Synthesis of Sulfated Carbohydrates Using Sulfuryl Imidazolium Salts

by

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AUTHOR'S DECLARATION

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.

I understand that my thesis may be made electronically available to the public.

Abstract

Sulfated polysaccharides are widespread in nature. These compounds are implicated in a wide variety of important biological processes such as blood clotting, cell adhesion, and cell–cell communication. However, detailed characterization of their specific biological roles has proved to be very challenging. One reason for this is that the synthesis of even relatively small sulfated oligosaccharides still remains a considerable challenge. A general approach to the synthesis of sulfated carbohydrates was examined in which the sulfate group is incorporated at the beginning of the syntheses as a protected sulfodiester. Towards this end, a series of modified sulfuryl imidazolium salts were prepared and examined as reagents for incorporating 2,2,2-trichloroethyl-protected sulfate esters into monosaccharides.. A more efficient sulfating agent was obtained by incorporating a methyl group at the 2-position of the imidazolium ring. *O*-Sulfations that required prolonged reaction times and a large excess of the original sulfuryl imidazolium salt (SIS) which bears no alkyl groups on the imidazolium ring, were more readily achieved using the new reagent. Direct regioselective incorporation of TCE-protected sulfates into monosaccharides was achieved using the new imidazolium salt. We have also shown that the new SIS can also be used for the direct disulfation of monosaccharides and that trisulfated monosaccharides can also be prepared from the disulfated compounds. SIS's bearing the TFE and phenyl groups, were readily prepared. In most instances, both TFE- and phenyl protected sulfated carbohydrates were easily prepared in good yields using SIS's. Deprotection of the TFE group from secondary sulfates in carbohydrates and aryl sulfates was achieved in excellent yields using NaN_3 in DMF. We applied the sulfate protecting group strategy towards the total synthesis of the tetrasaccharide portion of a

disulfated glycosphingolipid called SB_{1a}. Efficient routes were developed for the construction of the left- and right-hand protected disaccharide portions of the SB_{1a} tetrasaccharide.

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To my wife *Hend*

&

my children *Bahaa* and *Sara*

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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
DMTST	(dimethylthio) methylsulfonium trifluoromethanesulfonate
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
IDCP	iodonium dicollidine perchlorate
Im	imidazole
m	multiplet
MBn	methoxybenzyl
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
PMB	p-methoxybenzyl
py	pyridine
rt	room temperature
s	singlet
SAR	structure activity relationship
SIS's	sulfurylimidazolium salts
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
TMSOTf	trimethylsilyl triflate
Troc	2,2,2-trichloroethylcarbonyl
TTBP	tri-*tert*-butylpyrimidine

Chapter 1. Sulfated Carbohydrates: their biological functions, roles in drug development and chemical synthesis

1.1 Sulfated Carbohydrates

Carbohydrates encompass the most abundant group of natural products. They represent a unique family of polyfunctional compounds which can be chemically manipulated in a large number of ways. Carbohydrates form the major constituents of shells of insects, lobsters, crabs, and the supporting tissue of the plants and also they are found as parts of all cell walls. Oligosaccharides have many biological functions which include bacterial and viral adhesion to host tissues, tumor cell metastasis, leukocyte trafficking and associated inflammatory responses, clearance of materials from the blood stream, determination of blood group specificity, and regulation of hormone and enzyme activities.

The presence of negatively charged sulfate groups on a wide variety of carbohydrates from simple monosaccharides to complex polysaccharides provides many interesting and important compounds. The diversity of carbohydrate sulfates is attributed to the position(s) of the sulfate group(s) on the sugar ring, the nature of the carbohydrate and the presence of other functional groups, such as amines or uronic acids. An important consequence of this structural diversity is that each unique structure has the potential to be recognized by an individual receptor or enzyme, making sulfated oligosaccharides ideal for carrying information in complex biological systems. There are many important sulfated carbohydrates.[1] A handful of them, as well as recent developments in carbohydrate drug design, are presented below.

An important class of polysaccharides found in connective tissue and the extracellular matrix are the glycosaminoglycans (GAG's). GAG's are a large family of polydisperse anionic polysaccharides that bind numerous proteins of biological interest including antithrombin III and fibroblast growth factor. They are classified into seven structural groups, heparin, heparan sulfate, keratan sulfate, dermatan sulfate, hyaluronate and chondroitin 4- and 6-sulfates. Heparin and heparan sulfate are made essentially by the same process, but whereas heparin is only synthesised in mast cells, heparan sulfate is widely distributed through epithelial tissues. Heparan sulphate and heparin consist of alternating units of glucosamine (GlcN) with glucuronic acid (GlcA) or iduronic acid (IdoA). Heparan sulphate contains a greater proportion of GlcA, whereas heparin contains more IdoA. Both molecules are sulphated, but heparin is more highly *N*-sulfated and has a significant number of 3-*O*-sulfated *N*-sulfated glucosamine residues (Figure 1.1 and 1.2).[2-4]

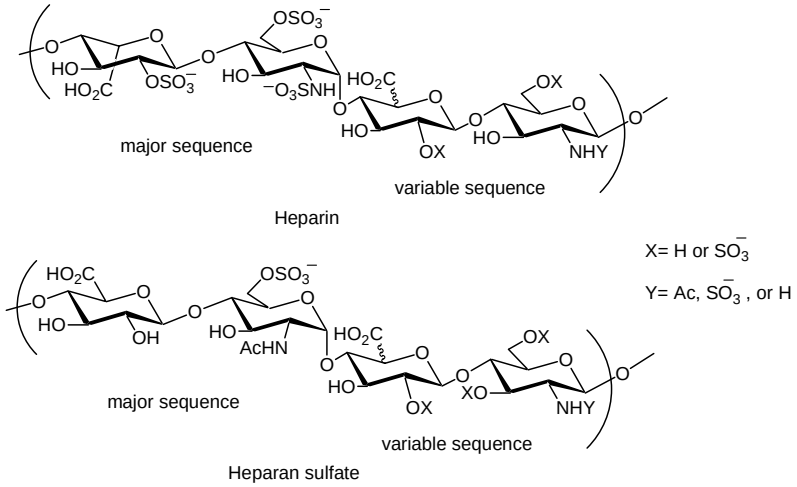


Figure 1.1. Major and minor disaccharide repeating units in heparin and heparan sulfate

Heparan sulphate GAG chains tend to exist as proteoglycan components — that is, tethered to a protein core. In this way, they are expressed on the surfaces of cells,

including those of the vascular endothelium and of circulating leukocytes, which provides a general net negative charge to these surfaces. Heparin, by contrast, is co-released with histamine from degranulating mast cells, and can dissociate from its protein core to exist as free GAG chains.[5-7]

More than 94 years ago, a medical student at Johns Hopkins University found that an extract of dog liver prolonged the time required for plasma to clot.[8] The extract, which was named heparin, remains the most important anticoagulant drug in current clinical use. The coagulation cascade is defined by Rosenberg and coworkers[9] as a series of linked, proteolytic reactions ultimately leading to the generation of thrombin. Once thrombin is formed, two pairs of fibrinopeptides are released from fibrinogen, and polymerization of the resulting fibrin leads to an insoluble clot.

After the understanding of the structure and properties of heparin, many efforts were made to demonstrate the structure-activity relationship of heparin. In the early 1980's, the studies of Lindahl and coworkers[10] on the identification of the consensus sequence of hexa- and octasaccharides, which were isolated by heparin degradation and affinity chromatography on immobilized AT-III, led to the structural elucidation of the unique pentasaccharide domain (**1.1** Figure 1.2), which now called the antithrombin III binding domain (ABD).[10]

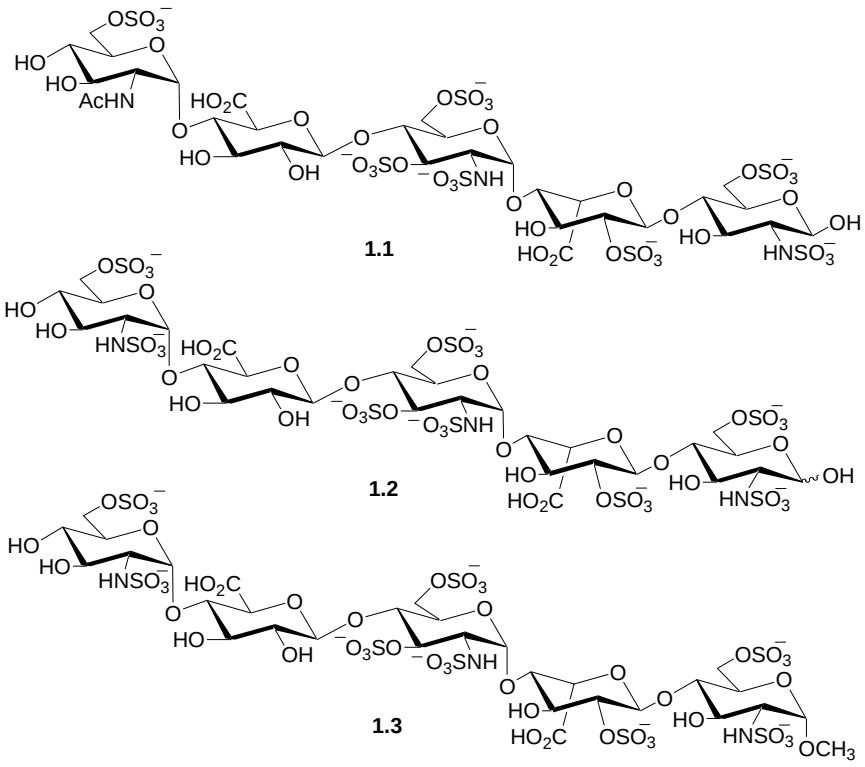


Figure 1.2. Heparin pentasaccharide and its synthetic analogs.

Interaction of heparin with antithrombin III (AT-III) led to a conformational change in the serine protease inhibitor, which allowed the loop with the reactive center to interact with coagulation enzymes, such as thrombin and factor Xa. The unique pentasaccharide domain—also called the antithrombin III binding domain (ABD)—should be present in the heparin chain to induce the conformational change in AT-III. The pentasaccharide stimulates exclusively the AT-III-mediated inactivation of factor Xa (anti-Xa activity), whereas longer heparin fragments comprising both the pentasaccharide domain (ABD) and a thrombin-binding domain (TBD) are required for stimulating antithrombin activity (Figure 1.3).[10]

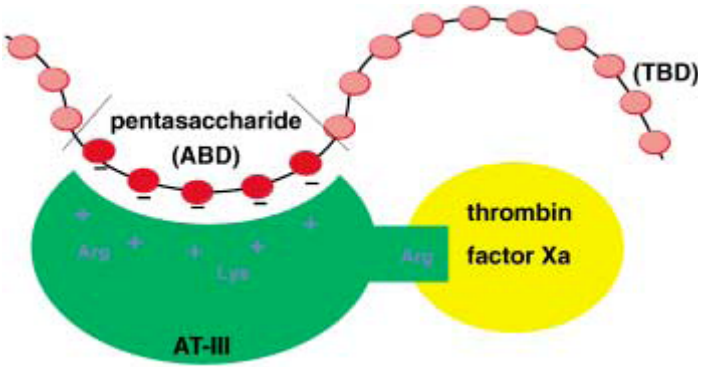


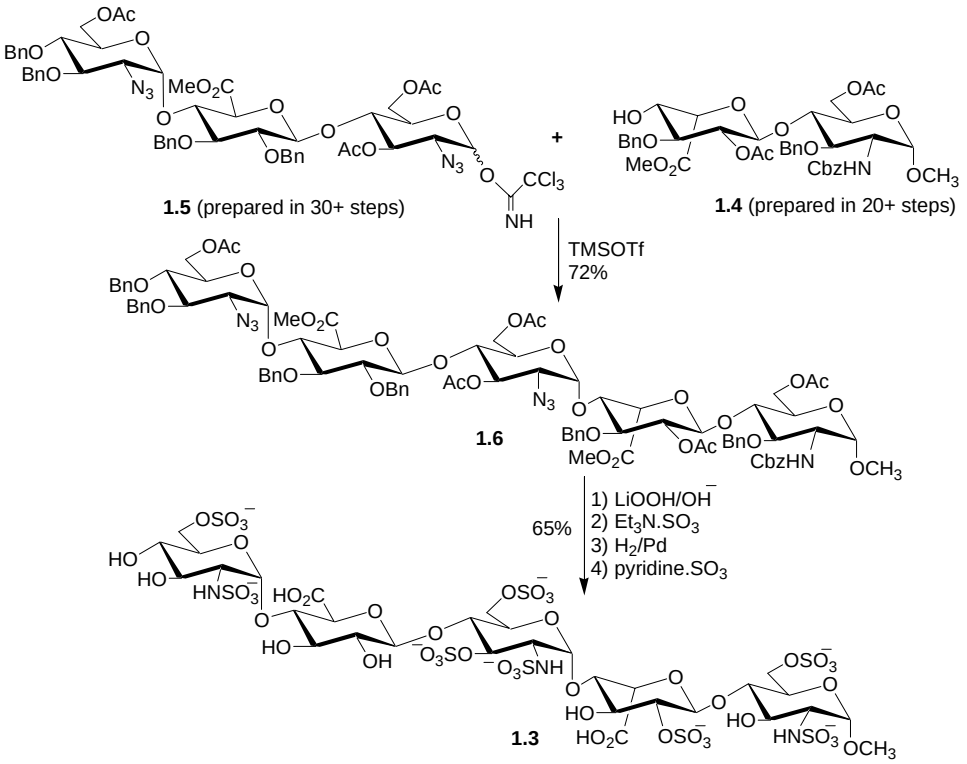
Figure 1.3 Interaction of heparin with antithrombin III (AT-III).

This Figure is taken from a review published by Petitou, M. and van Boeckel, C. A. A. *Angew. Chem.Int. Ed.* **2004**, *43*, 3118-3133.

In 1985, Petitou *et al.*,[11] synthesized the first pentasaccharides **1.2** as analogues of the active domain **1.1**, which contain an *N*-sulfate group instead of an *N*-acetyl group on the non-reducing end of the pentasaccharide. (Figure 1.2). More than 60 steps were required in the chemical synthesis of the first pentasaccharide **1.2**, which was isolated in extremely low yield and insufficient purity for use as a synthetic drug. The pentasaccharide showed anti-Xa activity but no antithrombin activity was observed.

In early 1987, the pentasaccharide **1.3** (Figure 1.2), which is closely related to the natural sequence and had been prepared by both Sanofi (now Sanofi-Synthelabo) and Organon companies was selected for further development.[12,13] The analogue **1.3** with a methyl group at the anomeric center was expected to be easier to synthesize and purify than the pentasaccharide **1.2**. In 1989 over 20 g of highly purified pentasaccharide **1.3** 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).[14] Arixtra is currently used for the prevention of venous

thromboembolic events following knee- or hip replacement surgery and after hip fractures. Arixtra is superior to natural heparin in that it requires a lower dosage, has a much longer half life ($t_{1/2}$ = 17 h, the half life of heparin is 1 h).[14] The synthesis of Arixtra is summarized in Scheme 1.1, where the orthogonal protecting group strategy was applied in which hydroxy groups to be sulfated are masked with acetyl groups, whereas those corresponding to the free hydroxy groups in the pentasaccharide are protected as benzyl ethers.[3] The fully protected pentasaccharide **1.6** is prepared by coupling trisaccharide donor **1.4** with disaccharide **1.5** (prepared in over 50 combined synthetic steps).[3,12] The locations that bear the sulfates are deprotected and then *O*-sulfated. The remaining benzyl and azido groups are removed by hydrogenolysis, and then selective *N*-sulfation affords the desired pentasaccharide **1.3**.



Scheme 1.1. Synthesis of Arixtra.

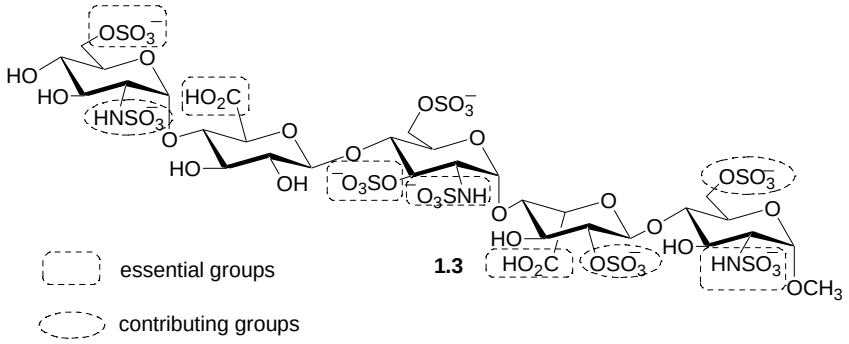


Figure 1.4. Essential sulfate groups in Arixtra

Many synthetic analogues of the unique pentasaccharide were prepared to help in understanding of the heparin-mediated activation of AT- III.[12] The results of the structural activity relationship of these analogues showed that, the heterogeneous pentasaccharide domain of heparin is the only polyanion identified so far that is able to trigger the conformational change in AT-III. There are two *N*-sulfate groups, and two essential carboxylate groups. Removal of one of these key sulfate or carboxylate groups leads to a nearly complete loss of activity, while the presence of two to three more sulfate groups in the pentasaccharide domain help to increase its biological activity. Moreover, the persulfated pentasaccharide displays no significant activity. The orientation of the key charged groups in space is important, since epimers display reduced activity. The activity was decreased when an essential sulfate group was replaced by a phosphate group, which means the type of charge is crucial. These results suggest that the essential charged groups of heparin interact with the complementary residues of AT- III through ordered hydrogen bonds. In addition, the hydroxyl groups of the pentasaccharide are not used as hydrogen bond donors in the interaction process, since methylation of the hydroxyl groups does not reduce the activity. The hydrogen atoms of the sulfated amino groups are

not important hydrogen bond donors, because N-sulfate can be replaced by sulfate without changing the activity. [12]

Among the synthetic analogues of the unique pentasaccharide are the two *O*-methylated analogues, Idraparinux **1.7** [3] and Idrabiotaparinux **1.8**[15] (Figure 1.5).

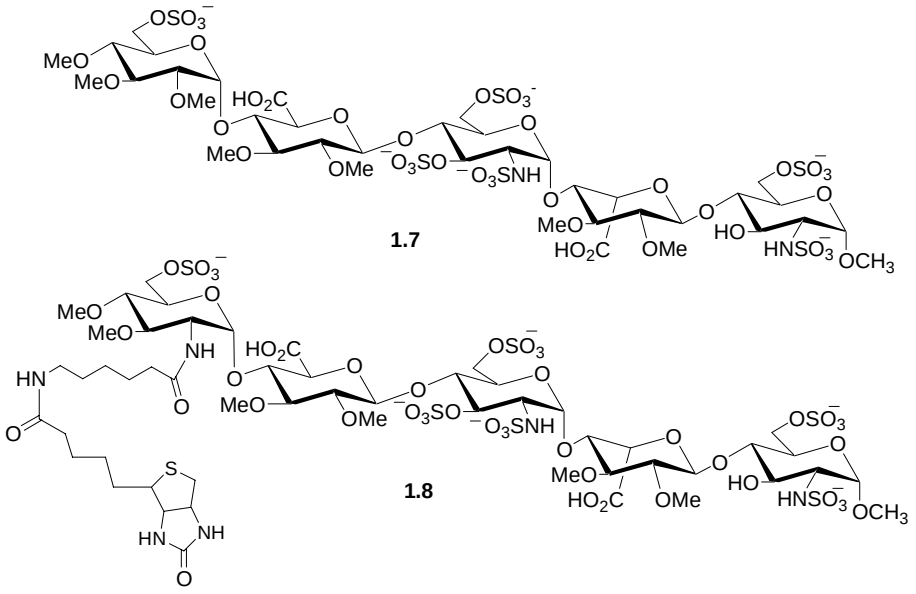
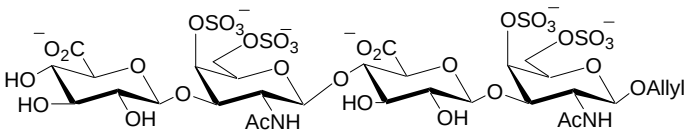


Figure 1.5. Idraparinux and Idrabiotaparinux

Idraparinux showed higher activity and higher bio-availability than both heparin and Arixtra, (t1/2 = 120 h), and by 2003 had reached phase III clinical trials. Due to the high activity of Idraparinux, major bleeding was observed in greater than 18% of the clinical trials.[15] To overcome this problem, a biotin moiety was tethered to the non-reducing end of **1.7** to give Idrabiotaparinux **1.8** (Figure 1.5). It is expected that the activity of Idrabiotaparinux can be controlled by the addition of avidin, and this process is currently in phase III clinical trials.[15] The development of Arixtra, Idraparinux and Idrabiotaparinux is considered to be one of the most exceptional achievements of modern drug design and development.

Another important class of naturally occurring sulfated oligosaccharides is chondroitin sulfate family of glycosaminoglycans. Chondroitin sulfates occur in many tissues as side chains of proteoglycans. They are found at the cell surface or intracellularly in secretory granules, as well as in various body fluids.[16,17] Chondroitin sulfates are linear copolymers formed from dimeric units composed of D-glucuronic acid (GlcA) and 2-acetamido-2- deoxy-D-galactose (GalNAc). In the major variants, the 4- and 6-*O*-positions of the Gal-NAc residues are found sulfonated. Chondroitin sulfates have many biological roles, such as cell recognition, [18] development of osteoarthritis,[19] inhibition of human C1q factor,[20] and AT-III-mediated anticoagulant activity.[21]

Hsieh-Wilson and coworkers, reported that tetrasaccharide **1.9** (Figure 1.6), a fragment of a chondroitin sulfate glycoaminoglycan, stimulates neuronal growth.[22] The authors mentioned that, the presence of the four sulfate residues was absolutely essential for the stimulatory activity.

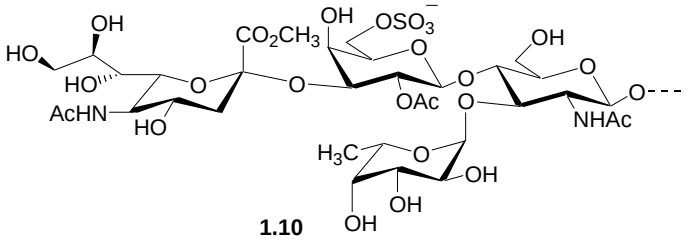


1.9

Figure 1.6. Chondroitin 4,6-*O*-sulfates

The discovery of the selectins in the 1980`s has generated intense interest in therapeutic potential of carbohydrates as anti-inflammatory agents. Selectins (E-, P-, and L-) are a family of adhesion molecules that mediates the interaction of circulating leukocytes with endothelial cells, a key step in their recruitment to sites of inflammation.[23] Potential ligands for these selectins include sialyl Lewis x, sialyl Lewis a, 6`-sulfo Lewis x, and 3`-sulfo Lewis x. [23b-e] The structure of 6`-sulfated sialyl Lewis x,

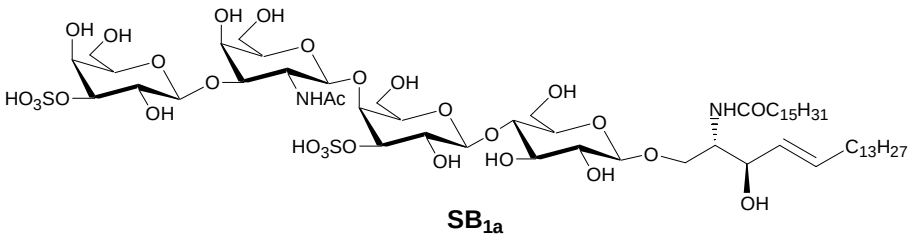
1.10 which is a main capping group[24] of the high endothelial venules (HEV) associated glycoprotein GLYCAM-1 is shown in Figure 1.7.



1.10

Figure 1.7. 6`-Sulfated sialyl Lewis x

Another example of a biologically active sulfated carbohydrate is the glycosphingolipid SB_{1A} (**1.11**, Figure 1.8). SB_{1A} is a 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.[25] It has been suggested that SB_{1A} is one of the most important cancer-associated carbohydrate antigens for HCC.[25] SB_{1A} will be covered in more detail in chapter 5.



SB_{1a}

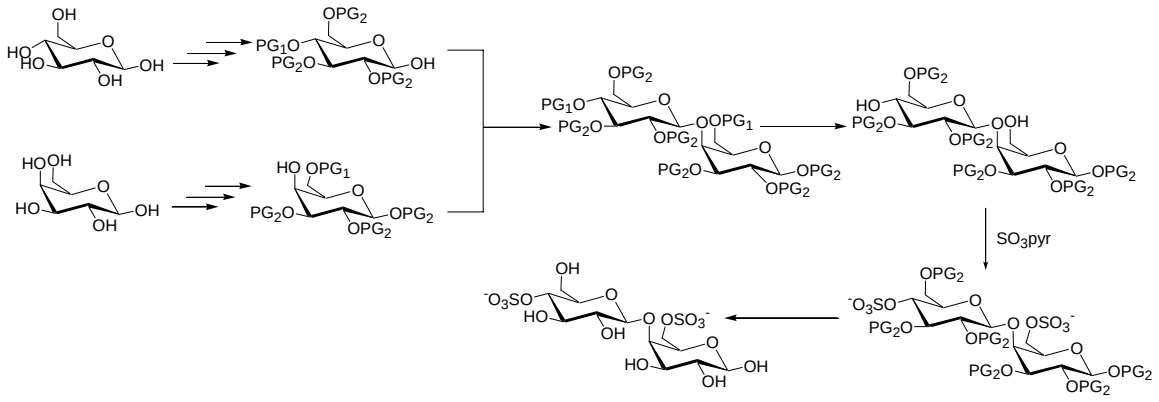
1.11

Figure 1.8. Structure of SB_{1A}

1.2 Synthesis of Sulfated Carbohydrates

Although sulfated carbohydrates are widespread, a clear comprehension of their specific roles is limited. These compounds are commonly isolated as complex mixtures from natural sources, which makes it difficult to determine the precise structure-activity relationships responsible for their biological functions and hence there is a need to synthesize deliberate portions of their structures.

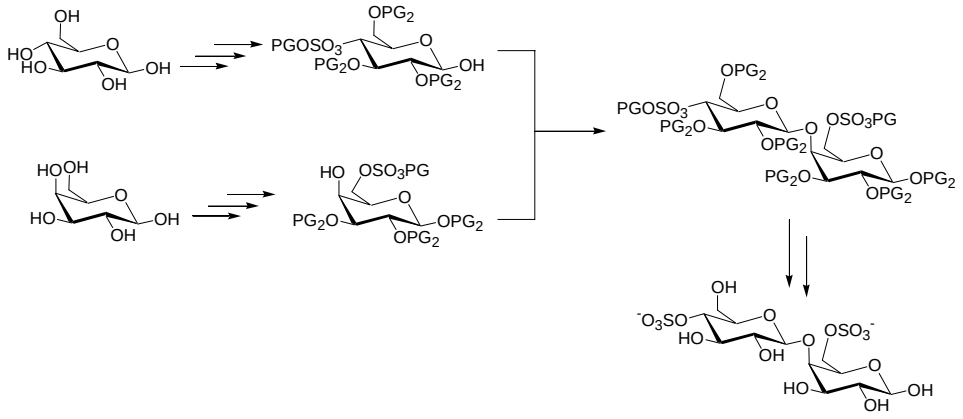
In the current approach, the synthesis of sulphated oligosaccharides typically involves first constructing a fully protected precursor in which the hydroxyl groups that ultimately bear the sulfate group(s) are protected in a manner orthogonal to those that will not be sulfated. The protecting groups on the hydroxyls that are to be sulfated are then removed, and the resulting free hydroxyl groups are sulfated, usually with a sulfur trioxide-amine or amide complex, and then all other protecting groups are removed to give the desired product (Scheme 1.2).



Scheme 1.2. Orthogonal protection strategy for the synthesis of sulphated sugars

Despite the success of the direct sulfation strategy, however, there are some drawbacks especially during the synthesis of complex molecules. First, good yields of the sulfation

reactions can be difficult to attain especially when multiple sulfations are necessary.[26] Second, the sulfated products are highly polar and can be difficult to purify for subsequent manipulations. Finally, additional protecting group manipulations are required at later stages of the synthesis. Due to these shortcomings, another strategy for the synthesis of sulfated carbohydrates appeared some time ago where the sulfate groups are introduced at the monosaccharide stage as a protected sulfate diester. Once the fully protected oligosaccharide is assembled, the hydroxyl and sulfate protecting groups are removed, and the desired sulfopolysaccharide is obtained (Scheme 1.3). This is an attractive approach because the initial products are uncharged, can be purified by the traditional chromatographic methods, and may be amenable to subsequent chemical manipulations. The key to this approach is finding a sulfate protecting group that is readily introduced, is compatible with the diverse conditions that are encountered during the synthesis of complex carbohydrates and, easily removed under mild conditions.



Scheme 1.3. Incorporation of a protected sulfate moiety.

1.3 Protecting Groups for the Sulfate Moiety

Sulfate esters can react with nucleophiles by S-O bond cleavage (pathway (c) in Figure 1.9) or C-O bond cleavage (pathways (a) and (b) in Figure 1.9).[27,28] Nucleophilic substitution by pathway (c) is usually slow unless the leaving group is good. A protecting group that would be removed by pathway (c) would probably have to be too good a leaving group to be practical for complex syntheses. Hence, the design of sulfate protecting groups has focused mainly on disfavouring attack by route (a) where R = carbohydrate (Figure 1.9), and favoring attack by route (b) where R' is the protecting group. Substitution by pathway (a) is also generally slow when R is a carbohydrate especially with the sulfate esters of secondary alcohols. Acid-labile protecting groups cannot be used as the deprotected products are also acid labile. Benzylic moieties, which are typically removed by hydrogenolysis, cannot be used as they are very labile to C-O bond cleavage (pathway (b)). Base labile protecting groups that are removed by basic hydrolysis (route (c)) are unlikely candidates for the reasons mentioned above. If a β-proton is available then elimination (route (d)) also occurs very readily under basic conditions so the absence of a β-proton is important. Taken together, these factors severely limit the number of protecting groups which can be used for the sulfate moiety. In the next section a survey of the different sulfate protecting groups that have been developed to date is presented.

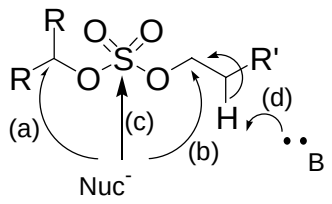
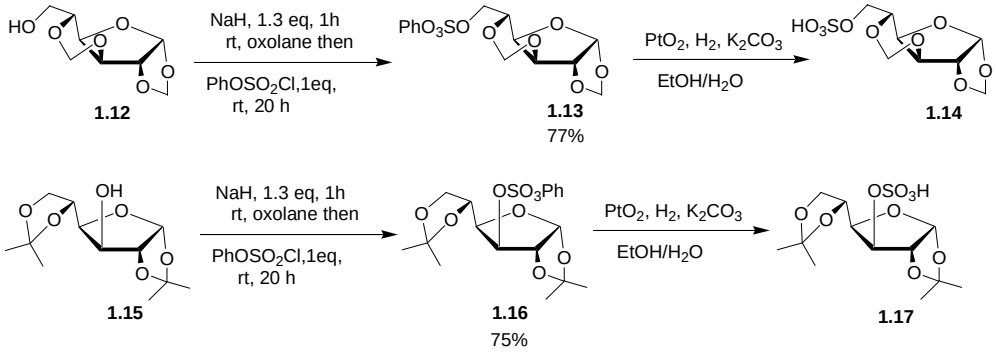


Figure 1.9. Possible routes for reactions of sulfate esters.

1.3.1 Phenyl Protection for Sulfate Monoesters

Penney and Perlin[29] were the first to explore the possibility of using a protecting group strategy for the synthesis of sulfated carbohydrates. They examined the phenyl group as a sulfate protecting group. The phenyl sulfate was introduced using phenyl chlorosulfate. Two simple monosaccharides were used as model systems, where the partially protected monosaccharides were treated with sodium hydride in oxalane followed by phenyl chlorosulfate to afford the corresponding phenyl sulfocarbohydrates **1.13** and **1.16** in a 77 % and 75% yield respectively (Scheme 1.4).



Scheme 1.4. Incorporation and deprotection of phenyl sulfates.

Stability studies of the phenyl-protected sulfate esters showed that, the phenyl sulfates were stable to variety of conditions including NaOMe at room temperature; 2:1 NH_4OH in pyridine; CsF in acetonitrile or methanol; KF and 18-crown-6. In addition, the 5,6-isopropylidine group in **1.16** could be hydrolyzed without affecting the phenyl group using mild acidic conditions such as TFA in $CHCl_3$ or cationic resin in water/oxolane at room temperature but not at elevated temperature. The authors also reported that the acetals could be removed using 1:1 Ac_2O/H_2SO_4 without affecting the

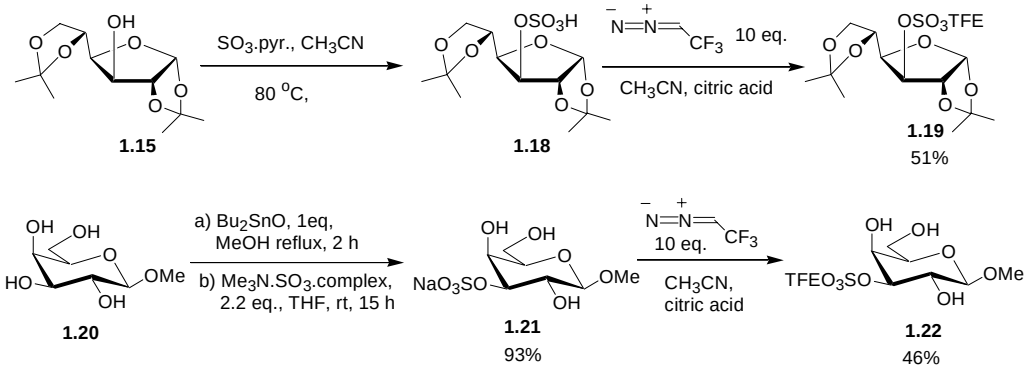
phenyl sulfate. On the other hand, unidentifiable products were formed upon treatment of **1.13** with TBAF in oxolane.

To remove the phenyl group, Penney and Perlin subjected the protected sulfates to a solution of potassium carbonate (3 eq.) and 12 wt. % platinum oxide/H_2 (Parr hydrogenator, 37 lb. in $^{-2}$) in ethanol-water for 20 h (Scheme 1.4). These conditions resulted in the hydrogenation of the phenyl ring to the cyclohexyl group, which was removed under the basic conditions to give the desired sulfated carbohydrates **1.14** and **1.17**.[29] A rather tedious purification then followed and the resulting sulfated products were obtained in low yields (< 50%). It was noted that 10 % desulfation had also taken place. Perlin and coworkers also examined selective sulfations with this methodology.[30,31] These studies will be discussed in more detail in chapter 3. Nevertheless, due to the low yields obtained during deprotection and the fact that partial desulfation occurs this protecting group has never been used in the synthesis of complex sulfated carbohydrates.

1.3.2. Trifluoroethyl Protection for Sulfate Monoesters

Due to the limitations of the phenyl protecting group, Proud *et al.*[28] offered an alternative. For both steric and electronic reasons, the authors expected trihaloethyl sulfate esters, to be stable to nucleophilic attack at the methylene carbon. They initially focused on the trichloroethyl (TCE) group, since it had previously been used for phosphate and carboxyl protection and can be removed selectively with Zn/AcOH.[32] The authors tried many conditions for the reaction of 2,2,2-trichloroethyl chlorosulfate with partially protected carbohydrates, but the reactions yields were too low to be of use, attributing this to steric reasons. Proud *et al.* [28] therefore switched to the trifluoroethyl

group as a protecting group. They started by treating the carbohydrates with 2,2,2-trifluoroethyl chlorosulfate, but the yields were poor. However, the authors were able to introduce the TFE-protected sulfates into the sugars using a two step approach in which the sulfate-unprotected sugar was prepared using sulfur trioxide-amine complexes followed by treatment with 2,2,2-trifluorodiazoethane to give the desired TFE-protected sulfocarbohydrates (Scheme 1.5). Other sulfated carbohydrates prepared by Proud et are shown in Figure 1.10.



Scheme 1.5. Two- step incorporation of TFE-protected sulfates

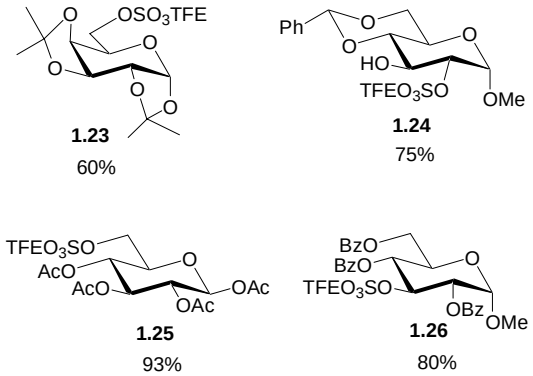
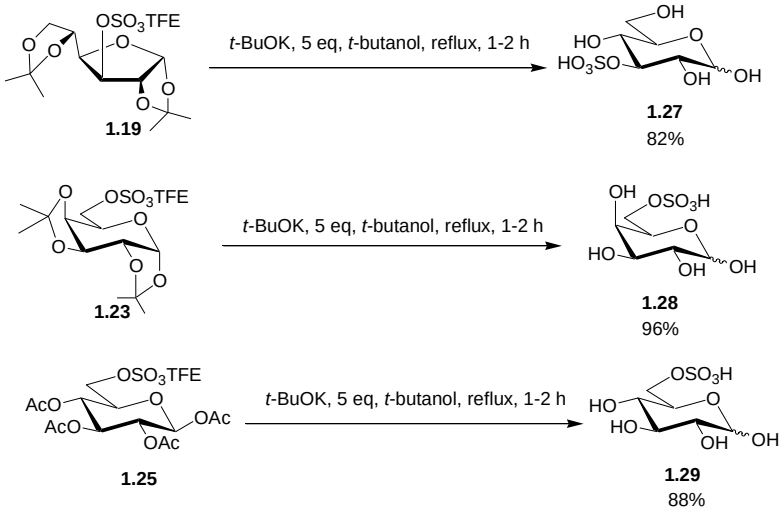


Figure 1.10. Trifluoroethyl esters of carbohydrate sulfates prepared by Proud et al.[28]

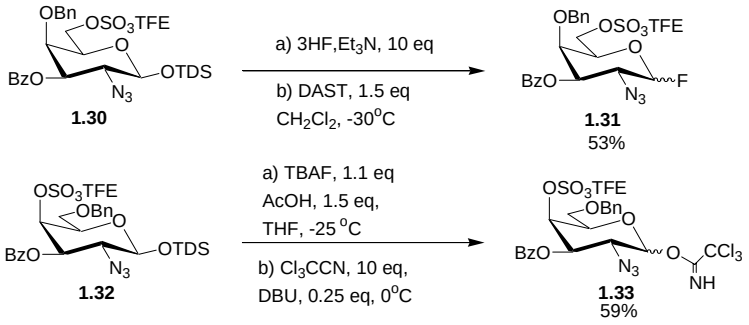
Stability studies by Proud *et al*. revealed that the TFE-protected sulfates are stable to a variety of conditions commonly used in carbohydrate chemistry such as TFA in EtOH, TBAF, hydrogenation, and NaOMe in MeOH at room temperature and apparently, at reflux. On the other hand, the TFE-group was removed by using potassium *t*-butoxide in refluxing *t*-butanol with the yields of deprotected products ranging from 82-96% (Scheme 1.6).



Scheme 1.6. Deprotection of the TFE-group.

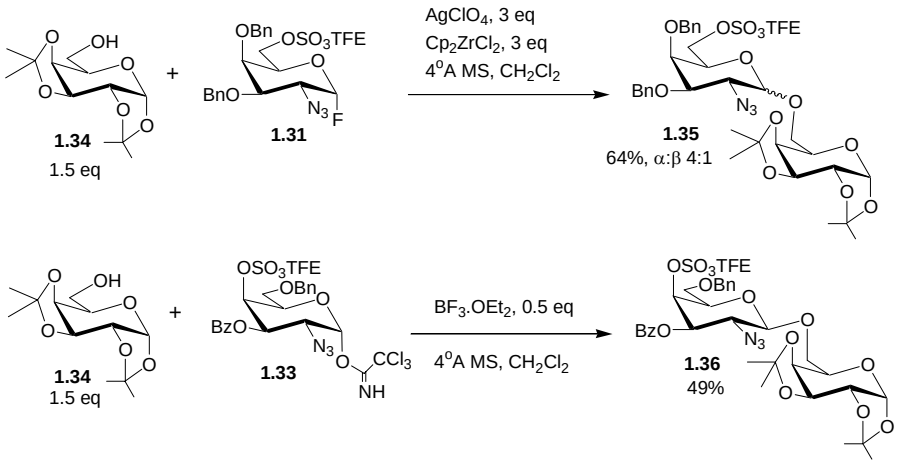
The next stage in the development of TFE-group as a protecting group for sulfated carbohydrates started in 2003 by Linhardt and coworkers[33] where they reported the use of the TFE group in the synthesis of fully differentiated hexosamine monosaccharides for the synthesis of GAG's. The TFE-protected sulfate was introduced into the monosaccharides in the same manner used by Proud *et al*.

The Linhardt group studied the preparation of some monosaccharides donors bearing the TFE sulfate moiety at the C-6 or C-4 position of the sugar. Both fluoride and trichloroacetimidate species were prepared (Scheme 1.7).



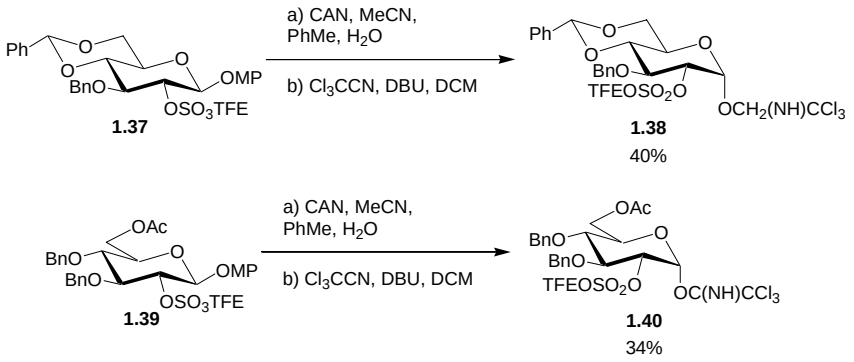
Scheme 1.7. Synthesis of monosaccharides donors bearing TFE sulfates.

The authors mentioned that, when TBAF was used to remove the anomeric thexyldisilyl (TDS) group on monosaccharides, the TFE-sulfate moiety at the 6-position acted as a good leaving group and 1,6-anhydro sugars was recovered as a side product. To overcome this problem, excess acetic acid was added to TBAF, or a milder reagent such as triethylamine trihydrofluoride was used. In addition, partial loss of the sulphate protecting group was observed under the basic conditions required to prepare trichloroacetimidate **1.33** (Scheme 1.7). Fluoride **1.31** and imidate **1.33** were used in the glycosylation reactions with simple donors having primary hydroxyl groups (Scheme 1.8), where the disaccharides were obtained in 64% and 49% yields.



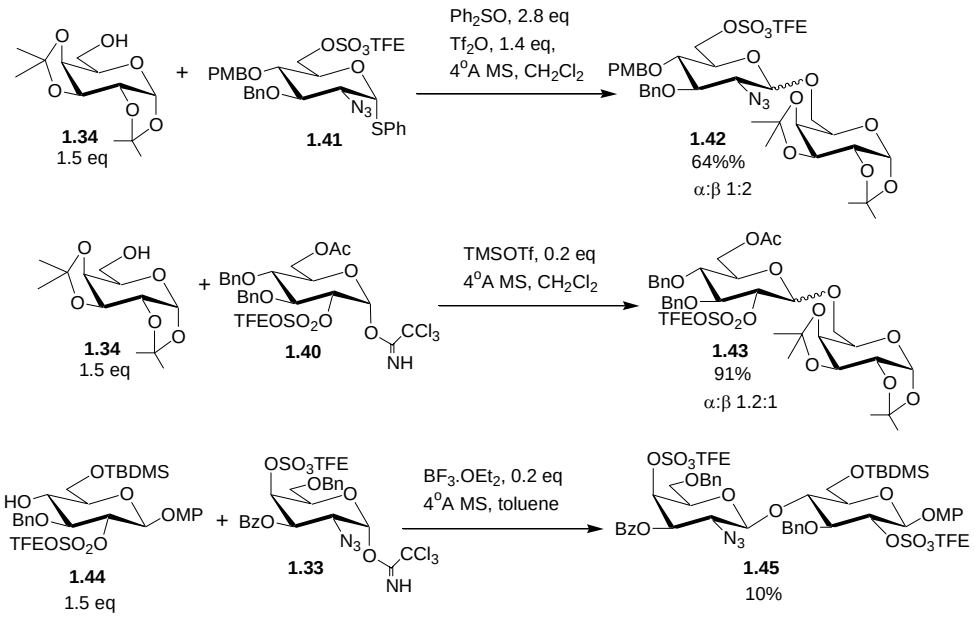
Scheme 1.8. Glycosylation with TFE-sulfated donors

In 2004, a second paper was published by the Linhardt group where more sulfo-protected monosaccharide donors (Scheme 1.9) and acceptors were prepared and investigated in glycosylation reactions (Schemes 1.10).[34]



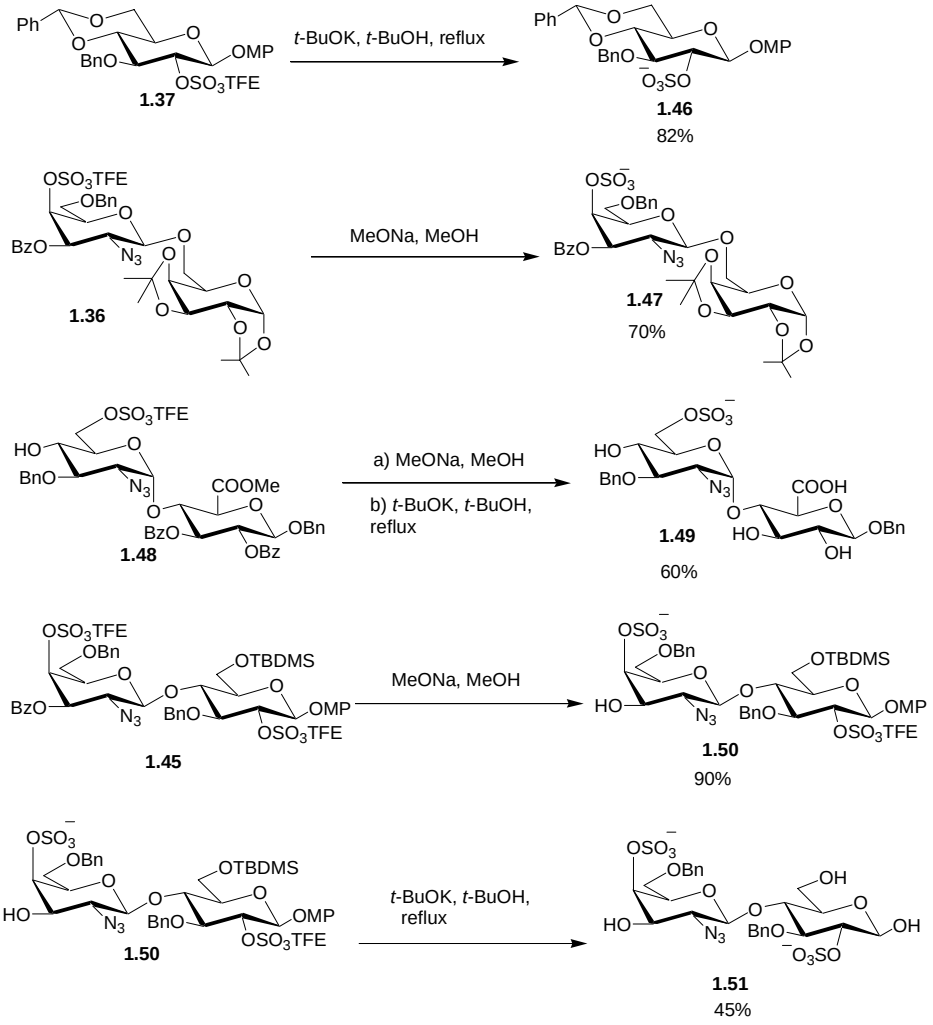
Scheme 1.9. Synthesis of glycosyl imidate donors

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. Reaction of imidate donor **1.40** with galactose acceptor **1.34** in DCM in presence of TMSOTf as promoter afforded the disaccharide **1.43** in 91% yield as a mixture of α and β in 1.2:1 ratio indicating that there is probably no anchimeric assistance by the TFE-protected sulfate during the glycosidation reaction (Scheme 1.10). Less reactive acceptors such as **1.44** gave the disaccharide product **1.45** in low yield.



Scheme 1.10. Synthesis of the disaccharides

Linhardt *et al.*[34] used the conditions of Proud et al (*t*-BuOK in refluxing *t*-BuOH)[28] for TFE-sulfate deprotection.[28] Although the TFE-group was deprotected from the monosaccharide **1.37** in 82% yield, disaccharide **1.36** decomposed under the same conditions. The authors found that NaOMe/MeOH could sometimes remove the TFE-group, such as in the synthesis of disaccharide **1.47** which was isolated in a 70 % yield (Scheme 1.11). This is in contrast to the report by Proud *et al*[28] that TFE-protected sulfates are stable to NaOMe/MeOH.



Scheme 1.11. Deprotection of TFE-protected sulfates

In some cases it was found that the TFE protecting group could be removed under the standard conditions (*t*-BuOK/*t*-BuOH) only after the complete removal of the ester groups with NaOMe/MeOH such as with **1.48** (Scheme 1.11). They applied stepwise deprotection for the 2,4-disulfate **1.45**, where the 4-*O*-sulfate was removed with NaOMe/MeOH to give the mono protected sulphate **1.50**, which was then subjected to *t*-BuOK/*t*-BuOH to deprotect the 2-*O*-position sulfate, providing the disaccharides **1.51** in only a 45% overall yield, with loss of the 6-OTBDMS and anomeric OMP protecting groups (Scheme 1.11).[34]

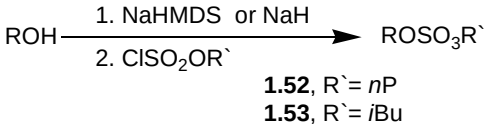
As we see from the above studies, the TFE protecting group has been used for the synthesis of variety of protected carbohydrate sulfate diesters, and has proved to be stable to a number of conditions commonly used in carbohydrates synthesis. On the other hand, this protecting group has some strong limitations. Introducing the TFE group involves the use of trifluorodiazoethane, a reagent that must be prepared fresh and is highly toxic and potentially explosive, and often the yields are inconsistent. Moreover, the harsh conditions that are usually employed for its removal, KO*t*-Bu in refluxing *t*-BuOH, can result in substrate decomposition and consequently low or moderate sulfate deprotection yields. Alternative protecting groups are required to overcome these limitations.

1.3.3. Neopentyl and Isobutyl Protection for Sulfate Monoesters

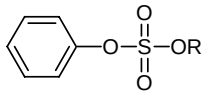
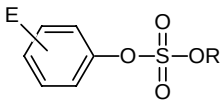
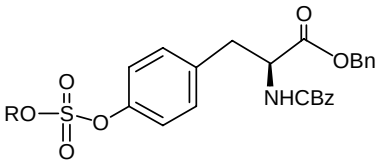
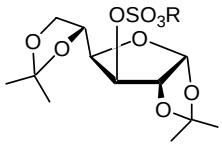
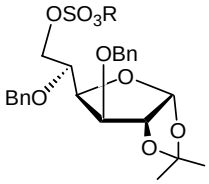
Widlanski *et al*.[35] described the synthesis of sulfate esters employing the neopentyl and isobutyl protecting groups. The selection of these two protecting groups was based on the fact that the neopentyl (nP) and isobutyl (*i*-Bu) groups are known to serve as good protecting groups for sulfonates.[35] The authors reported the synthesis of various neopentyl- and isobutyl-protected sulfate monoesters by treating alcohols and phenols with a slight excess of sodium bis (trimethylsilyl)amide (NaHMDS) in THF (20% DMPU present for nP, no DMPU for *i*-Bu) at –75 ^{o}C (for nP) or -15 ^{o}C (for *i*-Bu), followed by the addition of neopentyl chlorosulfate (1.1 eq.) or isobutyl chlorosulfate (5-10 eq.) then warming to rt and stirring for several hours. Under these conditions, a variety of sulfo-protected compounds including protected estrone sulfate (**1.56** and **1.57**) protected tyrosine sulfate **1.58**, and sulfocarbohydrates **1.59-1.61** were readily prepared in excellent yields (Table 1.1). The neopentyl esters were stable at room temperature for

several months while the isobutyl esters degraded at room temperature and had to be stored at – 20 ^{o}C.

Table 1.1. Preparation of neopentyl and isobutyl sulfate diesters.



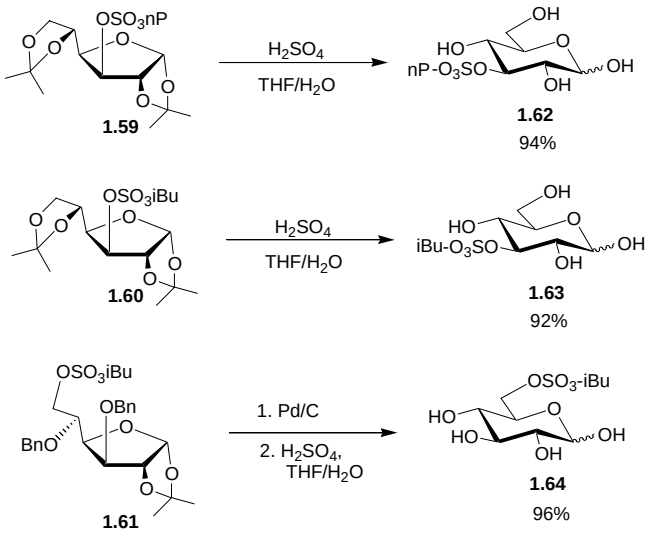
1.52, R`= *n*P
1.53, R`= *i*Bu

Entry	Product	Protecting group*	Yield (%)
1		**1.54** R= *n*P	95
		1.55 R= *iBu*	80
2		**1.56** R= *n*P	98
		1.57 R= *iBu*	82
3		**1.58** R= *n*P	99
4		**1.59** R= *n*P	95
		1.60 R= *iBu*	95
5		**1.61** R= *iBu*	86

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

Simpson and Widlanski[35] examined the stability of the neopentyl and isobutyl protected sulfate esters to acid and base by subjecting protected phenyl sulfates **1.54** and **1.55** to different concentrations of TFA and piperidine using NMR assays. The studies showed that both the isobutyl and neopentyl sulfate esters were stable to 50% TFA, with

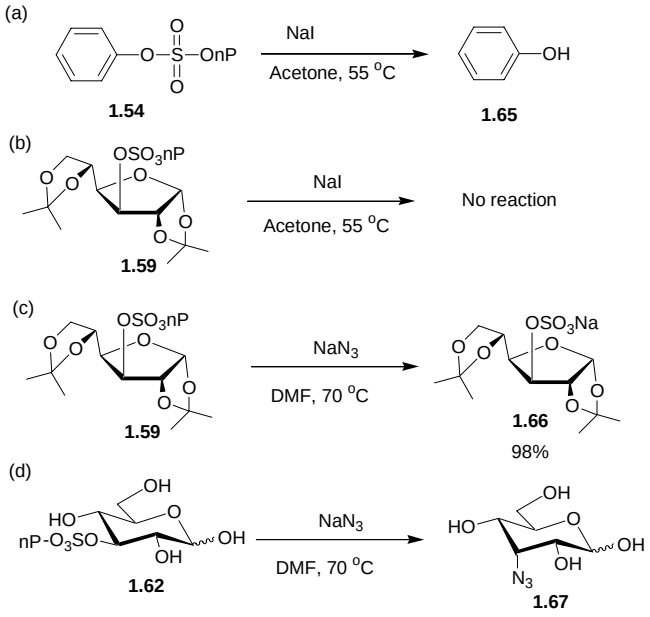
less than 10% degradation observed after 48 h. In addition, the neopentyl sulfate ester was stable to 20% piperidine in chloroform. On the other hand, the isobutyl sulfate ester was less tolerant, showing evidence of nucleophilic cleavage, even at 6% piperidine solution. From the stability studies, the authors concluded that both the neopentyl and isobutyl groups offer viable protection under highly acidic conditions, while the isobutyl protecting group was much less effective under basic or nucleophilic conditions. The stability of the alkyl sulfates was not investigated with TFA or piperidine; however, carbohydrate sulfates **1.59**, **1.60**, and **1.61** were subjected to H_2SO_4 in THF/H_2O to demonstrate their stability to acidic conditions (Scheme 1.12).



Scheme 1.12. Stability of alkyl protected carbohydrate sulfates

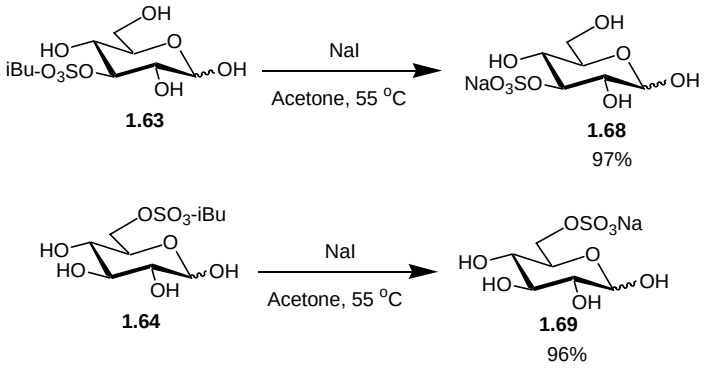
The authors studied the conditions required to cleave the neopentyl moiety by subjecting the protected sulfate esters to nucleophiles in polar solvents. Treating neopentyl sulfate **1.54** with sodium iodide in acetone yielded the desulfated phenol rather than cleavage of the neopentyl group (Scheme 1.13a), and no reaction was observed with the neopentyl protected glucose sulfate **1.59** (Scheme 1.13b). On the other hand, small nucleophiles such as azide and cyanide in warm DMF (60-70 ^{o}C) were reported to be

effective for removing the neopentyl protecting group in protected aryl sulfates **1.54**, **1.56**, and **1.58**, as well as the protected sulfate of diacetone-D-glucose **1.59** (Scheme 1.13c) in near quantitative yields. Treating the unprotected glucopyranose neopentyl diester **1.62** with sodium azide in DMF, however, led to the displacement of the entire protected sulfate group providing 3-azido-3-deoxy D-glucose **1.67** (Scheme 1.13d).



Scheme 1.13. Deprotection of neopentyl protected sulfate groups

The inconsistencies found during the deprotection of the neopentyl group prompted the authors to suggest that the neopentyl group is not useful for the protection for sulfates of primary or secondary alcohols of carbohydrates. On the other hand, as 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. Subjecting the isobutyl protected sulfate esters of unprotected hexoses **1.63** and **1.64** to sodium iodide in acetone at 55 °C provide the target sulfates **1.68** and **1.69** in excellent yields (Scheme 1.14).



Scheme 1.14. Deprotection of isobutyl protected sulfate groups

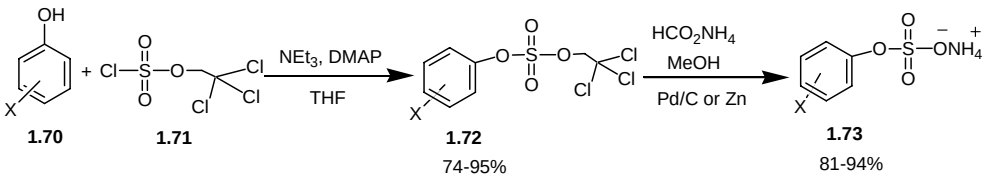
On the basis of the above studies, Simpson and Widlanski suggest that the neopentyl group is more suited as a protecting group for aryl sulfates. Since the *i*-Bu group is more readily removed than the nP group they suggested that it could be useful for the protection of alkyl sulfates though its lack of robustness would limit its use in complex syntheses. There are other issues with the *i*-Bu group. Its introduction required 5-10 equivalents of isobutyl chlorosulfate and the isobutyl esters degraded at room temperature and had to be stored at – 20 °C. Moreover, neither isobutyl chlorosulfate nor neopentyl chlorosulfate are particularly stable: they slowly decompose over several weeks to months even when stored under Ar at – 20 °C.[36] Moreover, no studies have been performed to test the isobutyl esters stability to many of the conditions encountered during carbohydrate syntheses nor has its introduction into carbohydrates been examined in the presence of other protecting groups other than ether and acetal protecting groups. The authors also suggested that isobutyl chlorosulfate would be effective for introducing sulfate monoesters at the last step in a synthesis and would be superiour to using sulfur trioxide complexes. This would probably not be the case for carbohydrates as it is introduced using a very strong base (NaHMDS) at -15 °C to rt, conditions where

protecting groups such as acyl groups (the most commonly used protecting groups in carbohydrate chemistry) could isomerize and other side reactions could occur. Thus, although this group might be useful in certain cases, it does not provide a significant alternative to the conventional sulfation methods. No further reports on the use of this group for the synthesis of sulfated carbohydrates has appeared since Widlanski and Simpson's initial report.

1.3.4 Trichloroethyl Protection for Sulfate Monoesters

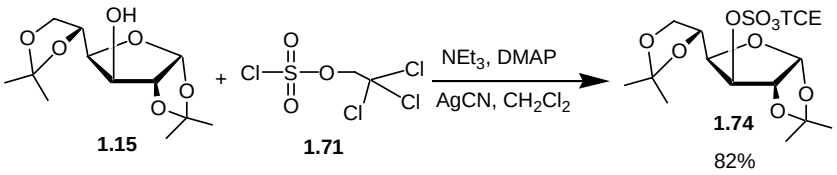
In 1997, Proud *et al*.[28] attempted to use the trichloroethyl (TCE) group as a sulfate protecting group for sulfated carbohydrates. The authors claimed they were unable to incorporate the TCE-protected sulfates into carbohydrates using 2,2,2-trichloroethyl chlorosulfate ($TCEOSO_2Cl$) in good yield so they switched to the TFE group as mentioned earlier. The TCE group has been used extensively for the protection of carboxylic acids. It is typically removed using Zn/AcOH. However, in order for it to be practical for sulfate protection new methodology would have to be developed for both its introduction (without using toxic and explosive diazo derivatives) and its removal as sulfate monoesters are acid labile.

In 2004 and 2006, the Taylor group came up with solutions to the above problems.[37,38] The authors demonstrated that trichloroethyl chlorosulfate (**1.71**) can successfully be used to prepare protected aryl sulfates **1.72** in high yields (Scheme 1.15).



Scheme 1.15. Preparation of aryl sulfates

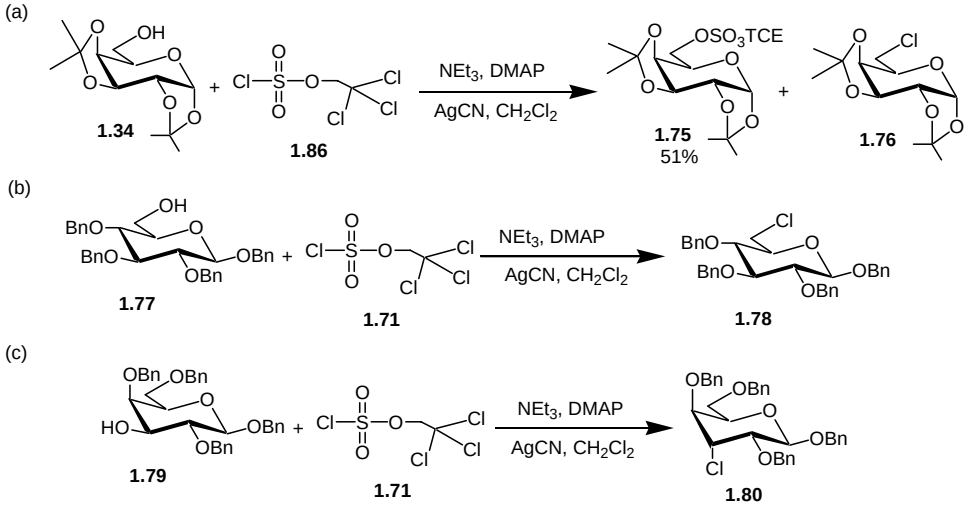
Most significantly, they demonstrated that the TCE group could be easily removed in excellent yields under mild conditions using Pd/C and ammonium formate, or using Zn and ammonium formate in methanol.[37] The success achieved in introducing and removing TCE-group into aryl sulfates encouraged the Taylor group to investigate the use of the TCE group for protecting alkyl sulfates, specifically carbohydrate sulfates. The authors reported that reagent **1.71** can be reacted with diacetone glucose **1.15** to give the sulfated product **1.74** in 82% yield (Scheme 1.16). 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.[28]



Scheme 1.16. TCE-protected sulfate moiety

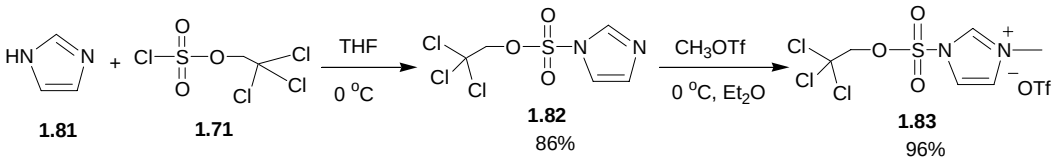
When the authors attempted to extend their methodology to other carbohydrates containing a primary hydroxyl group, some problems appeared. For example treating 1,2:3,4-di-*O*-isopropylidene galactose **1.34** with the TCE chlorosulfate **1.71** gave carbohydrate **1.75** in only a 51% yield (Scheme 1.17a). Analysis of the reaction products revealed that displacement of the TCE sulfate moiety by the liberated chloride ion to give chlorosugar **1.76** was a competing reaction. The chlorosugar by-products were also observed when the authors applied their methodology to benzyl-protected carbohydrates **1.77** and **1.79** (Scheme 1.17b,c) where the reactions proceeded very slowly, and the chlorosugars **1.78** and **1.80** were formed in varying amounts. Nevertheless, these results suggest that the desired compounds could be formed in good yield if a highly reactive

sulfating agent could be developed that did not liberate a nucleophilic species such as a chloride ion.



Scheme 1.17. Formation of chlorosugar by-products.

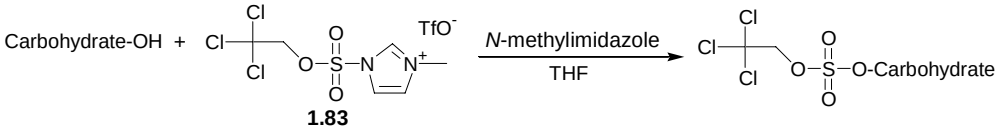
In 2006, Ingram and Taylor reported a new type of sulfating agent capable of introducing TCE-protected sulfates into monosaccharide building blocks. [38] The authors prepared the sulfuryl imidazolium triflate salt **1.83**, according to Scheme 1.18. Reagent **1.71** was added to an excess of imidazole in THF at 0 ^{o}C to afford TCE sulfuryl imidazole **1.82** in 86% yield. Treatment of TCE sulfuryl imidazole **1.82** with methyl triflate (1.0 eq.) in dry diethyl ether provided the imidazolium triflate salt **1.83** as a white precipitate which was obtained by simple filtration in 96% yield. No further purification was required. This was the first report describing the synthesis of a sulfuryl imidazolium salt. Compound **1.83** is a stable compound and can be stored indefinitely at 4 ^{o}C or – 20 ^{o}C. It has been stored at room temperature for months without any detectable decomposition.

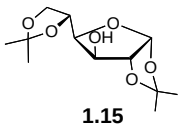
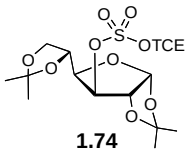
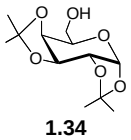
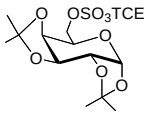
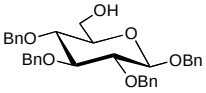
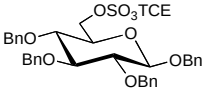
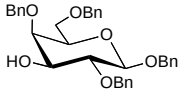
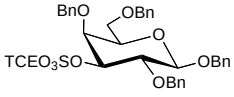
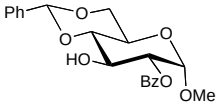
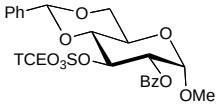
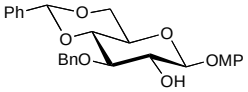
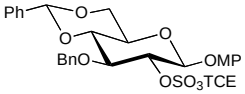
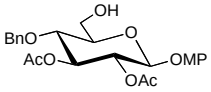
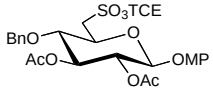
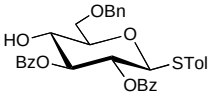
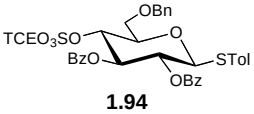
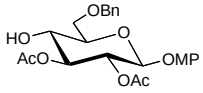
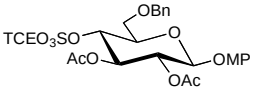


Scheme 1.18. Synthesis of TCE-sulfuryl imidazolium triflate reagent

Taylor and Ingram treated a variety of monosaccharide building blocks with reagent **1.83** (2.0–10.5 equiv) in the presence of *N*-methylimidazole (NMI) (2.5–11.6 equiv) in THF at room temperature for 16-48 h. The corresponding TCE-protected sulfocarbohydrates were isolated in yields ranging from 81 to 94% (Table 1.2). The authors found that, the presence of NMI was essential for all the reactions. Other bases (NEt_3, Hunig's base, pyridine, 2,6-lutidine, and piperidine) were considerably less effective.[38]

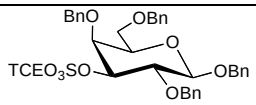
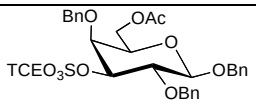
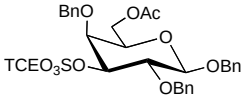
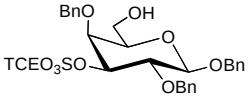
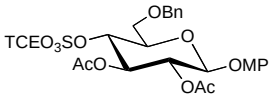
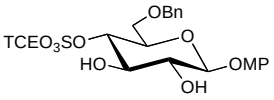
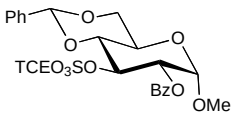
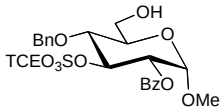
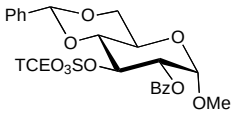
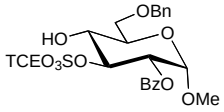
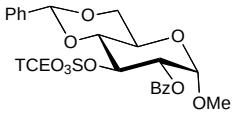
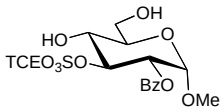
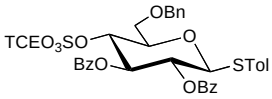
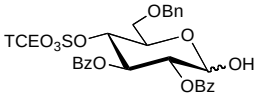
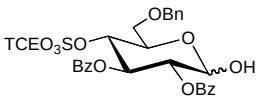
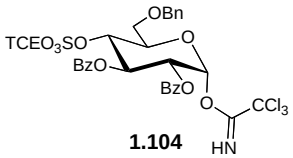
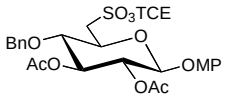
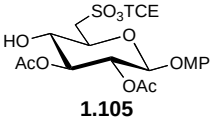
Table 1.2. Preparation of TCE-protected carbohydrate sulfates.



Entry	Monosaccharide	Sulfated Product	Yield (%)
1	 **1.15**	 **1.74**	90
2	 **1.34**	 **1.75**	87
3	 **1.77**	 **1.89**	75
4	 **1.79**	 **1.90**	94
5	 **1.84**	 **1.91**	94
6	 **1.85**	 **1.92**	90
7	 **1.86**	 **1.93**	81
8	 **1.87**	 **1.94**	91
9	 **1.88**	 **1.95**	76

Further manipulations of the sulfated carbohydrates indicated that the TCE-protected sulfates are stable to many of the conditions commonly encountered in carbohydrate chemistry (Table 1.3) such as selective 6-*O*-debenzylation and acetylation

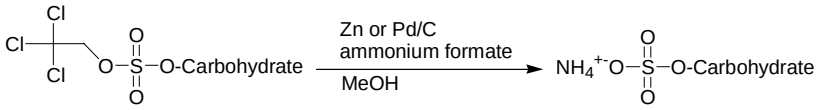
Table 1.3. Manipulations of TCE-protected carbohydrate sulfates[38]

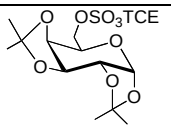
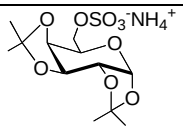
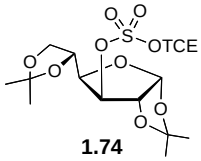
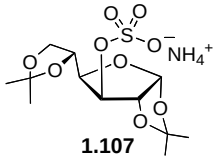
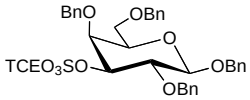
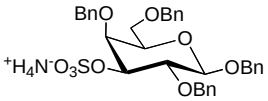
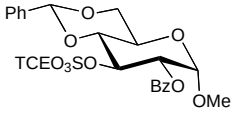
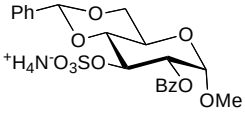
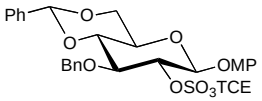
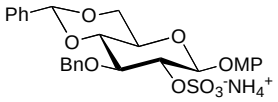
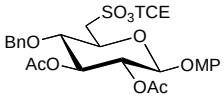
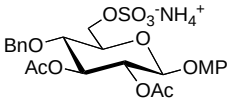
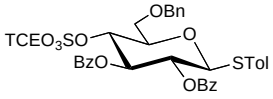
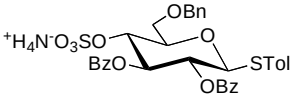
Entry	Substrate	Conditions	Product	Yield (%)
1	 **1.90**	$ZnCl_2$ (3.7 eq.), $AcOH/Ac_2O$, 3 h, rt	 **1.98**	95
2	 **1.96**	NaOMe (0.16 eq.) MeOH, 3 h, rt	 **1.99**	85
3	 **1.95**	CH_3COCl (3M in MeOH) 3 h, rt	 **1.100**	84
4	 **1.91**	$PhBCl_2$ (3.4 eq.), Et_3SiH (3.0 eq.), 4A MS, -78 °C, CH_2Cl_2, 1 h.	 **1.101**	96
5	 **1.91**	TfOH (3.4 eq.), Et_3SiH (3.0 eq), 4A MS, -78 °C, CH_2Cl_2, 1 h.	 **1.102**	87
6	 **1.91**	TsOH (0.1 eq.), CH_2Cl_2/MeOH, 45 °C, 16 h.	 **1.103**	94
7	 **1.94**	NBS (3.0 eq.), acetone, H_2O, 0 °C, 20 min	 **1.97**	74
8	 **1.97**	DBU (0.2 eq.), Cl_3CCN (16 eq.), -40 to -10 °C, CH_2Cl_2, 3 h.	 **1.104**	80
9	 **1.93**	NBS (3.5 eq.), $CaCO_3$ (5 eq.), CCl_4/H_2O, 250-W incandescent lamp	 **1.105**	91

with $ZnCl_2$/AcOH/Ac_2O, deacetylation with NaOMe in MeOH, benzylidene ring opening with either TfOH or $PhBCl_2$ in the presence of Et_3SiH, cleavage of the benzylidene group with TsOH, deprotection of the anomeric thiotolyl using *N*-bromosuccinimide (NBS) in acetone/water, formation of trichloroacetimidate with a free anomeric OH group using trichloroacetonitrile in the presence of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Although the ester group in **1.96** was deacetylated in 85% yield using catalytic NaOMe in MeOH, attempts to deacetylate **1.95**, which bears secondary acetate groups, under the same Zemplén conditions resulted in both deacetylation and loss of the TCE-sulfate. To overcome this issue, carbohydrate **1.95** was stirred in an acidic methanol to afford the corresponding deacetylated compound **1.100** in good yields (Table 1.3). On the other hand, attempts to remove the benzyl group in **1.93** selectively with H_2 and Pd catalyst, without affecting the TCE-protected sulfate group, were unsuccessful. However, the benzyl group in **1.93** was selectively cleaved to give **1.105** in 91% yield by irradiation with a 250-W lamp in the presence of NBS and $CaCO_3$.[39]

The TCE group was removed in very good yields by employing zinc–ammonium formate in methanol (Table 1.4). The 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. The authors also performed deprotection studies with Pd/C–ammonium formate; however, the yields were slightly lower than those with Zn.[38]

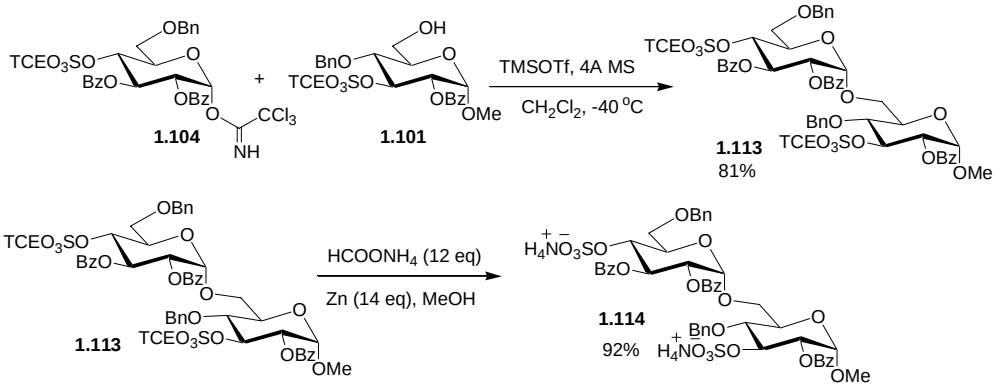
Table 1.4. Removal of the TCE protecting group with zinc– ammonium formate



Entry	Sulfated Monosaccharide	Deprotected Product	Yield (%)
1	 **1.75**	 **1.106**	99
2	 **1.74**	 **1.107**	94
3	 **1.90**	 **1.108**	97
4	 **1.91**	 **1.109**	96
5	 **1.92**	 **1.110**	96
6	 **1.93**	 **1.111**	91
7	 **1.94**	 **1.112**	94

As mentioned in the beginning of this chapter, Karst *et al*. removed the TFE moiety from sulfate groups in fully protected disaccharides in low to moderate yields.[34] Taylor *et al*. anticipated that this would not be an issue with the TCE group as it is removed under very mild conditions.[38] To illustrate this, the authors synthesised the

disaccharide **1.113** by coupling the trichloroacetimidate donor **1.104** with glycosyl acceptor **1.101** in the presence of TMSOTf to give disaccharide **1.113** in 81 % yield (Scheme 1.19). Subjecting the disaccharide **1.113** to zinc and ammonium formate in methanol gave the sulfodeprotected disaccharide **1.114** in 92 % isolated yield (Scheme 1.19).



Scheme 1.19. Synthesis of the disulfated disaccharide **1.113**

From the results obtained by Taylor *et al*.[38] it is clear that, reagent **1.83** represents a new class of highly potent sulfating agents and is the first new type of sulfating agent to appear in almost 50 years.

1.4 Summary and Thesis Outline

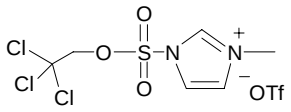
It is now well established that many distinct sulfated oligosaccharides have important biological roles. Moreover, of the few carbohydrate-based drugs that have been developed several are sulfated oligosaccharides and their activity depends mainly on the presence of the sulfate groups at specific locations within the oligosaccharides. There are no simple and efficient routes to obtain pure, well-defined fragments from naturally occurring sulfated oligosaccharides. Hence chemical synthesis of complex sulfated

carbohydrates is extremely important for studying and understanding their biological roles. This thesis focuses on my research contributions to the synthesis of sulfated carbohydrates. The ultimate goal is to devise a more efficient and reliable method by which such compounds can be prepared. *Chapter 2* describes the development of a second generation sulfuryl imidazolium salt and its application to the synthesis of TCE-protected sulfated monosaccharides. This new reagent allows for the incorporation of a protected sulfodiester into monosaccharides in higher yields than our previous first generation reagent. *Chapter 3* describes our investigations into the use of TCE sulfuryl imidazolium salts as reagents for the regioselective incorporation of trichloroethyl-protected sulfate esters into monosaccharides. We also examine the use of TCE sulfuryl imidazolium salts for the synthesis of multiply-sulfated monosaccharides. Chapter 4 covers the synthesis of sulfuryl imidazolium salts bearing the phenyl and TFE groups and the use of these salts in the synthesis of both phenyl- and TFE-protected sulfates. This chapter also includes a search for finding milder conditions for the removal of the TFE group. Finally, in chapter 5, we applied the methodology that we developed in chapters 2 and 3 to the synthesis of a complex disulfated tetrasaccharide that corresponds to the carbohydrate portion of SB_{1a}, an important cancer-associated carbohydrate antigen.

Chapter 2 The Second Generation of 2,2,2- Trichloroethoxy-Sulfuryl Imidazolium Salts.

2.1 Introduction and Objectives

As mentioned in Chapter 1, in 2004, Taylor *et al.* described the use of the 2,2,2-trichloroethyl (TCE) group as the first protecting group developed for aryl and alkyl sulfates.[37,38] The TCE-protected sulfate esters were prepared in a single step by the reaction of phenols or carbohydrates with 2,2,2-trichloroethyl chlorosulfate **1.71** or the corresponding imidazolium salt **1.83** (Figure 2.1) in the presence of triethylamine or *N*-methylimidazole. The resulting protected sulfates were stable to a variety of conditions, but were readily deprotected in excellent yields under neutral conditions with Pd/C–ammonium formate or zinc–ammonium formate.



1.83

Figure 2.1. Imidazolium salt **1.83.**

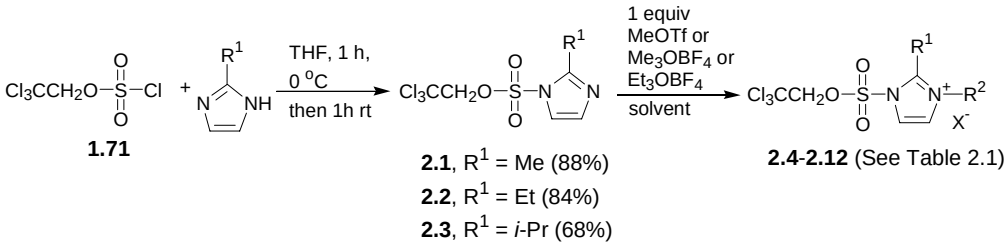
Although imidazolium salt **1.83** works well for most sulfations it does have some limitations. The sulfations must be carried out in THF as the triflate salt is poorly soluble in less polar solvents such as CH_2Cl_2 and chloroform. In THF its stability is limited and it is assumed that for the slower reactions, reagent **1.83** begins to break down in THF before the sulfation occurs, thus the addition of aliquots over extended periods of time is required for high yields. As a result, reactions involving poorly nucleophilic substrates require excess of reagent **1.83** and longer reactions time.[38] The objective of this work is to design sulfurylimidazolium sats (SIS's) with stability, solubility and sulfating properties that are superior to reagent **1.83**.

2.2 Results and Discussion

2.2.1 *Synthesis of Derivatives of 2,2,2-Trichloroethoxysulfuryl Imidazolium Triflate* ` **(1.83)**

This work was done in conjunction with Laura J. Ingram and Ahmed M. Ali former graduate students in the Taylor group. We anticipated that the solubility, stability and sulfating properties of SIS **1.83** could be improved by introducing alkyl groups on the imidazolium ring. Towards this end we prepared a series of SIS's all of which contained the TCE group yet had different alkyl groups at the 2- and 3-positions of the imidazolium ring.[40] In addition, the tetrafluoroborate counterion was also examined. The synthesis of the TCE-sulfurylimidazole derivatives was readily achieved by reacting reagent **1.71** with the appropriate imidazole derivative to give compounds **2.1**-**2.3**. Reaction of **2.1**-**2.3** with methyltriflate or trimethyl- or triethyloxonium tetrafluoroborate gave the SIS's (Table 2.1). When the product precipitated out of the reaction mixture pure SIS's were obtained in good yield simply by filtration. It is likely that the precipitation of the product is required to drive the formation of the sulfuryl imidazolium salts. Compounds **2.9**, **2.11**, and **2.12** 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, SIS's having the triflate counterion were obtained in higher yields than those having a tetrafluoroborate counterion (Table 2.1).

Table 2.1. Preparation of TCE Sulfuryl Imidazolium Salts.

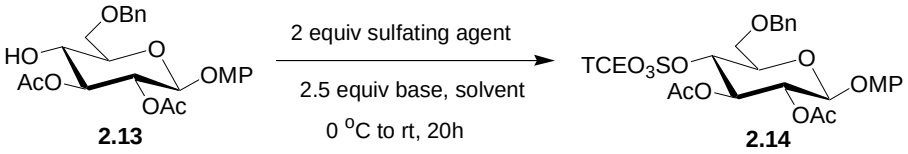


Product	R	R'	X^-	Yield (%)
2.4	Me	Me	TfO^-	99
2.5	Et	Me	TfO^-	98
2.6	iPr	Me	TfO^-	85
2.7	Me	Me	BF_4^-	85
2.8	Et	Me	BF_4^-	79
2.9	iPr	Me	BF_4^-	ND
2.10	Me	Et	BF_4^-	81
2.11	Et	Et	BF_4^-	ND
2.12	iPr	Et	BF_4^-	ND

We were unable to obtain the BF_4^- salt **2.9** which did not precipitate during the reaction while the corresponding TfO^- salt **2.6** was readily isolated in 85% yield (Table 2.1). Several of us encountered strong allergic reactions when we attempted to prepare and isolate the TfO^- analogues of compounds **2.10-2.12** and so these triflate salts were not pursued any further. All of the SIS's that were obtained in pure form were white powders and could be stored at 4 ^oC for years without any detectable decomposition. At the same time, SIS's were stable in a solution of $CDCl_3$ for several days as no detectable decomposition was observed by ^1H NMR analysis. As we expected, all of these SIS's exhibited good solubility in less polar solvents such as methylene chloride and chloroform which supports our assumptions.

Carbohydrate **2.13** was selected as a model substrate to test the sulfating ability of the new SIS's. Previously we found that sulfation of the 4-OH in carbohydrate **2.13** in THF requires 8.0 equiv of the original imidazolium salt **1.83**, and 8.5 equiv 1-methylimidazole (1-MeIm) added over a 72 hour period to obtain a 76% yield of carbohydrate **2.14**. The yield (56%) was even poorer in CH_2Cl_2 possibly due to the very limited solubility of **1.83** in this solvent. To evaluate the new SIS's compound **2.13** was treated with 2 equiv SIS in the presence of 2.5 equiv base in various solvents for 20 h after which the reaction was stopped and the yield of **2.14** was determined after purification (Table 2.2). Higher yields of **2.14** were obtained in CH_2Cl_2 as a solvent using SIS's **2.4**-**2.6** compared to THF (for example, table 2.2 entry 4 vs. 5). Due to their limited solubility in THF, we were unable to evaluate the sulfating ability of SIS's **2.7**-**2.10** in THF as a solvent. Good yields were obtained with SIS's **2.4** and **2.7**, which bear a 1,2-dimethylimidazolium group, using 1,2-dimethylimidazole (1,2-dMeIm) as the base (entries 5 and 16). The results showed that there is no significant effect of the counter ion (TfO^- or BF_4^-) on the yield. Low yields were obtained when the SIS contained an ethyl or isopropyl moiety at the 2-position of the imidazole ring (SIS's **2.5**, **2.6** and **2.10**) if these reactions were performed in which the base was the same as the leaving group of the respective SIS's (1-methyl-2-isopropylimidazole (1-Me-2-*i*PrIm), 1-methyl-2-ethylimidazole (1-Me-2-EtIm) (entries 8, 10, 12, 14, and 17). However, the yields were improved when 1,2-dMeIm was used as base (Table 2.2, entries 9, 11, 13, 15 and 18)

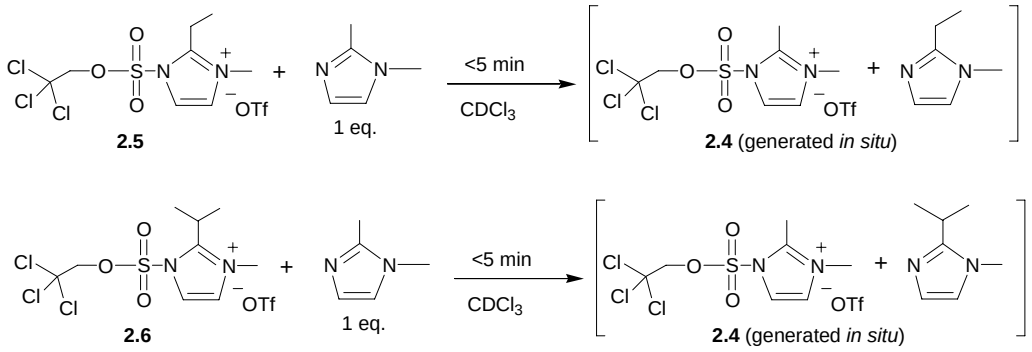
Table 2.2. Sulfating abilities of modified sulfuryl imidazolium salts **1.83**, **2.4**-**2.8**, **2.10.**



Entry	Reagent	Base	Solvent	Yield (%)
1	**1.83**	1-MeIm	THF	70
2	**1.83**	1-MeIm	CH_2Cl_2	56
3	**1.83**	1-MeIm	DMF	35
4	**2.4**	1,2-DiMeIm	THF	65
5	**2.4**	1,2-DiMeIm	CH_2Cl_2	80
6	**2.4**	1-Me-2-iPrIM	CH_2Cl_2	53
7	**2.4**	1,2-DiMeIm	DMF	40
8	**2.5**	1-Me-2-EtIm	THF	18
9	**2.5**	1,2-DiMeIm	THF	58
10	**2.5**	1-Me-2-EtIm	CH_2Cl_2	21
11	**2.5**	1,2-DiMeIm	CH_2Cl_2	68
12	**2.6**	1-Me-2-iPrIm	THF	18
13	**2.6**	1,2-DiMeIm	THF	54
14	**2.6**	1-Me-2-iPrIm	CH_2Cl_2	30
15	**2.6**	1,2-DiMeIm	CH_2Cl_2	60
16	**2.7**	1,2-DiMeIm	CH_2Cl_2	79
17	**2.8**	1-Me-2-Et	CH_2Cl_2	38
18	**2.8**	1,2-DiMeIm	CH_2Cl_2	78
19	**2.10**	1-Et-2-MeIm	CH_2Cl_2	45
20	**2.10**	1,2-DiMeIm	CH_2Cl_2	75

^{1}H NMR studies by Laura Ingram- a former PhD candidate in Taylor group- in $CDCl_3$ revealed that just one equiv of 1,2-dMeIm rapidly displaced the 1-Me-2-EtIm, or 1-Me-2-*i*PrIm from SIS's **2.5** and **2.6** within minutes thus forming in situ SIS **2.4** which is a better sulfating agent (Scheme 2.1). Even after several hours, ^{1}H NMR provided no evidence that the reverse reaction to the original compound was occurring, which indicates that

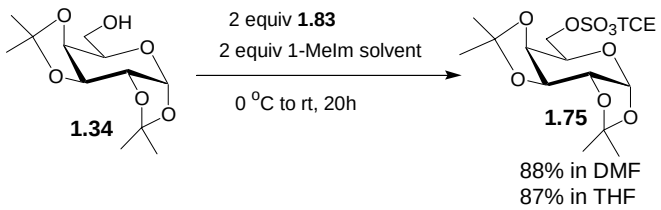
reagent **2.4** is more stable than **2.5** and **2.6**. This is possibly due to steric hindrance between the ethyl or isopropyl group at the 2-position of the imidazole ring, and the sulfamate moiety and methyl group.



Scheme 2.1. In situ imidazolium exchange in SIS's **2.5** and **2.6**.

We also found that a reduced yield of **2.14** was obtained using SIS **2.4** and 1-Me-2-*i*PrIm in CH_2Cl_2 as the base (entry 6) revealing that the low yields encountered with SIS's **2.5**, **2.6** and **2.10** with 1-Me-2-*i*PrIm or 1-Me-2-EtIm may in part be due to the added imidazole derivative itself which may be 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 SIS's **2.5**, **2.6** and **2.10** and 1-Me-2- *i*PrIm or 1-Me-2-EtIm than with SIS's **2.4** or **2.7** and 1,2-dMeIm. SIS **2.10**, which differs from SIS **2.7** only by the presence of an ethyl rather than methyl group on one of the nitrogens gave lower yields than SIS **2.7** (entry 16) when 1-ethyl-2-methyl imidazole (1-Et-2-MeIm) was used as base but almost the same yield when 1,2-dMeIm was used as base (entries 17 and 18) again suggesting that the 1-Et-2-MeIm group in SIS **2.10** was exchanging with 1,2-dMeIm. DMF was also examined as a solvent for SIS's **1.83** and **2.4** but low yields were obtained (entries 3 and 7). However, we found that DMF can be used as a solvent for less challenging sulfations. For example,

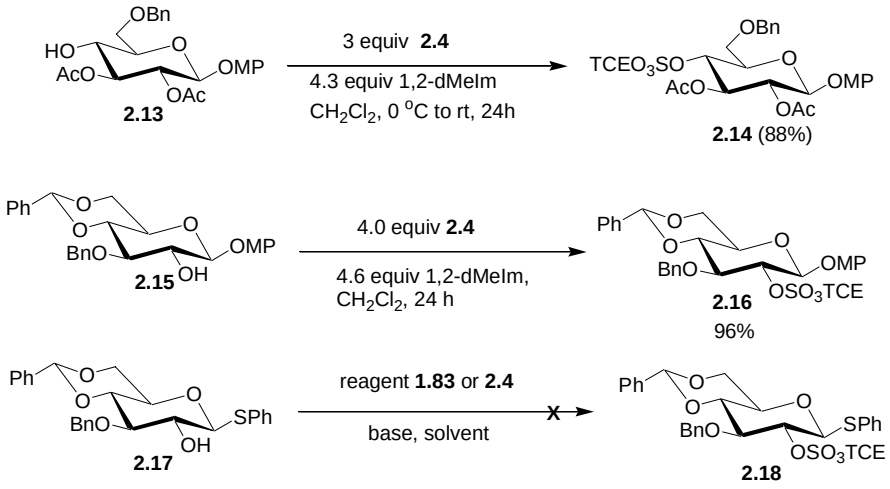
1:2,3:4-di-O-isopropylidene galactose **1.34** is sulfated with 2 equiv reagent **1.83** and 2 equiv 1-MeIm in very good yields in either THF or DMF (Scheme 2.2).



Scheme 2.2. Sulfation of carbohydrate **1.34** in DMF and THF

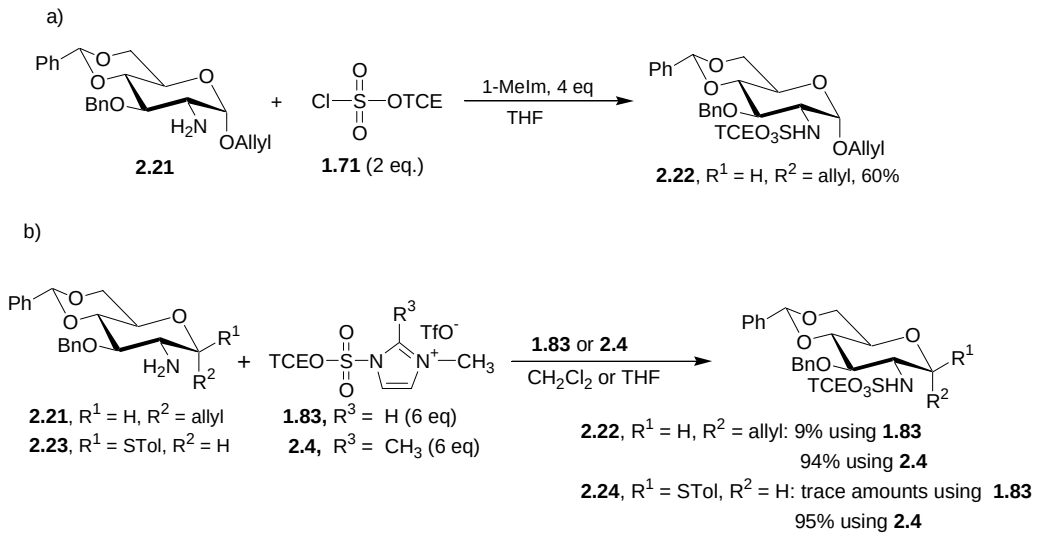
In general, SIS **2.4** can be readily prepared in very high yield and the best yields in Table 2.2 were obtained with this compound. We decided to use this reagent for subsequent studies. Further studies with SIS **2.4** and carbohydrate **2.13** revealed that an 88% yield of **2.14** could be obtained in 24 h using just 3 equiv reagent **2.4**, 4.3 equiv 1,2-dMeIm in CH_2Cl_2 (Scheme 2.3). Carbohydrate **2.16** was previously prepared in a 90% yield after subjecting compound **2.15** to 10.5 equiv SIS **1.83** and 11.6 equiv 1-MeIm for 28 h in THF.[38] However, using just 4 equiv SIS **2.4** and 4.6 equiv 1,2-dMeIm in CH_2Cl_2, carbohydrate **2.16** was obtained in a 96% yield after just 24 h. On the other hand, performing the reaction under the same conditions in THF gave a 40% yield of **2.16** with unreacted **2.15** still remaining after 24 h demonstrating that these reactions can be subject to a significant solvent effect. A complex mixture of products were obtained when we attempted to sulfate compound **2.17** which is the STol analogue of **2.15**, with either SIS **1.83** or **2.4** and we were unable to isolate the desired sulfated compound **2.18**. This was unexpected since the sulfation of the 4-OH of *p*-tolyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside was previously reported in high yield using reagent **1.83**.[38] one possible explanation is that, upon sulfation of compound **2.17** intramolecular

displacement of the TCE sulfate group by the sulfur atom occurs resulting in the formation of a reactive episulfonium ion.



Scheme 2.3. Improved sulfation reactions using reagent **2.4**

Laura Ingram has shown that reagent **2.4** is superiour to our previous sulfating agents **1.71** and **1.83** for performing *N*-sulfations.[40] For example, reaction of **2.21** with reagent **1.71** in the presence of 4 equiv 1,2-dMeIm gave product **2.24** in only a 60% yield (Scheme 2.4a). Sulfation of **2.21** using imidazolium salt **1.83** and 1-MeIm was unsuccessful in that a complex mixture of products were formed (as determined by TLC) and only a 9% yield of product **2.22** was isolated (Scheme 2.4b). The same was found for substrate **2.23** in that only a trace amount of product was obtained using reagent **1.83**. However, sulfations using reagent **2.4** gave the *N*-sulfated products in outstanding yields (Scheme 2.4b)



Scheme 2.4. Sulfation of glucose amine derivatives with reagents **1.71**, **1.83** and **2.4.**[40]

2.3 Summary and Future Work

A series of modified sulfuryl imidazolium salts was prepared and studied to overcome some of the limitations of reagent **1.83**. A more efficient sulfating agent **2.4**, was obtained by incorporating a methyl group at the 2-position of the imidazolium ring of **1.83**. *O*-Sulfations that required prolonged reaction times and a large excess of the original reagent **1.83**, were more readily achieved using reagent **2.4**. It is expected that this next generation of sulfating agent will find widespread use in the preparation of sulfated carbohydrates and other organosulfates. The next step in this work is the application of our sulfate protecting group strategy to the total synthesis of a multisulfated oligosaccharide and an example of such a synthesis will be given in Chapters 5.

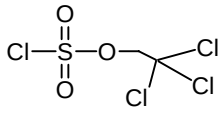
2.4 Experimental

2.4.1 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 ^{1}H 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.

2.4.2 Experimental Syntheses

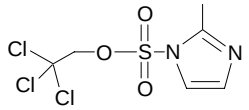


1.71

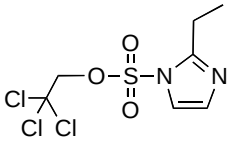
2,2,2-Trichloroethoxysulfuryl chloride (1.71). Procedure modified from the original synthesis.[41] Distilled sulfuryl chloride (20.0 mL, 0.250 mol) was added dropwise via syringe pump over 1 h to a solution of pyridine (20.5 mL, 0.250 mol) and 2,2,2-trichloroethanol (24.0 mL, 0.250 mol) in distilled Et_2O at –78 °C. The resulting slurry was stirred for an additional 1 h at –78 °C, and then stirred for 3 h at room temperature. The precipitate was removed by suction filtration and the filtrate was concentrated to a

crude oil. Purification by vacuum distillation afforded **1.71** 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).

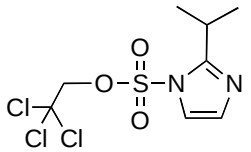
General procedure for the preparation of compounds 2.1-2.3 (Table 2.1). To a solution of appropriately 2-substituted imidazole (0.072 mol, 3.60 eq.) in dry THF (40 mL) at 0 ^{o}C was added dropwise a solution of 2,2,2-trichloroethoxysulfuryl chloride (**1.71**) (5.0 g, 0.02 mol, 1.00 eq.) in THF (50 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 (2.1). To a solution of 2-methyl imidazole (5.9 g, 0.072 mol, 3.60 equiv) in dry THF (40 mL) at 0 ^{o}C was added dropwise a solution of reagent **1.71** (5.0 g, 0.02 mol, 1.00 equiv) 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, the residue was washed with THF and the filtrate was concentrated under vacuum. The crude residue was purified by flash chromatography using (33:67 EtOAc/hexanes) to give **2.1** as a white solid (5.2 g, 88 %); Mp 53-55 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 1H, H_{imi}), 6.94 (s, 1H, H_{imi}), 4.65 (s, 2H, CH_2), 2.67 (s, 3H, $CH_{3\text{-}imi}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.4, 128.2, 120.1, 91.7, 80.0, 14.9; HRMS (EI^+): Calculate for $C_6H_7Cl_3N_2O_3S$ $(M)^+$ 291.9243, found 291.9244.



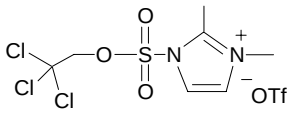
1-(2,2,2-Trichloroethoxysulfuryl) 2-ethyl imidazole (2.2). Prepared according to the general procedure. White solid (5.1 g, 84%). Mp 56-57 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, 1H, J = 1.8 Hz, H_{imi}), 6.95 (d, 1H, J = 1.5 Hz, H_{imi}), 4.61 (s, 2H, CH_2), 2.99 (q, 2H, J = 7.5 Hz, $CH_{2\text{-imi}}$), 1.36 (t, 3H, J = 7.5 Hz, $CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.2, 128.1, 120.0, 91.7, 80.0, 21.7, 11.3; HRMS (EI^+): Calculate for $C_7H_9Cl_3N_2O_3S$ $(M)^+$ 305.9399, found 305.9394.



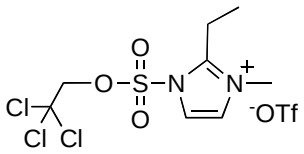
1-(2,2,2-Trichloroethoxysulfuryl) 2-isopropyl imidazole (2.3). Prepared according to the general procedure. White solid (4.4 g, 68%). Mp 67-69 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, 1H, J = 1.5 Hz, H_{imi}), 6.98 (s, 1H, H_{imi}), 4.64 (s, 2H, CH_2), 3.51 (sept, 1H, J = 6.8 Hz, CH_{imi}), 1.36 (d, 6H, J = 6.8 Hz, 2x$CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 128.1, 119.6, 91.7, 80.0, 27.5, 21.9; HRMS (EI^+): Calculate for $C_8H_{11}Cl_3N_2O_3S$ $(M)^+$ 319.9556, found 319.9555.

General procedure for the preparation of sulfuryl imidazolium triflate salts (Table 2.2, compounds 2.4-2.6). To a solution of the appropriate TCE-sulfurylimidazole (15 mmol, 1.0 equiv) in dry Et_2O (70 mL) at 0 ^{o}C was added methyltriflate (1.8 mL, 15 mmol, 1.0 equiv) dropwise. The reaction was stirred for 3 h at 0 ^{o}C during which time a

white precipitate formed. The mixture was filtered. The filter cake was washed with cold ether and the filtrate was cooled to – 20 ^{o}C and filtered. This second precipitate was washed with cold ether and then combined with the first precipitate which afforded sulfuryl imidazolium triflate salt as a white solid.

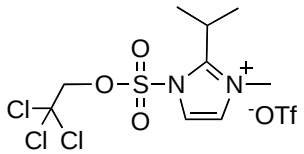


2,2,2-Trichloroethoxysulfuryl-(2-methyl)-*N*-methylimidazolium triflate (2.4). Prepared according to the general procedure**.** A fluffy white solid (6.8 g) in 99 % yield. We have prepared compound **2.4** in batches up to 75 g in excellent yield. We typically store compound **2.4** at 4 or –20 ^{o}C and have never detected any decomposition even after 6 months. We have also stored reagent **2.4** on the benchtop at room temperature for a month and not detected any decomposition. ^{1}H NMR (300 MHz, CD_3OD) δ 8.09 (d, 1H, J = 2.1 Hz, H_{imi}), 7.74 (d, 1H, J = 1.8 Hz, H_{imi}), 5.35 (s, 2H, CH_2), 3.92 (s, 3H, $CH_{3\text{-}imi}$), 2.91 (s, 3H, $CH_{3\text{-}imi}$); ^{13}C NMR (75 MHz, CD_3OD) δ 148.6, 123.52, 120.8, 120.4 (q, J_{CF} = 316.5 Hz, CF_3), 91.6, 82.0, 35.3, 10.5; ^{19}F NMR (282 MHz, CD_3OD) δ -79.8; HRMS (+ESI): Calculate for $(M\text{-}OTf)^+$ $C_7H_{10}Cl_3N_2O_3S^+$ 306.9478, found 306.9469.



1-(2,2,2-trichloroethoxysulfuryl) 2-ethyl 3-methylimidazolium triflate (2.5).

Prepared according to the general procedure. White solid (6.9 g, 98%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H, H_{imi}), 7.75 (s, 1H, H_{imi}), 5.37 (s, 2H, CH_2), 3.96 (s, 3H, $CH_{3\text{-imi}}$), 3.35 (q, 2H, J = 7.5 Hz, $CH_{2\text{-imi}}$), 1.39 (t, 3H, J = 7.5, Hz, $CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, CD_3OD) δ 151.7, 123.8, 121.0, 120.3 (q, J_{CF} = 317.25 Hz, CF_3), 91.6, 82.1, 35.3, 18.4, 9.2; ^{19}F NMR (282 MHz, CD_3OD) δ -79.8; HRMS (+ESI): Calculate for $C_8H_{12}Cl_3N_2O_3S^+$ $(M\text{-}OTf)^+$ 320.9634, found 320.9624.

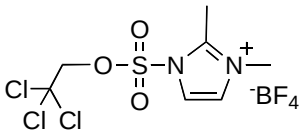


1-(2,2,2-trichloroethoxysulfuryl) 2- isopropyl 3-methylimidazolium triflate (2.6).

Prepared according to the general procedure. White powder (6.2 g, 85%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (s, 1H, H_{imi}), 7.69 (s, 1H, H_{imi}), 5.39 (s, 2H, CH_2), 4.09-4.00 (m,4H, CH_{imi} & $CH_{3\text{-imi}}$), 1.56 (d, 6H, J = 7.2 Hz, 2x$CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, CD_3OD) δ 152.9, 124.7, 121.4, 120.3 (q, J_{CF} = 315 Hz, CF_3), 91.6, 82.4, 37.1, 26.7, 17.0; ^{19}F NMR (282 MHz, CD_3OD) δ -79.7; HRMS (+ESI): Calculate for $C_9H_{14}Cl_3N_2O_3S^+$ $(M\text{-}OTf)^+$ 334.9791, found 334.9786.

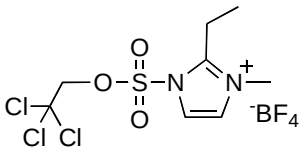
General procedure for the synthesis of substituted imidazolium tetraflouroborate salts 2.7 and 2.8.

A solution of compounds **2.1** or **2.2** (3.4 mmol, 1.00 equiv) in THF (12 mL) was added dropwise to an ice cooled suspension of trimethyloxonium tetrafluoroborate (0.5 g, 3.4 mmol, , 1.00 equiv) in 6 mL of THF. The reaction mixture was then allowed to come to room temperature and stirred O/N. The solvent then removed under vacuum and the resulting residue was triturated with a mixture of CH_2Cl_2:Et_2O (1:4) and resulting precipitate was filtered, and dried to give corresponding tertrafuoroborate salt as a white powder.



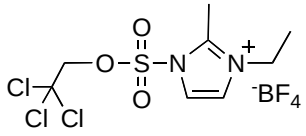
1-(2,2,2-trichloroethoxysulfuryl) 2,3-dimethylimidazolium tetrafluoroborate (2.7).

Prepared according to the general procedure. White powder (1.1 g, 85%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.07 (d, 1H, J = 2.2 Hz, H_{imi}), 7.73 (d, 1H, J = 2.2 Hz, H_{imi}), 5.33 (s, 2H, CH_2), 3.91 (s, 3H, $CH_{3\text{-imi}}$) , 2.90 (s, 3H, $CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, CD_3OD) δ 148.6, 123.6, 120.6, 91.7, 81.9, 35.3, 10.4; ^{19}F NMR (282 MHz, CD_3OD) δ -154.4; HRMS (+ESI): Calculate for $(M\text{-}BF_4)^+$ $C_7H_{10}Cl_3N_2O_3S^+$ 306.9478, found 306.9468.

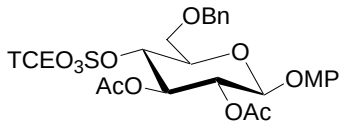


1-(2,2,2-trichloroethoxysulfuryl) 2-ethyl 3-methylimidazolium tetrafluoroborate (2.8). White powder (1.1 g, 79%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.19 (d, 1H, J = 1.9

Hz, H_{imi}), 7.93 (d, 1H, J = 2.0 Hz, H_{imi}), 5.51 (s, 2H, CH_2), 3.88 (s, 3H, $CH_{3\text{-imi}}$), 3.22 (q, 2H, J = 7.5 Hz, $CH_{2\text{-imi}}$), 1.25 (t, 3H, J = 7.5, Hz, $CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, CD_3OD) δ 151.8, 124.5, 121.7, 92.2, 82.1, 36.2, 18.8, 10.9; ^{19}F NMR (282 MHz, CD_3OD) δ -148.0; HRMS (+ESI): Calculate for $(M\text{-}BF_4)^+$ $C_8H_{12}Cl_3N_2O_3S^+$ 320.9634, found 320.9626.



1-(2,2,2-trichloroethoxysulfuryl) 3-ethyl 2-methylimidazolium tetrafluoroborate (2.10). A solution of compound 2.34 (0.5 g, 1.7 mmol, 1.00 equiv) in CH_2Cl_2 (6 mL) was added dropwise to a suspension of triethyloxonium tetrafluoroborate (0.32 g, 1.7 mmol, 1.00 equiv) in CH_2Cl_2 (3 mL). The reaction mixture was then heated at reflux for 1 hour, cooled to room temperature then solvent was removed under vacuum. The resulting residue was triturated with 5 mL of CH_2Cl_2:Et_2O (5:1) and the resulting precipitate was filtered, and dried to yield tetrafuoroborate salt **2.10** as a white powder (0.56 g, 81%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H, H_{imi}), 8.03 (s, 1H, H_{imi}), 5.54 (s, 2H, CH_2), 4.27 (q, 2H, J = 7.2 Hz, $CH_{2\text{-imi}}$), 2.88 (s, 3H, $CH_{3\text{-imi}}$), 1.42 (t, 3H, J = 7.2, Hz, $CH_{3\text{-imi}}$); ^{13}C NMR (75 MHz, CD_3OD) δ 148.4, 122.5, 121.8, 92.4, 81.9, 44.8, 14.4, 11.9; ^{19}F NMR (282 MHz, CD_3OD) δ -154.0; HRMS (+ESI): Calculate for $(M\text{-}BF_4)^+$ $C_8H_{12}Cl_3N_2O_3S^+$ 320.9634, found 320.9630.



General procedure for *O*-sulfation using reagent 1.98, 2.37-2.41 and 2.43 (Table 2.5, compound 2.14). To carbohydrate **2.13** (0.25 g, 0.54 mmol) in solvent (2.2 mL, 0.25 M) at 0 ^{o}C was added 1,2-dialkylimidazole (1.35 mmol) followed by the SIS (**1.83**, **2.4-2.8** or **2.10**, 1.08 mmol). The reaction was stirred at 0 ^{o}C, allowed to warm to room temperature by allowing the ice bath to melt, and then stirred for 18 h at room temperature. The reaction was diluted with CH_2Cl_2, (in case of using CH_2Cl_2 as a solvent) or the solvent was removed under vacuum (in case of using THF or DMF as a solvent) then the residue was dissolved in CH_2Cl_2, washed with brine, and dried over $MgSO_4$ and condensed to brown crude oil. Flash chromatography (1:5 EtOAc/hexanes) yielded **2.14** as a white amorphous solid. See Table 2.5 for yields. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.81 (m, 6H, OCH_3, H_5, H_6, $H_{6'}$), 4.58 (dd, 2H, J = 13.7, 12.2 Hz, CH_2Ph), 4.69 (AB system, 2H, J = 11.1 Hz , CH_2CCl_3), 4.98 (m, 2H, H1, H4), 5.21 (t, 1H, J = 9.2, 8.1 Hz, H3), 5.42 (t, 1H, J = 9.3 Hz, H2), 6.77 (d, 2H, J = 8.9 Hz, ArH), 6.93(d, 2H, J = 8.9 Hz, ArH), 7.31(m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 20.7, 55.6, 67.8, 71.4, 72.1, 73.1, 73.7, 79.3, 80.2, 92.5, 100.1, 114.6, 118.7, 127.8, 127.81, 128.4, 137.5, 150.8, 155.8, 169.3, 169.35; $[\alpha]_D^{25}$ =-40.7 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 688.0779 $(M+NH_4)^+$ $C_{26}H_{33}N\,Cl_3O_{12}S$ requires 688.0789.

Chapter 3 Multiple and Regioselective Introduction of Protected Sulfates into Carbohydrates Using Sulfuryl Imidazolium Salts

3.1 Introduction

In chapters one and two we demonstrated that trichloroethyl-protected sulfates could be introduced into monosaccharides in good yield using sulfuryl imidazolium salts. In this chapter we present our studies on the direct regioselective incorporation of trichloroethyl-protected sulphate groups into monosaccharides using trichloroethoxysulfuryl-(2-methyl)-*N*-methylimidazolium triflate. We also present the synthesis of multiply sulfated monosaccharides using the TCE-protected sulfate strategy.

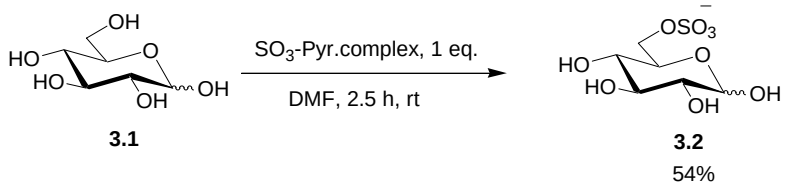
Regioselectivity is a major issue in carbohydrate chemistry as sugars contain several hydroxyl groups and it is often desirable to specifically functionalize or protect one or only selected ones during their synthesis. The hydroxyl groups in each carbohydrate differ in their reactivity depending on whether the hydroxyl groups are anomeric, primary, secondary, axial or equatorial. These differences in reactivity can sometimes be significant enough to achieve the desired product in one step without additional manipulations. Selective functionalization or protection of hydroxyl groups in carbohydrates can significantly reduce the number of steps in a synthesis.

Regioselective incorporation of sulfate groups into the sugars is usually achieved either by treating the sugar derivatives directly with sulfur trioxide-amines complex or more commonly, by reacting preformed stannanediyl acetals or stannyl ethers of carbohydrates with sulfur trioxide-amines complex. There are only a few examples in the literature in which protected sulfates were used for the regioselective incorporation of the sulfate groups into the monosaccharides. In the following section we present some

examples from the literature for the regioselective incorporation of the sulfate group into the carbohydrates.

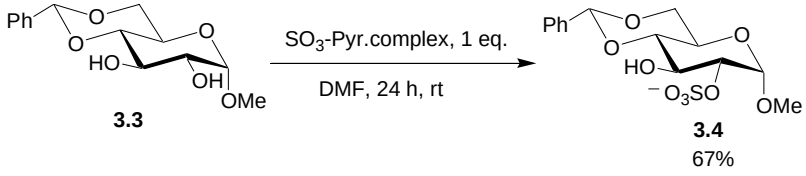
3.1.1 Direct Regioselective Incorporation of Sulfate Groups into Carbohydrates

In 1961, Guiseley and Ruoff [42] reported the direct regioselective sulfation of glucose. They prepared glucose-6-sulfate **3.2** in 54% yield by treating anhydrous glucose in dry DMF with SO_3-pyridine complex (1 equiv.) dropwise as a solution in DMF over 1.5 h at room temperature. 1,6-disulfoglucose was formed as byproduct (Scheme 3.1).



Scheme 3.1. Selective incorporation of a sulfate group into D-glucose.

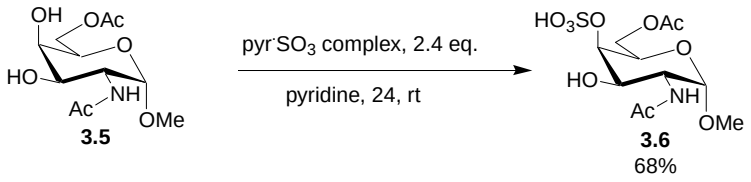
These workers also reported the selective sulfation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **3.3** at the 2-OH under the same conditions except the SO_3-pyridine complex was added over an 8 hour period at room temperature (Scheme 3.2).[43]



Scheme 3.2. Regioselective sulfation of methyl-α-glucopyranoside **3.3.**

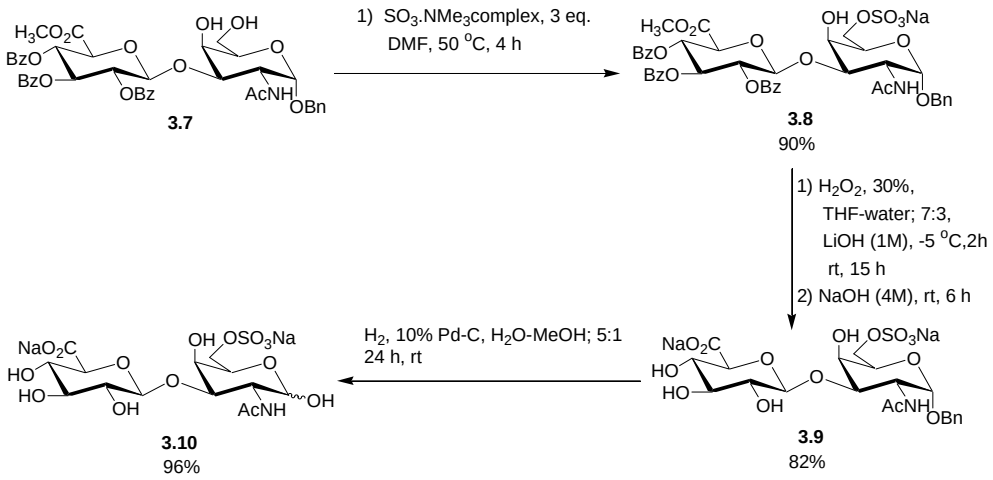
Since this initial report a number of groups have described the direct regioselective incorporation of sulfate groups into carbohydrates. Some examples are given below.

Although the secondary axial hydroxyl groups of carbohydrates usually undergo acylation less rapidly than the equatorial isomers, the 4-OH of the methyl 2-acetamido-2-deoxy-α-D-galactopyranoside **3.5** was selectively sulfated by Hirano with sulfur trioxide-pyridine complex in pyridine at room temperature in the presence of the free 3-OH in 68 % yield.[44]



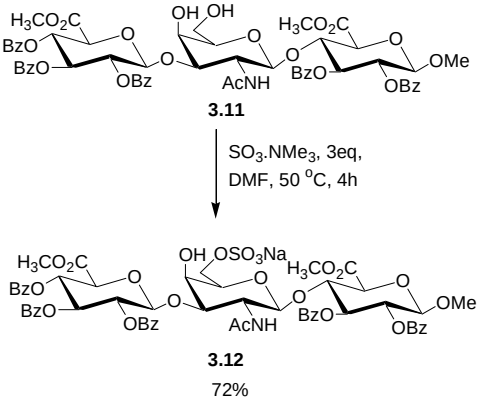
Scheme 3.3. Regioselective sulfation of galactose amine derivative **3.5**.

Several impressive examples of selective sulfations have been reported by Jacquinet and coworkers. In 1998, Jacquinet and coworkers[45] attempted to synthesise the disaccharide repeating units of chondroitin 6-sulfate using tin-mediated (see section 3.1.2 for a discussion on the use of tin–mediatedd selective sulfations) regioselective sulfation of the diol **3.7**, but low yields were obtained due to the difficulties they faced in removing the tin salts from the reaction mixture. By treating diol **3.7** with 3 equiv. sulphur trioxide-trimethyl amine complex in DMF at 50 ^{o}C for 4 hours, they were able to separate the 6-*O*-sulfo disaccharide **3.8** in an outstanding 90% yield. Traces of the 4,6-disulfated derivative was formed (< 2%) (Scheme 3.4).



Scheme 3.4. Regioselective sulfation of disaccharide **3.5.**

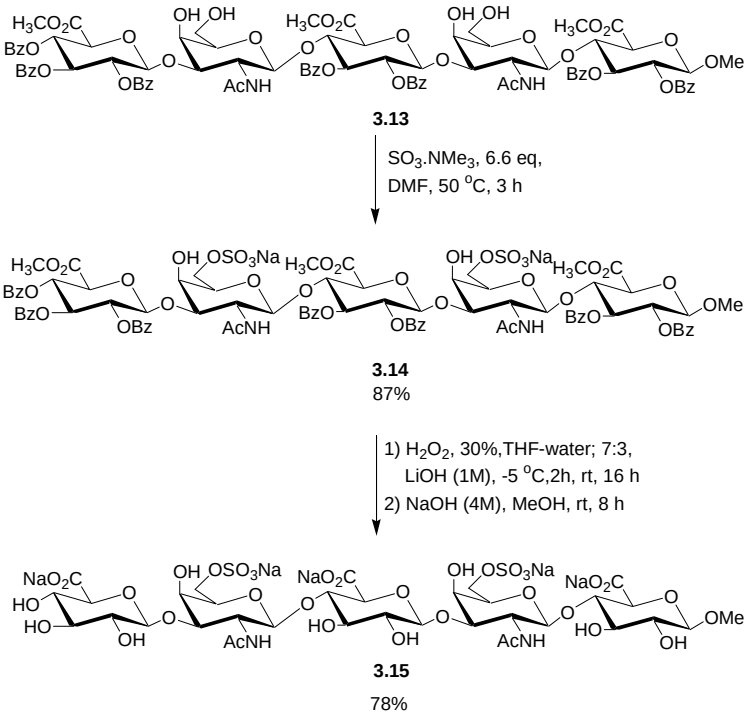
Belot and Jacquinet[46] applied the same procedure used in the synthesis of disaccharide **3.8** to the synthesis the chondroitin 6-sulfate trisaccharide **3.12**. The primary 6-OH was selectively sulfated over the axial 4-OH of the galactose amine moiety in 72% yield with sulfur trioxide-trimethyl amine complex in DMF at 50 °C (Scheme 3.5).



Scheme 3.5. Regioselective sulfation of trisaccharide **3.11.**

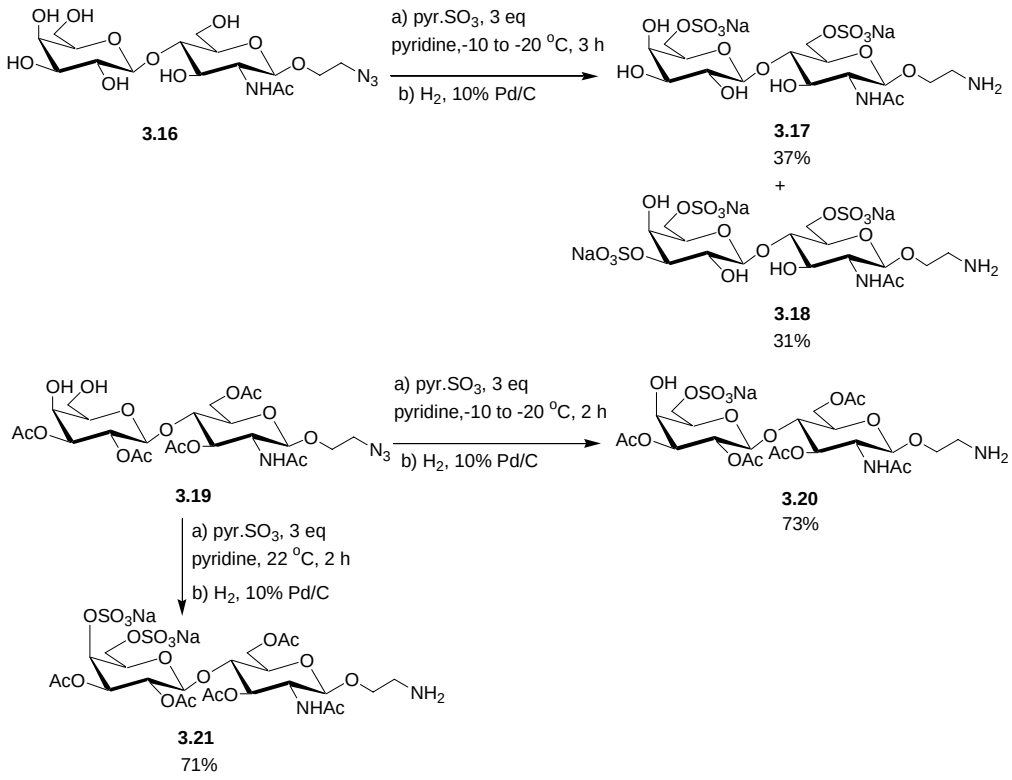
In 2000, Belot and Jaquinet[47] reported the total synthesis of chondroitin pentasaccharide derivatives *O*-sulfated at the C-4 or C-6 of the amino sugar moieties and

having a methyl β-D-glucopyranosiduronic acid at the reducing end, which represent structural elements of chondroitin 4- and 6- sulfate proteoglycans. The tetraol **3.13** was selectively sulfated with 6.6 equiv. sulfur trioxide-trimethyl amine complex in DMF at 50 ^{o}C for 3 hours, to give the disulfated pentasaccharide **3.14** in an 87% yield (Scheme 3.6).



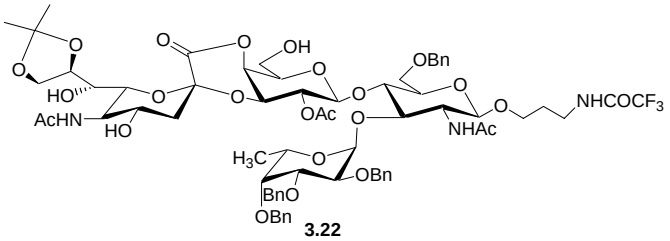
Scheme 3.6. Regioselective sulfation of pentasaccharide **3.11.**

Bovin et al.[48] described the synthesis of some mono-, di- and tri-*O*-sulfated *N*-acetyl lactosamines using sulfur trioxide-pyrinde complex in pyridine. The selective sulfation reactions were carried out at -10 to -20 ^{o}C, while the multiple sulfations were carried out at room temperature (Scheme 3.7).



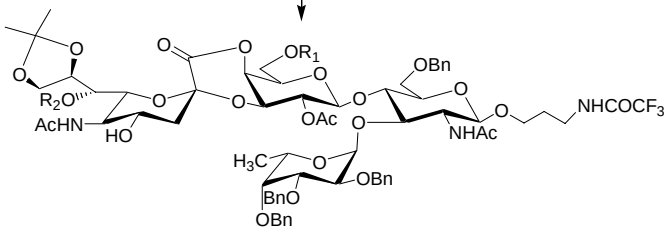
Scheme 3.7. Multiple and regioselective sulfation of disaccharides **3.16** and **3.19**.

SiaLe^x with a sulfate group at 6-OH of the galactose moiety has the highest-affinity ligand for siglec-8,[49] (siglec-8 is a sialic acid-binding immunoglobulin-like lectin that is expressed specifically by eosinophils) while SiaLe^x derivative with a sulfate group at the 6-OH of GlcNAc is known to be a specific receptor for the avian influenza viruses.[50] In 2009, Bovin and coworkers [51] used sulfur trioxide-pyridine complex in pyridine at -10 to -20 ^{o}C for the synthesis of SiaLe^x tetrasaccharide derivatives (Scheme 3.8).



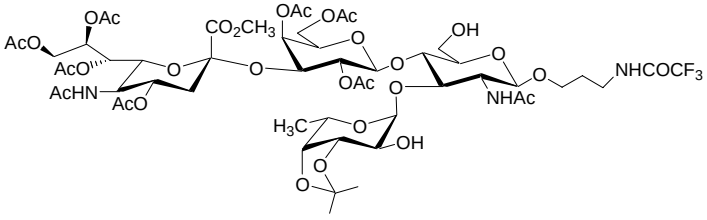
3.22

Py.SO_3, pyridine
-10 to -20 ^oC, 5 h



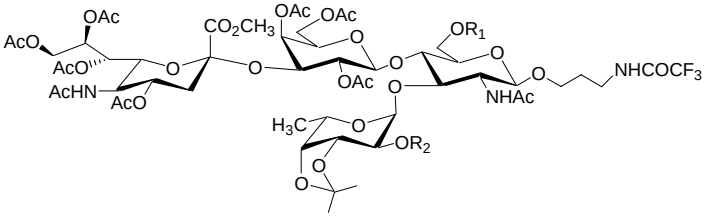
3.23; R_1= SO_3Na, R_2=H 51%

3.24; R_1=R_2= SO_3Na, 20%



3.25

Py.SO_3, pyridine
-10 to -20 ^oC, 1.5 h



3.26; R_1= SO_3Na, R_2= H 32%

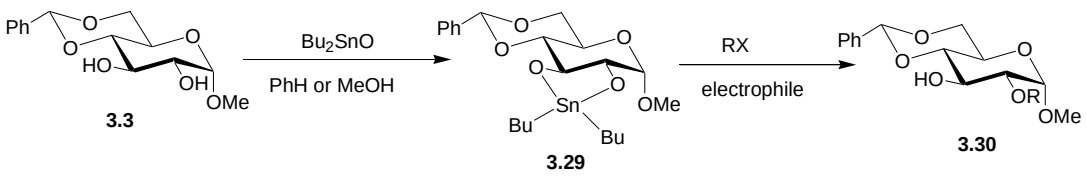
3.27; R_1=R_2= SO_3Na, 23%

3.28; R_1= H, R_2=SO_3Na, 3%

Scheme 3.8. Sulfation of $SiaLe^x$ tetrasaccharide derivatives **3.22** and **3.25.**

3.1.2 Tin Mediated Selective Sulfation of Carbohydrates

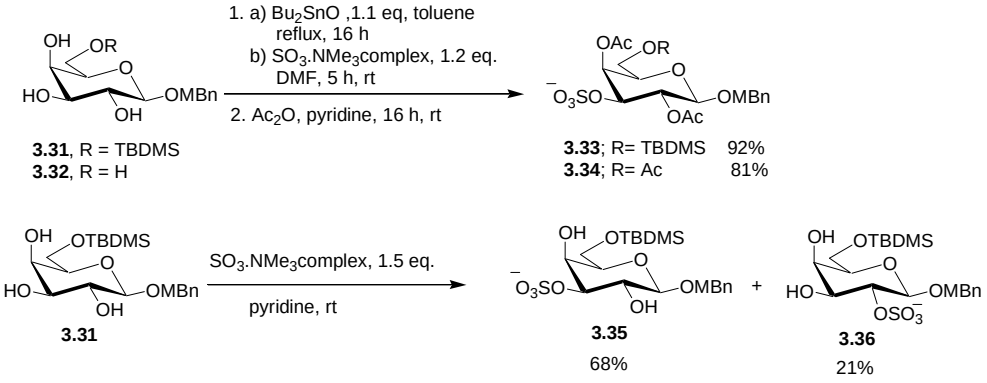
Organotin derivatives are widely used as intermediates in the regioselective substitution of the hydroxyl groups of sugars.[52] The reactions of electrophiles with organotin intermediates proceed much faster than with the parent diols and the reactions can be carried out under milder conditions. These reactions are commonly used in carbohydrate chemistry, as they give monosubstituted products with high regioselectivetiy.[52] The most common organotin (IV) carbohydrate derivatives are dialkylstannylene acetals which normally are formed by the reaction of dibutyltin oxide with the free diols (Scheme 3.9), and tributyltin ethers which can be formed by the reaction of bis(tributyltin)oxide with the alcohols.



Scheme 3.9. Formation and reaction of dialkylstannylene acetals with electrophiles

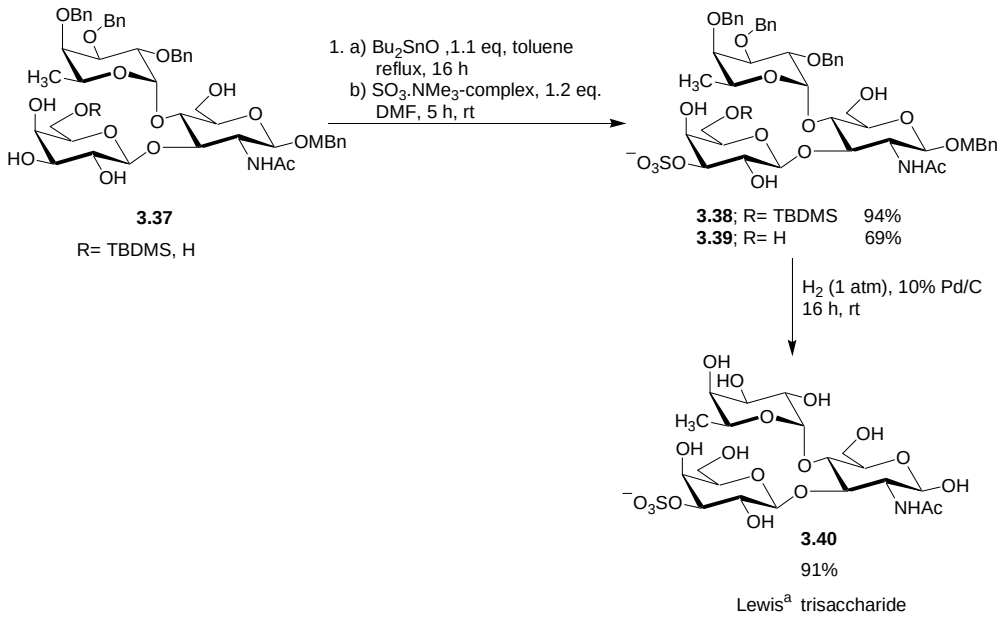
Although the two reagents give similar products, the dialkyltin reagents are usually preferred because of their lower toxicity.[53] The organotin derivatives of carbohydrates are normally prepared by heating a mixture of the bis(tributyltin) oxide or dibutyltin oxide and the sugar in toluene or benzene, with azeotropic removal of the water formed, or in methanol.[54] There are numerous examples in the literature describing tin-mediated regioselective sulfation of carbohydrates. A select few examples of this approach is presented below.

In 1994, Lubineau and Lemoine[55] reported that the stannylene derived from free or 6-protected β-D-galactopyranosides, gave the 3-*O*-sulfate in high yields upon treatment with sulfur trioxide-trimethyl amine complex in a one pot reaction, with excellent regioselectivity. When **3.31** or **3.32** was treated with Bu_2SnO in refluxing toluene, and the formed stannylene acetal was treated in situ with SO_3-NMe_3 complex in DMF at room temperature followed by acetylation with acetic anhydride-pyridine, the 3-O-sulfate derivatives **3.33** and **3.34** were obtained in 92% and 81% yield respectively (Scheme 3.10). In contrast, treatment of triol **3.31** with SO_3-NMe_3 complex in pyridine at room temperature, give a mixture of 3-*O*-sulfate (68%) and 2-*O*-sulfate (21%) derivatives.



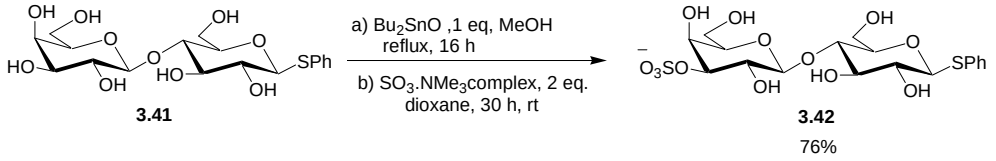
Scheme 3.10. Tin-mediated regioselective sulfation of galactoside **3.31** and **3.32**.

Lubineau applied this to the synthesis of a trisaccharide derived from Lewis[a], which is a sulfated oligosaccharide that has a high ligand affinity for E- and L-selectins.[55,56] By applying the tin-mediated regioselective sulfation methodology, Lubineau and Lemoine[55] were able to synthesis the 3`-O-sulfated Lewis[a] trisaccharide **3.40** in less steps by avoiding many protection-deprotection steps (Scheme 3.11**)**.



Scheme 3.11. Synthesis of Lewis[a] trisaccharide **3.40** via tin-mediated regioselective sulfation.

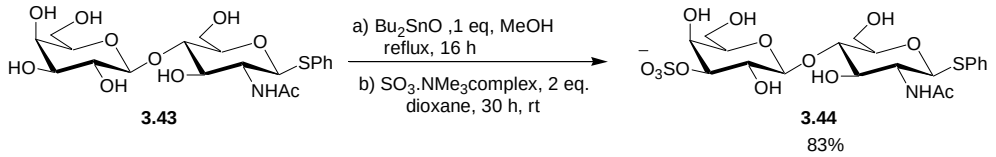
Flitsch *et al*.[57] reported the selective sulfation of thiophenylactoside **3.41** by treating it with dibutyltin oxide, followed by $SO_3{\cdot}NMe_3$ complex in dioxane to give the 3`-sulfated lactoside **3.42** in 76% yield and 10% of the 3`,6`-disulfated lactoside as a side product. By stirring lactoside **3.41** with $SO_3.\ NMe_3$ complex in dioxane without dibutyltin oxide, no product was observed due to the poor solubility of **3.41**. This confirms that the selectivity observed is due to the formation of the tin complex.



Scheme 3.12. Tin-mediated regioselective sulfation of lactoside **3.41**

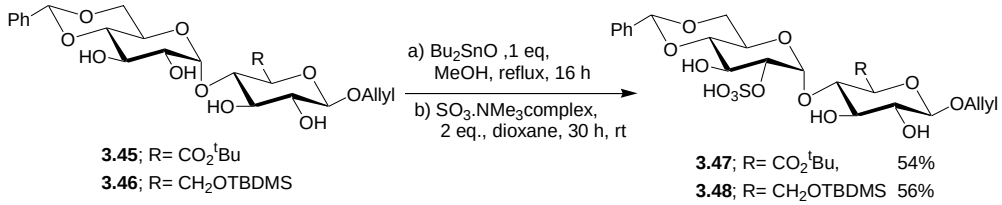
The 3`-sulfo-*N*-acetyllactosaminide **3.44** is useful in detecting high levels of serum α−1,3-L-fucosyltransferase in ovarian cancer patients,[58] as it is a selective substrate for this enzyme. Flitsch *et al*.[57] prepared the 3`-sulfo-*N*-acetyllactosaminide

3.44 in 83% yield from compound **3.43** using the same methodology without detecting of any side products (Scheme 3.13).



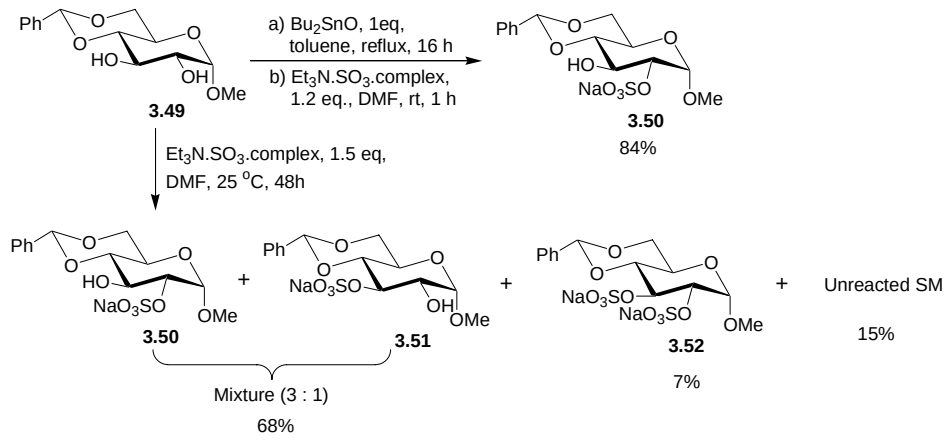
Scheme 3.13. Tin-mediated regioselective sulfation of lactosaminide **3.44.**

When Flitsch *et al*.[58] tried to apply the same regioselective sulfation procedure on maltosides (which contain no cis diols), they got mixtures of mono- and di-6- and 6`-sulfate esters. By protecting both primary hydroxyl groups as in compounds **3.45** and **3.46**, selective sulfation of the 2`-hydroxyl group was the major isolated product (Scheme 3.14). The increased selectivity of the 2`-hydroxyl may be due to the C1`-C2` cis dioxy configuration.[58]



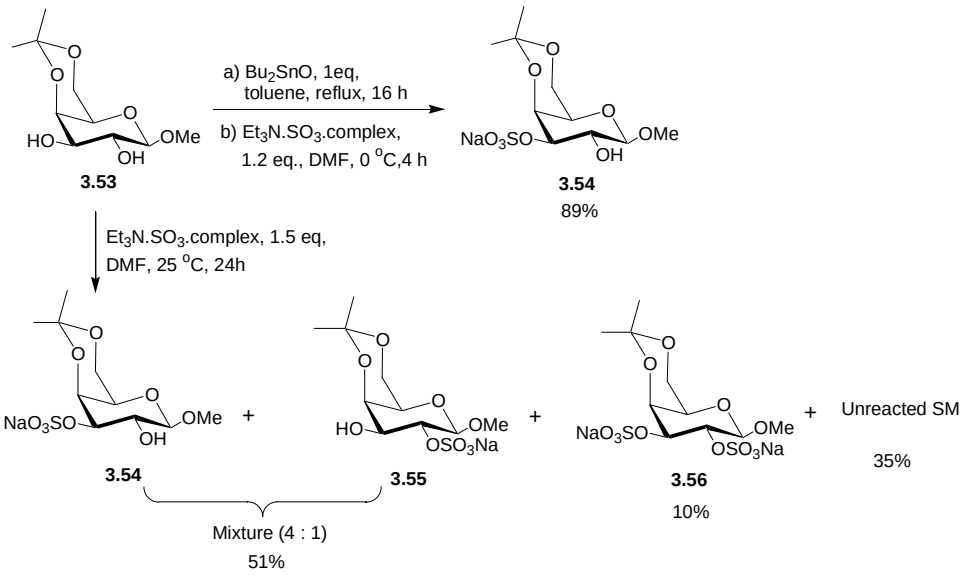
Scheme 3.14. Tin-mediated regioselective sulfation of maltosides **3.45** and **3.46**.

In 1994, Andrea Vasella and coworkers[59] reported the regioselective sulfation of dibutylstannanediyl acetals of partially protected hexoses. Treatment of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **3.49** with sulfur trioxide-triethyl amine complex in DMF at 25 ^oC for 48 hour, gave a mixture of mono- and disulfated derivatives. On the other hand treating glucopyranoside **3.49** with 1 equivalent of dibutyltin oxide in refluxing toluene, followed by sulfur trioxide-triethyl amine complex in DMF at room temperature, afforded only the 2-*O*-sulfate derivative in 84% yield (Scheme 3.15).

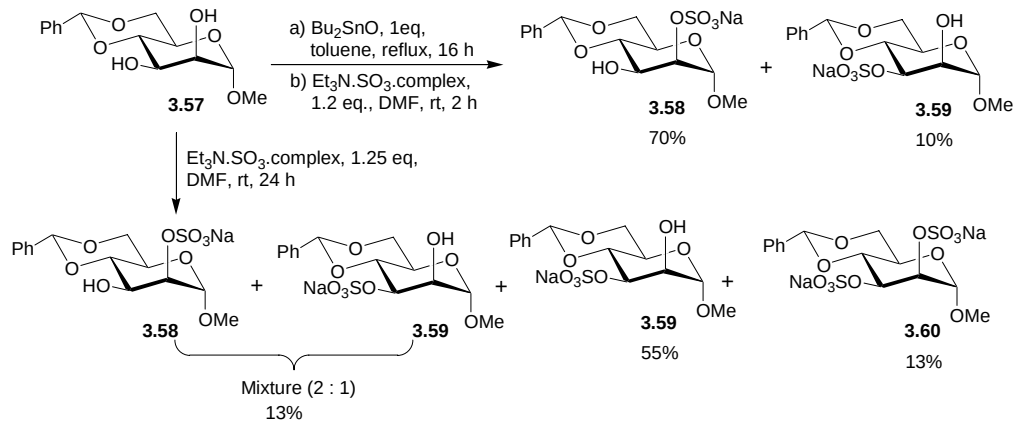


Scheme 3.15. Tin-mediated regioselective sulfation of glucopyranoside **3.49**

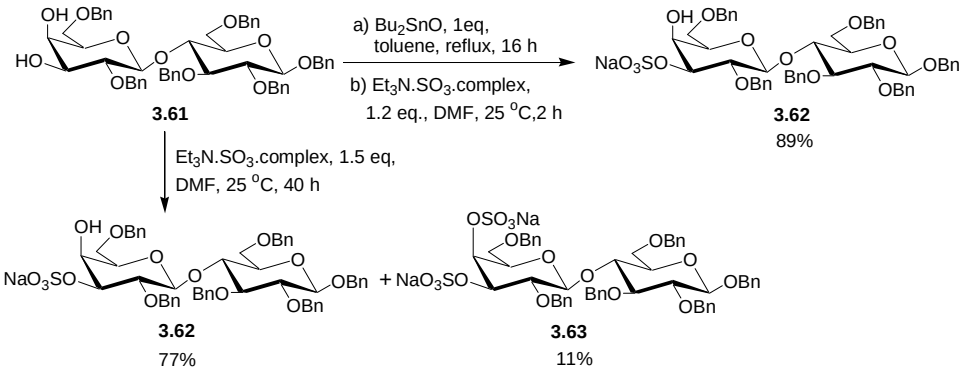
Vasella *et al.*[59] applied the same conditions to methyl 4,6-*O*-isopropylidene-β-D-galactcopyranoside **3.53** and methyl 4,6-*O*-isopropylidene-α-D-mannopyranoside **3.57**. They isolated the 3-*O*-sulfated galactopyranoside **3.54** and 2-*O*-sulfated mannopyranoside **3.58** in 89% and 70% yields respectively (Schemes 3.16 and 3.17). Good regioselectivity was achieved, when Vasella *et.al.*[59] applied the tin mediated regioselective sulfation procedure on the lactoside **3.61**, where the 3`-*O*-sulfated lactoside **3.62** was isolated as the only monosulfated product in 83% yield. In the absence of dibutyltin oxide, the reaction is slower and the 3`-*O*-sulfated lactoside **3.62** was isolated in 77% yield in addition to 11% of the disulfated derivatives (Scheme 3.18).



Scheme 3.16. Direct and tin-mediated regioselective sulfation of galactopyranoside **3.53.**

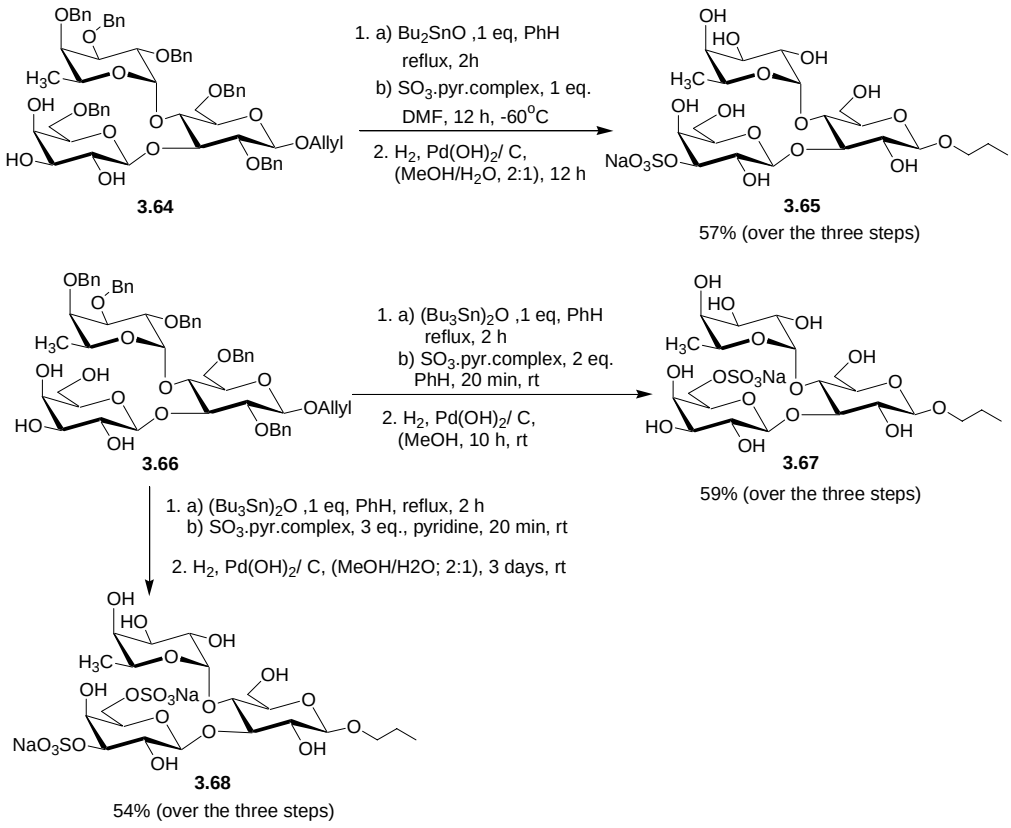


Scheme 3.17. Direct and tin-mediated regioselective sulfation of mannopyranoside **3.57**



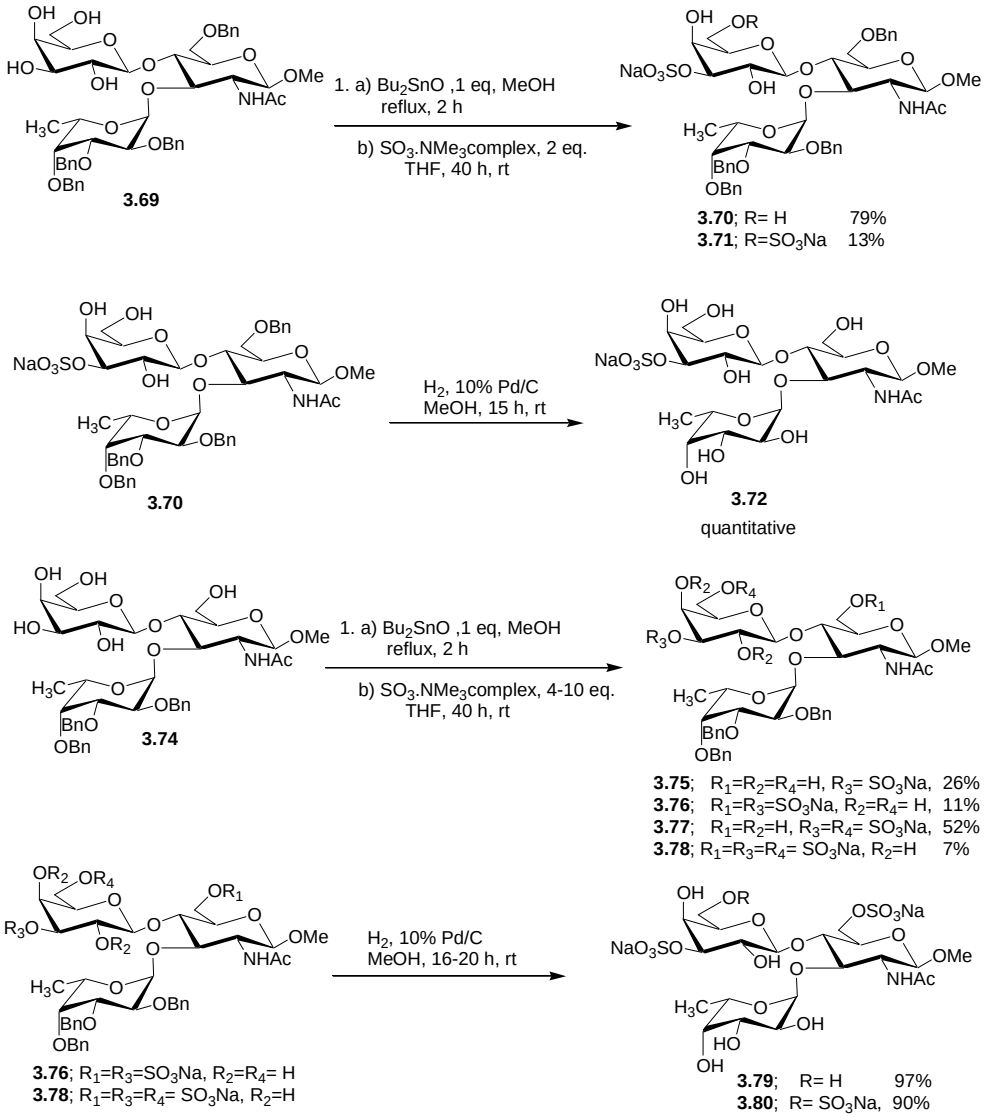
Scheme 3.18. Direct and tin-mediated regioselective sulfation of lactoside **3.61.**

In 1995, Kiessling *et al.*[60] used the tin mediated regioselective sulfation in the synthesis of three sulfated derivatives of Lewis a to test their binding affinity towards selectin (E, L and P-selectin). In the synthesis of compound **3.65**, sulfur trioxide-pyridine complex was added dropwise to a solution of **3.64** in DMF over 20 minutes at -60 ^{o}C (Scheme 3.19). Sulfation of compound **3.66** using $(Bu_3Sn)_2O$ followed by SO_3·pyr. complex in benzene at room temperature give the 6`-sulfated trisaccharide **3.67**. The 3`,6`-disulfate **3.68** was obtained when the sulfation reaction was carried out in pyridine (Scheme 3.19).



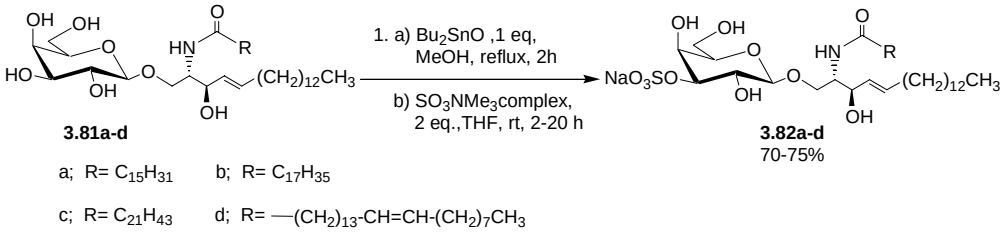
Scheme 3.19. Tin-mediated regioselective sulfation of Lewis a derivatives

Sinay and coworkers[61] used the stannylene procedure to synthesize some mono-, di- and trisulfated Lewis x trisaccharide derivatives (Scheme 3.20). Treatment of the stannylene acetal of **3.69** with 2 equivalents of $SO_3.NMe_3$ complex in THF for 40 hours at room temperature give the 3`-sulfated trisaccharide **3.70** in 79% yield in addition to 13% of the 3`,6`-disulfated derivative **3.71**. Different patterns of sulfation were obtained on subsequent sulfation of **3.74**. Compound **3.74** was treated with 1 equivalent of Bu_2SnO in refluxing methanol for 2 hours. After removing the solvent, the residue was treated with 2 equivalents of $SO_3.NMe_3$ complex in THF at room temperature for 40 hours. Then the solvent was evaporated and the residue was treated again with 2 equivalents of $SO_3.NMe_3$ complex in DMF at room temperature for 60 hours to give a mixture of sulfated products **3.75** (26%), **3.76** (11%), **3.77** (52%) and **3.78** (7%) (Scheme 3.20). Subjecting the sulfated product mixture (**3.75**-**3.78)** to 6 equivalents of sulfur trioxide-pyridine complex in DMF for 5 days at room temperature, afford the trisulfated derivative **3.78** in 66% yield (Scheme 3.20).



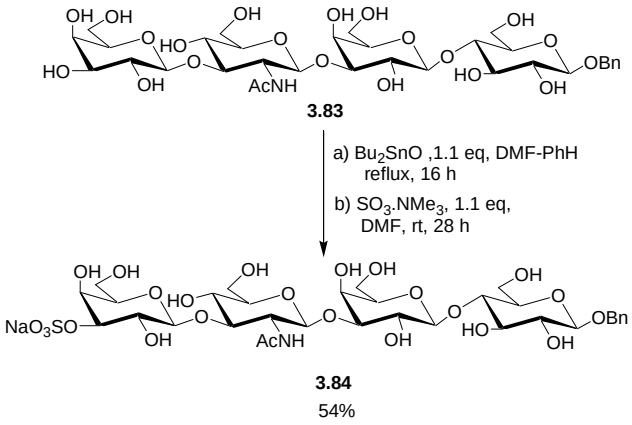
Scheme 3.20. Tin-mediated regioselective sulfation of Lewis x derivatives **3.69** and **3.74**.

Sulfatide, which is a mixture of 3-sulfated β-D-galactopyranosylceramides with different fatty acids attached to the ceramide moiety, is an antigen presented by CD1a protein. In 2002, Panza and coworkers[62] reported the tin-mediated regioselective sulfation synthesis of four different 3-O-sulfated galactosylceramides. The synthesized compounds differ in the type of fatty acid amide and the length of the hydrocarbon chain (Scheme 3.21).



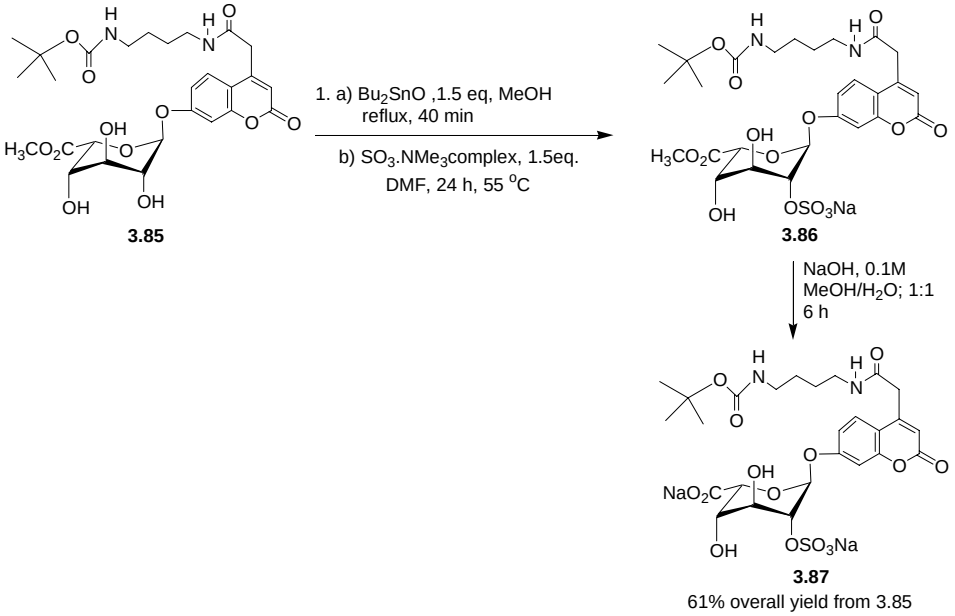
Scheme 3.21. Tin-mediated regioselective sulfation of sulfatide derivatives **3.81a-d**.

The stannylene procedure was used by Narvor *et.al*.[63] for the regioselective sulfation of the 3-OH of the terminal galactose during the chemoenzymatic synthesis of the 3-*O*-sulfated Lewis a pentasaccharide (Scheme 3.22).



Scheme 3.22. Tin-mediated regioselective sulfation of Lewis a tetrasaccharide **3.83**.

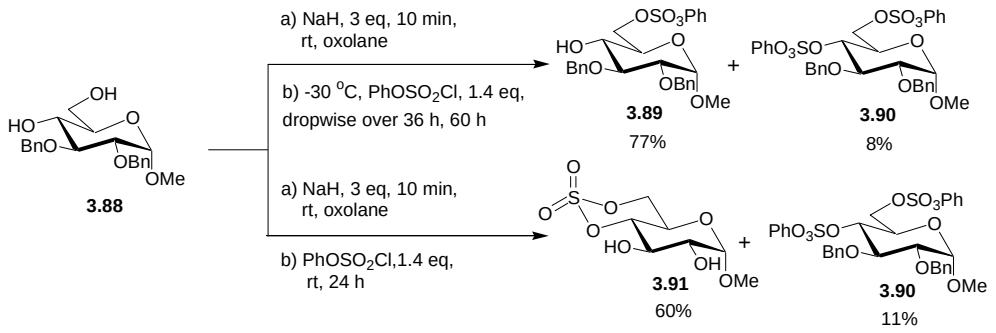
α-L-Iduronate glycosides containing a sulfate group at the 2-position are used for assaying iduronate-2-sulfatase, whose deficiency results in Hunter syndrome (mucopolysaccharidosis-II). Gelb and coworkers[64] applied the stannylene procedure in the regioselective sulfation of α-L-iduronate glycosides **3.87** (Scheme 3.23).



Scheme 3.23. Tin-mediated regioselective sulfation of α-L-iduronate glycoside **3.85**.

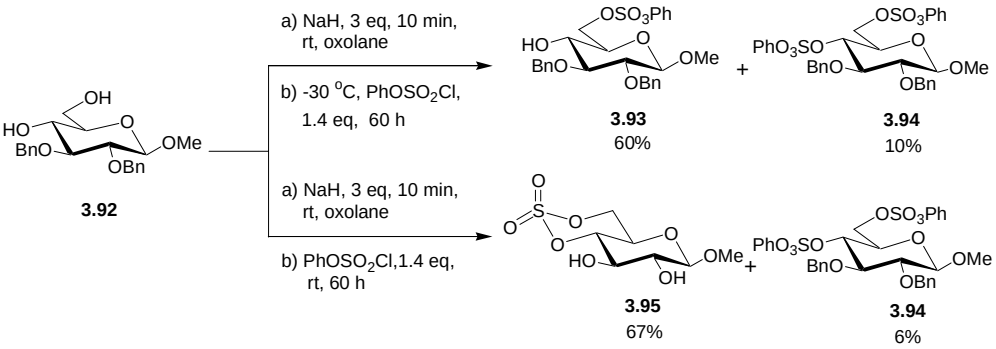
3.1.3 Selective Sulfation of Carbohydrates with Protected Sulfate Diesters

As discussed in Chapter 1, the phenyl group has been used by Perlin and coworkers as a sulfate protecting group for the synthesis of sulfated carbohydrates.[29] It is introduced using phenyl chlorosulfate ($PhOSO_2Cl$).[29] In 1989, Abdel-Malik and Perlin[30] reported the reaction of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside **3.88** with phenyl chlorosulfate (1.4 eq.) in dioxane in the presence of NaH at -30 °C, which gave a mixture of the 6-sulfate product **3.89** and the 4,6-bis-phenylsulfate product **3.90** in 77% and 8% yield respectively. When the reaction was carried out at room temperature, the 4,6-cyclic sulfate **3.91** was formed in 60% yield as the major product, in addition to 11% of the 4,6-bis-phenylsulfate **3.90.** No mono phenylsulfate product was found (Scheme 3.24).



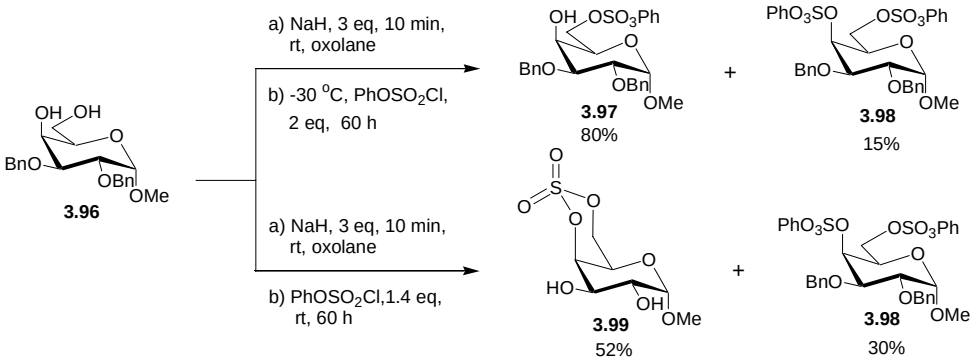
Scheme 3.24. Introduction of a phenyl-protected sulfate into α-glucopyranoside **3.88**.

Similar results were obtained in the reaction of methyl 2,3-di-*O*-benzyl-β-D-glucopyranoside and phenylchlorosulfate (Scheme 3.25).



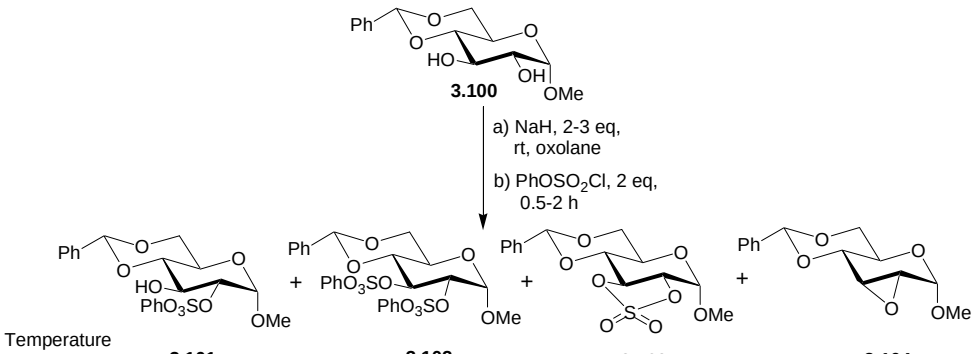
Scheme 3.25. Regioselective introduction of phenyl-protected sulfate into a β-glucopyranoside **3.92**.

The galactopyranosyl 4,6-diol **3.96** showed good regioselectivity upon treatment with phenyl chlorosulfate at -30 °C, where the 6-(phenylsulfate) was formed in 80% yield in addition to 15% of the disulfated derivatives. At room temperature, the 4,6-cyclic sulfate derivative was formed in 52% yield as well as 30% of the 4,6-di-(phenylsulfate) galactopyranoside **3.98** (Scheme 3.26).



Scheme 3.26. Regioselective introduction of a phenyl-protected sulfate into α-glactopyranoside **3.96**.

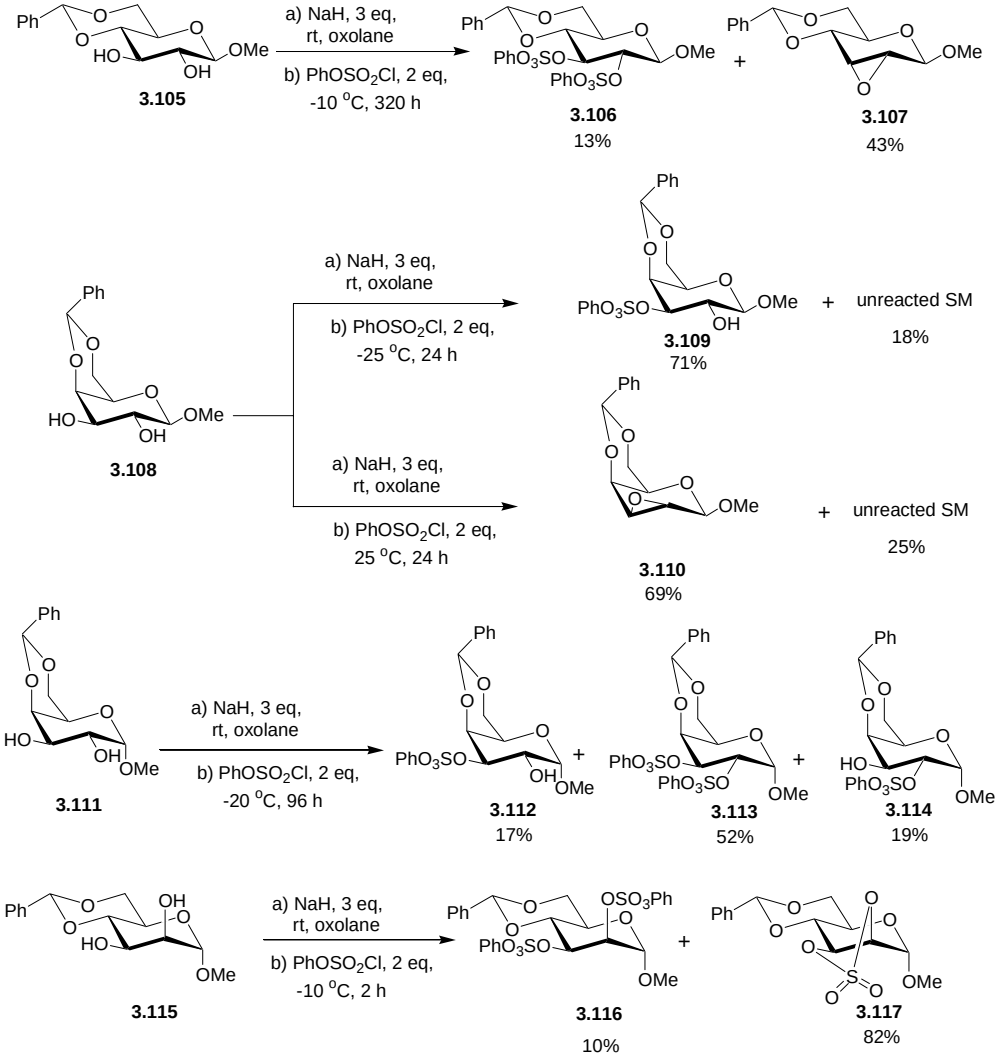
Perlin *et al*.[31] reported that phenylchlorosulfonate is more selective for the 2-OH than the 3-OH of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **3.100**, when the reactions were carried out over the temperature range of -25 to 0 °C. The 2-*O*-Phenyl sulfate was isolated in 68% yield at -25 °C. At higher temperatures (25 to 50 °C), the 2,3-cyclic sulfate was formed in yields ranging from 25 to 77%. 2,3-Diphenylsulfate **3.102** was formed as one of the products especially at high temperatures (10% yield at 25 or 50 °C), in addition to methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside which was isolated in 4 to 15% yield and its yield was increased with increasing reaction time (Scheme 3.27).



Temperature	3.101	3.102	3.103	3.104
-25 °C	68%	2%	5%	0%
25 °C	0%	10%	77%	4%
50 °C	0%	10%	46%	15%

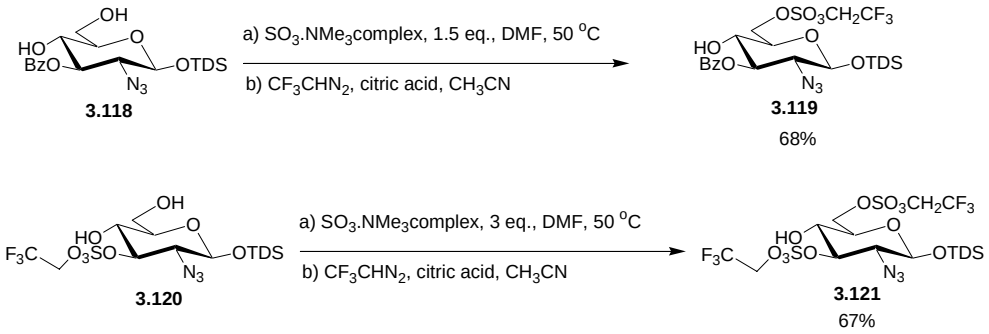
Scheme 3.27. Temperature-dependent introduction of phenyl-protected sulfates into **3.100**.

Perlin also treated different methyl 4,6-*O*-benzylidene-D-pyranosyl derivatives (β-D-glucopyranosyl (**3.105**), α- and β-D-galactopyranosyl **(3.108** and **3.111**) and α-D-mannopyranosyl (**3.115**)) with phenyl chlorosulfate and sodium hydride at different temperatures. The main products were mono-, di-sulfated and anhydro monosaccharides, depending on the temperature of the reaction (Scheme 3.28). Decent selectivity was obtained for the 3-OH with **3.108** as substrate.



Scheme 3.28. Incorporation phenyl-protected sulfates into benzylidene 2,3-diols **3.105, 3.108**, **3.11** and **3.115**.

As discussed in Chapter 1, the trifluoroethyl group has been used as a sulfate protecting group for the synthesis of sulfated carbohydrates.[28] It is introduced by reacting suflated carbohydrates with trifluorodiazoethane. In 2003, Linhardt and coworkers[33] reported the use of the TFE group in the synthesis of fully differentiated hexosamine monosaccharides. When diols **3.118** and **3.120** were submitted to selective 6-*O*-sulfation with sulfur trioxide-trimethyl amine complex followed by treatment with trifluorodiazoethane the TFE-protected sulfocarbohydrates **3.119** and **3.121** were obtained in 68 and 67% yield respectively (Scheme 3.29).



Scheme 3.29. Selective incorporation of a TFE-protected sulfate into carbohydrates **3.118** and **3.120**.

3.2 Objectives

It is clear from the above discussion that regioselective sulfation, either directly or using the tin-mediated approach, is a means by which sulfated carbohydrates can be prepared more efficiently. As discussed in chapters 1 and 2, the Taylor group has developed new sulfating agents based on sulfuryl imidazolium salts, such as reagent **2.4**, and shown that they can be used to prepare sulfated carbohydrates. However, regioselective sulfation of carbohydrates using these reagents had yet to be investigated. The primary objective of the work described in this chapter is to examine whether

trichloroethyl-protected sulfate esters could be regioselectively introduced directly into monosaccharides using reagent **2.4**. An additional objective is to examine whether reagent **2.4** can also be used to prepare multiply sulfated monosaccharides.

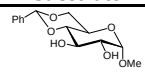
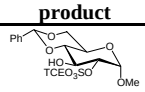
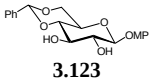
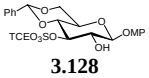
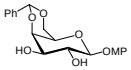
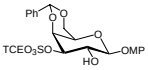
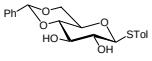
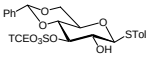
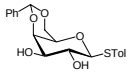
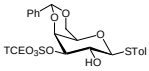
3.3 Results and Discussion

3.3.1 Regioselective Incorporation of TCE-Protected Sulfate into Monosaccharides

Our studies began with selective sulfations of 4,6-O-benzylidene acetals of galactosides and glucosides (2-OH versus 3-OH sulfations, Table 3.1). Adding 1.2 equiv of imiazolium salt **2.4** and 1.5 equiv of 1,2-dimethylimidazole (1,2-DiMeIm) in a single batch to a solution of α-glucoside **3.122** in methylene chloride gave the 2-sulfated product **3.127** as the major product in a 58% yield (entry 1). The 2,3-disulfated product was isolated in a 5% yield. Increasing the amount of reagent **2.4** and 1,2-DiMeIm to 2.0 and 2.5 equiv, respectively, and adding them as separate solutions in CH_2Cl_2 dropwise slowly to a solution of **3.122** in methylene chloride increased the yield of **3.127** to 68% (entry 1) and disulfated product to 15%. None of the 3-sulfated product was produced in either case. Alternatively, we found that a similar yield of **3.127** and disulfated product could be obtained by adding reagent **2.4** in a single portion to a solution of **3.122** and then introducing a solution of 1,2-DiMeIm slowly over several hours. Subjecting β-glycosides **3.123** and **3.124** and β-thioglycosides **3.125** and **3.126** to the same conditions (adding a solution of 2.5 equiv of 1,2-DiMeIm in methylene chloride slowly to a solution of the carbohydrate and 2.0 equiv of reagent **2.4**) led to the selective formation of the 3-*O*-monosulfated products **3.129**-**3.131** in good to excellent yields (entries 3-5) with the exception being glucoside **3.123**, which gave the 3-*O*-monosulfated product **3.128** in a 60% yield and the corresponding disuflated product in an 18% yield (entry 2). In most cases the monosuflated products were readily separated from disulfated products by silica gel chromatography except when glucoside **3.123** was the substrate. Attempts to increase

the yield of compound **3.128** by increasing the amount of sulfating agent and 1,2-DiMeIm did not result in a significant increase in isolated yield mainly because multiple

Table 3.1. Selective sulfations of 4,6-*O*-benzylidene acetals of galactosides and glucosides.

Entry	Substrate	Equiv Salt 2.4/ equiv 1,2-DiMeIm	Major product	% Yield major product
1	 **3.122**	1.2/1.5[a] 2.0/2.5[b]	 **3.127**	58[a] 68[b]
2	 **3.123**	2.0/2.5[a] 2.0/2.5[b]	 **3.128**	55[a] 60[b]
3	 **3.124**	2.0/2.5[b] 5.0/6.0[a]	 **3.129**	85[b] 92[a]
4	 **3.125**	2.0/2.5[b] 5.0/6.0[a]	 **3.130**	71[b] 78[a]
5	 **3.126**	2.0/2.5[b] 5.0/6.0[a]	 **3.131**	88[b] 94[a]

[a] The imidazolium salt **2.4** and the (1,2-DiMeIm) were added in a single portion to a solution of the carbohydrate in CH_2Cl_2, and the reactions were stirred 24-40 h. [b]1,2-DiMeIm was added dropwise as a solution in CH_2Cl_2 over 4-6 h to a solution of carbohydrate and imidazolium salt **2.4** in CH_2Cl_2 then the reactions were stirred 24-30 h.

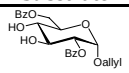
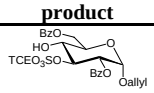
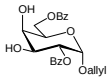
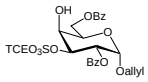
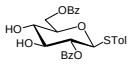
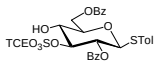
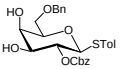
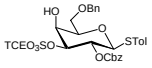
columns were required to separate the mono- and disulfated products. Both the *O*- and *S*-galactosides **3.124** and **3.126** exhibited better selectivity and higher yields than their glucoside counterparts **3.123** and **3.125**, and *S*-glycosides **3.125** and **3.126** gave better selectivity than *O*-glycosides **3.123** and **3.124**. Indeed, both galactosides **3.124** and **3.126** as well as both *S*-glycosides **3.125** and **3.126** gave excellent yields of the 3-*O*-monosulfated products even when subjected to a large excess of **2.4** and (1,2-DiMeIm) (entries 3-5), and only trace amounts of disulfated products or what appeared to be 2-*O*-monosulfated products were formed. The regioselectivity of acylation or sulfonation reactions involving 2,3-diols of 4,6-*O*-benzylidene glucopyranoside and

galactopyranoside substrates under basic conditions is dependent upon the reagents, the precise reaction conditions, and the stereochemistry and nature of the anomeric substituent.[65] However, in general, 4,6-*O*-benzylidene acetals of α-glucosides usually exhibit greater regioselectivity for the 2-OH,[65-68] while 4,6-*O*-benzylidene acetals of β-galactosides and, to a lesser extent, β-glucosides usually exhibit greater selectivity for the 3-OH.[69-71] 4,6-*O*-Benzylidene galactopyranosides usually exhibit greater regioselectivity than 4,6-*O*-benzylidene glucopyranosides.[65,66] The regioselectivity exhibited by carbohydrates **3.122-3.126** with reagent **2.4** is consistent with these general reactivity patterns. 4,6-*O* Benzylidene-β-glucopyranosides usually exhibit a lower degree of regioselectivity than 4,6-O-benzylidene-α-glucopyranosides[65,66] and this is also consistent with our results (entries 1 and 2). However, β-thioglucoside **3.125** exhibits very good regioselectivity for the 3-OH and was considerably greater than that exhibited by β-glucoside **3.123** (entries 2 and 4). Some regioselective 3-OH acylations of 4,6-*O*-benzylidene-β-thioglucopyranosides have been reported suggesting that this may be a general phenomenon with β-thioglucopyranosides.[72] In 2000, Box and Evans-Lora, conducted molecular modeling study on the 4,6-O-benzylidene-2,3-diols of different hexopyranoses.[73] The authors used the molecular mechanics module, QVBMM (quantized valence bond molecular mechanics) to simulate and evaluate the stereo-electronic effects of these diols. In the case of 2,3-diols α-glucopyranoside, the calculation showed that, the highest energy lone pair is on O-2 and has 1.69 kcal of repulsive electronic energy and this reflects the nucleophilic reactivity of the OH-2. On the other hand, in case of the β-glucopyranoside, the highest energy lone pair on O-2 and O-3 are 0.85 and 0.63 kcal respectively, and this explains the low regioselectivity of the

β-anomer of glucopyranoside.[73] In case of the β-galactopyranoside, the highest energy lone pair is on O-3, which explains the high regioselectivity found in the β-galactopyranoside series.

Next we examined selective sulfation of carbohydrates **3.132**-**3.135** that contain free 3- and 4-OH groups (Table 3.2). Adding solutions of reagent **2.4** (1.5 equiv) and (1,2-DiMeIm, 2.0 equiv) slowly to a solution of carbohydrate **3.132** resulted in the formation of mainly disulfated product. However, when a solution of 1,2-DiMeIm, (2.0 equiv) was added slowly to a solution of the carbohydrate, during which reagent **2.4** was added in three equal portions, followed by stirring for an additional 24 h, selective 3-*O*-sulfation of **3.132** was achieved in a 70% yield (entry 1, compound **3.136**), and only a trace amount of disulfated product and what appeared to be the 4-*O*-monosulfated product were detected. Sulfation of galactosyl derivative **3.133** under the same conditions gave the 3-*O*-sulfated compound **3.137** in a 77% yield (entry 2). Selective 3-*O*-sulfation of *S*-glucoside and *S*-galactoside derivatives **3.134** and **3.135** could also be achieved in 70% and 78% yields, respectively, using a similar approach (entries 3 and 4), though some disuflated product was obtained (approximately 10%). An 83% yield of the 3-*O*-monosulfated *S*-galactoside compound **3.139** was obtained by adding reagent **2.4** (6 equiv) in two portions over 8 h to a solution of **3.135** and 1,2-DiMeIm (7 equiv) followed by stirring for 20 h. Under these conditions the disulfated product was also formed in 15% yield.

Table 3.2. Selective sulfation of carbohydrates that contain free 3- and 4-OH groups.

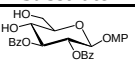
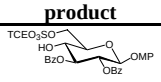
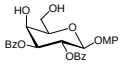
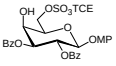
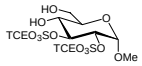
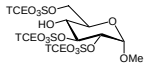
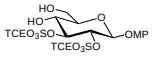
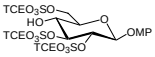
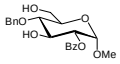
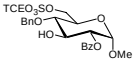
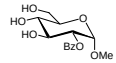
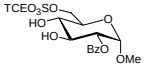
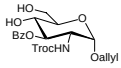
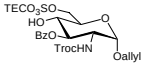
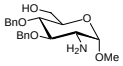
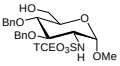
Entry	Substrate	Equiv Salt/ equiv 1,2-DiMeIm[a]	Major product	% Yield major product
1	 **3.132**	1.5/2.0	 **3.136**	70
2	 **3.133**	1.5/2.0	 **3.137**	77
3	 **3.134**	1.5/2.0	 **3.138**	70
4	 **3.135**	2.2/3.0 6.0/7.0	 **3.139**	78 83

[a]1,2-DiMeIm was added dropwise as a solution in CH_2Cl_2 over 6 h to a solution of the carbohydrate, while the imidazolium salt was added in 3 portions over 6 h.

Direct regioselective protection of 3,4-diols of galactosides is common with reaction preferably occurring on the less sterically hindered 3-OH, and the reaction of galactoside **3.128** with reagent **2.4** follows this pattern.[74] We also presented several examples of this pertaining to sulfation with SO_3 complexes in section 3.1.1. Several reports have appeared describing the regioselective protection (acylation, benzylation) of the 3-OH of 3,4-diols of 2-deoxy-2-amino glucosides in which the amino group is protected with an acyl or phthalimido group.[75,76] A report has appeared describing the selective benzylation of the 3-OH of a 3,4-diol of a 2-deoxy-2-azido glucosides.[77] However, we have been unable to find reports describing the protection of the 3-OH in 3,4-diols of glucosides with good regioselectivity.[78] Our results indicate that, at least for the sulfation reactions studied here, good selectivity can be achieved; however, further investigation will be required to determine if this level (or better) of selectivity can be achieved with other protecting groups and other 3,4-diols of glucosides.

The ability of reagent **2.4** to selectively sulfate the primary 6-OH over a secondary hydroxyl group by the slow addition of a solution of (1,2-DiMeIm) to a solution of the carbohydrate and reagent **2.4** was examined (Table 3.3). In most cases good selectivity was achieved (65-79% yield of 6-*O*-sulfated product) when one or two secondary OH's were present, with compound **3.140** (entry 1) being an exception that gave the 6-O-sulfated product in a 60% yield. Disulfated products were isolated in 15% and 10% yields with carbohydrates **3.140** and **3.141**, respectively. No products resulting from monosulfation of just the secondary hydroxyls were detected. For compounds bearing TCE sulfates at the 2- and 3-positions (entries 3 and 4), the 6-OH could be selectively sulfated over the 4-OH in good yield by direct addition of an excess of the base and sulfating agent in a single portion. Only trace amounts of what appeared to be tetrasulfated products were formed. Attempts to selectively monosulfate methyl α- and β-D-glucopyranoside using 1.2 equiv of reagent **2.4** and 1.5 equiv of 1,2-DiMeIm led to a mixture of sulfated products.

Table 3.3. Selective sulfation of primary hydroxyls in monosaccharides with reagent **2.4**.

Entry	Substrate	Equiv Salt/ equiv 1,2-DiMeIm	Major product	% Yield major product
1	 **3.140**	2.0/2.5[b]	 **3.148**	60
2	 **3.141**	2.0/2.5[b]	 **3.149**	68
3	 **3.142**	1.5/2.0[b] 3.0/4.0[a]	 **3.150**	68[b] 75[a]
4	 **3.143**	3.0/4.0[a]	 **3.151**	72
5	 **3.144**	1.5/2.0[c]	 **3.152**	68
6	 **3.145**	1.2/1.5[b]	 **3.153**	65
7	 **3.146**	1.5/2.0[b]	 **3.154**	79
8	 **3.147**	1.5/2.0[c]	 **3.155**	75

[a] The imidazolium salt and the 1,2-DiMeIm were added in a single portion to a solution of the carbohydrate in CH_2Cl_2, and the reactions were stirred 24-40 h. [b] 1,2-DiMeIm was added dropwise as a solution in CH_2Cl_2 over 4-6 h to a solution of carbohydrate and imidazolium salt in CH_2Cl_2 then the reactions were stirred 24-30 h. [c] 1,2-DiMeIm was added dropwise as a solution in CH_2Cl_2 over 6 h to a solution of the carbohydrate, while the imidazolium salt was added in 3 portions over 6 h.

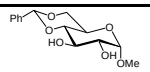
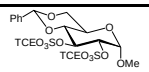
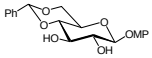
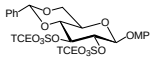
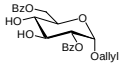
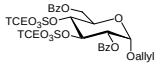
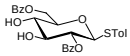
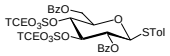
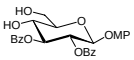
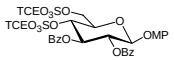
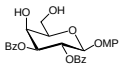
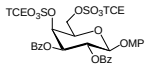
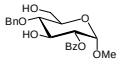
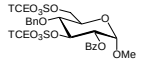
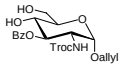
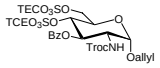
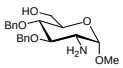
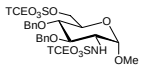
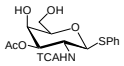
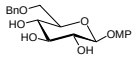
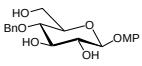
Finally, we examined glucosamine **3.147** (Table 3.3, entry 8) as a candidate for selective sulfation. We had previously found that reagent **1.83** does not readily sulfate amines yet is capable of sulfating alcohols in good yield, whereas reagent **2.4** readily sulfates both amines and alcohols.[40] Subjecting **3.147** to 1.5 equiv of **2.4** and 2.0 equiv of DMI gave the *N*-monosulfated product **3.155** in a 75% yield with a 10% yield of the disulfated product. Surprisingly, subjecting **3.147** to 1.5 equiv of reagent **1.83** and 2.0

equiv of 1-methylimidazole (1-MeIm) also gave the *N*-monosulfated product **3.155** in a 70% yield and only a trace amount of the disulfated product. Increasing the amount of reagent **1.83** and (1-MeIm) did not result in an increase in the yield of the N-sulfated product **3.155**, however, there was an increase in the amounts of unidentified byproducts formed as well as disulfated product as determined by TLC.

3.3.2. Multiple Introduction of TCE-Protected Sulfate into Monosaccharides

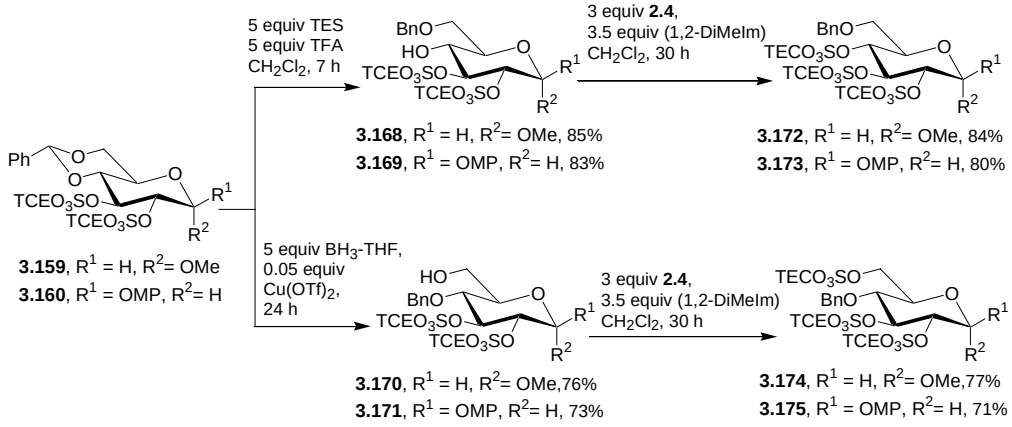
Polysaccharides often contain residues that bear more than one sulfate groups. Since disulfated products were sometimes formed during the selective sulfation studies mentioned above, we anticipated that disulfation of certain carbohydrates could be achieved in good yield using reagent **2.4**. As mentioned above, we were unable to obtain the disulfated products derived from 2,3-diols **3.124**-**3.126** and 3,4-diols **3.133** and **3.135**. However, 2,3-diols **3.122** and **3.123** and 3,4-diol **3.132** were disulfated in good yield using 3.5-5.0 equiv of reagent **2.4** and 4.0-6.0 equiv of 1,2-DiMeIm (entries 1-3). Surprisingly, 3,4-diol **3.134** gave disuflated product **3.161** in only a 45% yield. A considerable number of unidentified byproducts were formed as determined by TLC. Compounds **3.140**, **3.141**, **3.144**, **3.146**, and **3.147** were also all disulfated in good yield (entries 5-9). Attempts to disulfate compound **3.156** led to a complex mixture of products. It is possible that upon sulfation of the 6-OH group an intramolecular reaction occurs between the sulfur at the anomeric position and C-6 resulting in loss of the sulfate group at C-6 and formation of a reactive cyclic sulfonium ion.

Table 3.4. Multiple sulfations with reagent **2.4**.

Entry	Substrate	Equiv Salt 2.4/ equiv 1,2-DiMeIm	Product	% Yield
1	 **3.122**	5.0/6.0	 **3.159**	94
2	 **3.123**	3.5/4.0	 **3.160**	85
3	 **3.132**	6.0/7.0	 **3.161**	80
4	 **3.134**	5.0/6.0	 **3.162**	45
5	 **3.140**	6.0/7.0	 **3.163**	83
6	 **3.141**	6.0/7.0	 **3.164**	80
7	 **3.144**	5.0/6.0	 **3.165**	77
8	 **3.146**	5.0/6.0	 **3.166**	84
9	 **3.147**	5.0/6.0	 **3.167**	88
10	 **3.156**	5.0/6.0	Complex mixture	ND
11	 **3.157**		Complex mixture	ND
12	 **3.158**		Complex mixture	ND

Although we could readily prepare trisulfated compounds **3.150** and **3.151** (Table 3.3, entries 3 and 4) from their disulfated precursors **3.142** and **3.143**, attempts to

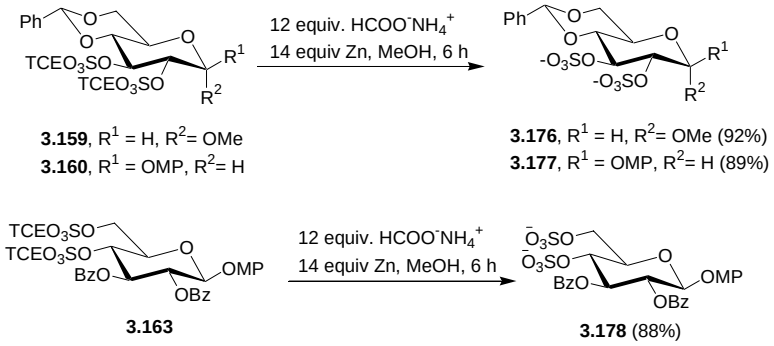
trisulfate triols **3.145**, **3.157**, and **3.158** as well as to tetrasulfate methyl α- and β-D-glucopyranoside under a variety of conditions were unsuccessful in that complex mixtures of sulfated products were obtained. However, trisulfated carbohydrates **3.172-3.175** could be prepared from disulflated carbohydrates **3.168-3.171** as outlined in Scheme 3.30. Subjecting disulfated compounds **3.159** and **3.160** to either triethylsilane/TFA or BH_3-THF/$Cu(OTf)_2$ gave compounds **3.168**- **3.171**, which were then sulfated using 3 equiv of SIS **2.4** and 3.5 equiv of 1,2-DiMeIm to give compounds **3.172-3.175** in yields ranging from 71% to 84%. Deprotection of the sulfate protecting groups in multisulfated carbohydrates can be readily achieved using Zn/ ammonium formate as illustrated for compounds **3.159**, **3.160**, and **3.163** (Scheme 3.30).



Scheme 3.30. Synthesis of trisulfated carbohydrates

3.3.3 Deprotection of TCE-Protected Sulfate in Multisulfated Carbohydrates

Deprotection of the sulfate protecting groups in multisulfated carbohydrates was readily achieved under mild conditions using Zn/ammonium formate as illustrated for compounds **3.159, 3.160** and **3.163** in (Scheme 3.31**)**.



Scheme 3.31. Deprotection of the sulfate group in multisulfated carbohydrates

3.4 Summary and Future Work

In conclusion, we have shown that the direct regioselective incorporation of TCE-protected sulfates into monosaccharides can be achieved using reagent **2.4**. The sulfated compounds were easily purified by flash chromatography. We have also shown that reagent **2.4** can also be used for the direct disulfation of monosaccharides and that trisulfated monosaccharides can also be prepared from the disulfated compounds. The TCE-protected sulphate was deprotected from multisulfated monosaccharides under mild conditions in excellent yields. We expect that the procedures outlined here will prove to be very useful for the multiple and regioselective sulfation of complex sulfated carbohydrates. An important future direction of this work will be to examine tin-mediated sulfations with reagent **2.4**.

3.5 Experimental

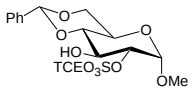
3.5.1 General Information

For general information regarding solvents, NMR, MS, refer to section 2.4.1 in Chapter 2. All commercially available reagents in chapter 3 were purchased from either Sigma Aldrich or Alfa Aesar.

3.5.2 Experimental Syntheses and Characterization

General procedure for the Selective Sulfation of Compounds 3.122-3.126, 3.140-3.143, 3.145, and 3.146. Method A: Reagent **2.4** (1.5 g, 3.32 mmol) and 1,2-DiMeIm (0.38 g, 3.98 mmol) were added in one portion to a solution of the appropriate monosaccharide (0.66 mmol, 1 equiv.) in CH_2Cl_2 (4 mL) at 0 ^oC (ice bath). The reaction was stirred, gradually allowed to warm to room temperature by allowing the ice bath to melt, and then stirred at room temperature until the reaction is complete (TLC, 1:2, EtOAc/hexanes), 24-40 h. The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and concentrated to crude brown oil. The crude residue was purified by flash chromatography (1:4, EtOAc/hexanes).

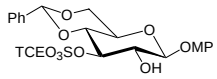
Method B: To the appropriate monosaccharide (0.66 mmol, 1 equiv.) in dry CH_2Cl_2 (2.0 mL) at 0 ^oC (ice bath) was added reagent **2.4** (0.61 g, 1.33 mmol) followed by the addition of a solution of 1,2-DiMeIm (0.16 g, 1.67 mmol) in CH_2Cl_2 (1 mL) over 6 h using a syringe pump. The ice bath was removed, and the reaction was allowed to warm to room temperature and left stirring until the reaction was complete by TLC (approximately 24 h). The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and concentrated to a crude brown oil. The crude residue was purified by flash chromatography (1:4, EtOAc/hexanes).



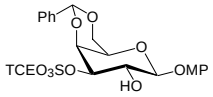
Methyl-2-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-α-D-glucopyranoside (3.127):

58% (method A) and 68% (method B) as a clear colorless syrup, (Table 3.1). ^1H NMR (300 MHz, $CDCl_3$) δ 2.41 (b, 1H, OH), 3.52-3.51 (m, 4H, OCH_3 , H4), 3.84-3.90 (m, 2H,

H6`, H5), 4.22-4.31 (m, 2H, H3, H6), 4.50 (dd, 1H, *J* = 9.4, 3.7 Hz, H2), 4.71, 4.82 (AB system, 2H, *J*= 10.7 Hz, CH_2CCl_3), 5.12 (d, 1H, *J* = 3.6 Hz, H1), 5.53 (s, 1H, CHPh), 7.41 (m, 3H, ArH), 7.43 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 61.7, 68.3, 68.6, 79.7, 81.1, 81.9, 92.5, 97.2, 102.0, 126.1, 128.3, 129.4, 136.5; $[\alpha]_D^{26}$ = -44.2 (c 1.0, $CHCl_3$); HRMS (ESI) *m/z* = 492.9910, $C_{16}H_{20}Cl_3O_9S$ $(M+H)^+$ requires 492.9894.

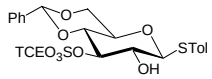


***p*-Methoxyphenyl-3-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-β-D-glucopyranoside (3.128):** 55% (method A) and 60% (method B) as a white solid, (Table 3.1). Mp 130-132 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 2.81, (d, 1H, *J* = 3.1 Hz, OH), 3.50 (ddd, 1H, *J* = 9.6, 9.5, 4.8 Hz, H5), 3.71-3.82 (m, 5H, OCH_3 , H6`, H4), 3.92 (dt, 1H, *J* = 8.9, 7.5, 3.1 Hz, H2), 4.31 (dd, 1H, *J* = 10.5, 9.9, 4.9 Hz, H6), 4.52-4.60 (AB system, 2H, *J* = 10.9 Hz, CH_2CCl_3), 4.81, 4.90 (m, 2H, H3, H1), 5.54 (s, 1H, CHPh), 6.82 (d, 2H, *J* = 8.9 Hz, ArH), 7.01(d, 2H, *J* = 9.0 Hz, ArH), 7.31 (m, 3H, ArH), 7.42 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 55.8, 65.1, 68.0, 71.9, 77.5, 79.6, 86.8, 93.4, 100.9, 101.8, 115.0, 118.5, 126.6, 128.6, 129.4, 137.4, 151.2, 155.3; $[\alpha]_D^{26}$ = 76.4 (c 1.0, $CHCl_3$); HRMS (ESI) *m/z* = 585.0154, $C_{22}H_{24}C_{l3}O_{10}S$ $(M+H)^+$ requires 585.0155.



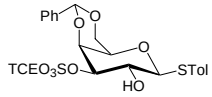
***p*-Methoxyphenyl-3-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-β-D-galactopyranoside (3.129):** 92% (method A) and 85% (method B) as a white solid, (Table 3.1). Mp 138-140 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 2.62, (d, 1H, *J* = 2.5 Hz, OH), 3.51 (br, 1H, H5), 3.72 (s, 3H, OCH_3), 4.02 (dd, 1H, *J* = 12.5, 5.4 Hz, H6`), 4.21-4.32 (m, 2H, H2, H6), 4.63 (d, 1H, *J* = 3.3 Hz, H4), 4.71-4.74 (m, 2H, H3, 1H of CH_2CCl_3), 4.81 (d, 1H, *J* = 7.7 Hz, H1

), 4.91 (d, 1H, *J*= 10.6 Hz, the second H of CH_2CCl_3), 5.52 (s, 1H, CHPh) 6.80 (d, 2H, *J* = 8.9 Hz, ArH), 7.02 (d, 2H, *J* = 8.9 Hz, ArH), 7.31 (m, 3H, ArH), 7.52 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 55.8, 65.5, 67.5, 68.5, 73.4, 79.7, 85.2, 93.2, 100.0, 101.1, 114.9, 118.3, 126.5, 128.6, 129.4, 138.3, 151.3, 155.1; $[\alpha]_D^{26}$ = 81.6 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 585.0168, $C_{22}H_{24}Cl_3O_{10}S$ $(M+H)^+$ requires 585.0156.



***p*-Tolyl-3-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-1-thio-β-D-glucopyranoside**

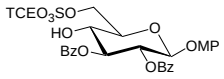
(3.130): 71% (method A) and 78% (method B) as a white solid, (Table 3.1). Mp 96-98 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (s, 3H, CH_3), 2.82 (s, 1H, OH), 3.53-3.91 (m, 4H, H5, H4, H2, H6`), 4.42 (dd, 1H, *J* = 10.3, 4.5 Hz, H6), 4.42-4.50 (m, 2H, CH_2CCl_3, H1), 4.82 (t, 1H, *J* = 8.9 Hz, H3), 5.42 (s, 1H, CHPh) 7.11-7.42 (m, 9H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.2, 68.4, 70.4, 70.7, 77.8, 79.7, 86.0, 88.7, 92.5, 102.2, 126.0, 126.2, 128.6, 129.6, 130.1, 134.3, 136.1, 139.5; $[\alpha]_D^{26}$ = 54.3 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 584.9981, $C_{22}H_{24}Cl_3O_8S_2$ $(M+H)^+$ requires 584.9978.



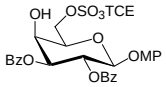
***p*-Tolyl-3-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-1-thio-β-D-galactopyranoside**

(3.131): 94% (method A) and 88% (method B) as a white solid, (Table 3.1). Mp 92-94 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.36, (s, 3H, CH_3), 2.68 (b, 1H, OH), 3.54 (s, 1H, H5), 3.39 (t, 1H, J=9.4, H2), 4.02, 4.35 (AB, 2H, *J*=12.4 Hz, H6`, H6), 4.48 (d, 1H, *J*=9.3 Hz, H1), 4.56 (d, 1H, *J*=2.1 Hz, H4), 4.66, 4.86 (AB, 2H, *J* =10.8 Hz, CH_2CCl_3), 4.72 (dd, 1H, *J* = 9.4, 2.7 Hz, H3), 5.52 (s, 1H, CHPh), 7.10 (d, 2H, *J* = 7.6 Hz, ArH), 7.41 (s, 5H, ArH), 7.57 (d, 2H, *J* = 7.6 Hz, ArH); ^{13}C NMR(75 MHz, CDCl3) δ 21.8, 65.1, 68.8, 69.3,

73.3, 78.3, 79.7, 84.8, 86.8, 92.6, 100.9, 125.2, 126.2, 126.4, 128.1, 129.6, 129.9, 133.1, 134.4, 134.6, 137.1, 139.0; $[\alpha]_D^{26}$ = 49.4 (c 1.0, CHCl3); HRMS (ESI) m/z = 584.9987, $C_{22}H_{24}Cl_3O_8S_2$ $(M + H)^+$ requires 584.9978.

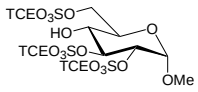


***p*-Methoxyphenyl-2,3-di-*O*-benzoyl-6-*O*-trichloroethylsulfo-β-D-glucopyranoside (3.148):** 60% (method B) as a colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (s, 4H, OCH_3 , OH), 4.02 (m, 2H, H5, H4), 4.62-4.81 (m, 4H, H6 , H6`, CH_2CCl_3), 5.23 (d, 1H, *J* = 7.8 Hz, H1), 5.52 (t, 1H, *J* = 9.3 Hz , H3), 5.74 (t, 1H, *J* = 9.2 Hz , H2), 6.73 (d, 2H, *J* = 8.8 Hz, ArH), 6.92 (d, 2H, *J* = 8.8 Hz, ArH), 7.47 (m, 6H, ArH), 7.91 (d, 2H, *J* = 7.3 Hz, ArH) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 68.9, 71.0, 71.8, 73.5, 76.7, 79.6, 92.3, 100.3, 114.6, 118.5, 128.4, 128.5, 128.9, 129.6, 129.9, 133.4, 133.8, 150.7, 155.8, 165., 167.6; $[\alpha]_D^{26}$ = -36.2 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 705.0370 $(M+H)^+$ $C_{29}H_{28}Cl_3O_{12}S$ requires 705.0367.

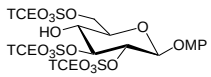


***p*-Methoxyphenyl-2,3-di-*O*-benzoyl-6-*O*-trichloroethylsulfo-β-D-glactopyranoside (3.149):** 68% (method B) as a white solid, (Table 3.3). Mp 71-73 ^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.7 (d, 1H, *J* = 4.5 Hz, OH at C2), 3.7 (s, 3H, OCH_3), 4.21 (t, 1H, *J* = 6.6 Hz, H5), 4.43 (s, 1H, H4), 4.60-4.71 (m, 4H, H6, H6`, CH_2CCl_3), 5.12 (d, 1H, *J* = 7.9 Hz, H1), 5.31- 5.40 (dd, 1H, *J* = 10.2, 2.9 Hz , H3), 5.73 (t, 1H, *J* = 10.1, 8.2 Hz , H2), 6.72 (d, 2H, *J* = 8.9 Hz, ArH), 6.91 (d, 2H, *J* = 8.9 Hz, ArH), 7.30 (m, 4H, ArH), 7.42 (t, 2H, ArH) 7.90 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 66.9, 69.0, 71.7, 71.9, 73.8, 76.7, 79.7, 92.3, 100.7, 114.6, 118.6, 128.4, 128.6, 129.1, 129.7, 129.9, 133.4, 133.7,

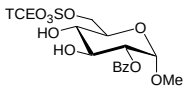
150.8, 155.8, 165.4, 165.7; $[\alpha]_D^{26}$ = 69.2 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 705.0394 $(M+H)^+$ $C_{29}H_{28}Cl_3O_{12}S$ requires 705.0367.



Methyl-2,3,6-tri-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.150): 75% (method A) and 60% (method B) as a colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.21 (s, 1H, OH), 3.42 (s, 3H, OCH_3), 3.90 (br, 2H, H5, H4), 4.53- 4.81 (m, 9H, H6, H6`, H2, $3CH_2CCl_3$), 5.04 (br, 1H, H3), 5.21 (d, 1H, *J* = 2.8 Hz, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 68., 68.6, 71.4, 78.0, 79.7, 80.2, 80.4, 83.4, 92.2, 92.2, 92.3, 96.3; $[\alpha]_D^{26}$ = 69 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 824.7010 $(M+H)^+$ $C_{13}H_{18}Cl_9O_{15}S_3$ requires 824.7005.

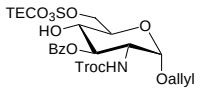


***p*-Methoxyphenyl-2,3,6-tri-*O*-trichloroethylsulfo-β-D-glucopyranoside (3.151):** 72% (method A) as a colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.09 (br, 1H, OH), 3.75 (s, 4H, OCH3, H5), 4.04 (t, 1H, *J*= 8.5 Hz, H4), 4.06-4.91 (m, 10H, H6, H6`, H2, $3CH_2CCl_3$, H3), 5.12 (d, *J* = 8.5 Hz, 1H), 5.21 (d, 1H, *J* = 6.7 Hz, H1), 6.81 (d, 2H, *J* = 8.9 Hz, ArH), 6.98 (d, 2H, = 8.9 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 68.2, 70.7, 72.5, 79.8, 80.1, 80.5, 80.8, 85.5, 92.2, 92.2, 92.3, 98.6, 114.9, 118.7, 149.5, 156.4; $[\alpha]_D^{26}$ = 77.2 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 916.7208 $(M+H)^+$ $C_{19}H_{23}Cl_9O_{16}S_3$ requires 916.7188.



Methyl-2-*O*-benzoyl-6-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.153): 65% (method B) as a colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.31 (br, 5H, OCH_3 , 2OH), 3.62 (t, 1H, *J* = 9.4 Hz, H4), 3.80 (d, 1H, *J* = 9.6 Hz, H5), 4.01 (t, 1H,

J = 9.6 Hz, H3), 4.62 (b, 2H, H6, H6`), 4.71 (b, 2H, CH_2CCl_3), 4.92 (dd, 1H, *J* = 9.6, 2.3 Hz , H2), 4.93 (d, 1H, *J* = 2.3 Hz , H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 68.5, 69.9, 71.8, 72.3, 73.4, 79.5, 92.4, 97.3, 128.4, 129.1, 129.8, 133.5, 166.4; $[\alpha]_D^{26}$ = 84.1 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 508.9836 $(M+H)^+$ $C_{16}H_{20}Cl_3O_{10}S$ requires 508.9843.

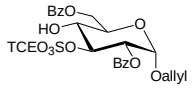


Allyl-2-deoxy-2-*N*-trichloroethoxyamido-3-*O*-benzoyl-6-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.154): 79% (method B) as a colorless syrup (Table 3.3). ^1H NMR (300 MHz, CDCl3) δ 3.12 (br, 1H, OH), 3.84 (t, 1H, *J*=9.3 Hz, H4), 4.05 (m, 2H, H5, H of allyl CH_2), 4.22 (m, 2H, H of allyl CH_2, H2), 4.46, 4.65 (AB system, 2H, *J* = 12.0 Hz, H6, H6`), 4.64 (s, 2H, CH_2CCl_3), 4.73 (s, 2H, Troc CH_2), 4.97 (d, 1H, *J*= 3.5 Hz, H1), 3.32 (m, 4H, oleifinic CH_2 of allyl, H3, NH), 5.91 (m, 1H, olefinic H), 7.43 (t, 4H, *J*=7.6 Hz, ArH), 7.56 (t, 1H, *J*= 7.5 Hz, ArH), 7.99 (d, 2H, *J*= 7.4 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 53.3, 68.8, 69.0, 69.7, 72.1, 74.4, 75.2, 79.6, 92.5, 95.0, 96.4, 119.0, 128.5, 128.6, 130.1, 132.7, 133.8, 154.2, 168.0; $[\alpha]_D^{26}$ = 69.2 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 707.9204, $C_{21}H_{24}NCl_6O_{11}S$ $(M+H)^+$ requires 707.9201.

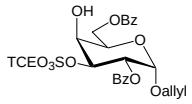
General procedure for the Selective Sulfation of Compounds 3.132-3.135, 3.143 and 3.147.

To the appropriate carbohydrate (0.23 mmol) in dry CH_2Cl_2 (2 mL) at 0 ^oC (ice bath) was added reagent **2.4** (0.053 g, 0.11 mmol), followed by the addition of a solution of 1,2-DiMeIm (0.16 g, 1.67 mmol) in CH_2Cl_2 (2 mL) over 8 h using a syringe pump. During the addition of the 1,2-DiMeIm two portions of reagent **2.4** (0.053 g, 0.11 mmol for each portion) was added after 3 and 6 h and the ice batch was removed after the initial 1 h. The reaction was left stirring until the reaction was complete by TLC (approx 24 h). The

reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to brown crude oil. The crude residue was purified with flash chromatography (1:4, EtOAc/hexanes).

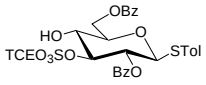


Allyl-2,6-di-*O*-benzoyl-4-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.136): 70% as a colorless syrup (Table, 3.2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (br, 1H, OH), 3.79 (t, 1H, *J*=9.4 Hz, H4), 4.03 (m, 2H, H5, 1H of allyl CH_2), 4.19 (AB, 1H, *J*=12.7 Hz, the second H of allyl CH_2), 4.46, 4.92 (AB system, 2H, *J*= 12.4 Hz, H6, H6`), 4.72 (s, 2H, CH_2CCl_3), 5.07-5.14 (m, 2H, H2, 1H of olefinic CH_2), 5.21-5.29 (m, 3H, 1H of olefinic CH_2, H3, H1), 5.77 (m, 1H, olefinic H), 7.41-7.61 (m, 6H, ArH), 8.09 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.01, 68.6, 69.0, 70.17, 70.91, 79.9, 84.73, 92.5, 95.23, 118.3, 128.6, 128.7, 129.0, 129.9, 130.1, 132.8, 133.7, 133.71, 165.7, 167.7; $[\alpha]_D^{26}$ =56.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z=639.0286, $C_{25}H_{26}Cl_3O_{11}S$ $(M+H)^+$ requires 639.0261.

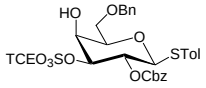


Allyl-2,6-di-*O*-benzoyl-4-*O*-trichloroethylsulfo-α-D-galactopyranoside (3.137): 77% as colorless syrup (Table 3.2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91 (d, 1H, *J*= 4.0 Hz, OH), 3.99, 4.15 (dd of AB system, 2H, *J* = 13.1, 5.8 Hz, allyl CH_2), 4.27 (t, 1H, *J*= 6.2 HZ, H5), 4.42-4.73 (m, 5H, H6, CH_2CCl_3, H4, H6`), 5.01- 5.32 (m,4H, olefinic CH_2, H1, H3), 5.56 (dd, 1H, *J*= 8.6, 3.3 Hz, H2), 5.79 (m, 1H, olefinic H), 7.44 (m, 4H, ArH), 7.58 (m, 2H, ArH), 8.04 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 62.5, 67.6, 67.6, 68.9, 79.8, 81.6, 92.2, 95.5, 118.1, 127.5, 128.5, 128.7, 129.0, 129.2, 129.7, 129.9, 132.9,

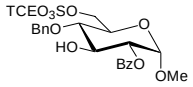
133.5, 133.6, 165.6, 166.6; $[\alpha]_D^{26}$ = 59.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 639.0261, $C_{25}H_{26}Cl_3O_{11}S$ $(M+H)^+$ requires 639.0251.



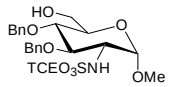
***p*-Tolyl-2,6-*O*-dibenzoyl-3-*O*-trichloroethylsulfo-1-thio-β-D-glucopyranoside (3.138):** 70% as colorless syrup (Table 3.2). ^1H NMR (300 MHz, $CDCl_3$) δ 2.25 (s, 3H, CH_3), 3.6-3.81 (m, 3H, OH, H5, H4), 4.53-4.61 (m, 3H, CH_2CCl_3, H6), 4.76-4.84 (m, 2H, H6`, H1), 4.97 (t, 1H, *J* = 9.5 Hz, H3), 5.19 (t, 1H, *J* = 9.5 Hz, H2), 6.95, 7.34 (AB system, 4H, *J* = 7.5 Hz, ArH), 7.41-7.60 (m, 6H, ArH), 8.01 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 62.9, 68.3, 69.5, 77.9, 79.9, 85.6, 88.0, 92.3, 126.8, 128.5, 129.06, 129.1, 129.6, 130.01, 130.1, 133.66, 133.69, 134.3, 138.9, 165.1, 167.3; $[\alpha]_D^{26}$ = 98.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 705.0177, $C_{29}H_{28}Cl_3O_{10}S_2$ $(M+H)^+$ requires 705.0189.



***p*-Tolyl-2-*O*-benzyloxycarbonyl-3-*O*-trichloroethylsulfo-6-*O*-benzyl-1-thio-β-D-galactopyranoside (3.139):** 78 and 83% as colorless syrup (Table 3.2). ^1H NMR (300 MHz, $CDCl_3$) δ 2.21 (s, 3H, CH_3), 3.0 (br, 1H, OH), 3.62 (t, 1H, *J* = 8.9 Hz, H5), 3.74 (m, 2H, H6, H6`), 4.51-4.62 (m, 6H, CH_2Ph, H4,H1, H3,1H of CH_2CCl_3), 4.72 (d, 1H, *J* = 10.7 Hz, 1H of CH_2CCl_3), 5.11-5.23 (m, 3H, H2, CH_2Ph), 7.01 (d, 2H, *J* = 7.8 Hz, ArH), 7.21-7.33 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 68.0, 69.4, 70.4, 70.8, 73.9, 76.0, 79.9, 84.9, 86.4, 92.4, 127.5, 127.8, 128.1, 128.3, 128.6, 128.6, 128.7, 129.7, 133.8, 134.7, 137.o, 138.8, 153.9; $[\alpha]_D^{26}$ = 95.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 721.0525, $C_{30}H_{32}Cl_3O_{10}S_2$ $(M+H)^+$ requires 721.0502.



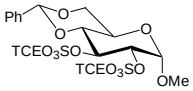
Methyl-2-*O*-benzoyl-4-*O*-benzyl-6-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.152): 68% as colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (d, 1H, *J* = 3.8 Hz, OH), 3.31 (s, 3H, OCH_3), 3.52 (t, 1H, *J* = 9.9 Hz, H4), 3.92 (m, 1H, H5)), 4.31 (dt, 1H, *J* = 9.4, 8.6, 3.7 Hz H3), 4.53 (m, 2H, H6, H6`), 4.61-4.70 (m, 3H, CH_2CCl_3, 1H of CH_2Ph), 4.81-4.91 (m, 2H, H2, 1H of CH_2Ph), 4.92 (d, 1H, *J* = 3.5 Hz, H1), 7.31-7.50 (m, 8H, ArH), 8.01 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 67.9, 72.3, 72.3, 73.7, 75.0, 76.9, 79.6, 92.5, 92.3, 97.0, 128.2, 128.2, 128.5, 1287, 129.3, 129.9, 133.5, 137.5, 166.3; $[\alpha]_D^{26}$ = 54.6 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 599.0295 $(M+H)^+$ $C_{23}H_{26}Cl_3O_{10}S$ requires 599.0312.



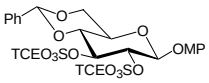
Methyl-3,4-di-*O*-benzyl-2-deoxy-2-*N*-trichloroethoxysulfamido-α-D-glucopy-ranoside (3.155): 75% as colorless syrup (Table 3.3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (t, 1H, *J* = 6.7 Hz, OH), 3.31 (s, 3H, OCH_3), 3.62-3.83 (m, 6H, H5, H4, H3, H2, H6, H6`), 4.51-4.50 (AB, 2H, *J* = 10.8 Hz, CH_2CCl_3), 4.61, 4.83 (AB, 2H, *J* = 10.9 Hz, CH_2Ph), 4.71-5.01 (m, 4H, CH_2Ph, H1, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.3, 58.2, 61.4, 71.1, 75.0, 75.6, 77.8, 78.1, 79.2, 93.3, 97.9, 128.0, 128.0, 128.5, 128.6, 137.7; $[\alpha]_D^{26}$ = 69.6 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 601.0957 $(M+NH4)^+$, $C_{23}H_{32}Cl_3NO_8S$ requires 601.0945.

General procedure for the preparation of multiply sulfated monosaccharides 3.159-3.167 (Table 3.4). To the appropriate carbohydrate (0.85 mmol) in CH_2Cl_2 (3.4 mL) at 0

^{o}C (ice bath) were added 1,2-DiMeIm (0.32 g, 3.4 mmol) and reagent **2.4** (1.16 g, 2.54 mmol). The ice bath was removed, and the reaction was allowed to warm to room temperature and then stirred for 15 h. After 15 h, additional aliquots of 1,2-DiMeIm (0.163 g, 1.7 mmol) and reagent **2.4** (0.778 g, 1.69 mmol) were added at room temperature. Upon completion by TLC (approximately 30 h) the reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and concentrated to crude brown oil. The crude residue was purified with flash chromatography (1:5, EtOAc/hexanes).

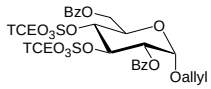


Methyl-2,3-di-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-β-D-gluco-pyranoside (3.159): 94% as a white solid (Table 3.4). Mp 128-130 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (s, 3H, OCH_3), 3.81-3.72 (m, 2H, H4, H6`), 3.79 (ddd, 1H, *J*=9.8, 9.7, 4.6 Hz, H5), 4.35 (dd, 1H, *J*=10.4, 4.6 Hz, H6), 4.42, 4.51 (AB system, 2H, *J* = 11.1 Hz, CH_2CCl_3), 5.68 (dd, 1H, *J*=9.3, 3.6 Hz, H2), 4.83, 4.91 (AB, 2H, *J*=11.0 Hz,CH_2CCl_3), 5.17 (t, 1H, *J*=9.6 Hz, H3), 5.25 (d, 1H, *J*=3.1 Hz, H1), 5.51 (s, 1H, CHPh), 7.35 (m, 3H, ArH), 7.46 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 62.3, 68.6, 78.53, 78.8, 79.5, 80.1, 80.4, 92.4, 92.4, 97.5, 102.8, 126.4, 128.7, 129.9, 135.8; $[\alpha]_D^{26}$ = 44.0 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z =702.8589, $C_{18}H_{20}Cl_6O_{12}S_2$ $(M + H)^+$ requires 702.8606.



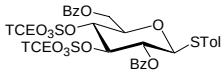
***p*-Methoxyphenyl-2,3-di-*O*-trichloroethylsulfo-4:6-*O*-benzylidine-β-D-gluco-pyranoside (3.160).** 85% as a white solid (Table 3.4). Mp 132-134 ^{o}C; ^{1}H NMR (300

MHz, $CDCl_3$) δ 3.61, (ddd, 1H, *J* = 9.4, 9.2, 4.8 Hz, H5), 3.70 (s, 3H, OCH_3), 3.71-3.90 (m, 2H, H4, H6`), 4.41-4.43 (m, 2H, H6, 1H of CH_2CCl_3), 4.42, 4.51 (AB, 2H, *J* = 11.1 Hz, CH_2CCl_3), 4.81-4.82 (m, 2H, CH_2CCl_3), 4.91-5.02 (m, 2H, H2, H3), 5.11 (d, 1H, *J* = 7.0 Hz, H1), 5.51 (s, 1H, CHPh), 6.8 (d, 2H, *J* = 9.0 Hz, ArH), 7.01 (d, 2H, *J* = 8.9 Hz, ArH),7.31 (m, 3H, ArH), 7.42 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 65.84, 68.2, 77.6, 80.1, 80.5, 81.2, 92.4, 92.6, 100.0, 102.6, 144.7, 119.1, 126.4, 128.7, 129.9, 135.6, 150.0, 156.3; $[\alpha]_D^{26}$ = -48.6 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 794.8896, $C_{24}H_{25}Cl_6O_{13}S_2$ $(M+H)^+$ requires 794.8868.

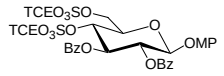


Allyl-2,6-di-*O*-benzoyl-3,4-di-*O*-trichloroethylsulfo-α-D-glucopyranoside

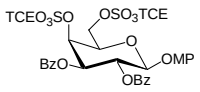
(3.161). 80% as a colourless syrup (Table 3.4). ^1H NMR (300 MHz, $CDCl_3$) δ 3.99, 4.18 (AB, dd, 2H, *J* = 12.9, 7.6 Hz, allylic CH_2), 4.32 (d, 1H, *J* = 9.6 Hz, H5), 4.52-4.57 (dd , 1H, *J* = 12.5, 3.9 Hz, H6`), 4.6-4.76 (AB, 2H, *J* = 11.1 Hz, CH_2CCl_3), 4.87 (m, 3H, H6, CH_2CCl_3), 5.06-5.12 (m, 2H, H4, H2), 5.19-5.25 (m, 3H, H1, olefinic CH_2), 5.49 (t, 1H, *J* = 9.1 Hz, H3), 5.75-5.77 (ddd, 1H, J = 6 Hz, oleifinic H), 7.42-7.49 (m, 4H, ArH), 7.58 (m, 2H, ArH), 8.06-8.13 (m, 4H, ArH);^{13}C NMR (75 MHz, $CDCl_3$) δ 61.7, 67.2, 69.4, 70.8, 78.1, 80.4, 80.6, 81.4, 92.2, 92.3, 94.7, 118.8, 128.4, 128.5, 128.6, 129.4, 129.7, 130.2, 132.3, 133.3, 133.9; $[\alpha]_D^{25}$ = 56.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 848.8996, $C_{27}H_{27}Cl_6O_{14}S_2$ $(M+H)^+$ requires 848.8973.



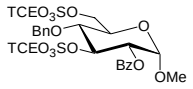
***p*-Tolyl-2,6-*O*-dibenzoyl-3,4-di-*O*-trichloroethylsulfo-1-thio-β-D-glucopyranoside (3.162):** 45% as a colourless syrup (Table 3.4). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.27 (s, 3H, CH_3), 4.02 (d, 1H, *J*= 8.1 Hz, H5), 4.53-4.57 (dd, 1H, *J* = 12.5, 4.3 Hz,H6), 4.11, 4.80 (AB system, 3H, *J*= 11.2 Hz, CH_2CCl_3, H1 overlapped with one proton of the CH_2CCl_3), 4.91 (s, 2H, CH_2CCl_3), 5.01-5.05 (m, 2H, H4, H6`), 5.23 (t, 1H, *J* = 9.2 Hz, H3), 5.32 (t, 1H, *J* = 9.4 Hz, H2), 6.92, 7.31 (AB system, 4H, *J*= 7.6 Hz, ArH), 7.53 (m, 4H, ArH), 7.65 (m, 2H, ArH), 8.01 (m, 4H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 61.8, 69.7, 75.5, 77.6, 80.6, 80.7, 84.1, 85.5, 92.3, 125.8, 128.5, 128.6, 128.8, 129.4, 129.7, 129.9, 130.2, 133.4, 133.9, 134.7, 139.3, 165.0, 165.7; $[\alpha]_D^{26}$ = 67.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 914.8701, $C_{32}H_{28}Cl_6O_{13}S_3$ $(M+H)^+$ requires 914.8801.



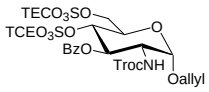
***p*-Methoxyphenyl-2,3-di-*O*-benzoyl-4,6-di-*O*-trichloroethylsulfo-β-D-glucopy-ranoside (3.163):** 83% as a colourless syrup (Table 3.4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (s, 3H, OCH_3), 4.11, (ddd, 1H, *J* = 6.9, 5.0, 2.2 Hz, H5), 4.51-4.62 (AB, 2H, *J* = 11.0 Hz, CH_2CCl_3), 4.72 (m, 4H, H6, CH_2CCl_3, H6`), 5.11 (t, 1H, *J* = 9.3 Hz, H4,), 5.23 (d, 1H, *J* = 7.6 Hz, H1), 5.61 (t, 1H, *J* =9.0, 8.0 Hz, H2), 5.65 (t, 1H, *J* = 9.3 Hz, H3), 6.72 (d, 2H, *J* = 8.8 Hz, ArH), 6.91 (m, 2H, ArH), 7.32 (m, 4H, ArH), 7.53 (m, 2H, ArH), 7.90 (m, 4H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.6, 70.3, 71.1, 71.2, 71.6, 78.0, 79.9, 80.4, 92.0, 92.3, 100.3, 114.7, 118.9, 128.2, 128.5, 128.6, 129.8, 130.1, 133.6, 133.9, 150.4, 156.1, 164.8, 165.6; $[\alpha]_D^{26}$ = 56.8 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 931.9343, $C_{31}H_{32}NCl_6O_{15}S_2$ $(M+NH_4)^+$ requires 931.9345.



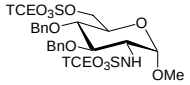
***p*-Methoxyphenyl-2,3-di-*O*-benzoyl-4,6-di-*O*-trichloroethylsulfo-β-D-galactopyranoside (3.164).** 80% as a colourless syrup (Table 3.4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (s, 3H, OCH_3), 4.12, (t, 1H, *J* = 6.3 Hz, H5), 4.61-4.72 (m, 5H, H6, H6`, CH_2CCl_3, 1H of CH_2CCl_3), 4.82 (the second half of the AB system, 1H, *J* = 11.0 Hz, 1H of CH_2CCl_3), 5.21 (d, 1H, *J* = 8.0 Hz, H1,), 5.42-5.41 (m, 2H, H4, H3), 5.90 (t, 1H, *J* = 9.0, 8.3 Hz, H2), 6.81 (d, 2H, *J* = 8.9 Hz, ArH), 6.92 (d, 2H, *J* = 8.9 Hz ArH), 7.31 (m, 4H, ArH), 7.51 (m, 2H, ArH), 7.92 (m, 4H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.6, 68.3, 70.2, 70.7, 71.1, 78.5, 79.9, 80.3, 92.1, 92.3, 100.8, 114.7, 118.8, 128.1, 128.5, 128.7, 129.7, 129.8 130.0, 133.6, 134.0, 150.5, 156.1, 165.1, 165.7; $[\alpha]_D^{26}$ =36.2 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 914.9109, $C_{31}H_{29}Cl_6O_{15}S_2$ $[M+H]^+$ requires 914.9079.



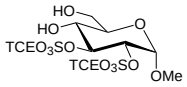
Methyl-2-*O*-benzoyl-4-*O*-benzyl-3,6-di-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.165): 77% as a colourless syrup (Table 3.4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.31 (s, 3H, OCH_3), 3.80, (t, 1H, *J* = 9.5 Hz, H4), 3.93 (d, 1H, *J* = 10.0 Hz, H5), 4.41-4.62 (m, 5H, H6, H6`, CH_2CCl_3, 1H of CH_2Ph), 4.70 (s, 2H, CH_2CCl_3), 4.91-5.12 (m, 3H, 1H of CH_2Ph, H1, H2), 5.43 (t, 1H, *J* = 9.3 Hz, H3), 7.31-7.42 (m, 7H, ArH), 7.50 (t, 1H, *J* = 7.3 Hz, ArH), 8.12 (d, 2H, *J* = 7.4 Hz ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.8, 68.0, 70.9, 71.3, 74.8, 75.4, 79.6, 80.0, 85.1, 92.0, 92.3, 96.9, 128.1, 128.5, 128.6, 128.8, 130.2, 130.3, 133.8, 136.2; $[\alpha]_D^{26}$ = 34.6 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 808.9057, $C_{25}H_{27}Cl_6O_{13}S_2$ $[M+H]^+$ requires 808.9024.



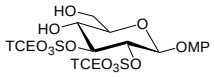
Allyl-2-deoxy-2-*N*-trichloroethoxyamido-3-*O*-benzoyl-4,6-di-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.166). 84% as a colourless syrup (Table 3.4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.09 (m, 1H, H of allyl CH_2), 4.21-4.29 (m, 3H, H of allyl CH_2, H5, H2), 4.41-4.47 (m, 2H, H6, 1H of CH_2CCl_3), 4.59-4.73 (m, 4H, 1H of CH_2CCl_3 , H6`, CH_2CCl_3), 4.77 (s, 2H, Troc CH_2), 4.92-5.0 (m, 2H, H4, H1), 5.28-5.37 (m, 3H, olefinic CH_2 of allyl, NH), 5.69 (t, 1H, *J* = 9.6 Hz, H3), 5.91 (m, 1H, olefinic H), 7.41 (t, 4H, *J*=7.6 Hz, ArH), 7.56 (t, *J*= 7.5 Hz , 1H, ArH), 8.03 (d, 2H, *J*= 7.4 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 54.1, 67.1, 69.6, 70.2, 70.4, 74.4, 78.4, 79.8, 80.3, 92.0, 92.4, 94.9, 96.2, 119.7, 128.3, 128.6, 130.2, 132.2, 134.0, 154.0, 166.2; $[\alpha]_D^{26}$ = 89.2 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z = 917.7842, $C_{23}H_{25}Cl_9NO_{14}S$ $(M+H)^+$ requires 917.7835.



Methyl-3,4-di-*O*-benzyl-6-*O*-trichloroethylsulfo-2-deoxy-2-*N*-trichloroethoxy-sulfamido-α-D-glucopyranoside (3.167): 88% as a colourless syrup (Table 3.4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 3H, OCH_3), 3.51-3.75 (m, 3H, H6, H4, H6`), 3.83-3.87 (dd, 1H, *J* = 10, 2.4 Hz, H5), 4.39-4.48 (m, 2H, H3, H2), 4.65-4.64 (m, 3H, CH_2CCl_3, 1H CH_2Ph), 4.69 (s, 2H, CH_2CCl_3), 4.85-4.91 (m, 3H, CH_2Ph, 1H of the CH_2Ph), 4.94 (d, 1H, *J* = 3.4 Hz, H1), 5.03 (br, 1H, NH), 7.24-7.38 (m, 10H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.7, 58.1, 68.6, 72, 75.3, 75.8, 77.1, 78.2, 79.3, 79.6, 92.5, 93.3, 97.9, 128.09, 128.12, 128.17, 128.3, 128.6, 137.0, 137.3; $[\alpha]_D^{26}$ = 72.3 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 810.9664, $C_{25}H_{33}N_2C_{l6}O_{11}S_2$ $[M+NH_4]^+$ requires 810.9657.



Methyl-2,3-di-*O*-trichloroethylsulfo-α-D-glucopyranoside (3.141). A suspension of carbohydrate **3.159** (0.35 g, 0.5 mmol) in CH_2Cl_2:MeOH (1:2, 2.4 mL CH_2Cl_2, 4.8 mL MeOH) in a round bottom flask equipped with a reflux condenser was heated to 40 ^oC (oil bath) and stirred until all starting material had dissolved. *p*-TsOH (0.009 g, 0.05 mmol) was added and the temperature was increased to 45 ^oC and the reaction was stirred until no starting material remained by TLC (24 h). The reaction was neutralized with NEt_3 and concentrated to a yellow oil. Flash chromatography (1:3, EtOAc:hexanes) provided compound **3.141** as a white solid (0.26 g, 85%). Mp 48-50 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 1.90 (t, 1H, *J* = 5.9 Hz, OCH_3 , OH), 3.21 (d, 1H, *J* = 4.5 Hz, OH), 3.41 (s, 3H, OCH_3), 3.72 (dd, 1H, *J* = 9.6, 5.5, 4.1 Hz, H5), 3.91-4.01 (m, 3H, H6, H6`, H4), 4.62 (dd, 1H, *J* = 9.7, 3.2 Hz, H2), 4.72-4.81 (m, 4H, $2CH_2CCl_3$), 5.01 (t, 1H, *J* = 9.4, 9.1 Hz , H3), 5.12 (d, 1H, *J* =2.7 Hz , H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 61.3, 68.9, 70.6, 78.5, 80.2, 80.2, 84.0, 92.2, 92.4, 96.3; $[\alpha]_D^{26}$ = 88.6 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 614.8289 $(M+H)^+$ $C_{11}H_{17}Cl_6O_{12}S_2$ requires 614.8293.

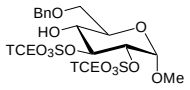


***p*-Methoxyphenyl-2,3-di-*O*-trichloroethylsulfo-β-D-glucopyranoside (3.142).** Compound **3.142** was prepared by the same procedure used for the preparation of compound **3.141**. Flash chromatography (1:3, EtOAc:hexanes) provided **3.142** as a white solid. 80% yield. Mp 62-64 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 2.11 (br, 1H, , OH), 3.52 (b, 2H, OH, H5), 3.80 (s, 3H, OCH_3), 3.91 (m, 2H, H6, H6`), 4.11 (m, 1H, *J* = 8.8, 8.4, 4.1 Hz, H4), 4.82-4.91 (m, 6H, H3, H2, $2CH_2CCl_3$), 5.11 (d, 1H, *J* = 7.0 Hz , H1), 6.81

(d, 2H, *J* = 8.9 Hz, ArH), 7.01 (d, 2H, *J* = 8.9 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 61.4, 68.9, 74.8, 80.3, 80.5, 80.5, 86.0, 92.3, 92.4, 98.7, 114.7, 118.5, 149.8, 156.1; $[\alpha]_D^{26}$ = 64.3 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 723.8809 $(M+NH_4)^+$ $C_{17}H_{24}Cl_6NO_{13}S_2$ requires 723.8820.

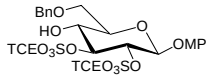
General procedure for the selective opening of the benzylidene acetal in compounds 3.159 and 3.160 using triethysilane/TFA (Scheme 3.30).[79]

To the fully protected sugar (0.976 mmol) in CH_2Cl_2 (6 mL) was added dropwise triethysilane (0.779 mL, 4.88 mmol) followed by trifluoroacetic acid (0.37 mL, 4.88 mmol). The reaction was stirred at room temperature for 9 h until there was no starting material remaining. The reaction was diluted with CH_2Cl_2, carefully quenched with triethylamine, and concentrated to crude syrup. The crude residue was purified with flash chromatography (1:5, EtOAc/hexanes).



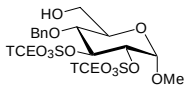
Methyl-2,3-di-*O*-trichloroethylsulfo-6-*O*-benzyl-β-D-glucopyranoside (3.168):

85% as a colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.21 (d, 1H, *J*=3.5 Hz, OH), 3.40 (s, 3H,OCH_3), 3.61 (ddd, 1H, *J*=10.9, 5.6, 5.2 Hz, H5), 3.81 (m, 2H, H6,H6`), 3.92 (ddd, 1H, *J*=9.0, 8.9, 3.4 Hz, H4), 4.63 (m, 3H, H2,CH_2Ph), 4.81 (m, 4H, $2CH_2CCl_3$), 5.01 (t, 1H, *J*=9.4 H3), 5.12 (d, 1H, *J*=3.0 Hz, H1), 7.32 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8, 69.0, 69.1, 70.5, 73.9, 78.5, 80.2, 83.7, 92.3, 92.6, 96.3, 127.8, 128.2, 128.6, 137.0; $[\alpha]_D^{25}$ =96.2 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z=704.8768, $C_{18}H_{23}Cl_6O_{12}S_2$ $(M+H)^+$ requires 704.8762.



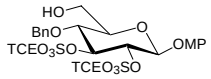
***p*-Methoxyphenyl-2,3-di-*O*-trichloroethylsulfo-6-*O*-benzyl-β-D-glucopyranoside (3.169): 83%** as a colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (br, 1H, OH), 3.60 (dd, 1H, *J* = 10.9, 4.3 Hz, H5), 3.77 (m, 5H, OCH_3, H6, H6`), 4.03 (br, 1H, H4), 4.58 (dd, 2H, *J* = 11.3, 9.9 Hz, CH_2Ph), 4.89 (m, 7H, $2CH_2CCl_3$, H3, H2, H1), 6.81 (m, 2H, ArH), 7.01 (m, 2H, ArH), 7.31 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 69.2, 70.6, 73.2, 73.8, 80.3, 80.4, 80.6, 85.6, 92.5, 99.1, 114.7, 119.0, 127.9, 128.2, 128.6, 137.0, 150.0, 156.2; $[\alpha]_D^{26}$ = 38.6 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 813.9177, $C_{24}H_{30}Cl_6NO_{13}S_2$ $(M+NH_4)^+$ requires 813.9289.

General procedure for the selective opening of the benzylidene acetal in compounds 3.159 and 3.160 using borane/tetrahydrofuran/copper(II) triflate (Scheme 3.30).[80] To a solution of borane/tetrahydrofuran (1 M in THF, 1.41 mL, 1.41 mmol) was added the appropriate carbohydrate (0.28 mmol) at room temperature under argon. The mixture was stirred for 10 min, and freshly dried copper (II) triflate (0.0051 g, 0.014 mmol) was added to the solution. After stirring for 24 h, the mixture was cooled to 0 ^{o}C (ice bath), and the reaction was quenched with triethylamine (0.1 mL, 0.7 mmol) and methanol (1 mL, caution: hydrogen gas was evolved). The resulting mixture was concentrated at reduced pressure followed by co-evaporation with methanol. The crude residue was purified by flash chromatography (1:4, EtOAc/hexanes).



Methyl-2,3-di-*O*-trichloroethylsulfo-4-*O*-benzyl-β-D-glucopyrano-side (3.170): 76%

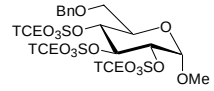
as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (s, 1H, OH), 3.42 (s, 3H,OCH_3), 3.69-3.86 (m, 4H, H6, H60, H5, H4), 4.61-4.65 (m, 2H, 1H of CH_2Ph, H2), 4.75-4.94 (m, 5H, 1H of CH_2Ph, $2CH_2CCl_3$), 5.15-5.21 (m, 2H, H3, H1), 7.34 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.56, 60.53, 70.78, 74.96, 75.21, 79.04, 80.24, 80.30, 83.38, 92.32, 92.64, 96.21, 128.3, 128.4, 128.6, 136.6; $[\alpha]_D^{26}$ = 68.6 (c 1.0, $CHCl_3$); HRMS (ESI)m/z =721.9020, $C_{18}H_{26}Cl_6NO_{12}S_2$ $(M+NH_4)^+$ requires 721.9027.



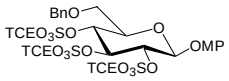
***p*-Methoxyphenyl-2,3-di-*O*-trichloroethylsulfo-4-*O*-benzyl-β-D-glucopyranoside (3.171):** 73% as colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.82 (br, 1H, OH), 3.41 (d, 1H, *J* = 9.1 Hz, H5), 3.7-3.9 (m, 5H, H6`, OCH_3, H6), 4.6 (d, 1H, *J* = 10.4 Hz, 1H of CH_2Ph), 4.72-5.01 (m, 7H, $2CH_2CCl_3$, $1HCH_2Ph$, H2, H3), 5.04 (d, 1H, *J* = 7.3 Hz, H1), 6.81 (d, 2H, *J* = 8.4 Hz, ArH), 6.92 (d, 2H, *J* = 8.4 Hz, ArH), 7.31 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 60.6, 74.8, 75.1, 75.2, 80.3, 80.5, 80.9, 84.8, 92.1, 92.6, 98.5, 114.8, 118.4, 128.4, 128.6, 136.5, 149.7, 156.1; $[\alpha]_D^{26}$ = 87.3 (c 1.0, $CHCl_3$); HRMS

(ESI) m/z = 813.9315, $C_{24}H_{30}Cl_6$ $NO_{13}S_2$ $(M+NH_4)^+$ requires 813.9290.

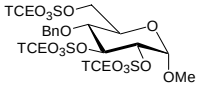
General procedure for the synthesis of trisulfated carbohydrates, compounds 3.172-3.175 (Scheme 3.30). Prepared according to the general procedure described above for the multiple sulfations. The appropriate carbohydrate (0.14 mmol), CH_2Cl_2 (2 mL), (1,2-DiMeIm) (0.07 g, 0.73 mmol), reagent **2.4** (0.3 g, 0.65 mmol), reaction time 30 h. The residue was purified with flash chromatography (1:4, EtOAc/hexanes).



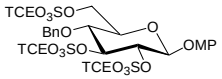
Methyl-2,3,4-tri-*O*-trichloroethylsulfo-6-*O*-benzyl-α-D-glucopyranoside (3.172): 84% as colourless syrup yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (s, 3H, OCH_3), 3.79-3.98 (m, 3H, H6, H6`, H5), 4.51-4.62 (AB, 2H, *J* = 11.3 Hz, CH_2Ph), 4.69 (m, 1H, H2), 4.81-4.92 (m, 6H, $3CH_2CCl_3$), 5.07-5.25 (m, 3H, H4, H3, H1), 7.34 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 66.7, 68.3, 73.7, 77.3, 78.0, 79.6, 79.7, 80.1, 80.3, 80.7, 80.8, 92.2, 92.4, 92.5, 95.9, 127.9, 128.0, 128.4, 137.2; $[\alpha]_D^{26}$ = 46.9 (c 1.0, $CHCl_3$); HRMS (ESI) m/z = 931.7767, $C_{20}H_{27}NCl_9O_{15}S_3$ $(M+NH_4)^+$ requires 931.7740.



***p*-Methoxyphenyl-2,3,4-tri-*O*-trichloroethylsulfo-6-*O*-benzyl-β-D-glucopyranoside (3.173):** 80% as colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.74- 3.79 (m, 4H, H5, OCH3), 3.90 (t, 1H, *J* = 9.1 Hz, H4), 4.37 (dd, 1H, *J* = 10.8, 4.2 Hz, H6`), 4.49 (d, 1H, *J* = 10.9 Hz, H6), 4.58-4.64 (m, 3H, CH_2Ph, 1H of CH_2CCl_3), 4.79-4.95 (m, 5H, 1H of CH_2CCl_3, $2CH_2CCl_3$), 5.03-5.08 (m, 2H, H2, H3), 5.13 (d, 1H, *J* = 7.3 Hz, H1), 6.86 (d, 2H, *J* = 8.9 Hz, ArH), 7.01 (d, 2H, *J* = 8.9 Hz, ArH), 7.41 (m, 5H, ArH); ^{13}C NMR (125 MHz, CDCl3) δ 55.6, 70.3, 72.2, 74.6, 75.81, 79.7, 80.3, 80.5, 80.7, 84.3, 92.2, 92.4, 92.5, 98.5, 114.9, 118.7, 128.8, 128.9, 128.9, 135.7, 149.4, 156.4; $[\alpha]_D^{26}$ = 86.2 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z =1006.7729, $C_{26}H_{28}Cl_9NO_{16}S_3$ $(M+H)^+$ requires 1006.7736.



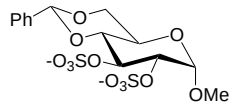
Methyl-2,3,6-tri-*O*-trichloroethylsulfo-4-*O*-benzyl-β-D-glucopyranoside (3.174): 77% as colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.44 (s, 3H, OCH_3), 3.72 (t, 1H, *J*=9.5, 9.1 Hz, H4), 3.94 (d, 1H, *J*=9.5 Hz, H5), 4.42-4.5 (m, 2H, H6, H6`), 4.55, 4.99 (AB system, 2H, *J*=10.5 Hz, CH_2Ph), 4.63-4.89 (m, 7H, H2, $3CH_2CCl_3$), 5.16-5.21 (m, 2H, H3, H1), 7.35 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 68.1, 70.8, 75.0, 75.6, 78.4, 79.7, 80.3, 80.5, 82.9, 92.2, 92.4, 92.5, 96.2, 128.6, 128.8, 135.8 $[\alpha]_D^{26}$ = 41.8 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z=914.7466, $C_{20}H_{24}Cl_9O_{15}S_3$ $(M + H)^+$ requires 914.7474.



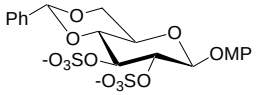
***p*-Methoxyphenyl-2,3,6-tri-*O*-trichloroethylsulfo-4-*O*-benzyl-β-D-glucopyranoside (3.177):** 71% as colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71- 3.88 (m, 5H, H5, OCH_3, H4), 4.3-4.56 (m, 2H, H6, H6`), 4.57-4.73 (m, 3H, CH_2Ph, 1H of CH_2CCl_3), 4.76-4.9 (m, 5H, 1H of CH_2CCl_3, $2CH_2CCl_3$), 4.97-5.09 (m, 2H, H3, H1), 6.8 (d, 2H, *J* = 8.8 Hz, ArH), 6.96 (d, 2H, *J* = 8.7 Hz, ArH), 7.36 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 70.3, 72.1, 74.6, 75.8, 97.7, 80.3, 80.4, 80.7, 84.4, 84.5, 92.2, 92.4, 92.5, 98.4, 114.9, 118.6, 128.8, 128.9, 135.7, 149.4, 156.4; $[\alpha]_D^{26}$ = 75.1 (c 1.0, $CHCl_3$); HRMS (+ESI) m/z =1006.7731, $C_{26}H_{28}$ Cl_9 $NO_{16}S_3$ $(M+H)^+$ requires 1006.7736.

General procedure for the deprotection of the sulphate moiety in compounds 3.176, 3.177, and 3.178 (Scheme 3.31). To a suspension of ammonium formate (0.105 g, 1.67 mmol) in MeOH (1.4 mL) was added the appropriate carbohydrate (0.14 mmol) followed by zinc dust (0.13 g, 1.98 mmol). The reaction was stirred for 6 h at room temperature

after which no starting material was detected by TLC. The reaction was filtered through Celite, and the filtrate was concentrated. The crude residue was purified by flash chromatography (20:4:1CH_2Cl_2/MeOH/NH_4OH) afforded a white solid that was lyophilized (3x) to yield a white powder.

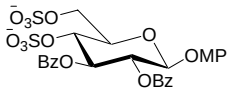


Methyl-2,3-di-*O*-sulfo-4:6-*O*-benzylidine-β-D-glucopyranoside (3.176): 92% as a white powder. ^{1}H NMR (300 MHz, DMSO-d6) δ 3.29 (s, 3H, OCH_3), 3.48-3.58 (m, 2H, H4, H5), 3.71 (t, 1H, *J* = 9.7 Hz, H6`), 3.94-3.99 (dd, 1H, *J* = 9.9, 3.1 Hz, H2), 4.12-4.16 (dd, 1H, *J*=10.16, 5.9 Hz, H6), 4.45 (t, 1H, *J*=9.3 Hz, H3), 5.09 (d, 1H, *J* = 3.1 Hz, H1), 5.54 (s, 1H, CHPh), 7.11 (br, 8H, $2NH_4$), 7.28 (m, 3H, ArH), 7.49 (m, 2H, ArH); ^{13}C NMR (75 MHz, DMSO-d6) δ 54.6, 62.6, 68.4, 74.9, 75.7, 80.0, 98.9, 100.9, 126.0, 127.4, 128.0, 137.7; $[\alpha]_D^{26}$ = 53.8 (c 1.0, H_2O); HRMS (ESI) m/z = 441.0168, $C_{14}H_{17}O_{12}S_2$ requires 441.0161.



***p*-Methoxyphenyl-2,3-di-*O*-sulfo-4:6-*O*-benzylidine-β-D-glucopyranoside (3.177):** 89% as a white powder. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 3.50 (t, 1H, *J* = 10.1, 9.26 Hz, H6`), 3.71 (m, 4H, OCH_3, H5), 4.16-4.25 (m, 2H, H6, H4), 4.32 (d, 1H, *J*= 6.9 Hz, H3), 4.41 (s, 1H, H2), 5.58 (s, 1H, CHPh), 5.76 (s, 1H, H1), 6.84, 6.92 (AB, 4H, *J* = 8.6 Hz, ArH), 7.12 (s, 8H, $2NH_4$), 7.36 (m, 5H, ArH); ^{13}C NMR (75 MHz, DMSO-*d*6) δ 55.7, 64.2, 69.5, 75.7, 76.6, 79.2, 98.0, 101.0, 115.0, 119.1, 126.8, 128.3, 129.1, 138.2, 150.8,

154.9; $[\alpha]_D^{26}$ = 77.2 (c 1.0, H_2O); HRMS (ESI) m/z = 533.0417, $C_{20}H_{21}O_{13}S_2$ requires 533.0424.



***p*-Methoxyphenyl-2,3-di-*O*-benzoyl-4,6-di-*O*-sulfo-β-D-glucopyranoside (3.178):** 88% as a white powder. ^{1}H NMR (500 MHz, DMSO-*d*6) δ 3.67 (s, 4H, OCH_3, H6`), 4.01 (t, 1H, *J* = 9.5 Hz, H5), 4.21 (t, 1H, *J* = 9.4 Hz, H4), 4.36 (d, 1H, *J* = 11.2 Hz, H6), 5.26 (t, 1H, *J* = 9 Hz, H2), 5.45 (d , 1H, *J* = 7.8 Hz, H1), 5.66 (t, 1H, *J* = 9.3 Hz, H3), 6.75, 6.88 (AA`BB`, 4H, *J* = 8.5, 8.4 Hz, ArH), 7.04 (s, 8H, $2NH_4$), 7.41 (m, 6H, ArH), 7.79 (d, 4H, *J* = 11.9 Hz, ArH); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 55.7, 66.4, 72.7, 73.2, 73.7, 74.3, 99, 114.9, 118.2, 128.5, 129.2, 129.3, 129.5, 129.9, 130.5, 133.1, 134, 151.2, 155.2, 165.1, 165.7; $[\alpha]_D^{26}$ = 76.3 (c 1.0, H_2O); HRMS (ESI) m/z = 653.0612, $C_{27}H_{25}O_{15}S_2$ requires 653.0635.

Chapter 4 - Preparation of Trifluoroethyl- and Phenyl-Protected Sulfates using Sulfuryl Imidazolium Salts

4.1 Introduction and Objectives

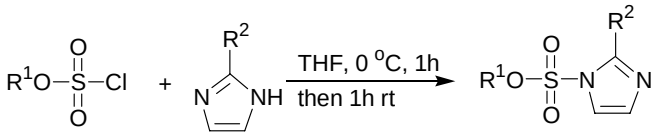
In previous chapters we demonstrated that trichloroethyl (TCE)-protected sulfates could be introduced into monosaccharides in good yield using sulfuryl imidazolium salts and that the TCE group has many of the characteristics that are required of a successful sulfate protecting group. However, as we mentioned in chapter 1, other groups have also been examined as sulfate PG's such as the phenyl, trifluoroethyl (TFE), neopentyl and isobutyl groups and some of these groups have useful properties, such as stability to certain hydrogenolysis conditions, which the TCE group lacks. Unfortunately, in some instances the methodology that had been developed for incorporating these groups into carbohydrates was far from optimal. For instance, the TFE group was installed by reacting the sulfate monoesters with trifluorodiazoethane, a reagent that must be prepared fresh and is a potentially explosive. Also, the conditions that had been developed for removing this group, potassium *tert*-butoxide in refluxing *tert*-butanol, were not very compatible with complex carbohydrate syntheses. There are several objectives to the work presented in this chapter. One is to determine what groups, in addition to the TCE group, are compatible with sulfuryl imidazolium salt formation. The second is to demonstrate that, in addition to the TCE group, other protecting groups can be introduced into carbohydrates using sulfuryl imidazolium salts. The third objective is to find better conditions for removing TFE groups from TFE-protected sulfated carbohydrates.

4.2. Results and Discussion

4.2.1 Synthesis of sulfuryl imidazolium salts

We initially examined the preparation of SIS's bearing moieties that have been studied as sulfate protecting groups such as the TFE, phenyl, isobutyl and neopentyl groups (Table 4.1). Thus, sulfuryl chlorides **4.1**-**4.4**[29,35,37,81] were reacted with imidazole or 2-methylimidazole to give compounds **4.5**-**4.12**. Compounds **4.5** and **4.6** formed by the reaction of imidazole with neopentyl and isobutylsulfuryl chloride were found to be very unstable and decomposed shortly after chromatographic purification. The 2-methylimidazole derivatives of **4.5** and **4.6**, compounds **4.7** and **4.8**, were slightly more stable yet still decomposed within 6-12 h after chromatography. In contrast those bearing the trifluoroethyl or phenyl groups were readily obtained suggesting that electron withdrawing groups on the ester portion are important for stability and electron donating alkyl groups decrease stability. However, sulfuryl imidazolides bearing electron donating aryl groups can be prepared as exemplified by the known compound **4.13** (Figure 4.1), which has an electron donating methoxy group at the 4-position of the phenyl ring and is readily prepared by reacting 4-methoxyphenol with sulfuryl diimidazole in the presence of a base.[82] We also found that the 4-methylmercapto derivative **4.14** (Figure 4.1) could prepared in a similar manner and is a stable compound.

Table 4.1. Yields of compounds 4.5-4.12.



4.1, R^1 = nPt R^2 = H or Me
4.2, R^1 = i-Bu
4.3, R^1 = TFE
4.4, R^1 = Ph

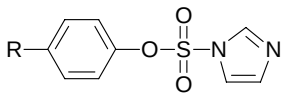
4.5-4.12

product	R^1	R^2	% Yield
4.5	nPt	H	0[a]
4.6	*i*-Bu	H	0[a]
4.7	nPt	CH_3	0[b]
4.8	*i*-Bu	CH_3	35[b,c]
4.9	TFE	H	84
4.10	TFE	CH_3	87
4.11	Ph	H	75
4.12	Ph	CH_3	85

[a]Decomposed within 1 h of chromatography.

[b]Decomposed within 6-12 h of chromatography.

[c]Contained 13 % 1,2-DiMeIm as impurity.



4.13, R = MeO
4.14, R = MeS

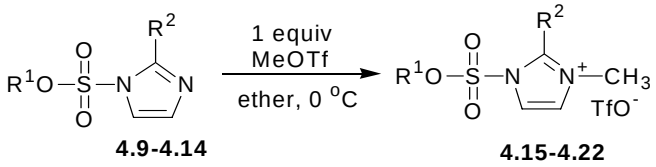
Figure 4.1. Sulfuryl imidizolates **4.13** and **4.14**.

Attempts to convert **4.7** and **4.8** into SIS's by treating them, immediately after purification by chromatography, with MeOTf in Et_2O resulted in the precipitation of a white powder which rapidly decomposed after filtration and drying under high vacuum. In contrast, subjecting compounds **4.9**-**4.12** to methyl triflate in ether resulted in the precipitation of SIS's **4.17**-**4.20** as white powders in high yield (**Table 4.2**) and these

compounds can be stored for at least two years at -20 ^{o}C with showing any detectable decomposition.

Although the *p*-methoxyphenyl and *p*-thiomethylphenyl groups have not been employed as sulfate protecting groups Prof. Scott Taylor subjected compounds **4.13** and **4.14** to MeOTf to determine if SIS's bearing an electron donating group on the phenyl ring could be prepared. As with SIS's **4.17-4.20**, the resulting SIS's **4.21** and **4.22** readily precipitated out of solution and were found to be very stable and can be stored at -20 ^{o}C for at least a year without any detectable decomposition.

Table 4.2. Yields of sulfuryl imidazolium salts **4.17-4.22.**



4.9-4.14 **4.15-4.22**

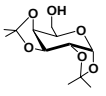
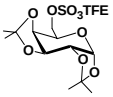
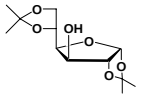
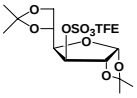
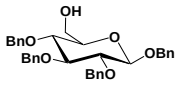
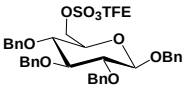
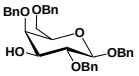
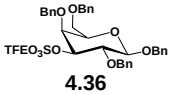
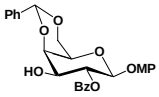
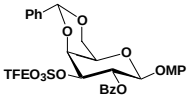
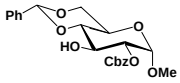
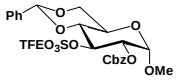
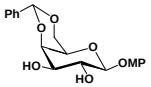
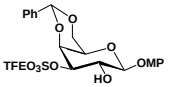
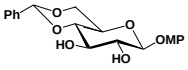
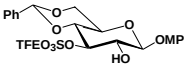
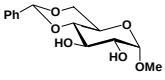
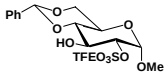
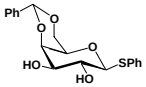
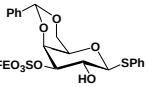
Product	R^1	R^2	% Yield
4.15	nPt	CH_3	0
4.16	*i*-Bu	CH_3	0
4.17	TFE	H	92
4.18	TFE	CH_3	91
4.19	Ph	H	92
4.20	Ph	CH_3	93
4.21	4-MeOPh	H	90
4.22	4-MeSPh	H	86

4.2.2 Sulfating Abilities of TFE/Phenyl Sulfuryl Imidazolium Salts 4.17-4.22

Introduction of TFE-protected sulfates into carbohydrates was examined using SIS's **4.17** and **4.18**. Subjecting carbohydrate **4.23** to 2 equiv **4.17** in the presence of 2.5

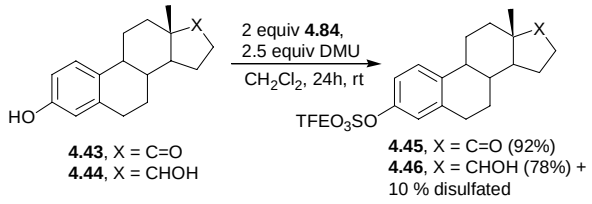
equiv *N*-methyl imidazole (1-MeIm) in THF gave sulfated carbohydrate **4.33** in a 45% yield. However, switching to 2,6-lutidine as base and performing the reaction in CH_2Cl_2 gave **4.33** in an 88% yield (entry 1). Using 2 equiv of SIS **4.18** in the presence of 2.5 equiv 1,2-dimethylimidazole, 1,2-DiMeIm in CH_2Cl_2 gave **4.33** in an 86% yield (entry 2). Carbohydrate **4.34** was obtained in an 80% yield using SIS **4.18** and 1,2-DiMeIm (entry 3). In contrast, carbohydrates **4.33** and **4.34** were prepared by Proud et al in 60 % and 51 % yields respectively using the pyr/SO_3/2,2,2-trifluorodiazoethane methodology.[28] In general, sulfations using SIS **4.18** and 1,2-DiMeIm gave higher yields than those using SIS **4.17** and 2,6-lutidine (entries 3-8). Further studies with SIS **4.18** using 1,2-DiMeIm as base revealed that most of the sulfated carbohydrates could be obtained in good yield though in some instances yields were modest even when a considerable excess of SIS and base were used with prolonged reaction times (entries 8 and 10). The TFE-protected sulfate group could be introduced selectively into 2,3-diols of benzylidene acetals in good yield by the slow addition of a solution of 1,2-DiMeIm to a solution of the carbohydrate and SIS **4.18** (entries 10-13). The selectivities are consistent with the intrinsic reactivities of the hydroxyl groups of the respective carbohydrates.[83]

Table 4.3. Synthesis of TFE-protected sulfocarbohydrates with sulfuryl imidazolium salts **4.17** and **4.18.**

entry	Substrate	Sulfating agent	Product	Yield (%)
1	 **4.23**	**4.17**	 **4.33**	45[a],88[b]
2	**4.23**	**4.18**	**4.33**	86[c]
3	 **4.24**	**4.17**	 **4.34**	70[b]
4	**4.24**	**4.18**	**4.34**	80[c]
5	 **4.25**	**4.17**	 **4.35**	77[b]
6	**4.25**	**4.81**	**4.35**	79[c]
7	 **4.26**	**4.17**	 **4.36**	26[b]
8	**4.26**	**4.18**	**4.36**	58[c]
9	 **4.27**	**4.18**	 **4.37**	80[c]
10	 **4.28**	**4.18**	 **4.38**	52[d]
11	 **4.29**	**4.18**	 **4.39**	75,[e] 89[f]
12	 **4.30**	**4.18**	 **4.40**	89[f]
13	 **4.31**	**4.18**	 **4.41**	85[g]
14	 **4.32**	**4.18**	 **4.42**	78,[e] 91[f]

[a]2.5 equiv **4.17**, 2 equiv NMI, THF, 24h. [b]2.0 equiv **4.17**, 1.1 equiv 2,6-lutidine CH_2Cl_2, 24h. [c]2 equiv **4.18**, 2.5 equiv 1,2-DiMeIm, CH_2Cl_2, 24-30 h. [d]5 equiv **4.18**, 6 equiv 1,2-DiMeIm, CH_2Cl_2, 72 h. [e]1.2 equiv **4.18**, 2 equiv 1,2-DiMeIm, CH_2Cl_2, 30 h. [f]4 equiv **4.18**, 5 equiv 1,2-DiMeIm, CH_2Cl_2, 30 h.

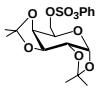
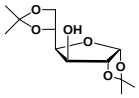
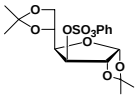
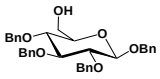
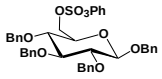
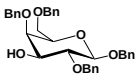
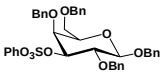
Using estrone (**4.43**) and estradiol (**4.44**) as model aryl substrates it was found that the TFE-protected sulfate group can also be introduced into aryl substrates using SIS **4.18** in good to excellent yields and relatively good selectivity could be achieved for the phenolic OH in estradiol (Scheme 4.1).



Scheme 4.1. Synthesis of TFE-protected estrone and estradiol 3-sulfates.

The synthesis of phenyl-protected sulfated carbohydrates were examined using reagents **4.19** and **4.20** and carbohydrates **4.25-4.28** (Table 4.4). Using reagent **4.19**/NMI (1-MeIm)or **4.20**/1,2-DiMeIm carbohydrates **4.47** and **4.48** were obtained in 90-95% yields. In contrast, carbohydrate **4.48** was prepared in a 75% yield by Penney and Perlin using NaH/$PhOSO_2Cl$.[29] Carbohydrates **4.49** and **4.50** which were obtained in a modest yield using reagent **4.19** were obtained in good yield using reagent **4.20**.

Table 4.4. Synthesis of phenyl-protected sulfocarbohydrates with sulfuryl imidazolium salts **4.19** and **4.20.**

Entry	Substrate	Sulfating agent	Product	Yield (%)
1		**4.19**		90[a]
	4.23		**4.47**	
2	**4.23**	**4.20**	**4.47**	95[b]
3		**4.19**		92[a]
	4.24		**4.48**	
4	**4.24**	**4.20**	**4.48**	93[b]
5		**4.19**		60[a]
	4.25		**4.49**	
6	**4.25**	**4.20**	**4.49**	80[b]
7		**4.19**		51[a]
	4.26		**4.50**	
8	**4.26**	**4.20**	**4.50**	75[b]

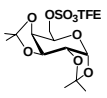
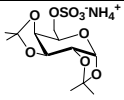
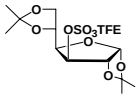
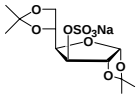
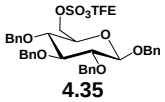
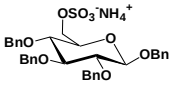
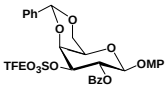
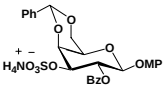
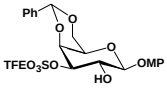
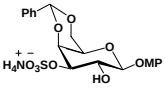
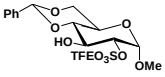
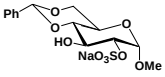
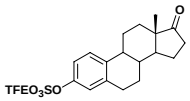
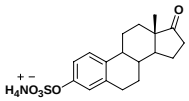
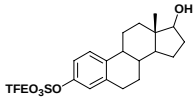
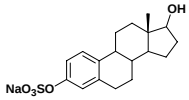
[a]2-2.5 equiv **4.19**, 2 equiv 1-MeIm, THF, 24 h. [b]2 equiv **4.20**, 2.5 equiv 1,2-DiMeIm, CH_2Cl_2, 24-30 h.

4.2.3 Deprotection of TFE-protected Sulfate Esters

The conditions that are most commonly employed for deprotecting TFE-protected sulfates are refluxing KO*t*-Bu in *t*-BuOH.[28,34] These harsh conditions have severely limited the use of the TFE moiety as a suflate protecting group as the deprotection yields are often low.[34] As part of our efforts to find more suitable conditions for removing this

group we evaluated NaN_3 in warm DMF since these conditions have been used for removing neopentyl groups from sulfates sometimes in very high yield.[35] Subjecting fully protected carbohydrates bearing secondary suflate groups, **4.34**, **4.37**, **4.39** and **4.41** to 1.4 equiv of NaN_3 in warm DMF (65-70 ^oC) for 10-16 h resulted in removal of the TFE group in high yields (Table 4.5). The crude products were passed through a small silica column using CH_2Cl_2/MeOH or CH_2Cl_2/MeOH/NH_4OH as eluent which effectively removed any contaminating NaN_3 and gave the desired deprotected sulfates as their sodium or ammonium salts.

Table 4.5. Deprotection of TFE-protected sulfates with NaN_3.

Substrate	Product	Yield (%)
 **4.33**	 **4.51**	65
 **4.34**	 **4.52**	90
 **4.35**	 **4.53**	55
 **4.37**	 **4.54**	95
 **4.39**	 **4.55**	88
 **4.41**	 **4.56**	95
 **4.45**	 **4.57**	96
 **4.46**	 **4.58**	94

However, deprotections of substrates bearing a primary sulfate group, **4.99** and **4.101**, proceeded in lower yields due to competing attack of the azide ion at C-6 followed by partial desulfation. This side reaction was also noted by Karst et al during the attempted removal of a TFE group from the 6-position of a fully protected disaccharide substrate using KO*t*-Bu in refluxing HO*t*-Bu though it appears that this problem can be reduced or eliminated when free OH groups are present in the substrate.[28,34] This side reaction was also found to occur during removal of the neopentyl group from neopentyl-protected glucose-3-sulfate though in this case complete loss of the sulfate group occurred.[35] The phenolic sulfates **4.45** and **4.46** were deprotected in almost quantitative yield within 1-3 h using 1.4 equiv NaN_3 in DMF at 65-70 ^oC.

4.3 Summary and Future Work

In summary, we have shown that SIS's bearing the TFE (compounds **4.17** and **4.18**) and phenyl (**4.19** and **4.20**) groups, two functionalities that have been used for the protection of suflate groups, can be readily prepared. SIS's bearing the electron donating neopentyl and isobutyl groups, two moieties that have also been used for the protection of sulfates, were found to be unstable and could not be isolated though SIS's bearing an electron-donating *p*-methoxyphenyl or *p*-thiomethylphenyl group were readily prepared and are stable compounds. In most instances, both TFE- and phenyl protected sulfates were easily prepared using reagents **4.17**-**4.20** though reagents **4.18** and **4.20** having a methyl group at the 2-position of the imidazole ring were, in general, superior sulfating agents in comparison to reagents **4.17** and **4.19** which lacked a methyl group at this position. In general, the use of SIS's **4.17**-**4.20** to prepare TFE- or phenyl-protected sulfates represent a significant improvement over the previous approaches to these

compounds. Deprotection of carbohydrate substrates bearing a primary sulfate group using NaN_3 in warm DMF proceeded in lower yields due to competing attack of the azide ion at C-6. However the TFE group can be removed from secondary sulfates in carbohydrates and aryl sulfates in excellent yields using NaN_3 in DMF, conditions that we believe are superior to the previous conditions using refluxing KO*t*-Bu in *t*-BuOH. Overall, these results make the TFE group a more viable alternative for sulfate protection which considered a viable approach to the synthesis of complex sulfated oligosaccharides. In addition, tin-mediated sulfations with reagent **4.18** could be examined in future.

4.4. Experimental

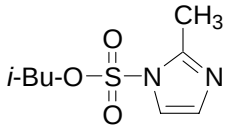
4.4.1 General Information

For general information regarding solvents, NMR, MS, refer to section 2.4.1 in Chapter 2. All commercially available reagents in chapter 4 were purchased from either Sigma Aldrich or Alfa Aesar. Compounds **4.13**, **4.14**, **4.21** and **4.22** were prepared by Prof. Taylor.

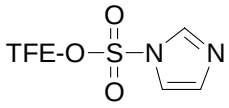
4.4.2 Experimental Syntheses and Characterization

General procedure for the preparation of compounds 4.8-4.12 (Table 4.1). To a solution of the appropriate imidazole derivative (0.165 mol, 3.0 equiv) in dry THF (60 mL) at 0 ^{o}C was added dropwise a solution of the appropriate sulfuryl chloride **4.1-4.4** (0.055 mol, 1.0 equiv) in THF (40 mL). The reaction was stirred at 0 ^{o}C for 1 h, warmed to room temperature, and stirred for an additional 1 h. The reaction mixture was filtered, the residue was washed with THF, and the filtrate was concentrated under vacuum. The

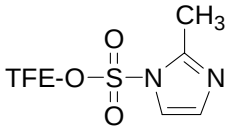
crude residue was purified by flash chromatography (1:2 EtOAc/hexanes) to give compounds **4.8-4.12** (Table 4.1).



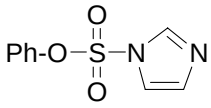
Isobutoxysulfuryl-(2-methyl)imidazole (4.8). Prepared according to the general procedures. Purified by flash chromatography (33:67 EtOAc/hexanes) to provide **4.8**, 35% as a clear, colorless oil containing approximately 13 % of 2-methylimidazole as impurity. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (d, 6H, J = 6.8 Hz), 1.94 (dt, 1H, J = 7.5, 6.8 Hz), 2.30 (s, 3H), 3.56 (d, 2H, J = 7.5 Hz), 6.73 (d, 1H, J = 1.3 Hz), 6.83 (d, 1H, J = 1.3Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.1, 19.9, 29.9, 53.5, 119.6, 121.2, 126.7, 144.5. We were unable to obtain a mass spectrum of this compound due to its rapid decomposition in the mass spectrometer.



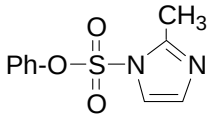
Trifluoroethoxysulfuryl imidazole (4.9). Prepared according to the general procedures. Flash chromatography (1:2, EtOAc:hexanes), 84% yield as colorless oil. ^{1}H NMR (300MHz, $CDCl_3$) δ 4.53 (q, 2H, J = 7.55 Hz, CH_2), 7.20 (s, 1H, Himi), 7.36 (s, 1H, Himi), 8.01 (s, 1H, Himi); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.9 (q, 1C, J = 150 Hz, CH_2CF_3), 117.8, 122 (q, 1C, J = 305.5 Hz, CF_3), 131.8, 136.9; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.5; HRMS (EI), 229.9966, $C_5H_5F_3N_2O_3S$ $(M)^+$, require 229.9973.



Trifluoroethoxysulfuryl–(2-methyl) imidazole (4.10). Prepared according to the general procedures. Flash chromatography (1:2, EtOAc:hexanes), 87% yield as colorless oil.^{1}H NMR (300 MHz, $CDCl_3$) δ 2.57 (s, 3H, CH_3), 4.44 (q, 2H, J = 7.55 Hz, CH_2), 6.90 (s, 1H),7.24 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 67.2 (q, 1C , J_{CF} = 150 Hz, CH_2CF_3), 120.0, 121 (q, 1C , J_{CF} = 316.5 Hz, CF_3), 128.3, 146.4; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.5; HRMS (EI^+), 244.0131 $C_6H_7F_3N_2O_3S$ $(M)^+$, require 244.0129.



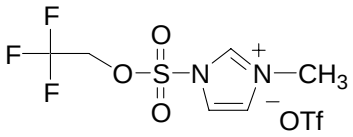
Phenoxysulfuryl imidazole (4.11). Prepared according to the general procedures. Flash chromatography (1:3, EtOAc:hexanes), 75% yield as white solid. Mp 32-33 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (m, 2H), 7.14 (s, 1H, Himi),7.32 (m, 4H), 7.74 (s, 1H, Himi); ^{13}C NMR (75 MHz, $CDCl_3$) δ 118.2, 121.9 (2C), 128.5, 130.19 (2C), 131.2, 137.3, 148.8; HRMS (EI), 224.0253, $C_9H_8N_2O_3S$ $(M)^+$, require 224.0356.



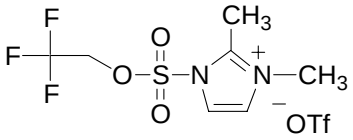
Phenoxysulfuryl-(2-methyl) imidazole (4.12). Prepared according to the general procedures. Flash chromatography (1:3, EtOAc:hexanes), 85% yield as white solid. Mp 32-33 ^{o}C. ^{1}H NMR (300MHz, $CDCl_3$) δ 2.20 (s, 3H), 6.71 (m, 3H), 6.93 (s, 1H, Himi),

7.15 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 120.3, 121.5, 127.9, 128.5, 130.2, 146.7, 148.9; HRMS (EI) , 238.0412, $C_{10}H_{10}N_2O_3S$ $(M)^+$, require 238.0412.

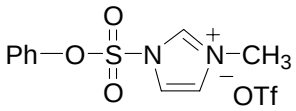
General procedure for the preparation of sulfuryl imidazolium salts, compounds 4.17-4.22 (Table 4.2). To a solution of the appropriate sulfuryl imidazole (0.04 mol, 1.0 equiv) in dry Et_2O (70 mL) at 0 ^oC was added methyl triflate (4.6 mL, 0.04 mol, 1.0 equiv) dropwise over 30 min. The reaction was stirred for 3 h at 0 ^oC during which time a white precipitate formed. The mixture was filtered. The filter cake was washed with cold ether, which afforded the imidazolium salts as a white solid (Table 4.2).



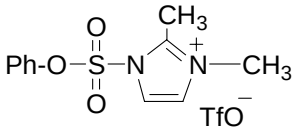
Trifluoroethoxysulfuryl-*N*-methylimidazolium triflate (4.17). Prepared according to the general procedures. White solid, 92%. Mp 103-104 ^oC; ^{1}H NMR (300 MHz, CD_3OD) δ 4.02 (s, 3H, CH_3), 5.25 (q, 2H, J = 7.8 Hz, CH_2), 9.95 (s,1H, Himi), 8.28 (s, 1H, Himi),7.36 (s, 1H, Himi), 8.01 (s, 1H, Himi); ^{13}C NMR (75 MHz, CD_3OD) δ 36.6, 69.8 (q, 1C, J = 150 Hz, CH_2CF_3), 121.0 (2q, 2C, $2CF_3$, J_{CF} = 305.5, 316.5 Hz, CF_3), 120.8, 126.1, 139.9; ^{19}F NMR (282 MHz, CD_3OD) δ −75.1, −79.9; HRMS (ESI), 245.0208, $C_6H_8F_3N_2O_3S$ $(M- OTf)^+$, require 245.0208.



Trifluoroethoxysulfuryl -(2-methyl)-*N*-methylimidazolium triflate (4.18). Prepared according to the general procedures. White solid, 91% yield. Mp 98-100 ^{o}C; ^{1}H NMR (300MHz, CD_3OD) δ 2.87 (s, 3H, CH_3-imi), 3.90 (s, 3H, CH_3), 5.16 (q, 2H, J = 7.55 Hz, CH_2), 7.70 (d, 1H, J = 2.3 Hz, Himi), 8.02 (d, 1H, J = 2.3 Hz, Himi),; ^{13}C NMR (75 MHz, CD_3OD) δ 10.4, 35.3, 69.3 (q, 1C , J_{CF} = 150 Hz, CH_2CF_3), 120.7, 121.0 (2q, 2C, $2CF_3$, J_{CF} = 303, 316.5 Hz, CF_3), 123.6, 148.8; ^{19}F-NMR (300 MHz, CD_3OD) δ –75.1, –79.7; HRMS (ESI), 259.0363, $C_7H_{10}F_3N_2O_3S$ (M- OTf)$^+$, require 259.0364.



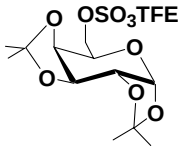
Phenoxysulfuryl-*N*-methylimidazolium triflate (4.19). Prepared according to the general procedures. White solid, 92% yield. Mp 87-88 ^{o}C; ^{1}H NMR (300MHz, CD_3OD) δ 4.05 (s, 3H), 7.26 (d, 2H, J = 6.7 Hz), 7.51 (m, 3H), 7.80 (s, 1H), 8.05 (s, 1H), 9.76 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 36.5, 120.5 (q, J = 316.5 Hz, CF_3), 120.9, 126, 129.3, 130.8, 139.5, 149.3; ^{19}F-NMR (282 MHz, CD_3OD) δ –79.5; HRMS(ESI), 239.0489, $C_{10}H_{11}N_2O_3S$ (M-OTf)$^+$, require 239.0490.



Phenoxysulfuryl-(2-methyl)-*N*-methylimidazolium triflate (4.20). Prepared according to the general procedure. White solid, 93% yield. Mp 79-80 ^{o}C; ^{1}H NMR (300 MHz,

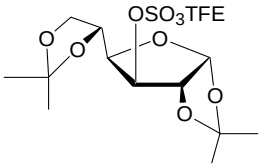
CD_3OD) δ 2.81 (s, 3H), 3.94 (s, 3H), 7.26 (d, 2H, *J* = 7.5 Hz), 7.49 (d, 3H, *J* = 7.5 Hz), 7.68 (s, 1H), 7.77 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 10.5, δ 35.5, 120.0 (q, 1C, *J* = 316.5 Hz, CF_3), 120.9, 122.5, 123.6, 129.3, 130.7, 148.7, 149.3; ^{19}F-NMR (282 MHz, CD_3OD) δ −79.5; HRMS (ESI), 253.0640, $C_{11}H_{13}N_2O_3S$ $(M\text{-}OTf)^+$, require 253.0647.

General procedure for the synthesis of TFE-protected sulfocarbohydrates using reagent 4.17 and 2,6-lutidine, compounds 4.33-4.36 (Table 4.3). To a solution of the appropriate carbohydrate **(**0.95 mmol) in CH_2Cl_2 (4.2 mL) at 0 ^oC (ice bath) was added 2,6-lutidine (0.123 ml, 1.05 mmol, 1.1 equiv) followed by reagent **4.17** (0.19 g, 0.48 mmol, 0.5 equiv). The reaction was stirred for 1 h at 0 ^oC and gradually allowed to warm to room temperature. After every two hours another 0.19 g of reagent **4.17** was added until the total was equal to 2 equiv and the reaction was stirred over night for a total of 24 h. The reaction was quenched with water, extracted with EtOAc, washed with brine, dried ($MgSO_4$) and concentrated to a crude brown oil. Flash chromatography (1:4, EtOAc:hexanes) gave the TFE-protected sulfocarbohydrates.

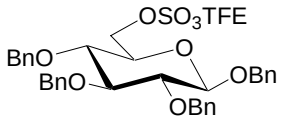


1,2:3,4-di-*O*-isopropylidene-6-*O*-trifluroroethylsulfo-α-D-glactopyranoside (4.33). Prepared from carbohydrate **4.23** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 88% yield as white solid. Mp 34-35 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30, (s, 6H, $2CH_3$), 1.41 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 4.09 (bd, 1H, *J* = 5.6 Hz, H5), 4.15 (dd, 1H, *J* = 7.7, 1.8 Hz, H4), 4.32 (t, 1H, *J* = 4.8 Hz, H2), 4.41

(dd, 2H, J = 5.8, 2.5 Hz, H6, H6`), 4.52- 4.63 (m, 3H, CH_2CF_3, H3), 5.49 (d, 1H, J = 4.6 Hz, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 24.72, 25.73, 25.55, 65.6, 66.6 (q, 1C, J_{CF} = 150 Hz, CH_2CF_3), 70.1, 70.4, 70.6, 72.5, 96.1, 109.1, 110.05, 121.6 (q, 1C, J_{CF} = 303 Hz, CF_3); ^{19}F-NMR (282 MHz, $CDCl_3$) δ –73.8; HRMS (ESI) m/z = 423.0952, $C_{14}H_{22}F_3O_9S$ $(M+H)^+$ requires 423.0937.

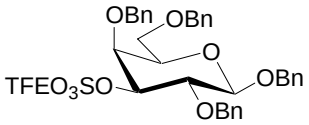


1,2:5,6-di-*O*-isopropylidene-3-*O*-trifluroroethylsulfo-α-D-glucopyranoside (4.34). Prepared from carbohydrate **4.24** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 70% yield as white solid. Mp 67-68 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30, (s, 6H, $2CH_3$), 1.39 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 4.18 (m, 4H, H6, H6`, H4, H5), 4.52 (m, 1H, 1H of CH_2CF_3), 4.82 (m, 1H, 1H of CH_2CF_3), 4.89 (bs, 1H, H2), 4.95 (bs, 1H, H3), 5.91 (bs, 1H, H1); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 26.1, 26.5, 26.8, 67 (q, J = 150 Hz, CH_2CF_3), 67.5, 71.7, 79.4, 82.5, 85.6, 105, 110.1, 112.8, 121 (q, J = 305.5 Hz, CF_3); ^{19}F-NMR (282 MHz, $CDCl_3$) δ –73.8; HRMS (ESI) m/z = 423.0945, $C_{14}H_{22}F_3O_9S$ $(M+H)^+$ requires 423.0937.



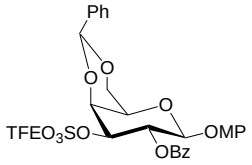
Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-trifluoroethylsulfo-β-D-glucopyranoside (4.35). Prepared from carbohydrate **4.25**[84] according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 77% yield as white solid. Mp 106-107 ^{o}C; ^{1}H

NMR (300 MHz, $CDCl_3$) δ 3.52 (m, 3H, H4,H2, H5), 3.71 (t, 1H, J = 8.6 Hz, H3), 4.41, 4.57 (AB system, 2H, *J* = 10.2 Hz, H6, H6`), 4.51-4.82 (m, 7H, CH_2CF_3, H1, $2CH_2$), 4.91-5.01 (m, 4H, $2CH_2$), 7.32 (m, 20H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.6 (q, 1C, *J* = 150 Hz, CH_2CF_3), 71.5, 72.2, 73.3, 74.9, 75.1, 75.7, 76.3, 82.0, 84.3, 102.4, 121 (q, 1C, *J* = 305.6 Hz, CF_3), 126.1, 127.1, 127.6, 127.7, 127.8, 127.85, 128.02, 128.05, 128.1, 128.2, 128.4, 128.47, 128.5, 128.6, 128.7, 136.8, 137.3, 138.1, 138.2; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.8; HRMS (ESI) m/z = 709.2281, $C_{36}H_{37}F_3O_9SLi$, $(M+Li)^+$ requires 709.2270.

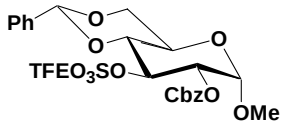


Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-trifluoroethylsulfo-β-D-galactopyranoside (4.36). Prepared from carbohydrate **4.26**[85] according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 26% yield as colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48-3.62, (m, 3H, H6,H6`, H5), 3.94 (t, 1H, J = 8.6 Hz, H2), 4.06 (m, 2H, CF_3), 4.24 (d, 1H, *J* = 2.4 Hz, H4), 4.51 (m, 7H, H1, H3, CH_2, 1H of CH_2), 4.83, 5.02 (AB system, 2H, *J* = 10.7 Hz, CH_2), 4.94 (d, 1H, *J* = 10.7 Hz, the other H of CH_2), 7.29 (m, 20H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 66.6 (q, 1C, *J* = 150 Hz, CH_2CF_3), 76.9, 71.1, 72.7, 73.5, 73.8, 75.3, 76.6, 85.7, 102.1, 122 (q, 1C, *J* = 305.6 Hz CF_3), 126.7, 127.4, 127.8, 127.9, 127.9, 128, 128.1, 128.2, 128.3, 128.3, 128.4, 128.5, 128.5, 136.8, 137.3, 137.5, 137.6; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.8; HRMS (ESI) m/z = 709.2281, $C_{36}H_{37}F_3O_9S$ Li$(M+Li)^+$ requires 709.2270.

General procedure for the synthesis of TFE-protected sulfocarbohydrates using reagent 4.18 and (1,2-DiMeIm), compounds 4.33-4.38 (Table 4.3). To a solution of the appropriate carbohydrate (0.62 mmol) in CH_2Cl_2 (5 mL) at 0^oC (ice bath) was added 1,2-DiMeIm (0.15 g, 1.56 mmol) and reagent **4.18** (0.51 g, 1.25 mmol). The ice bath was removed and the reaction allowed to warm to room temperature and then stirred for 30 h. The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to crude brown oil. Flash chromatography (1:4, EtOAc:hexanes) gave desired compounds (Table 4.3).

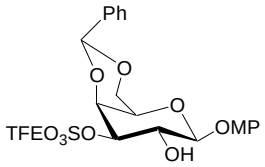


***p*-Methoxyphenyl 2-*O*-benzoyl-3-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-α-D-glac-topyranoside (4.37)**. Prepared from carbohydrate **4.27**[86] according to the general procedures. Flash chromatography (1:4, EtOAc:hexanes), 80% yield as a white solid. Mp 128-130 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 3.6o (bs, 1H, H5), 3.71 (s, 3H, OCH_3), 4.13, 4.41 (AB system, 2H, *J* = 12.5 Hz, H6, H6`), 4.23-4.34 (m, 2H, $CH_2CF_3$), 4.64 (d, 1H, *J* = 3.3 Hz, H4), 4.99 (dd, 1H, *J* = 10.2, 3.2 Hz, H3), 5.04 (d, 1H, *J* = 7.9 Hz, H1), 5.56 (s, 1H, CHPh), 5.88 (t, 1H, *J* = 9.6 Hz, H2), 6.73, 6.92 (AA`BB` system, 4H, *J* = 8.7, 8.5 Hz, ArH), 7.47 (m, 8H, ArH), 8.04 (d, 2H, *J* = 7.8 Hz ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 65.9, 67.2 (q, J_{CF} = 150 Hz, CH_2CF_3), 68.3, 68.7, 73.0, 82.1, 101.11, 101.5, 114.4, 119.4, 134 (q, J_{CF} = 304.5 Hz, CF_3)126.4, 128.4, 128.6, 129.6, 129.8, 133.6, 136.7, 150.9, 155.9, 164.9; ^{19}F-NMR (282 MHz, $CDCl_3$) δ –73.5; HRMS (ESI) m/z = 663.1111, $C_{29}H_{27}F_3O_{11}SNa$ $(M+Na)^+$ requires 663.1124.

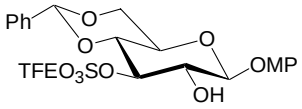


Methyl 2-*O*-benzyloxycarbonyl-3-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-α-D-glucoyranoside (4.38). Prepared from carbohydrate **4.28**[87] according to the general procedures. Flash chromatography (1:4, EtOAc:hexanes), 52% yield as White solid. Mp 112-114 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.40 (s, 3H, OCH_3), 3.71 (m, 2H, H6, H6`), 3.92 (m, 1H, H5), 4.32 (dd, 3H, H4, CH_2CF_3), 4.92 (dd, 1H, J = 9.1, 7.8 Hz, H3), 5.04 (d, 1H, J = 3.4, H1), 5.18 (t, 1H, J = 9.3, Hz, H2), 5.23 (s, 2H, CH_2Ph), 5.55 (s, 1H, CHPh), 7.49 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 62.1, 66.8 (q, 1C, J_{CF} = 150 Hz, CH_2CF_3), 68.4, 70.4, 73.7, 78.2, 80.9, 97.5, 101.9, 117 (q, J_{CF} = 305.6 Hz, CF_3), 123, 126.0, 126.7, 128.1, 128.2, 128.4, 128.5, 129.4, 134.4, 136.0, 153.9; ^{19}F-NMR (282 MHz, $CDCl_3$) δ –73.5; HRMS (ESI) m/z = 579.1156, $C_{24}H_{26}F_3O_{11}S$, $(M+H)^+$ requires 579.1148.

General procedure for the selective sulfation of compounds 4.39-4.42 (Table 4.3). To the appropriate carbohydrate (0.55 mmol) in dry CH_2Cl_2 (4 mL) at 0 ^{o}C (ice bath) was added reagent **4.18** (0.45 g, 1.1 mmol), followed by the addition of a solution of 1,2-DiMeIm (0.26 g, 2.77 mmol) in CH_2Cl_2 (2 mL) over 8 h using a syringe pump. During the addition of the 1,2-DiMeIm another portion of reagent **4.18** (0.45 g, 1.1 mmol) was added after 6 h and the ice bath was removed after the initial 1 h. The mixture was left stirring until the reaction was complete by TLC (approx 30 h). The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to brown crude oil. Flash chromatography (1:4, EtOAc:hexanes) gave the desired compounds (Table 4.3).

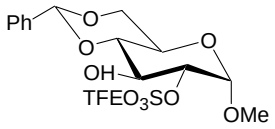


***p*-Methoxyphenyl 3-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-β-D-galactopyranoside (4.39).** Prepared from carbohydrate **4.29**[88] according to general procedures. Flash chromatography (1:4, EtOAc:hexanes), 89% yield as colourless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (b, 1H, OH), 3.41 (bs, 1H, H5), 3.72 (s, 3H, OCH_3), 4.03, 4.2 (AB system, 2H, J = 12.5 Hz, H6, H6`), 4.28 (t, $J$ = 8.2 Hz, H2), 4.49 (m, 2H, H4, 1H of $CH_2CF_3$), 4.66 (m, 3H, $J_{1,2}$ = 7.7 Hz, 1H of $CH_2CF_3$, H3, H1), 5.40 (s, 1H, CHPh), 6.9, 7.0 (AA`BB` system, 4H, J = 8.7, 8.5 Hz, ArH), 7.41 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 65.9, 67.2 (q, J_{CF} = 150 Hz, CH_2CF_3), 68.09, 68.6, 73.1, 83.4, 101.0, 102.2, 114.6, 119.0, 123 (q, J = 305.6 Hz, CF_3), 126.2, 128.2, 129.3, 137.0, 150.7, 155.8; ^{19}F NMR (282 MHz, $CDCl_3$) δ –73.8; HRMS (ESI) m/z = 537.1044 $C_{22}H_{24}F_3O_{10}S$, $(M+H)^+$ requires 537.1042.

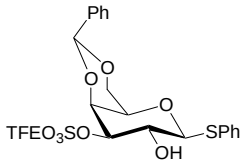


***p*-Methoxyphenyl 3-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-β-D-glucopyranoside (4.40).** Prepared from carbohydrate **4.30**[89] according to general procedures. Flash chromatography (1:4, EtOAc:hexanes), 89% yield as white solid. Mp 71-73 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.90 (b, 1H, OH at C2), 3.61 (m, 1H, J = 9.3, 4.8 Hz, H5), 3.82 (m, 5H, OCH_3, H4, H6`), 4.01 (t, J = 7.8 Hz, H2), 4.42 (m, 3H, H6, CH_2CF_3), 4.81 (t, 1H, J =

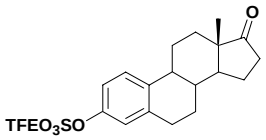
9.3 Hz, H3), 4.95 (d, 1H, *J* = 7.5 Hz, H1), 5.5 (s, 1H, CHPh), 6.80, 7.01 (AA`BB` system, 4H, *J* = 8.9, 8.7 Hz, ArH), 7.41 (m, 5H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.6, 66.0, 67.0 (q, 1C, J_{CF} = 150 Hz, CH_2CF_3), 68.4, 72.7, 77.7, 84.8, 102.0, 102.6, 114.7, 118.9, 122 (q, 1C, *J* = 305.6 Hz, CF_3), 126.0, 128.5, 129.6, 136.0, 150.5, 156.1; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.79; HRMS (ESI) m/z = 537.1063 $C_{22}H_{24}F_3O_{10}S$, $(M+H)^+$ requires 537.1042.



Methyl 2-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-α-D-glucopyranoside (4.41). Prepared from carbohydrate **4.31**[90] according to the general procedures. Flash chromatography (1:4, EtOAc:hexanes), 85% yield as colorless syrup; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.81 (b, 1H, OH at C3), 3.40 (s, 3H, OCH_3), 3.46 (t, 5H, *J* = 9.3 Hz, H4), 3.68 (dd, 1H, *J* = 10.2, 4.3 Hz, H6`), 3.81 (dd, 1H, *J* = 9.7, 4.3 Hz, H5), 4.16 (t, 1H, *J* = 9.1 Hz, H3), 4.28 (dd, 1H, *J* = 9.7, 4.3 Hz, H6), 4.46 (dd, 1H, *J* = 9.4, 3.4 Hz, H2), 4.51-4.72 (m, 2H, CH_2CF_3), 5.41 (d, 1H, *J* = 3.5 Hz, H1), 5.50 (s, 1H, CHPh), 7.42 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 61.7, 67.0 (q, J_{CF} = 150 Hz, CH_2CF_3), 68.2, 68.6, 81.1, 82.1, 97.2, 102.1, 122 (q, *J* = 305.6 Hz, CF_3), 126.2, 128.4, 129.5, 136.6; ^{19}F NMR (282 MHz, $CDCl_3$) δ −73.7; HRMS (ESI) m/z = 445.0796, $C_{16}H_{20}F_3O_9S$, $(M+H)^+$ requires 455.0780.



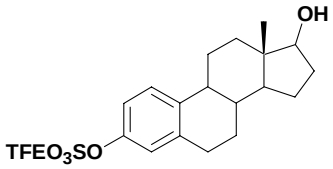
Phenyl 3-*O*-trifluoroethylsulfo-4:6-*O*-benzylidene-1-thio-a-D-galactopyranoside (4.42). Prepared from carbohydrate **4.32**[91] according to general procedures. Flash chromatography (1:4, EtOAc:hexanes), 91% yield as colorless syrup; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.57 (br, 1H, OH), 3.57 (bs, 1H, H5), 3.93 (t, 1H, *J* = 9.5 Hz, H2), 4.03, 4.39 (AB system, 2H, *J*= 12.4 Hz, H6, H6`), 4.4-4.68 (m, 5H, H4, H1, CH_2CF_3, H3), 5.51 (s, 1H, CHPh), 7.29 (m, 8H, ArH), 7.65 (d, 2H, *J* = 7.3 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 65.2, 67 (q, J_{CF} = 150 Hz, CH_2CF_3), 68.9, 69.6, 73.4, 84.8, 87.0, 101.1, 123 (q, J_{CF} = 305.5 Hz, CF_3),126.3, 128.2, 128.8, 129.1, 129.2, 129.4, 134.0, 137.0; ^{19}F-NMR (282 MHz, $CDCl_3$) δ –73.8; HRMS (ESI) m/z = 523.0720, $C_{21}H_{22}F_3O_8S_2$ $(M+H)^+$ requires 523.0708.



Synthesis of 3-TFE-protected estrone (Scheme 4.12, compound 4.45).

To a solution of estrone **4.43** (0.29 g, 1.1 mmol) in dry CH_2Cl_2 (4 mL) at 0 ^{0}C (ice bath) was added reagent **4.18** (0.86 g, 2.2 mmol) followed by the addition 1,2-DiMeIm (0.26 g, 2.75 mmol). The ice bath was removed after the initial 1h. The reaction was left stirring until the reaction was complete by TLC (approx 24 h). The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to brown crude oil. Flash chromatography (1:4, EtOAc:hexanes) gave compound **4.45** (0.42 g, 91%) as colorless syrup.^{1}HNMR (300 MHz, $CDCl_3$) δ 0.81 (s, 3H, CH_3), 1.41-1.56 (m, 6H), 1.92-2.11 (m,

4H), 2.26 (m, 1H), 2.38 (m, 1H), 2.49 (m, 1H), 2.92 (dd, 2H, *J*= 8.6, 4.0 Hz), 4.58 (q, 2H, *J*= 7.6.0 Hz, CH_2CF_3), 7.01 (m, 2H, ArH), 7.29 (d, 1H, *J*= 8.5 Hz, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.7, 21.5, 25.6, 26.0, 29.3, 31.4, 35.7, 37.7, 44.0, 47.8, 50.3, 67.5 (q, J_{CF} = 150 Hz, CH_2CF_3), 119.6, 120.8, 121.2 (q, J_{CF} = 306.4 Hz, CF_3), 127.1, 139.1, 139.8, 147.9; ^{19}F NMR (282 MHz, $CDCl_3$) δ −73.3; HRMS (EI) m/z = 432.1229, $C_{20}H_{23}F_3O_5S$ $(M)^+$ requires 432.1218.

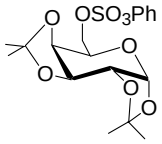


Synthesis of 3-TFE-protected estradiol (Scheme 4.12, compound 4.46).

To a solution of estradiol **4.44**, (0.3 g, 1.1 mmol) in dry CH_2Cl_2 (4 mL) at 0 ^{0}C (ice bath) was added reagent **4.18** (0.43 g, 1.1 mmol) followed by the addition of a solution of 1,2-DiMeIm (0.26 g, 2.75 mmol) in CH_2Cl_2 (2 mL) over 6 h using a syringe pump. During the addition of the DMI another portions of reagent **4.18** (0.43 g, 1.1 mmol) was added after 4 h and the ice bath was removed after the initial 1h. The reaction was left stirring until the reaction was complete by TLC (approx 24 h). The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to brown crude oil. Flash chromatography (1:4, EtOAc:hexanes) gave compound **4.46** as a colorless syrup (0.373 g, 78% of the monosulfated estradiol + 0.065 g, 10% of the disulfated derivative). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81 (s, 3H, CH_3), 1.21-1.52 (m, 7H), 1.60 (s, 1H), 1.73 (m, 1H), 1.91 (m, 2H), 1.98 (m, 1H), 2.14 (m, 1H), 2.33 (d, 1H, *J*= 13.0 Hz), 2.92 (t, 1H, *J*= 3.6 Hz), 3.71 (t, 1H, *J*= 8.4 Hz, H17), 4.64 (q, 2H, *J*= 7.6 Hz, CH_2CF_3), 7.01 (s, 1H, ArH), 7.06 (d, 1H, *J*= 8.5 Hz, ArH), 7.32 (d, 1H, *J*= 8.5 Hz, ArH); ^{13}C NMR (125 MHz,

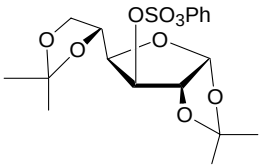
$CDCl_3$) δ 11.0, 23.1, 26.1, 26.8, 29.5, 30.5, 36.6, 38.1, 38.2, 43.1, 44.1, 50.0, 67.5 (q, J_{CF} = 150 Hz, CH_2CF_3), 81.7, 117.7, 120.8, 121.2 (q, J_{CF} = 308 Hz, CF_3), 127.1, 139.4, 140.4, 147.8; ^{19}F-NMR (282 MHz, $CDCl_3$) δ −73.3; HRMS (EI^+) m/z = 434.1364, $C_{20}H_{25}F_3O_5S$ $(M)^+$ requires 434.1375.

General procedure for synthesis of phenyl-protected sulfocarbohydrates using sulfuryl imidazolium salt 4.20 (Table 4.4, compounds 4.47-4.50). To a solution of the appropriate carbohydrate **(**0.96 mmol) in CH_2Cl_2 (4.2 mL) at 0 ^oC (ice bath) was added 2-methylimidazole (0.23 ml, 2.4 mmol) followed by reagent **4.20** (0.77 g, 1.92 mmol). The reaction was stirred for 1 h at 0 ^oC and gradually allowed to warm to room temperature. The reaction was stirred overnight (24 h), then diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to a crude brown oil. Flash chromatography (1:4, EtOAc:hexanes) gave the desired compounds.

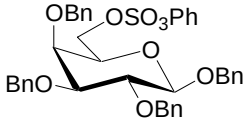


1,2:3,4-di-*O*-isopropylidene-6-*O*-phenylsulfo-α-D-galactopyranoside (4.47). Prepared from carbohydrate **4.23** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 95% yield as a white solid, Mp 77-79 ^oC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (2s, 6H, $2CH_3$), 1.42 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 4.15 (t, 1H, *J* = 6.6 Hz, H5), 4.21 (dd, 1H, *J* = 7.8, 1.8 Hz, H4), 4.32 (dd, 1H, *J* = 4.8, 2.4 Hz, H2), 4.47-4.56 (dd, 2H, *J* = 5.8, 2.5 Hz, H6, H6`), 4.62 (dd, 1H, *J* = 7.7, 2.2 Hz , H3), 5.51 (d, 1H, *J* = 4.9 Hz, H1), 7.32 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 24.8, 25.91, 25.94, 65.7,

70.2, 70.52, 70.59, 72.3, 96.1, 109.0, 109.8, 121.4, 127.4, 129.8, 150.3; HRMS (EI) m/z = 401.0905, $C_{17}H_{21}O_9S$ $(M\text{-}CH_3)^+$ requires 401.0906.

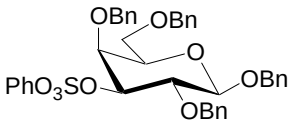


1,2:5,6-di-*O*-isopropylidene-3-*O*-phenylsulfo-α-D-glucopyranoside (4.48). Prepared from carbohydrate **4.24** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 93% yield as colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27, (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 4.18 (m, 4H, H6, H6`, H4, H5), 4.84 (d, 1H, *J* = 3.6 Hz, H2), 5.12 (d, 1H, *J* = 3.6 Hz, H3), 5.88 (d, 1H, *J* = 3.6 Hz, H1), 7.33 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.0, 26.1, 26.5, 26.8, 67.1, 71.5, 79.7, 82.5, 85.8, 104.8, 109.6, 112.7, 121.0, 121.1, 127.5, 127.6, 129.9, 130.0, 150.3; HRMS (ESI) m/z = 417.1216, $C_{18}H_{25}O_9S$ $(M+H)^+$ requires 417.1219.



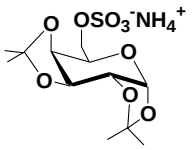
Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-phenylsulfo-β-D-glucopyranoside (4.49). Prepared from carbohydrate **4.25** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 80% yield as colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (m, 4H, H4,H2, H5, H3), 4.47-5.01 (m, 11H, H6, H6`, H1, $4CH_2$), 7.32 (m, 25H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 71.2, 72.4, 72.5, 74.9, 75.1, 75.7, 76.7, 82.0, 84.4, 102.3, 121.3, 127.4, 127.7, 127.8, 127.88, 128.0, 128.05, 128.2, 128.24, 128.44, 128.48, 128.5,

128.6, 130.0, 137.06, 138.23, 150.3; HRMS (ESI) m/z = 714.2742, $C_{40}H_{44}NO_9S$ $(M+NH_4)^+$ requires 714.2737.



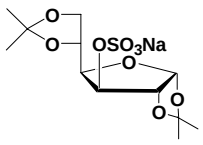
Benzyl 2,4,6-tri-*O*-benzyl-3-*O*-phenylsulfo-β-D-galactopyranoside (4.50). Prepared from carbohydrate **4.26** according to the general procedures. Flash chromatography (1:5, EtOAc:hexanes), 75% yield as colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (m, 3H, H6,H6`, H5), 4.01 (t, 1H, *J* = 8.6 Hz, H2), 4.31 (bs, 1H, H4), 4.42 (bs, 2H, CH_2), 4.62 (m, 2H, H1, H3), 4.71- 5.01 (m, 6H, $3CH_2$), 7.32 (m, 25H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.18, 71.2, 72.8, 73.5, 74.2, 74.9, 75.2 76.5, 86.3, 102.4, 121.2, 127.3, 127.6, 127.9, 127.94, 127.96, 128, 128.1, 128.2, 128.3, 128.37, 128.4, 128.5, 128.5, 129.8, 130.1, 137.0, 137.7, 137.8, 137.89, 150; HRMS (ESI) m/z = 714.2728, $C_{40}H_{44}NO_9S$ $(M+NH_4)^+$ requires 714.2737.

General procedure for the deprotection of TFE-protected sulfates with sodium azide (Table 4.5, compounds 4.51-4.58). To a solution of the TFE-protected carbohydrate (0.156 mmol) in DMF (1 mL) was added sodium azide (0.014 g, 0.21 mmol) and the reaction was heated at 70 ^{o}C (oil bath) until no starting material was detected by TLC (1-10 h). The solvent was removed by rotary evaporation. Flash chromatography of the residue (20:4:1 CH_2Cl_2/MeOH/NH_4OH) afforded the deprotected sulphate as a white solid (Table 4.5).



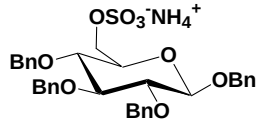
1,2:3,4-di-*O*-isopropylidene-6-*O*-sulfo-α-D-galactopyranoside (4.51).

Prepared according to the general procedures.Flash chromatography (10:2:0.5, CH_2Cl_2: MeOH:NH_4OH), 65% yield as a white solid in addition to 20% of the glucose 6-azido derivative. ^{1}H NMR (300 MHz, CD_3OD) δ 1.30, (s, 6H, $2CH_3$), 1.37 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 4.09 (m, 3H, H5,H6, H6`), 4.27 (m, 2H, H4, H3), 4.62 (dd, 1H, J = 7.3, 4.6 Hz, H2), 5.47 (d, 1H, J = 4.6 Hz, H1); ^{13}C NMR (75 MHz, CD_3OD) δ 23.2, 23.9, 24.9, 24.99, 66.5, 66.5, 70.4, 70.5, 70.8, 96.2, 108.7, 109.1; HRMS (ESI) m/z = 339.0753, $C_{12}H_{19}O_9S$ $(M-H)^+$ requires 339.0750.



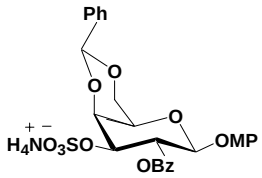
1,2:5,6-di-*O*-isopropylidene-3-*O*-sulfo-α-D-glucopyranoside (4.52).

Prepared according to the general procedures. Flash chromatography (10:2, CH_2Cl_2: MeOH), 90% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19, (s, 3H, CH_3), 1.21, (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 3.75 (t, 1H, J = 8.1 Hz, H6`), 3.89 (t, 1H, J = 8.1 Hz, H6), 4.19 (m, 2H, H5, H4), 4.39 (d, 1H, J = 2.2 Hz, H3), 4.72 (d, 1H, J = 3.6 Hz, H2), 5.91 (d, 1H, J = 3.6 Hz, H1); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.7, 26.5, 28.8, 27, 65.1, 73.4, 78.5, 79.7, 83.1, 104.8, 107.8, 111.1; HRMS (ESI) m/z = 339.0761, $C_{12}H_{19}O_9S$ $(M-H)^-$ requires 339.0750.



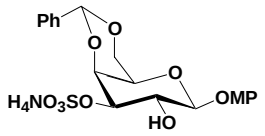
Benzyl 2,3,4-tri-*O*-benzyl-6-*O*- sulfo-α-D-glucopyranoside (4.53).

Prepared according to the general procedures. Flash chromatography (10:2:0.5, CH_2Cl_2: MeOH:NH_4OH), 50% yield as white solid in addition to 30% of the glucose 6-azido derivative. ^{1}H NMR (300 MHz, CD_3OD) δ 3.62 (t, 1H, *J* = 7.4 Hz, H4), 3.53 (m, 3H, H2, H5, H3), 4.32 (m, 2H, H6, H6`), 4.61 (d, 1H, *J* = 7.6 Hz, H1), 4.62-4.93 (m, 8H, $4CH_2$), 7.32 (m, 20H, ArH); ^{13}C NMR (75 MHz, CD_3OD) δ 51.4, 70.6, 72.7, 74.2, 74.3, 74.5, 74.9, 78.9, 82.1, 83.9, 102.0, 127.7, 127.9, 127.92, 128.0, 128.1, 128.16, 128.2, 128.29, 128.3, 128.6, 128.63, 128.72, 128.79, 129, 137.8, 138.4, 139.5; HRMS (ESI) m/z = 619.2006, $C_{34}H_{36}O_9S$ $(M-H)^-$ requires 619.2002.

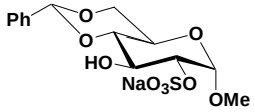


***p*-Methoxyphenyl 2-*O*-benzoyl-3-*O*-sulfo-4:6-*O*-benzylidene-β-D-galactopyranoside (4.54).** Prepared according to the general procedures. Flash chromatography (20:4:1 CH_2Cl_2/MeOH/NH_4OH), 95% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.60 (s, 3H, OCH_3), 3.91 (bs, 1H, H5), 4.08 (AB system, 2H, *J* = 12.4 Hz, H6, H6`), 4.61 (m, 2H, H4, H3), 5.35 (m, 2H, H1, H2), 5.61 (s, 1H, CHPh), 6.76, 6.86 (AA`BB` system, 4H, *J* = 8.8, 8.6 Hz, ArH), 7.04 (b, 4H, NH_4), 7.47 (m, 6H, ArH), 7.59 (t, 1H, *J*= 7 Hz, ArH), 7.95 (d, 2H, *J* = 7.8 Hz ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8, 66.5, 68.6, 70.0, 73.8, 74.6, 99.6, 100.3, 115.0, 118.1, 126.7, 128.4, 128.9, 129.2, 129.9, 130.5, 133.5,

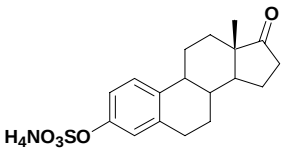
138.8, 151.2, 155.1, 165.5; HRMS (ESI$^-$) m/z = 557.1126, $C_{27}H_{25}O_{11}S$, $(M-H)^-$ requires 557.1118.



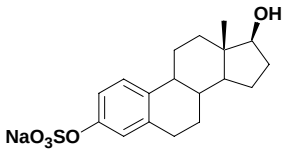
***p*-Methoxyphenyl 3-*O*-sulfo-4:6-*O*-benzylidene-β-D-galactopyranoside (4.55).** Prepared according to the general procedures. Flash chromatography (10:2:0.5, CH_2Cl_2: MeOH:NH_4OH), 88% as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.72 (m, 5H, OH, OCH_3, H5), 4.05 (m, 2H, H6, H6`), 4.35 (m, 2H, H4, H2), 5.03 (d, 1H, $J$ = 7.6 Hz, H1), 5.26 (bs, 1H, H3), 5.50 (s, 1H, CHPh), 6.81, 7.02 (AA`BB` system, 4H, J = 8.7, 8.5 Hz, ArH), 7.15 (b, 4H, NH_4), 7.42 (m, 5H, ArH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.8, 66.2, 68.7, 68.8, 74.7, 76.9, 100.1, 101.4, 114.8, 118.0, 126.7, 128.4, 129.1, 138.9, 151.5, 154.7; HRMS (ESI) m/z = 453.0850, $C_{20}H_{21}O_{10}S$, $(M-H)^-$ requires 453.0855.



Methyl 2-*O*-sulfo-4:6-*O*-benzylidene-α-D-glucopyranoside (4.56). Prepared according to the general procedures. Flash chromatography (10:2, CH_2Cl_2: MeOH), 95% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.27 (s, 3H, OCH_3), 3.43 (m, 1H, H5), 3.57 (dt, 1H, J = 9.6, 4.2 Hz, H3), 3.66 (m, 2H, H6, H6`), 3.95 (dd, 1H, J = 9.6, 3.4 Hz, H2), 4.13 (dd, 1H, J = 9.6, 4.2 Hz, H4), 4.85 (d, 1H, J = 3.4 Hz, H1), 5.12 (b, 1H, OH), 5.50 (s, 1H, CHPh), 7.36 (m, 5H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.3, 62.5, 68.5, 68.6, 76.8, 81.8, 99.1, 101.3, 126.8, 128.4, 129.3, 138.1; HRMS (ESI) m/z = 361.0599, $C_{14}H_{17}O_9S$, $(M-H)^-$ requires 361.0593.



Estrone-3-sulfate (4.57). Prepared according to the general procedures. Flash chromatography (10:2:0.5, CH_2Cl_2 : MeOH : NH_4OH), 94% yield as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 0.81 (s, 3H, CH_3), 1.31-1.50 (m, 6H), 1.72 (m, 1H), 1.89-2.01 (m, 3H), 2.12 (b, 1H), 2.38 (m, 2H), 2.75 (t, 2H, *J*= 8.6Hz), 6.85 (m, 2H, ArH), 7.01 (b, 4H, NH_4), 7.11 (d, 1H, *J*= 8.5 Hz, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9, 21.6, 25.9, 26.5, 29.5, 31.8, 35.8, 38.2, 44.0, 47.8, 50.0, 118.5, 120.9, 125.9, 134.7, 137.0, 151.7; HRMS (ESI) m/z = 349.1111 $C_{18}H_{21}O_5S$ $(M-H)^-$ requires 349.1110.



Estradiol-3-sulfate (4.58). Prepared according to the general procedures. Flash chromatography (10:2, CH_2Cl_2:MeOH), 96% yield as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 0.82 (s, 3H, CH_3), 1.21-1.52 (m, 7H), 1.63 (m, 1H), 1.91 (m, 1H), 2.01 (m, 2H), 2.18 (m, 1H), 2.33 (d, 1H, *J*= 13.0 Hz), 2.88 (t, 1H, *J*= 3.6 Hz), 3.67 (t, 1H, *J*= 8.4 Hz, H17), 7.04 (m, 2H, ArH), 7.25 (d, 1H, *J*= 8.5 Hz, ArH); ^{13}C NMR (125 MHz, CD_3OD) δ 10.2, 22.6, 26.1, 26.9, 29.1, 29.3, 36.6, 38.8, 42.9, 44.1, 49.9, 81.06, 118.3, 121.0, 125.5, 136.7, 137.4, 150.2; HRMS (ESI) m/z = 351.1272, $C_{18}H_{23}O_5S$ $(M-H)^-$ requires 351.1266.

Chapter 5. Towards the Total Synthesis of the Disulfated Tetrasaccharide Portion of SB_{1a}, a Carbohydrate Antigen Associated with Human Hepatocellular Carcinoma

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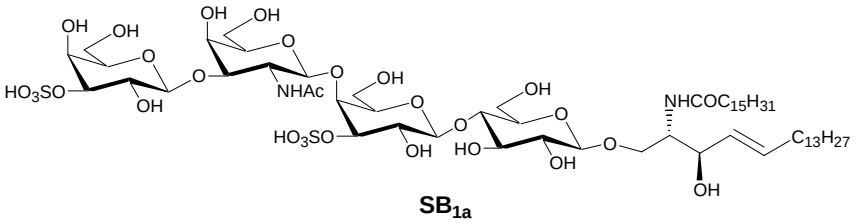
5.1 Introduction

As we mentioned in Chapter 2, a series of modified sulfuryl imidazolium salts (SIS's) were prepared and their sulfating abilities were studied. The most efficient sulfating agent, **2.4**, was obtained by incorporating a methyl group at the 2-position of the imidazolium ring of the original reagent **1.83**. The trichloroethyl-protected sulfates could be introduced into monosaccharides in excellent yields using reagent **2.4**. The TCE group withstands many of the conditions that are commonly encountered in carbohydrate chemistry and a simple disaccharide containing TCE-protected sulfates was prepared and the suflate groups deprotected in high yield (see chapter 1 section 1.3.4).[38] In Chapter 3, we showed that the direct regioselective incorporation of TCE-protected sulfates into monosaccharides can be achieved using reagent **2.4** and that the TCE group can be removed from di- and trisulfated monosaccharides under mild conditions in excellent yields. Our next objective is to demonstrate that our sulfate-protecting group strategy can be applied to the synthesis of a complex multisulfated oligosacharide. In this chapter we present our studies on the application of our sulfate protecting group strategy towards the synthesis of the tetrasaccharide portion of a disulfated glycosphingolipid called SB_{1a}.

In 1982, Tadano and coworkers isolated a novel disulfated tetraglycosylceramide from the lipid extract of rat kidney.[92] After extensive structural studies, Tadano and

coworkers proposed the structure of this glycolipid to be (HSO_3-3)Galβl-3GalNAcβ1-4(HSO_3-3)Galβl-4Glcβl-1Cer (**5.1** Figure 5.1).



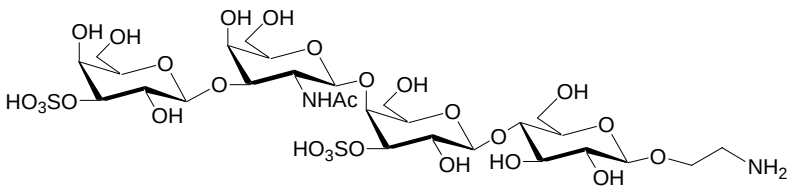
SB_{1a}

5.1

Figure 5.1. The structure of SB_{1a} (**5.1**)**.**

In 1988, Hiraiwa and coworkers raised two mouse monoclonal antibodies against human hepatocellular carcinoma (HCC) cells and an acidic glycolipid mixture prepared from the same cells.[93] The antigen recognized by both monoclonals was determined to be SB_{1a}. SB_{1a} was found to be strongly expressed on the surface of human HCC's but was not detectable in the acidic glycolipid fractions obtained from normal livers indicating that the SB_{1a} antigen is associated with human HCC.

Little is known about the function of SB_{1a}. In order to clarify the functions of SB_{1a} in detail, especially its involvement in the metastasis of human HCC and hence pursue carbohydrate-based anticancer vaccines for HCC, Li and coworkers undertook the first synthesis of the disulfated tetrasaccharide moiety of SB_{1a} (**5.2**, Figure 5.2). The ceramide moiety was replaced by a 2-aminoethyl spacer arm which could be used to facilitate formation of immunogenic glycoconjugates.[25]



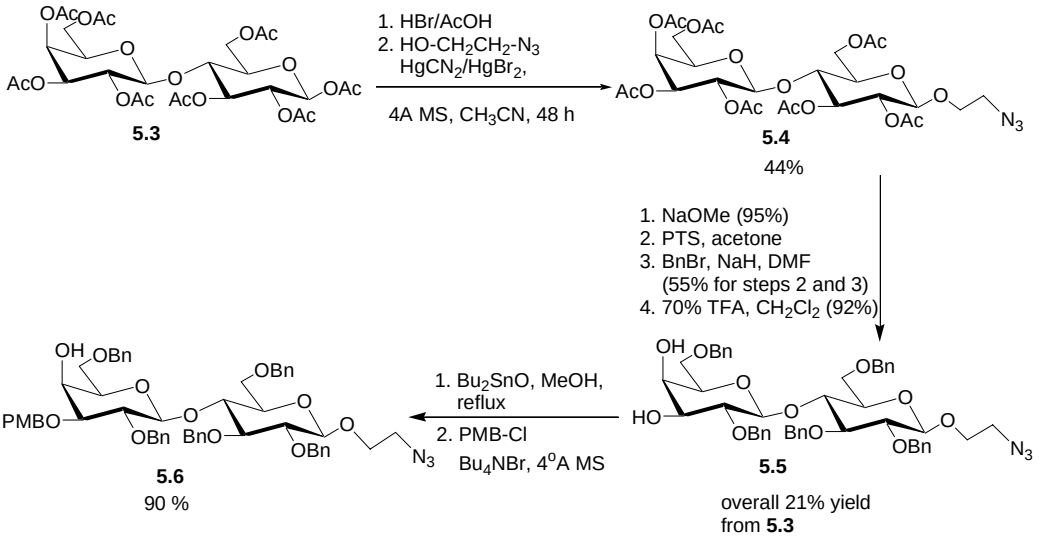
5.2

Figure 5.2. The tetrasaccharide portion of SB_{1a}.with a tether attached to the reducing end, prepared by Li et al.

Li *et al.* used a [2+1+1] approach to form the fully protected tetrasaccharide back bone. The orthogonal protecting group strategy was used where the hydroxyls that would ultimately contain the sulfate groups were orthogonally protected using *p*-methoxybenzyl (PMB) and monochloroacetate (MCA) moieties. 2-*N*-Phthalimido and 2-*O*-benzoyl protected donors were used to direct the required β-glycosidic linkages. Finally, benzyl ethers were used for permanent protection of the rest of the hydroxyl groups.

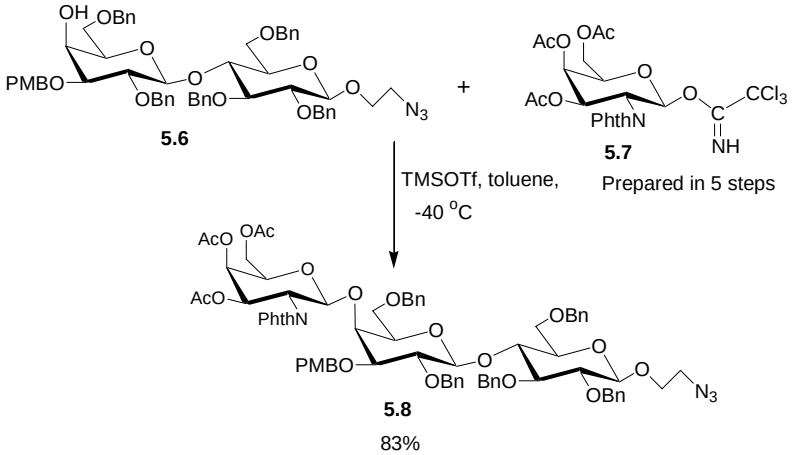
The authors started the synthesis of the SB_{1a} tetrasaccharide with the preparation of lactosyl acceptor **5.6** which had been previously prepared by Chernyak et al (Scheme 5.1).[94] No details were given for the synthesis of this compound by Li et al. Chernyak et al's synthesis along with the yields they obtained is shown Scheme 5.1. Treating lactose acetate derivative **5.3** with hydrogen bromide in acetic acid followed by 2-azidoethanol in the presence of mercuric cyanide and mercuric bromide afforded the 2-azidoethyl lactose derivative **5.4** in 44 % yield. Deacetylation of **5.4** with sodium methoxide gave the 2-azidoethyl lactose in 95% yield. The resulting material was subjected to *p*-TsOH in acetone then the crude product was treated with benzyl bromide and sodium hydride in DMF to afford the fully protected disaccharide in 55% yield (over the two steps). Removal of the acetal group was achieved in 92% yield by treating the fully protected disaccharide with 70% TFA in methylene chloride. The lactosyl diol **5.5** was prepared in

21% overall yield from **5.3**. Tin mediated selective alkylation of **5.5** afforded the 3`-*p*-methoxybenzyl ether derivative **5.6** in 90% yield (the overall yield of **5.6** is 19% over the 8 steps) (Scheme 5.1).[95,96]



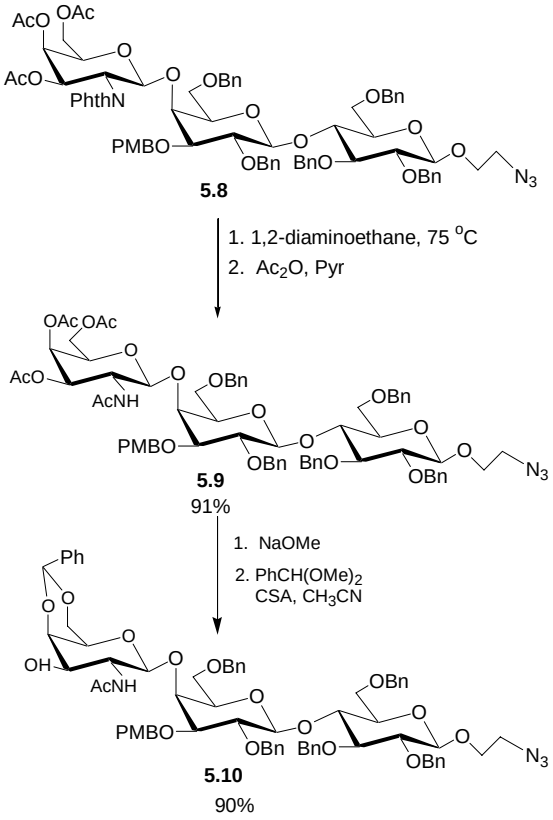
Scheme 5.1 Synthesis of the lactosyl acceptor **5.6**

Standard glycosylation of acceptor **5.6** and the glycosyl donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate **5.7** (prepared in 5 steps)[97] in toluene at −40 °C afforded the desired β-linked trisaccharide **5.8** in 83% yield (Scheme 5.2).



Scheme 5.2. Synthesis of the trisaccharide **5.8.**

Trisaccharide **5.8** was subjected to a series of protecting group manipulations including dephthaloylation using 1,2-diaminoethane in *n*-butanol at 75 ^{o}C followed by *N*-acetylation to give **5.9** in 91% yield. *O*-Deacetylation of **5.9** followed by installation of the 4,6-*O*-benzylidene acetal provided the trisaccharide acceptor **5.10** in 90% yield (compound **5.10** was obtained on 67% overall yield starting from disaccharide **5.6**) (Scheme 5.3).



Scheme 5.3 Synthesis of the trisaccharide acceptor **5.10**

With the trisaccharide acceptor **5.10** in hand, the authors first examined ethyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside **5.11** and the corresponding glycosyl bromide **5.12** (Figure 5.3) as the glycosyl donor for the assembly of the tetrasaccharide backbone. No reaction occurred when **5.11** and **5.10** were reacted

at room temperature employing $Bu_4NBr–CuBr_2$ as the promoter in nitromethane or DMF or when using methyl triflate as promotor in dichloromethane or diethyl ether. The authors then tried the glycosylation of the donor **5.12** with the trisaccharide acceptor **5.10** in the presence of silver triflate as a promoter, but again no desired tetrasaccharide was obtained, but the main product isolated was disaccharide **5.13** (Figure 5.3).

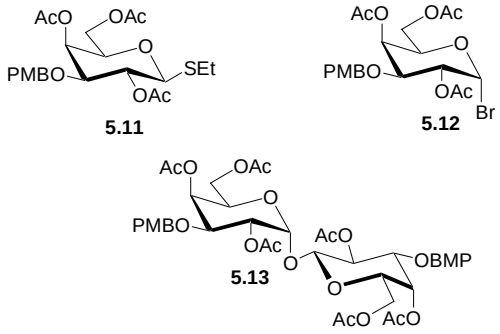
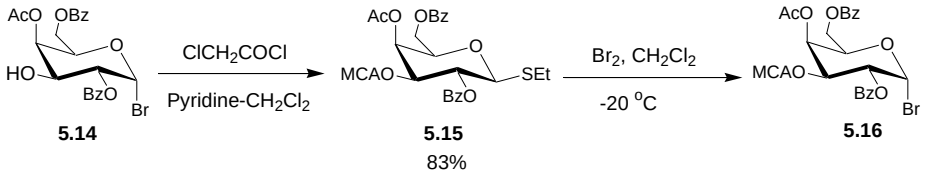


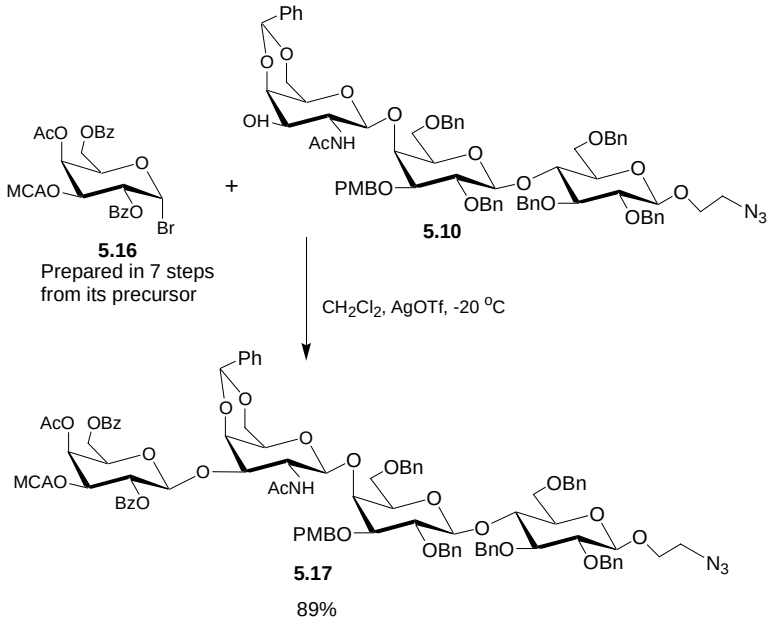
Figure 5.3. Glycosyl donors **5.11**, **5.12** and the disaccharide by-product **5.13**

After the above failures the authors then turned to glycosyl donor **5.16.** This donor was prepared by chloroacetylation of the known ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside **5.14**[98] (Compound **5.14** was prepared in 6 steps in 28% overall yield starting from galactose pentaacetate)[98] followed by the in situ transformation to the bromide using bromine in methylene chloride at -20 ^oC (Scheme 5.4). Compound **5.16** was prepared in 8 steps in 18 % overall yield starting from



Scheme 5.4 Synthesis of the glycosyl bromide **5.16**

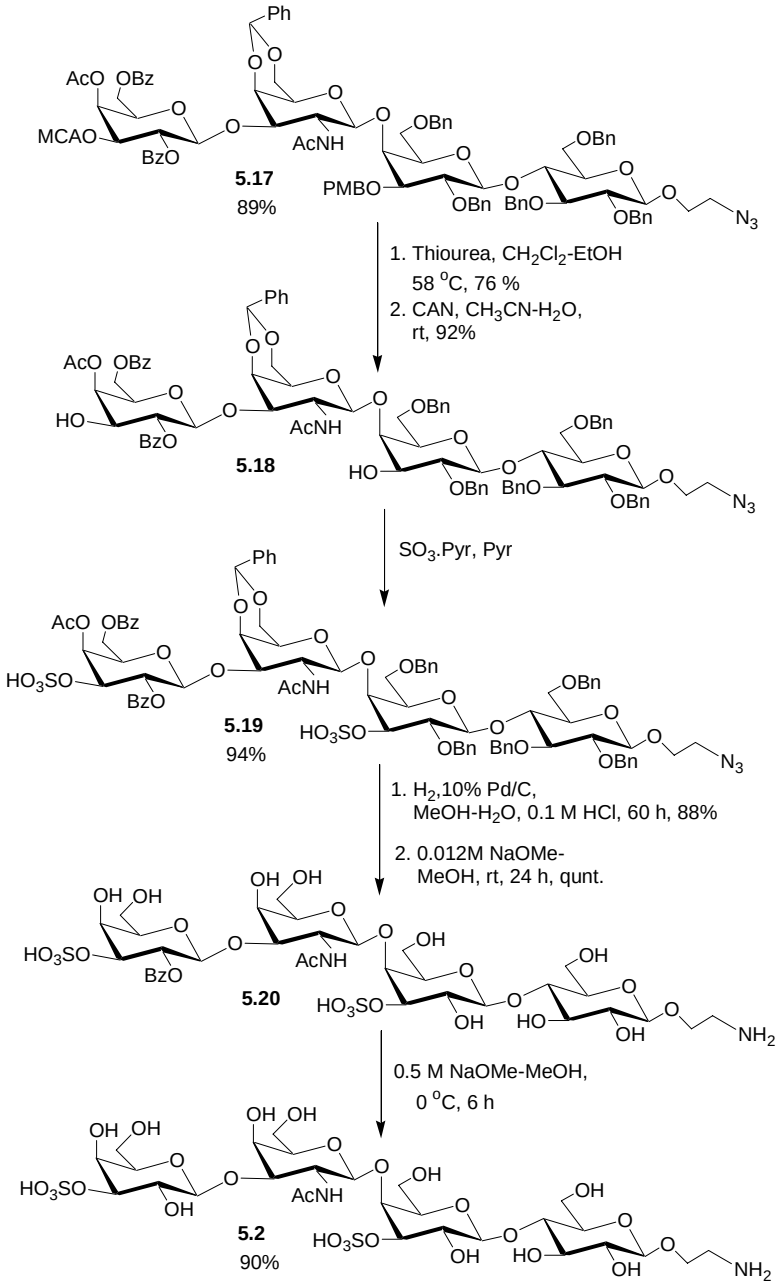
galactose pentaacetate. Surprisingly, when glycosyl bromide **5.16** was treated with the trisaccharide acceptor **5.10** in CH_2Cl_2 at -20 ^oC in the presence of silver triflate as promoter the tetrasaccharide **5.17** was obtained in 89% yield (Scheme 5.5).



Scheme 5.5 Synthesis of the protected tetrasaccharide **5.17**

The protected tetrasaccharide **5.17** was subjected to several transformations to give the target tetrasaccharide **5.2.** First, selective removal of the chloroacetyl group at the 3```-OH position was achieved in 76% yield by treating **5.17** with thiourea in CH_2Cl_2-EtOH at 58 ^oC in the presence of 2,6-lutidine. In the next step, the *p*-methoxybenzyl group at 3`-OH position was removed selectively with cerium(IV) ammonium nitrate (CAN), to give diol **5.18** in 92% yield (Scheme 5.6). Treatment of the diol **5.18** with sulfur trioxide·pyridine complex in pyridine furnished the disulfated tetrasaccharide **5.19** in 94% yield. Subjecting the disulfated tetrasaccharide **5.19** to catalytic hydrogenolysis, using Pd/C in different solvents (AcOH, 2:1 MeOH–AcOH) proceeded very slowly and gave low yields. The authors attributed this problem to some undefined inhibitory interaction of the catalyst with the aminoethyl fragment formed during the reaction.[99,100]

To over come this apparent inhibitory effect, the authors converted the amino group to its hydrochloride salt and then did the hydrogenolysis reaction which gave the target in 88% yield (Scheme 5.6).



Scheme 5.6 Completed synthesis of the SB_{1a} tetrasaccharide

Some difficulties were faced during the saponification of the ester groups on the left hand galactose moiety. Saponification with 0.012 M sodium methoxide in MeOH at room

temperature gave the 2```-*O*-benzoyl **5.20** where only the ester groups at 4```-OH and 6```-OH were hydrolysed. Increasing the base concentration and prolonging the reaction time only led to decomposition of the product. On the other hand, using ammonia in MeOH led to *O*-desulfation, and no *O*-deacylation was observed. The saponification of **5.20** was eventually achieved using 0.5 M sodium methoxide in MeOH at 0 °C for 6 h which gave compound **5.2** in 90% yield (Scheme 5.6).

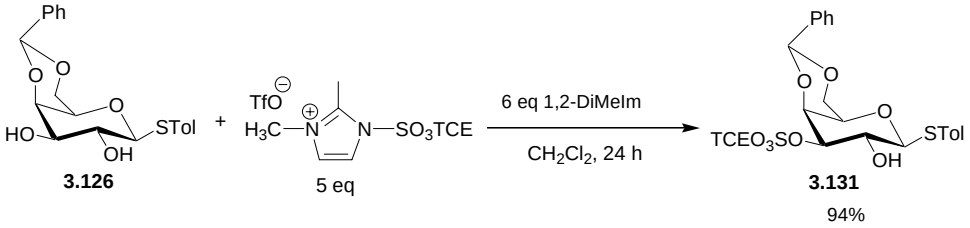
5.2 Objectives

Although Li *et al*.[25] achieved the synthesis of **5.2** their approach did have some shortcomings. Although good yields were eventually obtained for most steps some significant problems were encountered at various stages especially during the deprotection of the benzoyl groups at the end of the synthesis. Because the sulfate group was introduced using the traditional approach at the end of the synthesis, protecting group manipulations had to be performed in the latter stages of the synthesis to accommodate them. The objective of the work described in this chapter is to determine if a superior synthesis of SB_{1a} can be achieved using the sulfate protecting group strategy.

5.3 Results and Discussion

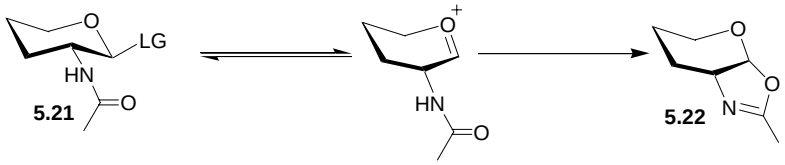
We chose the SB_{1a} tetrasaccharide as a target to test our sulfate protecting group approach to the synthesis of complex multisulfated oligosaccharides for several reasons. First, as we discussed in the introduction, it had been made before using traditional sulfation strategies. This was important since we wished to compare the synthesis of a complex sulfated oligosaccharide using the sulfate protecting group approach to the traditional approach. The second reason had to do with our selective sulfation studies described in Chapter 3. We had recently discovered that we were able to incorporate a

TCE-protected sulfate group selectively into the 3-OH of thiogalactosyl derivative **3.131** in the presence of a free 2-OH in excellent yields (Scheme 5.7).[83] We anticipated that this



Scheme 5.7. Selective sulfation of carbohydrate **3.126**.

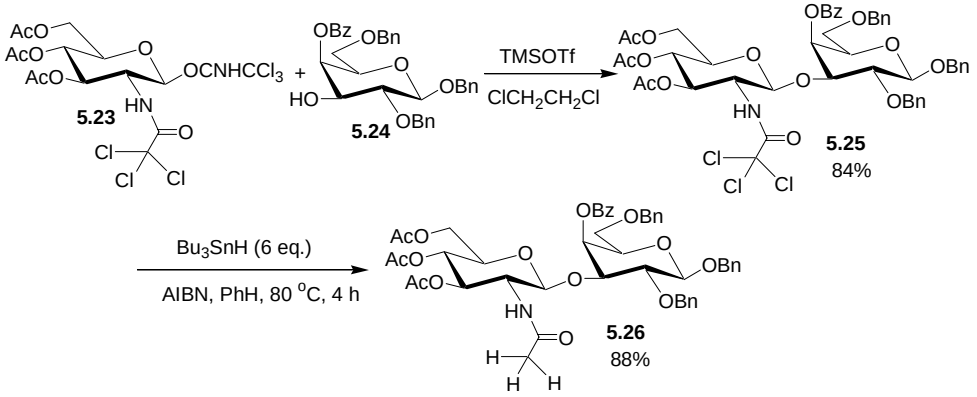
reaction would be very useful in the synthesis of sulfated oligosaccharides containing one or more galactosyl residues with a sulfate at the 3-position. SB_{1a} contains two galactosyl residues with a sulfate at the 3-position and so was a very good target for testing this. The third reason has to do with the *N*-acetyl group in residue 3. The *N*-acetyl group is found in many in oligo- and polysaccharides. However, carbohydrates bearing *N*-acetyl groups at the 2-position are usually not used as donors in glycosidation reactions since very stable oxazoline compounds can readily form upon donor activation (such as **5.22** in Scheme 5.8) which may prevent any glycosylation reactions from occurring.



Scheme 5.8. Stable oxazoline formation

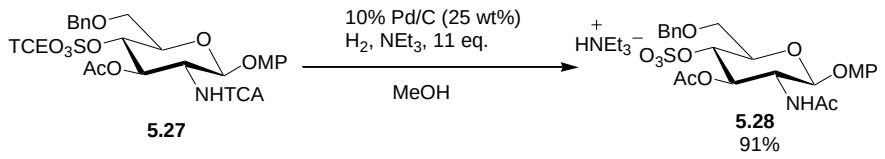
This is why Li et al used a phthalimido group to protect the amino group in residue 3 in their synthesis of SB_{1a} (Scheme 5.2). The phthalimido group participates during glycosidic bond formation between **5.6** and **5.7** but does not form stable oxazoline compounds. Hence, the glycosidic bond is formed and with the required β-

stereochemistry. The disadvantage of the phthalimido group is that it has to be removed and the resulting free amino group acetylated which increases the number of steps (as in Scheme 5.3). Other amino protecting groups besides the phthalimido group have also been used. One is the trichloroacetyl (TCA) group (Scheme 5.9).[101] This group also participates during glycosidic bond formation but does not form stable oxazoline compounds. The advantage of using this protecting group is that it can be *directly* converted to the *N*-acetyl group using tributylstannane and AIBN or by hydrogenolysis using Pd/C as catalyst in the presence of triethylamine.[101,102]



Scheme 5.9. 2-Trichloroacetamido-2-deoxyglucopyranosyl glycosyl donors.

Laura Ingram, a former graduate student in the Taylor group has shown that a monosaccharide containing a TCE-protected sulfate and a TCA-protected amide group can be simultaneously reduced/deprotected using H_2, 10 wt% Pd/C in MeOH containing

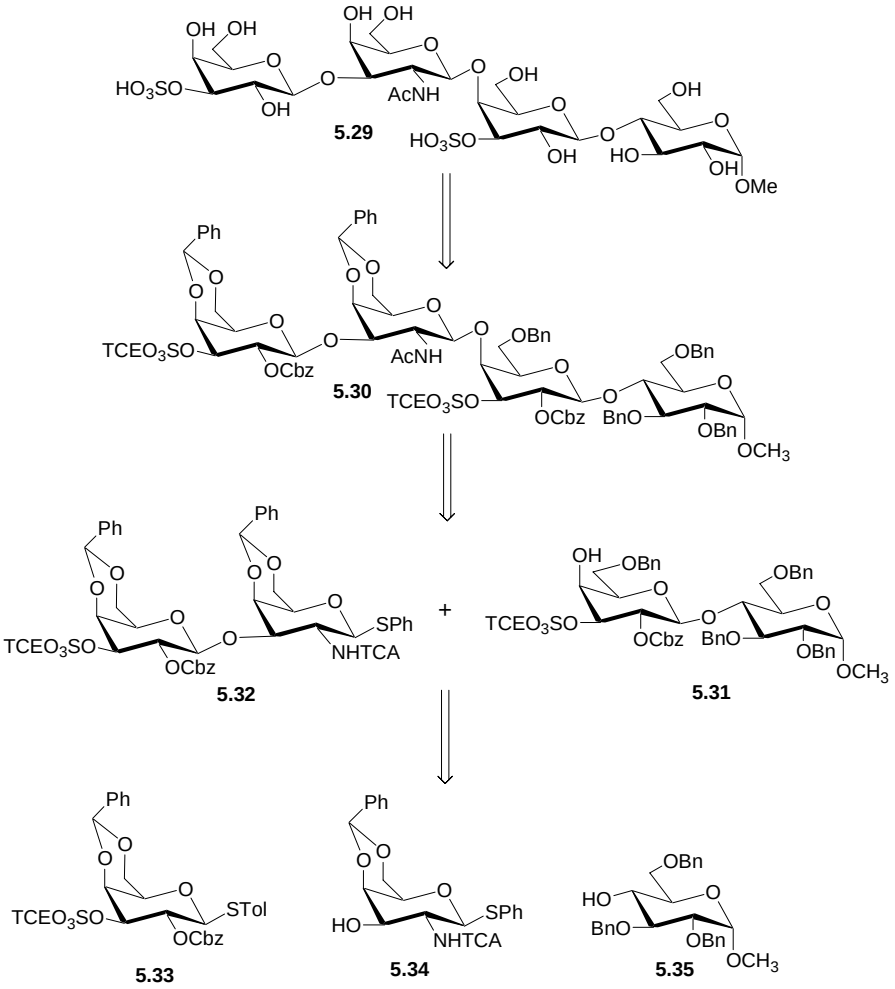


Scheme 5.10. Co-reduction and deprotection of trichloroacetamide and TCE groups

an excess of Et_3N (Scheme 5.10). We wanted to try this methodology on a more complex carbohydrate such as SB_{1a}. Indeed, we ultimately wanted to design a synthesis of SB_{1a} such that all of the protecting groups could be removed at the end of the synthesis in a single step by hydrogenolysis. By doing so we would avoid the difficulties encountered by Li et al during the removal of the benzoyl groups at the end of their synthesis. The obvious group for protecting the hydroxyl moieties that would not bear a sulfate group was the benzyl group either in the form of a benzyl ether or benzylidene acetal as it can be readily removed by hydrogenation. However, we could not use a benzyl ether at the 2-position of residues 2 and 4 since we required a participating group at these positions to get the desired β-stereochemistry. The carbobenzyloxy (Cbz) group offered itself as a possible alternative. Although the Cbz group is rarely used in carbohydrate chemistry it has been shown that it can be readily introduced into carbohydrates, can participate to give exclusively trans-1,2 linkages and can be removed by catalytic hydrogenolysis.[103-105] 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.[106] This issue will be discussed in more detail in a subsequent section in this chapter.

Our first synthetic strategy for the synthesis of SB_{1a} is shown in Scheme 5.11. We anticipated that the target tetrasaccharide **5.29** could be prepared from the fully protected precursor **5.30** (Scheme 5.11). The protecting groups in **5.30** (the benzyl ethers and benzylidene acetals, Cbz, TCE-protected sulfate and TCA-protected amide group) were chosen such that they could be removed in one step by catalytic hydrogenation/hydrogenolysis in the last step of the synthesis. Tetrasaccharide **5.30**

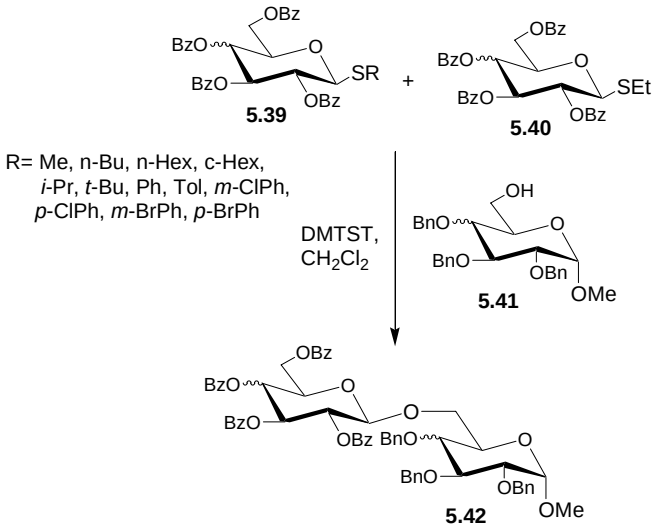
would ultimately be assembled from the disaccharides **5.31** and **5.32** which in turn would be synthesised from monomers **5.33**, **5.34** and **5.35** (Scheme 5.11). Monomer **5.33** would be easily prepared via our selective sulfation methodology and two of the residues would be derived from this monomer.



Scheme 5.11. Retrosynthesis of the tetrasaccharide of SB_{1a}

The formation of **5.32** involves the coupling of donor **5.33** and acceptor **5.34** both of which are thioglycosides. They clearly have different groups attached to the 2- and 3-positions and one is a phenyl thioglycosides while the other is a tolyl thioglycoside.

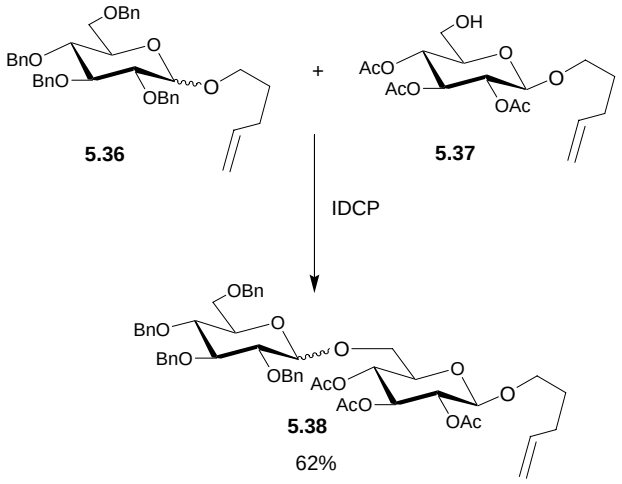
Thioglycoside reactivity can be manipulated by the introduction of different thiol aglycons.[107c] In 2002, Lahmann and Oscarson reported a study on the reactivity of thioglycosides with different aglycon moieties using competitive glycosylations. The authors used methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **5.41** as an acceptor and DMTST ((dimethylthio) methylsulfonium trifluoromethanesulfonate) as a promoter. [107c] Lahmann and Oscarson found that the reactivity depends on the electron donating properties of the aglycon (Scheme 5.12). [107c] The donor bearing the more electron donating group attached to the sulfur was more readily activated by the promotor and so was a better donor. On the basis of these results one would expect that carbohydrate **5.33** would be more readily activated than carbohydrate **5.34**. However other factors influence reactivity.



Scheme 5.12. A competitive glycosylation of thioglycosides with different aglycon

Protecting groups can also affect the reactivity of donors (and acceptors). In 1988, Fraser-Reid and coworkers, introduced the armed–disarmed concept to denote the influence of the protecting groups on the reactivity of the anomeric *n*-pentenyl group. The

authors reported that, *n*-pentenyl glycoside **5.36**, "armed" with the electron-donating benzyl groups, was selectively activated with IDCP (iodonium dicollidine perchlorate) to couple with *n*-pentenyl glycoside **5.37**, which is "disarmed" with the electron-withdrawing acetyl groups, where disaccharide **5.38** was formed in 62% yield without detection of the self coupling products of **5.37** (Scheme **5.13**). [107b]



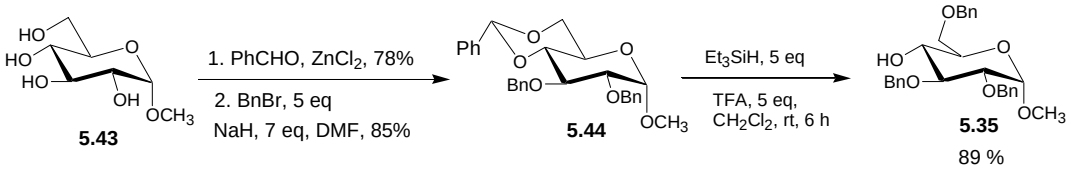
Scheme 5.13. A prototypical "armed-disarmed" glycosylation.

It is not known whether a trichloroacetamide (TCA) group at the 2-position in a donor (as in **5.34**) arms or disarms a donor. The chlorines are electron withdrawing which may decrease the reactivity of **5.34** relative to **5.33.** However, no studies have ever been conducted on the effect of a TCE-protected sulfate on the reactivity on a donor nor has the effect of a Cbz group been examined. The TCE-protected suflate in **5.33** will certainly be electron-withdrawing and deactivate it but is further away for the anomeric position than the TCA group in **5.34**. No matter what their respective reactivities, **5.33** will have to be activated in preference to **5.34** in order to obtain **5.32**. Should we witness

products resulting from the self-coupling of **5.34** during the formation of **5.32**, then modifications will have to be made to donor **5.33**.

*5.3.1 Synthesis of the Glucosyl acceptor **5.35***

Glucosyl acceptor **5.35** was prepared according to the reported literature procedures starting from methyl α-D-glucopyranoside (Scheme 5.14).[108] Methyl α-D-glucopyranoside **5.43** was stirred with a mixture of benzaldehyde and anhydrous zinc chloride at room temperature to afford the corresponding benzylidene acetal in 78% yield. Treatment of the diol with benzyl bromide and sodium hydride in DMF for 24 hour gives the fully protected methyl glucopyranoside **5.44** in 85% yield. Acceptor **5.35** was obtained in 89% yield by reductive opening of the benzylidene acetal **5.44** with Et_3SiH and TFA in CH_2Cl_2 (Scheme 5.14). The overall yield of **5.35** was 59%.

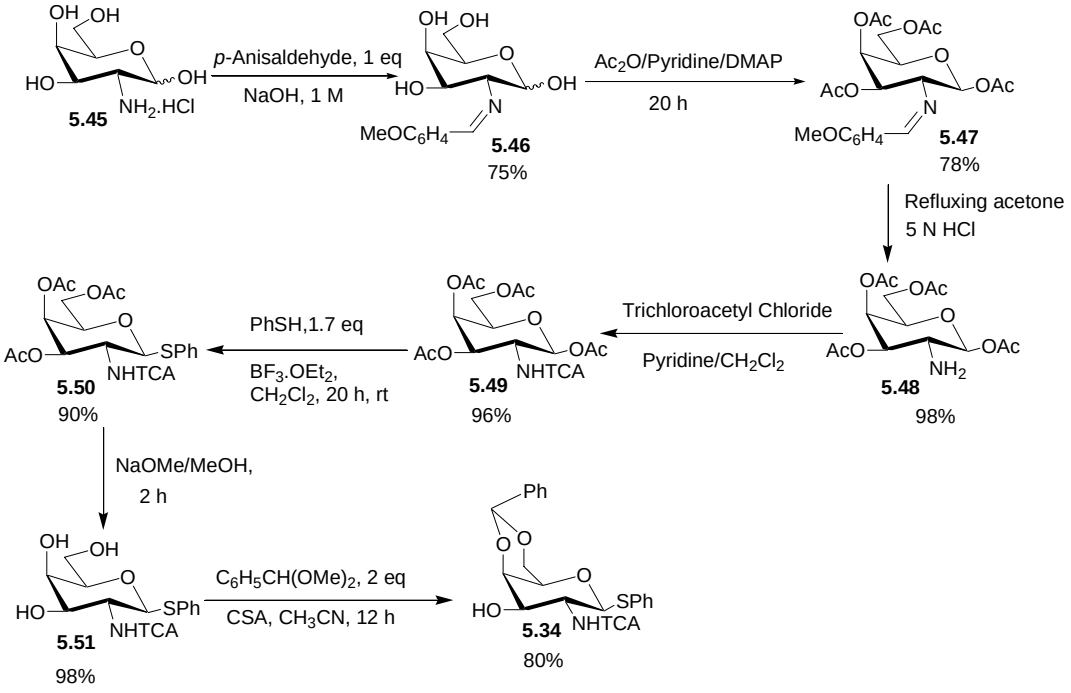


Scheme 5.14. Synthesis of glucosyl acceptor.

*5.3.2 Synthesis of the GalNTCA Acceptor **5.34***

The synthesis of acceptor **5.34** was straight forward starting from the commercially available galactose amine hydrochloride **5.45** using the known literature procedures.[109] Condensation of the amino group in **5.45** with *p*-anisaldehyde in presence of 1 M NaOH afforded **5.46** in 75% yield. Acetylation of **5.46** followed by the deprotection of the amino group give the free amino galactose amine **5.48** almost in quantitative yield. Protection of the amino functionality with TCA-group was achieved in 96% yield using trichloroacetyl chloride and pyridine in methylene chloride (Scheme

5.13). The fully protected galactose amine **5.49** was treated with BF_3OEt_2 in the presence of benzenethiol to give compound **5.50** in 90% yield. Removal of the remaining acetate protecting groups gave triol **5.51** in near quantitative yield. Treatment of the triol with benzaldehyde dimethyl acetal in the presence of catalytic amount of CSA in acetonitrile afforded the benylidene acetal acceptor **5.34** in 80% yield (Scheme 5.15). The overall yield of **5.34** was 39% (7 steps).

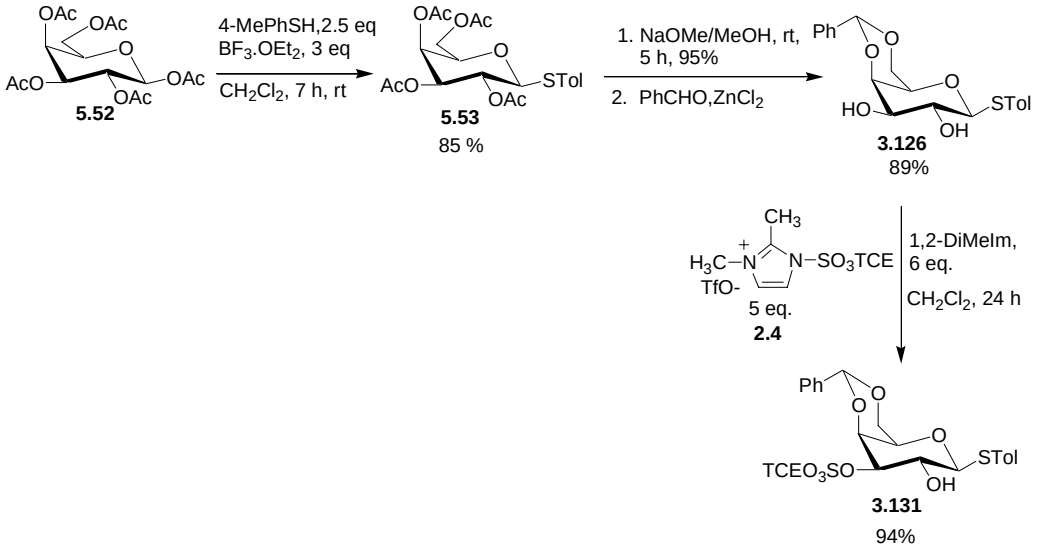


Scheme 5.15. Synthesis of galactose amine acceptor **5.34**.

*5.3.3 Synthesis of the galactose donor **5.33***

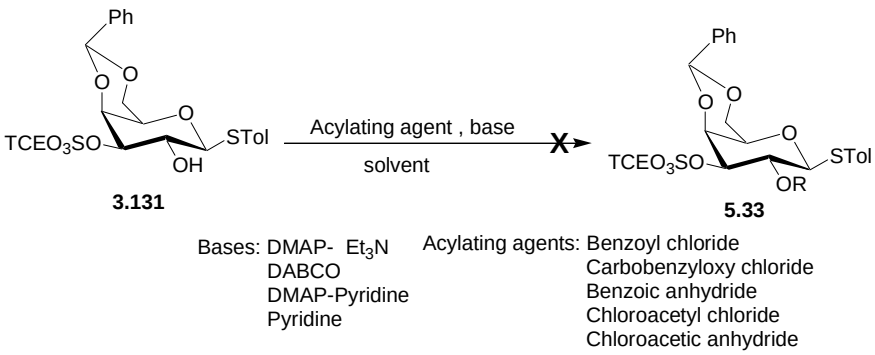
As we mentioned in the beginning of this chapter and chapter 3 we were able to introduce the TCE-protected sulfate selectively into the 3-OH of the galactose in the presence of 2-OH free in excellent yield (Scheme 5.7). The thiogalactose diol **3.126** was prepared in good yield starting from the commercially available galactose pentaacetate in three steps.[110] The TCE-protected sulfate was selectively introduced into the 3-OH in

94% yield by treating the diol **3.126** with the imidazolium salt **2.4** in methylene chloride in the presence of dimethyl imidazole 1,2-DiMeIm as a base (Scheme 5.16).



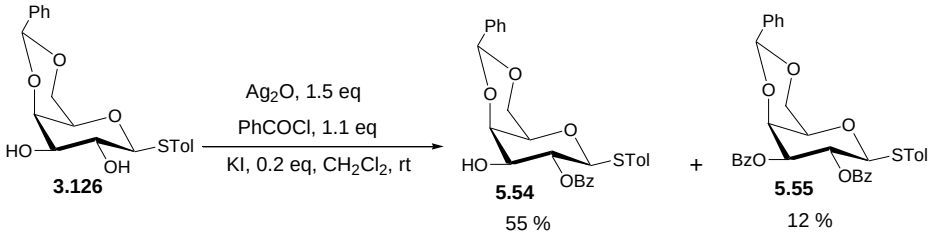
Scheme 5.16. Selective sulfation of galactose diol.

With the TCE-protected sulfate **3.131** in hand, we started looking for conditions for introducing the Cbz group into the 2-OH. Unfortunately, once the $TCESO_3$ moiety had been installed, the 2-OH of **3.131** appeared to be extremely unreactive. Under different conditions including different bases, solvents, and temperatures and after many trials, we were not able to introduce the Cbz-group into the desired donor and the starting material was isolated each time without observing the product. To insure that the problem was not due to the Cbz-group, we tried other acylating agents under the same conditions but unfortunately no product was isolated (Scheme 5.17).



Scheme 5.17 Different acylating conditions

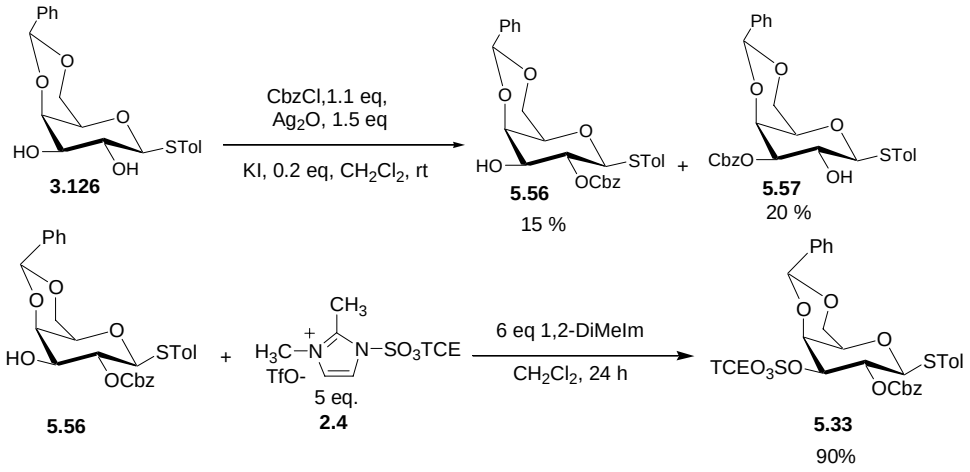
Since we were unable to introduce the Cbz-group into compound **3.131**, we decided to find an alternative route for the synthesis of the galactose donor **5.33**. In 2004, Shan Ye *et al*. reported that 2,3-diols of benzylidene acetal-protected galactosides could be selectively acylated at the 2-position using freshly prepared silver oxide and KI (Scheme 5.18).[111] The Cbz group was not examined. We decided to try these conditions



Scheme 5.18. Selective incorporation of benzoyl group

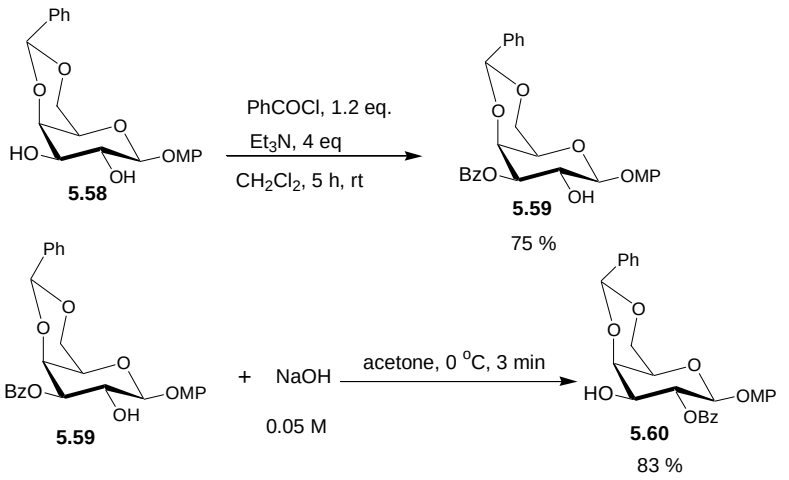
to introduce the Cbz-group into the 2-OH of diol **3.126**. If successful we would then install the TCE-SO_3 group at the 3-OH to complete the synthesis of **5.33**. When diol **3.126** was treated with CbzCl under the same condition described by Xin-Shan Ye,[111] the desired product **5.56** was isolated in relatively low yield (15%) after a tedious separation from the 3-O-Cbz derivative **5.57** which was isolated in a 20% yield. Compound **5.56**

was treated with imidazolium salt **2.4** and 1,2-dimethylimidazole (1,2-DiMeIm) in methylene chloride to afford the target donor **5.33** in 90% yield (Scheme 5.19).



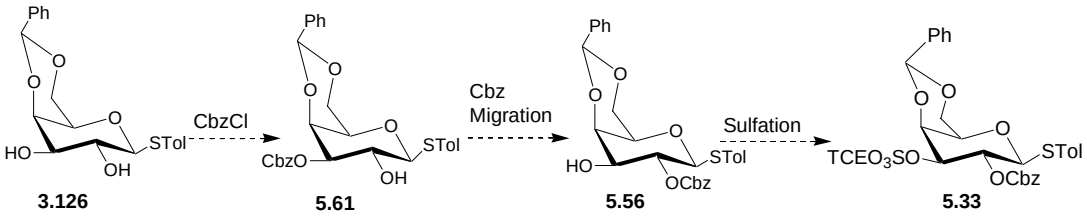
Scheme 5.19. Selective incorporation of Cbz- group.

Due to the low yield and the difficult purification of thiogalactoside **5.56**, we decided to search for a better route to **5.33**. In 2004, Jacquinet was able to selectively introduce a benzoyl protecting group at the 3-position of 2, 3-diol-4,6-*O*-benzylidene-galactose protected with OMP group at the anomeric position. The author was able to migrate the benzoyl group to the 2-position in good yield by subjecting it to a solution of NaOH in acetone-water (Scheme 5.20).[112] The migration took 3 minutes and the product precipitated during the reaction which probably helped in pushing the equilibrium towards the formation of 2-O-Bz.



Scheme 5.20. Selective benzoylation and benzoyl migration.

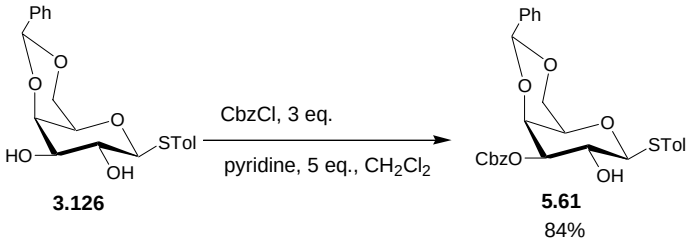
On the basis of the Jacquinet's work, another route for the synthesis of donor **5.33** was proposed in which the Cbz group would be selectively introduced into the 3-position of **3.126** and then migrated to the 2-position to provide **5.56**. Compound **5.56** would then be sulfated using imidazolium salt **2.4** (Scheme 5.21).



Scheme 5.21. Proposed route to donor **5.33**

This work was done by Laura Ingram a former colleague in the Taylor laboratory while she was performing studies on migration of Cbz-group in carbohydrates. Migration of a Cbz group from 3-OH to 2-OH (or vice versa) in a carbohydrate had never been demonstrated before. After trying many conditions, she was able to selectively introduce the Cbz group into the 3-OH of diol **3.126** by adding a solution of Cbz-Cl (2 equiv) in

methylene chloride dropwise over 1.5 hour to a solution of **3.126** and 2 eq. of pyridine. After stirring the reaction for 3 hour at room temperature, another equivalent of CbzCl was added dropwise over 30 minutes and the reaction was stirred for an additional hour. Compound **5.61** was isolated in 84% yield (Scheme 5.22). Compound **5.61** was subjected to a variety of conditions including those of Jaquinet condition but unfortunately, a mixture of the 3-OCbz **5.61** and 2-OCbz **5.56** isomers was obtained. The 2-OCbz isomer did not precipitate out of solution.

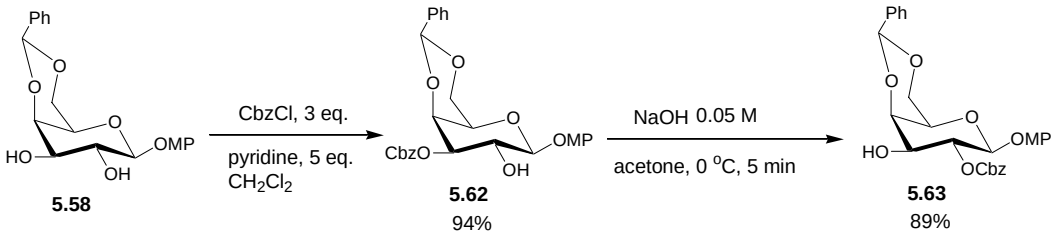


3.126 **5.61** 84%

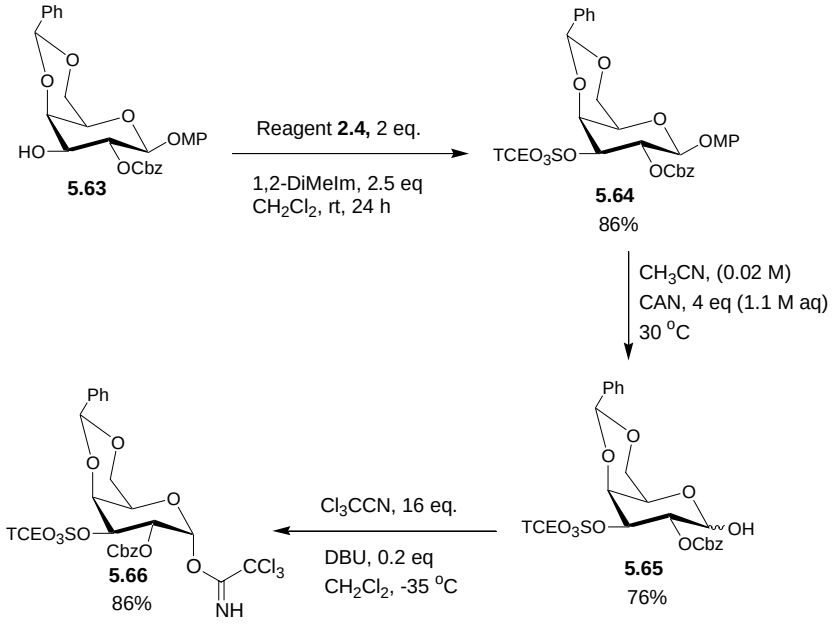
Scheme 5.22. Selective installation of the Cbz protecting group into **3.126**.

The benzoyl migration reaction reported by Jacquinet was performed on the same galactose derivative but he used an OMP group for the protection of the anomeric position instead of STol group in our case. In order to determine if the thiol moiety at the anomeric position was hindering the migration of the Cbz-group, Laura Ingram tried the migration reaction on the galactose derivative protected at the anomeric position with OMP-group using Jacquinet's migration conditions (Scheme 5.23). The Cbz-group was selectively introduced to the 3-OH of diol **5.58** using the same condition for the preparation of compound **5.61**. The desired product was isolated in 94% yield (Scheme 5.23). Subjecting compound **5.62** to the condition used by Jaquinet, the 2-OCbz galactose derivative **5.63** precipitated out from the reaction mixture and was isolated by filtration in 89% yield (Scheme 5.23).

After this achievement, our strategy for the synthesis of the TCE-protected sulfate galactose donor was changed. Compound **5.63** was sulfated in 86% yield using imidazolium salt **2.4** followed by removal of the OMP- group using CAN and acetonitrile at 30 ^{o}C to afford the hemiacetal **5.65** in 76% yield (Scheme 5.24). Treatment of the hemiacetal **5.65** with Cl_3CCN and DBU in dry methylene chloride at -35 ^{o}C give the trichloroactimidate donor **5.66** in 86% yield (Scheme 5.24). The synthesis of compounds **5.62-5.66** was done by Laura Ingram.

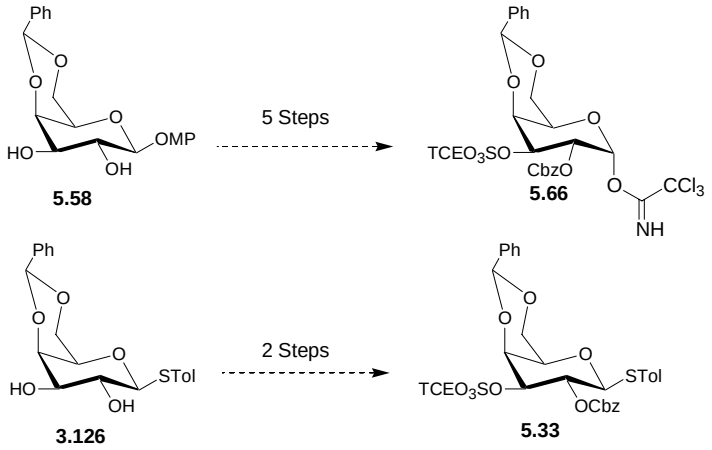


Scheme 5.23. Selective installation and migration of 3-*O*-Cbz protecting group in **5.62**.



Scheme 5.24. Synthesis of trichloroacetimidate donor **5.66**.

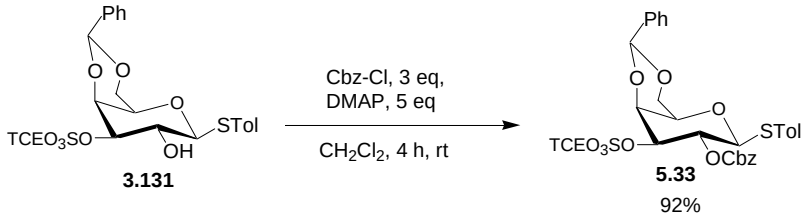
Although the synthesis of donor **5.66** was achieved in good yield, there were some drawbacks to this route. First, we missed the advantage of selectively incorporating the TCE-protected sulfate into diol **3.126**. Conseqently, the synthesis of donor **5.66** requires 5 steps starting from diol **5.58**, while the synthesis of **5.33** - if we were able to achieve it - requires two steps only (Scheme 2.25). Finally, we found that the yields for removal of the OMP from **5.64** were not consistent especially when the reaction was run on a large scale.



Scheme 5.25. Comparison between the number of steps required for synthesis of donors **5.66** and **5.33.**

Due to the above limitations, we decided to try again the synthesis of the thiogalactoside donor **5.33** by finding a suitable method for the installation of the Cbz-group at the 2-OH of the sulfated thiogalactoside **3.131**. After many attempts under a variety of conditions we eventually found that the Cbz-group could be successfully introduced into our target by adding 3 eq. of Cbz-Cl portionwise over 15 minutes to a solution of compound **3.131** and 5 eq. of DMAP in methylene chloride at room temperature (Scheme 5.24). The starting compound **3.131** was completely consumed within 4 hours, where the desired product **5.33** was isolated in 92% yield. In our

previous attempts we only used DMAP in catalytic quantities and in the presence of other bases. It is possible that when using a high concentration of DMAP the Cbz-Cl is converted into a more reactive acyl dimethylaminopyridinium ion.



Scheme 5.26. Incorporation of Cbz-group into the galactose donor

All of the building blocks required to prepare disaccharides **5.31** and **5.32** were now in hand (Figure 5.4). Only 15 steps were required for their preparation. We then started looking for glycosylation conditions that would enable us to build the desired disaccharides **5.31** and **5.32**.

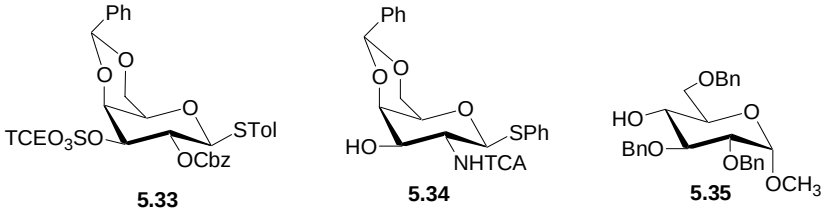
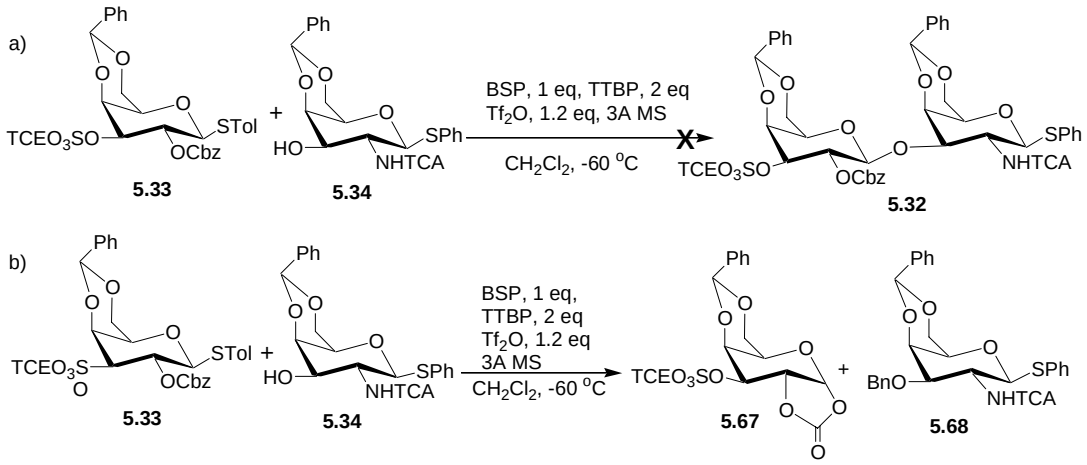


Figure 5.4. Building blocks for the tetrasaccharide of SB_{1a}

*5.3.4. Synthesis of disaccharide **5.32***

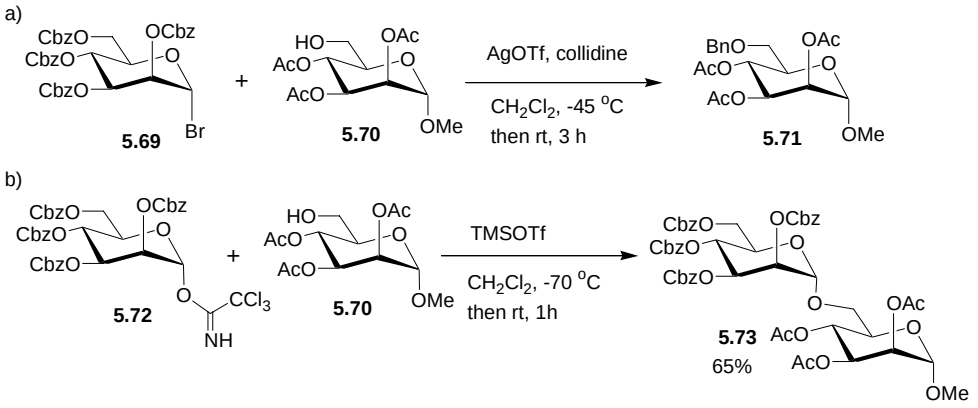
Among the wide variety of promoters that can be used for the activation of thioglycoside donors, we selected the conditions developed by Crich *et al.* which employ a combination of 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) as promoters. These were chosen since they have been shown to be very successful with both armed and disarmed thioglycosides.[113,114] For the synthesis of the left hand disaccharide, a mixture of thioglycoside donor **5.33**, BSP, and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in anhydrous methylene chloride in the presence of 3Å

molecular sieves were activated at low temperatures with Tf_2O. A few minutes after the addition of the Tf_2O, the galactose amine acceptor **5.34** was added (Scheme 5.27a). However, after analysis of the major isolated product, the spectroscopic data indicated that target disaccharide **5.32** was not formed. Instead, the major products isolated from the reaction were the cyclic carbonate **5.67** and benzylated acceptor **5.68** (Scheme 5.27b). No products resulting from dimerization of **5.34** were detected.



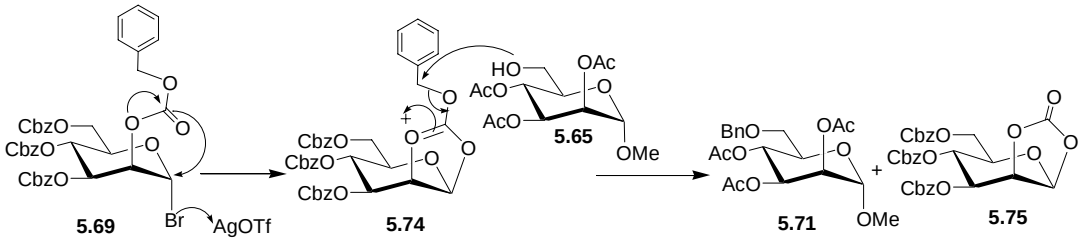
Scheme 5.27. Unsuccessful glycosylation using donor **5.33**

This was not entirely unexpected as Montero *et al.* reported the same type of benzyl group transfer reaction during the coupling of donor **5.69** with acceptor **5.70** in presence of AgOTf as a promoter. The benzylated acceptor **5.71** was formed as the only isolated product (Scheme 5.28a).[106] However, the authors mentioned that the target disaccharide was obtained in 65% yield by using trichloroacetimidate donor **5.72** instead of the bromide donor **5. 69** (Scheme 5.28b). Only the β-isomer was formed indicating that the Cbz group directed the formation of the trans-1,2 linkage. Thioglycoside donors bearing a Cbz group at the 2-position were not examined.



Scheme 5.28. Benzyl migration during the synthesis of disaccharide **5.73**[106]

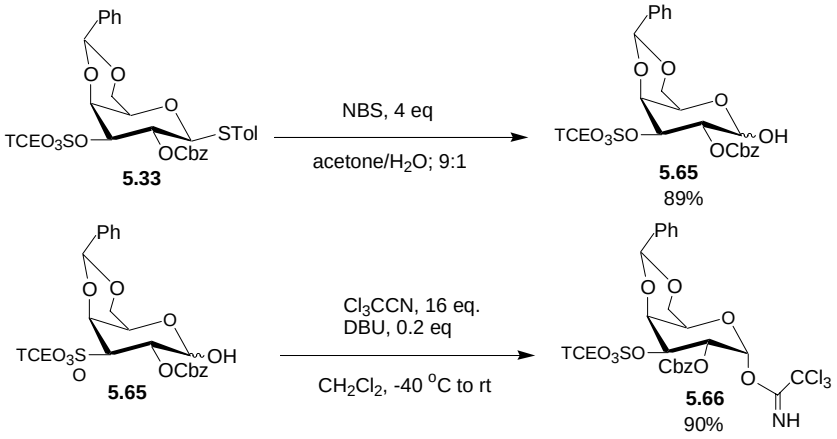
Montero *et al.* suggested a mechanism for the formation of the side product in that upon activation, the 2-*O*-Cbz group of the donor participates in the formation of the oxazolium ion **5.74**. The subsequent nucleophilic attack by the acceptor, however, did not occur at the anomeric position as required, but at the benzylic carbon in the Cbz protecting group, as shown in Scheme 5.27. The cyclic carbonate **5.75** was not isolated.[106]



Scheme 5.29. Mechanism for cyclic carbonate formation as proposed by Montero and coworkers[106]

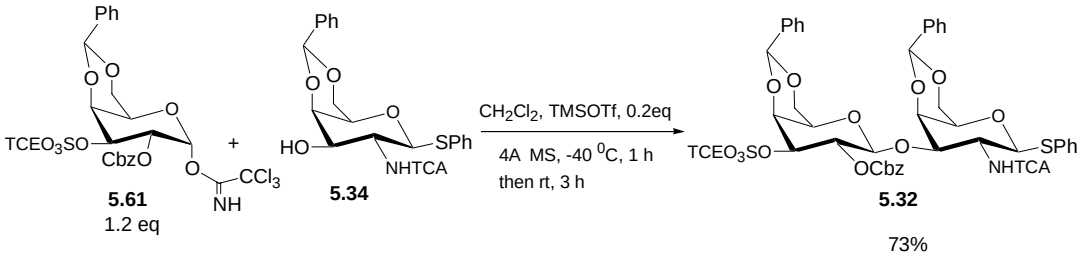
On the basis of Montereo and coworkers' results we decided to switch to the trichloroacetimidate glycosyl donor **5.66.** We had already prepared **5.66** from the OMP-glycoside **5.64** (Scheme 5.24). However, as we mentioned above, there were drawbacks

to that route. Therefore, we decided to see if we could prepare **5.66** more efficiently from thioglycoside **5.33**. Thioglycoside **5.33** was treated with NBS/H_2O to afford the corresponding hemiacetal **5.65** in an 89% yield. Subsequent treatment of hemiacetal **5.65** with a catalytic amount of DBU in the presence of trichloroacetonitrile in methylene chloride at -40 ^oC afforded imidate **5.66** in a 90 % yield (Scheme 5.30). This procedure was superior to our previous approach as the overall yield was higher and was reproducible upon scale-up. The overall yield of **5.66** starting from **3.126** was 50 % (7 steps).



Scheme 5.30. Synthesis of trichloroacetimidate donor **5.66**

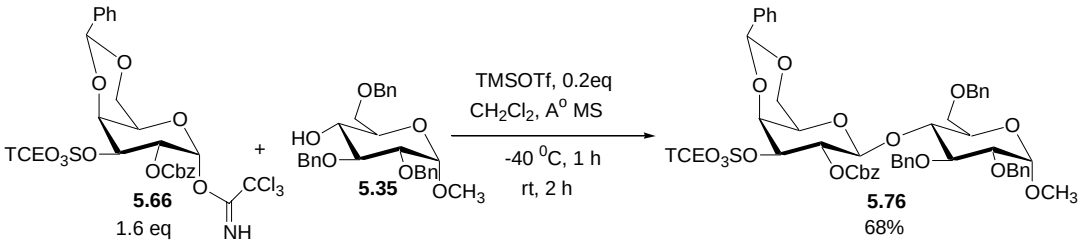
Trichloroacetimidate **5.66** and glycosyl acceptor **5.34** were treated with TMSOTf at -40 °C in anhydrous methylene chloride in presence of 4^oA molecular sieves to afford the desired disaccharide **5.32** in 73% yield with the required β-linkage (Scheme 5.31).



Scheme 5.31. Synthesis of disaccharide **5.32**.

5.3.5. Synthesis of right hand disaccharide acceptor of SB_{1a}

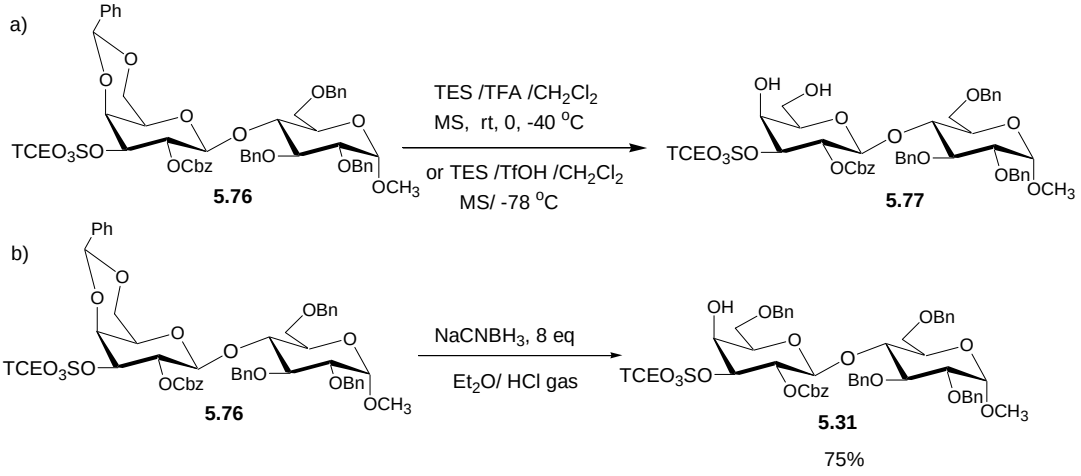
For the synthesis of disaccharide **5.76**, the trichloroacetimidate glycosyl donor **5.66** and the glucose acceptor **5.35** were subjected to a variety of glycosylation conditions including different promoters such as $BF_3.OEt_2$, TMSOTf, $HClO_4$-SiO_4, under a range of reaction temperature (-40 ^oC to room temperature) in different solvents such as methylene chloride and acetonitrile in presence of 4^{o}A molecular sieves. After many trials, the synthesis of target disaccharide **5.76** was achieved in 68% yield with the required β-linkage by treating a mixture of donor **5.66** and acceptor **5.35** in dry methylene chloride with 0.2 equivalent of TMSOTf at -40 ^oC. The glycosyl donor **5.66** was used in excess (1.6 eq.). Again, no benzyl migration or formation of cyclic carbonate were observed (Scheme 5.32).



Scheme 5.32. Synthesis of disaccharide **5.76**

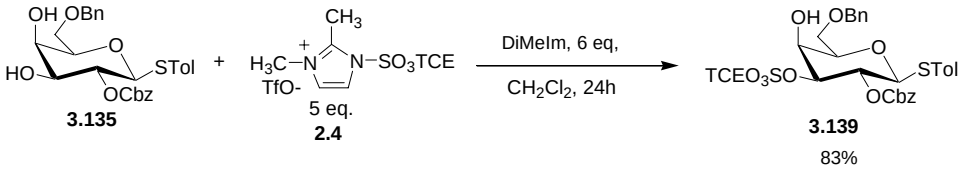
For selective ring opening/reduction of the benzylidene acetal, disaccharide **5.76** was subjected to a variety of conditions commonly used in carbohydrate chemistry for opening of the benzylidene acetals. Treatment of disaccharide **5.76** with Et_3SiH in methylene chloride in the presence of TFA at room temperature or TfOH at -78 ^oC led to the cleavage of the acetal, where the 4,6-diol was obtained as the major product (Scheme 5.33a). On the other hand, by treating disaccharide **5.76** with Et_3SiH/TFA at lower temperature, no significant reaction was observed and the starting material was isolated. A thorough literature search revealed that the majority of benzylidene acetals in galactose

derivatives were selectively opened and reduced (to give the free 4-OH) by passing dry HCl gas into a solution of the galactose derivative in the presence of $NaCNBH_3$.[115] Applying these conditions to disaccharide **5.76**, led to the regioselective opening of the benzylidene acetal where the desired disaccharide acceptor **5.31** was isolated in 75% yield (Scheme 5.33 b).

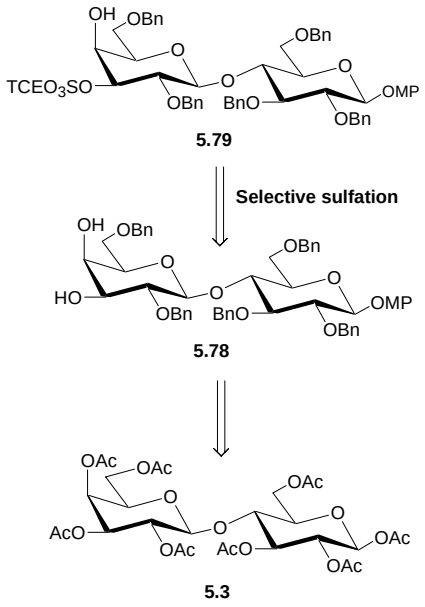


Scheme 5.33. Synthesis of disaccharide acceptor **5.31**

Some of the selective sulfation studies reported in Chapter 3 were being done concurrently with our SB_{1a} synthesis. While we were tackling the synthesis of SB_{1a}, we discovered that we were able to incorporate the TCE-protected sulfate selectively into the 3-OH of galactose derivative **3.135** in good yield (Scheme 5.34). It then occurred to us that we might be able to perform a selective sulfation on the 3-OH of the galactosyl portion of a lactose derivative and so have a potentially more efficient route (we will call this route # 2) to the right-hand disaccharide portion of the SB_{1a} tetrasaccharide. More specifically, the idea was to start with peracetylated lactose and convert it into protected disaccharide diol **5.78** using chemistry similar to that developed for the preparation of **5.5** (Scheme 5.1).[95,96] A selective sulfation would then give the desired disaccharide **5.79** (Scheme 5.35).



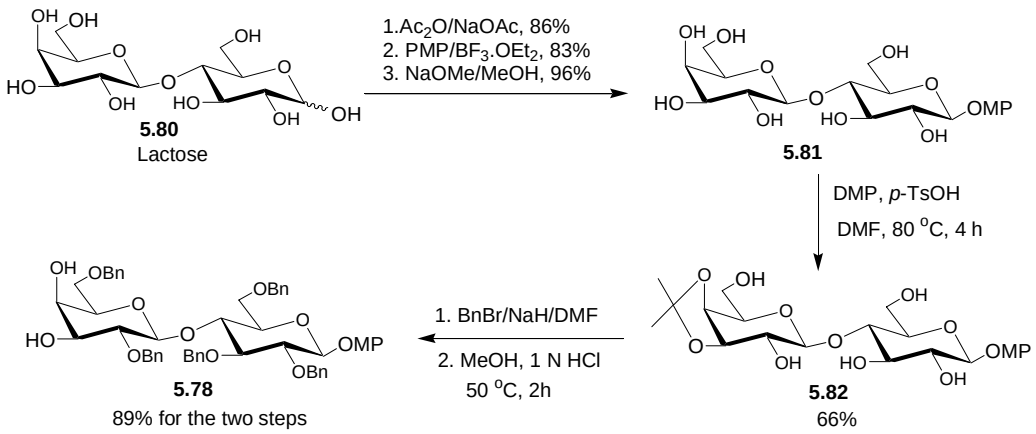
Scheme 5.34. Selective incorporation of TCE-protected sulfate.



Scheme 5.35. Alternative route to the synthesis of the right-hand disaccharide acceptor

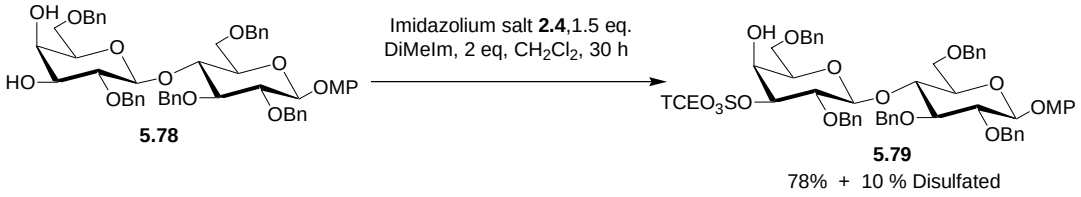
*5.3.6. Synthesis of lactosyl acceptor **5.79***

The lactose diol **5.78** was prepared in six steps starting from commercially available lactose according to the published procedure (Scheme 5.36).[116]



Scheme 5.36. Synthesis of lactose diol **5.78**

Treatment of lactose diol **5.78** with 1.5 equivalent of imidazoliun salt **2.4** in methylene chloride followed by the dropwise addition of 2 equivalent of 1,2-DiMeIm afforded the desired lactosyl acceptor **5.79** in 78% yield in addition to 10% of the disulfated derivative (Scheme 5.37).



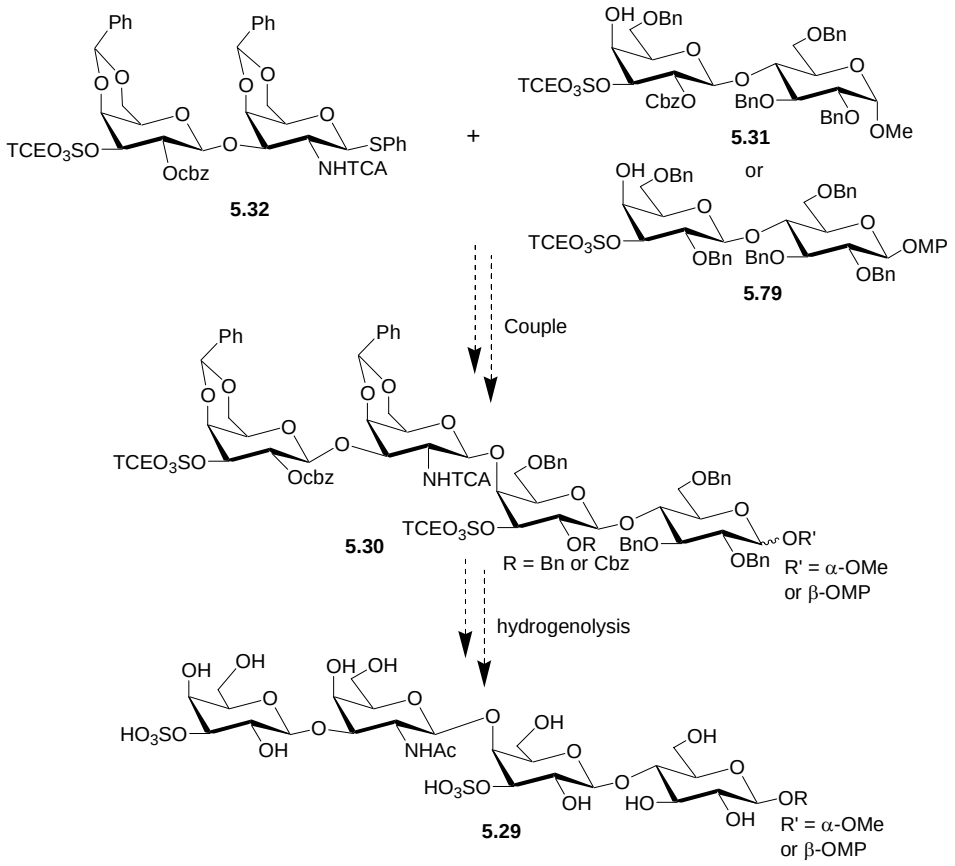
Scheme 5.37. Selective sulfation of lactose diol **5.78**

We now had two very good routes to the protected right-hand disaccharide portion of our target. In route # 2 disaccharide **5.79** was prepared in just 7 steps in an overall 31% yield which is very respectable. In route # 1, disaccharide **5.31** required 12 steps. The overall yield of **5.31** is difficult to compute since it is a convergent synthesis where donor **5.66** was prepared in an overall 50% yield over 7 steps and acceptor **5.35** in an overall 60% yield over 3 steps. The coupling of these two monomers (Scheme 5.32) and the subsequent ring opening of the resulting disaccharide (Scheme 5.33) proceeded in

yields of 68 % and 75 % respectively. So route # 2 is the superior route for the synthesis of this disaccharide. However, it is not the best approach for the synthesis of the target tetrasaccharide since *donor **5.66** has to be prepared no matter what route we chose for the synthesis of the right-hand disaccharide since it is required for the synthesis of the left-hand disaccharide **5.32*** (Scheme 5.31). So although route # 2 is a very efficient route to the protected right-hand disaccharide, it actually adds two additional steps onto the overall synthesis of the target tetrasaccharide.

5.3.7 Synthesis of the protected tetrasaccharide

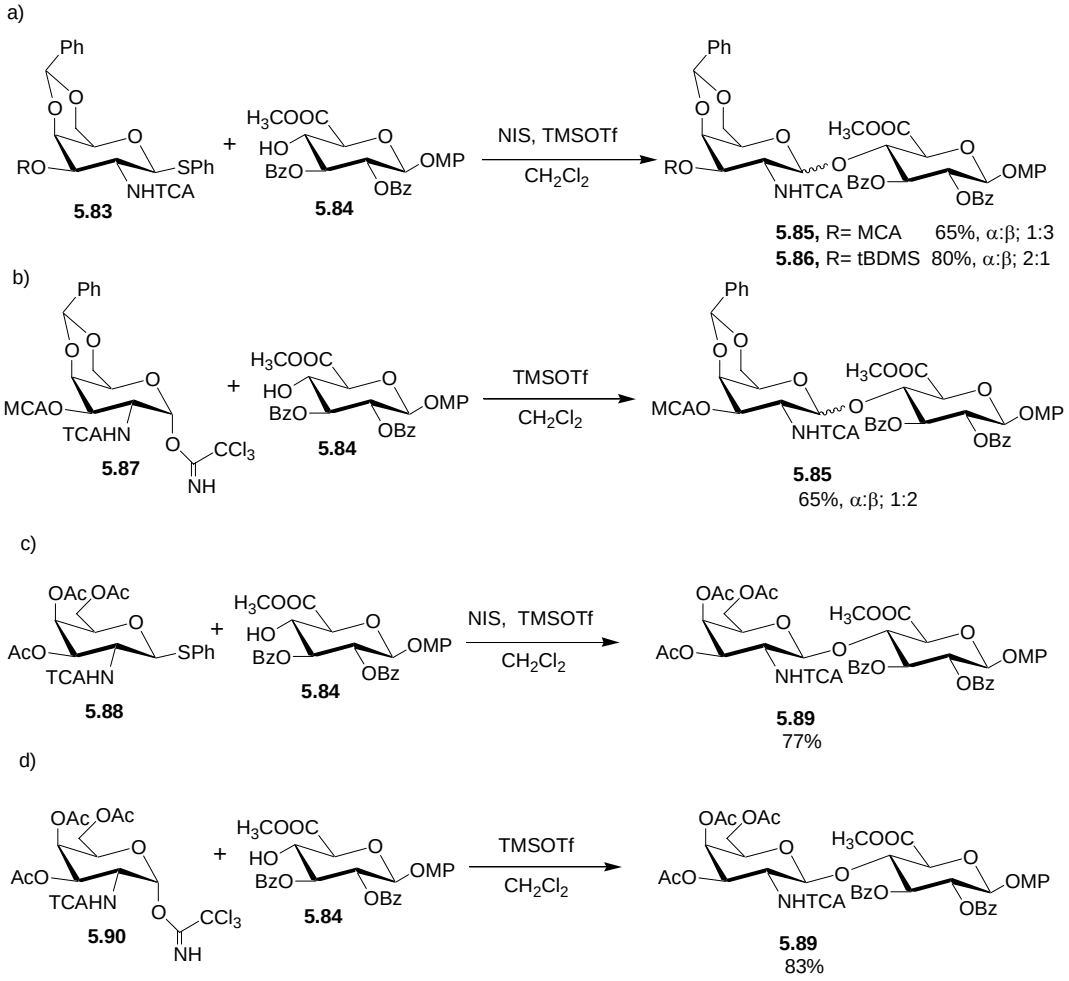
The next step is the formation of the protected tetrasaccharide using disaccharide donor **5.32** and disaccharide acceptors **5.31** or **5.79** (Scheme 5.38). We attempted to couple **5.32** to **5.31** under the conditions developed by Crich *et al.* which employ a combination of 1-benzenesulfinyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) as promoters. [113] However, so far we have not been able to obtain any product. The donor disappeared (by TLC) within few minutes, while the acceptor **5.31** remained. The promoter used in this method is a very powerful activator. Our donor is being rapidly activated but we believe that our acceptor is relatively unreactive. So the activated donor is decomposing before it reacts with the acceptor. We believe that a less powerful promotor may be necessary.



Scheme 5.38. Remaining steps for the synthesis of the SB_{1a} tetrasaccharide.

While these studies were in progress, we came across a paper by Belot and Jaquinet who performed an extensive study on the glycosylation of galactose donors **5.83** and **5.87** (Scheme 5.39). These two donors are in some ways similar to the reducing end of donor disaccharide **5.34** (Scheme 5.38).[117] The authors found that the coupling of these donors to acceptor **5.84** (a relatively unreactive acceptor) gave the disaccharide products as α and β mixtures (Scheme 5.39a and b). The authors attributed this to steric factors induced by the rigid 4,6- dioxolane ring in the 4,6-*O*-benzylidene-D-galactose derivatives which results in the formation of an incompatible pair in the transition state. In order to confirm that the loss of stereocontrol in the coupling reactions was not due to the lack of

participation of the trichloroacetamido group, the authors treated thioglycosides **5.83** and **5.88** with NIS–trimethylsilyl triflate for a short period in the absence of acceptor, where the corresponding silylated oxazoline **5.91** and the acetylated oxazoline **5.92** were isolated, respectively, as the major product, which indicating that participation of the 2-trichloroacetamido group is quite effective in both cases (Figure 5.5). On the other hand, when donors **5.88** and **5.99** were used the disaccharide **5.89** was obtained in good yield with only β-stereochemistry (Scheme 5.39c and d).



Scheme 5.39. Glycosylation of galactose amine donors

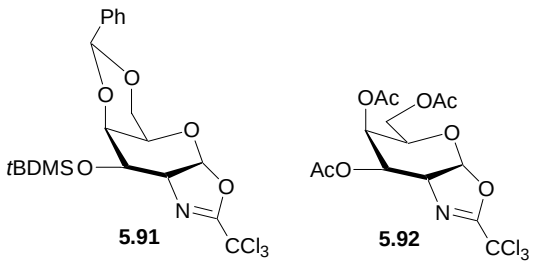


Figure 5.5. Oxazoline intermediates

Belot and Jaquinet's results suggest that it is possible that even if we get the coupling of **5.79** or **5.31** to **5.32** to proceed, we may end up with an α, β mixture of the protected tetrasaccharide **5.30**. Nevertheless, we will still attempt to couple **5.79** or **5.31** to **5.32** using other promoters though if we get an α-β mixture then we will probably have to remove the benzylidene acetals from **5.32** and replace them with other groups such as a Cbz group.

5.4 Summary and Future Work

Although we have not yet achieved the synthesis of the target tetrasaccharide several significant results have come from these studies so far. Efficient routes were developed for the construction of the left- and right-hand protected disaccharide portions of the SB_{1a} tetrasaccharide. In the case of the right-hand disaccharide two efficient routes were developed. Selective sulfations using reagent **2.4** were key reactions in both syntheses. The conditions we developed for installing the Cbz group in the relatively unreactive carbohydrate **3.131** may prove to be useful for installing the Cbz group in carbohydrates in general. Consistent with Montero's work, we found that the Cbz group installed at the 2-position of a donor can direct the formation of trans-1,2 linkages so long as a trichloroacetimidate donor is used. The thioglycoside donor **5.33** gave the cyclic

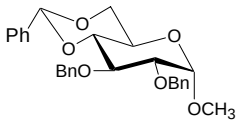
carbonate and benzyl-transfer products at least when Crich's conditions were used for forming the glycosidic bond. Whether this is always going to be the case for thioglycosides donors and/or whether it depends upon the conditions used to promote the glycosidation reaction are issues that are currently under study by the Taylor group. The synthesis of the target tetrasaccharide is currently being completed in the Taylor group. Only two steps remain. Should this synthesis be successful then it will have been accomplished in just 22 steps (using disaccharide **5.31**), eleven less than the synthesis described by Li et al. This will be a potent demonstration of the value of the sulfate-protecting group approach in the synthesis of complex, multisulfated oligosaccharides.

5.5 Experimental

5.5.1 General Considerations

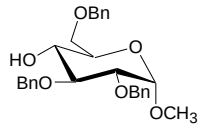
For all general considerations, see section 2.4.1 in Chapter 2.

5.5.2 Experimental Syntheses and Characterization

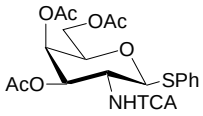


Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene –β-D-glucopyranoside (5.44). Prepared according to literature procedure.[107] Anhydrous $ZnCl_2$ (3.5 g, 25.8 mmol) was added to (15 mL, 147 mmol) of pure benzaldehyde and the solution was stirred for 10 minutes at room temperature. Methyl α-D-glucopyranoside (5.0 g, 25.7 mmol) was added to the reaction mixture and the reaction was stirred for 5 h at room temperature. The reaction was diluted with ethyl acetate (200 mL) and washed with water. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue

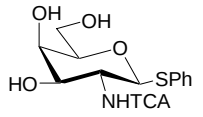
was crystallized from ethyl acetate/hexane to give (5.7 g, 78%) of the desired compound. All spectra are in agreement with literature data for this compound. The crystallized diol (5.0 g, 0.02 mol) was stirred with 60% NaH (1.8 g, 0.1 mol) in DMF at 0 ^{o}C for 30 minutes. Benzyl bromide (10 mL, 0.08 mol) was added dropwise to the reaction mixture and the reaction was stirred at room temperature for 24 hour. The reaction was quenched with methanol, and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate and washed with water and brine then dried over anhydrous sodium sulfate. Flash chromatography (EtOAc/Hexanes; 1: 3) afforded (6.95 g, 85%) of compound **5.44**. All spectra are in agreement with literature data for this compound.



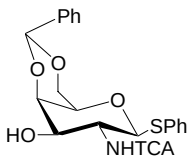
Methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.35). To the fully protected sugar **5.44** (5.0 g, 0.01 mol) in CH_2Cl_2 (100 mL) was added dropwise triethysilane (7.9 mL, 0.05 mol) followed by trifluoroacetic acid (3.85 mL, 0.05 mol). The reaction was stirred at room temperature for 9 h until there was no starting material remaining. The reaction was diluted with CH_2Cl_2, carefully quenched with triethylamine, and concentrated to crude syrup. Flash chromatography (1:4, EtOAc/hexanes) gave compound **5.35** as a colorless syrup (4.5 g, 89%). All spectra are in agreement with literature data for this compound.[107b]



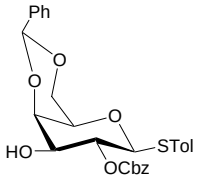
Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside (5.50). To tetraacetate **5.49** (15.0 g, 30.4 mmol) in freshly distilled CH_2Cl_2 at 0 °C was added benzene thiol (15.0 mL, 136 mmol), follwed by a dropwise addition of BF_3OEt_2 (15.0 mL, 118.0 mmol).The reaction 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 **5.50** as fine white needles (14.77 g, 90%). All spectra agree with literature data for this compound.[108]



Phenyl 2-deoxy-2-trichloroacetamido-1-thio-β-D-galactopyranoside (5.51). Na^0 metal (0.3 eq.) was added to **5.50** 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.

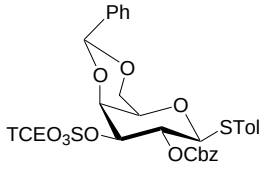


Phenyl 4.6-*O*-2-deoxy- 2-trichloroacetimido-1-thio -β-D-galactopyranoside (5.34). To a solution of **5.51** (4.0 g, 9.6 mmol) in acetonitrile (80 mL) was added benzaldehyde dimethyl acetal (5.0 mL, 28.8 mmol) and DL-10-camphorsulfonic acid (10 mol %) and the mixture stirred 16 h at room temperature. The solution was neutralized by addition of triethylamine, and concentrated to a crude yellow solid. The crude solid was purified by flash chromatography (1:1 EtOAc/Hexanes) to afford **5.34** (3.9 g, 80%). All spectra for **5.34** were in agreement with literature data.[108]

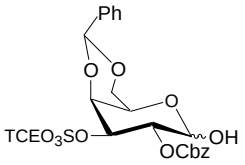


***p*-Tolyl 4,6-*O*-benzylidene-2-*O*- -carbobenzyloxy-1-thio-β-Dgalactopyranoside (5.56).** To carbohydrate **3.126** (0.5 g, 1.33 mmol) in CH_2Cl_2 (10 mL) was added freshly prepared Ag_2O (0.46 g, 2 mmol), KI (0.044 g, 0.27 mmol) followed by a dropwise addition of benzyl chloroformate (0.2 mL, 1.5 mmol). The reaction was stirred in dark for 24 h. The reaction mixture was filtered over cellite, and then the solvent was washed with water, brine and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the residue was purified by flash chromatography (EtOAc/Hexanes; 1:4) provided **5.56** as a white foam (0.1 g, 15%) in addition to (0.125 g, 20%) of the 3-*O*-Cbz derivative **5.57**. ^{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 (dd, 1H, $J_{3,2}$ = 9.7 Hz, $J_{3,4}$ =3.1 Hz, H3), 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,

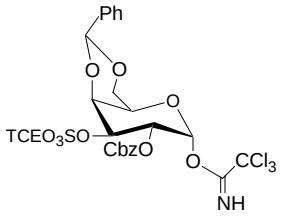
H1), 4.76 (dd, 1H, $J_{2,3} = J_{2,1} = 9.6$ Hz, H2), 5.14 (s, 2H, CO_2CH_2Ph), 5.44 (s, 1H, CHPh), 7.03 (d, 2H, *J*=7.9 Hz, ArH), 7.24-7.33 (m, 10H, ArH), 7.54 (d, 2H, *J*= 7.9 Hz, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 69.0, 69.8, 70.0, 72.4, 74.1, 75.5, 84.5, 101.4, 126.6, 127.0, 128.2, 128.3, 128.5, 128.6, 129.4, 129.5, 134.5, 135.2, 137.4, 138.4, 154.6. HRMS (+ESI) m/z = 509.1646 $C_{28}H_{29}O_7S$, $(M+H)^+$ requires 509.1634.



***p*-Tolyl 4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-1-thio-β-D-galactopyranoside (5.33).** To a solution of **3.131** (0.3 g, 0.51 mmol) and DMAP (0.3 g, 2.56 mmol) in CH_2Cl_2 (6 mL) at room temperature was added benzyl chloroformate (0.2 mL, 1.5 mmol) dropwise over 15 min. The reaction was stirred for 4 h, where 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 (EtOAc/Hexanes; 1:4) afforded **5.33** as a white solid (0.34 g, 92%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.57 (s, 1H, H5), 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 21.2, 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. HRMS (+ESI) m/z = 736.0609 $C_{30}H_{33}Cl_3NO_{10}S_2$, $(M+NH_4)^+$ requires 736.0611.



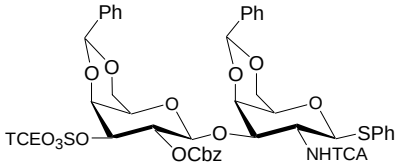
4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-β-D-galactose (5.65). NBS (0.39 g, 2.22 mmol) was added to **5.33** (0.4 g, 0.550 mmol) in acetone/ H_2O (9:1, 15 mL) at 0 °C. The reaction was stirred for 4 h at 0 °C then concentrated until turbidity developed. The remaining residue was dissolved in EtOAc, washed with H_2O, dried over $MgSO_4$ and concentrated to a yellow foam. Purification by flash chromatography (EtOAc/Hexanes; 1:1) afforded **5.65** as a white foam (0.3, 89%). The product was used directly in the preparation of **5.66** with no further characterization.



4,6-O-benzylidene-2-O-carbobenzyloxy-3-Otrichloroethoxysulfo-β-D-galactose trichloroacetimidate (5.66).

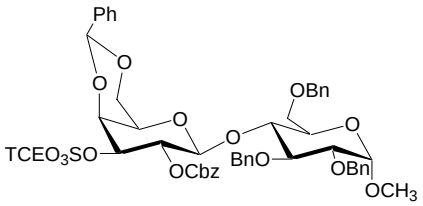
To hemiacetal **5.65** (0.4 g, 0.64 mmol) in CH_2Cl_2 (5.0 mL) at –40 °C was added trichloroacetonitrile (1.4 mL, 10.32 mmol) followed by a 1.0 M solution of DBU in CH_2Cl_2 (0.12 mL, 0.12 mmol). The reaction was stirred for 2 h and gradually warmed to room temperature. The solvent was evaporated and the residue was applied to a silica gel column (EtOAc/Hexanes; 1:4) where **5.66** was isolated as white foam (0.44 g, 90%). ^{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.



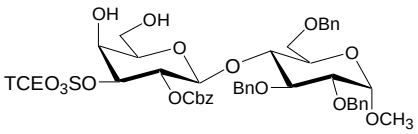
Phenyl 3-*O*-(4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-β-D-galactosyl)-4:6-*O*-2-deoxy-2-trichloroacetimido-1-thio-β-D-galactopyranoside (5.32). A mixture of imidate **5.66** (0.18 g, 0.25 mmol) and acceptor **5.34** (0.10 g, 0.2 mmol) and 4^{o}A molecular sieves were stirred in dry CH_2Cl_2 (3.0 mL) under argon for 1 h at room temperature. The solution was then cooled to -40 ^{o}C. A solution of 0.1 M TMSOTf (0.040 mL) was added, and the reaction was allowed to stir at -40 ^{o}C for 1 h and at rt for 3 h. The reaction mixture was then treated with triethylamine (0.08 mL), filtered through celite and concentrated. Purification by flash chromatography (EtOAc/Hexanes; 1:1) afforded (0.164 g, 73%) of the desired disaccharide **5.32**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.27(b, 1H, H5`), 3.46-3.59 (m, 2H, H5, H2), 3.92, 4.31 (AB, 2H, $J_{6`,6``}$ =12.3 Hz, H6Gal, H6`Gal), 4.01, 4.22 (AB, 2H, $J_{6,6`}$ =12.3 Hz, H6GalN, H6`GalN), 4.28 (m, 1H, H4), 4.51-4.61(m, 5H, H3, $CH_2CCl_3$, H3`, H4`), 4.72 (d, 1H, $J_{1,2}$ =7.8 Hz, H1`), 4.94, 5.10 (AB, 2H, *J*= 11.8 Hz, CH_2Ph), 5.15 (m, 1H, H2`under the CH of the CH_2Ph,),5.45 (d, 1H, $J_{1,2}$ =10 Hz, H1), 5.51 (s, 1H, CHPh), 5.52 (s, 1H, CHPh), 6.94 (d, 1H, $J_{NH,H2}$ =6.7 Hz, NH), 7.19-7.37 (m, 14H, ArH), 7.42 (m, 2H, ArH), 7.52 (m, 2H, ArH), 7.62 (d, 2H, *J*=6.7 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 53.1, 65.8, 68.5,

69.0, 70.1, 70.3, 72.3, 72.8, 74.4, 75.8, 79.9, 81.1, 83.2, 92.1, 92.3, 100.21, 100.27, 101.1, 126.27, 126.29, 128.11, 128.2, 128.4, 128.8, 129, 129.1, 129.5, 131.7, 133.5, 134.7, 136.6, 137.9, 153.8, 162.0.HRMS (+ESI) m/z = 1115.0393 $C_{44}H_{45}$ $Cl_6N_2O_{15}S_2$, $(M+NH_4)^+$ requires 1115.0393.

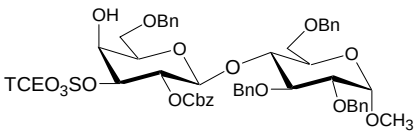


Methyl 4-*O*-(4,6-*O*-benzylidene-2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-β-D-galactosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (5.76). A mixture of imidate **5.66** (0.186 g, 0.25 mmol) and acceptor **5.35** (0.1 g, 0.22 mmol) and 4^{o}A molecular sieves were stirred in dry CH_2Cl_2 (3.0 mL) under argon for 1 h at room temperature. The solution was then cooled to -40 ^{o}C. A solution of 0.1 M TMSOTf (0.040 mL) was added, and the reaction was allowed to stir at -40 ^{o}C for 1 h and at rt for 2 h. The system was then treated with triethylamine (0.08 mL), filtered through celite and concentrated. Purification by flash chromatography (EtOAc/Hexanes; 1:1) afforded (0.155 g, 68%) of the desired disaccharide **5.76**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.91(b, 1H, H5`), 3.35 (s, 3H, $OCH_3$), 3.47 (m, 3H, H5, H2, H4`), 3.88 (m, 4H, H6Gl, H6Gal, H3, H6`Gal) 4.16 (d, 1H, $J_{6,6`}$= 12.5 Hz, H6`Gl), 4.34 (d, 1H, *J*=8.7 Hz, H1`-Gal), 4.41 (m, 3H, CH_2CCl_3, H4), 4.55 (d, 1H, *J*= 3.5 Hz, H1), 4.58-4.84 (m, 7H, H2`, 3$CH_2$Ph), 5.13 (dd, 1H, H3`under the CH_2Ph), 5.14, 5.24(AB, 2H, *J* =12.3 Hz CH_2Ph), 5.52 (s, 1H, CHPh), 7.19 (m, 3H, ArH), 7.34 (m, 18H, ArH), 7.46 (d, 4H, *J*=3.7 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 65.5, 67.6, 68.3, 69.6, 70.3, 72.5, 72.9, 73.3, 73.7, 76, 78.1, 79.2, 79.9, 80, 81.6, 92.5, 98.4, 100.3, 101.4, 126.5, 127.9, 128, 128.1, 128.2, 128.2, 128.3, 128.4, 128.5, 128.6,

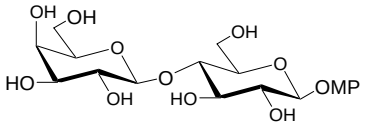
128.7, 128.8, 129.4, 134.9, 136.9, 138.1, 138.3, 138.9, 153.9.HRMS (+ESI) m/z = 1059.2205 $C_{51}H_{54}Cl_3O_{16}S$, $(M+H)^+$ requires 1059.2198.



Methyl 4-*O*-(2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-β-D-galactosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (5.77). To the fully protected disaccharide **5.76** (0.1 g, 0.09 mmol) in CH_2Cl_2 (2 mL) was added dropwise triethysilane (0.08 mL, 0.47 mmol) followed by trifluoroacetic acid (0.04 mL, 0.47 mmol). The reaction was stirred at room temperature for 6 h until there was no starting material remaining. The reaction was diluted with CH_2Cl_2, carefully quenched with triethylamine, and concentrated to crude syrup. Flash chromatography (1:1, EtOAc/hexanes) gave compound **5.77** as a white solid (0.073 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21 (b, 1H, OH), 2.72 (b, 1H, OH), 2.91(b, 1H, H5`), 3.35 (s, 3H, $OCH_3$), 3.47 (m, 3H, H5, H2, H4`), 3.88 (m, 4H, H6Gl, H6Gal, H3, H6`Gal) 4.16 (d, 1H, $J_{6,6`}$= 12.5 Hz, H6`Gl), 4.34 (d, 1H, *J*=8.7 Hz, H1`-Gal), 4.41 (m, 3H, CH_2CCl_3, H4), 4.55 (d, 1H, *J*= 3.5 Hz, H1), 4.58-4.84 (m, 5H, H3`, $2CH_2Ph$), 5.13 (t, 1H, *J*= 7.8 Hz, H2`), 5.14, 5.24(AB, 2H, *J* =12.3 Hz CH_2Ph), 7.19 (m, 3H, ArH), 7.34 (m, 18H, ArH), 7.46 (d, 4H, *J*=3.7 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 63.1, 67.3, 68.6, 69.6, 70.1, 71.8, 72.7, 73.4, 73.5, 75, 77.2, 78.7, 79.7, 79.9, 83.5, 92.5, 98.4, 100.2, 126.8, 127.4, 127.8, 128, 128.1, 128.2, 128.4, 128.5, 128.59, 128.66, 128.7, 134.9, 137.7, 138.1, 138.1, 139.5, 153.9.

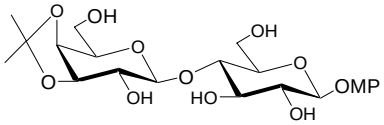


Methyl 4-*O*-(6-*O*-benzyl-2-*O*-carbobenzyloxy-3-*O*-trichloroethoxysulfo-β-D-galactosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (5.31). To the disaccharide **5.76** (0.1 g, 0.09 mmol) in dry diethyl ether (2 mL) was added $NaCNBH_3$ (0.05 g, 0.75 mmol) followed by dropwise addition of diethyl ether saturated with HCl until no effervescence. The reaction was stirred at room temperature for 4 h until there was no starting material remaining. The reaction was diluted with CH_2Cl_2, carefully quenched with triethylamine, and concentrated to crude syrup. Flash chromatography (1:1, EtOAc/hexanes) gave compound **5.31** as a white foam (0.075 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (b, 1H, OH), 2.91(b, 1H, H5`), 3.35 (s, 3H, $OCH_3$), 3.47 (m, 3H, H4`, H5, H2), 3.88 (m, 4H, H6Gl, H6Gal, H3, H6`Gal) 4.16 (d, 1H, $J_{6,6`}$= 12.5 Hz, H6`Gl), 4.34 (d, 1H, *J*=8.7 Hz, H1`-Gal), 4.41 (m, 3H, CH_2CCl_3, H4), 4.55 (d, 1H, *J*= 3.5 Hz, H1), 4.58-4.84 (m, 7H, H2`, 3$CH_2$Ph), 5.14 (dd, 1H, H3`under the CH_2Ph), 5.14- 5.24(m, 4H, *2*CH_2Ph), 7.2 (m, 3H, ArH), 7.36 (m, 18H, ArH), 7.46 (d, 4H, *J*=3.7 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 65.5, 67.6, 68.3, 69.6, 70.3, 72.5, 72.8, 72.9, 73.3, 73.7, 76, 78.1, 79.2, 79.9, 80, 81.6, 92.5, 98.4, 100.3, 126.5, 127.9, 128, 128.1, 128.2, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 129.4, 134.9, 136.9, 138.1, 138.3, 138.9, 153.9.HRMS (+ESI) m/z = 1061.2314 $C_{51}H_{56}Cl_3O_{16}S$, $(M+H)^+$ requires 1061.2235.



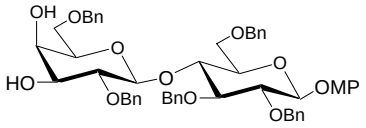
4-Methoxyphenyl β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside (5.81).

To a solution of peracetylated lactose **5.3** (30.0 g, 44.1 mmol) in freshly distilled CH_2Cl_2 (250 mL) at 0 °C was added 4-methoxyphenol (7.13 g, 57.5 mmol), follwed by a dropwise addition of BF_3OEt_2 (9.50 mL, 75.2 mmol).The reaction was allowed to gradually warm to room temperature and stirred 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 H_2O, dried over $MgSO_4$ and concentrated to a yellow solid. Recrystallization (EtOAc/Hexanes) afforded desired product as fine white crystalls (36.3 g, 83%). All spectra agree with literature data for this compound.[115] To a solution of the lactose heptaacetate-OMP (30 g, 40.4 mmol) in methanol-CH_2Cl_2; 1:1 (300 mL) was added Na° metal (0.35 g, 15 mmol). The reaction was stirred for 6 h, neutralized with Dowex H^+ resin, filtered and concentrated to a white solid (17.4 g, 96%). The resulting crude was used directly in the next step without any purification or characterization.

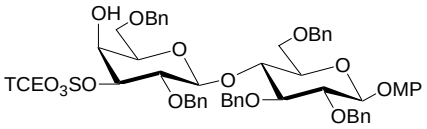


4-Methoxyphenyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside (5.82). To a solution of 4-methoxyphenyl β-D-galactopyranosyl-(1-4)-β-D-glucopyranoside **5.81** (15 g, 33.4 mmol) in DMF (80mL) 2,2-dimethoxypropane (10 mL)

and *p*-toluenesulfonic acid (200 mg) were added and the mixture was stirred for 4 hr at 80 ^{o}C, then neutralized with triethyl amine. The solvent was removed under vacuum and the residue was crystallized from ethanol to give **5.82** (11 g, 66%) as white needles.



4-Methoxyphenyl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1-4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.78). Prepared according to a modified literature procedure.[115] To a solution of **5.82** (8.0 g, 16.3 mmol) in dry DMF 80% NaH (3 g, 0.10 mol) was added in small portions at 0 ^{0}C. The reaction mixture was stirred for 30 min and benzyl bromide (12 mL, 0.10 mol) was added. After 6 hr, another portion of NaH (1.5 g, 0.05 mol) and benzyl bromide (6 mL, 0.05 mol) were added. The reaction was stirred at rt for another 10 h, then MeOH was added in order to decompose the excess of hydride. The solvent was evaporated, the residue was dissolved in DCM (300 mL), washed with distilled water (3 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The sirupy crude product (15 g) was used for the next step without purification. A stirred mixture of the fully protected isopropylidene lactose (15 g, 16 mmol) in methanol (200 mL) and 1M HCl (20 mL) was heated for 50 ^{o}C. After 2 hr, t.l.c. showed complete conversion, the solution was evaporated and the solid residue was crystallized from methanol to give **5.78** (13 g, 89% for two steps). All spectra agree with literature data for this compound.[115]



4-Methoxyphenyl 2,6-di-*O*-benzyl-6-*O*-trichloroethoxysulfo-β-D-galactopyranosyl-(1-4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.79).

To the diol **5.78** (0.5 g, 0.55 mmol) in dry CH_2Cl_2 (5 mL) at 0 ^oC (ice bath) was added reagent **2.4** (0.2 g, 0.41 mmol), followed by the addition of a solution of DiMeIm (0.1 g, 1.1 mmol) in CH_2Cl_2 (2 mL) over 6 h using a syringe pump. During the addition of the DiMeIm another portion of reagent **2.4** (0.2 g, 0.41 mmol) was added after 4 h and the ice batch was removed after the initial 1 h. The reaction was left stirring until the reaction was complete by TLC (approx 24 h). The reaction was diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$) and concentrated to brown crude oil. The crude residue was purified with flash chromatography (1:4, EtOAc/hexanes) to give the sulfated lactose **5.79** (0.48 g, 78%) in addition to (0.07 g, 10%) of the disulfated derivative. ^1H NMR (300 MHz, $CDCl_3$) δ 2.91 (b, 1H, OH at C4`), 3.22 (b, 1H, H5`), 3.45-3.8 (m, 11H, H5, H4`, H3, H2`, H6Gl, H6`Gl, $OCH_3$, H6 Gal, H6`Gal), 4.10 (t, 1H, *J*=9 Hz, H2), 3.41 (m, 6H, H1`, $CH_2CCl_3$, H4, $CH_2Ph$), 4.71 (m, 8H, $3CH_2$, H1, 1H of $CH_2Ph$), 5.01 (m, 2H, 1H of $CH_2Ph$, H3`), 6.81, 7.01 (AB, 2H, *J*= 12 Hz, ArH), 7.31 (m, 25 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.6, 67.5, 67.9, 68.7, 71.5, 73.3, 73.6, 75, 75.1, 75.4, 75.5, 76.4, 79.6, 82.6, 86.1, 92.6, 102.2, 102.8, 114.5, 118.5, 127.4, 127.7, 127.9, 128, 128.1, 128.2, 128.4, 128.41, 128.6, 137.3, 137.4, 138, 138.3, 139, 151.5, 155.3. HRMS (+ESI) m/z = 1126.2950 $C_{56}H_{63}Cl_3NO_{15}S$, $(M+NH_4)^+$ requires 1126.2984.

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