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Development of New Strategies for Expeditious Oligosaccharide Synthesis

Sophon Kaeothip

University of Missouri-St. Louis, sophonkaeothip@yahoo.com

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Development of New Strategies for Expeditious

Oligosaccharide Synthesis

by

Sophon Kaeothip

A dissertation submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy (Chemistry)

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Dissertation Committee

Prof. Alexei V. Demchenko, Ph. D. (Chairperson)

Prof. James S. Chickos, Ph. D.

Prof. Michael R. Nichols, Ph. D.

Prof. Rudolph E. K. Winter, Ph. D.

Abstract

Development of New Strategies for Expeditious Oligosaccharide Synthesis

Sophon Kaeothip

Doctor of Philosophy

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Prof. Alexei V. Demchenko, Chairperson

The important role that carbohydrates play in biology and medicine has been the major incentive for devising new methods for chemical and enzymatic glycosylation. The chemical approach to oligosaccharides involves multiple synthetic steps and the development of new strategies that allow for streamlining the synthesis of these complex biomolecules is a significant area of research.

The aim of this doctoral dissertation is to develop new reagents and efficient methodologies for oligosaccharide synthesis. It was found that siver(I) tetrafluoroborate can be used as a powerful activator of various glycosyl donors for oligosaccharide synthesis. This study evolved into the development of a very effective orthogonal strategy which allowed for the synthesis of a variety of oligosaccharide sequences with moderate stereoselectivity. Further studies showed that the improvement of stereoselectivity of glycosylation can be achieved in two different modes. First, we found that electron withdrawing protecting groups on the glycosyl acceptor can help to obtain excellent α-stereoselectivity. Second, the stereoselectivity of glycosidation of thioglycosides in the presence of bromine depends greatly on the anomeric orientation of

the leaving group. The mechanistic studies of this phenomenon were conducted using high field NMR and the developed methodology was applied to the synthesis of a range of therapeutically attractive carbohydrate-amino acid conjugates.

This dissertation is dedicated to my parents with

love and respect

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I wish to express extremely grateful to my parents and members in my family for their love, understanding, support and looking forward to my graduation.

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List of abbreviations

Å..Angstrom

Ac..Acetyl

Bn... Benzyl

BSP...Benzenesulfinyl piperidine

Bz .... Benzoyl

CMP...Cytidine monophosphate

COD...Cyclooctadienyl

Cp..Cyclopentadienyl

CSA...(±)10-Camphorsulfonic acid

d..Doublet

DAST...(Diethylamino)sulfur trifluoride

DBU...1,8-Diazabicycloundec-7-ene

DCC...*N,N*-dicyclohexylcarbodiimide

DCE.. 1,2-Dichloroethane

DCM .... Methylene chloride

DDQ...2,3-Dichloro-5,6-dicyanobenzoquinone

dd..Double of doublets

DEIPS..Diethylisopropylsilyl

Dimedone..5,5-dimethyl-1,3-cyclohexanedione

DIPEA..Diisopropylethylamine

DMF...*N,N*-Dimethylformamide

DMAP...Dimethylaminopyridine

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CHAPTER I

Introduction

1.1   Introduction. Complex carbohydrates, chemical glycosylation, and conventional oligosaccharide synthesis

Carbohydrates are the most abundant biomolecules on Earth, nevertheless, their chemistry and biology has been a "Cinderella" field. Our current knowledge about the key roles of these fascinating natural compounds is not yet complete. However, thanks to the explosive growth of the field of glycobiology in recent years, we already know that carbohydrates are involved in a broad range of vital biological processes, fertilization, anti-inflammation, immunoresponse, joint lubrication, antigenic determination, are only a few to mention.[1-4] Carbohydrates are also responsible for many damaging processes in our cells - bacterial and viral infections, development and growth of tumors, metastasis, tissue rejection, etc.[5] This area of science has stimulated major scientific efforts in the beginning of 21st century primarily due to the fact that many of these processes are directly associated with deadly diseases, such as AIDS, cancer, meningitis, septicemia, hepatitis, and malaria.

The elucidation of the exact mechanisms of the disease progression would be significantly facilitated if we had comprehensive knowledge of the structure, conformation, and properties of the associated carbohydrate molecules. Although glycoscientists have learned to selectively cleave, isolate, purify, and characterize certain classes of naturally occurring glycostructures, the availability of pure natural isolates in sufficient quantities remains low. In order to ensure the availability of pure complex carbohydrates, their chemical or enzymatic synthesis is of prime importance. It is well appreciated that a reliable and efficient synthesis could lead to useful glycostructures for structural studies and determination of their biological roles. In addition, the synthetic

approach can provide significant quantities and natural carbohydrates or their unnatural mimetics that are often required for investigating their therapeutic and diagnostic applications. Pharmaceutical and biotechnology companies have already demonstrated an interest in producing complex oligosaccharides or glycoconjugates, however, examples including heparin and its analogs,[6,7] oligosaccharide antibiotics,[8,9] carbohydrate-based vaccines,[10-14] and other therapeutic agents[15-17] are still rare. Therefore, the development of stereoselective methods and efficient strategies for the synthesis of complex carbohydrates is critical to the emerging areas of glycosciences.

The monomeric units in complex carbohydrates are connected via glycosidic linkages. The glycosidic linkage is obtained by means of the glycosylation reaction, arguably the most important and challenging reaction in carbohydrate chemistry. A typical chemical glycosylation is based on the nucleophilic displacement of a leaving group (LG) on the glycosyl donor by a hydroxyl moiety of the glycosyl acceptor (Scheme 1). The remaining hydroxyls of both units are temporarily masked with protecting groups. In spite of significant progress made in the area of glycoside synthesis, the necessity to form either a 1,2-*cis* or a 1,2-*trans*-glycosidic bond with *complete* stereoselectivity remains the main reason chemical *O*-glycosylation is ranked among the most challenging problems of modern synthetic chemistry. The achievement of high yields and complete stereocontrol is difficult due to the complexity of the glycosylation process that often proceeds along with a variety of side reactions. Various factors such as temperature, pressure, structure, conformation, solvent, promoter, steric hindrance, or leaving group can affect the stereoselectivity of glycosylation. Some of these factors influence the stereoselectivity dramatically, others only to certain extent;[18,19] undoubtedly, the

neighboring substituent at C-2 is one of the major players. If the use of a base-labile ester-protecting groups is permitted, 1,2-*trans* glycosides can be prepared with the assistance of a neighboring participating group.[20,21] These glycosylations proceed primarily via a bicyclic acyloxonium ion intermediate that can only accept the top face nucleophilic attack resulting in the stereoselective formation of a 1,2-*trans* glycoside. Many traditional glycosyl donors such as halides, thioglycosides, or *O*-imidates provide excellent stereoselectivity and high yields. However, if the use of an acyl participating group is impractical or impossible, the synthesis of 1,2-trans glycosides becomes cumbersome, which has stimulated the development of a number of alternative approaches.
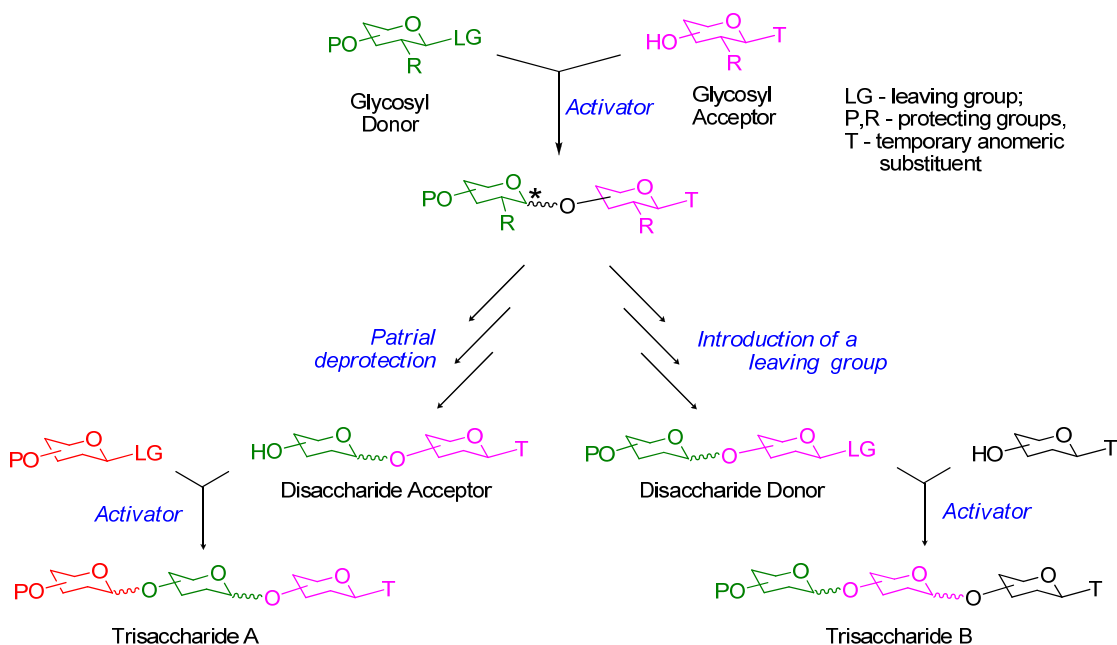
Scheme 1.1 Traditional linear oligosaccharide synthesis.

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Another important factor affecting the outcome of glycosylations is the nature of the anomeric leaving group. As a result, a large number of glycosyl donors have been developed.[22,23] In addition to traditionally used chlorides or bromides,[24,25] more recent glycosyl donors such as Schmidt's O-trichloroacetimidates,[26] Mukaiyama's fluorides,[27,28] alkyl/aryl thioglycosides pioneered by Ferrier, Nicolaou and Garegg,[29,30] Fraser-Reid's O-pentenyl glycosides,[31,32] and Danishefsky's glycal-epoxide system[33] have become valuable alternatives to the classic approaches.[34]

Moreover, a single-step glycosylation is not the only challenge researchers working on oligosaccharide synthesis face. Traditional linear approaches for the synthesis of oligosaccharides involve extensive protecting group manipulations between each glycosylation step.[35] Such additional manipulations increase the number of total synthetic steps and decrease the efficiency of the oligosaccharide assembly as reflected in dramatic drop in yield. This called for revisiting existing linear strategies for oligosaccharide synthesis and some significant improvements have already emerged.

1.2   Expeditious oligosaccharide synthesis: selective and chemoselective concepts

More recent strategies for oligosaccharide synthesis are typically based on the selective activation of one leaving group over another.[35,36] These expeditious approaches significantly shorten oligosaccharide assembly by minimizing the need for protecting group manipulations between glycosylation steps. Nicolaou's selective activation,[27] Fraser-Reid's armed-disarmed approach,[37] Danishefsky's glycal assembly,[33] Ogawa's orthogonal technique,[38,39] Roy's[40] and Boons'[41] active-latent concept, Wong's and Ley's programmable strategies,[42-45] are only few to mention.

One of the most conceptually attractive and practically efficient procedures, Fraser-Reid's *armed-disarmed approach,* is based on the principle of chemoselectivity. According to this principle, a benzylated (electronically activated, armed) glycosyl donor is chemoselectively activated over the acylated (electronically deactivated, disarmed) glycosyl acceptor bearing the same type of leaving group (Scheme 2).[46,47] The availability of a suitable mild promoter is essential to effectively differentiate the reactivity levels of the armed and disarmed species. At this stage, a 1,2-*cis*-linked disaccharide is preferentially obtained due to the general use of the ether-type arming substituent, a non-participating group. The obtained disaccharide can then be used for 1,2-*trans* glycosylation directly in the presence of a more potent promoter that can activate the disarmed leaving group. Initially developed for *O*-pentenyl glycosides,[32,47] this approach works with many other classes of stable glycosyl donors.[35] The armed-disarmed strategy thus offers an efficient tool for the synthesis of oligosaccharides with cis-trans glycosylation patterns. While the synthesis of cis-cis-linked derivatives is also possible (after additional reprotection OAc→OBn), the classic armed-disarmed strategy is not applicable to the synthesis of other oligosaccharide patterns, which significantly limited its versatility. This resulted in a number of improvement and various reactivity levels as well as inverse procedures that expand the scope of the armed-disarmed approach.
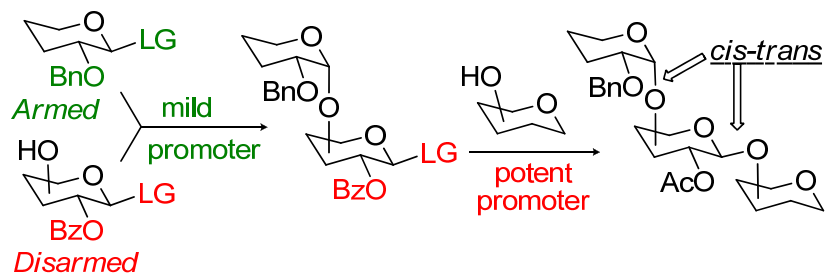
Scheme 1.2 Chemoselective activation strategy.

In principle, the use of the selective activation concept offers a more flexible oligosaccharide sequencing because this approach does not rely on the nature of the protecting groups. Instead, it requires a few leaving groups (LG^a, LG^b, LG^c, etc) that can be sequentially activated. Unfortunately, this relatively simple concept is limited to the number of available leavings groups compatible with the principle of sequential activation. This in part can be addressed by related semi-orthogonal and orthogonal strategies (vide infra).
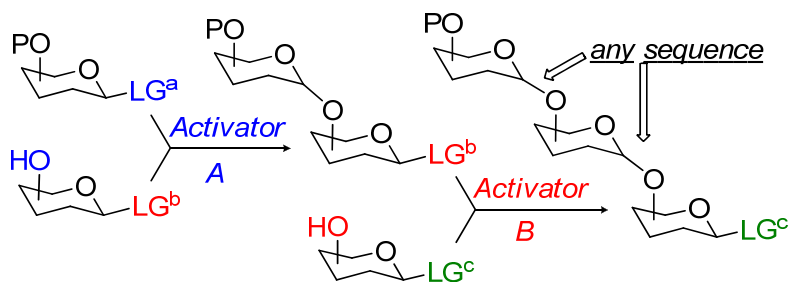
Scheme 1.3 Selective activation strategy.

This chapter is dedicated to a thorough overview of strategies making use of the principle of selective activation of one leaving group of another. The accumulated

knowledge has eventually translated into more advanced semi-orthogonal and orthogonal strategies that are also discussed below.

1.3   Selective activation

1.3.1   Glycosyl bromides and chlorides

The application of glycosyl bromides and chlorides as an effective glycosyl donor in the glycosylation reaction was first introduced by Michael followed by more versatile approach developed by Koenigs and Knorr.[48] Being relatively unstable, glycosyl chloride and bromide can only be selectively activated over other, more stable leaving groups, such as thio, seleno and *n*-pentenyl glycosides. The first application of glycosyl bromide in selective glycosylation strategy was reported by Zen[49] in the synthesis of isomaltose, isomaltotetraose and isomaltooctaose. Zen demonstrated that glycosyl bromide can be selectively activated over a thioglycoside acceptor in presence of silver carbonate (AgCO$_3$). This principle is illustrated by a two-step synthesis reported by Lonn (Scheme 1.4).[50,51] Accordingly, fucosyl bromide **1.1** was selectively activated over the *S*-ethyl leaving group of glycosyl acceptor **1.2**. The resulting disaccharide **1.3** is then coupled directly with glycosyl acceptor **1.4**. This was achieved in the presence of MeOTf and led to the formation of trisaccharide **1.5**. This example clearly illustrated the advantage of the selective activation strategy over the traditional approach that would imply additional protecting/leaving group manipulations after the first coupling step.
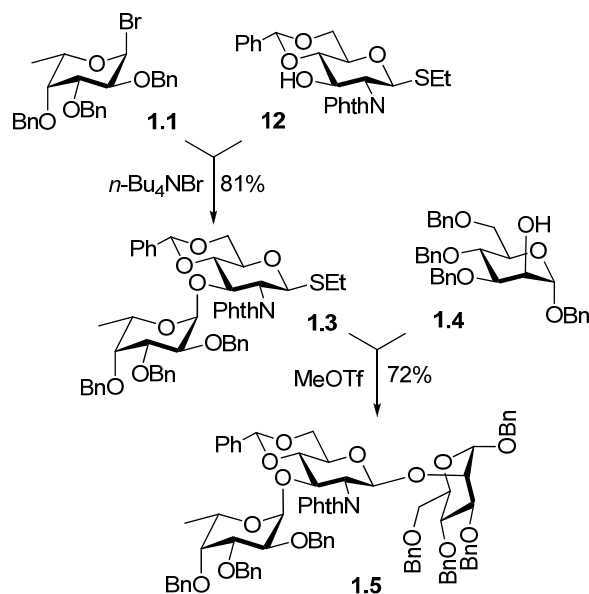
Scheme 1.4 Selective activation of glycosyl bromide over thioglycoside.

Stronger promoters for bromide activation, such as AgOTf, can be also successfully employed as required.[52,53] For example, as reported by Oscarson *et al.,* the synthesis of a tetrasaccharide of *Vibro cholerae* O139 began with AgOTf-promoted activation of bromide **1.6** over *S*-ethyl glycosyl acceptor **1.7** (Scheme 1.5).[54] The resulting disaccharide **1.8** was then subjected to a two-step deprotection sequence, which brings the elements of a conventional strategy. The resulting diol **1.10** is then glycosylated with glycosyl bromide **1.11** to afford tetrasaccharide **1.12**. All three glycosidic linkages were constructed using selective activation strategy although some interim protecting group manipulations were necessary.
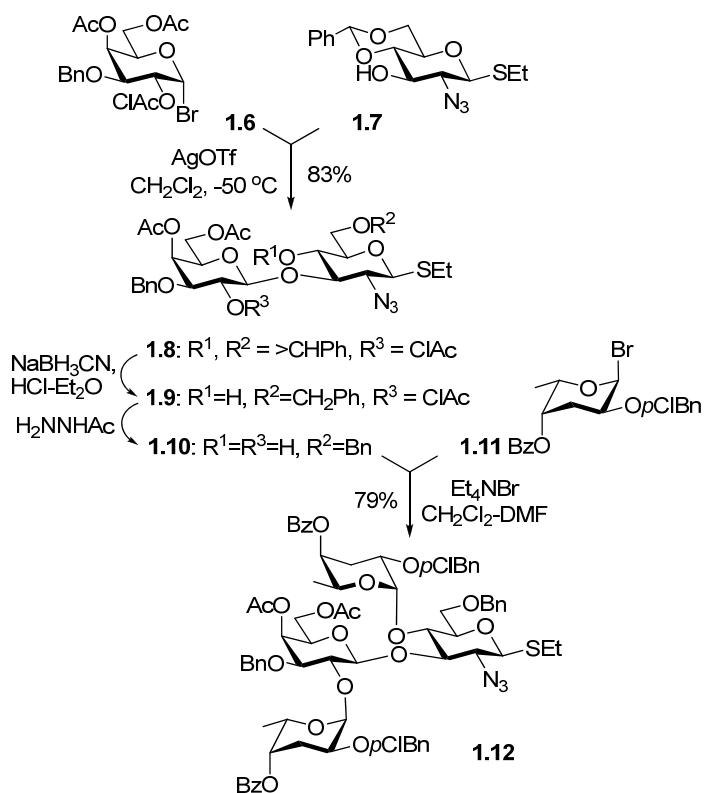
Scheme 1.5 Selective activation approach to the synthesis of a tetrasaccharide repeating unit of *V. cholerae* O139.

Kovac *et al.* showed that glycosyl bromide and chloride can be selectively activated with AgOTf in presence of anomeric actetate.[55] Pinto *et al.* described selective activation of glycosyl bromide donors over phenyl selenoglycoside acceptors, which was realized in the presence of AgOTf and γ-collidine.[56] Selective activation of glycosyl bromide over *n*-pentenyl glycoside acceptor in presence of AgOTf was demonstrated in the synthesis of an elicitor-active analog[57] and in the synthesis of glycophosphatidylinositol.[58] Mukaiyama and Kobashi introduced tri(1-pyrrolidino)phosphine oxide as an activator for glycosyl bromide suitable for conducting selective activations.[59] The activation takes place via glycosyl phosphonium bromide

intermediate. Scheme 1.6 illustrates the activation of glycosyl bromide **1.13** over thioglycoside **1.14** and glycosyl fluoride **1.15** leading to the respective disaccharides **1.16** and **1.17** in good yields.
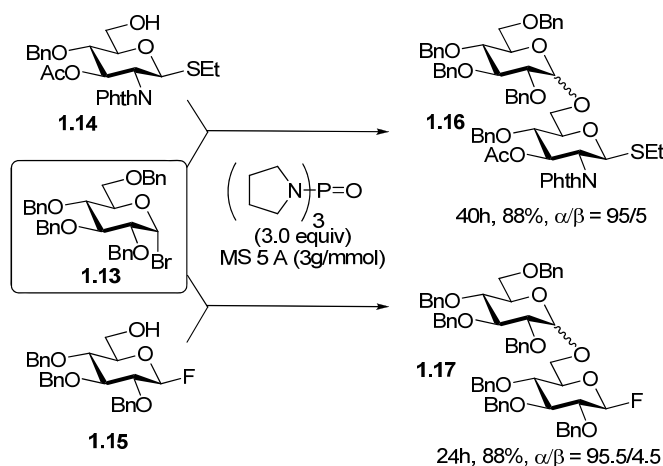
Scheme 1.6 Tri(1-pyrrolidineo)phosphine oxide as the activator of glycosyl bromides in selective activations.

Glycosyl chlorides can be also selectively activated with AgOTf in presence of thioglycosides. An example of this activation was employed in the synthesis of dodecasaccharide described by Ito and co-worker.[60,61] Selective activation of glycosyl chloride over glycosyl fluoride was also employed as depicted in Scheme 7.[62,63] Thus, sialyl chloride donor **1.18** was activated over sialyl fluoride acceptor **1.19** to afford a 2→8-linked disialoside in 49% yield.
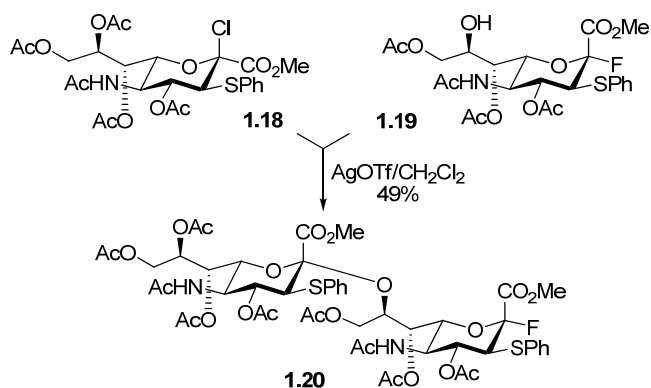
Scheme 1.7 Selective activation of glycosyl chloride over glycosyl fluoride.

Glycosyl iodides were also developed as an extension of the glycosyl chlorides and the glycosyl bromide.[64] The first glycosyl iodide was reported in 1910 by Fischer,[65] but it was mainly due to the efforts by Gervay-Hague *et al.* that glycosyl iodides found broad application in glycoside and oligosaccharide synthesis, including that via selective activation.[66,67] The glycosidation of glycosyl iodide **1.21** with glycosyl acetate acceptor **1.22** under neutral conditions (TBAI/DIPEA) gave disaccharide **1.23** with complete α-stereoselectivity (Scheme 1.8). Another example of glycosyl iodide utility is selective activations was reported by Oscarson.[68] Glycosyl iodide donor **1.21** was activated over thioglycoside acceptor **1.24** in the presence of triphenylphosphine oxide (Ph$_3$PO) to afford the corresponding disaccharide **1.25** with complete α-stereoselectivity.
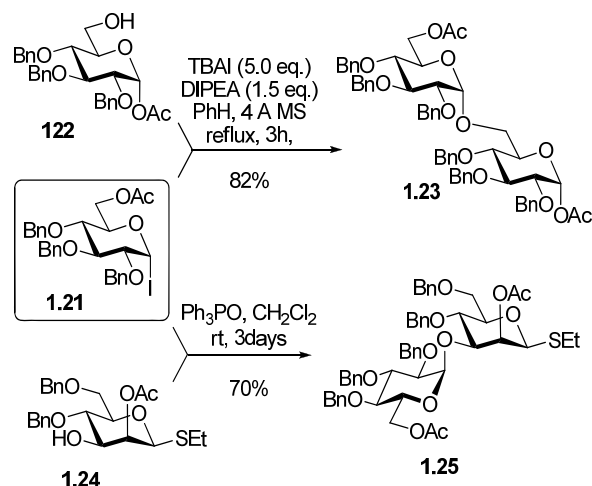
Scheme 1.8 Selective activation of glycosyl iodide **1.21** over glycosyl acetate **1.22** and thioglycoside **1.24**.

1.3.2 Glycosyl fluorides

Glycosyl fluorides were introduced by Mukaiyama *et al.* as glycosyl donors that can be activated with fluorophilic activator $SnCl_2$-$AgClO_4$.[69] Since then, glycosyl fluorides have become useful building blocks for oligosaccharide synthesis because they can be used both as glycosyl donors and glycosyl acceptors. It was established that the glycosyl fluorides are typically stable under basic as well as weakly acidic conditions required for protecting group manipulations. Glycosyl fluorides are also able to withstand typical conditions required for the activation of other anomeric halides (*vide supra*). Nicolaou and his co-workers extensively studied the application glycosyl fluorides in oligosaccharide and natural product synthesis.[70,71] It was proven that glycosyl fluorides can be activated in the presence of thioglycosides with a combination of $AgClO_4$ and $SnCl_2$. An example of the total synthesis of the tumor-associated Le[x] family of glycosphingolipids is depicted in Scheme 1.9.[72]
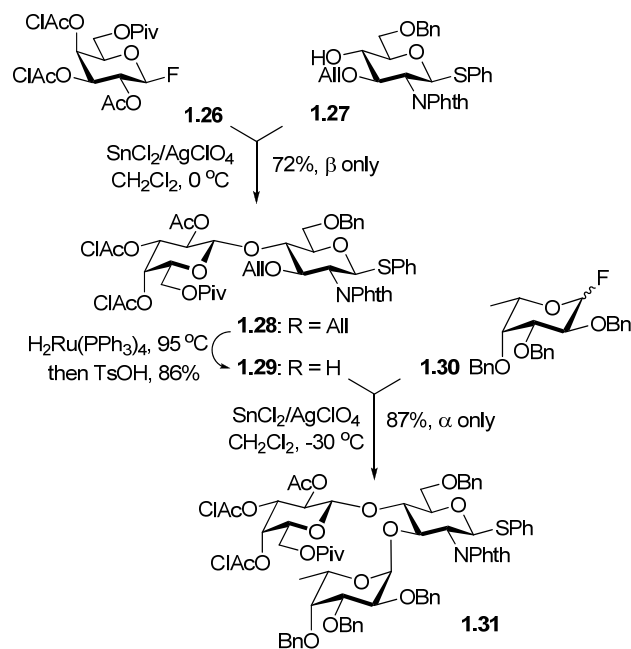
Scheme 1.9 Selective activation utilizing glycosyl fluoride as glycosyl donor.

As depicted in Scheme 1.10, a similar concept was applied to the synthesis of a tetrasaccharide containing two consecutive sialic acid units, which were also connected by selective activation (*vide supra*). Thus, the activation of glycosyl donor **1.20** over thioglycoside acceptor **1.32** was achieved in presence of AgOTf/SnCl$_2$ and resulted in the formation of tetrasaccharide **1.33**.[62,63]
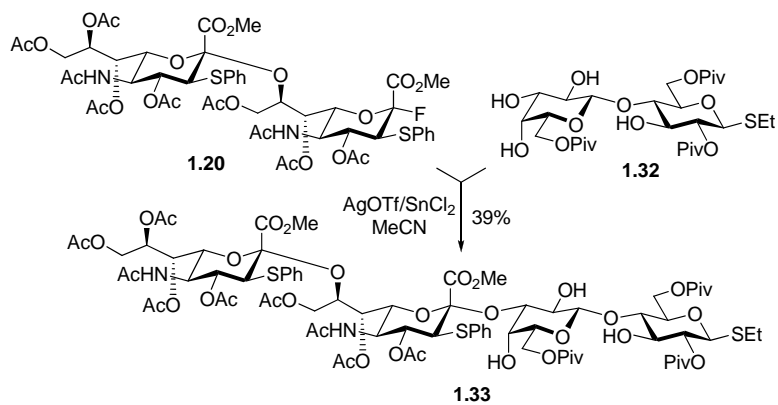
Scheme 1.10 Selective activation utilizing glycosyl fluoride as glycosyl donor.

In 2002, Mukaiyama *et al.* reported that a catalytic amount of protic acid, such as TfOH, HClO$_4$ or C$_4$F$_9$SO$_3$H is also suitable for the activation of fluoride donors over thioglycoside acceptors.[73] Danishefsky demonstrated that glycals also can tolerate activation of glycosyl fluorides in the presence of Sn(OTf)$_2$.[74,75] Nicolaou and co-workers have described a related glycosylation strategy based on two-stage activation concept, whereby thioglycoside is first converted into a glycosyl fluoride, which is then used as a glycosyl donor for coupling with a thioglycoside acceptor.[71,76-80]

1.3.3   *O*-Linked glycosyl donors

1.3.3.1 Hemiacetals (1-OH derivatives)

The direct glycosidation of 1-hydroxyl sugar is undoubtedly the most direct method of glycosylation. Reported by Fischer in 1901, this general approach has undergone a few stages of modifications and conceptual adjustments, and today, the dehydrative approach is arguably the most commonly used in application to oligosaccharide synthesis. In 1976, Leroux and Perlin developed the direct dehydrative glycosylation using triflic anhydride in the presence of a sterically hindered base, γ-collidine, and Bu$_4$NBr[81,82] The reaction proceeded via a stepwise activation of glycosyl donor **1.34** via the formation of the anomeric triflate followed by glycosyl bromide intermediates. Resultantly, glycosylation of glycosyl acceptor **1.35** bearing an anomeric acetate group afforded disaccharide **1.36** in 60% yield (Scheme 1.11a).
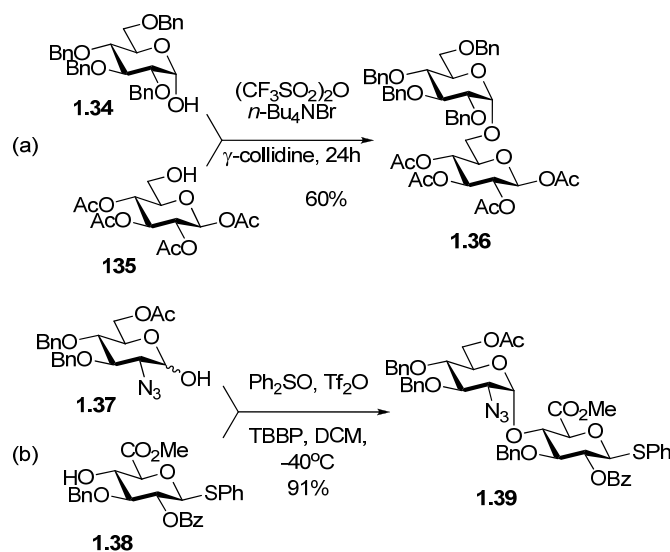
Scheme 1.11 Selective activation of hemiacetal over glycosyl acetate (a) and

thioglycoside (b).

In last few years, Gin and co-workers have developed a sulfonium-based electrophile activation of hemiacetal donor with treatment of Ph_2SO and Tf_2O. The in situ formation of the highly reactive sulfonium bistriflate provided the formation of the activated anomeric oxosulfonium intermediate. Van der Marel *et al.* then utilized the diphenyl sulfoxide protocol for selective glycosylation of hemiacetal with thioglycosides.[83,84] Thus, Ph_2SO/Tf_2O-mediated dehydrative condensation of 1-hydroxyl donor **1.37** with thioglycoside **1.38** afforded disaccharide **1.39** in 91%. This approach was then extended to the synthesis of heparin oligosaccharide (*vide infra*).[84]

1.3.3.2 Acyl and carbonyl derivatives

Advantages of the glycosyl ester donors in the carbohydrate synthesis are their easy preparation and relative chemical stability. The most representative anomeric functional group in this area is the acetyl group. In general, Lewis acids ($SnCl_4$, $FeCl_3$,

BF$_3$.OEt$_2$, TsOH) are used as promoters to activate glycosyl esters. To date, several glycosyl esters have been developed as efficient glycosyl donors, particularly for the synthesis of thioglycosides and glycosylation of simple alcohols. However, a number of applications to oligosaccharide synthesis via selective activation have also emerged. Below is a brief survey of relevant applications. Mukaiyama *et al.* utilized a preactivation-based glycosidation of glycosyl acetate **1.40** using iodotrimethylsilane followed by the addition of thioglycoside glycosyl acceptor **1.41** and phosphine oxide (Scheme 1.12a).[85] This glycosylation provided disaccharide **1.42** in good yields and high α-selectivity.
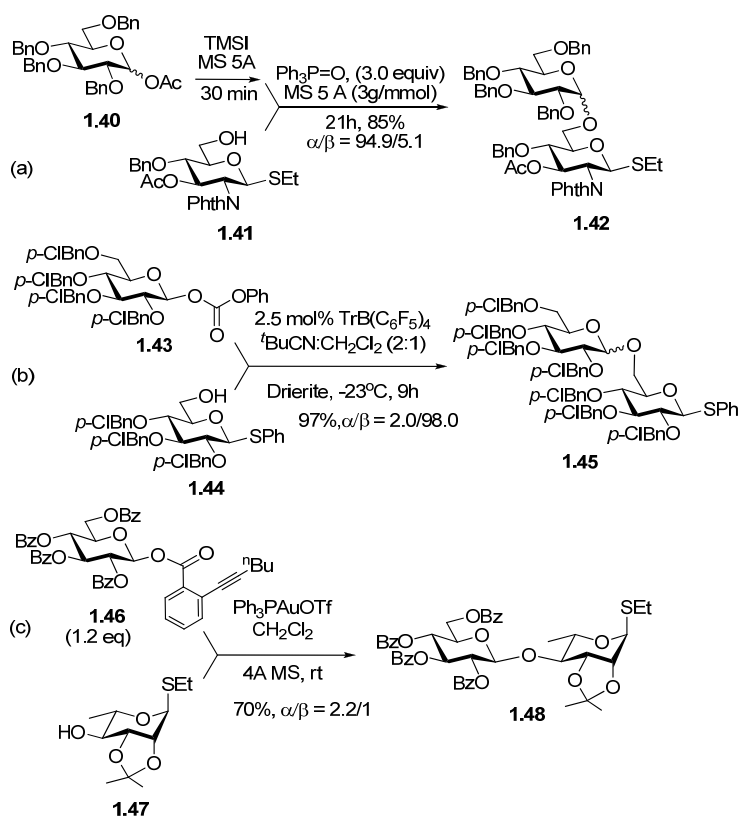
Scheme 1.12 Selective activation of glycosyl acetate **1.40** (a), carbonate **1.43** (b), and *ortho*-alkynylbenzoate **1.46** (c) over thioglycoside acceptors.

A example from work reported by Mukaiyama *et al.* involves selective activation of anomeric carbonate **1.43** over thioglycosyl acceptor **1.44** in the presence of TrB(C_6F_5)$_4$).[86,87] As depicted in Scheme 1.12b, the requisite disaccharide **1.45** was isolated in a nearly quantitative yield. Recently, Yu *et al.* introduced *ortho*-alkynylbenzoates as efficient glycosyl donors, which can be activated with catalytic amount of Ph$_3$PAuOTf.[88] The utility of glycosyl donor **1.46** was demonstrated selective activation of thioglycoside acceptor **1.47** to afford the disaccharide **1.48** (Scheme 1.12c).

1.3.3.3 *O*-Imidates

Glycosyl trichloroacetimidates, that over the years have become the most commonly used glycosyl donors, were introduced by Schmidt and co-workers. These are relatively unstable derivatives, and their glycosidation can be readily promoted by the catalytic amount of a mild Lewis acid. The selective activation of trichloroacetimidates over other glycosyl donors can be straightforwardly executed with TMSOTf[89] or BF$_3$-OEt$_2$.[90] For example, as a part of the synthesis of glycolipid of *Mycobacterium smegmatis,* TMSOTf-promoted coupling of trichloroacetimidate **1.49** and thioglycoside acceptor **1.50** afforded β(1→4) linked trisaccharide **1.51** in 71% yield (Scheme 1.13a).[89]
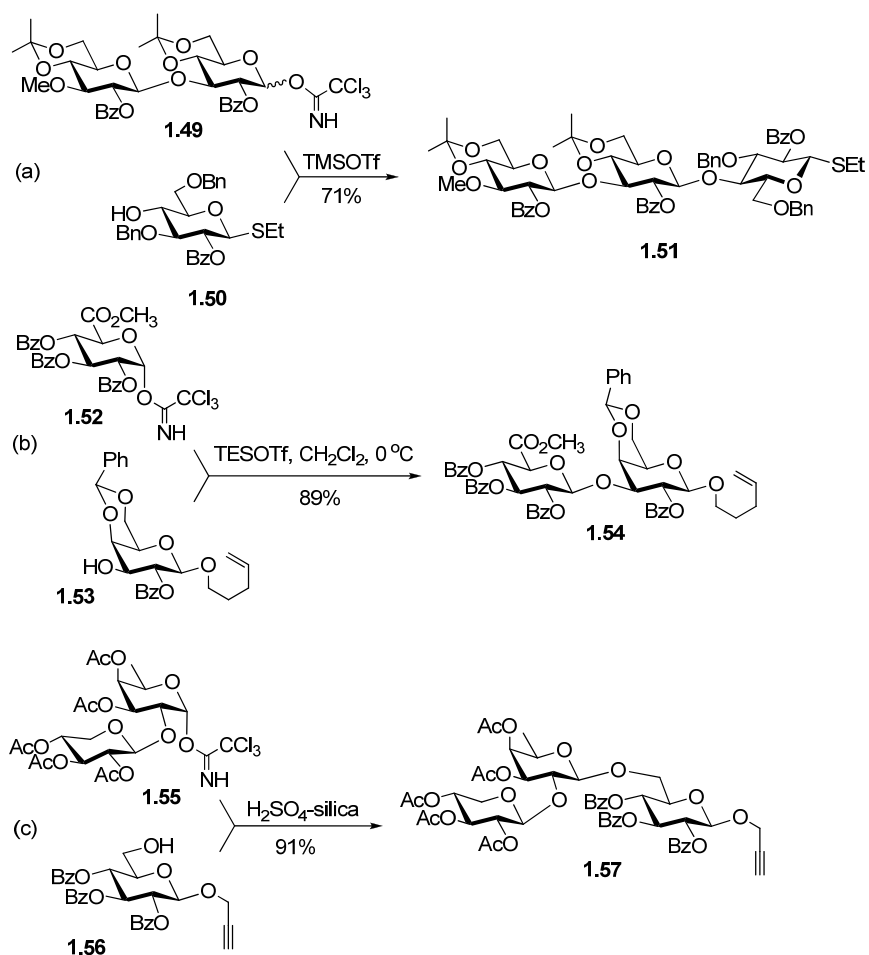
Scheme 1.13 Glycosyl trichloroacetimidates in oligosaccharide synthesis via selective activation over thioglycoside **1.50** (a), *n*-pentenyl glycoside **1.53** (b) and propargyl glycoside **1.56** (c).

The use of TESOTf for selective activation was also demonstrated in the glycosidation of glycosyl trichloroacetimidate donor with selenoglycoside acceptor, as described by Pinto *et al.*[56] A number of syntheses involving the activation of trichloroacetimidates over *O*-pentenyl glycosides also emerged.[91-93] For example, towards the synthesis of proteoglycan linkage region, trichloroacetimidate glycosyl donor **1.52** was selectively activated over with *n*-pentenyl glycosyl acceptor **1.53** in the presence of

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TESOTf. As depicted in Scheme 1.13b, disaccharide **1.54** was isolated in 89% yield.[92] Recently, Mukhopadhyay *et al.* showed that H_2SO_4-silica can be used as an activator to selectively activate trichloroacetimidate disaccharide donor **1.55** over propargyl glycosyl acceptor **1.56** leading to trisaccharide **1.57** in 91%.[94]

Similar glycosyl donors, (*N*-phenyl)trifluroacetimidates introduced by Yu and co-workers[95] can also be selectively activated over a range of glycosyl acceptors bearing stable anomeric leaving groups. For example, as reported by Seeberger *et al.*[96] activation of (*N*-phenyl)trifluoroacetimidate donor **1.58** over *n*-pentenyl glycosyl acceptor **1.59** was accomplished in the presence of TMSOTf (Scheme 1.14a). The resulting tetrasaccharide **1.60** was isolated in 80% yield and served as a precursor towards the synthesis of the repeating unit of *Bacillus Anthracis* cell wall polysaccharide.[96]
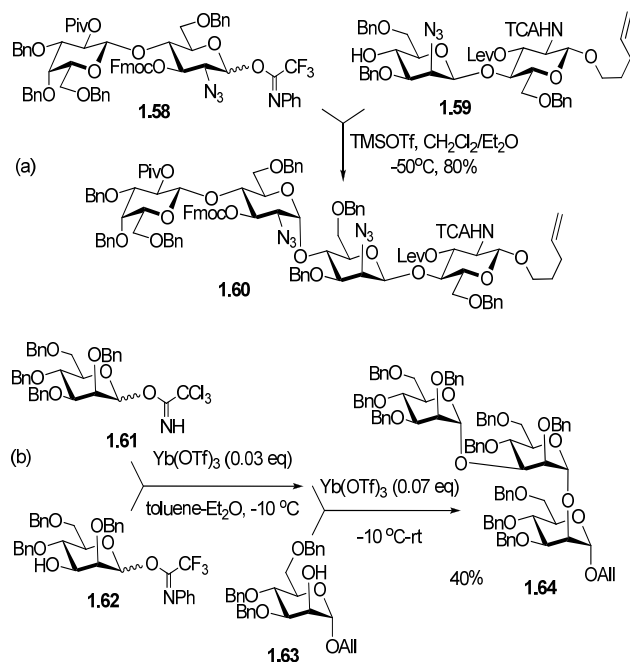
Scheme 1.14 Selective activation of (*N*-phenyl)trifluroacetimidate **58** over *n*-pentenyl glycoside **59** (a) and sequential activation of trichloroacetimidate **61** followed by the activation of (*N*-phenyl)trifluroacetimidate **62** (b).

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Iadonisi *et al.* demonstrated that the reactivity of glycosyl trichlro- and (*N*-phenyl)trifluoroacetimidates can be differentiated.[97] It was found that a mild promoter, such as Yb(OTf)₃, allows for selective activation of glycosyl trichloroactimidate building block **1.61** over glycosyl (*N*-phenyl)trifluoroacetimidate **1.62** (Scheme 1.14b). It should be noted that since both glycosyl donor and glycosyl acceptor bear the same protecting groups, the nature of this activation is clearly selective. The resulting disaccharide is then coupled with glycosyl acceptor **1.63** in a one-pot manner. This was affected by using additional quantities of the promoter and led to the formation of trisaccharide **1.64** in 40% yield over 2 steps.

Mukaiyama and co-workers reported selective activation of cyclic imidate donor, 6-nitro-2-benzothiazoate **1.65** over thioglycoside **1.66** or glycosyl fluoride acceptor **1.15**.[98] As depicted in Scheme 1.15, these HB(C₆F₅)₄-catalyzed selective activations resulted in the formation of disaccharides **1.67** and **1.68**, respectively, in good yields.
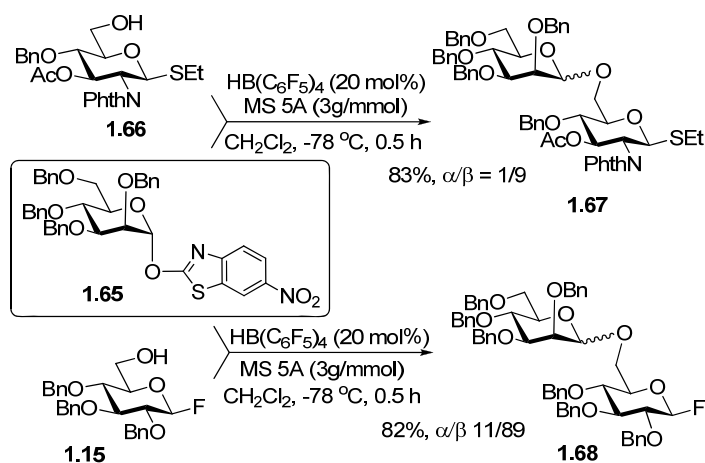
Scheme 1.15. Selective activation of 6-nitro-2-benzothiazoate **1.65** over thioglycoside **1.66** and glycosyl fluoride **1.15**.

1.3.3.4 O-P derivatives

Several glycosyl donors possessing a phosphorus atom in the leaving group at the anomeric center have also been investigated. Diethyl and dibenzyl glycosyl phosphites independently introduced by Schmidt and Wong, respectively, have found a wide application in glycoside and oligosaccharide synthesis. The activation of glycosyl phosphite requires only catalytic of TMSOTf (usually 10-20%), which allows for potential application in selective activations over a broad range of leaving groups, similarly to that of *O*-imidates *(vide supra)*. Thus, the selective activation of glycosyl phosphites over thioglycoside acceptors affected in the presence of TMSOTf was also reported.[99,100] For example, Nagorny demonstrated that the coupling of sialyl phosphite donor **1.69** and thioglycoside acceptor **1.70** was accomplished using TMSOTf as a promoter. The resultant trisaccharide product was obtained as a 3.5:1 mixture of anomers, which was acetylated and purified to furnish trisaccharide **1.71** as a pure α-anomer.[100] Danishefsky demonstrated that the coupling of glycosyl phosphites with glycals can be affected in the presence of TMSOTf.[101]
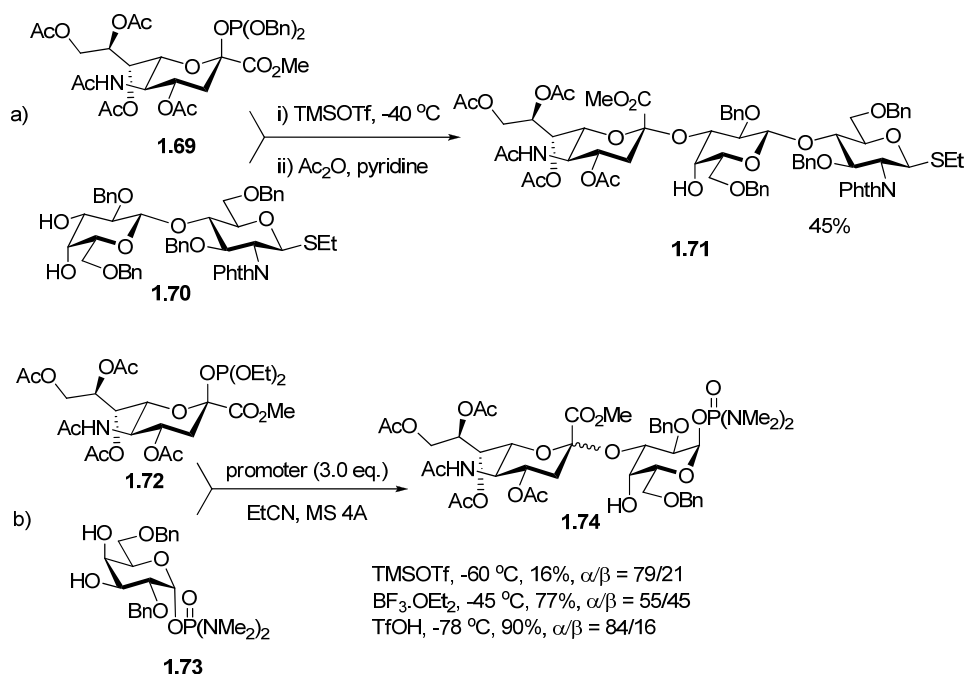
Scheme 1.16 Selective activation of glycosyl phosphite over thioglycoside (a) and

phosphorodiamidate (b).

Further expansion emerged with the development of glycosyl diphenyl phosphates,[102] glycosyl diphenylphosphineimidates,[103] and glycosyl phosphoroamidates.[104] Towards the synthesis of ganglioside GM3, sialyl phosphite **1.72** could be selectively activated over phosphorodiamidate acceptor **1.73** in the presence of TMSOTf, BF$_3$-OEt$_2$ or TfOH.[105] As depicted in Scheme 1.16b, the disaccharide **1.74** was isolated in excellent yield after fine-tuning of the reaction conditions.

In 2001, Seeberger and co-workers reinvented the use of glycosyl phosphates as glycosyl donors.[106,107] TMSOTf, TBSOTf, or BF$_3$.OEt$_2$ were all proved to be suitable promoters for the activation of glycosyl phosphates, also in oligosaccharide synthesis *via* selective activations. For example, selective activation of glycosyl phosphate **1.75** over thioglycoside acceptor **1.76** in the presence of TMSOTf resulted in the formation of

disaccharide **1.77** in 96% yield (Scheme 1.17). Similarly, glycosyl phosphate **1.78** was activated over *n*-pentenyl glycosyl acceptor **1.79** that resulted in the formation of disaccharide **1.80** in 82% yield.
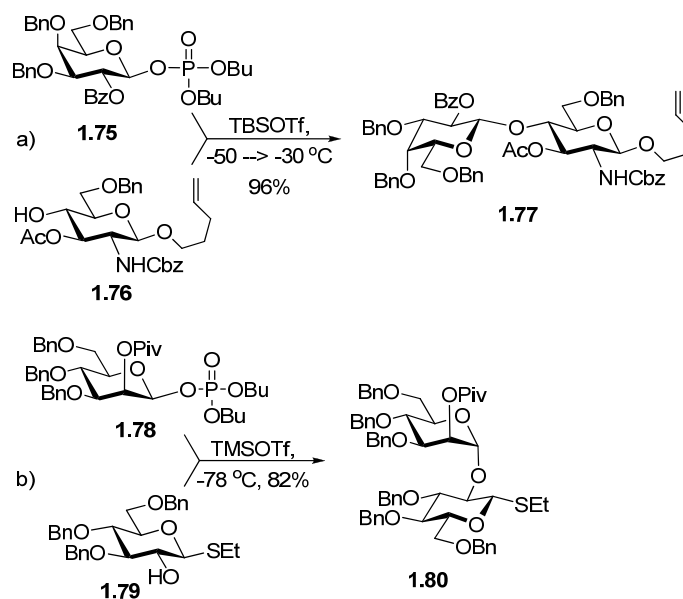
Scheme 1.17 Glycosyl phosphate in selective activation reported by Seeberger *et al.*

Along similar lines, Mukaiyama *et al.* glycosyl diphenylphosphinites as a glycosyl donors.[85] It was noted that this donor can be selectively activated over thioglycoside and glycosyl fluoride acceptors in the presence of MeI.

1.3.3.5 Transglycosylation (*n*-pentenyl, propargyl, hetaryl)

The use of *n*-pentenyl glycoside as glycosyl donors, was introduced by Fraser-Reid in 1988.[108] The activation of the leaving group is based on an electrophilic reagent, most commonly in the presence of IDCP, NIS/TfOH or NIS/TESOTf. Since these are essentially the same activation conditions as those for the activation of more common

thioglycosides, the use of *n*-pentenyl glycosides as glycosyl donors in selective activations is rare. For example, it was found that *n*-pentenyl glycoside **1.81** could be selectively activated with IDCP over disarmed thioglycoside **1.82** (Scheme 1.18a).[109] This coupling needs the additional enforcement by the electronic effects as only armed *n*-pentenyl glycoside could be activated over disarmed thioglycoside. Lopez *et al.* demonstrated that *n*-pentenyl glycosides can be selectively activated over glycosyl fluoride acceptors (vise infra).[110] More recently, Hotha *et al.* paired *n*-pentenyl glycoside with propargyl glycosides for selective glycosylation.[111] It was observed that *n*-pentenyl glycoside **1.84** could be activated over propargylated glycosyl acceptor **1.85** in the presence of NIS/TMSOTf (Scheme 1.18b). Resultantly, disaccharide **1.86** was obtained in 68% yield propargyl disaccharide in moderate yield.
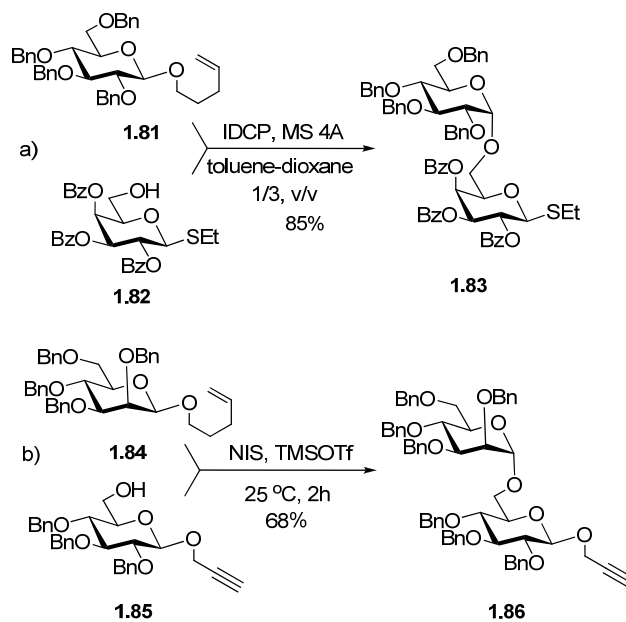
Scheme 18. Application of *n*-pentenyl glycosyl donors in selective glycosylation strategy.

The utility of 3-methoxy-2-pyridyl glycoside as a glycosyl donor in selective glycosylations was described by Hanessian *et al.*[112] As depicted in Scheme 1.19a, 3-methoxy-2-pyridyl glycosyl donor **1.87** was selectively activated over thioglycoside acceptor **1.88** in the presence of Cu(OTf)$_2$ and the resulting disaccharide **1.89** was obtained in 75% yield. Very recently, Hotha described that propargyl glycoside **1.90** can be selectively activated over *n*-pentenyl glycoside **1.91** in the presence of AuBr$_3$ at 65°C.[111] As depicted in Scheme 1.19b, disaccharide **1.92** was isolated in 65 % yield.
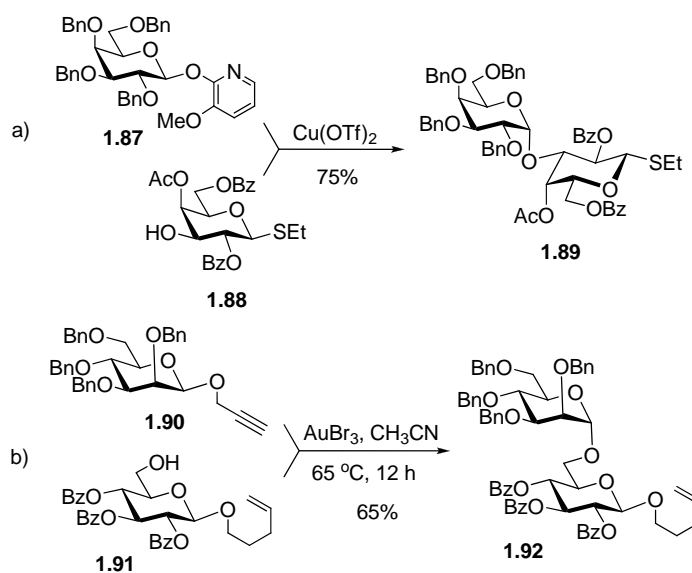
Scheme 1.19 Application of 3-methoxy-2-pyridyl (a), and propargyl glycosides (b) in selective activations.

Chenault *et al.* discovered that isopropenyl glycosides can be activated over *n*-pentenyl glycosides in the presence of TMSOTf.[113] Jensen and co-workers reported a glycosylation method employing methyl 2-hydroxy-3,5-dinitrobezoate glycosides (methyl 3,5-dinitrosalicylate, DISAL) as a glycosyl donors.[114] DISAL glycosyl donor

was applied to the synthesis of starch-related linear hexasaccharide whereas DISAL donor can be activated over thioglycoside acceptor in the presence of $LiClO_4$ and Li_2CO_3 as the promoter system.[115] Kim *et al.* discovered that 2-(benzyloxycarbonyl)benzyl (BCB) glycosides are perfectly stable, whereas the corresponding 2-(hydroxycarbonyl)benzyl (HCB) counterparts can be activated in presence of Tf_2O and DTBMP.[75] This phenomenon was applied to selective activation of 2-(hydroxycarbonyl)benzyl donor over 2-(benzyloxycarbonyl)benzyl acceptor. This activation, however, was performed using pre-activation conditions under which HCB was first treated with DTBMP (30 min), followed by the treatment with Tf_2O. After that, BCB glycosyl acceptor was added, therefore, formally this cannot be classified as the selective activation.

1.3.4   *S*-linked glycosyl donors

1.3.4.1 Sulfoxides, thiocyanates, xanthates, etc.

Glycosyl sulfoxides prepared from the corresponding thioglycosides via oxidation,[116] can be glycosidated in the presence of trifluoromethanesulfonic anhydride.[117] In 1994, van Boom *et al.* reported that TMSOTf and triethylphosphite (TEP) is an effective promoter system for the selective activation of phenylsulfenyl glycosides over phenylthioglycoside acceptors.[118] The synthesis of antigens Le^a, Le^b and Le^x utilizing the glycosyl sulfoxide method was reported by Kahne (Scheme 1.20).[119] Herein, Tf_2O in presence of 2,6-di-tert-butyl-4-methylpyridine (DTBMP) was used as activator. Thus, glycosidation of sulfoxide **1.93** over thenylthio glycoside **1.94** resulted in

the formation of disaccharide **1.95** in 86% yield. The latter was converted into a glycosyl

acceptor **1.96**, which was subjected to another round of selective glycosylation with

glycosyl sulfoxides **1.97**. The resulting trisaccharide **1.98** was obtained in 95% yield.
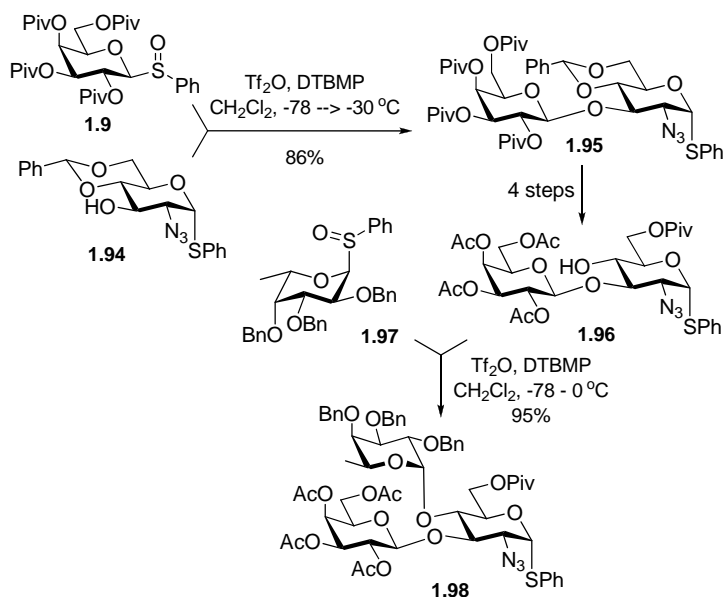
Scheme 1.20 Utilizing sulfoxide glycoside in selective activation as a part of the

synthesis of Le[a] antigen.

Kochetkov and co-workers showed that glycosyl thiocyanates can be selectively

activated over the 6-*O*-trityl thioglycoside acceptor.[120] As depicted in Scheme 1.21a,

thiocyanate **1.99** was selectively activated over thioglycoside **1.100** in the presence of

triphenylmethylium perchlorate (TrClO$_4$) as an activator. The resulting disaccharide

1.101 was obtained in 82% yield. Fügedi *et al.* introduced glycosyl 1-

piperidinecarbodithioates as glycosyl donors that can be activated in the presence of

AgOTf or TMSOTf.[121] As depicted in Scheme 1.21b, selective activation of 1-

piperidinecarbodithioate donor **1.102** over thioglycosides acceptor **1.103** was affected in

the presence of AgOTf and resulted in the formation of disaccharide **1.104**. Along similar lines, condensation of pentafuranosyl dithiocarbamate with 5-nitro-2-pyridyl thioglycoside in the presence of NIS/TfOH was described by Bogusiak and Szeja.[122]
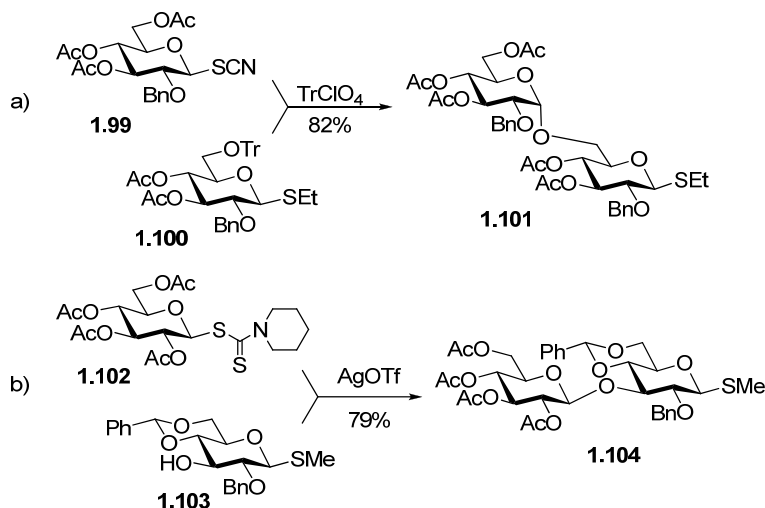
Scheme 1.21 Selective glycosidation of thiocyanate (a) and 1-piperidinecarbodithioate (b) over thioglycoside acceptors.

1.3.4.2 Thioimidates

The first wave of glycosyl thioimidates, compounds with a generic leaving group $SCR^1=NR^2$, included benzothiazolyl,[123] pyridin-2-yl,[124-126] pyrimidin-2-yl,[124,127] imidazolin-2-yl,[124] and 1'-phenyl-1*H*-tetrazolyl.[128] Although all of these derivatives have been tested as glycosyl donors and promising results have been obtained, the application of this class of compounds to selective activations emerged only much later. A few years ago, Demchenko *et al*. found that *S*-benzoxazolyl (SBox) and *S*-thiazolinyl (STaz) glycosides were sable toward many harsh reaction conditions associated with protective group manipulations.[129,130] In addition, these thioimidates could be glycosidated under a

range of mild reaction conditions and provided excellent anomeric stereoselectivity –
important traits for both glycoside synthesis and oligosacccharide assembly. The
particular versatility of thioimidates derives from the different modes that can be used for
their activation. Activations can take place either directly via the anomeric sulfur or
remotely via the nitrogen.[131,132] Further studies revealed that SBox and STaz glycosides
can be selectively activated over other classes of leaving groups. For example, AgBF₄ –
promoted activation of SBox glycoside **1.105** over *n*-pentenyl glycoside acceptor **1.106**
resulted in the formation of disaccharide **1.107** in 86% yield (Scheme 1.22a).[133]
Similarly, De Meo and Parker described that the SBox sialyl donor **1.108** can be activated
over SEt glycosyl acceptor **1.109** in the presence of AgOTf.[134] As shown in Scheme
1.22b, the resulting disaccharide **1.110** was obtained in 89% yield.
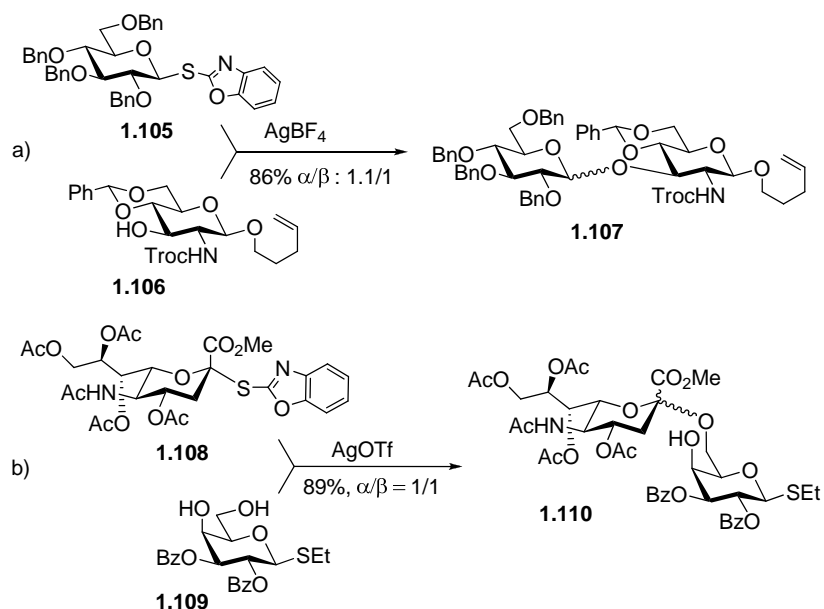
Scheme 1.22 SBox glycosides in selective activation strategy.

STaz glycosyl donor **1.111** was glycosidated selectively over thioglycoside acceptor **1.112** in the presence of AgBF$_4$.[133] As depicted in Scheme 1.23a, disaccharide **1.113** was isolated in 89% yield. SBox glycosyl donor **1.114** could be also selectively activated over STaz acceptor **1.115** in the presence of Bi(OTf)$_3$ to afford disaccharide **1.116** in 69% yield (Scheme 1.23b).[132]
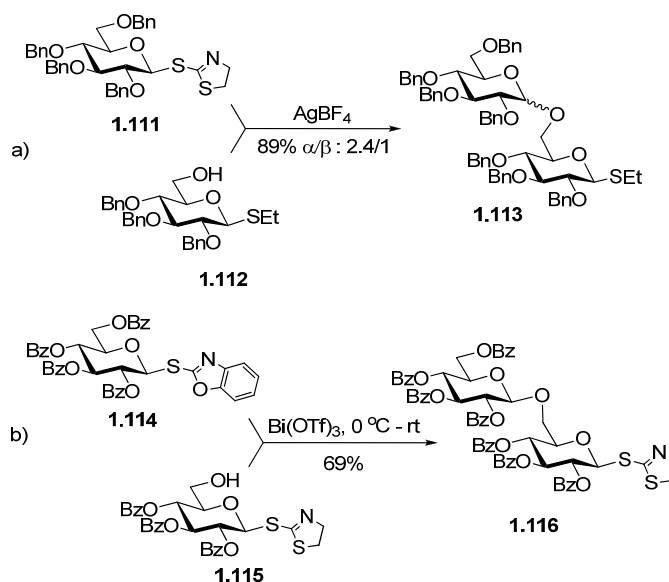
Scheme 1.23 STaz glycosides as glycosyl donors and acceptors in selective activations.

1.3.4.3 *S*-Alkyl and aryl glycosides

Alkyl and aryl thioglycosides have been widely studied as versatile building block for oligosaccharide synthesis due to their high chemical stability. They are also good intermediates to be transformed into a range of other glycosyl donors and can act as acceptors in glycosylation reactions, which make thioglycosides suitable for use in chemoselective, selective and iterative glycosylations. As shown by Ogawa *et al.*, selective activation of thioglycosides over glycosyl fluorides can be executed in the

presence of NIS and AgOTf as a promoter system.[38] In addition, other promoters including NIS/TfOH,[135] MeOTf,[60,61] and DMTST[136,137] are also capable of the selective activation of thioglycosides over fluorides. For example, selective activation of thioglycoside **1.117** over fluoride acceptor **1.118** led to the formation of trisaccharide **1.119** in 85% yield (Scheme 1.24a).[137] Selective activation of thioglycoside **1.120** over *S*-thiazolinyl glycosyl acceptor **1.115** could be affected in the presence of NIS in combination with a catalytic amount of TfOH.[129] As shown in Scheme 1.24b, this coupling resulted in the formation of disaccharide **1.116**. STaz glycosides can be also activated in the presence of the same activators, but this requires at least stoichiometric amount of TfOH.
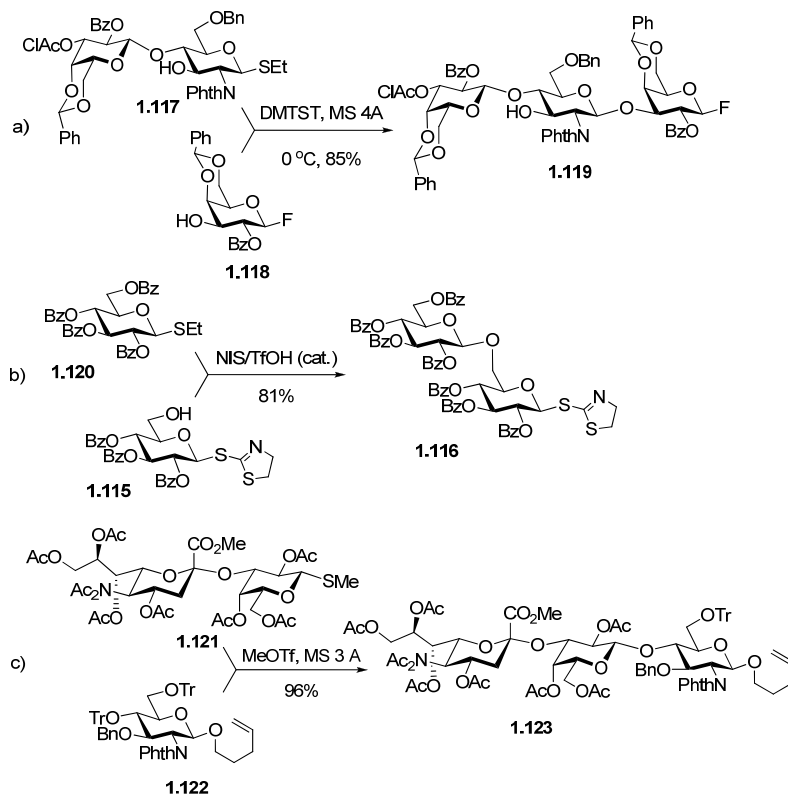
Scheme 1.24 Selective of thioglycosides over glycosyl fluoride (a), S-thiazolinyl (b), and *n*-pentenyl (c) glycosyl acceptors.

Boons reported that the glycosidation of thioglycoside **1.121** with di-*O*-trityl pentenyl glycosyl acceptor **1.122** can be achieved in the presence of MeOTf.[138] As shown in Scheme 1.24c, trisaccharide **1.123** was obtained in 96% yield. The regioselectivity observed is due to the observation reported by Kochetkov *et al.* that trityl ethers invert the reactivity of alcohols towards glycosylation.[139] Boons and co-workers also developed as interesting concept for oligosaccharide synthesis by demonstrating that the increase of steric hindrance around the active site of the leaving group has a dramatic effect on the leaving group ability.[140] Thus, it was shown that an *S*-ethyl glycoside can be selectively activated over dicyclohexylmethyl thioglycoside in the presence of NIS/TMSOTf at low temperature.

In the development of 4-acetoxy-2,2-dimethylbutanoate as a novel protecting group for oligosaccharide synthesis, Ensley demonstrated that the activation of thioglycosides over 1-*O*-acyl glycosyl acceptor can be performed in the presence of NIS/AgOTf.[141] It was also reported that glycals are stable under MeOTf-promoted activation of thioglycosides.[142] Recently, it was reported that *S*-tolyl rhamnopyranoside **1.124** can be selectively activated over propargyl glycosyl acceptor **1.125** in the presence of NIS/H_2SO_4-silica.[94] As shown in scheme 1.25, the resulting disaccharide **1.126** obtained in 87% yield, was converted to glycosyl acceptor **1.137**. The latter was then subjected to glycosylation with *S*-tolyl glycosyl donor **1.128** under the same reaction conditions. This sequence resulted in the formation of trisaccharide **1.129** related to the cytotoxic triterpenoid saponin in 83% yield.
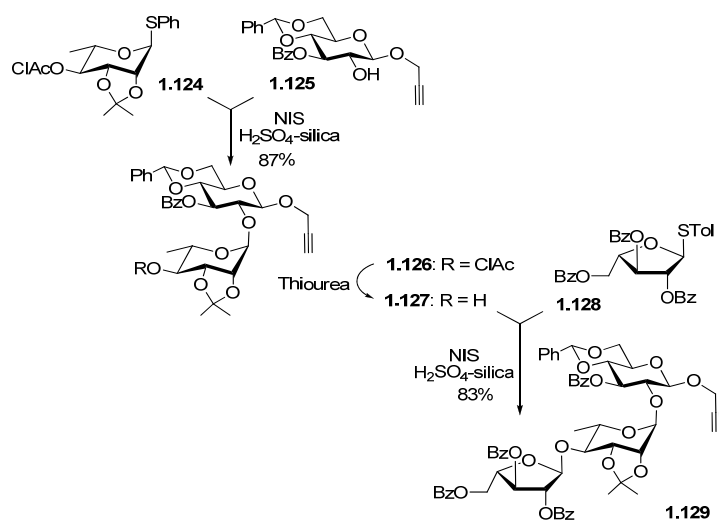
Scheme 1.25 Application of the selective activation of thioglycoside over propagyl in the synthesis of trisaccharide **1.129**.

1.3.5 Miscellaneous glycosyl donors (orthoesters, Se-glycosides, glycal/epoxides)

Anomeric phenylselenides, introduced by Pinto *et al.* in 1991, are very effective glycosyl donors.[143] The phenylseleno leaving group behaves similarly to that of thioglycosides with respect to their stability towards protecting group manipulations and lability towards electrophilic reagents. Very differently though, selenoglycosides can be activated with AgOTf and K_2CO_3, which allows for their selective activation in presence of thioglycosides.[56,143] In 2000, selenoglycosides are also more reactive towards MeOTf or NIS/TfOH than their thioglycoside counterparts.[144,145] Thus, Ley *et al.* reported selective activation of phenylseleno glycoside **1.130** over thioglycoside acceptor **1.131** in application to convergent synthesis of GPI anchor of *Trypanosoma brucei* (Scheme 1.26).[144] This coupling was performed in the presence of MeOTf and resulted in the formation of trisaccharide **1.132** in 75% yield. The latter was then converted into

glycosyl acceptor **1.133** which was glycosylated with selenoglycoside **1.134** again in the presence of MeOTf. This second selective activation resulted in the formation of pentasaccharide **1.135 i**n 75% yield. Recently, Fairbanks utilized electrochemistry for activation selenoglycoside donors, although selective glycosylation of selenoglycoside over thioglycoside acceptor gave low yields.[146]
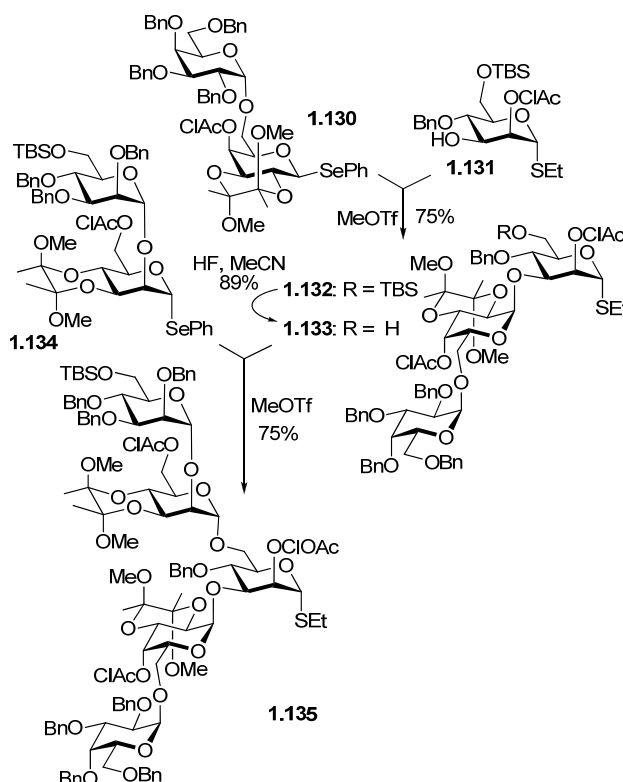
Scheme 1.26 Convergent synthesis of pentasaccharides **1.135** using selective activation of selenoglycosides over thioglycosides.

Yoshida *et al.* reported the use of telluroglycosides as glycosyl donors which can be activated either electrochemically[147,148] or in the presence of MeOTf, NIS/*t*-BuOCl, or NBS/TMSOTf.[149] The latter reaction conditions allowed for selective activation of telluroglycosides over thioglycoside acceptors.

Orthoesters have been used as useful glycosyl donors for 1,2-*trans* glycosylation for decades. Their activation is typically achieved in the presence of Lewis acid or mild electrophilic promoters, which permits their selective activation over a number of leaving groups of different classes. For example, it was demonstrated that ethylthio orthoester **1.136** can be activated over conventional thioglycoside **1.137** using NIS/TfOH as an activator (Scheme 1.27a).[150] The resulting disaccharide **1.138** was isolated in 75% yield. Lopez *et al.* reported a glycosylation approach in which *n*-pentenyl 1,2-orthoesters are activated over glycosyl fluoride acceptor using NIS and Yb(OTf)$_3$ as a promoter system.[110] Recently, Hotha *et al.* reported that *n*-pentenyl 1,2-orthoester **1.139** can be selectively activated over propargyl glycoside acceptor **1.85** in the presence of NIS/Yb(OTf)$_3$ to afford disaccharide **1.140** in 62% yield (Scheme 1.27b).[111] Along similar lines, propagyl 1,2-orthoester **1.141** can be selectively activated over *n*-pentenyl acceptor **1.142** in the presence with AuBr$_3$ leading to trisaccharide **1.143** (Scheme 1.27c).
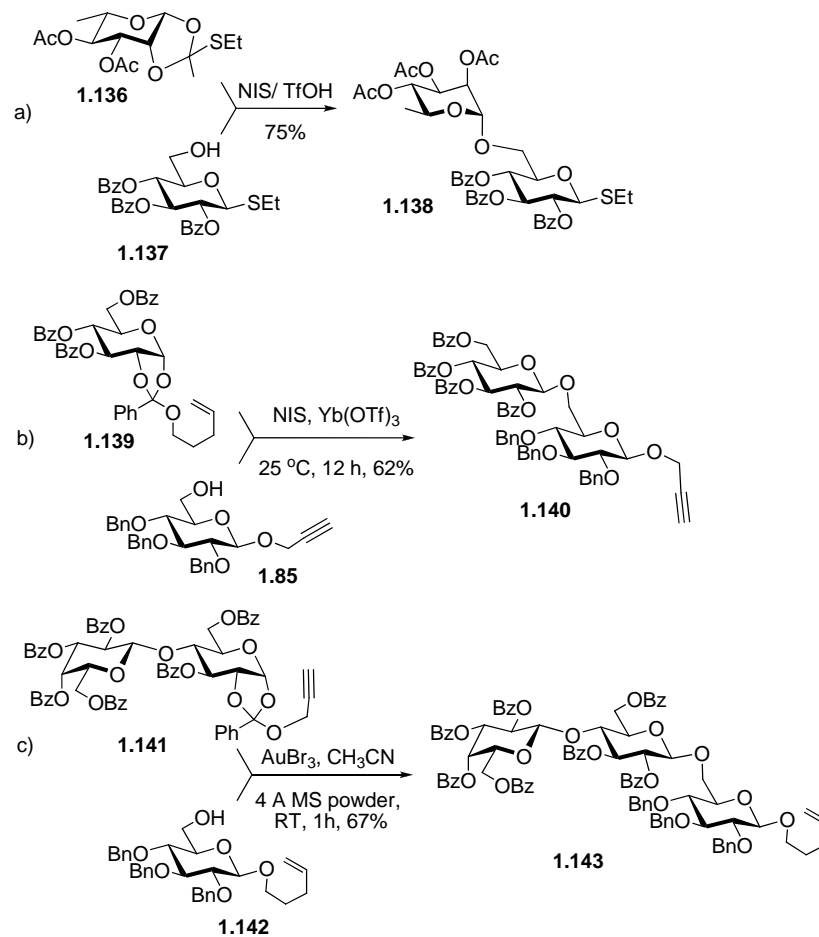
Scheme 1.27 Selective activation of orthoesters in oligosaccharide synthesis.

Cyanoethylidene approach to 1,2-*trans*-glycosylation of tritylated acceptors has been extensively studied by Kochetkov.[151] As reported by Boons, this approach allows for selective activation over *n*-pentenyl glycosyl acceptor in the presence of TrClO$_4$.[138,152] Backinowsky also reported that 1,2-*O*-(1-cyanoethylidene) glycoside can be activated with TrClO$_4$ in presence of thioglycoside acceptors described in synthesis of a "fully-carbohydrate" mannodendrimer.[153]

Danishefsky and his-coworkers developed a convenient method for the preparation of the 1,2-anhydro sugars from glycals using dimethyldioxirane (DMDO)[154]

as an effective epoxidation reagent.[155] For example, 1,2-anhydro sugar **1.144** generated from glycal **1.145** could be activated over glycal acceptor **1.146** in the presence of $ZnCl_2$ in THF to afford 1,2-*trans*-linked disaccharide **1.147** in 81% (Scheme 1.28). The epoxidation-glycosylation sequence can be then reiterated to yield larger oligosaccharides, although similarly to other pre-activation type of couplings, this approach cannot be classified as the formal oligosaccharide synthesis via selective activations.
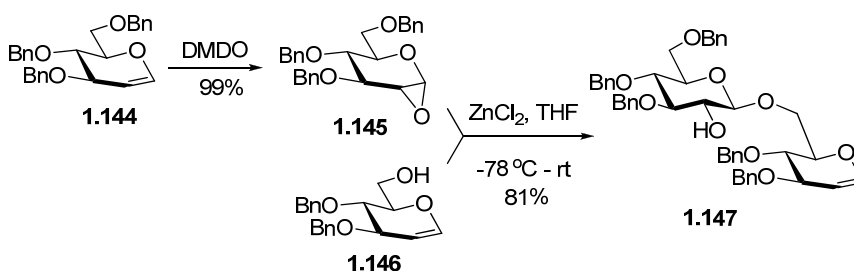
Scheme 1.28 Danishefsky's glycal-epoxide method for oligosaccharide synthesis.

1.4 Multistep sequences based on selective activations

Multistep oligosaccharide synthesis have been developed using selective activation strategy. In principle, the activation sequence can be continued providing beyond previously discussed couplings of building blocks equipped with LG^a and LG^b. The continuation requires the availability of a LG^c, which would withstand activation conditions for the LG^b. However, these elongated multi-step sequences are not yet routinely available (*vide infra*).[62,63] This can be circumvented by performing additional modifications between glycosylations that allow repetition of the sequence involving repetitive LG^a and LG^b activations. An example applying such selective-activation

strategy is the synthesis of heparin-like oligosaccharides reported by van der Marel and co-workers (Scheme 1.29).[84] The synthesis of pentasaccharide **1.157** began by utilizing the diphenyl sulfoxide—triflic anhydride dehydrative protocol developed by Gin[156] for the activation of the hemiacetal donor **1.148** over thioglycoside acceptor **1.149**. The phenylthio moiety of the resulting disaccharide **1.150** was then directly coupled with glycosyl acceptor **1.151** with the benzenesulfinyl piperidine (BSP)- triflic anhydride system.[157] The resulting trisaccharide **1.152** is then converted to the corresponding hemiacetal derivative **1.153**, which was selectively activated over thioglycoside acceptor **1.154** in the presence of diphenyl sulfoxide-triflic anhydride. The resulting tetrasaccharide **1.155** isolated in 51% yield was glycosidated with glycosyl acceptor **1.156** in the presence of the BSP/Tf$_2$O promoter system to furnish the target pentasaccharide **1.157** in 53% yield.
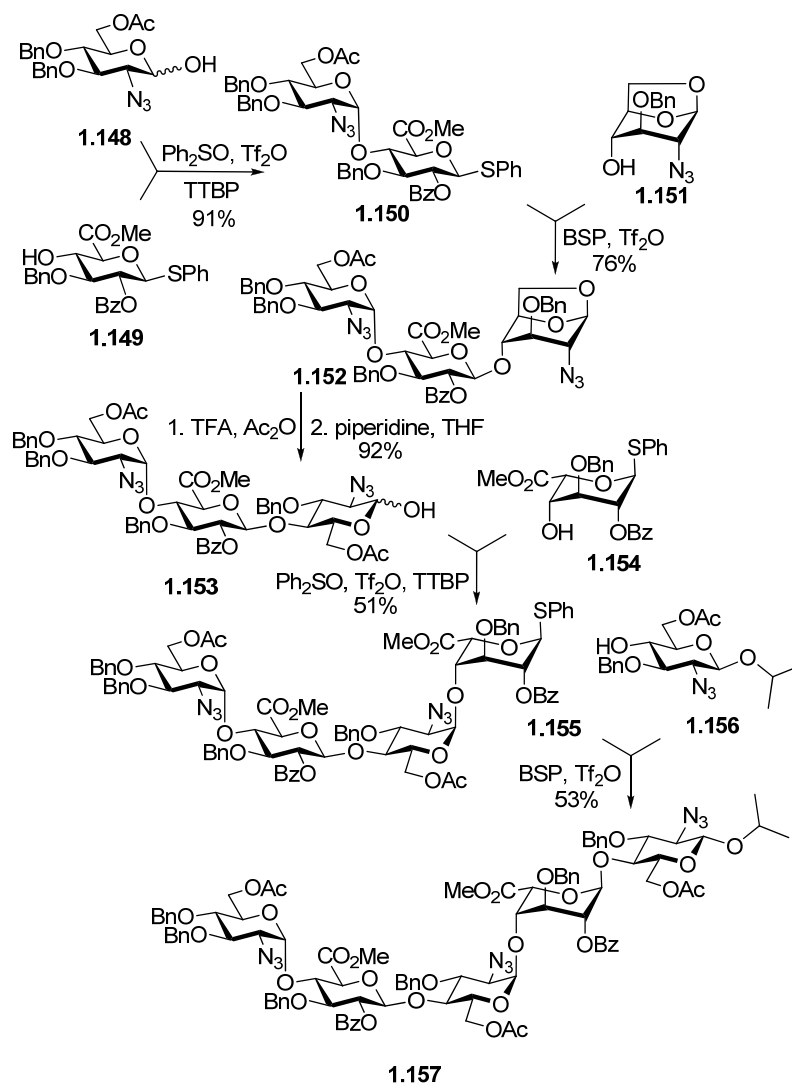
Scheme 1.29 Selective activation approach to the synthesis of heparin analogues.

An example with the involvement of three different leaving groups is depicted in Scheme 1.30, in which trichloroacetimidoyl is used as a LG^a, phenylselnyl as LG^b, and S-ethyl as LG^c.[158] Thus, activation of trichloroacetimidate **1.158** over selenoglycoside acceptor **1.159** was achieved in the presence of TMSOTf. The resulting disaccharide **1.160** isolated in 90% was then coupled with thioglycoside acceptor **1.112** in the presence of AgOTf and K_2CO_3 leading to trisacccharide **1.161** in 81% yield. In principle the

thioethyl moiety can be activated further. A similar sequence using bromide as LG[a], Selenoglycoside as LG[b] and thioglycosides as LG[c] was also reported.[56]
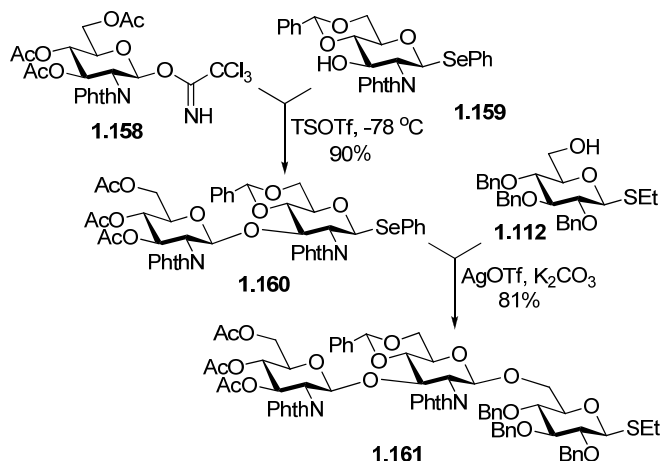
Scheme 1.30 Selective activation utilizing three different leaving group.

Another relevant example is the use of the *S*-benzoxazolyl (SBox) moiety as LG[a], *S*-ethyl as LG[b], and *O*-pentenyl as LG[c] depicted in Scheme 1.31.[159,160] The SBox glycosyl donor **1.114** was selectively activated over the SEt acceptor **1.82** in the presence of AgOTf to produce disaccharide **1.162** in 99% yield. The latter disaccharide was glycosidated with the *n*-pentenyl acceptor **1.163** in the presence of MeOTf to provide trisaccharide **1.164** in 90% yield. Finally, the leaving *n*-pentenyl moiety of the trisaccharide donor **1.164** was then activated with NIS/TMSOTf for the coupling with glycosyl acceptor **1.165**, resulting in the formation of tetrasaccharide **1.166** in 73% yield. A similar example included the following three-step activation sequence performed in a one-pot fashion: SBox as LG[a], *S*-thiazolinyl (STaz) as LG[b], and *S*-ethyl as LG[c].[161]
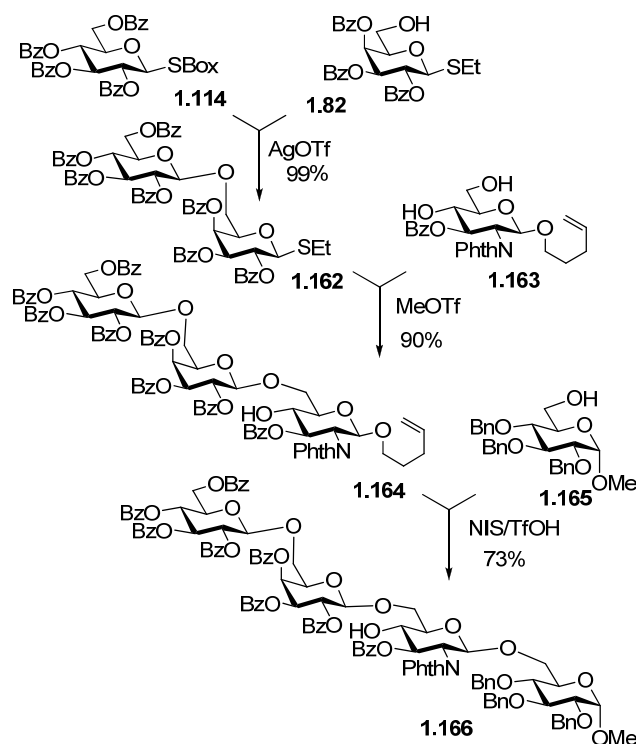
Scheme 1.31 Three-step SBox→ SEt → *n*-pent sequential selective activation.

Very recently, the synthesis of a β-(1→6)-linked hexasaccharide via the five-step selective activation sequence was employing six different leaving groups as depicted in Scheme 1.32.[162] The synthesis began with the selective activation of thiocyanate donor **1.167** over STaz glycosyl acceptor **1.115** using Cu(OTf)$_2$ as a promoter. The STaz leaving group of disaccharide **1.116** was then directly activated over SBox acceptor **1.168** with benzyl bromide to give trisaccharide **1.169**. The SBox moiety of **1.169** was then selectively activated over the fluoride acceptor **1.170** in the in presence of MeOTf. The resulting tetrasaccharide **1.170** was glycosidated with thioglycoside acceptor **1.137** in presence of AgClO$_4$/Cp$_2$ZrCl$_2$ to provide pentasaccharide **1.171**. Finally, the *S*-ethyl leaving group of **1.171** was selectively activated over the *n*-pentenyl acceptor **1.91** in the presence of MeOTf as a promoter to furnish the hexasaccharide **1.172** in 72% yield.[162]

42
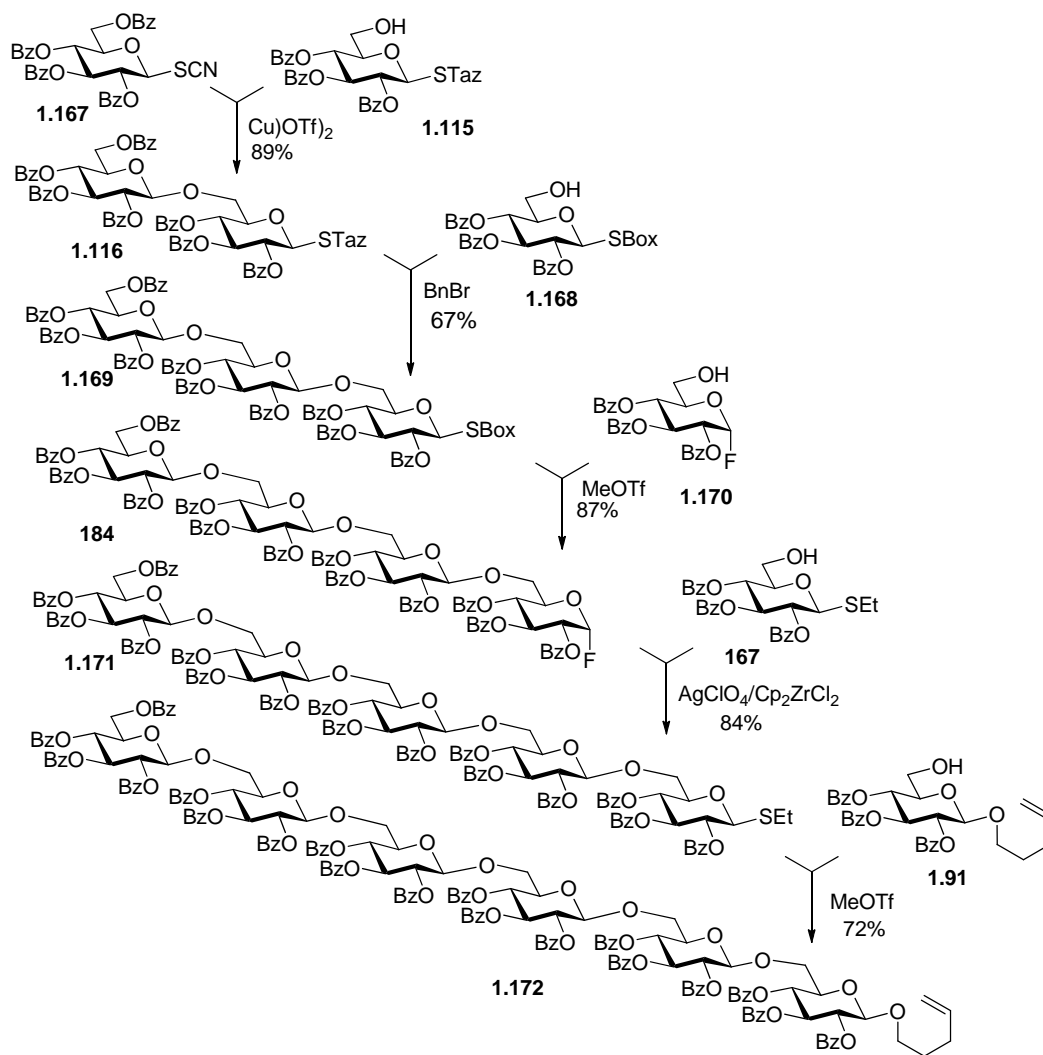
Scheme 1.32 Selective activation utilizing six different leaving groups in the synthesis of 1,2-*trans* hexasaccharide **1.172**.

1.5   Semi-orthogonal approach

The orthogonal approach makes use of two leaving groups LG[a] and LG[b] that can be independently activated one over another and vice versa (*vide infra*). A semi-orthogonal strategy is based on a similar principle, but it requires the additional enforcement by electronic effects, similarly to that of the "armed-disarmed concept". Overall, this concept combines advantages of both selective and chemoselective

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activation; its utility, however, is limited as the protecting groups also often determine the stereoselectivity as well.

Thus, it was demonstrated[109] that either armed or disarmed thioglycoside can be selectively activated over either armed or disarmed *n*-pentenyl glycosides in the presence of MeOTf. However, only armed *n*-pentenyl could then be activated over a disarmed thioglycoside acceptor using IDCP as a mild promoter. Thus, lactose *S*-ethyl donor **1.173** was selectively activated over the *n*-pentenyl moiety of glycosyl acceptor **1.142** in the presence of MeOTf. The resulting trisaccharide was isolated in 98% yield as depicted in Scheme 1.33. The *n*-pentenyl moiety of the latter was then subjected to selective/chemoselective activation over *S*-ethyl acceptor **1.82** in the presence of IDCP leading to tetrasaccharide 148 in 92% yield.
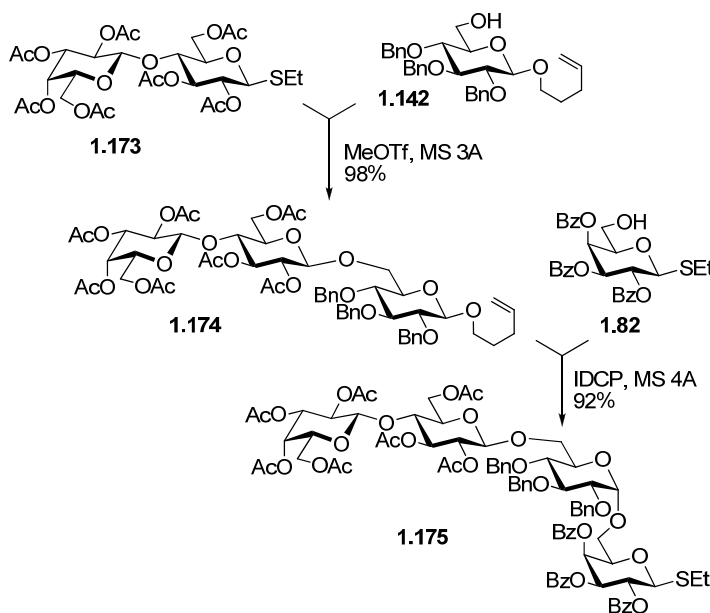
Scheme 1.33 Semi-orthoganality of thioglycoside and *n*-pentenyl leaving groups.

This semi-orthogonal concept was recently extended to another suitable pair of leaving groups, namely fluoride and *n*-pentenyl.[110] While either armed or disarmed glycosyl fluorides underwent smooth glycosylation with armed/disarmed *n*-pentenyl glycoside in presence of Yb(OTf)$_3$, only armed *n*-pentenyl glycosides could be activated over disarmed glycosyl fluorides in presence of IDCP. As depicted in Scheme 1.34, fluoride donor **1.176** was selectively activated over pentenyl glycosyl acceptor **1.177** in the presence of Yb(OTf)$_3$. The resulting *n*-pentenyl disaccharide was isolated in 68% yield. Conversely, the activation of armed *n*-pentenyl glycoside **1.179** was achieved selectively over fluoride acceptor **1.180** to afford disaccharide **1.181**. No activation of benzoylated *n*-pentenyl glycosides could be accomplished, whereas NIS/BF$_3$-E$_{t2}$O– promoted selective activation resulted in a poor yield (25%).
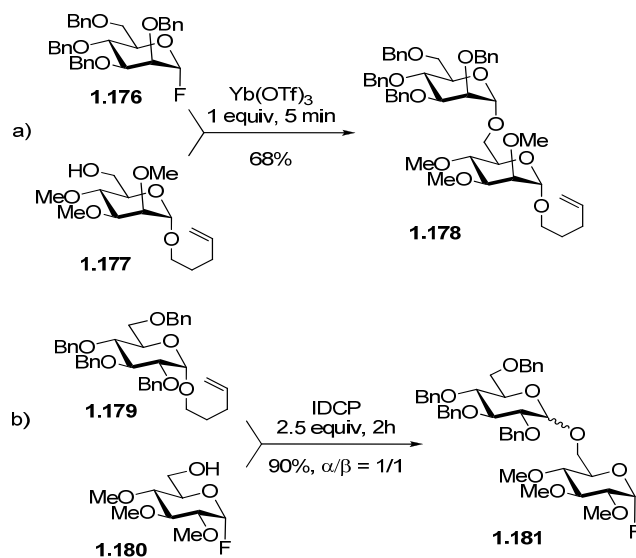
Scheme 1.34 Semi-orthogonality of *n*-pentenyl glycoside and glycosyl fluoride.

1.6   Orthogonal activation

The combination of two chemically distinct glycosylation reactions, in which one of the leaving groups is activated while the other one remains intact, and *vice versa,* has led to the discovery of the orthogonal strategy for oligosaccharide synthesis.[38] This unique, and virtually one of the most conceptually attractive techniques for expeditious oligosaccharide synthesis, requires the use of two orthogonal classes of glycosyl donors.[163,164] As with the selective activation strategy, at the first step the glycosyl donor bearing LG^a is activated over the glycosyl acceptor bearing LG^b. Uniquely to the orthogonal strategy, however, the LG^b is then activated over LG^a of the new glycosyl acceptor (Scheme 1.35). This activation sequence can then be reiterated to give straightforward access to larger oligosaccharides. Ideally, the orthogonal approach allows for an unlimited number of reiterations of the two orthogonal leaving groups LG^a and LG^b, which is conceptually very attractive. In practice, however, the efficiency of glycosidation is usually inversely correlated to the size of the glycosyl donor involved. And since this approach presumes the elongation of glycosyl donors, the yields are typically decreased dramatically at the later stage of the assembly.
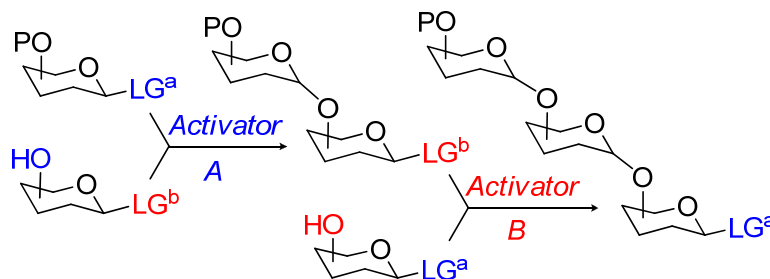
Scheme 1.35 Orthogonal strategy for oligosaccharide synthesis.

1.6.1   Fluorides and thioglycosides.

The classic variation of the orthogonal activation route introduced by Ogawa *et al.* involves building blocks bearing *S*-phenyl and fluoro leaving groups.[38] Phenyl thioglycoside donor **1.182** can be selectively activated over glycosyl fluoride acceptor **1.183** in the presence of NIS/AgOTf to afford the disaccharide **1.184** in 85% (Scheme 1.36). The fluoro leaving group of disaccharide **1.184** can then be activated over thioglycoside acceptor **1.185** in the presence $Cp_2Hf_2Cl_2$/AgOTf as a promoter system. The resulting trisaccharide **1.186** is obtained in 72% yield. This selective activation sequence can be then reiterated; to illustrate this, the trisaccharide donor **1.186** bearing *S*-phenyl was activated over the fluoride acceptor **1.183** to provide tetrasaccharide **1.187** in 66% yield.
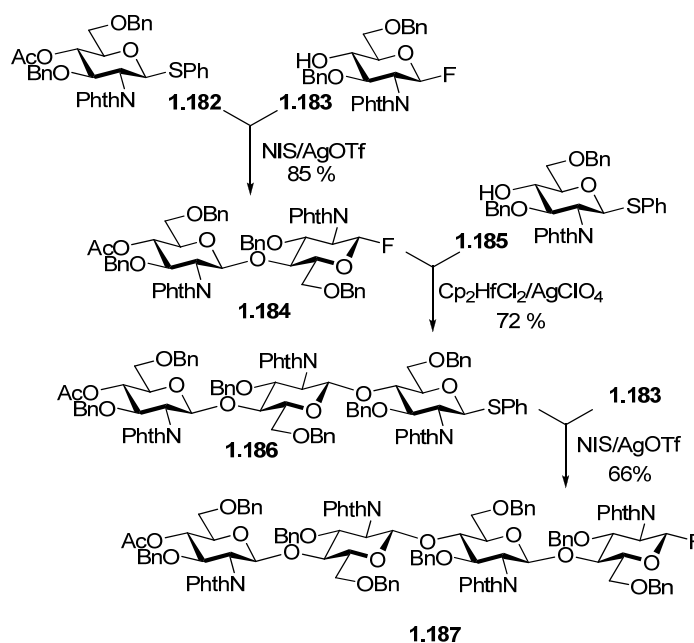
Scheme 1.36. Orthogonal activation of phenylthio glycosides and fluorides.

1.6.2   Thioglycoside and thioimidate

A combination of *S*-ethyl and *S*-thiazolinyl (STaz) leaving groups also represents a promising orthogonal pair. STaz glycosides can be activated selectively with AgOTf, whereas the ethyl thioglycoside can be activated with NIS and catalytic TfOH.[129] This finding was applied to the synthesis of a pentasaccharide derivative **1.192**, as depicted in Scheme 1.37.[165] Thioglycoside donor **1.188** was first activated over the STaz acceptor **1.115** in the presence of NIS/cat. TfOH. The resulting disaccharide **1.189** isolated in 98% yield was in turn activated over thioglycoside acceptor **1.137** with AgOTf to give trisaccharide **1.190** in 93% yield. The sequence was then reiterated as follows. Thioglycoside donor **1.190** was activated over STaz acceptor **1.115** in the presence of NIS/cat. TfOH. The resulting tetrasaccharide **1.191** was selectively activated over thioglycoside acceptor **1.137** by the addition of AgOTf to give pentasaccharide **1.192** in 59% yield.
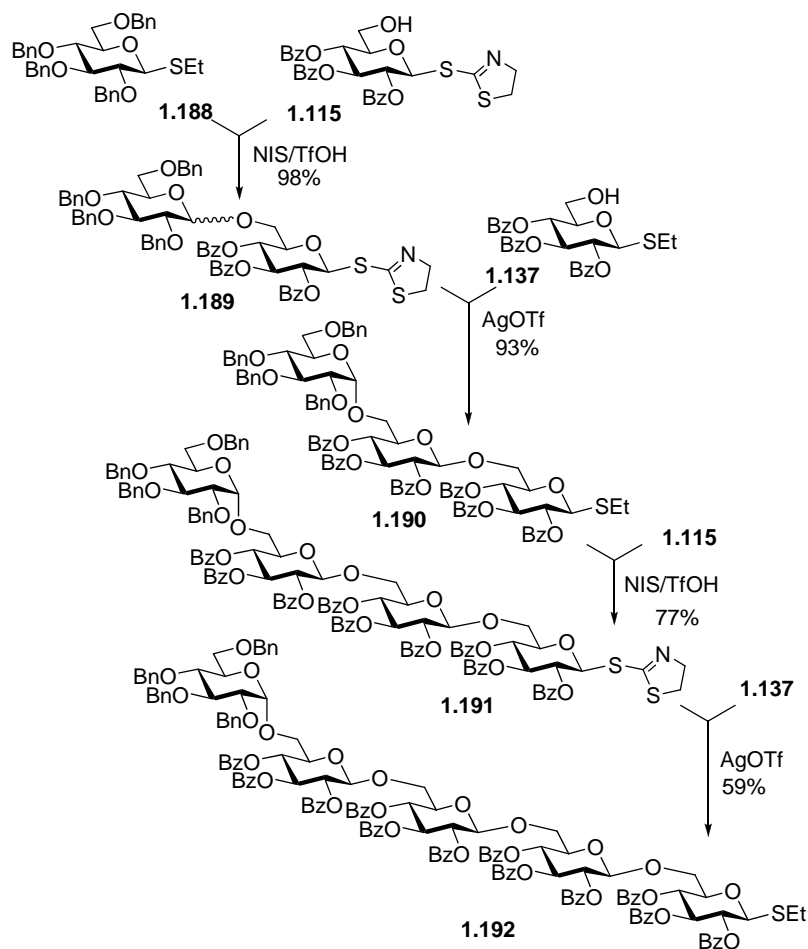
Scheme 1.37 Orthogonality of ethyl and thiazolinyl thioglycosides.

1.6.3 Thioimidate-only (SBox and STaz glycosides)

A related approach involving orthogonal activation of STaz and SBox glycosides has also recently emerged.[133] SBox glycosides can be selectively activated with Cu(OTf)$_2$[165] or Bi(OTf)$_3$[132] over the STaz leaving group. As shown in Scheme 1.38, STaz leaving group that is typically more stable than SBox towards a majority of thiophilic activation conditions, can be also selectively activated over SBox in the presence of relatively mild alkylation reagents, benzyl bromide or methyl iodide. Thorough mechanistic studies showed that the selective activation is based on the different

activation modes reactivity of these leaving groups. Thus, it was found that preferential glycosidation of a certain thioimidate is not simply determined by the strength of activating reagents; instead, the type of activation – direct (for SBox) vs. indirect (for STaz) – comes to the fore and plays the key role. Thus, activation of STaz donor **1.193** over SBox acceptor **1.168** in the presence of BnBr at 55 °C afforded disaccharide **1.194** in 76% yield. The latter could be then activated over STaz acceptor in the presence of Bi(OTf)$_3$ at 0 °C. The resulting trisaccharide **1.195** was isolated in 62% yield.
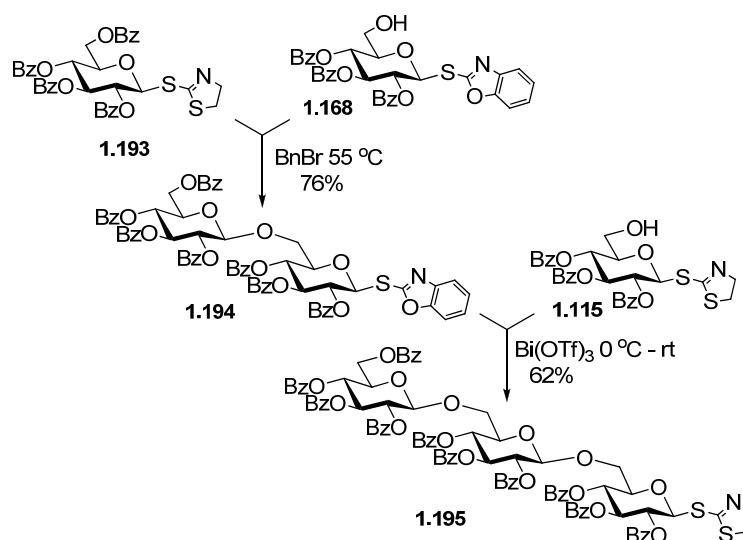
Scheme 1.38 Thioimidate-only orthogonal strategy.

1.6.4 *n*-Pentenyl and propargyl activation

Recently, Hotha *et al.* reported a new promising pair of leaving groups for orthogonal activation involving of propargyl and *n*-pentenyl glycosides (Scheme 1.39a).[111] According to this study, NIS/TMSOTf served as an efficient activator for selective activation of *n*-pentenyl glycoside **1.84** (Scheme 1.39a). In this case, either alkylated or acylated *O*-propagyl glycosyl acceptors **1.56** or **1.85**, respectively, worked

equally well. As the result of this selective activation, disaccharides **1.197** and **1.86** were isolated in 66 and 68% yield. On the other hand, propargyl glycosyl donor **1.90** can be selectively activated over propargyl glycosyl acceptor **1.91** in the presence of AuBr₃ to afford disaccharide in 65%. This activation works particularly well for benzoylated glycosyl acceptor **1.91** and as glycosylation of its benzylated counterpart **1.142** led to modest yield of the respective disaccharide **1.198**. This implies a more semi-orthogonal like activation (vide supra) rather than purely orthogonal.
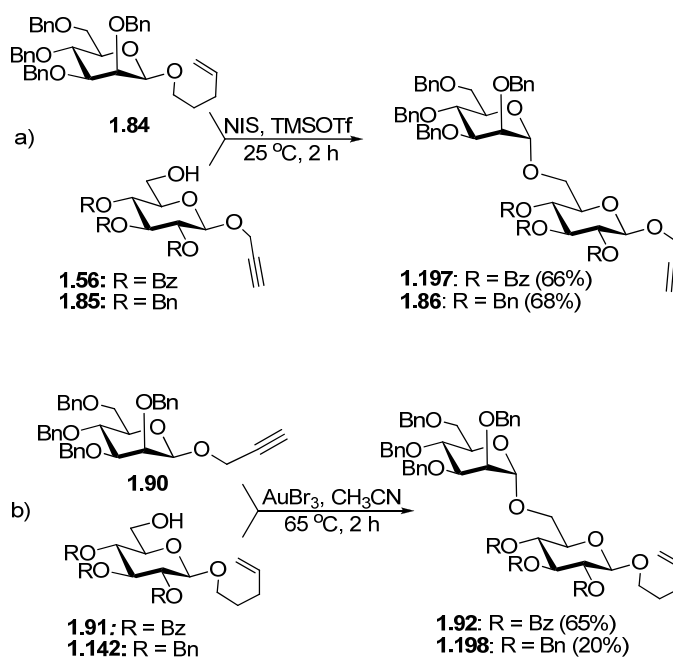
Scheme 1.39 Orthogonality of *n*-pentenyl vs. O-propargyl leaving groups.

1.7 Selective and orthogonal activations in modern high throughput technologies

1.7.1 Intramolecular glycosylations

An intramolecular aglycone delivery (IAD) approach first introduced by Barresi and Hindsgaul[166,167] and later by Stork and Kim[168,169] is the one of the most reliable methods to obtain β-mannosides. Ogawa *et al.* then reported the use of a *p*-methoxybenzyl (PMB) group at the C-2 position as the stereocontrolling tether for β-mannosylation.[170] The *p*-methoxybenzyl assisted IAD process was used as the key reaction for the construction of β-mannoside containing fragment that also involved selective activation concept (Scheme 40).[60,61] According to this approach, thioglycoside donor **1.199** was linked to fluoride acceptor **1.138** via DDQ-mediated tethering. The resulting construct **1.200** was subjected to treatment with MeOTf and DTBMP that allowed for selective activation of the *S*-methyl group over the fluoro group to afford β-mannoside **1.201**. The free hydroxyl group in **1.201** was then acetylated, and the resulting fluoride donor **1.202** was glycosylated with glycosyl acceptor **1.203**. The resulting trisaccharide **1.204** was obtained in 85% yield. In principle, the last step can also be considered selective activation, as *O*-allyl glycosides can be activated via a two-step one-pot isomerization-glycosidation sequence. A similar approach was also investigated using polymer support.[171]
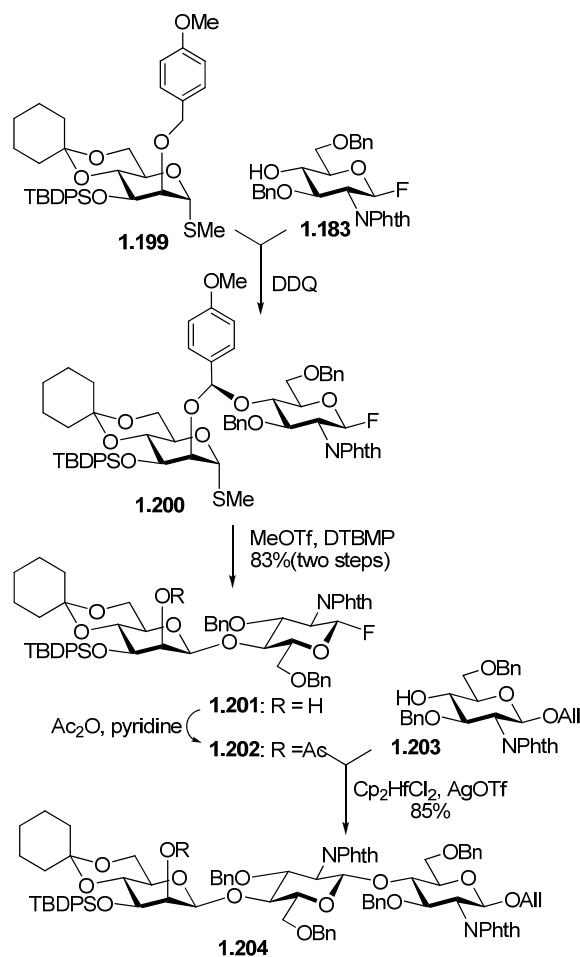
Scheme 1.40 *p*-Methoxybenzyl mediated intramolecular aglycone delivery and selective

activation concepts.

1.7.2 One-pot synthesis

Conceptually, one-pot strategies offer the shortest pathway to oligosaccharides, as the sequential glycosylation reactions are performed in a single flask (pot) and do not require purification of the intermediates. It is very important for a successful one-pot glycosylation that there is complete consumption of all building blocks and the high yield in each glycosylation step. In addition, the requirement for very high or preferably complete stereoselectivity in every glycosylation step becomes particularly important.

These will ensure that only the target oligosaccharide is being produced as the major product, thus providing high efficiency of this technique. Many variations of the one-pot strategy have been developed,[172,173] herein we focus our discussion on the approaches based on selective activation of one type of a leaving group over another. The use of selective activation strategy for the one-pot oligosaccharide sequencing is easy to envisage. As illustrated in Scheme 1.41, it requires a set of suitable leaving groups (LG^a, LG^b, LG^c) activators that would activate one leaving group over another: A (selectively activates LG^a), B (selectively activates LG^b), etc.
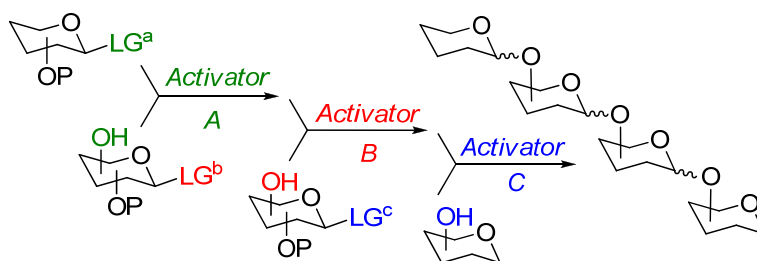
Scheme 1.41 A generic selective activation-based oligosaccharide synthesis in one pot.

The first one-pot synthesis based on the leaving group based activation concept was reported by Takahashi and co-workers.[57] As depicted in Scheme 1.42, this synthesis involved the use of trichloroacetimidate donor **1.205**, which was selectively activated over thioglycoside **1.206** with TMSOTf. The SPh moiety of the formed, but not isolated, tetrasaccharide was then glycosidated by adding glycosyl acceptor **1.207** in the presence of NIS/TfOH to provide hexasaccharide **1.208** in 50% yield over two steps. This approach incorporates advantages of the convergent block strategy according to which

pre-synthesized oligosaccharide building blocks *i.e.* **1.205** and **1.207** are converged together during the final assembly.
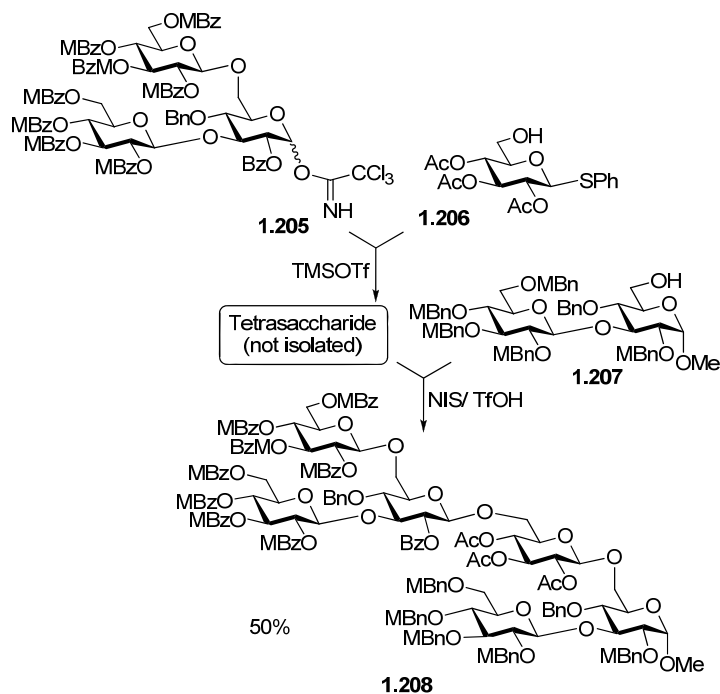
Scheme 1.42 Convergent one-pot synthesis by selective activation.

A number of related two-step one-pot syntheses have emerged, and the most representative examples are surveyed below. As depicted in Scheme 1.43a, reaction of bromide **1.209** and *S*-phenyl acceptor **1.210** was affected in the presence of silver triflate. The *S*-phenyl leaving group of the intermediate disaccharide was then glycosidated by adding glycosyl acceptor **1.211** and NIS. The resulting trisaccharide **1.212** was isolated in 84% yield over two steps.[174] In a similar fashion, a tetrasaccharide glycosyl glycerol which represents the core structure of a glycoglycerolipid of *M. taiwanesis,* based on a three-component one-pot strategy was synthesized as depicted in Scheme 1.43b.[175] A trisaccharide intermediate was formed from the glycosyl phosphite **1.213**, which was

selectively activated over selenoglycoside acceptor **1.214** in the presence of TMSOTf. Subsequent addition of acceptor **1.215** and NIS produced the target tetrasaccharide **1.216** in 46% yield over-all. Seeberger and co-workers presented an efficient one-pot synthesis of a pentasaccharide **1.220** shown in Scheme 1.43c.[176] Accordingly, disaccharide trichloroacetimidate **1.217** was activated with TMSOTf to react regioselectively at the C-4 hydroxyl group of the thioglycoside **1.218**. Subsequent addition of disaccharide acceptor **1.219**, followed by the addition of NIS/TfOH to promote the thioethyl group of the *in situ* formed trisaccharide completed the sequence. The requisite pentasaccharide **1.220** was isolated in 63% yield.
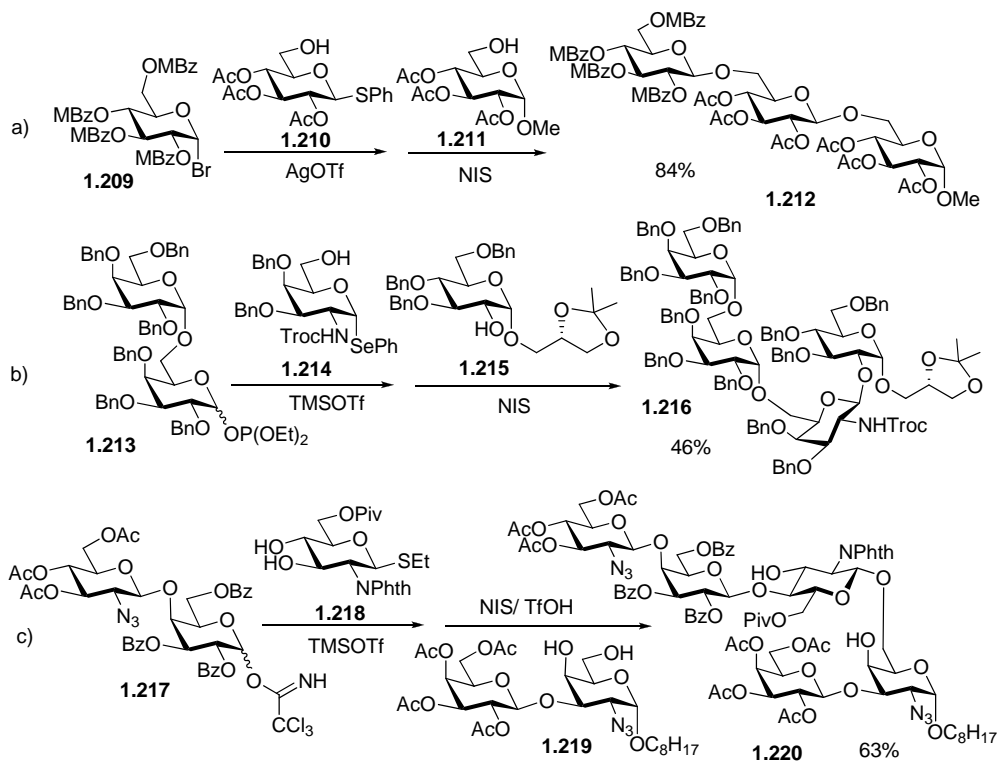
Scheme 1.43 Typical examples of two-step one-pot oligosaccharide syntheses.

A representative example involving three sequential glycosylation steps is shown in Scheme 1.44.[161] This three step activation was achieved by the stepwise activation of

S-benzoxazolyl (SBox) glycosyl donor **1.114** over *S*-ethyl glycosyl acceptor **1.82** by addition of AgOTf. Thioethyl moiety of the resulting disaccharide intermediate was then activated over the added S-thiazolinyl (STaz) acceptor **1.115** by the addition of NIS and catalytic TfOH. Finally, the STaz moiety of the trisaccharide intermediate was reacted with freshly added glycosyl acceptor **1.165** in the presence of additional quantity of AgOTf. As a result, the linear tetrasaccharide **1.221** was obtained in 73% yield over three steps.
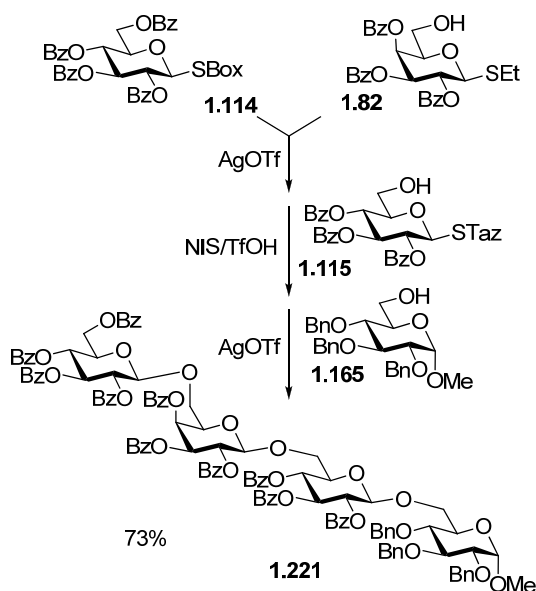
Scheme 1.44 One-pot synthesis of tetrasaccharide **1.221** by the three-step sequential selective activation.

Utilizing longer sequences of selective activations are not yet routinely available. Although a number of one-pot syntheses or large oligosaccharides has emerged, all are based on mixed convention, typically a combination of selective and chemo or regioselective sequential activations. For example, Ley's group reported the synthesis of a GPI anchor involving building blocks with well defined levels of reactivity (Scheme 1.45).[177] Thus,

armed and disarmed glycosyl fluorides (**1.222**, **1.223**), armed and disarmed selenoglycosides (**1.224, 1.225**) and thioglycoside **1.226** were all sequentially activated to provide the target linear pentamannoside **1.227** in 8% overall yield in four steps. Evidently the first and the third steps involved chemoselective activations, whereas the second and fourth steps involved selective activations of fluoride over SePh and SePh over SPh, respectively.
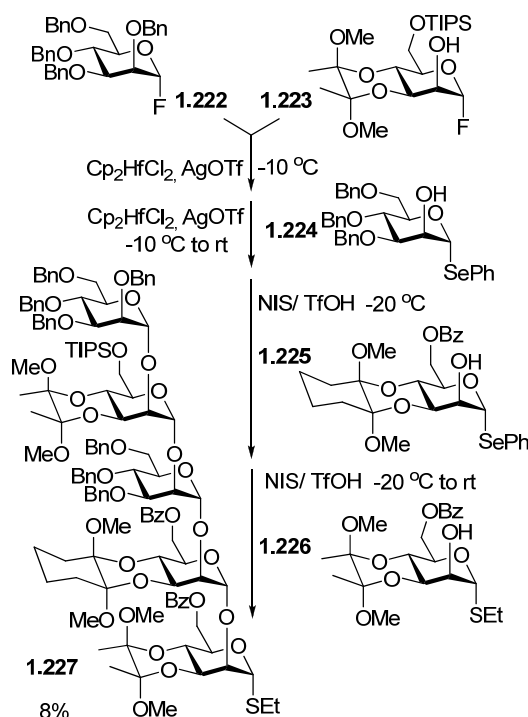
Scheme 1.45 Ley's synthesis via alternating chemoselective and selective couplings.

Mukaiyama reported an elaborate one-pot sequence that resulted in the formation of a phytoalexin elicitor heptasaccharide **1.232** in 48% yield.[178] As depicted in Scheme 1.46, the synthesis of heptasaccharide **1.232** involved a combination of sequential selective (fluoride **1.228** over thioglycoside **1.229**) and chemoselective activations (armed SEt disaccharide

intermediate over disarmed acceptor **1.230**), as well as convergent approach (4 + 3, building

block **1.231**), all in one pot.
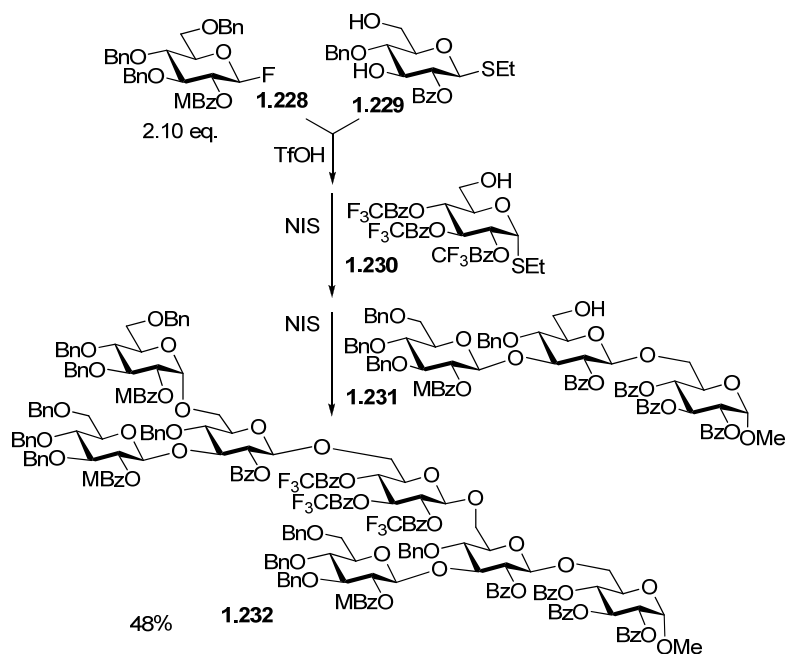
Scheme 1.46 Mukaiyama's one-pot synthesis involving the elements of orthogonal

activation.

A similar target was obtained by Takahashi in a six-step one-pot synthesis of

heptasaccharide using a Quest 210 manual synthesizer.[179] As depicted in Scheme 1.47, this

one-pot synthesis required the sequential addition of seven reaction components **1.233-1.239**

using four different leaving groups. Sequential activations employed herein included:

activation of glycosyl bromide **1.233** (activated with AgOTf over *S*-ethyl acceptor), ethyl

thioglycosides **1.234** and **1.235** (with MeOTf over fluoride acceptor **1.236**), tetrasaccharide

fluoride (with Cp$_2$HfCl$_2$/AgOTf over S-phenyl acceptor **1.237**) and phenyl thioglycosides

1.238 and hexasaccharide (with DMTST in the armed-disarmed fashion). The resulting

protected phytoalexin elicitor heptasaccharide **1.240** was isolated in 24% yield over six steps. To the best of our knowledge, this is the only selective activation-based synthesis that incorporates the elements of the orthogonal-like activations in one-pot (steps three and four).
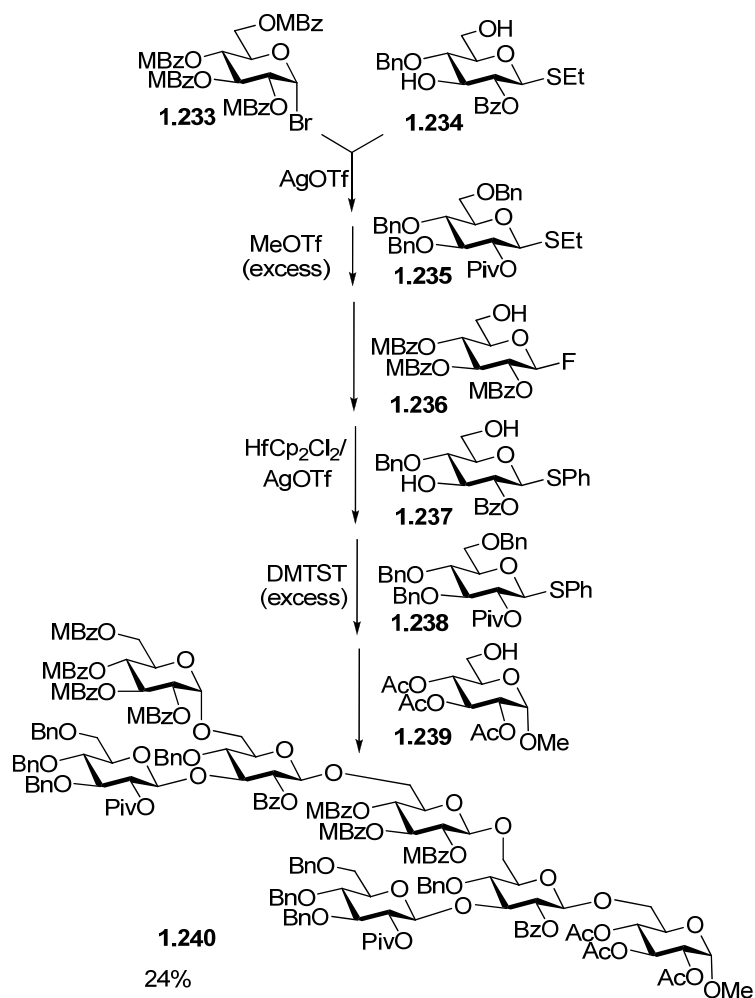
Scheme 1.47 Takahashi's synthesis using the elements of orthogonal activations.

1.7.3 Polymer-supported synthesis

Solid-phase synthesis has been developed and widely utilized in a routine preparation of oligopeptides and oligonucleotides, and is attracting renewed attention in connection with combinatorial chemistry. The use of polymer supports in oligosaccharide synthesis has also been attractive and several intriguing approaches have been reported. Most of the concepts involve glycosyl acceptor bound approach, with examples involving glycosyl acceptor bound strategies that allow for selective activations also known. For instance, Kahne *et al.* reported selective activation of sulfoxide donor **1.241** over the Merrifield resin –bound thioglycoside acceptor **1.242** in the presence of Tf$_2$O and DTBMP (Scheme 1.48).[180]   Upon completion of the sequencing that resulted in the formation of trisaccharide **1.245**, the thioglycoside linker is cleaved under mild conditions employing mercuric trifluoroacetate.
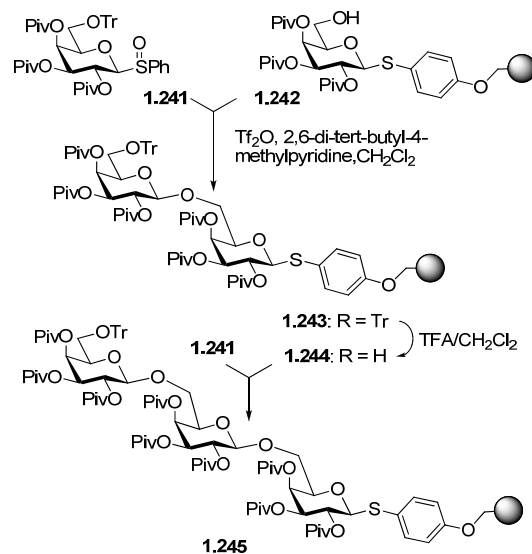
Scheme 1.48 Utilizing glycosyl sulfoxides in selective activations over thioglycoside bound to the polymer support.

An example of a donor-bound approach utilizing selective activations on solid phase synthesis is the synthesis of the Le^b blood group antigen reported by Seeberger and co-workers (Scheme 1.49).[181,182] Glycal connected to Merrifield's resin **1.246** was subjected to epoxidation with DMDO, followed by coupling with glycal acceptor **1.247** in the presence of ZnCl$_2$. Fucosyl fluoride donor **1.248** was then activated over polymer-bound glycal disaccharide **1.249** in the presence of Sn(OTf)$_2$ and DTBP. This steps incorporates the elements of a two-directional concept developed by Boons.[183,184] The resulting tetrasaccharide glycal **1.250** was then converted into the corresponding 2-amino thioglycoside **1.251**. This transformation allowed the continuation of the sequence that resulted in glycosidation of **1.251** with glycal acceptor **1.252**. The resulting pentasaccharide was then cleaved off of the polymer support to afford pentasaccharide **1.253** in 20% over-all. Selective activation of the SBox bound glycosyl donor over solution phase thioglycoside acceptor in the presence of TMSOTf was also reported.[185]
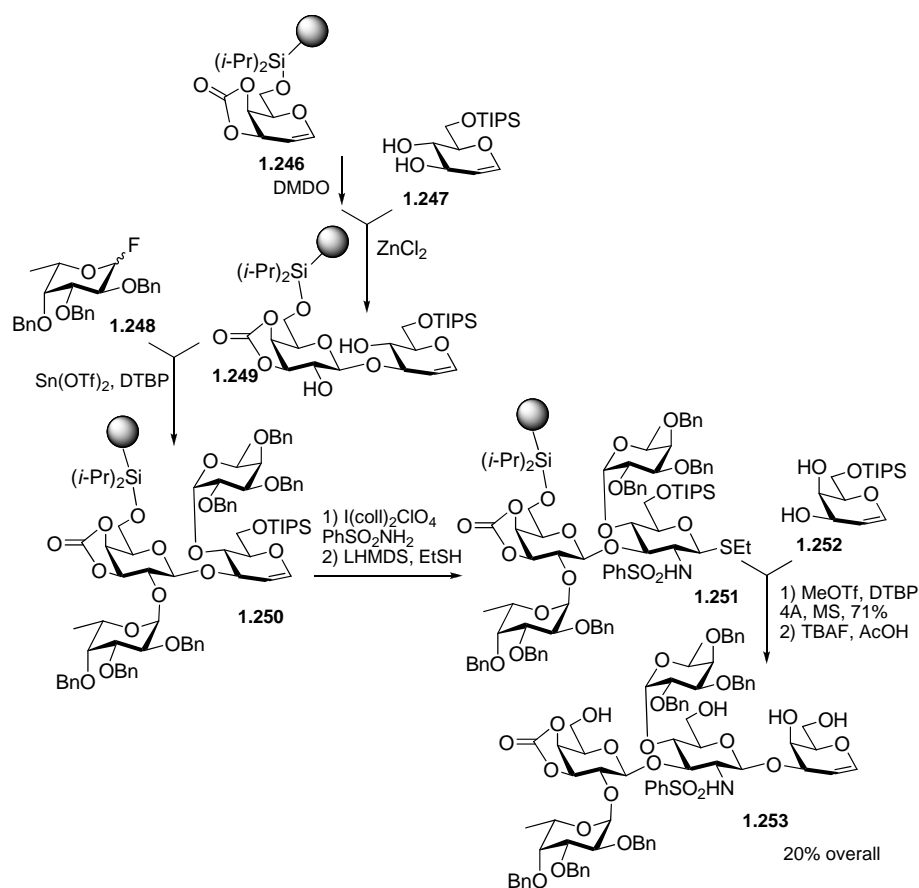
Scheme 1.49 Solid phase synthesis of Le^b blood-group antigen analog.

The application of an orthogonal strategy utilizing polymer support was also developed by Ogawa in 1996.[39] Ogawa reported a combination of chemistry on polymer supports and orthogonal glycosylation (thioglycosides and glycosyl fluorides) which resulted in the construction of oligosaccharide by minimize the number of operation. To illustrate this concept, a representative example reported by Kanie *et al.* (Scheme 1.50) is included.[186] According to this approach, polymer-bound glycosyl donor **1.254** was activated selectively over fluoride acceptor **1.15** in the presence of DMTST. The resulting disaccharide **1.255** was activated over *S*-phenyl acceptor **1.256** in the presence of

Cp$_2$Hf(OTf)$_2$. Finally, *S*-phenyl trisaccharide **1.257** was glycosidated with octanol in the presence of DMTST.
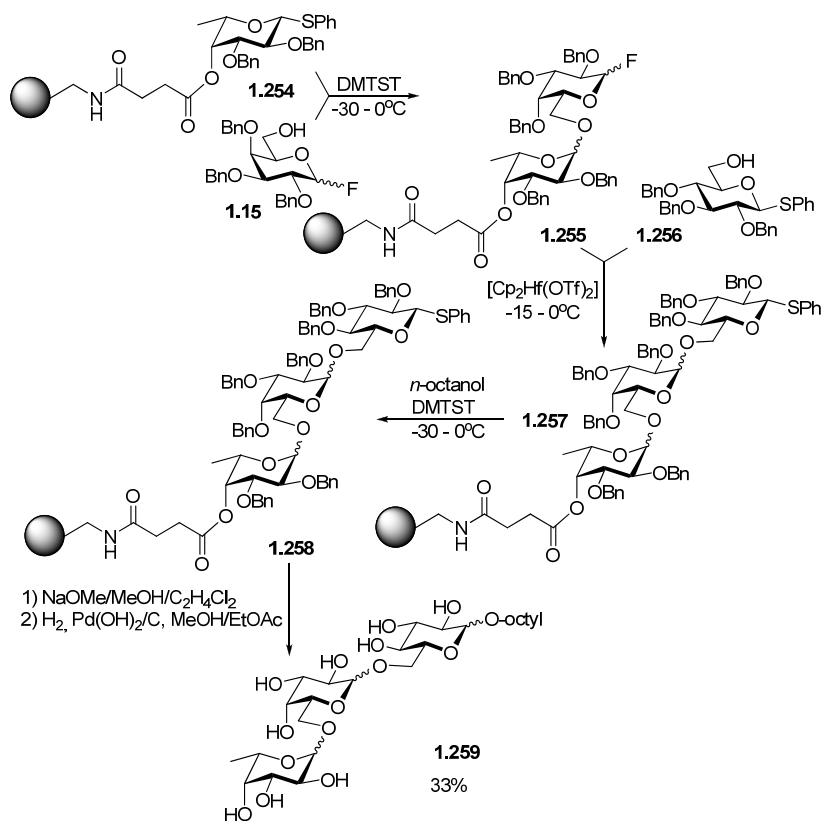
Scheme 1.50 Orthogonal glycosylation reactions on solid phase for synthesis of fucosyl galactose oligosaccharides.

1.8   Conclusions and future directions

It is critical to make complex carbohydrates more accessible to the general chemical, biochemical, and industrial audience to keep pace with the exploding area of glycobiology. This can be only achieved by the development of methods and strategies for efficient oligosaccharide synthesis that would be applicable for both laboratory and industrial preparation. A number of excellent strategies that offer a reasonably efficient route to oligosaccharide assembly have already emerged. Important discoveries surveyed

in this article have already allowed scientists to synthesize complex oligosaccharides and glycoconjugates for structural, biological, and medical studies. Nevertheless, carbohydrates of even moderate complexity still present a considerable challenge, even to experts. No expeditious strategy developed to date can offer an easy and reliable access to all full range of compounds of interest. Some strategies offer a very efficient approach to certain sequences, but in general each target still requires careful selection of methods, conditions, and strategies.

The development of efficient and general methods for the expeditious synthesis of complex carbohydrates will undoubtedly remain an important and active arena for scientific endeavours during the 21st century. In the coming years, glycoscientists are expected to have developed simple, efficient, and flexible approaches to oligosaccharide assembly that will complement existing methodologies and bring our ability to obtain complex oligosaccharides up to a significantly higher level. The recent innovations surveyed herein make contemporary glycoscientists well positioned to undertake further studies. Future developments may be expected to incorporate existing methodologies, and combinations thereof, while blending in new concepts and technologies.

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CHAPTER II

Evaluation of silver(I) tetrafluoroborate as a promoter for chemical glycosylation

Kaeothip, S.; Pornsuriyasak, P.; Demchenko, A .V. "Silver (I) tetrafluoroborate as a potent promoter for chemical glycosylation", *Tetrahedron Lett.* **2008**, 49, 1542-1545.

2.1   Introduction

With increasing demand for the synthesis of biologically important and therapeutically active oligosaccharides and glycoconjugates, efforts to expand the arsenal of glycosylation methods and techniques have emerged. In spite of significant recent advances in the areas of stereoselective glycosylation[1,2] and expeditious oligosaccharide synthesis,[3,4] the construction of complex oligosaccharides with high efficiency and complete stereoselectivity remains a difficult task. As a part of a program to develop new methods and strategies for glycochemistry, we became interested in glycosyl thioimidates, a class of glycosyl donors with the generic leaving group $SCR^1=NR^2$.[5,6] We have already reported the synthesis of *S*-benzoxazolyl (SBox)[7,8] and *S*-thiazolinyl (STaz)[9,10] glycosides and evaluated their properties in stereoselective glycosylations and expeditious oligosaccharide syntheses. Metal salt-based promoters were shown to provide efficient activation of the thioimidoyl moiety for glycosylation.[8,11] Among a variety of metal salts investigated, arguably, silver trifluoromethanesulfonate (AgOTf) was one of the best choices for the activation of the SBox and STaz moieties for a variety of synthetic applications. Beyond the scope of our own research program, AgOTf has been commonly employed as an activator for many other classes of glycosyl donors including glycosyl bromides,[12-14] chlorides,[15] trichloroacetimidates,[16] and seleno glycosides.[17] In spite of wide applicability and high versatility of AgOTf, there are some significant drawbacks that limit the usage of this promoter in synthesis. In a majority of applications, AgOTf requires fresh activation by repetitive co-evaporation with toluene followed by extended drying under vacuum directly prior to use. In addition, we noticed that the quality of commercial reagent may significantly vary depending on the supplier and the

batch. It should be noted that the use of AgOTf as an acidic additive in NIS-promoted glycosidation of thioglycosides does not require preactivation of AgOTf. Bearing in mind these drawbacks, we questioned if any other silver salts could be used to achieve an efficient glycosidation of a variety of glycosyl donors and would produce reproducible results without the requirement of the preactivation or azeotropic dehydration. Herein we present a report that demonstrates the use of silver tetrafluoroborate (AgBF$_4$) as a potent promoter for the activation of various types of glycosyl donors. It should be noted that AgBF$_4$ has already been used in glycosylations, yet these applications, including alcoholysis of glycosyl bromides,[18,19] synthesis of C-glycosides from chlorides,[20] glycosidation of 2-deoxy thioglycosides,[21] and α-stereoselective glycosidation of 1,2 anhydro sugars,[22] were scarce and scattered. In this context, Cp$_2$ZrCl$_2$–AgBF$_4$ in benzene has proven to be a powerful promoter combination for the activation of tetra-O-benzyl-D-mannosyl fluoride.[23]

2.2   Results and discussion

To generate a broad array of glycosyl donors we investigated whether thiazolinyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D glucopyranoside **2.5a**[10] could serve as a common precursor. These studies were successful and we discovered that the STaz glycoside **2.5a** can be directly converted into the following glycosyl donors: acetate **2.1**, bromide **2.2a**, chloride **2.8**, fluoride **2.9**, and hemiacetal **2.10** in excellent yields (84–95%, Scheme 2.1). Moreover, glycosyl bromide **2.2a** was then used as a precursor for the next synthetic steps to obtain benzoxazolyl, benzothiozolyl, and ethyl thioglycosides (**2.3a**, **2.4a**, and **2.6**), as well as n-pentenyl glycoside **2.7**—all with exclusive β stereoselectivity. The

hemiacetal derivative **2.10** was converted into the corresponding glycosyl trichloroacetimidate **2.11** as shown in Scheme 2.1.
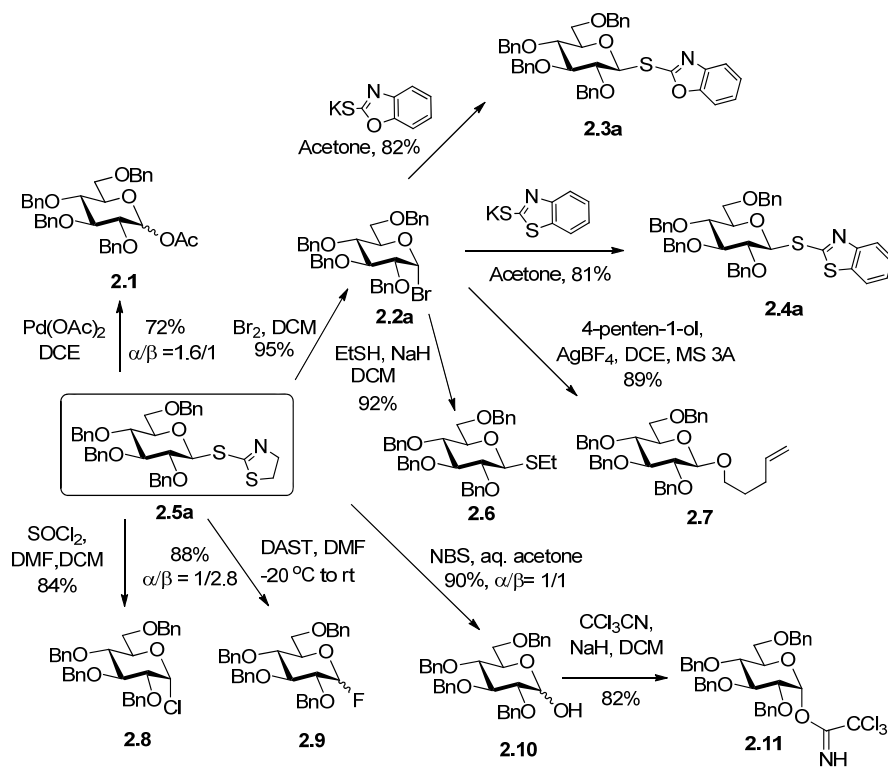
Scheme 2.1 Conversion of the a common STaz glycoside **2.5a** into a range of glycosyl donors

Synthesis of a range of differently protected glycosyl thioimidates has been similarly accessed and is depicted in Scheme 2.2. Yields given are over-all yields for two synthetic steps.
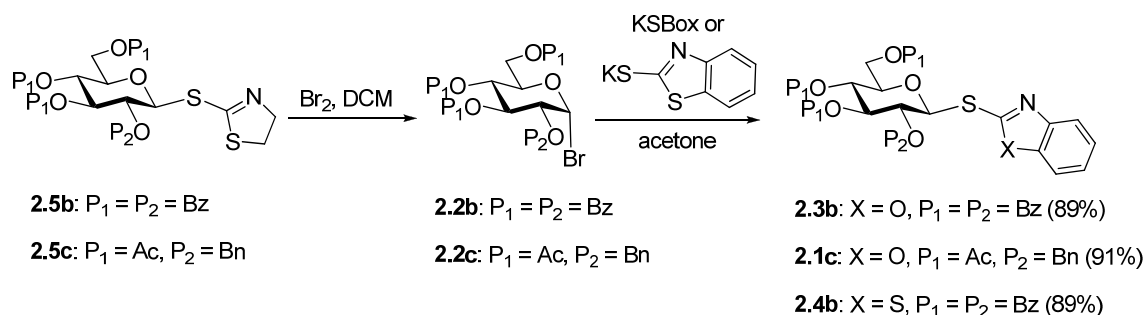
Scheme 2.2 Conversion of the STaz glycosides **2.5b,c** into thioimidates **2.3b,c** and

2.4b

Having synthesized a library of glycosyl donors, we performed a number of test glycosylation reactions using AgBF$_4$ as a promoter (Table 2.1). Since we wanted to determine whether the glycosylations could be performed without pre-activation of AgBF$_4$, all reactions were performed with commercial reagent as received, without further conditioning.

Glycosidation of glycosyl acetate **2.1** with glycosyl acceptor **2.13** in the presence of AgBF$_4$ did not proceed (entry 3). A similar coupling in the presence of BF$_3$–Et$_2$O, a conventional promoter for the activation of glycosyl acetates,[24] gave disaccharide **2.17a** (Fig. 2.1) in 91% yield, although it required 16 h for the reaction to complete. We determined that the reaction time of this reaction could be significantly reduced (16 h vs 5 h, entries 4 and 5) by adding 20–50 mol % of AgBF$_4$. In these couplings, even higher yield of **2.17a** (95%) and an improved stereoselectivity were detected.

Glycosidation of glycosyl bromides **2.2** and **2.2** could be efficiently accomplished with AgBF$_4$, although the coupling of per-benzoylated bromide **2.2**, prepared as shown in Scheme 2.2, with the sterically hindered acceptor **2.14** required prolonged reaction time

(entries 15–16). These results were comparable with the glycosylations using traditional bromide activators-silver carbonate,[25,26] or mercury salts.[27,28]

Table 2.1 AgBF$_4$ promoted glycosylations of glycosyl acceptor **2.12-2.15**
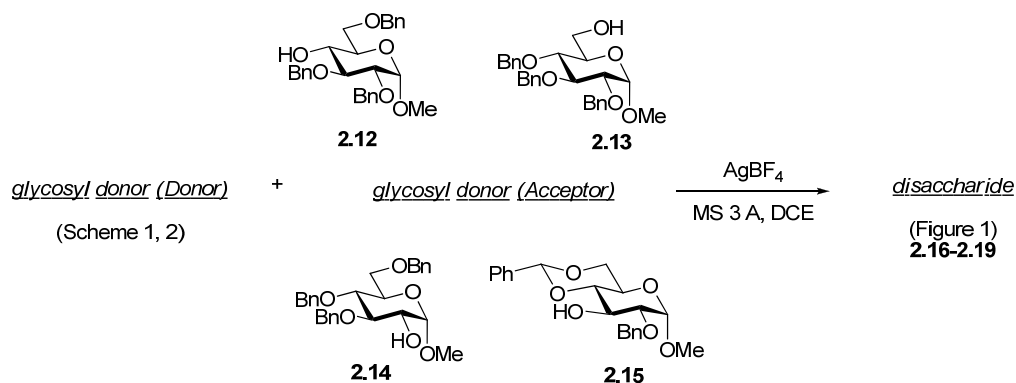
Entry	Donor	Acceptor	Time	Product	Yield (%)	α/β Ratio
1	**2.1**	**2.13**	24 h	**2.17a**	-[a]	-
2[b]	**2.1**	**2.13**	16 h	**2.17a**	91	1.4/1
3[c]	**2.1**	**2.13**	5 h	**2.17a**	95	2.0/1
4	**2.2a**	**2.13**	5 min	**2.17a**	92	2.4/1
5	**2.2b**	**2.13**	5 min	**2.17b**	96	β only
6	**2.2b**	**2.14**	24 h	**2.18b**	82	β only
7	**2.3a**	**2.13**	10 min	**2.17a**	89	1.2/1
8	**2.3b**	**2.13**	10 min	**2.17b**	91	β only
9	**2.3b**	**2.14**	15 min	**2.18b**	84	β only
10	**2.3c**	**2.13**	15 min	**2.17c**	91	1.6/1
11	**2.4a**	**2.15**	5 min	**2.19a**	87	7.4/1
12	**2.4b**	**2.13**	20 min	**2.17b**	90	β only

13	**2.5a**	**2.13**	5 min	**2.17a**	95	1.3/1
14	**2.5a**	**2.14**	5 min	**2.18a**	92	1.4/1
15	**2.5b**	**2.13**	10 min	**2.17b**	90	β only
16	**2.5c**	**2.13**	20 min	**2.17c**	75	1.2/1
17	**2.6**	**2.13**	24 h	**2.17a**	-[a]	-
18[d]	**2.6**	**2.13**	24 h	**2.17a**	91	1.2/1
19[e]	**2.6**	**2.13**	10 min	**2.17a**	92	1.2/1
20	**2.7**	**2.13**	24 h	**2.17a**	-[a]	-
21[d]	**2.7**	**2.13**	24 h	**2.17a**	89	1.0/1
22[e]	**2.7**	**2.13**	20 min	**2.17a**	76	1.2/1
23	**2.8**	**2.13**	5 min	**2.17a**	97	1.6/1
24	**2.8**	**2.12**	10 min	**2.16a**	91	1/3.4
25	**2.9**	**2.13**	20 h	**2.17a**	81	2.0/1
26	**2.9**	**2.15**	1.5 h	**2.19a**	81	2.3/1
27	**2.10**	**2.13**	24 h	**2.17a**	-[a]	-
28	**2.11**	**2.13**	5 min	**2.17a**	80	1/1.5
29	**2.11**	**2.12**	2 days	**2.16a**	65	1/5.4

[a] No product formation was detected; all glycosylations were promoted with $AgBF_4$ except the following.
[b] -3.0 equiv of $BF_3–Et_2O$ were used.
[c] -3.0 equiv of $BF_3–Et_2O$ and 0.5 equiv of $AgBF_4$ were used.
[d] -2.0 equiv of NIS was used.
[e] -2.0 equiv of NIS and 0.5 equiv of $AgBF_4$ were used.
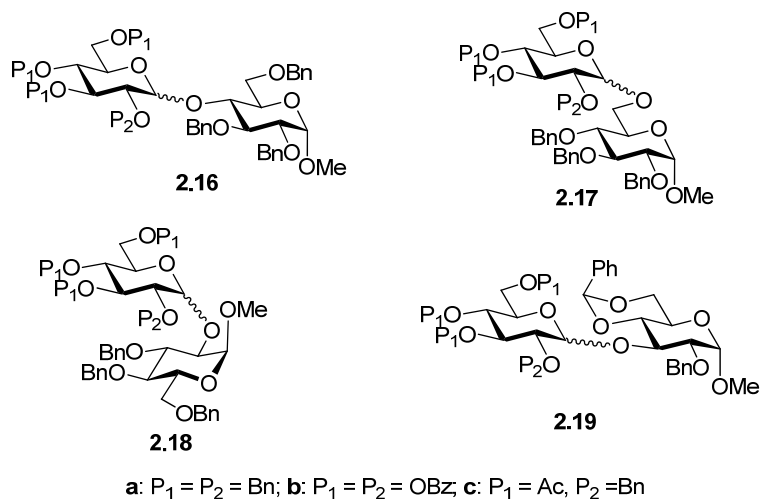
Figure 2.1 Structure of disaccharide **2.16-2.19**

Glycosidation of glycosyl thioimidates **2.3-2.5** was smoothly driven to completion in a matter of minutes (entries 7–16). All glycosylations proceeded with very high conversion yields, comparable or even exceeding those achieved in glycosylations promoted with preactivated AgOTf. For example, AgBF$_4$-promoted couplings depicted in entries 14 and 16 (Table 2.1) proceeded in 92% (90% with AgOTf) and 75% (70% with AgOTf) yield, respectively.[9] While the anchimerically assisted couplings with benzoylated glycosyl donors were β stereoselective, only fair α-stereoselectivity was achieved with 2-*O*-benzylated glycosyl donors. All couplings were performed in the neutral solvent since the stereoselectivity optimization was not a primary intention of these studies.

Promoters for thioglycoside and *n*-pentenyl glycoside activation include thiophilic reagents, among which NIS/TfOH or NIS/TMSOTf are arguably the most common.[29,30] Therefore, not surprisingly, the activation of stable glycosyl donors, thioglycoside **2.6** or *n*-pentenyl glycoside **2.7,** did not take place in the presence of AgBF$_4$. It should be noted

that NIS alone can also activate both *S*-ethyl and *n*-pentenyl glycosides, however, these transformations require prolonged reaction time (entries 18 and 21). Having decided to investigate whether these glycosyl donors can be activated by NIS/AgBF$_4$ system, we discovered that these coupling reactions proceeded very rapidly and provided the corresponding disaccharide in minutes (entries 19 and 22). We then demonstrated that AgBF$_4$ could be employed as a suitable promoter for glycosidation of chloride **2.8**, fluoride **2.9**, or trichloroacetimidate **2.11**, whereas the hemiacetal derivative **2.10** remained inert under these reaction conditions (entry 23-29).

Based on the results summarized in Table 2.1, we anticipated that it might be possible to activate glycosyl halides, *S*-benzothiazolyl, SBox, or STaz glycosides over the SEt or *n*-pentenyl moieties in the presence of AgBF$_4$. We have already reported that selective activation of SBox and STaz glycosides over SEt glycosyl acceptor can be achieved in the presence of AgOTf.[7,31] Also in this case, AgBF$_4$ was found to be capable of providing smooth couplings with consistently high yields (Table 2.2). We observed that glycosidation of glycosyl fluoride **2.9** was sluggish and the moderate to good yields presented in Table 2.2 are based on unreacted acceptor recovery (entries 1 and 5).

Table 2.2 Selective activation of various glycosyl donors over *n*-pentenyl acceptor **2.20**

and SEt acceptor **2.22**
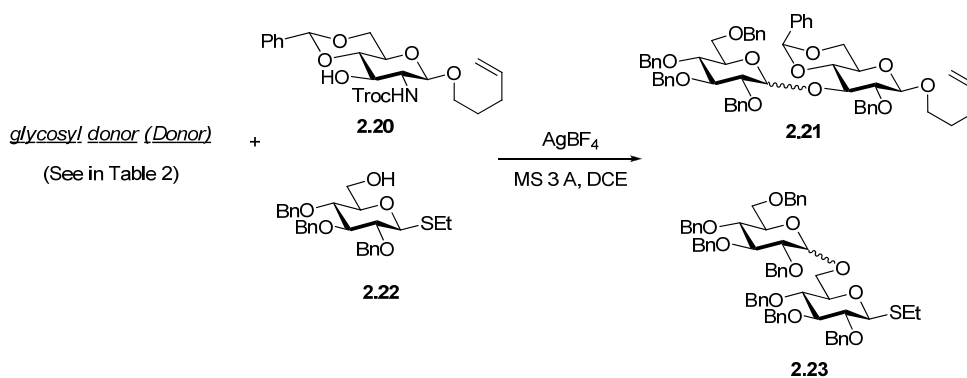
Entry	Donor	Acceptor	Time	Product	Yield (%)	α/β Ratio
1	**2.3a**	**2.20**	10 min	**2.21**	86	1.1/1
2	**2.4a**	**2.20**	20 min	**2.21**	85	3.3/1
3	**2.5a**	**2.20**	10 min	**2.21**	83	1/1.2
4	**2.9**	**2.20**	16 h	**2.21**	65a	1.6/1
5	**2.2a**	**2.22**	15 min	**2.23**	84	1.6/1
6	**2.3a**	**2.22**	20 min	**2.23**	87	2.01
7	**2.5a**	**2.22**	15 min	**2.23**	89	2.41
8	**2.8**	**2.22**	15 min	**2.23**	84	2.2/1
9	**2.9**	**2.22**	24 h	**2.23**	83a	2.41

ª This yield is based on the recovery acceptor.

Based on the results of the selective activations, we assumed that it could be possible to perform the activation sequence in a highly efficient one-pot fashion. Presumably, any building block that can be activated with AgBF$_4$ can be used as the glycosyl donor for the first activation step. We chose to investigate SBox and STaz

glycosides as well as glycosyl bromides as glycosyl donors. Glycosyl acceptors should withstand AgBF$_4$ activation, but in turn could be readily activated by addition of NIS. Either *S*-ethyl or *n*-pentenyl leaving groups could be used for this purpose. An example shown in Scheme 2.3 makes use of the STaz leaving group in **2.5a** for the first coupling step. At this stage the S-ethyl moiety of the glycosyl acceptor **2.24** remains inert. Upon the formation of the intermediate disaccharide, its SEt moiety can then be activated for the reaction with the newly added acceptor **2.13** by adding 2.0 equiv of NIS. As demonstrated above, NIS along with AgBF$_4$ (used in excess and is remaining from the previous step) serves as an efficient promoter for this type of glycosylation. As a result of this two-step activation, trisaccharide **2.25** was isolated in 72% yield. Other leaving group combinations mentioned above were also found to be capable of the one-pot activations and provided the corresponding trisaccharides in 55–70% overall yields.
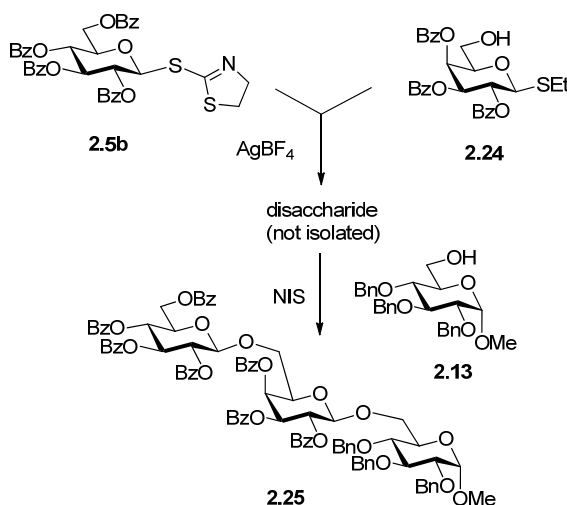
Scheme 2.3 One-pot glycosylation: synthesis of trisacharide **2.25**

2.3 Conclusion

In summary, we have identified silver tetrafluoroborate as an excellent promoter for the activation of various glycosyl donors, prepared from the common precursor bearing the STaz anomeric moiety. Easy handling and no requirement for azeotropic dehydration prior to the application makes $AgBF_4$ especially beneficial in comparison to the commonly used AgOTf. We also demonstrated that selective activation of glycosyl halides or thioimidates over thioglycosides or n-pentenyl glycosides could lead to simple one-pot syntheses via sequential selective activation of one leaving group over another. Therefore, we believe that $AgBF_4$ should be considered as a powerful and convenient alternative for silver(I) activated glycosylations.

2.4 Experiment part

2.4.1 General

Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. Dichloromethane and 1,2-dichloroethane were distilled from CaH_2 directly prior to application. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3Å). Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application.

AgBF$_4$ (Acros) was used as received. Optical rotations were measured at 'Jasco P-1020' polarimeter. ^{1}H-NMR spectra were recorded at 300 or 500 MHz, ^{13}C-NMR spectra were recorded at 75 or 125 MHz. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

2.4.2 Transformation of the STaz moiety into other leaving groups: synthesis of glycosyl donors

1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (2.1)

Pd(OAc)$_2$ (35 mg, 0.156 mmol) was added to a mixture of 2-thiazolinyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (**2.5a**, 50 mg, 0.078 mmol) and activated molecular sieves 3Å (150 mg) in dry (ClCH$_2$)$_2$ (0.5 mL). The reaction mixture was stirred under argon for 24 h at 55 °C. Upon completion, the solid was filtered-off and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane gradient elution) to afford 1-*O*-acetate **2.1** as a colorless syrup in 72% yield (α/β = 1.6/1). Analytical data for **2.1** were essentially the same as reported previously.[32]

2-Benzoxazolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2.3a)

A mixture of **2.4a** (50 mg, 0.078 mmol) and activated molecular sieves 3Å (40 mg) in dry CH$_2$Cl$_2$ (1.2 mL) was stirred under argon for 1 h. Freshly prepared solution of Br2 in dry CH$_2$Cl$_2$ (0.75 mL, 1/165, v/v) was then added dropwise over the period of 5 min at rt. Quantitative TLC estimates were made based on the accumulation of 2,3,4,6-tetra-*O*-

benzyl-α-D-glucopyranosyl bromide **2.2a**. After that, the solvent was evaporated under reduced pressure at rt. The residue containing crude **2.2a** was then treated dissolved in dry acetone (0.5 mL) and the potassium salt, KSBox, (22 mg, 0.117 mmol) and 18-crown-6 (4 mg, 0.012 mmol) were added. The reaction mixture was stirred under argon for 1 h at rt. After that, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL), 1% aq. NaOH (10 mL) and water (3 x 10 mL), the organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane gradient elution) to afford the title compound **2.3a** as a white foam in 82% yield. Analytical data for **2.3a** were essentially the same as reported previously.[8]

2-Benzothiazolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2.4a).

Crude glycosyl bromide **2.2a** (~48 mg, 0.078 mmol), obtained as described for the synthesis of **2.3a**, was dissolved in dry acetone (0.5 mL) and potassium salt of 2-mercaptobenzotriazole (24 mg, 0.117 mmol) and 18-crown-6 (4 mg, 0.012 mmol) were added. The reaction mixture was stirred under argon for 1 h at rt, then diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL), 1% aq. NaOH (10 mL), and water (3 x 10 mL). The organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane gradient elution) to afford the title compound **2.4a** as a white foam in 75% yield. Analytical data for **2.4a** were essentially the same as reported previously.[33]

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2.6)

A mixture of ethanethiol (17 μL, 0.225 mmol) and sodium hydride (1.0 mg, 0.025 mmol) was added to a solution of **2.2a** (~95 mg, 0.15 mmol), prepared as described for the synthesis of **2.3a**, in dry CH_2Cl_2 (1.0 mL) at 0 °C and the resulting mixture was stirred under argon for 1 h at rt. The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL), 20% aq. $NaHCO_3$ (10 mL), and water (3 x 10 mL), the organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl-acetate/hexane gradient elution) to afford the title thioglycoside **2.6** as a white foam in 92% yield. Analytical data for **2.6** were essentially the same as reported previously.[34]

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride (2.8)

To a stirred solution of **2.5a** (100 mg, 0.15 mmol) and dimethylformamide (6 μL, 0.075 mmol) in dry CH_2Cl_2 (1.2 mL) under argon, thionyl chloride (34 μL, 0.45 mmol) was added dropwise. The reaction mixture was kept for 1 h and then concentrated under the reduced pressure. The residue was purified by short-path silica gel column chromatography (ethyl acetate/hexane gradient elution) to afford the title compound **2.8** as a colorless syrup in 84% yield. Analytical data for **2.8** were essentially the same as reported previously.[35]

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranosyl fluoride (2.9)

To a stirred solution of **2.5a** (100 mg, 0.15 mmol) in THF (1.2 mL) diethylaminosulfur trifluoride (DAST, 72 μL, 0.55 mmol) was added at -30 °C under argon. The external

cooling was then removed and the reaction mixture was stirred for 24 h at rt. After that, the solution was cooled to -30 °C and methanol (0.1 mL) was added, the resulting mixture was warmed to rt and the volatiles were evaporated off *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and washed successively with water (10 mL), 20% aq. $NaHCO_3$ (10 mL), and water (3 x 10 mL). The organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by short-path silica gel column chromatography (ethyl acetate/hexane gradient elution) to afford the title fluoride **2.9** as a white foam in 88% yield (α/β = 1/2.8). Analytical data for **2.9** were essentially the same as reported previously.[36,37]

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (2.10)

A solution of **2.5a** (65 mg, 0.10 mmol) and N-bromosuccinimide (NBS) (45 mg, 0.20 mmol) in acetone/water (1.5 mL, 9/1, v/v) was stirred for 16 h at rt. The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL), 20% aq. $NaHCO_3$ (10 mL), and water (3 x 10 mL). The organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane gradient elution) to afford the title hemiacetal **2.10** as a colorless syrup in 90% yield (α/β = 1/1). Analytical data for **2.10** were essentially the same as for the commercial sample.

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate (2.11)

NaH (8 mg, 0.37 mmol) was added portionwise to a stirred solution of compound **2.10** (100 mg, 0.185 mmol) and trichloroacetonitrile (85 μL, 0.84 mmol) in dry CH_2Cl_2 (2

mL). After stirring for 3 h at rt, the reaction mixture was concentrated under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane gradient elution) to afford the title compound **2.11** as a colorless syrup in 82% yield. Analytical data for **2.11** were essentially the same as reported previously.[38]

2.4.3 General Glycosylation Procedures: Synthesis of *O*-Glycosides and Oligosaccharides.

Method A – Activation with AgBF₄.

A mixture containing the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(Cl_2CH_2)_2$ (2mL) was stirred under argon for 1.5 h. $BF_3.Et_2O$ (40 μL, 0.3 mmol) was added and the reaction mixture was stirred for 16 h at rt. It was then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford the disaccharide derivative.

Method B – Activation of glycosyl donor 2.1 with $BF_3.Et_2O$

A mixture containing the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(Cl_2CH_2)_2$ (2mL) was stirred under argon for 1.5 h. $BF_3.Et_2O$ (40 μL, 0.3 mmol) was added and the reaction mixture

was stirred for 16 h at rt. It was then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford the disaccharide derivative.

Method C- Activation of 2.1 with BF₃.Et₂O/AgBF₄. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(Cl_2CH_2)_2$ (2 mL) was stirred under argon for 1.5 h. $BF_3.Et_2O$ (40 μL, 0.33 mmol) and $AgBF_4$ (10 mg, 0.05 mmol) were added and the reaction mixture was stirred for 5 h at rt. Upon completion, it was diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford the disaccharide derivative.

Method D - Activation of 2.6 and 2.7 with NIS. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(Cl_2CH_2)_2$ (2 mL) was stirred for 1.5 h under argon. NIS (50 mg, 0.22 mmol) was then added and the reaction mixture was stirred for 24 h at rt. Upon completion, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL)

was washed with 20% aq. Na$_2$S$_2$O$_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane gradient elution) to afford the disaccharide derivative.

Method E - Activation of 2.7 and 2.8 with NIS/AgBF4. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in (Cl$_2$CH$_2$)$_2$ (2 mL) was stirred for 1.5 h under argon. NIS (50 mg, 0.22 mmol) and AgBF$_4$ (10 mg, 0.05 mmol) were added and the reaction mixture was stirred for 10-20 min at rt. Upon completion, the solid was filtered-off and the residue was washed with CH$_2$Cl$_2$. The combined filtrate (30 mL) was washed with 20% aq. Na$_2$S$_2$O$_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane gradient elution) to afford the disaccharide derivative.

Pent-4-en-1-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (2.7)

The title compound was synthesized from glycosyl bromide **2.2a**, obtained as described for the synthesis of **2.3a**, and 4-penten-1-ol by *Method A* in 89% yield as a white foam. Analytical data for **2.7** were essentially the same as reported previously.[39]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (2.16a)

The title compound was obtained by Method A from **2.1** and **2.12**[40] in 65% yield (α/β = 1/5.4) or **2.6** and **2.12** in 91% yield (α/β = 1/3.4). Analytical data for **2.16a** were essentially the same as reported previously.[41]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17a).

The title compound was obtained by *Method A* from glycosyl donors **2.2a**, **2.3a**, **2.5a**, **2.8**, **2.9** or **2.11** and glycosyl acceptor **2.13**[42] in 92% (α/β 2.4:1), 89% (α/β 1.2:1), 95% (α/β 1.3:1), 97% (α/β 1.6:1), 81% (α/β 2:1) and 80% (α/β 1.5:1) yield, respectively. (see Table 2.1). In addition, the title compound was obtained by *Method B* from **2.2** and **2.13** in 91% yield (α/β = 1.4/1), by *Method C* - from **2.1** and **2.13** in 95% yield (α/β = 2/1), by *Method D* obtained from **2.6** or **2.7** and **2.13** in 91% (α/β 1.2:1) and 89% (α/β 1:1) and by *Method E* obtained from **2.6** or **2.7** and **2.13** in 92% (α/β 1.2:1) and 76% (α/β 1.2:1). Analytical data for **2.17a** were essentially the same as reported previously.[43]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.17b): This compound was obtained by *Method A* from glycosyl donors **2.2b**, **2.3b**, **2.4b** or **2.5b** and glycosyl acceptor **2.13** in 96%, 91%, 90% and 90% yield, respectively. Analytical data for **17b** were essentially the same as reported previously.[44]

Methyl 6-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α -D-glucopyranoside (17c)

This compound was obtained by *Method A* from glycosyl donors **2.3c** or **2.5c** and glycosyl acceptor **2.13** in n 91% (α/β 1.6:1) and 75% (α/β 1.2:1), respectively. Analytical data for **2.17c** were essentially the same as reported previously.[10]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α -D-glucopyranoside (2.18a)

The title compound was obtained by *Method A* from glycosyl donor **2.5a** and glycosyl acceptor **2.14** in 92% yield (α/β = 1.4/1). Analytical data for **2.18a** were essentially the same as reported previously.[10]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α -D-glucopyranoside (2.18b)

This compound was obtained by *Method A* from glycosyl donor **2.2b** and glycosyl acceptor **2.14** in 82% yield. Analytical data for **2.18b** were essentially the same as reported previously.[10]

Methyl 2-*O*-benzyl-3-*O*-(2,3,4,6-tetra-O-benzyl-D-glucopyranosyl)-4,6-*O* - benzylidene-α-D-glucopyranoside (2.19a). This compound was obtained by *Method A* from glycosyl donors **2.4a** or **2.9** and glycosyl acceptor **2.15**[45] in 87 % (α/β 7.4:1) and 81% (α/β 2.3:1), respectively. Analytical data for **2.19a** were essentially the same as reported previously.[46]

Pent-4-en-1-yl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbamoyl)−β−D-galactopyranoside (2.21)

This compound was obtained by *Method A* from glycosyl donors **2.2a**, **2.4**, **2.5** or **2.9** and glycosyl acceptor **20**[47] in 86% (α/β 1.1:1), 85% (α/β 1.3:1), 83% (α/β 1.2:1) and 65% (α/β 1.6:1) yield, respectively. Analytical data for **2.21** were essentially the same as reported previously.[10]

Ethyl 6-*O*-(2,3,4,6-tetra-O-benzyl-D-glycopyranosyl)-2,3,4-tri-O-benzyl-1-thio−β-D-glucopyranoside (23)

This compound was obtained by *Method A* from glycosyl donors **2.2a**, **2.4a**, **2.5a**, **2.8** or **2.9** and glycosyl acceptor **2.22**[48] in 84% (α/β 1.6:1), 87% (α/β 2:1), 89% (α/β 2.4:1), 84% (α/β 2.2:1) and 83 (α/β 2.4:1) yield, respectively. (see Table 2.2). Analytical data for **2.23** were essentially the same as reported previously.[17]

Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.25)

This compound was obtained by one-pot glycosylation procedure in 55-70% overall yield (**22.b** 62%, **2.3b** 70%, **2.4b** 55%, and **2.5b** 72%). $[\alpha]_D^{24.8} = + 56.74$ ($c = 1$, CHCl$_3$); $R_f =$ 0.47 (ethyl acetate/hexane, 4.5/5.5, v/v); ^{1}H NMR: δ = 3.24 (s, 3H; OCH$_3$), 3.33 (m, 2H, H-2, 4), 3.51 (m, 2H, H-6a,5), 3.85 (dd, 1H, $J_{3,4} = $ 9.0 Hz, H-3), 3.87 (m, 1H, H-6a"), 3.93 (dd, 1H, H-6a), 4.01-4.10 (m, 3H, H-5',5",6b"), 4.32 (s, 1H; C*H*$_2$Ph), 4.33 (dd, 1H, H-6a'), 4.47 (d, 1H; C*H*$_2$Ph), 4.50 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 4.51 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 4.55 (d, 1H; C*H*$_2$Ph), 4.55 (dd, 1H, H-6b'), 4.69 (dd, 2H; C*H*$_2$Ph), 4.88 (d, 1H,

C*H*$_2$Ph), 4.91 (d, 1H, $J_{1",2"}$ = 7.8 Hz, H-1"), 5.52 (dd, 1H, $J_{2",3"}$ = 3.5 Hz, H-2"), 5.54 (dd, 1H, $J_{3',4'}$ = 7.8 Hz, H-3'), 5.65 (dd, 1H, $J_{4",5"}$ = 9.8 Hz, H-4"), 5.75 (dd, 1H, $J_{2',3'}$ = 8.0 Hz, H-2'), 5.84 (d, 1H, $J_{4',5'}$ = 2.5 Hz, H-4'), 5.86 (dd, 1H, $J_{3",4"}$ = 10.4 Hz, H-3"), 7.09-8.05 (m, 50H, aromatic); ^{13}C NMR: δ = 55.4, 63.0, 68.0, 68.4, 68.9, 69.6, 69.7, 70.0, 71.8, 72.1, 72.5, 73.0, 73.6, 74.7, 75.6, 77.4, 80.0, 80.1, 98.2, 101.3, 101.8, 127.7 (×2), 127.8, 128.0 (×2), 128.1, 128.2, 128.3, 128.3, 128.4 (×3), 128.5 (×2), 128.6 (×5), 128.6 (×6), 128.7 (×2), 128.7 (×2), 128.9, 129.0, 129.0, 129.3, 129.4, 129.5, 129.7, 129.9 (×2), 129.9 (×6), 130.0 (×3), 130.1 (×2), 133.3 (×2), 133.4, 133.5 (×2), 133.6, 133.6 (×2), 138.4,.138.6, 139.1, 165.1, 165.3, 165.6, 165.7, 166.0, 166.2. HR-FAB MS: calcd for C$_{90}$H$_{80}$O$_{23}$ [M+H]$^+$: 1559.4547 ; found: 1559.4550. (See Appendix; Figure A-1, A-2, and A-3)

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CHAPTER III

Unexpected orthogonalilty of *S*-benzoxazolyl and *S*-thiazolinyl glycosides:

Application to expeditious oligosaccharide assembly

3.1 Introduction

Traditional linear approaches to oligosaccharide assembly are often cumbersome and consequently the availability of complex glycostructures remains insufficient to address the challenges of modern glycosciences.P[1-3] Recent improvements in strategies for oligosaccharide assembly, have significantly shortened the number of synthetic steps required by minimizing protecting group manipulations between glycosylation steps.[4-9] One of the most flexible assembly strategies is the orthogonal concept.[10,11] Unlike the armed-disarmed approach,[12] the orthogonal activation is not reliant on the nature of the protecting groups, which can interfere with stereoselectivity. The only requirement for the orthogonal approach is a set of two orthogonal leaving groups and a pair of suitable activators. Unfortunately, this simple concept is still limited to the following two examples: Ogawa's *S*-ethyl and fluoride,[10,11] and the thioimidate *S*-thiazolinyl (STaz) and *S*-alkyl/aryl.[13,14] In addition, a related, albeit less flexible, semi-orthogonal approach with the use of *S*-ethyl and *O*-pentenyl glycosides was reported,[15] and was recently extended to fluoride/pentenyl leaving groups. *Overall, the orthogonal strategy is an excellent concept for flexible sequencing of oligosaccharides that remains under explored, with too few examples to become universal.* The excellent glycosyl donor properties of glycosyl thioimidates and their unique activation conditions have led to a number of useful developments for oligosaccharide synthesis, including the orthogonal approach.[13,14] From our previous studies we had determined the *S*-benzoxazolyl (SBox) glycosyl donors[16,17] to be significantly more reactive than their STaz counterparts,[13,14] and, although direct selective activations of the SBox donors over STaz acceptors were reported in the presence of Cu(OTf)$_2$,[13,14] no comprehensive side-by-side comparisons had been

performed.

3.2   Results and Discussion

With the main purpose of determining relative reactivity patterns of thioimidates, we set-up a series of glycosidations including STaz,[13,14] SBox,[16,17] and structurally related Mukaiyama's S-benzothiazolyl (SBaz) derivatives.[18] All reactions of benzylated glycosyl donors **3.1-3.3** with glycosyl acceptor **3.4** promoted with MeOTf were very effective, and disaccharide **3.5** was obtained in high yields (Table 3.1). Although the disparity amongst the reaction times was unremarkable, it was evident that glycosidation of the STaz glycoside **3.1** (entry 1) was about two times slower than that of its SBox counterpart **3.2** (entry 2), with the reactivity of the SBaz glycoside 3 (entry 3) somewhere in the middle.

To gain better control of the glycosylations and to achieve a more precise differentiation of reactivity between the different thioimidates, we turned to investigating other activators. Amongst these, results obtained with common alkylating (and acylating) reagents were particularly attractive. For example, MeI was only effective for glycosidation of per-benzylated STaz glycoside **3.1** (entry 4). Surprisingly, when these reaction conditions were applied to the glycosidation of expectedly more reactive SBox and SBaz glycosides, no glycosylation took place (entry 5). Also BnBr was only effective for glycosidation STaz derivative **3.1**, whereas glycosyl donors **3.2** or **3.3** gave only trace amounts of products (entries 6-8).

Table 3.1 Alkylation-initiated glycosidation of benzylated thioimidates **3.1-3.3**
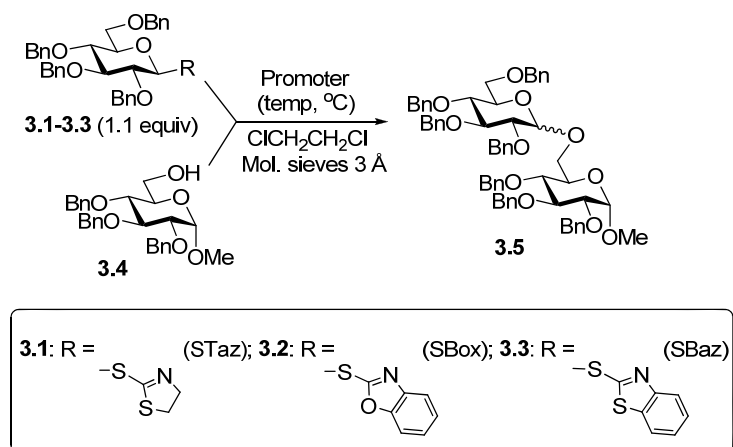
Entry	Donor	Promoter (equiv, temp)	Time	Product 3.5 Yield (%), α/β ratio
1	**3.1**	MeOTf (3, rt)	0.75 h	87%, 1.4/1
2	**3.2**	MeOTf (3, rt)	0.33 h	88%,1.6/1
3	**3.3**	MeOTf (3, rt)	0.58 h	89%, 1.6/1
4	**3.1**	MeI (9, rt)	120 h	89%, 8.0/1
5	**3.2** or **3.3**	MeI (9-15, rt)	120 h	no reaction
6	**3.1**	BnBr (3, 55 °C)	24 h	90%, 3.5/1
7	**3.2**	BnBr (3-9, 55°C)	120 h	traces
8	**3.3**	BnBr (3-9, 55°C)	120 h	no reaction

Subsequently, similar observations have been made with less reactive per-benzoylated glycosyl donors. Thus, all reactions of benzoylated glycosyl donors **3.6-3.8** with glycosyl acceptor **3.4** promoted with MeOTf were very effective, and disaccharide **3.9** was obtained in high yields (Table 3.2, entries 1-3). With MeI though, no glycosidation of benzoylated glycosyl donors **3.6-3.8** took place (entry 4). BnBr was only effective for the STaz derivative **3.6**, (entry 5), whereas glycosidation of glycosyl donors **3.7** or **3.8** gave no products (entry 6). It should be noted that all reactions with benzylated glycosyl donors (Table 3.1) were significantly faster than those with their benzoylated counterparts (Table 3.2).

Table 3.2 Alkylation-initiated glycosidation of benzoylated thioimidates **3.6-3.8**

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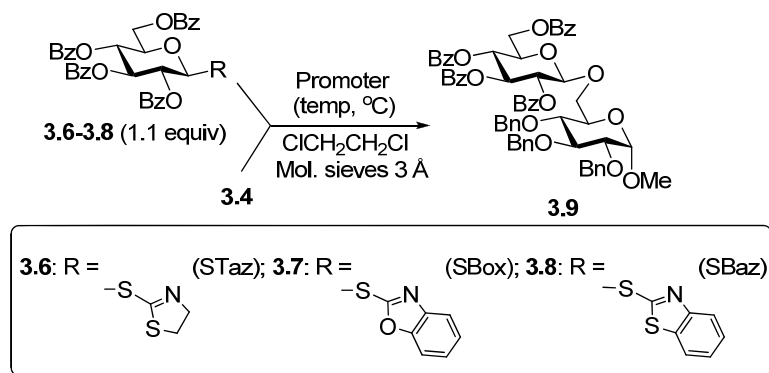
Entry	Donor	Promoter (equiv, temp)	Time	Product 3.9 Yield (%), α/β ratio
1	**3.6**	MeOTf (3, rt)	2 h	97%, β only
2	**3.7**	MeOTf (3, rt)	1 h	95%, β only
3	**3.8**	MeOTf (3, rt)	2 h	84%, β only
4	**3.6-3.8**	MeI (9-15, rt)	120 h	No reaction
5	**3.6**	BnBr (3, 55 °C)	72 h	79%, β only
6	**3.7** or **3.8**	BnBr (3-9, 55°C)	120 h	no reaction

This discovery signified the gap in our understanding of the thioimidate activation and created a basis for the development of the STaz-SBox orthogonal strategy. The uniqueness of this approach would be that both leaving groups employed are of essentially the same class. Although certain pathways for the activation of thioimidates were postulated (Scheme 3.1),[19-21] little proof had been available until our recent mechanistic study,[16,17] wherein we showed that MeOTf-promoted activation of the SBox glycosyl donor **3.10** proceeds via the anomeric sulfur atom (direct activation). This was confirmed by isolating the departed S-methylated aglycone MeSBox (**3.11**, Scheme 3.1).
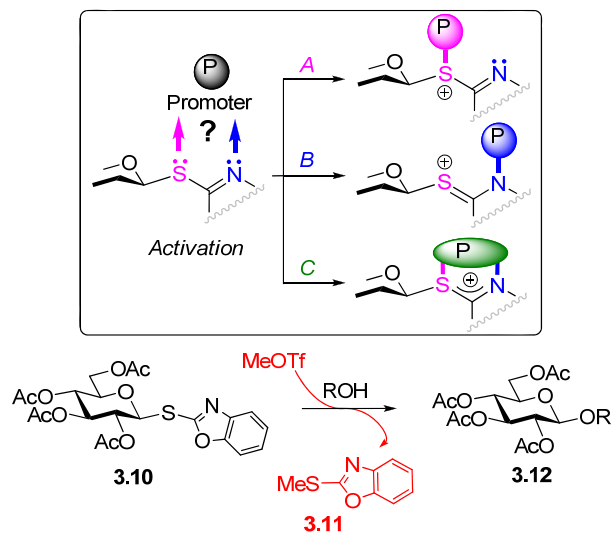
Scheme 3.1 Activation of thioimidates: a study with SBox glycoside **3.10**.

Since the exact nature of the STaz activation was not known, our working hypothesis for this study was based on our previous findings with the SBox moiety, taking into consideration the structural differences between the two moieties. We anticipated that the activation of the STaz moiety proceeds via the nitrogen (remote activation), as opposed to the direct activation of the SBox moiety. This remote activation of STaz would lead to a marginally slower reaction with a powerful promoter, such as MeOTf (refer to Tables 3.1 and 3.2). When weak alkylating reagents are used (MeI, BnBr), a powerful nucleophile is needed to replace the iodine or bromine, respectively. Evidently, this can be predominantly achieved with STaz glycosides that bear the reactive nitrogen atom, but not with the SBox glycosides, which can only be activated via the exocyclic sulfur[16,17] (Figure 3.1). For comparison, previous reports suggest that SPh glycosides do not react with MeI (direct activation), while S-pyridyl derivatives do (remote activation is postulated).[21]
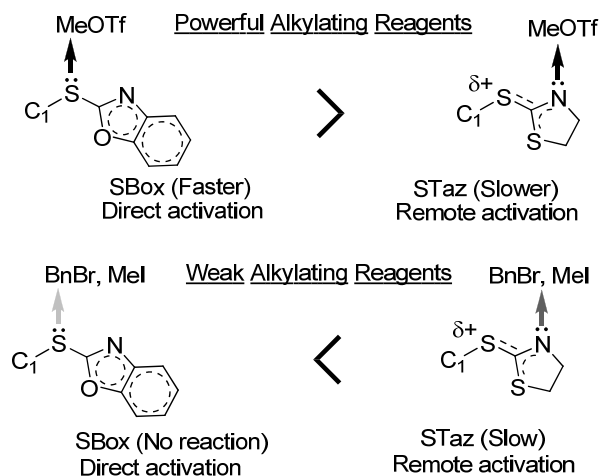
Figure 3.1 Working hypothesis for thioimidate activation.

The credibility of this working hypothesis was verified by a series of experiments in which glycosyl donor **3.1** was reacted with the standard acceptor **3.4** in the presence of BnBr (Scheme 3.2). Upon disappearance of **3.1**, the reaction mixture was concentrated in vacuo and the fastest moving UV-active spot, corresponding to the benzylated aglycone, was separated from the disaccharide product **3.5** by column chromatography on silica gel. The structure of the isolated alkylated aglycone was assigned as thioamide **3.13** (BnNTaz) by X-ray (Figure 3.2) and UV (λ_{max} = 277 nm, C=S).
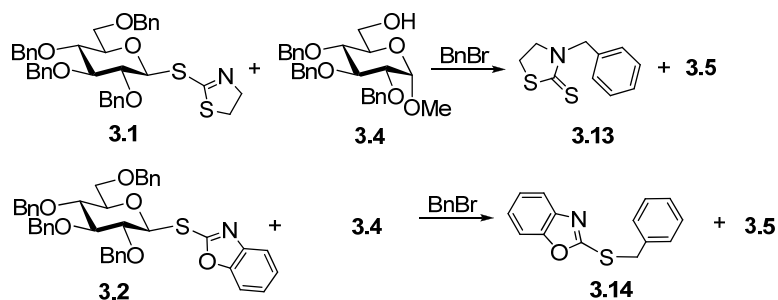
Scheme 3.2 Mechanism of thioimidate activation with BnBr.

A similar reaction between **3.2** and **3.4** was significantly slower, nevertheless, we succeeded in isolating the departed aglycone, which was assigned as thioimide **3.14** (BnSBox) by X-Ray (Figure 3.2) and UV (λ_{max} = 280, 287 nm, C=N), as anticipated. To exclude the impact of tautomerization of the products both reactions were monitored by HPLC (Figure 3.3).
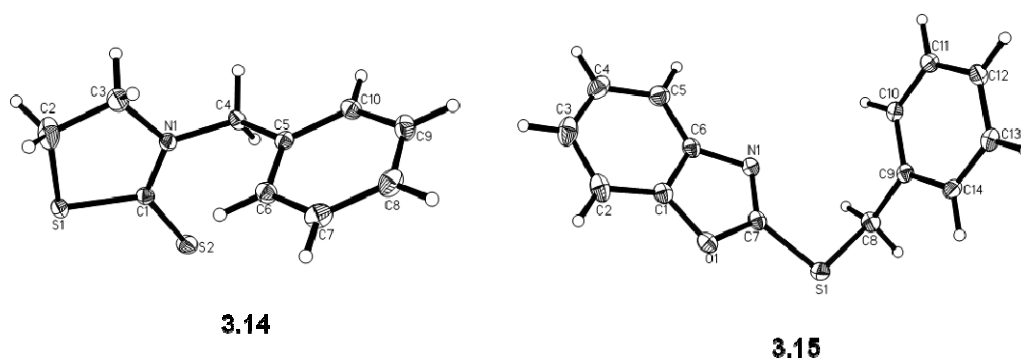
Figure 3.2 X-ray structure of compound **3.14** and **3.15**
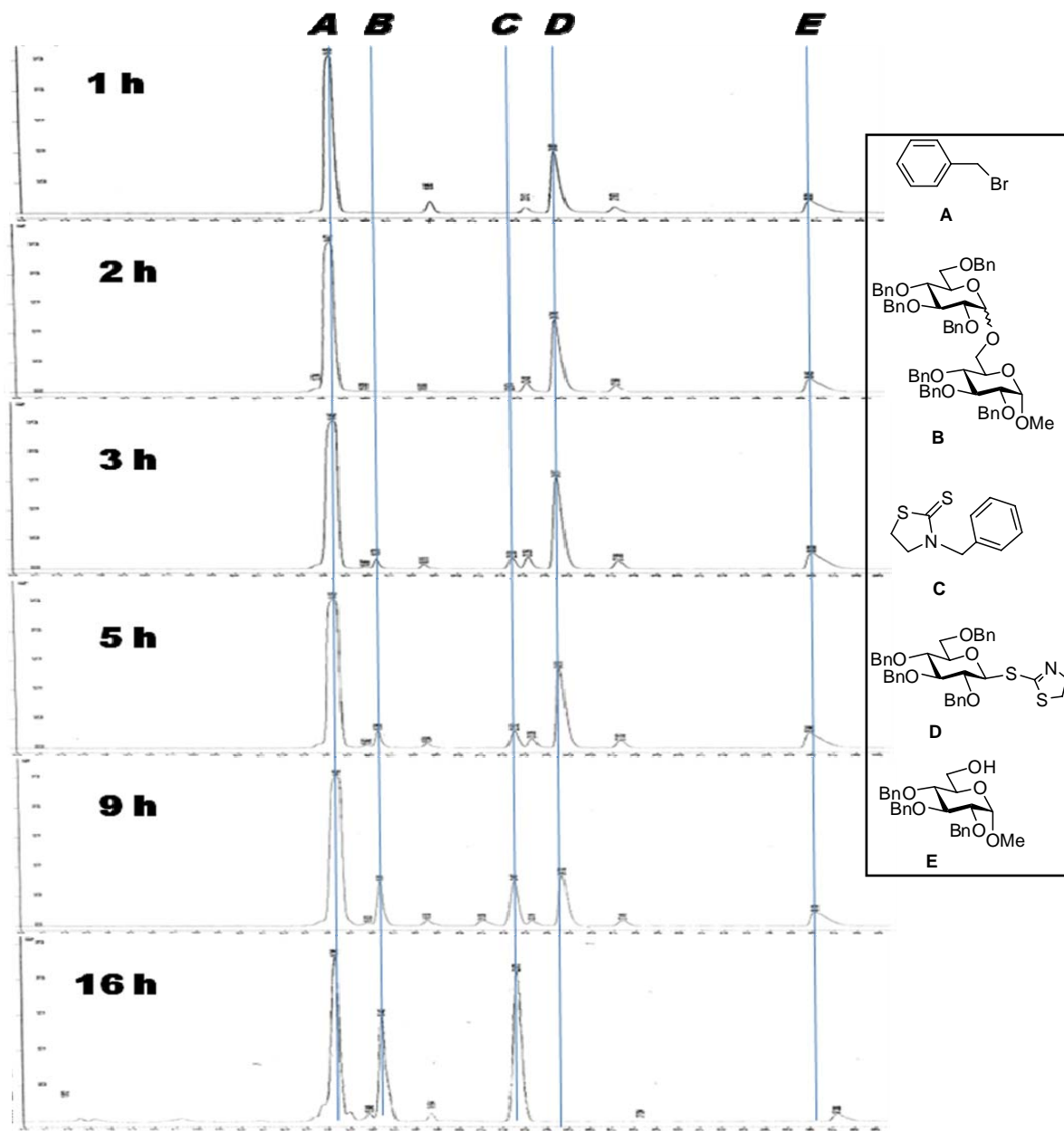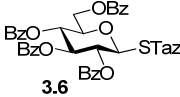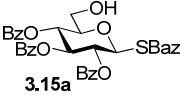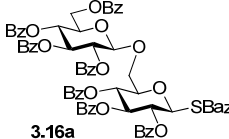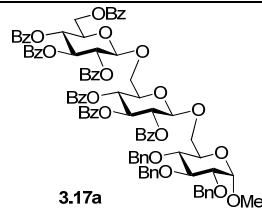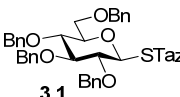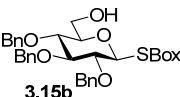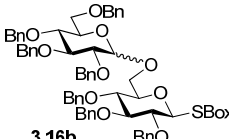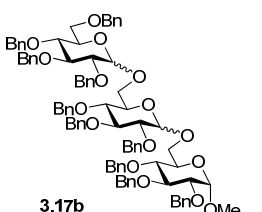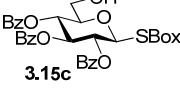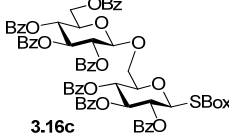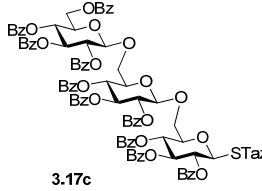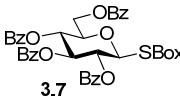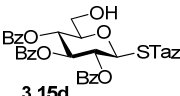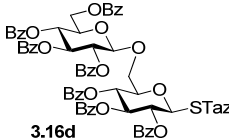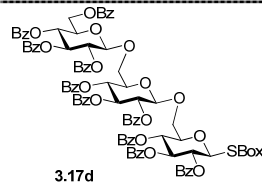
Figure 3.3 HPLC chromatograms of reaction mixture at 1h, 2h, 3h, 5h, 9h and 16h

With a better understanding of the mechanistic pathway, we were well positioned to undertake further studies of expeditious oligosaccharide assembly. First, the activation of STaz donors **3.1** and **3.6** over SBaz/SBox acceptors **3.15a-c** was investigated in the presence of BnBr or MeI. As a result, disaccharides **3.16a-c** were obtained in good yields (70-82%, Table 3.3). Conversely, SBox donor **3.7** was also activated over the STaz acceptor **3.15d**. This activation could be accomplished in the presence of a variety of activators, amongst which Bi(OTf)$_3$ was the most promising; disaccharide **3.16d** was obtained in 69% yield. The terminal SBox moieties of **3.16b** or **3.16c** could be glycosidated either with model acceptor **3.4** or with STaz acceptor (**3.15d**). Conversely, the STaz moiety of **3.16d** could be glycosidated with **3.4** or activated over the SBox acceptor (3.15c). The resulting trisaccharides **3.17a-d** were isolated in 62-92% yields (Table 3.3), with 1,6-anydro and hemiacetal being the major by-products identified. Among trisaccharides generated, **3.17c** and **3.17d** can be used in subsequent sequencing.

Table 3.3 Orthogonal activations for oligosaccharide synthesis
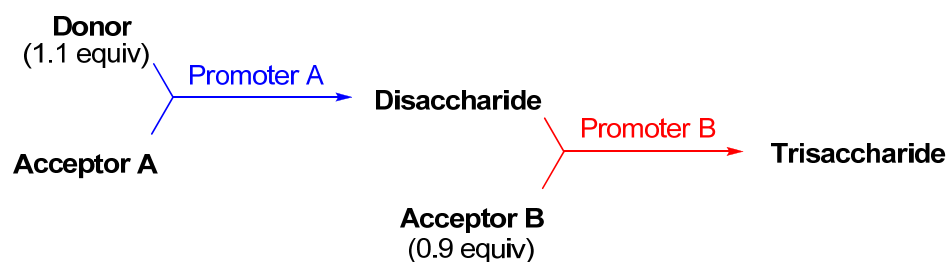
Entry	Donor	Acceptor A	Promoter A [a]	Disaccharide (yield, α/β)	Acceptor B	Promoter B [a]	Trisaccharide (yield, α/β)
1	**3.6**	**3.15a**	BnBr	**3.16a** (70%, β only)	**3.4**	AgBF$_4$	**3.17a** (75%, β only)
2	**3.1**	**3.15b**	MeI	**3.16b** (82%, 6/1)	**3.4**	AgOTf	**3.17b** (92%, 1.6/1)
3	**3.6**	**3.15c**	BnBr	**3.16c** (76%, β only)	**3.4**	AgOTf	**3.17a** (72%, β only)
4				**3.16c**	**3.15d**	Bi(OTf)$_3$	**3.17c** (62%, β only)
5	**3.7**	**3.15d**	Bi(OTf)$_3$	**3.16d** (69%, β only)	**3.4**	AgBF$_4$	**3.17a** (81%, β only)
6				**3.16d**	**3.15c**	BnBr	**3.17d** (67%, β only)

[a] – *Conditions*: All glycosylations were performed in 1,2-dichloromethane in the presence of molecular sieves (3 Å). Promoters: BnBr (3 equiv, 55 °C); AgBF$_4$ (3 equiv, rt); MeI (9 equiv, 55 °C); AgOTf (2 equiv, rt); Bi(OTf)$_3$ (3 equiv, 0 °C → rt). Reaction time: **3.16a** - 24 h, **3.16b** – 16 h, **3.16c** – 24 h, **3.16d** – 1h, **3.17a** – 30, 30, and 40 min (entries 1, 3, and 5, respectively), **3.17b** – 15 min, **3.17c** – 2 h, **3.17d** – 36 h.

The further study of this methodology was applied to the synthesis of a β-(1→6)-linked hexasaccharide *via* the five-step selective activation sequence which was employing six different leaving groups as depicted in Scheme 3.33. The synthesis began with the selective activation of thiocyanate donor **3.18** over STaz glycosyl acceptor **3.15d** using Cu(OTf)$_2$ as a promoter. The STaz leaving group of disaccharide **3.16d** was then directly activated over SBox acceptor **3.15c** with benzyl bromide to give trisaccharide **3.17d**. The SBox moiety of **3.17d** was then selectively activated over fluoride acceptor **3.19** in the in presence of MeOTf. The resulting tetrasaccharide **3.20** was glycosidated with thioglycoside acceptor **3.21** in presence of AgClO$_4$/Cp$_2$ZrCl$_2$ to provide pentasaccharides **3.22**. Finally, the *S*-ethyl leaving group of **3.22** was selectively activated over *n*-pentenyl acceptor **3.23** in the presence of MeOTf as a promoter to furnish the hexasaccharide **3.24** in 72% yield.[22]

3.3 Conclusions

In conclusion, a mechanistic study of the alkylation pathway for the activation of glycosyl thioimidates has led to the development of the "thioimidate-only orthogonal strategy". The synthesis of trisaccharides **3.17a-d** clearly illustrates the entirely orthogonal character of the SBox and STaz derivatives.
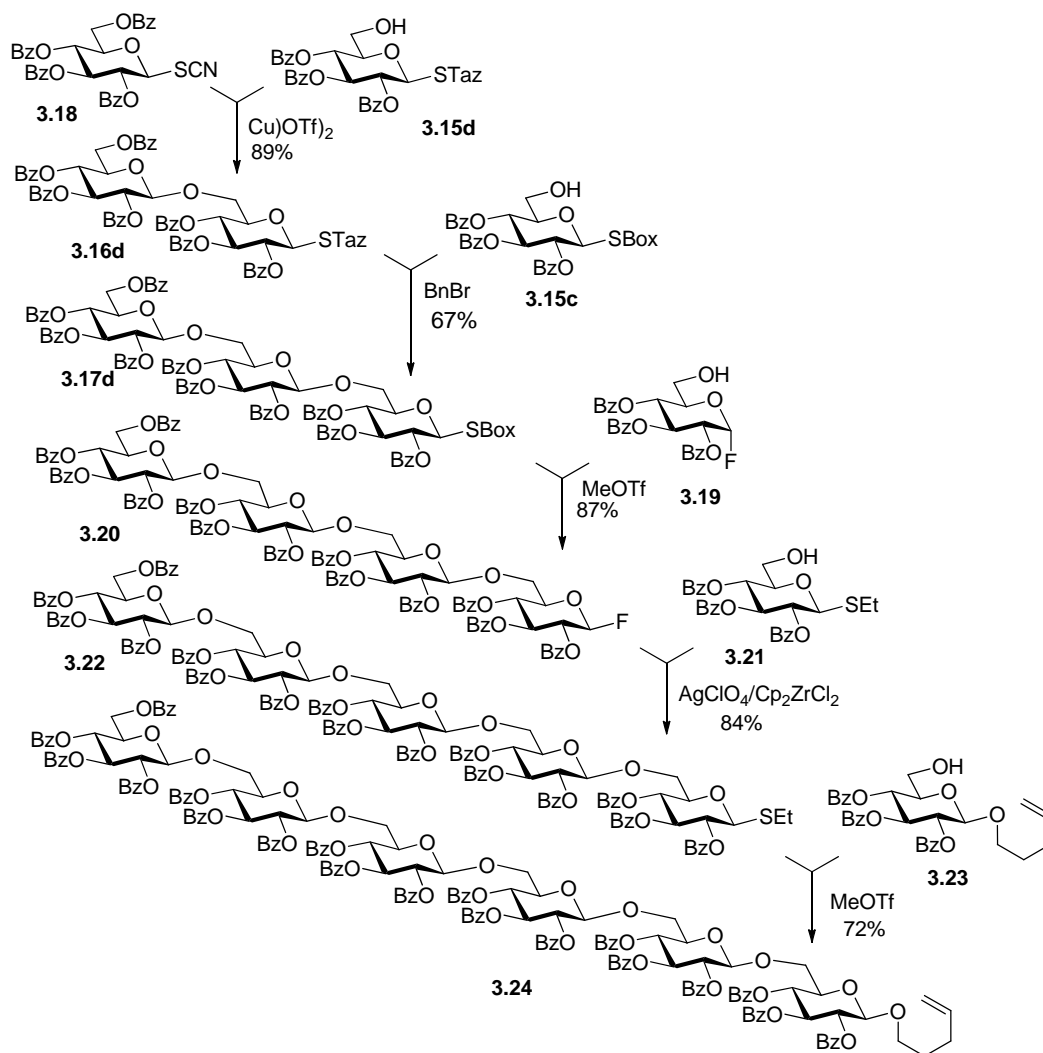
Scheme 3.3 Selective activation utilizing six different leaving groups employing SBox and STaz glycosides in the synthesis of 1,2-*trans* hexasaccharide.

3.4 Experimental part

3.4.1 General

Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol.

Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2, $ClCH_2CH_2Cl$, and MeCN were distilled from CaH_2 directly prior to application. Methanol was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3Å). Molecular sieves (3Å or 4Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. AgOTf (Acros) was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. ^{1}H-NMR spectra were recorded at 300 or 500 MHz, ^{13}C-NMR spectra were recorded at 75 or 125 MHz. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

3.4.2 Synthesis of glycosyl acceptor

Benzothiazol-2-yl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (3.15a)

Benzothiazol-2-yl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside[23] (550 mg, 1.1 mmol) was dissolved in methanol (5 mL) and the pH 8-9 was adjusted by careful addition of 1M solution of $NaOCH_3$ in MeOH (~0.1 mL). The reaction mixture was kept for 1 h at rt, then Dowex (H^+) was added until a neutral pH was reached. The resin was filtered off and washed with methanol (3 x 5 mL). The combined filtrate was concentrated *in vacuo* and dried. The residue was dissolved in dry pyridine (5.0 mL) and triphenylmethyl chloride (439 mg, 1.57 mmol) was added. The reaction mixture was left stirring for 16 h at rt. After that, the reaction mixture was cooled to 0 °C and benzoyl chloride (0.49 mL,

4.21 mmol) was added dropwise. The reaction mixture was allowed to gradually warm up. Upon stirring for 3 h at rt, the reaction was quenched with methanol (5 mL), co-evaporated with toluene (3 x 10 mL), then diluted with dichloromethane (20 mL) and washed with 1N HCl (10 mL), water (10 mL), sat. NaHCO$_3$ (10 mL), and water (3 x 10 mL). The organic layer was separated, dried with MgSO$_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate-toluene gradient elution). After that, the product was dissolved in dichloromethane (10 mL) containing one drop of water and trifluoroacetic acid (0.2 mL) was added dropwise. Upon completion, the reaction was then diluted with dichloromethane (10 mL), washed with saturated NaHCO$_3$ (10 mL) and water (3 x 15 mL). The organic layer was separated, dried with MgSO$_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate-toluene gradient elution) to obtain **3.15a** as yellow amorphous solid in 64% yield. $R_f = 0.35$ (ethyl acetate/toluene, 1.5/8.5, v/v); $[\alpha]_D^{23} = 0.48$ (c = 1.0, CHCl$_3$); ^{1}H NMR: δ, 2.75 (broad s, 1H, OH), 3.86 (m, 2H, H-6a, 6b), 4.06 (m, 1H, H-5), 5.62 (dd, 1H, $J_{4,5} = 4.7$ Hz, H-4), 5.74 (dd, 1H, $J_{2,3} = 4.5$ Hz, H-2), 5.97 (d, 1H, $J_{1,2} = 10.2$ Hz, H-1), 6.11 (dd, 1H, $J_{3,4} = 9.6$ Hz, H-3), 7.2- 8.10 (m, 19H, aromatic) ppm; ^{13}C NMR: δ, 60.6, 61,7, 69.4, 70.8, 74.0, 79.7, 84.4, 121.3, 122.5, 125.2, 126.6, 128.6 (×4), 128.7, 128.7 (×2), 128.8, 128.9, 129.9 (×2), 130.1 (×3), 133.6, 133.7, 134.0, 135.8, 152.9, 162.4, 165.4, 165.9, 166.1 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{34}$H$_{27}$NO$_8$S$_2$Na 664.1076, found 664.1085.

3.4.3   General glycosylation procedures

Method A.   A typical MeOTf - promoted glycosylation procedure: A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under argon for 1 h. MeOTf (0.33 mmol) was added and the reaction mixture was stirred for 20 min – 2 h. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with water (10 mL), saturated $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone/toluene gradient elution) to allow the corresponding disaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H NMR spectra.

Method B.   A typical MeI - promoted glycosylation procedure. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under argon for 1 h, then MeI (1.0-1.5 mmol) was added. The reaction mixture was stirred for 60 h at rt. Upon completion, the reaction mixture was diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL), the organic phase was separated, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to allow the corresponding di- or oligosaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H NMR spectra.

Method C – A typical BnBr- promoted glycosylation procedure: A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under argon for 1 h, then BnBr (0.33-0.99 mmol) was added. The reaction mixture was stirred for 24-36 h at 55 °C. Upon completion, the reaction mixture was diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL), the organic phase was separated, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford the corresponding di- or oligosaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^1H NMR spectra.

Method D – A typical Bi(OTf)₃- promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1 h. The reaction mixture was cooled down at 0°C and then $Bi(OTf)_3$ (0.22 mmol) was added into the solution. After that the reaction mixture was stirred for 1-2 h at rt. Upon completion, the reaction mixture was diluted with CH_2Cl_2, the solid was filtered-off, and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford a di- or oligosaccharide derivative.

Method E – A typical AgOTf- promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 1–2 h at rt and then diluted with CH_2Cl_2, the solid was filtered-off, and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford an oligosaccharide derivative.

Method F – A typical AgBF₄- promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1 h. $AgBF_4$ (0.22 mmol) was added and the reaction mixture was stirred for 2 h at rt and then diluted with CH_2Cl_2, the solid was filtered-off, and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford an oligosaccharide derivative.

Method G-MeOTf promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1 h. MeOTf (0.33 mmol) was then

added into the solution. After that the reaction mixture was stirred for 3-5 h at rt. Upon completion, the reaction mixture was diluted with CH_2Cl_2, the solid was filtered-off, and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford a di- or oligosaccharide derivative. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H-NMR spectra.

Method H-AgClO₄/Cp₂ZrCl promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1 h. $AgBF_4$ (0.22 mmol) and Cp_2ZrCl (0.22 mmol) were then added into the solution. After that the reaction mixture was stirred for 3-5 h at rt. Upon completion, the reaction mixture was diluted with CH_2Cl_2, the solid was filtered-off, and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to afford a di- or oligosaccharide derivative. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H-NMR spectra.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside (3.5). The title compound was obtained as a colorless syrup by a range of methods in 87-90% yield (see *Table 3.1* of the manuscript). Analytical data for **3.8** was in good agreement with those reported previously.[24]

Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl)-α-D-glucopyranoside (3.9). The title compound was obtained as a white foam by a variety of methods shown in *Table 3.2* in the manuscript in 79-97% yield. Analytical data for **3.9** was in good agreement with those reported previously.[25]

Benzothiazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (3.16a). The title compound was obtained by *Method C* from 2-thiazolinyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.6**)[14] and **3.15a** in 70% yield as a white foam. Analytical data for **3.16a**: R_f = 0.6 (ethyl acetate/toluene, 1/4, v/v); $[\alpha]_D^{24}$ +49.9 (*c* = 1, CHCl$_3$); ^{1}H NMR; δ, 3.58 (m, 2H, H-6a,6b), 4.12 (m, 2H, H-5,5'), 4.36 (dd, 1H, $J_{5',6b'}$ = 7.4 Hz, H-6b'), 4.48 (dd, 1H, $J_{5',6a'}$ = 2.8 Hz, $J_{6a',6b'}$ = 12.1 Hz, H-6a'), 4.60 (m, 1H, H-4), 5.44 (d, 1H, $J_{4',5'}$ = 8.7 Hz, H-4'), 5.72 (m, 2H, H-3,3'), 5.85 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.93 (dd, 1H, $J_{2',3'}$ = 9.4 Hz, H-2'), 5.95 (dd, 1H, $J_{2,3}$ = 5.0 Hz, H-2), 7.1-8.10 (m, 39H, aromatic); 13C NMR: δ, 63.1, 64.2, 67.7, 68.6, 69.3, 69.3, 70.8, 72.1, 74.3, 77.4, 84.6, 98.0, 121.2 (×2), 122. 5, 125.1, 125.5, 126.5, 126.7 (×2), 128.4 (×2), 128.4 (×2), 128.5 (×3), 128.6 (×2), 128.6 (×3), 128.7 (×2), 128.7 (×2), 128.9, 128.9, 129.0, 129.2 (×2), 129.4, 129.9 (×2), 129.9 (×5), 130.1 (×2), 130.1 (×3), 130.3 (×2), 133.1, 133.7 (×2), 133.7, 134.5, 135.9, 152. 9, 162.5,

164.5, 165.3 (×2), 165.9, 166.2 ppm; HR FAB MS [M+Na]$^+$ calcd for $C_{68}H_{53}NO_{17}S_2Na$ 1242.2653, found 1242.2683.

Benzoxazol-2-yl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (3.16b). The title compound was obtained from 2-thiazolinyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3.1**)[14] and benzoxazolyl 2,3,4-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3.15b**)[26] by *Method B* in 82% yield and *Method C* in 85% yield, as a colorless syrup. Analytical data for **3.16b**: R_f = 0.52 (ethyl acetate/hexane, 3/7, v/v); ^{1}H NMR; δ, 3.28 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 3.82 (dd, $J_{3',4'}$ = 9.2 Hz, H-3'), 3.42-3.82 (m, 10H, H-3, 4, 5, 6a, 6b, 2', 4', 5', 6a', 6b'), 4.43-4.95 (m, 14H, C*H*$_2$Ph), 4.98 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 5.09 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.38 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 7.10-7.65 (m, 39H, aromatic); ^{13}C NMR: δ, 65.5, 68.7, 70.4, 72.3, 73.5, 75.0, 75.3, 75.7, 75.8, 77.4, 79.8, 80.3, 80.8, 81.8, 85.0, 86.7, 97.3, 119.2, 124.4, 124.6, 127.6 (×2), 127.6 (×2), 127.7 (×2), 127.8 (×2), 127.8 (×3), 128.0 (×3), 128.0 (×3), 128.1 (×3), 128.3 (×3), 128.5 (×3), 128.5 (×3), 128.6 (×3), 128.6 (×3), 128.7 (×2), 137.7, 138.2, 138.4, 138.5, 138.7, 138.7, 138.8, 139.0, 142.0, 152.0, 161.7 ppm; HR FAB MS [M+Na]$^+$ calcd for $C_{68}H_{67}NO_{11}SNa$ 1128.4333, found 1128.4368.

Benzoxazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (3.16c). The title compound was obtained by *Method C* from 2-thiazolinyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.6**)[14] and benzoxazolyl 2,3,4-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.15c**)[27] in 76% yield as a colorless syrup. Analytical data for **3.16c**: R_f = 0.45 (ethyl acetate/hexane,

7/3, v/v); $[\alpha]_D^{24}= 48.5$ ($c = 1$, CHCl$_3$]; ^{1}H NMR; δ, 3.53 (dd, 1H, $J_{5,6b} = 8.3$ Hz, H-6b), 3.86 (dd, 1H, $J_{6a,6b} = 4.8$ Hz, H-6a), 4.12 (m, 1H, H-5'), 4.15 (m, 1H, H-5), 4.34 (dd, 1H, $J_{5,6b'} = 7.9$ Hz, H-6b), 4.47 (dd, 1H, $J_{6a',6b'} = 2.6$ Hz), 4.74 (m, 1H, H-2), 5.43 (d, 1H, $J_{4',5'} = 8.4$ Hz, H-4'), 5.68 (dd, 1H, $J_{4,5} = 9.8$ Hz, H-4), 5.72 (dd, 1H, $J_{3',4'} = 9.6$ Hz, H-3'), 5.90-5.99 (m, 3H, H-1, 1', 2'), 7.09-8.10 (m, 39H, aromatic); ^{13}C NMR: δ, 63.3, 64.4, 67.8, 68.8, 79.4, 71.0, 72.3, 74.5, 77.6, 84.3, 110.5, 119.2, 121.4, 124.9, 124.9, 126.9 (×2), 128.7 (×5), 128.8 (×5), 128.9 (×2), 129.0, 129.1, 129.2, 129.5, 129.5, 130.5 (×3), 130.1 (×5), 130.3 (×5), 130.5 (×2), 133.3, 133.7, 133.8 (×3), 133.9, 134.7, 141.9, 152.2, 164.7, 165.5, 165.5, 165.6, 166.1, 166.4 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{68}$H$_{53}$NO$_{18}$SNa 1226.2281, found 1226.2283.

2-Thiazolinyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (3.16d). The title compound was obtained by *Method D* from benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.7**)[17] and 2-thiazolinyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.15d**)[14] in 69% yield as a white foam. Analytical data for **3.16d** was in a good agreement with those reported previously.[28]

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-b-D-glucopyranosyl]-β-D-glucopyranoside (3.17a). The title compound was obtained as a colorless syrup by *Method E* from methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**3.4**)[29] and **3.16c** in 72% yield and *Method F* from **3.4** and **3.16a** in 75% yield and from **3.4** and **3.16d** in 81% yield. Analytical data for **3.17a** was

in a good agreement with those reported previously.[30]

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-D-glucopyranosyl]-α-D-glucopyranoside (3.17b). The title compound was obtained by *Method E* from **3.4** and **3.16b** in 92% yield as a white amorphous solid. Analytical data for α/β–**3.17b**; R_f = 0.48 (ethyl acetate/hexane, 3:7, v/v); ^{13}C NMR: δ, 55.4, 70.7, 70.8, 72.4, 72.5., 72,6, 73.5, 73.6, 73.6, 75.1, 75,1, 75.2, 75.6, 75.7, 75.7, 75.7, 75.8, 75.9, 77.4, 78.0, 80.0, 80.3, 80.3, 80.4, 80.5, 81.9, 82.1, 82.3, 82.4, 84.9, 97.3, 97.4, 97.5, 98.1, 98.2, 98.2, 103.7, 127.7, 127.7, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.5, 128.5, 128.6, 128.6, 138.2, 138.3, 138.4, 138.5, 138.6, 138.6, 138.7, 138.8, 138.9, 139.0, HR FAB MS [M+Na]$^+$ calcd for $C_{89}H_{94}O_{16}Na$ 1441.6440, found 1441.6437.

2-Thiazolinyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-1-thio-β–D-glucopyranoside (3.17c). The title compound was obtained by *Method D* from **16c** and **15d** in 62 % yield as a white foam. Analytical data for **17c**: R_f = 0.46 (ethyl acetate/toluene, 1/4, v/v); $[\alpha]_D^{21}$ = 5.84 (*c* = 1, CHCl$_3$); ^{1}H NMR; δ, 3.29-3.39 (m, 2H, CH$_2$S), 3.59 (m, 1H, H-6a'), 3.85 (m, 2H, H-5',6b"), 4.00 (m, 1H, 6a"), 4.13-4.29 (m, 2H, CH$_2$N), 4.44 (m, 2H, H-5, 6a'), 4.66 (m, 1H, H-6b), 4.67 (d, 1H, $J_{1",2"}$ = 7.8 Hz, H-1"), 5.04 (dd, 1H, $J_{4",5"}$ = 9.5 Hz, H-4"), 5.11 (dd, 1H, $J_{2",3"}$ = 7.8 Hz, H-2"), 5.21 (d, 1H, $J_{1',2'}$ = 7.9, H-1'), 5.27 (dd, 1H, $J_{2',3'}$ = 4.0 Hz, H-2'), 5.59-5.62 (m, 2H, H-3", 4'), 5.65 (dd, 1H, $J_{4,5}$ = 8.3 Hz, H-4), 5.77 (dd, 1H, $J_{2,3}$ = 7.1 Hz, H-2), 5.78 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.91 (dd, 1H, $J_{3,4}$ = 9.6 Hz,

H-3), 6.15 (dd, 1H, $J_{3',4'}$ = 9.7 Hz, H-3'), 7.15-8.10 (m, 50H, aromatic); ^{13}C NMR: δ, 58.8, 63.5, 64.4, 68.2, 68.4, 69.8, 69.9, 70.6, 70.8, 72.0, 72.3, 72.5, 72.9, 73.0, 74.2, 74.3, 83.5, 100.2, 101.5, 125.5 (×2), 127.2, 127.9, 128.4 (×2), 128.4 (×2), 128.5 (×2), 128.5 (×2), 128.5 (×3), 128.6 (×2), 128.6 (×4), 128.8 (×2), 128.8, 128.9 (×2), 129.0, 129.1, 129.1 (×3), 129.2 129.3, 129.4 (×2), 129.5, 129.8, 129.9 (×2), 130.0 (×3), 130.0 (×5), 130.1(×3), 130.1 (×2), 130.2 (×2), 130.3 (×2), 130.3 (×2), 133.3, 133.3, 133.4 (×2), 133.5 (×2), 133.6, 133.6, 165.2, 165.3, 165.4, 165.6 (×2), 166.0, 166.0, 166.4 ppm; HR FAB MS [M+Na]$^+$ calcd for $C_{91}H_{75}NO_{25}S_2Na$ 1668.3967, found 1668.4023. (See Appendix; Figure A-4, A-5, and A-6)

Benzoxazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-1-thio-β–D-glucopyranoside (3.17d). The title compound was obtained by *Method B* from **16d** and **15c** in 67% yield as a colorless syrup. Analytical data for **3.17d**: R_f = 0.48 (ethyl acetate/toluene, 1.5/8.5, v/v); $[\alpha]_D^{28}$= 47.4 (*c* = 1, CHCl$_3$); ^{1}H-n.m.r.: δ, 3.43-3.45 (m, 2H, H-6a, 6b), 3.66 (dd, 1H, $J_{5',6a'}$ = 5.5 Hz, $J_{6a',6b'}$ = 11.5 Hz, H-6a'), 3.84 (m, 1H, H-5'), 4.06-4.16 (m, 3H, H-5, 5'', 6b'), 4.30 (dd, 1H, $J_{5'',6a''}$ = 5.1 Hz, $J_{6a'',6b''}$ = 12.1 Hz, H-6a''), 4.54-4.58 (m, 2H, H-4, 6b''), 4.95 (d, 1H, $J_{1',2'}$ = 11.0 Hz, H-1'), 5.22 (dd, 1H, H-4'), 5.50-5.55 (m, 2H, H-2', 3'), 5.60-5.69 (m, 2H, H-3'', 4''), 5.71 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 5.77 (d, 1H, $J_{1'',2''}$ = 5.3 Hz, H-1''), 5.86 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.95 (d, 1H, $J_{2'',3''}$ = 9.5 Hz), 5.96 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 7.15-8.10 (m, 54H, aromatic); ^{13}C NMR: δ, 60.6, 63.0, 63.3, 68.1, 68.6, 69.3, 69.5, 69.98, 70.8, 71.7, 72.4, 72.4, 73.2, 74.3, 76.7, 77.6, 77.7, 84.1, 97.8, 102.0, 110.3, 119.1, 121.1, 124.6, 124.8, 126.6, 128.2 (×2), 128.4 (×2), 128.4 (×2), 128.5

(×4), 128.6 (×4), 128.8, 128.9, 129.0, 129.1, 129.1, 129.2, 129.4 (×3), 129.7, 129.8 (×2),

129.9 (×4), 130.0 (×4), 130.0 (×4), 130.0 (×4), 130.10 (×2), 130.3, 133.1, 133.2, 133.3

(×2), 133.4, 133.5, 133.6 (×2), 133.6, 134.4, 141.7, 152.0, 161.4, 164.5, 165.1, 165.2,

165.2, 165.4, 165.9, 166.0, 166.3 ppm; HR FAB MS [M+Na]$^+$ calcd for $C_{95}H_{75}NO_{26}SNa$

1700.4196, found 1700.4182. (See Appendix; Figure A-7, A-8, and A-9)

2,3,4-Tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride (3.20). This compound was obtained by

Method G from **3.17d** and 2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl fluoride (**3.19**)[31] in

87% yield. R_f = 0.42 (ethyl acetate/toluene, 1/9, v/v); [α]$_D^{25}$ = -2.02 (*c* = 1, CHCl$_3$); ^{1}H

NMR: δ, 3.54 (m, 1H), 3.59 (m, 1H), 3.86 (m, 3H), 4.00 – 4.08 (m, 3H), 4.14 (dd, 1H, *J*

= 12.1 Hz), 4.34 (m, 1H), 4.46 (dd, 1H, *J* = 4.9, 11.4 Hz), 4.60 (dd, 1H, *J* = 11.8 Hz),

4.76 (d, 1H, *J* =7.7 Hz), 4.82 (d, 1H, *J* = 7.9 Hz), 5.11 (d, 1H, *J* = 7.8 Hz), 5.21 (dd, 1H, *J*

= 9.8), 5.28 (dd, 1H, *J* = 9.3 Hz), 5.34 (d, ½ H, *J* = 6.2 Hz), 5.40 (dd, 1H, *J* = 9.7 Hz),

5.46 (m, 1.5 H), 5.53 (dd, 1H, *J* = 9.5 Hz), 5.62 – 5.70 (m, 3H), 5.75 (dd, 1H, *J* = 9.6 Hz),

5.79 – 5.85 (m, 2H), 6.16 (dd, 1H, *J* = 9.6 Hz), 7.22 – 8.10 (m, 65H, aromatic) ppm; ^{13}C

NMR: δ, 63.45, 68.14, 68.66, 68.91, 69.14, 69.67, 69.79, 70.24, 71.76, 71.98, 72.03,

72.40, 72.46, 72.78, 72.93, 73.03, 73.77, 74.24, 74.76, 100.87, 101.51, 101.71, 106.23,

128.36, 128.38, 128.46, 128.50, 128.52, 128.62, 128.67, 128.70, 128.73, 128.77, 128.83,

128.89, 128.92, 129.05, 129.07, 120.10, 129.16, 129.21, 129.57, 129.72, 129.80, 129.89,

129.96, 129.99, 130.02, 130.09, 130.18, 133.21, 133.25, 133.29, 133.35, 133.40, 133.44,

133.52, 133.65, 133.70, 133.77, 133.82, 134.05, 165.01, 165.11, 165.23, 165.26, 165.39,

165.43, 165.46, 165.73, 165.80, 165.89, 165.91, 166.00, 166.10, 166.32 ppm;

HRFABMS $[M+Na]^+$ calcd for $C_{115}H_{93}FO_{33}Na$ 2043.5481, found 2043.5452. (See

Appendix; Figure A-10, A-11, and A-12)

Ethyl O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-thio-D-glucopyranoside (3.22). This compound was obtained by *Method H* from glycosyl fluoride **3.20** and ethyl 2,3,4-tri-O-benzoyl-thio-β-D-glucopyranoside (**3.21**)[32] in 84% yield. R_f = 0.46 (ethyl acetate/toluene, 1.5/8.5, v/v); $[\alpha]_D^{26}$ = -9.32 (*c* = 1, CHCl₃); ^{1}H NMR: δ, 1.14 (t, 3H, SCH₂C*H*₃), 2.68 (m, 2H, SC*H*₂CH₃), 3.58 (m, 1H, *J* = 5.5, 6.2 Hz), 3.67 − 3.75 (m, 3H), 3.85 − 3.92 (m, 6H), 4.02 (dd, 1H, *J* = 10.8 Hz), 4.12 (m, 1H), 4.35 (m, 1H), 4.48 (dd, 1H, *J* = 5.5, 12.1 Hz), 4.57 (dd, 1H, *J* = 11.0 Hz), 4.70 (d, 1H, *J* = 10.0 Hz), 4.76 (d, 1H, *J* = 7.7 Hz), 4.86 (m, 2H), 5.16 (d, 1H, *J* = 7.9 Hz), 5.25 (dd, 1H, *J* =9.6 Hz), 5.33 (dd, 1H, *J* = 9.3 Hz), 5.39 (dd, 1H, *J* = 9.6 Hz), 5.43 − 5.56 (m, 7H), 5.70 (dd, 1H, *J* = 9.7 Hz), 5.76 − 5.87 (m, 2H), 5.90 − 5.95 (m, 2H), 6.23 (dd, 1H, *J* =9.7 Hz), 6.95 − 8.90 (m, 80H, aromatic) ppm; ^{13}C NMR: δ, 14.88, 31.14, 63.48, 67.95, 68.03, 69.00, 69.29, 60.74, 69.91, 70.12, 70.19, 70.74, 70.95, 72.18, 72.28, 72.42, 72.47, 72.77, 72.87, 72.98, 73.05, 73.76, 74.16, 74.66, 83.73, 101.20, 101.27, 101.39, 101.80, 128.19, 128.28, 128.35, 128.45, 128.49, 128.60, 128.67, 128.74, 128.82, 128.92, 128.94, 129.01, 129.05, 129.11, 129.18, 129.24, 129.28, 129.52, 129.69, 129.74, 129.77, 129.79, 129.82, 129.90, 129.96, 129.99, 130.05, 130.08, 130.12, 133.08, 133.19, 133.27, 133.35, 133.49, 133.65, 133.82, 165.20, 165.27, 165.30, 165.34, 165.38, 165.43, 165.58, 165.61, 165.76, 165.89,

165.91, 165.97, 166.29 ppm;   HRFABMS [M+Na]$^+$ calcd for $C_{144}H_{120}O_{41}SNa$ 2559.6923, found 2559.6938. (See Appendix; Figure A-13, A-14, and A-15)

4-Pentenyl O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside   (3.24).   This compound was obtained by *Method E* from thio pentasaccharide (**3.22**) and 4-pentenyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**3.23**)[33] in 72% yield. R_f = 0.50 (ethyl acetate/toluene, 2/8, v/v); $[\alpha]_D^{22}$ = -10.54 (c = 1, CHCl$_3$), 1H NMR; δ, = 1.41 – 1.58 (m, 4H), 1.89 (m, 2H), 3.28 (m, 1H), 3.55 (m, 2H), 3.71 (dd, 1H, J = 11.5 Hz), 3.75 – 3.81 (m, 5H), 3.90 - 4.07 (m, 7H), 4.12 (m, 1H), 4.31 (m, 1H), 4.47 (dd, 1H, J = 5.4, 12.1 Hz), 4.56 (dd, 1H, J = 2.6, 9.3 Hz), 4.64 (d, 1H, J = 7.9 Hz), 4.75 (d, 1H, J = 1.6 Hz), 4.79 - 4.87 (m, 6H), 5.12 (d, 1H, J = 7.8 Hz), 5.24 (dd, 1H, J = 9.7 Hz), 5.31 (dd, 1H, J = 1.8, 9.6 Hz), 5.38 - 5.55 (m, 9H), 5.58 – 5.63 (m, 1H), 5.68 (dd, 1H, J = 9.7 Hz), 5.67 (dd, 1H, J = 9.7 Hz), 5.86 (dd, 1H, J = 9.6 Hz), 5.90 -5.97 (m, 2H, J = 9.6 Hz), 6.06 (dd, 1H, J = 9.6 Hz), 6.16 (dd, 1H, J = 9.7 Hz), 6.90 – 8.00 (m, 95H, aromatic) ppm; ^{13}C NMR: δ, 28.71, 29.92, 30.01, 31.15, 63.04, 63.43, 67.64, 68.19, 69.80, 69.94, 70.09, 70.47, 70.51, 70.93, 72.12, 72.31, 72.26, 72.42, 72.85, 72.89, 72.99, 74.54, 89.86, 101.28, 101.30, 102.35, 101.44, 101.57, 115.02, 128.24, 128.34, 128.36, 128.41, 128.47, 128.50, 128.52, 128.65, 128.69, 128.75, 128.79, 128.98, 129.00, 129.02, 129.05, 129.08, 129.15, 129.18, 129.22, 129.28, 129.44, 129.72, 129.80, 129.82, 129.84, 129.86, 129.95, 129.99, 130.04, 130.08, 130.13, 130.16, 130.17, 165.23, 165,25, 165.27, 165.30, 165.37, 165.42, 165.46,

165.49, 165.62, 165.65, 165.74, 165.90, 165.91, 165.95, 166.31 ppm; HRFABMS [M+Na]$^+$ calcd for $C_{174}H_{146}O_{50}Na$ 3057.8780, found 3057.8748. (See Appendix; Figure A-16, A-17, and A-18)

3.4.3 Aglycone isolation

3-Benzyl-thiazolidine-2-thione (3.13). The title compound was isolated by column chromatography from the reaction mixture resulted from glycosylation between **3.1** and **3.7**. Analytical data for **3.13** was reported previously.[34] The structure of **3.13** was confirmed by UV spectrometry with absorption maximum at 277 nm.

2-(Benzylthio)-benzoxazole (3.14). The title compound was isolated by column chromatography from the reaction mixture resulted from glycosylation between **3.3** and **3.7**. Analytical data for **3.14** was reported previously.[35] The structure **of 3.14** was confirmed by UV spectrometry with two absorption maxima at 280 and 290 nm.[36]

3.4.5 HPLC Monitoring

The analyses were carried out on a Supelcosil LC-SI Semi Prep (250mm×10mm i.d., 5μm) (Supelco, Bellefonte, PA, USA) The mobile phase was ethyl acetate/hexane (30/70, v/v), under isocratic conditions. The sample analysis (injection volume 20 μL) was performed for 60 min at rt with the flow rate 1.0 mL/min and UV detection at 254 nm.

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CHAPTER IV

On the stereoselectivity of glycosidation of thiocyanates, thioimidates and

thioglycosides

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4.1   Introduction

The stereocontrolled introduction of glycosidic linkages[1,2] is arguably the most challenging aspect in the synthesis of oligosaccharides[3-9] and glycoconjugates.[10-12] While a 1,2-*trans* glycosidic linkage can be usually stereoselectively obtained by using the anchimeric assistance from the participatory neighboring substituent at C-2 (most commonly an ester[13] or recently introduced picolinyl),[14,15] the stereoselective formation of a 1,2-*cis* linkage still remains a notable challenge,[16] despite significant recent improvements.[17-19] In addition to the effect of protecting groups on the glycosyl donor, a myriad of other factors is known to have effect on the stereoselectivity of the chemical glycosylation;[20] and the effect of conformation,[21] solvent,[22-24] temperature,[25] metal coordination,[26] steric hindrance,[27,28] and remote participation[29-31] are only a few to mention. It is commonly believed that a typical glycosylation follows the unimolecular mechanism with the rate-determining step being the glycosyl acceptor attack on the intermediate formed as a result of the leaving group departure.[32] Nevertheless, occasionally the effect of a leaving group itself may also have an influence on the anomeric stereoselectivity. However, it is often unclear whether this effect is a result of a bimolecular or close-ion pair rather than the unimolecular displacement mechanism, or other factors affecting this complex process.

As part of a program to develop new protocols for stereocontrolled glycosylation, we have been investigating various classes of sulfur-based leaving groups ranging from conventional alkyl/aryl thioglycosides to relatively novel S-benzoxazolyl (SBox) or S-thiazolinyl (STaz) thioimidates. In particular, we were interested in a possible influence that different leaving groups may have on the outcome of glycosylations. While on a

number of occasions we managed to achieve very good to excellent stereoselectivities, particularly with the SBox glycosides, these results have been occasionally outshadowed by other classes of glycosyl donors. For example, glycosyl thiocyanates, introduced two decades ago, have proven to be very effective glycosyl donors for what then appeared to be "stereospecific 1,2-*cis* glycosylations".[33]

Indeed, in a series of publications, Kochetkov *et al.*, provided a convincing case of the power of this class of glycosyl donors.[33-37] Various hexose and pentose 1,2-*trans* thiocyanate glycosyl donors were found to provide 1,2-*cis* glycosides completely stereoselectively (or stereospecifically, as quoted in the original literature) with the only failure reported when the method was applied to the synthesis of β-mannosides.[38] Another attractive feature of this glycosylation approach is that the activation could be performed in the presence of a catalytic amount of promoter and at ambient temperature. While tritylated acceptors were used in most of the reported transformations, a complementary procedure involving an unprotected hydroxyl group was also developed.[35] The major drawback of this technique was modest yields obtained during both the synthesis of glycosyl thiocyanates and glycosidations thereof. This drawback was arguably related to the propensity of thiocyanates to isomerize into the corresponding isothiocyanates. The latter would remain inert under the glycosylation conditions leading to average to modest yields, which could be further diminished by the migration of acetyl substituents that have been used as protecting groups throughout the original reports.

4.2 Results and Discussion

When 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranosyl-thiocyanate **4.1**[37] was allowed to react with glycosyl acceptor **4.2**[39] in the presence of catalytic amount (0.2 equiv.) of triphenylmethyl perchlorate[40] (TrClO₄) in 1,2-dichloroethane (DCE) at ambient temperature, the corresponding 1,2-*cis* linked disaccharide **4.5**[37] was obtained with complete stereoselectivity (conservative estimation α/β > 25:1, although no evidence of the corresponding 1,2-*trans* anomer could be detected by ¹H NMR) yet only in a modest yield of 65% (Scheme 4.1).
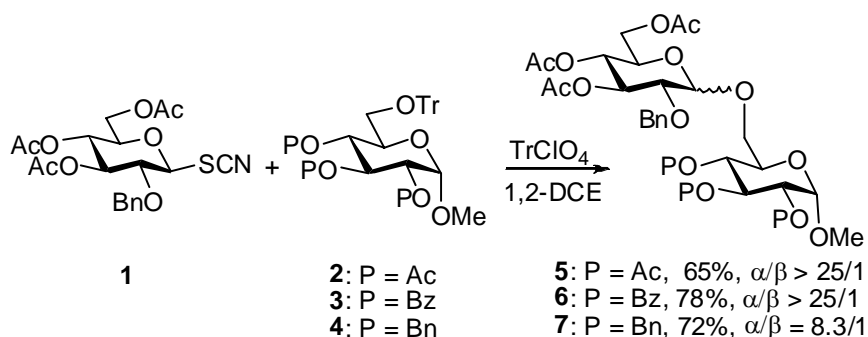
Scheme 4.1 Reaction of thiocyanate **4.1** with differently protected 6-O-trityl glycosyl acceptors **4.2-4.4**.

This result was in line with previously reported results for similar systems.[37] We assumed that the application of more stable benzoyl or benzyl protecting groups would enhance the overall stability of the glycosyl acceptor by reducing the acyl migration. Indeed, the disaccharide **4.6**,[41] derived from benzoylated glycosyl acceptor **4.3**,[42] was isolated in a significantly improved yield of 78% and with complete 1,2-*cis* stereoselectivity. However, when the structurally similar benzylated glycosyl acceptor **4**[43]

was employed, the corresponding disaccharide **4.7**[44] was obtained in a yield of 72%, but as a mixture of diastereomers (α/β = 8.3:1). This notable decrease in the stereoselectivity of glycosyl thiocyanates, which were previously described as glycosyl donors capable of the concerted stereospecific displacement, was intriguing. To the best of our knowledge, this was the first example wherein thiocyanate **4.1** of the D-gluco series gave the anomeric mixture, although prior to this study the stereoselectivity of glycosyl thiocyanates was only investigated with partially acetylated acceptors.[37] Hence, this a typical observation led us to a decision to reinvestigate glycosidations of thiocyanates in a greater detail. In principle, the effect of protecting groups on the reactivity of glycosyl acceptor has been noted.[16,45-47] It has been observed that strongly electron withdrawing ester protecting groups decrease electron density at the hydroxyl group (or triphenylmethoxy as in the examples depicted in Scheme 1) thus lowering its nucleophilicity and hence reactivity in glycosylation. However, still very little is understood about the actual effect that the glycosyl acceptor protection may have on stereoselectivity of glycosylation.[48] Basic knowledge in this area implies that an enhanced stereocontrol could be achieved with the less nucleophilic acceptors (such as **4.2** or **4.3**). Occasionally, a mismatch between donor-acceptor pair may result in the unexpected stereoselectivity outcome and/or reduced yields.[49-53] Hence, at first, we attributed the result obtained for disaccharide **4.7** to the increased reactivity of the glycosyl acceptor **4.4**, which implies that the glycosidation of glycosyl thiocyanate donors follows unimolecular rather than the earlier proposed concerted push-pool mechanism.[33,37]

In order to extend this finding, we employed common benzoylated (**4.10**)[42] and benzylated (**4.11**)[54] 6-OH glycosyl acceptors that were subjected to systematic investigation with the thiocyanate donor **4.1** along with a variety of other thioderivatives. As previously reported,[35] glycosyl thiocyanate **4.1** could be activated for reactions with non-tritylated acceptors in the presence of catalytic amount of TMSOTf. However, these rather sluggish reactions were commonly accompanied by the competing isomerization of glycosyl donor **4.1** into the corresponding isocyanate, which was reflected in low efficiency.[35] Although excellent stereoselectivity (α/β = 10.2:1) was observed with the benzoylated glycosyl acceptor **4.10**, the disaccharide **4.6** was obtained in only 45% yield (Entry 1, Table 1). The reaction with the more reactive glycosyl acceptor **4.11** was more rapid (36 vs. 48 h), which was also reflected in the improved yield of disaccharide (69%) yet displayed two-fold reduced stereoselectivity (Entry 2).

Since our key task was to compare glycosyl thiocyanates with other classes of thioglycosyl donors, such as thioimidates, we decided to search for the universal promoters of glycosylation that would activate both classes of glycosyl donors. If such promoters were available, one could standardize the reaction conditions across the spectrum of different classes of glycosyl donors to obtain comparable results by excluding the possibility of the promoter effect. Based on our previous reports, the most prominent results for glycosidation of *S*−benzoxazolyl (SBox, **4.8**)[41,55] and *S*-thiazolinyl (STaz, **4.9**)[44,56] glycosides have been obtained in the presence of AgOTf as the promoter. Hence, thiocyanate **4.1** was also investigated in the presence of stoichiometric amount of AgOTf. Relatively clean and fast activations (5–15 min) resulted in good yields for the coupling products **4.6** and **4.7** (78–84%, Entries 3 and 4). This result could serve as a

promising new beginning of comparative glycosylation studies with thiocyanates and thioimidates.

Table 4.1 Glycosylation of various glycosyl donors **4.1** (thiocyanate), 4.2 (*S*-benzoxazolyl) and **4.9** (*S*-thiazolinyl) with glycosyl acceptor **4.10** and **4.11**

1: R = SCN
8: R = SBox
9: R = STaz

10: P = Bz
11: P = Bn

6: P = Bz
7: P = Bn
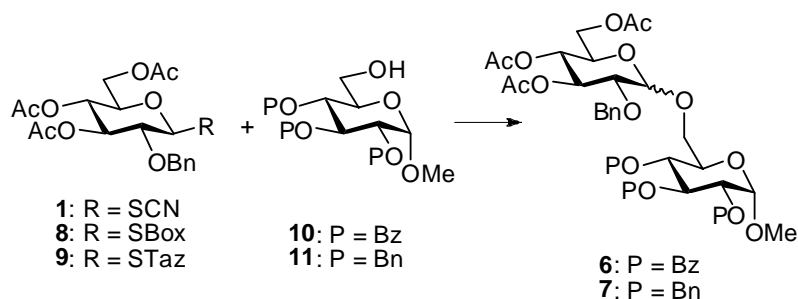
Entry	Donor	Acceptor	Cond's	Time	Product, Yield (%)	Ratio α/β
1	**1**	**10**	A	48 h	**6**, 45%	10.2/1
2	**1**	**11**	A	36 h	**7**, 69%	5.0/1
3	**1**	**10**	B	5 min	**6**, 84%	7.5/1
4	**1**	**11**	B	15 min	**7**, 78%	2.2/1
5	**8**	**10**	B	1 h	**6**, 92%	9.5/1
6	**8**	**11**	B	30 min	**7**, 84%	3.8/1
7	**9**	**10**	B	2 h	**6**, 89%	7.4/1
8	**9**	**11**	B	1.5 h	**7**, 81%	2.7/1

Reagents and conditions: All glycosylations were performed in 1,2-dichloroethane under argon at rt: A - TMSOTf (0.2 equiv); B - AgOTf (1.0 equiv for **1**, 2.0 equiv for **8** and **9**), 3Å molec. sieves

However, somewhat lower overall stereoselectivity was observed in comparison to that seen in TMSOTf-promoted glycosidations (Entries 1 and 2). Nevertheless, the

trend remained the same, and the disaccharide **4.6** derived from the benzoylated glycosyl acceptor **4.10** was obtained in a notably higher α stereoselectivity than its benzylated counterpart **4.7** from **4.11** (7.5:1 and 2.2:1, respectively). The latter result was particularly surprising considering the sturdy common knowledge that thiocyanates are amongst the most stereoselective glycosyl donors developed to date. Indeed, this example clearly illustrated that the effect of glycosyl acceptor and promoter of glycosylation cannot be underestimated and may have a prevailing effect on the reaction outcome.
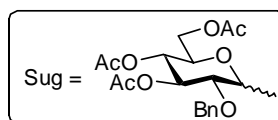
When SBox or STaz glycosides (**4.8** and **4.9**, respectively) have been investigated, a very similar trend has been observed, although these activations were slower than those of glycosyl thiocyanate **4.1**, and required at least 2.0 equivalents of AgOTf. Again, disaccharide **4.6** derived from the benzoylated glycosyl acceptor **10** was obtained with a much higher (approximately three-fold) α stereoselectivity (Entries 5 and 7) than its benzylated counterpart **4.7** from acceptor **4.11** (Entries 6 and 8). It should be noted that the rate of the reaction did not clearly correlate with the stereoselectivity observed: for example, slower glycosidations of the STaz derivative **4.9** provided lower stereoselectivity than that of the SBox glycoside **4.8**.

To gain a broader insight, a similar set of experiments have been performed using a variety of standard secondary glycosyl acceptors with the uniform benzoyl and benzyl protecting group pattern (**4.12**,[57] **4.14**,[58] **4.16**,[57] **4.18**,[59] **4.20**,[60] and **4.22**[61]). STaz glycosyl donor **4.9** was used in this comparative evaluation. As evident from the results presented in Table 4.2, the glycosylations of secondary glycosyl acceptors were in accordance with the observation made for primary glycosyl acceptors **4.10** and **4.11**. Thus, benzoylated glycosyl acceptors (**4.12**, **4.16** and **4.20**) gave consistently higher stereoselectivity (α/β∼

144

12:1, Entries 1, 3, and 5) for the formation of disaccharides (**4.13**, **4.17**, and **4.21**, respectively) than their benzylated counterparts (**4.14**, **4.18**, and **4.22**) provided for disaccharides **4.15**,[44] **4.19**, and **4.23**[44] ($\alpha/\beta \sim 6.5 - 9.3{:}1$, Entries 2, 4, and 6).

Table 4.2 The glycosylation of secondary acceptor **4.12**, **4.14**, **4.16**, **4.18**, **4.20**, and **4.22** with the STaz donor **4.9**

Glycosyl Donor 4.9	+	Glycosyl Acceptor 4.12, 4.14, 4.16, 4.18, 4.20, or 4.22	AgOTf (2 equiv) 3Å molec. sieves	Disaccharide 4.13, 4.15, 4.17, 4.19, 4.21, or4. 23

Entry	Acceptor	Time	Product, yield	Ratio α/β
1	**12**	16 h	**13**, 89%	11.7/1
2	**14**	14 h	**15**, 90%	6.8/1
3	**16**	12 h	**17**, 87%	12.1/1
4	**18**	8 h	**19**, 85%	6.5/1
5	**20**	12 h	**21**, 72%	12.0/1
6	**22**	6 h	**23**, 87%	9.3/1

4.12: P=Bz, R=H
4.13: P=Bz, R=Sug
4.14: P=Bn, R=H
4.15: P=Bn, R=Sug

4.16: P=Bz, R=H
4.17: P=Bz, R=Sug
4.18: P=Bn, R=H
4.19: P=Bn, R=Sug

4.20: P=Bz, R=H
4.21: P=Bz, R=Sug
4.23: P=Bn, R=H
4.23: P=Bn, R=Sug

Sug =
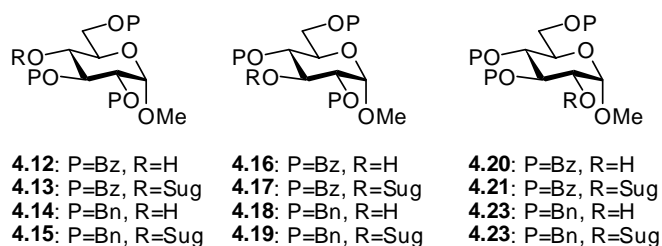
Since ethyl thioglycosides are inert in the presence of either TMSOTf or AgOTf, direct comparison of them with thiocyanates and thioimidates was not possible.

Nevertheless, we supposed that this general study may benefit from the indirect comparison with a common ethyl thioglycosides, even though its activation required entirely different activation protocol. Indeed, a very similar trend was achieved when ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside[62] was glycosidated in the presence of NIS–TfOH (or other common promoters for thioglycoside activation).[63-66] For example, disaccharide **4.6** derived from the benzoylated glycosyl acceptor **4.10** was obtained in a significantly higher α-stereoselectivity than its benzylated counterpart **4.7** (6.8:1 vs. 1.7:1, respectively). In addition, a very similar trend has been observed with per-benzylated STaz and SEt glycosyl donors (these results are not shown herein and will be reported elsewhere).

4.3   Conclusions

In conclusion, we observed that protecting groups on glycosyl acceptor have a profound effect on stereoselectivity of glycosylation. The effect of protecting groups of glycosyl acceptor on the stereoselectivity of glycosylation seemed very directly correlated to their electron-withdrawal power. Thus, we demonstrated that much higher α-stereoselectivity can be obtained with glycosyl acceptors bearing more electronwithdrawing benzoyl substituents in comparison to that of their benzylated conterparts. This improvement was consistently achieved with all classes of glycosyl donors investigated (thiocyanates, thioimidates, and thioglycosides) and was practically independent on the leaving group used. Both primary and secondary glycosyl acceptors showed the same trend; and these results were in line with generally accepted principles of the effect of protecting groups on glycosyl acceptor. The actual effect remains unclear,

but it is possible that a variety of factors may have to be considered: dipole moments of the entire molecule, dipole-dipole interactions, polarizability, donor-acceptor match-mismatch, the role of the promoter (and the activation mode), solvent, etc. Further investigation of the protecting and leaving group effects on stereoselectivity of glycosylation is currently underway in our laboratory.

4.4   Experimental part

4.4.1   General

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ^{o}C. 1, 2-Dichloroethane was distilled from CaH_2 directly prior to application in glycosylations. Molecular sieves (3Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to each application. AgOTf (Acros) was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to each application. ^{1}H-NMR spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C-NMR spectra were recorded at 75 MHz (Bruker Avance). HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

4.4.2 Typical glycosylation procedures

Method A. Typical AgOTf promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 200 mg) in $(ClCH_2)_2$ (2mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.11-0.22 mmol) was added and the reaction mixture was monitored by TLC. Upon completion (see Table), the reaction mixture was diluted with CH_2Cl_2 (20 mL), the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL), water (3 × 10 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/toluene gradient elution) to afford the corresponding disaccharide derivative.

Method B. TMSOTf promoted glycosylation procedure: A mixture the glycosyl donor (0.11 mmol) and glycosyl acceptor (0.10 mmol) and freshly activated molecular sieves (4 Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred for 1.5 h under argon. TMSOTf (0.022 mmol) was added and the reaction mixture was monitored by TLC. Upon completion, the reaction was quenched with a drop of pyridine. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 × 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/toluene gradient elution) to afford the corresponding disaccharide derivative.

Method C. NIS-TfOH promoted glycosidation of S-ethyl glycosides. A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in (ClCH$_2$)$_2$ (2 mL) was stirred for 1.5 h under argon. NIS (0.22 mmol) and TfOH (0.022 mmol) were added and the reaction mixture was monitored by TLC. Upon completion, the reaction mixture was diluted with CH$_2$Cl$_2$ (20 mL), the solid was filtered off and the residue was washed with CH$_2$Cl$_2$. The combined filtrate (30 mL) was washed with 20% aq. Na$_2$S$_2$O$_3$ (15 mL) and water (3 × 10 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate–toluene gradient elution) afford a disaccharide derivative.

Methyl 3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glycopyranosyl)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (4.17) was obtained from **9** and **16** in 89% yield (α/β = 11.7/1). Analytical data for **17**: R_f = 0.48 (ethyl acetate/toluene, 3/7, v/v); ^{1}H NMR: δ, 1.76, 1.78, 2.02 (3s, 9H, 3 × COCH$_3$), 3.27 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 6.46 (s, 3H; OCH$_3$), 3.76-3.87 (m, 3H, H-6a', 6b', ½ C*H$_2$*Ph), 4.06 (dd, 1H, $J_{5',6a'}$ = 10.4 Hz, $J_{5',6a'}$ = 5.3 Hz, H-5'), 4.17 (d, 1H, J^2 = 12.7 Hz, ½ C*H$_2$*Ph), 4.33 (m, 1H, H-5), 4.45 (dd, 1H, $J_{6a',6b'}$ = 14.4 Hz, H-6a'), 4.56-4.62 (m, 2H; H-3, 6b'), 4.74 (dd, 1H, $J_{4',5'}$ = 9.8 Hz, H-4'), 5.06 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1'), 5.10 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 5.22 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, H-3'), 5.36 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 5.68 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 6.91-8.06 (m, 20H, aromatic) ppm; ^{13}C NMR: δ, 20.7, 20.8, 20.9, 55.8, 61.8, 63.3, 67.8 (x2), 68.3, 71.4, 71.7, 72.5, 72.6, 76.3, 77.4, 97.3, 98.0, 127.9 (×2), 128.5 (×2), 128.6 (×2), 128.7 (×3), 129.9, 130.0 (×2), 130.7 (×2), 130.2 (×2), 133.3, 133.5, 133.6, 164.9, 165.8, 166.5,

169.7, 169.9, 170.8 ppm; HR-FAB MS: calcd for $C_{47}HB_{48}O_{17}Na$ [M+Na]$^+$: 907.2789 found: 907.2790.

Methyl 3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glycopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (4.19) was obtained from **9** and **18** in 90% yield (α/β = 6.8/1). Analytical data for **19**: R_f = 0.50 (ethyl acetate/toluene, 3/7, v/v); ^{1}H NMR: δ, 1.91, 1.94, 2.06 (3s, 9H, 3 × COCH$_3$), 3.35 (s, 3H, OCH$_3$), 3.52-3.79 (m, 3H, H-2, 2', 4), 3.76 (m, 1H, H-5), 3.89-3.93 (m, 2H, H-6a, 6b), 4.24 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.34-4.53 (m, 4H, H-6a', 6b', C*H*$_2$Ph), 4.59-4.68 (m, 4H, H-5', C*H*$_2$Ph), 4.73 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.84 (d, 1H, J^2 = 12.0 Hz, ½ C*H*$_2$Ph), 4.91 (dd, 1H, $J_{4',5'}$ = 10.3 Hz, H-4'), 5.49 (dd, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 5.60 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 6.90-7.35 (m, 20H, aromatic) ppm; ^{13}C NMR: δ, 20.9, 21.0, 21.1, 55.3, 62.0, 67.3, 68.6, 68.8, 70.0, 72.4, 72.8, 73.5, 73.7 (x2), 76.4, 77.4, 78.3, 78.9, 97.1, 97.7, 126.6 (×2), 127.4, 127.8 (×2), 127.9 (×2), 128.1 (×2), 128.8, 128.5 (×4), 128.6 (×2), 128.6 (×2), 128.8 (×2), 137.7, 137.9, 138.0, 138.6, 170.1, 170.3, 171.0 ppm; HR-FAB MS: calcd for $C_{47}H_{48}O_{17}Na$ [M+Na]$^+$: 907.2789 found: 907.2785.

Methyl 4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glycopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (4.13) was obtained from **9** and **12** in 87% yield (α/β = 12.1/1). Analytical data for **13**: R_f = 0.46 (ethyl acetate/toluene, 3/7, v/v); ^{1}H NMR: δ, 1.97, 2.01, 2.02 (3s, 9H, 3 × COCH$_3$), 3.37 (dd, 1H, $J_{2',3'}$ = 5.8 Hz, H-2'), 3.45 (s, 3H, OCH$_3$), 4.09 (m, 1H, H-5'), 4.16-4.26 (m, 3H, H-4, 6a', 6b'), 4.08 (m, 1H, $J_{5,6b}$ = 2.7 Hz, H-5), 4.66 (dd, 1H, H-6a), 4.78 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.85 (dd, 1H, $J_{4',5'}$ = 9.4

Hz, H-4'), 5.14 (d, 1H, $J_{1,2}$ = 2.1 Hz, H-1), 5.14-5.22 (m, 3H, H-1', CH_2Ph), 5.36 (dd, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 6.20 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3), 7.03-8.10 (m, 20H, aromatic) ppm; ^{13}C NMR: δ, 20.8 (×3), 55.7, 62.1, 63.6, 68.5, 68.6, 68.7, 71.7, 72.2, 72.4, 73.1, 75.7, 76.4, 77.4, 97.0, 98.0, 127.8 (×2), 128.0, 128.5 (×2), 128.6 (×3), 128.8 (×3), 129.2, 129.9 (×4), 130.1, 130.2 (×2), 133.3, 133.6 (×2), 137.7, 165.4, 166.3 (x2), 169.9, 170.0, 170.7 ppm; HR-FAB MS: calcd for $C_{47}H_{55}O_{14}$ [M+H]$^+$: 843.3592 found: 843.3594.

Methyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glycopyranosyl)-3,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (4.21) was obtained from **9** and **20** in 72% yield (α/β = 12/1). Analytical data for **21**: R_f = 0.40 (ethyl acetate/toluene, 3/7, v/v); ^{1}H NMR: δ, 1.83 (s, 3H; COCH_3), 1.99 (s, 6H; 2 × COCH_3), 3.51 (s, 3H; OCH_3), 3.53-3.60 (m, 2H, H-2', 5'), 3.87 (dd, 2H, $J_{6a',6b'}$ = 9.5 Hz, H-6a', 6b'), 3.94 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.39 (m, 1H, H-5), 4.46 (dd, 1H, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.57-4.60 (m, 2H, H-6b, ½ CH_2Ph), 4.64 (d, 1H, J^2 = 12.1 Hz, ½ CH_2Ph), 4.85 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 4.88 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.00 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1), 5.33 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, H-3'), 5.54 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 6.03 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 7.28-8.06 (m, 20H, aromatic) ppm; ^{13}C NMR: δ, 20.7, 20.8, 21.0, 56.0, 61.3, 63.3, 67.8, 68.0, 68.1, 69.8, 71.6, 72.0, 73.4, 77.1, 97.5, 97.6, 128.1 (×3), 128.4, 128.6 (×6), 128.8 (×3), 129.1, 129.7, 129.9 (×4), 130.1 (×2), 133.3 (×2), 133.6, 137.9, 165.6, 165.7, 166.4, 169.9, 170.0, 170.7 ppm; HR-FAB MS: calcd for $C_{47}H_{48}O_{17}Na$ [M+Na]$^+$: 907.2789 found: 907.2788

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CHAPTER V

Glycosidation of thioglycosides in the presence of bromine: mechanism, reactivity,

and stereoselectivity

_____

5.1 Introduction

Since the first glycosylation reactions were performed in the late 1800s, carbohydrate chemistry has evolved into a broad area of research that has persistently captured the interest of the scientific community. Nevertheless, the installation of the glycosidic linkage remains cumbersome due to the lack of understanding the mechanistic detail of glycosylation or the inability to translate such knowledge into practical application.[1,2] With significant recent advances in the rapidly expanding fields of glycosciences, the demand for reliable and stereocontrolled glycosylation technologies has increased. Owing to many recent breakthroughs in the field, the formation of many glycosidic bonds can be readily achieved;[3,4] however, it is the inability to effectively control the stereoselectivity of glycosylation that has proven to be the major synthetic hurdle.

Building upon our earlier discovery of the O-2/O-5 cooperative effect in glycosidation of superdisarmed glycosyl donors bearing 2-benzyl-3,4,6-triacyl protecting group pattern,[5] herein we present our study for the development of a highly stereocontrolled glycosylation using glycosyl bromides as glycosyl donors generated *in situ*. It has been our belief that a carefully controlled reaction pathway helps to ensure predictable, rather than serendipitous, outcome of chemical glycosylation, a remarkably complex and multi-step reaction of very high scientific and industrial significance as a vast majority of all sugars are bound via the glycosidic linkage. There are many complexities to consider when depicting the mechanism of the glycosylation reaction, and often a clear delineation between S_N1 and S_N2 nucleophilic substitution reactions is obscured.[6,7] Nevertheless, today it is generally presumed that the reaction conditions

favor that of a unimolecular S_N1 mechanism, and the reaction is thought to proceed through a total of four distinct steps: activation, dissociation, nucleophilic attack and deprotonation.[6]

Glycosyl bromides are arguably the best known and the most studied glycosyl donors to date. Historically being the first glycosyl donors investigated, glycosyl bromides are still in frequent use, particularly in glycosylations of simple aglycones. In the recent years, however, their utility for oligosaccharide synthesis has been diminished by the development of new classes of glycosyl donors. A very significant application of the accrued mechanistic and kinetic knowledge was the halide-ion catalyzed glycosylation developed by Lemieux *et al.* in 1975.[8] Through extensive theoretical studies, careful consideration of the reaction intermediates, and conformations thereof, it was found that a rapid equilibrium could be established between a relatively stable α-halide **A** and its more reactive β-counterpart **I**, by adding tetraalkylammonium bromide (Scheme 5.1). At first, the expulsion of α-halide **A** results in the formation of ion-pair **B**. Since no inverted product (**E**) is formed herein, it was concluded that the ion-pair **F** leading to the anomerized β-linked bromide **I** is a more energetically favorable pathway. The existence of alternate conformations for intermediates **G** and **H** *en route* to/from **I** was deemed necessary to form/activate the equatorial bond.[9] At this point, the highly unstable β-halide dissociates back into its ion pair (**I**→**G**), whereupon it quickly undergoes nucleophilic attack (**G**→**K**) to form the 1,2-*cis* product **L**. As an end result, nucleophilic substitution of the β-bromide **I** occurs favorably, whereas the *α*-bromide **A** anomerizes before glycosylation can occur.
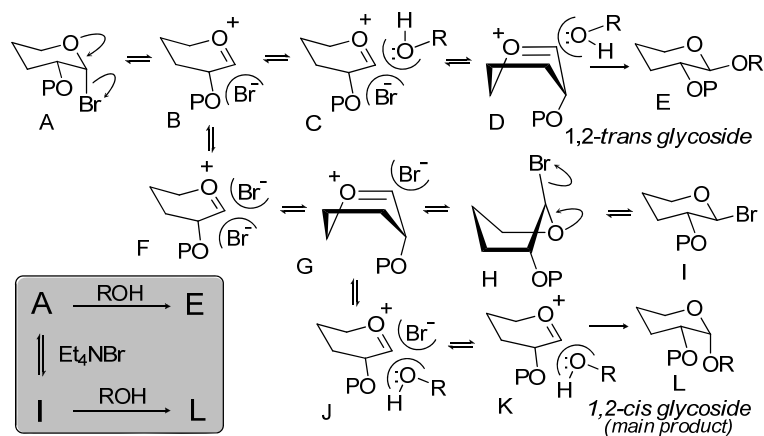
**Scheme 5.1** Lemieux's in situ anomerization concept.

Does this concept offer a practical application to synthesis of glycosides and oligosaccharides? Yes, but it lacks generality as it requires very reactive per-benzylated bromides (or iodides)[10] as glycosyl donors and provides satisfactory results mainly with the highly reactive series, L-fucose and D-galactose.
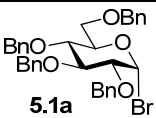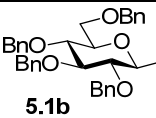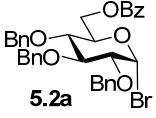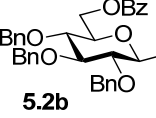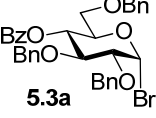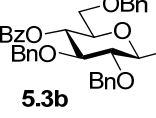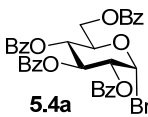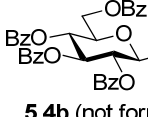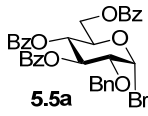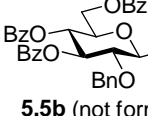
5.2   Results and Discussion

In our hands, this procedure was found less effective and thorough investigation of differently protected glucosyl bromides **5.1a-5.5a** in reactions with glycosyl acceptor **5.6**, showed it is it mainly applicable to glycosidation of per-benzylated glucosyl bromide **5.1a**. Herein and below, index **a** or **b** is used to designate α- or β-anomeric configuration in glycosyl donors, if known. Even then, the reaction was extremely sluggish and proceeded only half-way in 120 h (entry 1, Table 1). The stereoselectivity obtained for disaccharide **5.7** was good (α/β = 9/1), but far from being exclusive; and, in principle, the application of such uniformly protected building blocks in oligosaccharide synthesis is limited to the introduction of terminal units only. Our consecutive attempt to

differentiate the protecting group pattern by adding a single benzoyl substituent led to the significant decrease in the rate of glycosylation and yields (entries 2 and 3). Stereoselectivity and yields observed with 6-*O*-benzoyl bromide **5.2a** and 4-*O*-benzoyl bromide **5.3a** differed drastically indicating that the effect of the electron-withdrawal is very position dependable. Not surprisingly, all our attempts to activate the disarmed and superdisarmed bromides **5.4a** and **5.5a**, respectively, under Lemieux's inversion conditions have failed (entries 4 and 5).

Table 5.1 Glycosidation of differently protected glucosyl bromides **5.1a-5.5a** in the presence of Bu$_4$NBr.
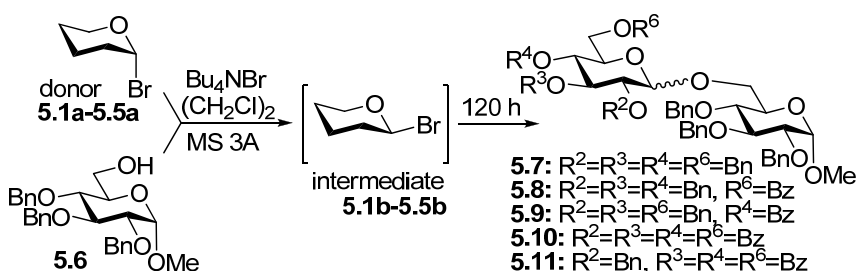
Entry	Glycosyl donor	Intermediate	Product (yield, α/β ratio)
1	**5.1a**	**5.1b**	**5.7** (50%, 9.0/1)
2	**5.2a**	**5.2b**	**5.8** (43%, 24/1)
3	**5.3a**	**5.3b**	**5.9** (15%, 6.2/1)

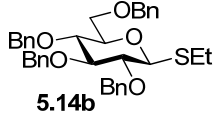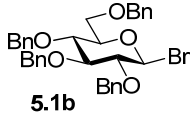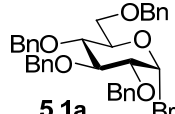|			
4	**5.4a**	**5.4b** (not formed)	**5.10** (no reaction)
5	**5.5a**	**5.5b** (not formed)	**5.11** (no reaction)

Interestingly, glycosyl bromide **5.5** generated from the corresponding β-ethylthio glycoside **5.12** in the presence of bromine reacted readily and provided disaccharide **5.11** as the α-linked diastereomer only (entry 1, Table 5.2). We also observed that no reaction would take place if the acceptor **5.6** was added at the later stage, when all thioglycoside **5.12** has been consumed. Strikingly, we also observed that bromide **5.5a** cannot be glycosidated directly by the addition of bromine (entry 2). These observations led us to a <u>working hypothesis</u> that *thioglycoside **5.12** is first converted into a highly reactive β-bromide **5.5b** that reacts readily with the glycosyl acceptor **5.6** to give the α-linked disaccharide **5.11** exclusively.* The literature precedent for this exists, and the formation of β-bromide from thioglycosides or selenium glycosides has been observed. In a majority of studies, however, the reaction of thioglycoside with bromine is used to generate the corresponding glycosyl bromide, which is usually seen as the α-anomer only upon isolation. Perhaps, in the absence of the glycosyl acceptor, the kinetic β-bromide rapidly equilibrates into the thermodynamically more stable α-anomer. If the glycosyl acceptor is present from the beginning, β-bromide **5.5b** can undergo direct nucleophilic displacement that arguably takes place via concerted bimolecular pathway resulting in the formation of α-linked glycoside only. Since the disaccharide **5.11** was

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isolated in only 28% yield along with 63% of α-bromide **5.5a**, we hypothesized that there is a competition between β-bromide glycosidation and anomerization, with both reactions being irreversible under these reaction conditions. *The α-bromide **5.5a** is stable under these reaction conditions and cannot be glycosidated,* which is significant as it helps to ensure complete stereoselectivity of such transformation.

Table 5.2 Comparative glycosidations of β-thioglycosides and their corresponding α-glycosyl bromides in the presence of bromine.
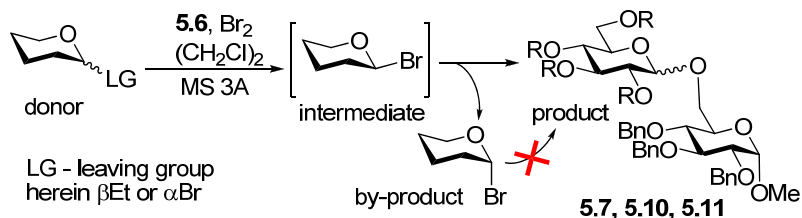
Entry	Glycosyl donor	Intermediate	Time	Product (yield, α/β ratio)
1	**5.12b** (OBz, BzO, BzO, BnO, SEt)	**5.5b** (OBz, BzO, BzO, BnO, Br)	16 h	**5.11** (28%, >25/1)
2	**5.5a** (OBz, BzO, BzO, BnO, Br)	**5b** (not formed)	16 h	**5.11** (no reaction)
3	**5.13b** (OBz, BzO, BzO, BzO, SEt)	**5.4b** (OBz, BzO, BzO, BzO, Br)	16 h	**5.10** (45%, β-only)
4	**5.4a** (OBz, BzO, BzO, BzO, Br)	**4b** (not formed)	16 h	**5.10** (no reaction)

5			10 min	**5.7** (71%, 2.2/1)
6		**5.1b**	10 min	**5.7** (67%, 1.5/1)

In a similar fashion, per-benzoylated thioglycoside **5.13b** was glycosidated in the presence of bromine to afford disaccharide **5.10** as β-linked product only in 45% yield (entry 3). Since α-bromide **5.4a** did not undergo glycosidation under these reaction conditions (entry 4), we conclude that the formation of **5.10** also takes place via the intermediacy of β-bromide **5.4b** only. Differently, since bromide **5.4b** is equipped with the participating group at C-2, it undergoes a two-step displacement via bicyclic acyloxonium ion (*vide infra*, see Scheme 3). Since no α-linked disaccharide formation was detected; this appears to be the only feasible route leading to the products of glycosylation, which copes well with the well-known effect of the neighboring protecting groups.

When a similar glycosylation was set up with per-benzylated thioglycoside **5.14b**, disaccharide **5.7** was readily obtained in a good yield of 71%, but as a mixture of diastereomers (entry 5). Also the corresponding α-bromide **5.1a** reacted readily under these reaction conditions (entry 6) providing nearly identical outcome to that obtained with thioglycoside **5.14b**. Perhaps, this reaction proceeds via the classic S_N1 pathway via the intermediacy of the flattened oxacarbenium ion (vide infra) resulting in scrambled stereoselectivity.[7]

In order to improve an outcome of the reaction between the superdisarmed thioglycoside **5.12b** and acceptor **5.6** in the presence of bromine leading to complete stereoselectivity, we attempted to delineate between two plausible reactions pathways, as follows. First, it is possible that the rate of formation of the diastereomeric bromides from the thioglycoside precursor are similar, but only the β-bromide then elaborates into the glycoside product while the α-counterpart remains intact. Second, it is also possible that the kinetic β-bromide forms exclusively first and then anomerizes into its thermodynamically more stable α-counterpart concomitantly with glycosidation. To differentiate between these two anticipated scenarios by which the glycosylation reaction may proceed we developed a toolkit for the reaction monitoring by ^{1}H NMR. First, we investigated the reaction of per-benzoylated β-thioglycoside **5.13b** with bromine, as depicted in Scheme 5.2. It was determined that α- and β-bromides **5.4a/5.4b** form rapidly (5 min) with a vast predominance of the latter (α/β = 1/11). This was followed by a relatively slow anomerization of β-bromide **5.4b** into its α-linked counterpart **5.4a** resulting in a nearly equal mixture of anomers with a slight predominance of **5.4a** after 16 h (see also entry 1 in Table 5.3).
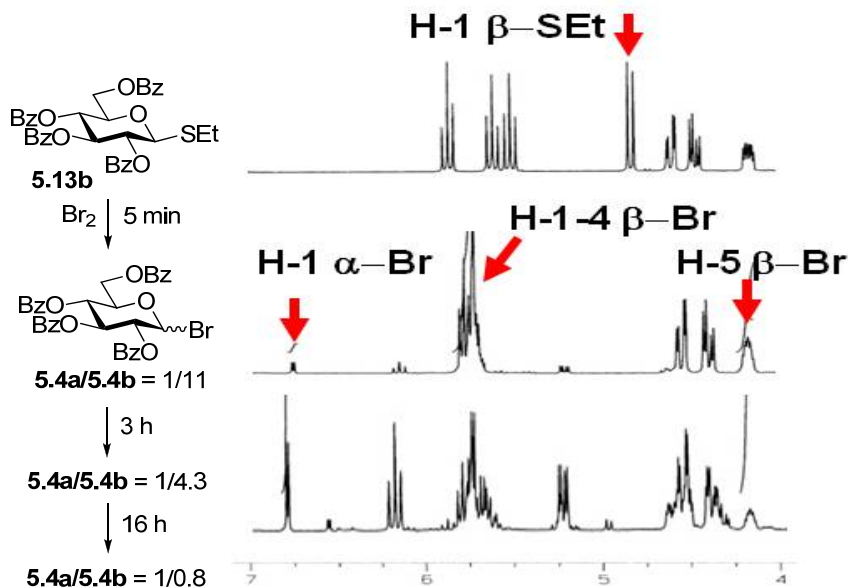
Scheme 5.2 In situ NMR monitoring of the reaction of thioglycoside **5.13b** with bromine.

Certainly, the predominant formation of β-bromide **5.4b** in this case can be attributed to the assistance of the neighboring benzoyl substituent at C-2. Hence, it was important to establish how the β-thioglycosides of the armed and superdisarmed series **5.14b** and **5.12b**, respectively, both bearing a non-participating 2-O-benzyl substituent, react. The results of this study are summarized in Table 5.3. The reaction of per-benzylated thioglycoside **5.14b** was very fast and by the time the first ^{1}H NMR was acquired, 5 min since the addition of bromine, α-bromide **5.1a** was present exclusively (entry 2, Table 5.3). Therefore, we attempted to perform the reaction monitoring at low temperature, which was performed as follows. Thioglycoside **5.14b** was dissolved in CDCl3, placed in the standard 5 mm NMR tube equipped with a septum, which was then placed into a dewar containing liquid nitrogen for 5 min. Bromine (1 equiv.) was added via syringe, the external cooling was removed, and the NMR tube was placed

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immediately into the magnet and the spectrum was recorded (approximately 5 min since Br$_2$ addition). This approach allowed us to detect an anomeric mixture of bromides **5.1a** and **5.1b** (α/β = 7.3/1), which then rapidly equilibrated into the pure α-bromide **5.1a** (entry 3, Table 5.3).

Next, the reaction of thioglycoside **5.12b** equipped with the superdisarming protecting group pattern (2-*O*-benzyl-3,4,6-tri-*O*-benzoyl) was investigated. As indicated by NMR, room temperature reaction resulted in the formation of a 2.1/1 mixture of bromides **5.4a/5.4b** with predominance of α-anomer **5.4a**. This mixture then began equilibrating slowly, and further accumulation of the α-anomer was monitored (entry 4, Table 5.3). The outcome of this study was that even though β-bromides only can react under these reaction conditions, the predominate formation of the α-bromide is a serious conceptual drawback of such an approach. This significantly diminishes the likelihood of further improvement of this reaction. For example, if one assumes that the β-anomer in a α/β = 2.1/1 mixture were to react entirely to form glycoside, the theoretical yield of such transformation based on the starting material cannot exceed 32%. This maximum yield correlates well with the actual observed yield of 28% for the formation of disaccharide **5.11** (see Table 5.2, entry 1), which corresponds to about 88% of conversion β-bromide **5.4b** into **5.11** leaving about 12% as a tribute to the competing anomerization process. This result is significant as it demonstrates that the glycosidation of **5.4b** is significantly faster than the competing anomerization into **5.4a**. Further evidence was acquired during the NMR-monitored glycosidation of **5.4a/5.4b** with CD$_3$OD as a glycosyl acceptor (Figure 5.1).
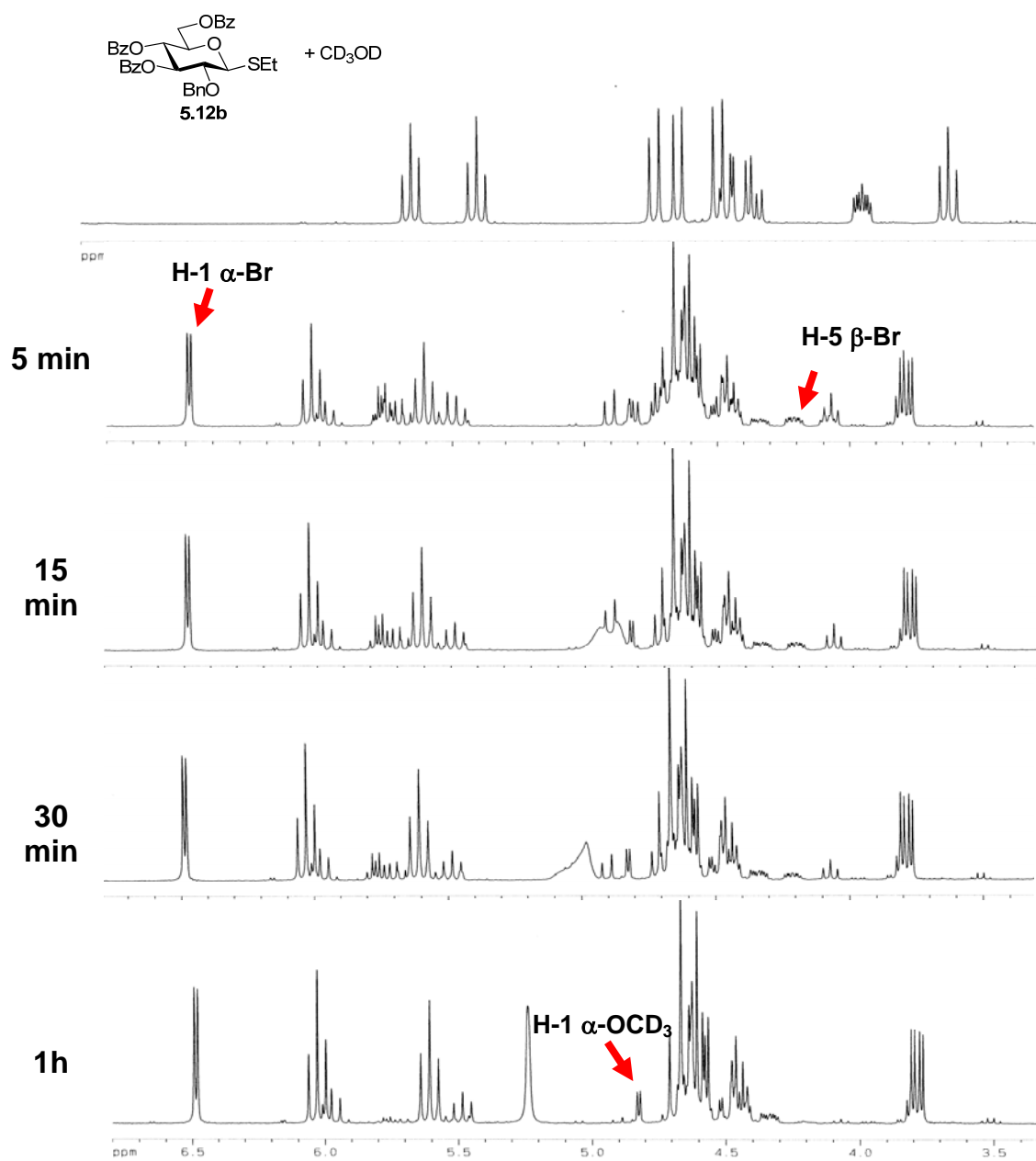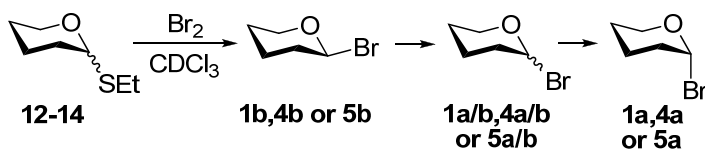
Figure 5.1 In situ NMR monitoring of the reaction of thioglycoside **5.12b** with bromine

in presence of CD$_3$OD.

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Table 5.3 Reactions of thioglycosides **5.12-5.14** with bromine: NMR monitoring of glycosyl bromide formation and anomerization.

Entry	Reaction	Temperature	Time recorded	Ratio α/β
1	**13b→4a/4b**	rt	5 min	1/10.7
			15 min	1/10.3
			30 min	1/9.0
			3 h	1/4.3
			16 h	1/0.8
2	**14b→1a/1b**	rt	5 min	>25/1
3	**14b→1a/1b**	Low (see text)	5 min	7.3/1
			10 min	8.9/1
			20 min	10.2/1
			30 min	12.7/1
4	**12b→5a/5b**	rt	5 min	2.1/1
			15 min	2.2/1
			1 h	2.4/1
5	**14a→1a/1b**	rt	5 min	>25/1
6	**14a→1a/1b**	Low (see text)	5 min	1/>25
			10 min	1/2.5
			15 min	1/0.9
			30 min	1/0.3
			1 h	>25/1
7	**12a→5a/5b**	rt	5 min	1/20
			15 min	1/17.3
			30 min	1/16.3
			3 h	1/11.5
			16 h	1/4.5

Since no Lemieux-like equilibrium could be established with the bromides of the unreactive series, the only possibility to improve the yield of glycosylation is to make sure that β-bromide is generated preferentially. In case of thioglycosides equipped with the neighboring participating group at C-2, such as **5.13**, or schematically represented as **A** in Scheme 5.3a, the β-thioglycoside undergoes a multi-step displacement via the intermediacy of the bicyclic acyloxonium ion **D**. Thus, upon interaction of **A** with bromine, active sulfonium intermediate **B** is generated. The promoter-assisted leaving group departure then results in the formation of oxacarbenium intermediate **C**. The leaving group departs as BrSEt that then disproportionates into ethyl disulfide (easily detectable by NMR) and bromine. Oxacarbenium ion **C** is then stabilized via the anchimeric assistance of the neighboring benzoyl group resulting in the formation of cyclic acyloxonium intermediate **D**. The formation of the latter can be used to explain the preferential formation of β-bromide **E** as the bottom; α-face of the ring is blocked for the bromide ion attack. In the case of β-thioglycosides **5.12** and **5.14**, schematically shown as **F** in Scheme 5.3b, there is no direct route that would lead to the stereoselective formation of the corresponding β-bromides. This reaction proceeds via the formation of oxacarbenium intermediate **G** and although β-bromide **H** is the kinetic product, our results show that more thermodynamically stable α-bromide formation predominates, with most β-bromide obtained in case of the superdisarmed series **5.5a/5.5b** (α/β = 2.1/1, see Table 5.3, entry 4).
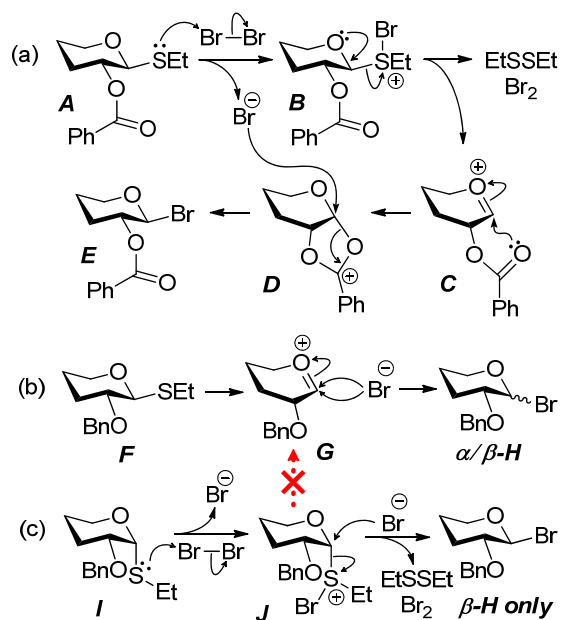
Scheme 5.3 Mechanistic rationale for the transformation of thioglycosides to bromides.

We conceptualized that the use of α-thioglycosides, generically shown as **I** in Scheme 5.3, as the starting material may offer a more direct route to obtain β-bromide **H**. Our underlined modified working hypothesis that has been adjusted as more experimental data was gathered is depicted in Scheme 5.3c. Thus, promoter-assisted activation of the leaving group of α-thioglycode **I** with bromine will lead to the formation of α-sulfonium ion **J**. Two possible pathways by which **J** may convert into bromides **H** can be assumed as follows. First, the formation of β-**H** takes place directly via the concerted nucleophilic (or close ion-pair) displacement of BrSEt leading to the complete inversion. Second, upon monomolecular departure of the activated leaving group in **J**, the reaction would proceed via the intermediacy of the oxocarbenium ion **G** that will result in the formation of the anomeric mixture of α/β-**H**, as it has been proven for reactions with β-thioglycosides **F**.

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To reveal the most likely reaction pathway by which α-ethylthio glycosides may elaborate into bromides, we obtained per-benzylated derivative **5.14a**. Disappointingly, an NMR experiment with **5.14a** performed at room temperature led to the formation of α-bromide **5.1a** exclusively (entry 5, Table 5.3). Very differently though, when this experiment was performed at low temperature (vide supra), β-bromide **5.1b** was formed nearly exclusively (α/β > 1/25, entry 6) with some trace amount of unreacted α-thioglycoside still remaining. After that, a rapid anomerization of **5.1b** resulted in complete anomerization into **5.1a** within 1 h of the addition of bromine. Assuming that the rate of this transformation would be very difficult to control, we obtained thioglycoside **5.12a** equipped with the super-disarming 2-benzyl-tri-benzoyl protecting group pattern. We anticipated that **5.12a** would provide the bromination and anomerization reaction rates that would be easier to comprehend. This in turn, may lead to the enhanced control of the subsequent glycosidation process.

Figure 5.2 shows a series of NMR spectra that have been acquired at different times of reaction (see also the summary in entry 7, Table 5.3). After recording the spectrum of the starting material **5.12a** (Figure 5.2a), bromine (1 equiv) was injected into the NMR tube. Subsequent NMR spectrum recorded at 5 min (Figure 5.2b) since the addition of Br_2 showed that bromides **5.5a/5.5b** formed in a ratio of α/β = 1/20 favoring the formation of β-anomer. The ratios were measured by comparison of the integral intensity of the signals corresponding to H-1 of α-bromide **5.5a** (6.48 ppm) and H-5 of β-bromide **5.5b** (4.20 ppm), which were clearly separable from the rest of the signals. At this time, about 24% of the starting material **5.12a** was still remaining, as judged by the integral intensity of its H-3 signal (5.96 ppm). This result indicates that the formation of

β-bromide takes place by the direct displacement of the activated leaving group (Figure 5.2c) rather than via the intermediacy of the oxacarbenium intermediate. Subsequent NMR spectra recorded at 15 and 30 min time points since the addition of bromine showed that the starting material was completely consumed (by 30 min), and the β-bromide began slow anomerisation into its more stable α-counterpart (Figure 5.2b and 5.2c). This result indicates that anomerization of the superdisarmed bromide **5.5b** is much slower than that observed for its armed, per-benzylated counterpart **5.1b**. Moreover, it **5.5b** anomerizes even slower that per-benzoylated disarmed bromide **5.4b**. Thus, the super-disarmed **5.5b** it is still present as the major anomer after 16 h ($\alpha/\beta = 1/4.5$), whereas **5.4b** has been succeeded by **5.4a** by the same time point ($\alpha/\beta = 1/0.8$, see entry 1, Table 5.3).
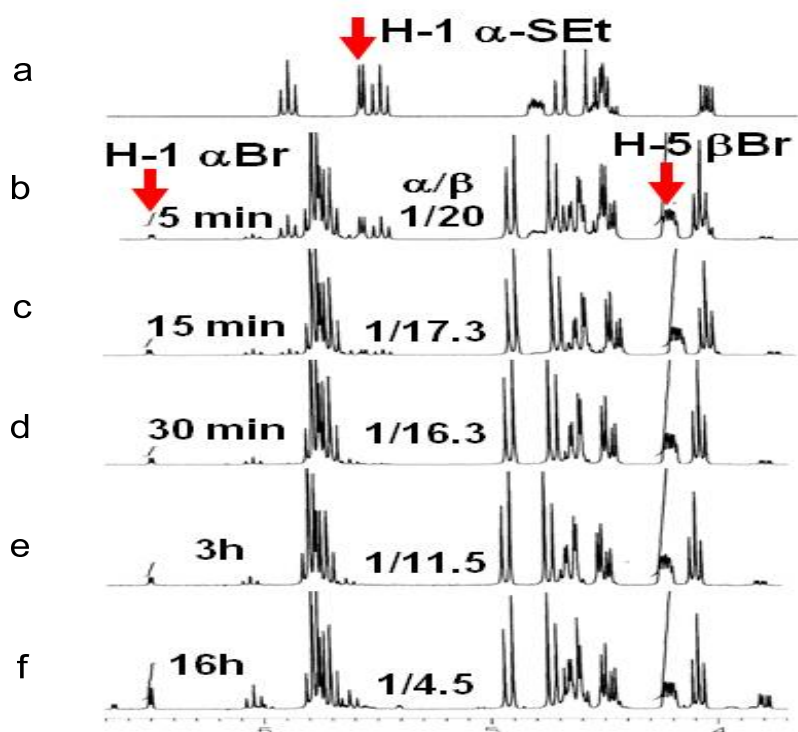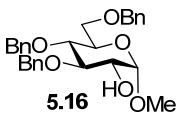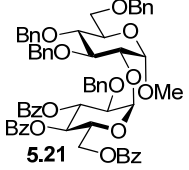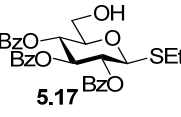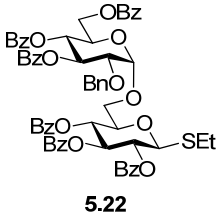

Figure 5.2 NMR monitoring of the conversion of thioglycoside **5.12a** into bromides **5a/5b** at rt.

Having established favorable reaction conditions for the formation of relatively stable β-bromide **5.5b** at room temperature, we turned our attention to studying its glycosidation. For this purpose we obtained a number of glycosyl acceptors ranging from simple aliphatic alcohols to secondary hydroxyls of monosaccharide acceptors. The results summarized in Table 5.4 clearly indicate the correlation between the reactivity of glycosyl acceptors and the yield: more reactive primary acceptors providing higher yields in glycosylations. In all cases, α-linked glycosides have been formed exclusively.

Table 5.4 Glycosidation of thioglycoside **5.12a** with various glycosyl acceptors
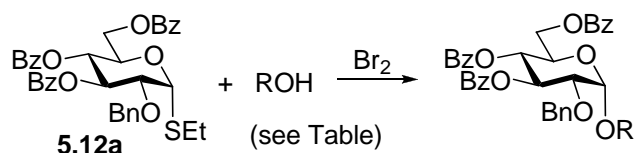
Entry	ROH	Time	Product	%yield, α/β ratio
1	MeOH	16 h	**5.18**	78%, >25/1P[a]
2	BnOH	16 h	**5.19**	75%, >25/1
3	**5.6**	16 h	**5.11**	67%, >25/1
4	**5.15**	48 h	**5.20**	35%, >25/1

5	**5.16**	48 h	**5.21**	27%, >25/1
6	**5.17**	24 h	**5.22**	42%, >25/1

Having achieved this promising outcome, we conceptualized that if an efficient equilibrium could be established between stable α-anomeric bromide **5.5a** and its more reactive b-linked counterpart **5.5b**, this would lead to the further improvement. Since the bromide **5.5a** cannot be anomerized in the presence of Bu$_4$NBr or Br$_2$ at room temperature (see Tables 5.1 and 5.2), it might be possible to anomerize it with the assistance of a metal-based promoter. As discovered in 1901 by Koenigs and Knorr[11] (and independently by Fischer and Armstrong[12]), glycosyl halides can be activated in the presence of Ag$_2$CO$_3$ or Ag$_2$O. The silver salt was used with the primary intention to scavenge the hydrogen halide by-product, and it was not until the early 1930's when it was realized that it plays an active role by assisting in leaving group departure.[13] This mild assistance resulted in the Koenigs-Knorr glycosylation reaction being very stereoselective and being one of the rare examples wherein a concerted bimolecular displacement occurs. Even in case of α-bromide equipped with the neighboring participating group, as noticed by Isbell,[14] the inversion product was obtained as a result

of concerted displacement, not participation due to the lack of the 1,2-orthoester formation.

Table 5.5 Attempts to anomerize unreactive α-bromide **5a** into its more reactive β-counterpart **5b**.
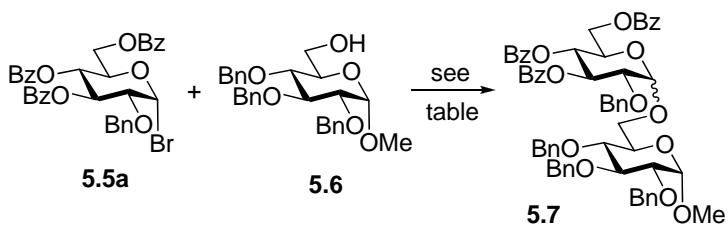

Entry	Promoter (equiv.)	Time	%yield, α/β ratio
1	TBAB (0.5-5)	72 h	No reaction
2	AgCO$_3$/TBAB (1-3/0.5-2)	72 h	No reaction
3	TBAB, 80 ^{o}C (1.0)	16 h	52% 5.6/1
4	80 ^{o}C	72 h	84% 7/1
5	KBr/18-crown-6 (1/0.5)	72 h	No reaction
6	HgBr$_2$ (1.0)	12 h	77% 9.3/1
7	HgBr$_2$/TBAB (1.0/0.5)	12 h	81% 9.5/1

It is possible that the anomerization can be achieved by using other heavy metal salt-based systems, although a delicate balance between reactivity and stereoselectivity should be kept in place to ensure success of this study. As shown by Helferich *et al.*,[15-17] mercury(II) salts offer a potent assistance for the leaving group departure. On the other

hand, Helferich's study serves as an important lesson to necessitate caution and find a balance between the reactivity and stereoselectivity as it was noted that faster reactions often result in a decreased stereoselectivity. Nevertheless, investigation of mercury-based reagents, such as mercury (II) bromide, seemed attractive as this reagent is capable of both assisting the leaving group departure and providing Br⁻. In order to prove the concept, we performed glycosidation of **5.5a** with **5.6** in the presence of HgBr₂ (1 equiv.) and obtained the expected disaccharide in 73% yield. Importantly, along with the notable increase of the yield, stereoselectivity remained high.

5.3   Conclusions
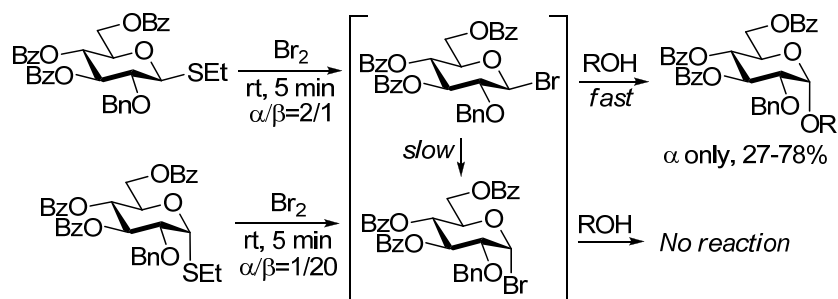
The conceptual similarity between this approach and the Lemieux method that was introduced in 1975 is undeniable. However, because no equilibrium between **5.5a** and **5.5b** can be established by the addition of tetraalkylammonium bromide, we believe that the concept described herein offers a novel complementary approach to glycosidation of relatively unreactive alkyl halides. The fact that α-bromide was found to be totally unreactive with glycosyl acceptors under these reaction conditions *is a very significant result* as it guarantees that its β-counterpart will be the only species en route to the

product. The yields achieved are similar to (or surpass) those achieved using traditional Lemieux method (see results in Table 5.1), but the complete stereoselectivity observed here is unmatched. Significantly, both simple aliphatic alcohols and the less reactive sugar acceptors provided complete α-stereoselectivity.

5.4 Experimental part

5.4.1 General

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). Preparative layer chromatography was performed on PLC silica gel 60 glass plates, Kieselgel 60 F_{254}, 1 mm (Merck). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at < 40 ^{o}C. CH_2Cl_2 and $ClCH_2CH_2Cl$ were distilled from CaH_2 directly prior to application. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. ^{1}H-n.m.r. spectra were recorded in $CDCl_3$ at 300 and 500 MHz, ^{13}C-NMR spectra were recorded in $CDCl_3$ at 75 and 125 MHz (Bruker Avance). HR FAB-MS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer, matrix *m*-nitrobenzyl alcohol, with NaI as necessary.

5.4.2   Synthesis of glycosyl donors

6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (5.2a)

The solution of ethyl 2,3,4-tri-*O*-benzyl-6-*O*-benzoyl-1-thio-β-D-galactopyranoside (0.5 g, 0.83 mmol) and activated molecular sieves (3 Å, 0.40 g) in CH_2Cl_2 12.5 mL) was stirred under argon for 1 h. Freshly prepared solution of Br_2 in CH_2Cl_2 (8 mL, 1/165, v/v) was then added and the reaction mixture was kept for 15 min at rt. After that, the solid was filtered-off and the filtrate was concentrated in vacuo at rt. The residue was purified by silica gel flash column chromatography (ethyl acetate-toluene gradient elution) to obtain compound **2a** as a white syrup in 59% yield (0.31 g, 0.50 mmol). R_f = 0.43 (ethyl acetate - hexane, 2/8, v/v); $[α]_D^{24}$ +122.56 (c = 1.0, CHCl$_3$); ^{1}H NMR: δ, 3.56 (ddd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.2, H-2), 3.73 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 4.11 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 4.27 (m, 1H, $J_{5,6a}$ = 3.02 Hz, H-5), 4.50-5.02 (m, 8H, 3×CH_2Ph, H-6a, 6b), 6.41 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 7.20-7.60 (m, 18H, aromatic), 7.90 (d, 2H, aromatic) ppm; ^{13}C NMR: δ, 62.6, 72.9, 73.9, 75.5, 76.1, 76.2, 77.6, 80.0, 82.2, 91.1, 128.1, 128.2 (×3), 128.3(×2), 128.4 (×3), 128.6 (×2), 128.7 (×4), 128.8 (×2), 129.8 (×3), 164.1 ppm. HR FAB MS [M+Na]$^+$ calcd for $C_{34}H_{33}O_6BrNa$ 639.1358, found 639.1332.

4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (5.3a)

The solution of ethyl 2,3,6-tri-O-benzyl-4-O-benzoyl-1-thio-β-D-galactopyranoside (0.5 g, 1.59 mmol) and activated molecular sieves (3 Å, 0.40 g) in CH_2Cl_2 (12.5 mL) was stirred under argon for 1 h. Freshly prepared solution of Br_2 in CH_2Cl_2 (8 mL, 1/165, v/v) was then added and the reaction mixture was kept for 15 min at rt. After that, the solid

was filtered-off and the filtrate was concentrated in vacuo at rt. The residue was purified by silica gel flash column chromatography (ethyl acetate-toluene gradient elution) to obtain compound **3a** as a white syrup in 70% yield (0.36 g, 0.58 mmol). R_f = 0.42 (ethyl acetate - hexane, 2/8, v/v); $[\alpha]_D^{24}$ +110.56 (c = 1.0, CHCl$_3$); ^{1}H NMR: δ, 3.55-3.72 (m, 3H, H-2, 6a, 6b), 4.16 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.34 (m, 1H, $J_{5,6a}$ = 3.7 Hz, H-5), 4.51 (dd, 2H, J = 2.5, 12.0 Hz, CH_2Ph), 4.77 (dd, 2H, J = 12.7 Hz, CH_2Ph), 4.88 (d, 1H, J^2 = 11.2 Hz, ½ CH_2Ph), 5.56 (dd, 1H, $J_{4,5}$ = 12.0 Hz, H-4), 4.47 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 7.13-7.50 (m, 17H, aromatic), 7.62 (m, 1H, aromatic), 8.00 (d, 2H, aromatic) ppm; ^{13}C NMR: δ, 67.9, 69.4, 73.1, 73.7, 74.1, 75.5, 79.3, 79.5, 91.4, 127.7, 127.8, 128.0 (×2), 128.2 (×3), 128.3 (×3), 128.3 (×4), 128.4 (×2), 128.5 (×2), 128.7 (×3), 129.6, 129.9 (×2), 133.4, 137.4, 137.5, 137.9, 165.2 ppm. HR FAB MS [M+Na]$^+$ calcd for C$_{34}$H$_{29}$O$_8$BrNa 639.1358, found 639.1343.

3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopuranosyl bromide (5.5a)

The solution of ethyl 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (1.0 g, 1.59 mmol) and activated molecular sieves (3 Å, 0.79 g) in CH$_2$Cl$_2$ (24 mL) was stirred under argon for 1 h. Freshly prepared solution of Br$_2$ in CH$_2$Cl$_2$ (15 mL, 1/165, v/v) was then added and the reaction mixture was kept for 15 min at rt. After that, the solid was filtered-off and the filtrate was concentrated in vacuo at rt. The residue was re-dissolved in CH$_2$Cl$_2$ and then washed with distill water. The organic phase was concentrated in *vacuo* at rt and then the residue was purified by silica gel column chromatography (ethyl acetate-toluene gradient elution) to obtain compound **1** as a white solid in 71% yield (0.76 g, 1.18 mmol). R_f = 0.52 (ethyl acetate - hexane, 4/6, v/v); $[\alpha]_D^{23}$ +65.21 (c = 1.0,

CHCl$_3$); ^{1}H NMR: δ, 3.78 (ddd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 4.45 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.54-4.70 (m, 4H, H-5, 6b, CH_2Ph), 5.60 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 6.03 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 6.48 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 7.22 – 7.57 (m, 14H, aromatic), 7.93 (dd, 4H, aromatic), 8.03 (dd, 2H, aromatic) ppm; ^{13}C NMR: δ, 62.3, 68.3, 72.3, 72.7, 76.7, 89.2, 128.1 (×2), 128.4 , 128.5 (×2), 128.5 (×2), 128.6 (×3), 128.7 (×3), 128.8, 129.5, 129.7, 129.9 (×4), 130.1 (×2), 133.3, 133.4, 133.7, 136.8, 165.4, 165.6, 166.3 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{34}$H$_{29}$O$_8$BrNa 667.0943, found 667.0970.

3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-β-D-glucopyranosyl bromide (5.5b)

The solution of ethyl 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-1-thio-α-D-glucopyranoside (0.03 mg, 0.04 mmol) in CD$_3$Cl (0.5 mL) in NMR tube was frozen in liquid nitrogen. After that, bromine solution 98% (2.4 μL, 0.04mol) was added directly to NMR tube. 1H NMR was recorded immediately. Selected analytical data for the β-isomer; ^{1}H NMR: δ, 4.07 (dd, 1H, $J_{2,3}$ =7.8 Hz, H-2), 4.20 (m, 1H, H-5), 4.46 (dd, 1H, $J_{5,6a}$ = 5.1 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.57 (dd, 1H, $J_{5,6b}$ = 3.1 Hz, H-6b), 4.72 (d, 1H, J^2 = 10.7 Hz, ½ CH_2Ph), 4.91 (d, 1H, J^2 = 10.77 Hz, ½ CH_2Ph), 5.67-5.81 (m, 3H, H-1, 3, 4), 7.19-8.05 (m, 20H, aromatic) ppm; ^{13}C NMR: δ, 63.0, 68.8, 74.8, 75.3, 76.4, 81.7, 82.6, 128.2, 128.4 (×2), 128.5 (×5), 128.6 (×4), 129.2, 129.6, 129.9 (×2), 130.0 (×3), 130.1 (×2), 136.9, 165.3, 165.6, 166.2 ppm.

5.4.3 General Glycosylation Procedures

Method A: typical TBAB (tetrabutyl ammonium bromide) promoted glycosylation procedure

A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under argon for 1 h., then TBAB (0.11 mmol) was added. The reaction mixture was stirred at rt and monitored with TLC. Upon completion, the reaction mixture was diluted with dichloromethane, the solid was filtered-off and the residue was washed with dichloromethane. The combined filtrate was washed with 20% aq. $NaHCO_3$ and water, the organic phase was separated, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to allow the corresponding disaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H-n.m.r. spectra.

Method B: typical Br$_2$ promoted glycosylation procedure

A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under argon for 1 h., then bromine solution, 99.8% (0.11 mmol) was added. The reaction mixture was stirred at rt and monitored with TLC. Upon completion, the reaction mixture was diluted with dichloromethane, the solid was filtered-off and the residue was washed with dichloromethane. The combined filtrate was washed with 20% aq. $NaHCO_3$ and water,

the organic phase was separated, dried with MgSO$_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to allow the corresponding disaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H-n.m.r. spectra.

Method C: typical Br$_2$ pre-activation glycosylation procedure

A mixture of the glycosyl donor (0.1 mmol) freshly activated molecular sieves (3Å, 200 mg) in (ClCH$_2$)$_2$ (2 mL) was stirred under argon for 1 h. Bromine solution 98% (0.1 mol) was added and the reaction mixture was stirred for 10 min at rt. After that, the solvent was removed out under *vacuo* at rt and dried in *vacuo* for 1 h. The solution of glycosyl acceptor (0.15 mmol of glycosyl in 2 mL (ClCH$_2$)$_2$ was added into the glycosyl flask and the reaction was stirred under argon for 3-5 days at rt. Upon completion, the reaction mixture was diluted with dichloromethane, the solid was filtered-off and the residue was washed with dichloromethane. The combined filtrate was washed with 20% aq. NaHCO$_3$ and water, the organic phase was separated, dried with MgSO$_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution) to allow the corresponding disaccharide. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in ^{1}H-n.m.r. spectra.

Methyl 6-*O*-(6-*O*-benzoyl-2,3,6,-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5.8)

This compound was obtained by *Method B* from compound **2a** and compound **6** in 45% yield as a white syrup (α/β = 24/1), R_f = 0.41 (ethyl acetate/hexane, 3/7, v/v); selected analytical data ^{1}H NMR: δ, 3.32 (ddd, 1H, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 9.6 Hz, H-2'), 3.46-3.54 (m, 3H, H-2, 4, 4'), 3.62 (m, 1H, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.72 (m, 2H, H-5, 6b), 3.90 (m 3H, J =9.8 Hz, H-3, 3',5'), 4.31 (dd, 1H, $J_{5',6a'}$ = 4.4 Hz, $J_{6a',6b'}$ = 11.9 Ha, H-6a'), 4.43 (dd, 1H, $J_{5',6b'}$ = 2.0 Hz), 4.47-4.64 (m, 7H, $J_{1',2'}$ = 3.3 Hz, H-1', 3×CH_2Ph), 4.70-4.75 (m, 2H, CH_2Ph), 4.81-4.93 (m, 5H, $J_{1,2}$ = 3.56 Hz, H-1, 3×CH_2Ph) ppm; δ, ^{13}C NMR : δ, 55.4, 63.6, 66.2, 69.1, 70.6.72.6, 73.5, 75,2 (×2), 75.9 (×2), 77.4, 78.0, 80.3, 80.4, 81.9, 82.3, 97.1, 98.1, 127.8 (×3), 127.9 (×2), 127.9 (×3), 127.9 (×2), 128.0 (×2), 128.1 (×2), 128.2 (×4), 128.4 (×3), 128.5 (×3), 128.5 (×3), 128.6 (×4), 129.8 (×3), 130.2, 133.2, 138.2, 138.3, 138.5 (×2), 138.7, 139.0, 166.4 ppm; HRFABMS [M+Na]$^+$ calcd for $C_{62}H_{54}O_{12}Na$ 1023.4295, found 1023.4257.

Methyl   6-*O*-(4-*O*-benzoyl-2,3,4,-tetra-*O*-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5.9)

This compound was obtained by *Method B* from compound **3a** and compound **6** in 15% yield as white syrup (α/β = 6.2/1), R_f = 0.41 (ethyl acetate/hexane, 3/7, v/v); Selected analytical data for the α-isomer ^{1}H NMR: δ, 3.38 (s, OCH$_3$), 3.47 (m, 2H, H-2', 4), 3.98-4.11 (m, 3H, H-3, 3', 5), 4.59 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 4.96 (d, 1H, $J_{1,2}$ = 2.5 Hz, H-1), 5.31 (dd, 1H, $J_{4',5'}$ = 9.45 Hz, H-4') ppm; ^{13}C NMR : δ, 55.3, 69.1, 69.3, 70.6, 71.1, 72.7, 73.6, 73.7, 75.1, 75.2, 75.9, 77.4, 78.1, 78.4, 79.9, 80.3, 82.4, 127.5 (×2), 127.6 (×2), 127.8, 127.8 (×3), 127.9 (×3), 128.1 (×2), 128.2 (×2), 128.2 (×2), 128.3 (×2), 128.3, 128.4, 128.5 (×3), 128.6 (×2), 128.6 (×3), 129.9 (×2), 130.1, 133.2, 138.0, 138.3, 138.5,

138.7, 139.0, 165.46 ppm; HRFABMS [M+Na]$^+$ calcd for $C_{62}H_{54}O_{12}Na$ 1023.4295, found 1023.4337.

Benzyl 3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (5.19) was obtained by *method B* from glycosyl donor and benzyl alcohol, as a clear film in 75% yield. Analytical data for **5.19** : R_f = 0.35 (ethyl acetate-toluene, 0.5/9,.5 v/v); $[α]_D^{26}$ +29.30 (*c* = 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ, 3.78 (ddd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 4.37-4.51 (m, 6H, C*H*$_2$Ph, H-5, 6a, 6b), 4.62 (d, 1H, J^2 = 12.3 Hz, ½ C*H*$_2$Ph), 4.98 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.99 (d, 1H, J^2 = 15.0 Hz, ½CH$_2$Ph), 5.49 (dd, 1H, $J_{4,5}$ = 9.93 Hz, H-4), 6.03 (dd, 1H $J_{3,4}$ = 9.7 Hz, H-3), 7.14-7.60 (m, 19H, aromatic), 7.91 (m, 4H, aromatic), 8.02 (d, 2H, aromatic) ppm; ^{13}C NMR (CDCl$_3$): δ, 31.4, 63.4, 68.1, 69.6, 70.1, 72.2, 77.4, 72.8, 95.5, 128.0 (×2), 128.1, 128.2, 128.4 (×2), 128.5 (×2), 128.6 (×5), 128.7 (×3), 128.8, 129.1, 129.9 (×3), 130.0 (×3), 130.1 (×2), 133.2, 133.3, 133.5, 136.9, 137.7, 165.7, 165.9, 166.4 ppm; HR-FAB MS calcd. for $C_{41}H_{36}O_9Na$ 695.2257, found 695.2244.

Ethyl 6-*O*-(2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzoyl-thio-β-D-glucopyranoside (5.22)

This compound was obtained by *Method B* from ethyl 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-1-thio-α-D-glucopyranoside and ethyl 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside in 42% yield as a white syrup, Analytical data for **5.22** : R_f= 0.62 (ethyl acetate/toluene, 1/9, v/v); $[α]_D^{25}$ +28.06 (*c* = 1.0, CHCl$_3$); ^{1}H NMR: δ, 1.39 (t, 3H, J = 7.4 Hz, C*H*$_3$CH$_2$S), 2.83 (m, 2H, CH$_3$C*H*$_2$S), 3.37 (d, 1H, $J_{6b,6a}$ = 10.7 Hz, H-6b), 3.79 (ddd, 1H, $J_{1',2'}$ = 3.3 Hz, $J_{2',3'}$ = 9.9 Hz, H-2'), 4.00 (dd, 1H, $J_{6a,6b}$ = 10.4 Hz, H-6a), 4.19 (dd, 1H, $J_{5,6a}$ = 8.3

Hz, H-5), 4.36 (dd, 1H, $J_{5',6a'}$ = 5.6 Hz, $J_{6a',6b'}$ = 12.1 Hz, H-6a'), 4.51–4.53 (m, 2H, H-5',

6b'), 4.56 (d, 1H, J^2 = 12.53 Hz, ½ CH_2Ph), 4.64 (d, 1H, J^2 = 12.54 Hz, ½ CH$_2$Ph), 4.85

(d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1'), 4.88 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 5.39 (dd, 1H, $J_{4',5'}$ = 9.6

Hz, H-4'), 5.48 (dd, 2H, J = 10.0 Hz, H-2, 4), 5.92 (dd, 1H, $J_{3',4'}$ = 9.4 Hz, H-3'), 6.0 (dd,

1H, $J_{3,4}$ = 9.8 Hz, H-3), 7.10–7.98 (m, 35H, aromatic) ppm; δ, ^{13}C NMR : δ, 14.3, 24.9,

63.1, 67.2, 68.1, 69.7, 69.9, 70.9, 72.1, 73.3, 74.3, 84.1, 89.6, 96.6, 125.5, 128.1 (×3),

128.4, 128.5 (×3), 128.5 (×4), 128.6 (×3), 128.6 (×2), 128.7 (×3), 128.9, 129.1, 129.2,

129.4, 129.8, 129.9 (×3), 130.0 (×2), 130.1 (×3), 130.1 (×3), 133.2, 133.3, 133.4, 133.4,

133.5, 133.7, 137.7 (×2), 165.3, 165.4, 165.6, 165.8, 165.9, 166.2 ppm; HRFABMS

[M+Na]$^+$ calcd for C$_{63}$H$_{56}$NO$_{16}$SNa 1123.3187, found 1123.3198.

Deuterated methoxy 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-α-D-glucopyranoside

This compound was obtained by *Method A* from ethyl 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl-1-

thio-α-D-glucopyranoside (1 equiv) and deuturated methanol (2 equiv) in 74 % yield as a

white syrup, R$_f$ = 0.5 (ethyl acetate/hexane, 4/6, v/v); [α]$_D^{25}$ +7.7 (*c* = 1.0, CHCl$_3$); ^{1}H

NMR: δ, 3.83 (ddd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 4.37 (m, 1H, H-5), 4.47 (dd,

1H, $J_{6a,6b}$ = 12.01 Hz, $J_{5,6a}$ = 5.5 Hz, H-6a), 4.58 (dd, 1H, H-6b), 4.67 (dd, J = 3.09, 12.49

Hz, CH_2Ph), 4.90 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 5.48 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 6.02 (dd,

1H, $J_{3,4}$ = 9.7 Hz, H-3), 7.30 (m, 4H, aromatic), 7.45 (m, 7H, aromatic), 7.55 (m, 3H,

aromatic), 7.97 (m, 4H, aromatic), 8.05 (d, 2H, aromatic) ppm; ^{13}C NMR.: δ, 63.4, 67.8,

70.1, 72.1, 73.3, 98.1, 128.1 (×3), 128.2, 128.5 (×2), 128.5 (×4), 128.6 (×2), 129.2, 129.8

(×3), 129.9 (×2), 130.0 (×2), 130.1 (×2), 133.2, 133.3, 133.5, 165.7, 165.8, 166.3 ppm;

HR FAB MS [M+Na]$^+$ calcd for C$_{35}$D$_3$H$_{29}$O$_9$Na 622.2129, found 622.2132.

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CHAPTER VI

Development of CD14-targeting therapeutics: synthesis and evaluation of

glucopyranoside-spacer-amino acid motifs

6.1   Introduction

Septicemia is a serious world-wide health problem associated with mortality rates of 40-60%.[1] It has been estimated that 1% of hospital patients and 20-30% of ICU patients develop sepsis. The cardiovascular consequences of septic shock resulting from bacterial infections include myocardial dysfunction that develops in nearly all patients,[2] vascular tone and permeability abnormalities, as well as abnormal oxygen delivery and metabolism. As a result, vital organs, such as the brain, heart, kidneys, and liver may be affected or may fail, and this is reflected in over 100,000 deaths annually in the US.[1] It is well established that septic shock is initiated by movement of bacterial endotoxin (or lipopolysaccharide, LPS) into the blood stream. LPS, a vital component of the outer leaflet of the gram-negative outer membrane, has been shown to be a principle mediator of the depression of left ventricular function and myocardial contractility.[3] LPS is comprised of three structural regions (O-antigen, core-oligosaccharide, and Lipid A), the Lipid-A region of which consists of a polyacylated glucosamine disaccharide and is largely responsible for the toxic activity.[4] The results of recent studies suggest that concurrent evidence of a proinflammatory response to LPS is by far more important than detection of LPS in circulation.[5,6] The effects of LPS are initiated after it interacts with a plasma LPS-binding protein (LBP), which has strong affinity for both the Lipid-A region of endotoxin and glycosylphosphatidyl inositol-anchored LPS receptor CD14 on mononuclear phagocytes. When LPS-LBP complex interacts with CD14 (and then TLR-4), the cells produce proinflammatory factors, such as tumor necrosis factor α (TNFα) (Figure 6.1).

Due to recent advances in the understanding of structure-function relationships for LPS, some generalizations regarding the most important structural determinants that result in the endotoxic activity of Lipid-A were made.[7] These determinants include the number and chain length of fatty acids (lipids). Typically, 1,4'-diphosphate disaccharides of the *E. coli* type are used (**6.1**, Figure 6.1), but their fair stability (chemical or *in vitro*) has been a major drawback in their synthesis and application. Although the exact role of the phosphate moieties is still unknown, the findings that showed 1-hydroxyl-4'-O-phosphate derivative as inactive gave rise to a belief that omitting at least one phosphate results in the entire loss of activity.[7,8]

In an effort to explore molecules that will bind with high affinity to CD14 without activating the inflammatory cascade, our laboratories have been designing simplified Lipid A analogs that lack the complexity of the highly lipidated diphosphorylated disaccharide core yet still maintain potent antagonistic activity against LPS. Herein we present the synthesis and unprecedented LPS-antagonistic activity of the methyl glucopyranoside-amino acid conjugates. This project was inspired by observations that structurally modified or totally unrelated to Lipid A can still exhibit potent antagonistic activity. Amongst a myriad of research articles that have been disseminated in the past years, the following three groundbreaking discoveries appeared to us to be of a particular inspiration and relevance to our own attempts.

First, as reported by Boons *et al.* strong binding and antagonistic effect was achieved even with 'phosphateless' *Rhizobium* syn-1 disaccharide **6.2**, which bears a lactone moiety at the reducing end (Figure 6.1).[9,10] This finding shows that one could avoid hurdles associated with labile phosphate group installation, handling, and storage.

Second, Shiozaki *et al.* showed that strong anti-LPS antagonistic response can be achieved even with disaccharide **6.3**, in which the non-reducing glucosamine is replaced with glucose. In addition, instead of chiral β-hydroxy/acyl esters, relatively simple alkyl chains were used at C-3 and 3' positions, whereas a β-ketoamide moiety is used at C-2.[8]
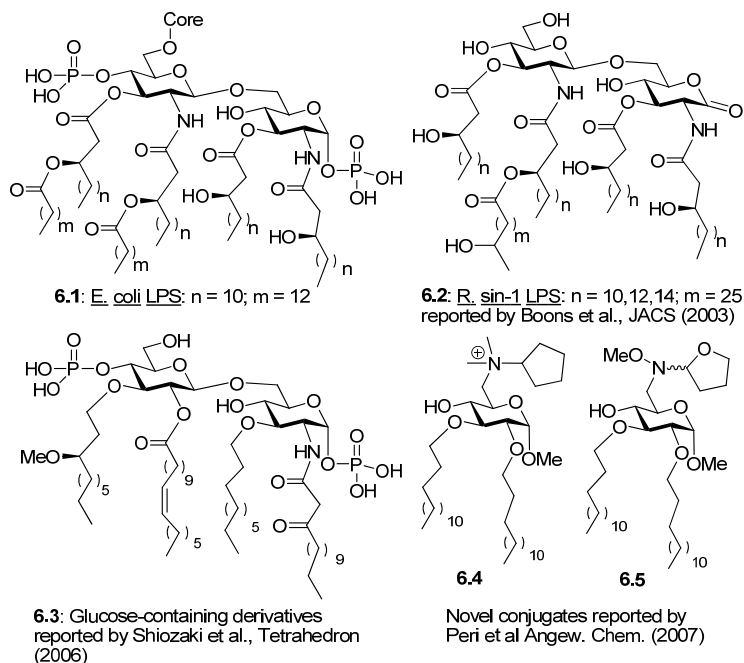

Figure 6.1 Structure of monsaccharide and disaccaharide Lipid A analogs

Third, Peri *et al.* demonstrated that even simple methyl glycoside-heterocycle conjugates **6.4** and **6.5** bearing alkyl chains instead of traditional lipids provide antagonistic activity.[11] To this end, we found it attractive to develop molecules that contain the following: a methyl glucoside core to simplify the synthesis, hydrophobic chains to facilitate membrane intercalation, and amino acids to provide an ionic character. Here we report a highly efficient synthesis of molecules of this type and biological studies of their endotoxic activity *in vitro*.

6.2   Results and Discussion

The synthesis began with the conversion of commercially available methyl α-D-glucopyranoside **6.6** into methyl 2,3-di-O-benzyl-α-D-glucopyranoside **6.7** via sequential 4,6-benzylidene acetal formation, 2,3-dibenzylation, and acetal cleavage accomplished in 67% overall yield (Scheme 6.1).   4,6-Diol **6.7** was then succinoylated at the primary position with succinic anhydride in the presence of 4-dimethylaminopyridine (DMAP) in pyridine to afford derivative **6.8** in 71 % yield.   The carboxyl moiety of the linker was then coupled with 3-hydroxyl of protected L-serine derivative **6.9**. This was accomplished using *N,N'*-diisopropylcarbodiimide (DIC) as the coupling reagent in the presence of DMAP in pyridine to afford conjugate **6.10** in 80% yield.   Having obtained the key construct **6.10**, we decided to pursue further protecting group transformations. The main driving force for these synthetic manipulations was to obtain a series of simple analogs that would allow us to investigate the effect of cationic and anionic character on LPS-antagonistic activity *in vitro*.   With this objective in mind, carboxylated compound **6.11** was obtained from **6.10** by cleavage of *tert*-butyl ester in presence of TFA/DCM in 81% yield.   Alternatively, the Fmoc protecting group could also be removed from compound **6.10** with piperidine in DMF to afford free amine **6.12**. Subsequently, we also obtained compound **6.13** having both carboxyl and amine groups unprotected. This was accomplished by the treatment of compound **6.12** with TFA/CH$_2$Cl$_2$ to give derivative **6.13** in 75% yield.
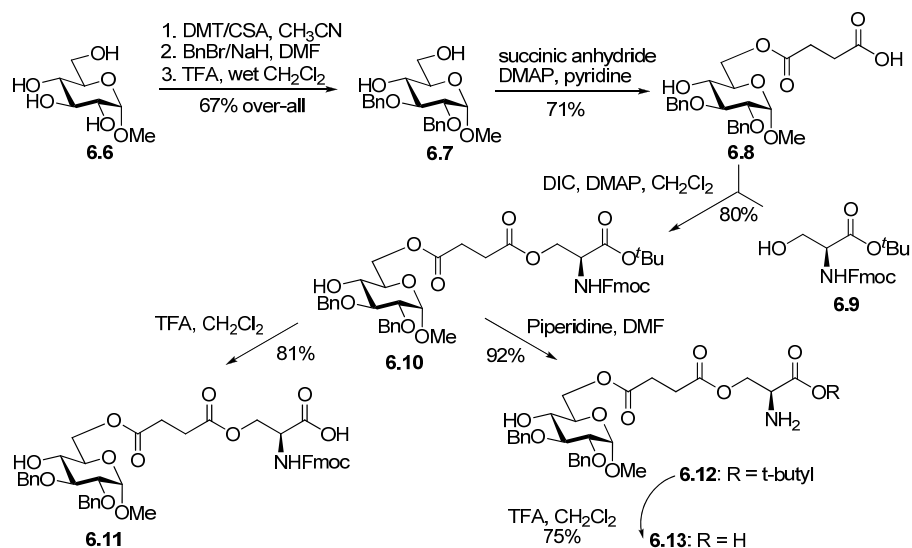
Scheme 6.1 Synthesis of monosaccharide Lipid analog **6.10-6.13**

The inhibitory activity of compounds **6.10-6.13** on LPS-induced TNFα production was investigated *in vitro* using THP-1 macrophages. Compounds **6.10, 6.12,** and **6.13** exhibited no inhibitory activity against LPS-induced TNFα production in the concentration range of 0.1 nM to 10 μM (Figure 6.2A-C). These compounds were also tested in the absence of LPS and demonstrated no agonist activity (data not shown). Compound **6.11** which has a free carboxylic group was able to significantly inhibit LPS-induced TNFα production at concentrations greater than 10 μM (Figure 6.3). Unfortunately, cell viability measurements using an XTT reduction assay indicated that compound **6.11** was toxic to the cells in the 30-100 μM range. Although there was a gap between inhibition and toxicity at 30 and 50 μM, the similarities between the inhibition and toxicity curves suggested that much of the antagonistic activity by **6.11** was related to toxicity.

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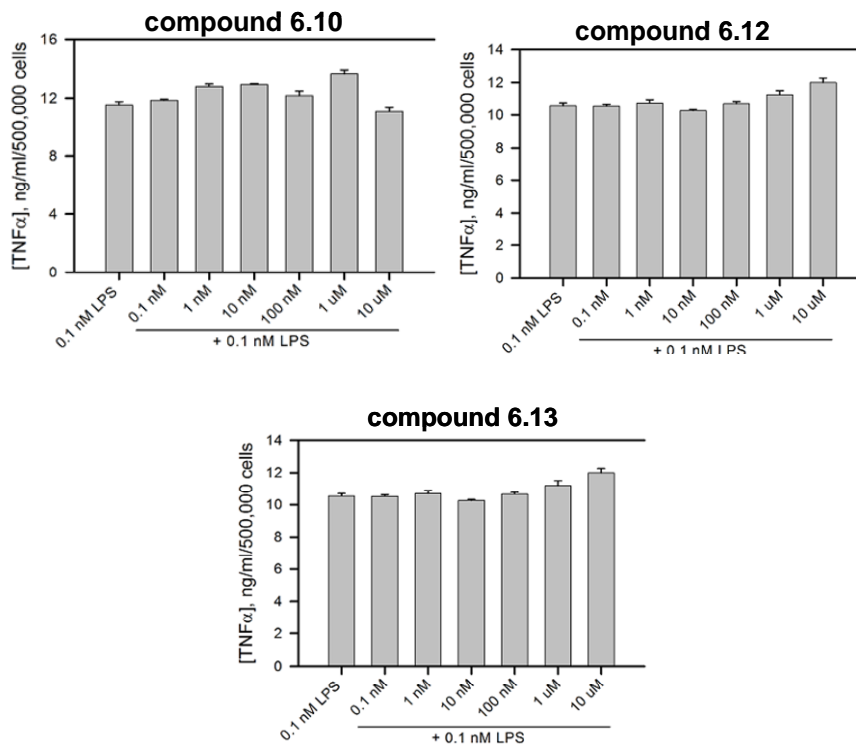
Figure 6.2 Antagonistic activity of compound **6.10**, **6.12** and **6.13** (Experimental work and data analysis done by Maria Udan in the laboratory of Dr. MR Nichols)
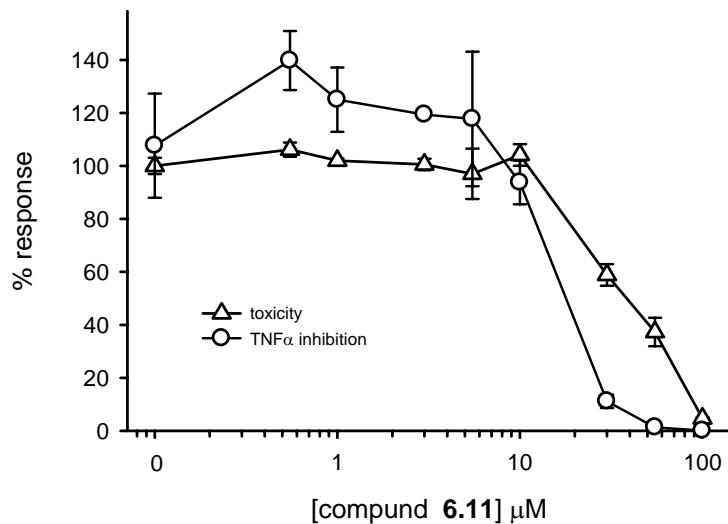
Figure 6.3 Antagonistic activity and toxicity of compound **6.11**(Experimental work and data analysis done by Geeta Paranajape in the laboratory of Dr. MR Nichols)

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With the purpose of gaining further insight into the effect of various substituents on endotoxic activity of monosaccharide-amino acid conjugates we obtained an analog of compound **6.11** in which benzyl groups have been replaced with acyl (myristoyl, C14) fatty acid chains. The synthesis of lipidated analog **6.19** was accomplished as depicted in Scheme 6.2.
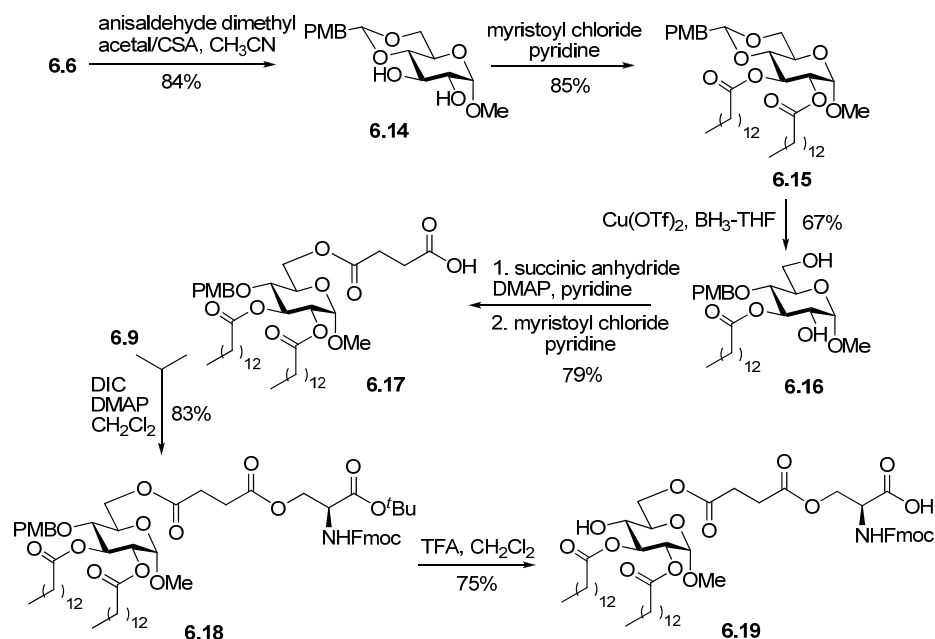
Scheme 6.2 Synthesis of monosaccharide Lipid A analog **6.19**

Methyl glycoside **6.6** was protected as 4,6-*O*-(*p*-methoxybenzylidene) acetal **6.14** by treatment with anisaldehyde dimethylacetal in presence of camphorsulfonic acid in 89% yield. The acylation of **6.14** with myristoyl chloride in the presence of pyridine furnished compound **6.15** in 85% yield. The benzylidene ring in **6.15** was reductively opened by treatment with BH$_3$-THF catalyzed with Cu(OTf)$_2$ to obtain unexpected product **6.16** lacking the C-2 acyl chain in 67% yield. The loss of the acyl chain was

rather unexpected, but this glitch was overcome by regioselective succinoylation with succinic anhydride in pyridine at the primary C-6 position followed by C-2 acylation with miristoyl chloride. This two-step one-pot procedure allowed us to obtain compound **6.17** in 79% yield. L-serine derivative **6.9** was linked to the carboxyl group of **6.17** of via DIC-mediated coupling in the presence of DMAP. The target monosaccharide-amino acid conjugate **6.18** was obtained in 83% yield. Acid treatment of the fully protected compound **6.18** led to concomitant cleavage of the *p*-methoxybenzyl (PMB) group at C-4 and *tert*-butyl ester. As a result, compound **6.19** was isolated in 75% yield.

The synthesis of alkylated analog **6.24** was accomplished as depicted in Scheme 6.3. Intermediate **6.14** was di-alkylated at C-2 and C-3 with myristyl bromide in presence of NaH to afford compound **6.20** in 72% yield. The benzylidene ring in **6.20** was reductively opened by treatment with BH_3-THF catalyzed with $Cu(OTf)_2$ to obtain product **6.22** in 87%. Acylation of **6.22** with succinic anhydride led to compound **6.22** in 89% yield. L-serine derivative 6.**9** was then linked to the carboxyl group of **6.22** via DIC-mediated coupling in the presence of DMAP. The resulting monosaccharide-amino acid conjugate **6.23** was obtained in 79% yield. Acid treatment of the fully protected compound **6.23** led to concomitant cleavage of the *p*-methoxybenzyl (PMB) group at C-4 and *tert*-butyl ester. As a result, target compound **6.24** was isolated in 81% yield.
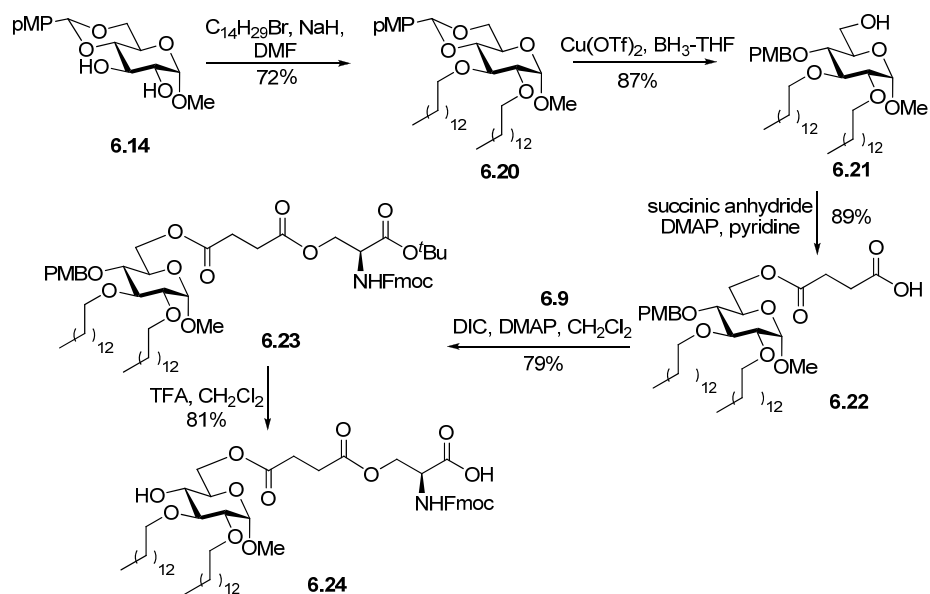
Scheme 6.3 Synthesis of monosaccharide Lipid A analog **6.24**

Having obtained compounds **6.19** and **6.24**, a lipidated and alkylated analogs of compound **6.11**, respectively, we were well positioned to perform comparative studies. In addition, as the comparison point, we obtained the standard positive control compound **6.4** as described by Peri *et al.*[11] A marked improvement in LPS-antagonistic ability was observed with the lipidated compound **6.19**. Inhibition of LPS-induced TNFα was found to begin at 550 nM. A reproducible biphasic response was consistently observed in the activity of **6.19**. This may indicate multiple binding sites on the macrophages, both higher and lower affinity for the antagonist compound. Eighty percent of the LPS response was blocked at 5 μM compound **6.19** with no observable toxicity. Cell viability began to be compromised at 10 μM and some agonist activity was found in the 10-30 μM range (Figure 6.4).
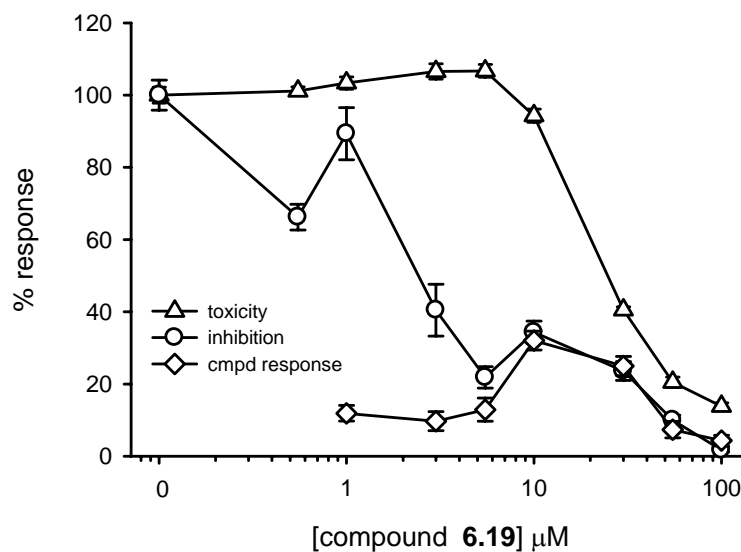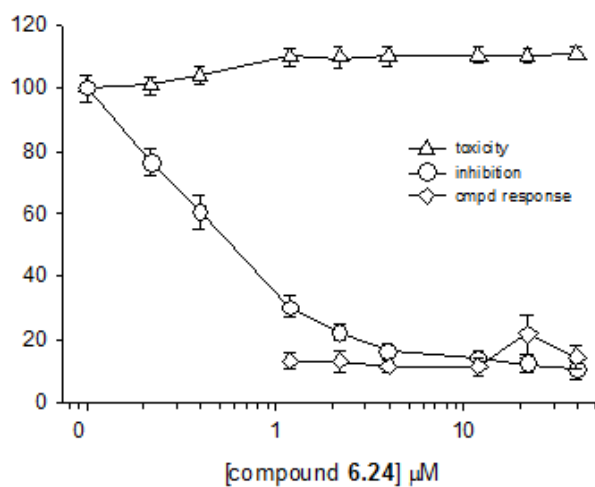
Figure 6.4 Biological activity test of compound **6.19** (Experimental work and data analysis done by Geeta Paranajape and Shana E. Terrill,in the laboratory of Dr. MR Nichols)

Compound **6.24** with alkyl chains rather than lipid chains as in **6.19** was found to be a very effective inhibitor of LPS-induced TNFα production in human macrophages. Seventy percent inhibition was observed at 1 μM and overall inhibition reached 90% at 40 μM. Compound **6.24** exhibited no toxicity or agonist activity in the tested 0.2 to 40 μM range. Curve fitting of the inhibition data in Figure 6.5 produced an IC_{50} value of 470 nM. These results were superior to those obtained with compound **6.4** [11] which had an inhibition range from 3-10 μM and began to show agonist activity at concentrations >10 μM (Figure 6.6). Compound **6.4** was only toxic at high concentrations.

Figure 6.5 Biological activity test of compound **6.24** (Experimental work and data analysis done by Geeta Paranajape and Shana E. Terrill,in the laboratory of Dr. MR Nichols
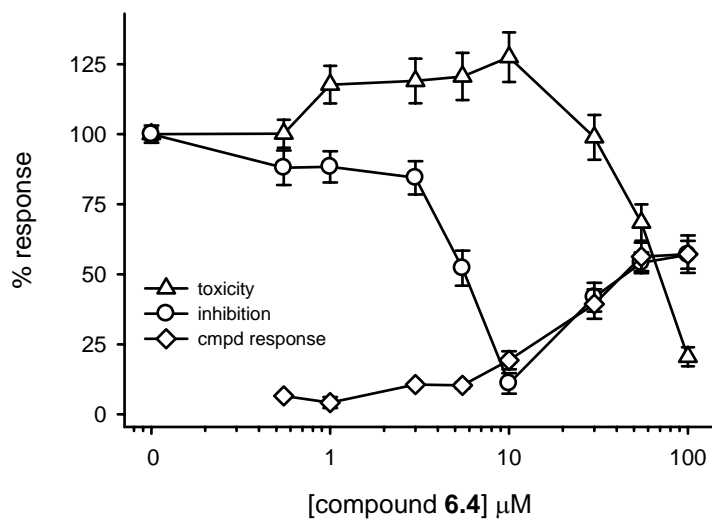
Figure 6.6 Biological activity test of compound **6.4** (Experimental work and data analysis done by Geeta Paranajape and Shana E. Terrill,in the laboratory of Dr. MR Nichols

**6.3	Conclusions**

In summary, studies described herein provide new information regarding structure-activity relationship of novel class of compounds mimicking classic Lipid-A analogs, and the scientific basis for future studies to prevent the deleterious effects of endotoxemia. We believe that the results of these studies will have far reaching impacts on the treatment of patients diagnosed with endotoxemia. As a consequence, we can foresee additional efforts to investigate the molecular mechanisms responsible for the antagonistic actions of the synthetic Lipid A analogs.

**6.4	Experimental Section**

**6.3.1.	General**. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh) and reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at $< 40\ ^{\circ}C$. CH_2Cl_2 was freshly distilled from CaH_2 prior to application. The solvent was kept in contact with the alloy until distilled for use. *N,N'*-Diisopropylcarbodiimide, 4-dimethylaminopyridine, trifluoroacetic acid, anhydrous *N,N*-dimethylformamide were purchased from Sigma-Aldrich and used as is. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over. Optical rotations were measured using 'Jasco P-1020' polarimeter. Unless noted otherwise, ^{1}H-NMR spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Avance) or at 500 MHz (Bruker ARX-500), ^{13}C-NMR spectra and two-dimensional experiments were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) or at 125

MHz (Bruker ARX-500). HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

Methyl 2,3-di-O-benzyl-6-O-(3-carboxypropanoyl)-α-D-glycopyranoside (6.8).

DMAP (32 mg, 0.26 mmol) and succinic anhydride (0.16 g, 1.56 mmol) were added to a stirred solution of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside **6.7** (0.5 g, 1.3 mmol) in dry pyridine (5 mL) at room temperature under argon. The reaction mixture was stirred for 16 h at room temperature under argon. After that, the reaction mixture was concentrated under the reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL) and washed successively with water (3 × 15 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give compound **6.8** (0.45 g, 0.95 mmol) as a colorless syrup in 71% yield. Analytical data for **6.8**: R_f = 0.43 (ethyl acetate); $[\alpha]_D^{22}$ +7.19 (*c* = 1.0, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$): δ, 2.60 (m, 4H, 2×CH$_2$), 3.38 (s, 3H, OCH$_3$), 3.44 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 3,51 (ddd, 1H, J = 3.5, 9.5 Hz, H-4), 3.73 (m, 1H, $J_{5,6b}$ = 4.8 Hz, H-5), 3.80 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-3), 4.26 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, H-6a), 4.42 (dd, 1H, H-6b), 4.61 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.65 (d, 1H, J^2 = 12.1 Hz, ½ C*H*$_2$Ph), 4.74-4.79 (m, 2H, C*H*$_2$Ph), 4.99(d, 1H, J = 11.31 Hz, ½ C*H*$_2$Ph), 7.26-7.36 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ, 55.39, 3.73, 69.37, 69.99, 73.34, 75.34, 79.65, 81.34, 98.26, 128.02, 128.12, 128.24, 128.49, 128.62, 128.68, 138.08, 138.63, 172.64, 177.19 ppm; HR FAB MS [M+Na]$^+$ calcd for $C_{25}H_{30}O_9Na$ 497.1788, found 497.1770.

Methyl 2,3-di-O-benzyl-6-O-(4-((S)-2-(9-fluorenylmethoxycarbonyl)amino-3-(tert-butoxy)-3-oxopropyl)oxy-4-oxobutanoyl)-α-D-glycopyranoside (6.10).

DMAP (12.7 mg, 0.01 mmol) and DIC (0.16 mL, 1.04 mmol) were added to a stirred solution of the *O-tert*-butyl-*N*-fluorenylmethoxycarbonyl-L-serine **6.9** (0.19 g, 0.52 mmol) in CH_2Cl_2 (4 mL). After 1 h, the carboxylic acid derivative **6.8** (0.29 g, 0.62 mmol) was added and the reaction was stirred for 5-9 h when TLC indicated no further conversion of starting material. The reaction was diluted with CH_2Cl_2 (20 mL), washed with distill water (2 × 15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to obtain compound **6.10** (0.34g, 0.41 mmol) as a white powder in 80 % yield. R_f = 0.34 (ethyl acetate-hexane 1/1, v/v); $[\alpha]_D^{27}$ +12.05 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.48 (s, 9H, *t*-Bu), 2.53 (broad s, OH), 2.66 (m, 4H, 2×CH_2), 3.37 (s, 3H, OCH_3), 3.42 (d, 1H, $J_{2,3}$ = 9.0, H-2), 3.73 (m, 1H, H-5), 3.78 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 4.25 (m, 2H, H-6b, SerC$^\beta$-*H*), 4.38 (m, 2H, H-6^a, SerC$^\beta$-*H*), 4.44 (m, 2H, C*H*$_2$Fmoc), 4.52 (m, 1H, SerC$^\alpha$-*H*), 4.61 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.64 (d, 1H, J^2 = 12.1 Hz, ½ C*H*$_2$Ph), 4.75 (dd, 2H, J = 12.0 Hz, C*H*$_2$Ph), 4.98 (d, 1H, J^2 = 11.33 Hz, ½ CH_2Ph), 4.75 (d, 1H, $J_{NH,CH}$ = 8.00 Hz, Ser-NH), 7.27-7.42 (m, 14H, aromatic), 7.62 (d, 2H, J = 7.4 Hz, aromatic), 7.77 (d, 2H, J = 7.5 Hz, aromatic) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.12, 29.09, 29.11, 47.30, 54.07, 55.48, 63.85, 64.81, 67.41, 69.38, 70.11, 73.40, 75.71, 79.74, 81.31, 83.29, 98.39, 120.17, 125.35, 125.40, 127.28, 127.91, 128.06, 128.16, 128.27, 128.68, 128.76, 138.18, 138.86, 141.48, 144.01, 144.05, 156.00, 168.51, 171.80, 172.60 ppm; HR FAB MS $[M+Na]^+$ calcd for $C_{47}H_{53}NO_{13}Na$ 862.3415, found 862.3432.

Methyl 2,3-di-O-benzyl-6-O-(4-((S)-2-(9-fluorenylmethoxycarbonyl)amino-2-carboxyethyl)oxy-4-oxobutanoyl)-α-D-glycopyranoside (6.11).

Methyl glucoside derivative **6.10** (0.25g, 0.29 mmol) was dissolved in TFA/CH$_2$Cl$_2$ (1/5 v/v, 2mL) and stirred for 2 h. Upon completion as assessed by TLC analysis, solvents were evaporated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ (5 mL) and neutralized with triethylamine (TEA). The solvent was removed under the reduced the pressure. The residue was subjected to column chromatography on silica gel (ethyl acetate-hexane 1/1, v/v) to afford carboxylic derivative **6.11** (0.18g, 0.24 mmol) as white foam in 81% yield. R_f = 0.5 (methanol-ethyl acetate 2/8, v/v)); $[\alpha]_D^{26}$ +22.48 (c = 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$:(CD$_3$)$_2$SO 4:2) : δ = 2.49 - 2.54 (m, 4H), 3.26 (s, 3H, OCH$_3$), 3.39 (m,2H), 3.57 (m,2H), 4.01 (broad s, 1H), 4.12 (m, 2H), 4.22 (m, 2H), 4.32 (dd, 2H, J = 6.6 Hz), 4.43 (d, 1H, J = 5.1 Hz), 4.60 (s, 2H), 4.76 (m, 2H), 7.24 – 7.43 (m, 10H, aromatic), 7.68 (d, 2H, J = 4.38 Hz, aromatic), 7.93 (d, 2H, J = 4.89 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$:(CD$_3$)$_2$SO 4:2): δ = 28.84, 28.99, 31.06, 47.03, 54.71, 54.83, 63.42, 63.90, 65.31, 65.92, 69.85, 70.20, 71.85, 74.50, 79.36, 81.46, 97.29, 120.47, 125.56, 127.44, 127.49, 127.87, 127.83, 127.90, 127.98, 128.33, 128.52, 138.94, 139.62, 141.06, 144.23, 156.05, 172.16, 172.23 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{43}$H$_{45}$NO$_{13}$Na 806.2789, found 806.2780. (See Appendix; Figure A-22, and A-23)

Methyl 2,3-di-O-benzyl-6-O-(4-((S)-2-amino-3-(tert-butoxy)-3-oxopropyl)oxy-4-oxobutanoyl)-α-D-glycopyranoside (6.12).

Piperidine (0.5 mL) was added dropwise to stirred solution of monosaccharide **6.10** (0.1 g, 0.12 mmol) in DMF (2.0 mL). After stirred for 20 min, TLC indicated complete

conversion of starting material. The solvents were removed in vacuo (co-evaporation with toluene). The residue was purified by flash column chromatography (ethyl acetate-hexane 1/1) to give the amine derivative **6.12** (67 mg, 0.1 mmol) as yellow syrup in 92% yield. R_f = 0.62 (methanol-ethyl acetate 1/9, v/v)); $[\alpha]_D^{26}$ +17.33 (c = 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) : δ = 1.49 (s, 9H, t-Bu), 2.52 – 2.66 (m, 3H), 2.73 – 2.78 (m, 1H), 3.02 (broad s, 1H, OH), 3.38 (s, 3H, OCH$_3$), 3.46 (d, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 3.50 (ddd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 3.75 (m, 2H, H-5, Ser-C$^\beta$*H*), 3.80 (m, 2H, H-3, Ser-C$^\beta$*H*), 4.18 (dd, 1H, $J_{6a,6b}$ = 10.19 Hz, H-6a), 4.46 (m, 1H, Ser-C$^\alpha$*H*), 4.52 (dd, 1H, $J_{5,6b}$ = 4.19 Hz, H-6b), 4.61 (d, 1H, $J_{1,2}$ = 3.53 Hz, H-1), 4.65 (d, 1H, J^2 = 12.5 Hz, ½ C*H*$_2$Ph), 4.77 (dd, 2H, J = 11.5 Hz, CH$_2$Ph), 4.99 (d, 1H, J^2 = 9.0 Hz, ½ C*H*$_2$Ph,) 6.49 (d, 1H, $J_{\text{NH,CH}}$ = 6.95 Hz, Ser-N*H*), 7.27 - 7.31 (m, 10H, aromatic) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 28.18, 29.76, 31.05, 31.11, 55.51, 55.68, 63.35, 63.63, 69.46, 69.88, 73.49, 75.98, 79.83, 81.50, 83.01, 98.47, 128.09, 128.17, 128.30, 128.41, 128.68, 128.72, 138, 22, 128.73, 169.60, 172.05, 173.06 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{32}$H$_{43}$NO$_{11}$Na 640.2734, found 640.2841.

Methyl 2,3-di-O-benzyl-6-O-(4-((S)-2-amino-2-carboxyethyl)oxy-4-oxobutanoyl)-α-D-glycopyranoside (6.13)

Methyl glucoside derivative **6.12** (0.1 g, 0.12 mmol) was dissolved in TFA/CH$_2$Cl$_2$ (1/5 v/v, 2 mL) and stirred for 2 h. Upon completion as assessed by TLC analysis, solvents were evaporated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ (5 mL) and neutralized with triethylamine (TEA). Solvents were removed under the reduced the pressure. The residue was subjected to column chromatography on silica gel (methanol-ethyl acetate

gradient elution) to obtain product **6.13** (50 mg, 0.09 mmol) as white foam in 75% yield.

R_f = 0.5 (methanol-ethyl acetate 2/8, v/v)); ^{1}H NMR (500 MHz, D$_2$O) : δ = 1.68 (m, 2H), 1.82 (m, 3H), 2.73 (m, 4H), 3.19 (dd, 3H, J = 5.7 Hz), 3.4 (s, 3H, OCH$_3$), 3.58 (ddd, 2H, J = 9.5, 10.0 Hz), 3.80 (dd, 1H, J = 9.2 Hz), 3.87 (d, 3H, J = 4.7 Hz), 4.33 - 4 .37 (m, 2H, J = 4.7 Hz), 4.54 (d, 1H, J = 10.9 Hz), 7.44 (s, 10H, aromatic) ppm; ^{13}C NMR (125 MHz, D$_2$O): δ = 21.87, 22.608, 29.54, 30.46, 44.94, 55.38, 62.43, 69.78, 73.36, 75.74, 79.13, 81.08, 97.82, 128.69, 128.78, 129.02, 129.11, 137.73, 137.96, 174.53, 175.12, 176.23 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{28}$H$_{35}$NO$_{11}$Na 584.2108, found 584.2104.

Methyl 4,6-*O*-(*p*-methoxybenzylidene)-2,3-di-O-tetradecanoyl-α-D-glucopyranoside (6.15).

Myristoyl chloride (2.34 mL, 8.67 mmol) and DMAP (70 mg, 0.57 mmol) were added to a stirred solution of methyl 4,6-*O*-*p*-anisylidene-α-D-glucoside **6.14**[1] (1 g, 2.89 mmol) in pyridine (15 mL) at 0°C. The mixture was stirred under an atmosphere of argon for 16 h. Upon completion, the reaction mixture was concentrated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ (30 mL), and washed with 1N HCl (2 × 15 mL), distill water (2 × 15 mL) and brine (15 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford derivative **6.15** (1.81 g, 2.48 mmol) as a white foam in 86% yield. R_f = 0.47 (ethyl acetate-hexane 3/7, v/v)); [α]$_D^{23}$ +23.56 (*c* = 1.0, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) : δ = 0.88 (t, 6H, J = 7.02 Hz, 2×CH$_3$), 1,26 (broad s, 40H, 20×CH$_2$), 1.59 (m, 4H, 2×CH$_2$), 2.29 (m, 4H, 2×CH$_2$), 3.40 (s, 3H, OCH$_3$),

3.62 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.75 (dd, 1H, $J_{6a,6b}$ = 10.2 Hz, H-6a), 3.78 (s, 3H, OCH$_3$), 3.92 (m, 1H, H-5), 4.28 (dd, 1H, $J_{5,6b}$ = 4.7 Hz, H-6b), 4.82 – 2.94 (m, 2H, H-1, 2), 5.46 (s, 1H, >C*H*Ph), 5.60 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 6.86 (d, 2H, *J* = 8.8 Hz, aromatic), 7.25 (d, 2H, *J* = 8.7 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ = 14.28, 22.86, 25.09, 25.28, 29.18, 29.22, 29.44, 29.47, 29.53, 29.63, 29.66, 29.83, 29.86, 32.09, 34.26, 34.46, 55.37, 55.49, 62.53, 68.76, 68.95, 71.58, 76.80, 77.23, 77.65, 79.46, 97.84, 101.62, 113.67, 127.59, 129.62, 160.22, 172.64, 173.39 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{43}$H$_{72}$O$_9$Na 755.5074, found 755.5090.

Methyl 4-*O*-*p*-methoxybenzyl-3-O-tetradecanoyl-α-D-glucopyranoside (6.16).

A 1 M solution of borane/tetrahydrofuran complex in tetrahydrofuran (5 mL, 5 mmol) was added to a benzylidene acetal **6.15** (0.75 g, 1.02 mmol) at room temperature under nitrogen. The mixture was stirred for 10 min, and freshly dried copper (II) trifluoromethanesulfonate (18 mg, 0.05 mmol) was added to the solution. After stirring for 2 hours the mixture was cooled down to 0 °C, and the reaction was quenched by sequential additions of triethylamine (TEA) (0.14 mL, 1 mmol) and methanol (1.8 mL). The resulting mixture was concentrated at reduced pressure followed by co evaporation with methanol. The residue was purified by flash column chromatography on silica gel to give compound **6.16** (0.35g, 0.67 mmol) as white solid in 67% yield. R_f = 0.40 (ethyl acetate-hexane 7/3, v/v)); [α]$_D^{21}$ +78.50 (*c* = 1.0, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) : δ = 0.88 (t, 3H, *J*= 7.02 Hz, CH$_3$), 1.24 (broad s, 20H, 10×CH$_2$), 1.63 (m, 2H, CH$_2$), 1.97 (broad s, 1H, OH), 2.23 – 3.82 (m, 3H, CH$_2$, OH), 3.41 (s, 3H, OCH$_3$), 3.52 -3.82 (m, 5H, H-2, 4, 5, 6a, 6b), 3.79 (s, 3H, OCH$_3$), 4.57 (dd, 2H, *J* = 12.4 Hz, C*H$_2$*Ph), 4.75 (d, 1H,

$J_{1,2}$ = 3.7 Hz, H-1), 5.30 (dd, 1H, J = 9.1 Hz, H-3), 6.85 (d, 2H, J = 8.6 Hz, aromatic),

7.20 (d, 2H, J = 8.6 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ = 14.30, 22.86,

25.07, 29.31, 29.48, 29.52, 29.64, 29.79, 29.82, 29.85, 32.09, 34.68, 55.41, 55.53, 61.67,

71.04, 71.87, 74.33, 74.96, 99.65, 114.02, 129.63, 130.10, 159.54, 174.29 ppm; HR FAB

MS [M+Na]$^+$ calcd for C$_{29}$H$_{48}$O$_8$Na 547.3247, found 547.3254.

Methyl 6-*O*-(3-carboxypropanoyl)-4-*O*-*p*-methoxybenzyl-2,3-di-*O*-tetradecanoyl-α-D-glucopyranoside (6.17).

DMAP (8.1 mg, 0.06 mmol) and succinic anhydride (67 mg, 0.67 mmol) were added to a

stirred solution of partially protected derivative **6.16** (0.35g, 0.67 mmol) in dry pyridine

(5 mL) at room temperature under argon. The reaction mixture was stirred for 16 h at

room temperature under argon. After 16 h, TLC indicated no further conversion of the

starting material. Myristoyl chloride (0.21 ml, 0.80 mmol) was added dropwise to the

reaction mixture. The reaction mixture was stirred for 2 h. Upon completion, the reaction

mixture was concentrated under reduced pressure, then the residue was dissolved in

CH$_2$Cl$_2$ (30 mL) and washed with 1N HCl (2 × 15 mL), distill water (2 × 15 mL) and

brine (15 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated in

vacuo. The residue was purified by silica gel column chromatography (ethyl acetate-

hexane gradient elution) to give compound **6.17** (0.43 g, 0.52 mmol) as white syrup in

79% yield. R_f = 0.55 (ethyl acetate-hexane 1/1, v/v); $[α]_D^{24}$ +42.45 (c = 1.0, CHCl$_3$); [1]H

NMR (300 MHz, CDCl$_3$) : δ = 0.87 (t, 6H, 2×CH$_3$), 1.25 (broad s, 40H, 20×CH$_2$), 1.59

(m, 4H, 2×CH$_2$), 2.26 (m,2H, CH$_2$), 2.33 (m, 2H, CH$_2$), 2.61 (m, 4H, 2×CH$_2$), 3.36 (s,

3H, OCH$_3$), 3.59 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 3.90 (m, 1H, H-5), 4.25 − 4.31 (dd, 2H,

$J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a, 6b), 4.44 (d, 1H, J^2 = 10.65 Hz, ½ CH_2Ph), 4.54 (d, 1H, J^2 = 10.65, ½ CH_2Ph), 4.87 (m, 2H, H-1, 2), 5.55 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 6.84 (d, 2H, J = 8.7 Hz, aromatic), 7.16 (d, 2H, J = 8.6 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ = 14.28, 22.85, 25.07, 28.82, 29.22, 29.35, 29.41, 29.45, 29.52, 29.60, 29.65, 29.81, 32.08, 34.20, 34.49, 34.30, 34.49, 55.37, 62.56, 68.56, 71.78, 71.89, 74.28, 75.72, 96.87, 114.04, 129.53, 129.76, 159.62, 171.67, 172.82, 173.68 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{47}$H$_{78}$O$_{12}$Na 857.5391, found 857.5410.

Methyl 6-O-(4-((S)-2-(9-fluorenylmethoxycarbonyl)amino-3-(tert-butoxy)-3-oxopropyl)oxy-4-oxobutanoyl)-2,3-di-O-tetradecanoyl-α-D-glucopyranoside (6.18).

DMAP (9.5 mg, 0.07 mmol) and DIC (0.12 mL, 0.78 mmol) were added to a stirred solution of the O-tert-butyl-N-fluorenylmethoxycarbonyl-L-serine **6.9** (0.15 g, 0.39 mmol) in CH$_2$Cl$_2$ (3 ml). After 1 h, the carboxylic acid derivative **6.17** (0.39 g, 0.46 mmol) was added and the reaction was stirred for 5-9 h when TLC indicated no further conversion of starting material to the major product. The reaction was diluted with CH$_2$Cl$_2$ (20 mL), washed with distill water (2 × 10 mL) and brine (10 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by silica gel flash column chromatography (ethyl acetate-hexane gradient elution) to obtain compound **6.18** (0.38 g, 0.32 mmol) as white solid in 83 % yield. R_f = 0.5 (ethyl acetate-hexane 3/7, v/v)); [α]$_D^{24}$ +25.72 (c = 1.0, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$) : δ = 0.88 (t, 6H, J = 6.4 Hz, 2×CH$_3$), 1.25 (broad s, 40H, 20×CH$_2$), 1.49 (s, 9H, t-Bu), 1.59 (m, 4H, 2×CH$_2$), 1.59 (m, 4H, 2×CH$_2$), 2.30 (m, 4H, 2×CH$_2$), 3.37 (s, 3H, OCH$_3$), 3.48 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 3.76 (s, 3H, OCH$_3$), 3.90 (m, 1H, H-5), 4.16-

4.26 (m ,3H, H-6a, 6b, Ser-C$^{\beta}$*H*), 4.35 − 4.55 (m, 6H, CH$_2$Ph, Ser-C$^{\alpha}$*H*, Ser-C$^{\beta}$*H*, C*H*$_2$Fmoc), 4.90 (m, 2H, H-1, 2), 5.58 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 5.82 (d, 1H, $J_{NH,CH}$ = 8.3 Hz, Ser-N*H*), 6.18 (d, 2H, J = 8.5 Hz, aromatic), 7.12 (d, 2H, J = 8.5 Hz, aromatic), 7.32 − 7.43 (m, 5H, aromatic), 7.63 (m, 2H, aromatic), 7.77 (d, 2H, J = 8.5 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ = 14.25, 22.81, 25.00, 28.03, 28.76, 28.97, 29.27, 29.35, 29.43, 29.47, 29.62, 29.77, 32.04, 34.19, 34.44, 47.22, 54.02, 55.30, 62.58, 67.37, 68.53, 71.68, 71.88, 74.27, 83.04, 96.83, 113.95, 120.09, 25.40, 127.23, 127.84, 129.28, 129.62, 141.39, 143.98, 155.98,159.54, 168.44, 171.50, 172.72, 173.53 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{69}$H$_{101}$NO$_{16}$Na 1222.7018, found 1222.7023.

Methyl 6-O-(4-((S)-2-(9-fluorenylmethoxycarbonyl)amino-2-carboxyethyl)oxy-4-oxobutanoyl)-2,3-di-O-tetradecanoyl-α-D-glucopyranoside (6.19).

Methyl glucoside derivative **6.18** (0.2 g, 0.16 mmol) was dissolved in TFA/CH$_2$Cl$_2$ (1/5 v/v, 4 mL) and stirred for 2 h. Upon completion as assessed by TLC analysis, solvents were evaporated in vacuo. The residue was dissolved in CH$_2$Cl$_2$ (5 mL) and neutralized with triethylamine (TEA). Solvent were removed under the reduced the pressure. The residue was subjected to column chromatography on silica gel (ethyl acetate-hexane 1/1, v/v) to obtain product **6.19** (0.13 g, 0.12 mmol) as white foam in 75% yield. R_f = 0.5 (methanol-ethyl acetate 1/9, v/v)); ^{1}H NMR (300 MHz, CDCl$_3$) : δ = 0.88 (t, 6H, J = 5.70 Hz, 2×CH$_3$), 1.25 (broad s, 40H, 20×CH$_2$), 1.58 (m, 4H, 2×CH$_2$), 2.33 (m, 4H, 2×CH$_2$), 3.27 (broad s, 4H, 2×CH$_2$), 3.27 (broad s, 1H, OH), 3.38 (s, 3H, OCH$_3$), 3.47 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 3.83 (m, 1H, H-5), 4.21 − 4.51 (m, 7H, H-6a, 6b, Ser-C$^{\beta}$*H*$_2$, Ser-C$^{\alpha}$*H*, C*H*$_2$Fmoc), 4.86 (m, 1H, H-2), 4.90 (broad s, 1H, H-1), 5.31 (dd, 1H, $J_{3,4}$ = 9.1

Hz, H-3), 5.96 (broad s, 1H, Ser-N*H*), 7.27 – 7.42 (m, 4H, aromatic), 7,61 (d, 2H, *J* = 6.9

Hz, aromatic), 7.76 (d, 2H, *J* = 7.5 Hz, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl$_3$): δ =

25.03, 25.08, 28.93, 29.26, 29.30, 29.44, 29.54, 29.66, 29.83, 29.86, 32.10, 34.24, 34.47,

47.20, 55.46, 62.91, 64.33, 67.64, 69.36, 69.57, 69.89, 71.16, 72.73, 77.42, 96.84, 114.10,

120.18, 124.95, 125.29, 127.28, 127.95, 141.44, 143.83, 143.89, 171.67, 171.78, 174.31,

174.58, 175.10 ppm; HR FAB MS [M+Na]$^+$ calcd for C$_{57}$H$_{85}$NO$_{15}$Na 1046.5817, found

1045.5822. (See Appendix; Figure A-24, and A-25)

6.3.2 Biological assays

The biological activity testings of Lipid A analogues were performed by Geeta

Paranajape, Shana E. Terrill, and Maria L. D. Udan in the laboratory of Dr. MR Nichols

with the standard experiments.

Preparation of compounds for cellular treatment. Synthetic compounds in solid form

were dissolved in tetrahydrofuran (THF), aliquotted in small volumes, vacuum-

centrifuged for 1 hour and stored at −80°C as dry compounds. For cellular treatment, an

aliquot was dissolved in an appropriate volume of dimethylsulfoxide (DMSO) (Fisher

Sci) to give a concentrated working stock solution. This stock solution was further diluted

in DMSO to give the desired concentration range. The final concentration of DMSO was

always maintained at 0.6%.

Cell culture. THP-1 cells were obtained from ATCC (Manassas, VA) and maintained in

RPMI-1640 culture medium (HyClone, Logan, UT) containing 2 mM L-glutamine, 25

mM HEPES, 1.5 g/L sodium bicarbonate, 10% fetal bovine serum (FBS) (HyClone), 50 units/mL penicillin, 50 μg/mL streptomycin (HyClone), and 50 μM β-mercaptoethanol at 37°C in 5% CO_2. For cellular assay, THP-1 monocytes were centrifuged and resuspended in a fresh growth medium to a cell density of 5 x 105 cells/ml. Cell were then seeded in a 48-well plate and differentiated into adherent macrophages by treatment with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) for 24 hours at 37°C in5% CO_2. The non adherent cells were removed and the adherent cells were washed and replenished with reduced FBS growth medium. For the two wells set aside for calculating percent differentiation, the cells were washed with PBS (Hyclone) prior to removal by 0.25% trypsin-EDTA. The adherent cells were counted under the microscope using hemocytometer. Percentage of adherent cells was determined by dividing the number of adherent cells by the number of plated cells. For the remaining wells, the cells were pre-incubated with LPS antagonist at different concentrations for 30 minutes followed by by addition of 10 nM ultra pure LPS for 6 h at 37°C in 5% CO_2. DMSO was used as a negative control. The medium was collected and stores at -20°C until analyzed by ELISA for TNFα production

XTT cell toxicity assay. Cellular toxicity was monitored using an XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] cell assay. Estimate of cellular metabolic activity was obtained by probing mitochondrial reduction of XTT (Sigma) which in turn reflected the toxicity in response to LPS-antagonists. The cells treated with LPS antagonists and ultrapure LPS were washed with PBS (Hyclone) and then further incubated with XTT and phenazine methosulfate (PMS) (Acros, Morris

Plains, NJ) to a final concentration of 0.33 mg/mL and 8.3 μM respectively for 2 h at 37C in 5% CO_2. The cellular toxicity was then assessed based on the absorbance measurement of the reduced XTT at 467 nm.

6.4   References:

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        Chem. Int. Ed. **2007**, *46*, 3308-3312.

APPENDIX

Selected NMR spectral data
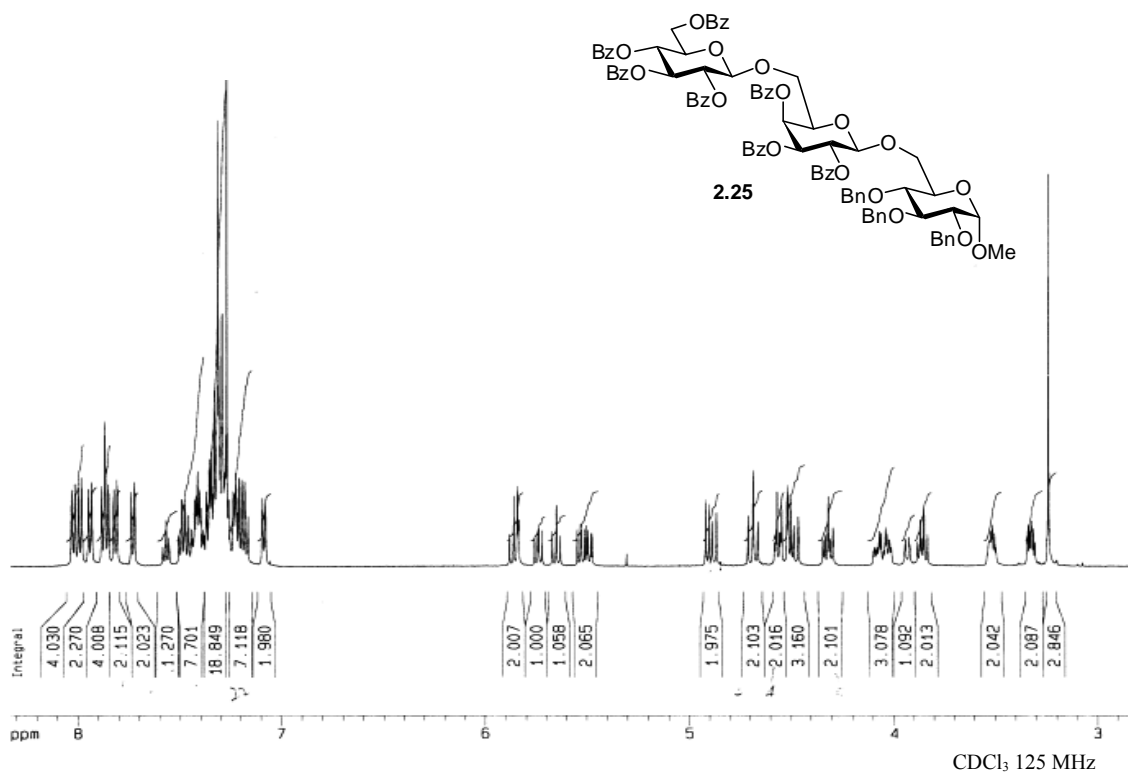
Figure A-1: ¹H NMR spectrum of Methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.25**)
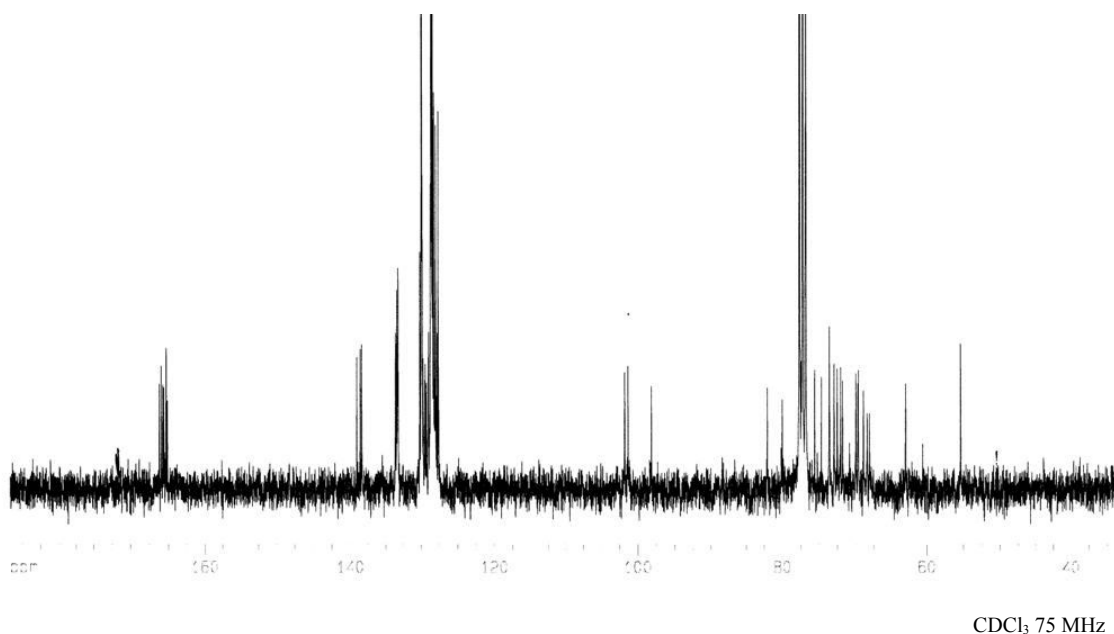
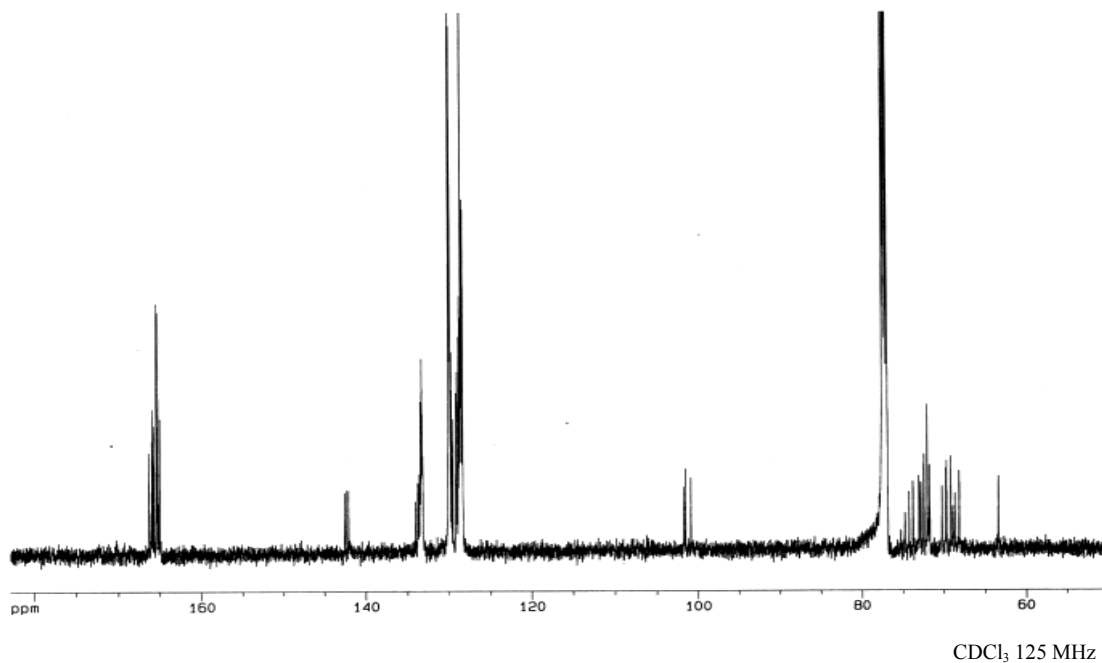Figure A-2: ¹³C NMR spectrum of Methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.25**)
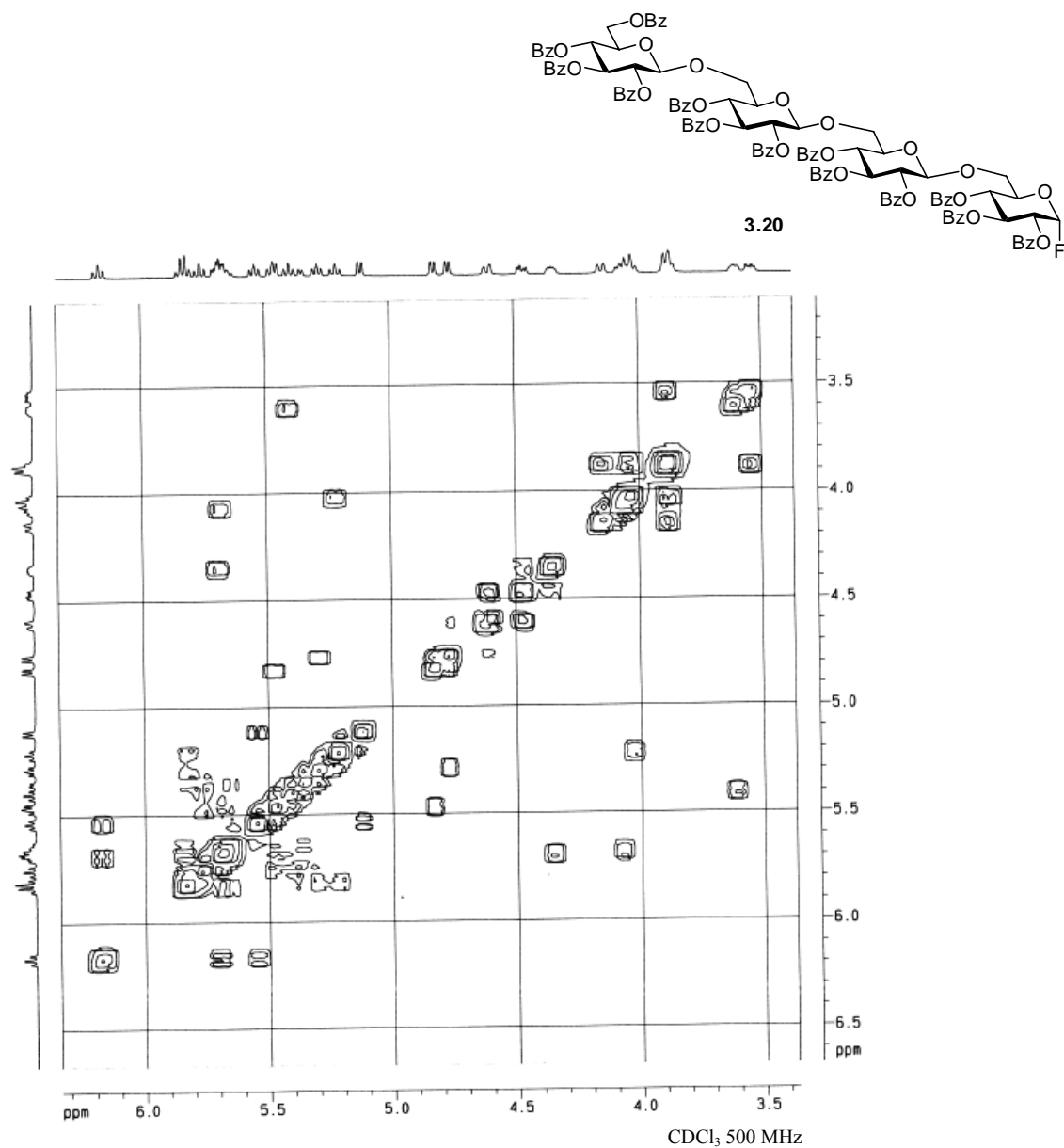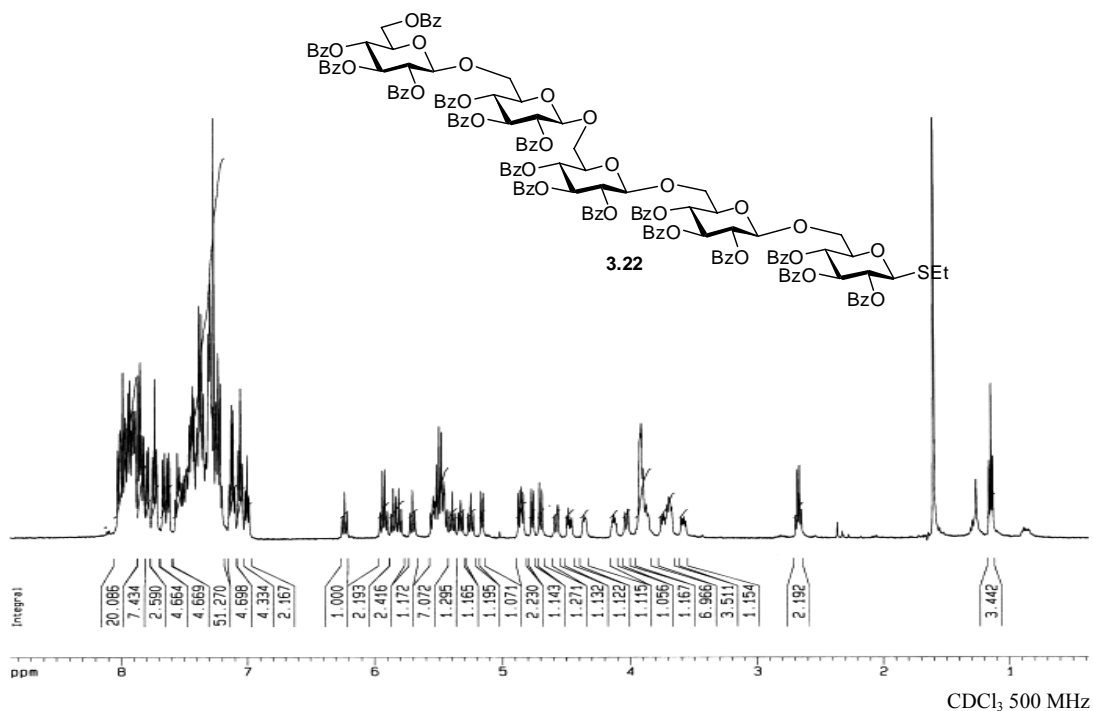
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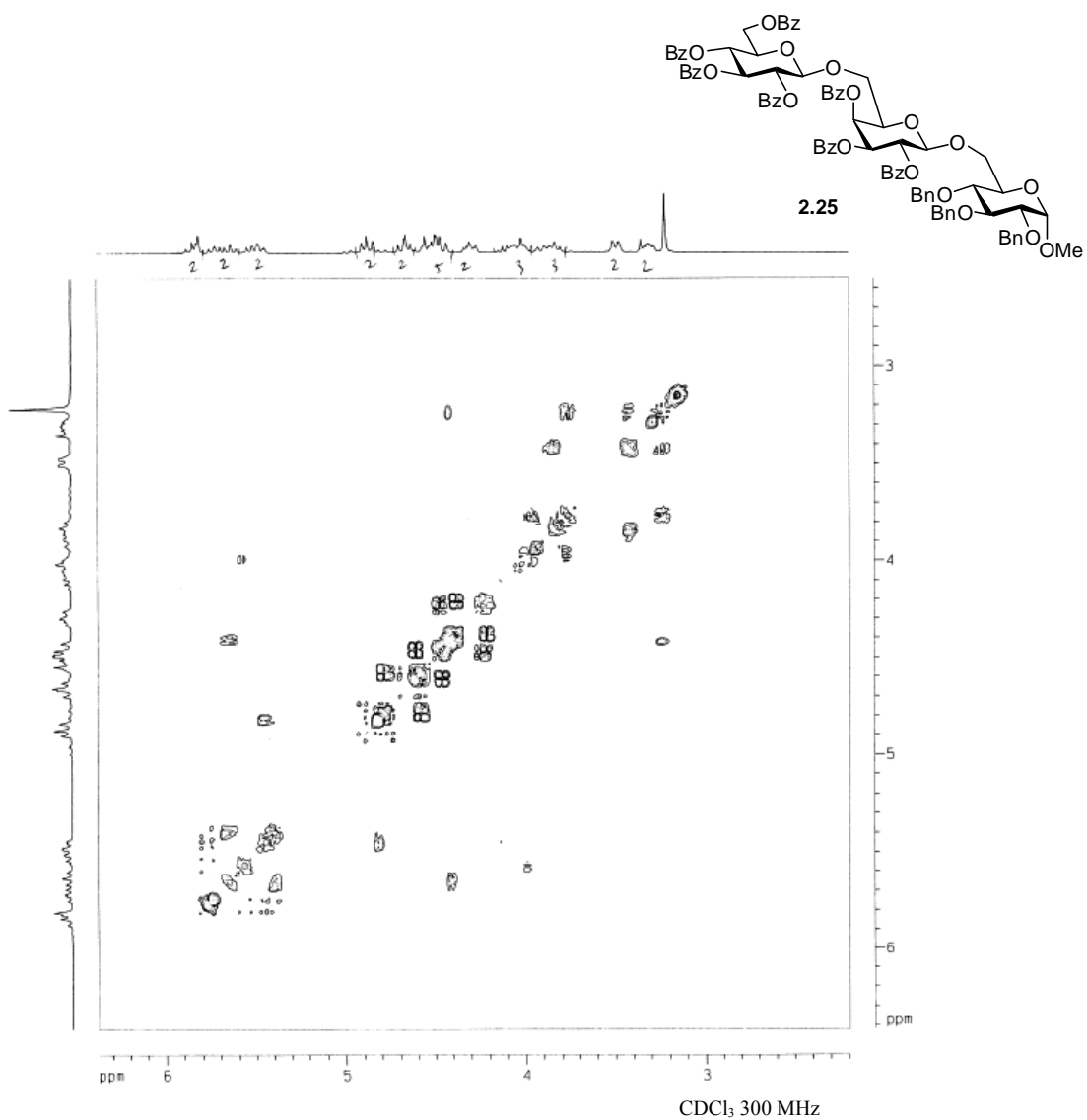
Figure A-3: 2-D NMR COSY spectrum of Methyl *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.25**)
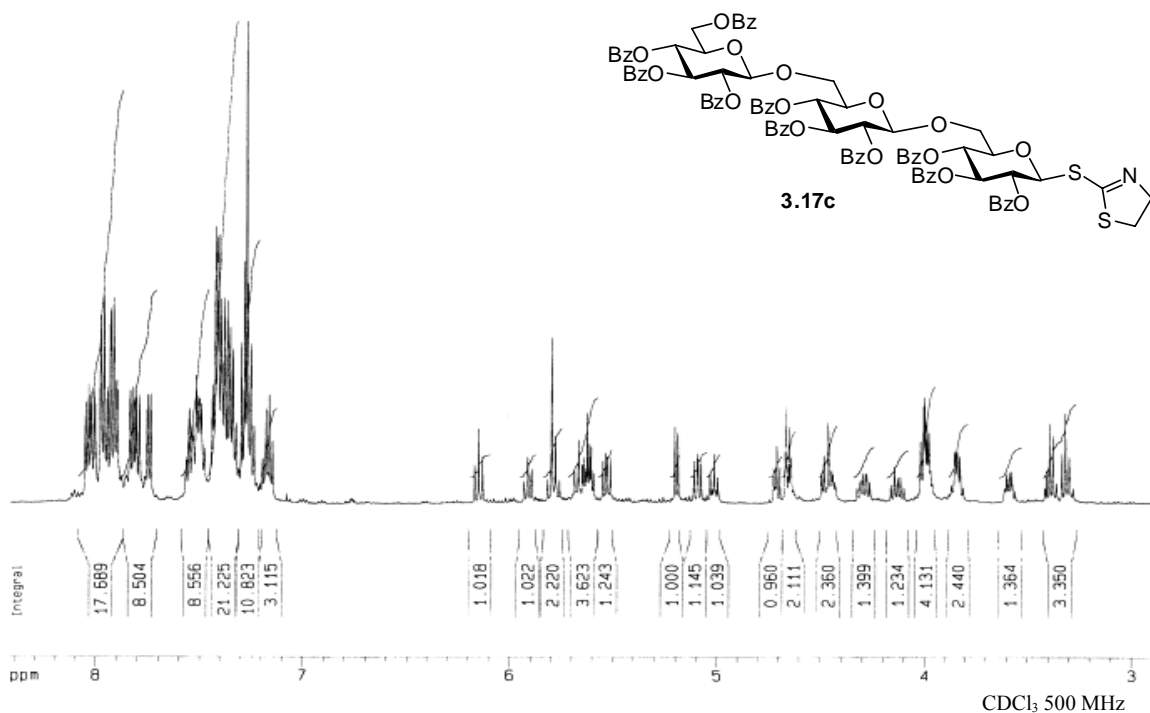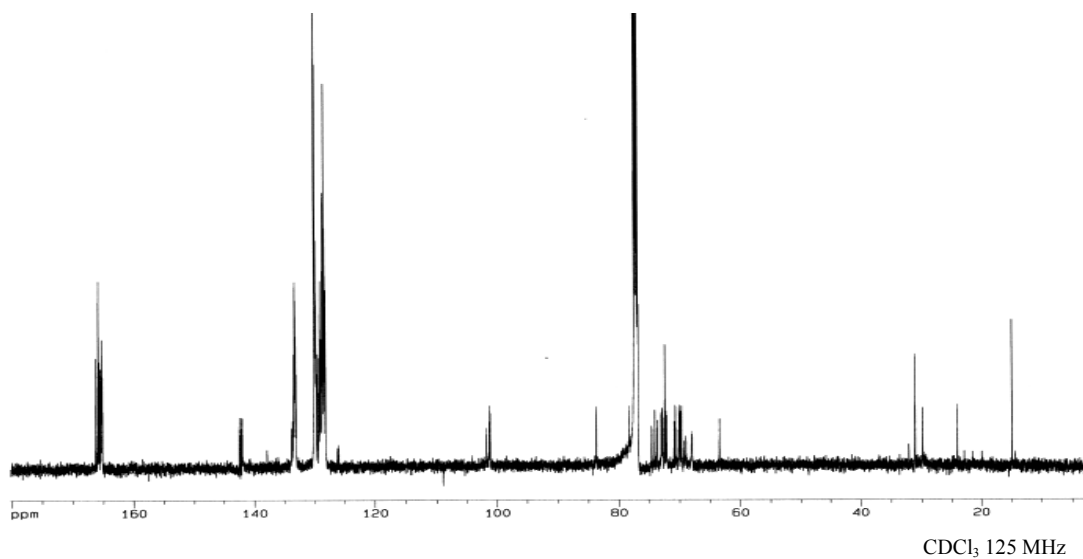
Figure A-4: ^{1}H NMR spectrum of 2-Thiazolinyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-1-thio-β−D-glucopyranoside (**3.17c**).
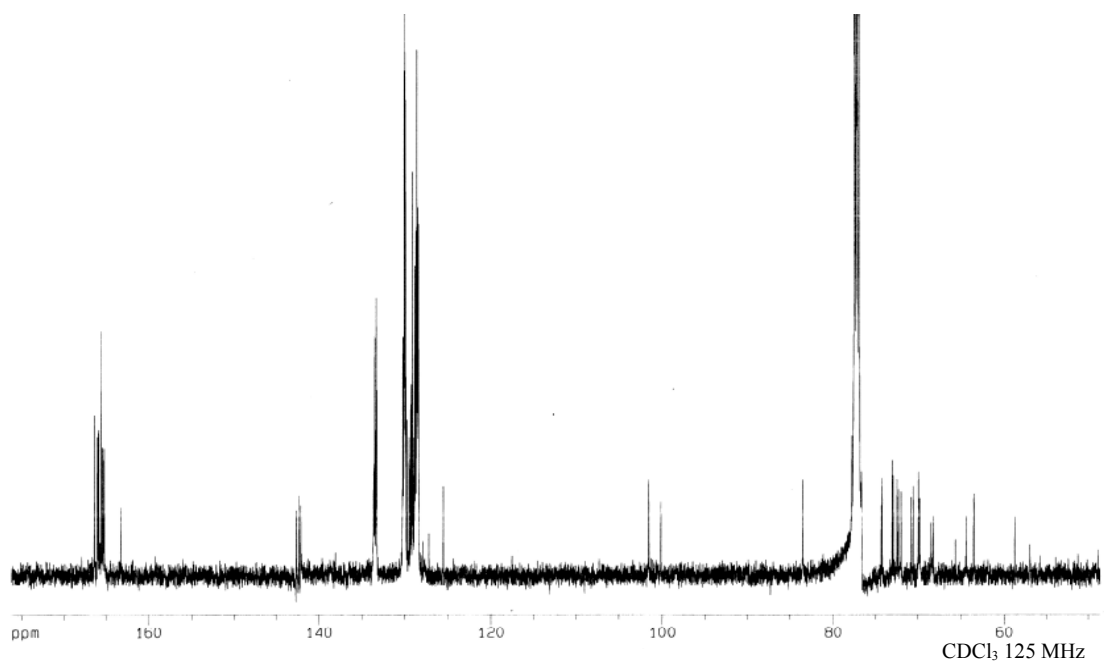
Figure A-5: ^{13}C NMR spectrum of 2-Thiazolinyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-1-thio-β−D-glucopyranoside (**3.17c**).
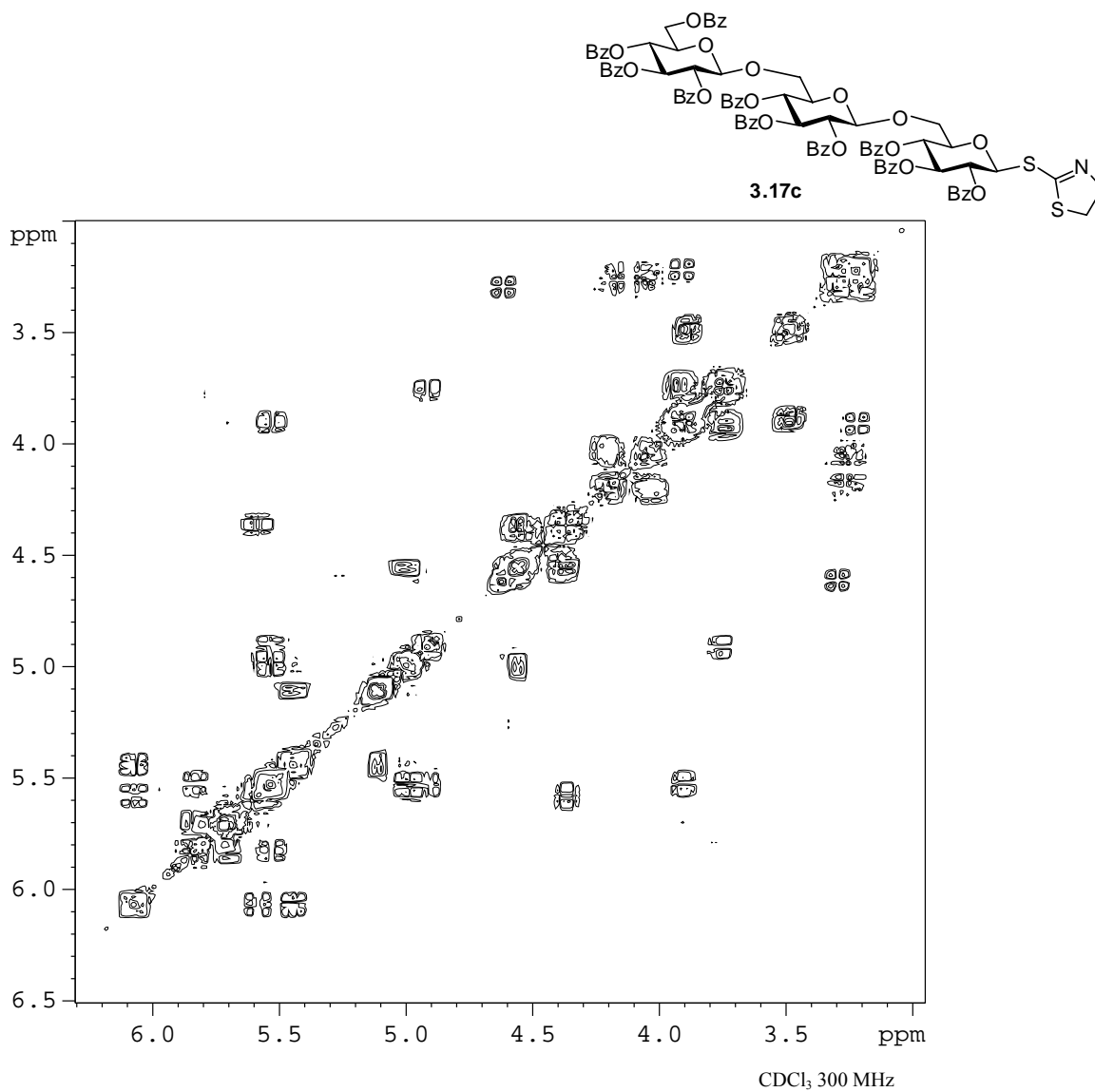
Figure A-6: 2-D NMR COSY spectrum 2-Thiazolinyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-1-thio-β–D-glucopyranoside (**3.17c**).
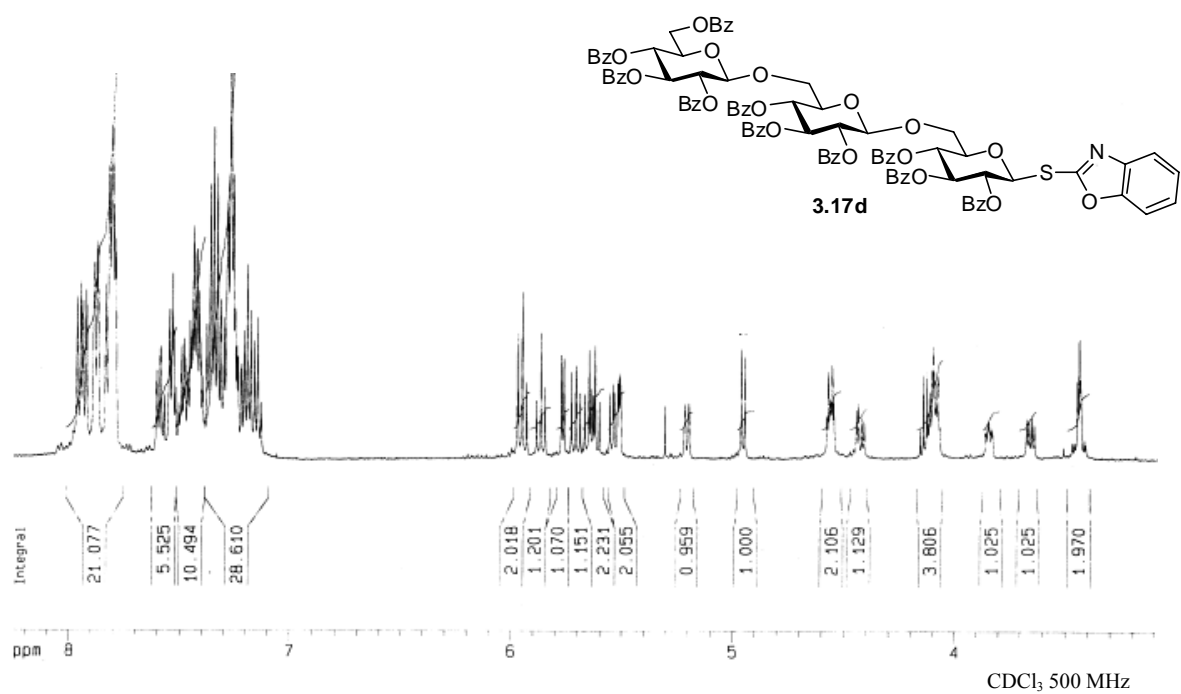
Figure A-7: ^{1}H NMR spectrum of Benzoxazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-1-thio-β−D-glucopyranoside (**3.17d**).
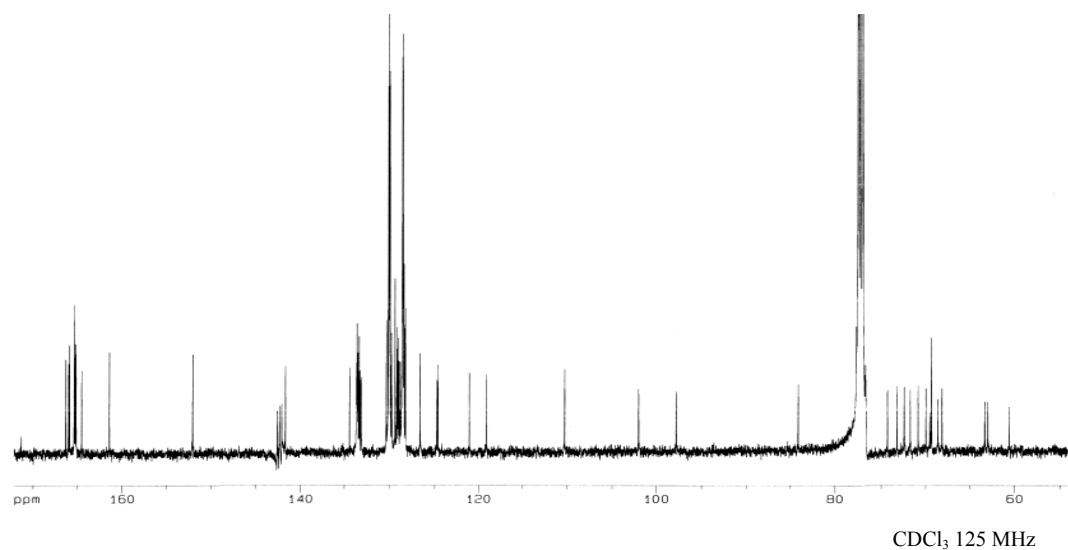
Figure A-8: ^{13}C NMR spectrum of Benzoxazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-1-thio-β−D-glucopyranoside (**3.17d**).

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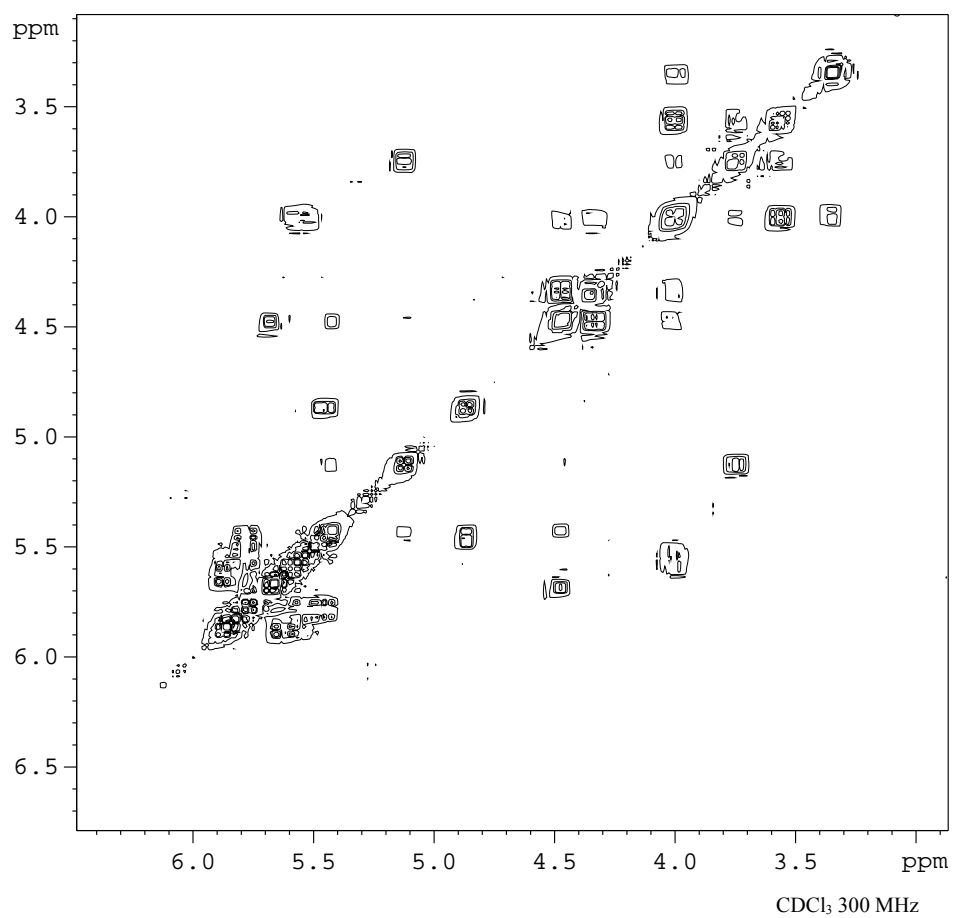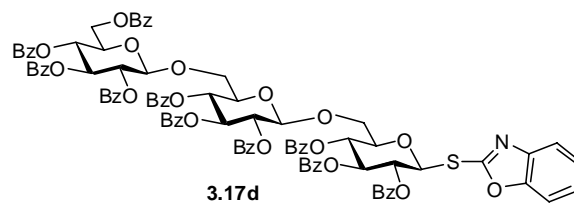
Figure A-9: 2-D NMR COSY spectrum of Benzoxazol-2-yl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-1-thio-β–D-glucopyranoside (**3.17d**).
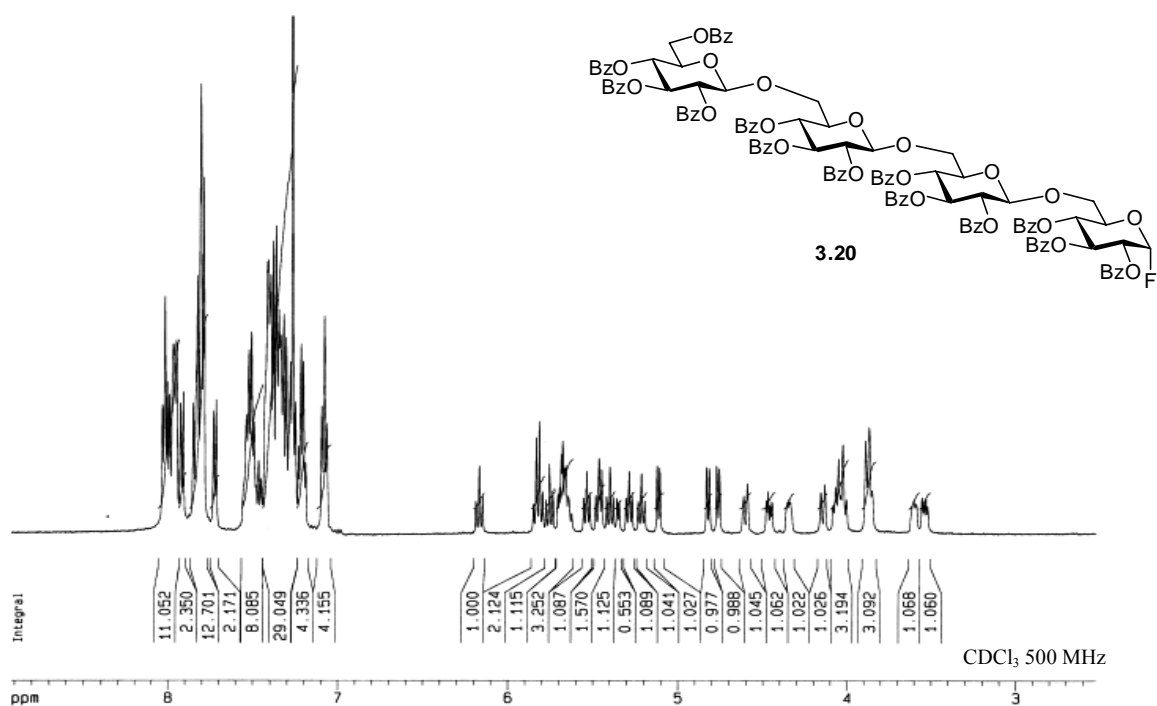
Figure A-10: ¹H NMR spectrum of 2,3,4-Tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride (**3.20**).
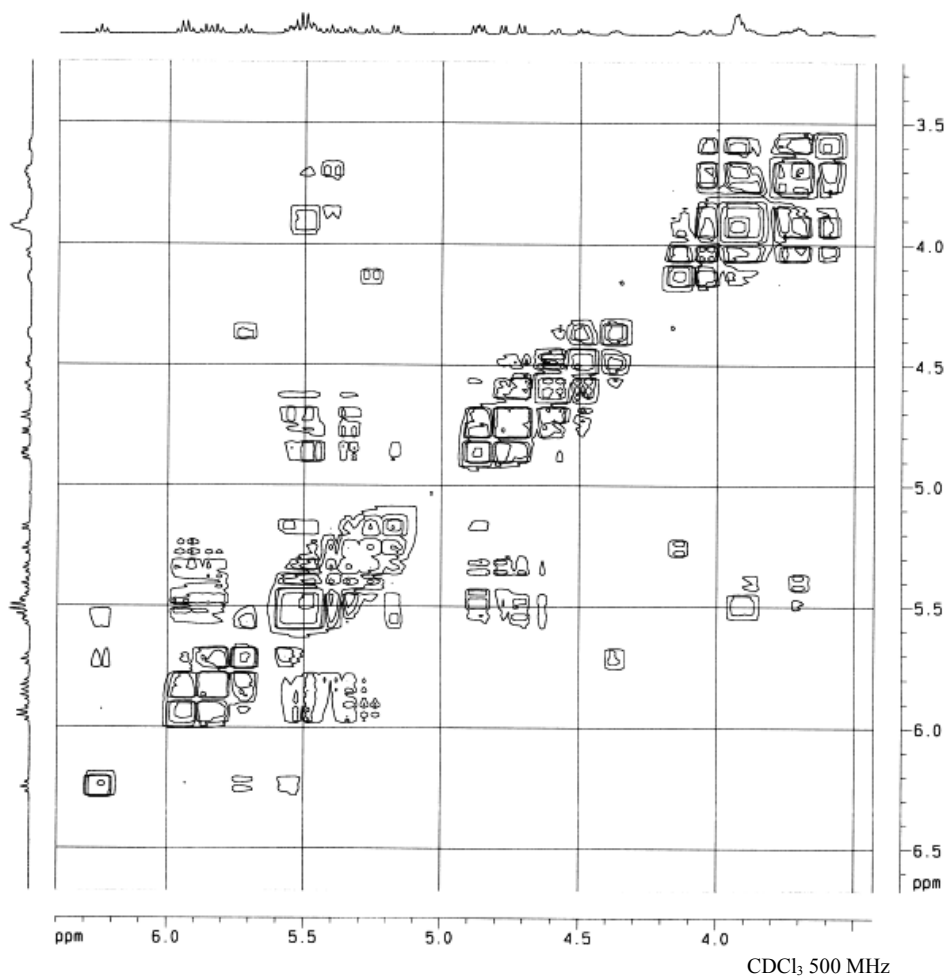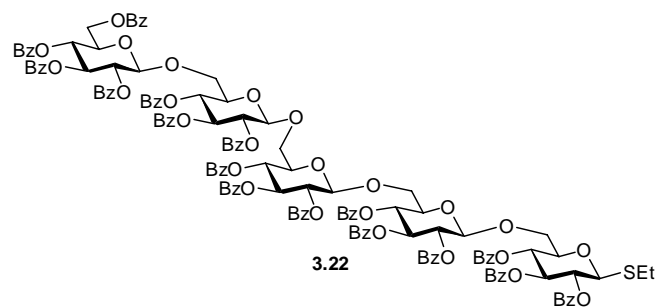

Figure A-11: ¹³C NMR spectrum of 2,3,4-Tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride (**3.20**).

Figure A-12: 2-D NMR COSY spectrum of 2,3,4-Tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl fluoride (3.20).

Figure A-13: ^{1}H NMR spectrum of Ethyl O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-thio-D-glucopyranoside (**3.22**).

Figure A-14: ^{13}C NMR spectrum of Ethyl O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-thio-D-glucopyranoside (**3.22**).
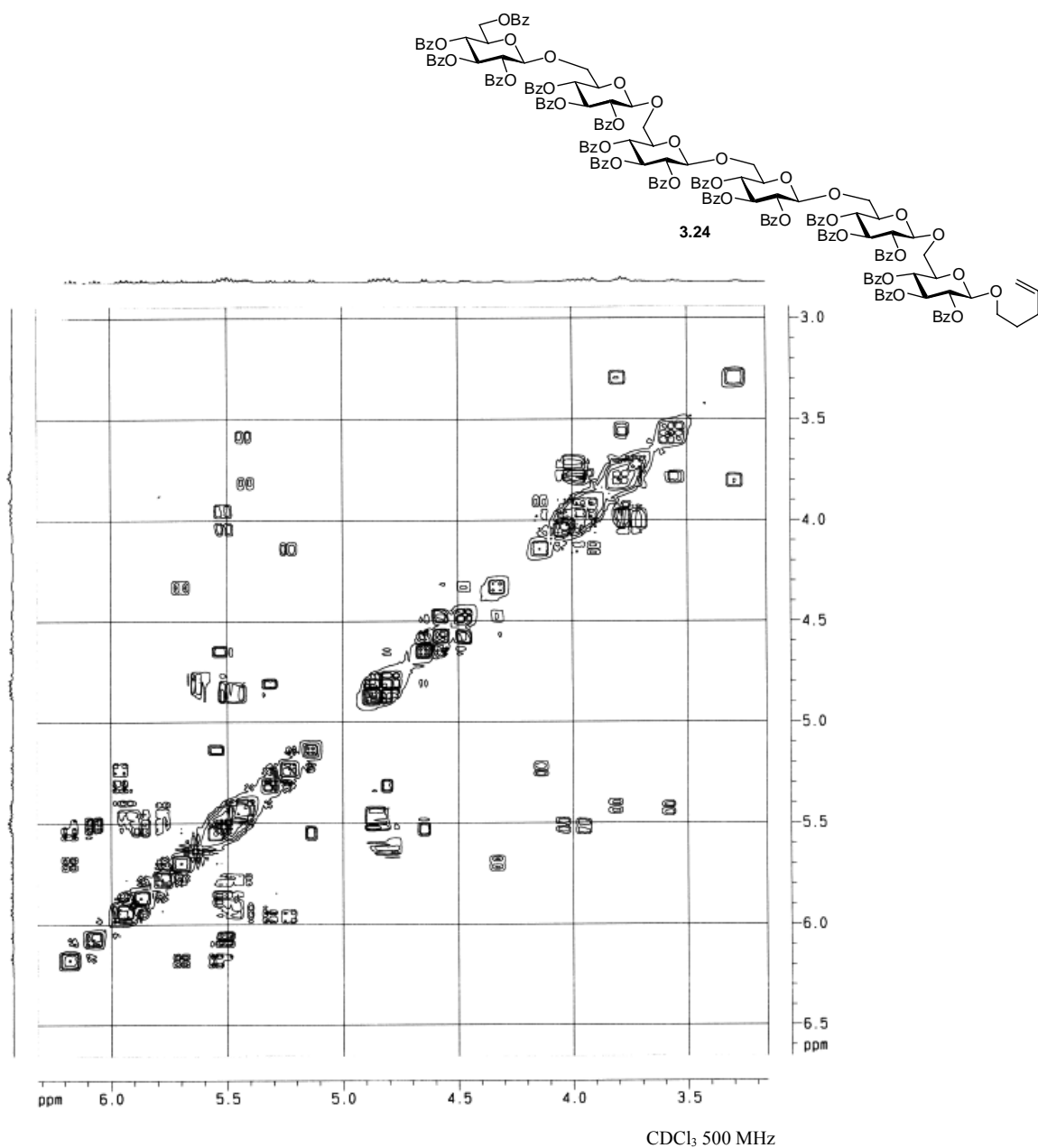
Figure A-15: 2-D NMR COSY spectrum of Ethyl *O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-thio-D-glucopyranoside (**3.22**).
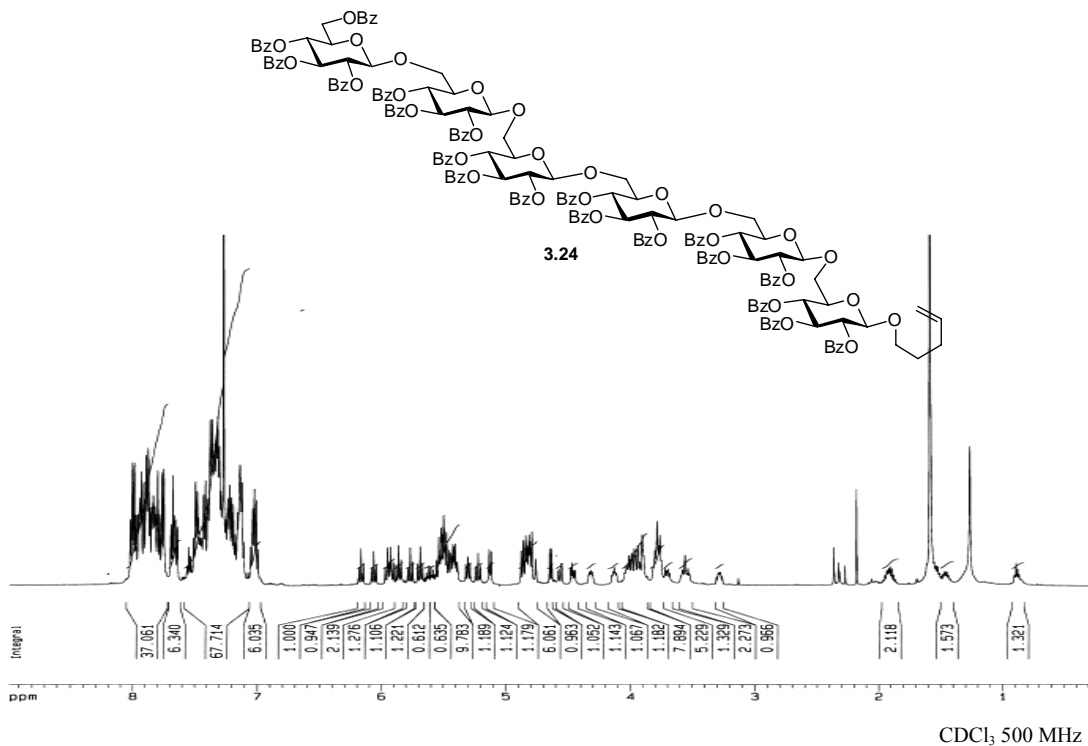
Figure A-16: ^{1}H NMR spectrum of 4-Pentenyl *O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl) (1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**3.24**).
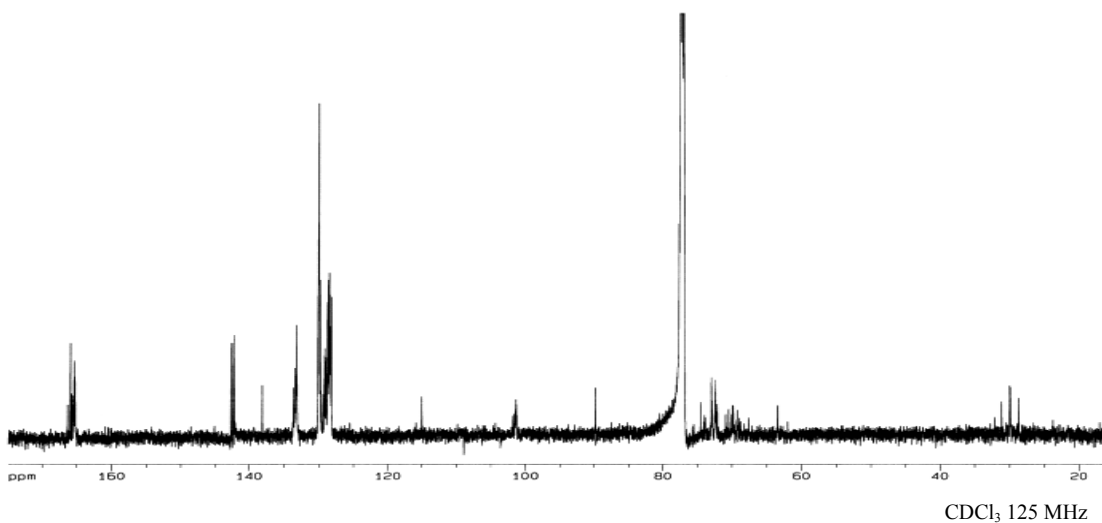

Figure A-17: ^{13}C NMR spectrum of 4-Pentenyl *O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl) (1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**3.24**).

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CDCl₃ 500 MHz

Figure A-18: 2-D NMR COSY spectrum of 4-Pentenyl *O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl) (1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**3.24**).

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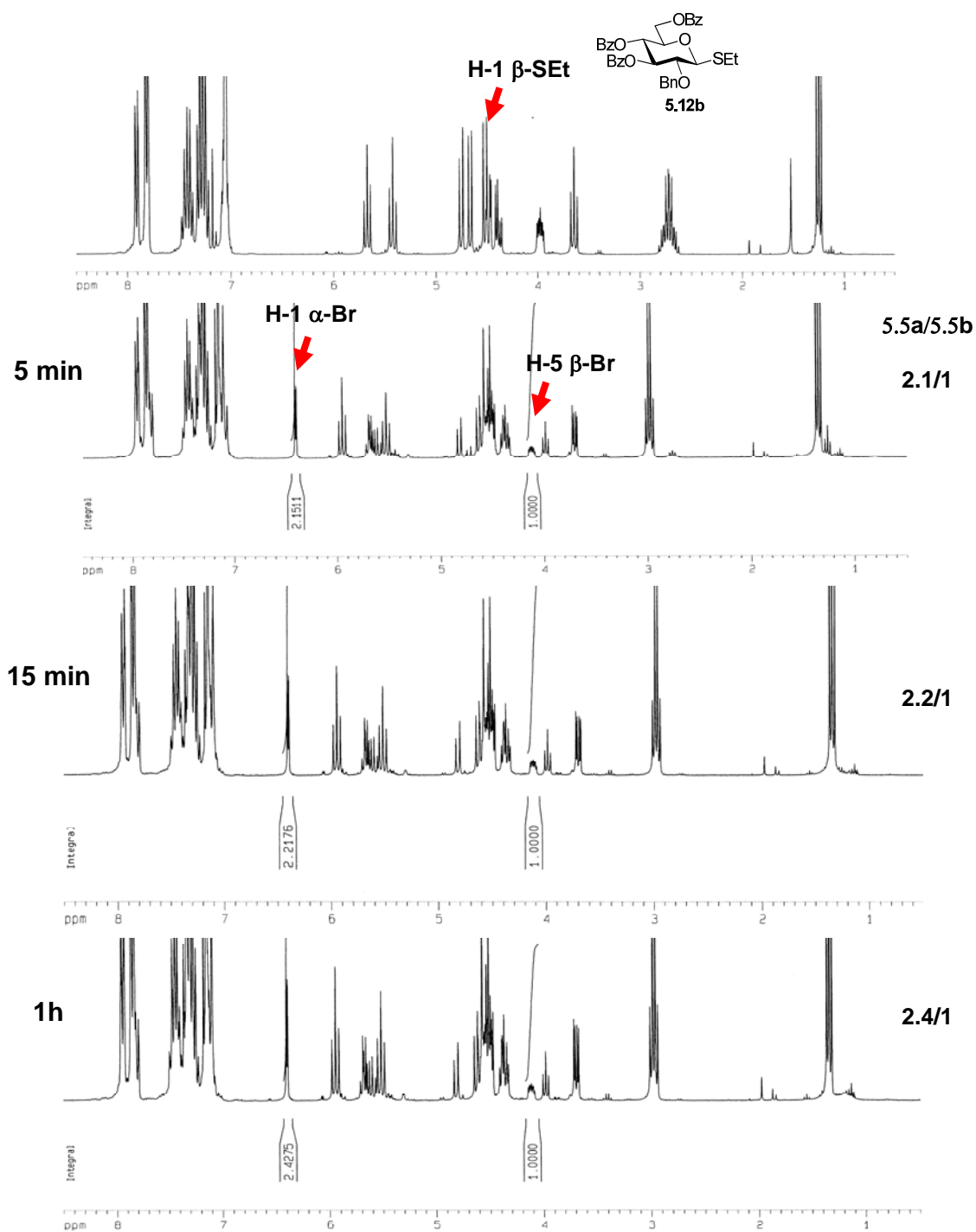
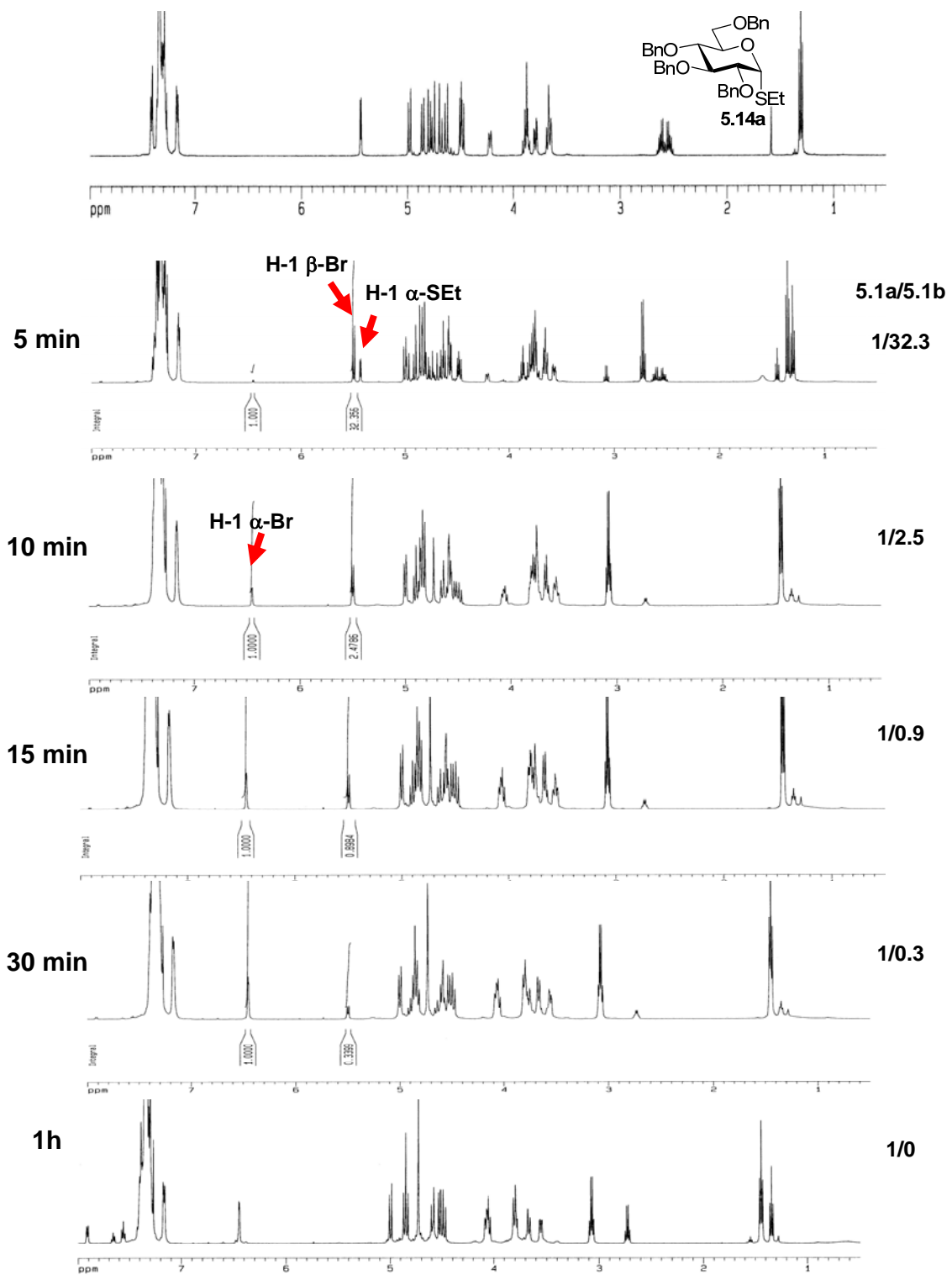Figure A-19: ^{1}H NMR monitoring of the conversion of thioglycoside **5.12b** into **5.5a/5.5b**.

Figure A-20: ^{1}H NMR monitoring of the conversion of thioglycoside **5.14a** into **5.1a/5.1b**.
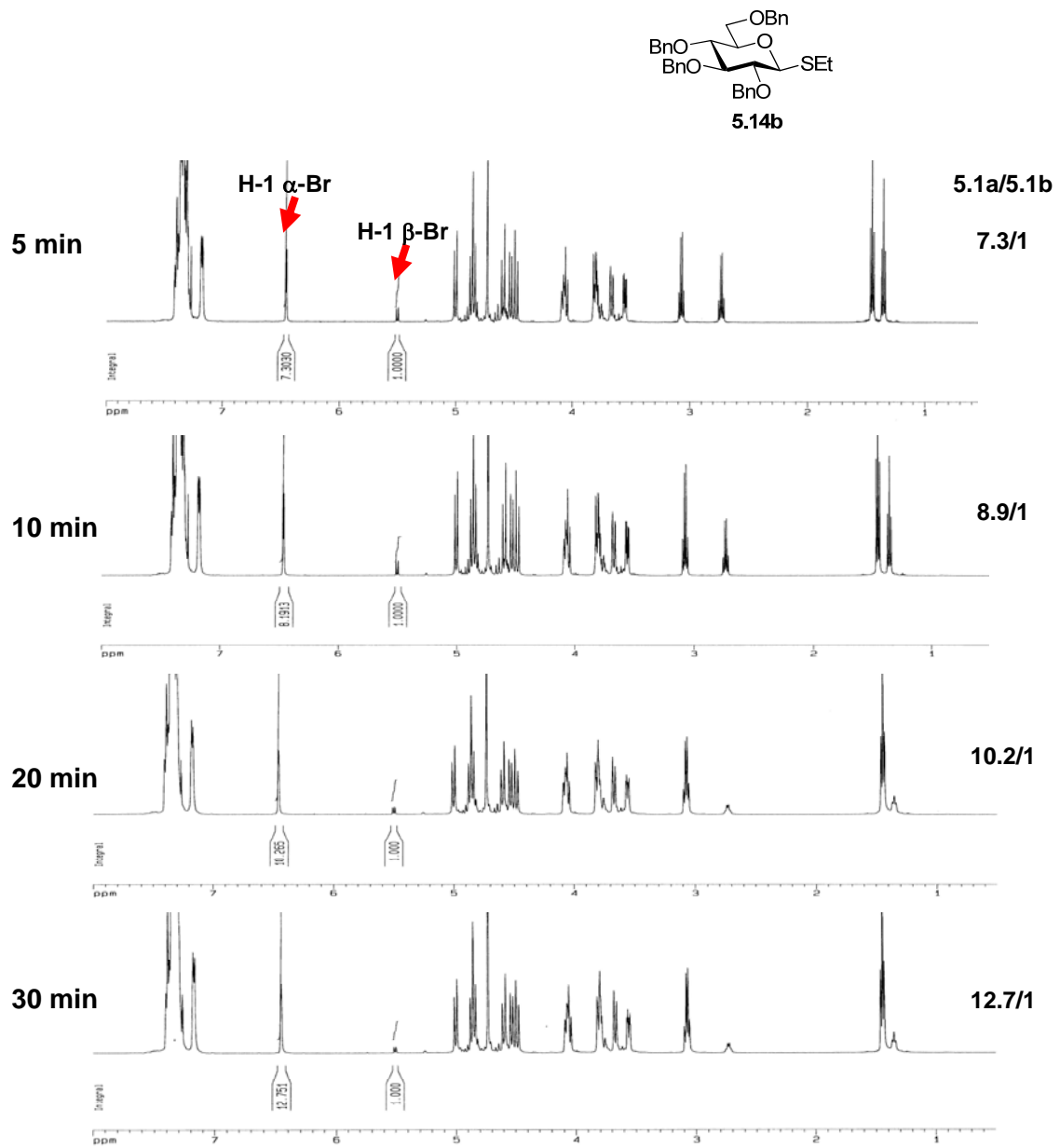
Figure A-21: ^{1}H NMR monitoring of the conversion of thioglycoside **5.14b** into **5.1a/5.1b**.
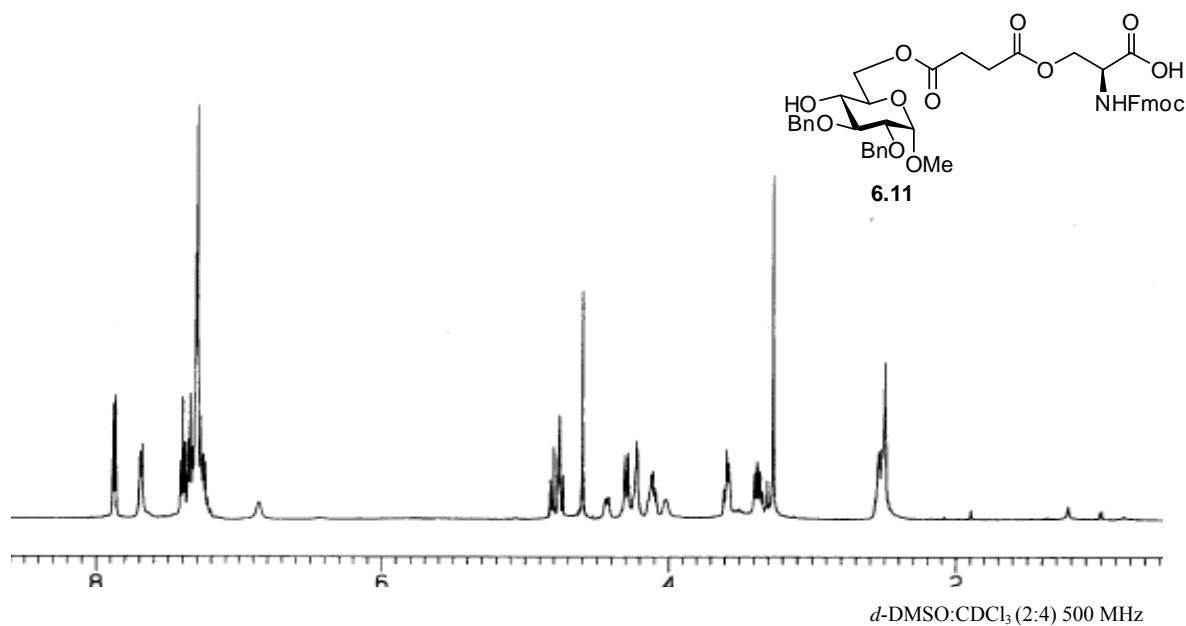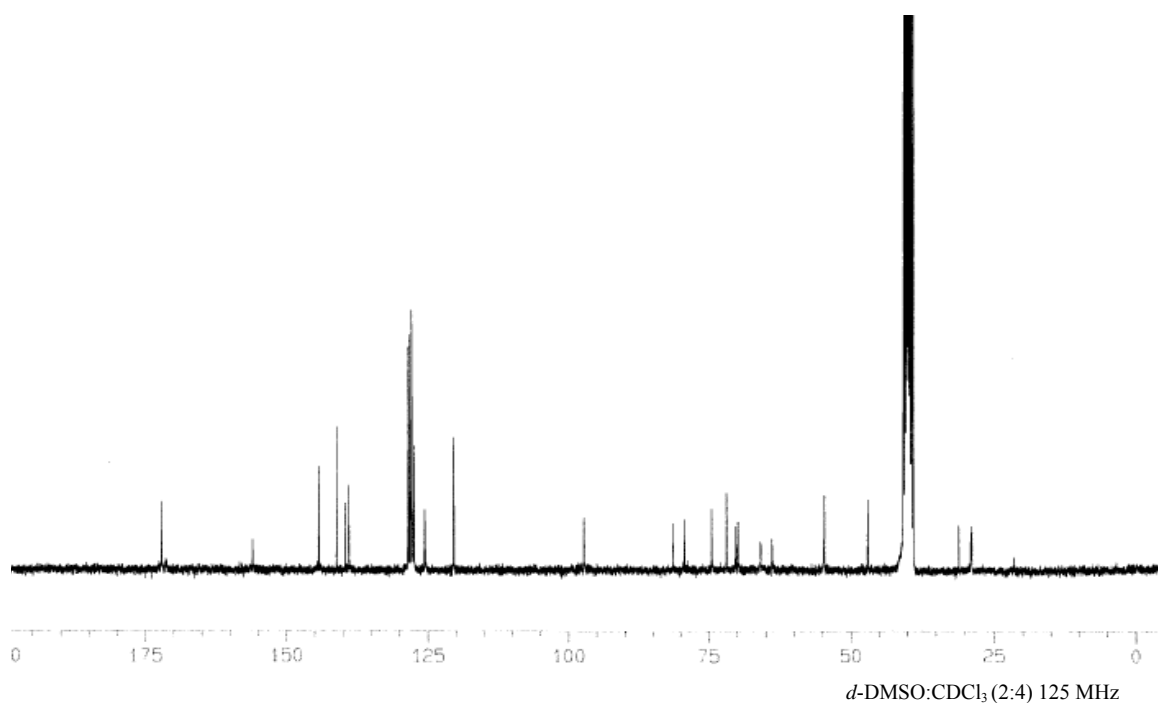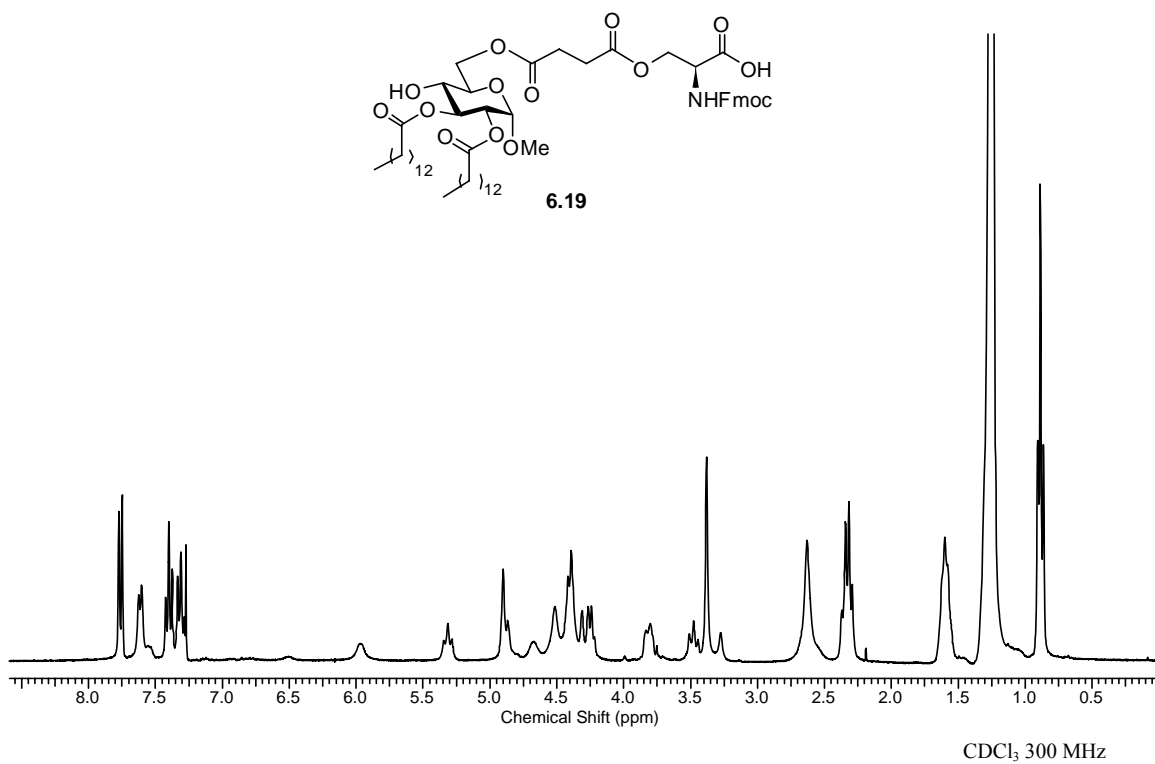
Figure A-22: ^{1}H NMR spectrum of Lipid A analog **6.11**.

Figure A-23: ^{13}C NMR spectrum of Lipid A analog **6.11**.

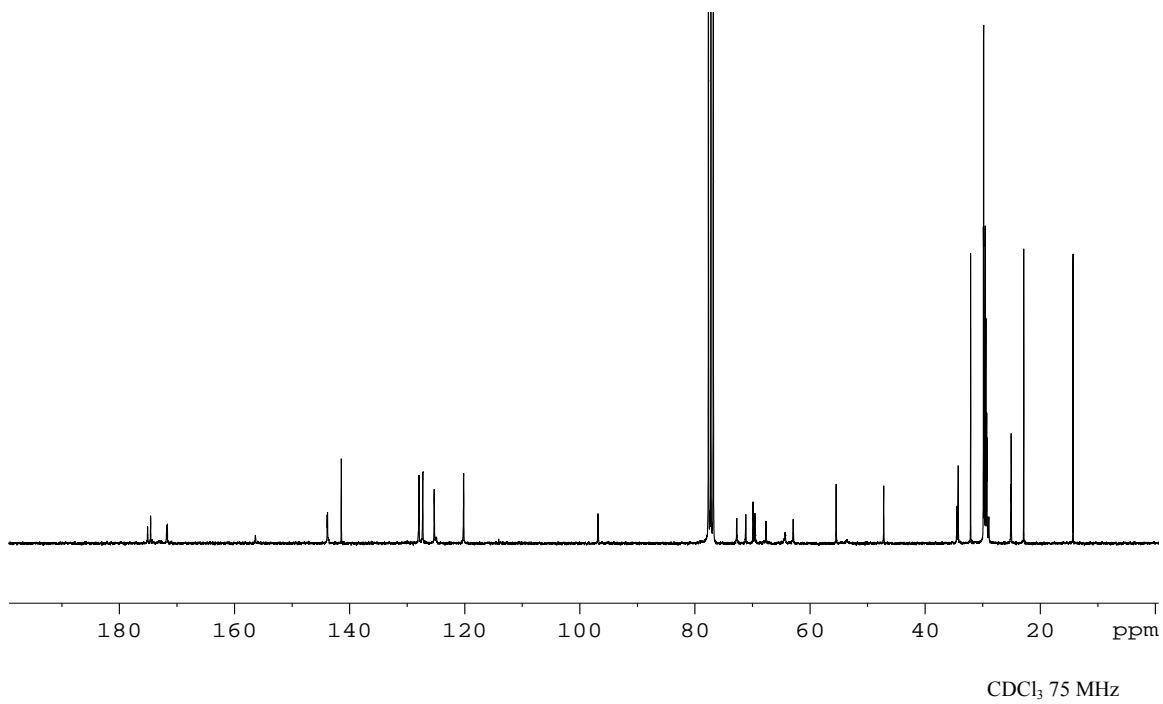Figure A-24: ^{1}H NMR spectrum of Lipid A analog **6.19**.

CDCl$_3$ 300 MHz

Figure A-25: ^{13}C NMR spectrum of Lipid A analog **6.19**.

CDCl$_3$ 75 MHz