (dd, 1H, $J_{6,6'}$ = 12.4 Hz, $J_{6,5}$ = 1.5 Hz, H6), 4.12 (d, 1H, $J_{4,3}$ = 3.6 Hz, H4), 4.36 (br-d, 1H, $J_{6',6}$ = 11.3 Hz, H6'), 4.66 (dd, 1H, $J_{3,2}$ = 10.6 Hz, $J_{3,4}$ = 3.6 Hz, H3), 5.52 (d, 1H, $J_{1,2}$ = 8.4 Hz,

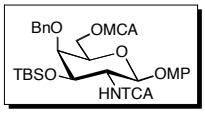
H1), 5.54 (s, 1H, CHPh), 6.74-6.79 (m, 2H, ArH), 6.93-7.02 (m, 3H, ArH, NH_2), 7.33-7.36 (m, 3H, ArH), 7.52-7.55 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ -4.5, 18.1, 25.7, 55.6, 56.8, 66.7, 68.7, 69.2, 76.0, 98.8, 100.7, 114.4, 119.7, 126.1, 128.1, 128.8, 137.8, 151.2, 155.6, 161.8; HRMS (+ESI) m/z = 649.1686, $C_{28}H_{40}Cl_3N_2O_7Si$ $(M+NH_4)^+$ requires 649.1670.



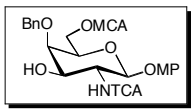
4-Methoxyphenyl 4-*O*-benzy-2-deoxy-3-*O*-*tert*-butyldimethylsilyl-2-trichloroacetamido-β-D-galactopyranoside (3.151).

To carbohydrate **3.150** (1.50 g, 2.37 mmol) in freshly distilled THF (7.5 mL) at rt was added $CoCl_2$ (0.90 g, 6.93 mmol) followed by BH_3THF (1.0 M soln. in THF, 7.1 mL, 7.1 mmol). When no starting material remains by TLC, (approximately 4 hours) the reaction is diluted with EtOAc, and the unused $CoCl_2$ is filtered off. The remaining filtrate is treated with aq. $NaBH_4$ (0.2 eq), and a second filtration is performed to remove the black precipitate. The resulting two phases are separated, the organic phase is washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated to a crude solid. Flash chromatography (98:2 CH_2Cl_2:MeOH) afforded pure **3.151** as a white waxy solid (1.21 g, 80%). ^1H NMR (300 MHz, $CDCl_3$) δ 0.13 (s, 3H, CH_3), 0.19 (s, 3H, CH_3), 0.93 (s, 9H, 3xCH_3), 1.71-1.75 (m, 1H, OH), 3.56-3.66 (m, 2H, H5, H6), 3.70 (s, 3H, OCH_3), 3.74-3.78 (m, 2H, H4, H6'), 4.05-4.14 (m, 1H, H2), 4.36 (br-d, 1H, $J_{3,2}$ = 8.0 Hz, H3), 4.56, 5.02 (AB, 2H, J = 11.4 Hz, CH_2Ph), 5.35 (d, 1H, $J_{1,2}$ = 8.2 Hz, H1), 6.70-6.73 (m, 2H, ArH), 6.90-6.97 (m, 2H, NH, ArH), 7.24-7.35 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$)

δ -5.0, -3.5, 17.9, 25.8, 55.6, 56.9, 61.9, 71.2, 74.9, 75.1, 76.2, 98.9, 114.5, 118.7, 127.9, 128.0, 128.5, 138.2, 151.2, 155.4, 161.2.

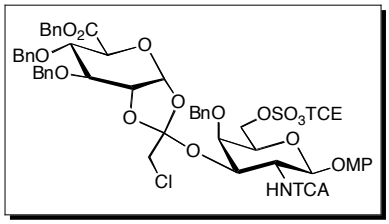


4-Methoxyphenyl 4-*O*-benzy-2-deoxy-6-*O*-chloroacetyl-3-*O*-*tert*-butyldimethylsilyl-2-trichloroacetamido-β-D-galactopyranoside (3.152). To carbohydrate **3.151** (0.780 g, 1.23 mmol) in CH_2Cl_2 (7.3 mL) at 0 °C was added NEt_3 (0.25 mL, 1.80 mmol) followed by chloroacetic anhydride (0.313 g, 1.83 mmol). The reaction was stirred for 30 min at 0 °C, then quenched with cold MeOH and concentrated to a crude brown solid. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **3.152** as a white waxy solid (0.785 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.13 (s, 3H, CH_3), 0.20 (s, 3H, CH_3), 0.93 (s, 9H, 3xCH_3), 3.72 (brs, 4H, OCH_3, H4), 3.80-3.82 (m, 1H, H5), 3.94 (s, 2H, CH_2Cl), 4.00-4.09 (m, 1H, H2), 4.17 (dd, 1H, $J_{6,6'}$ = 11.1 Hz, $J_{6,5}$ = 5.3 Hz, H6), 4.36-4.42 (m, 2H, H3, H6'), 4.56, 5.04 (AB, 2H, J = 11.3 Hz, CH_2Ph), 5.38 (d, 1H, $J_{1,2}$ = 8.1 Hz, H1), 6.72-6.75 (m, 2H, ArH), 6.93-6.96 (m, 3H, ArH, NH), 7.23-7.34 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ -5.0, -3.5, 17.9, 25.8, 40.6, 55.6, 56.9, 64.6, 71.4, 72.0, 75.1, 75.9, 92.4, 98.6, 114.4, 119.0, 127.9, 128.0, 128.5, 138.0, 151.2, 155.6, 161.9, 166.8; HRMS (+ESI) m/z = 727.1537, $C_{30}H_{43}Cl_4N_2O_8Si$ $(M+NH_4)^+$ requires 727.1543.



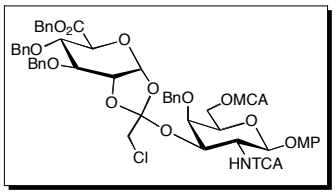
4-Methoxyphenyl 4-*O*-benzy-2-deoxy-6-*O*-chloroacetyl-2-trichloroacetamido-β-D-galactopyranoside (3.149). To carbohydrate

3.152 (0.500 g, 0.703 mmol) in dry THF (10 mL) was added 1.0 M TBAF in THF (0.77 ml, 0.77 mmol) dropwise. The reaction was stirred at room temperature for 2 h, then concentrated to a crude oil using minimal heat. Purification by flash chromatography (98:2 CH_2Cl_2/MeOH) afforded **3.149** as a white solid (0.311 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.76 (d, 1H, $J_{OH,3}$ = 8.4 Hz, OH), 3.78 (s, 3H, OCH_3), 3.84 (br-t, $J_{5,6}$ + $J_{5,6'}$ = 12.6 Hz, H5), 3.90 (brd, $J_{4,3}$ = 2.03 Hz, H4), 4.02 (s, 2H, CH_2Cl), 4.04-4.09 (m, 1H, H_2), 4.20-4.27 (m, 2H, H3, H6), 4.48 (dd, 1H, $J_{6',6}$ = 11.0 Hz, $J_{6',5}$ = 7.0 Hz, H6'), 4.79,4.85 (AB, 2H, J = 11.6 Hz, CH_2Ph), 5.09 (d, 1H, $J_{1,2}$ = 8.3 Hz, H1), 6.80-6.84 (m, 2H, ArH), 6.98-7.02 (m, 3H, NH, ArH), 7.36-7.41 (m, 5H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.6, 55.6, 57.0, 64.2, 70.9, 72.5, 75.1, 75.6, 92.4, 99.8, 114.6, 119.0, 128.37, 128.43, 128.8, 137.4, 151.0, 155.8, 162.9, 166.8;



Orthoester (3.155). A suspension of thioglycoside donor **3.142** (0.354 g, 0.547 mmol), BSP (0.126 g, 0.602 mmol), TTBP (0.272 g, 1.09 mmol) and freshly activated 3A MS (0.40 g) was stirred under argon in freshly distilled CH_2Cl_2 (3.7 mL) for 1 h at room temperature. The reaction mixture was cooled to –60 °C and stirred for an additional 30 min. Tf_2O (0.1 mL, 0.602 mmol) was added, and after 10-12 min, a solution of acceptor **3.69** (0.200 g, 0.273 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise. The reaction was stirred for 3 h, while the temperature gradually rose to 0 °C, until no starting acceptor **3.69** was detected using TLC. The reaction was filtered, diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$. The

organic layer was dried over Na_2SO_4 and concentrated to a greenish crude oil. Flash chromatography performed twice, (99:1 CH_2Cl_2/MeOH then 25:57 EtOAc/Hexanes) provided **3.141** as a white foam (0.299 g, 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (s, 3H, CH_3), 3.82, 3.90 (AB, 2H, J = 12.0 Hz, CH_2Cl), 3.81-3.91 (m, 3H, H2', H5', H5), 4.00-4.03 (m, 2H, H3, H4), 4.21 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4}$ = 4.3 Hz, H3'), 4.28 (br-s, 1H, H2), 4.50-4.53 (m, 3H, CH_2CCl_3, H4'), 4.57-4.65 (m, 5H, H6', H6', 3x1/2 CH_2Ph), 4.70-4.64 (m, 2H, 2x1/2 CH_2Ph), 4.94, 5.01 (AB, 2H, J = 12.1 Hz, CO_2CH_2Ph), 5.12 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H1'), 6.16 (d, 1H, $J_{1,2}$ = 3.4 Hz, H1), 6.99 (d, 2H, J = 9.0 Hz, ArH), 7.12 (d, 1H, $J_{NH,H2'}$ = 6.8 Hz, NH), 7.28 (d, 2H, J = 9.0 Hz, ArH), 7.34-7.24 (m, 20H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 47.0, 54.9, 55.6, 67.3, 69.9, 72.1 72.3, 72.5, 72.8, 72.9, 73.2, 73.8, 74.0, 74.9, 78.2, 79.6, 92.3, 92.4, 94.7, 98.5, 114.6, 119.1, 122.7, 128.0, 128.19, 128.21, 128.3, 128.5, 128.56, 128.58, 128.6, 128.7, 134.8, 136.8, 137.0, 137.6, 151.0, 155.8, 162.3, 169.3; HRMS (+ESI) m/z = 1269.1132, $C_{53}H_{56}Cl_7N_2O_{17}S$ $(M+NH_4)^+$ requires 1269.1119.



Orthoester (3.156). Prepared and isolated according to the procedure described for **3.155.** ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (s, 3H, OCH_3), 3.80-3.93 (m, 4H, CH_2Cl, H5, H2'), 3.95-3.96 (m, 2H, CH_2Cl), 3.99-4.02 (m, 2H, H3, H4), 4.14 (dd, 1H, $J_{6',6''}$ = 11.3 Hz, $J_{6',5}$ = 5.3 Hz, H6'), 4.29 (br-t, 1H, $J_{2,1}$+$J_{2,3}$ = 6.2 Hz, H2), 4.40 (dd, 1H, $J_{6'',6'}$ = 11.3 Hz, $J_{6'',5'}$ = 7.2 Hz, H6''), 4.54 (s, 2H, CH_2Ph), 4.58 (dd, 1H, $J_{3',2'}$ = 10.8 Hz, $J_{3',4'}$ = 2.6 Hz, H3'), 4.63-4.65 (m, 2H, H4', 1/2 CH_2Ph), 4.71 (d, 1H, J = 11.5 Hz, 1/2 CH_2Ph), 4.77, 5.10

(AB, 2H, J = 11.6 Hz, CH_2Ph), 4.95, 5.01 (AB, 2H, J = 12.1 Hz, CO_2CH_2Ph), 5.57 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H1'), 6.14 (d, 1H, $J_{1,2}$ = 3.5 Hz, H1), 6.80 (d, 2H, J = 9.0 Hz, ArH), 7.01 (d, 2H, J = 9.0 Hz, ArH), 7.14 (d, 1H, $J_{NH,2}$ = 6.8 Hz, NH), 7.22-7.24 (m, 3H, ArH), 7.28-7.29 (m, 4H, ArH), 7.34-7.40 (m, 16H, ArH), 7.41-7.44 (m, 2H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.6, 47.1, 55.1, 55.6, 64.5, 67.3, 70.2, 72.3, 72.7, 72.87, 72.94, 73.8, 73.9, 74.0, 74.7, 78.6, 92.3, 94.9, 98.7, 114.4, 119.5, 122.7, 127.7, 127.9, 127.98, 128.0, 128.17, 128.19, 128.2, 128.4, 128.49, 128.55, 128.59, 128.7, 128.8, 134.8, 136.8, 137.0, 137.9, 151.2, 155.8, 162.6, 166.8, 169.3; HRMS (+ESI) m/z = 1135.2122, $C_{53}H_{56}Cl_5N_2O_{15}$ $(M+NH_4)^+$ requires 1135.2123.

Chapter 4 – Studies on the Selective Introduction and Isomerization of the Carbobenzyloxy Carbonyl group in 2,3-diols of 4,6-*O*-Benzylidene Galactose Derivatives

4.1 Introduction

In addition to the synthesis of the chondroitin sulfate fragments described in chapter 3, the Taylor group is working towards the synthesis of another multisulfated oligosaccharide using the sulfate protecting group strategy, namely the synthesis of the tetrasaccharide portion of SB_{1A}. SB_{1A} (**4.1**, Figure 4.1), is glycosphingolipid carbohydrate antigen with a disulfated tetrasaccharide moiety that has shown to accumulate in both cultured and tissue-extracted human hepatocellular carcinoma (HCC) cell lines. Carbohydrate antigens are often expressed specifically to a certain type of tumor, and are not overexpressed or recognized by the immune system in normal tissues; thus oligosaccharide based antigens show a great deal of potential for application towards tumor immunotherapy.[84] It has been suggested that SB_{1A} is one of the most important cancer associated carbohydrate antigens for HCC.[84]

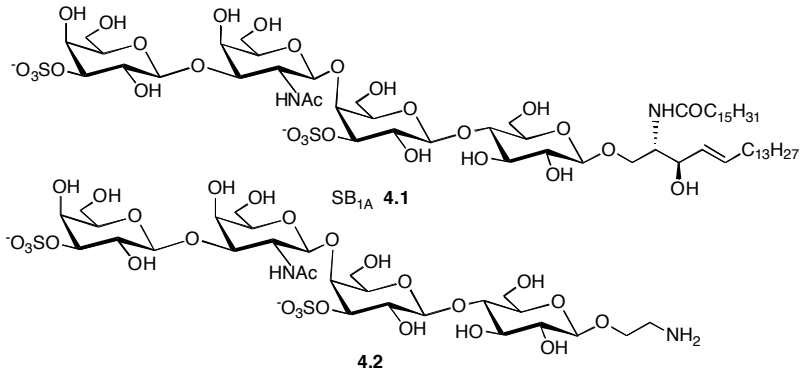
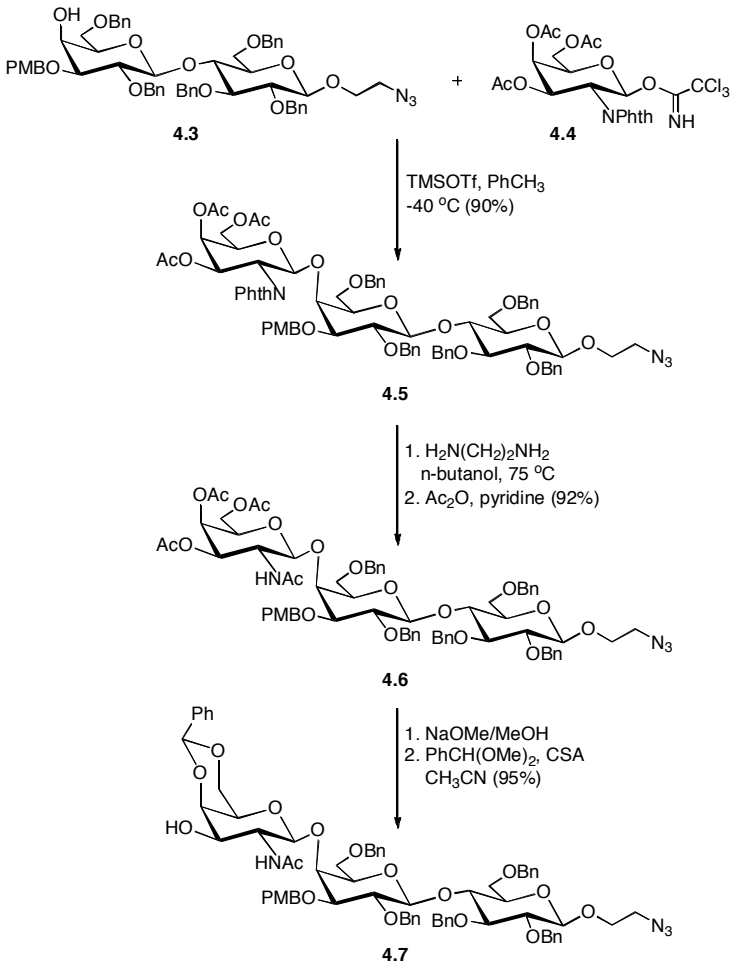


Figure 4.1. SB_{1A} (**4.1**) and its synthetic analog **4.2.**

In 2002, Zhong-Jun Li and coworkers reported the first synthesis of the disulfated tetrasaccharide moiety of SB_{1A} (**4.2**, Figure 4.1).[84] The authors used a [2+1+1] stepwise synthetic approach to assemble the fully protected target tetrasaccharide. The protecting group strategy was designed such that benzyl ethers were used for permanent protection until the end of the synthesis; 2-*N*-phthalimido and 2-*O*-benzoyl protected donors were used to direct the required β-glycosidic linkages, and the locations that would ultimately be sulfated were orthogonally protected using *p*-methoxybenzyl (PMB) and monochloroacetate (MCA) moieties.

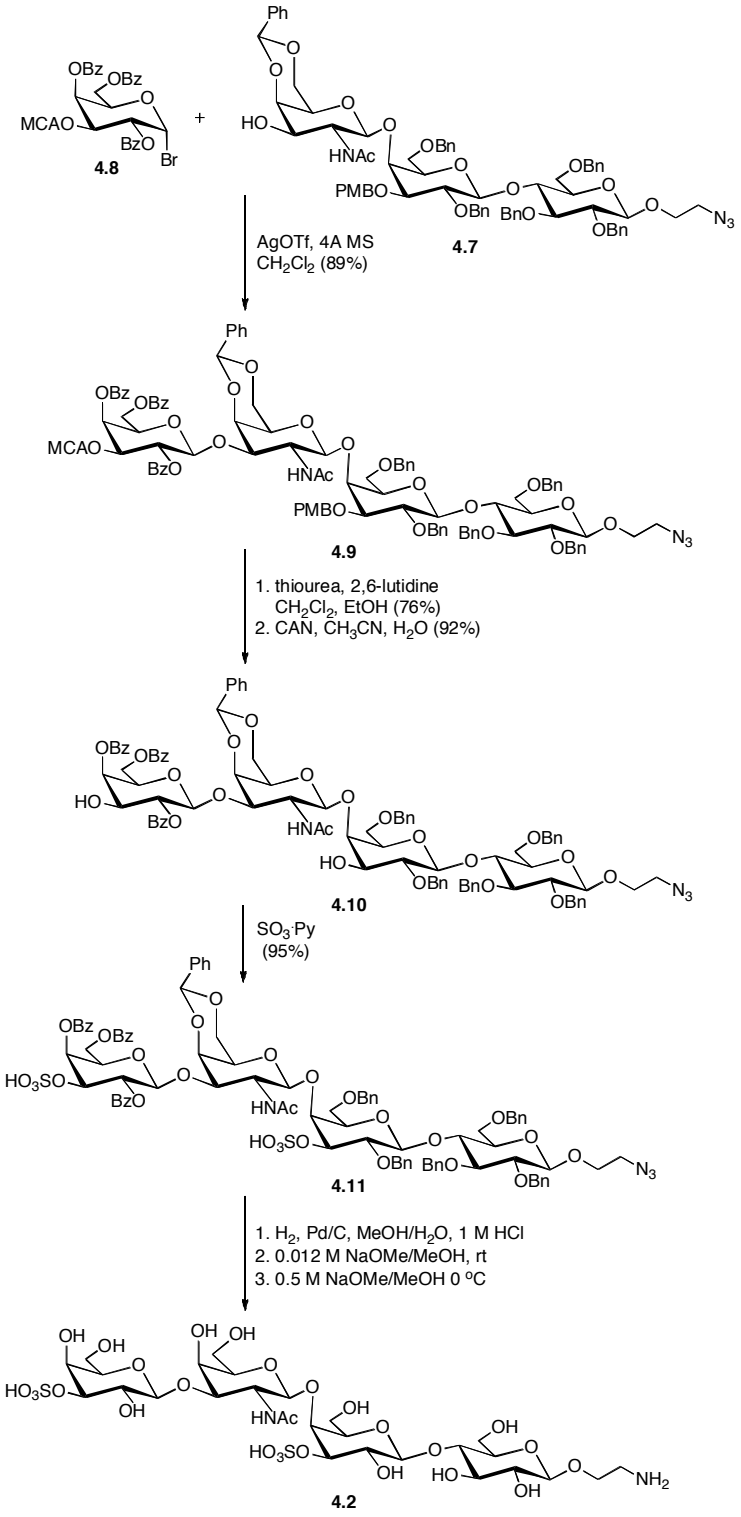
The synthesis of SB_{1A} began with the preparation of a previously reported[85] lactose derivative **4.3** (Scheme 4.1) which was coupled to the trichloroacetimidate donor **4.4** in the presence of TMSOTf to give trisaccharide **4.5** in an 83% yield. A series of protecting group manipulations on trisaccharide **4.5** including dephthaloylation and *N*-acetylation to give **4.6**, and subsequent *O*-deacetylation and installation of the 4,6-*O*-benzylidene acetal provided access to trisaccharide acceptor **4.7** in good yield.



Scheme 4.1. Preparation of SB_{1A} trisaccharide acceptor.

The trisaccharide acceptor **4.7** was then glycosylated with the glycosyl bromide donor **4.8** (several other donors were examined but only donor **4.8** gave the desired product) using AgOTf as a promoter at low temperature which gave the fully protected tetrasaccharide **4.9** in an 89% yield (Scheme 4.2). Selective removal of the monochloroacetate protecting group followed by oxidative cleavage of the PMB group provided diol **4.10** which was treated with the sulfur trioxide-pyridine complex to furnish

the disulfated tetrasaccharide **4.11** in a 95% yield. Removal of the benzyl groups in **4.11** was achieved in an 88% yield by catalytic hydrogenolysis in the presence of HCl (0.011 M in MeOH-H_2O, 10:1) over three days. These are somewhat unusual conditions for this reaction since sulfate monoesters are quite acid labile; however, there was no mention that any loss of the sulfate groups occurred during this step. Some difficulties were encountered in removing the benzoyl groups. Subjecting the debenzylated product to 0.012 M NaOMe in MeOH at room temperature only resulted in loss of the benzoyl group at the 6-position while the one at the 2-position was retained. Increasing the base concentration and prolonging the reaction time led to decomposition. Ammonia in MeOH resulted in desulfation. Nevertheless, they were eventually able to obtain target tetrasaccharide **4.2** by using 0.5 M NaOMe in MeOH at 0 ^{o}C for 6 h (reported 90% yield).



Scheme 4.2. Completed synthesis of the SB_{1A} tetrasaccharide.

We reasoned that the SB_{1A} tetrasaccharide would be a good target to evaluate the sulfate protecting group strategy for the preparation of sulfated oligosaccharides. While Li and coworkers' synthesis is an accomplishment in the preparation of sulfated oligosaccharides, it does have some shortcomings. The total number of steps was 33 if one takes into account the steps required to prepare the protected monosaccharides (not discussed). Over 8 different protecting group manipulations are required at the tri- and tetrasaccharide stage. Although the removal of the benzoyl groups at the end of the synthesis was eventually achieved in good yield considerable difficulties were encountered in this step. By designing a synthesis that incorporates a protected sulfate moiety at the beginning of the synthetic sequence, and by using a protecting group strategy in which all protecting groups are removed by hydrogenolysis, this step would no longer be required and some of protecting group manipulations would be avoided which would in turn decrease the number of synthetic steps in the preparation of the target.

We envisioned the target tetrasaccharide **4.12** being prepared from the fully protected precursor **4.13** (Figure 4.2). The target was designed to be fully protected with groups (including the TCE-protected SO_3 moieties) that can be removed under catalytic hydrogenation conditions in the last step of the synthesis. This includes the use of the carbobenzyloxy (Cbz) group, which we discussed to a limited extent, in chapter 3, at the 2-position of the galactose units. Tetrasaccharide **4.13** would ultimately be assembled from monomers **4.14**, **4.15** and **4.16**.

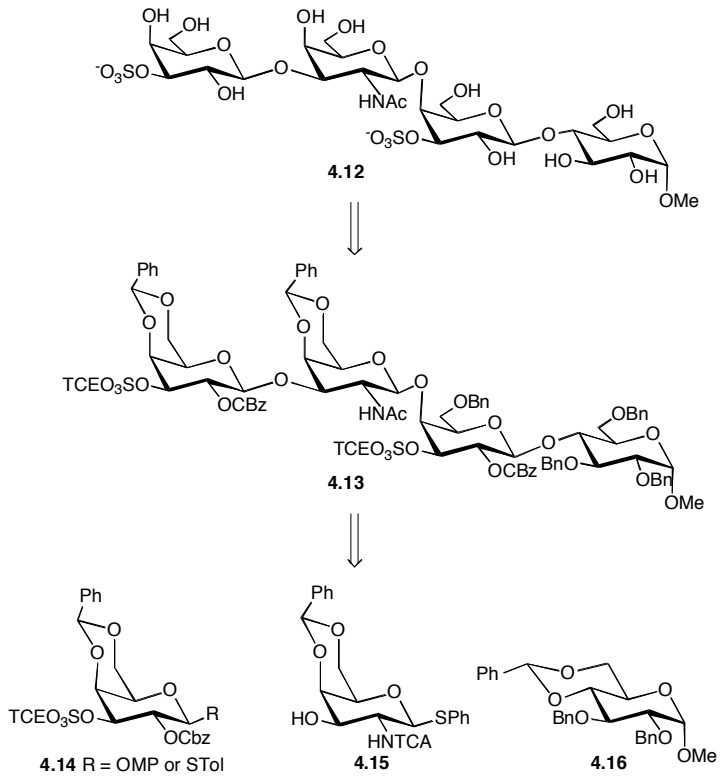
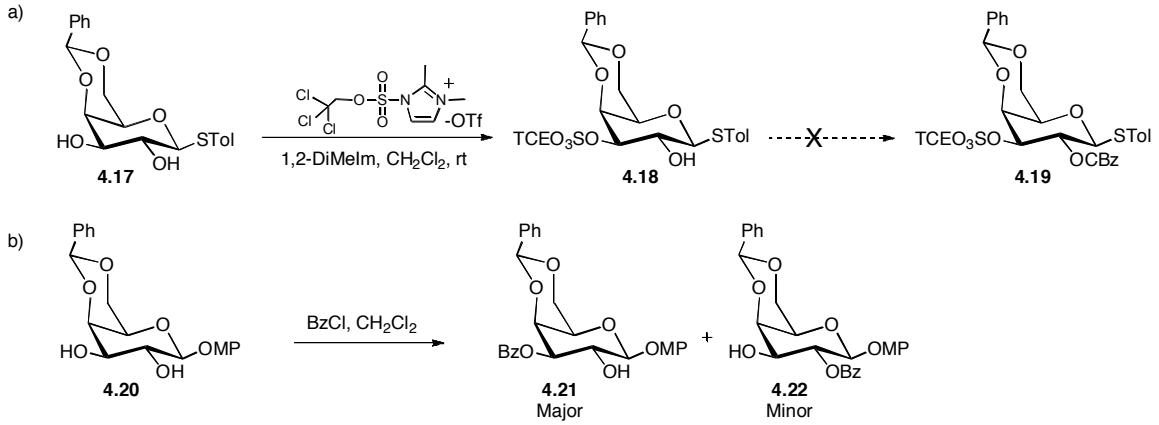


Figure 4.2. Retrosynthesis of SB_{1A}

This particular route was very appealing for several reasons. First of all, monomer **4.15** with either an SPh (**3.106**, chapter 3) or OMP group (**3.105**, chapter 3) at the anomeric position had already been prepared during our synthesis of chondroitin sulfate fragments that were discussed in chapter 3. Secondly, two of the residues could be derived for a single monomer (monomer **4.14**). Finally, we had anticipated that monomer **4.14** could be easily prepared via selective sulfation methodology recently developed in our laboratory by Ahmed Desoky, a colleague in the Taylor laboratory. While performing studies on the selective sulfation of partially protected gluco- and

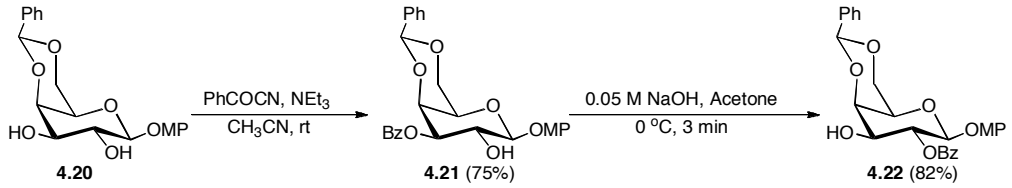
galactopyranosides, Ahmed Desoky had discovered that diol **4.17** could be selectively sulfated in 88% yield with 2.0 equivalents of SIS **2.40** in the presence of 2.5 equivalents of 1,2-dimethylimidazole to prepare 3-*O*-sulfate **4.18** (Scheme 4.3a). This finding was quite exciting, as in just one further synthetic step, the required sulfated monomer **4.14** could, in principle, be prepared. Unfortunately, once the $TCESO_3$ moiety had been installed, the 2-OH of **4.18** appeared to be extremely unreactive, and, at the time we began these studies, no conditions had been found to install the desired 2-*O*-Cbz group to prepare **4.19**. Interestingly, Ahmed Desoky had also observed that selective *O*-benzoylations of diol **4.17** followed the same selectivity pattern.



Scheme 4.3. Selective sulfation and benzoylation of diol **4.17** and **4.20.**

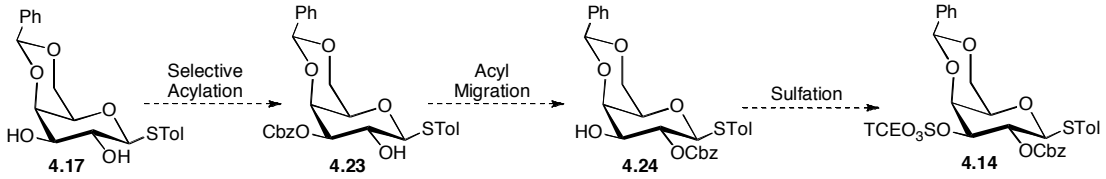
A literature search quickly revealed that other researchers had noted this selectivity pattern with 2,3-diols of 4,6-*O*-benzylidene-protected galactose derivatives. For example, in 2004, Jacquinet and co-workers reported the same selectivity pattern with OMP protected galactopyranoside **4.20**. The authors were able to selectively

introduce a benzoyl protecting group at the 3-position of **4.20** and then migrate the protecting group under basic conditions to provide **4.22** in good yields (Scheme 4.4).[86]



Scheme 4.4. Selective benzoylation and benzoyl migration as reported by Jacquinet[86]

Jacquinet's work lead us to investigate an alternative route to the desired SB_{1A} galactopyranoside donor, as described in Scheme 4.5. We proposed the possibility of selectively introducing a Cbz protecting group to the *3-O*-position of **4.17** giving **4.23**, and then migrate the Cbz group to the 2-*O*-position to provide **4.24**. Although 3→2 migration of a Cbz group in a carbohydrate had never been demonstrated before we anticipated that it should be achieveable using conditions that had already been developed for benzoyl group migration. Sulfation of **4.24** would then result in the target galactopyranoside donor **4.14.**



Scheme 4.5. Proposed route to donor **4.14**

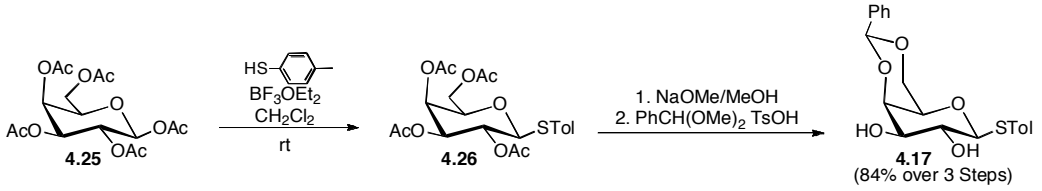
4.2 Objectives

The objectives of the work described in this chapter was to determine if a Cbz group could be introduced selectively into the 3-position of 2,3-diols of 4,6-*O*-benzylidene-protected galactose derivatives and if the Cbz group of the resulting 3-carbobenzyloxy carbonyl derivatives could be isomerized to the 2-position.

4.3 Results and Discussion

4.3.1 Selective Introduction and Isomerization of the Cbz group

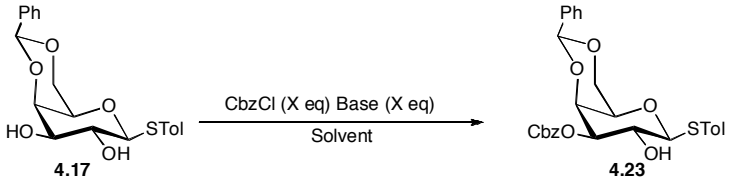
Diol **4.17** was prepared in an overall 84% yield in three high yielding steps starting from commercially available galactose tetraacetate (**4.25**) as described by Scheme 4.6. An anomeric protecting group exchange using 4-methylbenzenethiol and BF_3OEt_2 provided tetraacetate **4.26**. Removal of the acetate esters under Zemplén conditions followed by a selective installation of the 4,6-O-benzylidene acetal provided diol **4.17**.[87]



Scheme 4.6. Preparation of diol **4.17**

We next examined whether the Cbz protecting group could be selectively installed, and if it would follow the same selectivity patterns previously observed for sulfation and benzoylation of monomers **4.17** and **4.20**. Diol **4.17** was treated with benzyl chloroformate under a variety conditions as shown in table 4.1. Initial attempts to selectively install the Cbz group using triethylamine as a base (entries 1 and 2) were very sluggish, and after extended periods of time the conversion of **4.17** to product was very low. Under these conditions, a mixture of 2-*O*-Cbz and 3-O-Cbz compounds were obtained, with the latter being the major product. Changing the base to pyridine lead to better selectivity, with **4.23** being the only observed product (entry 3); however with only 1.2 eq. of CbzCl the conversion of starting material to product was again, quite low. Increasing the equivalents of CbzCl (entry 4), and drastically decreasing the rate of addition (entry 6) resulted in the preparation and isolation of target compound **4.23** in an easily reproducible 84% yield.

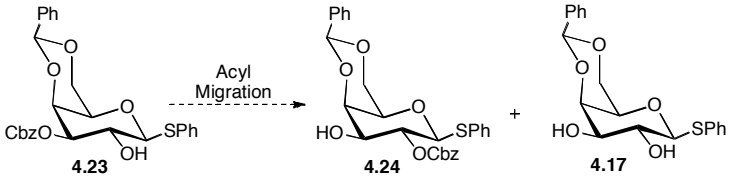
Table 4.1. Selective installation of the 3-O-Cbz protecting group



Entry	Solvent	Base (eq.)	CBzCl (eq.)	Results
1	CH_2Cl_2	NEt_3 (5.0)	1.2	Mostly **4.17**, trace **4.23** (major) + 2-O-CBz
2	CH_2Cl_2	NEt_3 (5.0)	3.0	Mostly **4.17**, trace **4.23** (major) + 2-O-CBz
3	CH_2Cl_2	Py (5.0)	1.2	Mostly **4.17**, trace 3.23 (only)
4	CH_2Cl_2	Py (5.0)	2.0+2.0	78 % **4.23**
5	--	Py (40.0)	2.0+2.0	60% **4.23** + unreacted **4.17**
6	CH_2Cl_2	Py (5.0)	2.0+1.0	84 % **4.23**

With **4.23** in hand we then set out to examine various conditions to see if the protecting group would undergo successful alkoxycarbonyl group migration to produce the 2-OCbz **4.24** (table 4.2). We began by attempting to migrate the Cbz protecting group under the same conditions which Jacquinet was able to migrate the 3-OBz in **4.21** to give **4.24** as previously shown in Scheme 4.4. Under the basic conditions, a mixture of 3-*O*-CBz **4.23**, migrated product **4.24** and diol **4.17** was observed (table 4.2, entry 1). The concentration and molar equivalents of NaOH were varied along with temperature and time (entries 2-6); however it appeared that an equilibrium between **4.23** and **4.24** was rapidly established that consisted of considerable quantities of both **4.23** and **4.24**. DBU was also examined in a variety of solvents (entries 7-12), and although some migration was observed, we could not find conditions that gave **4.24** as the dominant product.

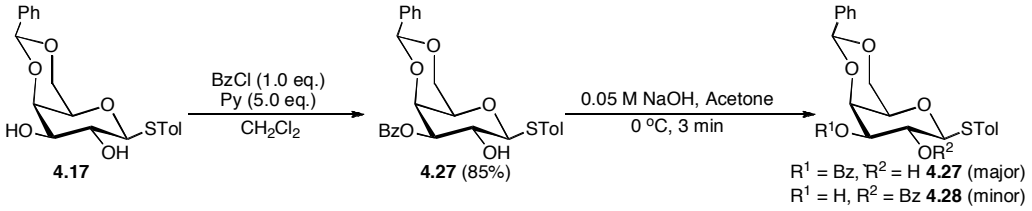
Table 4.2. Attempted 3-O-Cbz migrations with **4.23.**



Entry	Migration Conditions	Results
1	0.05 M NaOH (0.5 eq) / Acetone, 5 min, RT	**4.23** (major) **4.24** (minor) **4.17** (trace)
2	0.05 M NaOH (0.05 eq) / Acetone, 15 min, RT	**4.23** (major)**4.24** (minor) **4.17** (trace)
3	0.05 M NaOH (0.05 eq) / Acetone, 24 h, 0 ^{o}C to RT	**4.23** (major)**4.24** (minor) **4.17** (trace)
4	0.05 M NaOH (0.05 eq x 3) / Acetone, 9 h, 0 ^{o}C	**4.23** (major) **4.24** (minor) **4.17** (trace)
5	0.05 M NaOH (0.5 eq) / Acetone, 10 h, 0 ^{o}C	**4.23** (major)**4.24** (minor) **4.17** (trace)
6	0.5 M NaOH (5.0eq) / Acetone, 5 min, RT	**4.17**
7	DBU (0.5 eq) / Acetone, 4 h, RT	**4.23** (major) **4.24** (minor)
8	DBU (1.0 eq) / Acetone 24 h, RT	**4.23** (major) **4.24** (minor)
9	DBU (1.0 eq) / DCM, 24 h, RT	**4.23** (major) **4.24** (minor)
10	DBU (1.0 eq) / Et_2O, 24 h, RT	**4.23** (major) **4.24** (minor)
11	DBU (1.0 eq) / H_2O, 24 h, RT	**4.17**
12	DBU (1.0 eq) / MeOH, 24 h, RT	**4.17**
13	Silica Gel (0.05g/mg sugar), 16 h, RT	**4.23**

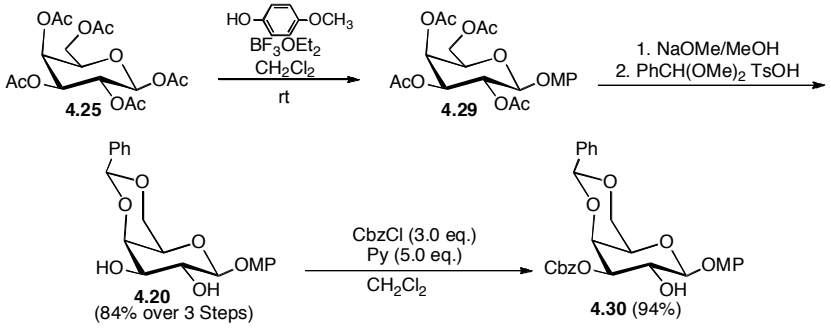
When Jacquinet performed the acyl group migration of the benzoyl protecting group on galactopyranoside **4.21** (Scheme 4.4) the process took just 3 minutes. The completely migrated **4.22** product precipitated out of solution and was isolated in good yields. It is possible that this product precipitation resulted in pushing the equilibrium to the 2-O-acyl side. With our system the migrated product did not precipitate out of solution. In our attempts, two variables differed from Jacquinet's. The acyl protecting

group was changed to a Cbz group, and the anomeric protecting group was changed from an OMP to a thioether. This brings up the question: Are the migration difficulties we encountered related to the Cbz protecting group itself, or are they a result of the thioglycoside function? In order to determine which variable was hindering the migration, the thioglycoside derivative of the 3-*O*-benzoylated **4.21**, compound **4.27**, was prepared and subjected to Jacquinet's migration conditions (Scheme 4.7).



Scheme 4.7. Benzoyl migration with thioglycoside **4.27**

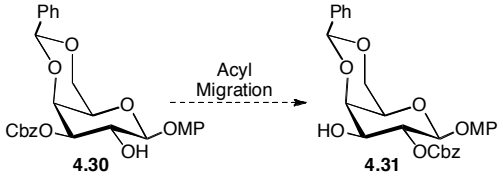
As previously observed with **4.23**, only trace amounts of the migrated product **4.28** were observed. The migrated thioglycoside product **4.28** did not precipitate out of solution as was reported for **4.22**. Presumably then, it is the product precipitation that shifts the equilibrium towards the migrated product, and above migration issues with **4.23** could be due to the anomeric thioglycoside, and are not necessarily a result of the 3-O-Cbz protecting group. To prove this, the OMP derivative of **4.23**, compound **4.30**, was prepared according to Scheme 4.8. The selective introduction of the Cbz group into monomer **4.20** proceeded particularly well using the same conditions described for compound **4.17**.



Scheme 4.8. Preparation of 3-*O*Cbz derivative **4.30**

Compound **4.30** was then subjected to the literature migration conditions (table 4.3). The solubility of this compound was found to be quite poor, so the literature migration conditions had to be modified to ensure that the **4.30** was in solution before the base catalyst was added. Fortunately, under these conditions, the 3-O-Cbz quickly migrated to the 2-position of the carbohydrate ring, and the resulting product **4.31** precipitated out of solution and was isolated in an 89% yield (table 4.3, entry 3).

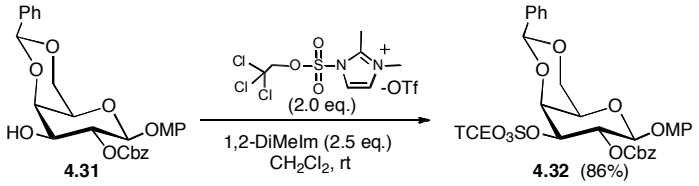
Table 4.3. Cbz migrations with OMP-protected carbohydrate **4.30.**



Entry	Migration Conditions	Results
1	0.05 M NaOH (0.5 eq) / Acetone (0.1 M), 5 min, 0 °C	All **4.30** precipitate
2	0.05 M NaOH (0.5 eq) dropwise / Acetone (0.1 M), 5 min, 0 °C	**4.31** 75%
3	0.05 M NaOH (0.5 eq) dropwise / Acetone (0.07 M), 5 min, 0 °C	**4.31** 89%

4.3.2 Installation of the Sulfate and Trichloroacetimidate group into monomer 4.31

With the desired 2-O-Cbz compound **4.31** in hand, we next looked at the sulfation of the 3-OH to provide fully protected **4.32**. As anticipated, the sulfation reaction using SIS **2.40** and 1,2-DiMeIm proceeded smoothly to give **4.32** in an 86% yield (Scheme 4.9).

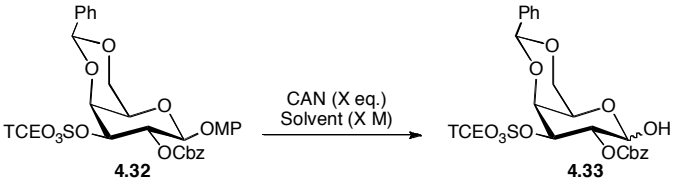


Scheme 4.9. 3-O-Sulfation of **4.31**

Because the target SB_{1A} donor had been modified from a thioglycoside to an OMP protected glycoside, the next steps to complete the synthesis include hemiacetal formation followed by installation of the trichloroacetimidate. When attempting to prepare hemiacetal **4.33** (table 4.4), using the standard CAN, CH_3CN/H_2O conditions, difficulties were encountered due to the insolubility of **4.32** (table 4.4, entries 1-7). In many situations, when a starting material is partially insoluble in a reaction medium, the material will slowly go into solution as the reaction progresses. In all water-acetonitrile solvent systems we examined however, **4.32** was extremely insoluble to the point where no reaction was even occurring. Starting carbohydrate **4.32** was soluble in CH_3CN; however as soon as any aqueous media was introduced into the reaction mixture **4.32** precipitated out of solution and no reaction occurred. A variety of reactant

concentrations and temperatures were examined using both H_2O and aqueous pH 7 buffer; however **4.32** would not remain in solution to allow for the reaction to proceed. Decomposition of **4.32** was observed when the CAN reagent was introduced to the reaction system before the aqueous media (entry 8). It was finally discovered that if carbohydrate **4.32** was dissolved acetonitrile at 30 °C, and then CAN was added as an aqueous solution, the target hemiacetal could be isolated in a 76 % yield (entry 12).

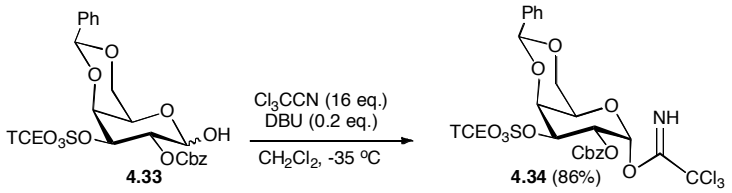
Table 4.4. Preparation of hemiacetal **4.34.**



4.32 **4.33**

Entry	Solvent	Conditions	CAN (eq.)	Result
1	CH_3CN/pH7 Buffer (1:1.5 0.04 M)	1h, 0°C	1.0 then 3.0	**4.32** ppt. (NR)
2	CH_3CN/pH7 Buffer (1:1 0.03 M)	1h, 0 °C	4.0 portionwise	**4.32** ppt. (NR)
3	CH_3CN/H_2O (1:1.5 0.04 M)	1h, 0 °C	4.0 portionwise	**4.32** ppt. (NR)
4	CH_3CN/H_2O (1:1.5 0.04 M)	1h, RT	4.0 at once	**4.32** ppt. (NR)
5	CH_3CN/pH7 Buffer (1:1 0.03 M)	1h, RT	4.0 at once	**4.32** ppt. (NR)
6	CH_3CN (0.03 M) then pH7 Buffer (0.04 M)	30°C	2.0 at once	**4.32** ppt. (NR)
7	CH_3CN (0.03 M) then H_2O (0.04 M)	30 °C	2.0 at once	**4.32** ppt. (NR)
8	CH_3CN (0.03M) *then CAN* then H_2O (0.04M)	30 °C	4.0 at once	Complex mixture
9	CH_3CN (0.03M) then H_2O (0.01M)	30 °C	4.0 at once	**4.32** ppt. (NR)
10	CH_3CN (0.03M)	30 °C	4.0 (0.55M aq.)	**4.33** 67%
11	CH_3CN (0.03M)	30 °C	4.0 (1.1M aq.)	**4.33** 67%
12	CH_3CN (0.02M)	30 °C	4.0(1.1M aq.)	**4.33** 76%

With hemiacetal **4.33** finally prepared, it was converted smoothly to the corresponding trichloroacetimidate donor **4.34** in an 86% yield (Scheme 4.10). Overall, the target 3-O-sulfated donor **4.34** was prepared in 8 steps and an overall 38% yield (89% average yield per step).



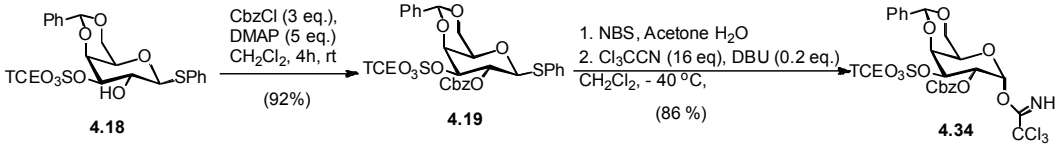
Scheme 4.10. Synthesis of trichloroacetimidate **4.34** from hemiacetal **4.33**.

4.4 Summary and Future Work

In this chapter we demonstrated that selective introduction of the Cbz group into the 3-position of 2,3-diols of 4,6-*O*-benzylidene-protected galactose derivatives can be achieved in high selectivity and yield. We also demonstrated that the Cbz group of the resulting 3-carbobenzyloxy derivatives could be isomerized to the 2-position under basic conditions. However conditions that allow the precipitation of the migrated product appear to be necessary for good yields. Future studies on this subject will be to determine if the selective introduction and subsequent isomerization can also be achieved using glucose-based substrates.

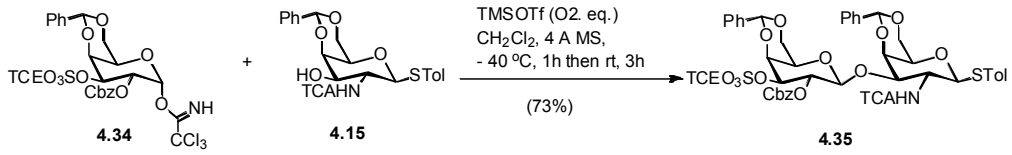
The complete total synthesis of SB_{1A} is now under investigation by Ahmed Desoky in the Taylor group. Subsequent to the work described in this chapter Mr. Desoky eventually developed conditions for the introduction of the Cbz group into

monomer **4.18** to give monomer **4.19** (Scheme 4.11) a reaction that we had previously found to be unachieveable (see Scheme 4.3a)



Scheme 4.11. Synthesis of monomer **4.19** and donor **4.34** starting from thioglycosides **4.18**.

Of particular note is that he has found that the coupling of monomer to **4.34** to monomer **4.15** proceeds in good yield (73 %, unoptimized) and no cyclic carbonate product was formed nor was any product obtained resulting from benzyl group migration (Scheme 4.12). *Thus, the problems that we encountered in chapter 3 with the Cbz group during glycosidic bond formation do not seem to be an issue with our synthesis of SB_{1A} at least so far.* Further studies to determine the factors that effect cyclic carbonate formation when using 2-*O*-Cbz-protected donors are in progress in the Taylor group.



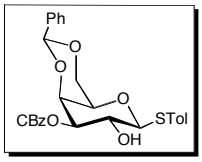
Scheme 4.12. Coupling of **4.34** to **4.15.**

4.5 Experimental

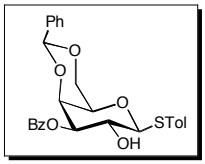
4.5.1 General Considerations

For all general considerations, see section 2.5.1 in Chapter 2.

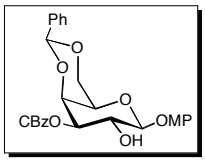
4.5.2 Experimental Syntheses and Characterization



p-Tolyl 4,6-*O*-benzylidene-3-*O*-carbobenzyloxy-1-thio-β-D-galactopyranoside (4.23). Pyridine (2.3 mL, 28.5 mmol) was added to a solution of carbohydrate **4.17**[87] (2.0 g, 5.76 mmol) in CH_2Cl_2. Benzyl chloroformate (1.6 mL, 11.4 mmol) was added dropwise using a syringe pump over 1.5 h. After 3 h, additional benzyl chloroformate (0.8 mL, 5.68 mmol) was added dropwise over 30 minutes. After an additional hour, no starting material was detected using TLC. The reaction was quenched with methanol and concentrated to a crude yellow oil. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) afforded **4.18** as a clear, colourless glassy compound (2.45 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.44 (br-s, 1H, OH), 3.55 (s, 1H, H5), 3.89 (br-t, $J_{2,3} = J_{2,1} = 9.6$ Hz, H2), 3.99 (d, 1H, $J_{6,6'} = 12.4$ Hz, H6), 4.34 (d, 1H, $J_{6',6} = 12.4$ Hz, H6'), 4.40 (d, 1H, $J_{4,3} = 2.9$ Hz, H4), 4.51 (d, 1H, $J_{1,2} = 9.5$ Hz, H1), 4.76 (dd, 1H, $J_{3,2} = 9.7$ Hz, $J_{3,4} = 3.1$ Hz, H3), 5.14 (s, 2H, CO_2CH_2Ph), 5.44 (s, 1H, CHPh), 7.03 (d, 2H, $J = 7.9$ Hz, ArH), 7.24-7.33 (m, 10H, ArH), 7.54 (d, 2H, $J = 7.9$ Hz, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 65.5, 69.1, 69.6, 70.0, 73.3, 78.3, 87.1, 100.9, 125.9, 126.5, 128.0, 128.2, 128.5, 129.0, 129.8, 134.4, 134.8, 137.6, 138.7, 154.6.

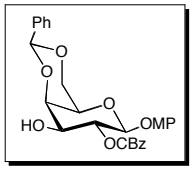


p-Tolyl 4,6-*O*-benzylidene-3-*O*-benzoyl-1-thio-β-D-galactopyranoside (4.27). To carbohydrate **4.17**[87] (0.5 g, 1.44 mmol) in CH_2Cl_2 (6.3 mL) was added pyridine (0.58 mL, 7.19 mmol) followed by a dropwise addition of benzoyl chloride (0.33 mL, 2.84 mmol). The reaction was stirred for 3h, then quenched with methanol and concentrated to a crude syrup. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **4.27** as a white solid (0.599 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.43 (s, 1H, OH), 3.65 (s, 1H, H5), 4.00-4.11 (m, 2H, H2, H6), 4.41 (dd, 1H, $J_{6',6}$ = 12.4 Hz, $J_{6',5}$ = 1.4 Hz, H6') 4.47 (d, 1H, $J_{4,3}$ = 3.2 Hz, H4), 4.60 (d, 1H, $J_{1,2}$ = 9.4 Hz, H1), 5.16 (dd, 1H, $J_{3,2}$ = 9.8 Hz, $J_{3,4}$ = 3.3Hz, H3), 5.46 (s, 1H, CHPh), 7.06 (d, 2H, J = 7.9 Hz, ArH), 7.31-7.53 (m, 7H, ArH), 7.48-7.53 (m, 3H, ArH), 8.01 (d, 2H, J = 7.1 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 65.8, 69.2, 69.9, 73.9, 75.3, 87.9, 100.8, 126.4, 126.6, 128.0, 128.4, 129.0, 129.7, 129.8, 130.0, 133.3, 134.0, 137.9, 138.5, 166.4.

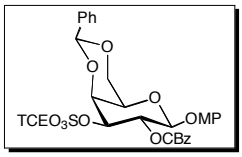


4-Methoxyphenyl 4,6-*O*-benzylidene-3-*O*-carbobenzyloxy-β-D-galactopyranoside (4.30). To a suspension of carbohydrate **4.20**[88] (1.0 g, 2.67 mmol) in CH_2Cl_2 was added pyridine (1.0 mL, 12.4 mmol) followed by a slow addition of benzyl chloroformate (1.2 mL, 8.53 mmol) via syringe pump over 2.5 h. The reaction was stirred for an additional 2 h, quenched with MeOH and concentrated to a crude syrup. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **4.30** as a white solid (1.28 g, 94%). %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.63 (d, 1H, $J_{OH,2}$ = 2.9 Hz, OH), 3.54 (br-s, 1H, H5),3.74 (s, 3H, CH_3), 4.04

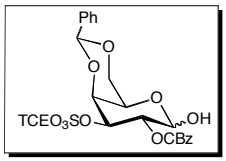
(dd, 1H, $J_{6,6'}$ = 12.5 Hz, $J_{6,5}$ =1.6 Hz, H6), 4.23-4.33 (m, 2H, H2, H6'), 4.46 (d, 1H, $J_{4,3}$ = 3.5 Hz, H4), 4.79-4.82 (m, 1H, H3), 4.82 (d, 1H, $J_{1,2}$ = 7.8 Hz, H1), 5.18 (s, 2H, CO_2CH_2Ph), 5.49 (s, 1H, CHPh), 6.78-6.82 (m, 2H, ArH), 7.02-7.07 (m, 2H, ArH), 7.29-7.38 (m, 7H, ArH), 7.46-7.49 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 66.4, 68.3, 68.9, 70.1, 73.1, 77.1, 100.9, 102.6, 114.5, 119.3, 126.3, 128.1, 128.3, 128.6, 129.0, 143.9, 137.5, 151.0, 154.8, 155.7.



4-Methoxyphenyl 4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-β-D-galactopyranoside (4.31). To carbohydrate **4.30** (0.60 g, 1.18 mmol) in acetone (15 mL) was added NaOH (0.05 M sol'n, 11.8 mL, 0.590 mmol). Within 5 minutes, **4.31** precipitated out of solution. The product was dissolved in EtOAc, washed with H_2O and the resulting organic layer was dried over $MgSO_4$. Purification by flash chromatography (99:1 CH_2Cl_2/MeOH) provided **4.31** as a white solid (0.531 g, 89%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.57 (br-s, 1H, OH), 3.52 (s, 1H, H5), 3.74 (s, 3H, CH_3), 3.84 (m, 1H, H3), 4.08 (d, 1H, $J_{6,6'}$ = 12.6 Hz, H6), 4.48 (d, 1H, $J_{4,3}$ = 3.7 Hz, H4), 4.63 (d, 1H, $J_{6',6}$ = 12.8 Hz, H6'), 4.82 (d, 1H, $J_{1,2}$ = 8.0 Hz, H1), 5.09-5.26 (m, 3H, H2, CO_2CH_2Ph), 5.56 (s, 1H, CHPh), 6.75 (d, 2H, J = 8.9 Hz, ArH), 6.90 (d, 2H, J = 8.9 Hz, ArH), 7.32-7.48 (m, 8H, ArH), 7.49-7.53 (m, 2H, ArH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.8, 66.7, 68.6, 69.3, 76.2, 99.6, 100.3, 114.8, 114.9, 118.1, 126.7, 128.4, 128.5, 128.8, 128.9, 129.2, 136.0, 138.8, 151.2, 154.8, 155.2.

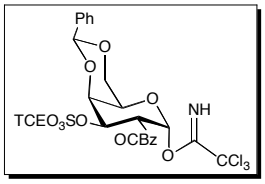


4-Methoxyphenyl 4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-(2,2,2-trichloroethoxysulfo)-β-D-galactopyranoside (4.32). To carbohydrate **4.31** (0.300 g, 0.590 mmol) in CH_2Cl_2 (4.8 mL) at room temperature was added 1,2-DiMeIm (0.145 g, 1.51 mmol) followed by **2.40** (0.540 g, 1.18 mmol). After 4 h, the reaction was concentrated to a yellow crude oil and applied directly to a silica gel column. Purification by flash chromatography afforded **4.32** as a white solid (0.364 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (s, 1H, H5), 3.74 (s, 3H, OCH_3), 4.08 (d, 1H, $J_{6,6'}$ = 12.4 Hz, H6). 4.35 (d, 1H, $J_{6',6}$ = 12.4 Hz, H6'), 4.57-4.67 (m, 3H, H4, CH_2CCl_3), 4.84 (dd, 1H, $J_{3,2}$ = 10.3 Hz, $J_{3,4}$ = 3.6Hz, H3), 4.88 (d, 1H, $J_{1,2}$ = 7.9 Hz, H1), 5.19, 5.26 (AB, 2H, J= 12.1 Hz, CH_2CO_2Ph), 5.44 (dd, $J_{2,3}$ = 10.3 Hz, $J_{2,1}$ = 8.1 Hz, H2), 5.57 (s, 1H, CHPh), 6.74 (d, 2H, J = 9.0 Hz, ArH), 6.85 (d, 2H, J = 9.0 Hz, ArH), 7.24-7.51 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 65.9, 68.6, 70.5, 71.9, 72.9, 80.0, 81.3, 92.4, 100.8, 101.4, 114.5, 119.3, 126.5, 128.4, 128.5, 128.69, 128.74, 129.6, 134.8, 136.7, 150.9, 153.9, 155.9.



4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-(2,2,2-trichloroethoxysulfo)-β-D-galactose (4.33). Carbohydrate **4.32** (0.500 g, 0.694 mmol) was stirred in a solution of CH_3CN (37 mL) at 30 °C until all starting material had gone into solution. An aqueous solution of CAN (1.5 g in 2.5 mL H_2O, 2.74 mmol) was then added in one portion. The reaction was immediately removed from heat and allowed to stir at rt for 4 h. Upon completion, the reaction was diluted with EtOAc and the resulting layers were separated. The organic

layer was collected, dried over $MgSO_4$ and concentrated to an orange crude syrup. Purification by flash chromatography (50/50 EtOAc/Hexane) followed by a short silica gel gravity column (75:25 benzene/EtOAc) yielded **4.33** as a white foam (0.324 g, 76%). The product was used directly in the preparation of **4.34** with no further characterization.



4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-(2,2,2-trichloroethoxysulfo)-β-D-galactose trichloroacetimidate (4.34). To hemiacetal **4.33** (0.2 g, 0.326 mmol) in CH_2Cl_2 (2.0 mL) at –35 °C was added trichloroacetonitrile (0.52 mL, 5.16 mmol) followed by a 1.0 M solution of DBU in CH_2Cl_2 (0.065 mL, 0.065 mmol). The reaction was stirred for 2 h and gradually warmed to –10 °C. The reaction mixture was then directly applied to a silica gel column, and **4.34** (0.212 g, 86%) was isolated as a white foam after purification by flash chromatography (100% CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.04 (s, 1H, H5), 4.13 (d, 1H, $J_{6,6'}$ = 11.8 Hz, H6), 4.35 (d, 1H, $J_{6',6}$ = 11.8 Hz, H6'), 4.68, 4.81 (AB, 2H, J = 10.7 Hz, CH_2CCl_3), 4.84 (d, 1H, $J_{4,3}$ = 3.0 Hz, H4), 5.18-5.24 (m, 3H, H2, CO_2CH_2Ph), 5.43 (dd, 1H, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, H3), 5.66 (s, 1H, CHPh), 6.89 (d, 1H, $J_{1,2}$ = 3.2 Hz, H1), 7.28-7.45 (m, 2H, ArH), 7.54-7.57 (m, 2H, ArH), 8.61 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.7, 68.5, 70.2, 70.6, 73.3, 78.1, 79.9, 92.4, 94.1, 101.1, 126.2, 128.4, 128.6, 128.7, 128.8, 128.9, 129.5, 134.4, 136.7, 153.6, 160.5.

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