Synthesis of caryophyllose-containing lipooligosaccharides from *Mycobacterium marinum*

by

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Abstract

"To understand something" is a perpetual theme in scientific research. In carbohydrate chemistry, we pursue to understand certain properties of sugar molecules. This is further described as the purpose of my research "understanding *Mycobacterium marinum* lipooligosaccharides' (LOSs) immnuogenic properties on a molecular level."

To start addressing this goal, LOSs from *M. marinum* have to be synthesized because they could not be isolated in pure and abundant quantity. These species are found in nature as a heterogeneous mixture of four major compounds, named LOS-I–IV, which all share LOS-I as a common structural motif. LOS-I is a pentasaccharide that contains a trehalose core with three hydroxyl groups acylated with fatty acids. In LOS-II and LOS-III, this pentasacccharide is modified by the addition of one or two residues of a rare branched monosaccharide, caryophyllose. LOS-IV consists of LOS-III further functionalized with a terminal *N*-acyl-D-fucosyl moiety.

This thesis describes my work focused on synthesizing *M. marinum* LOS I–IV. I will first discuss my studies on preparing the common triacylated trehalose core, which could be prepared by glycosylation between two glucose moieties and then acylation with synthesized, optically-pure lipids. Next, I outline my attempts to synthesize caryophyllose, which was hindered by a low-yielding key carbon–carbon bond formation step. This made the synthesis of LOS-II–IV not possible at this stage; however, my work suggested a better plan for the future. Finally, I will report the synthesis of a protected derivative of LOS-I, starting from the triacylated trehalose core, via its coupling with different glycosyl fluorides to give the pentasaccharide backbone. Unfortunately, the final deprotection was unsuccessful and thus the synthesis of LOS-I was not achieved. This led me to propose an alternate plan for synthesizing LOS-I and the remaining targets.

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List of Abbreviations

$[\alpha]_D$	Specific rotation (sodium D Line)
Å	Angstrom
Ac	Acetyl
ACN	Acetonitrile
Ac_2O	Acetic anhydride
AcOH	Acetic acid
Ac_2SGL	Diacylated sulfoglycolipid
AgOTf	Silver trifluromethanesulfonate
Ar	Aromatic
ax	Axial
9-BBN	9-Borabicyclo[3.3.1]nonane
$BF_3•Et_2O$	Boron trifluoride etherate
Bn	Benzyl
BnOH	Benzyl alcohol
BnBr	Benzyl bromide
br s	Broad singlet (NMR spectra)
Br_2	Bromine
Bz	Benzoyl
BzCl	Benzoyl chloride
CAN	Ammonium cerium(IV) nitrate

Car	Caryophyllose
CH_3OTf	Methyl trifluoromethanesulfonate
ClAc	Chloroacetyl
CSA	Camphorsulfonic acid
CsF	Cesium fluoride
CTAB	Cetyltrimethylammonium bromide
Cp_2HfCl_2	Bis(cyclopentadienyl)hafnium(IV) dichloride
$Cp_2Hf(OTf)_2$	Bis(cyclopentadienyl)hafnium(IV) bis(trifluoromethanesulfonate)
Cp_2ZrCl_2	Bis(cyclopentadienyl)zirconium(IV) dichloride
$Cu(OAc)_2$	Copper(II) acetate
d	Doublet (NMR spectra)
DAIB	(Diacetoxyiodo)benzene
DAST	(Diethylamino)sulfur trifluoride
DCC	Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
dd	Doublet of doublet (NMR spectra)
ddd	Doublet of doublet of doublet (NMR spectra)
DDQ	2,3-Dichloro-5,6-dicyano-*p*-benzoquinone
DIBAL	Diisobutylaluminum hydride
DIPEA	*N*,*N*-Diisopropylethylamine

DIPA	Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMB	3,4-Dimethoxybenzyl
DMF	*N*,*N*-Dimethylformamide
2,2-DMP	2,2-Dimethoxypropane
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine
EB	Electronic magnetic
EDC•HCl	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride
EI	Electron ionization
eq	Equatorial
ESI	Electrospray ionization
EtOAc	Ethyl acetate
EtOH	Ethanol
Et_2O	Diethylether
Et_3N	Triethylamine
FTICR	Fourier-transform ion cyclotron resonance
FTIR	Fourier-transform infrared
GPL	Glycosphingolipid
HMDS	Bis(trimethylsilyl)amine
HMBC	Heteronuclear multiple bond correlation spectroscopy

HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation spectroscopy
Hz	Hertz
IAD	Intramolecular aglycone delivery
^{i}Pr	Isopropyl
$(^{i}PrCO)_2O$	Isobutyric anhydride
IR	Infrared
LAM	Lipoarabinomannan
LDA	Lithium diisopropylamide
Lev	Levulinoyl
LevOH	Levulinic acid
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
m	Multiplet (NMR spectra)
M. canetti	*Mycobacterium canetti*
M. marinum	*Mycobacterium marinum*
M. smegmatis	*Mycobacterium smegmatis*
M. tuberculosis	*Mycobacterium tuberculosis*
MALDI	Matrix-assisted laser desorption/ionization
MHz	Megahertz

mp	Melting point
MS 3 Å	3 Angstrom molecular sieve
MS 4 Å	4 Angstrom molecular sieve
MTBC	*Mycobacterium tuberculosis* complex
NaHMDS	Sodium bis(trimethylsilyl)amide
NAP	2-Naphthylmethyl
NAPBr	2-(Bromomethyl)naphthalene
NBS	*N*-Bromosuccinimide
NH_4OAc	Ammonium acetate
$^{n}Bu_2SnO$	Di-*n*-butyltin oxide
^{n}BuLi , *n*-BuLi	*n*-Butyllithium
NIS	*N*-Iodosuccinimide
NMR	Nuclear magnetic resonance spectroscopy
Nuc	Nucleophile
$Pd_2(dba)_3$•$CHCl_3$	Tris(dibenzylideneacetone)dipalladium(0)–chloroform adduct
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladiuim(0)
PFP	Pentafluoroproprionyl
PGL	Phenolic glycolipid
Ph	Phenyl
Phth	Phthalimido
Pico	Picoloyl

PicoOH	2-Picolinic acid
Piv	Pivaloyl
PMB	*p*-Methoxybenzyl
PMBOH	*p*-Methoxybenzyl alcohol
PMP	*p*-Methoxyphenyl
PPh_3	Triphenylphosphine
ppm	Parts per million
p-TsOH	*p*-Toluenesulfonic acid
q	Quartet (NMR spectra)
R_f	Retention factor
s	Singlet (NMR spectra)
$Sc(OTf)_3$	Scandium (III) trifluoromethanesulfonate
SEt	Ethylthio
SL	Sulfolipid
STL	Succinoyl trehalose lipid
TBAB	Tetrabutylammonium bromide
TBAC	Tetrabutylammonium chloride
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDPS	*ter*t-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl

TBSOTf	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
^{t}Bu	*tert*-Butyl
^{t}BuOH	*tert*-Butanol
^{t}BuONa	Sodium *tert*-butoxide
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical
TES	Triethylsilane
TESO	Triethylsiloxyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
Tf_2O	Trifluoromethanesulfonic anhydride
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
THL	Trehalose lipid
THP	Tetrahydropyran
TIPS	Triisopropylsilyl
TIPSOTf	Triisopropylsilyl trifluoromethanesulfonate
TLC	Thin layer chromatography
TMCM	Trehalose corynomycolate

TMEDA	Tetramethylethylenediamine
TMS	Trimethylsilyl
TMSCl	Trimethylsilyl chloride
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TMU	Tetramethylurea
TLR2	Toll-like receptor 2
TOF	Time-of-flight
Tol	*p*-Tolyl
TolSH	4-Methylbenzenethiol
Tr	Trityl
TrCl	Trityl chloride
Ts	*p*-Toluenesulfonyl
TsCl	*p*-Toluenesulfonyl chloride
UV	Ultraviolet spectroscopy

Chapter 1 : Organic synthesis is the solution

We live in a world full of interesting structures. Structures that could be as large as astronomical entities or as small as unobservable single molecules that could only be described by formulae or spectroscopic data. As scientists, we are intrinsically trained or adapted to become interested and appreciate these structures. The reason behind this intrigued response might simply be: “This molecule looks cool, what does it do?”. The answer to this question may be that “This molecule triggers an immune response in the human body”. However, I was not satisfied with this answer, which then urged me to ask a deeper question: “Which part of this molecule is recognized by our immune system and how does it modulate our immune response?”. These questions could be answered by immunologists, which I was not trained to be, but as a chemist I could provide molecules to help produce an answer. The first step towards answering the question requires obtaining the molecule of interest or parts of the molecule of interest. Trained as an organic chemist, I rely heavily on organic synthesis to achieve this goal because these molecules could not be isolated in pure and abundant quantity. After obtaining the target molecules, biological assays could then be carried out to understand their properties, which may provide answers. Therefore, the title of my thesis “Synthesis of caryophyllose-containing lipooligosaccharides from *Mycobacterium marinum*” describes the features of my Ph.D. project: the molecule that we were interested in are caryophyllose-containing lipooligosaccharides and the method to access these molecules is organic synthesis. But before we get into the main content of my synthesis, I will describe where these interesting molecules were isolated from, *Mycobacterium marinum*.

1.1 *Mycobacterium marinum*

A species of *Mycobacterium* was first isolated in 1926 from different organs of saltwater fish in the Philadelphia Aquarium.[1] Another similar species was reported in 1942 but from freshwater fish.[2] Interestingly, another analogous species was isolated from human patients in 1951.[3] Different names were initially suggested for these organisms but it was later shown that they are all the same organism, *Mycobacterium marinum*[4] (**Figure 1.1**).

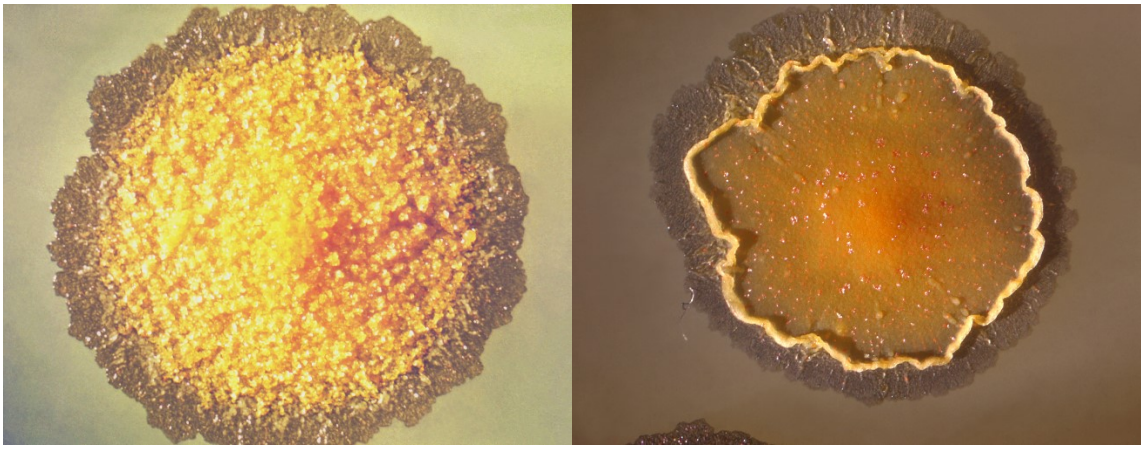

Figure 1.1. Single colonies of M. marinum.[5,6] Rough variant (left) and smooth variant (right).

The disease caused by *M. marinum*, aquarium granuloma, is probably less mentioned than the ones caused by other bacteria within the same genus. Tuberculosis[7] is caused by the *Mycobacterium tuberculosis* complex (MTBC):[8] *Mycobacterium tuberculosis* (mainly), *Mycobacterium bovis*, *Mycobacterium Africanum*, *Mycobacterium canetti* and *Mycobacterium microti*. Leprosy[9] is caused by *Mycobacterium leprae*[10] and *Mycobacterium lepromatosis*.[11] However, *M. marinum* is still prevalent in scientific research because it is closely related to *M. tuberculosis* (more than 85% nucleotide identity)[12] and causes a disease similar to tuberculosis in

fish and human.[13] These characteristics allowed *M. marinum* to serve as an alternative organism to study tuberculosis instead of using *M. tuberculosis*. Other advantages include short generation time (four to six hours)[14] and a suitable animal model (zebrafish)[15] for real-time monitoring of the infection[16] and analyzing host–pathogen interactions. These attributes can ultimately provide insights into the pathogenesis of tuberculosis[17,18] as well as allow for antitubercular drug screening.[19]

In addition to applying *M. marinum* in the research described above, the mycobacterial contents are also an interesting field of study. For example, *M. marinum* produces a class of molecules called lipooligosaccharides (LOSs) on their cell surface, different from *M. tuberculosis* which only produces the disaccharide that is present in the core of LOSs.[20] These unique molecules caught my attention.

1.2 Mycobacterial cell wall

Moving into the mycobacterial cell wall, there are various interesting molecules that are not found in humans and are different to bacteria (**Figure 1.2**). The cell wall itself is a complex structure that leads to mycobacteria not being classified as either Gram-positive nor Gram-negative. The mycobacterial cell wall has a single cell membrane and peptidoglycan, which is what Gram-positive bacteria have. However, the peptidoglycan can not be stained (with the Gram stain) because it is buried under other surface glycolipids. The negative staining result does not classify it as Gram-negative bacteria either, because mycobacteria do not have two cell membranes, and the surface glycolipids are different than lipopolysaccharides (LPSs). The classes of surface glycolipids in mycobacateria include lipoarabinomannan (LAM), an important virulence factor in

tuberculosis,[21] and extractable glycolipids: phenolic glycolipids[22] (PGLs), glycopeptidolipids[23] (GPLs) and lipooligosaccharides (LOSs).[24]

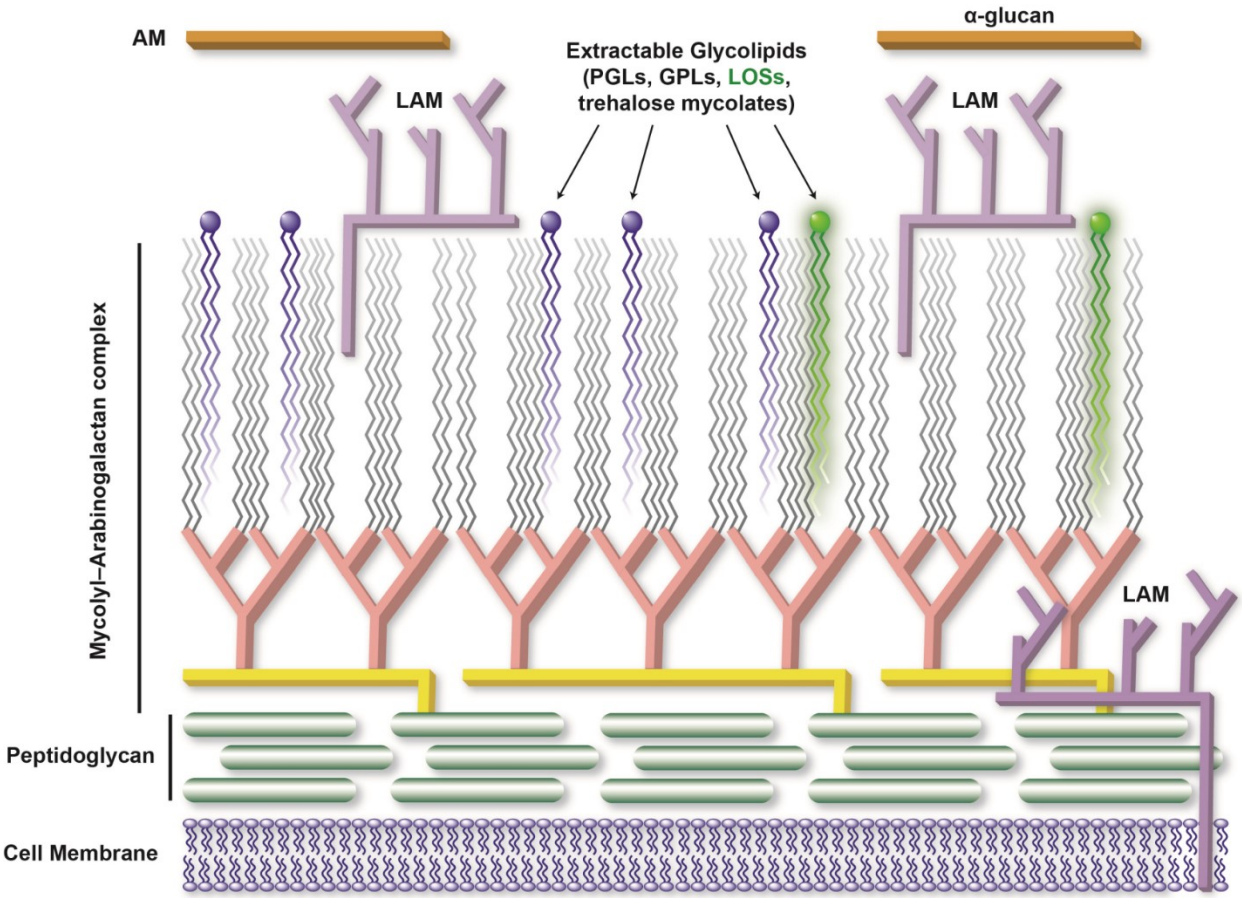

Figure 1.2. Schematic depiction of the mycobacterial cell wall. Figure originally provided by Mickey Richards. Reprinted with permission from John Wiley and Sons.[25]

1.2.1 Lipooligosaccharides

Mycobacterial LOSs are also different from Gram-negative bacterial LOSs. Gram-negative bacterial LOSs are lipopolysaccharides (LPS) without their O-antigen (O-polysaccharide) and are composed of three domains of molecules termed lipid A, inner core and outer core.[26] These LPSs

are antigens and modulate immune systems; for example, the lipid A component was recognized by Toll-like receptor 4 (TLR4) that activates inflammatory responses in human.[27] Mycobacterial LOSs are also recognized as antigens and immunomodulators but they do not have these structural features. They have different oligosaccharide compositions and vary within the same genus; the structures of known mycobacterial LOS were compiled in our publication.[25] Simplified selected examples are shown in **Figure 1.3** to demonstrate the various complexity of these molecules from strain-to-strain. Mycobacterial LOSs all have a common core of trehalose, a disaccharide made of two glucose units linked in an α,α-(1↔1) fashion. This trehalose core is also acylated with fatty acids at various positions. As mentioned earlier, the *M. tuberculosis* H37Rv strain produces the simplest LOS, a diacyltrehalose that sometimes is not even referred to as a LOS.[20] However, *M. canetti*, a member of MTBC, produces a distinct LOS that has eight sugar residues.[24] The sugar residues in many LOSs are not commonly found in nature. Examples of these are the molecules that we are interested in, the LOSs of *M. marinum*. The largest *M. marinum* LOS (shown in the figure) has two types of rare sugar residues: caryophyllose[28] (Car) and *N*-acyl-4-amino-D-fucose.[29] The details of the *N*-acyl moiety (NZx) is discussed in the next section.

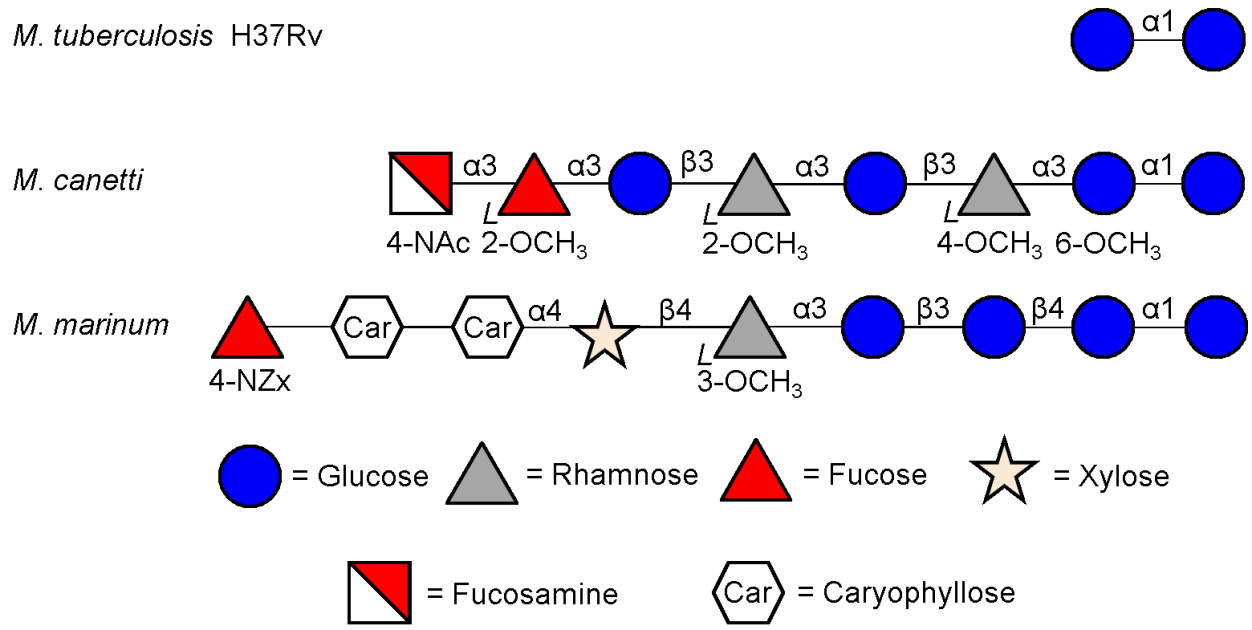

Figure 1.3. Simplified symbolic depiction of molecules of LOSs. Symbols used are those developed by Consortium for Functional Glycomics.[30] All default sugars are in D-pyranose form.

1.2.1.1 M. marinum lipooligosaccharides

The *M. marinum* LOSs (LOSs-I–IV) were isolated and elucidated by mass spectrometry and NMR spectroscopy and are summarized in **Figure 1.4**. In 2005, LOS-I was elucidated as a triacylated pentasaccharide by Guérardel and coworkers.[31] The trehalose core was shown to be acylated on the C-6, C-4 and C-2' positions but the structures of the lipids were not finalized at this point. The trehalose core was followed by two β-D-glucose units and an O-3 methylated α-L-rhamnose unit. Other species, containing a β-D-xylose unit attached to the α-L-rhamnose unit were reported in this paper but the complete structures of the remaining LOSs (LOSs-II–IV) were not elucidated. Moving forward to 2009, one of the unknown sugars was elucidated as α-D-caryophyllose, which was attached to the β-D-xylose unit. This discovery completed the structure

elucidation of LOS-II and III.[28] The structure of the last unknown sugar, *N*-acyl-4-amino-D-fucose, was elucidated in 2010 again by Guérardel and coworkers. The *N*-acyl moiety has four different structures, one of them could be either enantiomer, which resulted in LOS-IV as a mixture. The LOS-IV, which has the *N*-acyl moiety bearing a carboxylic acid group was termed "acidic LOS-IV" and represented about 95% of the total LOS-IV.[29] This carboxylic acid group in the *S* configuration was also the majority (70%) of the acidic LOS-IV, with the *R* configuration at this centre representing only 30% of the mixture of two compounds.[29] Finally, the fatty acid structures (R^1 and R^2) were characterized in 2011, but the absolute stereochemistry of the stereogenic centers was not assigned.[32]

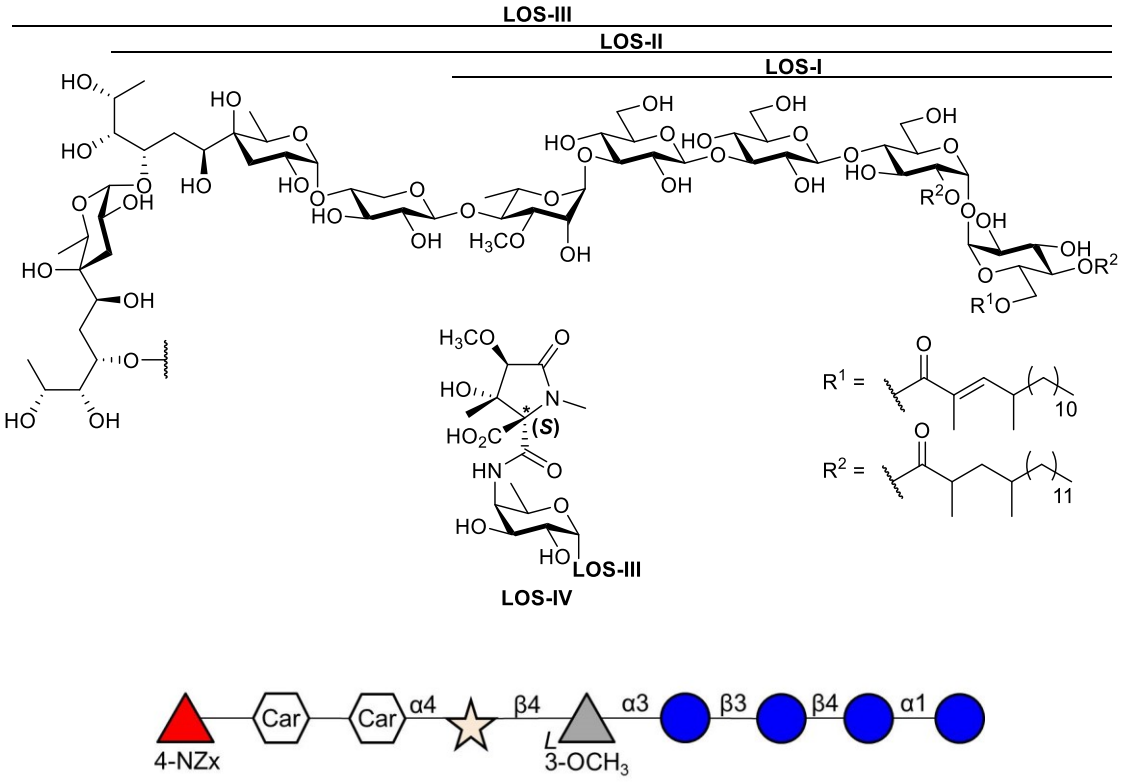

Figure 1.4. Major structures of LOSs in *M. marinum*.

During the course of structure elucidation, Guérardel and coworkers also became interested in the biosynthesis of LOSs in *M. marinum*. Some genes involved in the process were identified and mutants of *M. marinum* were grown to show the effect of particular genes on LOS production. One mutant was shown to have no LOSs produced,[32] another was shown to have no LOS-IV,[28] other mutants showed accumulation of certain LOSs.[33,34] These mutants that differ in the degree of LOSs production also made the study of understanding LOSs in host–pathogen interactions possible.[35] Compiling everything I have presented, some research on understanding structure–function studies of *M. marinum* LOSs has been achieved; however, not everything has been explored yet.

1.3 Purpose of research

Let us go back to the question I asked myself, "Which part of this molecule is recognized by our immune system and how does it modulate our immune response?". It is a difficult question to answer because the molecules needed are challenging to prepare in pure form. The research I presented above was mostly at the organism and cell level but, not on a molecular level. However, two research groups probed the interactions of *M. marinum* LOSs on a molecular level and that work was published in in 2009[28] and 2017.[35] In 2009, "purified" LOSs-I–IV (with possible heterogeneity within the same type of molecules presumed) were shown to inhibit pro-inflammatory responses of macrophages. However, the receptors that recognize the LOSs were not reported. In 2017, *M. marinum* mutants with different LOSs-I–IV proportions were found to have different effects of recognition by Toll-like receptor 2 (TLR2) molecules. The success of these two molecular level studies would prompt further studies.

1.3.1 The need for synthesizing *M. marinum* LOSs

An ideal study of "Understanding *M. marinum* LOSs' immunogenic properties on a molecular level" would need pure, single-form LOSs-I–IV that could only be made from chemical synthesis. The other part would be purified surface receptors,[36] like TLR2.

The need for synthesis is essential in this study. Purified LOS-I from *M. marinum* "gave a cluster of signals around *m*/*z* 1631 separated by 14 mass units."[31] Purified LOS-II contained "the mixture of two glycolipids LOS-II and LOS-II'."[28] No comments were made on the purity of purified LOS-III; purified LOS-IV was shown to be a mixture mentioned earlier.[29] Finally, the stereochemistry of the two fatty acids is also unknown.[32] Chemical syntheses of LOSs-I–IV would then primarily serve as a structure verification tool.

In addition, once the molecules are synthesized, biological assays could then be planned to understand their immunogenic properties as a function of their structure. The starting example would be measuring the binding affinities (recognition) to different receptors. The synthesized LOSs may also contribute to understanding its biosynthesis. These studies can only be unequivocally verified with the use of LOSs-I–IV in single forms, which is why synthesis of these LOSs is needed.

1.3.2 Targets of interest

The desired synthetic targets of this thesis are the four molecules LOSs-I–IV of *M. marinum* (**Figure 1.5**). I was successful in synthesizing a protected version of LOS-I and did work on the synthesis of the rare branched chain sugar (α-D-caryophyllose) in LOS-II–LOS-IV. With regard to the fatty acid moieties, the stereogenic centers in the two lipids (R^1 and R^2) were selected to have the *S* configuration after comparison with other molecules synthesized by mycobacteria.[37] In this thesis, Chapter 2 describes the synthesis of triacylated trehalose core common to LOS-I–

LOS-IV. Chapter 3 describes my attempt to synthesize a derivative of α-D-caryophyllose suitable for glycosylation. In Chapter 4, I report the key challenges in oligosaccharide synthesis and deprotection to access LOS-I. A final summary and future work are provided in Chapter 5. Instead of providing an extensive introduction here, each of Chapters 2–4 contain additional background information relevant to the work presented in that particular chapter.

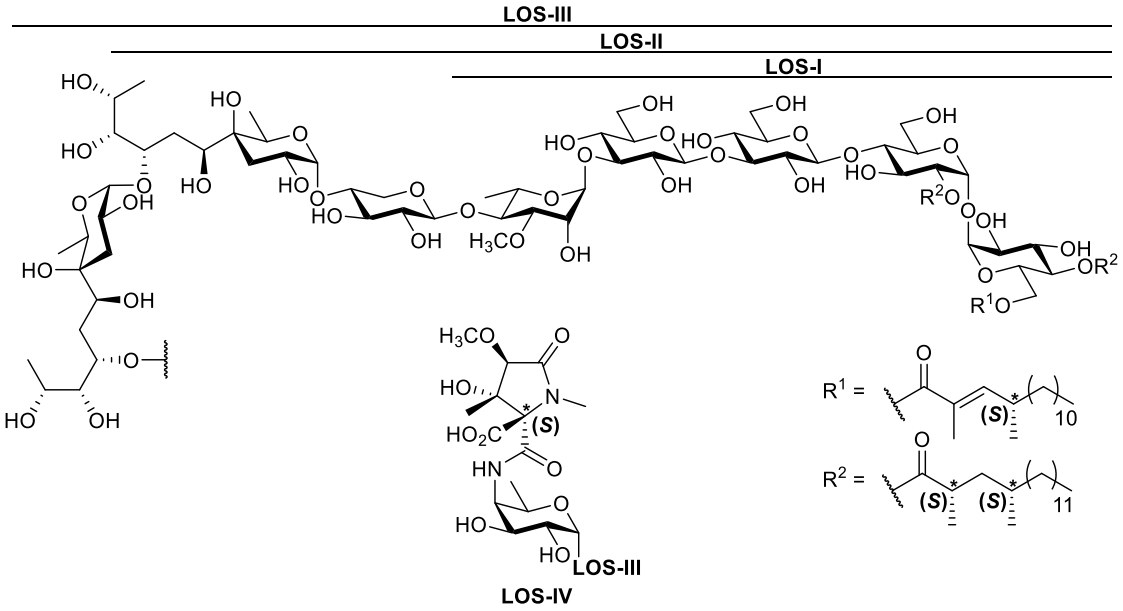

Figure 1.5. Desired synthetic targets of this thesis.

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Chapter 2 : Synthesis of the asymmetrically-substituted (acylated) trehalose moiety of *M. marinum* lipooligosaccharides

There are several challenges in constructing lipooligosaccharides (LOS) from *M. marinum*. In addition to stitching together (glycosylating) several carbohydrate building blocks, the synthesis of the individual building blocks is also difficult. One of these is caryophyllose, a rare monosaccharide with a C-4 branching moiety; its synthesis will be discussed in Chapter 3. Another is trehalose, a disaccharide whose synthetic challenges reside in establishing the glycosidic linkage and substitution patterns (if any). This chapter will describe previous studies on the synthesis of asymmetrically-acylated trehalose derivatives and also my synthesis of the disaccharide needed for the synthesis of all *M. marinum* LOSs.

2.1 Previous syntheses of asymmetrically-acylated trehalose derivatives

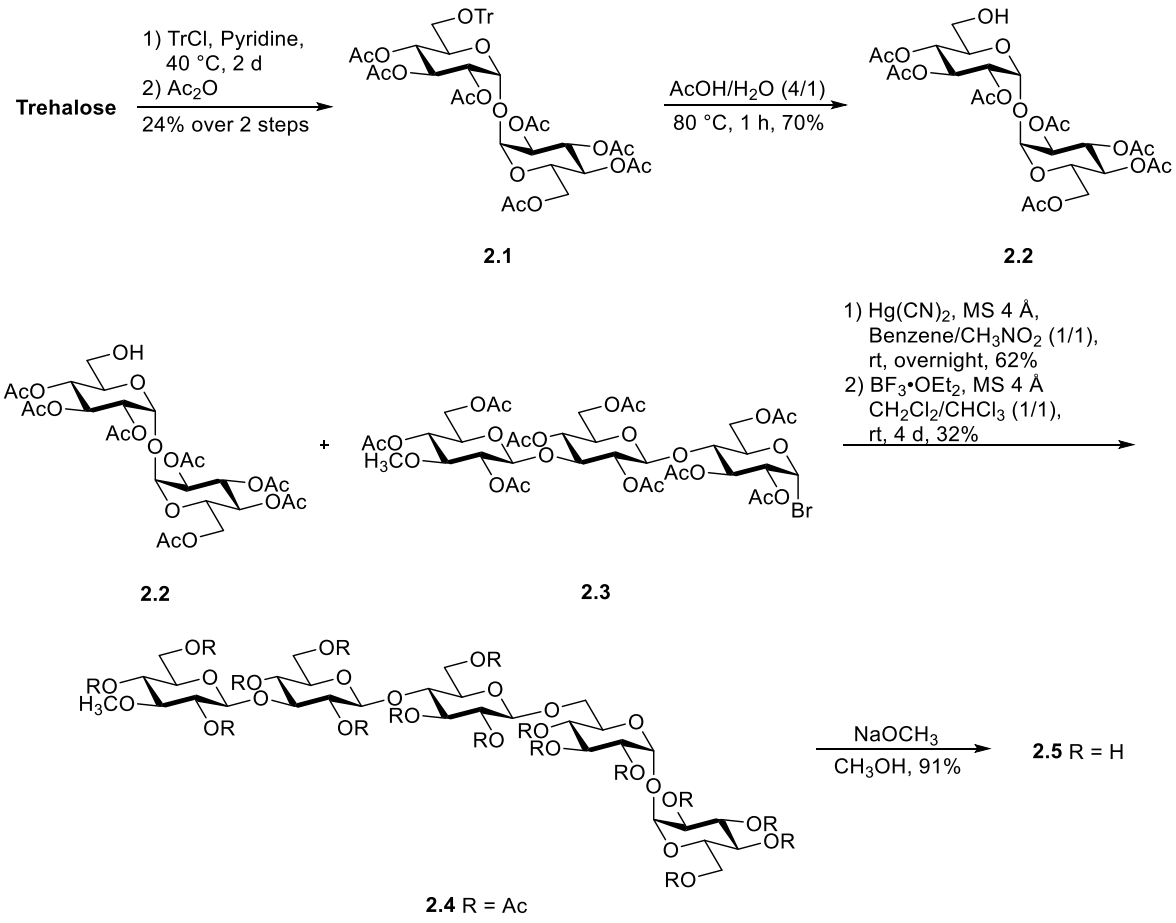
In the synthesis of asymmetrically-substituted (acylated) trehaloses, two methods have primarily been used: 1) desymmetrization of the trehalose moiety itself and 2) synthesis of the α,α-(1↔1) glycosidic linkage from two differentially-substituted monosaccharides. Establishing the α,α-(1↔1) glycosidic linkage can be further broken down into two categories: 1) the use of Intramolecular Aglycone Delivery (IAD) and 2) standard glycosylation conditions. In the following sections, I will describe the reported syntheses, as well as their advantages and disadvantages.

2.1.1 Synthesis of asymmetrically-substituted trehalose-derivatives via desymmetrizing trehalose

The methods and efforts undertaken to desymmetrize trehalose have been extensively reported in two reviews: one by Sarpe and Kulkarni in 2013,[1] the other by Wu and Wang in 2014.[2] Due to the extensive scope of both reviews, I will only briefly highlight methods that have been applied to the synthesis of oligosaccharides or to the synthesis of acylated trehalose moieties. There are two general approaches: 1) selective substitution at one of the two primary hydroxyl groups and 2) selective substitution of multiple hydroxy groups using acetal protecting groups. More details of applying these approaches to synthesize different types of trehalose-derivatives will be described in the following sections.

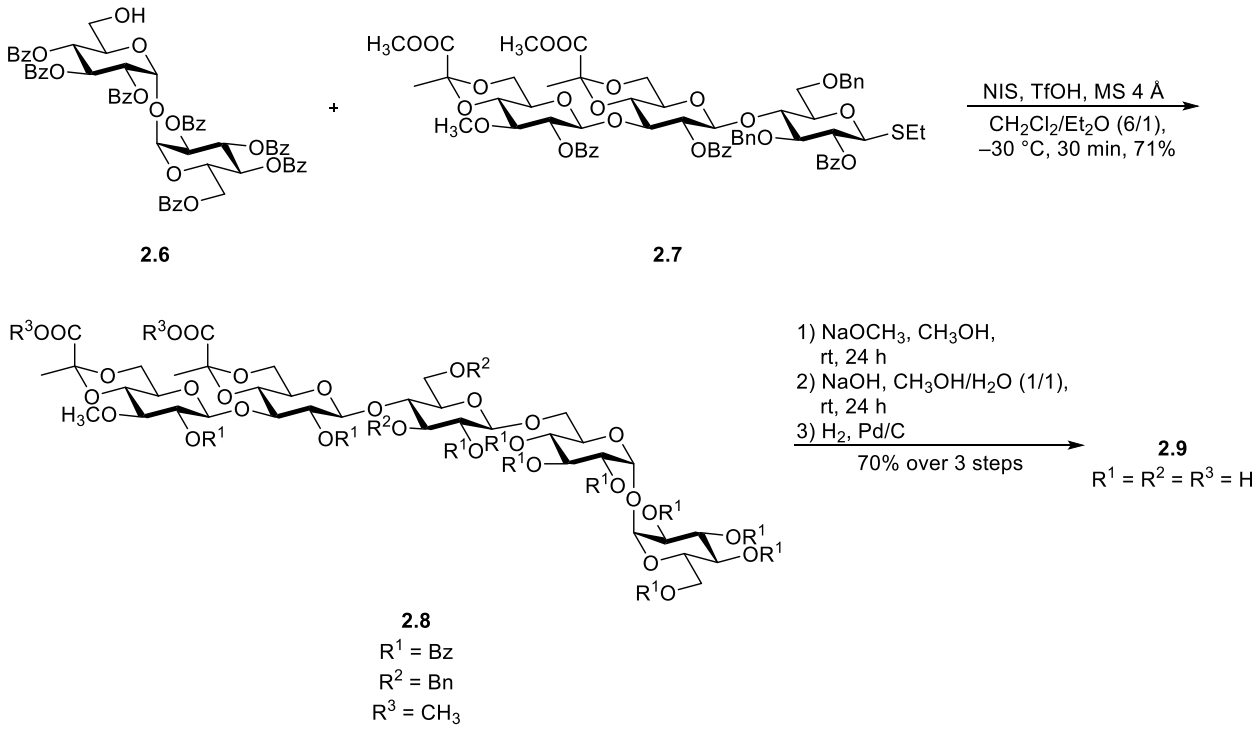
2.1.1.1 Synthesis of oligosaccharides containing trehalose lacking acyl groups

The first attempt to desymmetrize trehalose was reported by Helferich in 1948.[3] This approach was applied by two groups to synthesize oligosaccharides from *Mycobacterium smegmatis*.[4,5] As shown in **Scheme 2.1**, Szurmai et al.[6] started the synthesis by tritylating only one of the C-6-hydroxyl groups, followed by peracetylation to obtain the desymmetrized trehalose derivative **2.1**. The trityl group of **2.1** was then removed under acidic conditions to provide **2.2**. Glycosylation of **2.2** with glycosyl bromide **2.3** promoted by mercury(II) cyanide resulted in the formation of an orthoester, which was rearranged by treatment with boron trifluoride etherate giving pentasaccharide **2.4**. Deacylation of **2.4** led to the final product, **2.5**.



Scheme 2.1. Synthesis of a trehalose-containing pentasaccharide from *M. smegmatis* by Szurmai et al.[6]

Later, in 1993, Ziegler et al.[7] published their synthesis of a similar pentasaccharide containing pyruvate acetals. As depicted in **Scheme 2.2**, the same strategy used by Szurmai et al. was used to desymmetrize trehalose leading to **2.6**. Disaccharide **2.6** was glycosylated with thioglycoside **2.7** using *N*-iodosuccinimide and trifluorosulfonic acid to obtain the desired pentasaccharide **2.8**. A series of deprotections of **2.8** resulted in the final pentasaccharide **2.9**.

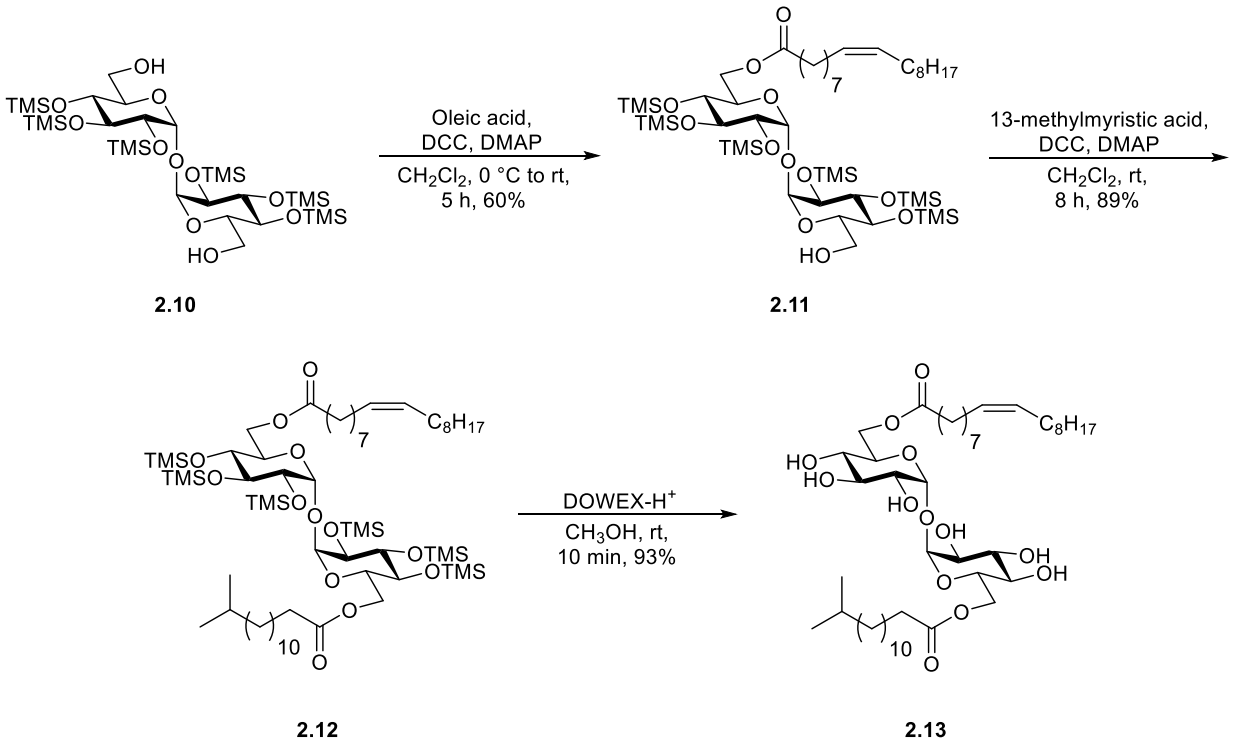


Scheme 2.2. Synthesis of a pyruvylated trehalose-containing pentasaccharide from *M. smegmatis* by Ziegler et al.[7]

2.1.1.2 Synthesis of molecules containing acylated trehalose residues

In the previous section, I showed how desymmetrizing trehalose paved a way for chemists to synthesize complex molecules containing this disaccharide. However, the final targets synthesized as shown in **Scheme 2.1** and **Scheme 2.2**, lack a major structural feature that is often found in trehalose-containing glycoconjugates: acylation. Incorporation of acyl groups onto trehalose complicates the synthetic strategy. However, several research groups took on this challenge. In this section, I will describe their work to synthesize acylated (or lipid containing), desymmetrized trehalose moieties.

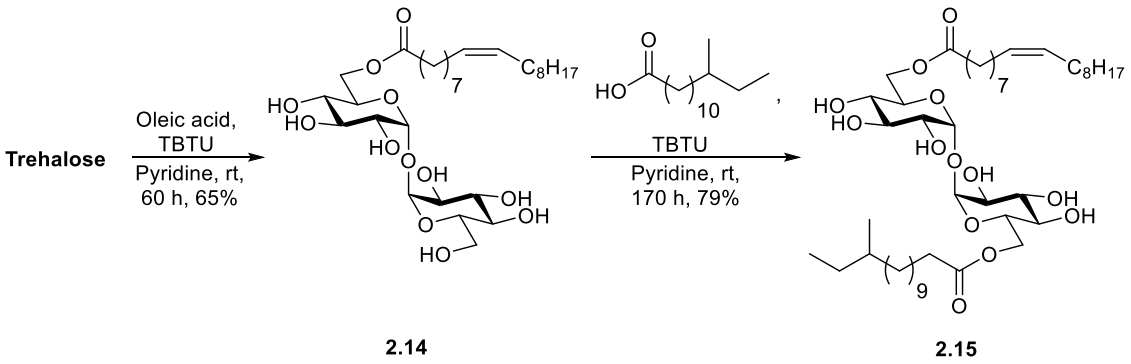
Sarpe and Kulkarni published their synthesis of the major maradolipid[8] in 2011 (**Scheme 2.3**).[9] The key desymmetrization step was the monoacylation of one of the primary hydroxyl groups of silylated trehalose **2.10** with oleic acid to obtain the acylated, desymmetrized derivative **2.11**. The second acylation, of the remaining primary hydroxyl group in **2.11**, with 13-methylmyristic acid gave **2.12**, which was converted to maradolipid **2.13** upon desilylation under acidic conditions.



Scheme 2.3. Sarpe and Kulkarni's synthesis of maradolipid **2.13**.[9]

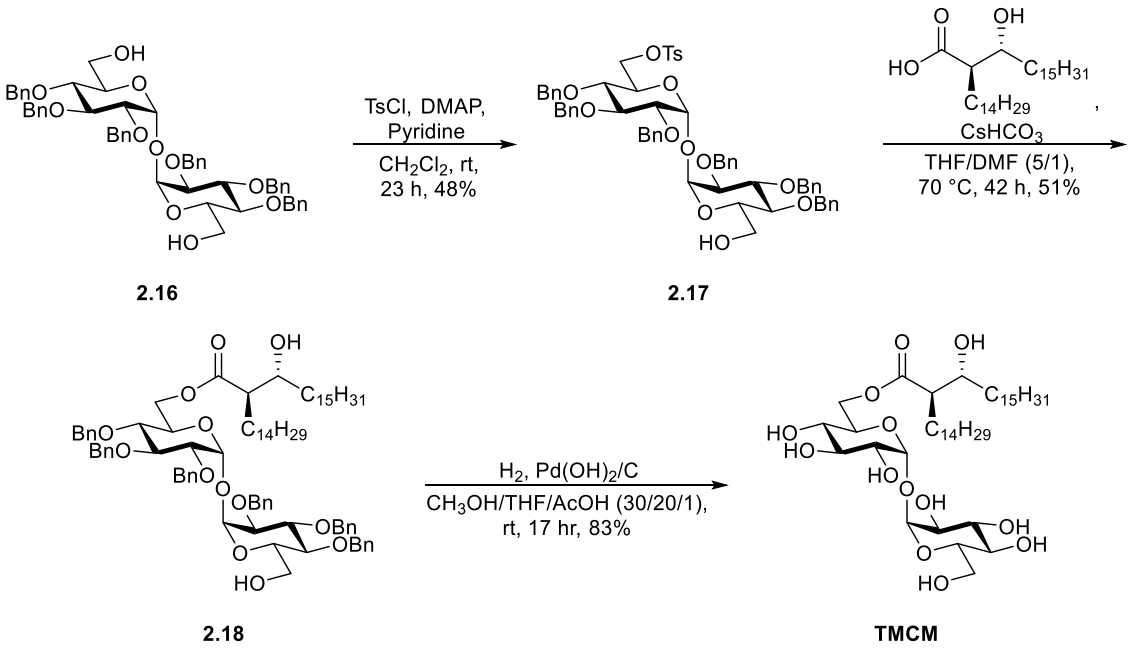
Two years later, in 2013, Paul et al. published a more efficient way to synthesize a different form of maradolipid and some of its analogues.[10] The key desymmetrization step (**Scheme 2.4**) was again the monoacylation of a primary hydroxyl group, but in this case a fully deprotected

trehalose was used as the starting material. The compound obtained, **2.14**, was then acylated on the other primary alcohol to produce maradolipid **2.15**.



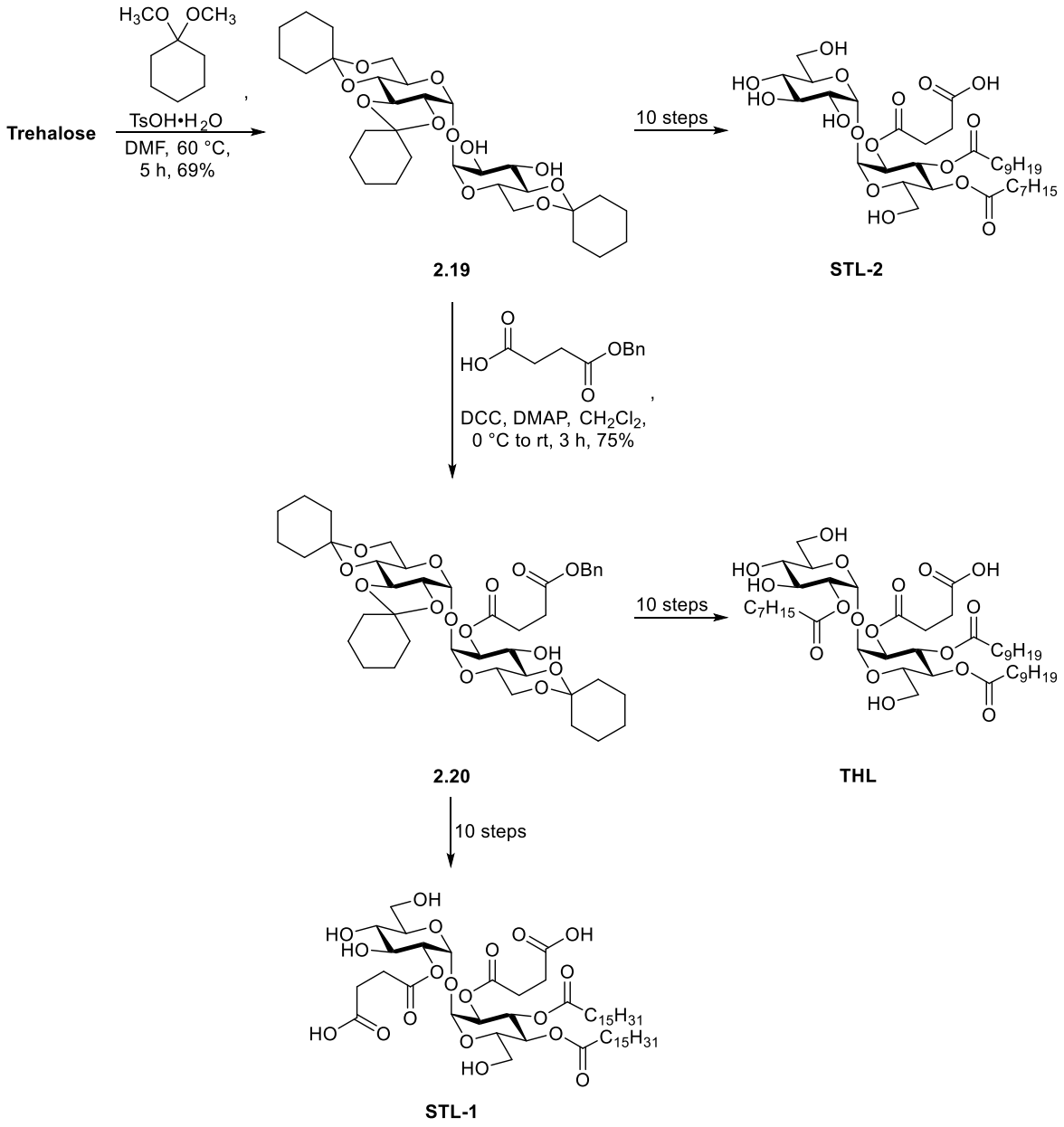
Scheme 2.4. Synthesis of maradolipid **2.15** by Paul et al..[10]

In addition to the acylation method (coupling an alcohol and an acid) just described, a different strategy, using a S_N2 displacement, was published by Yamaryo-Botte et al. to synthesize trehalose monocorynomycolate (TMCM).[11] As shown in **Scheme 2.5**, the key desymmetrization step was tosylation of one of the primary hydroxyl groups of **2.16** to obtain **2.17**. The tosylate was then displaced by an *in situ* generated cesium corynomycolate to obtain the acylated trehalose derivative **2.18**. Removal of the benzyl groups in **2.18** produced **TMCM**.



Scheme 2.5. Synthesis of **TMCM** by Yamaryo-Botte et al..[11]

So far, the work described in this section contains examples that only have lipids incorporated on the primary position of trehalose. However, derivatives with other acylation patterns have also been made. An example is provided in **Scheme 2.6**, which shows the syntheses of three succinoylated trehalose lipids (**STL-1**, **STL-2** and **THL**) by Jana et al. These compounds feature a desymmetrized trehalose core with lipids incorporated on positions other than the primary positions.[12] The first step is the key desymmetrization step: treatment of trehalose with cyclohexanone diethyl acetal, which resulted in the formation of tri-acetal **2.19**. This compound was then modified in ten steps to obtain **STL-2**. Monoacylation of **2.19** at the C-2 hydroxyl group provided **2.20**, which was converted to either **STL-1** or **THL** in ten steps each.



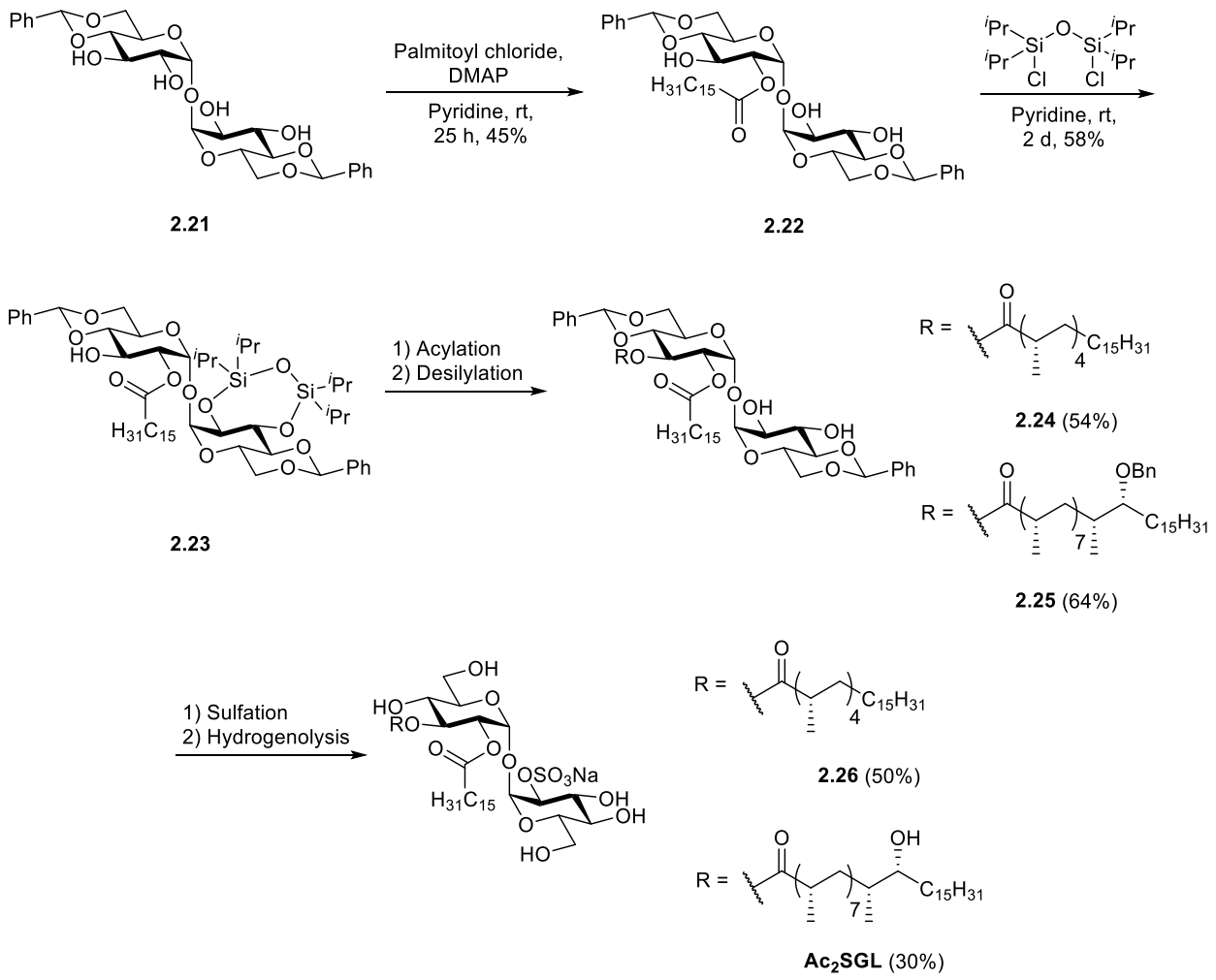
Scheme 2.6. Synthesis of succinoylated trehalose lipids **STL-1**, **STL-2** and **THL** by Jana et al..[12]

2.1.1.3 Synthesis of trehalose-containing sulfoglycolipids

Attaching lipids onto trehalose complicates the overall synthesis but the challenge does not end there. Sulfoglycolipids containing trehalose functionalized with both acyl and sulfate groups

have also been successfully synthesized. First identified in *Mycobacterium tuberculosis* in 2004,[13] many research groups have synthesized Ac_2SGL or analogues. Ac_2SGL is immunogenic and a possible target as a vaccine against tuberculosis.[14] The syntheses of this interesting molecule also requires the desymmetrization of trehalose.

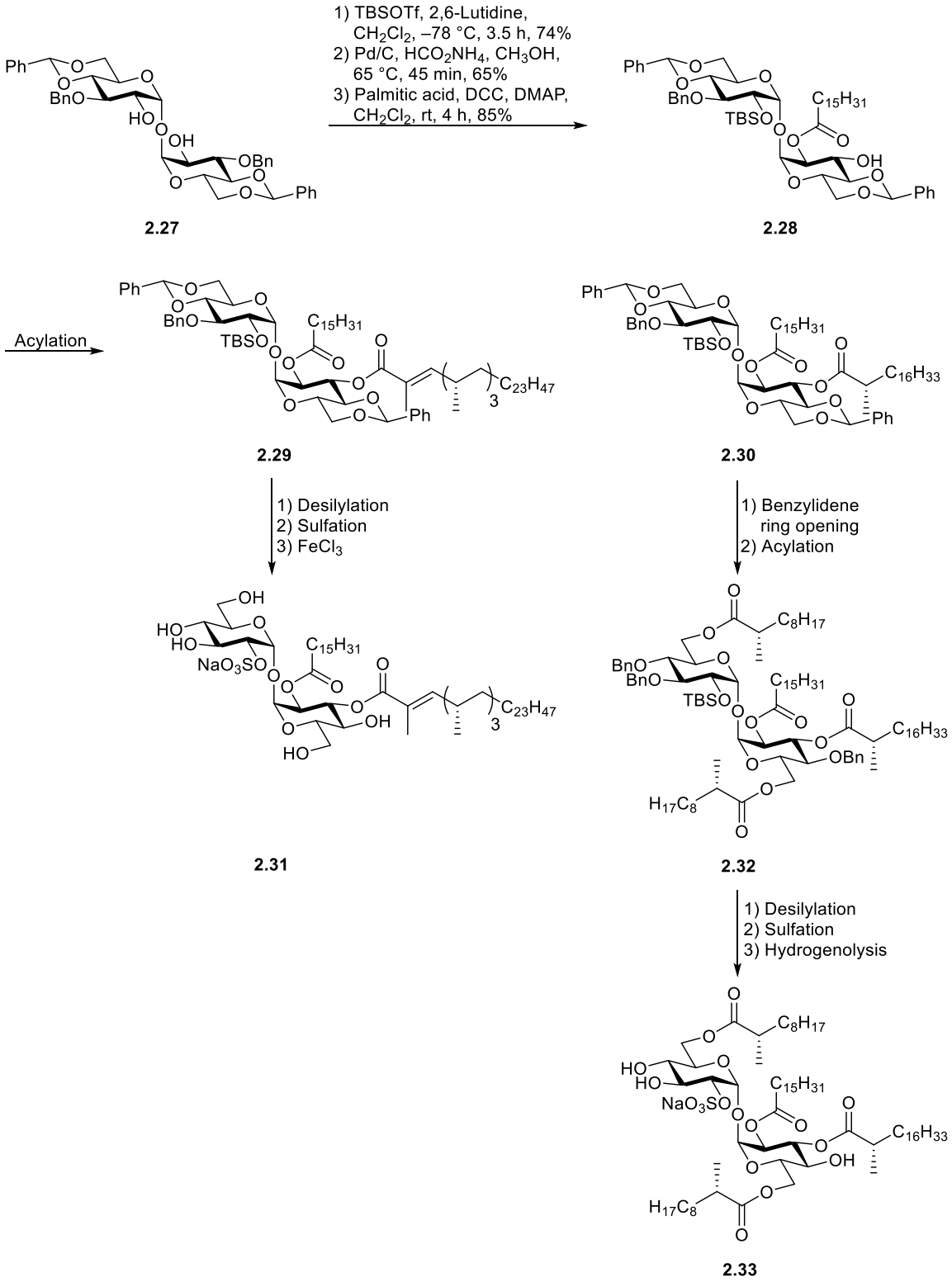
Summarized in **Scheme 2.7**, two groups, Guiard et al.[15] as well as Geerdink et al.[16] used the same desymmetrization strategy to obtain Ac_2SGL. Starting from the dibenzylidene protected trehalose derivative **2.21**, monoacylation of the C-2 hydroxyl group produced acylated trehalose **2.22**. The hydroxyl groups at C-2' and C-3' were then protected as a cyclic siloxane giving **2.23**. Acylation of **2.23** with various acids and then desilylation produced **2.24** (by Guiard et al.) or **2.25** (by Geerdink et al.). Both **2.24** and **2.25** were sulfated at the C-2' hydroxyl group and, after hydrogenolysis, **2.26** and **Ac_2SGL** were produced.



Scheme 2.7. Summary of two groups' work on the synthesis of **Ac$_2$SGL** and analogues.[15,16]

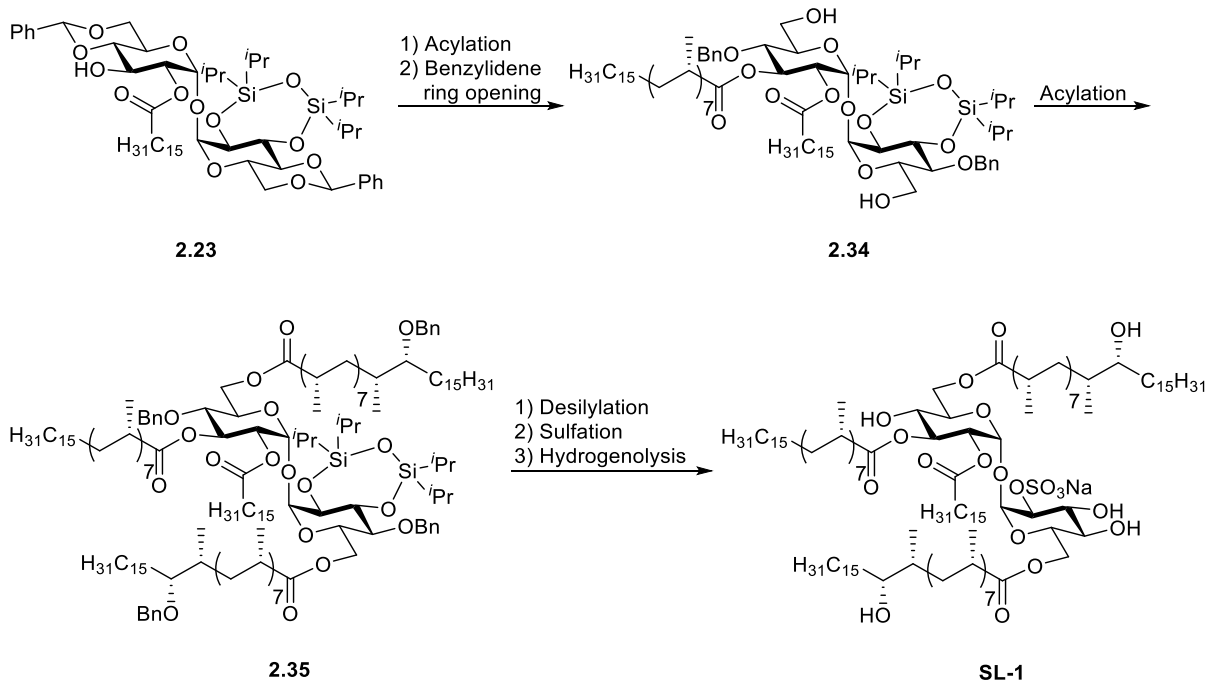
Using a similar route (**Scheme 2.8**) Gau et al. have synthesized Ac$_2$SGL analogues[17] and Lemétais et al.[18] have synthesized SL-1 analogues. Both syntheses involved desymmetrization of **2.27** by a series of reactions: silylation at the C-2 hydroxyl group, debenzylation at C-3', and then acylation of the C-2' hydroxyl group to obtain intermediate **2.28**. The routes diverged upon acylation of the C-3' hydroxyl group in **2.28** with various acids. In the synthesis by Gau et al., the acylation product **2.29** was desilylated, sulfated at the C-2 hydroxyl group, and then removal of benzyl ether and benzylidene acetal with $FeCl_3$ gave the desired product **2.31**. The acylation

product in the synthesis by Lemétais et al., **2.30**, was subjected to a regioselective benzylidene acetal opening, resulting in a molecule with two primary hydroxyl groups. These hydroxyl groups were further acylated to obtain the tetraacylated trehalose derivative **2.32**. Compound **2.32** was desilylated, sulfated at C-2, and then underwent hydrogenolysis to obtain SL-1 analogue **2.33**.



Scheme 2.8. Summary of two groups' synthesis of Ac_2SGL and SL-1 analogues.[17,18]

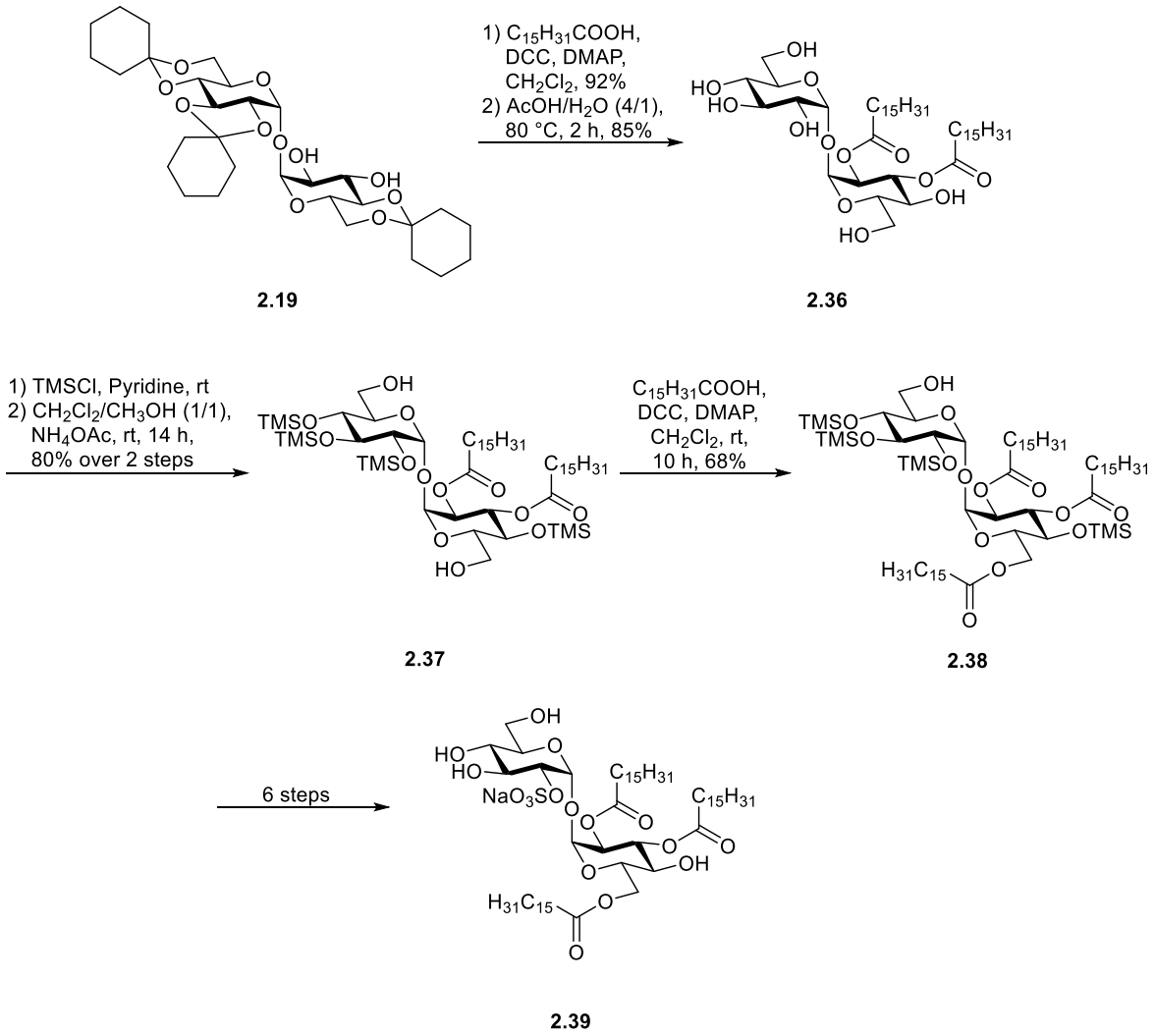
The synthesis of **SL-1** by Geerdink and Minnaard[19] used the desymmetrization approach shown in **Scheme 2.7**: the conversion of **2.21** to **2.23**. Afterwards, as shown in **Scheme 2.9**, compound **2.23** was acylated at the C-3 hydroxyl group, and the product was subjected to a regioselective benzylidene acetal opening to provide **2.34**. Diol **2.34** was acylated both positions to give **2.35**; subsequent desilylation, sulfation and hydrogenolysis provided **SL-1**.



Scheme 2.9. Synthesis of **SL-1** by Geerdink and Minnaard.[19]

Sarpe et al. also published syntheses of Ac_2SGL and SL-1 analogues[20], using an approach different from those previously described. They chose to attach simpler lipids, but their route enabled them to synthesize other challenging molecules, such as sulfolipid-3 analogues.[21] Their desymmetrization strategy was the same as that shown in **Scheme 2.6**, the formation of tri-acetal **2.19** in one step from trehalose. Starting from **2.19** (**Scheme 2.10**), acylation of the C-2 and C-3

hydroxyl groups followed by acetal hydrolysis produced diacyltrehalose derivative **2.36**. Persilylation of **2.36**, followed by partial desilylation produced **2.37**, containing two primary hydroxyl groups. Selective acylation of **2.37** provided the triacyltrehalose derivative **2.38**. After an additional six steps, which included sulfation of the C-2' hydroxyl group, **2.38** was successfully transformed into the desired sulfolipid-3 analogue **2.39**.



Scheme 2.10. Synthesis of sulfolipid-3 analogue by Sarpe et al..[21]

The work described above demonstrates that there are many ways of desymmetrizing trehalose. The application of this strategy to synthesizing complex trehalose-containing molecules, has allowed the synthesis of a number of complicated molecules containing asymmetrically-substituted trehalose moieties. There is another strategy to obtain asymmetrically-substituted trehalose derivatives: the establishment of the α,α-(1↔1) glycosidic linkage from two different monosaccharides. This approach has used IAD, as well as standard glycosylations, to form this tricky linkage.

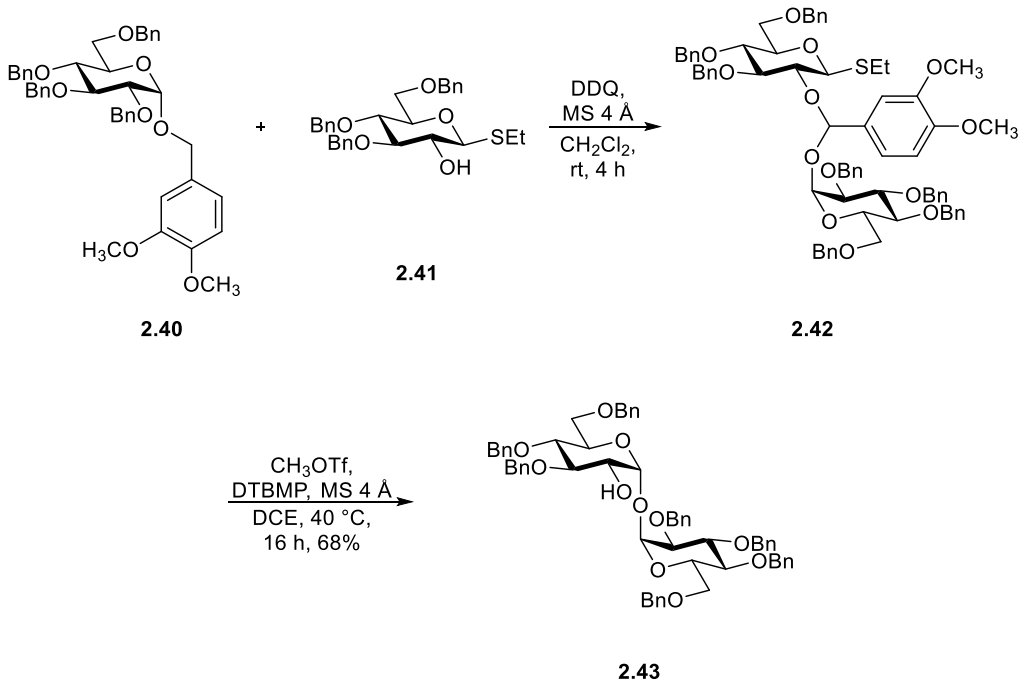
2.1.2 Synthesis of asymmetrically-substituted trehalose derivatives via glycosylation

2.1.2.1 Use of IAD to synthesize asymmetrically-substituted trehalose derivatives

IAD was first introduced by Barresi and Hindsgaul in 1991[22] to synthesize β-mannosides. Since then, this method has been applied to the formation of various types of challenging 1,2-*cis*-glycosidic linkages, including the α,α-(1↔1) glycosidic linkage in trehalose. IAD was reviewed by Ishiwata and co-workers in 2010[23] and by Jia and Demchenko in 2017.[24] A former group member Dr. Bing Bai also wrote a section about IAD in his Ph.D. thesis, which covered the various tethers that were used in IAD.[25] For the purpose of this thesis, the scope of this section will be limited to the formation of the α,α-(1↔1) glycosidic linkage in trehalose by IAD.

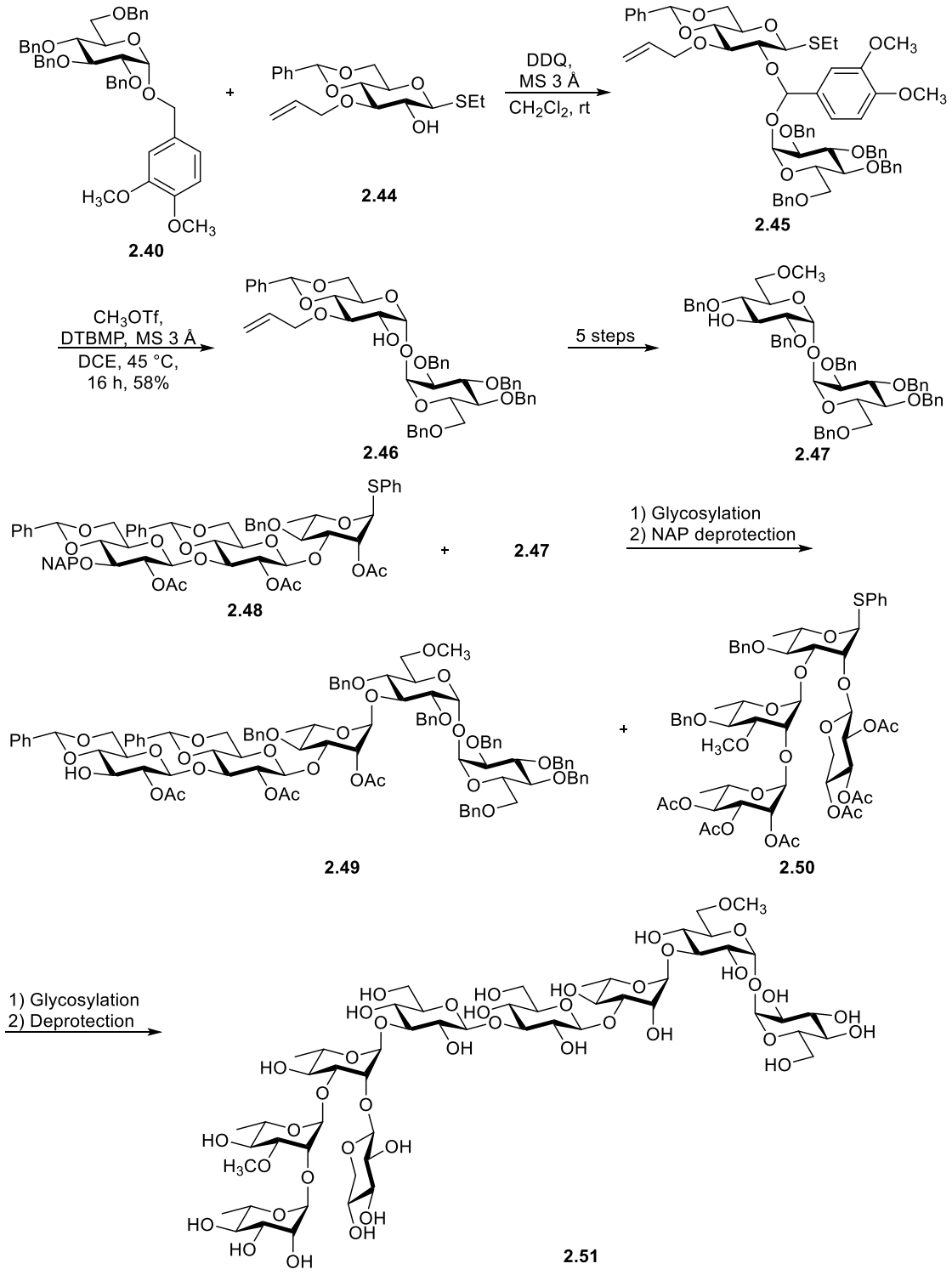
Although introduced in 1991, the use of IAD to form the α,α-(1↔1) glycosidic linkage in trehalose was not seen in the literature until Bertozzi and co-workers published their work in 2003.[26] As shown in **Scheme 2.11**, a 3,4-dimethoxybenzyl (DMB) group was attached to the anomeric position in **2.40**. The benzylic position in the DMB group was oxidized by 2,3-dicyano-5,6-dichloro-*p*-benzoquinone (DDQ) in the presence of **2.41**, which gave the tethered intermediate **2.42**. After activating the ethanethiol group in **2.42** with CH_3OTf, **2.42** rearranged with loss of the DMB group to produce the trehalose derivative **2.43** in 68% yield. The choice of the tether was

crucial. If the DMB was swapped with a 4-methoxybenzyl group, the final rearrangement step only gave 40% yield due to incomplete oxidation by DDQ. This strategy was applied by the same research group to a similar substrate in the synthesis of a sulfolipid-1 analogue.[27]



Scheme 2.11. Bertozzi and co-workers' IAD approach to synthesize trehalose derivatives.[26]

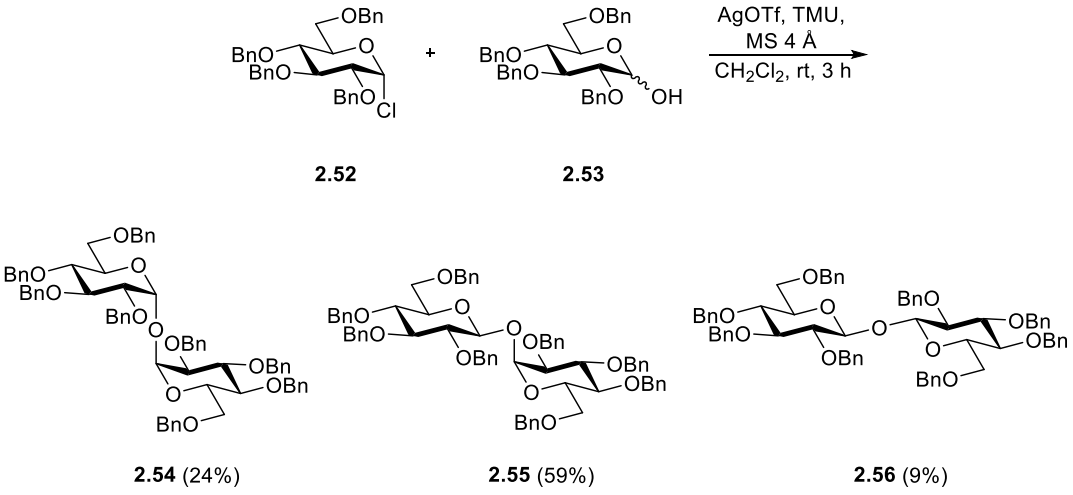
Chaube and Kulkarni later applied this strategy to a complex oligosaccharide[28] from *Mycobacterium gordonae* (strain 990) as illustrated in **Scheme 2.12**.[29] DMB-glycoside **2.40** was oxidatively coupled with **2.44** to obtain intermediate **2.45**, which was then converted to trehalose derivative **2.46**. Further transformation of **2.46** to glycosyl acceptor **2.47** was achieved in five steps. Glycosylation of disaccharide **2.47** with trisaccharide **2.48** provided a pentasaccharide, which, after the NAP group removal, gave alcohol **2.49**. A 4+5 glycosylation between **2.49** and thioglycoside **2.50**, followed by global deprotection, produced the desired nonasaccharide **2.51**.



Scheme 2.12. Chaube and Kulkarni's synthesis of a trehalose-containing oligosaccharide via IAD.[28]

2.1.2.2 Use of standard glycosylation to synthesize asymmetrically-substituted trehalose derivatives

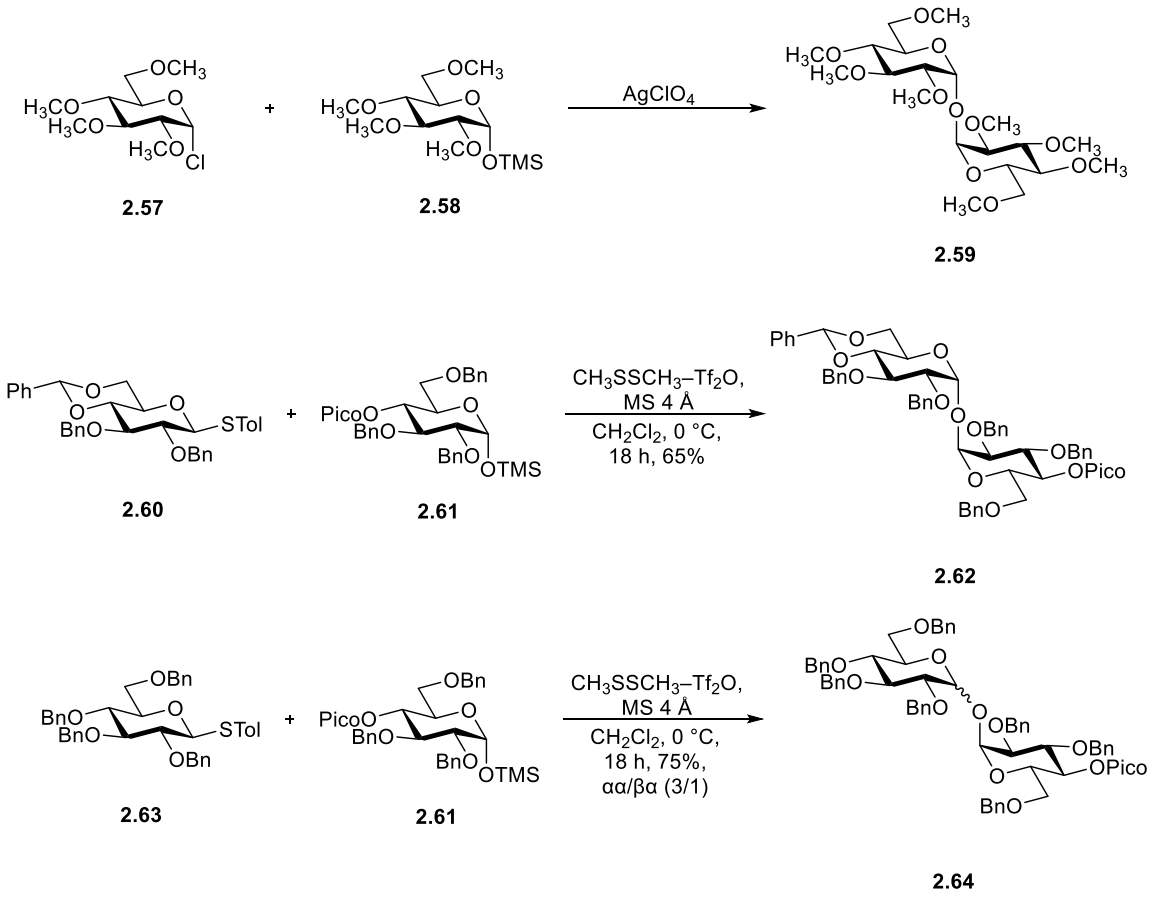
Using IAD requires two steps to form α,α-(1↔1) linkages; therefore, single step approaches like traditional glycosylation chemistry have also been explored. Establishing α,α-(1↔1) glycosidic linkages (not limited to trehalose) by standard glycosylation was extensively reviewed by Chaube and Kulkarni in 2012.[30] This section will focus on the examples for synthesizing asymmetrically-substituted trehalose derivatives. An example of the use of standard glycosylation conditions to make an α,α-(1↔1) glycosidic linkage in trehalose is shown in **Scheme 2.13**.[31] The general approach involves activation of a glycosyl donor (here glycosyl chloride **2.52**) in the presence of a glycosyl acceptor (in this case **2.53**) to generate the product. As depicted in the scheme, the yield of the desired α,α-(1↔1) product **2.54** was 24%, and the yields of undesired α,β-(1↔1) **2.55**, and β,β-(1↔1) **2.56** products were 59% and 9%, respectively.



Scheme 2.13. Example of a standard glycosylation to obtain trehalose derivatives.[31]

This example illustrates the principle challenge of using standard glycosylation to make trehalose derivatives: stereoselectivity. This is a bigger challenge when using hemiacetal glycosyl acceptors, as compared to alcohol acceptors, as up to four products can be generated, instead of two. The application of IAD to this problem was in response to that challenge. Another approach involves fixing the anomeric stereocenter of the acceptor to improve the stereoselectivity of the glycosylation (**Scheme 2.14**). This is often done through the use of a trimethylsilyl ether derivative. For example, when α-trimethylsilyl ether **2.58** was used in the glycosylation with glycosyl chloride **2.57**, the desired trehalose product **2.59** was produced as the major product.[32] This approach has also been used to synthesize asymmetrically-substituted trehalose derivatives such as **2.62**[33] and **2.64**[33] in 65 and 75% yields, respectively.

In addition to fixing the stereochemistry of the acceptor, glycosyl donors can also be manipulated to increase the stereoselectivity of the reaction. As illustrated in **Scheme 2.14**, the 4,6-*O*-benzylidene acetal protecting group in **2.60** is crucial to the stereoselectivity of its reaction with **2.61** leading to **2.62**. If **2.60** is swapped with **2.63**, in which the benzylidene acetal is replaced with two benzyl groups, glycosylation with the same acceptor **2.61** results in a diastereomeric mixture of α,α-(1↔1) and β,α-(1↔1) **2.64** in a 3:1 ratio. The enhanced stereoselectivity using the 4,6-*O*-benzylidene acetal protecting group was inspired by the work of Crich and co-workers on α-glucosylation reactions.[34,35]

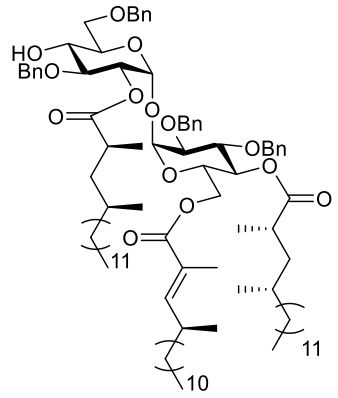


Scheme 2.14. Fixing stereochemistry of glycosyl acceptors for increasing stereoselectivity.

Comparison of the use of standard glycosylation conditions to IAD shows that the major differences are stereoselectivity and substrate scope. In the context of synthesizing trehalose, IAD is stereoselective, but the substrate scope is narrow. On the other hand, standard glycosylation offers the possibility of using a variety of substrates but is not stereoselective. Although better stereoselectivity was accomplished by tuning the substrates as in **Scheme 2.14**, this strategy might not be suitable for all targets. These issues must be considered in the synthesis of the asymmetrically-substituted (acylated) trehalose moiety in mycobacterial LOSs.

2.2 Synthesis of the target trehalose derivative

The target compound of this chapter is shown in **Figure 2.1**. The trehalose moiety **2.65** is the first disaccharide unit of *M. marinum* LOS (when the benzyl groups are replaced by hydrogens). It features three positions that are acylated (the C-4, C-6 and C-2'-hydroxyl groups) with two different lipids, and one position, the C-4' hydroxyl group, which must be available for glycosylation. When the structure was first elucidated, the stereochemistry of the stereocenters on the lipids were not defined.[36] However, when we compared its structure to the previous examples (e.g., **SL-1** in **Scheme 2.9**), we thought it would be best to adopt the same configuration as those and synthesize enantiomerically pure lipids where all stereocenters are of the *S* configuration.



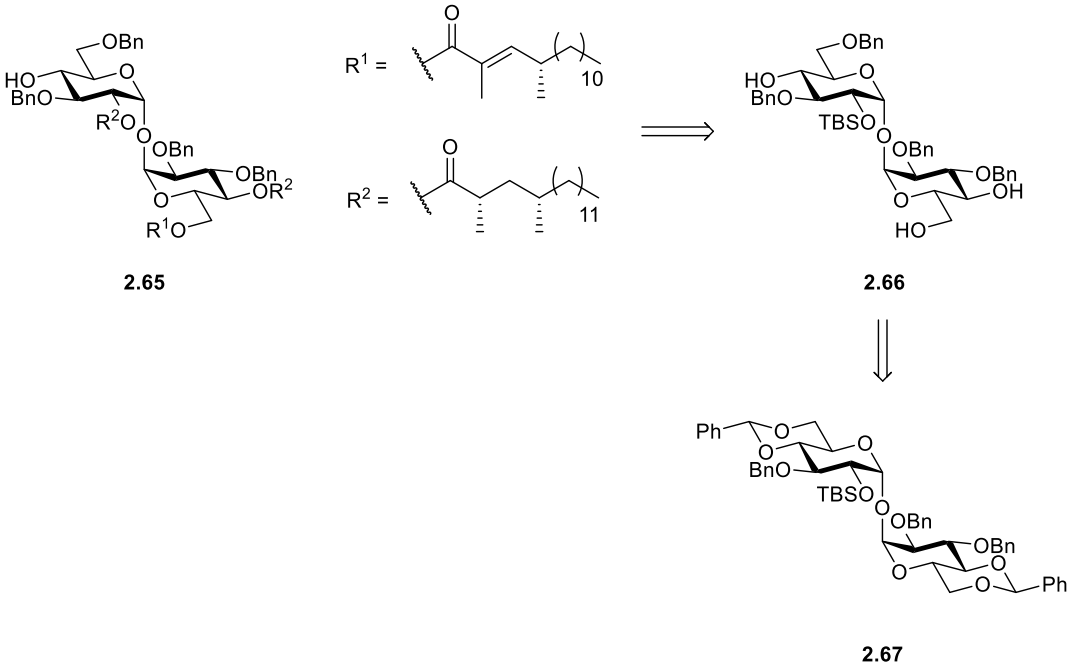
2.65

Figure 2.1. The target compound of this chapter, **2.65**.

2.2.1 Retrosynthetic analysis and plan

In considering an approach to synthesize **2.65**, the key decision was the strategy to establish the α,α-(1↔1) glycosidic linkage. Of the three methods presented above – desymmetrization of trehalose, IAD and standard glycosylation conditions – I chose the latter based on the following

analysis. The desymmetrization approach initially seemed attractive, as the α,α-(1↔1) glycosidic linkage was already installed. However, the challenge then becomes differentiating hydroxyl and protecting groups, which is not trivial. Although there were many examples discussed above, their lipids were incorporated in different positions than mine, which made the strategy less attractive. Nevertheless, a retrosynthetic analysis was made to assess this strategy (**Scheme 2.15**). Based on the literature, I envisioned that desymmetrized trehalose **2.67** could be synthesized, but the following step of differentiating the two benzylidene acetals to make **2.66** was anticipated to be difficult. Therefore, this method was not my first choice.



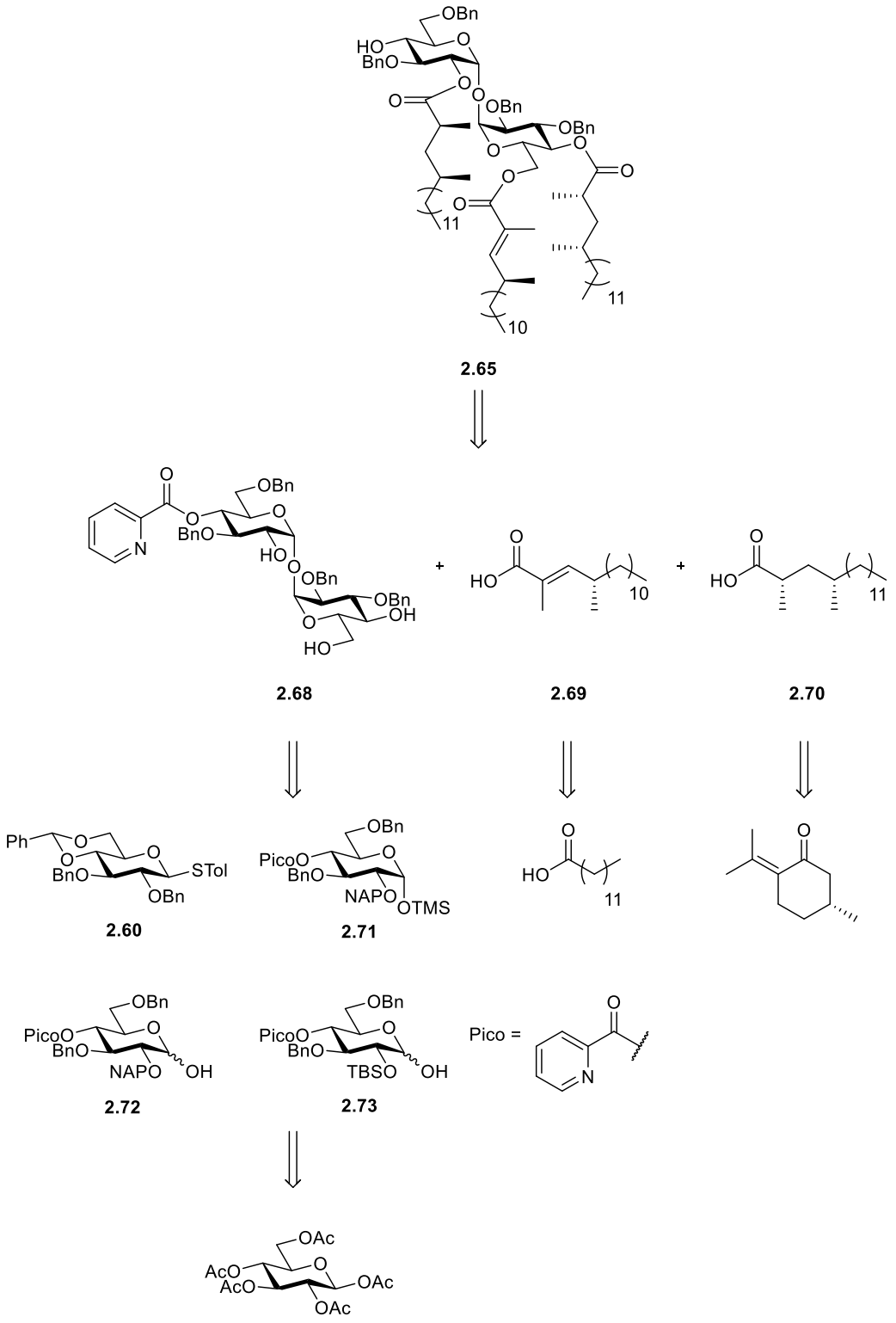
Scheme 2.15. Retrosynthetic analysis of using the trehalose desymmetrization strategy.

IAD served as the next attractive strategy because it is stereoselective when establishing the α,α-(1↔1) glycosidic linkage. However, a previous Ph.D. student in the group, Dr. Bing Bai, explored this approach to make other LOSs.[25] Bai explored several reactions, but this strategy did

not work well on his substrates, which were similar to mine. Therefore, this strategy was also not my first choice.

On the other hand, Dr. Bing Bai did have success using the standard glycosylation approach,[25] and therefore this strategy seemed like a good choice. Although stereoselectivity will be an inherent issue, I introduced ways to improve it in **Section 2.1.3**. The standard glycosylation strategy is generally more flexible, unlike trehalose desymmetrization and IAD. Should the reagents in **Scheme 2.14** not work on my substrate, there are other glycosylation reagents to try.

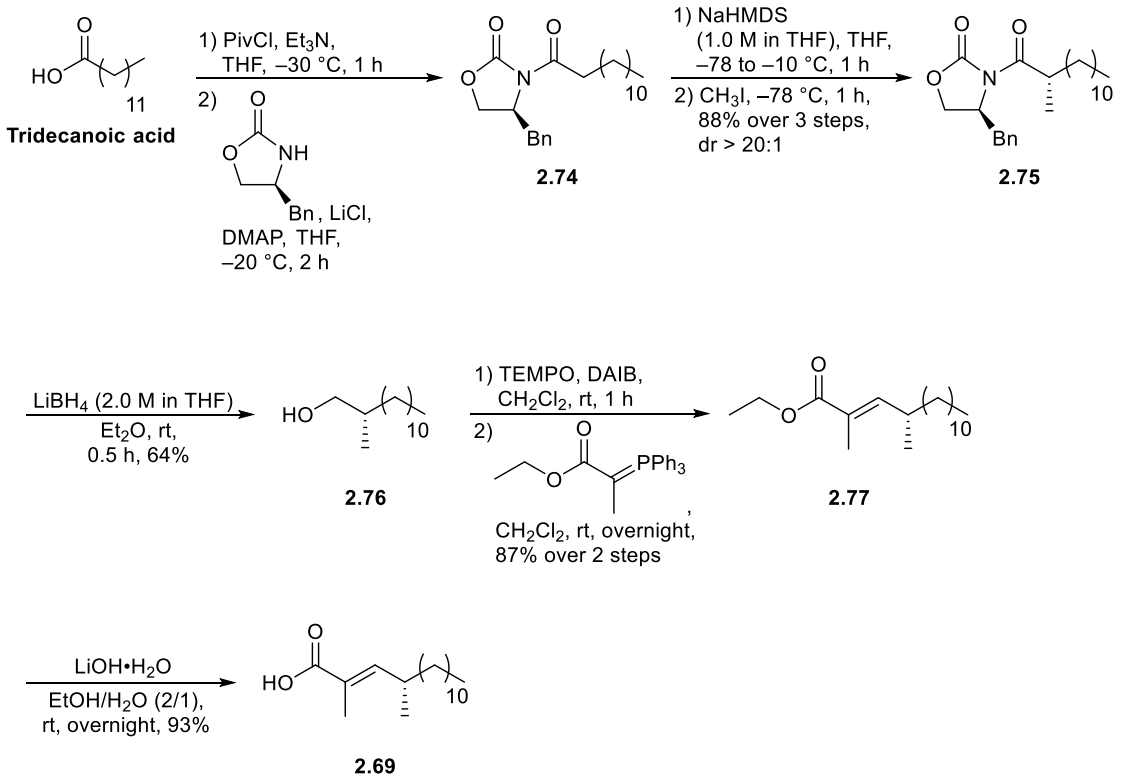
A retrosynthetic plan to **2.65** using this approach is shown in **Scheme 2.16**. I envisioned that the target could be synthesized from three building blocks: trehalose derivative **2.68**, the 17-carbon α,β-unsaturated carboxylic acid **2.69** and 18-carbon carboxylic acid **2.70**. Compound **2.68** bears a 2-picolinoyl (Pico) protecting group that can be removed in the presence of the other protecting groups to reveal the C-4' hydroxyl group for further glycosylation. This disaccharide could be synthesized using standard glycosylation with the strategy described in **Scheme 2.14** using the 4,6-*O*-benzylidene acetal-protected thioglycoside **2.60** and the α-trimethylsilyl-fixed glycosyl acceptor **2.71**. Should that approach fail, I envisioned that **2.68** could alternatively be synthesized from **2.60** and either of glycosyl acceptors **2.72** and **2.73**. These four molecules – **2.60**, **2.71**, **2.72** and **2.73** – could all be synthesized from β-D-glucose pentaacetate. Carboxylic acids **2.69** and **2.70** could be synthesized from commercially available tridecanoic acid and (*R*)-(+)-pulegone, respectively. Based on that retrosynthetic plan, I will describe my synthesis of **2.65**, starting from the preparation of carboxylic acids **2.69** and **2.70** and then describing the synthesis of trehalose derivatives **2.68** and **2.65**.



Scheme 2.16. Retrosynthetic plan to synthesize **2.65**.

2.2.2 Synthesis of the carboxylic acids 2.69 and 2.70.

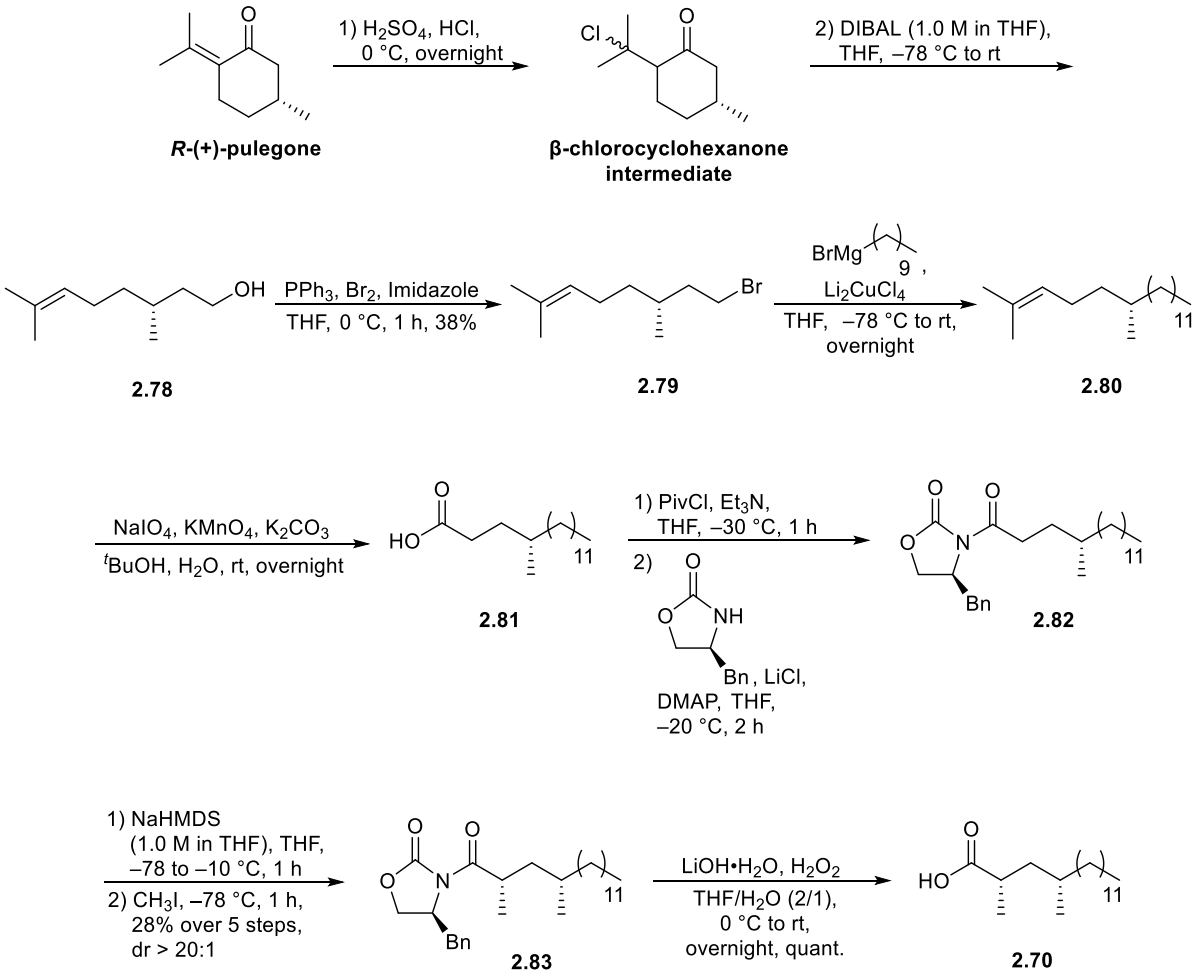
The synthesis of the 17-carbon α,β-unsaturated carboxylic acid **2.69** is shown in **Scheme 2.17**. First, tridecanoic acid was converted to a mixed anhydride using pivaloyl chloride and triethylamine at –30 °C. Then Evans' benzyl oxazolidinone was added to the mixed anhydride in the presence of dried lithium chloride and catalytic 4-(dimethylamino)pyridine (DMAP). The temperature was kept at –20 °C, different from the reported procedure[37] to minimize the formation of the pivaloyl oxazolidinone, which could not be separated from the desired acyloxazolidinone **2.74**. Once in hand, **2.74** was deprotonated by the addition of a solution of sodium bis(trimethylsilyl)amide at –78 °C. The mixture was warmed to –10 °C and the enolate was methylated upon the addition of iodomethane producing **2.75** in an 88% yield over three steps.[37] Compound **2.75** was treated with lithium borohydride to cleave the oxazolidinone and then further reduce the resultant aldehyde to alcohol **2.76** in 64% yield.[38] Alcohol **2.76** was then oxidized to the corresponding aldehyde using (diacetoxyiodo)benzene and catalytic TEMPO.[39] Subjecting this aldehyde to a Wittig reaction using a preformed ylide[40] produced α,β-unsaturated ester **2.77** in 87% yield over two steps. Hydrolysis of ester **2.77** to the carboxylic acid was very sluggish when sodium hydroxide was used. However, switching to lithium hydroxide monohydrate produced the target carboxylic acid **2.69** in a shorter time and in 93% yield, presumably due to the increased electrophilicity of the ester resulting from lithium ion-chelation.



Scheme 2.17. Synthesis of carboxylic acid **2.69**.

Scheme 2.18 shows the synthesis of the 18-carbon carboxylic acid **2.70**. The synthesis started with the conversion of (*R*)-(+)-pulegone to (*R*)-(+)-citronellol (**2.78**) using the published procedure.[41] This process involves passing *in situ* generated hydrochloride gas (by adding concentrated sulfuric acid to concentrated hydrochloric acid) into pulegone to generate a β-chlorocyclohexanone intermediate. Upon reduction of this ketone with DIBAL, an E1cb-type mechanism takes place to open the cyclohexane ring and eliminate the β-chloride generating citronellal, which is further reduced by DIBAL to produce **2.78**. Using the Appel reaction,[42] alcohol **2.78** was converted to citronellyl bromide **2.79** in 38% yield. Bromide **2.79** was coupled with freshly-made decyl magnesium bromide, using a preformed solution of dilithium

tetrachlorocuprate, to generate a mixture of alkene **2.80** and other products,[43] which were difficult to separate. After several rounds of column chromatography, a mixture of **2.80** and what was believed to be eicosane ($C_{20}H_{42}$, produced by dimerization of the Grignard reagent) in a ratio of 1 to 1 was isolated. Because there were no other alkenyl signals observed in the ^{1}H NMR spectrum, this mixture was subjected to oxidative cleavage using catalytic potassium permanganate. A stoichiometric amount of sodium periodate was necessary to regenerate permanganate(VII) to drive the reaction to completion to generate carboxylic acid **2.81**.[44] Unfortunately, like **2.80**, compound **2.81** also could not be obtained in pure form after several column chromatography or acid–base extraction attempts. Therefore, crude material **2.81**, with non-polar impurities, was subjected to the same reaction conditions[37] as those used to synthesize **2.74** and **2.75** (**Scheme 2.17**) to generate **2.82** and **2.83** (28% yield over five steps). Finally, cleaving the oxazolidinone moiety of **2.83** using hydrogen peroxide anion led to the desired 18-carbon carboxylic acid **2.70** in quantitative yield.[37]



Scheme 2.18. Synthesis of carboxylic acid **2.70**.

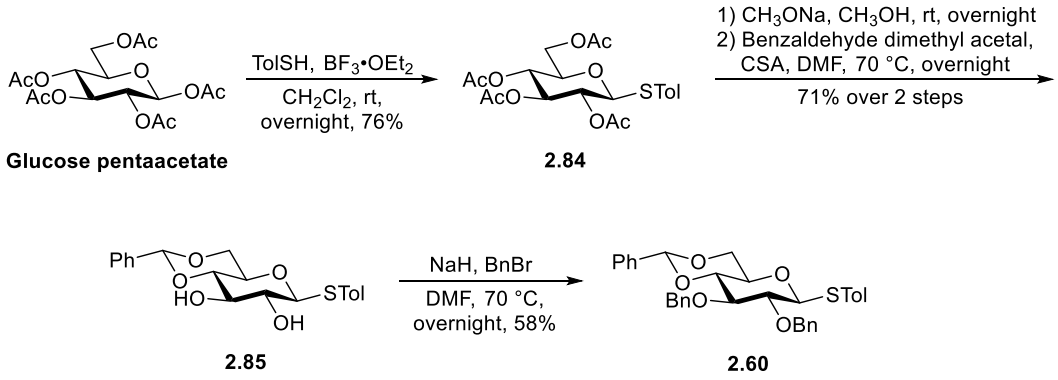
2.2.3 Synthesis of the target moiety 2.65

After the syntheses of the two carboxylic acids **2.69** and **2.70**, I synthesized the trehalose target compound **2.65**. In this section, I will first discuss establishing the α,α-(1↔1) glycosidic linkage to make the trehalose core. The second part will be focused on describing incorporating the lipids into the trehalose core and obtaining **2.65**.

*2.2.3.1 Establishing the α,α-(1↔1) glycosidic linkage in **2.65***

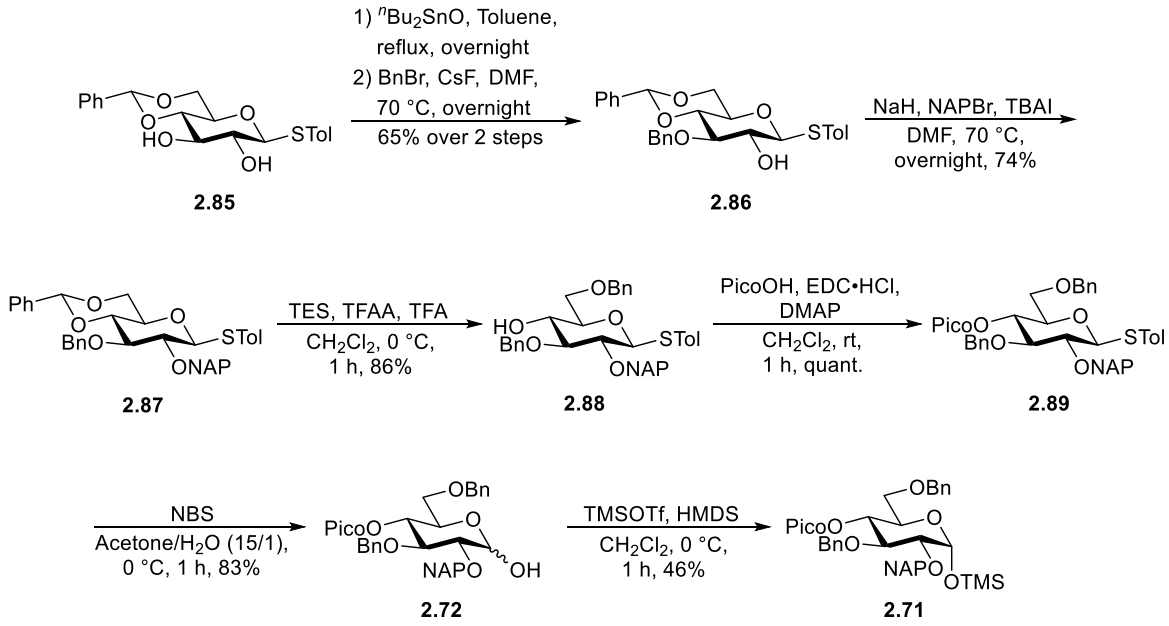
As depicted in **Scheme 2.16**, the strategy of establishing the α,α-(1↔1) glycosidic linkage employs a standard glycosylation approach using substrate-controlled stereoselectivity. This involved using glycosyl donor **2.60** and glycosyl acceptor **2.71**, which has structural features that we anticipated would lead to good α,α-(1↔1) selectivity. The 4,6-*O*-benzylidene in glycosyl donor **2.60** would restrict the conformations of activated **2.60** during glycosylation. This leads to a Curtin–Hammett scenario that favors the formation of the desired stereoisomer through a less stable but more reactive intermediate.[35] Two other glycosyl acceptors (**2.72** and **2.73**) served as backup alternatives, in case the glycosylation between **2.60** and **2.71** did not proceed as expected. Before describing the glycosylation studies between these molecules, the monosaccharide syntheses need to be discussed.

Shown in **Scheme 2.19** is the synthesis of glycosyl donor **2.60**. The β-anomer of glucose pentaacetate was reacted with 4-methylbenzenethiol using boron trifluoride etherate to generate thioglycoside **2.84** in 76% yield. The acetates on **2.84** were hydrolyzed under basic conditions to reveal the corresponding hydroxyl groups. Then, the C-4 and C-6 hydroxyl groups were converted to the 4,6-*O*-benzylidene acetal using benzaldehyde dimethyl acetal under acidic conditions generating **2.85** in 71% yield over two steps. The other hydroxyl groups on **2.85** were then converted to benzyl ethers to generate the desired glycosyl donor **2.60** in 58% yield. An advantage of this synthetic route is all three products could be purified by recrystallization (instead of column chromatography), which enabled me to obtain 20 grams of **2.60** efficiently.



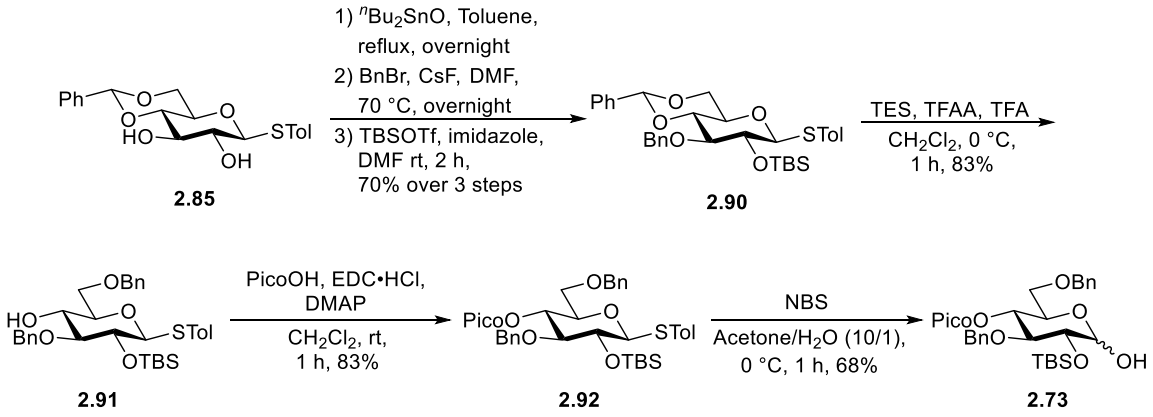
Scheme 2.19. Synthesis of 4,6-*O*-benzylidene protected glycosyl donor **2.60**.

Synthesis of glycosyl acceptors **2.71** and **2.72**, shown in **Scheme 2.20**, began with a regioselective benzylation of the C-3 hydroxyl group of **2.85** to form **2.86** in 65% yield over two steps.[45] This reaction involved forming a 3,4-*O*-stannnylidene acetal using dibutyltin oxide[46] then a nucleophilic displacement with benzyl bromide. During the displacement step, cesium fluoride was used to increase the yield presumably by the combination of two factors: 1) the cesium interacts with the bromide to make benzyl bromide more electrophilic 2) the fluoride coordinates to the tin acetal to make Sn–O more nucleophilic.[47] Then, the C-2 hydroxyl group of **2.86** was converted to the 2-naphthylmethyl ether via S_N2 reaction with 2-(bromomethyl)naphthalene to generate **2.87** in 74% yield.[48] The 4,6-*O*-benzylidene acetal of **2.87** was reductively and regioselectively opened using trifluoroacetic acid and triethylsilane to generate **2.88** in 86% yield.[49] The C-4 hydroxyl group of **2.88** was then coupled with 2-picolinic acid to generate **2.89** quantitatively. Thioglycoside **2.89** was then activated with *N*-bromosuccinimide and hydrolyzed to generate **2.72** in 83% yield. This hemiacetal was converted and isolated as the α-trimethylsilyl acceptor **2.71** using a combination of trimethylsilyl trifluoromethanesulfonate and bis(trimethylsilyl)amine in 46% yield.[33]



Scheme 2.20. Synthesis of glycosyl acceptors **2.71** and **2.72**.

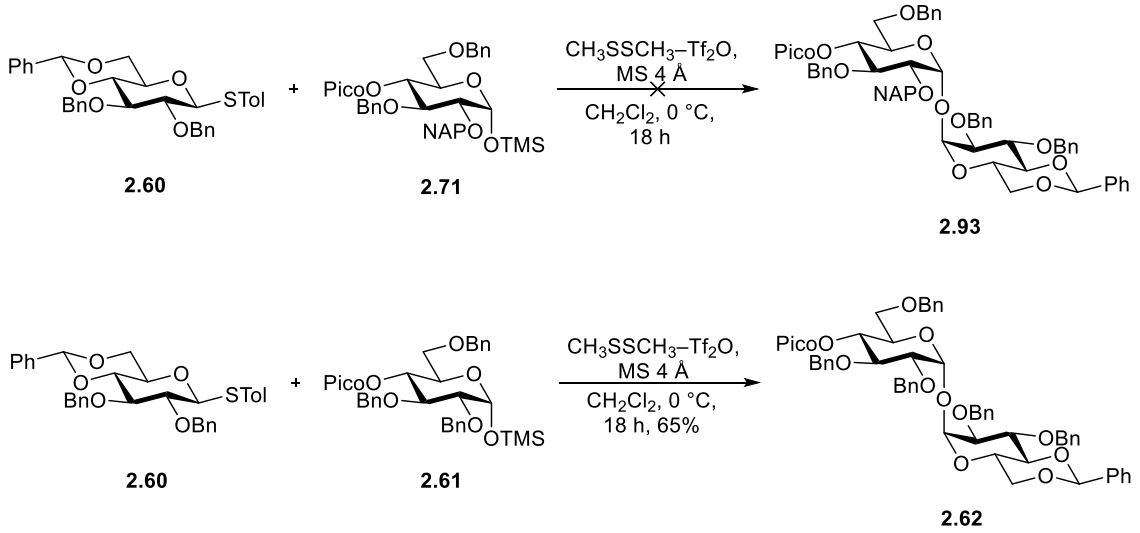
The synthetic route towards glycosyl acceptor **2.73** was almost identical to that used to prepare **2.71**. As shown in **Scheme 2.21**, the approach started with **2.85**, which was used in the route to **2.71**. The same regioselective benzylation was used on **2.85** to protect the C-3 hydroxyl group. Without purification between the steps, the C-2 hydroxyl group on **2.85** was then silylated with *tert*-butyldimethylsilyl trifluoromethanesulfonate to generate **2.90** in 70% yield over three steps. Starting from **2.90**, the same series of steps were carried out as done for the preparation of **2.88**, **2.89** and **2.72** to produce **2.91**, **2.92** and **2.73** in 83%, 83% and 68% yields, respectively.



Scheme 2.21. Synthesis of glycosyl acceptor **2.73**.

With glycosyl donor **2.60** and glycosyl acceptors **2.71**, **2.72** and **2.73** all in hand, I began glycosylation studies to establish the α,α-(1↔1) glycosidic linkage of the trehalose core. The first glycosylation was performed between glycosyl donor **2.60** and glycosyl acceptor **2.71** (**Scheme 2.22**). The reaction did not progress (monitored by TLC) under reported conditions[33] despite the similarities to a published transformation: the coupling of **2.60** and **2.61**. I then tried to identify the reasons for this problem. The corresponding authors suggested in a personal communication with me that the glycosyl acceptors might be contaminated with bis(trimethylsilyl)amine (a by-product from previous step) even after column chromatography. This was an issue they observed; however, I did not observe this by-product contamination with acceptor **2.71** by ^{1}H NMR spectroscopy. Another possibility was that the activator (CH_3SSCH_3–Tf_2O) was not formed, even though it was reported to form rapidly in an NMR tube or less than 30 minutes in a flask.[50] I elongated the formation time from 30 minutes to two hours, even though the reported color change was evident. Doing this did not alter the result of the glycosylation. The formation of the activator might also fail due to impure reagents. However, that was quickly ruled out by measuring the ^{1}H NMR

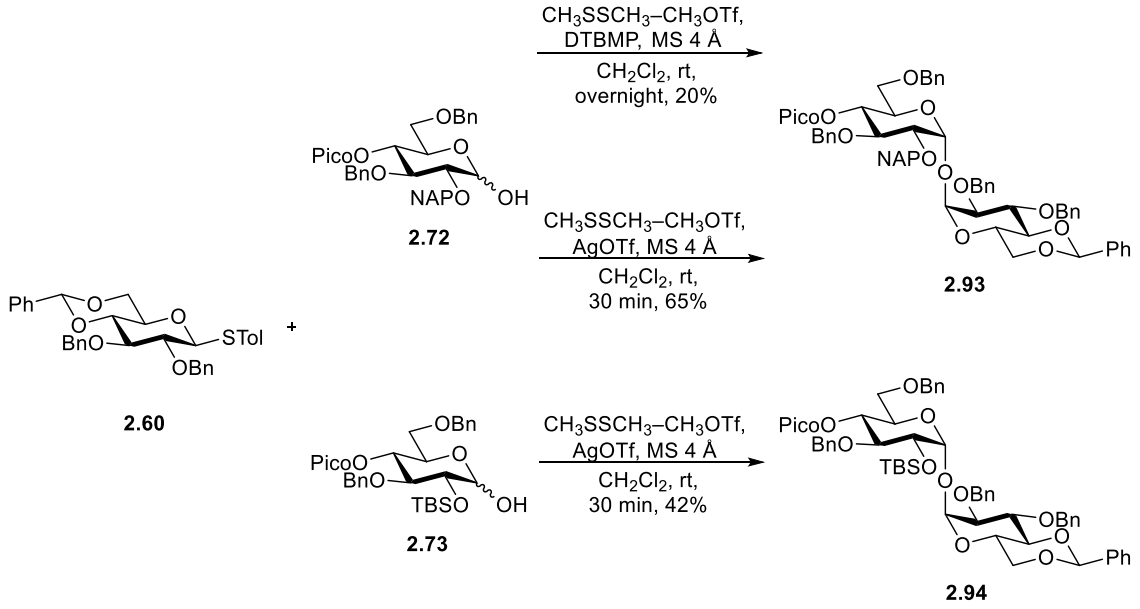
spectrum of the reagents, which showed them to be pure. Based on all of these results for this glycosylation, my hypothesis was that the anomeric trimethylsilyl group was cleaved during the reaction by a residual amount of water, which formed a more reactive glycosyl acceptor. This hypothesis led me to use glycosyl acceptors **2.72** and **2.73**, which I expected to be more reactive than **2.71**.



Scheme 2.22. Initial glycosylation study.

Glycosylations between glycosyl donor **2.60** and glycosyl acceptors **2.72** and **2.73** using (CH_3SSCH_3–CH_3OTf)[51] as the activator were successful although the yields were modest (**Scheme 2.23**). Glycosylation between **2.60** and **2.72** produced trehalose derivative **2.93** in 20% yield. This reaction was sluggish and low-yielding and so I tried to optimize it. By comparing it to glycosylations that use an activator and a Lewis acid (e.g., *N*-iodosuccinimide and silver trifluoromethanesulfonate), I thought that it might be missing a Lewis acid. Therefore, I replaced di-*tert*-butylmethylpyridine with AgOTf and the result was remarkable. Not only the reaction finished within 30 minutes, but the yield increased to 65%. The anomeric configuration in

trehalose **2.93** was verified by the two H-1 to H-2 coupling constants observed in ^{1}H NMR (4.2 and 3.5 Hz), indicating the α,α-configuration. These conditions were also applied to the glycosylation between **2.60** and **2.73**, which produced trehalose **2.94** in only 42% yield. The anomeric configuration in trehalose derivative **2.94** was also verified in the same way as for **2.93**, the two H-1 to H-2 coupling constants (3.6 and 3.6 Hz) indicated the α,α-configuration. Unfortunately, the yield was even lower (26%) during an attempt to upscale the reaction. Although a sufficient amount (2.9 g) of **2.94** was obtained, this glycosylation requires further improvement. Having formed the key linkage, the next goal was modifying **2.93** and **2.94** for attaching the lipids **2.69** and **2.70**, and obtaining the target trehalose derivative **2.65**.

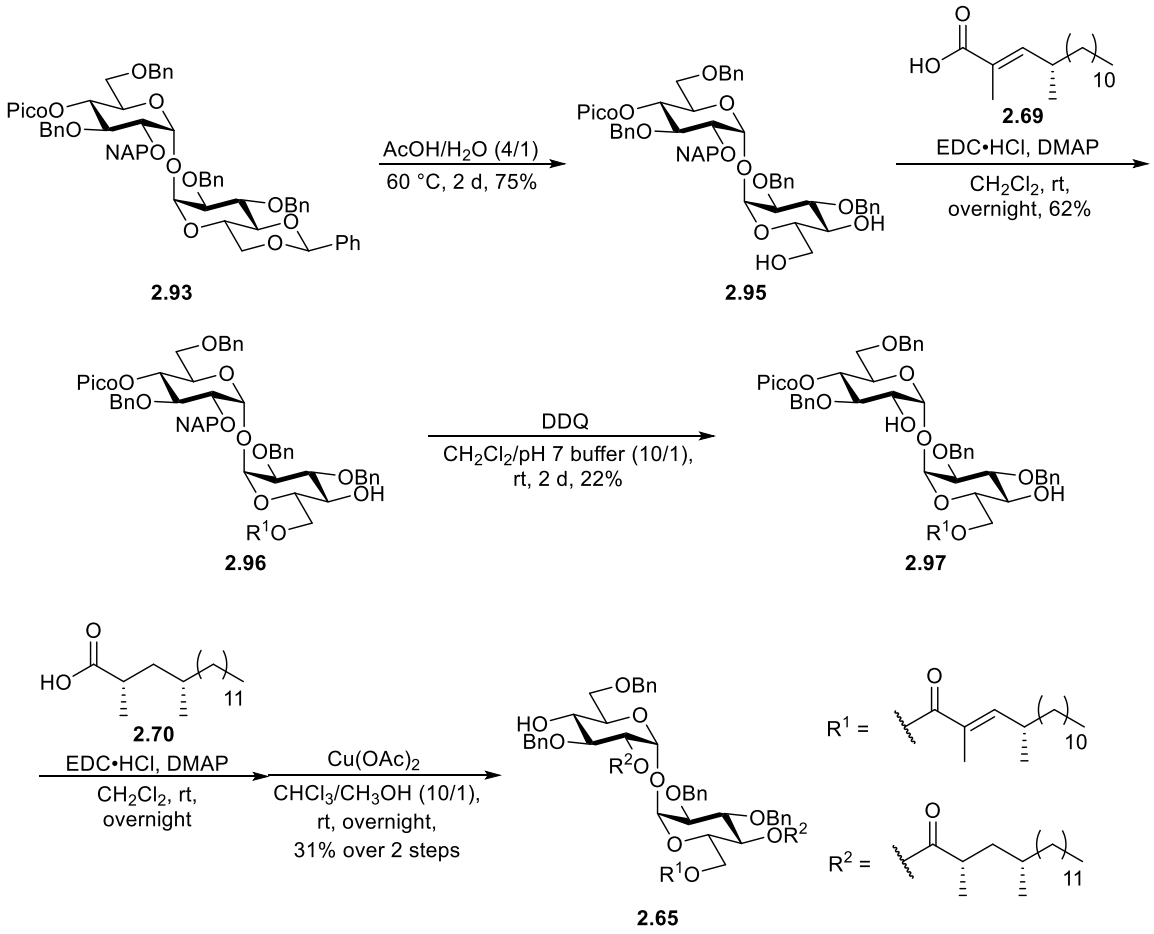


Scheme 2.23. Successful glycosylation to obtain trehalose derivatives **2.93** and **2.94**.

*2.2.3.2 Further modification of trehalose derivatives into target moiety **2.65***

Because there are two trehalose derivatives to discuss, I will start with **2.93** (**Scheme 2.24**). The benzylidene acetal in **2.93** was hydrolyzed to produce **2.95** in 75% yield, revealing two

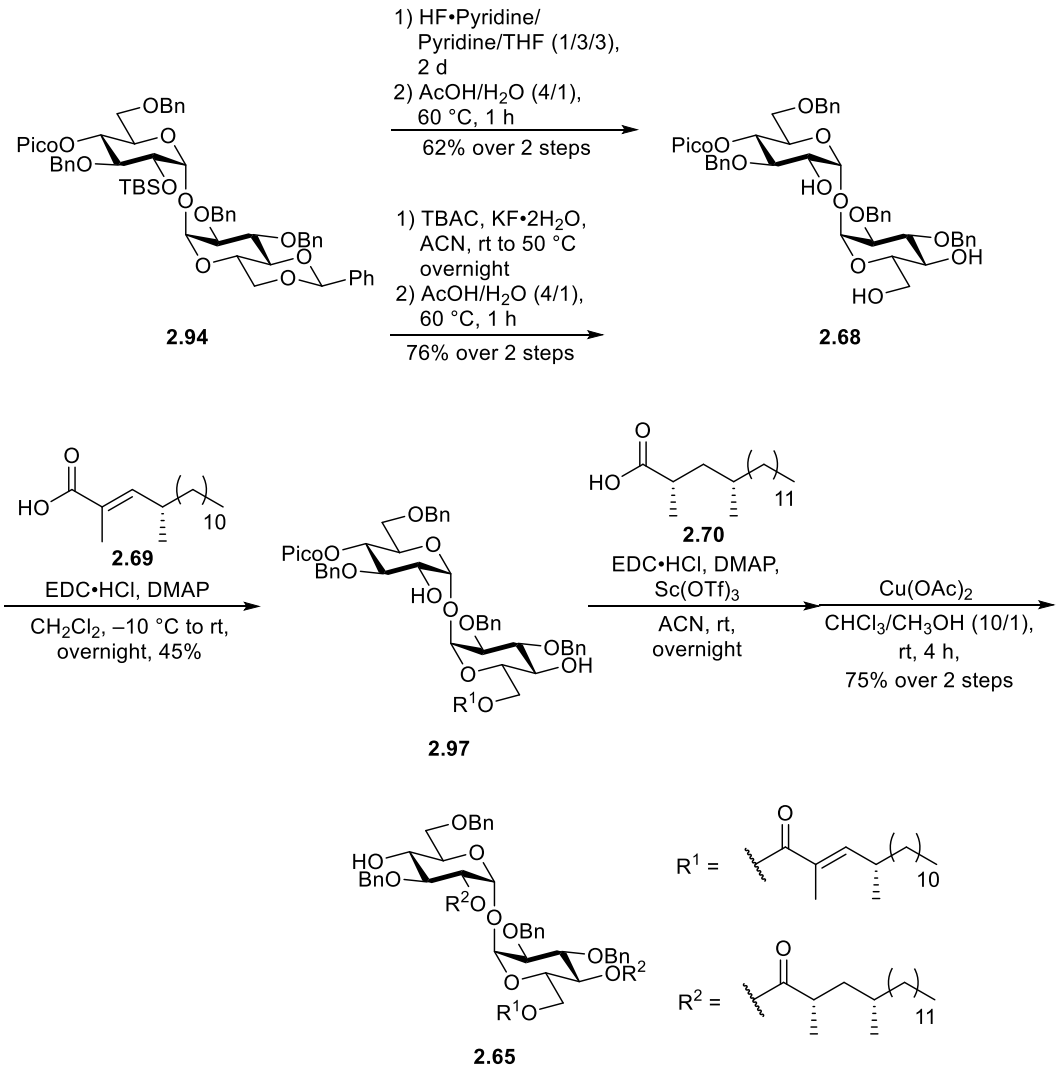
hydroxyl groups for coupling to the lipids. The primary alcohol (C-6 hydroxyl group) was selectively coupled with **2.69** to produce **2.96** in 62% yield. The regioselectivity was confirmed by the deshielding of the H-6 resonances in ^{1}H NMR spectrum in the acylated product (from 3.69 ppm in **2.95** to 4.09 and 4.48 ppm in **2.96**). Deprotecting the NAP group on **2.96** to produce **2.97** proved to be difficult, possibly due to steric hindrance. With the little amount of **2.97** I had, I tried to couple the two hydroxyl groups with lipid **2.70**. Unfortunately, the reaction was sluggish, and the product could not be separated from **2.70**. However, deprotecting the picoloyl after coupling with **2.70** separated the desired target **2.65** from **2.70**, but the overall yield was low (31%). This route was then abandoned, mainly due to the low-yielding NAP deprotection step. Therefore, I switched to **2.94**, intent on obtaining **2.65** from this intermediate instead.



Scheme 2.24. Obtaining the desired target **2.65** from **2.93**.

The synthesis began by deprotection of the TBS group on **2.94** with HF•Pyridine, which was also sluggish and never went to completion (**Scheme 2.25**). Similar to the cleavage of the NAP group on **2.96**, the reason might also be because it is sterically hindered. However, generating TBAF (a more reactive desilylating reagent) *in situ* with tetra-*n*-butylammonium chloride and KF[52] solved this problem. After deprotection of the benzylidene acetal, triol **2.68** was obtained in 76% over two steps. The primary, C-6, hydroxyl group of **2.68** was selectively coupled with **2.69** to produce **2.97** in 45% yield. The regioselectivity was also confirmed by the deshielding H-6 resonances in the ^{1}H NMR spectrum of the product, from 3.75, 3.82 ppm in **2.68** to 4.24, 4.55 ppm in **2.97**. The difficult coupling of **2.97** and **2.70** was solved by the addition of Sc(OTf)$_3$,[53] which

required that the reaction be carried out in acetonitrile to dissolve the Lewis acid. The product still could not be separated from **2.70**, but deprotection of the picoloyl group, as in previous case, solved the problem and gave the target **2.65** in 75% yield over two steps.



Scheme 2.25. Obtaining the desired target **2.65** from **2.94**.

2.3 Summary

In the chapter, I discussed the reported methods for establishing an α,α-(1↔1) glycosidic linkage to make trehalose derivatives. The methods include desymmetrizing trehalose, IAD and standard glycosylation. Further introduction of these methods included applications toward the preparation of acylated trehalose derivatives and trehalose-containing oligosaccharide, which informed my decision on how to best obtain my desired target **2.65,** an asymmetrically-acylated trehalose derivative. The key step to obtaining the trehalose core is the glycosylation between donor **2.60** and acceptor **2.73** using CH_3SSCH_3–CH_3OTf as the activator. The glycosylation was further improved by adding the Lewis acid AgOTf to obtain **2.94**. The protecting groups of the trehalose core were then modified and then the synthesized lipids **2.69** and **2.70** were attached to it to provide reasonable amounts of material (0.5 g of **2.65**). There is more that can be improved in the future, such as the large-scale glycosylation. A detailed future plan will be summarized in Chapter 5.

2.4 Experimental section

2.4.1 General methods

All reagents were purchased from commercial sources and used without further purification except LiCl, $Sc(OTf)_3$, AgOTf and $KF•2H_2O$, which were purified as described below. Dichloromethane, tetrahydrofuran, acetonitrile and *N*,*N*-dimethylformamide used in reactions as solvents were taken from a solvent purification system in which the solvents were purified by successive passage through columns of alumina, copper and molecular sieves under argon. All reactions were carried out in round bottom flasks with stir bars and capped with rubber septum. Thin layer chromatography was performed on silica gel 60 F254 (0.25 mm, Merck) glass plates. Spots were

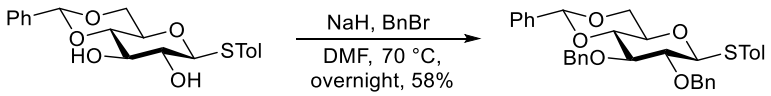
detected by UV light and by charring after treatment with a solution of either 1) ceric ammonium nitrate (0.5 g) and ammonium molybdate (12 g) in water (235 mL) and sulfuric acid (15 mL) or 2) potassium permanganate (1.5 g) and potassium carbonate (10 g) and 10% NaOH(aq) (1.25 mL) in water (200 mL). In the reaction work-up steps involving extractions, TLC were performed on combined organic layer and aqueous layer after extraction and before concentrating the combined organic layer. All column chromatography was performed on silica gel 60 (40–60 μm). Melting points were measured on a Gallenkamp apparatus and are not corrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter at the sodium D line (589 nm) at 21 ± 2 °C and are in units of (deg·mL)/(dm·g). FTIR spectra were run on Thermo Nicolet (Madison Wisconsin, USA) 8700 main bench with a Continuum FTIR microscope attached, and samples were cast from a chloroform solution onto an IR-transparent silicone wafer. ^{1}H NMR spectra were recorded at 400, 500, 600 and 700 MHz and the chemical shifts were referenced to $CHCl_3$ (7.26 ppm, $CDCl_3$). ^{1}H decoupled ^{13}C NMR spectra were recorded at 125 MHz and the chemical shifts were referenced to $CDCl_3$ (77.00 ppm, $CDCl_3$). High resolution EI mass spectra were recorded on Kratos Analytical MS-50G spectrometer; high resolution ESI mass spectra were recorded on Agilent Technologies 6220 spectrometer; high resolution MALDI mass spectra were recorded on Bruker 9.4 T Apex-Qe spectrometer.

Procedure for drying solids:

LiCl, $Sc(OTf)_3$ and AgOTf: The metal salt was transferred to a round bottom flask. The flask was attached to high vacuum and gently heated with a heat gun on a low setting (~250 °C). After the solids stopped bumping and the appearance changed from shiny to chalky, the flask was cooled to room temperature. The dried metal salt was then weighed quickly in air and then transferred to the reaction flask.

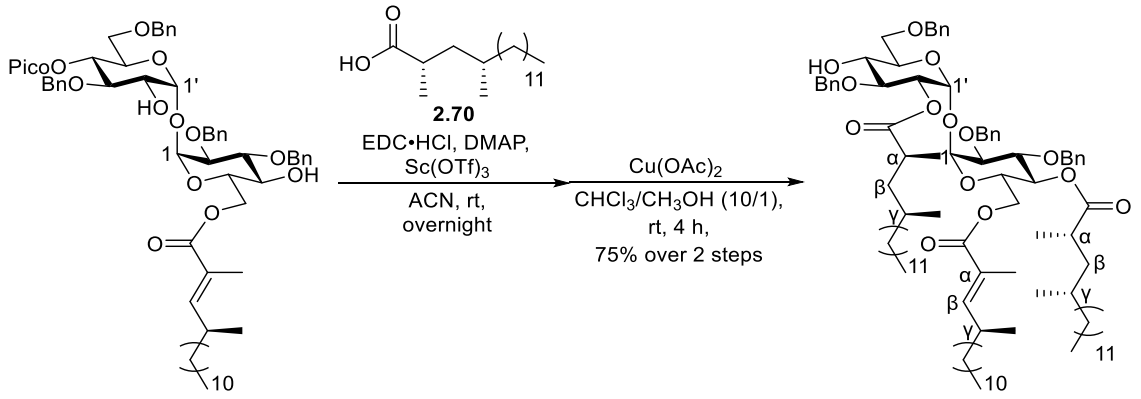
KF•2H_2O: KF•2H_2O was ground with a mortar and pestle and then transferred to a round bottom flask. The flask was attached to high vacuum and dried for two hours. Dried KF•2H_2O was then weighed quickly in air then transferred to the reaction flask.

2.4.2 Experimental procedures and spectroscopic data



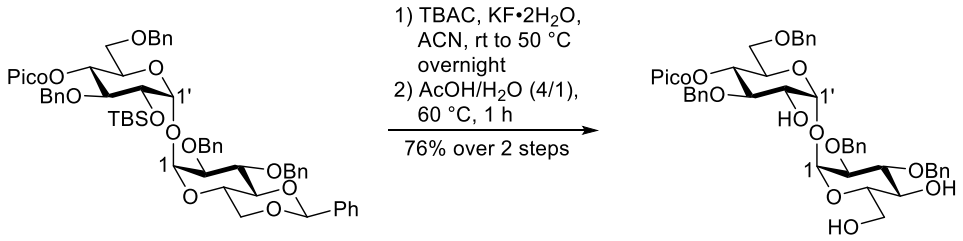
2.85 **2.60**

4-Methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (2.60). Thioglycoside **2.85** (24 g, 63 mmol, 1.0 equiv) was dissolved in DMF (150 mL) and then BnBr (30 mL, 0.25 mol, 4.0 equiv) was added. Under vigorous stirring, NaH (60% dispersion in mineral oil, 10 g, 0.25 mol, 4.0 equiv) was slowly added. The mixture was heated at 70 °C overnight at which point TLC (1:1 EtOAc–hexane) indicated no **2.85** remained. The mixture was cooled to room temperature, diluted with EtOAc (200 mL) and the excess NaH quenched by the addition of H_2O (50 mL) before being transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 500 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude product was purified by recrystallization (1:5 EtOAc–hexane, 720 mL) to give **2.60** (20.1 g, 58%) as beige feather-like needles; m.p. 115–116 °C. The ^{1}H and ^{13}C NMR data for **2.60** were identical to those reported.[54]



3',6'-di-*O*-Benzyl-2'-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (2.65). Trehalose derivative **2.97** (48 mg, 45 μmol, 1.0 equiv) and **2.70** (78 mg, 0.27 mmol, 6.0 equiv) were dissolved in acetonitrile (0.5 mL) and then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (52 mg, 0.27 mmol, 6.0 equiv), 4-(dimethylamino)pyridine (33 mg, 0.27 mmol, 6.0 equiv) and $Sc(OTf)_3$ (45 mg, 91 μmol, 2.0 equiv) were added. The mixture was stirred at room temperature overnight at which point TLC (1:4 EtOAc–hexane) indicated no **2.97** remained. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. This obtained crude product was dissolved in $CHCl_3$ (0.9 mL) and CH_3OH (0.09 mL). Next, $Cu(OAc)_2$ (12 mg, 68 μmol, 1.5 equiv) was added to the mixture and the mixture was stirred at room temperature for 4 h. The mixture was concentrated on a rotary evaporator and then purified by column chromatography (50 mL silica gel, 0:1→1:10 EtOAc–hexane) to give **2.65** (51 mg, 75%) as a transparent colorless film. $[\alpha]_D^{21}$ +45.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.33–7.22 (m, 20 H, ArH), 6.54 (d, *J* = 9.8 Hz, 1 H, lipid βH), 5.28 (d, *J* = 3.5 Hz, 1 H, H-1'), 5.23 (d,

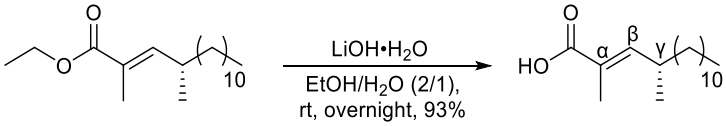
J = 3.5 Hz, 1 H, H-1), 5.10 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4), 4.97 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2'), 4.90 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.85 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.82 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.68 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.67–4.63 (m, 2 H, benzylic H), 4.52 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.48 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.11–3.96 (m, 6 H, H-6, H-5', H-3, H-3', H-5), 3.75 (dd, *J* = 9.8, 9.1 Hz, 1 H, H-4'), 3.65 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2), 3.58–3.57 (m, 2 H, 2 × H-6'), 2.58–2.43 (m, 3 H, lipid αH and γH), 2.41 (br s, 1 H, 4-OH), 1.81 (d, *J* = 2.1 Hz, 3 H, lipid αCH_3), 1.75–1.61 (m, 3 H), 1.41–0.93 (m, 68 H), 1.14 (d, *J* = 6.3 Hz, 3 H, lipid αCH_3), 1.07 (d, *J* = 6.3 Hz, 3 H, lipid αCH_3), 0.99 (d, *J* = 6.3 Hz, 3 H, lipid γCH_3), 0.89–0.86 (m, 9 H, lipid terminal CH_3), 0.82–0.80 (m, 6 H, lipid γCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 175.8 (ester C=O), 175.3 (ester C=O), 167.9 (ester C=O), 149.3 (lipid βC), 138.5, 138.2, 137.74, 137.72, 128.5, 128.4, 128.3, 127.8, 127.74, 127.68, 127.5, 127.3, 127.2, 125.5 (lipid αC), 91.6 (C-1'), 91.5 (C-1), 79.3 (C-3'), 79.0 (C-2), 78.8 (C-3), 74.9 (benzylic C), 74.4 (benzylic C), 73.7 (benzylic C), 73.3 (benzylic C), 72.1 (C-2'), 71.6 (C-4'), 70.4 (C-5'), 69.7 (C-6'), 69.5 (C-4), 68.6 (C-5), 62.4 (C-6), 41.4, 41.35, 41.29, 37.3 (lipid αC), 37.00 (lipid αC), 36.96, 36.8, 36.6, 33.3 (lipid γC), 31.9, 30.7, 30.53, 30.51, 30.1, 29.9, 29.81, 29.76, 29.72, 29.69, 29.68, 29.4, 27.5, 26.81, 26.77, 22.7, 19.8, 19.7, 19.6, 19.5, 17.84, 17.79, 17.6, 14.1 (lipid terminal CH_3), 12.5 (lipid αCH_3); IR (cast film): 3486, 2956, 2924, 2853, 1742, 1716, 1463, 1378, 1144, 1111, 1025 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{93}H_{144}NaO_{14}$, 1508.0448; found, 1508.0468.



2.94 **2.68**

3',6'-di-*O*-Benzyl-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-α-D-glucopyranoside (2.68). Trehalose derivative **2.94** (2.8 g, 2.8 mmol, 1.0 equiv) was dissolved in acetonitrile (28 mL) then tetra-*n*-butylammonium chloride (2.5 g, 8.9 mmol, 3.2 equiv) and KF•2H_2O (0.8 g, 8.3 mmol, 3.0 equiv) was added. The mixture was stirred at 50 °C overnight at which point TLC (1:2 EtOAc–hexane) indicated no **2.94** was left. The mixture was cooled to room temperature then diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $Na_2S_2O_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. Acetic acid (28 mL) and H_2O (7 mL) were added to the obtained crude product and then the mixture was stirred at 60 °C for 1 h. The mixture was dried by dissolving in toluene and evaporation (3 × 50 mL) and the resulting residue was then purified by column chromatography (150 mL silica gel, 0:1→1:1→4:1→1:0 EtOAc–hexane) to give **2.68** (1.7 g, 76%) as a transparent colorless film. $[\alpha]_D^{21}$ –11.4 (*c* 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.77 (d, *J* = 4.2 Hz, 1 H, picoloyl H), 8.01 (d, *J* = 7.8 Hz, 1 H, picoloyl H), 7.81 (dd, *J* = 7.8, 7.2 Hz, 1 H, picoloyl H), 7.49 (dd, *J* = 7.2, 4.2 Hz, 1 H, picoloyl H), 7.42–7.14 (m, 20 H, ArH), 5.47 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4'), 5.28 (d, *J* = 3.6 Hz, 1 H, H-1), 5.25 (d, *J* = 3.6 Hz, 1 H, H-1'), 5.10 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.82 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.77 (m, 2 H, benzylic H), 4.74 (d, *J* = 12.0 Hz, benzylic H), 4.64 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.48–4.41 (m, 3 H, H-5' and 2 × benzylic H), 4.14 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-3'), 3.98 (ddd, *J*

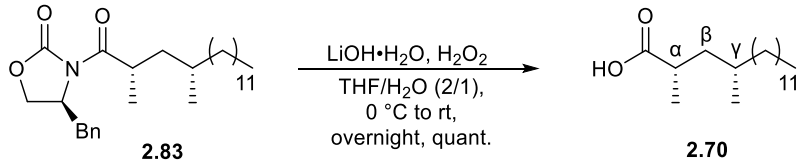
= 10.2, 4.8, 3.6 Hz, 1 H, H-5), 3.90 (dd, *J* = 9.0, 3.6 Hz, 1 H, H-2'), 3.85 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-3), 3.82 (dd, *J* = 11.4, 3.6 Hz, 1 H, H-6), 3.75 (dd, *J* = 11.4, 4.8 Hz, 1 H, H-6), 3.59 (dd, *J* = 10.2, 9.0 Hz, 1 H, H-4), 3.56 (dd, *J* = 9.6, 3.6 Hz, 1 H, H-2), 3.51 (dd, *J* = 10.8, 3.0 Hz, 1 H, H-6'), 3.48 (dd, *J* = 10.8, 4.8 Hz, 1 H, H-6'), 2.40 (br s, 1 H, 4-OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 164.2 (C=O), 149.8 (picoloyl C), 147.6, 138.7, 138.1, 137.8, 137.7, 137.0 (picoloyl C), 128.6, 128.37, 128.35, 128.2, 128.1, 127.9, 127.8, 127.74, 127.68, 127.66, 127.4, 127.0 (picoloyl C), 125.6 (picoloyl C), 95.0 (C-1'), 93.6 (C-1), 81.1 (C-3), 79.8 (C-3'), 78.8 (C-2), 75.2 (benzylic C), 74.8 (benzylic C), 73.7 (benzylic C), 72.4 (benzylic C), 72.1 (C-4'), 71.8 (C-2'), 71.5 (C-5), 70.4 (C-4), 69.8 (C-5'), 68.8 (C-6'), 62.4 (C-6); IR (cast film): 3091, 3031, 2924, 2859, 1729, 1605, 1454, 1370, 1090, 1029 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{46}H_{49}NNaO_{12}$, 830.3147; found, 830.3140.



2.77 **2.69**

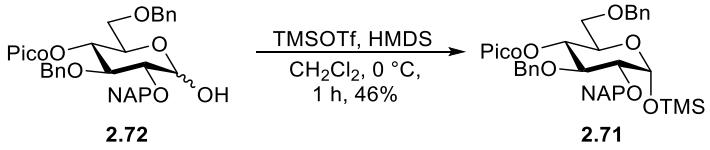
(*S*,*E*)-2,4-Dimethylpentadec-2-enoic acid (2.69). Ester **2.77** (2.4 g, 8.1 mmol, 1.0 equiv) was dissolved in EtOH (40 mL) and H_2O (20 mL). Lithium hydroxide monohydrate (1.4 g, 32 mmol, 4.0 equiv) was added and then the mixture was stirred at room temperature overnight at which point TLC (1:15 EtOAc–hexane) indicated no **2.77** was left. The mixture was acidified to pH 3 with 4 N $HCl_{(aq)}$ and then transferred to a separatory funnel. The aqueous layer was extracted with Et_2O (3 × 100 mL) then the organic extracts were combined, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:6→1:3 EtOAc–hexane) to give **2.69** (2.0 g, 93%) as a

transparent pale-yellow oil. $[\alpha]_D^{21}$ +24.1 (*c* 0.3, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 11.50 (br s, 1 H, COOH), 6.69 (dd, *J* = 10.2, 1.2 Hz, 1 H, βH), 2.53–2.46 (m, 1 H, γH), 1.84 (d, *J* = 1.2 Hz, 3 H, αCH_3), 1.40–1.25 (m, 20 H), 1.00 (d, *J* = 6.6 Hz, 3 H, γCH_3), 0.88 (dd, *J* = 7.2, 7.2 Hz, 3 H, terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 173.4 (C=O), 151.1 (βC), 125.4 (αC), 36.7, 33.5 (γC), 31.9, 29.8, 29.7, 29.65, 29.62, 29.57, 29.3, 27.5, 22.7 (γCH_3), 14.1 (terminal CH_3), 12.1 (αCH_3); IR (cast film): 2926, 2854, 2669, 2544, 1689, 1644, 1458, 1281 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M–H]^-$ calcd for $C_{17}H_{31}O_2$, 267.2330; found, 267.2337.



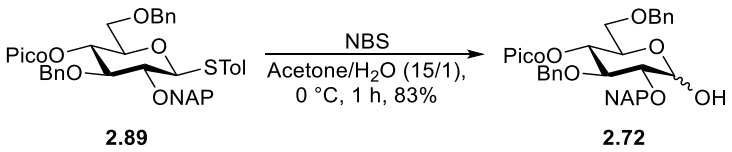
(2*S*,4*S*)-2,4-Dimethylhexadecanoic acid (2.70). Oxazolidinone **2.83** (1.5 g, 3.4 mmol, 1.0 equiv) was dissolved in THF (34 mL) and H_2O (17 mL) and then the reaction mixture was cooled to 0 °C. Lithium hydroxide monohydrate (0.72 g, 17 mmol, 5.0 equiv) and H_2O_2 (30% in water, 4.0 mL, 34 mmol, 10 equiv) were added and then the mixture was warmed to room temperature and stirred overnight at which point TLC (1:4 EtOAc–hexane) indicated no **2.83** was left. The mixture was acidified to pH 3 with 4 N $HCl_{(aq)}$ and then transferred to a separatory funnel. The aqueous layer was extracted with Et_2O (3 × 100 mL) then the organic extracts were combined, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:10→1:4 EtOAc–hexane) to give **2.70** (1.0 g, quantitative) as a transparent pale-yellow oil. $[\alpha]_D^{21}$ +1.8 (*c* 0.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 10.84 (br s, 1 H, COOH), 2.59–2.56 (m, 1 H, αH), 1.73

(ddd, J = 14.4, 9.0, 5.4 Hz, 1 H, βH), 1.48–1.44 (m, 1 H, γH), 1.32–1.26 (m, 21 H), 1.18 (d, J = 6.6 Hz, 3 H, αCH_3), 1.18–1.12 (m, 1 H, βH), 1.12–1.09 (m, 1 H), 0.89 (d, J = 6.6 Hz, 3 H, γCH_3), 0.88 (dd, J = 7.2, 7.2 Hz, 3 H, terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 182.4 (C=O), 41.3 (βC), 37.1 (αC), 37.0, 31.9, 30.7 (γC), 29.9, 29.70, 29.69, 29.67, 29.4, 26.8, 22.7, 19.6 (γCH_3), 17.8 (αCH_3), 14.1 (terminal CH_3); IR (cast film): 2957, 2924, 2854, 2667, 1707, 1465, 1379, 1291, 1241 cm^{-1}; HRMS–ESI–TOF (m/z): [M–H]$^-$ calcd for $C_{18}H_{35}O_2$, 283.2643; found, 283.2644.



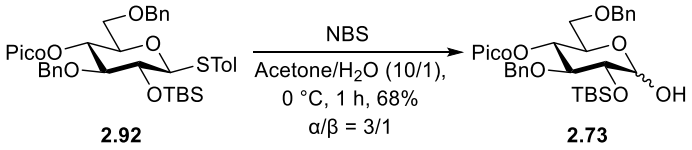
2.72 **2.71**

3,6-di-*O*-Benzyl-2-*O*-(2-naphthylmethyl)-4-*O*-picoloyl-1-*O*-trimethylsilyl-α-D-glucopyranose (2.71). Hemiacetal **2.72** (0.18 g, 0.29 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (3.7 mL) and the mixture was cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (5.3 μL, 29 μmol, 0.10 equiv) and bis(trimethylsilyl)amine (61 μL, 0.29 mmol, 1.0 equiv) were added and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with EtOAc and then saturated $NH_4Cl_{(aq)}$ was added. The mixture was transferred to a separatory funnel and the organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:3 EtOAc–hexane) to give **2.71** (91 mg, 46%) as white amorphous solid. The ^{1}H and ^{13}C NMR data for **2.71** were identical to those reported.[33]



2.89 **2.72**

3,6-di-*O*-Benzyl-2-*O*-(2-naphthylmethyl)-4-*O*-picoloyl-D-glucopyranose (2.72). Thioglycoside **2.89** (0.21 g, 0.30 mmol, 1.0 equiv) was dissolved in acetone (6 mL) and H_2O (0.4 mL) and then cooled to 0 °C. *N*-Bromosuccinimide (0.24 g, 14 mmol, 4.5 equiv) was added and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with EtOAc and saturated $NaHCO_{3(aq)}$ was added. The mixture was transferred to a separatory funnel and the organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 1:1→3:1 EtOAc–hexane) to give **2.72** as a transparent colorless film. The ^1H and ^{13}C NMR data for **2.72** were identical to those reported.[33]

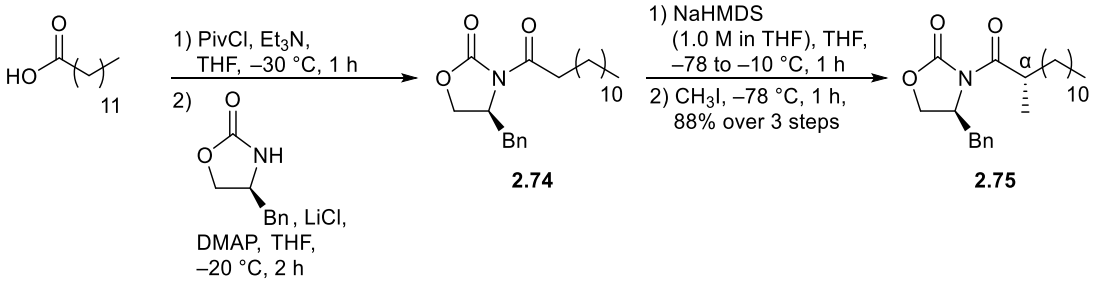


2.92 **2.73**

3,6-di-*O*-Benzyl-2-*O*-*tert*-butyldimethylsilyl-4-*O*-picoloyl-D-glucopyranose (2.73). Thioglycoside **2.92** (11 g, 16 mmol, 1.0 equiv) was dissolved in acetone (80 mL) and H_2O (8 mL) and then the solution was cooled to 0 °C. *N*-Bromosuccinimide (8.6 g, 48 mmol, 3.0 equiv) was added and then the mixture was stirred at 0 °C for 1 h. Although TLC (1:1 EtOAc–hexane) did not indicate full consumption of **2.92**, the mixture was diluted with EtOAc and then saturated $NaHCO_{3(aq)}$ before being transferred to a separatory funnel. The organic layer was washed with

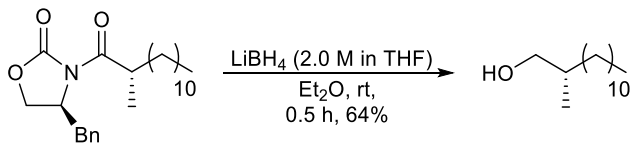
saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:2→1:1→2:1 EtOAc–hexane) to give **2.73** (6.3 g, 68%, α:β = 3:1) as a transparent colorless film. ^{1}H NMR (600 MHz, $CDCl_3$, α isomer, δ): 8.69 (m, 1 H, picoloyl H), 7.97 (d, *J* = 7.8 Hz, 1 H, picoloyl H), 7.76 (ddd, *J* = 7.8, 7.8, 1.8 Hz, 1 H, picoloyl H), 7.43 (ddd, *J* = 7.8, 4.8, 1.2 Hz, 1 H, picoloyl H), 7.23–7.02 (m, 10 H, ArH), 5.43 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4), 5.27 (dd, *J* = 3.6, 3.0 Hz, 1 H, H-1), 4.80 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.67 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.48–4.42 (m, 3 H, H-5 and benzylic H), 4.26 (br s, 1 H, 1-OH), 4.19 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-3), 3.87 (dd, *J* = 9.0, 3.6 Hz, 1 H, H-2), 3.60–3.58 (m, 1 H, H-6), 3.50 (dd, *J* = 10.8, 4.2 Hz, H-6), 0.91 (s, 9 H, *t*-butyl CH_3), 0.10 (s, 3 H, silyl CH_3), 0.09 (s, 3 H, silyl CH_3); ^{1}H NMR (600 MHz, $CDCl_3$, β isomer, δ): 8.70 (m, 1 H, picoloyl H), 9.30 (d, *J* = 7.8 Hz, 1 H, picoloyl H), 7.74 (m, 1 H, picoloyl H), 7.42 (m, 1 H, picoloyl H), 7.23–7.02 (m, 10 H, ArH), 5.34 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4), 4.78 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.69–4.63 (m, 2 H, H-1 and benzylic H), 4.48–4.42 (m, 2 H, benzylic H), 3.92–3.89 (m, 1 H, H-5), 3.77 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-3), 3.67 (d, *J* = 5.4 Hz, 1 H, 1-OH), 3.62–3.60 (m, 3 H, H-2 and H-6), 0.91 (s, 9 H, *t*-butyl CH_3), 0.15 (s, 3 H, silyl CH_3), 0.08 (s, 3 H, silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, α isomer, δ): 163.8 (picoloyl C=O), 149.5, 147.5, 138.4, 137.7, 136.9, 128.0, 127.9, 127.7, 127.6, 127.3, 127.0, 126.9, 125.4, 93.4 (C-1), 79.6 (C-3), 75.5 (benzylic C), 74.0 (C-2), 73.4 (benzylic C), 72.1 (C-4), 68.9 (C-6), 68.7 (C-5), 25.7 (*t*-butyl CH_3), 17.9 (*t*-butyl C), –4.63 (silyl CH_3), –4.73 (silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, β isomer, δ): 164.0 (picoloyl C=O), 149.7, 147.4, 138.0, 137.6, 136.8, 128.1, 127.9, 127.7, 127.6, 127.4, 127.1, 125.4, 97.6 (C-1), 82.9 (C-3), 76.1 (C-2), 75.4 (benzylic C), 73.5 (benzylic C), 73.1 (C-5), 72.4 (C-4), 69.5 (C-6), 25.9 (*t*-butyl CH_3), 18.2 (*t*-butyl C), –4.2 (silyl CH_3), –4.4 (silyl CH_3); IR (cast film): 3240, 3064, 3032, 2928, 2857, 1731, 1591, 1472,

1361, 1247, 1126, 1029 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{32}H_{41}NNaO_7Si$, 602.2545; found, 602.2542.



(*S*)-4-Benzyl-3-((*S*)-2-methyltridecanoyl)oxazolidin-2-one (2.75). Tridecanoic acid (4.1 g, 19 mmol, 1.0 equiv) was dissolved in THF (63 mL) and cooled to –30 °C. Trimethylacetyl chloride (2.6 mL, 21 mmol, 1.1 equiv) and Et_3N (9.2 mL, 66 mmol, 3.5 equiv) were added and the mixture was stirred at –30 °C for 1 h. The mixture was then warmed to –20 °C before LiCl (4.0 g, 95 mmol, 5.0 equiv), DMAP (0.23 g, 1.9 mmol, 0.10 equiv) and (*S*)-4-benzyloxazolidin-2-one (3.7 g, 21 mmol, 1.1 equiv) were added. The mixture was stirred at –20 °C for 2 h at which point TLC (1:6 EtOAc–hexane) indicated no progression of the reaction. The mixture was diluted with Et_2O and then transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:12 EtOAc–hexane) to give an inseparable mixture of **2.74** and pivaloyl oxazolidinone (7.4 g). HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{23}H_{35}NNaO_3$, 396.2509; found, 396.2509. This inseparable mixture (7.4 g) was dissolved in THF (66 mL) and the solution was then cooled to –78 °C. Sodium bis(trimethylsilyl)amide (1.0 M in THF, 24 mL, 24 mmol, 1.2 equiv) was added to the mixture and then the mixture was immediately put into a –10 °C bath and stirred for 1 h. The mixture was then

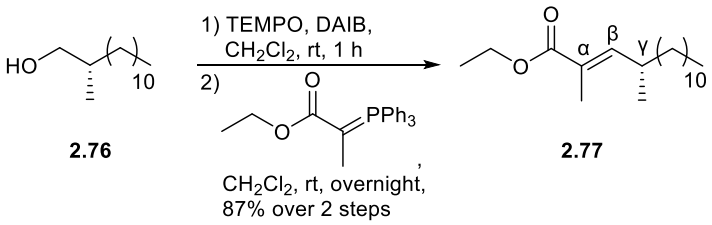
cooled to –78 °C, CH_3I (6.2 mL, 0.10 mol, 5.0 equiv) was added and the solution was stirred at –78 °C for 1 h. The mixture was diluted with Et_2O and saturated $NH_4Cl_{(aq)}$ and then transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:12 EtOAc–hexane) to give **2.75** (6.4 g, 88% over three steps) as a transparent colorless oil. $[\alpha]_D^{21}$ +55.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.35–7.31 (m, 2 H, ArH), 7.29–7.26 (m, 1 H, ArH), 7.22–7.21 (m, 2 H, ArH), 4.68 (dddd, *J* = 9.5, 7.5, 3.5, 3.0 Hz, 1 H, NCH, M of ABM system), 4.20 (dd, *J* = 9.0, 7.5 Hz, 1 H, OCH, A of ABM system), 4.16 (dd, *J* = 9.0, 3.0 Hz, 1 H, OCH, B of ABM system), 3.71 (qdd, *J* = 6.5, 6.0, 6.0 Hz, 1 H, αH), 3.27 (dd, *J* = 10.5, 3.5 Hz, 1 H, benzylic H), 2.77 (dd, *J* = 10.5, 9.5 Hz, 1 H, benzylic H), 1.76–1.70 (m, 1 H), 1.43–1.38 (m, 1 H), 1.29–1.25 (m, 18 H), 1.22 (d, *J* = 6.5 Hz, 3 H, αCH_3), 0.88 (dd, *J* = 7.0, 7.0 Hz, 3 H, terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 177.4 (oxazolidinone C=O), 153.0 (C=O), 135.4, 129.4, 128.9, 127.3, 66.0 (OCH_2), 55.4 (NCH), 37.9 (benzylic C), 37.7 (αC), 33.4, 31.9, 29.7, 29.64, 29.61, 29.58, 29.50, 29.3, 27.3, 22.7, 17.3 (αCH_3), 14.1 (terminal CH_3); IR (cast film): 2925, 2854, 1784, 1700, 1455, 1386, 1238, 1210 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{24}H_{37}NNaO_3$, 410.2666; found, 410.2667.



2.75 **2.76**

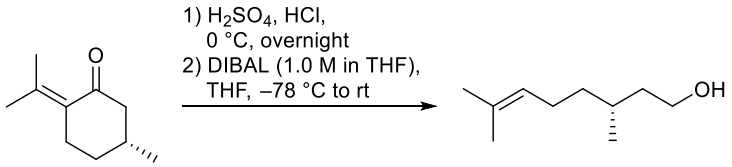
(*S*)-2-Methyltridecan-1-ol (2.76). Acyl oxazolidinone **2.75** (6.4 g, 17 mmol, 1.0 equiv) was dissolved in Et_2O (55 mL) and then $LiBH_4$ (2.0 M in THF, 8.3 mL, 17 mmol, 1.0 equiv) was added.

The mixture was stirred at room temperature for 0.5 h. The mixture was diluted with Et_2O and saturated 1N $HCl_{(aq)}$ and then transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:3 EtOAc–hexane) to give **2.76** (2.3 g, 64%) as a transparent colorless oil. $[\alpha]_D^{21}$ –9.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 3.51 (dd, *J* = 10.5, 5.5 Hz, 1 H, H-1), 3.42 (dd, *J* = 10.5, 6.5 Hz, 1 H, H-1), 1.64–1.56 (m, 1 H, H-2), 1.42–1.26 (m, 19 H), 1.13–1.07 (m, 1 H), 0.91 (d, *J* = 6.5 Hz, 3 H, 2-CH_3), 0.88 (dd, *J* = 7.0, 7.0 Hz, 3 H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 68.5 (C-1), 35.8 (C-2), 33.2, 31.9, 30.0, 29.70, 29.68, 29.67, 29.4, 27.0, 22.7, 16.6 (2-CH_3), 14.1 (CH_3); IR (cast film): 3356, 2925, 2854, 1466, 1378, 1039 cm^{-1}; HRMS–EI–Double focusing (EB) sector (*m*/*z*): $[M–H_2O]^{\bullet+}$ calcd for $C_{14}H_{28}$, 196.2191; found, 196.2190.



Ethyl (*S*,*E*)-2,4-dimethylpentadec-2-enoate (2.77). Alcohol **2.76** (2.3 g, 11 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (11 mL) before (diacetoxyiodo)benzene (3.8 g, 12 mmol, 1.1 equiv) and TEMPO (0.17 g, 1.1 mmol, 0.10 equiv) were added. The mixture was stirred at room temperature for 1 h. The reaction was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with saturated $Na_2S_2O_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The obtained crude aldehyde was dissolved in CH_2Cl_2 (13 mL) and then the prepared ylide[40] (3.9 g, 11 mmol, 1.0 equiv) dissolved

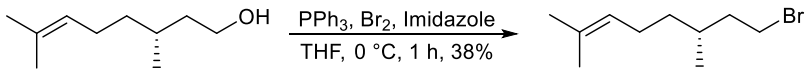
in CH_2Cl_2 (36 mL) was added. The mixture was stirred at room temperature until ^1H NMR spectroscopy indicated no aldehyde remained. This was done as the R_f of the intermediate aldehyde and ester product were the same, which made monitoring the reaction by TLC impossible. The mixture was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (150 mL silica gel, 0:1→1:40 EtOAc–hexane) to give **2.77** (2.4 g, 76%) as a transparent colorless oil. $[\alpha]_D^{21}$ +22.7 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 6.53 (dd, *J* = 10.0, 1.5 Hz, 1 H, βH), 4.19 (ddd, *J* = 7.0, 7.0, 7.0 Hz, 2 H, ester OCH_2), 2.50–2.44 (m, 1 H, γH), 1.83 (d, *J* = 1.5 Hz, 3 H, αCH_3), 1.42–1.25 (m, 23 H, OCH_2CH_3 and alkyl H), 0.99 (d, *J* = 6.5 Hz, 3 H, γCH_3), 0.88 (dd, *J* = 7.0, 7.0 Hz, 3 H, terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 168.5 (C=O), 148.2 (βC), 126.2 (αC), 60.4 (ester CH_2), 36.9, 33.3 (γC), 31.9, 29.8, 29.67, 29.65, 29.63, 29.60, 29.4, 27.5, 22.7 (γCH_3), 14.3 (ester CH_3), 14.1 (terminal CH_3), 12.5 (αCH_3); IR (cast film): 2926, 2854, 1713, 1650, 1465, 1251, 1104 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{19}H_{36}NaO_2$, 319.2608; found, 319.2613.



2.78

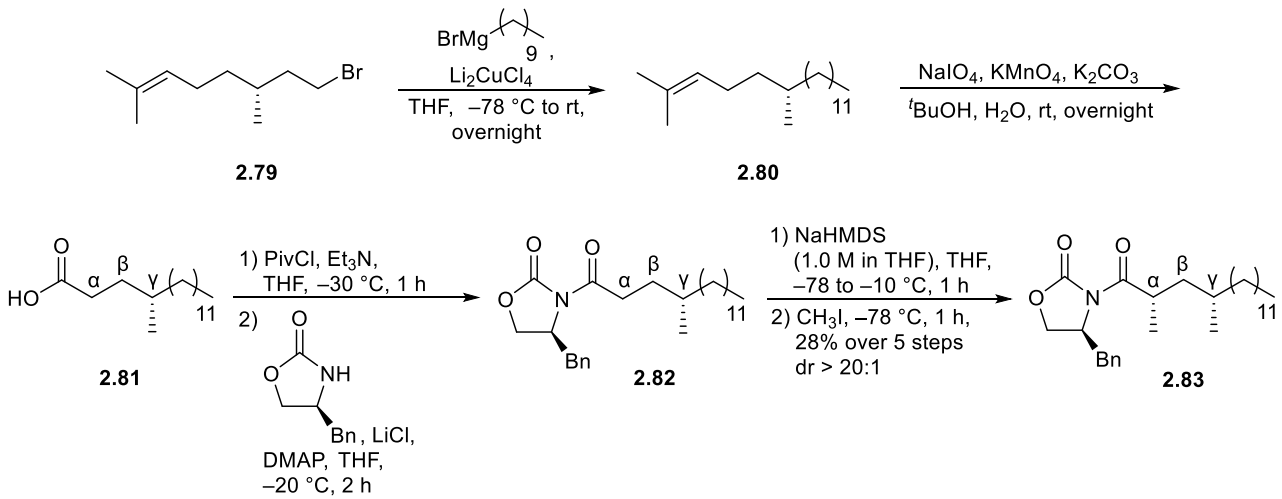
(*R*)-3,7-Dimethyloct-6-en-1-ol (2.78). (*R*)-(+)-Pulegone (85% technical grade, 15 g, 97 mmol, 1.0 equiv) was added to a Schlenk tube (24/40 joint, 18 cm in length × 2.5 cm in diameter). The tube was capped with a rubber septum and then an empty balloon was attached to the side arm. The tube was cooled to 0 °C then a cannula was inserted into the Schlenk flask and one end was below liquid level. The other end of the cannula was inserted into a round bottom flask containing

concentrated H_2SO_4 (70 mL) above the liquid level. The round bottom flask was cooled to 0 °C and then concentrated HCl (40 mL) was carefully dropwise added via syringe to generate HCl gas. The addition of concentrated HCl was carefully controlled through observing the bubbling at the end of the cannula inserted into the Schlenk tube. The cannula was removed from the Schlenk tube just before the last drop of concentrated HCl was added to the round bottom flask and then the mixture was stirred at 0 °C overnight. The mixture was warmed to room temperature and the crude HCl-adduct was obtained. The crude HCl adduct (7.5 g) was transferred from the Schlenk tube to a separate flask and dissolved in THF (125 mL) and then the mixture was cooled to –78 °C. Diisobutylaluminum hydride (1.0 M in THF, 85 mL, 2.1 equiv) was added dropwise to the mixture via an addition funnel. After the addition of DIBAL was complete, the mixture was stirred at –78 °C for 1 h and then the cooling bath was removed. The mixture was warmed and stirred at room temperature overnight at which point TLC (1:1 Et_2O–hexane) indicated no HCl adduct was left. The mixture was cooled to 0 °C then diluted with Et_2O and excess DIBAL was quenched with the addition of a saturated solution of Rochelle salt. The mixture was transferred to a separatory funnel and the organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:2 Et_2O–hexane) to give **2.78** (5.0 g) as a transparent colorless liquid. Given its low boiling point, **2.78** was not dried on high vacuum. The ^1H and ^{13}C NMR data for **2.78** were identical to those reported.[41]



2.78 **2.79**

(*R*)-8-Bromo-2,6-dimethyloct-2-ene (2.79). Imidazole (3.3 g, 48 mmol, 1.5 equiv) and PPh_3 (9.3 g, 36 mmol, 1.1 equiv) were dissolved in THF (40 mL) and then the mixture was cooled to 0 °C. Bromine (1.8 mL, 36 mmol, 1.1 equiv) was added dropwise and then the reaction mixture was stirred at 0 °C for 0.5 h. Then, **2.78** (5.0 g, 32 mmol, 1.0 equiv) dissolved in THF (40 mL) was added and the solution was stirred at 0 °C for 0.5 h. The mixture was diluted with Et_2O, excess bromine was quenched with the addition of saturated $Na_2S_2O_{3(aq)}$ and then the solution was transferred to a separatory funnel. The organic layer was washed with saturated $Na_2S_2O_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, hexane) to give **2.79** (2.7 g, 38%) as a transparent colorless liquid. Given its low boiling point, **2.79** was not dried on high vacuum. The ^1H and ^{13}C NMR data for **2.79** were identical to those reported.[42]



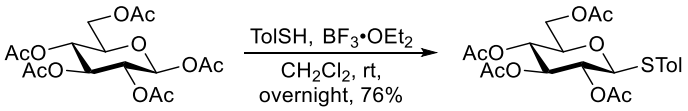
(*S*)-4-Benzyl-3-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)oxazolidin-2-one (2.83). A three-neck round bottom flask containing Mg (turnings, 1.8 g, 73 mmol, 6.0 equiv) was attached to a

condenser. A three-way valve was attached to the condenser then the whole apparatus was dried on high vacuum for 1 h and then purged with argon and stirred under argon with an argon balloon. Tetrahydrofuran (80 mL) and bromodecane (10 mL, 49 mmol, 4.0 equiv) was added to the flask then the mixture was heated to reflux. The reaction was initiated by heating the glass surface under the neck with a heat gun. Increasing condensation in the condenser was observed and the mixture gradually turned greyish brown. The mixture was stirred at reflux until the ^{1}H NMR spectra of mixture samples (quenched with 1N $HCl_{(aq)}$) indicated little bromodecane remained. This obtained Grignard reagent was cooled to room temperature then to –78 °C. In a separate flask containing **2.79** (2.7 g, 12 mmol, 1.0 equiv) and THF (10 mL) was cooled to –78 °C. The Grignard reagent was transferred to this flask via cannula and then Li_2CuCl_4 (0.10 M in THF, 24 mL, 2.4 mmol, 0.20 equiv) was added to the flask. The mixture was warmed and stirred at room temperature overnight at which point TLC (hexane) indicated no **2.79** remained. The mixture was cooled to 0 °C then diluted with Et_2O, saturated $NH_4Cl_{(aq)}$ was then added and the solution was transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, hexane) to give an inseparable mixture of **2.80** and a side-product though to be eicosane (3.2 g) in a 1 to 1 ratio as a transparent colorless liquid. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 5.11 (m, 1 H, H-3), 2.02–1.90 (m, 2 H, H-4), 1.69 (s, 3 H, vinyl CH_3), 1.61 (s, 3 H, vinyl CH_3), 1.40–1.34 (m, 1 H), 1.33–1.26 (m, 22 H), 1.15–1.07 (m, 2 H), 0.88 (dd, J = 7.2, 7.2 Hz, 3 H, terminal CH_3), 0.86 (d, J = 6.6 Hz, 3 H, 6-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 130.9 (C-2), 125.1 (C-3), 37.2, 37.0, 32.4, 31.9, 30.0, 29.74, 29.71, 29.67, 29.4, 27.0, 25.7 (vinyl CH_3), 25.6 (C-4), 22.7, 19.6 (6-CH_3), 17.6 (vinyl CH_3), 14.1 (terminal CH_3); HRMS–EI–Double focusing (EB) sector (m/z): $[M]^{\bullet+}$ calcd for $C_{20}H_{40}$, 280.3130; found, 280.3131.

Crude **2.80** and K_2CO_3 (3.08 g, 22.3 mmol, 1.94 equiv) was dissolved in *tert*-butanol (138 mL) and H_2O (206 mL). Sodium periodate (19 g, 90 mmol, 7.8 equiv), $KMnO_4$ (0.36 g, 2.3 mmol, 0.20 equiv) dissolved in H_2O (115 mL) was added to the mixture dropwise via an addition funnel. The mixture was stirred at room temperature for 2 h at which point TLC (hexane) indicated that no **2.80** remained and two major spots were observed. The mixture was then stirred at room temperature overnight at which point TLC (1:2 EtOAc–hexane) indicated the polar major spot of the previously two disappeared. Ethylene glycol (2.6 mL, 46 mmol, 4.0 equiv) was added to the mixture and then the mixture was concentrated on a rotary evaporator. During concentrating, the mixture gradually turned from purple to brown which indicated excess $KMnO_4$ was quenched and condensation stopped indicating some water remained. The mixture was acidified to pH 3 by the addition of 4 N HCl and then it was transferred to a separatory funnel. The aqueous layer was extracted with Et_2O (3 × 200 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:5 EtOAc–hexane) to give an inseparable mixture of **2.81** (2.3 g) as an oil. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 11.31 (br s, 1 H, COOH), 2.41–2.30 (m, 2 H, αH), 1.70–1.65 (m, 1 H, βH), 1.48–1.42 (m, 2 H, βH and γH), 1.32–1.28 (m, 21 H), 1.18–1.10 (m, 1 H), 0.89–0.87 (m, 6 H, γCH_3 and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 180.2 (C=O), 36.6, 32.3 (γC), 31.9 (αC), 31.8 (βC), 31.6, 29.9, 29.7, 29.4, 26.9, 22.7, 19.3 (γCH_3), 14.1 (CH_3); HRMS–ESI–TOF (*m*/*z*): $[M–H]^-$ calcd for $C_{17}H_{33}O_2$, 269.2486; found, 269.2481. Crude **2.81** (2.3 g) was dissolved in THF (21 mL) and cooled to –30 °C. Trimethylacetyl chloride (1.1 mL, 9.2 mmol, 1.1 equiv), Et_3N (4.1 mL, 29 mmol, 3.5 equiv) was added to the mixture then the was stirred at –30 °C for 1 h. The mixture was then warmed to –20 °C before LiCl (1.8 g, 42 mmol, 5.0 equiv), 4-dimethylaminopyridine (0.10 g, 0.84 mmol, 0.10 equiv) and

(*S*)-4-benzyloxazolidin-2-one (1.6 g, 9.2 mmol, 1.1 equiv) were added to the mixture. The mixture was stirred at –20 °C for 2 h at which point TLC (1:4 EtOAc–hexane) indicated no progression of the reaction. The mixture was diluted with Et_2O then transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:20→1:10 EtOAc–hexane) to give an inseparable mixture of **2.82** and pivaloyl oxazolidinone (2.8 g). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.35–7.32 (m, 2 H, ArH), 7.29–7.26 (m, 1 H, ArH), 7.22–7.20 (m, 2 H, ArH), 4.67 (dddd, *J* = 9.6, 7.8, 3.0, 3.0 Hz, 1 H, NCH, M of ABM system), 4.19 (dd, *J* = 9.0, 7.8 Hz, 1 H, OCH, A of ABM system), 4.16 (dd, *J* = 9.0, 3.0 Hz, 1 H, OCH, B of ABM system), 3.30 (dd, *J* = 13.2, 3.0 Hz, 1 H, benzylic H), 3.00 (ddd, *J* = 15.6, 10.2, 5.4 Hz, 1 H, αH), 2.89 (ddd, *J* = 15.6, 9.6, 6.0 Hz, 1 H, αH), 2.76 (dd, *J* = 13.2, 9.6 Hz, 1 H, benzylic H), 1.74–1.68 (m, 1 H, βH), 1.55–1.46 (m, 2 H, βH and γH), 1.34–1.26 (m, 21 H), 1.18–1.13 (m, 1 H), 0.92 (d, *J* = 6.0 Hz, 3 H, γCH_3), 0.88 (dd, *J* = 7.2, 7.2 Hz, 3 H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 173.7 (oxazolidinone C=O), 153.4 (C=O), 135.3, 129.4, 128.9, 127.3, 66.1 (OCH_2), 55.2 (NCH), 37.9 (benzylic C), 36.8, 33.4 (αC), 32.4 (γC), 31.9, 31.3 (βC), 30.0, 29.7, 29.6, 29.4, 27.0, 22.7, 19.4 (γCH_3), 14.1 (CH_3); HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{27}H_{43}NNaO_3$, 452.3135; found, 452.3135. This inseparable mixture of **2.82** (2.8 g) was dissolved in THF (13 mL) then cooled to –78 °C. Sodium bis(trimethylsilyl)amide (1.0 M in THF, 32 mL, 32 mmol, 1.2 equiv) was added to the mixture then the mixture was immediately put into a –10 °C bath and stirred for 1 h. The mixture was the cooled to –78 °C before CH_3I (2.0 mL, 32 mmol, 5.0 equiv) was added to the mixture. The mixture was stirred at –78 °C for 1 h, diluted with Et_2O and then saturated $NH_4Cl_{(aq)}$ was added before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and

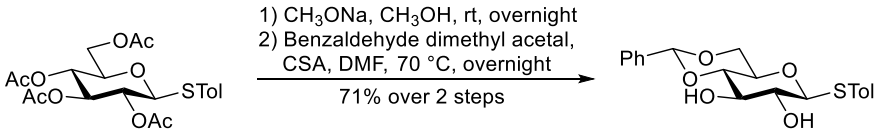
the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:20 EtOAc–hexane) to give **2.83** (1.5 g, 28% over 5 steps) as a transparent colorless oil. $[\alpha]_D^{21}$ +43.0 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.34–7.32 (m, 2 H, ArH), 7.29–7.26 (m, 1 H, ArH), 7.22–7.21 (m, 2 H, ArH), 4.68 (dddd, *J* = 9.6, 7.8, 3.0, 2.4 Hz, 1 H, NCH), 4.20 (dd, *J* = 9.0, 7.8 Hz, 1 H, OCH), 4.17 (dd, *J* = 9.0, 2.4 Hz, OCH), 3.90–3.84 (m, 1 H, αH), 3.26 (dd, *J* = 13.2, 3.0 Hz, 1 H, benzylic H), 2.76 (dd, *J* = 13.2, 9.6 Hz, 1 H, benzylic H), 1.85 (ddd, *J* = 13.8, 8.4, 5.4 Hz, 1 H, βH), 1.39–1.37 (m, 1 H, γH), 1.31–1.26 (m, 22 H), 1.22 (d, *J* = 7.2 Hz, 3 H, αCH_3), 1.16 (ddd, *J* = 13.8, 8.4, 6.0 Hz, 1 H, βH), 1.10–1.08 (m, 1 H), 0.88 (dd, *J* = 7.2, 6.6 Hz, 3 H, CH_3), 0.86 (d, *J* = 6.6 Hz, 3 H, γCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 177.5 (oxazolidinone C=O), 153.0 (C=O), 135.3, 129.5, 128.9, 127.3, 66.0 (OCH_2), 55.3 (NCH), 40.9 (βC), 37.9 (benzylic C), 37.0, 35.4 (αC), 31.9, 30.8, 30.0, 29.7, 29.4, 26.9, 22.7, 19.9 (γCH_3), 18.4 (αCH_3), 14.1 (CH_3); IR (cast film): 2925, 2854, 1785, 1701, 1456, 1386, 1350, 1210 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{28}H_{45}NNaO_3$, 466.3292; found, 466.3290.



2.84

4-Methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (2.84). β-D-Glucose pentaacetate (40 g, 0.10 mol, 1.0 equiv) and *p*-thiocresol (15 g, 0.12 mol, 1.2 equiv) were dissolved in CH_2Cl_2 (100 mL) then BF_3•OEt_2 (15 mL, 0.12 mol, 1.2 equiv) was added to the mixture. The mixture was stirred at room temperature overnight at which point TLC (2:3 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc (200 mL) and excess acid was quenched by the addition of 1 M NaOH (200 mL) and then the solution was

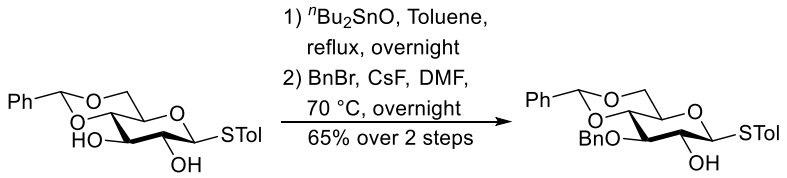
transferred to a separatory funnel. The organic layer was washed with 1 M NaOH, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by recrystallization from EtOH (95%, 200 mL) to give **2.84** (36 g, 76%) as white feather-like needles. The ^1H and ^{13}C NMR data for **2.84** were identical to those reported.[55]



2.84 **2.85**

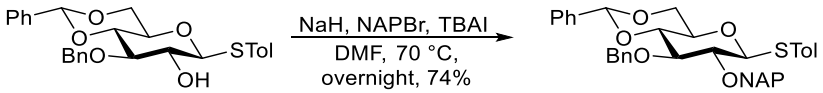
4-Methylphenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (2.85). Thioglycoside **2.84** (40 g, 88 mmol, 1.0 equiv) was suspended in CH_3OH (120 mL) then CH_3ONa (0.95 g, 18 mmol, 0.2 equiv) was added to the mixture. The mixture was stirred at room temperature overnight at which point TLC (1:4 CH_3OH–CH_2Cl_2) indicated no **2.84** remained. The excess base was quenched by the addition of CH_3OH-washed Amberlite® IR120 then the solution was filtered and the filtrate concentrated on a rotary evaporator. This crude reside was dried on high vacuum for 2 h and then dissolved in DMF (88 mL). Benzaldehyde dimethyl acetal (20 mL, 0.13 mol, 1.5 equiv) was added to this solution and then 10-camphorsulfonic acid was added until the pH was 1. The mixture was heated and stirred at 70 °C overnight at which point TLC (1:10 CH_3OH–CH_2Cl_2) indicated no further progression of the reaction. The mixture was cooled to room temperature and then diluted with EtOAc (200 mL). The acid was quenched by the addition of saturated $NaHCO_{3(aq)}$ then the solution was transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (600 mL silica

gel, 0:1→1:1 EtOAc–hexane) to give **2.85** (24 g, 71%) as a white amorphous solid. The ^{1}H and ^{13}C NMR data were identical to those reported.[56]



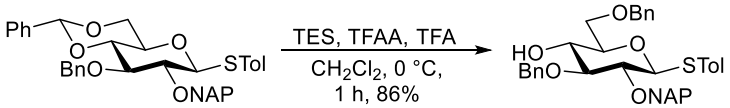
2.85 **2.86**

4-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (2.86). Thioglycoside **2.85** (2.0 g, 5.3 mmol, 1.0 equiv) and dibutyltin(IV) oxide (1.6 g, 6.4 mmol, 1.2 equiv) were suspended in toluene (53 mL). The flask was attached with Dean–Stark apparatus then the mixture was heated and stirred at reflux overnight. The mixture was cooled to room temperature then concentrated on a rotary evaporator and dried on high vacuum for 2 h. Next CsF (1.8 g, 12 mmol, 2.2 equiv) and DMF (13 mL) were added to the crude product. Benzyl bromide (1.3 mL, 11 mmol, 2.0 equiv) was added to the solution and then the mixture was sonicated to achieve minimal stirring. The mixture was heated and stirred at 70 °C overnight, during which time the mixture dissolved and a white suspension gradually formed. The mixture was cooled to room temperature then diluted with EtOAc (200 mL). The mixture was filtered over a pad of Celite® 545 and the filtrate transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 130 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (150 mL silica gel, 0:1→1:3 EtOAc–hexane) to give **2.86** (1.6 g, 65%) as a white amorphous solid. The ^{1}H and ^{13}C NMR data were identical to those reported.[57]



2.86 **2.87**

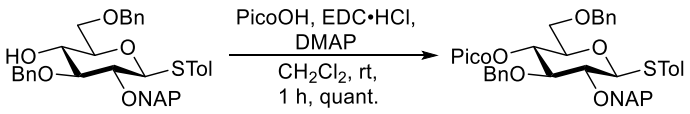
4-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (2.87). Thioglycoside **2.86** (1.6 g, 3.5 mmol, 1.0 equiv), 2-(bromomethyl)naphthalene (0.93 g, 4.2 mmol, 1.2 equiv and tetra-*n*-butylammonium iodide (0.13 g, 0.35 mmol, 0.10 equiv) were dissolved in DMF (8.7 mL). Sodium hydride (60% dispersion in mineral oil, 0.21 g, 5.2 mmol, 1.5 equiv) was added to the mixture then the solution was heated to and stirred at 70 °C overnight at which point TLC (1:3 EtOAc–hexane) indicated no **2.86** remained. The mixture was cooled to room temperature then diluted with EtOAc and the excess alkyl halide was quenched by the addition of H_2O. The solution was transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 90 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (150 mL silica gel, 0:1→1:6 EtOAc–hexane) to give **2.87** (1.6 g, 74%) as a white amorphous solid. The ^1H and ^{13}C NMR data were identical to those reported.[48]



2.87 **2.88**

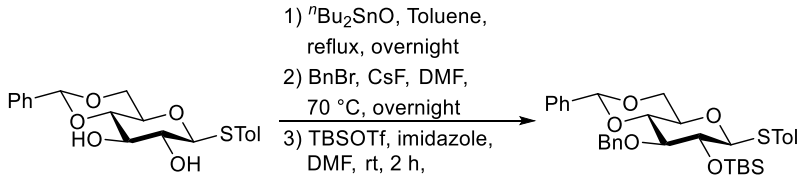
4-Methylphenyl 3,6-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (2.88). Thioglycoside **2.87** (0.62 g, 1.0 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (10 mL) and then triethylsilane (0.82 mL, 5.1 mmol, 5.0 equiv) and trifluoroacetic anhydride (0.43 mL, 3.1 mmol, 3.0 equiv) were added before the mixture was cooled to 0 °C. Trifluoroacetic acid (0.39 mL, 5.1

mmol, 5.0 equiv) was added dropwise to the solution and then the mixture was stirred at 0 °C for 1 h at which point TLC (1:2 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and the acid was quenched by the addition of saturated $NaHCO_{3(aq)}$ and the solution was then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (150 mL silica gel, 0:1→1:6→1:4 EtOAc–hexane) to give **2.88** (0.5 g, 86%) as a transparent colorless oil. $[\alpha]_D^{21}$ –14.1 (*c* 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.84–7.79 (m, 4 H, ArH), 7.57–7.56 (m, 1 H, ArH), 7.49–7.46 (m, 4 H, ArH), 7.37–7.28 (m, 10 H, ArH), 7.07–7.05 (m, 2 H, ArH), 5.09 (d, *J* = 10.8 Hz, 1 H, benzylic H), 4.93 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.90 (d, *J* = 10.8 Hz, 1 H, benzylic H), 4.81 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.67 (d, *J* = 9.6 Hz, 1 H, H-1), 4.60 (d, *J* = 12.0 Hz, benzylic H), 4.56 (d, *J* = 12.0 Hz, 1 H, benzylic H), 3.80 (dd, *J* = 11.2, 4.2 Hz, 1 H, H-6, A of ABM system), 3.77 (dd, *J* = 11.2, 4.8 Hz, 1 H, H-6, B of ABM system), 3.67 (dd, *J* = 9.0, 8.4 Hz, 1 H, H-4), 3.56 (dd, *J* = 9.0, 8.4 Hz, 1 H, H-3), 3.52 (dd, *J* = 9.6, 8.4 Hz, 1 H, H-2), 3.48 (ddd, *J* = 9.0, 4.8, 4.2 Hz, 1 H, H-5, M of ABM system), 2.32 (s, 3 H, $ArCH_3$), 2.56 (br s, 1 H, 4-OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.5, 137.9, 137.8, 135.5, 133.3, 133.1, 132.6, 129.8, 129.7, 128.6, 128.4, 128.1, 128.0, 127.90, 127.88, 127.71, 127.70, 127.68, 126.9, 126.2, 126.0, 125.9, 88.0 (C-1), 86.2 (C-3), 80.5 (C-2), 78.0 (C-5), 75.5 (benzylic C), 75.4 (benzylic C), 73.7 (benzylic C), 71.9 (C-4), 70.5 (C-6), 21.1 ($ArCH_3$); IR (cast film): 3462, 3057, 3026, 2915, 2886, 1602, 1494, 1454, 1363, 1127, 1064 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{38}H_{38}NaO_5S$, 629.2332; found, 629.2330.



2.88 **2.89**

4-Methylphenyl 3,6-di-*O*-benzyl-2-*O*-(2-naphthylmethyl)-4-*O*-picoloyl-1-thio-β-D-glucopyranoside (2.89). Thioglycoside **2.88** (0.53 g, 0.88 mmol, 1.0 equiv), 2-picolinic acid (0.32 g, 2.6 mmol, 3.0 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.25 g, 1.3 mmol, 1.5 equiv) and 4-(dimethylamino)pyridine (0.21 g, 1.8 mmol, 2.0 equiv) were dissolved in CH_2Cl_2 (9 mL). The mixture was stirred at room temperature for 1 h until TLC (1:3 EtOAc–hexane) indicated no **2.88** was left. The mixture was diluted with EtOAc then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (150 mL silica gel, 0:1→1:3→1:1 EtOAc–hexane) to give **2.89** (0.7 g, quantitative) as a transparent colorless oil. The ^1H and ^{13}C NMR data were identical to those reported.[33]

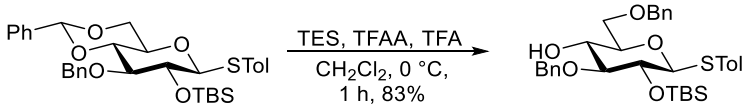


2.85 **2.90**

4-Methylphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.90). Thioglycoside **2.85** (13 g, 33 mmol, 1.0 equiv) and dibutyltin(IV) oxide (10 g, 40 mmol, 1.2 equiv) were suspended in toluene (100 mL). The flask was attached with

Dean–Stark apparatus and then the mixture was heated and stirred at reflux overnight. The mixture was cooled to room temperature then concentrated on a rotary evaporator and dried on high vacuum for 2 hours. After which time, CsF (11 g, 73 mmol, 2.2 equiv) and DMF (80 mL) was added to the crude. Benzyl bromide (8.0 mL, 67 mmol, 2.0 equiv) was added to the mixture then the mixture was sonicated to achieve minimal stirring. The mixture was heated and stirred at 70 °C overnight. Over this time period, the mixture dissolved and a white suspension gradually formed. The mixture was cooled to room temperature and then diluted with EtOAc (500 mL). The mixture was filtered over a pad of Celite® 545 and the filtrate was then transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 800 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator and dried on high vacuum for 2 h. The resulting crude product and imidazole (11 g, 0.17 mol, 5.0 equiv) were dissolved in DMF (80 mL) and then *tert*-butyldimethylsilyl trifluoromethanesulfonate (15 ml, 67 mmol, 2.0 equiv) was added to the mixture. The mixture was stirred at room temperature overnight at which point TLC (1:6 EtOAc–hexane) indicated the reaction was complete. The mixture was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 800 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, toluene) to give **2.90** (13 g, 70%) as a transparent colorless oil. $[\alpha]_D^{21}$ –58.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.42–7.25 (m, 12 H, ArH), 7.14–7.12 (m, 2 H, ArH), 5.54 (s, 1 H, benzylidene CH), 5.01 (d, *J* = 11.0 Hz, 1 H, benzylic H), 4.70 (d, *J* = 11.0 Hz, 1 H, benzylic H), 4.65 (d, *J* = 8.0 Hz, 1 H, H-1), 4.35 (dd, *J* = 10.5, 5.0 Hz, 1 H, H-6), 3.79 (dd, *J* = 10.5, 10.0 Hz, 1 H, H-6), 3.74 (dd, *J* = 9.5, 9.0 Hz, 1 H, H-4), 3.69–3.63 (m, 2 H, H-2 and H-3), 3.48 (ddd, *J* = 10.0, 9.5, 5.0 Hz, 1 H, H-5), 2.35 (s, 3 H, $ArCH_3$), 0.94 (s, 9 H, *t*-butyl CH_3), 0.19 (s, 3 H, silyl CH_3), 0.07 (s, 3

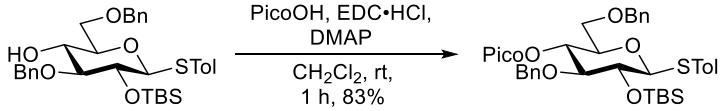
H, silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.6, 137.7, 137.3, 132.0, 130.5, 129.7, 128.9, 128.4, 128.22, 128.20, 127.84, 127.80, 127.6, 127.4, 126.0, 101.2 (benzylidene Ph*C*H), 90.8 (C-1), 83.3 (C-3), 82.1 (C-4), 74.5 (benzylic C), 73.8 (C-2), 69.9 (C-5), 68.8 (C-6), 26.2 (*t*-butyl CH_3), 21.1 ($ArCH_3$), 18.4 (*t*-butyl C), –3.5 (silyl CH_3), –4.2 (silyl CH_3); IR (cast film): 3034, 2954, 2927, 2883, 2856, 1493, 1454, 1368, 1249, 1132, 1095, 1030 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{42}NaO_5SSi$, 601.2414; found, 601.2411.



2.90 **2.91**

4-Methylphenyl 3,6-di-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (2.91). Thioglycoside **2.90** (13 g, 23 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (58 mL) and then triethylsilane (19 mL, 0.12 mol, 5.0 equiv) and trifluoroacetic anhydride (9.7 mL, 70 mmol, 3.0 equiv) were added to the mixture. The solution was cooled to 0 °C before trifluoroacetic acid (8.9 mL, 0.12 mol, 5.0 equiv) was added dropwise. After stirring at 0 °C for 1 h, TLC (1:3 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and the acid was quenched by the addition of saturated $NaHCO_{3(aq)}$ and the solution was then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:6 EtOAc–hexane) to give **2.91** (11 g, 83%) as a transparent colorless oil. $[\alpha]_D^{21}$ –81.2 (*c* 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.41–7.27 (m, 12 H, ArH), 7.05–7.04 (m, 2 H, ArH), 4.86 (d, *J* = 12.0 Hz, 1 H,

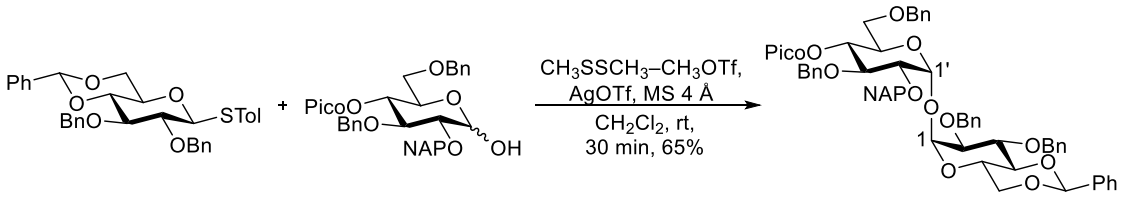
benzylic H), 4.81 (d, J = 12.0 Hz, 1 H, benzylic H), 4.56–4.51 (m, 3 H, H-1 and benzylic H), 3.74 (dd, J = 10.2, 4.8 Hz, 1 H, H-6), 3.71 (d, J = 10.2, 5.4 Hz, 1 H, H-6), 3.65 (ddd, J = 9.6, 9.0, 2.4 Hz, 1 H, H-4), 3.62 (dd, J = 9.6, 8.4 Hz, 1 H, H-2), 3.44 (ddd, J = 9.6, 5.4, 4.8 Hz, 1 H, H-5), 3.36 (dd, J = 9.0, 8.4 Hz, H-3), 2.40 (d, J = 2.4 Hz, 1 H, 4-OH), 2.30 (s, 3 H, $ArCH_3$), 0.96 (s, 9 H, *t*-butyl CH_3), 0.23 (s, 3 H, silyl CH_3), 0.11 (s, 3 H, silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.8, 137.9, 137.3, 131.9, 131.2, 129.6, 128.5, 128.4, 127.72, 127.70, 127.66, 90.3 (C-1), 87.2 (C-3), 77.8 (C-5), 75.1 (benzylic C), 73.6 (benzylic C), 73.4 (C-2), 72.6 (C-4), 70.8 (C-6), 26.2 (*t*-butyl CH_3), 21.1 ($ArCH_3$), 18.3 (*t*-butyl C), –3.4 (silyl CH_3), –3.8 (silyl CH_3); IR (cast film): 3472, 3063, 3031, 2953, 2927, 2884, 2857, 1494, 1454, 1361, 1251, 1140, 1059 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{33}H_{44}NaO_5SSi$, 603.2571; found, 603.2573.



2.91 **2.92**

4-Methylphenyl 3,6-di-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-4-*O*-picoloyl-1-thio-β-D-glucopyranoside (2.92). Thioglycoside **2.91** (11 g, 19 mmol, 1.0 equiv), 2-picolinic acid (4.7 g, 38 mmol, 2.0 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (5.5 g, 29 mmol, 1.5 equiv) and 4-(dimethylamino)pyridine (4.7 g, 38 mmol, 2.0 equiv) were dissolved in CH_2Cl_2 (48 mL). The mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica

gel, 0:1→1:6→1:3 EtOAc–hexane) to give **2.92** (11 g, 83%) as a transparent colorless oil. $[\alpha]_D^{21}$ –85.6 (*c* 0.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 8.68 (ddd, *J* = 4.8, 1.8, 1.2 Hz, 1 H, picoloyl H), 7.92 (d, *J* = 7.8 Hz, 1 H, picoloyl H), 7.72 (ddd, *J* = 7.8, 7.8, 1.8 Hz, 1 H, picoloyl H), 7.46–7.44 (m, 2 H, ArH), 7.41 (ddd, *J* = 7.8, 4.8, 1.2 Hz, 1 H, picoloyl H), 7.23–7.18 (m, 5 H, ArH), 7.11–7.02 (m, 7 H, ArH), 5.40 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-4), 4.76 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.71 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.63 (d, *J* = 9.0 Hz, 1 H, H-1), 4.45–4.41 (m, 2 H, benzylic H), 3.85 (ddd, *J* = 9.6, 6.0, 3.6 Hz, 1 H, H-5), 3.81 (dd, *J* = 9.0, 7.8 Hz, H-3), 3.76 (dd, *J* = 9.0, 7.8 Hz, 1 H, H-2), 3.68–3.63 (m, 2 H, H-6), 2.30 (s, 3 H, $ArCH_3$), 0.94 (s, 9 H, *t*-butyl CH_3), 0.23 (s, 3 H, silyl CH_3), 0.04 (s, 3 H, silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 164.2 (picoloyl C=O), 149.7 (picoloyl C), 147.4, 138.1, 137.3, 136.8 (picoloyl C), 131.7, 131.1, 129.7, 128.1, 128.0, 127.6, 127.3, 127.3, 127.1, 127.0 (picoloyl C), 125.5 (picoloyl C), 90.3 (C-1), 84.8 (C-3), 77.4 (C-5), 75.2 (benzylic C), 73.9 (C-2), 73.5 (benzylic C), 73.0 (C-4), 70.0 (C-6), 26.1 (*t*-butyl CH_3), 21.1 ($ArCH_3$), 18.2 (*t*-butyl C), –3.5 (silyl CH_3), –3.8 (silyl CH_3); IR (cast film): 3061, 3032, 2953, 2928, 2894, 2856, 1728, 1585, 1494, 1471, 1361, 1302, 1246, 1124, 1035 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{39}H_{47}NNaO_6SSi$, 708.2786; found, 708.2785.

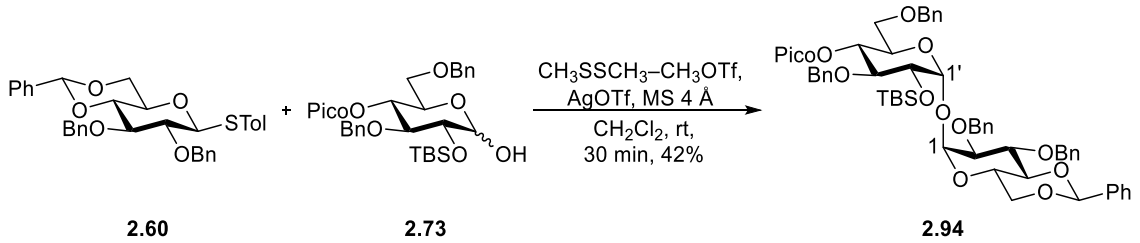


2.60 **2.72** **2.93**

3',6'-di-*O*-Benzyl-2'-*O*-(2-naphthylmethyl)-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (2.93). Glycosyl donor **2.60** (19 mg, 34

μmol, 2.0 equiv), glycosyl acceptor **2.72** (10 mg, 17 μmol, 1.0 equiv), AgOTf (35 mg, 0.14 mmol, 8.0 equiv) and molecular sieves 4 Å (powder, 110 mg) were suspended in CH_2Cl_2 (0.6 mL). In a separate vial equipped with a stir bar was added methyl trifluoromethanesulfonate (15 μL, 0.14 mmol, 8.0 equiv) and then the vial was cooled to 0 °C. Dimethyl disulfide (12 μL, 0.14 mmol, 8.0 equiv) was added to the vial and then the mixture was stirred at 0 °C for 1 h before CH_2Cl_2 (0.5 mL) was added to dissolve the solidified mixture. This solution was transferred via syringe to the flask containing glycosyl donor and acceptor. The mixture was stirred at room temperature for 30 min. The mixture was diluted with EtOAc and quenched with the addition of saturated $NaHCO_{3(aq)}$ then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (50 mL silica gel, 0:1→1:3→1:2 EtOAc–hexane) to give **2.93** (11 mg, 65%) as a transparent colorless film. $[\alpha]_D^{21}$ +62.7 (*c* 0.4, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 8.78 (d, *J* = 4.2 Hz, 1 H, picoloyl H), 7.95 (d, *J* = 7.7 Hz, 1 H, picoloyl H), 7.80–7.71 (m, 5 H, picoloyl H and ArH), 7.49–7.27 (m, 16 H, picoloyl H and ArH), 7.22–7.10 (m, 13 H, ArH), 5.58 (s, 1 H, benzylidene CH), 5.50 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4'), 5.26 (d, *J* = 3.5 Hz, 1 H, H-1'), 5.23 (d, *J* = 4.2 Hz, 1 H, H-1), 5.03 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.93 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.91–4.87 (m, 3 H, benzylic H), 4.78 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.74–4.71 (m, 2 H, benzylic H), 4.49 (ddd, *J* = 9.8, 4.2, 2.8 Hz, 1 H, H-5'), 4.44 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.39 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.35 (ddd, *J* = 9.8, 9.8, 4.9 Hz, 1 H, H-5), 4.27 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-3'), 4.24 (dd, *J* = 10.5, 4.9 Hz, 1 H, H-6), 4.20 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-3), 3.80 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2'), 3.71 (dd, *J* = 10.5, 9.8 Hz, 1 H, H-6), 3.67 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4), 3.63 (dd, *J* = 9.8, 4.2 Hz, 1 H, H-2), 3.44 (dd, *J* = 10.5, 2.8 Hz, 1 H, H-6'), 3.39 (dd, *J* = 10.5, 4.2 Hz, 1 H, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 164.0 (picoloyl

C=O), 149.8 (picoloyl C), 147.8, 138.9, 138.4, 138.1, 137.7, 137.5, 136.8 (picoloyl C), 135.2, 133.2, 133.0, 128.8, 128.34, 128.31, 128.24, 128.20, 128.12, 128.10, 128.0, 127.9, 127.8, 127.7, 127.6, 127.54, 127.51, 127.4, 127.3, 126.8, 126.7, 126.1, 126.0, 125.9, 125.8, 125.5 (picoloyl C), 101.2 (benzylidene Ph*C*H), 94.9 (C-1'), 94.3 (C-1), 82.5 (C-4), 79.2 (C-3'), 79.1 (C-2'), 78.6 (C-3), 78.5 (C-2), 75.4 (benzylic C), 75.3 (benzylic C), 73.64 (benzylic C), 73.62 (benzylic C), 73.4 (benzylic C), 71.8 (C-4'), 69.3 (C-5'), 69.1 (C-6), 68.4 (C-6'), 63.0 (C-5); IR (cast film): 3062, 3031, 2922, 2864, 1749, 1727, 1454, 1368, 1304, 1244, 1109, 1090, 1015, 993 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{64}H_{61}NNaO_{12}$, 1058.4086; found, 1058.4072.

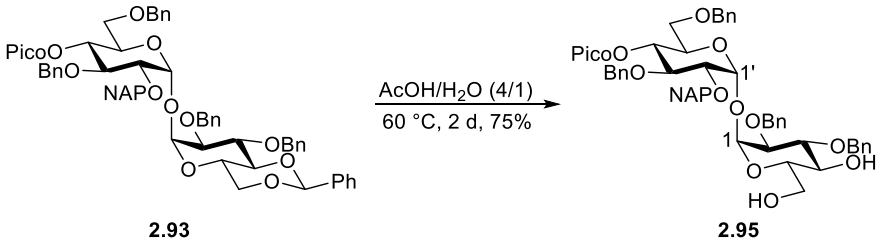


2.60 **2.73** **2.94**

3',6'-di-*O*-Benzyl-2'-*O*-*tert*-butyldimethylsilyl-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (2.94). Glycosyl donor **2.60** (71 mg, 0.13 mmol, 2.0 equiv), glycosyl acceptor **2.72** (37 mg, 64 μmol, 1.0 equiv), AgOTf (0.13 g, 0.51 mmol, 8.0 equiv) and molecular sieves 4 Å (powder, 260 mg) were suspended in CH_2Cl_2 (1.6 mL). In a separate vial equipped with a stir bar was added methyl trifluoromethanesulfonate (58 μL, 0.51 mmol, 8.0 equiv) then the vial was cooled to 0 °C. Dimethyl disulfide (46 μL, 0.51 mmol, 8.0 equiv) was added to the vial then the mixture was stirred at 0 °C for 1 hour. After which time, CH_2Cl_2 (1.0 mL) was added to the vial to dissolve the solidified mixture. This solution was transferred to the flask containing glycosyl donor and acceptor via syringe. The mixture was stirred

at room temperature for 30 min. The mixture was diluted with EtOAc and the acid was quenched by the addition of saturated $NaHCO_{3(aq)}$ and then the solution was transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (50 mL silica gel, 0:1→1:4→1:2 EtOAc–hexane) to give **2.94** (27 mg, 42%) as a transparent colorless film. $[\alpha]_D^{21}$ +40.0 (*c* 0.7, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 8.74 (d, *J* = 4.2 Hz, 1 H, picoloyl H), 7.79 (d, *J* = 7.8 Hz, 1 H, picoloyl H), 7.73 (ddd, *J* = 7.8, 7.8, 1.2 Hz, 1 H, picoloyl H), 7.50–7.49 (m, 2 H, ArH), 7.44 (ddd, *J* = 7.8, 4.2, 1.2 Hz, 1 H, picoloyl H), 7.42–7.26 (m, 10 H, ArH), 7.23–7.05 (m, 13 H, ArH), 5.56 (s, 1 H, benzylidene H), 5.45 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4'), 5.27 (d, *J* = 3.6 Hz, 1 H, H-1), 5.12 (d, *J* = 3.6 Hz, 1 H, H-1'), 5.00 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.90 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.81 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.80 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.72 (d, *J* = 12.0, 1 H, benzylic H), 4.67 (d, *J* = 11.4, 1 H, benzylic H), 4.46–4.39 (m, 3 H, H-5' and benzylic H), 4.27 (dd, *J* = 10.2, 4.8 Hz, 1 H, H-6), 4.21–4.17 (m, 2 H, H-5 and H-3), 4.09 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-3'), 3.90 (dd, *J* = 9.6, 3.6, 1 H, H-2'), 3.70 (dd, *J* = 10.2, 10.2 Hz, 1 H, H-6), 3.66 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-4), 3.62 (dd, *J* = 9.0, 3.6 Hz, 1 H, H-2), 3.40 (dd, *J* = 10.8, 2.4 Hz, 1 H, H-6'), 3.33 (dd, *J* = 10.8, 4.8 Hz, 1 H, H-6'), 0.93 (s, 9 H, *t*-butyl CH_3), 0.09 (s, 3 H, silyl CH_3), 0.07 (s, 3 H, silyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 163.9 (picoloyl C=O), 149.8 (picoloyl C), 147.7, 138.8, 138.5, 138.2, 137.8, 137.5, 136.7 (picoloyl C), 128.9, 128.3, 128.25, 128.18, 128.13, 128.0, 127.91, 127.90, 127.8, 127.51, 127.47, 127.40, 127.3, 127.0, 126.7, 126.2, 125.3 (picoloyl C), 101.6 (benzylidene Ph*C*H), 95.3 (C-1'), 93.8 (C-1), 82.8 (C-4), 79.5 (C-3'), 78.7 (C-2), 78.3 (C-3), 75.4 (benzylic C), 75.1 (benzylic C), 73.9 (benzylic C), 73.7 (benzylic C), 73.0 (C-2'), 72.3 (C-4'), 69.2 (C-5' and C-6), 68.6 (C-6'), 63.0 (C-5), 26.1 (*t*-butyl CH_3), 18.1 (*t*-butyl C), –4.1 (silyl CH_3), –4.8 (silyl CH_3); IR

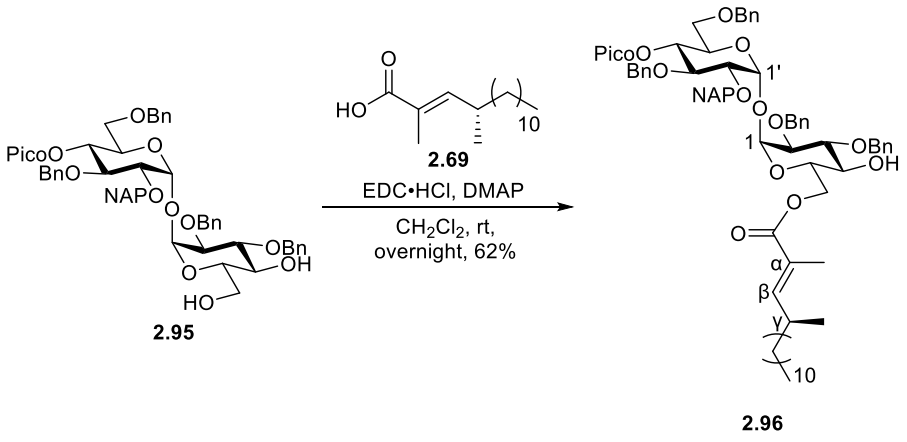
(cast film): 3064, 3031, 2930, 2859, 1750, 1727, 1454, 1367, 1304, 1246, 1112, 1090, 992 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{59}H_{67}NNaO_{12}Si$, 1032.4325; found, 1032.4326.



2.93 **2.95**

3',6'-di-*O*-Benzyl-2'-*O*-(2-naphthylmethyl)-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-α-D-glucopyranoside (2.95). Acetic acid (0.1 mL) and H_2O (25 μL) were added to trehalose derivative **2.93** (11 mg, 11 μmol, 1.0 equiv). The mixture was heated and stirred at 60 °C for 2 d at which point TLC (1:2 EtOAc–hexane) indicated no further progression of the reaction. The mixture was cooled to room temperature then dried by dissolution in and then concentration with toluene (3 × 5 mL) on a rotary evaporator. The crude reside was purified by column chromatography (50 mL silica gel, 0:1→1:1 EtOAc–hexane) to give **2.95** (7.7 mg, 75%) as a transparent colorless film. $[\alpha]_D^{21}$ +85.6 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ): 8.78 (d, *J* = 4.4 Hz, 1 H, picoloyl H), 7.97 (d, *J* = 8.0 Hz, 1 H, picoloyl H), 7.82–7.74 (m, 5 H, picoloyl H and ArH), 7.50–7.26 (m, 10 H, picoloyl H and ArH), 7.21–7.11 (m, 14 H, ArH), 5.48 (dd, *J* = 10.0, 10.0 Hz, 1 H, H-4'), 5.27 (d, *J* = 3.2 Hz, 1 H, H-1), 5.26 (d, *J* = 3.2 Hz, 1 H, H-1'), 5.11 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.92–4.83 (m, 4 H, benzylic H), 4.75 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.73 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.65 (d, *J* = 12.4 Hz, 1 H, benzylic H), 4.47 (ddd, *J* = 10.0, 4.4, 2.8 Hz, 1 H, H-5'), 4.44–4.37 (m, 2 H, benzylic H), 4.29 (dd, *J* = 10.0, 9.6 Hz, 1 H, H-3'), 4.13 (ddd, *J* = 9.6, 4.0, 4.0 Hz, 1 H, H-5), 3.95 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-3), 3.80 (dd, *J* = 9.6,

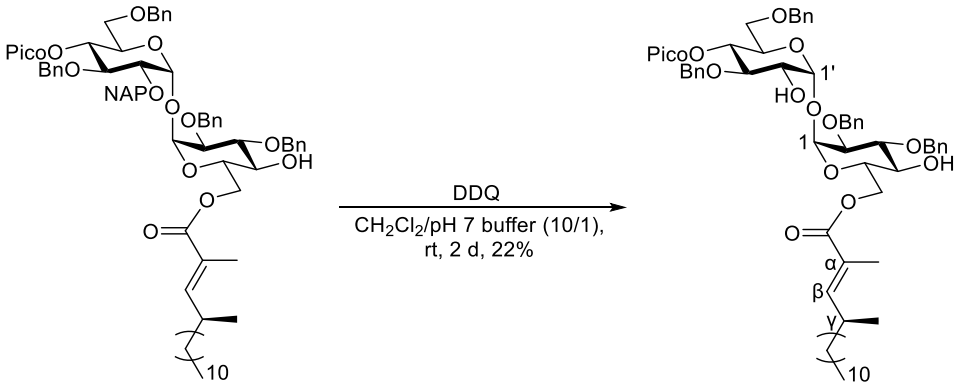
3.2 Hz, 1 H, H-2'), 3.69 (m, 2 H, H-6), 3.61 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4), 3.55 (dd, *J* = 9.6, 3.2 Hz, 1 H, H-2), 3.46 (dd, *J* = 10.2, 2.8 Hz, 1 H, H-6'), 3.41 (dd, *J* = 10.2, 4.4 Hz, 1 H, H-6'), 2.33 (br s, 1 H, 4-OH), 1.72 (br s, 1 H, 6-OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 164.1 (picoloyl C=O), 149.8 (picoloyl C), 147.8, 138.8, 138.3, 137.9, 137.7, 136.9 (picoloyl C), 135.3, 133.2, 133.0, 128.6, 128.3, 128.2, 128.13, 128.11, 128.0, 127.9, 127.83, 127.80, 127.64, 127.61, 127.60, 127.39, 127.35, 126.9, 126.6, 126.1, 126.0, 125.7, 125.6 (picoloyl C), 94.1 and 93.9 (C-1' and C-1), 81.0 (C-3), 79.1 (C-3'), 78.9 (C-2 and C-2'), 75.3 (benzylic C), 75.2 (benzylic C), 73.6 (benzylic C), 73.4 (benzylic C), 72.4 (benzylic C), 72.0 (C-4'), 71.3 (C-5), 70.6 (C-4), 69.4 (C-5'), 68.6 (C-6'), 62.4 (C-6); IR (cast film): 3461, 3062, 3030, 2925, 2870, 1746, 1730, 1586, 1497, 1454, 1361, 1306, 1289, 1244, 1106, 1056, 999 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{57}H_{57}NNaO_{12}$, 970.3773; found, 970.3792.



3',6'-di-*O*-Benzyl-2'-*O*-(2-naphthylmethyl)-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (2.96). Trehalose derivative **2.95** (30 mg, 32 μmol, 1.0 equiv), **2.69** (13 mg, 48 μmol, 1.5 equiv), *N*-(3-

dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (7.4 mg, 38 μmol, 1.2 equiv) and 4-(dimethylamino)pyridine (6.0 mg, 48 μmol, 1.2 equiv) were dissolved in CH_2Cl_2 (0.3 mL). The mixture was stirred at room temperature until TLC (1:2 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (50 mL silica gel, 0:1→1:3→1:2 EtOAc–hexane) to give **2.96** (24 mg, 62%) as a transparent colorless film. $[\alpha]_D^{21}$ +107.6 (*c* 0.05, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 8.78 (m, 1 H, picoloyl H), 7.96 (d, *J* = 8.4 Hz, 1 H, picoloyl H), 7.81–7.75 (m, 5 H, picoloyl H and ArH), 7.49–7.44 (m, 5 H, picoloyl H and ArH), 7.40–7.36 (m, 3 H, ArH), 7.31–7.26 (m, 3 H, ArH), 7.21–7.11 (m, 13 H, ArH), 6.56 (d, *J* = 10.5 Hz, 1 H, lipid βH), 5.49 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4'), 5.30 (d, *J* = 3.5 Hz, 1 H, H-1'), 5.29 (d, *J* = 3.5 Hz, 1 H, H-1), 5.05 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.94 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.90–4.86 (m, 3 H, benzylic H), 4.74–4.72 (m, 2 H, benzylic H), 4.68 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.49–4.47 (m, 2 H, H-5' and H-6), 4.42 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.38 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.30–4.27 (m, 2 H, H-5 and H-3'), 4.09 (dd, *J* = 12.6, 2.1 Hz, 1 H, H-6), 3.99 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-3), 3.80 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2'), 3.55 (dd, *J* = 9.1, 3.5 Hz, 1 H, H-2), 3.47 (ddd, *J* = 9.1, 9.1, 3.5 Hz, 1 H, H-4), 3.44 (dd, *J* = 10.5, 2.8 Hz, 1 H, H-6'), 3.39 (dd, *J* = 10.5, 4.2 Hz, 1 H, H-6'), 2.92 (d, *J* = 3.5 Hz, 1 H, 4-OH), 2.46–2.41 (m, 1 H, lipid γH), 1.80 (s, 3 H, lipid αCH_3), 1.38–1.19 (m, 20 H, lipid CH_2), 0.95 (d, *J* = 7.0 Hz, 3 H, γCH_3), 0.88 (dd, *J* = 7.0, 7.0 Hz, 3 H, lipid terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 169.1 (lipid C=O), 164.1 (picoloyl C=O), 149.8 (picoloyl C), 149.7 (lipid βC), 147.8 (lipid αC), 138.7, 138.3, 138.1, 137.7, 136.9 (picoloyl C), 135.2, 133.2, 133.0, 128.5, 128.3, 128.2, 128.12, 128.10, 128.02, 128.00, 127.83, 127.80, 127.7, 127.6, 127.5, 127.4,

127.3, 126.9, 126.5, 126.2, 126.0, 125.53 (picoloyl C), 125.50, 94.1 (C-1 and C-1'), 80.7 (C-3), 79.2 (C-3'), 79.1 (C-2'), 78.8 (C-2), 75.7 (benzylic C), 75.4 (benzylic C), 73.6 (benzylic C), 73.5 (benzylic C), 72.7 (benzylic C), 71.9 (C-4'), 70.5 (C-5), 70.2 (C-4), 69.4 (C-5'), 68.5 (C-6'), 63.2 (C-6), 36.7, 33.4 (lipid γC), 31.9, 29.72, 29.70, 29.64, 29.61, 29.60, 29.5, 29.3, 27.5, 22.7, 19.9 (lipid γCH_3), 14.1 (lipid terminal CH_3), 12.6 (lipid αCH_3); IR (cast film): 3484, 3062, 3031, 2925, 2854, 1749, 1713, 1454, 1363, 1305, 1289, 1244, 1105, 1060, 1001 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+H]^+$ calcd for $C_{74}H_{88}NO_{13}$, 1198.625; found, 1198.626.



2.96 **2.97**

3',6'-di-*O*-Benzyl-4'-*O*-picoloyl-α-D-glucopyranosyl-(1'↔1)-2,3-di-*O*-benzyl-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (2.97). Trehalose derivative **2.96** (14 mg, 11 μmol, 1.0 equiv) was dissolved in CH_2Cl_2 (0.22 mL) and H_2O (22 μL). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (5.2 mg, 23 μmol, 2.0 equiv) was added and then the mixture was stirred at room temperature for 2 d at which point TLC (1:2 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and

the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (50 mL silica gel, 0:1→1:2→1:1 EtOAc–hexane) to give **2.97** (2.7 mg, 22%) as a transparent colorless film. $[\alpha]_D^{21}$ +64.2 (*c* 0.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 8.77 (d, *J* = 3.6 Hz, 1 H, picoloyl ArH), 8.01 (d, *J* = 8.4 Hz, 1 H, picoloyl ArH), 7.81 (dd, *J* = 8.4, 7.8 Hz, 1 H, picoloyl ArH), 7.49 (dd, *J* = 7.8, 3.6 Hz, 1 H, picoloyl ArH), 7.44–7.27 (m, 7 H, ArH), 7.21–7.09 (m, 13 H, ArH), 6.59 (dd, *J* = 10.2, 1.2 Hz, 1 H, lipid βH), 5.47 (dd, *J* = 9.6, 9.6 Hz, 1 H, H-4'), 5.29 (d, *J* = 3.6 Hz, 1 H, H-1'), 5.26 (d, *J* = 4.2 Hz, 1 H, H-1), 5.05 (d, *J* = 10.8 Hz, 1 H, benzylic H), 4.90 (d, *J* = 10.8 Hz, 1 H, benzylic H), 4.77 (m, 2 H, benzylic H), 4.73 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.66 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.55 (dd, *J* = 12.0, 4.2 Hz, 1 H, H-6), 4.48–4.41 (m, 3 H, H-5' and benzylic H), 4.24 (dd, *J* = 12.0, 1.8 Hz, 1 H, H-6), 4.17–4.12 (m, 2 H, H-5 and H-3'), 3.90–3.87 (m, 2 H, H-3 and H-2), 3.56 (dd, *J* = 9.6, 3.6 Hz, 1 H, H-2'), 3.51–3.44 (m, 3 H, H-6' and H-4), 2.91 (br s, 1 H, 4-OH), 2.49–2.44 (m, 1 H, lipid γH), 2.11 (br s, 1 H, 2'-OH), 1.83 (d, *J* = 1.2 Hz, 3 H, lipid αCH_3), 1.37–1.25 (m, 20 H, lipid CH_2), 0.98 (d, *J* = 6.6 Hz, 3 H, lipid γCH_3), 0.88 (dd, *J* = 7.2, 6.6 Hz, 3 H, lipid terminal CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 169.1 (lipid C=O), 164.1 (picoloyl C=O), 149.8 (picoloyl C), 149.7 (lipid βC), 147.6 (lipid αC), 138.7, 138.1, 138.0, 137.7, 136.9 (picoloyl C), 128.6, 128.35, 128.32, 128.1, 127.9, 127.8, 127.70, 127.68, 127.6, 127.4, 127.0 (picoloyl C), 125.6 (picoloyl C), 94.8 (C-1), 93.6 (C-1'), 80.9 (C-3), 80.0 (C-3'), 78.7 (C-2'), 75.6 (benzylic C), 74.9 (benzylic C), 73.6 (benzylic C), 72.6 (benzylic C), 72.2 (C-4'), 71.9, (C-2), 70.4 (C-5), 70.2 (C-4), 69.7 (C-5'), 68.7 (C-6'), 63.4 (C-6), 36.7, 33.4 (lipid γC), 31.9, 29.74, 29.69, 29.65, 29.62, 29.60, 29.3, 27.5, 22.7, 20.0 (lipid γCH_3), 14.1 (lipid terminal CH_3), 12.6 (lipid αCH_3); IR (cast film): 3437, 3064, 3031, 2925, 2854, 1744, 1714, 1454, 1363, 1307, 1288, 1244, 1145, 1092, 1004 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+H]^+$ calcd for $C_{63}H_{80}NO_{13}$, 1058.5624; found, 1058.5641.

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Chapter 3 : Studies towards the synthesis of the caryophyllose moiety of *M. marinum* lipooligosaccharides

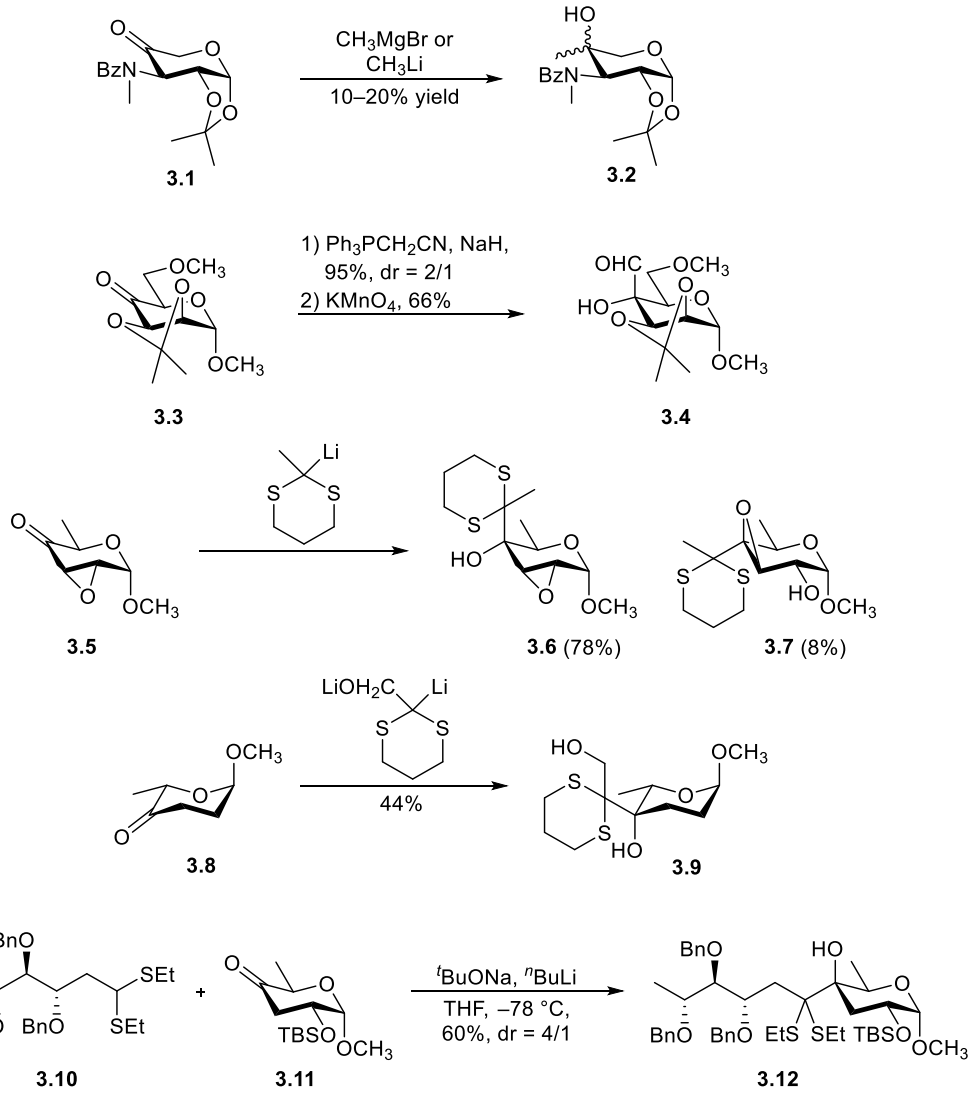
As described in Chapter 2, one of the challenges in constructing LOSs from *M. marinum* is the synthesis of individual building blocks. In Chapter 2, I summarized how I managed the difficult synthesis of the asymmetrically-substituted (acylated) trehalose. In this chapter, the synthetic work I carried out towards synthesizing caryophyllose, a rare monosaccharide with a C-4 branching moiety, will be described. The main challenge in synthesizing caryophyllose resides in the C–C bond forming step to install the branching moiety into the monosaccharide. Previous studies on C–C bond forming reactions used to install a C-4 branching moiety into monosaccharides, as well as the work I did leading to the synthesis of caryophyllose, will be described.

3.1 Previous syntheses of C-4 branched monosaccharides via C–C bond formation

The synthesis of branched sugars has been summarized by Grisebach and Schmid in 1972,[1] Yoshimura in 1984[2] and Chapleur and Chrétien in 1997.[3] Due to the extensive scope of these publications, I will only briefly highlight two methods that were applied to synthesis of C-4 branched monosaccharides in the pyranose ring form as they are relevant to caryophyllose.

3.1.1 Bond formation using carbon nucleophiles

The common methods that have been used to synthesize C-4 branched pyranosides involve the coupling of a carbon nucleophile (as the branch moiety) and a C-4 ketone on the pyranoside. Various types of nucleophiles have been used, including organolithium reagents,[4,5] Grignard reagents[6,7] and ylides.[8,9] Selected examples are shown in **Scheme 3.1**.



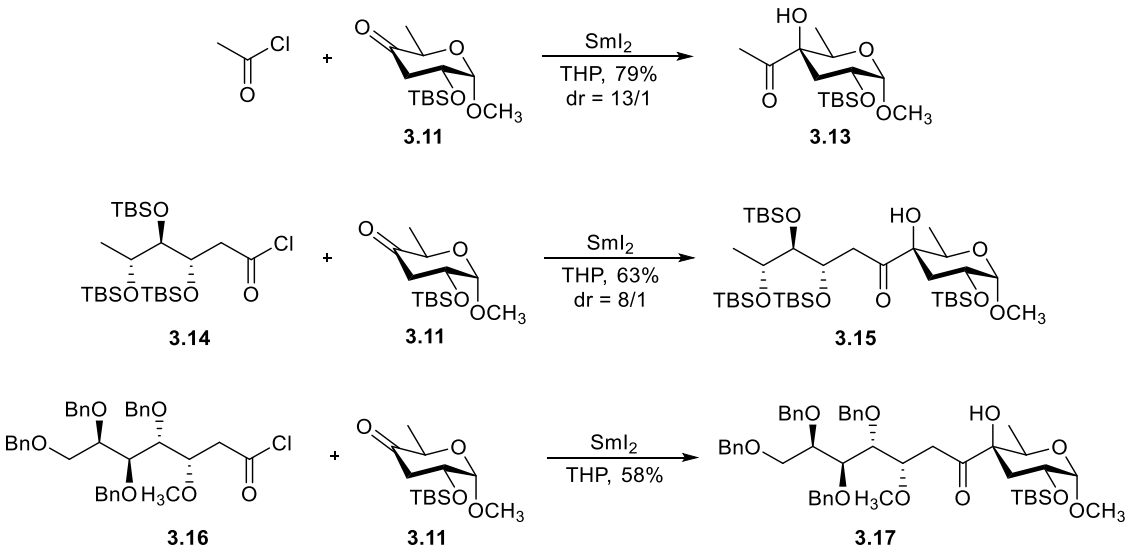
Scheme 3.1. Examples of synthesized C-4 branched pyranosides using carbon nucleophiles.[10–14]

In one case, ketone **3.1** was coupled with either methylmagnesium bromide or methyllithium to produce the methylated pyranoside **3.2** in 10–20% yield as a diastereomeric mixture (ratio not reported).[10] In another example, ketone **3.3** was coupled with a nitrile-functionalized ylide to form the alkene that was then oxidatively cleaved with $KMnO_4$ to form the branched sugar **3.4**.[11] In addition to short chain nucleophiles such as those used in the previous

two examples, bulkier nucleophiles such as lithiated dithioacetals have also been used. As an example, ketone **3.5** was coupled with lithiated 2-methyldithiane to produce dithioacetal **3.6** as the major diastereomer; the minor diastereomer underwent Payne rearrangement to produce dithioacetal **3.7**.[12] More complicated lithiated dithianes have also been used. Ketone **3.8** was coupled with dilithiated C-2 dihydroxylmethyl dithiane to produce branched pyranose **3.9** as the major product.[13] The most complicated nucleophile used in this type of reaction was during the coupling between dithioacetal **3.10** and ketone **3.11** to produce branched pyranose **3.12** as the major diastereomer.[14] This coupling was made possible due to the additive used in the formation of lithiated **3.10**. Deprotonation of C-2 substituted dithianes requires additives because the C-2 proton is more difficult to deprotonate than the C-2 proton on unsubstituted dithianes. Superior to TMEDA,[15] sodium *tert*-butoxide[16] was used with butyllithium, forming presumably butylsodium.[17] This "butylsodium" was proposed to be a stronger base than butyllithium to effectively deprotonate the C-2 proton of dithioacetal **3.10**.[14]

3.1.2 Bond formation using acyl radicals

The other method that has been used to synthesize C-4 branched pyranosides is the coupling between an acyl chloride (as the branching moiety) and a ketone on the C-4 position of the pyranoside. Summarized in **Scheme 3.2**, the coupling between acetyl chloride and ketone **3.11** mediated by a stochiometric amount of samarium(II) iodide as a reductant produced branched pyranoside **3.13**.[18] The same method was used to synthesize branched pyranoside **3.15**[18] and a protected derivative of a branched sugar (**3.17**) isolated from *Mycobacterium gastri*.[19] This coupling had to be carried out in tetrahydropyran, instead of the commonly used tetrahydrofuran, because side products derived from ring opening of tetrahydrofuran lowered the overall yield.[20]

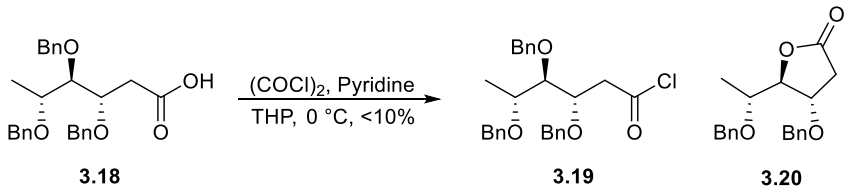


Scheme 3.2. Synthesis of C-4 branched pyranosides using acyl radicals.[18,19]

3.1.3 Summary of reported synthesis of C-4 branched pyranosides

After describing both methods that have been used to synthesize C-4 branched pyranosides, rationalizing the approach I chose for my synthesis of caryophyllose is necessary. Both methods were shown to be effective in synthesizing complex substrates (e.g., **3.12**, **3.15** and **3.17**). Using the samarium(II) iodide-mediated coupling to synthesize caryophyllose was appealing because it provided higher yields and selectivity than using carbon nucleophiles. However, it was reported that synthesizing the acyl chloride **3.19** led to side products. As shown in **Scheme 3.3**, attempting to convert carboxylic acid **3.18** to acyl chloride **3.19** only resulted in the formation of the cyclized lactone **3.20**; less than 10% of the desired **3.19** was isolated.[18] Formation of the unexpected lactone **3.20** eventually led the authors to synthesize acyl chloride **3.14**, which served as an alternative way to solve the problem. Although this kinetically-driven 5-member ring cyclization was not general (e.g. acyl chloride **3.16** was successfully made), this rapid cyclization was studied and then applied

to synthesize cyclic pentitols[21] and furanosides.[22] These reports made it difficult to predict whether the same undesired transformation would happen during synthesis. Therefore, this approach was not chosen, which left me with using carbon nucleophiles. Being one of the most studied and used extensively for C–C bond formations, carbon nucleophiles provided more reliability and flexibility when designing synthetic plans. I will later describe my synthesis towards caryophyllose, using this method in **Scheme 3.4**.



Scheme 3.3 Generated side product in attempt to synthesize acyl chloride **3.19**.[18]

3.2 Studies towards the synthesis of caryophyllose

Although the coupling method was chosen, there is a reason why I needed to develop a new synthetic route, instead of just repeating the reported synthesis: the required caryophyllose derivatives had to be suitable for glycosylation. As shown in **Figure 3.1**, the two protecting groups highlighted in pyranose **3.12** could not be modified separately, orthogonally, for lipooligosaccharide synthesis. The highlighted benzyl group in **3.12** cannot be regioselectively deprotected in the presence of other benzyl groups to reveal a hydroxyl group for glycosylation as a glycosyl acceptor. The same reason applies for the highlighted anomeric methoxy group in **3.12**. This methoxy group cannot be chemoselectively deprotected to reveal a hemiacetal, required to be converted into a glycosyl donor for glycosylation. These two protecting groups need to be switched to two orthogonal protecting groups as in the target moiety **3.21**. The highlighted triisopropylsilyl

group (TIPS) in **3.21** could be chemoselectively deprotected to reveal a hydroxyl group, becoming a glycosyl acceptor for glycosylation. The highlighted *p*-methoxybenzyl group (PMB) could also be chemoselectively deprotected to reveal a hemiacetal, to be converted into a glycosyl donor for glycosylation. With the target moiety **3.21** designed, the retrosynthetic plan will be explained.

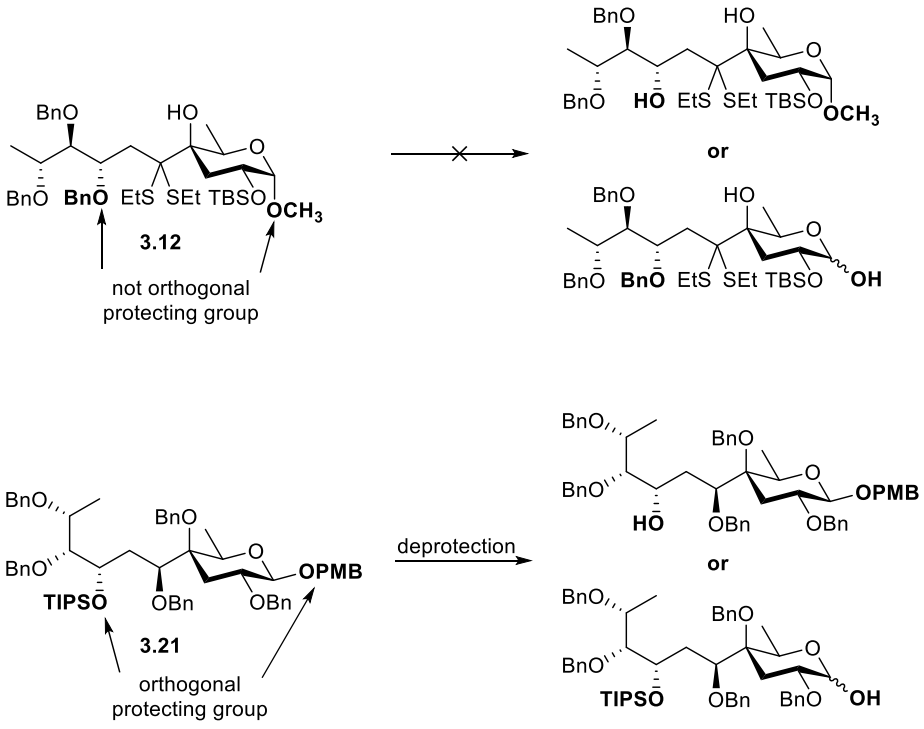
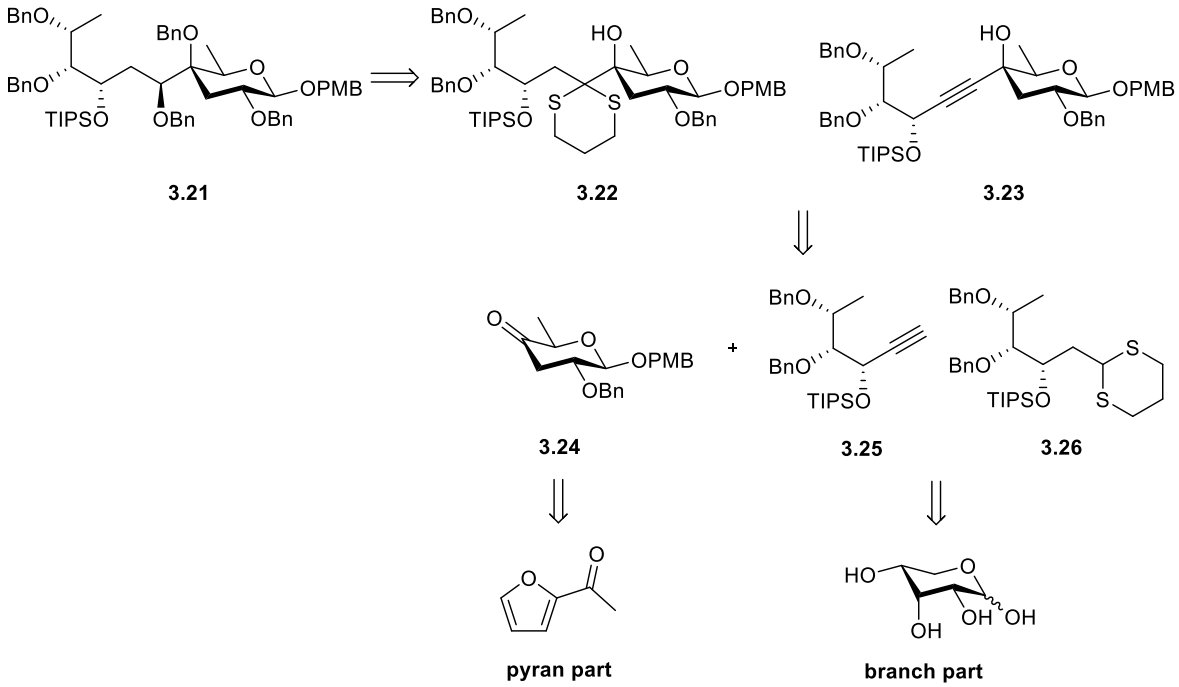


Figure 3.1. Comparison of a caryophyllose derivative synthesized in the literature[14] and one suitable for lipooligosaccharide synthesis.

3.2.1 Retrosynthetic analysis

The retrosynthetic plan of **3.21** is described in **Scheme 3.4**. The target could be synthesized via one or two intermediates, **3.22** or **3.23**. Pyranoside **3.22** was designed to be similar to pyranoside **3.12** (**Scheme 3.2**) so the coupling conditions used to synthesize the latter could be replicated without problems. That is, coupling of **3.24** and **3.26** would give **3.22**. However, in the

synthesis of **3.12**, the yield was modest (60%), which presumably comes from the bulk of dithioacetal **3.10**, which leads to a difficult coupling. I anticipated I might also obtain a low yield in joining **3.24** and **3.26**. Thus, pyranoside **3.23** was also designed, which could be obtained using a less bulky nucleophile (**3.25**). Alkyne **3.25** would also serve as an intermediate for synthesizing dithioacetal **3.26**. Both alkyne **3.25** and dithioacetal **3.26**, named as the 'branch part', could be synthesized from commercially available D-ribose. The other coupling partner, **3.24**, bearing a C-4 ketone, (the 'pyran part'), could be accessed from commercially available 2-acetylfuran.

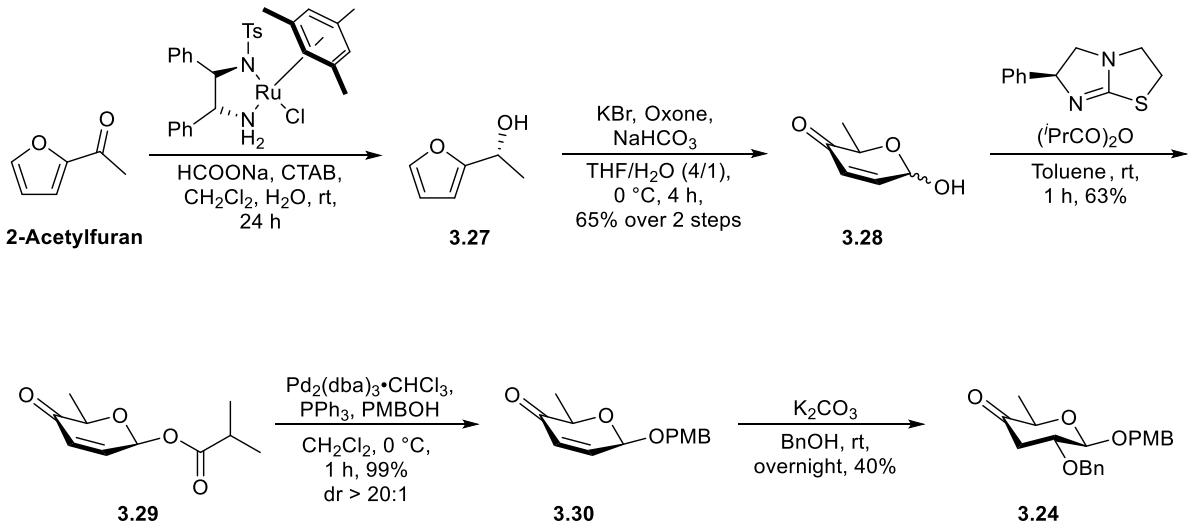


Scheme 3.4. Retrosynthetic plan of target moiety **3.21**.

3.2.2 Synthesis of coupling partners 3.24, 3.25 and 3.26

Synthesis of the pyran part **3.24** is shown in **Scheme 3.5**, starting with the reduction of 2-acetylfuran. A phase-transfer variation of the Noyori asymmetric transfer hydrogenation was

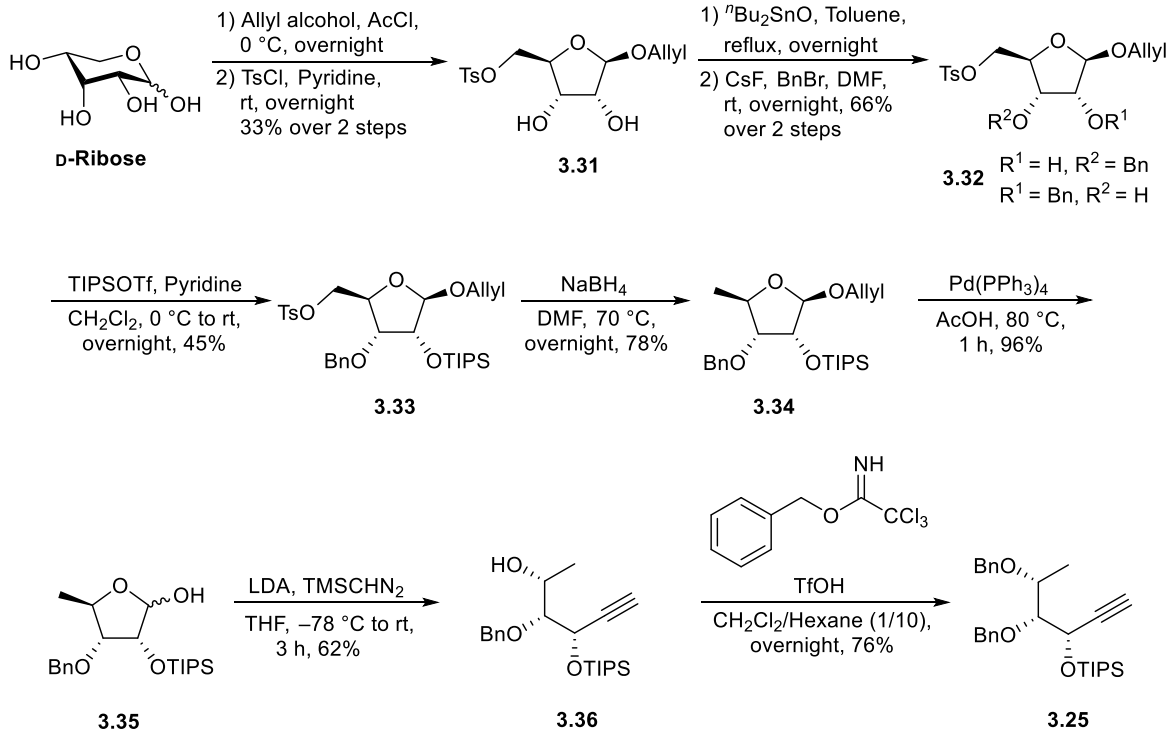
carried out to enantioselectively reduce the carbonyl group in 2-acetylfuran to produce alcohol **3.27**.[23] The phase-transfer reagent, cetyltrimethylammonium bromide (CTAB) was used to accelerate the reaction.[24] Without purification of **3.27**, a catalytic Achmatowicz rearrangement[25] was carried out to obtain an anomeric mixture of hemiacetal **3.28** in 65% yield over two steps.[26] The mixture of hemiacetal **3.28** was subjected to a catalytic diastereoselective acylation to resolve the anomers to obtain β-isomer **3.29** in 63% yield.[27] This yield was achieved after increasing the reaction concentration in an optimization attempt. The catalyst used in this reaction, (–)-tetramisole, served as a chiral base for this resolution.[28] Compound **3.29** anomerized slowly after purification; therefore, immediately after its formation, it was glycosylated with 4-methoxybenzyl alcohol in the presence of a palladium catalyst to produce β-glycoside **3.30** in 99% yield.[29] The final step involved a conjugate addition of benzyl alcohol to the α,β-unsaturated ketone in **3.30** to produce the desired 3,6-dideoxy-sugar **3.24** in 40% yield.[30] The addition of benzyl alcohol to **3.30** proceeded *anti* to the anomeric group, which is why the β-configuration (instead of α) had to be ensured in the previous steps. This reaction did not go to completion; therefore, the yield is only 40%, but the starting material could be recovered and subjected again to the reaction. The stereochemistry of **3.24** was validated by comparing the ^{1}H NMR spectra of **3.24** to a closely-related enantiomer, in which the anomeric group is a benzyl group.[30] The increased yield of the step, **3.28** to **3.29**, was crucial for this synthetic route to provide a sufficient quantity of **3.24** (2.0 g) for coupling studies.



Scheme 3.5. Synthesis of **3.24** (pyran part).

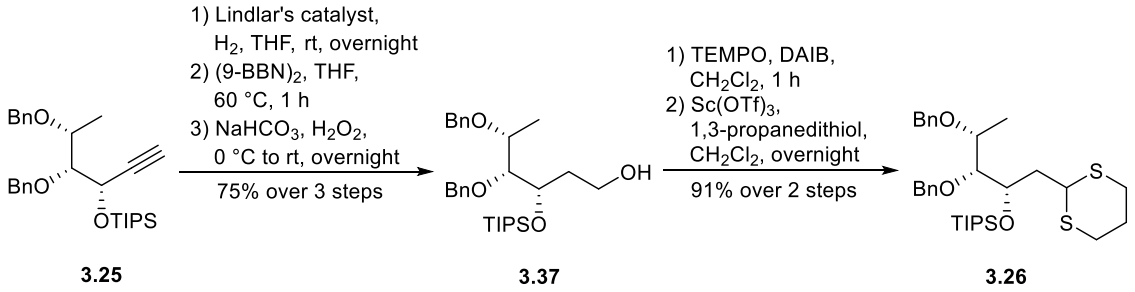
Having described the synthesis of the pyran part **3.24**, the synthesis of the branch part, **3.25**. will be discussed (**Scheme 3.6**). Ribose was glycosylated with allyl alcohol in the presence of *in situ* generated hydrogen chloride to produce allyl ribofuranoside. Without purification, the C-5 hydroxyl group was tosylated to produce **3.31** as the major product in 33% yield over two steps. The low yield resulted from the need to do multiple chromatography steps to purify the compound. The diol in **3.31** was monobenzylated *via* the nucleophilic attack of a formed tin acetal to benzyl bromide in the presence of cesium fluoride. However, this process was not regioselective on this substrate and therefore inseparable regioisomers of **3.32** were formed in 66% yield. Next, the remaining hydroxyl group in **3.32** was silylated and then the desired riboside **3.33** was isolated in 45% yield after purification. An HMBC experiment was used to determine the correct regioisomer after isolating **3.33**. A correlation signal between H-3 and the benzylic carbon observed in the HMBC spectrum indicated that the benzyloxy group was on O-3. Reduction of the tosyl group in riboside **3.33** proved to be difficult as using lithium aluminum hydride or the more reactive lithium

triethylborohydride generated little of the deoxygenated product **3.34**. However, sodium borohydride was effective in this reduction; therefore, riboside **3.34** was produced in 78% yield.[31] The anomeric allyl group of riboside **3.34** was deprotected with tetrakistriphenylphosphine palladium under acidic conditions to produce hemiacetal **3.35** in 96% yield. Next, **3.35** was converted to alkyne **3.36** in 62% yield using lithiated trimethylsilyldiazomethane.[32] The hydroxyl group on alkyne **3.36** was protected as a benzyl group under acidic conditions to produce alkyne **3.25** in 77% yield. If basic conditions were used in the benzylation step, silyl migration was observed. The only drawback of using acidic conditions in this case was that multiple column chromatography steps were needed to remove benzyl trichloroacetimidate from alkyne **3.25**.



Scheme 3.6. Synthesis of **3.25** (branched part).

The last coupling partner, dithioacetal **3.26**, was also synthesized from **3.25** in several steps as shown in **Scheme 3.7**. Alkyne **3.25** was semi-hydrogenated to the corresponding alkene using Lindlar's catalyst; then the alkene was subjected to hydroboration–oxidation to produce alcohol **3.37** in 75% yield over three steps. The hydroxyl group in **3.37** was oxidized to an aldehyde, which was protected as a dithioacetal by reaction with 1,3-propanedithiol and scandium triflate to produce **3.26** in 91% yield over two steps. Boron trifluoride etherate could not be used as the Lewis acid in this protection because silyl group cleavage was observed. Now that the syntheses of the coupling partners **3.24**, **3.25** and **3.26** has been presented, I will describe the coupling studies of these compounds.

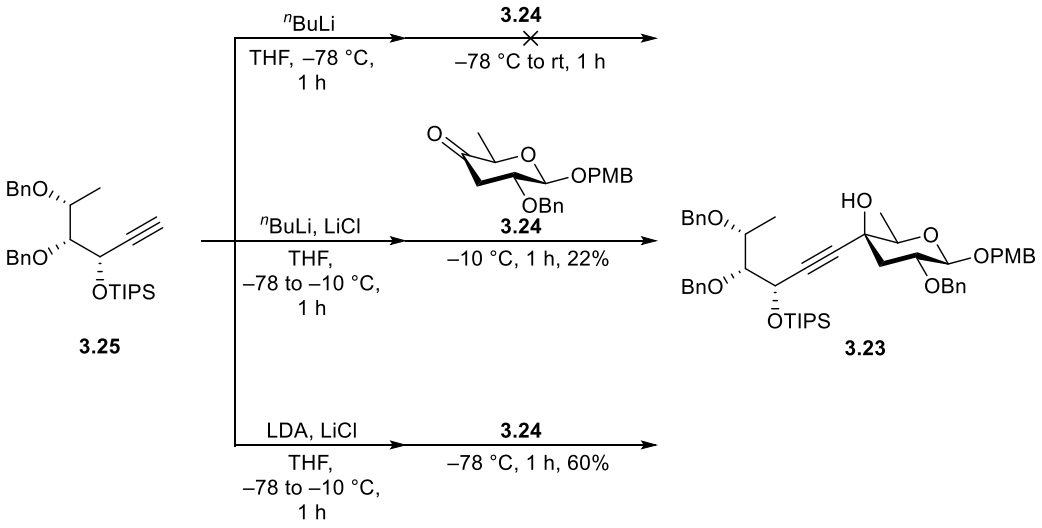


Scheme 3.7. Synthesis of **3.26** (branched part).

3.2.3 Coupling studies between 3.24, 3.25 and 3.26

The coupling studies are summarized in **Scheme 3.8**. In a first attempt, alkyne **3.25** was deprotonated at –78 °C using *n*-butyllithium and ketone **3.24** was added to the solution. However, TLC analysis indicated that no reaction had occurred; therefore, the solution was warmed to room temperature, at which point the reaction turned into a complex mixture. Most of the alkyne was recovered but little product **3.23** could be isolated. Theoretically, the alkyne should be easily deprotonated and the resulting acetylide could attack the ketone. However, this appears not to be

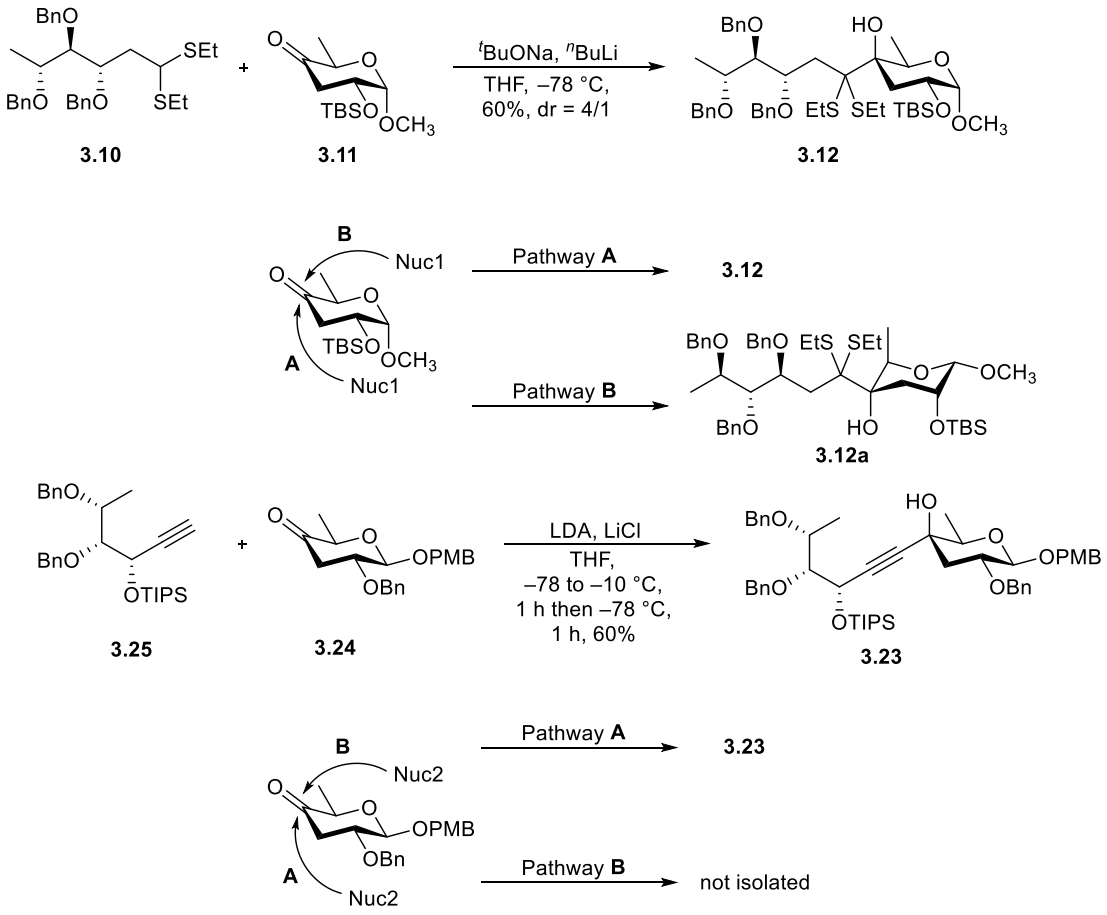
the case. I hypothesized that the problem resulted from the alkyne because the ketone is similar to **3.11** (**Scheme 3.1**), which had previously been shown to be susceptible to nucleophilic attack in the synthesis of **3.12**. The results I described above suggest that the alkyne is not fully deprotonated at –78 °C and that upon warming, even if it is deprotonated, the resulting acetylide is not nucleophilic enough to attack the ketone. My logic was then to try to deprotonate the alkyne at higher temperatures and enhance its nucleophilicity. Thus, I chose to add lithium chloride to the reaction. This "salt effect"[33] has various effects on organic reactions. It allows strong bases like lithium diisopropylamide[34] and methyllithium[35] to become more nucleophilic that can undergo conjugate additions with different Michael acceptors. Lithium diisopropylamide are described as aggregates in solutions; however, these salts disaggregate it into reactive monomers thus make it more nucleophilic.[34] Another explanation of enhanced nucleophilicity is that these salts form complexes with the nucleophile and electrophile, which then favor product formation.[34] To test out the idea, alkyne **3.25** was mixed with *n*-butyllithium and lithium chloride at –78 °C then the solution was warmed to –10 °C before ketone **3.24** was added. Under these conditions, alkyne **3.23** was produced, but only in 22% yield. The product was contaminated with a small amount of **3.24** and some side products resulting from butyllithium attack on the ketone were also observed. This problem was relatively easy to solve: 1) switching *n*-butyllithium to *in situ* generated lithium diisopropylamide, 2) using less **3.24** and precooling **3.24** to –78 °C before adding it to the deprotonated **3.25**. These changes increased the yield of product **3.23** to 60%.



Scheme 3.8. Coupling studies between **3.24** and **3.25**.

Glycoside **3.23** was isolated as a single diastereomer. Because it exists as a colorless film, not a solid, X-ray crystallography was not pursued to elucidate the orientation of the C-4 alkyne substituent. The substituent was assigned to occupy equatorial position after comparison of its NMR data to that reported for **3.12** (**Scheme 3.9**). To rationalize the result, I considered the possible pathways by which these reactions could proceed. During the coupling between **3.10** and **3.11**, deprotonated **3.10** (Nuc1) could preferentially attack **3.11** from the equatorial orientation (Pathway A) to generate **3.12** (the major isomer). However, the anomeric α-methoxy group might hinder attack via Pathway B; therefore, axial attack of Nuc1 on **3.11** (Pathway B) could generate the minor isomer of **3.12a**. Similar logic can be applied in the coupling between **3.24** and **3.25**. Preferential equatorial attack of lithiated **3.25** (Nuc2) on **3.24** (Pathway A) would generate **3.23**. Without an anomeric α-substituent, this pathway is less hindered compared to the reaction with **3.11**, which could explain the absence of a minor product (from Pathway B). In addition, Pathway B could be hindered by the anomeric β-substituent, resulting in a less favored pathway. Product

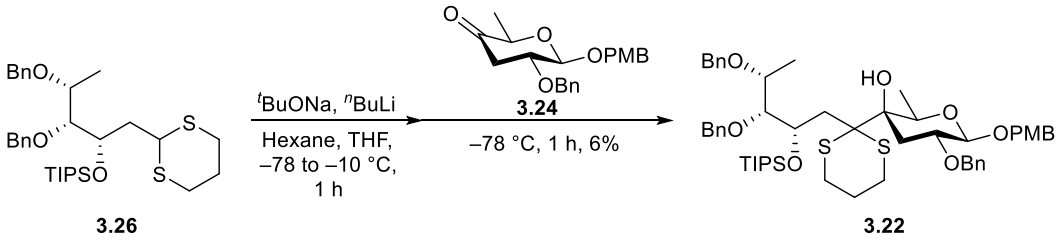
3.23 was also drawn in the $^4C_1$ conformation, as deduced from the coupling constant between H-1 and H-2 (8.4 Hz) in the ^{1}H NMR spectrum. Although these analyses cannot replace empirical data in determining the orientation of the C-4 substituent in the product of this reaction, I expect **3.23** to be the desired product.



Scheme 3.9. Theoretical explanation of C-4 alkyne substituent orientation in **3.23**.

The coupling between **3.24** and **3.26** (**Scheme 3.10**) was carried out using the described method, deprotonating dithiane **3.26** with a combination of *n*-butyllithium and sodium *tert*-butoxide;[14] however, the desired product **3.22** was only isolated in 6% yield. The reaction did not

proceed to completion even if excess dithioacetal **3.26** was used. Both **3.26** and **3.24** (60%, limiting reagent) were recovered. In an attempt to increase the yield, I extended the deprotonation time by doubling it to two hours. I also added an excess amount of sodium *tert*-butoxide because there might be residual sodium hydroxide. However, even with these changes, the yield was similar.



Scheme 3.10. Coupling studies between **3.26** and **3.24**.

Verification of **3.22** by NMR spectroscopy could not be completed as multiple resonances appeared as broad signals. This phenomenon could be explained by restricted bond rotation caused by the adjacent quaternary carbons. The orientation of the C-4 dithiane substituent was also not verified but it was assumed to be equatorial based on the analysis shown in **Scheme 3.9**. Lithiated **3.26** would attack **3.24** equatorially along a less hindered pathway. The chair conformation was expected to be in $^4C_1$ conformation, deduced from the coupling constant between H-1 and H-2 (7.7 Hz) in ^{1}H NMR spectrum after the bulky dithiane in **3.22** was deprotected to form ketone **3.41**, for which a clear NMR spectrum (**Figure 3.2**) was obtained.

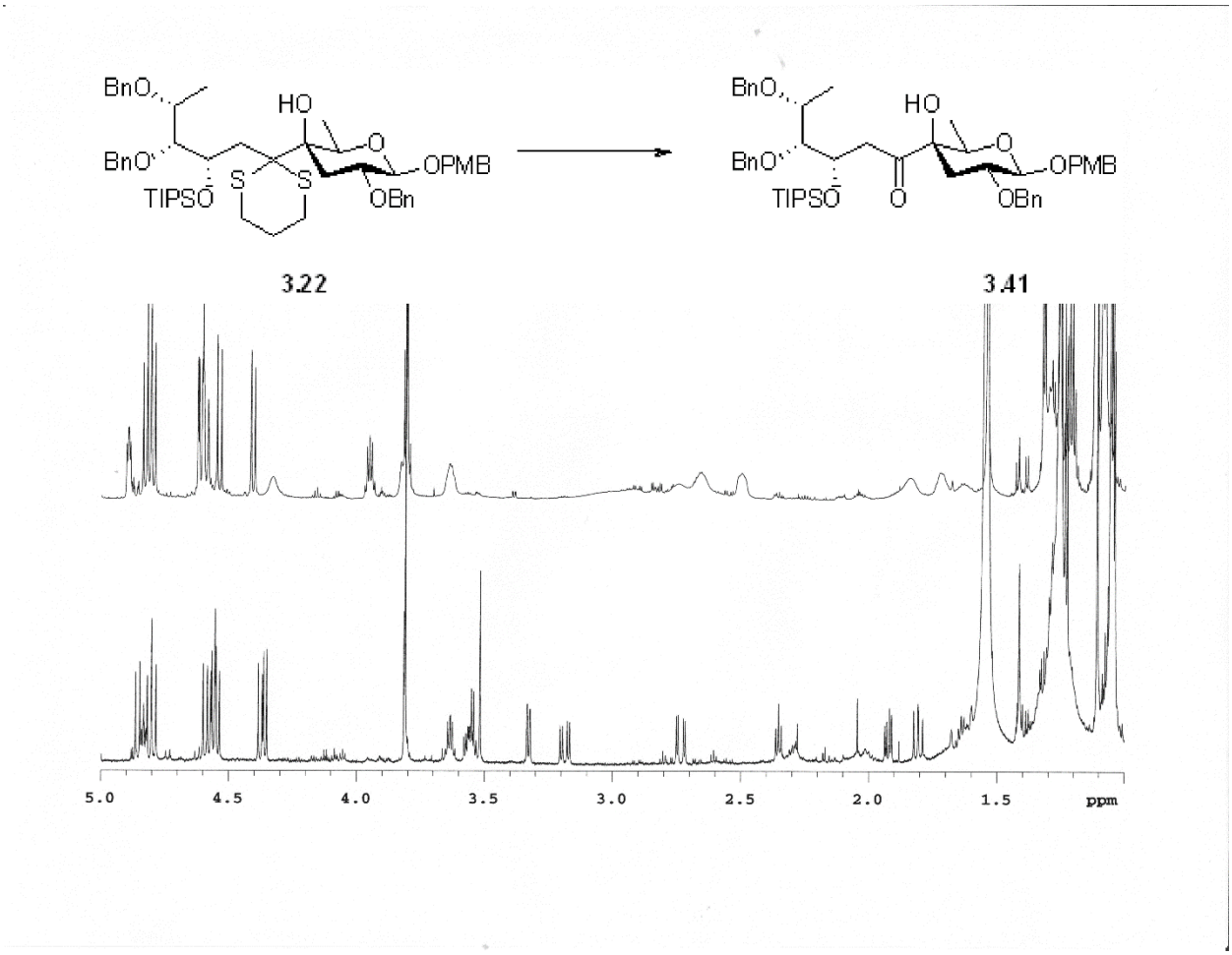


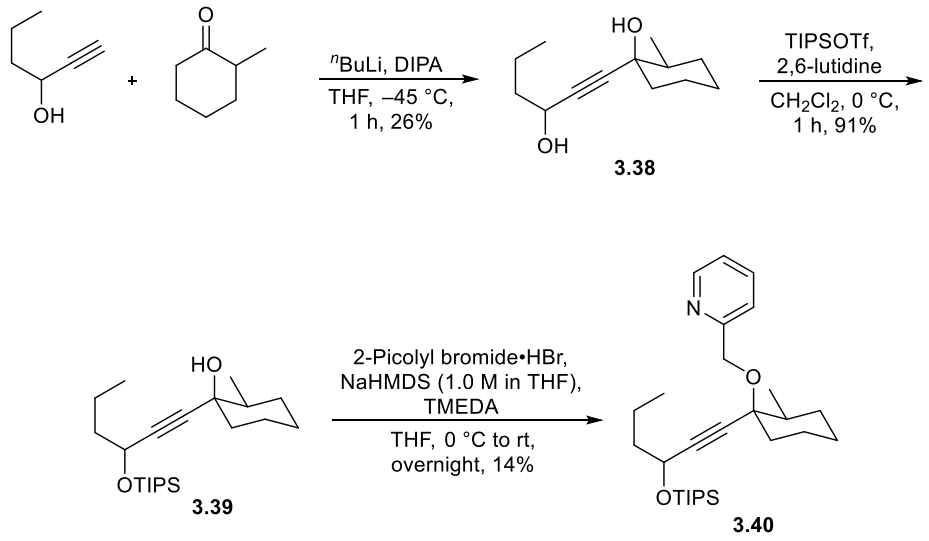
Figure 3.2. Comparison of ^{1}H NMR spectrum of **3.22** (top) and **3.41** (bottom).

Although the caryophyllose backbone was constructed, both **3.22** and **3.23** were isolated in limited quantities, 14 mg and 12 mg, respectively. It was therefore difficult to continue with further studies. However, I did try to go a bit further and test some reactions although the products were not well characterized.

3.2.4 Further studies

For **3.22**, the next step is to convert the internal alkyne to a ketone via hydroboration–oxidation. Although there was a reported procedure for this transformation on a complex

substrate,[36] I decided to plan a model study as I was unfamiliar with this chemistry and the substrate is quite different from **3.22**. The synthesized model compounds are shown in **Scheme 3.11**. Coupling of hexynol and methylcyclohexanone produced an inseparable mixture of diol **3.38** in 26% yield. The secondary alcohol of **3.38** was silylated to produce, in 91% yield, **3.39** also as an inseparable mixture. The tertiary alcohol in **3.39** was then protected as a picolyl group to produce an inseparable mixture of **3.40** in 14% yield. Using racemic materials, the relative configuration of all three products, **3.38**, **3.39** and **3.40** could not be identified at this stage. However, presumably a pair of diastereomers in a 1 to 1 ratio was found in all of the three products, as observed in the ^{1}H and ^{13}C NMR resonances.

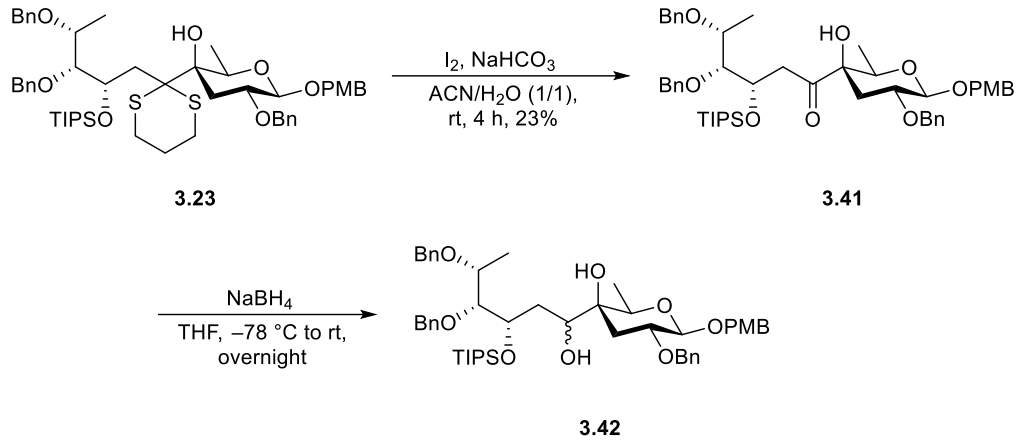


Scheme 3.11. Synthesis of model compounds for hydroboration–oxidation studies.

Alkyne **3.39** was subjected to hydroboration–oxidation conditions following the literature,[36] but no reaction occurred. I hypothesized that the lack of reaction was due to steric hinderance caused by the tertiary center and the triisopropylsilyl group, which interfered with the

approach of the bulky 9-borabicyclo[3.3.1]nonane reagent to the alkyne. Thus, a smaller borane reagent, borane dimethyl sulfide, was used. However, the reaction turned into a complex mixture, probably a mixture of regioisomers, that I could not isolate nor characterize. Based on this assumption, **3.40** was synthesized to improve the regioselectivity. The lone-pair of electrons on the nitrogen would coordinate to the borane resulting in a partial positive charge on the nitrogen. This partial positive charge would cause an electronic bias on the alkyne, creating a partial negative charge on the carbon closer to the ring. This partial negative charge will bind preferred to the boron of the borane, thus increasing the regioselectivity.[37] However, a complex mixture that could not be characterized was still obtained. This complex mixture might be multiple isomers generated from the diastereomeric starting materials, which made the results of the hydroboration–oxidation at this stage inconclusive. Given this outcome and the time constraints of my Ph.D. research, no further studies were executed on the original substrate **3.22**.

For the further transformation of **3.23**, the next few steps were familiar. Therefore, I decided to carry them out on **3.23** (**Scheme 3.12**). First dithioacetal **3.23** was hydrolyzed to ketone **3.41** using iodine and water in 23% yield.[37] TLC analysis indicated the reaction produced a complex mixture of products. I supposed that the anomeric PMB group was probably modified and this happened even in the presence of sodium bicarbonate as a buffer or when the pH was around neutral. Ketone **3.41** was reduced with sodium borohydride in THF to produce the protected caryophyllose derivative **3.42** in quantitative yield.[38] The product was isolated as a single diastereomer, but the stereochemistry of the hydroxyl group could not be elucidated as there is little material to derivatize and no similar substrate with which to compare. Thus, although I had prepared a caryophyllose derivative, I stopped my worked given time constraints. In addition, I planned to reconsider a new strategy that addresses the issues faced during the synthesis.



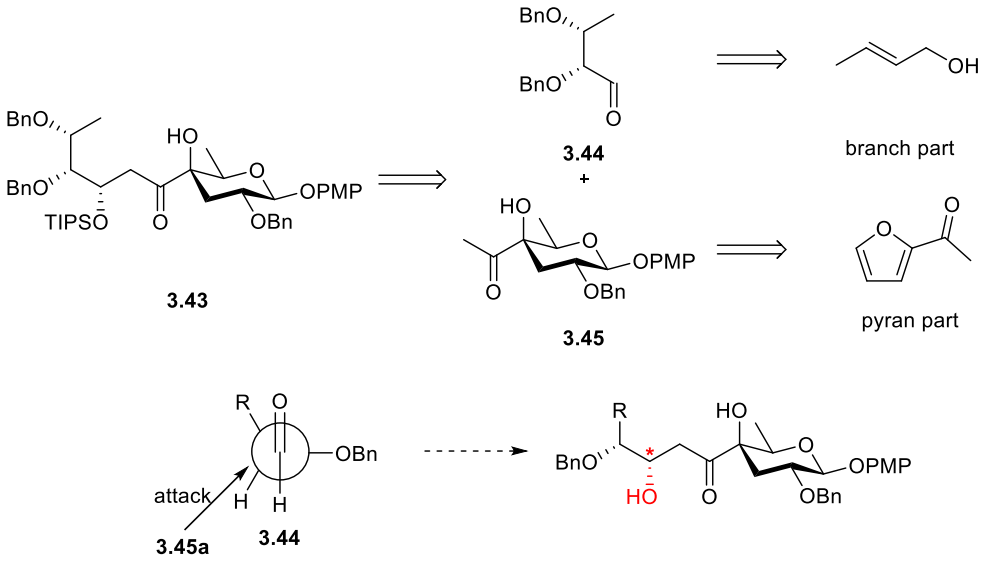
Scheme 3.12. Further studies with **3.23**.

3.3 Summary and future plan

In the previous sections, I described my progress towards synthesizing caryophyllose. The strategy involved coupling of the branched part with a C-4 ketone in the pyran part *via* nucleophilic addition. The pyran part **3.26** was synthesized in an efficient way, but the branched part, either **3.24** or **3.25**, was not. The coupling step was also challenging. Coupling between alkyne **3.24** and ketone **3.26** was initially low-yielding, but made to work using the "salt effect". However, coupling between dithioacetal **3.25** and ketone **3.26** was low yielding and could not be improved. The coupled products **3.22** and **3.23** were isolated and further studies were done as much as possible with the small amount of product obtained. In the end, caryophyllose backbone **3.42** was obtained but the stereochemistry could not be elucidated.

The major issues that were faced during the synthesis were: 1) the PMB group on the pyran part is labile to Lewis acids, 2) the synthesis of the branched part was not as efficient as the pyran part, 3) the coupling strategy was not optimal and 4) converting the installed functional group to a ketone after coupling was difficult. In the future, a new synthetic strategy could be employed as

proposed in **Scheme 3.13**. Caryophyllose backbone **3.43** could be obtained via a boron aldol condensation between aldehyde **3.44** and ketone **3.45**. This aldol condensation will take the advantage of the α-benzyloxy group in aldehyde **3.44**; which allowed the enolized ketone **3.45a** to attack from the favored path predicted by the polar Felkin-Anh model, to generate the desired stereocenter.[40] Aldehyde **3.44** could be synthesized from butenol in six steps, which is a lot shorter compared to my previous route. Ketone **3.45** could be synthesized from 2-acetylfuran in six steps (as in the route described above). This compound features an anomeric *p*-methoxyphenyl (PMP) group, which is more stable than a PMB group. The synthesized **3.43** could then be modified for oligosaccharide synthesis to construct the lipooligosaccharide.



Scheme 3.13. New synthetic plan for caryophyllose.

With my attempts to synthesize caryophyllose summarized, I will move on to Chapter 4, which describes a continuation of my work discussed in Chapter 2 to synthesize the LOS-I pentasaccharide.

3.4 Experimental section

3.4.1 General methods

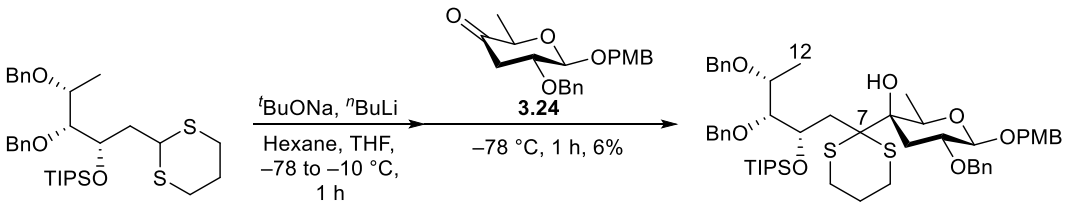
All reagents were purchased from commercial sources and used without further purification except LiCl and $Sc(OTf)_3$, which were purified as described below. *n*-Butyllithium was titrated with diphenylacetic acid before use. Diisopropylamine was freshly distilled from NaOH before use. Hexane used as reaction solvent was freshly distilled from CaH_2 before use. Dichloromethane, tetrahydrofuran, acetonitrile, toluene and *N*,*N*-dimethylformamide used in reactions as solvents were taken from a solvent purification system in which the solvents are purified by successive passage through columns of alumina, copper and molecular sieves under argon. All reactions were carried out in round bottom flasks with stir bars inside and capped with rubber septum. Thin layer chromatography was performed on silica gel 60 F254 (0.25 mm, Merck) glass plates. Spots were detected by UV light and charring after treatment with a solution of either 1) ceric ammonium nitrate (0.5 g) and ammonium molybdate (12 g) in water (235 mL) and sulfuric acid (15 mL) or 2) *p*-anisaldehyde (3.7 mL) and glacial acetic acid (1.5 mL) and concentrated H_2SO_4 (5 mL) in ethanol (135 mL). In the reaction work-up involving extractions, TLC were performed on combined organic layer and aqueous layer after extraction and before concentrating the combined organic layer. All column chromatography was performed on silica gel 60 (40–60 μm). Melting points were measured on a Gallenkamp apparatus and are not corrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter at the sodium D line (589 nm) at 21 ± 2 °C and are in units of (deg·mL)/(dm·g). FTIR spectra were run on Thermo Nicolet (Madison Wisconsin, USA) 8700 main bench with a Continuum FTIR microscope attached, and samples cast from a chloroform solution onto an IR- transparent silicone wafer. ^{1}H NMR spectra were recorded at 400, 500, 600 and 700 MHz, and the chemical shifts were referenced to $CHCl_3$ (7.26 ppm, $CDCl_3$). ^{13}C

NMR spectra were recorded at 125 MHz and are proton decoupled, and the chemical shifts were referenced to $CDCl_3$ (77.00 ppm, $CDCl_3$). High resolution EI mass spectra were recorded on Kratos Analytical MS-50G spectrometer; high resolution ESI mass spectra were recorded on Agilent Technologies 6220 spectrometer; MALDI mass spectra were recorded on Bruker 9.4 T Apex-Qe spectrometer.

Procedure for drying solids:

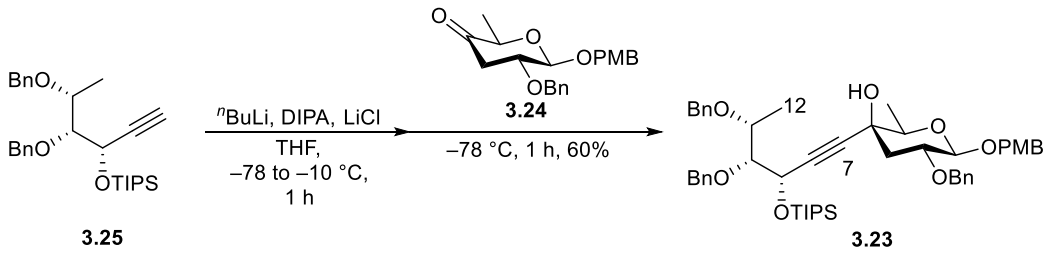
LiCl and $Sc(OTf)_3$: The metal salt was transferred to a round bottom flask. The flask was attached to high vacuum and gently heated with a heat gun. After the solids stopped bumping and the appearance changed from shiny to chalky, the flask was cooled to room temperature. The dried metal salt was then weighed quickly in air and then transferred to the reaction flask.

3.4.2 Experimental procedures and spectroscopic data



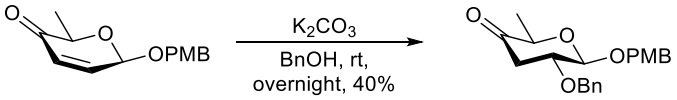
4-Methoxybenzyl 2-*O*-benzyl-3,6-dideoxy-4-*C*-(((2*S*,3*R*,4*R*)-3,4-bis(benzyloxy)-2-((triisopropylsilyl)oxy)pentyl)-1,3-dithian-2-yl)-β-D-xylopyranoside (3.22). Sodium *tert*-butoxide (19 mg, 0.20 mmol, 2.0 equiv) was suspended in hexane (0.45 mL) then the mixture was cooled to 0 °C. *n*-Butyllithium (1.5 M in hexane, 0.13 mL, 0.20 mmol, 2.0 equiv) was added then the mixture was stirred for 1 h at 0 °C and then for 1 h at room temperature. The mixture was then cooled to –78 °C before **3.26** (0.12 g, 0.20 mmol, 2.0 equiv) dissolved in THF (0.45 mL) was added. The mixture was warmed to –10 °C and stirred for 1 h and then cooled to –78 °C. Ketone

3.24 (31 mg, 87 μmol, 1.0 equiv) dissolved in THF (0.45 mL) was added and then the mixture was stirred for 1 h. The excess lithiated dithiane was quenched by the addition of saturated $NH_4Cl_{(aq)}$ and then diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:4 EtOAc–hexane) to give **3.22** (4.5 mg, 6%) as a transparent colorless film. $[\alpha]_D^{21}$ –5.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.37–7.20 (m, 17 H, ArH), 6.88–6.86 (m, 2 H, ArH), 4.90–4.89 (m, 1 H, H-9), 4.83 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.80 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.63–4.59 (m, 4 H, benzylic H), 4.54 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.41 (d, *J* = 10.5 Hz, 1 H, benzylic H), 4.33 (m, 1 H, H-1), 3.97–3.94 (m, 1 H, H-11), 3.83–3.80 (m, 4 H, H-10 and OCH_3), 3.64–3.63 (m, 1 H, H-5), 1.24–1.19 (m, 3 H, isopropyl CH), 1.12–1.08 (m, 18 H, isopropyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 159.2, 139.09, 139.89, 138.8, 129.9, 129.5, 128.2, 128.13, 128.11, 128.06, 127.7, 127.6, 127.43, 127.36, 127.2, 113.8, 104.0 (C-1), 86.2 (C-10), 75.7 (C-5), 75.2 (C-11), 72.7 (benzylic C), 72.6 (benzylic C), 72.2 (C-9), 71.2 (benzylic C), 70.0 (benzylic C), 55.3 (OCH_3), 29.7, 18.6, 18.5, 18.2, 17.1, 13.3; IR (cast film): 3500, 3031, 2940, 2865, 1731, 1613, 1514, 1454, 1366, 1249, 1109, 1041 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{53}H_{74}NaO_8S_2Si$, 953.4487; found, 953.4487.



4-Methoxybenzyl 2-*O*-benzyl-3,6-dideoxy-4-*C*-((3*S*,4*R*,5*R*)-4,5-bis(benzyloxy)-3-((triisopropylsilyl)oxy)hex-1-yn-1-yl)-β-D-xylopyranoside (3.23). Diisopropylamine (13 μL, 91 μmol, 4.0 equiv) and LiCl (3.9 mg, 91 μmol, 4.0 equiv) were dissolved in THF (0.1 mL). The mixture was cooled to –78 °C then *n*-butyllithium (1.52 M in hexanes, 60 μL, 87 μmol, 3.8 equiv) was added and then the mixture was stirred at –78 °C for 30 min. Alkyne **3.25** (43 mg, 91 μmol, 4.0 equiv) dissolved in THF (0.1 mL) was added to the mixture and then the solution was stirred at –10 °C for 30 min. The mixture was then cooled to –78 °C before ketone **3.24** (8.0 mg, 23 μmol, 1.0 equiv) dissolved in THF (0.1 mL) was added. The mixture was stirred for 1 h then the excess lithiated alkyne was quenched by the addition of saturated $NH_4Cl_{(aq)}$ and then diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:3 EtOAc–hexane) to give **3.23** (12 mg, 60%) as a transparent colorless film. $[\alpha]_D^{21}$ –30.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.38–7.37 (m, 2 H, ArH), 7.33–7.24 (m, 15 H, ArH), 6.88–6.87 (m, 2 H, ArH), 4.97 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.91 (d, *J* = 3.5 Hz, 1 H, H-9), 4.88 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.81 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.70 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.62–4.58 (m, 3 H, benzylic H), 4.43 (d, *J* = 8.4 Hz, 1 H, H-1), 4.42 (d, *J* = 11.2 Hz, 1 H, benzylic H), 3.81 (s, 3 H, OCH_3), 3.65–3.62 (m, 1 H, H-11), 3.58–3.51 (m, 3 H, H-10, H-2 and H-5), 2.42 (dd, *J* = 13.3, 4.9 Hz, 1 H, H-3eq), 2.01 (br s, 1 H, 4-OH), 1.74 (dd, *J* =

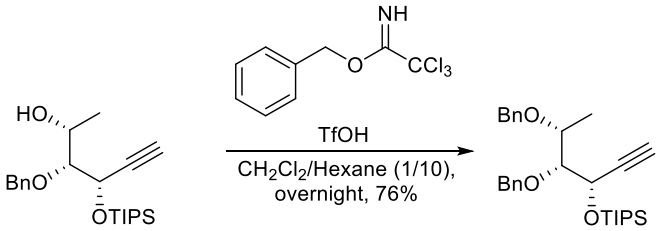
13.3, 11.9 Hz, 1 H, H-3ax), 1.28 (d, J = 6.3 Hz, 3 H, H-12), 1.26 (d, J = 6.3 Hz, 3 H, H-6), 1.15–1.06 (m, 21 H, isopropyl CH and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 159.2, 139.0, 138.6, 138.5, 130.0, 129.5, 128.30, 128.27, 128.1, 127.73, 127.71, 127.70, 127.5, 127.3, 113.8, 104.0 (C-1), 85.5 (C-10), 85.2 (C≡C), 84.6 (C≡C), 76.3 (C-5), 75.0 (C-11), 74.3 (benzylic C), 73.05 (benzylic C), 72.97 (C-2), 70.9 (benzylic C), 70.2 (benzylic C), 68.8 (C-4), 65.5 (C-9), 55.2 (OCH_3), 42.8 (C-3), 18.1 (isopropyl CH_3), 18.0 (isopropyl CH_3), 15.9 (C-12), 14.6 (C-6), 12.2 (isopropyl CH); IR (cast film): 3500, 3030, 2941, 2866, 1613, 1515, 1455, 1368, 1250, 1110, 1076 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{50}H_{66}NaO_8Si$, 845.4419; found, 845.4419.



3.30 **3.24**

4-Methoxybenzyl 2-*O*-benzyl-3,6-dideoxy-β-D-*erythro*-hex-4-ulo-pyranoside (3.24). Pyranone **3.30** (3.4 g, 14 mmol, 1.0 equiv) was dissolved in benzyl alcohol (7.1 mL, 68 mmol, 5.0 equiv) and then K_2CO_3 (0.19 g, 1.4 mmol, 0.10 equiv) was added. The mixture was stirred at room temperature overnight at which point TLC (1:6 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and then transferred to a separatory funnel. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:15 EtOAc–toluene) to give **3.24** (1.93 g, 40%) as a transparent colorless oil. $[\alpha]_D^{21}$ –20.2 (*c* 3.0, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ): 7.35–7.27 (m, 7 H, ArH), 6.91–6.89 (m, 2 H, ArH), 4.89 (d, J = 4.2 Hz, 1 H, H-1), 4.88 (d, J = 11.9 Hz, 1 H, benzylic H), 4.64 (d, J = 11.9 Hz, 1 H, benzylic H), 4.61 (d, J = 11.9 Hz, 1 H, benzylic H), 4.58 (d, J = 11.9 Hz, 1 H, benzylic H), 4.09 (q, J = 7.0 Hz, 1 H, H-5), 3.92 (ddd, J = 5.6, 4.9, 4.2 Hz, 1

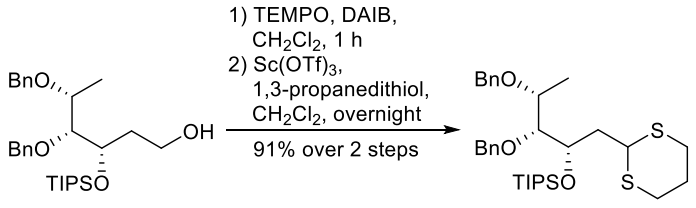
H, H-2), 3.82 (s, 3 H, OCH_3), 2.92 (dd, J = 9.1, 4.9 Hz, 1 H, H-3), 2.56 (dd, J = 9.1, 5.6 Hz, 1 H, H-3), 1.40 (d, J = 7.0 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 208.0 (ketone C=O), 159.4, 137.6, 129.6, 129.2, 128.4, 127.8, 127.7, 113.9, 100.2 (C-1), 76.2 (C-2), 75.6 (C-4), 71.6 (benzylic C), 70.0 (benzylic C), 55.2 (OCH_3), 40.7 (C-3), 16.5 (C-6); IR (cast film): 3031, 2937, 2870, 1731, 1613, 1514, 1455, 1367, 1249, 1064 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{21}H_{24}NaO_5$, 379.1516; found, 379.1517.



3.36 **3.25**

(((3*S*,4*R*,5*R*)-4,5-bis(benzyloxy)hex-1-yn-3-yl)oxy)triisopropylsilane (3.25). Alcohol **3.36** (96 mg, 0.26 mmol, 1.0 equiv) was dissolved in hexane (4.5 mL) and then benzyl 2,2,2-trichloroacetimidate (0.24 mL, 1.3 mmol, 5.0 equiv) was added. Next, a solution of triflic acid (4.5 μL, 52 μmol, 0.20 equiv) in CH_2Cl_2 (0.45 mL) was added then the mixture was stirred at room temperature overnight at which point TLC (1:6 EtOAc–hexane) indicated no **3.36** remained. The mixture was diluted with hexane and transferred to a separatory funnel. The organic layer was washed with 1 N $HCl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, toluene) to give **3.25** (91 mg, 76%) as a transparent colorless oil. $[\alpha]_D^{21}$ +18.8 (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.41–7.39 (m, 2 H, ArH), 7.33–7.30 (m, 6 H, ArH), 7.28–7.25 (m, 2 H, ArH), 5.03 (d, J = 11.4 Hz, 1 H, benzylic H), 4.94 (dd, J = 3.0, 2.4 Hz, 1 H, H-3), 4.73 (d, J = 11.4 Hz, 1 H, benzylic H), 4.60 (d, J = 11.4 Hz, benzylic H), 4.40 (d, J = 11.4 Hz,

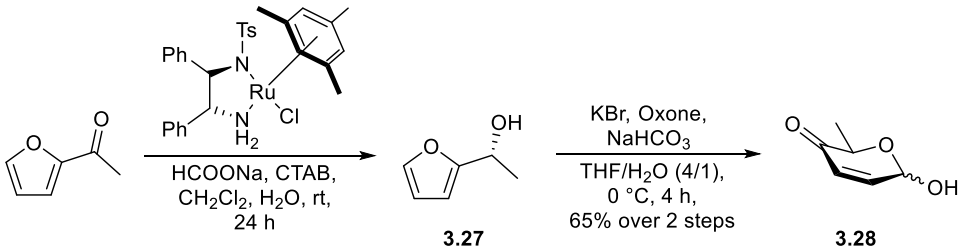
1 H, benzylic H), 3.74–3.70 (m, 1 H, H-5), 3.60 (dd, *J* = 7.2, 3.0 Hz, 1 H, H-4), 2.41 (d, *J* = 2.4 Hz, 1 H, H-1), 1.27 (d, *J* = 6.0 Hz, 3 H, H-6), 1.19–1.12 (m, 3 H, isopropyl CH), 1.11–1.06 (m, 18 H, isopropyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.8, 128.5, 128.3, 128.2, 127.7, 127.5, 127.4, 85.3 (C-4), 82.8 (C-2), 75.1 (C-5), 74.7 (benzylic C), 74.0 (C-1), 71.2 (benzylic C), 65.6 (C-3), 18.06 (isopropyl CH_3), 18.05 (isopropyl CH_3), 16.0 (C-6), 12.2 (isopropyl CH); IR (cast film): 3309, 3030, 2944, 2866, 1603, 1454, 1369, 1111, 1064 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{42}NaO_3Si$, 489.2795; found, 489.2789.



3.37 **3.26**

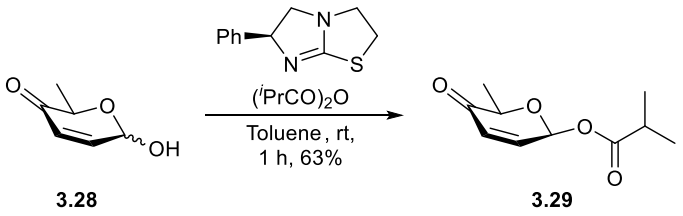
(((2*S*,3*R*,4*R*)-3,4-bis(benzyloxy)-1-(1,3-dithian-2-yl)pentan-2-yl)oxy)triisopropylsilane (3.26). Alcohol **3.37** (0.48 g, 0.99 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (2.0 mL) before (diacetoxyiodo)benzene (0.35 g, 1.1 mmol, 1.1 equiv) and TEMPO (15 mg, 99 μmol, 0.10 equiv) were added. The mixture was stirred at room temperature for 1 h then diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $Na_2S_2O_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The obtained crude aldehyde was dissolved in CH_2Cl_2 (10 mL) and then 1,3-propanedithiol (0.12 mL, 1.2 mmol, 1.2 equiv) and $Sc(OTf)_3$ (19 mg, 39 μmol, 0.04 equiv) were added. The mixture was stirred at room temperature overnight until TLC (1:5 EtOAc–hexane) indicated no aldehyde remained. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with 1 M $NaOH_{(aq)}$, H_2O, brine, dried over

anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:5 EtOAc–hexane) to give **3.26** (0.52 g, 91%) as a transparent colorless oil. $[\alpha]_D^{21}$ +4.2 (*c* 0.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.38–7.25 (m, 10 H, ArH), 4.88 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.66 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.58 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.55–4.52 (m, 1 H, H-2), 4.49 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.21 (dd, *J* = 6.6, 6.0 Hz, 1 H, SCHS), 3.66–3.61 (m, 1 H, H-4), 3.57 (dd, *J* = 7.2, 2.4 Hz, 1 H, H-3), 2.83–2.73 (m, 4 H, dithiane CH_2), 2.12–2.05 (m, 3 H, H-1 and dithiane CH_2), 1.89–1.84 (m, 1 H, dithiane CH_2), 1.30 (d, *J* = 6.0 Hz, 3 H, H-5), 1.15–1.06 (m, 21 H, isopropyl CH and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.9, 138.6, 128.21, 128.16, 127.82, 127.76, 127.4, 127.3, 86.4 (C-3), 75.4 (C-4), 74.2 (benzylic C), 71.1 (benzylic C), 70.4 (C-2), 44.4 (SCHS), 39.5 (C-1), 30.5 (dithiane C), 30.1 (dithiane C), 25.9 (dithiane C), 18.31 (isopropyl CH_3), 18.30 (isopropyl CH_3), 16.4 (C-5), 13.0 (isopropyl CH); IR (cast film): 3030, 2943, 2866, 1464, 1390, 1110, 1056 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{32}H_{50}NaO_3S_2Si$, 597.2863; found, 597.2863.



6-Hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one (3.28). The procedure for synthesizing **3.27** was carried out as reported[23] using 2-acetylfuran (3.62 g), sodium formate (11 g), RuCl[(*R*,*R*)-TsDPEN](mesitylene) (60.8 mg) and cetyltrimethylammonium bromide (1.18 g), CH_2Cl_2 (0.4 mL) in H_2O (53.4 mL). The obtained crude compound **3.27** was dissolved in THF (66 mL) and H_2O

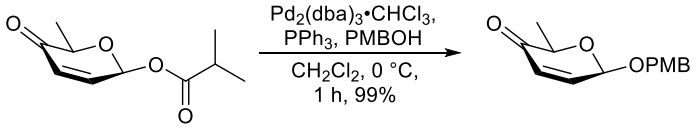
(17 mL), and then the mixture was cooled to 0 °C. Next, Oxone® (12 g, 39 mmol, 1.2 equiv), $NaHCO_3$ (1.4 g, 16 mmol, 0.50 equiv) and KBr (0.20 g, 1.7 mmol, 0.05 equiv) were added and then the mixture was stirred for 30 min. The mixture was decanted into a separatory funnel and diluted with EtOAc. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:6→1:3 EtOAc–hexane) to give **3.28** (2.75 g, 65%) as a transparent orange oil. The ^1H and ^{13}C NMR data for **3.28** were identical to those reported.[39]



3.28 3.29

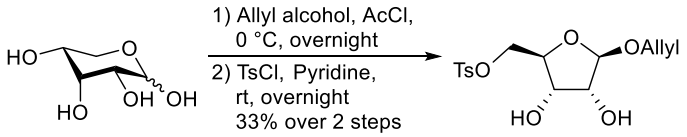
(2*S*,6*R*)-6-Methyl-5-oxo-5,6-dihydro-2*H*-pyran-2-yl isobutyrate (3.29). Pyranone **3.28** (2.8 g, 22 mmol, 1.0 equiv) was dissolved in toluene (14 mL) and then isobutyric anhydride (6.0 mL, 36 mmol, 1.6 equiv) and (–)-tetramisole[28] (0.22 g, 1.1 mmol, 0.05 equiv) was added. The mixture was stirred at room temperature for 1 h at which point TLC (1:3 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:9→1:6 EtOAc–hexane) to give **3.29** (2.73 g, 63%) as a transparent colorless oil. $[\alpha]_D^{21}$ +110.5 (*c* 0.7, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ): 6.86 (dd, *J* = 10.5, 2.8 Hz, 1 H, H-3), 6.55 (dd, *J* = 2.8, 0.7 Hz, 1 H, H-1), 6.21 (dd, *J* = 10.5, 0.7 Hz, 1 H, H-2), 4.37 (q, *J* = 7.0 Hz, 1 H, H-5), 2.60 (septet, *J* = 7.0 Hz, 1 H, isopropyl

CH), 1.49 (d, J = 7.0 Hz, 3 H, H-6), 1.21 (d, J = 7.0 Hz, 3 H, isopropyl CH_3), 1.20 (d, J = 7.0 Hz, 3 H, isopropyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 195.9 (ketone C=O), 175.3 (ester C=O), 143.4 (C-4), 128.1 (C-3), 87.6 (C-2), 75.7 (C-6), 34.0 (isopropyl CH), 18.64 (C-6), 18.60 (isopropyl CH_3); IR (cast film): 3060, 2981, 2940, 2879, 1750, 1703, 1634, 1471, 1387, 1106, 1050 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{10}H_{14}NaO_4$, 221.0784; found, 221.0784.



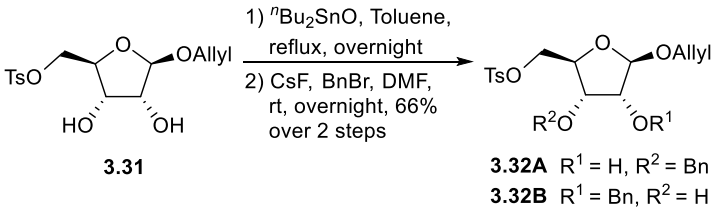
3.29 **3.30**

(2*R*,6*R*)-6-((4-Methoxybenzyl)oxy)-2-methyl-2*H*-pyran-3(6*H*)-one (3.30). Pyranone **3.29** (2.7 g, 14 mmol, 1.0 equiv) and 4-methoxybenzyl alcohol (3.4 mL, 28 mmol, 2.0 equiv) was dissolved in CH_2Cl_2 (14 mL) then the mixture was cooled to 0 °C. Next, $Pd_2(dba)_3$•$CHCl_3$ (0.36 g, 0.34 mmol, 0.025 equiv) and PPh_3 (0.36 g, 1.4 mmol, 0.1 equiv) were added then the reaction was stirred at 0 °C for 1 h. The mixture was concentrated on a rotary evaporator and then the crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:10 EtOAc–hexane) to give **3.30** (3.4 g, 99%) as a transparent colorless oil. $[\alpha]_D^{21}$ –58.2 (c 0.2, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ): 7.32–7.30 (m, 2 H, ArH), 6.91–6.89 (m, 2 H, ArH), 6.88 (dd, J = 10.5, 1.4 Hz, 1 H, H-3), 6.12 (dd, J = 10.5, 1.4 Hz, 1 H, H-2), 5.38–5.37 (m, 1 H, H-1), 4.87 (d, J = 11.9 Hz, 1 H, benzylic H), 4.62 (d, J = 11.9 Hz, 1 H, benzylic H), 4.23 (qd, J = 7.0, 0.7 Hz, 1 H, H-5), 3.81 (s, 3 H, OCH_3), 1.52 (d, J = 7.0 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 196.9 (ketone C=O), 159.6, 146.7 (C-3), 129.9, 128.9, 128.1 (C-2), 114.0, 94.0 (C-1), 75.3 (C-5), 69.8 (benzylic C), 55.3 (OCH_3), 17.3 (C-6); IR (cast film): 3051, 2938, 2837, 1699, 1613, 1515, 1464, 1374, 1250, 1057 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{14}H_{16}NaO_4$, 271.0941; found, 271.0943.



3.31

Allyl 5-*O*-tosyl-β-D-ribofuranoside (3.31). Ribose (18 g, 0.12 mol, 1.0 equiv) was suspended in allyl alcohol (300 mL) and then the mixture was cooled to 0 °C before acetyl chloride (6.0 mL, 84 mmol, 0.70 equiv) was added. The reaction was stirred at 0 °C overnight at which point TLC (9:1 CH_2Cl_2–CH_3OH) indicated no further progression of the reaction. The mixture was concentrated on a rotary evaporator before pyridine (180 mL) and *p*-toluenesulfonyl chloride (34 g, 0.18 mol, 1.5 equiv) were added. The mixture was stirred at room temperature overnight at which point TLC (20:1 CH_2Cl_2–CH_3OH) indicated no further progression of the reaction. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, hexane→50:1 CH_2Cl_2–CH_3OH) to give **3.31** (13.5 g, 33%) as a transparent yellow oil. $[\alpha]_D^{21}$ –34.0 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.80–7.79 (m, 2 H, ArH), 7.35–7.33 (m, 2 H, ArH), 5.82–5.75 (m, 1 H, allyl H), 5.22–5.18 (m, 1 H, allyl H), 5.16–5.13 (m, 1 H, allyl H), 4.94 (s, 1 H, H-1), 4.28–4.25 (m, 1 H, H-3), 4.19–4.15 (m, 1 H, H-5), 4.11–4.02 (m, 4 H, H-5, H-4, allyl H and H-2), 3.86–3.82 (m, 1 H, allyl H), 3.23 (m, 2 H, 2-OH and 3-OH), 2.44 (s, 3 H, $ArCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 145.1, 133.6 (allyl C), 132.6, 129.9, 128.0, 117.6 (allyl C), 106.1 (C-1), 80.3 (C-4), 74.9 (C-2), 72.1 (C-3), 70.4 (C-5), 68.2 (allyl C), 21.6 ($ArCH_3$); IR (cast film): 3447, 3085, 2928, 2878, 1598, 1451, 1360, 1177, 1097 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{15}H_{20}NaO_7S$, 367.0822; found, 367.0821.

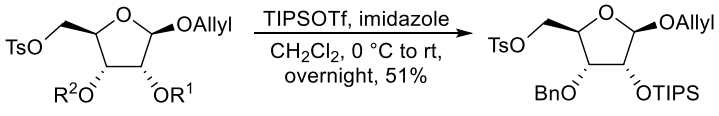


3.31

3.32A R^1 = H, R^2 = Bn
3.32B R^1 = Bn, R^2 = H

Allyl 3-*O*-benzyl-5-*O*-tosyl-β-D-ribofuranoside (3.32A) and **Allyl 2-*O*-benzyl-5-*O*-tosyl-β-D-ribofuranoside (3.32B).** Riboside **3.31** (14 g, 39 mmol, 1.0 equiv) and dibutyltin(IV) oxide (12 g, 47 mmol, 1.2 equiv) was suspended in toluene (260 mL). The flask was fitted with a Dean–Stark apparatus and then the mixture was heated and stirred at reflux overnight. The mixture was cooled to room temperature, concentrated on a rotary evaporator and dried on high vacuum for 2 h. Next, CsF (12 g, 78 mmol, 2.0 equiv) and DMF (100 mL) were added followed by benzyl bromide (10 mL, 86 mmol, 2.2 equiv). The mixture was sonicated and then heated and stirred at 70 °C overnight. Over the course of the reaction, the mixture dissolved and later a white suspension gradually formed. The mixture was cooled to room temperature and then diluted with EtOAc (200 mL). The mixture was filtered over a pad of Celite® 545 then transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 500 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:2 EtOAc–hexane) to give an inseparable mixture of **3.32A** and **3.32B** (11.3 g, 66%) as a transparent yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.81–7.78 (m, 4 H, ArH), 7.39–7.29 (m, 14 H, ArH), 5.82–5.75 (m, 2 H, OCH_2CH=CH_2), 5.23–5.19 (m, 2 H, OCH_2CH=CH_2), 5.17–5.15 (m, 2 H, OCH_2CH=CH_2), 4.98 (s, 1 H, BH-1), 4.96 (s, 1 H, AH-1), 4.71 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.61 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.55 (m, 2 H, benzylic H), 4.23–4.15 (m, 3 H, BH-5, AH-3 and BH-3), 4.11–4.01 (m, 8 H, BH-4, BH-5, 2 × AH-5, AH-4, AH-2 and 2 × allylic H), 3.86 (d, *J* = 4.8 Hz, 1 H, BH-2), 3.84–3.81 (m, 2 H, allylic H), 2.57 (d, *J* = 2.4 Hz, 1 H, A2-OH), 2.56 (d, *J* = 9.6 Hz, 1 H, B3-OH), 2.44 (s, 6 H, $ArCH_3$); ^{13}C

NMR (125 MHz, $CDCl_3$, δ): 144.9, 144.8, 136.8, 136.7, 133.7 ($OCH_2CH{=}CH_2$), 133.6 ($OCH_2CH{=}CH_2$), 132.89, 132.87, 129.9, 129.8, 128.7, 128.64, 128.60, 128.4, 128.3, 128.00, 127.98, 127.96, 127.6, 127.0, 117.7 ($OCH_2CH{=}CH_2$), 117.6 ($OCH_2CH{=}CH_2$), 106.5 (AC-1), 103.5 (BC-1), 81.6 (BC-2), 81.4 (AC-4), 79.3 (BC-4), 78.8 (AC-3), 73.10 (AC-2), 73.06 (benzylic C), 72.98 (benzylic C), 71.3 (BC-3), 70.3 (BC-5), 70.0 (AC-5), 68.2 (allylic C), 68.1 (allylic C), 21.6 ($ArCH_3$); HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{22}H_{26}NaO_7S$, 457.1291; found, 457.1291.

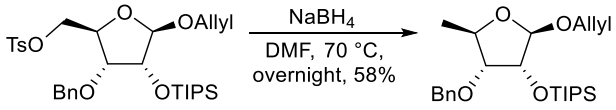


3.32A R^1 = H, R^2 = Bn
3.32B R^1 = Bn, R^2 = H

3.33

Allyl 3-*O*-benzyl-2-*O*-triisopropylsilyl-5-*O*-tosyl-β-D-ribofuranoside (3.33). A mixture of riboside **3.32A** and **3.32B** (11 g, 26 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (260 mL) and then the mixture was cooled to 0 °C. Triisopropylsilyl trifluoromethanesulfonate (8.4 mL, 31 mmol, 1.2 equiv) and imidazole (5.2 g, 78 mmol, 3.0 equiv) were added and the mixture was stirred at room temperature overnight at which point TLC (1:2 EtOAc–hexane) indicated no **3.32A** or **3.32B** remained. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:15 EtOAc–hexane) to give **3.33** (7.74 g, 51%) as a transparent colorless oil. $[\alpha]_D^{21}$ +25.1 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 7.78–7.77 (m, 2 H, ArH), 7.34–7.26 (m, 7 H, ArH), 5.84–5.74 (m, 1 H, $OCH_2CH{=}CH_2$), 5.24–5.20 (m, 1 H, $OCH_2CH{=}CH_2$), 5.17–5.15 (m, 1 H, $OCH_2CH{=}CH_2$), 4.86 (s, 1 H, H-1), 4.62 (d, *J* = 11.5 Hz, 1

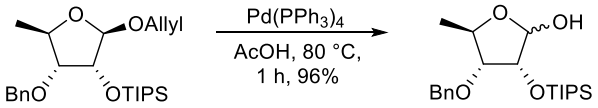
H, benzylic H), 4.37 (d, J = 11.5 Hz, 1 H, benzylic H), 4.28 (d, J = 4.0 Hz, 1 H, H-2), 4.23 (ddd, J = 7.5, 5.5, 3.0 Hz, 1 H, H-4), 4.15 (dd, J = 10.5, 3.0 Hz, 1 H, H-5), 4.08–4.04 (m, 1 H, allylic H), 3.99 (dd, J = 10.5, 5.5 Hz, 1 H, H-5), 3.95 (dd, J = 7.5, 4.0 Hz, 1 H, H-3), 3.85–3.81 (m, 1 H, allylic H), 2.42 (s, 3 H, $ArCH_3$), 1.09–1.03 (m, 21 H, isopropyl CH and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 144.7, 137.6, 133.9 ($OCH_2CH=CH_2$), 133.0, 139.8, 128.3, 128.0, 127.8, 127.7, 117.6 ($OCH_2CH=CH_2$), 106.5 (C-1), 78.8 (C-3), 77.9 (C-4), 74.4 (C-2), 72.7 (benzylic C), 70.1 (C-5), 68.0 (allylic C), 21.6 ($ArCH_3$), 17.9 (isopropyl CH_3), 12.5 (isopropyl CH); IR (cast film): 3032, 2943, 2867, 1599, 1464, 1367, 1178, 1097 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{31}H_{46}NaO_7SSi$, 613.2626; found, 613.2621.



3.33 **3.34**

Allyl 3-*O*-benzyl-5-deoxy-2-*O*-triisopropylsilyl-β-D-ribofuranoside (3.34). Riboside **3.33** (7.7 g, 13 mmol, 1.0 equiv) was dissolved in DMF (130 mL) before $NaBH_4$ (2.0 g, 52 mmol, 4.0 equiv) was added. The mixture was heated to 70 °C and stirred overnight at which point TLC (1:10 EtOAc–hexane) indicated no **3.33** remained. The mixture was diluted with diethyl ether and then the excess $NaBH_4$ was quenched by the addition of saturated $NH_4Cl_{(aq)}$ before being transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 500 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude reside was purified by column chromatography (300 mL silica gel, 0:1→1:20 EtOAc–hexane) to give **3.34** (4.3 g, 78%) as a transparent colorless oil. $[\alpha]_D^{21}$ +216.0 (c 0.03, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.35–7.27 (m, 5 H, ArH), 5.92–5.85 (m, 1 H, $OCH_2CH=CH_2$), 5.28–5.25 (m, 1

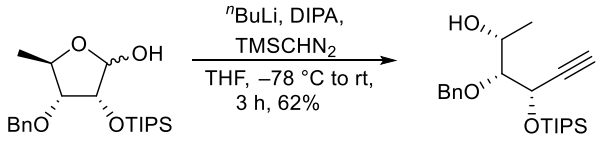
H, $OCH_2CH=CH_2$), 5.18–5.16 (m, 1 H, $OCH_2CH=CH_2$), 4.88 (s, 1 H, H-1), 4.71 (d, J = 11.4 Hz, 1 H, benzylic H), 4.48 (d, J = 11.4 Hz, 1 H, benzylic H), 4.32 (d, J = 4.2 Hz, 1 H, H-2), 4.24–4.18 (m, 2 H, H-4 and allylic H), 3.97–3.94 (m, 1 H, allylic H), 3.72 (dd, J = 7.2, 4.2 Hz, 1 H, H-3), 1.28 (d, J = 6.6 Hz, 3 H, H-5), 1.13–1.07 (m, 21 H, isopropyl CH and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.2, 134.4 ($OCH_2CH=CH_2$), 128.3, 127.7, 127.6, 117.2 ($OCH_2CH=CH_2$), 106.6 (C-1), 84.2 (C-3), 76.7 (C-4), 75.2 (C-2), 72.5 (benzylic C), 68.2 (allylic C), 20.4 (C-5), 18.0 (isopropyl CH_3), 12.5 (isopropyl CH); IR (cast film): 3030, 2943, 2867, 1464, 1378, 1170, 1065, 1015 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{24}H_{40}NaO_4Si$, 443.2588; found, 443.2591.



3.34 **3.35**

3-*O*-Benzyl-5-deoxy-2-*O*-triisopropylsilyl-D-ribofuranose (3.35). Riboside **3.34** (4.3 g, 10 mmol, 1.0 equiv) and $Pd(PPh_3)_4$ (5.9 g, 5.0 mmol, 0.50 equiv) were added to a flask that was then purged with argon. Acetic acid (68 mL) was added and then the mixture was heated to 80 °C and stirred for 1 h. After cooing to room temperature, the mixture was dried by dissolving in toluene and evaporation (3 × 100 mL) and the resulting residue was then purified by column chromatography (300 mL silica gel, 0:1→1:12→1:6 EtOAc–hexane) to give **3.35** (3.8 g, 96%, α/β = 2/5) as a transparent yellow film. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.36–7.27 (m, 10 H, ArH), 5.21 (d, J = 3.0 Hz, 1 H, βH-1), 5.14 (dd, J = 10.2, 4.2 Hz, 1 H, αH-1), 4.78 (d, J = 12.0 Hz, 1 H, benzylic H), 4.72 (d, J = 12.0 Hz, 1 H, benzylic H), 4.62 (d, J = 12.0 Hz, 1 H, benzylic H), 4.49 (d, J = 12.0 Hz, 1 H, benzylic H), 4.36 (qd, J = 6.6, 1.8 Hz, 1 H, αH-4), 4.29 (d, J = 4.2 Hz, 1 H,

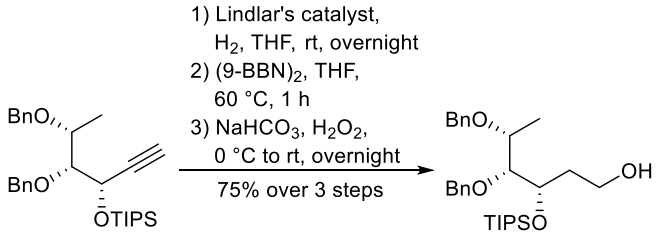
βH-2), 4.27 (dd, *J* = 4.8, 4.2 Hz, 1 H, αH-2), 4.22 (dq, *J* = 6.6, 6.0 Hz, 1 H, βH-4), 4.20 (d, *J* = 10.2 Hz, 1 H, α1-OH), 3.73 (dd, *J* = 6.6, 4.2 Hz, 1 H, βH-3), 3.55 (dd, *J* = 4.8, 1.8 Hz, 1 H, αH-3), 2.72 (d, *J* = 3.0 Hz, 1 H, β1-OH), 1.30 (d, *J* = 6.0 Hz, 1 H, βH-5), 1.18 (d, *J* = 6.6 Hz, 1 H, αH-5), 1.17–1.08 (m, 42 H, isopropyl CH and CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.1, 137.7, 128.4, 128.3, 128.0, 127.71, 127.65, 127.6, 102.4 (βC-1), 97.2 (αC-1), 83.6 (βC-3), 82.9 (αC-3), 77.7 (αC-4), 76.6 (βC-4), 73.1 (benzylic C), 72.8 (αC-2), 72.5 (benzylic C), 20.4 (αC-5), 20.2 (βC-5), 17.99 (isopropyl CH_3), 17.96 (isopropyl CH_3), 17.91 (isopropyl CH_3), 12.5 (isopropyl CH), 12.2 (isopropyl CH); HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{21}H_{36}NaO_4Si$, 403.2275; found, 403.2271.



3.35 **3.36**

(2*R*,3*R*,4*S*)-3-(Benzyloxy)-4-((triisopropylsilyl)oxy)hex-5-yn-2-ol (3.36). Diisopropylamine (3.8 mL, 27 mmol, 2.6 equiv) and THF (50 mL) was added to a flame dried flask and then the mixture was cooled to –78 °C. *n*-Butyllithium (1.4 M in hexane, 19 mL, 27 mmol, 2.6 equiv) was added and then the mixture was stirred at –78 °C for 30 min. Trimethylsilyldiazomethane (2.0 M in hexane, 6.7 mL, 13 mmol, 1.3 equiv) was then added and the mixture was stirred at –78 °C for 30 min. Next, **3.35** (3.9 g, 10 mmol, 1.0 equiv) dissolved in THF (50 mL) was added and then the cooling bath was removed. The mixture was stirred for 3 h while warming to room temperature, then the excess base was quenched by the addition of saturated $NH_4Cl_{(aq)}$. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on

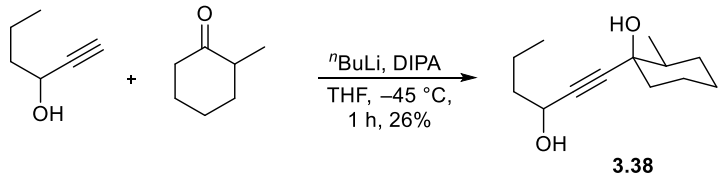
a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:9→1:6 EtOAc–hexane) to give **3.36** (2.1 g, 55%) as a transparent colorless oil. $[\alpha]_D^{21}$ +49.2 (*c* 0.4, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ): 7.29–7.27 (m, 5 H, ArH), 4.97 (d, *J* = 10.8 Hz, 1 H, benzylic H), 4.77 (dd, *J* = 4.2, 1.8 Hz, 1 H, H-4), 4.65 (d, *J* = 10.8 Hz, 1 H, benzylic H), 3.99 (dqd, *J* = 6.6, 6.0, 5.4 Hz, 1 H, H-2), 3.48 (dd, *J* = 6.6, 4.2 Hz, 1 H, H-3), 2.51 (d, *J* = 1.8 Hz, H-6), 2.24 (d, *J* = 5.4 Hz, 1 H, 2-OH), 1.26 (d, *J* = 6.0 Hz, 1 H, H-1), 1.23–1.17 (m, 3 H, isopropyl CH), 1.13–1.10 (m, 18 H, isopropyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.3, 128.3, 128.1, 127.7, 86.2 (C-3), 83.3 (C-5), 74.5 (benzylic C), 74.4 (C-6), 68.5 (C-2), 65.3 (C-4), 19.4 (C-1), 18.0 (isopropyl CH_3), 12.3 (isopropyl CH); IR (cast film): 3447, 3309, 3032, 2944, 2867, 1497, 1463, 1385, 1102, 1063 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{22}H_{36}NaO_3Si$, 399.2326; found, 299.2327.



3.24 **3.37**

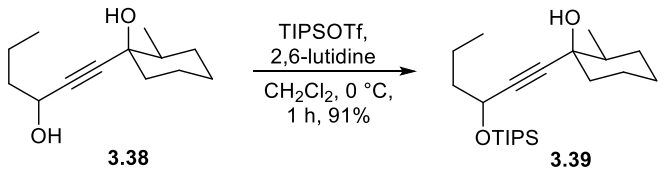
(3*S*,4*R*,5*R*)-4,5-Bis(benzyloxy)-3-((triisopropylsilyl)oxy)hexan-1-ol (3.37). Alkyne **3.24** (0.19 g, 0.42 mmol, 1.0 equiv) was dissolved in THF (4.2 mL) and then Lindlar's catalyst (5% Pd on $CaCO_3$, poisoned with lead, 89 mg) was added. The flask was fitted with a H_2 balloon and purged three times with H_2. The mixture was stirred at room temperature overnight at which point TLC (1:20 EtOAc–hexane) indicated no **3.24** remained. The mixture was purged three times with argon before being filtered through a pad of Celite® 545. The mixture was concentrated on a rotary evaporator and dried on high vacuum for 2 h. Next, the obtained crude alkene was dissolved in

THF (4.2 mL) before 9-borabicyclo[3.3.1]nonane dimer (0.26 g, 1.0 mmol, 2.5 equiv) was added. The mixture was heated to reflux and stirred for 1 h. After which time, the mixture was cooled to 0 °C before saturated $NaHCO_{3(aq)}$ (1.0 mL) and H_2O_2 (30% in H_2O, 0.5 mL) was added. The cooling bath was removed and then the mixture was stirred at room temperature overnight while warming to room temperature; at this point TLC (1:5 EtOAc–hexane) indicated no borane remained. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (100 mL silica gel, 0:1→1:10→1:5 EtOAc–hexane) to give **3.37** (0.14 g, 75%) as a transparent colorless film. $[\alpha]_D^{21}$ –14.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.36–7.26 (m, 10 H, ArH), 4.98 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.69 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.63 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.47–4.45 (m, 1 H, H-3), 4.39 (d, *J* = 11.2 Hz, 1 H, benzylic H), 3.91–3.87 (m, 1 H, H-1), 3.65–3.58 (m, 3 H, H-5, H-4 and H-1), 3.07 (br s, 1 H, 1-OH), 1.84–1.82 (m, 2 H, H-2), 1.31 (d, *J* = 5.6 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 138.4, 138.1, 128.4, 128.3, 128.1, 127.8, 127.6, 127.5, 86.5 (C-4), 75.6 (benzylic C), 75.1 (C-5), 71.2 (C-3), 70.7 (benzylic C), 57.9 (C-1), 35.4 (C-2), 18.23 (isopropyl CH_3), 18.18 (isopropyl CH_3), 16.4 (C-6), 12.6 (isopropyl CH); IR (cast film): 3448, 3032, 2944, 2867, 1497, 1454, 1383, 1109, 1066 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{46}NaO_4Si$, 509.3058; found, 509.3057.

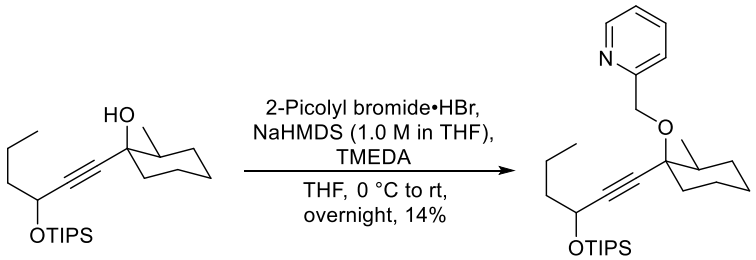


3.38

1-(3-Hydroxyhex-1-yn-1-yl)-2-methylcyclohexan-1-ol (3.38). Diisopropylamine (5.2 mL, 37 mmol, 4.2 equiv) was dissolved in THF (90 mL) and then the mixture was cooled to –45 °C. *n*-Butyllithium (1.6 M in hexane, 23 mL, 37 mmol, 4.2 equiv) was added and the mixture was stirred for 30 min before 1-hexyn-3-ol (2.0 mL, 18 mmol, 2.0 equiv) was added. The mixture was stirred for 30 min and then 2-methylcyclohexanone (1.1 mL, 9.0 mmol, 1.0 equiv) was added. After stirring for 1 h, TLC (1:1 EtOAc–hexane) indicated no further progression of the reaction. The excess base was quenched by the addition of saturated $NH_4Cl_{(aq)}$ and then the mixture was diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:6→1:3→1:2 EtOAc–hexane) to give **3.38** (0.50 g, 26%), a mixture of diastereomers as a transparent colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 4.42–4.39 (m, 1 H, propargylic H), 2.04–1.96 (m, 1 H), 2.04–1.96 (m, 5 H), 1.75–1.61 (m, 4 H), 1.04 (d, J = 7.2 Hz, 3 H, ring CH_3), 0.95 (dd, J = 7.2, 7.2 Hz, 3 H, chain CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 89.4, 84.1, 69.5, 62.28, 62.27, 40.51, 40.50, 39.99, 39.97, 39.3, 29.2, 25.0, 21.1, 18.5, 18.4, 16.1 (ring CH_3), 13.7 (chain CH_3); HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{13}H_{22}NaO_2$, 233.1512; found, 233.1512.



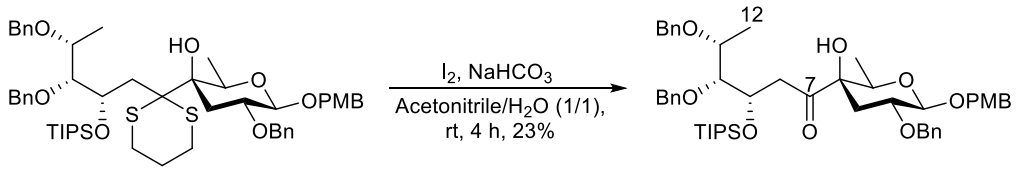
2-Methyl-1-(3-((triisopropylsilyl)oxy)hex-1-yn-1-yl)cyclohexan-1-ol (3.39). Diol **3.38** (0.50 g, 2.4 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (4.7 mL) and then the mixture was cooled to 0 °C. Triisopropylsilyl trifluoromethanesulfonate (0.76 mL, 2.8 mmol, 1.2 equiv) and 2,6-lutidine (0.41 mL, 3.5 mmol, 1.5 equiv) was added and then the mixture was stirred at 0 °C for 1 h. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:30 EtOAc–hexane) to give **3.39** (0.79 g, 91%), a mixture of diastereomers as a transparent colorless oil. ^{1}H NMR (700 MHz, $CDCl_3$, δ): 4.51–4.49 (m, 1 H, propargylic H), 1.98–1.95 (m, 1 H), 1.68–1.60 (m, 5 H), 1.59–1.43 (m, 5 H), 1.36–1.22 (m, 2 H), 1.16–1.10 (m, 3 H, isopropyl CH), 1.09–1.07 (m, 18 H, isopropyl CH_3), 1.04–1.03 (m, 3 H, ring CH_3), 0.93 (dd, J = 7.0, 7.0 Hz, 3 H, chain CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 88.31, 88.27, 84.92, 84.89, 69.52, 69.50, 62.9, 41.21, 41.20, 40.53, 40.51, 39.4, 39.3, 29.23, 29.21, 25.1, 21.1, 18.39, 18.37, 18.1 (isopropyl CH_3), 16.1 (ring CH_3), 13.9 (chain CH_3), 12.3 (isopropyl CH); HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{22}H_{42}NaO_2Si$, 389.2846; found, 389.2846.



3.39 **3.40**

2-(((2-Methyl-1-(3-((triisopropylsilyl)oxy)hex-1-yn-1-yl)cyclohexyl)oxy)methyl)pyridine (3.40). Alcohol **3.39** (60 mg, 0.16 mmol, 1.0 equiv) was dissolved in THF (0.8 mL) and the mixture was cooled to 0 °C. Sodium bis(trimethylsilyl)amide (1.0 M in THF, 0.49 mL, 0.49 mmol, 3.0 equiv) and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (30 μL, 0.16 mmol, 1.0 equiv) was added and then the mixture was stirred for 30 min before 2-picoloyl bromide•HBr (82 mg, 0.33 mmol, 2.0 equiv) was added and the cooling bath was removed. The mixture was stirred at room temperature overnight at which point TLC (1:10 EtOAc–hexane) indicated no further progression of the reaction. The excess base was quenched by the addition of saturated $NH_4Cl_{(aq)}$ and then the mixture was diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:10 EtOAc–hexane) to give **3.40** (10.3 mg, 14%), a mixture of diastereomers as a transparent colorless film. ^{1}H NMR (600 MHz, $CDCl_3$, δ): 8.51 (d, *J* = 5.4 Hz, 1 H, picolinyl H), 7.67 (dd, *J* = 7.8, 7.2 Hz, 1 H, picolinyl H), 7.52 (d, *J* = 7.8 Hz, 1 H, picolinyl H), 7.14 (dd, *J* = 7.2, 5.4 Hz, 1 H, picolinyl H), 4.80–4.76 (m, 1 H, benzylic H), 4.62 (d, *J* = 13.2 Hz, 1 H, benzylic H), 4.51–4.48 (m, 1 H, propargylic H), 2.22–2.20 (m, 1 H), 1.81–1.78 (m, 1 H), 1.65–1.61 (m, 3 H), 1.55–1.40 (m, 6 H), 1.33–1.28 (m, 2 H), 1.14–1.13 (m, 3 H, ring CH_3), 1.11–1.03 (m, 21 H, isopropyl CH and CH_3), 0.88 (dd, *J* = 7.2, 7.2 Hz, 3 H, chain CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 160.2, 148.5, 136.4, 121.8, 121.0, 87.49, 87.46, 85.2, 75.4, 66.02,

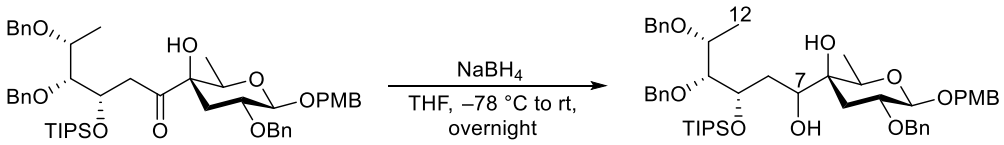
66.01, 62.9, 41.29, 41.27, 40.9, 34.69, 34.66, 29.4, 24.9, 21.1, 18.4, 18.3, 18.0 (isopropyl CH_3), 16.2 (ring CH_3), 13.9 (chain CH_3), 12.2 (isopropyl CH); HRMS–ESI–TOF (*m*/*z*): $[M+H]^+$ calcd for $C_{28}H_{48}NO_2Si$, 458.3449; found, 458.3446.



3.23 **3.41**

4-Methoxybenzyl 2-*O*-benzyl-3,6-dideoxy-4-*C*-(((2*S*,3*R*,4*R*)-3,4-bis(benzyloxy)-2-((triisopropylsilyl)oxy)pentyl)acetyl)-β-D-xylopyranoside (3.41). Dithiane **3.23** (2.9 mg, 3.1 μmol, 1.0 equiv) was dissolved in acetonitrile (30 μL) and saturated $NaHCO_{3(aq)}$ (30 μL) before iodine (3.2 mg, 13 μmol, 4.0 equiv) was added. The mixture was stirred for 4 h and diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with saturated $Na_2S_2O_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:4 acetone–hexane) to give **3.41** (0.6 mg, 23%) as a transparent colorless film. ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.35–7.22 (m, 17 H, ArH), 6.88–6.87 (m, 2 H, ArH), 4.86 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.84–4.82 (m, 1 H, H-9), 4.81 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.79 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.59 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.57 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.56 (d, *J* = 10.5 Hz, 1 H, benzylic H), 4.54 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.38 (d, *J* = 10.5 Hz, 1 H, benzylic H), 4.36 (d, *J* = 7.7 Hz, 1 H, H-1), 3.81 (s, 3 H, OCH_3), 3.67–3.62 (m, 1 H, H-11), 3.58–3.53 (m, 2 H, H-2 and H-5), 3.33 (dd, *J* = 7.7, 1.4 Hz, 1 H, H-10), 3.19 (dd, *J* = 18.9, 7.0 Hz, 1 H, H-8), 2.73 (dd, *J* = 18.9, 4.9 Hz, 1 H, H-8), 1.92 (dd, *J* = 12.6, 4.9

Hz, 1 H, H-3), 1.81 (dd, J = 12.6, 11.9 Hz, 1 H, H-3), 1.23 (d, J = 6.3 Hz, 3 H, H-12), 1.06–1.04 (m, 21 H, isopropyl CH and CH_3), 0.87 (d, J = 6.3 Hz, 3 H, H-6); HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{50}H_{68}NaO_9Si$, 863.4525; found, 863.4529.



3.41 **3.42**

4-Methoxybenzyl 2-*O*-benzyl-3,6-dideoxy-4-*C*-((3*S*,4*R*,5*R*)-4,5-bis(benzyloxy)-1-hydroxy-2-((triisopropylsilyl)oxy)hexyl)-β-D-xylopyranoside (3.42). Ketone **3.42** (0.6 mg, 0.7 μmol, 1 equiv) was dissolved in THF (7 μL) and then the mixture was cooled to –78 °C. $NaBH_4$ (0.11 mg, 2.9 μmol, 4.0 equiv) was added and then the cooling bath was removed. The mixture was stirred at room temperature overnight at which point TLC (1:4 EtOAc–hexane) indicated no **3.41** remained. The mixture was diluted with EtOAc and then the excess $NaBH_4$ was quenched by the addition of saturated $NH_4Cl_{(aq)}$ before being transferred to a separatory funnel. The organic layer was washed with saturated $NH_4Cl_{(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (20 mL silica gel, 0:1→1:10→1:4 EtOAc–hexane) to give **3.42** (0.6 mg, 99%) as a transparent colorless film. ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.35–7.22 (m, 17 H, ArH), 6.87–6.85 (m, 2 H, ArH), 4.86 (d, J = 11.2 Hz, 1 H, benzylic H), 4.85 (d, J = 11.2 Hz, 1 H, benzylic H), 4.83 (d, J = 11.2 Hz, 1 H, benzylic H), 4.68 (d, J = 11.2 Hz, 1 H, benzylic H), 4.62 (d, J = 11.2 Hz, 1 H, benzylic H), 4.61 (d, J = 11.2 Hz, 1 H, benzylic H), 4.60 (d, J = 11.2 Hz, 1 H, benzylic H), 4.41 (d, J = 11.2 Hz, 1 H, benzylic H), 4.40 (d, J = 8.4 Hz, H-1), 4.38–4.37 (m, 1 H, H-9), 3.88–3.87

(m, 1 H, H-7), 3.81 (s, 3 H, OCH_3), 3.79–3.76 (m, 1 H, H-5), 3.71–3.69 (m, 1 H, H-11), 3.64–3.62 (m, 1 H, H-10), 3.54–3.51 (m, 2 H, 7-OH and H-2), 1.96–1.92 (m, 2 H, H-8 and H-3), 1.70–1.60 (m, 2 H, H-8 and H-3); HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{50}H_{70}NaO_9Si$, 865.4681; found, 865.4677.

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Chapter 4 : Synthesis of *M. marinum* LOS-I using glycosyl fluorides as glycosyl donors

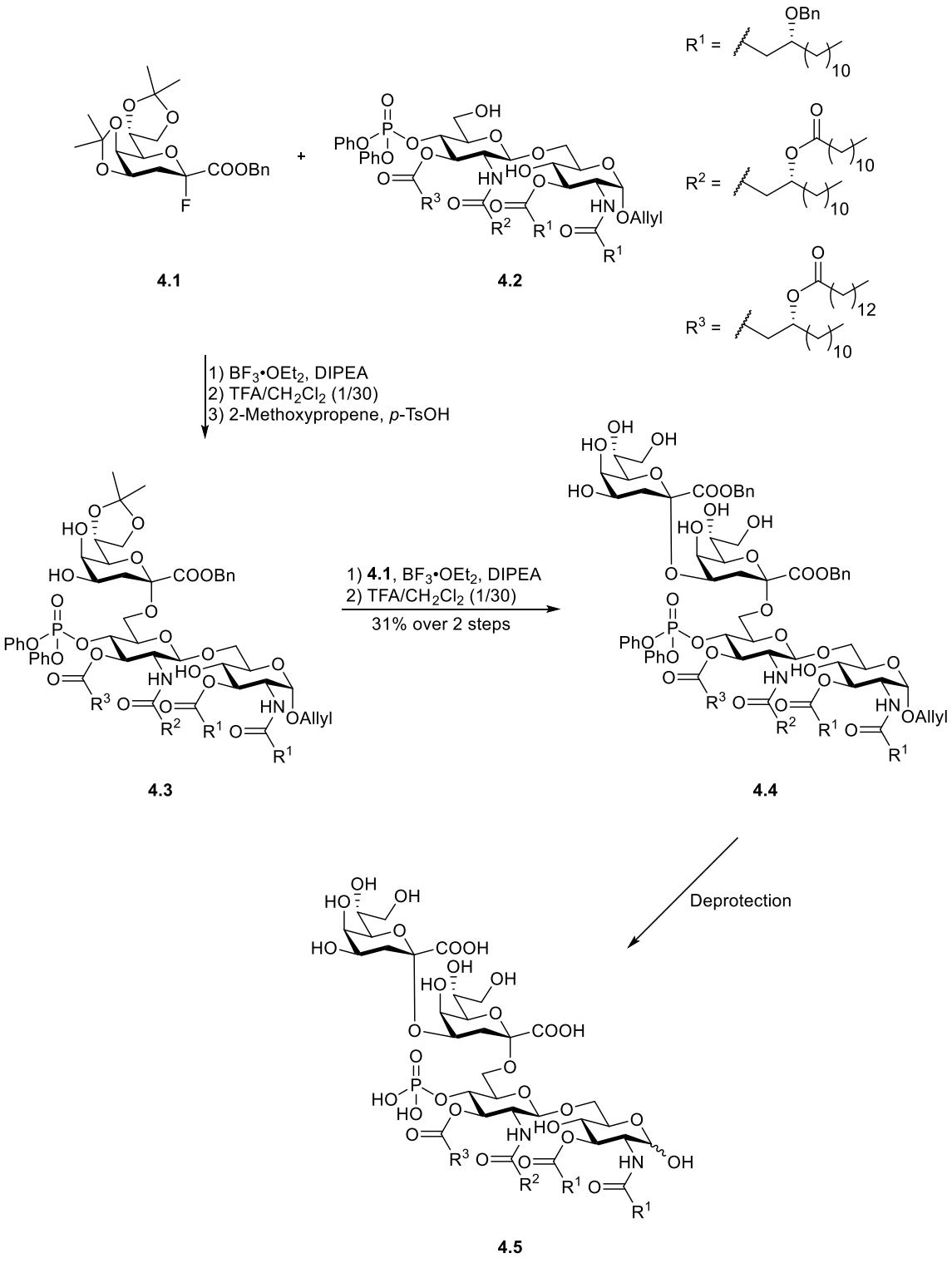
As described in Chapter 1, the challenges in constructing lipooligosaccharides (LOS) from *M. marinum* are 1) the synthesis of individual building blocks and 2) glycosylating those building blocks. Previously, in Chapter 2 and 3, I summarized the difficulties in synthesizing the building blocks. I will describe the glycosylations of the synthesized building blocks in this chapter. The challenges in glycosylation could be separated into 1) the method, that is, the type of glycosyl donor used and 2) the strategy, the coupling order of the glycosyl donors and acceptors. The method I chose is to use glycosyl fluorides as the donors, which will be rationalized in this Chapter. Continuing from Chapter 2, this chapter will describe previous syntheses of oligosaccharides using this method and then how this method influenced my synthesis of *M. marinum* LOS-I.

4.1 Previous syntheses of oligosaccharides using glycosyl fluorides as glycosyl donors

Early syntheses of glycosyl fluorides appeared in 1962[1] and 1966.[2] However, glycosyl fluorides were not used until 1981[3] to synthesize glycosidic bonds. Since then, their development as glycosyl donors has included new methods to synthesize glycosyl fluorides[4] and to couple them to glycosyl acceptors leading to various natural products[5] and oligosaccharides. These syntheses have been summarized in book chapters[6,7] and reviews.[8,9] Due to the extensive scope of these publications, only selected examples that involve the use of glycosyl fluorides in oligosaccharide syntheses will be highlighted to demonstrate their potential. The following examples were ranked in the number of sugar residues, from the least to the most and a brief summary of the advantages

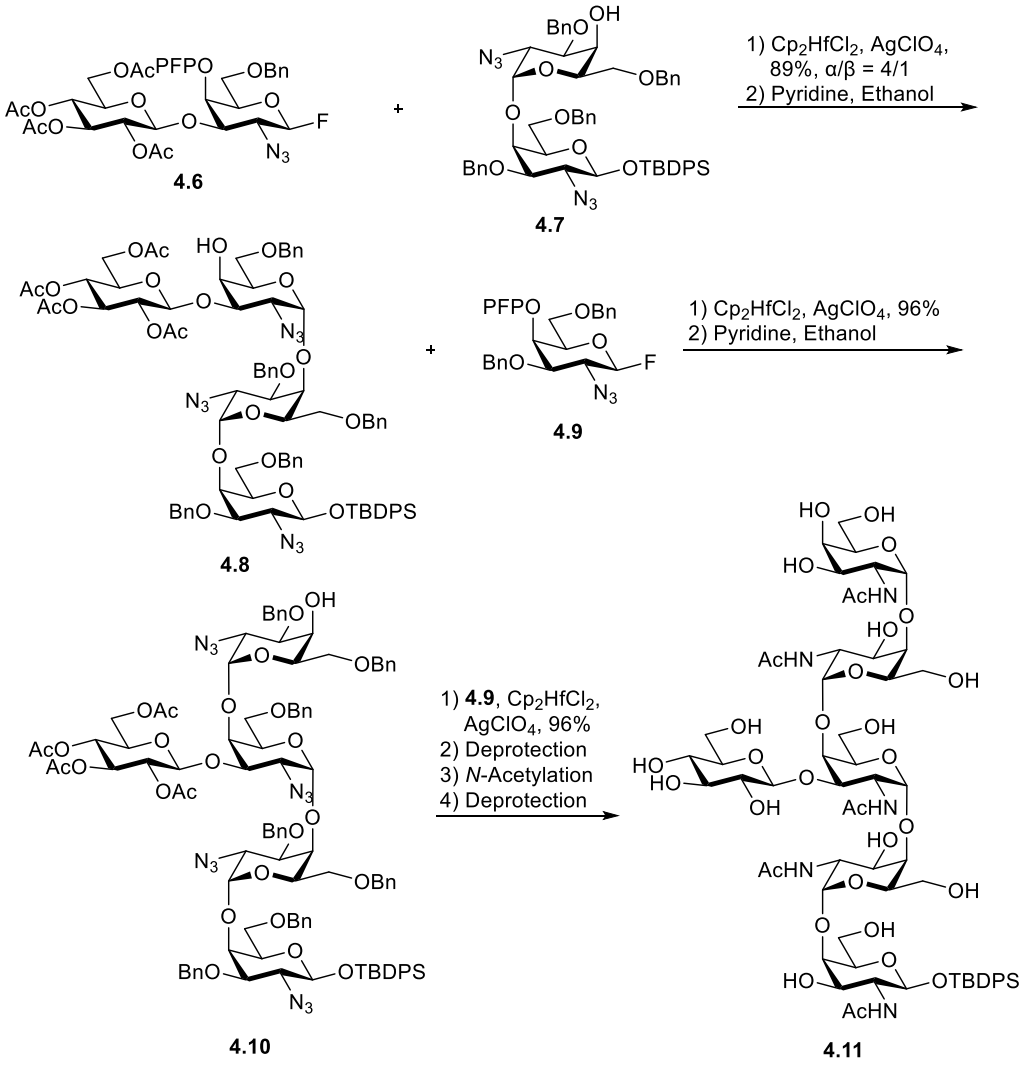
and disadvantages are discussed.

Two papers published in 1988[10,11] described the synthesis of a derivative of *Escherichia coli* lipopolysaccharide (LPS) (**Scheme 4.1**). Glycosyl fluoride **4.1** was used to glycosylate tetraacylated glycosyl acceptor **4.2** (no yields given) using boron trifluoride etherate as the activating reagent. Next, both isopropylidene ketals were hydrolyzed using trifluoroacetic acid and then the C-7" and 8" hydroxyl groups were reprotected as an isopropylidene ketal to give trisaccharide **4.3** as the desired glycosyl acceptor. Glycosylation between donor **4.1** and acceptor **4.3** followed by deprotection of isopropylidene groups gave tetrasaccharide **4.4** in 31% yield. Finally, after a series of deprotection steps, LPS derivative **4.5** was obtained.



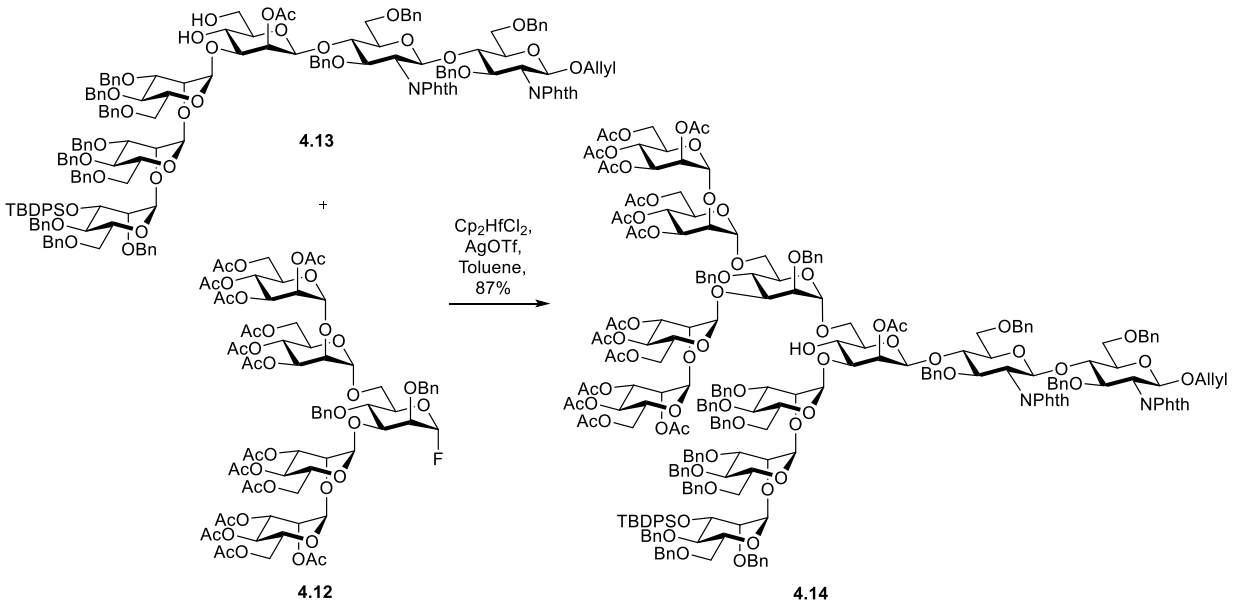
Scheme 4.1. Synthesis of *Escherichia coli* LPS derivative **4.5**.[10,11]

Another example is the synthesis of the *N*-linked glycan found in *Campylobacter jejuni* (**Scheme 4.2**).[12] Glycosyl fluoride **4.6** bearing a pentafluoropropionyl (PFP) group was used to glycosylate **4.7** in the presence of bis(cyclopentadienyl)hafnium dichloride and silver perchlorate in 89% yield. After deprotecting the PFP group, tetrasaccharide **4.8** was isolated. Glycosylation of acceptor **4.8** with glycosyl fluoride **4.9** and then deprotection of the PFP group gave pentasaccharide **4.10**. Repeating the glycosylation between glycosyl fluoride **4.9** and acceptor **4.10** gave, in 96% yield, a hexasaccharide, which was deprotected to give the glycan **4.11**.



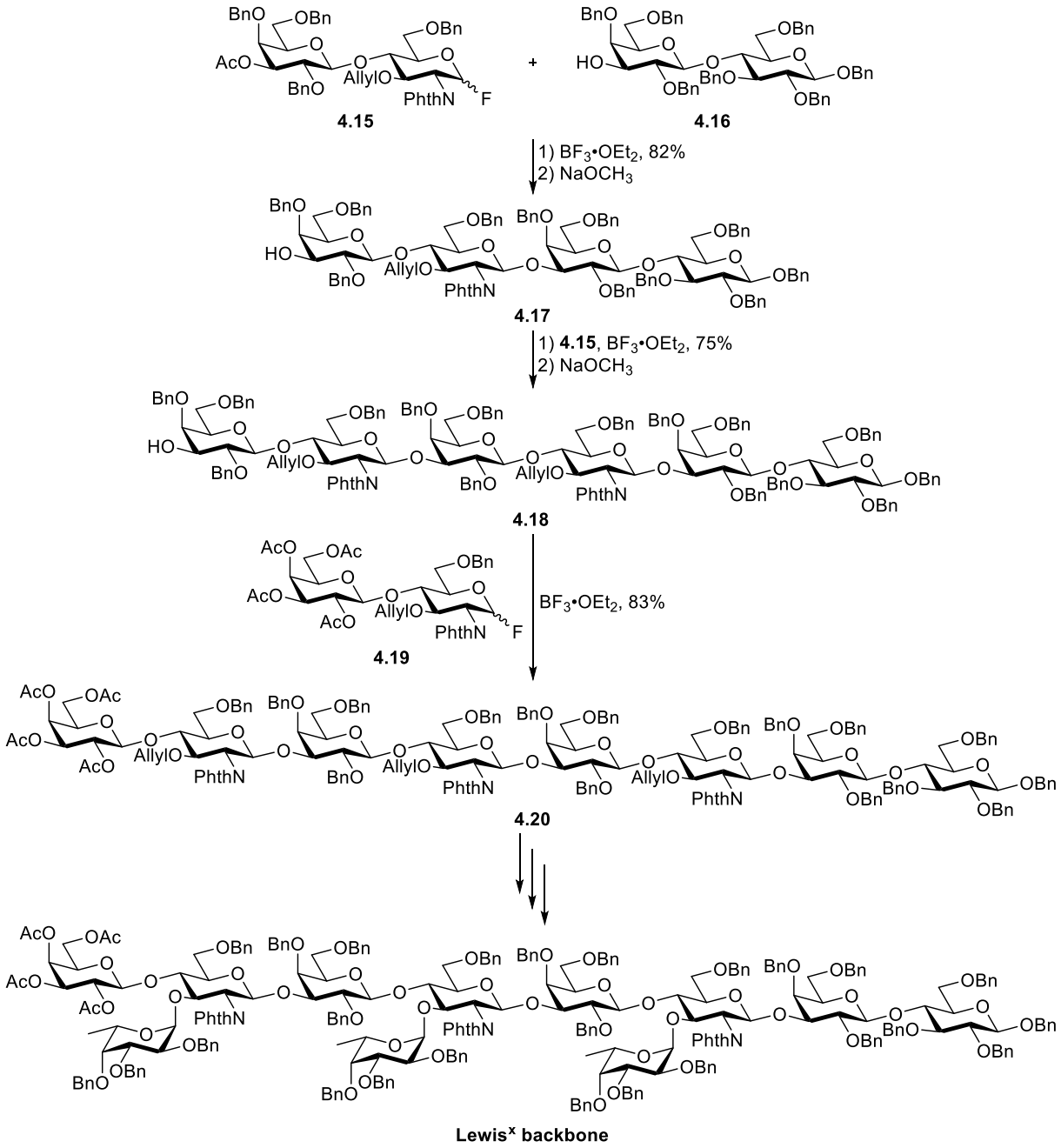
Scheme 4.2. Synthesis of *Campylobacter jejuni N*-linked glycan fragment.[12]

Continuing with the synthesis of *N*-linked glycans, a high-mannose type oligosaccharide was constructed using glycosyl fluorides as a key step (**Scheme 4.3**).[13] Glycosyl fluoride **4.12** was used to glycosylate hexasaccharide acceptor **4.13** in the presence of bis(cyclopentadienyl)hafnium dichloride and silver triflate to give undecasaccharide **4.14** in 87% yield.



Scheme 4.3. Key step in the synthesis of high-mannose type *N*-linked glycan.[13]

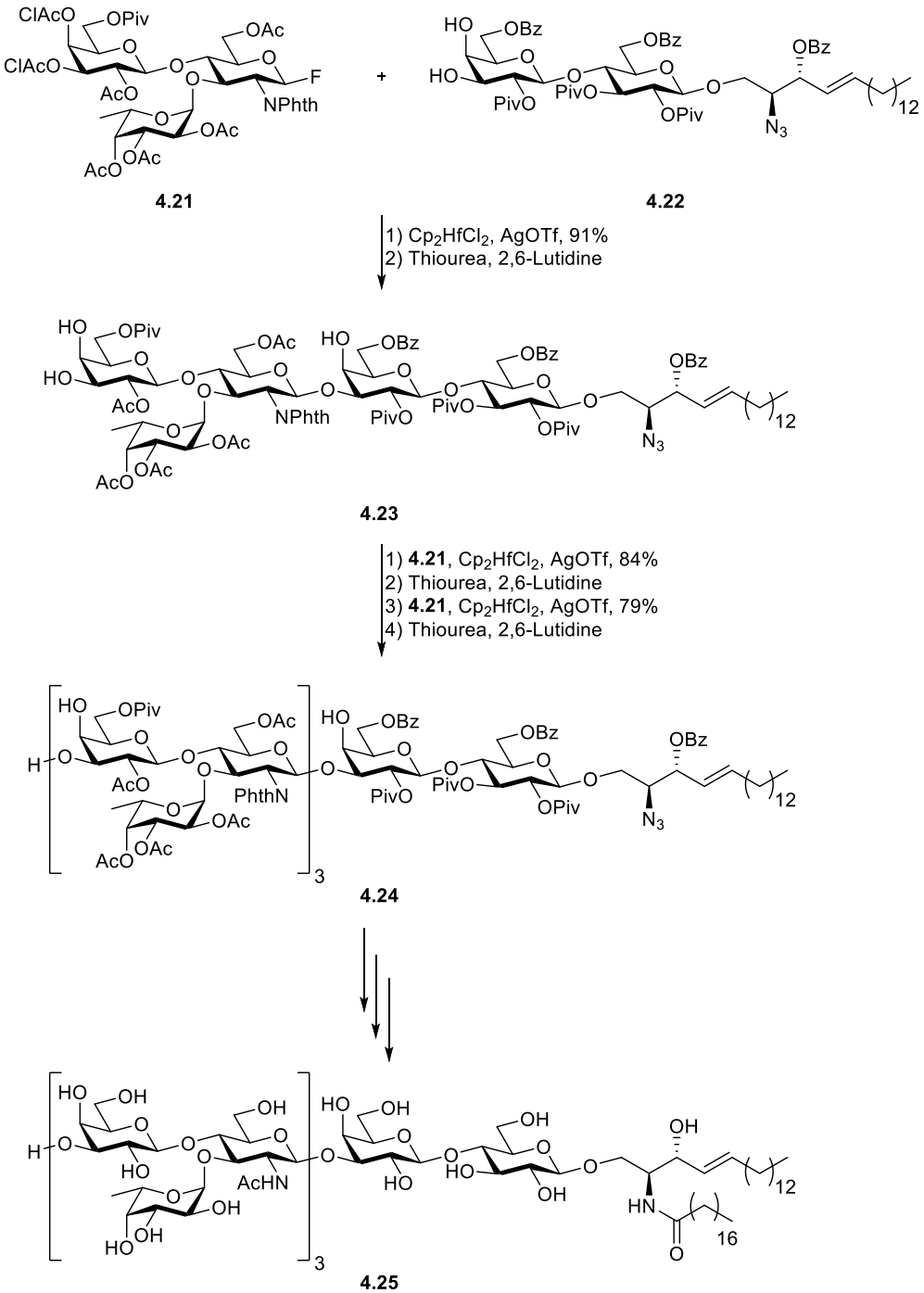
Two other examples are the synthesis of trimeric Lewis^x oligosaccharides. The first example synthesized the oligosaccharide backbone[14] whereas the second example completed the synthesis of trimeric Lewis^x glycosphingolipid. The first example (**Scheme 4.4**) started with the glycosylation between glycosyl fluoride **4.15** and glycosyl acceptor **4.16** to give a tetrasaccharide in 82% yield. After deacetylation, tetrasaccharide **4.17** was isolated and then again glycosylated with glycosyl fluoride **4.15** to give a hexasaccharide in 75% yield. Another deacetylation gave **4.18**, which was glycosylated with glycosyl fluoride **4.19** to give the desired octasaccharide **4.20** in 83% yield. Further glycosylations of octasaccharide **4.20** without using glycosyl fluorides as donors into trimeric Lewis^x backbone was achieved.



Scheme 4.4. Key steps in the synthesis of trimeric Lewis^x oligosaccharide.[14]

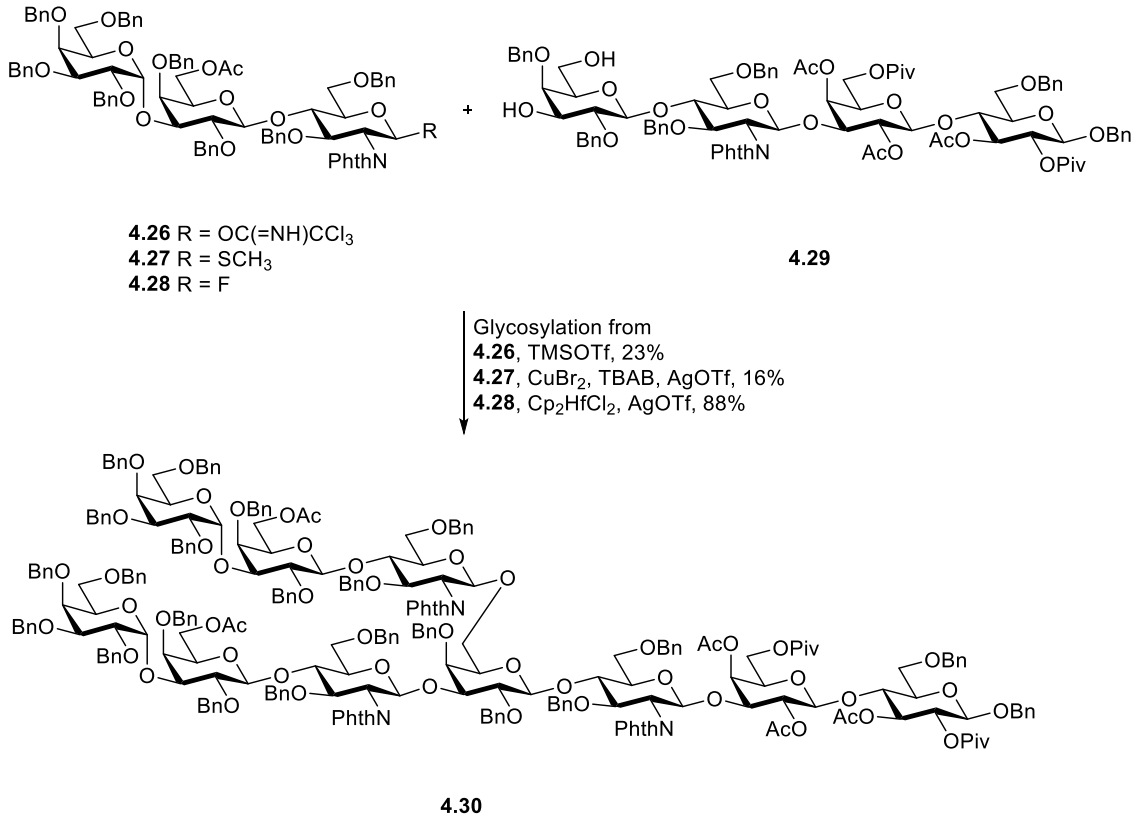
In contrast, the second example used glycosyl fluorides for all of the glycosylation steps to assemble the trimeric Lewis^x undecasaccharide (**Scheme 4.5**).[15] Glycosyl fluoride **4.21** was used to regioselectively glycosylate glycosyl acceptor **4.22** in 91% and then removal of the chloroacetyl

group gave pentasaccharide **4.23**. Pentasaccharide **4.23** underwent two successive glycosylations, in 84% and 79% yields, respectively, following chloroacetyl group deprotection to give the desired undecasaccharide **4.24**. Further modifications of **4.24** gave glycosphingolipid **4.25**.



Scheme 4.5. Synthesis of trimeric Lewis^x glycosphingolipid **4.25**.[15]

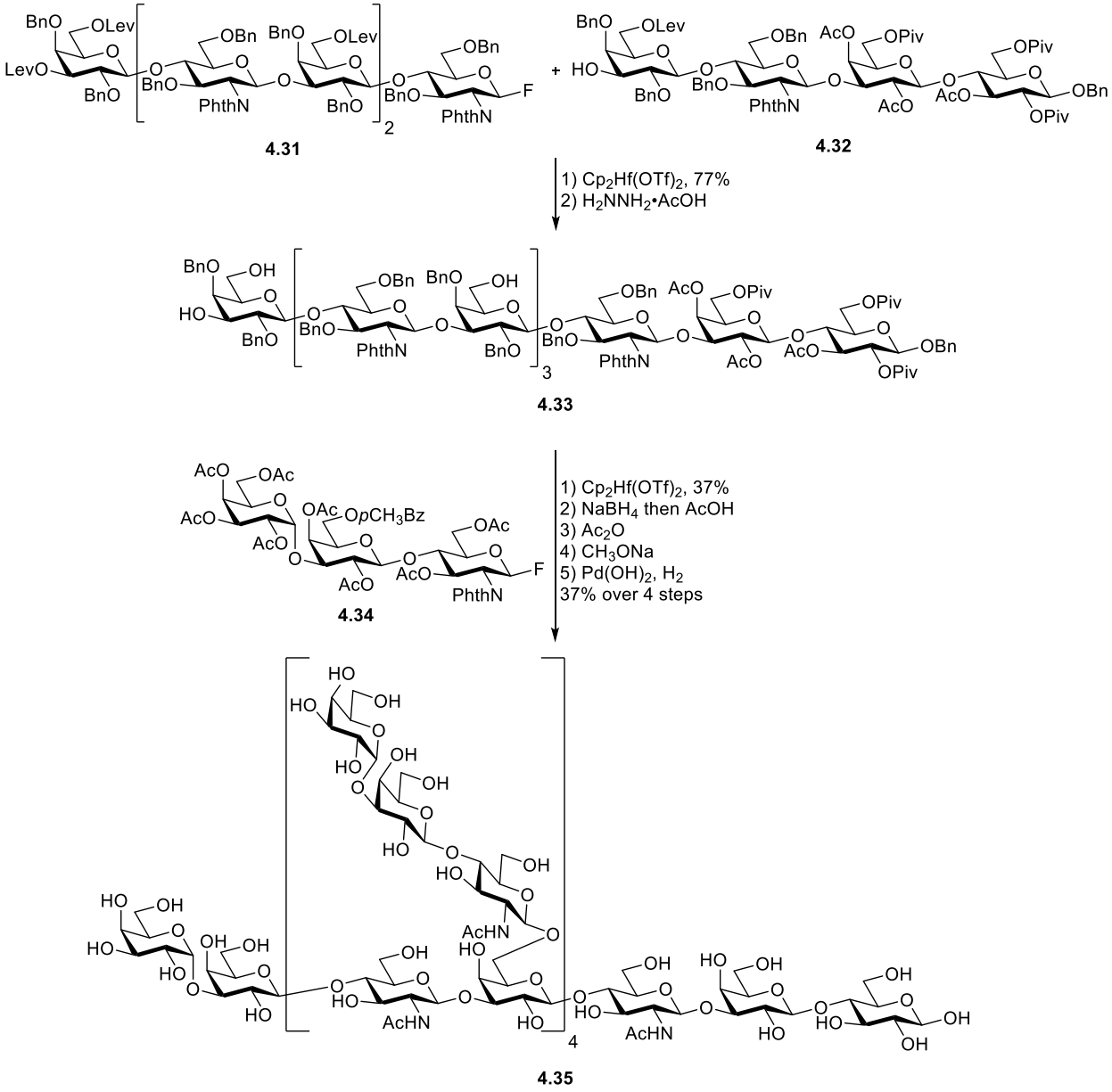
The last example I will describe is the synthesis of a 25-mer oligosaccharide, the glycan moiety of a glycosyl ceramide. Before starting this synthetic challenge, the authors carried out a glycosylation study with similar substrates (**Scheme 4.6**).[16] Thus, glycosylation between various trisaccharide donors (**4.26**, **4.27** and **4.28**) with tetrasaccharide acceptor **4.29** gave decasaccharide **4.30** in 23%, 16% and 88% yields, respectively. Because the best yield was achieved using **4.28**, they decided to use this donor for the synthesis of the 25-mer oligosaccharide.



Scheme 4.6. Glycosylation study with various glycosyl donors.[16]

Moving on to the synthesis of 25-mer oligosaccharide (**Scheme 4.7**),[17] glycosyl fluoride **4.31** was coupled with tetrasaccharide acceptor **4.32** in 77% yield and then removal of the levulinoyl groups gave decasaccharide **4.33**. The five hydroxyl groups in **4.33** were glycosylated

with glycosyl fluoride **4.34** (similar to **4.28**) to give the desired 25-mer in 37% yield. A series of deprotection steps gave the desired glycan **4.35** in 37% yield over four steps.



Scheme 4.7. Synthesis of 25-mer oligosaccharide **4.35**.[17]

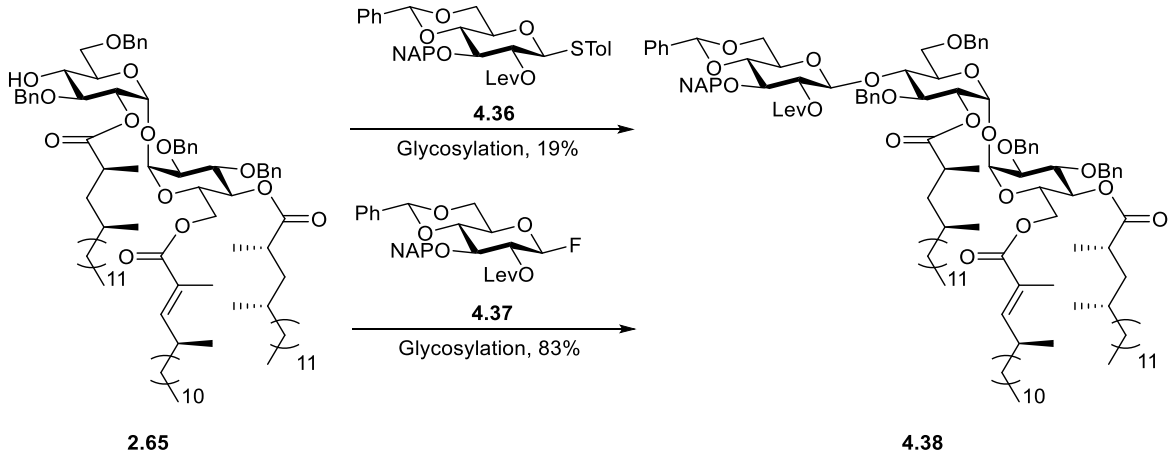
In summary, glycosyl fluorides are useful glycosyl donors in the syntheses of oligosaccharides. When synthesizing oligosaccharides, glycosylations between donors and

acceptors are usually more difficult when the coupling partners increase in size or have multiple ester (or other electron withdrawing) protecting groups. However, glycosyl fluorides bearing multiple monosaccharide residues (e.g. **4.12** and **4.31**) or esters (e.g. **4.21** and **4.34**) could still be successfully coupled with their corresponding acceptors, which were also oligosaccharides. The usefulness of glycosyl fluorides is also clear from the reactions shown in **Scheme 4.6**, which showed that using glycosyl fluoride **4.28** gave better results than using glycosyl trichloroacetimidate **4.26** and thioglycoside **4.27**. Aside from the advantages of using glycosyl fluorides as glycosyl donors, the disadvantages are the cost of the fluoride activating reagents. In the previous schemes, bis(cyclopentadienyl)hafnium dichloride and silver triflate are costly materials, and both reagents must be used stoichiometrically. Although switching the hafnium to zirconium reduced the cost, and some activating reagents could be used catalytically,[18] they have not been widely used oligosaccharide synthesis.

4.2 Synthesis of LOS-I using glycosyl fluorides as glycosyl donors

In the first paragraph of this chapter, I briefly discussed how glycosylation could be accomplished by considering the methods and the strategies that were used. Although I have shown the utility of glycosyl fluorides in oligosaccharide synthesis, my final decision on both the method and strategy was dictated by theory and experiments (**Scheme 4.8**). In an early experiment, I used thioglycoside donor **4.36** to glycosylate trehalose acceptor **2.65** but obtained the desired trisaccharide **4.38** only in 19% yield. The reason for such a low yield might be the coupling partners were not reactive enough; I anticipated that the acceptor **2.65** might be more likely to be the less reactive one due to the large lipid groups that hindered effective collisions between the glycosyl donor and acceptor. Trying to solve this problem I looked into the literature and luckily found **4.2** (**Scheme 4.1**), a complex disaccharide with four lipids attached that I considered less

reactive than **2.65** but similar in terms of protecting groups. Though yields were not shown, disaccharide **4.2** was successfully glycosylated with glycosyl fluoride **4.1**. This gave me enough reason to use a glycosyl fluoride donor in these reactions. Therefore, I tried the glycosylation between trehalose acceptor **2.65** and glycosyl fluoride **4.37**, which successfully gave desired trisaccharide **4.38** in 83% yield. These results align with those in **Scheme 4.6**. This result made me decide to use glycosyl fluorides donors as the glycosylation method. For the glycosylation strategy, I decided to add one monosaccharide at a time to reach the pentasaccharide LOS-I. I chose this approach for two reasons. One reason is monosaccharides could be obtained quickly in a large quantity, which could be used in excess quantities to ensure complete glycosylation of the more precious acceptor **2.65**. The other is there are no disaccharide (or larger) repeating units in LOS-I (unlike in **Scheme 4.5**); therefore, using a disaccharide (or bigger) does not provide a lot of synthetic advantages. Having decided on the method and strategy, I will describe the retrosynthesis of LOS-I.

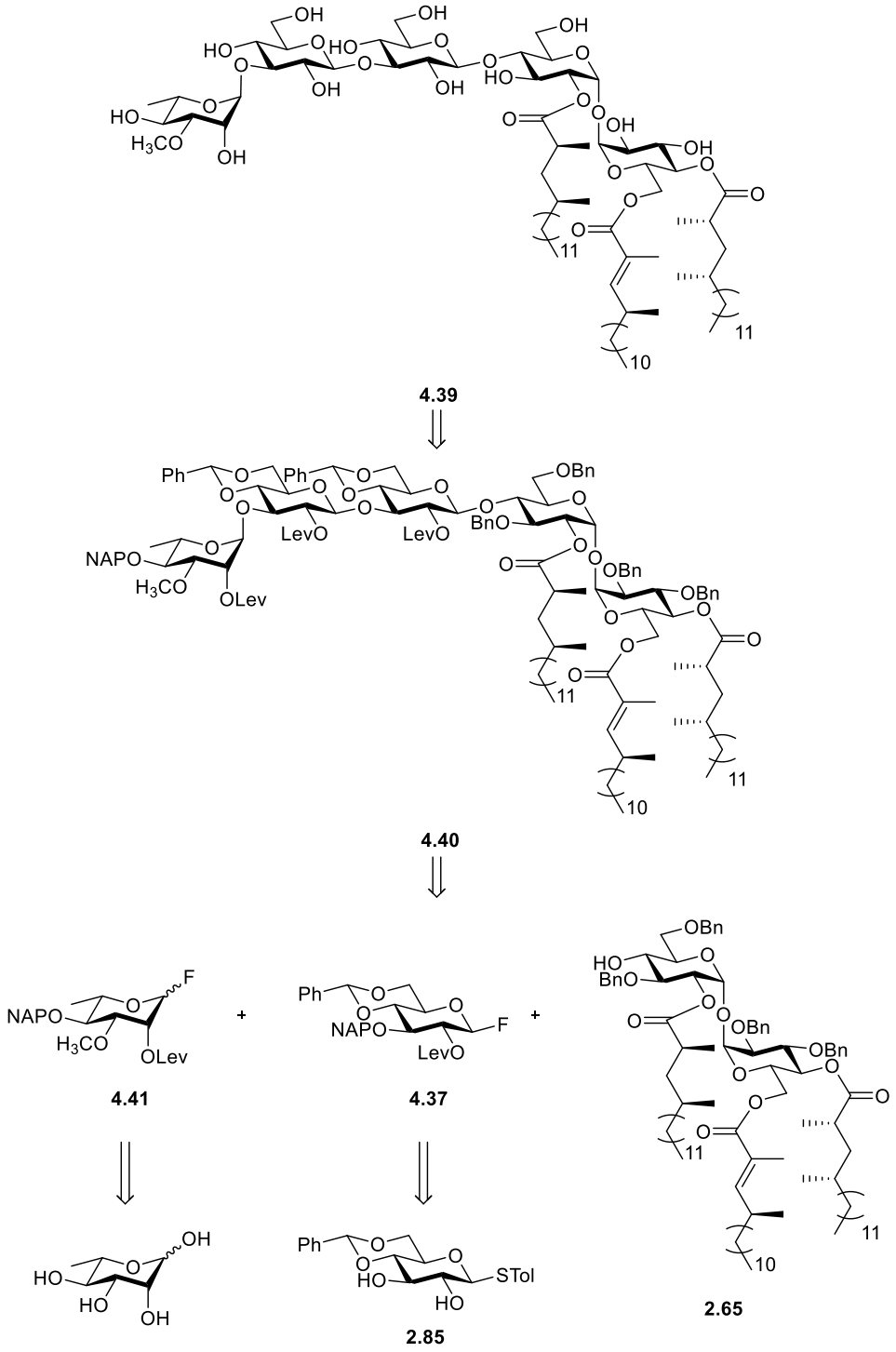


2.65 **4.38**

Scheme 4.8. Glycosylation with different glycosyl donors.

4.2.1 Retrosynthetic analysis of LOS-I

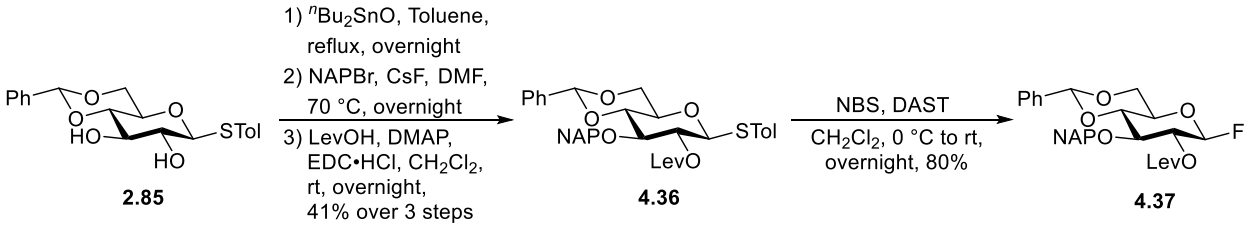
Shown in **Scheme 4.9** is the retrosynthesis of LOS-I **4.39**. The compound could be synthesized from the fully protected pentasaccharide **4.40**, which could be constructed by sequential glycosylations using glycosyl fluorides **4.37** and **4.41** and different glycosyl acceptors, starting from **2.65**. The three 1,2-*trans* glycosidic linkages in **4.40** could be established with the help of levulinoyl ester groups at C-2 in donors **4.37** and **4.41** *via* neighboring group participation during glycosylation. This specific ester was chosen because it could be deprotected without affecting the lipids. Glycosyl fluoride **4.37** and **4.41** could be synthesized from thioglycoside **2.85**[19] and L-rhamnose, respectively. With the retrosynthetic plan in hand, I will describe the syntheses of building blocks **4.37** and **4.41** and then the glycosylations to obtain **4.40** and then the attempted deprotection to achieve LOS-I **4.39**.



Scheme 4.9. Retrosynthetic analysis of LOS-I **4.39**.

4.2.2 Syntheses of monosaccharide building blocks

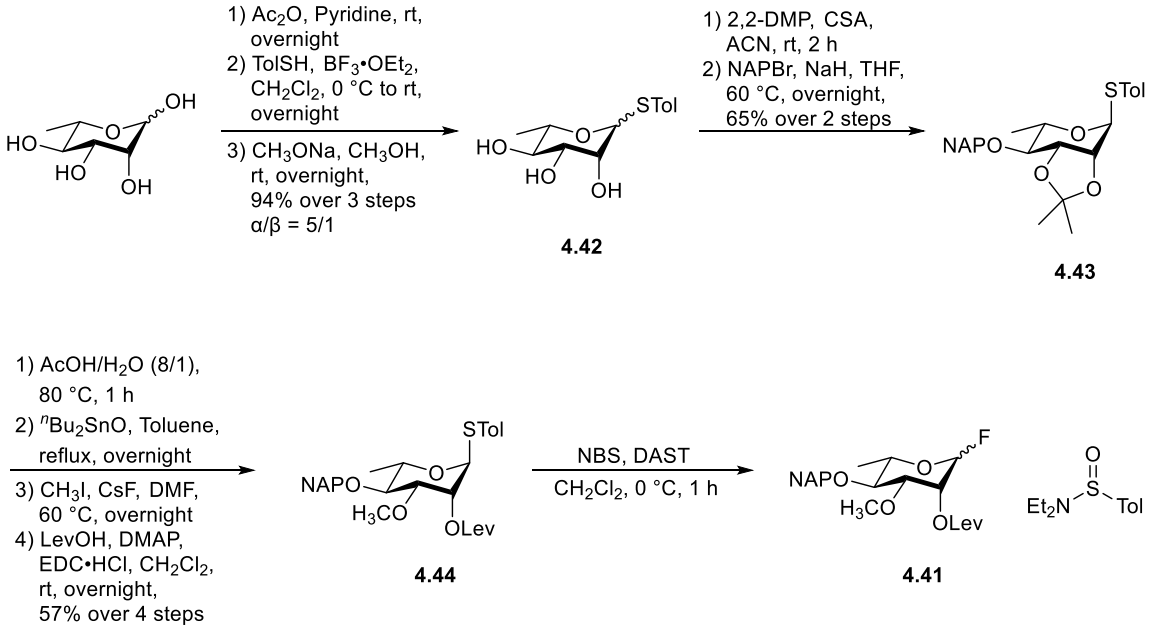
Synthesis of glycosyl fluoride **4.37** started with the previously synthesized **2.85** (**Scheme 4.10**). The C-3 hydroxyl group was regioselectively protected as a 2-naphthylmethyl ether *via* S_N2 attack on 2-(bromomethyl)naphthalene with a pre-formed 2,3-stannylidene acetal. The remaining C-2 hydroxyl group was then coupled with levulinic acid to give thioglycoside **4.36** in 41% yield over the three steps. Thioglycoside **4.36** was converted to the glycosyl fluoride **4.37** using *N*-bromosuccinimide and (diethylamino)sulfur trifluoride in 80% yield. This conversion was usually complete within one hour at 0 °C on a small scale (0.2 g of **4.36**) but required prolonged reaction time when 8.0 g of substrate **4.36** was used. Both **4.36** and **4.37** were purified by recrystallization, providing sufficient amount of materials for the oligosaccharide synthesis.



Scheme 4.10. Synthesis of glycosyl fluoride **4.37**.

The synthesis of glycosyl fluoride **4.41** is described in **Scheme 4.11**. L-Rhamnose monohydrate was peracetylated and then converted to the thioglycoside, which was further deacetylated to give **4.42** in 94% yield over three steps. The C-2 and C-3 hydroxyl groups of thioglycoside **4.42** were protected as an isopropylidene ketal using 2,2-dimethoxypropane under acidic conditions. The remaining C-4 hydroxyl group was protected as a 2-naphthylmethyl ether under basic conditions to give thioglycoside **4.43** in 65% yield over two steps. The isopropylidene ketal in thioglycoside **4.43** was cleaved using aqueous acetic acid under heat to reveal a diol, in which the C-3 hydroxyl group was then regioselectively methylated using a pre-formed

stannylidene acetal. The remaining C-2 hydroxyl group was coupled with levulinic acid to give **4.44** in 57% yield over four steps. Thioglycoside **4.44** was converted to glycosyl fluoride **4.41** using NBS and DAST but the product was inseparable from a sulfinimide byproduct even after multiple chromatographic purifications. The ratio of fluoride **4.41** to sufinimide was 10 to 1 and the yield of the fluoride was estimated to be 78%.

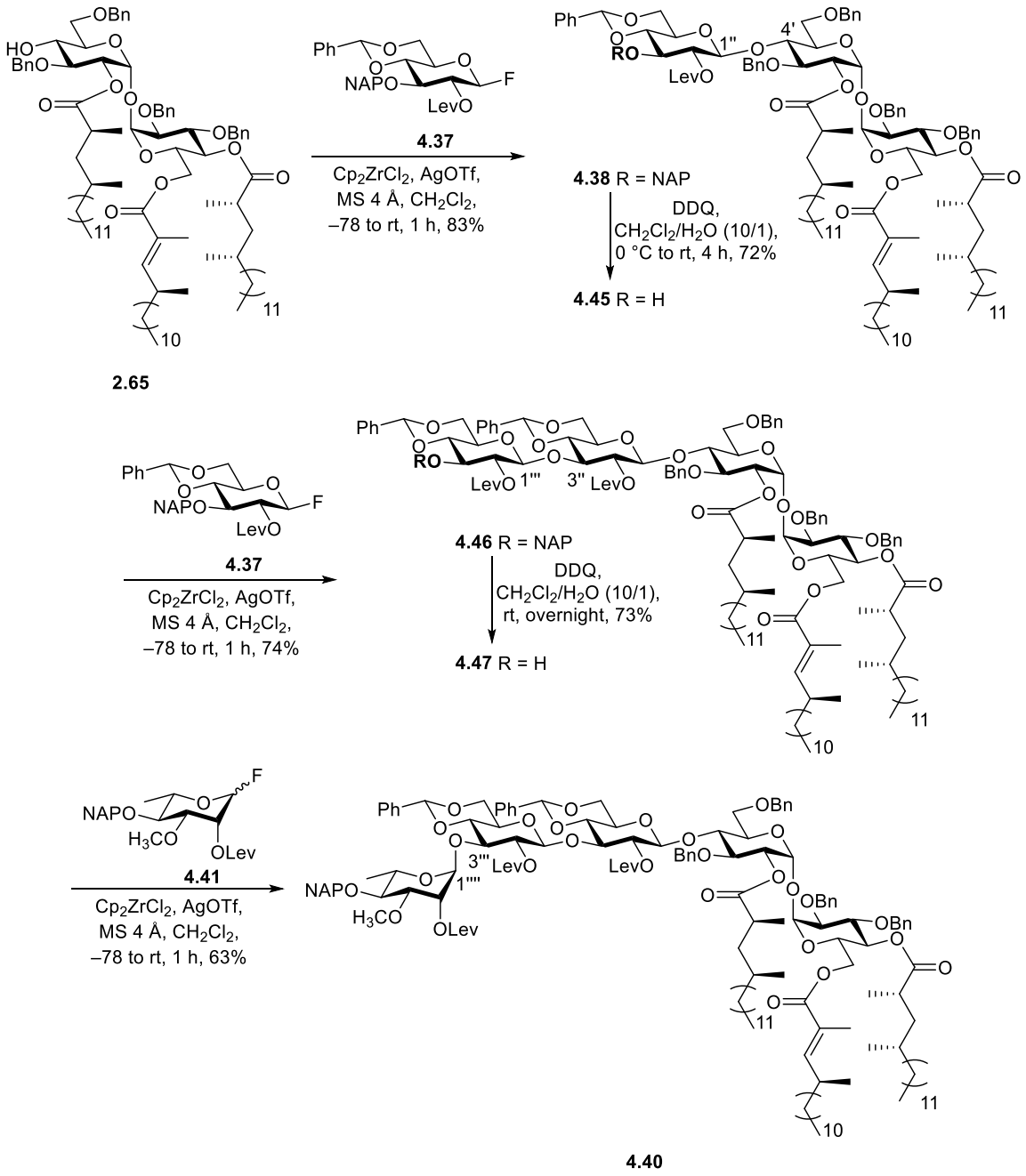


Scheme 4.11. Synthesis of glycosyl fluoride **4.41**.

4.2.3 Synthesis of LOS-I moiety

After both glycosyl fluorides were synthesized, the glycosylations were carried out (**Scheme 4.12**). Trehalose acceptor **2.65** was glycosylated with glycosyl fluoride **4.37** in the presence of bis(cyclopentadienyl)zirconium dichloride and silver triflate to give trisaccharide **4.38** in 83% yield. This result showed that zirconocene could be used as a cheaper alternative than hafnocene in oligosaccharide synthesis. The formed glycosidic linkage was verified by a correlation signal of H-1'' to C-4' in the HMBC spectrum; it was also assigned as a β-linkage,

from the coupling constant between H-1'' and H-2'' (7.7 Hz). The 2-naphthylmethyl ether in **4.38** was cleaved using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone to give the trisaccharide acceptor **4.45** in 72% yield. The trisaccharide acceptor **4.38** was glycosylated again with glycosyl fluoride **4.37** to give tetrasaccharide **4.46** in 74% yield. The formed glycosidic linkage was verified by a correlation signal of C-1''' to H-3'' in HMBC spectrum. The coupling constant between H-1''' and H-2''' could not be extracted due to resonance overlap; therefore, it could not be assigned as a β-linkage at this stage. Another deprotection of the 2-naphthylmethyl ether in tetrasaccharide **4.46** gave tetrasaccharide acceptor **4.47** in 73% yield; the coupling constant between H-1''' and H-2''' (7.7 Hz) confirmed the β-linkage installed in the glycosylation. The final glycosylation between tetrasaccharide acceptor **4.47** and glycosyl fluoride **4.41** gave the desired pentasaccharide **4.40** in slightly lower yield of 63% than the other glycosylations. However, increasing the equivalents of glycosyl fluoride, zirconocene and silver triflate did not increase the yield. The formed glycosidic linkage was verified by a correlation signal of C-1'''' to H-3''' in the HMBC spectrum; it was assigned as an α-linkage via the coupling constant between H-1'''' and C-1'''' (175.7 Hz) in the ^{1}H coupled HSQC spectrum.



Scheme 4.12. Synthesis of pentasaccharide **4.40**.

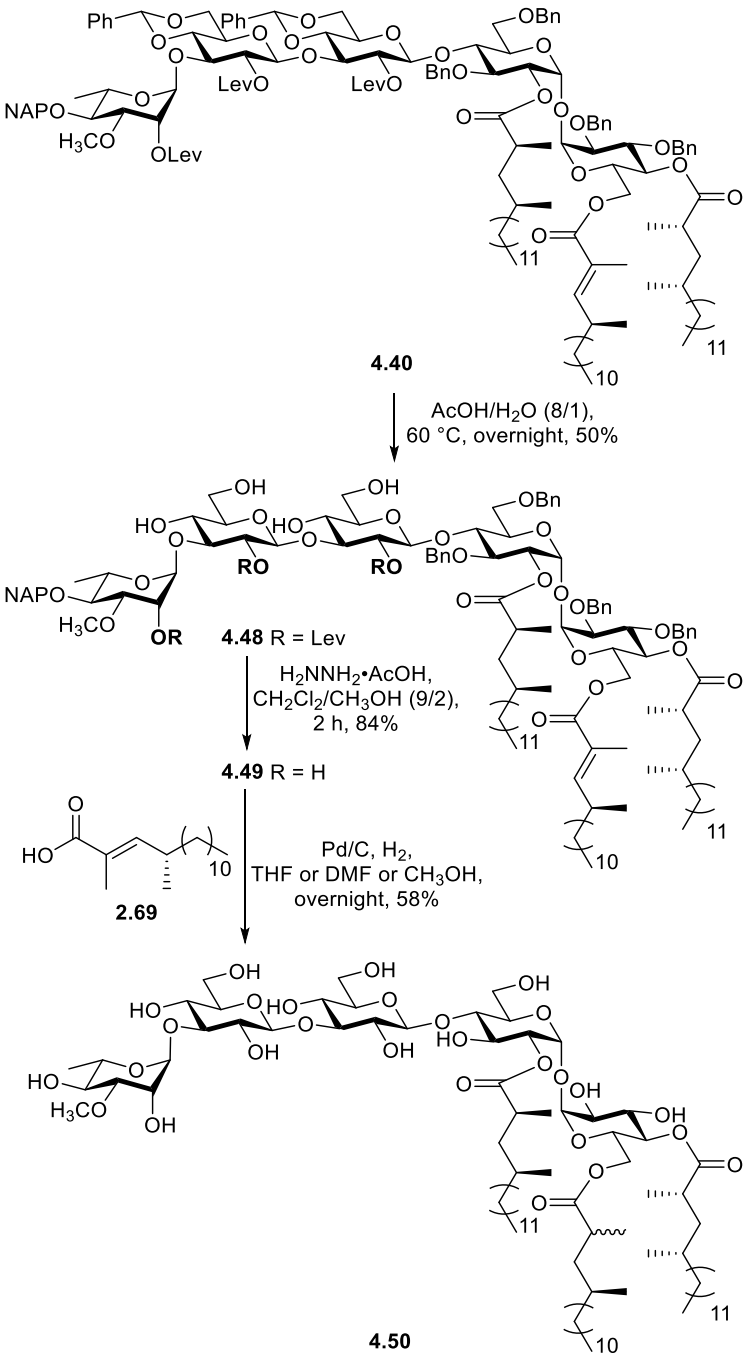
Having the pentasaccharide **4.40** in hand, the final few steps were its deprotection (**Scheme 4.13**). The two benzylidene acetals in **4.40** were deprotected by heating in aqueous acetic acid to give tetraol **4.48** in 50% yield. The reaction was sluggish and produced acetylated side products

after prolonged reaction periods. On the other hand, deprotecting the three levulinoyl groups on **4.48** using hydrazine acetate was straightforward, which gave pentasaccharide **4.49** in 84% yield.

The last step was hydrogenolysis of pentasaccharide **4.49**, which is challenging because it might hydrogenate the alkene on one of the lipids; therefore, more research was required. The goal was to deprotect four benzyl groups, one on a primary alcohol and three on secondary alcohols and a 2-naphthylmethyl group selectively in the presence of a trisubstituted alkene conjugated to an ester. Hydrogenolysis of benzyl ethers without hydrogenating alkenes has been reported in the literature. However, either only a benzyl group on a primary alcohol was present,[20] or the alkene was next to sterically congested moieties,[21] which made access of the catalyst to the substrate difficult. Taking these into account, it was not appealing to apply these conditions to **4.49**, a molecule with a 2-naphthylmethyl group, four benzyl groups and a non-sterically hindered alkene. There have been no comprehensive studies on chemoselective benzyl ether cleavage in the presence of alkenes. Going back to the basics, solvents play a role in hydrogenolysis of benzyl ethers; the use of tetrahydrofuran or acetic acid leads to faster in reaction rates.[23,24] Using acetic acid was not my first choice because it was reported that protic solvents are usually used during hydrogenation.[25]

In addition to control by the solvent, the idea of a "sacrificial" alkene was considered. The idea is that in the case that hydrogenation happens prior to hydrogenolysis, this sacrificial alkene would be hydrogenated instead of the alkene in **4.49**. This approach was applied to my problem, based on a successful attempt to deprotect two benzyl groups in the presence of an allylic ether using dissolving metal reduction; in this example, allyl ethyl ether was used as the sacrificial alkene.[26] The first experiment was carried out using lipid **2.69** as the sacrificial alkene and tetrahydrofuran as the solvent. The reaction was closely monitored by ^{1}H NMR spectroscopy to

prevent the sacrificial alkene **2.69** from being fully hydrogenated and more **2.69** was added if necessary. The hydrogenolysis was sluggish and only unreacted **4.49** and the 2-naphthylmethyl ether-deprotected pentasaccharide were isolated; luckily, the alkene was not hydrogenated in either molecule. At this point, I observed that the 2-naphthylmethyl ether deprotected pentasaccharide **4.49** was not readily dissolved in THF or chloroform. This solubility issue would prevent the hydrogenolysis from progressing;[22] therefore, more polar aprotic solvents should be used. I chose DMF. The reaction was closely monitored as previously mentioned and the hydrogenolysis did progress further, but no desired product was observed by TLC. Without other choices, I switched to methanol for hydrogenolysis. Even though sacrificial alkene **2.69** was still present, an inseparable mixture of desired LOS-I **4.39** and hydrogenated pentasaccharide **4.50** was isolated in an approximate ratio of 1 to 5. This mixture was then further hydrogenated without alkene **2.69** to obtain diastereomeric mixture of pentasaccharide **4.50** in 58% yield. Thus, the idea of using a sacrificial alkene to assist the chemoselectivity was unsuccessful.



Scheme 4.13. Deprotection of pentasaccharide **4.40**.

4.3 Summary

Glycosyl fluorides proved to be useful in oligosaccharide syntheses reported to date. Applying these species to the synthesis of a protected version of *M. marinum* LOS-I (**4.40**) was successful, as shown by sequential glycosylations between the trehalose acceptor **2.65** with glycosyl fluorides **4.37** and **4.41**. The benzylidene acetal and levulinoyl groups on pentasaccharide **4.40** were successfully deprotected; however, the final hydrogenolysis did not give the desired LOS-I **4.39** as a single product. My attempts to use a sacrificial alkene **2.69** to 'protect' the alkene in the target molecule was unsuccessful. A different strategy to synthesize the LOS-I of *M. marinum* will be discussed in the final chapter.

4.4 Experimental section

4.4.1 General methods

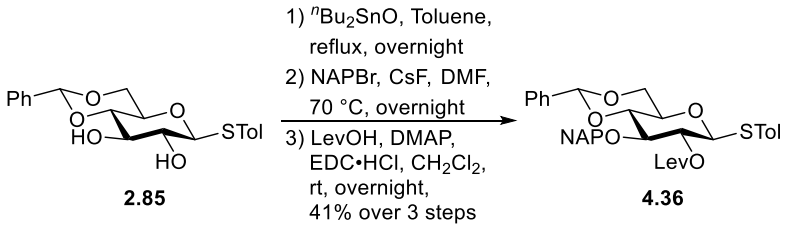
All reagents were purchased from commercial sources and used without further purification except AgOTf, which was purified as described below. Molecular sieves (4 Å, powder) were activated by heating the sieves in an oven at 300–320 °C overnight or flame-drying the sieves under high vacuum with a propane torch. Dichloromethane, tetrahydrofuran, acetonitrile, toluene and *N*,*N*-dimethylformamide used in reactions as solvents were taken from a solvent purification system in which the solvents were purified by successive passage through columns of alumina, copper and molecular sieves under argon. All reactions were carried out in round bottom flasks with stir bars inside and capped with rubber septa. Thin layer chromatography was performed on silica gel 60 F254 (0.25 mm, Merck) glass plates. Spots were detected by UV light and charring after treatment with a solution of either 1) ceric ammonium nitrate (0.5 g) and ammonium molybdate (12 g) in water (235 mL) and concentrated sulfuric acid (15 mL) or 2) concentrated sulfuric acid (5 mL) in ethanol (100 mL). In reaction work-ups involving extractions, TLC was performed on both the

combined organic layers and aqueous layers after extraction and before concentrating the combined organic layer. All column chromatography was performed on silica gel 60 (40–60 μm). Melting points were measured on a Gallenkamp apparatus and are not corrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter at the sodium D line (589 nm) at 21 ± 2 °C and are in units of (deg·mL)/(dm·g). FTIR spectra were run on Thermo Nicolet (Madison Wisconsin, USA) 8700 main bench with a Continuum FTIR microscope attached, and samples cast from a chloroform solution onto an IR-transparent silicone wafer. ^{1}H NMR spectra were recorded at 500, 600 and 700 MHz and the chemical shifts were referenced to $CHCl_3$ (7.26 ppm, $CDCl_3$). ^{13}C NMR spectra were recorded at 125 and 175 MHz and are proton decoupled; the chemical shifts were referenced to $CDCl_3$ (77.00 ppm, $CDCl_3$). High resolution ESI mass spectra were recorded on Agilent Technologies 6220 spectrometer; High resolution MALDI mass spectra were recorded on Bruker 9.4 T Apex-Qe spectrometer.

Procedure for AgOTf:

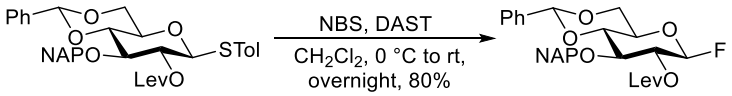
AgOTf was transferred to a round bottom flask. The flask was attached to high vacuum and gently heated with a heat gun. After the solids stopped bumping and the appearance changed from shiny to chalky, the flask was cooled to room temperature. The dried AgOTf was then weighed quickly in air and then transferred to the reaction flask.

4.4.2 Experimental procedure and spectroscopic data



4-Methylphenyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (4.36). Thioglycoside **2.85** (19 g, 49 mmol, 1.0 equiv) and dibutyltin(IV) oxide (15 g, 59 mmol, 1.2 equiv) was suspended in toluene (200 mL). The flask was fitted with Dean–Stark apparatus then the mixture was heated and stirred at reflux overnight. The mixture was cooled to room temperature then concentrated on a rotary evaporator and dried on high vacuum for 2 h. At that point, 2-(bromomethyl)naphthalene (22 g, 99 mmol, 2.0 equiv), CsF (15 g, 99 mmol, 2.0 equiv) and DMF (100 mL) were added to the crude residue and then the mixture was sonicated. The mixture was heated and stirred at 70 °C overnight, during which time the mixture dissolved and a white suspension gradually formed. The mixture was cooled to room temperature and diluted with EtOAc (1 L). The mixture was filtered over a pad of Celite® 545 and then transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 500 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator and then dried on high vacuum for 2 h. Levulinic acid (23 g, 0.20 mol, 3.0 equiv), 4-(dimethylamino)pyridine (24 g, 0.20 mol, 3.0 equiv), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (19 g, 99 mmol, 2.0 equiv) and CH_2Cl_2 (200 mL) were then added to the crude naphthylmethyl ether. The mixture was stirred at room temperature overnight at which point TLC (1:3 EtOAc–hexane) indicated no starting material remained. The mixture was then diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with

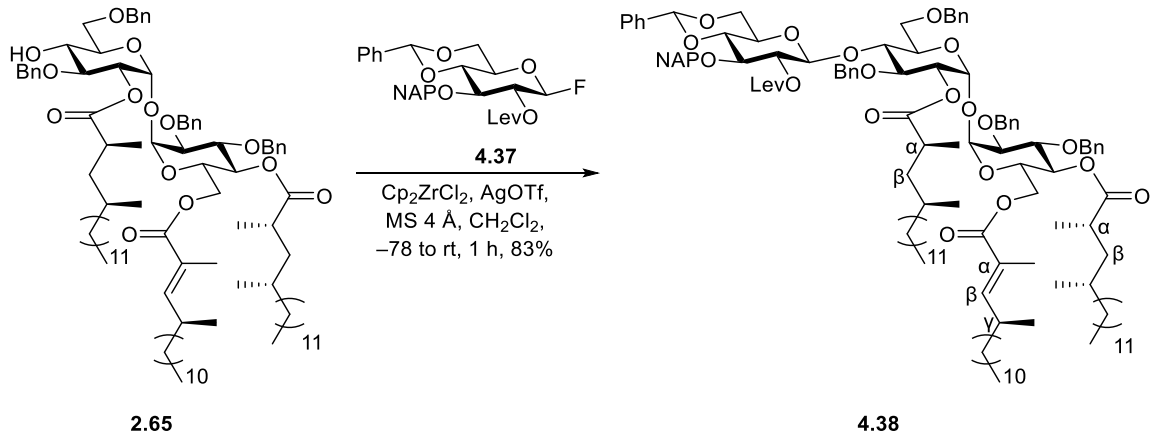
H_2O (3 × 200 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude product was purified by recrystallization from 95% EtOH (2 L) to give **4.36** (13 g, 41%) as white feather-like needles. mp 129–130 °C; $[\alpha]_D^{21}$ –0.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.81–7.73 (m, 4 H, ArH), 7.50–7.35 (m, 10 H, ArH), 7.12–7.10 (m, 2 H, ArH), 5.59 (s, 1 H, benzylidene H), 5.01 (dd, *J* = 10.2, 8.4 Hz, 1 H, H-2), 5.00 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.85 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.61 (d, *J* = 10.2 Hz, 1 H, H-1), 4.38 (dd, *J* = 10.8, 4.8 Hz, 1 H, H-6), 3.80 (dd, *J* = 10.8, 10.2 Hz, 1 H, H-6), 3.79 (dd, *J* = 9.0, 8.4 Hz, 1 H, H-3), 3.74 (dd, *J* = 9.6, 9.0 Hz, 1 H, H-4), 3.48 (ddd, *J* = 10.2, 9.6, 4.8 Hz, 1 H, H-5), 2.67–2.65 (m, 2 H, levulinoyl H), 2.58–2.48 (m, 2 H, levulinoyl H), 2.33 (s, 3 H, $ArCH_3$), 2.12 (s, 3 H, levulinoyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.1 (levulinoyl C=O), 171.2 (levulinoyl C=O), 138.5, 137.1, 135.6, 133.5, 133.2, 133.0, 129.7, 129.1, 128.3, 128.2, 128.0, 127.9, 127.7, 126.8, 126.1, 126.0, 125.9, 101.3 (benzylidene Ph*C*H), 87.1 (C-1), 81.3 (C-4), 79.6 (C-3), 74.4 (benzylic C), 71.8 (C-2), 70.5 (C-5), 68.6 (C-6), 37.8 (levulinoyl C), 29.8 (levulinoyl C), 28.0 (levulinoyl C), 21.2 ($ArCH_3$); IR (cast film): 3052, 2922, 2874, 1744, 1707, 1601, 1493, 1368, 1103, 1066 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{36}H_{36}NaO_7S$, 635.2074; found, 635.2075.



4.36 **4.37**

4,6-*O*-Benzylidene-2-*O*-levulinoyl-3-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl fluoride (4.37). Thioglycoside **4.36** (8.1 g, 13 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (76 mL) and then the mixture was cooled to 0 °C. (Diethylamino)sulfur trifluoride (3.5 mL, 26 mmol, 2.0 equiv) and *N*-bromosuccinimide (2.8 g, 16 mmol, 1.2 equiv) were added to the mixture and then the cooling

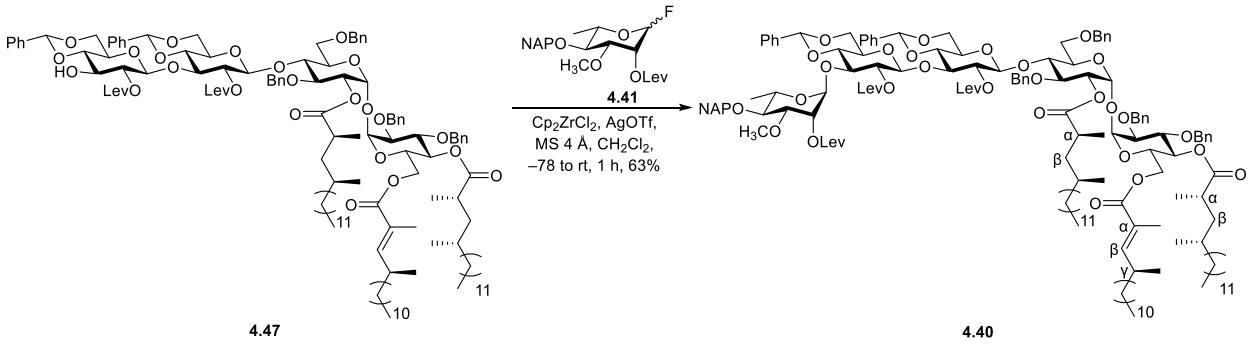
bath was removed. The mixture was stirred at room temperature overnight at which point TLC (1:3 EtOAc–hexane) indicated no **4.36** remained. The mixture was diluted with EtOAc and then saturated $NaHCO_{3(aq)}$ was added. The mixture was transferred to a separatory funnel and the organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine and dried over anhydrous $MgSO_4$. The solution was filtered and the filtrate was concentrated on a rotary evaporator. The crude product was purified by recrystallization (1:1 EtOAc–hexane, 300 mL) to give **4.37** (5.4 g, 80%) as white needles. mp 128–129 °C; $[\alpha]_D^{21}$ –9.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.83–7.73 (m, 4 H, ArH), 7.49–7.44 (m, 5 H, ArH), 7.41–7.38 (m, 3 H, ArH), 5.62 (s, 1 H, benzylidene H), 5.36 (dd, *J* = 54.0, 5.5 Hz, 1 H, H-1), 5.18 (ddd, *J* = 9.0, 6.0, 5.5 Hz, 1 H, H-2), 4.99 (d, *J* = 12.5 Hz, 1 H, benzylic H), 4.91 (d, *J* = 12.5 Hz, 1 H, benzylic H), 4.40 (dd, *J* = 10.5, 5.0 Hz, 1 H, H-6), 4.05 (dd, *J* = 8.5, 8.5 Hz, 1 H, H-4), 3.84 (dd, *J* = 10.5, 10.0 Hz, 1 H, H-6), 3.80 (dd, *J* = 8.5, 6.0 Hz, 1 H, H-3), 3.69–3.63 (m, 1 H, H-5), 2.70–2.67 (m, 2 H, levulinoyl H), 2.60–2.50 (m, 2 H, levulinoyl H), 2.14 (s, 3 H, levulinoyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.0 (levulinoyl C=O), 171.2 (levulinoyl C=O), 136.9, 135.2, 133.2, 133.0, 139.1, 128.3, 128.1, 127.9, 127.7, 126.9, 126.06, 126.01, 125.9, 106.6 (d, *J* = 217.6 Hz, C-1), 101.5 (benzylidene Ph*C*H), 80.2 (C-4), 77.5 (d, *J* = 5.6 Hz, C-3), 73.5 (benzylic C), 72.7 (d, *J* = 29.5 Hz, C-2), 68.7 (C-6), 65.6 (C-5), 37.7 (levulinoyl C), 29.7 (levulinoyl C), 27.8 (levulinoyl C); IR (cast film): 3027, 2917, 2872, 1744, 1710, 1603, 1498, 1369, 1097, 1073 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{29}H_{29}FNaO_7$, 531.1790; found, 531.1789.



2.65 **4.38**

4’’,6’’-*O*-Benzylidene-2’’-*O*-levulinoyl-3’’-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.38). Glycosyl fluoride **4.37** (0.35 g, 0.68 mmol, 2.0 equiv), trehalose acceptor **2.65** (0.50 g, 0.34 mmol, 1.0 equiv), silver trifluoromethanesulfonate (0.35 g, 1.4 mmol, 4.0 equiv) and 4 Å molecular sieves (powder, 0.7 g) were mixed with CH_2Cl_2 (7 mL). The mixture was cooled to –78 °C and then bis(cyclopentadienyl)zirconium(IV) dichloride (0.20 g, 0.68 mmol, 2.0 equiv) was added. The cooling bath was removed and then the mixture was stirred for 1 h. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:4 EtOAc–hexane) to give **4.38** (0.55 g, 80%) as a colorless film. $[\alpha]_D^{21}$ +31.3 (*c* 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.82–7.72 (m, 4 H, ArH), 7.47–7.45 (m, 4 H, ArH), 7.41–7.38 (m, 4 H, ArH), 7.33–7.27 (m, 12 H, ArH), 7.25–7.17 (m, 8 H, ArH), 6.54 (dd, *J* = 9.8, 0.7 Hz, 1 H, lipid βH), 5.38 (s, 1 H, benzylidene H), 5.28 (d, *J* = 3.5 Hz, 1 H, H-1’), 5.15

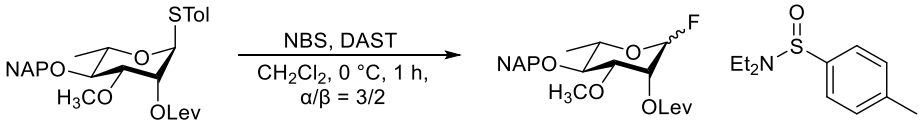
(d, J = 2.8 Hz, 1 H, H-1), 5.08 (dd, J = 9.8, 9.8 Hz, 1 H, H-4), 5.04–5.01 (m, 2 H, benzylic H and H-2'), 4.98–4.94 (m, 3 H, benzylic H and H-2''), 4.80 (d, J = 12.6 Hz, 1 H, benzylic H), 4.72–4.69 (m, 3 H, benzylic H), 4.67 (d, J = 12.6 Hz, 1 H, benzylic H), 4.62 (d, J = 12.6 Hz, 1 H, benzylic H), 4.50 (d, J = 7.7 Hz, 1 H, H-1''), 4.41 (d, J = 11.9 Hz, 1 H, benzylic H), 4.10 (dd, J = 12.6, 6.3 Hz, 1 H, H-6), 4.05–3.96 (m, 7 H, H-6, H-5', H-4', H-3', H-3, H-6'' and H-5), 3.75–3.74 (m, 1 H, H-6'), 3.61 (dd, J = 9.1, 2.8 Hz, 1 H, H-2), 3.59–3.54 (m, 3 H, H-4'', H-6' and H-3''), 3.12 (ddd, J = 9.8, 9.8, 4.9 Hz, 1 H, H-5''), 3.07 (dd, J = 9.8, 9.8 Hz, 1 H, H-6''), 2.52–2.32 (m, 6 H, 2 × lipid αH, lipid γH and 3 × levulinoyl H), 2.23–2.20 (m, 1 H, levulinoyl H), 1.86 (s, 3 H, levulinoyl CH_3), 1.80 (d, J = 0.7 Hz, 3 H, lipid αCH_3), 1.66–1.61 (m, 2 H, lipid βH), 1.37–0.95 (m, 75 H, lipid H and 3 × CH_3), 0.88–0.84 (m, 10 H, lipid H and 3 × CH_3), 0.80 (d, J = 7.0 Hz, 3 H, lipid CH_3), 0.73 (d, J = 7.0 Hz, 3 H, lipid CH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ): 205.6 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 171.3 (ester C=O), 167.9 (ester C=O), 149.4 (lipid βC), 139.2, 138.3, 137.8, 137.7, 137.3, 135.7, 133.2, 133.0, 129.0, 128.54, 128.51, 128.3, 128.2, 128.1, 128.00, 127.97, 127.92, 127.88, 127.80, 127.7, 127.4, 127.3, 127.0, 126.7, 126.4, 126.09, 126.06, 126.03, 125.9, 125.5, 101.1 (benzylidene Ph*C*H), 100.7 (C-1''), 92.1 (C-1), 91.7 (C-1'), 81.5 (C-4''), 78.9 (C-3), 78.5 (C-3''), 78.4 (C-2), 77.2–76.8 (C-4' and C-3' overlapped with $CDCl_3$), 75.0 (benzylic C), 74.3 (benzylic C), 74.1 (benzylic C), 73.8 (benzylic C), 73.7 (C-2''), 73.0 (benzylic C), 71.6 (C-2'), 70.9 (C-5'), 69.5 (C-4), 68.5 (C-5), 68.3 (C-6''), 67.5 (C-6'), 65.8 (C-5''), 62.4 (C-6), 41.6 (lipid βC), 41.3 (lipid βC), 37.4, 37.2, 36.89, 36.85, 36.79, 36.6, 33.3 (lipid γC), 31.9, 30.5, 30.4, 30.14, 30.08, 29.79, 29.75, 29.74, 29.71, 29.69, 29.67, 29.65, 29.5, 29.4, 27.7, 27.5, 26.8, 26.7, 22.7, 19.8, 19.6, 19.5, 17.7, 17.5, 14.1 (3 × CH_3), 12.5 (lipid αCH_3); IR (cast film): 3034, 2925, 2854, 1744, 1719, 1455, 1377, 1147, 1099 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{122}H_{172}NaO_{21}$, 1996.2283; found, 1996.2258.



2’’’’-*O*-Levulinoyl-3’’’’-*O*-methyl-4’’’’-*O*-(2-naphthylmethyl)-α-L-rhamopyranosyl-(1’’’’→3’’’)-4’’’,6’’’-*O*-benzylidene-2’’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’’→3’’)-4’’,6’’-*O*-benzylidene-2’’-*O*-levulinoyl-3’’-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.40). Glycosyl fluoride **4.41** (mixture, ca. 0.10 g, 0.24 mmol, 2.0 equiv), tetrasaccharide acceptor **4.47** (0.24 g, 0.12 mmol, 1.0 equiv), silver trifluoromethanesulfonate (0.31 g, 1.2 mmol, 10 equiv) and 4 Å molecular sieves (powder, 0.5 g) were mixed with CH_2Cl_2 (5 mL). The mixture was cooled to –78 °C and then bis(cyclopentadienyl)zirconium(IV) dichloride (87 mg, 0.30 mmol, 2.5 equiv) was added. The cooling bath was removed and then the mixture was stirred for 1 h. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was and concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:3→1:2 EtOAc–hexane) to give **4.40** (0.18 g, 63%) as a colorless film. $[\alpha]_D^{21}$ +8.1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.84–7.72 (m, 4 H, ArH), 7.49–7.27 (m, 25 H, ArH), 7.25–7.20 (m, 8 H, ArH), 6.54 (d, *J* = 9.8 Hz, 1 H, lipid βH), 5.36 (s, 1 H, benzylidene H), 5.31 (s, 1 H, benzylidene H), 5.28 (d, *J* = 3.5 Hz, 1 H, H-1’), 5.16 (d, *J* = 3.5 Hz,

1 H, H-1), 5.09–5.07 (m, 2 H, H-4 and H-2’’’’), 5.04–5.00 (m, 2 H, H-2’ and benzylic H), 4.97–4.94 (m, 3 H, H-2’’’ and benzylic H), 4.90 (dd, *J* = 8.4, 8.4 Hz, 1 H, H-2’’), 4.78–4.76 (m, 2 H, H-1’’’’ and benzylic H), 4.71–4.66 (m, 4 H, benzylic H), 4.63 (d, *J* = 7.7 Hz, 1 H, H-1’’’), 4.62 (d, *J* = 12.6 Hz, 1 H, benzylic H), 4.50 (d, *J* = 8.4 Hz, 1 H, H-1’’), 4.49 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.29 (dd, *J* = 10.5, 4.9 Hz, 1 H, H-6’’’), 4.10 (dd, *J* = 12.6, 6.3 Hz, 1 H, H-6), 4.06–3.96 (m, 8 H, H-5’, H-6, H-3, H-3’, H-4’, H-5’’’’, H-5 and H-6’’), 3.92 (dd, *J* = 9.1, 8.4 Hz, 1 H, H-3’’’), 3.82–3.81 (m, 1 H, H-6’), 3.78 (dd, *J* = 9.1, 8.4 Hz, 1 H, H-3’’), 3.68 (dd, *J* = 10.5, 9.8 Hz, 1 H, H-6’’’), 3.65–3.59 (m, 4 H, H-6’, H-3’’’’, H-2 and H-4’’’), 3.47–3.39 (m, 5 H, H-5’’’, H-4’’ and OCH_3), 3.26 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4’’’’), 3.13 (ddd, *J* = 9.8, 9.8, 4.9 Hz, 1 H, H-5’’), 3.05 (dd, *J* = 10.5, 9.8 Hz, 1 H, H-6’’), 2.88–2.77 (m, 2 H, levulinoyl H), 2.72–2.58 (m, 6 H, levulinoyl H), 2.57–2.42 (m, 6 H, levulinoyl H, 2 × lipid αH and γH), 2.30–2.26 (m, 1 H, levulinoyl H), 2.16 (s, 3 H, levulinoyl CH_3), 2.15 (s, 3 H, levulinoyl CH_3), 1.96 (s, 3 H, levulinoyl CH_3), 1.80 (s, 3 H, lipid αCH_3), 1.66–1.60 (m, 2 H, lipid βH), 1.36–0.95 (m, 75 H, lipid H and 3 × CH_3), 0.89–0.84 (m, 10 H, lipid H and 3 × CH_3), 0.81 (d, *J* = 6.3 Hz, 1 H, H-6’’’’), 0.80 (d, *J* = 7.0 Hz, 3 H, lipid CH_3), 0.72 (d, *J* = 6.3 Hz, 3 H, lipid CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.5 (levulinoyl C=O), 206.2 (levulinoyl C=O), 205.7 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 171.9 (ester C=O), 171.8 (ester C=O), 171.1 (ester C=O), 167.9 (ester C=O), 149.4 (lipid βC), 139.2, 138.3, 137.83, 137.81, 137.2, 136.9, 136.2, 133.3, 132.9, 129.0, 128.6, 128.5, 128.3, 128.2, 128.14, 128.10, 128.00, 127.97, 127.95, 127.93, 127.83, 127.78, 127.7, 127.4, 127.3, 127.0, 126.5, 126.3, 126.2, 126.0, 125.9, 125.8, 125.5, 101.8 (benzylidene Ph*C*H), 100.9 (benzylidene Ph*C*H), 100.6 (C-1’’), 100.1 (C-1’’’), 97.8 (C-1’’’’), 92.1 (C-1), 91.7 (C-1’), 80.2 (C-4’’’’), 79.3 (C-3’’’’), 79.0 (C-2), 78.9 (C-4’’’), 78.6 (C-4’’), 78.3 (C-4’), 77.3–76.7 (C-3, C-3’, C-3’’ and C-3’’’ overlapped with $CDCl_3$), 75.0 (benzylic C), 74.9 (benzylic C), 74.2 (benzylic C), 74.1 (C-2’’), 74.0 (benzylic

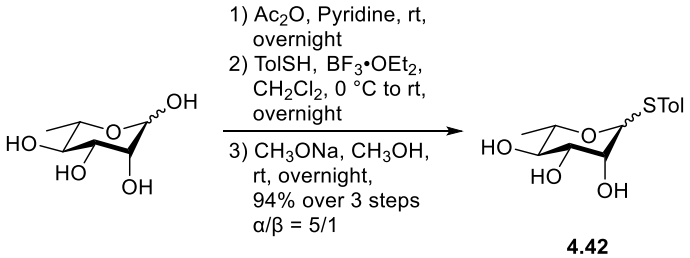
C), 73.8 (C-2’’’), 72.9 (benzylic C), 71.6 (C-2’), 71.0 (C-5’), 69.5 (C-4 and C-2’’’’), 68.9 (C-6’’’), 68.7 (C-6’’), 68.5 (C-5), 68.2 (C-5’’’’), 67.5 (C-6’), 66.3 (C-5’’’), 66.2 (C-5’’), 62.4 (C-6), 57.4 (OCH_3), 41.6 (lipid βC), 41.3 (lipid βC), 37.9, 37.5, 37.2, 36.9, 36.83, 36.78, 36.6, 33.3, 31.9, 30.5, 30.4, 30.14, 30.07, 29.8, 29.75, 29.71, 29.67, 29.6, 29.45, 29.36, 28.1, 27.5, 26.8, 26.7, 22.7, 19.8, 19.6, 19.5, 17.7, 17.5, 17.3 (C-6’’’’), 14.1 (3 ×CH_3), 12.5 (lipid αCH_3); IR (cast film): 3063, 2925, 2854, 1744, 1718, 1455, 1366, 1142, 1099 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{152}H_{210}NaO_{34}$, 2602.4596; found, 2602.4580.



4.44 **4.41**

2-*O*-Levulinoyl-3-*O*-methyl-4-*O*-(2-naphthylmethyl)-L-rhamopyranosyl fluoride (4.41). Thioglycoside **4.34** (0.16 g, 0.31 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (3.1 mL) and then the mixture was cooled to 0 °C. (Diethylamino)sulfur trifluoride (80 μL, 0.61 mmol, 2.0 equiv) and *N*-bromosuccinimide (66 mg, 0.37 mmol, 1.2 equiv) were added and then the mixture was stirred for 1 h. The mixture was diluted with EtOAc and then saturated $NaHCO_{3(aq)}$ was added before being transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:2 EtOAc–hexane) to give an inseparable mixture (0.11 g, 10:1) of **4.41** and the sulfonamide by-product as a colorless film. The ^1H and ^{13}C NMR data for the sulfinamide were identical to those reported.[27] ^1H NMR (600 MHz, $CDCl_3$, δ, α anomer): 7.84–7.80 (m, 4 H, ArH), 7.49–7.46 (m, 3 H, ArH), 5.50 (d, *J* = 49.2 Hz, 1 H, H-1), 5.42–5.43 (m, 1 H, H-2), 5.05 (d, *J* = 11.4 Hz, 1 H,

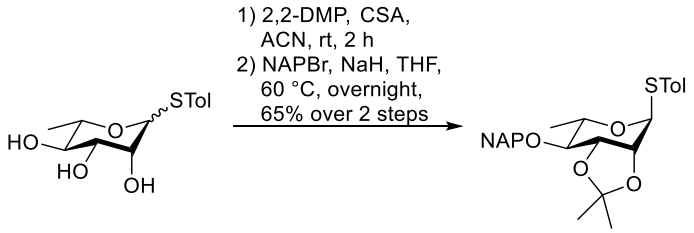
benzylic H), 4.80 (d, J = 11.4 Hz, 1 H, benzylic H), 3.93 (dq, J = 9.6, 6.0 Hz, 1 H, H-5), 3.69–3.67 (m, 1 H, H-3), 3.45–3.42 (m, 4 H, OCH_3 and H-4), 2.83–2.69 (m, 4 H, levulinoyl H), 2.19 (s, 3 H, levulinoyl CH_3), 1.36 (d, J = 6.0 Hz, 3 H, H-6); ^{1}H NMR (600 MHz, $CDCl_3$, δ, β anomer): 7.84–7.82 (m, 4 H, ArH), 7.50–7.45 (m, 3 H, ArH), 5.61–5.60 (m, 1 H, H-2), 5.34 (d, J = 48.6 Hz, 1 H, H-1), 5.03 (d, J = 11.4 Hz, 1 H, benzylic H), 4.79 (d, J = 11.4 Hz, 1 H, benzylic H), 3.55–3.51 (m, 1 H, H-5), 3.46–3.41 (m, 5 H, H-3, H-4 and OCH_3), 2.83–2.75 (m, 4 H, levulinoyl H), 2.20 (s, 3 H, levulinoyl CH_3), 1.42 (d, J = 6.6 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ, α anomer): 206.1 (levulinoyl C=O), 171.8 (levulinoyl C=O), 135.8, 133.3, 133.0, 128.1, 127.9, 127.7, 126.6, 126.1, 126.0, 125.9, 105.2 (d, J = 218.1 Hz, C-1), 79.3 (C-3), 79.0 (C-4), 75.4 (benzylic C), 70.1 (d, J = 2.8 Hz, C-5), 67.0 (d, J = 40.3 Hz, C-2), 57.6 (OCH_3), 37.9 (levulinoyl C), 29.8 (levulinoyl C), 27.9 (levulinoyl C), 17.9 (C-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ, β anomer): 206.1 (levulinoyl C=O), 172.0 (levulinoyl C=O), 135.6, 133.3, 133.0, 128.2, 127.9, 127.7, 126.7, 126.1, 126.0, 104.8 (d, J = 213.6 Hz, C-1), 81.3 (d, J = 7.3 Hz, C-3), 78.7 (C-4), 75.2 (benzylic C), 71.6 (d, J = 5.1 Hz, C-5), 66.9 (d, J = 17.5 Hz, C-2), 57.5 (OCH_3), 38.0 (levulinoyl C), 29.8 (levulinoyl C), 28.0 (levulinoyl C), 18.0 (C-6); IR (cast film): 3056, 2978, 2934, 2837, 1746, 1720, 1602, 1452, 1365, 1182, 1155, 1098, 1082 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{23}H_{27}FNaO_6$, 441.1684; found, 441.1682.



4.42

4-Methylphenyl 1-thio-L-rhamopyranoside (4.42). L-Rhamnose monohydrate (5.0 g, 27 mmol, 1.0 equiv) was suspended in acetic anhydride (30 mL, 0.27 mol, 10 equiv) and pyridine (30 mL).

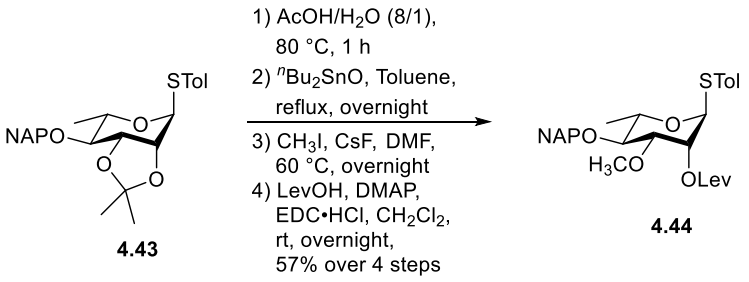
The mixture was stirred at room temperature overnight at which point TLC (2:3 EtOAc–hexane) indicated no rhamnose remained. The mixture was dried by dissolving in and evaporation with toluene (3 × 200 mL) and heptane (3 × 200 mL) on a rotary evaporator; the residue was then further dried on high vacuum for 2 h. Then, *p*-thiocresol (4.8 g, 38 mmol, 1.4 equiv) and CH_2Cl_2 (30 mL) were added to the crude tetraacetate and then the mixture was cooled to 0 °C. Boron trifluoride etherate (4.0 mL, 36 mmol, 1.2 equiv) was next added and then the mixture was warmed to room temperature and stirred overnight at which point TLC (2:3 EtOAc–hexane) indicated no tetraacetate remained. The mixture was diluted with EtOAc and the residual acid was quenched by the addition of 1 M $NaOH_{(aq)}$ before being transferred to a separatory funnel. The organic layer was washed with 1 M $NaOH_{(aq)}$ (3 × 200 mL), H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. Next, the crude thioglycoside was suspended in CH_3OH (60 mL) and sodium methoxide (1.5 g, 27 mmol, 1.0 equiv) was added. The mixture was stirred at room temperature overnight at which point TLC (10:1 CH_2Cl_2–CH_3OH) indicated no thioglycoside remained. The pH of the mixture was adjusted to 6–7 by the addition of CH_3OH-washed Amberlite® IR-120 (H^+) resin. The mixture was filtered and concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 1:0→20:1→10:1 CH_2Cl_2–CH_3OH) to give an anomeric mixture of **4.42** (7.0 g, 94%, α/β = 5/1) as a colorless syrup. The ^1H and ^{13}C NMR data for **4.42** were identical to those reported.[28]



4.42 **4.43**

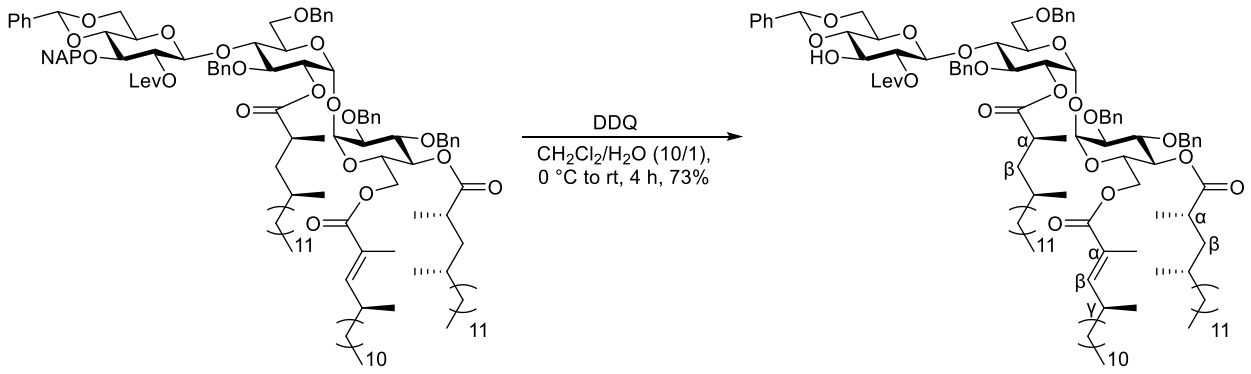
4-Methylphenyl 2,3-*O*-isopropylidene-4-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamopyranoside (4.43). Thioglycoside **4.42** (7.0 g, 26 mmol, 1.0 equiv) was dissolved in acetonitrile (65 mL) and then 2,2-dimethoxypropane (4.8 mL, 39 mmol, 1.5 equiv) and camphor-10-sulfonic acid (0.6 g, 3 mmol, 0.1 equiv) were added. The mixture was stirred at room temperature for 2 h and then the acid was quenched by the addition of saturated $NaHCO_{3(aq)}$. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator and then dried on high vacuum for 2 h. The crude material was dissolved in tetrahydrofuran (60 mL) and then 2-(bromomethyl)naphthalene (8.6 g, 39 mmol, 1.5 equiv) and NaH (60% dispersion in mineral oil, 1.6 g, 39 mmol, 1.5 equiv) were added. The mixture was heated and stirred at 60 °C overnight. The mixture was cooled to room temperature and then the excess NaH was quenched by the addition of H_2O. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:20 EtOAc–hexane) to give **4.43** (7.6 g, 65%) as a white amorphous solid. $[\alpha]_D^{21}$ –196.8 (*c* 0.4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 7.84–7.81 (m, 4 H, ArH), 7.51–7.45 (m, 3 H, ArH), 7.37–7.35 (m, 2 H, ArH), 7.13–7.11 (m, 2 H, ArH), 5.66 (s, 1 H, H-1), 5.07 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.81 (d, *J* = 12.0 Hz, 1 H, benzylic H), 4.37–4.21 (m, 2 H, H-2 and H-3), 4.18 (dq, *J* = 10.0, 6.5 Hz, 1 H, H-

5), 3.36–3.31 (m, 1 H, H-4), 2.33 (s, 3 H, $ArCH_3$), 1.51 (s, 3 H, isopropylidene CH_3), 1.39 (s, 3 H, isopropylidene CH_3), 1.25 (d, J = 6.5 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 137.8, 135.7, 133.2, 133.0, 132.5, 129.8, 129.7, 128.1, 127.9, 127.7, 126.8, 126.1, 126.0, 125.8, 109.4 (isopropylidene C), 84.2 (C-1), 81.5 (C-4), 78.5 (C-3), 77.3–76.7 (C-2 overlapped with $CDCl_3$), 73.1 (benzylic C), 66.1 (C-5), 28.0 (isopropylidene CH_3), 26.5 (isopropylidene CH_3), 21.1 ($ArCH_3$), 17.8 (C-6); IR (cast film): 3056, 2985, 2933, 2878, 1510, 1493, 1380, 1220, 1162, 1123, 1075 cm^{-1}; HRMS–ESI–TOF (m/z): $[M+Na]^+$ calcd for $C_{27}H_{30}NaO_4S$, 473.1757; found, 473.1757.



4-Methylphenyl 2-*O*-levulinoyl-3-*O*-methyl-4-*O*-(2-naphthylmethyl)-1-thio-α-L-rhamopyranoside (4.44). Thioglycoside **4.43** (7.6 g, 17 mmol, 1.0 equiv) was dissolved in acetic acid (84 mL) and H_2O (11 mL) before being heated and stirred at 80 °C for 1 h. The mixture was cooled to room temperature and dried by dissolving in and concentration with toluene (3 × 200 mL) on a rotary evaporator; the residue was further dried on high vacuum for 2 h. The crude material and dibutyltin(IV) oxide (5.0 g, 20 mmol, 1.2 equiv) was suspended in toluene (84 mL) before being heated and stirred at reflux overnight. The mixture was cooled to room temperature and concentrated on a rotary evaporator and then dried on high vacuum for 2 h. Next, CsF (5.1 g, 34 mmol, 2.0 equiv), DMF (42 mL) and CH_3I (2.1 mL, 34 mmol, 2.0 equiv) was added to the crude material. The mixture was heated and stirred at 60 °C overnight before being cooled to room temperature and diluted with EtOAc (500 mL). The mixture was filtered over a pad of Celite® 545

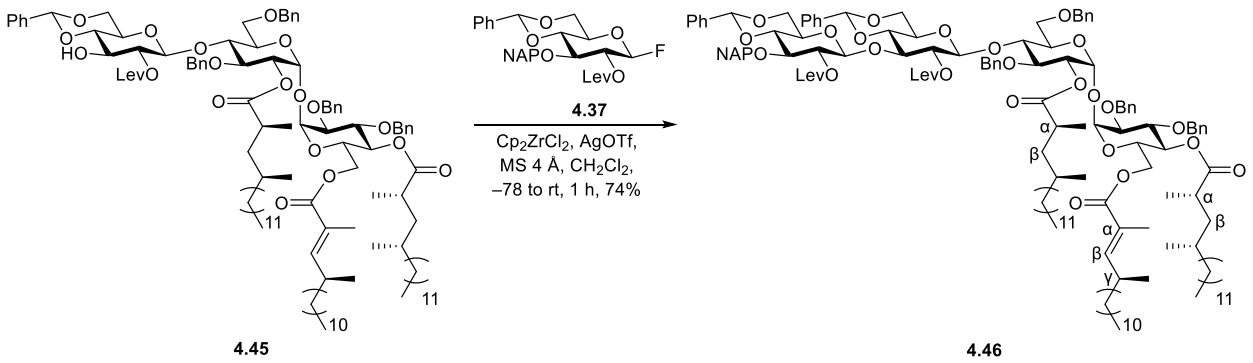
and transferred to a separatory funnel. The organic layer was washed with H_2O (3 × 500 mL), brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator and then dried on high vacuum for 2 h. Levulinic acid (3.9 g, 34 mmol, 2.0 equiv), 4-(dimethylamino)pyridine (4.1 g, 34 mmol, 2.0 equiv), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (6.4 g, 34 mmol, 2.0 equiv) and CH_2Cl_2 (42 mL) were then added to the crude methyl ether. The mixture was stirred at room temperature overnight at which point TLC (1:1 EtOAc–hexane) indicated no starting material remained. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (300 mL silica gel, 0:1→1:4→1:2→1:1 EtOAc–hexane) to give **4.44** (5.0 g, 57%) as a transparent pale-yellow syrup. $[\alpha]_D^{21}$ –143.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.85–7.82 (m, 4 H, ArH), 7.50–7.46 (m, 3 H, ArH), 7.35–7.34 (m, 2 H, ArH), 7.12–7.11 (m, 2 H, ArH), 5.56–5.55 (m, 1 H, H-2), 5.34 (s, 1 H, H-1), 5.07 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.81 (d, *J* = 11.4 Hz, 1 H, benzylic H), 4.26 (dq, *J* = 9.6, 6.0 Hz, 1 H, H-5), 3.68 (dd, *J* = 9.0, 3.0 Hz, 1 H, H-3), 3.48–3.44 (m, 4 H, H-4 and OCH_3), 2.79–2.74 (m, 2 H, levulinoyl H), 2.72–2.68 (m, 2 H, levulinoyl H), 2.32 (s, 3 H, $ArCH_3$), 2.18 (s, 3 H, levulinoyl CH_3), 1.35 (d, *J* = 6.0 Hz, 3 H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.2 (levulinoyl C=O), 171.9 (levulinoyl C=O), 137.9, 136.0, 133.3, 133.0, 132.3, 130.0, 129.8, 128.1, 127.9, 127.8, 126.6, 126.05, 126.00, 125.8, 86.3 (C-1), 80.5 (C-3), 80.2 (C-4), 75.4 (benzylic C), 70.4 (C-2), 68.8 (C-5), 57.4 (OCH_3), 38.0 (levulinoyl C), 29.8 (levulinoyl C), 28.1 (levulinoyl C), 21.1 ($ArCH_3$), 17.9 (C-6); IR (cast film): 3054, 2976, 2929, 2834, 1740, 1720, 1509, 1493, 1363, 1155, 1103, 1090 cm^{-1}; HRMS–ESI–TOF (*m*/*z*): $[M+Na]^+$ calcd for $C_{30}H_{34}NaO_6S$, 545.1968; found, 545.1967.



4.38 **4.45**

4’’,6’’-*O*-Benzylidene-2’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.45). Trisaccharide **4.38** (0.55 g, 0.28 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5.6 mL) and H_2O (0.56 mL) and then the mixture was cooled to 0 °C before 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (95 mg, 0.42 mmol, 1.5 equiv) was added. The mixture was warmed to room temperature and stirred for 4 h. The mixture was then diluted with EtOAc and saturated $NaHCO_{3(aq)}$ was added before being transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:4→1:2 EtOAc–hexane) to give **4.45** (0.37 g, 72%) as a colorless film. $[\alpha]_D^{21}$ +41.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.45–7.44 (m, 2 H, ArH), 7.41–7.27 (m, 17 H, ArH), 7.25–7.19 (m, 6 H, ArH), 6.55 (dd, *J* = 9.1, 1.4 Hz, 1 H, lipid βH), 5.35 (s, 1 H, benzylidene H), 5.29 (d, *J* = 3.5 Hz, 1 H, H-1’), 5.17 (d, *J* = 2.8 Hz, 1 H, H-1), 5.10 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4), 5.04–5.03 (m, 2 H, benzylic H and H-2’), 4.95 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.85 (dd, *J* = 9.1, 7.7 Hz, 1 H, H-2’’), 4.74–4.70 (m, 3 H, benzylic H), 4.68 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.64 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.61 (d, *J* = 7.7 Hz, 1 H,

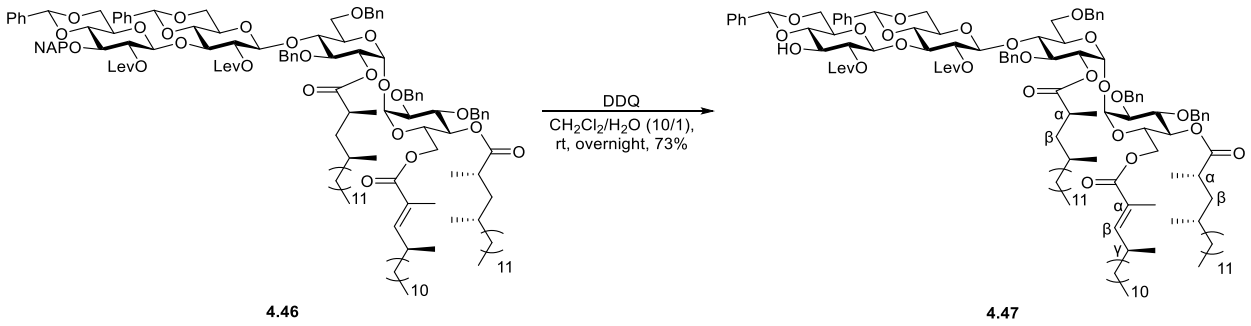
H-1’’), 4.11 (dd, *J* = 12.6, 6.3 Hz, 1 H, H-6), 4.06–3.97 (m, 6 H, H-5’, H-6, H-3’, H-4’ H-3 and H-5), 3.95 (dd, *J* = 9.1, 3.5 Hz, 1 H, H-6’’), 3.80–3.78 (m, 1 H, H-6’), 3.71 (ddd, *J* = 9.1, 9.1, 2.8 Hz, 1 H, H-3’’), 3.62 (dd, *J* = 9.1, 2.8 Hz, 1 H, H-2), 3.60 (d, *J* = 10.5 Hz, 1 H, H-6’), 3.40 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4’’), 3.13 (ddd, *J* = 9.1, 9.1, 3.5 Hz, 1 H, H-5’’), 3.10 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-6’’), 2.87 (d, *J* = 2.8 Hz, 1 H, 3’’-OH), 2.70–2.66 (m, 1 H, levulinoyl H), 2.57–2.41 (m, 5 H, levulinoyl H, 2 × lipid αH and γH), 2.24–2.20 (m, 1 H, levulinoyl H), 2.03 (s, 3 H, levulinoyl CH_3), 1.81 (d, *J* = 1.4 Hz, 3 H, lipid αCH_3), 1.67–1.61 (m, 2 H, lipid βH), 1.36–0.93 (m, 75 H, lipid H and 3 × CH_3), 0.89–0.84 (m, 10 H, lipid H and 3 × CH_3), 0.80 (d, *J* = 6.0 Hz, 3 H, lipid CH_3), 0.72 (d, *J* = 5.4 Hz, 3 H, lipid CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.7 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 172.0 (ester C=O), 167.9 (ester C=O), 149.4 (lipid βC), 139.1, 138.3, 137.9, 137.8, 136.9, 129.2, 128.6, 128.5, 128.29, 128.25, 128.0, 127.9, 127.7, 127.5, 127.3, 127.0, 126.4, 126.3, 125.5, 101.7 (benzylidene Ph*C*H), 100.5 (C-1’’), 92.1 (C-1), 91.7 (C-1’), 80.4 (C-4’’), 78.9 (C-4’), 78.7 (C-2), 77.3–76.7 (C-3 and C-3’ overlapped with $CDCl_3$), 75.3 (C-2’’), 75.0 (benzylic C), 74.3 (benzylic C), 73.8 (benzylic C), 73.1 (benzylic C), 72.5 (C-3’’), 71.7 (C-2’), 70.9 (C-5’), 69.5 (C-4), 68.6 (C-5), 68.2 (C-6’’), 67.6 (C-6’), 66.0 (C-5’’), 62.4 (C-6), 41.6 (lipid βC), 41.3 (lipid βC), 38.1 (levulinoyl C), 37.2, 36.9, 36.8, 36.6, 33.3 (lipid γC), 31.9, 30.5, 30.4, 30.13, 30.08, 29.8, 29.72, 29.68, 29.67, 29.6, 29.4, 27.9, 27.5, 26.8, 26.7, 22.7, 19.8, 19.6 (2 × CH_3), 17.8, 17.6, 14.1 (3 × CH_3), 12.5 (lipid αCH_3); IR (cast film): 3484, 3065, 2925, 2854, 1744, 1717, 1455, 1378, 1152, 1099 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{111}H_{164}NaO_{21}$, 1856.16573; found, 1856.16340.



4’’’,6’’’-*O*-benzylidene-2’’’-*O*-levulinoyl-3’’’-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl-(1’’’→3’’)-4’’,6’’-*O*-benzylidene-2’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.46). Glycosyl fluoride **4.37** (0.20 g, 0.40 mmol, 2.0 equiv), trisaccharide acceptor **4.45** (0.37 g, 0.20 mmol, 1.0 equiv), silver trifluoromethanesulfonate (0.21 g, 0.80 mmol, 4.0 equiv) and 4 Å molecular sieves (powder, 0.4 g) were mixed with CH_2Cl_2 (4 mL). The mixture was cooled to –78 °C and then bis(cyclopentadienyl)zirconium(IV) dichloride (0.12 g, 0.40 mmol, 2.0 equiv) was added. The cooling bath was removed and then the mixture was stirred for 1 h. The mixture was diluted with EtOAc and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:6→1:2 EtOAc–hexane) to give **4.46** (0.34 g, 74%) as a colorless film. $[\alpha]_D^{21}$ +21.1 (*c* 1.0 $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.80–7.72 (m, 4 H, ArH), 7.45–7.27 (m, 28 H, ArH), 7.25–7.19 (m, 5 H, ArH), 6.54 (dd, *J* = 9.8, 1.4 Hz, 1 H, lipid βH), 5.30 (s, 1 H, benzylidene H), 5.29 (d, *J* = 4.2 Hz, 1 H, H-1’), 5.22 (s, 1 H, benzylidene H), 5.16 (d, *J* = 2.8 Hz, 1 H, H-1), 5.08 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4), 5.03 (dd, *J* = 9.8, 4.2 Hz, 1 H,

H-2’), 5.01 (d, *J* = 10.2 Hz, 1 H, benzylic H), 4.98–4.94 (m, 3 H, H-2’’’ and benzylic H), 4.91 (dd, *J* = 8.4, 8.4 Hz, 1 H, H-2’’), 4.85 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.76 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.71–4.67 (m, 4 H, benzylic H and H-1’’’), 4.62 (d, *J* = 12.6 Hz, 1 H, benzylic H), 4.49–4.46 (m, 2 H, H-1’’ and benzylic H), 4.23 (dd, *J* = 10.5, 4.9 Hz, 1 H, H-6’’’), 4.10 (dd, *J* = 11.9, 5.6 Hz, 1 H, H-6), 4.05–3.95 (m, 7 H, H-6, H-5’, H-3, H-3’, H-4’, H-5 and H-6’’), 3.90 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4’’’), 3.80–3.76 (m, 2 H, H-6’ and H-3’’), 3.73 (dd, *J* = 9.1, 7.0 Hz, 1 H, H-3’’’), 3.65 (dd, *J* = 10.5, 10.5 Hz, 1 H, H-6’’’), 3.63–3.60 (m, 2 H, H-6’ and H-2), 3.46 (ddd, *J* = 9.1, 9.1, 5.6 Hz, 1 H, H-5’’’), 3.43 (d, *J* = 9.8, 9.8 Hz, 1 H, H-4’’), 3.13 (ddd, *J* = 9.8, 9.8, 4.9 Hz, 1 H, H-5’’), 3.04 (dd, *J* = 10.5, 9.8 Hz, 1 H, H-6’’), 2.64–2.62 (m, 2 H, levulinoyl H), 2.56–2.38 (m, 8 H, levulinoyl H, 2 × lipid αH and γH), 2.27–2.22 (m, 1 H, levulinoyl H), 2.08 (s, 3 H, levulinoyl CH_3), 1.85 (s, 3 H, levulinoyl CH_3), 1.80 (d, *J* = 1.4 Hz, 3 H, lipid αCH_3), 1.67–1.61 (m, 2 H, lipid βH), 1.37–0.95 (m, 75 H, lipid H and 3 × CH_3), 0.89–0.83 (m, 10 H, lipid H and 3 × CH_3), 0.80 (d, *J* = 7.0 Hz, 3 H, lipid CH_3), 0.72 (d, *J* = 6.3 Hz, 3 H, lipid CH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ): 206.2 (levulinoyl C=O), 205.6 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 171.4 (ester C=O), 171.1 (ester C=O), 167.9 (ester C=O), 149.4 (lipid βC), 139.2, 138.3, 137.81, 137.79, 137.3, 137.2, 135.8, 133.2, 132.9, 129.2, 128.9, 128.6, 128.5, 128.31, 128.28, 128.25, 128.20, 128.17, 128.05, 127.99, 127.9, 127.8, 127.6, 127.4, 127.3, 127.0, 126.6, 126.4, 126.2, 126.1, 126.04, 125.98, 125.8, 125.5, 101.2 (benzylidene Ph*C*H), 100.9 (benzylidene Ph*C*H), 100.6 (C-1’’), 99.7 (C-1’’’), 92.1 (C-1), 91.7 (C-1’), 80.6 (C-4’’’), 78.9 (C-4’), 78.7 (C-4’’), 78.4 (C-3’’’), 78.3 (C-2), 77.2–76.8 (C-3, C-3’ and C-3’’ overlapped with $CDCl_3$), 74.9 (benzylic C), 74.2 (benzylic C and C-2’’), 73.8 (benzylic C), 73.39 (benzylic C), 73.37 (C-2’’’), 72.9 (benzylic C), 71.6 (C-2’), 71.0 (C-5’), 69.5 (C-4), 68.7 (C-5), 68.5 (C-6’’’), 68.2 (C-6’’), 67.5 (C-6’), 66.3 (C-5’’), 65.8 (C-5’’’), 62.4 (C-6), 41.6 (lipid βC), 41.3 (lipid βC), 37.8, 37.5, 37.2, 36.9, 36.84,

36.79, 36.6, 33.3, 31.9, 30.5, 30.4, 30.14, 30.07, 29.78, 29.75, 29.71, 29.69, 29.67, 29.6, 29.4, 29.3, 27.7, 27.5, 26.8, 26.7, 22.7, 19.8, 19.6, 19.5, 17.7, 17.5, 14.1 (3 × CH_3), 12.5 (lipid αCH_3); IR (cast film): 3064, 2925, 2854, 1745, 1718, 1455, 1378, 1146, 1099 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{140}H_{192}NaO_{28}$, 2344.3492; found, 2344.3459.

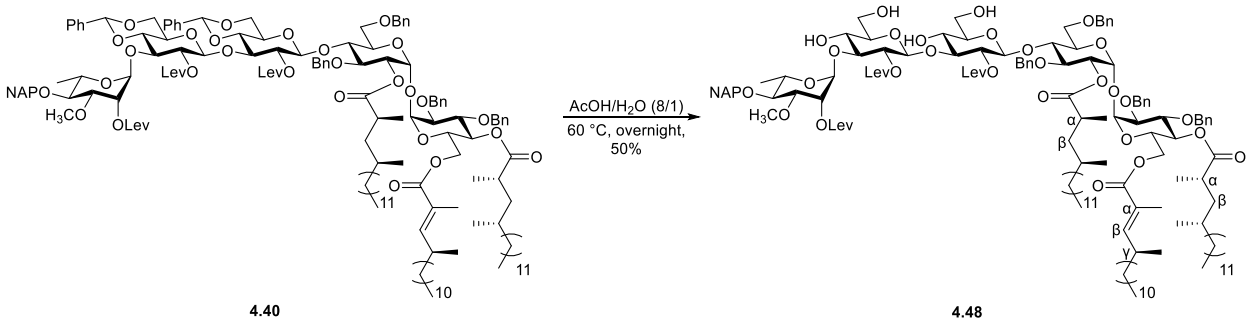


4.46 4.47

4’’’,6’’’-*O*-Benzylidene-2’’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’’→3’’)-4’’,6’’-*O*-benzylidene-2’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.47). Tetrasaccharide **4.46** (0.34 g, 0.15 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (1.5 mL) and H_2O (0.15 mL) and then 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (50 mg, 0.22 mmol, 1.5 equiv) was added. The mixture was stirred overnight at which point TLC (1:2 EtOAc–hexane) indicated no further progression of the reaction. The mixture was diluted with EtOAc and saturated $NaHCO_{3(aq)}$ was added before being transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_{3(aq)}$, H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (150 mL silica gel, 0:1→1:2→1:1 EtOAc–hexane) to give **4.47** (0.24 g, 73%) as a colorless film. $[\alpha]_D^{21}$ +34.0 (*c* 0.1, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ): 7.46–7.27 (m, 25 H,

ArH), 7.25–7.20 (m, 5 H, ArH), 6.54 (dd, *J* = 9.8, 1.4 Hz, 1 H, lipid βH), 5.322 (s, 1 H, benzylidene H), 5.320 (s, 1 H, benzylidene H), 5.29 (d, *J* = 4.2 Hz, 1 H, H-1'), 5.17 (d, *J* = 3.5 Hz, 1 H, H-1), 5.08 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4), 5.03 (dd, *J* = 9.8, 4.2 Hz, 1 H, H-2'), 5.00 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.95 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.91 (dd, *J* = 9.1, 8.4 Hz, 1 H, H-2''), 4.86 (dd, *J* = 8.4, 7.7 Hz, 1 H, H-2'''), 4.77 (d, *J* = 12.6 Hz, 1 H, benzylic H), 4.71–4.68 (m, 4 H, H-1''' and benzylic H), 4.62 (d, *J* = 12.6 Hz, 1 H, benzylic H), 4.51 (d, *J* = 8.4 Hz, 1 H, H-1''), 4.49 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.26 (dd, *J* = 10.5, 9.1 Hz, 1 H, H-6'''), 4.10 (dd, *J* = 11.9, 6.3 Hz, 1 H, H-6), 4.07–3.97 (m, 7 H, H-5', H-6, H-3, H-3', H-4', H-6'' and H-5), 3.89 (ddd, *J* = 8.4, 8.4, 3.5 Hz, 1 H, H-3'''), 3.81–3.77 (m, 2 H, H-6' and H-3''), 3.70–3.63 (m, 3 H, H-6''', H-4''' and H-6'), 3.61 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2), 3.46 (ddd, *J* = 9.1, 9.1, 3.5 Hz, 1 H, H-5'''), 3.43 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4''), 3.14 (ddd, *J* = 9.8, 9.1, 3.5 Hz, 1 H, H-5''), 3.06 (dd, *J* = 10.5, 9.8 Hz, 1 H, H-6''), 2.87–2.82 (m, 1 H, levulinoyl H), 2.77–2.74 (m, 2 H, 3'''-OH and levulinoyl H), 2.73–2.59 (m, 2 H, levulinoyl H), 2.55–2.42 (m, 6 H, levulinoyl H, 2 × lipid αH and γH), 2.33–2.28 (m, 1 H, levulinoyl H), 2.16 (s, 3 H, levulinoyl CH_3), 1.94 (s, 3 H, levulinoyl CH_3), 1.80 (d, *J* = 1.4 Hz, 3 H, lipid αCH_3), 1.67–1.61 (m, 2 H, lipid βH), 1.36–0.96 (m, 75 H, lipid H and 3 × CH_3), 0.89–0.83 (m, 10 H, lipid H and 3 × CH_3), 0.80 (d, *J* = 7.0 Hz, 3 H, lipid CH_3), 0.72 (d, *J* = 6.3 Hz, 3 H, lipid CH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ): 206.9 (levulinoyl C=O), 205.7 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 172.1 (ester C=O), 171.0 (ester C=O), 167.8 (ester C=O), 149.4 (lipid βC), 139.1, 138.3, 137.8, 137.2, 137.0, 129.1, 128.6, 128.5, 128.2, 128.2, 128.2, 128.03, 128.99, 127.93, 127.8, 127.4, 127.3, 127.0, 126.4, 126.2, 126.1, 125.4, 101.6 (benzylidene Ph*C*H), 101.0 (benzylidene Ph*C*H), 100.6 (C-1''), 99.8 (C-1'''), 92.1 (C-1), 91.7 (C-1'), 80.4 (C-4'''), 78.8 (C-4'), 78.7 (C-4''), 78.3 (C-2), 77.4 (C-3''), 77.2–76.8 (C-3 and C-3' overlapped with $CDCl_3$), 74.95 (C-2'''), 74.89 (benzylic C), 74.2 (benzylic C), 74.1 (C-2''), 73.8 (benzylic C), 72.9

(benzylic C), 72.3 (C-3’’’), 71.6 (C-2’), 71.0 (C-5’), 69.5 (C-4), 68.6 (C-5), 68.5 (C-6’’’), 68.2 (C-6’’), 67.5 (C-6’), 66.2 (C-5’’), 65.9 (C-5’’’), 62.3 (C-6), 41.6 (lipid βC), 41.3 (lipid βC), 38.3, 37.6, 37.2, 36.9, 36.83, 36.77, 36.6, 33.3, 31.9, 30.5, 30.4, 30.13, 30.06, 29.8, 29.74, 29.72, 29.70, 29.68, 29.67, 29.65, 29.63, 29.43, 29.35, 27.9, 27.6, 27.5, 26.75, 26.72, 22.7, 19.8, 19.55, 19.52, 17.7, 17.5, 14.1 (3 ×CH_3), 12.5 (lipid αCH_3); IR (cast film): 3469, 3033, 2925, 2854, 1745, 1717, 1455, 1378, 1148, 1099 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{129}H_{184}NaO_{28}$, 2204.2866; found, 2204.2866.

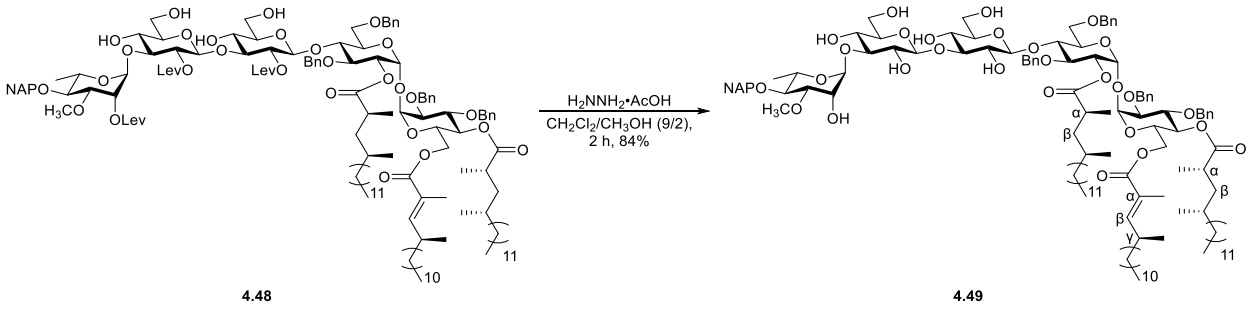


4.40 4.48

2’’’’-*O*-Levulinoyl-3’’’’-*O*-methyl-4’’’’-*O*-(2-naphthylmethyl)-α-L-rhamopyranosyl-(1’’’’→3’’’)-2’’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’’→3’’)-2’’-*O*-levulinoyl-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.48). Pentasaccharide **4.40** (0.12 g, 47 μmol, 1.0 equiv) was dissolved in acetic acid (0.8 mL) and H_2O (0.1 mL). The mixture was heated and stirred at 60 °C overnight at which point TLC (1:1 EtOAc–hexane) indicated no further progression of the reaction. The mixture was cooled to room temperature and was dried by dissolving in and evaporation with toluene (3 × 5 mL) on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:2→1:1 EtOAc–hexane) to give

4.48 (56 mg, 50%) as a colorless film. $[\alpha]_D^{21}$ +38.0 (*c* 0.03, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ): 7.83–7.78 (m, 4 H, ArH), 7.48–7.27 (m, 17 H, ArH), 7.25–7.19 (m, 6 H, ArH), 6.54 (dd, *J* = 9.8, 1.4 Hz, 1 H, lipid βH), 5.28 (d, *J* = 4.2 Hz, 1 H, H-1'), 5.21 (dd, *J* = 2.8, 2.1 Hz, 1 H, H-2''''), 5.18 (d, *J* = 3.5 Hz, 1 H, H-1), 5.09–5.03 (m, 3 H, H-4, H-2' and benzylic H), 5.00 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.95 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.84 (dd, *J* = 9.8, 8.4 Hz, 1 H, H-2'''), 4.81–4.77 (m, 4 H, H-1'''', H-2'' and benzylic H), 4.70–4.69 (m, 3 H, benzylic H), 4.62 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.46 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.46 (d, *J* = 7.7 Hz, 1 H, H-1'''), 4.39 (d, *J* = 8.4 Hz, 1 H, H-1''), 4.12–4.07 (m, 2 H, H-6 and H-5'), 4.05–3.97 (m, 7 H, H-6, OH, H-3', H-3, H-4', H-6''' and H-5), 3.94 (dq, *J* = 9.1, 6.3 Hz, 1 H, H-5''''), 3.81–3.76 (m, 2 H, H-6' and H-6'''), 3.66–3.60 (m, 4 H, H-3'''', H-6', H-3''' and H-2), 3.55–3.51 (m, 2 H, H-5''' and H-6''), 3.49–3.46 (m, 1 H, H-4'''), 3.43–3.40 (m, 4 H, OCH_3 and H-4''''), 3.38 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-3''), 3.30 (dd, *J* = 9.8, 9.1 Hz, 1 H, H-4''), 3.26 (br s, 1 H, OH), 3.10–3.07 (m, 1 H, H-5''), 3.04–3.02 (m, 1 H, H-6''), 2.88–2.83 (m, 1 H, levulinoyl H), 2.80–2.62 (m, 7 H, levulinoyl H), 2.59–2.41 (m, 6 H, levulinoyl H, 2 × lipid αH and γH), 2.28–2.24 (m, 1 H, levulinoyl H), 2.17 (s, 3 H, levulinoyl CH_3), 2.16 (s, 3 H, levulinoyl CH_3), 2.12 (br s 1 H, OH), 1.97 (s, 3 H, levulinoyl CH_3), 1.80 (d, *J* = 1.4 Hz, 3 H, lipid αCH_3), 1.67–1.60 (m, 3 H, lipid H), 1.37–0.96 (m, 77 H, H-6'''', lipid H and 3 × CH_3), 0.89–0.87 (m, 10 H, lipid H and 3 × CH_3), 0.79 (d, *J* = 7.0 Hz, 3 H, lipid CH_3), 0.72 (d, *J* = 6.3 Hz, 3 H, lipid CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 206.2 (2 × levulinoyl C=O), 205.6 (levulinoyl C=O), 175.8 (ester C=O), 175.3 (ester C=O), 171.9 (ester C=O), 171.7 (ester C=O), 171.1 (ester C=O), 167.8 (ester C=O), 149.4 (lipid βC), 138.8, 138.3, 137.8, 137.7, 135.8, 133.3, 133.0, 128.7, 128.5, 128.3, 128.2, 128.1, 128.0, 127.92, 127.87, 127.8, 127.7, 127.4, 127.3, 126.4, 126.10, 126.07, 125.9, 125.8, 125.4, 100.0 (C-1''), 99.9 (C-1'''), 99.6 (C-1''''), 92.0 (C-1), 91.7 (C-1'), 85.8 (C-3'''), 82.8 (C-3''), 79.4 (C-4'''' and C-3''''), 79.0 (C-

3’), 78.8 (C-2), 78.3 (C-3), 77.3–76.7 (C-4’ overlapped with $CDCl_3$), 75.9 (C-5’’’), 75.5 (C-5’’), 75.2 (benzylic C), 74.9 (benzylic C), 74.1 (benzylic C), 73.9 (benzylic C), 73.2 (benzylic C), 72.9 (C-2’’), 71.44 (C-2’), 71.39 (C-2’’’), 71.0 (C-5’), 70.4 (C-4’’’), 69.6 (C-4), 69.1 (C-5’’’’), 68.9 (C-4’’ and C-2’’’’), 68.5 (C-5), 67.4 (C-6’), 62.5 (C-6’’’), 62.4 (C-6), 62.0 (C-6’’), 57.6 (OCH_3), 41.6 (lipid βC), 41.3 (lipid βC), 38.0, 37.9, 37.6, 37.2, 36.81, 36.77, 36.6, 33.3, 31.9, 30.5, 30.4, 30.14, 30.06, 29.8, 29.70, 29.66, 29.65, 29.6, 29.42, 29.35, 28.0, 27.5, 26.8, 26.7, 22.7, 19.8, 19.58, 19.49, 18.0 (C-6’’’’), 17.6, 17.5, 14.1 (3 × CH_3), 12.5 (lipid αCH_3); IR (cast film): 3423, 3030, 2925, 2854, 1745, 1719, 1455, 1363, 1156, 1102 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{138}H_{202}NaO_{34}$, 2426.3970; found, 2426.3952.

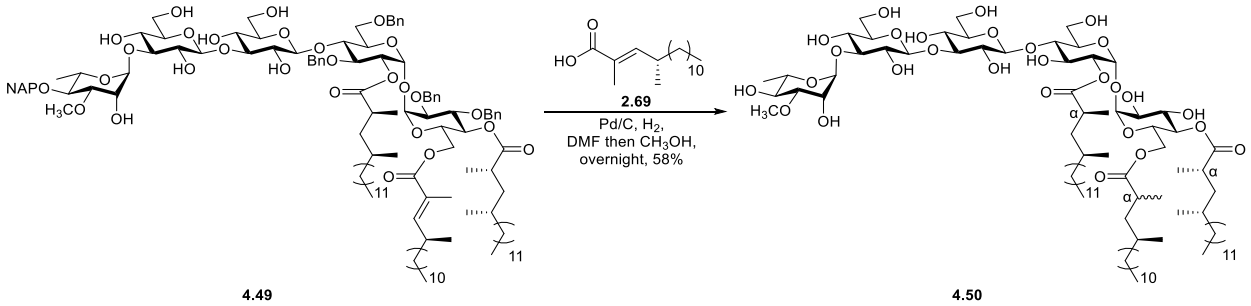


4.48 4.49

3’’’’-*O*-Methyl-4’’’’-*O*-(2-naphthylmethyl)-α-L-rhamopyranosyl-(1’’’’→3’’’)-β-D-glucopyranosyl-(1’’’→3’’)-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((*S*,*E*)-2,4-dimethylpentadec-2-enoyl)-α-D-glucopyranoside (4.49). Pentasaccharide **4.48** (56 mg, 23 μmol, 1.0 equiv) was dissolved in CH_2Cl_2 (0.36 mL) before hydrazine acetate (7.5 mg, 81 μmol, 3.5 equiv) dissolved in CH_3OH (40 μL) was added. The mixture was stirred at room temperature for 1 h and then another portion of hydrazine acetate (7.5 mg, 81 μmol, 3.5 equiv) dissolved in CH_3OH (40 μL) was added. The mixture was stirred at

room temperature for 1 h and then diluted with EtOAc before being transferred to a separatory funnel. The organic layer was washed with H_2O, brine, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated on a rotary evaporator. The crude residue was purified by column chromatography (50 mL silica gel, 0:1→1:1→2:1→4:1→1:0 EtOAc–hexane) to give **4.49** (41 mg, 84%) as a colorless film. $[\alpha]_D^{21}$ +37.5 (*c* 0.02, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ): 7.84–7.78 (m, 4 H, ArH), 7.49–7.45 (m, 3 H, ArH), 7.37–7.27 (m, 17 H, ArH), 7.24–7.23 (m, 3 H, ArH), 6.56 (dd, *J* = 10.5, 1.4 Hz, 1 H, lipid βH), 5.28 (d, *J* = 4.2 Hz, 1 H, H-1'), 5.18 (d, *J* = 3.5 Hz, 1 H, H-1), 5.13 (dd, *J* = 9.8, 9.8 Hz, 1 H, H-4), 5.11 (d, *J* = 1.4 Hz, 1 H, H-1''''), 5.01 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2'), 4.99 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.98 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.91 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.80 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.78 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.72 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.70 (d, *J* = 11.2 Hz, 1 H, benzylic H), 4.63 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.60 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.47 (d, *J* = 11.9 Hz, 1 H, benzylic H), 4.40 (d, *J* = 7.7 Hz, 1 H, H-1''), 4.42 (d, *J* = 7.0 Hz, 1 H, H-1'''), 4.20–4.19 (m, 1 H, H-2''''), 4.13–3.98 (m, 9 H, OH, H-3, H-3', H-4', H-5, H-5', H-5'''' and 2 × H-6), 3.93–3.91 (m, 1 H, H-6'''), 3.78–3.72 (m, 2 H, H-6''' and H-6'), 3.67 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-2), 3.65 (dd, *J* = 8.4, 3.5 Hz, 1 H, H-3''''), 3.61 (br s, 1 H, 4''-OH), 3.53–3.47 (m, 9 H, OCH_3, H-6'', H-4'''', H-2''', H-4''', H-3''' and H-6'), 3.44–3.42 (m, 1 H, H-5'''), 3.39 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4''), 3.33–3.30 (m, 2 H, H-2'' and H-6''), 3.24–3.22 (m, 2 H, H-3'' and OH), 3.16 (br s, 1 H, 2''-OH), 2.99–2.97 (m, 1 H, H-5''), 2.61 (br s, 1 H, 2''''-OH), 2.53–2.44 (m, 3 H, 2 × lipid αH and γH), 2.21 (br s, 1 H, 6'''-OH), 1.82 (d, *J* = 1.4 Hz, 3 H, lipid αCH_3), 1.68–1.63 (m, 6 H, lipid H), 1.60–1.59 (m, 1 H, 6''-OH), 1.37–0.98 (m, 74 H, H-6'''', lipid H and 3 × CH_3), 0.89–0.86 (m, 10 H, lipid H and 3 × CH_3), 0.81 (d, *J* = 7.0 Hz, 3 H, lipid CH_3), 0.69 (d, *J* = 7.0 Hz, 3 H, lipid CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 175.8 (ester C=O), 175.3 (ester C=O), 167.8 (ester C=O),

149.4 (lipid βC), 138.6, 138.1, 137.8, 137.3, 135.6, 133.3, 133.0, 128.6, 128.4, 128.34, 128.30, 128.2, 128.1, 128.0, 127.94, 127.88, 127.7, 127.65, 127.6, 127.4, 127.3, 126.6, 126.2, 126.0, 125.95, 125.9, 125.5, 104.6 (C-1’’’), 102.5 (C-1’’), 101.1 (C-1’’’’), 91.8 (C-1), 91.7 (C-1’), 88.1 (C-3’’), 86.9, 81.0 (C-3’’’’), 79.2, 79.1 (C-2), 79.0, 77.9, 76.9, 76.0, 75.4 (benzylic C), 75.0 (benzylic C), 74.1 (benzylic C), 73.8 (benzylic C), 73.5 (benzylic C), 73.4 (C-2’’), 73.0, 72.0 (C-2’), 70.5, 69.54 (C-4), 69.51, 68.9 (C-4’’), 68.8 (C-5’’’’), 68.6, 68.2 (C-2’’’’), 67.8 (C-6’), 62.4 (C-6’’’), 62.3 (C-6), 61.9 (C-6’’), 57.7 (OCH_3), 41.4 (lipid βC), 41.3 (lipid βC), 37.2, 36.9, 36.8, 36.6, 33.3 (lipid γC), 31.9, 30.53, 30.46, 30.10, 30.06, 29.8, 29.70, 29.67, 29.66, 29.4, 27.5, 26.8, 26.7, 22.7, 19.8, 19.5 (2 × CH_3), 17.9 (C-6’’’’), 17.7, 17.6, 14.1 (3 × CH_3), 12.5 (lipid αCH_3); IR (cast film): 3433, 3030, 2925, 2854, 1743, 1717, 1455, 1377, 1081, 1027 cm^{-1}; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{123}H_{184}NaO_{28}$, 2132.2866; found, 2132.2874.



4.49 4.50

3’’’’-*O*-Methyl-α-L-rhamopyranosyl-(1’’’’→3’’’)-β-D-glucopyranosyl-(1’’’→3’’)-β-D-glucopyranosyl-(1’’→4’)-3’,6’-di-*O*-benzyl-2’-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-α-D-glucopyranosyl-(1’↔1)-2,3-di-*O*-benzyl-4-*O*-((2*S*,4*S*)-2,4-dimethylhexadecanoyl)-6-*O*-((4*S*)-2,4-dimethylpentadecanoyl)-α-D-glucopyranoside (4.50). Pentasaccharide **4.49** (27 mg, 13 μmol, 1.0 equiv) and lipid **2.69** (17 mg, 64 μmol, 5.0 equiv) was dissolved in DMF (3.0 mL) and then palladium (10% on activated carbon, reduced, nominally 50% water wet, 27 mg) was added.

The flask was attached with a H_2 balloon and then the mixture was purged three times with H_2. The mixture was stirred at room temperature and lipid **2.69** (17 mg, 64 μmol, 5.0 equiv) was added, followed by purging the mixture three times with H_2 every 20 minutes for 8 h at which point TLC (10:1 $CHCl_3$–CH_3OH) indicated no **4.49** remained and no further progression of the reaction. The mixture was filtered over a pad of Celite® 545 and dried by dissolving in and evaporation with heptane (3 × 100 mL) on a rotary evaporator. The mixture was dissolved in CH_3OH (3.0 mL) and then palladium (10% on activated carbon, reduced, nominally 50% water wet, 27 mg) was added. The flask was attached with a H_2 balloon and then the mixture was purged three times with H_2. The mixture was stirred at room temperature for 6 h, lipid **2.69** (0.19 g, 0.69 mmol, 54 equiv) was added and the mixture was purged three times with H_2 and then stirred for 20 h. After which time, lipid **2.69** (0.19 g, 0.69 mmol, 54 equiv) was added and the mixture was purged three times with H_2 and then stirred for 8 h at which point TLC (5:1 $CHCl_3$–CH_3OH) indicated a major product spot. The mixture was filtered over a pad of Celite® 545 and concentrated on a rotary evaporator. The crude residue was purified by column chromatography (40 mL silica gel, 1:0→5:1 $CHCl_3$–CH_3OH) to give an inseparable mixture of LOS-I **4.39** and **4.50** (1:5 by ^1H NMR) as a colorless film. LOS-I **4.39** HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{84}H_{152}NaO_{28}$, 1632.03624; found, 1632.03651. The inseparable mixture of **4.39** and **4.50** was dissolved in CH_3OH (3.0 mL) and palladium (10% on activated carbon, reduced, nominally 50% water wet, 27 mg) was added. The flask was attached with a H_2 balloon and then the mixture was purged three times with H_2. The mixture was stirred at room temperature overnight at which point ^1H NMR spectroscopy indicated the absence of the alkenyl resonance. The mixture was filtered over a pad of Celite® 545 and concentrated on a rotary evaporator. The crude residue was purified by column chromatography (40 mL silica gel, 1:0→5:1 $CHCl_3$–CH_3OH) to give a diastereomeric mixture of

4.50 (12 mg, 58%, dr = 1:1 by ^{1}H NMR spectroscopy) as a colorless film. ^{1}H NMR (600 MHz, 2:1 $CDCl_3$–CD_3OD, δ): 5.01–5.00 (m, 1 H, H-1'), 4.92–4.91 (m, 1 H, H-1''''), 4.90–4.89 (m, 1 H, H-1), 4.68–4.63 (m, 1 H, H-4), 4.61–4.58 (m, 1 H, H-2'), 4.28–4.24 (m, 2 H, H-1''' and H-1''), 3.91–3.83 (m, 3 H, H-2'''', H-3' and H-6), 3.78–3.59 (m, 9 H, H-6, H-5', H-5, H-5'''', H-6'', H-6''', H-3 and 2 × H-6'), 3.48–3.42 (m, 3 H, H-6'', H-6''' and H-4'), 3.38–3.36 (m, 1 H, H-2), 3.31–3.14 (m, 13 H, H-3'', H-3''', OCH_3, H-4'''', H-4'', H-4''', H-2''', H-2'', H-3'''', H-5''' and H-5''), 2.41–2.28 (m, 3 H, 3 × lipid αH), 1.52–1.42 (m, 3 H, lipid H), 1.29–0.85 (m, 82 H, H-6'''', 3 × lipid CH_3 and H), 0.68–0.60 (m, 18 H, 6 × lipid CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ): 176.8 (ester C=O), 176.6 (ester C=O), 176.1 (ester C=O), 176.0 (ester C=O), 103.6 (C-1'''), 102.1 (C-1''), 100.8 (C-1''''), 92.9 (C-1), 90.7 (C-1'), 86.7 (C-3''), 83.2 (C-3'''), 80.1 (C-3''''), 79.4 (C-4'), 76.2 (C-5'' or C-5'''), 76.0 (C-5'' or C-5'''), 73.8 (C-2'' or C-2'''), 72.3 (C-2'' or C-2'''), 71.9 (C-2'), 71.3 (C-2), 70.9 (C-3 and C-4''''), 70.4 (C-5'), 69.9, 69.8 (C-4), 69.0 (C-3'), 68.5 (C-5), 68.2 (C-4'' and C-4'''), 68.0, 67.9 (C-5''''), 66.5 (C-2''''), 61.8 (C-6), 61.1 (C-6'''), 60.9 (C-6''), 60.4 (C-6'), 56.3 (OCH_3), 41.0, 40.94, 40.89, 40.2, 37.0, 36.84, 36.76, 36.60, 36.56, 36.4, 31.5, 30.3, 30.2, 30.0, 29.9, 29.6, 29.5, 29.3, 29.24, 29.18, 28.9, 26.4, 22.2, 19.05, 18.9, 18.6, 17.23, 17.17, 17.1, 16.7 (C-6''''), 16.3, 13.4; HRMS–MALDI–FTICR (*m*/*z*): $[M+Na]^+$ calcd for $C_{84}H_{154}NaO_{28}$, 1634.05189; found, 1634.05141.

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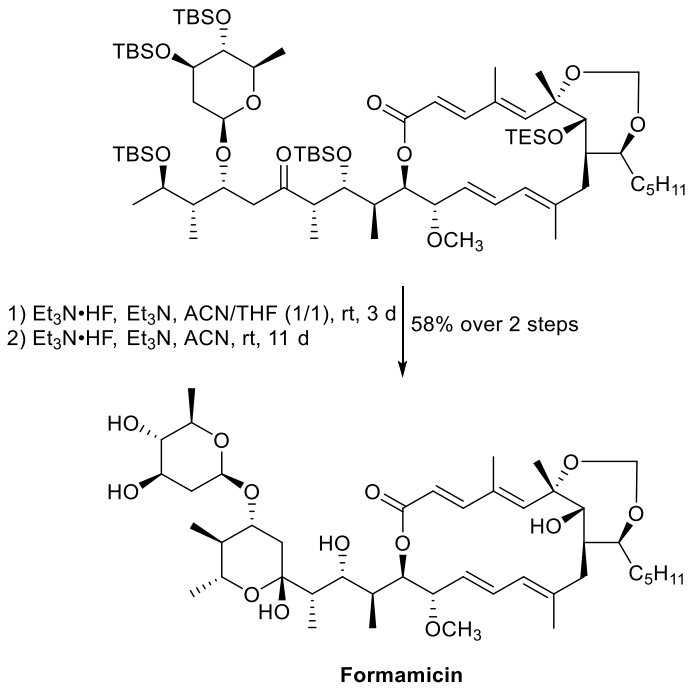
Chapter 5 : Summary and future work

My work in carbohydrate synthesis of more than six years was recorded in the previous four chapters and will be summarized in this last chapter. Chapter 1 stated the reason for synthesizing *M. marinum* LOSs: the molecules themselves are interesting and could be used to study host–pathogen interactions. The molecules were challenging to synthesize because they have moieties that are difficult to construct. In Chapter 2, the synthesis of the desired asymmetrically-triacylated trehalose core was described. Two optically pure fatty acids were synthesized and then attached to the trehalose core. The trehalose core was synthesized via a standard glycosylation between a glycosyl donor and acceptor, but the yields were lower on larger scales. This problem needs to be solved to produce larger quantities of materials for the LOSs synthesis. In Chapter 3, my attempts to synthesize a derivative of caryophyllose suitable for glycosylation were described. Following the literature, the key C–C bond formation step could not be optimized to produce desirable yields; therefore, a different bond formation strategy was suggested. In Chapter 4, the trehalose core synthesized in Chapter 2 was successfully glycosylated to reach the pentasaccharide core of *M. marinum* LOS-I. The key glycosylation method was to apply glycosyl fluorides as glycosyl donors. The final deprotection step used a different hydrogenolysis approach, but still gave a mixture of the desired LOS-I and alkene-reduced LOS-I. This final deprotection step needs to be solved to enable further pursuit of synthesizing LOSs-II–IV.

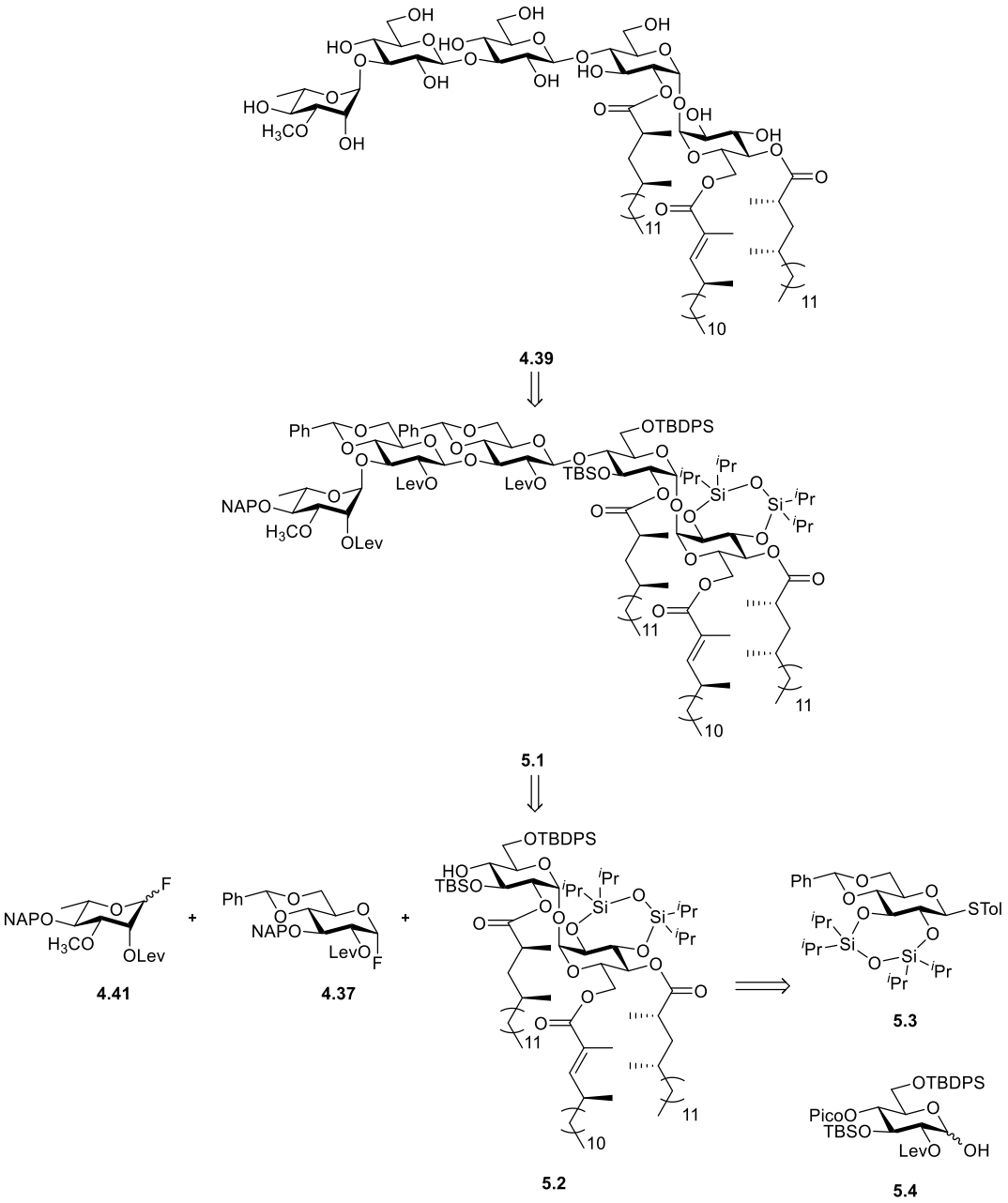
5.1 Short-term goal of synthesizing LOSs in *M. marinum*

The short-term goal is to solve the problems mentioned in the previous paragraph and successfully synthesize caryophyllose and LOS-I **4.39** (**Scheme 5.2**). I propose to revise the strategy so that the final deprotection step does not require hydrogenolysis. This will be accomplished by using silyl deprotection, which was featured in the final step of the synthesis of

formamicin (**Scheme 5.1**).[1] Therefore as shown in **Scheme 5.2**, the protecting groups will be switched from benzylic ethers to silyl ethers as in **5.1**. Pentasaccharide **5.1** could be constructed using glycosyl fluorides **4.37** and **4.41** as in Chapter 4 with glycosyl acceptor **5.2**. Trehalose **5.2** could be glycosylated with thioglycoside **5.3** and acceptor **5.4** using Crich's method.[2]

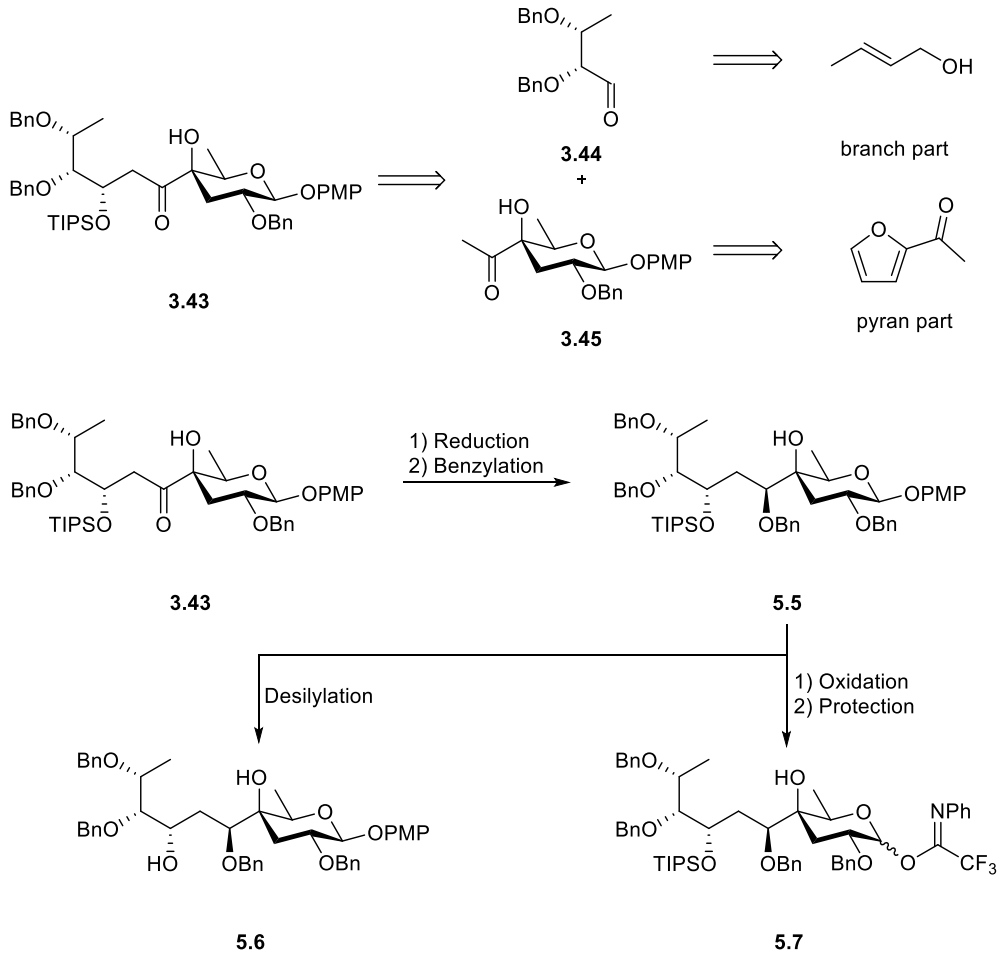


Scheme 5.1. Final desilylation step to generate formamicin.[1]



Scheme 5.2. Revised retrosynthetic plan of synthesizing LOS-I **4.39**.

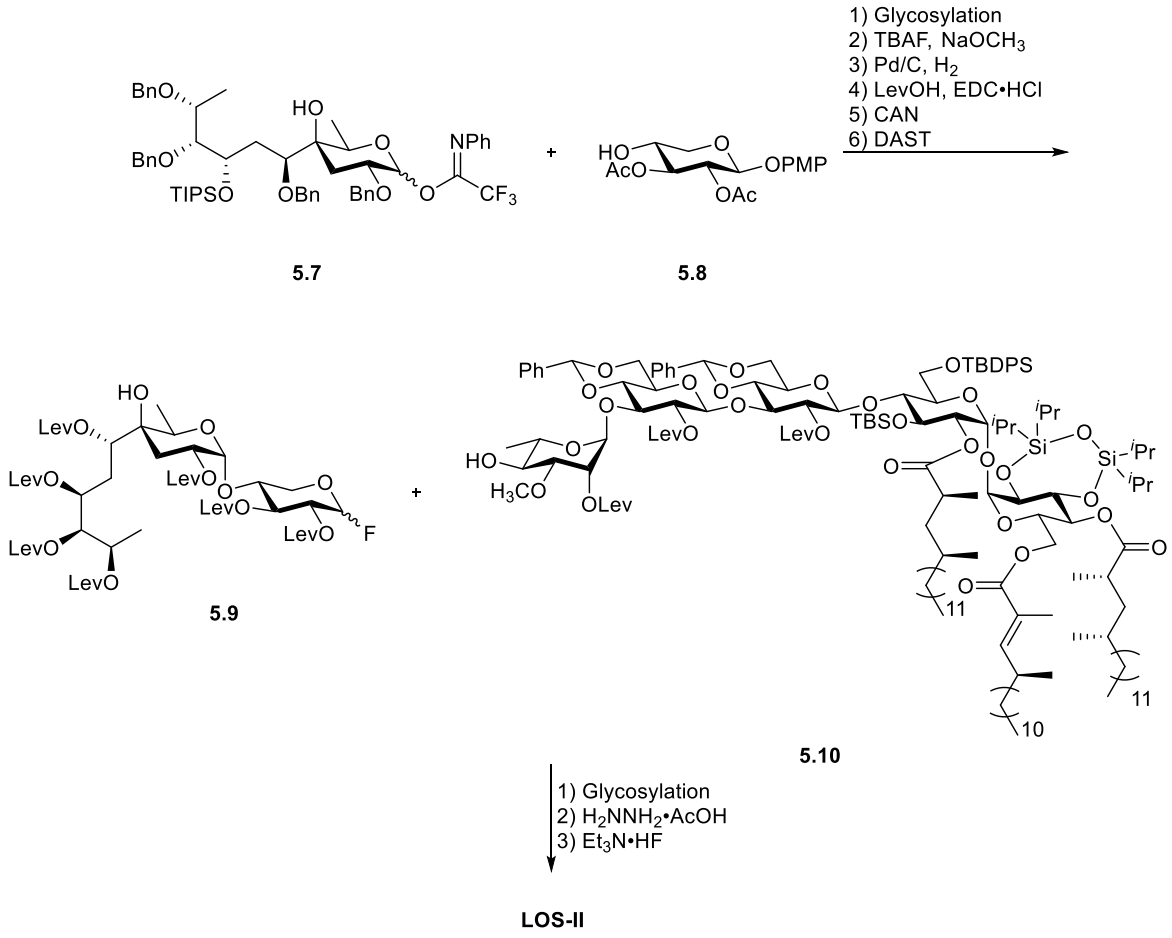
The revised plan for synthesizing caryophyllose was described in Chapter 3. Shown in **Scheme 5.3**, the desired caryophyllose backbone in **3.43** could be constructed via a boron aldol reaction between aldehyde **3.44** and ketone **3.45**. Once **3.43** was obtained, further steps to convert the caryophyllose into suitable glycosyl donors and acceptors could be carried out. The carbonyl group in ketone **3.43** could be reduced and the resulting alcohol then benzylated to give **5.5**. The silyl group of **5.5** could be deprotected to give glycosyl acceptor **5.6**. Alternatively, the anomeric *p*-methoxyphenyl group of **5.5** could be oxidatively deprotected and then converted to an imidate to serve as the glycosyl donor **5.7**. This concludes the short-term goals.



Scheme 5.3. Revised plan to synthesize a caryophyllose derivative suitable for glycosylation.

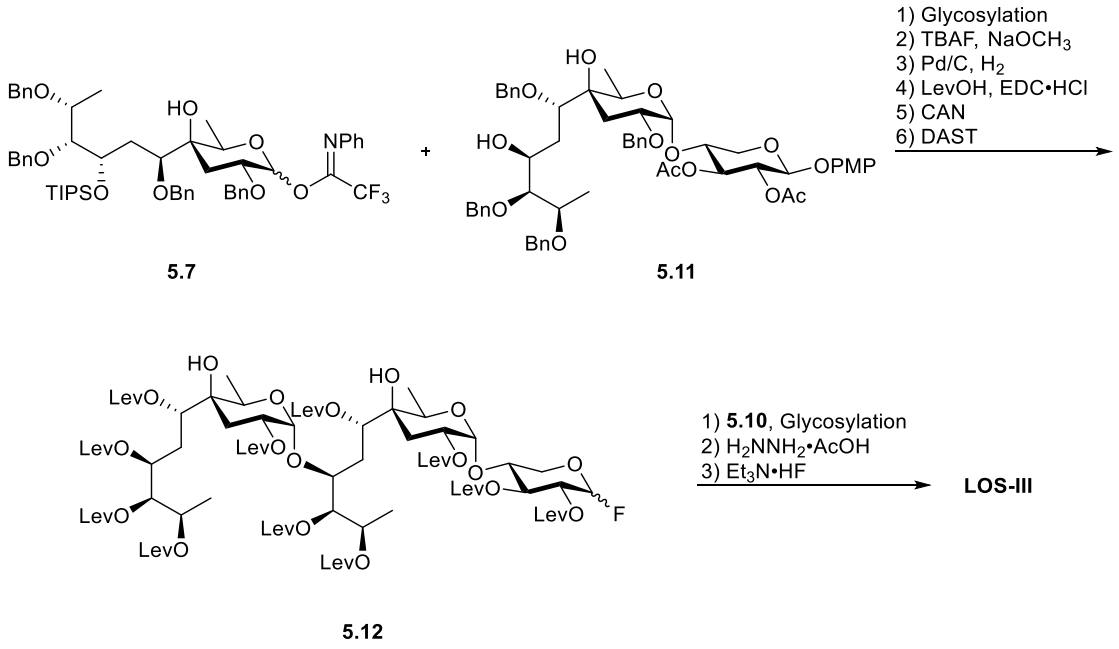
5.2 Long-term goal of synthesizing LOSs in *M. marinum*

The long-term goal will be focused on synthesizing LOS-II–IV. For the synthesis of LOS-II (**Scheme 5.4**), glycosyl donor **5.7** could be coupled with glycosyl acceptor **5.8** using reported conditions to hopefully maximize the α-selectivity.[3,4] The resulting disaccharide could be modified in few steps to give glycosyl fluoride **5.9**. The modification steps include deprotecting the acetyl and silyl groups using tetra-*n*-butylammonium fluoride and sodium methoxide, then debenzylation using hydrogenolysis. The revealed hydroxyl groups, possibly except the tertiary one, could be protected with levulinoyl esters. Next, converting the anomeric *p*-methoxyphenyl group to a fluoride using cerium ammonium nitrate then (diethylamino)sulfur trifluoride would give **5.9**. Glycosyl fluoride **5.9** could be coupled with pentasaccharide acceptor **5.10** and then, after deprotecting the levulinoyl esters using hydrazine acetate, the silyl groups using triethylamine–hydrogen fluoride, give LOS-II.



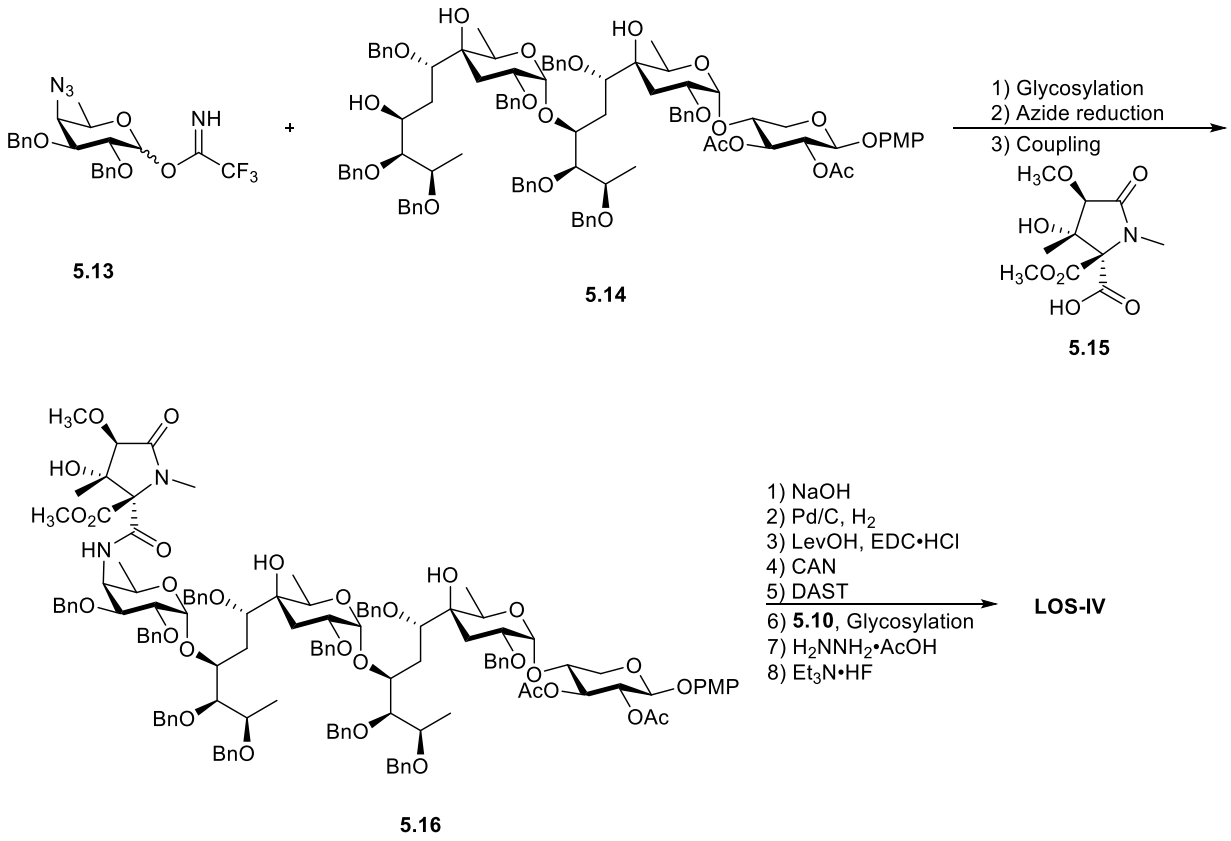
Scheme 5.4. Proposed synthetic plan for LOS-II.

The synthesis of LOS-III (**Scheme 5.5**) would be similar to the synthesis of LOS-II. Glycosyl donor **5.7** could be coupled with disaccharide acceptor **5.11** (obtained in a few steps after coupling **5.7** and **5.8**) and, after modification of the resulting trisaccharide (same steps as used for obtaining **5.9**, **Scheme 5.4**), would give glycosyl fluoride **5.12**. Glycosylation between glycosyl fluoride **5.12** and pentasaccharide acceptor **5.10** would produce the desired heptasaccharide, which would give the desired LOS-III after a series of deprotection steps as in **Scheme 5.4**.



Scheme 5.5. LOS-III synthetic plan.

The plan for LOS-IV would be similar to the previous schemes but with a few extra steps (**Scheme 5.6**). D-Fucosyl donor **5.13** (synthesized from glucose) could be coupled with trisaccharide acceptor **5.14** to give a tetrasaccharide. The azide in the tetrasaccharide could be reduced and then coupled with carboxylic acid **5.15**[5] to complete the *N*-acylfucosyl moiety in LOS-IV (compound **5.16**). The tetrasaccharide **5.16** could be modified by: 1) deprotecting the methyl ester of the carboxylic acid and the acetyl group using sodium hydroxide 2) debenzylation using hydrogenolysis 3) protect the hydroxyl groups with levulinoyl esters 4) convert to a glycosyl fluoride using CAN and DAST and then coupled with pentasaccharide acceptor **5.10**. The resulting oligosaccharide will undergo a series of deprotection steps as in **Scheme 5.4** to give LOS-IV.



Scheme 5.6. LOS-IV synthetic plan.

After having successfully synthesized LOSs-I–IV (**Figure 5.1**), we will carry out bioassays for the purpose of "understanding *M. marinum* LOSs' immunogenic properties on a molecular level" described in Chapter 1. Synthesizing these complex LOSs and what more could we find out about their protein-binding profiles will be another exciting journey to look forward to.

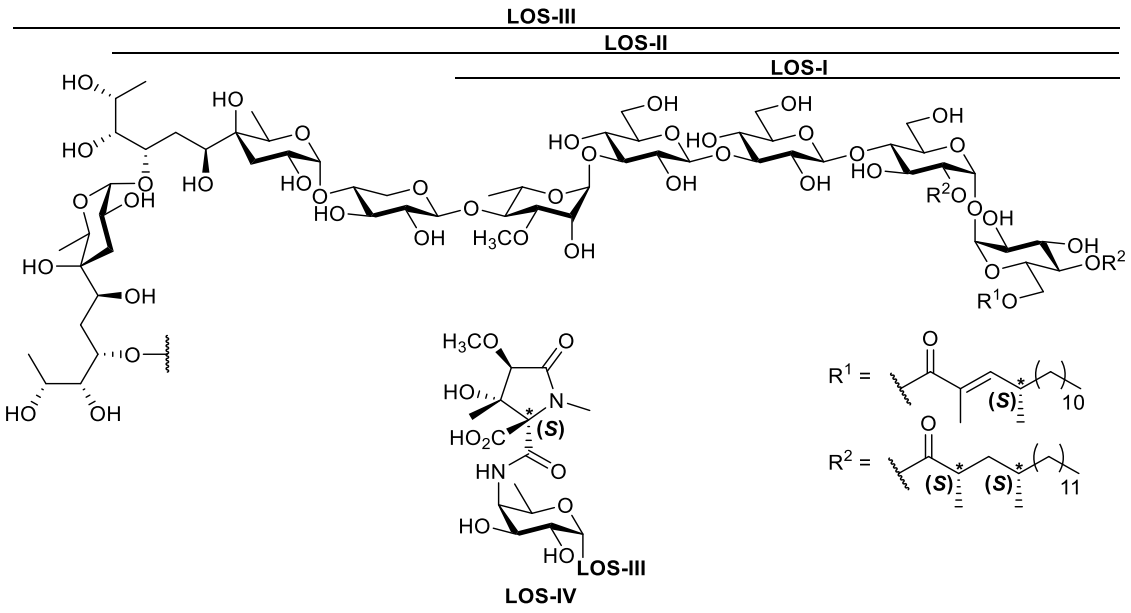


Figure 5.1. Desired synthetic targets LOS-I–IV of this thesis.

5.3 References

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