Studies Toward the Chemical Synthesis of Lacto-*N*-neotetraose

By

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To my loving wife, Kate

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

2'FL	2’-fucosyllactose
Å	angstrom
Ac	acetyl
Ac2O	acetic anhydride
AcOH	acetic acid
AgOTf	silver trifluoromethanesulfonate
$AlCl_3$	aluminum chloride
app	apparent
aq.	aqueous
$BF_3{\cdot}Et_2O$	boron trifluoride diethyl etherate
$BH_3{\cdot}N(CH_3)_3$	borane trimethylamine
Bn	benzyl
BnBr	benzyl bromide
BnOH	benzyl alcohol
br	broad
Bu_2SnO	dibutyltin oxide
°C	degrees Celsius
C. jejuni	*Campylobacter jejuni*
calcd.	calculated
$CDCl_3$	chloroform-*d*
CH_2Cl_2	dichloromethane
$(CH_3)_2C(OCH_3)_2$	2,2-dimethoxypropane
$(CH_3)_2N(CH_2)_3NH_2$	*N*,*N*-dimethylaminopropylamine
CH_3CN	acetonitrile

CH_3OH	methanol
Cl_3CCN	trichloroacetonitrile
$ClAc_2O$	chloroacetic anhydride
$CuSO_4$	copper (II) sulfate
δ	chemical shift in ppm
d	doublet
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DC-SIGN	dendritic cell-specific ICAM3-grabbing non-integrin
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhancement by polarization transfer
DMAP	4-dimethylaminopyridine
DMAPA	*N*,*N*-dimethylaminopropylamine
DMF	dimethylformamide
DMM	dimethylmaleoyl
DMTST	dimethyl(methylthio)sulfonium trifluoromethanesulfonate
dt	doublet of triplets
E. coli	*Escherichia coli*
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	ethyl
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
EtSH	ethanethiol
FT-IR	Fourier transform infrared spectroscopy

Fuc	fucose
g	gram(s)
Gal	galactose
Glc	glucose
GlcNAc	*N*-acetylglucosamine
Gnd·Cl	guanidinium chloride
h	hour(s)
H_2O	water
HBr	hydrobromic acid
HCl	hydrochloric acid
$Hg(OAc)_2$	mercury (II) acetate
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation
HMO	human milk oligosaccharide
Hz	Hertz
IDCP	iodine dicollidine perchlorate
ImH	imidazole
J	coupling constant
K_2CO_3	potassium carbonate
L	liter(s)
Lac	lactose
Lev	levulinyl
Le^x	Lewis X
LG	leaving group
LNnH	lacto-*N*-neohexaose
LNnO	lacto-*N*-neooctaose

LNnT	lacto-*N*-neotetraose
LNT	lacto-*N*-tetraose
LRMS	low-resolution mass spectrum
LST	sialyl Lewis tetrasaccharide
µ	micro
m	milli, multiplet (NMR)
M	moles per liter
MeCN	acetonitrile
MeOD-d_4	methanol-*d*
MeOH	methanol
MeOTf	methyl trifluoromethanesulfonate
$MgSO_4$	magnesium sulfate
MHz	megahertz
mol	mole(s)
MS	molecular sieves
ν	wavenumber
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
$NaOCH_3$	sodium methoxide
NaOH	sodium hydroxide
NaOMe	sodium methoxide
NEC	necrotizing enterocolitis
Neu5Ac	*N*-acetylneuraminic acid
NH_2NH_2·AcOH	hydrazine acetate
NH_3	ammonia
NIS	*N*-iodosuccinimide

NMR	nuclear magnetic resonance spectroscopy
obsd.	observed
OH	hydroxyl
P	protecting group
p	pentet
Ph	phenyl
Ph_2SO	phenyl sulfoxide
$PhCH(OCH_3)_2$	benzaldehyde dimethylacetal
PhSeOTf	phenylselenyl trifluoromethanesulfonate
Phth	phthalimido
PMB	*p*-methoxybenzyl
ppm	parts per million
p-TsOH	*p*-toluenesulfonic acid
pyr	pyridine
q	quartet
RT	room temperature
$SnCl_4$	tin (IV) chloride
t	triplet
TBAB	tetra-*n*-butylammonium bromide
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
TESH	triethylsilane
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid

THF	tetrahydrofuran
$TiCl_4$	titanium (IV) chloride
TIPS	triisopropylsilyl
TIPS-Cl	triisopropylsilyl chloride
TLC	thin-layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Troc	2,2,2-trichloroethoxycarbonyl
Troc-Cl	2,2,2-trichloroethyl chloroformate
UV	ultraviolet
wt	weight

CHAPTER I

BACKGROUND AND SIGNIFICANCE

Introduction

Human milk oligosaccharides (HMOs) are a diverse class of carbohydrates that are unique to human milk. Their original discovery in the late 19th century occurred at a crossroad between pediatric findings of the benefits associated with breastfeeding and the drive for chemists to understand the roles of the abundant carbohydrates found in human milk. Pediatricians found that breast-fed infants had lower mortality rates and lower occurrences of infectious diarrhea than bottle-fed infants.[1] Furthermore, with the knowledge that intestinal bacteria affect digestion in infants, Moro[2] and Tissier[3] each independently observed differences in fecal bacterial composition between breast-fed and bottle-fed infants.

At the same time as the discoveries in microbiology and pediatrics, chemists isolated "a different type of lactose" from human milk that was not present in bovine milk.[1] Refinement of the characterization process by Polonowski and Lespagnol identified the "different lactose" as complex oligosaccharides with unknown functions.[4] Research conducted in the 1920s by Schönfeld demonstrated that the whey fraction of human milk contained a factor that promoted the growth of *Bifidobacterium bifidus.*[5] Schönfeld's findings started a renaissance in

HMO research when collaborative studies between chemist Richard Kuhn and pediatrician Paul György conclusively identified HMOs as the "bifidus factor" and thereby demonstrated that HMOs contribute to the benefits of breastfeeding.[6]

Structure

HMOs are comprised of five monosaccharide building blocks: D-galactose (Gal, **1.1**), D-glucose (Glc, **1.2**), *N*-acetylglucosamine (GlcNAc, **1.3**), L-fucose (Fuc, **1.4**), and *N*-acetylneuraminic acid (Neu5Ac, **1.5**). Structurally, HMOs contain lactose (Lac; Galβ1-4Glc, **1.6**) at the reducing end and can be elongated by the ß1-3 or ß1-6 addition of lacto-*N*-biose (Galß1-3GlcNAc, **1.7**) or *N*-acetyllactosamine (Galß1-4GlcNAc, **1.8**). Lactose and elongated oligosaccharides can be fucosylated in α1-2, α1-3, or α1-4 linkages and/or sialylated in α2-3 or α2-6 linkages.

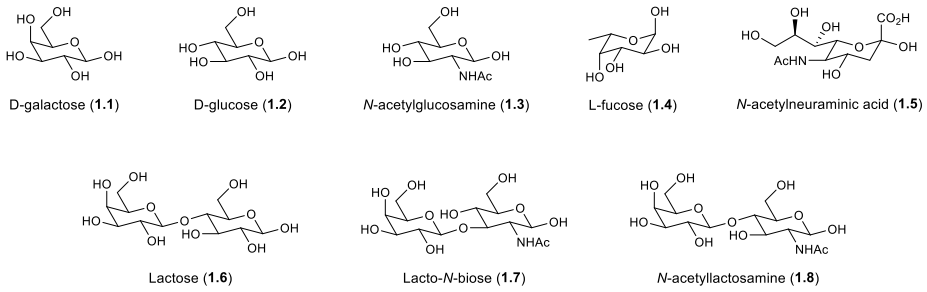

Figure 1.1. HMO constituents.

Beneficial Effects of Human Milk Oligosaccharides

Prebiotic effects

The pioneering studies by Kuhn and György established the precedent for HMOs as prebiotics. By definition, a prebiotic is an ingredient that allows specific changes in the composition or activity in the gastrointestinal microflora that provides benefits to the host's health.[7] To serve as such, the ingredient must satisfy three criteria:

1. Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption
2. Fermentation by intestinal microflora
3. Selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing

HMOs satisfy each of these criteria and serve to stimulate the growth of certain species of bifidobacteria and lactobacilli.[6a] There is substantial specificity for bacteria's ability to use HMOs as some strains, such as *Bifidobacterium longum* subspecies *infantis*, can thrive with HMOs as the singular carbohydrate source whereas others, such as *Bifidobacterium bifidum*, grow slower in their presence and produce degradation side products.[8] Furthermore, strain specificity for HMO metabolism extends even to HMO isomer variations. For example, *Bifidobacterium longum* and *Bifidobacterium breve* selectively metabolize lacto-*N*-tetraose (LNT; Galß1-3GlcNAcß1-3Galß1-4Glc, **1.9**) but not its isomer lacto-*N*-neotetraose (LNnT; Galß1-4GlcNAcß1-3Lac, **1.10**).[8b]

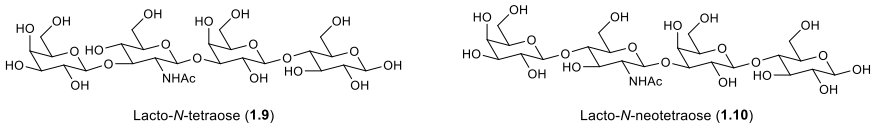

Figure 1.2. Comparative structures of lacto-*N*-tetraose and lacto-*N*-neotetraose.

Selective stimulation of gut-associated microbes creates a competitive environment wherein bacteria that benefit from HMO supplementation out-compete potentially harmful bacteria, such as *Escherichia coli* (*E. coli*) for nutrients. HMO fermentation by bifidobacteria and lactobacilli also results in the production of short-chain fatty acids which inhibit pathogen colonization by acidifying the intestinal environment.[9]

Antiadhesive antimicrobial effects

The selective metabolism of only approximately 10% of all HMOs suggests that HMOs have roles apart from their prebiotic effects.[10] The ability of many gut-associated pathogens, such as *E. coli*, *Campylobacter jejuni* (*C. jejuni*), and *Salmonella* strains, to cause illness is often dependent upon the microbes' ability to adhere to the epithelial surface of their host. Adherence is often mediated by lectin-glycan interactions wherein microbial lectins bind to glycans expressed on the host epithelium.[11] Because HMOs can have similar structures to epithelial glycans, they are able to deter pathogenic adhesion by serving as soluble, competitive ligands for the binding proteins. With no epithelial binding event, the bacteria are safely expelled from the body through the feces. The prevention of infection due to adhesion

deterrence is exemplified in *C. jejuni*, one of the world's major causes of diarrheal infection. *C. jejuni* binds to type 2 H-antigens (**1.11**), which are α1-2 fucosylated lactosamine residues. The presence of α1-2 fucosylated HMOs such as 2'-fucosyllactose (2'FL; Fucα1-2Galß1-4Glc, **1.12**) has been shown to inhibit *C. jejuni* binding to human epithelial mucosa *ex vivo* and an inverse correlation has been shown to exist between the concentration of 2'FL in breastmilk with the incidence of infant diarrhea.[12]

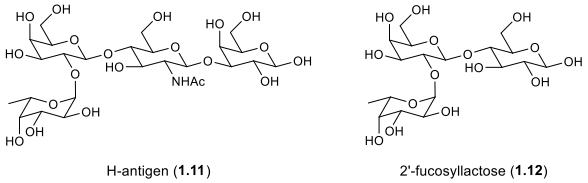

Figure 1.3. Comparative structure of H-antigen and 2'FL.

Instead of expressing lectins to bind to epithelial glycans, some microorganisms, such as viruses, express glycans to bind to host lectins. This glycan expression is true in the case of HIV. For HIV transmission across a mucosal membrane, the viral envelope glycoprotein gp120 must bind to DC-SIGN on human dendritic cells.[13] Although DC-SIGN binds high-mannose type glycans on gp120, it has a higher affinity for Lewis blood group antigens.[14] HMO expression mirrors the blood group antigen expression of the mother and, as a result, the high effective concentration of blood group antigens from HMOs can saturate DC-SIGN and inhibit mother-to-child HIV transmission.[1, 15]

Other associated benefits

HMOs have been associated with additional benefits that contribute to an infant's overall wellness and development. These include the abilities to modulate the immune system, alter surface glycan expression, and potentially supplement brain development. Immune system modulation is postulated to occur through multiple mechanisms. In 2004, *in vitro* experiments demonstrated that cord blood-derived T cell exposure to sialylated HMOs caused a shift from Th2 cytokine production to Th1 cytokines.[16] These observations, and the fact that approximately 1% of HMOs reach systemic circulation, provide evidence that HMOs may help balance T-cell differentiation and regulate low-level immunity.[1, 17]

Similar to their ability to inhibit pathogen adhesion, HMOs can reduce rolling leukocyte adhesion by binding to selectins—epithelial lectins that bind sialylated Lewis antigens.[18] Although leukocyte adhesion is critical for innate immunity, these findings may have implications in preventing or deterring the onset of necrotizing enterocolitis (NEC). NEC is an often fatal disorder among preterm infants and is partially characterized by significant increases in neutrophil activation and infiltration.[19] It has been observed that breast-fed infants have a lower risk of developing NEC and studies in rat models have provided evidence that HMOs may be the cause of these preventative effects.[20]

Apart from directly binding lectins to alter adhesion, HMOs can indirectly regulate microbe-host interactions by altering the expression of cell surface glycans. In a 2005 study by Angeloni and coworkers, it was found that exposure of Caco-2 intestinal cells to 3'sialyllactose resulted in the reduced expression of cell surface α2-3- and α2-6-linked sialylated glycans.

Further studies demonstrated that the reduced glycan expression was translatable to a reduction in pathogen adhesion and provided an observable 90% reduction of enteropathogenic *E. coli*.[21]

Evidence suggests that HMOs may provide dietary sialic acid in the form of Neu5Ac as an essential nutrient in brain development.[1, 22] It is understood that brain development depends on sialic acid-containing gangliosides and glycoproteins and that concentrations of sialic acid more than double within the first few years of life.[23] Though it remains generally unknown, because human milk contains a high concentration of sialylated oligosaccharides, it is possible that HMOs serve as the sialic acid source rather than glycolipids or glycoproteins.[24]

HMOs offer a myriad of benefits for infants, some of which may still need discovery. The difficulty in isolating appreciable amounts of single-entity oligosaccharides limits the depth of which their roles can be understood. It has therefore become necessary, and the aim of several research groups, to access these complex oligosaccharides through chemoenzymatic and synthetic means.

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CHAPTER II

CHEMICAL SYNTHESIS OF HUMAN MILK OLIGOSACCHARIDES

Introduction

Until groundbreaking discoveries in the 1970s, carbohydrates were considered a class of biomolecules of minimal importance and were viewed primarily as an energy source.[1] Following the discovery of sugar-nucleotide compounds and the increased understanding of polysaccharide biosynthesis, the importance of glycoconjugates and their oligosaccharide components were realized.[2] While glycoconjugate functions include facilitation of immune responses[3] and cellular recognition[4], glyconjugates are also associated with disease states such as tumor progression and metastasis.[4-5] The drive to understand these associations has compelled chemists toward the structure elucidation and preparation of oligosaccharides.

The chemical synthesis of carbohydrates is notably complex and the complexity is exacerbated by the necessity to join glycosidic units in both regio- and stereoselective manners.[6] Although no general reaction conditions exist for comprehensive oligosaccharide synthesis, contemporary methods allow for high-yielding production of oligosaccharides. Many of the oligosaccharides, as will be later reviewed, are HMOs.

The Glycosylation Reaction

The glycosylation reaction is the most fundamental, and often most experimentally challenging, reaction in carbohydrate chemistry. In the reaction, a glycosidic bond is formed between the anomeric center of a glycosyl donor and a nucleophilic moiety of a glycosyl acceptor. To ensure high levels of regio- and stereoselectivity, extensive protecting group manipulations are often employed to yield an acceptor with a single deprotected nucleophilic site, most commonly a free alcohol.[6]

The mechanism for a glycosylation reaction most generally favors a unimolecular S_N1 mechanism. A glycosyl donor bearing a latent leaving group (**2.1**) is activated in the first step of the reaction and displacement of the group results in the formation of an oxocarbenium ion (**2.2**). As the intermediate oxocarbenium ion is sp^2 hybridized, a potential problem arises in achieving stereoselectivity. The geometry of the intermediate makes nucleophilic attack theoretically possible from both the top (*trans*, ß- for the D-gluco series, **2.3a**) and bottom (*cis*, α-, **2.3b**) faces.[7]

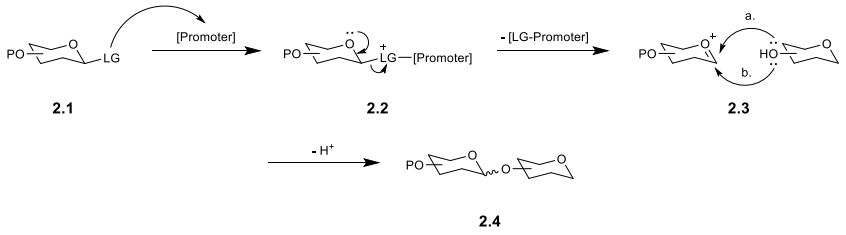


Figure 2.1. Glycosylation mechanism.

Stereoselectivity to yield a 1,2-*trans* glycosidic linkage is most often imparted via neighboring participation, generally by an acyl moiety (**2.6**).[8] These glycosylations proceed through an acyloxonium ion (**2.7**) formed by intramolecular stabilization of the oxocarbenium ion. The bicyclic intermediate blocks nucleophilic attack from the bottom face, giving rise to the 1,2-*trans* product (**2.7a**). In glycosylations using a C-2 acetyl participating group, potentially undesirable orthoester formation is observed occasionally as the result of nucleophilic attack on the acyloxonium species (**2.7b**). Formation of this product may be attributed to several causes including the use of strongly acidic conditions, non-polar solvents, and sterically hindered acceptors.[9] Isolation of orthoacetates (**2.9**) is generally inconsequential as subjection of the orthoacetate to acidic conditions can effect rearrangement to the glycosidic product[10] or hydrolysis to recoverable starting materials.[9b]

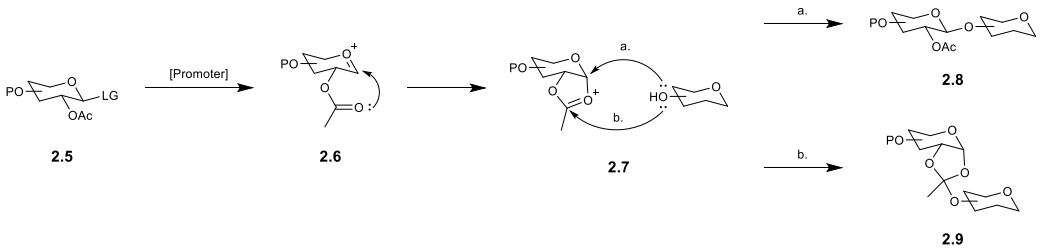
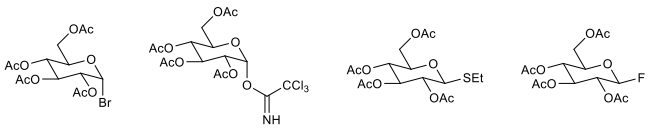


Figure 2.2. Neighboring group participation and orthoester formation.

Glycosyl Donors

Achieving high yields and good anomeric selectivity depends greatly on glycosyl donor selection. Traditionally, glycosylation methods used anomeric halides (**2.10**) as donors.[1, 11] The instability of these compounds and the harsh reaction conditions required for their preparation, however, lead to the development of newer methodologies in the 1970s and 1980s. Those methods lead to some of the most commonly used donors today[7]: trichloroacetimidates (**2.11**),[12] thioglycosides (**2.12**),[13] and glycosyl fluorides (**2.13**).[14] The glycosylations featured in this work used trichloroacetimidate and thioglycoside donors. These donors, therefore, will be discussed in greater depth.



2.10 2.11 2.12 2.13

Figure 2.3. Representative glycosyl donors.

Trichloroacetimidate Donors

Imidates were among the first donors developed as alternatives to glycosyl halides.[6, 15] The trichloroacetimidate, as developed by Schmidt and coworkers, was introduced as a facile method to synthesize α or ß donors from unprotected lactols.[16] Their preparation is facilitated by base-catalyzed addition of trichloroacetonitrile to the free anomeric alcohol. Base selection is key in determining the yield of α or ß products.[17] The use of sodium hydride or cesium carbonate favors formation of the thermodynamic α-glycosyl imidate whereas potassium carbonate favors the kinetic ß-product. DBU is also a frequently used base in the production of trichloroacetimidates and generally favors α-products.[18]

Activation of trichloroacetimidates (Figure 2.4a) occurs in the presence of catalytic amounts of Brønsted or Lewis acid, with the most common Lewis acids being TMSOTf and $BF_3{\cdot}Et_2O$.[18] Anomeric selectivity is influenced by the anomeric configuration of the donor, neighboring group participation, solvent effects, and thermodynamic or kinetic effects. The relatively high reactivity of trichloroacetimidates in glycosylation reactions can lead to side reactions or donor decomposition. In the presence of a weakly nucleophilic acceptor,

trichloroacetimidates can undergo a Chapman rearrangement (Figure 2.4b) to yield the corresponding trichloroacetamide (**2.19**).[19] The trichloroacetamide is not reactive under glycosylation conditions, but its formation may be circumvented by the use of *N*-phenyl trifluoroacetimidates.[20]

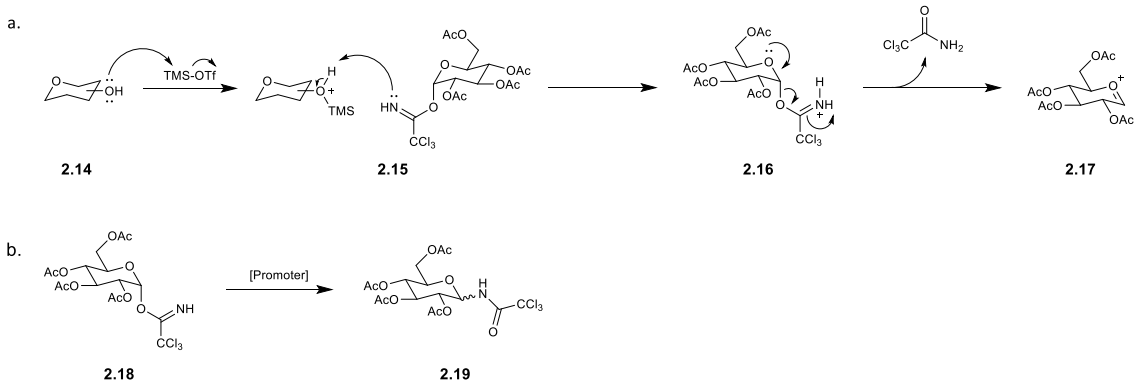


Figure 2.4. Activation of trichloroacetimidates (a) and the Chapman rearrangement (b).

Thioglycoside Donors

Due to their high chemical stability and facile activation in glycosylations, thioglycosides are another commonly used donor.[13a] Several methods exist for the formation of thioglycosides with the most frequently employed route being the Lewis acid-mediated thiolysis of peracetylated sugars.[21] Multiple Lewis acid catalysts have been reported for this method including $BF_3{\cdot}Et_2O$,[22] $SnCl_4$,[23] TMSOTf,[22a] and $TiCl_4$.[24] Thioglycosides are activated in

glycosylations through a variety of electrophilic promotors. The first report to use thioglycosides directly in glycosylations was by Ferrier and coworkers using $Hg(OAc)_2$ to form methyl glycosides from the corresponding phenyl thioglycosides.[25] Because of inconsistent yields when using heavy metal salts,[21] newer promoter systems were devised. Some of the most commonly used reagents include DMTST,[26] NIS/TfOH, NIS/TMSOTf,[27] MeOTf,[24a] IDCP,[28] PhSeOTf,[29] and Ph_2SO/Tf_2O.[30]

Anomeric control with thioglycosides generally depends on the presence of a C-2 participating group or solvent selection. In the presence of a participating group, 1,2-*trans* linkages will be favored while, in their absence, a mixture of anomers typically results. Participating solvents can also assist in directing anomeric selectivity (Figure 2.6). Diethyl ether favors formation of α-glycosides, because a ß-diethyl oxonium ion (**2.21**) is formed in complexation with the anomeric oxocarbenium ion.[21] In contrast, acetonitrile favors ß-product formation and proceeds through an α-nitrilium ion intermediate (**2.22**).[31] Thioglycosides, in addition to being tunable towards stereoselectivity, are amenable to reactivity and chemoselectivity tuning through application of arming and disarming principles.

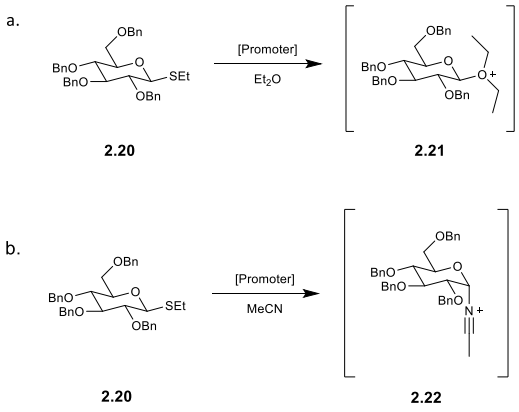


Figure 2.5. Solvent participatory effects.

Armed/Disarmed Principles

As alluded to in his 1982 review, "*Advances in Selective Chemical Syntheses of Complex Oligosaccharides*", Hans Paulsen first recognized that chemical glycosylations were a challenge that could not be met with universal conditions.[1] Though his original reported observations were cryptic, he later clarified that glycosylation reactions require a "match" between the coupling donor and acceptor to facilitate success. This concept, having been observed experimentally by Fraser-Reid and coworkers, was elaborated on and refined to develop the theory of "armed" and "disarmed" in glycoside synthesis.[32]

The initial theories relied on the observations that electron-withdrawing and electron-donating substituents on carbohydrates had a substantial effect on the rate and success of a glycosylation. In particular, electron-withdrawing acyl groups slowed, or even ceased, the progression of a reaction, whereas electron-donating alkyl groups increased the success of

reaction.[33] The electronic arming and disarming effects were compounded in 1991 when the Fraser-Reid group reported the ability of acetal protecting groups to exert torsional disarming effects.[34] These effects were attributed to the increased ring strain incurred when forming an oxocarbenium ion.

Armed and disarmed strategies are often used in the optimization of donors and acceptors. The judicious choice of protecting groups works to ensure proper reactivity and efficiency throughout multi-step syntheses. It is important to bear in mind while selecting protecting groups that the groups remain compatible throughout the continuing transformations. This compatibility signifies the importance of protecting group orthogonality.

Protecting Group Orthogonality

Orthogonal protection is commonly employed in organic syntheses to ensure that protecting groups can be selectively modified without affecting other functionalities.[35] Orthogonality is imparted through the use of protecting groups that are labile under differing conditions. For example, acetyl groups are base-labile and exhibit orthogonality to acetals which are hydrolyzed under acidic conditions. Benzyl ethers, on the other hand, are stable to both acidic and basic conditions and serve as a more permanent protecting group which can be removed toward the end of a synthesis via hydrogenolysis.

Orthogonal protecting groups in oligosaccharide synthesis allow for regioselective glycosylations by masking functional groups to prevent undesired products. When designing a synthesis, it is also important to consider the properties of the protecting group to promote the desired level of arming or disarming. Electronic and strain effects can be exploited to promote active-latent[36] and sequential one-pot glycosylations[37] but can also severely attenuate reactivity leading to minimal product formation. This project sought to apply an orthogonal protection approach in the synthesis of HMOs. The orthogonal design of the target tetrasaccharide, lacto-*N*-neotetraose, enables the future production of pentasaccharides and higher-order HMOs.

Strategies in the Synthesis of HMOs

The chemical synthesis of HMOs has garnered a noteworthy level of attention. As the most abundant tetrasaccharide structures in human milk, several research groups have synthesized LNT (**1.9**) and LNnT (**1.10**) as deprotected saccharides through chemical[37c, 38] and chemoenzymatic[39] means. LNnT has also served as a key intermediate in the syntheses of the Lewis pentasaccharides,[40] Le^X glycosphingolipids,[41] and higher-order oligosaccharides.[42]

A notable synthesis of LNnT was conducted in 1999 by Schmidt and coworkers[38d] to demonstrate the practical use of the dimethylmaleoyl (DMM)[43] protecting group. The synthetic procedure was furthered in 2000 in their syntheses of lacto-*N*-neohexaose (LNnH, **2.23**) and

lacto-*N*-neooctaose (LNnO, **2.24**).[42a] To demonstrate synthetic strategies in HMO synthesis, these syntheses will be discussed in greater detail.

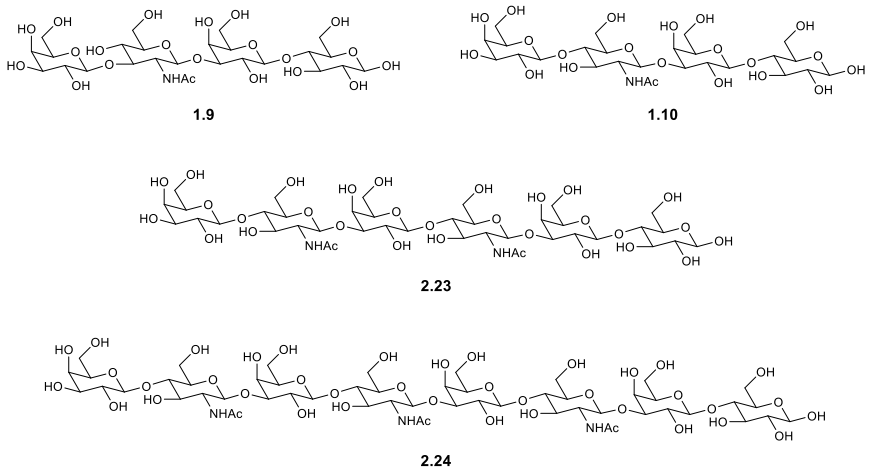
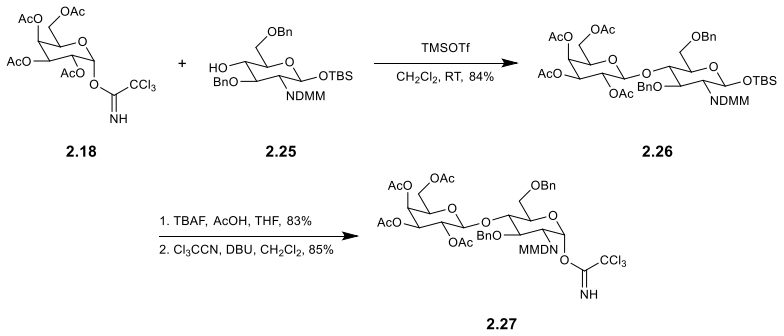


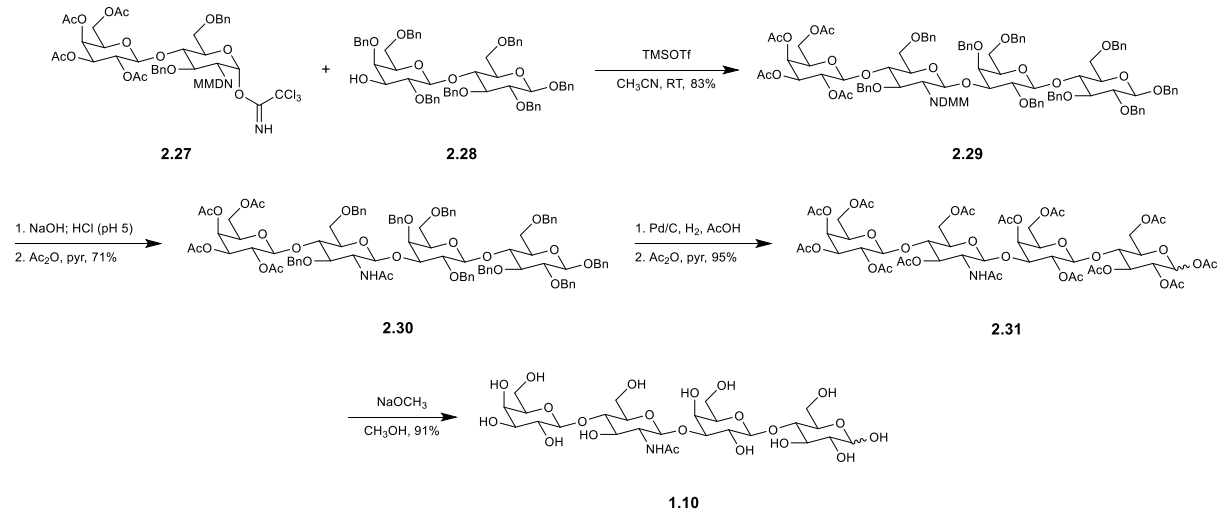
Figure 2.6. LNT, LNnT, & higher-order HMOs.

Syntheses of LNnT and its derivatives prior to 1999 were consistent in the glycosylation strategy of joining two disaccharides, an *N*-acetyllactosamine derivative and a lactose derivative.[44] Correspondingly, Schmidt's analysis envisioned a final linkage of DMM-protected lactosamine to a known perbenzylated lactosyl acceptor.[45] Synthetic studies toward lactosamine residue **2.27** were initiated through the glycosylation between galactosyl imidate[17] **2.18** and DMM-protected acceptor[43] **2.25** under catalytic treatment with TMSOTf to yield the ß-linked disaccharide in 84% yield. Subsequent TBS cleavage with TBAF[46] gave the anomeric alcohol, which was then converted to trichloroacetimidate donor **2.27**.



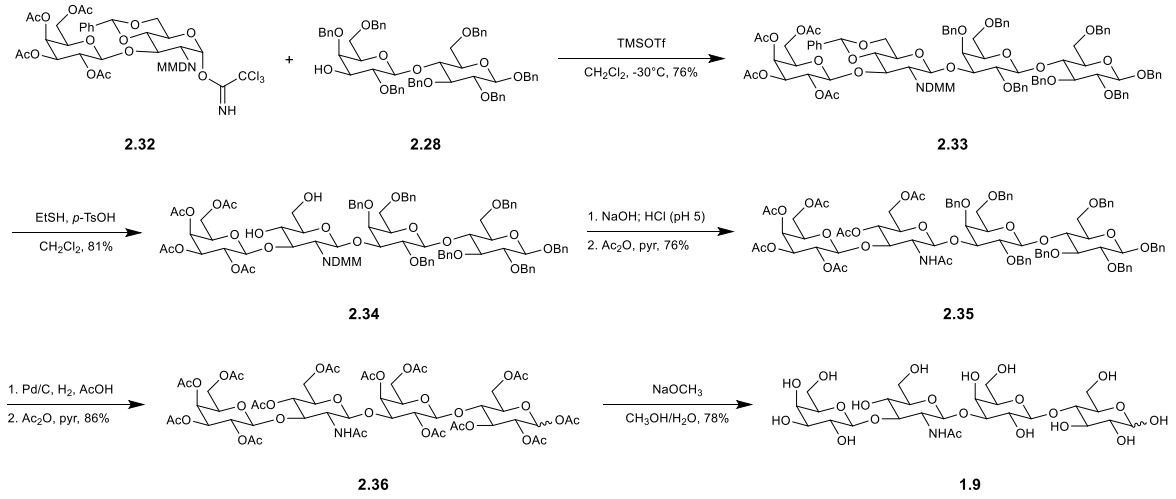
Scheme 2.1. Schmidt's lactosamine synthesis.

Synthesis continued with the glycosylation of donor **2.27**and acceptor **2.28** to yield tetrasaccharide **2.29** in 83% yield. ^{13}C NMR analysis confirmed the structure and ß-anomeric configurations by the presence of four signals at δ 100.2, 100.7, 102.7, and 102.8. Deprotection of **2.29** began by removal of the DMM group with NaOH followed by treatment with HCl to cleave the presumed butenolide intermediate. Acetylation of the liberated amine gave **2.30** in 71% yield. Sequential hydrogenolysis and acetylation yielded peracetylated compound **2.31** as a 3:2 α:ß mixture of anomers. The synthesis was completed by deacetylation[11b] to give **1.10** in 91% yield.



Scheme 2.2. Schmidt's completion of LNnT.

In the same report as their synthesis of LNnT, the Schmidt group reported using the DMM in the synthesis of LNT. Again coupling two disaccharides, known donor[43] **2.32** and acceptor **2.28** were treated with TMSOTf to give protected tetrasaccharide **2.33** in 76% yield. The benzylidene acetal was cleaved with *p*-TsOH and EtSH[47] to give **2.34**. Conversion of the DMM group to an acetyl was achieved via saponification and acidification followed by acetylation, yielding **2.35** in 76%. Catalytic hydrogenation of **2.35**, followed by peracetylation gave **2.36** as a 1:2 α:ß mixture of anomers. Deacetylation with sodium methoxide gave **1.9** in 78% yield.



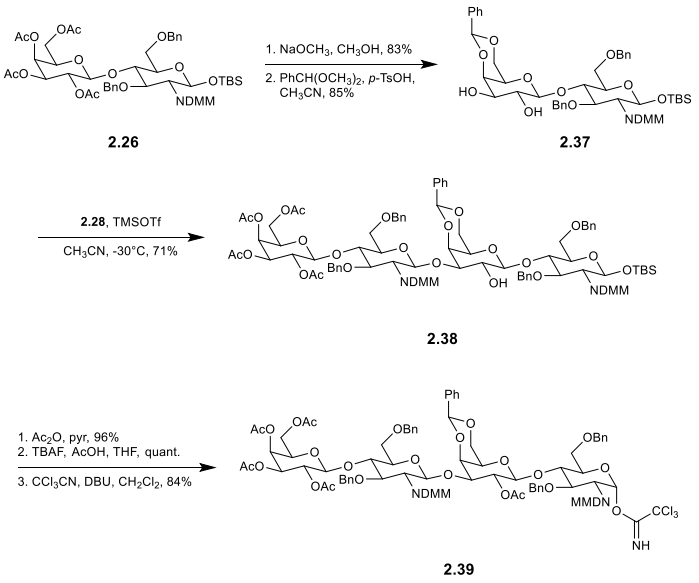
2.32 **2.28** **2.33**

2.34 **2.35**

2.36 **1.9**

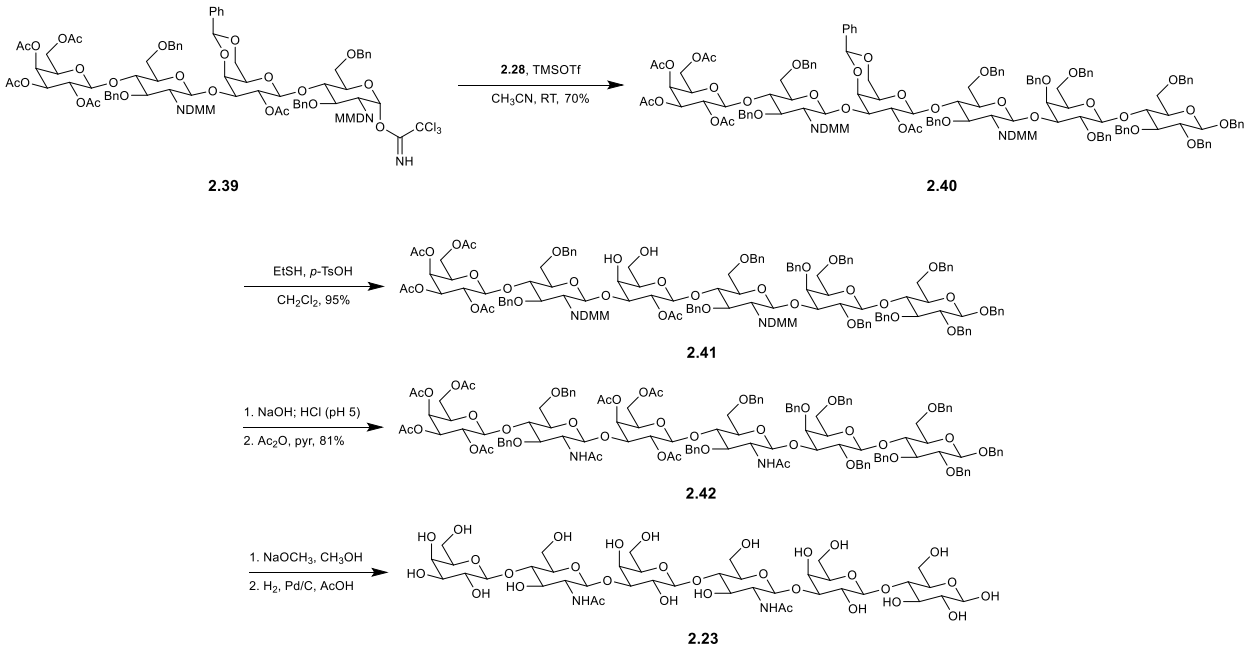
Scheme 2.3. Schmidt's synthesis of LNT.

Slight modification of lactosamine donor **2.27** permitted the Schmidt group to continue their syntheses of HMOs in the production of LNnH and LNnO.[42a] Lactosamine[38d] **2.26** was treated with sodium methoxide and the resulting tetraol was converted to benzylidene acetal **2.37**. TMSOTf-mediated glycosylation of **2.37** with trichloroacetimidate donor **2.27** regioselectively yielded tetrasaccharide **2.38**. The C-2' alcohol was acetylated and subsequent desilylation with TBAF in AcOH and reaction with Cl_3CCN and DBU gave donor **2.39**.



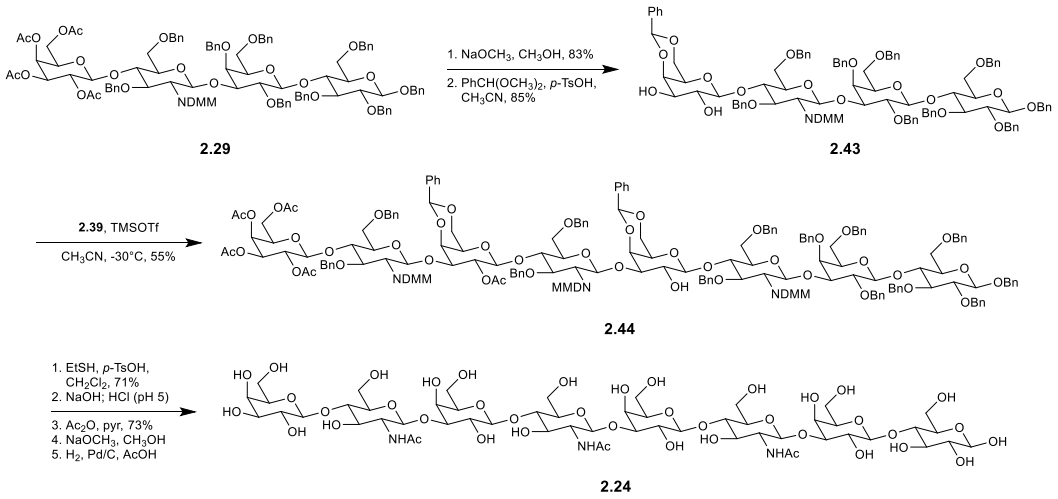
Scheme 2.4. Schmidt's synthesis of tetrasaccharide donor **2.39**.

Tetrasaccharide **2.39** and lactosyl acceptor **2.28** were glycosylated to yield hexasaccharide **2.40** in 70% yield. Treatment of **2.40** with EtSH and *p*-TsOH cleaved the benzylidene acetal to give diol **2.41**. The DMM groups were removed and the amines were acetylated to give **2.42** in 81% yield. Deacetylation and hydrogenolysis gave LNnH **2.23**, which was then peracetylated for structural assignment.



Scheme 2.5. Schmidt's completion of LNnH.

Synthesis of **2.23** provided the requisite intermediates for the facile synthesis of LNnO (**2.24**). Beginning with protected tetrasaccharide[48] **2.29**, sequential deacetylation and benzylidene acetal formation gave acceptor **2.43**. **2.43** was glycosylated with tetrasaccharide donor **2.39**, to yield octasaccharide **2.44** in 55%. Deprotection proceeded via cleavage of the benzylidene acetal and DMM groups, N-acetylation, deacetylation, and hydrogenolysis to give **2.24**. The octasaccharide was peracetylated in 83% yield for structural characterization.



Scheme 2.6. Schmidt's completion of LNnO.

Conclusion

Oligosaccharide syntheses are complex problems for which there is no single solution. Several factors must be considered when planning a synthesis to minimize synthetic transformations and control reactivity and selectivity. With all aspects well-accounted for, a well-executed carbohydrate synthesis can exemplify elegance and promote future studies within the field.

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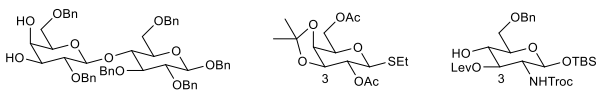
CHAPTER III

INVESTIGATIONS IN THE SYNTHESIS OF LACTO-*N*-NEOTETRAOSE

Synthetic Analysis and Early Investigations

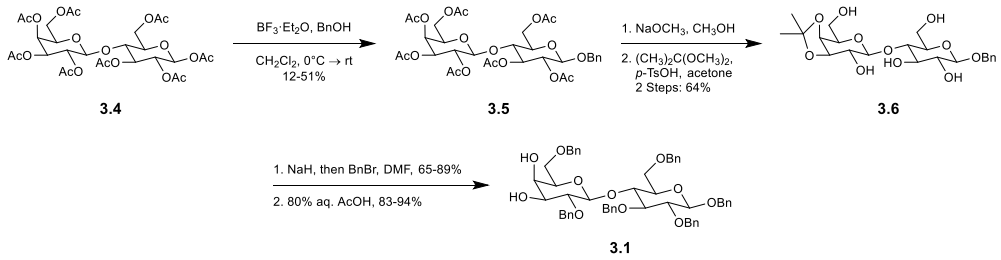
Consistent with earlier syntheses,[1] our synthesis of LNnT involved the joining of two disaccharide units: a protected lactosamine donor and known lactose acceptor **3.1**.[2] We envisioned the lactosamine donor to be the glycosylation product of a galactosyl donor and a selectively protected glucosamine acceptor. The glucosamine acceptor was designed to incorporate an anomeric group amenable to conversion into a donor. Our initial synthetic strategy used known galactosyl thioglycoside[3] **3.2** featuring a 3,4-acetonide which could be selectively hydrolyzed to ensure access to the C3 position. We also sought to use an orthogonally protected glucosamine acceptor[4] **3.3** to further facilitate modification at the glucosamine C3 upon removal of the Lev group.[5]



3.1 **3.2** **3.3**

Figure 3.1. Initial target donors and acceptors.

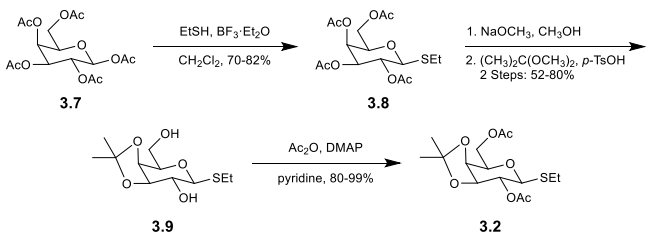
Synthetic efforts towards LNnT began with the production of lactose acceptor **3.1**.[2] Beginning with lactose octaacetate **3.4**, Lewis acid mediated glycosylation with benzyl alcohol yielded benzyl lactoside **3.5**.[6] Lactoside **3.5** was then deacetylated and subjected to acetonide-forming conditions, primarily resulting in the thermodynamic 3',4'-acetonide **3.6**. Perbenzylation of **3.6** followed by acidic hydrolysis of the acetonide gave lactose acceptor **3.1**.[2b, 7]



3.4 **3.5** **3.6** **3.1**

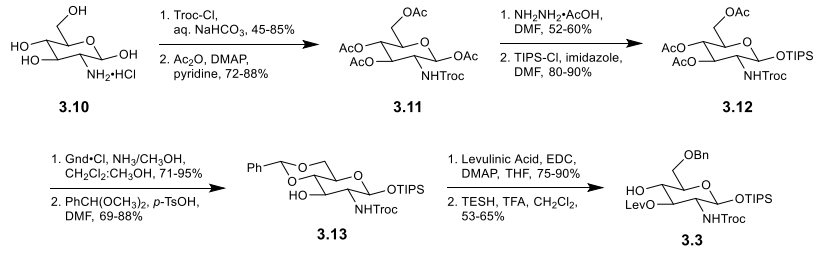
Scheme 3.1. Synthesis of lactose acceptor **3.1**.

Following completion of acceptor **3.1**, focus was shifted towards the synthesis of donor **3.2**.[3] Galactose petaacetate **3.7** was converted to ethyl thiogalactoside **3.8** by treatment with $BF_3{\cdot}Et_2O$ and ethanethiol.[8] Deacetylation[9] followed by 3,4-acetonide formation using 2,2-dimethoxypropane gave diol **3.9**. Final acetylation of **3.9** yielded donor **3.2**.



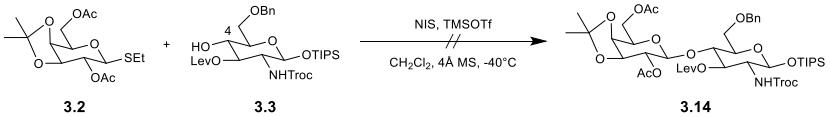
Scheme 3.2. Synthesis of donor **3.2**.

Finally, acceptor **3.3** was synthesized in eight steps starting from glucosamine hydrochloride **3.10**.[5] Aqueous Troc protection followed by peracetylation gave tetraacetate **3.11**,[10] which was then anomerically deacetylated[11] and silylated[12] to give TIPS glycoside **3.12**. **3.12** was then deacetylated[13] and protected as a benzylidene acetal[14] to yield **3.13**. Esterification with levulinic acid[15] and regioselective triethylsilane-mediated cleavage of the acetal[16] yielded acceptor **3.3**.



Scheme 3.3. Synthesis of acceptor **3.3**.

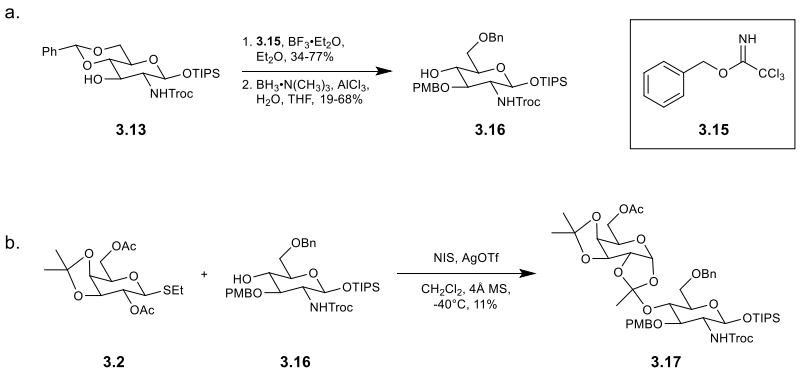
Glycosylation attempts to yield lactosamine **3.14** were largely unsuccessful and resulted in donor hydrolysis and acceptor recovery. We attributed these unsuccessful glycosylations to the weak nucleophilicity of the C4 alcohol on acceptor **3.3**. It was deemed possible that the Lev ester contributed an electron withdrawing effect, thereby decreasing the molecule's strength as an acceptor. To overcome this challenge, a PMB group was substituted at C3 to enhance nucleophilicity through electron donation.



3.2 **3.3** **3.14**

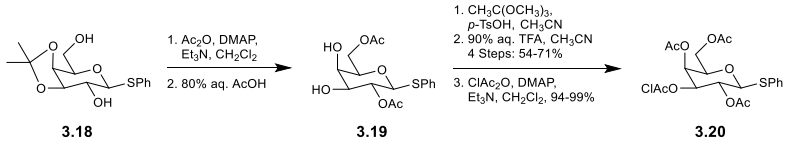
Scheme 3.4. Glycosylation attempts toward lactosamine **3.14**.

To synthesize acceptor **3.16**, **3.13** was PMB protected[17] and the benzylidene acetal was regioselectively cleaved.[18] Acidic sensitivity of the PMB ether resulted in only moderate yields of the desired acceptor. Glycosylations with this new acceptor did prove, however, to be slightly more successful, though yielding only the undesired orthoester (Scheme 3.5b).



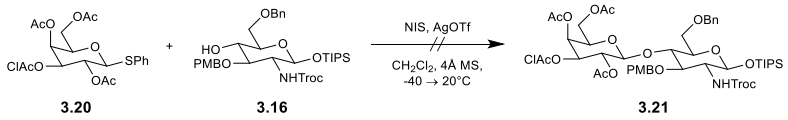
Scheme 3.5. Synthesis of acceptor **3.16** (a) and its use in glycosylation (b).

Because orthoester formation could be a solvable problem when sufficient quantities of material were obtained,[19] the low yields of the glycosylation were first addressed. Analysis of donor **3.2** revealed a potential disarming effect due to the acetonide.[20] To address this issue, a similar phenyl thioglycoside (**3.18**) was first prepared. Thioglycoside **3.18** was acetylated and the acetonide was hydrolyzed, revealing diol **3.19**. A two-step protocol involving orthoester formation followed by acidic hydrolysis gave selective acetylation at C4. Chloroacetylation at C3 gave donor **3.20** in high yields.



Scheme 3.6. Synthesis of donor **3.20**.

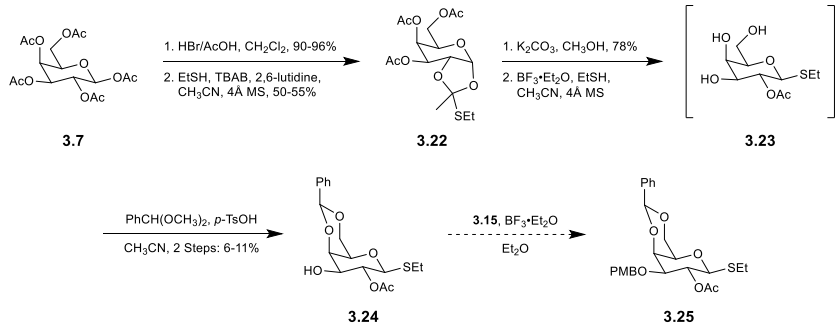
Glycosylation was attempted with donor **3.20** and acceptor **3.16**. Disappointingly, though not entirely unexpected, the modifications made to donor **3.2** decreased its reactivity due to electron withdrawing effects in spite of the decrease in torsional strain.



Scheme 3.7. Glycosylation attempts using donor **3.20**.

The galactosyl donor was redesigned to incorporate functionalities promoting electronic arming and neighboring group participation. Drawing inspiration from Fraser-Reid's work with *n*-pentenyl orthoesters[21] and previously demonstrated research in the formation and rearrangement of orthothioesters,[22] a synthetic scheme was devised through which a neighboring participating group could be maintained at C2 while permitting selective modification at C3, C4, and C6.

Beginning with **3.7**, conversion to the glycosyl bromide[23] and subsequent treatment with ethanethiol, TBAB, and 2,6-lutidine[22a, 22c] gave orthothioacetate **3.22**. **3.22** was deacetylated and the resulting triol was rearranged under Lewis acidic conditions[22b, 24] to yield intermediate **3.23**, which was converted *in situ* to the corresponding benzylidene acetal **3.23**. Low yielding production of **3.24** revealed that the synthesis of **3.25** would prove unfeasible. Though discouraging, this realization was concurrent with another critical finding.

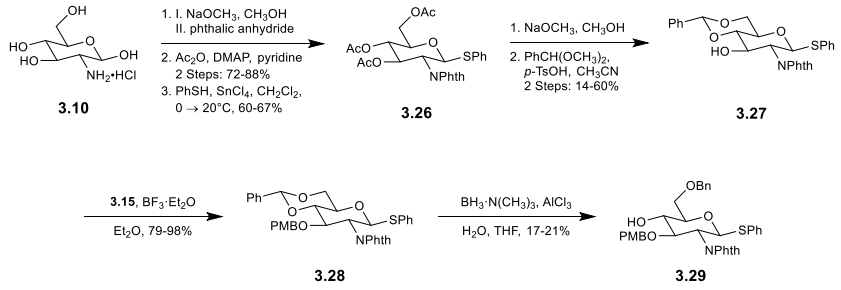


Scheme 3.8. Synthesis of donor **3.25**.

Contemporary Investigations

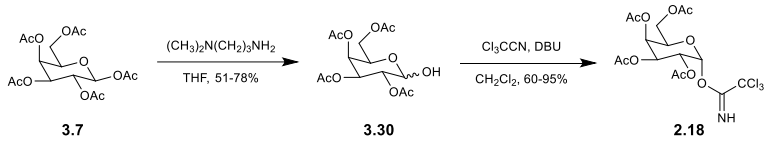
It has long been understood that the C4 alcohol of *N*-acetylglucosamine suffers from poor nucleophilicity.[25] This lack of reactivity has hampered the synthesis of biologically important glycoconjugates in which glycosidic linkages involve the C4-OH of *N*-acetylglucosamine are of critical importance.[26] Numerous strategies have been developed to overcome this low reactivity with the most successful being the use of 2-azido-2-deoxyglucopyranosides and *N*-phthalamidoglucosaminopyranosides. Reactivity studies conducted by David Crich and coworkers in 2001 investigated glycosylations with differentially protected glucosamine derivatives.[27] NMR investigations revealed that partially protected glucosamine derivatives containing an N-H bond contributed to an intramolecular hydrogen bond with the C4 oxygen atom. Despite reported success with Troc-protected glucosamine acceptors,[4c, 5] acceptor **3.16** was redesigned to incorporate phthalimide protection.

Starting from **3.10**, a three step protocol afforded phenyl thioglycoside **3.26**.[28] **3.26** was deacetylated and converted to benzylidene acetal **3.27** followed by PMB protection of the remaining alcohol to give **3.28**. Lastly, reductive acetal cleavage of PMB glycoside **3.28** yielded acceptor **3.29**.



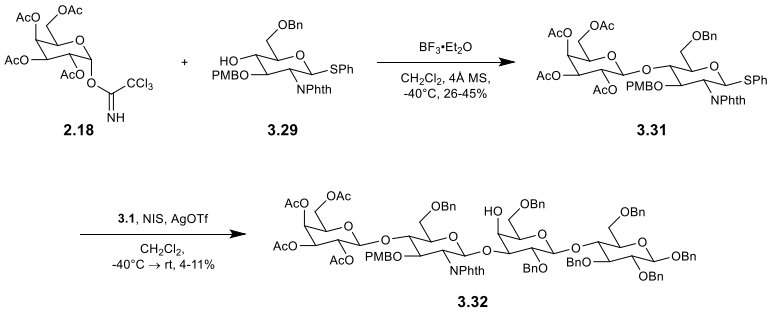
Scheme 3.9. Synthesis of acceptor **3.29**.

With access to a potentially more reactive acceptor, the galactosyl donor sequence was revised to minimize synthetic transformations by using known trichloroacetimidate donor **2.18**.[29] **2.18** was synthesized in two steps starting from **3.7**. Anomeric deacetylation of **3.7** using DMAPA[30] afforded lactol **3.30**, which was readily converted to **2.18** upon treatment with DBU and Cl_3CCN.[29]



Scheme 3.10. Synthesis of donor **2.18**.

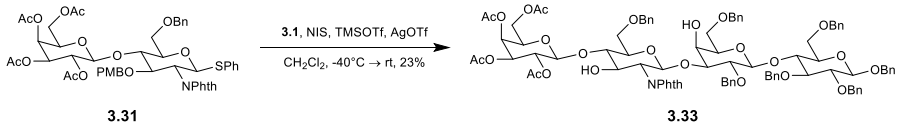
Upon completion of intermediates **2.18**, **3.29**, and **3.1**, synthesis was continued with the glycosylation of **2.18** and **3.29** to yield lactosamine donor **3.31**. Activation of imidate **2.18** with $BF_3{\cdot}Et_2O$ in the presence of acceptor **3.29** gave the desired disaccharide in moderately low yields. Glycosylations to yield protected tetrasaccharide **3.32** gave minimal results. Treatment of **3.1** and **3.31** with NIS and AgOTf yielded tetrasaccharide **3.32**.



Scheme 3.11. Lactosamine and LNnT glycosylations.

Future Directions

Glycosylations leading to the production of **3.32** were generally low yielding. Analysis of the side products revealed that the major side reaction was acidic hydrolysis of the PMB. As a result, we speculated that we could employ stronger Lewis acids in glycosylations to force PMB cleavage while increasing overall yields. Indeed, when catalytic amounts of TMSOTf were used, the yield was improved to 23%.

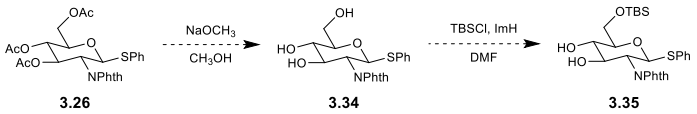


Scheme 3.12. LNnT glycosylation using TMSOTf.

The forcing conditions required to activate thioglycoside **3.31** left much to be optimized for promotion. The results were promising, though, that purposeful cleavage of the PMB could result in desirable product formation without side reactions at C3. Literature evidence suggests it may also be possible to selectively glycosylate at C4 of glucosamine in the absence of a protecting group on C3 because of lessened steric hindrance. In their studies toward one-pot glycosylations of HMOs, Madsen and coworkers found that their glycosylation protocol proceeded more efficiently in the absence of a C3 protecting group on glucosamine.[1a]

Regioselectivity remained unaffected and HMBC NMR data demonstrated a correlation between C1''' and H4'' on their target tetrasaccharide.

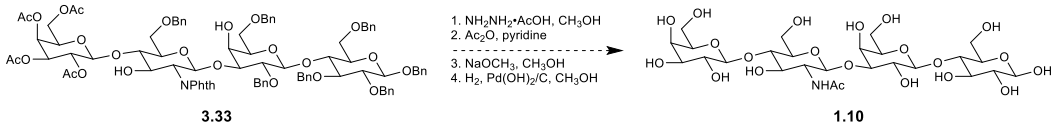
Moving forward, it may prove beneficial to eliminate the use of a C3 protecting group, thereby leaving an acceptor that can be synthesized as shown in Scheme 3.13. Thioglycoside **3.26** can be deacetylated to give triol **3.34**, which can then be silylated using Corey's conditions[12] to yield diol acceptor **3.35**.



3.26 **3.34** **3.35**

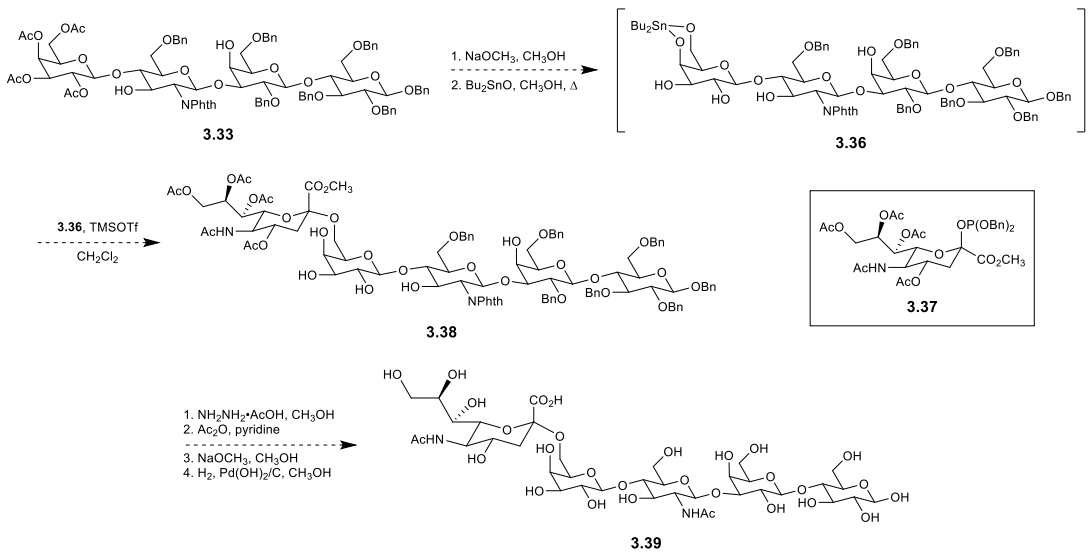
Scheme 3.13. Proposed silylated acceptor **3.35**.

Previously described glycosylations should hopefully yield protected tetrasaccharide **3.33**. **3.33** is a flexible intermediate that can be globally deprotected to arrive at **1.10** or selectively deprotected to further glycosylate toward human milk pentasaccharides. The anticipated scheme for global deprotection will incorporate phthalimide cleavage,[31] acetamide formation, deacetylation, and hydrogenolysis.[32]



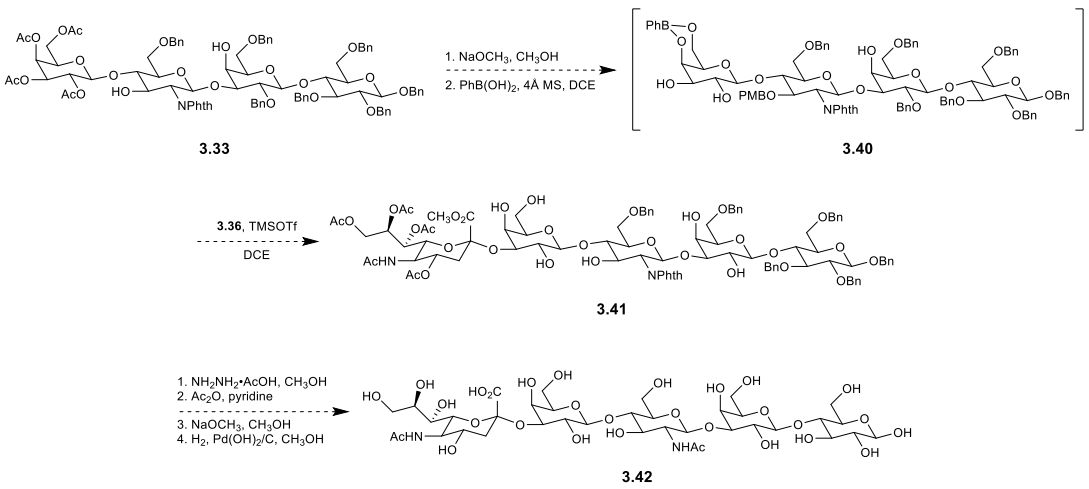
Scheme 3.14. Proposed LNnT deprotection sequence.

Synthesis of protected tetrasaccharide **3.33** allows for diversification through selective deprotection and glycosylation. For instance, deacetylation and sialylation can lead to the production of LS-tetrasaccharides C and D. Post-deacetylation, regioselective glycosylation can be achieved with the revealed tetraol through the use of stannylene and boronate acetals. The nucleophilicity of the primary C6''' alcohol is enhanced through the use of an intermediate stannylene acetal,[33] which, upon treatment with an appropriate sialyl donor such as **3.37**,[34] can yield protected LS-tetrasaccharide C **3.38**. Deprotection as previously described would yield pentasaccharide **3.39**.



Scheme 3.15. Proposed synthesis of LS-tetrasaccharide C.

Glycosylation to yield protected LS-tetrasaccharide D **3.41** would require selective sialylation at C3’’’. Both stannylene and boronate acetals have been demonstrated to give selective 3-O-glycosylation.[35] Formation of the thermodynamically favored 3’’’,4’’’-stannylene acetal enhances nucleophilicity of the 4’’’ alcohol. Alternatively, boronate acetals act as *in situ* protecting groups and prevent undesired reactions. Through application of these methods, it is anticipated that selectivity can be imparted to allow for the synthesis of LST D (**3.42**).



Scheme 3.16. Proposed synthesis of LS-tetrasaccharide D.

Conclusion

This chapter has detailed the progress our lab has made toward the synthesis of lacto-*N*-neotetraose (**1.10**). Several routes were employed throughout the synthesis and, though most of them proved only marginally successful, each attempt provided greater direction for the next step. It is our hope that continuation of the synthesis described herein will soon result in the completion of LNnT.

Experimental Methods

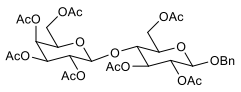
General Procedure: All moisture-sensitive reactions were performed in flame- or oven-dried round bottom flasks under an argon atmosphere. All air- and moisture-sensitive liquids were transferred via oven-dried stainless steel syringes or cannula. Reaction temperatures were monitored and controlled via thermocouple thermometer and corresponding hot plate stirrer. Flash column chromatography was performed as described by Still et. Al. using silica gel 230-400 mesh. Analytical thin-layer chromatography (TLC) was performed on Sorbtech Silica XHL UV254, glass backed, 250 μm plates and were visualized using UV, potassium permanganate stain, cerium ammonium molybdate stain, and anisaldehyde stain.

Materials: Solvents were obtained from Fischer Chemical or Sigma Alrich and dried as needed over 4Å or 3Å molecular sieves. N-iodosuccinimide was recrystallized from carbon tetrachloride and 1,4-dioxane. All other commercial reagents were used as received.

Instrumentation: ^{1}H NMR spectra were obtained on a Bruker 400 MHz or Bruker 600 MHz spectrometer with reporting relative to deuterated solvent signals. ^{1}H NMR spectral data are presented as follows: chemical shifts (δ ppm), multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, p=pentet, dd=doublet of doublets, dt=doublet of triplets, ddd=doublet of doublet of doublets, m=multiplet, br=broad, app=apparent), coupling constants (Hz), integration, assignment. Deuterated chloroform was calibrated to 7.26 ppm. Deuterated methanol was calibrated to 3.31 ppm. ^{13}C NMR spectra were obtained on a Bruker 100 or 150 MHz spectrometer with reporting relative to deuterated solvent signals. Deuterated chloroform was

calibrated to 77.16 ppm. Deuterated methanol was calibrated to 49.0 ppm. Assignments were based on homonuclear correlation measurements and DEPT measurements. Infrared spectroscopy was performed on a Nicolet IR 100 (Thermo Scientific, FT-IR). Low resolution mass spectrometry was performed with a Surveyor MSQ spectrometer (Thermo Scientific).

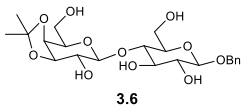
Preparative Procedures



3.5

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3R,4S,5R,6R)-4,5-diacetoxy-2-(acetoxymethyl)-6-(benzyloxy)tetrahydro-2H-pyran-3-yl)oxy) tetrahydro-2H-pyran-3,4,5-triyl triacetate (3.5). To a solution of lactose octaacetate (1.0 eq, 30 g, 44.2 mmol) in CH_2Cl_2 (221 mL) was added benzyl alcohol (2.3 eq, 10.6 mL, 102 mmol). The resulting solution was cooled to 0°C and to it was added $BF_3{\cdot}Et_2O$ (3 eq, 16.8 mL, 133 mmol). The reaction was allowed to warm to room temperature and stir for 16h. The reaction was quenched by the addition of $NaHCO_3$. The mixture was extracted and the organics were washed with water (2 x 200 mL), brine (1 x 200 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was crystallized from EtOH yielding **3.5** (15.46 g, 21.27 mmol, 48%) as a white solid: R*f* 0.54 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H, OBn), 5.34 (d, *J*=2.90 Hz, 1H, H-4'), 5.16 (t, *J*=9.28 Hz, 1H, H-2), 5.10 (dd, *J*=7.92, 10.40 Hz, 1H, H-2'), 4.97 (t, *J*=7.92 Hz, 1H, H-3), 4.95 (dd, *J*=1.96, 8.32 Hz, 1H, H-3'), 4.86 (d, *J*=12.30 Hz, 1H, C*H*$_2$Ph), 4.60 (d, *J*=12.30 Hz, 1H, C*H*$_2$Ph), 4.52 (dd, *J*=2.00, 11.33 Hz, 1H, H-6), 4.51 (d, *J*=7.90 Hz, 1H, H-1), 4.48 (d, *J*=7.90 Hz, 1H, H-1'), 4.15-4.04 (m, 3H, H-6, H-6', H-6'), 3.86 (t,

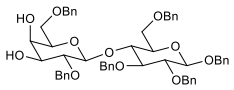
J=6.60 Hz, 1H, H-5'), 3.82 (t, *J*=9.60 Hz, 1H, H-4), 3.58 (ddd, *J*=2.00, 5.00, 9.90 Hz, 1H, H-5), 2.144 (s, 3H), 2.139 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.035 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.53, 170.49, 170.29, 170.20, 169.92, 169.76, 169.19, 136.79, 128.61, 128.19, 127.90, 101.21 (C-1'), 99.17 (C-1), 76.41 (C-4), 72.94 (C-2), 72.81 (C-5), 71.81 (C-3), 71.14 (C-3'), 70.87 (CH$_2$Ph), 70.84 (C-5'), 69.25 (C-2'), 66.75 (C-4'), 62.14 (C-6), 60.95 (C-6'), 21.04, 20.95, 20.83, 20.78, 20.66; LRMS $[M+H]^+$ $C_{33}H_{43}O_{18}$ calcd. 727.25, obsd. 727.26; IR (thin film) ν 1751, 1432, 1369, 1223, 1055 cm^{-1}. Characterization corresponded to literature data.[36]



3.6

(2R,3R,4R,5S,6R)-2-(benzyloxy)-5-(((3aS,4R,6S,7R,7aR)-7-hydroxy-4-(hydroxymethyl)-2,2-dimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyran-6-yl)oxy)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4-diol (3.6). To a solution of **3.5** (1.0 eq, 4.2 g, 5.78 mmol) in MeOH (58 mL) was added NaOMe (0.54 eq, 2 mL, 1.57 M, 3.14 mmol). The resulting solution was allowed to stir for 1.5h and was then neutralized by the addition of Dowex 50WX8 resin to neutral pH, filtered, and concentrated *in vacuo* yielding a crude oil (2.4 g, 5.55 mmol, 96%). The crude material was used without further purification or characterization. To a suspension of the polyol (1.0 eq, 1.0 g, 2.313 mmol) in acetone (23 mL) was added 2,2-dimethoxypropane (9.0 eq, 2.6 mL, 20.81 mmol) and p-toluenesulfonic acid (0.1 eq, 44 mg, 0.231 mmol), sequentially. The resulting slurry stirred for 16h and was then neutralized by the addition of Et_3N (50 µL). The solution was concentrated onto Celite and the crude material was purified by flash chromatography (1:0-9:1 CH_2Cl_2/MeOH) yielding acetonide **3.6** (699.5 mg, 1.48 mmol, 64%) as a white solid: R*f* 0.31 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz,

MeOD-d_4) δ 7.44-7.24 (m, 5H, OBn), 4.92 (d, *J*=11.8 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J*=11.8 Hz, 1H, C*H*$_2$Ph), 4.39 (d, *J*=7.8 Hz, 1H, H-1), 4.37 (d, *J*=8.2 Hz, 1H, H-1'), 4.19 (dd, *J*=2.12, 5.52 Hz, 1H, H-4'), 4.05 (dd, *J*=5.56, 7.24 Hz, 1H, H-3'), 3.95 (dd, *J*=2.08, 4.68 Hz, 1H, H-5'), 3.91 (dd, *J*=2.40, 9.70 Hz, 1H, H-6), 3.84 (dd, *J*=4.28, 12.12 Hz, 1H, H-6), 3.78 (dd, *J*=4.00, 11.64 Hz, 1H, H-6'), 3.75 (dd, *J*=4.60, 11.60 Hz, 1H, H-6'), 3.585 (t, *J*=8.88 Hz, 1H, H-4), 3.52 (t, *J*=8.88 Hz, 1H, H-3), 3.45 (t, *J*=7.8 Hz, 1H, H-2'), 3.40 (ddd, *J*=2.50, 4.20, 9.50 Hz, 1H, H-5), 3.315 (m, H-2, MeOD), 1.47 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 139.00, 129.28, 129.17, 128.72, 111.11 (*C*$(CH_3)_2$), 104.17 (C-1'), 103.14 (C-1), 80.98 (C-4), 80.86 (C-3'), 76.47 (C-5), 76.37 (C-3), 75.36 (C-5'), 75.06 (C-4), 74.89 (C-2), 74.46 (C-2'), 71.80 (*C*H$_2$Ph), 62.41 (C-6'), 61.90 (C-6), 28.40, 26.49; LRMS $[M+H]^+$ $C_{22}H_{33}O_{11}$ calcd. 473.20, obsd. 473.35; IR (thin film) ν 3354, 1379, 1280, 1068, 1040 cm^{-1}. Characterization corresponded to literature data.[37]

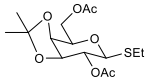


3.1

(2R,3R,4S,5R,6S)-5-(benzyloxy)-2-((benzyloxy)methyl)-6-(((2R,3R,4S,5R,6R)-4,5,6-tris(benzyloxy)-2-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4-diol (3.1). A suspension of NaH (5.5 eq, 281 mg, 60% wt, 7.02 mmol) in DMF (10 mL) was cooled to 0°C. To the suspension was added a solution of **3.6** (1.0 eq, 600 mg, 1.28 mmol) in DMF (2 mL). The resulting solution was allowed to stir at 0°C for 45 minutes, after which benzyl bromide (6 eq, 0.92 mL, 7.66 mmol) was added. The reaction was warmed to room temperature and stirred for 1h. The reaction was quenched by the addition of MeOH and diluted with EtOAc (5 mL) and water (20 mL). The organics were extracted and washed with water (5 x 100 mL), brine (1 x 50 mL), dried over $MgSO_4$, filtered,

and concentrated *in vacuo*. The crude oil was purified by flash chromatography (9:1-2:1 hexanes/EtOAc) yielding the perbenzylated acetonide (763 mg, 0.826 mmol, 65%) as a clear oil. A solution of the perbenzylated acetonide (1 eq, 762 mg, 0.825 mmol) in 80% aq. AcOH (8.25 mL) was heated to 75°C for 3h. Upon completion, the reaction was cooled to room temperature and was quenched by the addition of $NaHCO_3$. The solution was extracted with EtOAc (2 x 50 mL) and the organics were washed with water (2 x 50 mL), brined (1 x 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (4:1-1:1 hexanes/EtOAc) giving acceptor **3.1** (603.4 mg, 0.683 mmol, 83%) as a white solid: R*f* 0.13 (2:1 hexanes/EtOAc); ^{1}H NMR (400MHz, $CDCl_3$) δ 7.40-7.20 (m, 30H, 6 x OBn), 4.98 (d, *J*=10.80 Hz, 1H, C*H*$_2$Ph), 4.95 (d, *J*=11.80 Hz, 1H, C*H*$_2$Ph), 4.91 (d, *J*=10.90 Hz, 1H, C*H*$_2$Ph), 4.81 (d, *J*=11.60 Hz, 1H, C*H*$_2$Ph), 4.77 (d, *J*=11.00 Hz, 1H, C*H*$_2$Ph), 4.73 (d, *J*=10.90 Hz, 1H, C*H*$_2$Ph), 4.67 (d, *J*=11.60 Hz, 1H, C*H*$_2$Ph), 4.66 (d, *J*=12.10 Hz, 1H, C*H*$_2$Ph), 4.62 (d, *J*=12.10 Hz, 1H, C*H*$_2$Ph), 4.50 (d, *J*=7.70 Hz, 1H, H-1), 4.46 (d, *J*=12.00 Hz, 1H, C*H*$_2$Ph), 4.45 (d, *J*=11.80 Hz, 1H, C*H*$_2$Ph), 4.44 (d, *J*=7.10 Hz, 1H, H-1'), 4.39 (d, *J*=12.00 Hz, 1H, C*H*$_2$Ph), 4.02 (t, *J*=9.24 Hz, 1H, H-4), 3.95 (t, *J*=5.32 Hz, 1H, H-4'), 3.83 (dd, *J*=4.08, 10.92 Hz, 1H, H-6), 3.77 (dd, *J*=1.56, 10.80 Hz, 1H, H-6), 3.61 (dd, *J*=6.68, 10.08 Hz, 1H, H-6'), 3.59 (t, *J*=8.88 Hz, 1H, H-3), 3.50 (t, *J*=7.20 Hz, 1H, H-2), 3.49 (m, 1H, H-6'), 3.44 (m, 1H, H-3'), 3.42 (m, 1H, H-2'), 3.39 (ddd, *J*=1.60, 4.10, 9.60 Hz, 1H, H-5), 3.37 (dd, *J*=5.75, 5.88 Hz, 1H, H-5'), 2.46 (d, *J*=3.60 Hz, 1H, C_4'-OH), 2.38 (d, *J*=4.70 Hz, 1H, C_3'-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.29, 138.70, 138.50, 138.40, 138.13, 137.66, 128.67, 128.57, 128.53, 128.47, 128.42, 128.25, 128.21, 128.11, 128.06, 128.00, 127.87, 127.85, 127.75, 127.70, 127.41, 102.73 (C-1), 102.65 (C-1'), 83.01 (C-3), 81.97 (C-2), 80.18 (C-2'), 76.75 (C-4), 75.44 (*C*H$_2$Ph), 75.30 (*C*H$_2$Ph), 75.14 (*C*H$_2$Ph), 75.14 (*C*H$_2$Ph), 75.03 (*C*H$_2$Ph), 73.67 (C-3'),

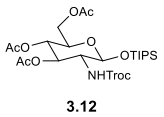
73.63 (*C*H_2Ph), 73.37 (*C*H_2Ph), 73.00 (C-5'), 71.12 (*C*H_2Ph), 68.93 (C-4'), 68.81 (C-6'), 68.43 (C-6); LRMS $[M+H]^+$ $C_{54}H_{59}O_{11}$ calcd. 883.41, obsd. 883.53; IR (thin film) ν 3465, 3062, 3029, 2870, 1496, 1453, 1363, 1093, 1061, 736, 697 cm^{-1}. Characterization corresponded to literature data.[2b]



3.2

((3aS,4R,6S,7R,7aS)-7-acetoxy-6-(ethylthio)-2,2-dimethyltetrahydro-4H-[1,3]dioxolo[4,5-c]pyran-4-yl)methyl acetate (3.2). To a solution of **3.7** (1.0 eq, 1.0 g, 2.56 mmol) in CH_2Cl_2 (8.54 mL) was added ethanethiol (1.4 eq, 0.26 mL, 3.59 mmol). The resulting solution was cooled to 0°C and to it was added $BF_3{\cdot}Et_2O$ (1.66 eq, 0.54 mL, 4.25 mmol). The reaction was allowed to warm to room temperature and stir for 2h. It was quenched by the addition of $NaHCO_3$ and diluted with water (10 mL). The organics were extracted and washed with water (2 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (1:4-2:3 hexanes/EtOAc) to yield thioglycoside **3.8** (752 mg, 1.92 mmol, 74.8%) as a clear oil. To a solution of **3.8** (1 eq, 655 mg, 1.67 mmol) in MeOH (16 mL) was added NaOMe (2 mL). The resulting solution stirred for 3h and was then neutralized by the addition of Dowex 50WX8 resin, filtered, and concentrated *in vacuo*. To a suspension of the corresponding tetraol residue (1.0 eq, 285 mg, 1.27 mmol) in 2,2-dimethoxypropane (6.4 mL) was added p-toluenesulfonic acid (0.03 eq, 7 mg, 0.04 mmol) and the resulting suspension stirred for 24h. Water (5 mL) was added to the solution and stirring continued for 15 minutes. The solution was cooled to 0°C for 20 minutes, followed by the addition of Et_3N (0.1 mL). The reaction mixture was returned to

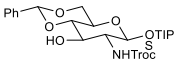
room temperature and diluted with water (5 mL) and EtOAc (5 mL). The aqueous solution was extracted with EtOAc (5 x 5 mL) and the organics were dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by flash chromatography (1:1-3:7 toluene:EtOAc) to give acetonide **S3** (174 mg, 0.518 mmol, 52%) as a white solid. To a solution of **3.9** (1 eq, 1.0 g, 3.78 mmol) in pyridine (19 mL) was added a catalytic amount of N,N-dimethylaminopyridine (1 crystal) and acetic anhydride (3.0 eq, 1.1 mL, 11.34 mmol), sequentially. The resulting solution stirred at room temperature for 16h and was then diluted with saturated $CuSO_4$ solution (10 mL), water (30 mL), and EtOAc (20 mL). The resulting mixture was extracted with EtOAc (3 x 20 mL), washed with water until blue color no longer remained, brined (1 x 20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (3:2 hexanes/EtOAc) yielding **3.2** (1.3 g, 3.73 mmol, 99%) as a clear oil: R*f* 0.65 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.00 (dd, *J*=6.56, 10.00 Hz, 1H, H-2), 4.34 (dd, *J*=4.92, 6.84 Hz, 1H, H-6), 4.33 (d, *J*=7.00 Hz, 1H, H-1), 4.19 (m, 1H, H-3), 4.18 (dd, *J*=5.36, 13.76 Hz, 1H, H-4), 3.98 (ddd, *J*=1.88, 5.12, 6.96 Hz, 1H, H-5), 2.69 (m, 2H, C*H*$_2$CH$_3$), 2.10 (s, 3H), 2.08 (s, 3H), 1.54 (s, 3H), 1.33 (s, 3H), 1.265 (t, *J*=7.48 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.95, 169.84, 110.88 (*C*(CH$_3$)$_2$), 82.93 (C-1), 76.88 (C-4), 74.41 (C-5), 73.76 (C-3), 71.47 (C-2), 63.77 (C-6), 27.80, 26.48, 24.48 (*C*H$_2$CH$_3$), 21.16, 20.99, 15.05 (CH$_2$*C*H$_3$); LRMS $[M+Na]^+$ $C_{15}H_{24}O_7SNa$ calcd. 371.11, obsd. 371.26; IR (thin film) ν 2985, 2935, 1745, 1373, 1226, 1084, 1048, 871, 838 cm^{-1}. Characterization corresponded to literature data.[38]



3.12

(2R,3S,4R,5R,6S)-2-(acetoxymethyl)-5-(((2,2,2-trichloroethoxy)carbonyl)amino)-6-((triisopropylsilyl)oxy)tetrahydro-2H-pyran-3,4-diyl diacetate (3.12). To a solution of **3.10** (1.0 eq, 10 g, 46.4 mmol) in water (93 mL) was added $NaHCO_3$ (3.0 eq, 11.7 g, 139 mmol) and stirring proceeded at room temperature for 1h. Trichloroethyl chloroformate (1.2 eq, 7.66 mL, 55.7 mmol) was added dropwise to the solution and the reaction continued stirring for 4h, after which a voluminous white solid had precipitated. The solid was filtered and dried for 16h. To a solution of the white solid in pyridine (155 mL) was added a catalytic amount of N,N-dimethylaminopyridine and the resulting solution was cooled to 0°C. Acetic anhydride (5.0 eq, 22 mL, 232 mmol) was added dropwise to the solution at 0°C and the reaction was returned to room temperature. The reaction stirred at room temperature for 4h and was then quenched by the addition of 1N HCl (100 mL). The mixture was diluted with EtOAc (300 mL) and the organics were extracted and washed with 1N HCl (5 x 100 mL), water (2 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, and concentrated *in vacuo* to give **3.11** (19.5 g, 37.3 mmol, 80%) as a white foam. A solution of **3.11** (1.0 eq, 66.2 g, 126.65 mmol) in DMF (125 mL) was cooled to 0°C. Hydrazine acetate (1.1 eq, 12.8 g, 139.3 mmol) was added to the solution at 0°C and it was returned to room temperature. The reaction stirred at room temperature for 16h and was then diluted with EtOAc (100 mL). The mixture was washed with water (5 x 300 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (2:1-1:1 hexanes/EtOAc) to yield the corresponding anomeric alcohol (40.7 g, 84.67 mmol, 67%, 2.2:1 α:β) as a white solid. The anomeric alcohol (1 eq, 7.04 g, 14.65 mmol) was dissolved in DMF (15 mL) and to it was added imidazole (2.0 eq, 2.0 g, 29.3 mmol)

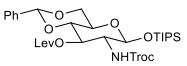
TIPS-Cl (1.2 eq, 3.77 mL, 17.58 mmol), sequentially. The resulting solution was allowed to stir for 16h and was then poured into cold water, resulting in the formation of a white precipitate. The solid was filtered and dissolved in EtOAc (100 mL). The solution was washed with water (2 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered, and concentrated onto Celite. The crude material was purified by flash chromatography (9:1-3:1 hexanes/EtOAc) to give **3.12** (7.5 g, 11.77 mmol, 80%) as a white solid: R*f* 0.62 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.235 (t, *J*=9.64 Hz, 1H, H-3), 5.04 (t, *J*=9.72 Hz, 1H, H-4), 5.02 (d, *J*=9.61 Hz, 1H, N*H*), 4.89 (d, *J*=7.80 Hz, 1H, H-1), 4.76 (d, *J*=11.88 Hz, 1H, troc CH_2), 4.61 (d, *J*=11.88 Hz, 1H, troc CH_2), 4.156 (d, *J*=4.10 Hz, 2H, H-6), 3.70 (dd, *J*=4.24, 9.80 Hz, 1H, H-5), 3.64 (dd, *J*=8.10, 9.20 Hz, 1H, H-2), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.15-1.01 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.95, 170.74, 169.65, 154.14, 96.06 (C-1), 95.42 (troc *C*Cl_3), 74.72 (troc *C*H_2), 72.14 (C-3), 71.88 (C-5), 69.18 (C-4), 62.63 (C-6), 58.59 (C-2), 20.77, 17.86, 17.82, 17.79, 12.25; LRMS $[M+H]^+$ $C_{24}H_{41}Cl_3NO_{10}Si$ calcd. 636.16, obsd. 636.67; IR (thin film) v 3291, 1747, 1706, 1557, 1234, 1036 cm^{-1}.



3.13

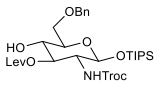
2,2,2-trichloroethyl ((2R,4aR,6S,7R,8R,8aS)-8-hydroxy-2-phenyl-6-((triisopropylsilyl) oxy)hexahydropyrano[3,2-d][1,3]dioxin-7-yl)carbamate (3.13). To a solution of **3.12** (1.0 eq, 10 g, 15.7 mmol) in 1:1 CH_2Cl_2:MeOH (156 mL) was added guanidine hydrochloride (5.0 eq, 7.5 g, 78 mmol), followed by 7N NH_3/MeOH (5.5 eq, 12.33 mL). The resulting solution was allowed to stir at room temperature for 18h, after which an additional 4.5 mL 7N NH_3/MeOH was added. Stirring continued at room temperature for 4h,

then the reaction was neutralized by the addition of Dowex 50WX8 resin, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (99:1-19:1 CH_2Cl_2/MeOH) to yield the intermediate triol (5.7 g, 11.16 mmol, 71%) as a white foam. The triol (1.0 eq, 5.7 g, 11.16 mmol) was dissolved in MeCN (37 mL) and to the resulting solution was added camphorsulfonic acid (0.1 eq, 260 mg, 1.116 mmol) and benzaldehyde dimethylacetal (1.25 eq, 2.1 mL, 13.9 mmol), sequentially. The reaction stirred at room temperature for 16h and was then quenched by the addition of Et_3N (0.3 mL). The solution was concentrated *in vacuo* and the crude oil was suspended in EtOAc (100 mL), washed with water (3 x 75 mL), brined (1 x 50 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography (9:1-4:1 hexanes/EtOAc) to yield **3.13** (5.9 g, 9.85 mmol, 88%) as a white foam: R*f* 0.7 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.47 (m, 2H, benzylidene Ph), 7.41-7.35 (m, 3H, benzylidene Ph), 5.55 (s, 1H, benzylidene C*H*Ph), 5.16 (d, *J*=6.10 Hz, 1H, N*H*), 4.94 (d, *J*=7.80 Hz, 1H, H-1), 4.74 (d, *J*=7.40 Hz, 1H, troc CH_2), 4.67 (d, *J*=7.40 Hz, 1H, troc CH_2), 4.295 (dd, *J*=3.10, 6.55 Hz, 1H, H-6), 4.07 (t, *J*=9.24 Hz, 1H, H-3), 3.79 (t, *J*=10.52 Hz, 1H, H-6), 3.58 (t, *J*=9.28 Hz, 1H, H-4), 3.475 (td, *J*=5.00, 9.80, 9.80 Hz, 1H, H-5), 3.40 (q, *J*=8.58 Hz, 1H, H-2), 2.93 (br, 1H, C_3-OH), 1.15-0.98 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.64, 137.16, 129.49, 128.52, 126.49, 102.13 (benzylidene *C*HPh), 96.13 (C-1), 95.33 (troc *C*Cl_3), 81.75 (C-4), 75.00 (troc *C*H_2), 71.00 (C-3), 68.71 (C-6), 66.32 (C-5), 61.38 (C-2), 29.86, 17.94, 17.88, 12.30; LRMS $[M+H]^+$ $C_{25}H_{39}Cl_3NO_7Si$ calcd. 598.16, obsd. 598.62; IR (thin film) ν 3330, 2942, 2866, 1717, 1548, 1463, 1385, 1100, 820, 698 cm^{-1}. Characterization corresponded to literature data.[4b]



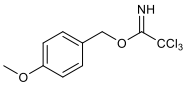
S1

(2R,4aR,6S,7R,8R,8aS)-2-phenyl-7-(((2,2,2-trichloroethoxy)carbonyl)amino)-6-((triisopropylsilyl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-8-yl 4-oxopentanoate (S1). To a solution of **3.13** (1.0 eq, 100 mg, 0.167 mmol) in CH_2Cl_2 (1.7 mL) was added levulinic acid (3.0 eq, 58 mg, 0.501 mmol), Et_3N (4.5 eq, 0.1 mL, 0.751 mmol), and a catalytic amount of N,N-dimethylaminopyridine. The resulting mixture was cooled to 0°C and to it was added EDC (3.0 eq, 96 mg, 0.501 mmol). The reaction stirred at 0°C for 2h, was warmed to room temperature, and stirred at room temperature for 1h. The reaction was diluted with CH_2Cl_2 (2 mL), washed with water (3 x 2 mL), brine (1 x 2 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography (1:0-7:3 hexanes/EtOAc) to yield ester **S1** (85.3 mg, 0.125 mmol, 75%) as a clear oil: R*f* 0.7 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.42 (m, 2H, benzylidene Ph), 7.37-7.32 (m, 3H, benzylidene Ph), 5.50 (s, 1H, benzylidene C*H*Ph), 5.30 (t, *J*=10.00 Hz, 1H, H-3), 5.28 (br, 1H, N*H*), 4.89 (d, *J*=7.80 Hz, 1H, H-1), 4.72 (s, 2H, troc CH_2), 4.26 (dd, *J*=4.96, 10.48 Hz, 1H, H-4), 3.78 (t, *J*=10.28 Hz, 1H, H-6), 3.71 (t, *J*=9.36 Hz, 1H, H-6), 3.66 (m, 1H, H-2), 3.47 (td, *J*=5.00, 9.70 Hz, 1H, H-5), 2.80-2.66 (m, 2H, lev CH_2CH$_2$COCH$_3$), 2.65-2.49 (m, 2H, lev CH_2COCH$_3$), 2.125 (s, 3H, lev CH_3), 1.13-0.97 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.15, 172.82, 154.5, 137.11, 129.18, 128.31, 126.33, 101.55 (benzylidene *C*HPh), 96.76 (C-1), 95.84 (troc *C*Cl$_3$), 78.97 (C-4), 74.88 (troc *C*H$_2$), 71.59 (C-3), 68.65 (C-6), 66.44 (C-5), 59.36 (C-2), 38.10 (lev *C*H$_2$CH$_2$COCH$_3$), 29.84 (lev *C*H$_2$COCH$_3$), 28.13 (lev *C*H$_3$), 17.86, 17.80, 12.21; LRMS [M+H]$^+$ $C_{30}H_{45}Cl_3NO_9Si$ calcd. 696.20, obsd. 696.40; IR (thin film) ν 3354, 2944, 2866, 1721, 1544, 1102, 821, 735, 699 cm^{-1}. Characterization corresponded to literature data.[4c]



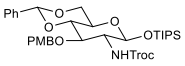
3.3

(2R,3S,4R,5R,6S)-2-((benzyloxy)methyl)-3-hydroxy-5-(((2,2,2-trichloroethoxy)carbonyl)amino)-6-((triisopropylsilyl)oxy)tetrahydro-2H-pyran-4-yl 4-oxopentanoate (3.3). A solution of **S1** (1.0 eq, 500 mg, 0.717 mmol) in CH_2Cl_2 (7.2 mL) was cooled to 0°C and to it was added triethylsilane (5.0 eq, 0.57 mL, 3.585 mmol) and TFA (5.0 eq, 0.27 mL, 3.585 mmol), sequentially. The resulting solution was warmed to room temperature and stirred for 2.5h. The reaction was quenched by the addition of $NaHCO_3$ solution, washed with water (2 x 10 mL), brined (1 x 10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude mixture was purified by flash chromatography (4:1-1:1 hexanes/EtOAc) to yield acceptor **3.3** (328 mg, 0.469 mmol, 65%) as a clear oil: R*f* 0.36 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.27 (m, 5H, OBn), 5.21 (d, *J*=9.50 Hz, 1H, N*H*), 5.04 (dd, *J*=9.08, 10.80 Hz, 1H, H-3), 4.785 (d, *J*=9.50 Hz, 1H, H-1), 4.73 (d, *J*=11.96 Hz, 1H, troc C*H*$_2$), 4.65 (d, *J*=11.96 Hz, 1H, troc C*H*$_2$), 4.57 (dd, *J*=11.96, 16.00 Hz, 2H, C*H*$_2$Ph), 3.80-3.72 (m, 3H, H-4, H-6), 3.64 (ddd, *J*=8.00, 9.70, 18.50 Hz, 1H, H-2), 3.53 (dt, *J*=4.24, 9.60 Hz, 1H, H-5), 3.31 (d, *J*=2.50 Hz, 1H, C_4-OH), 2.80-2.75 (m, 2H, lev C*H*$_2$CH$_2$COCH$_3$), 2.62-2.48 (m, 2H, lev C*H*$_2$COCH$_3$), 2.16 (s, 3H, lev CH_3), 1.14-1.00 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.45, 173.54, 154.37, 138.20, 128.51, 127.81, 127.71, 96.38 (C-1), 95.59 (troc *C*Cl_3), 75.76, 74.78, 74.33, 73.82, 70.56 (C-4), 70.21 (C-6), 58.14 (C-2), 38.41 (lev *C*H$_2$CH$_2$COCH$_3$), 29.88, 29.82, 28.36, 17.92, 17.86, 12.56, 12.27, 12.10; LRMS $[M+Na]^+$ $C_{30}H_{46}Cl_3NO_9SiNa$ calcd. 720.19, obsd. 720.57; IR (thin film) ν 3464, 3349, 2944, 1721, 1544, 1066, 817, 735 cm^{-1}. Characterization corresponded to literature data.[4c]



3.15

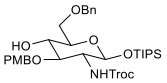
4-methoxybenzyl 2,2,2-trichloroacetimidate (3.15). A suspension of NaH (0.1 eq, 87 mg, 2.17 mmol, 60 % wt) in diethyl ether (6.8 mL) was cooled to 0°C. To the suspension was added 4-methoxybenzyl alcohol (1.0 eq, 2.7 mL, 21.7 mmol) and the resulting solution stirred for 20 minutes. Trichloroacetonitrile (1.2 eq, 2.6 mL, 26.1 mmol) was added to the solution and it was slowly returned to room temperature. The reaction stirred at room temperature for 2h and was then concentrated *in vacuo*. The crude oil was suspended in hexanes. A drop of methanol was added to the suspension and the solution stirred until salts precipitated. The solution was filtered through Celite and the resulting solution was concentrated *in vacuo* to yield imidate **3.15** (4.62 g, 21.7 mmol, 75%) as an orange liquid. The liquid was used in subsequent reactions without further purification. R*f* 0.7 (2:1 hexanes/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (s, 1H, N*H*), 7.375 (d, *J*=8.70 Hz, 2H, aromatic C*H*), 6.91 (d, *J*=8.70 Hz, 2H, aromatic C*H*), 5.275 (s, 2H, CH_2Ar), 3.82 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.75 (O*C*(NH)CCl_3), 159.83, 129.84, 127.63, 114.03, 91.61 (*C*Cl_3), 70.82 (*C*H_2Ar), 55.40 (*C*H_3). Characterization corresponded to reported literature data.[17]



S2

2,2,2-trichloroethyl ((2R,4aR,6S,7R,8R,8aS)-8-((4-methoxybenzyl)oxy)-2-phenyl-6-((triisopropylsilyl)oxy)hexahydropyrano[3,2-d][1,3]dioxin-7-yl)carbamate (S2). **3.13** (1.0 eq, 600 mg, 1.0 mmol) and **3.15** (1.2 eq, 339 mg, 1.2 mmol) were combined and coevaporated from benzene (2 x 2 mL). The compounds were dried under vacuum for 10 minutes and were then dissolved in diethyl ether (5 mL). TMSOTf (0.01 eq, 1.8 µL, 0.01 mmol) was added to the solution at room temperature and the reaction stirred for 20

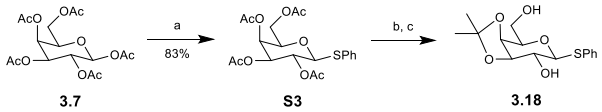
minutes. The reaction was quenched by the addition of Et_3N (10 µL) and was concentrated *in vacuo*. The crude residue was purified by flash chromatography (1:0-4:1 hexanes/EtOAc) to yield **S2** (505.3 mg, 0.703 mmol, 70%) as a white foam: R*f* 0.68 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.48 (m, 2H, benzylidene Ph), 7.43-7.36 (m, 3H, benzylidene Ph), 7.24 (d, *J*=8.60 Hz, 2H, PMB aromatic C*H*), 6.845 (d, *J*=8.60 Hz, 2H, PMB aromatic C*H*), 5.59 (s, 1H, benzylidene C*H*Ph), 4.98 (br d, *J*=7.50 Hz, 2H, H-1, PMB CH_2Ar), 4.82 (d, *J*=11.4 Hz, 1H, troc CH_2), 4.72-4.60 (m, 2H, N*H*, PMB CH_2Ar), 4.64 (d, *J*=11.40 Hz, 1H, PMB CH_2Ar), 4.29 (dd, *J*=5.00, 10.44 Hz, 1H, H-6), 3.95 (t, *J*=8.36 Hz, 1H, H-3), 3.80 (dd, *J*=5.98, 14.59 Hz, 1H, H-6), 3.79 (s, 3H, PMB CH_3), 3.73 (t, *J*=9.09 Hz, 1H, H-4), 3.44 (td, *J*=4.90, 9.56 Hz, 1H, H-5), 3.315 (q, *J*=8.16 Hz, 1H, H-2), 1.12-1.00 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.45, 137.55, 130.40, 130.10, 129.16, 128.44, 126.20, 113.93, 101.40 (benzylidene *C*HPh), 82.91 (C-1), 77.38 (C-4), 74.78 (C-3), 73.99 (troc *C*H_2), 68.84 (C-6), 66.14 (C-5), 60.68 (C-2), 55.40 (PMB *C*H_3), 17.895, 17.85, 12.30; LRMS $[M+H]^+$ $C_{33}H_{47}Cl_3NO_8Si$ calcd. 718.22, obsd. 718.35; IR (thin film) ν 3341, 2944, 2866, 1722, 1513, 1246, 1100, 820 cm^{-1}.



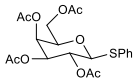
3.14

2,2,2-trichloroethyl ((2S,3R,4R,5S,6R)-6-((benzyloxy)methyl)-5-hydroxy-4-((4-methoxybenzyl)oxy)-2-((triisopropylsilyl)oxy)tetrahydro-2H-pyran-3-yl)carbamate (3.14). To a solution of **S2** (1.0 eq, 250 mg, 0.348 mmol) and $BH_3{\cdot}NMe_3$ (4.67 eq, 119 mg, 1.625 mmol) in THF (4 mL) was added a suspension of $AlCl_3$ (5.0 eq, 232 mg, 1.74 mmol) in THF (2 mL). The resulting solution stirred at room temperature for 15h, then water (10 µL) was added. The reaction continued stirring for 1h and was then quenched by the

addition of Rochelle's salt. The mixture was diluted with EtOAc (10 mL), washed with water (2 x 10 mL), brined (1 x 10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography (9:1-3:2 hexanes/EtOAc) to give **3.14** (16.9 mg, 7% recovery) and **S12** (159.4 mg, 0.221 mmol, 64%, 68% BRSM) as a clear oil: R*f* 0.125 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H, OBn), 7.28 (d, *J*=8.80 Hz, 2H, PMB aromatic C*H*), 6.88 (d, *J*=8.80 Hz, 2H, PMB aromatic C*H*), 5.00 (d, *J*=6.60 Hz, 1H, N*H*), 4.89 (d, *J*=7.40 Hz, 1H, H-1), 4.72 (s, 2H, CH_2Ar), 4.69 (s, 2H, troc CH_2), 4.60 (d, *J*=11.90 Hz, 1H, CH_2Ph), 4.55 (d, *J*=11.90 Hz, 1H, CH_2Ph), 3.80 (s, 3H, PMB CH_3), 3.79-3.68 (m, 4H, H-3, H-5, H-6), 3.46 (p, *J*=4.60 Hz, 1H, H-4), 3.32 (br d, *J*=8.40 Hz, 1H, H-2), 2.77 (s, 1H, C_4-OH), 2.69 (s, 1H), 1.15-0.98 (m, 21H, TIPS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.495, 154.05, 137.86, 130.56, 129.91, 128.59, 127.935, 127.79, 114.08, 95.55 (C-1), 79.91 (C-5), 74.71 (troc *C*H_2), 73.92 (*C*H_2Ph), 73.80 (PMB *C*H_2Ar), 73.625 (C-4), 73.51 (C-3), 70.99 (C-6), 59.77 (C-2), 55.405 (PMB *C*H_3), 50.44, 17.96, 17.91, 12.33; LRMS $[M+Na]^+$ $C_{33}H_{48}Cl_3NO_8SiNa$ calcd. 742.21, obsd. 742.44; IR (thin film) ν 3352, 2943, 2865, 1728, 1612, 1514, 1066, 818, 735, 694 cm^{-1}.

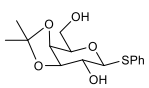


Scheme S1. Synthesis of thioglycoside **3.18**. a) PhSH, $SnCl_4$, CH_2Cl_2, rt, 45 min.; b) $NaOCH_3$, CH_3OH, rt, 4h; c) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH, acetone, rt, 24h.



S3

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(phenylthio)tetrahydro-2H-pyran-3,4,5-triyl triacetate (S3). To a solution of **3.7** (1.0 eq, 20.0 g, 51.2 mmol) in CH_2Cl_2 (256 mL) was added thiophenol (1.2 eq, 6.33 mL, 61.5 mmol) and the resulting solution was cooled to 0°C. $SnCl_4$ (2.0 eq, 12 mL, 102 mmol) was added to the solution at 0°C. The mixture was warmed to room temperature and stirred for 45 minutes. The reaction was quenched by the addition of saturated $NaHCO_3$ solution and saturated Rochelle's salt solution. The organics were extracted and washed with water (2 x 200 mL), brined (1 x 200 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (4:1-3:2 hexanes/EtOAc) to yield **S3** (18.7 g, 51.2 mmol, 83%) as a clear oil: R*f* 0.71 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.48 (m, 2H, SPh), 7.33-7.28 (m, 3H, SPh), 5.41 (dd, *J*=0.68, 3.28 Hz, 1H H-4), 5.235 (t, *J*=9.96 Hz, 1H, H-2), 5.05 (dd, *J*=3.32, 9.96 Hz, 1H, H-3), 4.71 (d, *J*=9.96 Hz, 1H, H-1), 4.19 (dd, *J*=7.00, 11.32 Hz, 1H, H-6), 4.11 (dd, *J*=6.16, 11.36 Hz, 1H, H-6), 3.93 (dt, *J*=0.72, 7.00, 7.00 Hz, 1H, H-5), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.49, 170.31, 170.17, 169.55, 132.68, 132.58, 129.02, 128.28, 86.74 (C-1), 74.54 (C-5), 72.18 (C-3), 67.38 (C-4), 67.34 (C-2), 61.75 (C-6), 20.97, 20.79, 20.76, 20.71; LRMS $[M+Na]^+$ $C_{20}H_{24}O_5SNa$ calcd. 463.09, obsd. 463.46; IR (thin film) ν 1751, 1480, 1439, 1369, 1224, 1083, 1055, 917, 747, 693 cm^{-1}. Characterization corresponded to literature data.[39]



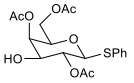
3.18

(3aS,4R,6S,7R,7aR)-4-(hydroxymethyl)-2,2-dimethyl-6-(phenylthio)tetrahydro-4H-[1,3]dioxolo[4,5-c]pyran-7-ol (3.18). To a solution of **S3** (1.0 eq, 18.7 g, 42.5

mmol) in MeOH (212 mL) was added NaOMe (5 mL). The resulting solution stirred at room temperature for 4h and was then neutralized by the addition of Dowex 50WX8 resin. The suspension was filtered and concentrated. The crude residue was suspended in acetone (211 mL) and to it was added 2,2-dimethoxypropane (1.6 eq, 8.38 mL, 67.6 mmol) and p-toluenesulfonic acid (0.1 eq, 800 mg, 4.2 mmol), sequentially. The resulting suspension stirred at room temperature for 24h and was then neutralized by the addition of Et_3N (1 mL). The crude residue was purified by flash chromatography (4:1-1:1 hexanes/EtOAc) to yield 3,4-acetonide **3.18** (7.4 g, 23.7 mmol, 56%) and undesired 4,6-acetonide (2.05 g, 6.56 mmol, 16%) as white foams. **3.18**: R*f* 0.16 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.51 (m, 2H, SPh), 7.35-7.29 (m, 3H, SPh), 4.74 (d, *J*=10.20 Hz, 1H, H-1), 4.19 (dd, *J*=2.16, 5.48 Hz, 1H, H-4), 4.11 (dd, *J*=5.64, 6.84 Hz, 1H, H-3), 3.99 (ddd, *J*=3.32, 7.00, 10.80 Hz, 1H, H-6), 3.88 (ddd, *J*=2.20, 3.92, 6.20 Hz, 1H, H-5), 3.81 (ddd, *J*=3.96, 9.36, 13.28 Hz, 1H, H-6), 3.57 (ddd, *J*=2.40, 6.92, 9.64 Hz, 1H, H-2), 2.48 (d, *J*=2.40 Hz, 1H, C_2-OH), 2.14 (dd, *J*=3.60, 9.48 Hz, 1H, C_6-OH), 1.42 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.72, 131.96, 129.25, 128.34, 110.68 ($C(CH_3)_2$), 87.98 (C-1), 79.29 (C-3), 77.20 (C-5), 74.04 (C-4), 71.66 (C-2), 62.80 (C-6), 28.17, 26.51; LRMS $[M+H]^+$ $C_{15}H_{21}O_5S$ calcd. 313.10, obsd. 313.19; IR (thin film) ν 3434, 3057, 2986, 2935, 2882, 1380, 1220, 1077, 1026, 871, 741, 693 cm^{-1}. Characterization corresponded to literature data.[40]

4,6-acetonide: R*f* 0.06 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68-7.63 (m, 2H, SPh), 7.35-7.28 (m, 3H, SPh), 4.46 (d, *J*=9.00 Hz, 1H, H-1), 4.18 (dd, *J*=1.04, 3.32 Hz, 1H, H-4), 4.025 (ddd, *J*=2.08, 12.84, 18.96 Hz, 2H, H-6), 3.65 (ddd, *J*=1.84, 9.12, 10.92 Hz, 1H, H-2), 3.61 (ddd, *J*=3.48, 9.08, 12.48 Hz, 1H, H-3), 3.43 (dd, *J*=1.60, 2.92 Hz, 1H, H-5), 2.54-2.51 (m, 2H, C_2-OH, C_3-

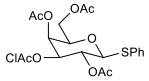
OH), 1.445 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.35, 131.16, 128.99, 128.21, 99.18 ($C(CH_3)_2$), 87.26 (C-1), 73.80 (C-3), 70.11 (C-5), 69.14 (C-2), 68.33 (C-4), 62.94 (C-6), 29.26, 18.81; LRMS $[M+H]^+$ $C_{15}H_{21}O_5S$ calcd. 313.10, obsd. 313.19; IR (thin film) ν 3424, 3057, 2991, 2939, 2881, 1380, 1197, 1092, 1067, 883, 826, 745, 693 cm^{-1}.



S4

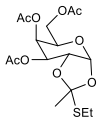
(2R,3R,4S,5R,6S)-2-(acetoxymethyl)-6-(phenylthio)-4-hydroxytetrahydro-2H-pyran-3,5-diyl diacetate (S4). To a solution of **3.18** (1.0 eq, 5.3 g, 14.87 mmol) in MeCN (149 mL) was added trimethyl orthoacetate (3.0 eq, 5.68 mL, 44.6 mmol) and p-toluenesulfonic acid (0.1 eq, 283 mg, 1.487 mmol), sequentially. The resulting solution stirred at room temperature for 45 minutes and was then quenched by the addition of $NaHCO_3$. The solution was extracted with CH_2Cl_2 (3 x 50 mL) and the organics were washed with water (2 x 50 mL), brined (1 x 50 mL), dried over $MgSO_4$, filtered, and concentrated. The crude oil was dissolved in MeCN (149 mL) and to the solution was added 90% aq. TFA (6.1 mL). The reaction stirred for 5 minutes and was then quenched by the addition of $NaHCO_3$. The aqueous solution was extracted with CH_2Cl_2 (3 x 50 mL) and the organics were washed with water (1 x 100 mL), brined (1 x 50 mL), dried over $MgSO_4$, filtered, and concentrated to give a crude white solid. The crude solid was recrystallized from petroleum ether/EtOAc to yield **S4** (4.17 g, 10.46 mmol, 71%) as a fluffy white solid: R*f* 0.38 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.49 (m, 2H, SPh), 7.33-7.28 (m, 3H, SPh), 5.345 (dd, *J*=0.72, 3.65 Hz, 1H, H-4), 5.01 (t, *J*=9.76 Hz, 1H, H-2), 4.68 (d, *J*=9.96 Hz, 1H, H-1), 4.175 (dd, *J*=6.12, 11.52 Hz, 1H, H-6), 4.14 (dd, *J*=2.80, 7.48 Hz, 1H, H-6), 3.875 (ddd, *J*=0.76, 6.32, 6.84 Hz, 1H, H-5), 3.86 (dd, *J*=6.68, 15.4 Hz, 1H, H-3), 2.43

(d, *J*=5.92 Hz, 1H, C_3-OH), 2.17 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.09, 171.05, 170.61, 132.73, 132.65, 129.01, 128.23, 86.26 (C-1), 74.95 (C-5), 72.59 (C-3), 70.94 (C-2), 70.02 (C-4), 62.36 (C-6), 21.16, 20.90, 20.86; LRMS $[M+Na]^+$ $C_{18}H_{22}O_8SNa$ calcd. 421.09, obsd. 421.15; IR (thin film) ν 3473, 1746, 1372, 1229, 1092, 1057, 744 cm^{-1}.



3.19

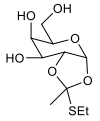
(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-4-(2-chloroacetoxy)-6-(phenylthio)tetrahydro-2H-pyran-3,5-diyl diacetate (3.19). A solution of **S4** (1.0 eq, 4.13 g, 10.37 mmol) in CH_2Cl_2 (104 mL) was cooled to 0°C and to it were sequentially added Et_3N (1.0 eq, 1.45 mL, 10.37 mmol), a catalytic amount of N,N-dimethylaminopyridine, and chloroacetic anhydride (1.5 eq, 2.66 g, 15.55 mmol). The resulting solution stirred at 0°C for 5 minutes and was then diluted with water (100 mL). The organics were extracted, washed with water (2 x 80 mL), brined (1 x 80 mL), dried over $MgSO_4$, filtered, and concentrated to yield **3.19** (4.9 g, 10.3 mmol, >98%) as a sticky white foam. The crude material was used without further purification. R*f* 0.78 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54-7.49 (m, 2H, SPh), 7.34-7.30 (m, 3H, SPh), 5.415 (dd, *J*=0.64, 3.20 Hz, 1H, H-4), 5.26 (t, *J*=9.92 Hz, 1H, H-2), 5.11 (dd, *J*=3.32, 9.92 Hz, 1H, H-3), 4.72 (d, *J*=9.96 Hz, 1H, H-1), 4.20 (dd, *J*=6.96, 11.36 Hz, 1H, H-6), 4.125 (dd, *J*=6.32, 11.36 Hz, 1H, H-6), 3.98-3.93 (m, 3H, H-5, chloroacetate C*H*$_2$Cl), 2.115 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.51, 170.47, 169.48, 166.74, 132.83, 132.32, 129.05, 128.415, 86.64 (C-1), 74.42 (C-5), 73.89 (C-3), 67.13 (C-4), 67.07 (C-2), 61.58 (C-6), 40.505 (chloroacetate *C*H_2Cl), 20.94, 20.77; LRMS $[M+Na]^+$ $C_{20}H_{23}ClO_9SNa$ calcd. 497.07, obsd. 497.44; IR (thin film) ν 1749, 1371, 1225, 1080, 1053, 747, 693 cm^{-1}.



3.22

(3aR,5R,6S,7S,7aR)-5-(acetoxymethyl)-2-(ethylthio)-2-methyltetrahydro-5H-[1,3]dioxolo[4,5-b]pyran-6,7-diyl diacetate (3.22). To a solution of **3.7** (1.0 eq, 30 g, 77 mmol) in CH_2Cl_2 (30 mL) was added 33% HBr/AcOH (30 mL), dropwise. The resulting solution stirred at room temperature for 30 minutes and was then poured over ice and diluted with water (100 mL). The solution was quenched by the addition of $NaHCO_3$ and diluted with CH_2Cl_2 (50 mL). The organics were extracted and washed with $NaHCO_3$ solution (2 x 50 mL), water (1 x 50 mL), and brine (1 x 50 mL). The solution was dried over $MgSO_4$, filtered, and concentrated. The crude product was crystallized from petroleum ether/diethyl ether to yield the anomeric bromide (30.3 g, 73.7 mmol, 96%) as a white solid. The galactosyl bromide (1.0 eq, 1.0 g, 2.43 mmol) and tetrabutylammonium bromide (0.1 eq, 78 mg, 0.243 mmol) were combined in a flask containing activated 4Å powdered molecular sieves (200 mg) and were suspended in MeCN (2.4 mL). To the suspension was added 2,6-lutidine (1.3 eq, 0.37 mL, 3.16 mmol) and the resulting suspension stirred at room temperature for 1h. Ethanethiol (2.0 eq, 0.36 mL, 4.86 mmol) was added to the solution and the reaction stirred at room temperature for 16h. The solution was diluted with water (2 mL) and EtOAc (2 mL). The organics were extracted and washed with water (2 x 5 mL), brined (1 x 5 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography (9:1-3:2 hexanes/EtOAc, 99:1 Et_3N) to yield orthothioacetate **3.22** (630 mg, 1.61 mmol, 66%) as a clear oil: R*f* 0.63 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 (d, *J*=5.00 Hz, 1H, H-1), 5.39 (dd, *J*=2.52, 3.60 Hz, 1H, H-3), 5.07 (dd, *J*=3.72, 6.52 Hz, 1H, H-2), 4.34-4.28 (m, 2H, H-4, H-5), 4.16-4.09 (m, 2H, H-6), 2.64 (qd, *J*=4.96, 7.08 Hz, 2H, C*H*$_2$CH$_3$), 2.10 (s, 3H), 2.06 (s, 3H), 2.056 (s,

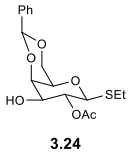
3H), 1.90 (s, 3H, orthothioacetate CH_3), 1.25 (t, *J*=7.44 Hz, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.61, 170.15, 169.93, 115.49 (orthothioacetate *C*(CH_3)SCH_2CH_3), 98.39 (C-1), 72.59 (C-5), 71.18 (C-2), 69.22 (C-4), 65.76 (C-3), 61.74 (C-6), 28.99 (orthothioacetate *C*H_3), 24.95 (orthothioacetate *C*H_2CH_3), 20.85, 20.68, 15.11 (orthothioacetate CH_2*C*H_3); IR (thin film) ν 2969, 2935, 1752, 1436, 1372, 1229, 1157, 1051, 915, 854 cm^{-1}. Characterization corresponded to literature data.[22a]



S5

(3aR,5R,6R,7S,7aR)-2-(ethylthio)-5-(hydroxymethyl)-2-methyltetrahydro-5H-[1,3]dioxolo[4,5-b]pyran-6,7-diol (S5). To a solution of **3.32** (1.0 eq, 416.5 mg, 1.06 mmol) in MeOH (5.3 mL) was added K_2CO_3 (0.1 eq, 15 mg, 0.106 mmol) and the resulting solution stirred at room temperature for 40 minutes. The reaction was concentrated *in vacuo* and the crude residue was purified by flash chromatography (7:3-9:1 CH_2Cl_2/MeOH, 99:1 Et_3N) to yield triol **S5** (172.4 mg, 0.647 mmol, 61%, 1:2 endo:exo) as a clear oil: R*f* 0.45 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, MeOD-d_4) δ 5.78 (d, *J*=5.20 Hz, 1H, H-1), 4.18 (t, *J*=5.60 Hz, 1H, H-2), 3.90-3.84 (m, 2H, H-4, H-5), 3.78-3.68 (m, 3H, H-3, H-6), 2.77 (q, 2H, endo orthothioacetate CH_2CH_3), 2.64 (dq, *J*=1.36, 7.56 Hz, 2H, exo orthothioacetate CH_2CH_3), 1.86 (s, 3H, orthothioacetate CH_3), 1.24 (t, *J*=7.44 Hz, 3H, exo orthothioacetate CH_2CH_3), 1.13 (t, *J*=7.24 Hz, 3H, endo orthothioacetate CH_2CH_3); ^{13}C NMR (100 MHz, MeOD-d_4) δ 115.81 (orthothioacetate *C*(CH_3)SCH_2CH_3), 99.95 (C-1), 77.79 (C-2), 75.11 (C-5), 72.73 (C-3), 68.22 (C-4), 62.26 (C-6), 47.22 (endo orthothioacetate *C*H_2CH_3), 29.43 (orthothioacetate *C*H_3), 25.46 (exo orthothioacetate *C*H_2CH_3), 15.58 (exo orthothioacetate CH_2*C*H_3), 10.48 (endo orthothioacetate

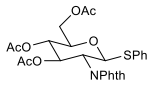
CH_2CH_3); IR (thin film) ν 3406, 2932, 1447, 1378, 1152, 1095, 1043, 971, 921, 861 cm^{-1}. Characterization corresponded to literature data.[24]



3.24

(2S,4aR,6S,7R,8S,8aR)-6-(ethylthio)-8-hydroxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl acetate (3.24). **S5** (1.0 eq, 172 mg, 0.646 mmol) was dissolved in MeCN (10.8 mL) and transferred to a flask containing activated 4Å powdered molecular sieves (1 g). The resulting suspension stirred at room temperature for 30 minutes, after which ethanethiol (0.21 eq, 10 µL, 0.135 mmol) was added and stirring continued for 30 additional minutes. TMSOTf (0.1 eq, 12 µL, 0.065 mmol) was added to the solution at room temperature and the reaction proceeded for 10 minutes. The solution was filtered through Celite and concentrated *in vacuo*. The crude thioglycoside was dissolved in MeCN (3.2 mL) and to the solution was added p-toluenesulfonic acid (0.25 eq, 31 mg, 0.161 mmol) and benzaldehyde dimethylacetal (1.25 eq, 0.121 mL, 0.807 mmol), sequentially. The reaction stirred at room temperature for 2h and was then heated to 60°C for 2h. The solution was returned to room temperature and was diluted with EtOAc (3 mL) and water (3 mL). The organics were extracted and washed with water (1 x 5 mL), brined (1 x 5 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography (7:3-3:7 hexanes/EtOAc) to yield benzylidene acetal **3.24** (64.9 mg, 0.183 mmol, 28%) as a white foam: R*f* 0.43 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52-7.47 (m, 2H, benzylidene Ph), 7.42-7.37 (m, 3H, benzylidene Ph), 5.55 (s, 1H, benzylidene C*H*Ph), 5.19 (t, *J*=9.68 Hz, 1H, H-2), 4.41 (d, *J*=9.90 Hz, 1H, H-1), 4.37 (dd, *J*=1.52, 12.60 Hz, 1H, H-6), 4.27 (dd, *J*=0.68, 3.76 Hz, 1H,

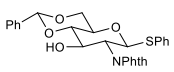
H-4), 4.04 (dd, *J*=1.76, 12.52 Hz, 1H, H-6), 3.75 (ddd, *J*=3.80, 9.50, 13.30 Hz, 1H, H-3), 3.53 (dd, *J*=1.44, 1.72 Hz, 1H, H-5), 2.91-2.81 (m, 1H, C*H*$_2$CH$_3$), 2.77-2.66 (m, 1H, C*H*$_2$CH$_3$), 2.46 (d, *J*=12.00 Hz, 1H, C$_3$-OH), 2.14 (s, 3H), 1.305 (t, *J*=7.48 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.50, 137.19, 129.42, 128.38, 126.48, 101.58 (benzylidene *C*HPh), 82.59 (C-1), 75.77 (C-4), 72.43 (C-3), 70.20 (C-2), 70.01 (C-5), 69.20 (C-6), 23.06 (*C*H$_2$CH$_3$), 21.11, 14.93 (CH$_2$*C*H$_3$); LRMS $[M+NH_4]^+$ $C_{17}H_{26}NO_6S$ calcd. 372.14, obsd. 372.16; IR (thin film) ν 3461, 2973, 2870, 1753, 1371, 1233, 1166, 1099, 1064, 993, 817, 731, 699 cm^{-1}.



3.26

(2R,3S,4R,5R,6S)-2-(acetoxymethyl)-5-(1,3-dioxoisoindolin-2-yl)-6-(phenylthio)tetra hydro-2H-pyran-3,4-diyl diacetate (3.26). To a suspension of **3.10** (1.0 eq, 30 g, 140 mmol) in MeOH (280 mL) was added NaOMe (1.0 eq, 100 mL, 1.4 M, 140 mmol) and the resulting mixture was allowed to stir 10 minutes. Phthalic anhydride (1.2 eq, 24.8 g) was added to the solution and it was heated to 60°C. The reaction proceeded at 60°C for 35 minutes, after which a white precipitate had formed. The suspension was returned to room temperature and the solid was filtered via vacuum filtration, washed with MeOH (50 mL), and allowed to dry for 16h yielding the phthalate (32.1 g, 104 mmol, 74.3%) as a white solid. The compound was used in the next reaction without further purification or characterization. The solid was dissolved in pyridine (208 mL) and to it was added a catalytic amount N,N-dimethylaminopyridine. The resulting mixture was cooled to 0°C. Acetic anhydride (5.0 eq, 49.0 mL, 519 mmol) was added to the solution dropwise and it was returned to room temperature and stirred for 16h. The reaction was diluted with water (300 mL) and EtOAc (200

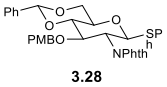
mL), washed with 2N HCl (5 x 100 mL), water (2 x 200 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was coevaporated with toluene (3 x 100 mL) to give the tetraacetate (20.4 g, 42.7 mmol, 41%, isomeric mixture) as a yellow foam. The foam was dissolved in CH_2Cl_2 (214 mL) and to it was added thiophenol (1.2 eq, 5.3 mL, 51.3 mmol). The resulting mixture was cooled to 0°C and $SnCl_4$ (2.0 eq, 10.0 mL, 85 mmol) was added. The reaction was allowed to warm to room temperature and stir for 3h. The reaction was quenched by the addition of saturated $NaHCO_3$ solution. The resulting mixture was washed with Rochelle's salt (1 x 100 mL), water (2 x 100 mL), brine (1 x 100 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude mixture was crystallized from petroleum ether/EtOAc yielding thioglycoside **3.26** (22.54 g, 25.6 mmol, 60%) as a yellow solid: R*f* 0.5 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (dd, *J*= 3.12, 5.48 Hz, 2H, NPhth), 7.76 (dd, *J*=3.04, 5.52 Hz, 2H, NPhth), 7.42 (dd, *J*=1.52, 7.36 Hz, 2H, SPh), 7.28 (m, 3H, SPh), 5.798 (dd, *J*=9.32, 10.04 Hz, 1H, H-3), 5.72 (d, *J*=10.60 Hz, 1H, H-1), 5.14 (dd, *J*=9.44, 9.96 Hz, 1H, H-4), 4.35 (t, *J*=10.36 Hz, 1H, H-2), 4.29 (dd, *J*=5.08, 12.20 Hz, 1H, H-6), 4.21 (dd, *J*=2.32, 12.24 Hz, 1H, H-6), 3.90 (ddd, *J*=2.30, 5.00, 10.20 Hz, 1H, H-5), 2.10 (s, 3H), 2.02 (s, 3H), 1.84 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 170.78, 170.25, 169.60, 133.44, 131.12, 129.05, 128.57, 123.87, 83.21 (C-1), 76.05 (C-5), 71.77 (C-3), 68.87 (C-4), 62.38 (C-6), 53.72 (C-2), 20.91, 20.77, 20.56; LRMS $[M+Na]^+$ $C_{26}H_{25}NO_9SNa$ calcd. 550.12, obsd. 550.29; IR (thin film) ν 1748, 1718, 1383, 1227, 1071, 1038, 721, 691 cm^{-1}. Characterization corresponded to reported literature data.[39]



3.27

2-((2R,4aR,6S,7R,8R,8aS)-8-hydroxy-2-phenyl-6(phenylthio) hexahydropyrano[3,2-d][1,3]dioxin-7-yl)isoindoline-1,3-dione (3.27). To a solution of **3.26** (1.0 eq, 13.4 g, 25.4 mmol) in MeOH (127 mL) was added NaOMe (1.0 eq, 19 mL, 1.3 M, 25.4 mmol). The resulting solution was allowed to stir 1.5h at room temperature. The reaction was neutralized by the addition of Dowex 50WX8 resin to neutral pH, filtered, and concentrated *in vacuo*. The triol (10 g, 24.9 mmol, 98%) was isolated as a yellow foam. To a solution of the glucosamine triol (1.0 eq, 5.07 g, 12.6 mmol) in CH_3CN (42 mL) was added benzaldehyde dimethyl acetal (1.25 eq, 2.37 mL, 15.8 mmol) and p-toluenesulfonic acid (0.1 eq, 0.24 g, 1.26 mmol), sequentially. The resulting solution was heated to 65°C and allowed to stir for 19h. The reaction was quenched by the addition of triethylamine (0.5 mL) and was returned to room temperature. The solution was diluted with EtOAc (50 mL), washed with water (2 x 50 mL), brine (1 x 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude reside was purified by flash chromatography (17:3-0:1 hexanes/EtOAc) to yield acetal **3.27** (3.73 g, 7.62 mmol, 60%) as a yellow foam: R*f* 0.85 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, CDCl3) δ 7.90 (br, 1H, NPhth), 7.85 (br, 1H, NPhth), 7.75 (dd, *J*=2.48, 5.24 Hz, 2H, NPhth), 7.48 (m, 2H, SPh), 7.38 (m, 5H, benzylidene Ph), 7.27 (m, 3H, SPh), 5.70 (d, *J*=10.50 Hz, 1H, H-1), 5.57 (s, 1H, benzylidene C*H*Ph), 4.65 (td, *J*=3.40, 9.88, 9.88 Hz, 1H, H-3), 4.41 (dd, *J*=4.80, 10.32 Hz, 1H, H-6), 4.34 (t, *J*=9.52 Hz, 1H, H-2), 3.83 (t, *J*=10.08 Hz, 1H, H-6), 3.71 (td, *J*=4.80, 9.40, 9.40 Hz, 1H, H-5), 3.61 (t, *J*=9.12 Hz, 1H, H-4), 2.45 (d, *J*=3.40 Hz, 1H, C_3-OH); ^{13}C (100 MHz, $CDCl_3$) δ 137.03, 134.41, 132.85, 131.87, 129.56, 129.12, 128.55, 128.29, 126.45, 124.04, 123.55, 102.15 (*C*HPh), 84.44 (C-1), 82.06 (C-4), 70.45 (C-5), 69.92 (C-3), 68.74 (C-6), 55.64 (C-2); LRMS $[M+H]^+$

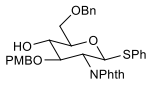
$C_{27}H_{24}NO_6S$ calcd. 490.13, obsd. 490.60; IR (thin film) ν 3478, 1774, 1713, 1387, 1091, 746, 719, 699 cm^{-1}. Characterization corresponded to reported literature data.[41]



3.28

2-((2R,4aR,6S,7R,8R,8aS)-8-((4-methoxybenzyl)oxy)-2-phenyl-6-(phenylthio) hexahydropyrano[3,2-d][1,3]dioxin-7-yl)isoindoline-1,3-dione (3.28). **3.27** (1.0 eq, 300 mg, 0.613 mmol) and **3.15** (5.0 eq, 0.86 mg, 3.06 mmol) were combined and coevaporated from benzene (2 x 2 mL) and placed under vacuum for 45 minutes. The compounds were dissolved in diethyl ether (3 mL) and the resulting solution was cooled to 0°C. $BF_3{\cdot}Et_2O$ (10µL) was added to the solution at 0°C and the reaction proceeded for 5 minutes. The reaction was quenched with $NaHCO_3$ and diluted with diethyl ether (2 mL). The ethereal layer was washed with water (2 x 2 mL), brine (1 x 2 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (9:1-7:3 hexanes/EtOAc) to yield **3.28** (295 mg, 0.48 mmol, 79%) as a yellow solid: R*f* 0.45 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J*=7.10 Hz, 1H, NPhth), 7.79-7.68 (m, 3H, NPhth), 7.63 (d, *J*=6.90 Hz, 1H, NPhth), 7.54 (dd, *J*=2.04, 7.92 Hz, 2H, SPh), 7.45-7.33 (m, 6H, SPh, benzylidene Ph), 7.28-7.23 (m, 3H, benzylidene Ph), 6.90 (d, *J*=8.60 Hz, 2H, OPMB), 6.37 (d, *J*=8.60 Hz, 2H, OPMB), 5.64 (s, 1H, benzylidene C*H*Ph), 5.63 (d, *J*=10.6 Hz, 1H, H-1), 4.71 (d, *J*=12.20 Hz, 1H, CH_2Ar), 4.46-4.40 (m, 3H, CH_2Ar, H-3, H-6), 4.26 (t, *J*=10.32 Hz, 1H, H-2), 3.89-3.68 (m, 3H, H-4, H-5, H-6), 3.62 (s, 3H, PhOCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.84, 167.33, 163.655, 158.96, 137.395, 134.02, 133.96, 132.84, 131.69, 129.95, 129.91, 129.24, 129.01, 128.40, 128.20, 126.16, 123.56, 123.28, 113.475, 101.415 (*C*HPh), 84.13 (C-1), 82.89 (C-5), 75.12 (C-3), 73.90 (*C*H_2Ar), 70.50 (C-4), 68.76

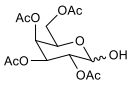
(C-6), 54.97 (C-2), 54.85 (PMB *C*H_3); LRMS $[M+H]^+$ $C_{15}H_{32}NO_7S$ calcd. 610.19, obsd. 610.37; IR (thin film) ν 3370, 3246, 1713, 1610, 1511, 1385, 1246, 1106, 831, 720, 648 cm^{-1}. Characterization corresponded to reported literature data.[42]



3.29

2-((2S,3R,4R,5S,6R)-6-((benzyloxy)methyl)-5-hydroxy-4-((4methoxybenzyl)oxy)-2-(phenylthio)tetrahydro-2H-pyran-3-yl)isoindoline-1,3-dione (3.29). To a solution of **3.28** (1.0 eq, 295 mg, 0.484 mmol) in THF (4 mL) was added $BH_3{\cdot}NMe_3$ (4.67 eq, 165 mg, 2.26 mmol). A suspension of $AlCl_3$ (5.0 eq, 323 mg, 2.42 mmol) in THF (4 mL) was added to the solution, followed by the addition of water (10 µL). The reaction stirred at room temperature for 20h and was then quenched by the addition of water. The solution was diluted with EtOAc (5 mL) and the mixture was washed with water (1 x 10 mL), saturated $NaHCO_3$ solution (1 x 10 mL), brine (1 x 10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (4:1-1:1 hexanes/EtOAc) yielding **3.29** (63.5 mg, 104 mmol, 21%) as a clear oil: R*f* 0.57 (1:1 hexanes/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, *J*=7.40 Hz, 1H, NPhth), 7.74-7.64 (m, 3H, NPhth), 7.38-7.29 (m, 7H, OBn, SPh), 7.22-7.16 (m, 3H, SPh), 6.95 (d, *J*=8.6 Hz, 2H, OPMB), 6.45. (d, *J*=8.6 Hz, 2H, OPMB), 5.55 (d, *J*=10.00 Hz, 1H, H-1), 4.64 (d, *J*=12.00 Hz, 1H, OCH_2Ar), 4.62 (d, *J*=11.36 Hz, 1H, CH_2Ph), 4.575 (d, *J*=11.96 Hz, 1H, CH_2Ph), 4.46 (d, *J*=12.00 Hz, 1H, OCH_2Ar), 4.26-4.18 (m, 2H, H-2, H-4), 3.85 (dd, *J*=5.00, 10.24 Hz, 1H, H-6), 3.83-3.74 (m, 2H, H-3, H-6), 3.70 (dd, *J*=4.80, 9.60 Hz, 1H, H-5), 3.62 (s, 3H, PhOCH_3), 2.81 (d, *J*=2.60 Hz, 1H, C_4-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.11, 167.39, 158.95, 137.81, 133.94, 132.49, 131.66, 130.25, 129.70, 128.89, 128.56, 127.92, 127.85, 123.50, 123.24,

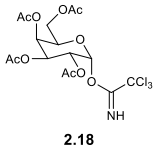
113.60, 83.65 (C-1), 79.43 (C-4), 78.05 (C-5), 74.22 (C-3), 74.07 (*C*H_2Ph), 73.82 (PMB *C*H_2Ar), 70.68 (C-6), 54.96 (PMB *C*H_3), 54.57 (C-2); LRMS $[M+H]^+$ $C_{35}H_{34}NO_7S$ calcd. 612.21, obsd. 612.37; IR (thin film) ν 3473, 1774, 1713, 1611, 1512, 1386, 1248, 1081, 720 cm^{-1}. Characterization corresponded to reported literature data.[42]



3.30

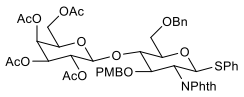
(2R,3S,4S,5R)-2-(acetoxymethyl)-6-hydroxytetrahydro-2H-pyran-3,4,5-triyl triacetate (3.30). To a solution of **3.7**(1.0 eq, 1.0 g, 2.6 mmol) in THF (13 mL) was added N,N-dimethylaminopropylamine (5.0 eq, 1.6 mL, 12.8 mmol) at room temperature and the resulting solution was allowed to stir 1.5h. The reaction was quenched by the addition of 1N HCl. The resulting mixture was diluted with EtOAc (20 mL), washed with 1N HCl (2 x 20 mL), water (2 x 50 mL), and brine (1 x 50 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (2:1-1:1 hexanes/EtOAc) to yield **3.30** (451 mg, 1.3 mmol, 51%, 5:1 α:β) as a white foam: R*f* 0.3 (1:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (t, *J*=3.52 Hz, 1H, H-1α), 5.48 (dd, *J*=1.0, 3.28 Hz, 1H, H-4α), 5.41 (dd, *J*=3.36, 10.8 Hz, 1.2H, H-3α, H-3β), 5.17 (ddd, *J*=1.0, 3.56, 10.8 Hz, 1H, H-2), 5.07 (m, 0.4H, H-2β, H-4β), 4.69 (ddd, *J*=3.04, 4.56, 9.0 Hz, 0.2H, H-1β), 4.47 (td, *J*=0.8, 6.56, 6.56 Hz, 1H, H-5α), 4.14 (t, *J*=6.76 Hz, 0.4H, H-6β), 4.10 (ddd, *J*=6.76, 11.28, 18.12 Hz, 2H, H-6α), 3.95 (dt, *J*=0.92, 6.6, 6.6 Hz, 0.2H, H-5β), 3.54 (d, *J*=9.08 Hz, 0.2H, C_1-OHβ) 2.97 (dd, *J*=1.16, 3.52 Hz, 1H, C_1-OHα), 2.16 (s, 0.6H), 2.15 (s, 3H), 2.11 (s, 0.6H), 2.10 (s, 3H), 2.05 (s, 3.6H), 2.00 (s, 0.6H), 1.99 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 170.66, 170.49, 170.37, 170.19, 96.21 (C-1β), 90.88 (C-1α), 71.28 (C-5β), 70.42 (C-2β), 68.40 (C-2α), 68.33 (C-4α), 67.34 (C-3α), 67.26

(C-3β), 66.49 (C-5α), 61.97 (C-6α), 61.60 (C-6β); LRMS: $[M+Na]^+$ $C_{14}H_{20}O_{10}Na$ calcd. 371.10, obsd. 371.61; IR (thin film) ν 3461, 1747, 1372, 1231, 1052, 736, 601 cm^{-1}. Characterization corresponded to reported literature data.[43]



2.18

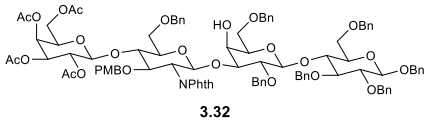
(2R,3S,4S,5R,6R)-2-(acetoxymethyl)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (2.18). To a solution of **3.30** (1.0 eq, 0.1 g, 0.3 mmol) in CH_2Cl_2 (1.4 mL) was added trichloroacetonitrile (5.0 eq, 0.3 mL, 1.4 mmol) followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (0.25 eq, 11 µL, 0.07 mmol). The resulting mixture was stirred at room temperature for 1h. The reaction was diluted with CH_2Cl_2 (2 mL), washed with water (2 x 5 mL), brine (1 x 5 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (17:3-7:3 hexanes/EtOAc, 99:1 Et_3N) yielding **2.18** (85 mg, 0.29 mmol, 60%) as a yellow foam: R*f* 0.7 (1:1 hexanes/EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, NH), 6.60 (d, *J*=3.48 Hz, 1H, H-1), 5.56 (dd, *J*=1.16, 3.08 Hz, 1H, H-4), 5.42 (dd, *J*=3.12, 10.88 Hz, 1H, H-3), 5.36 (dd, *J*=3.48, 7.36 Hz, 1H, H-2), 4.43 (dt, *J*=0.88, 6.80, 6.80 Hz, 1H, H-5), 4.12 (ddd, *J*=6.60, 11.32, 33.76 Hz, 2H, H-6), 2.16 (s, 3H), 2.02 (s, 3H), 2.014 (s, 3H), 2.009 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 170.43, 170.23, 170.21, 170.10, 161.08 (OC(NH)), 93.67 (C-1), 90.91 (CCl_3), 69.13 (C-5), 67.64 (C-3), 67.51 (C-4), 67.04 (C-2), 61.39 (C-6), 20.80, 20.77, 20.74, 20.68; IR (thin film) ν 3341, 1751, 1677, 1371, 1226, 1073, 797, 643 cm^{-1}. Characterization corresponded to reported literature data.[43]



3.31

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3S,4R,5R,6S)-2-((benzyloxy) methyl)-5-(1,3-dioxoisoindolin-2-yl)-4-((4-methoxybenzyl)oxy)-6-(phenylthio)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (3.31). **2.18** (1.5 eq, 76 mg, 0.154 mmol) and **3.29** (1.0 eq, 63 mg, 0.103 mmol) were coevaporated with benzene (3 x 2 mL) and placed under vacuum for 16h. The compounds were dissolved in CH_2Cl_2 (1 mL) and were transferred to a flask containing activated 4Å powdered molecular sieves (100 mg). The resulting suspension stirred at room temperature for 1h under argon atmosphere. The solution was then cooled to -40°C and a drop of $BF_3{\cdot}Et_2O$ was added. The reaction slowly returned to room temperature and stirred for 1.5h. The reaction was then quenched by the addition of triethylamine (3 drops) and was filtered through a plug of cotton. The organics were washed with water (2 x 2 mL), brine (1 x 2 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (4:1-1:1 hexanes/EtOAc) to yield recovered **3.29** (14.4 mg, 23%) as a clear oil and disaccharide **3.31** (25.5 mg, 0.027 mmol, 26%) as a clear oil: R*f* 0.7 (2:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J*=7.00 Hz, 1H, NPhth), 7.73-7.64 (m, 2H, NPhth), 7.60 (d, *J*=6.80 Hz, 1H, NPhth), 7.44-7.30 (m, 7H, OBn, SPh), 7.22-7.16 (m, 3H, SPh), 6.92 (d, *J*=8.60 Hz, 2H, OPMB), 6.36 (d, *J*=8.60 Hz, 2H, OPMB), 5.49 (d, *J*=10.20 Hz, 1H, H-1), 5.30 (dd, *J*=0.64, 3.40 Hz, 1H, H-4'), 5.155 (dd, *J*=8.00, 10.40 Hz, 1H, H-2'), 4.88 (dd, *J*=3.48, 10.40 Hz, 1H, H-3'), 4.76 (d, *J*=12.00 Hz, 1H, C*H*$_2$Ar), 4.67 (d, *J*=12.30 Hz, 1H, C*H*$_2$Ph), 4.625 (d, *J*=8.00 Hz, 1H, H-1'), 4.52 (d, *J*=12.10 Hz, 1H, C*H*$_2$Ar), 4.39 (d, *J*=12.30 Hz, 1H, C*H*$_2$Ph), 4.24 (t, *J*=8.06 Hz, 1H, H-3), 4.21-3.98 (m, 5H, H-2, H-4, H-5', H-6'), 3.81-3.74 (m, 2H, H-6), 3.69 (t, *J*=6.69 Hz, 1H, H-5), 3.59 (s, 3H, PhOC*H*$_3$), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.39, 170.24, 170.16, 169.975,

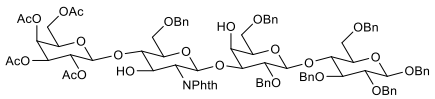
169.10, 158.53, 137.85, 133.57, 132.54, 132.05, 131.49, 130.51, 129.60, 128.70, 128.49, 127.88, 127.75, 123.23, 123.00, 113.15, 100.33 (C-1'), 90.65, 83.38 (C-1), 78.995, 77.89 (C-4), 77.15 (C-3), 74.15 (CH_2Ar), 73.51, 70.93 (C-3'), 70.47 (C-5), 69.42 (C-2'), 67.65 (C-6), 66.82 (C-4'), 60.63 (C-6'), 54.71 (C-2), 54.67 (PMB CH_3), 20.73, 20.59, 20.56, 20.49; LRMS $[M+Na]^+$ $C_{49}H_{51}NO_{16}SNa$ calcd. 964.28, obsd.964.57; IR (thin film) ν 3482, 1751, 1715, 1611, 1512, 1386, 1225, 1078, 721 cm^{-1}.



3.32

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3S,4R,5R,6S)-6-(((2S,3R,4S,5S,6R)-3-(benzyloxy)-6-((benzyloxy)methyl)-5-hydroxy-2-(((2R,3R,4S,5R,6R)-4,5,6-tris(benzyloxy)-2-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-4-yl)oxy)-2-((benzyloxy)methyl)-5-(1,3-dioxoisoindolin-2-yl)-4-((4-methoxybenzyl)oxy)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (19). **3.31** (1.0 eq, 25 mg, 0.027 mmol) and **3.1** (1.1 eq, 26 mg, 0.029 mmol) were combined and coevaporated from benzene (3 x 1 mL). The compounds were dried in a vacuum desiccator for 16h in the presence of phosphorus pentoxide. They were then dissolved in CH_2Cl_2 (0.26 mL) and transferred to a round bottom flask containing activated 4Å powdered molecular sieves (30 mg). The resulting suspension was allowed to stir at room temperature for 1.5 H and was then cooled to -40°C. NIS (1.5 eq, 7.2 mg, 0.032 mmol) and AgOTf (0.5 eq, 3.4 mg, 0.013 mmol) were sequentially added to the suspension at -40°C. The reaction was allowed to stir at -40°C for 2h and was then warmed to room temperature for 24h. Upon

completion, the reaction was quenched by the addition of Et_3N (10 µL). The solution was diluted with CH_2Cl_2 (1 mL). The reaction mixture was filtered through a cotton plug and was diluted with water (1 mL). The organics were extracted and washed with water (2 x 1 mL), brined (1 x 1 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified twice by preparatory TLC (20:1 CH_2Cl_2/CH_3OH; 1:1 hexanes/EtOAc) and flash chromatography (2:1-1:1 hexanes/EtOAc) to yield **3.32** (1.9 mg, 4%) as a residue: R*f* 0.52 (1:1 hexanes/EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.88 (d, *J*=8.5 Hz, 2H, OPMB), 6.33 (d, *J*=8.6 Hz, 2H, OPMB), 5.18 (H-2’’’), 5.35 (m, H-1’’), 5.32 (H-4’’’), 4.92(H-3’’’), 4.59 (d, *J*=8.0 Hz, 1H, H-1’’’), 4.34 (d, *J*=7.5 Hz, 1H, H-1), 4.26 (m, H-1’), 4.24 (H-2’’), 4.09 (H-6’’’), 4.04 (H-6’’’), 4.03(H-3’’, H-4’), 3.61(H-4’’), 3.86 (H-4), 3.75 (H-5’’’), 3.74 (H-5’’), 3.69 (H-6, H-6’’), 3.57 (s, 3H, PhOCH_3), 3.49 (H-6’), 3.41 (H-3’), 3.40 (H-2’, H-3), 3.39 (H-2), 3.38 (H-6’), 3.37 (H-6), 3.03 (H-5), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 102.3 (C-1), 101.8 (C-1’), 100.5 (C-1’’’), 98.7 (C-1’’), 83.6 (C-3), 83.0 (C-3’), 81.8 (C-2’), 81.7 (C-2), 78.1 (C-3’’, C-4’), 76.5 (C-2’’), 75.9 (C-4), 74.8 (C-5), 74.6 (C-4’’), 71.0 (C-3’’’), 70.6 (C-5’’, C-5’’’), 69.4 (C-2’’’), 68.1 (C-6), 67.8 (C-6’, C-6’’), 66.9 (C-4’’’), 60.8 (C-6’’’), 54.8 (PhO*C*H_3); LRMS $[M+Na]^+$ $C_{97}H_{103}NO_{27}Na$ calcd. 1736.66, obsd. 1736.86; IR (thin film) ν 2920, 2850, 1752, 1715, 1454, 1390, 1367, 1250, 1218, 1072, 741, 699 cm^{-1}.



3.33

(2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(((2R,3S,4R,5R,6S)-6-(((2S,3R,4S,5S,6R)-3-(benzyloxy)-6-((benzyloxy)methyl)-5-hydroxy-2-(((2R,3R,4S,5R,6R)-4,5,6-tris(benzyloxy)-2-((benzyloxy)methyl)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-4-yl)oxy)-2-((benzyloxy)methyl)-5-(1,3-dioxoisoindolin-2-yl)-4-hydroxytetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (3.33) **3.31** (1.0 eq, 300 mg, 0.318 mmol) and **3.1** (1.1 eq, 309 mg, 0.309 mmol) were combined and coevaporated from benzene (3 x 1 mL). The compounds were dried in a vacuum desiccator for 16h in the presence of phosphorus pentoxide. They were then dissolved in CH_2Cl_2 (3.2 mL) and transferred to a round bottom flask containing activated 4Å powdered molecular sieves (300 mg). The resulting suspension was allowed to stir at room temperature for 2 H and was then cooled to -40°C. NIS (1.2 eq, 86 mg, 0.382 mmol) and a drop of TMSOTf were sequentially added to the suspension at -40°C. The reaction was allowed to stir at -40°C for 2h and was then warmed to room temperature for 4h. An additional drop of TMSOTf was added and stirring continued for 1h, after which an additional 4 drops of TMSOTf were added and the reaction proceeded for 21h. AgOTf (0.5 eq, 41 mg, 0.159 mmol) and NIS (0.8 eq, 57 mg, 0.255 mmol) were added and stirring continued for 48h. Upon completion, the reaction was quenched by the addition of Et_3N (10 µL). The solution was diluted with CH_2Cl_2 (1 mL). The reaction mixture was filtered through a cotton plug and was diluted with water (5 mL). The organics were extracted and washed with water (2 x 5 mL), brined (1 x 5 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude oil was purified by flash chromatography (9:1 acetone/toluene) to yield 3.33 (118.3 mg, 23%) as a yellow oil: R*f* 0.56 (9:1 acetone/toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (d, *J*=9.03 Hz, 1H, H-1''), 4.51 (d, *J*=7.46 Hz, 1H, H-1'''), 4.37 (d,

J=7.46 Hz, 1H, H-1), 4.31 (d, *J*=8.71 Hz, 1H, H-1'); ^{13}C NMR (100 MHz, $CDCl_3$) δ 101.9 (C-1), 101.6 (C-1'), 101.1 (C-1'''), 98.4 (C-1'').

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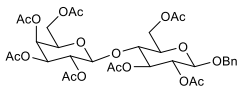
Appendix A1:

Spectra Relevant to Chapter III.



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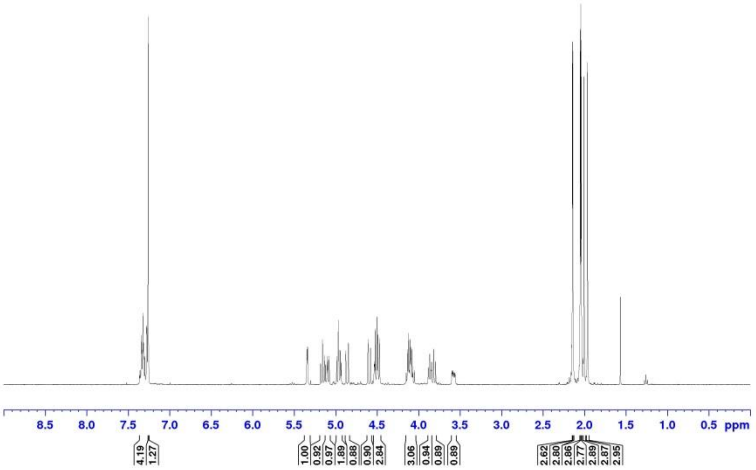


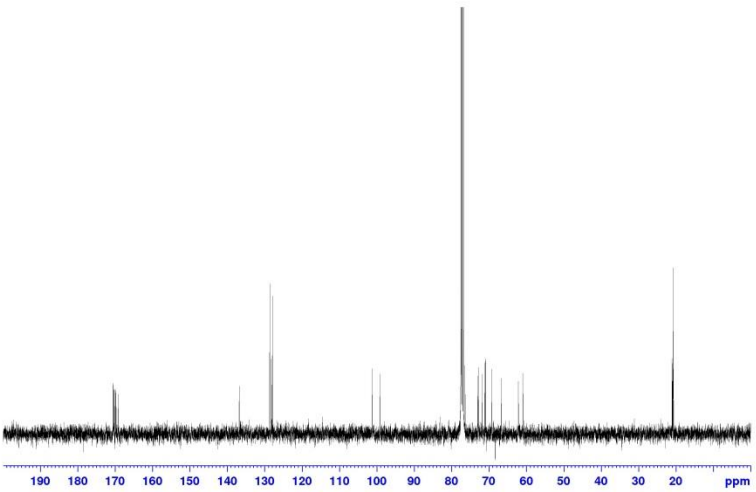
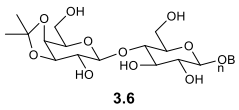


Figure A1.1. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.5**.



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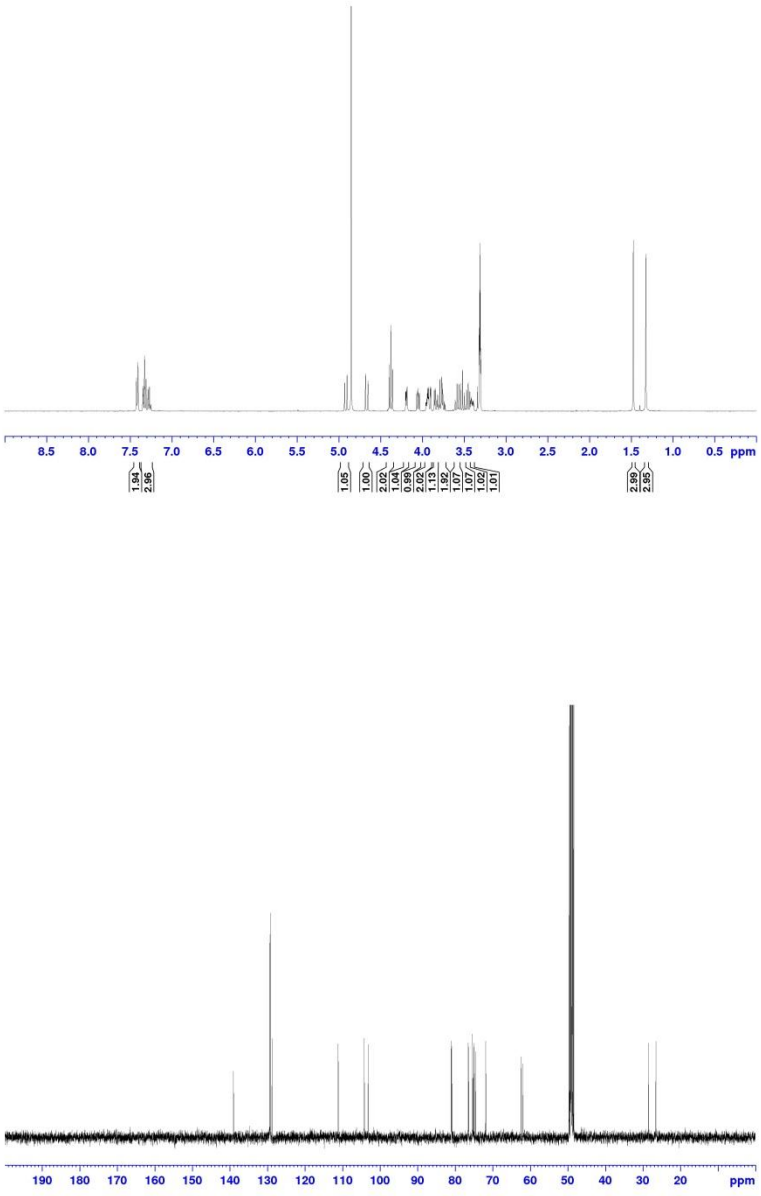
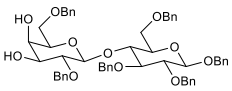


Figure A1.2. ^{1}H NMR (400 MHz, MeOD-d_4) and ^{13}C NMR (100 Mhz, MeOD-d_4) spectra of **3.6**.



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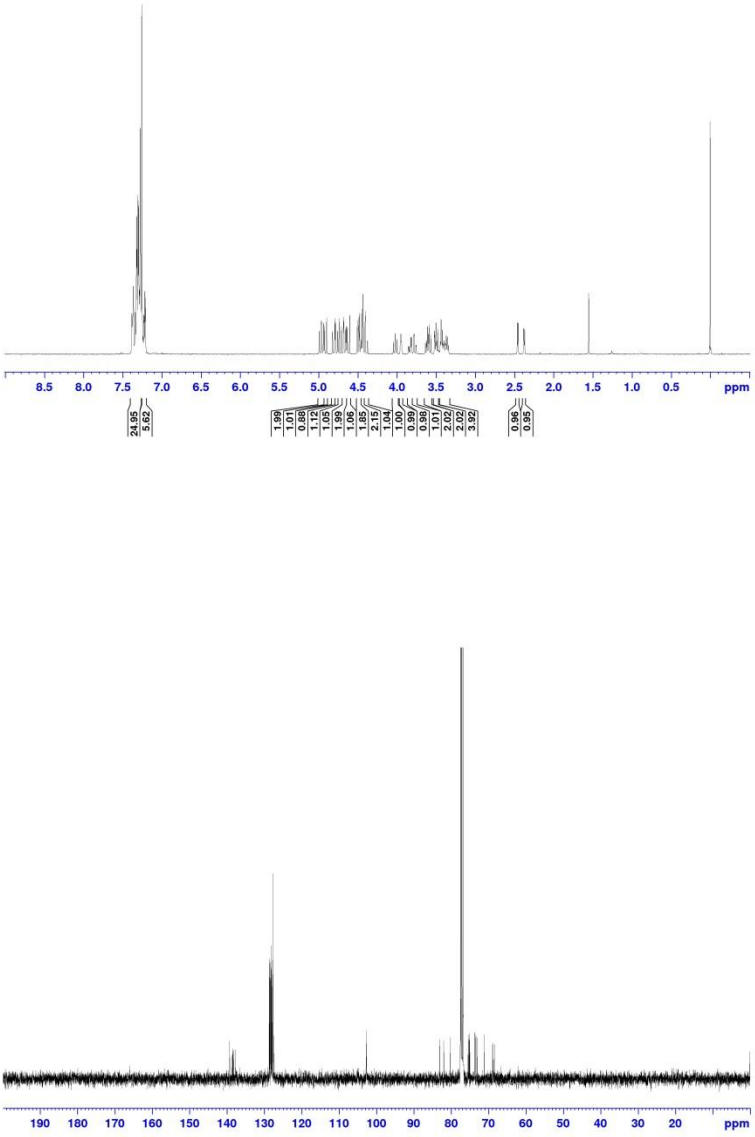
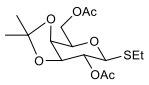


Figure A1.3. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.1**.



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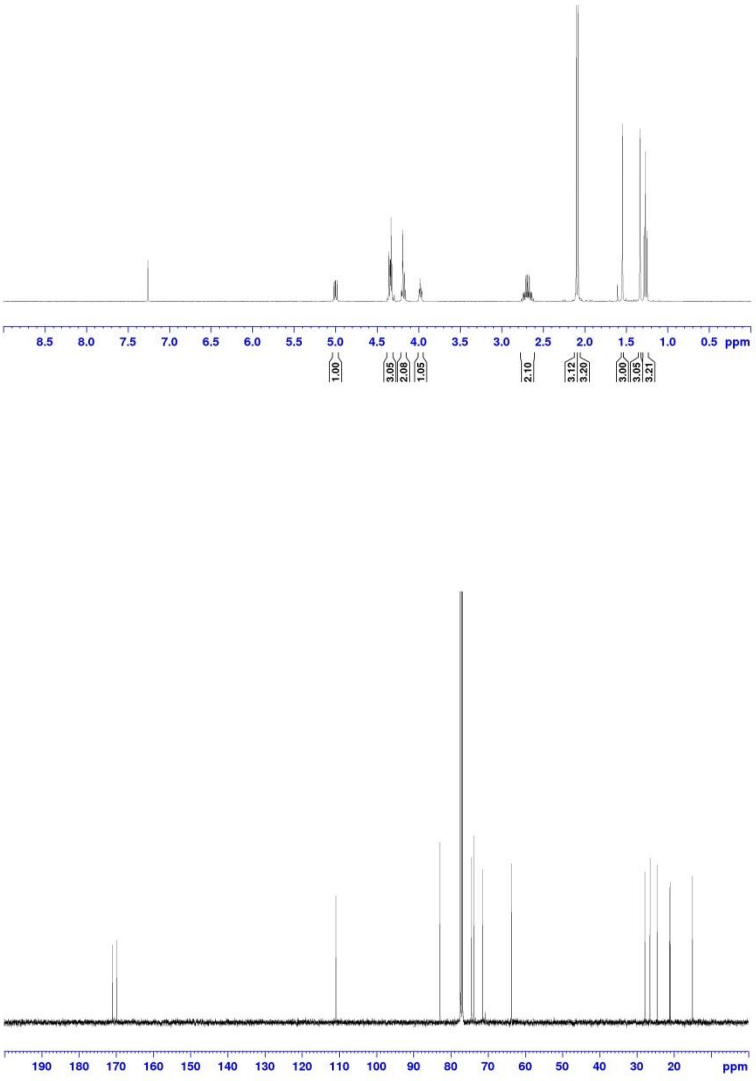
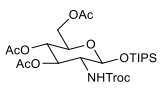


Figure A1.4. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.2**.



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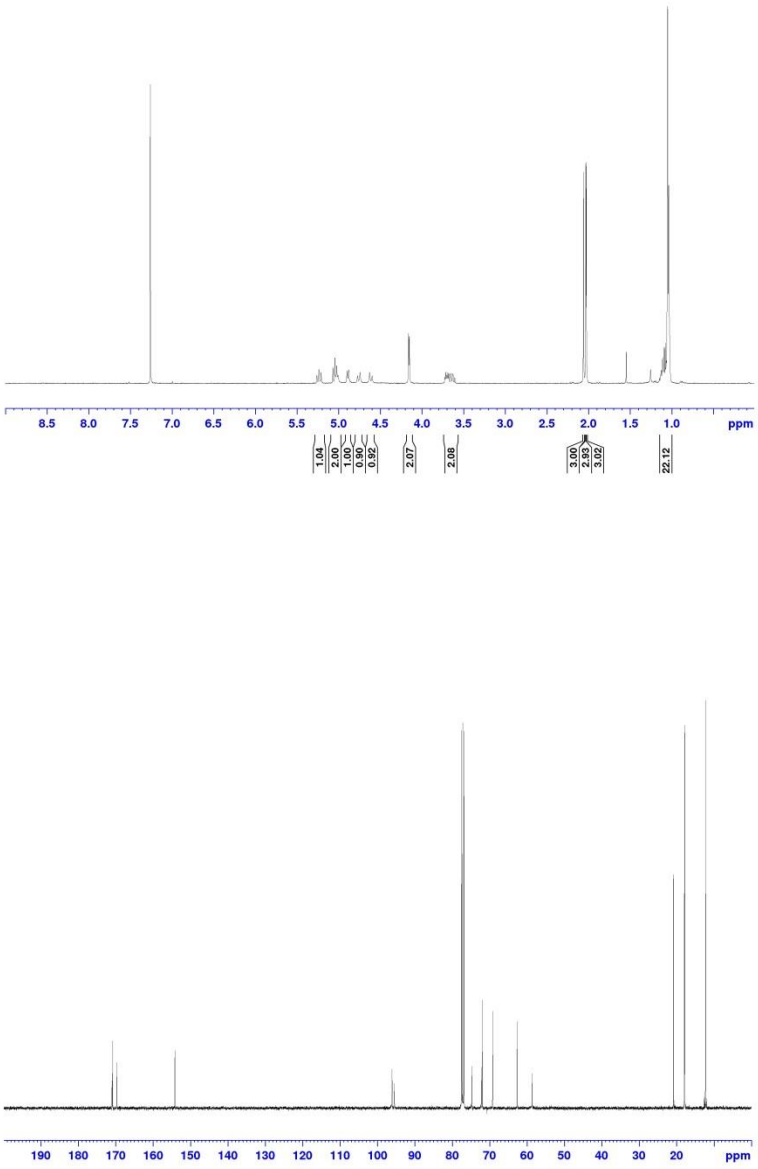
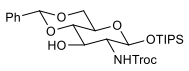


Figure A1.5. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.12**.



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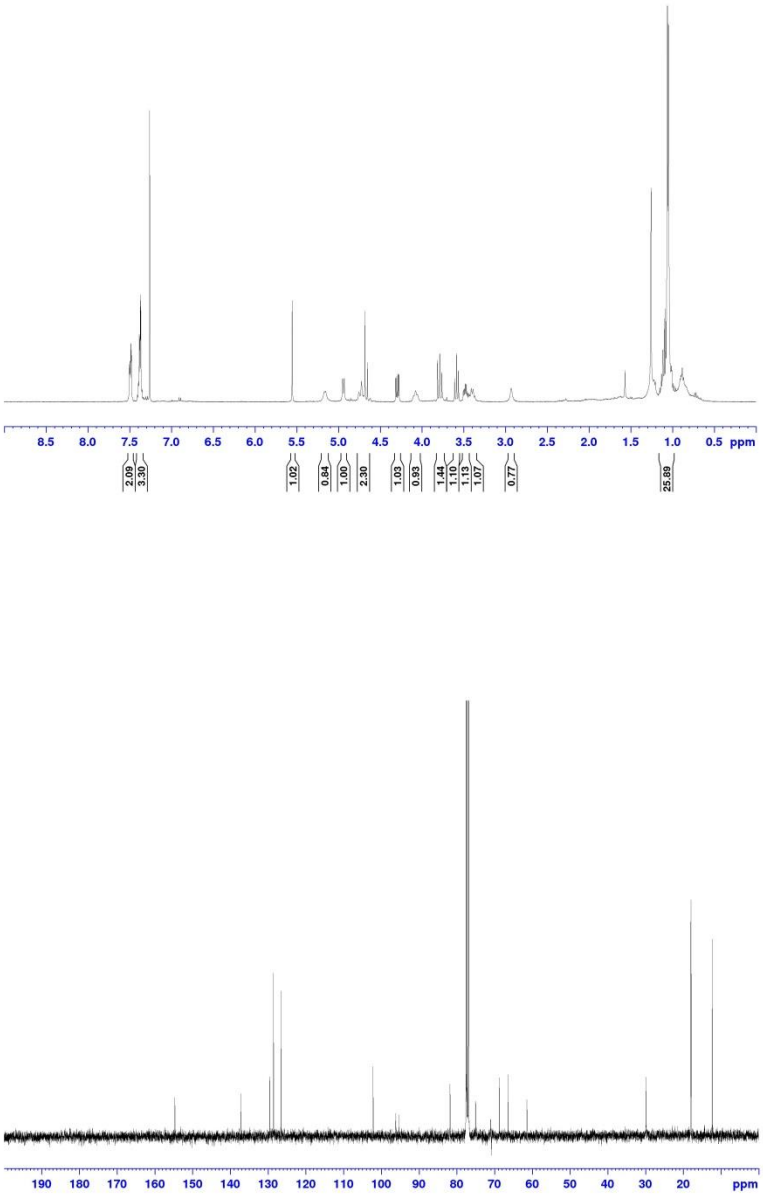
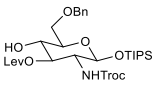


Figure A1.6. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.13**.



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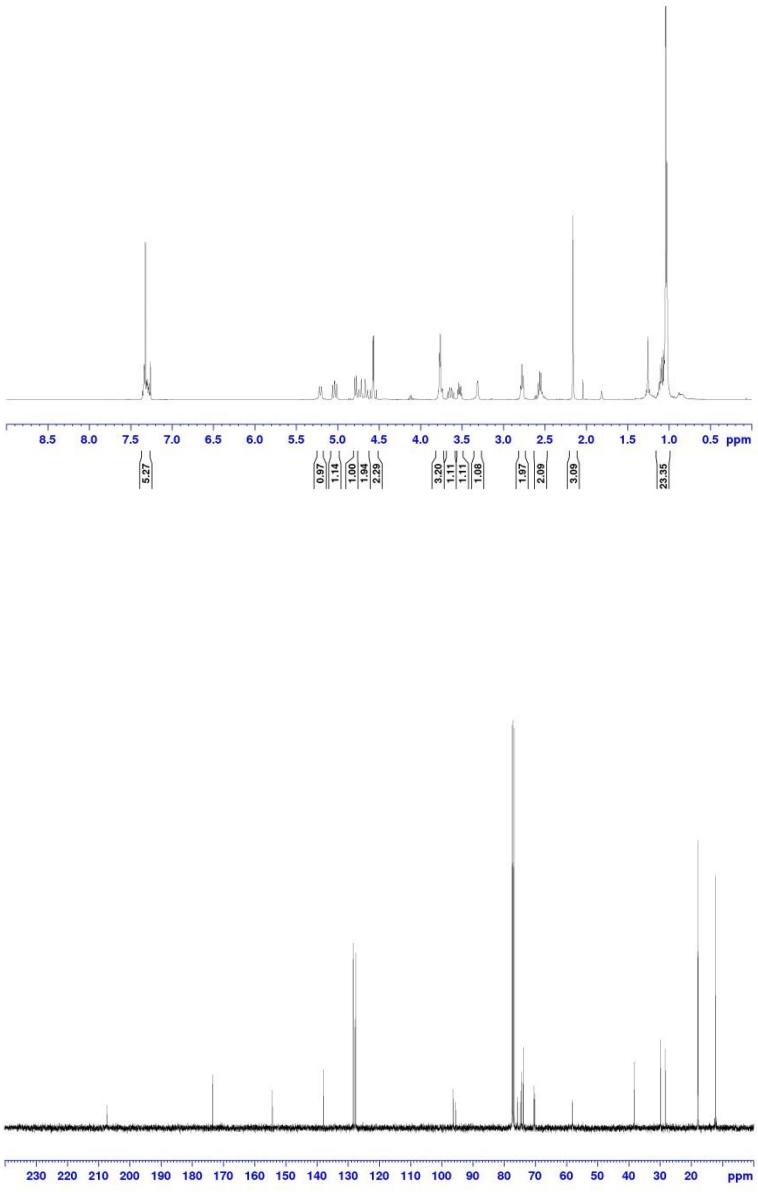
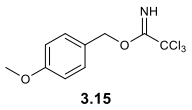
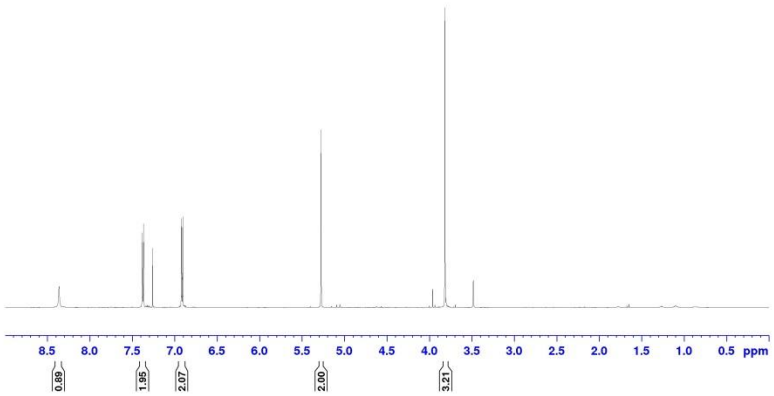


Figure A1.7. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.3**.



3.15



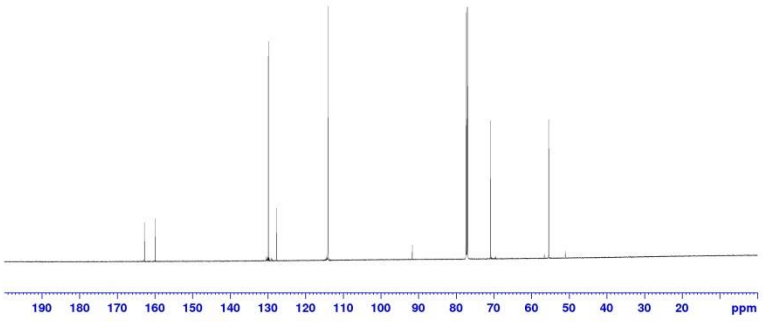
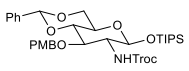


Figure A1.8. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.15**.



S2

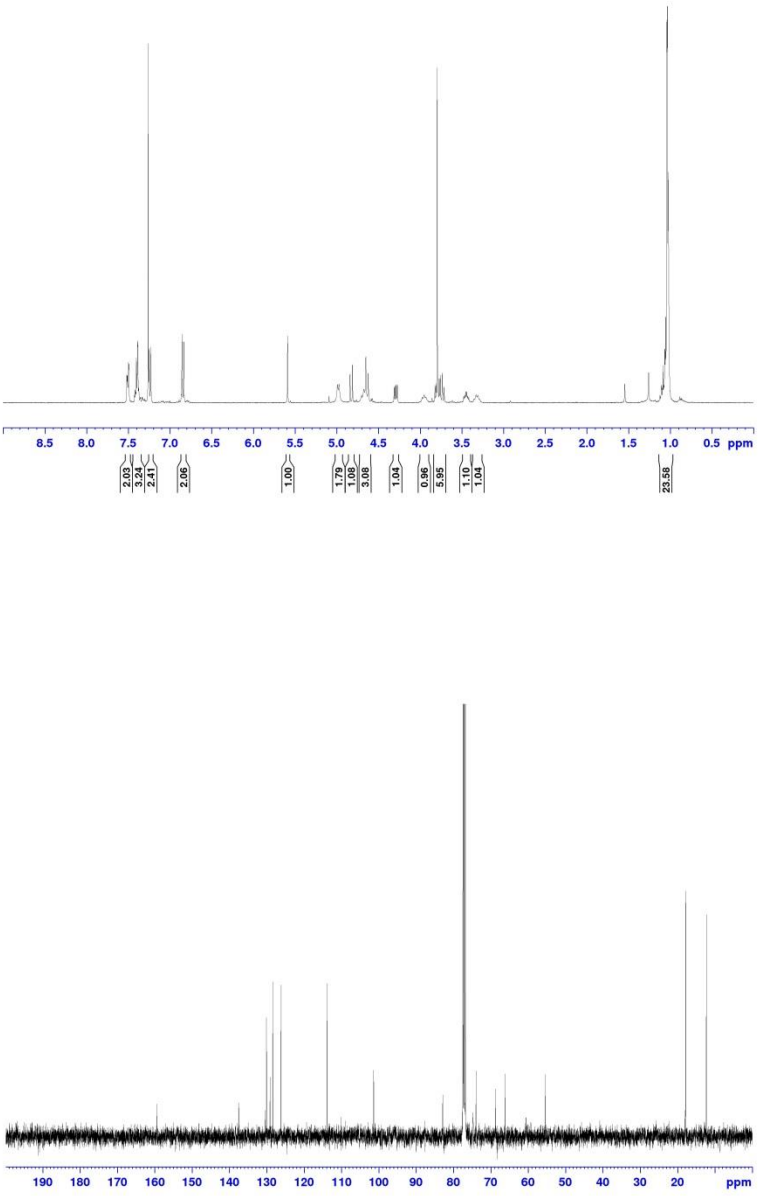
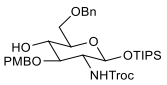


Figure A1.9. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **S2**.



3.14

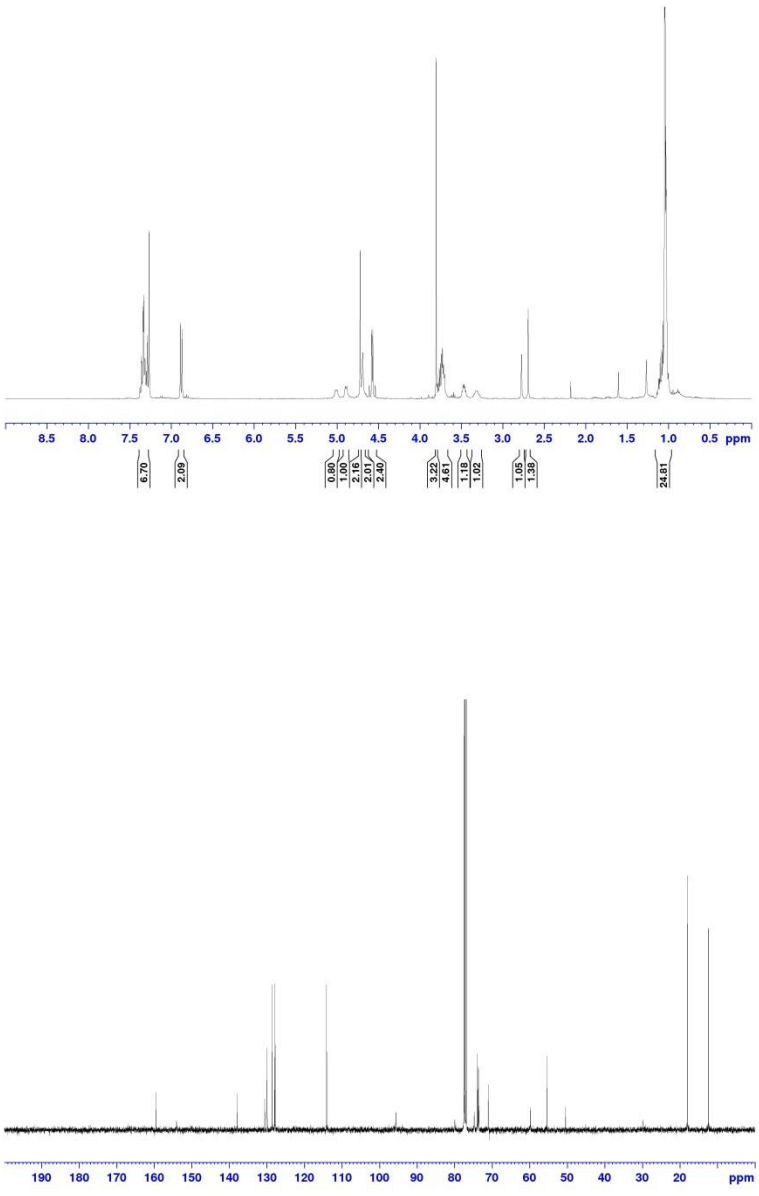
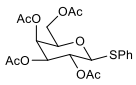


Figure A1.10. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.14**.



S3

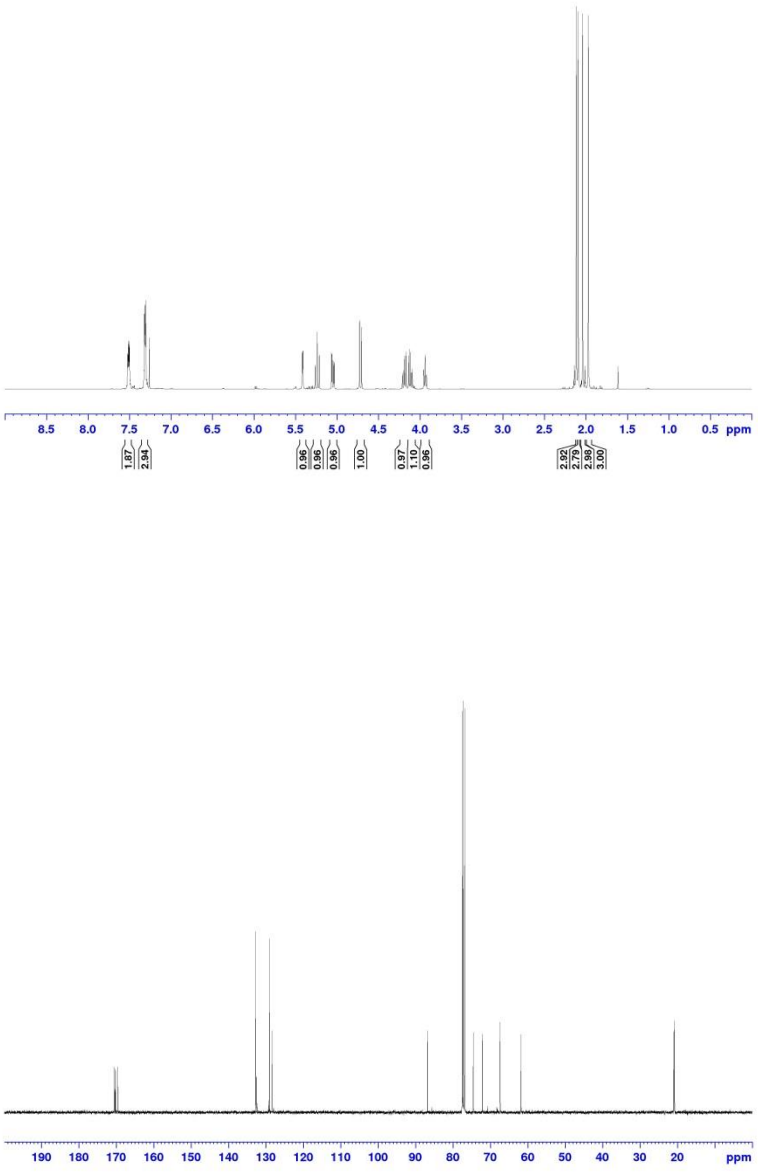
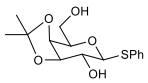


Figure A1.11. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **S3**.



3.18

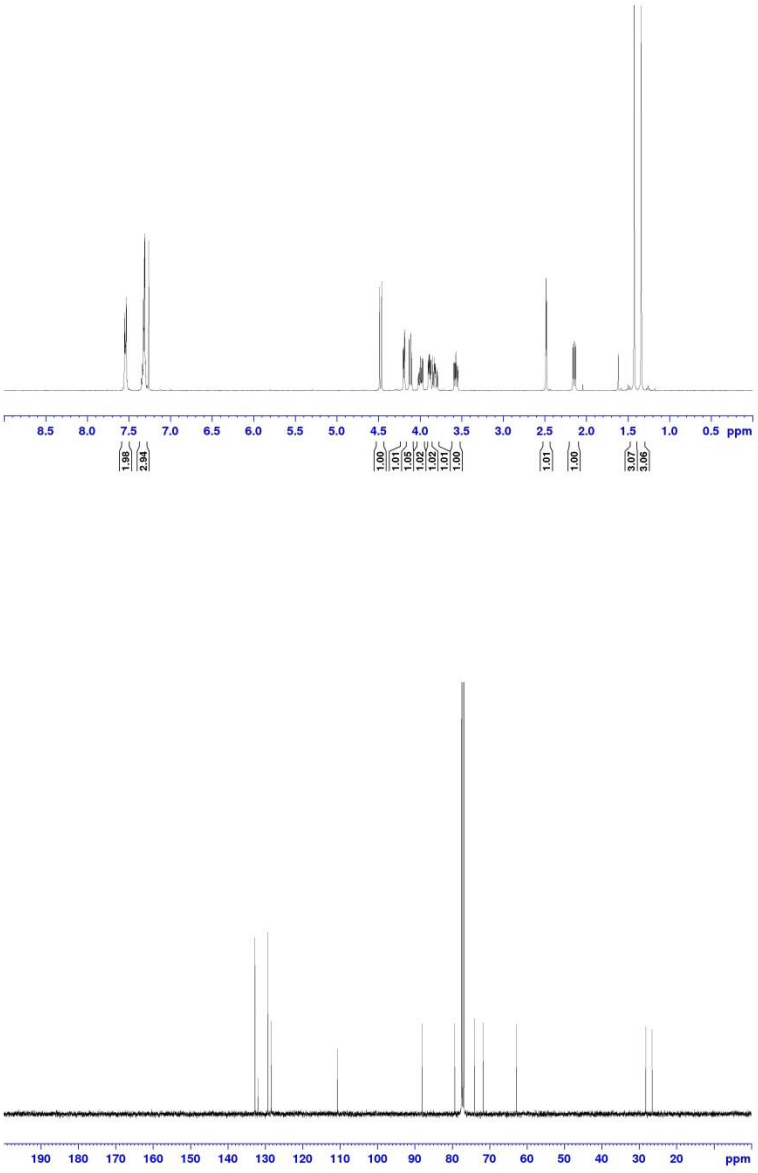
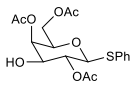


Figure A1.12. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.18**.



S4

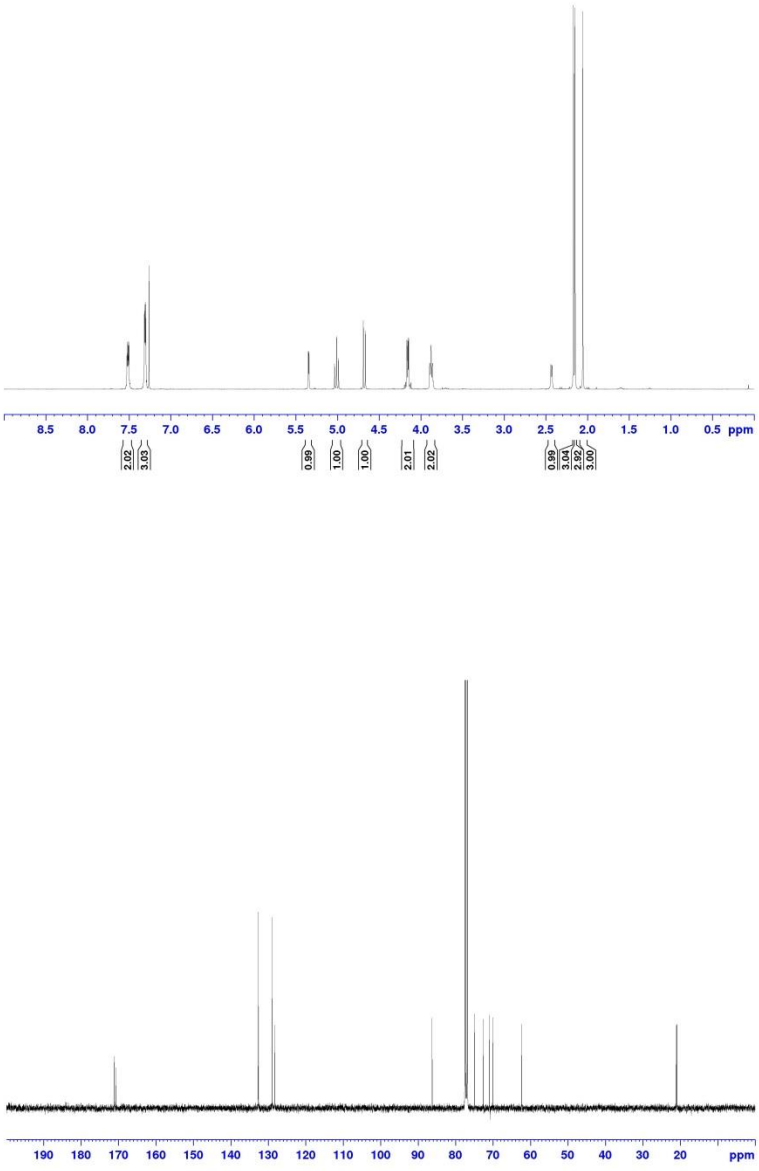
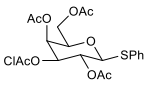


Figure A1.13. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **S4**.



3.19

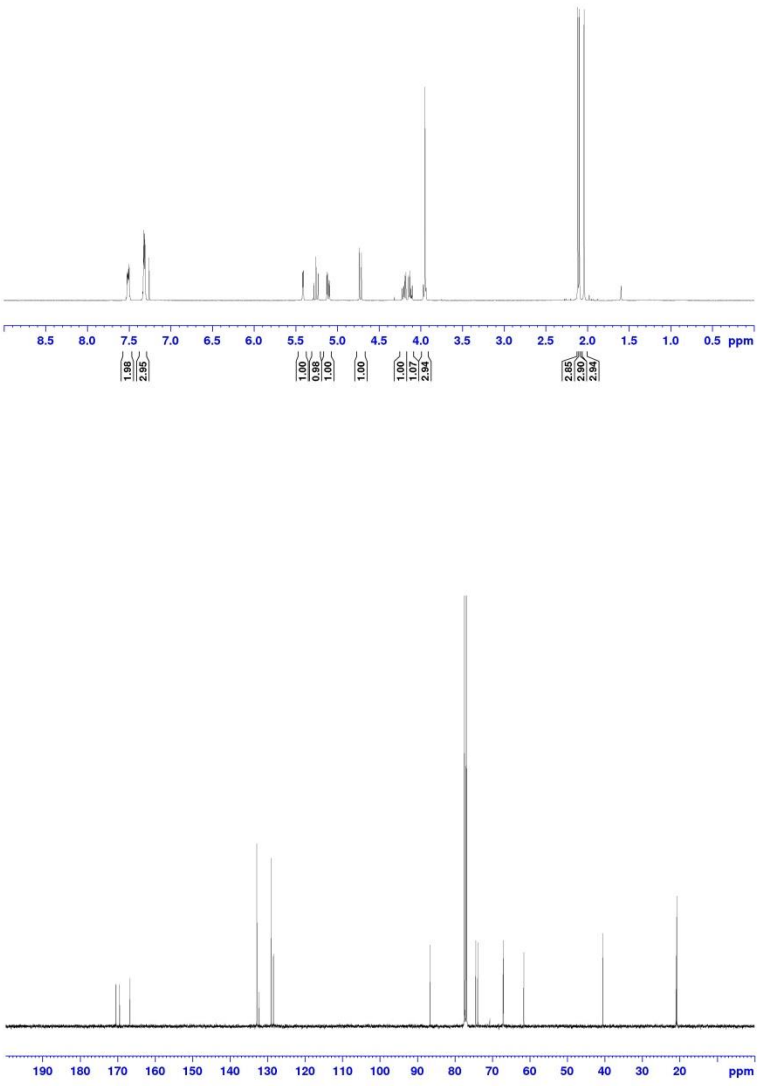
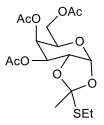
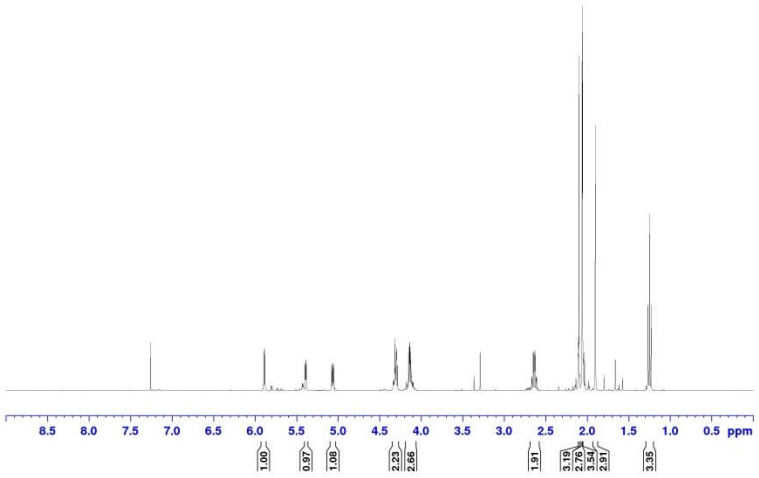


Figure A1.14. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.19**.



3.22



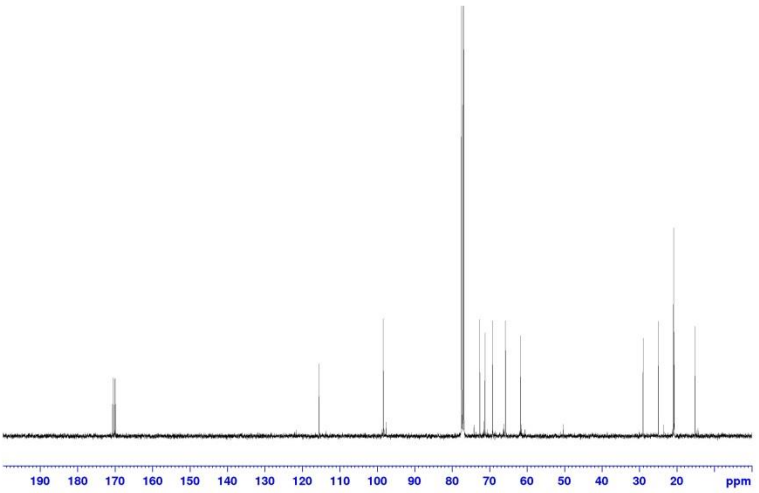
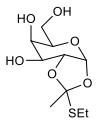


Figure A1.15. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.22**.



S5

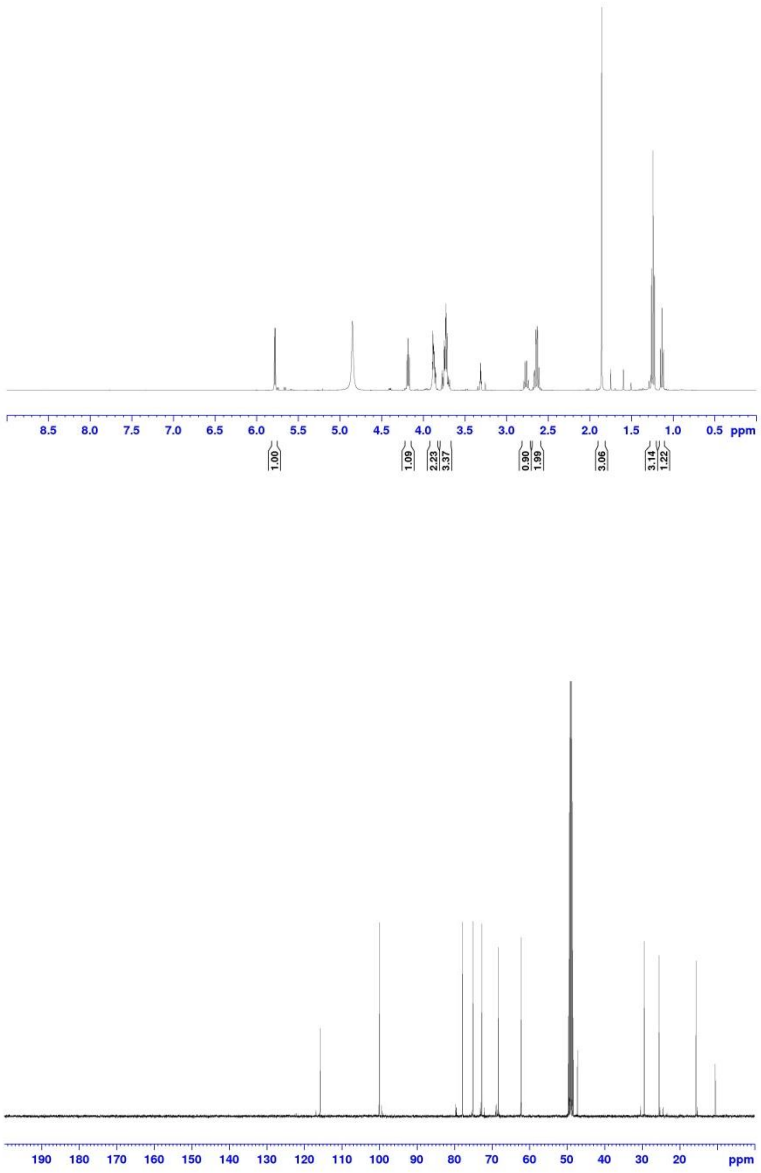
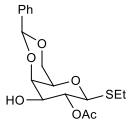


Figure A1.16. ^{1}H NMR (400 MHz, MeOD-d_4) and ^{13}C NMR (100 MHz, MeOD-d_4) spectra of **S5**.



3.24

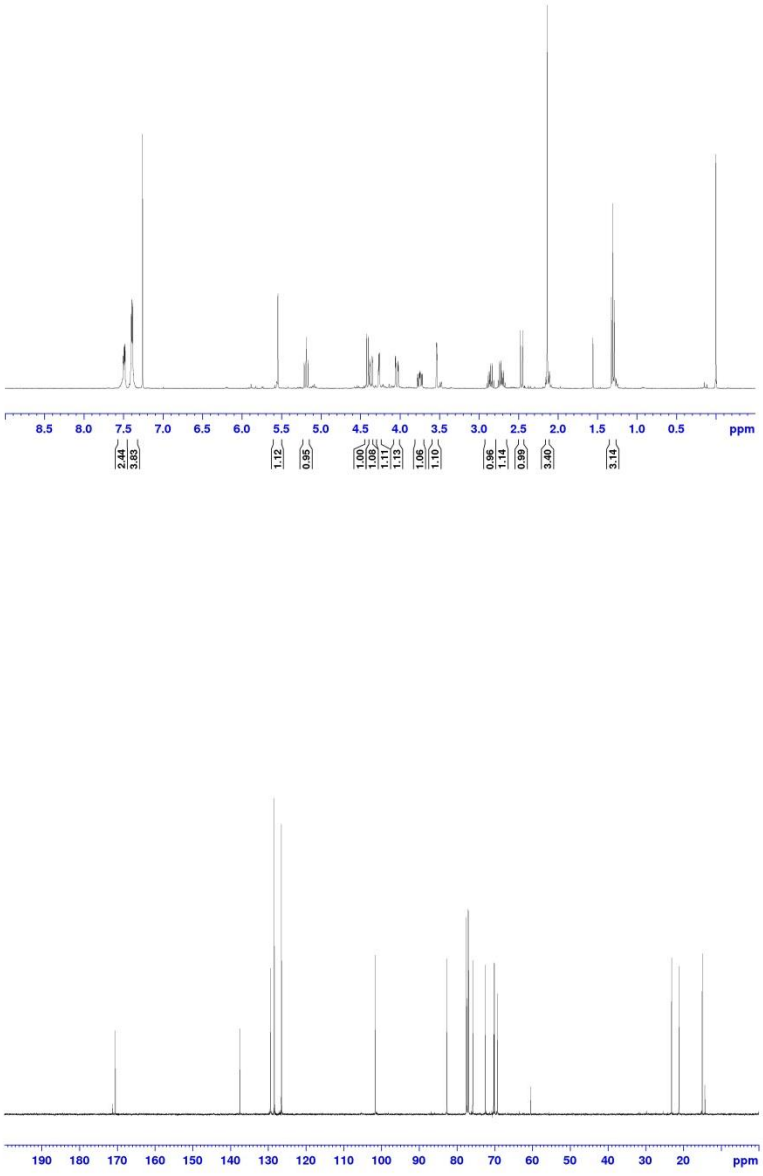
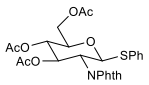


Figure A1.17. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.24**.



3.26

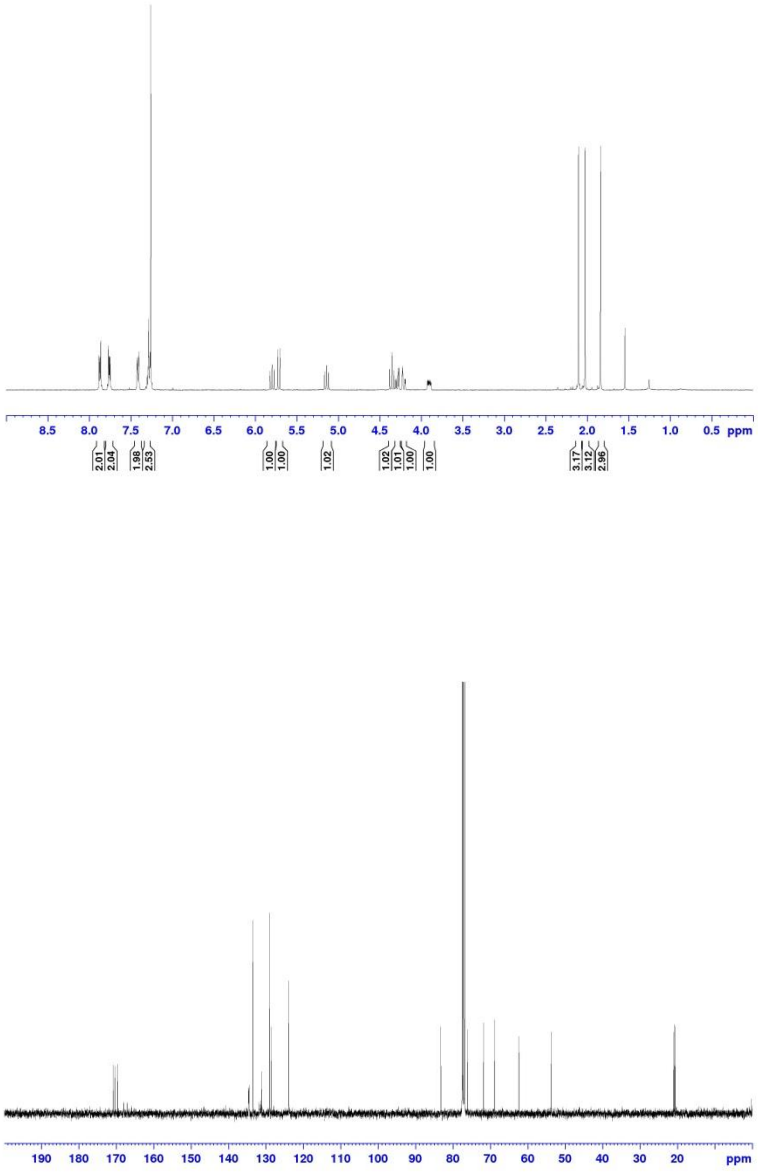
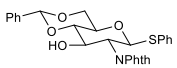


Figure A.18. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 Mhz, $CDCl_3$) spectra of **3.26**.



3.27

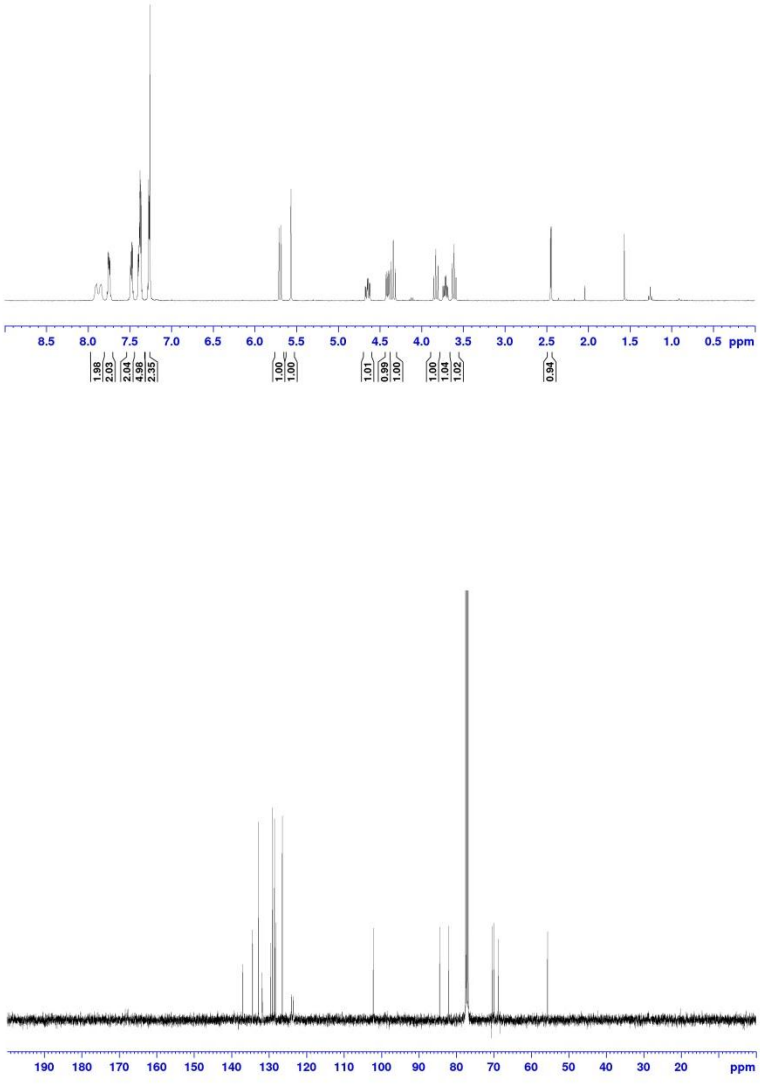
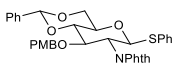


Figure A1.19. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.27**.



3.28

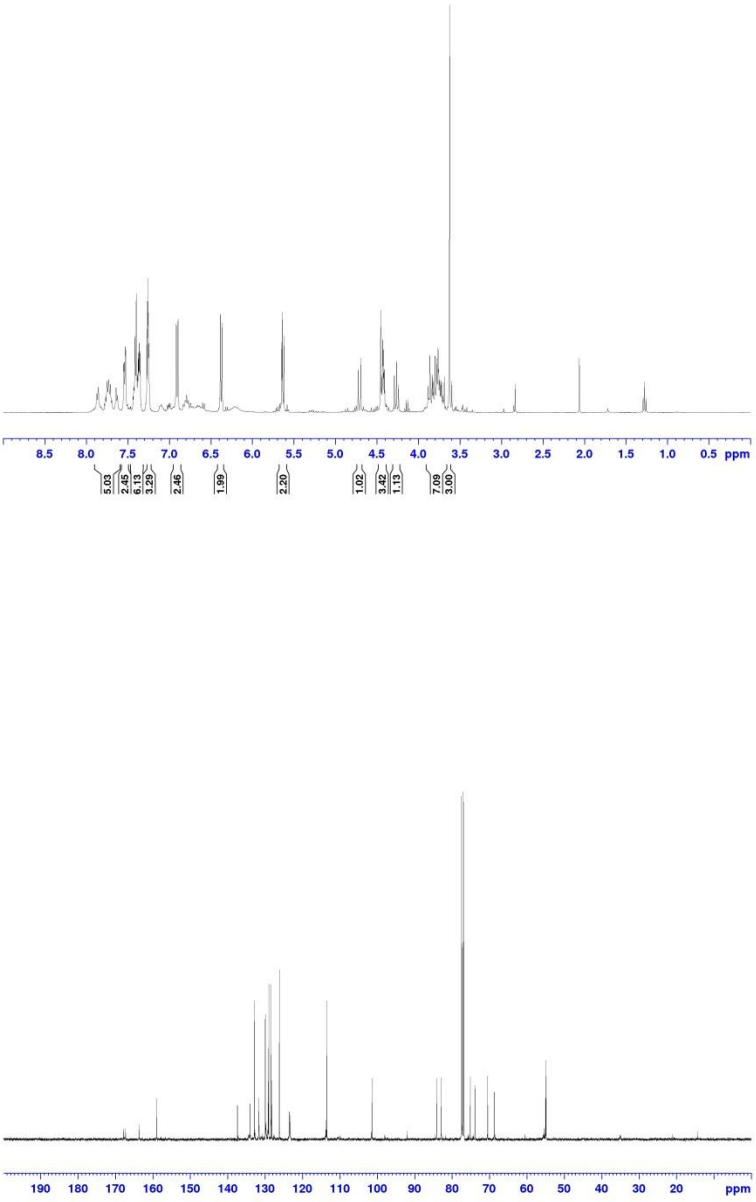
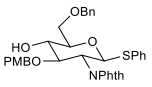


Figure A1.20. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.28**.



3.29

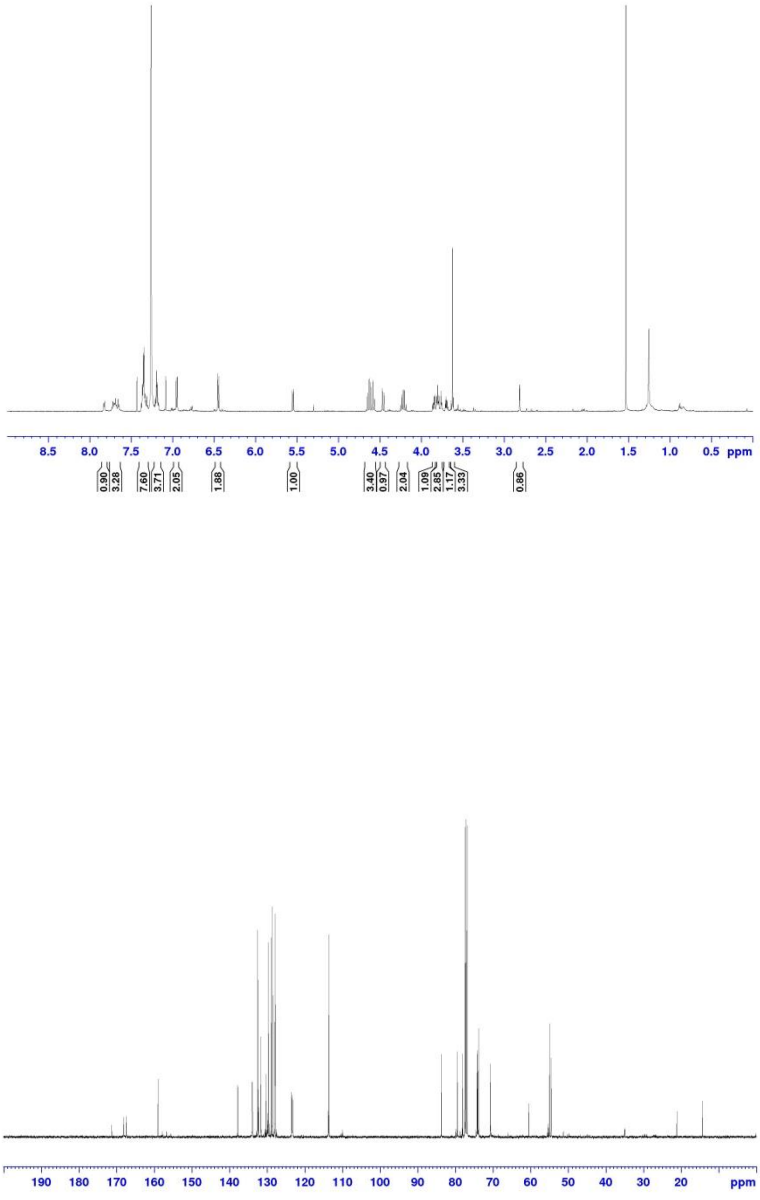
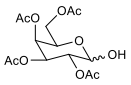


Figure A1.21. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.29**.



3.30

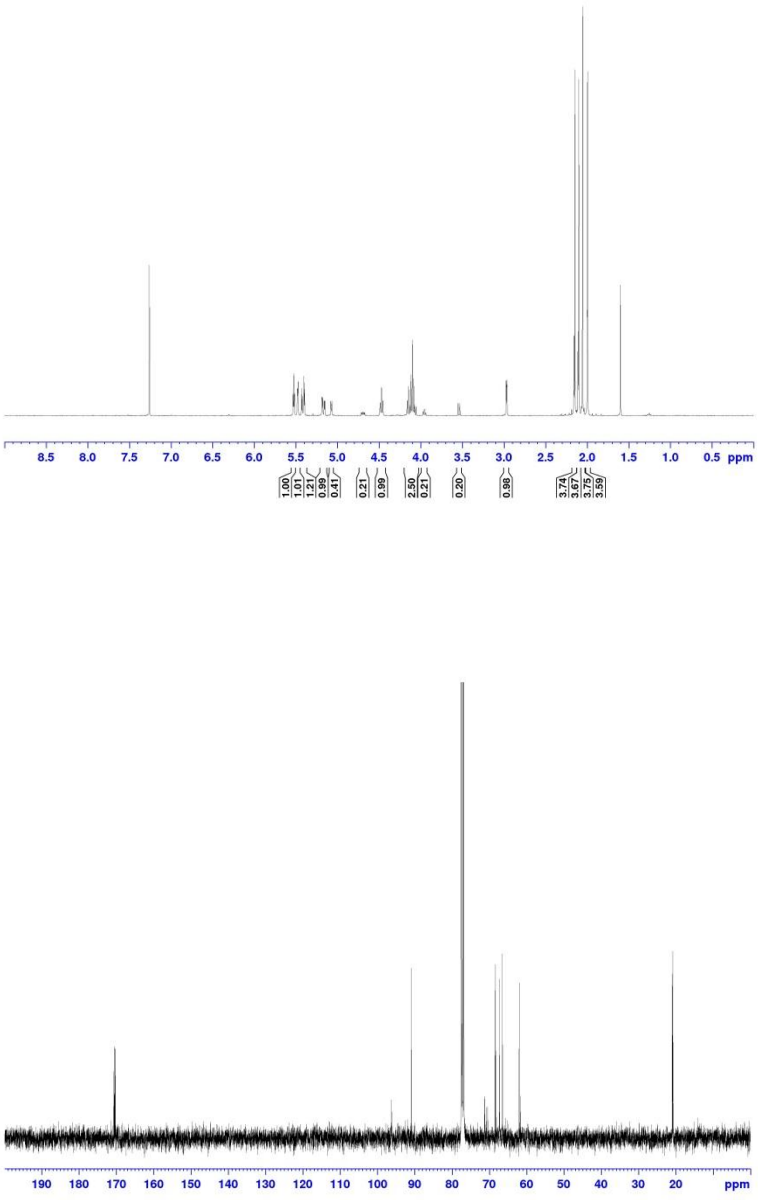
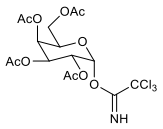


Figure A1.22. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.30**.



2.18

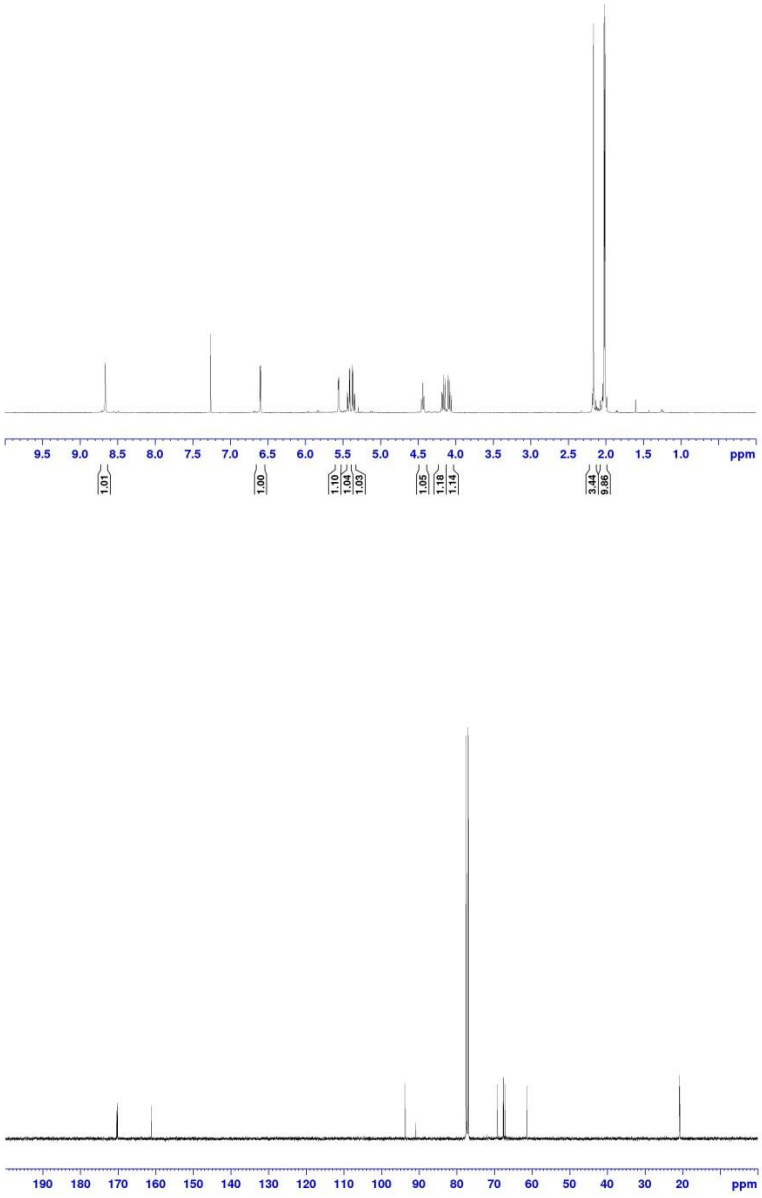
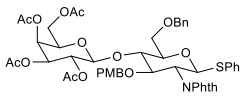


Figure A1.23. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **2.18**.



3.31

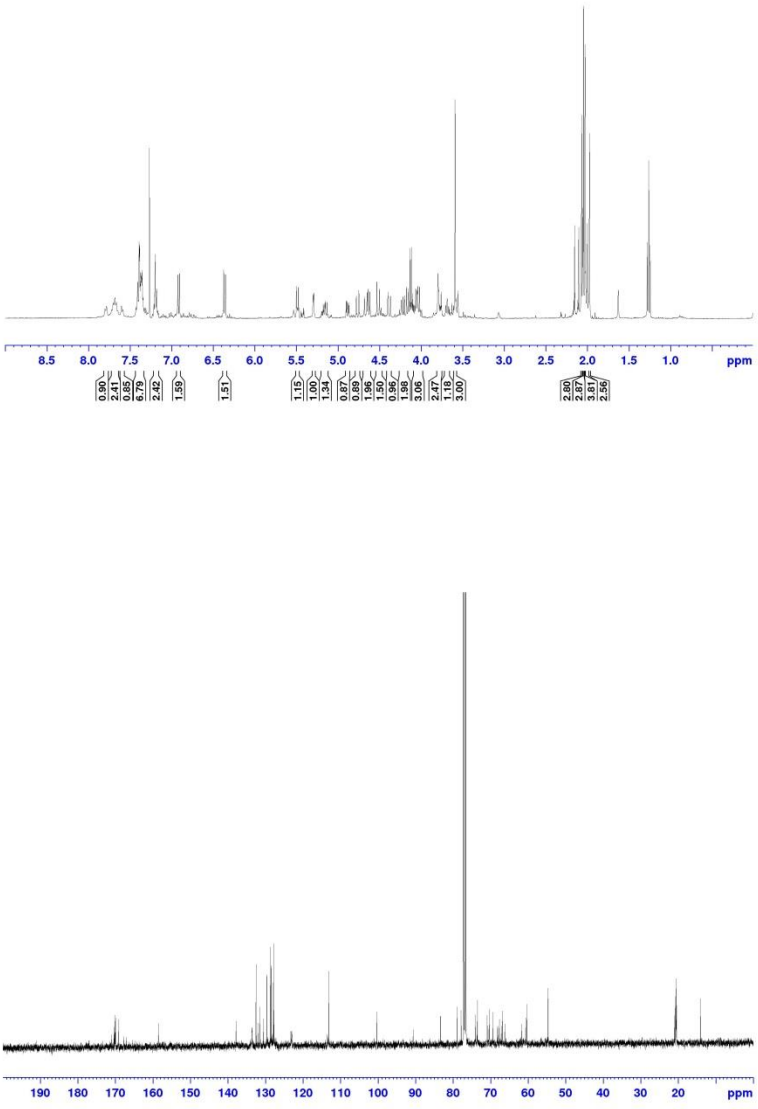
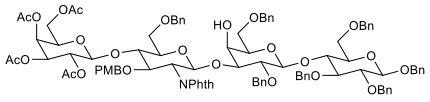
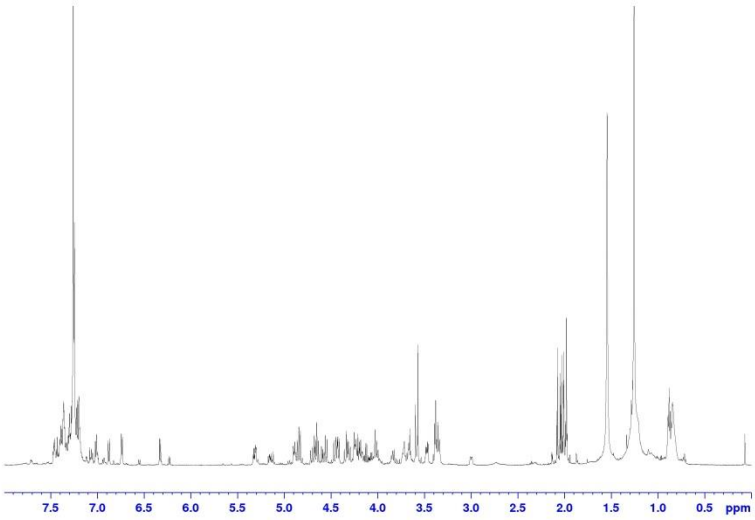


Figure A1.24. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.31**.



3.32



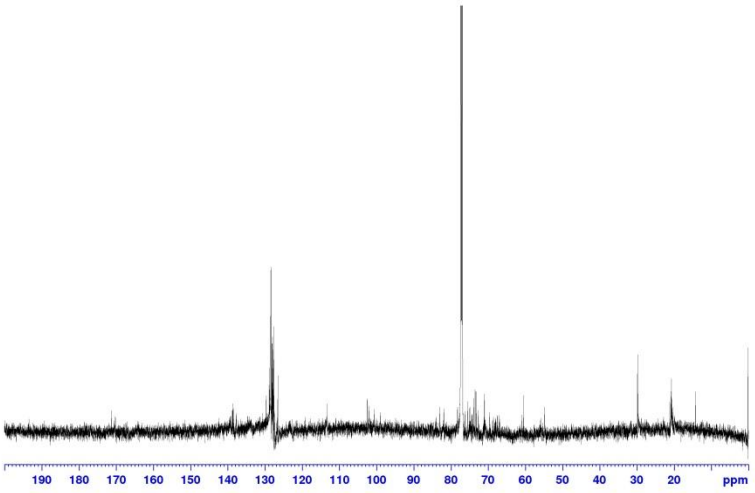
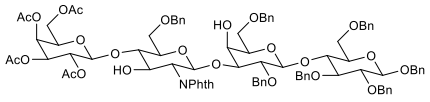


Figure A1.25. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectra of **3.32**.



3.33

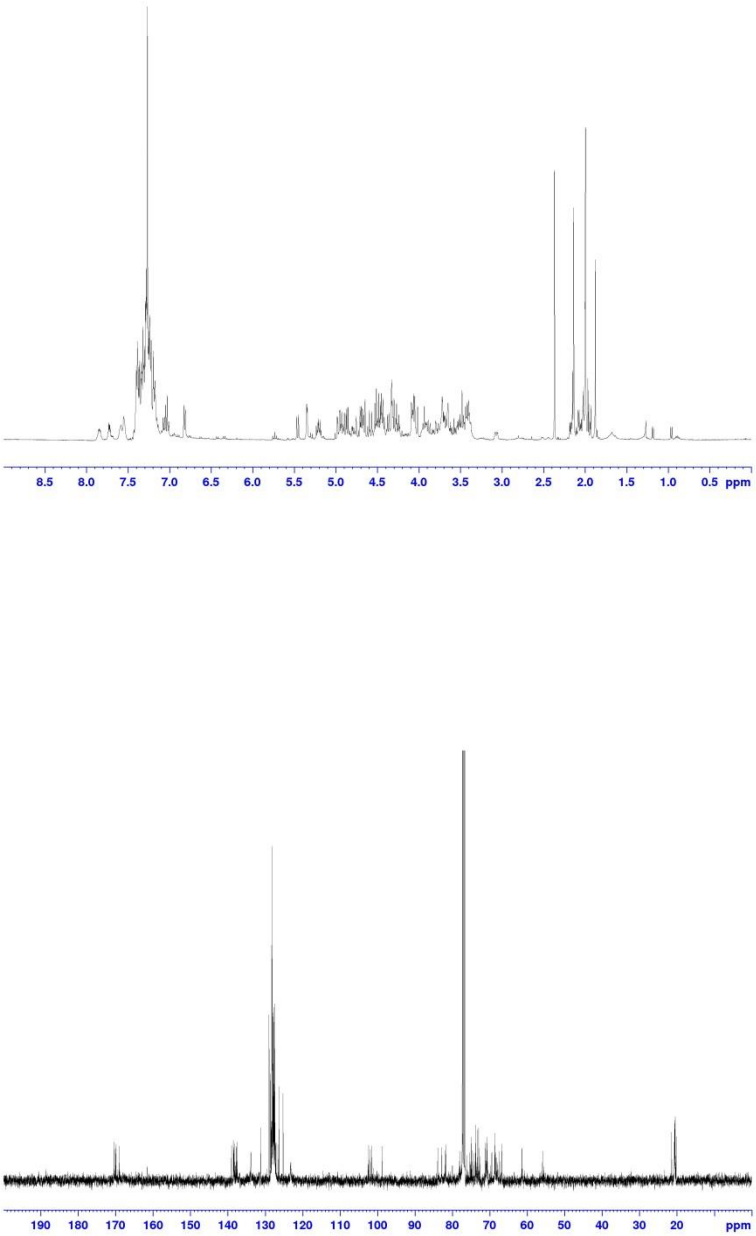


Figure A1.26. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3.33**.