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Novel Thioglycosides as Versatile Glycosyl Donors for Oligosaccharide Synthesis

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By

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ABSTRACT

Novel Thioglycosides as Versatile Glycosyl Donors for Oligosaccharide Synthesis

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This thesis is dedicated to the development of new methodologies for efficient synthesis of carbohydrate building blocks and their application to chemical glycosylation. *S*-Indolyl (SIn) anomeric moiety was investigated as a new leaving group. Understanding of the reaction pathways for the SIn moiety activation was achieved via the extended mechanistic study. The activation profile of indolylthio glycosides required large excess of activators. This drawback was partially addressed by the development of N-alkylated SInR derivatives. The activation process was studied by NMR and the increased understanding of the mechanism led to a discovery of different activation pathways taking place with SIn versus SInR derivatives. Also investigated was orthogonality of the SInR leaving groups versus other building blocks.

The synthesis of a common 3-OH glycosyl acceptor has been revisited and improved. This compound is a building block that is routinely synthesized by many research groups to be used in glycosylation refinement studies. This method provides consistent results and is superior to other strategies. Our studies culminated by the invention of 3,3-difluoroindolylthio (SFox) imidates. The synthesis and activation for glycosylation of these compounds has been studied. The SFox imidates showed high efficiency in 1,2-trans and 1,2-cis glycosylations.

I would like to sincerely dedicate this thesis to my family.

.

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First of all, I would like to thank my PhD mentor, advisor and my boss Professor Alexei V. Demchenko for accepting me as his PhD student in his "Glycoworld Lab". I am extremely grateful for all his supervision, guidance, support and patience during my PhD studies at University of Missouri-Saint Louis. Coming from Nepal, I did not have much practical skills to adapt quickly in the lab. He provided me enough opportunities and time to learn the necessary skills to be a real chemist. First year of my PhD just passed developing skills and working on my first project of my life-HSFox. However, my first project did not work well for me at that time and I switched to another project-Thioindole Leaving Group. It took me two and half years to complete that project and get my first ever paper published. Thank you for that. Secondly, I would like to thank and show my gratitude for all my graduate committee members; Prof. Keith Stine, Prof. Eike Bauer, Prof. Janet B. Wilking for their valuable guidance, comments and suggestion.

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LIST OF ABBREVIATIONS

Å .....Ångström

Ac .....Acetyl

Ac_2O.....Acetic anhydride

AcOH .....Acetic acid

AgOTf .....Silver(I) trifluoromethanesulfonate

Ag_2CO_3 .....Silver(I) carbonate

All .....Allyl

$BF_3 \cdot OEt_2$ .....Boron trifluoride etherate

BH_3•THF.....Borane tetrahydrofuran complex

Bn .....Benzyl

BnBr .....Benzyl bromide

Br_2 .....Bromine

Bz .....Benzoyl

BzCl.....Benzoyl chloride

CaH_2 .....Calcium hydride

$CDCl_3$ .....Deuterated chloroform

CD_3OD .....Deuterated methanol

$CHCl_3$ .....Chloroform

CH_2Cl_2 .....Dichloromethane

CH_3COCH_3 .....Acetone

$ClCH_2CH_2Cl$ 1,2-Dichloroethane

$Cu(OTf)_2$.....Copper(II) trifluoromethanesulfonate

d .....Doublet

dd .....Doublet of doublets

DIPEA .....*N,N*-Diisopropylethylamine

DMAP 4-Dimethylaminopyridine

DMF .....*N*,*N*-Dimethylformamide

EDC .....1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide

Et .....Ethyl

Et_2O .....Diethyl ether

EtOAc..... Ethyl acetate

Et_3SiH..... Triethylsilane

h ..... Hour(s)

$FeCl_3$……..Iron(III) chloride

HBr.....Hydrogen bromide

HCl .....Hydrogen chloride

H_2O.....Water

H_2NNH_2 H_2O .....Hydrazine hydrate

HR-ESI MS .....High Resolution Electrospray Ionization mass spectrometry

HR-FAB MS .....High Resolution Fast Atom Bombardment mass spectrometry

Hz .....Hertz

$LiAlH_4$ .....Lithium aluminum hydride

M .....Molar

m ..... Multiplet

Me ..... Methyl

MeCN ..... Acetonitrile

MeOH ..... Methanol

$MgSO_4$ .....Magensium sulfate

min ..... Minute(s)

MS ..... Molecular sieves

MW .....Molecular weight

m/z ..... Mass to charge ratio

Na .....Sodium

$NaBH_4$ .....Sodium borohydride

$NaCNBH_3$ .....Sodium cyanoborohydride

NaH .....Sodium hydride

$NaHCO_3$ .....Sodium bicarbonate

NaOAc .....Sodium acetate

NaOH ..... Sodium hydroxide

NaOMe ..... Sodium methoxide

$Na_2S_2O_3$ .....Sodum thiosulfate

NBS ..... *N*-Bromosuccinimide

NIS……..*N*-Iodosuccinimide

NMR ..... Nuclear magnetic resonance

OBox .....*O*-Benzoxazolyl

OFox……...…*O*-3,3-Difluoro-3*H*-indol-2-yl

Ph ..... Phenyl

PMB ..... *p*-Methoxybenzyl

ppm ..... Parts per million

Py .....Pyridine

R_f.....Retention factor

rt ..... Room temperature

s ..... Singlet

SBox .....*S*-Benzoxazolyl

SEt ..... *S*-Ethyl

SFox…….……....*S*-3,3-difluoro-3*H*-indol-2-yl

SIn……...*S*-Indolyl

SInAll…….….*S*-Allylindolyl

SInMe……..*S*-Methylindolyl

SPy…….……*S*-Pyridyl

SPyr.....*S*-Pyrimidyl

STaz……..*S*-Thiazolinyl

t ..... Triplet

TFA ..... Trifluoroacetic acid

Tf_2O .....Trifluoromethanesulfonic (triflic) anhydride

TfOH ..... Trifluoromethanesulfonic (triflic) acid

THF .....Tetrahydrofuran

TLC ..... Thin layer chromatography

TMS .....Trimethylsilyl

TMSOTf ..... Trimethylsilyl trifluoromethanesulfonate

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CHAPTER 1

Thioglycosides and glycosyl thioimidates in oligosaccharide synthesis

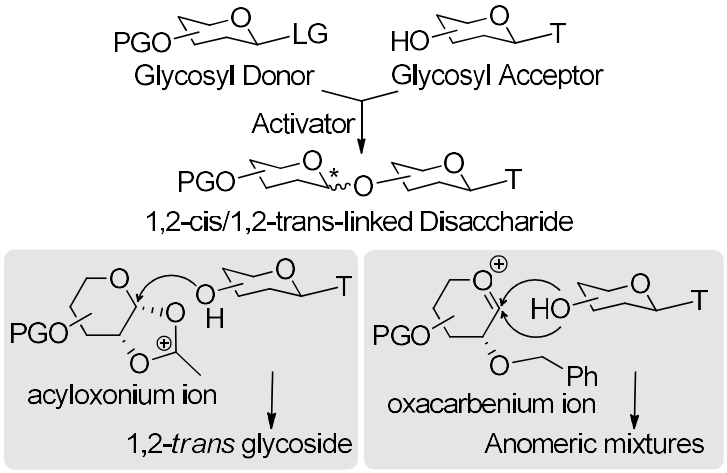
1.1. Introduction

Carbohydrates are the most ubiquitous biomolecules on the planet being metabolized in all living organisms as a crucial fuel source for life. Recently, the scientific world has witnessed a massive blast in glycobiology research wherein researchers are able to explore and enhance their insight into fundamental biological processes that carbohydrates are involved into such as fertilization, anti-inflammation, immunoresponse, joint lubrication, cell growth, antigenic determination etc.[1] Carbohydrates are also known to be involved in many deadly processes such as bacterial and viral infections, development of tumors, metastasis, tissue rejection, congenital disorders, AIDS, cancer, pneumonia, septicemia, hepatitis, malaria[2].[2-7] Detailed studies of such processes can only be possible upon easy access to complex glycostructures. Despite notable progresses, chemical synthesis remains a challenge, and demands for efficient methodologies for carbohydrate synthesis increase.

Glycans are comprised of an array of monosaccharide units connected via *O*-glycosidic linkages. This connection is achieved by means of a coupling reaction called glycosylation, wherein interaction occurs between two counterparts, glycosyl donor and glycosyl acceptor. A glycosyl donor is equipped with an anomeric leaving group (LG) whereas glycosyl acceptor has a free hydroxyl group while other active sites are temporarily blocked with protecting groups (PG, T). Nucleophilic attack from hydroxyl group become active along with the departure of leaving group of glycosyl donor with an aid of some promoter (Scheme 1.1).[8] Achieving 1,2-*trans*-stereoselectivity in glycosylation which often relies on the neighboring group participation from the 2-position. For a 2-acyl group, such assistance produces an acyloxonium ion intermediate that typically leads to 1,2-*trans*-linked glycosides

stereoselectively.[9] With a non-participating ether-type (2-*O*-benzyl) group, the reaction proceeds via an oxacarbenium ion. The nucleophilic attack on this flattened intermediate is often non-stereoselective, which makes the synthesis of 1,2-cis-linked glycosides more challenging.[8, 10]

Scheme 1.1. The outline of a typical glycosylation reaction.



T=PG, conventional synthesis
T=LG, chemoselective activation
T=LG′, selective or orthogonal activation

Factors including solvents, temperature, nature of the LG, activation conditions, and general structure of both reaction components have an effect on the general outcome of the glycosylation reaction. As such, a constant quest for searching new glycosyl donors is ongoing.[8] Starting from studies by Michael,[11] Fischer,[12] and Koenigs and Knorr,[13] glycosylation with glycosyl halides and hemiacetals as glycosyl donors have laid the foundation for further development of carbohydrate chemistry. Subsequent developments included the introduction of various new leaving groups including thioglycosides by Ferrier,[14] Nicolaou,[15] Garegg,[16] and others,[17] cyanoethylidene derivatives by Kochetkov,[18] *O*-imidates by Sinay[19] and Schmidt,[20] *S*-benzothiazolyl derivatives (SBaz) by Mukaiyama,[21] *S*-pyridyl and *S*-pyrimidin-2-yl derivatives by Hanessian[22] and Woodward,[23] and fluorides by Mukaiyama.[24]

[25-27]Along these general improvements, our laboratory contributed to the development of glycosyl thioimidates ($SCR_1=NR_2$).[28-30, 29,31]Among these *S*-benzoxazolyl (SBox), *S*-thiazolinyl (STaz), and S-benzimidazolyl (SBiz) leaving groups are the most versatile in terms of accessibility, reactivity, [32-35]and scope. Moreover, glycosyl thioimidate paved in the innovation of novel strategies like temporary deactivation concept, inverse armed-disarmed strategy, STICS: [36]surface-tethered iterative carbohydrate synthesis,[36] O-2/O-5 cooperative effect,[37] and the thioimidate-only orthogonal strategy.[38,39] This Chapter discusses key milestones of the methodology development with SBox, STaz, and SBiz thioimidates.

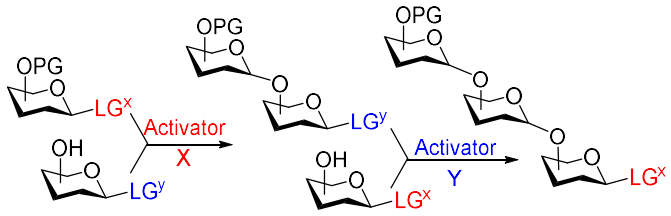
1.1.1. Thioglycosides and Thioimidates

Thioglycosides occur rarely in nature. One example of thioglycoside is antibiotic Lincomycin A. Lincomycin is used to treat severe bacterial infections in patients who cannot use penicillin antibiotics.[40] Fischer was the first to report the synthesis of thioglycosides in 1909.[41] Ferrier et al were the first to report the glycosylation reaction with thioglycosides as glycosyl donors in 1973.[14] Owing to the mild reaction conditions required for their activation, compatibility with many selective activation strategies, and high stability under different reaction conditions, thioglycosides are very versatile synthetic building blocks. They can be glycosidated with numerous acceptors, fit into various glycosylation strategies for chemoselective, orthogonal or one pot glycan synthesis.[42] Nonetheless, these donors require excess promoters and are prone to side reactions.[42] As a result, a dedicated worldwide effort has been applied to discovering new thioglycoside leaving group and methods for their activation.[42]

1.1.2. Orthogonal Activation Strategy

Introduced by Ogawa et al. to address some limitations seen in chemoselective glycosylation startegy,[43-46] orthogonal activation strategy[47] employed the independent activation of two anomeric leaving groups. First, glycosyl donor fitted with LG^x is activated over the glycosyl acceptor fitted with LG^y. Second, the resulting glycoside with LG^y acts as a donor and then activated over glycoside fitted with LG^x - at this time as a glycosyl acceptor. In principle, this sequence can be reiterated to obtain longer oligosaccharides. However, it is found that with increasing size of the glycosyl donor, the yields may deteriorate.

Scheme 1.2. Orthogonal Activation Strategy in Oligosaccharide Synthesis

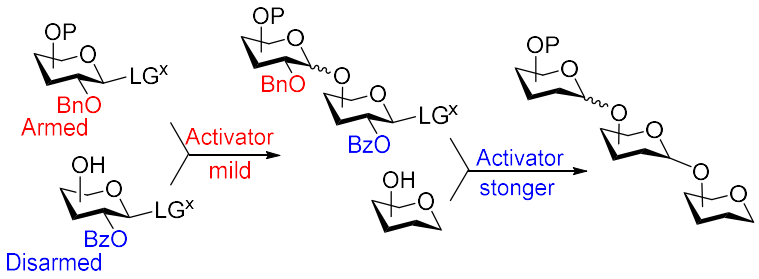


1.1.3. Armed-Disarmed Strategy

Armed-disarmed strategy was introduced by Fraser-Reid who originally employed n-pentenyl glycosides.[45] Difference in electronics between ester and ether protecting groups plays a key role in this chemoselective reaction (Scheme 1.3). As such, the electron donating benzylated donors are considered to be armed (electronically activated) in comparison to their electron withdrawing, benzoylated or acetylated disarmed (electronically deactivated) counterparts bearing the same leaving group. Thus, benzylated glycosyl donor can be activated chemoselectively in the presence of a mild promoter while the disarmed leaving group remain intact. Later on, the disarmed leaving group can also be activated with a suitable, typically more potent promoter. It should also be noted that the armed donor gives preferentially 1,2-

cis-linked products because it is benzylated at C-2. whereas the disarmed donors give 1,2-trans -linked products due to a participatory effect of the ester group at C-2.

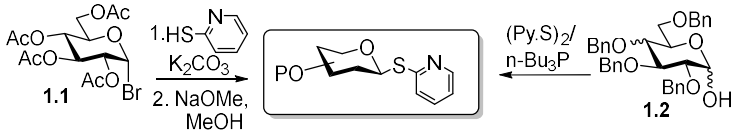
Scheme 1.3. Armed-disarmed Strategy in Oligosaccharide Synthesis



1.2. *S*-Pyridin-2-yl (SPy) derivatives

S-Pyridinyl glycosides were the first thioimidate leaving group employed in disaccharide synthesis that laid the foundation for various new thioimidate leaving groups onwards. Per-acetylated *S*-pyridinyl glucosyl donor was synthesized from the reaction of peracetylated glucosyl bromide **1.1** with 2-pyridine thiol and K_2CO_3 in a mixture of dry acetone / dry toluene solvent (Scheme 1.4).[22] Anomeric mixtures of per benzylated glucosyl and galactosyl S-Pyridinyl donors were synthesized from the reaction of respective 2,3,4,6-O-benzylated pyranosides **1.2** with $(PyS)_2$/ n-Bu_3P in dry dichloromethane.[48]

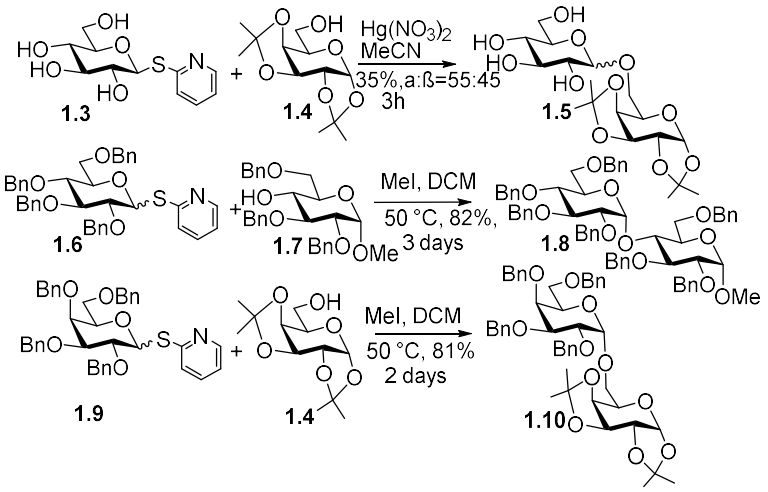
Scheme 1.4. Synthesis of the S-pyridin-2-yl glycosides.



Different promoter system like mercuric nitrate-$Hg(NO_3)_2$[22] and methyl iodide were used to activate 2-pyrindinyl glycosides (Scheme 1.5).[48] Reaction of S-pyridinyl donor with acceptor 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1.4**) with $Hg(NO_3)_2$ yielded disaccharide **1.5** in 35% (α/β = 55:45). Anomeric mixture of per-benzylated glucosyl donor

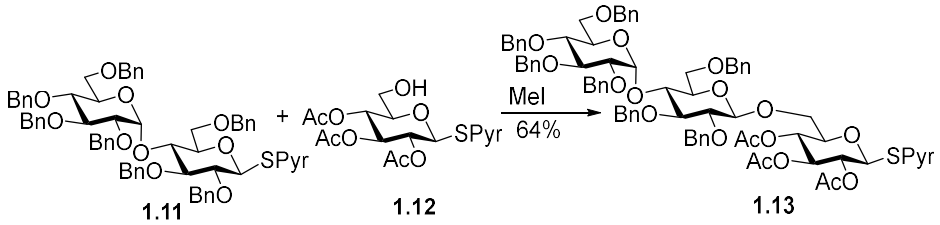
1.6 and acceptor **1.7** in presence of MeI at 50 °C yielded α-linked disaccharide **1.8.** Similarly, glycosylation of donor **1.9** and acceptor **1.4** in presence of MeI led to α-linked disaccharide **1.10**.

Scheme 1.5. Synthesis of disaccharides.



SPy glycoside was used successfully in chemoselective oligosaccharide synthesis following 'armed-disarmed' strategy. For example, armed *S*-pyridinyl benzylated disaccharide **1.11** and disarmed per-acetylated SPy acceptor **1.12** was reacted chemoselectively resulting in the formation of SPy trisaccharide **1.13**.[49]

Scheme 1.6. Armed-disarmed chemoselective synthesis of trisaccharide.[49]

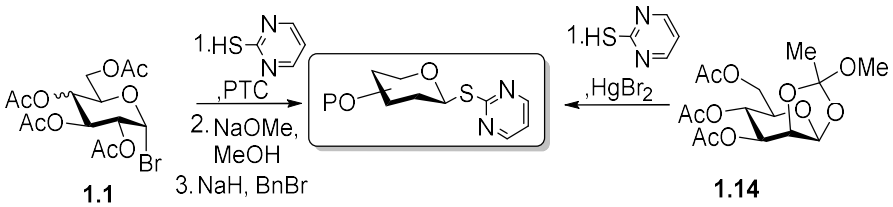


1.3. *S*-Pyrimidin-2-yl (SPyr) derivatives

Per benzylated S-pyrimidin-2-yl glucose was obtained from the reaction of corresponding 1-bromo per-acetylated glucose **1.1** and 2-mercaptopyrimidine under phase

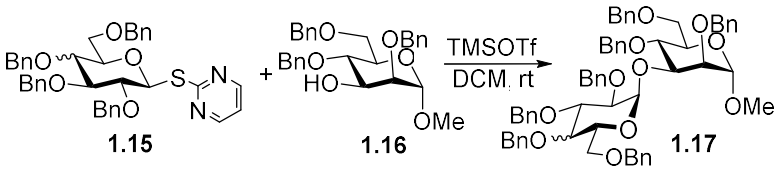
transfer conditions followed by deacetylation with NaOMe/MeOH and benzylation with NaH, BnBr in dry DMF (Scheme 1.7).[50] Similar protocol was followed for the synthesis of per-benzylated galactosyl pyrimidinyl donor. Per-acetylated SPyr glucosyl donor was synthesized by refluxing the reaction of 1,2-O-methyoxyethylidene-β-mannoside **1.14** with pyrimidinthiol in presence of $HgBr_2$.[51] Similar protocol was also followed for the synthesis of per-benzylated mannosyl SPyr donor.[51]

Scheme 1.7. Synthesis of the S-pyrimidin-2-yl glycosides



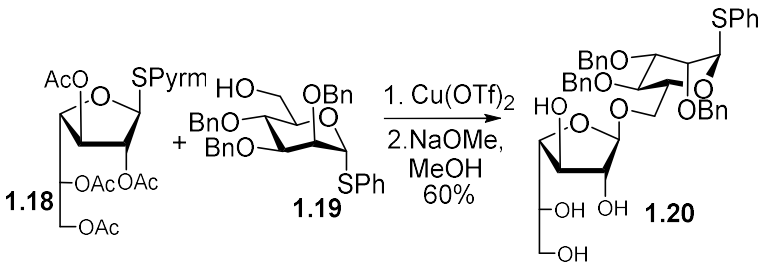
Promoter systems such as TMSOTf or AgOTf successfully activated SPyr leaving group in a typical glycosylation reaction. For example, reaction of per-benzylated SPyr donor **1.15** with acceptor **1.16** in presence of TMSOTf afforded disaccharide **1.17** in a stereoselective manner (Scheme 1.8).[50]

Scheme 1.8. Synthesis of disaccharides.



S-Pyrimidinyl donor could be selectively activated over S-phenyl leaving group. As such per-acetylated SPyr galactofuranosyl donor **1.18** was selectively activated over SPh glucosyl acceptor **1.19** in the presence of $Cu(OTf)_2$ followed by deacetylation to afford disaccharide **1.20** in a 60% yield (Scheme 1.9).

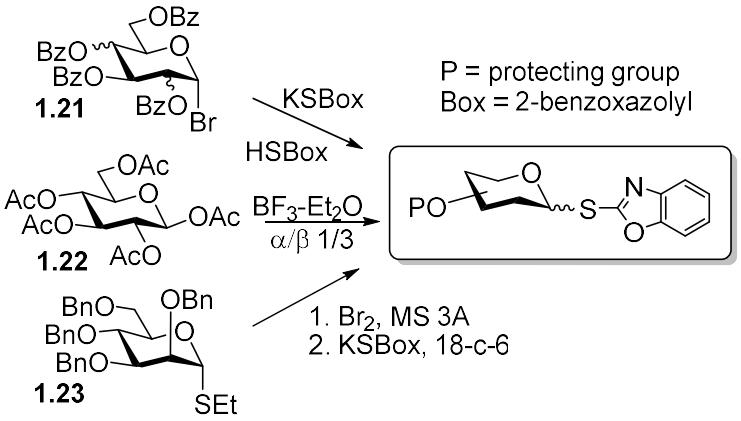
Scheme 1.9. Orthogonal activation of SPyr and SPh leaving groups.[52]



1.4. *S*-Benzoxazolyl (SBox) derivatives

Per-benzoylated SBox glucosyl donor was synthesized from the reaction of per-benzoylated glucosyl bromide **1.21** with KSBox and 18-crown-6 or HSBox and K_2CO_3 in dry acetone. Later, the protocol was also applied to D-galacto and D manno sugars (scheme 1.10).[33,53] Reaction of per-acetylated glucose **1.22** with HSBox in presence of BF_3-Et_2O also produces SBox glycosides (α/β = 1/3).[53] In addition, per benzylated thioglycoside **1.23** on reaction with Br_2 and then KSBox in the presence of 18-crown-6 also gave SBox glycoside.[54]

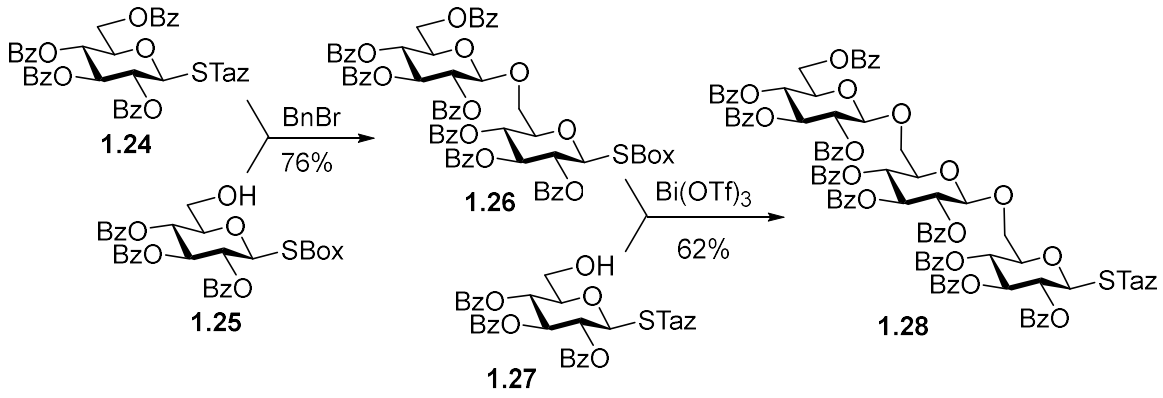
Scheme 1.10. Synthesis of the SBox glycosides.



Different promoter systems including AgOTf, $Cu(OTf)_2$, $Bi(OTf)_3$, $AgBF_4$, NIS/TfOH, TMSOTf, and MeOTf were capable of activating novel SBox glycosides efficiently in a typical glycosylation reaction. Application wise, SBox glycosides showed promising orthogonality

over other leaving groups like glycosyl alkoxyimidates[55] and STaz glycosides.[38] For example, sequential orthogonal activation of STaz building blocks **1.24** and SBox building block **1.25** afforded trisaccharide **1.26** as depicted in Scheme 1.11. Thus, in the presence of alkylating reagent BnBr, donor **1.24** was activated to produce SBox disaccharide **1.26** in 76% yield. The latter was then activated over STaz acceptor **1.27** in the presence of $Bi(OTf)_3$ to afford the trisaccharide **1.28** in 62% yield.

Scheme 1.11. Orthogonal activation of STaz and SBox leaving groups[38]

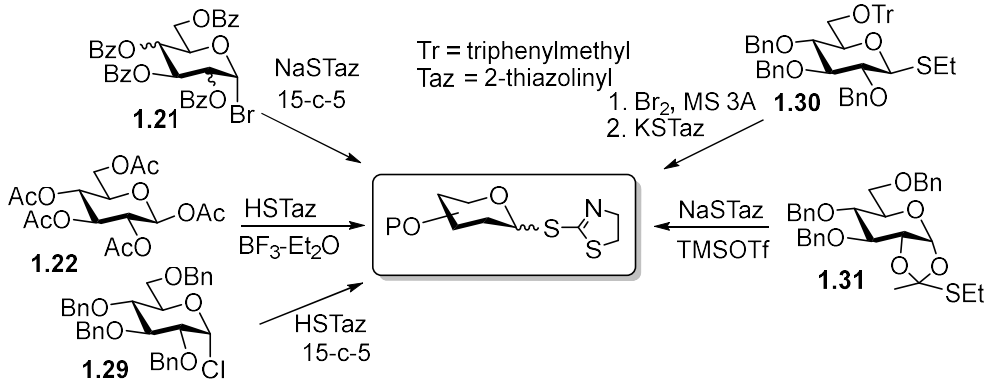


1.5. *S*-Thiazolinyl (STaz) derivatives

Per-benzoylated STaz glucosides were synthesized from the corresponding glucosyl bromides **1.21** with NaSTaz or KSTaz in the presence of a crown ether in dry acetone or acetonitrile.[32] Similarly, the protocol was extended to D-galacto, and D-manno series. Alternatively, STaz glycosides were also obtained directly from per-acetate **1.22** and HSTaz in the presence of BF_3-Et_2O.[32] Relatively high basicity of HSTaz (pK_a = 13.01) and the presence of a reactive nitrogen atom was often associated with the production of the *N*-linked glycoside and 1,2-dehydro derivatives as side products. As a result, anomeric chloride **1.29**,[32]

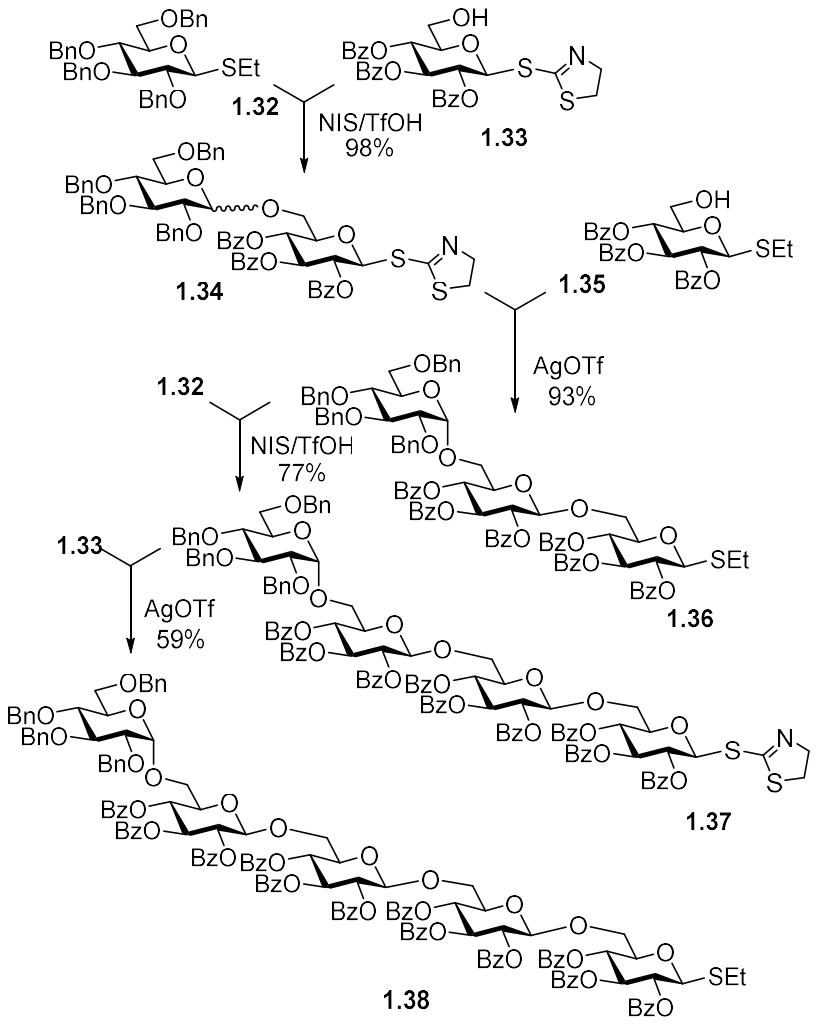
thioglycoside **1.30**,[56] and 1,2-orthoester **1.31** have also been investigated as a starting materials for the synthesis of STaz glycosides (Scheme 1.12).[57]

Scheme 1.12. Synthesis of STaz glycosides.



Exploration of STaz glycosides in glycosylation demonstrated that various promoters including AgOTf, MeOTf, NIS/TfOH (stoichiometric), benzyl bromide, methyl iodide, $AgBF_4$ and $Cu(OTf)_2$ are able to activate the STaz LG smoothly in a typical glycosylation.[32] Inertness of the STaz leaving group towards NIS and *catalytic* amount of TfOH, a typical promoter system for the activation of S-alkyl/aryl glycosides, led to the study of selective activation of STaz over ethylthio glycosides. As such, orthogonal reaction was carried for the synthesis of pentasaccharide **1.37** using AgOTf for the activation of STaz and NIS and catalytic TfOH for the activation of SEt (Scheme 1.5).[32,58] Accordingly, ethylthio glycoside **1.32** was activated over STaz glycoside **1.33** with NIS and catalytic TfOH to give the disaccharide **1.34** in 98% yield. The resulting disaccharide with anomeric STaz leaving group was then selectively activated over ethythio glycosyl acceptor **1.35** with AgOTf to give the trisaccharide **1.36** in 93% yield. The latter was then activated over glycoside **1.32** with NIS and catalytic TfOH to afford tetrasaccharide **1.37** in 77% yield. Finally, the STaz LG in tetrasaccharide **1.37** was activated over thioglycoside **1.33** with AgOTf to give pentasaccharide **1.38** in 59% yield.

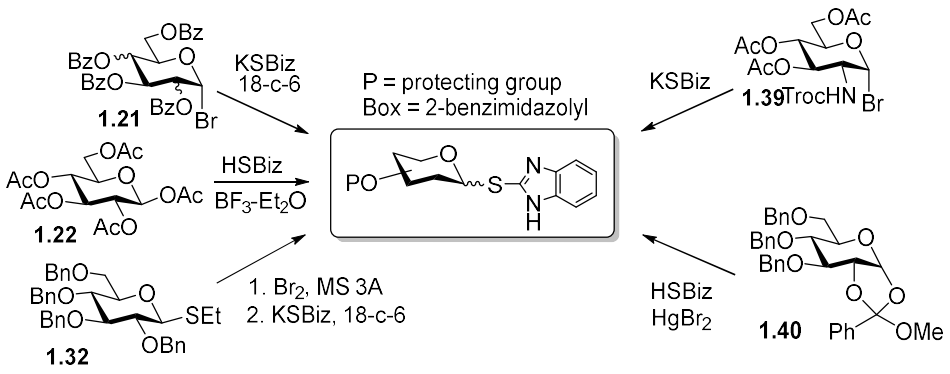
Scheme 1.13. Orthogonality of the STaz and SEt glycosides[58]



1.6. *S*-Benzimidazolyl (SBiz) derivatives

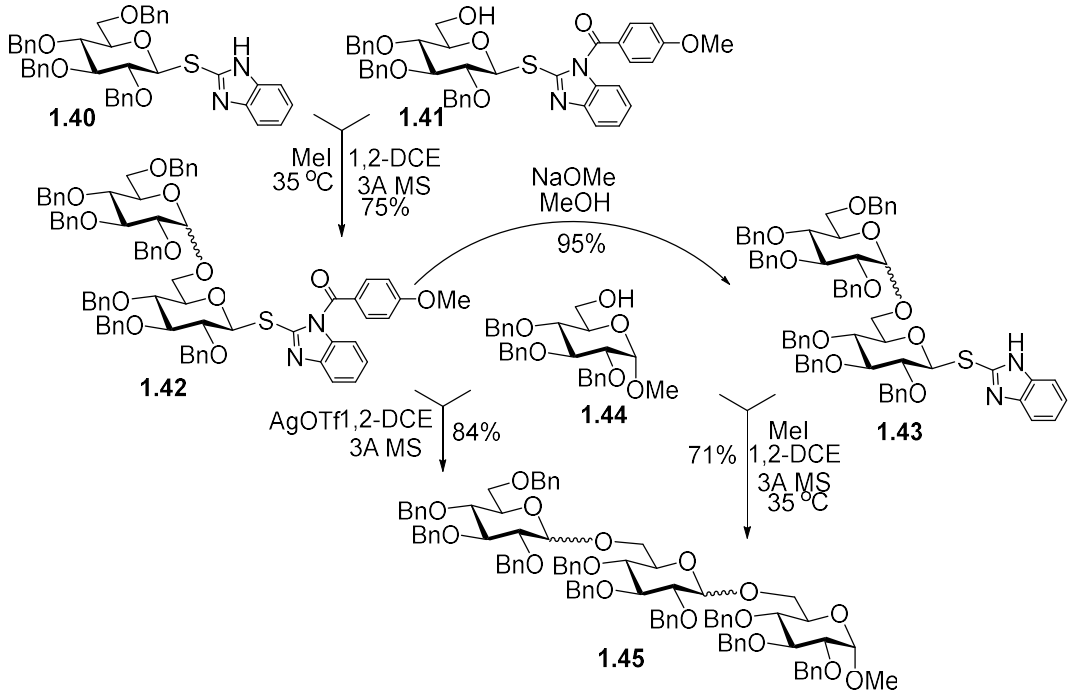
Per-benzoylated glycosyl SBiz donor was synthesized from the reaction of anomeric glycosyl bromide and KSBiz in presence of crown ether in dry acetonitrile and was applied for D-gluco, D-galacto and 2-deoxy-2-amino-D-gluco series.[59] Anomeric acetate **1.22**, thioglycoside **1.32**, and 1,2-orthoester **1.40** (Scheme 1.14) were also used as the starting material for the synthesis of SBiz glycosides.[59]

Scheme 1.14. Synthesis of SBiz glycosides



Next exploration of glycosylation showed SBiz leaving group was able to activate efficiently by promoter system such as AgOTf, $Cu(OTf)_2$, DMTST, MeI, AllBr, or BnBr. Interestingly, *N*-anisoylation of SBiz afforded *N*-anisoylated SBiz (SBizAn) that rendered unactive in presence of methyl iodide in glycosylation reaction. As a result, it was successfully used in active-latent-like glycosylations for oligosaccharide synthesis (Scheme 1.15), a strategy innovated by Roy et al.[60]

Scheme 1.15. Selective activation of the SBiz over SBizAn moieties.



Hence, in a typical glycosylation, SBiz glycoside become an active donor while SBizAn glycoside-a latent donor. As such, active glycosyl SBiz donor **1.40** and latent SBizAn acceptor **1.41** were coupled in presence of methyl iodide to afford disaccharide **1.42** in 75% yield. Latent SBizAn disaccharide was turned into active donor **1.42** by deanisoylation with NaOMe/MeOH. Finally, separate glycosylation of donor **1.42** and **1.43** with acceptor **1.44** in presence of AgOTf and MeI respectively afforded trisaccharide **1.45** in 84% and 71% yield. This approach was then extended to orthogonal activation and to studying N-alkylated SBiz imidates.[61,62]

1.7. Conclusions

Glycosyl thioimidates have become an essential part in modern day oligosaccharide synthesis. [63,64] Thioimidates can be prepared from a broad range of precursors. The activation of thioimidates for glycosylation is typically a high-yielding process, particularly with SBox imidates. Due to the polyfunctional character of the thioimidoyl LG, the activation profile is very versatile, and can be tuned as needed. The limited stability of glycosyl thioimidates toward some harsh reaction conditions limits their use. Therefore, the development of new classes of leaving groups with unique activation profile and stabilities close to conventional thioglycosides should enhance our synthetic capabilities. The subsequent Chapters detail the development of such leaving groups and their application in glycosylation.

1.8. References

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CHAPTER 2

Indolylthio glycosides as effective building blocks for chemical glycosylation

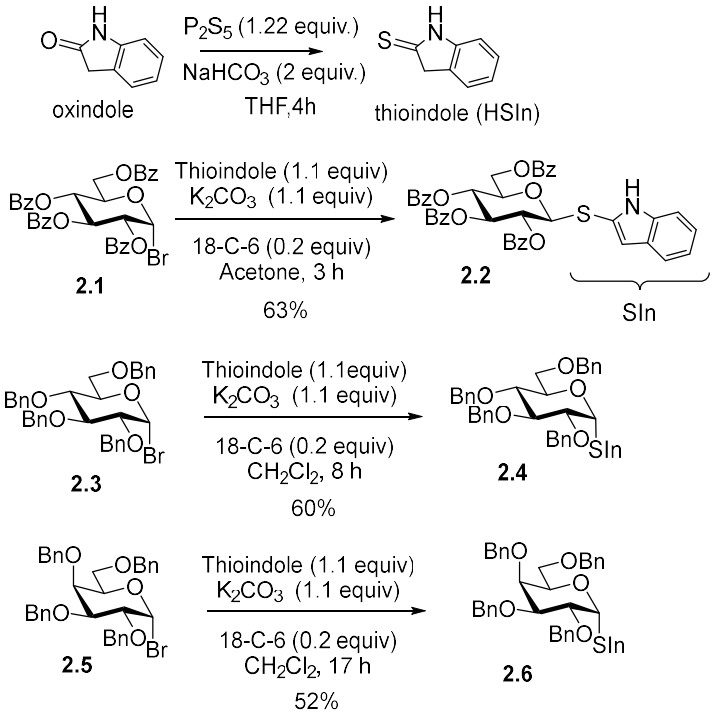
2.1. Introduction

Chemical synthesis of glycan sequences of even moderate complexity still remains a considerable challenge. As such, the development of efficient strategies for the oligosaccharide and glycoconjugate synthesis has been a demanding area of research effort worldwide.[35,65] A vast majority of glycan syntheses is nowadays accomplished using thioglycosides[26,41,66,67] and *O*-trichloroacetimidates (TCAI).[25,68,69] As a part of the ongoing research effort in our laboratory to develop versatile methods for chemical glycosylation and expeditious oligosaccharide synthesis, we became interested in glycosyl thioimidates, glycosyl donors equipped with $SCR_1=NR_2$ leaving group.[30] Among a variety of thioimidates studied by us and others, *S*-benzoxazolyl (SBox),[33] *S*-thiazolinyl (STaz),[32] and *S*-benzimidazolyl (SBiz)[59] moieties were found to be excellent building blocks for oligosaccharide synthesis. We also determined that thioimidates fit into practically all existing expeditious strategies for oligosaccharide synthesis due to the unique reaction conditions associated with their activation.[39] In addition, the glycosyl thioimidates led us to the development of conceptually new strategies for oligosaccharide synthesis: the inverse armed-disarmed strategy,[57] temporary deactivation concept,[56] O-2/O-5 cooperative effect,[37] coordination-assisted glycosylation,[70] and Surface-Tethered Iterative Carbohydrate Synthesis (STICS).[36] At the core of the study presented herein is the development of a new method for chemical glycosylation and expeditious oligosaccharide synthesis based on (1*H*-indol-2-yl)thio (*S*-indolyl, SIn) glycosides. These compounds are similar to thioimidates developed previously, but the study reported herein unexpectedly revealed that SIn glycosyl donors act neither as thioimidates nor as conventional alkyl/aryl thioglycosides in glycosylation.

2.2. Results and Discussion

The thioindole (HSIn) aglycone was obtained from commercially available oxindole (2-indolone) by means of thionation with P_2S_5 in tetrahydrofuran (THF).[71] HSIn was then reacted with a variety of bromide precursors in the presence of potassium carbonate and 18-crown-6 to afford the respective SIn glycosides. Thus, as depicted in Scheme 2.1, per-benzoylated glucosyl bromide **2.1**[72] was converted into SIn glycoside **2.2** in 63% yield. Per-benzylated glucosyl **2.3** and galactosyl bromide **2.5**, freshly prepared from the corresponding ethyl thioglycosides,[73-76] were converted into SIn glycosides **2.4** and **2.6** in 60 and 52% yield, respectively. We acknowledge that these yields are far from excellent, but would like to note that the yields listed herein are after crystallization.

Scheme 2.1. Synthesis of SIn glycosyl donors 2.2, 2.4 and 2.6

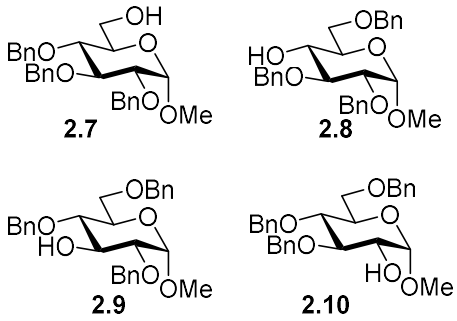


For X-ray structure determination of glucose derivative **2.2** and galactose derivative **2.6**, crystals of appropriate dimension were obtained by slow evaporation of ethyl acetate/hexane

solutions. Refer to the experimental section and the appendix for complete details of X-ray structure determination of compounds **2.2** and **2.6.**

With novel SIn glycosides **2.2, 2.4** and **2.6** in hand, we endeavored for investigation of their properties as glycosyl donors. To study the preliminary scope of this approach, we chose model glycosyl acceptors **2.7-2.10**[55] depicted in Figure 2.1. As possible activators, we decided to rely on the reaction conditions that worked well with thioimidates (AgOTf or $AgBF_4$), and thioglycosides (NIS/TfOH). To our great surprise, no activation took place when we attempted to react SIn donor **2.2** with reactive primary acceptor **2.7** in the presence of AgOTf at rt. Even large excess of 3.0 equiv and prolonged reaction time (24 h) produced no desired disaccharide **2.11**[77] (Table 2.1, entry 1). For comparison, the SBox LG could be smoothly and rapidly activated with 2.0 equiv of AgOTf.[33]

Figure 2.1. Standard glycosyl acceptors used in this study



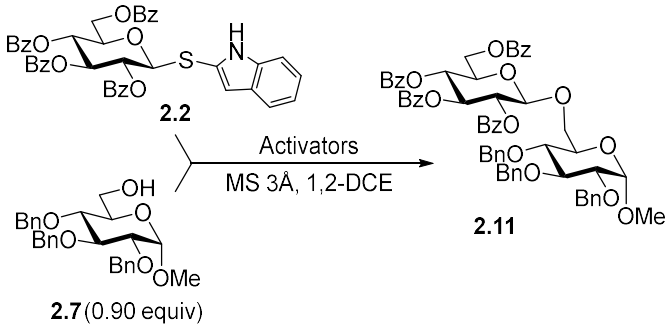
A drastic change was noted when $AgBF_4$, another effective activator for thioimidates,[78] was employed as the promoter for SIn donor **2.2**. Although this reaction required 5.0 equiv of $AgBF_4$, it could be smoothly driven to a completion in 5 h. As a result, disaccharide **2.11** was produced in a good yield of 85% (entry 2). In our prior experience, silver triflate and tetrafluoroborate act similarly,[78] and to explain this discrepancy in application to the SIn glycosides we came up with the following hypothesis. We thought that this could be due to

SIn glycosides being particularly sensitive to the marginal difference in the availability of Ag^+ ions between these two promoters. Therefore, we look at alternative sources of silver, and silver salt/Lewis acid cooperative promoter system recently developed by us for the activation of bromides[79,80] and chlorides[81] appealed to us as a possible solution. However, when donor **2.2** was coupled with standard acceptor **2.7** in the presence of Ag_2CO_3 (1.0 equiv) and TMSOTf (2.0 equiv) disaccharide **2.11** was produced only in a poor yield of 20% in 19 h (entry 3). The reaction was significantly accelerated in the presence of Ag_2CO_3 and a larger excess of TMSOTf (4.0 equiv), and disaccharide **2.11** was obtained in 87% yield in 2 h (entry 4). Being generally encouraged by this outcome, albeit realizing that large excess of TMSOTf is undesirable, we continued the search of alternative promoter systems.

Glycosidation of SIn donor **2.2** with acceptor **2.7** in presence of 2.0 equiv of NIS and 0.2 equiv of TfOH, a common promoter system used for the activation of alkyl/aryl thioglycosides,[82] in 1,2-dichloroethane (1,2-DCE) afforded disaccharide **2.11** only in a very modest yield of 36% (entry 5). Moreover, the reaction was very sluggish, appeared as something is hindering the activation of the SIn leaving group, and required 24 h to get to this stage. Increasing the amount of promoters, NIS to 4.0 equiv and TfOH to 0.8 equiv, again appealed as a possible solution. Under these reaction conditions, disaccharide **2.11** was obtained rather swiftly, in 1 h, in a significantly improved, albeit still unremarkable yield of 60% (entry 6). Further increase in the amount of TfOH to 1.0 equiv turned out to be the most advantageous for this promoter system. As shown in entry 7, disaccharide **2.11** was obtained in 72% yield. In a further search of the promoters, we replaced TfOH with TMSOTf. While somewhat similar, this modified promoter system allowed to achieve disaccharide **2.11** in 77% yield in 1.5 h. A gradual increase of the

amounts of promoters (entries 8-10) showed that 4.5 equiv of NIS and 1.0 equiv of TMSOTf are required to achieve this result.

Table 2.1. Optimization of glycosidation of SIn donor 2.2 with 6-OH acceptor 2.7

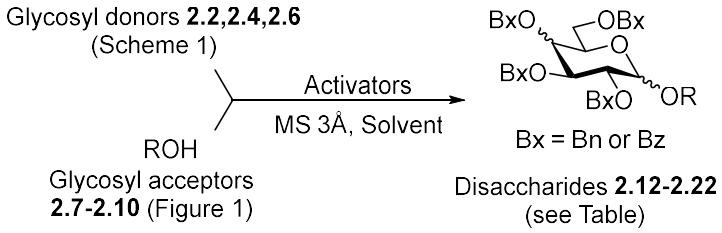


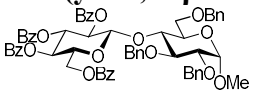
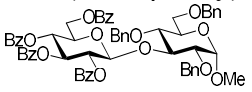
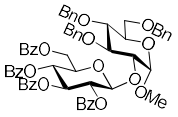
Entry	Conditions	Yield of 2.11
1	AgOTf (3.0 equiv), rt, 24 h	NR
2	$AgBF_4$ (5.0 equiv), rt, 5 h	85%
3	Ag_2CO_3 (1.0 equiv), TMSOTf (2.0 equiv), rt, 19 h	20%
4	Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), rt, 2 h	87%
5	NIS (2.0 equiv), TfOH (0.2 equiv), 0 °C-rt, 24 h	36%
6	NIS (4.0 equiv), TfOH (0.8 equiv), rt, 1 h	60%
7	NIS (4.0 equiv), TfOH (1.0 equiv), rt, 1 h	72%
8	NIS (2.0 equiv), TMSOTf (0.2 equiv), 0 °C-rt, 24 h	NR
9	NIS (4.0 equiv), TMSOTf (1.0 equiv), rt, 16 h	57%
10	NIS (4.5 equiv), TMSOTf (1.0 equiv), rt, 1.5 h	77%

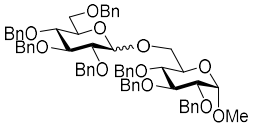
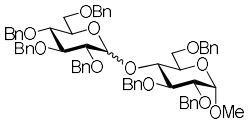
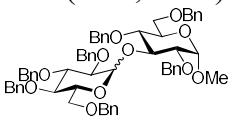
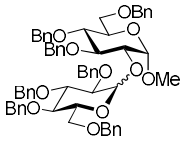
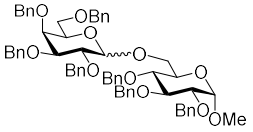
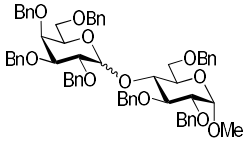
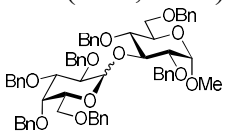
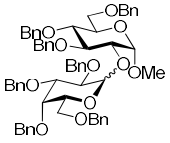
To expand the scope of this reaction, we extended our study to secondary glycosyl acceptors **2.8-2.10** (Figure 2.1). Glycosylation reaction of SIn glucosyl donor **2.2** with the secondary glycosyl acceptors **2.8-2.10** in presence of Ag_2CO_3 (1.0 equiv) and TMSOTf (4.0 equiv),

conditions that worked best for the primary acceptor **2.7** (see Table 2.1) were also successful herein. As shown in Table 2.2, the corresponding disaccharides **2.12**,[77] **2.13**,[55] and **2.14**[58] were obtained in commendable yields ranging between 69-79% (entries 1-3). Subsequently, we extended our study to glycosylation of per-benzylated donors **2.4** and **2.6** with primary and secondary acceptors **2.7-2.10**. This study included both silver-and iodonium-mediated pathways, and the reaction conditions were adjusted to account for differences in reactivity among the donor and acceptor combinations. Surprisingly, only slightly reduced amounts of promoters were permitted for the activation of supposedly much more reactive (armed) per-benzylated glycosyl donors.[83] Selected key results of this study are summarized in Table 2.2.

Table 2.2. Expanding the scope of glycosylation with SIn donors: synthesis of disaccharides 2.12-2.22



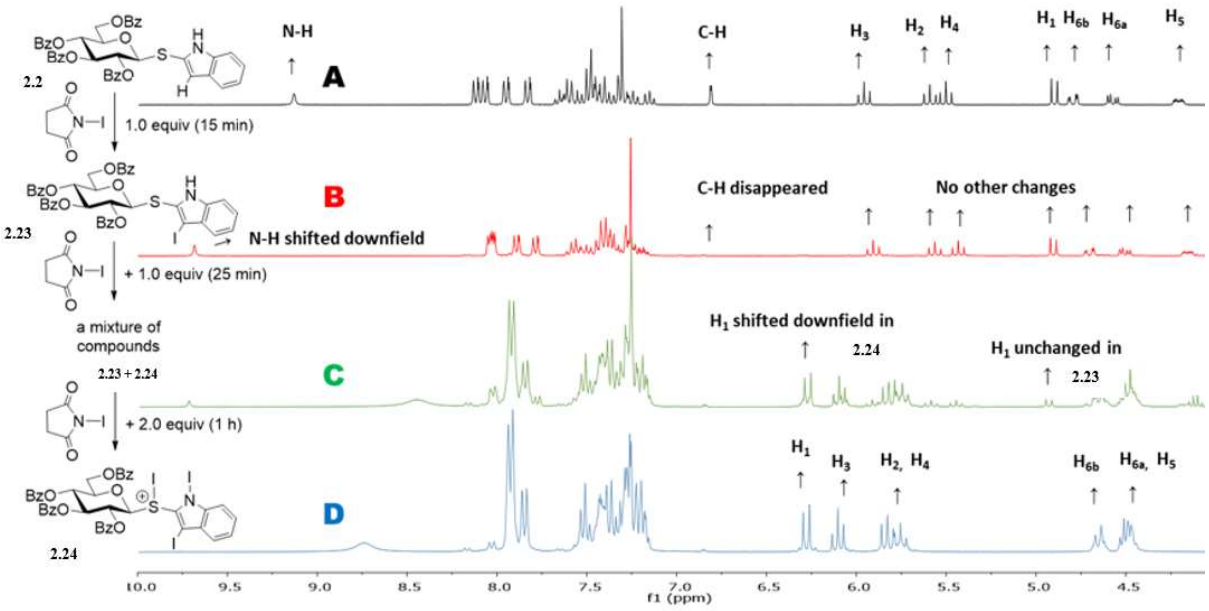
Entry	Donor + Acceptor, conditions	Product (yield, α/β ratio)
1	**2.2** + **2.8**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), 0 °C-rt, 20 h, 1,2-DCE	 **2.12** (69%, β only)
2	**2.2** + **2.9**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), 0 °C-rt, 4 h, 1,2-DCE	 **2.13** (78%, β only)
3	**2.2** + **2.10**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), 0 °C-rt, 4 h, 1,2-DCE	 **2.14** (79%, β only)

4	**2.4** + **2.7**, Ag_2CO_3 (1.0 equiv), TMSOTf (3.0 equiv), 0 °C-rt, 2 h, DCM	
5	**2.4** + **2.7**, NIS (4.0 equiv), TfOH (1.0 equiv), rt, 0.5 h, DCM	**2.15** (91%, 1.1/1) **2.15** (76%, 1/1.2)
6	**2.4** + **2.8**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 3 h, DCM	
7	**2.4** + **2.9**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 3 h, DCM	**2.16** (68%, 1/1.1) 
8	**2.4** + **2.10**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 3 h, DCM	**2.17** (70%, 1.4/1) 
9	**2.6** + **2.7**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), rt, 0.5 h, DCM	**2.18** (81%, 1.2/1) 
10	**2.6** + **2.7**, Ag_2CO_3 (1.0 equiv), TMSOTf (3.0 equiv), 0 °C-rt, 3 h, DCM	**2.19** (70%, 1/1.3) **2.19** (80%, 1/1.2)
11	**2.6** + **2.8**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 2 h, DCM	
12	**2.6** + **2.9**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 2 h, DCM	**2.20** (54%, 6.0/1) 
13	**2.6** + **2.10**, Ag_2CO_3 (1.0 equiv), TMSOTf (4.0 equiv), -70 °C-rt, 2 h, DCM	**2.21** (62%, 1.7/1)  **2.22** (75%, 2.0/1)

Being marginally more reactive than its per-benzoylated counterpart, benzylated donor **2.4** could be smoothly activated for the reaction with the primary acceptor **2.7** with Ag_2CO_3 (1.0 equiv) and the reduced amount of TMSOTf (3.0 equiv). As a result, disaccharide **2.15**[84] was obtained in 91% in 2 h (α/β = 1.1/1, entry 4). This series of experiments was performed in dichloromethane as the reaction solvent that would allow to start reactions at a low temperature (*vide infra*). Benzylated donor **2.4** could also be activated with NIS (4.0 equiv) and TfOH (1.0 equiv), essentially the same conditions used for the activation of the per-benzoylated SIn donor. As a result, disaccharide **2.15** was obtained in 76% yield in 30 min (α/β = 1/1.2, entry 5). We then investigated glycosidation of donor **2.4** with secondary glycosyl acceptors **2.8-2.10** in the presence of Ag_2CO_3 and TMSOTf. These reactions were slower, and to achieve faster reactions, we increased the amount of TMSOTf back to 4.0 equiv. To achieve higher yields, these reactions were started at -70 ºC, and then allowed to slowly warm to rt. This series of experiments produced the corresponding disaccharides **2.16**,[85] **2.17**,[86] and **2.18**[87] in good yields of 68-81% albeit unremarkable stereoselectivity (α/β from 1.4/1 to 1/1.1, entries 6-8). After that, we investigated glycosylations with SIn galactosyl donor **2.6** with the same series of glycosyl acceptors **2.7-2.10**. Glycosylation reaction of donor **2.6** with primary acceptor **2.7** in the presence of Ag_2CO_3 (1.0 equiv) and TMSOTf (4.0 equiv) afforded disaccharide **2.19**[88] in 70% yield in 30 min (α/β = 1/1.3, entry 9). Decreasing the amount of TMSOTf to 3.0 equiv afforded disaccharide **2.19** in an increased yield of 80% was observed with practically no change in stereoselectivity (entry 10). This reaction, however, required 3 h to complete, and to achieve practical rates with the secondary acceptors we increased the amount to TMSOTf to 4.0 equiv. Like in case of glucosyl donor **2.4**, glycosylations of galactosyl donor **2.6** with the secondary glycosyl acceptors **2.8-2.10** were started at -70 ºC, and then allowed to slowly warm

to rt. This series of experiments produced the respective disaccharides **2.20**,[89] **2.21,**[90] and **2.22**[91] in yields ranging between 54-75% with preferential α-stereoselectivity (α/β = 1.7-6/1, entries 11-13).

Scheme 2.2. NMR experiments with SIn donor 2.2 in the presence of NIS (1-4 equiv) to observe the formation of 2.23 and 2.24



After completing these series of experiments, we were still puzzled by the fairly low reactivity of SIn glycosides that required greater excess reagents than even stable alkyl/aryl thioglycosides. The requirement for using excess reagent resulted in unusually prominent side reactions that accompanied many glycosylations. Side products observed included silylated acceptors, hemiacetals, 1→1-linked disaccharides, and 1,6-anhydro sugars. To gain a better understanding of this glycosylation reaction and uncover its mechanism we turned our attention to performing a set of experiments monitored by NMR. First, we investigated the activation with NIS. For this purpose, SIn donor **2.2** was dissolved in $CDCl_3$, the solution was transferred into a standard 5 mm NMR tube, and ^1H NMR spectrum was recorded (Scheme

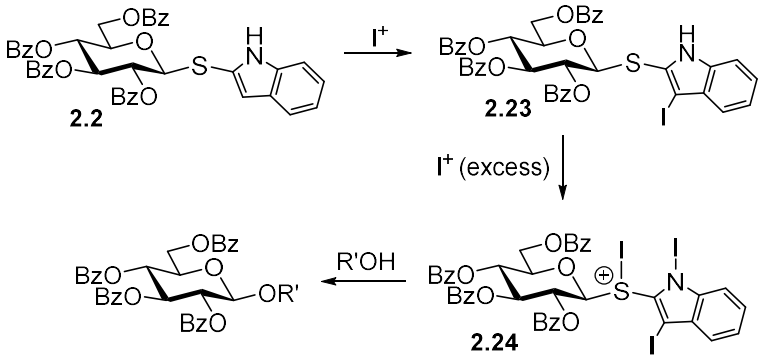
2.2A). NIS (1.0 equiv) was added and ^{1}H NMR spectrum was recorded in 15 min showing the formation of **2.23** as the only product (Scheme 2.2B).

While somewhat unanticipated, the formation of such intermediate is not surprising since the 3-position of the indole ring system is strongly nucleophilic and known to react easily with electrophiles of different kinds.[92,93] Compound **2.23** was sufficiently stable to be isolated through column chromatography and fully characterized (see the appendix section for details). The NH signal in **2.23** was noted to shift downfield, and the identity of this proton was confirmed by a separate deuterium exchange experiment that indicated the remanence of an exchangeable hydrogen on the N atom of **2.23** (see appendix for further details). Upon addition of the second equiv of NIS, ^{1}H NMR spectrum was recorded in 25 min showing the formation of a mixture of compounds (Scheme 2.2C). As suggested by the ^{1}H NMR spectrum, a partial conversion of compound **2.23** to a new compound, presumably **2.24**, took place. Only when total 4.0 equiv of NIS was added to the NMR tube, ^{1}H NMR spectrum recorded after 1 h showed the complete formation of intermediate **2.24** with no traces of **2.23** remaining (Scheme 2.2D). This explains the need for multiple equivalents of NIS to activate the SIn leaving group. The formation of the activated sulfonium intermediate **2.24** is supported by a $\delta\Delta$ 1.4 ppm downfield chemical shift of the H-1 signal, as indicated in Scheme 2.2C.

Based on the extended NMR monitoring our current understanding of the NIS-promoted reaction mechanism is as follows. Upon addition of 4.0 equiv of NIS and TfOH to the reaction mixture containing SIn donor **2.2** in 1,2-DCE the first equivalent of NIS gets consumed by the electrophilic addition of the iodonium ion at the remote C-3 position of the SIn aglycone to afford a stable intermediate **2.23** (Scheme 2.3). The presence of TfOH or TMSOTf) facilitates formation of the iodonium ion from NIS. The additional NIS is needed to convert **2.23** into the

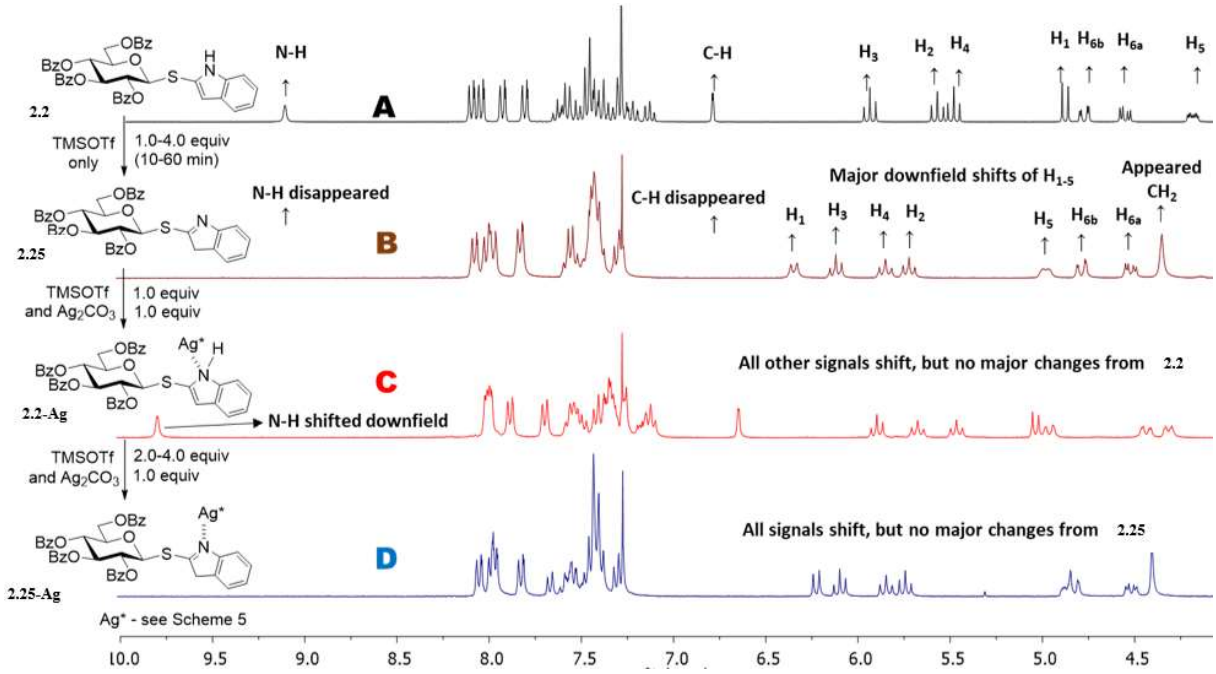
activated species **2.24** that results in the leaving group departure followed by glycosylation. While glycosidation of benzoylated SIn donor **2.2** proceeds via intermediacy of the acyloxonium intermediate leading to complete stereoselectivity, glycosidations of benzylated SIn donors **2.4** and **2.6** proceed via oxacarbenium ion intermediate. The formation of the later intermediate explains poor stereoselectivity of practically all uncontrolled glycosylations[10] taking place without the assistance of the neighboring group at C-2.

Scheme 2.3. Plausible mechanism of the NIS/TfOH-promoted reaction



To uncover the mechanism of glycosylation reactions proceeding under the cooperative silver salt-Lewis acid catalysis, we set up a series of experiments to monitor the interaction of SIn donor **2.2** with the promoter system by NMR. For this purpose, donor **2.2** was dissolved in $CDCl_3$, the solution was transferred into a standard 5 mm NMR tube, and ^1H NMR spectrum was recorded (Scheme 2.4A). TMSOTf (1.0 equiv) was added and ^1H NMR spectrum was recorded in 10 min showing the formation of thioimidate **2.25** (Scheme 2.4B). Formation of such a tautomer was somewhat unexpected. The same product was formed with other amounts of TMSOTf (up to 4.0 equiv). Thioimidate **2.25** was found to be stable in solution for at least one hour, it survives conventional aqueous work-up and evaporation. However, compound **2.25** tautomerizes back into SIn glycoside **2.2** during column chromatography on silica gel.

Scheme 2.4. NMR experiments with SIn donor 2.2 in the presence of TMSOTf and Ag_2CO_3 to study the mechanism



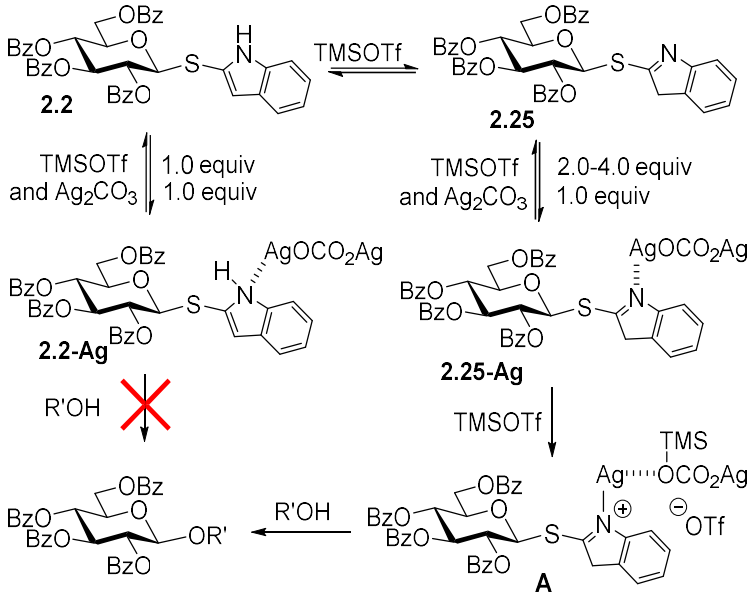
When Ag_2CO_3 (1.0 equiv) was added to the NMR tube containing **2.25** produced from **2.2** with 1 equiv of TMSOTf, compound **2.25** quickly tautomerizes back to compound **2.2**. Shown in Scheme 4C is a silver complex of **2.2** wherein the complexation has presumably occurred via the N-atom of indole. This mode of complexation is supported by a δΔ 0.7 ppm downfield chemical shift of the NH signal with practically no other changes observed in comparison to the uncomplexed **2.2** (Scheme 2.4A). The analogy is found in the mode of complexation taking place with other thioimidates. Thus, it was previously found that non-aromatic thioimidates, such as *S*-thiazolinyl (STaz),[32] complex silver and other metals via the nitrogen atom.[70,94] This mode of complexation is known to result in the remote activation with silver[95,96] or temporary deactivation with platinum or palladium salts.[56,97] Conversely, aromatic thioimidates such as *S*-benzoxazolyl (SBox)[33] and *S*-benzimidazolyl (SBiz)[61] complex silver strictly via the anomeric sulfur.[54,98] This differential activation, remote versus direct, allowed to devise a thioimidate-only orthogonal strategy for oligosaccharide synthesis.[38]

When Ag_2CO_3 (1.0 equiv) was added to the NMR tube containing **2.25** produced from **2.2** with 2.0 or 4.0 equiv of TMSOTf, imidate **2.25** remained intact. ^{1}H NMR spectrum recorded in 10 min showed the presence of compound **2.25** only, presumably as a complex with silver due to minor changes in chemical shifts observed (Scheme 2.4D). Since no glycosylation takes place until multiple equiv of TMSOTf are added, we believe that tautomerization of **2.2** to **2.25** needs to have occurred prior to glycosylation under these reaction conditions.

Based on extended NMR monitoring our current understanding of the reaction mechanism of glycosidation of **2.2** in the presence of TMSOTf and Ag_2CO_3 is as follows. In presence of excess of TMSOTf, glycosyl donor **2.2** is first tautomerized into its thioimidate counterpart **2.25**. We believe that the occurrence of this intermediate is the key for the successful activation to take place under these reaction conditions. While the formation of **2.2-Ag** complex in feasible, there is no feasible pathway for the SIn leaving group activation, neither remotely nor directly (Scheme 2.5). Silver carbonate then complexes with thioimidate **2.25** to produces complex **2.25-Ag** that is not yet sufficiently ionized to cause the leaving group departure. As proposed in our previous study of the cooperative silver/acid catalysis with glycosyl halides, after initial interaction of the donor with the silver salt, a strongly ionized species **A** are produced due to interaction with an acid. This intermediate will then cause the remote SIn leaving group departure. Depending on the protecting group at C-2, the subsequent glycosylation proceeds via an acyloxonium (SIn donor **2.2**) or oxacarbenium (SIn donors **2.4** and **2.6**) ion. Differently from our previous studies with glycosyl halides, wherein the glycosylations were driven by strong affinity of silver to halogens and the irreversible formation of the AgX bond, the activation of the SIn donors proceeding via the interaction of silver with the N-atom is significantly slower. It is also possible that silver carbonate will react

with excess TMSOTf first to generate a loosely bound silver cation species which are then attacked by the N-atom of thioimidate **2.25** to produce the ionized intermediate.

Scheme 2.5. Plausible mechanism of the Ag_2CO_3/TMSOTf-promoted reaction



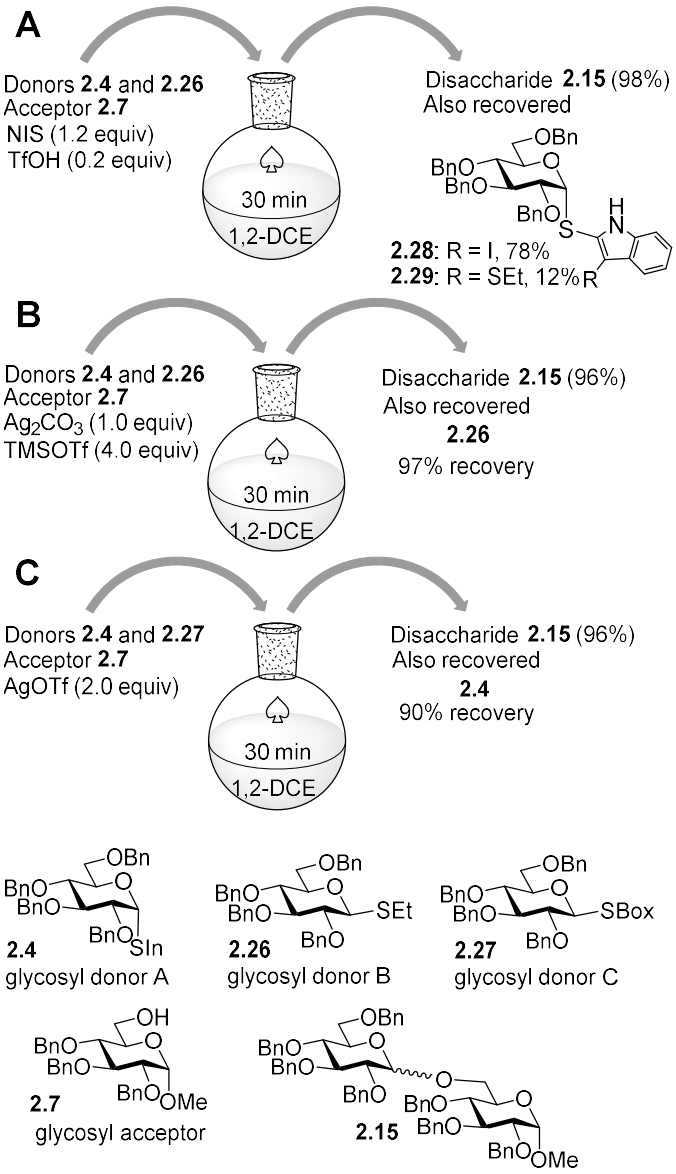
Given the specific set of reaction conditions needed for the activation of this new leaving group, the orthogonality of SIn donors in respect to other popular leaving groups, thioglycoside **2.26**[73] and thioimidate **2.27**,[37,54] was then investigated. For this purpose, we set up a series of comparative competition experiments wherein 1.0 equiv of SIn donor **2.4** was set to compete with 1.0 equiv other glycosyl donors for 2.0 equiv of acceptor **2.7** (Scheme 2.6). All reactions were stopped after 30 min, and all reaction components were isolated and characterized. First, we set up a comparative competition experiment wherein SIn donor **2.4** and ethylthio glycoside **2.26** were set to compete for acceptor **2.7** in the presence of NIS (1.2 equiv) and TfOH (0.2 equiv). These are typical reaction conditions for the activation of thioglycosides. Indeed, SEt donor **2.26** reacted entirely, whereas SIn donor **2.4** was recovered in the form of iodine and SEt adducts **2.28** and **2.29** that were isolated in the combined yield of 90% (Scheme 2.6A). This experiment ultimately confirmed slow and incomplete activation of the SIn glycosides

under the reaction conditions common for the activation of alkyl/aryl thioglycosides. Adduct **2.28** can be readily activated for subsequent glycosylation because it is an intermediate formed as the first step of the interaction of the SIn leaving group with the iodonium promoter. In the competition experiment it acts as a trap for excess I^+ remaining after the activation of the SEt leaving group. Upon this, adduct **2.29** was formed as a result of trapping SEt, and this compound can also be glycosidated under typical conditions for the SIn leaving group activation.

We then set up a comparative competition experiment wherein SIn donor **2.4** and ethylthio glycoside **2.26** were set to compete for acceptor **2.7** in the presence of Ag_2CO_3 and TMSOTf, newly developed reaction conditions for the activation of SIn glycosides. As a result, SIn donor **2.4** reacted completely, whereas SEt donor **2.26** was recovered nearly quantitatively (97% yield, Scheme 2.6B). This experiment ultimately confirmed that common thioglycosides cannot be activated under the reaction conditions that have been devised for the activation of SIn glycosides.

Finally, we set up a comparative competition experiment wherein SIn donor **2.4** and SBox imidate **2.27**[37,54] were set to compete for acceptor **2.7** in the presence of 2 equiv of AgOTf. These are common reaction conditions of the SBox leaving group activation[33,53] that were found practically ineffective with SIn glycosides, as determined over the course of the preliminary screening (*vide supra*, Table 2.2). As a result of this competition experiment, SBox donor **2.27** reacted completely, whereas SIn donor **2.4** was recovered in 90% yield (Scheme 2.6C). This series of experiments demonstrated that SIn glycosides possess different glycosyl donor properties than both thioglycosides and thioimidates to which they are structurally related to.

Scheme 2.6. Competition experiments of SIn glycoside 2.4 vs SEt and SBox donors 2.26 and 2.27



2.3. Conclusions

In summary, we developed a new class of glycosyl donors, indolylthio glycosides. This new glycosyl donor can be activated using a range of activators. Although the activation of the SIn leaving group requires excess reagents, their reactivity profile is interesting. The activation process was studied by NMR and the increased understanding of the mechanism led to a

discovery of orthogonality of the SIn leaving group versus thioglycosides and thioimidates. Further investigation of this leaving group in oligosaccharide synthesis, as well as structural and mechanistic studies are currently underway in our laboratory.

2.4. Experimental

2.4.1. *General methods.*

The reactions were performed using commercial reagents and the ACS grade solvents used for reactions were purified and dried in accordance with standard procedures. AgOTf was co-evaporated at least twice with dry toluene, dried in *vacuo* and stored under dark for better yield of glycosylated product. 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 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to 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 in $CDCl_3$ at 300 MHz, $^{13}C\{^1H\}$ NMR spectra were recorded at 75 MHz. The ^1H NMR chemical shifts are referenced to tetramethyl silane (TMS, $\delta_H = 0$ ppm) or $CDCl_3$ ($\delta_H = 7.26$ ppm) for ^1H 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 $CDCl_3$. Anomeric purity or anomeric ratios were accessed or calculated by integration of the relevant signals in their ^1H NMR spectra. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer.

1,3-Dihydro-2*H*-indole-2-thione (indoline-2-thione, HSIn) was obtained from oxindole and P_2S_5 following the previously described protocol[71] that was modified as follows. A mixture containing oxindole (500 mg, 3.76 mmol) and P_2S_5 (1.0 g, 4.6 mmol) in dry THF (25 mL) was stirred under argon for 10 min at rt. Sodium bicarbonate (631 mg, 7.5 mmol) was added portionwise, and the resulting mixture was stirred for 4 h at rt. After that, the volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (~200 mL) and washed with water (4 x 50 mL). The organic phase was separated, dried over dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dried in *vacuo* for 1 h. then dissolved in hot methanol (ca 50 °C). The resulting solution was allowed to slowly cool to rt to afford the title compound as a yellow crystalline solid in 64% yield. Analytical data for HSIn were in accordance with that previously reported.[71]

1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (2.2). HSIn (0.45 g, 3.34 mmol), anhydrous K_2CO_3 (0.46 g, 3.33 mmol) and 18-crown-6 (0.16 g, 0.61 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[72] (**2.1**, 2.0 g, 3.03 mmol) in dry acetone (50 mL), and the resulting mixture was stirred under argon at 50 °C for 3 h. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2 and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~150 mL), washed with 10% aq. $NaHCO_3$ (25 mL) and water (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) followed by crystallization from diethyl ether-hexanes to afford the title compound as colorless crystals in 63% yield (1.39 g, 1.904 mmol). Analytical data for **2.2:** R_f 0.60 (ethyl acetate/hexane, 3/7, v/v); m.p. 204-205 °C (diethyl ether-hexanes); $[\alpha]_D^{22}$ -

81.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 4.12-4.18 (m, 1H, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.6 Hz, H-5), 4.52 (dd, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.74 (dd, 1H, H-6b), 4.85 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 5.45 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 5.54 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 5.91 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 6.76 (s, 1H, =CH), 7.10-8.08 (m, 24H, aromatic), 9.08 (s, 1H, NH) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 62.3, 68.6, 70.6, 76.6, 76.9, 77.4, 84.1, 111.1, 112.3, 119.8, 120.6, 121.5, 123.0, 127.7, 128.2 (x2), 128.3 (x2), 128.4 (x2), 128.5 (x2), 128.6 (x2), 128.9, 129.3, 129.6 (x2), 129.7 (x2), 129.8 (x2), 129.9 (x2), 133.2, 133.3, 133.5, 137.9, 164.9, 165.2, 165.7, 166.6 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{42}H_{33}NO_9SNa]^+$ 750.1774; found: 750.1763.

1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (2.4). A mixture containing ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**2.26**,[73] 5.0 g, 8.55 mmol) and freshly activated molecular sieves (3 Å, 1.0 g) in CH_2Cl_2 (50 mL) was stirred under argon for 1 h at rt. Bromine (0.53 mL, 10.26 mmol) was added and the resulting mixture was stirred for 20 min at rt. After that, the volatiles were removed under reduced pressure, the residue was co-evaporated with dry toluene (2 x 20 mL), and dried in *vacuo* for 2 h. The resulting residue containing crude 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide **2.3** was dissolved in CH_2Cl_2 (60 mL), HSIn (1.4 g, 9.41 mmol), anhydrous K_2CO_3 (1.30 g, 9.41 mmol), and 18-crown-6 (0.45 g, 1.75 mmol) were added, and the resulting mixture was stirred under argon for 16 h at rt. The solids were filtered off through a pad of Celite and rinsed successively with CH_2Cl_2. The combined filtrate (~200 mL) as washed with water (50 mL), 10% aq. $NaHCO_3$ (250 mL), and water (2 x 50 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane 5% gradient elution) followed by crystallization from diethyl ether -hexanes to afford the title compound as colorless crystals in 60% yield (3.44 g, 9.27

mmol). Analytical data for **2.4**: R_f 0.55 (ethyl acetate/hexane, 1/4, v/v); m.p. 116-117 °C (diethyl ether-hexanes); $[\alpha]_D^{22}$ 159.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.29 (dd, 1H, $J_{6a,6b}$ = 10.2 Hz, H-6a), 3.48 (dd, 1H, $J_{4,5}$ = 8.8 Hz, H-4), 3.76-3.90 (m, 3H, H-2, 3, 6b), 4.44 (d, 1H, ^2J = 11.0 Hz, C*H*Ph), 4.56-4.82 (m, 7H, H-5, 3 x CH_2Ph, $J_{5,6a}$ = 8.3 Hz), 4.92 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.47 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1), 6.47-7.45 (m, 24H, aromatic), 6.60 (dd, 1H, =CH), 9.39 (s, 1H, NH) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 69.6, 70.7, 72.2, 73.6, 75.0, 75.8, 77.9, 79.2, 82.3, 86.9, 108.0, 110.6, 119.5, 119.7, 122.0, 126.4, 127.4, 127.6, 127.8, 127.9 (x5), 128.0 (x5), 128.3 (x2), 128.4 (x4), 128.6 (x2), 137.2, 137.3, 137.7, 137.8, 138.4 ppm; ESI-TOF $[M + H]^+$: calcd for $[C_{42}H_{42}NO_5S]^+$ 672.2784; found: 672.2792.

1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopyranoside (2.6). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[74] (5.0 g, 8.55 mmol) and freshly activated molecular sieves (3Å, 1.0 g) in CH_2Cl_2 (50 mL) were stirred under argon for 1 h at rt. Bromine (0.53 mL, 10.26 mmol) was added and the resulting mixture was stirred for 20 min at rt.[99] After that, the volatiles were removed under reduced pressure, the residue was co-evaporated with dry toluene (2 x 20 mL), and dried in *vacuo* for 2 h. The residue containing crude 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide **2.5** was dissolved in dichloromethane (60 mL), HSIn (1.40 g, 9.41 mmol), anhydrous K_2CO_3 (1.30 g, 9.41 mmol), and 18-crown-6 (0.45 g, 1.75 mmol) were added, and the resulting mixture was stirred under argon for 10 h at rt. The solids were filtered off through a pad of Celite and rinsed successively with CH_2Cl_2. The combined filtrate (~200 mL) as washed with water (50 mL), 10% aq. $NaHCO_3$ (250 mL), and water (2 x 50 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane 5% gradient elution) followed by crystallization from diethyl ether -hexanes to afford

the title compound as colorless crystals in 52% yield. Analytical data for **2.6**: R_f 0.40 (ethyl acetate/hexane, 1/4, v/v); m.p. 187-188 °C (diethyl ether-hexanes); $[\alpha]_D^{22}$ +108.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.24 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.80-3.90 (m, 3H, H-3, 4, 6b), 4.40 (dd, 1H, $J_{2,3}$ = 5.5 Hz, H-2), 4.32-4.96 (m, 9H, $J_{5,6a}$ = 2.3 Hz, H-5, 4 x CH_2Ph), 5.67 (d, 1H, $J_{1,2}$ = 5.5 Hz, H-1), 6.67-7.51 (m, 24H, aromatic), 6.67 (dd, 1H, =CH), 9.57 (s, 1H, NH) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 70.7, 72.5, 73.6, 73.7, 74.4, 74.8, 76.1, 79.2, 87.6, 108.1, 110.6, 119.4, 119.7, 121.9, 126.7, 127.4, 127.6 (x2), 127.7, 127.8 (x2), 127.9 (x5), 128.0, 128.3 (x4), 128.4 (x4), 128.7 (x2), 137.1, 137.8, 137.9 (x2), 138.4 ppm; ESI-TOF [M + H]$^+$: calcd for $[C_{42}H_{42}NO_5S]^+$ 672.2784; found: 672.2797.

2.4.2. General glycosylation procedures

Method A: Glycosylation in the presence of Ag_2CO_3 and TMSOTf. A mixture of a glycosyl donor (0.041-0.044 mmol), a glycosyl acceptor (0.037-0.040 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE or DCM (1.0 mL) was stirred under argon for 1 h at rt. The mixture was then cooled to 0 or -70 °C (see Tables), Ag_2CO_3 (0.041-0.044 mmol) and TMSOTf (0.165-0.178 mmol) were added, and the resulting mixture was stirred under argon for 1 h. After that, the external cooling was removed, the reaction mixture was allowed to warm to rt, and stirring was continued for the time indicated in Tables. The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the

corresponding disaccharide. If necessary, further purification was accomplished by size-exclusion column chromatography on Sephadex LH-20.

Method B: Glycosylation in presence of NIS and TfOH or TMSOTf. A mixture of a glycosyl donor (0.041-0.0044 mmol), a glycosyl acceptor (0.037-0.040 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE or DCM (1.0 mL) was stirred under argon for 1 h at rt. After that, NIS (0.165-0.178 mmol) and TfOH (0.041-0.044mmol) or TMSOTf (0.041 mmol) were added, and the resulting mixture was stirred for the time and temperature indicated in Tables. The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL) and H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution). If necessary, further purification was accomplished by size-exclusion column chromatography on Sephadex LH-20.

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.11). The title compound was obtained from donor **2.2** and acceptor **2.7**[55] by general glycosylation procedure in 87% yield as a white amorphous solid. Analytical data for **2.11** was in accordance with that previously reported.[77]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (2.12). The title compound was obtained from donor **2.2** and acceptor **2.8**[55] by the general glycosylation procedure in 69% yield as a clear syrup. Analytical data for **2.12** was in accordance with that previously reported.[77]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.13). The title compound was obtained from donor **2.2** and acceptor **2.9**[55]

by the general glycosylation procedure in 78% yield as a clear syrup. Analytical data for **2.13** was in accordance with that previously reported.[55]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.14). The title compound was obtained from donor **2.2** and acceptor **2.10**[55] by the general glycosylation procedure in 79% yield as a clear syrup. Analytical data for **2.14** was in accordance with that previously reported.[58]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.15). The title compound was obtained from donor **2.4** and acceptor **2.7** by the general glycosylation procedures in 91% yield (α/β = 1.1/1) as a colorless foam. Analytical data for **2.15** was in accordance with that previously reported.[84]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (2.16). The title compound was obtained from donor **2.4** and acceptor **2.8** by the general glycosylation procedure in 68% yield of **16** (α/β = 1/1.1) as a clear syrup. Analytical data for **2.16** was in accordance with that previously reported.[85]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.17). The title compound was obtained from donor **2.4** and acceptor **2.9** by the general glycosylation procedure in 70% yield (α/β = 1.4/1) as a clear syrup. Analytical data for **2.17** was in accordance with that previously reported.[86]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.18). The title compound was obtained from donor **2.4** and acceptor **2.10** by the general glycosylation procedure in 81% yield (α/β = 1.2/1) as a colorless foam. Analytical data for **2.18** was in accordance with that previously reported.[87]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.19). The title compound was obtained from donor **2.6** and acceptor **2.7** by the general glycosylation procedure in 70% yield (α/β = 1/1.3) as a clear syrup. Analytical data for **2.19** was in accordance with that previously reported.[88]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (2.20). The title compound was obtained from donor **2.6** and acceptor **2.8** by the general glycosylation procedure in 54% yield (α/β = 6.0/1) as a clear syrup. Analytical data for **2.20** was in accordance with that previously reported.[89]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.21). The title compound was obtained from donor **2.6** and acceptor **2.9** by the general glycosylation procedure in 62% yield (α/β = 1.7/1) as a clear syrup. Analytical data for **2.21** was in accordance with that previously reported.[90]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (2.22). The title compound was obtained from donor **2.6** and acceptor **2.10** by the general glycosylation procedure in 75% yield (α/β =2.0/1) as a clear syrup. Analytical data for **2.22** was in accordance with that previously reported.[91]

2.4.3. ^{1}H NMR monitoring experiments

*SIn glycoside **2.2** in the presence of NIS.* A solution of donor **2.2** (0.04 mmol) and NIS (0.04-0.16 mmol) in $CDCl_3$ (1.0 mL) was stirred in a round bottom flask under argon for 5 min at rt. The resulting solution was quickly transferred into a standard 5 mm NMR tube and ^{1}H NMR

spectrum was recorded at 15, 25, and 60 min timepoints. The recorded spectra are presented in Scheme 2.2 and the appendix.

*SIn glycoside **2.2** in the presence of TMSOTf.* A solution of donor **2.2** (0.0206 mmol) and TMSOTf (0.0206-0.0824 mmol) in $CDCl_3$ (1.0 mL) was stirred in a round bottom flask under argon for 5 min at rt. The resulting solution was quickly transferred into a standard 5 mm NMR tube and ^{1}H NMR spectrum was recorded at 15, 25, and 45 min timepoints. The recorded spectra are presented in Scheme 2.4 and the appendix.

*SIn glycoside **2.2** in the presence of TMSOTf and Ag_2CO_3.* A solution of donor **2.2** (0.0206 mmol) and TMSOTf (0.0206-0.0824 mmol) in $CDCl_3$ (1.0 mL) was stirred in a round bottom flask under argon for 5 min at rt. After that, Ag_2CO_3 (0.0206 mmol) was added and stirring was continued for 5 min at rt. The solid was filtered off, the filtrate was transferred into a standard 5 mm NMR tube, and ^{1}H NMR spectrum was recorded at 0.16, 0.25, 0.41, 0.75, and 1 h timepoints. The recorded spectra are presented in Scheme 2.4 and the appendix

2.4.3.1. Deuterium exchange experiment

A mixture of donor **2.2** (30 mg, 0.041 mmol) and molecular sieves (80 mg) in 1,2-DCE (1.0 mL) was stirred for 1 h under argon at room temperature. After that, *N*-iodosuccinimide (8.9 mg, 0.041 mmol) was added and stirring was continued for 17 h. TLC was checked at intervals of 5 minutes, 1, 2 and 5 h, no noticeable change was seen. After that, the reaction mixture was filtered through a pad of Celite, diluted with DCM (30 mL), washed with 10% aq. $Na_2S_2O_3.5H_20$. (10 mL), and water (2x10 mL). The organic phase was separated dried over sodium sulfate, filtered, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane gradient elution). ^{1}H NMR showed that the

compound corresponds to **2.23** (Figure A1, Spectrum B). Mass spectroscopy also proved the compound corresponds to **2.23** (HR-FAB MS $[M+Na]^+$: calcd for $[C_{42}H_{32}INO_9S+Na]^+$ 876.0842; found: 876.0734.

Deuterium exchange experiment was conducted by adding 4.0 μL of D_2O to a sample of the anticipated compound **2.23** in $CDCl_3$, the ^{1}H-NMR spectra again supported that the isolated compound to be **2.23** (Figure A1, Spectrum C) where disappearance of the N-H signal at 9.69 ppm was observed. The data acquired from ^{1}H NMR spectroscopy and mass spectrometry confirmed the formation of compound **2.23**.

2.4.4. Competition experiments

Experiment A. A mixture of ethylthio glucoside **2.26** (26.1 mg, 0.044 mmol), SIn glucoside **2.4** (30 mg, 0.044 mmol), acceptor **2.7** (41.5 mg, 0.089 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE (1.0 mL) was stirred under argon for 1 h at rt. Triflic acid (8.0 μL, 0.00894 mmol) and NIS (12.1 mg, 0.0536 mmol) were added, and the resulting mixture was stirred under argon for 30 min at rt. The solid was then filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to produce **2.15** in 98% yield. Also recovered were compounds **2.28** (HRMS $[M+Na]^+$: calcd for $[C_{44}H_{40}INO_5SNa]^+$ 820.1570, found: 820.1596) and **2.29** (HRMS $[M+Na]^+$: calcd for. $[C_{44}H_{45}NO_5S_2Na]^+$ 754.2637, found: 754.2645).

Experiment B. A mixture of ethylthio glucoside **2.26** (26.1 mg, 0.044 mmol), SIn glucoside **2.4** (30 mg, 0.044 mmol), glucosyl acceptor **2.7** (41.5 mg, 0.089 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE (1.0 mL) was stirred under argon for 1 h at room temperature. Ag_2CO_3 (12.3 mg, 0.044 mmol) was added and the resulting mixture was stirred for 5 min at rt. After that, TMSOTf (32.4 μL, 0.178 mmol) was added, and the resulting mixture was stirred under argon for 30 min at rt. The reaction was then quenched with triethyl amine (one drop). The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~ 30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to produce **2.15** in 96% yield. Also recovered was thioglycoside **2.26** in 97% yield.

Experiment C. A mixture of *S*-benzoxazolyl glucoside **2.27**[37,54] (30.1 mg, 0.045 mmol), SIn glucoside **2.4** (30 mg, 0.045 mmol) glucosyl acceptor **2.7** (41.5 mg, 0.089 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE (1.0 mL) was stirred under argon for 1 h at rt. After that, freshly activated AgOTf (10.7 mg, 0.089 mmol) was added, and the resulting mixture was stirred under argon for 20 min at rt. The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~ 30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane gradient elution) to produce **2.15** in 96% yield. Also recovered was SIn derivative **2.4** in 90% yield.

2.4.5. X-ray crystal structure determination for compounds 2.2 and 2.6

The obtained crystals were mounted on a MiTeGen cryoloop in random orientations. For compound **2.2**, preliminary examination and data collection were performed using a Bruker X8 Kappa Apex II Charge Coupled Device (CCD) Detector system single crystal X-Ray diffractometer equipped with an Oxford Cryostream LT device. All data were collected using graphite monochromated Mo Kα radiation (λ = 0.71073 Å) from a fine focus sealed tube X-Ray source. Preliminary unit cell constants were determined with a set of 36 narrow frame scans. Typical data sets consist of combinations of ϖ and ϕ scan frames with typical scan width of 0.5° and counting time of 10 seconds/frame at a crystal to detector distance of 4.0 cm.

For compound **2.6**, preliminary examination and data collection were performed using a Bruker Venture Duo Photon-II single crystal X-Ray diffractometer equipped with an Oxford Cryostream LT device. Data sets were collected using an Incoatec IμS micro-focus source (Cu) with multi-layer mirror optics. Preliminary unit cell constants were determined from a set of 180º fast ϕ scan frames (1 sec exposure, 1° scan). Typical data sets consist of combinations of ϖ and ϕ scan frames with typical scan width of 1.0° and counting time of 1 to 5 seconds/frame at a crystal to detector distance of 3.7 cm.

The collected frames were integrated using an orientation matrix determined from the narrow frame scans. Apex II and SAINT software packages[100] were used for data collection and data integration. Analysis of the integrated data did not show any decay. Final cell constants were determined by global refinement of reflections harvested from the complete data set. Collected data were corrected for systematic errors using SADABS[100] based on the Laue symmetry using equivalent reflections.

Structure solution and refinement were carried out using the SHELXTL-PLUS software package.[101] The structures were solved by direct methods and refined successfully in the monoclinic space group, $P2_1$. Full matrix least-squares refinements were carried out by minimizing $\Sigma\omega(F_o^2-F_c^2)^2$. The non-hydrogen atoms were refined anisotropically to convergence. All hydrogen atoms were treated using appropriate riding model (AFIX m3). Absolute structure determination resulted in Flack-x parameters of 0.02(4) and 0.011(11) for compounds **2.2** and **2.6**, respectively. Complete listings of positional and isotropic displacement coefficients for hydrogen atoms and anisotropic displacement coefficients for the non-hydrogen atoms are listed in the Appendix.

2.5. References

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CHAPTER 3

N-Alkylated analogues of indolylthio glycosides as glycosyl donors with enhanced activation profile

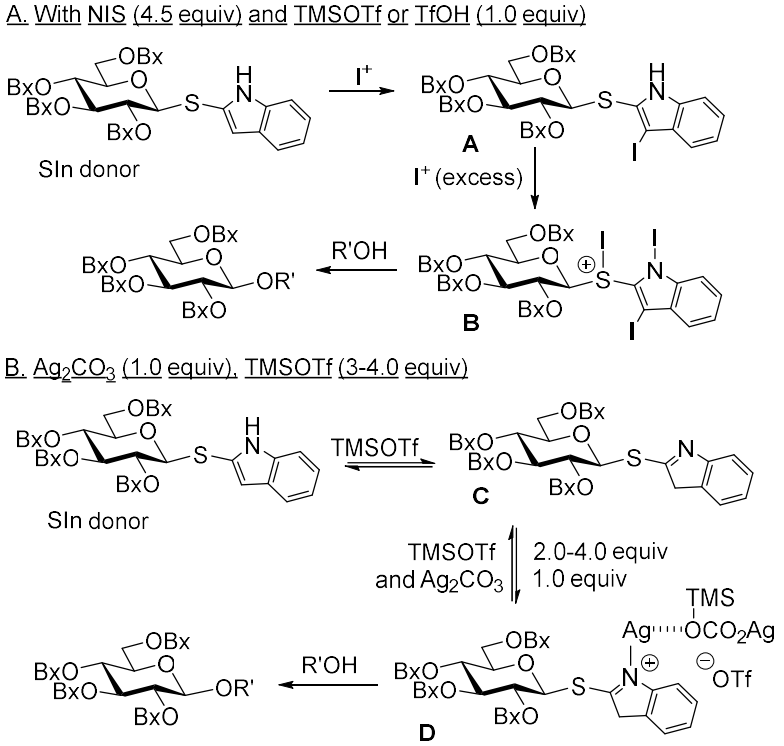
3.1. Introduction

Chemical synthesis of glycans and glycoconjugates remains challenging to chemists, and even advanced strategies and technologies can address this challenge only in part.[65] While most syntheses of glycans are accomplished using thioglycosides or *O*-imidates as glycosyl donors, the development of new efficient methods for chemical glycosylation remains a vibrant area of scientific quest. Following early work by Zinner, Mukaiyama, and Hanessian, our lab became interested in glycosyl thioimidates, glycosyl donors equipped with the $SCR_1=NR_2$ leaving group.[30] Among these, *S*-benzoxazolyl (SBox),[33] *S*-thiazolinyl (STaz),[32] and *S*-benzimidazolyl (SBiz)[59] imidates showed great levels of versatility and efficacy, both for single step glycosylations and expeditious strategies for glycan assembly.[39]

Our recent endeavor was desiccated to studying (1*H*-indol-2-yl)thio (*S*-indolyl, SIn) glycosides.[102] These compounds are structurally similar both to thioglycosides and to thioimidates, but in terms of reactivity act as neither due to their unusual activation profile. In fact, SIn glycosides were found to be fully orthogonal in respect to ethylthio glycosides.[102] The activation of the SIn leaving group for glycosylation persistently demanded large amounts of reagents, regardless of the activation method employed. For example, the activation required 5.0 equiv of $AgBF_4$ versus typical 2-3 equiv used for the activation of thioimidates.[78] Also *N*-iodosuccinimide (NIS, 4.5 equiv) and TMSOTf (1.0 equiv) or TfOH were needed for the SIn leaving group activation versus a typical NIS (2.0 equiv) and TMSOTf or TfOH (0.2 equiv) needed for the activation of alkyl/aryl thioglycosides.[26] Cooperative silver catalysis required Ag_2CO_3 (1.0 equiv) and TMSOTf (3.0-4.0 equiv), whereas only catalytic TMSOTf was needed in all of our previous applications of this reaction.[80]

These results were indicative of side processes that were taking place leading to the unusually high consumption of reagents. Reaction monitoring showed that in the presence of NIS, SIn glycosides only start to react upon consumption of 3.0 equiv of NIS, and the formation of intermediates **A** and **B** shown in Scheme 3.1A has been proven by NMR. A similar study performed in the presence of the silver salt and TMSOTf showed that the SIn leaving group should first tautomerize into a thioimidate **C**, and then be activated via intermediate **D** (Scheme 3.1B). This stepwise pathway was used to explain the demand for multiple equivalents of TMSOTf.

Scheme 3.1. A summary of previous mechanistic studies with SIn glycosyl donors.



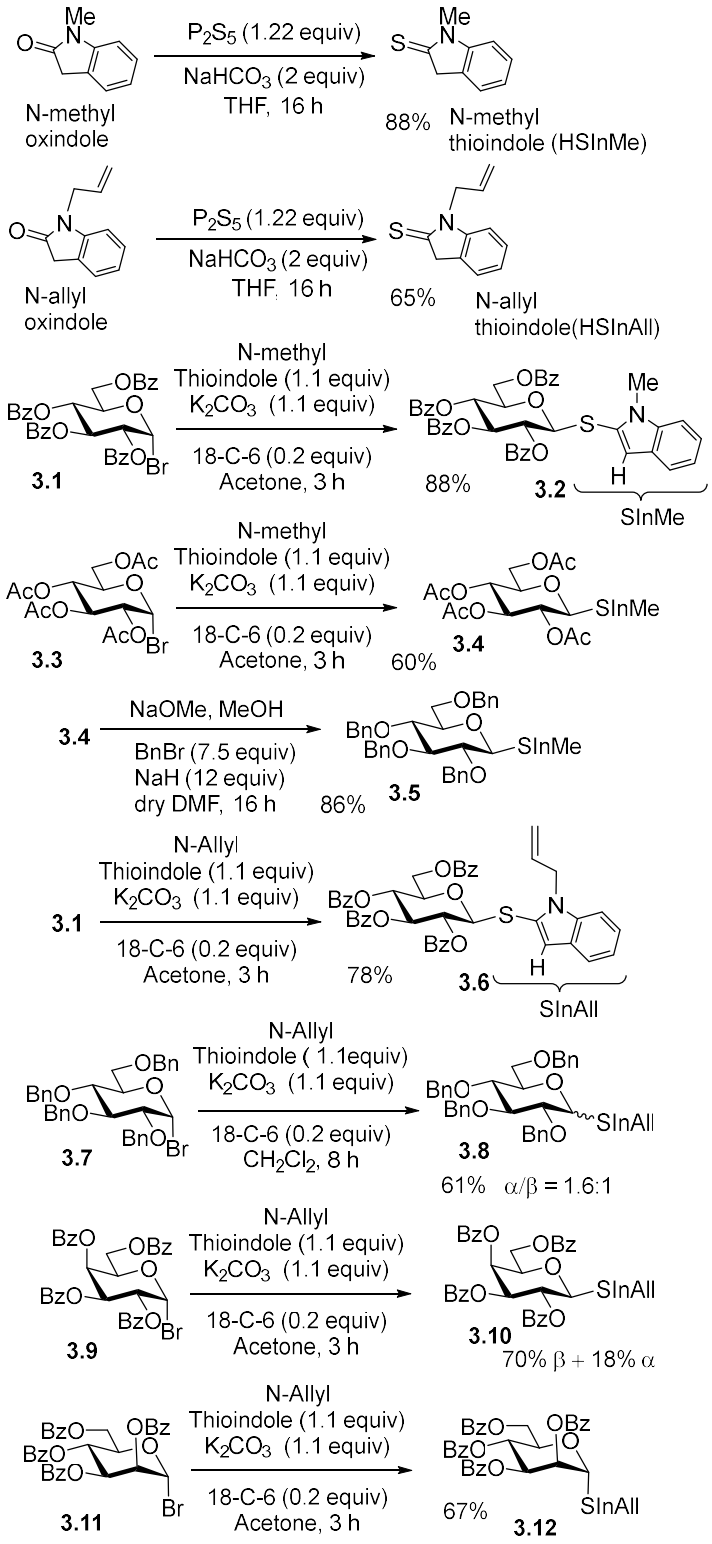
With acquiring preliminary understanding of the mechanistic pathways, we wondered whether blocking the nitrogen atom of the SIn leaving group would help to decrease the amounts of reagents required for the activation and hence allow for a more benign glycosylation reaction.

Reported herein is our first attempt to modify the SIn leaving group by introducing a stable *N*-alkyl substituent.

3.2. Results and Discussion

We have selected atom economical methyl and allyl protecting groups for this study. As shown in Scheme 3.2, *N*-substituted thioindoles were prepared from the respective *N*-substituted oxindoles[103] via thionation with P_2S_5 in tetrahydrofuran (THF).[71] *N*-Methyl thioindole and *N*-allyl thioindole were obtained in 88% and 65% yield, respectively. The leaving groups were then introduced by reactions of the respective *N*-alkylated thioindoles with glycosyl bromides in the presence of potassium carbonate and 18-crown-6 (18-C-6) in dry acetone. Thus, per-*O*-benzoylated glucosyl bromide **3.1**[72] was converted into SInMe glucosyl donor **3.2** in 88% yield. Similarly, per-*O*-acetylated SInMe derivative **3.4** was prepared from acetobromoglucose **3.3** in 60% yield. Per-*O*-benzylated SInMe glucosyl donor **3.5** was prepared from acetylated derivative **3.4** using conventional deacetylation - benzylation sequence. This two-step reprotection accomplished in 86% yield served as an indication that the new N-alkylated leaving groups are sufficiently stable to withstand strongly basic reaction conditions associated with these transformations. SInAll glucosyl donors **3.6** and **3.8** were prepared in 78% and 61% (α/β = 1.6:1) from glucosyl bromides **3.1** and **3.7**, respectively. SInAll galactosyl donor **3.10** and mannosyl donor **3.12** were synthesized from the corresponding per-*O*-benzoylated glycosyl bromides **3.9** and **3.11**, in 88% (separable α,β-mixture) and 67% (α-anomer), respectively (Scheme 3.2).[72]

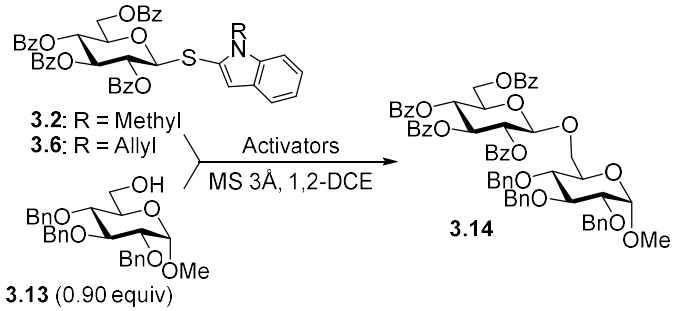
Scheme 3.2. Synthesis of protected SInMe and SInAll glycosides **3.2**, **3.4-3.6**, **3.8**, **3.10**, and **3.12**



With the novel SInR derivatives in hand, we probed their potential as glycosyl donors with primary glycosyl acceptor **3.13**.[55] The key results of this preliminary study are summarized in Table 1. We chose to explore the same activators as those used for regular (NH) thioindole glycosyl donors, *i. e.* NIS/TfOH, NIS/TMSOTf and Ag_2CO_3/TMSOTf.[102] Glycosidation of donor **3.2** with acceptor **3.13** in the presence of 3.0 equiv NIS and 0.8 equiv TfOH was somewhat sluggish (19 h) and disaccharide **3.14**[77] was obtained in a modest yield of 60% (entry 1). Reaction performed in the presence of 4.0 equiv of NIS and 1.0 equiv of TfOH rapidly (15 min) afforded disaccharide **3.14** in 75% yield (entry 2). Reaction in the presence of 4.5 equiv of NIS and 1.0 equiv of TMSOTf was even faster (10 min), and disaccharide **3.14** was obtained in 83% yield (entry 3). Glycosidation of donor **3.2** in the presence of 0.8 equiv of Ag_2CO_3 and 4.0 equiv of TMSOTf led to the formation of disaccharide **3.14** in 70% yield in 3.5 h (entry 4). It should be noted that while the results of these reactions were generally good, we saw no significant difference between the new *N*-methylated leaving group and the previously investigated SIn glycosides. A similar level of reagent consumption was observed, and all our attempts to perform reaction in the presence of lower amounts of activators resulted in sluggish reactions and/or low yields. Therefore, we turned our attention to studying glycosidation of *N*-allylated donor **3.6** with glycosyl acceptor **3.13** (Table 3.1). Starting with glycosylation in the presence of 2.0 equiv of NIS and 0.2 equiv of TMSOTf, typical conditions for the activation of alkyl/aryl thioglycosides, the activation of the SInAll leaving groups was sluggish and inefficient. Disaccharide **3.14** was obtained in only 29% yield in 16 h (entry 5). A steady increase of the amount of reagents led to improved outcomes of these reactions. Thus, reactions performed with 3.0 equiv of NIS and 0.8 or 1.0 equiv of TMSOTf rapidly (1-2 h) afforded disaccharide **3.14** in 68% or 89% yield (entries 6 and 7). We then attempted glycosylation in

the presence of 3.0 equiv of NIS and 0.4 equiv of TfOH. In this case, disaccharide **3.14** was obtained in 52% yield in 24 h (entry 8). Keeping the amount of NIS constant (3.0) and increasing the amount of TfOH to 0.8 and 1.0 equiv allowed us to improve yields of disaccharide **3.14** to 69% and 73%, respectively (entries 9 and 10). These somewhat slow reactions were stopped after 24 h. The optimal conditions were found to be 3.2 equiv of NIS and 1.0 equiv of TfOH which afforded disaccharide **3.14** in 94% in 1 h (entry 11). Glycosidation of donor **3.6** in the presence of 0.8 equiv of Ag_2CO_3 and 4.0 equiv of TMSOTf led to the formation of disaccharide **3.14** in 79% yield in 1 h (entry 12).

Table 3.1. Optimization of glycosidations of SInMe donor **3.2** and SInAll donor **3.6** with 6-OH acceptor **3.13**

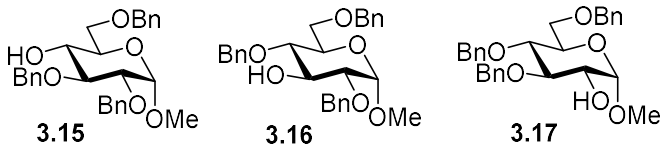


Entry	Conditions (equiv)	Yield of **3.14**
1	**3.2**, NIS (3.0), TfOH (0.8), rt, 19 h	60%
2	**3.2**, NIS (4.0), TfOH (1.0), rt, 15 min	75%
3	**3.2**, NIS (4.5), TMSOTf (1.0), rt, 10 min	83%
4	**3.2**, Ag_2CO_3 (0.8), TMSOTf (4.0), rt, 3.5 h	70%
5	**3.6**, NIS (2.0), TMSOTf (0.2), rt,16 h	29%
6	**3.6**, NIS (3.0), TMSOTf (0.8), rt, 2 h	68%
7	**3.6**, NIS (3.0), TMSOTf (1.0), rt, 1 h	89%

8	**3.6**, NIS (3.0), TfOH (0.4), rt, 24 h	52%
9	**3.6**, NIS (3.0), TfOH (0.8), rt, 24 h	69%
10	**3.6**, NIS (3.0), TfOH (1.0), rt, 24 h	73%
11	**3.6**, NIS (3.2), TfOH (1.0), rt, 1 h	94%
12	**3.6**, Ag_2CO_3 (1.0), TMSOTf (4.0), rt, 1 h	79%

Based on the preliminary experimentation, we chose NIS/TfOH activation conditions for both donors **3.2** and **3.6** in the ratios depicted in entries 2 and 11 (Table 3.1), respectively, to perform further studies. For expanding the scope of this reaction, we chose glycosyl donors **3.2**, **3.5**, **3.6**, **3.8**, **3.10**, and **3.12** (Scheme 3.2) and standard primary acceptor **3.13** as well as secondary glycosyl acceptors **3.15-3.17**[55] depicted in Figure 3.1. The results of this study are summarized in Table 3.2.

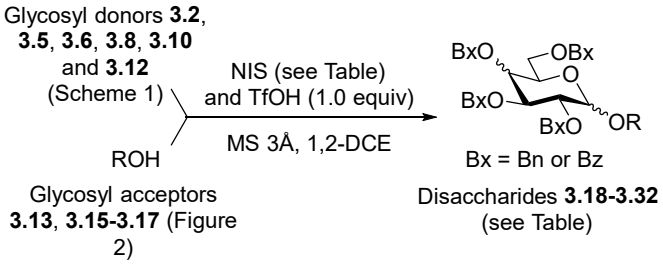
Figure 3.1. Standard glycosyl acceptors used in this study

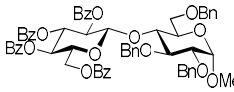
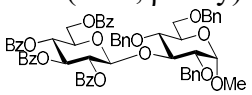
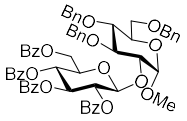
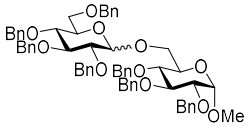
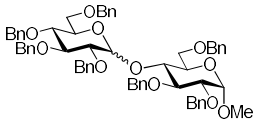
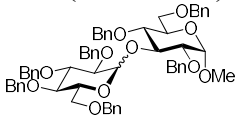


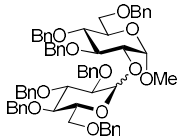
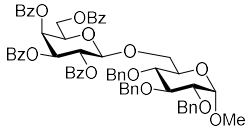
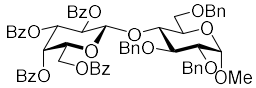
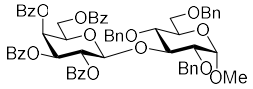
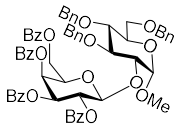
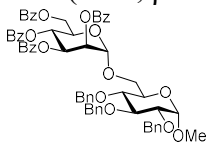
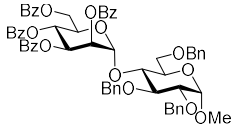
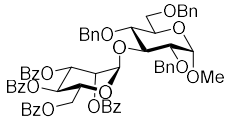
Glycosidation of per-*O*-benzoylated SInMe glucosyl donor **3.2** with glycosyl acceptors **3.15-3.17** gave the respective β-linked disaccharides **3.18-3.20**[55,58,77] in 79-85% yield (entries 1-3). Glycosylation of acceptor **3.13** with per-*O*-benzylated SInMe donor **3.5** in the presence of NIS (4.0 equiv) and TfOH (1.0) was swift (10 min) and efficient. As a result, disaccharide **3.21** was obtained in 92% yield in 10 min (entry 4). The high reactivity of per-*O*-benzylated (armed) donor **3.5** allowed us to decrease the amount of NIS to 3.0 equiv. In this case, a slower reaction (20 min) and a reduced yield (83%) for the formation of disaccharide **3.21** were recorded (entry 5). Glycosidation of SInMe donor **3.5** with secondary glycosyl acceptors **3.15-3.17** gave the

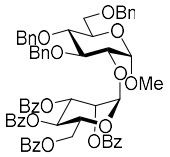
respective disaccharides **3.22-3.24** in 61-69% yields and poor stereoselectivity (entries 6-8). The low yields were attributed to the formation of unidentified side products. The reactions with donor **3.5** lack stereoselectivity due to a non-participating benzyl substituent at C-2.

Table 3.2. Expanding the scope of glycosylation with SInR donors: synthesis of disaccharides **3.18-3.32**



Entry	Donor + Acceptor, amount of NIS, time	Product (yield, α/β ratio)
1	**3.2** (SInMe) + **3.15**, NIS (4.0 equiv), 45 min	 **3.18** (79%, β only)
2	**3.2** + **3.16**, NIS (4.0 equiv), 45 min	 **3.19** (80%, β only)
3	**3.2** + **3.17**, NIS (4.0 equiv), 15 min	 **3.20** (85%, β only)
4	**3.5** (SInMe) + **3.13**, NIS (4.0 equiv), 10 min	 **3.21** (92%, 1.2/1)
5	**3.5** + **3.13**, NIS (3.0 equiv), 20 min	**3.21** (83%, 1.1/1)
6	**3.5** + **3.15**, NIS (4.0 equiv), 15 min	 **3.22** (61%, 1/1.2)
7	**3.5** + **3.16**, NIS (4.0 equiv), 15 min	 **3.23** (69%, 1.2/1)

8	**3.5** + **3.17**, NIS (4.0 equiv), 15 min	 **3.24** (67%, 1.2/1)
9	**3.6** (SInAll) + **3.15**, NIS (3.5 equiv), 3 h	**3.18** (68%, β only)
10	**3.6** + **3.16**, NIS (3.5 equiv), 1 h	**3.19** (75%, β only)
11	**3.6** + **3.17**, NIS (3.5 equiv), 45 min	**3.20** (87%, β only)
12	**3.8** (SInMe) + **3.13**, NIS (3.2 equiv), 10 min	**3.21** (84%, 1.3/1)
13	**3.8** + **3.15**, NIS (3.2 equiv), 20 min	**3.22** (75%, 1/1.3)
14	**3.8** + **3.16**, NIS (3.2 equiv), 10 min	**3.23** (76%, 1.1/1)
15	**3.8** +**3.17**, NIS (3.2 equiv), 10 min	**3.24** (83%, 1.5/1)
16	**3.10** (SInAll) +**3.13**, NIS (3.2 equiv), 1 h	 **3.25** (85%, β only)
17	**3.10** +**3.15**, NIS (3.5 equiv), 1 h	 **3.26** (92%, β only)
18	**3.10** + **3.16**, NIS (3.5 equiv), 1 h	 **3.27** (96%, β only)
19	**3.10** + **3.17**, NIS (3.5 equiv), 1 h	 **3.28** (96%, β only)
20	**3.12** (SInAll) + **3.13**, NIS (3.2 equiv), 1.5 h	 **3.29** (88%, α only)
21	**3.12** + **3.15**, NIS (3.5 equiv), 2.5 h	 **3.30** (89%, α only)
22	**3.12** + **3.16**, NIS (4.0 equiv), 2.5 h	 **3.31** (93%, α only)

23	**3.12** + **3.17**, NIS (3.5 equiv), 2.5 h	 **3.32** (92%, α only)

Glycosidation of per-*O*-benzoylated SInAll glucosyl donor **3.6** with acceptors **3.15-3.17** was conducted in the presence of NIS (3.5 equiv) and TfOH (1.0 equiv). These reactions required 45 min - 3 h to produce the respective β-linked disaccharides **3.18-3.20** in 68-87% yields (entries 9-11). Glycosidations of per-*O*-benzylated SInAll glucosyl donor **3.8** with acceptors **3.13** and **3.15-3.17** were conducted in the presence of a reduced amount of NIS (3.2 equiv) and TfOH (1.0 equiv). There reactions required only 10-20 min to afford the respective β-linked disaccharides **3.21-3.24** in 75-84% yields (entries 12-15). The lack of stereoselectivity in these reactions was also due to a non-participating benzyl ether substituent at C-2 of the donor.

We then turned to investigating glycosylations with benzoylated *N*-allyl galactosyl and mannosyl donors **3.10** and **3.12**, respectively. Glycosidation of galactosyl donor **3.10** with acceptor **3.13** in the presence of 3.2 equiv of NIS and 1.0 equiv of TfOH afforded β-linked disaccharide **3.25**[104] in 85% yield in 1 h (entry 16). Moving towards glycosylations with less reactive, secondary acceptors **3.14**-**3.16** we decided to increase the amount of NIS to 3.5 equiv keeping the amount of TfOH constant. This slight modification paid off, and the corresponding β-linked disaccharides **3.26-3.28**[105] were swiftly (1 h) obtained in excellent yields of 92-96% (entries 17-19). Glycosidation of mannosyl donor **3.12** with acceptor **3.13** in the presence of 3.2 equiv NIS and 1.0 equiv TfOH afforded α-linked disaccharide **3.29**[104] in 88% yield in 1.5 h (entry 20). Like in case of the galactosyl donor, we decided to increase the amount of NIS to 3.5 equiv keeping the amount of TfOH constant for glycosylations of less reactive secondary

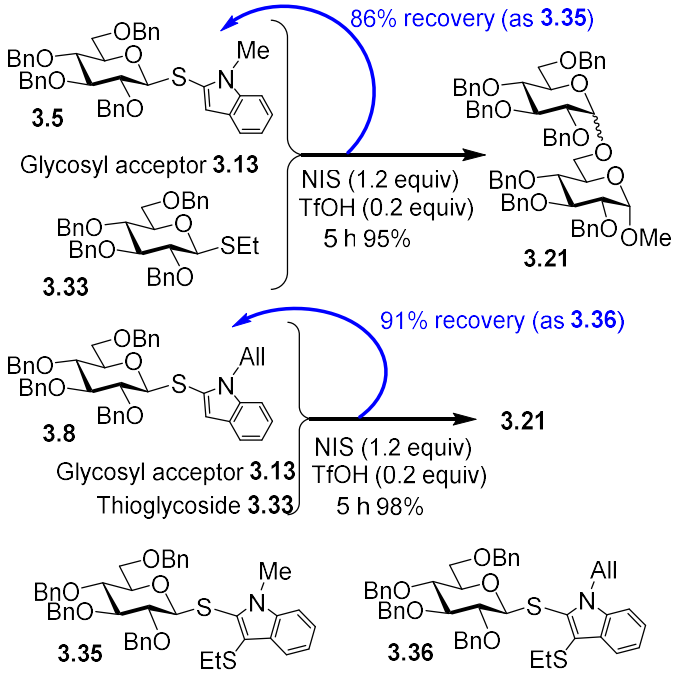
glycosyl acceptors **3.14**-**3.16**. As a result, the corresponding α-linked disaccharides **3.30**-**3.32**[104,106,107] were obtained in 89-93% yields in 2.5 h (entries 21-23).

Next, we advanced our studies toward testing possible orthogonality of SInR donors with glycosyl donors of other series. For this experimentation, we chose ethylthio glycoside **3.33**[73] and SBox imidate **3.34**.[54] In such comparative study, 1.0 equiv of a thioindolyl glycoside (SInMe **3.5** or SInAll **3.8**) were set to complete with 1.0 equiv of other donors (**3.33** or **3.34**) for 2.0 equiv of the primary glycosyl acceptor **3.13**. First competitive reaction was set between donor **3.5** and ethylthio glycoside **3.33** in the presence of NIS (1.2 equiv) and TfOH (0.2 equiv), conditions that are suitable for the activation of thioglycosides. Indeed, SEt donor **3.33** was consumed completely in 5 h while SInMe donor **3.5** was recovered as its SEt adduct **3.35** in 86% yield (Scheme 3.3). The formation of this adduct is a result of a capturing the departed leaving group. Disaccharide **3.21** was obtained in 95% yield that was calculated based on the acceptor recovery. A similar reaction of SInAll donor **3.8** and SEt donor **3.33** with glycosyl acceptor **3.13** led to a complete activation of SEt donor **3.33** while donor **3.8** was recovered as its SEt adduct **3.36** in 91% yield (Scheme 3.3). Disaccharide **3.21** was obtained in 98% yield. It should be noted that excellent yields obtained for the formation of disaccharide **3.21** is attributed, in part, to excess of donors used in these reactions.

Another competitive set of reactions was performed in the presence of Ag_2CO_3 and TMSOTf promoter system. Thioglycoside donor **3.33** was expected to remain unreactive under these reaction conditions. Indeed, the competitive reaction between donors **3.5** and **3.33** led to complete consumption of donor **3.5** in 15 min. SEt donor **3.33** was recovered in 92% yield, and disaccharide **3.21** was isolated in 98% yield (Scheme 3.4). A similar competition between donors **3.8** and **3.33** led to complete consumption of donor **3.8** in 15 min. As a result, donor

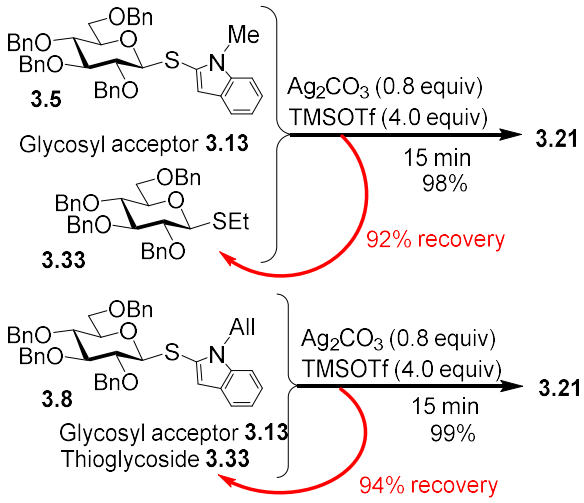
3.33 was recovered in 94% yield and disaccharide **3.21** was isolated in 99% yield (Scheme 3.4). These experiments signified that the thioindole analogs show entire orthogonality in respect to common ethylthio glycosides.

Scheme 3.3. Competition experiments between SInR and SEt that led to preferential activation of thioglycoside **3.33**

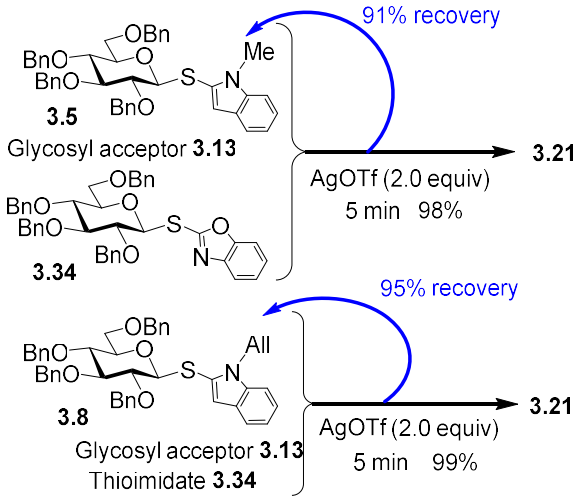


We also conducted competition experiments between thioindolyl glycosides and SBox imidate **3.34** in the presence of 2.0 equiv of AgOTf as promoter. These reaction conditions are standard for the activation of SBox glycosides,[53] but unmodified SIn glycosides reacted very slowly, even in the presence of a large access of AgOTf.[102] Thus, a competition reaction between SInMe donor **3.5** and SBox donor **3.34** resulted in a complete consumption of donor **3.34** in 5 min. As a result, donor **3.5** was recovered in 91% yield, and disaccharide **3.21** was obtained in 98% yield (Scheme 3.5). A similar competition reaction between SInAll donor **3.8** and SBox donor **3.34** led to complete consumption of donor **3.34** in 5 min. Unreacted donor **3.8** was recovered in 95% yield, and disaccharide **3.21** was isolated in 99% yield (Scheme 5).

Scheme 3.4. Competition experiments between SInR and SEt that led to the preferential activation of SInMe **3.5** or SInAll **3.8**



Scheme 3.5. Competition experiments between SInR and SBox building blocks that led to the preferential activation of SBox donor **3.34**



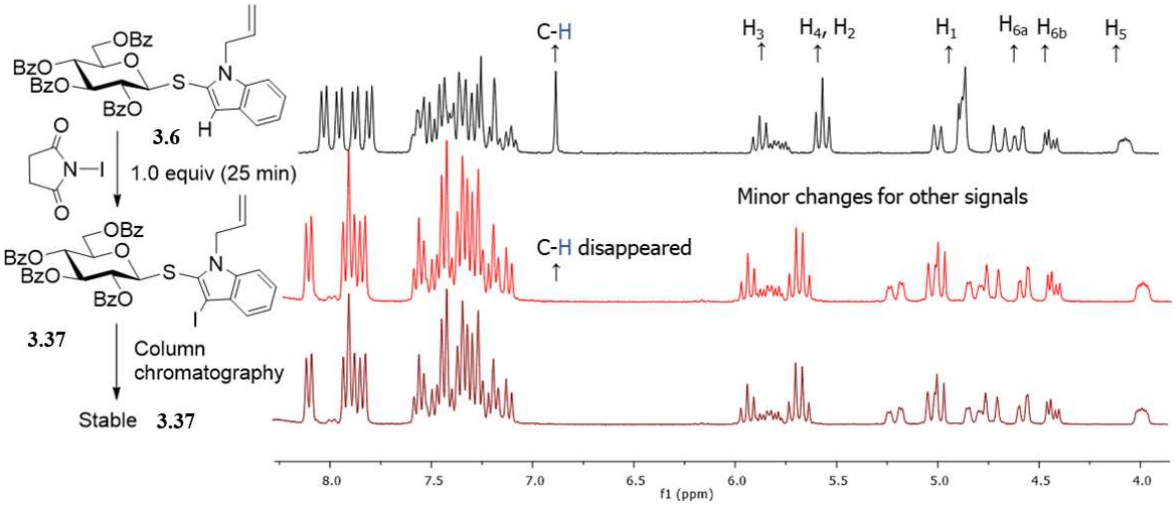
To gain understanding of the glycosylation reaction mechanism we performed a set of experiments monitored by NMR. First, we investigated the activation in the presence of NIS. For this purpose, SInAll donor **3.6** was dissolved in $CDCl_3$, the solution was transferred into a standard 5 mm NMR tube, and ^1H NMR spectrum was recorded (Scheme 3.6). NIS (1.0 equiv) was added and ^1H NMR spectrum was recorded in 25 min showing the formation of iodine

adduct **3.37** as the only product. A similar experiment performed with SInMe donor **3.2** cleanly produced analogous adduct **3.38** (structure shown in Scheme 3.7, see the appendix for the experiment details). Intermediates **3.37** and **3.38** are stable compounds, can be purified by column chromatography, were fully characterized by NMR, and their identity was confirmed by mass spectrometry.

To uncover the mechanism of glycosylation reaction proceeding under the cooperative silver salt-Lewis acid catalysis we also set up a series of NMR experiments. For this purpose, SInAll donor **3.6** was dissolved in $CDCl_3$, the solution was transferred into a standard 5 mm NMR tube, and ^1H NMR spectrum was recorded. TMSOTf (1.0 equiv) was added and ^1H NMR spectrum was recorded in 10 min showing the formation of TMS-adduct **3.39** (structure shown in Scheme 3.7, see the appendix for the experiment details). A similar experiment performed with SInMe donor **3.2** produced analogous adduct **3.40** (structure shown in Scheme 3.7). Intermediates **3.39** and **3.40** are relatively unstable compounds that produced very elusive NMR spectra. Upon attempt to purify them by column chromatography, they reverted to the respective starting materials **3.6** and **3.2**.

Based on this NMR monitoring our current understanding of the NIS-promoted reaction mechanism for the activation of SInR derivatives is as follows. Upon addition of NIS and TfOH to the reaction mixture containing SInAll donor **3.6** or SInMe donor **3.2** in 1,2-DCE, the first equivalent of NIS gets consumed by the electrophilic addition of the iodonium ion at the C-3 position of the SInR aglycone to afford stable intermediates **3.37** or **3.38**, respectively (Scheme 3.7A). The presence of TfOH facilitates the formation of the iodonium ion from NIS. The additional NIS is needed to convert **3.37**/**3.38** into the activated species **B'** that results in the leaving group departure followed by glycosylation.

Scheme 3.6. NMR experiment with SInAll donor **3.6** in the presence of NIS (1.0 equiv) to observe the formation of **3.37**

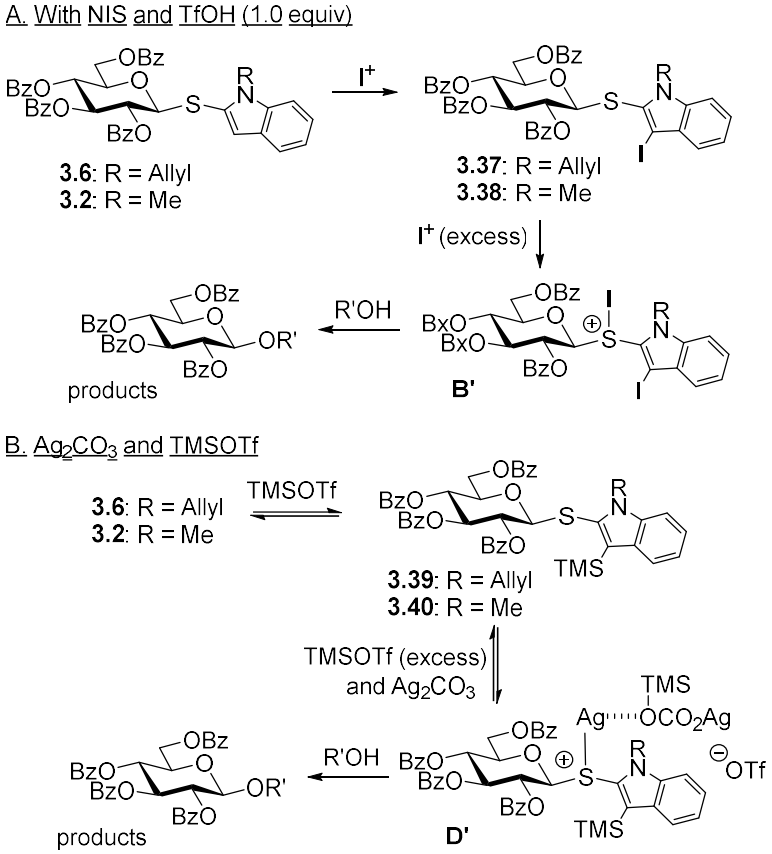


Note the key difference between intermediate **B'** and intermediate **B** that was observed with unprotected SIn derivatives in our previous work (see Scheme 3.1). This difference explains the reduced amount of NIS that was typically required for the activation of SInR donors (3.2 equiv) versus their unprotected SIn counterparts (4.5 equiv). While glycosidation of benzoylated SInR donors proceeds via the intermediacy of the acyloxonium intermediate leading to complete stereoselectivity, glycosidations of benzylated SInR donors proceed via oxacarbenium ion intermediate. The formation of the latter intermediate explains poor stereoselectivity of many uncontrolled glycosylations taking place without the assistance of the neighboring group at C-2.

Based on NMR monitoring our current understanding of the reaction mechanism of glycosidation of SInR donors **3.6** or **3.2** in the presence of TMSOTf and Ag_2CO_3 is as follows. Upon addition of the promoters to the reaction mixture containing SInAll donor **3.6** or SInMe donor **3.6** in 1,2-DCE, the first equivalent of TMSOTf gets consumed by the electrophilic

addition of TMS at the C-3 position of the SInR aglycone to afford elusive intermediates **3.39** or **3.40**, respectively (Scheme 3.7B). This step represents the key difference from the activation of unprotected SIn donors that were first tautomerized into its thioimidate counterpart (refer to Scheme 3.1).

Scheme 3.7. Plausible mechanistic pathways for the activation of SInR glycosyl donors.



Silver carbonate then complexes with adducts **3.39/3.40** to produce respective silver complexes, which become strongly ionized in the presence of access TMSOTf. In should be noted that the resulting intermediate **D'** signifies a drastic activation difference from the analogous intermediate **D** observed with SIn derivatives (see Scheme 3.1). Whereas SInR donors are activated via the anomeric sulfur, following the direct activation pathway like in

thioglycosides, SIn donors are activated via the endocyclic nitrogen atom of the leaving group, which represents the remote activation pathway, like in thioimidates.

3.3. Conclusions

In summary, we presented an extended study of a new class of glycosyl donors, indolylthio glycosides. Previously we observed that while the activation profile of the SIn leaving group is interesting, most reactions required large excess reagents. This drawback was partially addressed in the present study of *N*-alkylated SInR derivatives. The activation process was studied by NMR, and the increased understanding of the mechanism led to a discovery of different activation pathways taking place with SIn versus SInR derivatives. We also investigated orthogonality of the SInR leaving groups versus thioglycosides. Further investigation of the leaving groups of this class in application to the synthesis of glycans is currently underway in our laboratory.

3.4. Experimental

3.4.1. *General methods.*

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 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to 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. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C NMR spectra were recorded at 75 MHz. The ^{1}H NMR chemical shifts were referenced to tetramethyl silane (TMS, δ H = 0 ppm) or $CDCl_3$ (δ H= 7.26 ppm) for ^{1}H NMR spectra for solutions in $CDCl_3$. The ^{13}C NMR chemical shifts were referenced to the central signal of $CDCl_3$ (δ C = 77.00 ppm) for solutions in $CDCl_3$. HR FAB-MS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

3.4.2. Synthesis of HSInR aglycones

1-Methylindoline-2-one (N-methyl oxindole). The title compound was prepared from Isatin as previously described.[103] Analytical data for *N*-methyl oxindole were in accordance with that previously reported.[103]

1-Methylindoline-2-thione (N-methyl thioindole, HSInMe). The title compound was obtained following the previously described procedure,[71] which was modified as follows. *N*-Methyl oxindole (4.0 g, 27.2 mmol) was dissolved in dry tetrahydrofuran (100 mL) under argon. P_2S_5 (7.37 g, 33.16 mmol) was added followed by a portionwise addition of sodium bicarbonate (4.57 g, 54.6 mmol), and the resulting mixture was stirred under argon for 16 h at rt. After that, the volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (400 mL) and washed with water (3 × 50 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane 10% gradient elution) to afford the title compound in 88% yield. Analytical data for HSInMe: R_f= 0.45 (ethyl acetate/hexane, 2/3, v/v); ^{1}H NMR (300 MHz): δ 3.57 (s, 3H, NCH_3), 4.02 (s, 2H, CH_2), 6.93-7.34 (m, 4H,

aromatic) ppm; $^{13}C\{^{1}H\}$ NMR (75 MHz): δ 30.9, 48.7, 109.3, 123.6, 124.0, 127.7, 128.7, 146.2, 200.6 ppm; ESI-TOF $[M + H]^+$: calcd for $[C_9H_{10}NS]^+$ 164.0528; found: 164.0532.

1-Allylindolin-2-one (N-allyl oxindole). The title compound was prepared from Isatin using previously described procedure.[103] Analytical data for *N*-allyl oxindole were in accordance with that previously reported.[103]

1-Allylindolin-2-thione (N-allyl thioindole, HSInAll). *N*-allyl oxindole (2.0 g, 11.54 mmol) was dissolved in dry tetrahydrofuran (40 mL) under argon. P_2S_5 (3.12 g, 14.08 mmol) was added followed by a portionwise addition of sodium bicarbonate (1.93 g, 23.08 mmol), and the resulting mixture was stirred under argon for 16 h at rt. After that, the volatiles were removed under reduced pressure. The residue was dissolved in ethyl acetate (300 mL) and washed with water (3 × 40 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane 10% gradient elution) to afford the title compound in 65% yield. Analytical data for HSInAll: R_f = 0.50 (ethyl acetate−hexane, 1/4, v/v); ^1H NMR (300 MHz): δ 4.06 (s, 2H, CH_2C=S), 4.81 (d, 2H, NCH_2), 5.19-5.25 (m, 2H, =CH_2), 5.79-5.91 (m, 1H, CH=), 6.92-7.30 (m, 4H, aromatic) ppm; $^{13}C\{^{1}H\}$ NMR (75 MHz): δ 46.2, 48.6, 109.8, 118.0, 123.5, 123.8, 127.4, 128.5, 129.2, 145.2, 200.5 ppm. ESI-TOF $[M + H]^+$: calcd for $[C_{11}H_{12}NS]^+$ 190.0685; found: 190.0690.

3.4.3. Synthesis of SInR Glycosides

1-Methylindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (3.2). HSInMe (0.33 g, 2.02 mmol), anhydrous K_2CO_3 (0.28 g, 2.02 mmol), and 18-crown-6 (0.10 g, 0.37 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[108] (**3.1**, 1.21 g, 1.83 mmol) in dry acetone (32 mL), and the resulting mixture was stirred under argon at 50

°C for 3 h. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~150 mL), washed with 10% aq. $NaHCO_3$ (25 mL), and water (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 88% yield (1.17 g, 1.615 mmol). Analytical data for **3.2**: R_f = 0.3 (ethyl acetate/ diethyl ether, 4/6, v/v); $[\alpha]_D^{22}$ -20.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.73 (s, 3H, NCH_3), 4.06-4.12 (m, 1H, $J_{5,6a}$ = 5.3, $J_{5,6b}$ = 2.4 Hz, H-5), 4.43 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.59 (dd, 1H, H-6b), 4.89 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.51-5.59 (m, 2H, $J_{2,3}$ = 9.5 Hz, H-2, 4), 5.91 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 6.89 (s, 1H, =CH), 7.11-7.56 (m, 24H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 30.1, 62.8, 68.9, 70.4, 74.0, 76.3, 86.7, 109.9, 112.3, 112.4, 119.7, 120.8, 122.9, 125.1 (x2), 126.9 (x2), 128.2, 128.3, 128.4 (x3), 128.5 (x2), 128.6 (x2), 129.0, 129.3, 129.5, 129.6 (x2), 129.7 (x2), 129.8 (x2), 133.1, 133.2, 133.4, 138.6, 164.9 (x2), 165.7, 166.0 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{43}H_{35}NO_9SNa]^+$ 764.1925; found: 764.1916.

1-Methylindolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (3.4). HSInMe (1.3 g, 8.02 mmol), anhydrous K_2CO_3 (1.11 g, 8.02 mmol), and 18-crown-6 (0.38 g, 1.46 mmol) were added to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[108] (**3.3**, 3.00 g, 1.83 mmol) in dry acetone (50 mL), and the resulting mixture was stirred under argon at rt for 4 h. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2 and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (300 mL), washed with 10% aq. $NaHCO_3$ (30 mL) and water (2 x 30 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced

pressure. The residue was purified by column chromatography on silica gel (hexane - ethyl acetate 10% gradient elution) to afford the title compound as a white amorphous solid in 60% yield (2.15 g, 4.36 mmol). Analytical data for **3.4**: R_f = 0.45 (ethyl acetate/ hexane, 3/7, v/v); $[\alpha]_D^{22}$ -36.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 1.92-2.15 (m, 12 H, 4 x CH_3CO), 3.57-3.62 (m, 1H, H-5), 3.78 (s, 3H, NCH_3) 4.10-4.11 (m, 2H, H-6a, 6b), 4.51 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 4.90-5.05 (m, 2H, H-2, 4), 5.18 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 6.86 (s, 1H, =CH, indole), 7.09-7.62 (m, 4H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 20.4 (x2), 20.5, 20.7, 30.1, 61.6, 67.7, 69.7, 73.7, 75.6, 85.9, 109.8, 112.2 (x2), 119.7, 120.8, 122.9, 124.8, 126.8, 138.5, 169.2 (x2), 170.1, 170.3 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{23}H_{27}NO_9SNa]^+$ 516.1299; found: 516.1308.

1-Methylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (3.5). Compound **3.4** (2.0 g, 4.05 mmol) was dissolved in minimum amount of methanol (~25 mL), a 1 M solution of NaOMe in MeOH was added until pH >9, and the resulting mixture was stirred for 1 h at rt. The reaction mixture was neutralized with Dowex (H^+), the resin was filtered off, and rinsed successively with MeOH. The combined filtrate (~75 mL) was concentrated under reduced pressure, and the residue was dried in *vacuo* for 2 h. The crude residue was dissolved in dry DMF (24mL), NaH (1.94 g, 48.66 mmol) was added portionwise, followed by the dropwise addition of benzyl bromide (3.61 mL, 30.41 mmol), and the resulting mixture was stirred for 16 h at rt. The reaction mixture was poured into ice-water (50mL) and stirred for 30 min. The mixture was extracted with ethyl acetate/ diethyl ether (1:1, v/v, 3 x 100 mL), and the combined organic extract was washed with water (2 x 50 mL). The organic phase was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane, 5% gradient elution) to afford the title

compound as a white amorphous solid in 86% yield (2.4 g, 3.49 mmol). Analytical data for **3.5**: R_f = 0.55 (ethyl acetate/ hexane, 3/7, v/v); $[\alpha]_D^{22}$ -10.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.33 (dd, 1H, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.46-3.74 (m, 5H, H-2, 3, 4, 5, 6b), 3.80 (s, 3H, NCH_3), 4.41-4.53 (m, 4H, H-1, 3 x C*H*Ph), 4.43 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 4.81-4.89 (m, 3H, 3 x C*H*Ph), 5.02 (d, ^2J = 10.3 Hz, C*H*Ph), 6.87 (s, 1H, =CH, indole), 7.03-7.31 (m, 24H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 30.3, 68.6, 73.2 (x2), 74.8, 74.4, 75.2, 78.6, 80.4, 86.5, 88.2 (x2), 109.7, 110.9, 119.5, 120.5, 122.5, 126.9 (x2), 127.1 (x2), 127.5 (x3), 127.6 (x2), 127.8 (x2), 128.0 (x2), 128.3 (x3), 137.8 (x4), 137.9 (x2), 138.2 (x2), 138.4 (x2), ppm; ESI-TOF $[M + H]^+$: calcd for $[C_{43}H_{43}NO_5SNa]^+$ 708.2754; found: 708.2748.

1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (3.6). HSInAll (0.47 g, 2.5 mmol), anhydrous K_2CO_3 (0.35g, 2.5 mmol), and 18-crown-6 (0.12 g, 0.46 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-*D*-glucopyranosyl bromide[72] (**3.1**, 1.50 g, 2.27 mmol) in dry acetone (40 mL), and the resulting mixture was stirred under argon for 3 h at 50 °C. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~150 mL), washed with 10% aq. $NaHCO_3$ (25 mL) and water (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 78% yield (1.33 g, 1.71 mmol). Analytical data for **3.6**: R_f =0.6 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ -4.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 4.00-4.10 (m, 1H, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 2.3 Hz, H-5), 4.45 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.61 (dd, 1H, H-6b), 4.66-5.00 (m, 5H, H-1, NCH_2, $=CH_2$), 5.59 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.73-5.83 (m, 1H, CH=), 5.90 (dd, 1H, $J_{3,4}$

= 9.5 Hz, H-3), 6.90 (s, 1H, =CH, indole), 7.07-8.01 (m, 24H, aromatic) ppm; $^{13}C\{^{1}H\}$ NMR (75 MHz): δ 45.7, 62.8, 68.9, 70.4, 73.9, 76.3, 86.8, 110.4, 112.5 (x2), 115.9, 119.9, 120.8, 123.0, 125.1, 127.1, 128.2, 128.3 (x2), 128.4 (x4), 128.5 (x2), 128.9, 129.3, 129.5 (x2), 129.6 (x2), 129.7 (x2), 129.8 (x2), 133.1, 133.2, 133.4 (x2), 133.5, 137.8, 164.9 (x2), 165.6, 165.9 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{45}H_{37}NO_9SNa]^+$ 790.2081; found: 790.2074.

1-Allylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (3.8). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[73] (**3.33**, 1.10 g, 1.88 mmol) and freshly activated molecular sieves (3 Å, 0.75 g) in CH_2Cl_2 (50 mL) were stirred under argon for 1 h at rt. Bromine (0.12 mL, 2.26 mmol) was added, and the resulting mixture was stirred for 20 min at rt. After that, the volatiles were removed under reduced pressure, the residue was co-evaporated with dry toluene (2 x 20 mL), and dried in *vacuo* for 2 h. The residue containing crude 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide **3.7** was dissolved in CH_2Cl_2 (10 mL), HSInAll (0.39 g, 2.07 mmol), anhydrous K_2CO_3 (0.29 g, 2.068 mmol), and 18-crown-6 (0.1 g, 0.37 mmol) were added, and the resulting mixture was stirred under argon for 8 h at rt. The solids were filtered off through a pad of Celite and rinsed successively with CH_2Cl_2. The combined filtrate (~150 mL) as washed with water (30 mL), 10% aq. $NaHCO_3$ (25 mL), and water (2 x 30 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-toluene 2% gradient elution) to afford the title compound as a white amorphous solid in 61% yield (0.82 g, 1.15 mmol, α/β = 1.6:1). A small sample of α-**3.8** was separated for characterization purposes. Analytical data for α-**3.8**: R_f= 0.50 (ethyl acetate/ hexane, 3/7, v/v); $[\alpha]_D^{22}$ 68.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.59-3.71 (m, 3H, H-3, 5, 6b), 3.84-3.97 (m, 2H, H-2, 4), 4.38-4.42 (m, 1H, H-6a), 4.46-5.07 (m, 12H, $=CH_2$, NCH_2, 8 x C*H*Ph), 5.49

(d, 1H, $J_{1,2}$ = 4.4 Hz, H-1), 5.82-5.91(m, 1H, CH=), 6.79 (s, 1H, =CH indole), 7.07-7.51 (m, 24H, aromatic) ppm; $^{13}C\{^{1}H\}$ NMR (75 MHz): δ 45.6, 45.8, 67.9, 68.2, 68.6, 71.7, 72.4, 73.3 (x2),74.8, 75.0. 75.3, 75.6, 75.7, 76.0, 77.1, 78.8, 79.4, 80.7, 82.2, 86.6, 88.0, 88.5, 110.0, 110.1, 110.4, 110.5, 111.5, 115.8, 116.1, 119.7, 120.2, 120.3, 120.5, 120.6, 122.2, 122.5, 127.2, 127.6 (x4), 127.8 (x7), 128.2 (x4), 128.3 (x7), 133.4, 133.5, 133.7, 133.8, 137.4, 137.5, 137.7, 137.8, 137.9, 138.0, 138.2, 138.4) ppm; ESI-TOF $[M + H]^+$: calcd for $[C_{45}H_{45}NO_5SNa]^+$ 734.2916; found: 734.2927. Selected ^{1}H NMR (300 MHz) data for β-**3.8**: 3.33 (dd, H-5), 3.48-3.58 (m, H-2, 6a, 6b), 3.37-3.71 (m, H-3, 4), 4.39 (d, H-1), 5.82-5.91 (m, CH=), 6.89 (s, =CH indole), 7.07-7.51 (m, aromatic) ppm.

1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α- and β-D-galactopyranoside (α-3.10 and β-3.10). HSInAll (0.79 g, 4.20 mmol), anhydrous K_2CO_3 (0.58 g, 4.20 mmol) and 18-crown-6 (0.20 g, 0.76 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[72] (**3.9**, 2.50 g, 3.80 mmol) in dry acetone (66 mL), and the resulting mixture was stirred under argon for 3 h at 50 °C. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~250 mL), washed with 10% aq. $NaHCO_3$ (30 mL) and water (2 x 30 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford separate anomers of the title compound as white amorphous solid. Eluted first was α-**3.10** (18% yield, 0.53 g, 0.685 mmol) followed by elution of β-**3.10** (70% yield, 2.04 g, 2.66 mmol). Analytical data for α-**3.10**: R_f = 0.55 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ -4.20 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 4.48 (dd, 1H, $J_{6a,6b}$ = 11.6 Hz, H-6a), 4.61-4.68 (m, 3H, H-6b, NCH_2),

4.77-5.02 (m, 2H, =CH_2), 5.27 (dd, 1H, $J_{5,6a}$=5.2 Hz, H-5), 5.65-5.77 (m, 1H, =CH), 5.88-6.02 (m, 3H, CH=, H-1, 2, 3), 6.13 (m, 1H, =CH, H-4), 6.78 (s, 1H, =CH indole), 7.00-8.07 (m, 24H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 45.5, 62.6, 68.3, 68.5, 68.8, 38.9, 86.8, 109.9, 111.8, 116.3, 119.8, 120.5, 122.7, 125.7, 127.3, 128.2, 128.4 (x2), 128.5 (x2), 128.6, 128.8 (x4), 128.9, 129.3, 129.7, 129.8 (x2), 129.9 (x4), 132.9, 133.0, 133.2 (x2), 133.6 (x2), 137.6, 165.3, 165.4, 165.5, 166.1 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{45}H_{37}NO_9SNa]^+$ 790.2081; found: 790.2033. Analytical data for β-**3.10**: R_f = 0.50 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ -6.3 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz): δ 4.30-4.36 (m, 2H, , $J_{5,6b}$ = 8.5, $J_{6a,6b}$ = 13.3 Hz, H-5, 6a), 4.57 (m, 1H, H-6b), 4.73-4.94 (m, 3H, H-1, NCH_2), 5.00-5.07 (m, 2H, =CH_2), 5.59 (dd, 1H, H-3), 5.77-5.97 (m, 3H, $J_{2,3}$ = 6.9 Hz, H-2, 4, CH=), 7.05-8.03 (m, 25H, =CH indole, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 45.8, 62.3, 67.7, 67.9, 72.9, 75.1, 85.8, 110.6, 113.3, 115.9, 119.9, 121.2, 123.0, 124.3, 127.2, 128.2, 128.4 (x5), 128.5 (x2), 128.6 (x2), 129.1, 129.2, 129.6 (x5), 129.7 (x4), 133.2, 133.3 (x2), 133.6, 138.1, 165.0, 165.1, 165.4, 165.9 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{45}H_{37}NO_9SNa]^+$ 790.2081; found: 790.2076.

1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (3.12). HSInAll (0.79 g, 4.20 mmol), anhydrous K_2CO_3 (0.58 g, 4.20 mmol) and 18-crown-6 (0.20 g, 0.76 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[72] (**3.11**, 2.50 g, 3.80 mmol) in dry acetone (66 mL), and the resulting mixture was stirred under argon for 3 h at 50 °C. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~250 mL), washed with 10% aq. $NaHCO_3$ (30 mL) and water (2 x 30 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on

silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 67% yield (1.91 g, 2.54 mmol). Analytical data for **3.12**: R_f = 0.55 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ -49.0 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): 4.00-4.03 (m, 1H, $J_{5,6a}$ = 5.1 Hz, H-5), 4.49 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.71-4.90 (m, 3H, H-6b, NCH_2), 5.01-5.09 (m, 3H, H-1, $=CH_2$), 5.56 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3), 5.88-6.04 (m, 3H, H-4, $=CH_2$), 6.19 (dd, 1H, $J_{2,3}$ = 2.7 Hz, H-2), 6.92 (s, 1H, =CH indole), 7.08-8.07 (m, 24H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 45.7, 62.9, 66.2, 71.1 72.6, 86.7, 110.3, 111.5 (x2), 115.9, 119.9, 120.8, 123.0, 126.9, 127.2, 127.7, 128.2, 128.4 (x2), 128.5 (x3), 128.6 (x3), 128.8, 129.6 (x3), 129.7 (x4), 129.9 (x2), 133.1, 133.2, 133.4 (x2), 133.7, 133.8, 137.8, 165.2, 165.3, 165.5, 166.0 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{45}H_{37}NO_9SNa]^+$ 790.2081; found: 790.2073.

3.4.4. Synthesis of Disaccharides

General procedure for g*lycosylation in the presence of NIS and TfOH or TMSOTf.* A mixture of a glycosyl donor (0.040-0.044 mmol), a glycosyl acceptor (0.036-0.039 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE or DCM (1.0 mL) was stirred under argon for 1 h at rt. After that, NIS (0.122-0.182 mmol) and TfOH (0.040 mmol) or TMSOTf (0.040 mmol) were added, and the resulting mixture was stirred for the time indicated in Tables at rt. The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL) and H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel

(ethyl acetate-hexane gradient elution). If necessary, further purification was accomplished by size-exclusion column chromatography on Sephadex LH-20.

General procedure for glycosylation in the presence of Ag_2CO_3 and TMSOTf. A mixture of a glycosyl donor (0.040-0.044 mmol), a glycosyl acceptor (0.036-0.039 mmol), and molecular sieves (3 Å, 80 mg) in 1,2-DCE or DCM (1.0 mL) was stirred under argon for 1 h at rt. Ag_2CO_3 (0.035-0.042 mmol) and TMSOTf (0.168-0.175 mmol) were added, and the resulting mixture was for the time indicated in Tables at rt. The solid was filtered off through a pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the corresponding disaccharide. If necessary, further purification was accomplished by size-exclusion column chromatography on Sephadex LH-20.

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.17). The title compound was obtained from donor **3.2** and acceptor **3.13**[55] by general glycosylation procedure in 75% yield as a white amorphous solid. Analytical data for **3.17** was in accordance with that previously reported.[77]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.18). The title compound was obtained from donor **3.2** and acceptor **3.14**[55] by the general glycosylation procedure in 79% yield as a clear syrup. Analytical data for **3.18** was in accordance with that previously reported.[77]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.19). The title compound was obtained from donor **3.2** and acceptor **3.15**[55]

by the general glycosylation procedure in 80% yield as a clear syrup. Analytical data for **3.19** was in accordance with that previously reported.[55]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.20). The title compound was obtained from donor **3.2** and acceptor **3.16**[55] by the general glycosylation procedure in 85% yield as a clear syrup. Analytical data for **3.20** was in accordance with that previously reported.[58]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.21). The title compound was obtained from donor **3.5** and acceptor **3.13** by the general glycosylation procedures in 92% yield (α/β = 1.2/1) as a colorless foam. Analytical data for **3.21** was in accordance with that previously reported.[84]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.22). The title compound was obtained from donor **3.5** and acceptor **3.14** by the general glycosylation procedure in 61% yield (α/β = 1/1.2) as a clear syrup. Analytical data for **3.22** was in accordance with that previously reported.[85]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.23). The title compound was obtained from donor **3.5** and acceptor **3.15** by the general glycosylation procedure in 69% yield (α/β = 1.2/1) as a clear syrup. Analytical data for **3.23** was in accordance with that previously reported.[86]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.24). The title compound was obtained from donor **3.5** and acceptor **3.16** by the general glycosylation procedure in 67% yield (α/β = 1.2/1) as a colorless foam. Analytical data for **3.24** was in accordance with that previously reported.[87]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.25). The title compound was obtained from donor **3.10** and acceptor **3.13** by general glycosylation procedure in 85% yield as a white amorphous solid. Analytical data for **3.25** was in accordance with that previously reported.[104]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.26). The title compound was obtained from donor **3.10** and acceptor **3.14** by the general glycosylation procedure in 92% yield as a colorless amorphous solid. Analytical data for **3.26** was in accordance with that previously reported.[105]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.27). The title compound was obtained from donor **3.10** and acceptor **3.15** by the general glycosylation procedure in 96% yield as a colorless amorphous solid. Analytical data for **3.27:** R_f = 0.5 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ 5.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.25 (s, 3H, CH_3), 3.36 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-2), 3.56-3.70 (m, 4H, H-4, 5, 6a, 6b), 4.16 (d, 1H, ^2J = 12.1 Hz, C*H*Ph), 4.29-4.46 (m, 9H, H-1, 3, 5', 6a', 6b', 4 x C*H*Ph), 5.28 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.50 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 5.67 (dd, 1H, $J_{3',4'}$ = 7.5 Hz, H-3'), 5.87 (dd, 1H, $J_{2',3'}$ = 9.1 Hz, H-2'), 5.99 (dd, 1H, H-4'), 7.06-8.00 (m, 35H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 55.0, 61.4, 68.0, 68.3, 69.5, 70.5, 70.8, 71.6, 73.5, 73.7, 74.8, 75.1, 78.5, 80.6, 97.6, 97.7, 100.9, 127.5, 127.7 (x4), 128.0 (x4), 128.3 (x3), 128.4 (x5), 128.5 (x2), 128.7, 128.9, 129.3, 129.4, 128.8, 133.2, 137.8, 137.9, 138.5, 165.2, 165.4, 165.5, 165.6 ppm; ESI-TOF [M + Na]$^+$: calcd for $[C_{62}H_{58}O_{15}Na]^+$ 1065.3668; found: 1065.3629.

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.28). The title compound was obtained from donor **3.10** and acceptor **3.16**

by general glycosylation procedure in 96% yield as a colorless amorphous solid. Analytical data for **3.28:** R_f = 0.50 (ethyl acetate/ toluene, 0.5/9.5, v/v); $[\alpha]_D^{22}$ 53.3 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz): δ 3.42 (s, 3H, CH_3), 3.60-3.74 (m, 4H, H-4, 5, 6a, 6b), 3.97 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.33-4.67 (m, 9H, H-5', 6a', 6b', 6 x C*H*Ph), 5.09 (d, 1H, $J_{1,2}$ = 2.3 Hz, H-1), 5.15 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 5.58 (dd, 1H, $J_{3',4'}$ = 2.8 Hz, H-3'), 5.95-5.99 (m, 2H, H-2', 4'), 6.97-8.08 (m, 35H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 55.3, 55.4, 62.2, 68.1, 68.4, 69.6, 69.9, 71.4, 72.1, 73.4, 74.9, 75.2, 80.8, 82.2, 99.4, 102.7, 127.0 (x3), 127.5, 127.6, 127.7 (x3), 127.8, 128.0 (x4), 128.1 (x9), 128.5 (x6), 128.8, 128.9, 129.1, 129.7 (x5), 130.0 (x2), 133.2, 137.9, 138.0, 138.4, 165.0, 165.5, 165.6, 166.0 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{62}H_{58}O_{15}Na]^+$ 1065.3668; found: 1065.3627.

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.29). The title compound was obtained from donor **3.12** and acceptor **3.13** by general glycosylation procedure in 70% yield as a white amorphous solid. Analytical data for **3.29** was in accordance with that previously reported.[104]

***Methyl* 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.30).** The title compound was obtained from donor **3.12** and acceptor **3.14** by the general glycosylation procedure in 80% yield as a clear syrup. Analytical data for **3.30** was in accordance with that previously reported.[104]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.31). The title compound was obtained from donor **3.12** and acceptor **3.15** by the general glycosylation procedure in 93% yield as a clear syrup. Analytical data for **3.31** was in accordance with that previously reported.[106]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.32). The title compound was obtained from donor **3.12** and acceptor **3.16** by the general glycosylation procedure in 92 % yield as a clear syrup. Analytical data for **3.32** was in accordance with that previously reported.[107]

3.4.5. Competition Experiments

Experiment A. A mixture of ethylthio glucoside **3.33** (26.0 mg, 0.044 mmol), methyl thioindolyl glucoside **3.5** (30.0 mg, 0.044 mmol), acceptor **3.13** (40.6 mg, 0.087 mmol), and 3 Å molecular sieves (80 mg) in dichloroethane (1.0 mL) was stirred under argon for 1 h at rt. After that, triflic acid (0.78 μL, 0.0087 mmol) was added followed by the addition of NIS (11.8 mg, 0.0524 mmol), and the resulting mixture was stirred for 5 h at rt. The solid was filtered off through a pad of Celite, rinsed successively with dichloromethane, and the combined filtrate (~30 mL) was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane 5% gradient elution). The unreacted donor **3.5** was obtained as SEt adduct **3.35** in 86% yield. The identity of **3.35** was confirmed by ^{1}H NMR (see the SI) and mass spectrometry: ESI-TOF $[M + Na]^+$: Calcd for. $[C_{45}H_{47}NO_5S_2Na]^+$ 768.2788, found: 768.2743. A competition experiment with allyl thioindolyl glucoside **3.8** (30 mg, 0.042 mmol) instead of donor **3.5** was performed in a similar manner. The unreacted donor **3.8** was obtained as SEt adduct **3.36** in 91% yield. The identity of **3.36** was confirmed by ^{1}H NMR (see the SI) and mass spectrometry: ESI-TOF $[M+Na]^+$: Calcd for. $[C_{47}H_{49}NO_5S_2+Na]^+$ 794.2944, found: 794.2945.

Experiment B. A mixture of ethylthio glucoside **3.33** (26.0 mg, 0.044mmol), methyl thioindolyl glucoside **3.5** (30 mg, 0.044 mmol), glucosyl acceptor **3.13** (40.6 mg, 0.087 mmol), and 3 Å molecular sieves (80 mg) in dichloroethane (1.0 mL) was stirred under argon for 15 min at rt. After that, Ag_2CO_3 (9.6 mg, 0.035 mmol) and TMSOTf (31.6 μL, 0.175 mmol) were added, and the resulting mixture was stirred under argon for 15 min at rt. The reaction was quenched with triethyl amine (~0.2 mL), the solid was filtered off through a pad of Celite, rinsed successively with dichloromethane, and the combined filtrate (~30 mL) was washed with 10% aq. $Na_2S_2O_3$ (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane 5% gradient elution). The unreacted donor **3.33** was recovered in 92% yield and its identity was confirmed by ^{1}H NMR and mass spectroscopy. A competition experiment with allyl thioindolyl glucoside **3.8** (30 mg, 0.042 mmol) instead of donor **3.5** was performed in a similar manner. The unreacted donor **3.33** was recovered in 94% yield and its identity was confirmed by ^{1}H NMR and mass spectroscopy.

Experiment C. A mixture of *S*-benzoxazolyl glucoside **3.34** (29.5 mg, 0.044 mmol), methyl thioindolyl glucoside **3.5** (30.0 mg, 0.044 mmol), glucosyl acceptor **3.11** (40.6 mg, 0.087 mmol), and 3 Å molecular sieves (80 mg) in dichloroethane (1.0 mL) was stirred under argon for 1 h at rt. After that, freshly activated AgOTf (22.5 mg, 0.087 mmol) was added, and the resulting mixture was stirred for 5 min at rt. The solid was filtered off through a pad of Celite, rinsed successively with dichloromethane, and the combined filtrate (~30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane 5% gradient elution). The unreacted donor **3.5** was

recovered in 91% yield and its identity was confirmed by ^{1}H NMR and mass spectroscopy. A competition experiment with allyl thioindolyl glucoside **3.8** (30 mg, 0.042 mmol) instead of donor **3.5** was performed in a similar manner. The unreacted donor **8** was recovered in 95% yield and its identity was confirmed by ^{1}H NMR and mass spectroscopy.

3.4.6. ^{1}H NMR monitoring experiments

A typical experiment in the presence of NIS. A solution of SInMe donor **3.2** (0.0404 mmol) or SInAll donor **6** (0.0391 mmol) and NIS (0.0391-0.1173 mmol) in $CDCl_3$ (1.0 mL) was stirred in a RB flask under argon for 30 min at rt. The resulting solution was transferred into a standard 5 mm NMR tube and ^{1}H NMR spectra recorded at 10 min, 1 h, 16 h timepoints or as needed. The recorded spectra are presented in the appendix.

A typical experiment in the presence of TMSOTf. A solution of SInMe donor **3.2** (0.0404 mmol) or SInAll donor **3.6** (0.0391 mmol) and TMSOTf (0.040-0.1564 mmol) in $CDCl_3$ (1.0 mL) was stirred in a RB flask under argon for 5 min at rt. The resulting solution was transferred into a standard 5 mm NMR tube and ^{1}H NMR spectra recorded at 10 min, 30 min, 1 h, 16 h timepoints or as needed. The recorded spectra are presented in the appendix.

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CHAPTER 4

Streamlined access to carbohydrate building blocks: methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside

4.1. Introduction

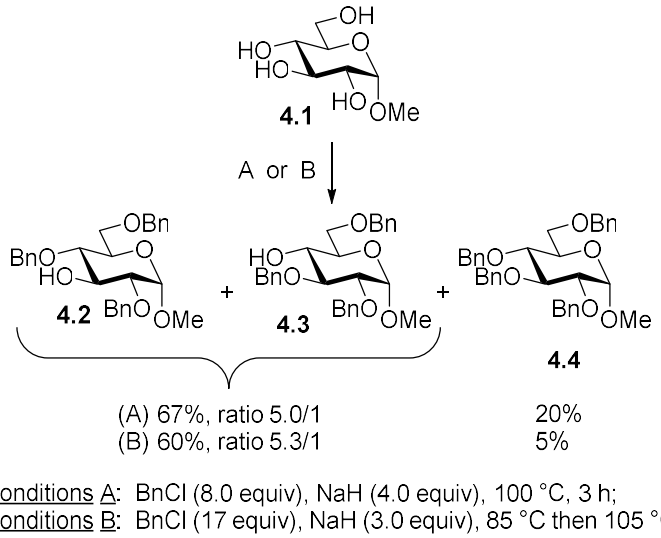
The development of accessible methods for glycan production is essential for further innovations and practical applications in all areas of glycosciences.[65] Poor accessibility to sugar building blocks hampers development of all synthetic methodologies and strategies platforms. *"Unlike the synthesis of peptides and oligonucleotides, there are no universal building blocks or methods for the synthesis of all glycans."*[109] Researchers experience significant setbacks because they have to continue to remake simple building blocks. As Seeberger noted *"differentially protected monosaccharide building blocks is currently the bottleneck for chemical synthesis."*[110] Some 15 years later, most bench time is still dedicated to making building blocks. In contribution to the global effort of the glycoscience community towards making building blocks more accessible, reported herein is the development of selectively protected building blocks of D-glucose as advanced synthetic intermediates.

D-Glucose, the predominant monosaccharide in Nature, is also among major components of bacterial glycans and the mammalian glycome.[111] D-Glucose building blocks are often first compounds tested in new reactions and applications. Every synthetic glycoscience lab makes glucose building blocks. Paradoxically, it remains the hardest sugar for selective protection due to its *trans-trans-trans*, all-equatorial 2,3,4-triol arrangement. Recently, we described a improved direct synthesis of glucose 1,3,4,6-tetra-*O*-acetate (2-OH glucose),[112,113] and herein we report a streamlined synthesis of 3-OH glucose. Also obtained alongside, albeit in minor quantities, is 4-OH glucose, another common building block.

4.2. Results and Discussion

Benzyl protecting group is widely used in carbohydrate chemistry due to its stability. However, the direct regioselective benzylation of polyols is fairly rare.[114] One such example was described by Koto *et al*. wherein methyl glucoside **4.1** was converted into methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside **4.2** in one step in 61% yield.[115] According to Koto *et al*., this was achieved by controlled heating with neat BnCl in the presence of NaH added portionwise. To adapt this procedure to a large-scale preparation of compound **4.2** that is used as a common glycosyl acceptor we attempted to reproduce this procedure. Benzylation of methyl α-D-glucopyranoside **4.1** was carried out with benzyl chloride in presence of sodium hydride for 3 h at 100 °C as depicted in Scheme 4.1. We indeed found that the main component of the reaction mixture was 3-OH derivative **4.2**, along with its 4-OH regioisomer **4.3**, and per-benzylated derivative **4.4**. The presence of the side products was not disclosed by Koto *et al*.

Scheme 4.1. Selective benzylation of methyl α-D-glucopyranoside 4.1



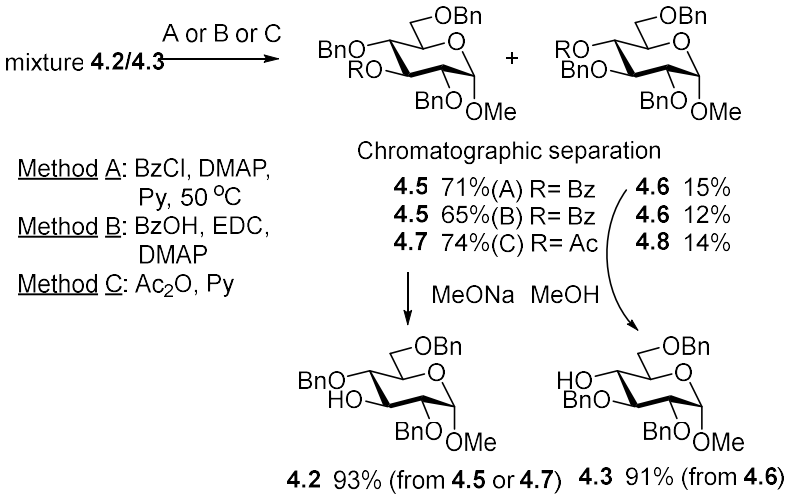
In an effort of separating the reaction mixture by column chromatography we managed to separate off compound **4.4** fairly easily, and it was isolated in 5 to 20% yield depending on the

reaction conditions employed. However, separation of regioisomers **4.2** and **4.3** was problematic. It should be noted that the chromatography solvent system reported by Koto, benzene/2-butanone, was neither available to us nor desirable for safety reasons. Our best attempt involved column chromatography using 0.2% gradient elution of ethyl acetate in dichloromethane. This allowed us to isolate pure **4.2** in 29% yield. A better separation of the mixture of compounds **4.2** and **4.3** by TLC was achieved using a ternary system comprising hexane/ DCM/ ethyl acetate (2.0/2.5/0.25, v/v/v). However, we could not achieve improved separation using column chromatography using this system.

To achieve a more reliable separation of the mixture of compounds **4.2** and **4.3**, we decided to derivatize the entire mixture. In the first route, benzoylation was chosen, which was affected using benzoyl chloride in the presence of *N*,*N*-dimethylaminopyridine (DMAP) in dry pyridine for 2.5 h at 50 °C. This afforded a mixture of compounds **4.5** and **4.6**, which was easily separated by column chromatography to afford 3-*O*-benzoylated compound **4.5**[116] in 71% yield. Also obtained was 4-*O*-benzoylated compound **4.6**[117] in 15% yield. Alternatively, benzoylation could be achieved using benzoic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and DMAP in 1,2-dichloroethane at 50 °C. Under these reaction conditions, compounds **4.5** and **4.6** were obtained in 65% and 12% yield, respectively.

Alternatively, acetylation of mixture of compounds **4.2** and **4.3** also allowed a straightforward chromatographic separation of the resulting mixture leading to 3-*O*-acetyl derivative **4.7** in 74% yield and its 4-*O*-acetyl counterpart **4.8**[118] in 14% yield (Scheme 4.2). Finally, deprotection of compounds **4.5**, **4.6** and **4.7** under Zemplen conditions produced the respective derivatives **4.2** and **4.3** in 91-93% yield as depicted in Scheme 4.2.

Scheme 4.2. Separation of the mixture of 4.2 and 4.3 via acylation-purification-saponification sequence



4.3. Conclusions

Reported herein is an improved synthesis of a common 3-OH glycosyl acceptor. Routinely synthesized by many research groups, this building block is commonly used as a model glycosyl acceptor. The only known direct synthesis by Koto lacks regioselectivity and could not be reproduced accurately because it relies on chromatography using hazardous solvents. This is the main reason others still synthesize 3-OH acceptor via multistep derivatization approaches.[119,120] While our synthetic approach relies on Koto's selective benzylation, it is followed by acylation-purification-deacylation sequence. These additional steps are straightforward and high-yielding, and the main payoff is in the simplified separation of the mixture of regioisomers. Also obtained, albeit in minor quantities, is 4-OH acceptor, another common building block.

4.4. Experimental Part

4.4.1. *General methods.*

The reactions were performed using commercial reagents. 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 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. $ClCH_2CH_2Cl$ (1,2-DCE) was distilled from CaH_2 directly prior to application. Optical rotations were measured at 'Jasco P-2000' polarimeter. ^1H NMR spectra were recorded in $CDCl_3$ at 300 or 400 MHz, ^{13}C NMR spectra were recorded at 75 or 100 MHz. The ^1H NMR chemical shifts are referenced to tetramethyl silane (TMS, δ_H = 0 ppm) or $CDCl_3$ (δ_H = 7.26 ppm) for ^1H NMR spectra for solutions in $CDCl_3$. The ^{13}C NMR chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.00 ppm) for solutions in $CDCl_3$. Compound purity or compound ratios were accessed or calculated by comparing of the integration intensities of the relevant signals in their ^1H NMR spectra. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer.

Methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (4.2) and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.3).

Conditions A. A 60% suspension of sodium hydride in mineral oil (1.6 g, 40.01 mmol) was added to a mixture containing methyl α-D-glucopyranoside (**4.1**, 5.0 g, 25.75 mmol) and benzyl chloride (23.7 mL, 206 mmol), and the resulting mixture was stirred under argon for 5 min at rt. After that, additional sodium hydride (60% suspension in mineral oil, 1.07 g, 26.76 mmol) was added, and the resulting mixture was heated for 3 h at 100 °C. Additional sodium

hydride (60% suspension in mineral oil, 1.50 g, 36.0 mmol) was then added, and the resulting mixture was heated for 2 h at 100 °C. The reaction mixture was allowed to cool to rt, poured into ice-water (100 mL) and stirred for 30 min. The mixture was extracted with CH_2Cl_2 (3 x 100 mL), and the combined organic extract was washed with water (2 x 50 mL). The organic phase was separated, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene, 5% gradient elution). Eluted first was methyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside **4.4** isolated in 20% yield (2.84 g, 5.12 mmol). Also eluted was a mixture of compounds **4.2** and **4.3** as a clear syrup in a combined yield of 67% yield (7.95 g, 17.11 mmol, ratio 5.0/1). Second separation of the mixture of compounds **4.2** and **4.3** (3.0 g, 6.46 mmol) by column chromatography on silica gel (ethyl acetate–dichloromethane, 0.2% gradient elution) allowed pure compound **4.2** as a clear syrup in 29% yield (0.88 g, 1.9 mmol). Analytical data for **4.2**: R_f = 0.45 (ethyl acetate/ hexane, 3/7, v/v); $[\alpha]_D^{20}$ +40.1 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ 2.36 (s, 1H, 3-OH), 3.33 (s, 3H, CH_3), 3.43 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.69 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 3.69-3.74 (m, 3H, H-5, 6a, 6b), 4.10 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.10-4.81 (m, 7H, H-1, 3 x C*H*$_2$Ph), 7.20-7.37 (m, 15H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): 55.1, 68.3, 69.5, 73.0, 73.4, 73.5, 74.5, 77.3,79.3, 97.4, 127.5, 127.6, 127.7 (x2), 127.8 (x2), 127.9, 128.0 (x2), 128.3 (x4), 128.5 (x2), 137.7, 137.7, 138.3 ppm; HRMS [M + Na]$^+$: calcd for $[C_{28}H_{32}O_6Na]^+$ 487.2097; found: 487.2098.

Conditions B. A 60% suspension of sodium hydride in mineral oil (1.03 g, 25,75 mmol) was added portionwise to a vigorously stirred mixture of methyl α-D-glucopyranoside (**4.1**, 10.1 g, 52 mmol) in benzyl chloride (100 mL, 869 mmol) under argon at 85°C. After 15 min, the reaction temperature was increased to 105 °C and additional NaH (60% in mineral oil, 5.24 g,

131 mmol) was added portionwise over a period of 15 min, and the resulting mixture was stirred for 3 h at 105 °C. After that, the reaction mixture was allowed to cool to rt and subjected to a work-up sequence described in Conditions A. The crude residue was purified by column chromatography on silica gel (hexane, hexane-toluene, ethyl acetate – toluene, 5% gradient elution). Eluted first was methyl 2,3,4,6-tetra-*O*-benzyl α-D-glucopyranoside **4.4** isolated in 5% yield (2.0 g, 2.44 mmol). Also eluted was a mixture of compounds **4.2** and **4.3** as a clear syrup in a combined yield of 60% yield (14.5 g, 31.1 mmol, ratio 5.3/1).

Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (4.5) and methyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.6).

Method A. *N*,*N*-dimethylaminopyridine (DMAP, 0.11 g, 0.86 mmol) and benzoyl chloride (3.6 g, 25.83 mmol) were added to a solution of compounds **4.2** and **4.3** (2.0 g, 4.3 mmol) in dry pyridine (25 mL), and the resulting mixture was stirred under argon for 2.5 h at 50 °C. After that, the reaction mixture was allowed to cool to rt, MeOH (~5 mL) was added, the volatiles were removed under reduced pressure, and the residue was co-evaporated with toluene (2 x 20 mL). The resulting residue was diluted with dichloromethane (~200 mL) and washed with water (2 x 50 mL). The organic phase was separated, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-dichloromethane, 1% gradient elution). Eluted first was compound **4.6** obtained as a clear syrup in 15% yield (0.37 g, 0.64 mmol). Also eluted was compound **4.5** obtained as a clear syrup in 71% yield (1.74 g, 3.06 mmol). Analytical data for **4.5**: R_f = 0.40 (ethyl acetate/ toluene, 15/85, v/v); $[\alpha]_D^{20}$ +10.4 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ 3.40 (s, 3H, CH_3), 3.61-3.75 (m, 2H, H-2, 6b), 3.76-3.83 (m, 3H, H-4, 5, 6a), 4.36 (d, 1H, ^2J = 10.7 Hz, C*H*Ph), 4.45-4.56 (m, 4H, 2 x CH_2Ph), 4.69 (d, 1H, ^2J = 12 Hz, C*H*Ph), 4.76 (d, 1H, $J_{1,2}$ =

3.4 Hz, H-1), 5.80 (dd, 1H, $J_{3,4}$ = 9 Hz, H-3), 6.97-8.03 (m, 20H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 55.2, 69.6, 72.5, 73.5, 74.2, 74.3, 75.8, 76.9, 76.9 , 97.7, 127.5, 127.6, 127.7, 127.8 (x2), 127.9 (x2), 128.0 (x2), 128.1 (x2), 128.2 (x4), 128.3 (x2), 129.6 (x2), 130.2, 132.8, 137.4, 137.6 (x2), 165.3 ppm; HRMS $[M + Na]^+$: calcd for $[C_{35}H_{36}O_7Na]^+$ 591.2359; found:591.2359. Analytical data for **4.6**: R_f= 0.55 (ethyl acetate/ toluene, 15/85, v/v); $[\alpha]_D^{20}$ -10.3 (*c* = 1.0, $CHCl_3$); ^1H n.m.r. (300 MHz): δ 3.44 (s, 3H, CH_3), 3.51-3.55 (m, 2H, H-6a, 6b), 3.69 (dd, 1H, $J_{1,2}$ = 3.5 Hz, H-2), 3.99 (m, 1H, H-5), 4.1 (dd, $J_{3,4}$ = 9.5 Hz, H-3), 4.47-4.85 (m, 7H, H-1, 3 x CH_2Ph), 5.35 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 7.96-7.10 (m, 20 H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 55.3, 68.8, 70.8 , 73.4, 73.5, 75.3, 77.2, 79.1, 79.4, 98.2, 127.3, 127.4, 127.6 (x2), 127.9 (x4), 128.0 (x3), 128.1 (x4), 128.2, 128.4 (x2), 129.6 (x2), 133.0, 137.6.0, 137.9 , 138.0.4, 165.2 ppm; HRMS $[M + Na]^+$: calcd for $[C_{35}H_{36}O_7Na]^+$ 591.2359; found: 591.2367.

Method B. Benzoic acid (6.26 g, 51.3 mmol), *N*,*N*-dimethylaminopyridine (DMAP, 0.418 g, 3.42 mmol), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 6.5 g, 34.2 mmol) were added to a solution of compounds **4.2** and **4.3** (7.95 g, 17.11 mmol) in 1,2-dichloroethane (35 mL), and the resulting mixture was stirred under argon for 96 h at 50 °C. The resulting mixture was allowed to cool to rt, diluted with dichloromethane (~400 mL), and washed with water (50 mL), Na_2CO_3 (2 x 50 mL), and water (2 x 50 mL). The organic phase was separated, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-toluene, 5% gradient elution). Eluted first was compound **4.6** obtained as a clear syrup in 12% yield (1.14 g, 2.0 mmol). Also eluted was compound **4.5** obtained as a clear syrup in 65% yield (6.31 g, 11.09 mmol).

Methyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (4.7) and methyl 4-*O*-aceyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.8).

Method C. Acetic anhydride (4.6 mL, 48.7 mmol) was added to a solution of compounds **4.2** and **4.3** (7.09 g, 15.3 mmol) in dry pyridine (30 mL), and the resulting mixture was stirred for 2 h at rt. After that, MeOH (~5 mL) was added, the volatiles were removed under reduced pressure, and the residue was co-evaporated with toluene (2 x 20 mL). The residue was purified by column chromatography on silica gel (ethyl acetate-hexane, 5% gradient elution). Eluted first was compound **4.8** obtained as a clear syrup in 14% yield (1.10 g, 2.17 mmol). Also eluted was compound **4.7** obtained as a clear syrup in 74% yield (5.75 g, 11.35 mmol). Analytical data for **4.8**: R_f = 0.40 (ethyl acetate/ hexane, 1/3, v/v); $[\alpha]_D^{22}$ +1.84 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (400 MHz): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81 (s, 3H, $COCH_3$), 3.40 (s, 3H, OCH_3), 3.44 (dd, 1H, $J_{6a,6b}$ = 10.7 Hz, H-6a), 3.49 (dd, 1H, H-6b), 3.58 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.81 (m, 1H, $J_{5,6a}$ = 5.0, $J_{5,6b}$ = 2.9 Hz, H-5), 3.91 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 4.48 (dd, 2H, ^2J = 11.2 Hz, C*H*Ph), 4.61 (d, 1H, *J* = 3.6 Hz, H-1), 4.71 (dd, 1H, ^2J = 12.1 Hz, CH_2Ph), 4.76 (dd, 1H, ^2J = 11.7 Hz, CH_2Ph), 5.02 (dd, 1H, $J_{4,5}$ = 10.2 Hz, H-4), 7.24–7.36 (m, 15H, aromatic) ppm; ^{13}C{^{1}H} NMR (100 MHz): δ 20.8, 55.4, 68.2, 69.0, 70.6, 73.5, 73.6, 75.3, 79.3, 79.6, 98.2, 127.6 (x2), 127.9 (x2), 128.0, 128.2, 128.4, 128.5, 137.9, 138.0, 138.7, 169.7 ppm; HRMS $[M + Na]^+$: calcd for $[C_{30}H_{34}NaO7]^+$ 529.2202; found: 529.2164. Analytical data for **7**: R_f = 0.50 (ethyl acetate/ hexane, 10/30, v/v); $[\alpha]_D^{22}$ +8.17 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.93 (s, 3H, $COCH_3$), 3.36 (s, 3H, OCH_3), 3.48 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 3.64 (dd, 1H, $J_{6a,6b}$ = 10.7 Hz, H-6a), 3.66 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.73 (dd, 1H, H-6b), 3.79 (m, 1H, $J_{5,6a}$ = 2.1, $J_{5,6b}$ = 3.3 Hz, H-5), 4.46 (dd, 2H, ^2J = 11.2 Hz, CH_2Ph), 4.54 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.59 (s, 2H, CH_2Ph), 4.70 (d, $J_{1,2}$ = 3.5 Hz, H-1), 5.51 (dd, 1H, $J_{3,4}$ = 9.6

Hz, H-3), 7.12-7.14 (m, 2H, aromatic), 7.24-7.34 (m, 13H, aromatic) ppm; $^{13}C\{^{1}H\}$ NMR (100 MHz): δ 21.1, 55.3, 68.2, 69.8, 72.8, 73.6, 74.3, 76.2, 77.5, 97.9, 127.8, 127.9, 128.5, 137.8, 138.0, 138.1, 169.9 ppm; HRMS $[M + Na]^+$: calcd for $[C_{30}H_{34}NaO7]^+$ 529.2202; found: 529.2153

Methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (4.2).

A 1 M solution of sodium methoxide in methanol (~25 mL) was added dropwise to a solution of compound **4.5** (6.31 g, 11.09 mmol) in methanol (120 mL) till pH > 9, and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was neutralized with Amberlite (H^+) ion exchange resin. The resin was filtered off and rinsed successively with methanol. The combined filtrate (~250 mL) was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane, 10% gradient elution) to afford the title compound as a clear syrup in 93% yield (4.79 g, 10.31 mmol). A similar protocol was applied to compound **4.7** (4.54 g, 8.97 mmol) that was converted in compound **4.2** in 93% yield (3.86g. 8.32 mmol).

Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (4.3).

A 1 M solution of sodium methoxide in methanol (5 mL) was added dropwise to a solution of compound **4.5** (1.14 g, 2.00 mmol) in methanol (45 mL) till pH > 9, and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was neutralized with Amberlite (H^+) ion exchange resin. The resin was filtered off and rinsed successively with methanol (7 x 10 mL). The combined filtrate (~75 mL) was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane, 10% gradient solution) to afford the title compound as a clear syrup in 91% yield (0.85 g, 1.83 mmol). Analytical data for **4.3**: R_f = 0.45 (ethyl acetate/ hexane, 3/7, v/v); $[\alpha]_D^{20}$ +7.1 (*c* = 1.0, $CHCl_3$);

^{1}H n.m.r. (300 MHz): δ 2.33 (s, 1H, 4-OH), 3.38 (s, 3H, CH_3), 3.55 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-2), 3.60-3.72 (m, 4H, H-4, 5, 6a, 6b), 3.78 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 4.51-5.02 (m, 7H, H-1, 3 x CH_2Ph), 7.25-7.37 (m, 15H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 55.2, 69.3, 69.8, 70.6, 73.1, 73.5, 75.4, 79.5, 81.4, 98.7, 127.6, 127.8, 127.9 (x2), 128.0 (x2), 128.1, 128.3 (x2), 128.4 (x4), 128.5 (x2), 137.9, 137.7, 138.7 ppm; HRMS $[M + Na]^+$: calcd for $[C_{28}H_{32}O_6Na]^+$ 487.2097; found: 487.2102.

4.5. References.

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CHAPTER 5

SFox imidates as versatile glycosyl donors for chemical glycosylation

5.1. Introduction

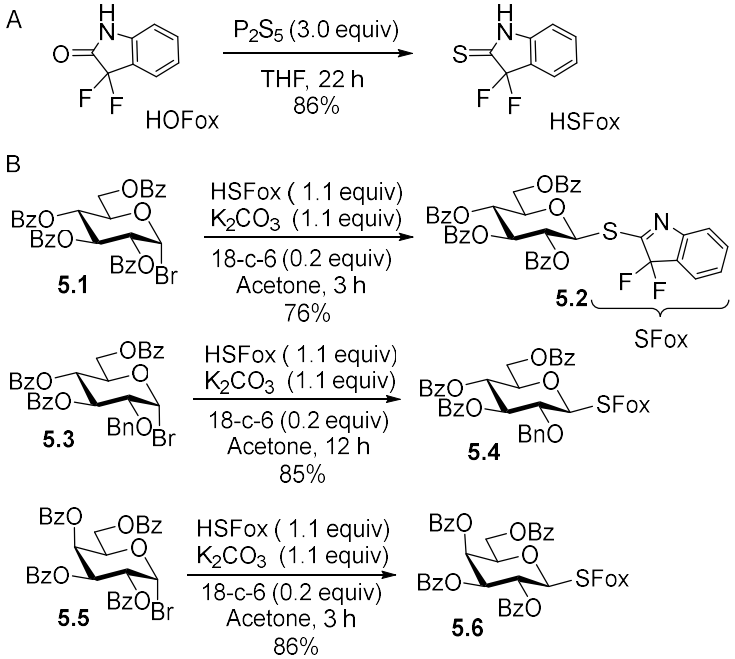
All glycans, both natural and synthetic, are connected via *O*-glycosidic linkages, but synthesizing these linkages by chemical methods remains challenging. Many methods for chemical glycosylation have been introduced, the complexity of the glycosylation reaction demands further improvement.[65] A majority of all glycosylations are performed with thioglycosides[26] and *O*-trichloroacetimidates (TCAI),[25] but *N*-phenyl trifluoroacetimidates (PTFAI) introduced by Yu and co-workers[121,122] have also been gaining a considerable niche amongst methods used for chemical glycosylation. Inspired by excellent results achieved with the PTFAI donors, previously we introduced the 3,3-difluoro-3*H*-indol-2-yl (OFox) leaving group. This leaving group comprised a hybrid structure between PTFAI and *S/O*-benzoxazolyl (SBox/OBox) leaving groups developed by our laboratory.[33,104] While investigating approaches to their synthesis, we observed that OFox imidates can be obtained and glycosidated in a catalytic regenerative fashion *in situ*. This led to the development of 3,3-difluoroindoline-2-one (HOFox)-mediated regenerative glycosylation approach wherein the OFox imidates were found to be key intermediates.[123] In accordance with the approach, thioglycoside precursors were first converted into the corresponding glycosyl bromides that were then converted into the OFox imidates in the presence of Ag_2O, and the OFox imidates were then activated with a catalytic Lewis acid. This approach was further refined in application of several glycoside and glycan targets.[107] More recently, we reported a direct conversion of thioglycosides via the regenerative approach that bypasses the intermediacy of bromides and eliminates the need for heavy metal-based promoters, such as Ag_2O. This direct regenerative activation of thioglycosides was achieved under neutral reaction conditions using only 1.0 equiv *N*-iodosuccinimide (NIS) and catalytic HOFox without acidic additives.[124]

Described herein is a continuation of this study with the main focus on the synthesis and glycosidation of various 3,3-difluoro-3*H*-indol-2-ylthio (SFox) imidates and their investigation as glycosyl donors for chemical glycosylation. SFox imidates represent a hybrid structure between OFox and conventional thioglycoside/thioimidates. Being thioimidates, SFox derivatives are expected to be more stable than their *O*-linked counterparts albeit more reactive than conventional alkyl/aryl thioglycosides.

5.2. Results and Discussion

HOFox was synthesized as described previously from the commercially available Isatin with diethylaminosulfur trifluoride (DAST) in anhydrous CH_2Cl_2 at room temperature.[123,125] As depicted in Scheme 5.1A, HOFox was then treated with P_2S_5 in tetrahydrofuran (THF) to afford 3,3-difluoroindoline-2-thione (HSFox) the in 86% yield 22 h.

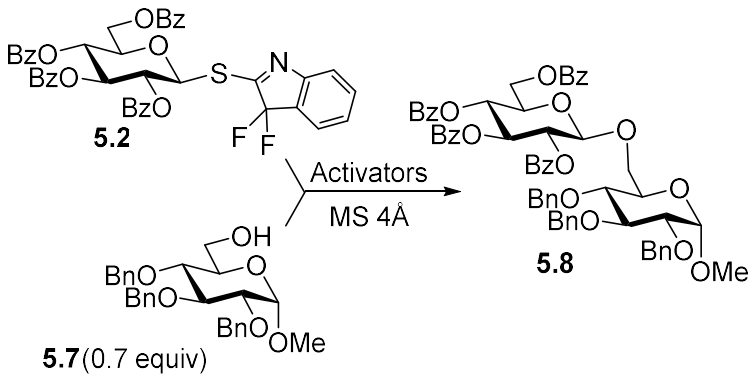
Scheme 5.1. Synthesis of HSFox aglycone (A) and SFox glycosyl donors **5.2**, **5.4**, and **5.6** (B).



Starting from glycosyl bromide precursors, several SFox glycosyl donors were prepared in the presence of potassium carbonate and 18-crown-6 (1.1 equiv each) in dry acetone. Thus, per-benzoylated glucosyl bromide **5.1**[72] was converted into SFox glucosyl donor **5.2** in 76% yield. SFox glucosyl donor **5.4** equipped with the superdisarming protecting group pattern (2-*O*-benzyl-3,4,6-tri-*O*-benzoyl)[83] was prepared from bromide precursor **5.3**[126] in 85% yield. Per-benzoylated SFox galactosyl donor **5.6** was synthesized from galactosyl bromide **5.5**[108] under similar reaction conditions in 86% yield.

With these novel glycosyl donors in hand, we performed a preliminary screening of reaction conditions for their activation in glycosylation using SFox donor **5.2** and standard glycosyl acceptor **5.7**.[55] The key results of this study that included identification of suitable promoters and determining the optimal amounts of promoters are summarized in Table 5.1. The initial set of activators of interest included NIS/TfOH, a common thiophilic promoter system that found broad application for the activation of alkyl/aryl thioglycosides.[82] Also selected were TMSOTf, AgOTf, and $FeCl_3$ as representative Lewis acids, and TfOH as a protic acid, all of which have found broad application in carbohydrate chemistry, including glycosylation reactions with reactive glycosyl imidates or halides. In the presence of 3.0 equiv of NIS and 0.2 equiv of TfOH, standard conditions for the activation of *S*-alkyl/aryl glycosides, glycosidation of SFox imidate donor **5.2** with glycosyl acceptor **7** lead to the formation of disaccharide **5.8**[77] in an excellent yield of 98% within 5 min (entry 1, Table 5.1). Glycosidation of SFox donor **5.2** with acceptor **5.7** was also possible in the presence of TfOH alone (1.0 equiv), and disaccharide **5.8** was obtained in 89% in 5 min (entry 2).

Table 5.1. Screening of reaction conditions for glycosidation of SFox donor **5.2** with 6-OH acceptor **5.7**

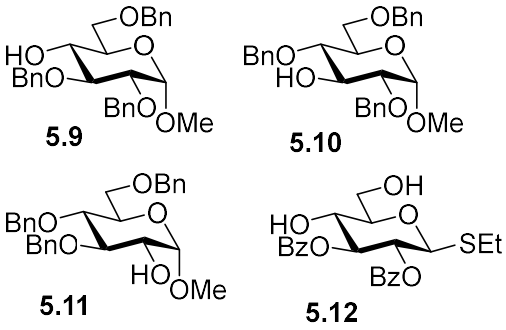


Entry	Conditions	Yield of **5.8**
1	NIS (3.0 equiv), TfOH (0.2 equiv), 1,2-DCE, rt, 5 min	98%
2	TfOH (1.0 equiv), DCM, rt, 5 min	89%
3	TMSOTf (1.0 equiv), DCM, rt, 5 min	97%
4	AgOTf (2.0 equiv), 1,2-DCE, rt, 5 min	99%
5	$FeCl_3$ (2.0 equiv), 1,2-DCE, rt, 18 h	89%
6	$FeCl_3$ (3.0 equiv), 1,2-DCE, rt, 9 h	95%

Next, we moved on to investigating suitability of popular Lewis acid promoters for the activation of the SFox leaving group. When 1.0 equiv of TMSOTf was used, disaccharide **5.8** was obtained in 97% yield in 5 min (entry 3). When a similar reaction of SFox donor **5.2** with acceptor **5.7** was performed in the presence of 2.0 equiv of AgOTf, disaccharide **5.8** was afforded in 99% within 5 min (entry 4). Glycosidation of SFox donor **5.2** with primary acceptor **5.7** in the presence of 2.0 equiv of $FeCl_3$ in 1,2 dichloroethane (1,2-DCE) afforded disaccharide

5.8 in a good yield of 89% in 18 h (entry 5). The reaction proceeded fast in the beginning, but then got stumbled. Increasing the amount of $FeCl_3$ to 3.0 equiv allowed to shorten the reaction time to 9 h and increase the yield of disaccharide **5.8** to 95% (entry 6). While all conditions listed in Table 5.1 provided excellent results, we decided to carry forward reactions in the presence of easily available and relatively stable TfOH and TMSOTf. For the new set of experiments we chose additional glycosyl acceptors **5.9-5.12**[55] depicted in Figure 5.1, and the results of this study are summarized in Table 5.2.

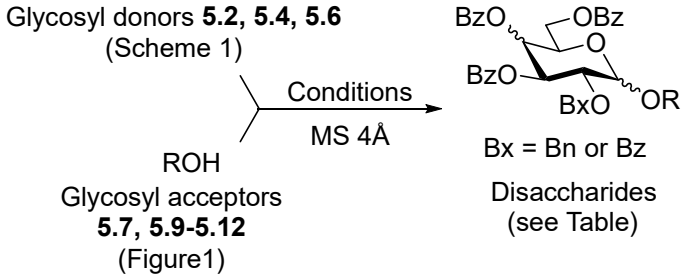
Figure 5.1. Additional glycosyl acceptors **5.9-5.12** used in this study

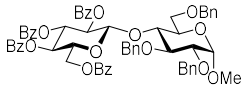


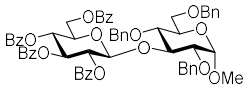
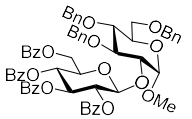
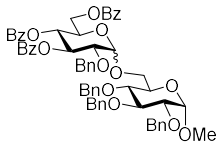
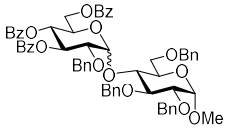
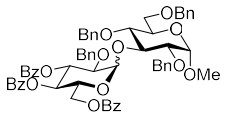
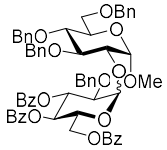
First set of experiments included glycosidation of SFox donor **5.2** with secondary glycosyl acceptors **5.9-5.11** in the presence of TfOH (1.0 equiv) using previously established reaction conditions. These experiments led to the formation of the respective disaccharides **5.13**[77], **5.14**,[55] and **5.15**[58] in good yields ranging between 72%-87% (Table 5.2, entries 1-3). The coupling with the secondary glycosyl acceptors were notably slower, 0.5-1 h in comparison to that with the primary acceptor **5.7** that we typically completed within 5 min. The rate of glycosidations of SFox donor **5.2** with secondary glycosyl acceptors **5.9-5.11** in the presence of TMSOTf (1.0 equiv) was also similar, and the synthesis of disaccharides **5.13-5.15** were completed in 25-60 min. The yields, however, were noticeably higher, and the disaccharides were obtained in excellent yields of 93-96% (entries 4-6).

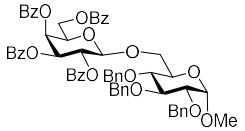
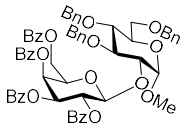
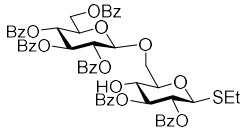
Subsequently, we extended our study to glycosylation of SFox glucosyl donor **5.4** equipped with the superdisarming protecting group pattern, 2-*O*-benzyl-3,4,6-tri-*O*-benzoyl. Not surprisingly, the reaction with donor **5.4** was much slower, and even glycosylation of the primary 6-OH acceptor **5.7** in presence of TfOH (1.0 equiv) required 20 h to produce disaccharide **5.16**[126] in 80%. The stereocontrol of this reaction was excellent (α/β > 25/1, entry 7). Glycosidations of donor **5.4** with the secondary glycosyl acceptors **5.9-5.11** in the presence of TfOH (1.0 equiv) required 20-26 h to complete. The resulting respective disaccharides **5.17**,[126] **5.18**,[126] and **5.19**,[126] were obtained in 48%-70% yield and with complete α-selectivity (entries 8-10). We then investigated how well donor **5.4** would react in the presence of TMSOTf (1.0 equiv). The reactions with glycosyl acceptors **5.7** and **5.11** produced the respective disaccharides **5.16** and **5.19** in 69-76% yield and complete stereoselectivity (entries 11 and 12).

Table 5.2. Expanding the scope of glycosylation with SFox donors: synthesis of disaccharides **5.13**-**5.22**



Entry	Reactants and conditions	Product (yield, α/β ratio)
1	**5.2** + **5.9,** TfOH (1.0 equiv), DCM, rt, 1 h	 **5.13** (72%, β only)

2	**5.2** + **5.10**, TfOH (1.0 equiv), DCM, rt, 30 min,	 **5.14** (73%, β only)
3	**5.2** + **5.11**, TfOH (1.0 equiv), DCM, rt, 30 min,	 **5.15** (87%, β only)
4	**5.2** + **5.9,** TMSOTf (1.0 equiv), DCM, rt, 1 h	**5.13** (93%, β only)
5	**5.2** + **5.10**, TMSOTf (1.0 equiv), , rt, 25 min	**5.14** (94%, β only)
6	**5.2** + **5.11**, TMSOTf (1.0 equiv), DCM, rt, 40 min	**5.15** (96%, β only)
7	**5.4** + **5.7**, TfOH (1.0 equiv), 1,2-DCE, rt, 20 h	 **5.16** (80%, α/β > 25/1)
8	**5.4 + 5.9,** TfOH (1.0 equiv), 1,2-DCE, rt, 26 h	 **5.17** (48%, α/β > 25/1)
9	**5.4** + **5.10**, TfOH (1.0 equiv), 1,2-DCE, rt, 20 h	 **5.18** (65%, α/β > 25/1)
10	**5.4 + 5.11,** TfOH (1.0 equiv), 1,2-DCE, rt, 20 h	 **5.19** (70%, α/β > 25/1)
11	**5.4** + **5.7**, TMSOTf (1.0 equiv), rt, 20 h, DCE	**5.16** (76%, α/β > 25/1)
12	**5.4** + **5.11**, TMSOTf (1.0 equiv), 1,2-DCE, rt, 21 h	**5.19** (69%, α/β > 25/1)

13	**5.6** + **5.7**, TfOH(1.0 equiv), 1,2-DCE, rt, 5 min	 **5.20** (98%, β only)
14	**5.6** + **5.11**, TfOH (1.0 equiv), 1,2-DCE, rt, 20 min	 **5.21** (88%, β only)
15	**5.6** + **5.7**, TMSOTf (1.0 equiv), 1,2-DCE, rt, 5 min	**5.20** (98%, β only)
16	**5.6** + **5.11**, TMSOTf (1.0 equiv), 1,2-DCE, rt, 10 min	**5.21** (97%, β only)
17	**5.2** + **5.12**, TfOH (0.8 equiv), 1,2-DCE, 0 °C, 5 min	 **5.22** (70%, β only)
18	**5.2** + **5.12**, TMSOTf (0.6 equiv), 1,2-DCE, 0 °C, 5 min	**5.22** (72%, β only)

After that, we investigated glycosidations of SFox galactosyl donor **5.6** with the primary and secondary glycosyl acceptors **5.7** and **5.11**, respectively. The reaction of donor **5.6** with acceptor **5.7** in the presence of TfOH (1.0 equiv) afforded β-linked disaccharide **5.20**[104] in 98% yield within 5 min (entry 13). The glycosidation of donor **5.6** with 2-OH acceptor **5.11** afforded β-linked disaccharide **5.21**[105] in 88% yield in 20 min (entry 14). Glycosylations of acceptors **5.7** and **5.11** with donor **5.6** in the presence of TMSOTf (1.0 equiv) were also swift and efficient. The respective disaccharides **5.20** and **5.21** were obtained in 5-10 min in 97-98% yields as pure β-anomers (entries 15 and 16).

Next, the applicability of SFox donors to selective glycosylation over SEt glycosyl acceptors was tested. For this study, SFox donor **5.2** was selectively activated over 2,3-di-O-benzoyl SEt

glycosyl acceptor **5.12**. This glycosylation in the presence of either TfOH (0.8 equiv) or TMSOTf (0.6 equiv) at 0°C gave SEt disaccharide **5.22** in 70-72% yield in 5 min with complete stereo-, regio-, and chemoselectivity (entries 17-18).

5.3. Conclusions

We invented a new class of glycosyl donors that were preliminary investigated in reactions with several glycosyl acceptors. These SFox donors can be activated with protic acids, Lewis acids, or thiophilic reagents. The unique activation profile allows for selective activation of SFox imidates over conventional thioglycosides.

5.4. Experimental

5.4.1. *General methods.*

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 5% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to 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. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C NMR spectra were recorded at 75 MHz. The ^{1}H NMR chemical shifts were referenced to tetramethyl silane (TMS, δ H = 0 ppm) or $CDCl_3$ (δ H= 7.26 ppm)

for ^{1}H NMR spectra for solutions in $CDCl_3$. The ^{13}C NMR chemical shifts were referenced to the central signal of $CDCl_3$ (δ C = 77.00 ppm) for solutions in $CDCl_3$. HR FAB-MS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

5.4.2. Synthesis of SFox imidates

Synthesis of 3,3-difluoroindoline-2-thione (3,3-difuoro-3*H*-thioindolyl-2-yl, HSFox).

P_2S_5 (10.44 g, 47.0 mmol) was added to a solution of 3,3-difluoroxindole (HOFox, 2.65 g, 15.66 mmol) in dry tetrahydrofuran (THF, 150 mL), and the resulting mixture was stirred under argon for 22 h at rt. After that, the solids were filtered off through a pad of Celite, rinsed successively with THF, and the combined filtrate (100 mL) was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane, 10% gradient elution) to afford the title compound in 86% yield (2.49 g, 13.47 mmol). Analytical data for HSFox: R_f = 0.50 (ethyl acetate−hexane, 1/4 v/v); ^{1}H NMR (300 MHz): δ 6.97-7.64 (m, 4H, aromatic), 9.37 (s, 1H, NH) ppm; $^{13}C\{^{1}H\}$ NMR (75 MHz): δ 109.4, 112.7, 123.4, 124.7, 125.8, 133.4, 142.2, 194.2 ppm.

3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (5.2).

HSFox (0.31 g, 1.66 mmol), anhydrous K_2CO_3 (0.23 g, 1.66 mmol), and 18-crown-6 (0.08 g, 0.30 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[108] (**5.1**, 1.0 g, 1.52 mmol) in dry acetone (30 mL), and the resulting mixture was stirred under argon at 50 °C for 3 h. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~150 mL), washed with 10% aq. $NaHCO_3$ (25 mL), and water (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4, filtered,

and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 76% yield (0.88 g, 1.15 mmol). Analytical data for **5.2**: R_f = 0.40 (ethyl acetate/ hexane, 1/1, v/v); $[\alpha]_D^{22}$ -74.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 4.41-4.48 (m, 2H, H-5, 6a), 4.65 (m, 1H, H-6a), 5.69-5.89 (m, 2H, H-1, 4), 6.04-6.13 (m, 2H, H-2, 3), 7.16-7.84 (m, 24H, aromatic) ppm; ^{13}C{^{1}H} NMR (75 MHz): δ 62.9, 69.2, 70.2, 73.8, 76.8, 80.9, 117.4, 120.2, 120.9, 123.0, 123.3, 124.3, 126.3, 128.0, 128.3 (x2), 128.4 (x4), 129.4 (x2), 129.5 (x2), 129.7 (x2), 129.9 (x4), 128.4 (x3), 129.9 (x2), 128.6 (x2), 129.0, 129.3, 129.5, 129.6 (x2), 129.7 (x2), 129.8 (x2), 133.1, 133.2, 133.4, 138.6, 164.9 (x2), 165.7, 166.0 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{42}H_{31}F_2NO_9SNa]^+$ 786.1585; found: 786.1587.

3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside (5.4).

A solution of 3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranosyl bromide (**5.3**,[127] 1.98 g, 3.10 mmol) in dry toluene (12 mL) was added dropwise to a solution of HSFox (0.63 g, 3.39 mmol) and anhydrous K_2CO_3 (0.47 g, 3.39 mmol) in dry acetone (48 mL) under argon. After that, 18-crown-6 (0.16 g, 0.62 mmol) was added and the resulting mixture was stirred under argon for 12 h at 50 °C. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL), washed with 10% aq. $NaHCO_3$ (15 mL) and water (2 x 15 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 85% yield (1.96 g, 2.61 mmol). Analytical data for **5.4**: R_f = 0.35 (ethyl

acetate/ hexane, 3/7, v/v); $[\alpha]_D^{22}$ 2.9 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz): δ 4.07 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 4.30-4.33 (m, 1H, $J_{5,6a}$ = 6.0 Hz, H-5), 4.40-4.46 (m, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.57-4.65 (m, 2H, H-6a, CHPh), 4.78 (d, 1H, ^2J = 10.8 Hz, C*H*Ph), 5.59 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 5.87-5.93 (m, 2H, H-1, 3), 7.11-7.96 (m, 24H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 63.1, 69.3, 75.7, 76.4, 77.4, 77.7, 77.8, 82.0, 75.6, 117.6, 120.4, 121.0, 123.0, 123.2, 124.4, 126.2 (x3), 128.0, 128.1 (x3), 128.3, 128.9, 128.6 (x2), 129.4 (x2), 129.6 (x3), 129.7, 132.6, 132.9, 133.0, 133.2, 133.3, 133.4, 136.3, 165.2, 165.3, 165.9, 171.6 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{42}H_{33}F_2NO_8SNa]^+$ 772.1792.; found: 772.1800.

3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (5.6). HSFox (0.37 g, 2.0 mmol), anhydrous K_2CO_3 (0.28 g, 2.0 mmol), and 18-crown-6 (0.09 g, 0.36 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[108] (**5.1**, 1.2 g, 1.82 mmol) in dry acetone (30 mL), and the resulting mixture was stirred under argon at 50 °C for 3 h. After that, the solids were filtered off through a pad of Celite, rinsed successively with CH_2Cl_2, and the combined filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (~150 mL), washed with 10% aq. $NaHCO_3$ (25 mL), and water (2 x 25 mL). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (toluene - ethyl acetate 1% gradient elution) to afford the title compound as a white amorphous solid in 76% yield (0.88 g, 1.15 mmol). Analytical data for **5.6**: R_f = 0.50 (ethyl acetate/ toluene, 1/19, v/v); $[\alpha]_D^{22}$ -147.6 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz): δ 4.47 (m, 3H, H-5, 6a, 6b), 5.80 (dd, 1H, $J_{4,5}$ = 3.3 Hz, H-3), 6.00-6.14 (m, 3H, H-1, 2, 4), 7.71-7.98 (m, 24H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 62.2, 67.8, 68.3, 72.3, 75.8, 81.1, 117.4, 120.2, 120.8, 122.9, 123.1, 124.2, 126.2, 127.7, 128.0, 128.1 (x2), 128.3 (x2), 128.5, 129.1

(x2), 129.3 (x2), 129.4 (x2), 129.5 (x2), 129.6 (x3), 129.7, 132.7, 133.0 (x3), 133.3 , 152.2, 165.1, 165.2, 165.3, 165.7 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{42}H_{31}F_2NO_9SNa]^+$ 786.1585; found: 786.1594.

Ethyl 2,3-di-*O*-benzoyl-1-thio-β-D-glucopyranoside (5.12).

A solution of ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[128] (**5.23**, 0.45 g, 0.864 mmol) in CF_3COOH/CH_2Cl_2 (5.0 mL 1/5, v/v) was kept for 8h at rt. After that, triethylamine was added (~3 mL, until pH ~ 7). The volatiles were removed under reduced pressure, and the residue was purified by column chromatography on silica gel (hexane - ethyl acetate, 10% gradient elution). Analytical data for **5.12**: R_f= 0.35 (ethyl acetate/ hexane, 1/1, v/v); $[\alpha]_D^{22}$ 105.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 1.19 (t, 3H, SCH_2CH_3), 2.63-2.72 (m, 2H, SCH_2CH_3), 3.59 (m, 2H, , $J_{5,6a}$ = 3.9 Hz, H-5), 3.84 (dd, 1H, , $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.96 (m, 2H, H-4, 6b), 4.75 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.38 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 5.56 (dd, 1H, $J_{3,4}$=9.3 Hz, H-3), 7.26-7.93 (m, 10H, aromatic) ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 14.6, 14.7, 24.2, 61.7, 68.9, 69.3, 70.3, 79.9, 83.3, 128.2 (x2), 129.0, 129.1, 129.5 (x2), 129.7 (x2), 133.0, 133.1, 133.2, 165.2, 165.2, 166.6 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{22}H_{24}NO_7SNa]^+$ 455.1140; found: 455.1141.

5.4.3. Synthesis of Disaccharides

General procedure for glycosylation in the presence of TfOH or TMSOTf. A mixture of a glycosyl donor (0.039-0.040 mmol), a glycosyl acceptor (0.026-0.028 mmol), and molecular sieves (4 Å, 80 mg) in 1,2-DCE or DCM (1.0 mL) was stirred under argon for 1 h at rt. After that, TfOH (0.039-0.04mmol) or TMSOTf (0.039-0.04 mmol) were added, and the resulting mixture was stirred for the time indicated in Tables at rt. The solid was filtered off through a

pad of Celite and rinsed successively with dichloromethane. The combined filtrate (~30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution). If necessary, further purification was accomplished by size-exclusion column chromatography on Sephadex LH-20.

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5.8). The title compound was obtained from donor **5.2** and acceptor **5.7**[55] by general glycosylation procedure in 97% yield as a white amorphous solid. Analytical data for **5.8** was in accordance with that previously reported.[77]

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (5.13). The title compound was obtained from donor **5.2** and acceptor **5.9**[55] by the general glycosylation procedure in 72% yield as a clear syrup. Analytical data for **5.13** was in accordance with that previously reported.[77]

Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (5.14). The title compound was obtained from donor **5.2** and acceptor **5.10**[55] by the general glycosylation procedure in 73% yield as a clear syrup. Analytical data for **5.14** was in accordance with that previously reported.[55]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (5.15). The title compound was obtained from donor **5.2** and acceptor **5.11**[55] by the general glycosylation procedure in 87% yield as a clear syrup. Analytical data for **5.15** was in accordance with that previously reported.[58]

Methyl 6-*O*-(3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5.16). The title compound was obtained from donor **5.4** and acceptor **5.7** by the general glycosylation procedures in 80% yield (α/β > 25/1) as a colorless foam. Analytical data for **5.16** was in accordance with that previously reported.[84]

Methyl 4-*O*-(3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (5.17). The title compound was obtained from donor **5.4** and acceptor **5.9** by the general glycosylation procedure in 48% yield (α/β = > 25/1) as a clear syrup. Analytical data for **5.17** was in accordance with that previously reported.[85]

Methyl 3-*O*-(3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (5.18). The title compound was obtained from donor **5.4** and acceptor **5.10** by the general glycosylation procedure in 65% yield (α/β = > 25/1) as a clear syrup. Analytical data for **5.18** was in accordance with that previously reported.[86]

Methyl 2-*O*-(3,4,6-tri-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (5.19). The title compound was obtained from donor **5.5** and acceptor **5.11** by the general glycosylation procedure in 70% yield (α/β = > 25/1) as a colorless foam. Analytical data for **5.19** was in accordance with that previously reported.[87]

Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (5.20). The title compound was obtained from donor **5.6** and acceptor **5.7** by general glycosylation procedure in 85% yield as a white amorphous solid. Analytical data for **5.20** was in accordance with that previously reported.[104]

Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (5.21). The title compound was obtained from donor **5.6** and acceptor **5.11**

by general glycosylation procedure in 96% yield as a colorless amorphous solid. Analytical data for **5.21** was in accordance with that previously reported.[129]

Ethyl 2,3-di-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-glucopyranosyl)-1-thio-β-D-glucopyranoside (5.22). A mixture of donor **5.2** (30.0 mg, 0.039 mmol), ethylthio glucoside acceptor **5.12** (11.9 mg, 0.028 mmol), and 4Å molecular sieves (80 mg) in dichloroethane (1.0 mL) was stirred under argon for 1 h at rt. After that, triflic acid (2.78 µL, 0.031 mmol)/TMSOTf (4.26 µL, 0.023 mmol) was added the resulting mixture was stirred for 5 min at 0°C. The mixture was neutralized with triethylamine. Then, the solid was filtered off through a pad of Celite, rinsed successively with dichloromethane, and the combined filtrate (~30 mL) was washed with H_2O (2 x 10 mL). The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/ hexane 5% gradient elution). Analytical data for **5.22**: R_f = 0.54 (ethyl acetate/toluene, 2.5/7.5, v/v); $[\alpha]_D^{22}$ 32.1 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz): δ 3.57-3.58 (m, 1H, H-5), 3.63-3.70 (m, 1H, H-4), 3.81-3.86 (dd, 1H, $J_{6a,6b}$ = 11.1 Hz, H-6a), 4.06-4.09 (m, 1H, $J_{5',6a'}$ = 5.5 Hz, H-5'), 4.17-4.20 (m, 1H, H-6b), 4.37-4.42 (dd, 1H, $J_{6a',6b'}$ = 12.2 Hz, H-6a'), 4.46 (d, 1H, $J_{1,2}$ = 9.1 Hz H-1), 4.63-4.67 (m, 1H, $J_{5,6a}$ = 4.4 Hz, H-5), 4.90 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 5.2-5.3 (m, 2H, H-2, 3), 5.46 (dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2'), 5.61 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 5.80 (dd, 1H, $J_{3',4'}$ = 9.5 Hz, H-3') ppm; $^{13}C\{^1H\}$ NMR (75 MHz): δ 14.6, 23.9, 62.6, 69.1, 69.3, 69.8, 70.0, 71.8, 72.2, 73.2, 72.7, 78.0, 79.4, 83.2, 101.5, 128.3 (x8), 128.6 (x4), 128.8, 128.9, 129.1, 129.4, 129.9 (x8), 129.9, 133.3 (x10), 165.0, 165.1 (x2), 165.7, 166.2, 167.2 ppm; ESI-TOF $[M + Na]^+$: calcd for $[C_{56}H_{50}O_{16}SNa]^+$ 1033.2717.; found: 1033.2722.

5.5. References

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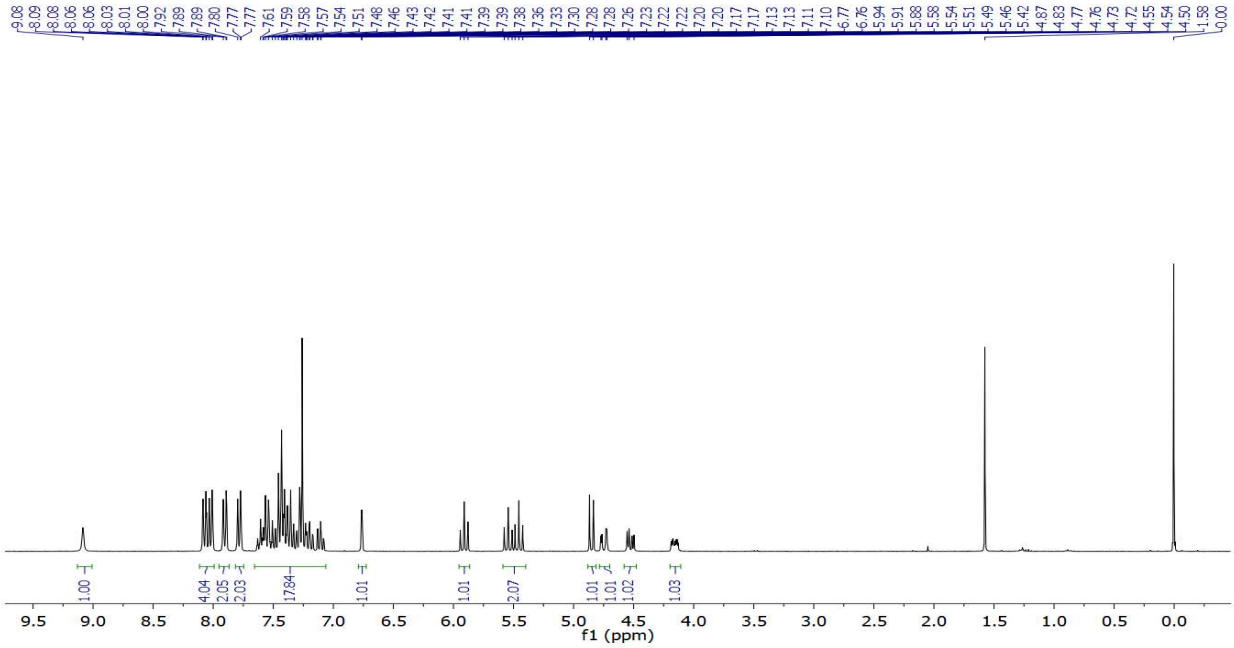
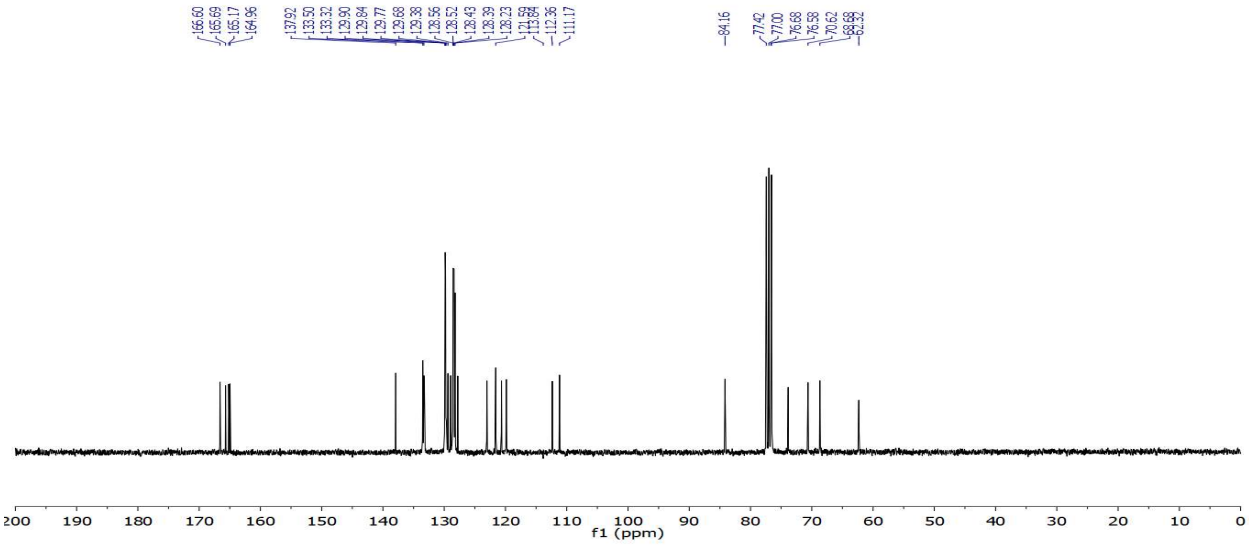
APPENDIX

2.2



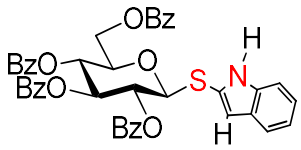
$CDCl_3$ 300 MHz

Figure A-1: ^{1}H NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**2.2**)

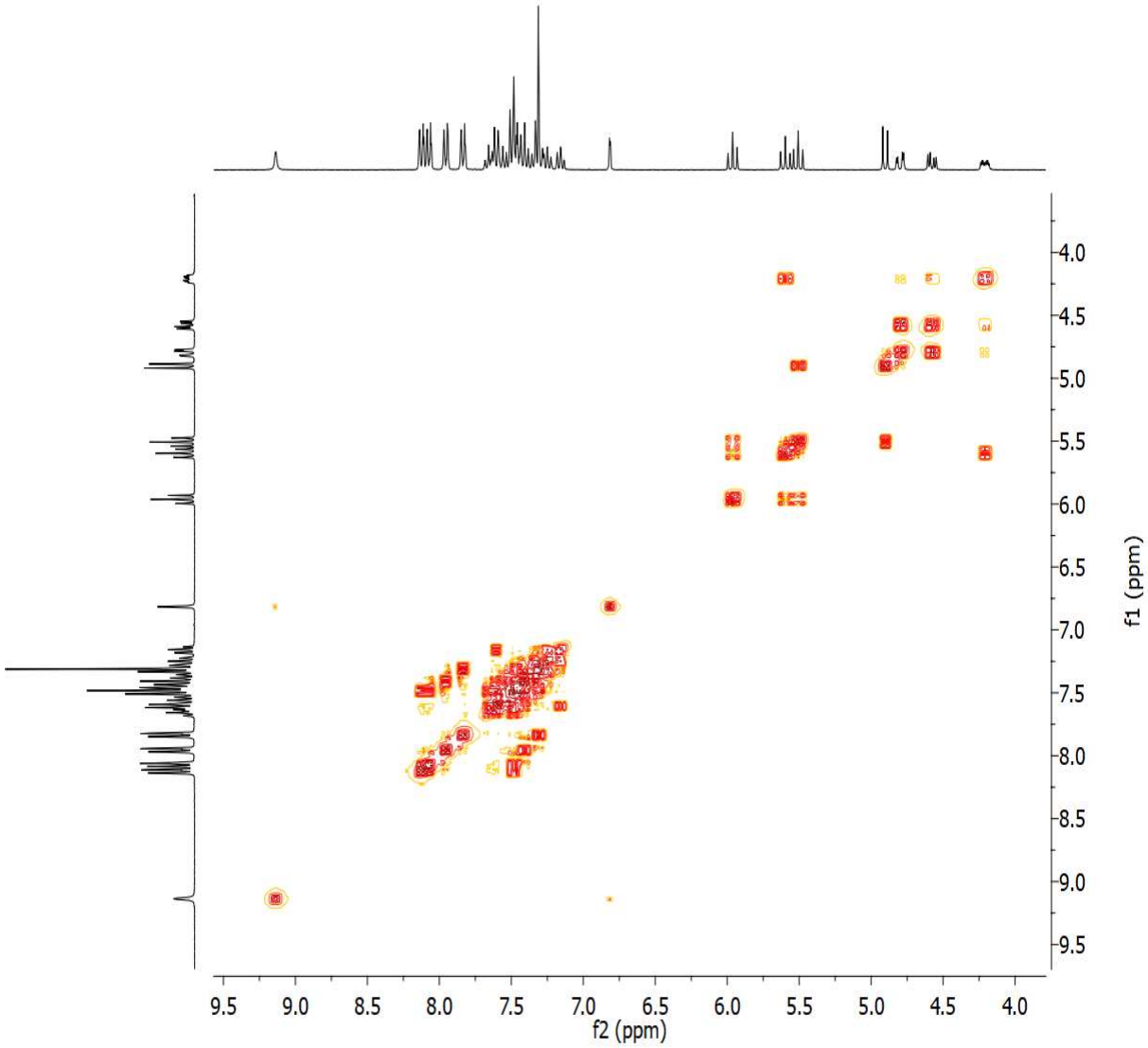


$CDCl_3$ 75 MHz

Figure A-2: ^{13}C NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**2.2**)

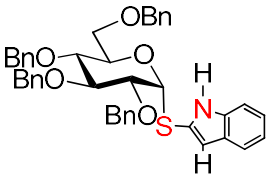


2.2

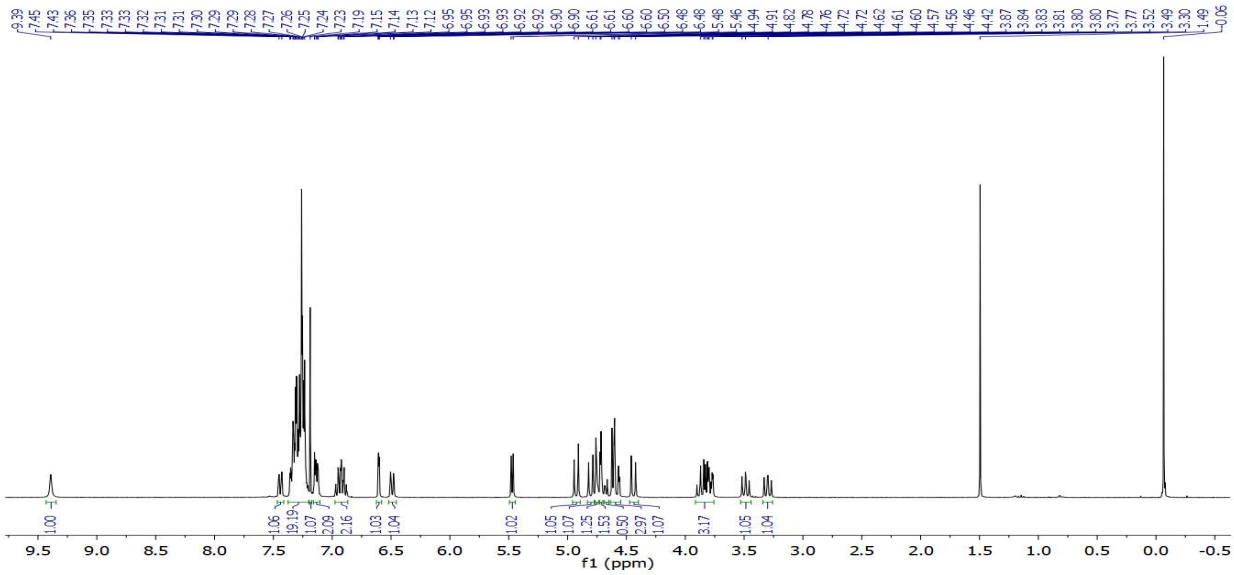


$CDCl_3$ 300 MHz

Figure A-3: 2-D NMR COSY spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**2.2**)

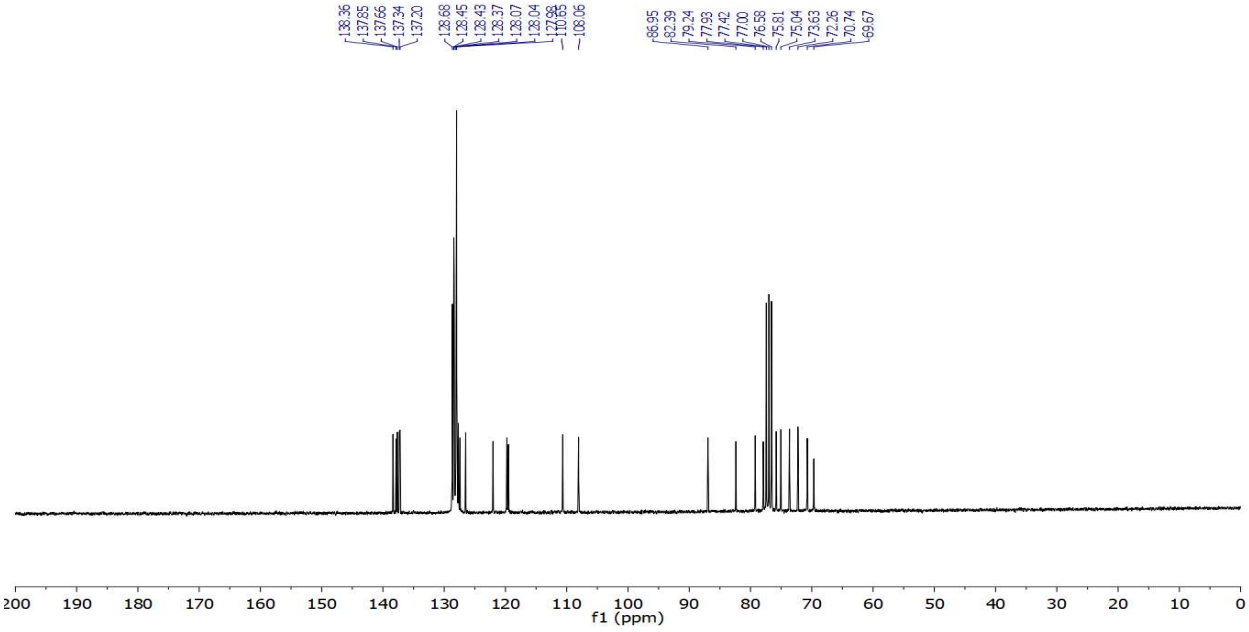


2.4



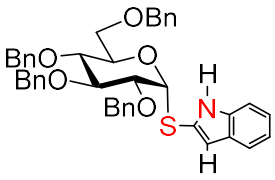
$CDCl_3$ 300 MHz

Figure A-4: ^{1}H NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**2.4**).

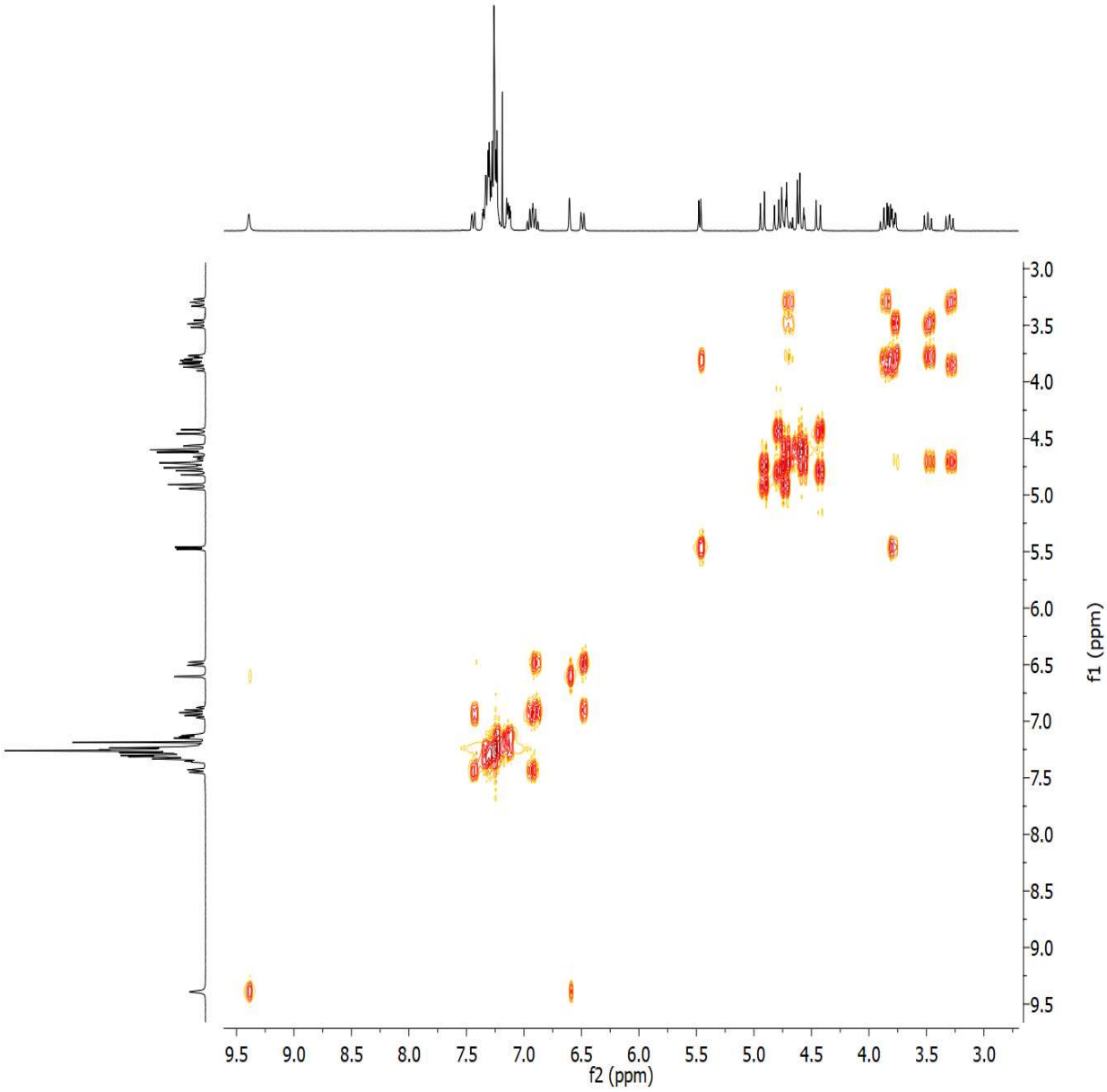


$CDCl_3$ 75 MHz

Figure A-5: ^{13}C NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**2.4**).

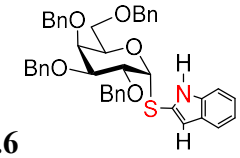


2.4

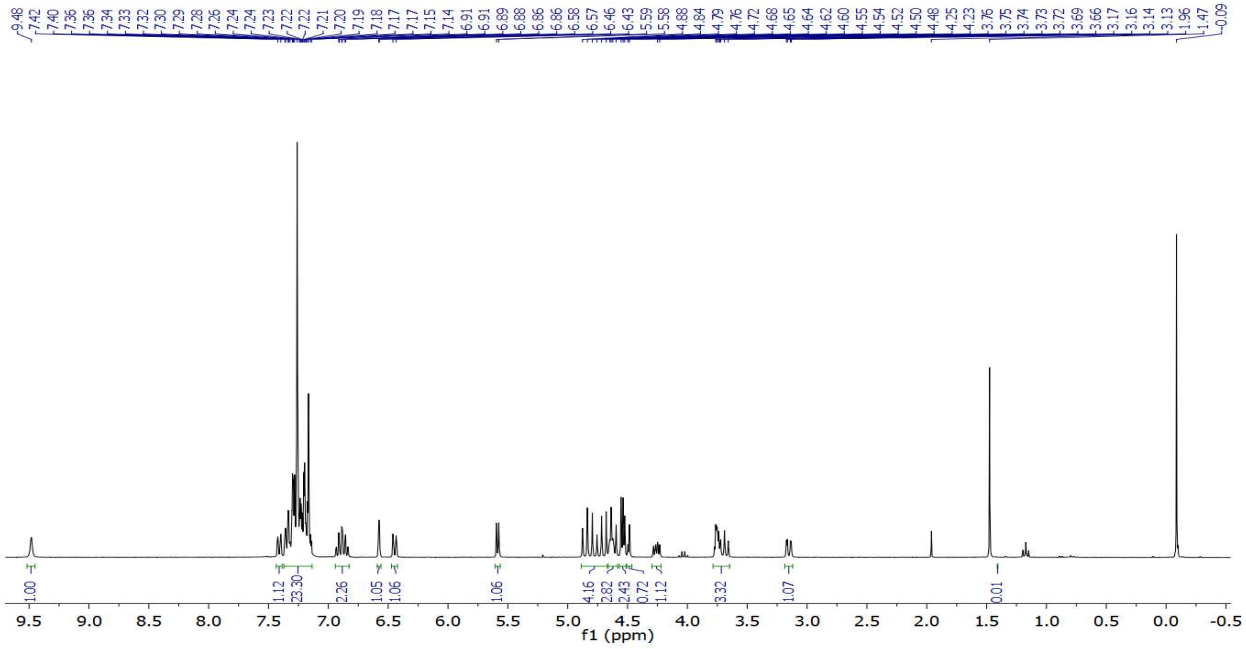


$CDCl_3$ 300 MHz

Figure A-6: 2-D NMR COSY spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**2.4**).

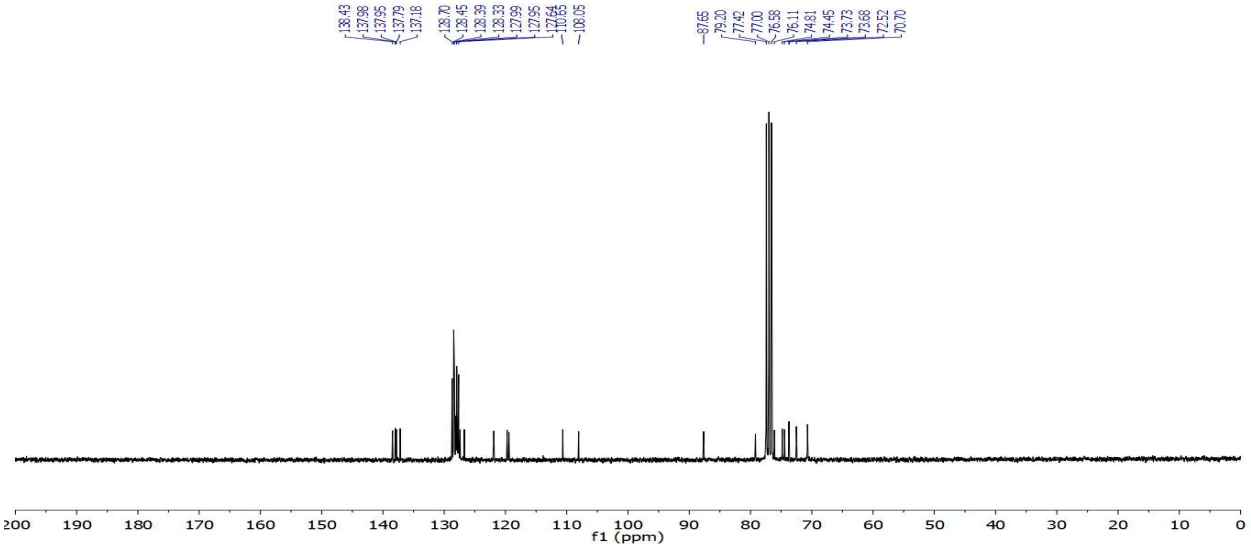


2.6



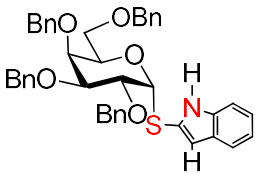
$CDCl_3$ 300 MHz

Figure A-7: ^{1}H NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopyranoside (**2.6**).

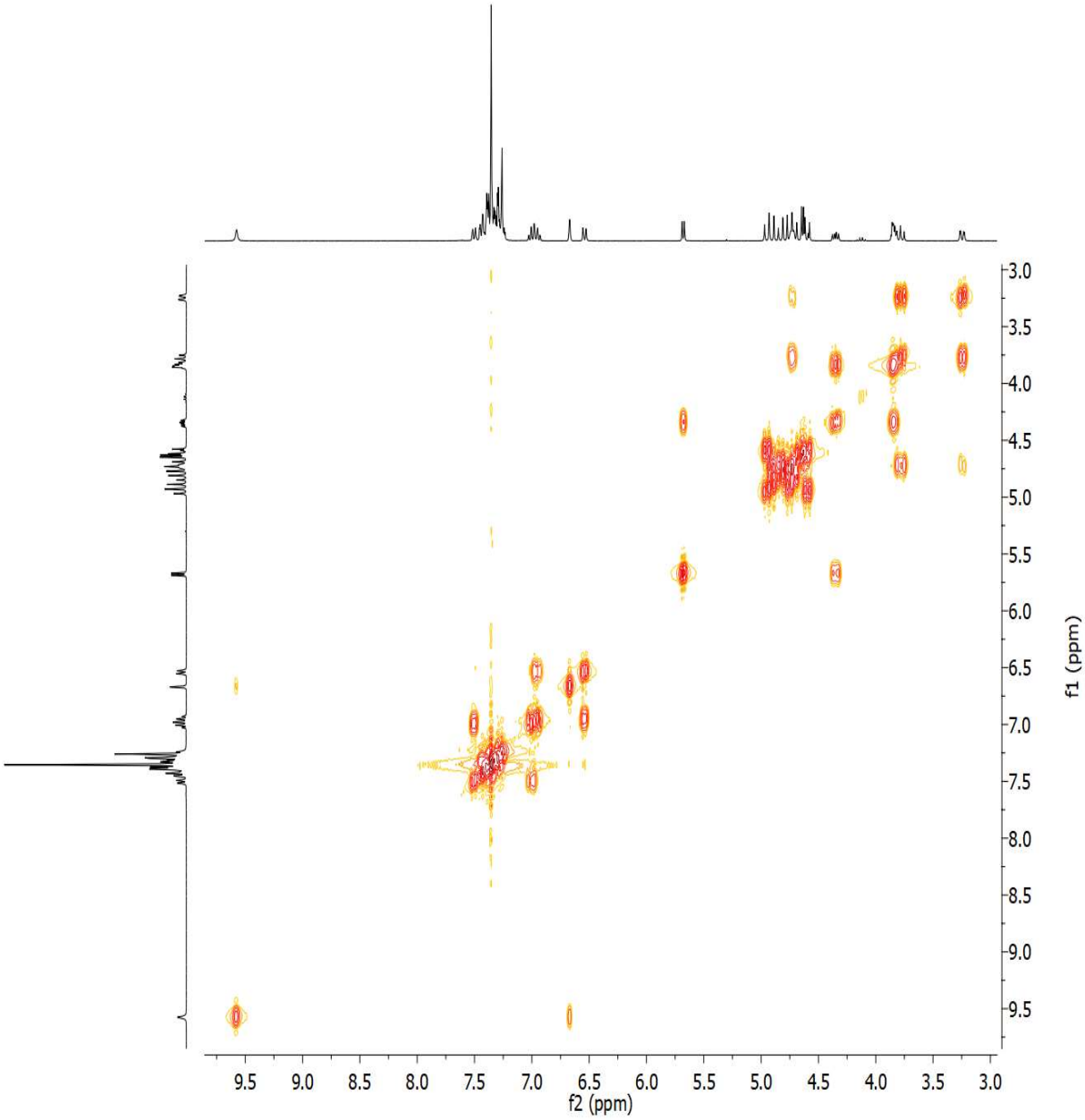


$CDCl_3$ 75 MHz

Figure A-8: ^{13}C NMR spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopyranoside (**2.6**).



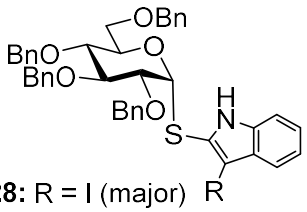
2.6



$CDCl_3$ 300 MHz

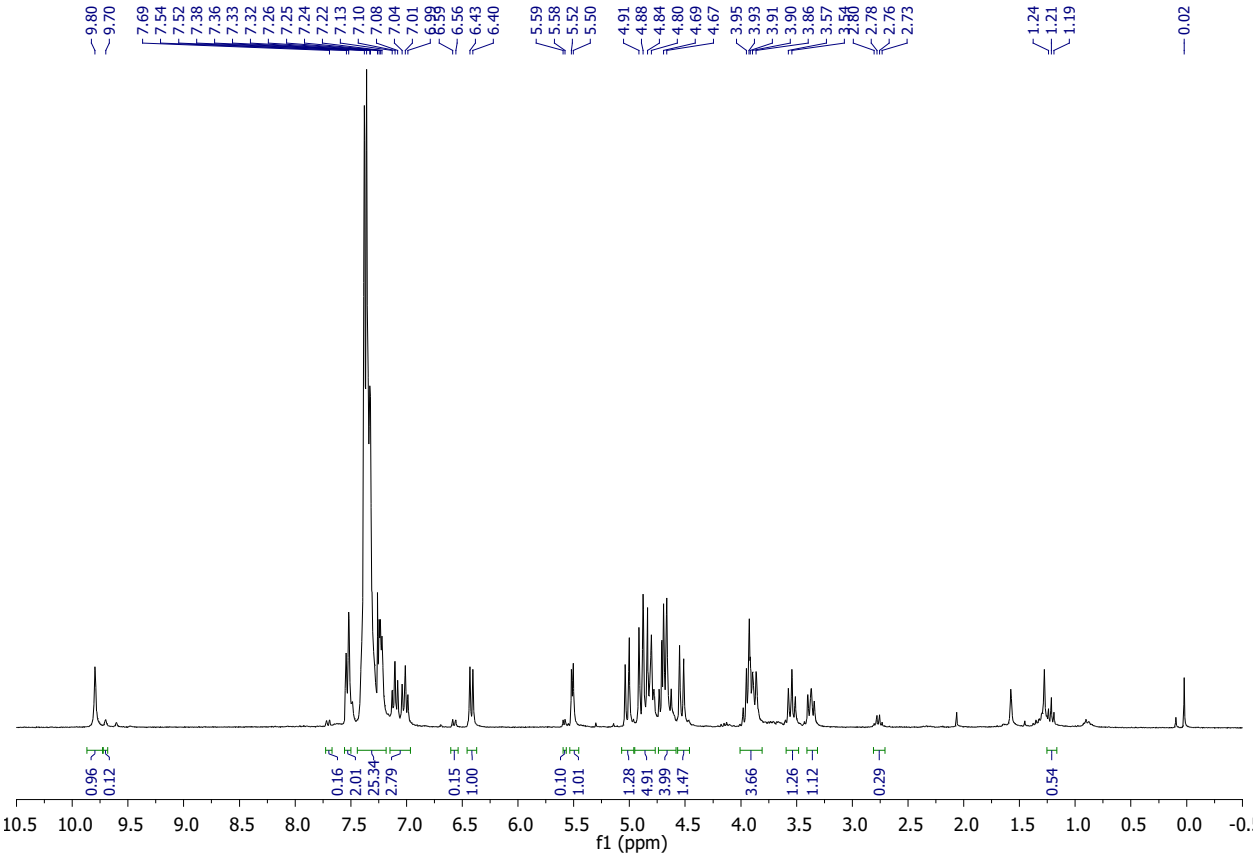
Figure A-9: 2-D NMR COSY spectrum of 1*H*-Indol-2-yl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopyranoside (**2.6**).

NMR spectra of recovered products from competition experiments



2.28: R = I (major)
2.29: R = SEt

^{1}H NMR ($CDCl_3$, 300 MHz)



$CDCl_3$ 300 MHz

Figure A-10: ^{1}H NMR spectrum of recovered donor from competition experiment (**2.28** and **2.29**).

Additional ^{1}H NMR monitored experiments ($CDCl_3$ 300 MHz)

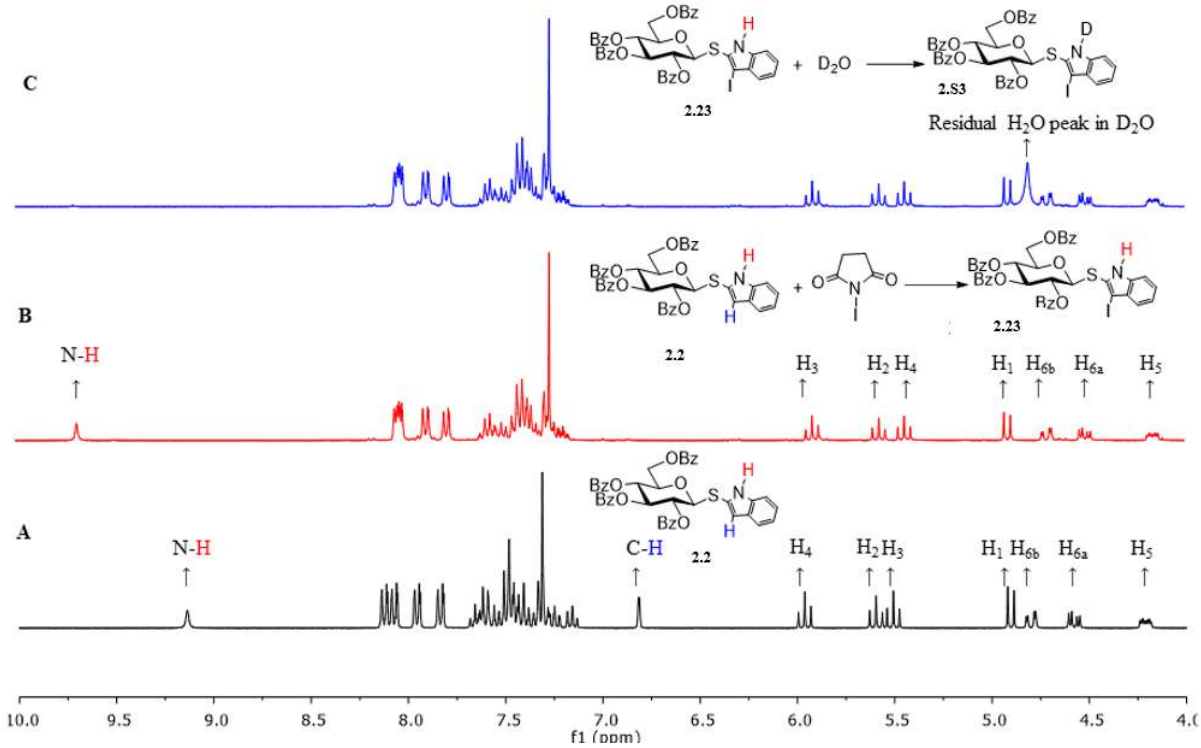


Figure A-11: Deuterium exchange experiment

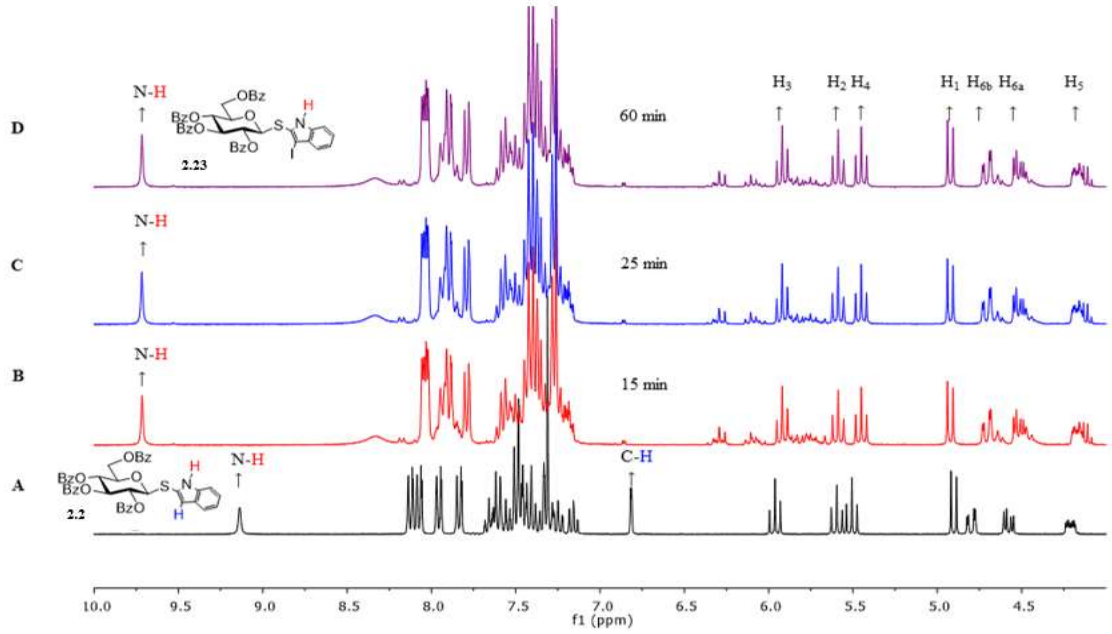


Figure A-12: ^{1}H NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 1 equiv. NIS at intervals of 15 min; C) 25 min; and D) 1 h.

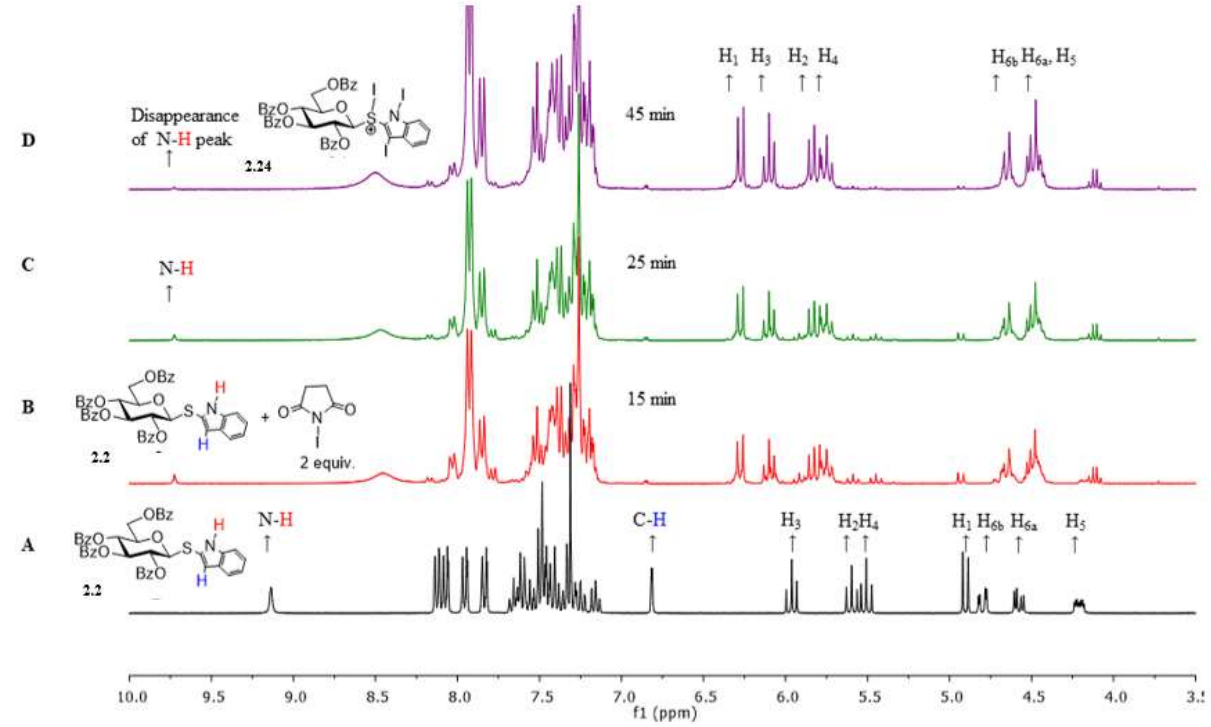


Figure A-13: ^{1}H- NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 2 equiv NIS at intervals of 15 min; C) in 25 min; D) in 45 min.

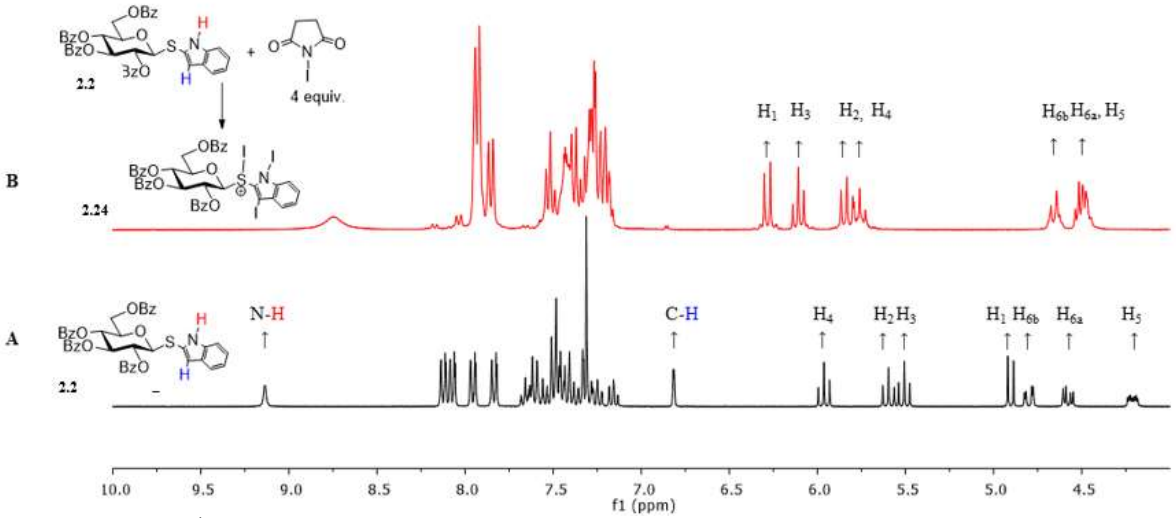


Figure A-14: ^{1}H NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of **4** equiv of NIS after 1 h.

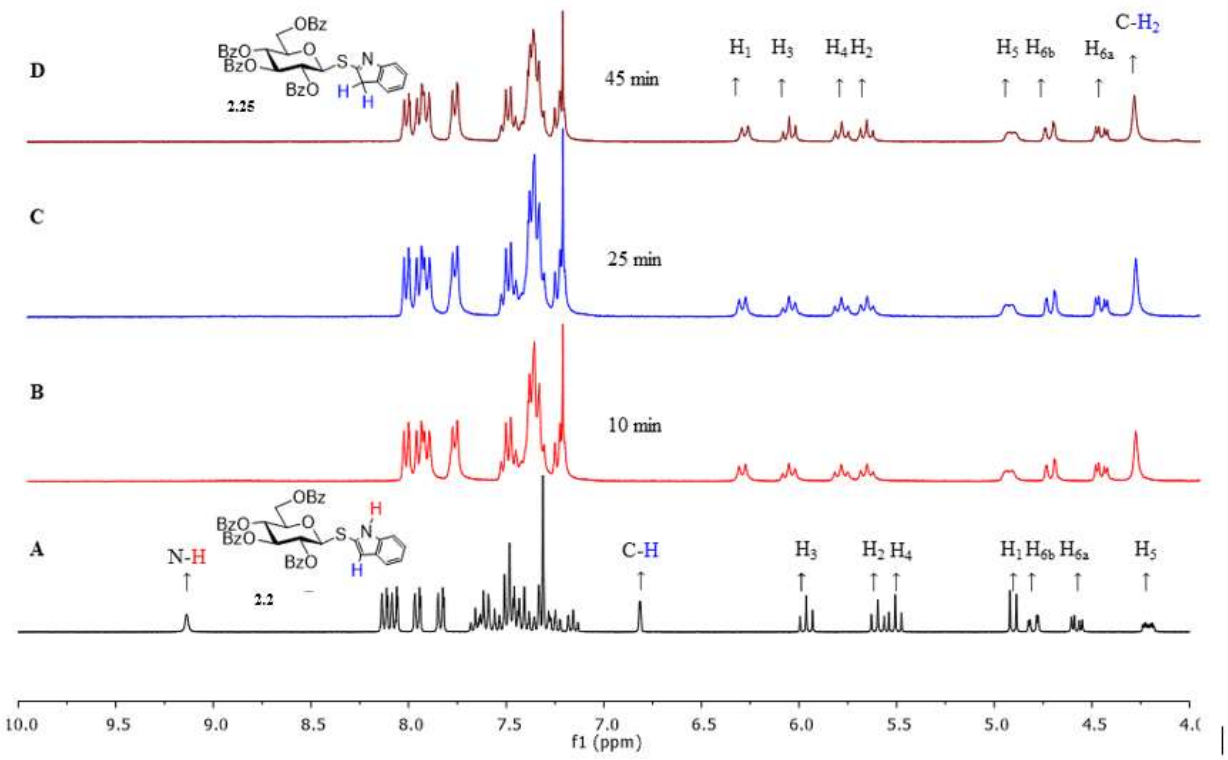


Figure A-15: ^{1}H NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 1 equiv TMSOTf at intervals of 10 min; C) in 25 min; D) in 45 min.

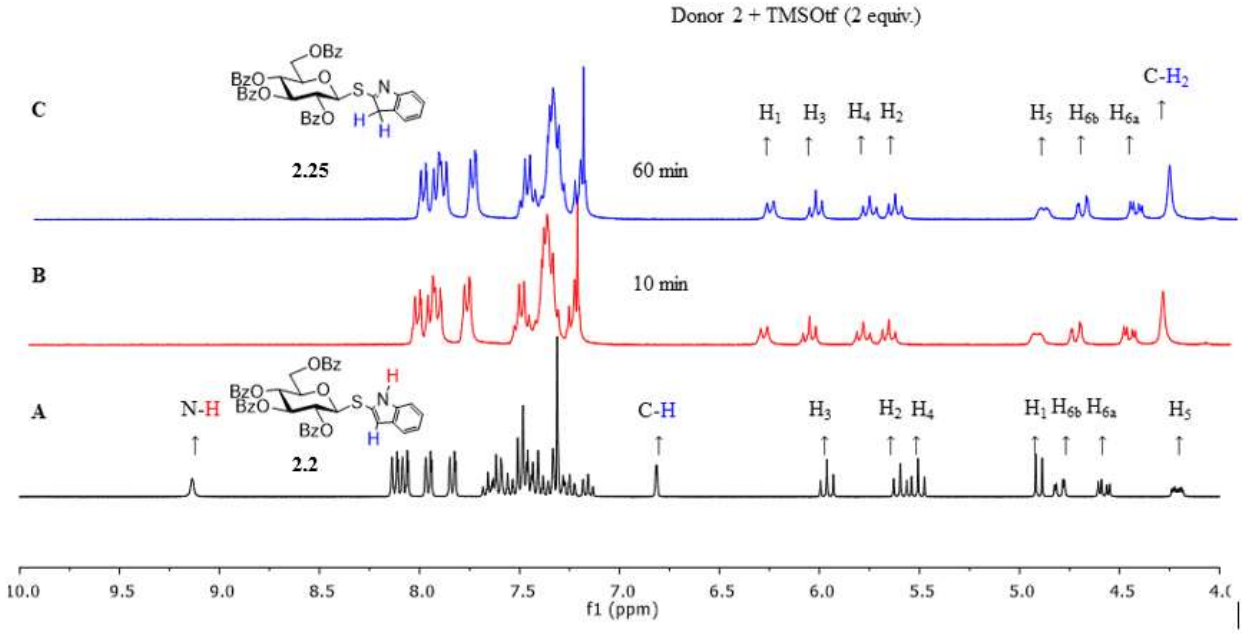


Figure A-16: ^{1}H- NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 2 equiv TMSOTf at intervals of 10 min; C) in 1 h.

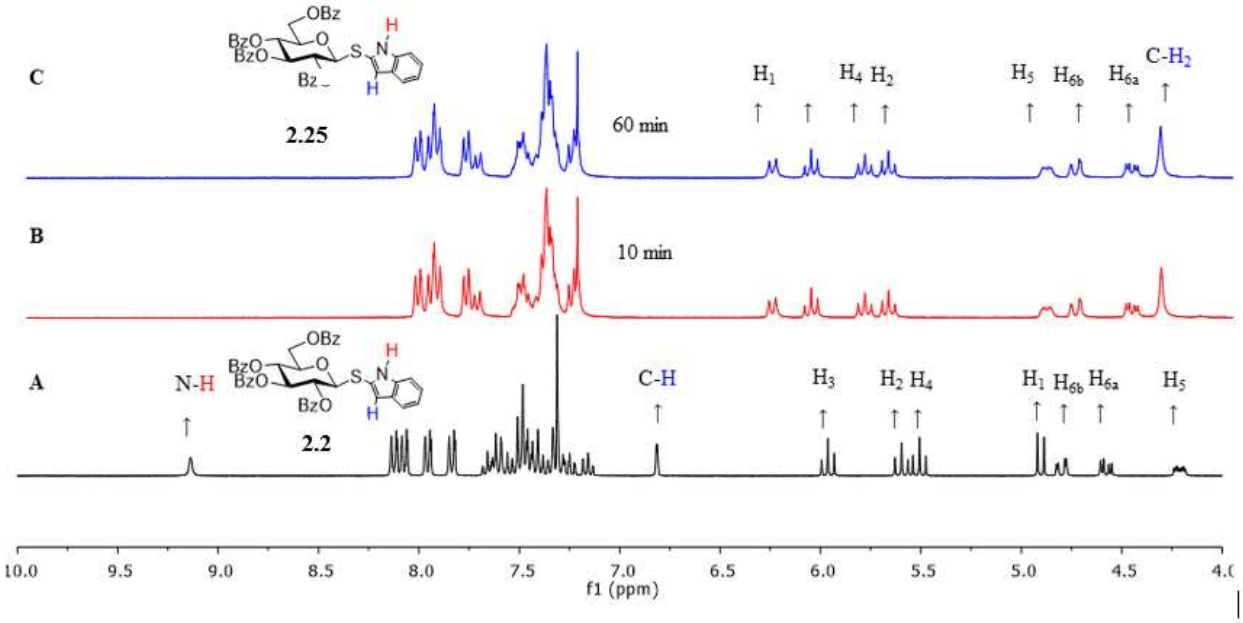


Figure A-17: ^{1}H NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 4 equiv of TMSOTf at intervals of 10 min; C) in 1 h.

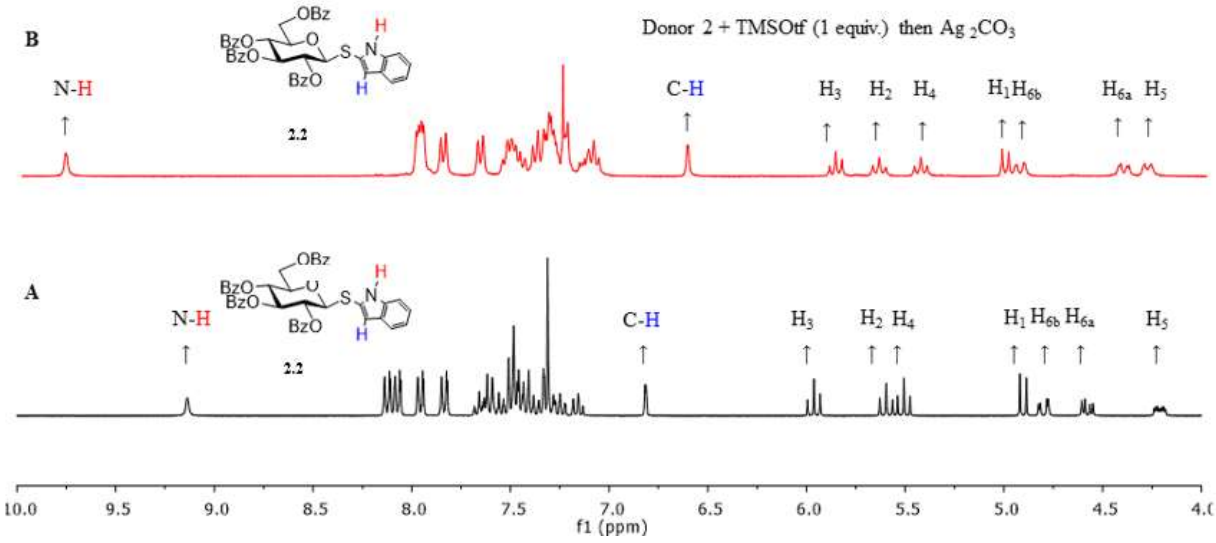


Figure A-18: ^{1}H NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 1 equiv. of TMSOTf and then Ag_2CO_3.

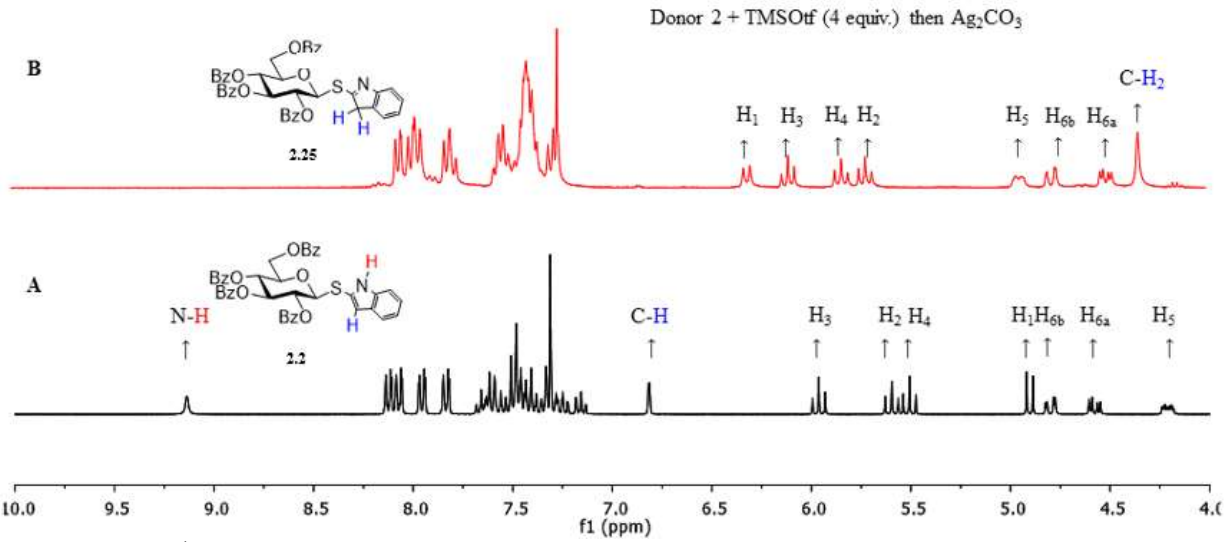


Figure A-19: ^{1}H- NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 4 equiv TMSOTf and then Ag_2CO_3.

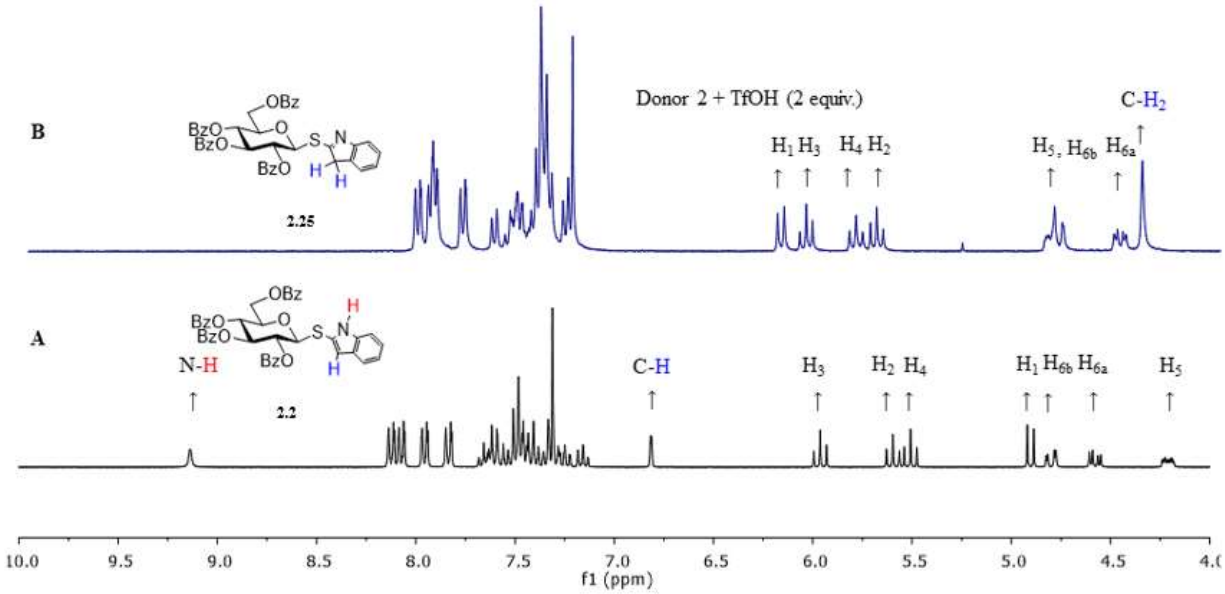


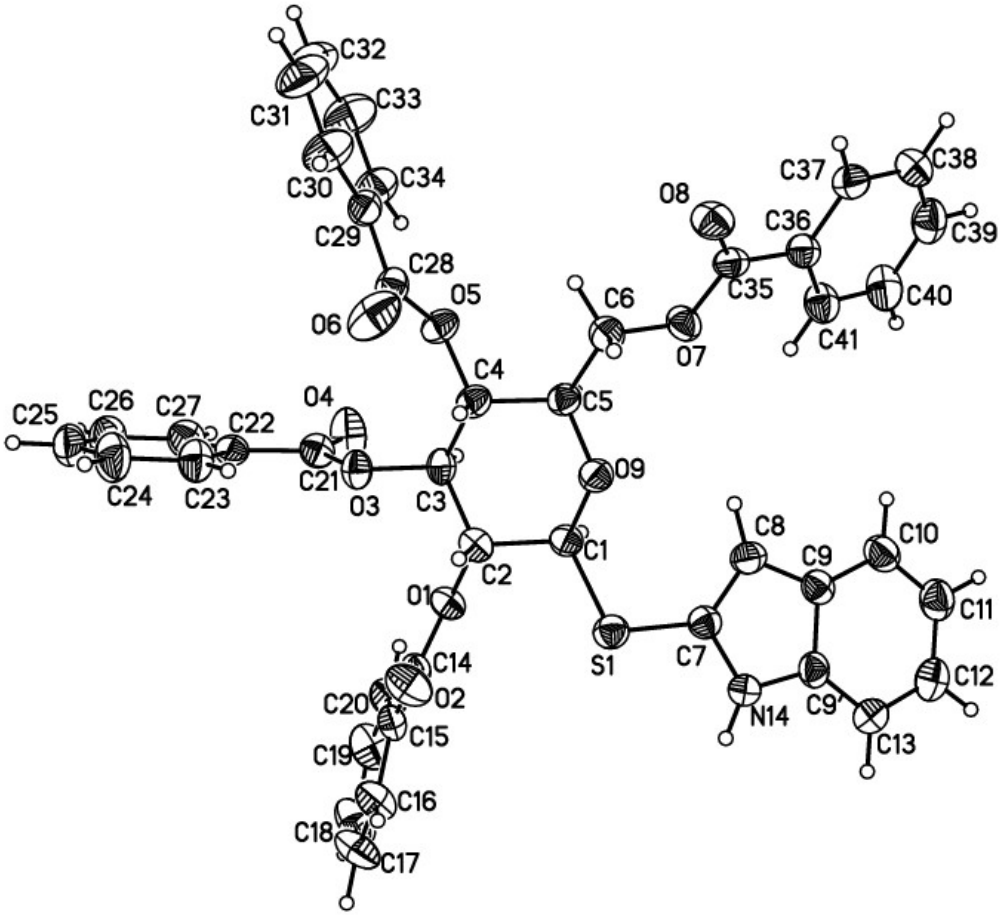
Figure A-20: ^{1}H- NMR spectra of A) glycosyl donor **2.2** in $CDCl_3$; B) glycosyl donor **2.2** in $CDCl_3$ in presence of 2 equiv TfOH.

X-Ray crystal structure determination for compound 2.2

Table A-1. Crystal data and structure refinement for 2.2 (d1119-180K).

Identification code	avd1119-180K/x8/GS2P54	
Empirical formula	C_{42} H_{33} N O_9 S	
Formula weight	727.75	
Temperature	180(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 5.9632(2) Å	□= 90°.
	b = 18.9516(9) Å	□= 95.889(2)°.
	c = 15.9602(7) Å	□ = 90°.
Volume	1794.18(13) $Å^3$	
Z	2	
Density (calculated)	1.347 Mg/m^3	
Absorption coefficient	0.150 mm^{-1}	
F(000)	760	
Crystal size	0.398 x 0.281 x 0.233 mm^3	
Theta range for data collection	2.149 to 27.530°.	
Index ranges	$-7\leq h\leq 7$, $-24\leq k\leq 24$, $-20\leq l\leq 20$	
Reflections collected	24279	
Independent reflections	8263 [R(int) = 0.0407]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9420 and 0.9063	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	8263 / 2 / 486	
Goodness-of-fit on F^2	1.041	
Final R indices [I>2sigma(I)]	R1 = 0.0412, wR2 = 0.0757	
R indices (all data)	R1 = 0.0659, wR2 = 0.0852	
Absolute structure parameter	0.02(4)	
Largest diff. peak and hole	0.146 and -0.179 $e.Å^{-3}$	

Figure A-21. Crystal projection view with 50% probability ellipsoids for compound 2.2:

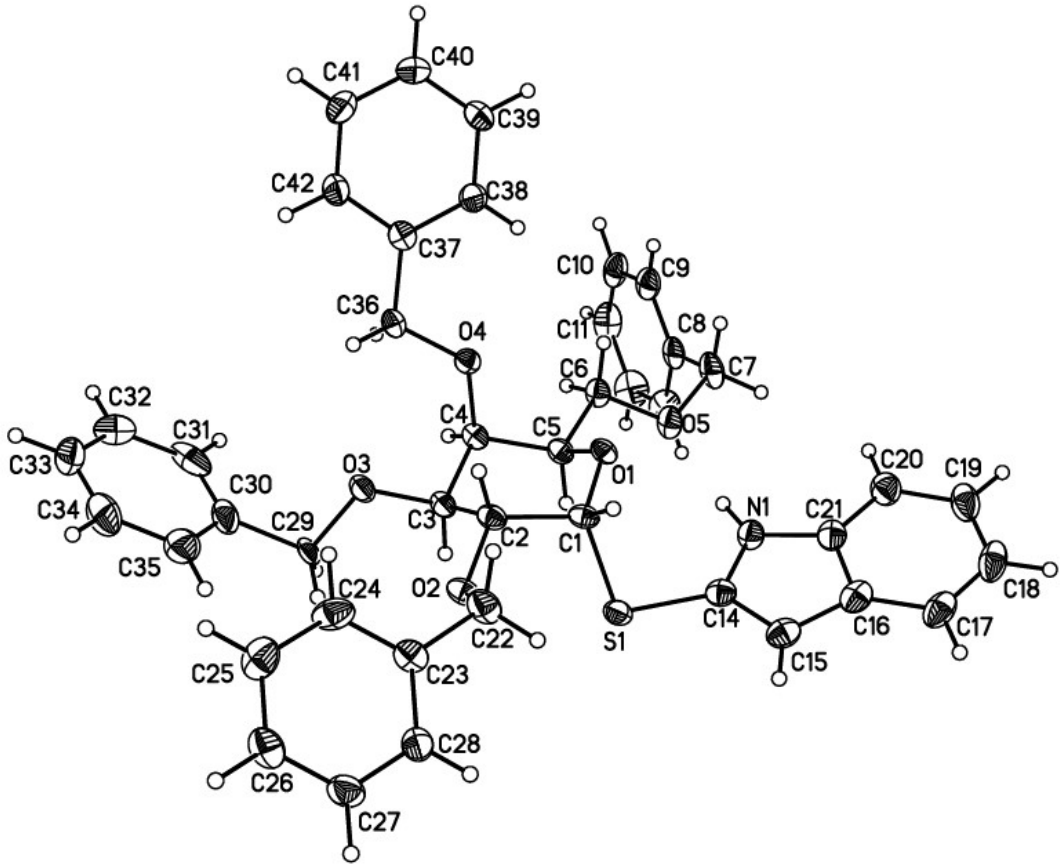


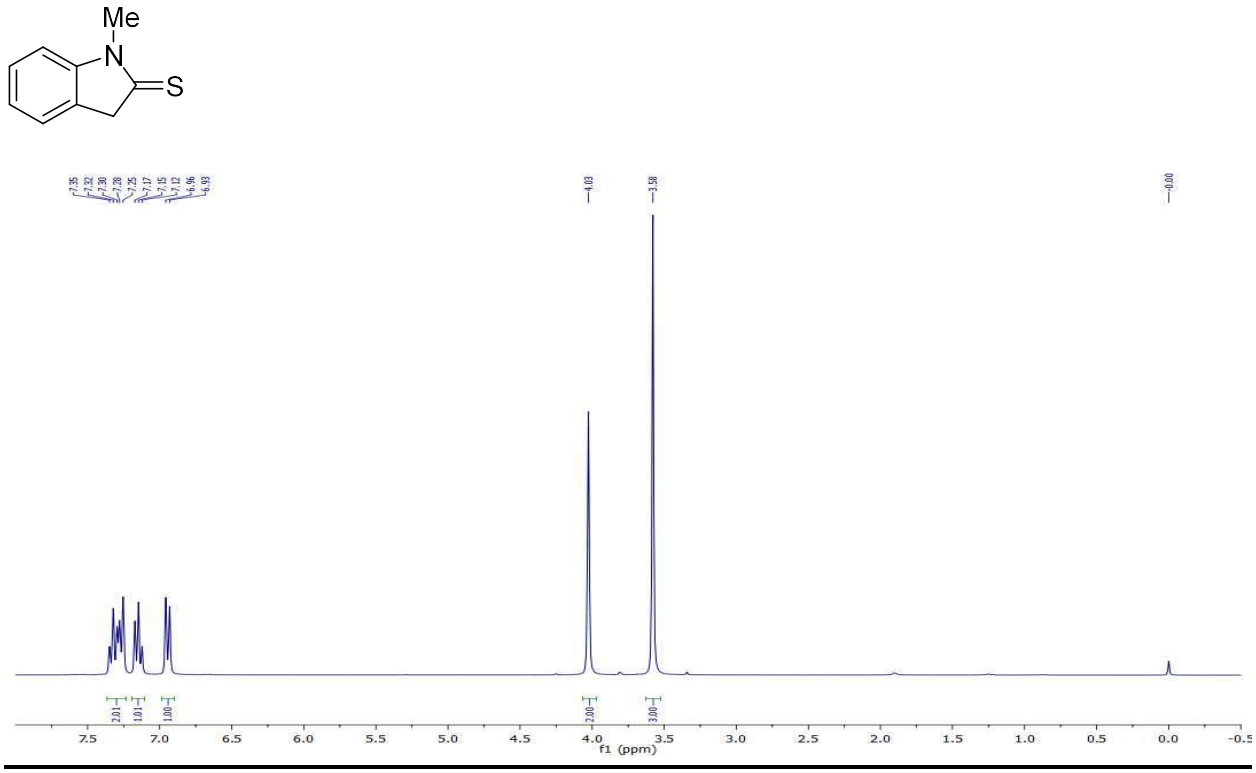
X-Ray crystal structure determination for compound 2.6

Table A-2. Crystal data and structure refinement for 2.6 (avd15819).

Identification code	d15819/lt/venture/Shrestha	
Empirical formula	C_{42} H_{41} N O_5 S	
Formula weight	671.82	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 11.9289(4) Å	□= 90°.
	b = 8.8193(3) Å	□= 100.021(2)°.
	c = 16.9246(6) Å	□ = 90°.
Volume	1753.38(11) $Å^3$	
Z	2	
Density (calculated)	1.272 Mg/m^3	
Absorption coefficient	1.194 mm^{-1}	
F(000)	712	
Crystal size	0.198 x 0.063 x 0.032 mm^3	
Theta range for data collection	2.651 to 74.608°.	
Index ranges	-14≤h≤14, -10≤k≤10, -21≤l≤20	
Reflections collected	25115	
Independent reflections	7005 [R(int) = 0.051]	
Completeness to theta = 67.679°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.8645 and 0.7030	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7005 / 4 / 452	
Goodness-of-fit on F^2	1.059	
Final R indices [I>2sigma(I)]	R1 = 0.0393, wR2 = 0.0872	
R indices (all data)	R1 = 0.0433, wR2 = 0.0896	
Absolute structure parameter	-0.011(11)	
Largest diff. peak and hole	0.344 and -0.171 e.$Å^{-3}$	

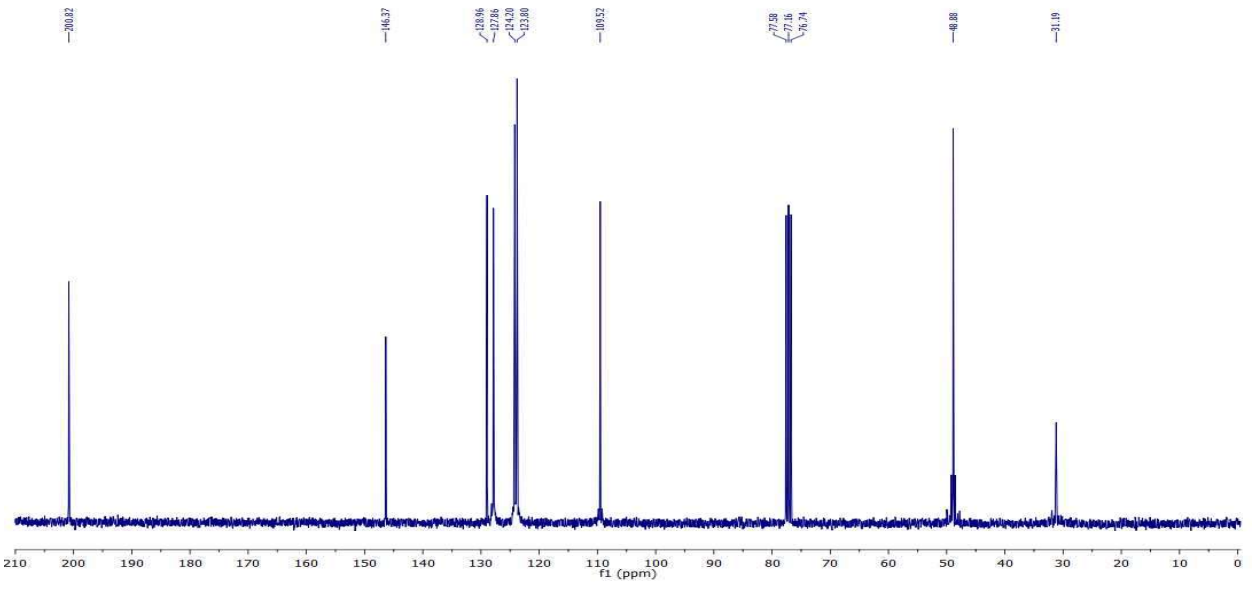
Figure A-22. Crystal projection view of compound 6 with 50% probability ellipsoids - disorder component omitted for clarity:





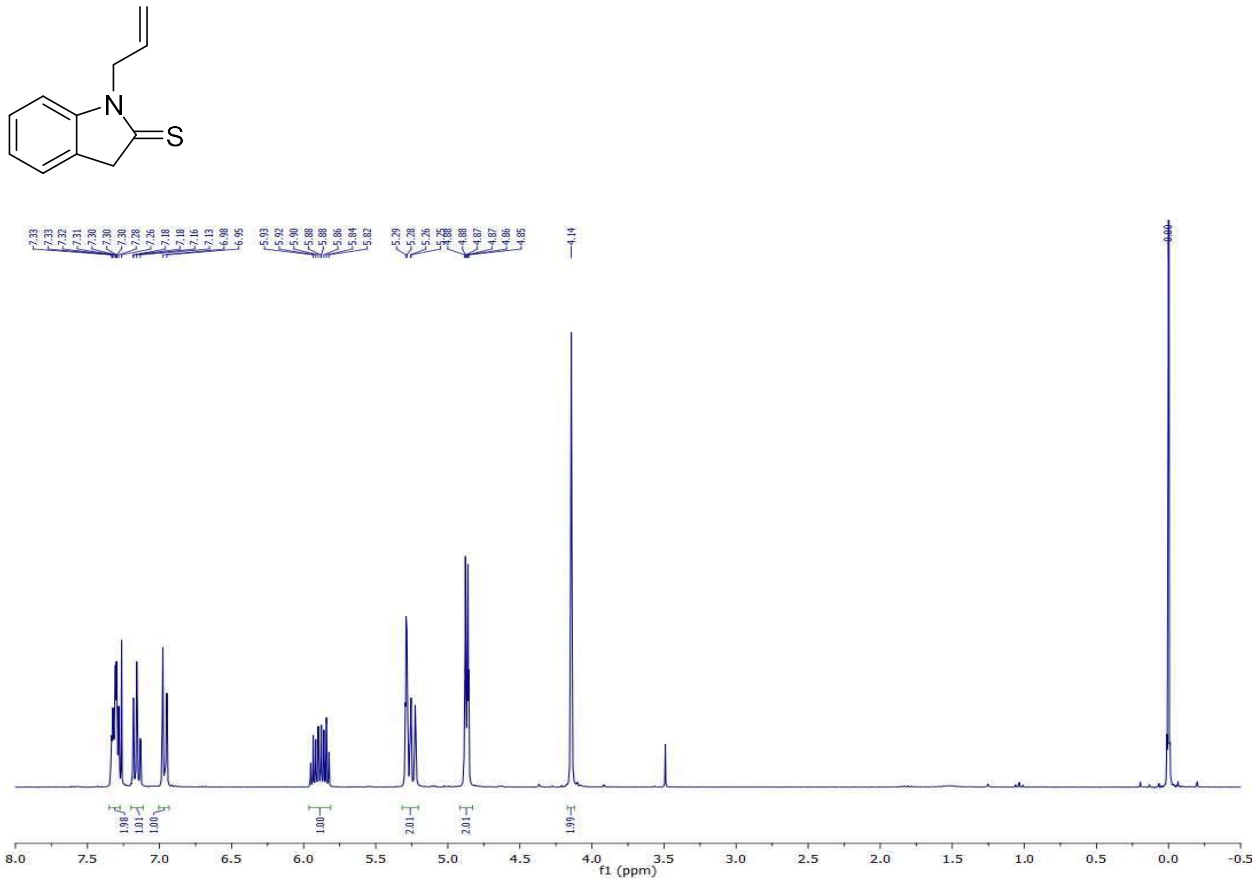
$CDCl_3$ 300 MHz

Figure A-23: ^{1}H- NMR spectra of 1-Methylindoline-2-thione (N-methyl thioindole, HSInMe).



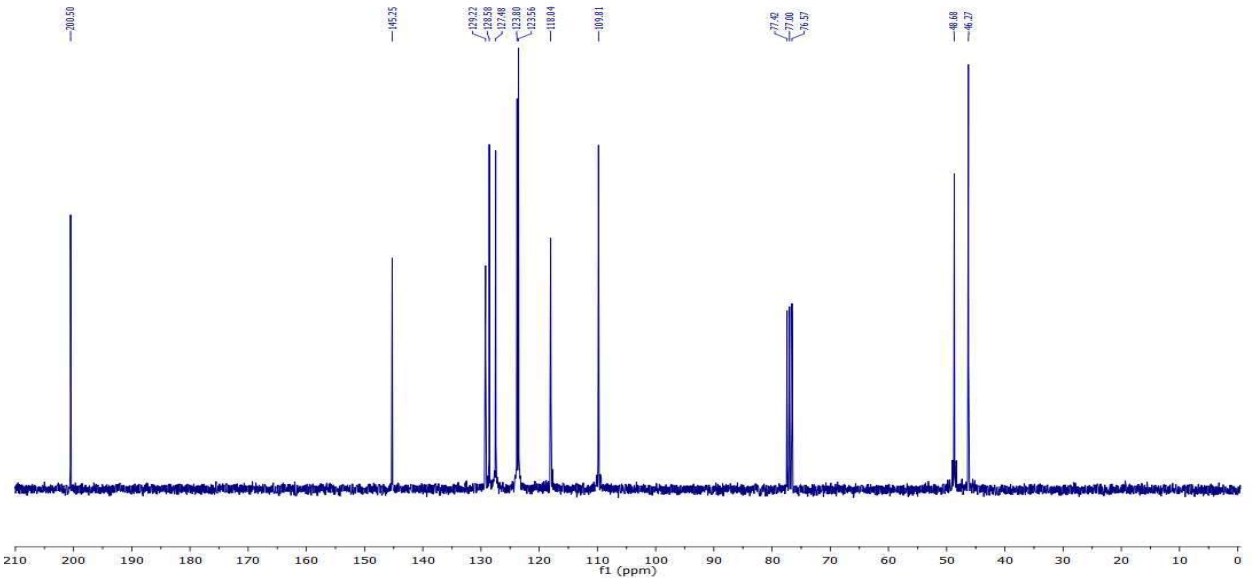
$CDCl_3$ 75 MHz

Figure A-24: ^{13}C NMR spectrum of 1-Methylindoline-2-thione (N-methyl thioindole, HSInMe).



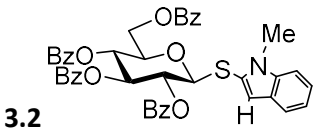
$CDCl_3$ 300 MHz

Figure A-25: ^{1}H- NMR spectra of 1-Allylindolin-2-thione (N-allyl thioindole, HSInAll).

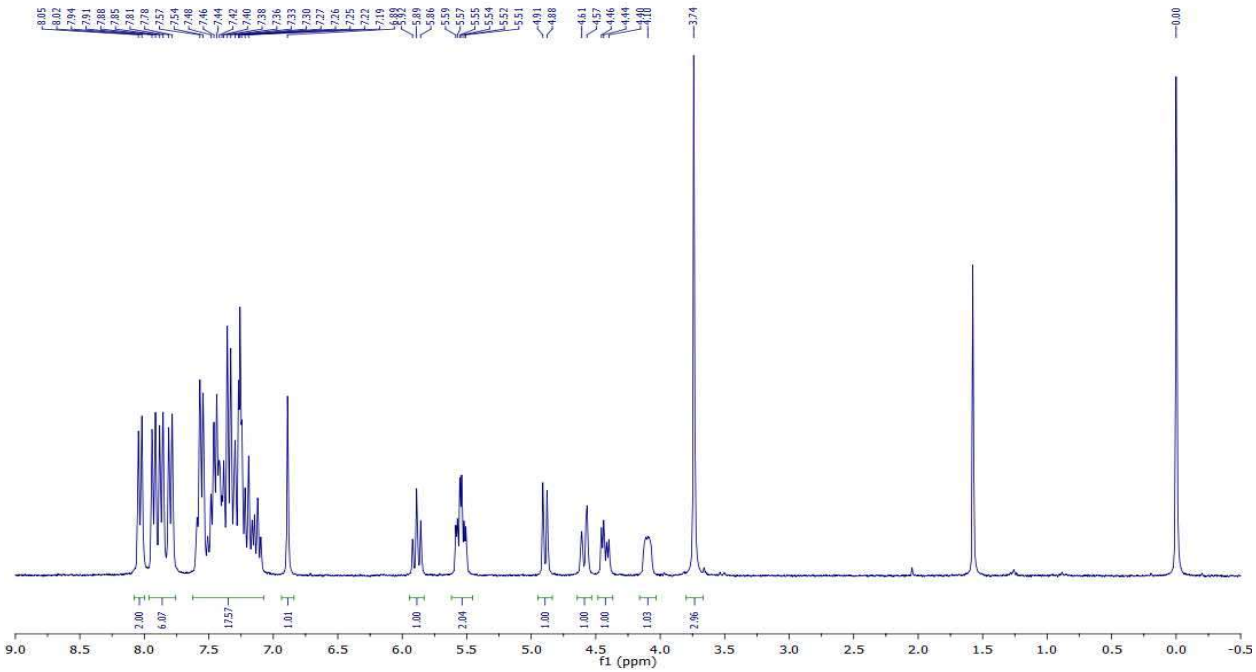


$CDCl_3$ 75 MHz

Figure A-26: ^{13}C NMR spectrum of 1-Allylindolin-2-thione (N-allyl thioindole, HSInAll).

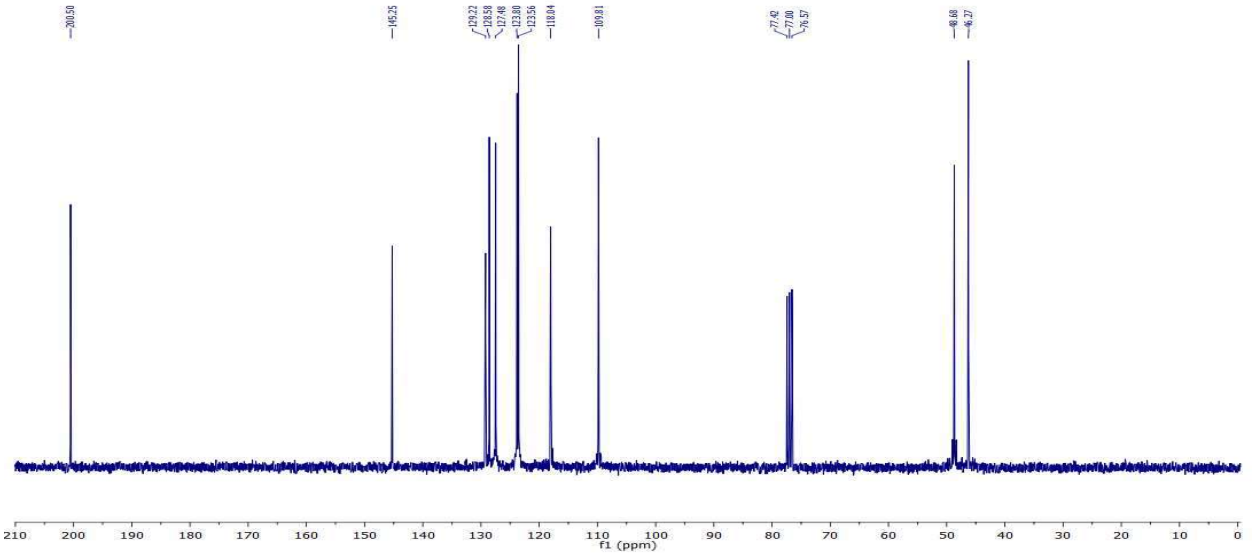


3.2



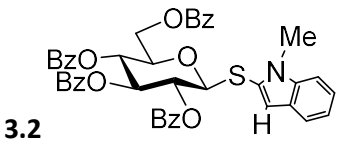
$CDCl_3$ 300 MHz

Figure A-27: ^{1}H- NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.2**).

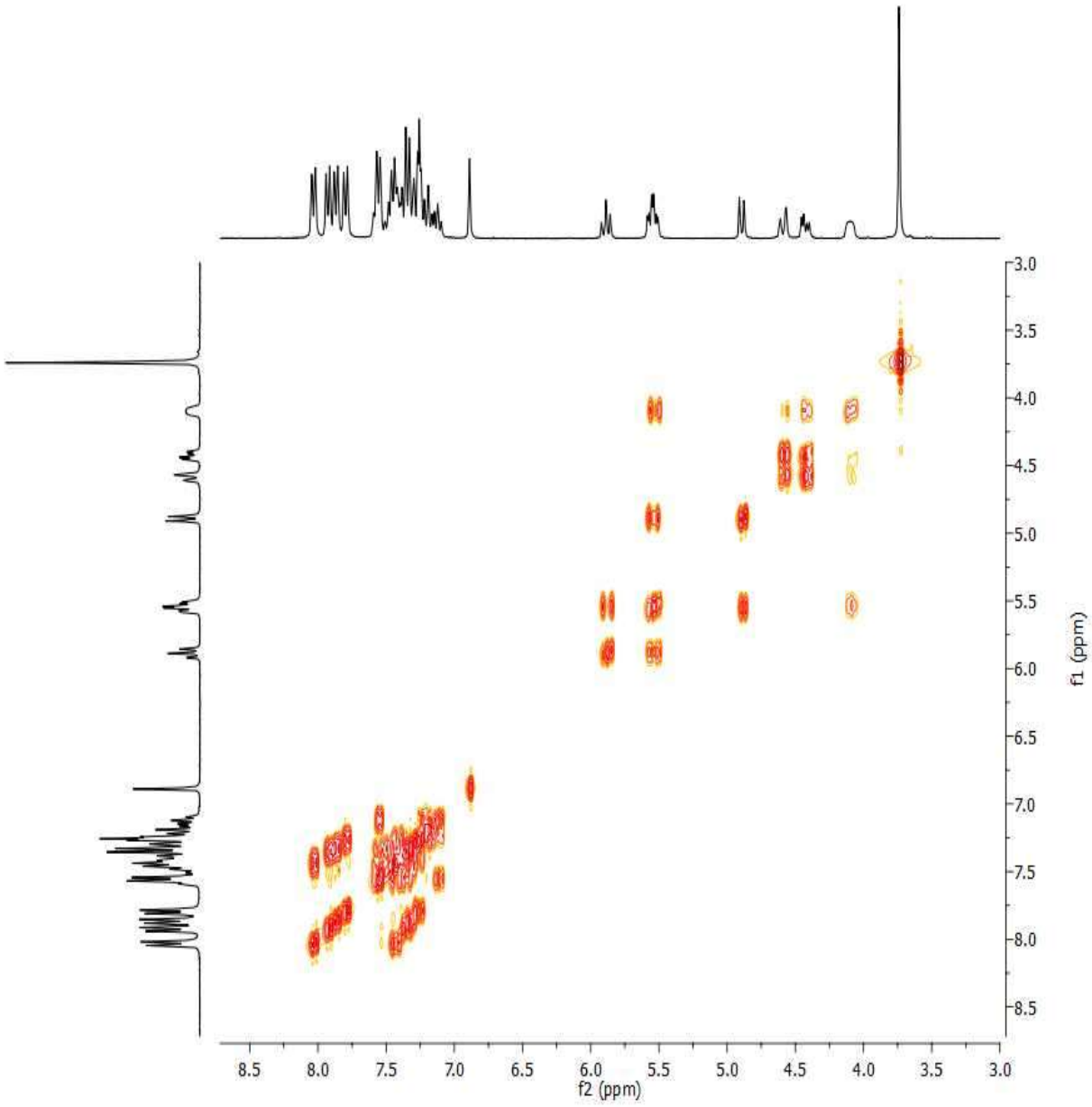


$CDCl_3$ 75 MHz

Figure A-28: ^{13}C NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.2**).

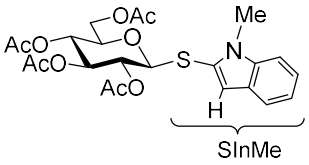


3.2

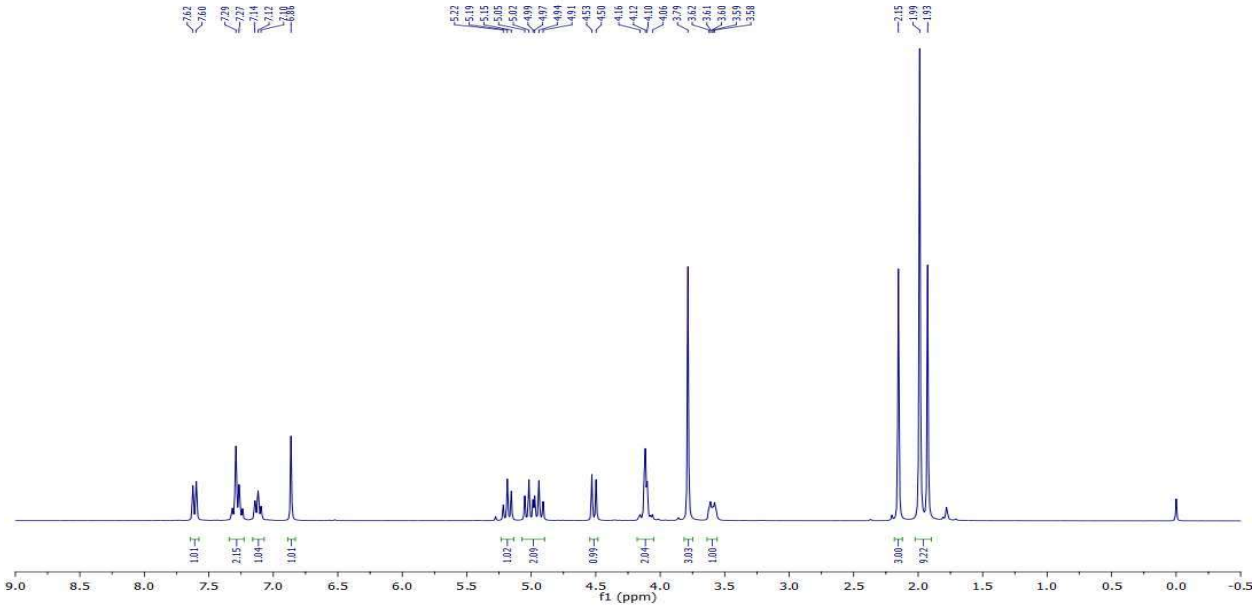


$CDCl_3$ 300 MHz

Figure A-29: 2-D NMR COSY spectrum of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.2**).

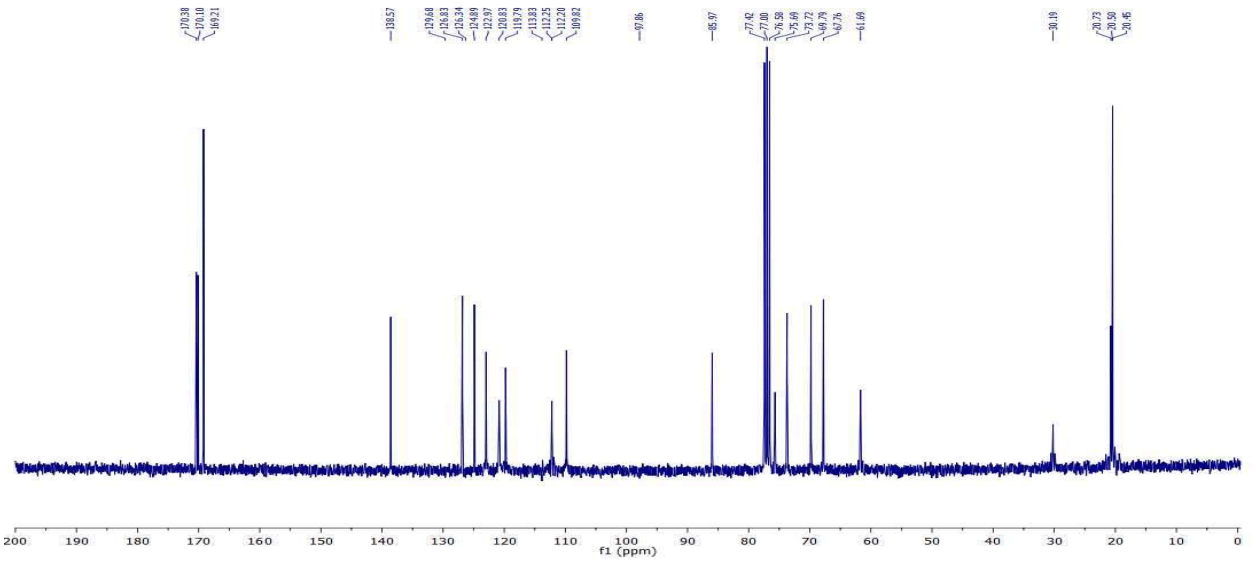


3.4



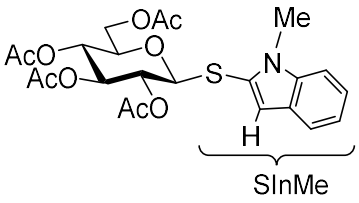
$CDCl_3$ 300 MHz

Figure A-30: ^{1}H- NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**3.4**).

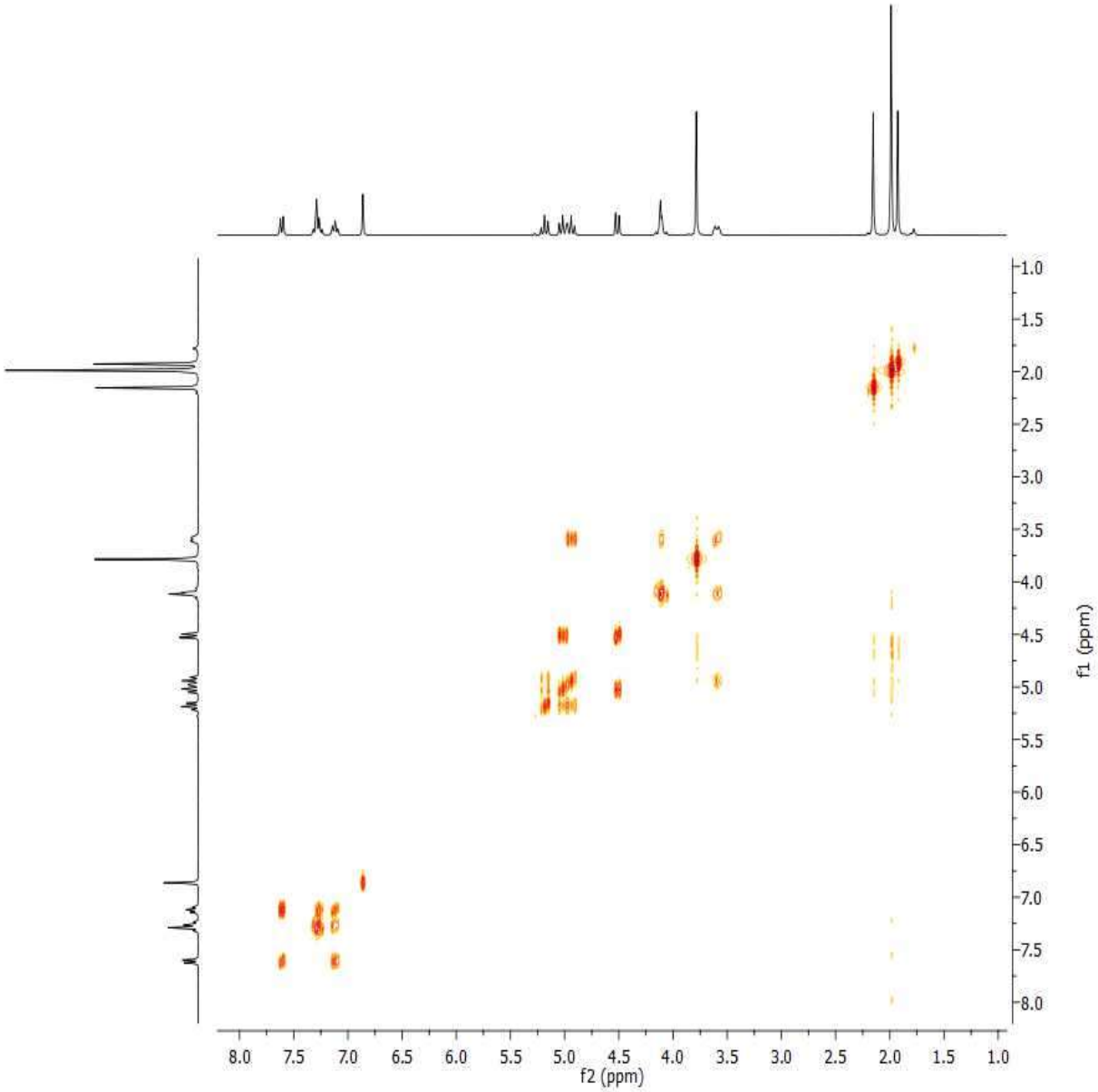


$CDCl_3$ 75 MHz

Figure A-31: ^{13}C NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**3.4**).

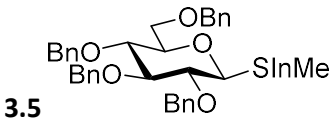


3.4

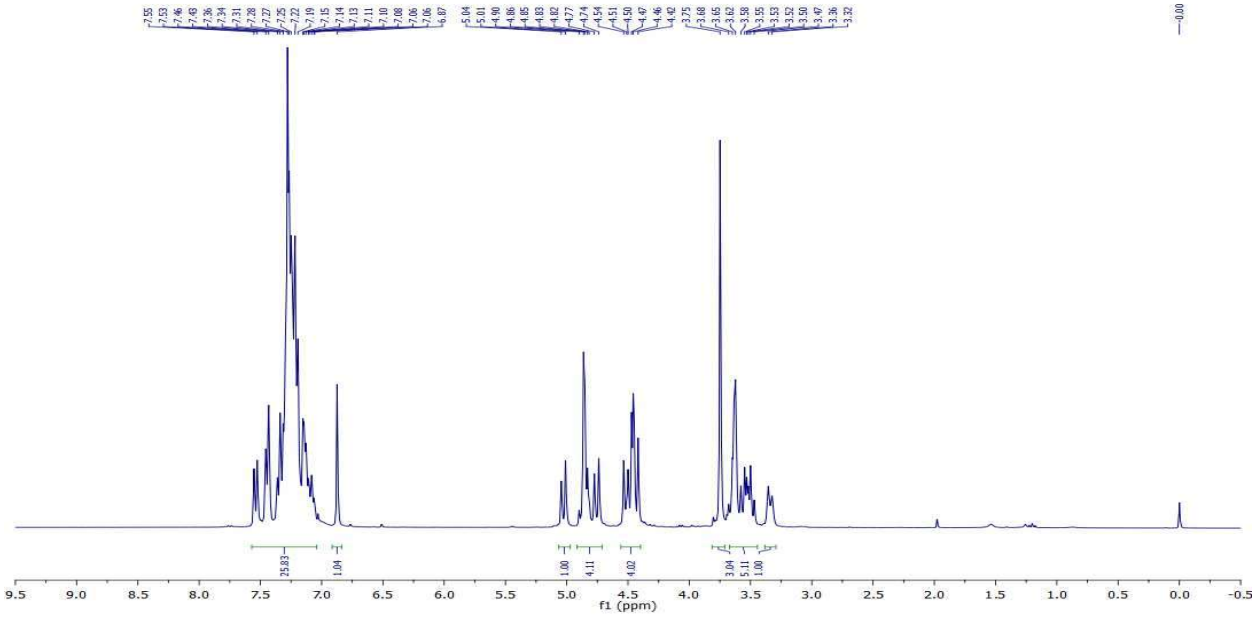


$CDCl_3$ 300 MHz

Figure A-32: 2-D NMR COSY spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**3.4**).

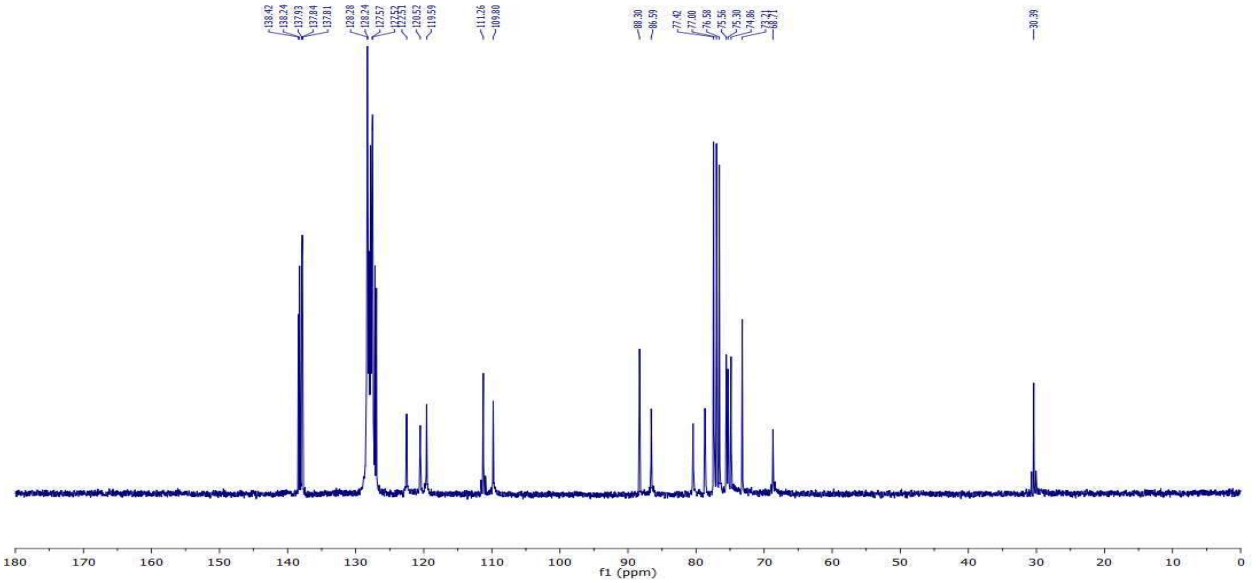


3.5



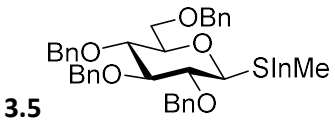
$CDCl_3$ 300 MHz

Figure A-33: ^{1}H- NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3.5**).

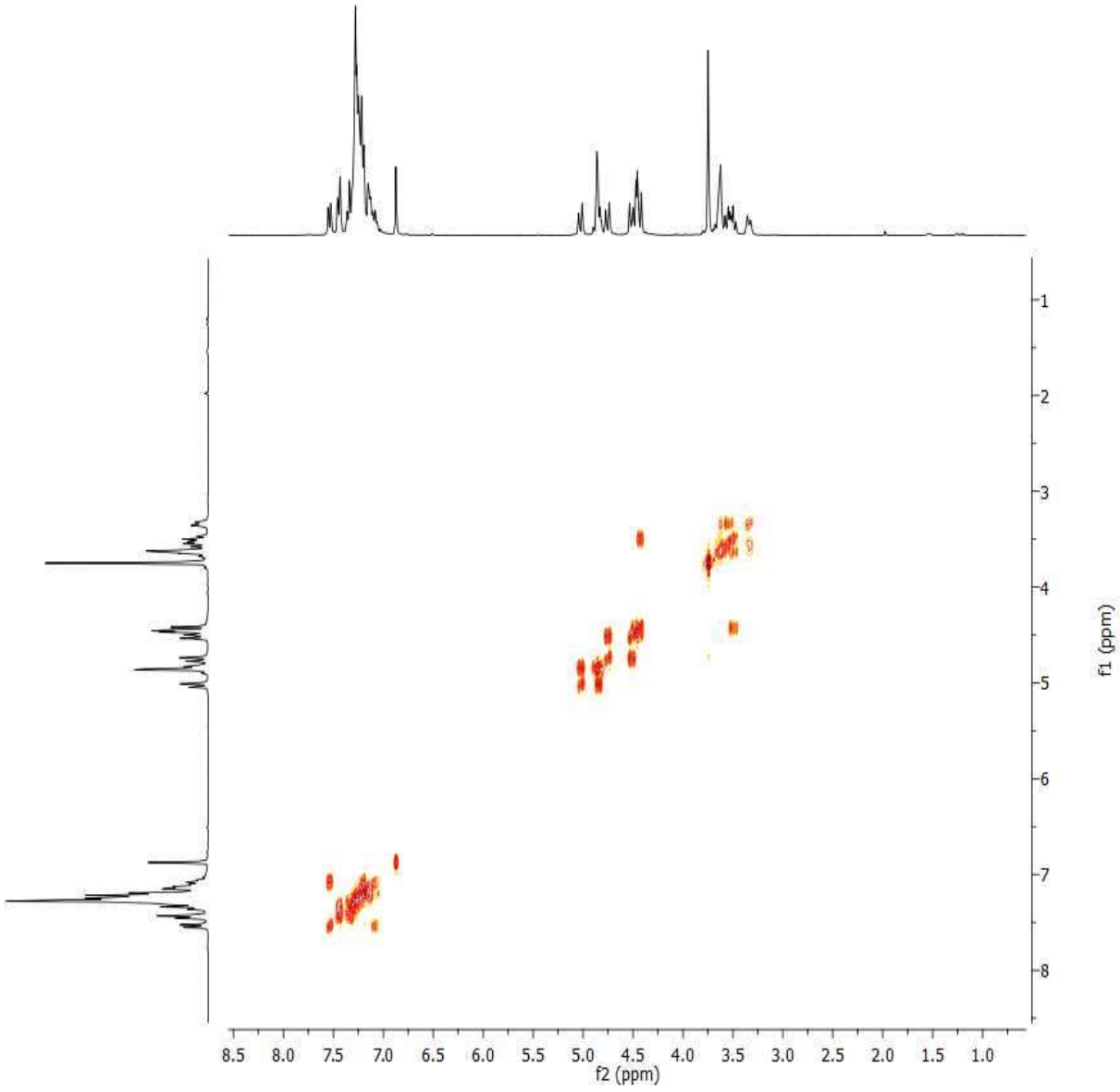


$CDCl_3$ 75 MHz

Figure A-34: ^{13}C NMR spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3.5**).

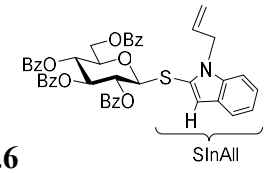


3.5

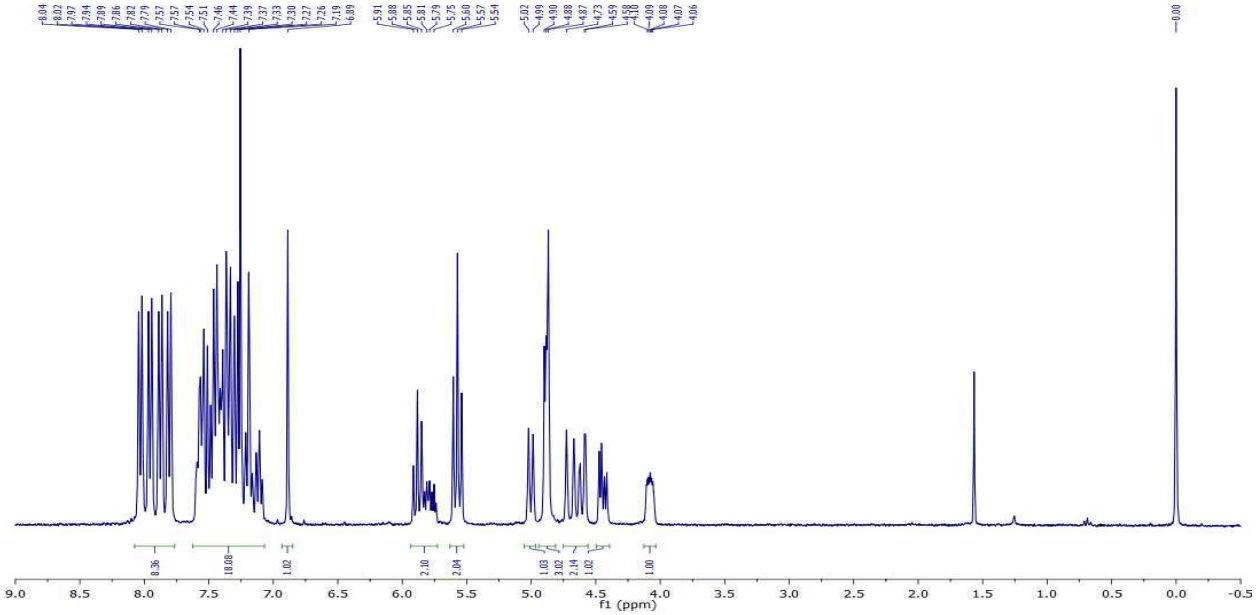


$CDCl_3$ 300 MHz

Figure A-35: 2-D NMR COSY spectra of 1-Methylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**3.5**).

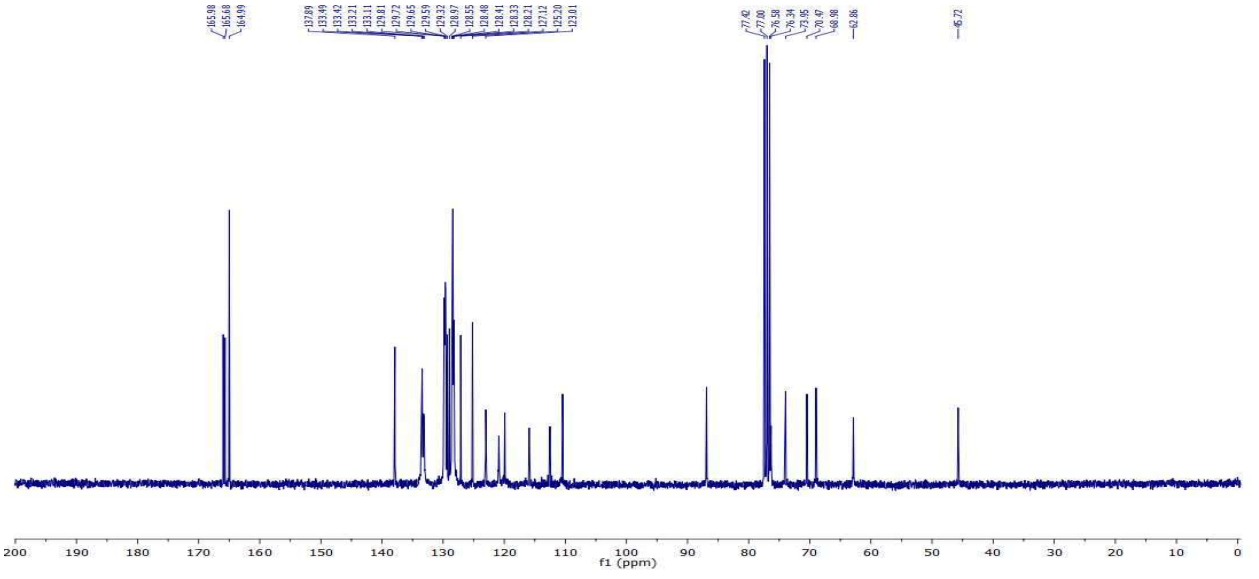


3.6



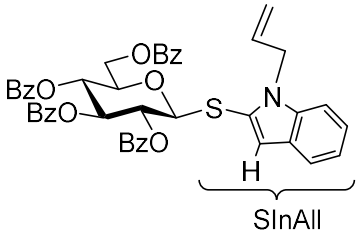
$CDCl_3$ 300 MHz

Figure A-36: ^{1}H- NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.6**).

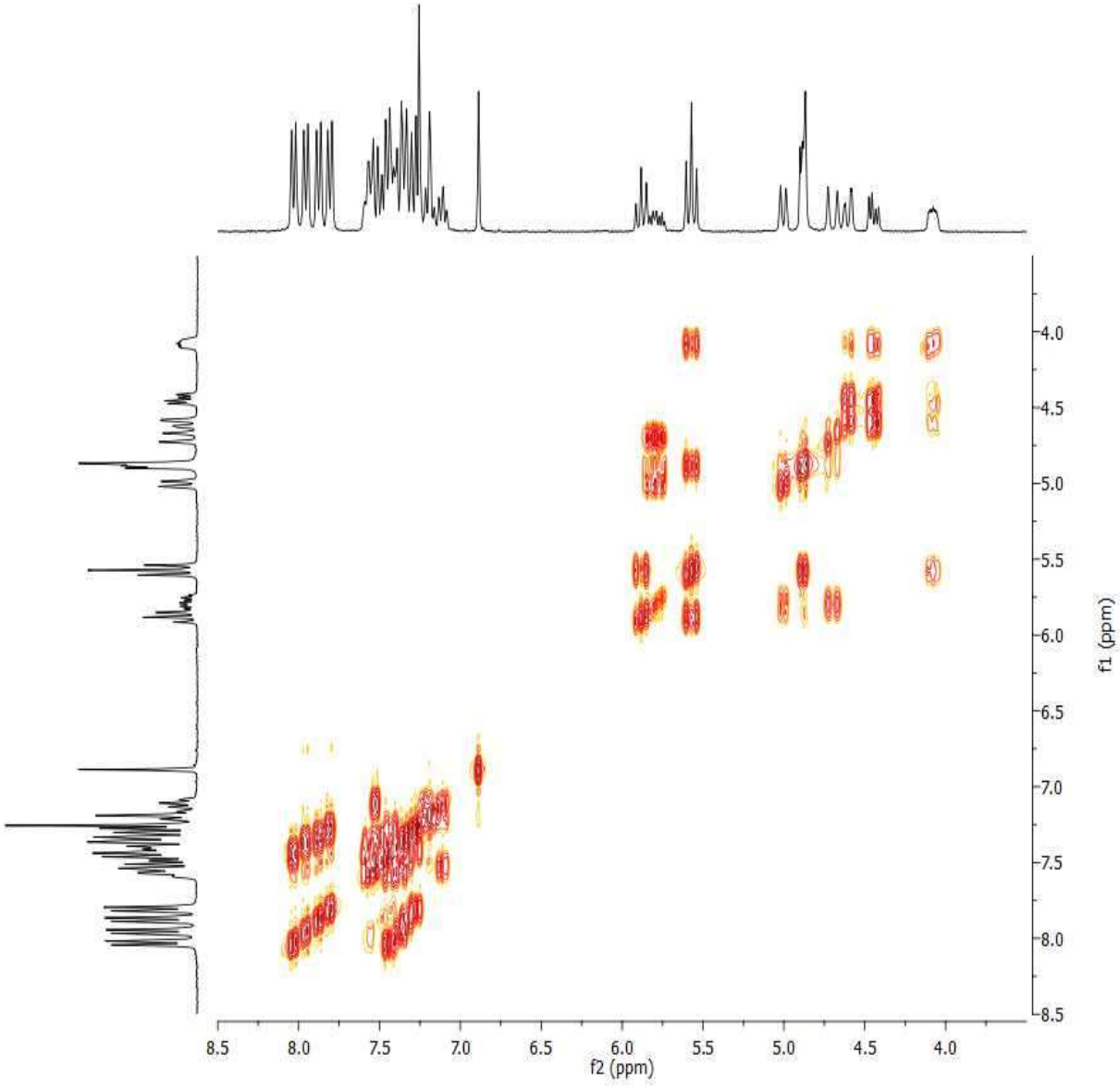


$CDCl_3$ 75 MHz

Figure A-37: ^{13}C NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.6**).

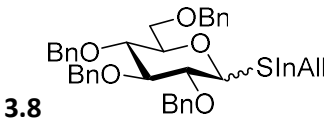


3.6

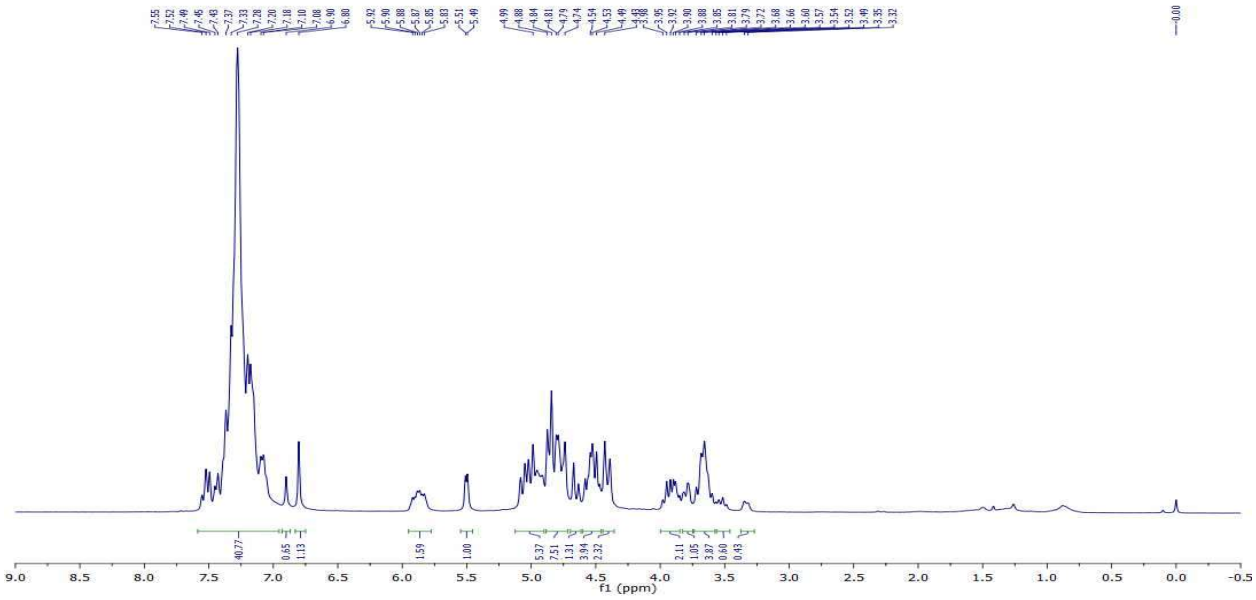


$CDCl_3$ 300 MHz

Figure A-38: 2-D NMR COSY spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**3.6**).

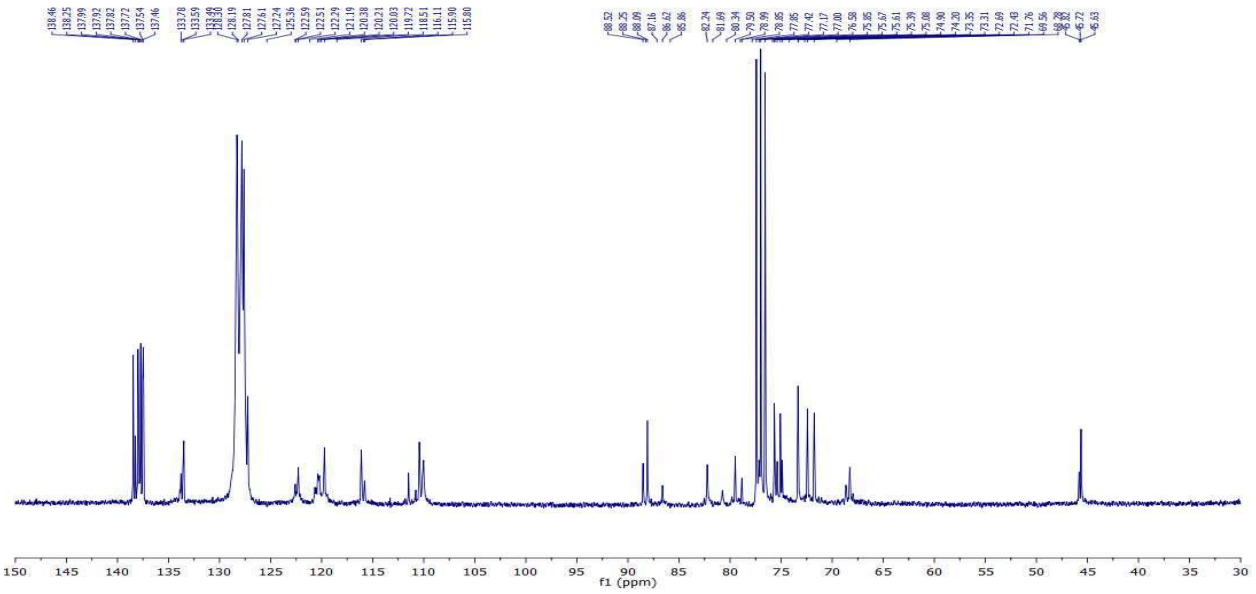


3.8



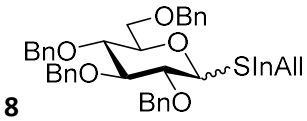
$CDCl_3$ 300 MHz

Figure A-39: ^{1}H- NMR spectra of 1-Allylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**3.8**).

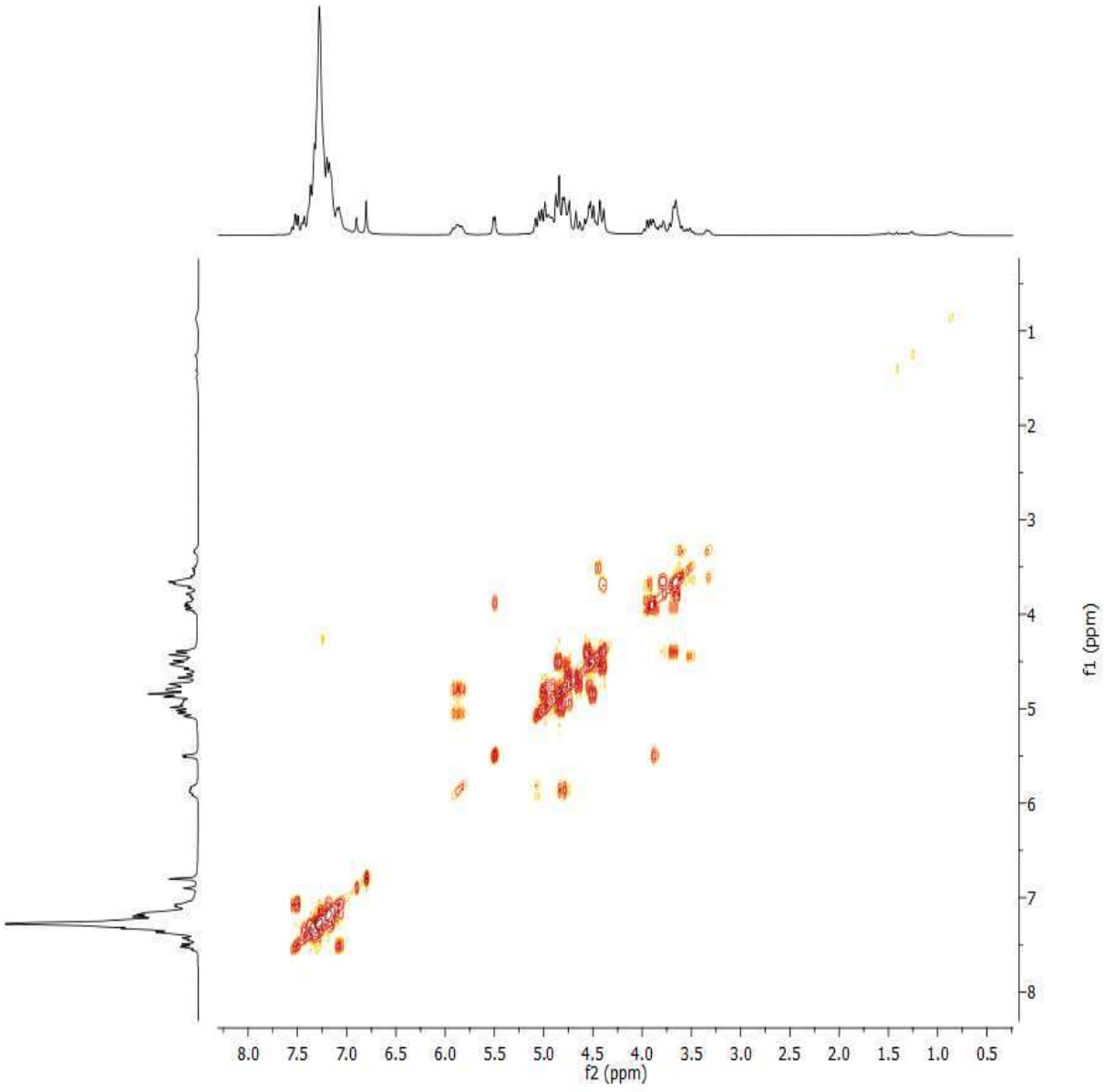


$CDCl_3$ 75 MHz

Figure A-40: ^{13}C NMR spectra of 1-Allylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**3.8**)

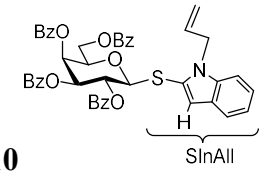


8

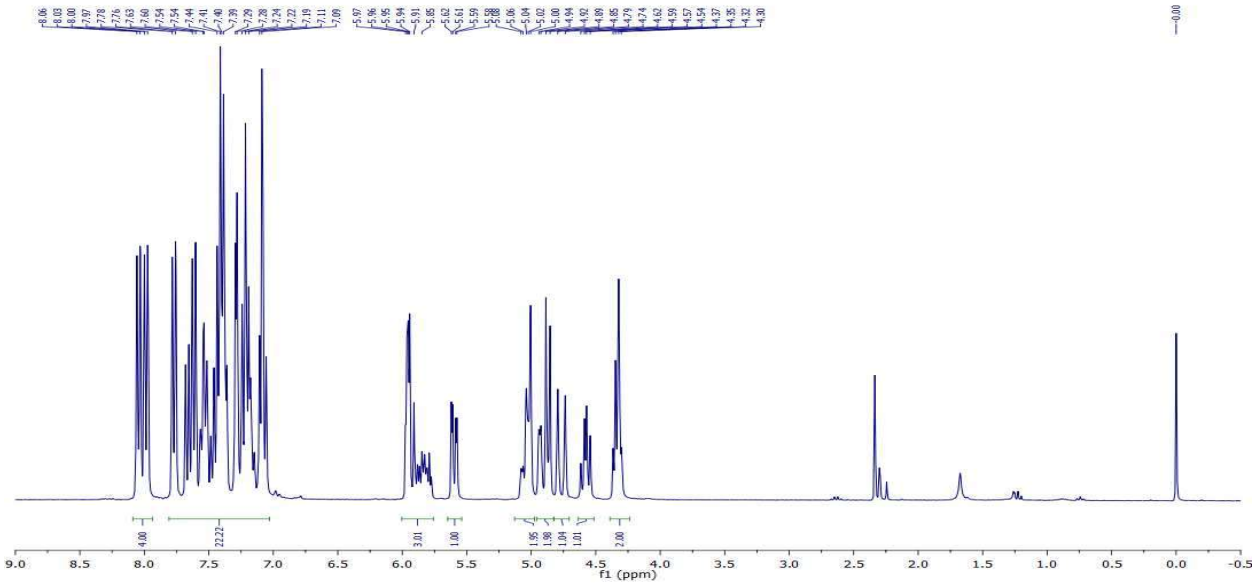


$CDCl_3$ 300 MHz

Figure A-41: 2-D NMR COSY spectra of 1-Allylindolyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranoside (**3.8**)

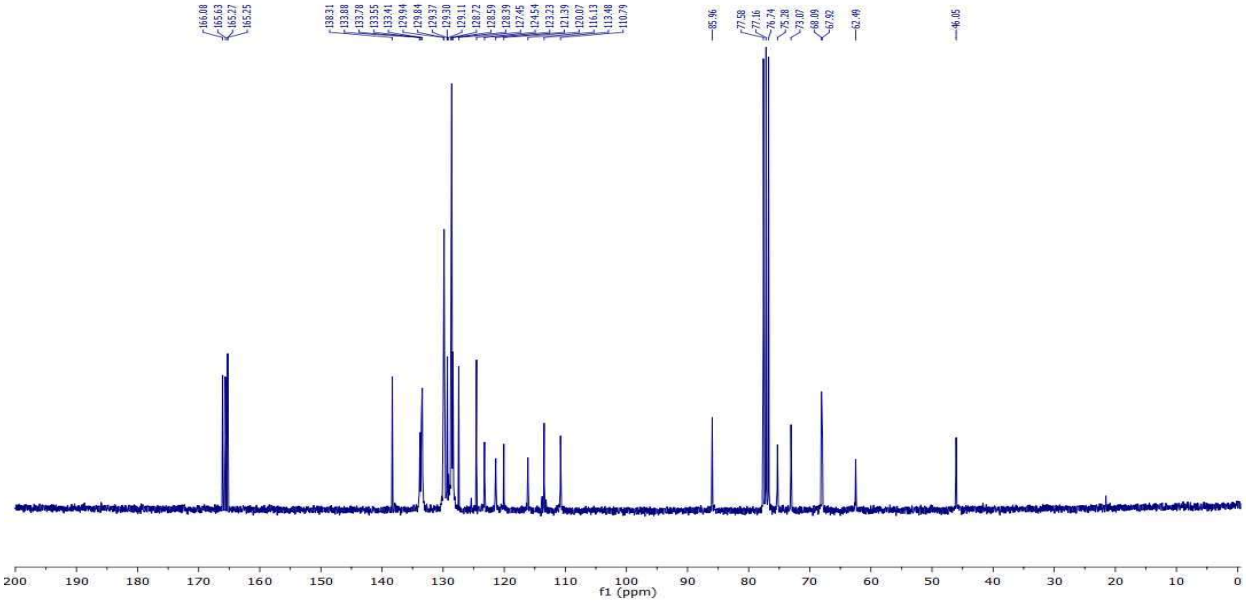


β-3.10



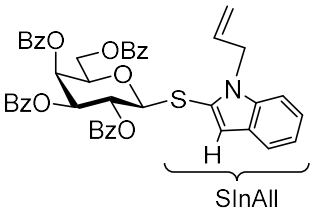
$CDCl_3$ 300 MHz

Figure A-42: ^{1}H- NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (β-**3.10**).

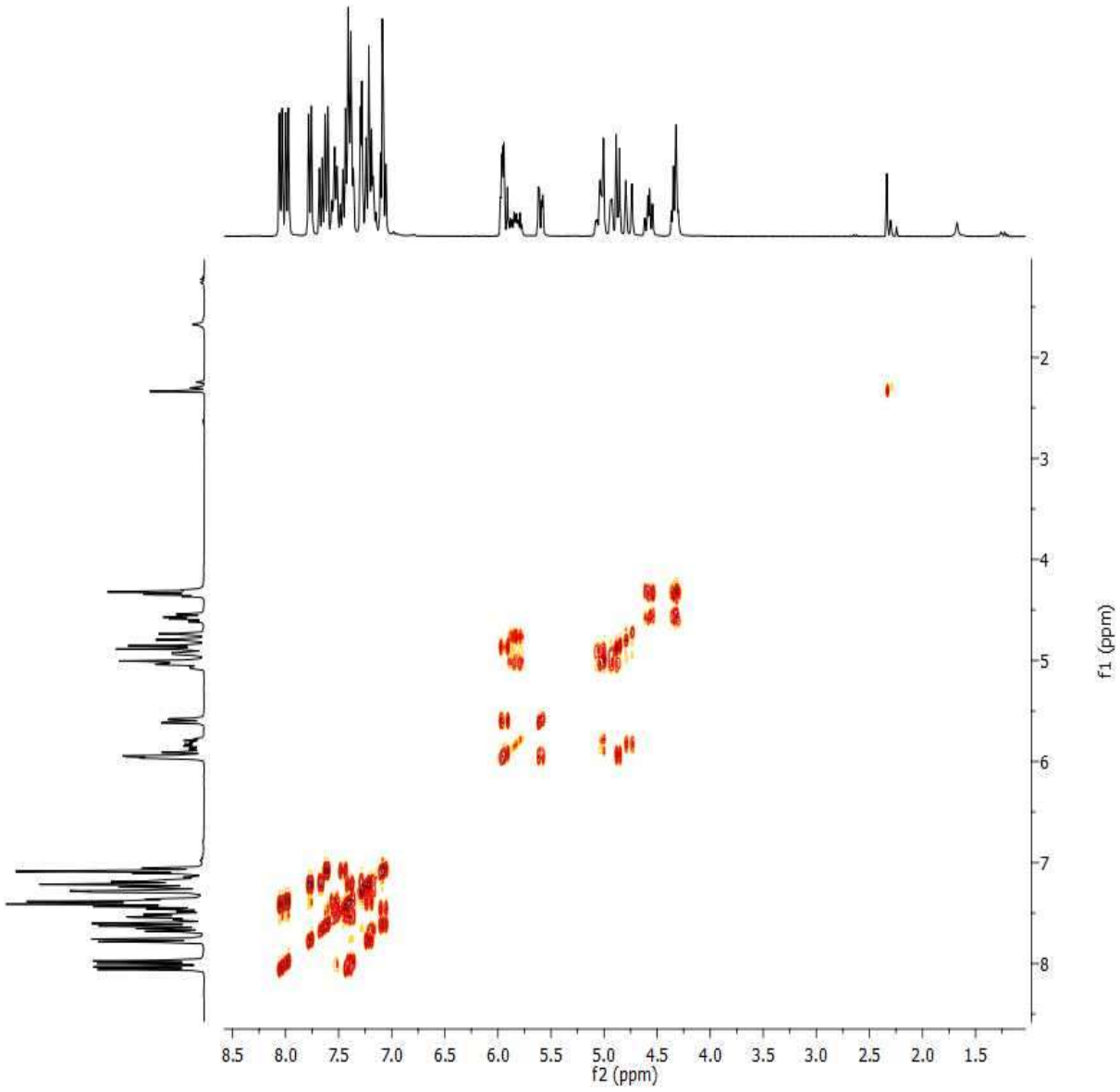


$CDCl_3$ 75 MHz

Figure A-43: ^{13}C NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio- β-D-galactopyranoside (β-**3.10**).

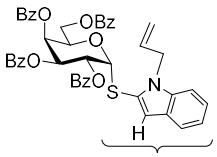


β-3.10

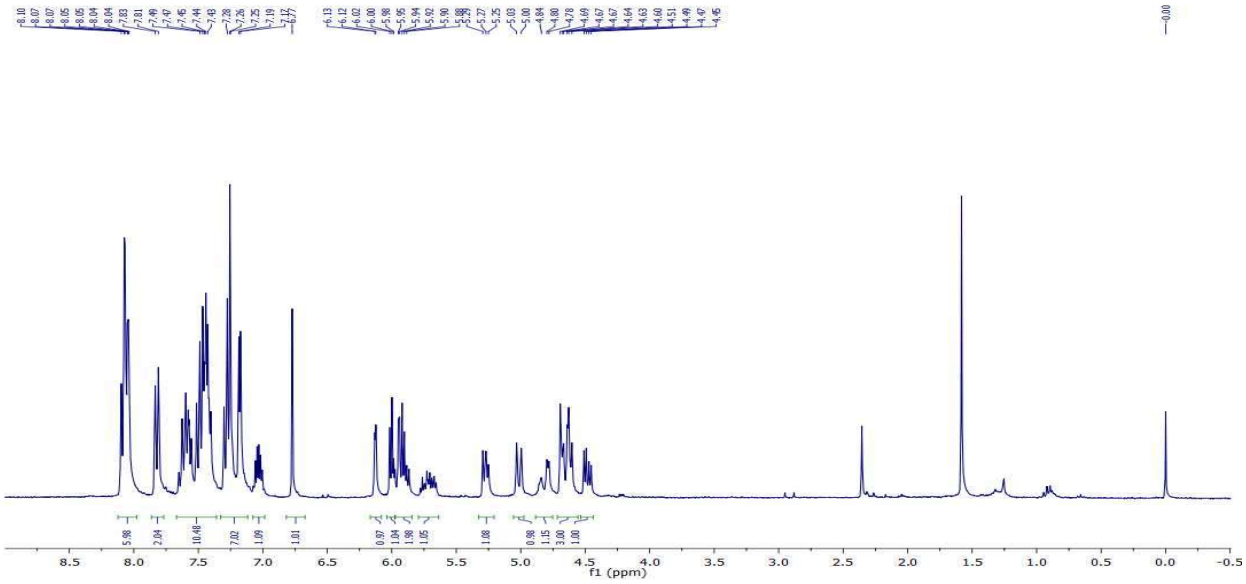


$CDCl_3$ 300 MHz

Figure A-44: 2-D NMR COSY spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (β-**3.10**).

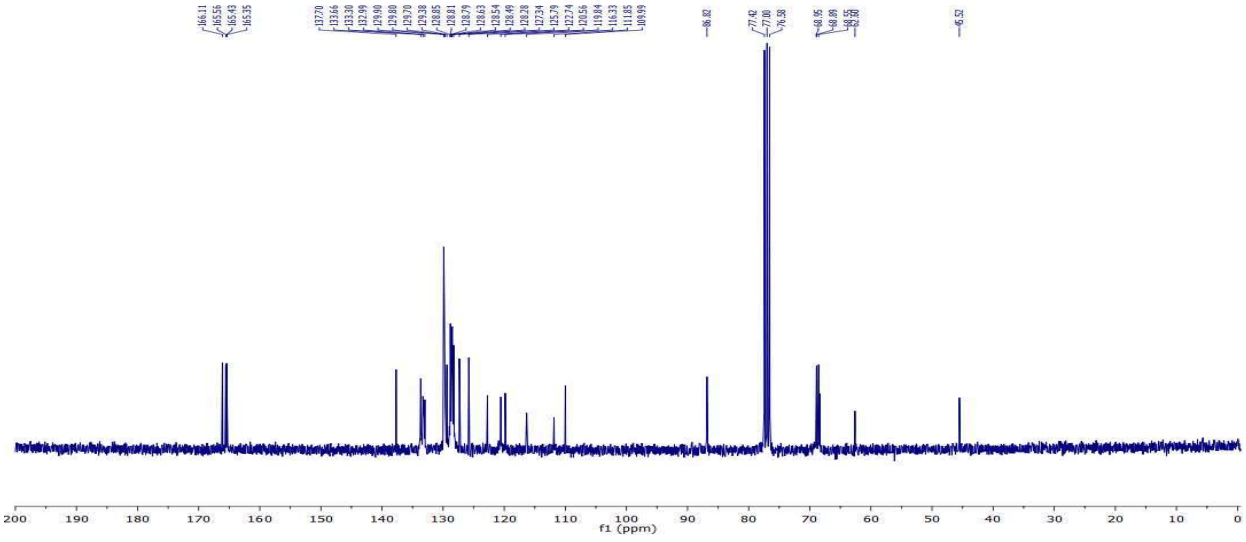


α-3.10



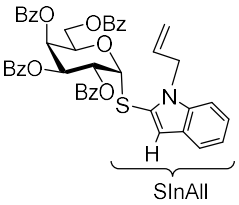
$CDCl_3$ 300 MHz

Figure A-45: ^{1}H- NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-galactopyranoside (α-**3.10**).

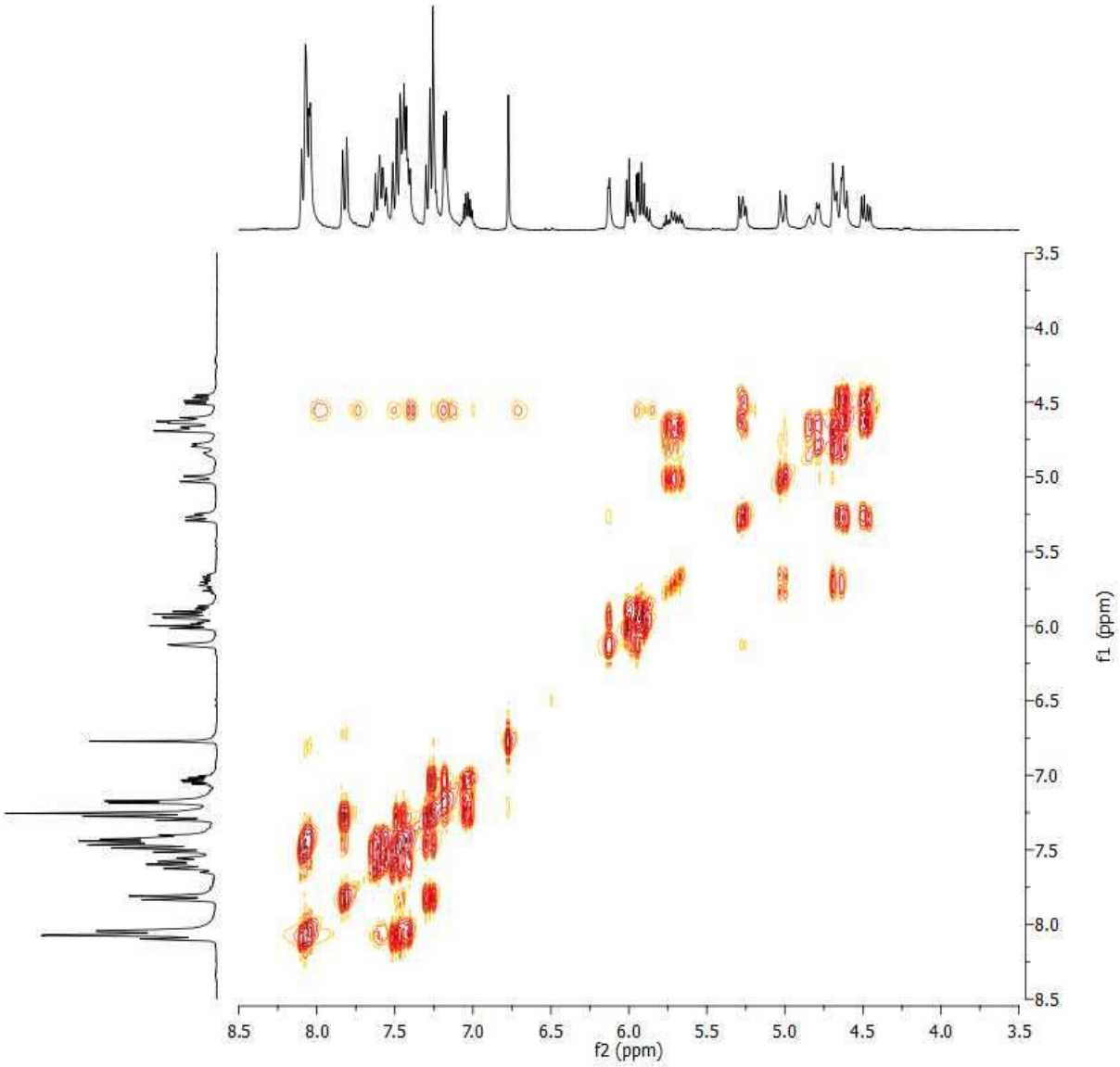


$CDCl_3$ 75 MHz

Figure A-46: ^{13}C NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-galactopyranoside (α-**3.10**).

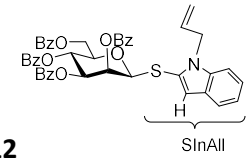


α-3.10

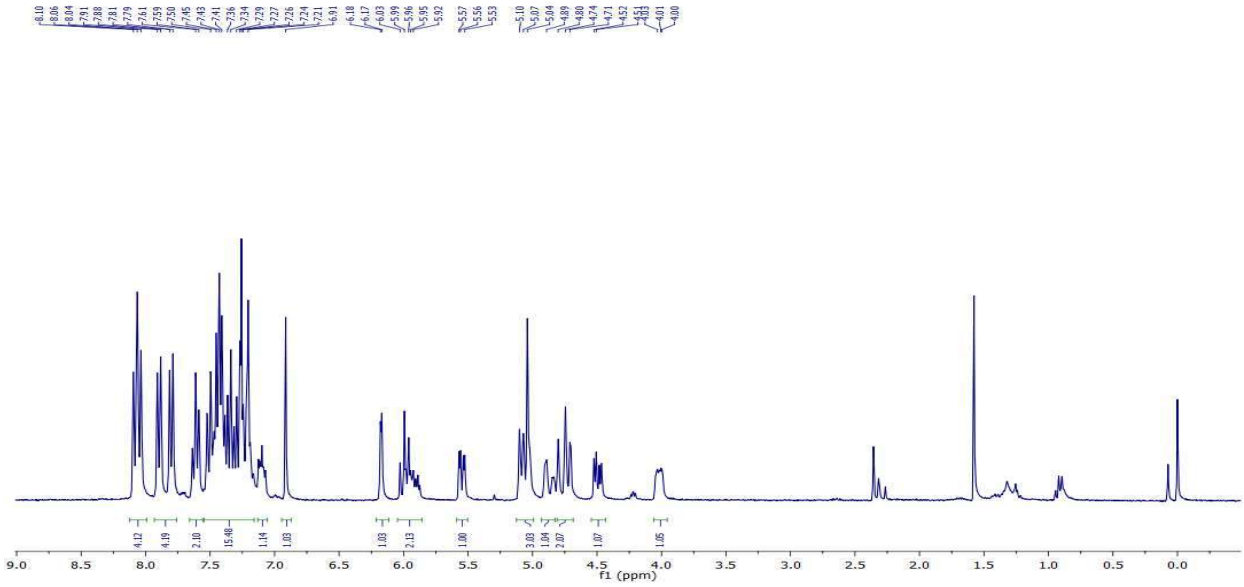


$CDCl_3$ 300 MHz

Figure A-47: 2-D NMR COSY spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-galactopyranoside (β-**3.10**).

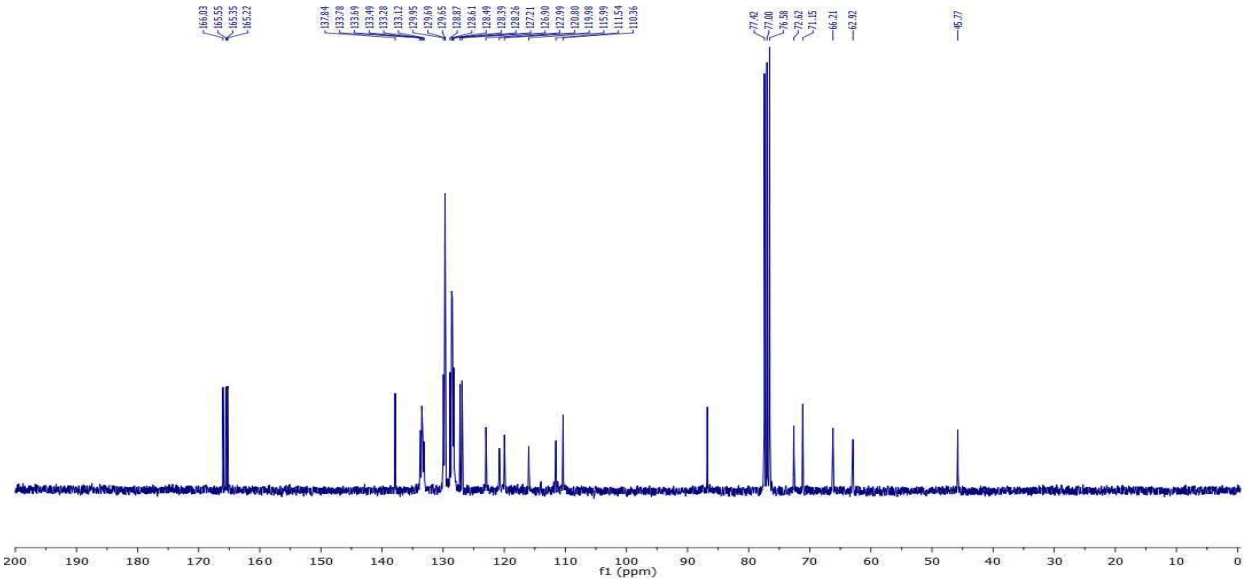


3.12



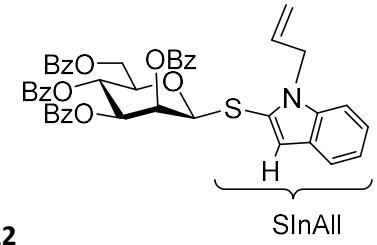
$CDCl_3$ 300 MHz

Figure A-48: ^{1}H- NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (**3.12**).

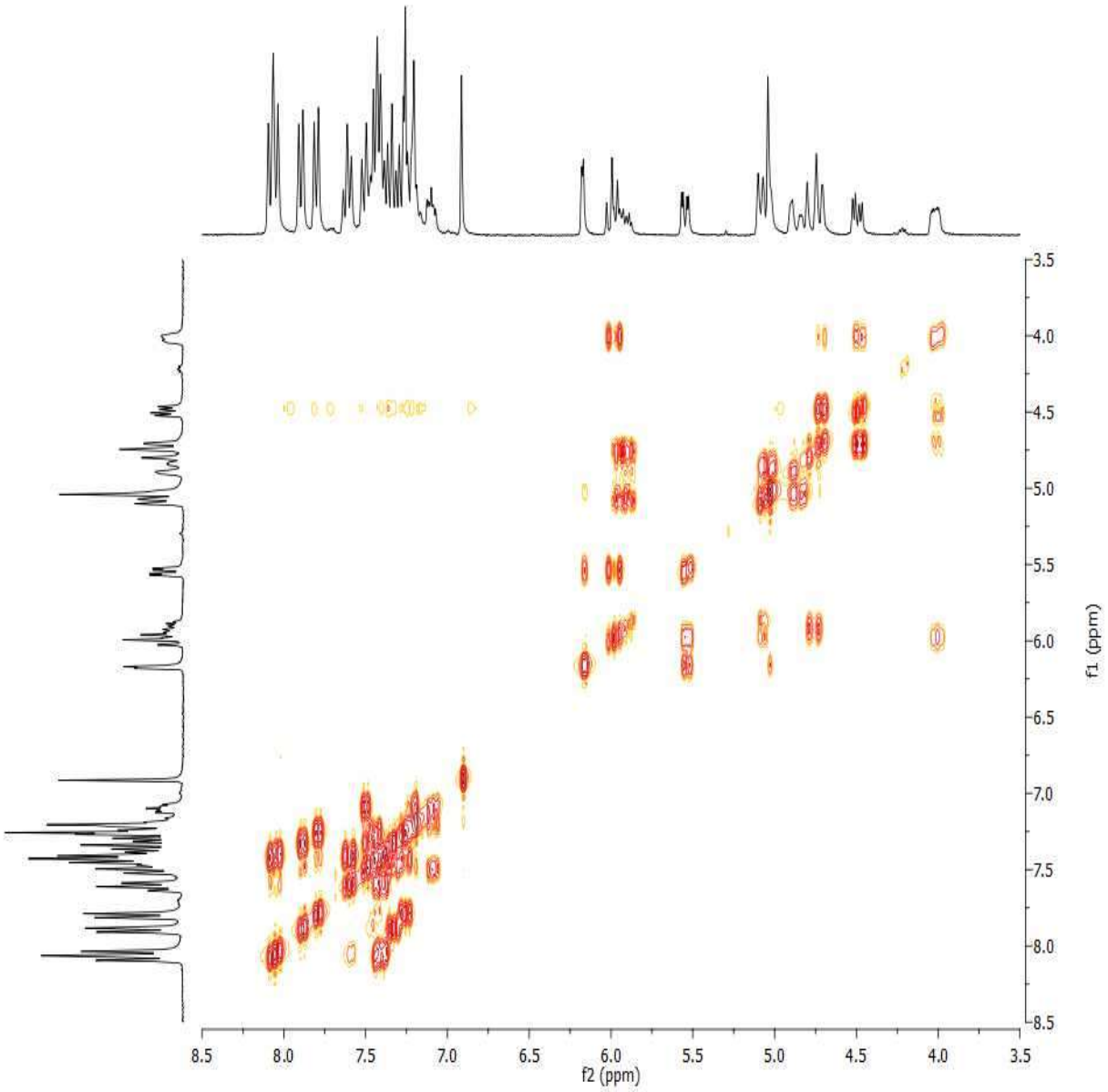


$CDCl_3$ 75 MHz

Figure A-49: ^{13}C NMR spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (**3.12**).



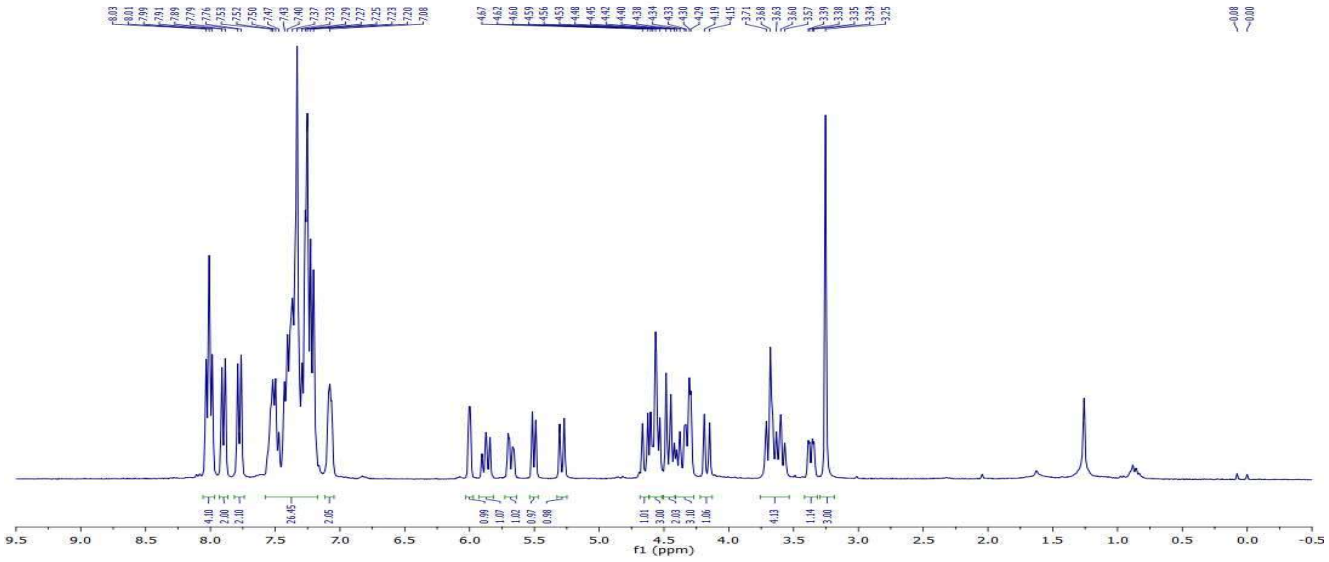
12



$CDCl_3$ 300 MHz

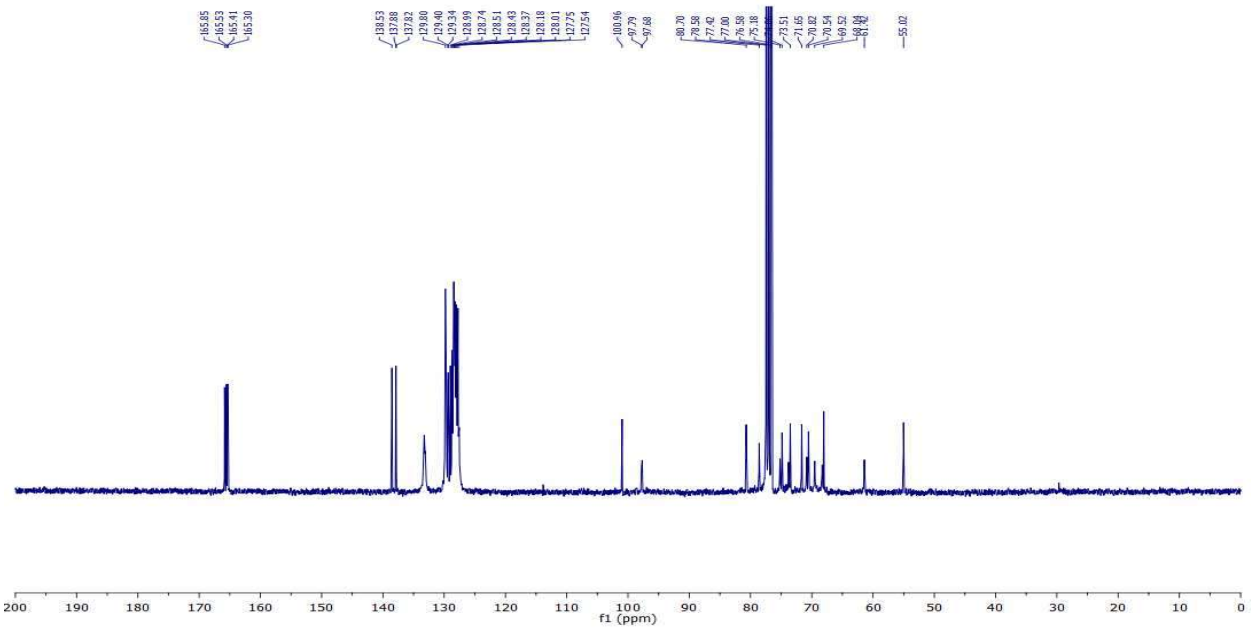
Figure A-50: 2-D NMR COSY spectra of 1-Allyllindolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (**3.12**).

3.27



$CDCl_3$ 300 MHz

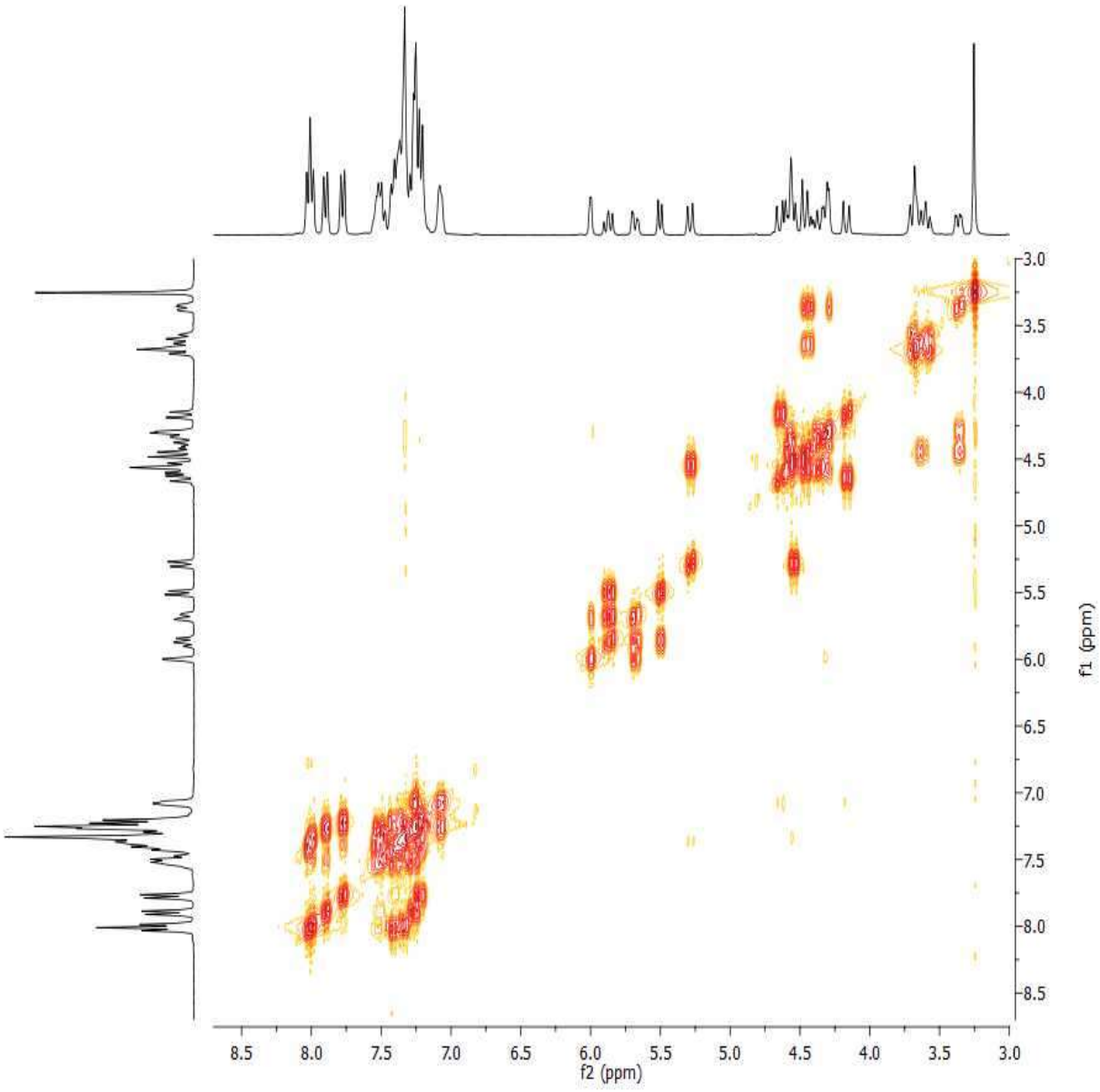
Figure A-51: ^{1}H- NMR spectra of Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.27**).



$CDCl_3$ 75 MHz

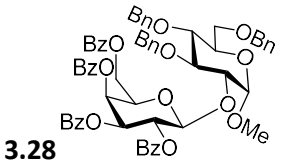
Figure A-52: ^{13}C NMR spectra of Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.27**).

3.27

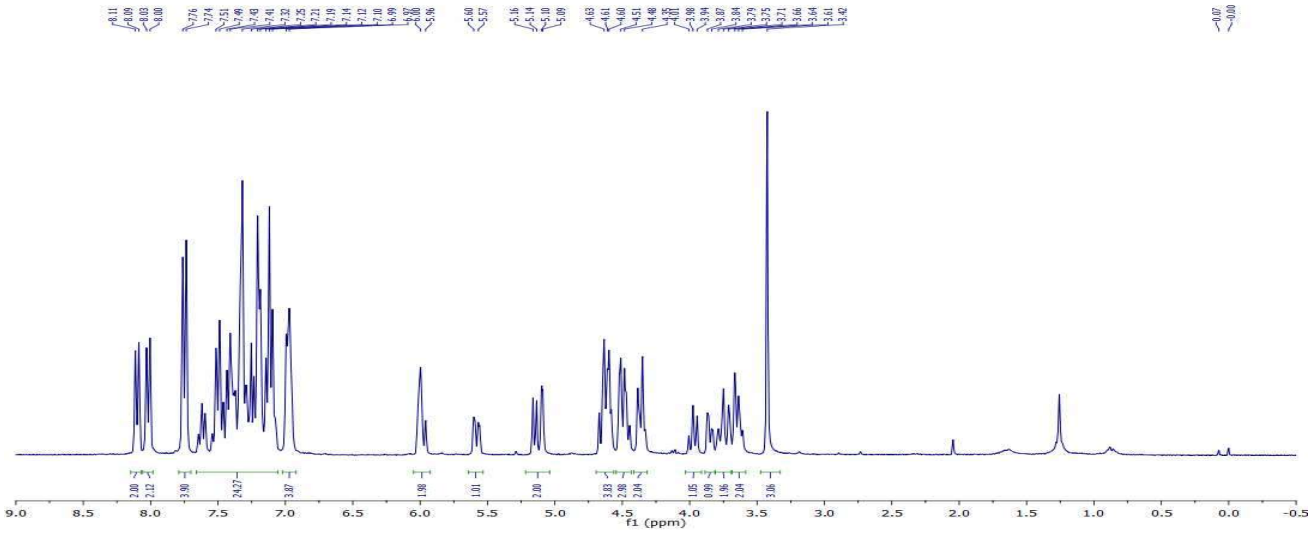


$CDCl_3$ 300 MHz

Figure A-53: 2-D NMR COSY spectra of Methyl 3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.27**).

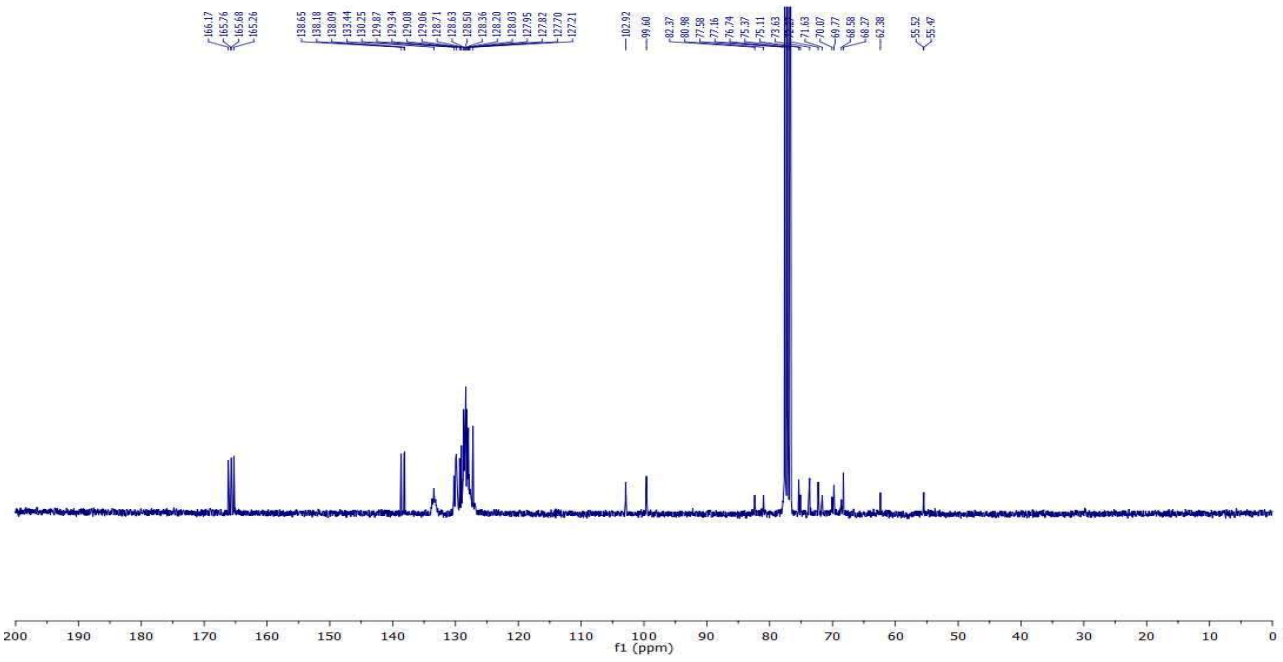


3.28



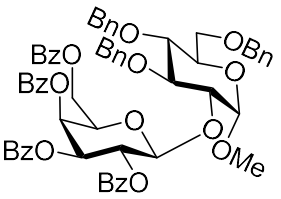
$CDCl_3$ 300 MHz

Figure A-54: ^{1}H- NMR spectra of Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.28**).

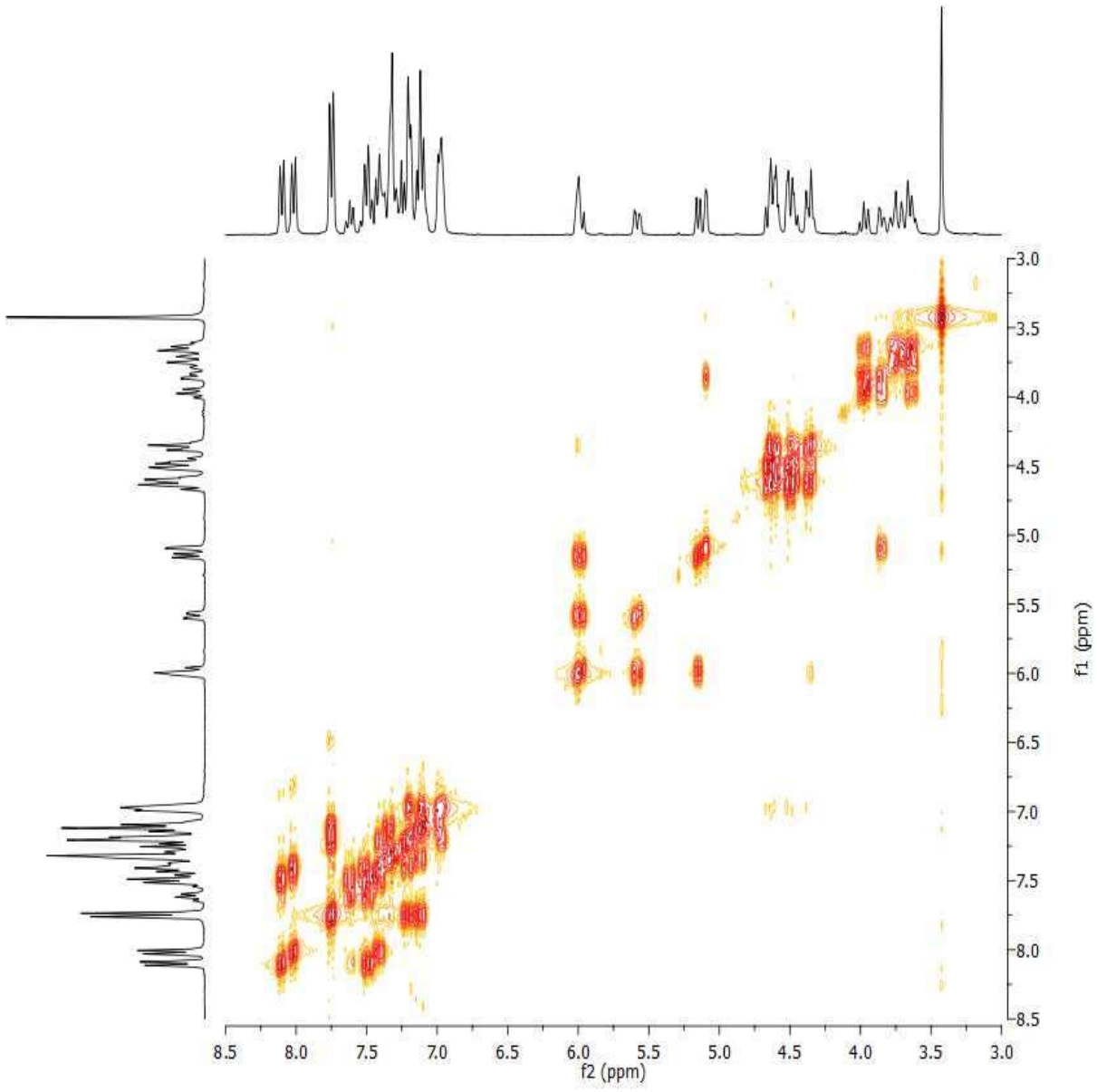


$CDCl_3$ 75 MHz

Figure A-55: ^{13}C NMR spectra of Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.28**).



3.28



$CDCl_3$ 300 MHz

Figure A-56: 2-D NMR COSY spectra of Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**3.28**).

[1]H NMR monitored experiments ($CDCl_3$ 300 MHz)

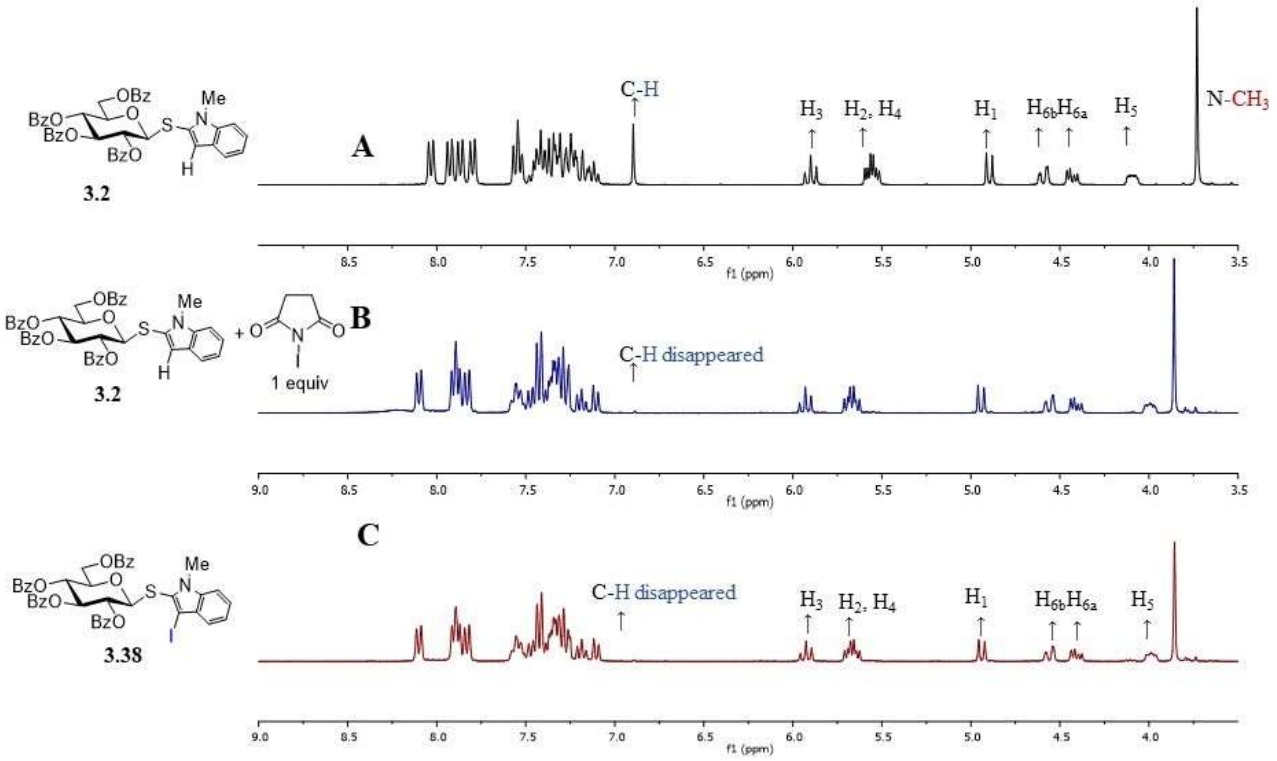


Fig A-57: [1]H- NMR spectra in $CDCl_3$ of A) Glycosyl donor **3.2**; B) Glycosyl donor **3.2** and 1 equiv. NIS in $CDCl_3$ 10 minutes; C) After column.

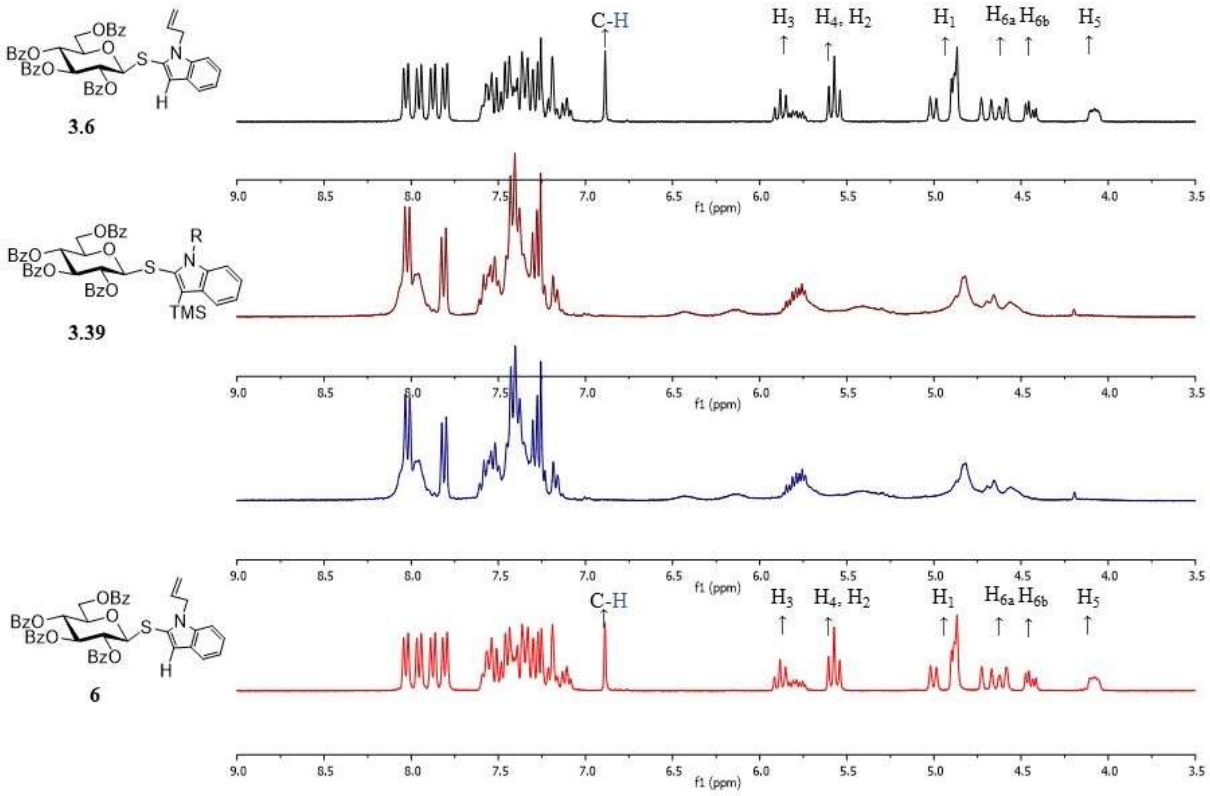
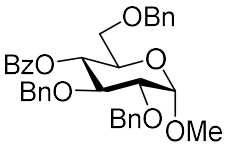
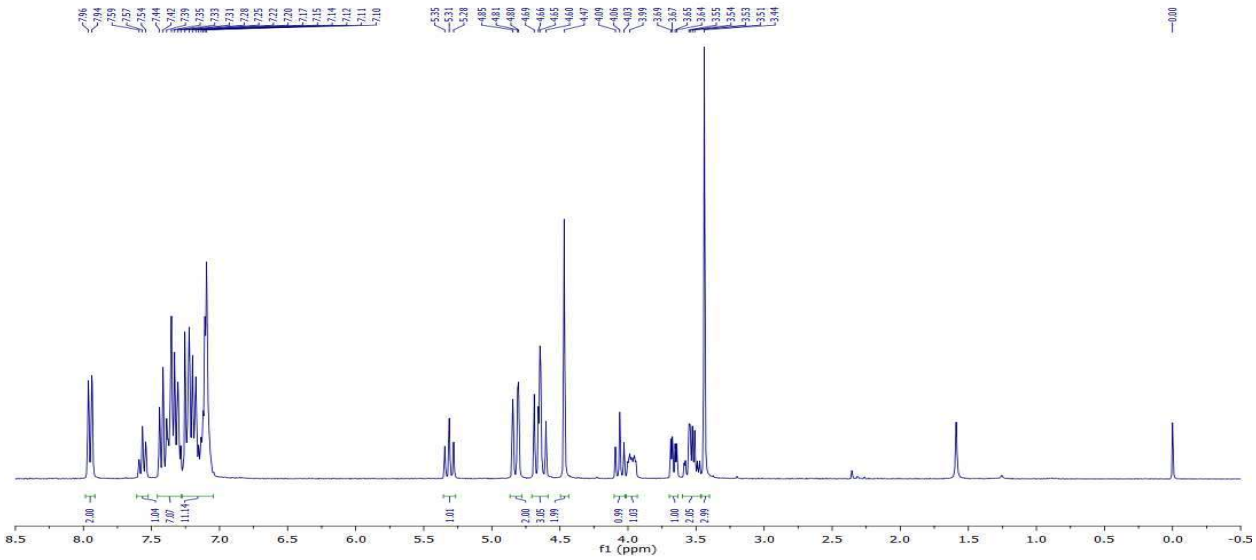


Fig A-58: 1H- NMR spectra in $CDCl_3$ of A) Glycosyl donor **3.6**; B) Glycosyl donor **3.6** and 1 equiv. TMSOTf in $CDCl_3$ 10 minutes; C) in 30 min ; D) After column

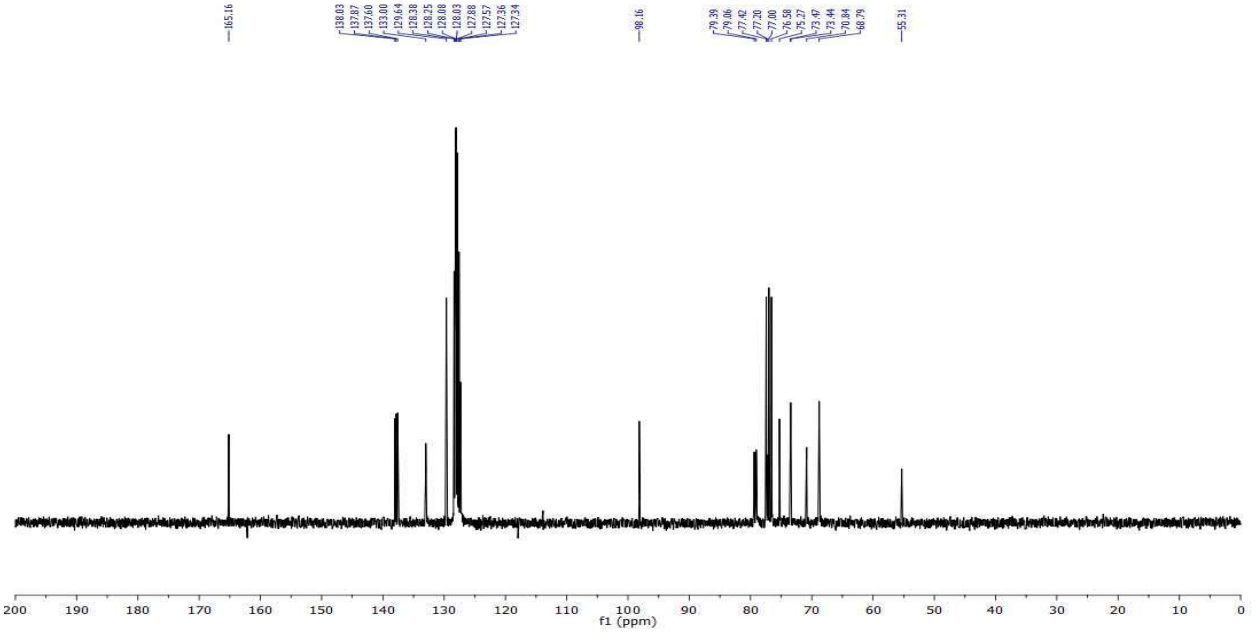


4.6



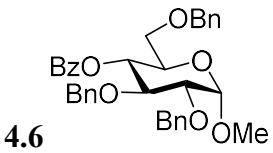
$CDCl_3$ 300 MHz

Figure A-59: ^{1}H- NMR spectra of Methyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.6**).

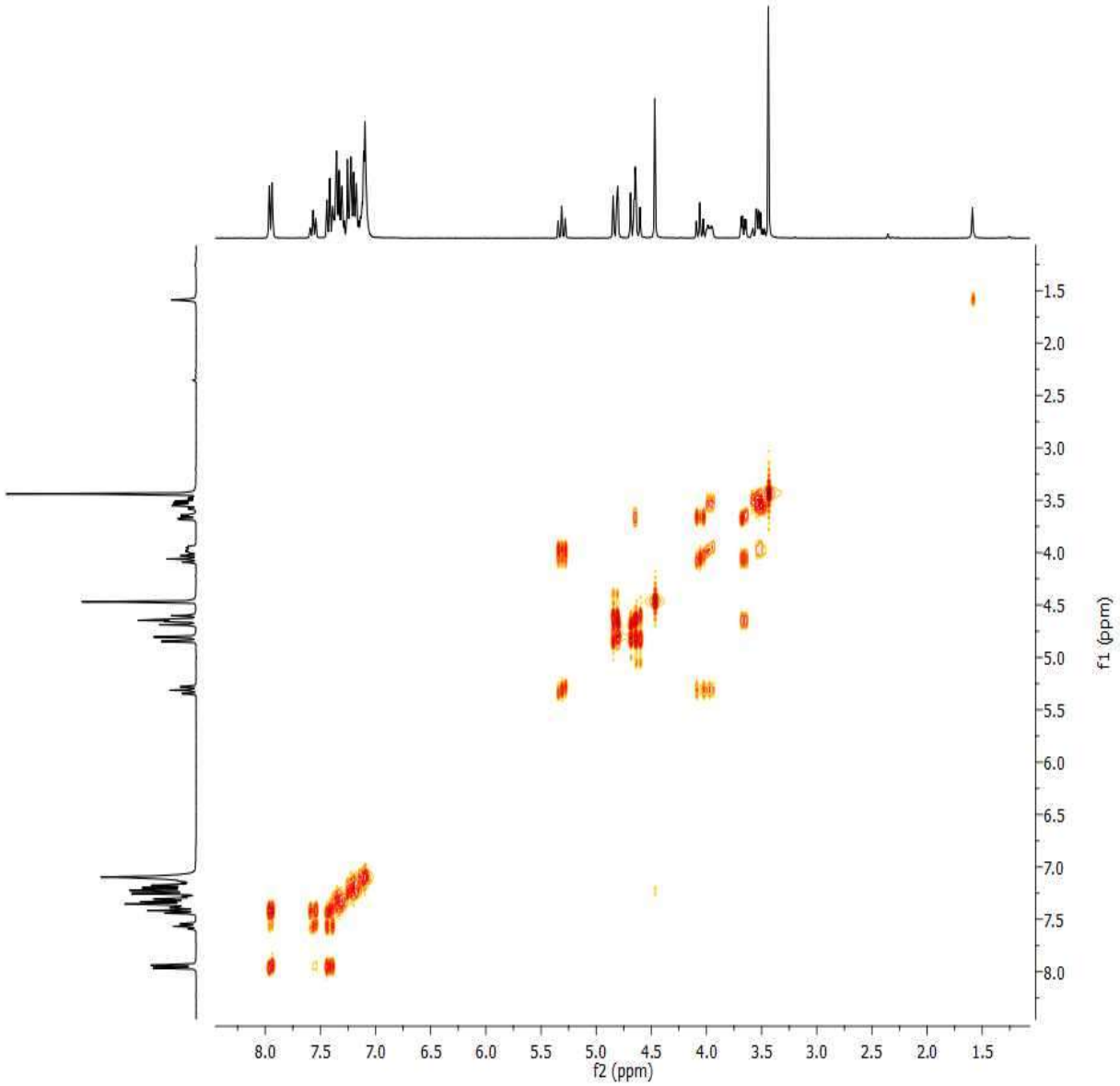


$CDCl_3$ 75 MHz

Figure A-60: ^{13}C NMR spectra of Methyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.6**).



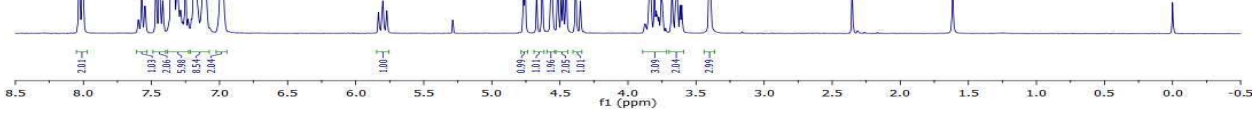
4.6



$CDCl_3$ 300 MHz

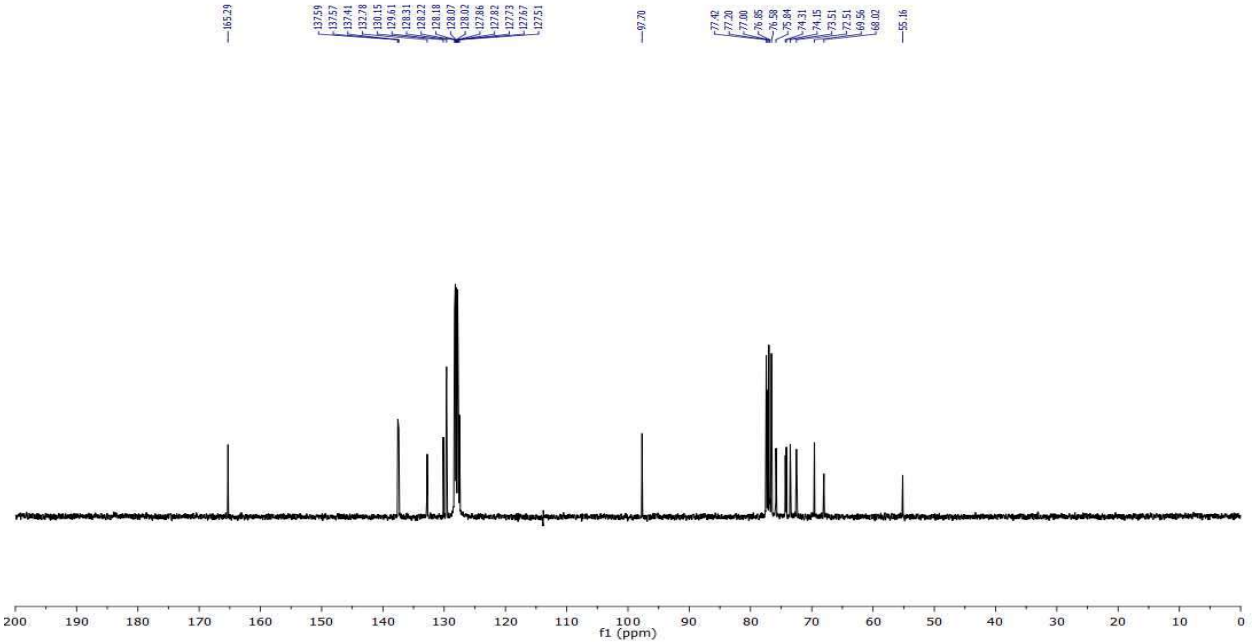
Figure A-61: 2-D NMR COSY spectra of Methyl 4-*O*-benzoyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.6**).

4.5



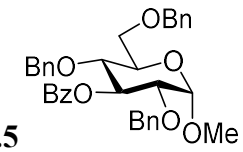
$CDCl_3$ 300 MHz

Figure A-62: ^{1}H- NMR spectra of Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.5**).

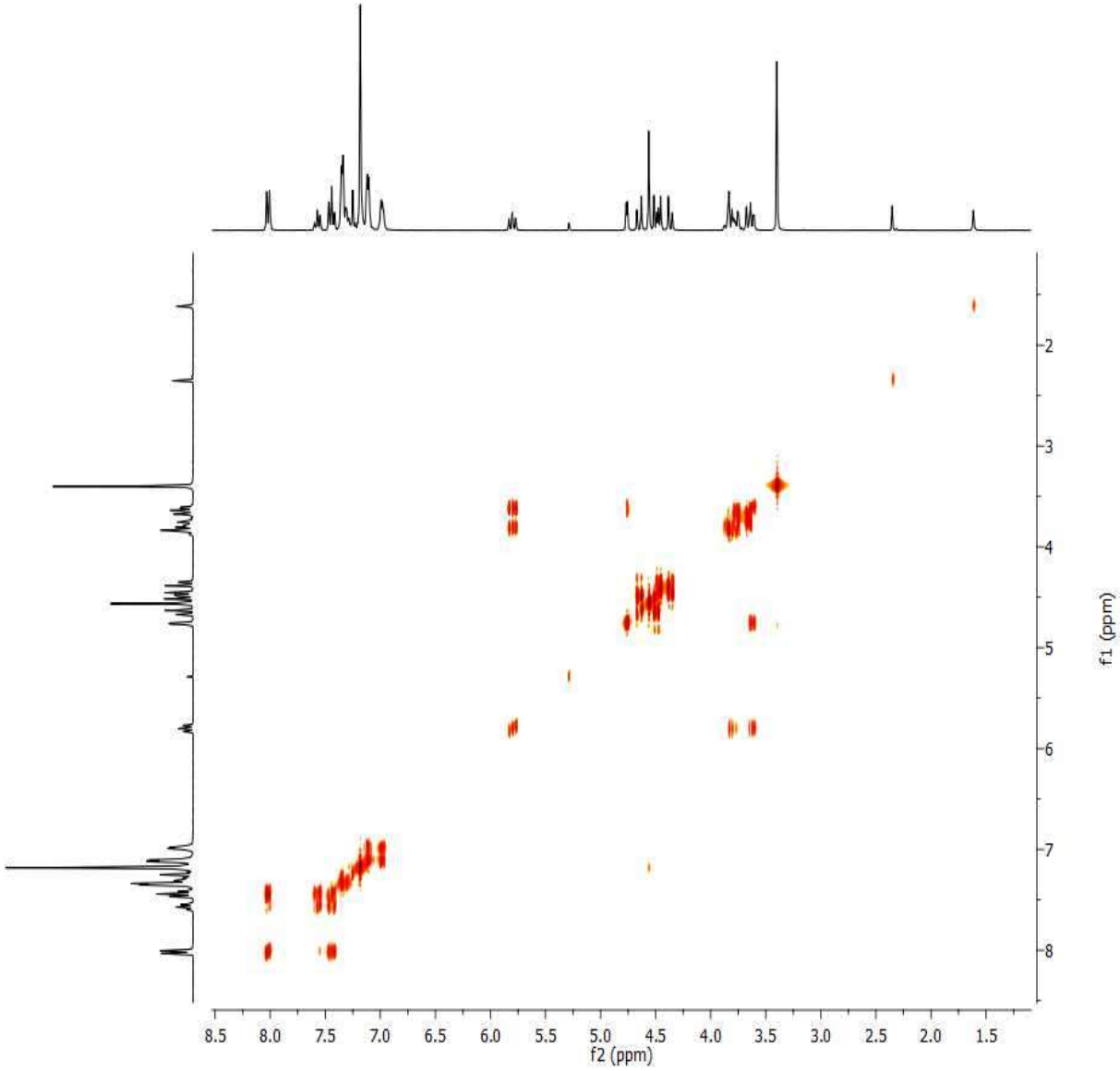


$CDCl_3$ 75 MHz

Figure A-63: ^{13}C NMR spectra of Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.5**).



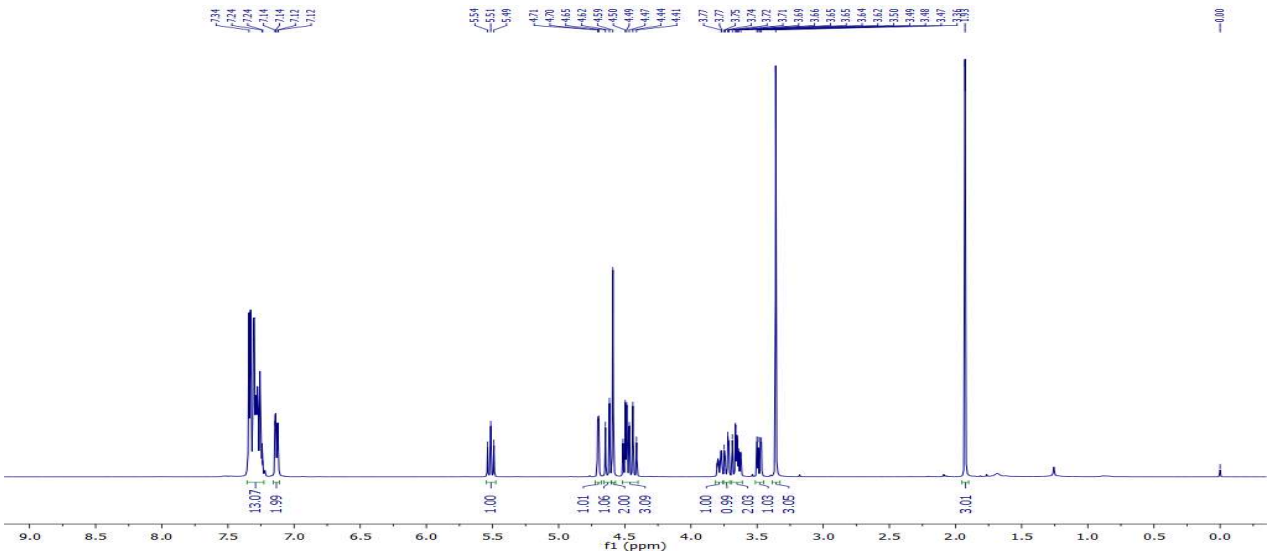
4.5



$CDCl_3$ 300 MHz

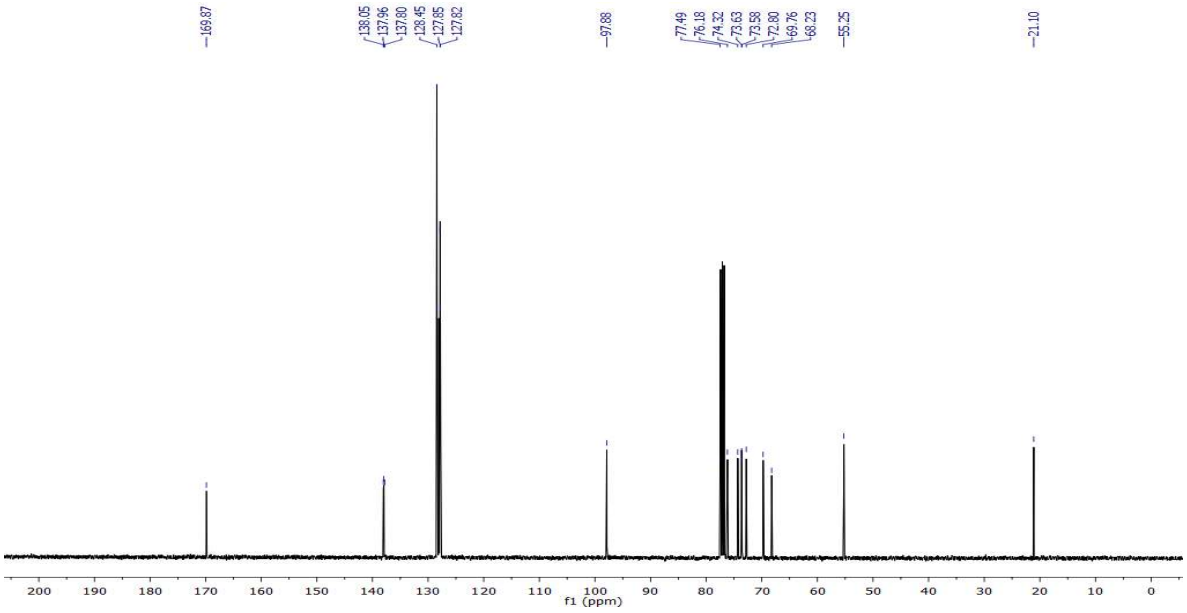
Figure A-64: 2-D NMR COSY spectra of Methyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.5**)

4.7



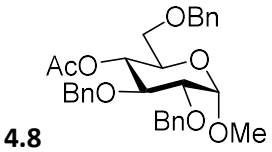
$CDCl_3$ 300 MHz

Figure A-65: ^{1}H- NMR spectra of Methyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.7**).

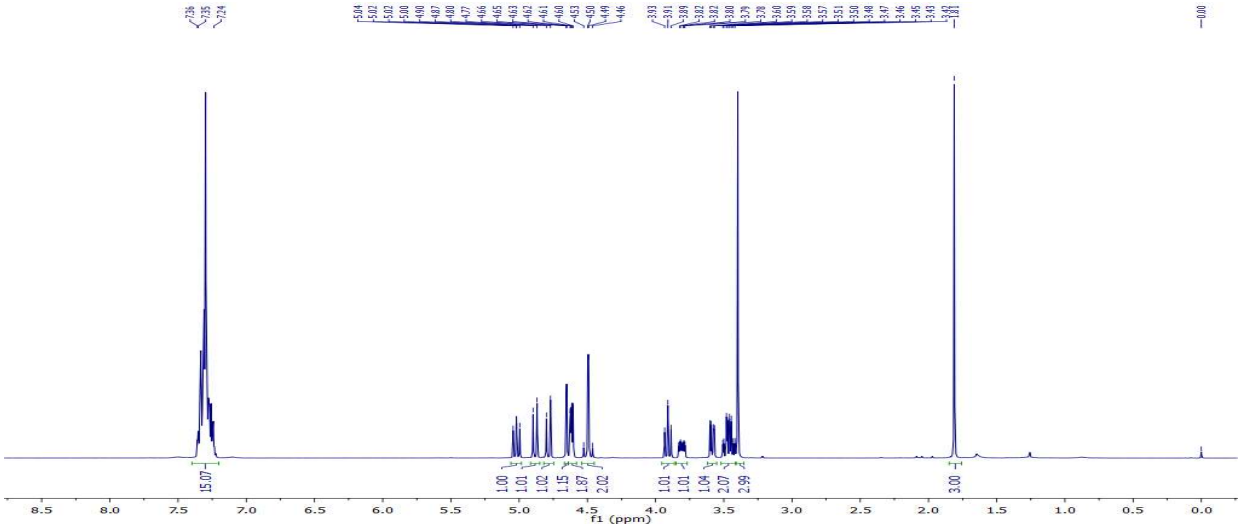


$CDCl_3$ 75 MHz

Figure A-66: ^{13}C NMR spectra of Methyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.7**).

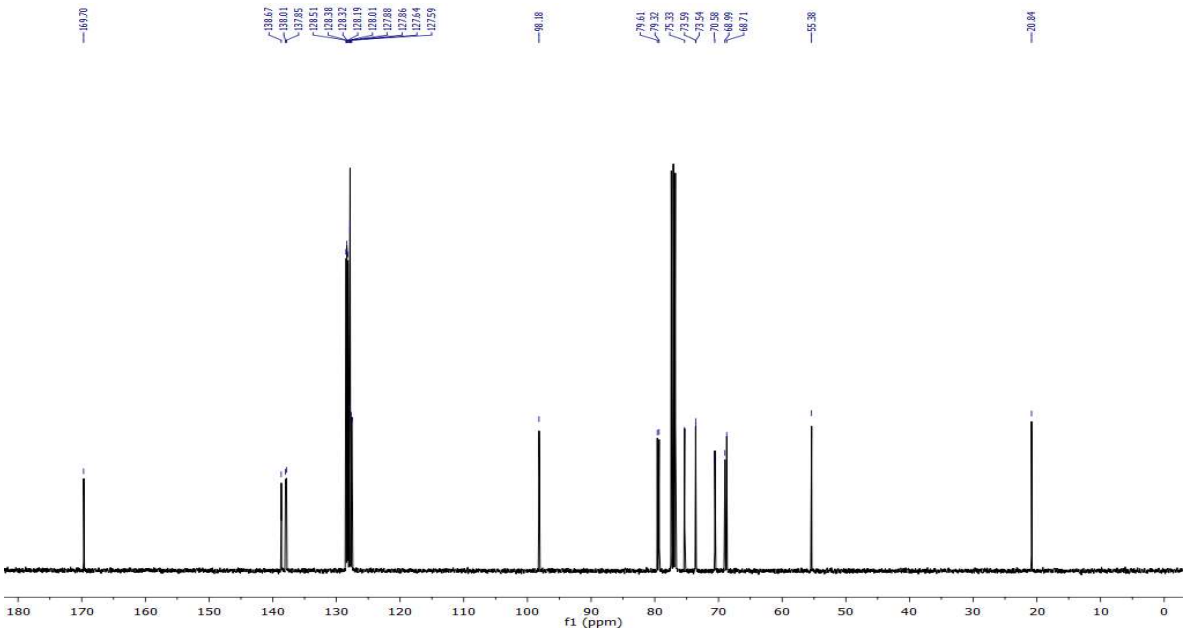


4.8



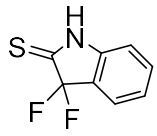
$CDCl_3$ 300 MHz

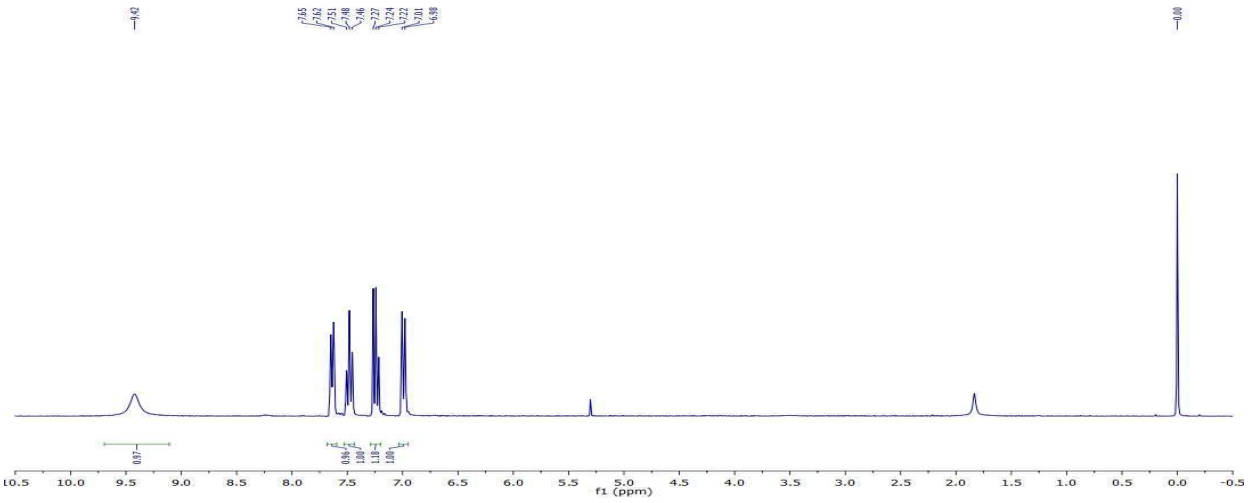
Figure A-67: ^{1}H- NMR spectra of Methyl 4-*O*-aceyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.8**).



$CDCl_3$ 75 MHz

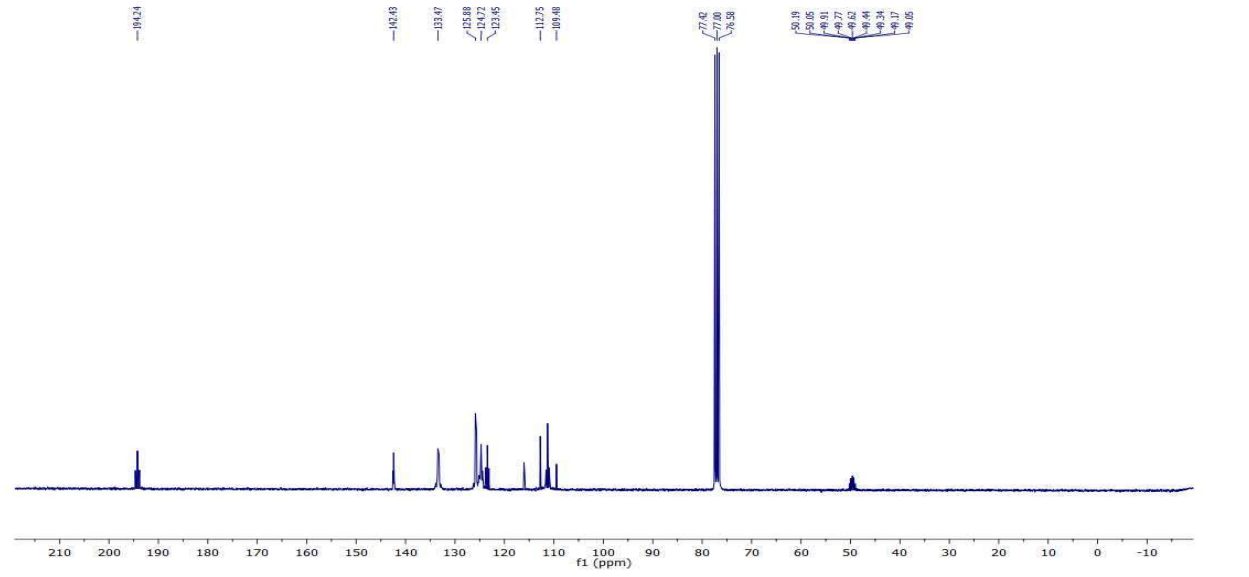
Figure A-68: ^{13}C NMR spectra of Methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**4.8**).





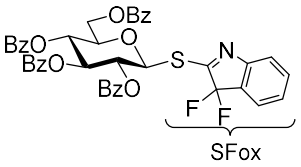
$CDCl_3$ 300 MHz

Figure A-69: ^{1}H- NMR spectra of 3,3-difluoroindoline-2-thione (3,3-difuoro-3H-thioindolyl-2-yl, HSFox).

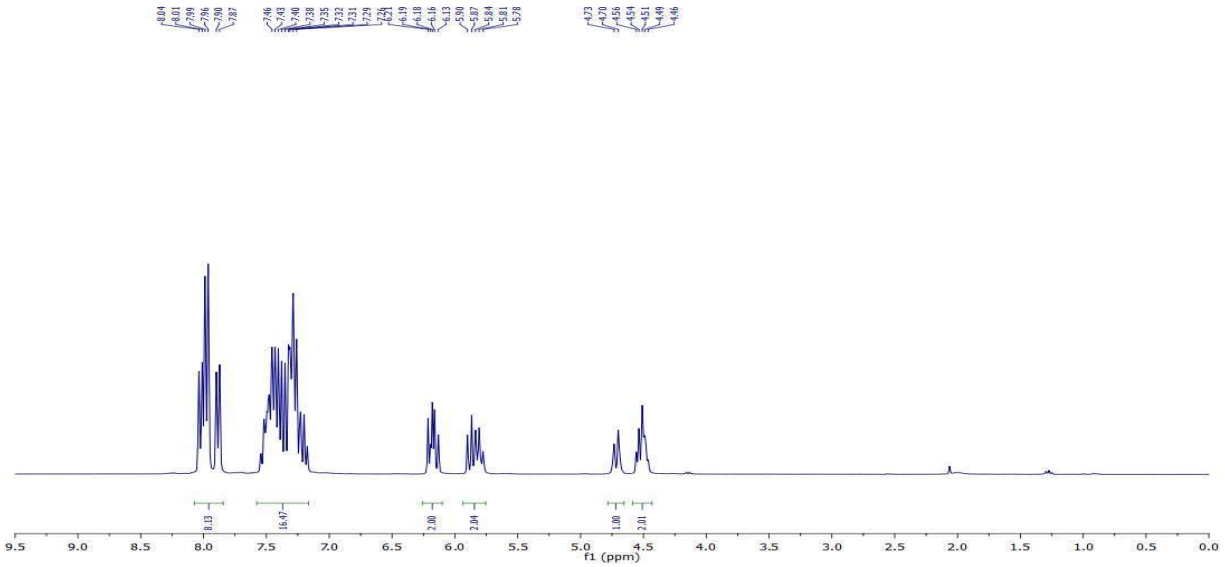


$CDCl_3$:MeOD (9:1) 75 MHz

Figure A-70: ^{13}C NMR spectra of 3,3-difluoroindoline-2-thione (3,3-difuoro-3H-thioindolyl-2-yl, HSFox).

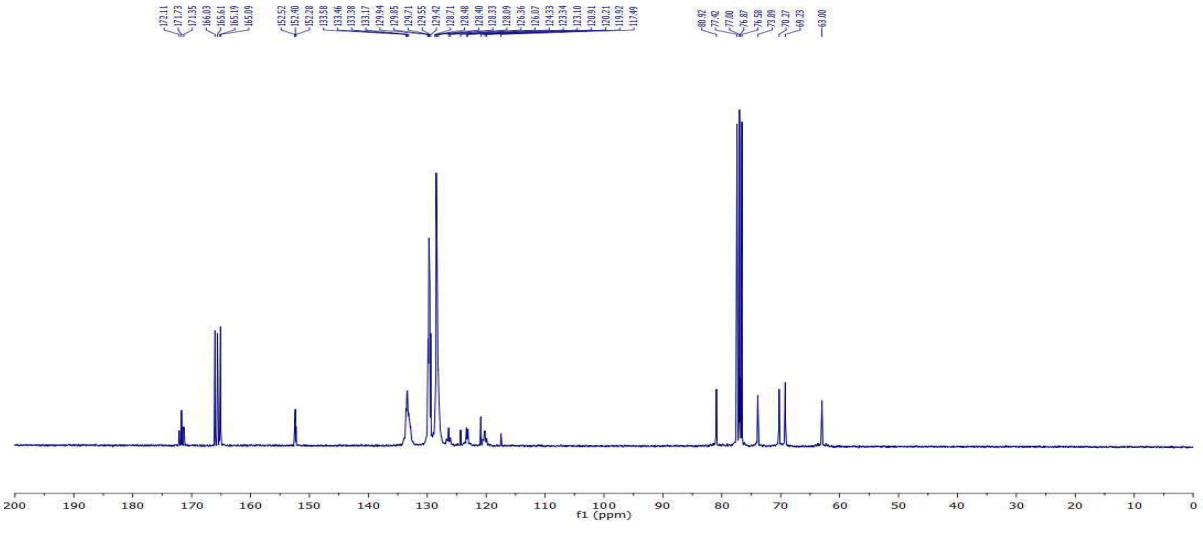


5.2



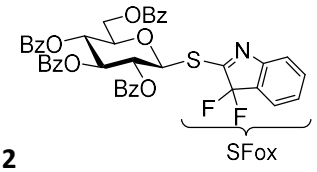
$CDCl_3$ 300 MHz

Figure A-71: ^{1}H- NMR spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**5.2**).

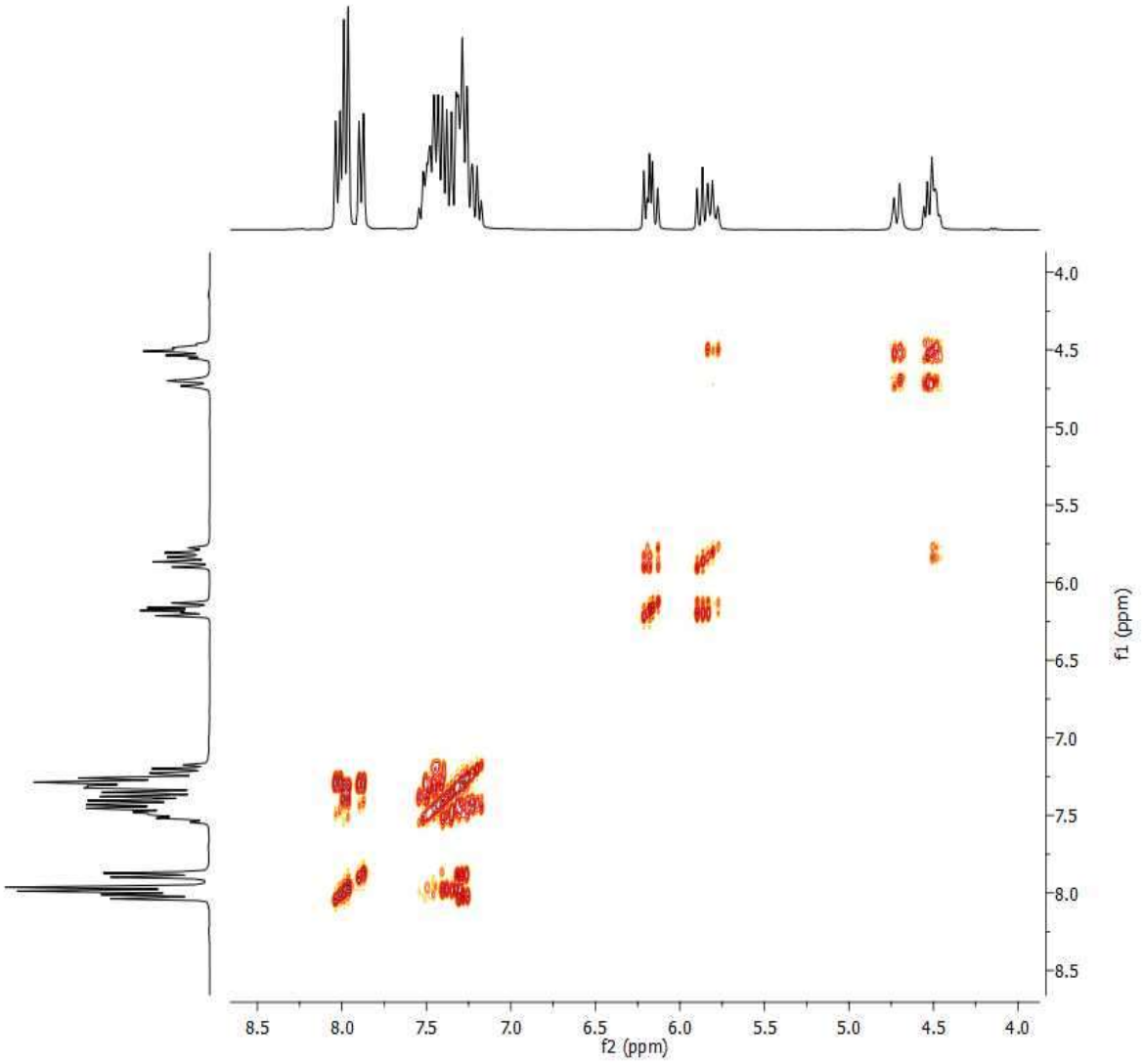


$CDCl_3$ 75 MHz

Figure A-72: ^{13}C NMR spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**5.2**).

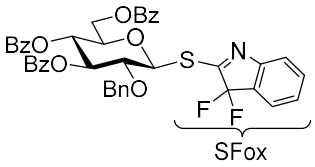


5.2

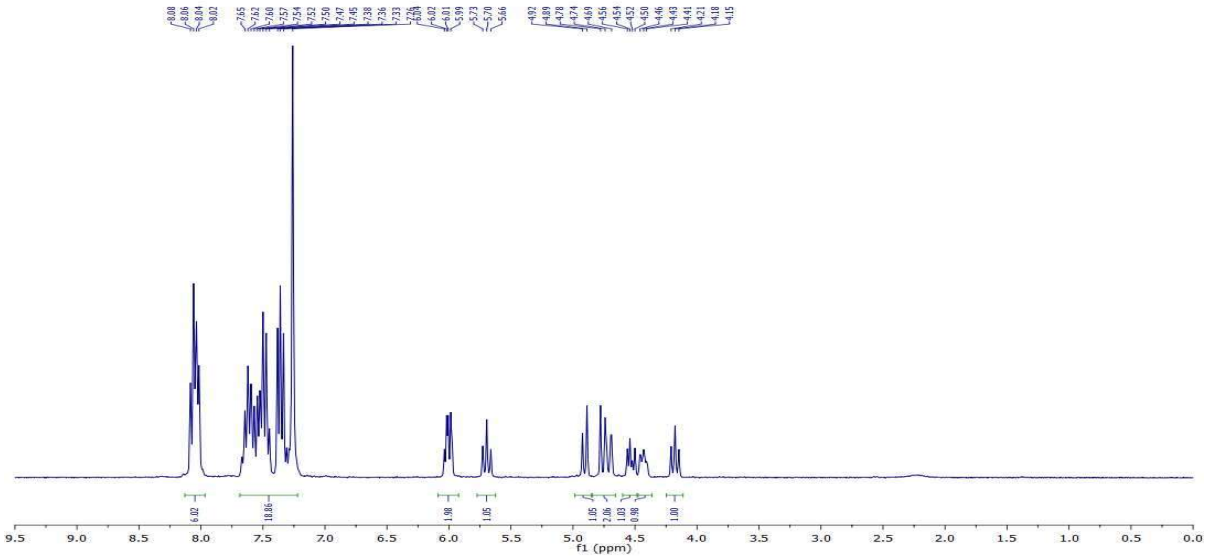


$CDCl_3$ 300 MHz

Figure A-73: 2-D NMR COSY spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**5.2**).

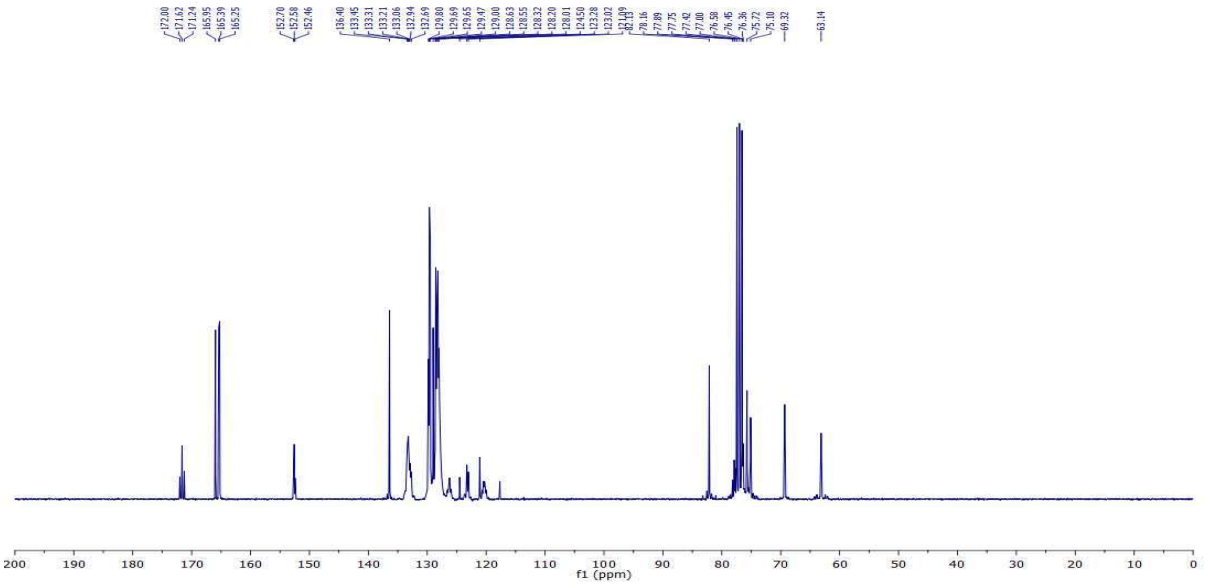


5.4



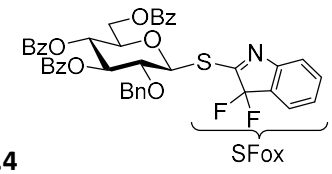
$CDCl_3$ 300 MHz

Figure A-74: ^{1}H- NMR spectra of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-O-benzoyl-2-O-benzyl-1-thio-β-D-glucopyranoside (**5.4**)

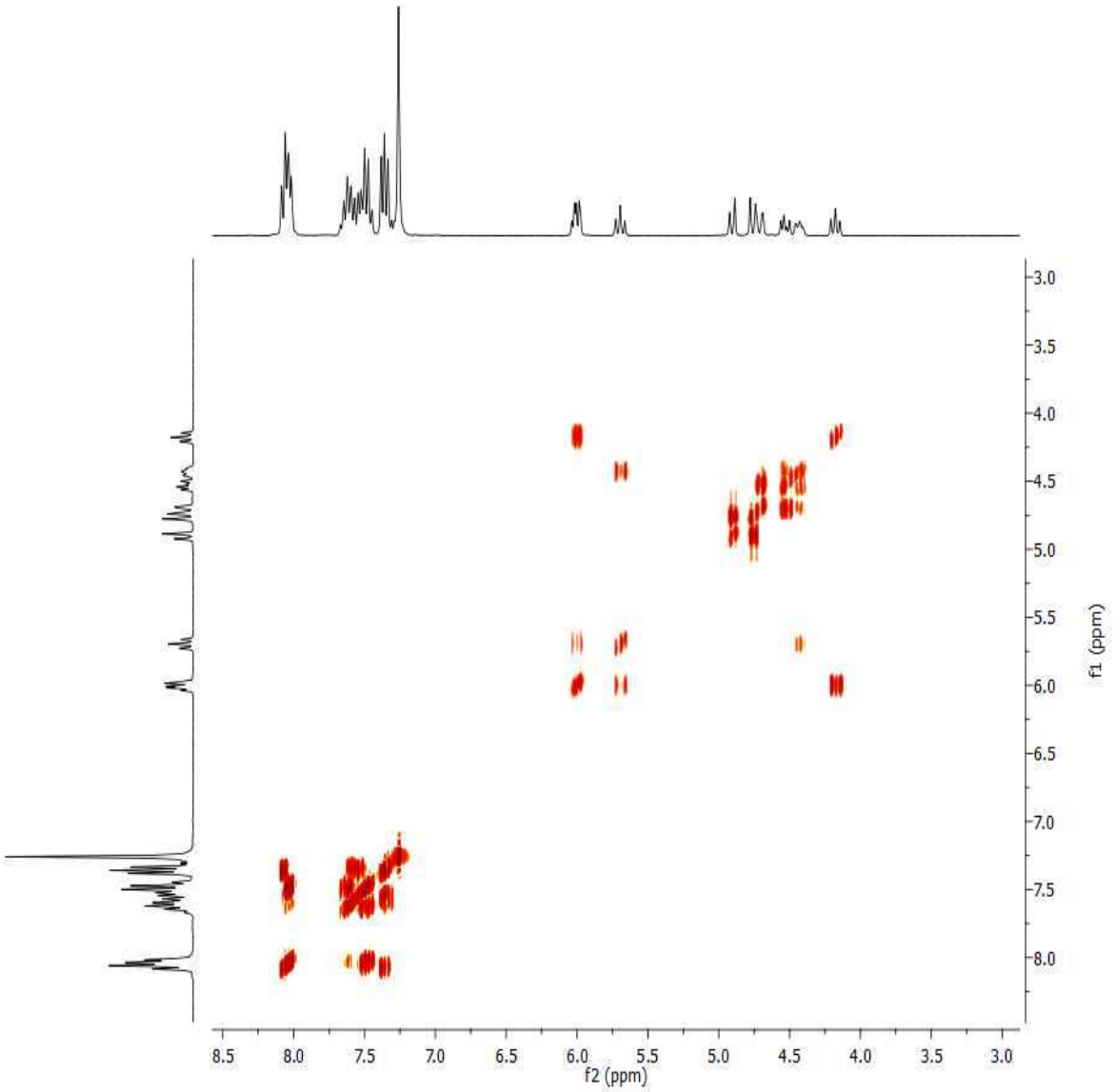


$CDCl_3$ 75 MHz

Figure A-75: ^{13}C NMR spectra of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-O-benzoyl-2-O-benzyl-1-thio-β-D-glucopyranoside (**5.4**)

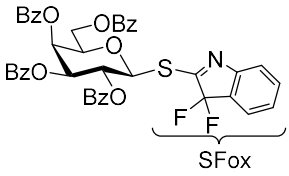


5.4

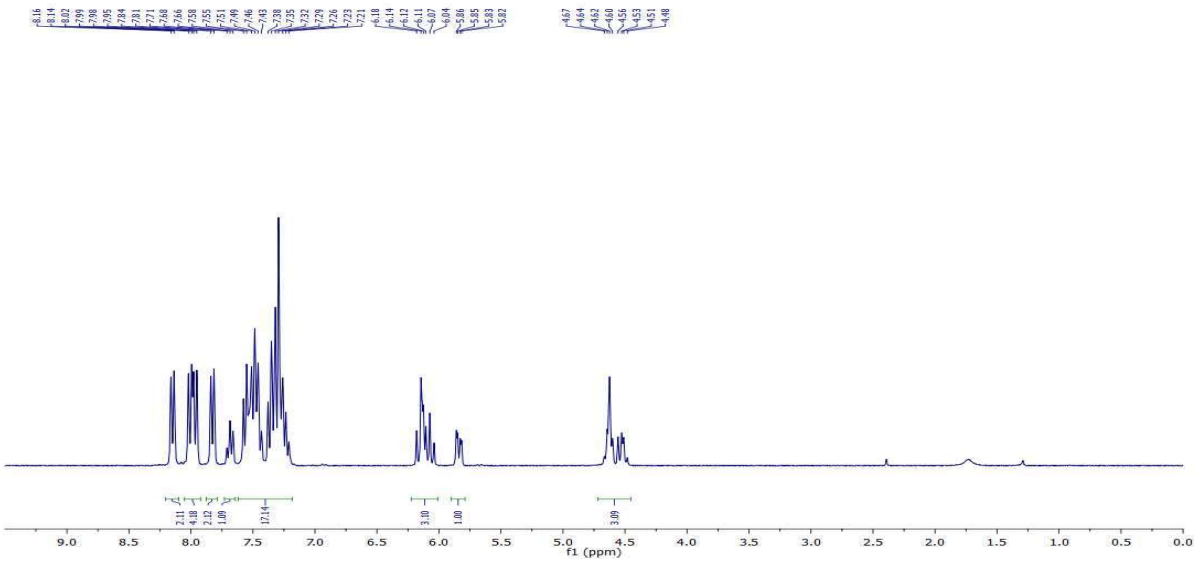


$CDCl_3$ 300 MHz

Figure A-76: 2-D NMR COSY spectra of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-O-benzoyl-2-O-benzyl-1-thio-β-D-glucopyranoside (**5.4**)

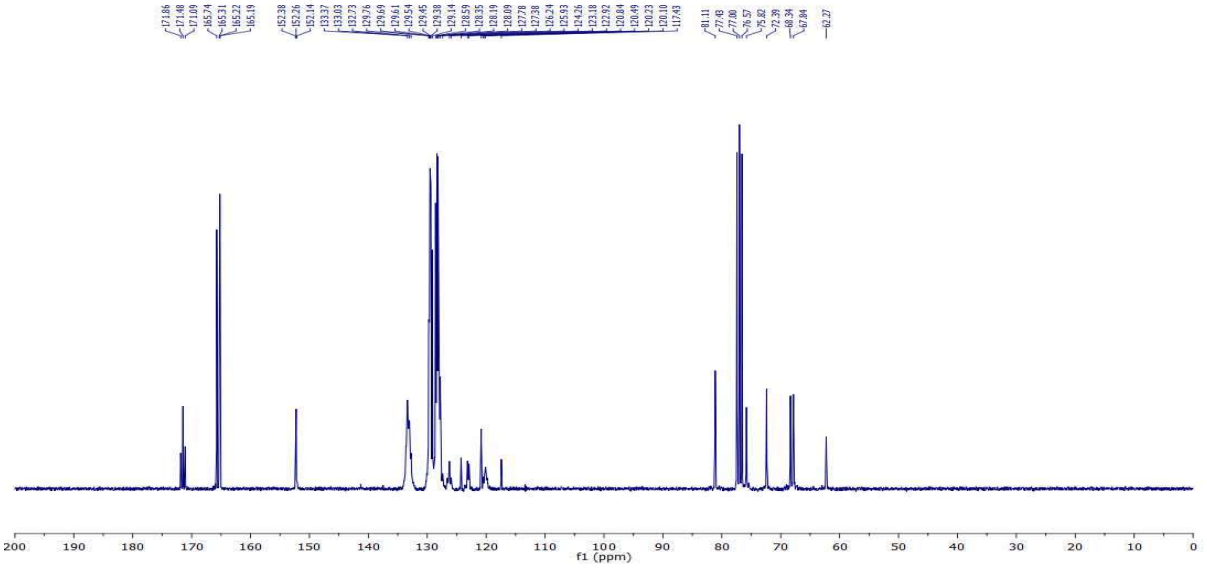


5.6



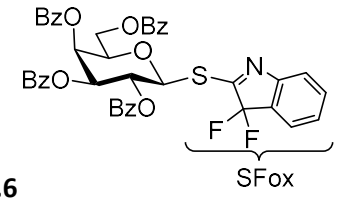
$CDCl_3$ 300 MHz

Figure A-77: ^{1}H- NMR spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**5.6**).

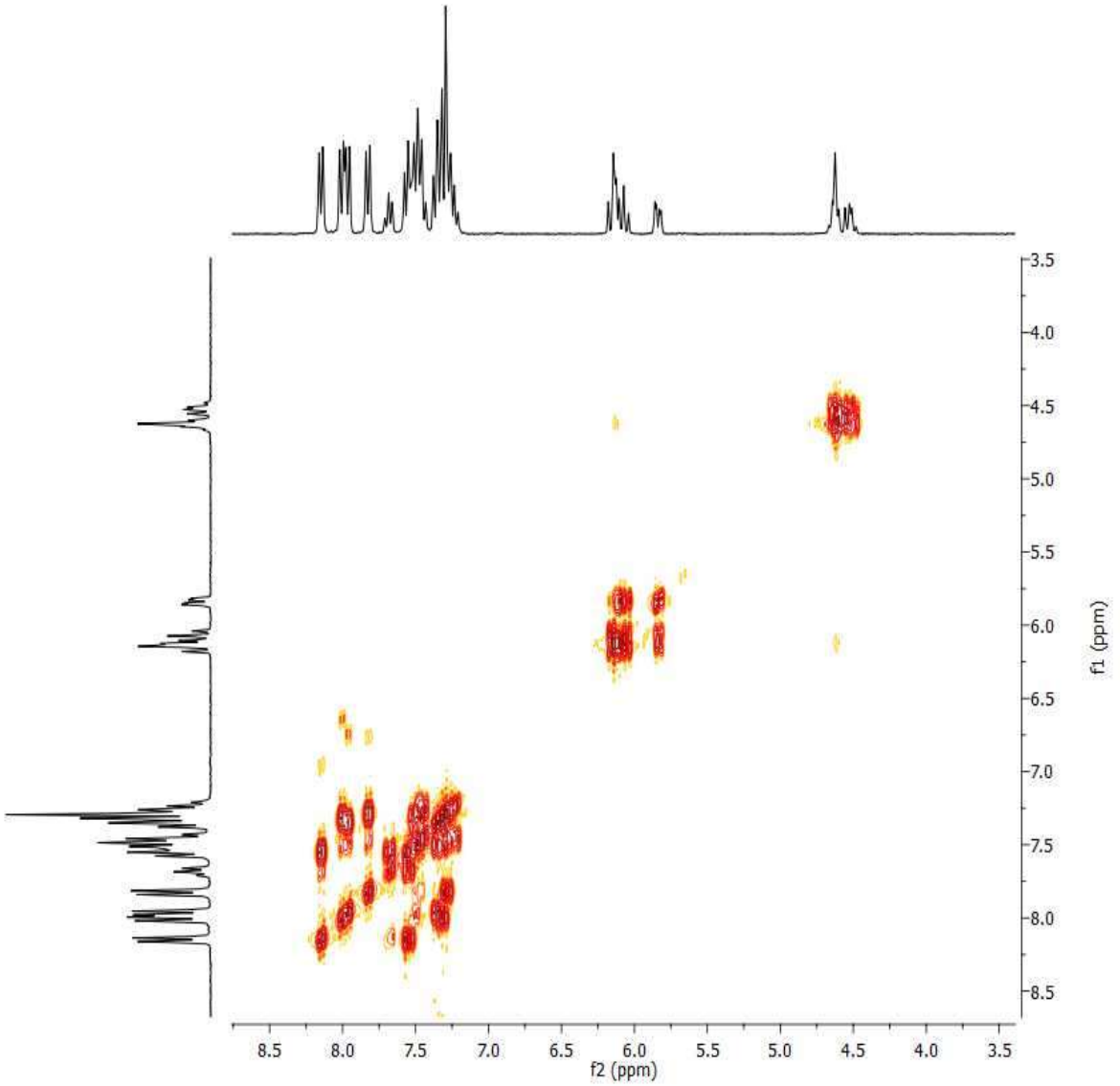


$CDCl_3$ 75 MHz

Figure A-78: ^{13}C NMR spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**5.6**).

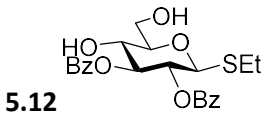


5.6

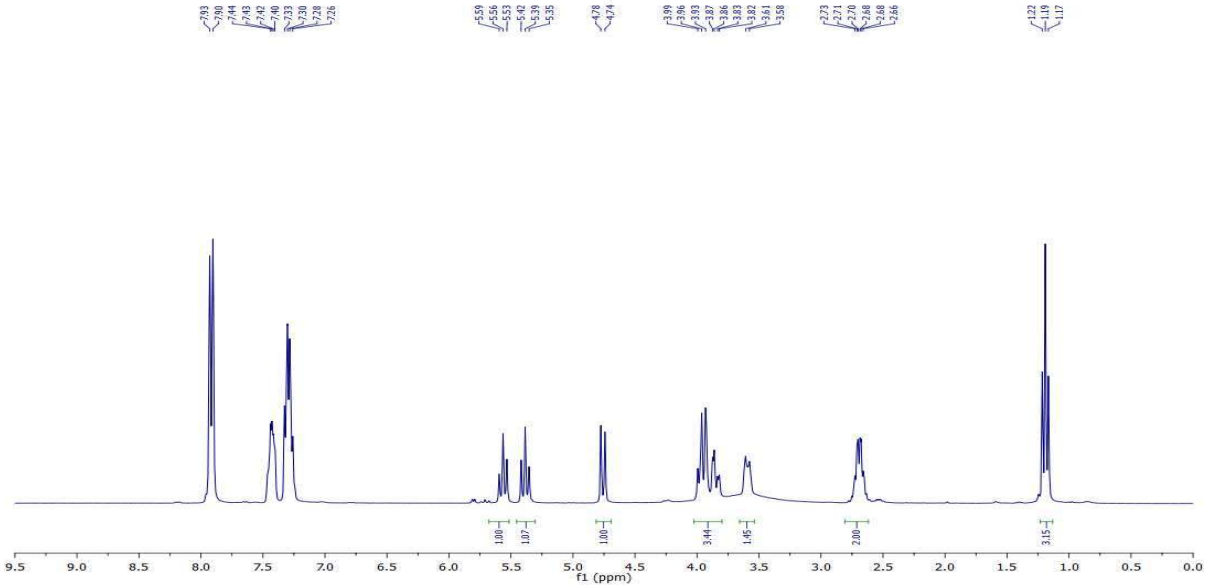


$CDCl_3$ 300 MHz

Figure A-79: 2-D NMR COSY spectra of 3,3-Difluoro-3H-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**5.6**).

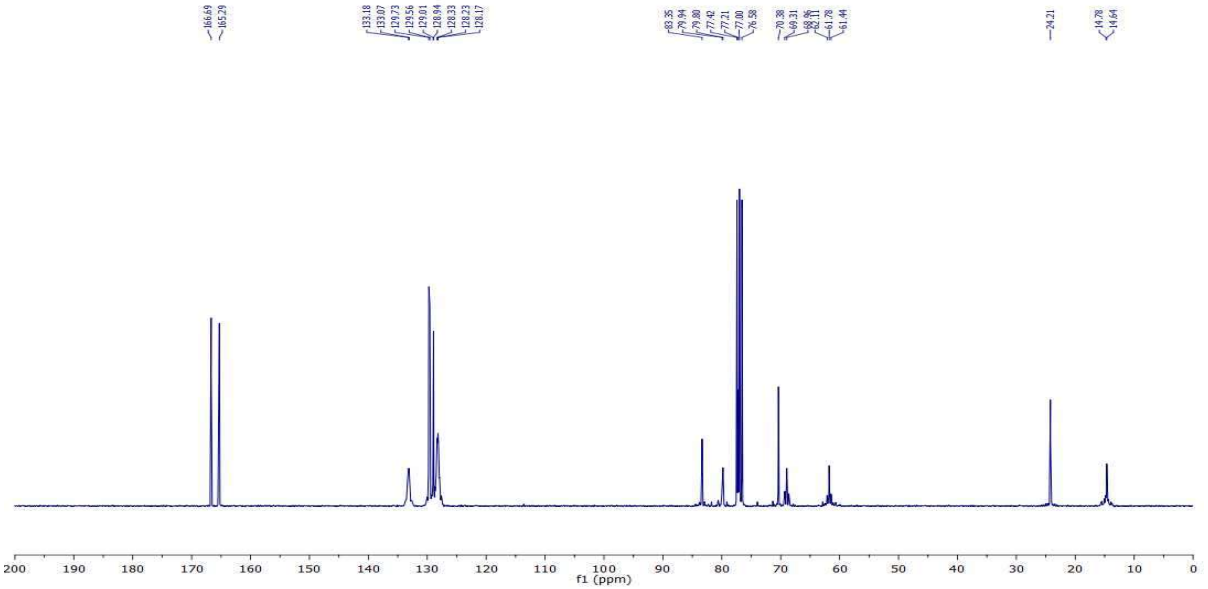


5.12



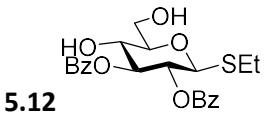
$CDCl_3$ 300 MHz

Figure A-80: ^{1}H- NMR spectra of ethyl 2,3-di-O-benzoyl-1-thio-β-D-glucopyranoside (**5.12**).

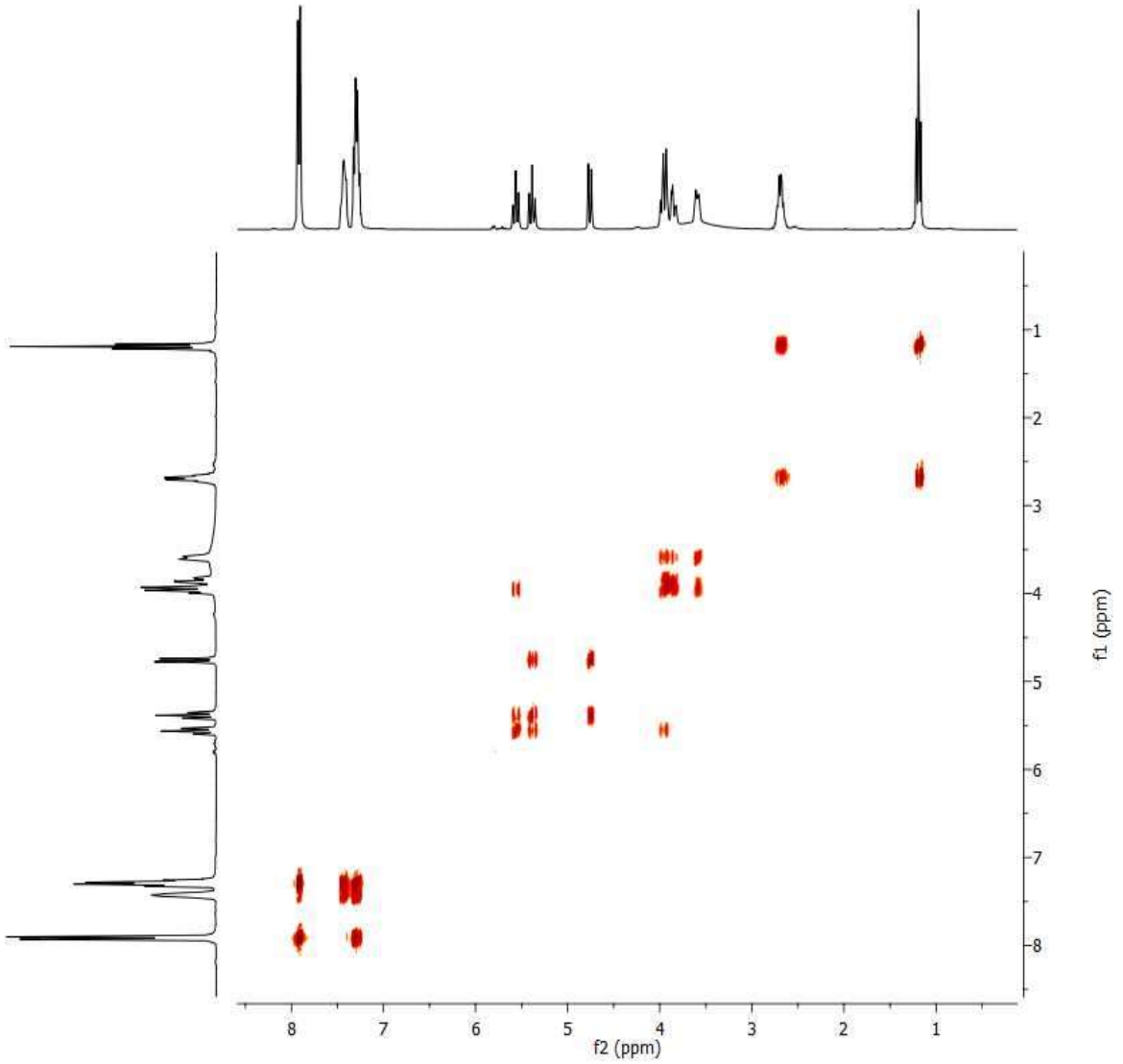


$CDCl_3$ 75 MHz

Figure A-81: ^{13}C NMR spectra of ethyl 2,3-di-O-benzoyl-1-thio-β-D-glucopyranoside (**5.12**).

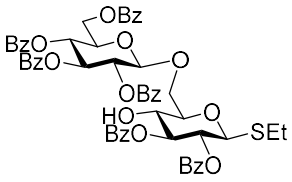


5.12

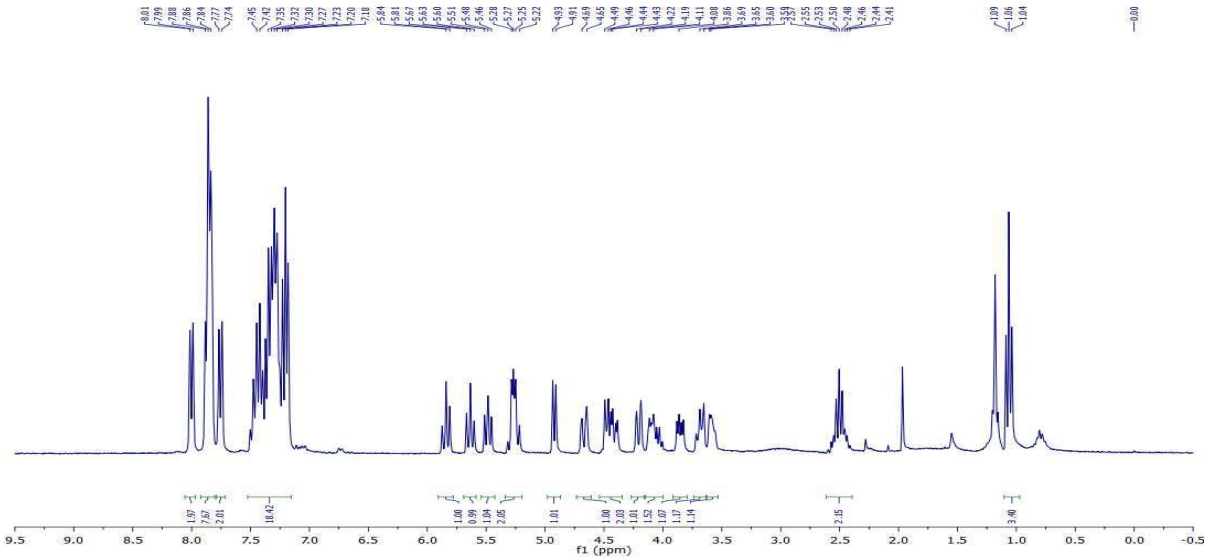


$CDCl_3$ 300 MHz

Figure A-82: 2-D NMR COSY spectra of ethyl 2,3-di-O-benzoyl-1-thio-β-D-glucopyranoside (**5.12**).

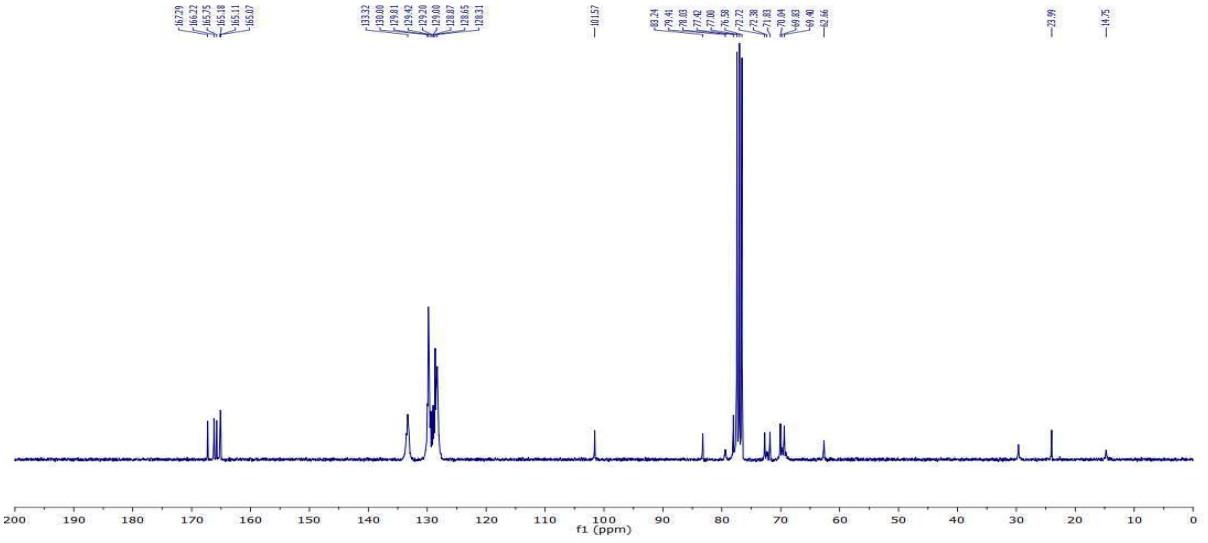


5.22



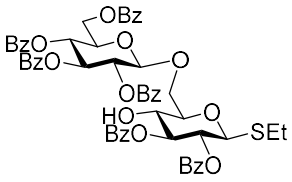
$CDCl_3$ 300 MHz

Figure A-83: ^{1}H- NMR spectra of Ethylthio 2,3-di-O-benzoyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-glucopyranosyl)-1-thio-β-D-glucopyranoside **(5.22).**

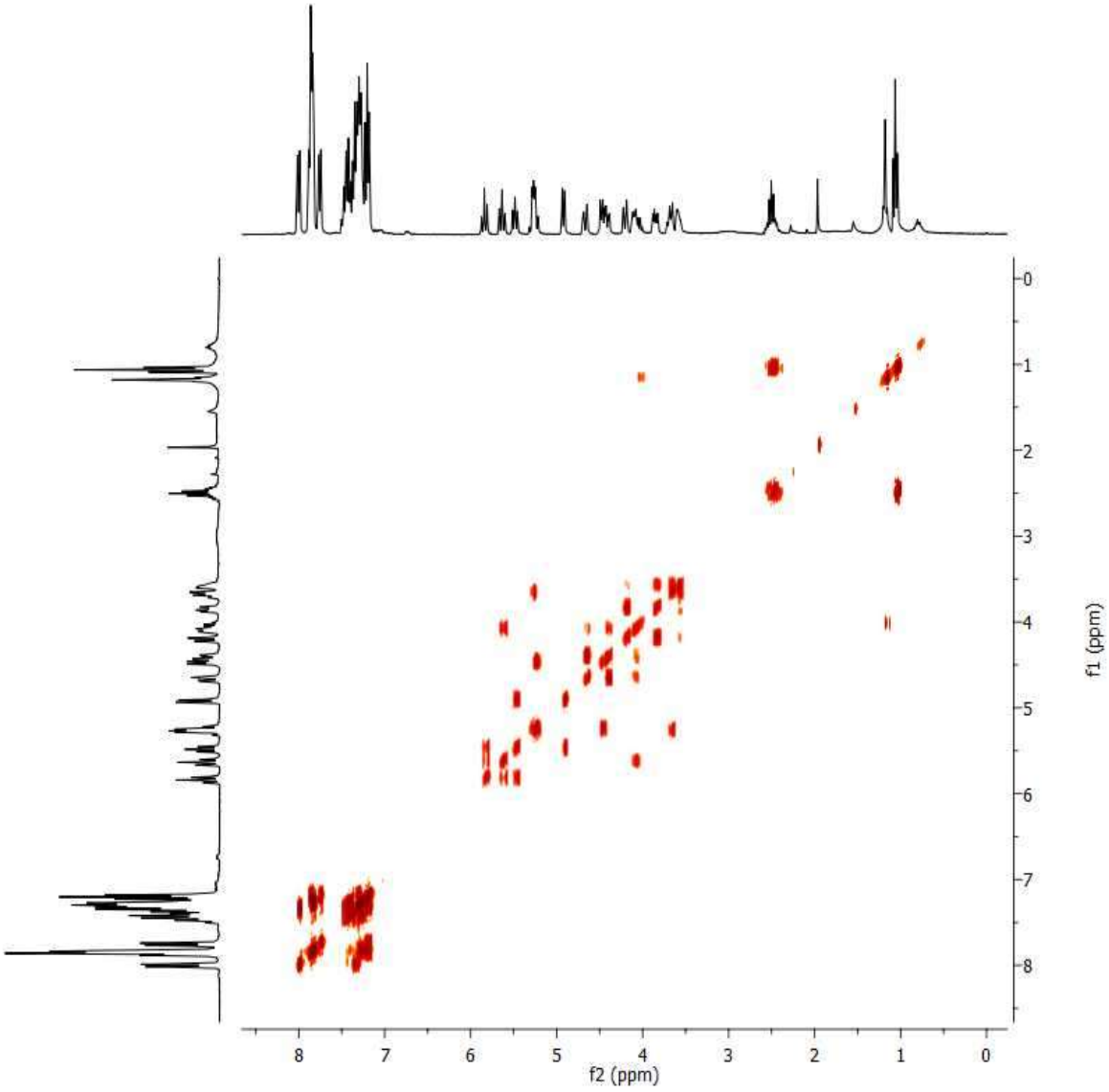


$CDCl_3$ 75 MHz

Figure A-84: ^{13}C NMR spectra of Ethylthio 2,3-di-O-benzoyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-glucopyranosyl)-1-thio-β-D-glucopyranoside **(5.22).**



5.22



$CDCl_3$ 300 MHz

Figure A-85: 2-D NMR COSY spectra of of Ethylthio 2,3-di-O-benzoyl-6-O-(2,3,4,6-tetra-*O*-benzoyl-glucopyranosyl)-1-thio-β-D-glucopyranoside **(5.22).**