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Chemical Synthesis of Oligosaccharides from Human Milk

Mithila Dulanjalee Bandara Weerasooriya Mudiyanselage
University of Missouri-St. Louis, wdbhhf@mail.umsl.edu

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By

W. M. Mithila D. Bandara

Master of Science (Chemistry), University of Missouri-St. Louis, May 2016

Bachelor of Science (Chemistry) Hons., University of Peradeniya, Sri Lanka, January 2013

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Dissertation Committee

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

Prof. James S. Chickos, Ph.D.

Prof. Keith J. Stine, Ph.D.

Prof. Janet B. Wilking, Ph.D.

ABSTRACT

Chemical Synthesis of Oligosaccharides from Human Milk

W. M. Mithila D. Bandara

Doctor of Philosophy, University of Missouri – St. Louis

Prof. Alexei V. Demchenko, Advisor

Human milk oligosaccharides (HMO) are a family of structurally related glycans that are highly abundant in breast milk. Oligosaccharide fraction is the third largest solid component in human milk after lactose and lipids. There is an accumulating evidence that HMO can provide significant benefits to the breast-fed infants. However, understanding of the exact HMO functions is still incomplete due to the lack of individual compounds in sufficient quantities. Therefore, development of expeditious strategies for the chemical synthesis of HMO has been increasingly important.

Among all the methods available for oligosaccharide synthesis, armed-disarmed strategy introduced by Fraser-Reid is based on chemoselective activation of different building blocks. Later, the scope of this armed disarmed strategy was broadened by the introduction of other reactivity levels that included superarmed glycosyl donors. One of those was invented by Bols and co-workers wherein and the superarming property was achieved by the conformational change to the glycosyl donor. The other type of glycosyl donors was introduced by our lab wherein the superarming was achieved using conventions of the O2/O5 cooperative effect (electronic effect).

Presented herein is the expansion of our previous work on the investigation of hybrid glycosyl donors that combine aforementioned conformational and electronic effects. The major emphasis of this study was to compare the reactivity of differently protected S-ethyl and S-phenyl donors by competition studies. The applicability of the developed glycosyl donors in one-pot oligosaccharide synthesis has been demonstrated. This ultimately led us to the development of versatile chemical strategies for the synthesis of HMO. Reported herein is the synthesis of common core HMO including lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), lacto-N-hexaose (LNH), and lacto-N-neohexaose (LNnH).

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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 carbonate

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

BH_3•THF.....Borane tetrahydrofuran complex

Bn .....Benzyl

BnBr .....Benzyl bromide

Br_2 .....Bromine

Bz .....Benzoyl

BzCl.....Benzoyl chloride

BzCN.....Benzoyl cyanide

CaH_2 .....Calcium hydride

CCl3CN..... Trichloroacetonitirle

$CDCl_3$ .....Deuterated chloroform

CD_3COCD_3..... Deuterated acetone

CH_2Cl_2 .....Dichloromethane

CH_3CN.....Acetonitrile

$ClCH_2CH_2Cl$ 1,2-Dichloroethane

$Cu(OAc)_2$.....Copper(II) acetate

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

d .....Doublet

DBU.....1,8-Diazabicyclo[5.4.0]undec-7-ene

dd .....Doublet of doublets

DMAP 4-Dimethylaminopyridine

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

DMTST.....Dimethyl(methylthio)sulfonium trifluoromethanesulfonate

D_2O.....Deuterium oxide

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

Et .....Ethyl

Et_2O .....Diethyl ether

EtOAc..... Ethyl acetate

EtOH..... Ethanol

EtSH .....Ethane thiol

Fmoc..... Fluorenylmethyloxycarbonyl

Gal .....Galactose

Glc .....Glucose

GlcNAc.....N-Acetylglucosamine

h ..... Hour(s)

HAD.....H-Bond-mediated aglycone delivery

HCl .....Hydrogen chloride

H_2O.....Water

HMO.....Human milk oligosaccharide(s)

$HOPO(OBu)_2$ .....Dibutyl phosphate

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

IDCP.....Iodonium(di-γ-collidine)perchlorate

LNB.....Lacto-N-biose

LG.....Leaving group

LNH.....Lacto-N-hexaose

LNnH.....Lacto-N-neohexaose

LNnT .....Lacto-N-neotetraose

LNT .....Lacto-N-tetraose

M .....Molar

MeOTf.....Methyl trifluoromethanesulfonate

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

$NaCNBH_3$ .....Sodium cyanoborohydride

NaH .....Sodium hydride

$NaHCO_3$ .....Sodium bicarbonate

NaOH ..... Sodium hydroxide

NaOMe ..... Sodium methoxide

NBn..... ortho-Nitrobenzyl

NIS.....N-Iodosuccinimide

$Na_2S_2O_3$ .....Sodum thiosulfate

NMR ..... Nuclear magnetic resonance

NPhth .....Phthalimido

Pd/C .....Palladium on carbon

PFBz..... Pentafluorobenzoyl

Ph ..... Phenyl

Pico..... Picoloyl

ppm ..... Parts per million

Py .....Pyridine

RDS.....Rate determining step

R_f…..... Retention factor

RRV.....Relative reactivity values

rt ..... Room temperature

s ..... Singlet

SBox .....*S*-Benzoxazolyl

SBiz ..... *S*-Benzimidazolyl

SEt ..... *S*-Ethyl

S_N1.....Nucleophilic substitution unimolecular

S_N2.....Nucleophilic substitution bimolecular

SPh.....S-Phenyl

STaz..... S-Thiazolinyl

STol..... S-Tolyl

t ..... Triplet

TBDMS ..... *tert*-Butyldimethylsilyl

TBDMSOTf..... *tert*-Butyldimethylsilyl trifluoromethanesulfonate

TBS.....*tert*-Butyldimethylsilyl

TBAI.....*tert*-Butylammonium iodide

TFA ..... Trifluoroacetic acid

Tf_2O.....Trifluoromethanrsulfonic anhydride

TfOH ..... Trifluoromethanesulfonic (triflic) acid

THF .....Tetrahydrofuran

TIPS .....Triisopropylsilyl

TLC ..... Thin layer chromatography

TMS .....Trimethylsilyl

TMSCl.....Trimethylsilyl chloride

TMSOTf.....Trimethylsilyl trifluoromethanesulfonate

TolSCl.....Toluenesulfonyl chloride

TsOH ..... *p*-Toluenesulfonic acid

TTBP.....2,4,6-Tri-*tert*-butylpyrimidine

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CHAPTER 1

Application of armed, disarmed, superarmed and superdisarmed building blocks in stereocontrolled glycosylation and expeditious oligosaccharide synthesis

M. D. Bandara, J. P. Yasomanee, and A. V. Demchenko. Application of Armed, Disarmed, Superarmed, and Superdisarmed Building Blocks. In Stereocontrolled Glycosylation and Expeditious Oligosaccharide Synthesis. Selective Glycosylations: Synthetic Methods and Catalysts, C. S. Bennett (Ed.). Willey-VCH, **2017**, 29-58.

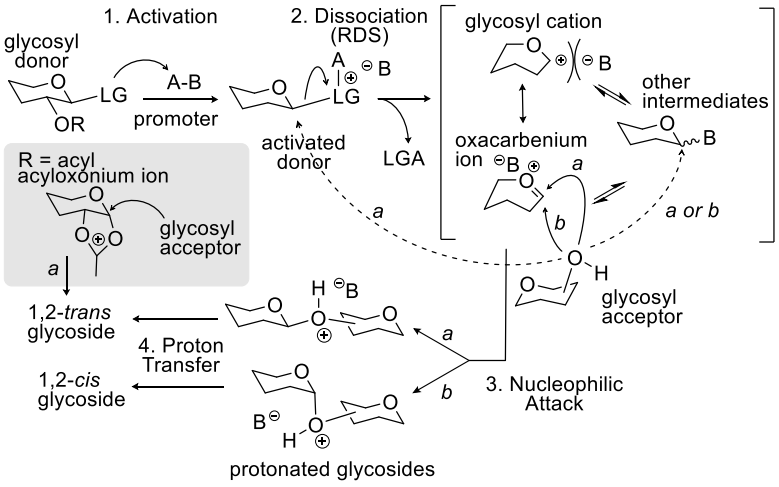
1.1. Introduction: chemical synthesis of glycosides and oligosaccharides

From the building blocks of nature to disease-battling therapeutics and vaccines, carbohydrates have had a broad impact on evolution, society, economy, and human health. Numerous applications of these essential molecules in many areas of science and technology exist, foremost of which can be found in the areas of therapeutic-agent and diagnostic-platform development. Although carbohydrate oligomers, oligosaccharides or glycans, are desirable for biological and medical communities, these molecules remain very challenging targets for chemists. Amongst a number of hurdles including functionalization, elaborate protecting and leaving group manipulations, tedious purification and sophisticated structure analysis, it is glycosylation, a coupling reaction performed between two monosaccharide units, that has proven particularly challenging to chemists. Nature flawlessly performs this reaction to obtain complex glycans and glycoconjugates.[1,2] Chemical glycosylation, however, remains challenging even with the aid of recent methodological breakthroughs[3-8] and modern technologies.[9-17]

Many methods for chemical glycosylation have been developed, but it is the inability to control the stereoselectivity that has proven to be the major hurdle. The glycosylation typically follows a unimolecular S_N1 mechanism[18,19] via four distinct steps: activation, dissociation, nucleophilic attack, and proton transfer (Scheme 1.1).[18] In the case of a non-participating ether-type substituent at C-2, glycosylation proceeds via an oxacarbenium ion.[5] The intermediacy of the flattened oxacarbenium ion typically results in the formation of anomeric mixtures in which 1,2-*cis* glycosides[20] (for D-gluco/galacto series) are slightly favored due to the anomeric effect.[21,22] The goal of stereocontrolling glycosylation has been approached in many ways and much effort dedicated to

developing new leaving groups (LG in schemes) and refining the reaction conditions. We know that leaving groups, temperature, promoter/additives, and the reaction solvent may have a significant effect on the reactivity of the reactants and the stereoselectivity of glycosylation.[23] However, since these factors still often fail to adequately control the outcome of many glycosylations that tend to proceed via the oxacarbenium ion, studies are refocusing on gaining a better understanding of the reaction mechanism.

Protecting groups are extensively used in carbohydrate chemistry to mask additional sites of reactivity in polyfunctional compounds, including both donors and acceptors. Protecting groups, however, can affect the glycosylation in a variety of other ways. As stated by Fraser-Reid "protecting groups do more than protect."[24] Amongst the best known effects of protecting groups is the neighboring acyl group participation,[25] which remains one of the most powerful modes for controlling the stereoselectivity of glycosylation reaction (protecting groups do more than protect). A vast majority of 1,2-*trans* glycosides is obtained from glycosyl donors equipped with a 2-acyl protecting group. These reactions proceed via the intermediacy of a cyclic acyloxonium ion, which

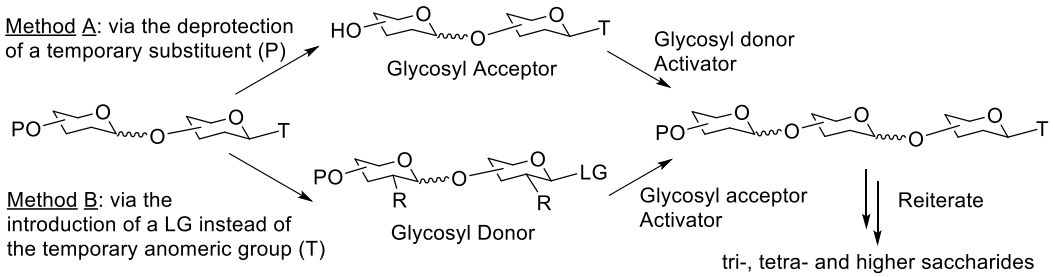


Scheme 1.1. Outline of the chemical glycosylation.

is then opened with the glycosyl acceptor from the opposite (trans) face (Scheme 1.1).

In addition, protecting groups may have a profound effect on the conformation and stereoelectronics of the starting material, key reaction intermediates, and the products.[26,27] In the recent years dedicated studies of these intermediates have led to the development of many stereocontrolled reactions, and the synthesis of β-mannosides via anomeric triflates by Crich and co-workers is arguably the best example of such a study.[28-36] Nevertheless, some linkages and targets remain challenging due to the requirement to achieve complete stereocontrol in each and every step,[37,38] and suppress side reactions.[23,39]

Beyond that, the synthesis of oligosaccharides may require additional synthetic steps between glycosylations. In accordance with the traditional oligosaccharide synthesis, the disaccharide intermediate should be converted into either a glycosyl acceptor or donor of the second generation. As depicted in Scheme 1.2, this can be accomplished via deprotection (Method A) or introduction of a leaving group instead of a temporary anomeric substituent (Method B), respectively. The modified disaccharide building blocks can then be reacted with other glycosyl donors or acceptors, resulting in the formation of a tri- or larger saccharide if the convergent approach is incorporated. These synthetic steps can be then reiterated to obtain larger oligosaccharides.



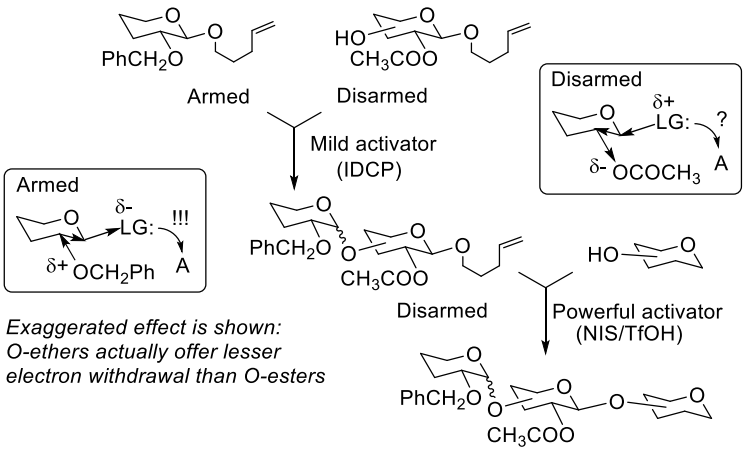
Scheme 1.2. Outline of conventional approaches to oligosaccharide synthesis.

A large number of additional synthetic steps between each glycosylation step typically lead to reduced yields and overall efficiency of the oligosaccharide assembly. Consequently, the past quarter of the century has witnessed the development of new strategies for oligosaccharide synthesis, amongst which selective and chemoselective concepts prevail.[40] Synthetic strategies based on selective activations make use of different leaving groups that are sequentially activated and traditional selective activation,[41,42] two-step activation,[41,43-45] active-latent concept,[46-49] and orthogonal strategy[50] are just a few examples of such approaches.[51] Another general direction in expeditious oligosaccharide synthesis involves chemoselective activations. This strategy is based on the so-called armed-disarmed strategy introduced by Fraser-Reid et al.[52] Building blocks used in chemoselective activations utilize only one type of a leaving group, and the building block reactivity is adjusted by the choice of protecting groups (protecting groups do more than protect). The next subchapter introduces this general strategy and subsequent sections elaborate on the recent progress that has been made in the area of tuning the reactivity of building blocks and their application in stereoselective glycosylation and chemoselective oligosaccharide synthesis.

1.2. Fraser-Reid's armed-disarmed strategy for oligosaccharide synthesis.

Although the effect of protecting groups on reactivity has been known for many decades,[53] Fraser-Reid was the first to describe a new mode by which the differential properties of protecting groups could be exploited.[54] It was noticed that ester protecting groups reduce the reactivity (disarm) of the n-pentenyl leaving group, in comparison to that of its alkylated (armed) counterpart. One explanation for this phenomenon is that the

increased electron-withdrawal of ester groups decrease the electron density (nucleophilicity) of the anomeric heteroatom. This translates into a reduced leaving group ability, and works with the leaving groups capable of either direct (thioglycosides) or remote activation (n-pentenyl).[55] To differentiate the reactivity, mild reaction conditions are required and iodonium(di-γ-collidine)perchlorate (IDCP) was found to be a suitable mild activator for the armed O-pentenyl glycosyl donors allowing for direct chemoselective coupling between an activated (armed) glycosyl donor and a deactivated (disarmed) glycosyl acceptor. The disaccharide is then used directly in subsequent glycosidation, but the activation of its disarmed leaving group may require a stronger activator (NIS/TfOH in case of pentenyl leaving group). In a more general sense, the differentiation can be achieved by modulating the reaction conditions that in addition of the choice of promoter, reaction temperature or solvent could be exploited.[56] Discovered with *n*-pentenyl glycosides, this armed-disarmed strategy ultimately proved to be of a general nature, and has been applied to many other classes of glycosyl donor (see subsequent sections).[57]



Scheme 1.3. Fraser-Reid's armed-disarmed strategy

A different rationalization of the arming and disarming effects has emerged with the discovery of the "O-2/O-5 cooperative effect."[58] In case of the armed donors, it is believed that the oxacarbenium ion intermediate is stabilized by integrated assistance from the lone pair of electrons on the adjacent ring oxygen (O-5, Figure 1.1). In case of the disarmed donors, it is believed that the oxacarbenium ion intermediate is stabilized by charge distribution via acyloxonium ion intermediate formed through the acyl type protecting group at O-2. The realization of the O-2/O-5 cooperative effect in glycosylation led to the discovery of electronically superarmed and superdisarmed glycosyl donors and acceptors (vide infra). Reinforcing early work by Isbell,[59] Crich *et al*. emphasized that the 1,2-*trans* orientation of the 2-O-acyl and S-benzoxazolyl (SBox) leaving group is required for the anchimeric assistance to occur.[60] A similar conclusion was reached by Bols and Demchenko for S-phenyl glycosides (*vide infra*).[61] Presumably, the stabilization takes place via the concerted displacement of the leaving group.

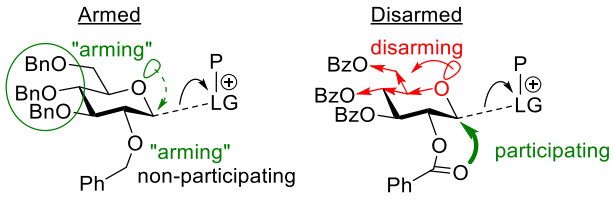
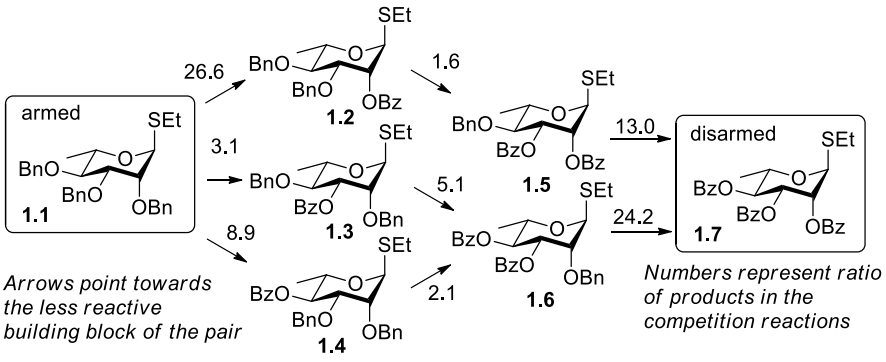


Figure 1.1. O-2/O-5 Cooperative effect in glycosylation.

1.3. Many reactivity levels exist between the armed and disarmed building blocks

From the early days, the researchers were devising different approaches to quantifying the relative reactivity of different building blocks. Following the pioneering study by Fraser-Reid dedicated to determining relative reactivities of variously protected

pairs of the n-pentenyl glycosides,[53] Ley and co-workers introduced a technique wherein building block reactivity could be "tuned."[62] In a series of competitive experiments, wherein two glycosyl donors were set to compete for one glycosyl acceptor, a series of relative reactivity ratios were established (Scheme 1.4). For instance, the greatest disarming effect was seen from the 2-benzoyl substituent in compound **1.2** in comparison to that of 3-benzoyl and 4-benzoyl substituents (**1.3** and **1.4**). Di-benzoylated glycosyl donors **1.5** and **1.6** were less reactive than their mono-benzoylated counterparts and, the disarmed per-benzoylated donor **1.7** being the least reactive in this series.



Scheme 1.4. Intermediate reactivity of a series of partially benzoylated rhamnosides.

Wong and co-workers devised a comprehensive approach wherein a broad library of glycosyl donors and acceptors was assigned relative reactivity values (RRVs).[63] The determination of RRVs was made using tolyl thioglycoside donors in the presence of an NIS/TfOH promoter system. More recently, Hung and Wong created a comprehensive database of RRVs for the series of D-glucose building blocks (Figure 1.2).[64] According to their database, some tri-benzylated acceptors **1.9**-**1.12** showed similar or even higher RRVs in comparison to that of the armed donor **1.8**. Not surprisingly, RRVs of mono-

benzoylated donors **1.13-1.16** was lower, but still much higher than that of the disarmed acceptor **1.17** or disarmed donor **1.18**. The application of this approach to chemoselective oligosaccharide synthesis and determination of the RRVs of silylated donors will be discussed below.

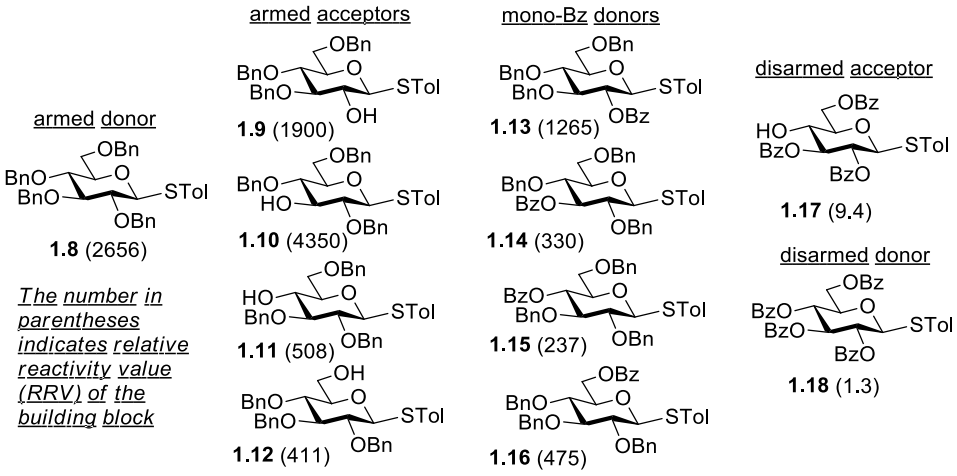
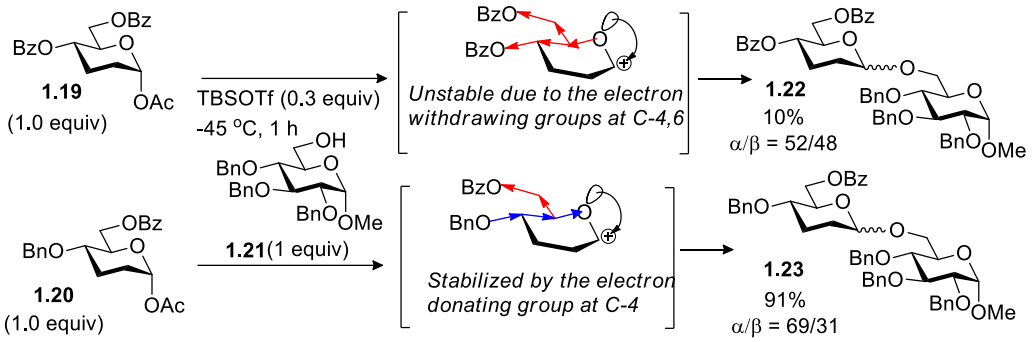


Figure 1.2. RRVs of differently protected STol glycosyl donors and acceptors.

Toshima *et al.* studied the effect of remote protecting groups of glycosyl donors of the 2,3-dideoxy series on the reactivity in glycosylations.[65] For this purpose, glycosylation reactions of glycosyl acetate donors **1.19** and **1.20** with acceptor **1.21** were performed in the presence of several Lewis and protic acid activators including TMSOTf, TBSOTf, BF_3-OEt_2, TfOH, and momorillonite K-10 (MK-10). It was found that glycosidation of donor **1.20** yielded disaccharide **1.23** with excellent yield while donor **1.19** with 4,6-dibenzoyl protection gave disaccharide **1.22** in low yield under the same reaction conditions. A similar reactivity profile was determined for 4-benzoyl-6-benzyl donor.[65]



Scheme 1.5. Disarming effects on 2,3-dideoxy donors.

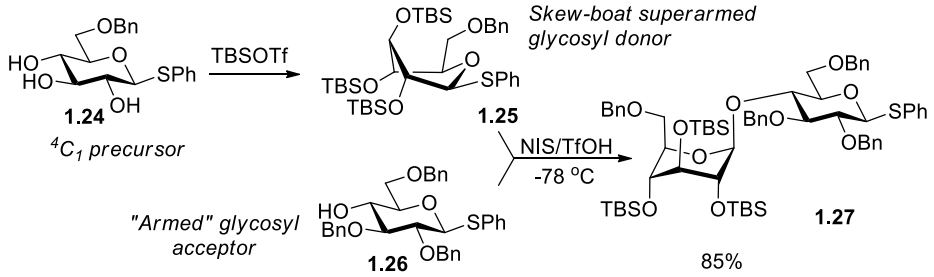
Although most reactivity levels in the studies surveyed in this section fall between the traditional armed and disarmed building blocks, Wong's and Hung's studies revealed a number of building blocks that extend beyond this boundary. For example, 2-hydroxyl galactoside[63] or 3-hydroxyl glucoside[64] were found to be 3 and 1.5 times more reactive than their respective per-benzylated counterparts. This important discovery led to a new direction in studying building blocks, and a variety of new reactivity levels ranging from more reactive than the armed ones (superarmed) to even less reactive than the disarmed ones (superdisarmed) has been discovered. The studies arising from these two new directions will be discussed in the subsequent two subsections, respectively.

1.4. Modes for enhancing the reactivity: superarmed building blocks

Uniformly protected per-benzylated glycosyl donors have become the benchmark for describing the armed glycosyl donors, or reactivity levels associated with it. Over the years, benzyl groups had been almost exclusively used as arming ether protecting groups until Demchenko and co-workers showed that 2-O-picolinyl group has similar electronic properties and can also be used in chemoselective armed-disarmed activations.[66]

Uniquely, the picolinyl group is capable of affecting the stereoselectivity, which makes it suitable as an "arming participating group." More recently, o-cyano and o-nitrobenzyl have been introduced as arming participating groups.[67,68]

Other recent improvements have revealed the reactivity levels that far exceed the reactivity of traditional uniformly benzylated armed building blocks. These "superarmed" glycosyl donors have further expanded the versatility of the armed-disarmed concept. Introduced by Bols for describing the reactivity of conformationally armed building blocks, the term superarmed is now used to describe all building blocks that are more reactive than per-benzylated armed building blocks. Building upon early studies of the effect of conformational changes on reactivity[69] Bols and co-workers hypothesized that the conformational change required to obtain a flattened oxacabenium intermediate that exists in a half-chair conformation will be facilitated in the axial-rich donor. If this conformational change could be facilitated, the activation energy of the rate determining step (RDS) should decrease, and the donor reactivity would be enhanced. The conformational change of SPh glucoside **1.24** was induced via creating steric congestion with *tert*-butyldimethylsilyl (TBS) groups at the C-2, 3 and 4 positions, resulting in a skew-boat conformation of **1.25** (Scheme 1.6).[69-73]



Scheme 1.6. Conformational superarming: conformation change leads to increased reactivity.

As a result, glycosyl donor **1.25** showed an estimated 20-fold increase in reactivity in comparison to the per-benzylated donor. This was ultimately translated into the direct chemoselective coupling of donor **1.25** with the armed acceptor **1.26** in the presence of NIS/TfOH at -78 °C to give disaccharide **1.27** in a high yield.[71,72]

The RRVs of partially silylated STol glycosyl donors reported by Hung and Wong clearly reinforce Bols' findings that the reactivity may vary drastically depending on the number of silyl groups present and their location on the sugar ring.[64] For example, mono-silylated donors **1.28-1.31** express much higher reactivity than the standard armed donor **8** (Figure 1.3). Both TBS and their tri-isopropylsilyl (TIPS) counterparts have been studied and the latter showed a marginally higher reactivity across the range of all mono-silylated derivatives studied. Di-silylated thioglycosides **1.32-1.35**, in which silyl groups were remotely positioned to each other, showed 2 to 6 times higher RRVs compared to

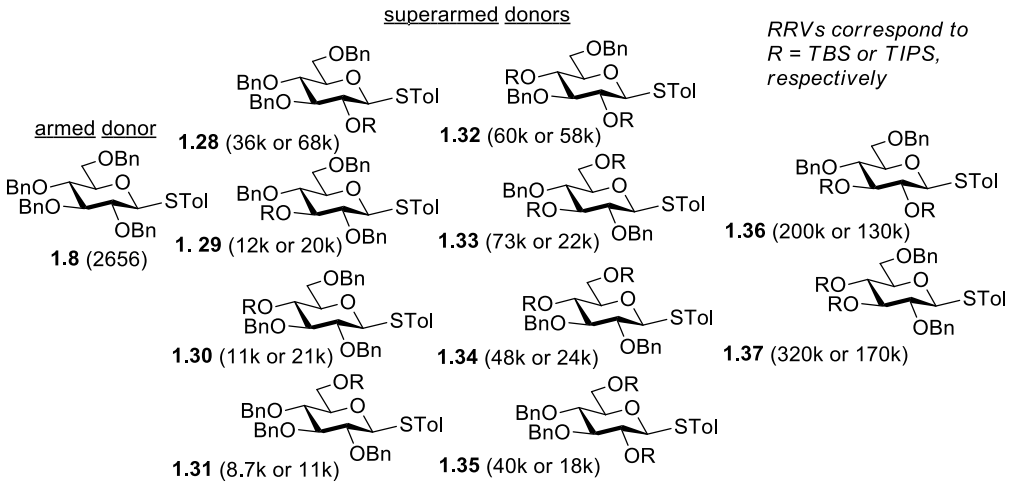
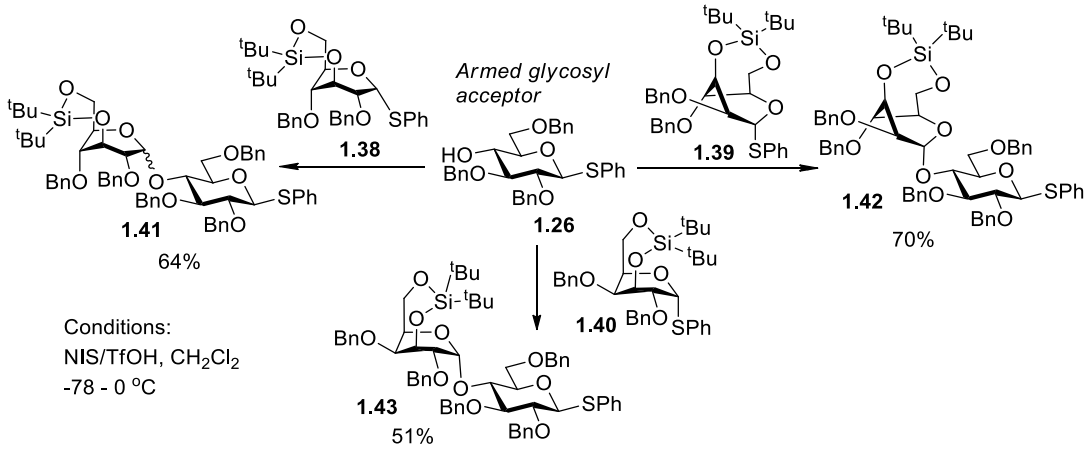


Figure 1.3. RRVs of partially silylated superarmed glycosyl donors.

their mono-silylated counterparts. The greater reactivity enhancement was detected for di-silylated derivatives **1.36** and **1.37** in which the two silyl substituents were placed at

the neighboring trans-vicinal positions of the ring, 2,3 and 3,4 respectively. Interestingly, TBS substituents were more arming than TIPS in all di-silylated donors **1.32-1.37**.

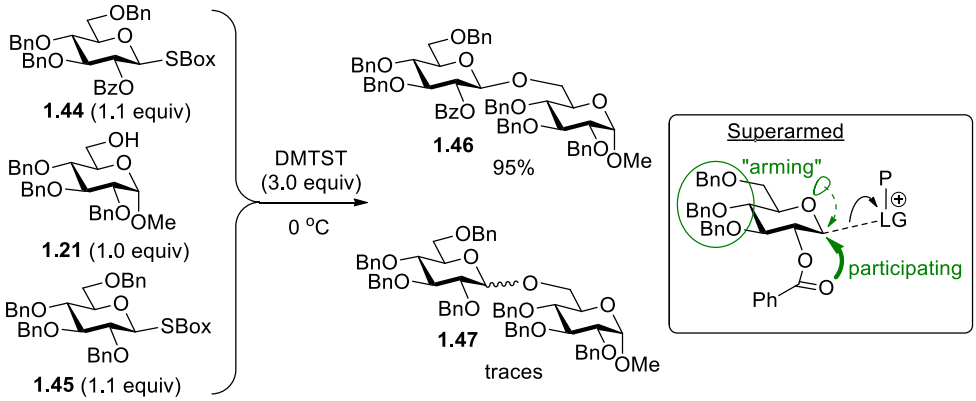
The scope of conformational superarming was broadened by the investigation of a series of glycosyl donors in which the axial-rich conformation was achieved via strategic tethering. Building upon their earlier studies of 2,4-diol tethering with di-*tert*-butyl silylene[72] and Yamada's 3,6-O-(o-xylylene)-bridging,[74] Bols and co-workers devised a series of novel glycosyl donors. Under this, 3,6-di-tert-butyl silylene tethering in gluco-, manno-, galacto-, and 2-azido-gluco pyranosides have been investigated.[75] All of these donors were found to adopt axial-rich $B_{1,4}$ boat or $^{3}S_{1}$ skew boat conformations. To determine the relative reactivity, direct activations of the new donors over the armed tri-benzylated acceptor **1.26** have been conducted. As depicted in Scheme 1.7, chemoselective glycosylations with donors **1.38**-**1.40** afforded disaccharides **1.41**-**1.43** in 51-70% yields with preferential α-selectivity.



Scheme 1.7. Conformational superarming via 3,6-silylene tethering.

Demchenko and co-workers took a different approach for superarming glycosyl donors. The superarming of S-benzoxazolyl (SBox) and SEt glycosyl donors was based

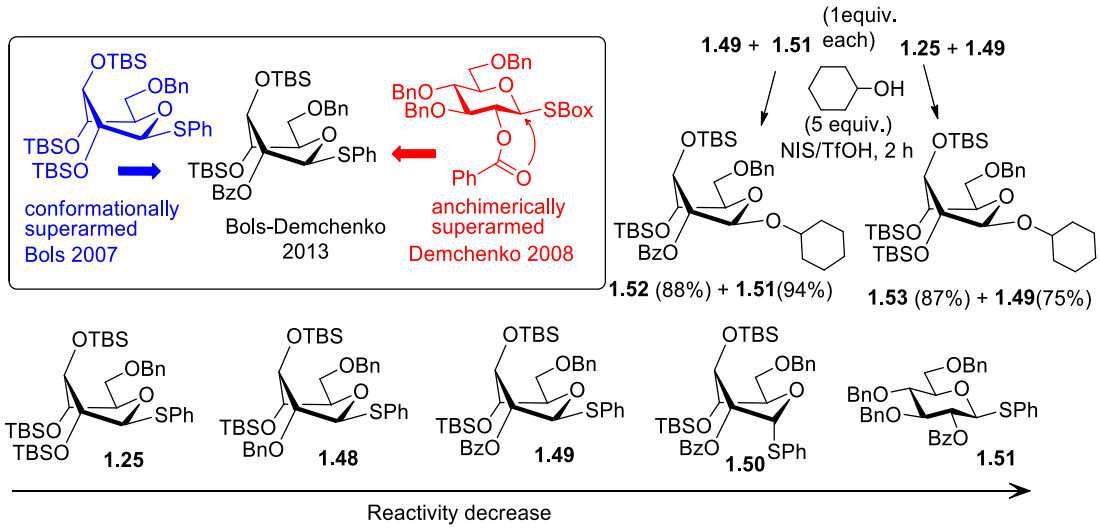
on the O-2/O-5 cooperative effect in glycosylation (*vide infra*).[58] In this scenario, it is believed that the oxacarbenium ion intermediate is stabilized by the cooperative assistance from both the lone pair of electrons on the adjacent ring oxygen (O-5) and charge distribution via acyloxonium ion intermediate formed through the acyl type protecting group at O-2 (Scheme 1.8). The 2-O-benzoyl-3,4,6-tri-O-benzyl derivatives gain extra stabilization upon activation through this O-2/O-5 cooperative mechanism and becomes a superarmed donor. As a result of the competitive glycosylation upon activation with dimethyl(thiomethyl)sulfonium trifluoromethanesulfonate (DMTST), it was observed that donors equipped with 2-OBz-3,4,6-tri-OBn pattern are 10-20 times more reactive than their armed counterparts.[76-78] Thus, a competitive reaction of donors **1.44** and **1.45** with acceptor **1.21** gave disaccharide **1.46** derived from the superarmed donor **1.44** in 95%. Meanwhile, the disaccharide **47** derived from the armed donor **45** was found only in trace amounts.



Scheme 1.8. Superarmed (1.44) and armed (1.45) glycosyl donors in the competitive glycosylation.

These results clearly showed that the superarmed donor is much more reactive than the armed counterpart. Further studies by the Demchenko group showed that the same trend of reactivity appears upon changing the leaving group. While efficient differentiation of glycosyl donors of the S-ethyl series could be efficiently achieved in the presence of iodine as promoter, the reactivity difference was notably lower for glycosyl donors of the SPh, S-Tolyl, STaz (S-thiazolinyl), and O-pentenyl series.[78]

Through a collaboration between the Bols and Demchenko groups, the two different approaches to superarm glycosyl donors were combined in one universal platform. Glycosylations with 2-OBz-3,4-di-OTBS donor **1.49** was swift, high yielding and β-stereoselective (Scheme 1.9).[61] In order to determine the relative reactivity of the new hybrid donor **1.49** in comparison to the previously investigated superarmed donors, a series of competitive experiments have been performed.



Scheme 1.9. Superarming by combined neighboring and conformational effects.

Thus, a competition experiment between the hybrid donor **1.49** and the electronically super-armed donor **1.51** showed a 88% conversion of donor **1.49** to glycoside **1.52**

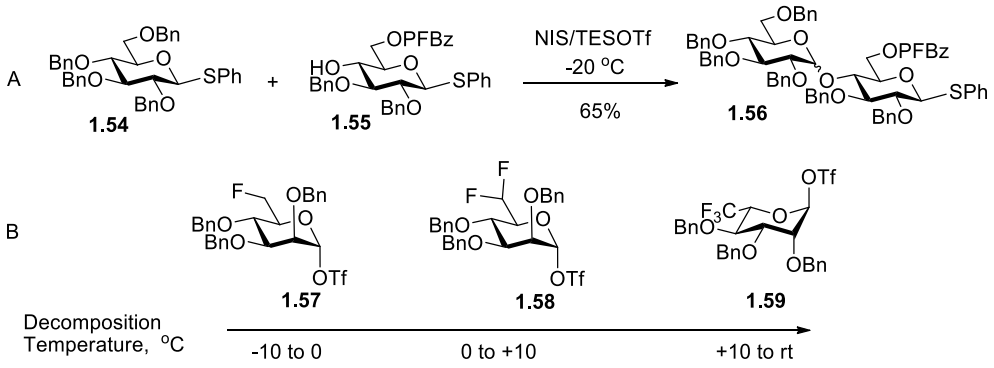
whereas unreacted donor **1.51** was recovered in 94%. When a similar competition experiment was performed between donors **1.49** and **1.25**, a higher conversion of donor **1.25** to **1.53** has been observed. A significance of the anomeric configuration brought up by Crich[60] was further reinforced by this comparison study that showed higher reactivity of donor **1.49** than its β-linked counterpart **1.50**. It is also believed that the flipped skew-boat conformation of the donor may also diminish the anchimeric assistance due to the non-antiperiplanar nature of the 2-OBz group to the anomeric leaving group.

On the other hand, α-configured glycosyl donors equipped with a non-participating group at C-2 typically by far exceed the reactivity of their β-counterparts.[61,79] This controversy reinforces the power of the anchimeric assistance that is able to invert the reactivity of α- and β-thioglycosides. Over the course of this study the order of relative reactivity of various glycosyl donors was determined. It was also learned that the conformational arming is a very powerful tool to increase the reactivity and achieve excellent yields. The anchimeric superarming effects are weaker, but the participation ensures high 1,2-*trans* selectivity, which was unavailable with other conformationally superarmed donors. A recent relevant study revealed that SEt glycosyl donors follow a very similar relative reactivity trend, but all SEt glycosides are marginally more reactive than their SPh counterparts.[80]

1.5. Modes for decreasing the reactivity: superdisarmed building blocks

Standard disarmed building blocks are uniformly protected with benzoyl groups (per-benzoates). Madsen *et al* have revealed that building blocks can be deactivated by placing a single electron-withdrawing group at a remote position.[81,82] Thus, they have

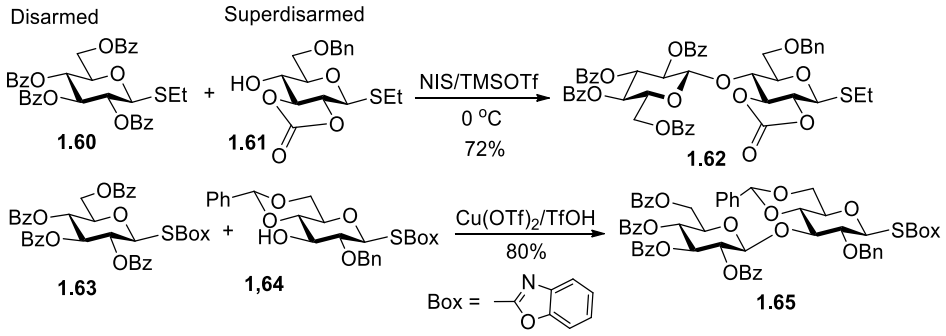
shown that 6-O-pentafluorobenzoyl (PFBz) group disarms the leaving group of **1.55**. The disarming effect is sufficient to selectively activate per-benzylated armed donor **1.54** over acceptor **55** to obtain disaccharide **1.56** (Scheme 1.10A). This concept was further extended to disarming building blocks of the galacto, manno and manosamine series. Crich and co-worker have investigated the disarming effect that 6-fluoro-6-deoxy glycosyl donors in terms of reactivity, selectivity and stability of the intermediate glycosyl triflates. Thus, it was observed that di- and tri-fluorinated D- or L-mannosyl triflates were more stable than their mono-fluorinated counterpart. This was accessed by comparing the decomposition temperatures specific to each glycosyl triflate as depicted in Scheme 10B.[83]



Scheme 1.10. Deactivation by strong electron withdrawal from C-6.

The effect of cyclic acetals and ketals has also been studied towards expanding the scopes of armed- disarmed strategy. Fraser-Reid *et al.*[84] and Ley[85] *et al.* have shown that the presence of such cyclic protecting groups can deactivate the sugar derivative by increasing the rigidity of the sugar ring and thereby locking the $^4C_1$ chair conformation that may interfere with the formation of the flattened oxacarbenium ion.[84,86] Extensive studies have led to the realization that reactivity of building blocks protected with cyclic

groups can even be lower than that of the corresponding per-benzoylated disarmed building blocks. Thus, Boons and co-workers have shown that presence of the cyclic 2,3-carbonate group disarms the thioglycosidic donor more than per-acylation, making the former superdisarmed.[87] As a result, disarmed per-benzoylated glycosyl donor **1.60** could be selectively activated over the superdisarmed acceptor **1.61** to yield disaccharide **1.62** in a good yield (Scheme 1.11). Along similar lines, Demchenko *et al.* showed that even a conventional benzylidene acetal groups can superdisarm building blocks of the SBox series.[58]

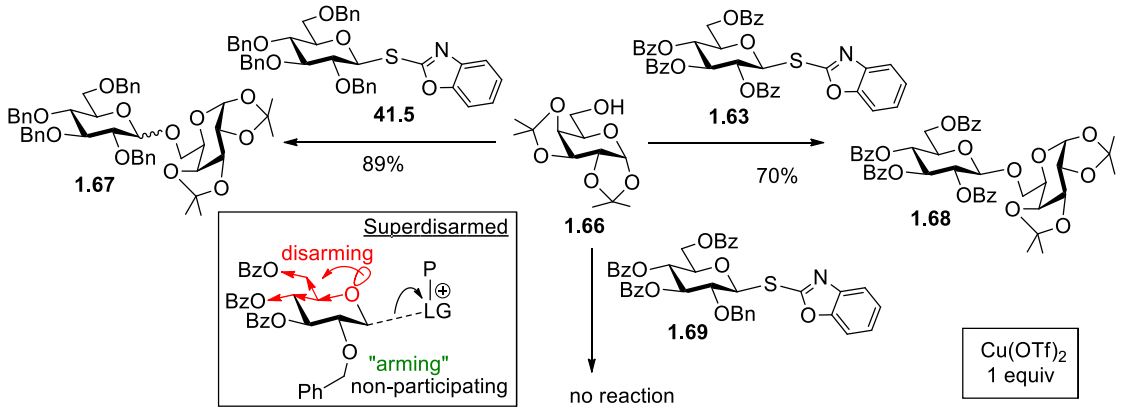


Scheme 1.11. Chemoselective activation of disarmed donors over acceptors bearing fused rings.

The Bols group brought up the fact that the disarming effect of cyclic acetals can be not only due to the ring rigidity, but also due to the electron withdrawing effect of C-6 group which is further enriched by its orientation.[88] From model studies, they have concluded that the torsional effect, which greatly depends on the substituent orientation, plays a role in disarming the sugar moieties. More recently, Crich and co-workers investigated the effect of a 4,6-O-alkylidene acetal or its 7-carba analog on the rates of hydrolysis of methyl and 2,4-dinitrophenyl galactopyranosides in which the methoxy

group adopts either an equatorial or an axial position according to the configuration.[89] This study reinforced previous findings, and it was determined that the alkylidene acetal leads to decreased rates of hydrolysis with respect to comparable systems lacking the cyclic protecting group. A combination of the two effects, torsional and electronic, may be one of the reasons the donors containing the fused ring systems tend to be less reactive than the pure-electronically disarmed, acylated building blocks. In contrast, 7-carba analogs had practically no effect on the rates of hydrolysis.

Demchenko *et al* discovered that S-benzoxazolyl (SBox) glycosyl donor protected with arming benzyl at C-2 and disarming benzoyl groups at C-3, 4 and 6 shows greatly diminished reactivity compared to both the armed per-benzylated and disarmed per-benzoylated glycosyl donors.[58]

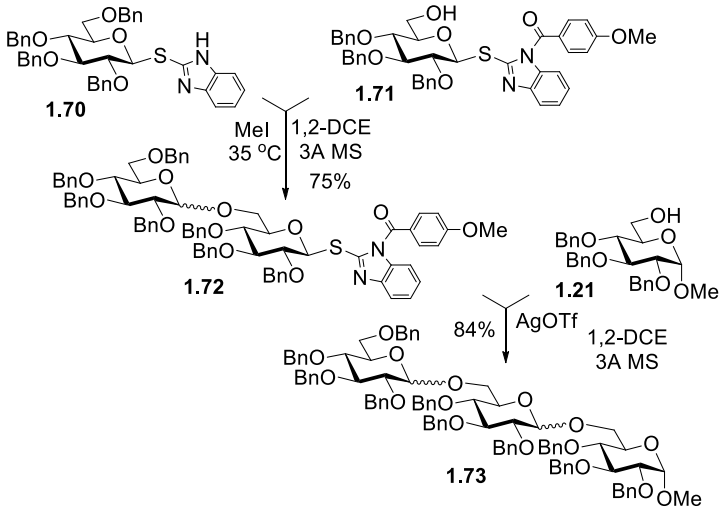


Scheme 1.12. Electronically superdisarmed building blocks.

While glycosylation of acceptor **1.66** with SBox donors **1.45** and **1.63** in the presence of copper(II) triflate gave the respective disaccharides **1.67** and **1.68** in good yields, donor **1.69** remained totally unreactive. Interestingly, this observation was contradictory to previously predicted higher reactivity for such 2-O-benzylated glycosides compared to

disarmed per-benzoylated donors.[62,63] This discrepancy was rationalized by the "O-2/O-5 cooperative effect".[58] In this application, the carbocation stabilization can neither be achieved from the endocyclic ring oxygen (O-5) as in the armed glycosyl donors, nor from O-2 as in disarmed donors. As a result, this combination gave rise to the "superdisarming" protecting group pattern overall.

Demchenko and co-workers discovered a conceptually new way of disarming the leaving groups. The conceptual difference of this approach from Fraser-Reid's armed-disarmed approach is that herein the disarming is achieved by acylation of the leaving group itself, not by introducing the neighboring acyl substituents in the sugar moiety. This was investigated in application to *S*-benzimidazolyl (SBiz) leaving group versus N-anisoylated SBiz.[90] First, SBiz donor **1.70** was activated with MeI over the disarmed acceptor **1.71** to afford disaccharide **1.72** (Scheme 1.13).



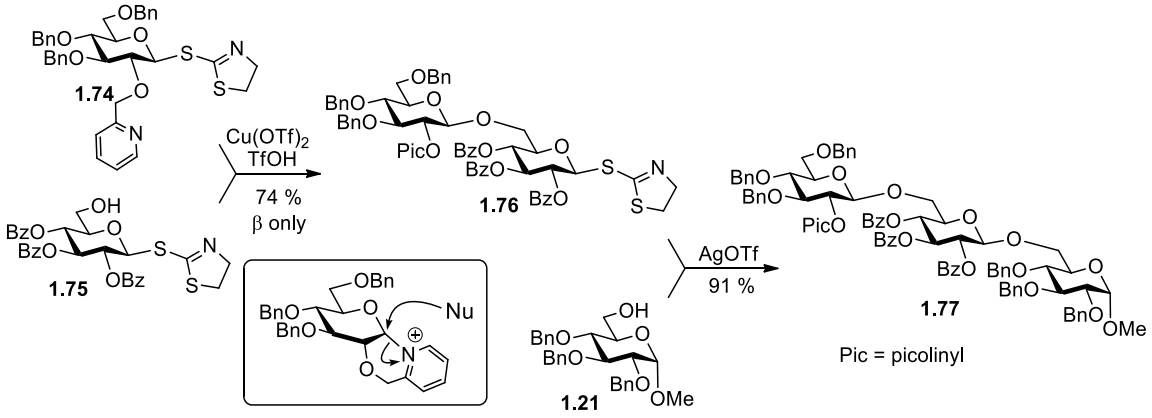
Scheme 1.13. Disarming by placing an acyl substituent on the leaving group.

The disarmed *N*-anisoylated SBiz disaccharide was then glycosylated with the glycosyl acceptor **1.21** in the presence of AgOTf to give the trisaccharide **1.73** in 84% yield. It was

noted that benzylated and benzoylated SBiz imidates can also be activated in the conventional armed-disarmed fashion.

1.6. Application of armed and disarmed building blocks in stereocontrolled glycosylation

Armed and disarmed building blocks follow general stereoselectivity trends in glycosylations: armed per-benzylated derivatives provide some α-stereoselectivity, whereas disarmed benzoylated ones give complete β-selectivity due to the participation of 2-benzoyl. As aforementioned, picolinyl can be used as an arming group at C-2, but the chemoselective activation of the 2-O-picolinylated donors leads to 1,2-*trans* glycosides, inverse stereoselectivity in comparison to that achieved with traditional benzylated armed glycosyl donors. Thus, upon activation with $Cu(OTf)_2$ the armed S-thiazolinyl (STaz) glycosyl donor **1.74** gives the stable cyclic intermediate.



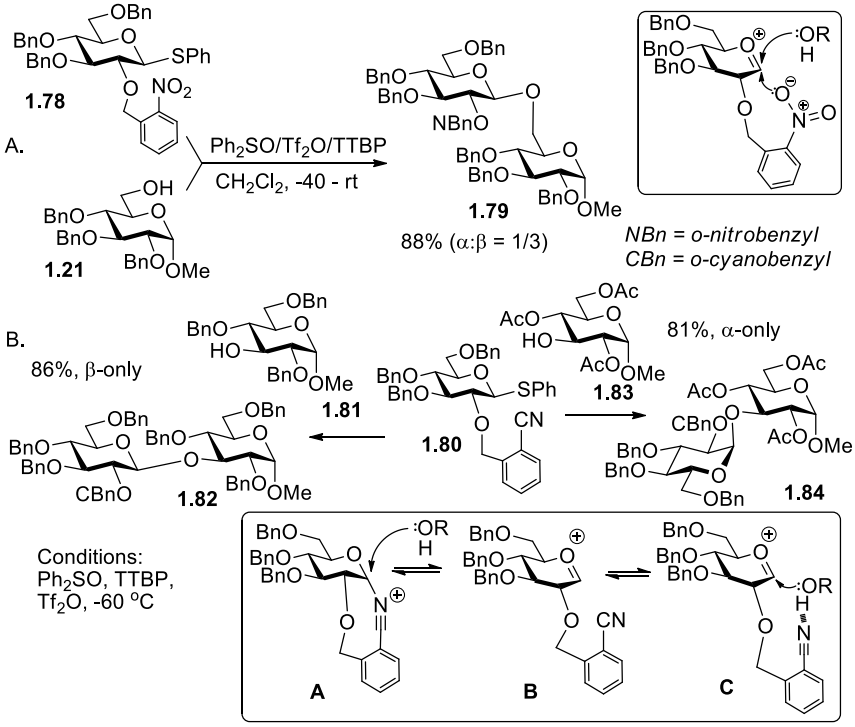
Scheme 1.14. Arming participating picolinyl group mediated 1,2-*trans*-glycosylation.

The latter is subsequently glycosylated with the disarmed STaz acceptor **1.75** to give 1,2-*trans*-linked disaccharide **1.76** in 74% yield (Scheme 1.14). The resulting disarmed disaccharide donor **1.76** has been further glycosylated with the acceptor **1.21** in the presence of stronger promoter AgOTf to obtain the *trans*-*trans*-linked trisaccharide **1.77** in 91% yield.[66]

Mlynarski and co-workers investigated ortho-nitrobenzyl (NBn) as an arming participating group.[67] They theorized that 2-O-ortho-nitrobenzyl will participate in glycosylation by stabilizing the oxacarbenium ion intermediate and hence blocking the bottom face from the nucleophilic attack (Scheme 1.15A). The activation of glucosyl donor 1.**78** with Ph_2SO/Tf_2O/TTBP (2,4,6-tri-tert-butylpyrimidine) afforded disaccharide **1.79** in a high yield and preferential 1,2-*trans* selectivity. Liu and co-workers investigated another arming participating group, o-cyanobenzyl (CBn) at C-2 position of a glycosyl donor.[68]

The interesting feature of this glycosylation method is that a single glycosyl donor can yield either α- or β-linked products depending on the nature of the glycosyl acceptor. It is believed that the dual directing effect of o-nitrobenzyl group is due to the equilibrium in reaction intermediates **A-C** (Scheme 1.15B). Thus, the activation of donor **1.80** will result in the formation of oxacarbenium ion **B** that is stabilized via *cis*-nitronium ion **A**. The latter will direct the nucleophilic attack from the top face hence offering β-directing effect that is preferred with reactive electron-rich glycosyl acceptors. Another mode by which o-cyanobenzyl group can react is via H-bond-mediated aglycone delivery (HAD). Discovered with remote picolinyl and picoloyl groups, the HAD method has already yielded a number of highly selective syntheses and applications.[91-100] A

similar HAD action can be envisaged for 2-O-ortho-cyanobenzyl group (see intermediate **C** in Scheme 1.15B). It was concluded that this mechanism of action leading to high α-selectivity is preferred with electron-deficient acceptors.

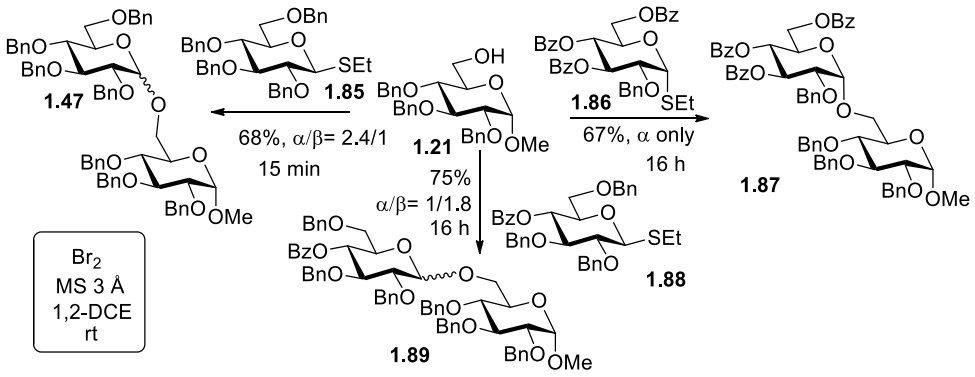


Scheme 1.15. Use of O-nitrobenzyl and O-cyanobenzyl arming participating groups.

In the context of the stereoselective synthesis of α-glycosides, electronically superdisarmed glycosyl donors, which are also 2-O-benzylated, often provide higher stereoselectivity than their per-benzylated counterparts.[20] A valuable application of superdisarmed SEt donors has emerged with the synthesis and glycosidation of glycosyl sulfonium salts as activated intermediates of thioglycoside glycosidation. The mode of activation of thioglycosides has been proposed multiple times although direct evidence could only be acquired with superdisarmed thioglycosides due to high stability of the

intermediate. Thus, direct alkylation of a superdisarmed ethylthio glycoside with MeOTf led to a sulfonium salt that could be isolated and characterized by NMR.[79] A number of other anomeric sulfonium salts, most notably that derived from the reaction with dimethyl(methylthio)sulfonium triflate (DMTST) was obtained.[79] Various experiments with simple alcohol acceptors showed high 1,2-*cis* stereoselectivity of glycosidation of sulfonium salt intermediates.

Bromine-activated glycosylation of thioglycosides introduced by Demchenko *et al*. gave high stereoselectivity only with superdisarmed thioglycosides.[101] Thus, it was demonstrated that the 1,2-*trans* glycosyl bromide is the only intermediate leading to products while the 1,2-*cis* bromide remains unreactive and the oxacarbenium intermediate does not form. Resultantly, the nucleophilic displacement of β-bromide takes place in a concerted bimolecular fashion leading to exclusive α-stereoselectivity. For instance, 3,4,6-tri-O-benzoyl-2-O-benzyl thioglycoside **1.86** was coupled with acceptor **1.21** in the presence of bromine to afford disaccharide **1.87** with exclusive 1,2-*cis* selectivity in 67% yield (Scheme 1.16).

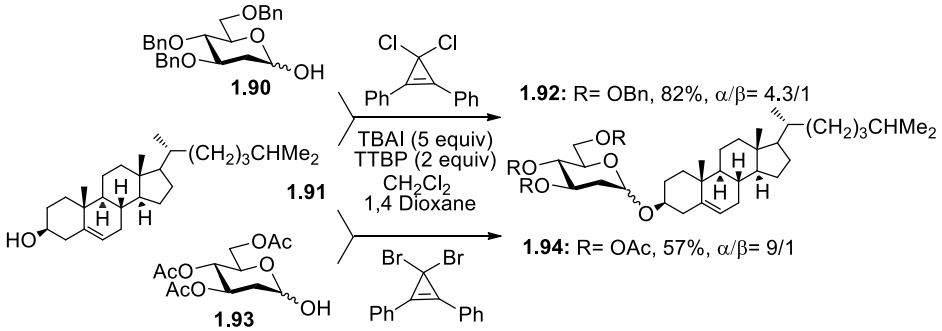


Scheme 1.16. Bromine-mediated activation of S-ethyl donors.[101]

The use of α-thioglycoside starting material was found advantageous for generating β-bromide intermediate. The average yield, which is due to the competing isomerization of

β- to α-bromide, could be further improved by using $HgBr_2$ as the co-promoter, but this could also decrease the stereoselectivity. For comparison, armed donor **1.85** or 4-benzoyl donor **1.88**, gave no stereoselectivity for the formation of the respective disaccharides **1.47** and **1.89**.

Bennett and co-workers devised new promoter systems that gave high α-selectivity for both armed and disarmed glycosyl donors of the 2-deoxy series **1.90** and **1.93**, respectively (Scheme 1.17).[102] NMR monitoring showed that the armed hemiacetal donor **1.90** is first converted into to the respective glycosyl chloride intermediate by the action of 3,3-dichloro-1,2-diphenylcyclopropene promoter. The latter is then converted into a mixture of α- and β-glycosyl iodides with TBAI, but predominantly the more reactive β-iodide reacts with glycosyl acceptor **1.91**. Presumably, this displacement proceeds in the S_N2 fashion leading to glycosides in high α-selectivity. Interestingly, 3,3-dichloro-1,2-diphenylcyclopropene promoter failed to activate the disarmed donor **1.93** due to the high stability of the intermediate chloride. In this case, 3,3-dibromo-1,2-diphenylcyclopropene was found to be an effective promoter that provided glycosides **1.94** in excellent stereoselectivity.



Scheme 1.17. Highly stereoselective glycosylation with armed and disarmed 2-deoxy donors.

Superarmed glycosyl donors allow to achieve various selectivities depending on their structure and the mode of superarming. Conformationally superarmed donor **1.25** introduced by Bols et al is capable of providing high stereoselectivity that was attributed to the steric hindrance from the bulky 2-O-silyl substituents.[69-72] Depicted in Scheme 1.6 is a β-stereoselective glycosylation of acceptor **1.26** that afforded disaccharide **1.27** in a high yield of 85% and complete selectivity. In application of the synthesis of rhamnosides, a drastic temperature effect on stereoselectivity was observed. The conformationally superarmed rhamnosyl donors produced modest β-selectivity at low temperatures, but increasing the temperature gave excellent α-selectivity.[103]

Table 1.1. Survey of stereoselectivity in glycosylation with different donors.

Type of donors	Stereoselectivity	Ref
Armed per-benzyl	*Cis*/*trans*-mixtures, mainly 1,2-*cis*	**52,54,104,105**
Disarmed per-benzoyl	Complete 1,2-*trans*	**52,54,104,105**
Armed with 2-O-arming participating group	Complete 1,2-*trans*	**66-68,106**
Electronically superdisarmed	Moderate or high 1,2-*cis*	**58,101**
Superdisarmed by fused ring systems	High or complete 1,2-*cis**	**31,34,107-111**
Conformationally superarmed	*Cis*/*trans*-mixtures	**70,71**
Conformationally and anchimerically superarmed	Complete 1,2-*trans*	**61**
Anchimerically superarmed	Complete 1,2-*trans*	**76-78**
Conformational superarming by tethering	High 1,2-*cis*	**75**

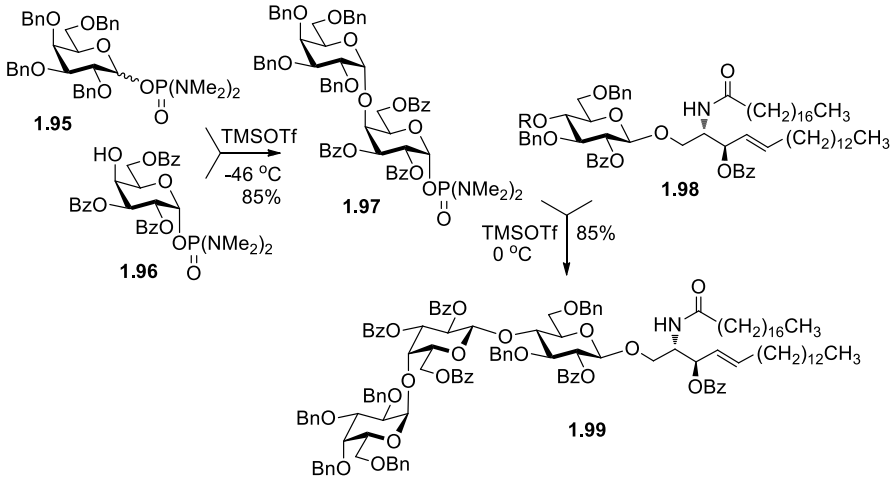
* - high stereoselectivity can be achieved in 4,6-benzylidene or 2,3-cyclocarbonyl/ oxazolidinone systems. However, little has been investigated in the context of the chemoselective oligosaccharide synthesis.

A relaxed stereoselectivity obtained with conformationally superarmed donors was addressed by the introduction of a hybrid donor **1.49** in which the superarming was achieved by the combined anchimeric and conformational factors (2-O-Bz-3,4-di-O-TBS protection). All glycosylations with donor **1.49** were completely β-selective[61] as well as the electronically superarmed donors bearing 2-O-Bz-tri-O-Bn protection.[76-78] Very differently, glycosyl donors in which the superarming is achieved by 3,6-silicon tethering, such as donor **1.38**, provide predominant α-selectivity, which was attributed to the steric hindrance of the top face of the oxacarbenium intermediate.[75] Table 1.1 surveys currently known modes for arming/ disarming and stereoselectivities that can be achieved by using these approaches.

1.7. Application of armed/superarmed and disarmed building blocks in chemoselective oligosaccharide synthesis.

The expeditious preparation of complex oligosaccharides remains a significant challenge to synthetic organic chemistry. The combined demands of regio- and stereoselectivity in glycosidic bond formation, has led to complex synthetic schemes and extensive protecting group manipulations. As aforementioned, the use of a chemoselective activation strategy avoids such extraneous manipulations, thus offering significant advantages for expeditious glycoside synthesis. Since the glycosidation of 2-O-acylated glycosyl donors typically proceeds via the formation of the bicyclic acyloxonium intermediate, the overall two-step armed-disarmed activation sequence leads to a *cis*-*trans*-patterned trisaccharide. Starting with Fraser-Reid's pentenyl-based synthesis,[54] a number of relevant examples have emerged.[57] For instance, Hashimoto *et*

al. activated the armed galactosyl donor **1.95** over the disarmed galactosyl acceptor **1.96** with TMSOTf at –46 °C to obtain disaccharide **1.97** in 85% yield. The latter was then glycosidated with glycosyl acceptor **1.98** in the presence of TMSOTf at 0 °C to provide the requisite *cis-trans*-sequenced trisaccharide **1.99** in 85% yield.[112] While this synthesis is highly stereoselective, a majority of glycosylation with armed donors suffers from low stereoselectivity.



Scheme 1.18. Armed-disarmed synthesis[112] of glycosphingolipid 1.99.

While the traditional armed-disarmed strategy provides a straightforward access to *cis-trans* sequenced trisaccharides, other sequences cannot be directly accessed. It was quickly realized that for this excellent concept to become universally applicable it should be expanded to a broader range of linkages, protecting group patterns, and oligosaccharide sequences. Major improvements in this direction that have emerged in the past decade are summarized in Table 1.2. With the discovery of other levels of reactivity, a more flexible synthesis of a variety of oligosaccharide sequences using the chemoselective activation has become possible.

Table 1.2. A survey of oligosaccharide sequences that can be obtained by chemoselective activation.

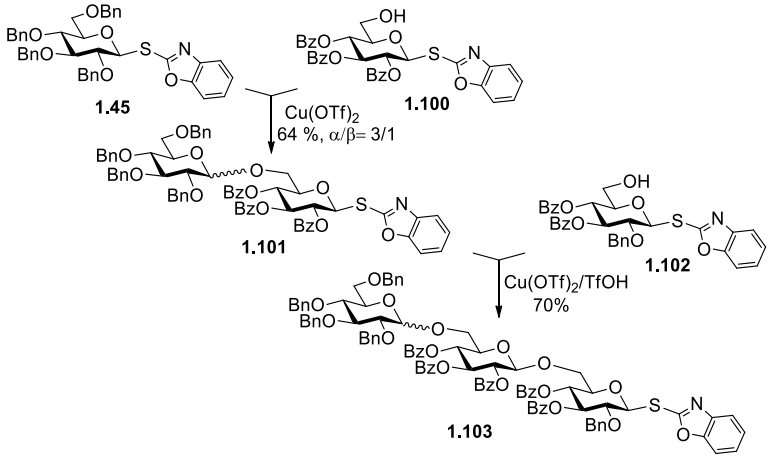
Sequence*	Building blocks needed	Ref
Cis-trans	Traditional armed → disarmed	**54,112**
Cis-trans-cis	Armed → disarmed → superdisarmed	**58**
Cis-trans-trans	Programmable strategy	**63**
Cis-cis	Armed → disarmed with the interim reprotection	**113,114**
	Armed → torsionally or anchimerically superdisarmed	**84,106**
	Armed → 6-PFB disarmed	**82**
Trans-trans	2-Pico armed → traditional disarmed (Scheme 14)	**66,106**
	Anchimerically superarmed → armed	**77**
Trans-cis	2-Pico armed → superdisarmed	**106**
	Anchimerically superarmed → disarmed	**77**
Trans-cis-trans	Conformationally superarmed → armed → disarmed	**70**
	Anchimerically superarmed → armed → disarmed	**78**

* - practically any sequence can be achieved with the use of selective activations based on building blocks with different leaving groups. Preactivation concept pioneered by Huang and Ye is another way to achieve flexible sequencing.

For example, *cis-trans* oligosaccharide sequence obtained through the traditional armed and disarmed donor could be extended to *cis-trans-cis* sequence by adding a superdisarmed acceptor to this combination.[58] However, if the extension to another *trans*-linkage is desired, Wong's programmable strategy is the only way to achieve the chemoselective synthesis of *cis-trans-trans* oligosaccharides.[63] With many reactivity

levels, the programmable strategy can be applied to many other targets and sequences and some representative examples will be discussed below.

A number of approaches have been developed for the synthesis of *cis*-*cis* sequenced oligosaccharides.[82,84,106,113,114] Arming participating 2-O-picolinyl and other similar groups can simplify the syntheses wherein the *trans*-linkage needs to be introduced first.[66-68,106] For instance, activation of 2-picolinyl donor over disarmed or superdisarmed acceptors can be used to obtain *trans*-*trans* or *trans*-*cis* oligosaccharide sequences, respectively.[106] The *trans*-cis-*trans* sequence has been obtained by the combination of either conformationally superarmed or anchimerically superarmed donor with armed and disarmed acceptors. [70,78] Preactivation-based strategies, which tend to be classified as selective rather than chemoselective, also allow for obtaining many of these sequences. A few examples of such sequences are discussed below.

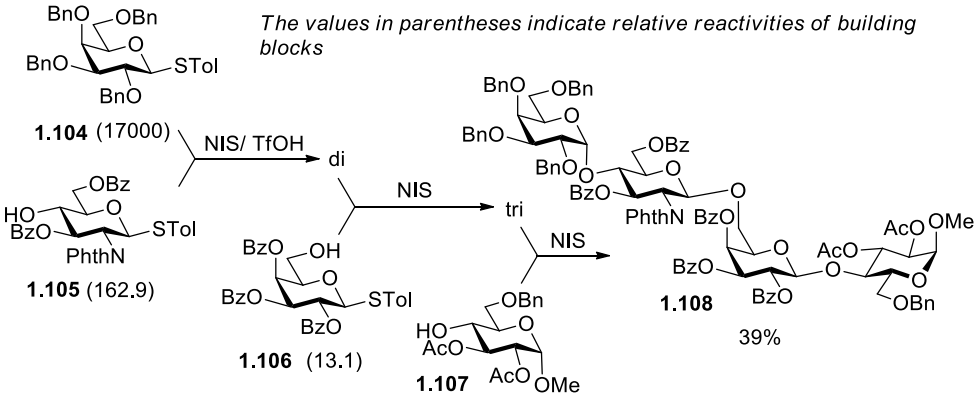


Scheme 1.19. Sequential activation of armed → disarmed → superdisarmed building blocks.[58]

With the discovery of the anchimerically superdisarmed building blocks, it is now possible to produce the *cis*-*trans*-*cis* oligosaccharide sequence.[58] Thus, it was

demonstrated that disarmed disaccharide **1.101**, obtained by classic armed-disarmed approach from building blocks **1.45** and **1.100**, could be chemoselectively activated over superdisarmed building block **1.102** (Scheme 1.19). This was affected in the presence of $Cu(OTf)_2$/TfOH to produce trisaccharide **1.103** (70% yield),[58] which can be used for further glycosylations directly.

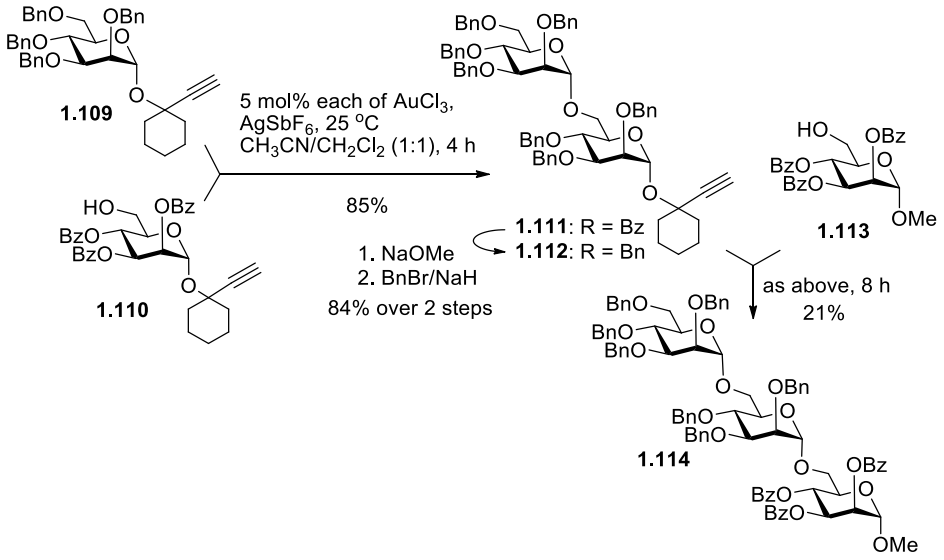
The programmable strategy revealed many reactivity levels, which allow to modulate building blocks to obtain various sequences. An example of a sequence wherein no other chemoselective approaches could be used is depicted in Scheme 1.20. This approach was conducted in one-pot with no isolation and characterization of intermediate oligosaccharides. Armed glycosyl donor **1.104** was chemoselectively activated over glycosyl acceptor **1.105** in the presence of NIS/TfOH. The resulting disaccharide intermediate was then reacted with added disarmed glycosyl acceptor **1.106** to form the trisaccharide intermediate that was then glycosidated with added glycosyl acceptor **1.107** to provide *cis-trans-trans*-linked tetrasaccharide **1.108** in 39% overall yield.[63]



Scheme 1.20. Sequential activation in one-pot for the synthesis of *cis-trans-trans*-linked tetrasaccharide.

Van Boom and co-workers invented a two-step method, glycosylation and protecting group manipulation, to obtain *cis-cis*-linked oligosaccharides.[113] Here, after the

first armed-disarmed activation step the resulting disaccharide was reprotected (OBz → OBn) prior to the subsequent glycosidation. A more recent example of this strategy wherein propargyl mannosyl donors have been used is shown in Scheme 1.21.[114] Glycosylation of the armed mannosyl donor **1.109** with disarmed acceptor **1.110** was performed in the presence of 5 mol % of $AuCl_3$ and $AgSbF_6$ in CH_3CN/CH_2Cl_2 (1/1) at 25 °C. As a result, the disarmed disaccharide **1.111** obtained in 85% yield was then reprotected with benzyls to obtain the armed disaccharide **1.112** in 84% yield. The glycosylation between disaccharide **1.112** and disarmed acceptor **1.113** was performed under the same conditions to obtain the desired trisaccharide **1.114** in 21% yield.

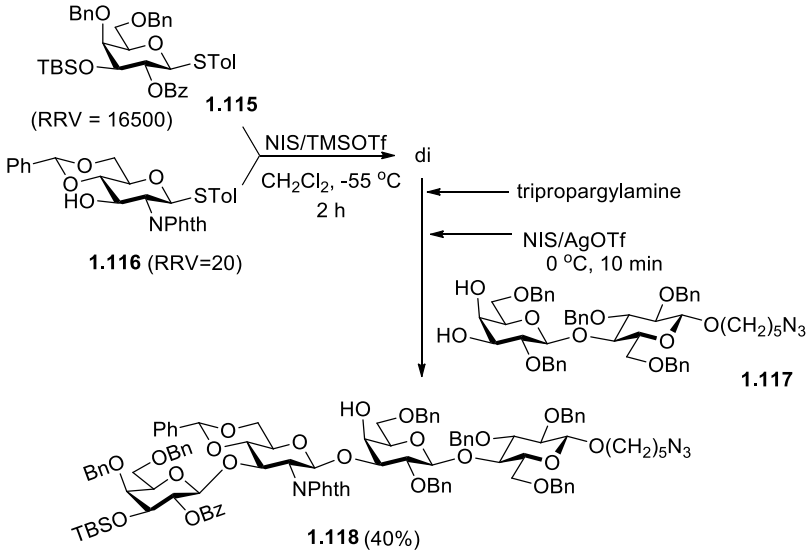


Scheme 1.21. Synthesis of *cis*-*cis*-patterned trisaccharide via the interim reprotection.

The utilization of the cooperative effect allows for the direct synthesis of *cis*-*cis*-linked oligosaccharides, similar to that discussed previously. In this application, the sequential activation of armed per-benzylated glycosyl donor over superdisarmed 3,4-di-O-benzoyl-2-O-benzyl protected STaz glycosyl acceptor led to the *cis*-*cis*-linked

oligosaccharide.[106] The presence of the remote 6-O-pentafluorobenzoyl group disarms the glycosyl acceptor and also facilitates the synthesis of *cis*-*cis*-linked sequences.[82]

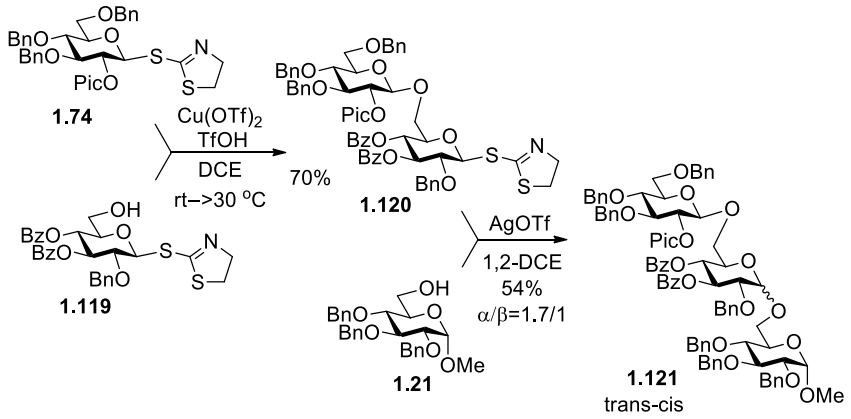
As aforementioned, the application of the 1,2-*trans*-directing picolinyl functionality of armed glycosyl donor in activation over the standard disarmed glycosyl acceptor will allow for the synthesis of *trans*-*trans*-patterned oligosaccharides (see Scheme 1.14). The programmable strategy was also applied to the synthesis of *trans*-*trans* linked oligosaccharide as shown in Scheme 1.22.[64] Coupling of thioglycoside **1.115** and acceptor **1.116** in the presence of NIS/TMSOTf followed by quenching the activator with tripropargylamine and glycosylation with the lactosyl diol **1.117** in the presence of NIS/AgOTf afforded tetrasaccharide **1.118** in 40% yield in one pot.



Scheme 1.22. **Programmable strategy for the synthesis of *trans*-*trans*-linked oligosaccharide.**

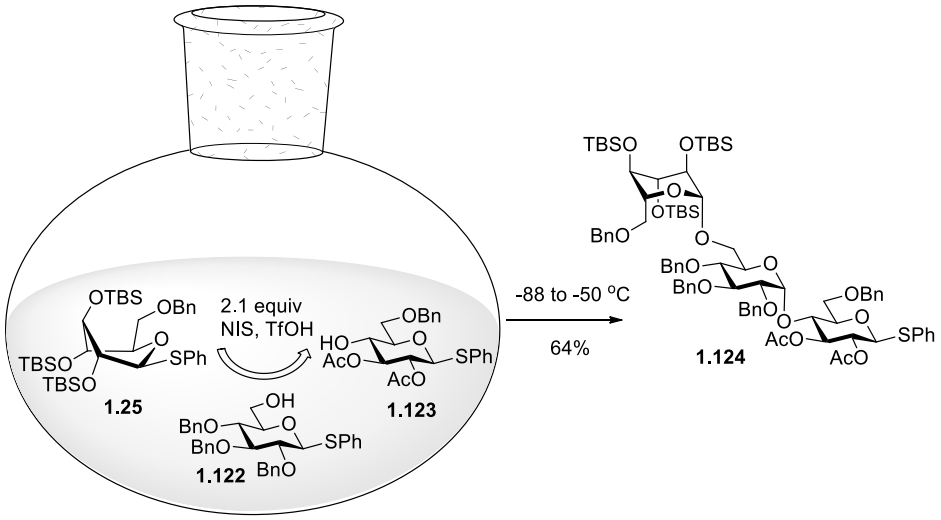
The application of the *trans*-directing 2-O-picolinylated armed glycosyl donors in activation over the superdisarmed acceptors allows to obtain a *trans*-*cis* glycosylation pattern, which is opposite to the traditional armed-disarmed methodology.[106] Thus,

glycosylation between S-thiazolinyl donor **1.74** and the disarmed acceptor **1.119** in the presence $Cu(OTf)_2$/TfOH afforded *trans*-linked disaccharide **1.120** in 70% yield. Disaccharide **1.120** was then glycosylated with acceptor **1,21** in the presence of AgOTf to give the anticipated inverse-patterned *trans-cis*-linked trisaccharide **1.121** in 54% yield (Scheme 1.23).



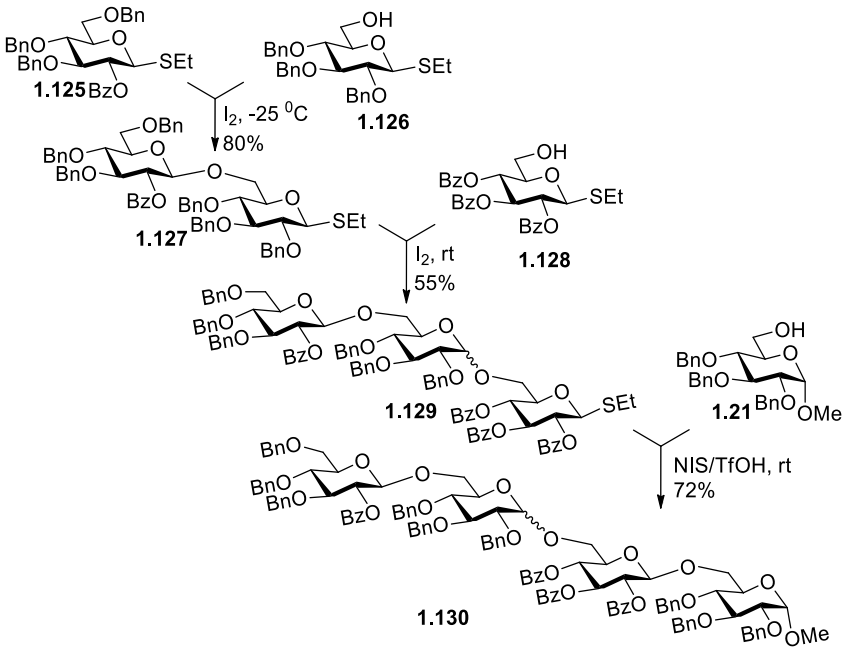
Scheme 1.23. Sequential activation of picolinylated armed → superdisarmed building blocks: synthesis of *trans-cis*-linked trisaccharide 1.121.

The conformational superarming concept has been successively proven by a one-pot glycosylation reaction performed between three building blocks **1.25**, **1.122**, and **1.123,** which were placed in the same reaction vessel from the beginning (Scheme 1.24).[70] In this method, it is essential that all reaction components, not only glycosyl donors (**1.25** and **1.122**), but also glycosyl acceptors (**1.122** and **1.123**) have differential reactivity. The superarmed glycosyl donor **1.25** will be glycosylated with the more reactive primary glycosyl acceptor **1.122**. The resulting disaccharide intermediate with then react with the remaining glycosyl acceptor **1.123** to yield the desired trisaccharide **1.124** in 64% in the one-pot fashion.



Scheme 1.24. Superarmed → armed → disarmed activation for the one-pot synthesis.

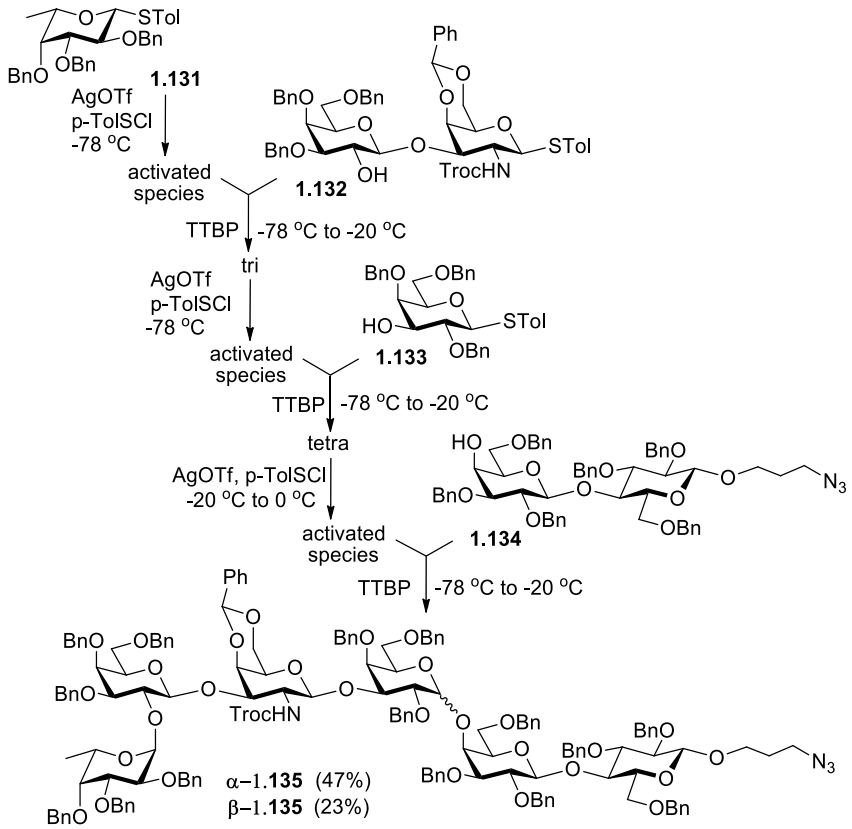
A similar sequence was obtained with the electronically superarmed glycosyl donors, but in this approach a more conventional stepwise synthesis was performed. Thus, disaccharide **1.127** was obtained in 80% yield from the glycosylation between superarmed glycosyl donor **1.125** and armed acceptor **1.126** upon activation with iodine at -25 °C (Scheme 1.25). The resulting disaccharide **1.127** was glycosylated with the disarmed acceptor **1.128** in the presence of iodine at room temperature to afford trisaccharide **1.129** in 55% yield. Finally, trisaccharide **1.129** was glycosylated with glycosyl acceptor **1.21** in the presence of NIS/ TfOH to obtain the desired tetrasaccharide **1.130** composed of the *trans-cis-trans* sequence.



Scheme 1.25. Synthesis of tetrasaccharide with alternating *trans-cis* linkages.

Preactivation concept is independent of the building-block reactivity since the leaving group of the glycosyl donor is first converted into a highly reactive species (preactivation) and then the acceptor is added.[115] Although this approach involves additional steps because the glycosylation herein is a two-step reaction, it offers more flexibility with the leaving and/or protecting groups. A relevant example illustrating this excellent concept in application to the synthesis of the tumor associated carbohydrate antigen Globo H hexasaccharide is shown in Scheme 1.26.[116] The fucosyl donor **1.131** was preactivated at -78 ^{o}C with p-TolSCl/AgOTf and then the first acceptor **1.132** was added along with a sterically hindered base TTBP. The temperature was then raised to -20 ^{o}C to obtain the trisaccharide intermediate. Upon complete disappearance of acceptor **1.132**, the reaction mixture was cooled again to -78 ^{o}C followed by the sequential addition of AgOTf, p-TolSCl, galactose acceptor **1.133**, TTBP and then warming up the

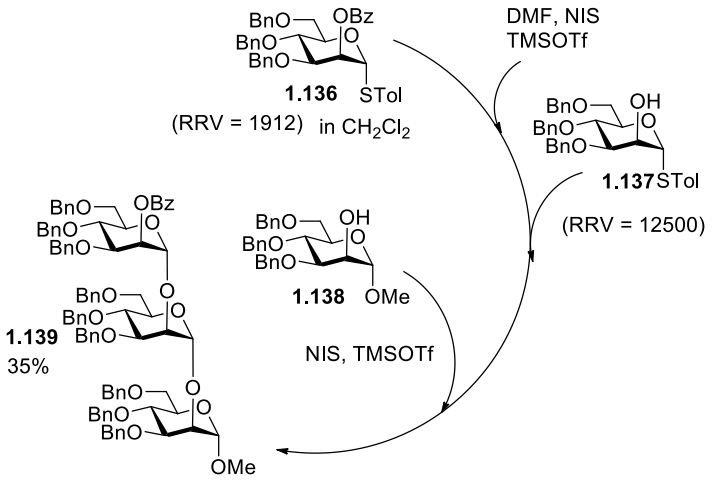
reaction to -20 °C. After complete disappearance of the acceptor **1.133**, the temperature was lowered to -78 °C and the sequence was reiterated for glycosylation of lactoside **1.134**. The resulting Globo H hexasaccharide α-**1.135** was isolated in 47% overall yield based on the four-component one-pot reaction within 7 h.



Scheme 1.26. **One-pot synthesis of Globo H hexasaccharide based on preactivation.**

Mong *et al*. investigated the possibility of using DMF-modulated glycosylation concept[117] in so-called disarmed-armed glycosylation, which is also based on preactivation.[118] In accordance with this approach, the glycosyl donor produces β-glycosyl imidinium triflate in the presence of DMF. It has been shown that dioxalenium ion and β-imidinium triflate are in equilibrium and the acceptor can react with both of these species resulting in an α/β-mixture of glycosides. The β-selectivity is inversely

correlated to the amount of DMF, and pure β-selectivity was achieved with 1.2 equiv of DMF.[117] Since DMF modulated glycosylations induce a preactivation step, this method opens the glycosyl donor to iterative glycosylations.



Scheme 1.27. **DMF modulated disarmed–armed iterative glycosylation**

To demonstrate the applicability of this method in oligosaccharide synthesis several different trisaccharides, including the one-pot synthesis of the α-(1,2)-linked trisaccharide **1.139** shown in Scheme 1.27, were obtained. Gildersleeve *et al.* have shown that the aglycone transfer side reaction may occur when the preactivation method is applied for the glycosylations of armed acceptors with the disarmed donors.[119] A number of approaches including the use of sterically hindered leaving groups (aglycones)[119-121] have been invented to overcome the aglycone transfer in such preactivation glycosylations.

1.8. Conclusions and Outlook

Since the first glycosylation reactions performed in the late 1800s, carbohydrate chemistry has evolved into a broad area of research that has persistently captured the

interest of the scientific community. With recent advances in the rapidly expanding fields of glycosciences, the demand for reliable and stereocontrolled glycosylation methods has increased. Nevertheless, the installation of the glycosidic linkages and the assembly of oligosaccharide sequences remain cumbersome due to the lack of understanding of the mechanistic detail of glycosylation or the inability to translate such knowledge into practical execution. A number of excellent strategies that offer a reasonably efficient route to oligosaccharide assembly have already emerged and the armed-disarmed approach for chemoselective oligosaccharide synthesis is undoubtedly amongst them. The search of new concepts continues, and the field of armed-disarmed glycosylations enjoyed an explosive expansion. New reactivity levels have been revealed and a few new concepts for glycosyl donor activation have been introduced and tested in armed-disarmed strategies.[122-124] Many new sequences can now be achieved directly, but in a majority of application, one needs to take care of protecting groups: protecting groups do more than protect. Although recent advancements discussed in this Chapter have already significantly expanded the scope of the armed-disarmed methodology it is clear that further development of efficient and general methods for the expeditious synthesis of complex carbohydrates will remain an important and active arena for scientific endeavors of the 21st century.

1.9. References

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CHAPTER 2

Conformationally superarmed S-ethyl glycosyl donors as effective building blocks for chemoselective oligosaccharide synthesis in one pot

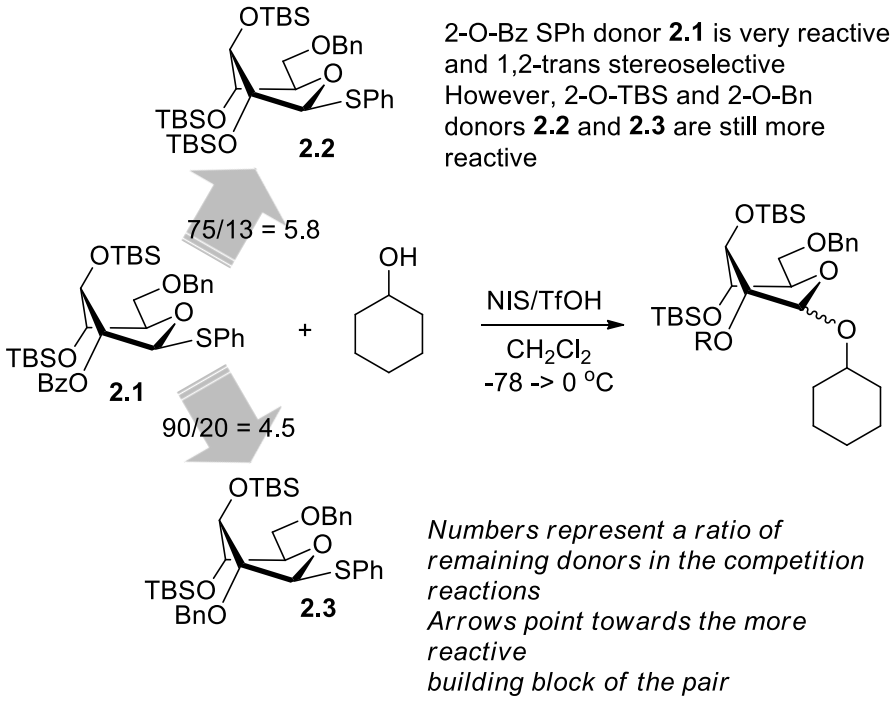
M. D. Bandara, J. P. Yasomanee, N. P. Rath, C. M. Pedersen, M. Bols, and A. V. Demchenko . "Conformationally Superarmed S-Ethyl Glycosyl Donors as Effective Building Blocks for Chemoselective Oligosaccharide Synthesis in One Pot." Organic & Biomolecular Chemistry, **2017**, 15 (3), 559-563.

2.1. Introduction

Mechanistic challenges of the chemical glycosylation reaction have been consistently capturing the attention of the synthetic community.[1-5] Many classes of glycosyl donors have been developed[6,7] and many strategies for oligosaccharide synthesis have emerged.[8-10] Among the methods and strategies available, the development of the armed-disarmed strategy for chemoselective oligosaccharide synthesis occupies an important niche.[11-13] Reactivity tuning of various series of thioglycosides has been reported and applied to the synthesis of a variety of oligosaccharide sequences.[14-19] Beyond the traditional scope of the armed-disarmed strategy, superarmed and superdisarmed building blocks have also been identified and studied.[20] Bols and co-workers developed an approach to superarm glycosyl donors by changing the equatorial-rich $^4C_1$ conformation to an axial-rich conformation.[21-25] These conformational changes were induced by creating steric congestion with *tert*-butyldimethylsilyl (TBS) or related bulky protecting groups at the C-2, 3 and 4 positions of S-phenyl (SPh) glucosides, resulting in a skew-boat conformation. These donors showed a 20-fold increase in reactivity as compared to the armed per-O-benzylated counterparts.[23] The Demchenko group also reported superarmed S-benzoxazolyl (SBox) and S-ethyl (SEt) glycosyl donors, but the superarming was based on the O2/O5-cooperative effect in glycosylation.[26] Thus, it was demonstrated that donors equipped with 2-O-benzoyl-3,4,6-tri-O-benzyl protecting group pattern are 10 times more reactive than their armed counterparts.[27-29]

With the two different approaches to superarm glycosyl donors, our groups jointly developed a 2-O-benzoyl donor **2**.**1** with 3,4-di-O-TBS protection (Scheme 2.1).

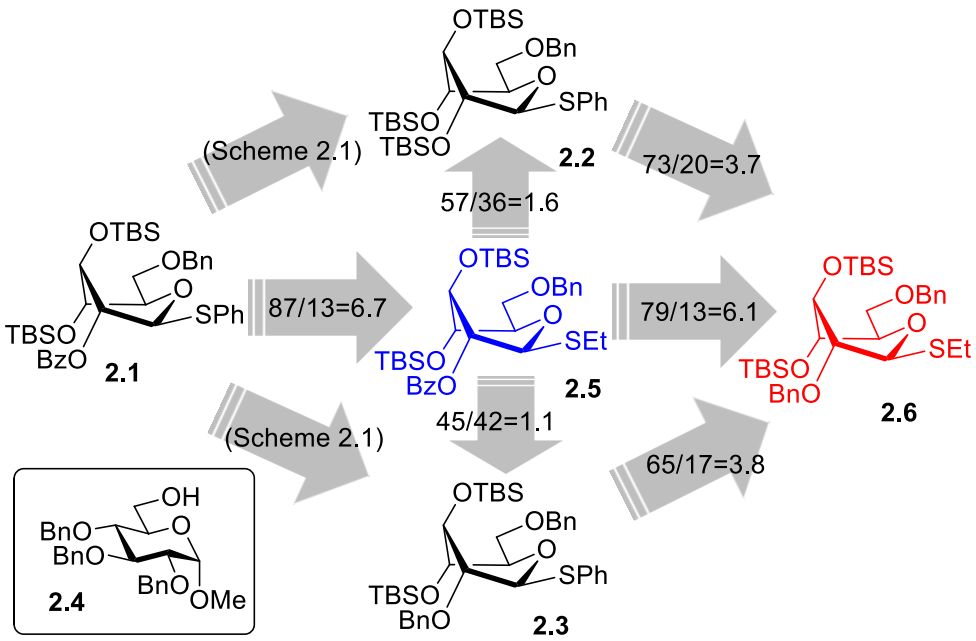
Over the course of that study we learned that conformational arming is a powerful tool to increase the reactivity and achieve excellent yields and the 2-O-benzoyl substituent ensures complete 1,2-trans stereoselectivity.[30] The anchimeric superarming effects in the conformationally modified donor **2.1** are significantly weaker to the extent that 2-O-benzoylated SPh donor **2.1** is 5.8 and 4.5 times less reactive than its 2-O-TBS and 2-O-benzylated counterparts **2.2** and **2.3**, respectively (Scheme 2.1). Although glycosylations with the hybrid donor **2.1** were swift, high yielding and β-stereoselective,[30] we feared that the reduced reactivity could translate into the decreased efficacy of these building blocks in application to sequential chemoselective glycosylations in one-pot. This led us to a hypothesis that the use of a more reactive S-ethyl leaving group[31,32] would help us to develop a complementary superarmed glycosyl donor with a superior reactivity profile while still maintaining β-stereoselectivity.



Scheme 2.1. The relative reactivity of the conformationally superarmed S-phenyl glycosyl donors.[30]

2.2. Results and discussion

Right off the start, when donor **2.1** was subjected to the competition experiment with the equally protected SEt donor **2.5**, a much higher reactivity of the latter has been detected. The competition experiments for this study were conducted following essentially the same experimental conditions and ratios as in our previous study.[30] Two glycosyl donors, used in equimolar amounts (1.0 equiv. each), were set to compete for excess glycosyl acceptor **2.4**[33] (2.0 equiv.) in the presence of NIS (1.0 equiv.) and TfOH (0.1 equiv.) at -78 °C. The use of low temperature, that was allowed to gradually increase over the course of the reaction, and the use of a very limited amount of the promoter helped to maintain workable reaction rates. All competition experiments were quenched after 1 h and the remaining glycosyl donors were isolated and quantified.



Numbers represent a ratio of remaining donors in the competition reactions.
Arrows point towards the more reactive building block of the pair

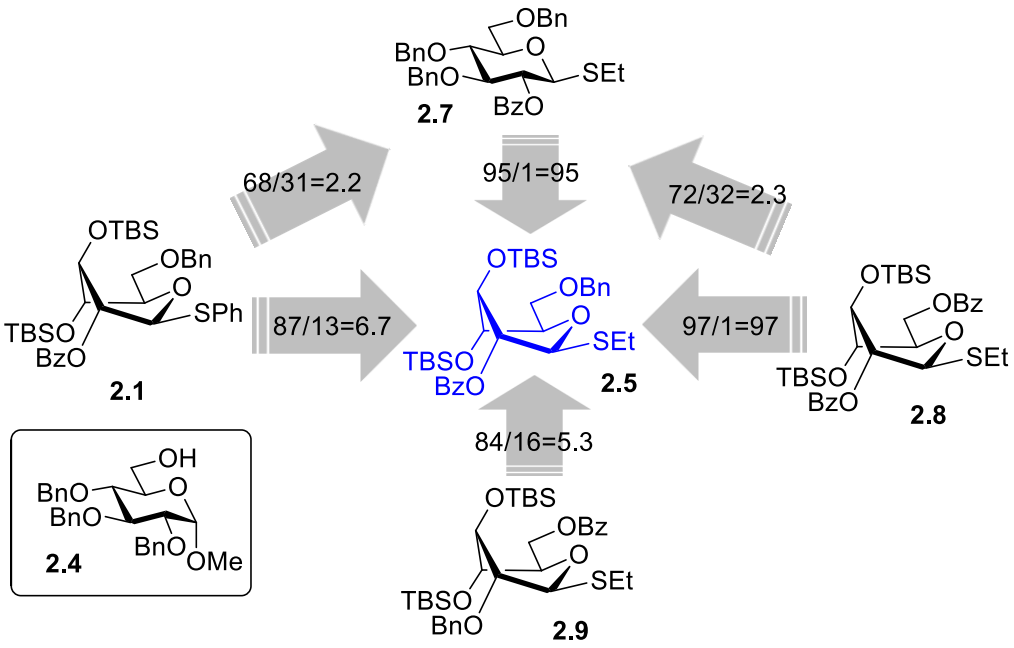
Scheme 2.2. The relative reactivity of S-phenyl versus S-ethyl glycosyl donors of the superarmed series.

Thus, as a result of the first competition experiment, SPh donor **2.1** remained as the major monosaccharide component of the mixture and was isolated in 87% yield, whereas only 13% of SEt donor **2.5** was remaining (Scheme 2.2). This translates into the 1/6.7 reactivity ratio between the two donors, or in other words, the SEt donor **2.5** is 6.7 times more reactive than its SPh counterpart **2.1**.

Subsequent competition experiments led to a realization that 2-O-benzoyl SEt donor **2.5** is nearly as reactive as 2-O-benzyl SPh donor **2.3** (1/1.1) and even only slightly less reactive than the most reactive superarmed 2-O-TBS protected SPh donor **2.2** known to date (1/1.6, Scheme 2). To explore the reactivity limits of superarmed glycosyl donors of the SEt series, we obtained donor **2.6** equipped with 2-O-benzyl protecting group. The competition experiment with equally protected SPh donor **2.3** led to a realization of the higher reactivity of donor **2.6** (**2,3**/**2.6** = 1/3.8). Along similar lines, we determined that donor **6** is 3.7 times more reactive than the 2-O-TBS SPh donor **2.2**.[30]

Being encouraged by the first series of comparative experiments, we decided to investigate the new hybrid donor **2.5** in the context of other SEt donors. For this study we obtained anchimerically superarmed derivative **2.7** along with two conformationally superarmed donors equipped with 6-O-benzoyl and 2,6-di-O-benzoyl protections, **2.8** and **2.9**, respectively. The first competition experiment between donors **2.5** and **2.7** provided a very impressive reactivity difference: donor **2.5** was 95 times more reactive that donor **2.7** (Scheme 2.3). This result is more indicative of the superior reactivity of **2.5** rather than poor reactivity of **2.7**. The latter is still superarmed because it is much more reactive than its per-benzylated counterpart. In addition, donor **2.7** is 2.2 times more reactive than the previously developed hybrid SPh donor **2.1**. Moreover, compound **2.7** is also 2.3

more reactive than the conformationally superarmed SEt donor **2.8** equipped with two benzoyl groups at O-2 and O-6.



Numbers represent a ratio of remaining donors in the competition reactions.
Arrows point towards the more reactive building block of the pair

Scheme 2.3. The effect of the conformational and electronic superarming in the series of S-ethyl glycosyl donors.

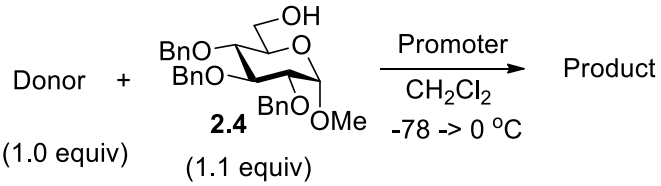
A comparison of donors **2.5** and **2.8** showed a very significant deactivating effect of 6-O-benzoyl in comparison with 6-O-benzyl, the only structural difference between the two donors.[34] Thus, 6-O-benzyl donor **2.5** was 97 times more reactive than its 6-O-benzoylated counterpart **2.8**. Donor **2.5** was also found to be 5.3 times more reactive than donor **2.9** with the reverse positioning of benzyl and benzoyl substituents: 2-O-benzyl, 6-O-benzoyl.

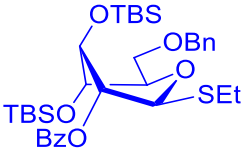
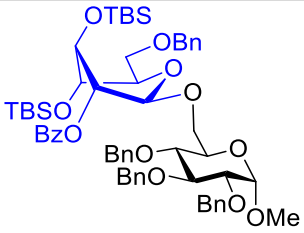
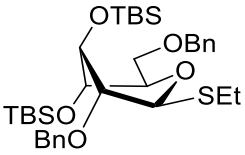
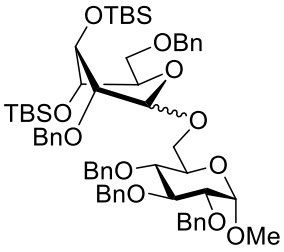
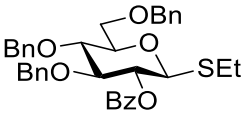
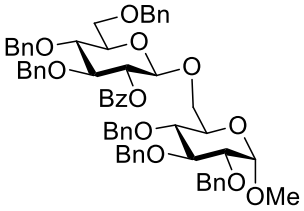
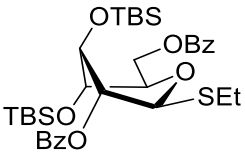
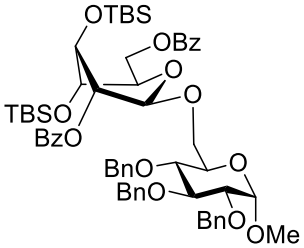
With this comprehensive set of competitive experiments, we began investigating the glycosyl donor properties of compounds **2.5**-**2.8** with the model acceptor **2.4**. After screening a number of promoters for the activation of thioglycosides,

we chose NIS/TfOH and DMTST. These reaction conditions offered a good balance of reactivity, selectivity and yields. Other promoters, including iodine that was successfully used in our previous study of the anchimerically superarmed SEt donors, let to decreased yieds resulting from high rates of major side reactions: TBS cleavage and/or SEt hydrolysis. Thus, NIS/TfOH-promoted coupling between donor **2.5** and acceptor **2.4** swiftly (20 min) produced disaccharide **2.10** in 83% yield and complete β-stereoselectivity (entry 1, Table 2.1). Practically the same outcome was achieved in the DMTST-promoted reaction listed in entry 2. NIS/TfOH-promoted activation of donor **2.6** gave disaccharide **2.11** in 81% yield (entry 3). In this case, the reaction was non-stereoselective due to the absence of neighboring group participation. DMTST was less effective and TBS groups showed high propensity to cleavage. As a result, disaccharide **2.11** was obtained in a poor yield of 21% (entry 4). The outcome of this reaction could be improved (44% yield) using only a slight excess DMTST (1.3 equiv.).

Glycosidation of the anchimerically superarmed donor **2.7** was successful in case of either NIS/TfOH or DMTST-promoted activations. Disaccharide **2.12** was obtained with complete β-stereoselectivity in 82 or 85% yield, respectively (entries 5 and 6). In case of donor **2.8**, only NIS/TfOH gave a practical result, whereas DMTST showed a high level of competing processes. Thus, NIS/TfOH-promoted activation of **2.8** produced disaccharide **2.13** in 85% yield and complete β-stereoselectivity (entry 7). The conformational properties of disaccharide **2.10** were studied by X-ray crystallography (Figure 2.1). The crystals of **2.10** were obtained by slow evaporation of aq. MeOH. The skew-boat conformation of disaccharide **2.10** was deduced from the X-ray data and was consistent with altered coupling constants obtained from its ^{1}H NMR spectrum.

Table 2.1. Glycosylation of acceptor 2.4 with different superarmed SEt donors.



entry	donor	conditions,[a] time	product (yield,% α/β ratio)
1	 **2.5**	A, 20 min	 **2.10** (83, β-only)
2	**2,5**	B, 15 min	**2.10** (85, β-only)
3	 **2.6**	A, 15 min	 **2.11** (81, 1.0/1)
4	**2.6**	B, 10 min	**2.11** (21, 0.9/1)[b]
5	 **2.7**	A, 30 min	 **2.12** (82, β-only)
6	**2.7**	B, 20 min	**2.12** (85, β-only)
7	 **2.8**	A, 30 min	 **2.13** (85, β-only)

[a] – Conditions A: NIS/TfOH (1.3 equiv), 3 Å mol sieves; B: DMTST (2.0 equiv), 4 Å mol sieves; [b] - the yield is impacted by fair stability of the TBS groups (see text)

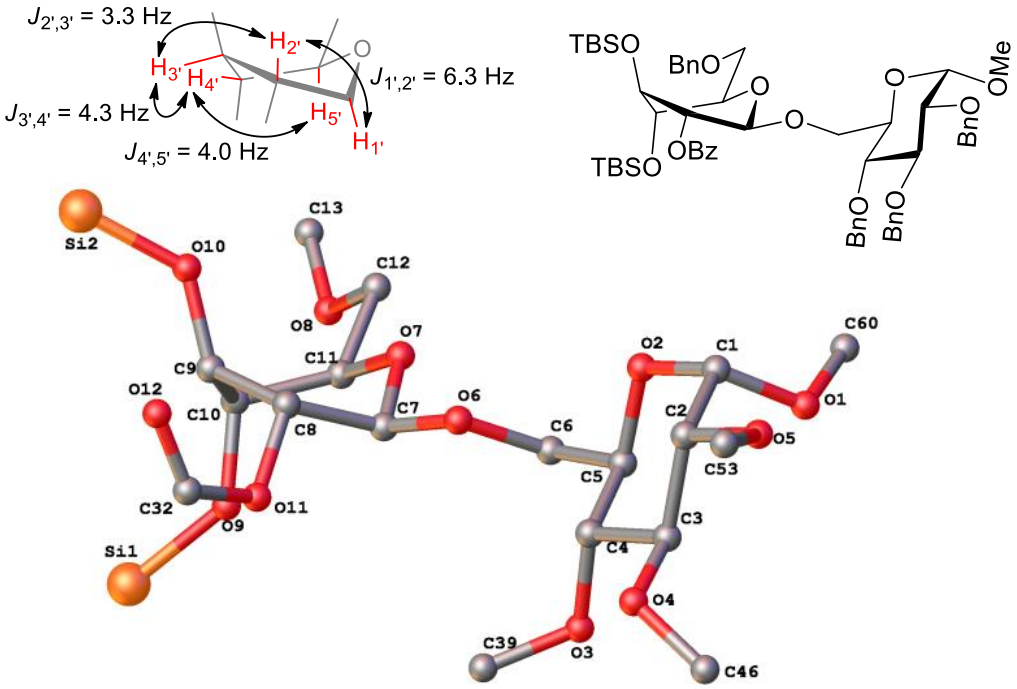
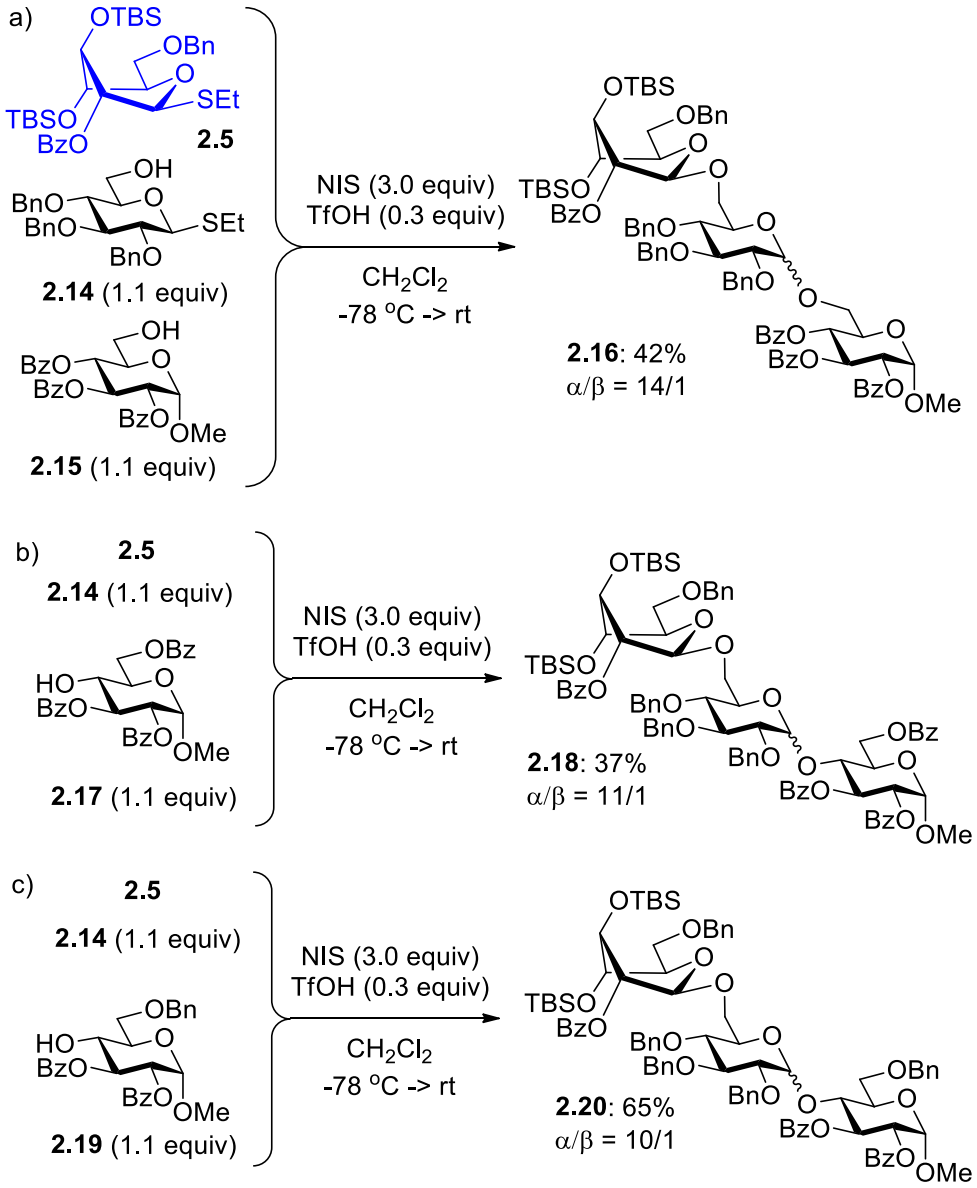


Figure 2.1. The X-ray structure of disaccharide 2.10 (hydrogens and protecting groups have been omitted for clarity)

With a series of glycosyl donors of differential reactivity, we began studying the applicability of this method to the one-pot oligosaccharide synthesis.[35] With a number of different concepts for the one-pot synthesis, we chose one-pot/one-addition method, the pure fashioned approach wherein all building blocks are present from the beginning. Invented by Kahne,[36] and further explored by Fraser-Reid[37] and Bols[21], this approach requires fine tuning of reactivity for differentiation of all reaction components. The general idea underpinning this approach is that the more reactive donor will react with the more reactive acceptor (hydroxyl). Subsequently, the second-step coupling will involve the coupling between the less reactive donor with the less reactive acceptor.

With these considerations, we chose highly reactive donor **2.5** to couple with the reactive 6-OH in benzylated building block **2.14** equipped with the anomeric SEt group. Fast first-step reaction will permit the sequential (rather than competitive) activation of the SEt leaving group of the intermediate disaccharide in reaction with the less reactive acceptor **2.15** (Scheme 2.4).

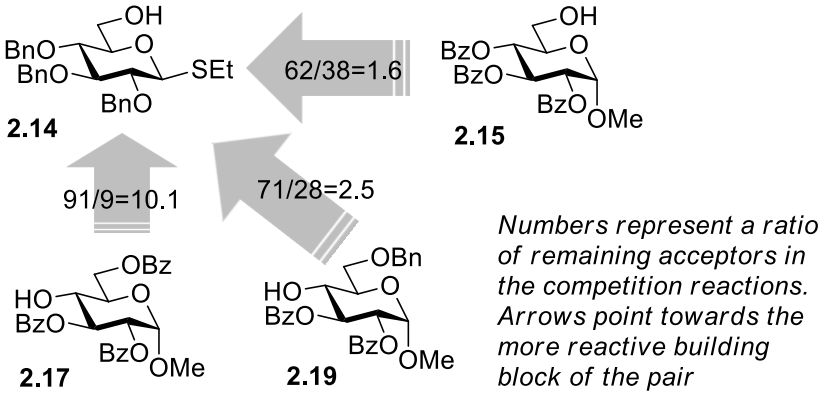


Scheme 2.4. One-pot one-addition synthesis of trisaccharides 2.16, 2.18 and 2.20.

Building block **2.14** is the key reaction component in the mixture because it is set to react both as the more reactive acceptor and then as the less reactive donor. The role of the highly reactive superarmed donor is also essential to ensure that the first coupling step is swift. The synthesis of trisaccharide **2.16** was conducted from building blocks **2.5**, **2.14** and **2.15** that were mixed together and NIS/TfOH were added. As a result, compound **2.16** was obtained in one pot in 42% yield and high stereoselectivity (α/β = 14/1, Scheme 2.4). A substantial quantity of the cross-coupled disaccharide resulting from the reaction between **2.5** and **2.15** indicated that the reactivity difference between primary hydroxyls in **2.15** and **2.16** is insufficient to ensure effective one-pot coupling. A simple competitive experiment set up between the two acceptors and donor **2.5** showed that **2**.**14** is only 2.1.6 times reactive than **2.15** (Scheme 2.5).

To improve the outcome of the one-pot synthesis we prepared secondary acceptor **2.17** that was deactivated by surrounding benzoyl substituents.[38,39] The competition experiment showed that primary acceptor **2.14** is 10.1 times more reactive than its secondary counterpart **2.17** (Scheme 2.5). Theorizing that this reactivity difference will be sufficient, we set up the synthesis of trisaccharide **2.18** from building blocks **2.5**, **2.14** and **2.17** that were mixed and NIS/TfOH were added. As a result, trisaccharide **2.18** was obtained in one pot in 37% yield and high stereoselectivity (α/β = 11/1, Scheme 2.4). No cross-coupled disaccharide was found in the reaction mixture, but attempts to push the reaction to completion promoted competitive TBS group hydrolysis. In a further search of suitable building blocks for the one-pot synthesis, we obtained acceptor **2.19** benzylated at C-6. This acceptor is only 2.5 times less reactive than its primary counterpart **2.14** (Scheme 2.5). Nevertheless, this reactivity difference was sufficient for

the synthesis of trisaccharide **2.20** in a good yield of 65% and high stereoselectivity (α/β = 10/1, Scheme 2.4) showing the utility of this approach and also the necessity to fine-tune all reaction components.



Scheme 2.5. The relative reactivity of glycosyl acceptors 2.14, 2.15, 2.17 and 2.19.

2.3. Conclusions

We have developed a series of superarmed SEt glycosyl donors that were applied to stereoselective glycosylations and multi-step oligosaccharide synthesis in one-pot. Further application of these highly reactive compounds to glycosylation of various glycosyl acceptors in solution and on solid supports is currently underway in our laboratories.

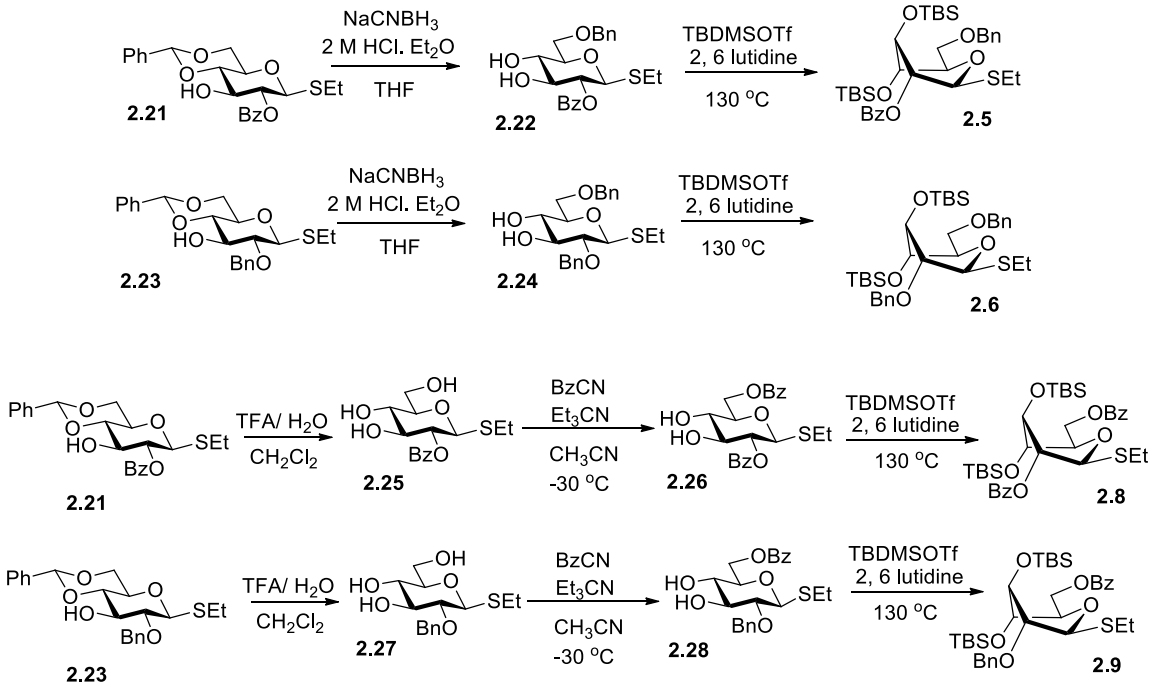
3.4 Experimental

3.4.1 General methods

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV

light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ^{o}C. CH_2Cl_2 was distilled from CaH_2 directly prior to application. Acetonitrile was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 or 4Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured using a polarimeter. ^{1}H NMR spectra were recorded at 300 or 600 MHz, ^{13}C NMR spectra were recorded at 75 MHz or 150 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual $CHCl_3$ (δ_H = 7.24 ppm). The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.23 ppm). HRMS determinations were made with the use of a mass spectrometer with FAB ionization and ion-trap detection.

3.4.2 Preparation of glycosyl donors 2.1, 2.2, 2.3, 2.5, 2.6, 2.8, 2.9



Scheme 2.6. Preparation of glycosyl donors 2.5, 2.6, 2.8, 2.9.

Ethyl 2-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (2.22). A mixture of ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-**D**-glucopyranoside (**2.21**,[40] 2.56 g, 6.15 mmol) and molecular sieves (3 Å, 3.0 g) in dry THF (80 mL) was stirred under argon for 1 h at rt. $NaCNBH_3$ (5.15 g, 81.8 mmol) and a 2 M solution of HCl in diethyl ether (40.9 mL, 81.8 mmol) were added and the resulting mixture was stirred for 30 min at rt. After that, the volatiles were removed under reduced pressure and the residue was diluted with CH_2Cl_2 (~150 mL). The solid was filtered off through a pad of Celite and rinsed successively with CH_2Cl_2. The combined filtrate (~250 mL) was washed with water (50 mL), sat. aq. $NaHCO_3$ (50 mL), and water (3 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound in 82% yield (2.11 g, 5.04 mmol) as a white amorphous solid. Analytical data for **2.22**: R_f = 0.25 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{27}$ -54.4 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.21 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.68 (m, 2H, CH_2CH_3), 3.48 (m, 1H, H-5), 3.65-3.78 (m, 3H, H-3, 4, 6b), 3.90 (s, 1H, OH), 3.82 (s, 1H, OH), 4.51 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.55 (br. s, 2H, CH_2Ph), 5.06 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 7.29-8.04 (10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.2, 24.1, 70.1, 71.8, 73.0, 73.7, 76.7, 78.7, 83.3, 127.9 (×3), 128.5 (×4), 129.7, 130.1 (×2), 133.4, 137.7, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{22}H_{26}NaO_6S^+$ 441.1348, found 441.1334.

Ethyl 2,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (2.24). A mixture of ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-**D**-glucopyranoside (**2.23**,[41] 1.58 g, 3.92 mmol) and molecular sieves [42] in dry THF (65 mL) was stirred under argon for 1 h at rt. $NaCNBH_3$

(3.29 g, 52.0 mmol) and a 2 M solution of HCl in diethyl ether (26.1 mL, 52.0 mmol) were added and the resulting mixture was stirred for 30 min at rt. After that, the volatiles were removed under reduced pressure and the residue was diluted with CH_2Cl_2 (~50 mL). The solid was filtered off through a pad of Celite and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and water (3 x 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound in 98% yield (1.65 g, 4.07 mmol) as a white amorphous solid. Analytical data for **2.24**: R_f = 0.25 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{26.6}$ -28.7 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.32 (t, 3 H, *J* = 7.4 Hz, CH_2CH_3), 2.76 (m, 2H, CH_2CH_3), 3.05 (s, 1H, OH), 3.26 (dd, 1H, $J_{2,3}$ = 8.6 Hz, H-2), 3.41-3.62 (m, 3H, H-3, 4, 5), 3.67-3.79 (m, 2H, H-6a, 6b), 4.46 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 4.57 (dd, 2H, ^2J = 13.7 Hz, CH_2Ph), 4.81 (dd, 2H, ^2J = 10.9 Hz, CH_2Ph), 7.19-7.50 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.3, 25.3, 70.6, 72.0, 73.8, 75.3, 77.8,78.2, 80.9, 85.0, 127.9 (×2), 128.0, 128.3, 128.5 (×2), 128.7 (×2), 128.8 (×2), 137.9, 138.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{22}H_{28}NaO_5S^+$ 427.1554, found 427.1555.

Ethyl 2-*O*-benzoyl-1-thio-β-D-glucopyranoside (2.25). Compound **2.21** (0.71 g, 1.70 mmol) was dissolved in a mixture of trifluoroacetic acid in wet CH_2Cl_2 (20 mL, 2/0.2/17.8, v/v/v) and the resulting mixture was stirred for 30 min at rt. After that, the reaction mixture was neutralized with trimethylamine (~3 mL) and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on

silica gel (methanol - dichloromethane gradient elution) to afford the title compound in 86% yield (0.48 g, 1.46 mmol) as a white amorphous solid. Analytical data for **2.25**: R_f = 0.27 (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{19.9}$ -8.1 (c = 1, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ, 1.18 (t, 3H, *J* = 7.5 Hz, CH_2CH_3), 2.70 (m, 2H, CH_2CH_3), 3.38-3.47 (m, 2H, H-5, 4), 3.64-3.73 (m, 2H, H-3, 6a), 3.89 (dd, 1H, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 1.8 Hz, H-6b), 4.65 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.97 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 7.45-8.00 (m, 5H, aromatic) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ, 15.2, 24.9, 62.8, 71.6, 74.5, 77.4, 82.2, 84.6, 129.5 (×2), 130.7 (×2), 131.5, 134.3, 167.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{15}H_{20}NaO_6S^+$ 351.0879, found 351.0875.

Ethyl 2-*O*-benzyl-1-thio-β-D-glucopyranoside (2.27). Compound **2.23** (0.55 g, 1.36 mmol) was dissolved in a mixture of trifluoroacetic acid in wet CH_2Cl_2 (20 mL, 1.5/0.2/14.8, v/v/v) and the resulting mixture was stirred for 30 min at rt. After that, the reaction mixture was neutralized with trimethylamine (~2.5 mL) and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound in 85% yield (0.36 g, 1.16 mmol) as a white amorphous solid. Analytical data for **2.27**: R_f = 0.35 (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{21.5}$ -45.4 (c = 1, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ, 1.17 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.65 (m, 2H, CH_2CH_3), 3.07 (dd, 1H, $J_{2,3}$ = 8.8 Hz, H-2), 3.11-3.26 (m, 2H, H-4, 5), 3.39 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 3.54 (dd, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.74 (dd, 1H, $J_{5,6b}$ = 2.1 Hz, H-6b), 4.36 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 4.70 (s, 2H, CH_2Ph), 7.10-7.32 (m, 5H, aromatic) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ, 15.5, 25.5, 63.0, 71.8, 76.2, 79.8, 82.0, 82.9, 85.9, 128.8, 129.3

(×2), 129.5 (×2), 139.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{15}H_{22}NaO_5S^+$ 337.1086, found 337.1082.

Ethyl 2,6-di-*O*-benzoyl-1-thio-β-D-glucopyranoside (2.26). A solution of BzCN (0.08 g, 0.81 mmol) in dry CH_3CN (10.0 mL) was added dropwise to a solution of **2.25** (0.19 g, 0.58 mmol) and triethyl amine (6.0 mL) in dry CH_3CN (10.0 mL), and the resulting mixture was stirred under argon for 3 h at -30 °C. MeOH (~1 mL) was added and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound in 70% yield (0.175 g, 0.41 mmol) as a white amorphous solid. Analytical data for **2.26**: R_f = 0.37 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{26.6}$ -7.7 (c = 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.24 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.71 (m, 2H, CH_2CH_3), 3.16 (d, 1H, *J* = 3.9 Hz, OH), 3.52 (d, 1H, *J* = 3.1 Hz, OH), 3.57-3.72 (m, 2H, $J_{5,6b}$ = 4.2 Hz, H-4, 5), 3.84 (ddd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.57 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, 6a), 4.63 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.74 (dd, 1H, H-6b), 5.11 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2), 7.51-8.04 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.2, 24.3, 63.9, 70.7, 73.0, 76.7, 78.2, 83.6, 128.7 (×4), 129.6, 129.7, 130.1 (×2), 130.2 (×2), 133.6, 133.7, 166.5, 167.5 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{22}H_{24}NaO_7S^+$ 455.1141, found 455.1145.

Ethyl 6-*O*-benzoyl-2-*O*-benzyl-1-thio-β-D-glucopyranoside (2.28). A solution of BzCN (0.138 g, 1.02 mmol) in dry CH_3CN (15.0 mL) was added dropwise to a solution of **2.27** (0.305 g, 0.970 mmol) and triethylamine (10.0 mL) in dry CH_3CN (15.0 mL) at -30 °C and the resulting mixture was stirred under argon for 3 h at that temperature. After that,

MeOH (1.0 mL) was added to the reaction mixture and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford the title compound in 75% yield (0.30 g, 0.727 mmol) as a white amorphous solid. Analytical data for **2.28**: R_f = 0.34 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21.5}$ -31.3 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.30 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.75 (m, 2H, CH_2CH_3), 3.11 (br. s, 1H, OH), 3.26 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 3.39-3.56 (m, 3H, H-4, 5, OH), 3.58-3.64 (m, 1H, H-3), 4.48 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 4.51-4.60 (m, 2H, H-6a, 6b), 4.80 (dd, ^2J = 10.9 Hz, CH_2Ph), 8.73-7.89 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.3, 25.3, 64.3, 70.2, 75.3, 77.8, 78.0, 80.9, 85.1, 128.3, 128.5 (×2), 128.6 (×2), 128.8 (×2), 129.8, 130.0 (×2), 138.0, 133.4, 167.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{22}H_{24}NaO_7S^+$ 441.1347, found 441.1346.

Phenyl 2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-lucopyranoside (2.1). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[30]

Phenyl 6-*O*-benzyl-2,3,4-tri-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.2). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[22]

Phenyl 2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.3). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[30]

Ethyl 2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.5). TBSOTf (2.7 mL, 12.0 mmol) was added to a solution of **2.22** (1.67 g, 4.0 mmol) in 2,6-lutidine (12.0 mL) and the resulting mixture was heated at 130 ^{o}C for 1 h. After that, the reaction mixture was allowed to cool to rt, diluted with ethyl acetate (~250 mL), and washed with 1 M aq. HCl (3 × 50 mL), water (50 mL), sat. aq. $NaHCO_3$ (50 mL), and brine (2 x 50 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound in 92% yield (2.35 g, 3.64 mmol) as a colorless syrup. Analytical data for **2.5**: R_f = 0.71 (ethyl acetate/ toluene, 1/9, v/v); $[\alpha]_D^{26.9}$ -3.7 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.05, 0.06, 0.07, 0.08 (4 s, 12H, 2 x $SiMe_2$), 0.83, 0.85 (2 s, 18H, 2 x Si^{t}Bu), 1.25 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.71 (m, 2H, CH_2CH_3), 3.65 (dd, 1H, $J_{6a,6b}$ = 9.7 Hz, H-6a), 3.74-3.82 (m, 2H, H-4, 6b), 3.84-3.91 (m, 2H, $J_{5,6a}$ = 6.6 Hz, H-3, 5), 4.58 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 4.85 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 5.13 (dd, 1H, $J_{2,3}$ = 4.1 Hz, H-2), 7.22-8.04 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.2, -4.0, -3.6, -3.4, 0.2, 15.2, 18.2 (×2), 24.6 (×3), 26.1 (×3), 71.0, 71.5, 73.6, 74.5, 75.8, 81.6, 81.7, 127.8, 127.8 (×2), 128.5 (×4), 130.2 (×2), 130.3, 133.3, 138.5, 165.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{34}H_{54}NaO_6SSi_2^+$ 669.3078, found 669.3064.

Ethyl 2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.6). TBSOTf (1.05 mL, 4.60 mmol) was added to a solution of **2.24** (0.85 g, 1.53 mmol) in 2,6-lutidine (5.0 mL) and the resulting mixture was heated at 130 ^{o}C for 1 h. After that, the reaction mixture was allowed to cool to rt, diluted with ethyl acetate (~100 mL) and washed with 1 M aq. HCl (3 × 20 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL) and brine (2 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound in 90% yield (0.87g, 1.37 mmol) as a colorless syrup. Analytical data for **2.6**: R_f = 0.75 (ethyl acetate/ toluene, 1/9, v/v); $[\alpha]_D^{26.8}$ -26.4 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.04, -0.01, 0.01, 0.03 (4 s, 12H, 2 x $SiMe_2$), 0.80, 0.84 (2 s, 18H, 2 x Si^{t}Bu), 1.26 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.69 (m, 2H, CH_2CH_3), 3.33 (dd, 1H, $J_{2,3}$ = 3.6 Hz, H-2), 3.56 (dd, 1H, $J_{5,6a}$= 6.6 Hz, $J_{6a,6b}$ = 9.6 Hz, H-6a), 3.62-3.84 (m, 4H, H-3, 4, 5, 6b), 4.51 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 4.51 (dd, 2H, ^2J = 10.9 Hz, CH_2Ph), 4.74 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 7.17-7.37 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.2, -3.9, -3.5, -3.4, 15.3, 18.2 (×2), 25.3, 26.1 (×3), 26.3 (×3), 71.3, 71.7, 73.4 (×2), 76.5, 81.3, 82.7, 82.8, 127.6, 127.7, 127.8 (×4), 128.3 (×2), 128.5 (×2), 138.6, 138.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{34}H_{56}NaO_5SSi_2^+$ 655.3285, found 655.3291.

Ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (2.7). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[43]

Ethyl 2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.8). TBSOTf (1.13 mL, 4.93 mmol) was added to a solution of 2.26 (0.71 g, 1.64 mmol) in 2,6-lutidine (4.0 mL) and the resulting mixture was heated at 130 ^{o}C for 1 h. After that, the reaction mixture was allowed to cool to rt, diluted with ethyl acetate (~100 mL) and washed with 1 M aq. HCl (3 × 15 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and brine (2 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound in 89% yield (0.96 g, 1.46 mmol) as a colorless syrup. Analytical data for 2.**8**: R_f = 0.75 (ethyl acetate/ toluene, 1/9, v/v); $[\alpha]_D^{26.9}$ -0.3 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.02, -0.01, -0.01, 0.00 (4 s, 12H, 2 x $SiMe_2$), 0.75, 0.79 (2 s, 18H, 2 x Si^{t}Bu), 1.12 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.58 (m, 2H, CH_2CH_3), 3.75 (dd, 1H, $J_{4,5}$ = 5.4 Hz, H-4), 3.84-3.91 (m, 2H, $J_{3,4}$ = 5.4 Hz, $J_{5,6a}$ = 7.0 Hz, $J_{5,6b}$ = 4.6 Hz, H-3, 5), 4.36 (dd, 1H, $J_{6a,6b}$ = 11.4 Hz, H-6a), 4.60 (dd, 1H, H-6b), 4.78 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ = 4.9 Hz, H-2), 7.40-8.05 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.2, -3.9, -3.4, -3.2, 15.2, 18.2 (×2), 24.8, 26.1 (×3), 26.2 (×3), 65.2, 71.5, 74.1, 75.6, 79.7, 81.9, 128.5 (×2), 128.6 (×2), 129.8 (×4), 130.2 (×2), 130.3, 133.3, 165.7, 166.5 ppm. HR-FAB MS $[M+Na]^+$ calcd for $C_{34}H_{52}NaO_7SSi_2^+$ 683.2870, found 683.2877.

Ethyl 6-*O*-benzoyl-2-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.9). TBSOTf (0.30 mL, 1.28 mmol) was added to a solution of **2.28** (0.18 g, 0.429 mmol) in 2,6-lutidine (3.0 mL) and the resulting mixture was heated at 130

°C for 1 h. After that, the reaction mixture was allowed to cool to rt, diluted with ethyl acetate (~60 mL) and washed with 1 M aq. HCl (3 × 5 mL), water (5 mL), sat. aq. $NaHCO_3$ (5 mL) and brine (2 x 5 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound in 86% yield (0.24g, 0.37 mmol) as a colorless syrup. Analytical data for **2.9**: R_f = 0.75 (ethyl acetate/ toluene, 1/9, v/v); $[\alpha]_D^{21.6}$ -0.8 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.05-0.02 (4 s, 12H, 2 x $SiMe_2$), 0.80, 0.82 (2 s, 18H, 2 x Si^{t}Bu), 1.18 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.62 (m, 2H, CH_2CH_3), 3.32 (dd, 1H, $J_{2,3}$ = 4.0 Hz, H-2), 3.71 (dd, 1H, H-4), 3.78-3.86 (m, 2H, $J_{3,4}$ = 5.0 Hz, H-3, 5), 4.32 (dd, 1H, $J_{5,6a}$ = 7.4 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.52 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, H-6b), 4.65 (dd, ^2J = 11.1 Hz, CH_2Ph), 4.74 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 7.10 – 8.01 (m, 10H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.2, -3.8, -3.3, -3.2, 15.3, 18.2, 18.3, 25.4, 26.1 (×3), 26.4 (×3), 65.6, 71.7, 73.6, 76.5, 79.4, 82.5, 82.9, 127.6, 127.8 (×2), 128.3 (×2), 128.5 (×2), 129.8 (×2), 130.2, 133.2, 138.5, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{34}H_{54}NaO_6SSi_2^+$ 669.3077, found 669.3087.

2.4.3 Synthesis of glycosyl acceptors 2.4, 2.14, 2.15, 2.17, 2.19

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.4). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[33,44]

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (2.14). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[45]

Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (2.15). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[46]

Methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (2.17). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[47]

Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (2.19). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[48]

2.4.4. General procedures for competition experiments

Donor competition experiments. A mixture of two glycosyl donors (0.035 mmol each), glycosyl acceptor **2.4** (0.071 mmol), and freshly activated molecular sieves (3 Å, 150 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 16 h at rt. The mixture was cooled to -78 °C, NIS (0.035 mmol) and TfOH (0.0035 mmol) were added, and the resulting mixture was stirred under argon for 1 h. During this time, the temperature of the reaction mixture was allowed to gradually increase to 0 °C. After that, triethylamine (~ 0.1 mL) was

added, the solid was filtered off, and rinsed successively with CH_2Cl_2. The combined filtrate (~60 mL) was washed with sat. aq. $NaHCO_3$ (5 mL), 10% aq. $Na_2S_2O_3$ (5 mL) and water (3 x 10 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) and the amount of unreacted donors quantified.

Acceptor competition experiments. A mixture of two glycosyl acceptors (0.042 mmol each), glycosyl donor **2.5** (0.038 mmol), and freshly activated molecular sieves (3 Å, 150 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 16 h at rt. The mixture was cooled to -78 °C, NIS (0.038 mmol) and TfOH (0.0038 mmol) were added, and the resulting mixture was stirred under argon for 1 h. During this time, the temperature of the reaction mixture was allowed to gradually increase to 0 °C. After that, triethylamine (~0.1 mL) was added, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~60 mL) was washed with sat. aq. $NaHCO_3$ (5 mL), 10% aq. $Na_2S_2O_3$ (5 mL) and water (3 x 10 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) and the amount of unreacted acceptors quantified.

2.4.5. Synthesis of disaccharides

Method A. General glycosylation procedure in the presence of NIS/TfOH. A mixture of glycosyl donor (0.039 mmol), glycosyl acceptor (0.043 mmol), and freshly activated molecular sieves (3Å, 60 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 16 h at rt. The mixture was cooled to -78 °C, NIS (0.051 mmol) and TfOH (0.0039 mmol) were

added, and the resulting mixture was stirred under argon for 15-30 min (see Table 1 of the article). During this time, the temperature of the reaction mixture was allowed to increase gradually. After that, triethylamine (~0.1 mL) was added, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~60 mL) was washed with sat. aq. $NaHCO_3$ (5 mL), 10% aq. $Na_2S_2O_3$ (5 mL) and water (3 x 10 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the corresponding disaccharide derivative.

Method B. General glycosylation procedure in the presence of DMTST. A mixture of glycosyl donor (0.039 mmol), glycosyl acceptor (0.043 mmol), and freshly activated molecular sieves (4Å, 60 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 16 h at rt. The mixture was cooled to -78 °C, DMTST (0.051-0.078 mmol, see Table 1 of the article) was added, and the resulting mixture was stirred under argon for 10-20 min (see Table 1 of the article). During this time, the temperature of the reaction mixture was allowed to increase gradually. After that, triethylamine (~0.1 mL) was added, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~60 mL) was washed with sat. aq. $NaHCO_3$ (5 mL), 10% aq. $Na_2S_2O_3$ (5 mL) and water (3 x 10 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the corresponding disaccharide derivative.

Methyl 6-*O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.10). The title compound was prepared from donor **2.5** and acceptor **2.4** by Method A or B in 83 or 85% yield, respectively, as a colorless crystalline solid. Analytical data for **2.10**: R_f = 0.43 (ethyl acetate/ toluene, 1/9, v/v); m.p. 111-112.5 °C (methanol/water); $[\alpha]_D^{26.9}$ +3.2 (c = 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 0.01, 0.02, 0.04, 0.12 (4 s, 12H, 2 x $SiMe_2$), 0.84 (s, 18H, 2 x Si^{t}Bu), 3.24 (s, 3H, OCH_3), 3.44-3.49 (m, 2H, $J_{2,3}$ = 9.3 Hz, H-2, 4), 3.67-3.73 (m, 3H, H-5, 6a, 6a′), 3.77 (dd, 1H, $J_{6a',6b'}$ = 9.7 Hz, H-6b′), 3.82 (dd, 1H, $J_{4',5'}$ = 4.0 Hz, H-4′), 3.86 (dd, 1H, $J_{3',4'}$ = 4.3 Hz, H-3′), 3.91 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 3.95 (m, 1H, $J_{5',6a'}$ = 3.6 Hz, $J_{5',6b'}$ = 6.4 Hz, H-5′), 4.16 (br. d, 1H, J = 8.9 Hz, H-6b), 4.49 (dd, 2H, ^2J = 10.7 Hz, CH_2Ph), 4.50 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.53 (dd, 2H, ^2J = 10.8 Hz, CH_2Ph), 4.67 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.82 (dd, 2H, ^2J = 11.0 Hz, CH_2Ph), 4.94 (d, 1H, $J_{1',2'}$ = 6.3 Hz, H-1′), 5.11 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, H-2′), 7.07-7.99 (m, 25H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, -4.3, -4.1 (×2), -3.8, 18.1, 18.2, 26.0 (×6), 55.2, 67.9, 69.8, 71.1, 71.2, 73.5, 73.6, 75.1 (×2), 75.7 (×2), 77.6, 79.5, 79.9, 82.2, 98.2, 100.2, 127.6, 127.7 (×4), 127.8, 128.0 (×5), 128.3 (×2), 128.4 (×3), 128.5 (×4), 128.6 (×2), 130.0 (×2), 130.1, 133.1, 138.4 (×2), 138.5, 139.1, 165.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{80}NaO_{12}Si_2^+$ 1071.5085, found 1071.5066.

Methyl 6-*O*-(2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.11). The title compound was prepared from donor **6** and acceptor **2.4** by Method A or B in 81 or 21% yield, respectively, as a colorless syrup. Selected analytical data for α-**11**: R_f = 0.50 (ethyl acetate/ toluene, 1/9,

v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 3.28 (s, 3H, OCH_3), 3.29 (dd, 1H, H-2′) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, 96.7 (C-1), 98.1 (C-1′) ppm; Selected analytical data for β-**2.11**: R_f = 0.50 (ethyl acetate/ toluene, 1/9, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 3.36 (s, 3H, OCH_3) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, 98.2 (C-1), 102.6 (C-1′) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{82}NaO_{11}Si_2^+$ 1057.5293, found 1057.5288.

Methyl *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.12). The analytical data of **2.12** was in accordance with that previously described.[49]

Methyl 6-*O*-(2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.13). The title compound was prepared from donor **2.8** and acceptor **2.4** by Method A in 85% yield as a colorless syrup. Analytical data for **2.13**: R_f = 0.60 (ethyl acetate/ toluene, 1/9, v/v); $[\alpha]_D^{26.9}$ +12.5 (c = 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 0.01, 0.05, 0.10, 0.14 (4 s, 12H, 2 x $SiMe_2$), 0.84, 0.86 (2 s, 18H, 2 x Si^{t}Bu), 3.16 (s, 3H, OCH_3), 3.44 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 3.46 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 3.64-3.67 (m, 2H, H-5, 6a), 3.85 (dd, 1H, $J_{4',5'}$ = 3.7 Hz, H-4'), 3.88 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 3.93 (dd, 1H, $J_{3',4'}$ = 3.7 Hz, H-3′), 4.08 (m, 1H, H-5), 4.14 (d, 1H, $J_{6a,6b}$ = 9.3 Hz, H-6b), 4.49 (dd, 2H, ^{2}J = 10.9 Hz, CH_2Ph), 4.50 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.57-4.59 (m, 2H, H-6a′, 6b′), 4.66 (dd, 2H, ^{2}J = 11.8 Hz, CH_2Ph), 4.82 (dd, 2H, ^{2}J = 10.9 Hz, CH_2Ph), 4.91 (d, 1H, $J_{1',2'}$ = 4.4 Hz, H-1′), 5.06 (dd, 1H, $J_{2',3'}$ = 4.4 Hz, H-2′), 7.11-8.05 (m, 25H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, -4.4, -4.1 (×2), -3.8, 18.2 (×2), 26.0 (×6), 29.9, 55.0, 65.6, 67.8, 69.7, 70.7, 73.5, 73.7, 74.6, 75.1,

75.7, 77.6, 79.9, 82.2, 98.0, 100.0, 127.6, 127.8, 128.0 (×5), 128.3 (×2), 128.4 (×2), 128.5 (×4), 128.6 (×4), 129.8 (×2), 130.0, 130.1 (×2), 130.4, 133.2 (×2), 138.4 (×2), 139.2, 165.5, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{78}NaO_{13}Si_2^+$ 1085.4878, found 1085.4913.

3.4.6. One-pot one-addition trisaccharide synthesis

General procedure. A mixture of glycosyl donor **2.5** (0.038 mmol), glycosyl donor/acceptor **2.14** (0.038 mmol), glycosyl acceptor **2.15**, **2.17** or **2.19** (0.042 mmol), and freshly activated molecular sieves (3 Å, 150 mg) in CH_2Cl_2 (2.0 mL) was stirred under for 16 h at rt. The mixture was cooled to -78 °C, NIS (0.116 mmol) and TfOH (0.0116 mmol) were added, and the resulting mixture was stirred under argon for 5 h. During this time, the temperature of the reaction mixture was allowed to gradually increase to rt. After that, triethylamine (~0.1 mL) was added, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~60 mL) was washed with sat. aq. $NaHCO_3$ (5 mL), 10% aq. $Na_2S_2O_3$ (5 mL) and water (3 x 10 mL). The organic layer was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on Sephadex LH-20 (methanol – dichlorometane, 1/1, v/v) to afford the corresponding trisaccharide derivative **2.16**, **2.18** or **2.20**.

Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (2.16). The title compound was prepared from building blocks **2.5**, **2.14**, and **2.15** in accordance with the general procedure in 42% yield as a

colorless syrup. Analytical data for α-**2.16**: R_f = 0.46 (ethyl acetate/ toluene, 1/9, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, -0.01, 0.03, 0.05, 0.12 (4 s, 12H, 2 x $SiMe_2$), 0.80, 0.83 (2 s, 18H, 2 x Si^{t}Bu), 3.35-3.39 (m, 4H), 3.40 (d, 1H), 3.48 (dd, 1H), 3.56 (t, 1H), 3.60-3.66 (m, 2H), 3.72 (dd, 1H), 3.76-3.82 (m, 2H), 3.86-3.89 (m, 1H), 3.90-3.93 (m, 1H), 4.06 (dd, 1H), 4.15 (d, 1H), 4.23-4.28 (m, 1H), 4.39-4.47 (m, 3H), 4.52-4.58 (m, 2H), 4.67 (dd, 3H), 4.85 (d, 1H), 4.92 (d, 1H), 5.05 (dd, 2H), 5.20 (dd, 2H), 5.48 (t, 1H), 7.95-6.12 (m, 40H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, -4.3, -4.1 (×2), -3.8, 18.2 (×2), 26.1 (×6), 29.9, 55.9, 67.8, 68.7, 69.0, 70.1, 70.7, 70.9, 71.2, 72.4, 73.4, 74.5, 74.6, 75.0, 75.1, 75.2, 75.6, 79.0, 82.4, 84.7, 96.9, 100.1, 104.2, 127.8 (×2), 127.9 (×2), 128.2 (×2), 128.4 (×7), 128.5 (×6), 128.6 (×3), 129.2, 129.3, 129.5, 129.9 (×2), 130.1 (×2), 130.1 (×2), 130.1 (×2), 133.1, 133.23, 133.5, 138.2, 138.6, 138.8, 139.0, 165.4, 165.6, 166.0 (×2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{87}H_{104}NaO_{19}Si_2^+$ 1545.6400, found 1545.6368.

Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (2.18). The title compound was prepared from building blocks **2.5**, **2.14** and **2.17** in accordance with the general procedure in 37% yield as a colorless syrup. Analytical data for α-2.**18**: R_f = 0.55 (ethyl acetate/ toluene, 1/9, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, -0.03, 0.00, 0.03, 0.08 (4 s, 12H, 2 x $SiMe_2$), 0.81 (s, 18H, 2 x Si^{t}Bu), 3.24 (dd, 1H), 3.40 (s, 3H), 3.45-3.50 (m, 1H), 3.64-3.56 (m, 2H), 3.70-3.76 (m, 1H), 3.79-3.89 (m, 4H), 3.95 (d, 1H), 4.05 (d, 1H), 4.19 (dd, 3H), 4.31 (d, 1H), 4.39 (d, 1H), 4.48-4.71 (m, 6H), 4.79 (d, 1H), 4.84 (d, 1H), 5.05 (m, 1H), 5.08 (d,

1H), 5.12 (d, 1H), 5.18 (dd, 1H), 6.18 (dd, 1H), 7.01-8.04 (m, 40H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, -4.4, -4.3, -4.2, -4.0, 18.1, 18.2, 26.0(×6), 29.9, 55.5, 63.5, 67.6, 68.9, 70.8, 71.0, 71.4, 72.3, 72.4, 73.1, 73.5, 75.0, 75.3, 75.4, 75.5, 75.8, 79.1, 79.8, 81.4, 96.9, 98.9, 99.9, 127.4, 127.6, 127.7 (×4), 127.9 (×4), 128.0 (×2), 128.3 (×4), 128.4 (×4), 128.5 (×2), 128.6 (×4), 129.9 (×2), 130.00 (×2), 130.2 (×2), 133.0, 133.1, 133.3, 133.4, 138.2, 138.4, 138.6, 139.1, 165.3, 165.6, 166.1, 166.2 ppm. HR-FAB MS $[M+Na]^+$ calcd for $C_{87}H_{104}NaO_{19}Si_2^+$ 1545.6400, found 1545.6366.

Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (2.20). The title compound was prepared from building blocks **2.5**, **2.14** and **2.19** in in accordance with the general procedure in 65% yield as a colorless syrup. Analytical data for α-**18**: R_f = 0.60 (ethyl acetate/ toluene, 1/9, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 0.01, 0.05, 0.05, 0.12 (4 s, 12H, 2 x $SiMe_2$), 0.83, 0.84 (2s, 18H, 2 x Si^{t}Bu), 3.18 (dd, 1H), 3.41 (s, 3H), 3.51 (dd, 1H), 3.65-3.73 (m, 2H), 3.76 (dd, 1H), 3.79-3.92 (m, 6H), 3.92-4.05 (m, 4H), 4.20-4.27 (m, 2H), 4.35 (d, 1H), 4.46 (s, 2H), 4.50 (dd, 2H), 4.54-4.63 (m, 3H), 4.87 (d, 1H), 4.97 (d, 1H), 5.07 (d, 1H), 5.07 (d, 1H), 5.15-5.22 (m, 2H), 6.11 (dd, 1H), 7.04-7.94 (m, 40H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, -4.4, - 4.3, -4.2, -4.0, 18.1, 18.2, 26.0 (×6), 29.9, 55.5, 67.9, 68.7, 70.4, 70.8, 71.1 (×2), 72.3, 72.5, 72.7, 73.4, 73.5, 74.9, 75.0, 75.3, 75.4, 75.8, 79.4, 79.8, 81.5, 97.0, 98.4, 100.0, 127.7 (×2), 127.8 (×3), 127.9 (×2), 128.0 (×2), 128.1 (×2), 128.4 (×11), 128.5 (×2), 129.4, 129.9 (×2), 130.0 (×2), 130.1 (×2), 130.3, 133.0,

133.1, 133.4, 138.3, 138.4, 138.5, 138.6, 139.1, 165.3, 165.9, 166.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{87}H_{106}NaO_{118}Si_2^+$ 1531.6608, found 1531.6576.

2.4.7. X-ray structure determination of disaccharide 2.10

A crystal of approximate dimensions 0.324 x 0.121 x 0.098 mm^3 was mounted on MiTeGen cryoloops in a random orientation. 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 15 seconds/frame at a crystal to detector distance of 4.0 cm. The collected frames were integrated using an orientation matrix determined from the narrow frame scans. Apex II and SAINT software packages (*Bruker Analytical X-Ray, Madison, WI, 2010*) 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 (*Bruker Analytical X-Ray, Madison, WI, 2010*) based on the Laue symmetry using equivalent reflections.

Crystal data and intensity data collection parameters are listed in Table 1S. Structure solution and refinement were carried out using the SHELXTL- PLUS software package.[50] The structure was solved by direct methods and refined successfully in the

space group $P2_1$. Full matrix least-squares refinements were carried out by minimizing $\Sigma w(Fo^2-Fc^2)^2$. The non-hydrogen atoms were refined anisotropically to convergence. All hydrogen atoms were treated using appropriate riding model (AFIX m^3). The final residual values and structure refinement parameters are listed in Table 1S. Several motifs are disordered in this structure. The disorder was modeled with partial occupancy atoms and rigid body restraints (RIGU). Absolute structure determination was confirmed with a Flack x of -0.02(11).

Complete listings of positional and isotropic displacement coefficients for hydrogen atoms, anisotropic displacement coefficients for the non-hydrogen atoms are listed as supplementary material (Tables 2S and 4S). Table of calculated and observed structure factors are available in electronic format. The structural data have been deposited with Cambridge Crystallographic Data center with the CCDC number 1509777.

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CHAPTER 3

The chemical synthesis of human milk oligosaccharides: lacto-N-neotetraose (Galβ1→4GlcNAcβ1→3Galβ1→4Glc)

M. D. Bandara, K. J. Stine, and A. V. Demchenko. The chemical synthesis of human milk oligosaccharides: lacto-N-neotetraose (Galβ1→4GlcNAcβ1→3Galβ1→4Glc). Carbohydr. Res., 2019, 483, 107743; DOI: 10.1016/j.carres.2019.107743

3.1. Introduction

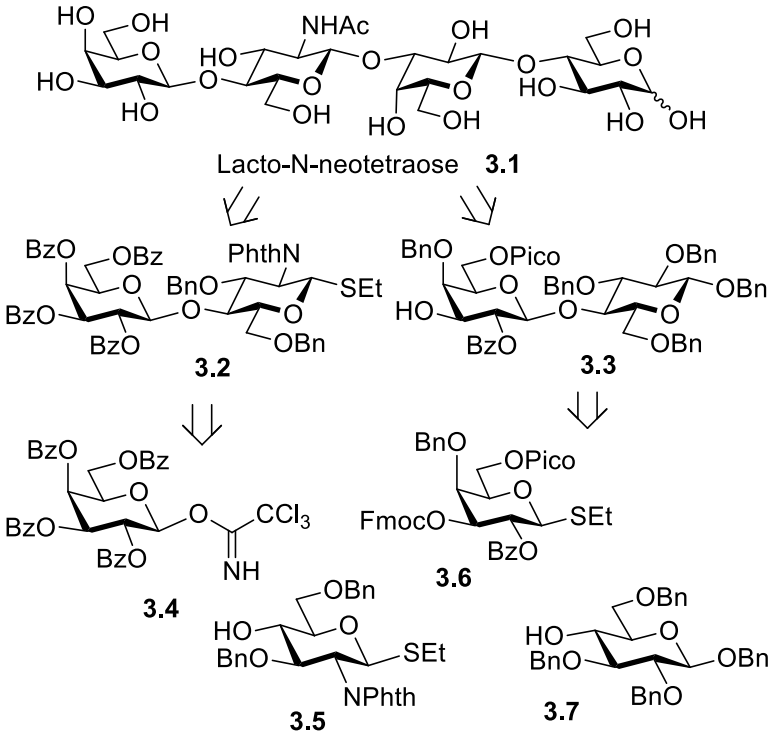
Carbohydrates are involved in many processes and are referred to as the "*essential molecules of life*".[1] Our life begins with fertilization, which takes place via carbohydrate-protein recognition.[2] Our journey with sugars continues with human milk that becomes the ideal first food.[3] Glycans present in human milk can provide prebiotic effects,[4] function as antimicrobial agents,[5-13] and provide necessary nutrients for the development of the brain and cognition of infants.[14] Thanks to advances in glycosciences, we already know that Human Milk Oligosaccharides (HMO) are a unique and diverse family of glycans.[15-17] Structures of 162 HMO have been elucidated,[18-22] but our understanding of how HMO function is far from complete and is severely hampered by the lack of simple, efficient and cost effective methods for synthesizing glycans. Despite many efforts to prepare HMO enzymatically[23-42] or chemically,[43-54] their availability in pure form remains poor. Adding HMO to infant formulas could be beneficial for infants' health,[55-58] but HMO are challenging to produce and purify, and exact roles of individual HMO remain unknown.[59-61] Only two simple glycans have been approved for infant formulas in the US and Europe, and three more HMO entered clinical trials.[62]

One of the two approved HMO structures is lacto-N-neotetraose **3.1** (LNnT), which is a linear tetrasaccharide comprising a Galβ1→4GlcNAcβ1→3Galβ1→4Glc sequence shown in Scheme 3.1. More specifically, LNnT contains lactose disaccharide (Galβ1→4Glc) at the reducing end elongated by N-acetyllactosamine (Galβ1→4GlcNAc).

3.2. Results and discussion

During the incipient stage of the synthesis of LNnT **3.1**, we decided to perform a convergent (2+2) synthetic strategy. Thus, per our retrosynthetic analysis, we chose the

protected lactosamine donor **3.2**, containing a suitable leaving group (LG), and the regioselectively protected lactose acceptor **3.3**. The latter was designed as a universal precursor for the synthesis of other HMO sequences elongated at C-2 and/or C-6 of the galactose unit. The access to those sequences is enabled by temporary protecting groups, benzoyl and picoloyl (Pico). Another key aspect in our design is the use of benzyl groups as semi-permanent protecting groups, whereas a majority of previous syntheses used acetyl groups that are prone to migration leading to side products.

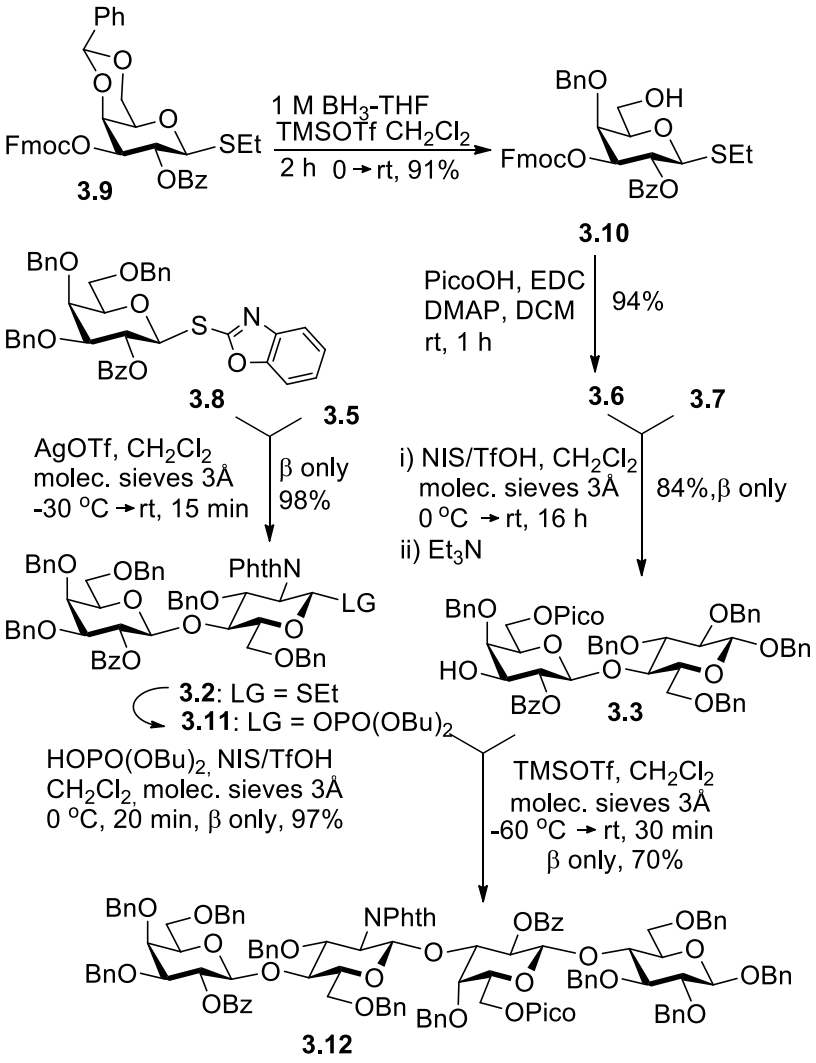


Scheme 3.1. Retrosynthesis analysis of LNnT 3.1.

The projected synthesis of lactosamine donor **3.2** involved coupling of galactosyl trichloroacetimidate donor **34** with glucosamine acceptor **3.5**. To achieve the synthesis of lactose precursor **3.3**, we projected the coupling between the orthogonally protected galactose donor **3.6** and tetrabenzylated glucose 4-OH acceptor **3.7**. The protecting groups

in donor **3.6** were chosen to provide access to the universal lactose acceptor precursor that would be suitable for glycosylation at C-3 via Fmoc removal (like in case of acceptor **3.3**), C-6 via OPico removal, or provide access to 3,6-branched HMO sequences.

For the synthesis of lactosamine building block **3.2** we obtained known trichloroacetimidate donor **3.4**.[63] Also known glucosamine thioglycoside acceptor **3.5** was obtained following a seven-step protocol similar to that previously described method.[64] Unfortunately, coupling between donor **3.4** and acceptor 3.**5** in the presence of TMSOTf was not straightforward as anticipated. Instead of the desired product **3.2**, the major product was tetrabenzoylated ethylthio galactoside, presumably generated through an aglycone transfer side reaction. Gildersleeve *et al.* has done a careful mechanistic study on the aglycone transfer reactions.[65] According to their mechanistic picture, several factors play a role in determining whether transfer occurs. One is the electronic, armed or disarmed, nature of the donor and the acceptor. Based on that, Gildersleeve *et al.* hypothesized that any thioglycoside could undergo transfer as long as the oxacarbenium ion or the glycosyl intermediate derived from the thioglycoside is more stable than the oxacarbenium ion derived from the glycosyl donor. In further investigation of our coupling reaction, we came to realization that the aglycone transfer practically always takes place in reactions between disarmed galactose donors, regardless of the leaving group, and glucosamine acceptor **3.5**. The same issue was reported by Wang,[31] and the solution was found in applying galactosyl bromide as the donor that produced the respective lactosamine disaccharide with moderate yield of 70%.



Scheme 3.2. Convergent synthesis of protected LNnT 3.12.

In order to eliminate the aglycone transfer side reaction and to obtain the desired disaccharide **3.2** in high yield, we first investigated whether galactose trichloroacetimidate donor[66] equipped with the superarming 2-benzoyl-3,4,6-tri-benzyl pattern[67,68] would be advantageous. This reaction indeed produced disaccharide **3.2**, and no aglycone transfer was detected in this case. However, multiple by-products have been detected that resulted in only a fair yield of disaccharide **3.2**. At this point we decided to screen other leaving

groups and determined that the superarmed S-benzoxazolyl (SBox) thioimidate **3.8**[67,68] provides a superior combination of simplicity, efficiency and yields for the synthesis of lactosamine, which is a major constituent in many HMO. Thus, selective activation of the SBox leaving group in glycosyl donor **3.8** over thioethyl anomeric moiety of glycosyl acceptor **3.5** was achieved in the presence of silver trifluoromethanesulfonate (AgOTf). This glycosylation afforded the desired β-linked lactosamine disaccharide **3.2** in excellent yield of 98% (Scheme 3.2).

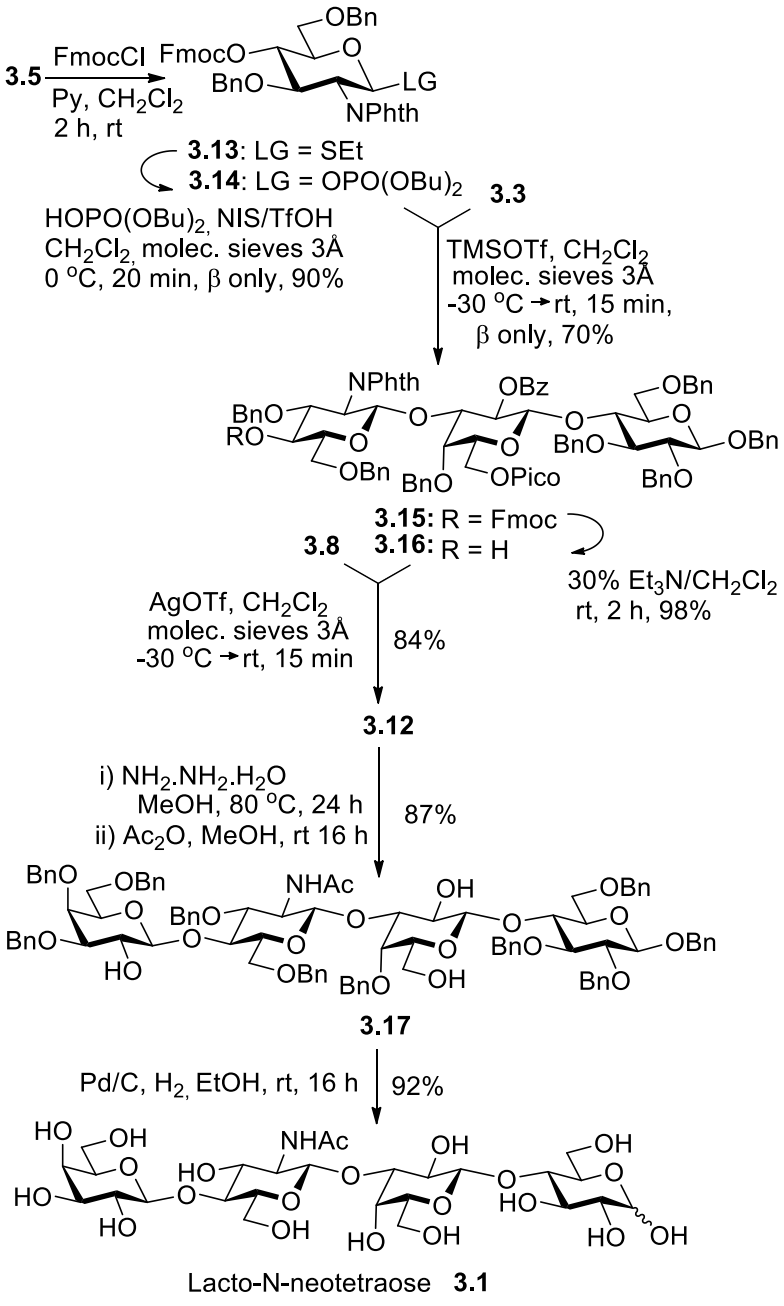
Having achieved success in the synthesis of lactosamine disaccharide **3.2**, we turned our attention to the synthesis of lactose derivative **3.3**. The synthesis of galactose donor **3.6** started from known precursor **3.9**[69] that was converted to the intermediate **3.10** via the reductive regioselective opening of the benzylidene acetal by reaction with 1 M BH_3 in THF in the presence of catalytic TMSOTf in 91% yield. Subsequently, the 6-hydroxyl derivative **3.10** was reacted with picolinic acid (PicoOH) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) to afford 6-picoloyl derivative **3.6** in 94% yield. The synthesis of the 4-OH acceptor **3.7** followed previously reported procedure.[70] The coupling of donor **3.6** and acceptor **3.7** was carried out by the activation of the thioethyl leaving group with *N*-iodosuccinimide (NIS) and TfOH in the presence of molecular sieves (3 Å). Subsequent deprotection of the Fmoc group with triethyl amine (Et_3N) in one pot led to the desired disaccharide acceptor **3.3** in 84% yield.

With the key disaccharide building blocks **3.2** and **3.3** in hand, we began to assemble the target tetrasaccharide sequence using the convergent strategy. The coupling of the disaccharide donor **3.2** bearing the SEt leaving group was very sluggish, and despite

all attempts to push the reaction to completion, significant amounts of both the donor and the acceptor remained. To increase the reactivity of the glycosyl donor counterpart, we converted thioglycoside **3.2** into a phosphate donor **3.11** in 97% yield via a one-step protocol developed by Seeberger.[71] The coupling of phosphate donor **3.11** with lactose acceptor **3.2** was then conducted in the presence of TMSOTf as depicted in Scheme 3.2. As a result, tetrasaccharide **3.14** was obtained in a good yield of 70% with complete β-stereoselectivity.

With a very minimal strategic adjustment to our synthetic scheme, we were also able to investigate whether the linear synthetic approach is able to offer any advantage for the synthesis of the target tetrasaccharide sequence. For this purpose, we converted building block **3.5** into its 4-*O*-Fmoc derivative **3.13** that was subsequently converted into phosphate donor **3.14**. The linear synthesis started by glycosylation between donor **3.14** and lactose acceptor **3.3** in the presence of TMSOTf to afford the intermediate trisaccharide **3.15** in 70% yield. The Fmoc protecting group was removed with 30% Et_3N in CH_2Cl_2 and glycosylation of the resulting trisaccharide acceptor **3.16** with SBox donor **3.8** in the presence of AgOTf afforded the desired β-linked tetrasaccharide **3.12** in 87% yield.

With the key tetrasaccharide intermediate **3.12** which was obtained via both convergent and linear synthesis methods, we then endeavored a series of deprotection steps to obtain the target LNnT tetrasaccharide **3.1**. Deprotection of the phthalimido and the ester groups was achieved by refluxing with NH_2NH_2-H_2O in MeOH, and the treatment with acetic anhydride in MeOH furnished tetrasaccharide **3.17** in 87% yield. Finally, the remaining benzyl groups were removed by hydrogenation the presence of 10% palladium on charcoal in wet ethanol to obtain the target trisaccharide **3.1** in 92% yield.



Scheme 3.3. The linear synthesis of tetrasaccharide 3.12 and its deprotection to obtain LNnT 3.1.

3.3. Conclusions

In summary, the total synthesis of lacto-N-neotetraose has been completed using both linear and convergent synthesis approaches. Both approaches employed the universal

lactose building block **3.3** and offered similar efficiency. In accordance with the convergent approach, lactosamine building block **3.11** needed for glycosylation of acceptor **3.3** was obtained in three steps from monosaccharide building blocks **3.5** and **3.8** followed by the introduction of the phosphate leaving group. As a result, the convergent assembly of tetrasaccharide **3.12** was achieved in 66% yield over three steps. The linear synthesis of **3.12** involved glycosylation of acceptor **3.3** with donor **3.14**, interim Fmoc deprotection, followed by glycosylation with **3.8**. As a result, the linear assembly of tetrasaccharide **3.12** was also achieved in three synthetic steps in 57% yield overall. Along the way, we have developed new synthetic protocols for different glycosidic linkages. Notably, the donor and acceptor protecting group and the leaving group combinations were found to be of paramount significance to successful coupling. The discovery of innovative methods and accessible technologies that will offer new capabilities for obtaining individual HMO will help to improve understanding their roles and boost practical applications. Further synthetic studies of HMO are underway in our laboratory.

3.4. Experimental

3.4.1. General methods

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh) and Sephadex G-25 size exclusion resin, reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ^{o}C. CH_2Cl_2 was distilled

from CaH_2 directly prior to application. Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured using a Jasco polarimeter. ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 151 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual TMS (δ_H = 0.00 ppm) for solutions in $CDCl_3$ or the signal of the residual D_2O (δ_H = 4.79 ppm) for solutions in D_2O. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.16 ppm) for solutions in $CDCl_3$ or the central signal of CD_3COCD_3 δ_C = 29.84 ppm) for solutions in D_2O. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer.

3.4.2. Preparation of monosaccharide building blocks 3.10, 3.6, 3.13. 3.14

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside (3.10). A 1 M solution of BH_3 in THF (48 mL, 48 mmol) was added to a solution of ethyl 2-*O*-benzoyl-3-*O*-fluorenylmethoxycarbonyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[69] (6.15 g, 9.62 mmol) in CH_2Cl_2 (50 mL). The resulting mixture was cooled to 0 ºC, TMSOTf (0.87 mL, 4.81 mmol) was added, and the resulting mixture was stirred under argon 2 h. During this time, the reaction was allowed to gradually increase to rt. After that, the reaction was quenched with Et_3N (~2 mL) and MeOH (~5 mL), and the resulting mixture was concentrated *in vacuo.* The residue was diluted with CH_2Cl_2 (~500 mL) and washed with sat. aq. $NaHCO_3$ (50 mL) and water (2 x 50 mL). The

organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white foam in 91% yield (5.61 g, 8.75 mmol). Analytical data for **3.10**: R_f = 0.27 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22}$ +31.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.23 (t, 3H, *J* = 7.5 Hz, CH_2CH_3), 2.75 (m, 2H, CH_2CH_3), 3.56 (m, 1H, *J* = 5.4, 8.4, 11.2 Hz, H-6a), 3.67 (m, 1H, *J* = 6.1 Hz, H-5), 3.85 (m, 1H, H-6b), 4.04 (br d, 1H, H-4), 4.08 (d, 1H, *J* = 7.4 Hz, $OCOCH_2CH$), 4.29 (m, 2H, $OCOCH_2CH$), 4.60 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.67 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 5.07 (dd, 1H, $J_{3,4}$ = 2.9 Hz, H-3), 5.76 (t, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 7.03-8.10 (m, 18H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.9, 24.0, 46.5, 61.7, 68.5, 70.3, 73.5, 74.9, 79.0, 79.2, 84.0, 120.1, 125.0, 125.2, 125.4, 127.2 (×2), 127.9, 128.2, 128.3 (×2), 128.6 (×4), 129.1, 129.6, 130.0, 133.4, 137.6, 141.2, 141.3, 142.9, 143.3, 154.6, 165.4 ppm; ESI TOF LCMS $[M+NH_4]^+$ calcd for $C_{37}H_{40}NO_8S$ 658.2475, found 658.2477.

Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-picoloyl-1-thio-β-D-galactopyranoside (3.6). Picolinic acid (1.21 g, 9.83 mmol), EDC (0.90 g, 9.83 mmol), and DMAP (0.18 g, 1.31 mmol) were added to a stirring solution of **310** (4.20 g, 6.55 mmol) in CH_2Cl_2 (60 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~500 mL) and washed with water (50 mL), 1% aq. HCl (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white form in 94% yield (5.30 g, 6.14 mmol). Analytical data for **3.6**: R_f = 0.41 (ethyl

acetate/hexane, 3/2, v/v); $[\alpha]_D^{23}$ +36.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.22 (t, 3H, *J* = 7.5 Hz, CH_2CH_3), 2.73 (m, 2H, CH_2CH_3), 4.06 (m, 2H, H-5, $OCOCH_2CH$), 4.15 (d,1H, H-4), 4.28 (m, 2H, $OCOCH_2CH$), 4.47 (dd, 1H, $J_{5,6a}$ = 11.2 Hz, $J_{6a,6b}$ = 6.5 Hz, H-6a), 4.61 (dd, 1H, H-6b), 4.68 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1) 4.73 (dd, 2H, ^2J = 11.4 Hz, CH_2Ph), 5.13 (dd, 1H, $J_{3,4}$ = 2.8 Hz, H-3), 5.79 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 7.00-8.82 (m, 22H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.0, 24.1, 46.5, 63.8, 68.5, 70.3, 73.6, 75.2, 76.0, 79.0, 83.9, 120.1, 125.0, 125.2, 125.4, 127.2 (×3), 127.9 (×2), 128.0, 128.5 (×5), 128.6 (×2), 129.5, 130.0 (×2), 133.4, 137.1, 137.4, 141.2, 141.3, 142.8, 143.3, 147.6, 150.1, 154.6, 164.7, 165.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{43}H_{39}NNaO_9S$ 768.2243, found 768.2247.

Ethyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (3.13). FmocCl (1.06 g, 4.12 mmol) was added to a solution of ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[64] (**3.5**, 1.10 g, 2.06 mmol) in CH_2Cl_2 (20 mL) and pyridine (0.42 mL), and the resulting mixture was stirred under argon for 2 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~250 mL) and washed with 1% aq. HCl (40 mL) and water (2 x 40 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white form in 87% yield (1.35 g, 1.79 mmol). Analytical data for **3.13**: R_f = 0.53 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ +75.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.17 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.54-2.75 (m, 2H, CH_2CH_3), 3.68 (br d, 2H, $J_{6a,6b}$ = 4.5 Hz, H-6a, 6b), 3.86 (m, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 4.5 Hz, H-5), 4.13 (t, 1H, *J* = 7.1 Hz,

OCOCH$_2$C*H*), 4.27-4.44 (m, 2H, OCOC*H*$_2$CH), 4.30 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 4.44 (dd, 2H, ^2J = 12.2 Hz, C*H*$_2$Ph), 4.50 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 4.55 (dd, 2H, ^2J = 12.0 Hz, C*H*$_2$Ph), 4.99 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 5.27 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 6.78-7.83 (m, 22H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.2, 46.9, 54.6, 69.9, 70.0, 73.6, 74.4, 77.3 (×2), 77.8, 81.3, 120.2 (×2), 123.4, 123.7, 124.8, 125.1, 125.2, 127.2, 127.3 (×2), 127.5, 127.7 (×3), 127.9 (×2), 128.0, 128.1 (×2), 128.4 (×2), 131.7, 134.1, 137.6, 138.1, 141.4 (×2), 143.2, 143.4, 154.4, 167.3, 168.1 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{45}H_{41}NNaO_8S$; 778.2451, found 778.2457.

Di-*O*-butyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxy-carbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (3.14). A mixture of thioglycoside **3.13** (0.30 g, 0.39 mmol), dibutyl hydrogen phosphate (0.23 mL, 1.19 mmol), and freshly activated molecular sieves (3 Å, 0.6 g) in CH_2Cl_2 (7.0 mL) was stirred under argon for 1 h at rt. The reaction mixture was cooled to 0 ºC, NIS (0.17 g, 0.78 mmol) and TfOH (7.0 µL, 0.08 mmol) were added, and the resulting mixture was stirred for 20 min at 0 ºC. After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL) and sat. aq. $NaHCO_3$ (15 mL), and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a clear syrup in 90% yield (0.31 g, 0.35 mmol). Analytical data for **3.14**: R_f = 0.45 (ethyl acetate/hexane, 1/4, v/v); $[\alpha]_D^{23}$ +55.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.69, 0.83 (2 t, 6H, 2 x O(CH$_2$)$_3$C*H*$_3$), 1.03 (m, 2H, *J* = 7.3, 14.4 Hz, O(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.18-1.34 (m, 4H, O(CH$_2$)$_2$C*H*$_2$CH$_3$,

$OCH_2CH_2CH_2CH_3$), 1.42-1.56 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.61-3.80 (m, 4H, H-6a, 6b, $OCH_2CH_2CH_2CH_3$), 3.84-4.03 (m, 3H, H-5, $OCH_2CH_2CH_2CH_3$), 4.13 (t, 1H, J = 7.0 Hz, $OCOCH_2CH$), 4.29-4.43 (m, 3H, H-2, $OCOCH_2CH$), 4.44 (dd, 2H, ^2J = 12.4 Hz, CH_2Ph), 4.51 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 4.58 (m, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 5.08 (t, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 5.84 (t, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 6.76-7.82 (m, 22H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.5, 13.6, 18.4, 18.6, 31.9 (d, J = 7.1 Hz), 32.0 (d, J = 7.2 Hz), 46.8, 55.9 (d, J = 8.2 Hz), 67.9 (d, J = 6.2 Hz), 68.1 (d, J = 6.3 Hz), 69.1, 70.1, 73.5, 73.6, 74.4, 76.4, 76.5, 94.1 (d, J = 4.5 Hz), 120.2 (×2), 123.5, 125.0, 125.2, 127.3 (×2), 127.5, 127.7 (×4), 127.8 (×3), 128.0 (×2), 128.1 (×3), 128.4 (×3), 131.6, 134.0 (×2), 137.6, 137.9, 141.4 (×2), 143.1, 143.4, 154.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{51}H_{54}NNaO_{12}P$ 926.3281, found 926.3286.

3.4.3. Synthesis of oligosaccharides 3.2, 3.3, 3.11, 3.15, 3.16, 3.12

Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3.2). A mixture of benzoxazolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[67,68] (3.8, 0.20 g, 0.29 mmol), acceptor 3.5 (0.12 g, 0.22 mmol), and freshly activated molecular sieves (3Å, 600 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h at rt. The reaction mixture was cooled to -30 °C, freshly conditioned AgOTf (0.15 g, 0.58 mmol) was added, and the resulting mixture was stirred for 15 min. The solids were filtered-off rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel

(acetone - toluene gradient elution) to afford the title compound as a white foam in 98% yield (0.23 g, 0.22 mmol). Analytical data for 3.2: R_f = 0.54 (acetone/toluene, 1/9, v/v); $[\alpha]_D^{23}$ +28.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.11 (t, 3H, *J* = 7.4 Hz, CH_2CH_3), 2.45-2.67 (m, 2H, CH_2CH_3), 3.34-3.70 (m, 7H, H-3′, 5, 5′, 6a, 6a′, 6b, 6b′), 4.03 (m, 2H, H-4, 4′), 4.15-4.66 (m, 11H, H-1′, 2, 3, 8 x CHPh), 4.66 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.91 (d, 1H, ^2J = 12.1 Hz, CHPh), 4.97 (d, 1H, ^2J = 11.6 Hz, CHPh), 5.13 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.65 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′), 6.73-7.98 (m, 34H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.0. 24.0, 54.9, 68.0, 68.2, 71.4, 72.6 (×2), 73.4 (×2), 73.6, 74.6, 74.8, 77.4, 78.1, 79.0, 79.8, 81.1, 100.8, 123.4, 123.5, 126.8, 127.4, 127.6 (×2), 127.7 (×2), 127.8 (×7), 127.9 (×4), 128.0 (×2), 128.2 (×2), 128.4 (×6), 128.5 (×3), 129.9 (×2), 131.7, 133.2, 133.9, 137.8, 138.1, 138.4, 138.8, 138.9, 165.2, 167.7, 168.0 ppm; ESI TOF LCMS $[M+NH_4]^+$ calcd for $C_{64}H_{67}N_2O_{12}S$ 1087.4415, found 1087.4426.

Benzyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3.3). A mixture of donor **3.6** (0.82 g, 0.96 mmol), benzyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[70] (**3.7**, 0.40 g, 0.74 mmol), and freshly activated molecular sieves (3Å, 2.0 g) in CH_2Cl_2 (20 mL) was stirred under argon for 2 h at rt. The reaction mixture was cooled to 0 ºC, NIS (0.43 g, 1.96 mmol) and TfOH (17 µL, 0.19 mmol) were added, and the resulting mixture was stirred for 16 h. Over this time, the reaction temperature was allowed to reach rt. After that, Et_3N (6.0 mL) was added, and the resulting mixture was stirred for 1 h at rt. The solid was filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL) and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and

concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 84% yield (0.61 g, 0.62 mmol). Analytical data for **3.3**: R_f = 0.50 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{23}$ -7.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.39 (d, 1H, J = 9.4 Hz, OH), 3.29 (m, 1H, $J_{5,6a}$ = 1.4 Hz, $J_{5,6b}$ = 3.8 Hz, H-5), 3.45 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.57 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 3.62 (dd, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.65-3.72 (m, 3H, $J_{3',4'}$ = 3.8 Hz, H-3′, 5′, 6b), 3.93 (br d, 1H, H-4′), 3.96 (dd, 1H, $J_{4,5}$ = 9.1 Hz, H-4), 4.33-4.42 (m, 3H, H-6a′, 6b′, C*H*Ph), 4.41 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.58 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.61 (d, 1H, ^2J = 12.2 Hz, C*H*Ph), 4.70 (d, 1H, ^2J = 11.0 Hz, C*H*Ph), 4.82-4.75 (m, 4H, $J_{1',2'}$ = 8.0 Hz, H-1′, 3 x C*H*Ph), 4.86-4.89 (m, 2H, 2 x C*H*Ph), 5.01 (d, 1H, ^2J = 10.9 Hz, C*H*Ph), 5.27 (dd, 1H, $J_{2',3'}$ = 10.0, H-2′), 7.12-8.82 (m, 34H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 63.1, 68.2, 71.2, 72.1, 73.5, 73.5, 74.6, 74.7, 75.0, 75.4, 75.8, 76.4, 76.8, 81.9, 82.8, 100.2, 102.6, 125.4, 124.4, 127.2, 127.4, 127.7, 127.8 (×3), 127.9 (×2), 128.0 (×2), 128.2 (×6), 128.4 (×2), 128.5 (×2), 128.6 (×4), 128.7 (×2), 129.6, 129.9 (×2), 133.5, 137.1, 137.5, 137.8, 138.3, 138.6, 139.1, 147.6, 150.1, 164.5, 166.7 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{60}H_{59}NNaO_{13}$ 1024.3884, found 1024.3878.

Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (3.11). A mixture of compound 3.2 (0.285 g, 0.282 mmol), dibutyl hydrogen phosphate (0.17 mL, 0.847 mmol), and freshly activated molecular sieves (3 Å, 0.5 g) in CH_2Cl_2 (5.0 mL) was stirred under argon for 1 h at rt. The mixture was cooled to 0 °C, NIS (0.126 g, 0.564 mmol) and TfOH (5.0 μL, 0.056 mmol) were added, and the resulting mixture was stirred for 20 min at 0 °C.

After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL), sat. aq. $NaHCO_3$ (15 mL), and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an oily syrup in 97% yield (0.33 g, 0.273 mmol). Analytical data for 3.11: R_f = 0.35 (acetone/toluene,1/9, v/v); $[\alpha]_D^{23}$ +31.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.71, 0.86 (2 t, 6H, 2 x $O(CH_2)_3CH_3$), 1.03 (m, 2H, $O(CH_2)_2CH_2CH_3$), 1.37-1.20 (m, 4H, $O(CH_2)_2CH_2CH_3$, $OCH_2CH_2CH_2CH_3$), 1.52 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.48-3.59 (m, 6H, H-3′, 5, 6a, 6a′, 6b, 6b′), 3.64-3.80 (m, 3H, H-5′, $OCOCH_2CH$), 3.84-4.00 (m, 2H, $OCOCH_2CH$), 4.06 (br d, 1H, $J_{3',4'}$ = 2.8 Hz, H-4′), 4.13 (dd, 1H, $J_{4,5}$ = 8.9 Hz, H-4), 4.21-4.63 (m, 8H, H-2, 3, 6 x C*H*Ph), 4.66-4.74 (m, 3H, $J_{1',2'}$ = 7.7 Hz, H-1′, 2 x C*H*Ph), 4.97 (d, 1H, ^2J = 12.2 Hz, C*H*Ph), 5.04 (d, 1H, ^2J = 11.6 Hz, C*H*Ph), 5.69 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 5.75 (dd, 1H, J = 8.2, 7.1 Hz, H-1), 6.80-8.05 (m, 34H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.4, 13.6, 18.3, 18.5, 31.8 (d, J = 7.0 Hz), 32.0 (d, J = 7.2 Hz), 56.0, 56.1, 67.4, 67.7 (d, J = 6.0 Hz), 67.9 (d, J = 6.3 Hz), 68.2, 71.4, 72.4, 72.6, 73.4, 73.5 (x2), 74.5, 74.7, 75.1, 76.4, 76.8, 79.7, 94.1 (d, J = 4.9 Hz), 100.7, 123.3, 125.4, 126.9, 127.4, 127.6 (×2), 127.7, 127.8 (×6), 127.9, 128.0 (×6), 128.2 (×2), 128.3, 128.4 (×2), 128.5 (×6), 129.1, 129.8, 129.9, 131.6, 133.2, 133.9, 137.8, 138.0, 138.1, 138.8 (x2), 165.1, 167.6 (×2) ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{70}H_{76}NNaO_{16}P$ 1240.4799, found 1240.4826

Benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxy-carbonyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-

galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3.15). A mixture of donor 3.14 (0.20 g, 0.22 mmol), acceptor 3.2 (0.17 g, 0.17 mmol), and freshly activated molecular sieves (3Å, 600 mg) in CH_2Cl_2 (10 mL) was stirred under argon for 2 h at rt. The mixture was cooled to -30 ^oC, TMSOTf (80 μL, 0.44 mmol) was added, and the resulting mixture was stirred for 15 min. The reaction mixture was then diluted with CH_2Cl_2, the solid was filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 70% yield (0.20 g, 0.12 mmol). Analytical data for 3.15: R_f = 0.52 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{23}$ +10.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.90 (m, 1H, J = 9.2 Hz, H-5), 3.25 (dd, 1H, J = 10.0 Hz, H-6a), 3.32 (dd, 1H, $J_{2,3}$ = 8.9 Hz, H-2), 3.36-3.39 (m, 2H, H-3, 6b), 3.63-3.72 (m, 4H, H-3′, 5′, 6a″, 6b″), 3.81(dd, 1H, H-4), 3.88 (m, 1H, H-5″), 4.03 (br d, 1H, $J_{3',4'}$ = 1.9 Hz, H-4′), 4.11 (t, 1H, J = 6.9 Hz, $OCOCH_2CH$), 4.15-4.27 (m, 5H, H-1, 2″, 6a′, 2 x C*H*Ph), 4.31-4.37 (m, 3H, H-6b, $OCOCH_2CH$), 4.42 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.44-4.49 (m, 4H, H-3″, 3 x C*H*Ph), 4.56-4.68 (m, 5H, 5 x C*H*Ph), 4.78-4.82 (m, 2H, 2 x C*H*Ph), 4.89-4.94 (m, 2H, H-4″, C*H*Ph), 5.05 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 5.19 (d, 1H, $J_{1'',2''}$ = 8.4 Hz, H-1″), 5.30 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2′), 6.62-8.86 (m, 56H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 46.0, 55.6, 63.7, 67.4, 69.7, 69.9, 70.9, 71.9 (x2), 72.9, 73.4, 73.6 (×2), 74.1, 74.2, 74.8, 74.9, 75.4, 75.7, 76.0, 76.3, 80.0, 81.5, 82.5, 99.4, 100.1, 102.4, 120.0 (×2), 124.9, 125.0, 125.3, 126.7, 127.1 (×2), 127.2, 127.3 (×2), 127.4, 127.5, 127.6 (×4), 127.7 (×3), 127.8, 127.9 (×11), 128.0 (×3), 128.1, 128.2 (×11), 128.4 (×3), 128.5

(×2), 128.6 (×3), 129.3, 129.6, 132.8, 136.8, 137.4 (×2), 137.6, 138.2, 138.4, 138.5, 138.9, 141.2, 141.3, 143.0, 143.2, 147.7, 149.8, 154.2, 164.2, 164.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{103}H_{94}N_2NaO_{21}$ 1718.6280, found 1718.6223.

Benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3.16). Compound 3.15 (200 mg, 0.118 mmol) was dissolved in a solution of Et_3N in CH_2Cl_2 (7.0 mL, 1/165, v/v), and the resulting solution was stirred for 2 h at rt. The resulting mixture was concentrated *in vacuo,* and the residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 98% yield (170.3 mg, 0.116 mmol). Analytical data for 3.16: R_f = 0.55 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ -5.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.92 (m, 1H, *J* = 1.8, 3.4, 10.0 Hz, H-5), 3.28 (dd, 1H, H-6a), 3.34 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 3.37-3.42 (m, 2H, H-3, 6b), 3.67-3.84 (m, 7H, H-3′, 4, 4″, 5′, 5″, 6a″, 6b″), 4.02 (br d, 1H, $J_{3',4'}$ = 2.2 Hz, H-4′), 4.16-4.29 (m, 5H, H-1, 2″, 6a′, 6b′, C*H*Ph), 4.37 (dd, 1H, *J* = 6.2, 11.2 Hz, H-3″), 4.41 (d, 1H, ^2J = 12.1 Hz, C*H*Ph), 4.45-4.70 (m, 9H, $J_{1',2'}$ = 8.0 Hz, H-1′, 8 x C*H*Ph), 4.81 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.82 (d, 1H, ^2J = 11.4 Hz, C*H*Ph), 4.93 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.08 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 5.22 (d, 1H, $J_{1'',2''}$ = 8.2 Hz, H-1″), 5.33 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′), 6.63-8.81 (m, 48H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.7, 63.8, 67.6, 70.7, 71.0, 72.0, 73.4, 73.9 (×4), 74.3, 74.5, 74.9, 75.0, 75.5, 75.9, 76.1, 78.6, 80.1, 81.6, 82.6, 99.7, 100.3, 102.5, 122.8, 123.4, 125.4, 126.9, 127.2, 127.5, 127.5, 127.6 (×3), 127.7 (×3), 127.8 (×2), 128.0 (×5), 128.1 (×4), 128.2 (×5), 128.3 (×6), 128.4 (×2), 128.6 (×2),

128.7 (×5), 129.4, 129.7, 130.9, 131.4, 132.9, 133.4, 133.5, 137.0, 137.5, 138.1, 138.3, 138.6, 139.0, 147.8, 149.9, 164.4, 164.4, 167.2, 168.1 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{88}H_{84}N_2NaO_{19}$ 1495.5566, found 1495.5579.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3.12). ***A convergent (2+2) approach.*** A mixture of donor 3.11 (128.3 mg, 0.105 mmol), acceptor 3.3 (80.0 mg, 0.081 mmol), and freshly activated molecular sieves (3Å, 400 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h at rt. The mixture was cooled to -60 ºC, TMSOTf (29 μL, 0.162 mmol) was added, and the resulting mixture was stirred for 30 min. During this time the reaction temperature was allowed to gradually increase to -30 ºC. The reaction mixture was then diluted with CH_2Cl_2, the solid was filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 70% yield (146.6 mg, 0.073 mmol). ***A linear approach.*** A mixture of donor 3.8[67,68] (42.5 mg, 0.062 mmol), acceptor 3.16 (70.0 mg, 0.0475 mmol), and freshly activated molecular sieves (3Å, 250 mg) in CH_2Cl_2 (4.0 mL) was stirred under argon for 2 h at rt. The mixture was cooled to -30 ºC, freshly conditioned AgOTf (31.8 mg, 0.124 mmol) was added, the external cooling was removed, and the resulting mixture was stirred for 15 min. During this time the reaction temperature was

allowed to gradually increase to -23 °C. The reaction mixture was then diluted with CH_2Cl_2, the solid was filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 84% yield (80.0 mg, 0.0398 mmol). Analytical data for 3.12: R_f = 0.50 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +11.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.89 (m, 1H, *J* = 1.9, 3.4, 9.9 Hz, H-5), 3.24 (dd, 1H, H-6a), 3.28-3.46 (m, 7H, H-2, 3, 5″, 5‴, 6a‴, 6b, 6b‴), 3.49 (dd, 1H, *J* = 2.8, 10.1 Hz, H-3‴), 3.53 (dd, 1H, $J_{5'',6a''}$ = 0.8 Hz, H-6a″), 3.56-3.61 (m, 2H, H-3′, 5′), 3.66 (dd, 1H, $J_{5'',6b''}$ = 3.8 Hz, H-6b″, 3.78 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 3.95-3.99 (m, 3H, H-4′, 4″, 4‴), 4.12-4.32 (m, 10H, H-1, 2″, 3″, 6a′, 6b′, 5 x C*H*Ph), 4.40-4.49 (m, 4H, H-1′, 3 x C*H*Ph), 4.50-4.58 (m, 4H, 4 x C*H*Ph), 4.60-4.67 (m, 3H, $J_{1''',2'''}$ = 7.9 Hz, H-1‴, 2 x C*H*Ph), 4.79 (m, 2H, 2 x C*H*Ph), 4.85 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.90 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 4.95 (d, 1H, C*H*Ph), 5.08 (m, 2H, $J_{1'',2''}$ = 8.1 Hz, H-1″, C*H*Ph), 5.28 (dd, 1H, $J_{2',3'}$ = 10.0, H-2′), 5.61 (dd, 1H, $J_{2''',3'''}$ = 10.0 Hz, H-2‴), 6.59-8.70 (m, 68H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 29.8, 56.0, 63.9, 67.6, 68.0, 68.3, 71.0, 71.4, 71.9, 72.0, 72.5, 72.6, 73.4, 73.5, 73.6, 74.3, 74.6 (x2), 74.0, 75.0, 75.1, 75.6, 76.1, 76.2, 76.8, 77.8, 79.8, 80.1, 80.3, 81.7, 82.6, 99.7, 100.4, 101.0, 102.5, 122.8, 123.3, 125.5, 126.7, 126.8, 127.2, 127.4 (×2), 127.5 (×2), 127.6, 127.7 (×7), 127.8 (×8), 127.9 (×4), 128.0 (×4), 128.1 (×6), 128.3 (×6), 128.4 (×5), 128.5 (×5), 128.6 (×3), 129.4, 129.7, 130.0 (×2), 131.0, 131.5, 132.9, 133.3, 137.0, 137.6, 137.8, 138.0 (×2), 138.3, 138.7, 138.8 (×2), 138.9, 139.0, 147.8, 149.9, 164.3,

164.4, 165.2, 167.2, 167.8 ppm; ESI TOF LCMS $[M+H]^+$ calcd for $C_{122}H_{117}N_2O_{25}$ 2010.7979, found 2010.7988.

3.4.4. Deprotection of tetrasaccharide 3.12

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3.17). Compound 3.12 (78.0 mg, 0.038 mmol) was dissolved in MeOH (5.0 mL), NH_2NH_2-H_2O (167 μL, 3.42 mmol) was added, and the resulting mixture was kept for 24 h at reflux. After that, the volatiles were removed under reduced pressure, and the residue was dried *in vacuo* for 3 h. The crude residue was dissolved in a mixture of Ac_2O/MeOH (2.0 mL, 1/1, v/v) and the resulting mixture was stirred for 12 h at rt. After that, the volatiles were removed under reduced pressure. The residue was diluted with CH_2Cl_2 (50 mL), and washed with water (10 mL), 1 M HCl (10 mL), and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 87% yield (53.1 mg, 0.033 mmol). Analytical data for 3.17: R_f = 0.67 (acetone/toluene, 2/3, v/v); $[\alpha]_D^{22}$ +12.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.75 (s, 3H, C*H*$_3$CO). 2.94-4.03 (m, 24H, H-2, 2′, 2″, 2‴, 3, 3′, 3″, 3‴, 4, 4′, 4″, 4‴, 5, 5′, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″, 6b‴), 4.30 (dd, 2H, ^2J = 11.7 Hz, C*H*$_2$Ph), 4.43-4.50 (m, 3H, H-1, 1′, 1‴), 4.52-4.73 (m, 11H, 11 x C*H*Ph), 4.80-4.96 (m, 7H, 7 x C*H*Ph), 4.99 (d, 1H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 5.61 (d, 1H, *J* = 7.4 Hz, N*H*COCH$_3$), 7.16-7.40 (m, 50H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.6, 57.2, 61.8, 68.3, 68.8, 69.0, 71.3,

71.9, 72.1, 72.3, 72.8, 73.5, 73.6 (×3), 73.7, 74.3 (×2), 74.7, 74.8, 74.9, 75.0, 75.1, 75.3, 77.1, 77.2, 80.2, 82.0, 82.2, 83.2 (×2), 102.2, 102.9, 103.2, 103.4, 127.4, 127.5 (×3), 127.6 (×2), 127.7 (×3), 127.8 (×3), 127.9 (×5), 128.0 (×7), 128.1 (×2), 128.3 (×6), 128.4 (×5), 128.5 (×4), 128.6 (×7), 137.6 (×2), 137.9 (×2), 138.1, 138.2, 138.4, 138.6, 138.8 (x2), 138.9, 139.1, 170.9 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{96}H_{105}NNaO_{21}$ 1631.7110, found 1631.7130.

***O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (LNnT, 3.1).** A 10% Pd on charcoal (150 mg) was added to a solution of 3.17 (53.0 mg, 0.033 mmol) in EtOH/H_2O (5.0 mL, 4/1, v/v), and the resulting mixture was stirred under hydrogen atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~40 mL) was concentrated in *vacuo,* and the residue was purified by column chromatography on Sephadex G-25 (water elution) to afford the title compound as a white amorphous solid in 92% yield (21.4 mg, 0.030 mmol). Selected analytical data for 3.1:[72] R_f = 0.32 (chloroform/methanol/water, 2/1/0.4, v/v/v); ^{1}H NMR (600 MHz, D_2O): δ, 2.01 (s, 3H), 3.24-3.28 (m, 1H), 3.88-3.49 (m, 29H), 3.89-3.96 (m, 4H), 4.14 (d, 1H, J = 3.3 Hz), 4.42 (d, 1H, J = 7.9 Hz), 4.46 (d, J = 7.8 Hz, 1H), 4.64 (d, 1H, J = 8.0 Hz), 4.68 (dd, 1H, J = 2.1, 8.4 Hz,), 5.20 (d, 1H, J = 3.8 Hz) ppm; ^{13}C NMR (151 MHz, D_2O): δ, 22.5, 55.5, 60.2, 60.3, 60.4, 61.3, 61.4, 68.7 (x2), 68.9, 70.3 (x2), 70.4, 70.5, 71.3, 71.5 (×2), 71.7, 72.5, 72.8, 74.1, 74.7, 74.9, 75.1, 75.2, 75.7, 78.5, 78.6, 78.7, 82.4, 92.2, 96.1, 103.1, 103.2 (x2), 103.3, 103.4, 175.2 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{26}H_{45}NNaO_{21}$ 730.2382, found 730.2392.

3.5. References

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CHAPTER 4

The chemical synthesis of human milk oligosaccharides: lacto-*N*-tetraose (Galβ1→3GlcNAcβ1→3Galβ1→4Glc)

M. D. Bandara, K. J. Stine, and A. V. Demchenko. The chemical synthesis of human milk oligosaccharides: lacto-N-tetraose (Galβ1→3GlcNAcβ1→3Galβ1→4Glc). *Carbohydr. Res.*, **2019**, 486, 107824; DOI: 10.1016/j.carres.2019.107824; PMID: 31585319

4.1. Introduction

Carbohydrates are essential biomolecules that become our first food.[1] Oligosaccharides present in human milk (HMO) can supply building blocks for the development of the infants' cognition,[2] act as prebiotics[3] and antimicrobials.[4,5] Thanks to advances in glycosciences, chemical structures of 162 HMO have been elucidated to date,[6,7] but our understanding of how HMO function is incomplete.[8,9] All HMO are composed of five monosaccharides: including glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), fucose, and sialic acid.[10] Many efforts to prepare HMO enzymatically or chemically have been reported,[11-13] and importance of including HMO in infant formulas has been acknowledged.[14]

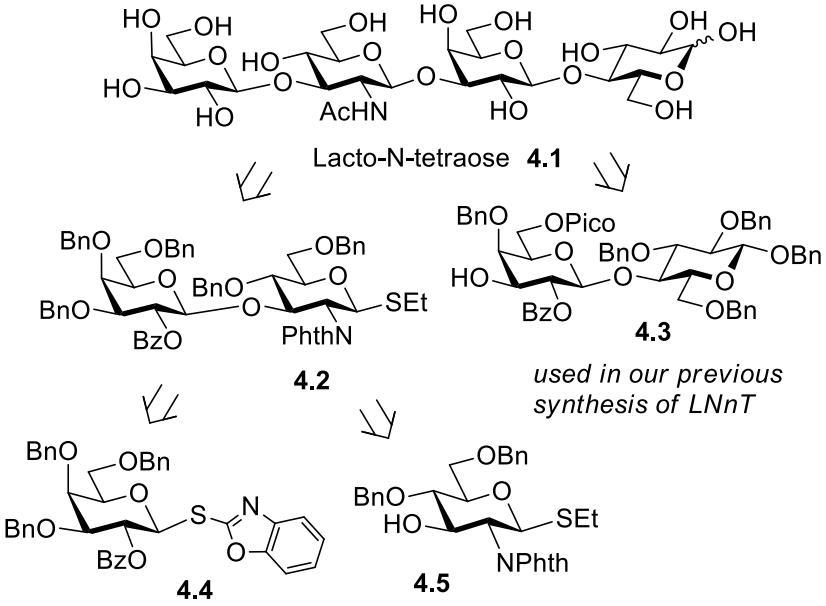
The total amount and composition of HMO varies between women and are dependent on maternal genetics, environment, and geographic location.[15] Lacto-N-tetraose (LNT) **4.1** represents one of the most common and abundant core structures and is classified as a type I HMO. It comprises a Galβ1→3GlcNAcβ1→3Galβ1→4Glc sequence shown in Scheme 4.1. More specifically, LNT is a linear tetrasaccharide wherein the reducing end lactose disaccharide (Galβ1→4Glc) is elongated with lacto-N-biose disaccharide residue (Galβ1→3GlcNAc). Chemical[16-18] and enzymatic syntheses[19] of LNT have been reported, and several of its derivatives have been synthesized using chemical synthesis in solution and on solid phase.[20-24] Despite being one of the most abundant HMO core structures in human milk, LNT is not yet available in large quantities and at reasonable prices for research and application.

Previously, we reported the total synthesis of lacto-N-neotetraose that has been completed using both linear and convergent approaches (see Chapter 3).[25] Along the way,

we developed the synthesis of key building blocks, accessed scalability, and refined coupling procedures to obtain different glycosidic linkages and sequences. Notably, the donor and acceptor protecting/leaving group combinations were found to be key parameters. In further synthetic studies of HMO in our lab reported herein is the synthesis of LNT **4.1**.

4.2. Results and discussion

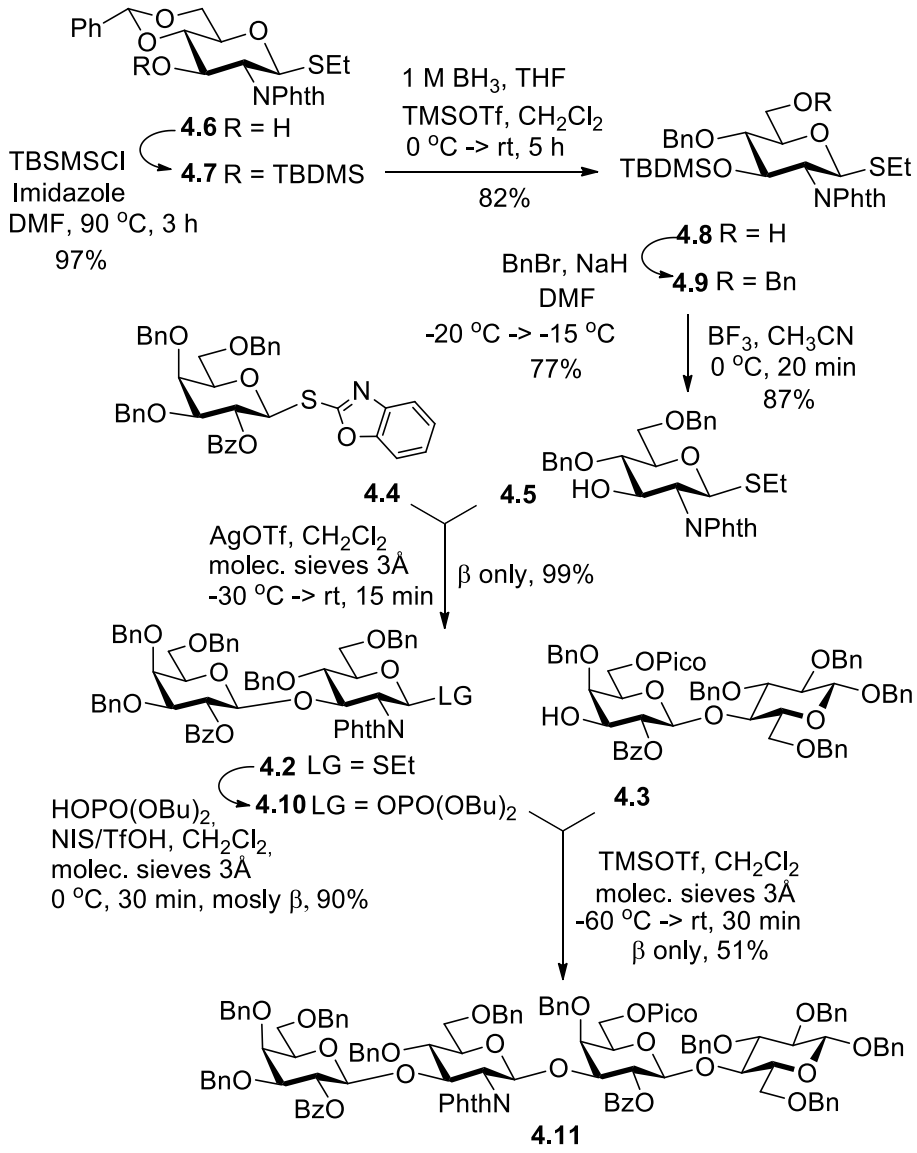
First, we decided to investigate a convergent (2+2) synthetic strategy, according to which we chose to converge the protected lacto-*N*-biose donor **4.2** and lactose acceptor **4.3**.[25] Based on our previous synthetic endeavors and preliminary refinement of reaction conditions (Chapter 3),[25] for the synthesis of disaccharide **4.2** we chose superarmed S-benzoxazolyl (SBox) galactosyl donor **4.4**[29,30] and glucosamine acceptor **4.5**. SBox galactosyl donor **4.4** was very instrumental in avoiding the unwanted aglycone transfer side reaction[12,26] that was taking place in other previously investigated building blocks.[25]



Scheme 4.1. Retrosynthesis analysis of LNT 4.1.

The synthesis of glucosamine thioglycoside acceptor **4.5** was achieved from known building block **4.6** as depicted in Scheme 4.2.[27] First, precursor **4.6** was reacted with tert-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole in DMF at 90 ºC to obtain compound **4.7** in 97% yield. The latter was then converted to intermediate **4.8** via the reductive regioselective opening of the benzylidene acetal by reaction with 1 M BH_3 in THF in the presence of catalytic TMSOTf in 82% yield. Subsequently, 6-OH in compound **4.8** was benzylated with BnBr in the presence of NaH in DMF to afford compound **4.9** in 77% yield. In order to minimize the formation of side products, temperature control is highly important in this reaction (see the experimental part for further details). Finally, the silyl group of compound **4.9** was removed with BF_3-Et_2O in CH_3CN at 0 ºC to afford the desired glucosamine thioglycoside acceptor **4.5** in 87% yield.

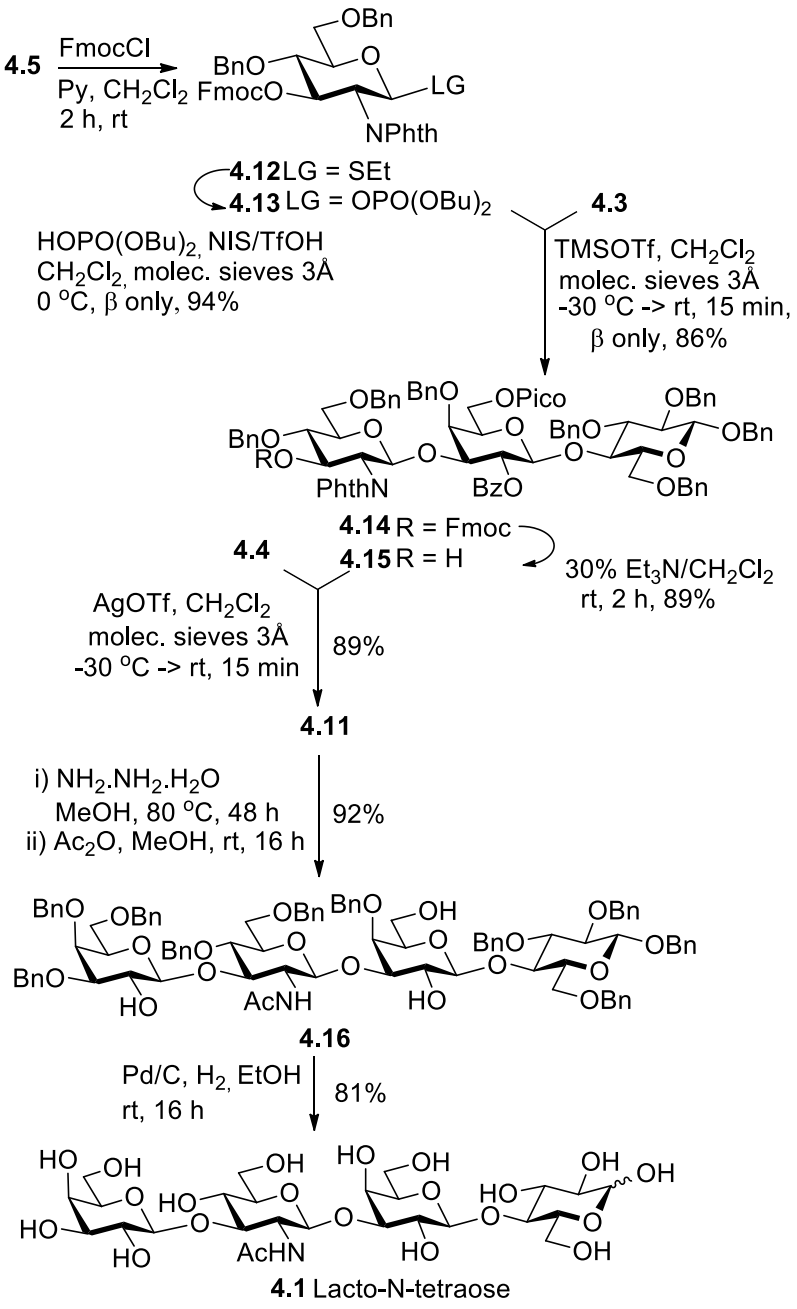
We next turned our attention to the assembly of LNB disaccharide **4.2**. Selective activation of the SBox leaving group in glycosyl donor **4.4** over thioethyl anomeric moiety of acceptor **4.5** was achieved in the presence of silver trifluoromethanesulfonate (AgOTf) to afford β-linked disaccharide **4.2** in excellent yield of 99%. Direct coupling of disaccharide **4.2** with acceptor **4.3** was proven inefficient, so we chose to employ the corresponding phosphate donor **4.10**. This was accomplished in 90% yield via an efficient one-step protocol developed by Seeberger and co-workers.[28] The coupling of phosphate donor **4.10** with lactose acceptor **4.3** in the presence of TMSOTf was more successful, and tetrasaccharide **4.11** was obtained in a moderate yield of 51% with complete β-stereoselectivity.



Scheme 4.2. Convergent synthesis of protected LNT 4.11.

Despite numerous attempts involving modifying the reaction condition, and replacing the glycosyl donor with O- and S-imidoyl leaving groups, we failed to improve the outcome of this reaction. We were also unable to elucidate structures of by-products forming alongside the desired tetrasaccharide **4.11**. Not being satisfied with the outcome

of the convergent synthesis, we were curious to investigate whether the linear approach would be more successful in achieving a better outcome.



Scheme 4.3. The linear synthesis of tetrasaccharide 4.11 and its deprotection to obtain LNT 4.1.

Only a minimal strategic adjustment was required, and this involved conversion of building block **4.5** into its 4-O-Fmoc derivative **4.12** that was subsequently transformed into phosphate donor **4.13**. Glycosylation between donor **4.13** and lactose acceptor **4.3** in the presence of TMSOTf afforded trisaccharide **4.14** in 86% yield. The Fmoc protecting group was removed with 30% Et_3N in CH_2Cl_2 and glycosylation of the resulting trisaccharide acceptor **4,15** with SBox donor **4.4** in the presence of AgOTf afforded the desired β-linked tetrasaccharide **4.11** in 89% yield. Overall, the three-step linear assembly of **4.11** involving glycosylation of acceptor **4.3** with glycosyl donor **4.13**, interim Fmoc deprotection, followed by glycosylation with donor **4.4** proceeded with 68% overall yield for the synthesis of tetrasaccharide **4.11**. In contrast, the convergent approach was much less efficient, 45% over three steps, primarily due to the very low-yielding last coupling step between disaccharides **4.3** and **4.10**.

With the key tetrasaccharide intermediate **4.11** we endeavored to carry out its deprotection steps to obtain the target LNT tetrasaccharide **4.1**. Deprotection of the phthalimido and the ester groups was performed in the presence of NH_2NH_2-H_2O in refluxing MeOH. Subsequent N-acetylation with acetic anhydride in MeOH furnished tetrasaccharide intermediate **4.16** in 92% yield. Subsequently, benzyl ethers were hydrogenated the presence of 10% Pd/C in wet ethanol to afford the target trisaccharide **4.1** in 81% yield.

4.3. Conclusions

In summary, the total synthesis of lacto-N-tetraose has been completed using both linear and convergent synthesis approaches. The linear approach was significantly more

effective in this application. Along the way, we have developed new synthetic protocols for different glycosidic linkages. Notably, the donor and acceptor protecting group and the leaving group combinations were found to be of paramount significance to successful glycosylations. The protecting groups in precursors used for the synthesis of the key building block **4.3** were chosen to provide access to variable glycosylation sites. In this application, 3'-OH acceptor **4.3** was achieved via the Fmoc group removal,[25] but the same precursors could also be used to achieve 6'-OH via the OPico group removal, or provide access to 3',6'-diol for the synthesis of branched HMO. We expect that new methods for obtaining individual HMO will boost practical applications of these important biomolecules.

4.4. Experimental

4.4.1. General methods

The reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh) and Sephadex G-25 size exclusion resin, reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ºC. CH_2Cl_2 was distilled from CaH_2 directly prior to application. Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured

using a Jasco polarimeter. ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 151 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual TMS (δ_H = 0.00 ppm) for solutions in $CDCl_3$ or the signal of the residual D_2O (δ_H = 4.79 ppm) for solutions in D_2O. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.16 ppm) for solutions in $CDCl_3$ or the central signal of CD_3COCD_3 δ_C = 29.84 ppm) for solutions in D_2O. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer.

4.4.2. Preparation of monosaccharide building blocks 4.7, 4.8, 4.5, 4.12. 4.13

Ethyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4.7). TBDMSCl (0.37 g, 2.44 mmol) and imidazole (0.16 g, 2.44 mmol) were added to a solution of ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[27] (4.**6**, 0.54 g, 1.22 mmol) in DMF (7.0 mL) and the resulting mixture was heated at 90 ºC for 3 h. After that, the reaction mixture was cooled to rt, diluted with CH_2Cl_2 (~250 mL) and washed with water (40 mL), sat. aq. $NaHCO_3$ (40 mL), and water (40 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white form in 97% yield (0.65 g, 1.18 mmol). Analytical data for **4.7**: R_f = 0.60 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{23}$ -2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.28, -0.12 (2 s, 6H, 2 x SiCH_3), 0.59 (s, 9H, Si^{t}Bu), 1.19 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.69 (m, 2H, CH_2CH_3), 3.59 (t, 1H, $J_{4,5}$ = 8.8 Hz, H-4), 3.71 (m, 1H, $J_{5,6a}$ = 10.0 Hz, $J_{5,6b}$ = 4.4 Hz, H-5), 3.81 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, H-

6a), 4.32 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 4.39 (dd, 1H, H-6b), 4.67 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 5.37 (d, 1H, $J_{1,2}$ = 10.7 Hz, H-1), 5.54 (s, 1H, *CH*Ph), 7.31-7.94 (m, 9H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -5.1, -4.0, 15.0, 17.8, 24.2, 25.5 (×3), 56.7, 68.8, 70.6, 70.7, 81.9, 82.8, 102.1, 123.3, 123.8, 126.5 (×2), 128.3 (×2), 129.2, 131.7, 131.9, 134.3, 134.4, 137.2, 167.7, 168.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{29}H_{37}NNaO_6SSi$ 578.2009, found 578.1997.

Ethyl 4-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (4.8). A 1 M solution of BH_3 in THF (43 mL, 43 mmol) was added to a solution of **4.7** (4.80 g, 8.66 mmol) in CH_2Cl_2 (45 mL). The resulting solution was cooled to 0 °C, TMSOTf (0.78 mL, 4.33 mmol) was added, and the resulting mixture was stirred for 5 h while the reaction temperature was allowed to gradually increase to rt. After that, the reaction was quenched with Et_3N (~2 mL) and MeOH (~5 mL), and the volatiles were removed *in vacuo.* The residue was diluted with CH_2Cl_2 (~500 mL), washed with sat. aq. $NaHCO_3$ (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a clear syrup in 82% yield (3.80 g, 7.06 mmol). Analytical data for **4.8**: R_f = 0.80 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ +27.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.38, 0.00 (2 s, 6H, 2 x SiCH_3), 0.76 (s, 9H, Si^{t}Bu), 1.20 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.21 (m, 1H, OH), 2.68 (m, 2H, CH_2CH_3), 3.52-3.67 (m, 2H, H-4, 5), 3.73 (m, 1H, H-6a), 3.94 (m, 1H, H-6b), 4.27 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 4.56 (dd, 1H, $J_{3,4}$ = 8.0 Hz, H-3), 4.80 (dd, 2H, ^2J = 11.7 Hz, CH_2Ph), 5.40 (d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), 7.27-7.95 (m, 9H,

aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.6, -4.0, 15.0, 17.7, 24.3, 25.7 (×3), 56.8, 62.0, 73.3, 74.7, 79.7 (×2), 81.2, 123.3, 123.7, 127.3 (×2), 127.6, 128.4 (×2), 131.7, 132.1, 134.3 (×2), 138.1, 167.6, 168.8 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{29}H_{39}NNaO_6SSi$ 580.2165, found 580.2163.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (4.9). NaH (0.51 g, 0.021 mmol) was added portionwise to a cooled (-20 ºC) solution of **4.8** (3.80 g, 7.06 mmol) in DMF (30 mL) and the resulting mixture was stirred under argon at -20 ºC until gas evolution has ceased. After that, BnBr (1.06 mL, 9.18 mmol) was added and the resulting mixture was stirred for 6 h at -15 ºC. The reaction mixture was cooled to -40 ºC and glacial acetic acid (~2 mL) was added dropwise. The resulting mixture was allowed to attain rt, then diluted with EtOAc (~500 mL) and washed with water (50 mL), sat. aq. $NaHCO_3$ (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a colorless syrup in 77% yield (3.65 g, 5.42 mmol). Analytical data for **4.9**: R_f = 0.80 (ethyl acetate/hexane,3/7, v/v); $[\alpha]_D^{23}$ +39.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.36, 0.00 (2 s, 6H, 2 x SiCH_3), 0.79 (s, 9H, Si^{t}Bu), 1.26 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.73 (m, 2H, CH_2CH_3), 3.63-3.71 (m, 2H, H-4, 5), 3.77-3.86 (br d, 2H, H-6a, 6b), 4.35 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.58 (dd, 1H, $J_{3,4}$ = 7.6 Hz, H-3), 4.64 (d, 2H, ^2J = 9.6 Hz, CH_2Ph), 4.79 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 5.38 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 7.21-7.99 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, -4.6, -4.1, 15.1, 17.7, 23.9, 25.8 (×3), 56.8, 69.0, 73.4, 73.5, 74.6, 79.5, 80.0, 80.8, 123.2, 123.7, 127.1 (×2),

127.4, 127.6, 127.8 (×2), 128.4 (×4), 131.8, 132.2, 134.3 (×2), 138.3, 138.4, 167.7, 168.8 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{36}H_{45}NNaO_6SSi$ 670.2635, found 670.2633.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4.5). BF_3-Et_2O (1.05 mL, 8.29 mmol) was added to a solution of **4.9** (5.07 g, 7.54 mmol) in dry CH_3CN (90 mL) and the resulting mixture was stirred under argon for 20 min at 0 °C. After that, the reaction was quenched with sat. aq. $NaHCO_3$ (5 mL), and the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~500 mL) and washed with brine (2 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white amorphous solid in 87% yield (3.49 g, 6.54 mmol). Analytical data for **4.5**: R_f = 0.30 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{22}$ +7.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.20 (t, 3H, J = 7.4 Hz, CH_2CH_3). 2.36 (d, 1H, J = 4.5 Hz, OH), 2.67 (m, 2H, CH_2CH_3), 3.58-3.69 (m, 2H, H-4, 5), 3.75-3.85 (m, 2H, H-6a, 6b), 4.24 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 4.48 (m, 1H, H-3), 4.63 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.70 (m, 2H, ^2J = 11.5 Hz, CH_2Ph), 5.29 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 7.15-7.90 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.1, 55.7, 69.0, 72.8, 73.6, 74.8, 79.3 (×2), 81.2, 123.4, 123.8, 127.8, 127.9 (×2), 128.0 (×2), 128.1, 128.5 (×2), 128.7 (×2), 131.7, 131.8, 134.2 (×2), 138.2 (×2), 168.1, 168.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{30}H_{31}NNaO_6S$ 556.1770, found 556.1767.

Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (4.12). FmocCl (3.88 g, 15.04 mmol) was added to a solution of **4.5**

(2.08 g, 3.90 mmol) in CH_2Cl_2 (50 mL) and pyridine (1.72 mL) and the resulting mixture was stirred under argon for 2 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~500 mL) and washed with 1 M aq. HCl (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as a white amorphous solid in 92% yield (2.70 g, 3.58 mmol). Analytical data for **4.12**: R_f = 0.40 (ethyl acetate/hexane,2/3, v/v); $[\alpha]_D^{23}$ +55.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.22 (t, 3H, J = 7.4 Hz, CH_2CH_3), 2.70 (m, 2H, CH_2CH_3), 3.73-3.86 (m, 4H, H-5, 6a, 6b, $OCOCH_2CH$), 3.91-4.16 (m, 3H, H-4, $OCOCH_2CH$), 4.47 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 4.62 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.64 (dd, 2H, ^2J = 11.2 Hz, CH_2Ph), 5.46 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.75 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 7.07-7.88 (m, 26H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 15.1, 24.2, 46.5, 54.1, 68.8, 70.3, 73.6, 75.0, 76.6, 78.5, 79.2, 81.0, 120.0 (×2), 123.7, 123.8, 125.1, 125.3, 127.3 (×2), 127.8 (×4), 127.9 (×4), 128.4 (×2), 128.5 (×2), 131.3, 131.8, 134.1, 134.4, 137.8, 138.2, 141.1, 141.2, 143.0, 143.3, 154.8, 167.5, 168.0 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{45}H_{41}NNaO_8S$ 778.2451, found 778.2451.

Di-*O*-butyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (4.13). A mixture containing thioglycoside **4.12** (0.50 g, 0.66 mmol), dibutyl hydrogen phosphate (0.39 mL, 1.99 mmol), and molecular sieves (3 Å, 1.0 g) in CH_2Cl_2 (10 mL) was stirred under argon for 1h. The mixture was cooled to 0 °C, NIS (0.29 g, 1.32 mmol) and TfOH (10 μL, 0.13 mmol) were added, and the resulting mixture was stirred under argon for 20 min at 0 °C. After that, the solids were filtered off

and washed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL), sat. aq. NaHCO3 (15 mL), and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white form in 94% yield (0.56 g, 0.62 mmol). Analytical data for **4.13**: R_f = 0.45 (ethyl acetate/hexane,1/4, v/v); $[\alpha]_D^{23}$ +43.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.71, 0.85 (2 t, 6H, 2 x $O(CH_2)_3CH_3$), 1.07 (m, 2H, $O(CH_2)_2CH_2CH_3$), 1.22-1.37 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.48-1.60 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.67-4.17 (m, 11H, 2 x $OCH_2CH_2CH_2CH_3$, H-4, 5, 6a, 6b, $OCOCH_2CH$, $OCOCH_2CH$), 4.49 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 4.60 (dd, 2H, ^2J = 11.9 Hz, CH_2Ph), 4.64 (dd, 2H, ^2J =11.2 Hz, CH_2Ph), 5.80 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 6.02 (d, 1H, $J_{1,2}$ = 8.3, H-1), 6.97-7.86 (m, 22H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.5, 13.6, 18.4, 18.6, 31.9 (d, J = 7.0 Hz), 32.0 (d, J = 7.2 Hz), 46.4, 55.3 (d, J = 8.9 Hz). 67.9 (d, J 6.1 Hz), 68.1 (d, J = 6.0 Hz), 70.3, 73.6, 74.9, 75.2, 76.0, 76.8, 77.3, 93.9 (d, J = 4.6 Hz), 120.0 (×2), 123.6, 125.0, 125.2, 125.4, 127.2 (×2), 127.7 (×3), 127.8 (×3), 127.9 (×3), 128.3, 128.4 (×3), 128.5 (×3), 129.1, 134.2, 137.6, 137.9, 141.1, 141.2, 143.0, 143.2, 154.6 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{51}H_{54}NNaO_{12}P$ 926.3281, found 926.3285.

4.4.3. Synthesis of oligosaccharides 4.2, 4.10, 4.14, 4.15, 4.11

Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (4.2). A mixture of benzoxazolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[29,30] (**4.4**, 0.20 g, 0.29 mmol), acceptor 5 (0.12 g, 0.22 mmol), and freshly activated molecular sieves (3Å,

600 mg) in CH_2Cl_2 (7 mL) was stirred under argon for 2 h. The reaction mixture was cooled to -30 °C, and freshly conditioned AgOTf (0.15 g, 0.58 mmol) was added. The resulting mixture was stirred for 15 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off, and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 99% yield (0.23 g, 0.22 mmol). Analytical data for **4.2**: R_f = 0.55 (acetone/toluene, 1/9 v/v); $[\alpha]_D^{23}$ +34.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.09 (t, 3H, *J* = 7.4 Hz, CH_2CH_3). 2.56 (m, 2H, CH_2CH_3), 3.30-3.51 (m, 4H, H-3′, 5′, 6a′, 6b′), 3.57-3.72 (m, 2H, H-4, 5), 3.77 (br d, 2H, *J* = 2.1 Hz, H-6a, 6b), 3.94 (br d, 1H, $J_{3',4'}$ = 2.5 Hz, H-4′), 4.26 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 4.29 (d, 1H, *J* = 12.0 Hz, CHPh), 4.30 (dd, 1H, $J_{2,3}$ = 10.3, H-2), 4.44 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.454.64 (m, 5H, 5 × CHPh), 4.83 (dd, 1H, $J_{3,4}$ = 8.0 Hz, H-3), 4.91 (d, 1H, *J* = 11.3 Hz, CHPh), 5.06 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.09 (d, 1H, *J* = 10.5 Hz, CHPh), 5.52 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 6.92-7.78 (m, 34H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.9, 23.7, 54.8, 67.8, 69.2, 71.7, 72.5, 72.8, 73.3, 73.4, 73.5, 74.8, 75.0, 77.4, 77.8, 79.5, 80.3, 81.0, 100.7, 127.2, 127.5 (×3), 127.6 (×2), 127.8 (×4), 128.0 (×3), 128.1 (×6), 128.2 (×3), 128.3 (×3), 128.4 (×3), 128.5 (×3), 130.0 (×2), 130.3, 131.5, 132.8, 134.0, 137.5, 138.0, 138.3, 138.7 (×2), 165.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{64}H_{63}NNaO_{12}S$ 1092.3969, found 1092.3981.

Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (4.10). A mixture of compound 2 (0.236 g, 0.234 mmol), dibutyl hydrogen phosphate (0.14 mL, 0.802 mmol), and freshly activated molecular sieves (3 Å, 0.5 g) in CH_2Cl_2 (5.0 mL) was stirred under argon for 1 h at rt. The mixture was cooled to 0 °C, NIS (0.104 g, 0.468 mmol) and TfOH (4.15 μL, 0.047 mmol) were added, and the resulting mixture was stirred for 20 min at 0 °C. After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with 10% aq. $Na_2S_2O_3$ (15 mL) and sat. aq. $NaHCO_3$ (15 mL), and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an oily syrup in 94% yield (0.267 g, 0.219 mmol). Analytical data for 4.10: R_f = 0.35 (acetone/toluene,1/9, v/v); $[\alpha]_D^{23}$ +26.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.67 (t, 3H, J = 7.2 Hz, $O(CH_2)_3CH_3$), 0.81 (t, 3H, J = 7.3 Hz, $O(CH_2)_3CH_3$), 0.90-1.06 (m, 2H, $O(CH_2)_2CH_2CH_3$), 1.11-1.31 (m, 4H, $O(CH_2)_2CH_2CH_3$, $OCH_2CH_2CH_2CH_3$), 1.38-1.53 (m, 2H, $OCH_2CH_2CH_2CH_3$), 3.31-3.96 (m, 13H, H-3′, 4, 4′, 5, 5′, 6a, 6b, 6a′, 6b′, 2 x $OCOCH_2(CH)_2CH_3$), 4.20 (d, 1H, ^2J = 11.7 Hz, C*H*Ph), 4.24-4.32 (m, 3H, H-2, 2 x C*H*Ph), 4.43-4.54 (m, 5H, H-1, 4 x C*H*Ph), 4.59 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.91 (m, 2H, H-3, C*H*Ph), 5.09 (d, 1H, ^2J = 10.5 Hz, C*H*Ph), 5.51 (dd, 1H, $J_{2',3'}$ = 8.8 Hz, H-2′), 5.69 (dd, 1H, $J_{1,2}$ = 7.4 Hz, H-1) 6.94-7.74 (m, 36H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.5, 13.6, 18.3, 18.6, 31.8 (d, J = 7.1 Hz). 32.0 (d, J = 7.2 Hz), 56.1, 56.2, 67.6, 67.8 (d, J = 4.5 Hz), 68.0 (d, J = 6.4 Hz), 68.6, 71.7, 72.5, 72.8, 73.3, 73.5 (×2), 74.8, 74.9, 75.6, 76.2, 80.2, 94.1 (d, J = 4.6 Hz), 100.8, 123.5, 127.3, 127.5 (×2), 127.6 (×3), 127.7, 127.8 (×3),

128.0 (×7), 128.1 (×4), 128.2 (×4), 128.3 (×2), 128.4 (×2), 128.5 (×2), 130.0, 130.2, 131.5, 132.7, 134.0, 137.5, 138.0, 138.1, 138.6, 138.7, 165.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{70}H_{76}NNaO_{16}P$ 1240.4799, found 1240.4829.

Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.14). A mixture of donor **4.13** (0.14 g, 0.15 mmol), acceptor **4.3**[25] (0.12 g, 0.12 mmol) (reference from LNnT paper), and freshly activated molecular sieves (3Å, 450 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h. The mixture was cooled to -30 ºC, TMSOTf (56 μL, 0.31 mmol) was added, and the resulting mixture was stirred for 15 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 86% yield (0.17 g, 0.10 mmol). Analytical data for **4.14**: R_f = 0.45 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{23}$ +11.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.91 (ddd, 1H, *J* = 1.8, 3.0, 9.9 Hz, H-5), 3.27 (dd, 1H, *J* = 9.5 Hz, H-6a), 3.33 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 3.38-3.42 (m, 2H, H-3, 6b), 3.69-3.75 (m, 3H, H-3′, 5′, $OCOCH_2CH$), 3.79-3.85 (m, 4H, H-4, 5″, 6a″, 6b″), 3.87-3.93 (m, 2H, H-4″, $OCOCH_2CH$), 4.02 (dd, 1H, *J* = 10.5, 7.2 Hz, $OCOCH_2CH$), 4.11 (br d, 1H, *J* = 2.4 Hz, H-4′), 4.18-4.24 (m, 2H, H-6a′, C*H*Ph), 4.27 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.34-4.40 (m, 2H, H-2″, 6b′), 4.42-4.49 (m, 3H,

$J_{1',2'}$ = 8.1 Hz, H-1′, 2 x C*H*Ph), 4.5-4.69 (m, 7H, 7 x C*H*Ph), 4.80 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.81 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.92 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.12 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 5.36 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′), 5.44 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1″), 5.66 (dd, 1H, *J* = 8.9, 10.7 Hz, H-3″), 6.81-8.73 (m, 56H, aromatic) ppm; ^{13}C NMR (151 MHz, cdcl$_3$): δ, 46.4, 55.2, 63.8, 67.6, 68.9, 70.3, 71.0, 71.9, 72.0, 73.5 (×2), 73.6 (×2), 74.4, 74.7, 75.0 (×2), 75.2, 75.6, 76.2, 76.6, 80.6, 81.7, 82.6, 99.4, 100.4, 102.6, 120.0, 125.0, 125.3, 125.5, 126.9, 127.2 (×2), 127.3, 127.6 (×2), 127.7 (×3), 127.8 (×3), 127.9 (×5), 128.0, 128.1 (×9), 128.3 (×6), 128.4 (×3), 128.5 (×3), 128.6 (×9), 128.7 (×3), 129.4, 129.8, 132.9, 137.0, 137.6 (×2), 137.9, 138.3, 138.7 (×2), 139.0, 141.1, 141.2, 142.9, 143.3, 147.8, 150.0, 154.6, 164.5, 164.5 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{103}H_{94}N_2NaO_{21}$ 1718.6280, found 1718.6222.

Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.15). Compound **4.14** (135 mg, 0.0796 mmol) was dissolved in a mixture of Et_3N in CH_2Cl_2 (5.0 mL, 3/7, v/v) and the resulting solution was stirred for 2 h at rt. After that, the reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 89% yield (104.8 mg, 0.0711 mmol). Analytical data for **4.15**: R_f = 0.55 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{22}$ -11.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.45 (d, 1H, *J* = 4.6 Hz, OH), 2.94 (ddd, 1H, *J* = 8.9 Hz, H-5), 3.29 (dd, 1H, *J* = 10.3 Hz, H-6a), 3.34 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.39-3.44 (m, 2H, H-3, 6b), 3.61 (dd, 1H, $J_{3'',4''}$ = 9.1 Hz, $J_{4'',5''}$ = 9.1 Hz, H-4″), 3.67-3.76 (m, 3H, H-3′, 5″,

6a″), 3.78-3.88 (m, 3H, H-4, 5′, 6b″), 4.11 (br s, 1H, H-4′), 4.13-4.26 (m, 3H, H-2″, 6a′, C*H*Ph), 4.29 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.36 (dd, 1H, *J* = 6.1, 11.0 Hz, H-6b′), 4.44-4.52 (m, 4H, $J_{1',2'}$ = 8.0 Hz, H-1′, 3″, 2 x C*H*Ph), 4.55-4.70 (m, 6H, 6 x C*H*Ph), 4.74 (d, 1H, ^2J = 11.4 Hz, C*H*Ph), 4.82 (d, 2H, ^2J = 12.0 Hz, C*H*Ph × 2), 4.94 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.14 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 5.22 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1″), 5.34-5.39 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 7.00-8.75 (m, 48H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 56.8, 63.9, 67.5, 69.1, 71.0 (×2), 72.0 (×2), 73.4, 73.6 (×2), 74.3, 74.9 (×3), 75.1, 75.6, 76.1, 79.2, 80.3, 81.6, 82.6, 99.7, 100.3, 102.5, 125.4, 126.9, 127.2, 127.5, 127.7 (×3), 127.8 (×3), 127.9, 128.0 (×8), 128.1 (×4), 128.2 (×3), 128.3 (×6), 128.4 (×3), 128.5 (×3), 128.6 (×6), 128.7 (×3), 129.4, 129.6, 132.9, 133.6, 137.0, 137.5, 137.9, 138.1, 138.2, 138.6, 138.8, 139.0, 147.7, 149.9, 164.4 (×2) ppm; ESI TOF LCMS $[M+H]^+$ calcd for $C_{88}H_{85}N_2O_{19}$ 1473.5747, found 1473.5757.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.11). ***Convergent method.*** A mixture of donor **4.10** (80.0 mg, 0.065 mmol), acceptor **4.3** (49.8 mg, 0.050 mmol),[25] and freshly activated molecular sieves (3Å, 400 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h. The mixture was cooled to -60 °C, TMSOTf (24 μL, 0.131 mmol) was added, and the resulting mixture was stirred for 30 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2

x 10 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 51% yield (66.6 mg, 0.033 mmol). ***Linear method.*** A mixture of benzoxazolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside[29,30] (**4.4**, 13.3 mg, 0.0194 mmol), acceptor **4.15** (22 mg, 0.0149 mmol), and freshly activated molecular sieves (3Å, 100 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 2 h. The reaction mixture was cooled to -30 ºC, freshly conditioned AgOTf (10.0 mg, 0.0387 mmol) was added, and the resulting mixture was stirred for 15 min while the temperature was allowed to increase gradually. The reaction mixture was then diluted with CH_2Cl_2, the solids were filtered off and was rinsed successively with CH_2Cl_2. The combined filtrate (~30 mL) was washed with sat. aq. $NaHCO_3$ (7 mL) and water (2 x 7 mL) The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 89% yield (26.7 mg, 0.0132 mmol). Analytical data for **4.11**: R_f = 0.45 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.85 (ddd, 1H, J = 9.6 Hz, H-5), 3.19 (dd, 1H, J = 10.2 Hz, H-6a), 3.25-3.37 (m, 6H, H-2, 3, 3‴, 6a‴, 6b, 6b‴), 3.43 (m, 1H, H-5‴), 3.58-3.66 (m, 3H, H-3′, 4″, 5′), 3.70 (m, 1H, H-5″), 3.84-3.75 (m, 3H, H-4, 6a″, 6b″), 3.88 (br d, 1 H, J = 1.9 Hz, H-4‴), 3.98 (s, 1H, H-4′), 4.14-4.30 (m, 9H, H-1, 1‴, 2″, 6a′, 6b′, 4 x C*H*Ph), 4.33 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.41-4.60 (m, 9H, 9 x C*H*Ph), 4.66 (d, 1H, ^2J = 11.0 Hz, C*H*Ph), 4.78-4.82 (m, 3H, H-3″, 2 x C*H*Ph), 4.89 (d, 2H, ^2J = 11.0 Hz, 2 x C*H*Ph), 4.95 (d, 1H, ^2J = 11.7 Hz, C*H*Ph), 5.00 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1″), 5.06 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 5.17 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2′), 5.45 (dd, 1H, J

$_{2'',3''}$ = 8.9 Hz, H-2‴), 6.75-8.69 (m, 68H, aromatic) ppm: ^{13}C NMR (151 MHz, $CDCl_3$): δ, 29.8, 31.1, 56.0, 63.8, 67.4, 67.8, 69.5, 71.0, 71.8, 72.0 (× 2), 72.6, 73.2, 73.5 (× 2), 73.6, 74.4, 74.8, 74.9, 75.0, 75.1, 75.2, 75.6, 75.8 (× 2), 76.1, 79.4, 80.2, 81.6, 82.6, 99.3, 100.4, 100.5, 102.6, 122.7, 123.7, 125.4, 126.8, 127.3, 127.4 (×2), 127.5 (×4), 127.6 (×2), 127.7, 127.8 (×7), 127.9, 128.0 (×3), 128.1 (×7), 128.2 (×8), 128.3 (×4), 128.4 (×7), 128.5 (×6), 128.6 (×2), 128.8 (×2), 129.6, 129.8, 130.0, 130.2, 130.9, 131.3, 132.7, 133.0, 133.4, 134.6, 136.9, 137.5, 137.6, 138.0, 138.1, 138.2, 138.5, 138.7, 138.8, 139.0, 147.8, 150.0, 164.3, 164.4, 165.5, 166.3, 168.4 ppm; ESI TOF LCMS $[M+H]^+$calcd for $C_{122}H_{117}N_2O_{25}$ 2010.7979, found 2010.7963.

4.4.4. Deprotection of tetrasaccharide 4.11

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4.16). Compound **4.11** (59.0 mg, 0.029 mmol) was dissolved in MeOH (3.0 mL), NH_2NH_2-H_2O (130 μL, 2.64 mmol) was added, and the resulting mixture was heated at 90 ^oC for 24 h. After that, the volatiles were removed under reduced pressure, and the residue was dried *in vacuo* for 3 h. The crude residue was dissolved in a mixture of Ac_2O and MeOH (2.0 mL, 1/1, v/v) and the resulting mixture was stirred for 12 h at rt. The volatiles were removed under reduced pressure, the residue was diluted with CH_2Cl_2 (50 mL), and washed with sat. aq. $NaHCO_3$ (10 mL) and 1 M HCl (10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-

white amorphous solid in 92% yield (42.9 mg, 0.026 mmol). Analytical data for **4.16**: R_f = 0.50 (acetone/toluene, 3/7 v/v); $[\alpha]_D^{23}$ +70.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.81 (s, 3H, C*H*$_3$CO), 2.95-4.03 (m, 24H, H-2, 2′, 2″, 2‴, 3, 3′, 3″, 3‴, 4, 4′, 4″, 4‴, 5, 5′, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″,6b‴), 4.23 (d, 1H, ^2J = 11.8 Hz, C*H*Ph), 4.30-4.58 (m, 10H, H-1, 1′, 1‴, 7 x C*H*Ph), 4.60-4.96 (m, 11H, 11 x C*H*Ph), 5.01-5.04 (m, 2H, H-1″, C*H*Ph), 6.48 (d, 1H, *J* = 6.3 Hz, N*H*COCH$_3$), 7.12-7.37 (m, 50H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.7, 58.4, 61.9, 68.2, 68.8, 69.4, 71.3, 71.6, 71.9, 72.1, 72.8, 73.5, 73.7 (×3), 73.8, 74.3, 74.7, 74.8, 74.9, 75.1 (×2), 75.2, 75.4, 76.6, 77.1, 81.9, 82.1, 83.0, 83.1, 83.7, 101.8, 102.9, 103.1, 104.5, 127.4, 127.5, 127.6 (×3), 127.7 (×2), 127.8 (×3), 127.9 (×4), 128.0 (×7), 128.2 (×8), 128.3 (×6), 128.4 (×4), 128.5 (×6), 128.7 (×3), 128.8 (×2), 137.6, 138.0 (×2), 138.1, 138.2, 138.4, 138.6, 138.7, 138.9, 139.0, 172.3 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{96}H_{105}NNaO_{21}$ 1631.7110, found 1631.7121.

***O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (4.1, LNT).** 10% Pd on carbon (125 mg) was added to a solution of tetrasaccharide 16 (40 mg, 0.025 mml) in 80% aq. EtOH (5.0 mL), and the resulting mixture was stirred under hydrogen atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~40 mL) was concentrated in *vacuo*. The residue was purified by size exclusion column chromatography on Sephadex G-25 using water as the eluent to afford the title compound as a white amorphous solid in 81% yield (14.3 mg, 0.020 mmol). Analytical data for **4.1**: R_f = 0.30 (chloroform/methanol/water, 2/1/0.4, v/v/v); ^{1}H NMR (600 MHz, D_2O): δ, 2.01 (s, 3H, C*H*$_3$CO), 3.24-3.51 (m, 2H), 3.49-3.96 (m, 33H), 4.14 (d,

1H, *J* = 3.3 Hz), 4.43 (d, 2H, *J* = 7.8 Hz), 4.65 (d, 1H, *J* = 8.0 Hz), 4.72 (dd, 1H, *J* = 2.5, 8.4 Hz), 5.21 (d, 1H, *J* = 3.8 Hz) ppm; ^{13}C NMR (151 MHz, D_2O): δ, 22.6, 55.0, 60.3, 60.4, 60.8, 61.3, 61.4, 68.7, 68.8, 68.9, 70.4, 70.5, 71.0, 71.5, 71.8, 72.8, 74.1, 74.7, 75.1, 75.2, 75.5, 75.6, 78.6, 78.7, 82.3, 82.4, 92.2, 96.0, 96.1, 102.9, 103.2, 103.3, 103.8, 175.3 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{26}H_{45}NNaO_{21}$ 730.2382, found 730.2361.

4.5. References

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CHAPTER 5

The chemical synthesis of human milk oligosaccharides: lacto-*N*-hexaose

Galβ1→3GlcNAcβ1→3

[Galβ1→4GlcNAcβ1→6] Galβ1→4Glc

5.1. Introduction

Thanks to advances in glycosciences in recent years, the importance of human milk oligosaccharides (HMO) as the essential source of antimicrobials[1,2] and prebiotics[3] has come to the fore. Biosynthesis of HMO follows a unique structural blue print. All glycan chains contain lactose (Galβ1→4Glc) at their reducing end, which can be elongated by the addition of a β1-3- or β1-6-linked lacto-N-biose (Galβ1→3GlcNAc, type 1 chain) or lactosamine (Galβ1→4GlcNAc, type 2 chain). Elongation of lactose via a β1-6 linkage introduces chain branching, and these structures are designated as *iso*-HMO, whereas linear structures are commonly designated as *para*-HMO. Lactose or the elongated oligosaccharide chain can also be fucosylated or sialylated.[4] Structures of many HMO are known,[5,6] many HMO sequences have already been prepared enzymatically and/or chemically,[7-9] but the exact roles of a majority of individual HMO remain unknown.[10,11]

With expectation that the development of reliable synthetic methods for obtaining individual HMO will boost our understanding the roles of these important biomolecules, we previously reported the total syntheses of lacto-N-neotetraose (LNnT, Chapter 3)[12] and lacto-N-tetraose (LNT, Chapter 4).[13] In further synthetic endeavors into the field of HMO, we became interested in studying more challenging, branched structures, such as lacto-N-hexaose (LNH),[14,15] whose exact biological roles are yet unknown. LNH **5.1** represents one of the most common core sequence and it is structurally derived from LNT **5.2** (Figure 5.1).

LNH comprises the reducing end lactose disaccharide (*AB*, Figure 5.1), common for all HMO, which is branched at the galactose residue (*B*). More specifically, it is elongated with lacto-N-biose disaccharide residue (*CD*) at C-3 of Gal, and also with N-

acetyllactosamine residue (*EF*) at C-6 of Gal. While many chemical[16-18] and enzymatic syntheses[19] of LNT are known, to the best of our knowledge, neither chemical nor enzymatic synthesis of LNH have yet been reported. We came across an article by Schmidt and co-worker describing the synthesis of a "*branched hexasaccharide related to lacto-N-hexaose*."[20] However, the actual molecule obtained therein is lacto-N-neohexaose, a different HMO core sequence. Human milk isolates of LNH are available on the market, but this common HMO is not yet available in large quantities and at accessible cost for mainstream research and application. Reported herein as an efficient and versatile method for the scalable synthesis of LNH **5.1** and, by extension, LNT **5.2**.

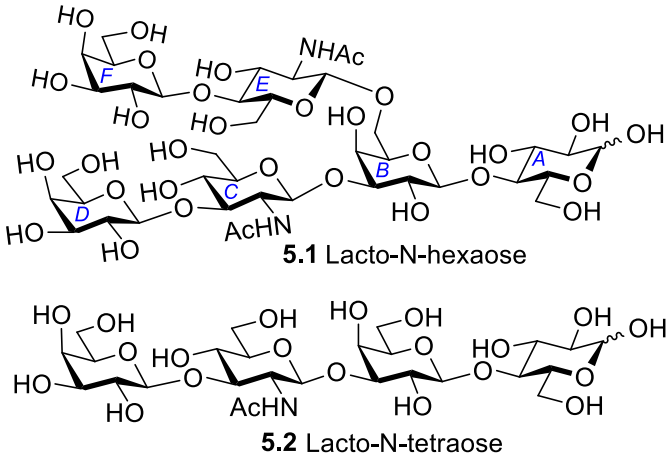
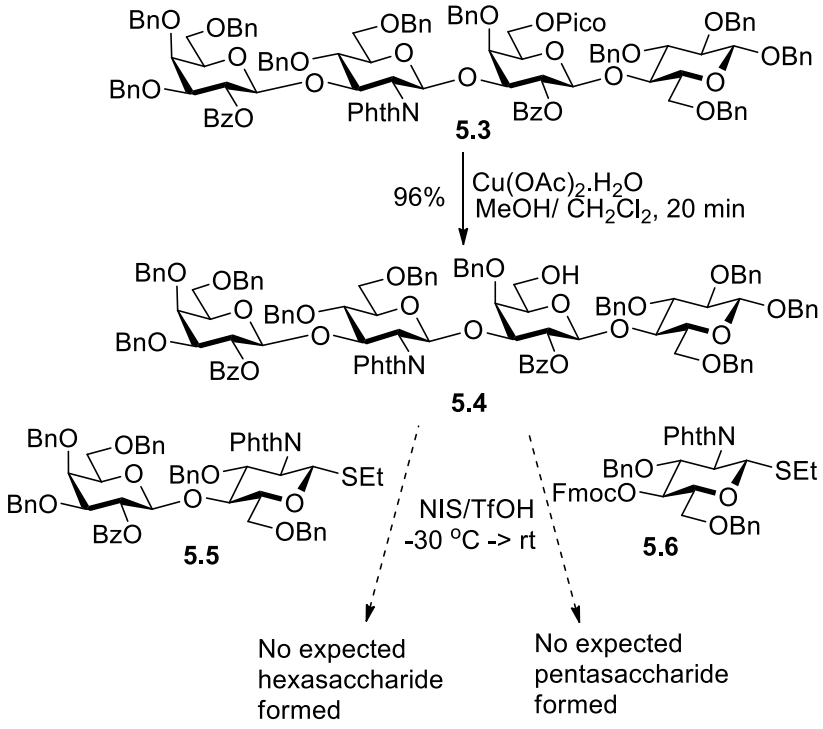


Figure 5.1. Chemical structures of LNH 5.1 and LNT 5.2.

5.2. Results and discussion

In the previous Chapter, we described the syntheses of LNT **5.2** via convergent and linear approaches.[13] The protecting groups in the tetrasaccharide intermediate **5.3**[13] were strategically placed to allow for the synthesis of other derivatives. Thus, we assumed that selective removal of 6′-*O*-picoloyl (Pico) substituent in **5.3** would provide a straightforward access to acceptor **5.4** suitable for subsequent branching. To pursue this

route, the deprotection of 6′-O-Pico in **5.3** was affected in the presence of $Cu(OAc)_2$-H_2O in MeOH/CH_2Cl_2 and tetrasaccharide **5.4** was obtained in 96% yield. To our disappointment, all attempts to glycosylate acceptor **5.4** with lactosamine thioglycoside donor **5.5**[12] were largely unsuccessful and practically no desired hexasaccharide was formed. Instead, a large number of unidentified side products derived from the donor have been isolated, and the acceptor remained practically intact.

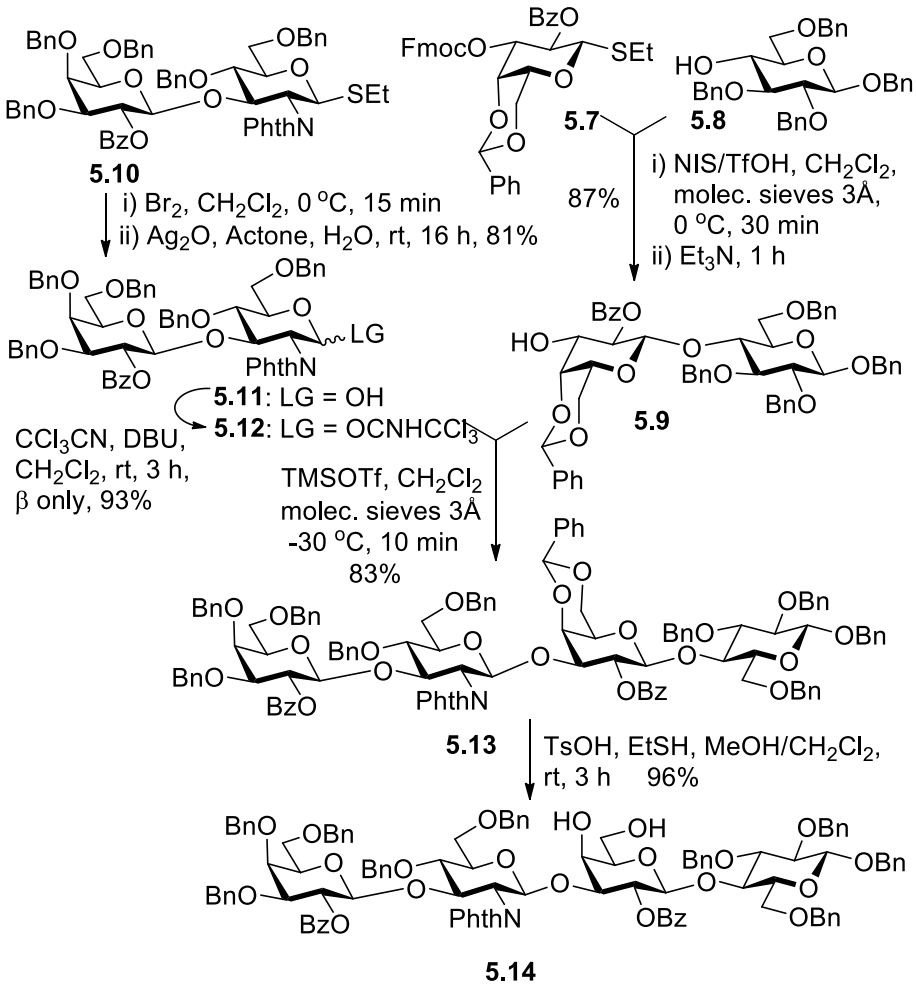


Scheme 5.1. First attempted assembly of LNH 5.1.

Recalling our previous endeavors with the synthesis of HMO wherein the nature of glycosyl donor and acceptor was found to be of paramount significance, we attempted to employ the corresponding lactosamine phosphate and trichloroacetimidate donors. Despite extended study, these attempts to access the backbone of LNH were also unsuccessful. In further attempts to enhance the efficiency of this coupling we applied less bulky

glucosamine thioglycoside donor **5.6** and its phosphate and trichloroacetimidate analogues. Although the formation of minor amounts of the desired pentasaccharide were observed by mass spectroscopy, these glycosylations were deemed unsuccessful. Having explored all possibilities available to us, we came to a conclusion that the bulky benzyl group at the 4′-OH position of acceptor **5.4** is hindering the glycosylation at the 6′-OH position.

In order to decrease the steric hindrance, we changed our strategy and employed building block **5.7** protected with 4,6-*O*-benzyliene acetal instead.[21] We envisaged that if the benzylidene acetal is removed at the tetrasaccharide stage, the resulting 4,6-diol will offer a far more accessible glycosyl acceptor site for glycosylation with donor **5.5**.



Scheme 5.2. Convergent synthesis of acceptor 5.14.

With this strategic adjustment, we coupled donor **5.7** with 4-OH acceptor **5.8**[22] in the presence of N-iodosuccinimide (NIS) and TfOH. The resulting disaccharide was subjected to deprotection of the Fmoc group with triethylamine in one pot affording the desired disaccharide acceptor **5.9** in 87% yield over two steps. We then attempted to glycosylate acceptor **5.9** with thioglycoside donor **5.10**. This reaction was sluggish and inefficient, and despite all attempts to push the reaction to completion, significant amounts of acceptor remained. After a quick screening of other leaving groups, we discovered that trichloroacetimidate donor **5.12** offers an effective building block to glycosylation acceptor **5.9**. Glycosyl donor **5.12** was prepared from thioglycoside precursor **5.10** that was first converted into the corresponding hemiacetal **5.11** via anomeric bromination using Br_2 in CH_2Cl_2 followed by the hydrolysis of the bromide using Ag_2O in wet acetone in 81% over two steps. Next, the installation of the trichloroacetimidate group was performed using trichloroacetonitirle (CCl_3CN) in the presence of 1,8-diazabicylco[5.4.0]undec-7-ene (DBU) to afford the lacto-N-biose trichloroacetimidate **5.12** in 93% yield. Glycosylation of acceptor **5.9** with donor **5.12** was conducted in the presence of catalytic TMSOTf and the desired tetrasaccharide **5.13** was obtained in a good yield of 83% with complete β-stereoselectivity. It is noteworthy that the corresponding glycosyl phosphate donor gave a comparable result in this application.

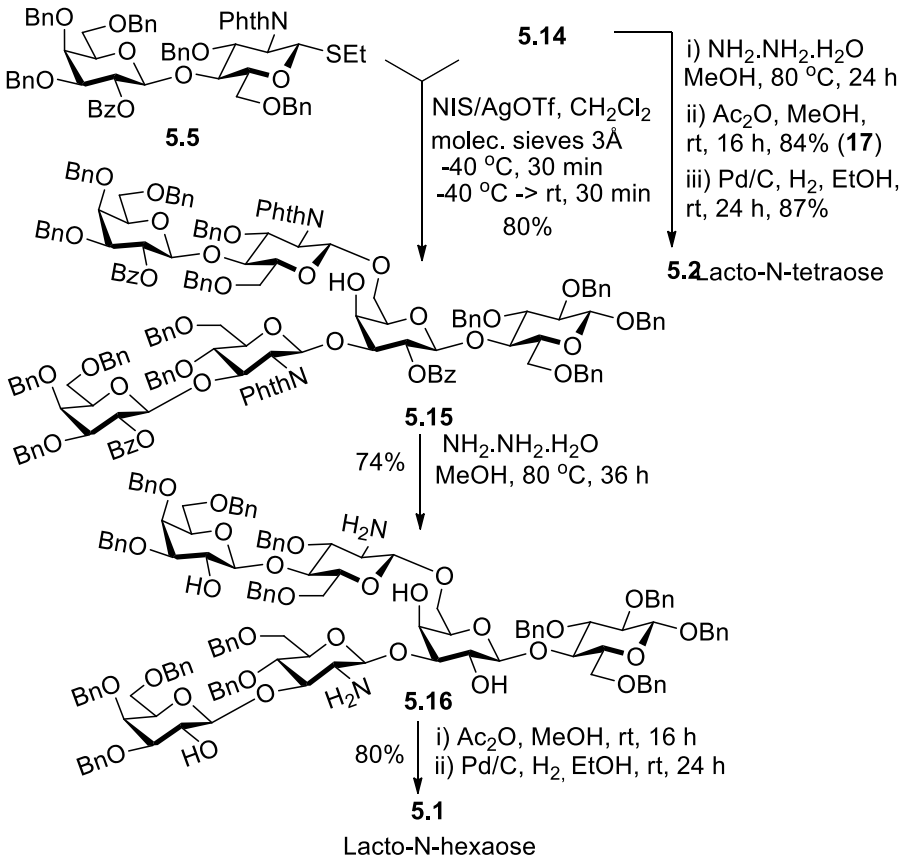
Subsequent deprotection of the 4′,6′-O-benzylidene acetal in intermediate **5.13** was somewhat low-yielding in the presence of TFA in wet DCM, traditional conditions for benzylidene cleavage. Inspired by work by Williams and Sit, wherein isopropylidene ketal was removed using p-toluenesulfonic acid (TsOH) and ethanedithiol in chloroform,[23] we

conducted this reaction in the presence of TsOH and EtSH in MeOH/CH_2Cl_2. As a result, tetrasaccharide diol **5.14** was obtained in 96% yield.

Next, we attempted glycosylation of tetrasaccharide diol **5.14** with lactosamine thioglycoside donor **5.5**. Even this coupling with a more accessible glycosyl acceptor was not as straightforward as we had initially hoped for. Standard reaction conditions employing NIS/TfOH as promoters produced the desired hexasaccharide **5.14** in a modest yield of 51%. Although this result was significantly better than our previous attempt of glycosylation of acceptor **5.4**, we wanted to make an effort to further improve the outcome of this challenging coupling. With this intention, we identified the major side reactions hampering the yield of the product: fairly rapid hydrolysis of lactosamine donor **5.5** and the formation of the 1-4-linked hexasaccharide **5.18** alongside the anticipated 1-6-linked counterpart. To suppress these side reactions, we employed milder promoters dimethyl(thiomethyl)sulfonium triflate (DMTST)[24,25] and NIS/AgOTf.[26] The former promoter provided a comparable result that that achieved with NIS/TfOH. With the latter promoter, known for providing a slower release of the iodonium ion, minimal side products resulting from hydrolysis of donor **5.5** were observed. After careful refinement of the reaction conditions, we achieved the desired protected LNH precursor **5.15** in a commendable yield of 80%. This result was obtained by conducting the reaction at -40 ºC for 30 min, and then allowing the reaction temperature to gradually increase over the course of the additional 30 min period.

With the protected intermediate **5.15** in hand, we then endeavored a series of deprotection steps to obtain the target LNH hexasaccharide. Deprotection of the phthalimido and NH_2NH_2-H_2O in MeOH to afford intermediate **5.16** in 74% yield. Column

purification was found necessary before the subsequent step: N-acetylation of **5.16** with acetic anhydride in MeOH. Finally, the benzyl groups were removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol to obtain target LNH **5.1** in 80% over two steps.



Scheme 5.3. Convergent synthesis of LNH 5.1 and LNT 5.2

We also performed deprotection of the key tetrasaccharide intermediate **5.14** to obtain LNT **5.2**. This was achieved via deprotection of the phthalimido and ester groups in the presence of NH_2NH_2-H_2O in refluxing MeOH followed by N-acetylation with acetic anhydride in MeOH to furnish partially protected tetrasaccharide intermediate **5,17** in 84%

yield. Subsequently, benzyl ethers were removed by hydrogenation in the presence of 10% Pd/C in wet ethanol to afford target LNT **5.2** in 87% yield.

5.3. Conclusions

The first total synthesis of lacto-N-hexaose has been completed using a convergent 2+2+2 strategy. This approach employed preassembled lactose, lactosamine, and lacto-N-biose building blocks. Along the way, we have also obtained lacto-N-tetraose core sequence. It has been acknowledged that including HMO to infant formulas could be beneficial for infants' health,[27-30] but HMO are challenging to produce and purify, and exact roles of individual HMO remain unknown.[10,11,31] Only two simple HMO have been approved for infant formulas, and three more entered clinical trials.[32] With expectation that new methods for reliable synthesis of individual HMO will boost practical applications of these important biomolecules further synthetic studies of HMO are described in the subsequent Chapter.

5.4. Experimental

5.4.1. General methods

Reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh) and Sephadex G-25 size exclusion resin, reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ^{o}C. CH_2Cl_2 was distilled from CaH_2 directly

prior to application. Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured using a Jasco polarimeter. ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 151 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual TMS (δ_H = 0.00 ppm) for solutions in $CDCl_3$ or the signal of the residual D_2O (δ_H = 4.79 ppm) for solutions in D_2O. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.16 ppm) for solutions in $CDCl_3$ or the central signal of CD_3COCD_3 δ_C = 29.84 ppm) for solutions in D_2O. Accurate mass spectrometry determinations were performed using an Agilent 6230 ESI TOF LCMS mass spectrometer.

5.4.2. Synthesis of oligosaccharides 5.4, 5.9, 5.11, 5.12, 5.13, 5.14, 5.15, 5.18.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.4). $Cu(OAc)_2$-H_2O (15.3 mg, 0.084 mmol) was added to a solution of benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[13] (**5.3**, 113.0 mg, 0.056 mmol) in MeOH/CH_2Cl_2 (8.0 mL, 1/3, v/v) and the resulting mixture was stirred for 20 min at rt. Next, the reaction mixture was diluted with CH_2Cl_2 (~100 mL) was washed with H_2O (15 mL), sat. aq. $NaHCO_3$ (15 mL) and water (2 x 15 mL). The organic phase was

separated, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 96% yield (102.3 mg, 0.053 mmol). Analytical data for **5.4**: R_f = 0.60 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ +5.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.84 (ddd, 1H, *J* = 8.9 Hz, H-5), 3.10-3,45 (m, 11H, H-2, 3, 3‴, 5″, 5‴, 6a, 6a″, 6a‴, 6b, 6b″, 6b‴), 3.54-3.60 (m, 2H, H-3′, 4″), 3.69-3.77 (m, 3H, 4, 5″, 6a″), 3.80 (br d, 1H, $J_{3',4'}$ = 2.2 Hz, H-4′), 3.87-3.89 (m, 2H, H-4‴, 6b′), 4.13-4.18 (m, 2H, H-1′, 2″), 4.20-4.30 (m, 6H, H-1, 1‴, 2 x C*H*$_2$Ph), 4.42-4.59 (m, 10H, 5 x C*H*$_2$Ph), 4.67 (d, 1H, ^2J = 11.1 Hz, C*H*Ph), 4.78-4.91 (m, 6H, H-3″, 5 x C*H*Ph), 4.98 (d, 1H, $J_{1'',2''}$ = 8.4 Hz, H-1″), 5.08 (d, 1H, ^2J = 10.5 Hz, C*H*Ph), 5.12 (dd, 1H, $J_{1',2'}$ = 8.1, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.46 (dd, 1H, $J_{1''',2'''}$ = 8.7, $J_{2''',3'''}$ = 9.4 Hz, H-2‴), 6.73-7.66 (m, 64H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 56.0, 62.0, 67.3, 67.8, 69.7, 71.0, 71.7, 72.0, 72.5, 72.7, 73.2, 73.5 (×2), 73.6 (×2), 74.3, 74.6, 74.8, 74.9, 75.0, 75.2, 75.4, 75.8, 75.9, 76.0, 77.6, 79.8, 80.2, 81.2, 82.7, 99.3, 100.5 (×2), 102.6, 114.0, 122.7 (×2), 127.5 (×3), 127.6 (×4), 127.7 (×2), 127.8 (×5), 127.9 (×4), 128.0 (×2), 128.1 (×6), 128.2 (×9), 128.3 (×9), 128.4 (×2), 128.5 (×4), 128.6 (×5), 129.0 (×2), 129.6, 129.8, 130.0, 130.1, 130.8, 131.3, 132.7, 133.0, 133.4, 133.6, 137.5, 137.6, 138.0, 138.2, 138.5, 138.6, 138.7, 138.8, 164.3, 165.5, 166.3, 168.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{116}H_{113}NNaO_{24}$ 1927.7584, found 1927.7613.

Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.9). A mixture of ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside[21] (**5.7**, 0.30 g, 0.45

mmol), benzyl 2,3,6-tri-*O*-benzyl-β-D-gulcopyranoside[33] (**5.8**, 0.19 g, 0.36 mmol), and freshly activated molecular sieves (3 Å, 1.0 g) in CH_2Cl_2 (10 mL) was stirred under argon for 2 h at rt. The reaction mixture was cooled to 0 ºC, NIS (0.21 g, 0.94 mmol) and TfOH (18 µL, 0.09 mmol) were added, and the resulting mixture was stirred for 30 min. After that, the reaction mixture was warmed to rt, Et_3N (~ 4.5 mL) was added, and the resulting mixture was stirred for 1 h at rt to achieve complete deprotection of the Fmoc group. Next, the solids were filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with sat. aq. $NaHCO_3$ (15 mL), 10% aq. $Na_2S_2O_3$ (15 mL), and water (2 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 87% yield (0.28 g, 0.31 mmol). Analytical data for **5.9**: R_f = 0.35 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{23}$ -15.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$); δ, 2.51 (d, 1H, J = 11.4 Hz, OH), 3.11 (br s, 1H, H-5′), 3.28 (ddd, 1H, H-5), 3.49 (dd, 1H, $J_{2,3}$ = 9.1 Hz, H-2), 3.59-3.67 (m, 3H, H-3, 3′, 6a), 3.71 (dd, 1H, $J_{5,6b}$ = 4.2, $J_{6a,6b}$ = 11.0, Hz, H-6b), 3.87 (dd, 1H, $J_{5',6a'}$ = 1.5, $J_{6a',6b'}$ = 12.5 Hz, H-6a′), 3.96 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4), 4.11 (br d, 1H, $J_{3',4'}$ = 3.6 Hz, H-4′), 4.21 (d, 1H, $J_{6a',6b'}$ = 12.5 Hz, H-6b′), 4.45 (dd, 2H, ^2J = 12.2 Hz, CH_2Ph), 4.43 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.45 (dd, 2H, ^2J = 12.2 Hz, CH_2Ph), 4.74 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 4.76 (d, $J_{1',2'}$ = 8.1 Hz, H-1′), 4.81 (dd, 2H, ^2J = 10.9 Hz, CH_2Ph), 4.97 (dd, 2H, ^2J = 10.8 Hz, CH_2Ph), 5.32 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-2′), 5.51 (s, 1H, C*H*Ph), 7.16-8.05 (m, 30H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 66.7, 68.4, 68.8, 71.2, 72.0, 72.2, 72.4, 73.4, 73.6, 74.6, 75.0, 75.6, 75.8, 77.8, 100.8, 101.6, 102.5, 126.6 (×2), 127.4, 127.6, 127.8 (×2), 127.9 (×2), 128.0 (×2), 128.1 (×2), 128.2 (×2), 128.3 (×4), 128.4 (×2), 128.5 (×4),

128.6 (×2), 129.3, 129.8, 129.9 (×2), 133.4, 137.5, 137.6, 138.4, 138.6, 139.1, 166.0 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{54}H_{54}NaO_{12}$ 917.3513, found 917.3521.

***O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β-D-glucopyranose (5.11).** A freshly prepared solution of Br_2 in DCM (6.5 mL, 1/165, v/v) was added to a pre-chilled solution of ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[13] (**5.10**, 0.73 g, 0.68 mmol) in CH_2Cl_2 (9.0 mL) and the resulting mixture was stirred for 15 min at 0 ºC. After that, the volatiles were evaporated under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (3 × 10 mL) and dried in *vacuo* for 2 h. The crude residue was dissolved in acetone (20 mL), water (1.0 mL) and Ag_2CO_3 (0.09 g, 0.34 mmol) were added, and the resulting mixture was stirred in the absence of light for 16 h at rt. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 81% yield (0.56 g, 0.55 mmol). Analytical data for β-**5.11**: R_f = 0.35 (acetone/toluene, 1/4, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.86 (d, 1H, J = 8.8 Hz, OH), 3.30 (m, 1H, H-5′), 3.38-3.45 (m, 3H, H-3′, 6a′, 6b′), 3.60-3.65 (m, 2H, H-4, 5) 3.72-3.74 (m, 2H, H-6a, 6b), 3.94 (br d, 1H, H-4′), 4.06 (dd, 1H, J = 8.7, 10.7 Hz, H-2), 4.19 (d, 1H, ^2J = 11.6 Hz, C*H*Ph), 4.26-4.33 (m, 2H, CH_2Ph), 4.36-4.61 (m, 7H, H-1′, 3 × CH_2Ph), 4.85 (dd, 1H, H-3), 4.90 (d, 1H, ^2J = 11.4 Hz, C*H*Ph), 5.07 (m, 1H, H-1), 5.47-5.52 (dd, 1H, H-2′), 6.94-7.70 (m, 34H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 57.8, 67.8, 68.9, 71.7, 72.6, 73.3, 73.6, 73.7, 74.8 (×2), 75.0, 75.2, 76.4, 77.2,

80.2, 93.0, 100.7, 127.3, 127.5 (×2), 127.6 (×2), 127.8, 127.9, 128.0 (×3), 128.1 (×11), 128.3 (×5), 128.5 (×3), 128.6 (×3), 130.0, 130.2, 131.5, 132.8, 134.1, 137.6, 138.0, 138.1, 138.6, 138.8, 165.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{62}H_{59}NNaO_{13}$ 1048.3884, found 1048.3893.

***O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (5.12).** CCl_3CN (1.01 mL, 10.14 mmol) and DBU (7.15 μL, 0.05 mmol) were added to a solution of compound 11 (0.52 g, 0.51 mmol) in CH_2Cl_2 (15 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, the reaction mixture was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 93% yield (0.47 g, 0.40 mmol). Analytical data for **5.12**: R_f = 0.60 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{24}$ +52.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.27-3.50 (m, 4H, H-3′, 5′, 6a′, 6b′), 3.79 (m, 4H, H-4, 5, 6a, 6b), 3.94 (dd, 1H, J = 2.6 Hz, H-4′), 4.25 (dd, 2H, ^2J = 11.5 Hz, CH_2Ph), 4.30 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.43-4.53 (m, 5H, H-1′, 2, 3 × C*H*Ph), 4.58 (dd, 2H, ^2J = 12.2 Hz, CH_2Ph), 4.88-4.97 (m, 2H, H-3, C*H*Ph), 5.10 (d, 1H, ^2J = 10.7 Hz, C*H*Ph), 5.53 (dd, 1H, $J_{1',2'}$ = 7.9, $J_{2',3'}$ = 10.0 Hz, H-2′), 6.22 (d, 1H, $J_{1,2}$ = 8.9 Hz, H-1), 6.83-7.87 (m, 34H, aromatic), 8.48 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 54.7, 67.8, 68.4, 71.7, 72.5, 72.8, 73.3, 73.4, 73.5, 74.8, 74.9, 76.1, 76.6, 76.8, 80.1, 90.4, 94.0, 100.8, 123.5, 127.3, 127.5 (×3), 127.6 (×2), 127.7, 127.9, 128.0 (×4), 128.1 (×6), 128.2 (×3), 128.3 (×3), 128.4 (×3), 128.5 (×3), 129.1, 130.0, 130.2, 131.3, 132.8, 134.1, 137.5, 138.0, 138.1, 138.6 (×2), 160.8,

163.4, 165.3, 167.6 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{64}H_{58}C_{l3}NNaO_{14}$ 1193.2966, found 1193.2968.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.13). A mixture of donor **5.12** (0.23 g, 0.19 mmol), acceptor **5.9** (0.13 g, 0.15 mmol), and freshly activated molecular sieves (3 Å, 600 mg) in CH_2Cl_2 (7.0 mL) was stirred under argon for 2 h at rt. The reaction mixture was cooled to -30 ºC, TMSOTf (8.20 μL, 0.04 mmol) was added, and the resulting mixture was stirred for 10 min while the reaction temperature was allowed to increase gradually. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 83% yield (0.27 g, 0.12 mmol). Analytical data for **5.13**: R_f = 0.55 (acetone/toluene, ¼, v/v); $[\alpha]_D^{22}$ -4.0 (*c* 1.0, $CHCl_3$): ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.89 (m, 1H, *J* = 8.8 Hz, H-5), 3.04 (br s, 1H, H-5′), 3.21-3.58 (m, 11H, H-2, 3, 3′, 3‴, 5‴, 6a, 6a″, 6a‴, 6b, 6b″, 6b‴), 3.70-3.84 (m, 3H, H-4, 4″, 5″), 3.89-3.90 (m, 2H, H-4′, 4‴), 4.11-4.32 (m, 8H, H-1‴, 2″, 3″, 6a′, 6b′, 3 x C*H*Ph), 4.34 (d, 1H, ^2J = 11.6 Hz, C*H*Ph), 4.40 (d, 1H, ^2J = 8.1 Hz, C*H*Ph), 4.43-4.49 (m, 2H, H-1′, C*H*Ph), 4.51 (d, 1H, ^2J = 12.0 Hz, C*H*Ph), 4.55-4.70 (m, 5H, 5 x C*H*Ph), 4.77-4.88 (m, 4H, H-1, 3 x C*H*Ph), 4.91 (d, 1H, ^2J = 11.4 Hz, C*H*Ph), 5.02 (d, 1H, ^2J = 10.7 Hz, C*H*Ph), 5.06-5.09 (m, 2H, H-1″, C*H*Ph), 5.17 (dd, 1H, $J_{1',2'}$ =

8.2, $J_{2',3'}$ = 10.0 Hz, H-2′), 5.44-5.51 (m, 2H, H-2‴, C*H*Ph), 6.93-7.72 (64H, m, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.5, 66.7, 67.7 (×2), 68.5, 70.0, 71.0, 71.1, 71.7, 72.5, 73.2, 73.4, 73.5 (×2), 73.6, 74.3, 74.8 (×2), 74.9, 75.2, 75.7, 75.8, 75.9, 76.7, 77.6, 77.8, 80.0, 81.7, 83.0, 99.2, 100.3, 100.6, 100.7, 102.4, 125.4, 126.5 (×2), 127.2, 127.4 (×2), 127.5 (×3), 127.7, 127.8 (×6), 127.9 (×2), 128.0 (×8), 128.1 (×4), 128.2 (×10), 128.3 (×3), 128.4 (×4), 128.5 (×8), 128.6 (×2), 129.1 (×2), 129.5, 129.6 (×2), 130.0 (×2), 130.1, 132.7, 132.8, 137.5 (×2), 137.9, 138.0 (×2), 138.2, 138.3, 138.4, 138.6 (×2), 139.0, 163.3, 164.1, 165.4 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{116}H_{111}NNa_2O_{24}$ 974.3663, found 974.3645.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.14). TsOH (15.49 mg, 0.09 mmol) and EtSH (57 μL, 0.81 mmol) were added to a solution of compound **5.13** (0.25 g, 0.13 mmol) in MeOH/CH_2Cl_2 (10 mL, 1/1, v/v) and the reaction mixture was stirred for 3 h at rt. The reaction was then quenched with triethylamine (~0.5 mL) and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white amorphous solid in 96% yield (0.23 g, 0.12 mmol). Analytical data for **5.14**: R_f = 0.55 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +23.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCll_3$): δ, 2.66 (br s,1H, H-5′), 2.87 (m, 1H, H-5), 3.19-3.38 (m, 9H, H-2, 3, 3‴, 5‴, 6a, 6a′, 6a″, 6b, 6b′), 3.42-3.46 (m, 3H, H-3′, 6a‴, 6b‴), 3.50 (dd, 1H, $J_{2'',3''}$ = 9.1 Hz, H-2″), 3.61-3.71 (m, 3H, H-3″, 5″, 6b″), 3.76-3.78 (m, 2H, H-4, 4″), 3.87 (br d, 1H, *J* =

1.5 Hz, H-4‴), 3.96 (br s, 1H, H-4′), 4.12-4.31 (m, 9H, H-1, 1′, 1‴, 3 x C*H*$_2$Ph), 4.42-4.68 (m, 6H, 3 x C*H*$_2$Ph), 4.77-4.89 (m, 5H, H-1″, 2 x C*H*$_2$Ph), 5.00-5.05 (m, 2H, C*H*$_2$Ph), 5.08 (dd, 1H, $J_{2',3'}$ = 8.9 Hz, H-2′), 5.45 (1H, dd, $J_{2''',3'''}$ = 8.9 Hz, H-2‴) 6.89-7.74 (m, 59 H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.5, 62.2, 67.4, 67.8, 69.0, 69.4, 71.0, 71.3, 71.8, 72.6, 73.3, 73.5, 73.6 (×2), 73.7 (×2), 74.3, 74.8, 74.9, 75.0, 75.2, 75.6, 76.0, 76.3, 77.3, 80.1, 80.2, 81.4, 82.6, 98.8, 100.3 (×2), 102.6, 127.5 (×3), 127.6 (×4), 127.7, 127.8 (×3), 127.9 (×4), 128.0, 128.1 (×7), 128.2 (×10), 128.3 (×8), 128.4 (×8), 128.5 (×3), 128.6 (×5), 129.4, 129.6 (×2), 130.0 (×2), 130.2, 132.8, 137.5, 137.6, 138.0 (×2), 138.2, 138.4, 138.6, 138.7, 164.2, 165.4 ppm; ESI TOF LCMS $[M+2Na]^{+2}$ calcd for $C_{109}H_{107}NNa_2O_{24}$ 930.3506, found 930.3488.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.15). A mixture of donor **5.5**[12] (15.3 mg, 0.0143 mmol), acceptor **5.14** (20.0 mg, 0.011 mmol), and freshly activated molecular sieves (3 Å, 100 mg) in CH_2Cl_2 (2.0 mL) was stirred under argon for 2 h at rt. The reaction mixture was cooled to -40 ^{o}C, NIS (4.95 mg, 0.022 mmol) and freshly conditioned AgOTf (1.4 mg, 0.005 mmol) were added, and the resulting mixture was stirred for 30 min at -40 ^{o}C. The external cooling was removed, and the reaction mixture was stirred for additional 30 min during which the reaction temperature was allowed to increase gradually. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate

(~30 mL) was washed with sat. aq. $NaHCO_3$ (7 mL), $Na_2S_2O_3$ (7 mL), and water (2 x 7 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 80% yield (24.8 g, 0.0088 mmol). Analytical data for **5.15**: R_f = 0.65 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{22}$ +22.9 (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, monosaccharide residues *A-F* are designated in the order shown in Figure 5.1; the assignment of signals for terminal galactose residues *D* and *F* can be interchanged): δ, 2.67 (br d, 1H, *J* = 1.5 Hz, H-5^B), 2.70-2.76 (ddd, 1H, 5^C), 2.85 (ddd, 1H, *J* = 9.8, *J* = 2.8 Hz, H-5^A), 3.02 (ddd, 1H, *J* = 9.6 Hz, H-5^E), 3.14-3.18 (m, 2H, H-6a^A, 6b^A), 3.23-3.77 (m, 20H, H-2^A, 3^A, 3^B, 3^D, 3^F, 4^A, 4^B, 4^C, 5^D, 5^F, 6a^B, 6a^C, 6a^D, 6a^E, 6a^F, 6b^B, 6b^C, 6b^D, 6b^E, 6b^F), 3.86 (br d, 1H, *J* = 2.4 Hz, H-4^D), 3.97-4.08 (m, 5H, H-2^C, 2^E, 3^E, 4^E, 4^F), 4.20-4.36 (m, 11H, H-1^A, 1^B, 1^D, 4 x C*H*$_2$Ph), 4.39-4.68 (m, 16H, H-1^C, 1^F, 3^C, 13 x C*H*Ph), 4.74 (d, 1H, ^2J = 10.4 Hz, C*H*Ph), 4.80 (m, 2H, C*H*$_2$Ph), 4.88 (m, 3H, H-1^E, C*H*$_2$Ph), 4.96 (m, 2H, C*H*$_2$Ph), 5.05 (dd, 1H, *J* = 8.3, 9.5 Hz, H-2^B), 5.42 (dd, 1H, *J* = 8.4, 9.3 Hz, H-2^D), 5.63 (dd, 1H, *J* = 8.0, 10.0 Hz, H-2^F), 6.75-7.98 (m, 93H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.3, 55.8, 65.9, 67.1, 67.6, 67.8, 67.9, 68.2, 68.8, 71.0, 71.5, 71.6, 71.8 (×2), 72.6, 72.7 (×2), 73.3 (×3), 73.6 (×6), 74.3, 74.6 (×4), 74.7, 74.8, 74.2 (×2), 75.4, 75.7, 75.9, 77.6, 79.9, 80.2 (×2), 81.7, 82.8, 97.8, 98.2, 100.0, 100.2, 100.8, 102.6, 123.2, 123.8, 126.8, 127.2, 127.3, 127.4, 127.5 (×2), 127.6, 127.7 (4), 127.8(×10), 127.9 (×4), 128.0 (×6), 128.1 (×16), 128.2 (×13), 128.3 (×6), 128.4 (×6), 128.5 (×6), 128.6 (×9), 129.5, 130.0 (×2), 130.2, 131.4, 132.1, 132.6, 132.7, 133.3, 134.0, 134.5, 137.5, 137.8, 137.9 (×2), 138.1, 138.2 (×2), 138.4, 138.7 (×2), 138.9, 139.0 (×2), 139.1, 164.0,

165.2, 165.4, 167.6, 168.0 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{171}H_{164}N_2NaO_{36}$ 2845.0995, found 2845.0975.

Also isolated were minor quantities of **benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.18).** Analytical data for **5.18**: R_f = 0.55 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +40.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, monosaccharide residues *A-F* are designated as in 15; the assignment of signals for terminal galactose residues *D* and *F* can be interchanged): δ, 2.78 (ddd, 1H, *J* = 1.4, 8.1 Hz, H-5^A), 3.04-3.68 (m, 24H, H-2^A, 2^C, 3^A, 3^B, 3^D, 3^F, 4^C, 5^B, 5^C, 5^D, 5^E, 5^F, 6a^A, 6b^A, 6a^B, 6b^B, 6a^C, 6b^C, 6a^D, 6b^D, 6a^E, 6b^E, 6a^F, 6b^F), 3.73 (dd, 1H, $J_{3A,4A}$ = 9.2 Hz, H-4A), 3.77-3.81 (m, 2H, H-4^D, 4^E), 3.98 (d, 1H, $J_{1D,2D}$ = 7.8 Hz, H-1^D) 4.05 (br d, 1H, $J_{3F,4F}$ = 1.1 Hz, H-4^F), 4.04 (d, 1H, ^2J = 11.8 Hz, C*H*Ph), 4.10-4.14 (m, 2H, C*H*$_2$Ph), 4.16-4.24 (m,7H, H-1^A, 1^B, 2^E, 2 x C*H*$_2$Ph), 4.25-4.34 (m, 4H, H-4^B, 3 x C*H*Ph), 4.36-4.47 (m, 6H, 3 x C*H*$_2$Ph), 4.50-4.57 (m, 3H, H-3^C, C*H*$_2$Ph), 4.60-4.71 (m, 5H, H-1^F, 3^E, 3 x C*H*Ph), 4.75-4.84 (m, 3H, H-2^B, C*H*$_2$Ph), 4.86-4.95 (m, 3H, H-1^C, C*H*$_2$Ph), 4.96-5.02 (m, 3H, 3 x C*H*Ph), 5.44 (dd, 1H, $J_{2D,3D}$ = 10.1 Hz, H-2^D), 5.46 (d, 1H, $J_{1E,2E}$ = 8.5 Hz, H-1^E), 5.64 (dd, 1H, $J_{1F,2F}$ = 8.4, $J_{2F,3F}$ = 9.5 Hz, H-2^F), 6.66-7.91 (m, 93H, aromatic); ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.3, 55.9, 67.7, 67.8, 68.2, 68.7, 69.1, 71.0, 71.1, 71.4, 71.6, 72.4, 72.5 (×3), 72.8, 73.9, 73.4 (×2), 73.6 (×4), 73.8, 74.2, 74.3, 74.4, 74.7, 74.8, 75.0 (×2), 75.6, 75.7, 75.9, 77.2, 77.3, 77.6, 78.6, 80.0, 80.1, 81.6, 82.1, 82.6, 98.8, 99.9, 100.0 (×2), 101.2, 102.6, 122.3 (×2), 122.8 (×2), 126.6 (×2), 127.0, 127.3 (×4), 127.5 (×2), 127.6

(×3), 127.7 (×2), 127.8 (×4), 127.9 (×4), 128.0 (11), 128.1 (×9), 128.2 (×7), 128.3 (×5), 128.4 (×12), 128.5 (×5), 128.6 (×6), 129.2, 129.5, 129.9, 130.0, 130.3, 130.6, 130.7, 132.2, 132.7, 133.1, 133.2, 133.4, 133.5, 137.5, 137.7, 137.8, 138.0 (×2), 138.3, 138.5, 138.7, 138.9 (×2), 139.4 (×2), 163.1, 164.6, 165.0, 165.3, 168.4 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{171}H_{164}N_2NaO_{36}$ 2845.0995, found 2845.1057.

5.4.3. Deprotection of oligosaccharides 5.15 and 5.14

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-amino-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.16). Compound **5.15** (65.0 mg, 0.023 mmol) was dissolved in NH_2NH_2-H_2O/MeOH (3.0 mL, 1/2, v/v) and the resulting mixture was kept for 36 h at reflux. After that, the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as an off-white amorphous solid in 74% yield (37.2 mg, 0.016 mmol). Selected analytical data for **5.16**: R_f = 0.50 (MeOH/CH_2Cl_2, 1/9, v/v); $[\alpha]_D^{22}$ +9.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, monosaccharide residues *A*-*F* are designated in the order shown in Figure 1; the assignment of signals for terminal galactose residues *D* and *F* can be interchanged): δ, 2.65-4.11 (m, 37H, H-1^A, 2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 4^A, 4^B, 4^C, 4^D, 4^E, 4^F, 5^A, 5^B, 5^C, 5^D, 5^E, 5^F, $6a^A$, $6a^B$, $6a^C$, $6a^D$, $6a^E$, $6a^F$, $6b^A$, $6b^B$, $6b^C$, $6b^D$, $6b^E$, $6b^F$), 4.17-4.23 (m, 3H, 3 x C*H*Ph), 4.28 (d, 1H, C*H*Ph), 4.43-4.51 (m, 9H, H-1^B, 1^C, 1^D, 1^E, 1^F, 2 x CH_2Ph), 4.55-4.74 (m, 12H, 6 x CH_2Ph), 4.79-4.93 (m, 6H, 3 x CH_2Ph),

5.02-5.08 (m, 4H, 2 x C*H*$_2$Ph), 7.11-7.38 (m, 70H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 56.8, 57.3, 67.4, 68.1 (×2), 68.4, 68.6, 68.8, 71.3, 72.2, 72.3 (×3), 72.6, 73.0, 73.6 (×2), 73.4 (×2), 73.5 (×3), 73.7, 73.8, 74.6 (×3), 74.7 (×3), 74.7 (×2), 75.0, 75.1, 75.2, 75.7, 76.5, 76.8, 82.1 (×3), 82.4, 83.6, 83.9, 102.8, 103.0, 103.2 (×2), 103.8, 107.2, 127.3 (×3) 127.4 (×3), 127.5 (×3), 127.6 (×3), 127.8 (×17), 127.9 (×3), 128.0 (×8), 128.1 (×3), 128.2 (×8), 128.4 (×9), 128.5 (×8), 128.6 (×4), 137.7, 137.9, 138.0, 138.1 (×2), 138.4, 138.7, 138.8, 138.9, 139.0, 139.3 (×2) ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{134}H_{148}N_2NaO_{29}$ 2273.0099, found 2273.0114.

***O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (5.1).** Compound **5.16** (23.0 mg, 0.009 mmol) was dissolved in a mixture of Ac_2O/MeOH (2.0 mL, 1/1, v/v) and the resulting mixture was stirred for 16 h at rt. Then, the volatiles were removed under the reduced pressure. The residue was diluted with CH_2Cl_2 (~50 mL) and washed with sat. aq. $NaHCO_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, concentrated under reduced pressure and dried *in vacuo* for 3 h. The crude residue was dissolved in EtOH/H_2O (3.0 mL, 4/1, v/v), 10% Pd on charcoal (75 mg) was added, and the resulting mixture was stirred under hydrogen for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~40 mL) was concentrated in *vacuo*. The residue was purified by column chromatography on Sephadex G-25 (water elution) to afford the title compound as a white amorphous solid in 80% yield (7.6 mg, 0.007 mmol). The spectral data for LNH

5.1 was in agreement with that reported previously.[15] Selected analytical data for **5.1**: R_f = 0.50 (chloroform/methanol/water, 2/1/0.4, v/v/v); ^{1}H NMR (600 MHz, D_2O): δ, 2.00, 2.03 (2 s, 6H, 2 x CH_3CO), 3.27 (dd, 1H, *J* = 8.1, 9.0 Hz), 3.43-3.99 (m, 46H), 4.12 (d, 1H, *J* = 3.3 Hz), 4.41 (dd, 2H, *J* = 5.8, 7.8 Hz), 4.44 (d, 1H, *J* = 7.8 Hz), 4.62 (dd, 1H, *J* = 4.2, 7.9 Hz), 4.64 (d, 1H, *J* = 8.0 Hz), 4.70 (dd, 1H, *J* = 2.7, 8.4 Hz), 5.19 (d, 1H, *J* = 3.7 Hz) ppm; ^{13}C NMR (151 MHz, D_2O): δ, 22.58, 22.77, 55.06, 55.37, 60.38, 60.86, 61.38, 68.75, 68.80, 68.89, 69.05, 70.23, 70.25, 70.35, 71.03, 71.31, 71.55, 71.77, 72.82, 72.85, 73.82, 74.20, 74.74, 75.07, 75.10, 75.55, 75.63, 75.70, 78.72, 79.25, 79.35, 82.02, 82.40, 92.15, 96.07, 101.33, 102.87, 103.24, 103.34, 103.84, 174.85, 175.29 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{40}H_{68}N_2NaO_{31}$ 1095.3704, found 1095.3705.

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5.17). Compound **5.14** (30.0 mg, 0.016 mmol) was dissolved in NH_2NH_2-H_2O/MeOH (2.5 mL, 1/4, v/v) and the resulting mixture was kept for 24 h at reflux. After that, the volatiles were removed under the reduced pressure. The residue was dissolved in MeOH/CH_2Cl_2 (~5 mL, 1/9, v/v) and filtered through a pad of silica gel eluting with MeOH/CH_2Cl_2 (1/9, v/v). The combined filtrate (~50 mL) was concentrated under the reduced pressure and dried in *vacuo* for 3 h. The crude residue was dissolved in Ac_2O/MeOH (2.0 mL, 1/1, v/v) and the resulting mixture was stirred for 12 h at rt. Then, the volatiles were removed under reduced pressure. The residue was diluted with CH_2Cl_2 (50 mL) and washed with sat. aq. $NaHCO_3$ (10 mL) and brine (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in *vacuo*. The

residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in 84% yield (20.3 mg, 0.013 mmol). Analytical data for **5.17**: R_f = 0.65 (acetone/toluene, 2/3, v/v); $[\alpha]_D^{22}$ +28.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.91 (3H, s, CH_3CO), 3.15-4.13 (m, 24H, H-2, 2′, 2″, 2‴, 3, 3′, 3″, 3‴, 4, 4′, 4″, 4‴, 5, 5′, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″, 6b‴), 4.20 (dd, 2H, ^2J = 11.7 Hz, CH_2Ph), 4.35-4.49 (m, 7H, H-1, 1′, 1‴, 2 x CH_2Ph), 4.52-4.74 (m, 7H, 7 x C*H*Ph), 4.82 (d, 1H, ^2J = 12.1 Hz, C*H*Ph), 4.85-4.94 (m, 4H, H-1″, 3 x C*H*Ph), 4.98 (d, 1H, ^2J = 10.6 Hz, C*H*Ph), 6.44 (d, 1H, *J* = 7.0 Hz, NH), 7.04-7.37 (m, 45H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.7, 56.6, 62.4, 68.3, 68.4, 68.7, 69.5, 70.5, 71.1, 71.5, 72.5, 73.0, 73.4, 73.5, 73.6, 73.8, 74.2 (×2), 74.7, 74.8, 74.9, 75.1, 75.5, 77.9, 80.4, 82.3 (×2), 83.1, 83.8, 100.7, 102.8, 103.0, 104.3, 127.3, 127.5 (×2), 127.7 (×2), 127.8 (×8), 127.9 (×4), 128.0 (×3), 128.1 (×2), 128.2 (×6), 128.3 (×2), 128.4 (×4), 128.5 (×7), 128.6 (×4), 137.8, 138.0, 138.1, 138.3 (×2), 138.4, 138.6, 138.7, 139.2, 172.2 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{89}H_{99}NNaO_{21}$ 1540.6607, found 1540.6627.

***O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (5.2).** 10% Pd on charcoal (75 mg) was added to a solution of **5.17** (20.0 mg, 0.013 mmol) in EtOH/H_2O (3.0 mL, 4/1, v/v), and the resulting mixture was stirred under hydrogen for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~40 mL) was concentrated in *vacuo,* and the residue was purified by column chromatography on Sephadex G-25 (water elution) to afford the title compound as a white amorphous solid in 87% yield (7.9 mg, 0.011 mmol). Analytical data for **5.2** was in

agreement with that reported previously:[13] R_f = 0.30 (chloroform/methanol/water, 2/1/0.4, v/v/v); ESI TOF LCMS $[M+Na]^+$ calcd for $C_{26}H_{45}NNaO_{21}$ 730.2382, found 730.2392.

5.5. References

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CHAPTER 6

The chemical synthesis of human milk oligosaccharides: lacto-*N*-neohexaose (Galβ1→4GlcNAcβ1→)$_2$ 3,6Galβ1→4Glc

6.1. Introduction

Human milk oligosaccharides (HMO) are complex glycans that are highly abundant in human milk that have been associated with many beneficial effects via various mechanisms. A woman can secrete a distinct HMO composition profile that varies geographically and may even substantially vary between different mothers.[1] All HMO sequences are variations of the specific structural blueprint, but all glycans composed of lactose (Galβ1→4Glc) at their reducing end. The lactose unit can be elongated at the galactose moiety at the C-3 position with lacto-N-biose (Galβ1→3GlcNAc, type 1 chain), which prevents further elongation in this direction. The galactose moiety of the reducing-end lactose can also be elongated by lactosamine (Galβ1→4GlcNAc, type 2 chain) units. And this elongation, which can take place either at the C-3 or C-6 position of galactose, is open for further oligosaccharide chain extension. In addition, the HMO core structures can be fucosylated and/or sialylated.[2] Structures of many HMO are known, and many have already been prepared enzymatically and/or chemically, but the exact biological mechanisms of action of a majority of individual HMO remain unknown due to the lack of well-defined HMO in sufficient quantities.[3]

With the expectation that developing reliable synthetic methods for obtaining individual HMO will boost further innovations in this area, we have reported the total syntheses of a number of glycans. Our initial efforts included syntheses of two linear tetrasaccharide structures (*para*-HMO): lacto-N-neotetraose (LNnT) (Chapter 3)[4] and lacto-N-tetraose (LNT) (Chapter 4).[5] In Chapter 5, we communicated the first synthesis of branched (*iso*-HMO) hexasaccharide, lacto-N-hexaose (LNH).[6] Reported herein is our

further advancement in the field of HMO dedicated to the synthesis of another branched hexasaccharide, lacto-N-neohexaose (LNnH).

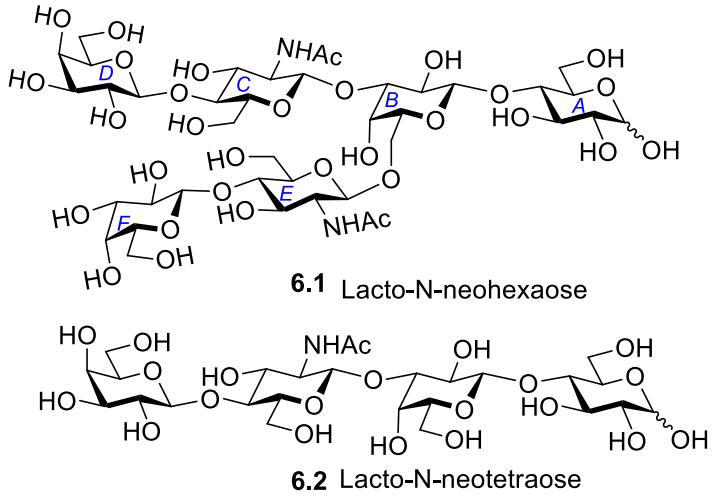


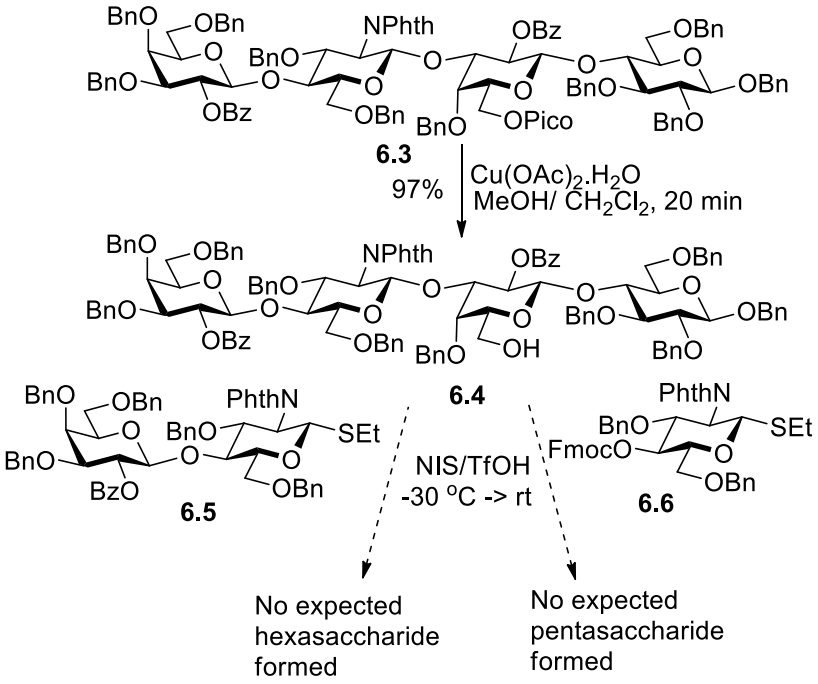
Figure 6.1. Chemical structures of LNH 6.1 and LNT 6.2.

LNnH **6.1** is structurally derived from LNnT **6.2**, and represents one of the most common HMO core structures.[7] LNnH comprises the reducing end lactose disaccharide (*AB*, Figure 6.1), common for all HMO, which is branched at the galactose residue (*B*). More specifically, it is elongated with N-acetyllactosamine disaccharide residue (*CD*) at C-3 of Gal, and also again with N-acetyllactosamine residue (*EF*) at C-6 of Gal. Many chemical[4,8,9] and enzymatic syntheses[3,10,11] of LNnT are known. LNnH has been synthesized enzymatically,[3,12] and some spacer-containing, protected LNnH sequences have been chemically synthesized using polymer supports.[13] However, to the best of our knowledge, no chemical synthesis of LNnH has yet emerged. Human milk isolates of LNnH are available from a variety of commercial vendors, but this common HMO is not yet available in large quantities and at accessible cost for mainstream research and

application. Reported herein as an efficient method for the scalable chemical synthesis of LNnH **6.1** and, by extension, LNnT **6.2**.

6.2. Results and discussion

Recently we have synthesized LNnT **6.2** using convergent and linear assembly strategies.[4] The protecting groups in the tetrasaccharide **6.3** obtained over the course of our previous work[4] were strategically placed to allow for the synthesis of other derivatives. For instance, during the incipient stage of the synthesis we assumed that selective removal of 6′-*O*-picoloyl (Pico) substituent in **6.3** would provide a straightforward access to acceptor **6.4** suitable for subsequent branching. Indeed, this would have offered a very straightforward access to LNnH.



Scheme 6.1. First attempted assembly of LNnH 6.1.

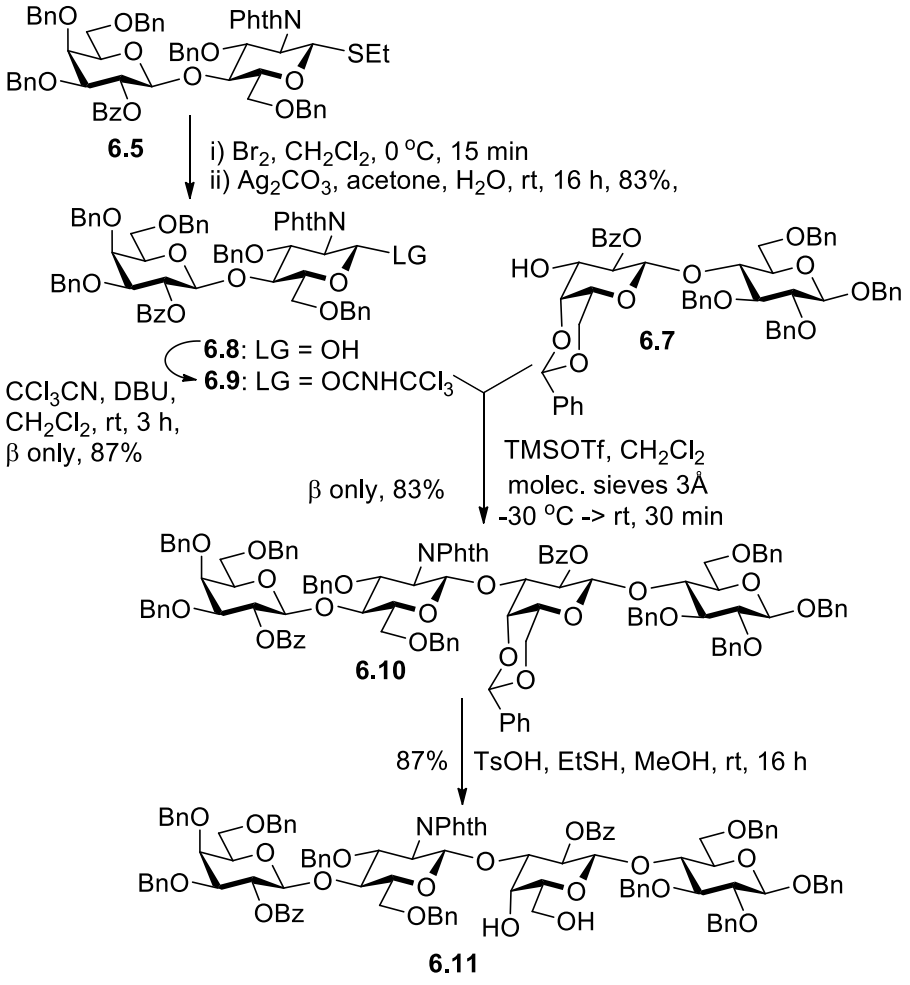
To follow this lead, the deprotection of 6′-O-Pico in **6.3** was affected in the presence of $Cu(OAc)_2$-H_2O in $MeOH/CH_2Cl_2$, and tetrasaccharide **6.4** was obtained in 97% yield. To our disappointment, all attempts to glycosylate acceptor **6.4** with lactosamine thioglycoside donor **6.5**[4] were largely unsuccessful and practically no desired hexasaccharide was formed. Instead, a large number of unidentified side products derived from the donor have been isolated, and the acceptor remained practically intact.

Recalling our previous endeavors with the synthesis of HMO wherein the nature of glycosyl donor and acceptor was found to be of paramount significance, we attempted to employ the corresponding lactosamine phosphate and trichloroacetimidate donors. Despite extended study, these attempts to access the backbone of LNnH were also unsuccessful. In further attempts to enhance the efficiency of this coupling we applied less bulky glucosamine thioglycoside donor **6.6** and its phosphate and trichloroacetimidate analogues. Although the formation of minor amounts of the desired pentasaccharide were observed by mass spectroscopy, these glycosylations were largely unsuccessful. Having explored all possibilities available to us, we came to a conclusion that the bulky benzyl group at the 4′-OH position of acceptor **6.4** is hindering the glycosylation at the 6′-OH position.

It should be noted that a similar conclusion was made over the course of our preliminary attempts to synthesize LNH.[6] In order to decrease the steric hindrance, we decided to follow the same strategy as that developed for the modified synthesis of LNH, in which we employed the lactose building block **6.7** protected with 4,6-*O*-benzyliene acetal.[6] Over the course of our previous work with similar sequences, we observed that if the benzylidene acetal is removed at the tetrasaccharide stage, the resulting 4,6-diol will

offer a far more accessible glycosyl acceptor site for glycosylation. This would be essential for our route employing the glycosylation with bulky disaccharide donor **6.5**.

With this strategic adjustment, we attempted to glycosylate acceptor **6.7** with thioglycoside donor **6.5**. This reaction was sluggish and inefficient, and despite all attempts to push the reaction to completion, significant amounts of the acceptor remained. After a quick screening of other leaving groups, we discovered that trichloroacetimidate donor **6.9** offers an effective building blocks to glycosylate acceptor **6.7**.



Scheme 6.2. Convergent synthesis of acceptor 6.11.

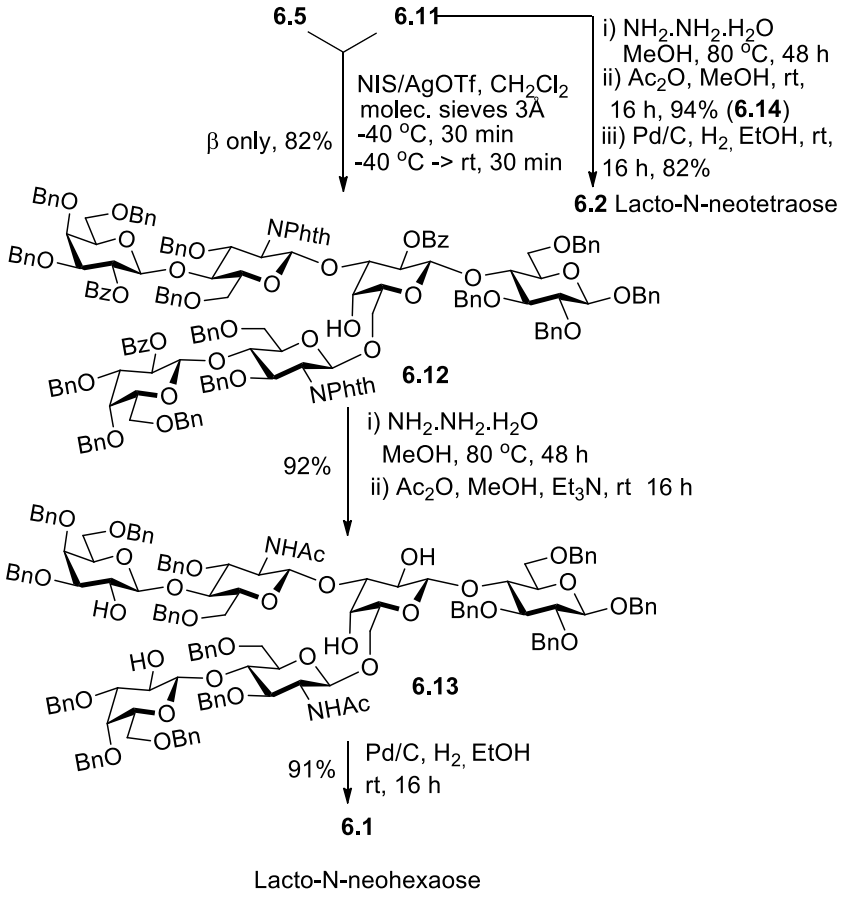
Glycosyl donor **6.9** was prepared from thioglycoside precursor **6.5** that was first converted into the corresponding hemiacetal **6.8** in 83% over two steps involving the anomeric bromination with Br_2 in CH_2Cl_2 followed by hydrolysis of the bromide in the presence of Ag_2CO_3 in wet acetone. Next, installation of the trichloroacetimidate group was performed using trichloroacetonitirle (CCl_3CN) in the presence of 1,8-diazabicylco[5.4.0]undec-7-ene (DBU) to afford lacto-N-biose trichloroacetimidate donor **6.9** in 87% yield. Glycosylation of acceptor **6.7** with donor **6.9** was conducted in the presence of catalytic TMSOTf and the desired tetrasaccharide **6.10** was obtained in a good yield of 83% with complete β-stereoselectivity. It is noteworthy that the corresponding glycosyl phosphate donor[4] gave a comparable result in this application. However, the activation of the glycosyl phosphate donor demanded 2 equiv of TMSOTf along with prolonged reaction time. As a result, product **6.10** was contaminated with tetrasaccharide **6.11**, which was the result of benzylidene cleavage. While this offers a possibility of obtaining **6.11** directly from disaccharide building blocks in one pot, we have not explored this potential advantage.

The deprotection of the 4′,6′-O-benzylidene acetal in intermediate **6.10** was somewhat low-yielding in the presence of TFA in wet DCM, hence we did the reaction in the presence of TsOH and EtSH in $MeOH/CH_2Cl_2$, an approach adapted in our previous work.[6] As a result, tetrasaccharide diol **6.11** was obtained in 87% yield.

Next, we attempted glycosylation of tetrasaccharide diol **6.11** with lactosamine thioglycoside donor **6.5**. Even glycosylation of this more accessible glycosyl acceptor was not as straightforward as we had hoped for. Standard reaction condition employing NIS/TfOH as promoters produced the desired hexasaccharide **6.12** in an unremarkable

yield of 57%. Although this result was a significant improvement in respect to our initial attempts to glycosylate acceptor **6.4**, wherein the yields rarely exceeded 25%, we endeavored to pursue further improvement the outcome of this challenging reaction. With this intention, we identified the major side reactions hampering the yield of the product: fairly rapid hydrolysis of lactosamine donor **6.5** and the formation of the 1→4-linked hexasaccharide alongside the desired 1→6-linked counterpart **6.12**. The formation of somewhat unexpected 1→4-linked hexasaccharide was the main reason we were unable to introduce lactosamine units at C-3 and C-6 positions concomitantly. To suppress these side reactions, we investigated milder promoters, dimethyl(thiomethyl)sulfonium triflate (DMTST)[14,15] and NIS/AgOTf.[16] The former promoter provided a comparable result that that achieved with NIS/TfOH. With the latter promoter, known for providing a slower release of the iodonium ion, minimal side products were observed. After careful refinement of the reaction conditions, we achieved the desired protected LNnH precursor **6.12** in a commendable yield of 82%. This result was obtained by conducting the reaction at -40 ^{o}C for 30 min, and then allowing the reaction temperature gradually increase over the course of the additional 30 min period.

With the protected intermediate **6.12** in hand, we then endeavored to carry out a series of deprotection steps to obtain the target LNnH hexasaccharide **6.1**. Deprotection of the phthalimido and ester groups was achieved by refluxing compound **6.12** with NH_2NH_2-H_2O in MeOH, followed by N-acetylation affected with acetic anhydride in MeOH/Et_3N to furnish hexasaccharide **6.13** in 92% yield. Finally, the benzyl groups were removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol to obtain target LNnH **6.1** in 91% yield.



Scheme 6.3. Convergent synthesis of LNnH 6.1 and LNnT 6.2.

We also performed deprotection of the key tetrasaccharide intermediate **6.11** to obtain LNnT **6.2**. This was achieved via deprotection of the phthalimido and ester groups in the presence of NH_2NH_2-H_2O in refluxing MeOH followed by N-acetylation with acetic anhydride in MeOH to furnish partially protected tetrasaccharide intermediate **6.14** in 94% yield. Subsequently, benzyl ethers were removed by hydrogenation in the presence of 10% Pd/C in wet ethanol to afford LNnT **6.2** in 82% yield.

6.3. Conclusions

In summary, the first chemical synthesis of lacto-N-neohexaose has been completed using a convergent 2+2+2 strategy. This approach employed preassembled lactose and lactosamine building blocks. Along the way, we have also obtained the lacto-N-neotetraose core sequence. With expectation that new methods for reliable synthesis of individual HMO will boost practical applications of these important biomolecules, further synthetic studies of HMO are underway in our laboratory.

6.4. Experimental

6.4.1. General methods

Reactions were performed using commercial reagents and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh) and Sephadex G-25 size exclusion resin, reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ºC. CH_2Cl_2 was distilled from CaH_2 directly prior to application. Molecular sieves (3Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured using a Jasco polarimeter. ^{1}H NMR spectra were recorded at 300 MHz or 600 MHz, and ^{13}C NMR spectra were recorded at 75 MHz or 151 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual TMS (δ_H = 0.00 ppm) for solutions in $CDCl_3$ or the signal of the

residual D_2O (δ_H = 4.79 ppm) for solutions in D_2O. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.16 ppm) for solutions in $CDCl_3$ or the central signal of CD_3COCD_3 δ_C = 29.84 ppm) for solutions in D_2O. Accurate mass spectrometry determinations were performed using Agilent 6230 ESI TOF LCMS mass spectrometer

6.4.2. Synthesis of oligosaccharides 6.4, 6.8, 6.9, 6.11, 6.12

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.4). $Cu(OAc)_2$-H_2O (15.3 mg, 0.084 mmol) was added to a solution of benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[4] (**6.3**, 22.0 mg, 0.011 mmol) in MeOH/CH_2Cl_2 (3.0 mL, 1/3, v/v) and the resulting mixture was stirred for 20 min at rt. Next, the reaction mixture was diluted with CH_2Cl_2 (~50 mL) was washed with H_2O (10 mL), sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound as an off-white amorphous solid in 97% yield (20.9 mg, 0.011 mmol). Analytical data for **6.4**: R_f = 0.60 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ +14.9 (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ, 2.88 (m, 1H, J = 9.5 Hz, H-5), 3.09-3.63 (m, 15H, 2, 3, 3′, 3‴, 5′, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″, 6b‴), 3.74-3.78 (m, 2H, H-4, 4′), 3.89-3.95 (dd, 1H, $J_{4'',5''}$ = 8.9 Hz, H-4″), 3.98 (d, 1H, $J_{3''',4'''}$ = 1.6 Hz, H-4‴), 4.14-4.30

(m, 9H, H-1, 1′, 2″, 3″, 5 × C*H*Ph), 4.42-4.59 (m, 7H, 7 × C*H*Ph), 4.61-4.70 (m, 3H, H-1‴, CH_2Ph), 4.79-4.82 (dd, 2H, CH_2Ph), 4.84-4.88 (dd, 2H, CH_2Ph), 4.98 (dd, 2H, CH_2Ph), 5.06 (d, 1H, $J_{1'',2''}$ = 8.4 Hz, H-1″), 5.24 (dd, 1H, $J_{1',2'}$ = 8.2, $J_{2',3'}$ = 9.9 Hz, H-2′), 5.64 (dd, 1H, $J_{1''',2'''}$ = 8.1, $J_{2''',3'''}$ = 9.8 Hz, H-2‴), 6.57-8.12 (m, 64H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 56.1, 61.9, 67.5, 68.1, 68.5, 71.0, 71.4, 71.9, 72.5, 72.7, 73.4 (×2), 73.5, 73.6, 74.3, 74.5, 74.6, 74.7 (×2), 74.8, 74.9, 75.8, 76.0, 76.3, 77.0, 78.4, 79.8, 80.5, 81.4, 82.7, 99.7, 100.6, 101.2, 102.5, 122.8, 123.2, 126.7, 127.4 (×2), 127.5, 127.6 (×2), 127.7 (×3), 127.8 (×8), 127.9 (×5), 128.0 (×4), 128.1 (×5), 128.2 (×3), 128.3 (×6), 128.4 (×8), 128.5 (×4), 128.6 (×3), 128.9 (×2), 129.4, 129.7, 130.0, 130.1, 130.9, 131.5, 132.9, 133.2, 133.4, 137.6, 137.8, 138.0, 138.1, 138.2, 138.6, 138.8 (×2), 138.9, 164.4, 165.2, 167.3, 167.8 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{116}H_{113}NNaO_{24}$ 1927.7584, found 1927.7586.

***O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (6.8).** A freshly prepared solution of Br_2 in DCM (2.5 mL, 1/165, v/v) was added to a pre-chilled solution of ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[4] (**6.5**, 0.28 g, 0.27 mmol) in CH_2Cl_2 (4.0 mL) and the resulting mixture was stirred for 15 min at 0 °C. After that, the volatiles were removed under reduced pressure. The residue was co-evaporated with CH_2Cl_2 (3 × 10 mL) and dried in *vacuo* for 2 h. The crude residue was dissolved in acetone (5.0 mL), water (0.25 mL) and Ag_2CO_3 (0.04 g, 0.14 mmol) were added, and the resulting mixture was stirred in the absence of light for 16 h at rt. After that, the solids were filtered-off and rinsed successively with

CH_2Cl_2. The combined filtrate (~150 mL) was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 83% yield (0.23 g, 0.22 mmol). Analytical data for β-**6.8**: R_f = 0.35 (acetone/toluene, 1/4, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.34 (d, 1H, J = 8.4 Hz, OH), 3.36-3.65 (m, 7H, H-3′, 5, 5′, 6a, 6a′, 6b, 6b′), 3.97-4.08 (m, 3H, H-2, 4, 4′), 4.22-4.63 (m, 10H, $J_{1',2'}$ = 7.9 Hz, H-1′, 3, 3 × CH_2Ph, 2 × C*H*Ph), 4.91 (d, 1H, ^2J = 12.1 Hz, C*H*Ph), 4.97 (d, 1H, ^2J = 11.6 Hz, C*H*Ph), 5.19 (dd, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 5.62 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, H-2′), 6.73-7.99 (m, 34H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 57.6, 67.8, 68.2, 71.4 (×2), 72.5, 72.6, 73.4, 73.6 (×2), 74.6 (×2), 74.7, 77.4, 79.8, 93.0, 100.7, 123.6, 126.8, 127.5, 127.6 (×2), 127.8 (×7), 127.9 (×3), 128.0 (×2), 128.1 (×2), 128.2 (×2), 128.3, 128.4 (×2), 128.5 (×5), 129.1, 129.9 (×2), 130.0 (×2), 131.7, 133.2, 133.9, 137.8, 138.0, 138.1, 138.8, 139.0, 168.2 (×3) ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{62}H_{59}NNaO_{13}$ 1048.3884, found 1048.3875.

***O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (6.9).** CCl_3CN (0.86 mL, 8.58 mmol) and DBU (5.63 μL, 0.04 mmol) were added to a solution of compound **6.8** (0.44 g, 0.43 mmol) in CH_2Cl_2 (8.0 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 87% yield (0.43 g, 0.37 mmol). Analytical data for **6.9**: R_f = 0.65 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +52.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.44-3.73 (m, 7H, H-3′, 5, 5′, 6a, 6a′, 6b, 6b′), 4.01 (br d,

1H, $J_{3',4'}$ = 2.4 Hz, H-4′), 4.16 (dd, 1H, $J_{3,4} = J_{4,5}$ = 8.8 Hz, H-4), 4.27-4.75 (m, 11H, H-1′, 2, 3, 4 × CH_2Ph), 4.92 (d, 1H, ^2J = 12.2 Hz, C*H*Ph), 4.98 (d, 1H, ^2J = 11.6 Hz, C*H*Ph), 5.65 (dd, 1H, $J_{1',2'}$ = 8.1 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 6.28 (d, 1H, J = 8.5 Hz, H-1), 6.73-8.00 (m, 34H, aromatic), 8.46 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 54.7, 67.2, 68.2, 71.4, 72.4, 72.7, 73.4, 73.5, 73.6, 74.6, 74.8, 75.4, 76.6, 76.9, 79.8, 90.5, 94.2, 100.6, 123.4 (×2), 125.4, 126.9, 127.4, 127.6 (×2), 127.7, 127.8 (×4), 127.9 (×3), 128.0 (×3), 128.2 (×4), 128.3, 128.4 (×2), 128.5 (×3), 128.6 (×2), 129.1, 129.9 (×3), 131.5, 133.2, 133.9, 137.8, 138.0, 138.2, 138.8 (×2), 160.9, 165.1, 167.6 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{64}H_{58}Cl_3NNaO_{14}$ 1193.2966, found 1193.2967.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.10). A mixture of donor **6.9** (0.15 g, 0.13 mmol), acceptor **6.7**[6] (0.09 g, 0.01 mmol), and freshly activated molecular sieves (3 Å, 450 mg) in CH_2Cl_2 (5.0 mL) was stirred under argon for 2 h at rt. The mixture was cooled to -30 °C, TMSOTf (5.50 μL, 0.03 mmol) was added, and the resulting mixture was stirred for 10 min while the reaction temperature was allowed to increase gradually. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL) and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 83% yield (0.21 g, 0.11 mmol). Analytical data for **6.10**: R_f

= 0.55 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{22}$ +7.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.92-3.00 (m, 2H, H-5, 5′), 3.28-3.65 (m, 13H, H-2, 3, 3′, 3‴, 4′, 5″, 5‴, 6a, 6a″, 6a‴, 6b, 6b″, 6b‴), 3.78-3.89 (m, 2H, H-4, 4″), 4.01 (br s, 1H, H-4‴), 4.09 (br d, 1H, *J* = 11.9 Hz, H-6a′), 4.12-4.28 (m, 6H, H-2″, 3″, 6b′, 3 × C*H*Ph), 4.29-4.34 (m, 3H, H-1, CH_2Ph), 4.39 (d, 1H, ^2J = 11.5 Hz, C*H*Ph), 4.45-4.59 (m, 5H, H-1′, 2 × CH_2Ph), 4.63- 4.72 (m, 4H, H-1⁗, 3 × C*H*Ph), 4.81-4.89 (m, 3H, 3 × C*H*Ph), 5.01 (dd, 2H, CH_2Ph), 5.20 (d, 1H, *J* = 7.6 Hz, H-1″), 5.26-5.32 (dd, 1H, $J_{2',3'}$ = 8.7 Hz, H-2′), 5.39 (s, 1H, >C*H*Ph), 5.64-5.71 (dd, 1H, $J_{2''',3'''}$ = 8.7 Hz, H-2‴), 6.72-8.13 (m, 64H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.6, 66.7, 68.0, 68.2, 68.6, 69.2, 71.0, 71.1, 71.5, 72.6, 72.8, 73.3, 73.5, 73.6 (×2), 74.3, 74.6 (×4), 74.9, 75.3, 75.6, 75.7, 77.0, 78.5, 79.0, 80.0, 81.8, 83.1, 99.6, 100.6, 100.8, 101.2, 102.5, 126.4 (×2), 126.7, 127.1, 127.5 (×4), 127.7 (×6), 127.8 (×3), 127.9 (×7), 128.0 (×6), 128.1 (×6), 128.2 (×3), 128.3 (×6), 128.4 (×5), 128.5 (×5), 128.6 (×4), 129.4, 129.5 (×2), 130.0, 130.1 (×2), 132.7, 133.3 (×2), 137.6, 137.8, 138.0, 138.1, 138.2, 138.4, 138.7, 138.8 (×2), 139.2, 164.1, 165.2 ppm; ESI TOF LCMS $[M+2Na]^{+2}$ calcd for $C_{116}H_{111}NNa_2O_{24}$ 974.3662, found 974.3644.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.11). TsOH (10.8 mg, 0.06 mmol) and EtSH (38 μL, 0.54 mmol) were added to a solution of compound **6.10** (0.17 g, 0.09 mmol) in MeOH/CH_2Cl_2 (7.0 mL, 1/1, v/v). and the resulting mixture was stirred for 3 h at rt. The reaction was then quenched with triethylamine (~0.5 mL) and the volatiles were removed under reduced pressure. The residue was purified by column

chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white amorphous solid in 87% yield (0.14 g, 0.08 mmol). Analytical data for **6.11**: R_f = 0.50 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{22}$ +36.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 2.85 (br s, 1H, H-5′), 2.95 (br d, 1H, *J* = 9.6 Hz, H-5), 3.21-3.65 (m, 14H, H-2, 3, 3′, 3‴, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″, 6b‴), 3.80 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 3.85 (dd, 1H, $J_{4'',5''}$ = 9.0 Hz, H-4″), 3.90 (s, 1H, H-4′), 4.00 (s, 1H, H-4‴), 4.10-4.59 (m, 14H, H-1, 1′, 2″, 3″, 5 × C*H*$_2$Ph), 4.61-4.71 (m, 4H, H-1‴, 3 × C*H*Ph), 4.80-4.87 (m, 3H, 3 × C*H*Ph), 4.90 (d, 1H, ^2J = 10.3 Hz, C*H*Ph), 4.97 (d, 1H, ^2J = 11.7 Hz, C*H*Ph), 5.13 (d, 1H, $J_{1'',2''}$ = 8.4 Hz, H-1″), 5.23 (dd, 1H, $J_{2'',3''}$ = 10.0 Hz, H-2′), 5.65 (dd, 1H, $J_{2'',3''}$ = 9.6 Hz, H-2‴), 6.66-8.07 (m, 59H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.5, 62.0, 67.6, 68.2, 68.3, 68.4, 71.0, 71.1, 71.5, 72.6, 72.7, 73.4 (×2), 73.6 (×3), 74.3 (×2), 74.6, 74.7, 74.8, 74.9, 75.7, 76.4, 78.3, 79.8, 81.4, 81.6, 82.7, 99.0, 100.3, 101.2, 102.5, 126.8, 127.5 (×3), 127.6, 127.7 (×3), 127.8 (×8), 127.9 (×6), 128.0 (×2), 128.1 (×3), 128.2 (×7), 128.3 (×6), 128.4 (×6), 128.5 (×3), 128.6 (×7), 129.2, 129.4, 130.0 (×2), 132.7, 133.4, 137.6, 137.7, 138.0 (×2), 138.2, 138.6, 138.7, 138.8, 138.9, 164.2, 165.2 ppm; ESI TOF LCMS $[M+2Na]^{+2}$calcd for $C_{109}H_{107}NNa_2O_{24}$ 930.3506, found 930.3489.

Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.12). A mixture of donor **6.5**[4] (38.3 mg, 0.035 mmol), acceptor **6.11** (50.0 mg, 0.0275 mmol), and freshly activated molecular

sieves (3 Å, 100 mg) in CH_2Cl_2 (4.0 mL) was stirred under argon for 2 h at rt. The mixture was then cooled to -40 °C, NIS (12.3 mg, 0.055 mmol) and freshly conditioned AgOTf (3.5 mg, 0.013 mmol) were added, and the resulting mixture was stirred for 30 min at -40 °C. After that, the reaction mixture was stirred for additional 30 min during which the reaction temperature was allowed to increase gradually. The solids were then filtered-off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with sat. aq. $NaHCO_3$ (10 mL), $Na_2S_2O_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as a white foam in 82% yield (63.6 mg, 0.0227 mmol). Analytical data for **6.12**: R_f = 0.60 (acetone/toluene, 1/4 v/v); $[\alpha]_D^{22}$ +34.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 3.93 (m, 1H, OH), 4.78 (br s, 1H, H-5^B), 5.00 (m, 1H, H-5^E), 5.06 (m, 1H, *J* = 9.3 Hz, H-5^C), 5.24-5.72 (m, 22H, H-2^A, 3^B, 3^D, 3^F, 4^A, 4^B, 4^E, 5^A, 5^D, 5^F, 6a^A, 6a^B, 6a^C, 6a^D, 6a^E, 6a^F, 6b^A, 6b^B, 6b^C, 6b^D, 6b^E, 6b^F), 5.78 (dd, 1H, *J* = 8.7, 10.7 Hz, H-3^E), 5.91-6.15 (m, 9H, H-2^C, 2^E, 3^A, 3^C, 4^C, 4^D, 4^F, C*H*$_2$Ph), 6.28-6.39 (m, 7H, H-1^A, 1^F, 5 × C*H*Ph), 6.43-6.61 (m, 14H, 1^B, 1^D, 1^E, 11 × C*H*Ph), 6.66-6.69 (dd, 2H, C*H*$_2$Ph), 6.77 (dd, 2H, C*H*$_2$Ph), 6.83-6.89 (m, 4H, 2 × C*H*$_2$Ph), 6.96 (d, 1H, $J_{1C,2C}$ = 8.4 Hz, H-1C), 7.01 (dd, 2H, C*H*$_2$Ph), 7.24 (dd, 1H, $J_{1B,2B}$ = 8.3, $J_{2B,3B}$ = 9.5 Hz, H-2^B), 7.66-7.69 (m, 2H, H-2^D, 2^F), 8.77-10.14 (m, 93H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 55.2, 55.6, 64.6, 65.9, 67.3, 67.9, 68.1, 68.2, 70.6, 71.0, 71.2, 71.4 (×2), 72.4, 72.5, 72.6, 72.7, 73.3, 73.4, 73.5, 73.6 (×5), 73.7, 73.8, 74.2, 74.3, 74.6 (×2), 74.7 (×3), 75.0, 75.6, 75.9, 76.3, 77.1, 77.3, 79.8, 81.6, 81.9, 82.8, 97.3, 97.9, 100.2, 100.6 (×2), 102.5, 123.2, 123.9, 126.9 (×2), 127.4 (×2), 127.5 (×2), 127.6 (×2), 127.7 (×3), 127.8 (×11), 127.9 (×12), 128.1 (×5), 128.2

(×6), 128.3 (×9), 128.4 (×8), 128.5 (×10), 128.6 (×8), 129.0, 129.2, 129.4, 129.9 (×2), 130.0 (×2), 130.9, 131.2 (×2), 131.9, 132.5, 133.1, 133.4, 133.7, 133.9, 134.6 (×3), 137.7, 137.9, 138.0, 138.1, 138.2 (×2), 138.4, 138.7, 138.8 (×3), 138.9, 139.0, 164.0, 165.2, 165.4, 166.6, 167.0, 168.0, 168.1 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{171}H_{164}N_2NaO_{36}$ 2845.0995, found 2845.0944.

6.4.3. Deprotection of oligosaccharides 6.11 and 6.12

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.13). Compound **6.12** (72.0 mg, 0.025 mmol) was dissolved in NH_2NH_2-H_2O/MeOH (3.0 mL, 1/2, v/v), and the resulting mixture was kept for 36 h at reflux. After that, the volatiles were removed under reduced pressure. The residue was dissolved in MeOH/CH_2Cl_2 (~5 mL, 1/9, v/v) and filtered through a pad of silica gel eluting with MeOH/CH_2Cl_2 (1/9, v/v). The combined eluate (~50 mL) was concentrated under reduced pressure and dried in *vacuo* for 3 h. The crude residue was dissolved in Ac_2O/MeOH/Et_3N (2.0 mL, 1/1/0.1, v/v/v) and the resulting mixture was stirred for 12 h at rt. The volatiles were then removed under reduced pressure. The residue was diluted with CH_2Cl_2 (~50 mL), and washed with sat. aq. $NaHCO_3$ (10 mL) and brine (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (acetone - toluene gradient elution) to afford the title compound as an off-white amorphous solid in

92% yield (54.7 mg, 0.023 mmol). Analytical data for **6.13**: R_f = 0.60 (acetone/toluene, 2/3, v/v); $[\alpha]_D^{24}$ +11.8 (*c* 1.0, $CHCl_3$); 1.74, 1.78 (2 s, 6H, 2 x $COCH_3$), 2.96-3.97 (m, 36H, H-2^A, 2^B, 2^C, 2^D, 2^E, 2^F, 3^A, 3^B, 3^C, 3^D, 3^E, 3^F, 4^A, 4^B, 4^C, 4^D, 4^E, 4^F, 5^A, 5^B, 5^C, 5^D, 5^E, 5^F, $6a^A$, $6a^B$, $6a^C$, $6a^D$, $6a^E$, $6a^F$, $6b^A$, $6b^B$, $6b^C$, $6b^D$, $6b^E$, $6b^F$), 4.20-4.98 (m, 34H, H-1^A, 1^B, 1^C, 1^D, 1^E, 1^F, 14 x CH_2Ph), 5.34, 5.62 (2 d, 2H, 2 x NH), 7.12-7.38 (m, 70H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.6, 23.7, 55.4, 56.0, 67.8, 68.0, 68.2 (×2), 68.6, 68.7, 70.8, 71.2, 71.9, 72.2, 72.3 (×3), 72.9, 73.0, 73.3, 73.4 (×4), 73.5 (×6), 73.9, 74.0, 74.3, 74.6, 74.7, 74.8, 75.0, 75.1, 75.6, 76.8, 77.0, 79.6, 80.3, 81.9, 82.4, 83.4, 101.0, 101.3, 102.7, 102.8, 103.3, 103.4, 127.2, 127.5 (×2) 127.6 (×4), 127.7 (×6), 127.8 (×6), 127.9 (×8), 128.0 (×12), 128.2 (×5), 128.3 (×3), 128.4 (×6), 128.5 (×15), 128.6 (×2), 137.5, 137.8, 137.9, 138.0 (×2), 138.1, 138.2, 138.4 (×2), 138.8 (×2), 139.0, 139.1, 139.2, 170.3, 171.2 ppm; ESI TOF LCMS $[M+Na]^+$calcd for $C_{138}H_{152}N_2NaO_{31}$ 2357.0310, found 2357.0316.

***O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (6.1, LNnH).** 10% Pd on charcoal (200 mg) was added to a solution of **6.13** (70.0 mg, 0.030 mmol) in EtOH/H_2O (7.0 mL, 4/1, v/v), and the resulting mixture was stirred under hydrogen atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~50 mL) was concentrated under reduced pressure. The residue was purified by size-exclusion chromatography on Sephadex G-25 (water elution) to afford the title compound as a white amorphous solid in 91% yield (29.2 mg, 0.027 mmol). Selected analytical data for **6.1**:[12] R_f = 0.50

(chloroform/methanol/water, 2/1/0.4, v/v/v); ^{1}H NMR (600 MHz, D_2O): δ, 1.99, 2.02 (2 s, 6H, $COCH_3$), 3.25 (dd, 1H, *J* = 8.1, 9.1 Hz), 3.47-3.59 (m, 9H), 3.61-3.64 (m, 2H), 3.65-3.84 (m, 22H), 3.92 (m, 7H), 4.11 (d, 1H, *J* = 3.3 Hz), 4.39 (d, 1H, *J* = 7.9 Hz), 4.43 (m, 2H), 4.59 (d, 1H, *J* = 7.9 Hz), 4.62 (d, 1H, *J* = 8.0 Hz), 4.66 (d, 1H, *J* = 8.3 Hz), 5.18 (d, 0.5H, *J* = 3.7 Hz) ppm; ^{13}C NMR (151 MHz, D_2O): δ, 22.5, 22.8, 55.3, 55.5, 60.2 (×2), 60.4, 61.4, 68.7, 68.9, 69.0, 70.2 (×2), 70.3, 71.3, 71.5, 71.7, 72.5, 72.8 (×2), 73.8 (×2), 74.2, 74.7, 74.9, 75.0, 75.1, 75.7, 78.4, 78.6, 79.2, 79.3, 82.1, 92.1, 96.0, 101.3, 103.1, 103.2 (×2), 103.3, 174.9, 175.2 ppm; ESI TOF LCMS $[M+H]^+$calcd for $C_{40}H_{69}N_2O_{31}$ 1073.3884, found 1073.3859.

Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6.14). Compound **6.11** (47.0 mg, 0.026 mmol) was dissolved in NH_2NH_2-H_2O/MeOH (2.5 mL, 1/4, v/v), and the resulting mixture was kept for 24 h at reflux. After that, the volatiles were removed under reduced pressure. The residue was dissolved in MeOH/CH_2Cl_2 (~5 mL, 1/9, v/v) and filtered through a pad of silica gel eluting with MeOH/CH_2Cl_2 (1/9, v/v). The combined eluate (~50 mL) was concentrated under reduced pressure and dried in *vacuo* for 3 h. The crude residue was dissolved in Ac_2O/MeOH (2.0 mL, 1/1, v/v), and the resulting mixture was stirred for 12 h at rt. The volatiles were then removed under reduced pressure. The residue was diluted with CH_2Cl_2 (~50 mL) and washed with sat. aq. $NaHCO_3$ (10 mL) and brine (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (acetone -

toluene gradient elution) to afford the title compound as an off-white amorphous solid in 94% yield (37.0 mg, 0.024 mmol). Analytical data for **6.14**: R_f = 0.65 (acetone/toluene, 2/3, v/v); $[\alpha]_D^{20}$ +20.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.73 (s, 3H, $COCH_3$), 2.85-4.01 (m, 24H, H-2, 2′, 2″, 2‴, 3, 3′, 3″, 3‴, 4, 4′, 4″, 4‴, 5, 5′, 5″, 5‴, 6a, 6a′, 6a″, 6a‴, 6b, 6b′, 6b″, 6b‴), 4.30 (dd, 2H, ^2J = 11.7 Hz, CH_2Ph), 4.43-4.61 (m, 9H, H-1, 1′, 1‴, 3 x CH_2Ph), 4.62-4.71 (m, 4H, 2 x CH_2Ph), 4.82-4.95 (m, 7H, H-1″, 3 x CH_2Ph), 5.64 (d, 1H, *J* = 7.5 Hz, NH), 7.19-7.37 (m, 45H, aromatic) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): δ, 23.6, 56.7, 62.6, 68.3, 68.6, 68.7, 68.8, 70.9, 71.3, 71.9, 72.4, 72.8, 73.4, 73.6 (×2), 73.7, 74.1, 74.3 (×2), 74.7, 74.8, 75.0, 75.1, 75.3, 77.1, 79.6, 82.0, 82.1, 82.9, 83.1, 101.5, 102.8 (×2), 103.4, 127.5 (×3), 127.6 (×2), 127.8 (×4), 127.9, 128.0 (×15), 128.3 (×6), 128.4 (×5), 128.5 (×7), 128.6 (×2), 137.6, 137.8, 137.9, 138.1 (×2), 138.4, 138.8 (×2), 139.0, 171.3 ppm; ESI TOF LCMS $[M+Na]^+$ calcd for $C_{89}H_{99}NNaO_{21}$ 1540.6607, found 1540.6625.

***O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (6.2, LNnT).** 10% Pd on charcoal (75 mg) was added to a solution of **6.14** (30.0 mg, 0.019 mmol) in EtOH/H_2O (4.0 mL, 4/1, v/v), and the resulting mixture was stirred under hydrogen atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol and water. The combined filtrate (~50 mL) was concentrated under reduced pressure. The residue was purified by size exclusion chromatography on Sephadex G-25 (water elution) to afford the title compound as a white amorphous solid in 82% yield (11.0 mg, 0.015 mmol). Analytical data for **6.2** was in agreement with that reported previously:[4] R_f = 0.30

(chloroform/methanol/water, 2/1/0.4, v/v/v); ESI TOF LCMS $[M+Na]^+$ calcd for $C_{26}H_{45}NNaO_{21}$ 730.2382, found 730.2381.

6.5. References

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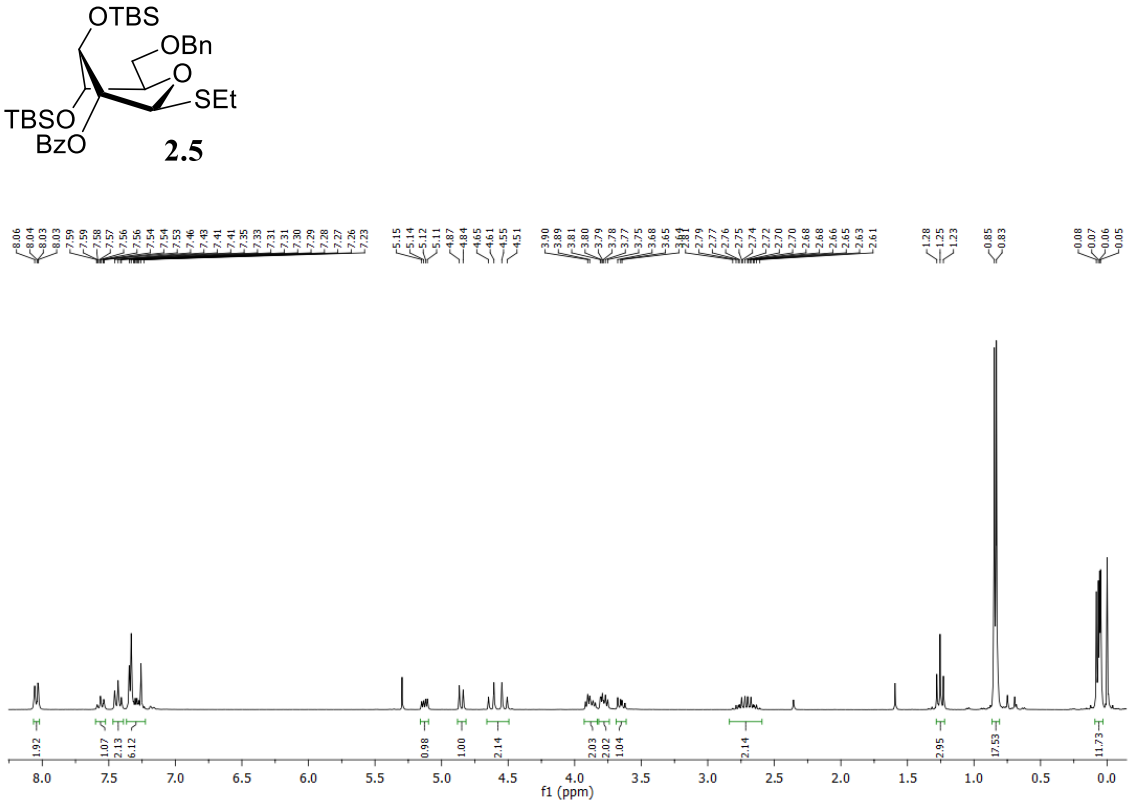
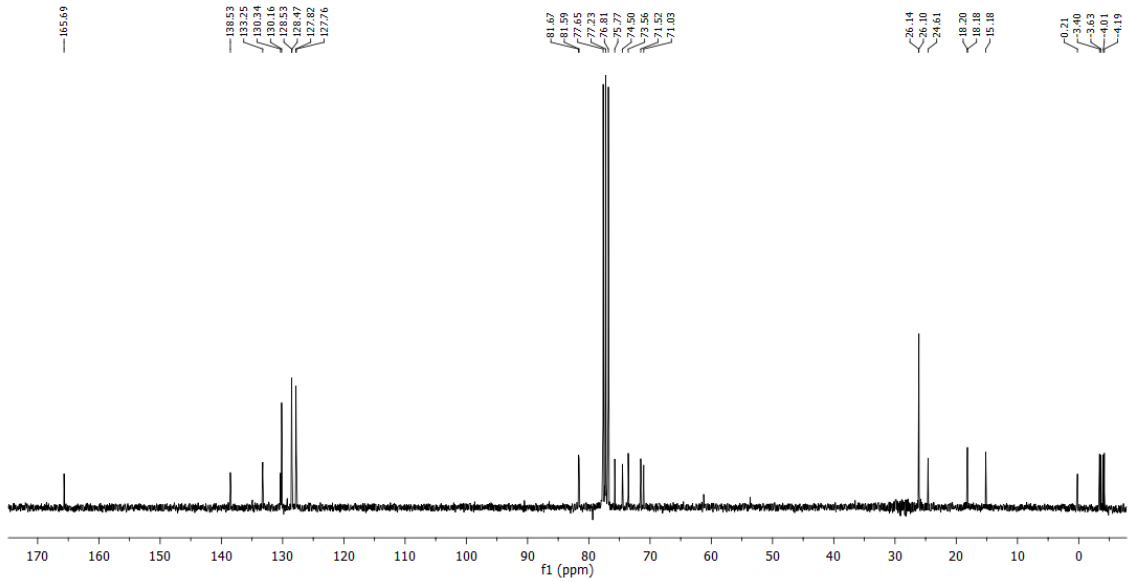
APPENDIX



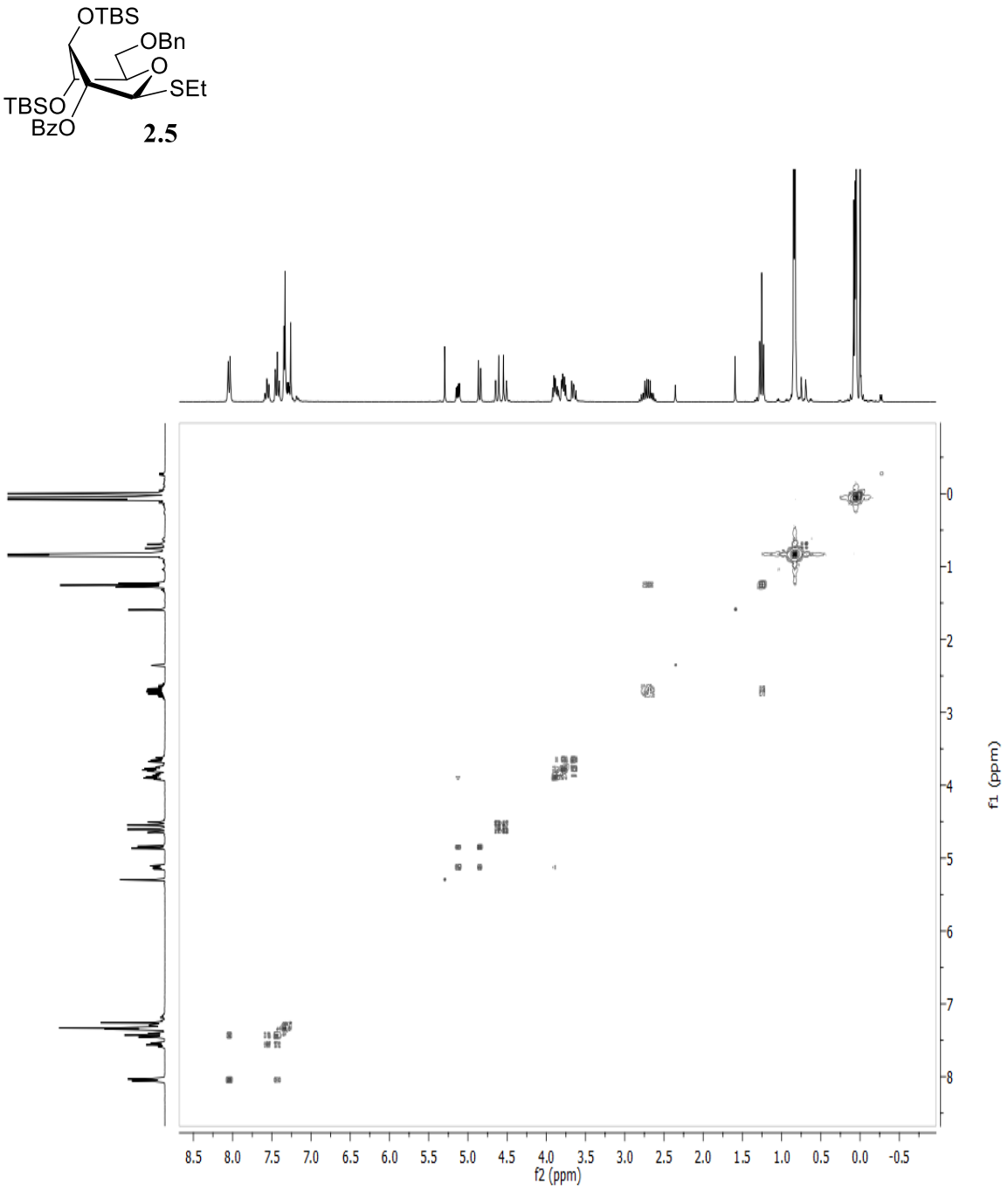
$CDCl_3$ 300 MHz

Figure A-1: ^{1}H NMR spectrum of Ethyl 2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.5**)



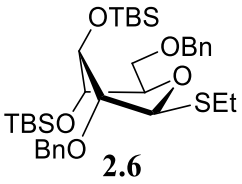
$CDCl_3$ 75 MHz

Figure A-2: ^{13}C NMR spectrum of Ethyl 2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.5**)



$CDCl_3$ 300 MHz

Figure A-3: 2-D NMR COSY spectrum of Ethyl 2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.5**)



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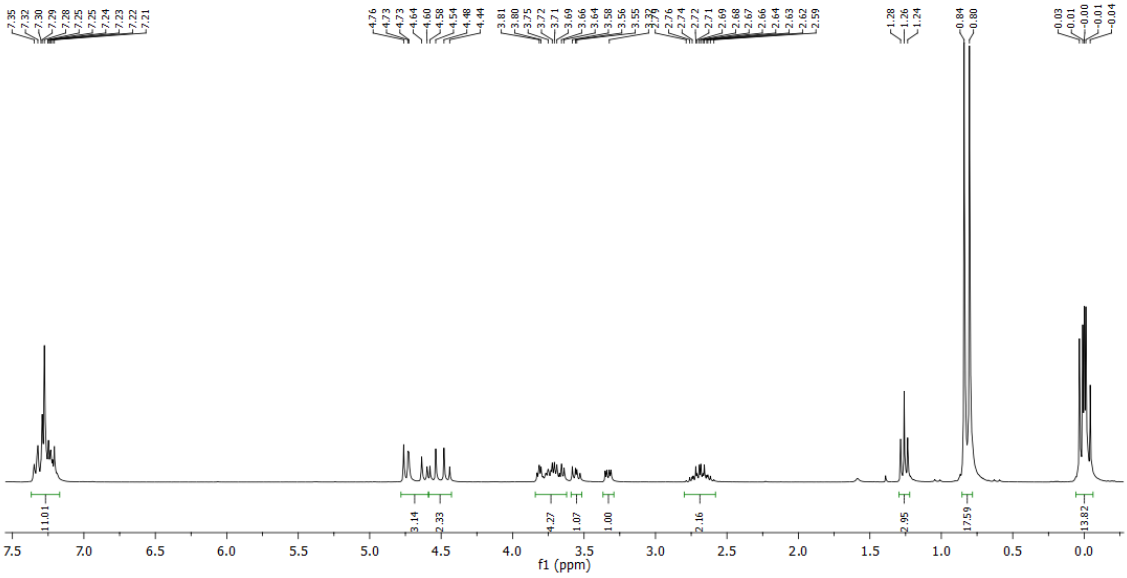
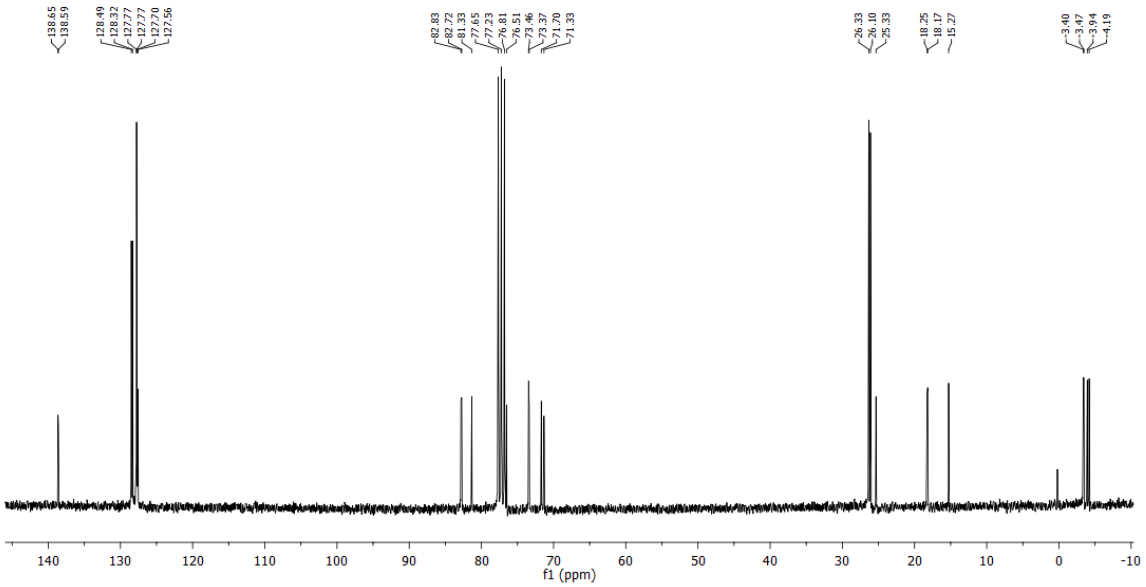


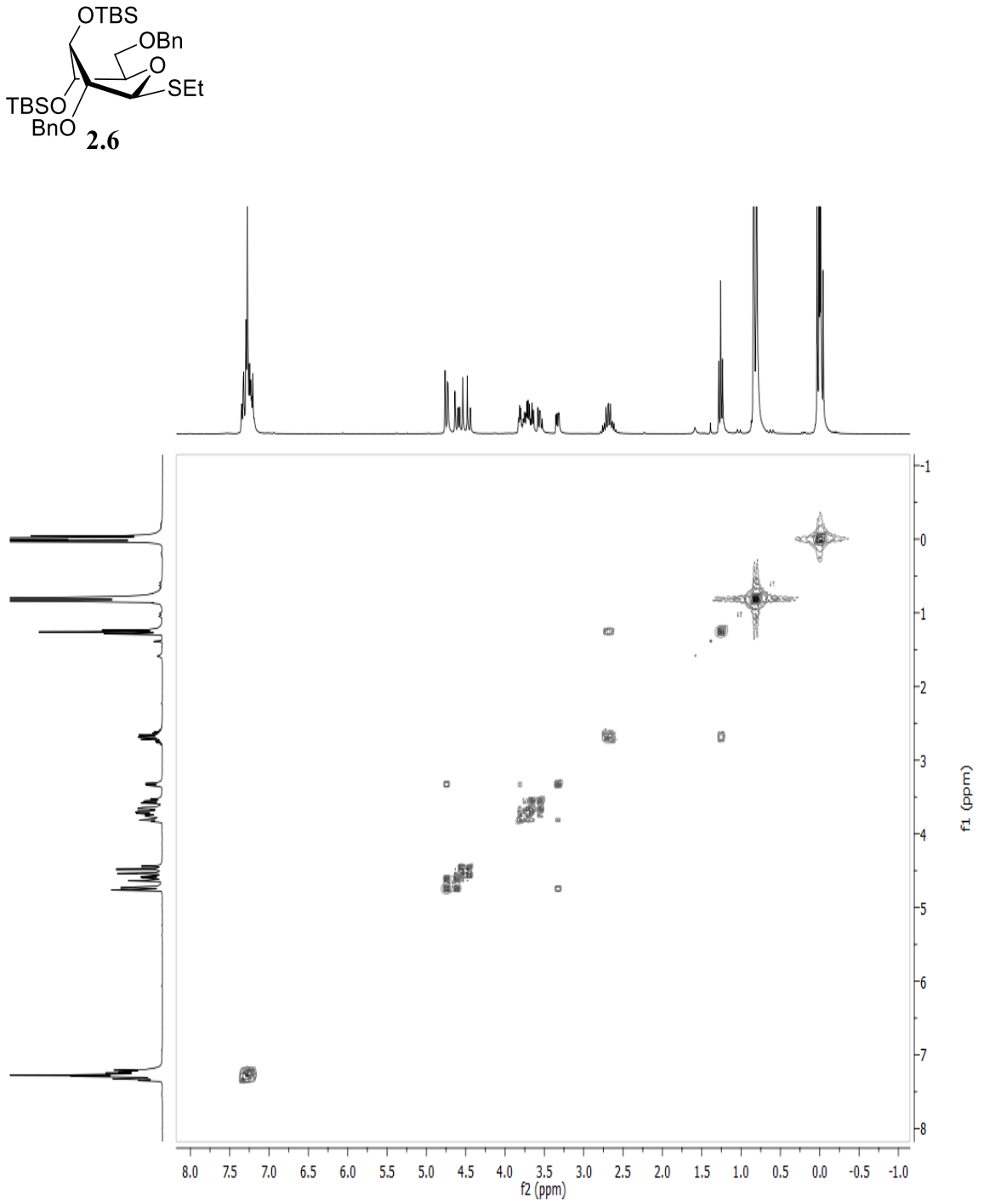
$CDCl_3$ 300 MHz

Figure A-4: ^{1}H NMR spectrum of Ethyl 2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.6**)



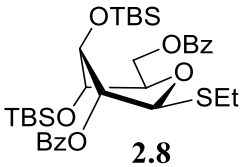
$CDCl_3$ 75 MHz

Figure A-5: ^{13}C NMR spectrum of Ethyl 2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.6**)



$CDCl_3$ 300 MHz

Figure A-6: 2-D NMR COSY spectrum of Ethyl 2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.6**)



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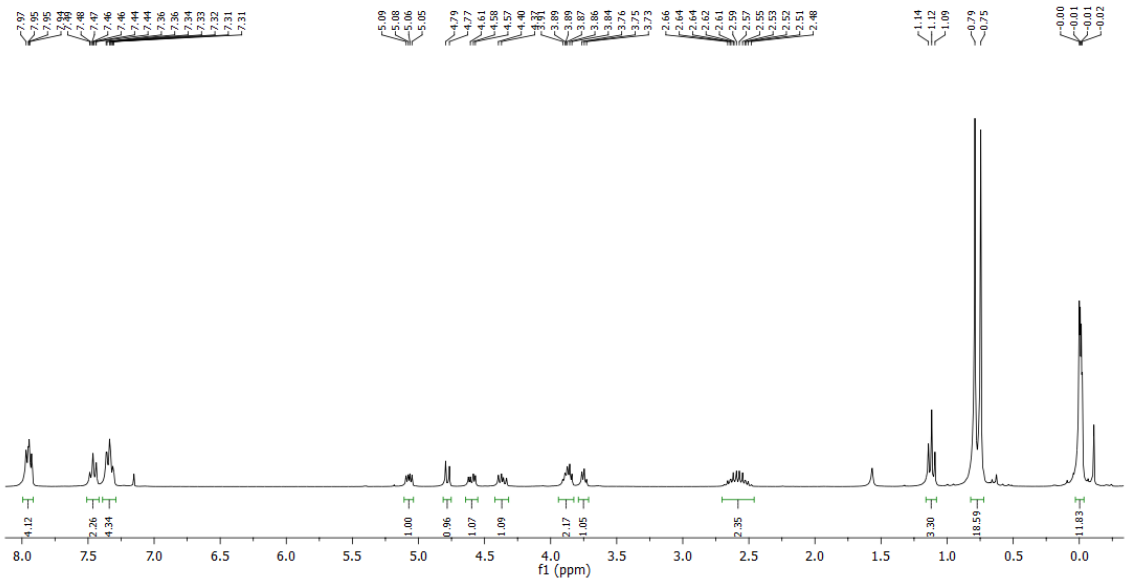
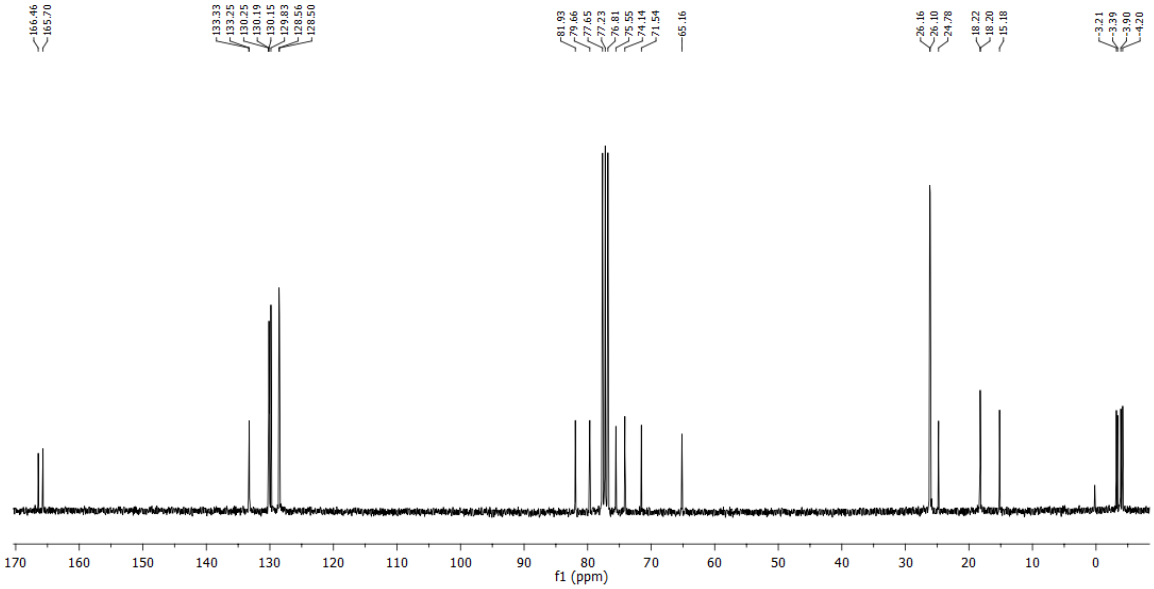


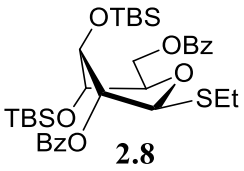
$CDCl_3$ 300 MHz

Figure A-7: ^{1}H NMR spectrum of Ethyl 2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.8**)



$CDCl_3$ 75 MHz

Figure A-8: ^{13}C NMR spectrum of Ethyl 2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.8**)



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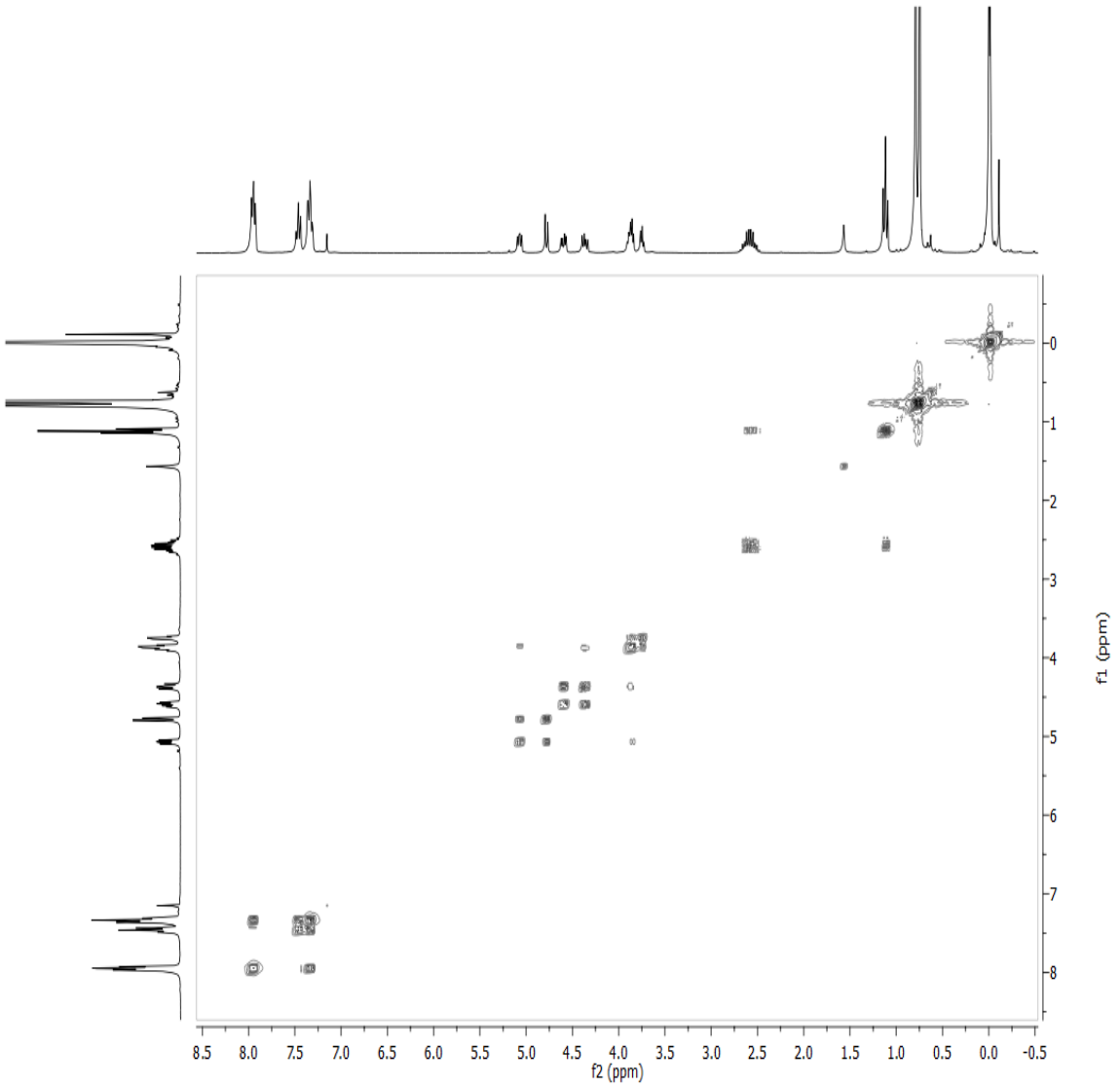
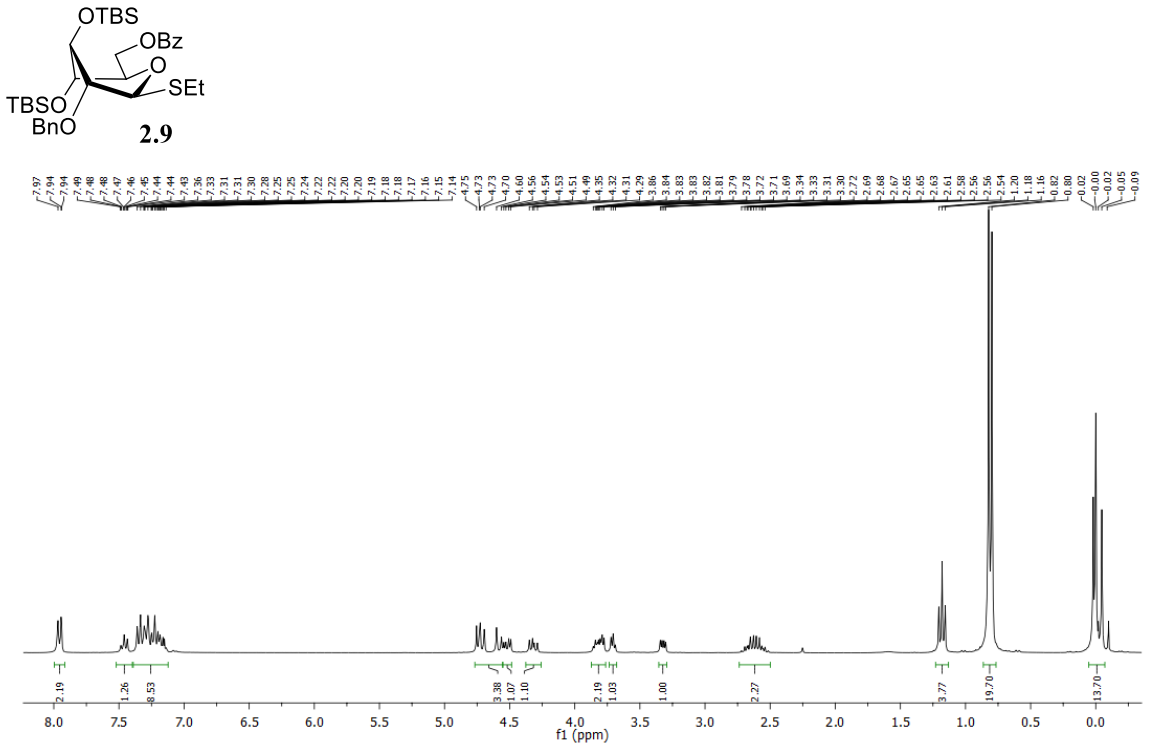


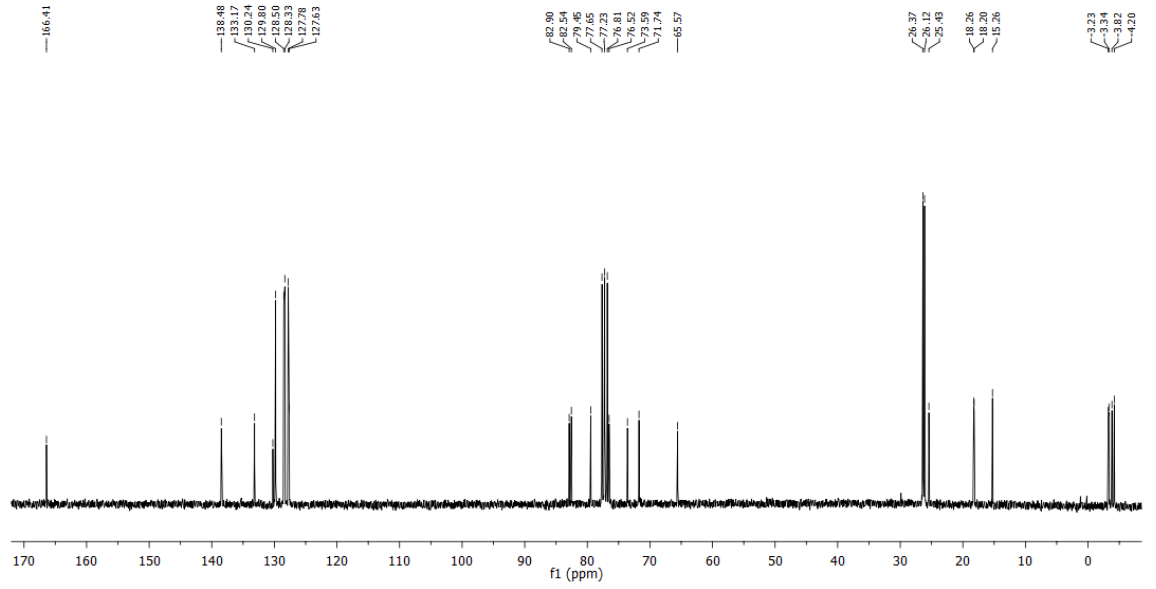
$CDCl_3$ 300 MHz

Figure A-9: 2-D NMR COSY spectrum of Ethyl 2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.8**)



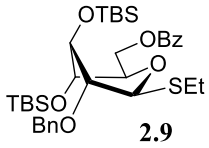
$CDCl_3$ 300 MHz

Figure A-10: ^{1}H NMR spectrum of Ethyl 6-*O*-benzoyl-2-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.9**)



$CDCl_3$ 75 MHz

Figure A-11: ^{13}C NMR spectrum of Ethyl 6-*O*-benzoyl-2-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.9**)



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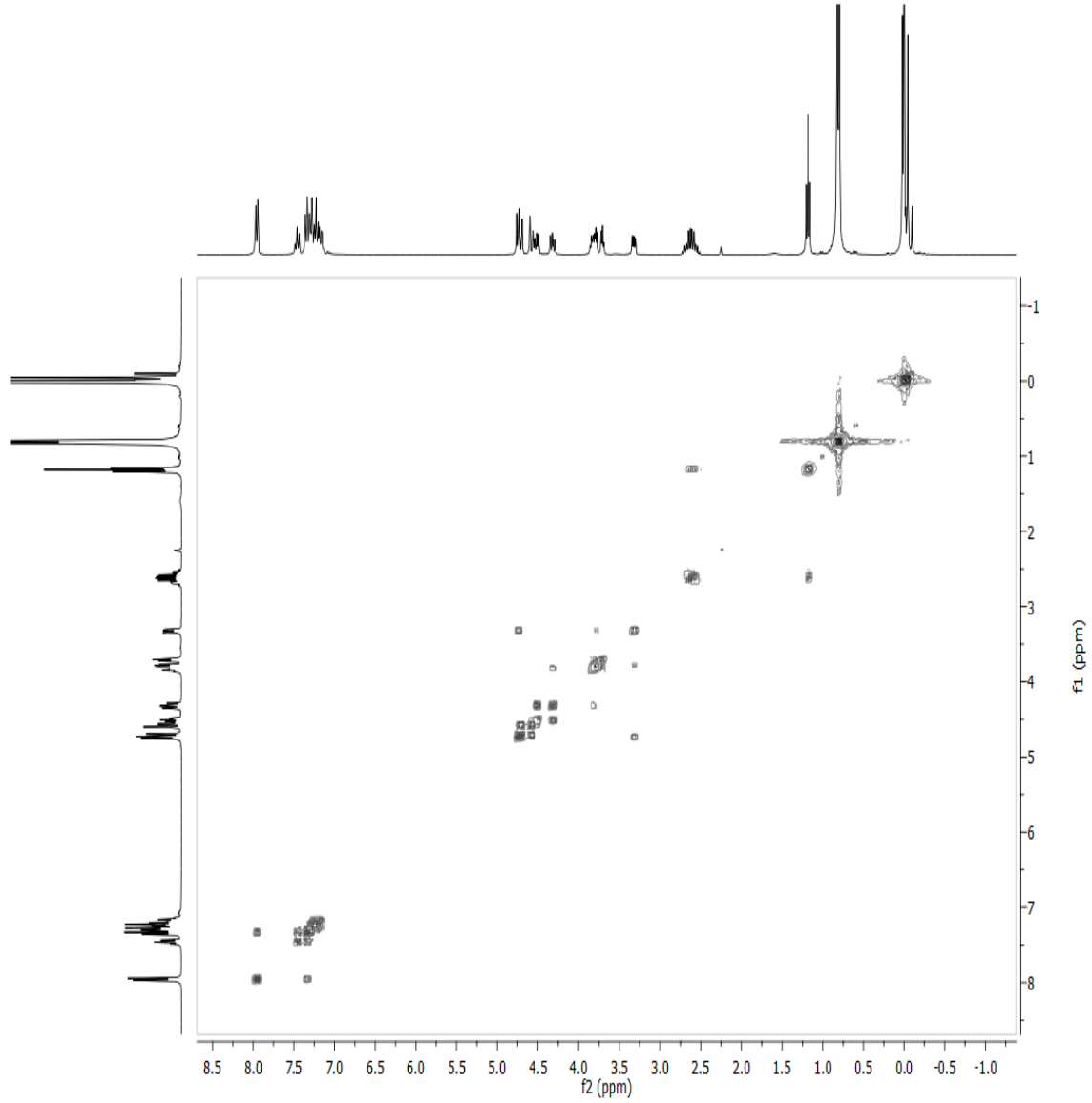
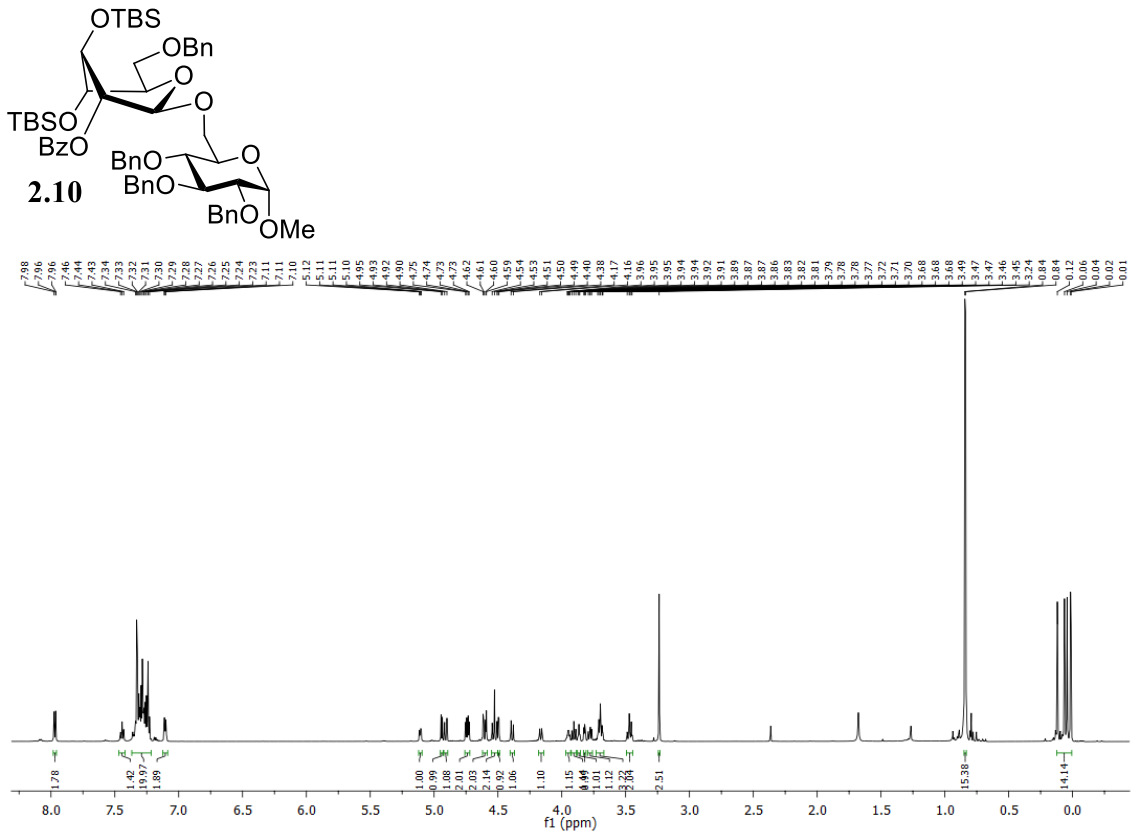


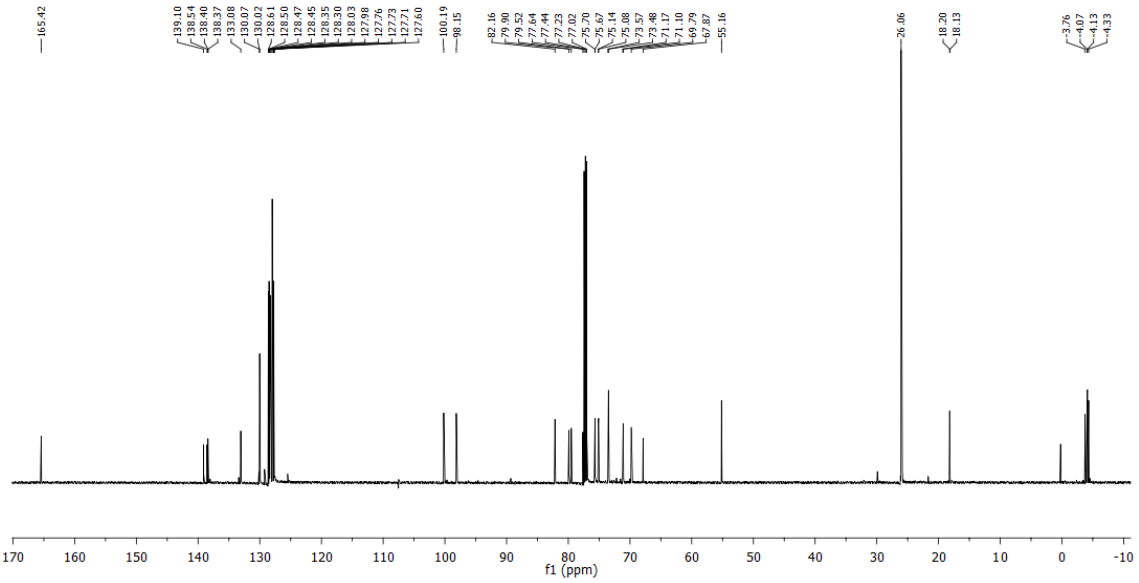
$CDCl_3$ 300 MHz

Figure A-12: 2-D NMR COSY spectrum of Ethyl 6-*O*-benzoyl-2-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**2.9**)



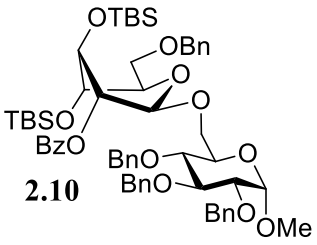
$CDCl_3$ 600 MHz

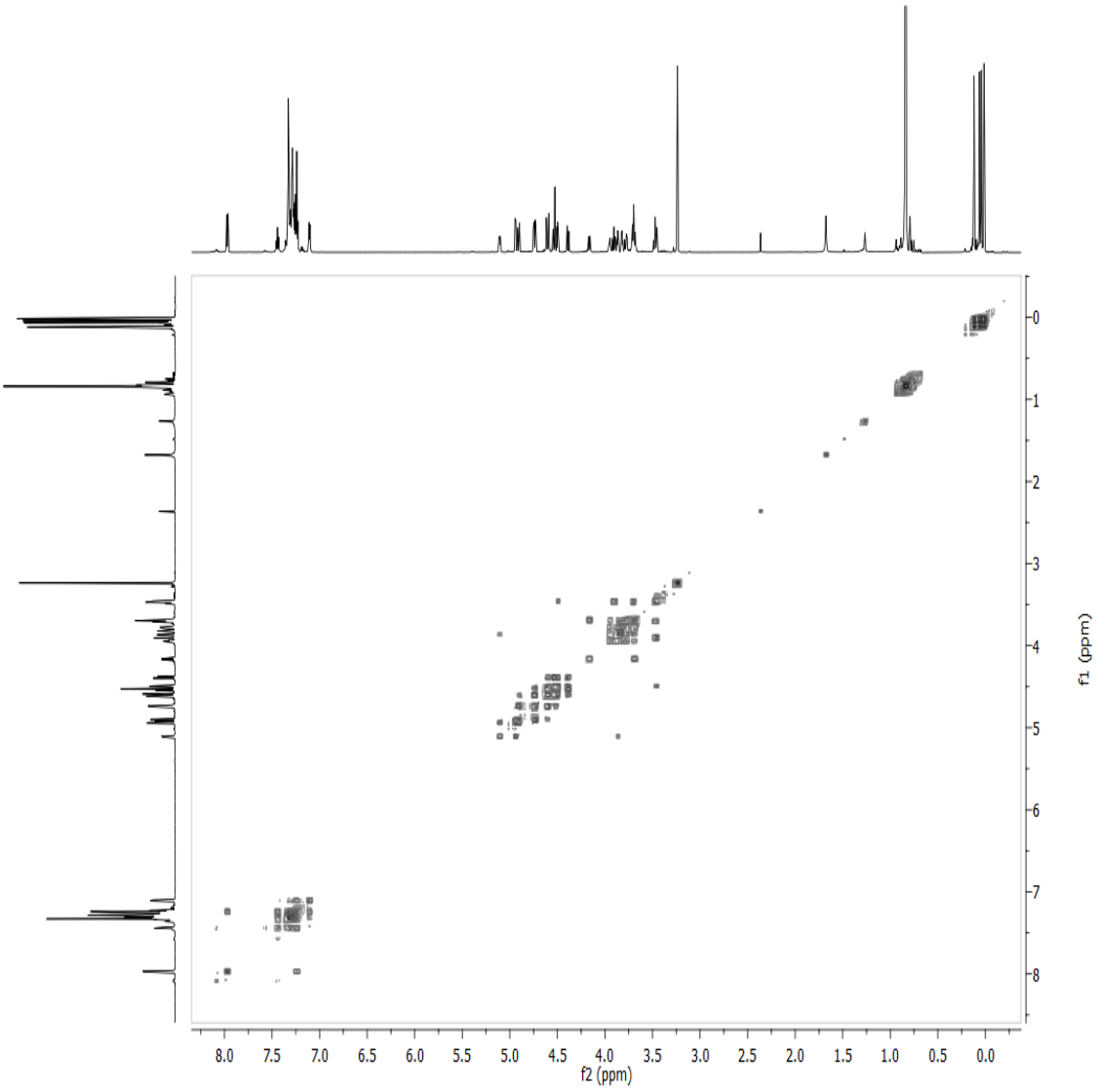
Figure A-13: ^{1}H NMR spectrum of Methyl 6-*O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.10**)



$CDCl_3$ 150 MHz

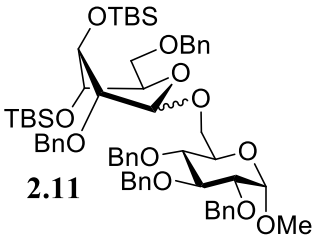
Figure A-14: ^{13}C NMR spectrum of Methyl 6-*O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.10**)





$CDCl_3$ 600 MHz

Figure A-15: 2-D NMR COSY spectrum of Methyl 6-*O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.10**)



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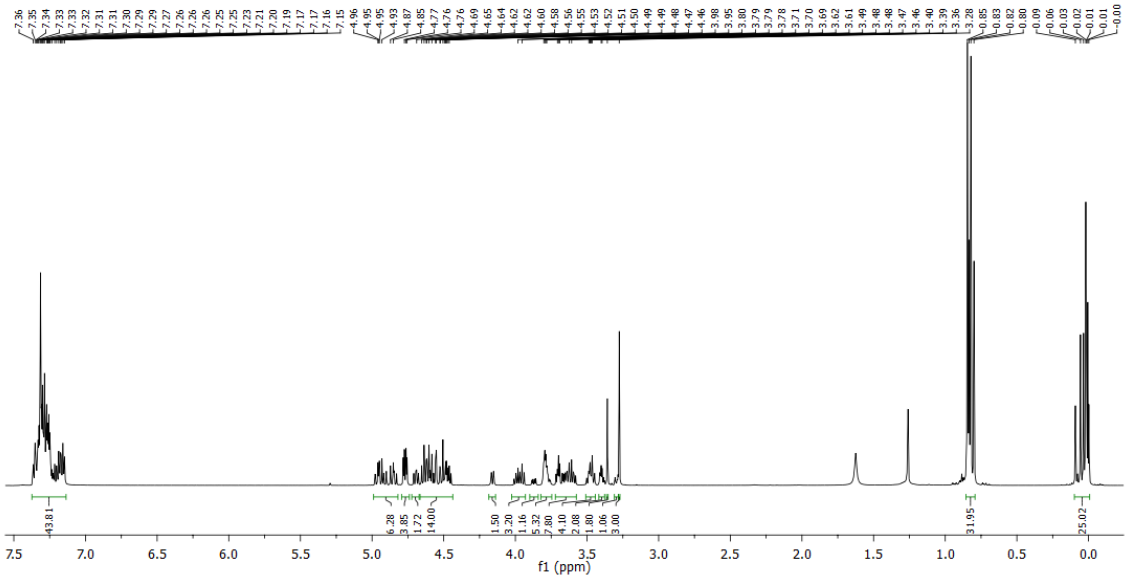
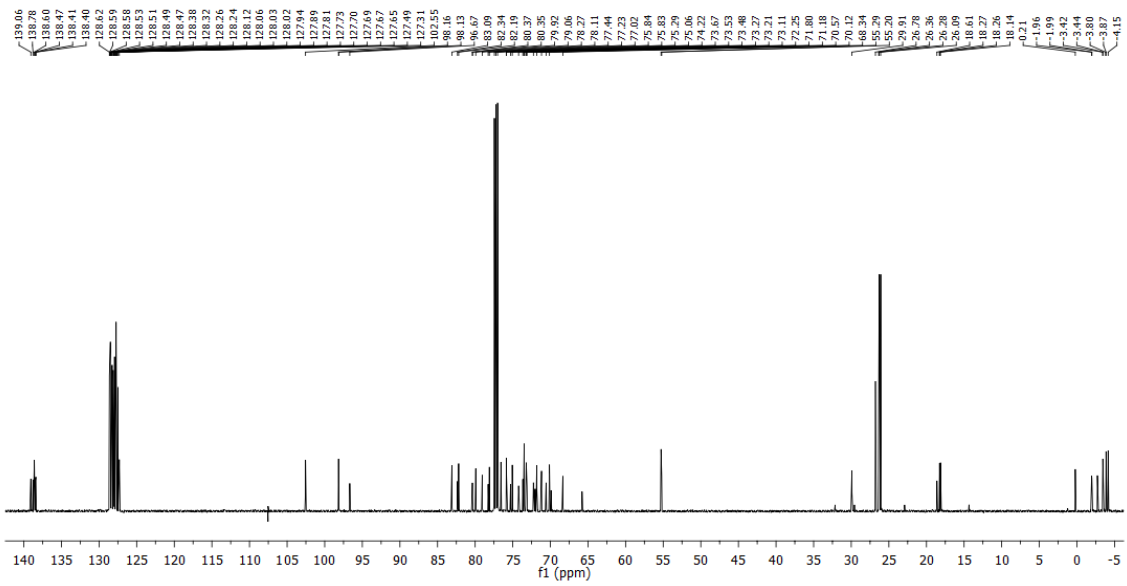


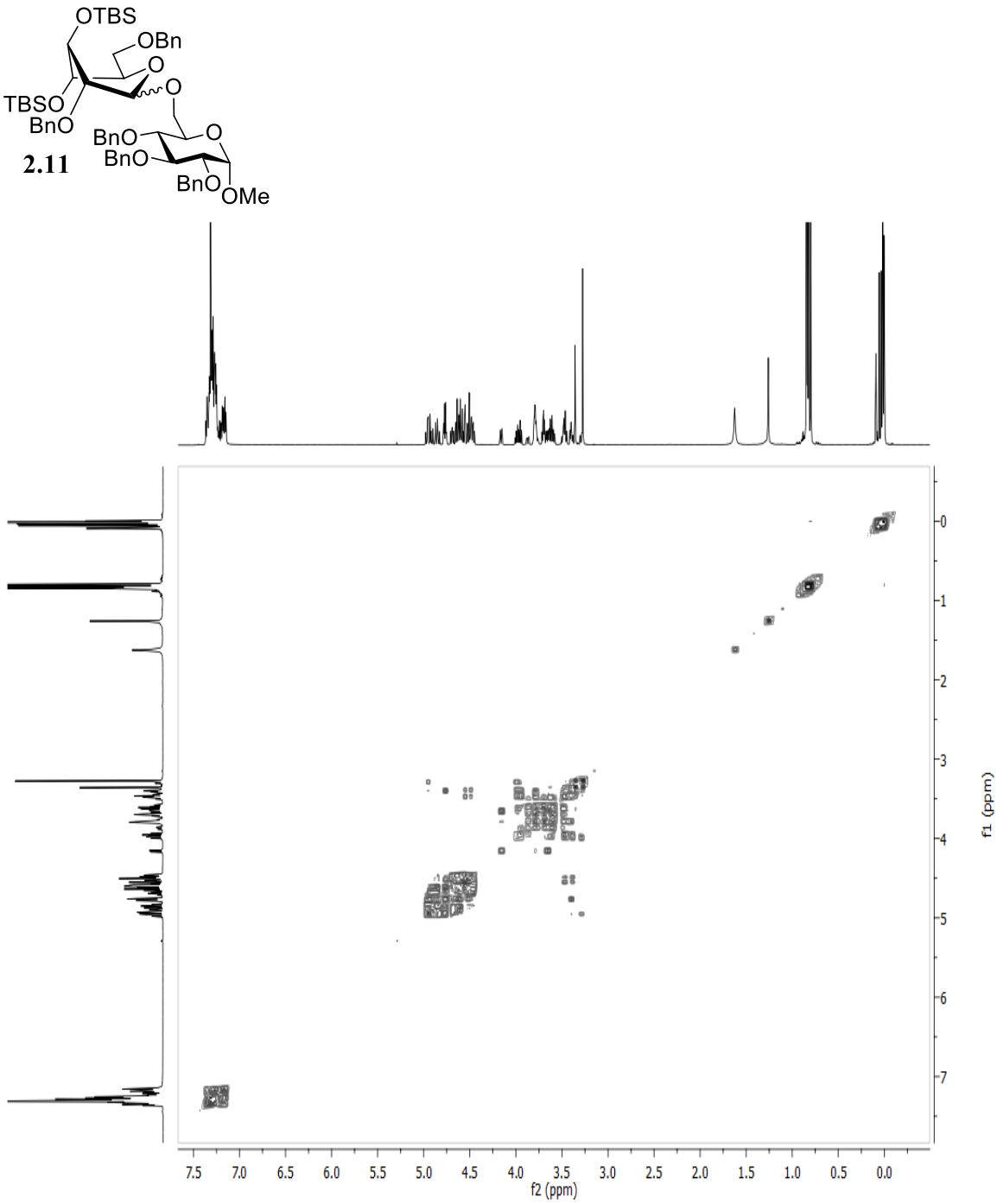
$CDCl_3$ 600 MHz

Figure A-16: ^{1}H NMR spectrum of Methyl 6-*O*-(2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.11**)



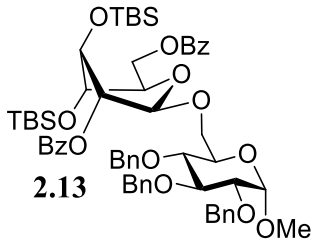
$CDCl_3$ 150 MHz

Figure A-17: ^{13}C NMR spectrum of Methyl 6-*O*-(2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.11**)



$CDCl_3$ 600 MHz

Figure A-18: 2-D NMR COSY spectrum of Methyl 6-*O*-(2,6-di-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-α/β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.11**)



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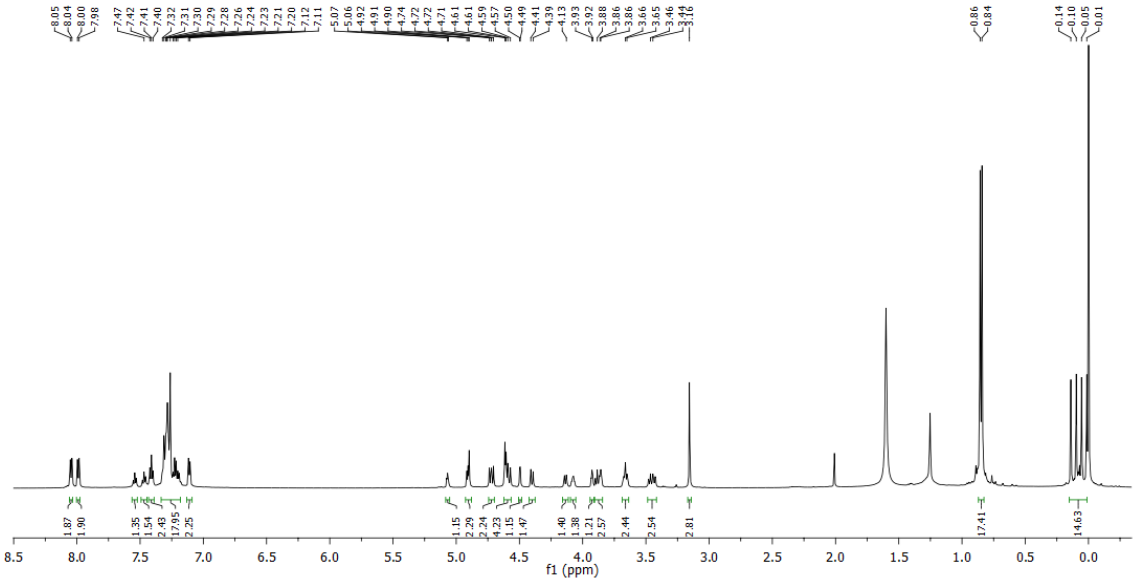
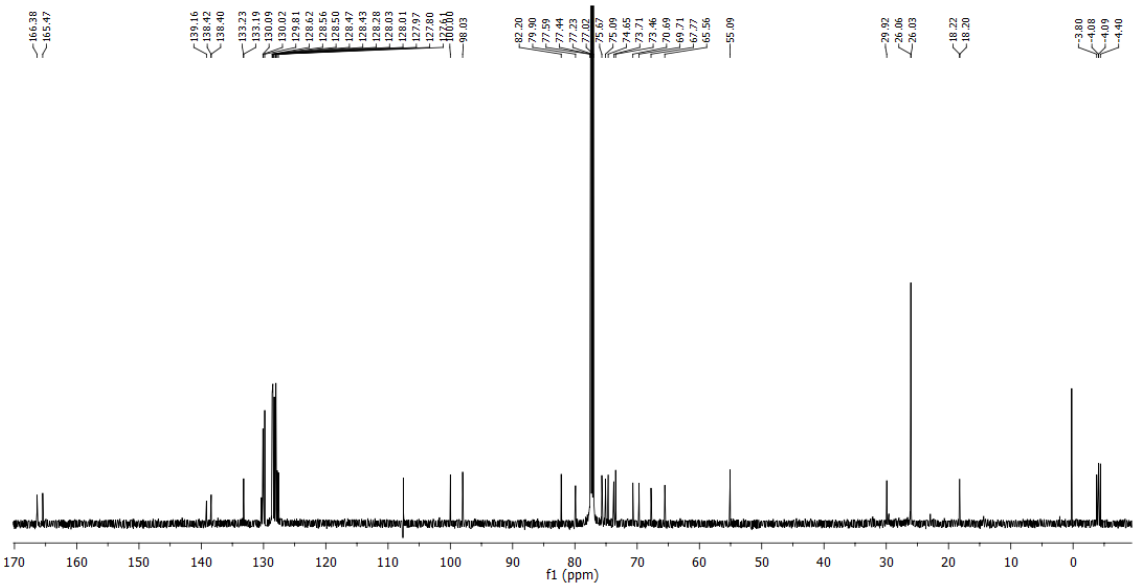


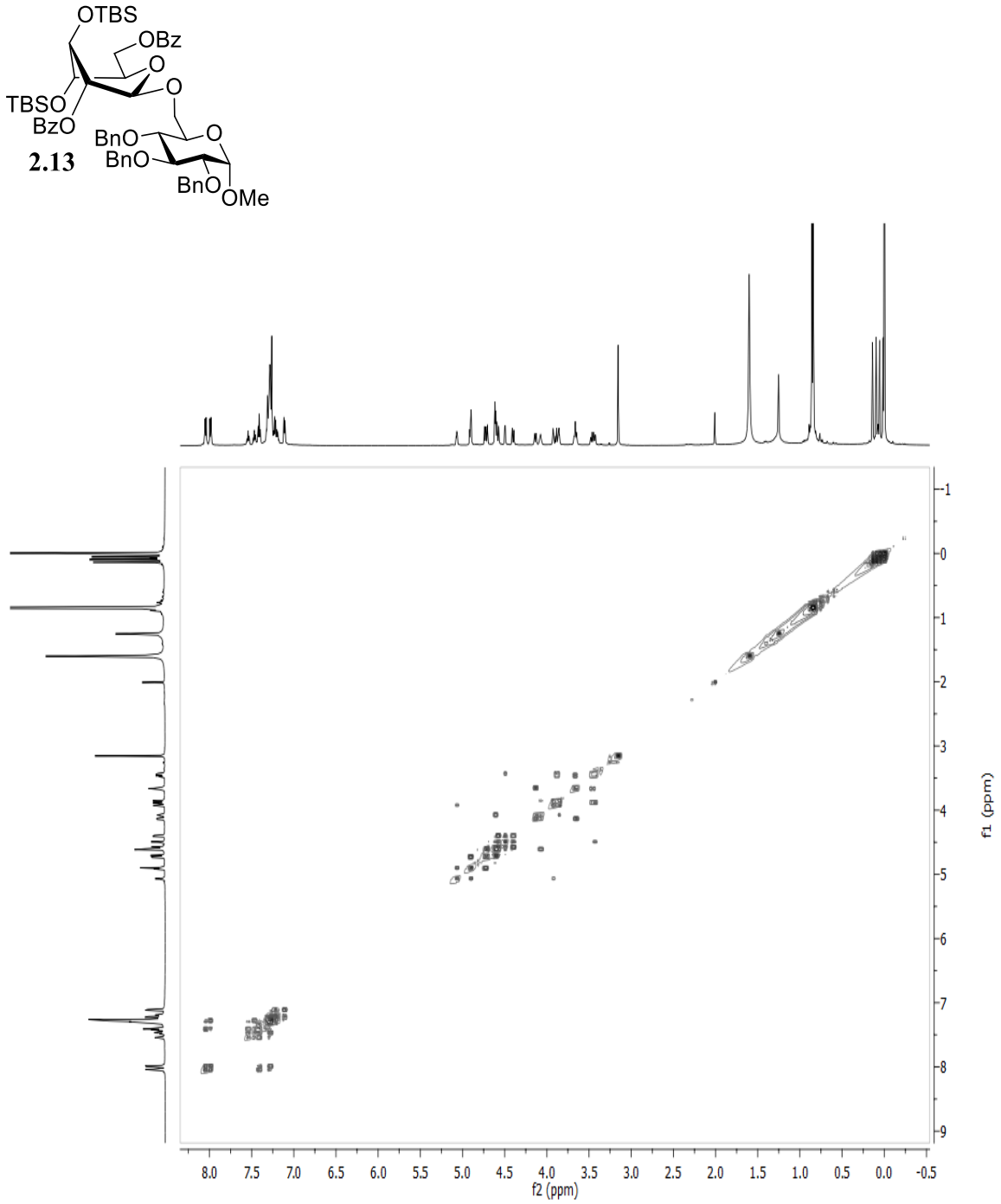
$CDCl_3$ 600 MHz

Figure A-19: ^{1}H NMR spectrum of Methyl 6-*O*-(2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.13**)



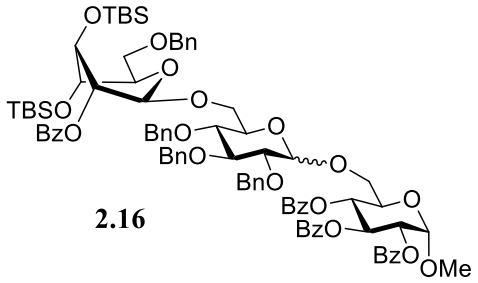
$CDCl_3$ 150 MHz

Figure A-20: ^{13}C NMR spectrum of Methyl 6-*O*-(2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.13**)



$CDCl_3$ 600 MHz

Figure A-21: 2-D NMR COSY spectrum of Methyl 6-*O*-(2,6-di-*O*-benzoyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2.13**)



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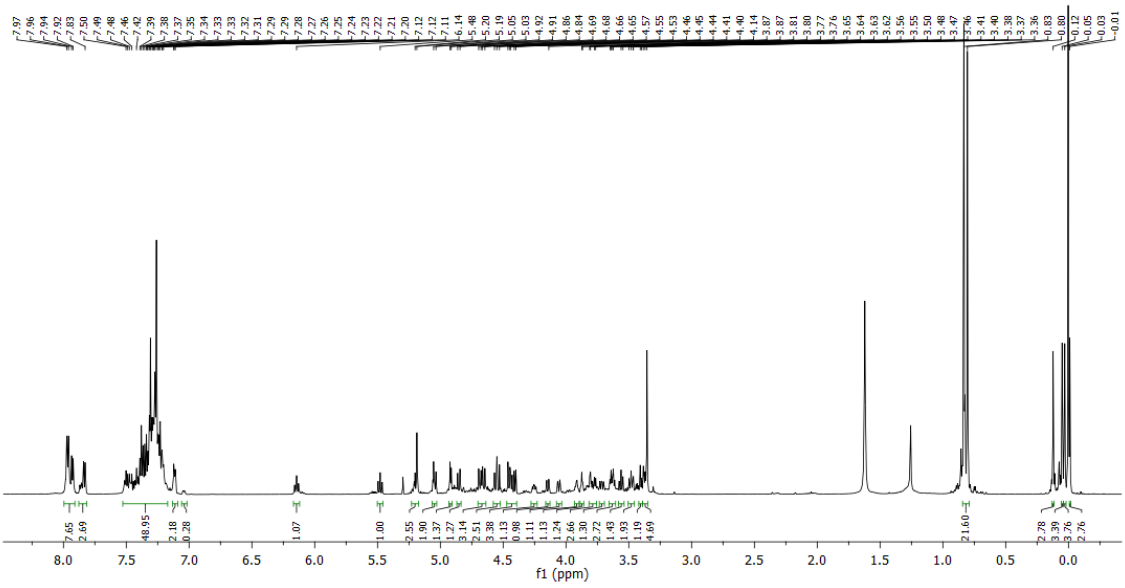
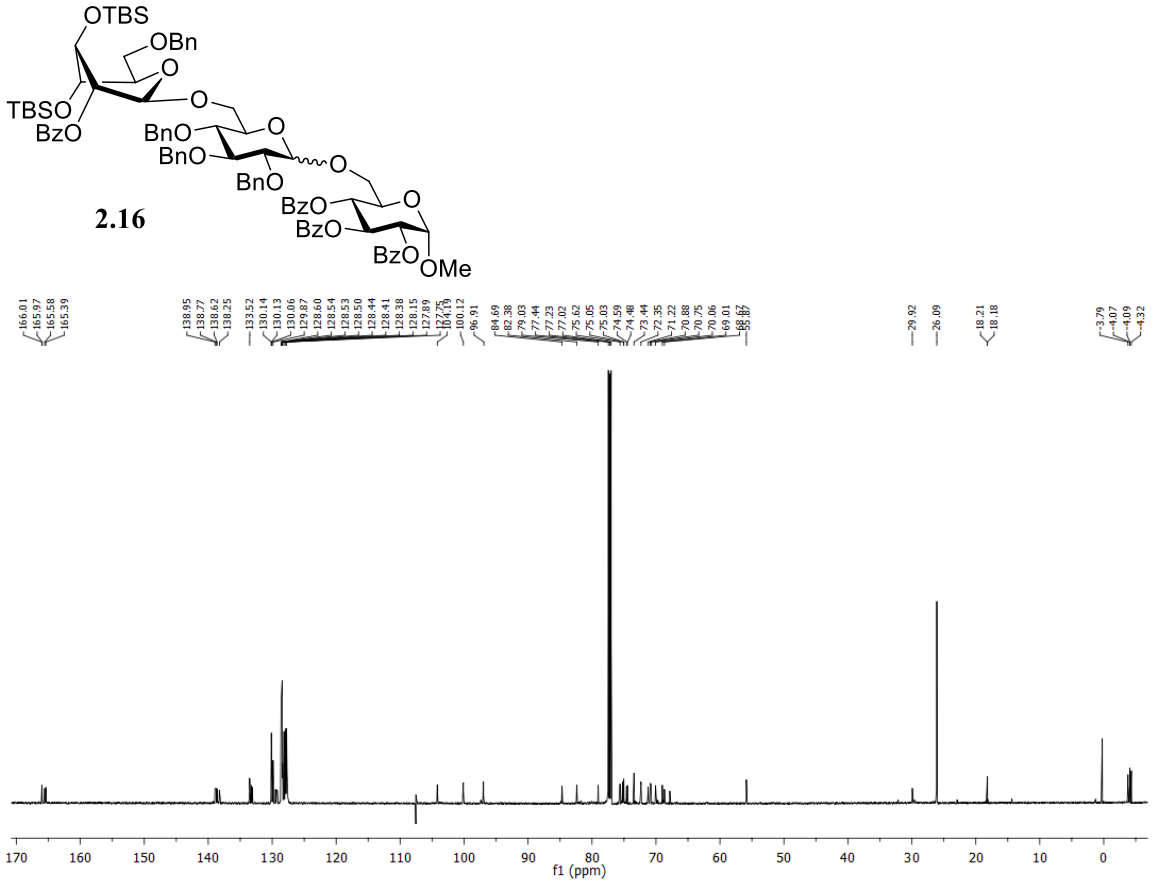


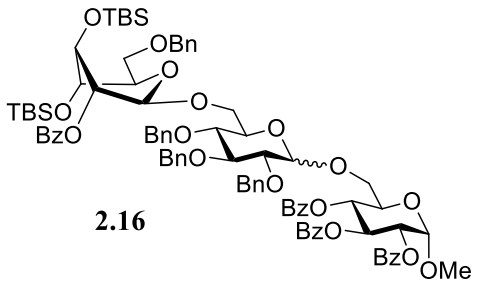
$CDCl_3$ 600 MHz

Figure A-22: ^{1}H NMR spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (**2.16**)

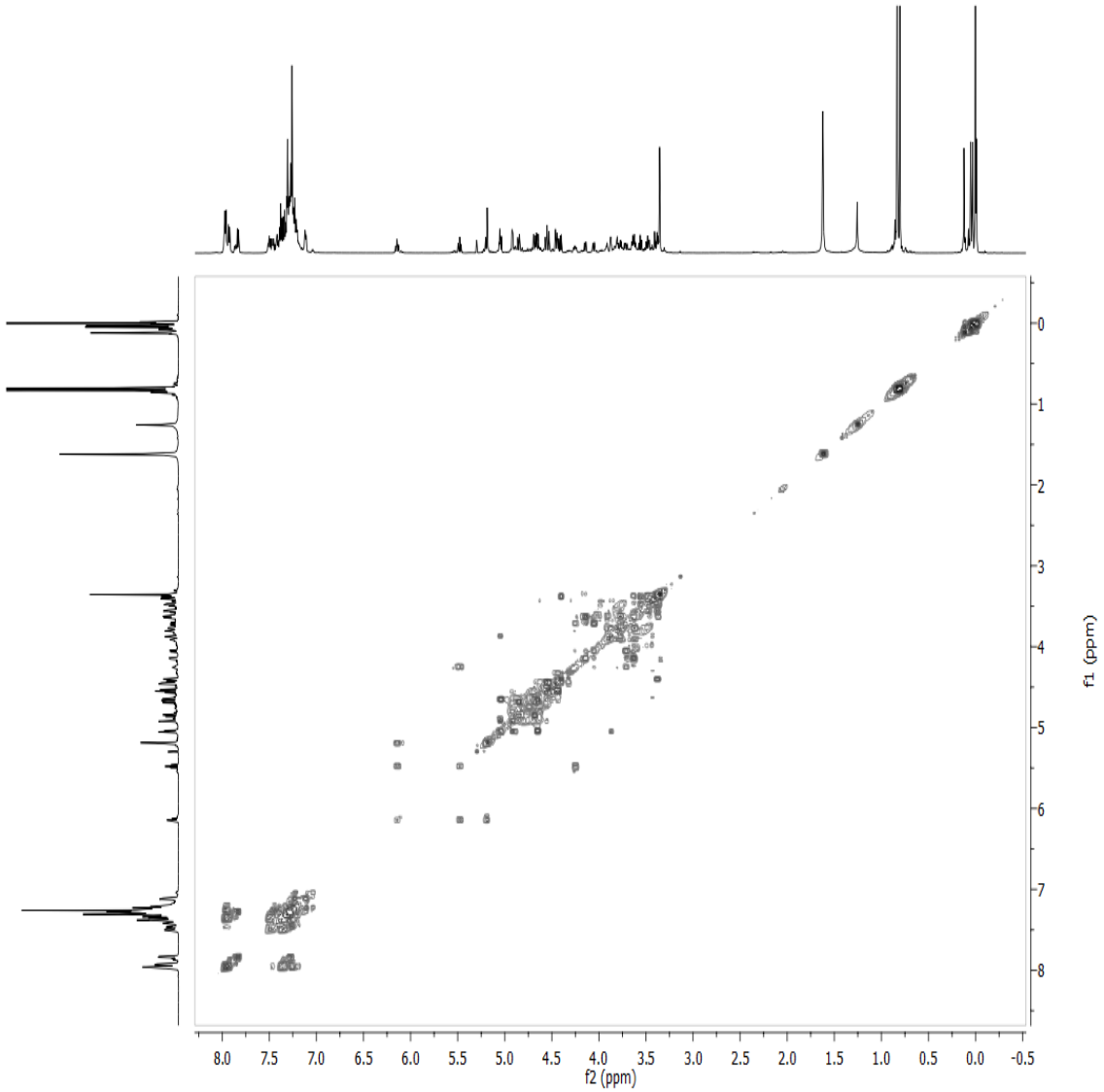


$CDCl_3$ 150MHz

Figure A-23: ^{13}C NMR spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (**2.16**)

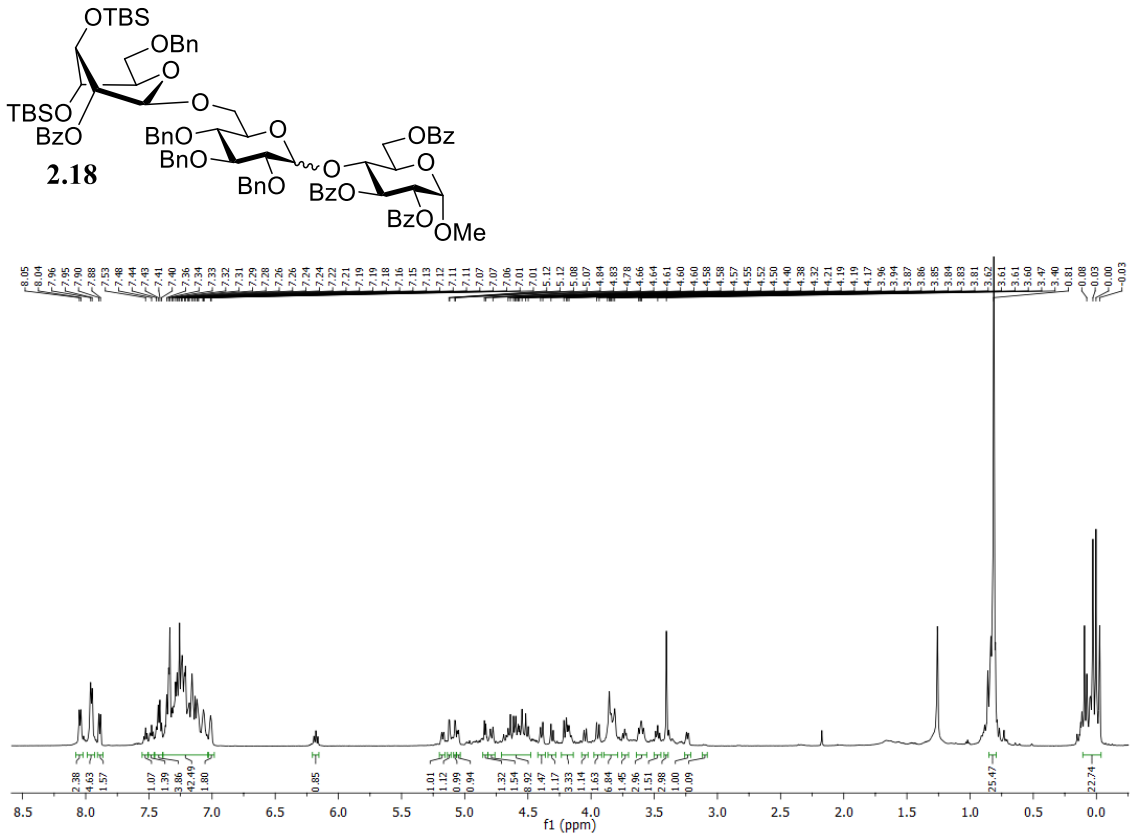


2.16



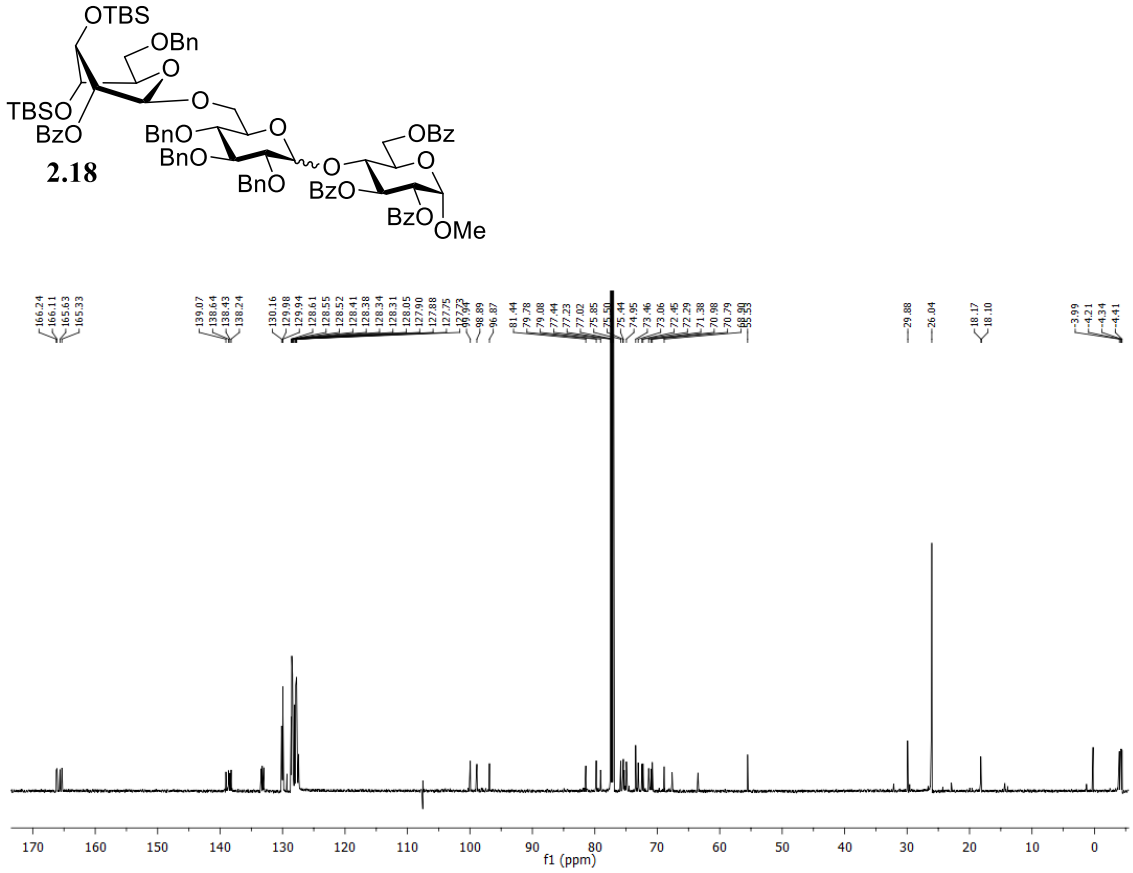
$CDCl_3$ 300 MHz

Figure A-24: 2-D NMR COSY spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (**2.16**)



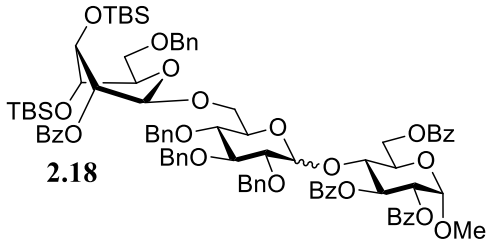
$CDCl_3$ 300 MHz

Figure A-25: ^{1}H NMR spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**2.18**)

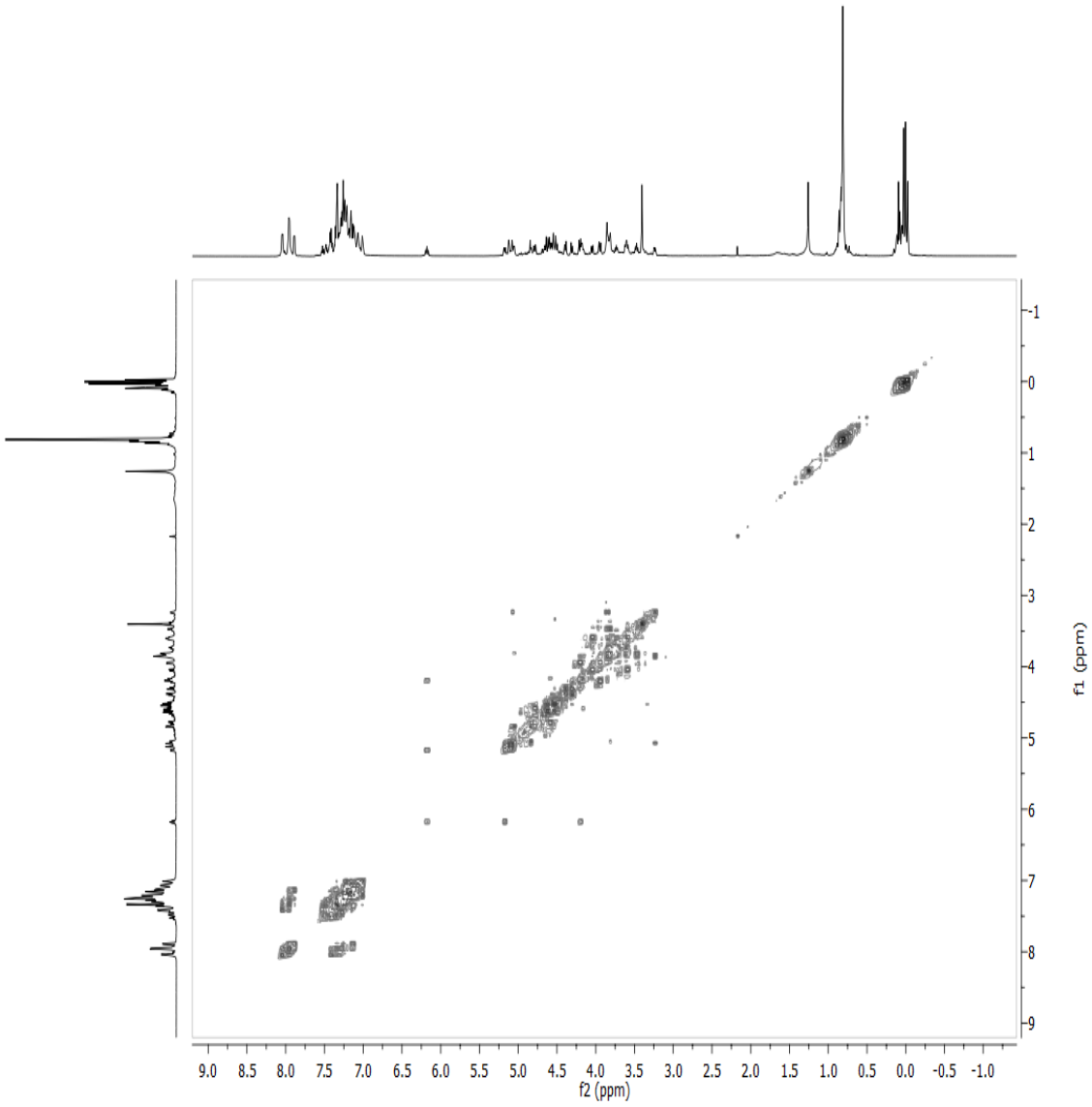


$CDCl_3$ 150 MHz

Figure A-26: ^{13}C NMR spectrum of 3 Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**2.18**)

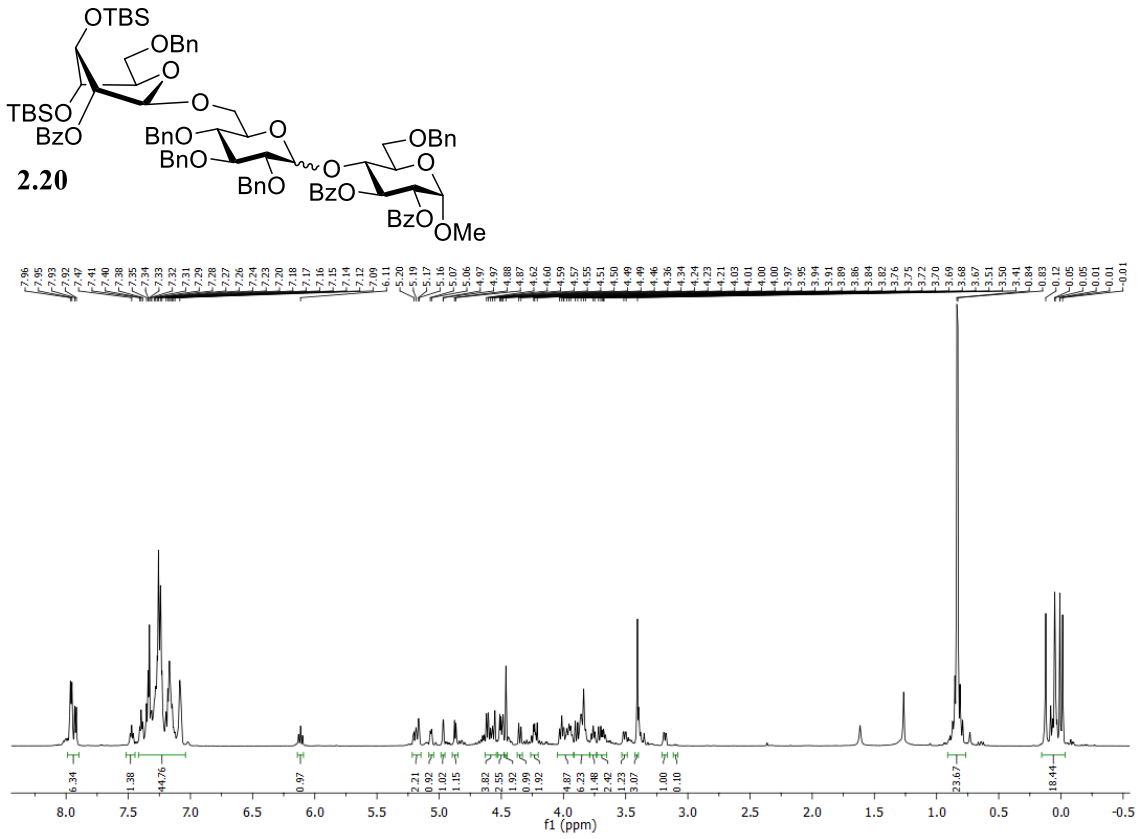


2.18



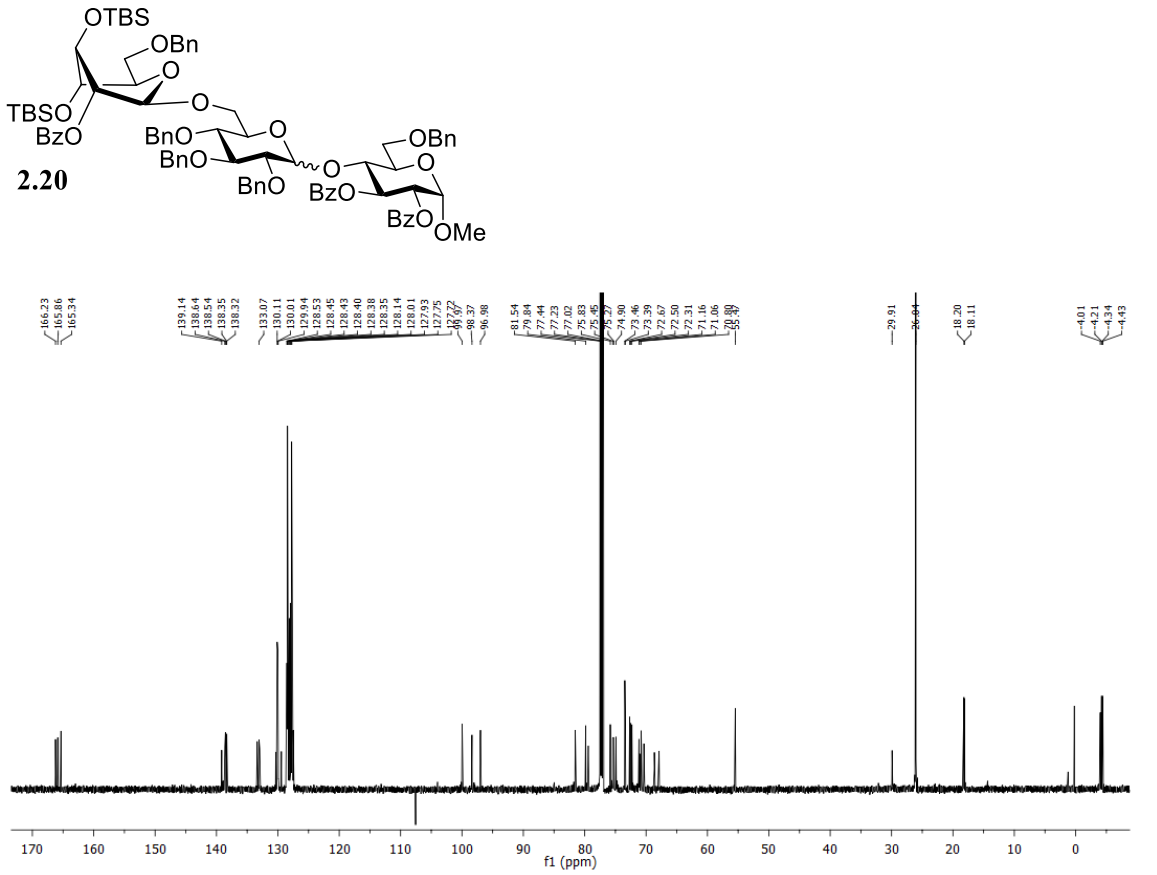
$CDCl_3$ 600 MHz

Figure A-27: 2-D NMR COSY spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**2.18**)



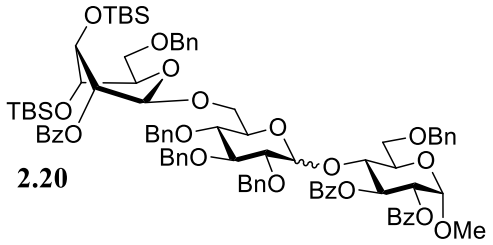
$CDCl_3$ 300 MHz

Figure A-28: ^{1}H NMR spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**2.20**)

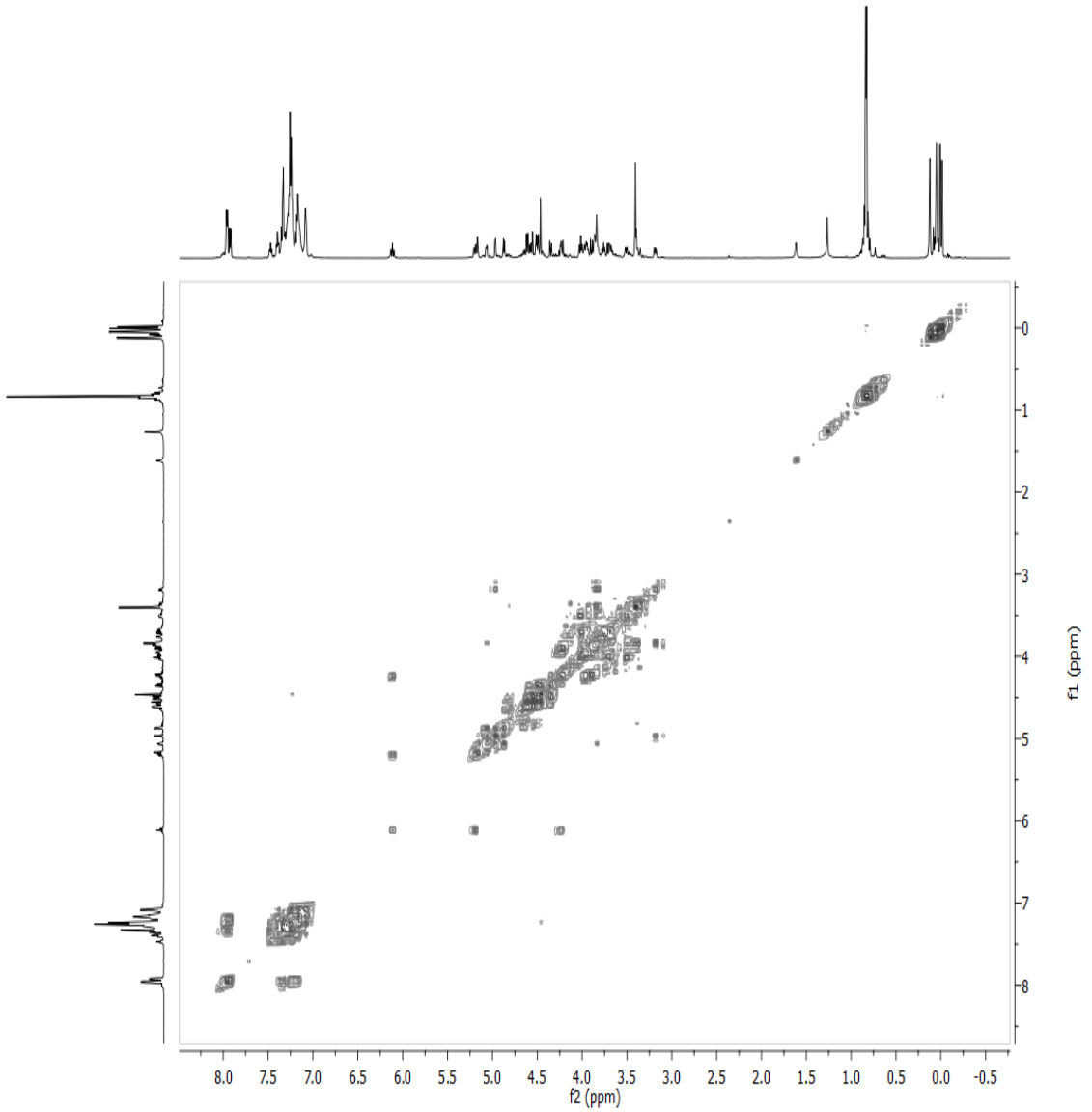


$CDCl_3$ 150 MHz

Figure A-29: ^{13}C NMR spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**2.20**)

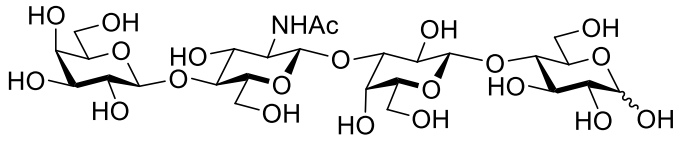


2.20

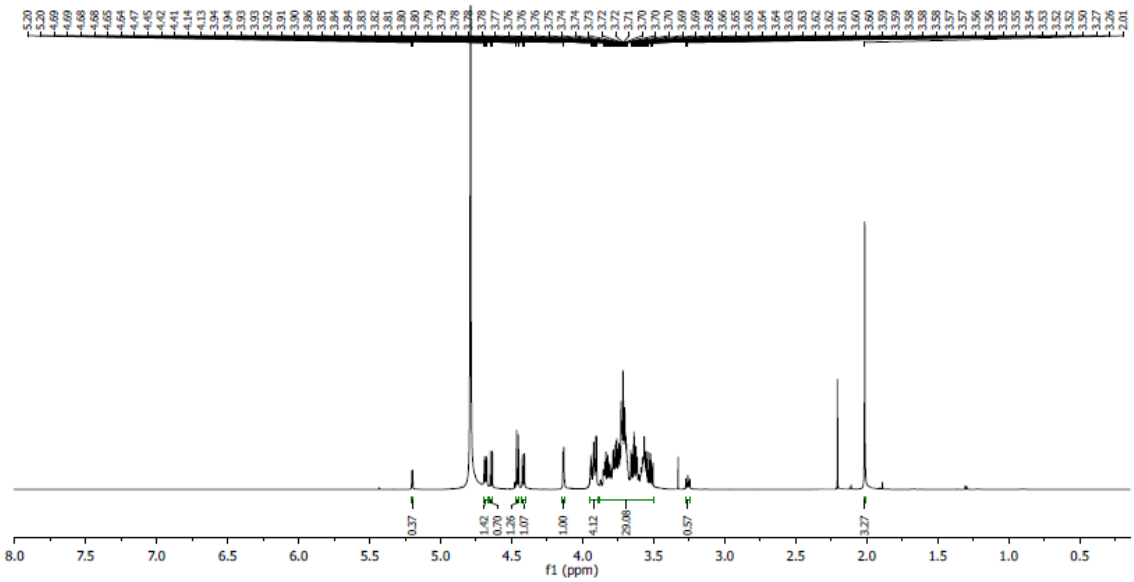


$CDCl_3$ 600 MHz

Figure A-30: 2-D NMR COSY spectrum of Methyl *O*-(2-*O*-benzoyl-6-*O*-benzyl-3,4-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-glucopyranoside (**2.20**)

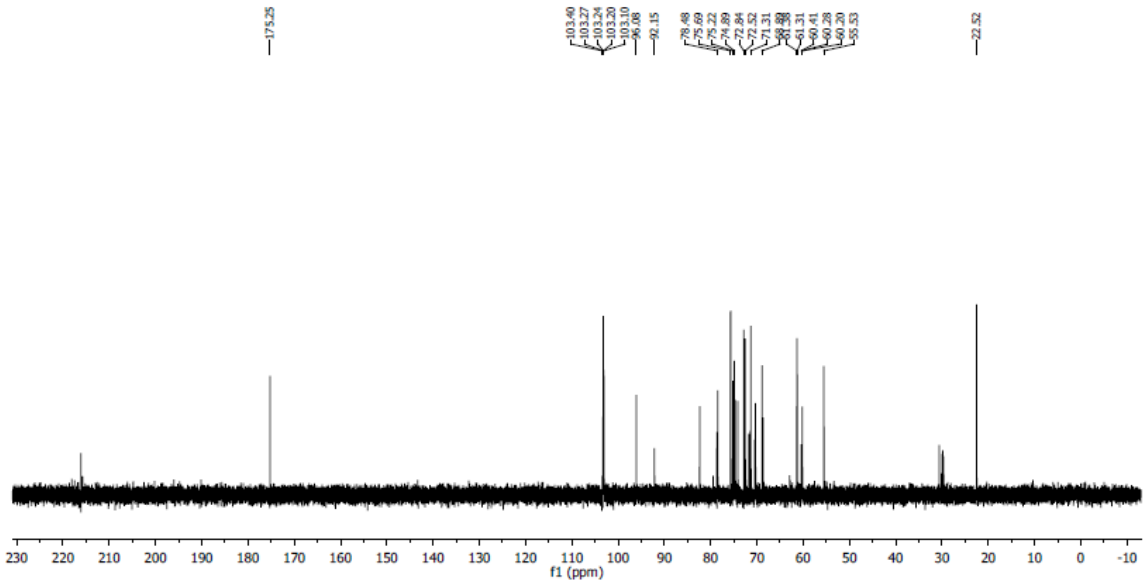


3.1



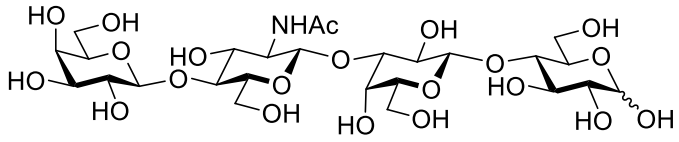
D_2O 600 MHz

Figure A-31: ^{1}H NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**3.1**, LNnT)

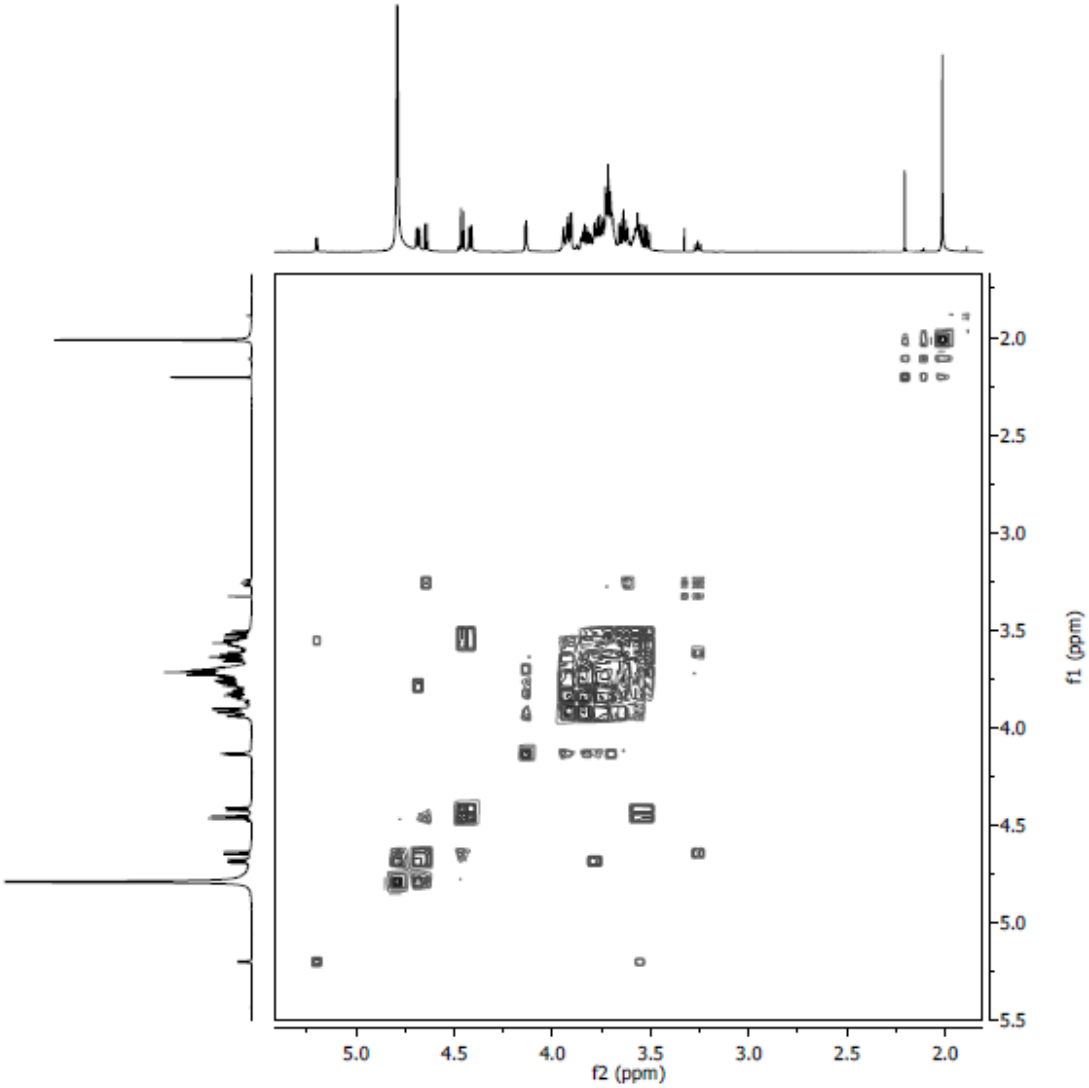


D_2O 151 MHz

Figure A-32: ^{13}C NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**3.1**, LNnT)



3.1



D_2O 600 MHz

Figure A-33: 2-D NMR COSY spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**3.1**, LNnT)

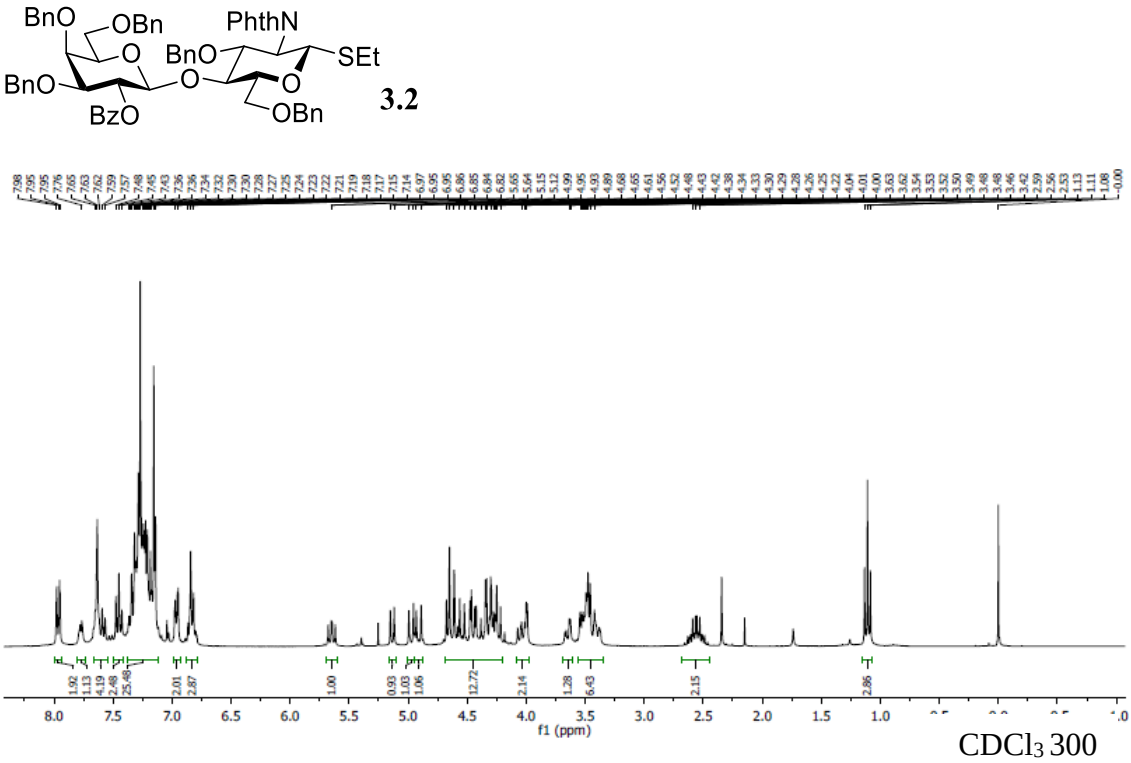


Figure A-34: ^{1}H NMR spectrum of Ethyl O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3.2**)

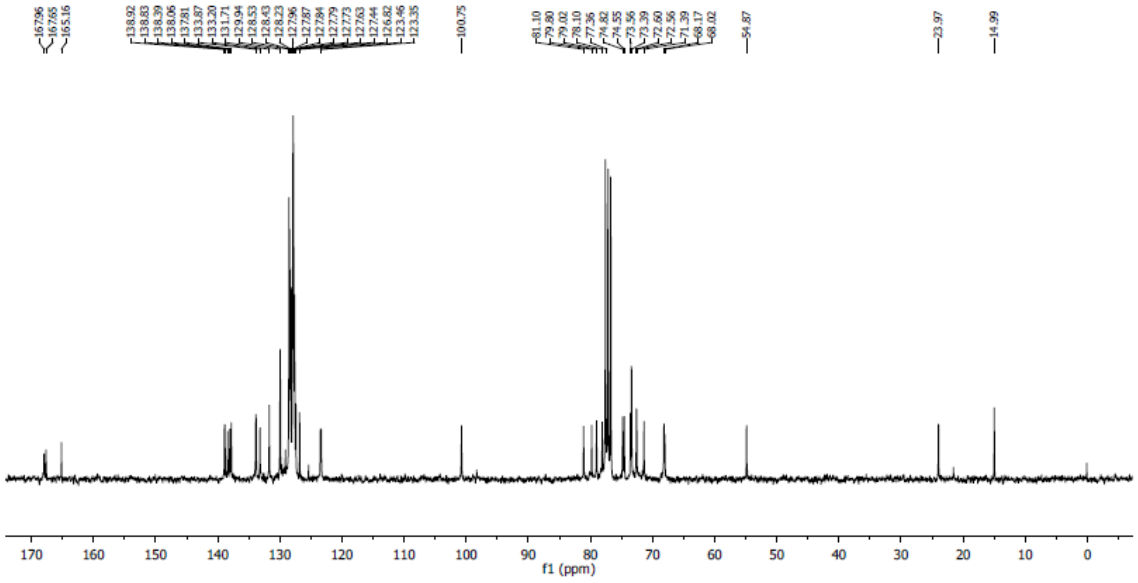
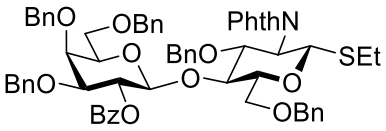
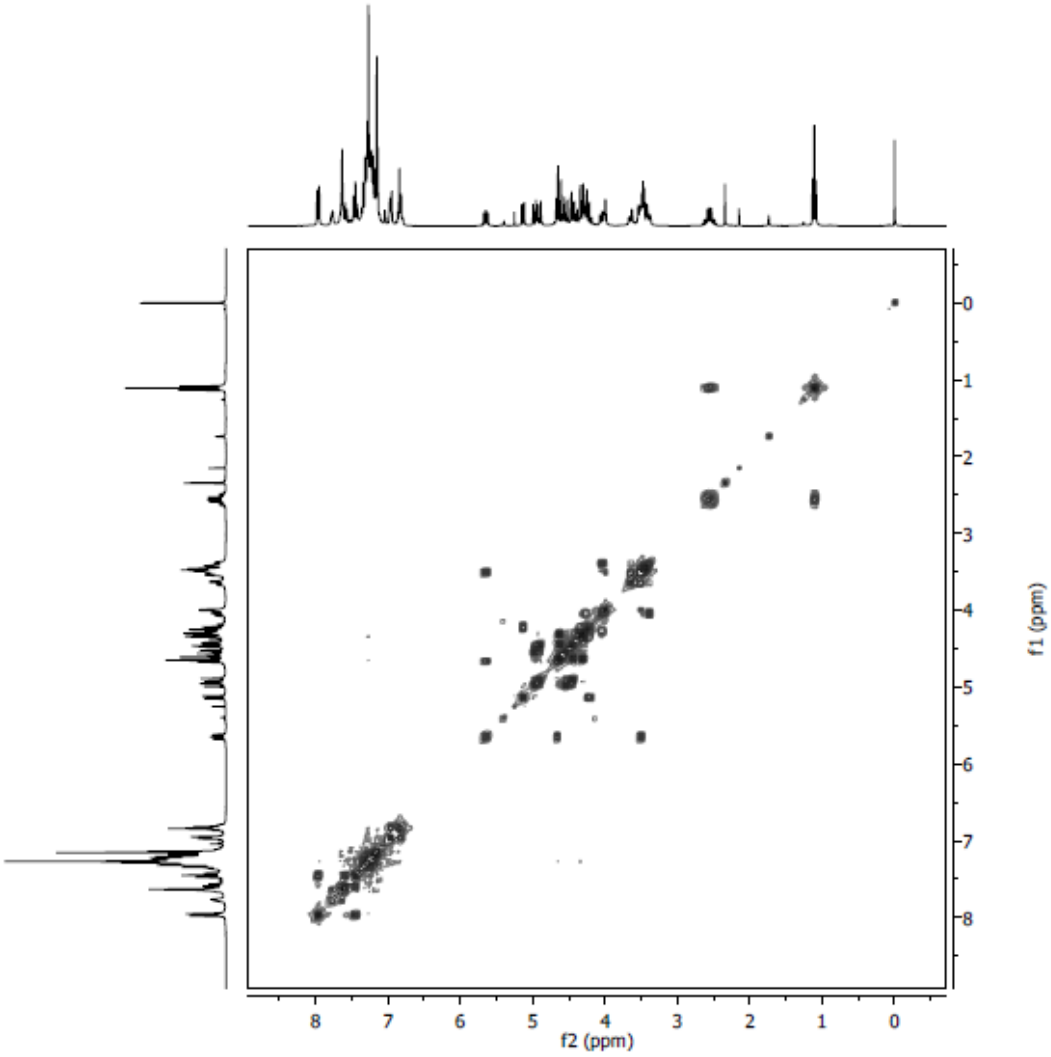


Figure A-35: ^{13}C NMR spectrum of Ethyl O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3.2**)

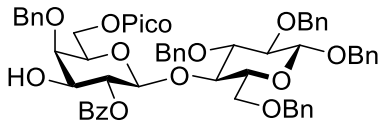


3.2



$CDCl_3$ 300 MHz

Figure A-36: 2-D NMR COSY spectrum of Ethyl O-(2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3.2**)



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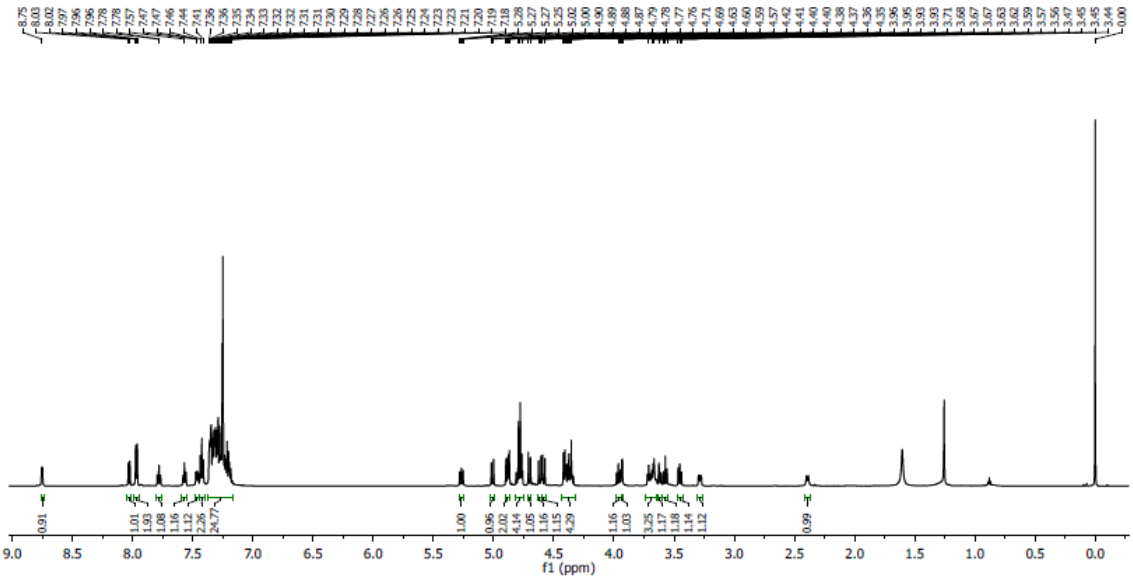
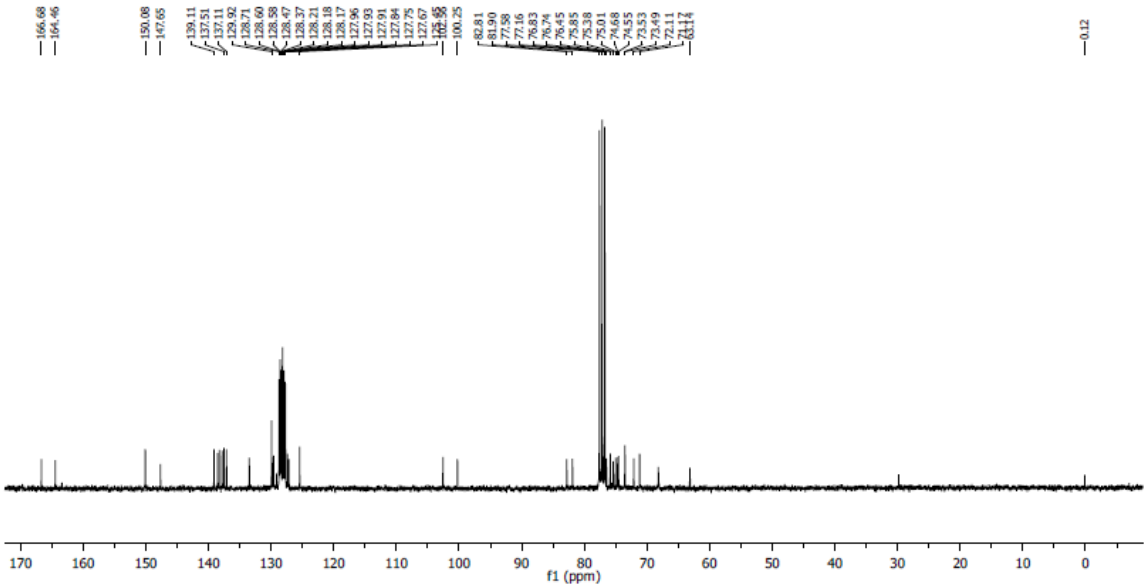


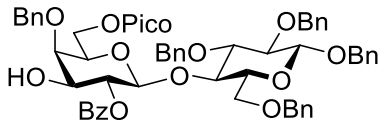
$CDCl_3$ 600 MHz

Figure A-37: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.3**)



$CDCl_3$ 75 MHz

Figure A-38: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.3**)



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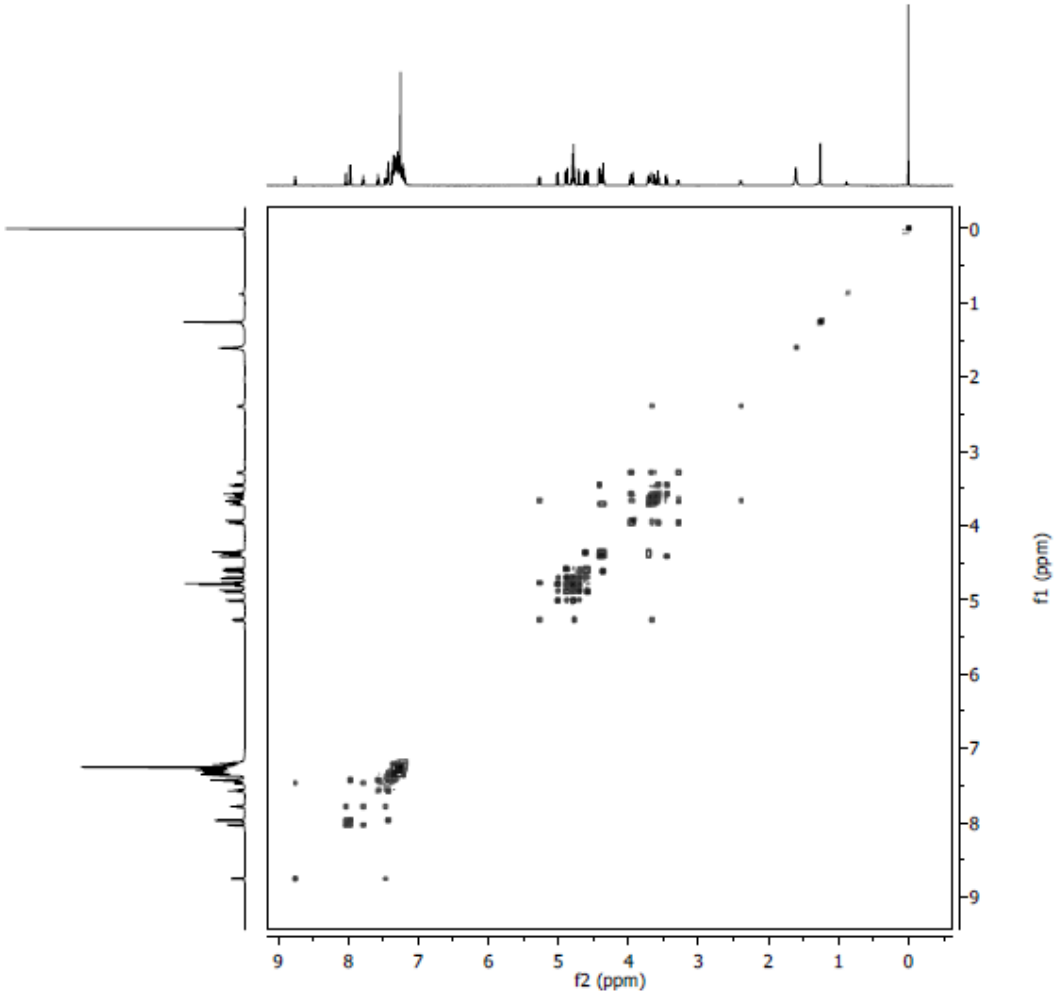
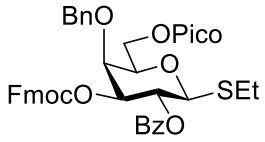
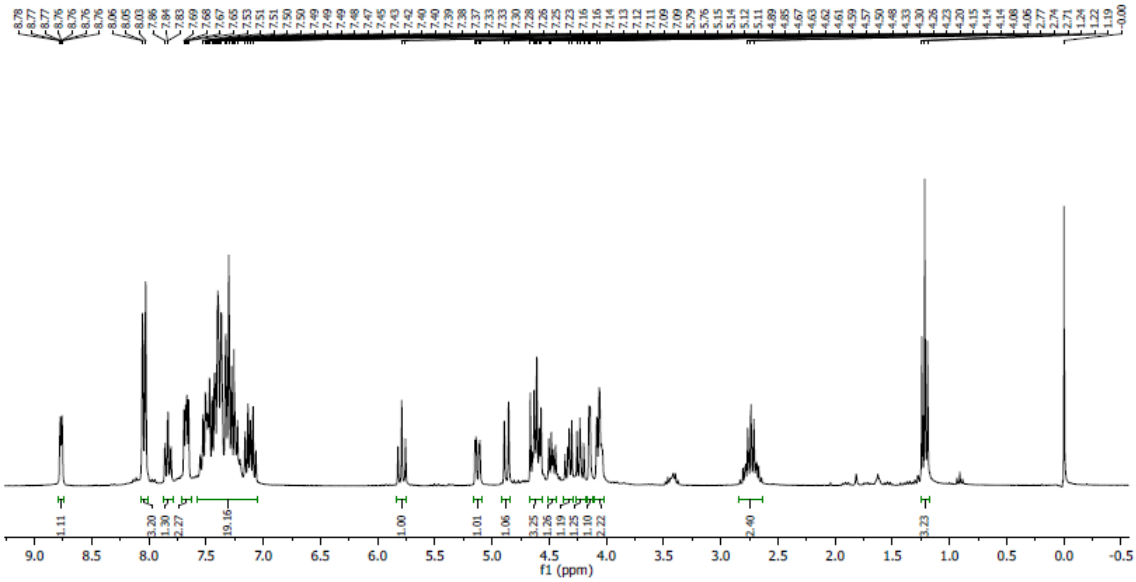


$CDCl_3$ 600 MHz

Figure A-39: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.3**)

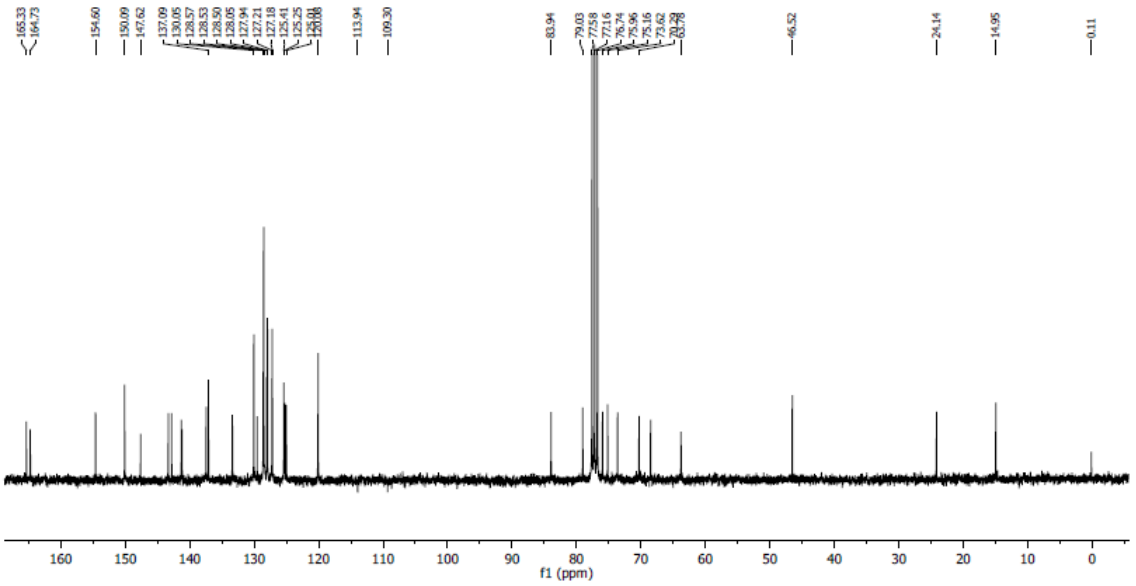


3.6



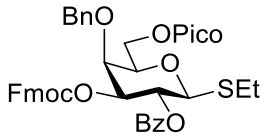
$CDCl_3$ 300 MHz

Figure A-40: ^{1}H NMR spectrum of Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-picoloyl-1-thio-β-D-galactopyranoside (**3.6**)

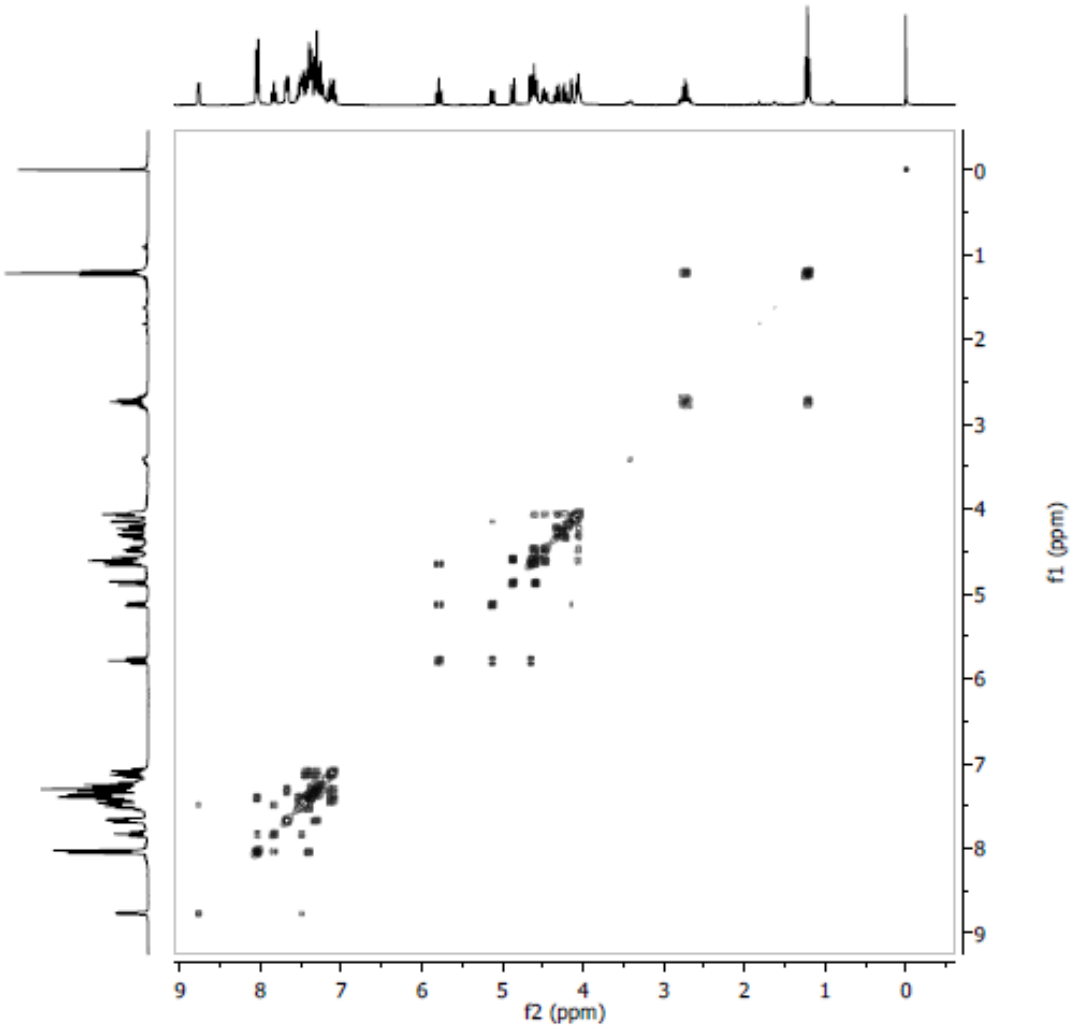


$CDCl_3$ 75 MHz

Figure A-41: ^{13}C NMR spectrum of spectrum of Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-picoloyl-1-thio-β-D-galactopyranoside (**3.6**)

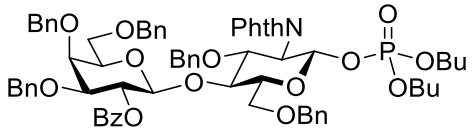


3.6

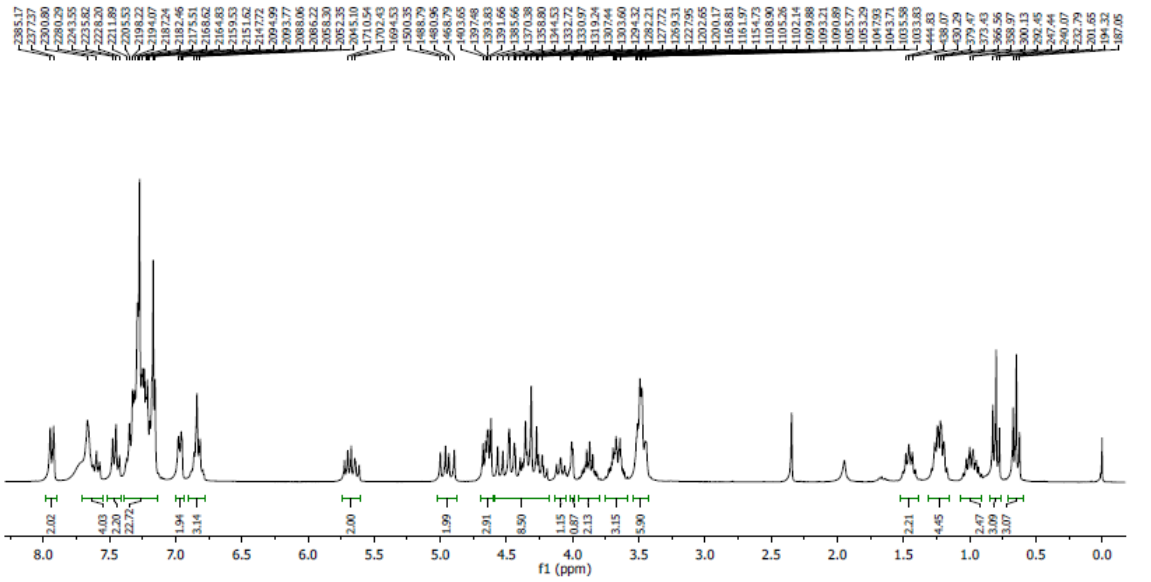


$CDCl_3$ 300 MHz

Figure A-42: 2-D NMR COSY spectrum of spectrum of Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-picoloyl-1-thio-β-D-galactopyranoside (**3.6**)

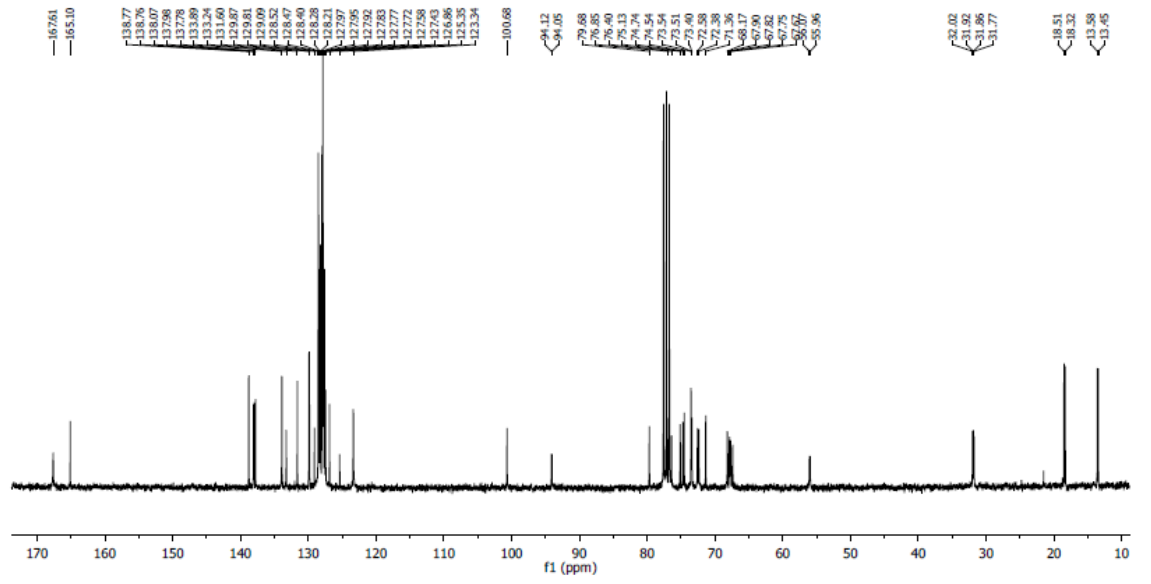


3.11



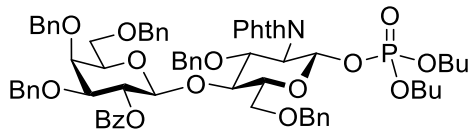
$CDCl_3$ 300 MHz

Figure A-43: ^{1}H NMR spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**3.11**)

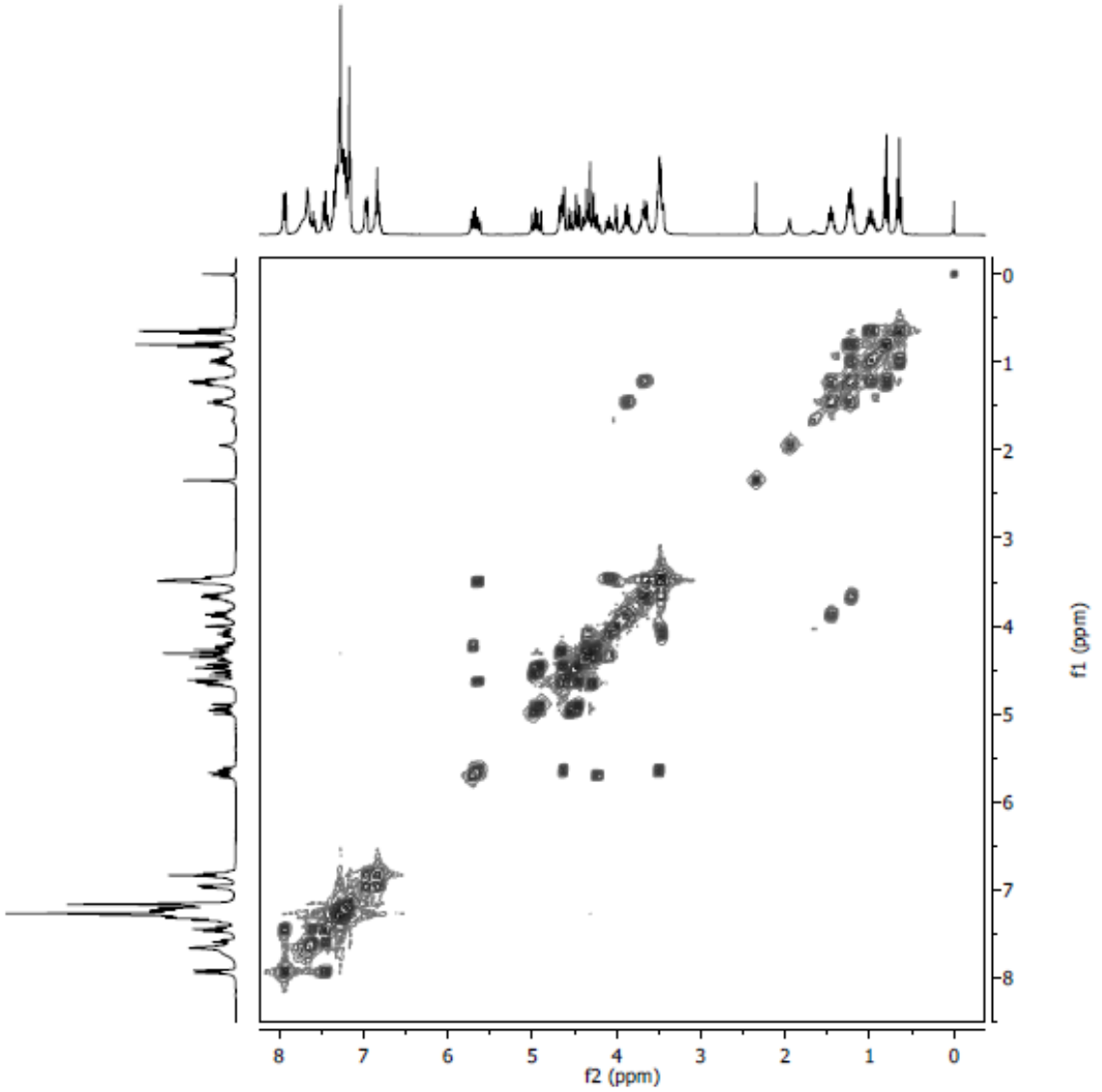


$CDCl_3$ 75 MHz

Figure A-44: ^{13}C NMR spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**3.11**)

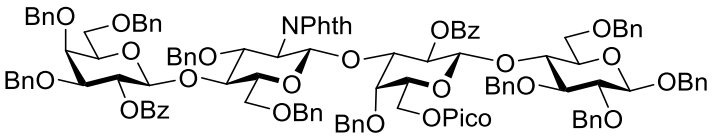


3.11

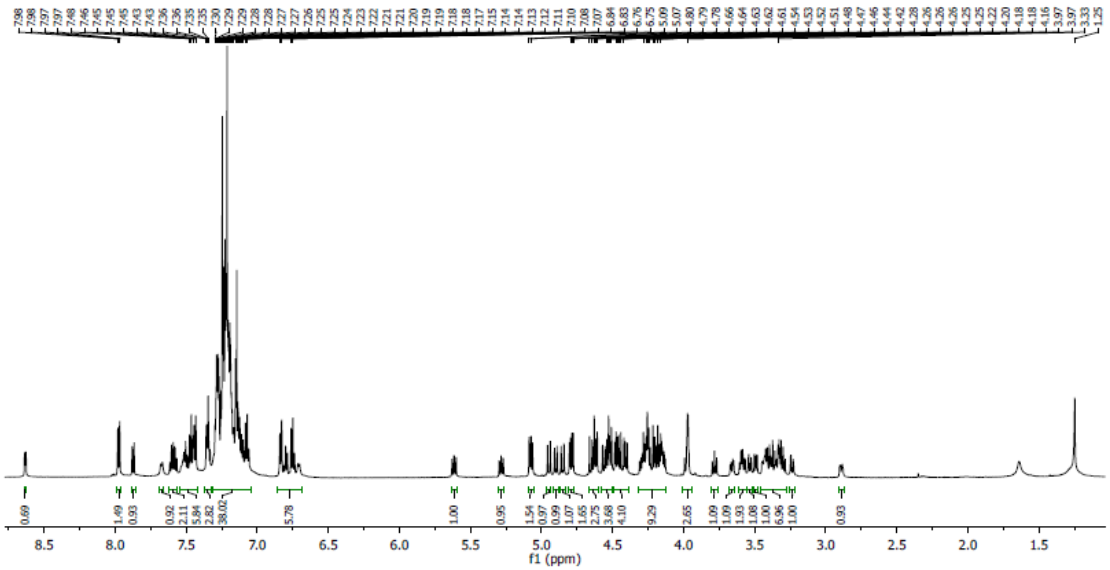


$CDCl_3$ 300 MHz

Figure A-45: 2-D NMR COSY spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**3.11**)

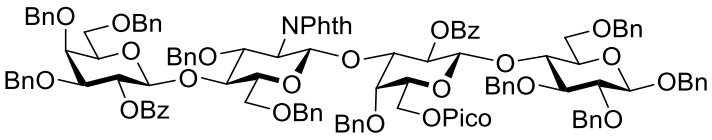


3.12

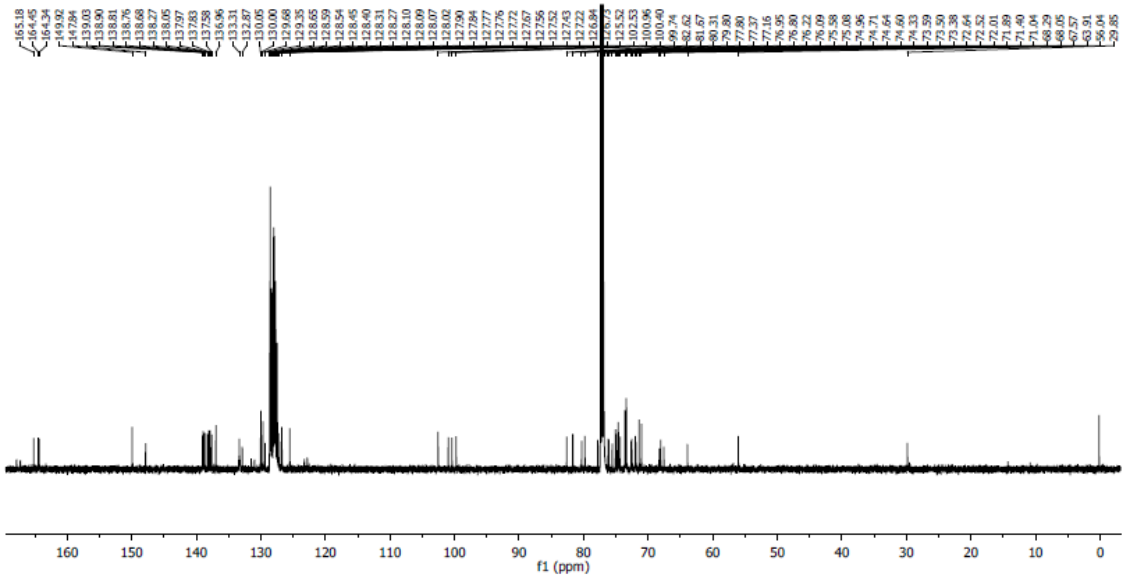


$CDCl_3$ 600 MHz

Figure A-46: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.12**)

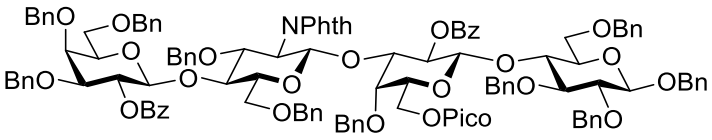


3.12

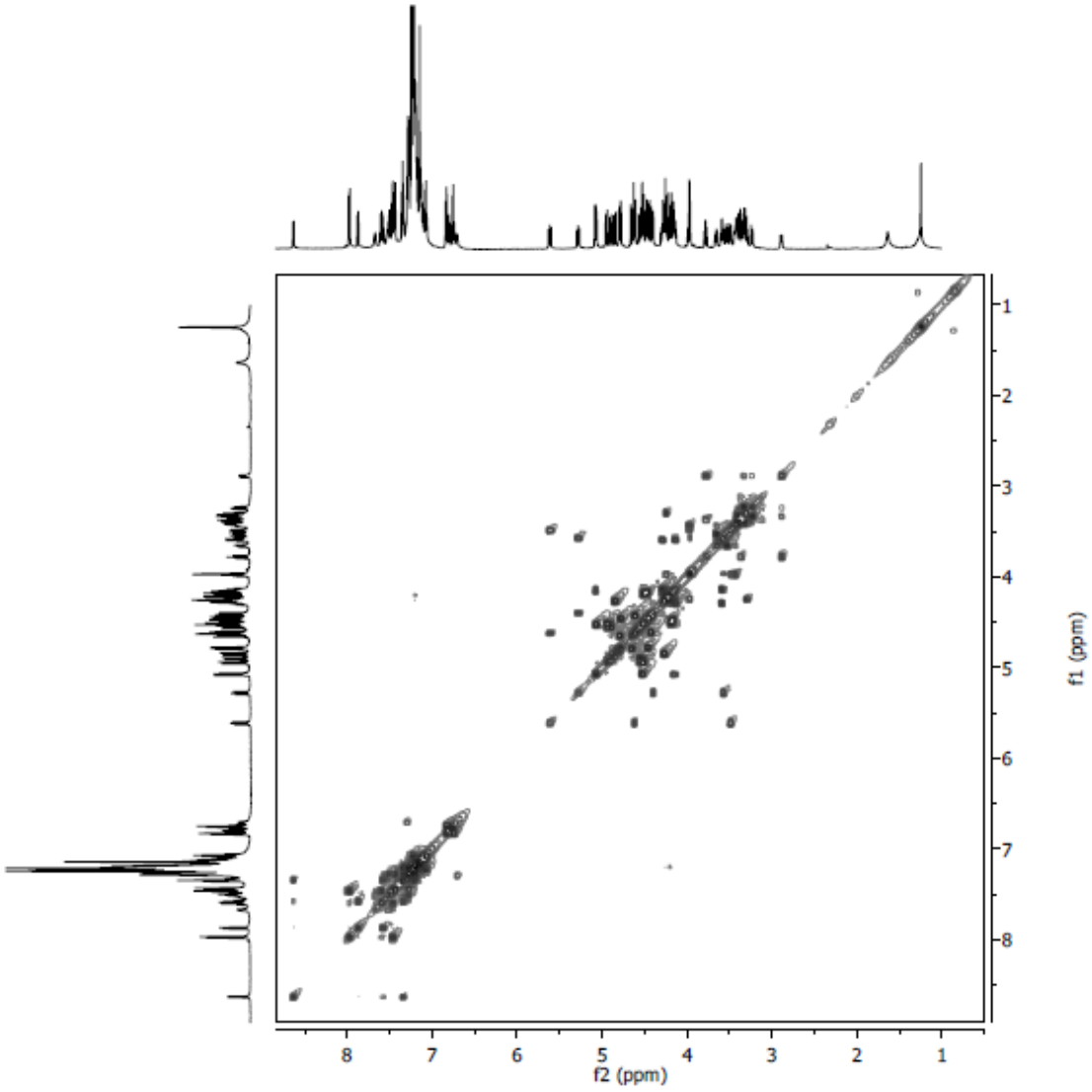


$CDCl_3$ 151 MHz

Figure A-47: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.12**)

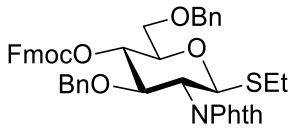


3.12

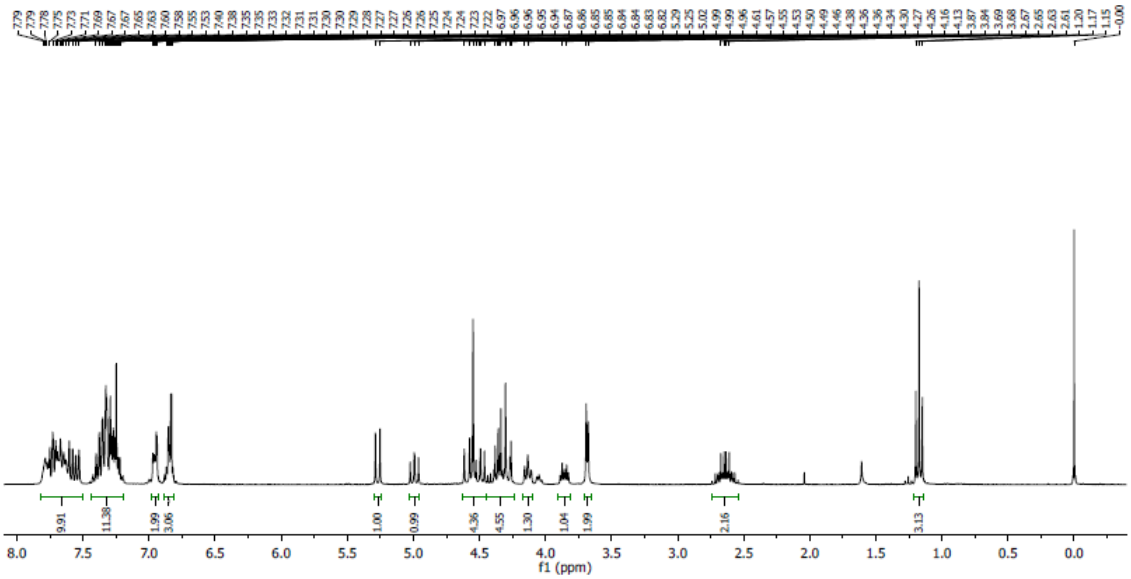


$CDCl_3$ 600 MHz

Figure A-48: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.12**)

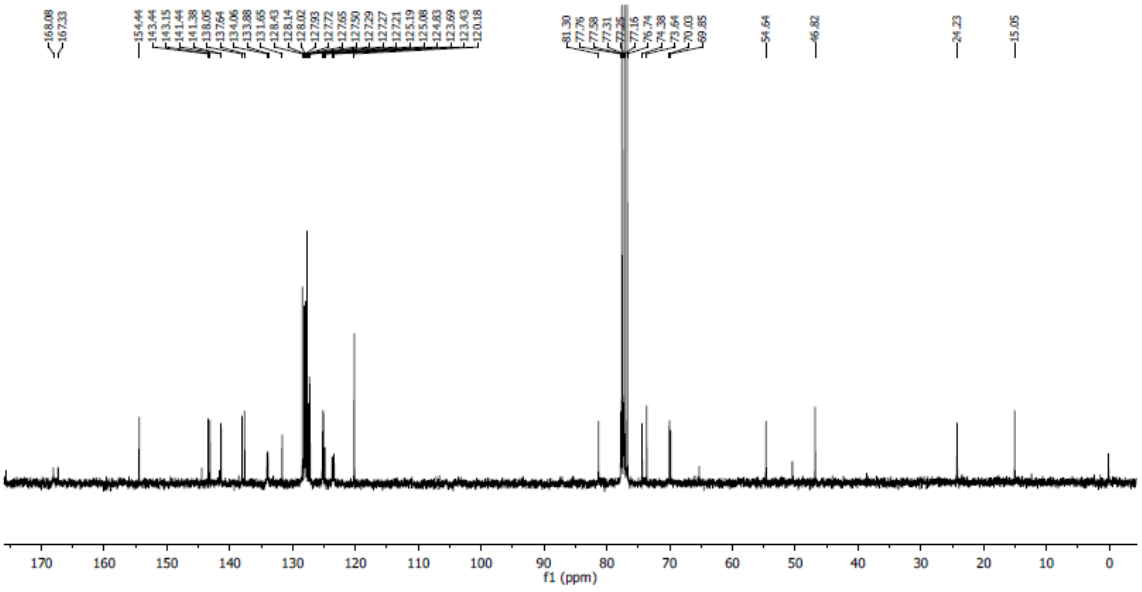


3.13



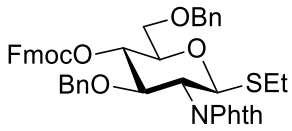
$CDCl_3$ 300 MHz

Figure A-49: ^{1}H NMR spectrum of Ethyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**3.13**)

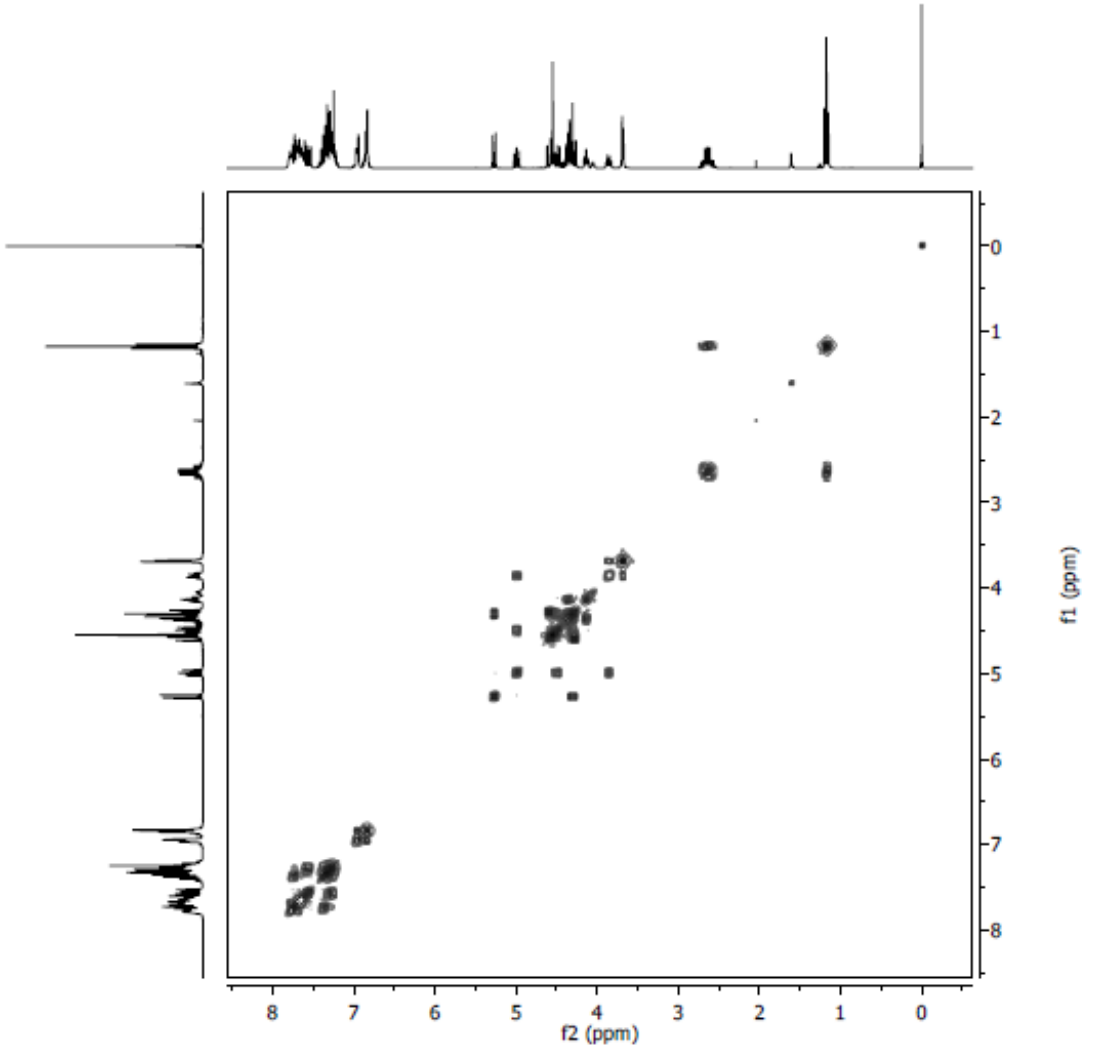


$CDCl_3$ 75 MHz

Figure A-50: ^{13}C NMR spectrum of Ethyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**3.13**)

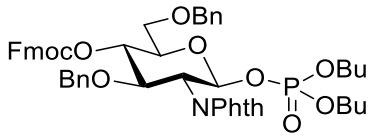


3.13

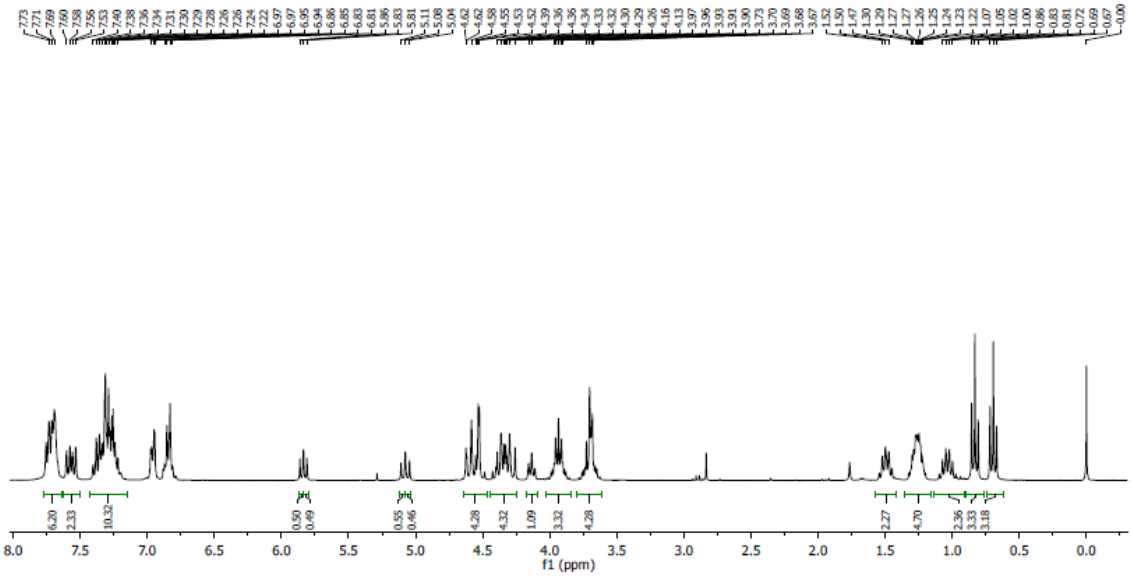


$CDCl_3$ 300 MHz

Figure A-51: 2-D NMR COSY spectrum of Ethyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**3.13**)

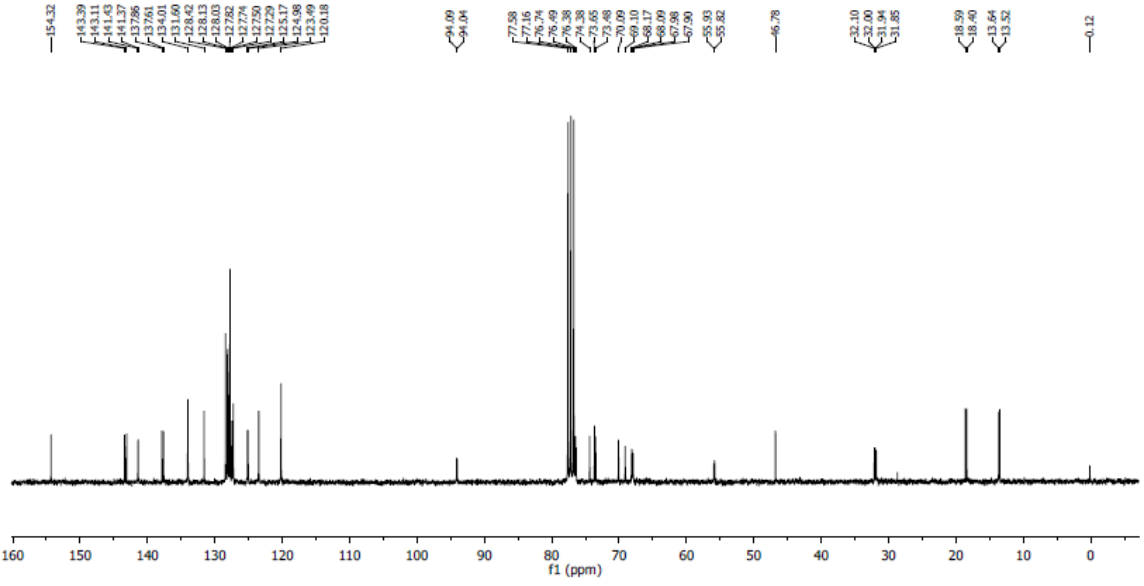


3.14



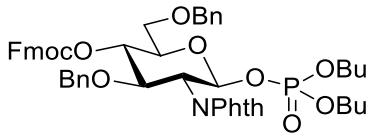
$CDCl_3$ 300 MHz

Figure A-52: ^{1}H NMR spectrum of Di-*O*-butyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxy-carbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**3.14**)

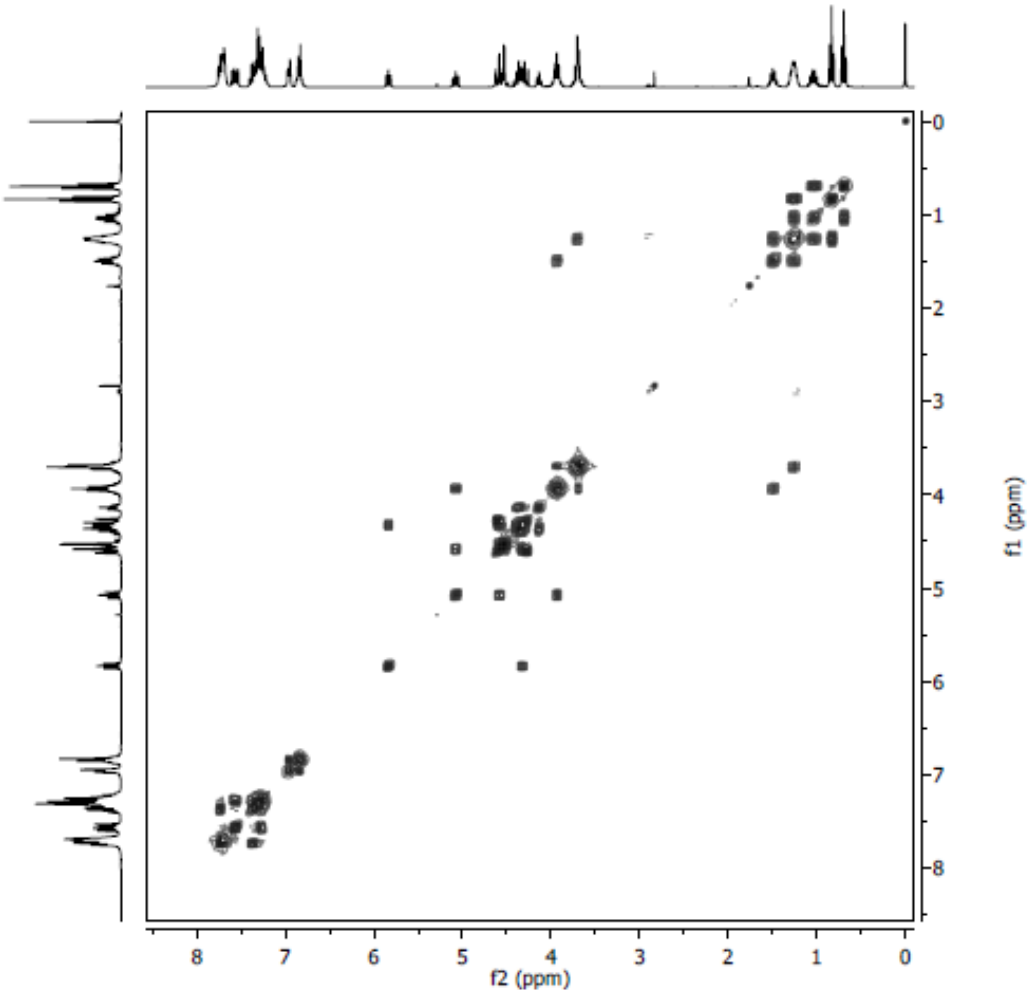


$CDCl_3$ 75 MHz

Figure A-53: ^{13}C NMR spectrum of Di-*O*-butyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxy-carbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**3.14**)

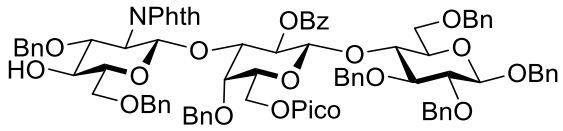


3.14

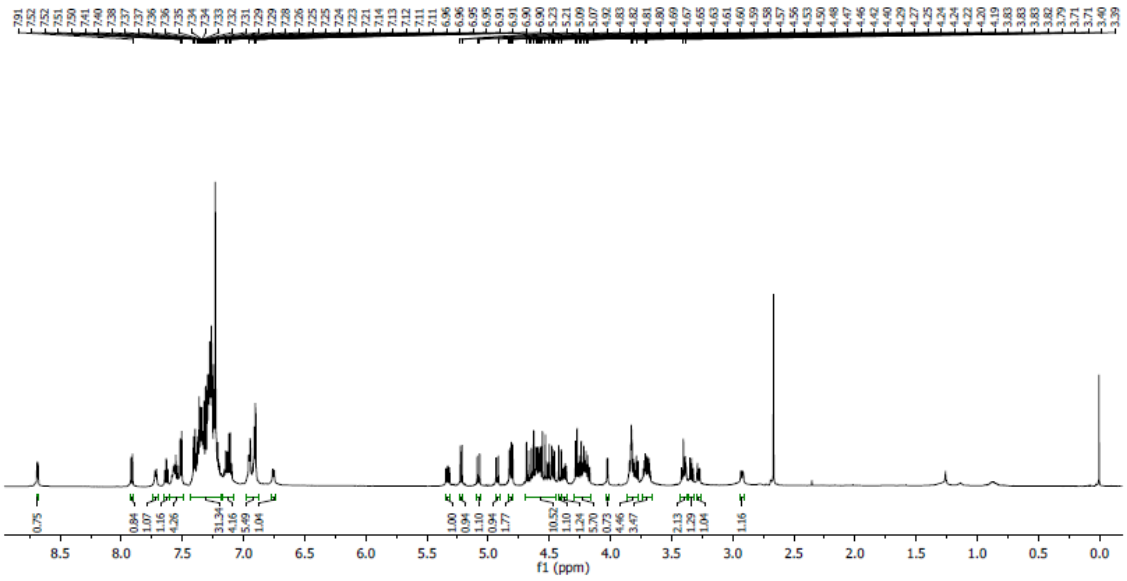


$CDCl_3$ 300 MHz

Figure A-54: 2-D NMR COSY spectrum of Di-*O*-butyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxy-carbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**3.14**)

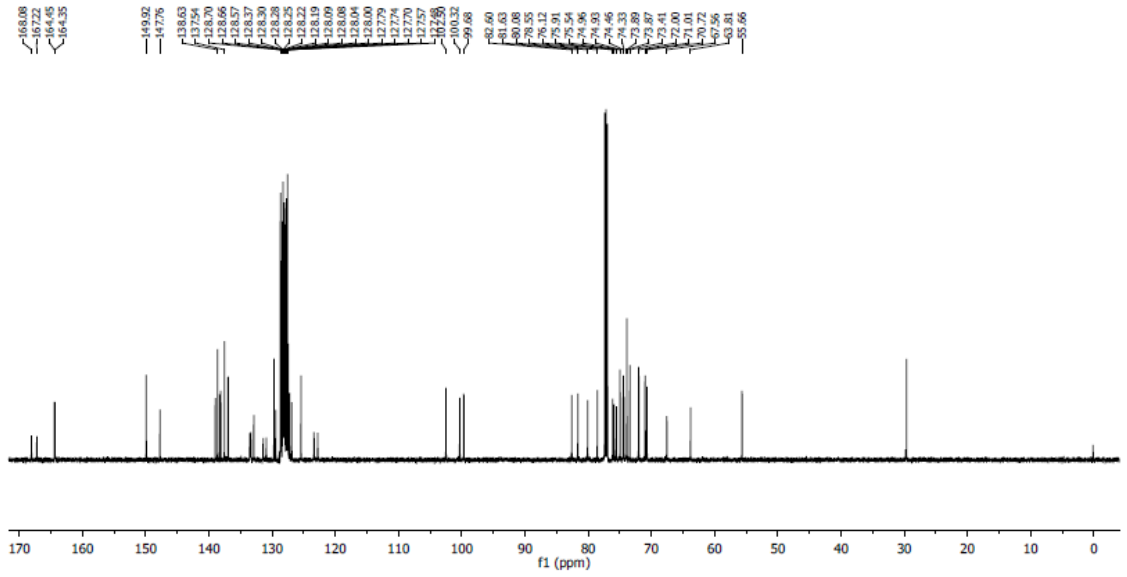


3.16



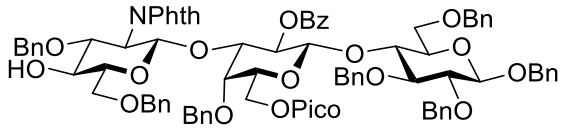
$CDCl_3$ 600 MHz

Figure A-55: ^{1}H NMR spectrum of Benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.16**)

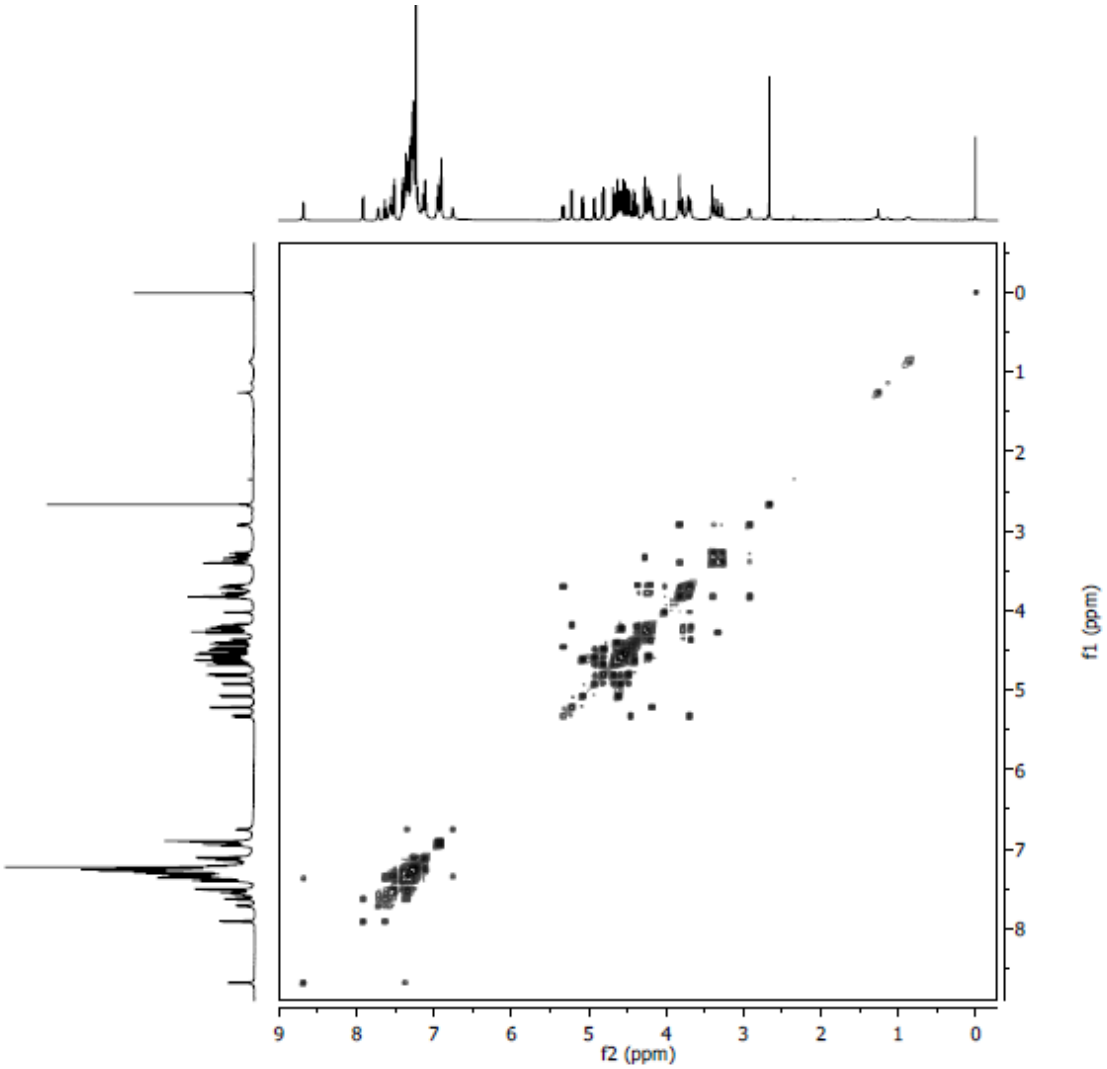


$CDCl_3$ 151 MHz

Figure A-56: ^{13}C NMR spectrum of Benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.16**)

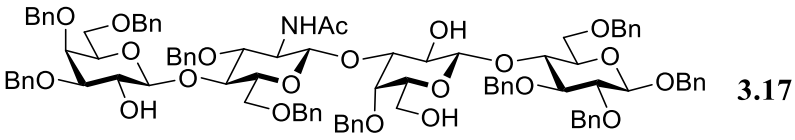


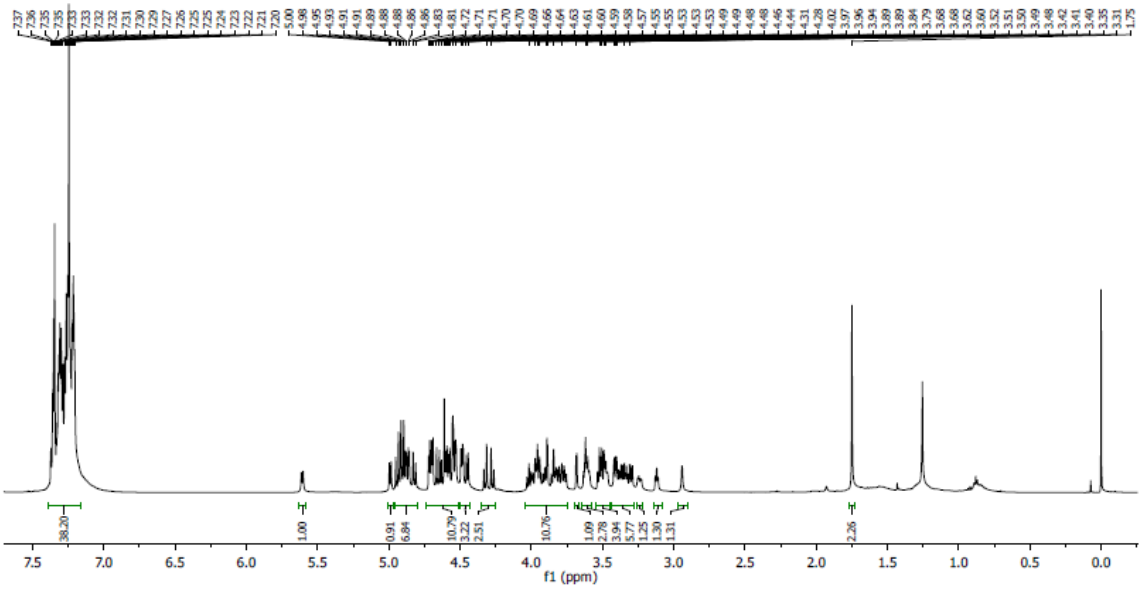
3.16



$CDCl_3$ 600 MHz

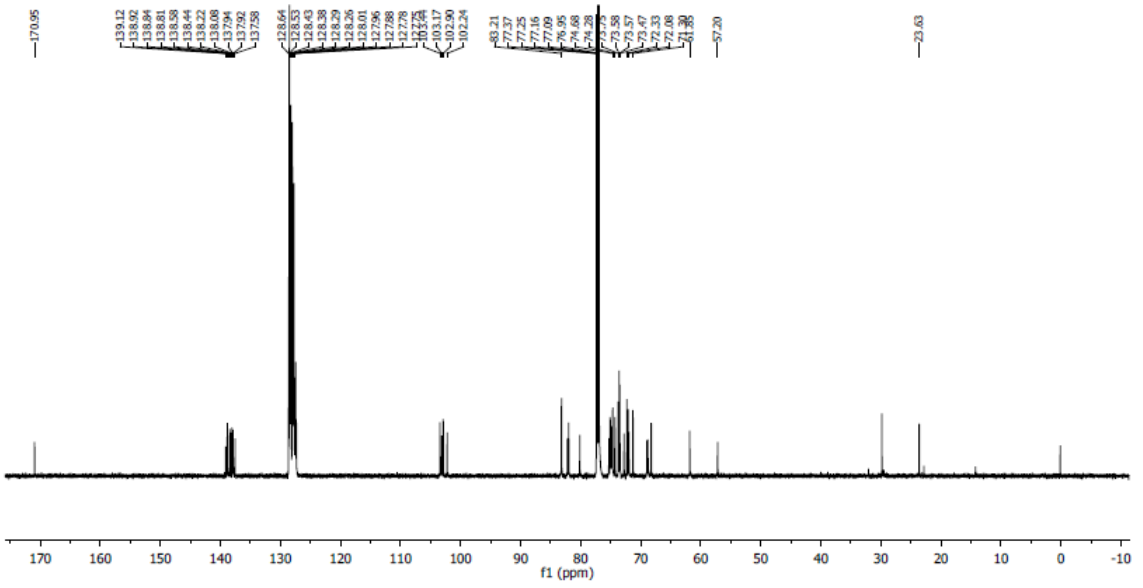
Figure A-57: 2-D NMR COSY spectrum of Benzyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.16**)





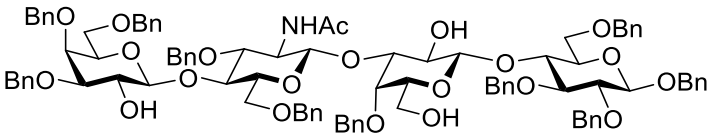
$CDCl_3$ 600 MHz

Figure A-58: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.17**)

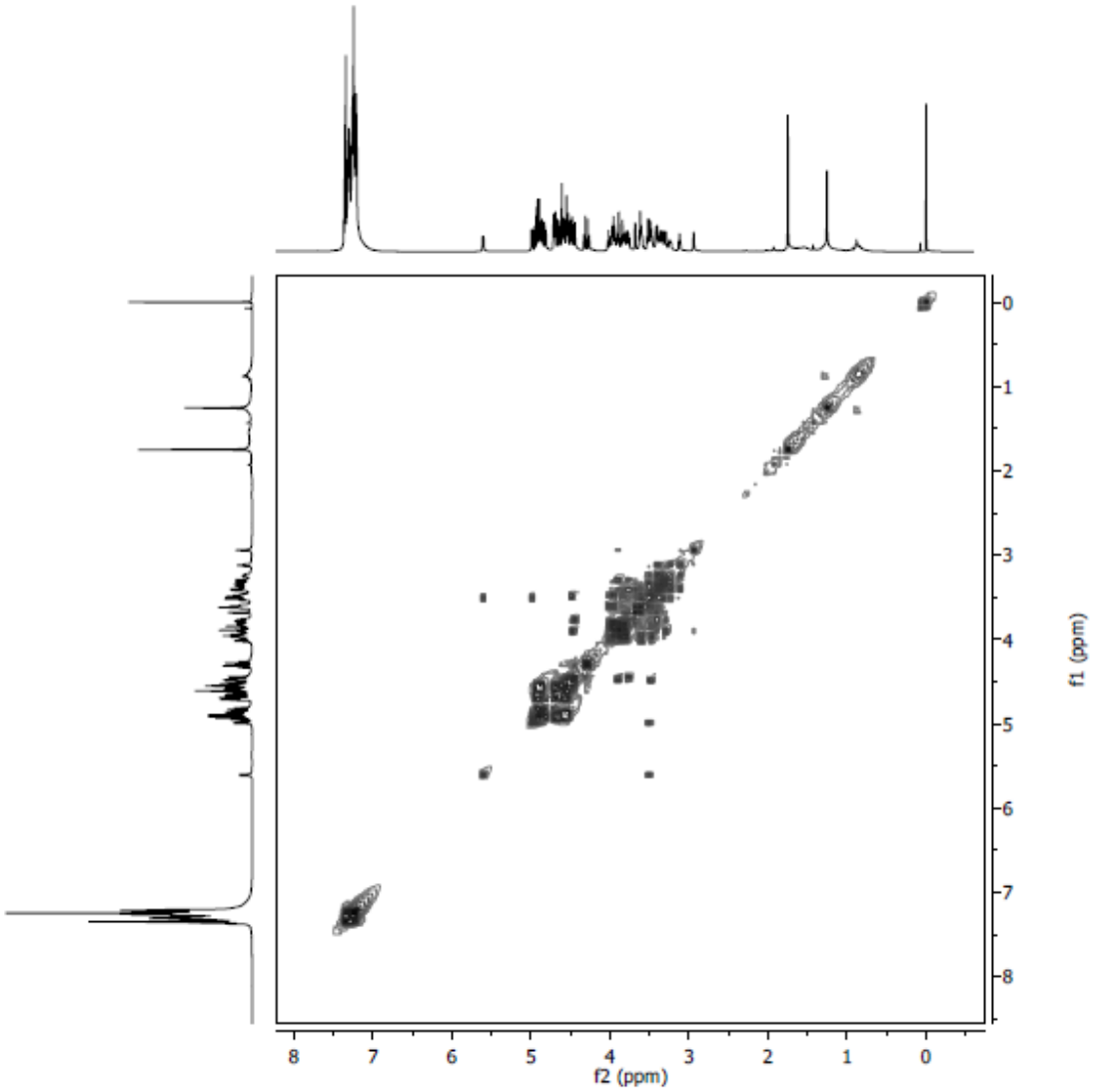


$CDCl_3$ 151 MHz

Figure A-59: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.17**)

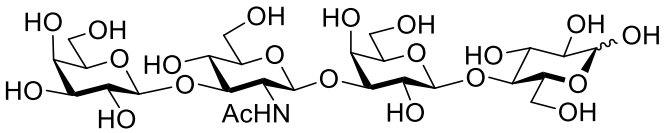


3.17

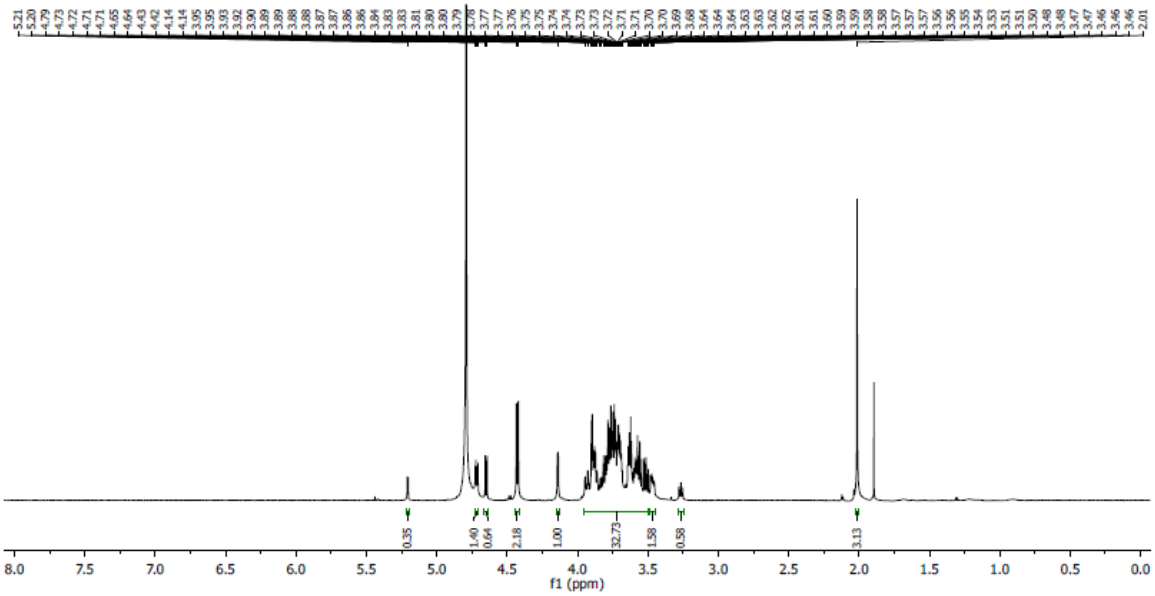


$CDCl_3$ 600 MHz

Figure A-60: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**3.17**)

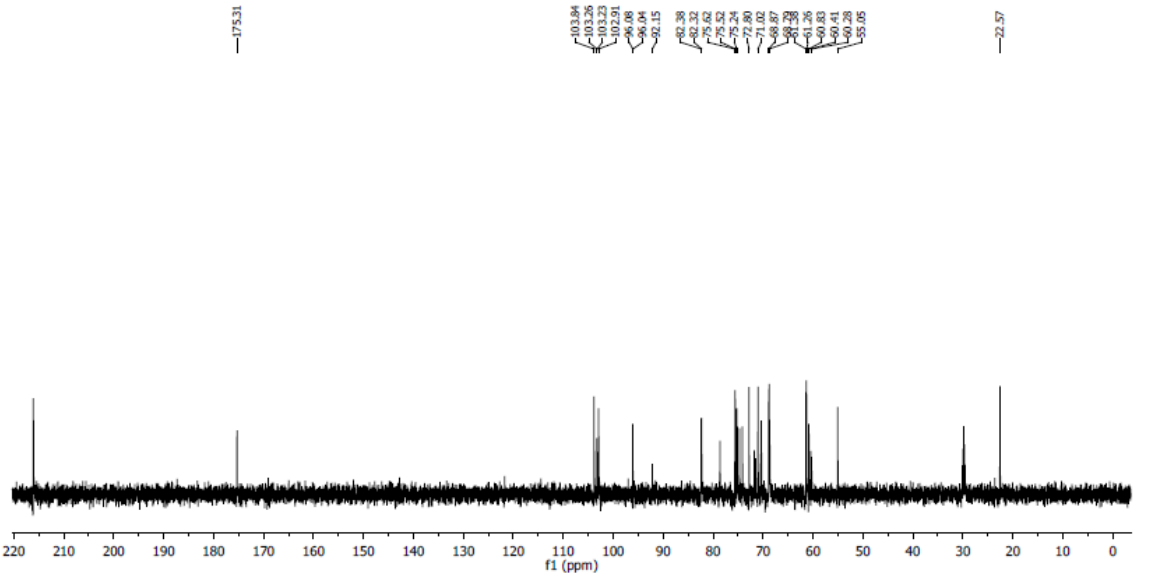


4.1



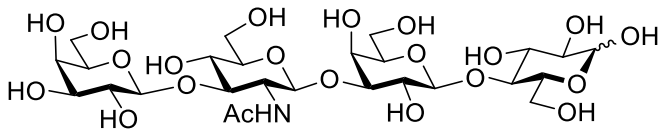
D_2O 600 MHz

Figure A-61: ^{1}H NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**4.1, LNT**)

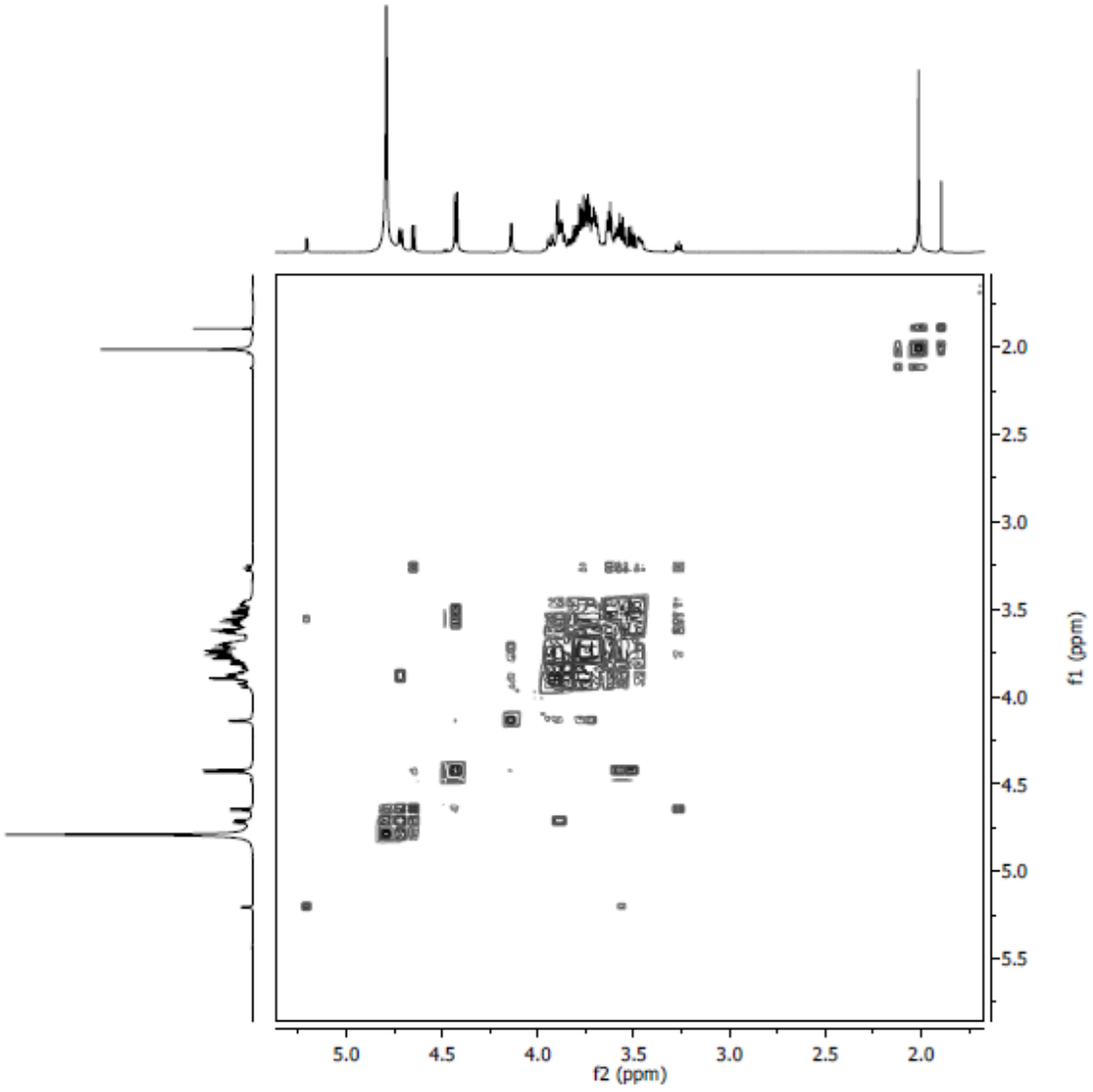


D_2O 151 MHz

Figure A-62: ^{13}C NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**4.1, LNT**)

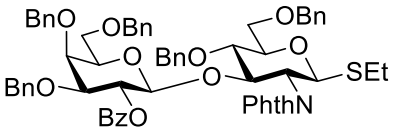


4.1

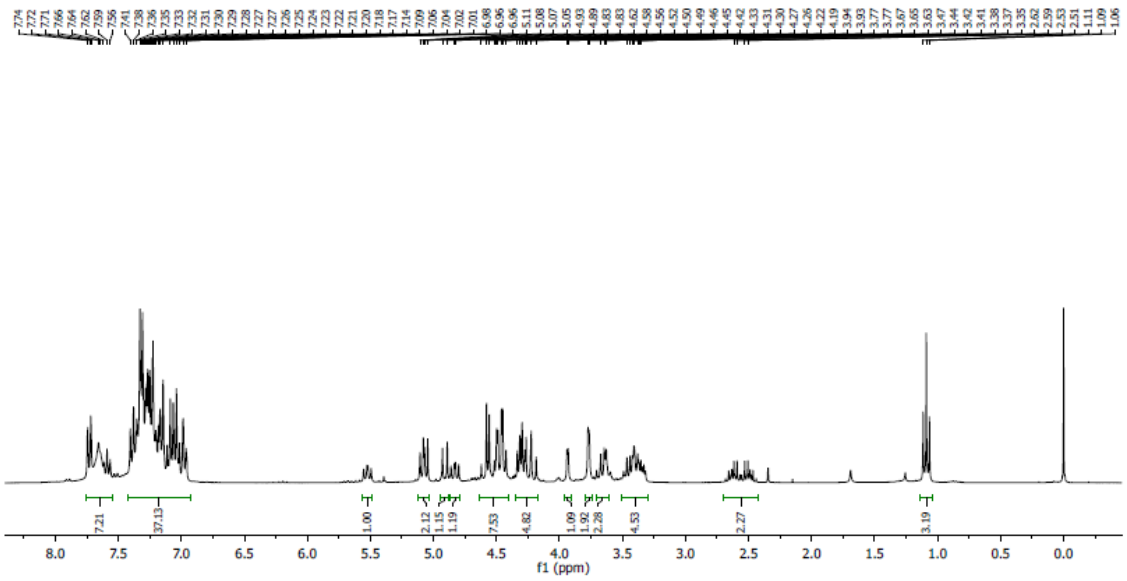


D_2O 600 MHz

Figure A-63: 2-D NMR COSY spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-D-glucopyranose (**4.1, LNT**)

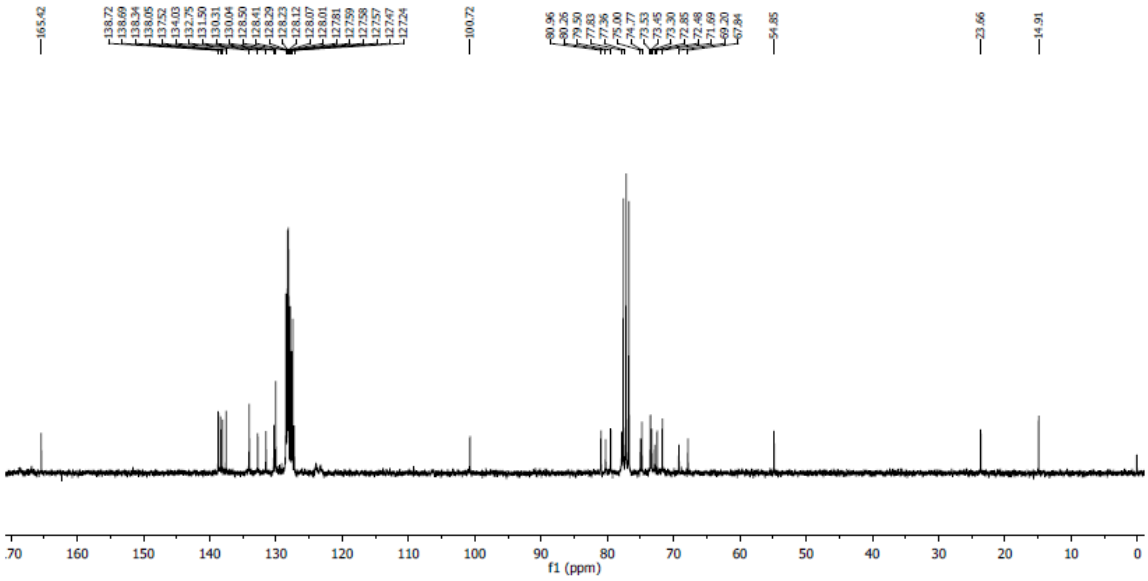


4.2



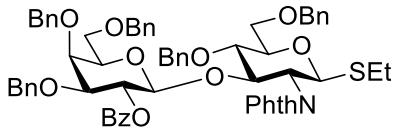
$CDCl_3$ 300 MHz

Figure A-64: ^{1}H NMR spectrum of Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4.2**)

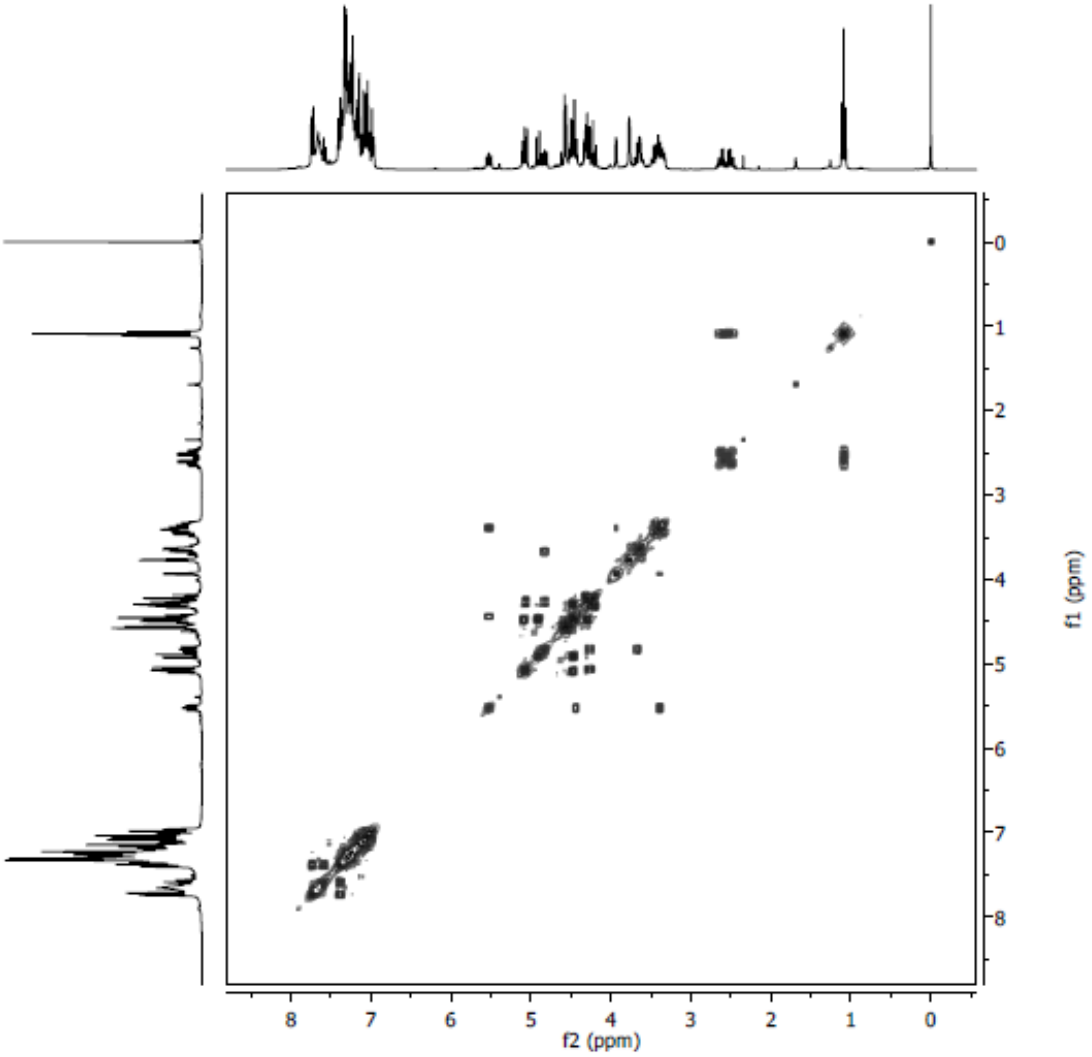


$CDCl_3$ 75 MHz

Figure A-65: ^{13}C NMR spectrum of of Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4.2**)

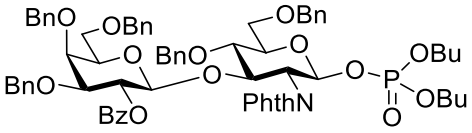


4.2

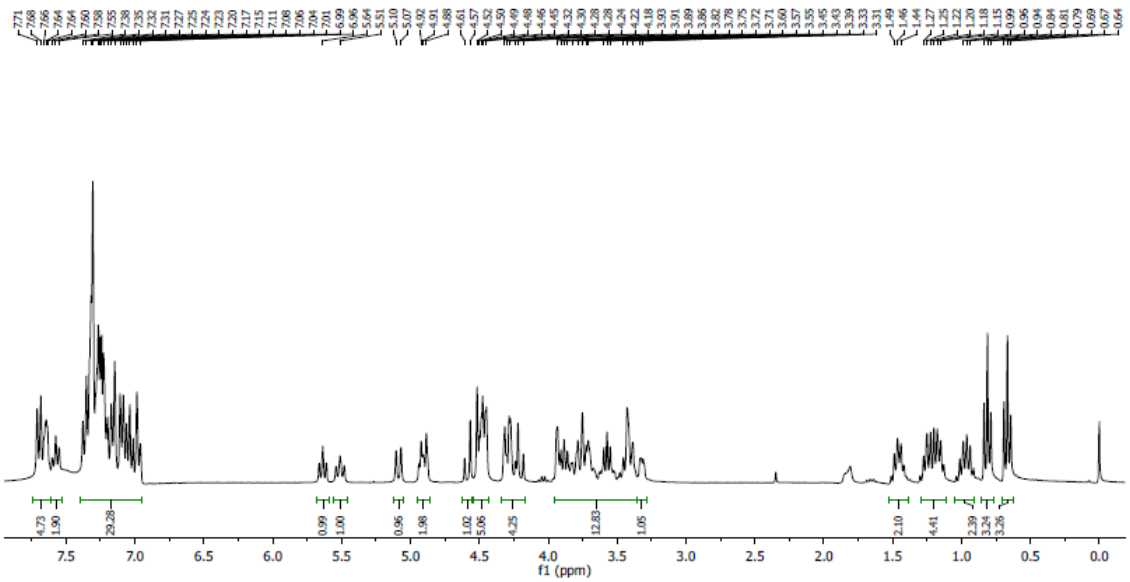


$CDCl_3$ 300 MHz

Figure A-66: 2-D NMR COSY spectrum of of Ethyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4.2**)

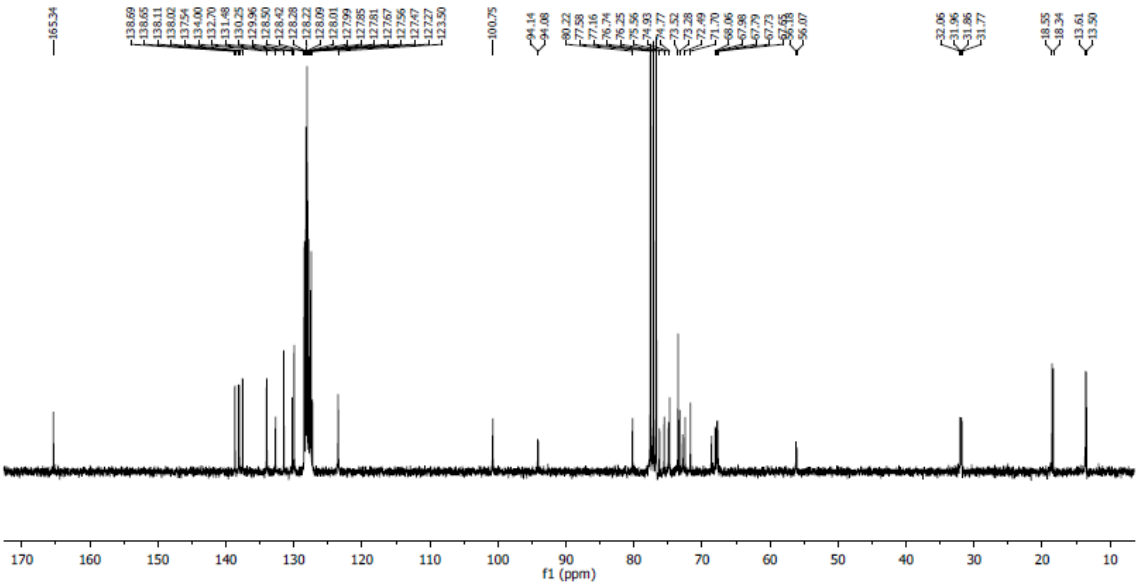


4.10



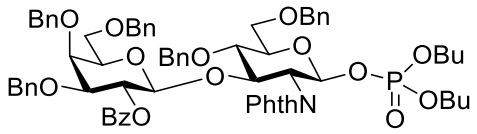
$CDCl_3$ 300 MHz

Figure A-67: ^{1}H NMR spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**4.10**)

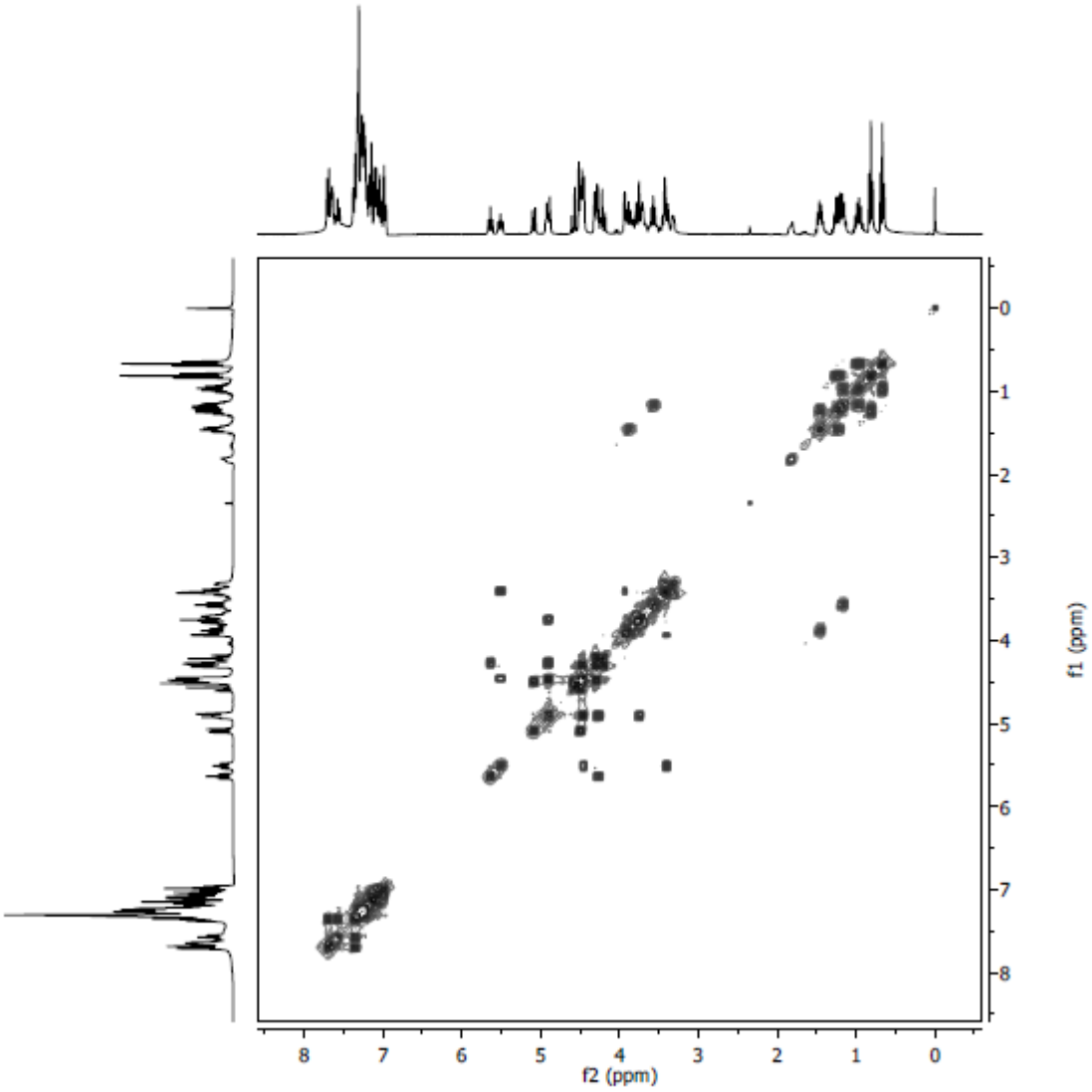


$CDCl_3$ 75 MHz

Figure A-68: ^{13}C NMR spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**4.10**)

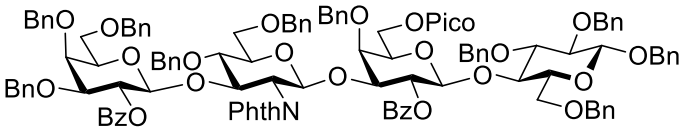


4.10

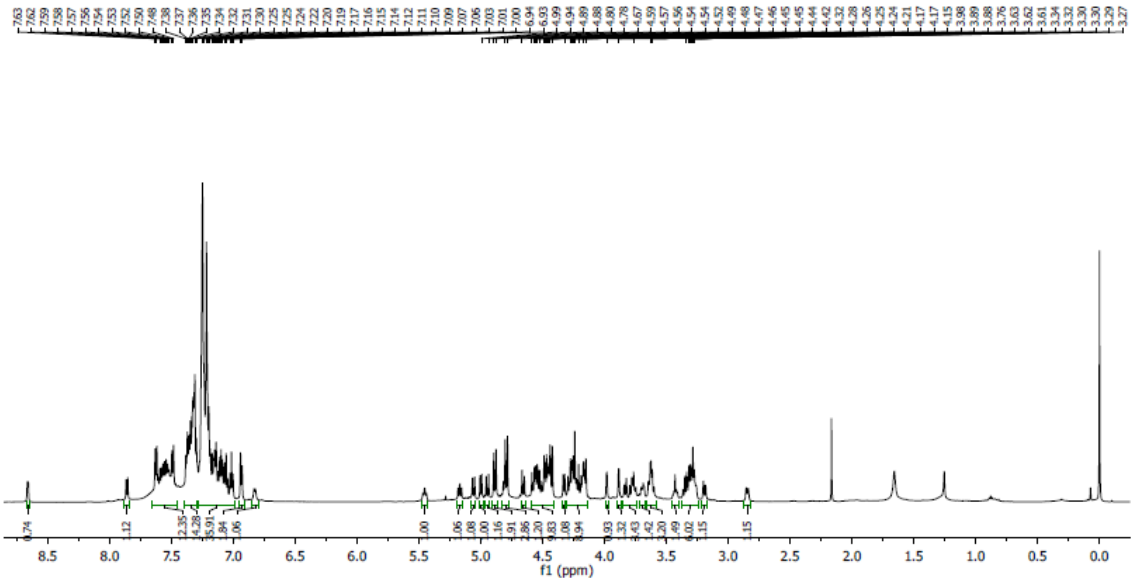


$CDCl_3$ 300 MHz

Figure A-69: 2-D NMR COSY spectrum of Di-*O*-butyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**4.10**)

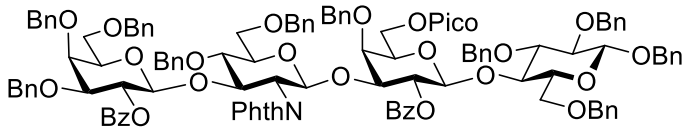


4.11

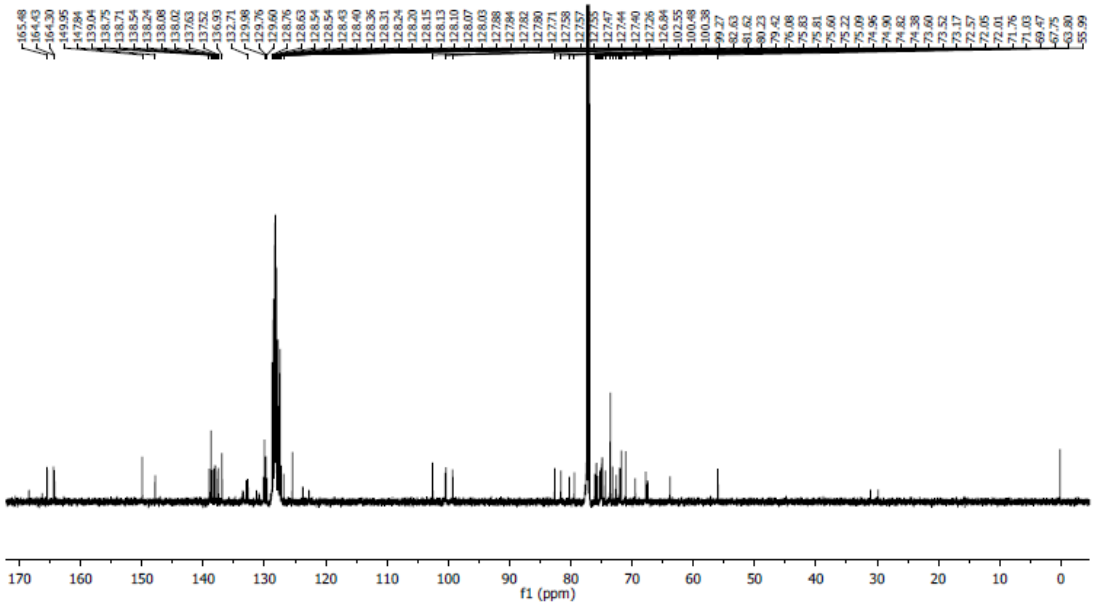


$CDCl_3$ 600 MHz

Figure A-70: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.11**)

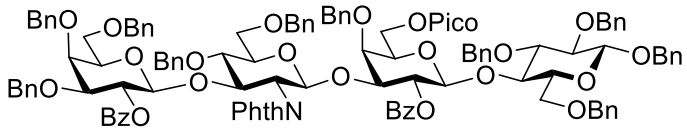


4.11

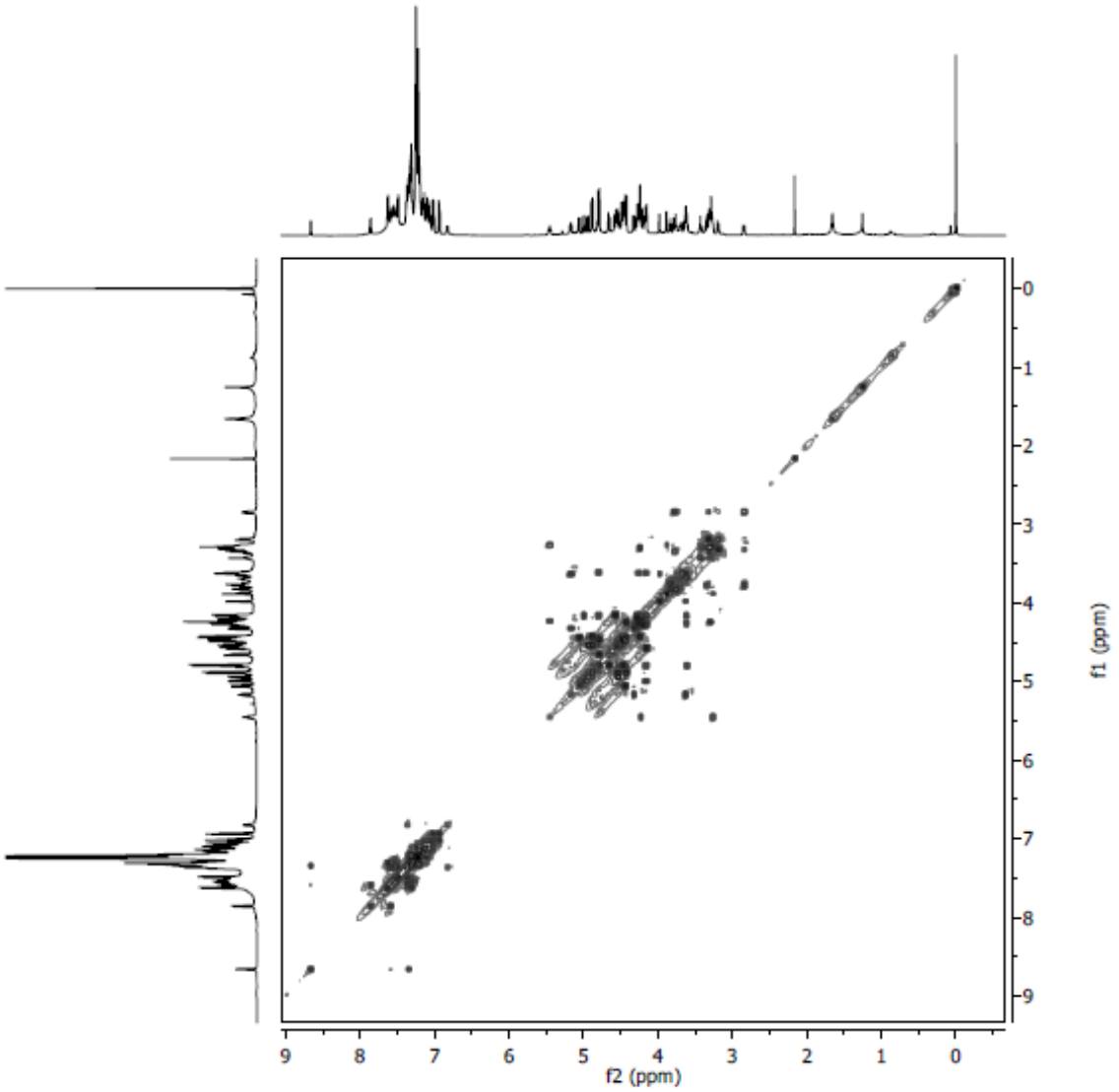


$CDCl_3$ 151 MHz

Figure A-71: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.11**)

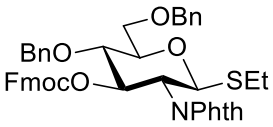


4.11

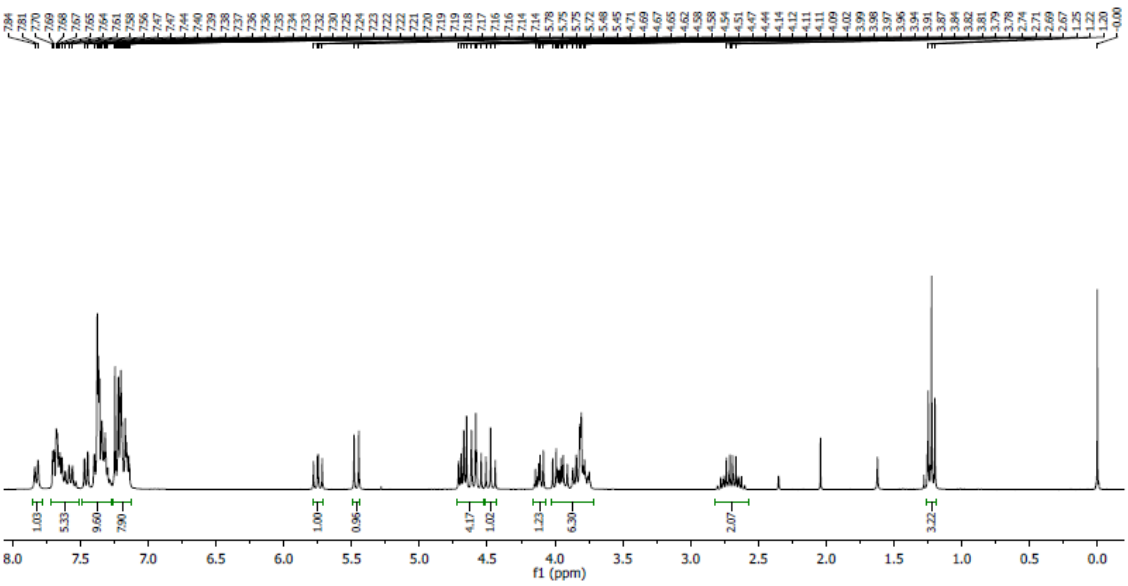


$CDCl_3$ 600 MHz

Figure A-72: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthlimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.11**)

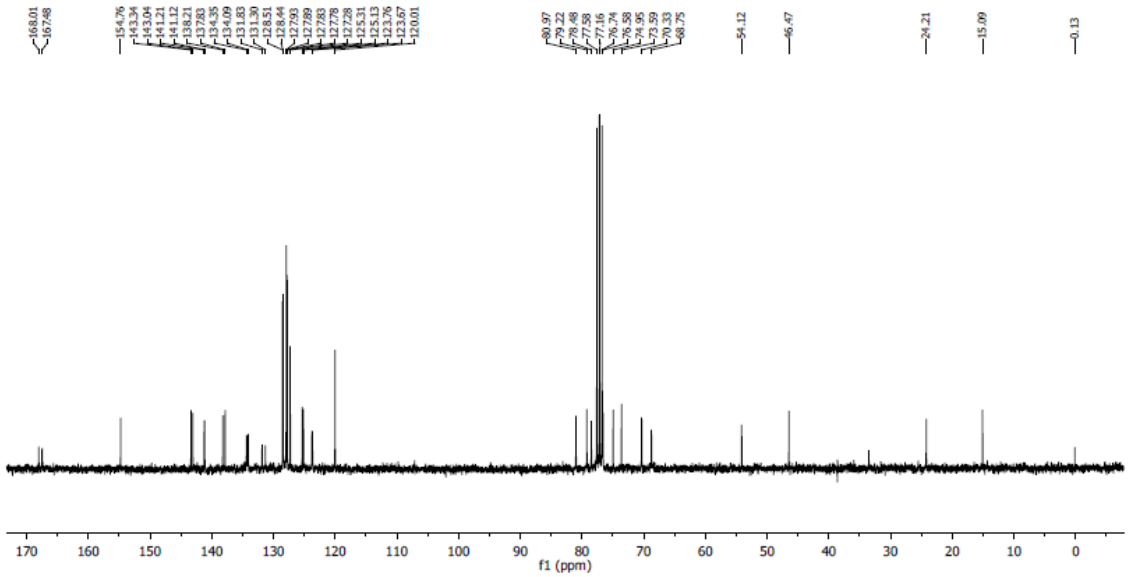


4.12



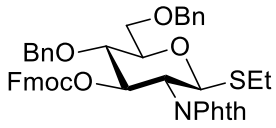
$CDCl_3$ 300 MHz

Figure A-73: ^{1}H NMR spectrum of Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**4.12**)

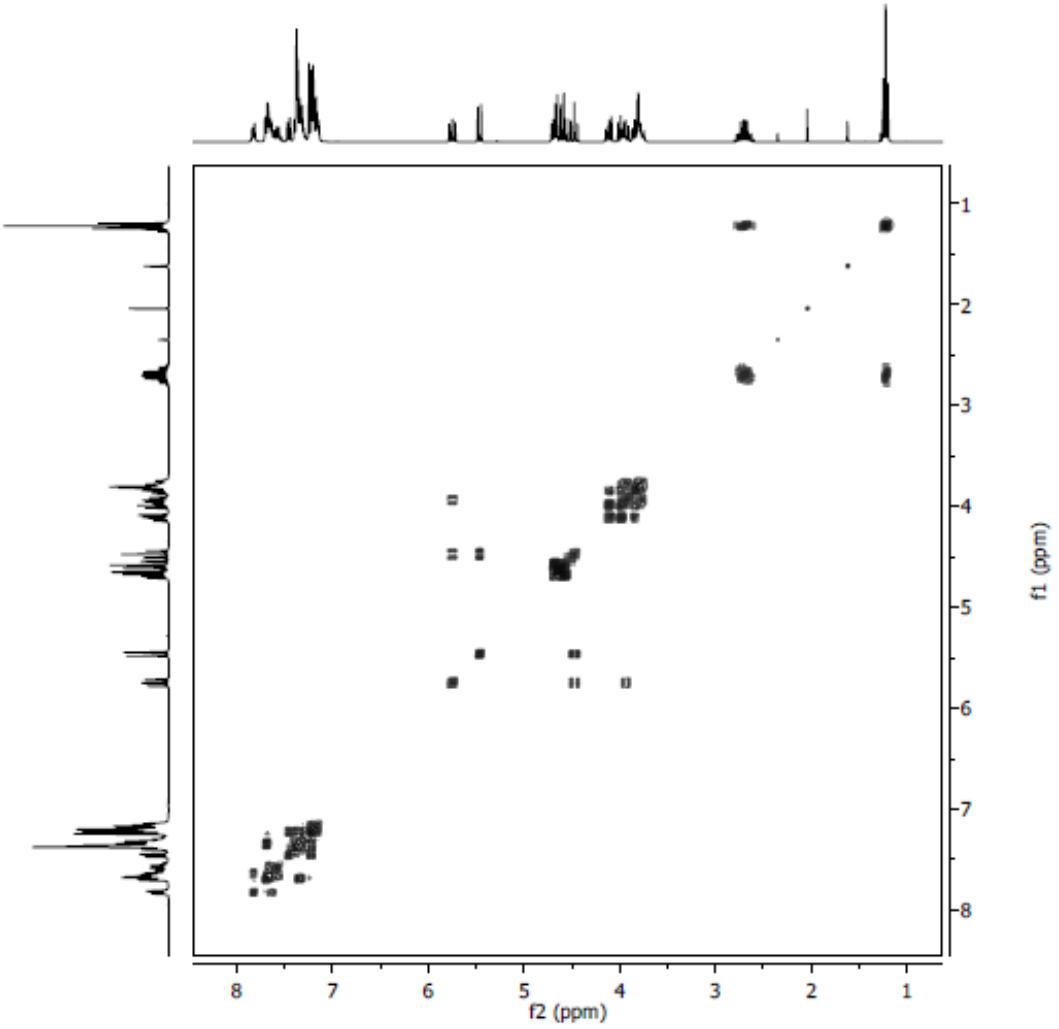


$CDCl_3$ 75 MHz

Figure A-74: ^{13}C NMR spectrum of Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**4.12**)

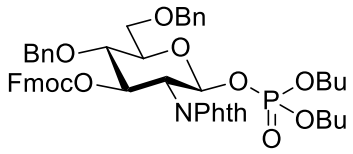


4.12

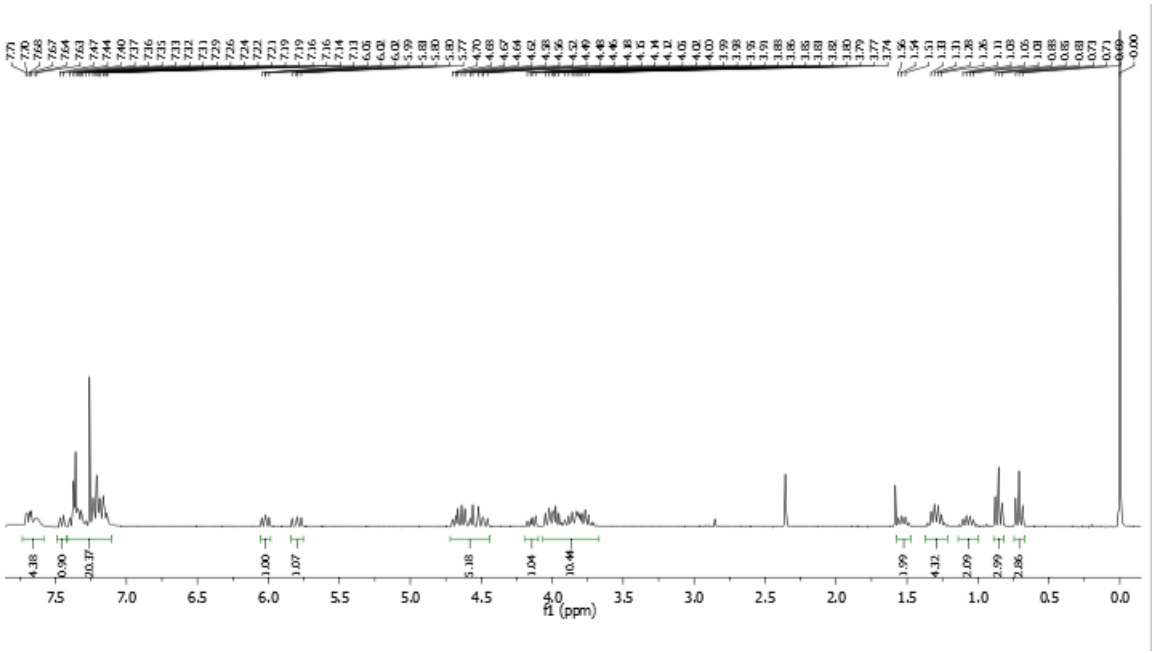


$CDCl_3$ 300 MHz

Figure A-75: 2-D NMR COSY spectrum of Ethyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-1-thio-β-D-glucopyranoside (**4.12**)

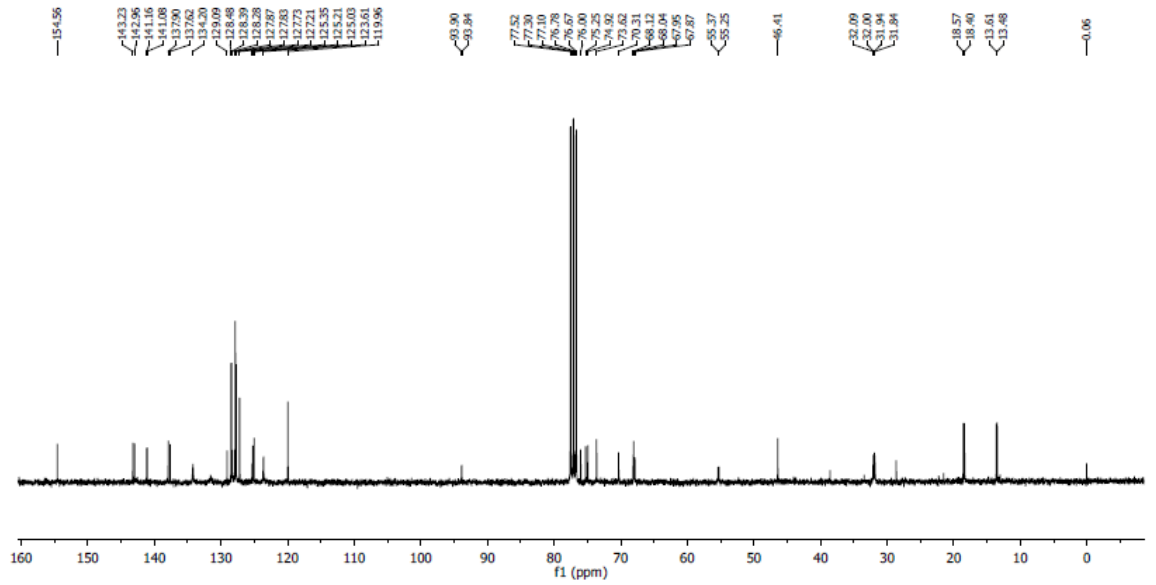


4.13



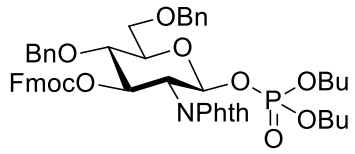
$CDCl_3$ 300 MHz

Figure A-76: ^{1}H NMR spectrum of Di-*O*-butyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**4.13**)

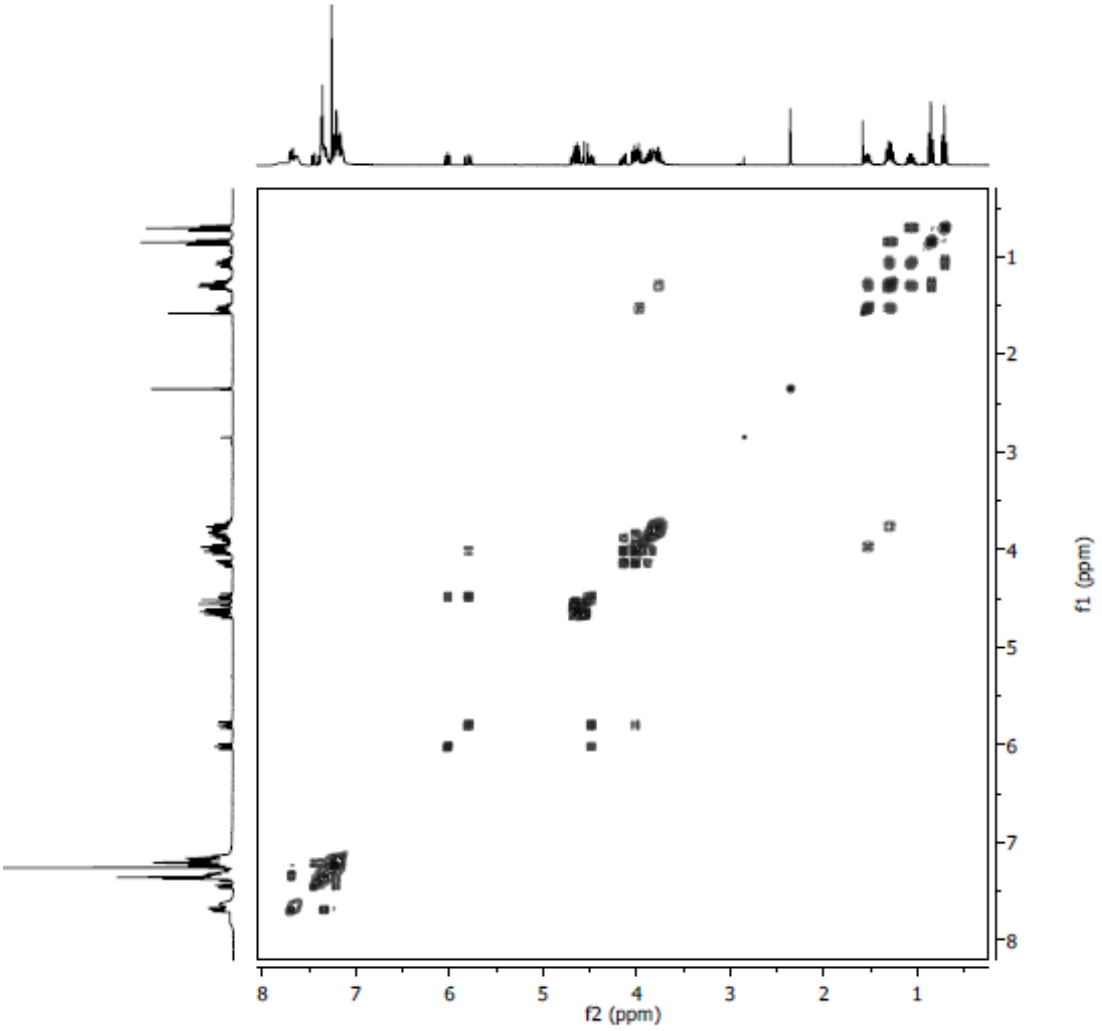


$CDCl_3$ 75 MHz

Figure A-77: ^{13}C NMR spectrum of Di-*O*-butyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**4.13**)

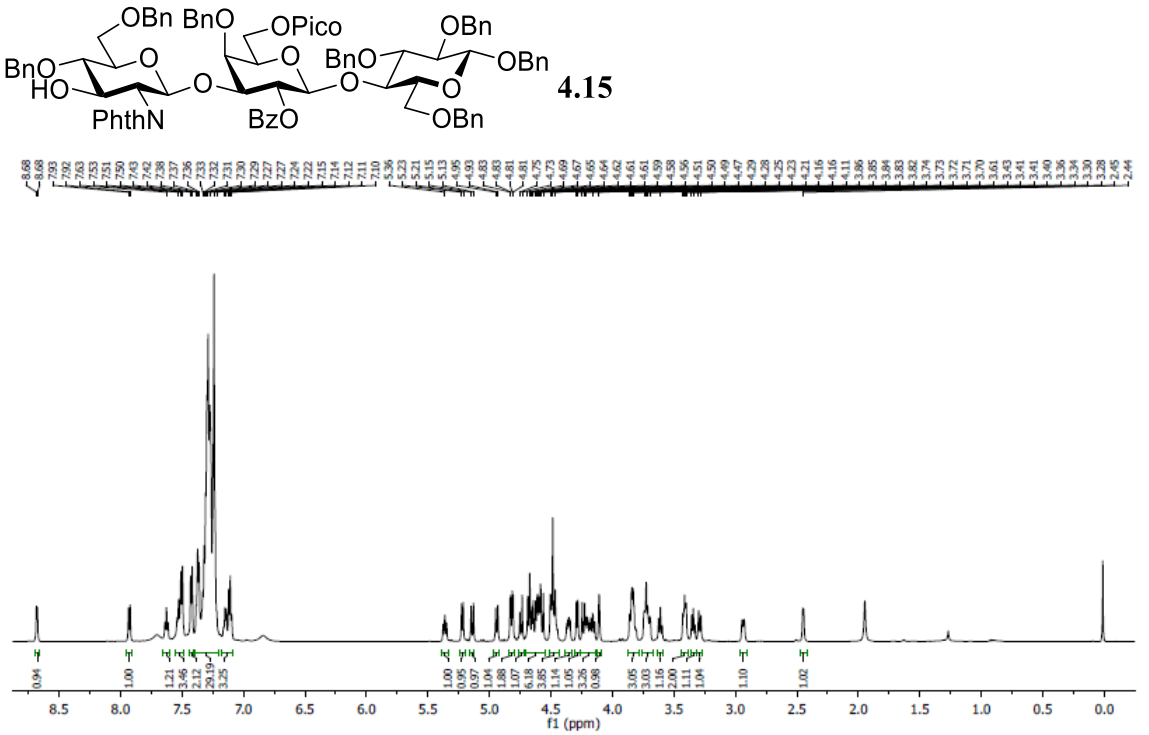


4.13



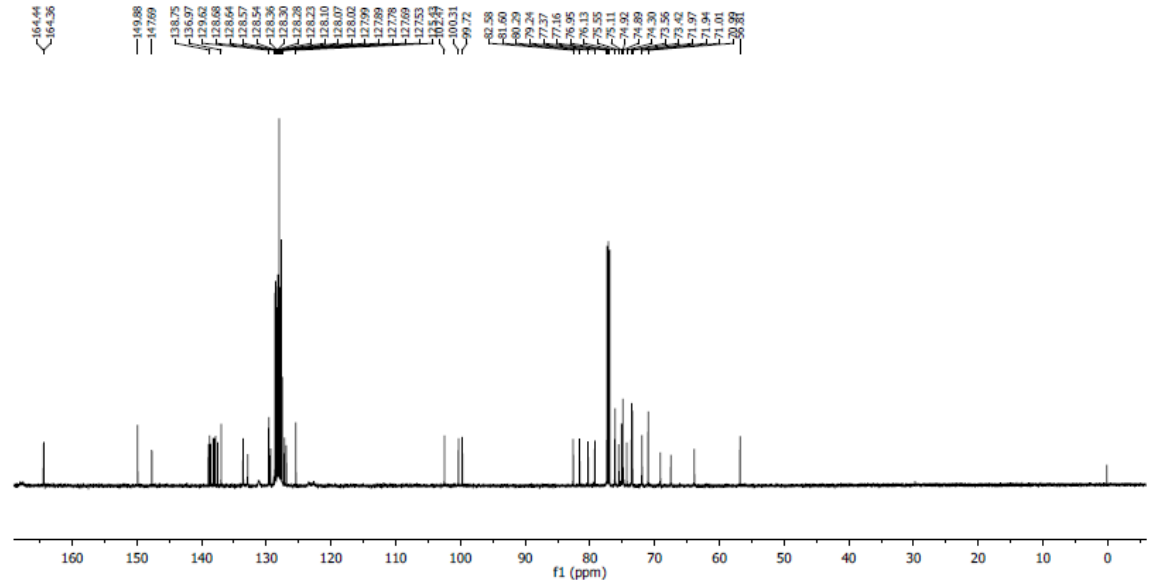
$CDCl_3$ 300 MHz

Figure A-78: 2-D NMR COSY spectrum of Di-*O*-butyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-fluorenylmethoxycarbonyl-2-phthalimido-β-D-glucopyranosyl phosphate (**4.13**)



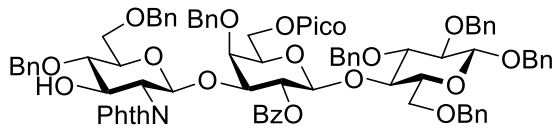
$CDCl_3$ 600 MHz

Figure A-79: ^{1}H NMR spectrum of Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.15**)

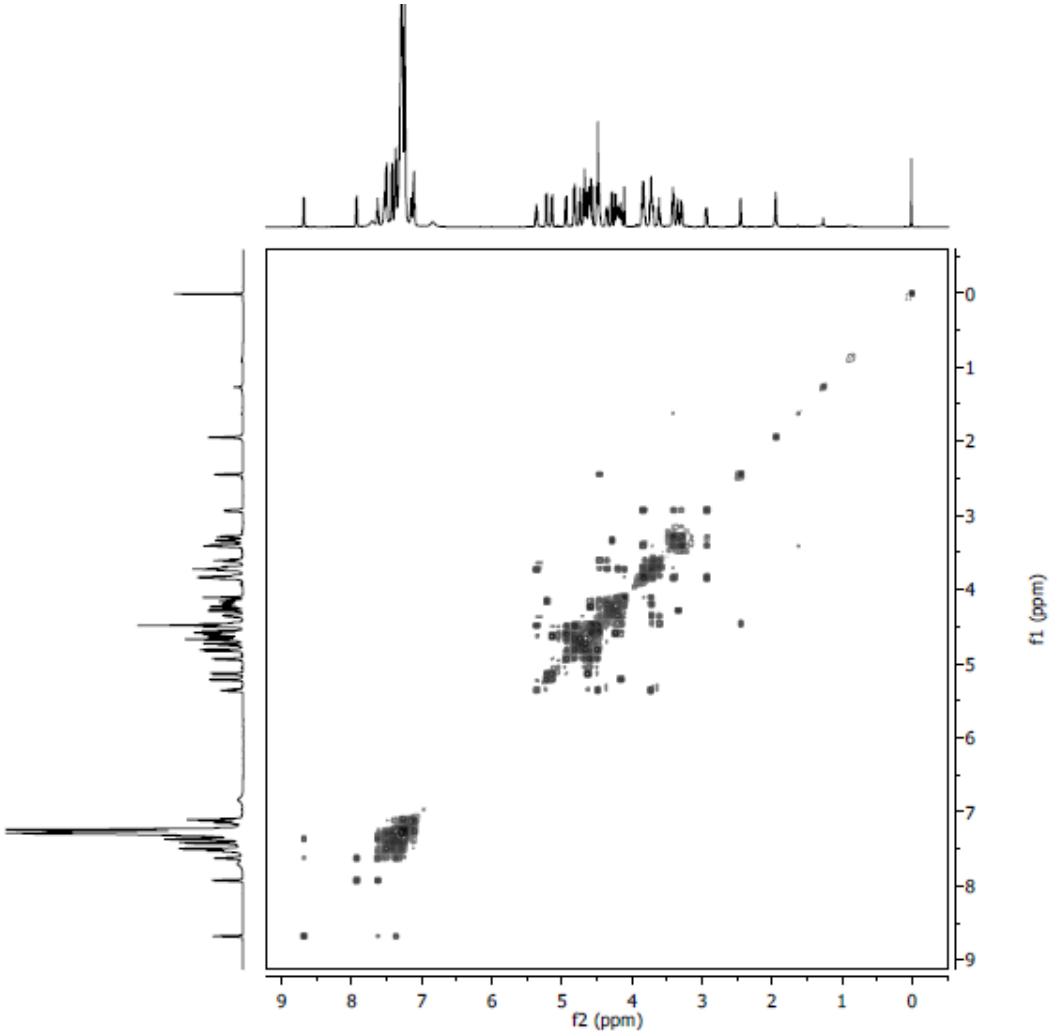


$CDCl_3$ 151 MHz

Figure A-80: ^{13}C NMR spectrum of Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.15**)

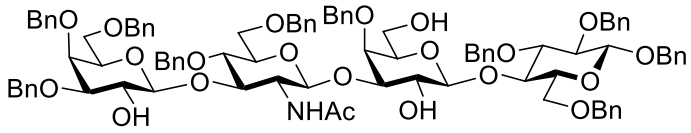


4.15

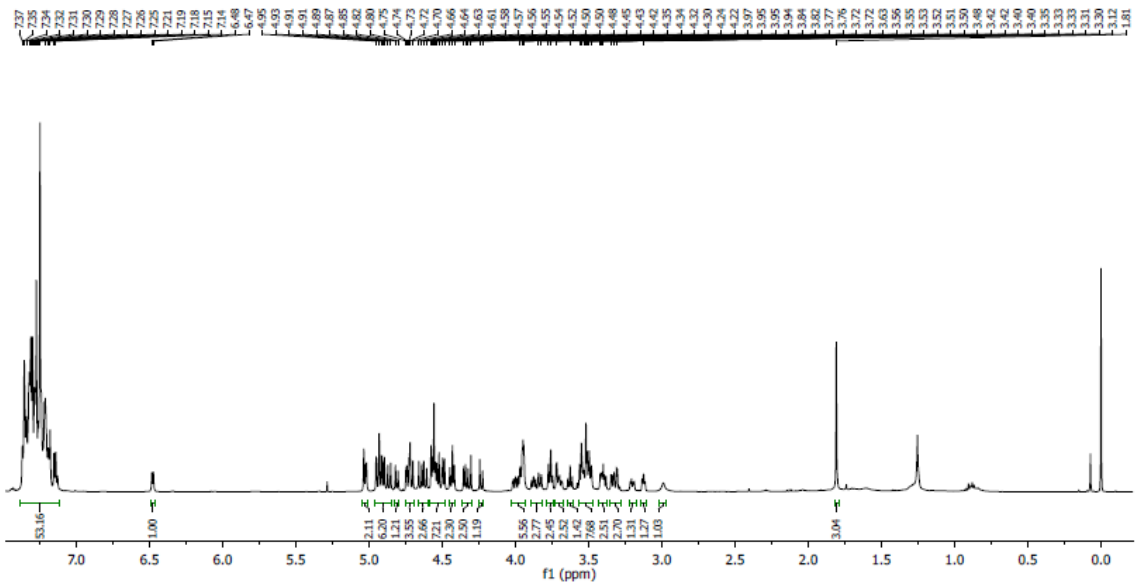


$CDCl_3$ 600 MHz

Figure A-81: 2-D NMR COSY spectrum of Benzyl *O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-6-*O*-picoloyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.15**)

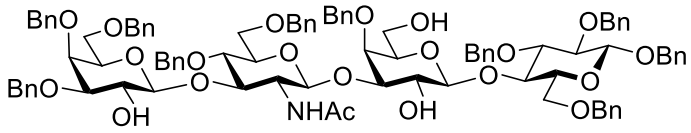


4.16

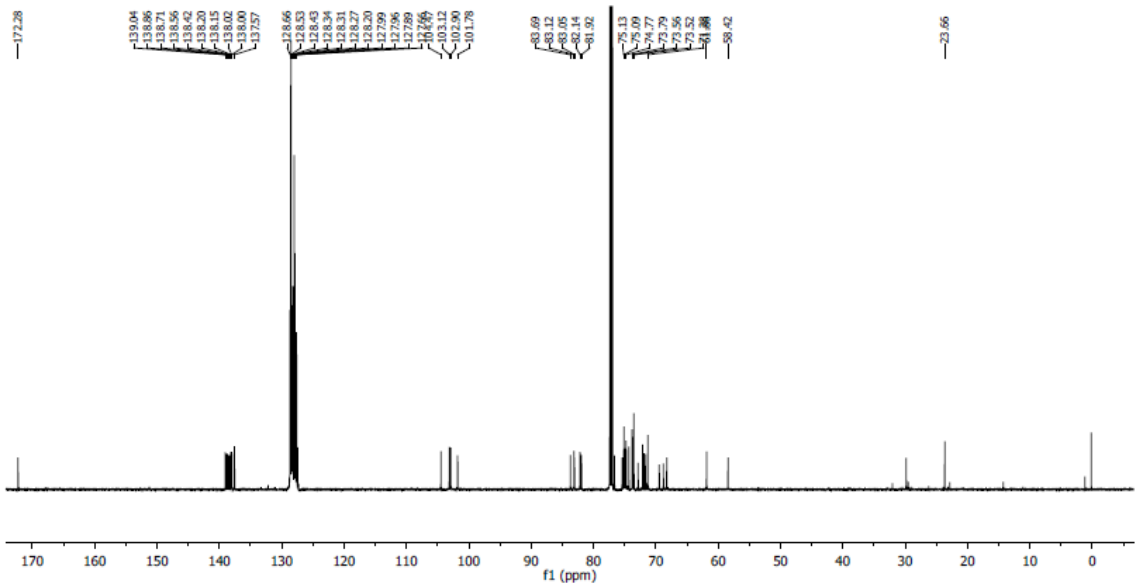


$CDCl_3$ 600 MHz

Figure A-82: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.16**)

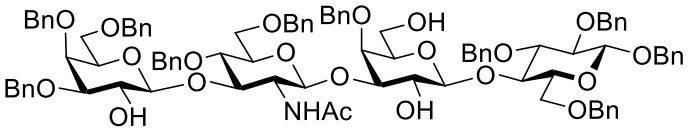


4.16

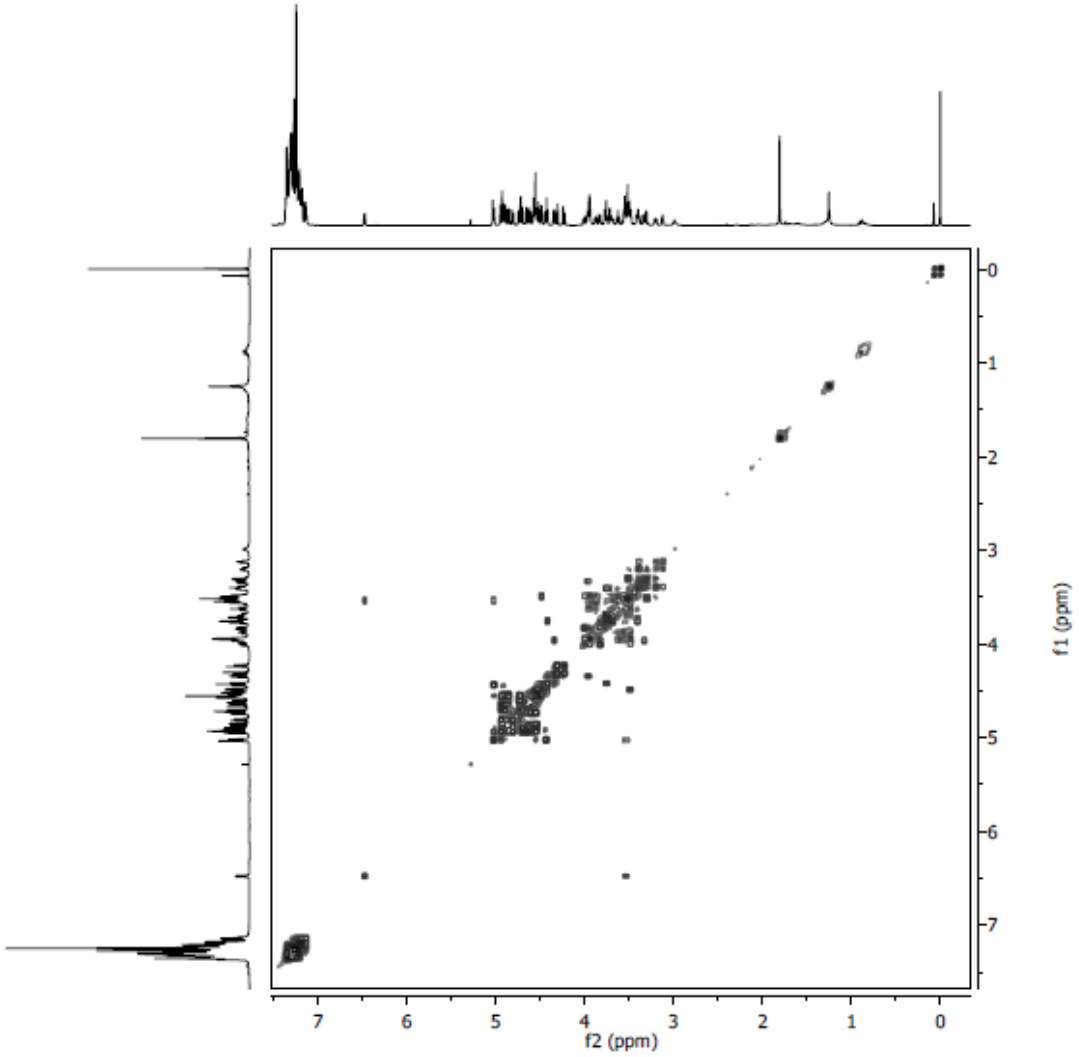


$CDCl_3$ 151 MHz

Figure A-83: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.16**)

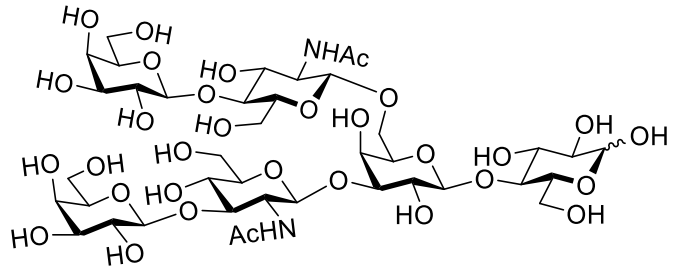


4.16

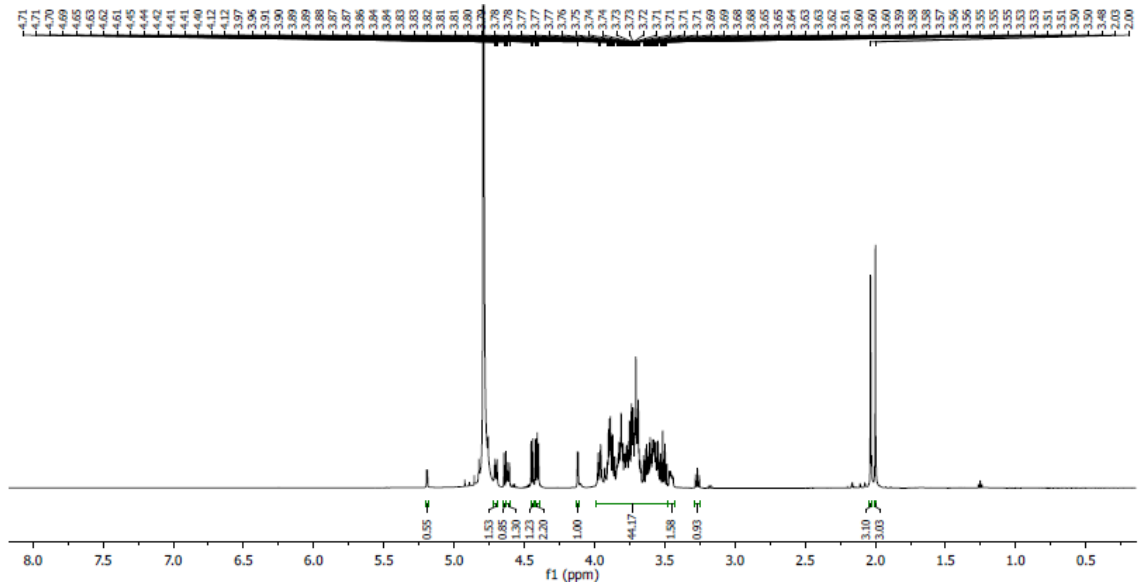


$CDCl_3$ 600 MHz

Figure A-84: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4.16**)

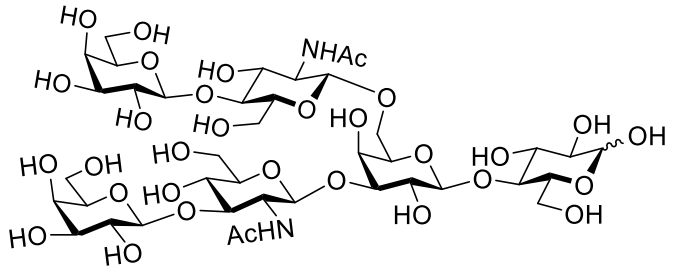


5.1

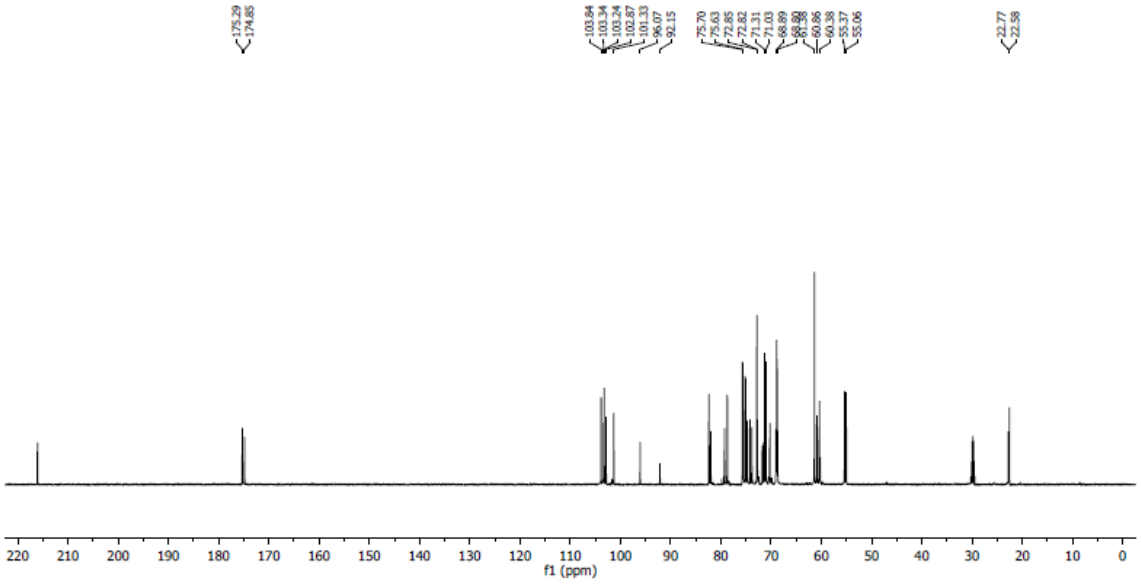


D_2O 600 MHz

Figure A-85: ^{1}H NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**5.1**)

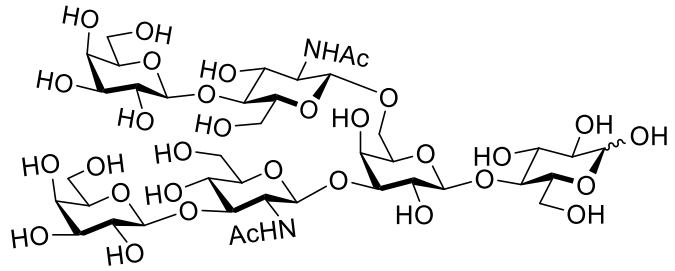


5.1

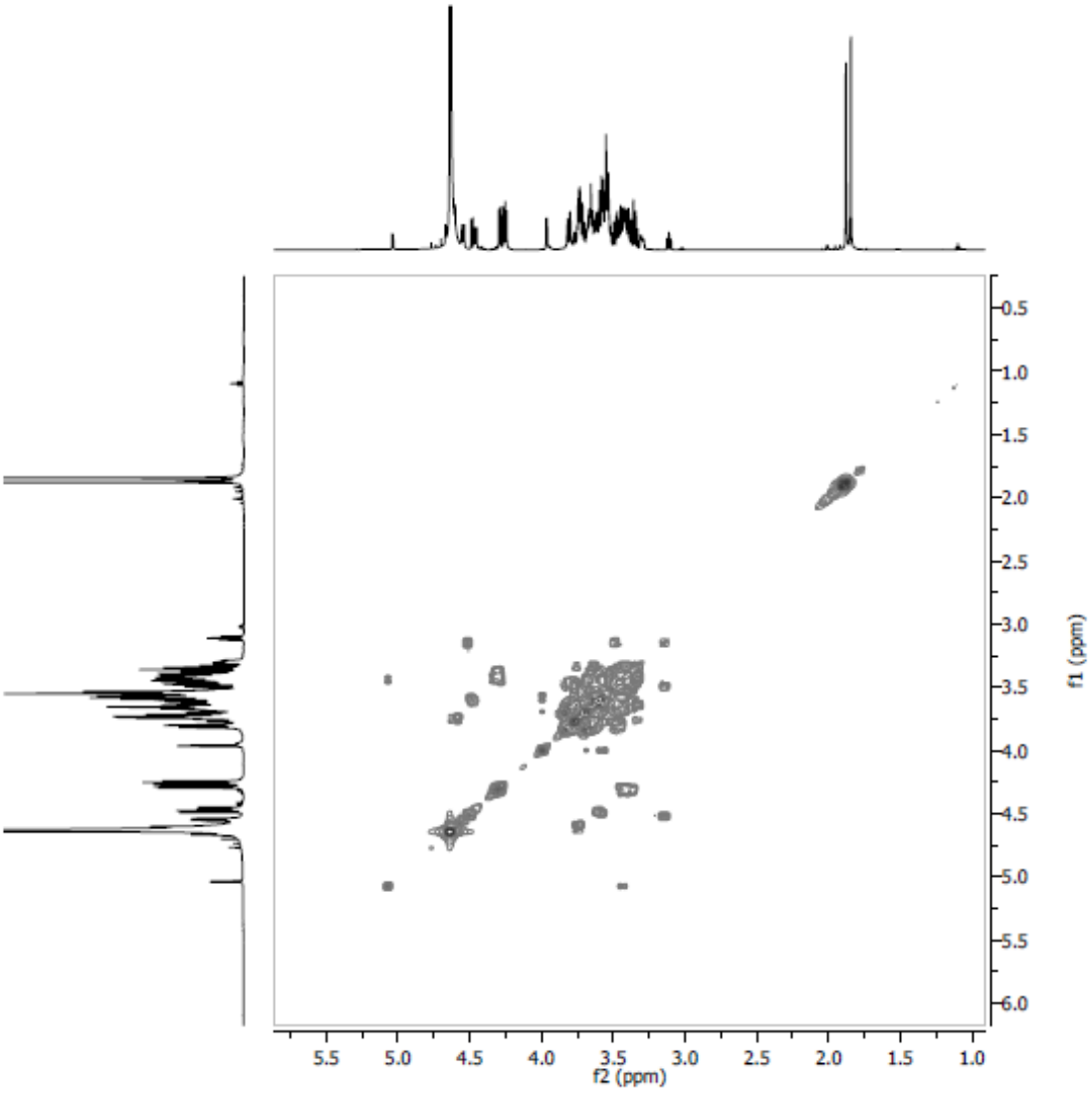


D_2O 151 MHz

Figure A-86: ^{13}C NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**5.1**)

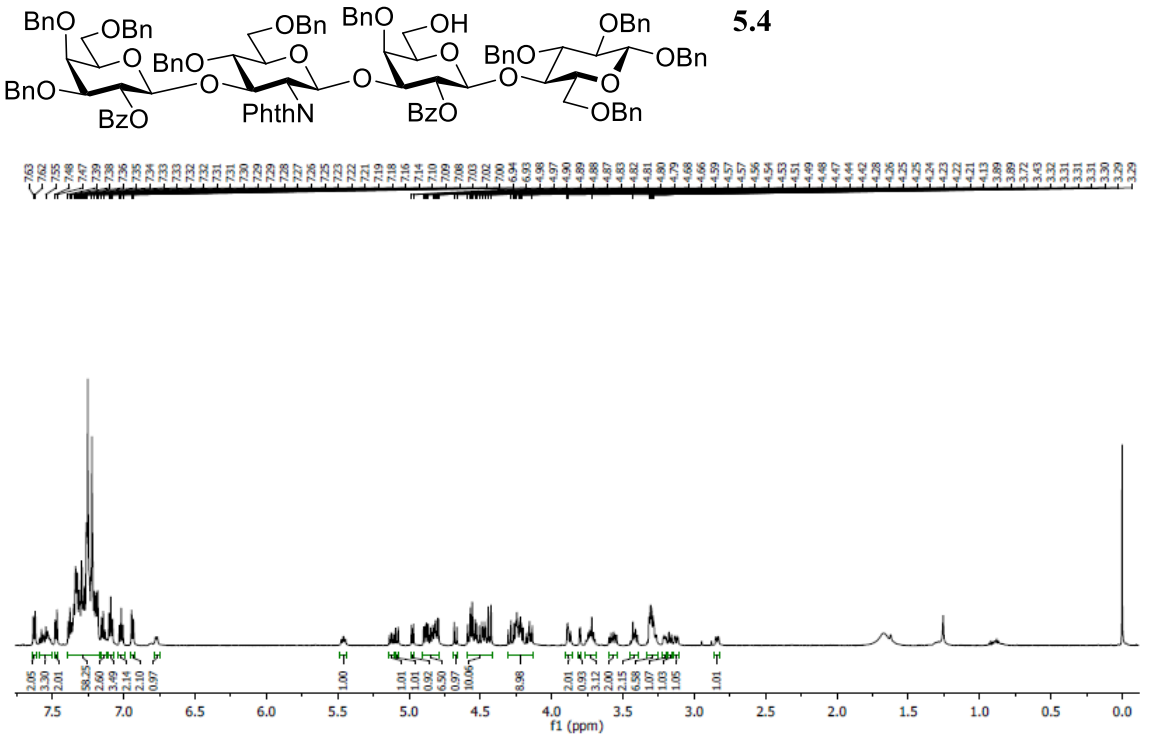


5.1



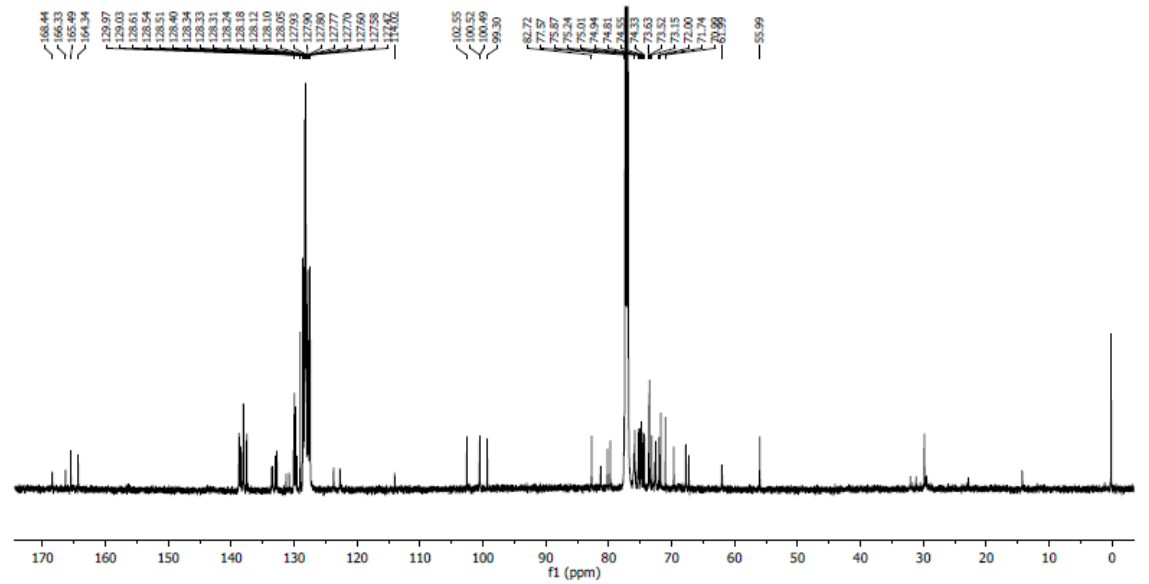
D_2O 600 MHz

Figure A-87: 2-D NMR COSY spectrum of *O*-(β-D-Galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**5.1**)



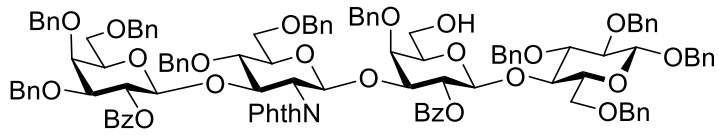
$CDCl_3$ 600 MHz

Figure A-88: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.4**)

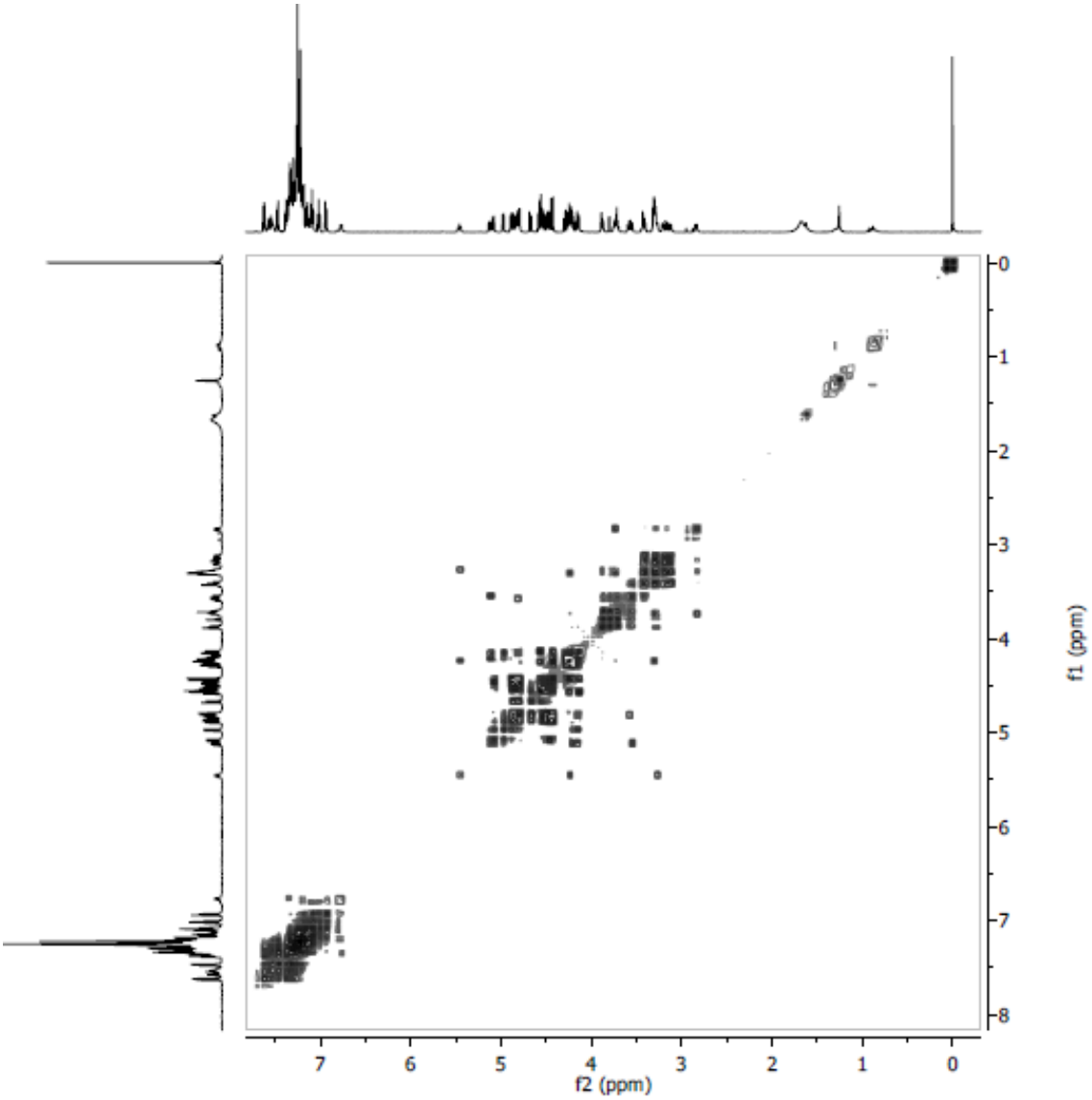


$CDCl_3$ 151 MHz

Figure A-89: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.4**)

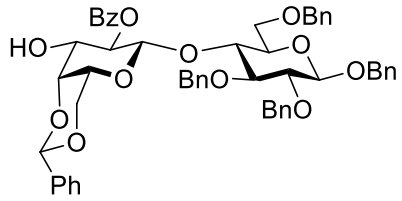


5.4

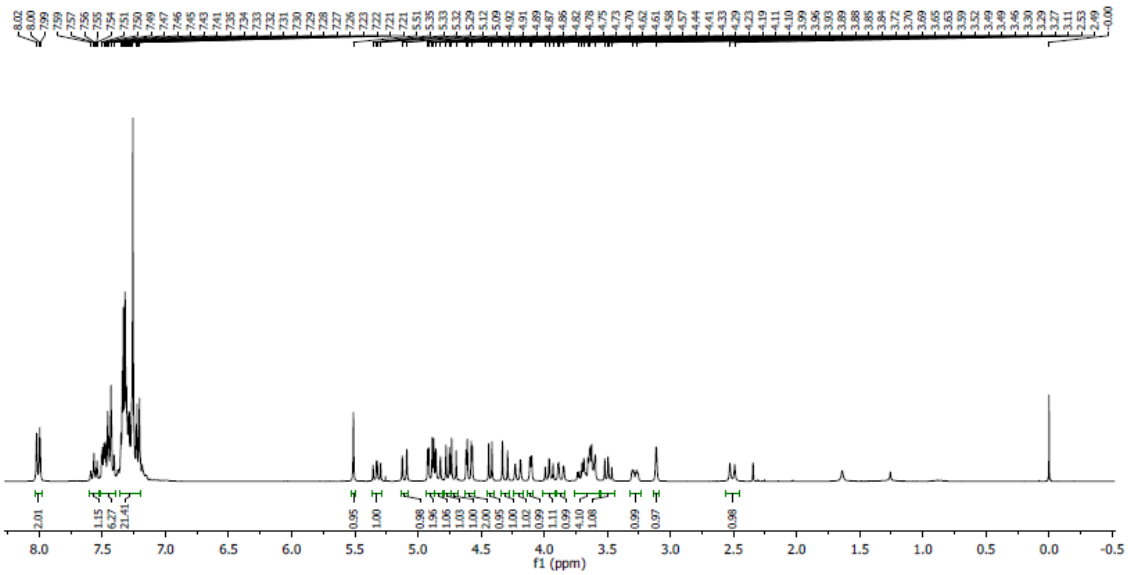


$CDCl_3$ 600 MHz

Figure A-90: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.4**)

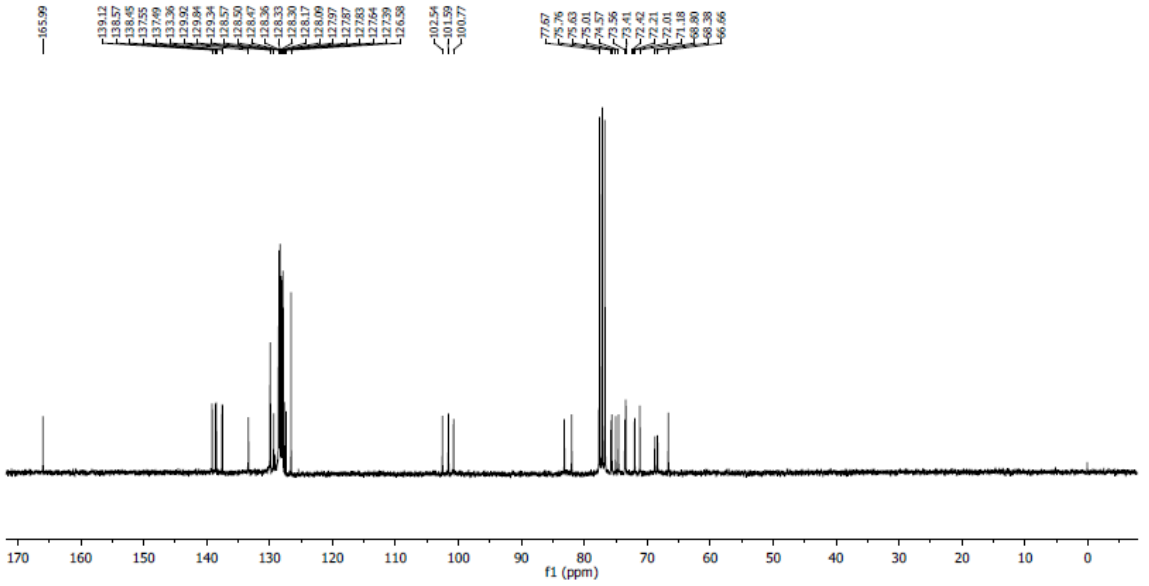


5.9



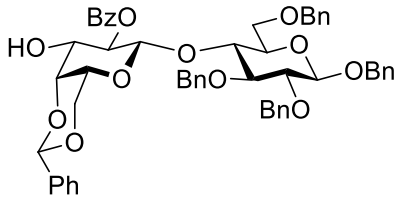
$CDCl_3$ 300 MHz

Figure A-91: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.9**)

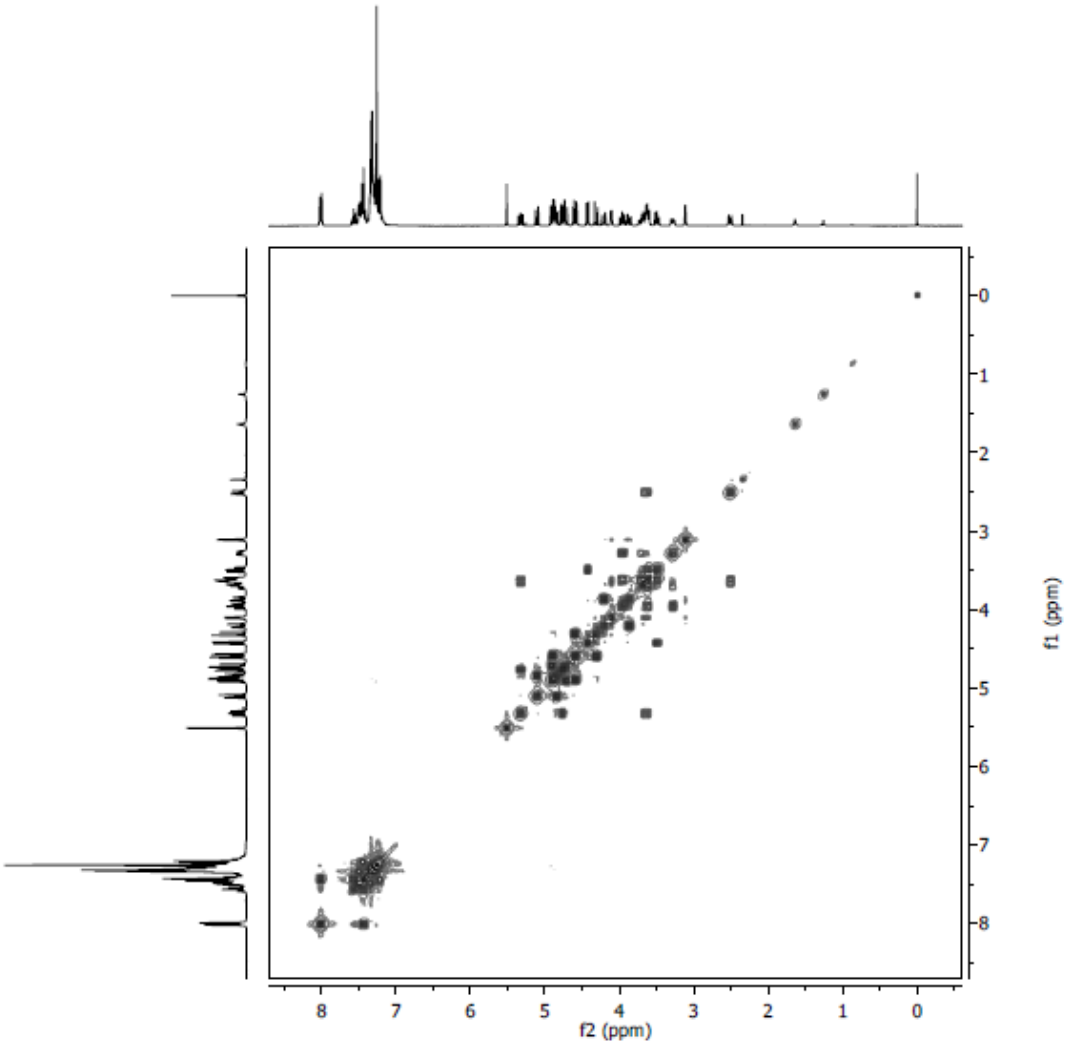


$CDCl_3$ 75 MHz

Figure A-92: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.9**)

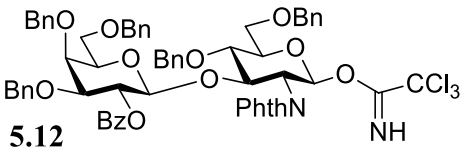


5.9

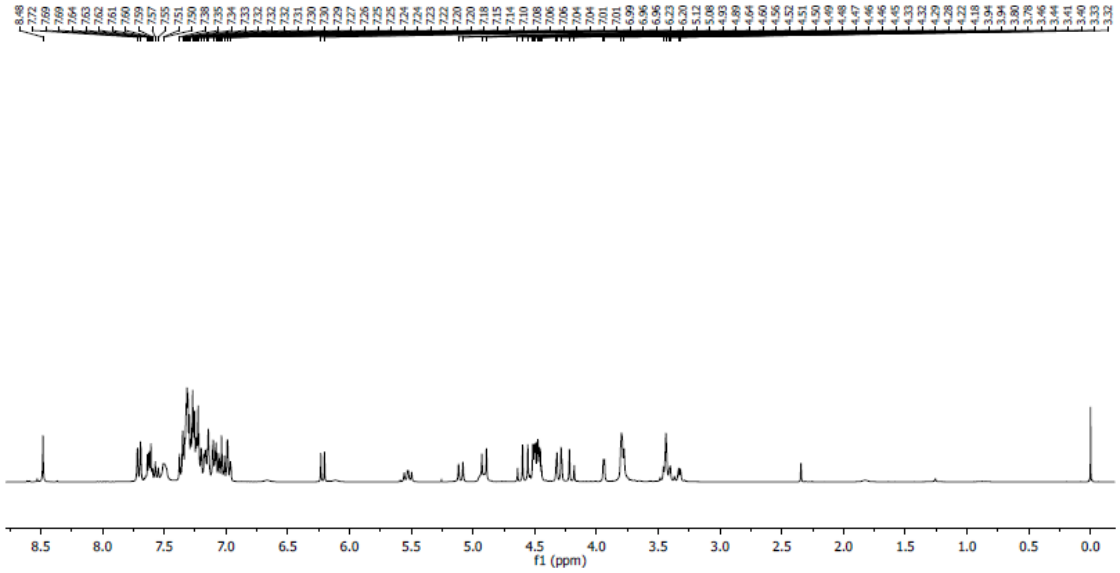


$CDCl_3$ 300 MHz

Figure A-93: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.9**)

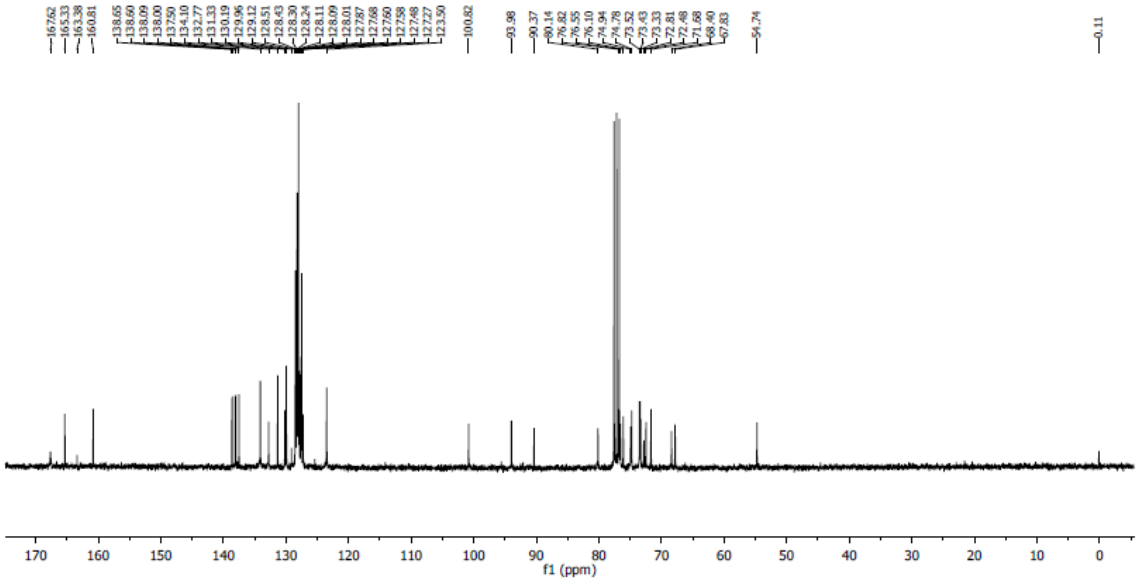


5.12



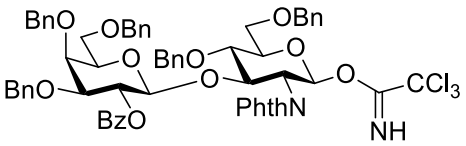
$CDCl_3$ 300 MHz

Figure A-94: ^{1}H NMR spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5.12**)

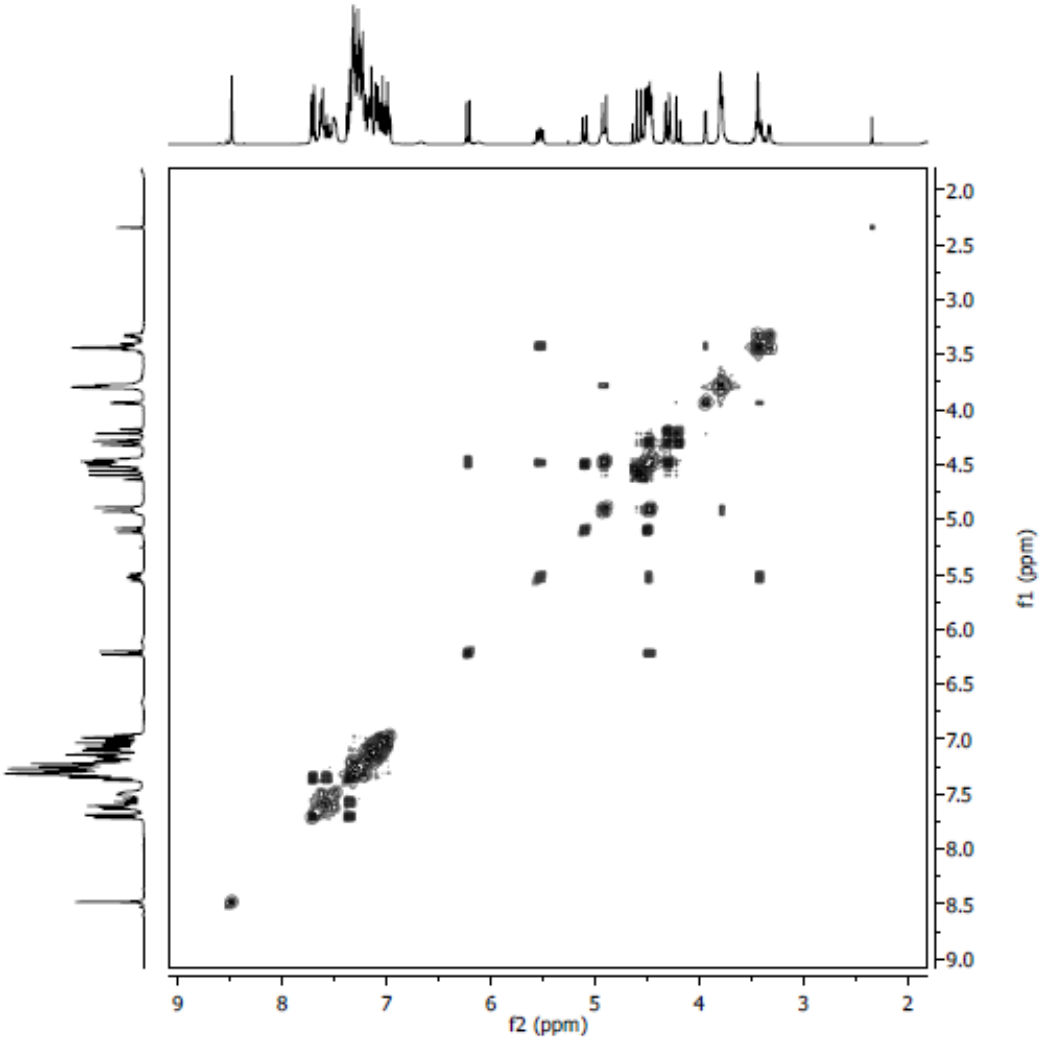


$CDCl_3$ 75 MHz

Figure A-95: ^{13}C NMR spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5.12**)

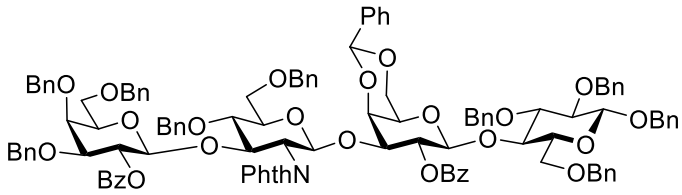


5.12

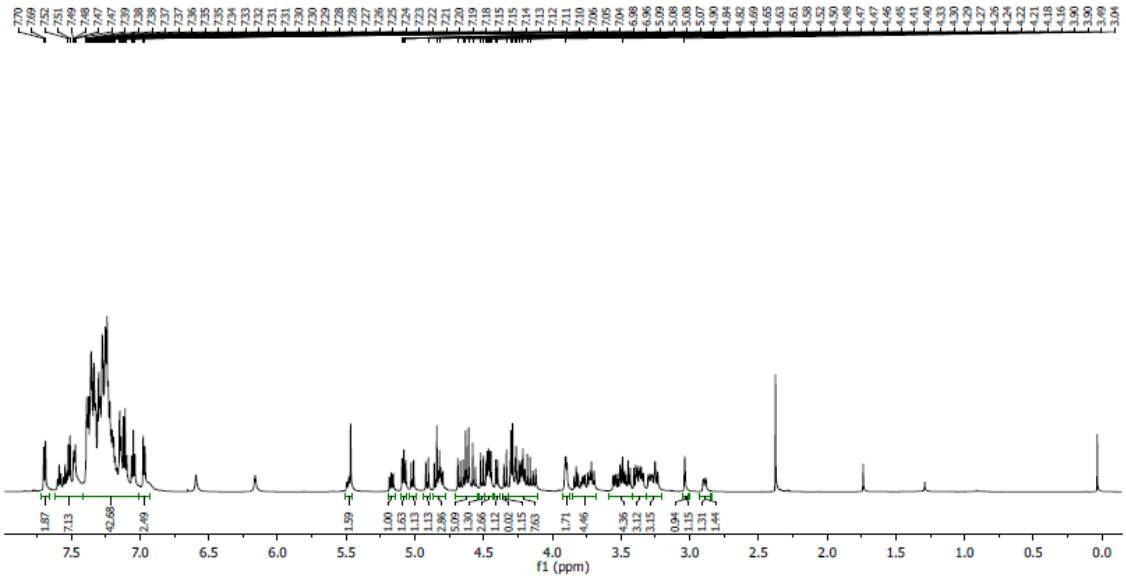


$CDCl_3$ 300 MHz

Figure A-96: 2-D NMR COSY spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5.12**)

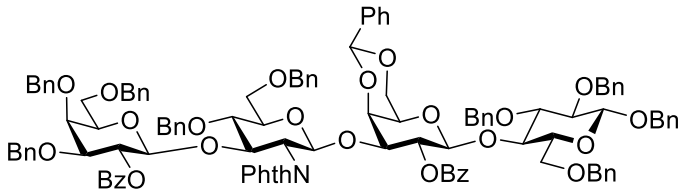


5.13

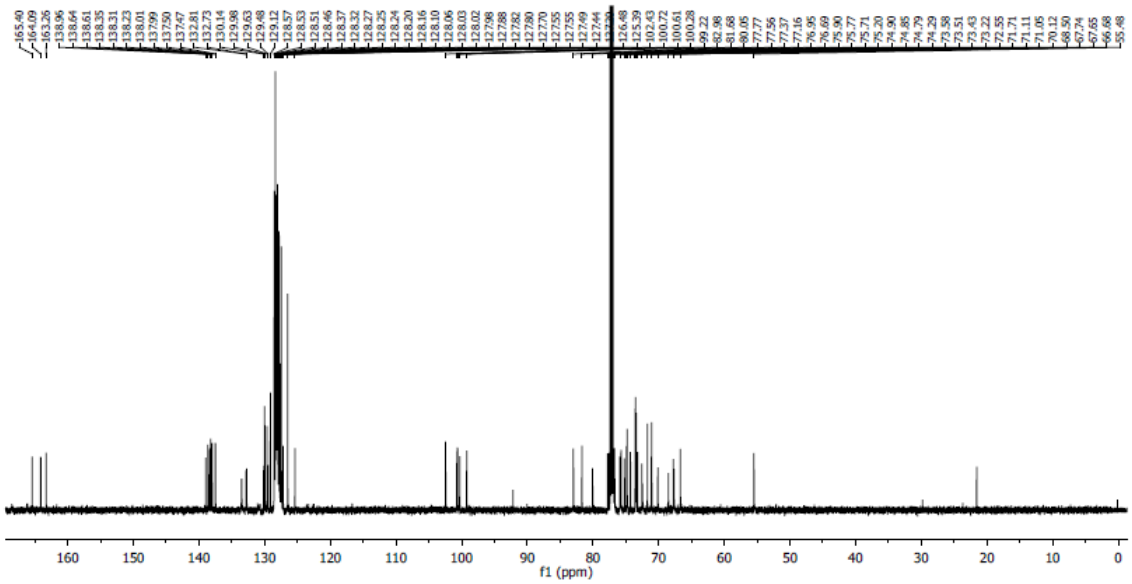


$CDCl_3$ 600 MHz

Figure A-97: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.13**)

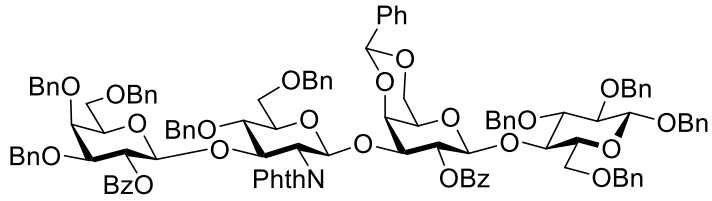


5.13

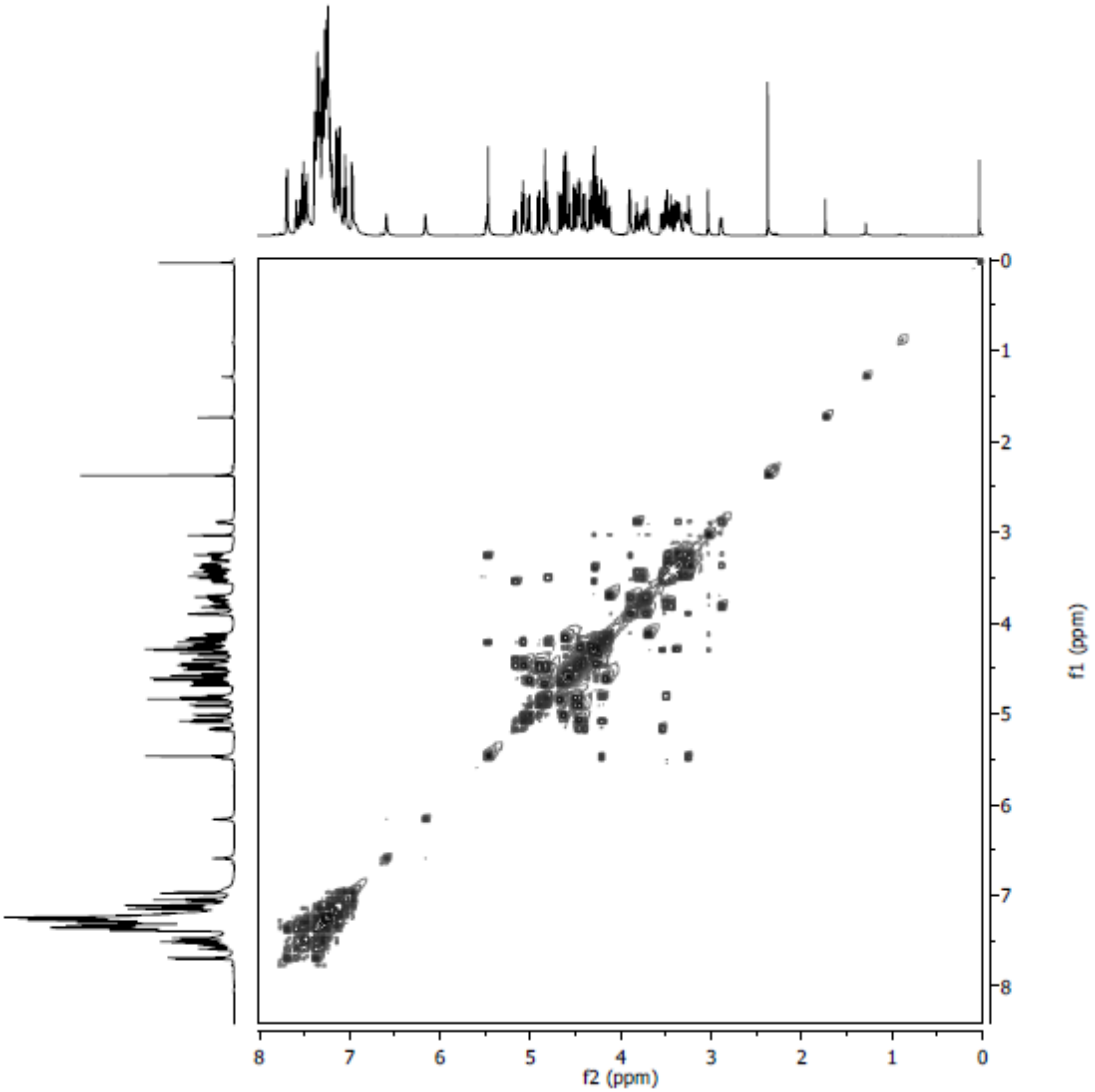


$CDCl_3$ 151 MHz

Figure A-98: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.13**)

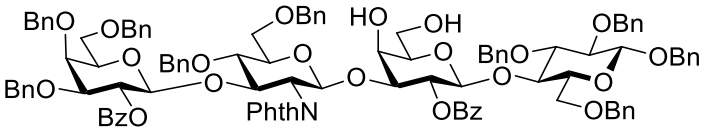


5.13

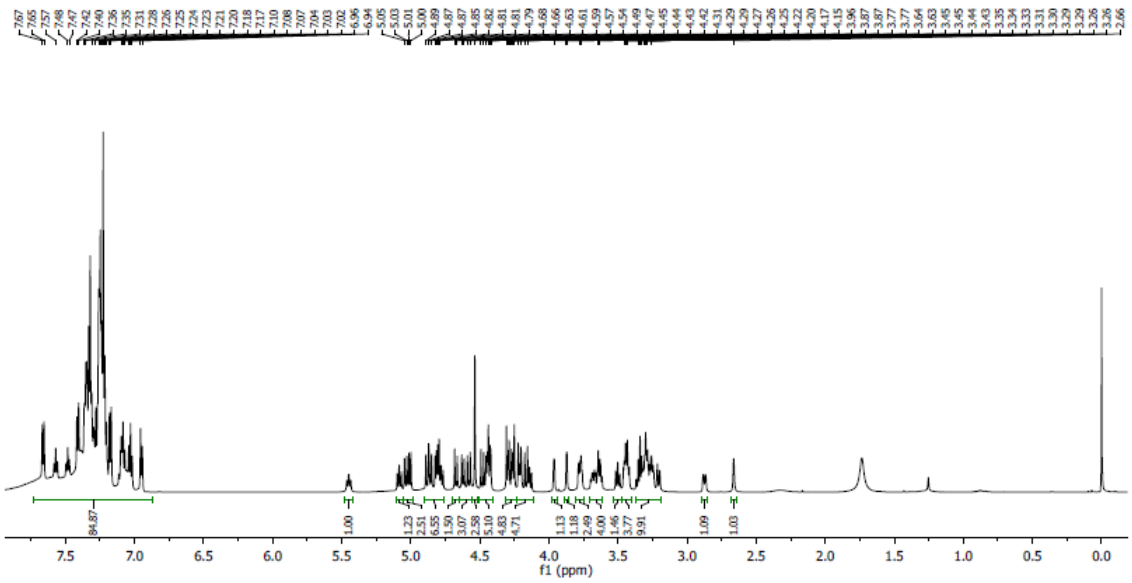


$CDCl_3$ 600 MHz

Figure A-99: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.13**)

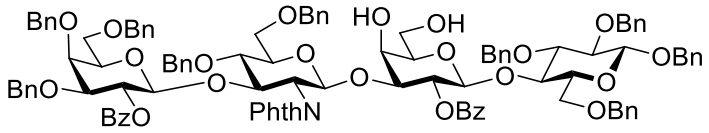


5.14

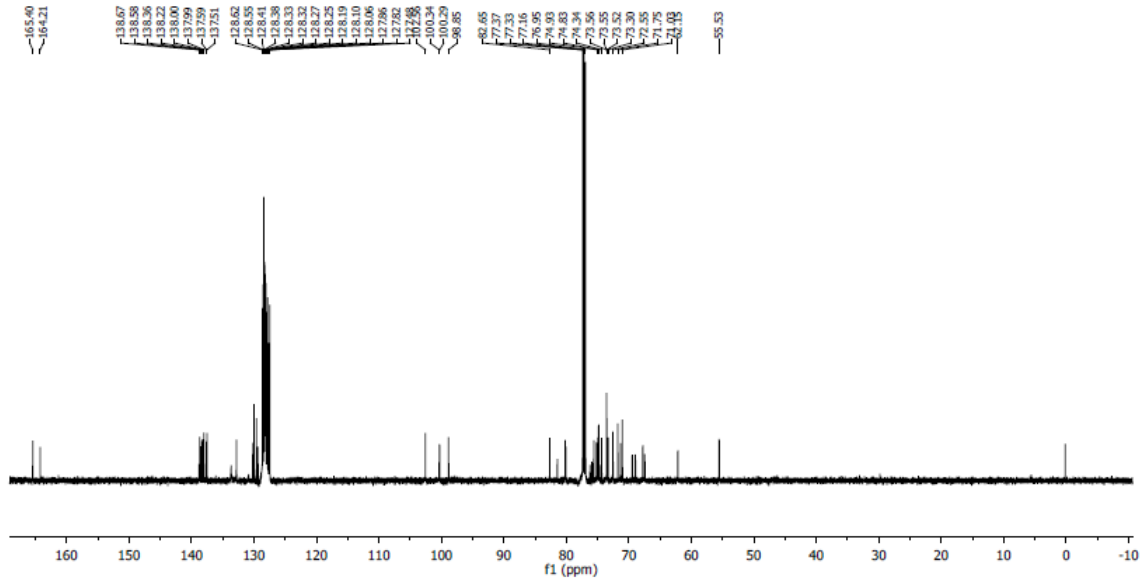


$CDCl_3$ 600 MHz

Figure A-100: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.14**)

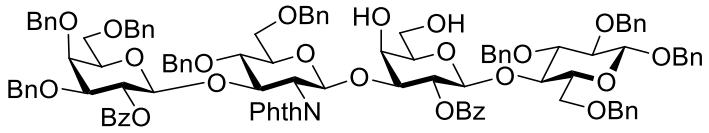


5.14

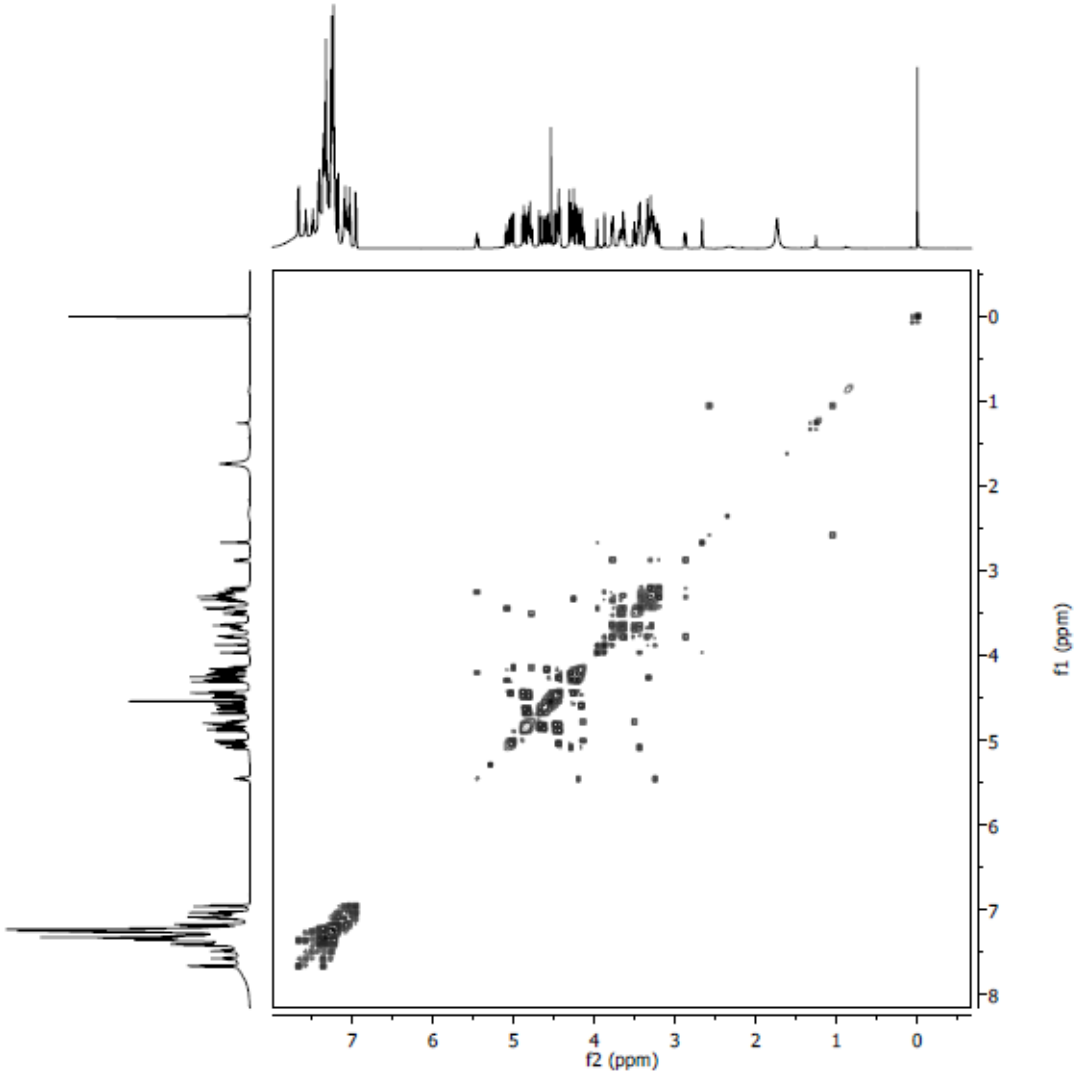


$CDCl_3$ 151 MHz

Figure A-101: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.14**)

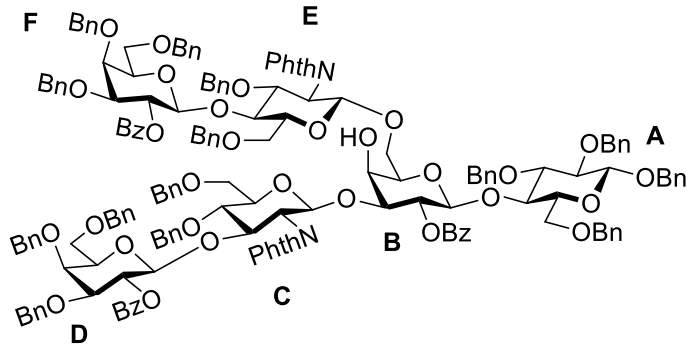


5.14

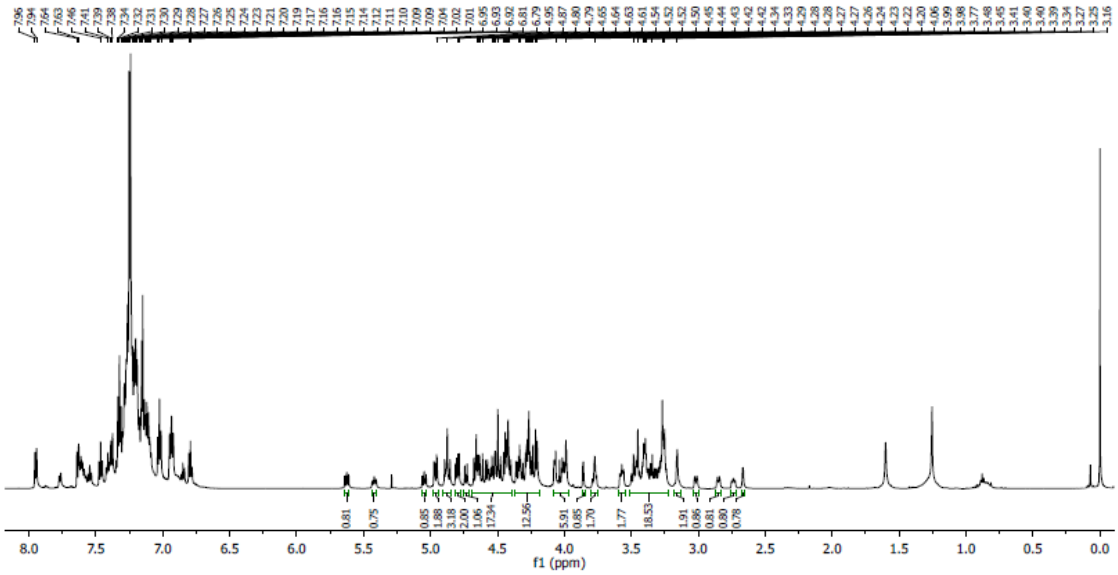


$CDCl_3$ 600 MHz

Figure A-102: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.14**)

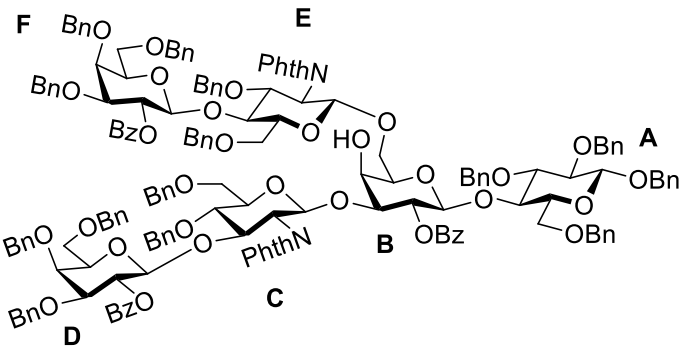


5.15

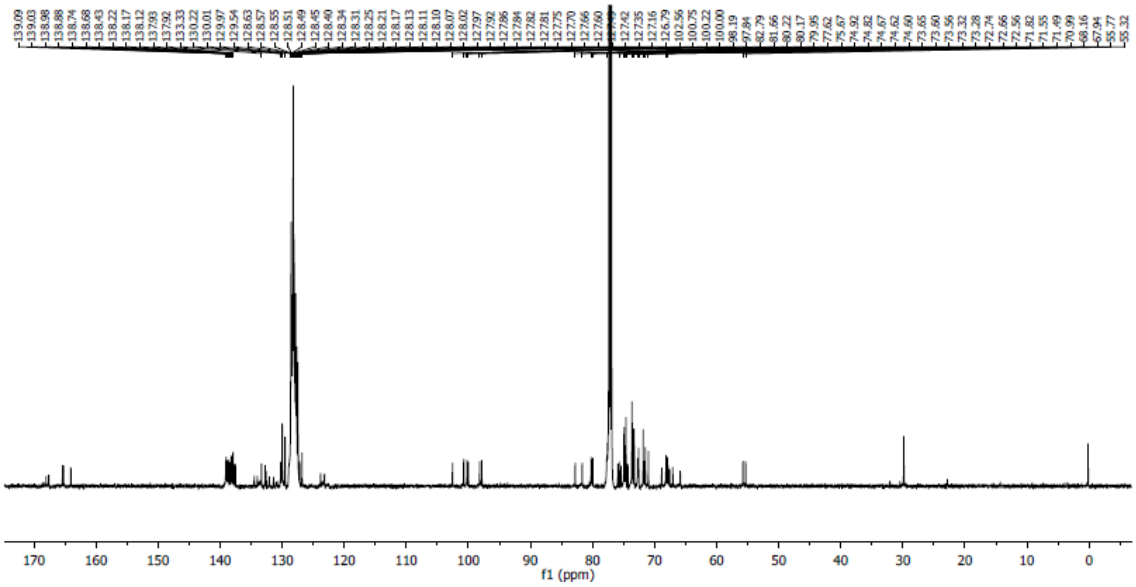


$CDCl_3$ 600 MHz

Figure A-103: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.15**)

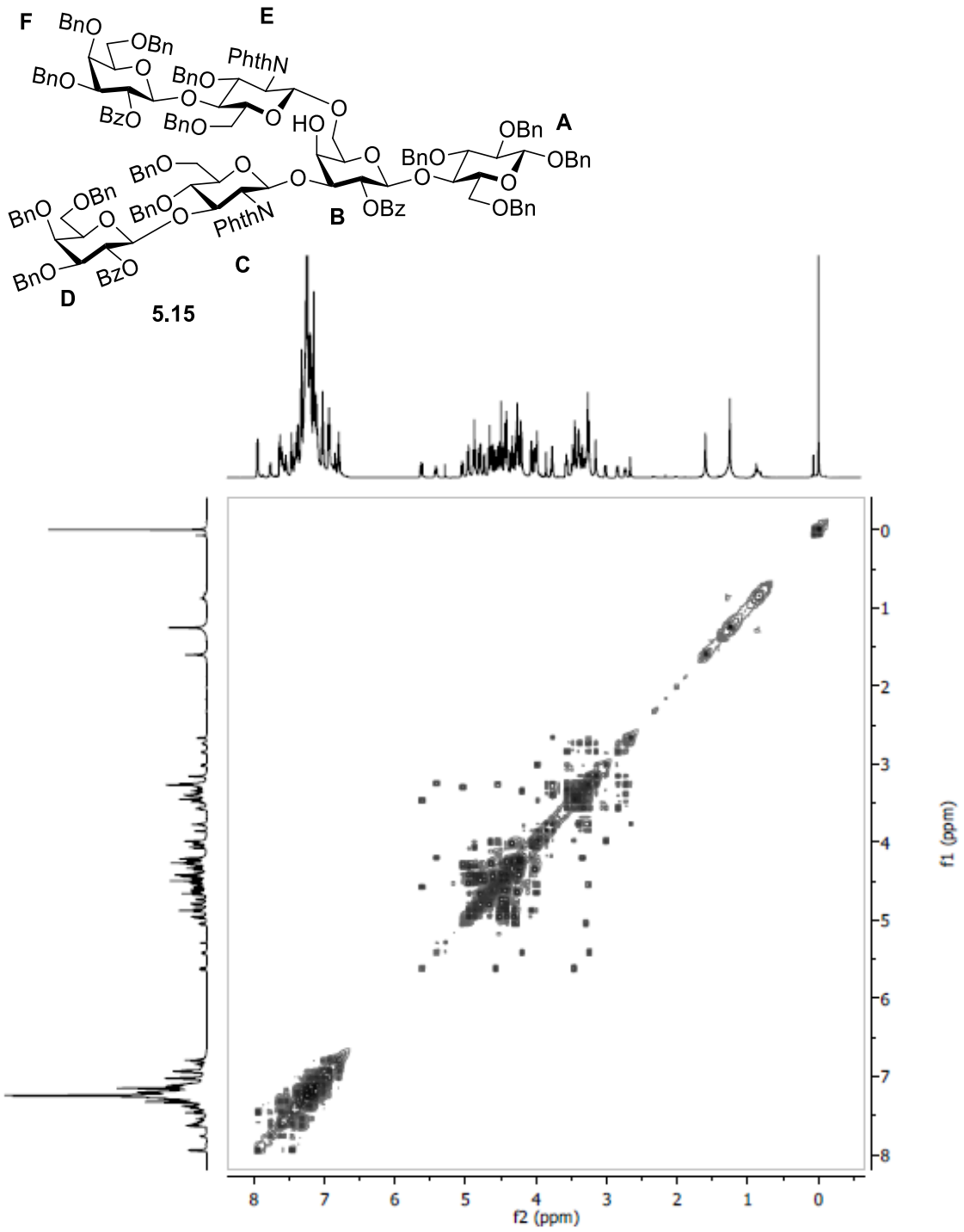


5.15



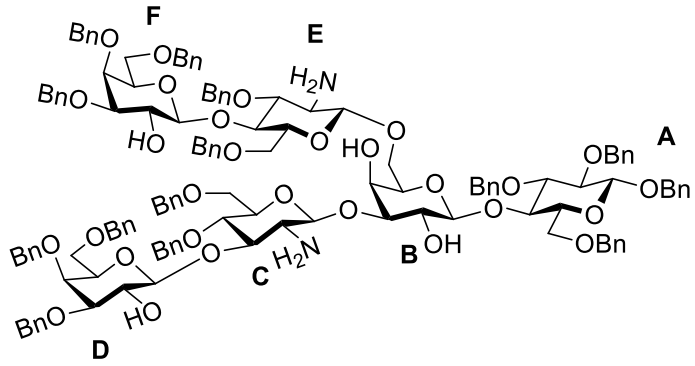
$CDCl_3$ 151 MHz

Figure A-104: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.15**)

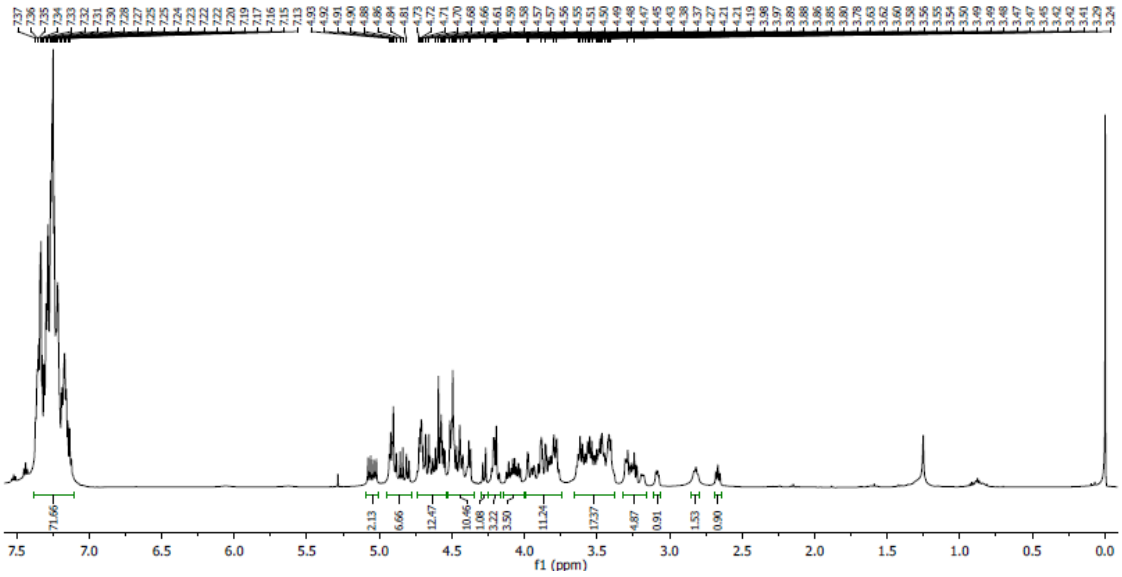


$CDCl_3$ 600 MHz

Figure A-105: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.15**)

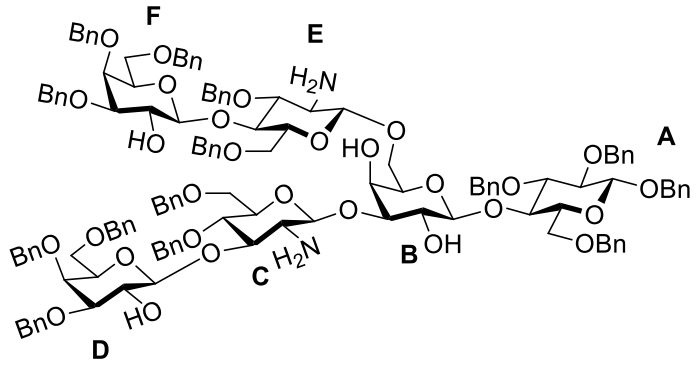


5.16

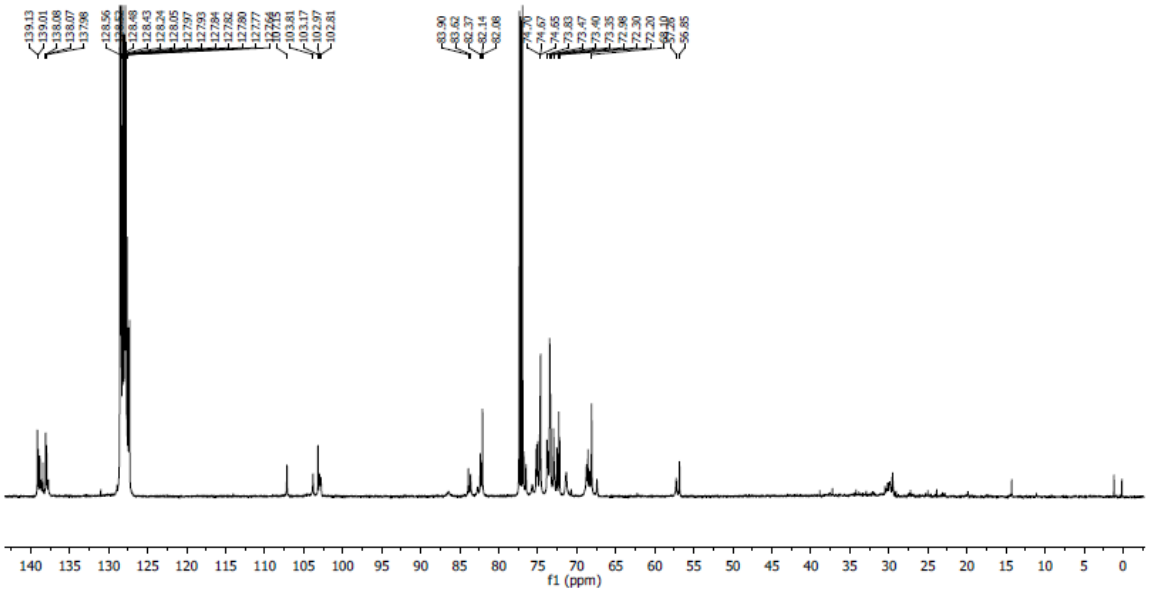


$CDCl_3$ 600 MHz

Figure A-106: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-amino-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.16**)

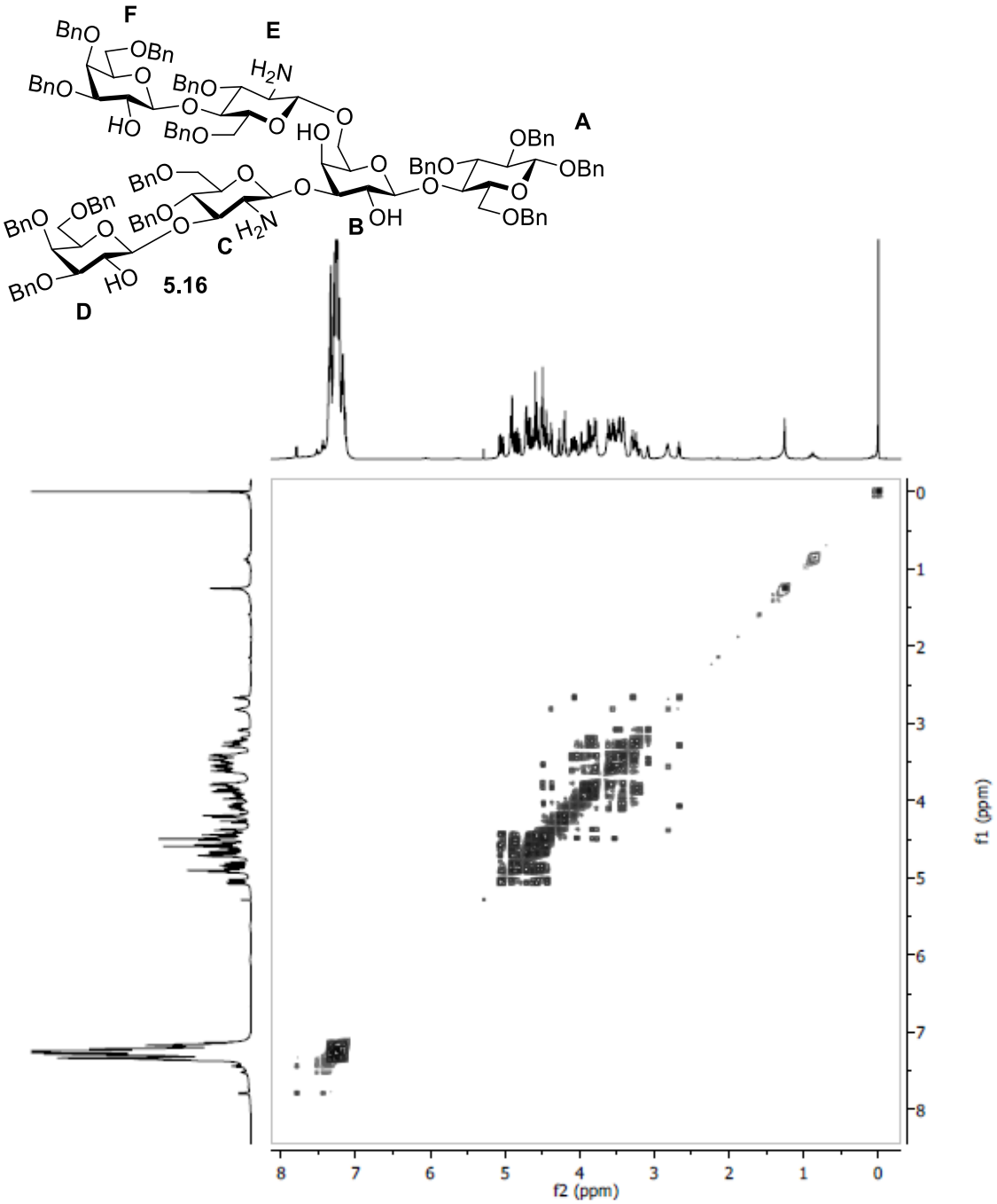


5.16



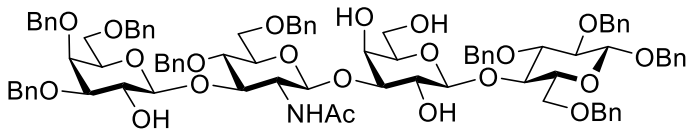
$CDCl_3$ 151 MHz

Figure A-107: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-amino-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.16**)



$CDCl_3$ 600 MHz

Figure A-108: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-amino-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.16**)



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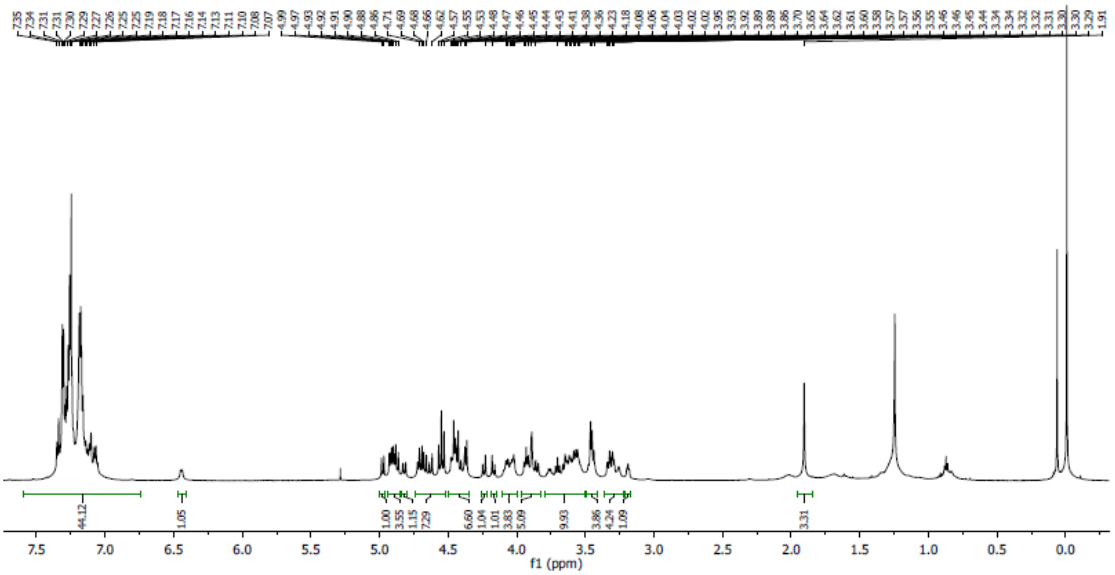
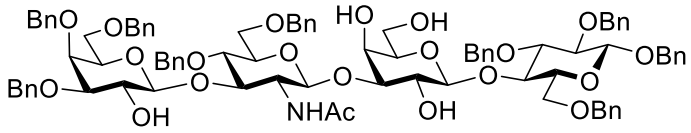
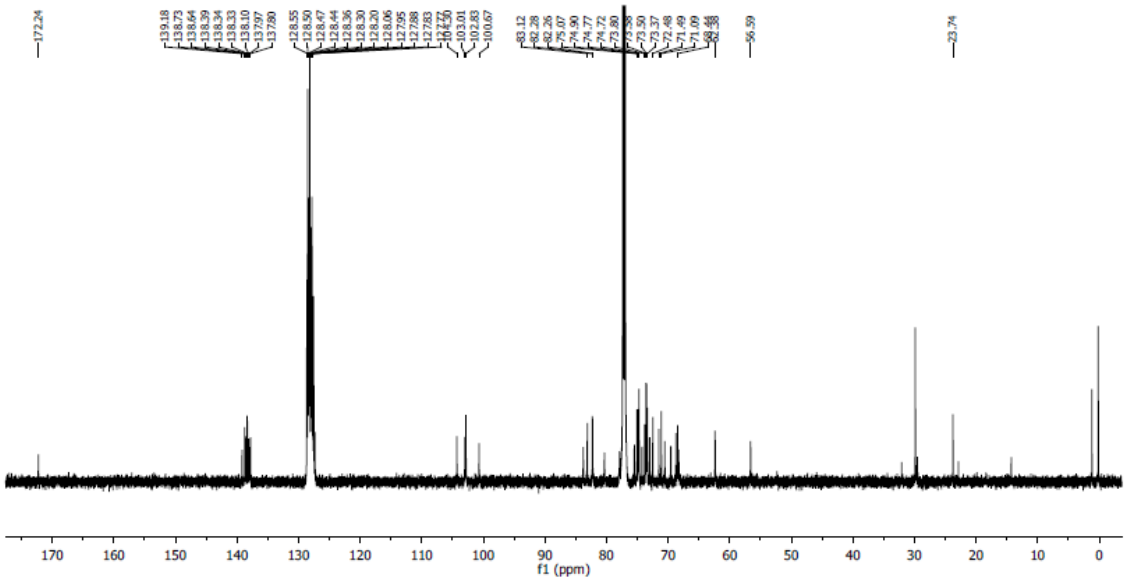


$CDCl_3$ 600 MHz

Figure A-109: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.17**)

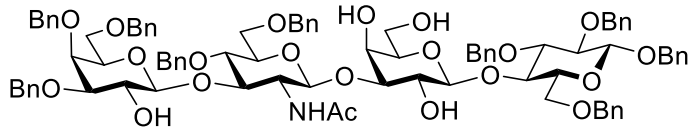


5.17

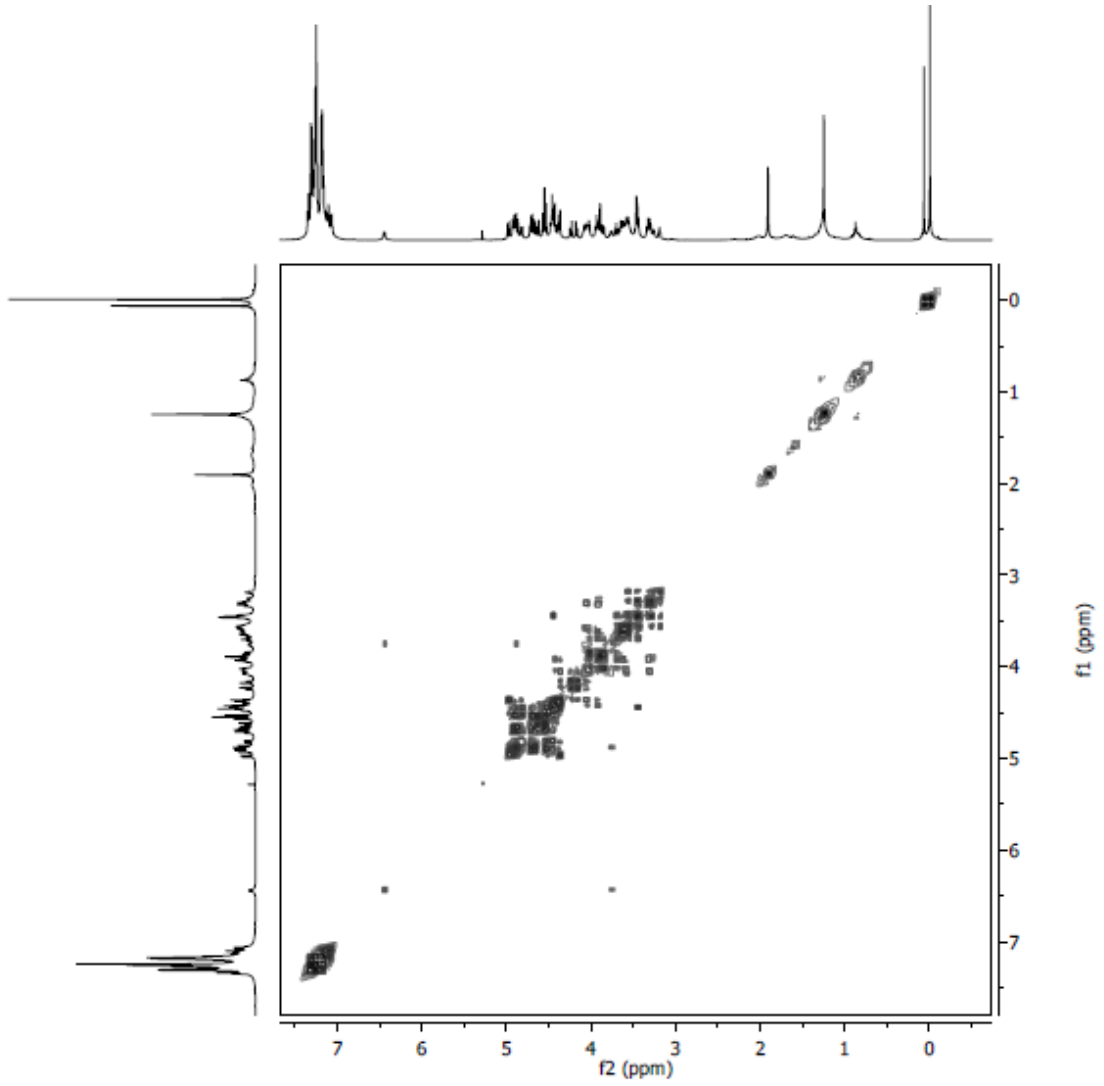


$CDCl_3$ 151 MHz

Figure A-110: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.17**)

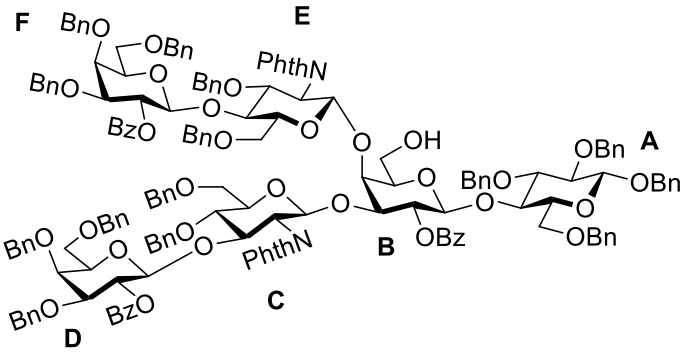


5.17

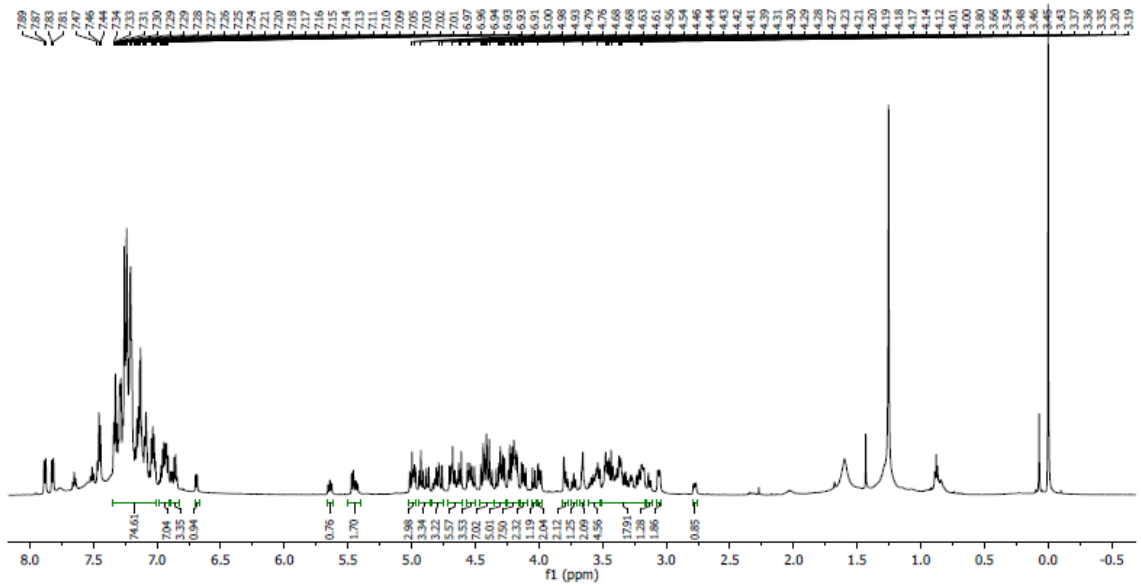


$CDCl_3$ 600 MHz

Figure A-111: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.17**)

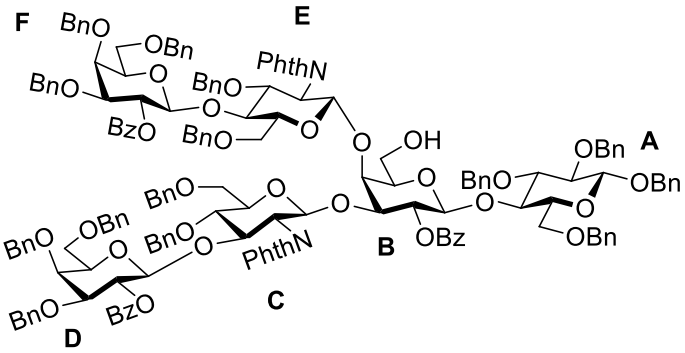


5.18

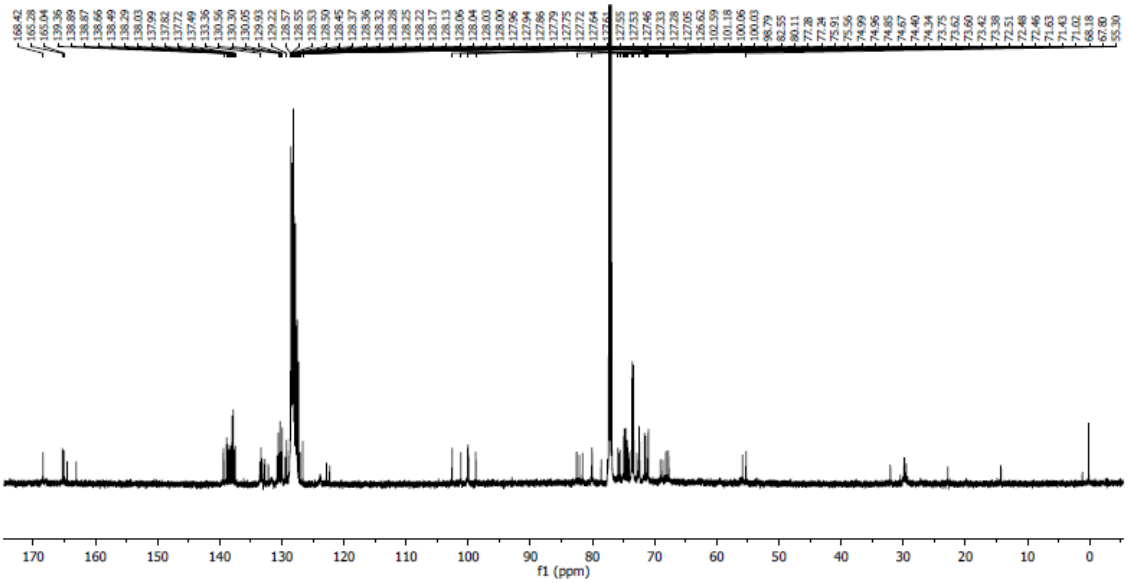


$CDCl_3$ 600 MHz

Figure A-112: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.18**)

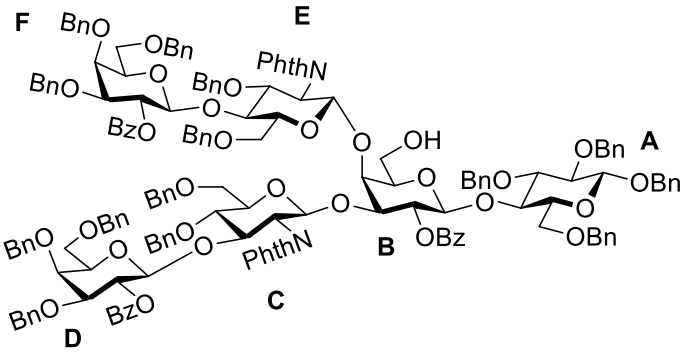


5.18

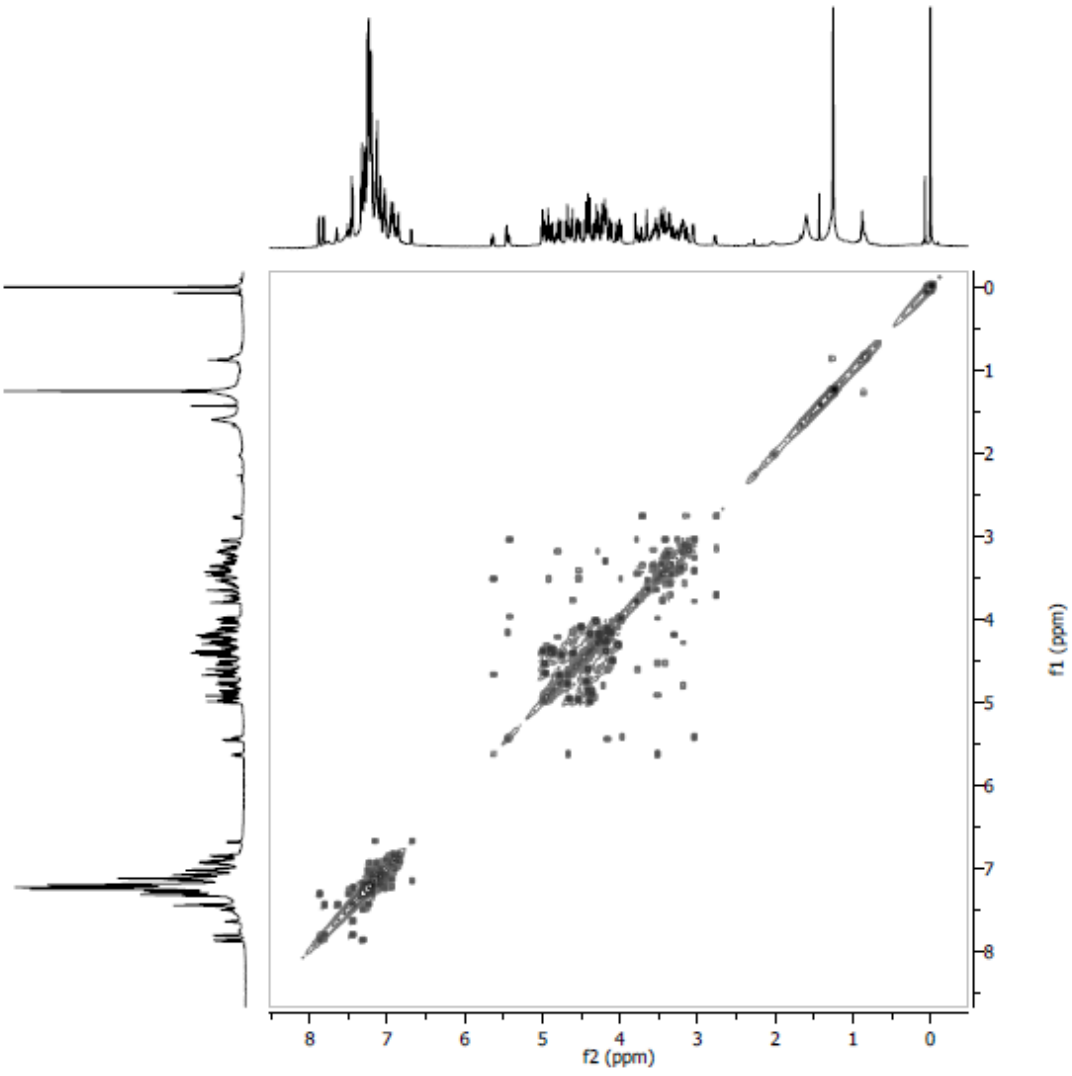


$CDCl_3$ 151 MHz

Figure A-113: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.18**)

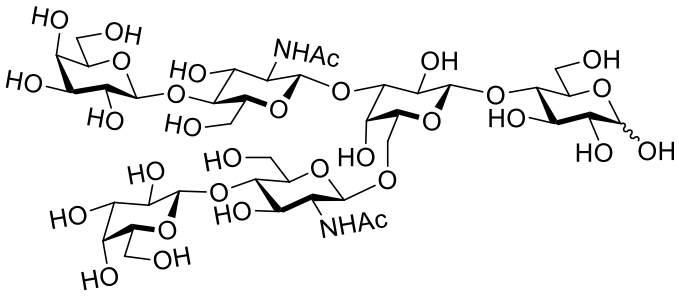


5.18

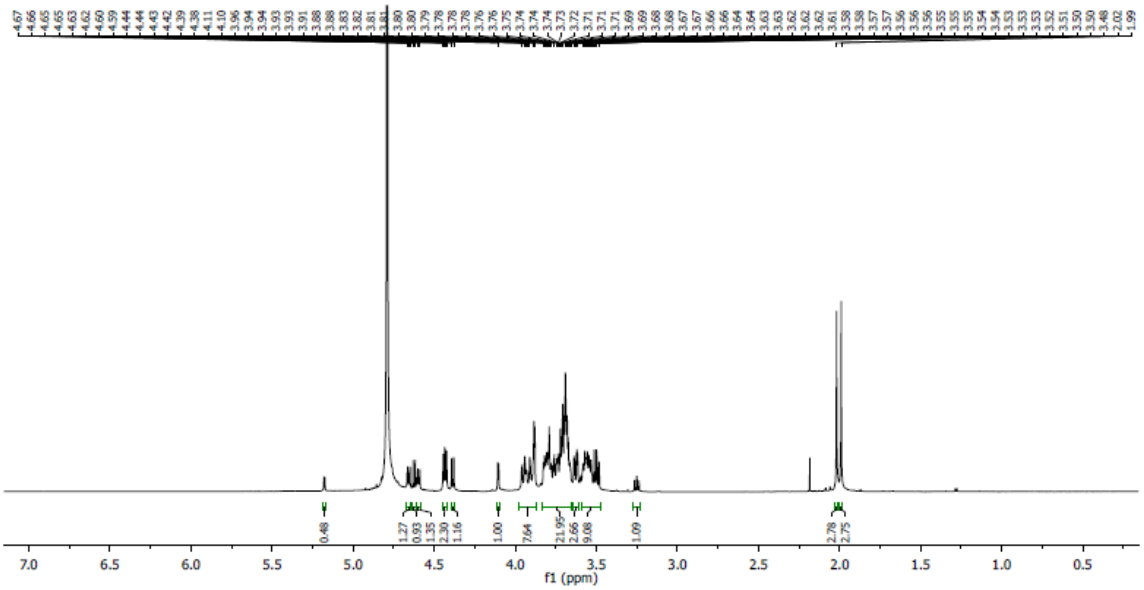


$CDCl_3$ 600 MHz

Figure A-114: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-O-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5.18**)

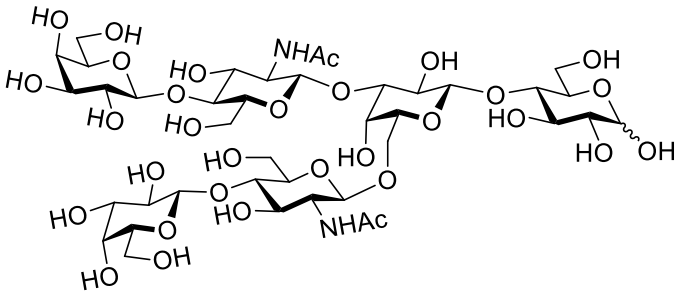


6.1

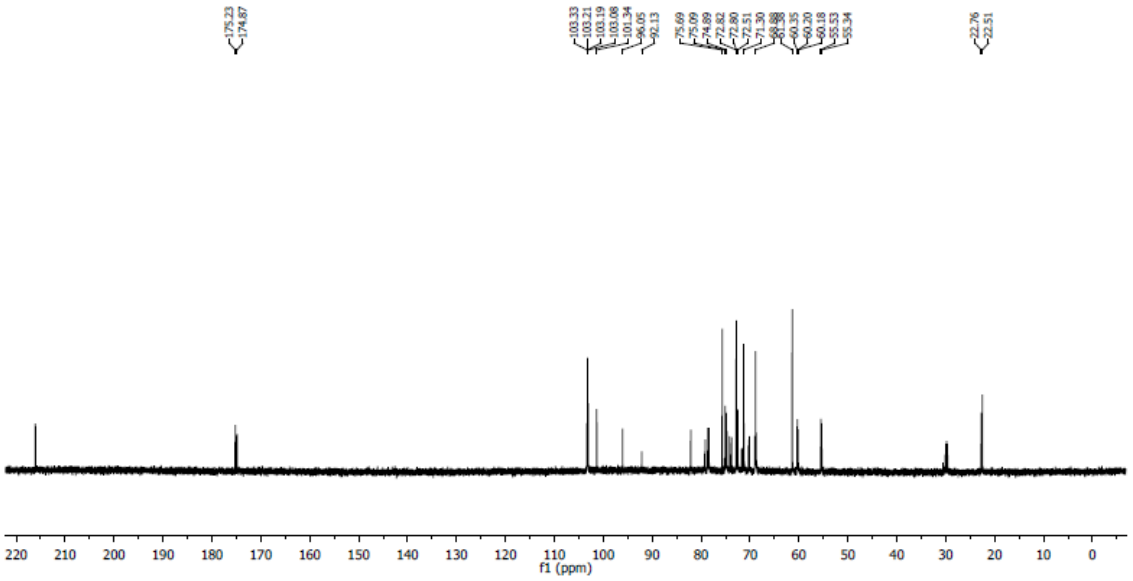


D_2O 600 MHz

Figure A-115: ^{1}H NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**6.1**)

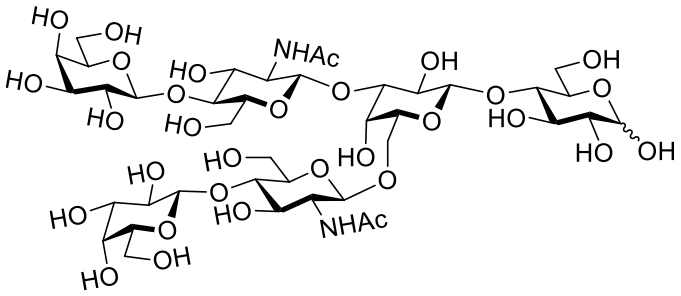


6.1

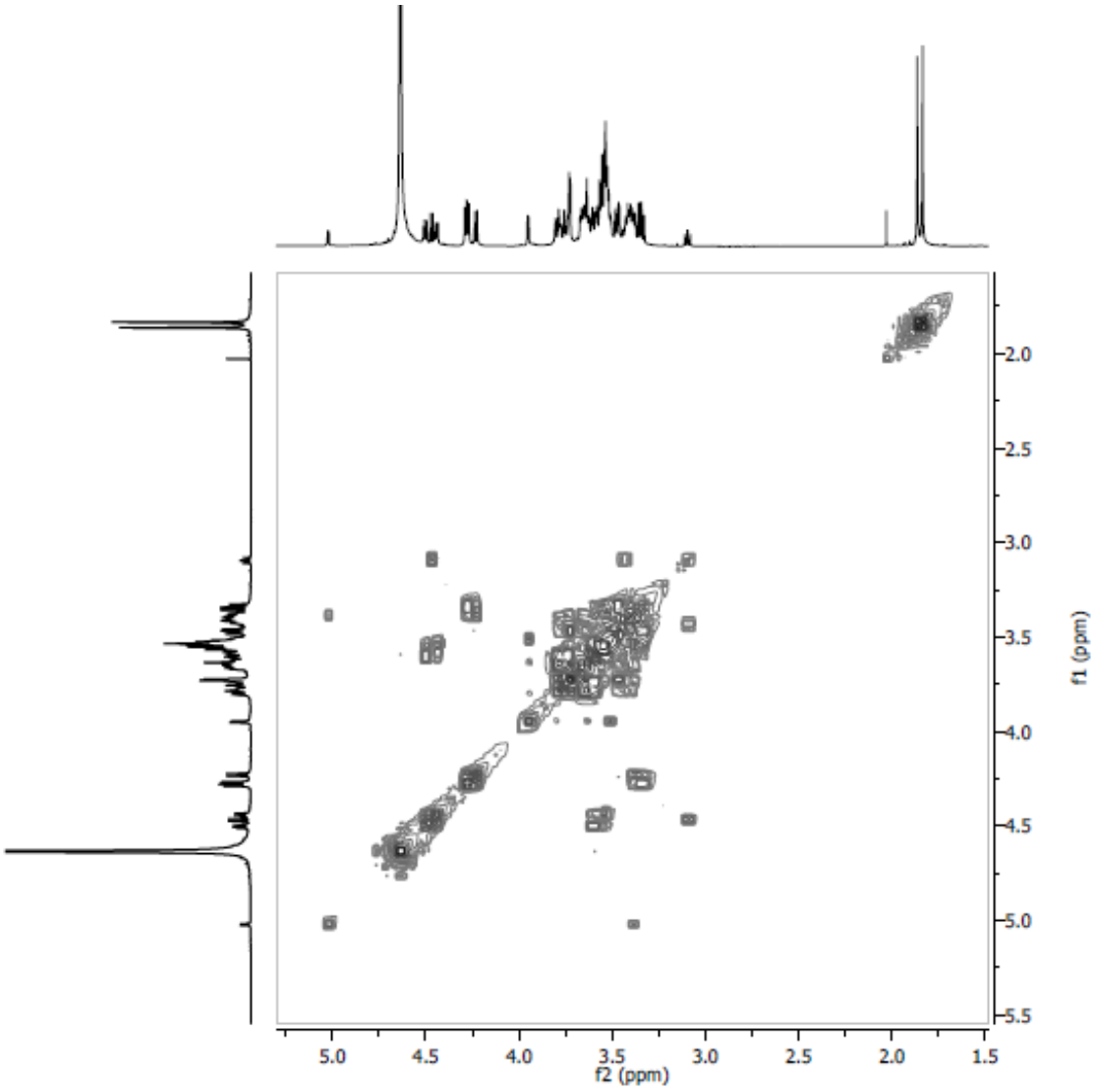


D_2O 151 MHz

Figure A-116: ^{13}C NMR spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**6.1**)

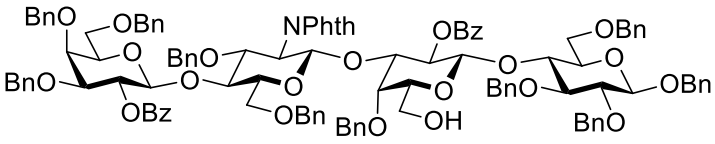


6.1

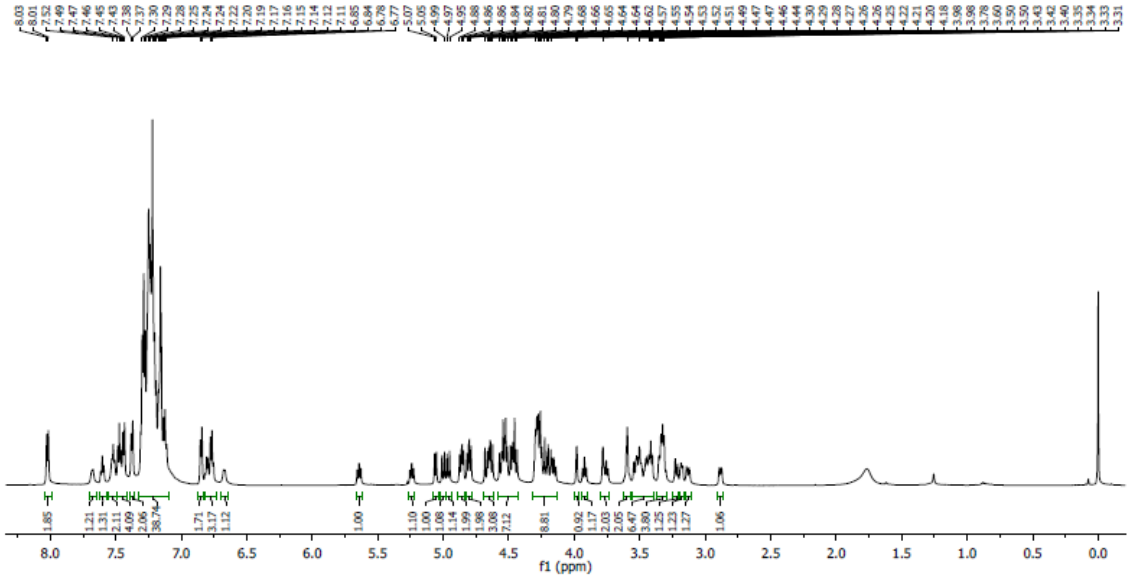


D_2O 600 MHz

Figure A-117: 2-D NMR COSY spectrum of *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranose (**6.1**)

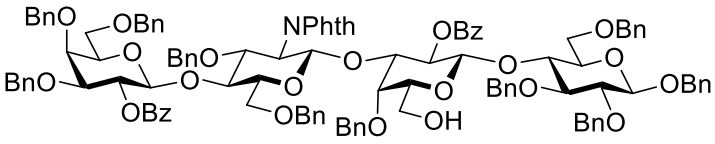


6.4

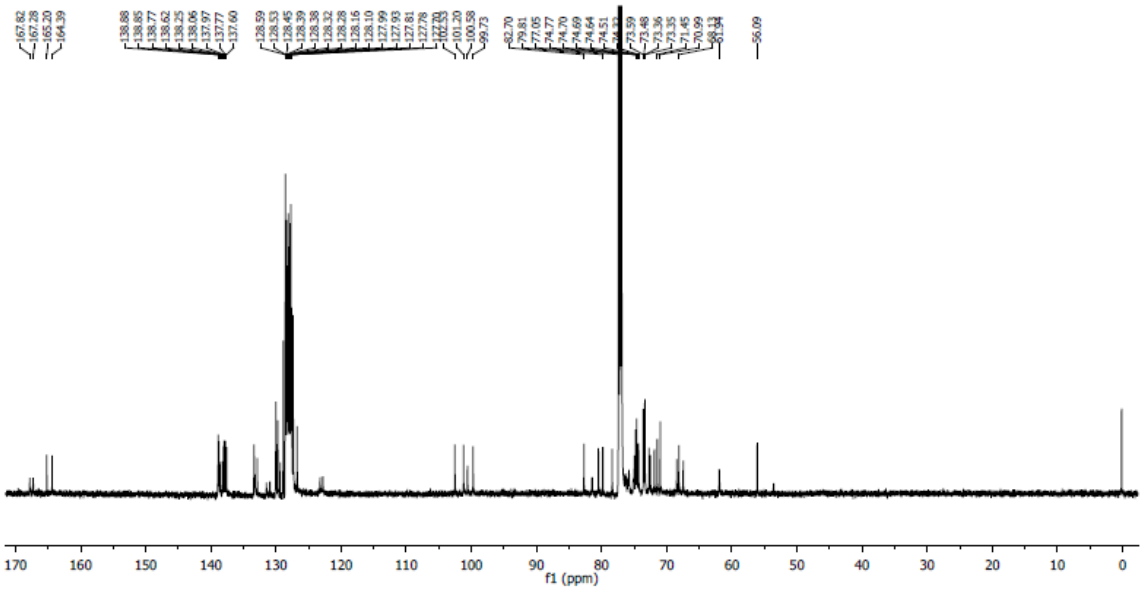


$CDCl_3$ 600 MHz

Figure A-118: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.4**)

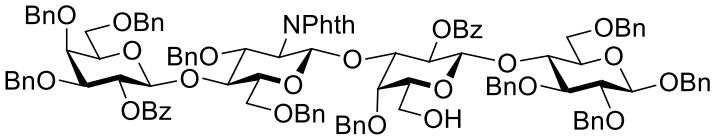


6.4

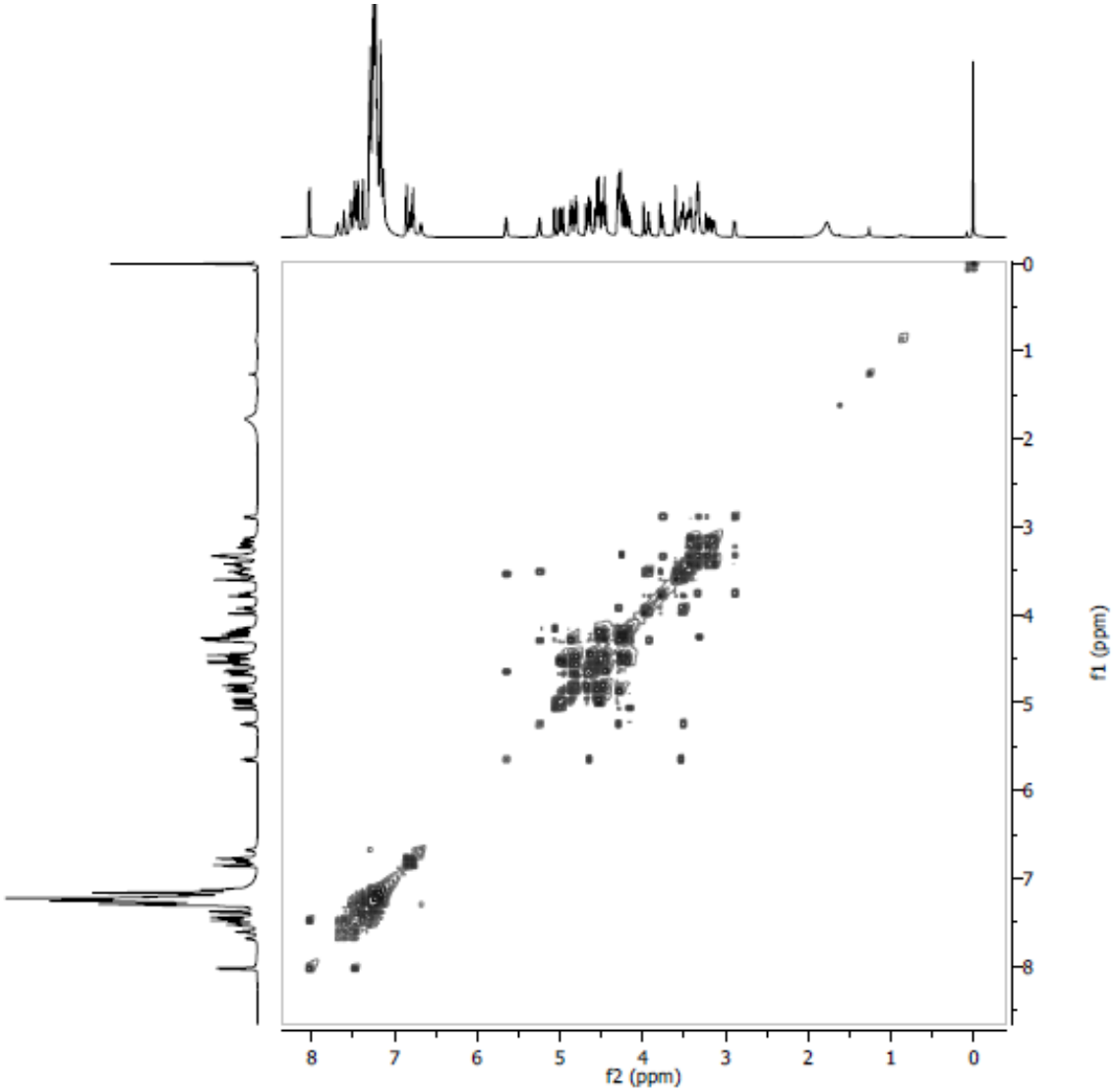


$CDCl_3$ 151 MHz

Figure A-119: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.4**)

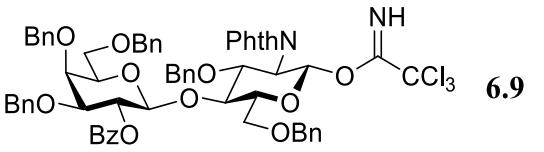


6.4

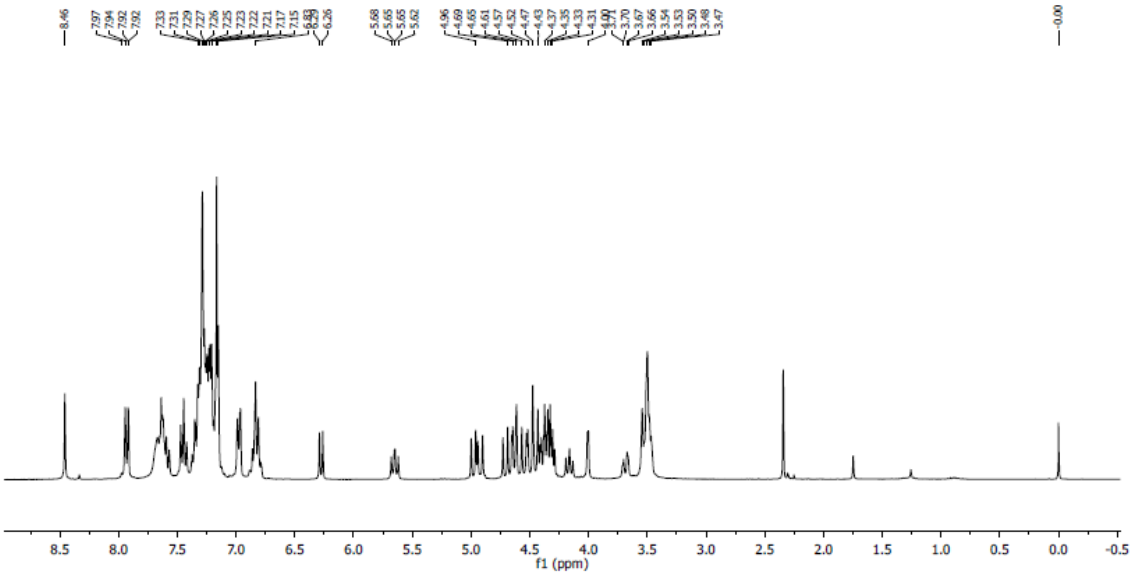


$CDCl_3$ 600 MHz

Figure A-120: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.4**)

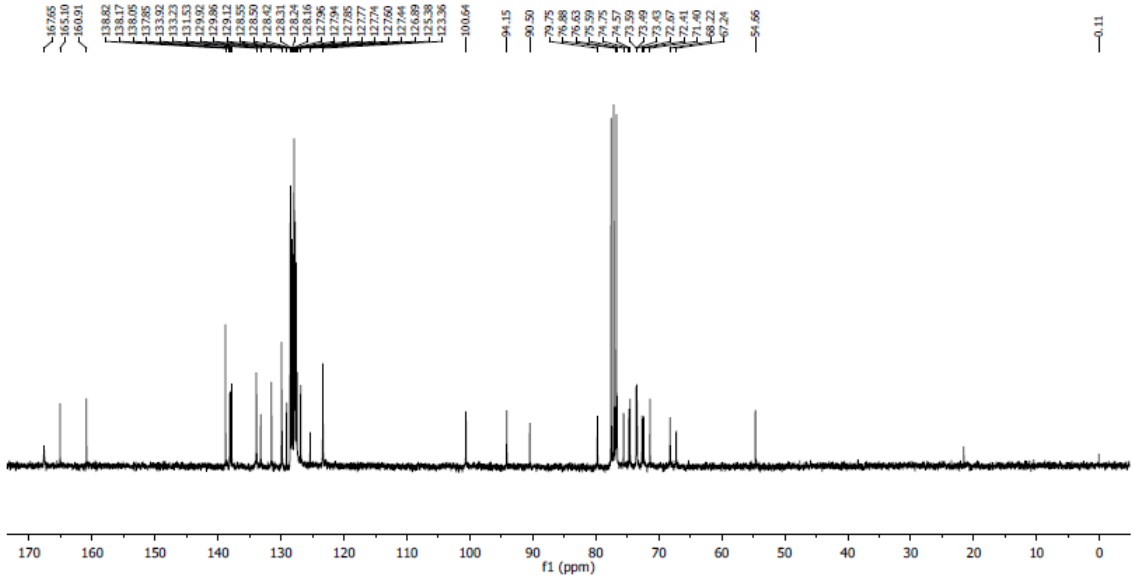


6.9



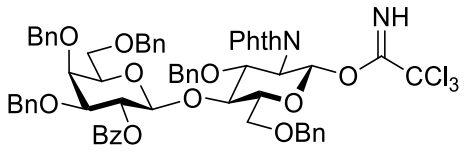
$CDCl_3$ 300 MHz

Figure A-121: ^{1}H NMR spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**6.9**)

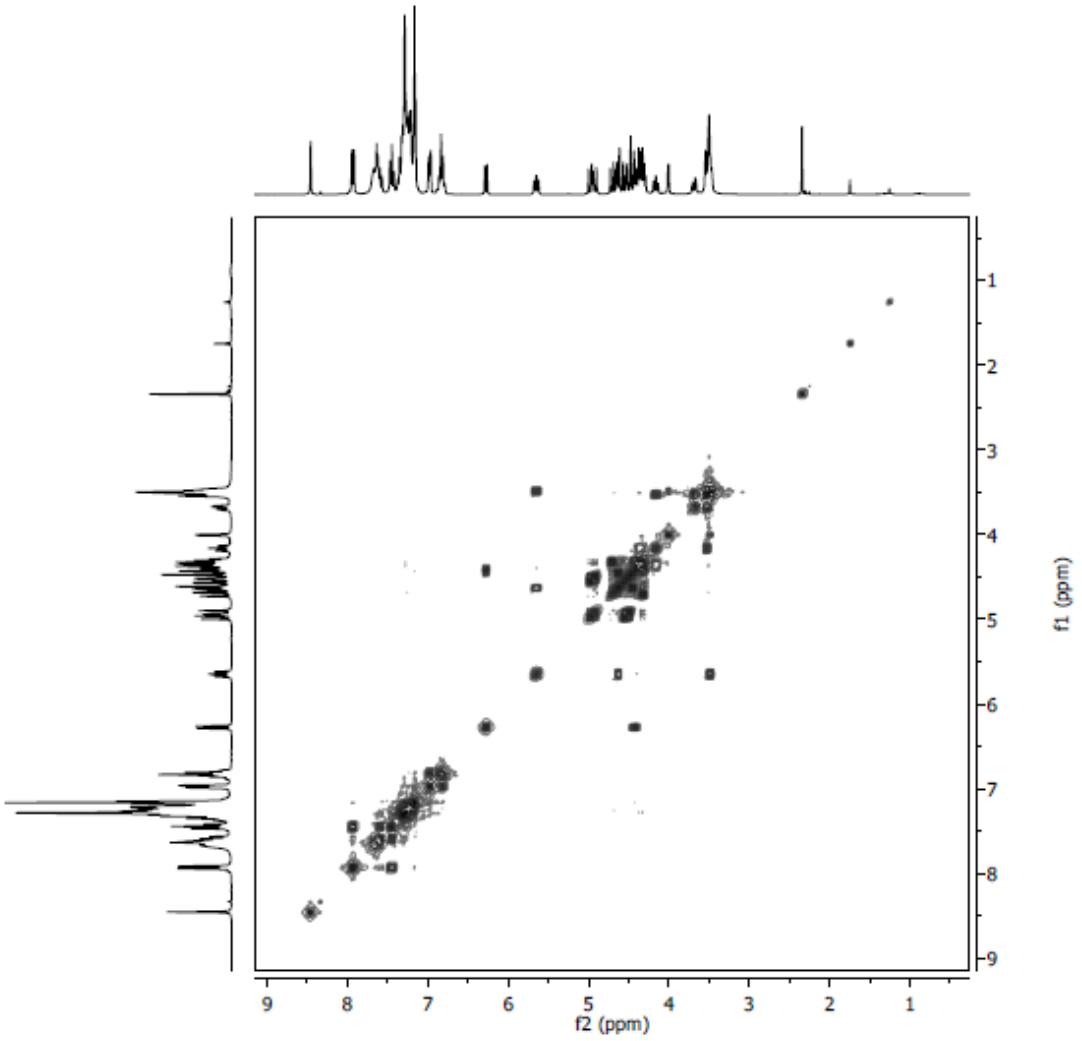


$CDCl_3$ 75 MHz

Figure A-122: ^{13}C NMR spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**6.9**)

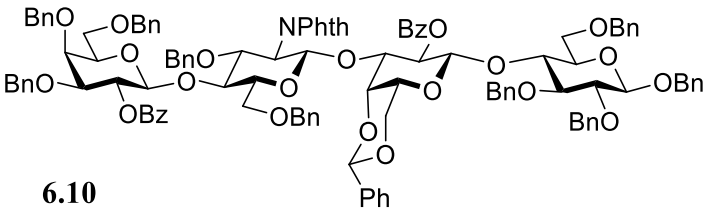


6.9

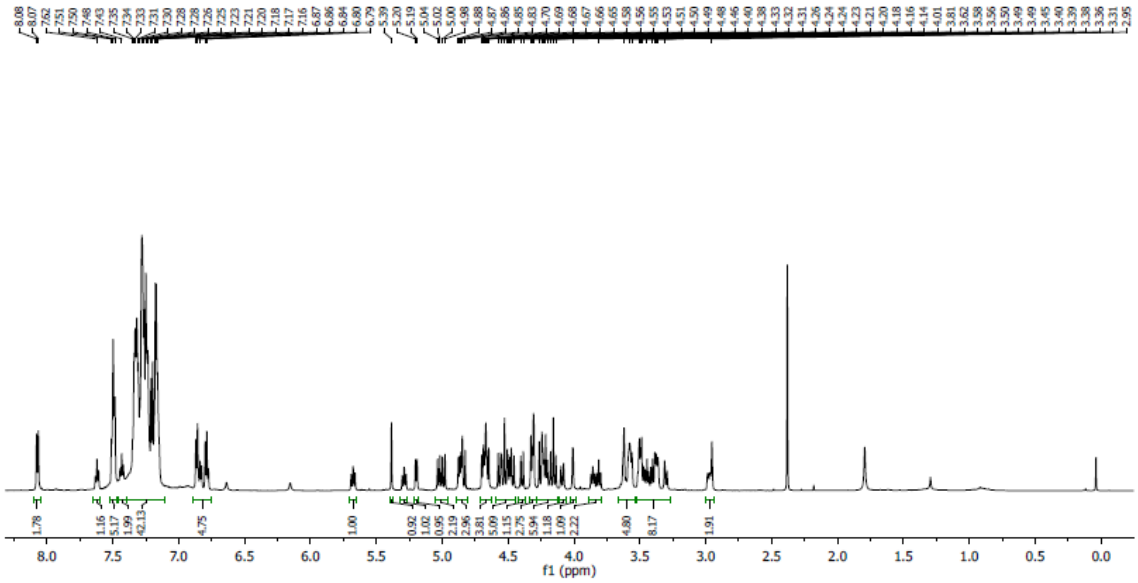


$CDCl_3$ 300 MHz

Figure A-123: 2-D NMR COSY spectrum of *O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**6.9**)

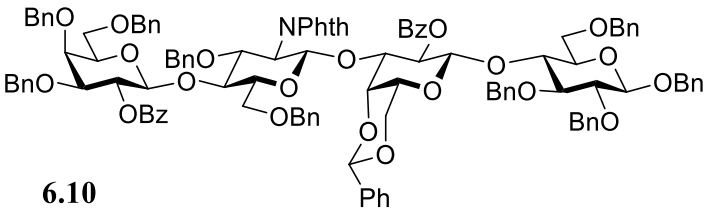


6.10

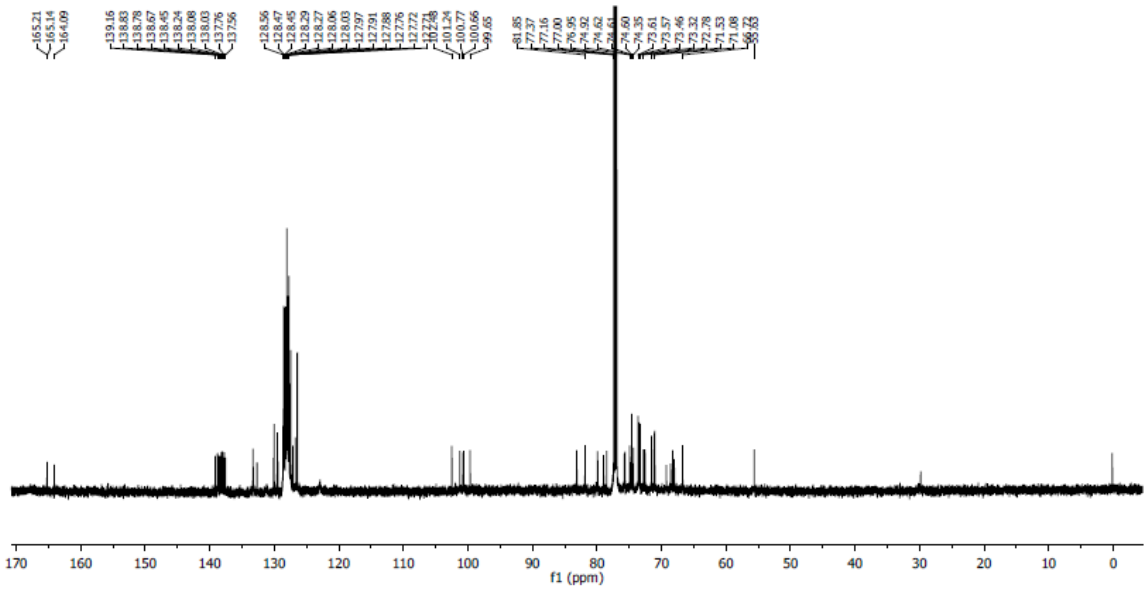


$CDCl_3$ 600 MHz

Figure A-124: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.10**)

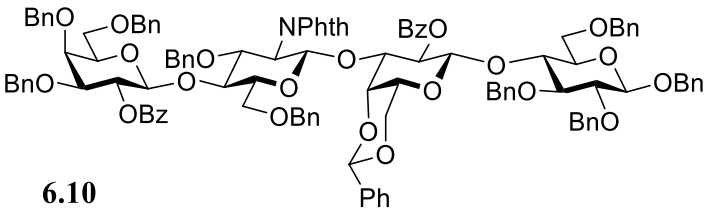


6.10

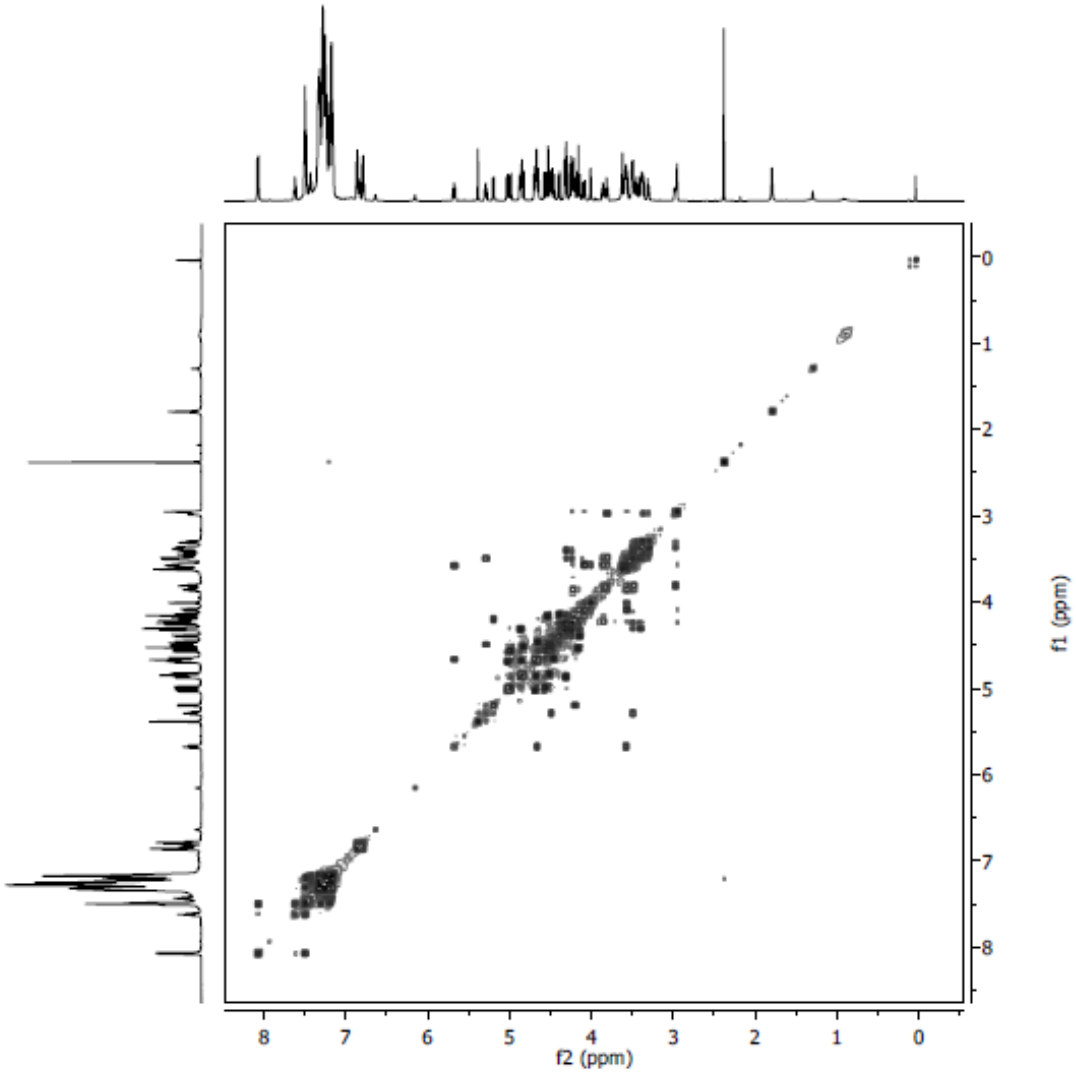


$CDCl_3$ 151 MHz

Figure A-125: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.10**)

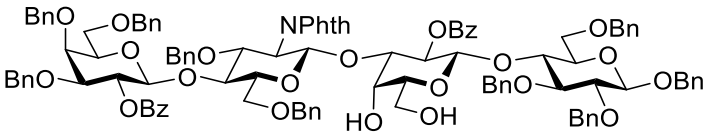


6.10

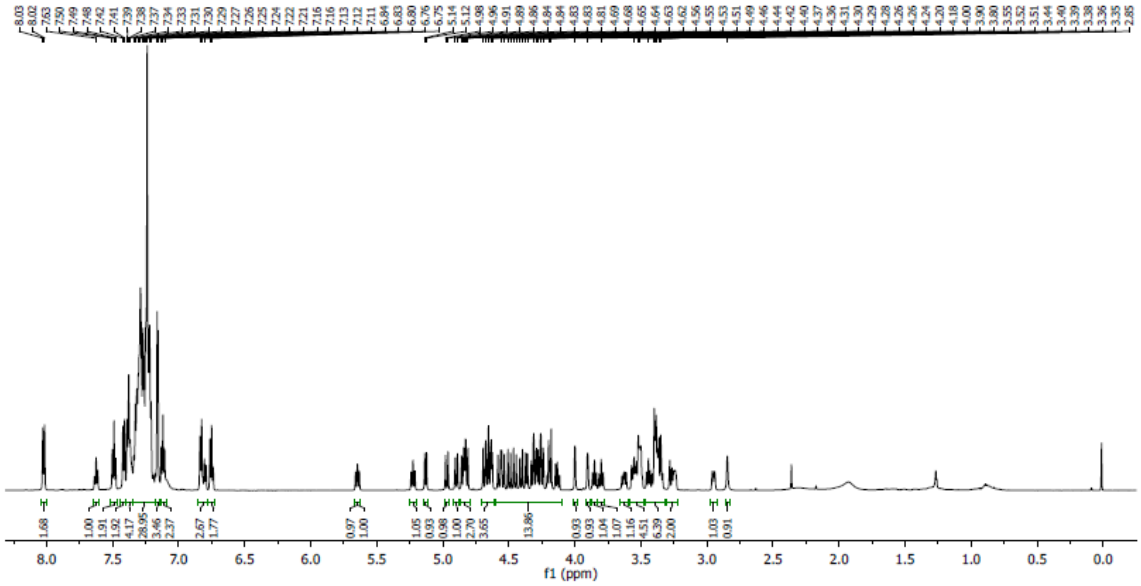


$CDCl_3$ 600 MHz

Figure A-126: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.10**)

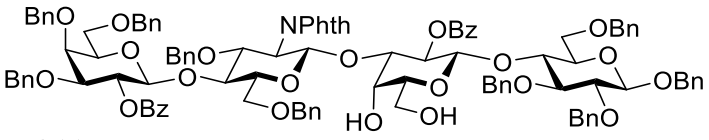


6.11

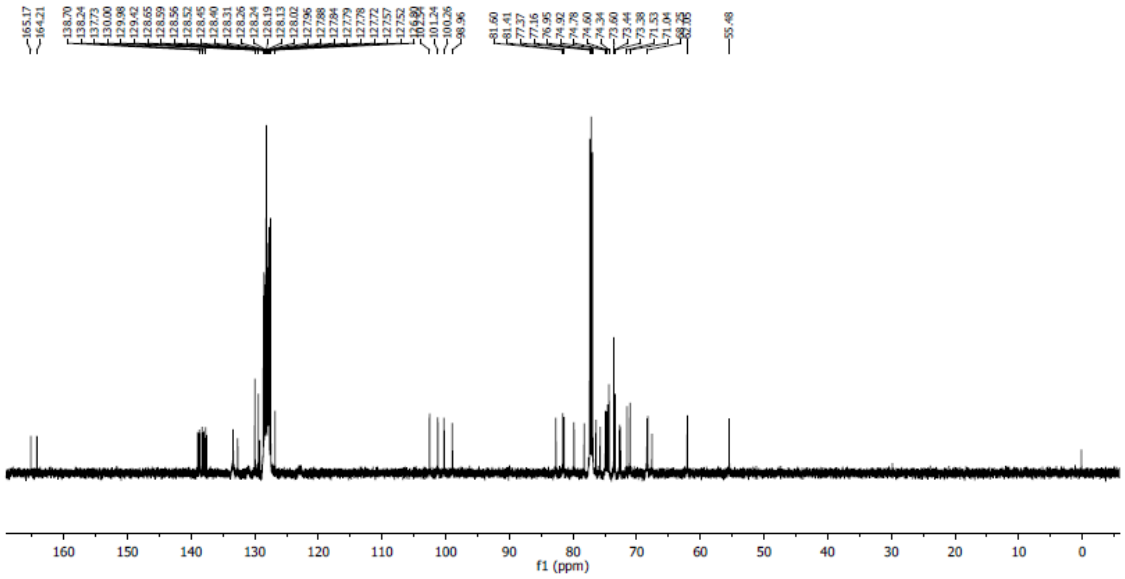


$CDCl_3$ 600 MHz

Figure A-127: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.11**)

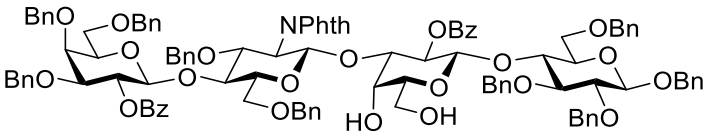


6.11

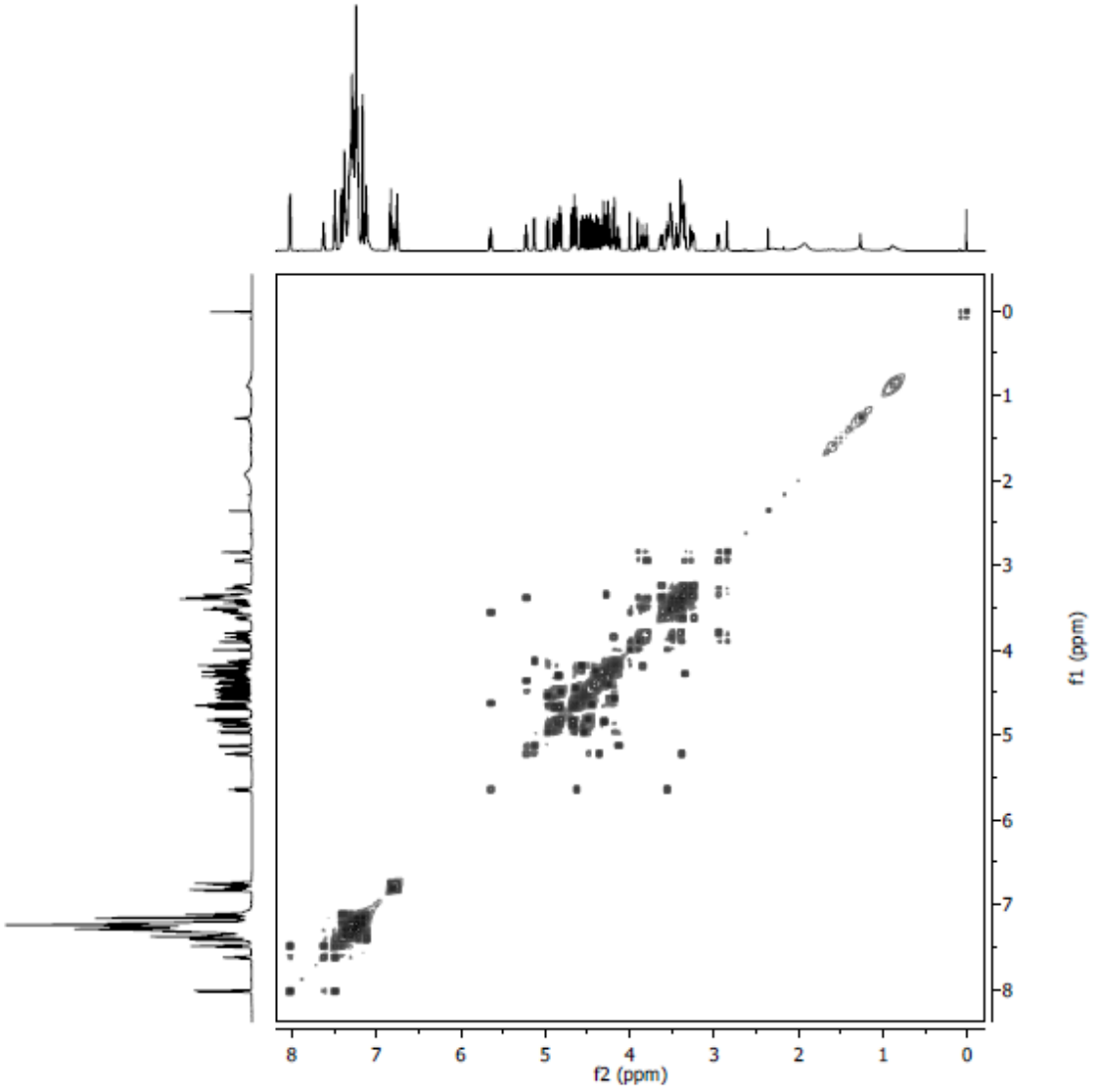


$CDCl_3$ 151 MHz

Figure A-128: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.11**)

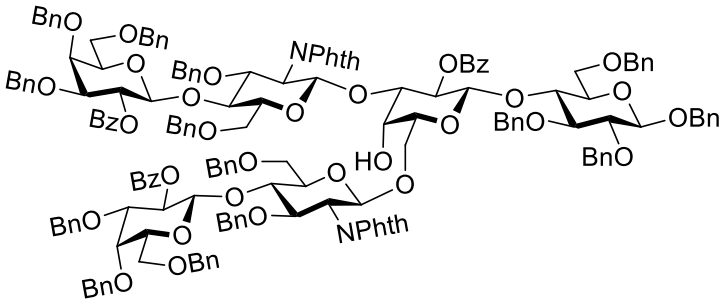


6.11

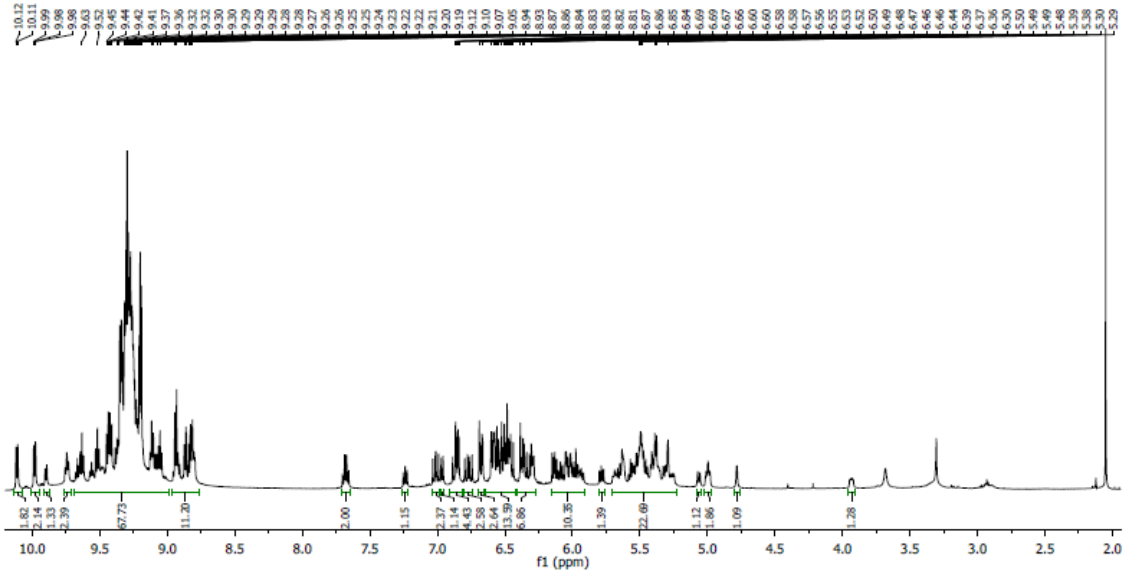


$CDCl_3$ 600 MHz

Figure A-129: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.11**)

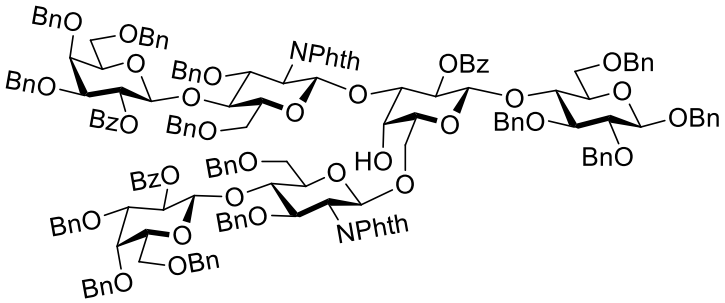


6.12

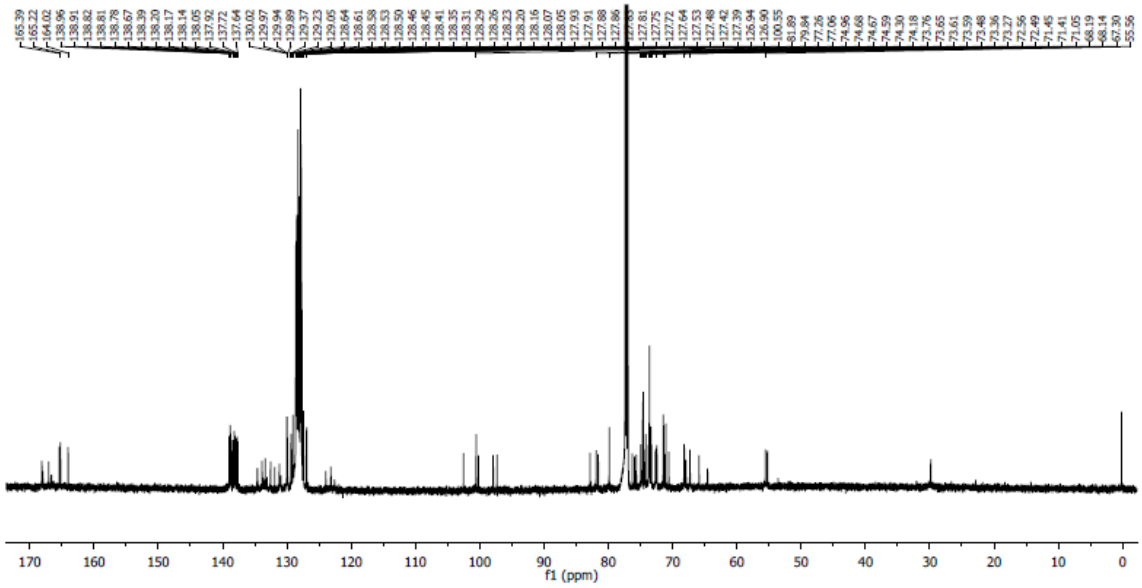


$CDCl_3$ 600 MHz

Figure A-130: ^{1}H NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.12**)

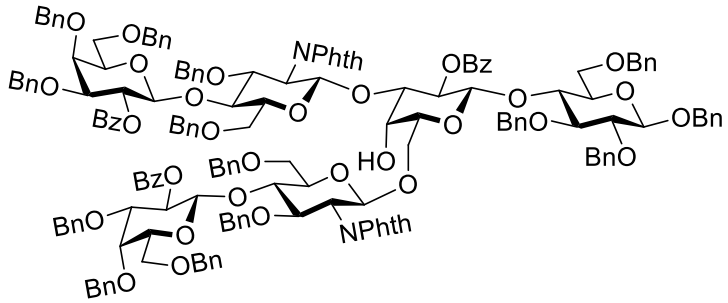


6.12

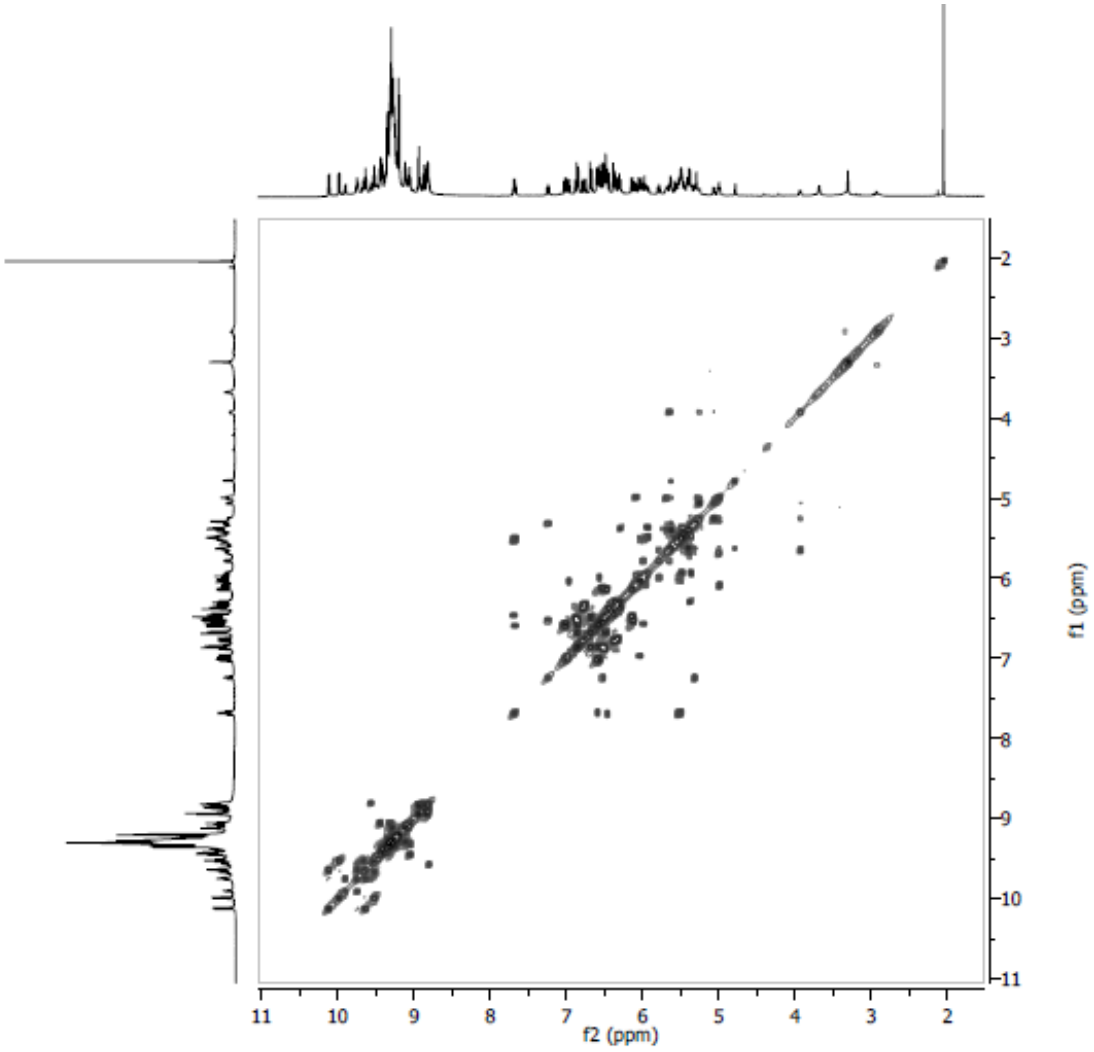


$CDCl_3$ 151 MHz

Figure A-131: ^{13}C NMR spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.12**)

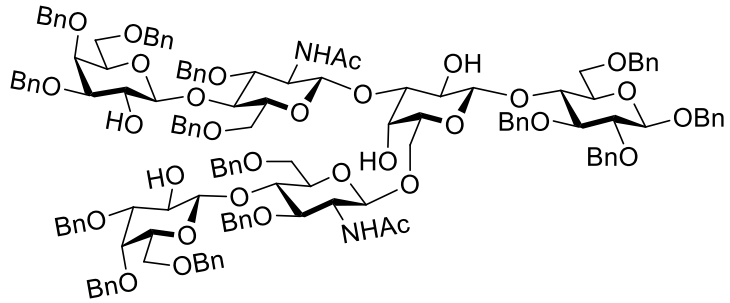


6.12

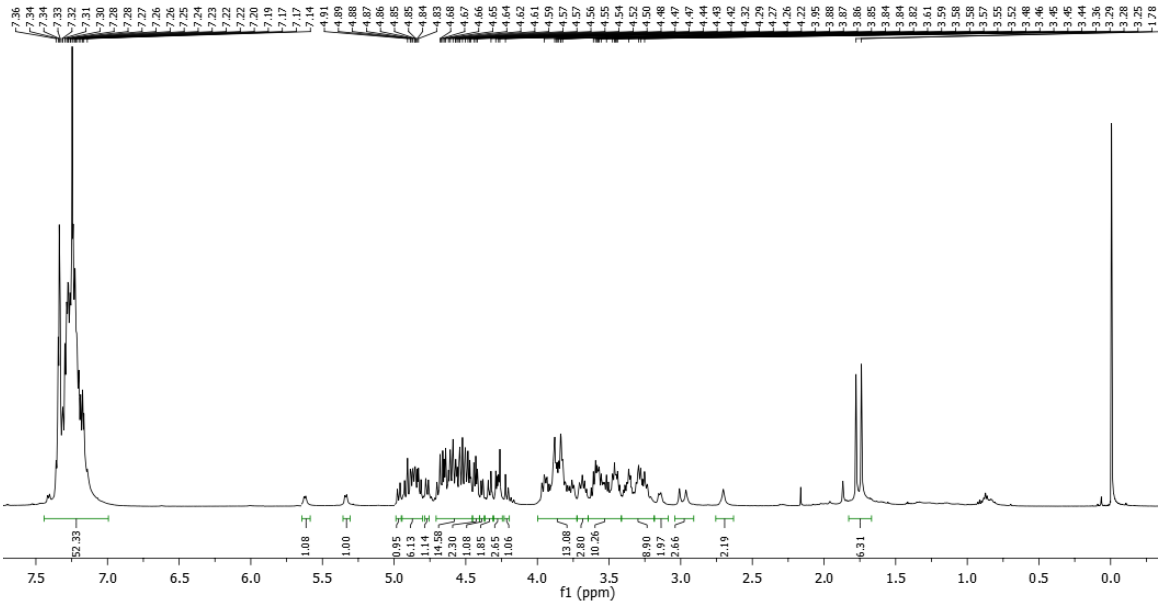


$CDCl_3$ 600 MHz

Figure A-132: 2-D NMR COSY spectrum of Benzyl *O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-[*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→6)]-*O*-(2-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.12**)

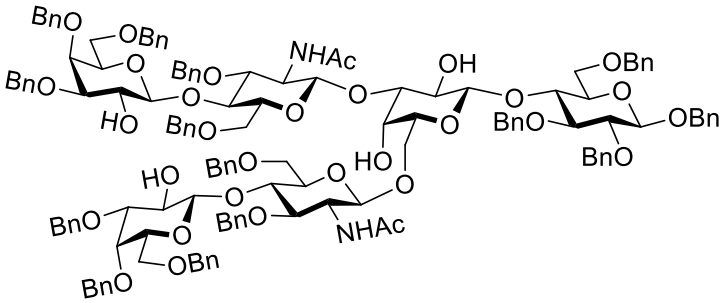


6.13

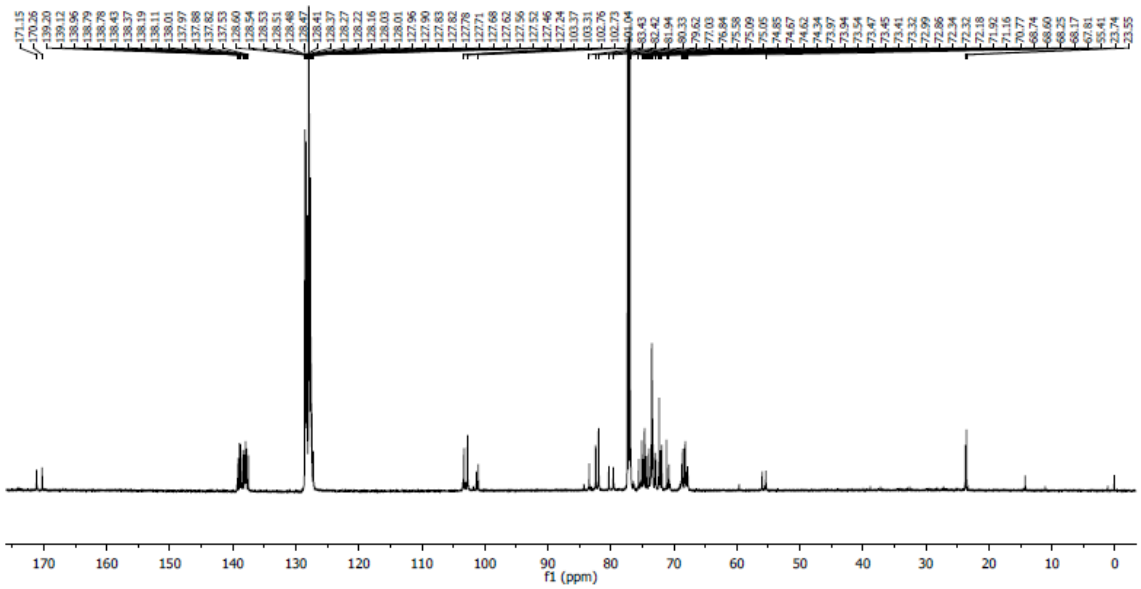


$CDCl_3$ 600 MHz

Figure A-133: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.13**)

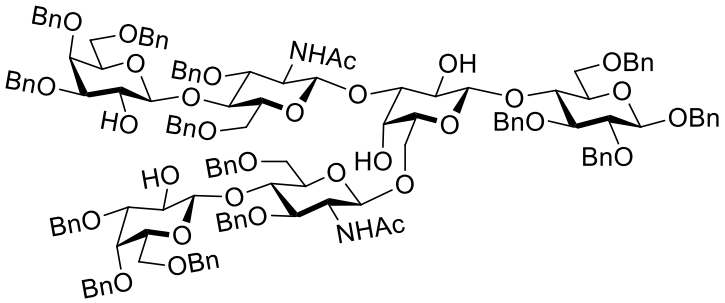


6.13

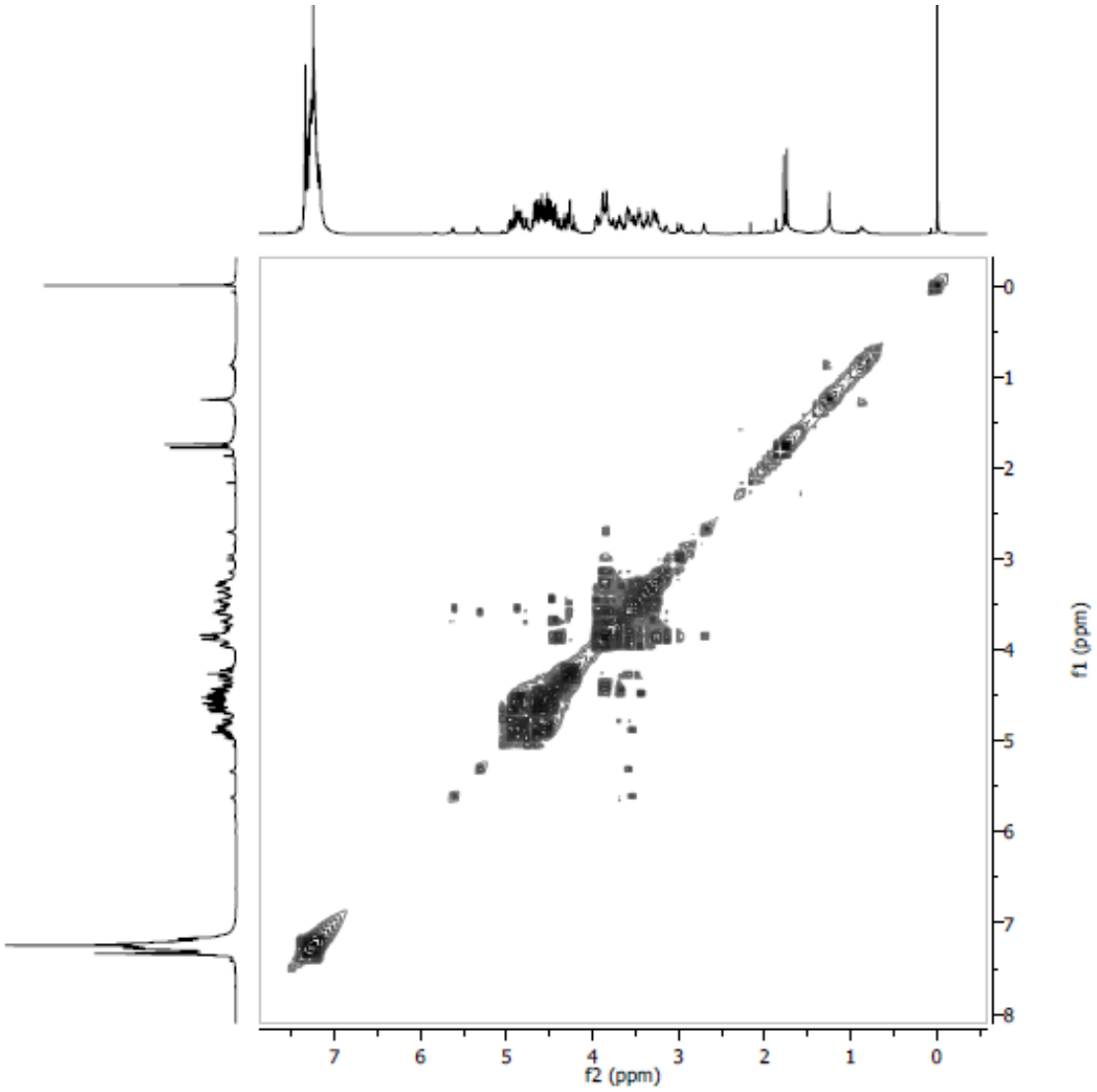


$CDCl_3$ 151 MHz

Figure A-134: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.13**)

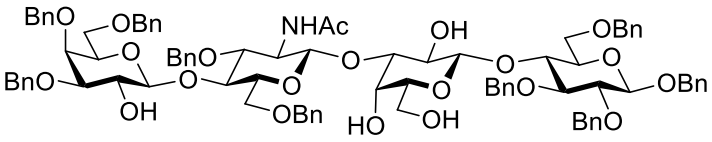


6.13

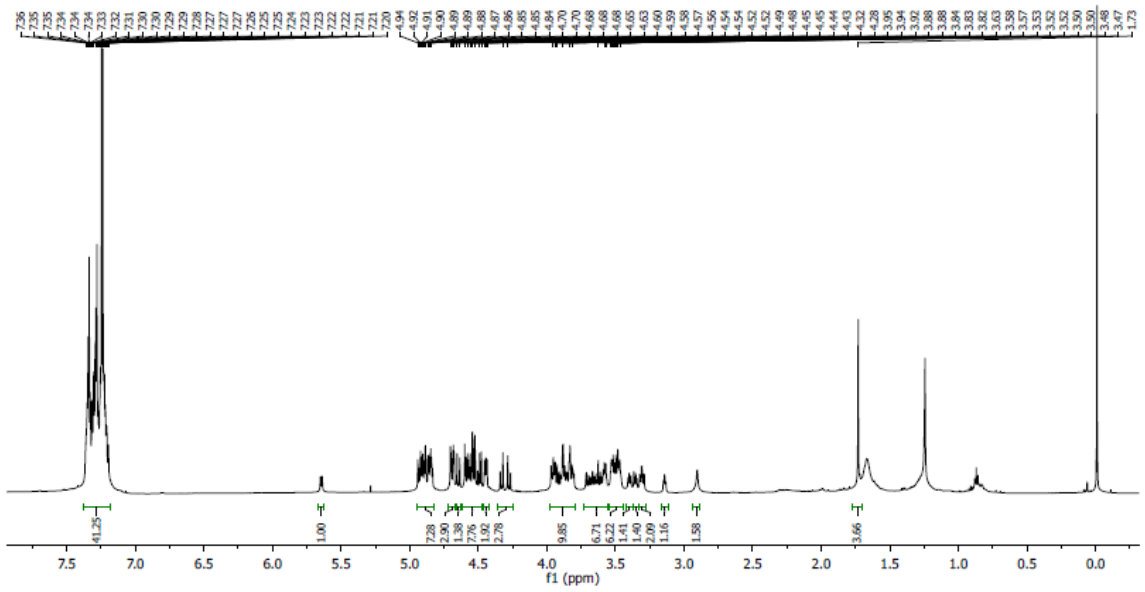


$CDCl_3$ 600 MHz

Figure A-135: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-[*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.13**)

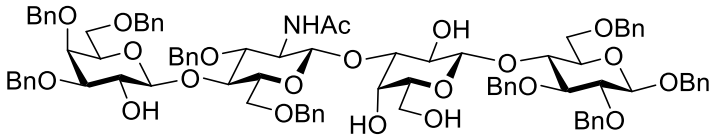


6.14

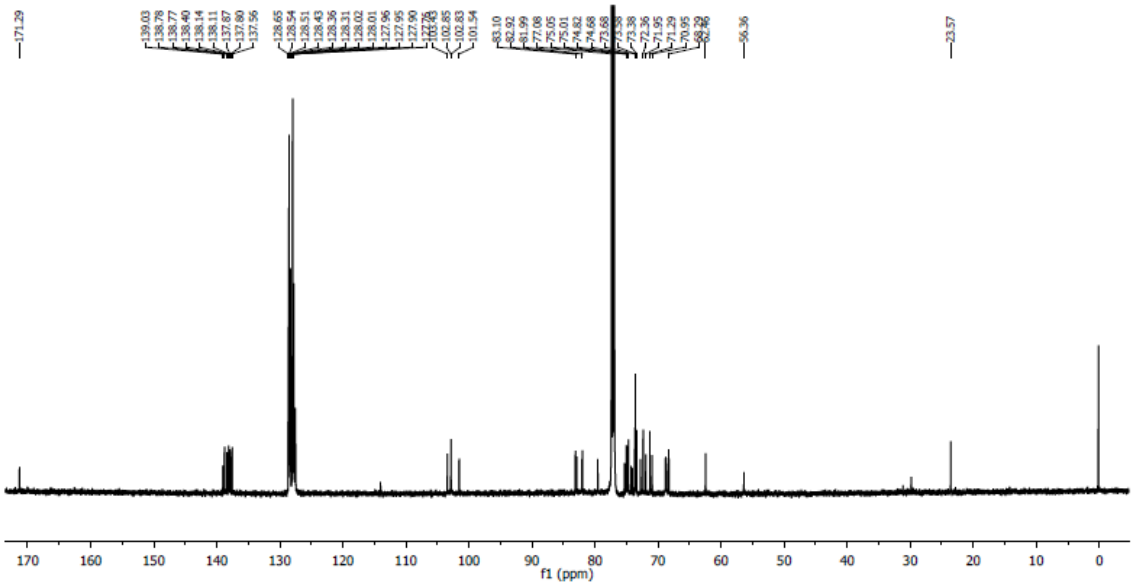


$CDCl_3$ 600 MHz

Figure A-136: ^{1}H NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.14**)

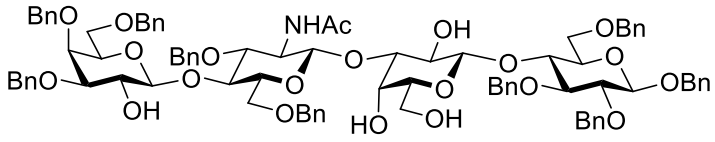


6.14

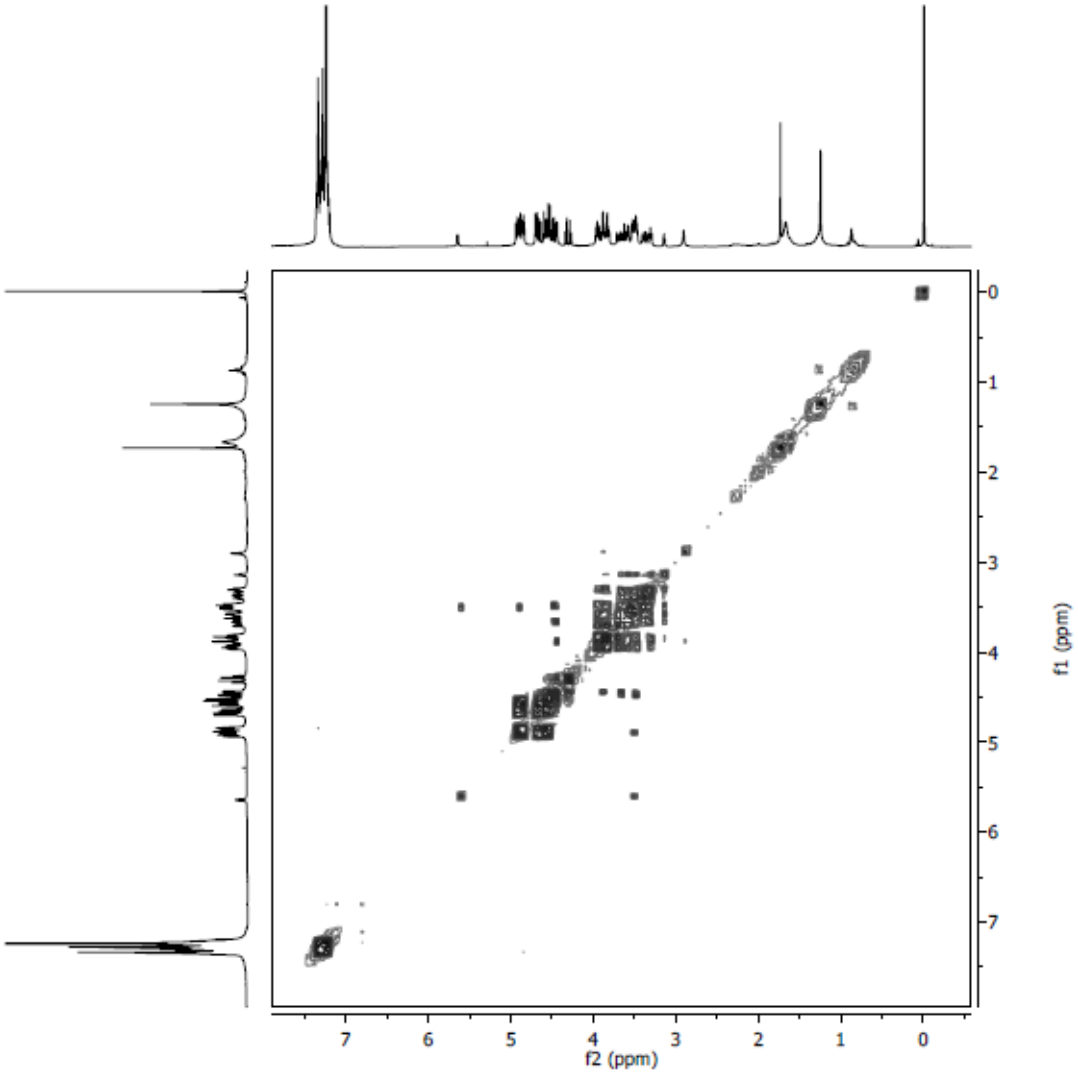


$CDCl_3$ 151 MHz

Figure A-137: ^{13}C NMR spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.14**)



6.14



$CDCl_3$ 600 MHz

Figure A-138: 2-D NMR COSY spectrum of Benzyl *O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6.14**)