New Methods for the Synthesis, Activation, and Application of Thioglycosides

Samira Escopy

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New Methods for the Synthesis, Activation, and Application of Thioglycosides

By

Samira Escopy

Master of Science (Chemistry), Southern Illinois University – Edwardsville, Aug 2017
Bachelor of Science (Chemistry), Taibah University, Medina – Saudi Arabia, Jan 2011

A Dissertation
Submitted to the Graduate School of the
UNIVERSITY OF MISSOURI – ST. LOUIS
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
in
CHEMISTRY with an emphasis on Organic Chemistry

December 2021

Dissertation Committee
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Prof. Eike B. Bauer, Ph.D.
Prof. Cristina De Meo, Ph.D. (SIUE)
Prof. Keith J. Stine, Ph.D.
ABSTRACT

New Methods for the Synthesis, Activation, and Application of Thioglycosides

Samira Escopy
Doctor of Philosophy, University of Missouri – St. Louis
Prof. Alexei V. Demchenko, Advisor

From their ubiquitous presence in Nature to their vital roles in biology and medicine, carbohydrates (sugars or glycans) are essential molecules of life, which are made and/or utilized by every living organism. Our cells are coated with sugars that are involved in almost every biological process and defensive mechanism in our body. To mention some of their crucial biological functions, carbohydrates are essential source of energy, they participate in blood coagulation, immune defense, cell growth, cell-cell interaction, and anti-inflammatory processes. Understanding of glycan functions and structure is crucial for the development of vaccines and therapeutics. Producing complex carbohydrates in sufficient quantities and purity and making them available for the general medical, chemical, and industrial audience is a goal that could only be achieved by the development of efficient strategies for their production. Among a variety of methodologies available for glycan synthesis, thioglycosides, sugars equipped with the anomeric sulfur atom, have been the most commonly used synthetic building blocks to date. A wide variety of reactions with thioglycosides have been developed, some of which are reviewed in the introductory part (Chapter 1). However, the field still lacks accessible methodologies that would provide efficient, high yielding, and stereoselective outcome of reactions, all in one universal platform.

Presented herein are our efforts dedicated to the development of accessible and reliable strategies for the synthesis, activation, and application of thioglycosides, from traditional manual reactions performed in flasks to sophisticated, fully automated technologies. Over the course of this study, a new method for the synthesis of thioglycosides has been established (Chapter 2). The synthesized thioglycosides have been investigated in the context of novel palladium-catalyzed activation pathways (Chapter 3) and applied for streamlining the regenerative glycosylation strategy (Chapter 4). The developed methods and strategies have then been implemented into the high performance liquid chromatography equipment-based automation (HPLC-A) platform. Differently from the previously developed HPLC-A approaches, which were exclusively based on the solid-phase synthesis, reported herein is a solution-phase automation (Chapter 5). We expect that new methods, strategies, and technologies described herein will open now exciting avenues for the synthesis and application of complex carbohydrate molecules.
ACKNOWLEDGEMENTS

“Who does not thank the people is not thankful to God”

First and foremost, I am extremely grateful and thankful to my Ph.D. advisor, Professor Alexei V. Demchenko for his invaluable supervision and support during the course of my Ph.D. studies. Thank you for pushing me, believing in me, and accepting me in the Glycoworld as I am (wild Samira). I have learned and grown a lot throughout this journey. Thank you for being the Glycoworld parent who always cared for my success and supported me to achieve this milestone of my life. Words are not enough to describe how I feel about being a Glycoworldian, but I am very proud to be part of the Glycoworld family, surrounded by great people and an amazing mentor.

I would like to specially thank Dr. Cristina De Meo, who was a main part of my success. I would not make it to this point without her kind heart, encouragement, and support. I also would like to thank my dissertation committee including Prof. Eike B. Bauer, and Prof. Keith J. Stine for all the recommendations. Also, I am thankful to the Saudi Arabian Cultural Mission for providing me with the graduate fellowship during this Ph.D., and the Department of Chemistry and Biochemistry at UMSL.

Special thanks also go to Dr. Yashapal Singh, who was my lab supervisor during the first year of my Ph.D. studies, for providing guidance and feedback throughout my research work. I also would like to thank all the past and current Glycoworld group members, Dr. Crystal O’Neil, Dr. Tinghua Wang, Dr. Satsawat Visansirikul, Dr. Michael P. Mannino, Dr. Mithila Bandara, Dr. Matteo Panza, Dr. Scott Geringer, Dr. Catherine Alex, Dr. Gustavo Kashiwagi, Melanie Shadrick, Daniel Hoard, Ashley Dent, Mariya Novakova, Nicholas P. Forsythe, and Faranak Pooladian for their support and the fun times in the lab.

Finally, to my everything, my family, thank you for your patience and support. This thesis is as much an attribute to you as to me. To all of my friends, thank you for your listening ears, motivation, and moral support. I dedicate this thesis to all of my family and friends, including the people whom I lost during this journey, my grandfather Kheder Salem Alraddadi and my best friend Tamader Abdullah Aljafal.

Thank you again to everyone who made this thesis and me possible
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Å</td>
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<tr>
<td>Ac</td>
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<td>Ac₂O</td>
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<td>Acetic acid</td>
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<td>ACS</td>
<td>American Chemical Society</td>
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<td>Biz</td>
<td>Benzimidazolyl</td>
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Bn ................................................................. Benzyl
BnBr .............................................................. Benzyl bromide
Box .............................................................. Benzoazolyl
Br2 .............................................................. Bromine
BrCCl3 .......................................................... Bromotrichloromethane
Bu4NF .......................................................... Tetra-\textit{n}-butylammonium fluoride
Bu4NI .......................................................... Tetra-\textit{n}-butylammonium iodide
\textit{tert}-BuOH ................................................. \textit{tert}-Butanol
Bu3SnH .......................................................... Tributylltin(IV) hydride
Bu2SnO .......................................................... Dibutyltin(IV) oxide
Bz ................................................................. Benzoyl
BzCl ............................................................. Benzoyl chloride
CaH2 ........................................................... Calcium hydride
CBr4 ............................................................. Carbon tetrabromide
CDCl3 .......................................................... Deuterated chloroform
CD3OD ........................................................ Deuterated methanol
CF3CO2H ...................................................... Trifluoroperacetic acid
CHCl3 .......................................................... Chloroform
C3H3Br ........................................................ Propargyl bromide
C3H5Br ........................................................ Allyl bromide
CH3COCH3 .................................................. Acetone
C3H5MgCl ...................................................... Allyl magnesium chloride
ClCH2CH2Cl .................................................. 1,2-Dichloroethane
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<td>Sodium azide</td>
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NaOAc ................................................................. Sodium acetate
NaOH .......................................................... Sodium hydroxide
NaOMe .................................................. Sodium methoxide
Nap ......................................................... Naphthyl
Na$_2$S$_2$O$_3$ .................................................. Sodium thiosulfate
NBS ......................................................... N-Bromosuccinimide
NIS .......................................................... N-Iodosuccinimide
NH$_4$OAc .............................................. Ammonium acetate
NMR ....................................................... Nuclear magnetic resonance
NPhth ......................................................... Phthalimido
NSC .......................................................... o-Nitrobenzenesulfonyl chloride
Pd/C .......................................................... Palladium on carbon
PdBr$_2$ ............................................................ Palladium bromide
PCB .......................................................... 4-Chlorobenzyl
Pt ................................................................. Platinum
Pb(ClO$_4$)$_2$ .............................................. Lead(II) perchlorate
Ph ............................................................. Phenyl
PhSH ......................................................... Thiophenol
Pico .......................................................... Picoloyl
PMB .......................................................... p-Methoxybenzyl
PMBM ...................................................... p-Methoxybenzyloxymethyl
ppm .......................................................... Parts per million
Pr ............................................................. Propyl
Py  ............................................................................................................................. Pyridine
Rf ................................................................. Retention factor
rt ............................................................................................................. Room temperature
s ................................................................................................................................. Singlet
Sc(OTf)₃ .................................................................................................................... Scandium(III) triflate
Sm(OTf)₃ .................................................................................................................... Samarium(III) triflate
SnCl₄ ................................................................. Tin(IV) chloride
S₂N₁ ........................................................................... Nucleophilic substitution unimolecular
S₂N₂ ........................................................................... Nucleophilic substitution bimolecular
T .................................................................................................................................. Triplet
Taz ...................................................................................................................... Thiazolinyl
TBAF ..................................................................... Tetra- n-butyl ammonium fluoride
TBAI ..................................................................... Tetra- n-butyl ammonium iodide
TBDMSH ................................................................. tert-Butyldimethylsilane
TBDMSOTf ....................................................... tert-Butyldimethylsilyl trifluoromethanesulfonate
TBS/TBDMS ................................................................. tert-Butyldimethylsilyl
TFA ...................................................................................................... Trifluoroacetic acid
Tf₂O ................................................................. Trifluoromethanesulfonic (triflic) anhydride
TfOH ................................................................. Trifluoromethanesulfonic (triflic) acid
THF ............................................................................................................. Tetrahydrofuran
TLC .......................................................................................... Thin layer chromatography
TMS ................................................................. Trimethylsilyl
TMSCl ................................................................. Trimethylsilyl chloride
TMSOTf ................................................................. Trimethylsilyl trifluoromethanesulfonate
Tol.........................................................................................................................p-Tolyl
TolSCl .................................................................................................................4-Toluenesulfonyl chloride
TsCl ......................................................................................................................p-Toluenesulfonyl chloride
TsOH .................................................................................................................p-Toluenesulfonic acid
TTBP ..................................................................................................................2,4,6-Tri-tert-butylpyrimidine
UV .........................................................................................................................Ultraviolet
Yb(OTf)₃ ...........................................................................................................Ytterbium(III) trifluoromethanesulfonate
ZnCl₂ ..................................................................................................................Zinc chloride
ZrCl₄......................................................................................................................Zirconium(IV) chloride
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CHAPTER 1

Transition Metal-Mediated
Glycosylation with Thioglycosides
1.1 Introduction

Existing as the most abundant class of compounds, carbohydrates are abundant in nature and important to medicine. With improved understanding of functions of carbohydrates, the demand for reliable chemical synthesis technologies has increased, thus elevating the priority for improving our experimental capabilities. The development of new reactions for obtaining complex glycans with total stereoselectivity and excellent purity has been the driving force of many recent developments in the field. Glycosylation, the most fundamental reaction with carbohydrates, remains a focus of research for many glycoscientists around the globe. Chemical $\text{O}$-glycosylation involves a nucleophilic displacement of the leaving group (LG) of the glycosyl donor with the hydroxyl group of the glycosyl acceptor (Scheme 1.1A). A typical glycosylation reaction begins with the interaction of the leaving group with the electrophilic promoter (E). As a result, ionized species are formed, and upon the departure of the LG, subsequent steps take place leading to glycosides. The development of new stereodirecting auxiliaries, leaving groups and activators to help the leaving group dissociation and enhance stereoselectivity has been a vibrant area of research in the past four decades.

Among the leaving groups developed, thioglycosides and related compounds have arguably been the most popular substrates. The soft nature of the sulfur atom means that these compounds would be relatively stable under a majority of harsh reaction conditions associated with protecting group manipulations, although they are readily activated in the presence of mild thiophilic reagents. Among a plethora of known thiophilic promoters, transition metals have been used for decades. In fact, the first known method for thioglycoside activation reported by Ferrier in 1973 involves mercury(II) salts.
Nevertheless, only recently the focus has been shifting to implementing transition metals into glycosylation reactions, either as a sole promoter or as a co-promoter.\textsuperscript{16-18} Many transition metal salts are air and moisture stable, can be used at ambient temperatures, and in some cases offer powerful means for controlling stereoselectivity.\textsuperscript{19} This review specifically focuses on metal-mediated \textit{O}-glycosidations of glycosyl donors equipped with the anomeric sulfur-based leaving groups. Three main themes covered by the review are shown in \textbf{Scheme 1.1}. These include: the activation via direct interaction between the anomeric sulfur with the metal (B); remote activation wherein the metal interacts with the remote heteroatom or a functional group (C); cooperative catalysis wherein the metal interacts with a co-promoter to generate active electrophilic species that then activate the LG (D).

\textbf{Scheme 1.1. A typical chemical \textit{O}-glycosylation (A) and the scope of this review (B-D)}
1.2 Early studies that shaped the field of transition metal-mediated activation of thioglycosides

Alkyl/aryl thioglycosides are among the most common classes of synthetic carbohydrates used both as key intermediates for regioselective modification of monosaccharides and as building blocks for the assembly of glycans. Thioglycosides are stable toward many reaction conditions, easily accessible, can be used both as glycosyl donors and glycosyl acceptors, and easily fit into a variety of expeditious strategies for glycan synthesis.\textsuperscript{13,20,21} The discovery of thioglycosides dates back to 1910 when Fischer and Delbrück first described their synthesis.\textsuperscript{22} However, it was not until 1973, when seminal work by Ferrier sparked the interest for using thioglycosides as glycosyl donors.\textsuperscript{15} These first activations of per-benzylated, now known as the armed, phenylthio (SPh) glycoside 1.1 were performed in the presence of mercury(II) salts. Hg(OAc)\textsubscript{2} was used for methanolysis and HgSO\textsubscript{4} was used for glycosylation of a sugar acceptor. These mercury salts were used as sole activators, and the respective products were obtained in promising yields and complete stereoselectivities. As depicted in Scheme 1.2A, glycosidation of thioglycoside donor 1.1 with diacetone galactose acceptor 1.2 in the presence of a freshly powdered HgSO\textsubscript{4} in refluxing THF produced disaccharide 1.3 in 54\% yield with $\alpha$-stereoselectivity, as determined by optical rotation.\textsuperscript{15}

Subsequently, Van Cleve reported the use of Hg(OBz)\textsubscript{2} as a more efficient mercury(II) salt activator in comparison to the previously used powdered HgSO\textsubscript{4}. This approach led to a successful synthesis of cholesteryl 2,3,4,6-tetra-$O$-benzyl-$\alpha$-D-glucopyranoside.\textsuperscript{23} Several years later, Garegg et al. successfully glycosidated per-benzoylated, now known as disarmed, SPh donor 1.4 with glycosyl acceptor 1.5 in the
presence of PhHgOTf. As a result, disaccharide 1.6 was obtained in an excellent yield of 91% (Scheme 1.2B).  

Scheme 1.2. First examples of activation of phenylthio glycosides as glycosyl donors

These early attempts to activate thioglycosides have immense historical significance. However, inconsistency in glycosylation yields, high toxicity, and stoichiometric loading of mercury(II) salt led to further developments in the field. Among these, exploration of thiohetaryl leaving groups is of particular relevance to the topic of this review. In 1979, Mukaiyama reported glycosidation of a glycosyl donor equipped with S-benzothiazolyl (SBaz) leaving group.  

Per-acetylated SBaz glycosides were first synthesized by Zinner, but remained largely unexplored till Mikayiama’s glycosylations that were performed in the presence of Cu(OTf)$_2$. These reactions were performed in diethyl ether and in most cases glycosylations proceeded with high yields and good $\alpha$-stereoselectivity. For example, the reaction of SBaz donor 1.7 with glycosyl acceptor 1.8 in the presence of Cu(OTf)$_2$ resulted in the formation of disaccharide 1.9 in an excellent
yield of 92% ($\alpha/\beta = 4.0/1$, **Scheme 1.3A**). The activation mode was not known at the time although the formation of active intermediates by interaction of metal salts with the ring nitrogen was mentioned by Mukaiyama *et al.*\(^{25}\)

Shortly thereafter, in 1980, Hanessian *et al.* investigated 2-pyridyl, 2-pyrimidyl, and 2-imidazolinyl glycosides as glycosyl donors that can be activated either using an acid catalyst or by using metal-based electrophilic promoter.\(^{27}\) The acid-catalyzed activation was achieved by using methanesulfonic acid, *p*-toluenesulfonic acid, etc. Thus, glycosidation of unprotected 2-pyridyl glycoside \(^{1.10}\) with 2-propanol to produce the respective glycoside \(^{1.11}\) was completed in minutes. The activation was assumed to take place via the remote nitrogen atom, and this type of activation was named “remote activation.” For metal ion-based activation, either AgNO\(_3\) or Hg(NO\(_3\))\(_2\) were employed. The activation was assumed to take place via coordination of the leaving group with the metal ion via both S and N atoms, as shown in **Scheme 1.3B**.

Woodward *et al.* then reported the application of S-pyrimidyl and S-pyridyl 2-deoxyglycosides to the synthesis of Erythromycin A.\(^{28}\) These activations were performed in the presence of AgOTf and Pb(ClO\(_4\))\(_2\) to activate pyrimidyl and pyridyl thioglycosides, respectively. These promoters outperformed other investigated activators, such as Cu(II) and Hg(II). Shortly thereafter, Pb(II)-mediated activation of S-pyridyl glycoside was successfully applied by Wuts and co-worker for the synthesis of Avermecin disaccharide.\(^{29}\) AgOTf-mediated activation of S-pyridyl disaccharide donor was successfully applied by Hanessian *et al.* in the total synthesis of (+)-Avermectin B\(_{1a}\).\(^{30}\) Thus, glycosyl donor \(^{1.12}\) was coupled with aglycone \(^{1.13}\) to yield the desired glycoside
1.14 in 72\% as $\alpha$-anomer (Scheme 1.3C). Additional remote activation approaches will be discussed in Section 1.4.

**Scheme 1.3. Early studies involving thiohetaryl leaving groups**

These early attempts to activate thioglycosides with mercury and hetaryl thioglycosides with other metal salts led to the identification of non-metallic electrophilic promoters that transformed the quest in the field in the mid-1980’s. While still used in stoichiometric amounts, NBS, MeOTf, DMTST, sulfenate ester/Lewis acid,
IDCP, and NIS/(Lewis) acid were the first wave of electrophilic promoters that immediately boosted the application of thioglycosides and allowed the achievement of unprecedented levels of efficacy and versatility. These activations are largely outside the scope of this review, but some cooperative systems do involve transition metal salts. Garegg et al. were the first to report the use of methylsulfenyl bromide (MSB)/AgOTf or o-nitrobenzenesulfenyl chloride (NSC)/AgOTf as efficient cooperative systems to activate disarmed thioglycosides at ambient temperature. For instance, the coupling of disarmed methylthio glycoside 1.15 with glycosyl acceptor 1.16 in the presence of MSB/AgOTf afforded disaccharide 1.17 in an excellent yield of 94% (Scheme 1.4). Along similar lines, Fraser-Reid et al. introduced an NIS/AgOTf promoter system that generates electrophilic iodonium species for the activation of O-pentenyl glycosides. Additional cooperative activation approaches will be discussed in Section 1.5.

**Scheme 1.4. Early studies involving cooperative promoter systems**

Another general direction in the field that emerged at about the same time involves a two-step activation approach. Accordingly, thioglycosides are first converted into other classes of glycosyl donors, and then the latter are activated. Thus, Nicolaou et al. reported the activation of phenylthio glycosides via fluorides using DAST/NBS or HF-Pyridine/NBS combinations, followed by the activation of the resulting fluorides with SnCl2/AgClO4. Ogawa et al. reported that methyl and ethyl thioglycosides can be
converted into the corresponding bromides and then activated in the presence of CuBr₂/Bu₄NBr/AgOTf reagent combination.⁴⁴ Other silver salts and HgBr₂ were investigated, but produced lower yields. Similarly, Bundle and co-workers reported Br₂/AgOTf promoted activation of thioglycosides that also proceeds via the intermediacy of glycosyl bromides.⁴⁵ Since these two step activations involve glycosyl halides as intermediates, these approaches are not covered by the scope of this review. However, the reaction conditions reported by Ogawa⁴⁴ imply that Cu(II) is capable of pooling the S-alkyl anomic moiety. For further discussion of the Cu(II)-promoted activation of S-alkyl glycosides, refer to the Dondoni-Marra approach (vide infra).

1.3 Direct activation via the interaction of a transition metal with the anomic sulfur

Following early efforts to perform direct activation of S-alkyl/aryl glycosides with Hg(II) salts described in the previous subsection, it became apparent that S-alkyl/aryl glycosides of fully oxygenated hexoses are too stable to undergo efficient glycosidation. Thioglycosides of 2-deoxysugars, however, are much more reactive and this enhanced reactivity was utilized in HgCl₂/CdCO₃-promoted activation of ethylthio glycosides for the preparation of Digitoxin and its derivatives by Wiesner and co-workers.⁴⁶,⁴⁷ Hirama and co-workers established a direct activation system using AgPF₆ as a sole promoter for deoxysugars.⁴⁸ The method has been exemplified by the reaction of thioglycoside 1.18 with kedarcidin acceptor 1.19 in the presence of AgPF₆/DTBMP at 0 °C. This glycosylation cleanly afforded the desired glycoside 1.20 in 80% yield as a single α-anomer (Scheme 1.5A). This method was extended to the synthesis of several other
complex glycosides including fully elaborated kedarcidin chromophore\textsuperscript{49} and duanorubicin analogues.\textsuperscript{50-52} More recent applications of the Hirama protocol include the synthesis of a branched trisaccharide and a non-reducing end hexasaccharide fragments of Saccharomycin B.\textsuperscript{53,54}

The hybrid solution/solid-phase synthesis of disaccharides from SEt glycosides promoted by Cu(OTf)\textsubscript{2} have been reported by Dondoni and co-workers in 2005.\textsuperscript{55} In accordance with their protocol, thioglycoside \textbf{1.21} was reacted with excess of an acceptor to afford the corresponding disaccharide \textbf{1.22} or \textbf{1.23} in high yields albeit with poor stereoselectivity (\textbf{Scheme 1.5B}). Once the solution-phase glycosylation is completed, the unreacted acceptor is separated using a solid-phase reaction/purification sequence. Via a personal communication with Professor Marra, we have learned the following. First, the choice of solvent is essential because reaction proceeds much faster in acetonitrile (or CH\textsubscript{3}CN/CH\textsubscript{2}Cl\textsubscript{2}) than in pure CH\textsubscript{2}Cl\textsubscript{2}. This is because Cu(OTf)\textsubscript{2} is highly soluble in CH\textsubscript{3}CN and the solvent was suspected to act as a ligand judged by the reaction mixture color change. Second, it is possible that reactions in CH\textsubscript{3}CN proceed through a single-electron transfer from sulfur to copper mechanism whereas in CH\textsubscript{2}Cl\textsubscript{2} the copper salt acts as a thiophilic metal. Third, these activation conditions are better suited for the "armed" glycosyl donors such as benzylated thioglycoside \textbf{1.21}.

While observations of the direct activation of $S$-alkyl/aryl glycosides remained scarce, the Demchenko group developed a variety of $S$-hetaryl and thioimidoyl leaving groups.\textsuperscript{56-58} Prior to the beginning of the 21\textsuperscript{st} century, glycosyl thioimidates have been viewed as a useful albeit somewhat insignificant variation of the thioglycoside glycosidation methodology. Because of this, beyond early work by Mukaiyama,
Hanessian, Woodward and a few others, *vide supra*, glycosyl thioimidates received very little attention, in part due to marginal stability toward protecting group manipulation that had been assumed.

**Scheme 1.5. Examples of Ag(I) and Cu(II) promoted activations of reactive S-aryl/alkyl glycosides**

Over the last two decades, the thioimidate method has evolved into a very robust approach to glycosylation.\textsuperscript{56,57,59} With the introduction of novel leaving groups, it has become apparent that thioimidates can withstand many reaction conditions associated with protecting group manipulations.\textsuperscript{60,61} In addition, these thioimidates are easily
accessible from a variety of simple precursors, can be glycosidated under a range of different reaction conditions and readily fit into a variety of glycan assembly strategies. Dedicated mechanistic studies performed with these derivatives allowed for clear differentiation between direct or remote activation pathways. The activation mode was highly conserved and leaving group structure dependent.62-65

Per-acetylated S-benzoazoyl (SBox) derivatives were first synthesized by Zinner66,67 and, decades later, the Schmidt group68 and the Demchenko group were the first to report glycosidation of differently protected SBox glycosides.61,69 These studies showed that SBox leaving group can be activated using a broad range of promoters including AgOTf, Cu(OTf)₂, Bi(OTf)₃,63 AgClO₄,70 AgBF₄, NIS/TfOH, TMSOTf, and MeOTf. Due to these fairly unique set of reaction conditions, selective activation of SBox over a range of other leaving groups has been investigated. For example, the glycosidation of SBox glycosyl donor 1.24 with acceptor 1.25 in the presence of AgOTf afforded disaccharide 1.26 in 98% yield with complete α-stereoselectivity (Scheme 1.6A).

SBox glycosides were subjected to extended mechanistic study and the following conditions all showed that these glycosyl donors follow the direct activation pathway: NIS/TfOH,62 MeOTf,62 BnBr,63 AgOTf-BINAP complex (Scheme 1.6).62 The activation pathway was concluded based on the structure of the departed aglycone using NMR, IR, UV spectroscopy as well as X-ray crystallography. This methodology has been extended to the synthesis of a variety of targets71 including furanosides by Szeja et al72 and sialosides by De Meo et al.73,74 Mainly thanks to the mechanistic studies, it was also possible to activate SBox glycosides in the presence of the S-thiazolinyl (STaz) leaving
group that undergoes remote activation. Thus, selective activation of SBox donor 1.27
over STaz acceptor 1.28 in the presence of Bi(OTf)₃ produced disaccharide 1.29 in 69% yield (Scheme 1.6B).

**Scheme 1.6. SBox and SBiz glycosides in selective glycosylation**

Per-acetylated S-benzimidazolyl (SBiz) glycosides were also first reported by Zinner.⁷⁵ Ferrieres and co-workers reported phosphorylation⁷⁶,⁷⁷ and glycosidation of unprotected SBiz donors.⁷⁸ Subsequently, Demchenko and co-workers elaborated the SBiz moiety into a new platform for active-latent⁶⁴,⁷⁹ and orthogonal glycosylations.⁶⁵ It was determined that activation of the SBiz leaving group can be readily achieved with the use of AgOTf, Cu(OTf)₂, AgClO₄,⁷⁰ and DMTST. SBiz glycosides were also shown to undergo direct activation when reacted with alkylating reagents.⁶⁴ Experiments to
understand their activation pathway were performed with BnBr as the activator. The S-benzylated aglycone isolated upon completion of glycosylation reactions indicated that SBiz glycosides follow the direct activation pathway. Chemoselective activation of the electronically superarmed SBiz glycosyl donor 1.30 over benzoylated (disarmed) glycosyl acceptor 1.31 was also investigated in the presence of AgOTf. As a result, disaccharide 1.32 was produced in 10 min in 67% yield (Scheme 1.6C).

The renewed interest to studying the direct activation of S-alkyl glycosides emerged in 2013 with the discovery by Pohl and co-workers that bismuth(V)-based reagents can act as promoters for propylthio (SPr) glycoside activation.\(^8^0\) Ph\(_3\)Bi(OTf)\(_2\) is soluble in many organic solvents, stable under oxygen atmosphere, and anhydrous conditions. The scope of the newly developed protocol has been demonstrated with a variety of donors and acceptors, ranging from primary to secondary alcohols. For example, when propyl thioglycoside 1.33 was coupled with glycosyl acceptor 1.34 in the presence of Ph\(_3\)Bi(OTf)\(_2\) disaccharide 1.35 was produced in an excellent yield of 91% (Scheme 1.7A). Interestingly, during control studies, it was found that Bi(III) was ineffective in glycosylations. A more detailed mechanistic studies of Ph\(_3\)Bi(OTf)\(_2\) promoted glycosylations revealed that the anomerization of the β-SPr donor was an essential step during glycosylation.\(^8^1\) In addition, a combination Ph\(_3\)Bi(OTf)\(_2\) with a thiol additive was able to activate less reactive per-acetylated uronic acid-derived thioglycosides, which could not be activated with Ph\(_3\)Bi(OTf)\(_2\) alone.\(^8^2\) For example, galacturonate 1.36 showed good reactivity in the reaction with fluororous-tagged glycosyl acceptor 1.37 in the presence of Ph\(_3\)Bi(OTf)\(_2\) and propane thiol as an additive to afford the corresponding product 1.38 in 71% yield (Scheme 1.7B).
Scheme 1.7. New methodologies for the direct activation of S-alkyl/aryl thioglycosides

Kartha et al. reported a new strategy employing In(OTf)$_3$ as a single promoter for the activation of conventional thioglycosides.$^{83}$ Through the application of an eco-friendly solvent-free conditions involving ball milling (BM) technique, the authors demonstrated that glycosylation of armed thiogalactoside 1.39 with acceptor 1.2 could be
promoted by catalytic In(OTf)\textsubscript{3} (25 mol%). As a result, disaccharide \textbf{1.40} was obtained in a moderate yield of 61\% (\textbf{Scheme 1.7C}). In case of more conventional glycosylations in solution, a co-promoter (NIS) was necessary to initiate the activation. A similar yield albeit very different stereoselectivity was observed in this reaction. A possible mechanism suggests that the nucleophilicity of sulfur atom (thioglycoside \textbf{1.39}) is sufficient to complex with the metal center in In(OTf)\textsubscript{3} allowing the formation of an activated intermediate \textbf{A} (\textbf{Scheme 1.7}).

Sureshan \textit{et al.} described the activation of a variety of thioglycosides using AuCl\textsubscript{3} or AuBr\textsubscript{3} catalysts without any co-promoter.\textsuperscript{84} For example, the glycosylation of thioglycoside \textbf{1.41} with secondary acceptor \textbf{1.42} in presence of catalytic AuBr\textsubscript{3} provided glycoside \textbf{1.23} in a high yield of 92\% (\textbf{Scheme 1.7D}). The authors proposed a mechanistic pathway via the direct coordination of Au(III) with the sulfur atom in thioglycosides (\textbf{B}). Once the LG departs, a formation of di-sulfide is demonstrated although no detailed mechanistic studies were presented to support this pathway. Originally, the authors illustrated that 3-5 mol % would be sufficient for the catalytic activation, however, soon thereafter, a correction appeared stating that a substoichiometric amount of Au(III) salt (0.8 equiv) was actually required for the activation.\textsuperscript{84}

Photochemistry is a well-established field with a plethora of applications for organic transformations\textsuperscript{85} including glycosylation.\textsuperscript{86} The first application of photochemically induced transformation of a thioglycoside was established by Griffin \textit{et al} in 1990. The photoinduced cleavage of the C-S bond in SPh thioglycosides was performed in the presence of 1,4-dicyanonaphthalene (DCN).\textsuperscript{87} This methodology has
been applied to a number of substrates and targets.\textsuperscript{88} The first application of a transition metal in photocatalyzed $O$-glycosidation of thioglycosides was reported in 2013 by Bowers and co-workers. Both $\text{Ru(bpy)}_3\text{Cl}_2$ and $\text{Ir[dF(CF}_3\text{)ppy]}_2(\text{dtbbpy})\text{PF}_6$ photocatalysts have been investigated in the presence of bromotrichloromethane ($\text{BrCCl}_3$) or carbon tetrabromide ($\text{CBr}_4$) as co-oxidants.\textsuperscript{89} For example, glycosidation of thioglycoside 1.43 with acceptor 1.44 under irradiation with LED blue light afforded product 1.45 in 71\% yield in moderate selectivity (\textbf{Scheme 1.8A}). Mechanistic studies suggested that the reaction occurs through decomposition of an oxidatively generated sulfur radical cation followed by thiol radical reduction as a side product.

In 2015, Ye and co-workers established a direct photoinduced activation of thioglycosides in the presence of Cu(OTf)$_2$ as an oxidant and in the absence of a photosensitizer. Under these reaction conditions and the UV light irradiation, the C-S glycosidic bond in $S$-tolyl (STol) glycosides undergoes homolytic cleavage to produce a glycosyl radical. The latter is then oxidized in the presence of Cu(OTf)$_2$ to form the oxacarbenium ion intermediate that reacts with a glycosyl acceptor. For example, irradiation of the disarmed thioglycoside 1.46 in the presence of Cu(OTf)$_2$ with protected amino acid 1.47 acceptor glycoside 1.48 was obtained in 86\% yield (\textbf{Scheme 1.8B}). A wide range of substrates and protecting groups are compatible with this protocol leading to excellent yields.\textsuperscript{90} In a follow up paper, the group reported an efficient photocatalyzed $O$-glycosylation of thioglycosides in presence of $\text{Ru(bpy)}_3(\text{PF}_6)_2$ as the photocatalyst, Umemoto’s reagent 1.51 as the radical source, and Cu(OTf)$_2$ as an additive.\textsuperscript{91}
Scheme 1.8. Photochemical induced direct activation of thioglycosides.

Mechanistic studies demonstrated that these light-driven glycosylations proceeded through CF₃-radical. Different sulfur-based leaving groups could be efficiently activated, and a rapid pre-activation-based one-pot sequential construction of oligosaccharides was demonstrated. In a continued work, thiosialosides were investigated for possible α-selective O-sialylation under the above reported conditions. Indeed, excellent stereoselectivity and good to excellent yields were the key results with a range of
alcohols. For example, sialylation of acceptor 1.50 with donor 1.49 under photocatalyzed conditions afforded disaccharide 1.52 in 75% yield with exclusive $\alpha$-selectivity (Scheme 1.8C).

More recently, Ye and co-workers further explored this methodology in application to the synthesis of a tumor-associated KH-1 antigen core nonasaccharide. This elegant work demonstrated the first successful application of photoinduced pre-activation glycosylation protocol toward the assembly of complex oligosaccharides. Umemoto’s reagent along with Cu(OTf)$_2$ were the key reagents utilized, and comparable yields and selectivity to traditional methods were the key findings. Ragains and co-workers prepared O-glycosides from 4-(4-methoxyphenyl)but-3-enyl thioglucoside donors by UV irradiation. The preliminary studies were conducted with Umemoto’s reagent in the presence of Ru(bpy)$_3$-(BArF)$_2$, however, extended experimentation showed that the presence of a photocatalyst was unnecessary. Both alkyl- and arylthio glycosides were inert under these reaction conditions.94

1.4. Remote activation via the interaction of a transition metal with the remote heteroatom or a remote functional group

Following early efforts to perform remote activation of S-pyridyl/pyrimidyl and similar leaving groups described in Section 1.2, a variety of applications of the earlier Hanessian and Woodward approaches have emerged.95-98 In more recent applications, mild and effective AgOTf has been implemented as the promoter.99,100 As an expansion of the remote activation studies, a variety of other S-leaving groups have emerged at the beginning of the 1990s. Glycosyl dithiocarbonates (xanthates) were introduced by Sinay
and Marra.\textsuperscript{101,102} Their activation was conducted in the presence of Cu(OTf)\textsubscript{2} alone. For example, xanthate donor 1.53 was used for an efficient synthesis of 2-amino-2-deoxyglycoside 1.55. Thus, glycosidation of 1.53 with acceptor 1.54 in the presence of Cu(OTf)\textsubscript{2} afforded trisaccharide 1.55 in 80\% yield for the α-anomer (Scheme 1.9A).

Scheme 1.9. A new wave of glycosyl donors for remote activation.

Szeja and co-workers, used AgOTf to activate glycofuranosyl xanthates\textsuperscript{103} with or without polar additives such as hexamethylphosphoramide (HMPA) that were found to enhance α-stereoselectivity.\textsuperscript{104} Other applications of this methodology have recently been reported.\textsuperscript{59,105,106} A variety of similar leaving groups including phosphorodithioates,\textsuperscript{107-109} 1-piperidinecarbodithioates,\textsuperscript{110} and dithiocarbamates,\textsuperscript{111} have been developed. Among
other leaving groups that emerged at about the same time is 1’-phenyl-1H-tetrazolyl glycosides that were introduced by Ogura and co-workers.\textsuperscript{112} For instance, when glycosylation of disaccharide acceptor 1.57 was performed with glycosyl donor 1.56 in the presence of AgOTf, trisaccharide 1.58 was obtained in 71\% yield (\textbf{Scheme 1.9B}).

At around the same time, sulfoxides\textsuperscript{113} as glycosyl donors activated in the presence of triflic anhydride were introduced by Kahne\textsuperscript{,114} but their activation with transition metals has not emerged till a decade later when Wipf \textit{et al.} illustrated that the combination of Cp\textsubscript{2}ZrCl\textsubscript{2}/AgClO\textsubscript{4} can serve as an efficient activator. Thus, glycosylation of acceptor 1.60 with donor 1.59 in the presence of Cp\textsubscript{2}ZrCl\textsubscript{2}/AgClO\textsubscript{4} afforded product 1.61 in 72\% yield (\textbf{Scheme 1.9C}).\textsuperscript{115} Soon thereafter, Chung \textit{et al.} reported the activation of glycosyl sulfoxides in the presence of Yb(OTf)\textsubscript{3} or Eu(OTf)\textsubscript{3}.\textsuperscript{116} At the time, the utility of these methodologies were limited to armed sulfoxides. Subsequently, a cooperative activation of glycosyl sulfoxides (armed and disarmed) by AuCl\textsubscript{3}/AgOTf catalysis has been demonstrated by Vankar and co-workers.\textsuperscript{117}

Introduced by Ley and co-workers\textsuperscript{118,119} glycosyl sulfones were mostly used as glycosyl donors for C-glycosylations.\textsuperscript{120,121} Examples of \textit{O}-glycosylations remained rare until studies by Lowary and co-workers who introduced samarium(III) triflate mediated activation of glycosyl 2-pyridylsulfones.\textsuperscript{122} For example, the coupling of furanosyl sulfone donor 1.62 with SEt acceptor 1.63 in the presence of Sm(OTf)\textsubscript{3} afforded disaccharide 1.64 in 71\% yield. The latter is equipped with the SEt leaving group that can be used in subsequent activations directly (\textbf{Scheme 1.10A}).
Scheme 1.10. Activation of glycosyl sulfones.

Norsikian et al established a novel MW-assisted procedure wherein armed glycosyl sulfone donors were activated in the presence of scandium(III) triflate. Thus, mannosyl sulfone donor 1.65 was glycosidated with iso-propanol in the presence of Sc(OTf)$_3$ to give the corresponding product 1.66 in 85% yield predominantly as the $\alpha$-anomer (Scheme 1.10B). It was suggested that the activation may occur via the intermediacy of a Sc(III) complex comprising both the acceptor and the donor. Subsequent cleavage of the C–S bond would lead to the oxacarbenium/scandium arenesulfinate ion pair followed by glycosylation. The released arylsulfinic acid is neutralized with TTBP. All manno-, gluco-, and galactosyl sulfone donors showed good reactivity, however, the reaction was particularly efficient for $\alpha$-mannosylation and less selective with other sugar series. These results were rationalized by Sc(OTf)$_3$ mediated anomerization occurring post-glycosylation.
Other important compounds for remote activation are S-thiazolinyl (STaz) derivatives that were introduced by Descotes and co-workers as glycosyl donors that can be activated with HgNO₃. Further investigation into the glycosylation protocol toward disaccharide and oligosaccharide synthesis was reported by Demchenko et al. Activators such as AgOTf, MeOTf, NIS/TfOH, benzyl bromide, methyl iodide, AgBF₄, and Cu(OTf)₂ have been found to be suitable for efficient STaz activation for glycosylation and glycan synthesis. For example, chemoselective activation of armed STaz donor 1.67 over disarmed acceptor 1.68 in the presence of Cu(OTf)₂ afforded product 1.69 predominantly as the α-anomer. A subsequent glycosylation of acceptor 1.34 with 1.69 promoted by AgOTf gave trisaccharide 1.70 in a good yield of 83% (Scheme 1.11A).

Scheme 1.11. STaz derivatives in synthesis.
An interesting observation that STaz glycosides can participate in stable non-ionizing transition metal complexes led to the development of a temporary deactivation concept. As depicted in Scheme 1.11B, deactivation of compound 1.71 was accomplished by coordinating its STaz functionality into a PdBr₂ complex 1.72. The activation of the STaz moiety of glycosyl donor 1.73 over the temporarily deactivated STaz moiety of acceptor 1.72 then achieved in the presence of MeOTf to form disaccharide complex 1.74. The latter was then decomplexed by treatment with NaCN to afford disaccharide 1.75, which could be used in subsequent transformations. The temporary deactivation concept was further expanded by the development of a new generation of leaving groups capable of a bidentate, and therefore more stable, complexation mode with the deactivating reagent PdBr₂. It was also found that the complexation between STaz leaving group to platinum(IV) can lead to enhanced stereoselectivity in glycosylations.

Thiocyanates are also capable of remote activation. Best known for their activations with trityl perchlorate or TMSOTf as reported by Kochetkov and co-workers, these glycosyl donors can also be activated with AgOTf. The latter conditions allow for much improved yields albeit lower stereoselectivity of glycosylations. N-Phenyltrifluorothioacetimidate leaving group activation was established in the presence of AgOTf among other reagents. S-Glycosyl O-methyl phenylcarbamothioates (SNea) can be efficiently activated with Cu(OTf)₂, and the activation of various derivatives of SNea could also be effected with Cu(OTf)₂, Bi(OTf)₃, or AgOTf.

High affinity of gold catalysis to alkyne functionality is a relatively new research direction in glycosylation chemistry. First reported by Hotha et al. for the activation of O-
propargyl glycosides, it was not until 2012 when Yu et al. reported the use of gold catalysts with thioglycosides. For example, glycosylation of acceptor 1.2 with donor 1.76 in presence of Au(I) catalyst gave disaccharide 1.77 along with the orthoester byproduct in 99% combined yield (Scheme 1.12A). Zhu et al. also reported the stereoselective synthesis of 2-deoxy and 2,6-dideoxy-α-glycosides using novel 2-deoxy-S-but-3-ynyl thioglycoside donors under Au(I)-catalysis. For example, when 2-deoxy thioglycoside donor 1.78 was coupled with acceptor 1.5 in the presence of Au(I) catalyst product 1.79 was obtained in 96% yield albeit with modest selectivity (Scheme 1.12B).

A variety of glycosyl acceptors could be efficiently glycosylated under these reaction conditions. Considering the high alkynophilicity of gold, the authors suggested that Au(I) would coordinate with the triple bond. This will facilitate cyclization of the leaving group via the attack of a sulfur atom. The resulting sulfonium ion intermediate A will then undergo cleavage of the glycosidic bond followed by a glycosylation. Subsequently, the authors investigated S-but-3-ynyl thioglycoside donors under Au(III)-catalysis. Although high yield and α-selectivity were obtained with 2-deoxy sugars, regular hexoses and 2-aminosugars required higher catalyst loading. Further experimentation showed that gem-dimethyl S-but-3-ynyl thioglycoside donors were much more efficient in these systems, probably due to the Thorpe-Ingold effect. For instance, the synthesis of disaccharide 1.81 was accomplished in a high yield (90%) by glycosylating acceptor 1.5 with gem-dimethyl S-but-3-ynyl amino sugar donor 1.80 in the presence of AuCl3/AgOTf system (Scheme 1.12C).
Scheme 1.12. Gold(I)-catalyzed activation of thioalkynyl leaving groups.

**Proposed Mechanism:**

\[
\text{Proposed Mechanism:} \quad \begin{array}{c}
\text{A} \\
\text{B} \\
\text{C} \\
\text{D} \\
\text{E}
\end{array}
\]

**Possible side reactions with unreactive ROH**

- **B** (isomerization)
- **C** (formal [1,3]-shift)
Recently, Zhang et al. demonstrated that an improvement to Yu’s approach can be achieved by employing (2-ethynylphenyl)thio leaving group, rather than alkyne terminus substituent ($^t$Bu). Using electron-withdrawing 4-chlorobenzyl groups (PCB) and performing the activation with a less acidic promoter $i$PrAu$^+$ allows the achievement of efficient stabilization of the sulfonium intermediate hence favoring the $S_N2$-like glycosylation pathway. After screening several counterions, NTf$_2^-$ was found to be the most efficient, hence the reactions were conducted in the presence of $i$PrAuCl/AgNTf$_2$. This methodology was successfully applied to the synthesis of $\alpha$-D-glucosides, wherein good to excellent $\alpha$-selectivity was achieved. For example, glycosidation of thioglucoside donor 1.82 with benzyl alcohol in the presence of $i$PrAuCl/AgNTf$_2$ produced glycoside 1.83 in a good yield of 80% and high $\alpha$-selectivity ($\alpha/\beta = 19/1$, Scheme 1.12D).

Further investigation of the reaction scope showed that the nucleophilicity and steric bulk of the glycosyl acceptor could significantly affect the reaction outcome because benzothiophene aglycone could compete for the activated intermediate B, resulting in its inversion. Additionally, a formal [1,3]-shift of intermediate B leading to unreactive C-glycoside C could also take place in glycosylations of unreactive glycosyl acceptors.

Very recently, Ikeuchi et al. investigated activation of SPh glucosides through a cationic Au(I)-mediated intramolecular migration of the LG into the alkyne moiety of the 2-O-propargyl substituent. It has been demonstrated that 3,6-O-EDB-bridge forces $\beta$-thioglucoside 1.84 into the $5_S$ conformation (Scheme 1.12E), which helps both the activation by placing the LG and 2-O-propargyl in close proximity to each other and $\alpha$-stereoselectivity. For example, when acceptor 1.85 was glycosylated with $\beta$-thioglucoside
donor 1.84 in the presence of catalytic (4-F₃CC₆H₄)₃PAuNTf₂, glycoside 1.86 was obtained in 57% yield mainly as the α-anomer (Scheme 1.12E). It was suggested by the authors that the intramolecular migration of the SPh group make occur to the either side of the triple bond on 2-O-propagyl group (exo- or endo- pathways shown for the intermediate D). Moderate to good yields and high α-stereoselectivities have been obtained in glycosylations of a variety of primary, secondary, and tertiary glycosyl acceptors using this method.

Jensen and co-workers recently reported the novel ortho-(methylthio)benzyl thioglycosides as effective donors capable of the Cu(OTf)₂ mediated remote activation pathway. It has been proposed that having two sulfur atoms, the anomic one and o-SMe functionalized benzyl, the activation would occur through a metal chelating effect. After investigating various metal triflates, only Cu(II) and Fe(III) salts were found to be effective, at which point the authors decided to proceed with less hygroscopic Cu(OTf)₂. Different o-SMe functionalized benzyl thioglycoside donors (glucose, galactose, and L-sugars) have been investigated with a variety of glycosyl acceptors. A very efficient application of this method to a one-pot two-step synthesis of trisaccharide 1.89 has also been described. Thus, o-SMe functionalized thioglycoside donor 1.87 was activated in presence of Cu(OTf)₂ to couple with acceptor 1.88. Following the formation of the disaccharide intermediate, acceptor 1.34 and NIS were added to afford the target trisaccharide 1.89 in 48% yield overall (Scheme 1.13A).

Demchenko and co-workers introduced indolylthio glycosyl (SIn) donors that can be activated with AgBF₄ and Ag₂CO₃/TMSOTf among other conditions. Several glycosyl donor – acceptor combinations have been investigated, among these,
glycosylation of 2-OH acceptor 1.91 with armed SIn glucosyl donor 1.90 in presence of 
$\text{Ag}_2\text{CO}_3$/TMSOTf produced product 1.92 in 81% yield (Scheme 1.13B). Although 
activation of the moiety SIn required excess reagents, activation was found to be 
completely orthogonal in respect to building blocks equipped with SEt and SBox leaving 
groups. Competition and NMR experiments suggested possible involvement of the 
remote $N$-atom during activation.

Scheme 1.13. New leaving groups for remote activation.

1.5. Cooperative catalysis wherein a metal first reacts with other reagents to 
generate a promoter

In addition to the originally introduced cooperative activation systems by Garegg 
et al. MSB/AgOTf$^{41}$ or Fraser-Reid et al. NIS/AgOTf (for $O$-pentenyl glycosides)$^{42}$
many other similar approaches have emerged over the years. The key developments in
the area are summarized in Table 1.1 wherein we have also included lanthanides and main group metal salts for comprehensiveness.

**Table 1.1. Overview of the cooperative systems developed for the activation of thioglycosides.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>LG</th>
<th>Promoter</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SEt</td>
<td>MSB-AgOTf</td>
<td>Garegg et al. (1988)&lt;sup&gt;41&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MSC-AgOTf</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SEt, STol</td>
<td>NIS-AgOTf</td>
<td>Bundle and Lowary (1995)&lt;sup&gt;143,144&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>SCSOEt</td>
<td>MSB-AgOTf</td>
<td>Magnusson et al. (1995-6)&lt;sup&gt;145,146&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PSB-AgOTf</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>SMe, SPh</td>
<td>PhIO-SnCl&lt;sub&gt;2&lt;/sub&gt;-AgClO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Kusumoto et al. (1996)&lt;sup&gt;147&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PhIO-BiCl&lt;sub&gt;3&lt;/sub&gt;-AgClO&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>SMe, SEt, SPh</td>
<td>IX-AgOTf (X = Cl, Br)</td>
<td>Magnusson et al. (2001)&lt;sup&gt;148,149&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>SEt</td>
<td>NIS-Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Ferrieres et al. (2003)&lt;sup&gt;150&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>SPh</td>
<td>NIS-Yb(OTf)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Chung et al. (2003)&lt;sup&gt;151&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>STol</td>
<td>p-TolSstCl-AgOTf</td>
<td>Ye et al. (2004)&lt;sup&gt;152&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>SMe, SEt, SPh</td>
<td>NBS-Bi(OTf)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Iadonisi et al (2007)&lt;sup&gt;153&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>SEt, STol</td>
<td>BDMS-AgOTf</td>
<td>Ye et al. (2008)&lt;sup&gt;152,154&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>SEt, SPh</td>
<td>p-NO&lt;sub&gt;2&lt;/sub&gt;PhSCl-AgOTf</td>
<td>Crich et al. (2008)&lt;sup&gt;155&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>SPh</td>
<td>NIS-Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Jensen et al. (2010)&lt;sup&gt;156&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>SEt, SPh, STol</td>
<td>NIS-La(OTf)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Mukhopadhyay et al. (2010)&lt;sup&gt;157&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>STol</td>
<td>DMTPSB&lt;sup&gt;a&lt;/sup&gt;-AgOTf</td>
<td>Ye et al. (2011)&lt;sup&gt;158&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>SPh</td>
<td>MSB-AgClO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Demchenko et al. (2014)&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIS-In(OTf)&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>STol, SPh</td>
<td>IX-In(OTf)&lt;sub&gt;3&lt;/sub&gt; (X = Cl, Br)</td>
<td>Fukase et al (2014)&lt;sup&gt;159&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PhIO-In(OTf)&lt;sub&gt;3&lt;/sub&gt;, etc</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>SEt, SPh</td>
<td>MSH-Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Bernardes et al. (2017)&lt;sup&gt;160&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NBS-Cu(OTf)&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>- DMTPSB: O,O-dimethylthiophosphonosulfenyl bromide
In addition, a number of approaches based on other concepts of cooperative catalysis have emerged. Thus, Li and co-workers were first to report a cobalt-propargyl cation induced glycosidation of thioglycosides in the presence of a Lewis acid. The utility of this method relies on the \textit{in situ} formation of a cobalt-propargyl cation through the activation of $\text{Co}_2(\text{CO})_6$-propargylated glycosyl acceptor. During the examination of a suitable Lewis acid, the authors found that TMSOTf was the most promising to activate cobalt species in terms of yield and reaction time. A variety of armed and disarmed STol donors were tested with primary and secondary propargylated acceptors. In all cases, moderate to good yields were obtained, however a lack of stereoselectivity, without C-2 participation, was observed. For example, glycosidation of armed STol donor 1.93 with propargylated glucoside acceptor 1.94 in the presence of TMSOTf provided disaccharide 1.35 in 88% yield albeit with no selectivity (Scheme 1.14A). It was proposed that in presence of TMSOTf a small amount of cobalt-propargyl cation is produced. The latter is then attacked by the anomeric sulfur atom causing dissociation of the STol leaving group.

Recently, an elegant work to activate thioglycosides via rhodium(II) and Brønsted acid-catalyzed glycosylation has been presented by Wan and co-workers. This reaction proceeds Rh(II)-catalyzed glycosyl sulfonium ylide formation in the presence of diazo-compound. Both one-pot and stepwise activation protocols have been investigated. For instance, the coupling of thioglucoside donor 1.95 with acceptor 1.96 was performed in one-step activation mode to afford disaccharide 1.97 in a good yield of 86% (Scheme 1.14B). It was proposed that the diazo-compound decomposes in the first catalytic cycle promoted by $\text{Rh}_2(\text{oct})_4$, which forms rhodium carbenoid D. The latter is attacked by the sulfur atom of the glycosyl donor, which leads to regeneration of Rh(II) catalyst, and the
resulting glycosyl ylide is protonated with Brønsted acid into sulfonium ion intermediate E. The latter undergoes the leaving group departure following by the glycosidic bond formation.

**Scheme 1.14. Cooperative catalysis S-alkylation pathways.**

1.6. Conclusions and outlook

The discovery of a mercury(II) salt in 1973 as an activator of thioglycosides opened the door toward many applications of these excellent building blocks. Group 11 transition metals, including silver and copper, were first attempts towards more benign methods of activation. More recent studies have focused on gold(I) and gold(III) complexes, which have proven to be effective activators for thioglycosides. Cobalt,
palladium and rhodium-based systems also found their application, although these approaches require further investigation to determine their scope. Moreover, post-transition metals, tin, bismuth, and indium are excellent Lewis acid activators due to their low toxicity and price. Metal-photocatalysis which activated thioglycosides via a single electron transfer (SET) mechanism is another promising albeit underexplored new direction.

The discovery of metal catalysts for the activation of thioglycosides opened a new venue for further significant developments en route to more stereoselective reactions and less toxic conditions. These new reactions have already been demonstrated in the context of orthogonal glycosylation strategies, chemoselective activation, synthesis of antibiotics and glycans among other applications. Some new reactions allow for controlling the anomeric selectivity by tuning the nature of the metal, ligands and the complexation mode.

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CHAPTER 2

Triflic Acid-Mediated Synthesis of Thioglycosides

2.1 Introduction

Carbohydrates are the most abundant biomolecules that play crucial roles in many biological processes and their involvement in all diseases has been proven. However, the stereoselective synthesis of complex carbohydrates is still a challenge in glycosciences. The key aspect of the oligosaccharide assembly is the attachment of various monosaccharide units via a glycosidic bond. The linkage is constructed by a chemical glycosylation reaction that involves a nucleophilic displacement of a leaving group of the glycosyl donor with a hydroxyl moiety of the glycosyl acceptor in the presence of an activator.

Since their invention in 1909 by Fisher, thioglycosides have become key building blocks both for modification of monosaccharides and for construction of glycans. Numerous methods for the preparation of thioglycosides have been established. The most commonly employed pathway is Lewis acid-mediated thioglycosidation of per-acetylated sugars in the presence of stoichiometric amounts (2-5 equiv) of TMSOTf, BF$_3$•Et$_2$O, ZrCl$_4$, SnCl$_4$, etc. A variety of other approaches such as one-pot acetylation-thioglycosidation of unprotected sugars, including Bronsted-acid-mediated reactions are also known. Although many of these conditions provide good stereoselectivity and yields, excess of promoter requirement indicates the limitation of this methodology. Herein we report that even a sub-stoichiometric amount of triflic acid can promote thioglycosidation of per-acetates of different sugar series.
2.2 Results and discussion

Our first attempts to standardize the reaction conditions involved common glucose pentaacetate \( \text{2.1} \) that was set to react with 2.0 equiv of ethanethiol (EtSH) in dichloromethane at 0 °C. The key results of this study are surveyed in Table 2.1. Thus, when catalytic amount of triflic acid (0.2 equiv) was applied at 0 °C, thioglucoside \( \text{2.2}\) was afforded in 26% yield in 4 h (entry 1). Prolonged experiments showed that the starting material did not consume even after 24 h, and the product yield remained practically the same. Increasing the triflic acid to 50 mol % produced thioglycoside \( \text{2.2}\) in a respectable yield of 70% in 3 h (entry 2). These experiments were started at 0 °C, and the temperature was allowed to gradually increase after 1 h. Further increment in the amount of triflic acid to 80 mol % produced thioglucoside \( \text{2.2}\) in an excellent yield of 94% in 1 h (entry 3). Since many other thioglycosylations demand low temperature to maintain the stereoselectivity we investigated the temperature effect on the outcome of the TfOH-promoted reaction. When essentially the same reaction in the presence of 0.8 equiv of TfOH was set at rt, excellent yield of 97% albeit compromised stereoselectivity (\( \alpha/\beta = 1/5.0 \)) were achieved (entry 4). This result confirms that low reaction temperature is required for maintaining complete \( \beta \)-stereoselectivity of TfOH-catalyzed thioglycosidation of \( \text{2.1} \).

A Further increase in the amount of triflic acid to 1.0 and 1.2 equiv reduced the reaction time to 35 and 20 min, but the yields declined to 87 and 75%, respectively (entries 5 and 6). We hence concluded that the reaction in the presence of 0.8 equiv TfOH (entry 3) offers the most advantageous combination of the reaction efficiency and rate.

For comparison, when the same amount of boron trifluoride diethyl etherate (BF\(_3\)•Et\(_2\)O)
was used, thioglycoside 2.2 was obtained in a modest yield of 55% after 24 h (entry 7). This result is consistent with a common method of thioglycosylation that demands excess BF$_3$•Et$_2$O to drive this reaction to completion.$^{21,30}$

**Table 2.1. Optimization of the reaction conditions for thioglycosidation of penta-acetate 2.1 with ethanethiol**

<table>
<thead>
<tr>
<th>Entry</th>
<th>$T$ °C</th>
<th>Catalyst (equiv.)</th>
<th>Time</th>
<th>Yield of 2.2, ratio α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0→rt</td>
<td>TfOH (0.2)</td>
<td>4 h</td>
<td>26%, β only</td>
</tr>
<tr>
<td>2</td>
<td>0→rt</td>
<td>TfOH (0.5)</td>
<td>3 h</td>
<td>70%, β only</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>TfOH (0.8)</td>
<td>1 h</td>
<td>94%, β only</td>
</tr>
<tr>
<td>4</td>
<td>rt</td>
<td>TfOH (0.8)</td>
<td>45 min</td>
<td>97%, 1/5.0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>TfOH (1.0)</td>
<td>35 min</td>
<td>87%, β only</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>TfOH (1.2)</td>
<td>20 min</td>
<td>75%, β only</td>
</tr>
<tr>
<td>7</td>
<td>0→rt</td>
<td>BF$_3$•OEt$_2$ (0.8)</td>
<td>24 h</td>
<td>55%, β only</td>
</tr>
</tbody>
</table>

Having standardized the reaction conditions for the synthesis of ethylthio glucoside 2.2, we moved to expand the scope of this reaction to other sugar series and other aglycone types. The key results of this study are summarized in Table 2.2. Thus, the reaction of galactose pentaacetate 2.3 with ethanethiol in the presence of 0.8 equiv of TfOH afforded thiogalactoside 2.4$^{36,37}$ in 90% yield in 30 min (entry 1). Expectedly, the reaction with much less reactive mannose penta-acetate 2.5 was slow, and ethylthio mannoside 2.6$^{26,37}$ was obtained in only 26% yield. To achieve the preparative outcome of this reaction, the amount of TfOH was increased to 2.0 equiv. In this case, thiomannoside 2.6 was isolated in a respectable yield of 85% (entry 2). Even under these
fortified conditions, the reaction remained fairly sluggish and required 8 h to complete. Also 2-phthalimido glucose tetra-acetate \( \text{2.7}^{38} \) required similar reaction conditions (2.0 equiv of TfOH) to produce the corresponding ethylthio glycoside \( \text{2.8}^{38,39} \) in 73% yield (\( \beta \)-only) in 4 h. When the amount of TfOH was increased to 2.5 equiv, this reaction produced thioglucoside \( \text{2.8} \) in 96% in 45 min (entry 3). Per-acetylated sialic acid \( \text{2.9}^{40} \) produced the corresponding ethylthio sialoside \( \text{2.10}^{41,42} \) in 70% yield (\( \alpha/\beta = 1/1.0 \)) in 45 min (entry 4). Our standard reaction conditions (0.8 equiv of TfOH) were also employed for thioglycosidation of lactose octa-acetate \( \text{2.11}^{43-45} \) to produce thiolactoside \( \text{2.12}^{46} \) in 70% yield in 6 h (entry 5).

We then investigated glycosylation of other common thiols, thiophenol and \( p \)-thiocresol, to generate SPh and STol glycosides, respectively. Glucose per-acetate \( \text{2.1} \) smoothly reacted with thiophenol (2.0 equiv) under the standard conditions in the presence of 0.8 equiv TfOH at 0 °C. As a result, the desired phenylthio glucoside \( \text{2.13}^{34} \) was obtained in 77% yield in 1.5 h (entry 6). Galactose per-acetate \( \text{2.3} \) very readily reacted with thiophenol under these conditions affording phenylthio galactoside \( \text{2.14}^{18,37} \) in 88% yield in 30 min (entry 7). Thioglycosidation of mannose per-acetate \( \text{2.5} \) again required excess TfOH because the reaction under the standard conditions yielded only 31% of thiomannoside \( \text{2.15}^{18,47} \). In contrast, when this reaction was repeated in the presence of 2.0 equiv TfOH, phenylthio mannoside \( \text{2.15} \) was obtained in 75% yield (entry 8). As in case of ethanethiol, the reaction was sluggish and required 9 h to complete. The introduction of the SPh anomeric group to sialic acid also required 2.0 equiv of TfOH, but it was rather swift (45 min). As a result, phenylthio sialoside \( \text{2.16}^{18,49} \) was obtained in 66% yield as an anomeric mixture (\( \alpha/\beta = 1/2.0 \), entry 9).
Table 2.2. TfOH-promoted thioglycosidation of acetylated hexoses 2.1, 2.3, 2.5, 2.7, sialic acid 2.9, and lactose 2.11

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions 0→rt</th>
<th>Product (yield, α/β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>EtSH, TfOH (0.8 equiv), 30 min</td>
<td>2.4 (90%, β only)</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>EtSH, TfOH (2.0 equiv), 8 h</td>
<td>2.6 (85%, α only)</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>EtSH, TfOH (2.5 equiv), 45 min</td>
<td>2.8 (96%, β only)</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>EtSH, TfOH (2.0 equiv), 45 min</td>
<td>2.10 (70%, 1/1.0)</td>
</tr>
<tr>
<td>5</td>
<td>2.11</td>
<td>EtSH, TfOH (0.8 equiv), 6 h</td>
<td>2.12 (70%, β only)</td>
</tr>
<tr>
<td>6</td>
<td>2.1</td>
<td>PhSH, TfOH (0.8 equiv), 1.5 h</td>
<td>2.13 (77%, β only)</td>
</tr>
<tr>
<td>7</td>
<td>2.3</td>
<td>PhSH, TfOH (0.8 equiv), 30 min</td>
<td>2.14 (88%, β only)</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>PhSH, TfOH (2.0 equiv), 9 h</td>
<td>2.15 (75%, α only)</td>
</tr>
</tbody>
</table>
First thioglycosylations with $p$-thiocresol showed that the standard conditions provide somewhat lower efficiency than that seen for reaction with EtSH and PhSH. For instance, the reaction of glucose per-acetate $\text{2.1}$ with $p$-thiocresol (2.0 equiv) in the presence of 0.8 equiv TfOH at 0 °C provided STol glucoside $\text{2.17}^{26,34,50}$ in a modest yield of 70%. The utility of this reaction was enhanced by increasing the amount of TfOH to stoichiometric (1.0 equiv). We have also observed that these reactions can be successfully performed at rt. When glucose penta-acetate $\text{2.1}$ was reacted with $p$-thiocresol under these modified conditions, thioglycoside $\text{2.17}$ was obtained in 88% yield in 30 min (entry 10). Galactose per-acetate $\text{2.3}$ also very readily reacted with $p$-thiocresol affording tolylthio galactoside $\text{2.18}^{50,51}$ in 87% yield in 30 min (entry 11). Even thioglycosidation
of mannose per-acetate 2.5 was very efficient under these conditions producing tolylthio mannoside 2.19\textsuperscript{27,33} in 88% yield in 2.5 h (entry 12). The introduction of the STol anomeric group to sialic acid 2.9 under these reaction conditions produced moderate efficiency for the synthesis of tolylthio sialoside 2.20\textsuperscript{33} (69%, \(\alpha/\beta = 1/3.0\)), but it was rather swift (45 min). When this coupling was performed in the presence of excess TfOH (2.0 equiv) at rt, tolylthio sialoside 2.20 was obtained both in a higher yield and higher stereoselectivity (85%, \(\alpha/\beta = 1/4.0\), entry 13). Also, lactose octa-acetate 2.11 reacted smoothly under similar conditions (1.0 equiv of TfOH at rt) producing tolylthio lactoside 2.21\textsuperscript{33} in 4 h in a good yield of 80% (\(\alpha/\beta = 1/10.0\), entry 14).

We then briefly investigated a possibility of expanding this methodology to the synthesis glycosyl thioimidates that found some synthetic utility in the recent years. The synthesis S-thiazolinyl (STaz) imidate, was also deemed possible, but required excess of both HSTaz and TfOH, up to 3.5 equiv each. The key results of this study are summarized in Table 2.3. Glucose per-acetate 2.1 produced the desired thioimidate 2.22\textsuperscript{34,52} in 76% yield in 6 h (entry 1). Galactose per-acetate 2.3 afforded STaz galactoside 2.23\textsuperscript{52} in 76% yield in 5 h (entry 2). Reaction of mannose per-acetate 2.5 again required longer reaction time (16 h), but we managed to obtain STaz mannoside 2.24\textsuperscript{52} in 66% yield (entry 3). It should be noted that all of these reactions were completely stereoselective (Table 3), whereas previous syntheses of STaz imidates from per-acetates in the presence of excess BF\textsubscript{3}•Et\textsubscript{2}O at high temperature often led to anomeric mixtures.\textsuperscript{52}
Table 2.3. TfOH-promoted synthesis of STaz imidates 2.22-2.24 from per-acetates.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Time</th>
<th>Product (yield, α/β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1</td>
<td>6 h</td>
<td>2.22 (76%, β only)</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>5 h</td>
<td>2.23 (76%, β only)</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>16 h</td>
<td>2.24 (66%, α only)</td>
</tr>
</tbody>
</table>

2.3 Conclusions

In conclusion, we developed a simple methodology for the preparation of thioglycosides promoted by triflic acid. Many reactions still required stoichiometric TfOH, typical range from 0.8 equiv for SEt introduction to 3.5 equiv for STaz imidate synthesis. Our initial attempts to lower the amount of TfOH led to sluggish reactions (16-24 h or longer) and modest yields due to the inability to fully consume the starting material. The scope of this approach was investigated and found to be consistently effective for the synthesis of various thioglycosides in application to different sugar series. Complete stereoselectivity, high yields, and relatively fast reaction rates have been achieved. We have also demonstrated the compatibility of the developed protocol to multi-gram preparation of thioglycosides. We have also explored a possibility of conducting this reaction in the absence of molecular sieves. While most reactions were
successful even without molecular sieves, the reaction yields were generally 10-20% lower due to competing hydrolysis leading to unreactive hemiacetal/hemiketal side products.

2.4 Experimental

2.4.1 General experimental

The reactions were performed using commercial reagents. 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$_2$Cl$_2$ was distilled from CaH$_2$ directly prior to application. 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. $^1$H-NMR spectra were recorded in CDCl$_3$ at 300 MHz.

2.4.2 Synthesis of thioglycosides

A general procedure for thioglycosidation of per-acetylated compounds 2.1, 2.3, 2.5, 2.7, 2.9 and 2.11. TfOH (0.8-3.5 equiv) was added dropwise to a mixture containing a thiol (2.0 equiv or 3.5 equiv for HSTz), per-acetate (1.0 equiv) in anhydrous CH$_2$Cl$_2$ (10 mL per gram of per-acetate) and freshly activated molecular sieves (3 Å) and the resulting mixture was stirred under argon for the time and at the temperature specified in tables. After that, the reaction mixture was diluted with CH$_2$Cl$_2$ and subjected to conventional aqueous work-up. The organic layer was separated, dried, and concentrated
under reduced pressure. The residue was purified either by crystallization (Et₂O-hexanes, mostly with Glc and Gal derivatives) or by column chromatography for most Man and aminosugar derivatives (EtOAc-hexanes gradient elution) to give the target thioglycosides. Anomeric ratios (or anomeric purity) were determined by comparison of the integral intensities of relevant signals in ¹H NMR spectra.

**Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (2.2).** A mixture of per-acetate 2.1 (0.43 g, 1.10 mmol), ethanethiol (0.16 mL, 2.20 mmol), and freshly activated molecular sieves (3 Å, 0.21 g) in anhydrous CH₂Cl₂ (34 mL) was stirred under argon for 15 min at rt. The mixture was cooled to 0 °C, TfOH (78.2μL, 0.88 mmol) was added dropwise, and the resulting mixture was stirred for 1 h at 0 °C. After that, the solids were filtered off through a pad of Celite and washed successively with CH₂Cl₂. The combined filtrate (~200 mL) was washed with sat. aq. NaHCO₃ (70 mL) and water (3 × 70 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. Crystallization (Et₂O-hexanes) for 16 h at 4 °C afforded the title compound (0.39 g, 90%) as a white crystalline solid. The analytical data for 2.2 was in agreement with that reported previously.³⁴,³⁵

**A large-scale synthesis of 2.2.** A mixture of per-acetate 2.1 (15.0 g, 38.4 mmol), ethanethiol (5.55 mL, 76.8 mmol), and freshly activated molecular sieves (3 Å, 2.25 g) in anhydrous CH₂Cl₂ (300 mL) was stirred under argon for 15 min at rt. The mixture was cooled to 0 °C, TfOH (2.72 mL, 30.7 mmol) was added dropwise, and the resulting mixture was stirred for 30 min at 0 °C. After that, the solids were filtered off through a pad of Celite and washed successively with CH₂Cl₂. The combined filtrate (~500 mL) was washed with sat. aq. NaHCO₃ (100 mL) and water (3 × 100 mL). The organic phase
was separated, dried with MgSO$_4$, and concentrated \textit{in vacuo}. Crystallization (Et$_2$O-hexane) for 16 h at 4 °C afforded the title compound (13.2 g, 87%) as a white solid.

The analytical data for \textit{2.2} was in agreement with that reported previously.$^{34,35}$ Spectral data for \textit{2.2}: $^1$H NMR (CDCl$_3$): $\delta$, 1.25 (t, 3H, CH$_2$CH$_3$), 2.01, 2.03, 2.06, 2.08 (4 s, 12H, 4 × COCH$_3$), 2.69-2.75 (m, 2H, CH$_2$CH$_3$), 3.69–3.75 (m, 1H, J$_{5,6a}$ = 5.0 Hz, H-5), 4.15 (dd, 1H, J$_{6a,6b}$ = 12.3 Hz, H-6a), 4.22 (dd, 1H, H-6b), 4.46 (d, 1H, J$_{1,2}$ = 10.0 Hz, H-1), 5.04 (dd, 1H, J$_{2,3}$ = 9.9 Hz, H-2), 5.09 (dd, 1H, J$_{4,5}$ = 9.6 Hz, H-4), 5.20 (dd, 1H, J$_{3,4}$ = 8.9 Hz, H-3) ppm; $^{13}$C-NMR (CDCl$_3$): $\delta$, 14.8, 20.6, 20.7, 20.8, 24.2, 62.1, 68.2, 69.7, 73.8, 75.8, 76.6, 83.5, 169.4, 169.5, 170.2, 170.7 ppm.

\textbf{Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-\textbeta-D-galactopyranoside (2.4).} The title compound was synthesized as described for the synthesis of \textit{2.2}. The analytical data for \textit{2.4} was in agreement with that reported previously.$^{36,37}$ Spectral data for \textit{2.4}: $^1$H NMR (CDCl$_3$): $\delta$, 1.25 (t, 3H, CH$_2$CH$_3$), 1.98, 2.03, 2.05, 2.13 (4 s, 12H, 4 × COCH$_3$), 2.73 (m, 2H, CH$_2$CH$_3$), 3.89 (dt, 1H, J$_{5,6}$ = 6.6 Hz, H-5), 4.12 (m, 2H, H-6a, 6b), 4.8 (d, 1H, J$_{1,2}$ = 9.9 Hz, H-1), 4.98 (dd, 1H, J$_{3,4}$ = 3.3 Hz, H-3), 5.23 (dd, 1H, J$_{2,3}$ = 10.0 Hz, H-2), 5.42 (dd, 1H, J$_{4,5}$ = 1.0 Hz, H-4) ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 14.8, 20.6, 20.7, 20.8, 24.4, 61.3, 67.0, 67.1, 71.8, 74.3, 84.0, 169.4, 169.6, 170.1, 170.2, 170.4 ppm.

\textbf{Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-\textalpha-D-mannopyranoside (2.6).} The title compound was synthesized as described for the synthesis of \textit{2.2}. The analytical data for \textit{2.6} was in agreement with that reported previously.$^{26,37}$ Spectral data for \textit{2.6}: $^1$H NMR (CDCl$_3$): $\delta$, 1.30 (t, 3H, CH$_2$CH$_3$), 1.99, 2.03, 2.04, 2.11 (4 s, 12H, 4 × COCH$_3$), 2.59-2.72 (m, 2H, CH$_2$CH$_3$), 4.09 (dd, 1H), 4.31 (dd, 1H), 4.49 (m, 1H), 5.24-5.37 (m, 4H) ppm; $^{13}$C NMR
(CDCl₃): δ, 14.8, 20.6, 20.7, 20.8, 25.4, 62.3, 66.3, 68.8, 69.4, 71.1, 76.6, 82.8, 169.7, 169.8, 170.0, 170.6 ppm.

**Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.8).**

The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.8 was in agreement with that reported previously. Spectral data for 2.8: ¹H NMR (CDCl₃): δ, 1.22 (t, 3H, CH₂CH₃), 1.87, 2.04, 2.11 (3 s, 9H, 3 × COCH₃), 2.72 (m, 2H, CH₂CH₃), 3.90 (m, 1H, H-5), 4.18 (dd, 1H, H-6a), 4.28-4.44 (m, 2H, H-2, 6b), 5.20 (dd, 1H, H-4), 5.50 (d, 1H, J₁,₂ = 10.6 Hz, H-1), 5.85 (dd, 1H, H-2), 7.74 – 7.90 (m, 4H, aromatic) ppm; ¹³C NMR (CDCl₃): δ 14.8, 20.4, 20.6, 20.8, 24.3, 53.6, 62.2, 68.8, 71.5, 75.8, 81.1, 123.74, 131.1, 131.5, 134.3, 134.4, 167.1, 167.8, 169.5, 170.1, 170.7 ppm.

**Methyl (ethyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-non-2-ulopyranosid)onate (2.10).** The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.10 was in agreement with that reported previously. Selected spectral data for 2.10: ¹H NMR (CDCl₃); δ, 2.70 (dd, J₃eq,₃ax =12.3 Hz, H-3eqα), 2.57 (dd, J₃eq,₃ax =13.1 Hz, H-3eqβ) ppm.

**Ethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-1-thio-β-D-glucopyranoside (2.12).** The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.12 was in agreement with that reported previously. Spectral data for 2.12: ¹H NMR (CDCl₃): δ, 1.25 (t, 3H, CH₂CH₃), 1.96-2.16 (7 s, 21H, 7 × COCH₃), 2.68 (m, 2H, CH₂CH₃), 3.62 (m, 1H, H-5), 3.80 (dd, 1H, H-4), 3.87 (m, 1H, H-5'), 4.05-4.16 (m, 3H), 4.44-4.51 (m, 3H), 4.90-4.98 (dt, 2H, H-3, 3’), 5.11 (dd, 1H, H-2'), 5.22 (dd, 1H, H-2), 5.35 (dd, 1H, H-4') ppm; ¹³C NMR (CDCl₃): δ,
14.8, 20.5, 20.6 (×2), 20.7, 20.8, 22.6, 24.2, 60.7, 62.2, 66.5, 69.0, 70.2, 70.6, 70.9, 71.4, 73.7, 76.2, 76.6, 83.4, 101.0, 168.9, 169.0, 169.7 (×2), 170.0, 170.1, 170.6 ppm.

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (2.13). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.13 was in agreement with that reported previously.\(^{34}\) Spectral data for 2.13: \(^1\)H NMR (CDCl\(_3\)): \(\delta, 1.96, 1.98, 2.02, 2.04\) (4 s, 12 H, \(4 \times \text{COCH}_3\)), 3.68-3.73 (m, 1H, \(J_{5,6a} = 5.1\) Hz, H-5), 4.15 (dd, 1H, \(J_{6a,6b} = 12.1\) Hz, H-6a), 4.21 (dd, 1H, H-6b), 4.66 (d, 1H, \(J_{1,2} = 10.1\) Hz, H-1), 4.91 (dd, 1H, \(J_{2,3} = 9.2\) Hz, H-2), 5.01 (dd, 1H, \(J_{4,5} = 9.9\) Hz, H-4), 5.23 (dd, 1H, \(J_{3,4} = 9.3\) Hz, H-3), 7.26-7.49 (m, 5H, aromatic) ppm; \(^{13}\)C NMR (CDCl\(_3\)): \(\delta, 20.8\) (×2), 20.9 (×2), 62.3, 68.4, 70.1, 74.1, 75.9, 85.9, 128.6, 129.1 (×2), 131.8, 133.3 (×2), 169.4, 169.6, 170.3, 170.7 ppm.

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (2.14). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.14 was in agreement with that reported previously.\(^{18,37}\) Spectral data for 2.14: \(^1\)H NMR (CDCl\(_3\)): \(\delta, 1.96, 2.02, 2.08, 2.10\) (4 s, 12 H, \(4 \times \text{COCH}_3\)), 3.93 (m, 1H, \(J_{5,6} = 6.6\) Hz, H-5), 4.12 (m, 2H, H-6a,b), 4.7 (d, 1H, \(J_{1,2} = 9.9\) Hz, H-1), 5.03 (dd, 1H, \(J_{3,4} = 3.3\) Hz, H-3), 5.23 (dd, 1H, \(J_{2,3} = 10.0\) Hz, H-2), 5.42 (dd, 1H, \(J_{4,5} = 1.0\) Hz, H-4), 7.28-7.52 (m, 5H, aromatic) ppm; \(^{13}\)C NMR (CDCl\(_3\)): \(\delta, 20.6\) (×2), 61.6, 67.1, 71.9, 71.3, 76.6, 86.5, 128.1, 128.8 (×2), 132.4 (×2), 132.5, 169.4, 169.6, 170.1, 170.2, 170.4 ppm.

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (2.15). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.15 was in agreement with that reported previously.\(^{18,47}\) Spectral data for 2.15: \(^1\)H NMR (CDCl\(_3\)): \(\delta,
2.01, 2.05, 2.08, 2.16 (4 s, 12H, 4 × COCH₃), 4.10 (m, 1H), 4.30 (dd, 1H), 4.51-4.57 (m, 1H), 5.29-5.36 (m, 2H, H-2, 3), 5.50 (d, 1H, H-1), 5.42 (dd, 1H, H-4), 7.28-7.52 (m, 5H, aromatic) ppm; ¹³C NMR (CDCl₃): δ, 20.6 (×2), 20.8, 62.3, 66.2, 70.8, 71.7, 85.6, 128.1, 129.0 (×2), 132.0 (×2), 133.2, 169.6, 169.7 (×2), 169.8, 170.4 ppm.

**Methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-non-2-ulopyranosid)onate (2.16).** The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.16 was in agreement with that reported previously.⁴⁸,⁴⁹ Selected spectral data for β-2.16: ¹H NMR: δ, 1.90, 1.96, 2.04, 2.07, 2.10 (5 s, 15H, 5 × COCH₃), 2.68 (dd, J₃eq,3ax =14.0 Hz, H-3eq), 2.00 (dd, 1H, H-3ax), 4.49 (dd, 1H, J⁹a,⁹b = 10.4 Hz, H-9a), 4.60 (dd, H-9b), 5.40 (n, 1H, H-4), 5.60 (d, 1H, J⁵,NH = 10.7 Hz, NH), 7.20-7.60 (m, 5H, aromatic) ppm; Selected spectral data for β-16: ¹H NMR: δ, 2.81 (dd, J₃eq,3ax = 13.1 Hz, H-3eq), 2.09 (dd, 1H, H-3ax).

**p-Methylphenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (2.17).** The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.17 was in agreement with that reported previously.¹⁵ Spectral data for 2.17: ¹H NMR (CDCl₃): δ, 1.98, 2.01, 2.08, 2.09 (4 s, 12H, 4 × COCH₃), 2.35 (s, 3H, CH₃Ar), 3.67-3.74 (m, 1H, J⁵,⁶a = 5.2 Hz, H-5), 4.17 (dd, 1H, J⁶a,⁶b = 12.0 Hz, H-6a), 4.20 (dd, 1H, H-6b), 4.61 (d, 1H, J₁,₂ = 10.1 Hz, H-1), 4.91 (dd, 1H, J₂,₃ = 9.3 Hz, H-2), 5.03 (dd, 1H, J₄,₅ = 9.6 Hz, H-4), 5.18 (dd, 1H, J₃,₄ = 9.3 Hz, H-3), 7.10-7.42 (m, 4H, aromatic) ppm; ¹³C NMR (CDCl₃): δ, 20.6, 20.7, 20.8, 21.2, 62.1, 68.3, 69.9, 74.1, 75.8, 76.7, 85.8, 127.6, 129.7 (×2), 133.9 (×2), 138.8, 169.3, 169.4, 170.2, 170.6 ppm.
\(p\)-Methylphenyl 2,3,4,6-tetra-O-acetyl-1-thio-\(\beta\)-D-galactopyranoside (2.18). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.18 was in agreement with that reported previously.\(^{50,51}\) Spectral data for 2.18: \(^1\)H NMR (CDCl\(_3\)): \(\delta\), 1.97, 2.05, 2.10, 2.12 (4 s, 12H, 4 \(\times\) COCH\(_3\)), 2.34 (s, 3H, CH\(_3\)Ar), 3.91 (m, 1H, \(J_{5,6} = 6.6\) Hz, H-5), 4.14 (m, 2H, H-6a, 6b), 4.65 (d, 1H, \(J_{1,2} = 9.9\) Hz, H-1), 5.03 (dd, 1H, \(J_{3,4} = 3.3\) Hz, H-3), 5.22 (dd, 1H, \(J_{2,3} = 10.0\) Hz, H-2), 5.41 (dd, 1H, \(J_{4,5} = 1.0\) Hz, H-4), 7.10-7.45 (m, 4H, aromatic) ppm; \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\), 20.7 (x2), 20.9, 21.2, 61.6, 67.3, 72.0, 74.3, 86.9, 128.6, 129.6 (x2), 133.1 (x2), 138.4, 169.3, 170.1, 170.2, 170.3 ppm.

\(p\)-Methylphenyl 2,3,4,6-tetra-O-acetyl-1-thio-\(\alpha\)-D-mannopyranoside (2.19). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.19 was in agreement with that reported previously.\(^{27,33}\) Spectral data for 2.19: \(^1\)H NMR (CDCl\(_3\)): \(\delta\), 1.99, 2.08, 2.10, 2.14 (4 s, 12H, 4 \(\times\) COCH\(_3\)), 2.35 (s, 3H, CH\(_3\)Ar), 4.03 (dd, 1H), 4.30 (dd, 1H), 4.50 (m, 1H), 5.29-5.35 (m, 2H), 5.40 (d, 1H, \(J_{1,2} = 1.0\) Hz, H-1), 5.47 (dd, 1H), 7.10-7.45 (m, 4H, aromatic) ppm; \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\), 20.6, 20.7 (x2), 20.8, 21.1, 62.4, 66.3, 69.3, 70.8, 85.9, 128.7, 129.9 (x2), 132.6 (x2), 138.4, 169.7, 169.8, 169.9, 170.1, 170.5 ppm.

Methyl (\(p\)-methylphenyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-D-glycero-D-galacto-non-2-ulopyranosid)onate (20). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.20 was in agreement with that reported previously.\(^{33}\) Selected spectral data for \(\beta\)-2.20: \(^1\)H NMR (CDCl\(_3\)): \(\delta\), 1.90, 1.96, 2.04, 2.08, 2.11 (5 s, 15H, 5 \(\times\) COCH\(_3\)), 2.34 (s, 3H, CH\(_3\)Ar), 2.65 (dd, \(J_{3eq,3ax} = 9.5\) Hz, H-3eq), 3.61 (s, 3H, OCH\(_3\)), 5.39 (m, 1H, H-4), 6.02 (d, 1H, NH), 7.14-7.33 (d, 4H,
aromatic ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 20.7, 20.8 ($\times$2), 21.0, 21.1, 23.2, 29.2, 37.2, 49.2, 49.4, 52.6, 52.7, 62.2, 67.5, 67.6, 68.7, 68.9, 69.9, 73.0, 76.5, 87, 88.7, 128.7, 128.8, 128.9 ($\times$3), 136.6 ($\times$3), 168.2, 170.1, 170.2, 170.3, 170.8, 171.0 ppm.

$p$-Methylphenyl $O$-(2,3,4,6-tetra-$O$-acetyl-$\beta$-$D$-galactopyranosyl)-(1→4)-2,3,6-tri-$O$-acetyl-1-thio-$\beta$-$D$-glucopyranoside (21). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.21 was in agreement with that reported previously.$^{33}$ Spectral data for 2.21: $^1$H NMR (CDCl$_3$): $\delta$, 1.92-2.14 (7 s, 21H, 7 × COCH$_3$), 2.31 (s, 3H, CH$_3$Ar), 3.63 (m, 1H), 3.72 (dd, 1H), 3.84 (dd, 1H), 4.09-4.22 (m, 3H), 4.48-4.75 (m, 3H), 4.83 (m, 1H), 4.93 (dd, 1H), 5.11 (dd, 1H), 5.21 (dd, 1H), 5.33 (dd, 1H), 7.09-7.49 (m, 4H, aromatic) ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 14.8, 20.5, 20.6 ($\times$2), 20.8 ($\times$2), 21.0, 21.1, 29.6, 60.2, 62.0, 66.5, 68.6, 70.9, 73.8, 75.4, 85.9, 101.0, 127.6, 129.8 ($\times$2), 133.3 ($\times$2), 138.6, 169.0, 169.3, 169.5, 169.7, 170.1 ($\times$2) ppm.

1,3-Thiazolin-2-yl 2,3,4,6-tetra-$O$-acetyl-1-thio-$\beta$-$D$-glucopyranoside (2.22). The title compound was synthesized as described for the synthesis of 2.2. The analytical data for 2.22 was in agreement with that reported previously.$^{34,52}$ Spectral data for 2.22: $^1$H NMR (CDCl$_3$): $\delta$, 1.98, 2.01, 2.03, 2.06 (4 s, 12H, 4 × CH$_3$CO), 3.35 (t, 2H, CH$_2$N), 3.76-3.83 (m, 1H, $J_{5,6a} = 2.3$ Hz, $J_{5,6b} = 4.5$ Hz, H-5), 4.09-4.30 (m, 4H, H-6a, 6b, CH$_2$S), 5.08 (dd, 1H, $J_{4.5} = 9.5$ Hz, H-4), 5.13 (dd, 1H, $J_{2.3} = 8.3$ Hz, H-2), 5.21 (dd, 1H, $J_{3.4} = 8.3$ Hz, H-3), 5.41 (d, 1H, $J_{1.2} = 10.4$ Hz, H-1) ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 20.5 ($\times$2), 20.6, 20.7, 35.3, 61.7, 64.2, 68.1, 67.8, 69.3, 73.8, 76.0, 83.0, 162.7, 169.5, 170.1,170.6 ppm.

1,3-Thiazolin-2-yl 2,3,4,6-tetra-$O$-acetyl-1-thio-$\beta$-$D$-galactopyranoside (2.23). The title compound was synthesized as described for the synthesis of 2.2. The analytical data
for **2.23** was in agreement with that reported previously. Spectral data for **2.23**: $^1$H NMR (CDCl$_3$): $\delta$, 1.99, 2.04, 2.06, 2.13 (4 s, 12H, $4 \times$ COCH$_3$), 3.40 (t, 2H, CH$_2$N), 4.02 (m, 1H, H-5), 4.10-.35 (m, 4H, H-6a, 6b, CH$_2$S), 5.10 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 5.31 (dd, 1H, $J_{2,3} = 10.1$ Hz, H-2), 5.45 (s, 1H, H-4), 5.46 (d, 1H, $J_{1,2} = 10.0$ Hz, H-1) ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 20.5, 20.6, 20.7, 20.8, 35.2, 61.1, 64.1, 66.7, 67.0, 71.7, 74.7, 83.4, 162.8, 169.6, 169.9, 170.2, 170.3 ppm.

**1,3-Thiazolin-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (2.24).** The title compound was synthesized as described for the synthesis of **2.2**. The analytical data for **2.24** was in agreement with that reported previously. Spectral data for **2.24**: $^1$H NMR (CDCl$_3$): $\delta$, 1.96, 2.01, 2.05, 2.13 (4 s, 12H, $4 \times$ COCH$_3$), 3.39 (t, 2H, CH$_2$N), 4.04-4.13 (m, 2H, H-5, H-6b), 4.16-4.38 (m, 3H, H-6a, CH$_2$S), 5.11 (dd, 1H, H-3), 5.32 (dd, 1H, H-4), 5.42 (dd, $J_{2,3} = 3.2$ Hz, 1H, H-2), 6.20 (d, $J_{1,2} = 1.3$ Hz, 1H, H-1) ppm; $^{13}$C NMR (CDCl$_3$): $\delta$, 20.6, 20.7 (×2), 20.8, 35.5, 62.0, 64.0, 65.7, 69.3, 70.6, 71.39, 82.6, 161.2, 169.3, 169.5 (×2), 170.6 ppm.

### 2.5 References


(27) Chatterjee, D.; Paul, A.; Rajkamal, R.; Yadav, S. Cu(ClO4)2·6H2O catalyzed solvent free per-O-acetylation and sequential one-pot conversions of sugars to thioglycosides. *RSC Adv.* 2015, 5, 29669-29674.


CHAPTER 3

Palladium(II)-Assisted Activation of Thioglycosides

3.1 Introduction

Improved understanding of the roles of glycans in various biological processes earned this class of molecules a unique stature in contemporary research. To elucidate specific functions of carbohydrates, the availability of analytically pure glycans is essential. However, practically all aspects of the synthesis and purification of complex carbohydrates remain challenging. Numerous approaches have been developed for the installation of glycosidic linkages. Among known glycosyl donors, halides, imidates and thioglycosides are the most common. First reported by Fischer in 1916, thioglycosides are very stable compounds and many are already commercially available. However, thioglycosides can be readily activated using thiophilic promoter systems, and are known to fit as building blocks into various strategies for glycan synthesis. A significant effort has been dedicated to the development of activators for the glycosidation of thioglycosides including metal salts, halogens, organosulfur reagents, alkylating reagents, photo-activators with or without heavy metal additives, and single electron-transfer activators. Nevertheless, many of these approaches have pitfalls, and the quest for better activators continues.

Transition metal catalysis is a relatively new trend in synthesis to replace toxic chemicals and establish greener reaction conditions. This approach has also found its application in glycochemistry. Specifically, a new class of glycosyl donors having alkyne-containing aglycones as leaving groups have gained popularity due to their high affinity to non-toxic Au(I), Au(III) and other transition metal salts. However, these methods require specialized (designed) leaving groups that have been specifically purposed to be compatible with these activation conditions. Activation of conventional
thioglycosides through the direct coordination of a green post-transition metal salt with the anomeric sulfur was first reported by Pohl et al. who employed a sub-stoichiometric amount of Ph$_3$Bi(OTf)$_2$. Subsequently, Sureshan et al. demonstrated the direct activation of thioglycosides using a sub-stoichiometric amount of AuCl$_3$ at ambient temperature. Zhu et al. also showed that propargyl thioglycosides are activated through the direct coordination of Au(III) to the sulfur atom rather than the remote pathway via the alkyne functionality. As a part of our efforts toward the development of novel methods for glycosylation, herein we report first activation of alkyl/aryl thioglycosides with palladium bromide (PdBr$_2$).

3.2 Results and discussion

Palladium catalysis is among commonly used methods in organic chemistry, and its application in glycochemistry is also known. In our previous endeavors, we encountered Pd(II) and Pt(IV) mediated reactions that served as the basis for the development of the temporary deactivation approach, metal-complexation directed stereoselective glycosylations, and glycosyl donors with switchable stereoselectivity. However, the direct utility of these metal salts in activation of thioglycosides did not occur to us, because every time the complexation took place with the involvement of the anomeric sulfur, those thioglycoside complexes appeared deactivated rather than activated. For example, complexes A–C, in all of which Pt/Pd atom was coordinated via the anomeric sulfur were stable and could not be activated for glycosylation using traditional thioglycoside activators (Figure 3.1). In contrast,
complexes D-G, in which Pt/Pd atom was coordinated away from the anomeric sulfur, could be activated for glycosylation with thioglycoside promoters.

**Figure 3.1.** Representative platinum group metal complexes synthesized in our lab.

Regardless of their structure, no complexes showed propensity to be activated on their own, without added thioglycoside activators. This is why we were greatly surprised when a reaction between ethylthio galactosyl donor 3.1 and glycosyl acceptor 3.2 produced disaccharide 3.3 in the presence of only 20 mol % of PdBr₂ (see Table 3.1, entry 1). The yield of disaccharide 3.3 was rather modest (12%), and the reaction was very sluggish (72 h), but an important precedent was set, and this result has served as a proof that PdBr₂ is capable of activating standard thioglycosides for glycosylation.

Encouraged by this observation, albeit dissatisfied with the rate and the yield of the reaction, we decided to investigate the mode of interaction of PdBr₂ with glycosyl donor 3.1 using NMR. For this purpose, a solution of thiogalactoside 3.1 in CDCl₃ was placed into a standard NMR tube, PdBr₂ (1.0 equiv) was added, and ¹H NMR spectrum was recorded after 12 h. As evident from Figure 3.2, a new set of signals has appeared
along with signals of donor 3.1, which were still predominant in the spectrum. This result suggested the formation of 1-PdBr₂, a complex between Pd(II) and thiogalactoside 3.1 via bidentate coordination. Pd-metal center appeared to be coordinated with the anomeric sulfur atom as evident from a downfield shift and division of the S-methylene protons that appeared at 2.90 ppm (marked as H\(^a\)) and 3.19 ppm (H\(^b\)) in 1-PdBr₂. The second complexation site appeared to be the oxygen atom at C-6, as evidenced by a prominent downfield shift of 6-O-benzylic protons to 5.39 ppm (marked as H\(^c\)) and 5.80 ppm (H\(^d\)) in 1-PdBr₂.

Figure 3.2. \(^1\)H NMR study of PdBr₂ complexation with thioglycoside 3.1 in CDCl₃.

Some other minor shifts were also been noted, and these observations were fully consistent with the previously reported polydentate complexes of transition metals.\(^{56,57}\) However, based on the NMR monitoring, nothing really implied that the activation is taking place in this environment. Perhaps, the process is different in the presence of a nucleophile, but it was clear that other, more effective activation modes, need to be engaged to enhance rates and yields of this glycosylation reaction. Analogy with published work\(^{64-66}\) made us believe that additives such as alkynes may be suitable for this purpose. After preliminary screening, propargyl bromide was chosen as the preferred
additive. Glycosylation between donor 3.1 and acceptor 3.2 in the presence of catalytic PdBr$_2$ (0.20 equiv) and C$_3$H$_7$Br (1.0 equiv) in CH$_2$Cl$_2$ at rt was more rapid than the previous reaction with PdBr$_2$ alone. When the reaction was stopped after 24 h, disaccharide 3.3 was isolated in an improved yield of 31% (Table 3.1, entry 2). Increasing the amount of PdBr$_2$ to 0.50 and 0.80 equiv showed an increase in yields of disaccharide 3.3 to 39 and 60%, respectively, in 24 h (entries 3 and 4). When a similar glycosylation reaction was conducted in the presence of a stoichiometric amount of PdBr$_2$, disaccharide 3.3 was obtained in an excellent yield of 96% yield (entry 5).

To confirm the active role of propargyl bromide in the latter glycosylation reaction promoted with stoichiometric PdBr$_2$, a similar reaction was conducted in the absence of propargyl bromide. This reaction was notably slower and required 48 h to afford disaccharide 3.3 in 76% yield (entry 6). Setting up a similar reaction at 60 °C helped to reduce the reaction time to 18 h albeit the yield of disaccharide 3.3 remained practically the same (77%, entry 7). This preliminary reaction optimization study suggested that the promoter and the additive both are required in a stoichiometric amount to produce disaccharide 3.3 in an excellent yield and in a reasonable reaction time. We have tried activation with several other palladium(II) salts including PdI$_2$, PdCl$_2$ and Pd(OAc)$_2$. However, these reactions were notably slower and lead to lower yields of the products.
Table 3.1. PdBr$_2$-mediated glycosidation of donor 3.1 with glycosyl acceptor 3.2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>PdBr$_2$ (equiv)</th>
<th>C$_3$H$_3$Br (equiv)</th>
<th>Conditions</th>
<th>Yield of 3.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>-</td>
<td>72 h, rt</td>
<td>12%</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>1.0</td>
<td>24 h, rt</td>
<td>31%</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>1.0</td>
<td>24 h, rt</td>
<td>39%</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>1.0</td>
<td>24 h, rt</td>
<td>60%</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>1.0</td>
<td>24 h, rt</td>
<td>96%</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>-</td>
<td>48 h, rt</td>
<td>76%</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>-</td>
<td>18 h, 60 °C</td>
<td>77%</td>
</tr>
</tbody>
</table>

Having optimized the reaction conditions, we proceeded to studying differently protected glycosyl donors and acceptors of other sugar series. For the purity of comparison, all reactions were stopped after 24 h, but some yields could be improved if the reactions were kept longer. Glycosylation conducted between glycosyl donor 3.1 and 6-OH glycosyl acceptor 3.4$^{67}$ deactivated by benzoyl ester groups afforded disaccharide 3.5$^{68}$ in a good yield of 75% (Table 3.2, entry 1). Glycosidation of donor 3.1 with more hindered, secondary 3-OH glycosyl acceptor 3.6$^{62}$ and cholesterol 3.8 produced the corresponding products 3.7$^{63}$ and 3.9 in modest yields of 37-43% (entries 2 and 3). A notable drop in the yield of glycosides produced from the secondary hydroxyls compared to the primary ones suggests that the glycosylation rates also depend on the
nucleophilicity of glycosyl acceptors. All these reactions were completely \( \beta \)-stereoselective due to the participatory effect of the 2-O-benzoyl group in donor 3.1.

Subsequently, we extended our glycosylation study to glycosyl donors of other series. Glycosidation of per-benzylated galactosyl donor 3.10\(^{19,69}\) with the primary glycosyl acceptor 3.2 was smooth and efficient. This reaction produced disaccharide 3.11\(^{70}\) in a high yield of 81% albeit with low stereoselectivity (\( \alpha/\beta = 1.5/1 \), entry 4). Glycosidation of mannosyl donor 3.12\(^{71}\) and glucosyl donor 3.14\(^{72}\) with 6-OH acceptor 3.2 produced very different results. Glycosidation of thiomannoside 3.12 was relatively slow, which was reflected in a modest yield of 50% for disaccharide 3.13\(^{73}\) (entry 5). However, glycosidation of thioglucoside 3.14 was much swifter, and disaccharide 3.15\(^{70}\) was produced in a good yield of 80% (entry 6). The drop in the yields of disaccharides 3.13 and 3.15 compared to that of 3.3 (96%) could be due to the relative reactivity of different sugar series.\(^{74,75}\)

It has also occurred to us that the reactivity of glycosyl donors seems to be the key for success because unreactive glucosamine donor 3.16\(^{76}\) and per-benzoylated (disarmed) glucosyl donor 3.18\(^{77}\) were practically ineffective in glycosidations with glycosyl acceptor 3.2 under these reaction conditions. As a result, disaccharides 3.17 and 3.19\(^{78}\) were obtained in 23 and 10% yield, respectively, even after 48 h (entries 7 and 8). In contrast, glycosidation of per-benzylated (armed) glucosyl donor 3.20\(^{79}\) with acceptor 3.2 was much more effective. Disaccharide 3.21\(^{70}\) was obtained in 61% yield albeit with low stereoselectivity due to the lack of a participating group at C-2 (\( \alpha/\beta = 2.0/1 \), entry 9). A practically identical result was obtained in glycosylation between \( S \)-tolyl (STol) donor 3.22\(^{80}\) and glycosyl acceptor 3.2 producing disaccharide 3.21 in 60% (entry 10). This
result indicates that common STol donors can also be activated with PdBr$_2$ in the presence of propargyl bromide, just like their SEt counterparts.

With the acquired knowledge of a very slow activation of the disarmed donor 3.18 (see entry 8), we hypothesized that the established reaction conditions would allow for chemoselective armed-disarmed activation of thioglycoside building blocks. To verify the viability of this hypothesis we activated armed thioglycoside 3.20 over disarmed thioglycoside acceptor 3.23. Although, we investigated reactions with up to 2.0 equiv of PdBr$_2$, this chemoselective reaction was slow, which translated into a poor yield of SEt disaccharide 3.24 ($\alpha/\beta = 2.0/1$, entry 11). We then attempted to activate the armed S-tolyl donor 3.22 over the disarmed glycosyl acceptor 3.25 bearing the same leaving group. Again, a slow reaction even with 2.0 equiv PdBr$_2$ was observed, and disaccharide 3.26 was produced in 35% yield ($\alpha/\beta = 2.5/1$, entry 12). However, glycosidation of armed SEt thioglycoside 3.20 with the disarmed S-tolyl acceptor 3.25 was much more effective in the presence of 2.0 equiv PdBr$_2$, and disaccharide 3.26 was obtained in a good yield of 73% ($\alpha/\beta = 2.5/1$, entry 13). To expand the utility of this approach, we activated the STol leaving group of the resulting disarmed disaccharide 3.26 in the presence of a more powerful promoter system NIS/TfOH to form trisaccharide 3.27 in 71% (entry 14). This synthesis represents a conventional two-step armed-disarmed sequence for streamlined oligosaccharide synthesis. Glycosidation of thioglycoside 3.1 with allyl glycoside acceptor 3.28 was also attempted to investigate the applicability of this approach in selective activation strategies wherein one class of the leaving group is activated over another. This reaction proceeded smoothly and disaccharide 3.29 was obtained in a good yield of 78% (entry 15).
Table 3.2. Broadening the scope of the PdBr$_2$-assisted glycosylation with various donors and acceptors.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>Acceptor</th>
<th>Product, yield, $\alpha/\beta$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Donor 3.1" /></td>
<td><img src="image" alt="Acceptor 3.4" /></td>
<td><img src="image" alt="Product 3.5" />, 75%, $\beta$ only</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Donor 3.1" /></td>
<td><img src="image" alt="Acceptor 3.6" /></td>
<td><img src="image" alt="Product 3.7" />, 43%, $\beta$ only</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Donor 3.1" /></td>
<td><img src="image" alt="Acceptor 3.8" /></td>
<td><img src="image" alt="Product 3.9" />, 37%, $\beta$ only</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Donor 3.10" /></td>
<td><img src="image" alt="Acceptor 3.2" /></td>
<td><img src="image" alt="Product 3.11" />, 81%, 1.5/1</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Donor 3.12" /></td>
<td><img src="image" alt="Acceptor 3.2" /></td>
<td><img src="image" alt="Product 3.13" />, 50%, $\alpha$ only</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Donor 3.14" /></td>
<td><img src="image" alt="Acceptor 3.2" /></td>
<td><img src="image" alt="Product 3.15" />, 80%, $\beta$ only</td>
</tr>
</tbody>
</table>
$^a$ reaction time 48 h; $^b$ PdBr$_2$ (2.0 equiv); $^c$ performed in the presence of NIS (2.0 equiv)/TfOH (0.2 equiv), 15 min.
It should be noted that the latter example represents selective activation, because the allyl group in compound 3.29 can be activated for subsequent chain elongation. In this context, this type of activation reaction cannot be performed in the presence of other thioglycoside activators (except methyl triflate) due to their cross reactivity with olefins.

While many further steps are still needed to fully understand the main driving forces and the substrate scope of this reaction, our current working hypothesis of the reaction mechanism is as follows. As depicted in Scheme 3.1, upon complexation with PdBr₂, thioglycoside donor will form complex H that is fairly stable. Complex H forms only partially and probably exists in equilibrium with the glycosyl donor. We observed that it will revert to the starting material in the absence of a nucleophile or upon work-up.

This was proven by performing a separate experiment in the absence of a glycosyl acceptor. In the presence of a glycosyl acceptor (R’OH), complex H will slowly produce the R’O-glycoside product. We hypothesize that in the presence of propargyl bromide additive, complex H will undergo oxidative addition to form complex I (or similar). The latter is expected to be much more reactive than H, and will fall apart with the formation of an oxacarbenium (or acyloxonium if an ester group is present at C-2) intermediate J. In the presence of R’OH it will readily produce the R’O-glycoside product, but in the absence of the nucleophile, it may produce glycosyl bromide or other by-products. However, the formation of glycosyl bromide is not detected during regular glycosylations with the glycosyl acceptor present from the beginning.
Scheme 3.1. Anticipated PdBr$_2$-promoted reaction pathway in the presence of propargyl bromide additive.

Indeed, a test reaction of 3.1 with PdBr$_2$ in the presence of propargyl bromide monitored by NMR showed the formation of glycosyl bromide as the main product (Figure 3.3A). In contrast, glycosyl bromide was not observed in the presence of allyl bromide (C$_3$H$_5$Br), which supports the important role of propargyl bromide in the activation process (Figure 3.3B). Since no other side products have been isolated from these reactions, the exact fate of reagents is yet to be determined. By analogy with published work and our own spectroscopic investigation, we postulate that the departed leaving group precipitates as Pd$_6$(SEt)$_{12}$ or a similar cluster,$^{88}$ and allene produces oligomeric brominated alkenes.$^{89}$ These products are removed during the standard work-up procedure.
3.3 Conclusions

A new method for the activation of thioglycosides has been developed. The activation with PdBr$_2$ can be sluggish, but it accelerates significantly in the presence of propargyl bromide additive that forms a more reactive reaction intermediate, and possibly acts as the leaving group scavenger. A preliminary mechanistic analysis and studying the
complexation modes relied on $^1$H NMR spectroscopy. Upon standardizing the basic reaction conditions, further examination of various thioglycosides has been performed. In most cases, our activation system was effective at room temperature, but the reaction time and the product yield were dependent on the reactivity of the donor and acceptor. Chemoselective and selective activation schemes have been investigated and successfully applied in a two-step synthesis of a trisaccharide. Further optimization of the reaction conditions and its application in automated synthesis of glycans are currently underway in our laboratory.

3.4 Experimental

3.4.1 General methods

All chemicals used were reagent grade and used as supplied. The ACS grade solvents used for reactions were purified and dried in accordance with standard procedures. 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$_2$Cl$_2$ was distilled from CaH$_2$ directly prior to the application. 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-2000’ polarimeter. Unless noted otherwise, $^1$H NMR spectra were recorded at 300 MHz, $^{13}$C NMR spectra were recorded at 75 MHz. The $^1$H NMR chemical shifts are referenced to tetramethylsilane ($\delta_H = 0$ ppm) or CHCl$_3$ ($\delta_H = 7.26$ ppm) for $^1$H NMR spectra for
solutions in CDCl₃. The $^{13}$C NMR chemical shifts are referenced to the central signal of CDCl₃ ($\delta_C = 77.00$ ppm) for solutions in CDCl₃. The HRMS analysis was performed using Agilent 6230 ESI TOF LC/MS mass spectrometer.

### 3.4.2 Synthesis of building blocks

**Ethyl 2-O-benzoyl-3,4,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (3.1)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{61}\)

**Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.2)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{62}\)

**Methyl 2,3,4-tri-O-benzoyl-α-D-glucopyranoside (3.4)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{67}\)

**Methyl 2,4,6-tri-O-benzyl-α-D-glucopyranoside (3.6)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{62}\)

**Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside (3.10)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{19,69}\)

**Ethyl 2-O-benzoyl-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (3.12)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{71}\)

**Ethyl 2-O-benzoyl-3,4,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (3.14)** was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{72}\)
Ethyl 4,6-di-O-benzyl-2-deoxy-3-O-fluorenlymethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (3.16) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{76}\)

Ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (3.18) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{77}\)

Ethyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (3.20) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{79}\)

Tolyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (3.22) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{80}\)

Ethyl 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (3.23) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{81}\)

Tolyl 2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (3.25) was synthesized as reported previously and its analytical data was in accordance with that previously described.\(^{82}\)

Allyl 2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (3.28). A mixture containing 3,6-di-O-acetyl-2-azido-4-O-benzyl-2-deoxy-α-D-glucopyranosyl bromide\(^{90}\) (1.77 g, 4.0 mmol), allyl alcohol (0.50 mL, 6.0 mmol), and molecular sieves (4 Å, 2.0 g) in acetonitrile (20 mL) under argon was cooled to -40 °C. Mercury(II) cyanide (1.01 g, 4.0 mmol) was added, the external cooling was removed, the resulting mixture was allowed
to warm to rt, and stirred for additional 2.5 h at rt. After that, the solids were filtered off through a pad of Celite and washed successively with DCM. The combined filtrate (~150 mL) was concentrated under reduced pressure and dried in vacuo. The crude residue was dissolved in MeOH (40 mL), a 1 M solution of NaOMe in MeOH was added dropwise to pH ~9, and the resulting mixture was stirred for 22 h at rt. After that, the reaction mixture was neutralized with Dowex (H+). The resin was filtered off and rinsed successively with MeOH. The combined filtrate (~80 mL) was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – hexanes gradient elution). Fractions containing β-3.25 were combined, concentrated under reduced pressure, and the residue was recrystallized from ethyl acetate - hexanes to afford the title compound as white crystals in 55% yield (0.74 g, 2.2 mmol). Also eluted from the column was α-3.25 that was obtained in 12% yield. Analytical data for β-3.25: Rf = 0.35 (ethyl acetate/hexane, 2/3, v/v); [α]D21 -15.7 (c = 1, CHCl3); m.p. 121-122 °C (ethyl acetate – hexanes); 1H NMR (300 MHz, CDCl3): δ 1.88 (m, 1H, 6-OH), 2.39 (s, 1H, 3-OH), 3.29-3.35 (m, 2H, H-2, 5), 3.47-3.53 (m, 2H, H-3, 4), 3.73-3.80 (m, 1H, H-6a), 3.87-3.93 (m, 1H, H-6b), 4.12 (dd, 1H, OCH2aCH=), 4.35-4.41 (m, 2H, J1,2 = 8.1 Hz, H-1, OCH2bCH=), 4.71 (dd, 2H, CH2Ph), 5.23 (dd, 1H, J = 10.7 Hz, CH=CH2a), 5.32 (dd, 1H, J = 17.0 Hz, CH=CH2b), 5.90-5.99 (m, 1H, CH=CH2), 7.17-7.59 (m, 5H, aromatic) ppm; 13C NMR (75 MHz, CDCl3): δ 61.7, 66.1 (×2), 70.6, 74.8, 75.1 (×2), 101.0, 118.0, 128.1 (×2), 128.2, 128.6 (×2), 133.2, 137.8 ppm; HR-FAB MS [M+Na]+ calcd for C16H21N3O5Na 358.1379, found 358.1401.
3.4.3 Synthesis of O-glycosides

General procedure for PdBr$_2$-C$_3$H$_5$Br assisted glycosidation of thioglycosides. A mixture of thioglycoside precursor (30 mg, 0.05–0.04 mmol), glycosyl acceptor (0.04–0.03 mmol) and freshly activated molecular sieves (3 Å, 90 mg) in dry CH$_2$Cl$_2$ (1.0 mL) was stirred under argon for 1 h at rt. After that, propargyl bromide (C$_3$H$_5$Br, 0.05 mmol) followed by palladium bromide (PdBr$_2$, 0.05–0.01 mmol) were added and the reaction mixture was stirred for 24 h. The solids were filtered off through a pad of Celite and rinsed successively with CH$_2$Cl$_2$. The combined filtrate (~20 mL) was washed with H$_2$O (2 × 5 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–hexanes gradient elution) to afford a glycoside derivative in yields listed in tables. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in $^1$H NMR spectra.

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.3) was obtained from thioglycoside 3.1 and glycosyl acceptor 3.2 by the general glycosylation method in 96% yield as a clear film. Analytical data for 3.3 was in accordance with that reported previously.$^{63}$

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (3.5) was obtained from thioglycoside 3.1 and glycosyl acceptor 3.4 by the general glycosylation method in 75% yield as a white amorphous solid. Analytical data for 3.5 was in accordance with that reported previously.$^{68}$

Methyl 3-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (3.7) was obtained from thioglycoside 3.1 and glycosyl
acceptor 3.6 by the general glycosylation method in 43% yield as a clear film. Analytical data for 3.7 was in accordance with that reported previously.63

Cholesteryl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl (3.9) was obtained from thioglycoside 3.1 and cholesterol 3.8 by the general glycosylation method in 37% yield as a white amorphous solid. Analytical data for 3.9: \( R_f = 0.70 \) (ethyl acetate/hexane, 2/3, v/v); \([\alpha]_D^{22} +0.6 \) (c = 1, CHCl₃); \(^1\)H NMR (300 MHz, CDCl₃): \( \delta \), 0.64 (s, 3H), 0.84-0.90 (m, 14H), 0.96-1.10 (m, 8H), 1.25 (s, 3H), 1.31-1.55 (m, 10H), 1.74-2.11 (m, 5H), 3.44 (m, 1H), 3.60-3.66 (m, 4H, H-3ʹ, 5ʹ, 6aʹ, 6bʹ), 3.99 (d, 1H, \( J_{4ʹ,5ʹ} = 2.2 \) Hz, H-4ʹ), 4.47-4.50 (m, 3H, 3x CHPh), 4.56 (d, 1H, \( J_{1ʹ,2ʹ} = 7.9 \) Hz, H-1ʹ), 4.62-4.68 (m, 2H, 2x CHPh), 4.98 (d, 1H, \( J = 11.7 \) Hz, CHPh), 5.19 (d, \( J = 5.0 \) Hz, 1H), 5.59 (dd, 1H, \( J_{2ʹ,3ʹ} = 8.0 \) Hz, H-2ʹ), 7.13-7.60 (m, 18H, aromatic), 8.01 (d, 2H, \( J = 7.8 \) Hz, aromatic) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃): \( \delta \) 11.9, 18.6, 19.2, 20.9, 22.5, 22.6, 22.8, 23.7, 24.2, 27.9, 28.2, 29.4, 29.7, 31.7, 31.8, 35.7, 36.1, 36.6, 37.2, 38.7, 39.4, 39.7, 42.2, 50.0, 56.0, 56.6, 68.7, 71.5, 72.1, 73.5 (×2), 74.3, 76.5, 79.3, 79.9, 100.2, 121.5, 127.5 (×2), 127.6, 127.8 (×2), 127.9 (×2), 128.1 (×2), 128.2 (×2), 128.4 (×4), 129.7, 130.0, 132.8, 137.6, 137.8, 138.2, 138.4, 140.7, 165.3 ppm; HR-FAB MS [M+Na]⁺ calcd for C₆₁H₇₈O₇Na 945.5645, found 945.5662.

Methyl 6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.11) was obtained from thioglycoside 3.10 and glycosyl acceptor 3.2 by the general glycosylation method in 81% yield (\( \alpha/\beta = 1.5/1 \)) as a colorless syrup. Analytical data for 3.11 was in accordance with that reported previously.70

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.13) was obtained from thioglycoside 3.12 and glycosyl
acceptor 3.2 by the general glycosylation method in 50% yield as a colorless syrup. Analytical data for 3.13 was in accordance with that reported previously. 

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.15) was obtained from thioglycoside 3.14 and glycosyl acceptor 3.2 by the general glycosylation method in 80% yield as a clear film. Analytical data for 3.15 was in accordance with that reported previously.

Methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenylmethoxycarbonyl-2-phthalamido-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.17) was obtained from thioglycoside 3.16 and glycosyl acceptor 3.2 by the general glycosylation method as a clear film in 23% yield. Analytical data for 3.17: R$_f$ = 0.50 (ethyl acetate/hexane, 2/3, v/v); [α]$_D$$^2$1 +10.0 (c = 1, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 3.14 (s, 3H, OCH$_3$), 3.26 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 3.36 (dd, 1H, $J_{2,3}$ = 3.7 Hz, H-2), 3.63-3.67 (m, 2H, H-5, 6a), 3.74-3.91 (m, 6H, 3, 4', 5', 6a', 6b', OCOCH$_2$CH), 3.99 (dd, 1H, OCOCH$_2$H$_a$), 4.09-4.16 (m, 3H, 6b, OCOCH$_2$H$_b$, CHPh), 4.34 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.39 (d, 1H, $^2$J = 10.1 Hz, CHPh), 4.45 (dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 4.54-4.73 (m, 7H, 7 x CHPh), 4.83 (d, 1H, $^2$J = 10.7 Hz, CHPh), 5.39 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.70 (dd, 1H, $J_{3',4'}$ = 8.8 Hz, H-3'), 7.01-7.70 (m, 37H, aromatic) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 46.3, 54.8 (×2), 68.6, 69.2, 70.2, 73.3, 73.4, 74.7, 74.9, 75.6 (×2), 79.6, 81.8, 97.8, 98.1, 113.8, 119.8 (×2), 123.3 (×2), 124.9, 125.1, 127.1 (×2), 127.5 (×2), 127.6 (×5), 127.7 (×6), 127.8 (×2), 127.9 (×2), 128.0 (×4), 128.2 (×5), 128.3 (×4), 128.4 (×2), 133.8 (×2), 137.6, 137.7, 138.0, 138.1, 138.6, 140.9, 141.0, 142.8, 143.2, 154.6, 167.2, 168.1 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{71}$H$_{67}$NO$_{14}$Na 1180.4460, found 1180.4550.
Methyl 6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.19) was obtained from thioglycoside \(3.18\) and glycosyl acceptor \(3.2\) by the general glycosylation method in 10% yield as a clear film. Analytical data for \(3.18\) was in accordance with that reported previously.\(^7\)

Methyl 6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.21) was obtained from thioglycosides \(3.20\) or \(3.22\) and glycosyl acceptor \(3.2\) by the general glycosylation method in 61 and 60% yield (\(α/β = 2.0/1\)) as a colorless syrup. Analytical data for \(3.21\) was in accordance with that reported previously.\(^7\)

Ethyl 6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (3.24) was obtained from thioglycoside \(3.20\) and glycosyl acceptor \(3.23\) by the general glycosylation method in 26% yield (\(α/β = 2.0/1\)) as a colorless syrup. Analytical data for \(3.24\) was in accordance with that reported previously.

Tolyl 6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-1-thio-β-D-glucopyranoside (3.26) was obtained from thioglycosides \(3.20\) or \(3.22\) and glycosyl acceptor \(3.25\) by the general glycosylation method in 73 or 35% yield (\(α/β = 2.5/1\)) as a colorless syrup. Analytical data for \(3.26\) was in accordance with that reported previously.\(^8\)

Methyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (3.27). A mixture of thioglycoside \(3.26\) (0.016 mmol), glycosyl acceptor \(3.4\) (0.015 mmol) and freshly activated molecular sieves (3 Å, 52 mg) in dry CH\(_2\)Cl\(_2\) (1.0 mL) was stirred under argon for 1 h at rt. After that, NIS (0.03 mmol) followed by TfOH (0.3 µL, 0.003 mmol) were
added, and the reaction mixture was stirred for 15 min at rt. The solids were filtered off through a pad of Celite and rinsed successively with CH$_2$Cl$_2$. The combined filtrate (~20 mL) was washed sat. aq. Na$_2$S$_2$O$_3$ (5 mL), sat. aq. NaHCO$_3$ (5 mL) and brine (2 × 5 mL). The organic phase was separated, dried with MgSO$_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate–hexanes gradient elution) to afford the title compound in 71% yield (0.011 mmol) as a white amorphous solid. Selected analytical data for α-3.27: R$_f$ = 0.45 (ethyl acetate/hexane, 2/3, v/v); $^1$H NMR (300 MHz, CDCl$_3$): δ 3.03 (s, 3H, OCH$_3$), 3.39 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2”), 3.50-3.87 (m, 9H, H-3", 4", 5", 6a, 6a", 6b", 6b’), 4.01-4.09 (m, 3H, 5, 5’, 6b), 4.33-4.69 (m, 7H, H-1”", 6 x CHPh), 4.78-4.85 (m, 2H, H-1, CHPh), 4.88-5.00 (m, 3H, H-1’, 2, CHPh), 5.31 (dd, 1H, $J_{4,5}$ = 9.0 Hz, H-4), 5.48-5.54 (m, 2H, H-2’, 4’), 5.83 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, H-3’), 5.98 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 7.11-7.51 (m, 38H, aromatic), 7.74-7.98 (m, 12H, aromatic) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 54.1, 66.2, 67.1, 67.4, 67.5 (×2), 68.4, 68.8, 69.2, 70.9, 71.2, 71.9, 72.0, 72.4, 73.8, 74.5, 95.4, 96.4, 100.3, 112.9, 126.5, 126.6 (×2), 126.7 (×6), 126.8 (×2), 126.9, 127.0 (×6), 127.2 (×5), 127.3 (×3), 127.4, 127.8 (×2), 127.9, 128.0 (×2), 128.1, 128.2 (×2), 128.5 (×2), 128.6 (×2), 128.8, 128.9 (×3), 131.0, 132.0 (×2), 132.1(×4), 132.3 (×4), 132.4, 136.9, 137.0, 137.1, 137.2, 137.2, 137.5, 137.8, 138.0, 164.1, 164.2 (×2), 164.7 (×2), 164.8 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{89}$H$_{82}$O$_{22}$Na 1525.5196, found 1525.5174.

**Allyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (3.29)** was obtained from thioglycoside 3.1 and glycosyl acceptor 3.28 by the general glycosylation method in 78% yield as a white
amorphous solid. Analytical data for 3.29: \( R_f = 0.55 \) (ethyl acetate/hexane, 2/3, v/v); 
\([\alpha]_D^{21} +11.8 \) (c = 1, CHCl₃); \(^1\)H NMR (300 MHz, CDCl₃): \( \delta \) 3.17-3.26 (m, 2H, H-2, 5), 3.36-3.42 (m, 2H, H-3, 4), 3.57-3.66 (m, 5H, H-3', 5', 6a, 6a', 6b'), 3.76 (dd, 1H, OCH₂.CH=), 3.98-4.03 (m, 2H, H-4', OCH₂.CH=), 4.12-4.18 (m, 2H, H-1, 6b), 4.39-4.70 (m, 7H, H-1', 6 x CHPh), 4.96 (d, 1H, \(^2\)J = 11.6 Hz, CHPh), 5.09 (dd, 1H, \( J = 10.0 \) Hz, CH=CH₂), 5.16 (dd, 1H, \( J = 17.7 \) Hz, CH=CH₂), 5.61-5.74 (m, 2H, H-2', CH=CH₂), 7.14-7.56 (m, 25H, aromatic), 7.96 (d, 2H, \( J = 7.7 \) Hz, aromatic) ppm; \(^{13}\)C NMR (75 MHz, CDCl₃): \( \delta \) 65.9 (×2), 67.4, 68.4, 69.5, 71.5, 71.6, 72.2, 73.5, 73.6, 74.4 (×2), 74.6, 75.2, 79.8, 100.3, 101.3, 117.4, 118.5, 127.6 (×4), 128.1 (×4), 128.2 (×2), 128.3 (×5), 128.4 (×3), 129.8 (×3), 130.0, 130.2, 132.9, 133.1, 134.0, 137.5, 137.7, 137.8, 138.3, 165.1 ppm; HR-FAB MS \([M+Na]^+\) calcd for C₅₀H₅₃N₃O₁₁Na 894.3578, found 894.3587.

### 3.4.4 \(^1\)H NMR experiments

\(^1\)H-NMR study of PdBr₂ complexation with thioglycoside 3.1 in CDCl₃ (Figure 3.2).
To a solution of 3.1 (10 mg, 0.01 mmol) in 0.5 mL CDCl₃, PdBr₂ (4.4 mg, 0.01 mmol) was added, and the mixture stirred at rt for 12 h, then the sample was immediately taken for NMR analysis.

\(^1\)H-NMR study of PdBr₂ with thioglycoside 3.1 in presence of C₃H₅Br in CDCl₃ (Figure 3.3, A). To a solution of 3.1 (10 mg, 0.01 mmol) in 0.5 mL CDCl₃, C₃H₅Br (1.5µL, 0.01 mmol) and PdBr₂ (4.4 mg, 0.01 mmol) were added, and the mixture stirred at rt for 12 h, then the sample was immediately taken for NMR analysis.

\(^1\)H-NMR study of PdBr₂ with thioglycoside 3.1 in presence of C₃H₅Br (allyl bromide) in CDCl₃ (Figure 3.3, B). To a solution of 3.1(10 mg, 0.01 mmol) in 0.5 mL
CDCl₃, C₃H₅Br (1.4µL, 0.01 mmol) and PdBr₂ (4.4 mg, 0.01 mmol) were added, and the mixture stirred at rt for 12 h, then the sample was immediately taken for NMR analysis.

3.5 References


(26) Crich, D.; Smith, M. 1-Benzenesulfinyl piperidine/trifluoromethanesulfonic anhydride: a potent combination of shelf-stable


CHAPTER 4

A Streamlined Regenerative Glycosylation Reaction: Direct, Acid-Free Activation of Thioglycosides

4.1 Introduction

From their ubiquitous presence in nature to vital roles in biology, complex carbohydrates are essential molecules made by every living organism. The structure diversity and complexity of carbohydrates have intrigued glycoscientists for decades.\textsuperscript{1} Given that glycosylation is one of the most fundamental modifications of carbohydrates, enzymatic and chemical synthesis of glycosidic linkages remains a focus of many research programs around the world. Among significant advances made in the area, automated synthesis using polymer supports has come to the fore as a powerful means to synthesize glycans.\textsuperscript{2} Many glycosylations in solution make use of thioglycosides,\textsuperscript{3} but glycosylations on solid supports commonly demand highly reactive imidates\textsuperscript{4-6} or phosphates\textsuperscript{7} as glycosyl donors to offset challenges related to the mismatch between reactive solution-based vs. their unreactive solid-phase-immobilized counterparts. The use of thioglycosides as glycosyl donors for automated solid-phase synthesis has also been reported,\textsuperscript{8} and a recent Glyconeer procedure comprises the treatment of a supported acceptor with 2 x 5.0 equiv thioglycoside donor in the presence of 2 x 5.5 equiv of NIS and 2 x 0.2 equiv of TfOH for 2 x 30 min.\textsuperscript{9} With the critical issue of making common glycosyl donors commercially available, the use of thioglycosides is attractive due to their superior stability, but a fairly low reactivity profile and the requirement for stoichiometric promoters leaves ample room for improvement.

As a part of the program to develop new methods and strategies for chemical glycosylation, we reported a regenerative glycosylation reaction concept.\textsuperscript{10} In accordance with this concept, 3,3-difluoroxindole (HOFox) reacts with a stable precursor to form a highly reactive OFox imidate (Scheme 4.1).\textsuperscript{11} The latter will then react with the glycosyl
acceptor while regenerating HOFox aglycone. The released HOFox will mediate the next catalytic cycle to obtain a new OFox donor, etc. In our original study, we reacted S-ethyl glycoside with Br$_2$ to form glycosyl bromide. The latter reacts very slowly in the presence of Ag$_2$O, but in the presence of HOFox gives reasonable reaction rates (2-3 h) with only 10 mol % of HOFox and a catalytic Lewis acid (BF$_3$-Et$_2$O or TMSOTf). The concentration of reactive glycosyl donor can be controlled by the amount of HOFox added. This regenerative concept differs from the two-step activation$^{12,13}$ and Huang’s preactivation strategy$^{14,15}$ in the way that the reactive donor is generated in only small amounts. This approach was successfully applied in oligosaccharide synthesis.$^{16}$ However, the two-step conversion, a stoichiometric generation of the glycosyl bromide from the thioglycoside precursor, followed by the heterogeneous activation of the bromide intermediate in the presence of Ag$_2$O, remained a drawback of this approach. Ultimately, this hampers its application in polymer supported or flow-through approaches. Reported herein is a direct conversion of thioglycosides via the regenerative approach that bypasses the intermediacy of glycosyl bromides and eliminates the need for heavy metal-based promoters.

**Scheme 4.1. Regenerative glycosylation approach.**
4.2 Results and discussion

When activated with \( N \)-iodosuccinimide (NIS) only, many thioglycosides react slowly or do not react at all, even in the presence of a large excess of NIS (2.0-3.0 equiv). This is typically improved by using strong Lewis (TMSOTf) or protic acids (TfOH) as co-promoters. Our preliminary experimentation showed that some reactive thioglycosides can be converted to OFox imidates using only 1.0 equiv NIS and catalytic HOFox without the acidic additives. The formation of OFox intermediates can easily be monitored by TLC and NMR (Figure A-16). Once formed, the OFox imidates rapidly react with the acceptor while liberating HOFox aglycone that will mediate the next catalytic cycle to obtain reactive OFox intermediate, etc. In our opinion, the observation that 1.0 equiv NIS and catalytic HOFox can activate thioglycosides in a regenerative fashion offers a promising venue for developing a new glycosylation reaction. This discovery also hints on feasibility of the activation of thioglycosides under neutral conditions offering a broader functional and protecting group compatibility.

Therefore, we endeavored to optimize these reaction conditions. We began these studies by investigating a highly reactive thiogalactoside donor 4.1\(^{17}\) in reactions with partially benzylated primary glycosyl acceptor 4.2\(^{18}\). These results are summarized in Table 4.1. These highly reactive substrates were able to react in the presence of only 1.0 equiv NIS even in the absence of the acidic additive producing disaccharide 4.3\(^{19}\) in 74% yield (entry 1). However, this reaction slowed down after 3.5 h, and was incomplete even after 12 h indicating that these reaction conditions are insufficient for effective glycosylation. When glycosidation of thiogalactoside 4.1 with glycosyl acceptor 3.2 was repeated in the presence of NIS (1.0 equiv) and catalytic HOFox aglycone (0.05 equiv)
the reaction was smoothly driven to completion in 6 h and disaccharide \(4.3\) was obtained in an excellent yield of 91% (entry 2). Upon increasing the amount of HOFox to 0.10 equiv, no significant change in the reaction time and yield was observed (entry 3).

**Table 4.1. NIS/HOFox mediated glycosidations of donor 4.1 with acceptor 4.2.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>HOFox (equiv)</th>
<th>TMSOTf (equiv)</th>
<th>Time</th>
<th>Yield of 4.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>--</td>
<td>3.5-12 h</td>
<td>74%</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>--</td>
<td>6 h</td>
<td>91%</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>--</td>
<td>6 h</td>
<td>90%</td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>--</td>
<td>3.5 h</td>
<td>92%</td>
</tr>
<tr>
<td>5</td>
<td>0.30</td>
<td>--</td>
<td>3 h</td>
<td>90%</td>
</tr>
<tr>
<td>6</td>
<td>0.50</td>
<td>--</td>
<td>1.5 h</td>
<td>91%</td>
</tr>
<tr>
<td>7</td>
<td>0.20</td>
<td>0.05</td>
<td>15 min</td>
<td>72%</td>
</tr>
</tbody>
</table>

Further, increasing the amount of HOFox to 0.20 equiv decreased the reaction time to 3.5 h, and disaccharide \(4.3\) was obtained in 92% yield (entry 4). Realizing the effect of catalyst loading, we increased the amount of HOFox to 0.30 and 0.50 equiv. These adjustments led to a decreased reaction time to 3 and 1.5 h, respectively (entries 5 and 6). Based on these results, we chose to perform all subsequent experimentation using 20 mol % of HOFox. In our opinion, these reaction conditions offer the best balance between the amount of catalyst, reaction times, and product yields. We note that
TMSOTf (or TfOH) is not required in these reactions but adding as little as 0.05 equiv TMSOTf can expedite both the generation of the OFox imidate and its activation. To exemplify this, we conducted glycosylation in the presence of HOFOx and 0.05 equiv of TMSOTf. This reaction proceeded quickly and produced disaccharide 4.3 in 15 min, albeit moderate yield of 72% (entry 7).

After optimization of the basic reaction conditions, we investigated glycosidation of glycosyl donor 4.1 with other glycosyl acceptors that differ in their reactivity. The key results of this study are summarized in Table 4.2. Glycosidations of donor 4.1 were first conducted with hindered secondary glycosyl acceptors 4.4, 4.6 and 4.8. The respective disaccharides 4.5, 4.7 and 4.9 were obtained within 3-4 h in moderate to good yields of 42-71% (entries 1-3). Glycosidation with partially benzoylated deactivated primary 6-OH acceptor 4.10 was also performed. This reaction gave the corresponding disaccharide 4.11 in a moderate yield of 58% within 4 h (entry 4). In further expansion, reactive 6-OH galactosyl acceptor 4.12 was glycosylated, and disaccharide 4.13 was obtained in a good yield of 76% (entry 5). Glycosylation of reactive aliphatic acceptors such as primary acceptor n-butanol 4.14 and more sterically hindered tertiary acceptor 1-adamantanol 4.16 under the aforementioned reaction conditions proceeded smoothly and produced glycosides 4.15 and 4.17 nearly quantitatively (99%) in relatively short reaction times of 30 min and 45 min, respectively (entries 6 and 7).
Table 4.2. HOFox-catalyzed glycosidation of thiogalactoside donor 4.1 with various glycosyl acceptors

<table>
<thead>
<tr>
<th>Entry</th>
<th>ROH</th>
<th>Time</th>
<th>Products, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="ROH 4.4" /></td>
<td>3 h</td>
<td><img src="image" alt="Products 4.5" /> 53%</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="ROH 4.6" /></td>
<td>4 h</td>
<td><img src="image" alt="Products 4.7" /> 71%</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="ROH 4.8" /></td>
<td>3 h</td>
<td><img src="image" alt="Products 4.9" /> 42%</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="ROH 4.10" /></td>
<td>4 h</td>
<td><img src="image" alt="Products 4.11" /> 58%</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="ROH 4.12" /></td>
<td>4 h</td>
<td><img src="image" alt="Products 4.13" /> 76%</td>
</tr>
</tbody>
</table>
After constructing different types of β-galactosidic linkages, we moved on to expand this acid-free glycosylation method to glycosidations of thioglycosides of other sugar series. For that, we first proceeded with glycosidation of per-benzylated thiogalactoside donor 4.18 with acceptor 4.2 under standard reaction conditions. This glycosylation afforded disaccharide 4.19 in a good yield of 80% in 2 h, albeit with no stereoselectivity (α/β = 1.2/1, entry 1, Table 4.3). Glycosylation of the secondary 3-OH glycosyl acceptor 4.6 produced the corresponding disaccharide 4.20 in a lower yield of 58% in 4 h (α/β = 1.2/1, entry 2). Glycosidations of less reactive mannosyl donor 4.21 under identical reaction conditions were slower. Thus, glycosylation of 6-OH acceptor 4.2 produced α-linked disaccharide 4.22 in 50% yield after 12 h (entry 3). Glycosylation of n-butanol 4.14 afforded α-linked glycoside 4.23 in 51% yield in 10 h (entry 4). Some of the glycosylation reactions were repeated with increased amount of promoter system (NIS/HOFox), however no significant improvement was noticed. We then moved on to investigate glycosyl donors with the D-gluco configuration. Glycosylation of glucosyl donor 4.24 proceeded smoothly and afforded disaccharide 4.25 in 87% in 4 h (entry 5). Glycosidation of benzylated glucosyl donor 4.26 with acceptor 4.2 produced disaccharide 4.27 in 67% yield in 6 h (α/β = 2.0/1, entry 6).
After investigating the activation of various ethylthio glycosides, we were curious to see whether other thioglycoside leaving groups would act accordingly. For this study we selected the S-tolyl (STol) leaving group because these glycosyl donors are better suited for large-scale preparations,\textsuperscript{28,29} can be readily preactivated,\textsuperscript{30-32} and a comprehensive reactivity database, compiled by Wong specifically for STol glycosides, is available.\textsuperscript{33-37} Glycosidation of tolylthio glucosyl donor 4.28\textsuperscript{38} with acceptor 4.2 proceeded smoothly and afforded disaccharide 4.27 in an excellent yield of 97\% in 6 h ($\alpha/\beta = 1.1/1$, entry 7). Glycosidation of glucosyl donor 4.28 with galactosyl acceptor 4.12 was somewhat slower, which was reflected in a lower yield (72\%) of disaccharide 4.29\textsuperscript{39} obtained in 8 h ($\alpha/\beta = 1.0/1$, entry 8). Glycosylation with less reactive secondary 4-OH acceptor 4.8 proceeded even slower, and the corresponding disaccharide 4.30\textsuperscript{40} was obtained in 40\% yield in 10 h ($\alpha/\beta = 1/1.1$, entry 9). Glycosylation of donor 4.28 with 1-adamantanol 4.16 gave the corresponding glycoside 4.31\textsuperscript{41} in an excellent yield of 91\% yield in 4 h ($\alpha/\beta = 1.0/1$, entry 10).

In further expansion of the approach, we investigated conformationally super-armed donor 4.32\textsuperscript{42} equipped with silyl protecting groups. Glycosidation of glucosyl donor 4.32 with acceptor 4.2 smoothly afforded the desired disaccharide 4.33\textsuperscript{42} in an excellent yield of 93\% with predominant $\alpha$-stereoselectivity ($\alpha/\beta = 5.0/1$, entry 11). No loss of TBS protecting groups, which was noted as a major side reaction in our previous unrelated study,\textsuperscript{42} was noted in NIS/HOFox-promoted reactions. Glycosidation of relatively unreactive 2-phthalimido-protected aminosugar donor 4.34 with electronically deactivated glycosyl acceptor 4.10 was also proven feasible. However, for the best results, 2.0 equiv NIS was necessary, and even then, the reaction required 24 h
and disaccharide 4.35 was obtained in 40% yield. To demonstrate the efficacy of the new reaction, we attempted selective activation of the SET leaving group in donor 4.1 over the S-thiazolinyl (STaz) anomeric group of acceptor 4.36. To obtain a better outcome, we used increased amounts of the promoters, 1,2 equiv NIS and 0.5 equiv HOFox. Under these reaction conditions, disaccharide 4.37 equipped with the anomeric STaz leaving group was obtained in 65% in 16 h (entry 13).

**Table 4.3. Expanding the scope of HOFox-catalyzed glycosylation with various donors and acceptors of other sugar series.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor</th>
<th>ROH</th>
<th>Product, time, yield, ratio α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.18</td>
<td>4.2</td>
<td>4.19, 2 h, 80%, 1.2/1.0</td>
</tr>
<tr>
<td>2</td>
<td>4.18</td>
<td>4.6</td>
<td>4.20, 4 h, 58%, 1.2/1.0</td>
</tr>
<tr>
<td>3</td>
<td>4.21</td>
<td>4.2</td>
<td>4.22, 12 h, 50%, α only</td>
</tr>
</tbody>
</table>
4.21

4.23, 10 h, 51%, α only

4.24

4.25, 4 h, 87%, β only

4.26

4.27, 6 h, 67%, 2.0/1.0

4.28

4.27, 6 h, 97%, 1.1/1.0

4.29, 8 h, 72%, 1.0/1.0

4.30, 10 h, 40%, 1.0/1.1

4.31, 4 h, 91%, 1.0/1.0
4.3 Conclusions

In summary, presented herein is the discovery of the direct activation of thioglycosides in the regenerative fashion. We demonstrated that in most cases, only 1.0 equiv NIS and catalytic HOFox (0.2 equiv) are needed to promote glycosidation of various thioglycosides. One advantage of this approach is that it does not require a strong acid as a co-promoter, which allows to maintain neutral reaction conditions during glycosylation. One potential disadvantage of this approach is that it is much less efficient with poorly reactive substrates, glycosylation of which would require the use of additional quantities of HOFox and/or Lewis acid additives. We believe that this approach would be particularly advantageous with the HPLC pump-based reagent delivery automated set-up that is being developed in our labs.
4.4 Experimental

4.4.1 General experimental

The reactions were performed using commercial reagents, and the ACS grade solvents used for reactions were purified and dried in accordance with standard procedures. Column chromatography was performed on silica gel 60 (70–230 mesh); reactions were monitored by TLC on Kieselgel 60 F254. 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. CH₂Cl₂ was distilled from CaH₂ directly prior to the application. Molecular sieves (3 Å), used for reactions, were crushed, and activated in vacuo at 390 °C for 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-2000” polarimeter. ¹H NMR spectra were recorded at 300 MHz, ¹³C NMR spectra were recorded at 75 MHz. The ¹H NMR chemical shifts are referenced to tetramethylsilane (TMS, δC = 0 ppm) for ¹H NMR spectra for solutions in CDCl₃. The ¹³C NMR chemical shifts are referenced to the central signal of CDCl₃ (δC = 77.00 ppm) for solutions in CDCl₃. HRMS analysis was performed using Agilent 6230 ESI TOF LC/MS mass spectrometer.

4.4.2. Synthesis of reagents and building blocks

3,3-Difluoroxindole (HOFox) was obtained from Isatin and DAST as previously described, and its analytical data were in accordance with that previously reported.¹⁰,⁴⁴

2-Thiazolinyl 2-O-benzoyl-3,4-di-O-benzyl-1-thio-β-D-galactopyranoside (4.36). A solution of ethyl 2-O-benzoyl-3-O-benzyl-4,6-O-benzylidene-1-thio-β-D-
galactopyranoside (4.38, 590 mg, 1.16 mmol) and activated molecular sieves 3 Å (885 mg) in dry CH₂Cl₂ (20 mL) was stirred under argon for 1 h at rt. The mixture was cooled to 0 °C, Br₂ (0.077 mL, 1.51 mmol) was added, and the resulting mixture was kept for 15 min at 0 °C. After that, the volatiles were removed under reduced pressure at rt. The crude residue was dissolved in dry MeCN (10 mL), NaSTaz (650 mg, 4.6 mmol) was added, and the resulting mixture was stirred under argon for 2 h at rt. After that, the solid was filtered-off and rinsed successively with toluene. The combined filtrate (~35 mL) was washed with 1% aq. NaOH (~15 mL) and water (3 x 15 mL). The organic phase was separated, dried over MgSO₄, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford 2-thiazolinyl 2-O-benzoyl-3-O-benzyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (4.39) as a clear film in 51% yield over two steps (330 mg). Analytical data for 4.39: Rf = 0.50 (ethyl acetate/hexane, 2/3, v/v); [α]D²⁰ +6.9 (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 3.22-3.29 (m, 2H, CH₂N), 3.63 (s, 1H, H-5), 3.93 (dd, 1H, J₃,₄ = 3.3 Hz, H-3), 4.06 (d, 1H, J₆ₐ,₆₅b = 12.2 Hz, 6a), 4.28-4.47 (m, 4H, H-4, 6b, CH₂S), 4.68 (dd, 2H, CH₂Ph), 5.54 (s, 1H, CHPh), 5.75 (dd, 1H, J₂,₃ = 9.5 Hz, H-2), 6.18 (d, 1H, J₁,₂ = 9.3 Hz, H-1), 7.24-7.26 (m, 4H, aromatic), 7.40-7.61 (m, 9H, aromatic), 7.99 (d, 2H, J = 7.2 Hz, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 28.6, 51.3, 68.4, 68.5 (×2), 69.0, 71.4, 73.0, 77.2, 82.8, 101.1, 126.2 (×2), 127.7 (×2), 127.9, 128.3 (×2), 128.4 (×5), 129.2, 130.0 (×2), 133.4, 137.4, 137.5, 165.9 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₀H₂₉NO₆S₂Na 586.1334, found 586.1339.

A 1 M solution of BH₃ in THF (3.0 mL) was added dropwise to a solution of 4.39 (290 mg, 0.51 mmol) in CH₂Cl₂ (9 mL). The resulting solution was cooled to 0 °C, TMSOTf
(4.6 µL, 0.025 mmol) was added dropwise, and the resulting mixture was stirred for 1 h at 0 °C. The reaction mixture was then allowed to warm to rt and stirred for additional 1 h. After that, the reaction was quenched with NaHCO$_3$ (~2 mL), diluted with CH$_2$Cl$_2$ (100 mL), and washed with sat. aq. NaHCO$_3$ (50 mL) and water (2 × 50 mL). The organic phase was separated, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the title compound as a white amorphous solid in 53% yield (150 mg, 0.27 mmol). Analytical data for 4.36: R$_f$ = 0.30 (ethyl acetate/hexane, 2/3, v/v); [α]$_{20}$ = +117.8 (c = 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 3.22-3.26 (m, 2H, CH$_2$N), 3.61-3.70 (m, 2H, H-5, 6a), 3.81 (m, 1H, H-6b), 3.89 (dd, 1H, $J_{3,4}$ = 2.5 Hz, H-3), 3.97 (d, 1H, $J_{4,5}$ = 2.0 Hz, H-4), 4.16-4.34 (m, 2H, CH$_2$S), 4.59-4.72 (m, 3H, CH$_2$Ph, CPh), 5.02 (d, 1H, $^2$J = 11.7 Hz, CPh), 5.30 (s, 1H, OH), 5.76 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 6.11 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 7.21-7.26 (m, 4H, aromatic), 7.37-7.59 (m, 9H, aromatic), 7.99 (d, 2H, $^2$J = 7.7 Hz, aromatic) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 28.5, 51.4, 61.8, 69.5, 72.5 (×2), 74.4, 77.1, 77.3, 80.1, 83.1, 127.7 (×3), 127.9, 128.1, 128.4 (×4), 128.5 (×2), 129.1, 129.9 (×3), 133.4, 137.2, 137.9, 166.0 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{30}$H$_{31}$NO$_6$S$_2$Na 588.1490, found 588.1501.

4.4.3 Synthesis of glycosides

Typical Glycosylation Procedure. A mixture of thioglycoside precursor (30.0 mg, 0.03–0.05 mmol), glycosyl acceptor (0.02–0.04 mmol), and freshly activated molecular sieves (3 Å, 90 mg) in dry CH$_2$Cl$_2$ (1.0 mL) was stirred under argon for 1 h at rt. After that, N-iodosuccinimide (NIS, 0.03–0.05 mmol) and HOFox (0.005–0.02 mmol) were
added, and the reaction mixture was stirred for the time specified in Tables 1-3. The solids were filtered off through a pad of Celite and rinsed successively with CH$_2$Cl$_2$. The combined filtrate (~20 mL) was washed with sat. aq. Na$_2$S$_2$O$_3$ (5 mL), sat. aq. NaHCO$_3$ (5 mL) and brine (2 × 5 mL). The organic phase was separated, dried with MgSO$_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate–hexanes gradient elution) to afford a glycoside product in yields listed in Tables 1-3 and below. Anomeric ratios (if applicable) were determined by comparison of the integral intensities of relevant signals in $^1$H NMR spectra.

**Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (4.3)** was obtained from thioglycoside 4.1$^{17}$ and glycosyl acceptor 4.2$^{18}$ by the general glycosylation method as a clear film in 92% yield. Analytical data for 4.3 was in accordance with that reported previously.$^{19}$

**Methyl 2-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (4.5)** was obtained from thioglycoside 4.1 and glycosyl acceptor 4.4$^{18}$ by the general glycosylation method as a clear film in 53% yield. Analytical data for 4.5: $R_f = 0.60$ (ethyl acetate/hexane, 2/3, v/v); [α]$_D^{21}$ +62.9 (c = 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDC$_3$): δ 3.34 (s, 3H, OCH$_3$), 3.55-3.72 (m, 9H, H-3, 5, 6a, 6b, 2ʹ, 4ʹ, 5ʹ, 6aʹ, 6bʹ), 3.87 (dd, 1H, $J_{3',4'} = 9.2$ Hz, H-3ʹ), 4.01 (d, 1H, $J_{4,5} = 2.7$ Hz, H-4), 4.32 (d, 1H, $^2J = 10.9$ Hz, CHPh), 4.40-4.47 (m, 5H, 5 x CHPh), 4.57-4.63 (m, 5H, 5 x CHPh), 4.81 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.91 (d, 1H, $J_{1',2'} = 3.0$ Hz, H-1ʹ), 4.98 (d, 1H, $^2J = 11.8$ Hz, CHPh), 5.75 (dd, 1H, $J_{2,3} = 9.1$ Hz, H-2), 6.94-6.99 (m, 4H, aromatic), 7.06-7.45 (m, 29H, aromatic), 7.81 (d, 2H, $J = 7.8$ Hz, aromatic) ppm; $^{13}$C NMR (75
MHz, CDCl$_3$): δ, 55.2, 68.4, 69.6, 71.6, 71.7, 72.3, 73.4, 73.5, 74.4, 74.8, 75.0, 76.5, 80.1, 80.4, 81.0, 99.5, 102.5, 126.9, 127.1 (×2), 127.4, 127.6 (×6), 127.7 (×4), 127.9 (×6), 128.1 (×5), 128.2 (×5), 128.3 (×2), 128.5 (×2), 129.8 (×3), 132.6, 137.3, 137.6, 137.9, 138.1, 138.4, 138.7, 165.1 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{62}$H$_{64}$O$_{12}$Na 1023.4295, found 1023.4312.

**Methyl 3-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (4.7)** was obtained from thioglycoside 4.1 and glycosyl acceptor 4.6$^{18}$ by the general glycosylation method as a clear film in 71% yield. Analytical data for 4.7 was in accordance with that reported previously.$^{19}$

**Methyl 4-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (4.9)** was obtained from thioglycoside 4.1 and glycosyl acceptor 4.8$^{18}$ by the general glycosylation method as a clear film in 42% yield. Analytical data for 4.9 was in accordance with that reported previously.$^{19}$

**Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (4.11)** was obtained from thioglycoside 4.1 and glycosyl acceptor 4.10$^{20}$ by the general glycosylation method as a white amorphous solid in 58% yield. Analytical data for 4.11: R$_f$ = 0.50 (ethyl acetate/hexane, 2/3, v/v); [α]$_D^{21}$+28.5 (c = 1, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): δ 3.00 (s, 3H, OCH$_3$), 3.53-3.65 (m, 5H, H-3, 5, 6a, 6a', 6b), 3.98-4.04 (m, 2H, J$_{4,5}$ = 2.2 Hz, J$_{6a',6b'}$ = 11.1 Hz, H-4, 6b'), 4.16 (dd, 1H, J$_{5',6a'}$ = 8.7 Hz, J$_{5',6b'}$ = 9.7 Hz, H-5'), 4.32-4.61 (m, 6H, H-1, 5 x C$_{6}$H$_{5}$), 4.80 (d, 1H, J$_{1',2'}$ = 3.6 Hz, H-1'), 4.95 (d, 1H, $^2$J = 11.6 Hz, C$_{6}$H$_{5}$), 5.06 (dd, 1H, J$_{2',3'}$ = 10.2 Hz, H-2'), 5.27 (dd, 1H, J$_{4',5'}$ = 9.7 Hz, H-4'), 5.66 (dd, 1H, J$_{2,3}$ = 8.7 Hz, H-2), 6.03 (dd, 1H, J$_{3',4'}$ = 9.8 Hz, H-3'), 7.17-7.59 (m, 27H, aromatic), 7.78 (d, 2H, J = 7.8 Hz, aromatic) 7.85 (d,
2H, $J = 7.9$ Hz, aromatic), 7.90 (d, 2H, $J = 7.7$ Hz, aromatic), 8.01 (d, 2H, $J = 7.8$ Hz, aromatic) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 30.9, 54.8, 68.2, 68.5, 69.5, 70.4, 71.6, 71.7, 72.0, 72.2, 73.5, 74.4, 76.5, 79.6, 96.1, 101.9, 127.5, 127.6 ($\times$4), 127.8 ($\times$2), 127.9 ($\times$3), 128.1 ($\times$4), 128.2 ($\times$4), 128.3 ($\times$6), 128.4 ($\times$2), 128.7, 129.0, 129.2, 129.6 ($\times$2), 129.8 ($\times$4), 130.2, 132.9, 133.2, 133.3, 137.6, 137.7, 138.3, 165.3, 165.4, 165.5, 165.6, 165.7 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{62}$H$_{58}$O$_{15}$Na 1065.3673, found 1065.3691.

6-O-(2-O-Benzoyl-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranosyl)-1,2:3,4-di-O-isopropylidene-\(\alpha\)-D-galactopyranose (4.13) was obtained from thioglycoside 4.1 and glycosyl acceptor 4.12 by the general glycosylation method as a clear film in 76% yield. Analytical data for 4.13 was in accordance with that reported previously.$^{21}$

\textit{n-Butyl 2-O-benzoyl-3,4,6-tri-O-benzyl-\(\beta\)-D-galactopyranoside (4.15)} was obtained from thioglycoside 4.1 and \textit{n}-butanol 4.14 by the general glycosylation method as a white amorphous solid in 99% yield. Analytical data for 4.15: $R_f = 0.70$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{21} +3.6$ ($c = 1.0$, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.69 (t, 3H, CH$_3$), 1.18-1.49 (m, 4H, 2 x CH$_2$), 3.43 (m, 1H, OCH$_a$), 3.62-3.73 (m, 4H, H-3, 5, 6a, 6b), 3.85 (m, 1H, OCH$_b$), 4.01 (d, 1H, $J_{4.5} = 2.7$ Hz, H-4), 4.41-4.46 (m, 4H, $J_{1.2} = 9.1$ Hz, H-1, 3 x CHPh), 4.65 (dd, 2H, CH$_2$Ph), 4.99 (d, 1H, $J = 11.7$ Hz, CHPh), 5.63 (dd, 1H, H-2), 7.14-7.60 (m, 18H, aromatic), 8.03 (d, 2H, $J = 7.5$ Hz, aromatic) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 13.5, 18.8, 29.6, 31.3, 68.6, 69.3, 71.5, 71.9, 72.3, 73.5, 74.3, 79.8, 101.5, 127.4, 127.5, 127.6 ($\times$2), 127.8, 127.9 ($\times$2), 128.1 ($\times$2), 128.2 ($\times$3), 128.3 ($\times$2), 128.4 ($\times$2), 129.7 ($\times$2), 130.3, 132.8 ($\times$2), 137.6, 137.8, 138.4, 165.3 ppm; HR-FAB MS [M+Na]$^+$ calcd for C$_{38}$H$_{42}$O$_7$Na 633.2828, found 633.2870.
1-Adamantyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (4.17) was obtained from thioglycoside 4.1 and 1-adamantanol 4.16 by the general glycosylation method as a white amorphous solid in 99% yield. Analytical data for 4.17: R\textsubscript{f} = 0.70 (ethyl acetate/hexane, 2/3, v/v); [α]\textsubscript{D}\textsuperscript{21} +24.7 (c = 1.0, CHCl\textsubscript{3}); \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 1.45-1.78 (m, 12H, Ada), 2.02 (s, 3H, Ada), 3.56-3.69 (m, 4H, H-3, 5, 6a, 6b), 3.98 (d, 1H, J\textsubscript{4,5} = 2.6 Hz, H-4), 4.40-4.52 (m, 3H, 3 x C\textsubscript{H}Ph), 4.62 (dd, 2H, C\textsubscript{H}\textsubscript{2}Ph), 4.75 (d, 1H, J\textsubscript{1,2} = 7.9 Hz, H-1), 4.98 (d, 1H, J\textsubscript{2,3} = 9.8 Hz, H-2), 7.11-7.60 (m, 18H, aromatic), 8.01 (d, 2H, J = 7.7 Hz, aromatic) ppm; \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 30.7 (×5), 36.3 (×3), 42.4 (×4), 69.3, 71.6, 72.3, 73.6, 73.7, 74.5, 75.0, 80.3, 94.5, 127.7 (×2), 127.8 (×2), 127.9, 128.0 (×2), 128.3 (×2), 128.4 (×3), 128.6 (×2), 129.9 (×3), 130.6, 132.9, 137.9, 138.1, 138.7, 165.3 ppm; HR-FAB MS [M+Na]\textsuperscript{+} calcd for C\textsubscript{44}H\textsubscript{48}O\textsubscript{7}Na 711.3298, found 711.3307.

Methyl 6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (4.19) was obtained from thioglycoside 4.18 and glycosyl acceptor 4.2 by the general glycosylation method as a clear film in 80% yield (α/β = 1.2/1). Analytical data for 4.19 was in accordance with that reported previously.\textsuperscript{22}

Methyl 3-O-(2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (4.20) was obtained from thioglycoside 4.18 and glycosyl acceptor 4.6 by the general glycosylation method as a clear film in 58% yield (α/β = 1.2/1). Analytical data for 4.20 was in accordance with that reported previously.\textsuperscript{19}

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (4.22) was obtained from thioglycoside 4.21 and
glycosyl acceptor 4.2 by the general glycosylation method as a colorless syrup in 50% yield. Analytical data for 4.22 was in accordance with that reported previously. 24

\textit{n-Butyl 2-O-benzoyl-3,4,6-tri-O-benzyl-\alpha-D-mannopyranoside (4.23)} was obtained from thioglycoside 4.21 and \textit{n-butanol 4.14} by the general glycosylation method as a colorless syrup in 51% yield. Analytical data for 4.23 was in accordance with that reported previously. 25

\textbf{Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-\beta-D-glucopyranosyl)-2,3,4-tri-O-benzyl-\alpha-D-glucopyranoside (4.25)} was obtained from thioglycoside 4.24 and glycosyl acceptor 4.2 by the general glycosylation method as a clear film in 87% yield. Analytical data for 4.25 was in accordance with that reported previously. 22

\textbf{Methyl 6-O-(2,3,4,6-tetra-O-benzyl-\alpha/\beta-D-glucopyranosyl)-2,3,4-tri-O-benzyl-\alpha-D-glucopyranoside (4.27)} was obtained from thioglycoside 4.26 and glycosyl acceptor 4.2 by the general glycosylation method as a clear film in 97% yield (\(\alpha/\beta = 1.1/1\)). Analytical data for 4.27 was in accordance with that reported previously. 22

\textbf{6-O-(2,3,4,6-Tetra-O-benzyl-\alpha/\beta-D-glucopyranosyl)-1,2:3,4-di-O-isopropylidene-\alpha-D-galactopyranose (4.29)} was obtained from thioglycoside 4.28 and glycosyl acceptor 4.12 by the general glycosylation method as a clear film in 72% yield (\(\alpha/\beta = 1.0/1\)). Analytical data for 4.29 was in accordance with that reported previously. 39

\textbf{Methyl 4-O-(2,3,4,6-tetra-O-benzyl-\alpha/\beta-D-glucopyranosyl)-2,4,6-tri-O-benzyl-\alpha-D-glucopyranoside (4.30)} was obtained from thioglycoside 4.28 and glycosyl acceptor 4.8 by the general glycosylation method as a clear film in 40% yield (\(\alpha/\beta = 1/1.1\)). Analytical data for 4.30 was in accordance with that reported previously. 40
1-Adamantyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside (4.31) was obtained from thioglycoside 4.28 and 1-adamantanol 4.16 by the general glycosylation method as a white amorphous solid in 91% yield (α/β = 1.0/1). Analytical data for 4.31 was in accordance with that reported previously.41

Methyl 6-O-(6-O-benzoyl-2-O-benzyl-3,4-di-O-tert-butyldimethylsilyl-α/β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (4.33) was obtained from thioglycoside 4.3242 and glycosyl acceptor 4.2 by the general glycosylation method as a colorless syrup in 93% yield (α/β = 5.0/1). Analytical data for 4.33 was in accordance with that reported previously.42

Methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (4.35) was obtained from thioglycoside 4.3443 and glycosyl acceptor 4.10 by the general glycosylation method as a clear film in 40% yield. Analytical data for 4.35: Rf = 0.60 (ethyl acetate/hexane, 2/3, v/v); [α]D21 +120.0 (c 1, CHCl3); ¹H NMR (300 MHz, CDCl3): δ 3.04 (s, 3H, OCH₃), 3.61 (dd, 1H, J₅',₆ₐ' = 7.8 Hz, J₆ₐ',₆ₖ' = 10.7 Hz, 6a'), 3.58-3.76 (m, 3H, H-5, 6a, 6b), 3.85-4.03 (m, 3H, H-4, OCOCH₂CH), 4.07-4.18 (m, 3H, H-5', 6b', OCOCH₂ CH), 4.40-4.65 (m, 5H, H-2, 2 x CH₂Ph), 4.70 (d, 1H, J₁',₂' = 3.6 Hz, H-1'), 5.07 (dd, 1H, J₂',₃' = 10.2 Hz, H-2'), 5.29 (dd, 1H, J₄',₅' = 9.9 Hz, H-4'), 5.40 (d, 1H, J₁,₂ = 8.4 Hz, H-1), 5.71 (dd, 1H, J₃,₄ = 8.9 Hz, H-3), 6.03 (dd, 1H, J₃',₄' = 9.8, H-3'), 7.11-7.51 (m, 28H, aromatic), 7.67-7.93 (m, 9H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 29.7, 46.3, 54.4 (×2), 68.0, 68.2, 69.3 (×2), 70.2 (×2), 71.9, 73.4, 74.7, 77.2, 96.3, 98.8, 114.2, 119.8 (×4), 125.0 (×2), 125.2 (×2), 127.1 (×4), 127.6 (×2), 127.8 (×3), 128.1 (×2), 128.2 (×2), 128.3 (×7), 128.6, 128.9, 129.1, 129.6 (×3), 129.7 (×4), 129.8 (×3), 133.0, 133.2,
133.3, 137.6, 137.9, 140.9, 141.0, 142.9, 143.2, 154.6, 165.1, 165.6 (×2) ppm; HR-FAB MS [M+Na]^+ calcd for C_{71}H_{61}NO_{17}Na 1222.3837, found 1222.3858.

2-Thiazolinyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-O-benzoyl-3,4-di-O-benzyl-1-thio-β-D-galactopyranoside (4.37) was obtained from thioglycoside 4.1 and glycosyl acceptor 4.36 by the general glycosylation method as a clear film in 65% yield. Analytical data for 4.37: R_f = 0.50 (ethyl acetate/hexane, 2/3, v/v); [α]_D^{21}+25.8 (c = 1.0, CHCl_3); _1^H NMR (300 MHz, CDCl_3): δ 3.10-3.19 (m, 2H, C_H_2N), 3.55-3.69 (m, 7H, H-3, 5, 6a, 6b, 3', 5', 6a'), 3.95 (d, 1H, J_{4',5'} = 2.4 Hz, H-4'), 3.99-4.03 (m, 3H, H-4, 6b', SCH_a), 4.18 (d, 1H, 2J = 12.4 Hz, CHPh), 4.26 (m, 1H, SCH_b), 4.34 (d, 1H, 2J = 12.1 Hz, CHPh), 4.42 (s, 2H, CH_2Ph), 4.56 (d, 1H, J_{1,2} = 7.9 Hz, H-1), 4.61-6.71 (m, 4H, 2x CH_2Ph), 4.84 (d, 1H, 2J = 11.2 Hz, CHPh), 5.01 (d, 1H, 2J = 11.9 Hz, CHPh), 5.57-5.68 (m, 2H, J_{2,3'} = 9.6 Hz, J_{2,3} = 9.6 Hz, H-2, 2'), 5.95 (d, 1H, J_{1',2'} = 9.3 Hz, H-1'), 7.01-7.57 (m, 31H, aromatic), 7.91 (d, 2H, J = 7.5 Hz, aromatic), 8.04 (d, 2H, J = 7.5 Hz, aromatic) ppm; _1^3C NMR (75 MHz, CDCl_3): δ 29.7 (×2), 30.9, 71.5 (×2), 71.6, 71.7, 71.8, 72.1, 72.4, 73.5, 73.6, 74.6, 74.8, 75.3, 79.6, 79.8, 83.0, 101.1, 113.8, 127.4 (×4), 127.5 (×2), 127.6, 127.7 (×3), 127.9 (×3), 128.1 (×6), 128.2 (×5), 128.3, 128.4 (×2), 128.5 (×2), 129.2, 129.8 (×2), 129.9 (×4), 133.2, 137.4, 137.5, 137.7, 138.4 (×2), 165.2, 165.9 ppm; HR-FAB MS [M+Na]^+ calcd for C_{64}H_{63}NO_{12}S_2Na 1124.3684, found 1124.3717.

4.5 References


CHAPTER 5

HPLC-Based Automated Synthesis of Glycans in Solution
5.1 Introduction

With improved understanding of functions of glycans, the demand for robust methods to produce both natural glycans and their mimetics has increased. Due to significant advances in chemical synthesis, many glycosidic bonds can now be achieved by using both classical methods and novel technologies. Nevertheless, traditional chemical synthesis of glycans remains among the top challenges of synthetic chemistry. Reactions that proceed with high rates, complete conversion, flawless stereoselectivity, and would work with a broad range of substrates remain rare. In addition, carbohydrate synthesis requires relevant training and qualifications, so it is practically impossible to implement these reactions in non-specialized labs. This significantly hampers the development in glycosciences, whereas other biopolymers, peptides and oligonucleotides, can be produced on demand by machines. Efforts to automate solution synthesis of glycans using parallel synthesizers have been reported by Takahashi, Pohl, and Nokami. Being still relatively unexplored, these approaches may offer viable alternatives to automated enzymatic syntheses being developed by Wong, Chen, Wang, and Boons.

Solid-phase synthesis is also automation amenable, which was nicely demonstrated in 2001 by Seeberger who adapted a peptide synthesizer to glycan synthesis. In 2012, Seeberger reported “the first fully automated solid-phase oligosaccharide synthesizer.” This synthesizer was then commercialized as Glyconeer 2.1 in 2014, and an updated version has also recently (2021) emerged. Also in 2012, our labs reported HPLC-based automation (HPLC-A) of solid-phase synthesis. The general idea for developing the HPLC-A is that a computer interface coupled with
standard HPLC components will allow recording a successful automated sequence as a computer program. This recorded sequence can then be accurately reproduced anywhere by anybody, even non-specialists, who may not have expertise to do conventional synthesis. Although the original HPLC-A (Generation A) offered operational convenience, substantially faster reaction times, UV detector reaction monitoring, it remained largely manual. Further improvements emerged with the implementation of a standard HPLC autosampler for delivering the promoter for glycosylations (Generation B). Although this set-up was applied to a variety of glycan sequences including branched N-glycan core structure, the semi-manual aspect of the HPLC-A remained because switching between the reaction and discharge modes required operator intervention. To address this, we recently introduced Generation C HPLC-A where we implemented a standard two-way split valve as a mode for complete, “press-of-a-button,” automation. The entire sequence of reactions consisting of glycosylations with Fmoc deprotections in between, followed by the off-resin cleavage was performed by delivering standard HPLC-grade ACS-spec solvents. Depicted in Scheme 5.1A, this set-up allowed the operator to add all reagents in the autosampler tray, press the button to start the automation sequence, leave the lab for 12 h, and upon return find the synthesized glycan in the collection flask.

As the 21st century unfolds with rapid changes in demographics, ways of learning, computation, and automation, new challenges in research and development emerge. Unexpected pandemic in 2020 revealed our unpreparedness, which resulted in many research labs shutting down completely for many weeks and even months. Even after reopening, many labs were operating using new social distancing, reduced work hours,
and time shift protocols. This period was particularly challenging for graduate student researchers who were unable to go to labs, but still needed to show progress in order to advance and graduate with minimal delays.

These challenges persuaded us to repurpose our HPLC-A platform to solution-phase synthesis and extend it to the repetitive batch synthesis. Like in our previous set-up, the operator adds all reagents in the autosampler, presses the button to start the repetitive automation sequence, leaves the lab, and upon return finds products of multiple reactions. The modular character of HPLC allows for implementing new attachments and accessories using the plug-in approach and modulating the reaction modes by computer programming. We envisaged that to enable multiple glycosylations with the single press of a button would require the following adjustments shown in Scheme 5.1B. Based on equipment used for Generation C HPLC-A,\textsuperscript{37,38} the new system was supplemented by an automated fraction collector. Having the fraction collector would allow for collecting multiple products obtained as a result of a sequence of reactions, as opposed to the single product that was previously collected in the flask. To enable the recirculation mode, along with the waste and product collection modes, we have implemented a new four-way split valve instead of the previously used two-way split valve. Since we were not using solid-phase based approach in this application, the Omnifit glass chromatography column that was previously used to hold polymeric resin, was loaded with activated beads of 3 Å molecular sieves. The column was integrated into the system, pump intake lines A and B were used for delivering standard HPLC-grade ACS-spec solvents needed for glycosylation and washing, DCM and MeOH, respectively. To minimize hydrolysis, a common side reaction of glycosylations, activated beads of 3 Å molecular sieves were
added to the DCM solvent bottle. Pump line D which was dedicated to recirculation and the preparative autosampler was used to deliver all necessary reagents (donors, acceptors, and promoters) to the system. The software was programmed in the way that allowed to perform a fully automated solution-phase glycosylation with the single press of the software start button.

**Scheme 5.1. Generation C HPLC-A on solid-phase (A) and HPLC-A in solution (B).**
5.2 Results and discussion

With the developed new automation circuit, we started from refining basic glycosylation conditions. This preliminary experimentation was performed with common thioglycoside 5.1 and trichloroacetimidate 5.2 donors and primary glycosyl acceptors 5.3 and 5.4. To establish a benchmark, we first conducted glycosidation of thioglucoside 5.1 with glycosyl acceptor 5.3 using conventional manual approach. The activation was achieved in the presence of N-iodosuccinimide (NIS, 2.0 equiv) and trifluoromethanesulfonylic acid (TfOH, 0.2 equiv), a common promoter system for the activation of thioglycosides, to afford disaccharide 5.5 in 93% yield within 15 min (Table 5.1, entry 1). When a similar reaction was performed using the automated delivery of all reagents with the autosampler followed by recirculation for 60 min and washing/collection for 15 min, disaccharide 5.5 was obtained in a disappointing yield of 25% (entry 2). Nevertheless, we were encouraged by this first attempt and turned our attention to refining the reaction conditions. Certainly, moving directly from manual to automated synthesis may not always be straightforward as previously shown by Pohl and Bennett.

A notable improvement in the reaction outcome was achieved by increasing the amount of TfOH with all other parameters remaining constant. Thus, when either 0.3 or 0.5 equiv of TfOH were delivered followed by recirculation for 60 min, disaccharide 5.5 was obtained in good yields of 77 or 84%, respectively (entries 3 and 4). On the other hand, decreasing the recirculation time to either 30 or 10 min while keeping all other reaction parameters constant led to a decline in the yields of product 5.5 to 83% and 20%, respectively (entries 5 and 6). When our best reaction conditions (entry 4) were applied to
glycosylation of a less reactive, benzoylated glycosyl acceptor 5.4, the respective disaccharide 5.6\(^{45}\) was obtained in 67% yield (entry 7). This prompted us to conduct further optimization of the reaction conditions. Increasing the excess of glycosyl donor 5.1 to 2.0 and 3.0 equiv resulted in an increase of the yield of product 5.6 to 79 and 87%, respectively (entries 8 and 9). With an ultimate goal of developing universal reaction that would work with all glycosyl donors and glycosyl acceptors, we decided to perform all of our glycosylations with 3-fold excess of the donor.

**Table 5.1. Refinement of basic parameters of HPLC-A glycosylations in solution.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Donor (equiv) + Acceptor</th>
<th>Promoter (equiv), Time</th>
<th>Product, Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^a)</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.2), 15 min</td>
<td>5.5, 93%</td>
</tr>
<tr>
<td>2</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.2), 60 min</td>
<td>5.5, 25%</td>
</tr>
<tr>
<td>3</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.3), 60 min</td>
<td>5.5, 77%</td>
</tr>
<tr>
<td>4</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.5), 60 min</td>
<td>5.5, 84%</td>
</tr>
<tr>
<td>5</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.5), 30 min</td>
<td>5.5, 83%</td>
</tr>
<tr>
<td>6</td>
<td>5.1 (1.25) + 5.3</td>
<td>NIS (2.0)/TfOH (0.5), 10 min</td>
<td>5.5, 20%</td>
</tr>
<tr>
<td>7</td>
<td>5.1 (1.25) + 5.4</td>
<td>NIS (2.0)/TfOH (0.5), 60 min</td>
<td>5.6, 67%</td>
</tr>
<tr>
<td>8</td>
<td>5.1 (2.0) + 5.4</td>
<td>NIS (2.0)/TfOH (0.5), 60 min</td>
<td>5.6, 79%</td>
</tr>
<tr>
<td>9</td>
<td>5.1 (3.0) + 5.4</td>
<td>NIS (2.0)/TfOH (0.5), 60 min</td>
<td>5.6, 87%</td>
</tr>
<tr>
<td>10(^b)</td>
<td>5.2 (3.0) + 5.3</td>
<td>TMSOTf (0.5), 60 min</td>
<td>5.5, 88%</td>
</tr>
</tbody>
</table>

\(^a\) – standard manual glycosylation; \(^b\) – this reaction was repeated three times by an untrained high school student who achieved yields of 84, 86, and 86%
We then investigated glycosidation of trichloroacetimidate 5.2 with glycosyl acceptor 5.3 in the presence of trimethylsilyltrifluoromethanesulfonate (TMSOTf, 0.5 equiv). This reaction was smooth and efficient, and the desired product 5.5 was obtained in 88% yield (entry 10). To ensure transferability of our automated system, a series of glycosylations between glycosyl donor 5.2 and glycosyl acceptor 5.3 were performed by an untrained sophomore high school student. The student has managed to reproduce this reaction in 84, 86, and 86% yield proving high level of reproducibility not only between different experiments, but also different operators.

With success of our preliminary experimentation, we extended our approach to investigating other glycosyl acceptors. One of potential advantages of this automated system is the ability to perform several glycosylations with a single “press of a button.” Hence, glycosidation of donor 1.1 with four selected glycosyl acceptors 5.7-5.10 was performed in one repetitive automation sequence. All automated glycosylations performed with the single press of a button were successful, and the respective disaccharides 5.11-5.14 were obtained in 65-72% yields as depicted in Scheme 5.2. Overall, the entire sequence comprising four glycosylation reactions was completed within 5 h, and the individual reaction mixtures were separated in individual collection vessels with the appropriately programmed fraction collector.

The scope of our automated glycosylation was further explored with a variety of glycosyl donors and glycosyl acceptors of other series. The summary of these reactions is listed in Table 5.2. Thus, benzylated (armed) thioglycoside 5.15 showed excellent efficiency in reaction with glycosyl acceptor 5.3. As a result, disaccharide 5.16 was obtained in 90% yield ($\alpha/\beta = 2.0/1$, entry 1).
Scheme 5.2. HPLC-A synthesis of multiple disaccharides 5.11-5.14 with a single press of a button.

Per-benzoylated (disarmed) thiogalactoside 5.17 was then glycosidated with acceptor 5.3 to afford disaccharide 5.18 in 78% yield (entry 2). This efficiency was on a par with that observed with benzoylated thioglucoside donor 5.1 (see Table 5.1). The automated glycosidation of per-acetylated thiomannoside 5.19 with 6-OH benzoylated glycosyl acceptor 5.4 afforded product 5.20 in 74% (entry 3). Glycosidation of per-benzylated thiomannoside 5.21 with secondary glycosyl acceptor 5.7 was also promising and product 5.22 was obtained in 72% yield (entry 4). Even the recently developed 3-picoloylated mannosyl donor 5.23, which is capable of stereocontrolling glycosylations via the H-bond-mediated aglycone deliver pathway, showed excellent reactivity and stereoselectivity in reaction with glycosyl acceptor 5.4. As a result,
disaccharide 5.24 was obtained in a high yield of 95% with impressive $\beta$-manno stereoselectivity ($\alpha/\beta = 1/21$, entry 5). In comparison, the manual synthesis reported previously was performed at low temperature, required much longer reaction time, and resulted in a lower yield.

**Table 5.2. Broadening the scope of the HPLC-A in solution.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Glycosyl donor</th>
<th>Glycosyl acceptor</th>
<th>Promoter (eq.)</th>
<th>Product, Yield, Ratio $\alpha/\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.15</td>
<td>5.3</td>
<td>NIS (2.0) TfOH (0.5)</td>
<td>5.16, 90%, 2.0/1</td>
</tr>
<tr>
<td>2</td>
<td>5.17</td>
<td>5.3</td>
<td>NIS (2.0) TfOH (0.5)</td>
<td>5.18, 78%, $\beta$ only</td>
</tr>
<tr>
<td>3</td>
<td>5.19</td>
<td>5.4</td>
<td>NIS (2.0) TfOH (0.5)</td>
<td>5.20, 74%, $\alpha$ only</td>
</tr>
<tr>
<td>4</td>
<td>5.21</td>
<td>5.7</td>
<td>NIS (2.0), TfOH (0.5)</td>
<td>5.22, 72%, $\alpha$ only</td>
</tr>
</tbody>
</table>
5 5.23 NIS (2.0) TfOH (0.5) 5.24, 95%, 1/21

6 5.25 NIS (2.0) HOFox (1.0) 5.26, 69%, β only

7 5.27 NIS (2.0) TfOH (0.5) 5.5, 86%, β only

8 5.28 NIS (2.0) TfOH (0.5) 5.5, 87%, β only

9 5.29 TfOH (3.0) 5.16, 51%, 3.0/1

10 5.2 5.30 TMSOTf (0.5) 5.31, 78%, β only

11 5.32 5.33 TMSOTf (0.5) 5.34, 80%, 2.1/1

12 5.34 5.35 TMSOTf (1.0) 5.36, 79%, 10.0/1
We then investigated activation of ethylthio glucoside 5.25 for reaction with glycosyl acceptor 5.3 under the previously developed regenerative conditions (NIS and HOFox). This approach that allows for thioglycoside activation under neutral reaction conditions afforded product 5.26 in 69% yield (entry 6). Other classes of thioglycosides were also found to be suitable for HPLC-A glycosylations in solution. Both SPh glucoside 5.27 and STol glucoside 5.28 produced a similar outcome in reactions with acceptor 5.3. Thus, disaccharide 5.5 was obtained in good yields of 86-87% (entries 7 and 8). S-Benzoxazolyl donor 5.29 was intentionally investigated in the presence of TfOH as the sole activator. Known for its high reactivity in the presence of silver triflate or NIS/TfOH promoters, protic acid-promoted activation for reaction with glycosyl acceptor 5.3 was also proven possible and disaccharide 5.16 was obtained in 51% with (α/β = 3.0/1, entry 9).

The developed HPLC-A platform was further investigated toward selective activation, wherein trichloroacetimidate donors 5.2 and 5.32 were selectively activated over thioglycoside acceptors 5.30 and 5.33. Both automated glycosylations were successful, and the respective products were obtained in good yields: 5.31 (78%, entry 10) and 5.34 (80%, α/β = 2.1/1, entry 11). Glycosidation of highly reactive OFox imidate 5.35 and allylphenyl glucoside 5.37 with glycosyl acceptor 5.3 was conducted in the presence of TMSOTf. As a result, the respective disaccharide 5.36 and 5.16 were obtained in 78-79% yields with preferential α-stereoselectivity (entries 12-13).
Scheme 5.3. HPLC-A synthesis of multiple di- and trisaccharides 5.12, 5.43-5.47 by different methods with a single press of a button.

With the universally proven platform for HPLC-A glycosylation in solution, we endeavored on batch glycosylations of a set of glycosyl acceptors with various glycosyl donors under different reaction conditions, all with the single press of a button. For this study, the autosampler tray was loaded with randomly chosen glycosyl donors 5.2, 5.38-5.41 equipped with a variety of leaving groups including phosphate and pentenyl as well as disaccharide donor. It should be noted that none these glycosyl donors were preliminary investigated in the single step HPLC-A glycosylations in solution. Also
loaded were glycosyl acceptors 5.3, 5.4, 5.8, 5.42, and necessary reagents, NIS, TfOH and TMSOTf. The HPLC-A software was then programmed to perform six sequential glycosylations in one batch. As a result, we obtained disaccharides 5.12, 5.43-5.45 in 75-88% yields and trisaccharides 5.46 and 5.47 in 61-70% yields (Scheme 5.3).

5.3 Conclusions

In conclusion, a new solution-phase automation set-up based on HPLC has been developed. A variety of glycosyl donors and acceptors were investigated for glycosylation. Multiple glycosylation were successfully performed with the “press of a button” mode. We also showcased how the new automated method can be accurately reproduced by non-specialists (untrained high school student). A very promising result achieved in the HAD directed β-mannosylation may open new exciting directions for further developments.

5.4 Experimental

5.4.1 General experimental

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. HPLC grade solvents used for automation were utilized without purification. Column chromatography was performed on silica gel 60 (70–230 mesh) or using flash purification system Biotage Isolera One, reactions were monitored by TLC on Kieselgel 60 F254. 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. CH2Cl2 was distilled from
CaH$_2$ directly prior to application. Molecular sieves (3 Å) beads, used in an Omnifit glass chromatography column, were activated in vacuo at 390 °C for 16 h prior to application. Optical rotations were measured using a Jasco ‘P-2000’ polarimeter. $^1$H NMR spectra were recorded at 300 MHz, $^{13}$C NMR spectra were recorded at 75 MHz. The $^1$H NMR chemical shifts are referenced to tetramethylsilane (TMS, $\delta_H = 0.00$ ppm) for $^1$H NMR spectra for solutions in CDCl$_3$. The $^{13}$C NMR chemical shifts are referenced to the central signal of CDCl$_3$ ($\delta_C = 77.00$ ppm) for solutions in CDCl$_3$. HRMS determinations were made using Agilent 6230 ESI TOF LC/MS mass spectrometer. Agilent 1260 infinity II HPLC System, Agilent 1260 Variable Wavelength UV−vis detector and Agilent 1260 Infinity II Analytical and Preparative scale fraction collector were used to assemble the automated solution-phase synthesizer.

5.4.2. Synthesis of reagents and glycosides

3,3-Difluoroxindole (HOFox) was obtained from Isatin and DAST as previously described, and its analytical data were in accordance with that previously reported.$^{71}$

Methyl 6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.5) was obtained from thioglycosides 5.1,$^{39}$ 5.27$^{56}$ or 5.28$^{57}$ and trichloroacetimidate donor 5.2$^{40}$ with glycosyl acceptor 5.3$^{41}$ by NIS/TfOH and TMSOTf activation sequences in 84%, 86%, 87% and 88% yield, respectively, as a syrup. ESI-HRMS [M+Na]$^+$ calcd for C$_{62}$H$_{58}$NaO$_{15}$ 1065.3673, found 1065.3679. Analytical data for 5.5 was in accordance with that reported previously.$^{43}$

Methyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (5.6) was obtained from thioglycoside 5.1 and glycosyl acceptor 5.4$^{42}$
by NIS/TfOH activation sequence in 87% yield as a clear film. ESI-HRMS [M+Na]^+ calcd for C_{62}H_{52}NaO_{18} 1107.3051, found 1107.3083. Analytical data for 5.6 was in accordance with that reported previously.\textsuperscript{45}

Methyl 2-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (5.11) was obtained from thioglycoside 5.1 and glycosyl acceptor 5.7\textsuperscript{41} by NIS/TfOH activation sequence in 71% yield as a clear film. ESI-HRMS [M+Na]^+ calcd for C_{62}H_{58}NaO_{15} 1065.3673, found 1065.3679. Analytical data for 5.11 was in accordance with that reported previously.\textsuperscript{46}

Methyl 3-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (5.12) was obtained from thioglycoside 5.1 and trichloroacetimidate 5.2 with glycosyl acceptor 5.8\textsuperscript{41} by NIS/TfOH and TMSOTf activation sequences in 72% and 75% yield, respectively, as a syrup. ESI-HRMS [M+Na]^+ calcd for C_{62}H_{58}NaO_{15} 1065.3673, found 1065.3713. Analytical data for 5.12 was in accordance with that reported previously.\textsuperscript{41}

Methyl 4-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (5.13) was obtained from thioglycoside 5.1 and glycosyl acceptor 5.9\textsuperscript{41} by NIS/TfOH activation sequence in 65% yield as a clear film. ESI-HRMS [M+Na]^+ calcd for C_{62}H_{58}NaO_{15} 1065.3673, found 1065.3713. Analytical data for 5.13 was in accordance with that reported previously.\textsuperscript{46}

6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (5.14) was obtained from thioglycoside 5.1 and glycosyl acceptor 5.10 by NIS/TfOH activation sequence in 72% yield as an amorphous solid. ESI-HRMS
[M+Na]$^+$ calcd for C$_{46}$H$_{46}$NaO$_{15}$ 861.2735, found 861.2763. Analytical data for 5.14 was in accordance with that reported previously.$^{45}$

**Methyl 2,3,4-tri-O-benzyl-6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-α-D-glucopyranoside (5.16)** was obtained from thioglycoside 5.15,$^{47}$ SBox donor 5.29$^{58}$ or allylphenyl 5.37$^{64}$ with glycosyl acceptor 5.3 by NIS/TfOH and TfOH or TMSOTf activation sequences in 90% (α/β = 2.0/1), 51% (α/β = 3.0/1) and 78% (α/β = 1.5/1) yield, respectively, as a syrup. ESI-HRMS [M+Na]$^+$ calcd for C$_{62}$H$_{66}$NaO$_{11}$ 1009.4503, found 1009.4553. Analytical data for 5.16 was in accordance with that reported previously.$^{48}$

**Methyl 6-O-(2,3,4,6-tetra-O-benzoyl-β-D-galacopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.18)** was obtained from thioglycoside 5.17$^{49}$ and glycosyl acceptor 5.3 by NIS/TfOH activation sequence in 78% yield as a clear film. ESI-HRMS [M+Na]$^+$ calcd for C$_{62}$H$_{58}$NaO$_{15}$ 1065.3673, found 1065.3690. Analytical data for 5.18 was in accordance with that reported previously.$^{50,63}$

**Methyl 6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (5.20)** was obtained from thioglycoside 5.19$^{51}$ and glycosyl acceptor 5.4 by NIS/TfOH activation sequence in 74% yield as a clear film. ESI-HRMS [M+Na]$^+$ calcd. for C$_{42}$H$_{44}$NaO$_{18}$ 859.2421, found 859.2390. Analytical data for 5.20 was in accordance with that reported previously.$^{52}$

**Methyl 2-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (5.22)** was obtained from thioglycoside 5.21$^{52}$ and glycosyl acceptor 5.7 by NIS/TfOH activation sequence in 72% yield as a clear film. ESI-HRMS [M+Na]$^+$calcd for C$_{62}$H$_{66}$NaO$_{11}$ 1009.4503, found 1009.4505. Analytical data for 5.22 was in accordance with that reported previously.$^{53}$
Methyl 6-O-(2-azido-4,6-O-benzylidene-2-deoxy-3-O-picoloyl-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.24) was obtained from thioglycoside 5.23\textsuperscript{54} and glycosyl acceptor 5.3 by NIS/TfOH activation sequence in 95% yield ($\alpha/\beta = 1/21$) as a clear film. ESI-HRMS [M+Na]$^+$ calcd for C\textsubscript{47}H\textsubscript{48}N\textsubscript{4}O\textsubscript{11}Na 867.3217 found 867.3225. Analytical data for 5.24 was in accordance with that reported previously.\textsuperscript{54}

Methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.26) was obtained from thioglycoside 5.25\textsuperscript{55} and glycosyl acceptor 5.3 by NIS/HO\textsubscript{Fox} activation sequence in 69% yield as a clear film. ESI-HRMS [M+Na]$^+$ calcd for C\textsubscript{62}H\textsubscript{64}NaO\textsubscript{12} 1023.4296, found 1023.4297. Analytical data for 5.26 was in accordance with that reported previously.\textsuperscript{48}

Ethyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-1-thio-β-D-galactopyranoside (5.31) was obtained from trichloroacetimidate donor 5.2 and glycosyl acceptor 5.30\textsuperscript{60} by TMSOTf activation sequence in 78% yield as a clear film. ESI-HRMS [M+Na]$^+$ calcd for C\textsubscript{63}H\textsubscript{54}NaO\textsubscript{17}S 1137.2980, found 1137.2988. Analytical data for 5.31 was in accordance with that reported previously.\textsuperscript{62}

$p$-Tolyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (5.34) was obtained from trichloroacetimidate donor 5.32\textsuperscript{59} and glycosyl acceptor 5.33\textsuperscript{61} by TMSOTf activation sequence in 80% yield ($\alpha/\beta = 2.1/1$) as a clear film. ESI-HRMS [M+Na]$^+$ calcd for C\textsubscript{68}H\textsubscript{64}NaO\textsubscript{13}S 1143.3965, found 1143.3970. Analytical data for 5.34 was in accordance with that reported previously.\textsuperscript{63}

Methyl 6-O-(3,4,6-tri-O-acetyl-2-O-benzyl-α/β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.36) was obtained from OFox imidate donor 5.35\textsuperscript{59} and glycosyl acceptor 5.3 by TMSOTf activation sequence in 79% yield ($\alpha/\beta = 10.0/1$) as a syrup. ESI-
HRMS [M+Na]^+ calcd for C_{47}H_{54}NaO_{14} 865.3412, found 865.3414. Analytical data for 5.36 was in accordance with that reported previously.\(^{59}\)

**Methyl 2,3,4-tri-O-benzoyl-6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (5.43)** was obtained from pentenyl donor 5.38\(^{65}\) and glycosyl acceptor 5.4 by NIS/TfOH activation sequence in 85% yield as a clear film. ESI-HRMS [M+Na]^+ calcd for C_{46}H_{46}NaO_{15} 1065.3674, found 1065.3715. Analytical data for 5.43 was in accordance with that reported previously.\(^{69}\)

**Methyl 2,3,4-tri-O-benzyl-6-O-(2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosyl)-α-D-glucopyranoside (5.44)** was obtained from thioglycoside 5.39\(^{47}\) and glycosyl acceptor 5.3 by NIS/TfOH activation sequence in 88% yield (α/β = 1.2/1) as a syrup. ESI-HRMS [M+Na]^+ calcd for C_{62}H_{66}NaO_{11} 1009.4503, found 1009.4520. Analytical data for 5.44 was in accordance with that reported previously.\(^{48}\)

**Methyl 6-O-(2,3,4-tri-O-benzoyl-6-O-fluorenymethoxycarbonyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.45)** was obtained from phosphate donor 5.40\(^{66}\) and glycosyl acceptor 5.3 by TMSOTf activation sequence in 83% yield as a white amorphous solid. Analytical data for 5.45: R\(_f\) = 0.60 (ethyl acetate/hexane, 2/3, v/v); [α]_D\(^{22}\) = -15.7 (c = 1.0, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 8.12 (d, 2H, J = 7.8 Hz, aromatic), 7.92 (d, 2H, J = 7.5 Hz, aromatic), 7.82 (d, 2H, J = 7.7 Hz, aromatic), 7.76 (d, 2H, J = 7.4 Hz, aromatic), 7.24–7.58 (m, 38H, aromatic), 5.85–5.95 (m, 2H, H-3’, 4’), 5.69 (dd, 1H, J_{2’,3’} = 7.5 Hz, H-2’), 5.19 (d, 1H, J_{1’,2’} = 1.7 Hz, H-1’), 5.01 (dd, J\(^2\) = 10.9 Hz, 2H, CH\(_2\)Ph), 4.67–4.87 (m, 4H, 2 x CH\(_2\)Ph), 4.64 (d, 1H, J\(_{1,2}\) = 4.6 Hz, 1H, H-1), 4.19–4.44 (m, 6H, H-5’, 6a’, 6b’, COOC\(_2\)CH\(_2\)H), 3.74–4.06 (m, 4H, H-3, 5, 6a, 6b), 3.52–3.61 (m, 2H, H-2, 4), 3.44 (s, 3H, OCH\(_3\)) ppm; \(^{13}\)C NMR (75 MHz,
Methyl  \( O-(2,3,4,6\text{-tetra-}\text{O-benzoyl}-\beta\text{-D-galactopyranosyl})-(1\rightarrow6)-O-(2,3,6\text{-tri-}\text{O-benzoyl-}\beta\text{-D-glucopyranosyl})-(1\rightarrow6)-2,3,4\text{-tri-}\text{O-benzyl-}\alpha\text{-D-glucopyranoside} \) (5.46) was obtained from trichloroacetimidate lactose donor 5.41 and glycosyl acceptor 5.3 by TMSOTf activation sequence in 70% yield as a white amorphous solid. ESI-HRMS [M+Na]\(^+\) calcd for C\(_{70}\)H\(_{64}\)O\(_{16}\)Na 1183.4193, found 1183.4110. Analytical data for 5.46 was in accordance with that reported previously.

Ethyl  \( O-(2,3,4,6\text{-tetra-}\text{O-benzoyl}-\beta\text{-D-galactopyranosyl})-(1\rightarrow6)-O-(2,3,6\text{-tri-}\text{O-benzoyl-}\beta\text{-D-glucopyranoside} \) (5.47) was obtained from trichloroacetimidate lactose donor 5.41 and glycosyl acceptor 5.42 by TMSOTf activation sequence in 61% yield as a white amorphous solid. Analytical data for 5.47: \( R_f = 0.40 \) (ethyl acetate/hexane, 2/3, v/v); \([\alpha]_D^{22} = +33.1 \) (c = 1.0, CHCl\(_3\)); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta 7.89\text{--}7.98 \) (m, 12H, aromatic), 7.71–7.81 (m, 5H, aromatic), 7.49–7.65 (m, 11H, aromatic), 7.11–7.45 (m, 22H, aromatic) 5.64–5.85 (m, 4H, H-2ʹʹ, 3ʹ, 3, 4ʹ), 5.22–5.46 (m, 4H, H-2, 2ʹ, 3ʹʹ, 4), 4.83–4.85 (m, 2H, H-1, 1ʹʹ), 4.53–4.60 (m, 2H, J\(_{6a,\prime\prime,6b\prime\prime} = 9.1\) Hz, H-1ʹ, 6aʺ), 4.43 (dd, 1H, H-6bʺ), 4.22 (dd, J\(_{4,5} = 9.3\) Hz, 1H, H-4ʹ), 3.65–3.94 (m, 7H, H-5, 5ʹ, 5ʺ, 6a, 6aʹ, 6b, 6bʹ), 2.50 (m, 2H, SCH\(_2\)), 1.07 (t, 3H, SCH\(_2\)CH\(_3\)) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta 165.5, 165.4, 165.3, 165.2 \) (×2), 165.1 (×3), 165.0,
164.5, 133.4 (×2), 133.2 (×5), 133.0 (×4), 132.9 (×2), 132.8, 129.9, 129.7 (×4), 129.6 (×3), 129.3, 129.2, 129.1 (×7), 128.9, 128.7, 128.5 (×3), 128.3 (×14), 128.2 (×5), 128.0 (×2), 114.1, 101.1 (×2), 100.9, 83.3, 78.0 (×3), 75.9 (×3), 73.8 (×2), 72.7, 71.7 (×2), 71.2, 70.4, 69.7, 69.0, 67.4, 23.9, 14.6 ppm; ESI-HRMS[M+Na]$^+$ calcd for C$_{90}$H$_{76}$O$_{25}$SNa1611.4289, found 1611.4247.

5.4.3 Set-up of the HPLC-A solution-phase synthesizer

The HPLC-A based solution-phase synthesizer has been assembled using:

- 1260 Agilent Infinity II series Quaternary Pump.
- Variable Wavelength Detector with Dual-Wavelength Mode.
- Preparative autosampler from 1260 Infinity series. Equipped with 900µL preparative loop and one tray holding 100 x 2.0 mL vials numbered from 1 to 100.
- 4-way 10-port Quick Change Valve from 1260 Infinity series.
- Analytical and preparative scale fraction collector from 1260 Infinity II series. Equipped with three trays holding 15 x 6 mL vials each.
- Omnipit Solvent Plus 50 mm column filled with activated molecular sieves 3Å beads.

The glycosylation sequences are programmed using Chemstation software and the autosampler programming option.
5.4.4 Preparation of the reagent vials

All solutions were freshly prepared using the HPLC grade solvents and kept at room temperature for the duration of the synthesis.

**Donor:** a solution of glycosyl donor (0.02-0.05 mmol) in CH$_2$Cl$_2$ (0.30 mL, for S-glycosides, 0.35 mL, for O-glycosides) was prepared in Agilent 2 mL screw top vial.

**Acceptor:** a solution of glycosyl acceptor (0.008-0.018 mmol) in CH$_2$Cl$_2$ (0.30-0.35 mL, as mentioned above) was prepared in Agilent 2 mL screw top vial.

**Promoters:** a solution of TMSOTf (0.02-0.06 mmol) in CH$_2$Cl$_2$ (0.19 mL), a solution of TfOH (0.02-0.03 mmol) or HOFox aglycone (0.05 mmol) in CH$_2$Cl$_2$ (0.19 mL) and NIS (0.09-0.10 mmol) in CH$_2$Cl$_2$:Dioxane (0.2 mL, 1/1, v/v) were prepared in separate Agilent 2 mL screw top vials.
Washing solution: CH$_2$Cl$_2$ (2 mL) prepared in Agilent 2 mL screw top vial. MeOH (2 mL) prepared in Agilent 2 mL screw top vial if needed.

5.4.5 The automated solution-phase assembly of oligosaccharides

All the synthesis of oligosaccharides were performed under standard sequences (Figure 5.2) as well as solvents amount, and ratios according to the general methods.

**Glycosylation.** The sequence is comprehensive of washing of the lines pre glycosylation for 5 min with CH$_2$Cl$_2$. The donor, acceptor and promoter were drawn, then ejected in a one empty vial as depicted in Fig 5.2B. The latter mixture then was drawn and injected. The reaction was recirculated for 60 min. The programming of the components of the synthesizer is reported in Fig 5.2. A represents pump timetable, B represents the autosampler programming, C represents the valve timetable (the valve was on position 2 during recirculation, switched to position 4 during collection), D represents variable wave-length detector (VWD), E represents the analytical and preparative scale fraction collector timetable.

**Washing.** The sequence is comprehensive of washing off left mixture after each glycosylation with CH$_2$Cl$_2$ delivered by pump line A. The programming of the components of the synthesizer for washing is reported in Figure 5.3. A represents pump timetable, B represents the autosampler programming, C represents the valve timetable, switched on and off between positions 2 and 4 during the run/wash, D represents the variable wave-length detector (VWD) and E represents the analytical and preparative scale fraction collector timetable.
Figure 5.2. Settings of the components of the HPLC-A solution-phase synthesizer during glycosylation. A) The pump timetable. B) The autosampler programming sequence. C) The valve timetable. D) The variable wave-length detector (VWD) including UV trace for the glycosylation at 250 nm. E) The analytical and preparative scale fraction collector timetable

A)

![Pump Timetable Diagram]

B)

![Autosampler Programming Sequence Diagram]
**Detection.** Representative UV trace for the glycosylation sequence is depicted in Figure 5.2, D. The glycosylation trace shows high absorbance during the injection. However, once pump line D is saturated with the reaction mixture, a steady absorbance is progressed during recirculation followed by collection.

**5.4.6 General glycosylation procedure for the automated synthetic cycle of oligosaccharides**

The volume of solvents in vials is constant, hence the concentration of donor, acceptor and promoter varies depending on the equivalents required for the glycosylation. The Omnifit column is filled with activated molecular sieves 3 Å beads, activated and replaced every other week.

**From S-glycosides**

**Automation module:** The vials were prepared according to the general methods and organized as depicted below. Vial 1 contains donor (30 mg, 3.0 equiv), vial 2 contains acceptor (0.008-0.018 mmol, 1.0 equiv), vial 3 contains NIS (2.0 equiv) and vial 4 contains TfOH or HOFox (0.5 equiv, 1.0 equiv, respectively). Vial 5 is used for mixing. Vial A contains CH$_2$Cl$_2$ (2.0 mL) for washing. Repeated for a number of glycosylations as needed.

**Automation sequence:**

- Glycosylation 60 min
- Washing 15 min
Vial tray organization:

From \(\textit{O}\)-glycosides

\textbf{Automation module:} The vials were prepared according to the general methods and organized as depicted below. Vial 1 contains donor (30 mg, 3.0 equiv), vial 2 contains acceptor (0.008-0.018 mmol, 1.0 equiv), vial 3 contains TMSOTf (0.5-1.0 equiv) or TfOH (3.0 equiv) and vial 4 is used for mixing. Vial A contains CH\(_2\)Cl\(_2\) (2.0 mL) for washing. Repeated for a number of glycosylations as needed.

\textbf{Automation sequence:}

- Glycosylation 60 min
- Washing 15 min
Vial tray organization:

Post automation: Reaction mixtures were collected from the fraction collector, washed, and evaporated under reduced pressure. Purified by column chromatography or flash purification system Biotage Isolera One.

5.5 References


APPENDIX
**Figure A-1:** $^1$H NMR spectrum of cholesteryl 2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranoside (3.9).

**Figure A-2:** $^{13}$C NMR spectrum of cholesteryl 2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranoside (3.9).
Figure A-3: 2-D NMR COSY spectrum of cholesteryl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (3.9).
Figure A-4: $^1$H NMR spectrum of methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenlymethoxycarbonyl-2-phthalimido-$\beta$-D-glucopyranosyl)-2,3,4-tri-O-benzyl-$\alpha$-D-glucopyranoside (3.17).

Figure A-5: $^{13}$C NMR spectrum of methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenlymethoxycarbonyl-2-phthalimido-$\beta$-D-glucopyranosyl)-2,3,4-tri-O-benzyl-$\alpha$-D-glucopyranoside (3.17).
Figure A-6: 2-D NMR COSY spectrum of methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenlymethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (3.17).
**Figure A-7:** $^1$H NMR spectrum of methyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (3.27).

**Figure A-8:** $^{13}$C NMR spectrum of methyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (3.27).
**Figure A-9:** 2-D NMR COSY spectrum of methyl 2,3,4,6-tetra-<i>O</i>-benzyl-α/β-<i>D</i>-glucopyranosyl-(1→6)-2,3,4-tri-<i>O</i>-benzoyl-β-<i>D</i>-glucopyranosyl-(1→6)-2,3,4-tri-<i>O</i>-benzoyl-α-<i>D</i>-glucopyranoside (3.27).
Figure A-10: $^1$H NMR spectrum of allyl 2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (3.28).

Figure A-11: $^{13}$C NMR spectrum of allyl 2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (3.28).
Figure A-12: 2-D NMR COSY spectrum of allyl 2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (3.28)
Figure A-13: $^1$H NMR spectrum of allyl 6-$O$-(2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2-azido-4-$O$-benzyl-2-deoxy-$\beta$-D-glucopyranoside (3.29).

Figure A-14: $^{13}$C NMR spectrum of allyl 6-$O$-(2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2-azido-4-$O$-benzyl-2-deoxy-$\beta$-D-glucopyranoside (3.29).
Figure A-15: 2-D NMR COSY spectrum of allyl 6-\(O\)-(2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranosyl)-2-azido-4-\(O\)-benzyl-2-deoxy-\(\beta\)-D-glucopyranoside \((3.29)\).
**Figure A-16:** $^1$H NMR spectrum of the \textit{in situ} OFox formation from donor 4.28 (20 min in CDCl$_3$ at rt in the absence of a glycosyl acceptor).
**Figure A-17:** $^1$H NMR spectrum of 2-thiazolinyl 2-$O$-benzoyl-3-$O$-benzyl-4,6-$O$-benzylidene-1-thio-$\beta$-D-galactopyranoside (4.39).

**Figure A-18:** $^{13}$C NMR spectrum of 2-thiazolinyl 2-$O$-benzoyl-3-$O$-benzyl-4,6-$O$-benzylidene-1-thio-$\beta$-D-galactopyranoside (4.39).
**Figure A-19:** 2-D NMR COSY spectrum of 2-thiazolinyl 2-\(O\)-benzoyl-3-\(O\)-benzyl-4,6-\(O\)-benzylidene-1-thio-\(\beta\)-D-galactopyranoside (4.39).
Figure A-20: $^1$H NMR spectrum of 2-thiazolinyl 2-\textit{O}-benzoyl-3,4-di-\textit{O}-benzyl-1-thio-\textit{\textbeta}-D-galactopyranoside (4.36).

Figure A-21: $^{13}$C NMR spectrum of 2-thiazolinyl 2-\textit{O}-benzoyl-3,4-di-\textit{O}-benzyl-1-thio-\textit{\textbeta}-D-galactopyranoside (4.36).
Figure A-22: 2-D NMR COSY spectrum of 2-thiazolinyl 2-\textit{O}-benzoyl-3,4-di-\textit{O}-benzyl-1-thio-\textbeta-D-galactopyranoside(4.36)
Figure A-23: $^1$H NMR spectrum of methyl 2-\(O\)-(2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranosyl)-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-glucopyranoside (4.5).

Figure A-24: $^{13}$C NMR spectrum of methyl 2-\(O\)-(2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranosyl)-3,4,6-tri-\(O\)-benzyl-\(\alpha\)-D-glucopyranoside (4.5).
Figure A-25: 2-D NMR COSY spectrum of methyl 2-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (4.5).
Figure A-26: $^1$H NMR spectrum of methyl 6-$O$-(2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2,3,4-tri-$O$-benzoyl-$\alpha$-D-glucopyranoside (4.11).

Figure A-27: $^{13}$C NMR spectrum of methyl 6-$O$-(2-$O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2,3,4-tri-$O$-benzoyl-$\alpha$-D-glucopyranoside(4.11).
Figure A-28: 2-D NMR COSY spectrum of methyl 6-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (4.11).
**Figure A-29:** $^1$H NMR spectrum of butyl 2-O-benzoyl-3,4,6-tri-O-benzyl-$\beta$-D-galactopyranoside (4.15).

**Figure A-30:** $^{13}$C NMR spectrum of butyl 2-O-benzoyl-3,4,6-tri-O-benzyl-$\beta$-D-galactopyranoside (4.15).
Figure A-31: 2-D NMR COSY spectrum of butyl 2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranoside (4.15).
Figure A-32: $^1$H NMR spectrum of 1-adamantyl 2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranoside (4.17).

Figure A-33: $^{13}$C NMR spectrum of 1-adamantyl 2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranoside (4.17).
Figure A-34: 2-D NMR COSY spectrum of 1-adamantyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (4.17).
Figure A-35: $^1$H NMR spectrum of methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (4.35).

Figure A-36: $^{13}$C NMR spectrum of methyl 6-O-(4,6-di-O-benzyl-2-deoxy-3-O-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside (4.35).
**Figure A-37:** 2-D NMR COSY spectrum of 6-$\text{O}$-(4,6-di-$\text{O}$-benzyl-2-deoxy-3-$\text{O}$-fluorenymethoxycarbonyl-2-phthalimido-$\beta$-D-glucopyranosyl)-2,3,4-tri-$\text{O}$-benzoyl-$\alpha$-D-glucopyranoside (4.35).
Figure A-38: $^1$H NMR spectrum of 2-thiazolinyl 6-$(2-O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2-$O$-benzoyl-3,4-di-$O$-benzyl-1-thio-$\beta$-D-galactopyranoside (4.37).

Figure A-39: $^{13}$C NMR spectrum of 2-thiazolinyl 6-$(2-O$-benzoyl-3,4,6-tri-$O$-benzyl-$\beta$-D-galactopyranosyl)-2-$O$-benzoyl-3,4-di-$O$-benzyl-1-thio-$\beta$-D-galactopyranoside (4.37).
Figure A-40: 2-D NMR COSY spectrum of 2-thiazolinyl 6-\(O\) (2-\(O\)-benzoyl-3,4,6-tri-\(O\)-benzyl-\(\beta\)-D-galactopyranosyl)-2-\(O\)-benzoyl-3,4-di-\(O\)-benzyl-1-thio-\(\beta\)-D-galactopyranoside (4.37).
Figure A-41: $^1$H NMR spectrum of Methyl 6-O-(2,3,4-tri-O-benzoyl-6-O-fluorenylmethoxycarbonyl-$\alpha$-D-mannopyranosyl)-2,3,4-tri-O-benzyl-$\alpha$-D-glucopyranoside (5.45).

Figure A-42: $^{13}$C NMR spectrum of methyl 6-O-(2,3,4-tri-O-benzoyl-6-O-fluorenylmethoxycarbonyl-$\alpha$-D-mannopyranosyl)-2,3,4-tri-O-benzyl-$\alpha$-D-glucopyranoside (5.45).
Figure A-43: 2-D NMR COSY spectrum of methyl 6-O-(2,3,4-tri-O-benzoyl-6-O-fluorenylmethoxycarbonyl-α-D-mannopyranosyl)-2,3,4-tri-O-benzyl-α-D-glucopyranoside (5.45).
Figure A-44: $^1$H NMR spectrum of ethyl 2,3,4,6-tetra-$O$-benzoyl-$\beta$-D-galactopyranosyl-(1$\rightarrow$6)-2,3,6-tri-$O$-benzoyl-$\beta$-D-glucopyranosyl-(1$\rightarrow$6)-2,3,4-tri-$O$-benzoyl-$\beta$-D-glucopyranoside (5.47).

Figure A-45: $^{13}$C NMR spectrum of ethyl 2,3,4,6-tetra-$O$-benzoyl-$\beta$-D-galactopyranosyl-(1$\rightarrow$6)-2,3,6-tri-$O$-benzoyl-$\beta$-D-glucopyranosyl-(1$\rightarrow$6)-2,3,4-tri-$O$-benzoyl-$\beta$-D-glucopyranoside (5.47).
Figure A-46: 2-D NMR COSY spectrum of ethyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,6-tri-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside (5.47).