Studies on the Synthesis of Bacterial Glycans

by

Lei Wang

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Chemistry
University of Alberta

Abstract

Glycans play essential roles in the life cycle of bacteria and often modulate interactions with their environment. Chemical synthesis provides a useful tool to access structurally well-defined bacterial glycans. These molecules can serve as probes for elucidating the biological function of these glycans and can also be helpful tools in providing an understanding of their biosynthesis. This thesis is focused on the synthesis of three types of bacterial glycans: rhamnolipids, lipooligosaccharides and lipopolysaccharide biosynthetic intermediates.

In the first project, I focused on the synthesis of four rhamnolipid analogs. Rhamnolipids can modulate the immune response of hosts that they infect. However, the structural features in these molecules required for their immunomodulatory activity is not well understood. The molecules consist of one or more rhamnose residues linked to an ester-containing lipid. Because the ester functionality is relatively unstable in biological systems, I was interested in making analogs in which this functionality was replaced with more stable species as they may have better biological activities due to longer survival times. In this study, I synthesized amide, ketone, ether and hydrocarbon rhamnolipid derivatives.

In the second project, a convergent strategy was developed for the stereoselective synthesis of four unusual *N*-acylated monosaccharides, which are fragments of

lipooligosacchride IV (LOS IV) from *Mycobacterium marinum*. A critical substrate-controlled cyclization of an amino acid derived oxazolidine was the key step for synthesis of three of the targets. The fourth target was assembled from 3-butynol; the key step was a one-pot oxidation–cyclization–oxidation of a Boc-protected amino alcohol that led to the formation of lactam. This work represents the first synthesis of these unusual motifs, which have been shown to be essential to the bioactivity of LOS IV.

In Chapter 4, I synthesized large, lipid-linked glycans related to lipopolysaccharide biosynthetic intermediates in *Escherichia coli* O9a. Our targets are structures that contain from 2, 6, 10, 14 or 18 tetrasaccharide repeating units, linked through a pyrophosphate moiety to farnesol. The approach I developed involves assembly of the carbohydrate chain, formation of a glycosyl phosphate intermediate, coupling with a lipid phosphate and finally deprotection. Using this approach, I successfully synthesized targets with two and six repeating units, containing 11 and 27 monosaccharide residues, respectively.

Preface

This thesis is an original work by Lei Wang. The synthetic work described in Chapter 3 has been published as: Wang, L.; Dong, M.; Lowary, T. L. Synthesis of unusual *N*-acylated aminosugar fragments of *Mycobacterium marinum* lipooligosaccharide IV. *J. Org. Chem.* **2015**, *80*, 2767−2780. I was responsible for the synthesis and characterization of all of the target molecules. Mr. Mengyao Dong was responsible for the scale up synthesis of intermediates needed for one of the target molecules. The manuscript was written jointly between me and Dr. Todd L. Lowary.

The work described in Chapter 2 and Chapter 4 were done solely by me and have not been published.

Dedicated to My Wife Ms. Yue Wu and My Son Edwin J. Wang

Acknowledgements

First, I would like to thank my supervisor, Dr. Todd L. Lowary, who has provided an immense amount of support and guidance over the past five years. Todd introduced me to the fantastic carbohydrate world and created a wonderful environment for us. I am fortunate to have the opportunity to work in his group.

I would like to thank Dr. Dennis Hall, Dr. Liang Li, Dr. Frederick G. West and Dr. Jianglong Zhu, my Ph. D committee members, for their guidance and helpful suggestions as this thesis. In addition, Dr. Randy Whittal, Bela Reiz, Jing Zheng, Wayne Moffat, Ed Fu, Dr. Angelina Morales-Izquierdo, Dr. Ryan McKay, Mark Miskolzie and Dr. Robert McDonald were instrumental in helping me obtain the compound characterizations required for my project. I am also thankful for all the help from graduate student assistant Anita Weiler and administrative assistant Lynne Lechelt.

I am very lucky to have the chance to work with friendly lab mates during these years, and every member of the Lowary group deserves my acknowledgement. I have learned so much from all of the former and current members, and our friendships will be valued forever. An extra-special thanks to Dr. Maju Joe and Ryan Sweeney, who helped me to double check my thesis. Finally, I am grateful to my parents and my wife Yue Wu for their love and support.

A special thank goes to Alberta Innovates-Technology Future and the U of A Faculty of Graduate Studies and Research for funding.

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List of Abbreviations

$[\alpha]_D$	specific rotation (sodium D Line)
11-mer	undecasaccharide
27-mer	heptacosasaccharide
Å	Angstrom
ABC transporter	ATP-binding cassette transporter
Ac	acetyl
AgOTf	silver trifluromethanesulfonate
AH	asymmetric hydrogenation
AIDS	acquired immune deficiency syndrome
ATH	asymmetric transfer hydrogenation
app	apparent
Ar	aromatic
ATP	adenosine triphosphate
BDA	butane-2,3-diacetal
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
bpy	2,2′-bipyridine

br s	broad singlet (NMR spectra)
BSA	bovine serum albumin
BSP	1-benzenesulfinyl piperidine
Bz	benzoyl
C. jejuni	*Campylobacter jejuni*
CALB	*Candida antarctica* lipase B
CAN	ceric ammonium nitrate
Car	caryophyllose
CD40	cluster of differentiation 40
CDI	carbonyldiimidazole
core-OS	core-oligosaccharide
CPS	capsular polysaccharide
CSA	camphorsulfonic acid
d	doublet (NMR spectra)
DAIB	dimethylaminoisoborneol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DFT	density functional theory
DGDG	digalactosyldiacylglycerol

DIBAL-H	diisobutylaluminum hydride
DIEA	*N*,*N*-diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
E. coli	*Escherichia coli*
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI	electrospray ionization
Fuc	fucose
Gal	galactose
Gal*f*	galactofuranose
Gal*p*	galactopyranose
GalNAc	*N*-acetylgalactosamine
GDP	guanosine diphosphate
Glc	glucose
GlcA	glucuronic acid
GlcNAc	*N*-acetylglucosamine
Glc*p*	glucopyranose
GT	glycosyltransferase
H. pylori	*Helicobacter pylori*

HASP	hydrophobically assisted switching phase
hBD-2	humanbeta-defensin-2
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HDTC	hydrazine dithiocarbonate
Hep	heptose
HMPA	hexamethylphosphoramide
HRMS	high-resolution mass spectrometry
ICAM-1	intercellular adhesion molecule-1
IL	Interleukin
IM	inner membrane
Kdo	3-deoxy-D-manno-oct-2-ulosonic acid
L, D-Hep	L-glycero-D-manno-heptose
Lev	levulinoyl
LiHMDS	lithium bis(trimethylsilyl)amide
LOS	lipooligosaccharide
LPS	lipopolysaccharide
Lpt	lipopolysaccharide transport
m	multiplet (NMR spectra)
M. bovis	*Mycobacterium. bovis*

M. canettii	*Mycobacterium. canettii*
M. gastri	*Mycobacterium gastri*
M. kansasii	*Mycobacterium kansasii*
M. leprae	*Mycobacterium. leprae*
M. marinum	*Mycobacterium marinum*
M. tuberculosis	*Mycobacterium tuberculosis*
M. ulcerans	*Mycobacterium. ulcerans*
MALDI-TOF	matrix-assisted laser desorption/ionization tim-of-flight
MAMP	microbe-associated molecular pattern
Man	mannose
Man*p*	mannopyranose
MCP-1	monocyte chemoattractant protein 1
*m*CPBA	3-chloroperbenzoic acid
MGDG	monogalactosyldiacylglycerol
MeOPN	O-methyl phosphoramidate
NADPH	β-Nicotinamide adenine dinucleotide phosphate (reduced)
NeuNAc	*N*-acetylneuraminic acid
NHC	*N*-heterocyclic carbene

NIS	*N*-iodosuccinimide
NMO	*N*-methylmorpholine-*N*-oxide
NMPTC	*N*-(p-methylphenylthio)-ε-caprolactam
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
OM	outer membrane
O-PS	O-polysaccharide
ORTEP	oak ridge thermal-ellipsoid plot
OS	oligosaccharide
P. aeruginosa	*Pseudomonas aeruginosa*
p-TsOH	*para*-toluenesulfonic acid
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PG	peptidoglycan
PGL	Phenolic glycolipid
Ph	phenyl
PMP	*p*-methoxyphenyl
PP	pyrophosphate
PPEtN	pyrophosphorylethanolamine
PPh_3	triphenylphosphine

PPi	pyrophosphate
ppm	parts per million
PRR	pattern recognition receptor
q	quartet (NMR spectra)
Qui4NAc	4-acetamido-4,6-dideoxy-D-glucose
R_f	retention factor
Rha	rhamnose
Rha*p*	rhamnopyranose
RL	rhamnolipid
RP-18	C_{18} reversed-phase
s	singlet (NMR spectra)
Sharpless AD	Sharpless asymmetric dihydroxylation
SQDG	sulfoquinovosyldiacylglycerol
SYNPHOS	[(5,6),(5',6')-bis(ethylenedioxy)biphenyl-2,2'-diyl]bis(diphenylphosphine)
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate

TBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TCA	trichloroacetic acid or trichloroacetimidate
TDP	thymidine diphosphate
TEA	triethylamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TLR	toll-like receptor
TMSCN	trimethylsilyl cyanide
TMSET	trimethylsilylethyl
TMSQD	*O*-trimethylsilylquinidine
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TNF-α	tumor necrosis factor α
Tol	*p*-tolyl
TPAP	tetrapropylammonium perruthenate
Troc	2,2,2-trichloroethoxycarbonyl

UDP	uridine diphosphate
UDP-Gal	uridine diphosphate galactose
UDP-GalNAc	uridine diphosphate *N*-acetylgalactosamine
UDP-Glc	uridine diphosphate glucose
UDP-GlcNAc	uridine diphosphate *N*-acetylglucosamine
Und	undecaprenol
WT	Wild type
Xyl*p*	xylopyranose

Chapter 1: Introduction

1.1 General introduction to bacterial glycans

Bacteria produce carbohydrate-containing molecules (glycans) with a huge amount of structural diversity. These glycans play essential roles in the life cycles of bacteria and often modulate interactions with their environment. Glycans are composed of monosaccharides linked through glycosidic bonds. To survive, bacteria have developed complex membrane structures containing different types of glycans. The bacterial membrane can act as a channel to facilitate the absorption of nutrients and the secretion of waste. More importantly, this membrane can protect bacteria from the host and their environment.[1,2]

Lipopolysaccharides (LPS), capsular polysaccharides (CPS), lipooligosaccharides (LOS) and glycolipids are widespread on the surface of bacterial cell walls. Typically, LOS contain a small number (2–20) of monosaccharide residues connected by glycosidic bonds and one or more lipids. LPS are large molecules consisting of a lipid and a linear or branched polysaccharide (more than 20 monosaccharide residues).[3] Both LOS and LPS are typically associated with the cell wall of the bacteria through their lipid domain. Bacteria also produce other glycolipids that are classified as either LOS or LPS. CPS are large polysaccharides found on the cell surface of bacterial species.[4] In the sections below, I provide additional information about these structures.

1.1.1 Lipopolysaccharide

The cell wall of Gram-negative bacteria (Figure 1-1) possesses a structure with three parts: the inner membrane, the periplasm and the outer membrane.[3] The outer membrane is divided into two lipid layers, the inner leaflet and the outer leaflet. Lipopolysaccharide (LPS) is usually located on the outer leaflet of the outer membrane.[3] As shown in Figure 1-1, the LPS molecule consists of three domains: the lipid A, the core-oligosaccharide (core-OS), which can be divided into an inner and an outer part, and the O-polysaccharide (O-PS) or O-antigen, which is specific for each bacterial strain.[5] Lipid A is a glucosamine-based phospholipid. The fatty acids in lipid A are essential for anchoring the molecule in the outer leaflet of the outer membrane through hydrophobic interactions.[5] The highly conserved inner part of the core-OS generally consists of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptose (L,D-Hep) residues.[6] The outer core consists predominately of hexoses with limited structural diversity, usually glucose (Glc), galactose (Gal) and *N*-acetyl-glucosamine (GlcNAc). The O-PS domain is mainly made up of repeating units, which show a lot of structural diversity.[5] There is a high variability in the monosaccharide constituents, glycosidic bond linkage position and stereochemistry, the size of repeating units and chain length, and the non-carbohydrate substituents (e.g., acetylation). These features make it one of the most complicated naturally-occurring glycans.

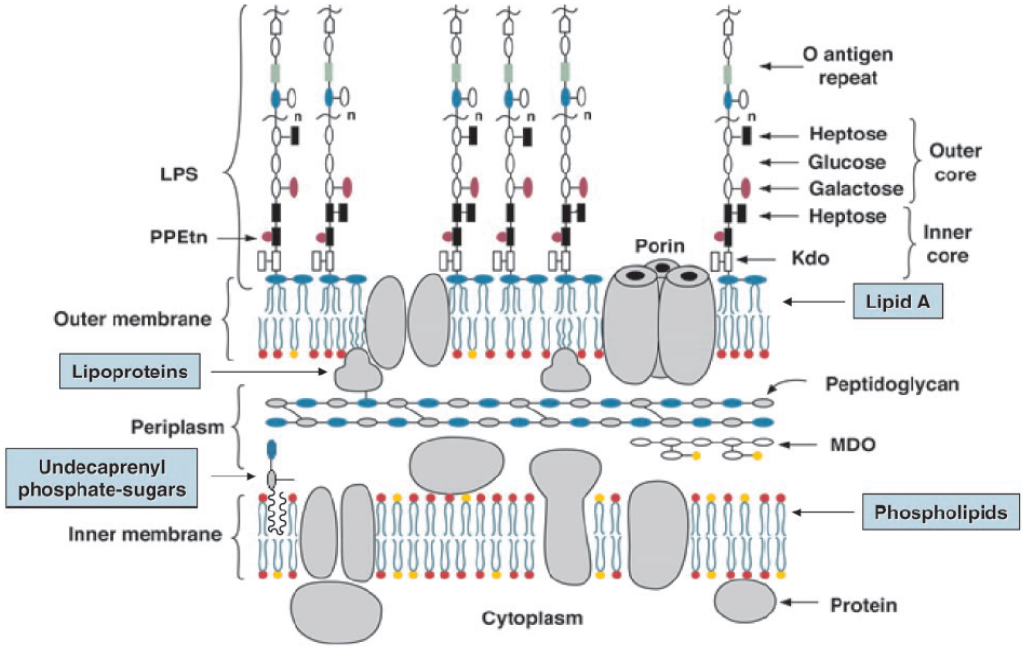


Figure 1-1: The structure of the Gram-negative bacterial cell wall. Reprinted with permission from Raetz, C. R.; Whitfield, C. *Annu. Rev. Biochem*. **2002**, *71*, 635–700.[5]

1.1.2 Capsular polysaccharide

Many bacteria produce protective structures on their surfaces, which are known as capsules. A major component of the capsule are large polysaccharides, known as capsular polysaccharide (CPS). CPS is not only widely found in different Gram-negative bacteria, but it is also present in Gram-positive bacteria.[4] CPS is located on the outermost layer of the cell wall, and they are often the first structure encountered by the host immune system. As a result, CPS is significant for the interaction between a bacterium and its environment.[4]

Like LPS, CPS is also composed of repeating units, containing one or more monosaccharide residues.[7] Both homopolymers and heteropolymers are possible. For example, the *E. coli* K1 antigen is a homopolymer consisting of α-(2→8)-linked *N*-acetylneuraminic acid (NeuNAc).[8] On the other hand, the *E. coli* K5 antigen is a heteropolymer containing both glucuronic acid (GlcA) and GlcNAc in a β-D-GlcA-(1→4)-α-D-GlcNAc-(1→4)- disaccharide repeating unit.[9] Some CPS may be substituted with other groups. For example, the *C. jejuni* NCTC 11168 (HS:2) CPS repeating unit (Figure 1-2) contains 2-amino-1,3-propanediol and MeOPN moieties on the Glc*p*A and galactofuranose (Gal*f*) residues, respectively.[10] The same CPS can be assembled by two different bacteria. For example, the *Neisseria meningitides* group B CPS is identical to that of *E. coli* K1 CPS.[11] CPS has several biological functions, such as prevention of desiccation and resistance to host immunity.[7]

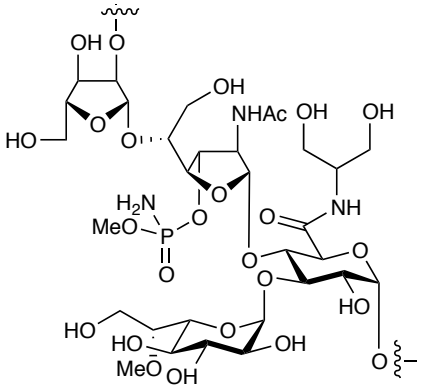


Figure 1-2: Structure of *C. jejuni* NCTC 11168 (HS:2) CPS repeating unit.[10]

1.1.3 Lipooligosaccharides

Lipooligosaccharides (LOS) are a class of oligosaccharides that contain a small number (2–20) of monosaccharide residues. Their highly diversified structures play an important role in maintaining the structural integrity of bacterial cell walls and allowing the organism to adapt to the host's immune system.[5] There are many types of LOS, but I will focus the discussion here only on one family relevant to my thesis research.

Mycobacterial LOS are antigenic compounds found in a variety of mycobacterial species including *Mycobacterium smegmatis*, *Mycobacterium kansasii*, *Mycobacterium gastri*, *Mycobacterium malmoense* and *Mycobacterium marinum*.[12] All mycobacterial LOS contain a core made up two glucopyranose residues attached head-to-head (α-D-Glc*p*-(1↔1)-α-D-Glc*p*, a structure also known as α,α'-trehalose, Figure 1-3), which serves as an anchor for acylation and further species-specific glycosylation. The first mycobacterial LOS was isolated in 1983.[13] To date, more than 30 different mycobacterial LOSs have been reported.[12] Variation in the LOS structure presents the host with a different set of antigens, which can better protect the bacteria. For instance, nine LOSs are produced by *M. kansasii* and four LOSs have been isolated from *M. gastri* (Figure 1-3). Although some studies demonstrating that mycobacterial LOS are immunomodulatory have been published,[14] a detailed understanding of the biological function of LOSs remains unclear.

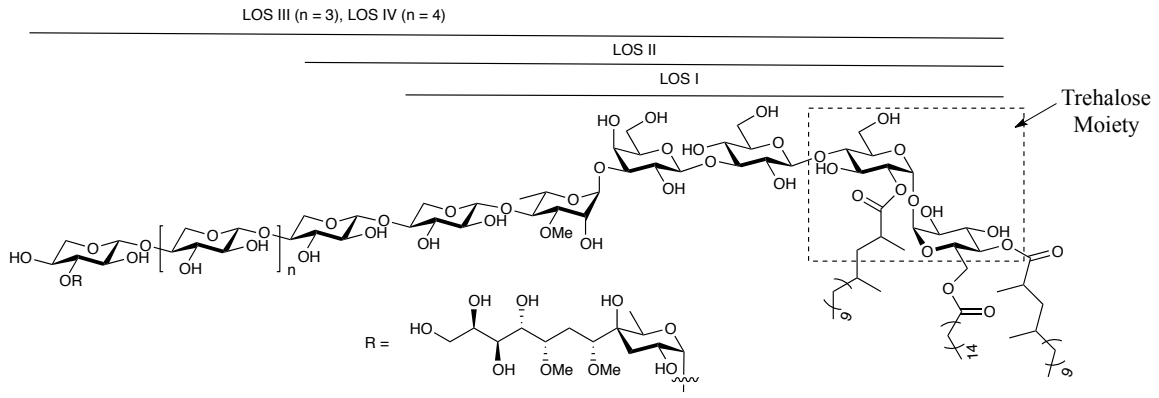


Figure 1-3: Structures of LOS from *Mycobacterium gastri*.[12]

1.1.4 Bacterial glycolipids

Glycolipids are hybrid molecules constructed from hydrophilic carbohydrates and hydrophobic aliphatic chains that show the unique physicochemical properties and bioactivities. Several classes of bacterial glycolipids, such as glycoglycerolipids, phenolic glycolipids and rhamnolipids, have been identified as bacterial membrane compounds.

1.1.4.1 Glycoglycerolipids

Glycoglycerolipids are membrane constituents of bacteria. In general, the glycoglycerolipid structure contains two adjacent acyl groups and a mono- or oligosaccharide attached to the glycerol backbone (Figure 1-4).[15] A variety of glycoglycerolipids have been identified in marine bacteria. For example, monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and

sulfoquinovosyldiacylglycerol (SQDG) are common glycoglycerolipids in marine bacteria (Figure 1-4).[15] Glycoglycerolipids often have different biological activities, such as anti-tumor, anti-viral, and anti-inflammatory activities.[15]

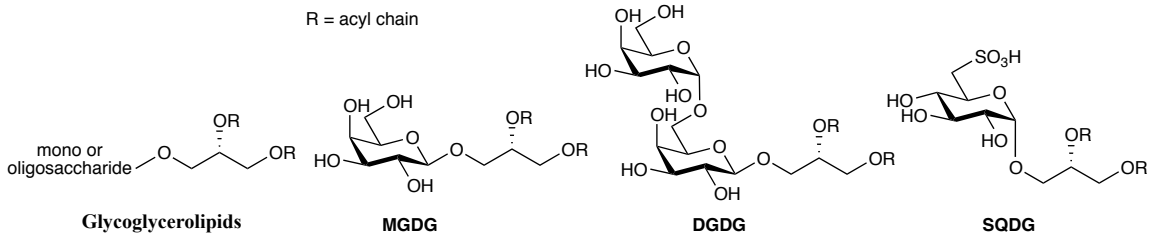


Figure 1-4: Structures of typical bacterial glycoglycerolipids.[15]

1.1.4.2 Phenolic glycolipids

Phenolic glycolipids (PGLs) consist of a conserved lipid core and a variable oligosaccharide moiety. The lipid core is composed of phenol-phthiocerol and two mycocerosic acids linked via ester groups.[16] Phenolic glycolipids (PGLs) are found in a variety of mycobacteria, such as *M. tuberculosis*, *M. leprae*, *M. bovis*, *M. ulcerans*, *M. marinum*, *M. kansasii*, *M. gastri* and *M. canettii*.[17] They play an important role in cell wall permeability and protection against the host immune response.[17] The structure of the phenolic glycolipid PGL-tb1 from *Mycobacterium tuberculosis* is shown in Figure 1-5.

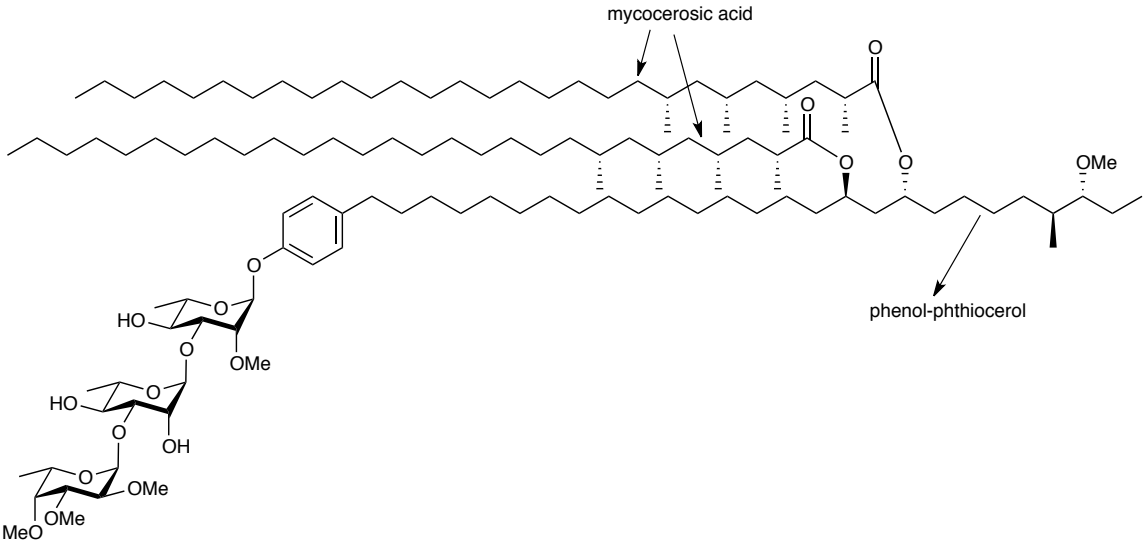


Figure 1-5: Structure of phenolic glycolipid PGL-tb1 from *Mycobacterium tuberculosis*.[18]

1.1.4.3 Rhamnolipids

Rhamnolipids (RLs, Figure 1-6), amphiphilic glycolipid biosurfactants, were first found in the opportunistic microorganism *Pseudomonas aeruginosa*.[19] As of today, more than sixty of rhamnolipid derivatives have been identified, most of them are produced by *Pseudomonas* and *Burkholderia* species.[19] These molecules consist of one or two rhamnopyranose (Rha*p*) monosaccharides attached to a lipid containing two β-hydroxy-alkanoate residues. The chemical structure of the first identified rhamnolipid is α-L-Rha*p*-(1→2)-α- L-Rha*p*-β-hydroxydecanoyl-β-hydroxydecanoate.[20]

RLs are efficient biosurfactants and have been used in the bioremediation of organic and heavy-metal contaminated sites,[21,22] as well as additives for food and cosmetics.[23] RLs can form micelles with organic compounds, which increases their water solubility and helps absorption by bacteria.

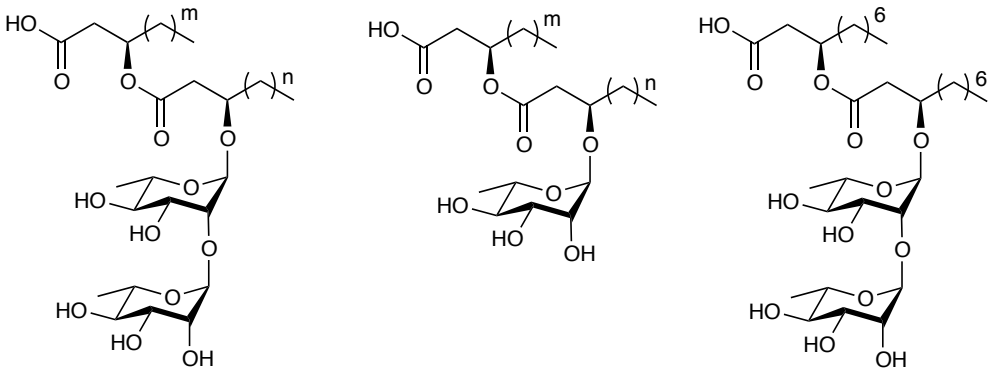


Figure 1-6: Structures of rhamnolipids from *Pseudomonas aeruginosa.*[19]

1.2 Structural role and immunomodulatory activity of bacterial glycans

1.2.1 Structural role of bacterial glycans

One role of bacterial glycans is structural, allowing the organism to maintain their cell wall. For example, LPS and CPS (both discussed above) as well as peptidoglycan contribute to the structural integrity of the bacteria.

The peptidoglycan is a polymer consisting of linear glycans and short peptides. It is a major structural component of bacteria. Antibiotics, such as penicillin, can inhibit the

biosynthesis of peptidoglycan, which results in the inhibition of bacterial growth through weakening of the cell wall.[24]

As the major constituent of Gram-negative bacterial outer membrane, LPS can stabilize the overall membrane structure.[5] Changes in LPS structure affect this structure and three LPS types have been identified in different mutant organisms: smooth, rough and deep-rough. Smooth LPS is LPS with full-length O-chains, whereas rough LPS has just the core-oligosaccharide and lipid A. Deep-rough LPS lacks the O-chain and heptose residues of the inner core.[6] Bacterial mutants with deep-rough LPS have changes in surface hydrophilicity, resulting in greater sensitivity to hydrophobic molecules in the environment.[5] Thus, by changing the structure and composition of the outer membrane, deep-rough LPS mutants have less stable bacterial cell membranes.

As the outermost layer of bacteria, capsular polysaccharides (CPS) can help keep the stability of the cell membrane by protecting the bacteria from desiccation.[7] It is thought this results from the highly hydrated nature of CPS.[25,26]

1.2.2 Immunomodulatory role of bacterial glycans

Microbe-associated molecular pattern (MAMP) recognition is a fundamental process in the immune response of plants and animals.[27] A large number of molecules can serve as MAMPs, including bacterial lipopolysaccharides (LPS) and glycolipids. The cells of the innate immune system of plants and animals possess pattern recognition receptors (PRRs),

which can recognize MAMP molecules.[27] Toll-like receptors (TLRs), a class of PRRs, can recognize structurally conserved MAMP molecules derived from microbes.[28] For example, LPS stimulates human innate immune responses through the Toll-like receptor 4 (TLR-4).[29] Once recognized, MAMP molecules trigger signaling pathways leading either to inflammation as a result of the production of cytokines or the production of antimicrobial compounds.[30] In the following sections, I will discuss in more detail how the molecules introduced above interact with the host immune system.

1.2.2.1 Immune stimulatory activity of lipopolysaccharide

As mentioned in the previous section, LPS can stimulate human innate immune responses through the PRR Toll-like receptor 4 (TLR-4).[31] The O-antigens and core-OS are not responsible for the immune-stimulatory activity of LPS; only lipid A possesses this activity.[32] In particular, the phosphate group and acyloxy acyl moieties of lipid A are needed to trigger a response.[33] Lipid A binding to TLR4 promotes the secretion of cytokines involved in inflammation, such as Tumor Necrosis Factor α (TNF-α) and Interleukin 1-β (IL1-β).[5]

1.2.2.2 Immune inhibition activity of PGLs

In addition to LPS, bacterial glycolipids also can play a role in the innate immune response. For example, PGLs produced by *M. tuberculosis* can affect the pro-inflammatory response by regulating the release of cytokines.[34] Loss of PGL formation correlates with an increase in the release of the pro-inflammatory TNF-α, IL-6 and IL-12 *in vitro*.[34] Furthermore, the inhibition of these cytokines was observed when the amount of PGL increased.[34] This pro-inflammatory inhibition effect has recently confirmed in studies that showed that PGL from *Mycobacterium marinum* prevents the release of pro-inflammatory cytokines (TNF-α and IL-12).[35]

In recent work, a series of synthetic *Mycobacterium tuberculosis* PGL analogues (Figure 1-7, **1.1**–**1.3**), differing from the native PGL (Figure 1-5) by possessing a simplified lipid algycone, were shown to inhibit the release of proinflammatory cytokines (TNF-α, IL-1b, IL-6, MCP-1).[36] Similar studies with *M. kansasii* PGLs showed inhibition activity of the same cytokines.[37] These results led to the discovery of an active anti-inflammatory compound **1.4**, which could be a potential candidate for further optimization.[37] All of these synthetic PGL analogues showed Toll-like Receptor 2 (TLR-2)-mediated activity. Structure–activity relationship studies indicated that the methylation pattern on the PGL analogues greatly affected the activity.[36,37]

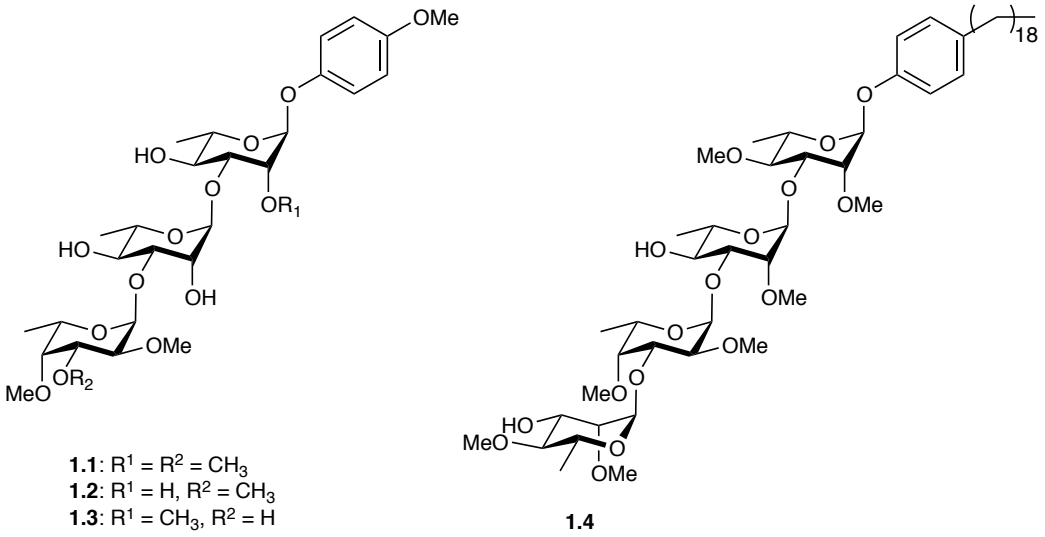


Figure 1-7: Representative structures of synthetic PGL analogues that inhibit pro-inflammatory cytokine formation.[36,37]

1.2.2.3 Immunomodulatory activity of LOS

In addition to PGLs, LOS from *Mycobacterium marinum* were also found to inhibit the secretion of TNF-α.[38] This finding, combined with the fact that the loss of TNF-α led to an increase of death in *M. marinum*-infected zebrafish,[39] resulted in the conclusion that LOSs play an important role in immunomodulatory activity during infection.

1.2.2.4 Immunomodulatory activity of rhamnolipids

In contrast to PGLs and LOS, rhamnolipids exhibit strong stimulation of TNF-α production.[40] The stimulating activity of rhamnolipids can be inhibited by incubation with

inhibitors of cytokine inducers.[40] The recognition of rhamnolipids by TLR-2 and TLR-4 was investigated and it was shown that neither receptor was involved in the process that led to the production of TNF-α by rhamnolipids. As of today, the receptor for rhamnolipids is still unclear.

1.3 Importance of chemical synthesis of bacterial glycans

It is usually very difficult, or impractical, to isolate a single glycoform (a molecule consisting of a single glycan) from natural complex glycan mixtures in quantities suitable for biological investigations. For example, LPS isolated from Gram-negative cell wall membranes contain a series of O-PS with different numbers of repeating units. In the case of glycoproteins, endoglycosidases can be used to modify them to obtain homogeneous glycoforms from isolate mixtures.[41] However, as of today, no practical method allows the cleavage of LPS to afford homogeneous glycoforms from the native O-PS. Therefore, chemical synthesis provides an alternative method to obtain pure glycans with enough material for biological testing. These synthetic molecules have served as useful biological probes. In the following section I provide a couple of examples of the synthesis of complex bacterial glycans and their uses as probes of biological processes.

1.3.1 Chemical synthesis of bacterial glycans as probe molecules

In many cases, the use of fragments of larger molecules is an efficient approach to probe biological function. For instance, to investigate the immune response against the bacteria *Shigella dysenteriae* type 1 and *Escherichia coli* O148, Pozsgay and coworkers synthesized three oligosaccharide fragments (Figure 1-8, **1.5**–**1.7**) of *E. coli* O148, up to a dodecasaccharide, as well as their bovine serum albumin (BSA) conjugates. [42] Interestingly, the BSA conjugate of the shorter oligosaccharide **1.5** did not react with serum generated by vaccination with the polysaccharide. The conjugates of larger octa- and dodecasaccharides **1.6** and **1.7** showed better reactivity.[42] These results support the hypothesis that the serum antibodies have a large binding site, which requires a large glycan ligand.

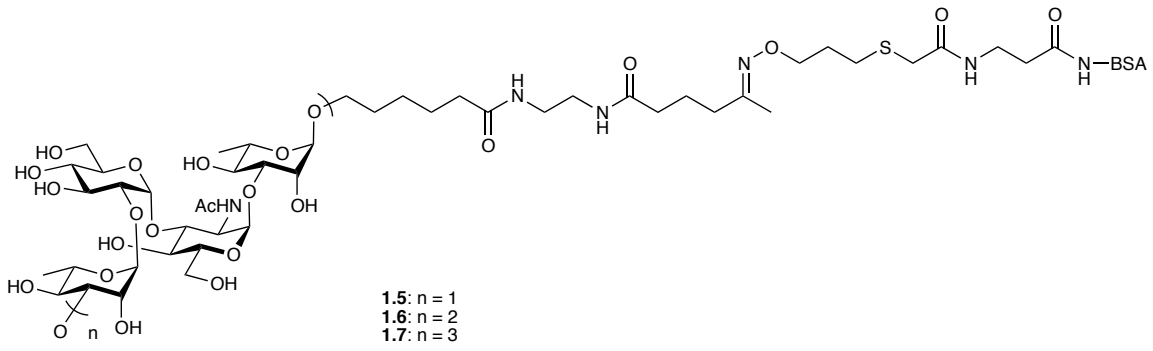
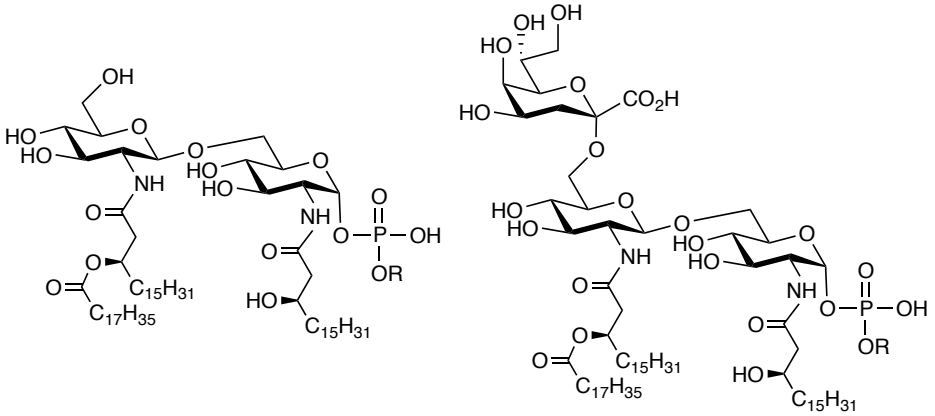


Figure 1-8: Structure of *E. coli* O148 oligosaccharide fragment BSA conjugates **1.5**–**1.7**.[42]

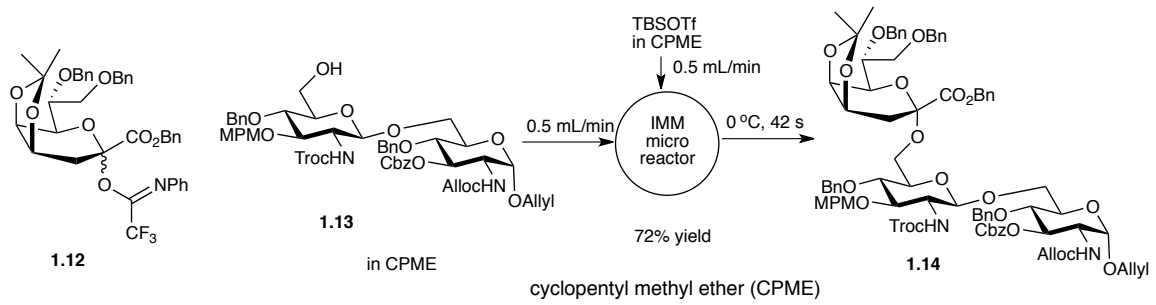
As mentioned above, lipid A plays an essential role in immunomodulatory activity. To probe this in more detail, Fukase and coworkers synthesized four *Helicobacter pylori* lipid A and lipid A–Kdo analogs (Figure 1-9: **1.8**–**1.11**) with or without the ethanolamine group on the 1-phosphate moiety.[43] The key step was the glycosylation between Kdo donor **1.12** and lipid A acceptor **1.13** (Scheme 1-1). To avoid the formation of a glycal byproduct resulting from elimination of the Kdo donor **1.12**, they used microfluidic glycosylation. It was expected that glycal formation could be suppressed due to efficient removal of the heat from the reaction in a microfluidic reactor.[43] The desired trisaccharide **1.14** was obtained in 72% yield with high α-stereoselectivity. The formation of the glycal was suppressed and only 1.5 equivalents of Kdo donor was needed. The immunomodulatory activities of these analogs showed potent IL-18 and IL-12 inducing activities.[43]



1.8: R = H
1.9: R = $CH_2CH_2NH_2$

1.10: R = H
1.11: R = $CH_2CH_2NH_2$

Figure 1-9: Structures of four *Helicobacter pylori* lipid A and lipid A-Kdo analogs synthesized by Fukase and coworkers.[43]

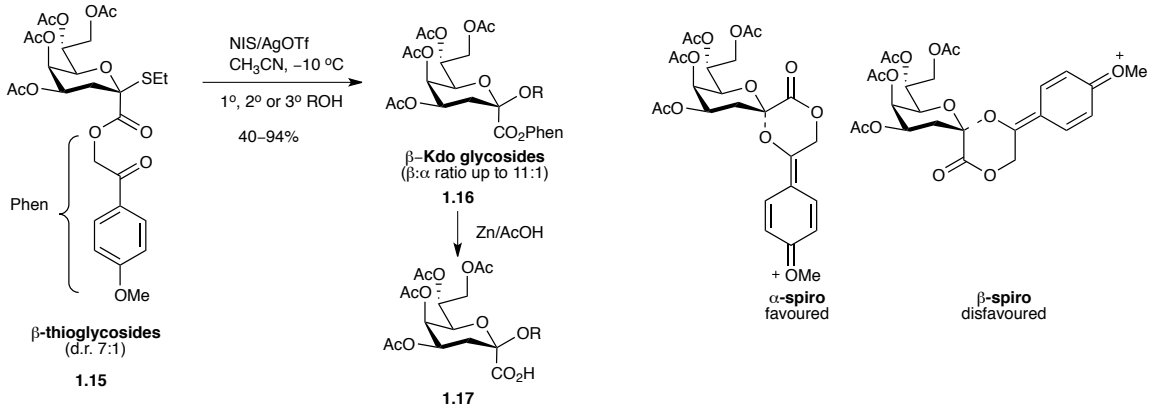


Scheme 1-1: Microfluidic glycosylation of the Kdo donor 1.12 and acceptor 1.13.[43]

1.3.2 Development of new chemistry related to bacterial glycans

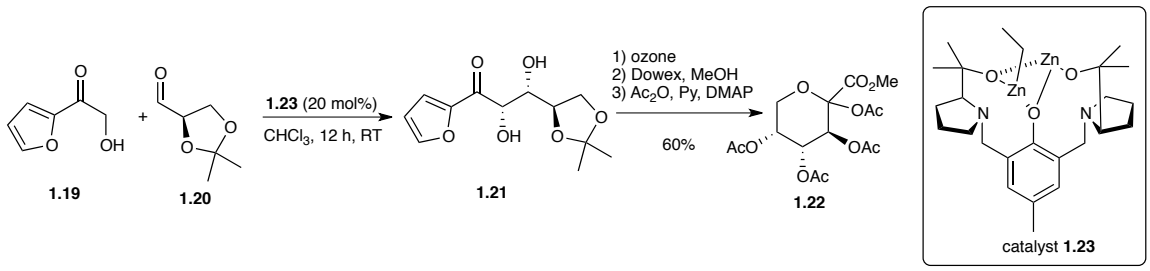
Often in the synthesis of bacterial glycans, the development of new chemistry is required. I provide here three examples of this from the recent literature. The LPS inner cores contain Kdo glycosides with the α-configuration. On the other hand, β-Kdo glycosides are found in CPS and extracellular exopolysaccharides from Gram-negative bacteria, β-Kdo glycosides are important for the integrity of these molecules.[44] Traditional Kdo donors (thioglycosides or imidates) mainly afford the α-glycosides. To provide β-Kdo glycosides in better yield, Gauthier and coworkers recently reported novel methodology for the stereoselective synthesis of β-Kdo glycosides via a 4′-methoxyphenacyl (Phen) auxiliary group (Scheme 1-2).[44] Using *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) to promote the glycosylations, a series of Kdo glycosides was synthesized in good yields and β-selectivities. DFT calculations at the B3LYP/6-311++G (2d,2p) level of theory suggest that a spirocyclic intermediate is involved in these reactions and that the α-spiro intermediate is more stable than the β-spiro intermediate. Thus, the authors proposed that

this reaction follows the α-spiro intermediate route leading to the β-glycoside via displacement by the alcohol.[44]



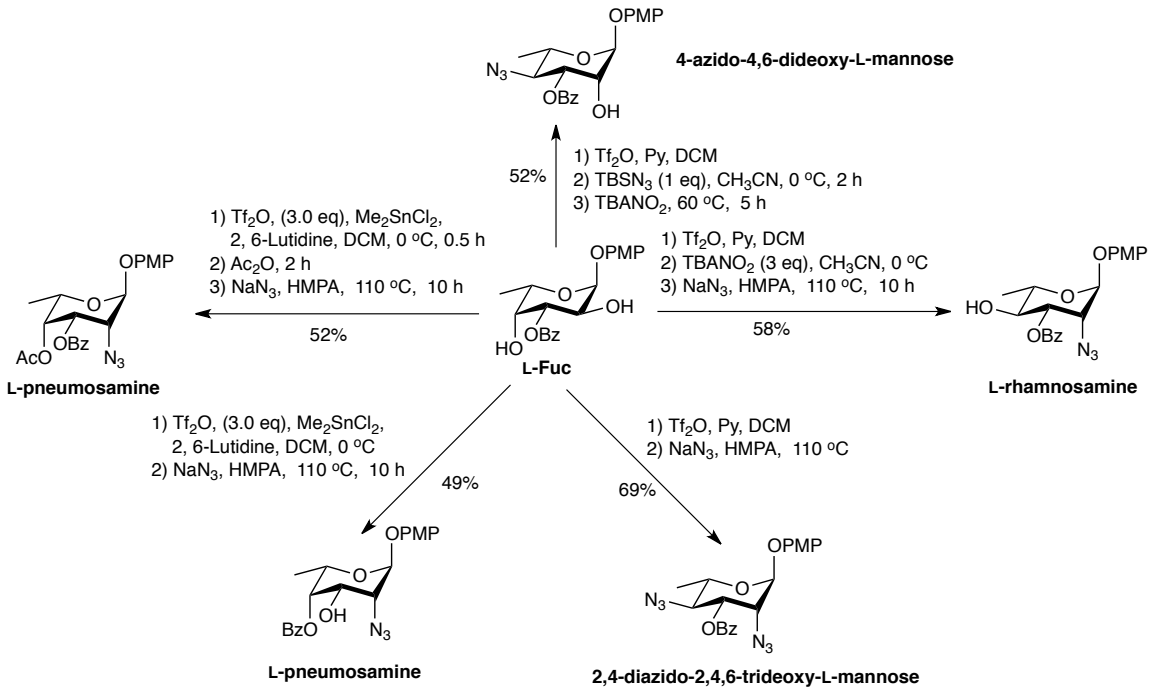
Scheme 1-2: Phen-auxiliary group mediated synthesis of β-Kdo glycosides[44]

Stereoselective aldol reactions are critical for *de novo* carbohydrate synthesis. For example, they have been used in the synthesis of D-*arabino*-hex-2-ulosonic acid, an important monosaccharide that is found in *Acetobacter* LPS.[45] Mylnarski recently reported a *syn*-selective aldol reaction between 2-(hydroxyacetyl)furan **1.19** and optically pure D- or L-glyceraldehyde derivative **1.20** to afford intermediate diol **1.21** in 86% yield. The stereoselectivity of this reaction was controlled by the catalyst **1.23**. Ozonolysis of the furan ring and deprotection of the isopropylidene acetal followed by acetylation delivered the protected form of D-*arabino*-hex-2-ulosonate **1.22** in 60% overall yield in three steps.

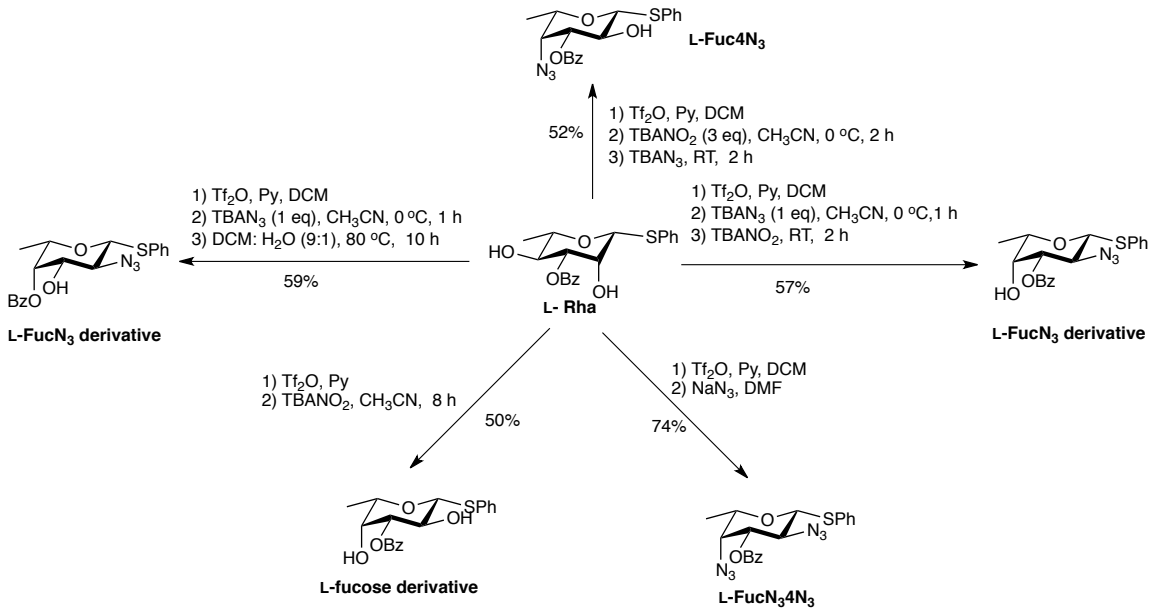


Scheme 1-3: *De novo* synthesis of D-*arabino*-hex-2-ulosonic acid[45]

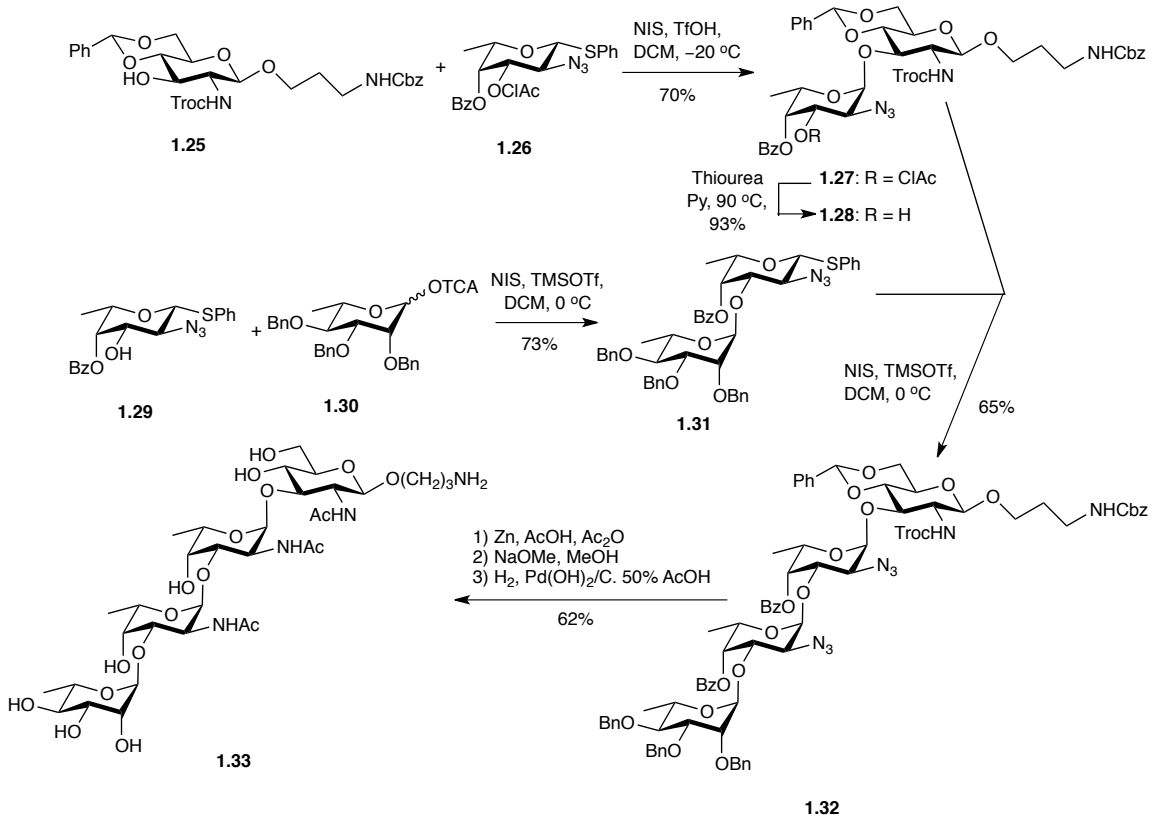
Several rare deoxy amino L-sugars, such as L-rhamnosamine and L-fucosamine, are present in bacterial oligosaccharides. These rare sugar residues cannot be obtained in large quantities from natural sources and thus chemical synthesis is the only method to obtain structurally well-defined and homogeneous glycans containing these sugars. To achieve this, Sanapala and Kulkarni reported a general method to synthesize unusual deoxy amino L-sugars starting from L-rhamnose and L-fucose.[46] As shown in Schemes **1-4** and **1-5**, the stereochemistry of the O2 or O4 positions of L-rhamnose and L-fucose can be inverted via regioselective monotriflation and subsequent substitution by tetrabutylammonium nitrite ($TBANO_2$) or sodium azide (NaN_3). Then, the orthogonally-protected deoxy amino L-sugar building blocks **1.26** and **1.29** can be stereoselectively assembled to obtain the amine functionalized tetrasaccharide of *Yersinia enterocolitica* O50 strain 3229 (Scheme **1-6**).



Scheme 1-4: Synthesis of rare deoxy amino L-sugars from a L-fucose derivative[46]



Scheme 1-5: Synthesis of rare deoxy amino L-sugars from a L-rhamnose derivative[46]



Scheme 1-6: Synthesis of tetrasaccharide of *Yersinia enterocolitica* O50 strain 3229[46]

1.4 Overview of thesis research

As discussed previously, bacterial glycans play essential roles in the life cycles of bacteria and often modulate interactions with their environment. Synthetic versions of these glycans can serve as probes for elucidating their biological function and mode of action. In this thesis, I will describe three projects in the field of chemical synthesis of bacterial glycans for biological investigations.

Project 1. Rhamnolipids are glycolipid biosurfactants that can modulate the immune response of the hosts that they infect.[40] However, the structural features in these molecules

required for their immunomodulatory activity is not well understood. In my research, I was particularly interested in understanding the importance of the ester group in rhamnolipids on the immune response. This is because compared to many other organic functional groups, the ester is relatively unstable in biological systems. Compounds that contain more stable groups in place of the ester may have better biological activities. In Chapter 2, I will present the synthesis of four rhamnolipid derivatives, including amide, ketone, ether and hydrocarbon analogs (Figure 1-10). A series of different approaches were used to access the different target molecules, via synthesis of a lipid precursor and glycosylation. The biological evaluation of these compounds is being done by other group members.

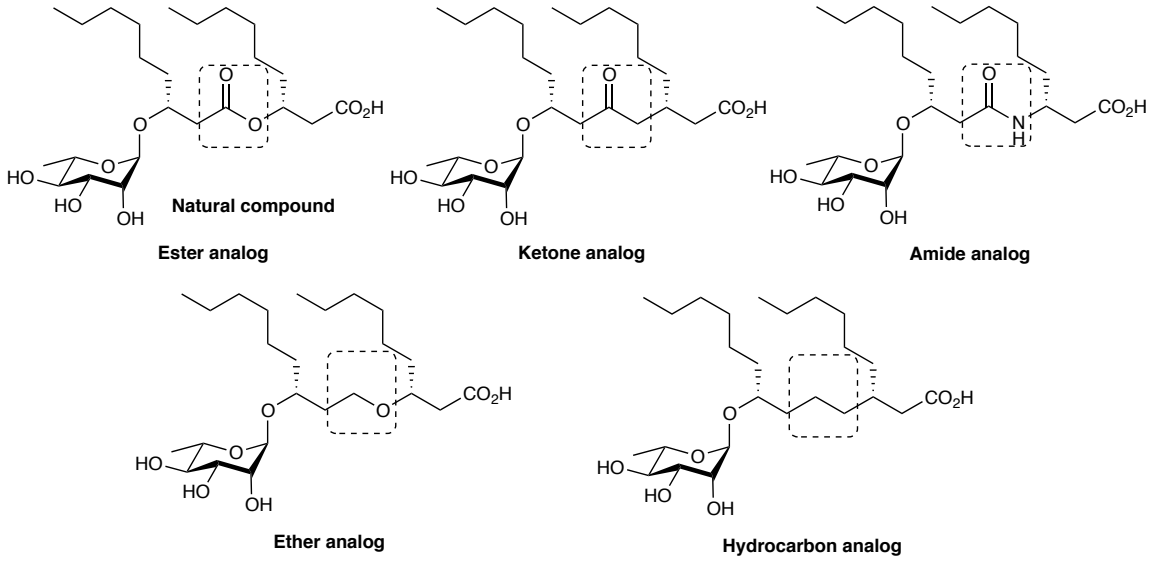


Figure 1-10: Structures of a rhamnolipid and the ketone, amide, ether and hydrocarbon analogs synthesized in this thesis

Project 2. Lipooligosaccharides are major components in many mycobacterial species. LOS-IV (Figure 1-11), which has been isolated from *Mycobacterium marinum,* contains an unusual *N*-acylated 4-amino-galactopyranose residue.[14] The C4 position of the terminal monosaccharide is substituted by one of four related *N*-acyl groups. These terminal *N*-acylated dideoxy-aminogalactose derivatives are essential for the biological function of LOS-IV, including macrophage activating properties.[14] From a synthetic chemistry perspective, these *N*-acylated dideoxy-aminogalactoses are structurally interesting molecules. In Chapter 3, a general route for synthesizing these *N*-acylated dideoxy-aminogalactose residues will be discussed. The molecules were assembled by *N*-acylation of a dideoxy-aminogalactose residue with carboxylic acid derivatives obtained starting from the chiral pool.

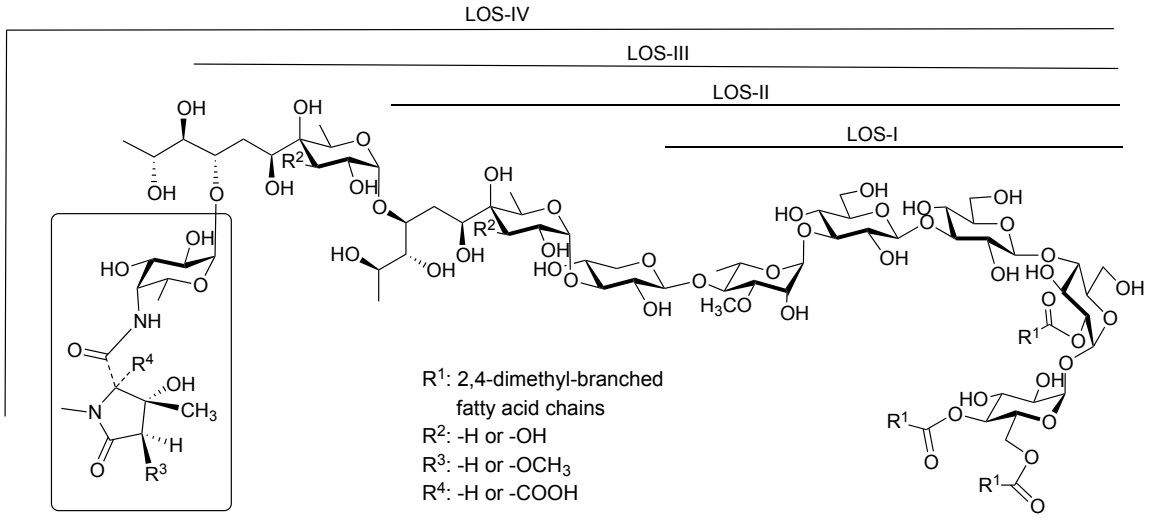


Figure 1-11: Structures of *Mycobacterium marinum* LOS-IV; the *N*-acylated dideoxy-aminogalactose derivatives relevant to this thesis are highlighted in the box.

Project 3. Bacterial Lipopolysacchride (LPS) is a complex and important immunomodulatory molecule and its biosynthesis has been a topic of interest for decades. Probing LPS biosynthetic pathways has often involved the chemical synthesis of various small fragments of the larger structure (e.g., the O-chain repeating unit) and then subsequent biochemical investigations with these molecules. In many cases, the use of these small fragments to probe the biosynthesis is a viable approach. However, we postulate that to study some biosynthetic processes much larger synthetic glycans will be necessary. In Chapter 4, I will describe my work to synthesize large, lipid-linked intermediates related to LPS O-chain biosynthesis in *Escherichia coli* O9A. Our targets are structures that contain 2 and 6 tetrasaccharide repeating units, linked through a pyrophosphate moiety to farnesol (Figure 1-12). The approach developed involves assembly of the carbohydrate chain, formation of a glycosyl phosphate intermediate, coupling with a lipid phosphate and finally deprotection. This approach can provide multi-milligram amounts of the compounds.

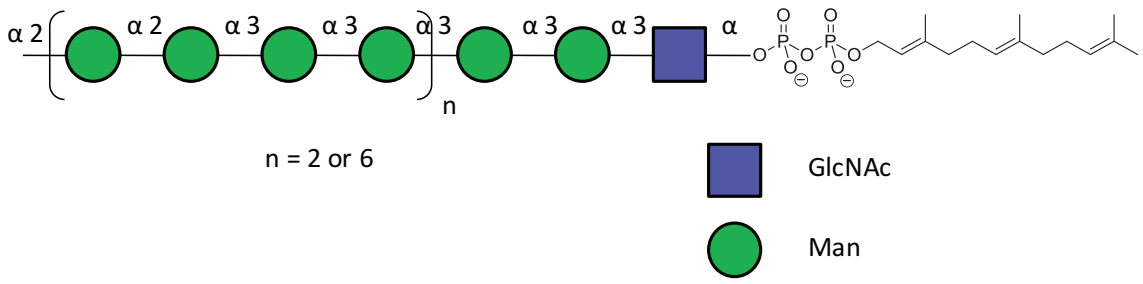


Figure 1-12: Structures of LPS O-chain fragments in *Escherichia coli* O9A

Chapter 2

2.1 Rhamnolipids

Rhamnolipids (RLs) were initially found in the opportunistic microorganism *Pseudomonas aeruginosa*.[19] As detailed in the introduction, rhamnolipids contain one or two rhamnose (Rha) residues and one or two β-hydroxy-alkanoate residues. To date, four RL derivatives have been reported (Figure 2-1): di-Rha-di-lipid, mono-Rha-di-lipid, di-Rha-mono-lipid and mono-Rha-mono-lipid. Among these, two forms are primarily produced by *P. aeruginosa*: mono-Rha-di-lipid and di-Rha-di-lipid.[19] Furthermore, an uncommon di-Rha-di-lipid RL has been characterized, which is mono-acylated at the 2-hydroxyl group of the second Rha residue (Figure 2-1).[47]

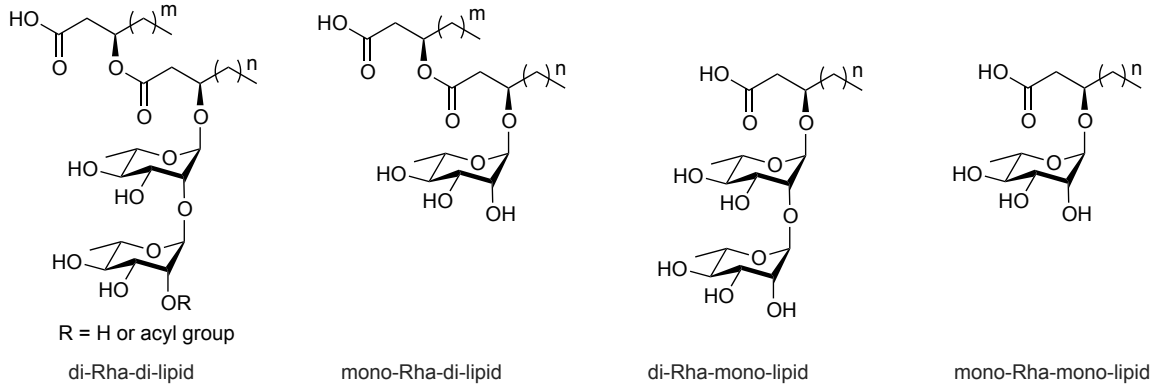


Figure 2-1: The structures of the naturally-occurring rhamnolipid derivatives reported to date.[47]

2.1.1 Rhamnolipids as stimulators of human and animal immunity

Our interest in RLs arises from the fact that these bacterial glycolipids stimulate the production of Tumor Necrosis Factor α (TNF-α), which is an inflammatory cytokine. In earlier studies, Brandenburg and coworkers showed that RL-2,2_{14} (**2.1**, Figure 2-2) exhibits strong stimulation of TNF-α.[40] To probe the mechanism of this activation, RL binding to the cell surface receptors TLR-2 and TLR-4, which interact with a number of other glycolipid antigens, was investigated. These studies revealed that neither TLR-2 and TLR-4 were involved in the production of TNF-α by RLs. To date, the receptor involved remains unknown.

Synthetic RL derivatives have also been investigated for their immunomodulatory activity.[48] As shown in Figure 2-2-A, these RLs differ by the number of lipid chains, chain length and stereochemistry, number of Rha residues (1 or 2) and the presence of charged (CO_2H) or neutral (CH_2OH) groups.[48] This structure–activity relationship (SAR) study showed that the number of Rha residues (**2.8** and **2.9**) was not critical for activity (Figure 2-2-B). The number and the length of lipids had the greatest impact on the activity. As the number of lipids increased to three (**2.6**), reduced biological activity was observed. Either increasing (**2.3**) or decreasing (**2.2**) the length of two lipid chains also led to a decrease in biological activity. Interestingly, a slight reduction in activity was observed if the length of only one lipid chain (external chain **2.5** or internal chain **2.4**) was decreased. A large

reduction in activity was observed if the carboxyl group was changed to CH_2OH (**2.10**), or if the stereogenic center in the external lipid was changed (**2.7**).[48]

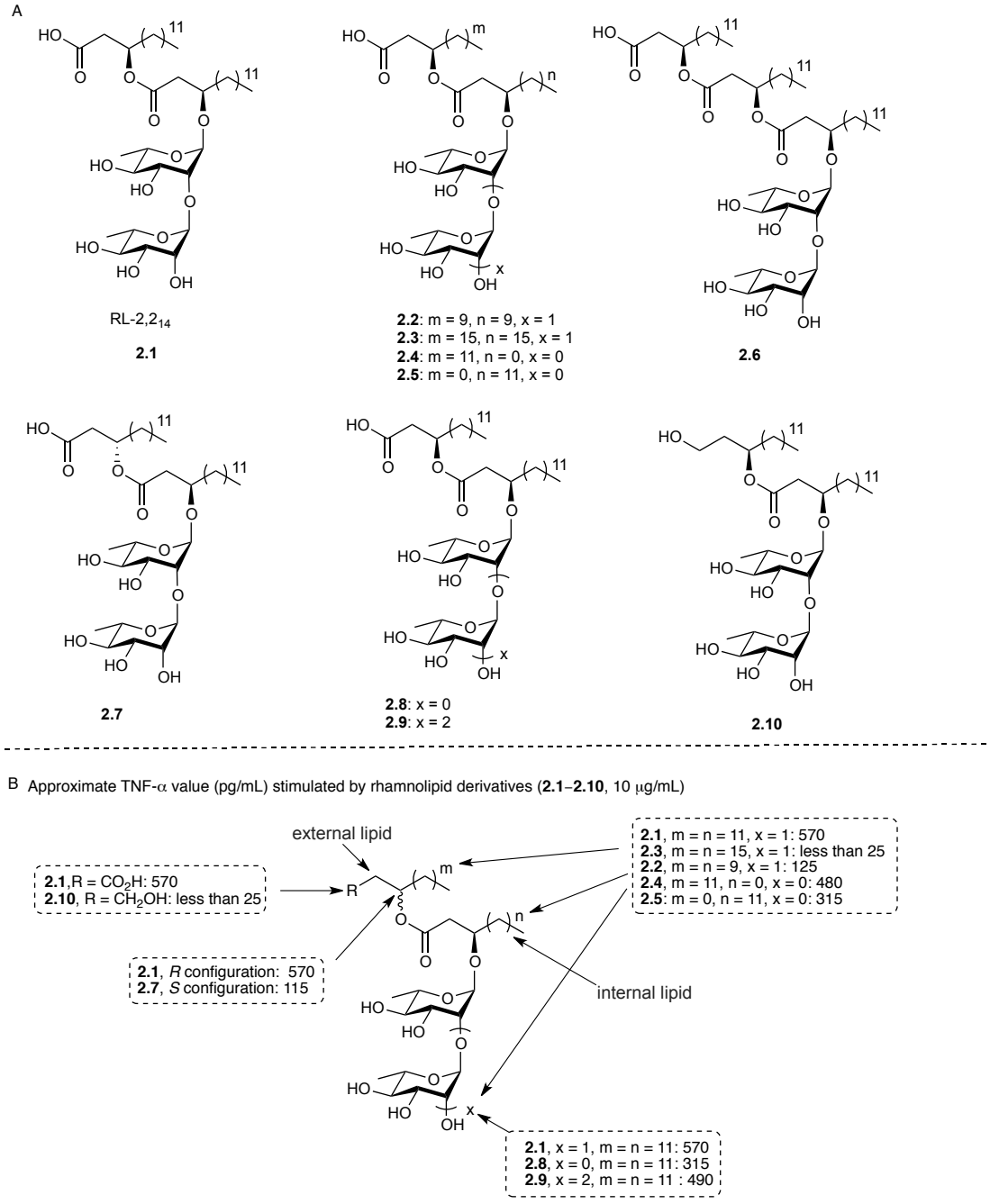
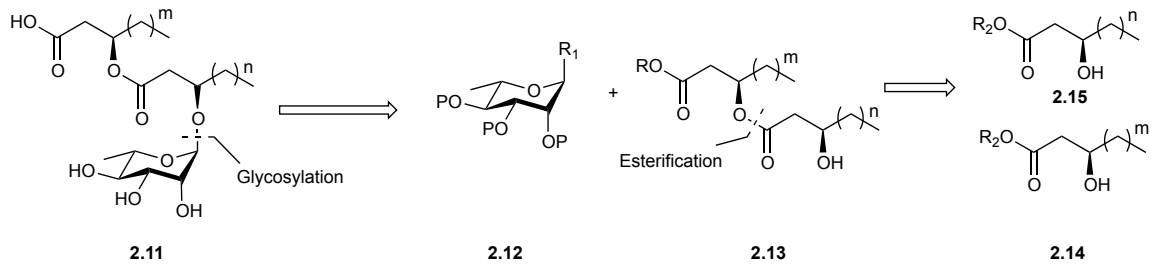


Figure 2-2: A) The structures of synthetic RLs prepared by Brandenburg and coworkers. B) TNF-α secretion after stimulation with synthetic RLs.[48]

Furthermore, biologically inactive synthetic-RLs (**2.3**, **2.6**, **2.10**) have been tested for their ability to prevent LPS-induced cytokine production.[49] The studies showed that the addition of an excess of biologically inactive RLs to LPS results in an inhibition of LPS-induced TNF-α production.[49] In addition to directly inducing cytokines as a microbe-associated molecular pattern (MAMP) molecule, RLs can also enhance the recognition of other MAMPs by the human immune system.[50] One example is the antimicrobial peptide human beta-defensin-2 (hBD-2), which is active against Gram-negative bacteria.[51]

2.1.2 Synthetic efforts towards rhamnolipids

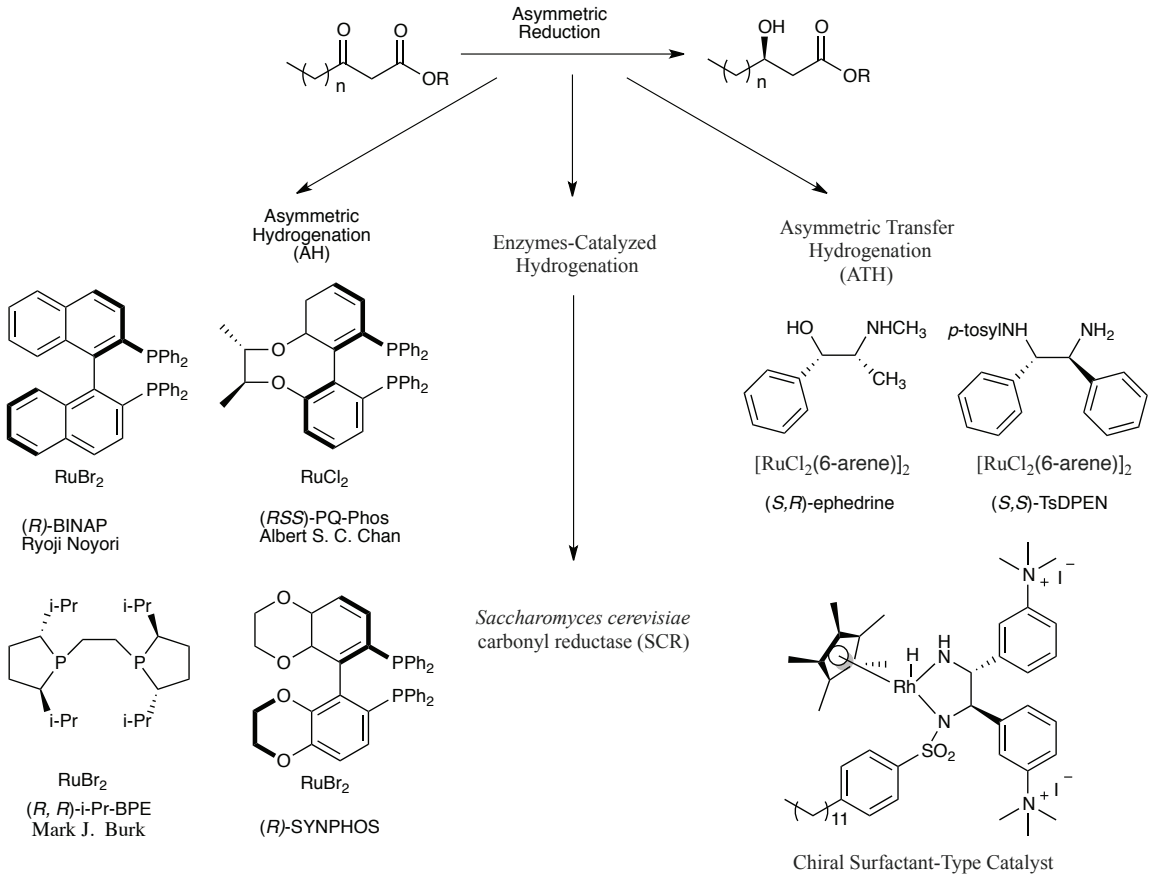
To date, there have been limited studies on the chemical synthesis of RLs. The routes reported involve the general retrosynthetic analysis outlined in Scheme 2-1. The disconnection of the anomeric C–O bond in **2.11** affords protected rhamnoside donor **2.12** and diester **2.13**, which can be synthesized through an esterification reaction between two β-hydroxy esters (**2.14** and **2.15**). Because the synthesis of these compounds requires the preparation of enantiomerically pure β-hydroxy esters, I will first summarize general approaches to these molecules.



Scheme 2-1: Retrosynthetic analysis of RLs synthesized to date.

2.1.2.1 Synthesis of β-hydroxy esters

The most efficient and convenient route to chiral aliphatic β-hydroxy esters is the asymmetric reduction of β-ketoesters. As shown in Scheme 2-2, significant progress has been made in this area and the approaches include metal-catalyzed asymmetric hydrogenation (AH), asymmetric transfer hydrogenation (ATH) and enzyme-catalyzed asymmetric reduction.



Scheme 2-2: Asymmetric reduction of β-ketoesters

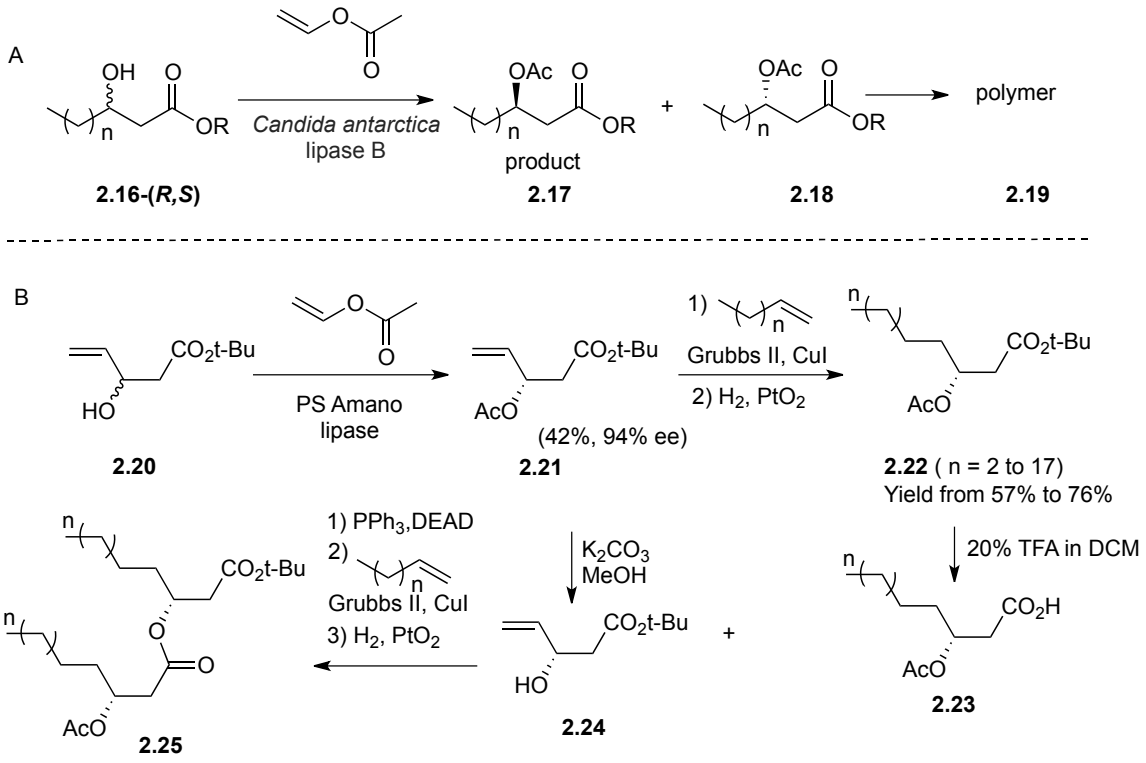
The famous Noyori Ru-BINAP catalysts (Scheme 2-2) can catalyze the AH of β-ketoesters with high enantioselectivity (>98% ee).[52] However, high temperatures and high hydrogen pressures are usually required for reasonable catalytic rates to be observed. Chan and coworkers developed the (*RSS*)-PQ-Phos ligand for the hydrogenation of β-ketoesters at room temperature with excellent yield and enantioselectivity (Scheme 2-2).[53] A disadvantage of this method is that it can only use aromatic and short chain aliphatic ketoesters as substrates. Burk and coworkers[54] designed the *i*-Pr-BPE-Ru catalyst that

allows the highly enantioselective hydrogenation of β-ketoesters under mild conditions. This method is also suitable for long chain substrates.[54] In addition, the Ru-SYNPHOS catalyst can also use long chain β-ketoesters as substrates with excellent enantioselectivity (>99% ee).[55]

Compared to AH, the enantioselectivity of ATH of β-ketoesters is modest. When (*S,R*)-ephedrine and (*S,S*)-TsDPEN $[RuCl_2(\eta 6\text{-arene})]_2$ are used as ATH catalysts for the preparation of β-hydroxyesters, the ee values are around 50% for short chain substrates (Scheme 2-2).[56] Lin and coworkers[57] developed a chiral surfactant-based catalyst and applied it to the ATH of long-chain aliphatic β-ketoesters in water. Quantitative conversion and good enantioselectivity (up to 86%–91% ee) were observed.

Asymmetric synthesis with biocatalysts is environmentally friendly. *Saccharomyces cerevisiae* (Bakers yeast) carbonyl reductase shows high enantioselectivity for many ketones, including β-ketoesters, although the yields are modest (52%–84%).[58,59] An alternative to enzymatic reduction is the enzymatic resolution of racemic mixtures using lipases, especially *Candida antarctica* lipase B (CALB).[60] As shown in Scheme 2-3-A, both of the substrates (**2.16**-*R* or *S*) can act as acyl acceptors to afford **2.17** and **2.18**. However, under these conditions, ester **2.18**, with the *S* configuration, is converted to polyester **2.19**, which facilitates purification of **2.17**. Using this method, compound **2.17** can be obtained as an enantiopure isomer.[60] Similarly, PS Amano lipase can resolve racemate **2.20** to afford diester **2.21** in 42% yield and 94% ee (Scheme 2-3-B).[61] Chain

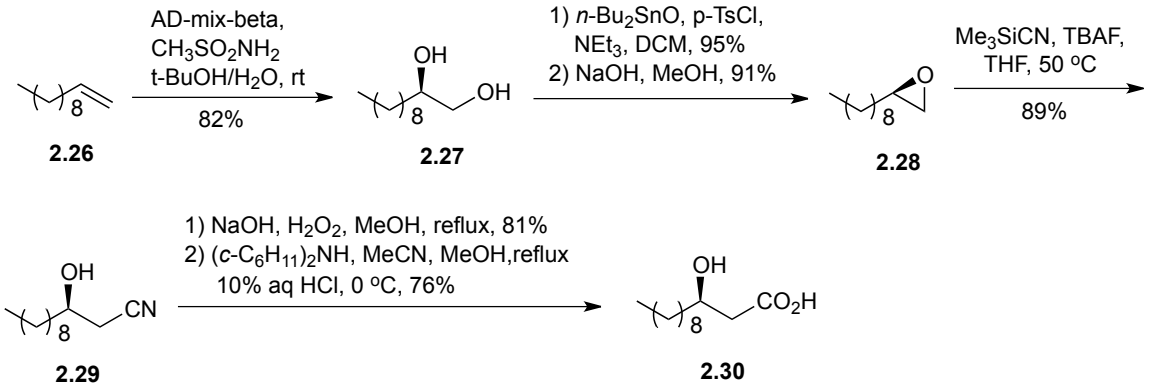
elongation by Grubbs II catalyzed cross-metathesis and then hydrogenation led to diester **2.22**, which could be selectively deprotected under acidic conditions to afford acid **2.23**. Alternatively, the acetyl protecting group was selectively removed under weakly basic conditions to give β-hydroxy ester **2.24**. Then, Mitsunobu reaction, Grubbs II catalyzed chain elongation and hydrogenation led to **2.25**, a key intermediate for RL synthesis (see below).[61]



Scheme 2-3: A) Enzymatic resolution of the β-hydroxy ester racemates using lipases. B) Synthesis of RL lipids using enzymatic resolution and additional transformations.[60,61]

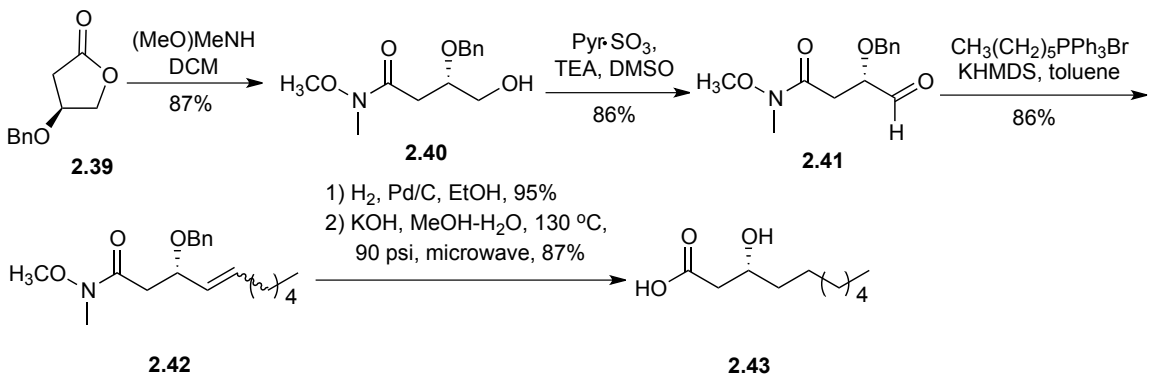
Epoxides, good electrophiles, are also key intermediates for the synthesis of β-hydroxy esters or acids.[62,63,64] As shown in Scheme 2-4, regioselective opening of epoxide **2.28**

using trimethylsilyl cyanide (TMSCN) and *tetra*-*n*-butylammonium fluoride (TBAF) in anhydrous THF at 50 °C gave cyanoalcohol **2.29** in 89% yield. Hydrolysis of intermediate **2.29** affords the desired β-hydroxy acid **2.30**.[62]



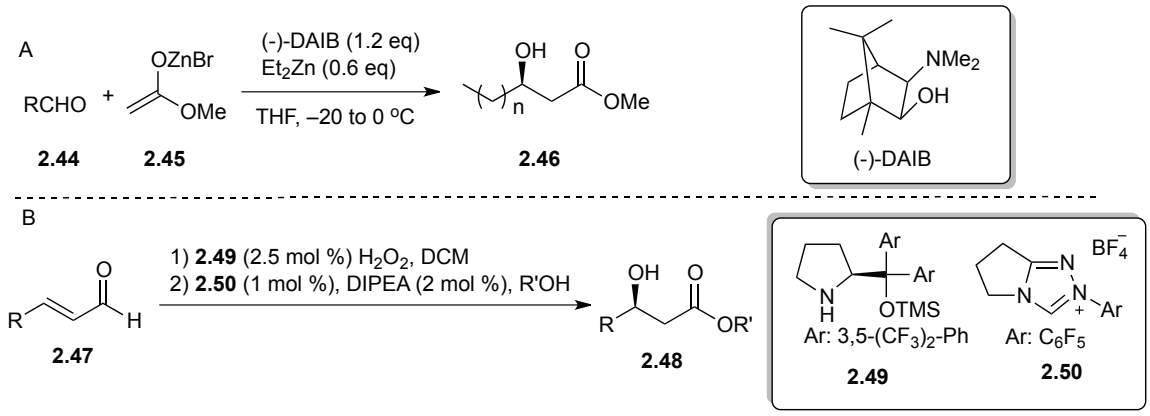
Scheme 2-4: Epoxides as intermediates for the synthesis of β-hydroxy esters or acids.[62]

The Wittig reaction can be used to elongate the lipid chain in β-hydroxy acids. For example, Pohl and coworkers[65] used *N*,*O*-dimethylhydroxylamine to open the benzyl-protected lactone ring (Scheme 2-5). Then, the primary hydroxyl group was oxidized via Parikh–Doering oxidation to afford aldehyde **2.41**. After Wittig reaction and deprotection, β-hydroxy acid **2.43** was obtained in good yield.



Scheme 2-5: Using the Wittig reaction to elongate the alkyl chain in β-hydroxy acids.[65]

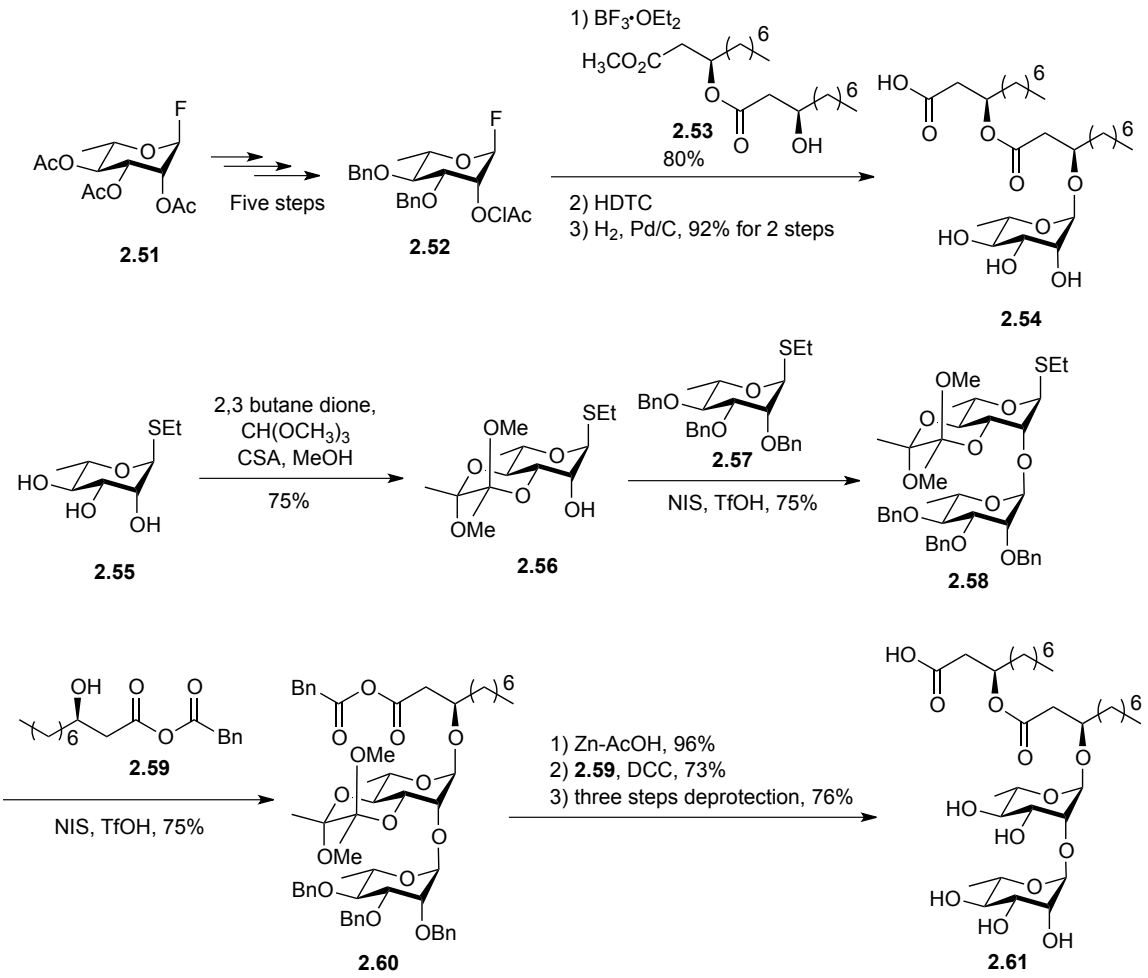
The synthetic strategies mentioned above need several steps to afford β-hydroxy esters or acids. Two methods have been developed to obtain the target compound in only one step. The Reformatsky reaction produces β-hydroxy esters via the addition of organozinc enolates to aldehydes or ketones.[66] (-)-*N*,*N*-Dimethylaminoisoborneol ((-)-DAIB) was found to be a good ligand for an asymmetric version of this reaction (Scheme 2-6-A). Products of up to 79% ee were obtained with aliphatic aldehydes.[66] Another example is the organocatalytic reaction shown in Scheme 2-6-B. An organocatalyzed one-pot reaction has been developed by Jørgensen and coworkers. In this work, they used proline derivative **2.49** as a catalyst to afford the Michael addition intermediate. Then, *N*-heterocyclic carbene (NHC)-catalyzed esterification led to β-hydroxy esters with excellent enantioselectivity.[67]



Scheme 2-6: One step synthesis of β-hydroxy esters using A) A Reformatsky reaction or B) An organocatalytic approach.[66,67]

2.1.2.2 Progress in the synthesis of rhamnolipids

Van Boom and co-workers reported the first synthesis of a rhamnolipid in 1988.[68] As shown in Scheme 2-7, the rhamnosyl donor **2.52** was synthesized from rhamnosyl fluoride **2.51** in five steps. Then, BF_3•Et_2O-promoted glycosylation with diester **2.53** afforded the protected rhamnolipid in 80% yield. After deprotection, rhamolipid **2.54** was obtained in 92% yield.

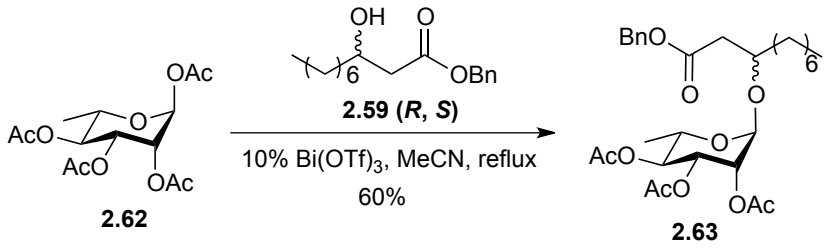


Scheme 2-7: Rhamnolipid syntheses by van Boom and coworkers.[68,69]

The drawback of this route is the preparation of the fluoride donor **2.52**. Therefore, they redesigned the synthetic route to use rhamnose thioglycosides **2.57** and **2.58** as the donors.[69] This allowed the more facile synthesis of a disaccharide-containing rhamnolipid. Thus, chemoselective *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) promoted glycosylation between **2.56** and **2.57** afforded a 75% yield of **2.58**. This disaccharide donor was then coupled with lipid **2.59**, also promoted by NIS/TfOH, to give

the protected rhamnolipid **2.60** in 75% yield. After deprotection of the anhydride, the resulting carboxylic acid was coupled with **2.59** by treatment with DCC to give the fully protected rhamnolipid derivative. Three deprotection steps afforded the final rhamnolipid **2.61** in 76% yield.

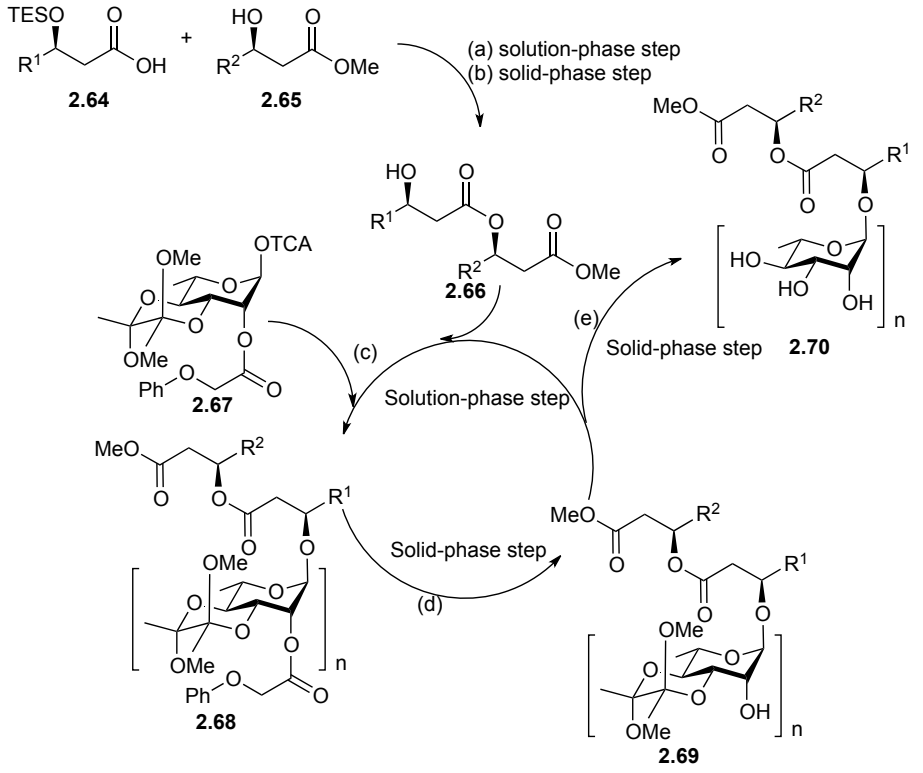
Although van Boom's route gave the desired product, a disadvantage of this synthesis is the long route needed for the preparation of the rhamnosyl donors. Therefore, Polt and coworkers[70] proposed to use rhamnose peracetate **2.62** as the donor, which can be synthesized from L-rhamnose in one step. Glycosylation of β-hydroxy ester **2.59 (*R*,*S*)** with **2.62** in the presence of 10% $Bi(OTf)_3$ produced rhamnoside **2.63** in 60% yield.



Scheme 2-8: Synthesis of rhamnolipid **2.63** by Polt and coworkers.[70]

To develop a SAR of rhamnolipid immunomodulatory activity, Rademann and coworkers[48] synthesized an array of rhamnolipid analogues with differences in lipid size, stereochemistry, functional group and the number of Rha residues. A hydrophobically assisted switching phase (HASP) synthesis was used to synthesize this library (Scheme 2-9). After the esterification of **2.64** and **2.65**, C_{18} reversed-phase silica gel (RP-18) was added to the reaction mixture. All monoester starting materials were removed by filtration

with methanol and water. After deprotection, washing and filtration, the pure 3-hydroxy diester (**2.66**) was desorbed with dichloromethane. Diester **2.66** was then glycosylated with the rhamnose donor **2.67** and the product **2.68** was transferred again to the RP-18 solid support. Removal of protecting groups gave the product **2.70**.[48] Compound **2.69** could be glycosylated in a second (or third) HASP reaction cycle to afford disaccharide or trisaccharide-containing rhamnolipids. This strategy yielded pure products without the need for lengthy purification steps.[48]



a) EDC, cat.DMAP, CH_2Cl_2, 12 h, addition of RP-support, evaporation, washing steps (MeOH/H_2O 80:20), 97.5%.
b) 5% TFA, MeOH/H_2O (80:20), washing steps (MeOH/H_2O 80:20), $MgSO_4$, desorption (CH_2Cl_2), 98%.
c) 1.3 equiv donor **2.67**, 0.05 equiv of TMSOTf, CH_2Cl_2, 30 min, neutralization (DIPEA), evaporation, washing steps (MeOH/H_2O 80:20), 94%.
d) 40 equiv aqueous $MeNH_2$, MeOH/THF (1:1), 1 h, washing steps (MeOH/H_2O 80:20), 93%.
e) TFA/H_2O (90:10), 3X10 min, washing steps (MeOH/H_2O 80:20), $MgSO_4$, desorption (CH_2Cl_2), 98%.

Scheme 2-9: Synthesis of rhamnolipid methyl esters using a hydrophobically assisted switching phase (HASP) approach.[48]

2.1.3 Research objective and retrosynthetic analysis

As mentioned in Chapter 1, four rhamnolipid derivatives (Figure 2-3), including amide **2.71**, ether **2.72**, hydrocarbon **2.73** and ketone **2.74** analogs, were targeted for synthesis to understand how these structural changes influenced their immunomodulatory activity. The retrosynthetic analysis of **2.71**–**2.74** is outlined in Scheme 2-10. The disconnection of the glycosidic bond in **2.71**–**2.74** gives rise to glycosyl donor **2.75** and the corresponding di-lipid intermediates (**2.76**, **2.77**, **2.81**–**2.83**). The generation of the amide di-lipid **2.76** could be achieved by the coupling of β-amino ester **2.78** and the β-hydroxy acid obtained from ester **2.79**. The synthesis of ether di-lipid **2.77** could be started with displacement of the iodide **2.80** with the β-hydroxy ester obtained from **2.79**. Compound **2.80** could also be synthesized from β-hydroxy ester **2.79** in several steps. For the preparation of **2.73**, the hydrocarbon di-lipid **2.81** could be generated by a Wittig reaction between aldehyde **2.84** and a triphenylphosphonium ylide produced from iodide **2.80**. The C=O bond in ketone **2.74** could be generated by opening of the six-membered ring lactones **2.82** or **2.83** and oxidation of the resulting hydroxyl group. The synthesis of lactones **2.82** or **2.83** could be achieved by regioselective β-hydroxylation of **2.81** and in situ lactonization.

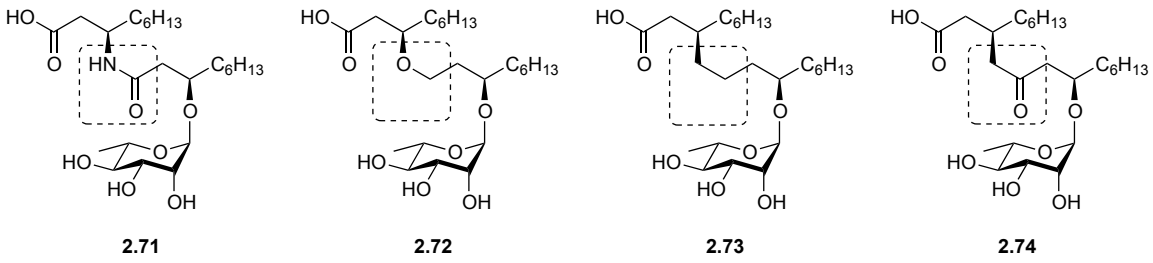
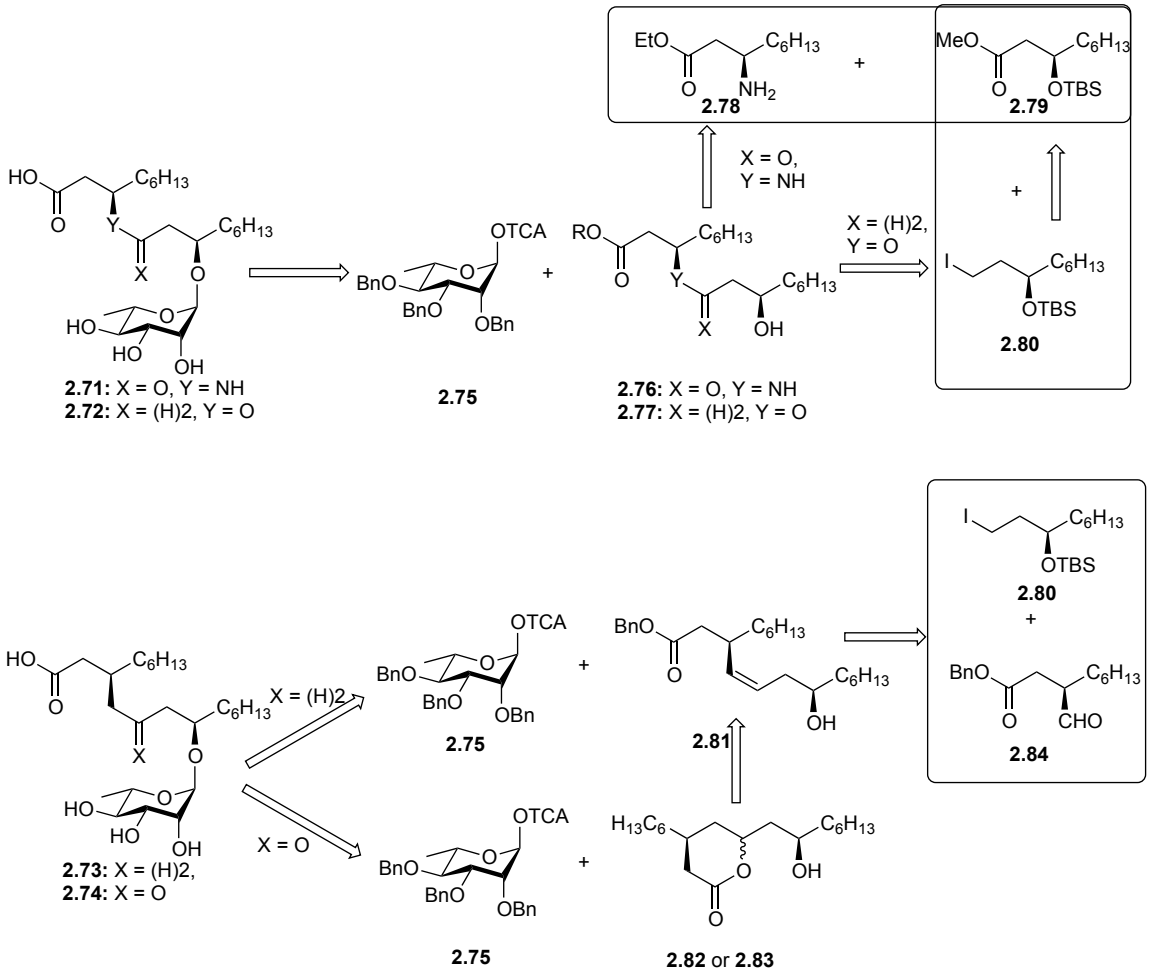


Figure 2-3: The structures of four rhamnolipid derivatives **2.71–2.74**.

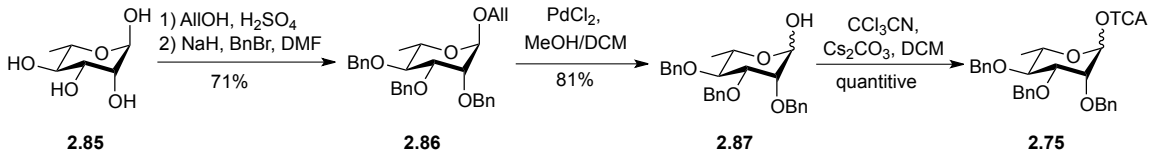


Scheme 2-10: Retrosynthetic analysis of **2.71–2.74**.

2.2 Results and discussion

2.2.1 Synthesis of rhamnosyl trichloroacetimidate donor 2.75

The synthesis of rhamnosyl donor **2.75** (Scheme 2-11) began with Fisher glycosylation of L-rhamnose **2.85** with allyl alcohol, followed by benzylation of all of the free hydroxyl groups to afford **2.86** in 71% yield. Removal of the anomeric allyl group by using $PdCl_2$ in methanol and dichloromethane afforded the hemiacetal **2.87** in 81% yield. Conversion of the hemiacetal **2.87** to the rhamnosyl trichloroacetimidate **2.75** was achieved quantitatively by treatment with trichloroacetonitrile and cesium carbonate.

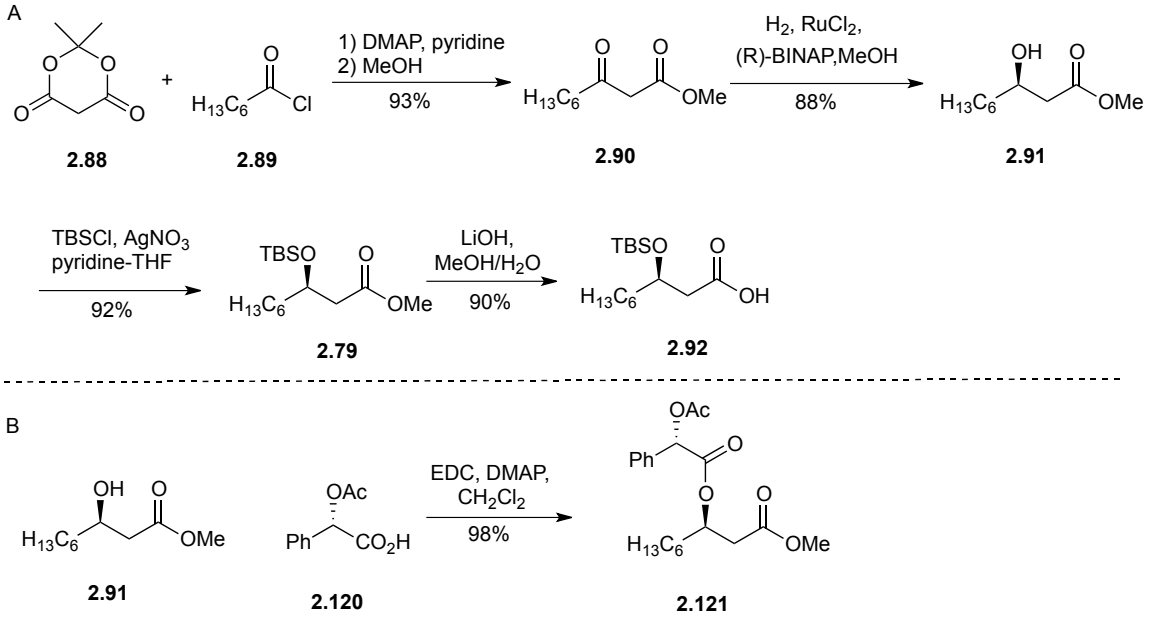


Scheme 2-11: Synthesis of rhamnosyl trichloroacetimidate donor **2.75**.

2.2.2 Synthesis of β-hydroxy ester 2.79 and the corresponding acid 2.92

The synthesis of the enantiomerically pure β-hydroxy ester **2.79** (Scheme 2-12-A) started with C-acylation of Meldrum's acid (**2.88**) with acid chloride **2.89** and then hydrolysis/decarboxylation to afford the β-keto ester **2.90** in 93% yield. Then, hydrogenation of the β-ketoester using Noyori's catalyst (Ru-BINAP) produced the enantiomerically pure β-hydroxy ester **2.91** in 88% yield and 93% ee. To determine the ee,

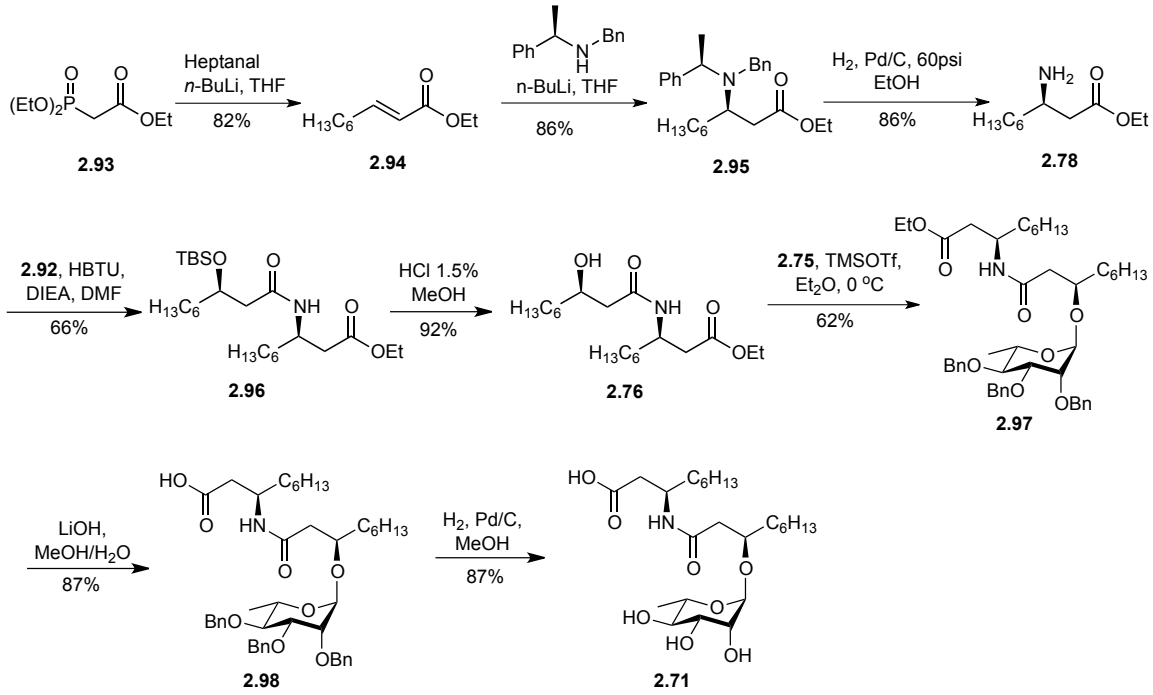
the free hydroxyl group of **2.91** was coupled with (*S*)-(+)-*O*-acetylmandelic acid **2.120** (Scheme 2-12-B). Then, the OCH_3 signals of **2.121** in the ^1H NMR spectra were integrated to determine the ratio between the (*R*)-β-hydroxyester and (*S*)-β-hydroxyester. Protection of the hydroxyl group of **2.91** was achieved in 92% yield by reaction with tert-butyldimethylsilyl chloride (TBSCl) and silver nitrate ($AgNO_3$) in pyridine and THF. Saponification of the methyl ester **2.79** was achieved by treatment with lithium hydroxide monohydrate ($LiOH \cdot H_2O$) giving the free acid **2.92** in 90% yield.



Scheme 2-12: A) Synthesis of β-hydroxy ester **2.79** and carboxylic acid **2.92**. B) Preparation of the mandelic acid derivative of **2.91** used to determine the ee of the Noyori reduction.

2.2.3 Synthesis of the amide rhamnolipid analog 2.71

The key intermediate in the synthesis of **2.71** was amide **2.76**. This compound was prepared starting with a Horner–Wadsworth–Emmons reaction between heptanaldehyde and the anion obtained by treatment of phosphonoacetate **2.93** with *n*-BuLi (Scheme 2-13). This reaction provided an 82% yield of the α, β-unsaturated ester **2.94**. Next, 1,4-addition of lithium (*R*)-*N*-benzyl-*N*-phenylethylamide to **2.94** afforded **2.95** in 86% yield and 95% de.[71] To determine the de, hydrogenolysis of **2.95** (at 60 psi) led to cleavage of the *N*-benzyl and *N*-phenylethyl groups to give the corresponding (*R*)-β-amino ester **2.78** in 86% yield. The free NH_2 group of **2.78** was protected with a tosyl (Ts) group, and then chiral HPLC was used to separate two enantiomers of the Ts-protected derivative **2.78**. The ratio between (*R*)-β-amino ester and (*S*)-β-amino ester was 97.5:2.5. The *R* stereochemistry of β-amino ester **2.78** was assumed based on the reference method.[71]

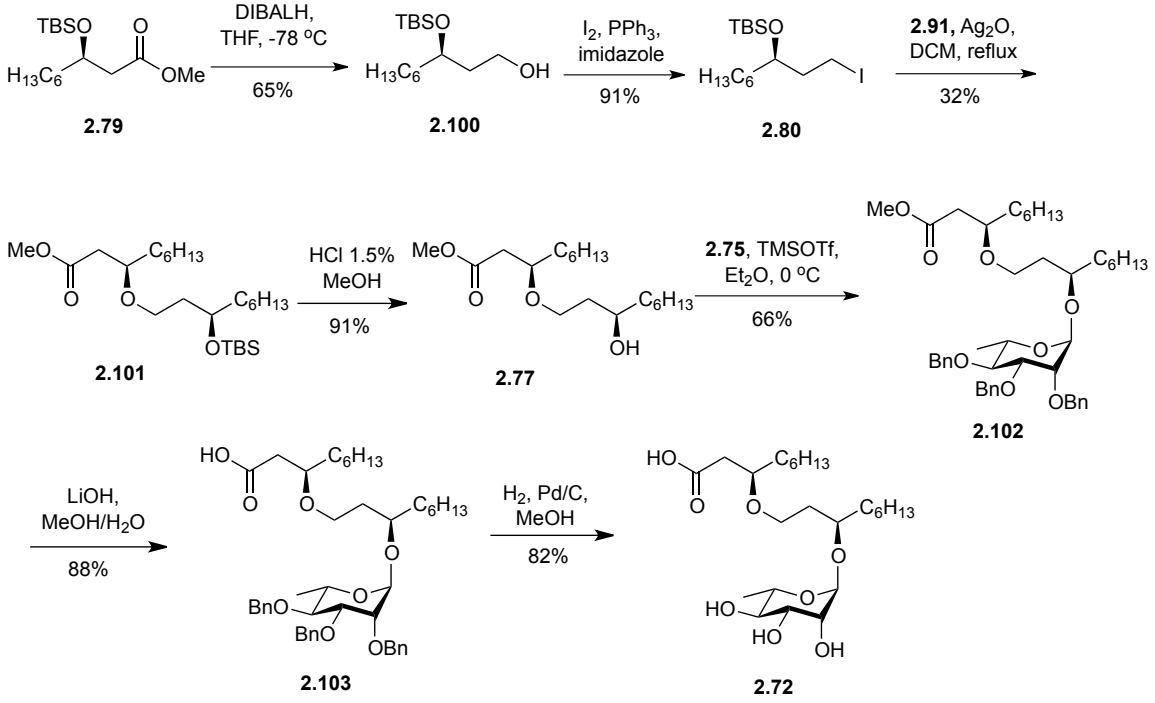


Scheme 2-13: Synthesis of amide rhamnolipid analog **2.71**.

Having established the stereochemistry of **2.78**, its coupling with TBS-protected β-hydroxy-acid **2.92** was carried out by treatment with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and DIEA to afford **2.96** in 66% yield. The resulting amide **2.96** was treated with 1.5% HCl in methanol, which led to the removal of the silyl ether affording **2.76** in 92% yield. Then, TMSOTf-promoted glycosylation of **2.76** with trichloroacetimidate **2.75** gave a 3:1 α:β mixture of protected rhamnolipids. After purification, the α-rhamnolipid **2.97** was obtained in 62% yield. The one-bond C–H coupling constant ($^1J_{\text{C-1, H-1}}$) was used to assign the α and β isomers. The value for the major product is 170.2 Hz, which indicates the α-stereochemistry.[72] Two deprotection steps, ester hydrolysis and hydrogenolysis, led to the amide rhamnolipid analog **2.71** in 76% yield.

2.2.4 Synthesis of ether rhamnolipid analog 2.72

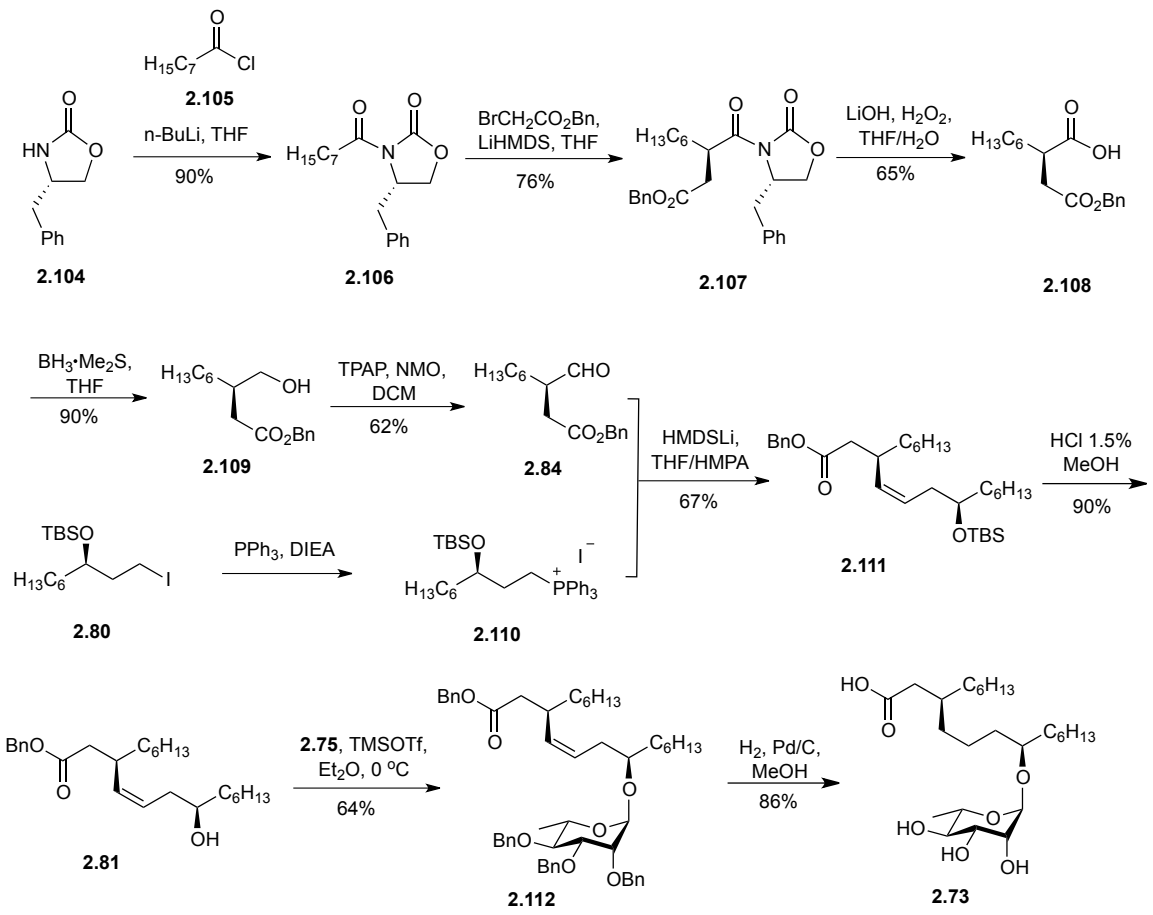
The synthesis of ether analog **2.72** (Scheme 2-14) started with the reduction of ester **2.79** to afford primary alcohol **2.100** in 65% yield. Treatment of **2.100** with iodine, PPh_3 and imidazole, resulted in its conversion, in 91% yield, into iodide **2.80**. I first explored the use of NaH to alkylate **2.91** with **2.80**, but compound **2.91** decomposed under these strongly basic conditions. I next investigated the use of silver(I) oxide-assisted O-alkylation of **2.91** with **2.80**. A major side-product in this reaction was dimerization of **2.80**, which presumably arose from the hydrolysis of **2.80** and displacement of iodide by the resulting alcohol to form the ether. The yield of the desired product **2.101** was only 32%. Nevertheless, with **2.101** in hand, I moved forward. Removal of the TBS protecting group proceeded in 91% yield to give alcohol **2.77**. TMSOTf-mediated glycosylation of **2.77** with trichloroacetimidate donor **2.75** afforded a 3.5:1 α:β mixture of protected rhamnolipid**s**. After purification, the desired α-rhamnolipid was obtained in 62% yield and the α-stereochemistry of **2.102** was determined by $^{1}J_{C\text{-}1,\ H\text{-}1}$ value (170.4 Hz). After saponification and hydrogenolysis, the ether linked target **2.72** was obtained in 72% yield over the two steps.



Scheme 2-14: Synthesis of ether rhamnolipid analog **2.72**.

2.2.5 Synthesis of the hydrocarbon rhamnolipid analog 2.73

The enantiopure α-alkyl aldehyde **2.84** (Scheme 2-15) is a key substrate for a Wittig reaction to obtain **2.111**, which was used in the synthesis of **2.72**. To prepare **2.84**, I first investigated a method reported by MacMillan and coworkers, which involves an enantioselective α-alkylation of aldehydes by using both a photoredox catalyst $Ru(bpy)_3Cl_2$ and an imidazolidinone organocatalyst.[73] However, when I used the aldehyde required for the target (*n*-octanal) as a substrate, the enantioselectivity was modest (~77% ee).



Scheme 2-15: Synthesis of hydrocarbon rhamnolipid analog **2.73**.

Thus, I decided to adopt the Evans asymmetric alkylation method (Scheme 2-15)[74] to prepare this substrate. To do this, (*S*)-*N*-*n*-octanoyl-4-benzyloxazolidinone **2.106**, prepared from *n*-octanoyl chloride **2.105** and 4-(*S*)-benzyloxazolidinone **2.104**, was treated with benzyl 2-bromoacetate to afford **2.107** in 76% yield. The stereochemistry of the α-position was assumed based on the stereochemical model for this auxiliary.[75] Removal of the oxazolidinone auxiliary with LiOH in the presence of 30% H_2O_2 gave the chiral carboxylic

acid **2.108** in 65% yield. The acid moiety of **2.108** was chemoselectively reduced with $BH_3 \cdot Me_2S$ in THF to afford an 88% yield of alcohol **2.109**.

The next step was to oxidize alcohol **2.109** to the corresponding aldehyde **2.84**. Initially, the Swern oxidation was used for this purpose. However, the value of the optical rotation for the product was zero, which suggested that the stereogeneic centre had been epimerized during the oxidation. To confirm that **2.84** was a mixture of two compounds, it was reacted with (2*S*, 4*S*)-(+)-pentanediol, which generates an acetal with the aldehyde.[73] The crude ^{1}H NMR spectrum of this acetal showed two diastereomers in a 1:1 ratio indicating that racemization had occurred during oxidation. The base used during the Swern oxidation, diisopropylethylamine, is presumably the cause of the epimerization.

To prevent epimerization, the less basic Ley–Griffith oxidation, which involves tetrapropylammonium perruthenate (TPAP) and the co-oxidant *N*-methyl morpholine oxide (NMO), was used to oxidize the primary hydroxyl group in **2.109** to aldehyde **2.84**. The crude ^{1}H NMR spectrum of the acetal produced from the reaction between **2.84** and (2*S*, 4*S*)-(+)-pentanediol, showed only one diastereomer, indicating that no epimerization had occurred during this oxidation.

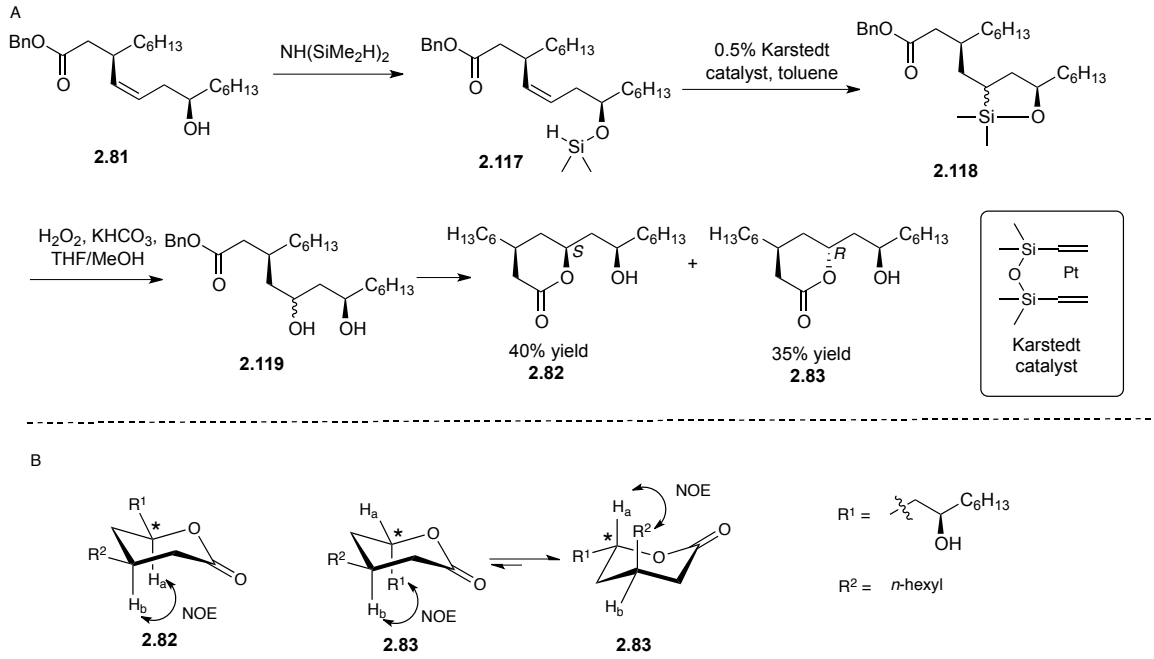
With the aldehyde in hand, a phosphonium salt was generated from iodide **2.80** using triphenylphosphine (Ph_3P) in toluene with Hünig's base as the additive.[76] The desired salt **2.110** was produced smoothly and, conveniently, it could be used after removal of the excess triphenylphosphine with *n*-pentane and no further purification steps. The

phosphonium ylide was generated by reaction of LiHMDS in THF/HMPA with **2.110**. After the addition of aldehyde **2.84,** the *Z*-alkene **2.111** was isolated in 67% yield from aldehyde **2.84**. After deprotection of the silyl ether using HCl in methanol, the resulting alcohol **2.81** was glycosylated with **2.75** to afford a 3.5:1 mixture of protected rhamnolipids. After purification, α-rhamnolipid **2.112** was obtained in 64% yield and the α-stereochemistry was confirmed by the $^{1}J_{\text{C-1, H-1}}$ value (170.1 Hz). Treatment of **2.112** with hydrogen over Pd/C led to reduction of the double bond and removal of all of the benzyl groups to generate the hydrocarbon analog **2.73** in 86% yield.

2.2.6 Synthesis of ketone rhamnolipid analog 2.74

To synthesize the ketone analog **2.74**, alkene **2.81**, which was used for hydrocarbon analog synthesis, could also be employed. As shown in Scheme 2-16-A, I reasoned that if compound **2.81** could be converted to a 1,3-diol (e.g., **2.119**), the newly formed hydroxyl group could be automatically protected by forming a six-membered ring lactone (**2.82** or **2.83**). To explore this possibility, I used an approach reported by Li and Roush, who developed an intramolecular hydrosilylation method for β,δ-unsaturated alcohols using Karstedt's catalyst.[77] The detailed reaction sequence is illustrated in Scheme 2-16. First, alcohol **2.81** was silylated and then by using 0.5 mol % Karstedt's catalyst in toluene, an intramolecular carbon–silicon bond formation process led to **2.118**. Subsequent oxidation

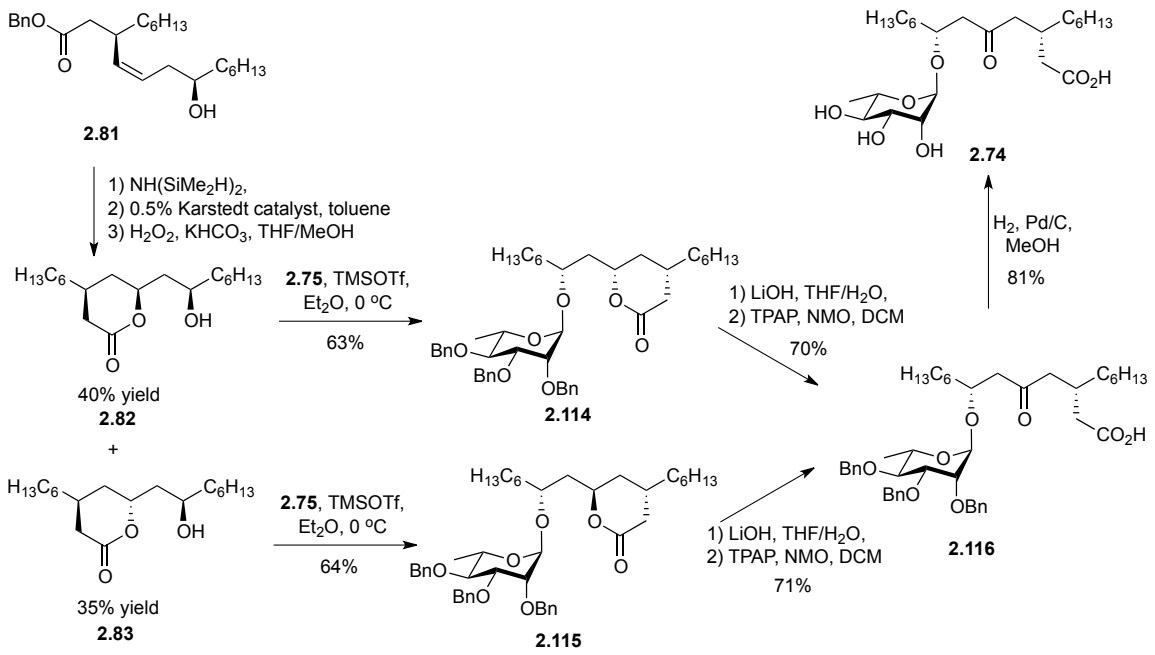
under basic conditions resulted in the formation of lactones **2.82** and **2.83** in a combined 75% yield.



Scheme 2-16: A) Intramolecular hydrosilylation–lactone formation sequence on **2.81** using Karstedt's catalyst. B) Using NOESY to confirm the newly formed stereocenters of **2.82** and **2.83**.

NOESY was used to assign the stereochemistry of the newly formed stereocenter in these lactones. As shown in Scheme 2-16-B, I assumed that the six-membered ring lactones (**2.82** and **2.83**) prefer chair conformations. A NOESY experiment of **2.83** showed no interaction between H_a (δ 4.68 ppm) and H_b (δ 2.04 ppm). However, a strong interaction was observed between H_a (δ 4.68 ppm) and the CH_2 group (δ 1.41 ppm) on the *n*-hexyl group. These results suggest that in **2.83** H_a and H_b are *trans* to each other and that the

newly formed stereocentre is *R*. A NOESY experiment of **2.82** showed strong interaction between H_a (δ 4.50 ppm) and H_b (δ 1.94 ppm). Thus, the newly formed stereocentre of **2.82** has the opposite (*S*) stereochemistry. As outlined below, these two lactones could be separated and both could be used in accessing the products. An advantage of this reaction sequence is that the newly formed hydroxyl group can be automatically protected via lactone formation. The other hydroxyl group remains free and can be glycosylated (Scheme 2-17).



Scheme 2-17: Synthesis of ketone analog **2.74**.

Once the lactones **2.82** and **2.83** were produced, TMSOTf-promoted glycosylation between **2.75** and acceptor **2.82** afforded a 4:1 mixture of protected rhamnolipids. The α-

rhamnolipid **2.114** was purified in 63% yield and the $^{1}J_{C\text{-}1,\ H\text{-}1}$ value (168.4 Hz) confirmed the α-stereochemistry of **2.114**. Alternatively, the diastereomer **2.115** was formed in 64% yield via glycosylation between **2.75** and acceptor **2.83** and the α:β selectivity was also 4:1. After opening the lactones in **2.114** and **2.115** using LiOH in THF and water, the free hydroxyl groups could be oxidized by the Ley–Griffith reagent to afford ketone **2.116**. Both saponification/oxidation procedures proceeded in essentially identical yields (70%). The material obtained from each **2.114** and **2.115** was combined and carried forward. After global deprotection by Pd/C catalyzed hydrogenolysis, the ketone analog **2.74** was produced in 81% yield.

2.3 Conclusions

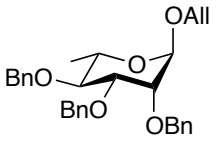
In summary, this chapter describes the synthesis of four rhamnolipid analogs, in which the ester linkage connecting the two lipid chains in the natural compound is replaced with an amide, ether, hydrocarbon, or ketone group (compounds **2.71**–**2.74**). To prepare **2.71**, the key step was an enantioselective 1,4-addition to α:β unsaturated ester **2.94**, which provided the (*R*)-β-amino ester **2.78**. Subsequent amidation of **2.78** with a β-hydroxy-acid, glycosylation and deprotection gave the amide-linked target **2.71**. The key step in the synthesis of the ether analog **2.72** is a Ag_2O-assisted O-alkylation of β-hydroxy-ester **2.91**, which successfully provided the compound **2.101**. Further elaboration (glycosylation and

deprotection) gave **2.72**. The synthesis of the hydrocarbon (**2.73**) and ketone (**2.74**) analogs share the same key intermediate, **2.111**, which could be generated via a Wittig reaction, using an aldehyde (**2.84)** produced from a Ley–Griffith oxidation. Glycosylation of **2.81** and hydrogenation led to **2.73**. For the ketone analog **2.74**, I adopted a four-step reaction sequence, involving silyl ether formation, hydrosilylation, oxidative cleavage and lactone formation, to synthesize the lactone acceptors **2.82** and **2.83** from **2.111**. Then, glycosylation, lactone ring opening and the late stage Ley–Griffith oxidation led to the ketone functional group for final target **2.74**. The biological evaluation of these compounds will be done by other group members.

2.4 Experimental section

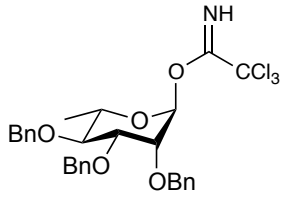
General Methods: Reactions were carried out in oven-dried glassware. All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless stated otherwise, all reactions were carried out at r.t. under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with cerium molybdate stain or potassium permanganate stain. Unless otherwise indicated, all column chromatography was performed on silica gel 60 (40–60 μM). The ratio between

silica gel and crude product ranged from 100 to 50:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS–8060, which is manufactured by Iatron Laboratories (Tokyo). Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of deg·mL(dm·g)$^{-1}$. ^{1}H NMR spectra were recorded at 400, 500 or 700 MHz, and chemical shifts are referenced to either TMS (0.0 ppm, $CDCl_3$) or HOD (4.78 ppm, CD_3OD). ^{13}C NMR spectra were recorded at 100, 125, or 150 MHz, and ^{13}C chemical shifts are referenced to internal $CDCl_3$ (77.23 ppm, $CDCl_3$), external dioxane CD_3OD (48.9 ppm, CD_3OD). In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were concentrated under vacuum at < 40˚C (bath). Electrospray mass spectra (time-of-flight analyzer) were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. Electron impact ionization mass spectra were recorded on samples dissolved in CH_2Cl_2.

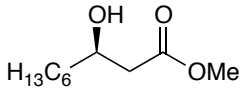


Allyl 2,3,4-tri-*O*-Benzyl-α-L-rhamnopyranoside (2.86): A solution of L-rhamnose monohydrate (5.0 g, 30.5 mmol) in dried allyl alcohol (50 mL) and concentrated H_2SO_4 (0.4 mL) was stirred at 100 °C for 1 h. The mixture was cooled to r.t. and then, K_2CO_3 (0.4 g) was added to neutralize the solution. After removing allyl alcohol *in vacuo*, the resulting syrup was partially purified by chromatography (gradient 10 → 40% CH_2Cl_2 in CH_3OH)

to afford crude allyl α-L-rhamnopyranoside. This crude residue was taken up in DMF (200 mL) and treated with BnBr (26 g, 152 mmol). The reaction mixture was cooled (−30 °C) and treated with NaH (6.7 g as a 60% dispersion in mineral oil, 167 mmol) and then warmed to r.t. and stirred for 12 h. The solution was cooled to 0 °C , the reaction quenched by the addition of CH_3OH (50 mL) and the mixture was concentrated. The residue was taken up in CH_2Cl_2 washed with brine, dried with Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 5→7% EtOAc in hexane) to afford **2.86** (10.3 g, 71% yield) as a foam. R_f 0.45 (7:1 hexane–EtOAc); $[\alpha]_D = -14.5$ (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.40–7.27 (m, 15 H), 5.84 (dddd, 1 H, *J* = 17.0, 10.5, 6.0, 5.0 Hz), 5.22 (app dq, 1 H, *J* = 17.0, 1.5 Hz), 5.16 (app dq, 1 H, *J* = 10.5, 1.6 Hz), 4.96 (d, 1 H, *J* = 10.8 Hz), 4.81 (d, 1 H, *J* = 1.6 Hz), 4.78 (d, 1 H, *J* = 12.2 Hz), 4.73 (d, 1 H, *J* = 12.2 Hz), 4.66 (d, 1 H, *J* = 10.8 Hz), 4.64 (s, 2 H), 4.13 (app ddt, 1 H, *J* = 13.0, 5.0, 1.5 Hz), 3.92 (app ddt, 1 H, *J* = 13.0, 6.0, 1.5 Hz), 3.90 (dd, 1 H, *J* = 9.5, 3.0 Hz), 3.82 (dd, 1 H, *J* = 3.0, 2.0 Hz), 3.73 (dq, 1 H, *J* = 9.5, 6.2 Hz), 3.64 (app t, 1 H, *J* = 9.5 Hz), 1.35 (d, *J* = 6.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 140.62, 140.61, 140.4, 135.8, 130.3, 130.0, 129.9, 129.6, 129.5, 119.1, 99.2, 82.5, 82.2, 77.4, 76.9, 74.8, 74.2, 70.1, 69.7, 20.0; HRMS (ESI) calcd for (M+Na) $C_{30}H_{34}NaO_5$: 497.2304. Found: 497.2310. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of this material were in good agreement with those reported.[78]

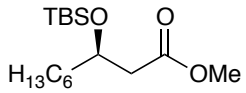


2,3,4-tri-*O*-Benzyl-α-L-rhamnopyranosyl trichloroacetimidate (2.75): To a solution of **2.86** (1.2 g, 2.53 mmol) in CH_3OH (20 mL) and CH_2Cl_2 (20 mL) at r.t. was added $PdCl_2$ (106 mg, 0.6 mmol). The mixture was stirred at r.t. for 16 h. After completion of the reaction, the mixture was filtered through a plug of Celite and the filtrate was concentrated and the resulting residue was purified by chromatography (25% EtOAc in hexane) to give **2.87** (0.89 g, 81% yield) as a white solid. A slurry of **2.87** (100 mg, 0.23 mmol), Cl_3CCN (331 mg, 2.3 mmol), and Cs_2CO_3 (373 mg, 1.15 mmol) in CH_2Cl_2 (10 mL) was stirred for 3.5 h. The reaction mixture was filtered through a short pad of Celite and the pad was washed with dry CH_2Cl_2. The filtrate was concentrated to give 130 mg of a slightly yellowish oil, which was immediately used in the glycosylation step without further purification.



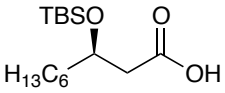
Methyl 3-(*R*)-hydroxy-nonanoate (2.91): To a solution of methyl-3-oxo-nonanoate **2.90** (1.0 g, 5.4 mmol) in dry CH_3OH (25 mL) was added (*R*)-BINAP-$RuCl_2$ (12 mg, 0.015 mmol). The reaction mixture was placed in a hydrogenation autoclave and pressurized with H_2 (55 PSI) and brought to 60 °C for 12 h. After cooling, the reaction mixture was diluted

with brine, extracted with CH_2Cl_2 and subsequently washed with water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 16→20% EtOAc in hexane) to afford **2.91** (0.89 g, 88% yield, 93% ee) as a colorless oil. The enantioselectivity was determined by coupling **2.91** with (*S*)-(+)-*O*-acetylmandelic acid. Then, the OCH_3 signals in ^1H NMR spectra of the resulting mixture were integrated to determine the ratio between the (*R*)-β-hydroxy-ester (3.64 ppm) and (*S*)-β-hydroxy-ester (3.37 ppm). Data for **2.91**: R_f 0.47 (3:1 hexane–EtOAc); $[\alpha]_D = -17.0$ (*c* 0.4, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 4.04–3.97 (m, 1 H), 3.72 (s, 3 H), 2.84 (d, 1 H, *J* = 4.2 Hz), 2.52 (dd, 1 H, *J* = 16.4, 3.2 Hz), 2.41 (dd, 1 H, *J* = 16.4, 9.0 Hz), 1.56–1.27 (m, 10 H), 0.88 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 173.1, 67.6, 51.3, 40.7, 36.1, 31.3, 28.8, 25.0, 22.2, 13.6; HRMS (ESI) calcd for (M+Na) $C_{10}H_{20}NaO_3$: 211.1310. Found: 211.1307.



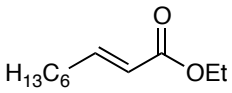
Methyl (*R*)-3-*tert*-butyldimethylsilyloxy-nonanoate (2.79): A solution of β-hydroxy ester **2.91** (0.77 g, 4.09 mmol) in pyridine–THF (2:1, 30 mL) was treated with $AgNO_3$ (0.77 g, 4.51 mmol) and TBSCl (0.68 g, 4.51 mmol) and the mixture was stirred at r.t. for 4 h. The mixture was filtered and concentrated. The residue was dissolved in CH_2Cl_2 (50 mL) followed by washing with 1M of HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by

column chromatography (gradient 5→10% EtOAc in hexane) to afford **2.79** (1.14 g, 92% yield) as a colorless oil; R_f 0.83 (3:1 hexane–EtOAc); $[\alpha]_D$ = –19.0 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.16–4.09 (m, 1 H), 3.67 (s, 3 H), 2.46 (dd, 1 H, *J* = 14.6, 7.0 Hz), 2.41 (dd, 1 H, *J* = 14.6, 5.8 Hz), 1.51–1.27 (m, 10 H), 0.88 (t, 3 H, *J* = 7.2 Hz), 0.87 (s, 9 H), 0.06 (s, 3 H), 0.03 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 171.9, 69.1, 51.0, 42.1, 37.2, 31.4, 28.9, 25.4, 24.5, 22.2, 17.6, 13.6, –4.9, –5.2; HRMS (ESI) calcd for (M+Na) $C_{16}H_{34}NaO_3Si$: 325.2175. Found: 325.2170.

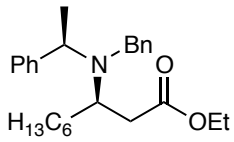


(*R*)-3-*tert*-butyldimethylsilyloxy-nonanoic acid (2.92): A solution of **2.79** (0.43 g, 1.4 mmol) in CH_3OH–water (4:1, 25 mL) was treated with $LiOH•H_2O$ (0.3 g, 7.11 mmol) and the mixture was stirred at 50 °C overnight. After cooling to r.t., the reaction mixture was acidified to pH 5 by the addition of 1 M HCl, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by chromatography (gradient 5→10% EtOAc in hexane) to afford **2.92** (0.37 g, 90% yield) as a colorless oil. R_f 0.44 (3:1 hexane–EtOAc); $[\alpha]_D$ = +3.5 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.13–4.07 (m, 1 H), 2.55 (dd, 1 H, *J* = 15.0, 5.2 Hz), 2.49 (dd, 1 H, *J* = 14.6, 5.8 Hz), 1.57–1.27 (m, 10 H), 0.90 (s, 9 H), 0.89 (t, 3 H, *J* = 7.2 Hz), 0.11 (s, 3 H), 0.09 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 177.2,

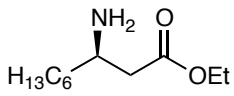
69.1, 41.2, 36.7, 31.3, 28.8, 25.3, 24.8, 22.2, 17.6, 13.6, –4.9, –5.3; HRMS (ESI) calcd for (M+Na) $C_{15}H_{32}NaO_3Si$: 311.2018. Found: 311.2025.



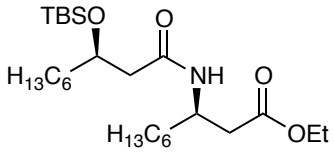
(*E*)-ethyl 2-nonenoate (2.94): To a solution of triethylphosphonoacetate (16.0 g, 71.0 mmol) in THF (90 mL) at −78 °C was added 1.6 M *n*-BuLi in hexane (44 mL, 71.0 mmol). The reaction mixture was stirred for 30 min and a solution of heptanaldehyde (6.2 g, 54.0 mmol) in THF (90 mL) was added dropwise at −78 °C. The resulting mixture was stirred at −78 °C for 1.5 h and then saturated NH_4Cl solution was added to quench the reaction. After the reaction mixture was warmed to r.t., the product was extracted with ether and the organic layer was washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 0.3→0.5% EtOAc in hexane) to afford **2.94** (7.6 g, 75% yield) as a colorless oil. R_f 0.20 (hexane); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 6.97 (dt, 1 H, J = 15.5, 7.0 Hz), 5.81 (dt, 1 H, J = 15.5, 1.5 Hz), 4.18 (q, 2 H, J = 7.2 Hz), 2.22–2.16 (m, 2 H), 1.49–1.23 (m, 11 H), 0.89 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.4, 149.1, 120.8, 59.7, 31.8, 31.2, 28.4, 27.6, 22.1, 13.9, 13.6; HRMS (ESI) calcd for (M+H) $C_{11}H_{21}O_2$: 185.1542. Found: 185.1551. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of this material were in good agreement with those reported.[79]



(*R*)-ethyl 3-(benzyl((*R*)-1-phenylethyl)amino)nonanoate (2.95) To a solution of (*R*)-*N*-benzyl-1-phenylethanamine (0.41 g, 1.95 mmol) in THF (12 mL) was added *n*-BuLi (2.5 M in hexane, 0.73 mL, 1.83 mmol) at −78 °C and the reaction mixture was stirred for 30 min. A solution of **2.94** (0.3 g, 1.22 mmol) in THF (4 mL) was added dropwise at −78 °C and the resulting mixture was stirred for 2.5 h. A solution of 4-bromo-2,6-di-*tert*-butylphenol (1.04 g, 3.66 mmol) in THF (4 mL) was added, the mixture was warmed to r.t. and then the solvent was evaporated. The crude material was washed with brine and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 0→0.3% EtOAc in hexane) to afford **2.95** (0.41 g, 86% yield) as a colorless oil. $[\alpha]_D = +7.0$ (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.44–7.42 (m, 2 H), 7.36–7.20 (m, 8 H), 4.05 (dq, 1 H, *J* = 10.8, 7.2 Hz), 3.99 (dq, 1 H, *J* = 10.8, 7.2 Hz), 3.84 (q, 1 H, *J* = 7.0 Hz), 3.80 (d, 1 H, *J* = 14.8 Hz), 3.55 (d, 1 H, *J* = 14.8 Hz), 3.34–3.27 (m, 1 H), 2.06 (dd, 1 H, *J* = 14.4, 4.8 Hz), 2.00 (dd, 1 H, *J* = 14.4, 8.0 Hz), 1.56–1.47 (m, 2 H), 1.34 (d, 3 H, *J* = 7.0 Hz), 1.32–1.22 (m, 8 H), 1.19 (t, 3 H, *J* = 7.2 Hz), 0.89 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 172.5, 142.9, 141.4, 127.9, 127.8, 127.6, 127.5, 126.4, 126.2, 59.7, 57.6, 53.7, 49.6, 36.5, 33.1, 31.5, 28.9, 26.6, 22.3, 19.3, 13.8, 13.7; HRMS (ESI) calcd for (M+H) $C_{26}H_{38}NO_2$: 396.2897. Found: 396.2889.

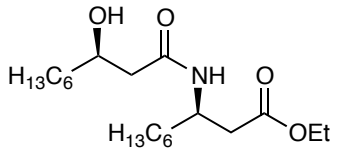


(*R*)-ethyl 3-aminononanoate (2.78): To a solution of **2.95** (2 g, 5.0 mmol) in ethanol (30 mL) was added 10% palladium on charcoal (100 mg) and the mixture was hydrogenolysed (hydrogen pressure: 60 psi) at r.t. for 16 h. The mixture was filtered and the solution was concentrated to afford the target compound **2.78** (0.94 g, 92% yield). R_f 0.20 (EtOAc); $[\alpha]_D$ = –4.9 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.15 (q, 2 H, *J* = 7.2 Hz), 3.21–3.15 (m, 1 H), 2.46 (dd, 1 H, *J* = 15.6, 4.0 Hz), 2.25 (dd, 1 H, *J* = 15.6, 8.8 Hz), 1.38–1.25 (m, 11 H), 0.89 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 172.6, 60.5, 48.5, 41.4, 36.8, 31.7, 29.2, 25.9, 22.6, 14.2, 12.0; HRMS (ESI) calcd for (M+H) $C_{11}H_{24}NO_2$: 202.1802. Found: 202.1801.



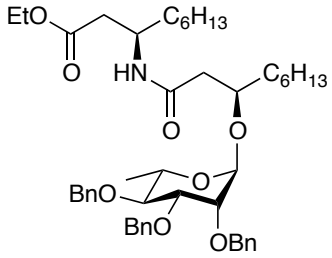
(*R*)-ethyl 3-((*R*)-3-((*tert*-butyldimethylsilyl)oxy)nonanamido)nonanoate (2.96): To a solution of **2.78** (110 mg, 0.54 mmol) in DMF (5 mL) was added **2.92** (150 mg, 0.52 mmol), HBTU (250 mg, 0.78 mmol) and DIEA (100 mg, 0.78 mmol). The mixture was stirred at r.t. overnight and then water was added and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (silica gel, gradient 10→12.5% EtOAc in hexane) to afford **2.96** (162 mg, 66% yield) as a colorless oil. R_f 0.42 (6:1

hexane–EtOAc); $[\alpha]_D$ = +16.3 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 6.60 (d, 1 H, *J* = 8.4 Hz), 4.29–4.22 (m, 1 H), 4.16–4.09 (m, 2 H), 4.02 (app tt, 1 H, *J* = 6.2, 4.8 Hz), 2.52 (dd, 1 H, *J* = 15.6, 5.6 Hz), 2.46 (dd, 1 H, *J* = 15.6, 6.0 Hz), 2.42 (dd, 1 H, *J* = 14.8, 4.8 Hz), 2.30 (dd, 1 H, *J* = 14.8, 4.8 Hz), 1.56–1.48 (m, 4 H), 1.37–1.24 (m, 19 H), 0.90 (s, 9 H), 0.89–0.86 (m, 6 H), 0.094 (s, 3 H), 0.091 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 171.1, 170.0, 69.5, 60.0, 45.5, 43.6, 38.8, 36.1, 33.9, 31.3, 28.9, 28.6, 25.7, 25.4, 25.3, 22.2, 22.1, 17.6, 13.8, 13.6, –5.1; HRMS (ESI) calcd for (M+H) $C_{26}H_{54}NO_4Si$: 472.3817. Found: 472.3815.



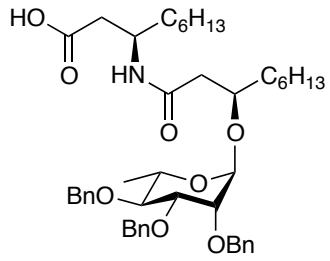
(*R*)-ethyl 3-((*R*)-3-hydroxynonanamido)nonanoate (2.76): A solution of **2.96** (516 mg, 1.09 mmol) in 1.5% HCl in CH_3OH (40 mL) was stirred for 1 h. The reaction mixture was neutralized by the addition of saturated aqueous $NaHCO_3$, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 20→50% EtOAc in hexane) to afford **2.96** (360 mg, 92% yield) as a colorless oil. R_f 0.40 (1:1 hexane–EtOAc); $[\alpha]_D$ = +4.9 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 6.22 (d, 1 H, *J* = 9.2 Hz), 4.29–4.21 (m, 1 H), 4.18–4.10 (m, 2 H), 3.99–3.93 (m, 1 H), 3.76 (d, 1 H, *J* = 3.2 Hz), 2.56 (dd, 1 H, *J* = 15.6, 4.8 Hz), 2.44 (dd, 1 H, *J* = 15.6, 6.0 Hz), 2.34 (dd, 1

H, *J* = 14.8, 2.8 Hz), 2.22 (dd, 1 H, *J* = 14.8, 9.2 Hz), 1.55–1.40 (m, 4 H), 1.34–1.24 (m, 19 H), 0.90–0.86 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 171.7, 171.5, 68.4, 60.4, 45.7, 42.7, 38.2, 36.5, 33.8, 31.4, 31.3, 28.8, 28.6, 25.7, 25.1, 22.2, 22.1, 13.76, 13.66, 13.63; HRMS (ESI) calcd for (M+H) $C_{20}H_{40}NO_4$: 358.2952. Found: 358.2949.



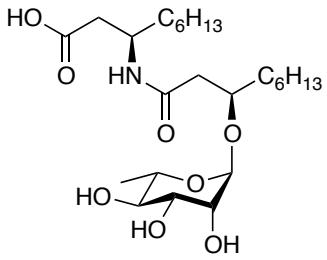
Ethyl (*R*)-3-*N*-[2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-(*R*)-3′-*O*-nonanamido]nonanoate (2.97): A mixture of acceptor **2.76** (40 mg, 0.11 mmol), trichloroacetimidate donor **2.75** (130 mg, 0.22 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous Et_2O (10 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C, and then TMSOTf (10 μL) was added. The solution was stirred for 1 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→20% EtOAc in hexane) to afford **2.97** (53 mg, 62% yield) as a colorless oil; R_f 0.55 (2:1 hexane–EtOAc); $[\alpha]_D$ = +2.7 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.26 (m, 15 H), 6.39 (d, 1 H, *J* = 9.2 Hz), 4.92 (d, 1 H, *J* = 10.8 Hz), 4.84 (d, 1 H, *J* = 2.0 Hz), 4.77 (d, 1 H, *J* = 12.8 Hz), 4.71 (d, 1 H, *J* = 12.8 Hz), 4.65–4.63 (m, 3 H), 4.24–4.16 (m, 1 H), 4.12–3.93 (m, 3 H), 3.82 (dd, 1 H, *J* = 9.2, 3.2 Hz), 3.72 (dd, 1 H, *J* = 3.0, 2.0 Hz),

3.71–3.64 (m, 1 H), 3.60 (app t, 1 H, J = 9.2 Hz), 2.53 (dd, 1 H, J = 16.0, 5.6 Hz), 2.43 (dd, 1 H, J = 16.0, 4.8 Hz), 2.40 (dd, 1 H, J = 14.4, 6.0 Hz), 2.32 (dd, 1 H, J = 14.4, 5.6 Hz), 1.49–1.41 (m, 4 H), 1.31 (d, 3 H, J = 6.4 Hz), 1.34–1.21 (m, 16 H), 1.17 (t, 3 H, J = 7.2 Hz), 0.90 (t, 3 H, J = 7.2 Hz), 0.87 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.1, 169.8, 138.74, 138.69, 138.3, 128.39, 128.36, 128.29, 128.1, 127.9, 127.5, 96.8, 80.6, 80.2, 75.3, 75.0, 74.4, 72.9, 72.2, 68.5, 60.5, 45.7, 42.6, 38.2, 34.1, 33.0, 31.7, 29.3, 29.0, 26.2, 24.9, 22.64, 22.57, 18.0, 14.14, 14.11, 14.06; HRMS (ESI) calcd for (M+Na) $C_{47}H_{67}NNaO_8$: 796.4759. Found: 796.4749.



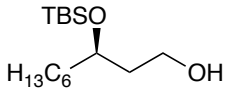
(*R*)-3-*N*-[2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-(*R*)-3′-*O*-nonanamido]nonanic acid (2.98): A solution of **2.97** (50 mg, 0.06 mmol) in CH_3OH–water (4:1, 5 mL) was treated with $LiOH•H_2O$ (25 mg, 0.6 mmol) and was stirred at 50 °C overnight. After cooling to r.t., the reaction mixture was acidified to pH 5 by the addition of 1 M HCl, diluted with water and then extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 50→70% EtOAc in hexane) to afford **2.98** (42 mg, 87% yield) as a colorless oil. R_f 0.13 (1:1 hexane–EtOAc); $[\alpha]_D$ = –16.4 (*c* 0.1, CH_2Cl_2);

^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.45–7.28 (m, 15 H), 5.51 (d, 1 H, *J* = 9.6 Hz), 5.02 (d, 1 H, *J* = 10.8 Hz), 4.80 (d, 1 H, *J* = 11.4 Hz), 4.78 (d, 1 H, *J* = 12.4 Hz), 4.77 (d, 1 H, *J* = 2.0 Hz), 4.67 (d, 1 H, *J* = 10.8 Hz), 4.66 (d, 1 H, *J* = 11.4 Hz), 4.65 (d, 1 H, *J* = 12.4 Hz), 4.49–4.39 (m, 1 H), 4.26–4.20 (m, 1 H), 4.05 (dd, 1 H, *J* = 8.4, 2.8 Hz), 3.71–3.65 (m, 3 H), 3.60 (app t, 1 H, *J* = 9.2 Hz), 2.56 (dd, 1 H, *J* = 13.6, 3.6 Hz), 2.37 (dd, 1 H, *J* = 14.8, 2.4 Hz), 2.12 (dd, 1 H, *J* = 14.8, 9.6 Hz), 2.07 (dd, 1 H, *J* = 13.6, 10.4 Hz), 1.44–1.36 (m, 4 H), 1.31 (d, 3 H, *J* = 6.4 Hz), 1.32–1.21 (m, 19 H), 0.90 (t, 3 H, *J* = 7.2 Hz), 0.86 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.6, 170.3, 139.2, 138.6, 137.3, 129.2, 128.8, 128.71, 128.69, 128.5, 128.4, 128.10, 128.08, 127.8, 95.1, 81.8, 80.1, 76.7, 75.3, 73.4, 72.4, 72.1, 67.5, 47.5, 42.4, 40.8, 36.5, 32.2, 32.1, 32.0, 29.8, 29.4, 26.2, 24.8, 22.94, 22.88, 18.1, 14.5, 14.4; HRMS (ESI) calcd for (M–H) $C_{45}H_{62}NO_8$: 744.4481. Found: 744.4478.



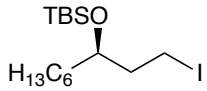
(*R*)-3-*N*-[α-L-rhamnopyranosyl-(1→3)-(*R*)-3′-*O*-nonanamido]nonanic acid (2.71): A solution of **2.98** (60 mg, 0.08 mmol) in CH_3OH (10 mL) and acetic acid (0.2 mL) was treated with palladium on charcoal (10%, 50 mg) and subjected to a hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The

residue was subjected to chromatography (Iatrobeads 6RS-8060, gradient 10→40% CH_3OH in CH_2Cl_2) to yield **2.71** (32 mg, 87% yield) as a colorless oil. $[\alpha]_D = -30.5$ (*c* 0.1, CH_2Cl_2); ^1H NMR (700 MHz, CD_3OD, δ_H) 4.79 (s, 1 H, H-1), 4.25–4.22 (m, 1 H, NHC*H*), 4.11–4.08 (m, 1 H, OC*H*CH_2), 3.74 (br s, 1 H, H-2), 3.68 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.66–3.62 (m, 1 H, H-5), 3.34 (app t, 1 H, *J* = 9.5 Hz, H-4), 2.49 (dd, 1 H, *J* = 14.0, 7.5 Hz, C*H*$_2$CONH), 2.40 (dd, 1 H, *J* = 15.0, 5.0 Hz, C*H*$_2$$CO_2H$), 2.36 (dd, 1 H, *J* = 15.0, 8.0 Hz, C*H*$_2$$CO_2H$), 2.33 (dd, 1 H, *J* = 14.0, 5.5 Hz, C*H*$_2$CONH), 1.55–1.43 (m, 4 H, 2 x CH_2), 1.37–1.27 (m, 16 H, 8 x CH_2), 1.24 (d, 3 H, *J* = 6.0 Hz, H-6), 0.90 (t, 3 H, *J* = 6.4 Hz, CH_3), 0.89 (t, 3 H, *J* = 6.4 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 172.9 (C=ONH), 171.5 (C=OOH), 99.8 (C-1), 74.9 (O*C*HCH_2), 74.1 (C-4), 72.7 (C-2), 72.2 (C-3), 70.5 (C-5), 48.2 (NH*C*H), 43.0 (*C*H_2C=ONH), 42.1 (*C*$H_2$$CO_2H$), 36.0 ($CH_2$), 33.9 ($CH_2$), 33.03 ($CH_2$), 32.98 ($CH_2$), 30.57 ($CH_2$), 30.31 ($CH_2$), 27.1 ($CH_2$), 25.8 ($CH_2$), 23.7 ($CH_2$), 17.9 (C-6), 14.4 ($CH_3$); HRMS (ESI) calcd for (M+Na) $C_{24}H_{45}NNaO_8$: 498.3037. Found: 498.3038.



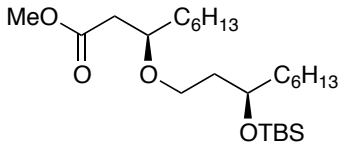
(*R*)-3-((*tert*-butyldimethylsilyl)oxy)-1-nonanol (2.100): Diisobutylaluminium hydride (DIBALH) (10 mL of a 1 M solution in hexane, 9.9 mmol) was added dropwise to a stirred solution of **2.79** (1 g, 3.3 mmol) in dry THF (25 mL) that had been pre-cooled to –78 °C. The resulting mixture was stirred at –78 °C for 2 h, then quenched by the addition of 1M

HCl. Ethyl acetate was then added to extract the target compound. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 10→16% EtOAc in hexane) to afford **2.100** (0.56 g, 65% yield) as a colorless oil. R_f 0.55 (4:1 hexane–EtOAc); $[\alpha]_D = -16.6$ (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 3.91 (ddd, 1 H, *J* = 12.6, 6.4, 4.2 Hz), 3.87–3.81 (m, 1 H), 3.75–3.68 (m, 1 H), 2.51 (t, 1 H, *J* = 5.2 Hz), 1.83 (dddd, 1 H, *J* = 14.4, 8.4, 5.0, 4.0 Hz), 1.65 (ddt 1 H, *J* = 14.4, 6.0, 4.4 Hz), 1.55–1.50 (m, 2 H), 1.32–1.26 (m, 8 H), 0.90 (s, 9 H), 0.89 (t, 1 H, *J* = 7.2 Hz), 0.10 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 71.7, 60.0, 37.2, 36.4, 31.4, 29.0, 25.4, 24.9, 22.2, 17.6, 13.7, –4.8, –5.1; HRMS (EI) calcd for (M–*t*-Bu) $C_{11}H_{25}O_2Si$: 217.1624. Found: 217.1622.

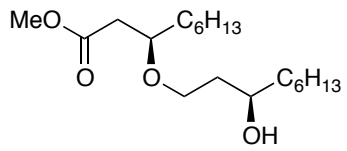


(*R*)-3-((*tert*-butyldimethylsilyl)oxy)-1-nonyl iodide (2.80): To a solution of the alcohol **2.100** (0.57 g, 2.09 mmol) in THF (30 mL) at 0 °C were added Ph_3P (0.82 g, 3.14 mmol), imidazole (0.43 g, 6.29 mmol) and I_2 (0.8 g, 3.14 mmol). After stirring for 1 h, the resulting solution was diluted with ether and washed with a saturated $Na_2S_2O_3$ solution. The aqueous layer was extracted with ether and the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 0→5% EtOAc in hexane) to afford **2.80** (0.74 g, 91% yield) as a colorless oil. R_f 0.84 (20:1 hexane–EtOAc); $[\alpha]_D = -27.1$ (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H)

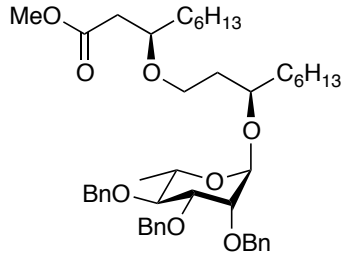
3.75–3.69 (m, 1 H), 3.28–3.17 (m, 2 H), 2.00–1.91 (m, 2 H), 1.47–1.43 (m, 2 H), 1.33–1.26 (m, 8 H), 0.90–0.88 (m, 12 H), 0.09 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 71.8, 40.5, 36.5, 31.4, 29.0, 25.5, 24.5, 22.2, 17.7, 13.7, 3.0, –4.7, –4.8; HRMS (EI) calcd for (M–*t*-Bu) $C_{11}H_{24}IOSi$: 327.0641. Found: 327.0641.



(*R*)-methyl 3-((*R*)-3-(((*tert*-butyldimethylsilyl)oxy)nonyl)oxy)nonanoate (2.101): To a stirred solution of alcohol **2.91** (109 mg, 0.58 mmol) in CH_2Cl_2 (10 mL) was added iodide **2.80** (200 mg, 0.73 mmol) and Ag_2O (1.7 g, 7.3 mmol). The reaction mixture was then heated at reflux for 1 day in the absence of light. After cooling to r.t., the solid was separated by filtration through a pad of Celite and the filtrate was concentrated. The resulting residue was purified by chromatography (gradient 0→5% EtOAc in hexane) to afford **2.101** (82 mg, 32% yield) as a colorless oil. R_f 0.32 (20:1 hexane–EtOAc); $[\alpha]_D$ = –6.7 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 3.78–3.68 (m, 2 H), 3.67 (s, 3 H), 3.52–3.47 (m, 2 H), 2.53 (dd, 1 H, *J* = 15.0, 7.0 Hz), 2.40 (dd, 1 H, *J* = 15.0, 5.6 Hz), 1.70–1.64 (m, 2 H), 1.56–1.26 (m, 20 H), 0.90–0.87 (m, 15 H), 0.045 (s, 3 H), 0.044 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.3, 76.4, 69.7, 66.2, 51.5, 39.7, 37.4, 37.3, 34.5, 31.9, 31.8, 29.5, 29.4, 25.9, 25.2, 25.1, 22.64, 22.61, 18.1, 14.10, 14.08, –4.4, –4.5; HRMS (ESI) calcd for (M+Na) $C_{25}H_{52}NaO_4Si$: 467.3527. Found: 467.3521.

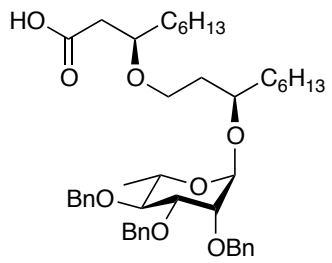


(*R*)-methyl 3-(((*R*)-3-hydroxynonyl)oxy)nonanoate (2.77): A solution of **2.101** (200 mg, 0.45 mmol) in 1.5% HCl in CH_3OH (40 mL) was stirred for 1 h. The reaction mixture was neutralized by the addition of saturated aqueous $NaHCO_3$, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 10→12% EtOAc in hexane) to afford **2.77** (135 mg, 91% yield) as a colorless oil. R_f 0.39 (4:1 hexane–EtOAc); $[\alpha]_D$ = +1.1 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 3.75–3.70 (m, 3 H), 3.69 (s, 3 H), 3.62–3.59 (m, 1 H), 2.66 (br s, 1 H), 2.53 (dd, 1 H, *J* = 15.0, 7.6 Hz), 2.44 (dd, 1 H, *J* = 15.0, 5.0 Hz), 1.70–1.62 (m, 2 H), 1.58–1.26 (m, 20 H), 0.88 (t, 3 H, *J* = 7.0 Hz), 0.87 (t, 3 H, *J* = 7.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ_C) 172.2, 76.9, 71.6, 68.4, 51.7, 39.4, 37.4, 36.6, 34.0, 31.8, 31.7, 29.4, 29.3, 25.6, 25.0, 25.1, 22.61, 22.57, 14.08, 14.03; HRMS (ESI) calcd for (M+Na) $C_{19}H_{38}NaO_4$: 353.2662. Found: 353.2655.

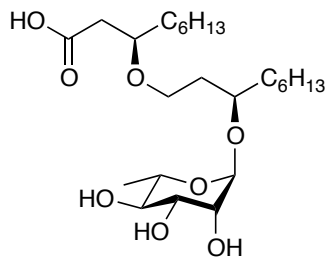


Methyl (*R*)-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-(*R*)-3-hydroxynonyl]nonanoate (2.102): A mixture of acceptor **2.77** (130 mg, 0.4 mmol),

trichloroacetimidate donor **2.75** (578 mg, 1.0 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous Et_2O (10 mL) and stirred for 10 min. The solution was then cooled to 0 °C, and then TMSOTf (10 μL) was added. The solution was stirred for 1 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 1→2% EtOAc in hexane) to afford **2.102** (193 mg, 66% yield) as a colorless oil; R_f 0.42 (5:1 hexane–EtOAc); $[\alpha]_D$ = +38.9 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.38–7.26 (m, 15 H), 4.94 (d, 1 H, *J* = 10.8 Hz), 4.78 (d, 1 H, *J* = 12.4 Hz), 4.77 (d, 1 H, *J* = 2.0 Hz), 4.70 (d, 1 H, *J* = 12.4 Hz), 4.66–4.61 (m, 3 H), 3.82 (dd, 1 H, *J* = 9.4, 3.2 Hz), 3.76–3.72 (m, 1 H), 3.70–3.66 (m, 2 H), 3.65 (s, 3 H), 3.64–3.60 (m, 2 H), 3.50–3.43 (m, 2 H), 2.50 (dd, 1 H, *J* = 15.0, 7.2 Hz), 2.40 (dd, 1 H, *J* = 15.0, 5.6 Hz), 1.73–1.63 (m, 2 H), 1.55–1.21 (m, 23 H), 0.91 (t, 3 H, *J* = 7.2 Hz), 0.88 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.3, 138.75, 138.69, 138.4, 128.36, 128.35, 128.33, 128.1, 128.0, 127.71, 127.68, 127.58, 127.52, 96.9, 80.7, 80.2, 76.5, 75.34, 75.24, 75.20, 72.8, 72.3, 68.3, 66.1, 51.5, 39.7, 35.2, 34.4, 33.3, 31.84, 31.80, 29.46, 29.36, 25.2, 24.6, 22.67, 22.62, 18.0, 14.14, 14.10; HRMS (ESI) calcd for (M+Na) $C_{46}H_{66}NaO_8$: 769.4650. Found: 769.4645.

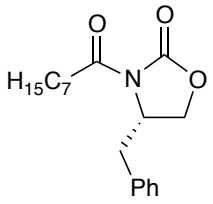


(*R*)-3-*O*-[2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-(*R*)-3-hydroxynonyl]nonanic acid (2.103): A solution of **2.102** (80 mg, 0.11 mmol) in CH_3OH–water (4:1, 10 mL) was treated with $LiOH•H_2O$ (45 mg, 1.1 mmol) and was stirred at 50 °C overnight. After cooling to r.t., the reaction mixture was acidified to pH 5 by the addition of 1 M HCl, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 20→25% EtOAc in hexane) to afford **2.103** (69 mg, 88% yield) as a colorless oil. R_f 0.23 (3:1 hexane–EtOAc); $[\alpha]_D = -16.6$ (*c* 0.9, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.43–7.31 (m, 15 H), 5.00 (d, 1 H, *J* = 10.5 Hz), 4.82–4.66 (m, 6 H), 3.93 (dd, 1 H, *J* = 9.0, 3.0 Hz), 3.81–3.76 (m, 1 H), 3.73–3.65 (m, 5 H), 3.46–3.41 (m, 1 H), 2.46 (dd, 1 H, *J* = 15.0, 3.5 Hz), 2.41 (dd, 1 H, *J* = 15.0, 8.0 Hz), 1.77–1.59 (m, 3 H), 1.48–1.17 (m, 22 H), 0.94 (t, 3 H, *J* = 7.2 Hz), 0.91 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.5, 138.6, 138.3, 137.8, 128.45, 128.43, 128.38, 128.37, 128.1, 128.0, 127.8, 127.6, 95.2, 81.1, 79.9, 75.9, 75.1, 72.9, 72.3, 67.9, 66.8, 39.9, 34.9, 34.8, 32.6, 31.80, 31.77, 29.5, 29.2, 25.5, 24.4, 22.63, 22.59, 18.1, 14.12, 14.08; HRMS (ESI) calcd for (M–H) $C_{45}H_{63}O_8$: 731.4528. Found: 731.4529.

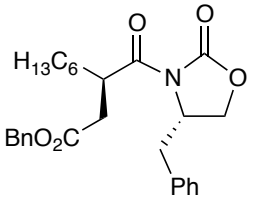


Methyl (*R*)-3-*O*-[α-L-rhamnopyranosyl-(1→3)-(*R*)-3-hydroxynonyl]nonanic acid (2.72): A solution of **2.103** (60 mg, 0.08 mmol) in CH_3OH (10 mL), and acetic acid (0.2 mL) was treated with palladium on charcoal (10%, 50 mg) and subjected to a hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was subjected to chromatography (Iatrobeads 6RS-8060, gradient 10→40% CH_3OH in CH_2Cl_2) to yield **2.72** (31 mg, 82% yield) as a colorless oil. $[\alpha]_D = -42.8$ (*c* 0.8, CH_2Cl_2); ^{1}H NMR (700 MHz, CD_3OD, δ_H) 4.75 (d, 1 H, *J* = 1.5 Hz, H-1), 3.75 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2), 3.74–3.70 (m, 2 H, OC*H*CH_2, OC*H*CH_2), 3.66–3.63 (m, 1 H, H-5), 3.63 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.59 (app dt, 1 H, *J* = 9.5, 6.0 Hz, C*H*$_2$O), 3.54 (app dt, 1 H, *J* = 9.5, 6.6 Hz, C*H*$_2$O), 3.36 (t, 1 H, *J* = 9.5 Hz, H-4), 2.46 (dd, 1 H, *J* = 15.0, 7.5 Hz, C*H*$_2$CO), 2.39 (dd, 1 H, *J* = 15.0, 5.5 Hz, C*H*$_2$CO), 1.79–1.70 (m, 2 H, C*H*$_2$$CH_2$O), 1.57–1.48 (m, 4 H, 2 x CH_2), 1.41–1.27 (m, 16 H, 8 x CH_2), 1.25 (d, 3 H, *J* = 6.0 Hz, H-6), 0.90 (t, 3 H, *J* = 7.0 Hz, CH_3), 0.89 (t, 3 H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 176.1 (CO_2H), 100.5 (C-1), 78.0 (*C*HCH_2CO_2H), 76.3 (O*C*HCH_2CH_2), 74.0 (C-4), 72.8 (C-2), 72.5 (C-3), 70.2 (C-5), 67.2 (OCH_2), 40.9 (*C*H_2CO_2H), 36.4 (CH_2), 35.6 (CH_2), 34.5 (CH_2), 33.0 (CH_2), 30.58 (CH_2),

30.53 (CH_2), 26.4 (CH_2), 25.9 (CH_2), 23.7 (CH_2), 18.1 (C-6), 14.5 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{24}H_{46}NaO_8$: 485.3085. Found: 485.3085.

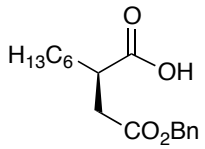


(*S*)-4-Benzyl-3-octanoyloxazolidin-2-one (2.106): (*S*)-Benzyl-2-oxazolinone (0.5 g, 2.8 mmol) was dissolved in THF (10 mL) under an argon atmosphere, *n*-BuLi (1.6 M in hexane, 1.2 mL, 3.08 mmol) was added, and the mixture was stirred for 1 h at –78 °C. To the mixture was added *n*-octanoyl chloride **2.105** (500 mg, 3.08 mmol), the mixture was stirred for 2 h at r.t. before being quenched by the addition of satd. aq. NH_4Cl. The aqueous layer was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 10→16% EtOA in hexane) to afford **2.106** (0.77 g, 90% yield) as a colorless oil. R_f 0.37 (4:1 hexane–EtOAc); $[\alpha]_D$ = +56.4 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.38–7.31 (m, 5 H), 5.15 (d, 1 H, *J* = 12.4 Hz), 5.13 (d, 1 H, *J* = 12.4 Hz), 2.93–2.88 (m, 1 H), 2.78 (dd, 1 H, *J* = 16.8, 9.0 Hz), 2.51 (dd, 1 H, *J* = 16.8, 5.4 Hz), 1.73–1.67 (m, 1 H), 1.58–1.52 (m, 1 H), 1.37–1.24 (m, 8 H), 0.88 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ_C) 180.9, 171.7, 135.7, 128.5, 128.3, 128.2, 66.5, 41.1, 35.6, 31.7, 31.6, 29.0, 26.8, 22.6, 14.0; HRMS (ESI) calcd for (M+Na) $C_{18}H_{25}NNaO_3$: 326.1727. Found: 326.1720.

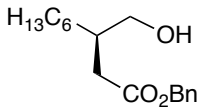


Benzyl 4-[(*S*)-4-benzyl-2-oxooxazolidin-3-yl]-3-hexyl-4-oxobutanoate (2.107): To a solution of **2.106** (5 g, 16.5 mmol) in THF (199 mL) was added LiHMDS (1.0 M in THF, 19.8 mL, 19.8 mmol) at –78 °C, and the mixture was stirred for 20 min at 0 °C. To this solution was added dropwise benzyl bromoacetate (4.9 g, 21.4 mmol) in THF (50 mL) over 90 min at –78 °C. The reaction mixture was stirred for 30 min at 0 °C and then for 2 h at r.t. before being quenched by the addition of satd. aq. NH_4Cl. The aqueous layer was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 4→10% EtOAc in hexane) to afford **2.107** (5.65 g, 76% yield) as a colorless oil. R_f 0.41 (4:1 hexane–EtOAc); $[\alpha]_D$ = +33.1 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.22 (m, 10 H), 5.12 (s, 2 H), 4.66–4.62 (m, 1 H), 4.27 (dddd, 1 H, *J* = 13.4, 7.6, 5.8, 4.0 Hz), 4.16 (ddd, 1 H, *J* = 9.0, 7.6, 1.0 Hz), 4.12 (dd, 1 H, *J* = 9.0, 2.8 Hz), 3.25 (dd, 1 H, *J* = 13.6, 3.2 Hz), 2.99 (dd, 1 H, *J* = 17.0, 10.8 Hz), 2.63 (dd, 1 H, *J* = 17.0, 4.0 Hz), 2.50 (dd, 1 H, *J* = 13.6, 10.0 Hz), 1.73–1.67 (m, 1 H), 1.53–1.46 (m, 1 H), 1.37–1.24 (m, 8 H), 0.89 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 175.8, 171.9, 153.1, 135.79, 135.77, 129.5, 128.9, 128.6, 128.32, 128.27, 127.2, 66.6, 65.9, 55.6, 39.2, 37.3, 35.9, 32.2,

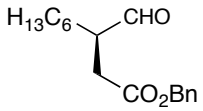
31.6, 29.2, 26.8, 22.6, 14.0; HRMS (ESI) calcd for (M+Na) $C_{27}H_{33}NNaO_5$: 474.2251. Found: 474.2248.



(*R*)-3-Benzyloxycarbonyl-2-hexylpropanoic acid (2.108): To a solution of **2.107** (1 g, 2.2 mmol) in THF–H_2O (48 mL:12 mL) were added 30% aq. H_2O_2 (1.25 g, 11 mmol) and a solution of LiOH·H_2O (138 mg, 3.3 mmol) in H_2O (6 mL). The mixture was stirred for 2 h at 0 °C, then acidified with 1 M HCl and warmed to r.t.. The aqueous layer was extracted with EtOAc. The organic phase was washed with satd. aq. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (4→25% EtOAc in hexane) to afford **2.108** (0.42 g, 65% yield) as a colorless oil. R_f 0.20 (3:1 hexane–EtOAc); $[\alpha]_D$ = +9.8 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.41–7.33 (m, 5 H), 5.18 (d, 1 H, *J* = 12.5 Hz), 5.15 (d, 1 H, *J* = 12.5 Hz), 2.96–2.91 (m, 1 H), 2.81 (dd, 1 H, *J* = 17.0, 9.0 Hz), 2.54 (dd, 1 H, *J* = 17.0, 5.0 Hz), 1.76–1.69 (m, 1 H), 1.61–1.55 (m, 1 H), 1.39–1.27 (m, 8 H), 0.91 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 180.6, 171.8, 135.7, 128.6, 128.3, 128.2, 66.6, 41.0, 35.7, 31.7, 31.60, 31.57, 29.1, 26.8, 22.61, 22.57, 14.0; HRMS (ESI) calcd for (M–H) $C_{17}H_{23}O_4$: 291.1602. Found: 291.1598.

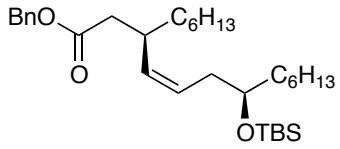


(*R*)-Benzyl-3-hydroxymethyl-nonanoate (2.109): To a solution of acid **2.108** (860 mg, 2.95 mmol) in dry THF cooled in an ice-salt bath, BH_3•Me_2S complex (354 μL, 3.54 mmol) was added by syringe. The mixture was warmed to r.t. and stirred for 24 h. The reaction was quenched by water (0.3 mL) with caution at 0 °C, before solid K_2CO_3 (500 mg) was added and the mixture was stirred for 2 min. The aqueous layer was extracted with Et_2O. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 4→25% EtOAc in hexane) to afford **2.109** (735 mg, 90% yield) as a colorless oil. R_f 0.33 (3:1 hexane–EtOAc); $[\alpha]_D$ = –1.2 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.33 (m, 5 H), 5.15 (s, 2 H), 3.67 (ddd, 1 H, *J* = 11.0, 5.5, 5.0 Hz), 3.52 (app dt, 1 H, *J* = 11.0, 6.5 Hz), 2.48 (dd, 1 H, *J* = 15.5, 7.5 Hz), 2.42 (dd, 1 H, *J* = 15.5, 5.5 Hz), 2.09–2.04 (m, 1 H), 1.75 (t, 1 H, *J* = 5.5 Hz), 1.41–1.24 (m, 10 H), 0.89 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.6, 135.9, 128.6, 128.28, 128.27, 66.3, 65.7, 37.9, 36.9, 31.7, 31.2, 29.4, 26.8, 22.6, 14.0; HRMS (ESI) calcd for (M+Na) $C_{17}H_{26}NaO_3$: 301.1774. Found: 301.1766.



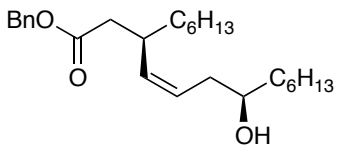
(*R*)-Benzyl-3-carbonyl-nonanoate (2.84): To a mixture of **2.109** (180 mg, 0.65 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (5 mL) were added NMO (114 mg, 0.98 mmol)

and TPAP (22.7 mg, 0.065 mmol) at r.t. The mixture was stirred for 6 h and then the mixture was concentrated and the crude product was purified by chromatography (gradient 10→20% EtOAc in hexane) to afford **2.84** (51 mg, 85% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.71 (d, 1 H, J = 1.0 Hz), 7.40–7.30 (m, 5 H), 5.13 (s, 2 H), 2.88–2.81 (m, 1 H), 2.76 (dd, 1 H, J = 16.5, 8.0 Hz), 2.45 (dd, 1 H, J = 16.5, 5.0 Hz), 1.77–1.69 (m, 1 H), 1.52–1.44 (m, 1 H), 1.37–1.23 (m, 8 H), 1.41–1.24 (m, 10 H), 0.88 (t, 3 H, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 202.5, 171.4, 135.3, 128.2, 127.9, 127.8, 66.2, 47.3, 32.7, 31.2, 28.8, 28.2, 26.3, 22.1, 13.6. Given the instability of this compound to oxidation, no mass spec data could be obtained.



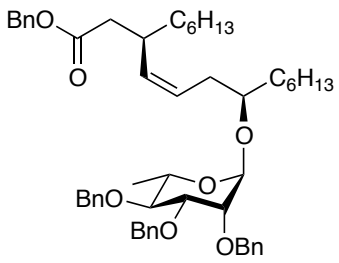
(3*R*,7*R*,*Z*)-Benzyl 7-((*tert*-butyldimethylsilyl)oxy)-3-hexyltridec-4-enoate (2.111): Iodide **2.80** (600 mg, 1.56 mmol), PPh_3 (610 mg, 2.34 mmol) and DIEA (1.4 g, 10.9 mmol) were heated in a sealed flask at 90 °C for 12 h. After cooling to r.t., DIEA was removed with *n*-pentane *in vacuo* and the residue was resuspended in dry *n*-pentane. After 1 min, the *n*-pentane was removed by pipette (repeated twice). After removal of the remaining solvent *in vacuo* the residue was dissolved in 30 mL of THF and LiHMDS (1.0 M in THF, 1.5 mL, 1.5 mmol) were added at –78 °C. After stirring for 15 min, a solution of 1.5 mL of HMPA and 1.5 mL of THF was added dropwise at –78 °C followed by the dropwise

addition of a solution of aldehyde **2.84** (360 mg, 1.3 mmol) in THF (5 mL). The mixture was stirred for 15 min at –78 °C and for 1 h at r.t. before the addition of satd. aq. $NaHCO_3$ solution. Then, the mixture was warmed to r.t. and the solution was extracted with ether. The organic phase were dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (gradient 1→4% EtOAc in hexane) to afford **2.111** (450 mg, 67% yield) as a colorless oil. R_f 0.40 (20:1 hexane–EtOAc); $[\alpha]_D$ = +10.8 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.37–7.31 (m, 5 H), 5.46 (appdt, 1 H, *J* = 10.5, 7.0 Hz), 5.18 (apptt, 1 H, *J* = 10.5, 2.0 Hz), 5.11 (d, 1 H, *J* = 12.5 Hz), 5.06 (d, 1 H, *J* = 12.5 Hz), 3.67–3.63 (m, 1 H), 2.86–2.79 (m, 1 H), 2.38 (dd, 1 H, *J* = 14.5, 6.5 Hz), 2.25 (dd, 1 H, *J* = 14.5, 8.0 Hz), 2.24–2.21 (m, 1 H), 2.17–2.12 (m, 1 H), 1.42–1.19 (m, 20 H), 0.89–0.86 (m, 15 H), 0.04 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.4, 136.1, 133.9, 128.5, 128.2, 128.1, 127.1, 72.2, 66.1, 40.7, 36.9, 35.4, 34.6, 31.9, 31.8, 29.5, 29.4, 27.2, 25.9, 25.4, 22.7, 22.6, 18.1, 14.11, 14.09, –4.3, –4.5; HRMS (ESI) calcd for (M+Na) $C_{32}H_{56}NaO_3Si$: 539.3891. Found: 539.3883.



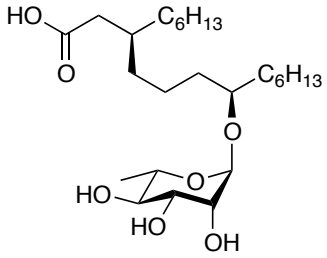
(3*R*,7*R*,*Z*)-benzyl 3-hexyl-7-hydroxytridec-4-enoate (2.81): A solution of **2.111** (500 mg, 0.39 mmol) in 1.5% HCl in CH_3OH (30 mL) was stirred for 1 h. The reaction mixture was neutralized by the addition of satd. aq. $NaHCO_3$, diluted with water and extracted with

CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 10→12% EtOAc in hexane) to afford **2.81** (350 mg, 90% yield) as a colorless oil. R_f 0.41 (5:1 hexane–EtOAc); $[\alpha]_D$ = +3.6 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.39–7.32 (m, 5 H), 5.48 (app dt, 1 H, *J* = 10.5, 7.5 Hz), 5.29 (app tt, 1 H, *J* = 10.5, 1.5 Hz), 5.11 (d, 1 H, *J* = 12.5 Hz), 5.09 (d, 1 H, *J* = 12.5 Hz), 3.65–3.61 (m, 1 H), 2.91–2.86 (m, 1 H), 2.44 (dd, 1 H, *J* = 15.0, 5.5 Hz), 2.27 (dd, 1 H, *J* = 15.0, 9.0 Hz), 2.27–2.18 (m, 2 H), 1.46–1.24 (m, 20 H), 0.90 (t, 3 H, *J* = 7.2 Hz), 0.88 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 172.6, 136.0, 135.7, 128.5, 128.3, 128.2, 126.3, 71.3, 66.2, 40.6, 36.7, 35.43, 35.39, 34.4, 31.9, 31.8, 29.4, 27.1, 25.8, 22.6, 14.09, 14.08; HRMS (ESI) calcd for (M+Na) $C_{26}H_{42}NaO_3$: 425.3026. Found: 425.3024.



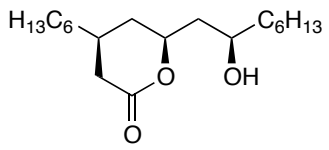
(3*R*,7*R*,4*Z*)-Benzyl-3-*n*-hexyl-7-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-tridec-4-enoate (2.112): A mixture of acceptor **2.81** (130 mg, 0.32 mmol), trichloroacetimidate donor **2.75** (337 mg, 0.58 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous Et_2O (8 mL) and stirred for 10 min. The solution was then cooled to 0 °C and then TMSOTf (10 μL) was added. The solution was stirred for 1 h before Et_3N (0.5 mL)

was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 5→10% EtOAc in hexane) to afford **2.112** (169 mg, 64% yield) as a colorless oil; R_f 0.55 (5:1 hexane–EtOAc); $[\alpha]_D$ = +4.9 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.39–7.28 (m, 20 H), 5.40 (app dt, 1 H, *J* = 11.0, 7.0 Hz), 5.18 (app t, 1 H, *J* = 11.0 Hz), 5.11 (d, 1 H, *J* = 12.0 Hz), 5.07 (d, 1 H, *J* = 12.0 Hz), 4.95 (d, 1 H, *J* = 11.0 Hz), 4.79 (d, 1 H, *J* = 12.5 Hz), 4.78 (d, 1 H, *J* = 1.5 Hz), 4.70 (d, 1 H, *J* = 12.5 Hz), 4.68–4.61 (m, 3 H), 3.84 (dd, 1 H, *J* = 9.5, 3.0 Hz), 3.83–3.77 (m, 1 H), 3.72 (dd, 1 H, *J* = 3.0, 1.5 Hz), 3.62 (app t, 1 H, *J* = 9.5 Hz), 3.55–3.49 (m, 1 H), 2.85–2.77 (m, 1 H), 2.38 (dd, 1 H, *J* = 15.0, 6.5 Hz), 2.33–2.14 (m, 3 H), 1.39–1.12 (m, 23 H), 0.92 (t, 3 H, *J* = 7.2 Hz), 0.87 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 172.4, 138.8, 138.7, 138.4, 136.1, 134.3, 128.5, 128.3, 128.2, 128.1, 128.06, 128.04, 127.7, 127.66, 127.59, 127.51, 126.6, 97.7, 80.7, 80.3, 78.1, 75.4, 75.2, 72.8, 72.3, 68.3, 66.1, 40.6, 35.3, 34.5, 33.4, 31.9, 31.8, 29.5, 29.4, 27.2, 25.1, 22.71, 22.66, 17.9, 14.15, 14.10; HRMS (ESI) calcd for (M+Na) $C_{53}H_{70}NaO_7$: 841.5014. Found: 841.5000.

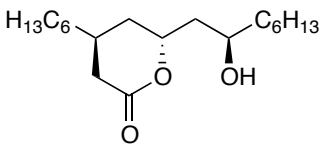


(3*R*,7*R*,4*Z*)-Benzyl-3-*n*-hexyl-7-(α-L-rhamnopyranosyl)-tridec-4-enoate (2.73): A solution of **2.112** (70 mg, 0.085 mmol) in CH_3OH (10 mL) and acetic acid (0.2 mL) was

treated with palladium on charcoal (10%, 50 mg) and subjected to a hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was subjected to chromatography (Iatrobeads 6RS-8060, gradient 10→40% CH_3OH in CH_2Cl_2) to yield **2.73** (34 mg, 86% yield) as a colorless oil. $[\alpha]_D = -38.6$ (*c* 1.1, CH_2Cl_2); ^1H NMR (700 MHz, CD_3OD, δ_H) 4.75 (d, 1 H, *J* = 1.5 Hz, H-1), 3.75 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2), 3.67 (app dq, 1 H, *J* = 9.5, 6.0 Hz, H-5), 3.63 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.61–3.58 (m, 1 H, OC*H*CH_2), 3.37 (app t, 1 H, *J* = 9.5 Hz, H-4), 2.23 (dd, 1 H, *J* = 15.0, 6.5 Hz, C*H*$_2$CO), 2.19 (dd, 1 H, *J* = 15.0, 7.0 Hz, C*H*$_2$CO), 1.85–1.81 (m, 1 H, C*H*CH_2CO_2H), 1.53–1.26 (m, 26 H, 13 x CH_2), 1.24 (d, 3 H, *J* = 6.0 Hz, H-6), 0.90 (t, 3 H, *J* = 7.0 Hz, CH_3), 0.89 (t, 3 H, *J* = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 177.5 (C=O), 100.6 (C-1), 78.9 (O*C*HCH_2CH_2), 74.0 (C-4), 72.9 (C-2), 72.5 (C-3), 70.2 (C-5), 40.2 (*C*H_2CO_2H), 36.2 (*C*HCH_2CO_2H), 36.0 (CH_2), 35.4 (CH_2), 35.1 (CH_2), 34.4 (CH_2), 33.01 (CH_2), 33.00 (CH_2), 30.68 (CH_2), 30.63 (CH_2), 27.7 (CH_2), 26.0 (CH_2), 23.73 (CH_2), 23.70 (CH_2), 18.1 (C-6), 14.46 (CH_3), 14.45 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{25}H_{48}NaO_7$: 483.3292. Found: 483.3293.



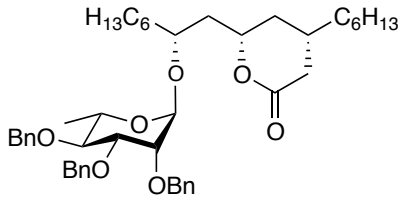
2.82



2.83

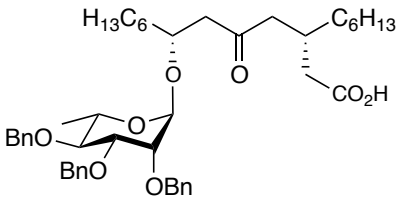
(4*S*,6*S*)-4-hexyl-6-((*R*)-2-hydroxyoctyl)tetrahydro-2*H*-pyran-2-one (2.82) and (4*S*,6*R*)-4-hexyl-6-((*R*)-2-hydroxyoctyl)tetrahydro-2*H*-pyran-2-one (2.83): A mixture of alcohol **2.81** (335 mg, 0.83 mmol) and $(HMe_2Si)_2NH$ (295 μL, 1.66 mmol) was stirred at r.t. overnight. The excess disilazane was removed under vacuum to provide the desired silane as a colorless oil. The silane was then dissolved in anhydrous toluene (9 mL) and cooled to 0 °C. To the resulting solution was slowly added Karstedt's catalyst (82 mg, 0.0041 mmol) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 3 h and then warmed to r.t. The solvent was removed under vacuum to provide crude **2.118** as a light yellow oil. The compound was then dissolved in THF–CH_3OH (7 mL:7 mL). To the solution was added $KHCO_3$ (415 mg, 4.15 mmol) followed by slow addition of 30% H_2O_2 (1.87 mL, 16.6 mmol) at r.t. The reaction mixture was stirred at r.t. overnight. The reaction was quenched by the careful dropwise addition of an satd aq sodium thiosulfate solution (10 mL) and the solution was then diluted with satd aq $NaHCO_3$ solution (10 mL) and finally extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated under 40 °C for 1 h to ensure all of the ester **2.119** were converted to lactone. The resulting residue was purified by chromatography (gradient 10→12% EtOAc in hexane) to afford **2.82** (110 mg, 40% yield) and **2.83** (97 mg, 35% yield) as

colorless oils. Data for **2.82**: R_f 0.26 (5:2 hexane–EtOAc); $[\alpha]_D$ = +8.1 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 4.50 (dddd, 1 H, *J* = 14.5, 8.0, 5.5, 3.0 Hz), 3.87–3.83 (m, 1 H), 2.71 (ddd, 1 H, *J* = 17.5, 6.0, 1.8 Hz), 2.08 (dd, 1 H, *J* = 17.5, 10.5 Hz), 2.04 (dddd, 1 H, *J* = 14.0, 4.5, 3.0, 2.0 Hz), 2.00 (br s, 1 H), 1.97–1.92 (m, 1 H), 1.88 (ddd, 1 H, *J* = 14.5, 9.0, 8.0 Hz), 1.74 (ddd, 1 H, *J* = 14.5, 5.5, 3.5 Hz), 1.51–1.27 (m, 21 H), 0.90 (t, 3 H, *J* = 7.2 Hz), 0.90 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ_C) 171.1, 79.6, 69.4, 43.3, 37.7, 36.40, 36.39, 35.3, 31.8, 31.7, 31.6, 29.3, 29.2, 26.3, 25.4, 22.6, 14.07, 14.05; HRMS (ESI) calcd for (M+Na) $C_{19}H_{36}NaO_3$: 355.2557. Found: 355.2553. Data for **2.83**: R_f 0.27 (5:2 hexane–EtOAc); $[\alpha]_D$ = +1.9 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 4.68 (app tdd, 1 H, *J* = 10.0, 4.0, 3.0 Hz), 3.99–3.96 (m, 1 H), 2.58 (dd, 1 H, *J* = 16.0, 6.0 Hz), 2.22 (dd, 1 H, *J* = 16.0, 9.5 Hz), 2.06–2.00 (m, 1 H), 1.83–1.77 (m, 2 H), 1.71 (br s, 1 H), 1.64 (ddd, 1 H, *J* = 14.0, 5.5, 4.0 Hz), 1.56 (ddd, 1 H, *J* = 14.5, 10.0, 2.7 Hz), 1.49–1.28 (m, 20 H), 0.902 (t, 3 H, *J* = 7.2 Hz), 0.901 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ_C) 172.9, 74.1, 67.6, 43.0, 38.1, 36.1, 35.8, 34.0, 31.8, 31.7, 29.4, 29.2, 27.1, 26.7, 25.5, 22.6, 14.07, 14.05; HRMS (ESI) calcd for (M+Na) $C_{19}H_{36}NaO_3$: 355.2557. Found: 355.2550.



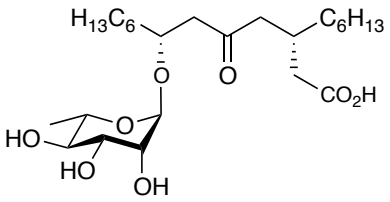
(4*S*,6*S*)-4-hexyl-6-((*R*)-2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)tetrahydro-2*H*-pyran-2-one (2.114): A mixture of acceptor **2.82** (100 mg, 0.30 mmol), trichloroacetimidate donor **2.75** (314 mg, 0.54 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous Et_2O (8 mL) and stirred at for 10 min. The solution was cooled to 0 °C, and then TMSOTf (10 μL) was added. The solution was stirred for 1 h and then Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 5→16% EtOAc in hexane) to afford **2.114** (138 mg, 63% yield) as a colorless oil; R_f 0.31 (5:1 hexane–EtOAc); $[\alpha]_D$ = –19.3 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.36–7.25 (m, 15 H), 4.92 (d, 1 H, *J* = 11.0 Hz), 4.77 (d, 1 H, *J* = 12.0 Hz), 4.75 (d, 1 H, *J* = 1.5 Hz), 4.67 (d, 1 H, *J* = 12.0 Hz), 4.64–4.61 (m, 3 H), 4.36–4.32 (m, 1 H), 3.78 (dd, 1 H, *J* = 9.0, 3.0 Hz), 3.71–3.66 (m, 2 H), 3.62–3.58 (m, 2 H), 2.66 (ddd, 1 H, *J* = 17.5, 6.0, 1.5 Hz), 2.05–1.97 (m, 3 H), 1.88–1.84 (m, 1 H), 1.61 (ddd, 1 H, *J* = 14.0, 7.0, 5.0 Hz), 1.42–1.20 (m, 24 H), 0.89 (t, 3 H, *J* = 7.2 Hz), 0.87 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.3, 138.6, 138.5, 138.2, 128.4, 128.38, 128.37, 128.1, 128.0, 127.8, 127.7, 127.67, 127.61, 97.2, 80.4, 79.9, 77.7, 75.4, 75.1, 74.2, 72.9, 72.3, 68.7, 40.9, 36.5, 36.4, 35.1, 33.3, 31.8, 31.7, 31.6, 29.4, 29.2, 26.3, 24.7, 22.7, 22.6, 18.1, 14.13, 14.07; HRMS (ESI) calcd for (M+Na) $C_{46}H_{64}NaO_7$: 751.4544. Found: 751.4544.

(4*S*,6*R*)-4-hexyl-6-((*R*)-2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)tetrahydro-2*H*-pyran-2-one (2.115): A mixture of acceptor **2.83** (60 mg, 0.18 mmol), trichloroacetimidate donor **2.75** (188 mg, 0.32 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous Et_2O (8 mL) and stirred for 10 min. The solution was then cooled to 0 °C and TMSOTf (10 μL) was added. The solution was stirred for 1 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 5→16% EtOAc in hexane) to afford **2.114** (84 mg, 64% yield) as a colorless oil; R_f 0.30 (5:1 hexane–EtOAc); $[\alpha]_D = -16.2$ (*c* 0.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.39–7.29 (m, 15 H), 4.94 (d, 1 H, *J* = 11.0 Hz), 4.82 (d, 1 H, *J* = 2.0 Hz), 4.79 (d, 1 H, *J* = 12.0 Hz), 4.72 (d, 1 H, *J* = 12.0 Hz), 4.66 (d, 1 H, *J* = 11.0 Hz), 4.64(s, 2 H), 4.53–4.49 (m, 1 H), 3.89–3.85 (m, 1H), 3.80 (dd, 1 H, *J* = 9.0, 3.0 Hz), 3.70 (dd, 1 H, *J* = 3.0, 2.0 Hz), 3.68–3.61 (m, 2 H), 2.53 (dd, 1 H, *J* = 16.0, 6.0 Hz), 2.14 (dd, 1 H, *J* = 16.0, 10.0 Hz), 2.01–1.96 (m, 1 H), 1.76–1.70 (m, 1 H), 1.59–1.53 (m, 1 H), 1.38–1.23 (m, 24 H), 0.92 (t, 3 H, *J* = 7.2 Hz), 0.89 (t, 3 H, *J* = 7.2 Hz); HRMS (ESI) calcd for (M+Na) $C_{46}H_{64}NaO_7$: 751.4544. Found: 751.4539.



(3*S*,7*R*)-3-hexyl-7-(2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl)-5-oxo-tridecanoic acid (2.116): A solution of **2.114** (70 mg, 0.096 mmol) in CH_3OH–water (4:1, 25 mL) was treated with $LiOH•H_2O$ (40 mg, 0.96 mmol) and was stirred overnight. The reaction mixture was acidified to pH 5 by the addition of 1 M HCl, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtere and concentrated to afford the alcohol intermediate in CH_2Cl_2, which was used immediately without further purification. To a mixture of the alcohol intermediate and 4 Å molecular sieves in dry CH_2Cl_2 were added NMO (22 mg, 0.19 mmol) and TPAP (4 mg, 0.011 mmol) at r.t. The mixture was stirred for 6 h and then concentrated and the crude product was purified by chromatography (gradient 30→50% EtOAc–hexane) to afford **2.116** (51 mg, 71% yield) as a colorless oil. Compound **2.115** (50 mg, 0.069 mmol) was converted to **2.116** (36 mg, 70% yield) using same method. $[\alpha]_D = -25.6$ (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, CD_3OD, δ_H) 7.37–7.24 (m, 15 H), 4.85 (d, 1 H, *J* = 11.0 Hz), 4.77 (d, 1 H, *J* = 1.5 Hz), 4.69 (d, 1 H, *J* = 12.0 Hz), 4.62 (d, 2 H, *J* = 12.0 Hz), 4.57 (d, 1 H, *J* = 12.0 Hz), 4.55 (d, 1 H, *J* = 12.0 Hz), 4.07–4.03 (m, 1 H), 3.73 (dd, 1 H, *J* = 9.5, 3.0 Hz), 3.68 (dd, 1 H, *J* = 3.0, 1.5 Hz), 3.64–3.59 (m, 1 H), 3.50 (app t, 1 H, *J* = 9.5 Hz), 2.68 (dd, 1 H, *J* = 16.0, 7.5 Hz), 2.50 (dd, 1 H, *J* = 16.0, 5.0 Hz), 2.48 (d, 2 H, *J* = 6.5 Hz), 2.32–2.28 (m, 3 H), 2.238 (d, 1 H, *J* = 6.5 Hz), 2.237 (d, 1 H, *J* = 7.0 Hz), 1.41–1.20 (m, 23 H), 0.91 (t, 3 H, *J*

= 7.2 Hz), 0.86 (t, 3 H, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, CD_3OD, δ_C) 209.1, 175.3, 138.6, 138.4, 138.1, 128.1, 128.01, 127.98, 127.8, 127.68, 127.66, 127.5, 127.3, 127.2, 96.3, 80.0, 79.4, 75.1, 74.5, 73.5, 72.4, 71.7, 68.2, 38.0, 33.7, 32.9, 31.53, 31.50, 30.6, 29.1, 29.0, 26.3, 24.4, 22.28, 22.24, 16.9, 13.04, 12.99; HRMS (ESI) calcd for (M–H) $C_{46}H_{63}O_8$: 743.4528. Found: 743.4533.



(3*S*,7*R*)-3-hexyl-7-(α-L-rhamnopyranosyl)-5-oxo-tridecanoic acid (2.74): To a solution of **2.116** (65 mg, 0.087 mmol) in CH_3OH (10 mL), and acetic acid (0.2 mL) was treated with palladium on charcoal (10%, 50 mg) and subjected to a hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was purified by chromatography (Iatrobeads 6RS-8060, gradient 10→40% CH_3OH–CH_2Cl_2) to give **2.74** (33 mg, 81% yield) as a colorless foam. $[\alpha]_D = -45.1$ (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, CD_3OD, δ_H) 4.76 (d, 1 H, *J* = 1.5 Hz, H-1), 4.14–4.11 (m, 1 H, OC*H*CH_2), 3.72 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2), 3.57 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.55 (dq, 1 H, *J* = 9.5, 6.0 Hz, H-5), 3.34 (app t, 1 H, *J* = 9.5 Hz, H-4), 2.75 (dd, 1 H, *J* = 16.0, 7.5 Hz, C*H*$_2$CHCH$_2$CO$_2$H), 2.56 (dd, 1 H, *J* = 16.0, 5.0 Hz, OCHC*H*$_2$C=O), 2.54 (dd, 1 H, *J* = 17.0, 7.0 Hz, C*H*$_2$CHCH$_2$CO$_2$H), 2.51 (dd, 1 H, *J* = 17.0, 6.5 Hz, C*H*$_2$CHCH$_2$CO$_2$H), 2.35–2.32 (m, 1 H, C*H*CH$_2$CO$_2$H), 2.29 (dd, 1 H, *J* = 15.0, 6.0 Hz, C*H*$_2$CO$_2$H), 2.24 (dd,

1 H, J = 15.0, 7.0 Hz, CH_2CO$_2$H), 1.51–1.26 (m, 20 H, 10 x CH_2), 1.23 (d, 3 H, J = 6.0 Hz, H-6), 0.90 (t, 3 H, J = 7.0 Hz, CH_3), 0.89 (t, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 210.7 (C=O), 176.8 (C=OOH), 100.4 (C-1), 74.9 (O*C*HCH$_2$), 74.0 (C-4), 72.7 (C-2), 72.4 (C-3), 70.3 (C-5), 49.2 (*C*H$_2$C=O), 49.1 (*C*H$_2$C=O), 39.4 (*C*H$_2$CO$_2$H), 35.3 (CH_2), 34.6 (CH_2), 32.95 (CH_2), 32.94 (CH_2), 30.51 (CH_2), 30.45 (CH_2), 27.7 (CH_2), 25.9 (CH_2), 23.70 (CH_2), 23.67 (CH_2), 18.0 (C-6), 14.4 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{25}H_{46}NaO_8$: 497.3085. Found: 497.3086.

Chapter 3

Synthesis of Unusual *N*-Acylated Aminosugar Fragments of *Mycobacterium marinum* Lipooligosaccharide IV

A portion of this Chapter has been published:

Wang, L.; Dong, M.; Lowary, T. L. *J. Org. Chem.* **2015**, *80*, 2767–2780.

3.1 Background

Mycobacterial lipooligosaccharides (LOSs) are antigenic cell surface glycolipids found in the cell wall complex of mycobacteria. They are produced by a range of mycobacteria, including *Mycobacterium smegmatis*, *Mycobacterium kansasii*, *Mycobacterium gastri*, *Mycobacterium malmoense* and *Mycobacterium marinum*.[12]

3.1.1 The structures of *M. marinum* lipooligosaccharides (LOS)

M. marinum, a waterborne organism, is responsible for infections primarily in immune-deficient individuals such as AIDS patients.[80] This organism produces four LOSs (LOS I–IV, Figure 3-1), which like all other mycobacterial LOSs have a trehalose (α-D-Glc-(1→1)-α-D-Glc) core.[14] LOS-I is a pentasaccharide with the structure of α-L-Rha-(1→3)-β-D-Glc-(1→3)-β-D-Glc-(1→3)-α-D-Glc-(1→1)-α-D-Glc. The heptasaccharide LOS-II consists of LOS-I with an additional disaccharide motif (Car-(1→4)-β-D-Xyl*p*) attached to the C-4 hydroxyl group of the Rha*p* residue. This disaccharide contains caryophyllose (Car) an unusual branched-chain monosaccharide originally identified in the lipooligosaccharide of *Pseudomonas caryophylli*.[81,82] *M. marinum* LOS-III contains an additional caryophyllose residue (Car) attached to the side chain of the caryophyllose in LOS II. Finally, *M. marinum* LOS-IV is terminated with one of four *N*-acylated 4-amino-4,6-dideoxy-galactopyranose residues.[14] The *N*-acyl substituent in these molecules all have a common γ-lactam core, with different modifications at C-4 (CO_2H or H) or C-2 (MeO or

H). These differences results in two acidic (**3.1** and **3.2**, Figure 3-2) and two neutral (**3.3** and **3.4**) compounds. The acidic derivatives **3.1** and **3.2** represent ~95% of the total LOS-IV derivatives.[14]

In addition to the glycan residues, the LOS of *M. marinum* contain two types of acyl chains, 2,4-dimethylhexadecanoate and 2,4-dimethyl-2-pentadecenoate which differ by the presence of unsaturation. The structure and location of the fatty acyl chains on the trehalose moiety in these molecules were determined by Guérardel and coworkers.[83] Three hydroxyl groups of the trehalose moiety (those at C-4, C-6 and C-2′) are substituted by fatty acyl chains. The unsaturated 2,4-dimethyl-2-pentadecenoic acid and saturated 2,4-dimethylhexadecanoic acid are attached to C-6 and C-4 hydroxyl groups on the reducing-end terminal Glc*p* residue, respectively. The C-2 hydroxyl group of the second Glc*p* residue is substituted by 2,4-dimethylhexadecanoic acid.[83]

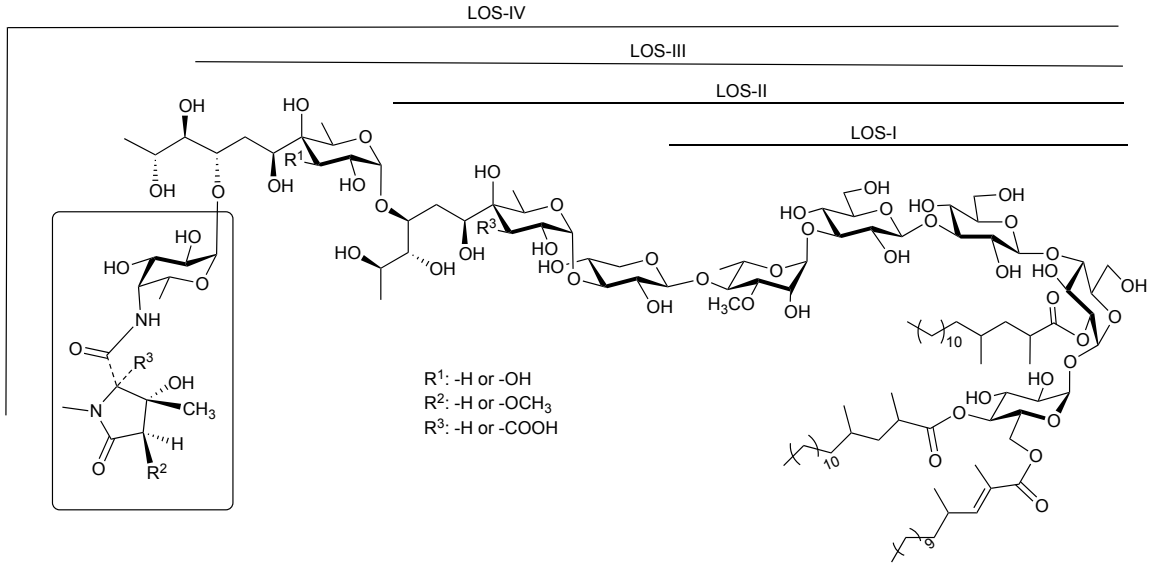


Figure 3-1: Structures of *M. marinum* Lipooligosaccharides I–IV.[14]

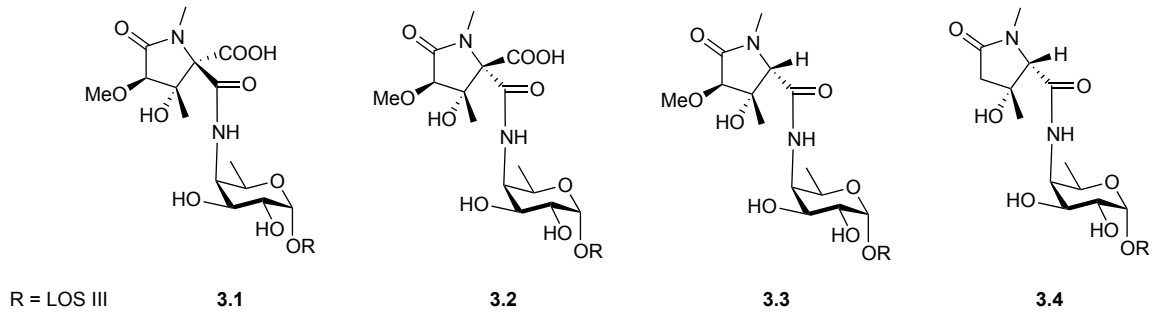


Figure 3-2: Structures of the *N*-acylated-4,6-dideoxy-galactopyranose residues present in *M. marinum* LOS-IV.[14]

3.1.2 Immunomodulatory activity of *M. marinum* LOSs

As cell surface glycolipids, LOSs are able to directly interact with the host immune system.[84] For example, LOS from *M. marinum* inhibit the secretion of the pro-inflammatory cytokine TNF-α in human macrophages.[38] The disruption of TNF-α secretion is involved in the ability of the organism to form granulomas and spread within the host.[39] Thus, by inhibiting TNF-α secretion, LOSs can influence the course of the infection.

M. marinum LOS-IV was also found to induce the expression of both intercellular adhesion molecule-1 antigen (ICAM-1) and CD40 on the surface of macrophages.[14] The expression of both ICAM-1 and CD40 are important for the formation of granulomas. On the other hand, LOS-III, which does not have the *N*-acylated 4-amino-4,6-dideoxy-galactopyranose residue, cannot induce the expression of ICAM-1 and CD40. In addition, only LOS-IV (not LOS-I to LOS-III) can stimulate IL-8 secretion from macrophages, a

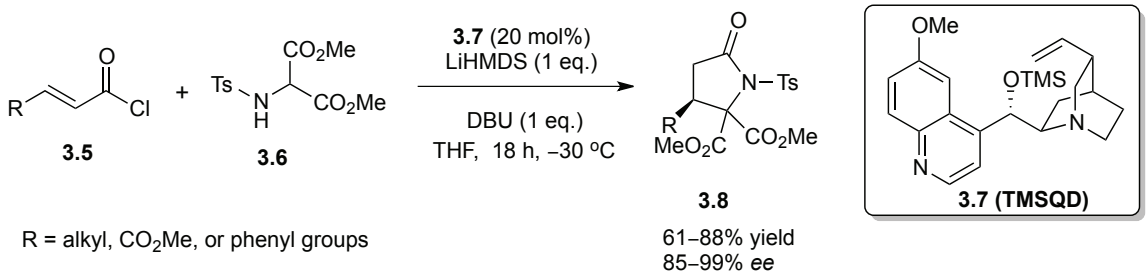
chemokine that also plays an important role in granuloma formation.[14] Thus, the terminal *N*-acylated dideoxygalactose residue of *M. marinum* LOS-IV appears to be necessary for these important immunomodulatory activities. However, removal of three acyl groups on the trehalose moiety completely suppressed these effects.[14] Therefore, not only the oligosaccharide part of LOS-IV, but also the lipid moiety, plays a crucial role for cell surface antigen-inducing activity.[14] Access to fragments of LOS-IV containing only the *N*-acylated 4-amino-4,6-dideoxygalactose motif would allow further studies of these effects to be studied. For this reason, we chose to undertake the synthesis of molecules incorporating these structures.

3.1.3 Synthesis of highly functionalized pyrrolidinone derivatives

As shown in Figure 3-2, the target *N*-acylated 4-amino-4,6-dideoxygalactose residues contain two substructures: a 4-amino-4,6-dideoxy-galactopyranose residue and a highly functionalized pyrrolidinone (γ-lactam) derivative. To synthesize these molecules, the key challenge is access to the pyrrolidinone moiety. In the sections below, I will discuss in more detail previous syntheses of structures relevant to pyrrolidinone derivatives.

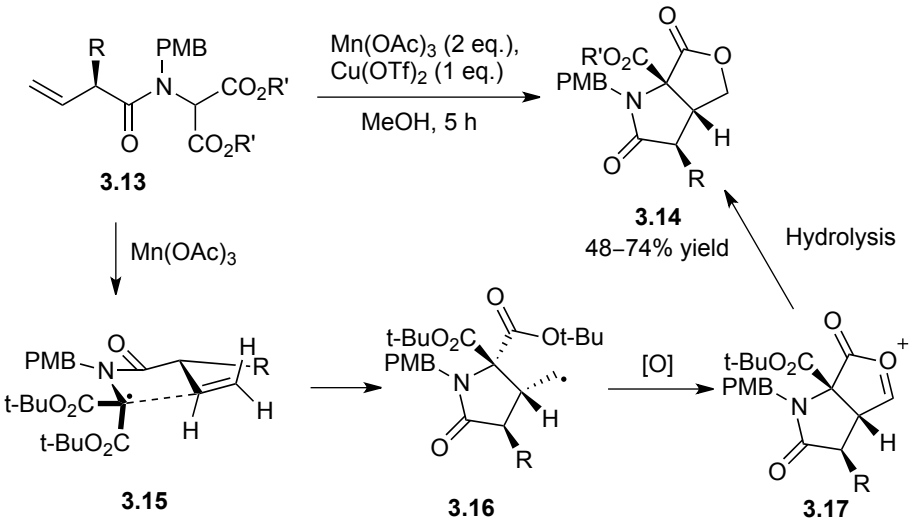
Michael addition/lactamization processes involving α,β-unsaturated acid chlorides with α-aminomalonates are one of the most direct methods for the synthesis of pyrrolidinones. For example, Romo and coworkers reported the first direct organocatalytic asymmetric synthesis of pyrrolidinones from α,β-unsaturated acid chlorides and α-

aminomalonates.[85] As shown in Scheme 3-1, when 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) was used as an acid scavenger and *O*-trimethylsilylquinidine (TMSQD) was used as the organocatalyst, tri-substituted pyrrolidinones **3.8** were obtained in modest yields but with good enantioselectivities.



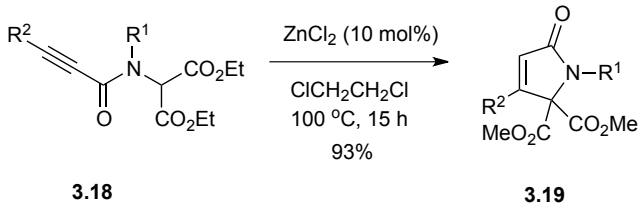
Scheme 3-1: Organocatalytic asymmetric synthesis of pyrrolidinone derivatives.[85]

Using a different approach, Burton and coworkers[86] reported a diastereoselective synthesis of lactone-fused pyrrolidinones **3.14** using an oxidative radical cyclization of amides **3.13** (Scheme 3-2). The authors proposed that the mechanism most likely involves single electron oxidation of **3.13** in the presence of $Mn(OAc)_3$ to afford malonyl radical **3.15**. Cyclization of the radical **3.15** occurs stereoselectively to give an adduct radical **3.16** via a five-membered ring transition state. Finally, single electron oxidation of **3.16** and trapping by the adjacent oxygen atom gives oxacarbenium ion **3.17**. Hydrolysis of **3.17** affords the product **3.14** (Scheme 3-2).



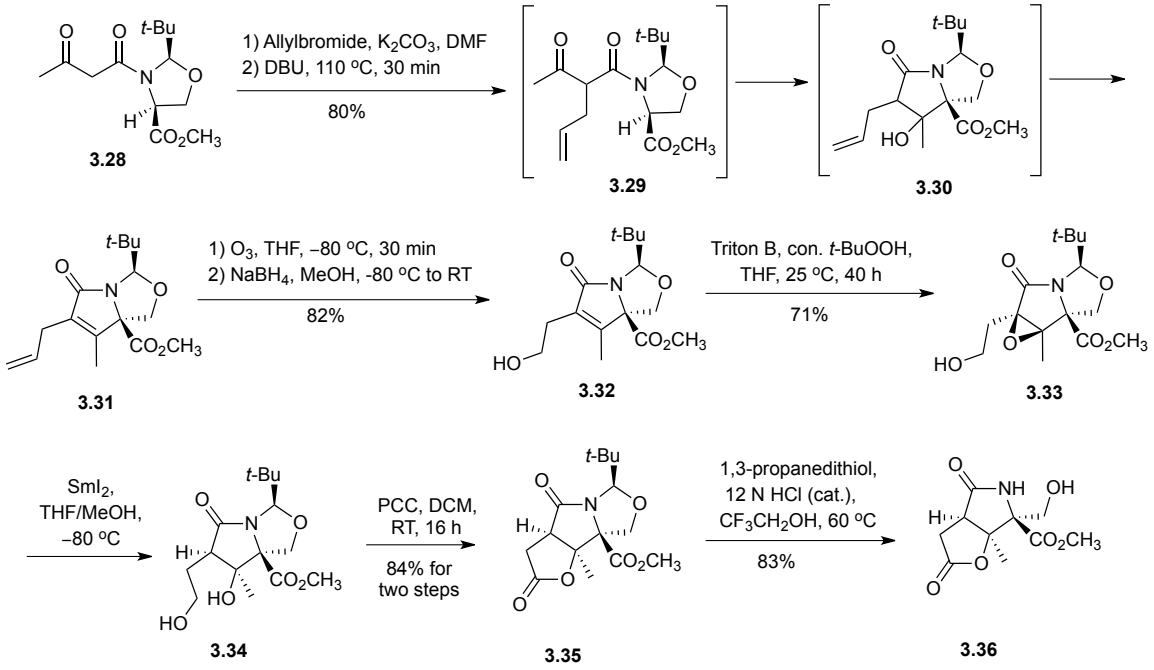
Scheme 3-2: Synthesis of lactone-fused pyrrolidinones via oxidative radical cyclization.[86]

Alkynyl amidomalonates can also be used as intermediates for the synthesis of pyrrolidinone derivatives. Hess and Burton[87] reported a convenient method to synthesize unsaturated pyrrolidinones **3.19** via zinc(II)-catalyzed 5-*endo*-dig cyclization of α-amidomalonates **3.18** (Scheme 3-3). The double bond in **3.19** can be oxidized to give various highly-functionalized pyrrolidinone derivatives.



Scheme 3-3: Zinc(II)-catalyzed 5-*endo*-dig cyclization of alkynyl amidomalonates.[87]

A final example is the synthesis of the highly functionalized pyrrolidinone derivative **3.36** via base-promoted cyclization of β-keto amide **3.28** (Scheme 3-4).[88] One-pot alkylation and cyclization of **3.28** in the presence of allyl bromide and K_2CO_3, followed by dehydration promoted by DBU, afforded **3.31** in 80% yield. Ozonolysis of **3.31** and subsequent $NaBH_4$ reduction gave alcohol **3.32**. Epoxidation of **3.32** promoted by Triton B (benzyltrimethylammonium hydroxide) in concentrated *t*-BuOOH solution provided **3.33**. Then, SmI_2 (samarium(II) iodide) promoted regio- and stereoselective reductive oxirane ring cleavage generated diol **3.34**, which was then oxidized to γ-lactam-γ-lactone **3.35** using pyridinium chlorochromate (PCC). After deprotection, pyrrolidinone derivative **3.36** was obtained in 83% yield. In designing a route to the target molecules, I used key steps in this route to synthesize the motifs present in **3.1**–**3.3**.



Scheme 3-4: Enantioselective synthesis of γ-lactam-γ-lactone **3.36**.[88]

3.1.4 Research objective and retrosynthetic analysis

As mentioned in section 3.1.3.1, the terminal *N*-acylated monosaccharide residue in LOS-IV confers important biological functions to it. The biological activity of these motifs, in conjunction with their intriguing structure, motivated us to develop a synthetic route that would provide these compounds in a form that could be used for future investigations of their function. In this Chapter, I describe the first (and to date only) stereoselective synthesis of these unusual *N*-acylated monosaccharides. These molecules were synthesized bearing an aminooctyl aglycone (**3.41**–**3.44**, Figure 3-3), a group that provides a convenient handle for conjugation, for example, to proteins for the generation of monoclonal antibodies.

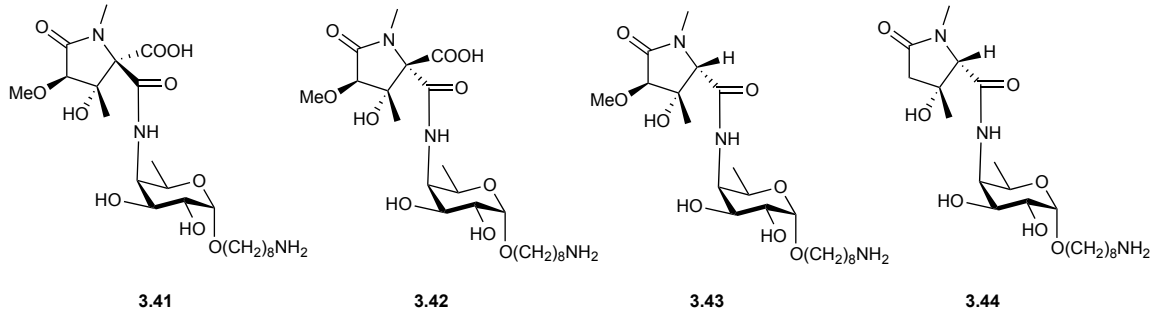
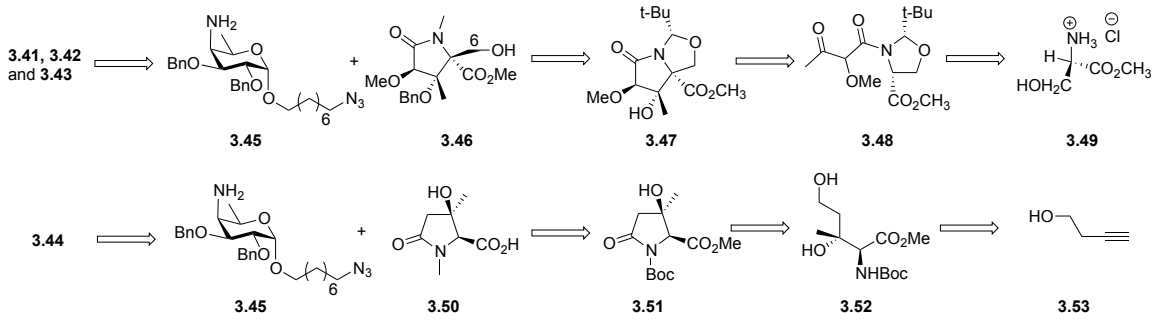


Figure 3-3: Structures of unusual *N*-acylated monosaccharides synthetic targets.

These targets feature a structure consisting of a highly substituted γ-lactam connected through an amide bond to a 4-amino-4,6-dideoxy-D-galactopyranoside moiety. My retrosynthetic analysis of **3.41**–**3.43** and **3.44** is outlined in Scheme 3-5. The disconnection of the amide bond in compounds **3.41**–**3.43** affords protected 4-amino-4,6-dideoxy-

galactose derivative **3.45** and three γ-lactams, which could be accessed from the same key intermediate **3.46**. Compound **3.46** could be produced from bicyclic oxazolidine–pyrrolinone **3.47** through a series of functional group transformations. In turn, **3.47** could be accessed by stereoselective cyclization of **3.48**, which is accessible from D-serine **3.49**. With regard to the synthesis of **3.44**, the same disconnection of the amide bond could lead to amine **3.45** and lactam **3.50**. Following a series of functional group manipulations, protection and deprotection steps, Boc protected lactam **3.51** could be converted to N-methyl lactam **3.50**. The key step is the conversion of the highly functionalized α-amino acid ester **3.52**[90] to lactam **3.51**.



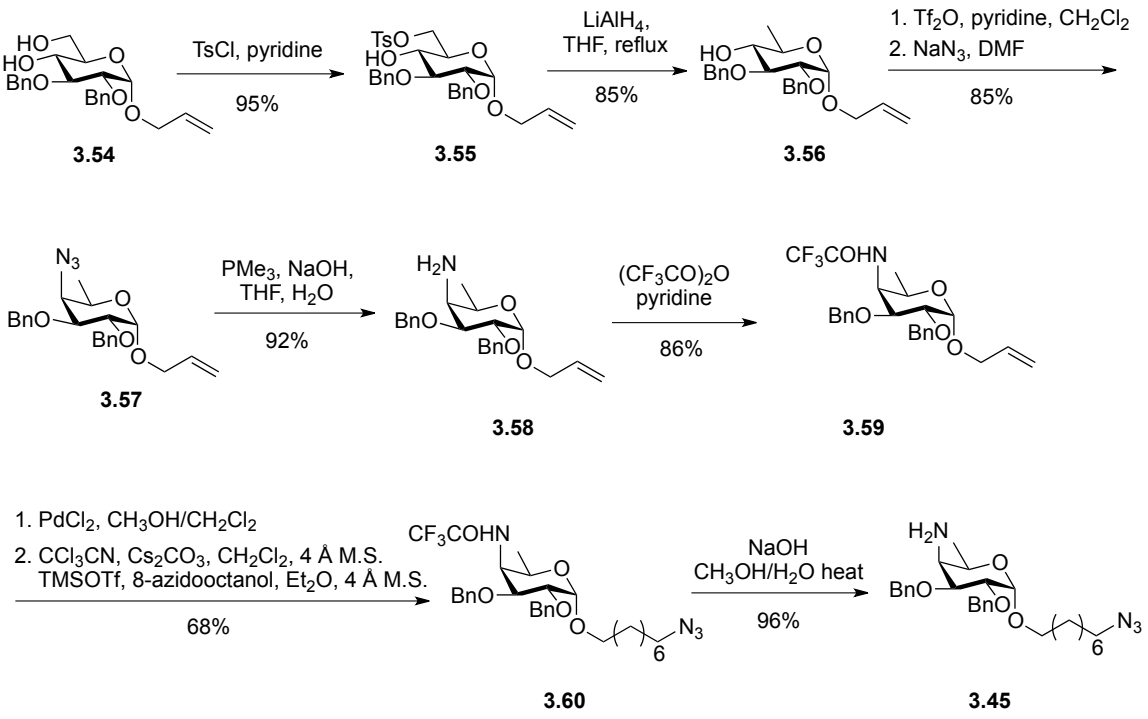
Scheme 3-5: Retrosynthetic analysis of target compounds **3.41**–**3.44**.

3.2 Results and discussion

3.2.1 Synthesis of monosaccharide 3.45

The synthesis of monosaccharide **3.45** is shown in Scheme 3-6. Glucopyranoside **3.54**[89] was treated with tosyl chloride, leading to regioselective tosylation of the C-6

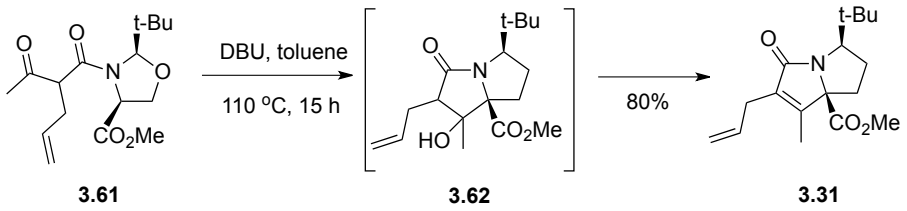
hydroxyl group to form **3.55** in 95% yield. Subsequent reduction of **3.55** by $LiAlH_4$ provided 6-deoxysugar **3.56** in 85% yield. Triflation of **3.56** followed by azide substitution at room temperature provided, in 85% overall yield, azide **3.57**. Azide reduction by the Staudinger reaction and trifluoroacetylation of the resulting amine provided the corresponding trifluoroacetamide derivative **3.59** in 79% yield over the two steps. The anomeric allyl group in **3.59** was then removed by treatment with a catalytic amount of $PdCl_2$ in a solution of methanol and CH_2Cl_2 to afford the hemiacetal, which, upon exposure to trichloroacetonitrile, Cs_2CO_3 and 4 Å molecular sieves in anhydrous CH_2Cl_2, gave the desired trichloroacetimidate donor. Glycosylation of the trichloroacetimidate donor with 8-azidooctanol in the presence of TMSOTf was α-selective affording **3.60** in a 68% overall yield from **3.59**. Finally, the trifluroacetyl protecting group was removed under basic conditions to afford 8-azidooctyl 4-amino-4,6-dideoxy-galactopyranoside (**3.45**) in 96% yield.



Scheme 3-6: Synthesis of 8-azidooctyl 4-amino-4,6-dideoxy-α-D-galactopyranoside **3.45**.

3.2.2 Synthesis of of key lactam precursor 3.47

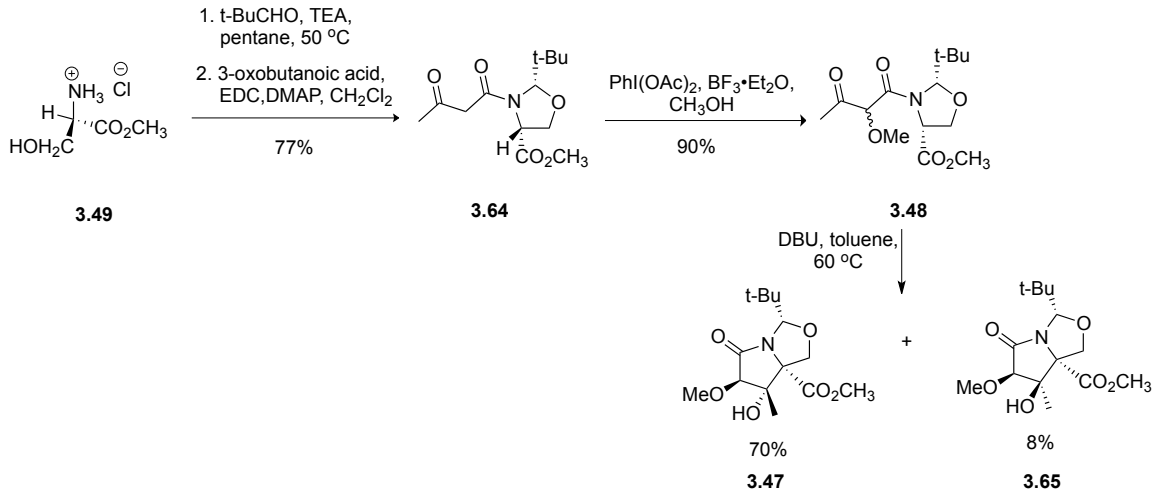
After the successful synthesis of **3.45**, I turned my attention to the preparation of the protected lactam derivative **3.47**. The key issue was to control the two newly formed stereocenters at the α and β positions of the lactam. In previous work, Ling and co-workers (Scheme 3-7)[88] treated α-alkyl-β-keto amide **3.61** with DBU at 110 °C, which led to a cyclization–dehydration sequence forming α, β-unsaturated lactam **3.31**.



Scheme 3-7: Synthesis of α,β-unsaturated lactam **3.31** from **3.61**.

Inspired by this reaction, I hypothesized that if α-methoxy-β-keto amide **3.48** (Scheme 3-8) was reacted with DBU, a product with the hydroxyl group *cis* to ester would be formed because of H-bonding with the carbonyl group. In addition, I anticipated that the methoxy group would be *trans* to the hydroxy group due to a dipole effect. Thus, I started my synthetic work from D-serine methyl ester hydrochloride **3.49**. This α-amino ester was condensed with pivaldehyde to afford an oxazolidine intermediate that was directly coupled with acetoacetic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) giving β-keto amide **3.64** in 77% overall yield. Iodosobenzene diacetate-mediated oxidation of **3.64** in methanol provided the α-methoxy-β-keto amide **3.48** in 90% yield. DBU-promoted cyclization of **3.48** was carried out as reported by Ling and coworkers; however, in my hands, it was necessary to use a lower temperature (60 °C) to avoid dehydration following cyclization. After 12 hours, two diastereomeric cyclized products were formed (ratio 9:1 from the ^{1}H NMR spectrum of the crude product), which could be separated by chromatography. The NOESY spectrum of the major product (see Appendix) indicated that

the CH_3 resonance (δ 1.33 ppm) showed a strong interaction with the H-1 (δ 4.95 ppm) and H-2 (δ 3.95 ppm) resonances (Figure 3-4). In contrast, the NOESY spectrum of the minor product (see Appendix) indicated that the CH_3 resonance (δ 1.30 ppm) showed a strong interaction with the H-4′ (δ 4.29 ppm) and CO_2CH_3 (δ 3.80 ppm) resonances . Thus these NOESY experiments confirmed that the major product (70% isolated yield) was the desired intermediate **3.47** and the minor product (8% isolated yield) was the undesired stereoisomer (**3.65**) where the hydroxyl and methoxy groups are *cis*. X-ray crystallographic analysis of **3.47** (Figure 3-5) further confirmed the structure of the major product.



Scheme 3-8: Synthesis of bicyclic oxazolidine **3.47**.

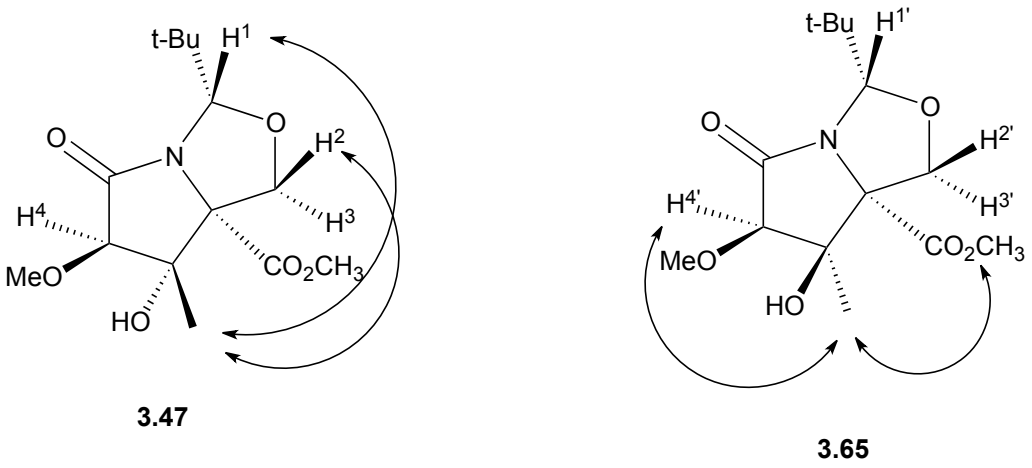


Figure 3-4: NOE effects in **3.47** and **3.65**.

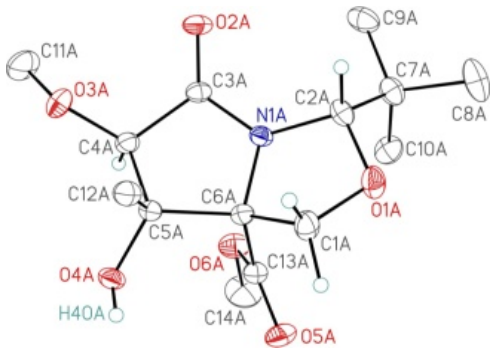


Figure 3-5: ORTEP of the X-ray crystal structure of **3.47** depicting the absolute stereochemistry.

The next step was to protect the tertiary alcohol in **3.47** as a benzyl ether. Mindful of the anticipated base-sensitivity of **3.47**, initial attempts to benzylate this tertiary alcohol were carried out under acidic conditions (TMSOTf and benzyl trichloroacetimidate). However, these attempts were unsuccessful and so I turned my attention to base-promoted

alkylation. Treatment of alcohol **3.47** with NaH and benzyl bromide (2 equiv) led to the formation of two benzylated compounds (ratio 24:71) and a trace amount of elimination by-product **3.71** (Table 3-1). The NOESY spectrum of minor product (see Appendix) showed a strong interaction between the resonacnes for the CH_3 group (δ 1.40 ppm) and H-1 (δ 4.96 ppm) (Figure 3-6). In contrast, the NOESY spectrum of the major product (see Appendix) indicated that the CH_3 resonance (δ 1.30 ppm) showed a strong interaction with the H-4′ (δ 4.42 ppm) and CO_2CH_3 (δ 3.84 ppm) resonances. Thus, these NOESY experiments confirmed that the major product was **3.70**, in which the substituents α and β to the carbonyl group were *cis*. Presumably, upon deprotonation of alcohol with base, the resulting anion undergoes a reversible retro-aldol reaction (Scheme 3-9). Although, upon recyclization both stereoisomers can be formed in this process, the *cis* intermediate **3.68** would be expected to react faster with benzyl bromide given dipolar effects, leading to a majority of **3.70**.

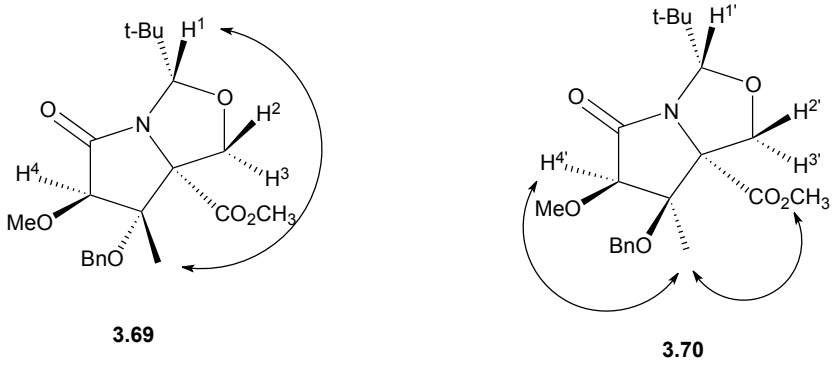
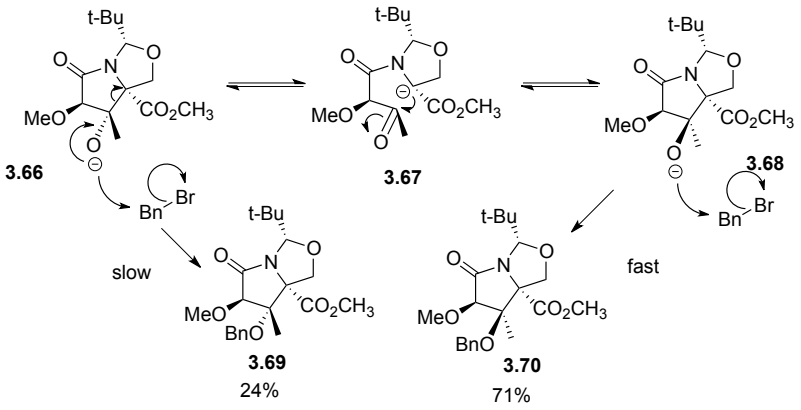


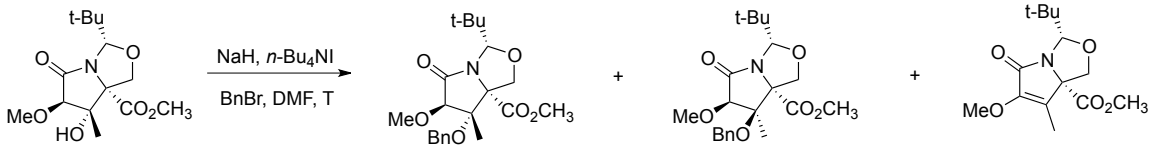
Figure 3-6: NOE effects in **3.69** and **3.70**.



Scheme 3-9: Proposed mechanism for the formation of by-product **3.70**.

I imagined that using a higher concentration of benzyl bromide would allow the trapping of alkoxide **3.66** before ring opening. Gratifyingly, as outlined in Table 3-1, increasing the ratio of BnBr to DMF to 1:2, led to a 3:2 ratio of the desired (**3.69**) to undesired (**3.70**) stereoisomers. Further increasing the ratio of BnBr to DMF to 3:1 and decreasing the temperature to –15 °C gave a 9:1 mixture of **3.69** and **3.70**.

Table 3-1: Protection of tertiary alcohol in bicyclic intermediate **3.47**[a]



3.47 **3.69** **3.70** **3.71**

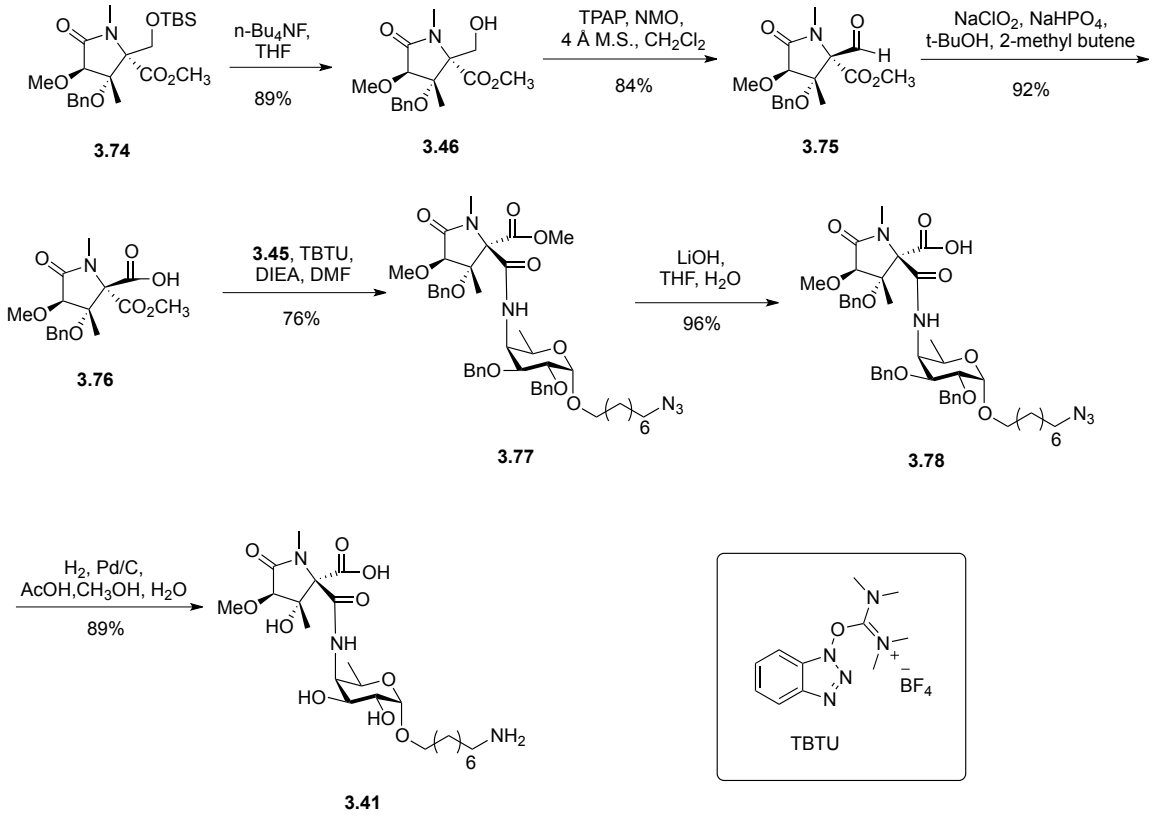
Entry	T (°C)	BnBr:DMF	Products distribution (NMR yield[b])		
			3.69	**3.70**	**3.71**
1	0	2 equiv of BnBr	24%	71%	5%
2	0	1:2	60%	40%	–
3	0	3:1	80%	20%	–
4	–15	3:1	90%	10%	–

[a]Reaction conditions: **3.47** (1 equiv), NaH (1.5 equiv), *n*-Bu_4NI (1.2 equiv)

[b]Yields were obtained by integration of CO_2CH_3 protons of the products observed in the crude ^1H NMR spectrum.

3.2.3 Synthesis of target 3.41

After the successful alkylation of the tertiary alcohol, a series of functional group manipulations converted **3.69** into the key intermediate **3.46** (Scheme 3-10). Acidic conditions were used to cleave the oxazolidine ring in 81% yield. The primary alcohol product of this reaction, **3.72**, was then converted to *tert*-butyldimethylsilyl (TBS) ether **3.73** and then *N*-methyl amide **3.74** in 83% yield over the two steps. Finally, *n*-Bu_4NF deprotection of the TBS ether provided **3.46** in 89% yield.



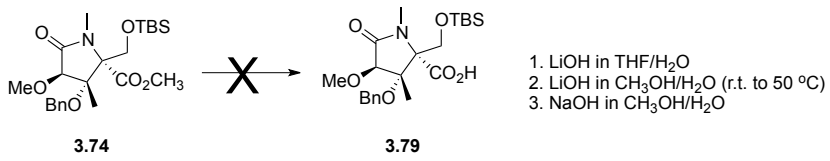
Scheme 3-10: Synthesis of target **3.41**.

With **3.46** in hand, I examined a number of different oxidation conditions (Jones, pyridinium dichromate(PDC) in wet DMF, $RuCl_3$ and $NaIO_4$, (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO) and $NaClO/NaClO_2$) to oxidize the primary alcohol to the corresponding carboxylic acid. Unfortunately, none of these methods afforded the desired product and therefore a two-step approach was explored. Ley oxidation (tetrapropylammonium perruthenate (TPAP)/*N*-methylmorpholine-N-Oxide (NMO)) afforded aldehyde **3.75** in 84% yield; subsequent Pinnick oxidation easily converted aldehyde **3.75** to carboxylic acid **3.76** in 92% yield. Amidation of the carboxylic

acid **3.76** with the amine **3.45** led to **3.77** in 76% yield. The methyl ester was then hydrolyzed by treatment with LiOH in THF and water to furnish **3.78** (96% yield). Global debenzylation and reduction of **3.78** was achieved in a mixture of methanol, water and acetic acid (10:1:0.1) under a H_2 atmosphere with 10% Pd/C as the catalyst to give an 89% yield of **3.41**.

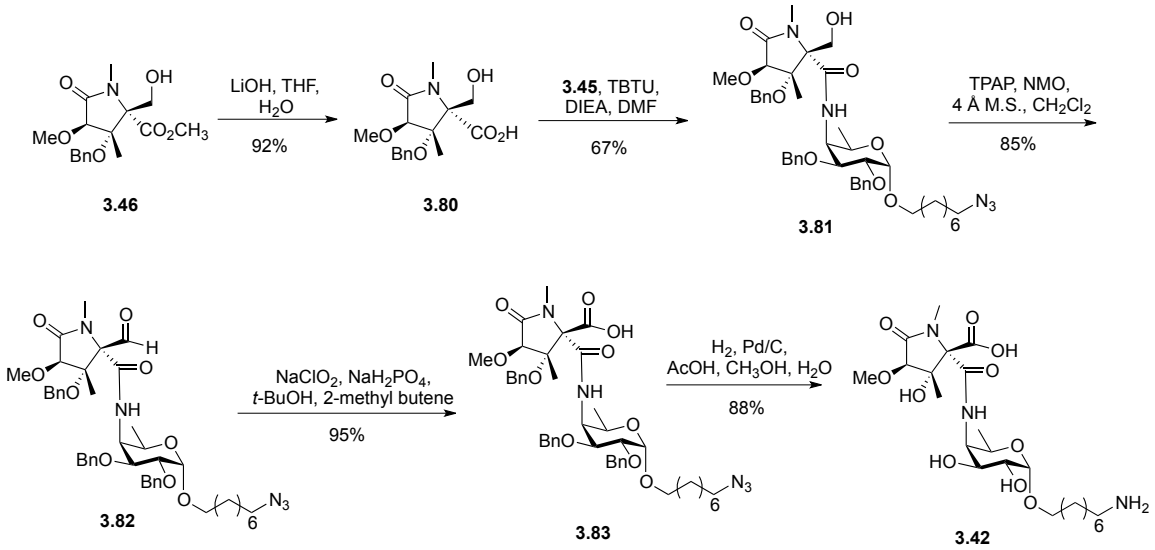
3.2.4 Synthesis of target 3.42

To synthesize **3.42**, an inverted order for the oxidation and amidation sequence was performed. The initial plan was to hydrolyze the ester in **3.74** to afford an acid **3.79** that could be coupled to amine **3.45** (Scheme 3-11). Unfortunately, presumably due to steric effects arising from the TBS protecting group, a number of different conditions (e.g., LiOH in THF/H_2O, LiOH in CH_3OH/H_2O (r.t. to 50 °C), NaOH in CH_3OH/H_2O) only afforded trace amounts of desired product. Thus, I decided to try to hydrolyze the key intermediate **3.46**, which possesses a free hydroxyl group (Scheme 3-12). Compound **3.46** was easily hydrolyzed by treatment with LiOH in THF and water at room temperature to afford acid **3.80** in 92% yield. TBTU-promoted amidation of **3.80** with amine **3.45** led to a 67% yield of **3.81**. Late-stage Ley oxidation (TPAP/NMO) and then Pinnick oxidation straightforwardly afforded acid precursor **3.83** in 80% yield over two steps. Finally, global debenzylation and azide reduction provided **3.42** in 88% yield. Like **3.41**, compound **3.42** demonstrated atropisomerism in CD_3OD with a ratio about 5:1 (Figure 3-7-D).



3.74 **3.79**

Scheme 3-11: Attempts to hydrolyze ester **3.74**.



3.46 **3.80** **3.81** **3.82** **3.83** **3.42**

Scheme 3-12: Synthesis of target **3.42**.

When **3.41**, which was homogeneous based on HRMS analysis, was dissolved in CD_3OD, the ^1H NMR spectrum showed a mixture of two species in a 4:3 ratio (Figure 3-7-A). Similarly, compound **3.42** showed a mixture of two species in CD_3OD with a ratio about 5:1 (Figure 3-7-D).

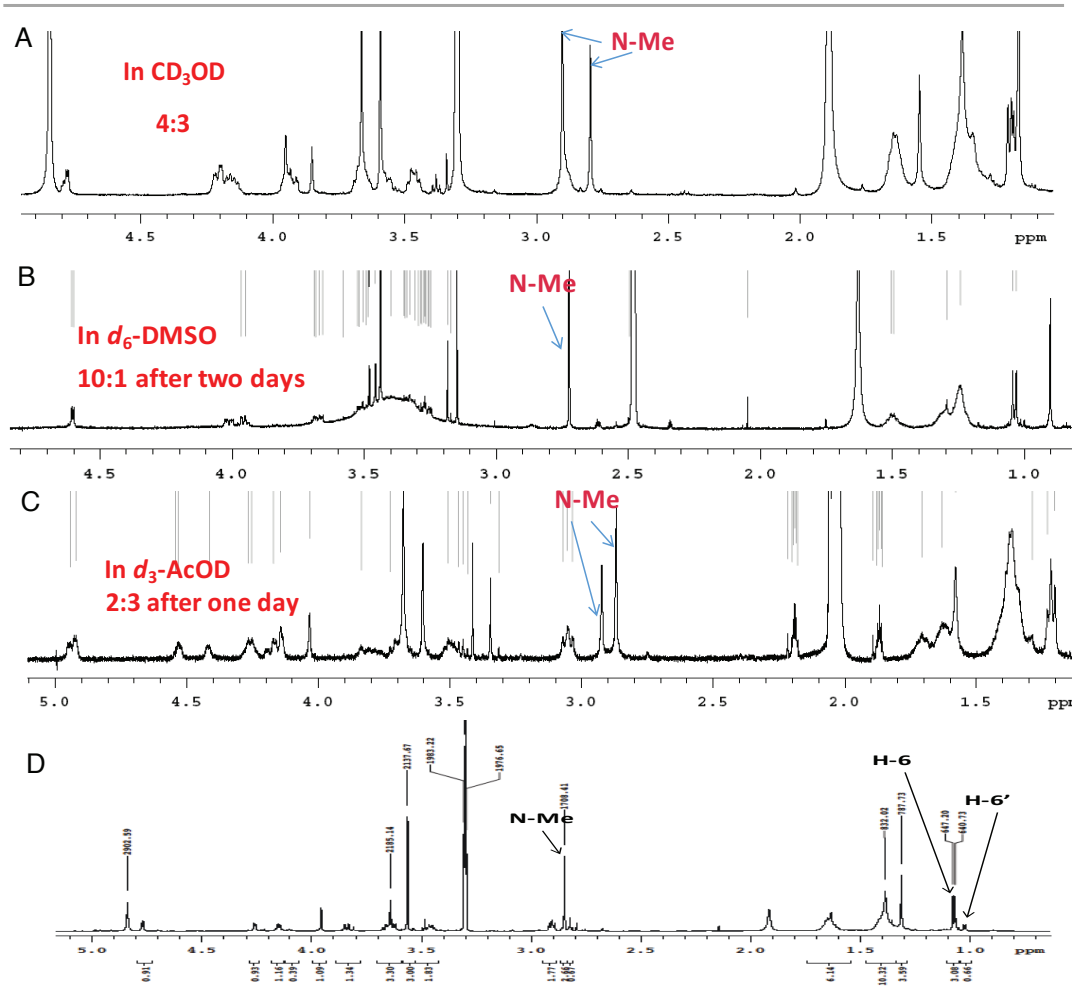


Figure 3-7:A) ^{1}H NMR spectra of **3.41** in CD_3OD. B) ^{1}H NMR spectra of **3.41** in DMSO-d_6. C) ^{1}H NMR spectra of **3.41** in AcOD-d_3. D) ^{1}H NMR spectra of **3.42** in CD_3OD

In the reference paper, Guérardel and coworkers reported the NMR data of LOS-IV using D_2O as solvent.[14] To compare with the reference NMR data, we tried to use D_2O as solvent for **3.41** and **3.42**. Although **3.41** and **3.42** cannot be fully dissolved in water, I could still obtain the NMR data after a long experiment. Interestingly, compound **3.41** and **3.42** also showed two species in D_2O. The ratio between two species for **3.41** and **3.42**

(Figure 3-8) were 1.25:1 and 1.65:1, respectively. ^{1}H and ^{13}C chemical shifts of lactam-galactose moiety in **3.41**, **3.42** and LOS-IV are summarized in Table 3-2 and Table 3-3.

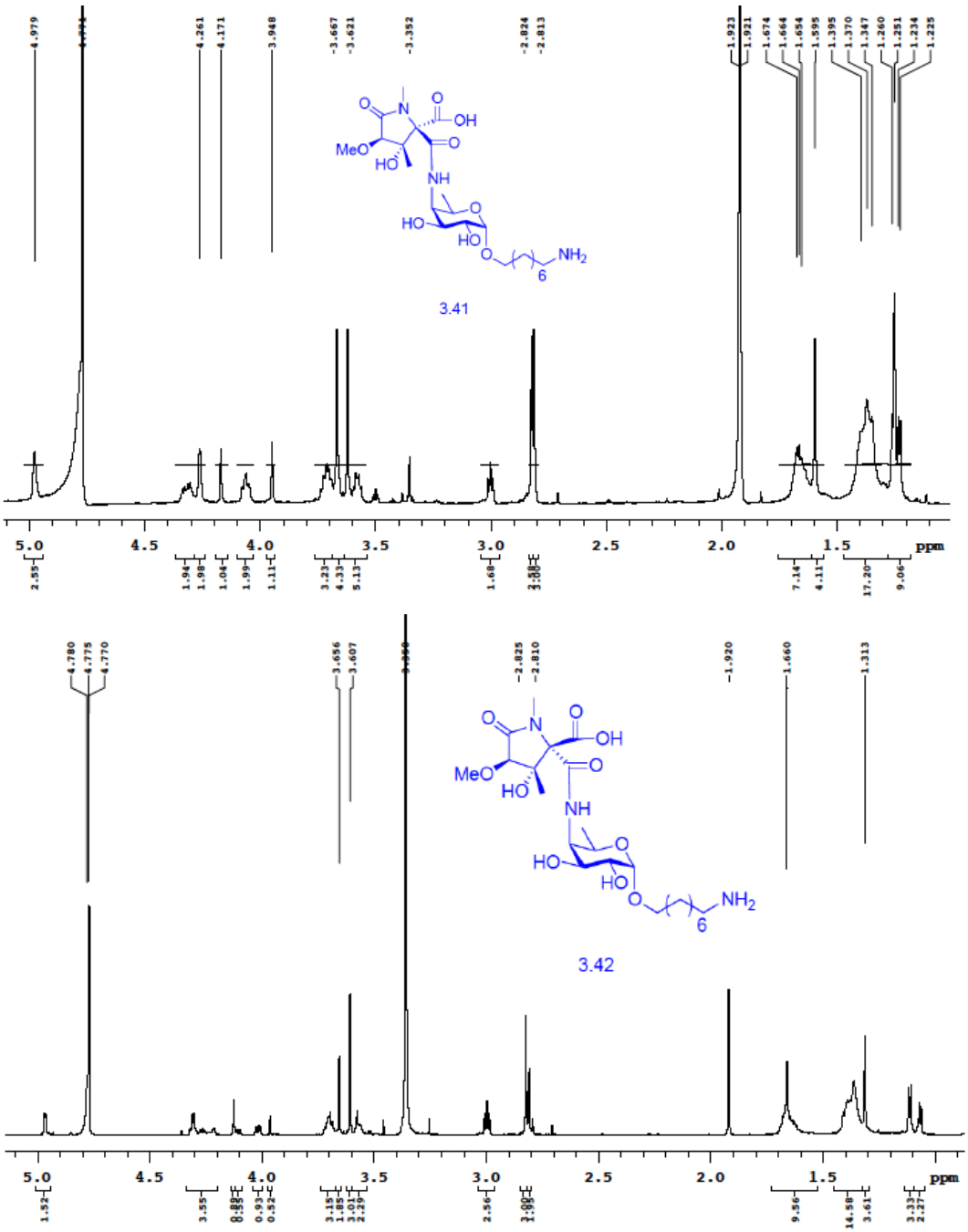


Figure 3-8: ^{1}H NMR spectra of **3.41** and **3.42** in D_2O.

Table 3-2: ^{1}H and ^{13}C chemical shifts (in ppm) of of lactam moiety in **3.41**, **3.42** and LOS-IV (measured in D_2O at 300 K)

		C-N	C-OH	CH-O	C=ONMe	O-CH_3	N-CH_3	CH_3	CO_2H	NH-C=O
3.41	^{1}H-NMR			3.95		3.67	2.81	1.59		
major	^{13}C-NMR	80.3	77.2	84.1	175.7	61.8	30.7	23.4	172.6	169.0
3.41	^{1}H-NMR			4.17		3.62	2.82	1.25		
minor	^{13}C-NMR	82.3	73.8	85.6	174.9	61.0	30.8	19.2	172.3	169.5
3.42	^{1}H-NMR			4.13		3.61	2.82	1.31		
major	^{13}C-NMR	80.7	80.3	85.3	175.0	60.8	30.8	19.1	172.2	170.1
3.42	^{1}H-NMR			3.96		3.66	2.81	1.66		
minor	^{13}C-NMR	82.2	77.2	83.7	176.3	61.6	30.7	22.8	171.8	169.9
LOS-IV	^{1}H-NMR			4.20		3.60	2.80	1.32		
Major	^{13}C-NMR	81.9	80.5	85.1	175.4	61.1	31.0	19.6	173.2	170.3
LOS-IV	^{1}H-NMR			3.96		3.65	2.81	1.63		
minor	^{13}C-NMR	83.4	77.3	84.2	176.4	61.9	31.0	23.5	172.3	170.6

Table 3-3: ^{1}H and ^{13}C chemical shifts (in ppm) of of galactose moiety in **3.41**, **3.42** and LOS-IV (measured in D_2O at 300 K)

		CH-1	CH-2	CH-3	CH-4	CH-5	CH-6
3.41	^{1}H-NMR	4.98	3.70	4.07	4.26	4.29–4.34	1.23
major	^{13}C-NMR	99.3	69.4	70.0	55.5	66.5	17.0
3.41	^{1}H-NMR	4.98	3.67	4.05	4.26	4.29–4.34	1.25
minor	^{13}C-NMR	99.3	69.7	70.2	55.8	66.0	17.4
3.42	^{1}H-NMR	4.97	3.69	4.02	4.31	4.29–4.32	1.11
major	^{13}C-NMR	99.28	70.3	70.0	56.0	66.0	16.8
3.42	^{1}H-NMR	4.97	3.71	4.10	4.22	4.25–4.28	1.07
minor	^{13}C-NMR	99.35	70.3	69.7	55.9	66.1	16.7
LOS-IV	^{1}H-NMR	5.06	3.71 or 3.73	4.11	4.38 or 4.40	4.33	1.18
	^{13}C-NMR	102.7	69.9	69.2	55.7 or 55.6	66.3	16.7

One possibility is that **3.41** undergoes transamidation reaction to form **3.42** (Figure 3-9-A). However, the ^{1}H NMR and ^{13}C NMR spectra of **3.41** and **3.42** are different. Therefore, the two species in the NMR spectra for **3.41** and **3.42** are not an equilibrating mixture of these two compounds. Another possibility is that **3.41** undergoes a retro-aldol reaction or retro-Michael reaction (Figure 3-9-B and Figure 3-9-C) to form epimers. If **3.41** was epimerized under retro-aldol or retro-Michael reaction, more than two epimers would be formed. In addition, the epimers formed by **3.41** and **3.42** should be same. However, as shown in Figure 3-8, only two species were shown in in the NMR spectra for both compounds. The difference in the ^{1}H NMR and ^{13}C NMR spectra of **3.41** and **3.42** exclude the epimerization possibility.

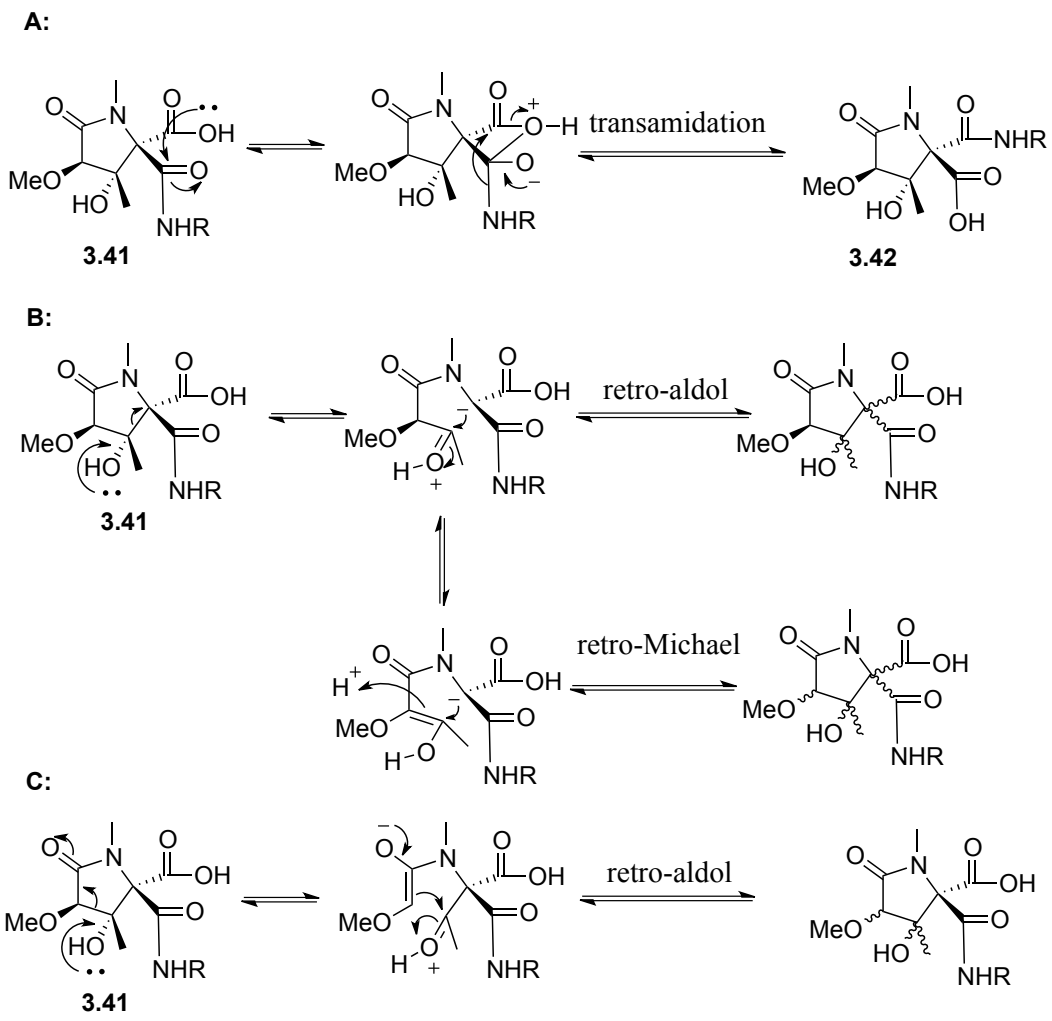
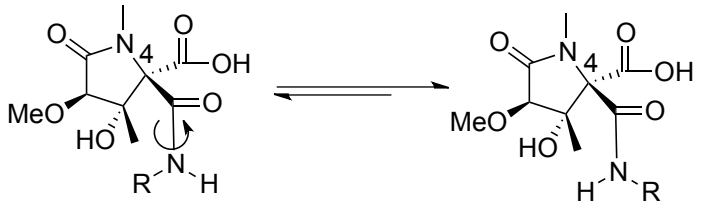


Figure 3-9: Three possible reactions involving compound **3.41** and **3.42**.

When the solvent for **3.41** was changed to DMSO-d_6, the ratio changed to 10:1 after two days (Figure 3-7-B). When AcOD-d_4 was used as solvent, the ratio changed to 2:3 after one day and did not change further (Figure 3-7-C). Recovery of the samples dissolved in DMSO-d_6 and AcOD-d_3 and redissolution in CD_3OD, resulted in a 4:3 mixture of isomers. These experiments suggest that compound **3.41** is not the mixture of two diastereomers. After deprotection, *N*-acylated monosaccharide **3.41** may exist as a mixture of two

rotamers about the C–C bonds to the quaternary carbon attached to the nitrogen, with solvent-dependent populations (Figure 3-10-B). Another possibility is that monosaccharide **3.41** exists as a mixture of two atropisomers about the C-4 amide bond (Figure 3-10-A). Attempts to resolve the rotamers or atropisomers into a single species using elevated (95 °C) temperature NMR experiments in D_2O did not result in significant changes in the distributions of the two rotamers. It is likely that the barrier between two rotamers or atropisomers is very high. Interestingly, in the report detailing the structure of these LOSs from *M. marinum*, two species were identified in ^{1}H NMR spectra of LOS IV.[14] The authors proposed that two species in ^{1}H NMR came from two compounds, which are differentiated by the stereochemistry at C-4 position on lactam and these two compounds were inseparable. Then, I found that two species in the ^{1}H NMR and ^{13}C NMR spectra for **3.42** match the two 'compounds' reported in reference paper. It is possible that the two species of NMR data in reference paper belongs to one compound, which also shows two rotamers or atropisomers in D_2O. Because the amide bond at C-4 position on lactam is a secondary amide, the barrier between two atropisomers should be low. Therefore, it is more likely that **3.41** and **3.42** may exist as a mixture of two rotamers about the C–C bonds to the quaternary carbon attached to the nitrogen.

Atropisomers



Rotamers

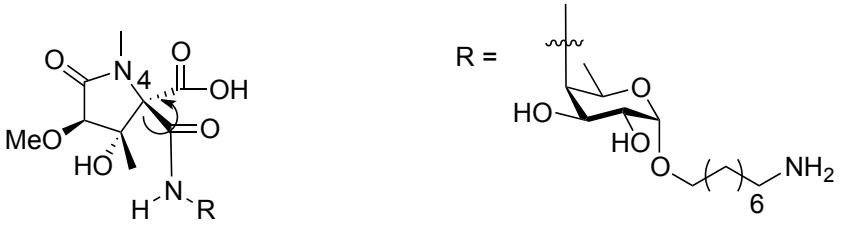
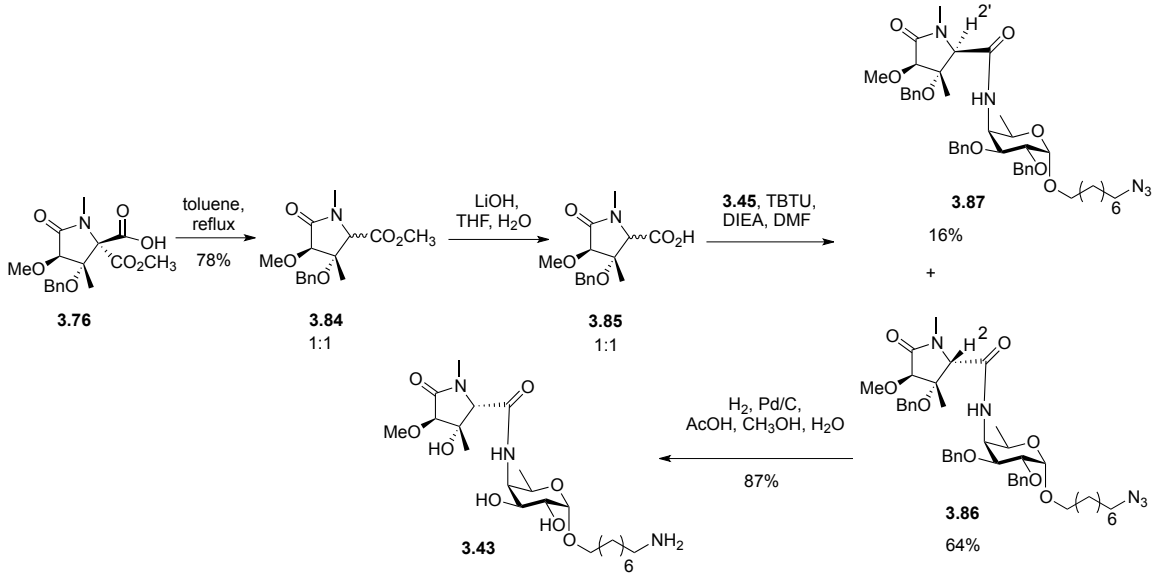


Figure 3-10: Possible rotamers or atropisomers for **3.41**.

3.2.5 Synthesis of target 3.43

To synthesize **3.43,** dicarboxylic acid monoester **3.76** (Scheme 3-13) was used as the starting material. Decarboxylation of **3.76** in toluene at reflux afforded ester **3.84** as a 1:1 mixture of diasteroisomers in 78% yield. These compounds were inseparable using silica gel column chromatography and were therefore carried forward as a mixture. Hydrolysis of **3.84** by treatment with LiOH in THF and water afforded the corresponding carboxylic acids **3.85** (also as an inseparable mixture of diastereomers), which were coupled with amine **3.45**. The resulting two amidation products, **3.86** and **3.87**, were separated by column chromatography to give major and minor products in a 4:1 ratio. NOESY

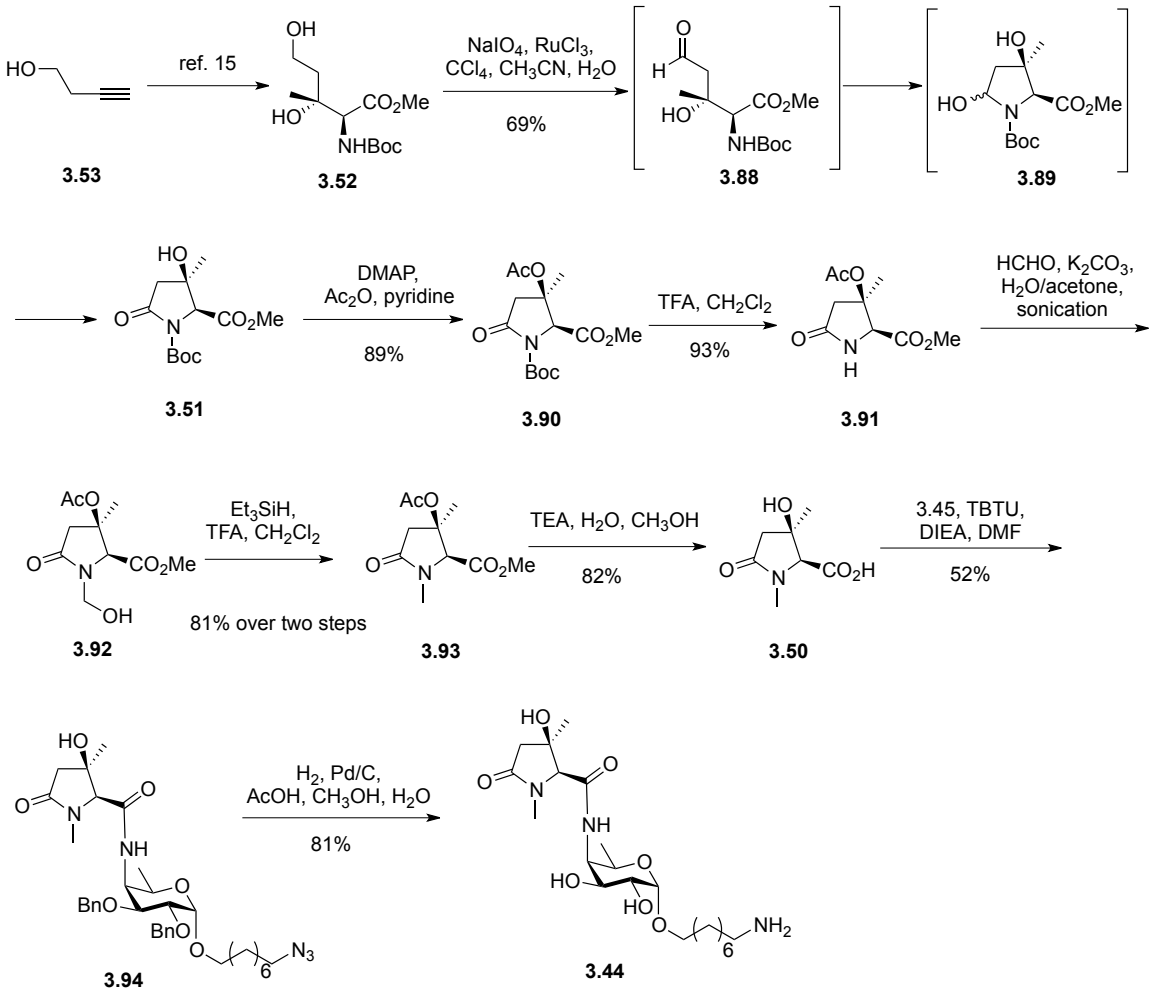
experiments (see Appendix) showed that the interaction between the CH_3 (δ 1.52 ppm) and H-2′ (δ 3.90 ppm) in the major product was stronger than the interaction between the CH_3 (δ 1.38 ppm) and H-2′ (δ 4.03 ppm) in the minor product. These results suggest that **3.86**, with a *cis* relationship between benzyl ether and amide groups, was formed as the major product (64% yield calculated based on amine **3.45**). Compound **3.87** was formed as a minor product in 16% yield. Unreacted **3.85** was also isolated at the end of the reaction. These yields presumably arise from differences in reactivities between the two stereoisomeric acids in the amidation reaction. After deprotection in a mixture of methanol, water and acetic acid (10:1:0.1) under a H_2 atmosphere with 10% Pd/C as the catalyst, **3.43** was obtained in 87% yield. Unlike the **3.41** and **3.42**, *N*-acylated monosaccharide **3.43** did not show any atropisomeric effects in CD_3OD, which is presumably attributed to more efficient amide bond rotation due to the lack of an adjacent carboxylic acid group.



Scheme 3-13: Synthesis of target **3.43**.

3.2.6 Synthesis of target 3.44

The synthesis of **3.44** (Scheme 3-14) began with 3-butynol (**3.53**), which was converted to α-amino acid ester **3.52** following the route reported by Qin and co-workers.[90] Treatment of **3.52** with $NaIO_4$ and $RuCl_3$ led to a cascade oxidation–cyclization–oxidation sequence, which produced *N*-Boc protected lactam **3.51** in 69% yield. Protecting the tertiary alcohol of **3.51** with an acetyl group and removing the Boc group afforded lactam **3.91** in 82% yield over two steps.



Scheme 3-14: Synthesis of target **3.44**.

Because lactam **3.91** was anticipated to be unstable in basic solution, milder conditions were chosen for the *N*-methylation step. Thus, *N*-hydroxymethylation of lactam **55** was achieved using paraformaldehyde in acetone in the presence of K_2CO_3 and water with sonication. To reduce the hemiaminal **3.92** to an *N*-methyl group (i.e., compound **3.93**), I initially explored the use of Pd/C catalyzed hydrogenation at atmospheric pressure in the presence of trifluoroacetic acid. However, this reaction was very slow and only a trace amount of product was formed. I found, however, that the hemiaminal **3.92** could be converted to **3.93** by treatment with triethylsilane and trifluoroacetic acid at r.t.. *N*-Methyl amide **3.93** was obtained in 81% yield over two steps from **3.91**.

Hydrolysis of methyl and acetate esters in **3.93** failed when LiOH was used in THF and water; the major product formed was elimination of the acetate to give the α,β unsaturated carboxylic acid. Fortunately, the use of milder conditions (1:2:2 Et_3N–H_2O–CH_3OH), led to the desired compound **3.50** in 82% yield. Amidation of the carboxylic acid **3.50** with the amine **3.45** was sluggish and led to **3.94** in modest 52% yield. Given the ease with which the amidations leading to **3.41**–**3.43** were carried out, the relatively low yield and sluggishness of the reaction were surprising. However, attempts to improve the yield of the product by changing the reaction conditions (e.g., EDC and *N,N*-diisopropylethylamine (DIEA)) were unsuccessful. Debenzylation and reduction of **3.94** was achieved in a mixture of methanol, water and acetic acid (10:1:0.1) under a H_2

atmosphere with 10% Pd/C as the catalyst to afford **3.44**. Similar to **3.43**, the ^{1}H NMR spectrum of **3.44** also did not show evidence of atropisomerism.

3.3 Summary

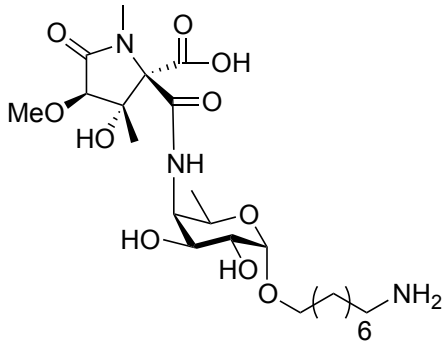
In summary, an efficient convergent strategy was developed for the synthesis of four unusual *N*-acylated monosaccharide fragments (**3.41**–**3.44**) present in the LOS-IV from *M. marinum*. The general approach to the targets involved the formation of lactam intermediates (**3.76**, **3.80**, **3.85** and **3.50**), which were coupled to amino sugar **3.45** and the resulting product deprotected. Monosaccharide **3.45** was prepared via a conventional route. The lactam moieties required for the targets were assembled via two approaches. A key feature of the sequence leading to the lactam precursors needed for the synthesis of **3.41**–**3.43** was the construction of highly substituted oxazolidine–pyrrolinone bicyclic ring system **3.47** through a substrate controlled stereoselective cyclization of α-methoxy-β-keto amide **3.48**. This reaction installed the two key stereocenters of the lactam moiety in a single step. A different approach was developed to synthesize the lactam needed for the preparation of target **3.44**. A cascade oxidation–cyclization–oxidation sequence of amino acid **3.52** was used to construct the core lactam **3.51**. Due to the decomposition under strong basic conditions, a milder approach (hemiaminal formation with parafomaldehyde and K_2CO_3 followed by reaction with triethylsilane and trifluoroacetic acid) was used to furnish

the *N*-methyl lactam **3.50**. The routes developed here will be useful in preparation of building blocks needed for the synthesis of the complete LOS-IV molecule. In addition, **3.41**–**3.44** have been synthesized in a form for conjugation to appropriate proteins and/or probes and hence represent useful biochemical tools. Immunochemical work with these compounds is ongoing.

3.4 Experimental section

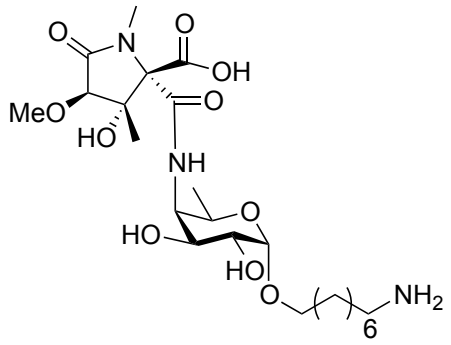
General Methods: Reactions were carried out in oven-dried glassware. All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina under nitrogen. Unless stated otherwise, all reactions were carried out at r.t. under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with Hanessian's Stain or potassium permanganate stain. Unless otherwise indicated, all column chromatography was performed on silica gel 60 (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS–8060, which is manufactured by Iatron Laboratories (Tokyo). Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of $deg \cdot mL(dm \cdot g)^{-1}$. ^{1}H NMR spectra were recorded at 500 or 700 MHz, and chemical shifts are referenced to either TMS (0.0 ppm, $CDCl_3$) or HOD (4.78 ppm, CD_3OD). ^{13}C NMR spectra were

recorded at 125, 150 or 175 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23 ppm, $CDCl_3$), external dioxane CD_3OD (48.9 ppm, CD_3OD). In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were concentrated under vacuum at < 40˚C (bath). Electrospray mass spectra (time-of-light analyzer) were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl.



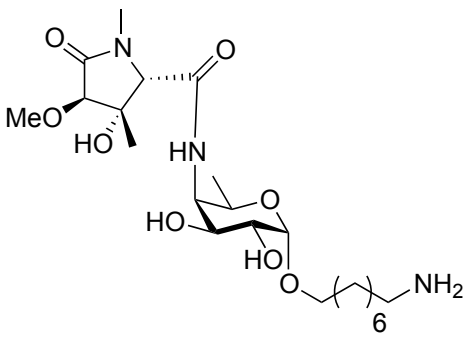
8-Azidooctyl 4-[(2′*R*,3′*S*,4′*R*)-3′-(hydroxy)-4′-methoxy-2′-carboxyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-4,6-dideoxy-α-D-galactopyranoside (**3.41**): To a solution of **3.78** (30 mg, 0.037 mmol) in CH_3OH (5 mL), water (0.5 mL) and acetic acid (0.1 mL) was treated with palladium on charcoal (10%, 10 mg) and subjected to hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was subjected to chromatography (C18 column, gradient 0→50% CH_3OH in H_2O) to yield **3.41** (17 mg, 89% yield) as a white solid. $[\alpha]_D$ = + 28.6 (*c* 0.3, CH_3OH); The NMR data showed that there were two atropisomers in CD_3OD in a 4:3 ratio. ^1H NMR (500 MHz, CD_3OD, δ_H) 4.80 (d, 1 H, *J* = 3.5 Hz, H-1 (major)), 4.78 (d,

1 H, *J* = 3.5 Hz, H-1 (minor)), 4.22–4.13 (m, 2H, H-4 and H-5), 3.95 (s, 1 H, CH_3OCH (major)), 3.96–3.90 (m, 1 H, H-3), 3.85 (s, 1 H, CH_3OCH (minor)), 3.69–3.66 (m, 2 H, octyl OCH_2 and H-2), 3.66 (s, 3 H, OCH_3 (minor)), 3.55–3.59 (m, 1 H, H-2), 3.49–3.44 (m, 1 H, octyl OCH_2), 2.91–2.88 (m, 2 H, CH_2NH_2), 2.90 (s, 3 H, NCH_3 (major)), 2.80 (s, 3 H, NCH_3 (minor)), 1.68–1.60 (m, 4 H, CH_2 x 2), 1.55 (s, 3 H, CCH_3 (minor)), 1.44–1.32 (m, 8 H, CH_2 x 4), 1.21 (d, 3 H, *J* = 6.5 Hz, H-6 (major)), 1.18 (d, 3 H, *J* = 6.5 Hz, H-6 (minor)), 1.17 (s, 3 H, CCH_3 (major)); ^{13}C NMR (125 MHz, CD_3OD, δ_C) for major atropisomer 173.3 ($CH_3NC=O$), 167.9 (HNC=O), 99.0 (C-1), 85.2 (CH_3OCH), 78.9 (CH_3COH), 72.5 (CCO_2H), 70.1 (C-3), 69.1 (C-2), 68.0 (OCH_2CH_2), 64.5 (C-5), 58.8 (OCH_3), 54.8 (C-4), 39.3 (CH_2NH_2), 29.2 to 28.5 (NCH_3 and CH_2 x 6), 17.7 (CCH_3), 16.3 (C-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) for minor atropisomer 174.2 ($CH_3NC=O$), 168.6 (HNC=O), 99.0 (C-1), 83.2 (CH_3OCH), 75.7 (CH_3COH), 69.9 (C-3), 69.2 (C-2), 68.1 (CCO_2H), 67.9 (OCH_2CH_2), 64.9 (C-5), 59.6 (OCH_3), 54.7 (C-4), 39.3 (CH_2NH_2), 29.2 to 28.5 (NCH_3 and CH_2 x 6), 22.0 (CCH_3), 16.1 (C-6); HRMS (ESI) calcd for (M+Na) $C_{23}H_{41}N_3NaO_{10}$: 542.2684. Found: 542.2678.



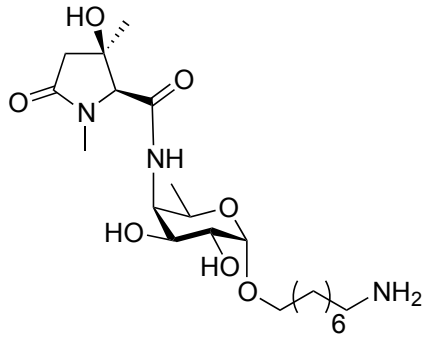
8-Azidooctyl 4-[(2′*S*,3′*S*,4′*R*)-3′-(hydroxy)-4′-methoxy-2′-carboxyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-4,6-dideoxy-α-D-galactopyranoside (**3.42**): To a solution of **3.83** (15 mg , 0.018 mmol) in CH_3OH (5 mL), water (0.5 mL) and acetic acid (0.1 mL) was added palladium on charcoal (10%, 7 mg) and subjected to hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was subjected to chromatography (C18 column, 0→50% CH_3OH in H_2O) to yield **3.42** (8 mg, 88% yield) as a white soild. $[\alpha]_D$ = + 117.8 (*c* 0.3, CH_3OH); The NMR data showed that there were two atropisomers in CD_3OD in a 5:1 ratio.^{1}H NMR for major atropisomer: ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.77 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.26 (dd, 1 H, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.15 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 3.96 (s, 1 H, CH_3OCH), 3.84 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.67–3.62 (m, 2 H, octyl OCH_2 and H-2), 3.56 (s, 3 H, OCH_3), 3.46 (dt, 1 H, J = 10.0, 6.5 Hz, octyl OCH_2), 2.91 (t, J = 8.0 Hz, 2 H, CH_2NH_2), 2.85 (s, 3 H, NCH_3), 1.67–1.60 (m, 4 H, CH_2 x 2), 1.42–1.36 (m, 8 H, CH_2 x 4), 1.31 (s, 3 H, CCH_3), 1.07 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR for major atropisomer: ^{13}C NMR (125 MHz, CD_3OD, δ_C) 173.7

($CH_3NC=O$), 169.1 (HNC=O), 99.0 (C-1), 84.3 (CH_3OCH), 79.1 (CH_3COH), 70.3 (C-2), 69.8 (C-3), 67.9 (octyl OCH_2), 64.3 (C-5), 58.9 (OCH_3), 55.0 (C-4), 39.3 (CH_2NH_2), 29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 28.6 (NCH_3), 27.2 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 17.8 (CCH_3), 15.7 (C-6); HRMS (ESI) calcd for (M+Na) $C_{23}H_{41}N_3NaO_{10}$: 542.2684. Found: 542.2677.



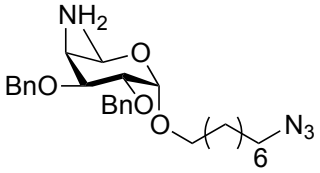
8-Azidooctyl 4-[(2′*S*,3′*S*,4′*R*)-3′-(hydroxy)-4′-methoxy-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-4,6-dideoxy-α-D-galactopyranoside (**3.43**): A solution of **3.86** (15.0 mg, 0.019 mmol) in CH_3OH (5 mL), water (0.5 mL) and acetic acid (0.1 mL) was treated with palladium on charcoal (10%, 8 mg) and subjected to hydrogen atmosphere for 20 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was triturated with CH_2Cl_2 to yield **3.43** (8.1 mg, 0.016 mmol, 87% yield) as a white solid. $[\alpha]_D$ = + 118.3 (*c* 0.3, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD, , δ_H) 4.78 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.34 (dd, 1 H, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.15 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.06 (s, 1 H, CH_3OCH), 4.01 (s, 1 H, CHC=ONH), 3.87 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.67 (dt, 1 H, *J* = 9.5, 7.0 Hz, octyl OCH_2), 3.62 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.59 (s, 3 H, OCH_3), 3.45 (dt, 1 H, *J* = 9.5, 6.5 Hz, octyl OCH_2), 2.90 (t, 2 H, *J* = 7.5 Hz, CH_2NH_2), 2.75 (s, 3 H, NCH_3), 1.68–1.62 (m, 4 H, CH_2 x 2), 1.43–1.38 (m, 8 H, CH_2 x 4), 1.37 (s, 3 H, CCH_3), 1.11 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 172.8 (CH_3NC=O), 170.0 (HNC=O), 98.9 (C-1), 84.1 (CH_3OCH), 76.1 (CH_3COH), 70.1 (CHC=ONH), 69.4 (C-3), 69.2 (C-2), 67.9 (octyl OCH_2), 64.1 (C-5), 58.9 (OCH_3), 54.7 (C-4), 39.2 (CH_2NH_2),

29.0 (CH_2), 28.8 (CH_2), 28.7 (CH_2), 27.8 (NCH_3), 27.2 (CH_2), 25.9 (CH_2), 25.7 (CH_2), 20.5 (CCH_3), 15.6 (C-6); HRMS (ESI) calcd for (M+H) $C_{22}H_{42}N_3O_8$: 476.2966. Found: 476.2961.



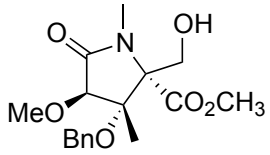
8-Azidooctyl 4-[(2′*S*,3′*R*)-3′-(hydroxy)-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-4,6-dideoxy-α-D-galactopyranoside (**3.44**): A solution of **3.94** (5.1 mg, 0.0077 mmol) in CH_3OH (5 mL), water (0.5 mL) and acetic acid (0.1 mL) was treated with palladium on charcoal (10%, 5 mg) and subjected to a hydrogen atmosphere for 8 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue was triturated with CH_2Cl_2 to afford **3.44** (2.8 mg, 81% yield) as a white solid. $[\alpha]_D$ = + 89.0 (*c* 0.1, CH_3OH); ^{1}H NMR (700 MHz, CD_3OD, δ_H) 8.02 (d, 1 H, *J* = 10.5 Hz, NH), 4.77 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.33–4.35 (m, 1 H, H-4), 4.14 (q, 1 H, $J_{5,6}$ = 6.5 Hz, H-5), 4.09 (s, 1 H, C*H*C=ONH), 3.85 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.66 (dt, 1 H, *J* = 10.0, 7.0 Hz, octyl OCH_2), 3.59 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.44 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OCH_2), 2.90 (t, 2 H, *J* = 7.5 Hz, CH_2NH$_2$), 2.76 (s, 3 H, NCH_3), 2.61 (d, 1 H, *J* = 16.5 Hz, CH_2C=O), 1.31 (d, 1 H, *J* = 16.5 Hz, CH_2C=O), 1.68–1.60 (m, 4 H,

CH_2 x 2), 1.49 (s, 3 H, CCH_3), 1.44–1.36 (m, 8 H, CH_2 x 4), 1.12 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 176.4 (CH_3NC=O), 171.5 (HNC=O), 100.5 (C-1), 74.3 (*C*HC=ONH), 73.2 (CH_3*C*OH), 70.9 (C-3 or C-2), 70.8 (C-3 or C-2), 69.4 (octyl O*C*H_2), 65.2 (C-5), 56.0 (C-4), 46.2 (*C*H_2C=O), 40.8 (*C*H_2NH_2), 30.5 (*C*H_2), 30.3 (*C*H_2), 30.2 (*C*H_2), 29.1 (NCH_3), 28.9 (C*C*H_3), 28.6 (*C*H_2), 27.4 (*C*H_2), 27.2 (*C*H_2), 17.1 (C-6); HRMS (ESI) calcd for (M+H) $C_{21}H_{40}N_3O_7$: 446.2861. Found: 446.2860.



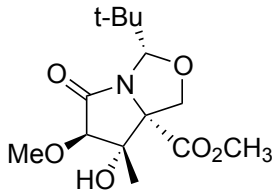
8-Azidooctyl 4-amino-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galacatopyranoside (3.45) To a solution of **3.60** (350 mg, 0.59 mmol) in CH_3OH (16 mL) at r.t. was added NaOH (aq.) (4 mL, 1 N, 4 mmol). The mixture was heated at reflux for 4 days, before being cooled, diluted with water and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford **3.45** (281 mg, 96% yield) as a colorless oil. R_f 0.17 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 42.3 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.42–7.30 (m, 10 H, ArH), 4.83 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.79 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$, C-3), 4.77 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.72 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$, C-3), 4.69 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.05 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 3.89 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 3.78 (dd, 1 H, $J_{2,3}$ =

10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.65 (dt, 1 H, J = 10.0, 7.0 Hz, octyl OCH_2), 3.47 (dt, 1 H, J = 10.0, 7.0 Hz, octyl OCH_2), 3.29 (t, 2 H, J = 7.0 Hz, CH_2N_3), 3.20 (dd, 1 H, $J_{3,4}$ = 4.0 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 1.68–1.60 (m, 4H, CH_2 x 2), 1.48 (br, 2H, NH_2), 1.42–1.37 (m, 8H, CH_2 x 4), 1.26 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) δ 138.8 (Ar), 128.4 (Ar), 128.3 (Ar), 127.8 (Ar), 127.6 (Ar), 127.63 (Ar), 127.61 (Ar), 97.4 (C-1), 78.6 (C-3), 75.5 (C-2), 73.1 (Ph*C*H_2, C-2), 72.3 (Ph*C*H_2, C-3), 68.1 (O*C*H_2CH_2), 65.1 (C-5), 53.4 (C-4), 51.5 (*C*H_2N_3), 29.4 (*C*H_2), 29.3 (*C*H_2), 29.1 (*C*H_2), 28.8 (*C*H_2), 26.7 (*C*H_2), 26.1 (*C*H_2), 16.8 (C-6); HRMS (ESI) calcd for (M+H) $C_{28}H_{41}N_4O_4$: 497.3122. Found: 497.3113.



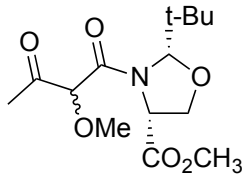
(2*S*,3*S*,4*R*)-methyl 3-(benzyloxy)-2-(hydroxymethyl)-4-methoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylate (3.46): To a solution of **3.74** (46 mg, 0.10 mmol) in THF (5 mL) was added *n*-Bu_4NF solution (0.5 mL, 1.0 M, 0.50 mmol) dropwise at r.t.. The mixture was stirred for 3 h. Then water was added and the mixture was extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 30→50% EtOAc in hexane) to afford **3.46** (30 mg, 89% yield) as a white solid. R_f 0.32 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 82.1 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ

7.36–7.26 (m, 5 H, ArH), 4.67 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.53 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.13 (d, 1 H, J = 12.5 Hz, CH_2OH), 4.10 (s, 1 H, CH_3OCH), 4.02 (d, 1 H, J = 12.5 Hz, CH_2OH), 3.75 (s, 3 H, CO_2CH_3), 3.73 (s, 3 H, OCH_3), 2.92 (s, 3 H, NCH_3), 1.48 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.3 (NC=O), 170.9 (CO_2CH_3), 138.1 (Ar), 128.3 (Ar), 127.5 (Ar), 126.8 (Ar), 83.4 ($PhCH_2OC$), 82.2 (CH_3OCH), 74.5 (CCO_2CH_3), 66.0 (PhCH_2), 62.5 (CH_2OH), 59.3 (OCH_3), 52.7 (CO_2CH_3), 27.7 (NCH_3), 12.8 (CCH_3); HRMS (ESI) calcd for (M+Na) $C_{17}H_{23}NNaO_6$: 360.1418. Found: 360.1420.



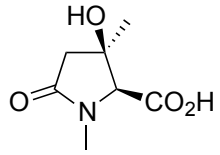
(3*S*,6*R*,7*S*,7a*S*)-methyl 3-(*tert*-butyl)-7-hydroxy-6-methoxy-7-methyl-5-oxohexahydropyrrolo[1,2-c]oxazole-7a-carboxylate (3.47): To a solution of **3.48** (1.50 g, 5.0 mmol) in dry toluene (100 mL) was added 1,8 diazabicyclo[5.4.0]undec-7-ene (DBU, 0.38 g, 2.5 mmol) and the mixture was heated at 60 °C for 12 h and then cooled to r.t. The mixture was concentrated and the crude product was purified by chromatography (gradient 9→13% EtOAc in hexane) to afford **3.47** (1.12 g, 70% yield) as a white solid. R_f 0.52 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 35.8 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.95 (s, 1 H, C$H(CH_3)_3$), 4.69 (d, 1 H, J = 9.5 Hz, OCH_RH_SC), 4.61 (s, 1 H, CH_3OCH), 3.95 (d, 1 H, J = 9.5 Hz, OCH_RH_SC), 3.85 (s, 3 H, CO_2CH_3), 3.70 (s, 3 H, OCH_3), 2.12 (s, 1 H, OH),

1.33 (s, 3 H, CCH_3), 0.91 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.0 (NC=O), 171.2 (CO_2CH_3), 96.1 ($CH(CH_3)_3$), 85.6 (CH_3OCH), 81.2 ($HOCCH_3$), 75.8 (CCO_2CH_3), 69.0 (OCH_RH_SC), 59.8 (OCH_3), 52.8 (CO_2CH_3), 36.4 ($C(CH_3)_3$), 24.9 ($C(CH_3)_3$), 18.6 (CCH_3); HRMS (ESI) calcd for (M+Na) $C_{14}H_{23}NNaO_6$: 324.1418. Found: 324.1419.



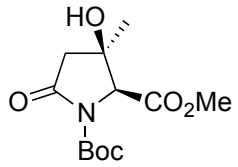
(2*S*,4*R*)-methyl 2-(*tert*-butyl)-3-(2-methoxy-3-oxobutanoyl)oxazolidine-4-carboxylate (3.48): To a stirred suspension of $PhI(OAc)_2$ (1.3 g, 7.2 mmol) in anhydrous CH_3OH (25 mL) at r.t. was added BF_3•OEt_2 (0.9 mL, 7.2 mmol). After the solution became clear, compound **3.64** (1.5 g, 5.5 mmol) in CH_3OH (3 mL) was added dropwise and the mixture was stirred at r.t. for 16 h. At that point, half of the solvent was removed and the BF_3•OEt_2 was quenched by the addition of a satd aq solution of $NaHCO_3$. The mixture was then extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (gradient 0→25% EtOAc in hexane) to yield **3.48** (1.5 g, 90% yield, 96:4 = keto–enol tautomers, 3:1 for two inseparable diastereoisomeric α-methoxy-β-keto amides) as a colorless oil; R_f 0.20 (3:1 hexane–EtOAc); $[\alpha]_D$ = + 55.8 (*c* 0.5, CH_2Cl_2); NMR data for major isomer: ^1H NMR (500 MHz, $CDCl_3$, δ_H) 5.31 (s, 1 H, $CH(CH_3)_3$), 5.30 (dd, 1 H, *J* = 7.0, 1.5 Hz, $CHCO_2CH_3$),

4.73 (s, 1 H, C*H*OCH_3), 4.60 (dd, 1 H, *J* = 8.5, 1.5 Hz, OCH_2), 3.98 (dd, 1 H, *J* = 8.5, 7.0 Hz, OCH_2), 3.82 (s, 3 H, CO_2CH_3), 3.51 (s, 3 H, $CHOCH_3$), 2.34 (s, 3 H, CH_3C=O), 0.94 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 207.0 (CH_3C=O), 170.1 (CO_2CH_3), 167.9 (NC=O), 97.1 ($CH(CH_3)_3$), 86.8 ($CHOCH_3$), 67.7 (OCH_2), 58.2 ($CHCO_2CH_3$), 57.5 (OCH_3), 52.7 (CO_2CH_3), 37.2 ($C(CH_3)_3$), 26.8 (CH_3C=O), 25.7 ($C(CH_3)_3$); NMR data for minor isomer: ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.34 (s, 1 H, $CH(CH_3)_3$), 4.79 (dd, 1 H, *J* = 7.0, 2.0 Hz, $CHCO_2CH_3$), 4.63 (s, 1 H, $CHOCH_3$), 4.46 (dd, 1 H, *J* = 8.5, 2.0 Hz, OCH_2), 3.94 (dd, 1 H, *J* = 8.5, 7.0 Hz, OCH_2), 3.81 (s, 3 H, CO_2CH_3), 3.49 (s, 3 H, $CHOCH_3$), 2.31 (s, 3 H, CH_3C=O), 0.99 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 202.5.0 (CH_3C=O), 170.0 (CO_2CH_3), 168.6 (NC=O), 97.6 ($CH(CH_3)_3$), 88.8 ($CHOCH_3$), 68.8 (OCH_2), 59.3 ($CHCO_2CH_3$), 58.6 (OCH_3), 52.5 (CO_2CH_3), 37.1 ($C(CH_3)_3$), 27.0 (CH_3C=O), 26.0 ($C(CH_3)_3$); HRMS (ESI) calcd for (M+H) $C_{14}H_{24}NO_6$: 302.1598. Found: 302.1597.



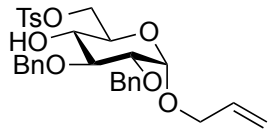
(2*S*,3*R*)-3-hydroxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylic acid (3.50): Compound **3.93** (80 mg, 0.35 mmol) was dissolved in Et_3N (5 mL), CH_3OH (10 mL) and water (10 mL) and the mixture was stirred at r.t. over night. Then the solution was concentrated, and the resulting residue was subjected to chromatography (latrobeads 6RS-

8060, gradient 10%→50% CH_3OH–CH_2Cl_2) to yield **3.50** (49 mg, 82% yield) as a white solid; R_f 0.2 (2:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +11.6 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 3.98 (s, 1 H, NC*H*), 2.83 (s, 3 H, NCH_3), 2.56 (d, 1 H, *J* = 17.0 Hz, C*H*$_2$C=O), 2.41 (d, 1 H, *J* = 17.0 Hz, C*H*$_2$C=O), 1.50 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 176.1 (NC=O), 170.0 (*C*O_2H), 75.0 (N*C*H), 73.0 (CH_3*C*OH), 46.6 (*C*H_2C=O), 29.4 (NCH_3), 28.1 (C*C*H_3); HRMS (ESI) (M-H) calcd for $C_7H_{10}NO_4$: 172.0615. Found: 172.0618.



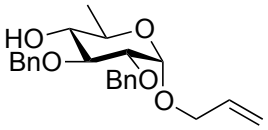
(2*S*,3*R*)-1-*tert*-butyl 2-methyl 3-hydroxy-3-methyl-5-oxopyrrolidine-1,2-dicarboxylate (3.51): Compound **3.52**[90] (0.91g, 3.31 mmol) was dissolved in CCl_4 (15 mL), CH_3CN (15 mL), and water (18 mL). With vigorous stirring, $NaIO_4$ (2.12 g, 9.9 mmol) and $RuCl_3 \cdot H_2O$ (30 mg) were added at 0 °C. The mixture was stirred at r.t. for 1 h, and then EtOAc (30 mL) was added. The mixture was washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (gradient 50→150% EtOAc in hexane) to give **3.51** as a colorless liquid (0.61 g, 69% yield). R_f 0.3 (4:3 EtOAc–Hexane); $[\alpha]_D$ = + 0.19 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.34 (s, 1 H, NC*H*), 3.84 (s, 3 H, OCH_3), 2.90 (d, 1 H, *J* = 17.0 Hz, COC*H*$_2$), 2.60 (d, 1 H, *J* = 17.0 Hz, COC*H*$_2$), 2.26 (br, 1 H, OH), 1.52 (s, 9 H, $C(CH_3)_3$), 1.45 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.6 (NC=O), 169.1 (*C*O_2CH_3),

149.0 ($CO_2C(CH_3)_3$), 84.1 ($OC(CH_3)_3$), 70.8 (CH_3COH), 69.6 (NCH), 52.5 (OCH_3), 46.4 (CH_2), 28.2 (CCH_3), 27.9 ($C(CH_3)_3$); HRMS (ESI) calcd for (M+Na) $C_{12}H_{19}NNaO_6$: 296.1105. Found: 296.1105.



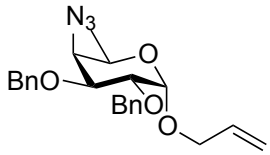
Allyl 2,3-di-*O*-benzyl-6-*O*-tosyl-α-D-glucopyranoside (3.55): To a solution of allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**3.54**, 1.60 g, 4.07 mmol) in anhydrous pyridine (10 mL) was added TsCl (1.16 g, 6.10 mmol) at 0 °C. After being stirred for 12 h at r.t., the mixture was diluted with EtOAc (30 mL), washed with 1 N HCl, a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 20→25% EtOAc in hexane) to afford **3.55** (2.14 g, 95% yield) as a colorless oil. R_f 0.45 (2:1 hexane–EtOAc); $[\alpha]_D$ = + 38.1 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.80 (d, 2 H, *J* = 8.5 Hz, ArH), 7.39–7.22 (m, 12 H, ArH), 5.92 (dddd, 1 H, *J* = 17.0, 10.0, 6.5, 5.0 Hz, CH_2=C*H*), 5.32 (app dq, 1 H, *J* = 17.0, 1.5 Hz, CH_2=CH), 5.24 (app dq, 1 H, *J* = 10.0, 1.0 Hz, CH_2=CH), 5.02 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-3), 4.79 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.74 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-2), 4.71 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-3), 4.65 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-2), 4.28–4.22 (m, 2 H, H-4 and H-5), 4.13 (app ddt, 1 H, *J* = 13.0, 5.0, 1.5 Hz, CH_2=CHCH_2), 3.97 (app ddt, 1 H, *J* = 13.0, 6.5, 1.0 Hz, CH_2=CHCH_2), 3.79 (app t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.79 (dd, 1

H, $J_{6R,6S}$ = 10.0 Hz, $J_{5,6}$ = 2.5 Hz, H-6), 3.49 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 3.45 (dd, 1 H, $J_{6R,6S}$ = 10.0 Hz, $J_{5,6}$ = 3.0 Hz, H-6), 2.45 (s, 3 H, $PhCH_3$), 2.23 (d, 1 H, J = 3.0 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 144.8 (Ar), 138.7 (Ar), 137.9 (Ar), 133.5 ($CH{=}CH_2$), 133.0 (Ar), 129.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 118.5 ($CH{=}CH_2$), 95.6 (C-1), 81.1 (C-3), 79.5 (C-2), 75.4 ($PhCH_2$, C-3), 73.0 ($PhCH_2$, C-2), 69.5 (C-6), 69.2 and 68.9 (C-4 and C-5), 68.4 ($CH_2CH{=}CH_2$), 21.7 (CH_3PhSO_2); HRMS (ESI) calcd for (M+Na) $C_{30}H_{34}NaO_8S$: 577.1867. Found: 577.1864.



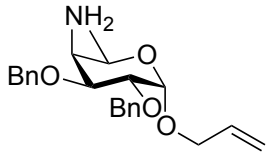
Allyl 2,3-di-*O*-benzyl-6-deoxy-α-D-glucopyranoside (3.56): Tosylate **3.55** (2.10 g, 3.79 mmol) was dissolved in THF (30 mL) and $LiAlH_4$ (288 mg, 7.58 mmol) was added. The reaction mixture was heated at reflux for 3 h. After completion of the reaction, the $LiAlH_4$ was quenched by slowly adding the mixture to ice, then the mixture was filtered through Celite. The filter cake was washed with EtOAc, and the resulting cloudy solution was filtered again through Celite. The organic phase was washed with 1 N HCl, a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 10→15% EtOAc in hexane) to afford **3.56** (1.25 g, 85% yield) as a colorless oil. R_f 0.39 (4:1 hexane–EtOAc); $[\alpha]_D$ = + 70.4 (*c* 0.2, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.41–7.29 (m, 10 H, ArH), 5.98 (dddd, 1 H,

J = 17.0, 10.0, 6.5, 5.0 Hz, CH_2=C*H*), 5.37 (app dq, 1 H, J = 17.0, 1.0 Hz, CH_2=CH), 5.27 (app dq, 1 H, J = 10.0, 1.0 Hz, CH_2=CH), 5.08 (d, 1 H, J = 11.5 Hz, PhCH_2, C-3), 4.81 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.76 (d, 1 H, J = 12.0 Hz, PhCH_2, C-2), 4.73 (d, 1 H, J = 11.5 Hz, PhCH_2, C-3), 4.70 (d, 1 H, J = 12.0 Hz, PhCH_2, C-2), 4.20 (app ddt, 1 H, J = 13.0, 5.0, 1.0 Hz, CH_2=CHCH_2), 4.05 (app ddt, 1 H, J = 13.0, 6.5, 1.0 Hz, CH_2=CHCH_2), 3.81 (app t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 3.75 (dq, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 3.57 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 3.21 (app td, 1 H, $J_{4,5}$ = $J_{3,4}$ = 9.5 Hz, $J_{4,OH}$ = 2.0 Hz, H-4), 2.19 (d, 1 H, J = 2.0 Hz, OH), 1.27 (d, 3 H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.8 (Ar), 138.1 (Ar), 133.9 (*C*H=CH_2), 128.7 (Ar), 128.5 (Ar), 128.1 (Ar), 128.0 (Ar), 127.92 (Ar), 127.91 (Ar), 118.1 (CH=*C*H_2), 95.5 (C-1), 81.3 (C-3), 80.1 (C-2), 75.4 (C-4), 75.3 (Ph*C*H_2, C-3), 72.8 (Ph*C*H_2, C-2), 68.2 (*C*H_2CH=CH_2), 67.1 (C-5), 17.7 (C-6); HRMS (ESI) calcd for (M+Na) $C_{23}H_{28}NaO_5$: 407.1829. Found: 407.1825.



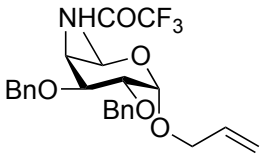
Allyl 4-azido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (3.57): To a solution of **3.56** (1.25 g, 3.25 mmol) and pyridine (3 mL) in anhydrous CH_2Cl_2 at 0 °C was added Tf_2O (1.37 g, 4.88 mmol) slowly. After being stirred for 1 h, the mixture was diluted with CH_2Cl_2, washed with water, a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated to obtain the crude triflate, which was dissolved into DMF (15

mL). Excess NaN_3 (845 mg, 13 mmol) was added and the mixture was stirred overnight at r.t. After completion of the reaction, the mixture was filtered through Celite and the residue was washed with EtOAc. The mixture was concentrated and the resulting residue was purified by chromatography (gradient 4→6% EtOAc in hexane) to afford **3.57** (1.13 g, 85% yield) as a colorless oil. R_f 0.61 (4:1 hexane–EtOAc); $[\alpha]_D$ = + 85.1 (*c* 0.4, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.44–7.29 (m, 10 H, ArH), 5.95 (dddd, 1 H, *J* = 17.0, 10.0, 6.0, 5.0 Hz, CH_2=C*H*), 5.34 (d, 1 H, *J* = 17.0 Hz, C*H*$_2$=CH), 5.25 (d, 1 H, *J* = 10.0 Hz, C*H*$_2$=CH), 4.89 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-3), 4.84 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.80 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.79 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-3), 4.68 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.12 (dd, 1 H, *J* = 12.5, 5.0 Hz, CH_2=CHC*H*$_2$), 4.10 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.03 (dd, 1 H, *J* = 12.5, 6.0 Hz, CH_2=CHC*H*$_2$), 4.01 (q, 1 H, $J_{5,6}$ = 6.5 Hz, H-5), 3.89 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 3.75 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4), 1.26 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.4 (Ar), 138.3 (Ar), 133.9 (*C*H=CH_2), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 118.1 (CH=*C*H_2), 96.3 (C-1), 78.1 (C-3), 76.0 (C-2), 73.6 (Ph*C*H_2, C-2), 73.2 (Ph*C*H_2, C-3), 68.5 (*C*H_2CH=CH_2), 65.2 (C-4), 64.5 (C-5), 17.3 (C-6); HRMS (ESI) calcd for (M+Na) $C_{23}H_{27}N_3NaO_4$: 432.1894. Found: 432.1893.



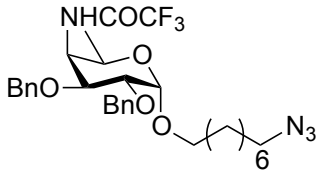
Allyl 4-amino-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (3.58): To a solution of **3.57** (1.05 g, 2.56 mmol) in THF (40 mL) at r.t. was added NaOH (1 M, 10 mL). Then a solution of PMe_3 (10.0 mL, 1M in THF, 10.0 mmol) was added dropwise. The mixture was stirred at r.t. for 10 h. After completion of the reaction, the mixture was diluted with water, extracted with CH_2Cl_2. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 0→2% CH_3OH in EtOAc) to afford **3.58** (0.91 g, 92% yield) as a colorless oil. R_f 0.17 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 98.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.42–7.29 (m, 10 H, ArH), 5.97 (dddd, 1 H, *J* = 17.0, 10.0, 6.5, 5.0 Hz, CH_2=C*H*), 5.36 (apt dq, 1 H, *J* = 17.0, 1.5 Hz, CH_2=CH), 5.25 (apt dq, 1 H, *J* = 10.0, 1.0 Hz, CH_2=CH), 4.83 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.81 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-2), 4.79 (d, 1 H, *J* = 11.5 Hz, PhCH_2, C-3), 4.72 (d, 1 H, *J* = 11.5 Hz, PhCH_2, C-2), 4.68 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-3), 4.17 (apt ddt, 1 H, *J* = 13.0, 5.0, 1.0 Hz, CH_2=CHCH_2), 4.06 (q, 1 H, $J_{5,6}$ = 6.5 Hz, H-5), 4.05 (apt ddt, *J* = 13.0, 6.5, 1.5 Hz, 1H, CH_2=CHCH_2), 3.92 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 3.78 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.22 (d, 1 H, $J_{3,4}$ = 4.0 Hz, H-4), 2.06 (br, 2 H, NH_2), 1.26 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 138.6 (Ar), 134.1 (*C*H=CH_2), 128.4 (Ar), 128.3 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 117.9 (CH=*C*H_2), 96.2 (C-1), 78.3 (C-3), 75.3 (C-2), 73.2

(Ph*C*H$_2$, C-2), 72.4 (Ph*C*H$_2$, C-3), 68.3 (*C*H$_2$CH=CH$_2$), 65.2 (C-5), 53.5 (C-4), 16.7 (C-6); HRMS (ESI) calcd for (M+H) $C_{23}H_{30}NO_4$: 384.2169. Found: 384.2165.



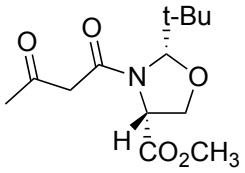
Allyl 4-trifluoroacetamido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (3.59): To a solution of **3.58** (590 mg, 1.5 mmol) in anhydrous pyridine (15 mL) at 0 °C was added trifluoroacetic anhydride (594 mg, 3.0 mol) dropwise. The mixture was slowly warmed to r.t. and stirred for 6 h. After completion of the reaction, the mixture was concentrated under vacuum and diluted with CH_2Cl_2. The organic solution was washed with 1 N HCl, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 10→15% EtOAc in hexane) to afford **3.59** (634 mg, 86% yield) as a colorless oil. R_f 0.48 (4:1 hexane–EtOAc); $[\alpha]_D$ = + 71.1 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.30 (m, 10 H, ArH), 6.32 (d, 1 H, *J* = 10.0 Hz, NH), 5.96 (dddd, 1 H, *J* = 17.0, 10.0, 6.5, 5.0 Hz, CH_2=C*H*), 5.37 (apt dq, 1 H, *J* = 17.0, 1.5 Hz, C*H*$_2$=CH), 5.28 (apt dq, 1 H, *J* = 10.0, 1.0 Hz, C*H*$_2$=CH), 4.84 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.83 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.83 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.68 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.60 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.57 (dd, 1 H, $J_{4,NH}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-4), 4.23 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.19 (apt ddt, 1 H, *J* = 13.0, 5.0, 1.5 Hz, CH_2=CHC*H*$_2$), 4.07 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz,

H-3), 4.06 (apt ddt, 1 H, J = 13.0, 6.5, 1.0 Hz, CH_2=CHCH_2), 3.47 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 1.18 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 158.0 (q, $^2J_{C,F}$ = 37.5 Hz, C=O), 138.1 (Ar), 137.9 (Ar), 133.5 (*C*H=CH_2), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 118.4 (CH=*C*H_2), 115.9 (q, $^1J_{C,F}$ = 287.5 Hz, CF_3), 96.3 (C-1), 76.2 (C-3), 75.1 (C-2), 73.4 (Ph*C*H_2, C-2), 72.1 (Ph*C*H_2, C-3), 68.8 (*C*H_2CH=CH_2), 63.8 (C-5), 52.1 (C-4), 16.4 (C-6); HRMS (ESI) calcd for (M+Na) $C_{25}H_{28}F_3NNaO_5$: 502.1812. Found: 502.1808.



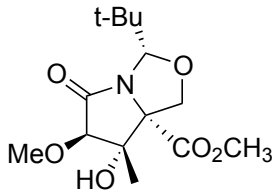
8-Azidooctyl 4-trifluoroacetamido-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (3.60): To a solution of **3.59** (500 mg, 1.04 mmol) in CH_3OH (10 mL) and CH_2Cl_2 (10 mL) at r.t. was added $PdCl_2$ (18 mg, 0.10 mol, 0.1 equiv). The mixture was stirred at r.t. for 16 h. After completion of the reaction, the mixture was filtered through a plug of Celite and the filtrate was concentrated to obtain crude 4-trifluoroacetamido-2,3-di-*O*-benzyl-4,6-dideoxy-D-galacatopyranose, which was carried forward without further purification. This crude product was dissolved in anhydrous CH_2Cl_2 (20 mL) with 4 Å molecular sieves and this mixture was treated with trichloroacetonitrile (1.5 g, 10.4 mmol) and Cs_2CO_3 (676 mg, 2.08 mmol). The mixture was stirred at r.t. for 6 h and then filtered through Celite. The filtrate was concentrated to obtain the corresponding glycosyl

trichloroacetimidate, which was dissolved in Et_2O (5 mL) and added to a mixture of 8-azidooctanol (355 mg, 2.08 mmol) and 4 Å molecular sieves in Et_2O (5 mL). The mixture was cooled to 0 °C and TMSOTf (15 μL) was added and the solution was stirred at 0 °C for 1 h. The TMSOTf was quenched by the addition of Et_3N (1 mL) and the solution was concentrated. The residue was purified by chromatography (gradient 10→15% EtOAc in hexane) to afford **3.60** (415 mg, 68% yield) as a colorless oil. R_f 0.58 (4:1 hexane–EtOAc); $[\alpha]_D$ = + 66.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.30 (m, 10 H, ArH), 6.33 (d, 1 H, *J* = 10.0 Hz, NH), 4.84 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.83 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.76 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.67 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.60 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.57 (ddd, 1 H, $J_{4,NH}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.21 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.05 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.65 (dt, 1 H, *J* = 10.0, 7.0 Hz, octyl OC*H*$_2$), 3.47 (dt, 1 H, *J* = 10.0, 7.0 Hz, octyl OC*H*$_2$), 3.46 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.29 (t, 2 H, *J* = 7.0 Hz, CH_2N_3), 1.70–1.60 (m, 4H, CH_2 x 2), 1.43–1.34 (m, 8H, CH_2 x 4), 1.18 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 158.0 (q, $^2J_{C,F}$ = 37.5 Hz, C=O), 138.3 (Ar), 137.9 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 115.9 (q, $^1J_{C,F}$ = 287.5 Hz, CF_3), 97.5 (C-1), 76.2 (C-3), 75.3 (C-2), 73.3 (Ph*C*H$_2$, C-2), 72.1 (Ph*C*H$_2$, C-3), 68.7 (O*C*H$_2$CH$_2$), 63.6 (C-5), 52.1 (C-4), 51.5 (*C*H$_2$N$_3$), 29.4 (*C*H$_2$), 29.3 (*C*H$_2$), 29.1 (*C*H$_2$), 28.8 (*C*H$_2$), 26.7 (*C*H$_2$), 26.1 (*C*H$_2$), 16.5 (C-6); HRMS (ESI) calcd for (M+Na) $C_{30}H_{39}F_3N_4NaO_5$: 615.2765. Found: 615.2754.



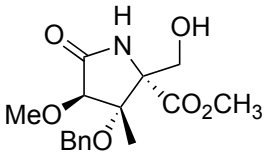
(2*S*,4*R*)-methyl 2-(*tert*-butyl)-3-(3-oxobutanoyl)oxazolidine-4-carboxylate (**3.64**): To a stirred suspension of L-serine methyl ester hydrochloride (3.23 g, 20.8 mmol) in pentane (100 mL) were added *t*-butyl aldehyde (2.32 g, 27.0 mmol) and Et_3N (2.73 g, 27.0 mmol) at r.t. The mixture was heated at reflux for 15 h using a Dean-Stark apparatus. The resulting mixture was cooled to r.t., filtered, and the cake was washed with pentane (2 x 50 mL). The filtrate was concentrated to afford crude product as clear oil, which was used in the next step without further purification. To a solution of the crude product in dry CH_2Cl_2 (100 mL) at 0 °C were added acetoacetic acid (2.55 g, 25.0 mmol), EDC hydrochloride (4.8 g, 25.0 mmol) and DMAP (0.25 g, 2.1 mmol). The mixture was warmed to r.t. and stirred for 16 h, before being diluted with water and extracted with CH_2Cl_2. The organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 20→50% EtOAc in hexane) to afford **3.64** (4.3 g, 77% yield) as a mixture of keto–enol tautomers (1.7: 1 ratio). R_f 0.16 (3:1 hexane–EtOAc); $[\alpha]_D$ = + 46.3 (*c* 0.4, CH_2Cl_2); NMR data for keto form: ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.35 (s, 1 H, C*H*$(CH_3)_3$), 4.66 (d, 1 H, *J* = 6.0 Hz, C*H*CO_2CH_3), 4.56 (d, 1 H, *J* = 8.0 Hz, OC*H*$_2$), 4.08–4.03 (m, 1H, OC*H*$_2$), 3.82 (s, 3 H, CO_2CH_3), 3.73 (s, 2 H, CH_2C=ON), 2.34 (s, 3 H, CH_3C=O), 0.93 (s, 9 H, C$(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 202.6 (CH_3C=O), 170.0 (CO_2CH_3), 168.0 (NC=O), 96.7 (*C*H$(CH_3)_3$), 67.8 (OCH_2), 59.4

($CHCO_2CH_3$), 52.7 (CO_2CH_3), 51.9 ($CH_2C{=}ON$), 37.4 ($C(CH_3)_3$), 30.7 ($CH_3C{=}O$), 25.8 ($C(CH_3)_3$); NMR data for enol form: ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.13 (s, 1 H, $CH(CH_3)_3$), 4.50 (d, 1 H, J = 8.5 Hz, OCH_2), 4.08–4.03 (m, 1 H, OCH_2), 3.83 (s, 3 H, CO_2CH_3), 2.01 (s, 3 H, $CH_3C{=}O$), 0.97 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 176.5 (NC=O), 170.4 (CO_2CH_3), 89.6 ($CH(CH_3)_3$), 67.9 (OCH_2), 52.8 (CO_2CH_3), 37.7 ($C(CH_3)_3$), 25.9 ($C(CH_3)_3$), 22.0 ($CH_3C{=}O$); HRMS (ESI) calcd for (M+H) $C_{13}H_{22}NO_5$: 272.1492. Found: 272.1487.



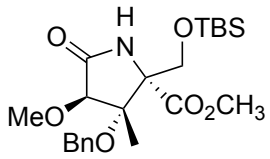
(3*S*,6*R*,7*S*,7a*S*)-methyl 7-(benzyloxy)-3-(*tert*-butyl)-6-methoxy-7-methyl-5-oxohexahydropyrrolo[1,2-c]oxazole-7a-carboxylate (3.69): To a solution of **3.47** (222 mg, 0.73 mmol) in DMF (1 mL) and benzyl bromide (3 mL) was added *n*-Bu_4NI (323 mg, 0.87 mmol). The mixture was cooled to –15 °C and NaH (44 mg, 60% in mineral oil, 1.09 mmol) was added in two portions. After 1 h, the mixture was slowly warmed to 0 °C and a satd aq solution of NH_4Cl (5 mL) was added dropwise. Thereafter, the mixture was extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 9→14% EtOAc in hexane) to afford **3.69** (245 mg, 85% yield) as a white solid. R_f 0.50

(3:1 hexane–EtOAc); $[\alpha]_D$ = + 36.6 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.37–7.29 (m, 5 H, ArH), 4.96 (s, 1 H, C*H*$(CH_3)_3$), 4.78 (d, 1 H, *J* = 9.5 Hz, OCH_RH$_S$C), 4.77 (s, 1 H, CH_3OC*H*), 4.59 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.52 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.05 (d, 1 H, *J* = 9.5 Hz, OCH$_R$$H_S$C), 3.71 (s, 3 H, CO_2CH_3), 3.70 (s, 3 H, OCH_3), 1.59 (s, 3 H, CCH_3), 0.91 (s, 9 H, C$(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.3 (NC=O), 171.0 (*C*O_2CH_3), 137.8 (Ar), 128.3 (Ar), 127.6 (Ar), 127.1 (Ar), 95.9 (*C*H$(CH_3)_3$), 85.9 (PhCH_2O*C*), 85.5 (CH_3O*C*H), 75.7 (*C*CO_2CH_3), 69.1 (O*C*H$_R$H$_S$C), 67.0 (Ph*C*H_2), 59.2 (OCH_3), 52.7 (CO_2*C*H_3), 36.5 (*C*$(CH_3)_3$), 24.9 (C(*C*$H_3)_3$), 13.9 (C*C*H_3); HRMS (ESI) calcd for (M+Na) $C_{21}H_{29}NNaO_6$: 414.1887. Found: 414.1885.



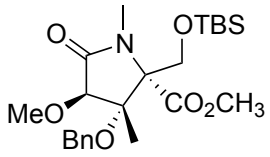
(2*S*,3*S*,4R)-methyl 3-(benzyloxy)-2-(hydroxymethyl)-4-methoxy-3-methyl-5-oxopyrrolidine -2-carboxylate (3.72): To a solution of **3.69** (620 mg, 1.58 mmol) in CF_3CH_2OH (4.0 mL) were added 1,3-propanedithiol (4.0 mL) and HCl (12 N, 60 μL). The mixture was stirred at 60 °C for 2 h, cooled, concentrated and the resulting crude product was purified by chromatography (gradient 75→100% EtOAc in hexane) to afford **3.72** (414 mg, 81% yield) as a white solid; R_f 0.18 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 61.7 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.36–7.23 (m, 5 H, ArH), 6.27 (br, 1 H, NH), 4.60 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.50 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.25 (dd, 1 H, *J* = 11.0, 5.5 Hz,

CC*H*$_2$OH), 4.04 (s, 1 H, CH$_3$OC*H*), 3.78 (dd, 1 H, *J* = 11.0, 5.5 Hz, CC*H*$_2$OH), 3.75 (s, 3 H, CO$_2$CH$_3$), 3.67 (s, 3 H, OCH$_3$), 2.25 (t, 1 H, *J* = 5.5 Hz, OH), 1.45 (s, 3 H, CCH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$, δ_C) 172.9 (NC=O), 171.0 (*C*O$_2$CH$_3$), 137.9 (Ar), 128.3 (Ar), 127.6 (Ar), 126.8 (Ar), 84.4 (PhCH$_2$O*C*), 82.3 (CH$_3$O*C*H), 72.1 (*C*CO$_2$CH$_3$), 65.8 (Ph*C*H$_2$), 64.6 (C*C*H$_2$OH), 59.3 (OCH$_3$), 52.8 (CO$_2$*C*H$_3$), 12.7 (C*C*H$_3$); HRMS (ESI) calcd for (M+Na) $C_{16}H_{21}NNaO_6$: 346.1261. Found: 346.1259.



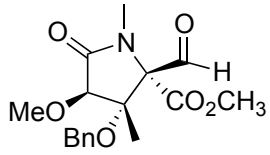
(2*S*,3*S*,4*R*)-methyl 3-(benzyloxy)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methoxy-3-methyl-5-oxopyrrolidine-2-carboxylate (3.73): To a solution of **3.72** (60.0 mg, 0.19 mmol) in CH_2Cl_2 (5.0 mL) were added imidazole (19.4 mg, 0.28 mmol) and TBSCl (42 mg, 0.28 mmol). The mixture was stirred at r.t. for 12 h. Thereafter, the organic phase was washed with brine, dried over Na_2SO_4, filtered, concentrated, and subjected to chromatography (gradient 20→25% EtOAc in hexane) to yield **3.73** (73.6 mg, 92% yield) as a colorless oil; R_f 0.26 (3:1 hexane–EtOAc); $[\alpha]_D$ = + 26.9 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) δ 7.31–7.21 (m, 5 H, ArH), 6.20 (br, 1 H, NH), 4.57 (d, 1 H, *J* = 11.4 Hz, PhC*H*$_2$), 4.47 (d, 1 H, *J* = 11.4 Hz, PhC*H*$_2$), 4.23 (d, 1 H, *J* = 8.8 Hz, CC*H*$_2$OTBS), 3.87 (s, 1 H, CH$_3$OC*H*), 3.69 (d, 1 H, *J* = 8.8 Hz, CC*H*$_2$OTBS), 3.76 (s, 3 H, CO$_2$CH$_3$), 3.63 (s, 3 H, OCH$_3$), 1.41 (s, 3 H, CCH$_3$), 0.85 (s, 9 H, SiC(CH$_3$)$_3$), 0.05 (s, 3 H, SiCH$_3$),

0.04(s, 3 H, $SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.5 (NC=O), 170.5 (CO_2CH_3), 138.0 (Ar), 128.3 (Ar), 127.5 (Ar), 126.8 (Ar), 83.8 ($PhCH_2OC$), 82.2 (CH_3OCH), 73.0 (CCO_2CH_3), 65.49 ($PhCH_2$), 65.48 (CCH_2OTBS), 59.2 (OCH_3), 52.4 (CO_2CH_3), 25.6 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), 12.7 (CCH_3), −5.5 ($SiCH_3$) , −5.7($SiCH_3$); HRMS (ESI) calcd for (M+Na) $C_{22}H_{35}NNaO_6Si$: 460.2126. Found: 460.2125.



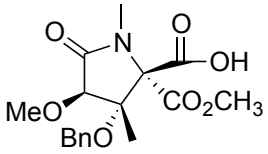
(2*S*,3*S*,4*R*)-methyl 3-(benzyloxy)-2-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylate (3.74): To a solution of **3.73** (50 mg, 0.11 mmol) in DMF (3 mL) was added CH_3I (162 mg, 1.14 mmol). Then the mixture was cooled to 0 °C and NaH (11 mg, 60% in mineral oil, 0.28 mmol) was added. After 1 h, a satd aq solution of NH_4Cl (5 mL) was added dropwise and the mixture was extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 20→25% EtOAc in hexane) to afford **3.74** (46 mg, 90% yield) as a colorless oil. R_f 0.33 (3:1 hexane–EtOAc); $[\alpha]_D$ = + 31.3 (*c* 0.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.35–7.25 (m, 5 H, ArH), 4.64 (d, 1 H, *J* = 11.5 Hz, $PhCH_2$), 4.52 (d, 1 H, *J* = 11.5 Hz, $PhCH_2$), 4.23 (d, 1 H, *J* = 11.0 Hz, CCH_2OTBS), 4.01 (s, 1 H, CH_3OCH), 3.98 (d, 1 H, *J* = 11.0 Hz, CCH_2OTBS), 3.69 (s, 3 H, OCH_3), 3.68 (s, 3 H, CO_2CH_3), 2.94 (s, 3 H, NCH_3), 1.41 (s, 3 H, CCH_3), 0.89 (s, 9 H,

$SiC(CH_3)_3$), 0.105 (s, 3 H, $SiCH_3$), 0.098(s, 3 H, $SiCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.5 (NC=O), 170.1 (CO_2CH_3), 138.3 (Ar), 128.3 (Ar), 127.4 (Ar), 126.8 (Ar), 83.2 ($PhCH_2OC$), 82.4 (CH_3OCH), 75.2 (CCO_2CH_3), 65.8 ($PhCH_2$), 63.1 (CCH_2OTBS), 59.3 (OCH_3), 52.2 (CO_2CH_3), 28.4 (NCH_3), 25.6 ($SiC(CH_3)_3$), 18.0 ($SiC(CH_3)_3$), 13.3 (CCH_3), −5.7 ($SiCH_3$) , −5.9($SiCH_3$); HRMS (ESI) calcd for (M+Na) $C_{23}H_{37}NNaO_6Si$: 474.2282. Found: 474.2283.

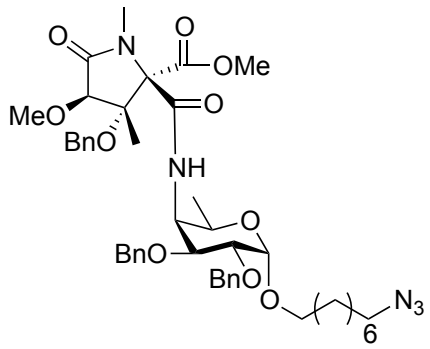


(2*R*,3*S*,4*R*)-methyl 3-(benzyloxy)-2-formyl-4-methoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylate (3.75): To a mixture of **3.46** (90 mg, 0.27 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (8 mL) were added NMO (47 mg, 0.40 mmol) and TPAP (4.9 mg, 0.014 mmol) at r.t. The mixture was stirred for 6 h and then concentrated. The crude product was purified by chromatography (gradient 20→25% EtOAc in hexane) to afford **3.75** (76 mg, 84% yield) as a colorless oil. R_f 0.65 (3:1 hexane–EtOAc); $[\alpha]_D$ = + 89.2 (*c* 0.4, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 10.08 (s, 1 H, CHO), 7.38–7.27 (m, 5 H, ArH), 4.64 (d, 1 H, *J* = 14.5 Hz, $PhCH_2$), 4.56 (d, 1 H, *J* = 14.5 Hz, $PhCH_2$), 4.18 (s, 1 H, CH_3OCH), 3.79 (s, 3 H, CO_2CH_3), 3.68 (s, 3 H, OCH_3), 2.84 (s, 3 H, NCH_3), 1.34 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 194.3 (CHO), 172.5 (NC=O), 167.8 (CO_2CH_3), 137.5 (Ar), 128.5 (Ar), 127.8 (Ar), 127.0 (Ar), 84.4 ($PhCH_2OC$), 82.3

(CH_3OCH), 80.2 (CCO_2CH_3), 66.9 ($PhCH_2$), 59.4 (OCH_3), 53.2 (CO_2CH_3), 28.9 (NCH_3), 14.3 (CCH_3); HRMS (ESI) calcd for (M+Na) $C_{17}H_{21}NNaO_6$: 358.1261. Found: 358.1260.

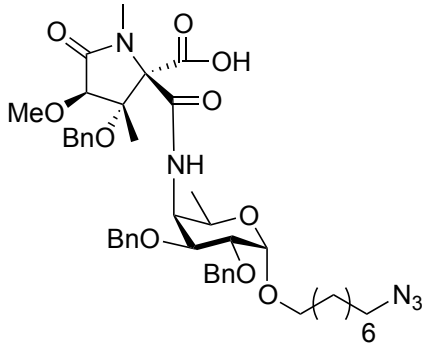


(2*R*,3*S*,4*R*)-3-(benzyloxy)-4-methoxy-2-(methoxycarbonyl)-1,3-dimethyl-5-oxopyrrolidine-2-carboxylic acid (3.76): A solution of **3.75** (155 mg, 0.44 mmol) in *t*-BuOH (5 mL) and 2-methyl-2-butene (3 mL) was treated with a freshly prepared solution of $NaClO_2$ (396 mg, 4.4 mmol, 10 equiv) in 20% aqueous NaH_2PO_4 (3 mL) at r.t. The mixture was stirred for 2 h and then water was added and the aqueous phase was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford **3.76** (149 mg, 92% yield) as a white solid. R_f 0.55 (3:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = + 46.6 (*c* 0.1, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.41–7.27 (m, 5 H, ArH), 4.74 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.58 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.11 (s, 1 H, CH_3OCH), 3.88 (s, 3 H, CO_2CH_3), 3.74 (s, 3 H, OCH_3), 2.90 (s, 3 H, NCH_3), 1.52 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.1 (NC=O), 171.2 (CO_2CH_3), 164.6 (CO_2H), 137.3 (Ar), 128.5 (Ar), 127.9 (Ar), 127.0 (Ar), 84.3 ($PhCH_2OC$), 82.5 (CH_3OCH), 78.0 (CCO_2CH_3), 66.8 ($PhCH_2$), 59.5 (OCH_3), 54.5 (CO_2CH_3), 28.8 (NCH_3), 15.2 (CCH_3); HRMS (ESI) calcd for (M+Na) $C_{17}H_{21}NNaO_7$: 374.1210. Found: 374.1212.



8-Azidooctyl 4-[(2′*R*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-2′-(methoxycarbonyl)-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (**3.77**): To a solution of **3.45** (40 mg, 0.08 mmol) in DMF (5 mL) was added **3.76** (35 mg, 0.10 mmol), TBTU (35 mg, 0.11 mmol) and DIEA (16 mg, 0.12 mmol). The mixture was stirred at r.t. over night and then water was added and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 30→50% EtOAc in hexane) to afford **3.77** (50 mg, 76% yield) as a colorless oil. R_f 0.52 (1:1 hexane–EtOAc); $[\alpha]_D$ = + 128.3 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20 (d, 1 H, *J* = 10.0 Hz, NH), 7.41–7.25 (m, 15 H, ArH), 4.88 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.85 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.83 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.69 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.69 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.61 (dd, 1 H, $J_{NH,4}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-4), 4.59 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.53 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.21 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.03 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, 1H, H-3), 3.95 (s, 1 H, CH_3OCH), 3.72 (s, 3 H, CO_2CH_3), 3.67 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.65 (s, 3 H, OCH_3), 3.61 (dd, 1 H, $J_{2,3}$ =10.0 Hz,

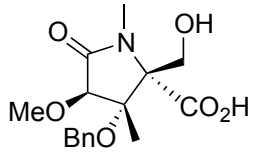
$J_{1,2}$ = 4.0 Hz, H-2), 3.50 (dt, 1 H, J = 10.0, 6.5 Hz, octyl OCH_2), 3.29 (t, 2 H, J = 7.0 Hz, CH_2N_3), 2.69 (s, 3 H, NCH_3), 1.69−1.62 (m, 4 H, CH_2 x 2), 1.51 (s, 3 H, CCH_3), 1.43−1.34 (m, 8 H, CH_2 x 4), 1.20 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.3 (CH_3NC=O), 169.5 (CO_2CH_3), 164.7 (HNC=O), 138.7 (Ar), 138.6 (Ar), 137.8 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 127.8 (Ar), 127.6 (Ar), 127.4 (Ar), 126.9 (Ar), 97.5 (C-1), 83.7 ($PhCH_2OCCH_3$), 82.3 (CH_3OCH), 79.3 (CCO_2CH_3), 77.5 (C-3), 75.5 (C-2), 73.1 ($PhCH_2$, C-2), 71.8 ($PhCH_2$, C-3), 68.4 (octyl OCH_2), 66.2 ($PhCH_2O$ lactam), 64.0 (C-5), 59.3 (OCH_3), 53.1 (CO_2CH_3), 51.9 (C-4), 51.5 (CH_2N_3), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 29.0 (NCH_3), 28.8 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 17.3 (C-6), 14.8 (CCH_3); HRMS (ESI) calcd for (M+Na) $C_{45}H_{59}N_5NaO_{10}$: 852.4154. Found: 852.4138.



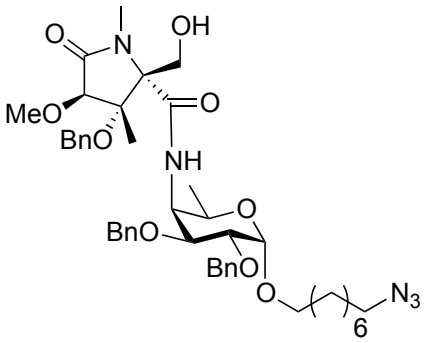
8-Azidooctyl 4-[(2′*R*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-2′-carboxyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (**3.78**): To a solution of **3.77** (45 mg, 0.054 mmol) in THF (5 mL) and water (5 mL) was added LiOH monohydrate (34 mg, 0.81 mmol) at r.t.. The mixture was

stirred at r.t. for 16 h. Then 1 M HCl was added to adjust the pH to 1. The mixture was diluted with water (5 mL) and extracted with EtOAc. The organic phases was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford **3.78** (42 mg, 96% yield) as a colorless oil. R_f 0.65 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = + 106.7 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.20 (m, 15 H, ArH), 6.61 (d, 1 H, *J* = 10.0 Hz, NH), 4.87 (d, 1 H, *J* = 10.5 Hz, PhC*H*$_2$, C-3), 4.84 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.79 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.66 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.65 (d, 1 H, *J* = 10.5 Hz, PhC*H*$_2$, C-3), 4.64 (dd, 1 H, $J_{NH,4}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-4), 4.48 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.29 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.26 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.0 Hz, H-5), 4.21 (s, 1 H, CH_3OCH), 4.05 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 3.67 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.61 (s, 3 H, OCH_3), 3.50 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.43 (dd, 1 H, $J_{2,3}$ =10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.29 (t, 2 H, *J* = 7.0 Hz, CH_2N_3), 2.91 (s, 3 H, NCH_3), 1.70–1.61 (m, 4 H, CH_2 x 2), 1.45–1.34 (m, 8 H, CH_2 x 4), 1.32 (s, 3 H, CCH_3), 1.19 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.3 (CH_3NC=O), 170.9 (HNC=O), 167.0 (*C*O_2H), 138.0 (Ar), 137.7 (Ar), 137.6 (Ar), 128.4 (Ar), 128.32 (Ar), 128.31 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5 (Ar), 126.9 (Ar), 97.2 (C-1), 84.5 (CH_3OCH), 82.6 ($PhCH_2OCCH_3$), 77.4 (C-3), 77.2 (*C*CO_2CH_3), 75.8 (C-2), 73.3 (Ph*C*H$_2$, C-2), 72.9 (Ph*C*H$_2$, C-3), 68.8 (octyl O*C*H$_2$), 66.4 (Ph*C*H_2O lactam), 63.6 (C-5), 59.6 (OCH_3), 53.3 (C-4), 51.5 (*C*H_2N_3), 31.0 (NCH_3), 29.4

(CH$_2$), 29.3 (CH$_2$), 29.1 (CH$_2$), 28.8 (CH$_2$), 26.7 (CH$_2$), 26.1 (CH$_2$), 17.2 (C-6), 15.7 (CCH$_3$); HRMS (ESI) calcd for (M-H) $C_{44}H_{56}N_5O_{10}$: 814.4033. Found: 814.4029.

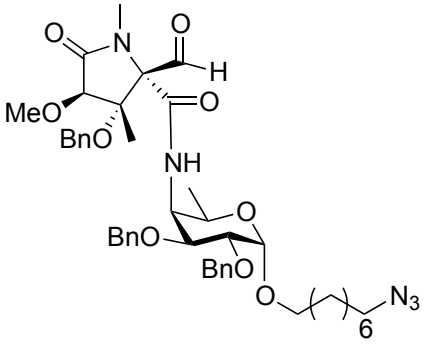


(2*S*,3*S*,4*R*)-3-(benzyloxy)-2-(hydroxymethyl)-4-methoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylic acid (3.80): To a solution of **3.46** (200 mg, 0.59 mmol) in THF (8 mL) and water (8 mL) was added LiOH monohydrate (124 mg, 2.95 mmol) at r.t. The mixture was stirred at r.t. for 16 h. Then 1 M HCl was added to adjust the pH to 1. The mixture was diluted by water and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford **3.80** (175 mg, 0.54 mmol, 92% yield) as a white solid. R_f 0.63 (3:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = + 58.2 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.27–7.21 (m, 5 H, ArH), 4.63 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.49 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.10 (s, 1 H, CH_3OCH), 4.09 (d, 1 H, J = 12.5 Hz, CH_2OH), 3.99 (d, 1 H, J = 12.5 Hz, CH_2OH), 3.65 (s, 3 H, OCH_3), 2.89 (s, 3 H, NCH_3), 1.44 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.4 (CO_2H), 172.6 (NC=O), 138.0 (Ar), 128.3 (Ar), 127.5 (Ar), 126.7 (Ar), 83.4 ($PhCH_2OC$), 81.9 (CH_3OCH), 74.6 (CCO_2CH_3), 66.0 (PhCH_2), 62.2 (CH_2OH), 59.4 (OCH_3), 27.8 (NCH_3), 12.8 (CCH_3); HRMS (ESI) (M+Na) calcd for $C_{16}H_{21}NNaO_6$: 346.1261. Found: 346.1256.



8-Azidooctyl 4-[(2′*S*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-2′-hydroxymethyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (**3.81**): To a solution of **3.80** (60 mg, 0.12 mmol) in DMF (5 mL) was added compound **3.45** (39 mg, 0.12 mmol), TBTU (51 mg, 0.16 mmol) and DIEA (23 mg, 0.18 mmol). The mixture was stirred at r.t. over night. Then water was added and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (gradient 30→70% EtOAc in hexane) to afford **3.81** (64 mg, 67% yield) as a colorless oil. R_f 0.39 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 121.1 (*c* 0.5, CH_2Cl_2,); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.40–7.23 (m, 15 H, ArH), 6.01 (d, 1 H, *J* = 10.0 Hz, NH), 4.78 (d, 1 H, *J* = 10.5 Hz, PhC*H*$_2$, C-3), 4.72 (d, 1 H, *J* = 12.5 Hz, PhC*H*$_2$, C-2), 4.69 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.66 (d, 1 H, *J* = 12.5 Hz, PhC*H*$_2$, C-2), 4.62 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$O lactam), 4.59 (d, 1 H, *J* = 10.5 Hz, PhC*H*$_2$, C-3), 4.57 (ddd, 1 H, $J_{NH,4}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.50 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$O lactam), 4.02 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.01 (dd, 1 H, *J* = 12.5, 3.0 Hz, C*H*$_2$OH), 3.94 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.85 (s, 1 H, CH_3OCH), 3.82 (dd, 1 H, *J* = 12.5, 8.0 Hz, C*H*$_2$OH), 3.66 (s, 3 H, OCH_3),

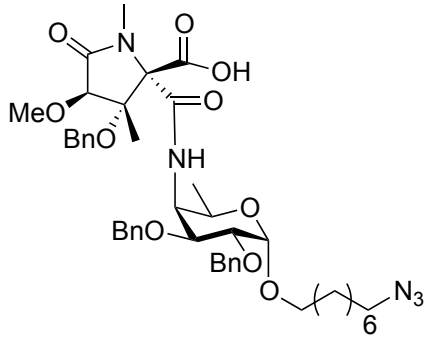
3.58 (dt, 1 H, J = 13.5, 6.5 Hz, octyl OCH_2), 3.44 (dt, 1 H, J = 13.5, 6.5 Hz, octyl OCH_2), 3.29 (t, 2 H, J = 7.0 Hz, CH_2N_3), 3.28 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 2.71 (s, 3 H, NCH_3), 1.64–1.60 (m, 4 H, CH_2 x 2), 1.52 (s, 3 H, CCH_3), 1.42–1.32 (m, 8 H, CH_2 x 4), 0.73 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.5 (CH_3NC=O), 169.7 (HNC=O), 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 128.4 (Ar), 128.327 (Ar), 128.321 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 97.0 (C-1), 82.9 (CH_3OCH), 81.3 ($PhCH_2OCCH_3$), 77.2 (C-3), 75.7 (CCH_2OH), 74.9 (C-2), 72.6 ($PhCH_2$, C-2), 71.9 ($PhCH_2$, C-3), 68.4 (octyl OCH_2), 66.2 ($PhCH_2O$ lactam), 63.9 (C-5), 62.9 (CH_2OH), 59.4 (OCH_3), 51.5 (CH_2N_3), 51.0 (C-4), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 28.3 (NCH_3), 26.7 (CH_2), 26.1 (CH_2), 16.4 (C-6), 13.2 (CCH_3); HRMS (ESI) calcd for (M+H) $C_{44}H_{60}N_5O_9$: 802.4386. Found: 802.4376.



8-Azidooctyl 4-[(2′*R*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-2′-formyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (**3.82**): To a mixture of **3.81** (60 mg, 0.075 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (8 mL) were added NMO (18 mg, 0.15 mmol) and TPAP (2.6 mg,

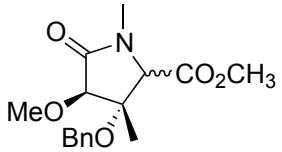
0.0075 mmol) at r.t. The mixture was stirred for 6 h and then the mixture was concentrated and the crude product was purified by chromatography (gradient 30→50% EtOAc in hexane) to afford **3.82** (51 mg, 0.064 mmol, 85% yield) as a colorless oil. R_f 0.76 (1:1 hexane–EtOAc); $[\alpha]_D$ = + 124.3 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 10.09 (s, 1 H, CHO), 7.32–7.22 (m, 15 H, ArH), 6.43 (d, 1 H, *J* = 10.0 Hz, NH), 4.75 (d, 1 H, *J* = 10.5 Hz, PhCH_2, C-3), 4.67 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.65 (d, 1 H, *J* = 11.5 Hz, PhCH_2O lactam), 4.62 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-2), 4.60 (ddd, 1 H, $J_{NH,4}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.54 (d, 1 H, *J* = 10.5 Hz, PhCH_2, C-3), 4.53 (d, 1 H, *J* = 12.0 Hz, PhCH_2, C-2), 4.47 (d, 1 H, *J* = 11.5 Hz, PhCH_2O lactam), 4.06 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 3.94 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.91 (s, 1 H, CH_3OCH), 3.60 (dt, 1 H, *J* = 10.7, 7.0 Hz, octyl OCH_2), 3.56 (s, 3 H, OCH_3), 3.44 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OCH_2), 3.29 (t, 2 H, *J* = 7.0 Hz, CH_2N_3), 3.25 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 2.75 (s, 3 H, NCH_3), 1.66–1.60 (m, 4 H, CH_2 x 2), 1.46 (s, 3 H, CCH_3), 1.42–1.32 (m, 8 H, CH_2 x 4), 0.87 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 197.8 (CHO), 172.5 (CH_3NC=O), 165.7 (HNC=O), 138.3 (Ar), 138.2 (Ar), 137.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 97.0 (C-1), 84.2 ($PhCH_2OCCH_3$), 81.5 (CH_3OCH), 80.6 (*C*CHO), 76.5 (C-3), 75.2 (C-2), 72.7 (PhCH_2, C-2), 71.8 (PhCH_2, C-3), 68.5 (octyl OCH_2), 66.3 (PhCH_2O lactam), 63.8 (C-5), 59.1 (OCH_3), 51.5 (CH_2N_3), 51.4 (C-4), 29.4 (NCH_3), 29.3

(CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 16.5 (C-6), 14.4 (CCH_3); HRMS (ESI) calcd for (M+H) $C_{44}H_{58}N_5O_9$: 800.4229. Found: 800.4214.



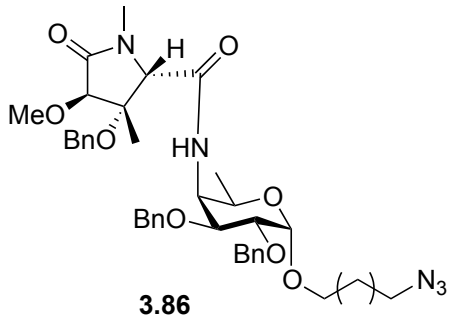
8-Azidooctyl 4-[(2′*S*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-2′-carboxyl-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (3.83): To a solution of **3.82** (45 mg, 0.056 mmol) in *t*-BuOH (5 mL) and 2-methyl-2-butene (3 mL) was treated with a freshly prepared solution of $NaClO_2$ (99 mg, 1.1 mmol, 20 equiv) in 20% aqueous NaH_2PO_4 (3 mL) at r.t. The mixture was stirred for 2 h and then water was added and the aqueous phase was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford **3.83** (43 mg, 95% yield) as a colourless oil. R_f 0.38 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = + 80.8 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD, δ_H) δ 7.36–7.21 (m, 15 H, ArH), 4.78 (d, 1 H, *J* = 10.5 Hz, PhCH_2, C-3), 4.72 (d, 1 H, *J* = 13.0 Hz, PhCH_2O lactam), 4.68 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.54 (d, 1 H, $J_{3,4}$ = 4.0 Hz, H-4), 4.49 (d, 1 H, *J* = 11.5 Hz, PhCH_2O lactam), 4.42 (d, 1 H, *J* = 10.5 Hz, PhCH_2, C-3), 4.29 (d, 1 H, *J* = 11.5 Hz, PhCH_2, C-2), 4.21 (d, 1 H, *J* = 11.5 Hz, PhCH_2, C-2), 4.13 (q, 1 H, $J_{5,6}$ = 6.5 Hz, H-5), 4.11 (s, 1 H,

CH_3OCH), 3.91 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.63 (dt, 1 H, J = 13.0, 6.5 Hz, octyl OCH_2), 3.44 (s, 3 H, OCH_3), 3.40−3.36 (m, 2 H, octyl OCH_2 and H-2), 3.24 (t, 2 H, J = 7.0 Hz, CH_2N_3), 2.89 (s, 3 H, NCH_3), 1.63−1.52 (m, 4 H, CH_2 x 2), 1.41−1.28 (m, 8 H, CH_2 x 4), 1.30 (s, 3 H, CCH_3), 1.08 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 172.6 (CH_3NC=O), 168.1 (HNC=O), 141.6 (Ar), 141.0 (Ar), 140.8 (Ar), 130.4 (Ar), 130.2 (Ar), 130.1 (Ar), 130.0 (Ar), 129.5 (Ar), 129.4 (Ar), 129.0 (Ar), 128.6 (Ar), 99.4 (C-1), 86.1 ($PhCH_2OCCH_3$), 85.5 (CH_3OCH), 79.0 (C-3), 78.7 (C-2), 74.8 ($PhCH_2$, C-2), 73.6 ($PhCH_2$, C-3), 70.2 (octyl OCH_2), 68.0 ($PhCH_2O$ lactam), 66.5 (C-5), 61.0 (OCH_3), 54.8 (C-4), 53.3 (CH_2N_3), 31.3 (CH_2), 31.2 (NCH_3), 31.1 (CH_2), 30.8 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 18.0 (CCH_3), 16.6 (C-6); HRMS (ESI) calcd for (M-H) $C_{44}H_{56}N_5O_{10}$: 814.4033. Found: 814.4027.

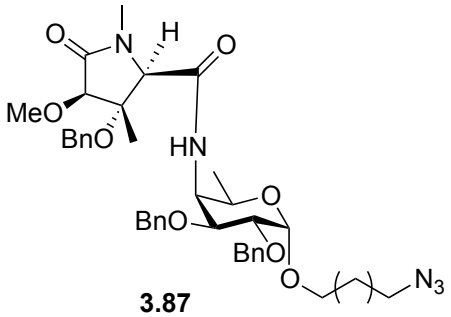


(3*S*,4*R*)-methyl 3-(benzyloxy)-4-methoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylate (**3.84**): A solution of **3.76** (250 mg , 0.71 mmol) in toluene (25 mL) was heated at reflux for 24 h. Then the mixture was concentrated and the crude product was purified by chromatography (gradient 10→25% EtOAc in hexane) to afford **3.84** (170 mg, 0.55 mmol, 78% yield) as a 1:1 mixture of inseparable diastereomers. These are defined below as *cis* and *trans*, to describe the relationship between the carboxymethyl and benzyloxy

groups. R_f 0.35 (2:3 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.29 (m, 10 H, ArH (*cis* and *trans*)), 4.69–4.60 (m, 4 H, $PhCH_2O$(*cis* and *trans*)), 4.25 (s, 1 H, CH_3OCH(*cis*)), 4.22 (s, 1 H, $CHCO_2CH_3$(*trans*)), 3.99 (s, 1 H, $CHCO_2CH_3$(*cis*)), 3.86 (s, 1 H, CH_3OCH(*trans*)), 3.85 (s, 3 H, CO_2CH_3(*trans*)), 3.72 (s, 3 H, OCH_3(*cis*)), 3.71 (s, 3 H, CO_2CH_3(*cis*)), 3.68 (s, 3 H, OCH_3(*trans*)), 2.88 (s, 3 H, NCH_3(*trans*)), 2.85 (s, 3 H, NCH_3(*cis*)), 1.55 (s, 3 H, CCH_3(*cis*)), 1.38 (s, 3 H, CCH_3(*trans*)); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.8 (NC=O(*cis*)), 171.4 (NC=O(*trans*)), 169.6 (CO_2CH_3(*cis*)), 168.9 (CO_2CH_3(*trans*)), 138.1 (Ar), 138.0 (Ar), 128.5 (Ar), 128.3 (Ar), 127.7 (Ar), 127.5 (Ar), 127.1 (Ar), 126.8(Ar), 83.5 (CH_3OCH(*trans*)), 82.5 (CH_3OCH(*cis*)), 81.6 ($PhCH_2O$), 81.4 ($PhCH_2O$), 70.1 ($CHCO_2CH_3$(*trans*)), 68.8 ($CHCO_2CH_3$(*cis*)), 66.3 ($PhCH_2$), 65.7 ($PhCH_2$), 59.4 (OCH_3), 59.3 (OCH_3), 52.5 (CO_2CH_3), 52.4 (CO_2CH_3), 29.2 (NCH_3(*cis*)), 28.9 (NCH_3(*trans*)), 18.0 (CCH_3(*cis*)), 14.8 (CCH_3(*trans*)); HRMS (ESI) calcd for (M+Na) $C_{16}H_{21}NNaO_5$: 330.1312. Found: 330.1312.



3.86



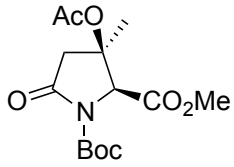
3.87

8-Azidooctyl 4-[(2′*S*,3′*S*,4′*R*)-3′-(benzyloxy)-4′-methoxy-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside

(**3.86**): To a solution of **3.84** (47 mg, 0.16 mmol) in THF (10 mL) and water (10 mL) was added LiOH monohydrate (67 mg, 1.6 mmol) at r.t. The mixture was stirred at r.t. for 16 h. Then 1 M HCl was added to adjust the pH to 1. The mixture was diluted with water and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford crude acid **3.85**. Then, to a solution of **3.45** (40 mg, 0.080 mmol) in DMF (5 mL) was added the above crude acid, TBTU (38 mg, 0.12 mmol) and DIEA (16 mg, 0.12 mmol). The mixture was stirred at r.t. over night. Water was then added and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (gradient 30→70% EtOAc in hexane) to afford **3.86** (39.8 mg, 64% yield) and **3.87** (9.8 mg, 16% yield). Data for **3.86**: R_f 0.45 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 124.2 (*c* 0.4, CH_2Cl_2,); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.36–7.21 (m, 15 H, ArH), 5.85 (d, 1 H, *J* = 10.5 Hz, NH), 4.75 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.69 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.68 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 4.67 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.60 (ddd, 1 H, $J_{4,NH}$ = 10.5 Hz, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.56 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$, C-2), 4.52 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$, C-3), 4.50 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$O lactam), 4.05 (s, 1 H, CH_3OCH), 4.04 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 3.94 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.90 (s, 1 H, C*H*C=ONH), 3.63 (s, 3 H, OCH_3), 3.58 (dt, 1 H, *J* = 10.0, 7.0 Hz, octyl OC*H*$_2$), 3.42 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.27 (t, 2 H, *J* = 7.0 Hz, CH_2N_3), 3.26 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.5 Hz, H-2), 2.70 (s,

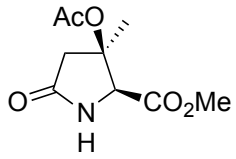
3 H, NCH_3), 1.63–1.58 (m, 4 H, CH_2 x 2), 1.52 (s, 3 H, CCH_3), 1.39–1.28 (m, 8 H, CH_2 x 4), 0.90 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (150 MHz, $CDCl_3$, δ_C) 171.8 (CH_3NC=O), 167.9 (HNC=O), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 128.3 (Ar), 128.2 (Ar), 128.18 (Ar), 128.0 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 97.1 (C-1), 82.7 (CH_3OCH), 80.9 ($PhCH_2OCCH_3$), 76.2 (C-3), 75.5 (C-2), 72.8 (PhCH_2, C-2), 72.4 (*C*HC=ONH), 71.5 (PhCH_2, C-3), 68.4 (octyl OCH_2), 66.5 (PhCH_2O lactam), 64.0 (C-5), 59.3 (OCH_3), 51.5 (CH_2N_3), 51.0 (C-4), 29.4 (NCH_3), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 18.3 (CCH_3), 16.6 (C-6); HRMS (ESI) calcd for (M+H) $C_{43}H_{58}N_5O_8$: 772.4280. Found: 772.4279. Data for **3.87**: R_f 0.49 (1:1 hexane–EtOAc); $[\alpha]_D$ = + 118.3 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) δ 7.44–7.26 (m, 15 H, ArH), 6.62 (d, 1 H, *J* = 10.0 Hz, NH), 4.92 (d, 1 H, *J* = 11.0 Hz, PhCH_2, C-3), 4.85 (d, 1 H, *J* = 12.5 Hz, PhCH_2, C-2), 4.79 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.70 (d, 1 H, *J* = 12.5 Hz, PhCH_2, C-2), 4.67 (d, 1 H, *J* = 11.0 Hz, PhCH_2, C-3), 4.57 (d, 1 H, *J* = 11.5 Hz, PhCH_2O lactam), 4.54 (ddd, 1 H, $J_{4,NH}$ = 10.5 Hz, $J_{3,4}$ = 4.0 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 4.53 (d, 1 H, *J* = 11.5 Hz, PhCH_2O lactam), 4.15 (qd, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 1.5 Hz, H-5), 4.03 (s, 1 H, C*H*C=ONH), 4.01 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.0 Hz, H-3), 3.66 (dt, 1 H, *J* = 10.0, 7.0 Hz, octyl OCH_2), 3.57 (s, 1 H, CH_3OCH), 3.50 (dt, 1 H, *J* = 10.0, 6.5 Hz, octyl OCH_2), 3.47 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.42 (s, 3 H, OCH_3), 3.29 (t, 2 H, *J* = 7.0 Hz, CH_2N_3), 2.87 (s, 3 H, NCH_3), 1.69–1.61 (m, 4H, CH_2 x 2), 1.38 (s, 3 H, CCH_3), 1.42–1.33 (m, 8 H, CH_2 x 4), 1.12 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.1 (CH_3NC=O),

168.8 (HNC=O), 138.7 (Ar), 138.5 (Ar), 137.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5 (Ar), 127.4 (Ar), 127.1 (Ar), 97.5 (C-1), 83.7 (CH_3OCH), 80.4 ($PhCH_2OCCH_3$), 77.6 (C-3), 75.4 (C-2), 73.0 ($PhCH_2$, C-2), 72.8 (CHC=ONH), 71.9 ($PhCH_2$, C-3), 68.5 (octyl OCH_2), 65.4 ($PhCH_2O$ lactam), 64.0 (C-5), 59.3 (OCH_3), 51.5 (CH_2N_3), 51.2 (C-4), 29.4 (CH_2), 29.3 (CH_2), 29.1 (NCH_3), 29.1 (CH_2), 28.8 (CH_2), 26.7 (CH_2), 26.1 (CH_2), 16.9 (C-6), 14.1 (CCH_3),; HRMS (ESI) calcd for (M+H) $C_{43}H_{58}N_5O_8$: 772.4280. Found: 772.4270.

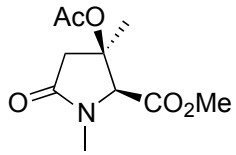


(2*S*,3*R*)-1-*tert*-butyl 2-methyl 3-acetoxy-3-methyl-5-oxopyrrolidine-1,2-dicarboxylate (3.90): To a solution of **3.51** (0.46 g, 1.7 mmol) in pyridine (15.0 mL) and Ac_2O (15.0 mL) were added DMAP (21 mg, 0.17 mmol). The mixture was stirred at r.t. for 12 h. After completion of the reaction, the mixture was concentrated and diluted with CH_2Cl_2 (30 mL). The organic phase was washed with 1N HCl, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 20→40% EtOAc in hexane) to yield **3.90** (472 mg, 89% yield) as a white solid; R_f 0.65 (4:3 EtOAc–hexane); $[\alpha]_D = -18.9$ (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.56 (s, 1 H, NC*H*), 3.83 (s, 3 H, OCH_3), 3.11 (d, 1 H, *J* = 17.5 Hz, $COCH_2$), 2.98 (d, 1 H, *J* = 17.5 Hz, $COCH_2$), 2.02 (s, 3 H, CH_3C=O), 1.78 (s, 3 H, CCH_3), 1.52 (s, 9 H, $C(CH_3)_3$); ^{13}C

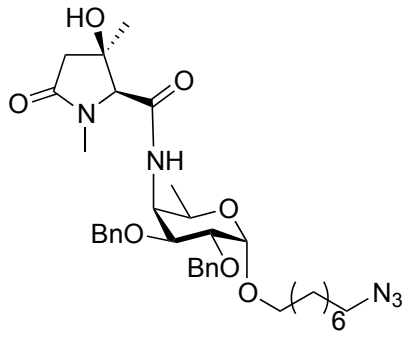
NMR (125 MHz, $CDCl_3$, δ_C) 169.8 (NC=O), 169.0 (CH_3C=O), 168.8 (CO_2CH_3), 148.9 ($CO_2C(CH_3)_3$), 84.4 ($OC(CH_3)_3$), 77.1 (CH_3COAc), 68.6 (NCH), 52.5 (OCH_3), 44.3 (CH_2), 27.9 ($C(CH_3)_3$), 24.8 (CCH_3), 21.4 (CH_3C=O); HRMS (ESI) calcd for (M+Na) $C_{14}H_{21}NNaO_7$: 338.1210. Found: 338.1208.



(2*S*,3*R*)-methyl 3-acetoxy-3-methyl-5-oxopyrrolidine-2-carboxylate (3.91): To a solution of **3.90** (0.36 g, 1.7 mmol) in CH_2Cl_2 (30.0 mL) was added TFA (5.0 mL) at 0 °C. The mixture was stirred at r.t. for 2 h. Then, the mixture was concentrated, diluted with CH_2Cl_2 (40 mL), washed with a satd aq solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 100→300% EtOAc in hexane) to yield **3.91** (227 mg, 93% yield) as a white solid; R_f 0.15 (4:3 EtOAc–Hexane); $[\alpha]_D$ = + 5.7 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.52 (br s, 1 H, NH), 4.21 (s, 1 H, NC*H*), 3.82 (s, 3 H, OCH_3), 3.09 (d, 1 H, *J* = 18.0 Hz, $COCH_2$), 2.65 (d, 1 H, *J* = 18.0 Hz, $COCH_2$), 2.00 (s, 3 H, CH_3C=O), 1.85 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 174.7 (NC=O), 169.5 (CH_3C=O), 169.0 (CO_2CH_3), 82.9 (CH_3COAc), 66.1 (NCH), 52.5 (OCH_3), 42.2 (CH_2), 23.6 (CCH_3), 21.7 (CH_3C=O); HRMS (ESI) calcd for (M+Na) $C_9H_{13}NNaO_5$: 238.0686. Found: 238.0686.



(2*S*,3*R*)-methyl 3-acetoxy-1,3-dimethyl-5-oxopyrrolidine-2-carboxylate (3.93) To a solution of **3.91** (0.2 g, 0.93 mmol) in acetone (25 mL) was added paraformaldehyde (0.1 g, 4.4 mmol), potassium carbonate (20 mg) and water (0.2 mL) at r.t. The mixture was placed in a sonication bath. After 3 h, the solution was filtered, concentrated and the residue was purified by chromatography on (gradient 50→25% hexane in EtOAc) to afford hemiaminal **3.92** (190 mg). To this hemiaminal in $CHCl_3$ (25 mL) was added TFA (2.0 mL) and Et_3SiH (2.0 mL). This mixture was stirred at r.t. over night. Thereafter, the organic phase was washed with a satd aq solution of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 50→25% hexane in EtOAc) to yield **3.93** (172 mg, 81% yield over two steps) as a white solid; R_f 0.25 (4:3 EtOAc–hexane); $[\alpha]_D$ = –5.1 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.09 (s, 1 H, NC*H*), 3.82 (s, 3 H, OCH_3), 2.97 (d, 1 H, *J* = 17.0 Hz, COCH_2), 2.85 (s, 3 H, NCH_3), 2.79 (d, 1 H, *J* = 17.0 Hz, COCH_2), 2.00 (s, 3 H, CH_3C=O), 1.76 (s, 3 H, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.2 (NC=O), 169.2 (CH_3C=O), 168.8 (CO_2CH_3), 79.1 (CH_3COAc), 72.3 (N*C*H), 52.5 (OCH_3), 42.8 (CH_2), 28.8 (NCH_3), 25.1 (CCH_3), 21.5 (CH_3C=O); HRMS (ESI) calcd for (M+Na) $C_{10}H_{15}NNaO_5$: 252.0842. Found: 252.0840.



8-Azidooctyl 4-[(2′*S*,3′*R*)-3′-(hydroxy)-1′,3′-dimethyl-5′-oxopyrrolidine-2′-carboxamido]-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-galactopyranoside (**3.94**) To a solution of **3.45** (6.0 mg, 0.012 mmol) in DMF (2 mL) was added the **3.50** (4.1 mg, 0.024 mmol), TBTU (9.6 mg, 0.030 mmol) and DIEA (3.9 mg, 0.030 mmol). The mixture was stirred at r.t. over night. Then water was added and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (silica gel saturated with Et_3N, gradient 50–16% hexane/EtOAc) to afford **3.94** (4.0 mg, 52% yield) as a colorless oil. R_f 0.21 (2:3 hexane–EtOAc); $[\alpha]_D$ = + 129.3 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.39–7.27 (m, 10 H, ArH), 5.75 (d, 1 H, J = 10.5 Hz, NH), 4.84 (d, 1 H, J = 12.0 Hz, PhCH_2, C-2), 4.79 (d, 1 H, J = 10.0 Hz, PhCH_2, C-3), 4.65 (dd, 1 H, $J_{NH,4}$ = 10.5 Hz, $J_{3,4}$ = 4.5 Hz, H-4), 4.64 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.62 (d, 1 H, J = 10.0 Hz, PhCH_2, C-3), 4.62 (d, 1 H, J = 12.0 Hz, PhCH_2, C-2), 4.19 (q, 1 H, $J_{5,6}$ = 6.5, Hz, H-5), 4.00 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.65 (s, 1 H, C*H*C=ONH), 3.62 (dt, 1 H, J = 10.0, 7.0 Hz, octyl OCH_2), 3.50 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.0 Hz, H-2), 3.45 (dt, 1 H, J = 10.0, 6.5 Hz,

octyl OCH_2), 3.27 (t, 2 H, J = 7.0 Hz, CH_2N_3), 3.07 (br s, 1 H, OH), 2.78 (s, 3 H, NCH_3), 2.11 (d, 1 H, J = 16.5 Hz, CH_2C=O), 1.95 (d, 1 H, J = 16.5 Hz, CH_2C=O), 1.66–1.55 (m, 4 H, CH_2 x 2), 1.42 (s, 3 H, CCH_3), 1.39–1.24 (m, 8 H, CH_2 x 4), 1.18 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 173.4 (CH_3NC=O), 168.8 (HNC=O), 138.2 (Ar), 137.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 97.6 (C-1), 77.7 (C-3), 74.8 (C-2), 73.7 (*C*HC=ONH), 73.5 (Ph*C*H_2, C-2), 72.8 (Ph*C*H_2, C-3), 68.6 (octyl O*C*H_2), 63.7 (C-5), 51.7 (C-4), 51.5 (*C*H_2N_3), 44.5 (*C*H_2C=O), 29.7 (*C*H_2), 29.4 (*C*H_2), 29.3 (*C*H_2), 29.1 (NCH_3), 28.9 (C*C*H_3), 28.7 (*C*H_2), 26.7 (*C*H_2), 26.0 (*C*H_2), 16.8 (C-6); HRMS (ESI) calcd for (M+H) $C_{35}H_{50}N_5O_7$: 652.3705. Found: 652.3695.

Chapter 4

4.1 Background

Escherichia coli is a gram-negative bacterium that is present in the large intestine of mammals. In most cases, *E. coli*, a non-pathogenic organism whose presence is normal in the gut of humans and animals, is a benefit for the host's life.[91] However, some *E. coli* strains can cause severe intestinal and urinary tract infections.[92,93,94] As I mentioned in the introduction, the lipopolysaccharide (LPS) of gram-negative bacteria plays an important role in infections by these organisms. In the next few sections, I will discuss LPS in more detail.

4.1.1 Structure of *E. coli* LPS

4.1.1.1 Structure of *E. coli* Lipid A and the core oligosaccharide

Like other Gram-negative bacteria, *E. coli* LPS consists of three parts: Lipid A, the core-oligosaccharide (OS) and the O-antigen.[5] The most abundant lipid A species in *E. coli* (Figure 4-1-A) is composed of a bisphosphorylated disaccharide, β-D-Glc*p*N-4-P-(1→6)-α-D-Glc*p*N-l-P with four attached β-hydroxy acyl chains.[95] Two of these chains, on the C-2' amino group and C-3' hydroxyl group, are esterified to dodecanoic and tetradecanoic acid, respectively. In some cases, the β-hydroxy-acyl chain on the C-2 amino group of the first GlcNAc residue is further acylated with palmitic acid.[5] The C-6' hydroxyl group is the linkage position for the core-OS.

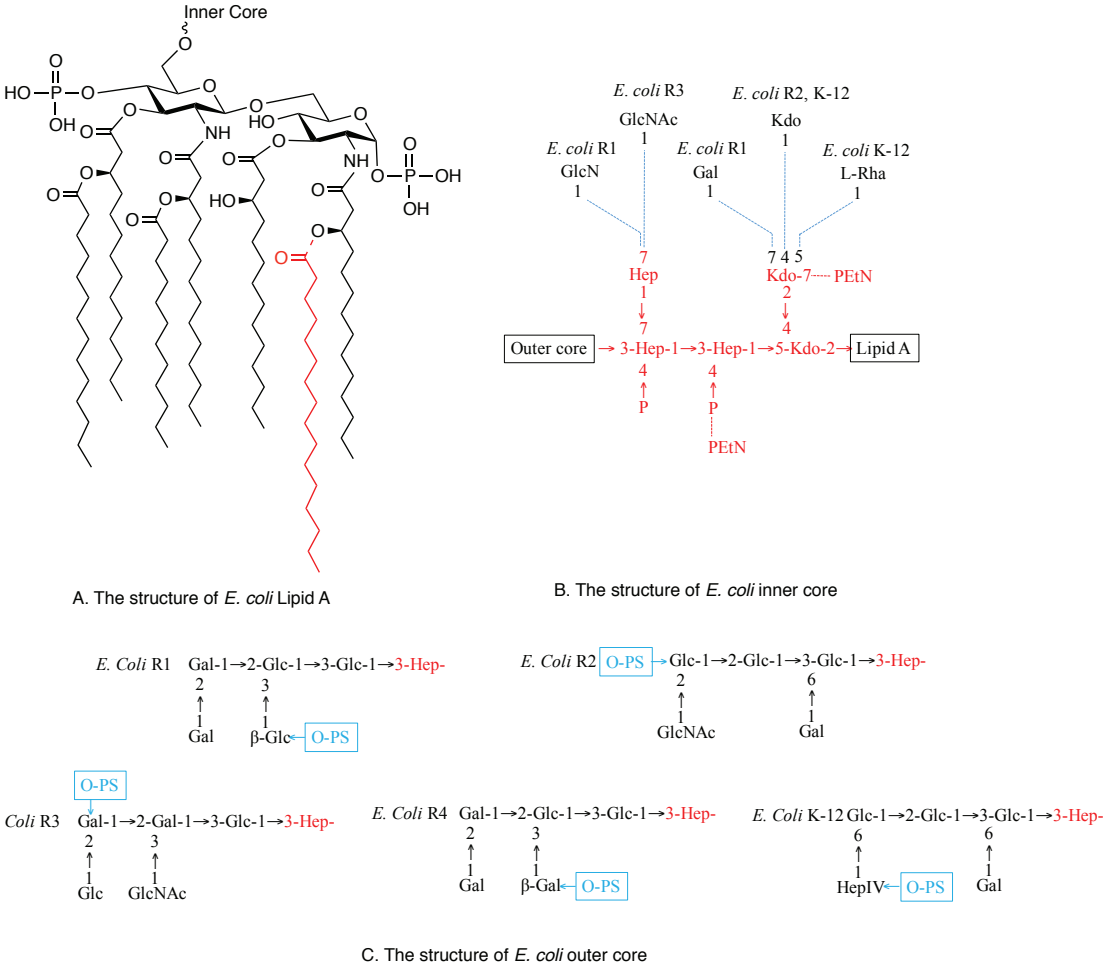


Figure 4-1: Structure of *E. coli* LPS. A) Structure of Lipid A. B) Structure of *E. coli* inner core. C) Structures of *E. coli* outer core. Figure 4-1-B and Figure 4-1-C were reprinted with permission from: Raetz, C. R.; Whitfield, C. *Annu. Rev. Biochem*. **2002**, *71*, 635–700.[5]

As shown in Figures 4-1-B and 4-1-C, in *E. coli* there are five known core types (R1, R2, R3, R4, and K-12).[5] These core types have different structures; however, they share a

common pentasaccharide, α-Hep-(1→7)-α-Hep-(1→3)-α-Hep-(1→5)-[α-Kdo-(2→4)]- α-Kdo backbone in the inner core (shown in red in Figure 4-1-B). The first and third residues in the backbone are connected to Lipid A and outer core, respectively. The sugar residues of the inner core are often substituted by other monosaccharides (e.g., GlcN, GlcNAc, Gal, Kdo or L-Rha), phosphate (P) or pyrophosphorylethanolamine (PEtN).[5] The outer core of *E. coli* LPS shows more structural diversity and this is what determines the core type species. All of the *E. coli* outer cores have a Glc residue linked to the terminal Hep residue of the inner core. For the other core species, Glc, Gal and GlcNAc, linked in different ways, are the major components of the outer core pentasaccharides.[5] The outer core region links the inner core and the O-antigen.

4.1.1.2 Structure of *E. coli* LPS O-polysaccharide

The O-antigen (or O-polysaccharide, O-PS) region shows much more structural diversity than the core-OS. In *E. coli*, more than 180 different O-antigens have been identified.[96,97] The detailed structures of all *E. coli* O-antigens for which structures have been deteremined are available at the *E. coli* database[96] (ECODAB), which is accessible at http://nevyn.organ.su.se/ECODAB/. The common monosaccharides in *E. coli* LPS are D-Glc, D-GlcNAc, D-Gal, D-GalNAc, D-Man, and L-Rha. A number of unusual sugars (pentoses, deoxyhexoses and heptoses) are also found in *E coli* O-PS.[96] The repeating units of the O-PS can be either linear or branched and typically contain 3–6 monosacchrides;

however most contain 3–5 sugar residues. The first monosaccharide residue of the O-PS, which is connected to the outer core, is highly conserved. This monosaccharide is a 2-acetamidosugar and is most commonly either D-GlcNAc (~70% of all strains) or D-GalNAc (~30%). In addition, D-FucNAc (6-deoxy-D-GalNAc) has been found as the first residue of the LPS from *E. coli* strain O45.[98] The second sugar attached to this 2-acetamido sugar is usually linked to O-3; however, in some cases it is linked to O-4.[99,100,101,102] The identity of the second sugar can be a broad range of monosaccharides. The structure relevant to my thesis is the O9a serogroup (Figure 4-2). In this O-PS, the GlcNAc is attached to two mannose residues linked to the O-3 of the GlcNAc, forming an α-D-Man-(1→3)-α-D-Man-(1→3)-β-D-GlcNAc structure. The O-PS repeating unit is attached to O-3 of the terminal mannose.[103] This trisaccharide, which links the core oligosaccharide and the O-PS repeating unit, is referred to as the 'adaptor–primer' region.[104,105] The structures of O-PS from other *E. coli* strains are shown in Table 4-1.

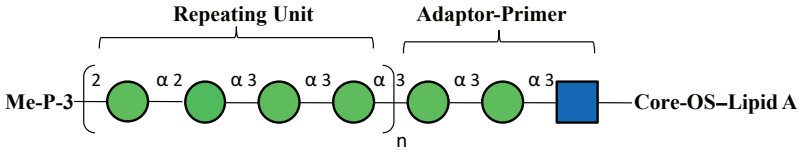
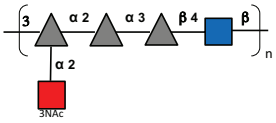
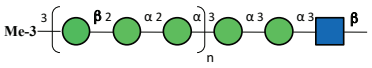
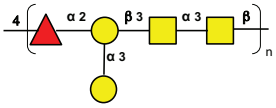
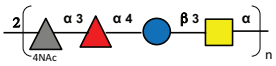


Figure 4-2: Structure of *E. coli* O9a LPS O-PS.

The structure is drawn using the Consortium for Functional Glycomics symbolic nomenclature (green circles = Man; blue squares = GlcNAc)[106]

Table 4-1: Structures of selected O-PS in *E. coli.* LPS[a]

Serogroup	Structure
O2	
O8	
O86	
O157	

[a]. The structures are drawn using the Consortium for Functional Glycomics symbolic nomenclature (green circles = Man; blue squares = GlcNAc; grey triangles = Rha; red squares = FucNAc; yellow squares = GalNAc; yellow circles = Gal; blue circles = Glc; red triangles = Fuc)

4.1.2 Biosynthesis of *E. coli* LPS.

4.1.2.1. Biosynthesis of LipidA and the core-OS.

E. coli LPS biosynthesis starts from the assembly of the Kdo_2-Lipid A domain, which is synthesized in nine enzymatic steps in the cytoplasm on an inner membrane bound structure (Figure 4-3).[3,107] Once produced, this Kdo_2–Lipid A intermediate serves as a substrate for the sequential addition of sugar residues (heptoses and hexoses) by glycosyltransferases to give first Hep_3Kdo_2–Lipid A and then the Lipid A–core-OS. Finally,

this structure is transported to the periplasmic face of the inner membrane by an ABC transporter.[108,109]

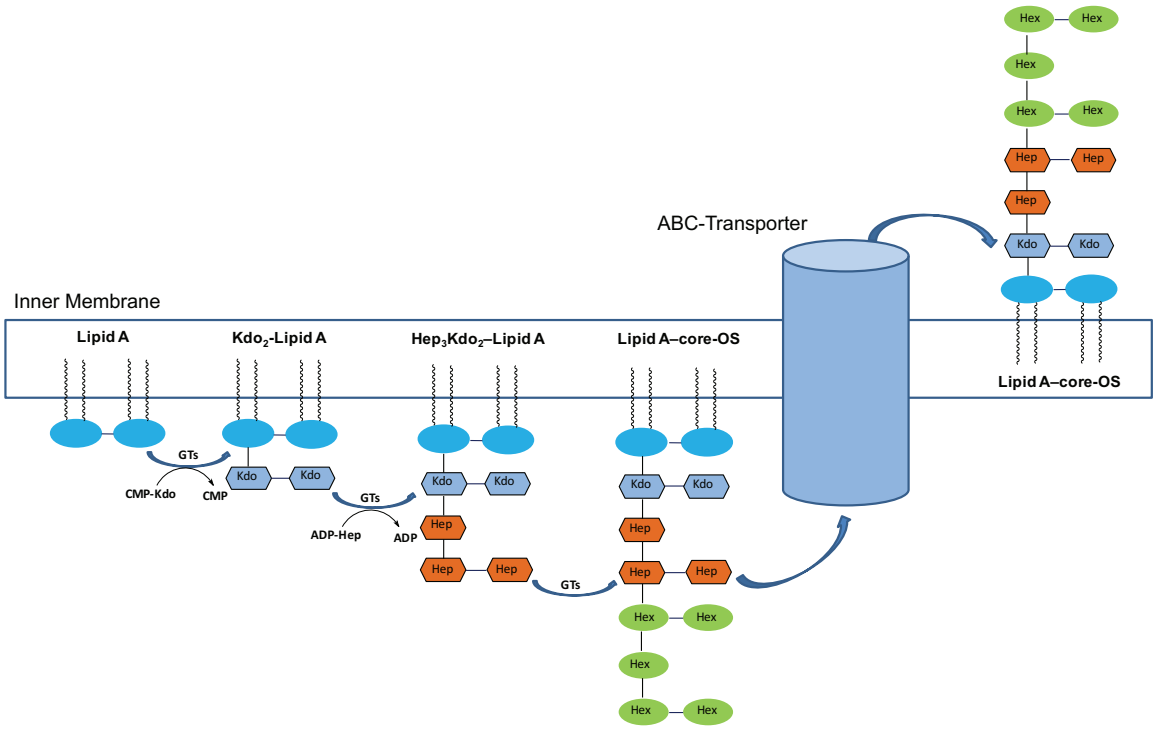


Figure 4-3: Biosynthesis and transport of Lipid A–core-OS

4.1.2.2. Biosynthesis of the O-PS.

O-PSs are synthesized by inner membrane enzymes. Three pathways are known to carry out this process: 1) the Wzy-dependent pathway, 2) the ABC-transporter-dependent pathway and 3) the synthase-dependent pathway.[3] All three pathways share the same initiation reaction, the transfer of *N*-acetylhexosamine-1-phosphate to an undecapreonol

lipid carrier (und-P) to form *N*-acetylhexosamine-PP-Und, but after that step the processes differ.[110]

The Wzy-dependent pathway is responsible for the biosynthesis of most heteropolymeric O-PS. In the Wzy-dependent pathway (Figure 4-4), the individual repeating units are synthesized in the cytoplasm and then transported to the periplasm where they are polymerized into the O-PS by the polymerase Wzy.[111,112] Finally, the completed O-PS are attached to the Lipid A–core OS[113] to form the final LPS molecule, which can be further transported to the outer membrane.[114]

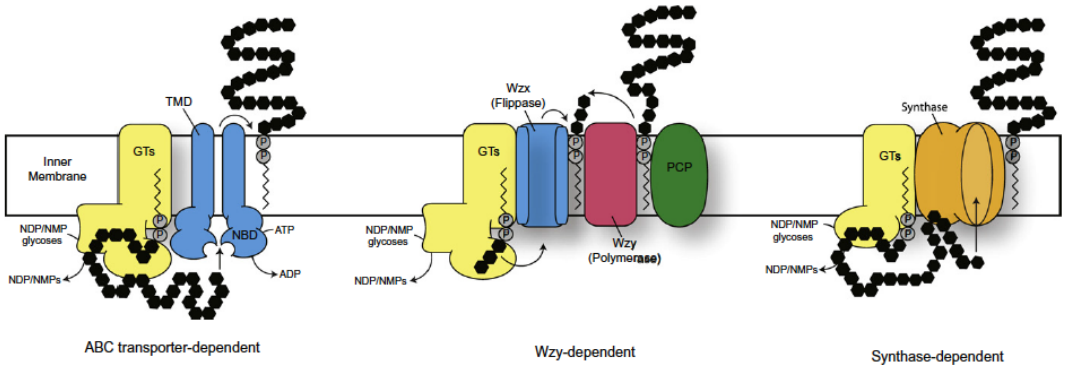


Figure 4-4: Mechanisms of O-PS biosynthesis: ABC transporter-dependent, Wzy-dependent and Synthase-dependent pathway. Reprinted with permission from: Greenfield, L.K; Whitfield, C. *Carbohydr. Res.* **2012**, *365*, 12–24.[3]

Homopolymeric O-PS biosynthesis generally uses the ABC-transporter-dependent pathway. In this case, the entire O-PS chain is assembled on the cytoplasmic side of the

inner membrane and then transferred to the periplasm by an ABC transporter. Once it is in the periplasm it is attached to the Lipid A–core OS and then transported to the outer membrane.[3]

The detailed mechanism of synthase-dependent pathway is still unclear.[3] It has been proposed that a bifunctional synthase can act both as glycosyltransferases to synthesize the O-PS on the cytoplasmic side of the inner membrane and as a transporter to flip the O-PS across the inner membrane.[3]

My project focuses on an O-PS prepared via the ABC transporter pathway. In the following sections I will provide additional detail on that process, with a focus on the particular system of interest, the O9a O-PS.

4.1.2.3 ABC transporter-dependent pathway for the synthesis of the O9a O-PS

Biosynthesis of the *E. coli* O8, O9 and O9a O-PSs follows the ABC transporter-dependent pathway.[3] These O-PS are linear homopolymers of mannose that differ in the linkage sequence and number of monosaccharide residues in the repeating units (Figure 4-5-A). In these organisms, O-PS biosynthesis is intiated by the transfer of GlcNAc-phosphate to undecaprenol phosphate catalyzed by the glycosyltransferase WecA.[3] This process occurs on the cytoplasmic side of the inner membrane. The undecaprenol starting material and the GlcNAc-containing product are embedded in the membrane. Chain extension of the O-PSs also occurs on the cytoplasmic side of the inner membrane using the product of the WecA

reaction. As shown in Figure 4-5-B, three mannosyltransferases, WbdC, WbdB, and WbdA elongate the O-PSs.[115] The enzyme WbdC transfers a single α-D-mannose residue from GDP-mannose to the C-3 hydroxyl group of GlcNAc-PP-Und **4.2** (Figure 4-5-C).[115] Then, WbdB, transfers two α-(1→3)-linked mannose residues to Man-GlcNAc-PP-Und **4.3** to form a tetrasaccharide **4.4**.[115] The three glycosylations catalyzed by WbdC and WbdB are highly conserved for biosynthesis of the adaptor domain of the *E. coli* O8, O9 and O9a O-PS.[3]

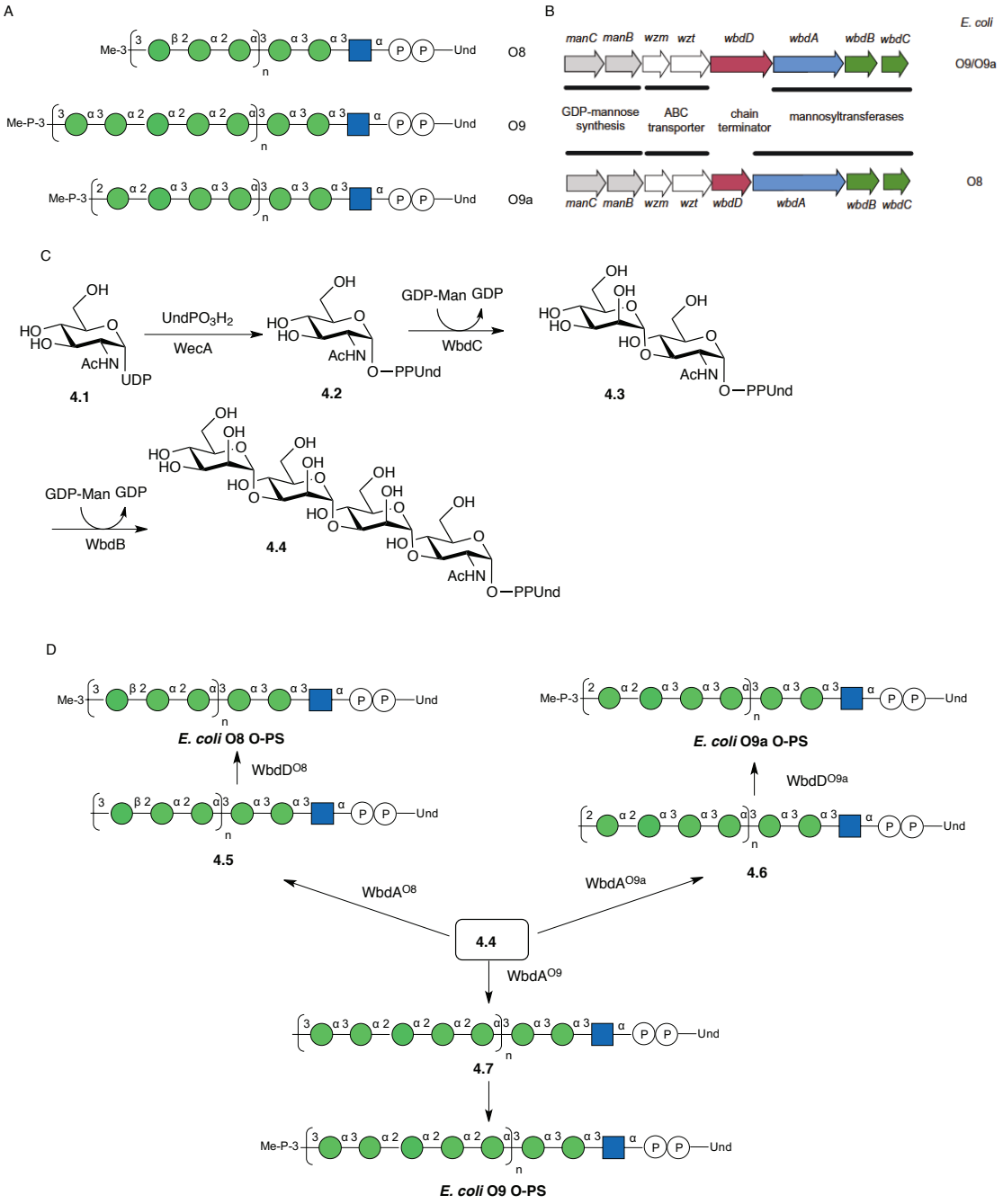


Figure 4-5: Biosynthesis of *E. coli* O8, O9 and O9a O-polysaccharide (O-PS). A) Structures of the O-PS. B) Biosynthetic gene clusters. C) Biosynthesis of the primer–adaptor 4.4. D) Chain elongation and termination. Figure 4-5-B was reprinted with permission from: Greenfield, L.K; Whitfield, C. *Carbohydr. Res.* **2012**, *365*, 12–24.[3]

The final mannosyltransferase, WbdA, is a serotype-specific enzyme that is responsible for the biosynthesis of the repeating unit.[116] The O8, O9 and O9a organisms all possess a different WbdA, which controls the final structure of the product (Figure 4-5-D). In O9a, $WbdA^{O9a}$ consists of two domains, an N-terminal domain (WbdA(N) and a C-terminal domain (WbdA(C)).[117] It has been shown that WbdA(N) is an α-(1→2)-mannosyltransferase and WbdA(C) possesses α-(1→3)-mannosyltransferase activity.[116] *In vitro* reactions using a synthetic primer–adaptor trisaccharide (α-Man*p*-(1→3)-α-Man*p*-(1→3)-β-Glc*p*NAc-PP-C_{13}) as an acceptor and $WbdA^{O9a}$ alone afforded O-PS products with the correct α-(1→2) and α-(1→3) linkages.[118] Therefore, this bifunctional enzyme is solely responsible for the assembly of the repeating unit structure and WbdB and WbdC are not involved. Similarly, $WbdA^{O8}$ with three functionally active domains can synthesize trisaccharide repeating units containing α-(1→2), α-(1→3), and β-(1→2)-mannopyranose residues.[29]

4.1.2.4 WbdD controlled chain termination in the O8, O9, O9a O-PS.

As shown in Figure 4-5-A, these O-PSs terminate with either a methyl (O8) or methyl-phosphate (O9 and O9a) residue at their non-reducing end.[3] Chain termination in these O-PS is controlled by the protein WbdD, which catalyzes the addition of a methyl or phosphomethyl group to the terminal Man residue (Figure 4-5-D).[119] In the O8 serotype, $WbdD^{O8}$ is a methyltransferase that transfers a methyl group to the C-3 hydroxyl group of

the terminal Man residue using S-adenosylmethionine as the methyl donor. WbdD protiens from the O9 and O9a serotypes are bifunctional kinase/methyltransferases; the phosphorylation reaction is responsible for chain termination.[119] *In vitro* reactions confirmed that purified $WbdD^{O9a}$ catalyzes a phosphorylation–methylation sequence using a synthetic O9a O-PS repeating unit as an acceptor.[120] This non-reducing end modification of the O-PS by WbdD prevents further chain elongation by WbdA. In contrast, $WbdD^{O9a}$ mutants without kinase/methyltransferase activity can synthesize O-PS; however, the O-PS without this terminal modification cannot be exported across the inner membrane.[119]

Two models, a "molecular clock" and a "molecular ruler", are usually used to explain the mechanism of polysaccharide length regulation. In the "molecular clock" model, chain length is controlled by the duration of the extension process. In contrast, in the "molecular ruler" model, a protein, or series of protiens, can measure the length of the O-PS chain and stop its extension. The biosynthesis of the O-PS happens on the membrane, and, in the molecular ruler model, at some point the chain length of the O-PS is long enough that its terminal residue of O-PS reaches the active site of the terminating enzyme, in this case WbdD. At this point, the enzyme can terminate chain extension by adding a methyl or phosphomethyl group.[121]

For the O9a system, Whitfield and co-workers proposed a "variable geometry model" in which the stoichiometry of the WbdA–WbdD complex also plays an important role in controlling the length distribution of the O-PS.[121] Using the *E. coli* O9a O-PS as a prototype,

they demonstrated that O-PS chain lengths decrease with increasing WbdD concentration. On the other hand, O-PS chain lengths decrease when the WbdA concentration declines.

In the crystal structure of WbdD,[122,123] an extended coiled-coil domain is present.[124] This coiled-coil domain acts as a ruler to measure the length of the O-PS (Figure 4-6). Through making a series of mutant WbdD enzymes, it was shown that O-PS length can be controlled by changing the length of the coiled-coil domain.[124] Thus, it was proposed that for the O9a O-PS, the coiled-coil domain of WbdD and the stoichiometry of WbdA and WbdD together controls chain length.[124]

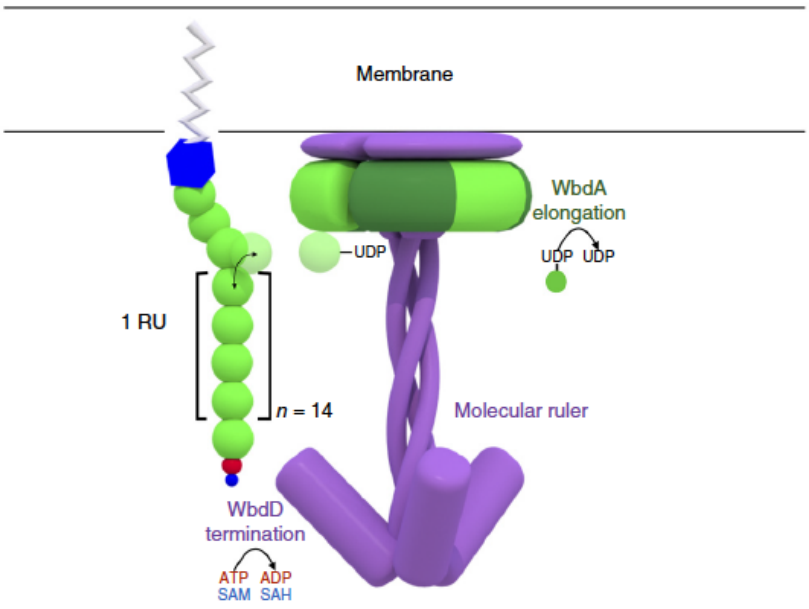


Figure 4-6: Model for *E. coli* O9a O-PS chain elongation and termination. Reprinted with permission from: Hagelueken, G.; Clarke, B. R.; Huang, H.; Tuukkanen, A.; Danciu, I.; Svergun, D. I.; Hussain, R.; Liu, H.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Mol. Biol.* **2015**, *22*, 50–56.[124]

After the terminal modification of the O-PS chain, the polysaccharide is transported from the inner leaflet of the inner membrane to the periplasm by an ABC transporter. Then, the O-PS is connected to the Lipid A–core and finally transported to the outer leaflet.[3]

4.1.3 Chemical synthesis of *E. coli* O-PS oligosaccharides

The O-PS of *E. coli* LPSs are a large group of complex glycans.[94] To date, there have been no total syntheses of any full length O-PS (from any organism) linked either to undecaprenol pyrophosphate lipid or the core-OS–Lipid A domain. With regard to large molecules, one paper describes the synthesis of a 12-mer of *E. coli* O148 O-PS with three repeating units (Figure 4-7).[42] Moreover, a few papers have reported the synthesis of repeating units of some *E. coli* O-PSs.[42,125,126,127,128,129,130,131] Examples of these molecules are shown in Figure 4-7. In the sections below, I will discuss in more detail the synthesis of structures relevant to the O8, O9 and O9a O-PS.

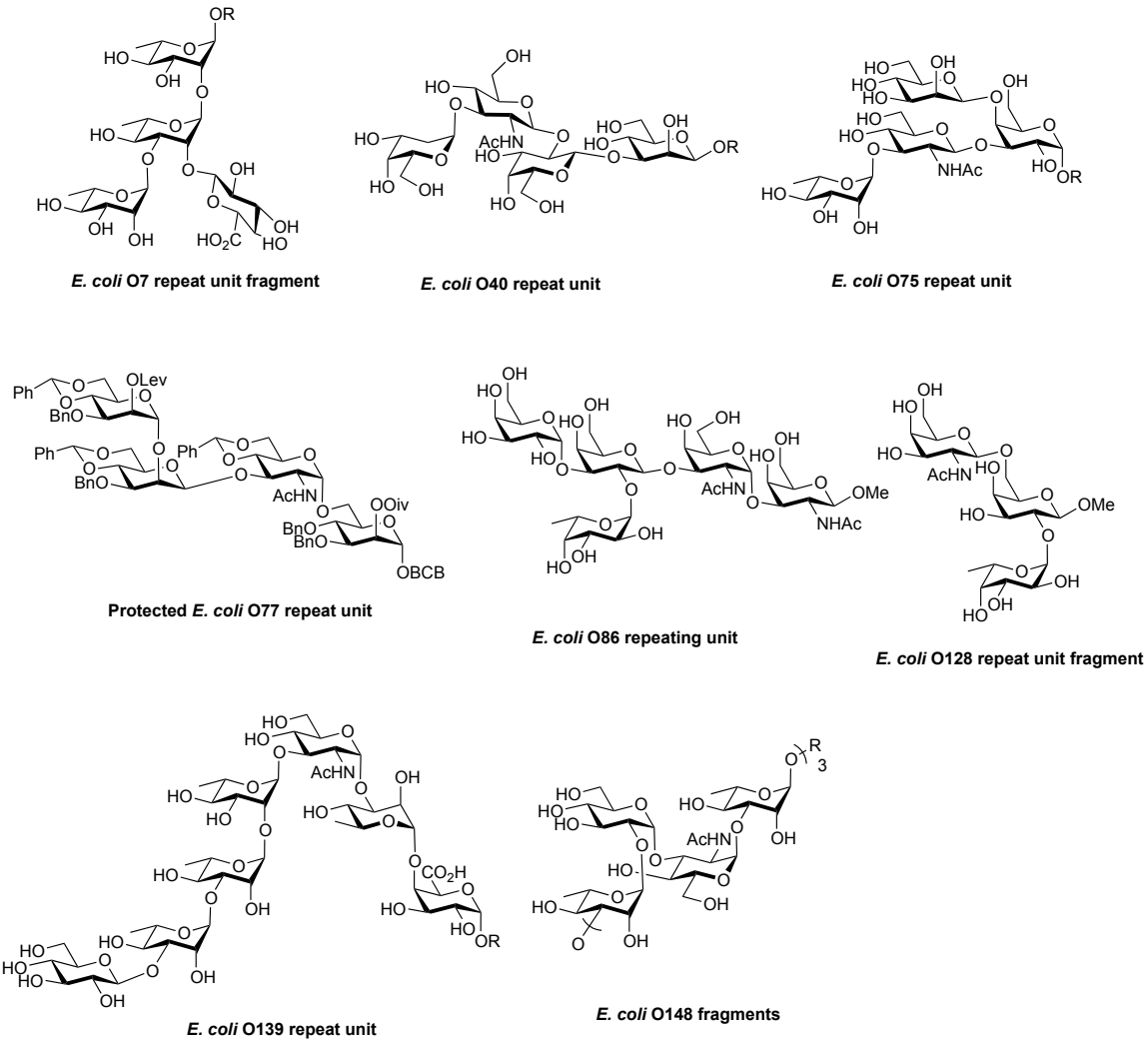
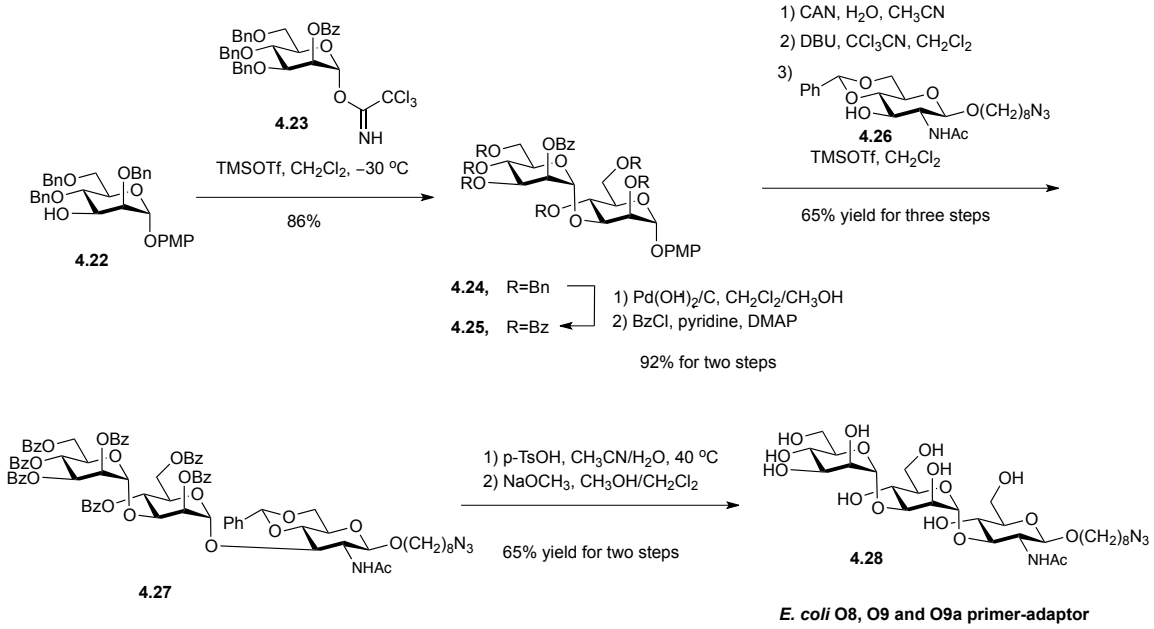


Figure 4-7: Selected examples of *E. coli* O-antigen repeating units that have been chemically synthesized.[37-44]

4.1.3.1 Synthesis of the *E. coli* O8, O9 and O9a primer–adaptor motif.

The trisaccharide moiety α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-β-D-Glc*p*NAc is the "primer–adaptor" of *E. coli* O8, O9 and O9a LPS O-PS. Lowary and coworkers[132] reported

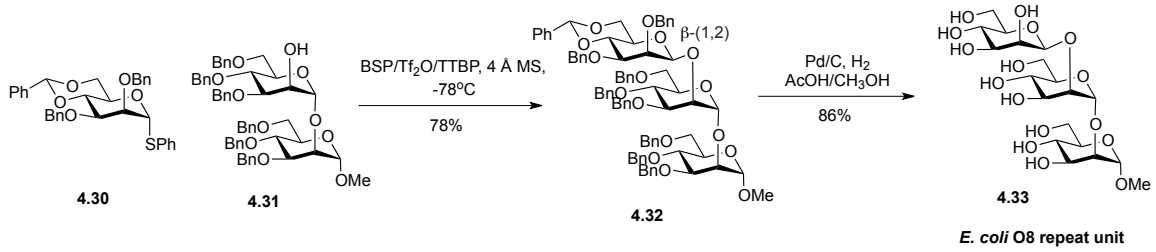
the synthesis of this trisaccharide moiety bearing an 8-azidooctyl aglycone, a group that provides a convenient handle for conjugation (Scheme 4-1). Glycosylation of acceptor **4.22** with donor **4.23** in presence of TMSOTf led to the desired disaccharide **4.24** in 86% yield. The *p*-methoxyphenyl (PMP) protected disaccharide **4.25** was converted to the trichloroimidate donor, which was glycosidated with acceptor **4.26** to give trisaccharide **4.27** in 65% yield. After deprotection, the primer–adaptor trisaccharide **4.28** was obtained in 65% yield.



Scheme 4-1: Synthesis of the *E. coli* O8, O9 and O9a primer–adaptor trisaccharide moiety 4.28.[132]

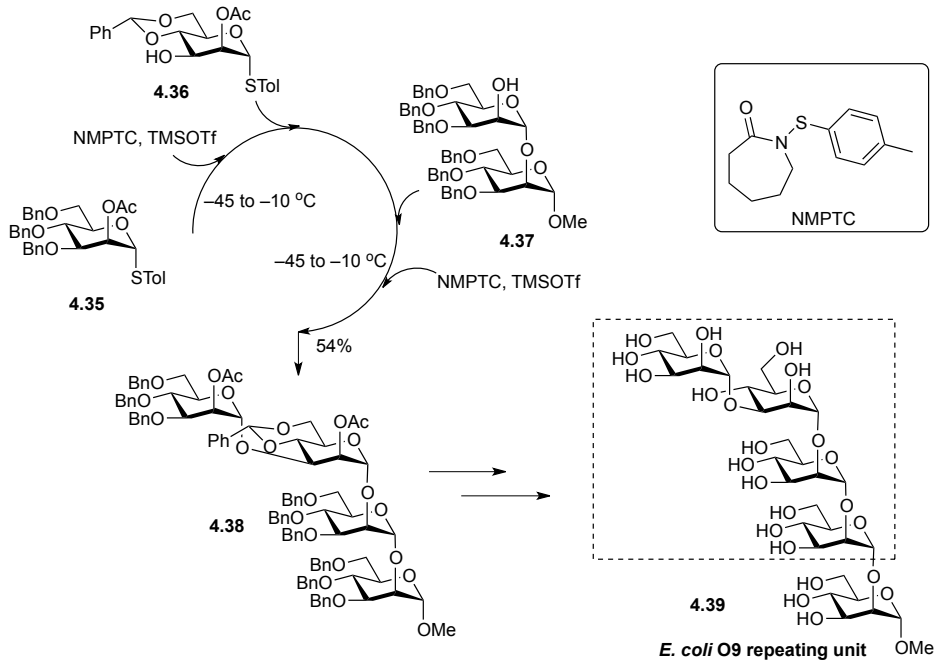
4.1.3.2 Synthesis of the *E. coli* O8, O9 and O9a repeating unit structures.

The synthesis of the *E. coli* O8 O-PS trisaccharide repeating unit has been reported by Ghosh and coworkers (Scheme 4-2).[133] The key step is the β-(1→2)-mannosylation of **4.31** using thioglycoside donor **4.30** to afford trisaccharide **4.32**. Global deprotection of **4.32** afforded *E. coli* O8 repeating unit **4.33** in 86% yield.



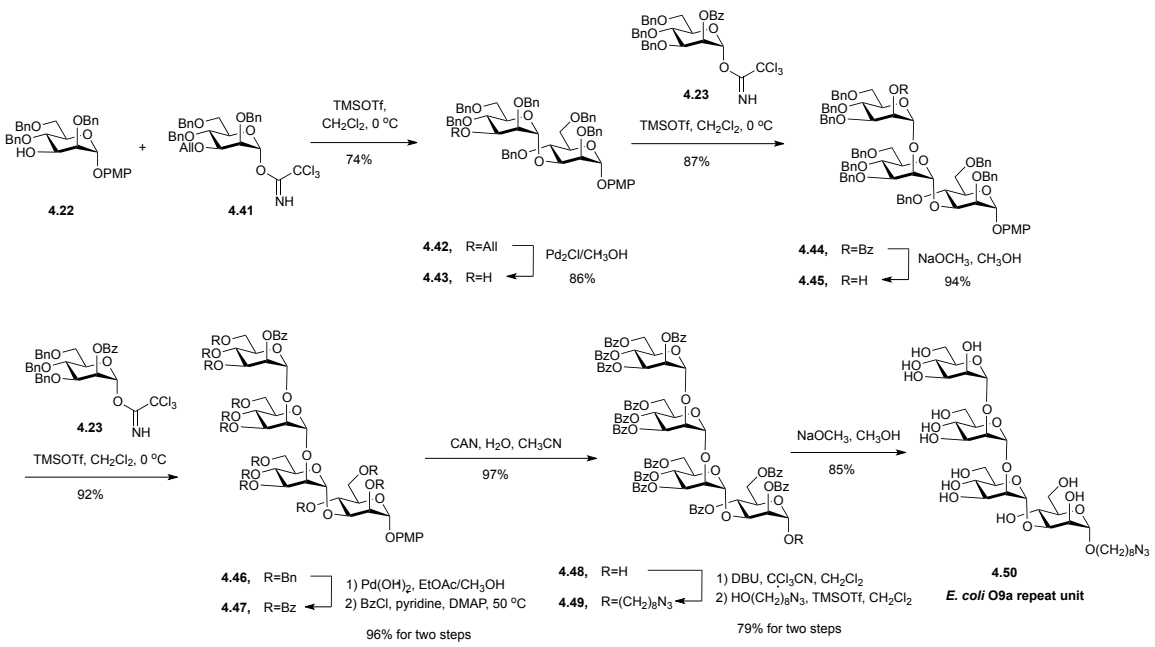
Scheme 4-2: Synthesis of *E. coli* O8 repeating unit **4.33**.[133]

Maity and Ghosh[134] reported a one-pot synthesis of tetrasaccharide **4.38** (Scheme 4-3), which is related to the pentasaccharide repeating unit of the *E. coli* O9 O-PS. The key feature of this work is the use of two sequential glycosylation steps mediated by *N*-(p-methylphenylthio)-ε-caprolactam (NMPTC) and TMSOTf in one pot. This process afforded the target tetrasaccharide **4.38** in 54% overall yield.



Scheme 4-3: Synthesis of an *E. coli* O9 repeating unit related tetrasaccharide **4.38**.[134]

The synthesis of the *E. coli* O9a O-PS repeating unit was reported by Lowary and coworkers.[135] Sequential glycosylations of acceptor **4.22** with trichloroacetimidate donors **4.41** and **4.23** afforded tetrasaccharide **4.46** (Scheme 4-4). After protecting group manipulation, the PMP group was removed at a late stage. The corresponding anomeric hydroxyl group was converted to a trichloroacetimidate donor, which was used to glycosylate 8-azido-1-octanol to afford tetrasaccharide **4.49** in 79% yield. After deprotection, the *E. coli* O9a O-PS repeating unit with an 8-azidooctyl aglycone **4.50** was obtained in 85% yield.



Scheme 4-4: Synthesis of the *E. coli* O9a repeating unit related tetrasaccharide **4.50**.[135]

4.2 Research objective – synthesis of polysaccharides as probes of LPS biosynthesis

As described above, the biosynthesis of the O-PS in *E. coli* serotype O9a has been investigated and many of the steps are understood. These investigations were significantly helped by the availability of small synthetic fragments of these larger structures. However, having access to large, more complex molecules that better represent the natural structures would be valabule tools. For example, they could be used as ligands in crystallization trials with WbdD to probe the mechanism by which chain length is controlled in this system. In addition, they could be used as substrates for WbdA to determine if there are changes in the way these larger compounds are recognized compared to their shorter counterparts. Perhaps most interestingly they could be used to study the recognition of these compounds by the ABC transporter that tranports the final lipid-linked O-PS across the inner membrane to the periplasm. This 'flipping' process is very poorly understood and it is likely that short oligosaccharide fragments of the O-PS will not be effective probes of this process.

To provide molecules that could probe these questions, we designed a series of large *E. coli* O9a O-PS fragments to synthesize. At the outset of the project, the goal was to make molecules with 2, 6, 10, 14 and 18 repeating units. I thought that completing all of them as part of my thesis was not likely. Nevertheless, my major goal was to develop a robust route that could be applied to all of the target molecules and then use it to make at least two of the targets. In this chapter, I describe the preparation of O9a O-PS fragments containing 2

(**4.51**) and 6 (**4.52**) tetrasaccharide repeating units connected via the primer–adaptor trisaccharide to farnesyl pyrophosphate (Figure 4-8). These molecules contain 11 and 27 monosaccharide residues, respectively. Given the large size of the targets and the presence of the farnesyl pyrophosphate lipid, their synthesis presents a significant challenge.

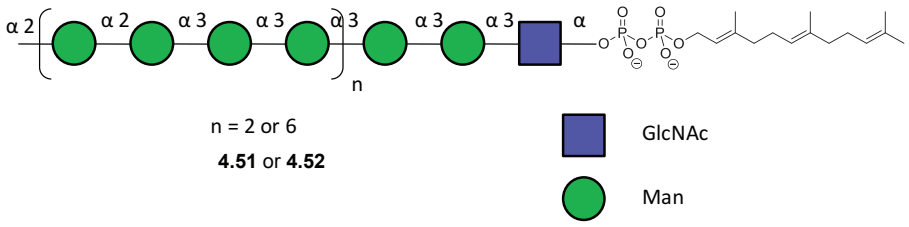


Figure 4-8: Structures of LPS O-chain fragments in *E. coli* O9a synthesized in the work described in this chapter.

4.3 Results and discussion

4.3.1 Retrosynthetic strategy and protecting groups selection

The retrosynthetic analysis of the *E. coli* O9a O-PS targets is shown in Figure 4-9. I proposed that farnesol pyrophosphate could be attached to the protected O-PSs **4.56** and **4.57** at a late stage. I envisioned two possible routes to synthesize **4.56** and **4.57**.

One route (Route I, Figure 4-9-A) includes a trisaccharide primer–adaptor (**4.62**) and a tetrasaccharide repeating unit (**4.61**). Trisaccharide **4.62** could be obtained from glucosamine-based building block **4.71** and mannose thioglycoside **4.70**. The tetrasaccharide could be synthesized from four different protected mannose-based building blocks **4.66**, **4.67**, **4.68**, and **4.69**.

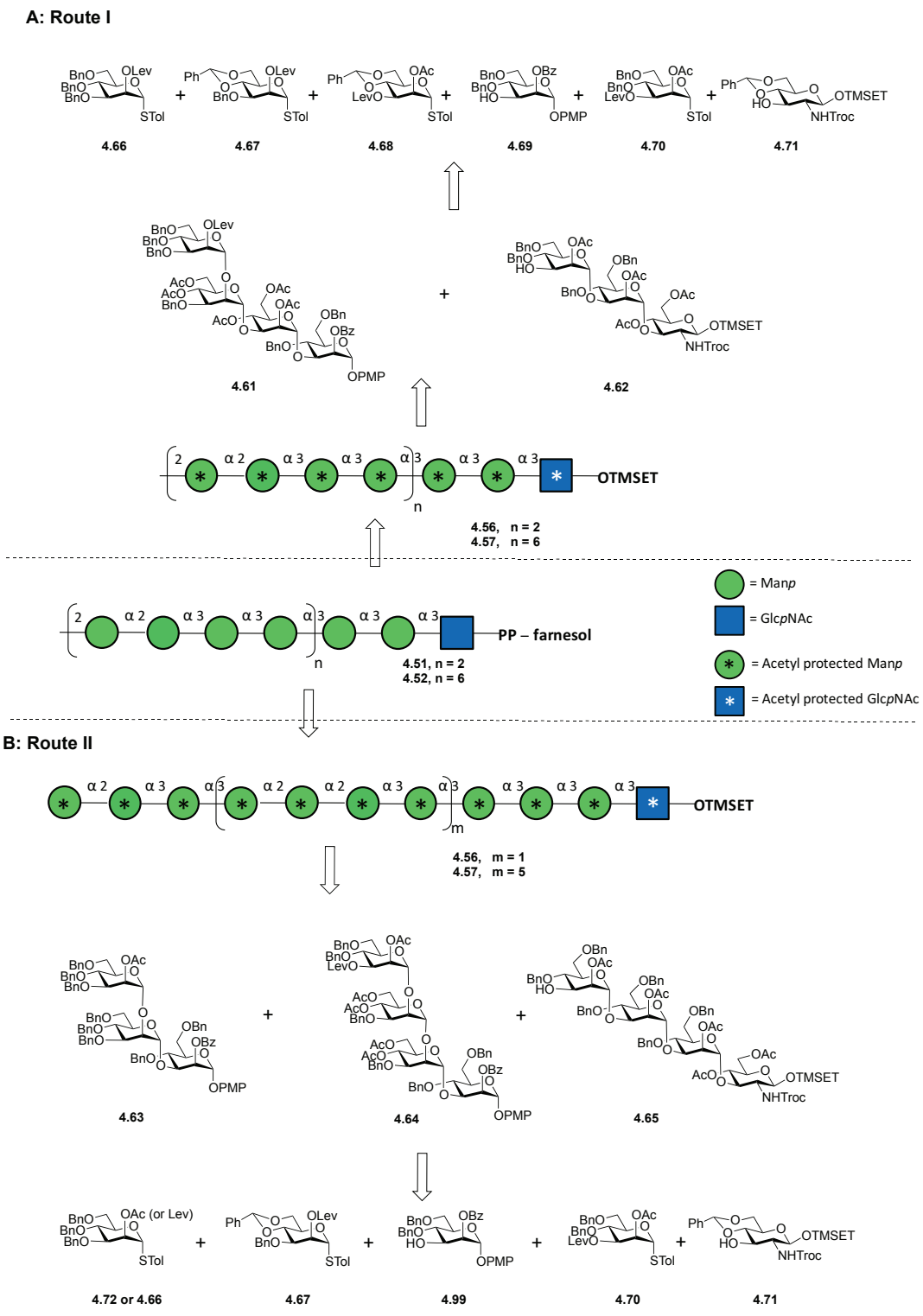


Figure 4-9: Retrosynthesis of *E. coli* O9a O-PS fragments. A) Retrosynthetic Route I. B)

Retrosynthetic Route II

The second route (Route II, Figure 4-9-B) adopts a different strategy, involving a tetrasaccharide primer–adaptor (**4.65**), a tetrasaccharide repeating unit (**4.64**), and a trisaccharide cap (**4.63**). Five mannose- or glucosamine-based building blocks (**4.72** or **4.66**, **4.67**, **4.69**, **4.70**, and **4.71**) are needed to obtain primer–adaptor **4.65**, repeating unit **4.64**, and cap **4.63**.

Access to large amounts of the tetrasaccharide repeat units (**4.61** or **4.64**) is required to assemble the *E. coli* O9a O-PS. Therefore, the first thing I needed to do was to compare possible routes leading to **4.61** or **4.64** and then their elongation into larger structures. I will provide a detailed analysis for one of these intermediates (**4.64**); a similar analysis was done for the design of the other oligosaccharide building blocks.

Protecting groups greatly influence glycosylation reactivity, glycosylation stereoselectivity and deprotection efficiency. In particular, the reactivity of a glycosyl donor and a glycosyl acceptor can be tuned through choice of protecting groups.[136] Because of this, one of my first goals was to determine the protecting groups to be present in oligosaccharide donors and acceptors to be used building the chains. Those choices would determine the monosaccharide building blocks to be synthesized. The key chain elongation reaction for Route II is shown in Figure 4-10. This is a reaction between donor **4.117** and acceptor **4.133**. Both of these compounds can be prepared from **4.64**.

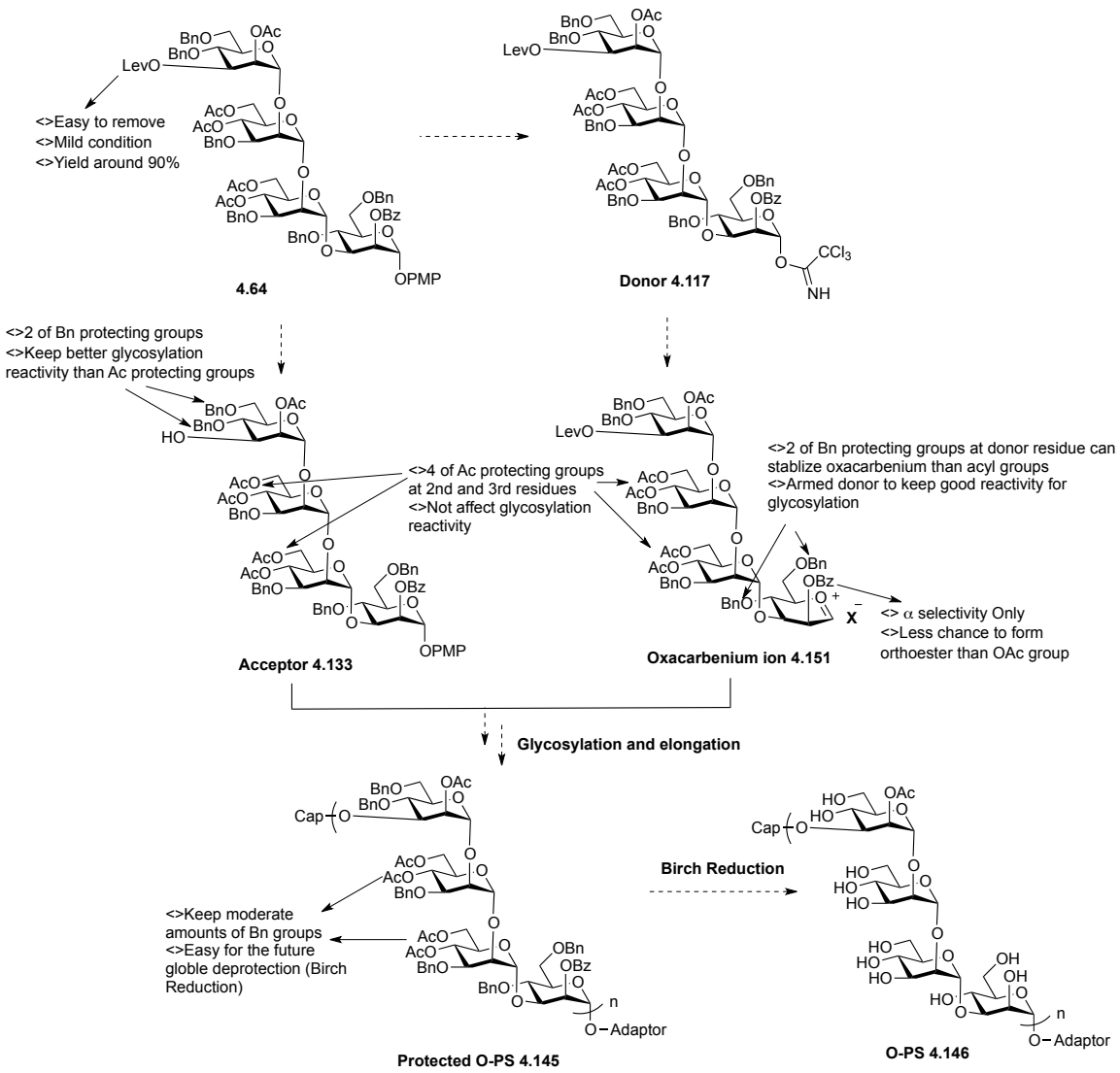


Figure 4-10: Protecting group selection for repeating units.

With regard to the donor **4.117**, Fraser-Reid first proposed the "armed/disarmed" concept, wherein glycosyl donors containing 2-O-ether ("armed") protecting groups were more reactive than those with 2-O-ester ("disarmed") groups.[137] Compared to the alkyl protecting groups, electron withdrawing groups (e.g., Ac or Bz) destabilize oxacarbenium ion intermediates (in this case **4.117**) and slow down glycosylations. Thus, I chose two

benzyl groups to protect the hydroxyl groups at C-4 and C-6 on the first mannose residue from the reducing end (**4.117**, Figure 4-10). This is the residue that will form the oxacarbenium ion in the glycosylation. In addition, neighboring group participation of an acyl group at C-2 in a glycosyl donor (**4.117**, Figure 4-10) can control the selectivity of a glycosylation leading to the formation of a 1,2-*trans* glycoside, the stereochemistry needed for my targets. Therefore, acetyl and benzoyl groups were considered as protecting groups for this position. However, a common problem in glycosylations with 2-*O*-acylated donors is the formation of 1,2-orthoester byproducts. Acetyl groups are more prone to orthoester formation than benzoyl groups and so I chose benzoyl groups for protection of the C-2 hydroxyl group at the reducing end of **4.117**.

Having settled on the protecting groups for the reducing end of the molecule, I considered the non-reducing end, which would serve as an acceptor (**4.133**) in the chain elongation. Electron-withdrawing acyl groups on an acceptor residue decrease the nucleophilicity of hydroxyl groups on the same ring; thus, acceptors with acyl protecting groups are less reactive than those with alkyl protecting groups. Based on this reason, I chose two benzyl groups to protect the hydroxyl groups at C-4 and C-6 on the fourth mannose residue (**4.133**, Figure 4-10). I also needed to select a 'temporary' protecting group for the C-3 on nonreducing end in **4.64**. This group would be present in donor **4.117**, but then would be removed after glycosylation to provide a hydroxyl group that could undergo further reaction. The Lev ester drew my attention because it is orthogonal to acyl,

Troc, TMS-ethyl, PMP, and benzyl groups, other groups that will be used the synthesis of these targets. In addition, its deprotection by hydrazine acetate gives the desired products in excellent yield.

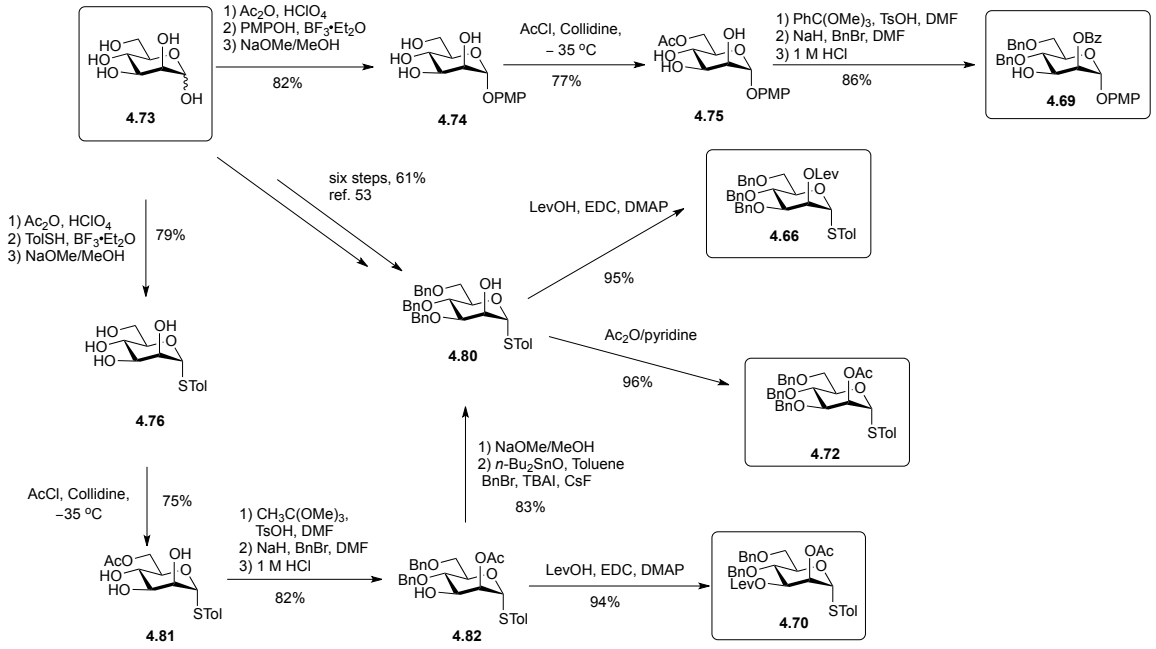
With regard to the choice of the protecting groups on the internal residues in **4.64** (and also **4.117** and **4.113**), I anticipated the following things: 1) that the protecting groups on the second and third mannose residues would not greatly affect the glycosylation reactivity and 2) that removal of a large number of benzyl groups from complex oligosaccharides at the end of the the synthesis could be difficult. Therefore, I chose acetyl groups to protect the groups at C-4 and C-6 hydroxyl groups on the second and third mannose residues. The choice of benzyl groups for the C-3 hydroxyl groups was made because of the ease of the synthesis of the monosaccharides used to assemble compounds leading to **4.64** (see details below).

Using a similar anlaysis process, I designed the other oligosaccharide building blocks show in Figure 4-9 (e.g., **4.61**, **4.65**).

4.3.2 Synthesis of O-polysaccharide

4.3.2.1 Synthesis of mannose and glucosamine related building blocks required for the synthesis of 4.61 and 4.64.

Once the protecting groups of the repeating units **4.61** and **4.64** were determined, I focused my attention on the synthesis of building blocks needed to assemble these tetrasaccharides. I first addressed the synthesis of building block **4.69** (Scheme 4-5), which would become the reducing end residue of **4.61** and **4.64**.



Scheme 4-5: Synthesis of building blocks **4.66**, **4.69**, **4.70** and **4.72**.

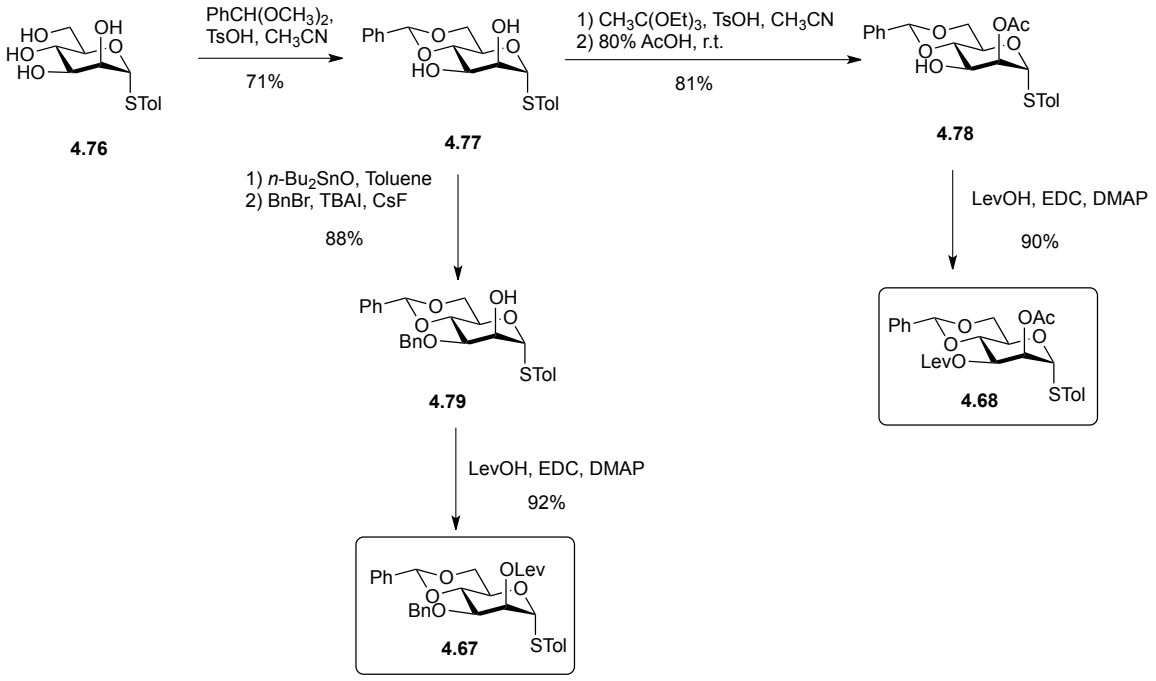
The synthesis of **4.69** started from the PMP glycoside **4.74**, which was prepared from D-mannose (**4.73**) in three steps as previously reported.[138] Regioselective acetylation of the primary hydroxyl group in **4.74** afforded the 6-*O*-acetylated mannoside **4.75**, which

was confirmed byNMR analysis (a downfield shift in the two H-6 resonances from 3.71 and 3.78 ppm to 4.22 and 4.46 ppm were seen). Reaction of triol **4.75** with trimethylorthobezoate and acid allowed for the efficient formation of the 2,3-*O*-orthoester intermediate, which was then directly benzylated and then the orthoester was subsequently opened to give the desired alcohol **4.69**.[139] The introduction of the acetyl group on O-6 in **4.74** was necessary to ensure good regioselectivity in the orthoesterification step. However, the acetate ester could conveniently be cleaved and the resulting hydroxyl group alkylated under the basic conditions of the benzylation reaction. Using the same method, the known protected mannoside **4.82** was readily synthesized from tetraol **4.76** (also prepared from D-mannose) in four steps and 62% yield. The donor **4.70**, which would serve as the non-reducing end residue in tetrasaccharide **4.64** was then obtained in 94% yield by protection of the C-3 hydroxyl group in **4.82** as a levulinate ester via treatment with levulinic acid, *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP).

The synthesis of donors **4.66** and **4.72**, key intermediates for both Route I and Route II, began with protected thioglycoside **4.80** (Scheme 4-5), which was obtained from D-mannose in six steps in 61% yield as previously reported.[140] Intermediate **4.80** can also be obtained from **4.82** by deacetylation and regioselective benzylation of the C-3 hydroxyl group in 83% yield. Due to sharing the same intermediate (**4.82**) with synthesis of building block **4.70**, this approach is more convergent than the former one. The C-2 hydroxyl group

in **4.80** was protected as a levulinate ester giving **4.66** in 95% yield. Altermatively, acetylation of **4.80** afforded building block **4.72** in 96% yield.

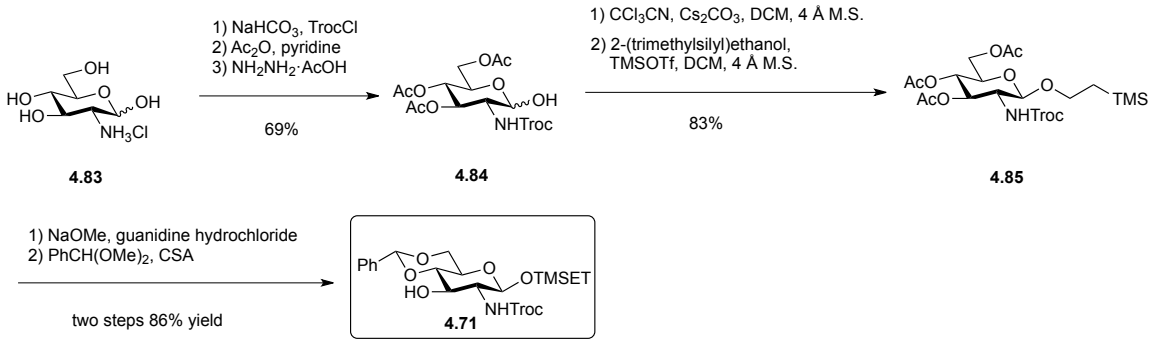
Synthesis of building blocks **4.67** and **4.68**, which are needed for Route II and Route I, respectively, began from the 4,6-*O*-benzylidene-protecting thioglycoside **4.77** (Scheme 4-6). Compound **4.77** was obtained from **4.76** in 71% yield upon treatment with benzlidene dimethyl acetal and *p*-toluenesulfonic acid[141] With this intermediate in hand, compound **4.68** was synthesized following a two-step sequence. Diol **4.77** was treated with triethylorthoacetate and *p*-toluenesulfonic acid to form a 2,3-*O*-orthoester followed by its selective opening in acidic conditions to afford **4.78** in 81% yield.[142] Donor **4.68** was obtained by the protection of the C-3 hydroxy as a levulinate ester in 90% yield. Alternatively, regioselective benzylation of the C-3 hydroxyl group in diol **4.77** was achieved by treatment with *n*-Bu_2SnO in toluene at reflux, followed by the addition of BnBr, TBAI and CsF at 110 °C. This reaction provided benzyl ether **4.79** in 85% yield. The C-2 hydroxyl group of **4.79** was protected as a levulinate ester to afford a 92% yield of building block **4.67**.



Scheme 4-6: Synthesis of building blocks **4.67** and **4.68**.

The synthesis of acceptor **4.71**, which is needed for both Route I and Route II, began with D-glucosamine hydrochloride (**4.83**, Scheme 4-7). First, the amino group was protected as a trichloroethyl carbamate and the remaining hydroxyl groups were acetylated in Ac_2O and pyridine. Next, the anomeric acetyl group was selectively removed by treatment with hydrazine acetate to afford **4.84** in 69% yield over the three steps. Compound **4.84** was treated with trichloroacetonitrile (CCl_3CN) and cesium carbonate (Cs_2CO_3) to provide the corresponding trichloroacetimidate. The imidate was then treated with 2-(trimethylsilyl)ethanol and TMSOTf to afford an 83% yield of monosaccharide **4.85**, which was confirmed by comparison with the ^1H NMR spectroscopic and mass spectrometric data reported previously.[143] From monosaccharide **4.85**, building block **4.71**,

was synthesized by deacetylation and protection of the C-4 and C-6 hydroxyl groups as a benzylidene acetal (86% over the two steps).



Scheme 4-7: Synthesis of building block **4.71**.

4.3.2.2 Synthesis of two repeating units and comparison of routes

Having established the synthesis of the building blocks, I turned my attention to comparing the routes to synthesize the two different tetrasaccharides, **4.61** (Route I) and **4.64** (Route II). In the routes developed, my strategy was to prepare two tetrasaccharides, **4.91** and **4.96**, which were then converted to the key building blocks **4.61** and **4.64**, respectively by manipulation of the protecting groups (Figure 4-10). In the paragraphs below, the synthesis of **4.91** and **4.96** is described.

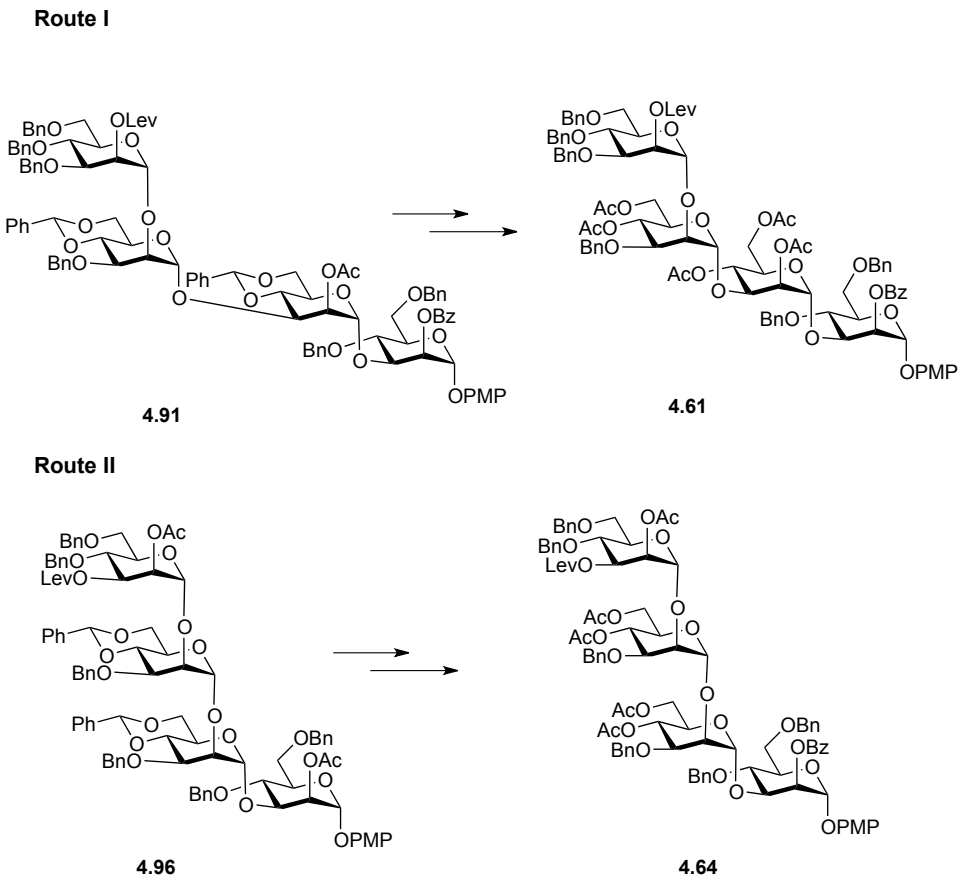
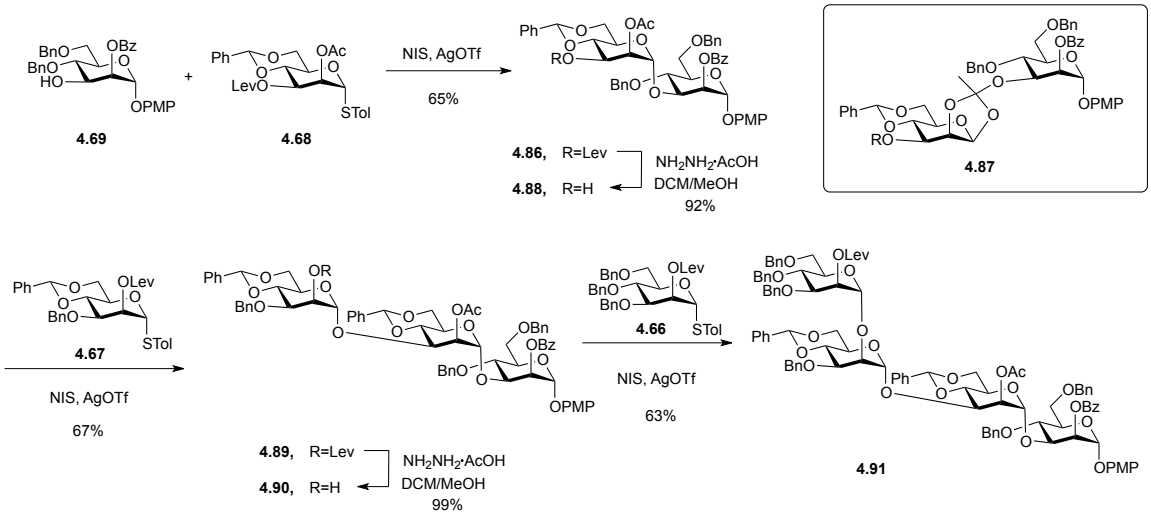


Figure 4-11: Structures of tetrasaccharides **4.91/4.96** and their relationship to **4.61/4.64**.

I first investgated the preparation of tetrasaccharide **4.91** (Scheme 4-8), which is the key building block for Route I. The synthesis started by coupling of glycosyl acceptor **4.69** to a mannose thioglycoside donor **4.68** mediated by NIS (1.3 equiv.) and AgOTf (0.1 equiv.). The product was produced in 45% yield, mainly due to the formation of the 1,2-othorester **4.87** (25%). To improve the yield, a larger amount of AgOTf (0.35 equiv.) was added. Under these more acidic conditions, disaccharide **4.86** was isolated in 65% yield. Chemoselective removal of the levulinate group using hydrazine acetate gave acceptor **4.88**

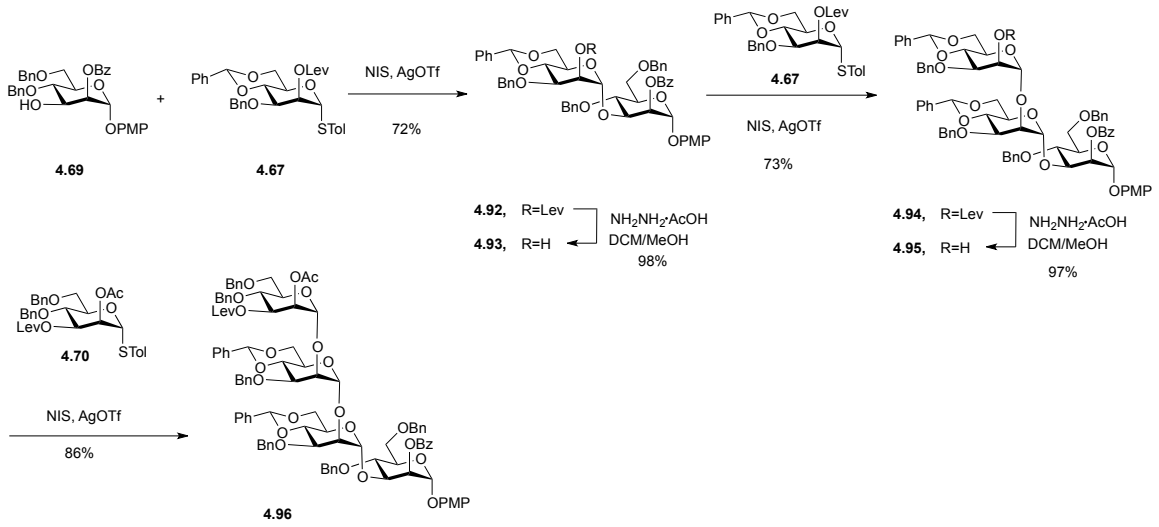
in 92% yield. Next, acceptor **4.88** and donor **4.67** were coupled through an NIS/AgOTf-promoted glycosylation to afford trisacchride **4.89** in 67% yield. After removing the levulinate group, another NIS/AgOTf-mediated glycosylation was performed to couple trisaccharide acceptor **4.90** and thioglycoside **4.66** to give tetrasaccharide **4.91**. This reaction proceeded in 63% yield. In all glycosylations, the stereochemistry of the newly formed glycosidic linkage was determined by measuring the $^{1}J_{C\text{-}1,H\text{-}1}$. These values were 171–176 Hz, as expected for an α-linkage.[144]



Scheme 4-8: Synthesis of repeating unit **4.91** for Route I.

I next focused on the synthesis of tetrasaccharide **4.96**, which was needed for Route II. Like the preparation of **4.61**, this synthesis involved an alternating series of NIS/AgOTf-promoted glycosylations and levulinate protecting group removals with hydrazine acetate (Scheme 4-9). First, glycosylation of **4.69** with thioglycoside **4.67** afforded a 72% yield of disaccharide **4.92**, which was in turn deprotected, giving acceptor **4.93**. Then, glycosylation

of **4.93** with the same thioglycoside donor (**4.67**) gave the trisaccharide **4.94** in 73% yield. Elongation of this trisaccharide to the tetrasaccharide **4.96** was achieved by reaction of **4.95** with monosaccharide donor **4.70**. This reaction proceeded in 86% yield to give the expected fully protected tetrasaccharide **4.96**. As was done for the synthesis of **4.91**, the α-stereochemistry of the glycosylation reactions leading to **4.96** was determined from the $^{1}J_{C\text{-}1,H\text{-}1}$ values of the newly introduced mannose residues, which were in the range of 172–175 Hz.[144]



Scheme 4-9: Synthesis of repeating unit **4.96** for Route II.

The synthesis of tetrasaccharides **4.91** and **4.96** is summarized in Table 4-2. For the the intermediate needed for Route I, **4.91**, four building blocks are needed. In contrast, only need three building blocks are needed to prepare **4.96**, the intermediate needed for Route II. In addition, compared with the low overall yield (27%) of three glycosylation

steps for the synthesis of **4.91**, the overall yield (45%) for the synthesis of repeating unit **4.96** was much higher.

Table 4-2: Comparison of synthetic routes to tetrasaccharides **4.91** and **4.96**

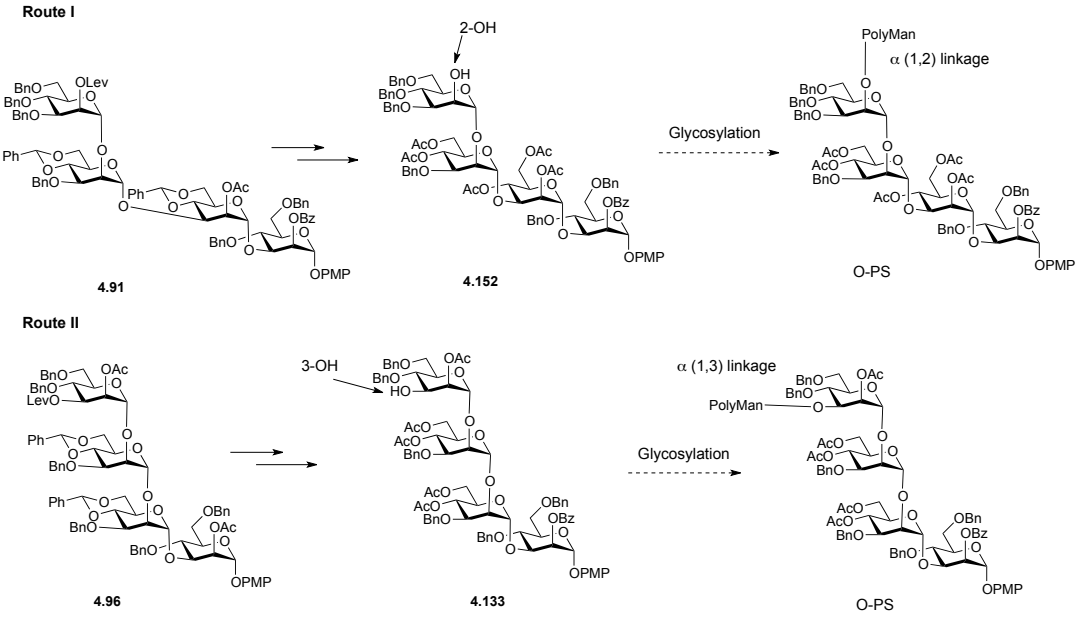
	No. of monosaccharide BBs for RU[a]	Overall yield for three glycosylation steps[b]	Free hydroxyl position as acceptor at the fourth residue
4.91	4	27%	2-OH, less reactive
4.96	3	45%	3-OH, more reactive

[a]BB = Building block; RU = Repeating Unit;

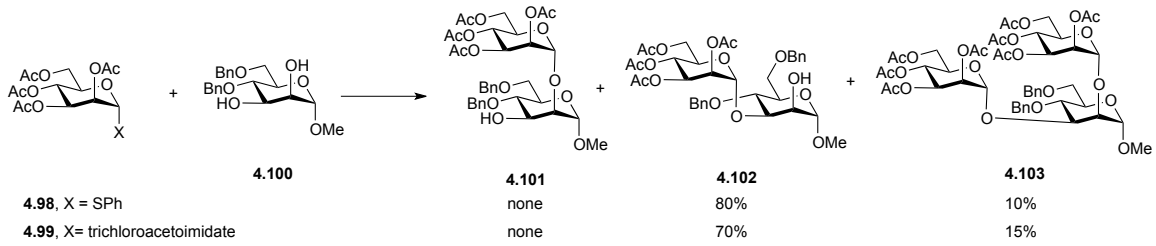
[b]The yield for removal of levulinate groups is similar in all cases and so these reactions are not considered in the analysis.

I then considered the relative glycosylation reactivities that could be expected in both routes in the reactions leading to longer oligomers of the repeating units (Scheme 4-10). In Route I, chain extension would involve the formation of an α-(1→2) glycosidic linkage. In Route II, this process would require generation of an α-(1→3)-linkage. In a previous study the relative reactivity between the C-2 and C-3 hydroxyl groups on mannose has been investigated.[145] In that study, using diol **4.100** (Scheme 4-11) as an acceptor, the equatorial C-3 hydroxyl group was shown to be more reactive than the axial C-2 hydroxyl group. In this case, only the α-(1→3)-disaccharide **4.102** and trisaccharide **4.103** were isolated in reactions between with two different donors (**4.98** and **4.99**); no α-(1→2)-

disaccharide (**4.101**) was observed. After these considerations, I decided to focus on Route II to assemble the targets. Although Route II looks more complicated, it bears three advantages: 1) fewer building blocks are needed for the preparation of the tetrasaccharide bulidng block **4.96**; 2) there is a higher yield in the synthesis of **4.96** compared to **4.91** and 3) the key chain extension process will involve reactions at the more reactive C-3 hydroxyl group.

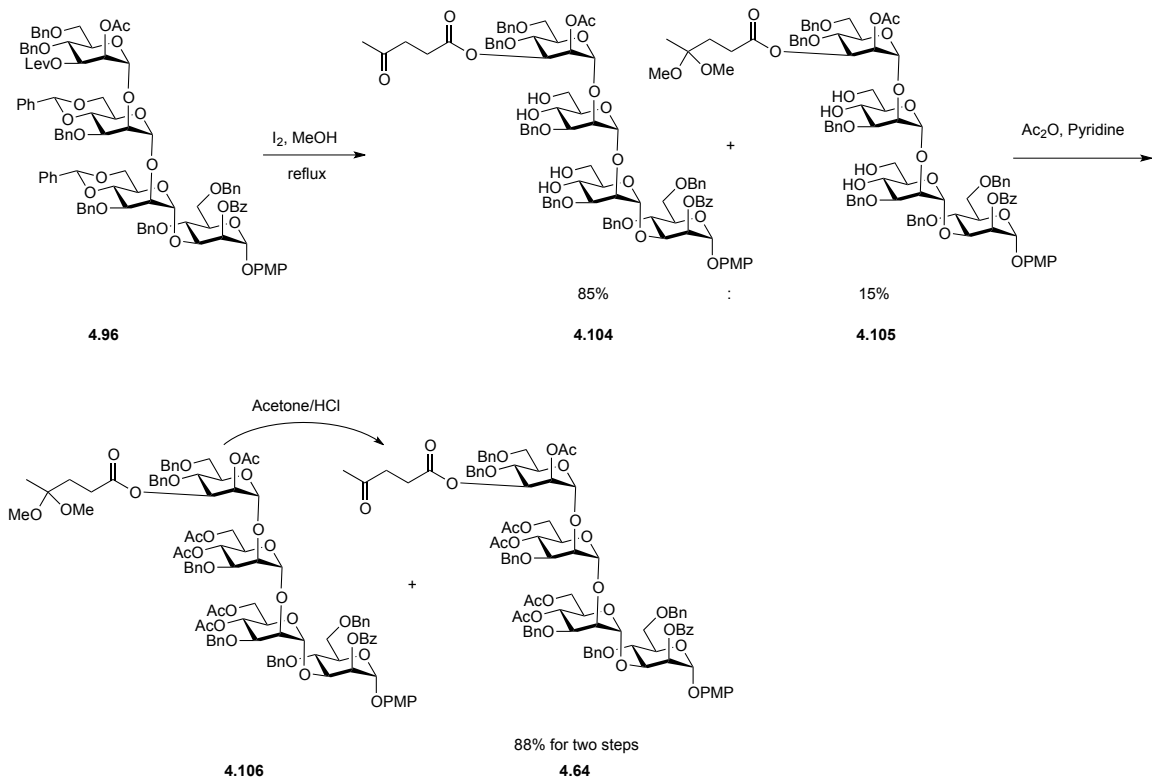


Scheme 4-10: Comparison of the key chain extension reactions for Route I and Route II.



Scheme 4-11: The relative reactivities between the C-2 and C-3 hydroxyl groups on glycosylations with mannoside acceptor **4.100**.

Having decided to procced using Route II, the next step was to convert the two benzylidene acetals in **4.96** to acetyl protecting groups. This was done to simplify the deprotection steps at the end of the synthesis. Initially I explored the use of 4:1 acetic acid–water at 80 °C to hydrolyze the two benzylidene acetals in **4.96**. Unfortunately, the yield for this reaction was only about 60% and a spot at the baseline in the TLC of the reaction was seen, presumably resulting from degradation of the starting material or product. However, these acetals could be removed by reaction with iodine in methanol at reflux. The crude ^{1}H NMR spectrum of the reaction mixture and mass spectrometric data showed that there was about 15% of by-product **4.105** (Scheme 4-12), which was formed by conversion of the ketone in the levulinate ester to a ketal. These compounds are inseparable. Therefore, the mixture of compound **4.104** and by-product **4.105** was treated with Ac_2O and pyridine to give **4.106** and **4.64**. The residue was then dissolved in a 2% solution of HCl in acetone to hydrolyze the ketal in **4.106** to afford the desired product **4.64** in 88% yield over two steps. The $^{1}J_{\text{C-1, H-1}}$ values of the four mannose residues in **4.64** (176.2, 174.9, 172.9 and 172.9 Hz) confirmed the α-stereochemistry.[144]

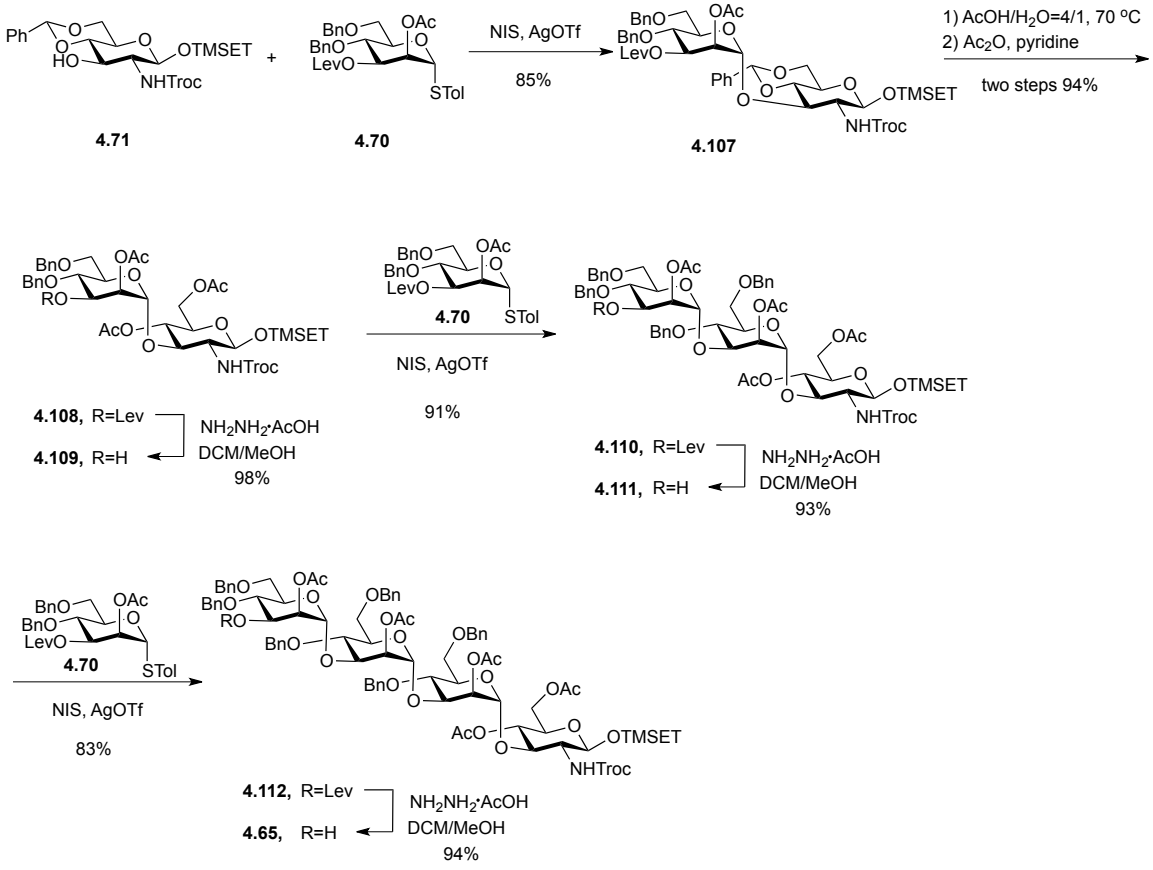


Scheme 4-12: Synthesis of tetrasaccharide **4.64** from **4.96**.

4.3.2.3 Synthesis of the primer–adaptor domain

For the synthesis of the target O-PS using Route II, the protected primer–adaptor tetrasaccharide **4.65** was needed for the reducing end of the molecule (Scheme 4-13). The synthesis started by coupling of glycosyl acceptor **4.71** to mannose thioglycoside donor **4.70** mediated by NIS and AgOTf, which gave disaccharide **4.107** in 85% yield. Next, the benzylidene acetal in **4.107** was hydrolyzed and the resuling diol was acetylated leading to the formation of, in 94% overall yield, **4.108**. Removal of the levulinate ester with hydrazine acetate gave disaccharide alcohol **4.109**, which was subsequently glycosylated

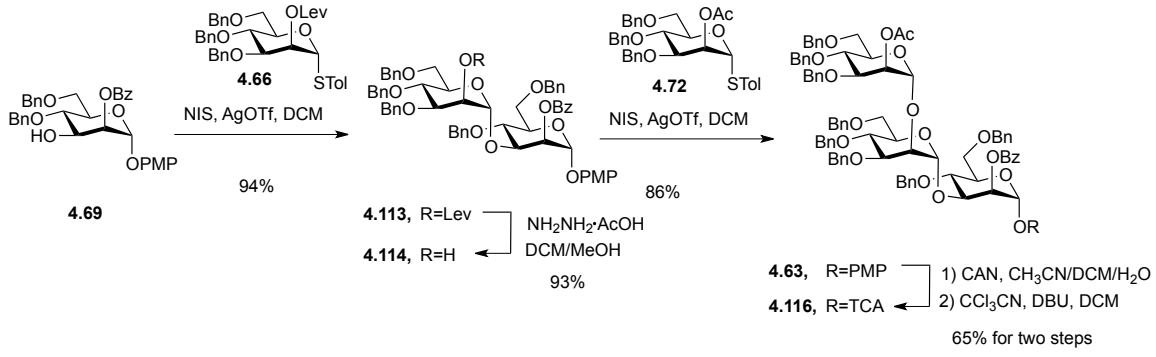
with the donor **4.70** to afford trisaccharide **4.110** in 91% yield. After removal of the levulinate ester group (93% yield), trisaccharide acceptor **4.111** was coupled with donor **4.70** to afford an 83% yield of tetrasaccharide **4.112**. Finally, cleavage of the levulinate ester afford the primer–adaptor tetrasaccharide **4.65** in 94% yield. The $^{1}J_{\text{C-1, H-1}}$ value for the GlcNAc residue (162.2 Hz) confirmed the β-stereochemistry on the reducing end of tetrasaccharide **4.65**. The three $^{1}J_{\text{C-1, H-1}}$ values for the mannose residues (176.8, 172.3, 172.3 Hz) confirmed the α-stereochemistry of these linkages.[144]



Scheme 4-13: Synthesis of primer–adaptor building block **4.65**.

4.3.2.4 Synthesis of the capping motif

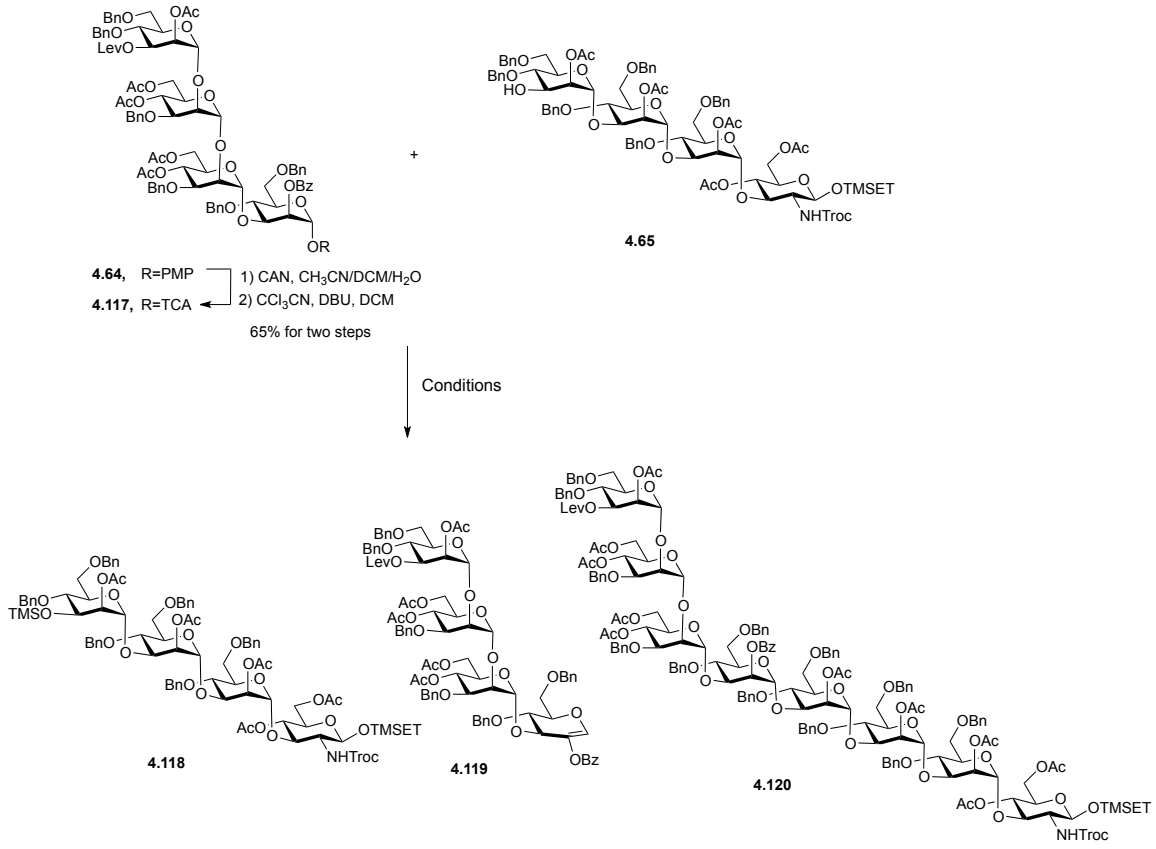
The last piece needed for the undecasaccharide target **4.56** was the trisaccharide cap at the nonreducing end. As outlined in the retrosynthetic analysis above, trisaccharide **4.63** (Scheme 4-14) was the precursor to this piece of the molecule. To synthesize **4.63**, NIS–AgOTf-promoted glycosylation of monosaccharide **4.69** with thioglycoside **4.66** gave disaccharide **4.113** in 94% yield. Subsequent treatment with hydrazine acetate led to the formation of alcohol **4.114** in 93% yield. Then, another glycosylation, this time using donor **4.72**, enabled the conversion of **4.114** to trisaccharide **4.63** in 86% yield. The three $^{1}J_{\text{C-1, H-1}}$ values of the glycosidic linkages (175.0, 171.8 and 172.4 Hz) confirmed the α-stereochemistry of three mannose residues in trisaccharide **4.63**. Finally, a two-step functional group transformation sequence (from OPMP to O-trichloroacetimidate) led to the activated donor **4.116** in 65% yield. The synthesis of the capping species needed for the 27-residue target (**4.57**) is discussed below.



Scheme 4-14: Synthesis of trisaccharide cap **4.116**.

4.3.2.5 Exploration of glycosylation conditions

With the primer–adaptor tetrasaccharide **4.65**, repeating unit tetrasaccharide **4.64**, and trisaccharide cap **4.116** in hand, I turned my attention to assembling the full oligosaccharide and then the prearation of the smaller of the two targets, undecasaccharide **4.56**. I first investigated the glycosylation between these tetrasaccharide **4.64** and **4.65** (Scheme 4–15).



Scheme 4-15: Exploring glycosylation conditions between primer–adaptor tetrasaccharide **4.65** and repeating unit tetrasaccharide **4.117**.

To achive this, it was necessary to convert **4.64** into an activatable species and I chose trichloroacetimidate **4.117** as the donor. This compound was prepared by selective cleavage of the PMP group in **4.64** and subsequent reaction of the resulting hemiacetal with trichloroacetonitrile in the presence of DBU. The desired compound **4.117** was obtained in 65% overall yield. Glycosylation between donor **4.117** and acceptor **4.65** was then explored (Table 4-3). When 0.4 equiv. of TMSOTf was used as promoter (Entry 1), two by-products, **4.118** (35%) and **4.119** (30%), were obtained and the yield for desired product was modest (52%). By-products **4.118** and **4.119** come from acceptor **4.65** and donor **4.117**, respectively. I concluded that under these conditions, the hydroxyl group on **4.65** reacted with the TMSOTf promotor to form the TMS protected by-product **4.118**, which cannot be glycosylated. As a result, some of the trichloroacetimidate **4.117** has no substrate to glycosylate and it eliminates to **4.119**. The chromatographic mobilities of the desired product **4.120** and glycal **4.119** were very close, so it was hard to separate them by column chromatography. To minimize the formation of by-products, I reduced the amount of TMSOTf to 0.2 equiv. (Entry 2). The yield for **4.120** was improved to 65%, however, I still obtained **4.118** (30%) and **4.119** (15%). This suggests that the silylation of **4.117** is rapid and that using a more sterically-demanding Lewis acid as the promotor might result in less of these two byproducts. When using 0.4 equiv. of TBSOTf, the yield of the desired product **4.120** was greatly improved, to 86%, and no by-products **4.118** and **4.119** were formed. Although the newly formed H-1 resonances cannot be identified in the one-dimensional ^{1}H

NMR spectrum due to the overlap, all of the $^{1}J_{C\text{-}1,\ H\text{-}1}$ values could be measured from the ^{1}H-coupled HSQC spectrum. The $^{1}J_{C\text{-}1,\ H\text{-}1}$ value for the GlcNAc residue (161.7 Hz) confirmed the β-stereochemistry on the reducing end of **4.120**. The coupling constants of mannose residues were 175.0, 175.0, 174.3, 174.3, 174.3, 173.6, and 172.2 Hz, which confirmed the α-stereochemistry of the seven mannose residues in **4.120**.[144] Given this finding, I used TBSOTf as the promotor for all trichloroacetimidate glycosylations I did in the synthesis of the targets (see below).

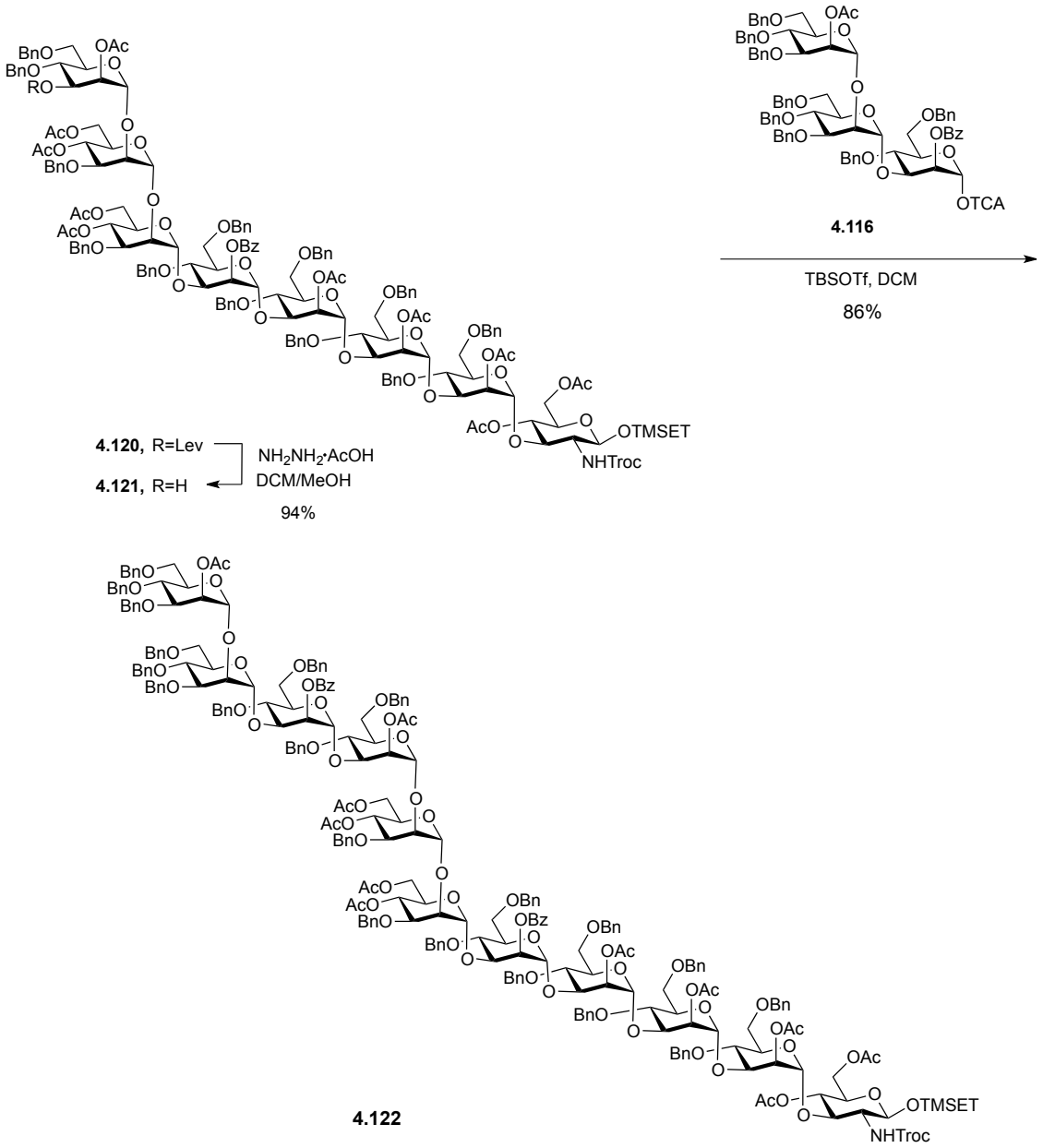
Table 4-3：Conditions explored for the glycosylation reactions between **4.117** and **4.65**

entry	conditions	By-product **4.118** (%)	By-product **4.119** (%)	Product **4.120** (%)
1	Donor **4.117** (1.2 eq.), acceptor **4.65** (1.0 eq.), TMSOTf (0.4 eq.)	35%	30%	52%
2	Donor **4.117** (1.0 eq.), acceptor **4.65** (1.2 eq.), TMSOTf (0.2 eq.)	30%	15%	65%
3	Donor **4.117** (1.0 eq.), acceptor **4.65** (1.2 eq.), TBSOTf (0.4 eq.)	trace	trace	86%

4.3.2.6 Synthesis of undecasaccharide (11-mer)

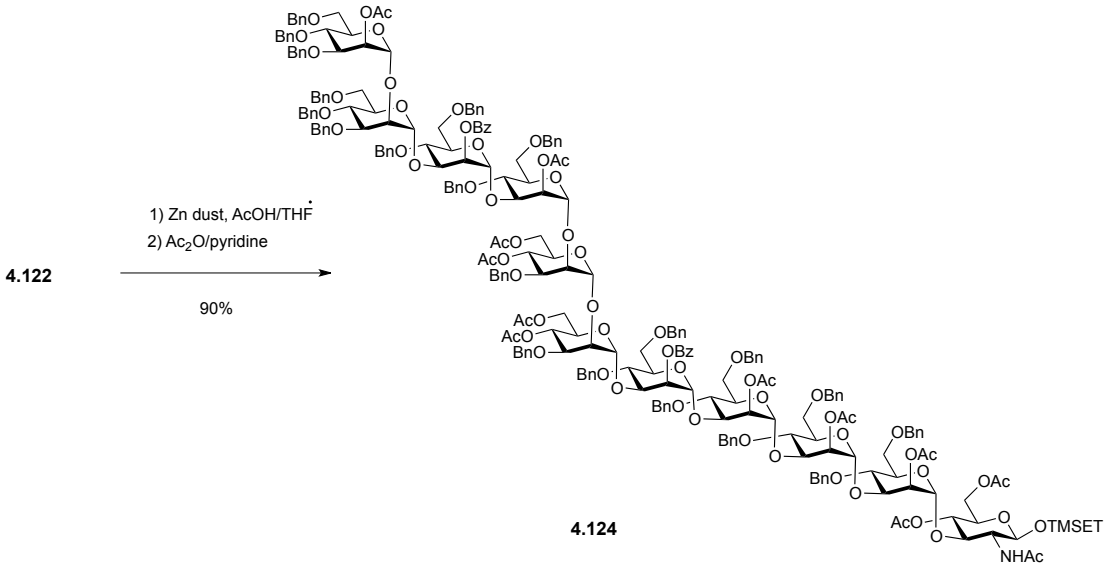
With a synthetic approach (TBSOTf-promoted glycosylation) for the construction of octasaccharide **4.120** deteremined, I turned my attention to undecasaccharide **4.124** (Scheme 4-16). Cleavage of the levulinate protecting group in **4.120** under standard conditions (hydrazine acetate) gave octasaccharide alcohol **4.121** (94% yield), which was

subsequently reacted with an excess of trichloroacetimidate **4.116** to afford the undecasaccharide **4.122**. This [8 + 3] glycosylation provided **4.122** in 86% yield.



Scheme 4-16: Assembly of undecasaccharide **4.122**.

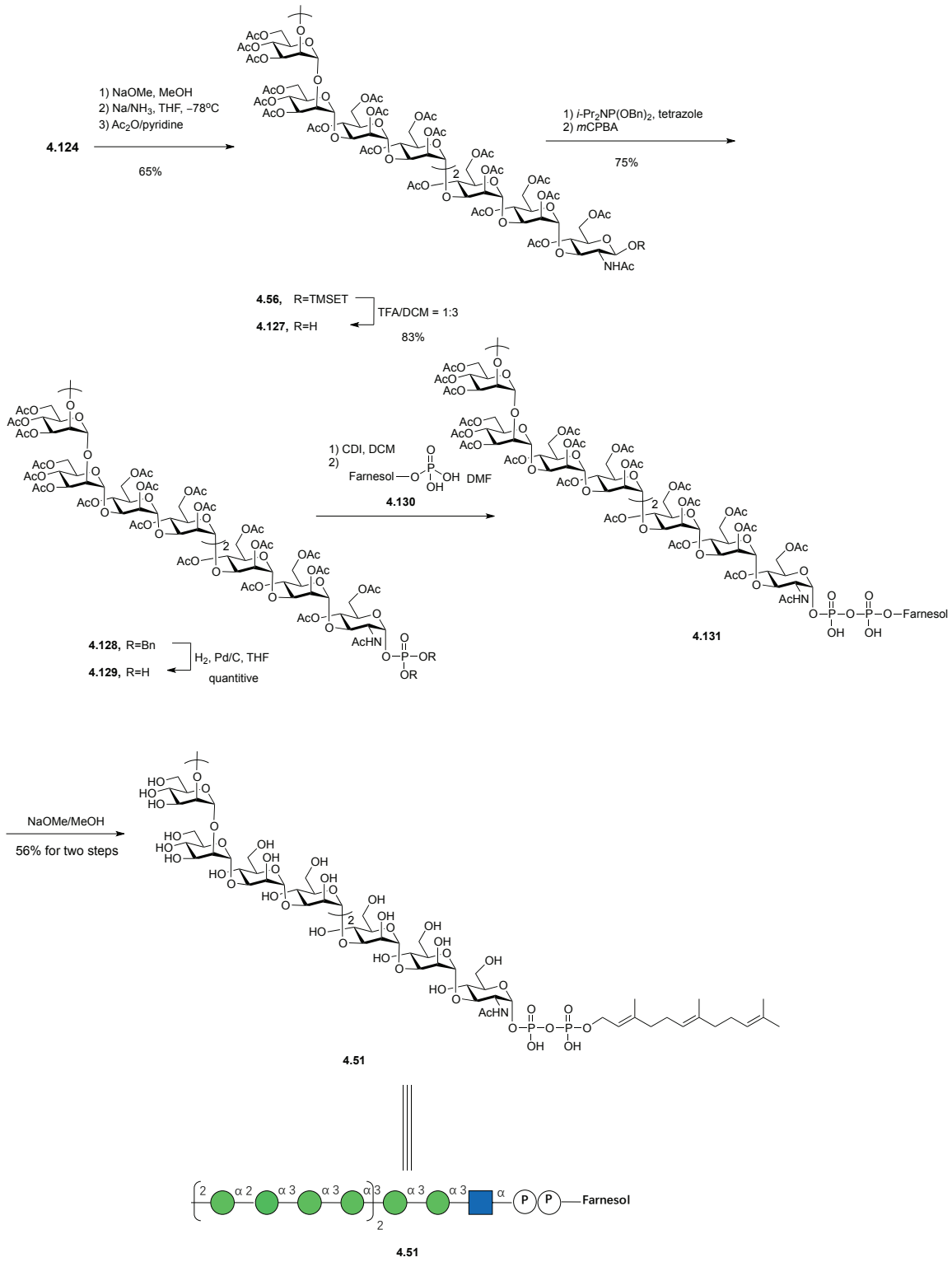
After all of the monosaccharide residues were in place, the focus became changing the functional group on the nitrogen atom, introduction of the phosphate and lipid moiety and the final deprotection. The Troc group in **4.122** was removed via a reductive elimination process, which employed activated zinc in AcOH/THF, to afford a crude product with a free amine group (Scheme 4-17). A common problem of this reaction is the formation of the dichloroethoxy carbamate by-product, which can be minimized by using freshly activated zinc dust. After *N*-acetylation using acetic acid and pyiridine, the Troc protecting group was successfully converted to an acetyl group to give undecasaccharide **4.124** in 90% yield.



Scheme 4-17: Conversion of the *N*-Troc group in **4.122** to an NHAc group.

It was necessary at this stage to remove all of the benzyl ether protecting groups and replace them with acetate groups. This would simplify the deprotection at the end of the

synthesis to a single ester cleavage step with an easy to remove byproduct (methyl acetate). It can be difficult to remove large numbers of benzyl groups in large oligosaccharides using hydrogenolysis; so, I chose to use Birch reduction conditions (Scheme 4-18).



Scheme 4-18: Synthesis of undecasaccharide lipid phosphate **4.51**.

The Birch reduction is a strongly basic reaction and so under these conditions all of the acyl groups are cleaved. It should then be possible to cleave all of the protecting groups in a single step before replacing them with acetate esters. However, after exploring this reaction, I found it more convenient to first cleave the acyl groups, purifying the molecule and then do the dissolving metal reduction. This approach has been used for the synthesis of other moleceules.[146, 147] Therefore, compound **4.124** was treated with sodium methoxide to remove all of the acetyl and benzoyl groups. This partically-deprotected intermediate was taken further to remove benzyl groups under Birch conditions giving a fully deprotected oligosaccharide, which was then acetylated to afford **4.56** in 65% yield over the three steps.

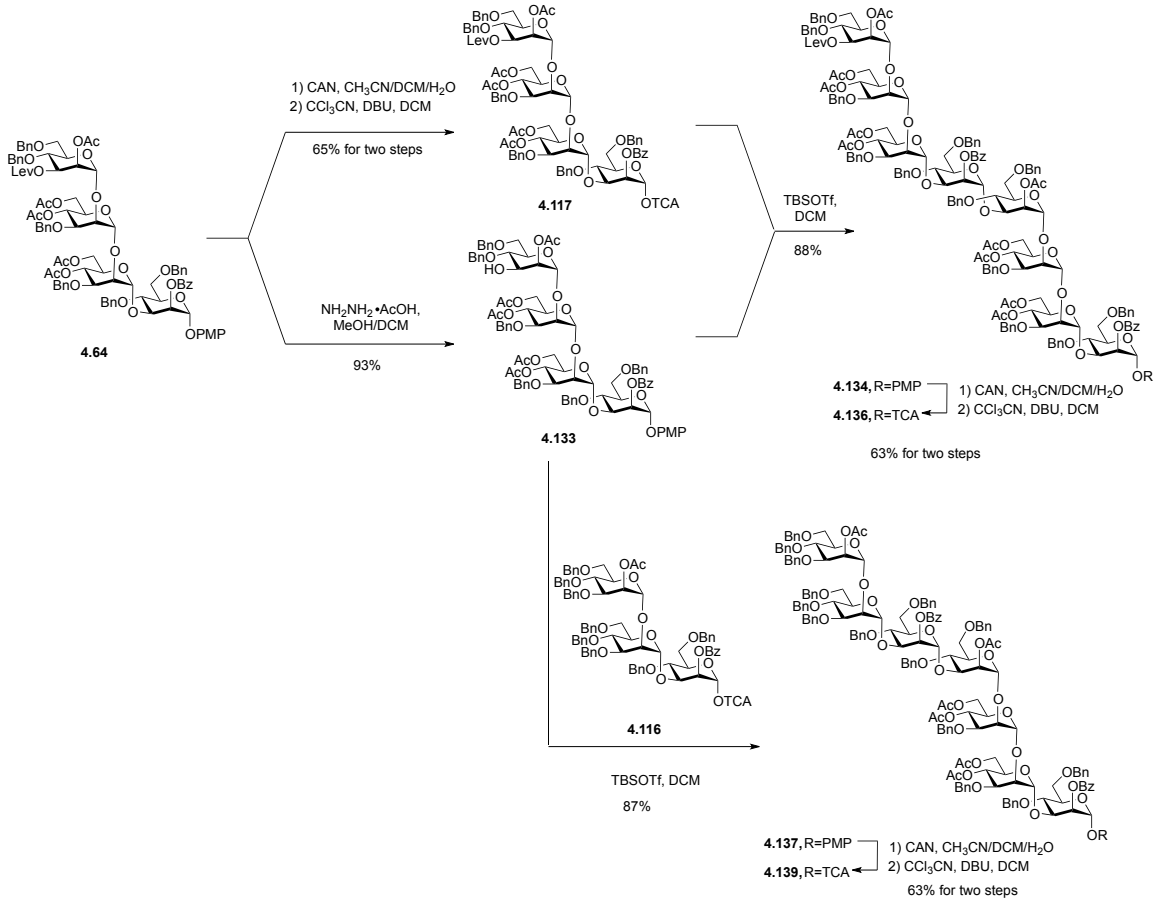
The final steps in the synthesis involved the introduction of the lipid phosphate moiety. To do this, the anomeric TMSET protecting group was removed upon treatment of **4.56** with 25% of TFA in anhydrous dichloromethane to give hemiacetal **4.127** in 83% yield. The resulting hemiacetal **4.128** was treated with dibenzyl *N*,*N*-diisopropyl phosphoramidite and tetrazole to afford a phosphite intermediate, which was oxidized by *m*-CPBA to give a 75% overall yield of glycosyl phosphate **4.128**. After hydrogenolysis of the benzyl groups on the phosphate, the resulting glycosyl phosphate **4.129** was coupled to farnesol phosphate (**4.130**) using a carbonyldiimidazole (CDI)-mediated phosphoesterification. The product, **4.131**, was then deacetylated with catalytic sodium

methoxide in methanol affording the farnesol pyrophosphate-linked oligosaccharide **4.51** in 56% yield from **4.128** over the three steps.

4.3.2.7 Synthesis of building blocks for preparation of the 27-mer target.

Having made the smallest of the two targets, I moved to the preparation of the larger target, **4.52**, with 27 sugar residues. The approach detailed above could be used to make larger oligosaccharides. However, an issue was how to best carry out the chain extension. Using tetrasaccharide **4.64** as a donor for oligosaccharide assembly would allow chain extension only by one repeating unit in each glycosylation. This would be a slow process. I therefore decided to synthesize an octasaccharide donor (a dimer of **4.64**) to facilitate the chain extension. To do this (Scheme 4-19), a tetrasaccharide acceptor (**4.133**) was obtained in 93% yield by removal of the levulinate group in **4.64**. Next, trichloracetimidate donor **4.117** was used to glycosylate **4.133** promoted by TBSOTf to afford the desired octasaccharide **4.134** in 88% yield. Although the newly formed H-1 resonances cannot be identified in the one-dimensional ^{1}H NMR spectrum due to the overlap, all of the $^{1}J_{\text{C-1, H-1}}$ values could be measured from the C-H-coupled HSQC spectrum. These coupling constants were 176.0, 176.0, 175.6, 175.6, 175.5, 173.3, 173.2 and 173.1 Hz, which confirm the α-stereochemistry of the eight mannose residues in **4.134**.[144] This result confirmed that the TBS-promoted (1→3) glycosylation between two Man residues is α-selective. With a route to the octasaccharide in place, it was converted to the

trichloroacetimidate donor **4.136** by selective cleavage of the PMP group and subsequent reaction of the resulting hemiacetal with trichloroacetonitrile in the presence of DBU.



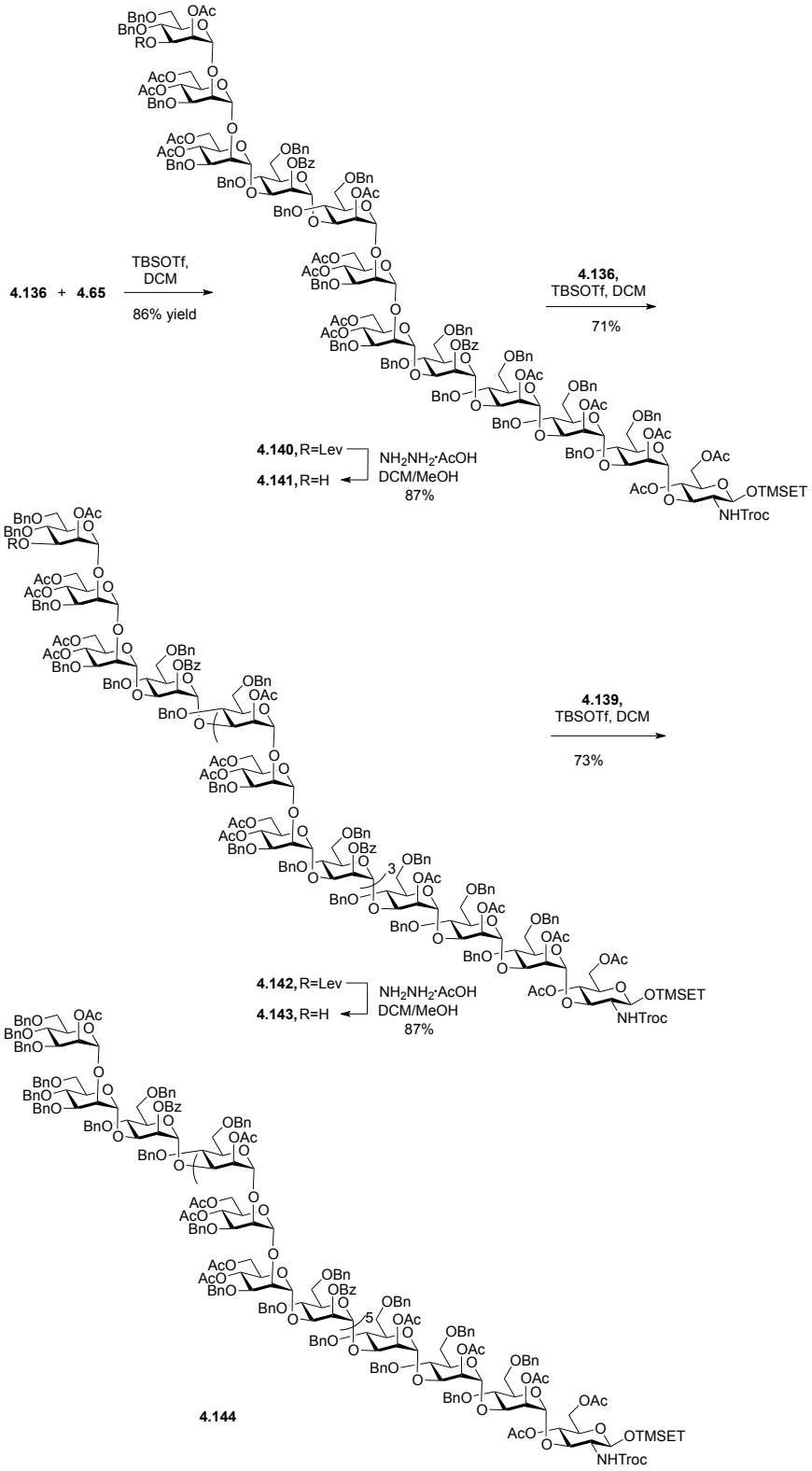
Scheme 4-19: Synthesis of octasaccharide **4.136** and heptasaccharide **4.139** donors.

With both the primer–adaptor tetrasaccharide **4.65** and octasaccharide donor **4.136** in hand, the 20-mer could be synthesized by following a 4 + 8 + 8 sequence. To obtain the desired 27-mer, I needed to synthesize a heptasaccharide donor (**4.139**, Scheme 4-19), which was analogus to the trisaccharide 'cap' needed for the undecasaccharide target. As shown in Scheme 4-19, glycosylation of tetrasaccharide acceptor **4.133** with trisaccharide donor **4.116** in presence of TBSOTf led to the desired heptasaccharide **4.137** in 87% yield.

The compound could be converted to heptasaccharide donor **4.92** in 63% overall yield using the same method used for the synthesis of octasaccharide donor **4.136**: PMP glycoside cleavage and conversion of the product hemiacetal to the trichloroacetimidate.

4.3.2.8 Assembly of the 27-mer

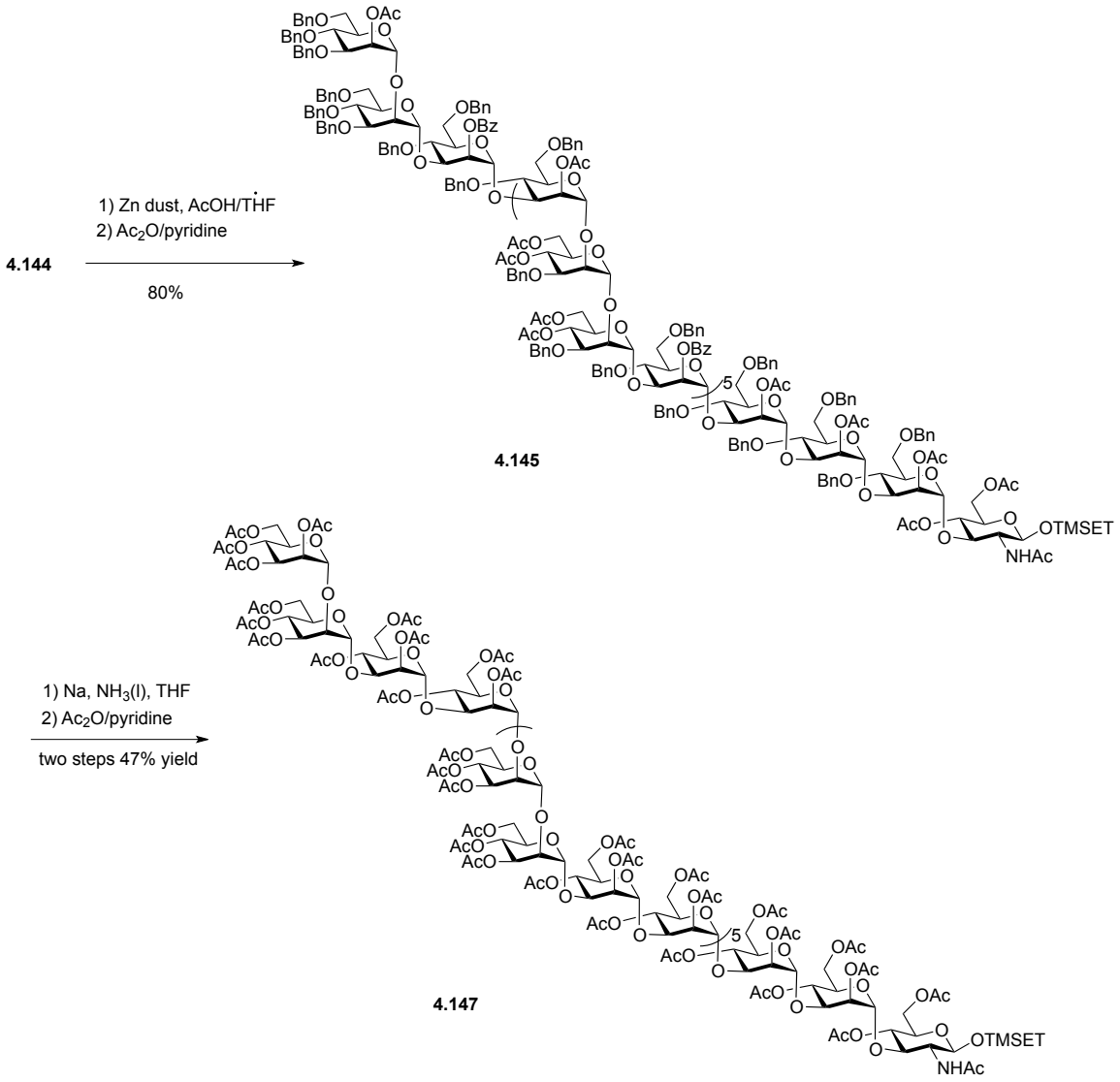
Having established the synthesis of three required building blocks (primer–adaptor tetrasaccharide **4.65**, octasaccharide donor **4.136** and heptasaccharide donor **4.139**), the assembly of the 27-mer was achieved using a 4 + 8 + 8 + 7 reaction sequence. As shown in Scheme 4-20, the [4 + 8] glycosylation between primer–adaptor acceptor **4.65** and octasaccharide donor **4.136** using TBSOTf as the promotor proceeded in 86% yield. When I explored the subsequent deprotection of the levulinate ester on the 12-mer **4.140**, I found that this reaction was very slow at room temperature. In the ^{1}H NMR spectrum of 12-mer **4.140**, the resonance for H-3 of the terminal mannose residue (the hydrogen adjacent to the levulinate ester) appears at 5.37 ppm as a doublet of doublets (J = 9.5, 3.5 Hz). This signal can be easily identified in the crude ^{1}H NMR spectrum of the mixture and this method was used to follow the reation as it was not possible to do so by chromatography. After four hours at room temperature, the spectrum showed that only 15% of the levulinate group was removed. To achieve full deprotection, I used a rotary evaporator to concentrate the reaction mixture and then kept the flask rotating for 30 min at 40 °C. Under these conditions, the crude ^{1}H NMR spectrum showed complete disappearance of the peak at 5.37 ppm, suggesting 100% conversion, and the yield of



Scheme 4-20: Assembly of protected 27-mer **4.144**.

4.141 was 87% after purification. Having successfully solved this deprotection problem, I tried the TBSOTf-mediated glycosylation again using the 12-mer acceptor **4.141** and octasaccharide donor **4.136** to obtain an oligosaccharide with 20 monosaccharide residues. However, it was necessary to extend the reaction time to 12 hours at room temperature to give the desired 20-mer **4.142**. Compared to the glycosylation reactions described above, the yield for [12 + 8] glycosylation was relatively low (71%). The levulinate ester group on **4.142** was removed by using the same method for deprotection as that used on the 12-mer **4.140**. Finally, using 20-mer **4.140** as the acceptor and heptasaccharide **4.139** as the donor, TBSOTf-promotied glycoslation generated the desried 27-mer **4.97** in 73% yield.

With the oligosaccharide core of the molecule assembled, the next step was the exchange of the Troc group on the nitrogen for an acetate (Scheme 4-21). This could be achieved with the same reaction sequence used for the synthesis of the undecasaccharide target: removal of Troc group using activated zinc in acetic acid and then *N*-acetylation with acetic anhydride and pyridine. Compound **4.145**, was obtained in 80% yield after these transformations.



Scheme 4-21: Protecting group transformations of **4.144** to afford **4.147**.

After successful construction of 27-mer **4.145**, deprotection by Birch reduction to remove all of the benzyl ethers was investigated (Scheme 4-21). Initially, I removed all acetate and benzoate groups of **4.145** using sodium methoxide in methanol to afford a partially deprotected product as I did for the undecasaccharide target. However, this deacylated product was not soluble in THF, the solvent for the Birch reduction. Therefore,

it was necessary to use the fully protected molecule **4.145** in this step. As mentioned earlier, under these strongly basic reaction conditions, all of the acyl protecting groups would be removed together with the benzyl ethers. When I followed the conditions used for undecasaccharide (Table 4-4, Entry 1), the ratio between the desired product and by-product(s), in which the 2-(trimethylsilyl)ethyl (TMSEt) group appeared to had been lost, was 1:1.5 as determined from the ^{1}H NMR spectrum of the crude reaction mixture. The exact structure of the byproducts was impossible to determine by ^{1}H NMR spectroscopy and mass spectroscopy of the crude mixtures showed a number of species with molecular weights lower than that of the desired

Table 4-4： Conditions explored for Birch reduction of **4.145**.

Entry	Reaction temp. (°C)	Reaction time (h)	Quench method	Product : by-product[a]
1	–70	4	Amberlite IR-120 resin	1:1.5
2	–70	4	NH_4Cl	1:1.5
3	–70	1.5	Amberlite IR-120 resin	1:0.5
4	–80	1.5	Amberlite IR-120 resin	1:0

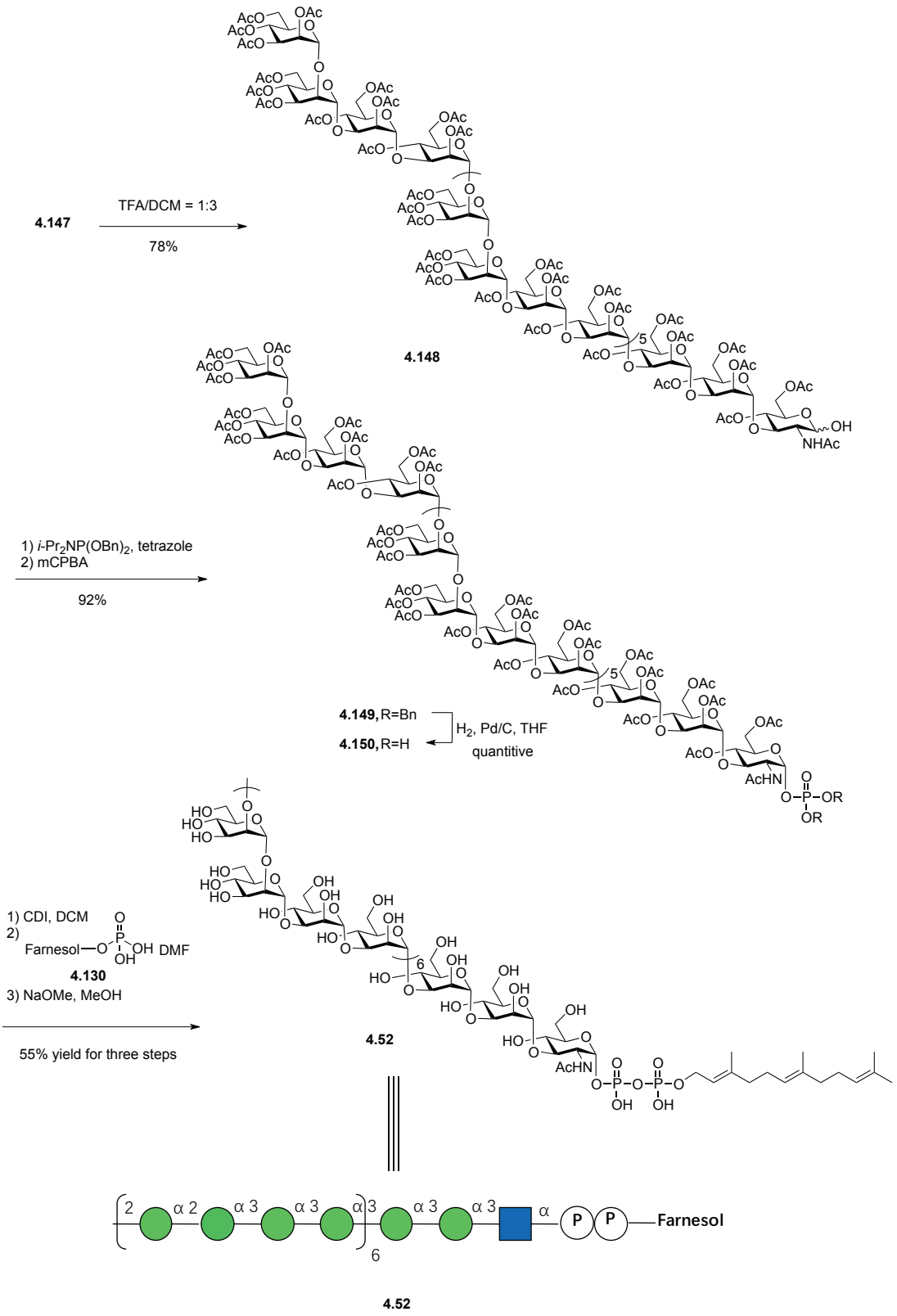
[a] Ratios were obtained from the ^{1}H NMR spectrum of the crude product. The integration of TMS group (0.08 ppm) was assigned to 9. The total amount of anomeric hydrogens of Man residues (5.10–5.50 ppm) were then integrated. In theory, there are 26 hydrogens from 5.10 ppm to 5.50 ppm. The ratio was obtained from this equation: Ratio = (Found anomeric Number – 26):26.

product. In addition to dramatically lowering the yield, the product and by-products were impossible to separate.

Initially, I thought that the TMSEt group might be removed when I quenched the mixture using Amberlite IR-120 resin, which is strongly acidic. However, when I used the NH_4Cl to quench the reaction mixture (Entry 2), the ratio between the product and by-product did not change. In addition, when the product and by-product mixture was mixed with Amberlite IR-120 resin in methanol and water overnight, the ratio did not change. These results indicate that the quenching method was not the reason for by-product formation. Then, I shortened the reaction time to 1.5 h (Entry 3) and the ratio became to 1:0.5. Finally, I discovered that decreasing the reaction temperature to –80 ^{o}C (Entry 4) resulted in only a trace amount of the by-product being produced. Under these conditions, the two step yield for the Birch reduction and acetyl protection (using acetic anhydride and pyridine) was improved to 47%.

Finally, with a fully acetylated molecule in hand, I proceeded to the preparation of the target **4.52** (Scheme 4-22). Removal of the TMSEt group using TFA in dichloromethance gave oligosaccharide **4.148** in 78% yield. This compound was then treated with tetrazole and dibenzyl *N*,*N*-diisopropylphosphoramidite to generate the corresponding phosphite intermediate, which was subsequently oxidized by *m*-CPBA to afford, in 92% yield, oligosaccharide phosphate **4.149**. After removal of the benzyl groups on phosphate **4.149** by hydrogenolysis, coupling between the resulting glycosyl 1-phosphate **4.150** and farnesol

phosphate (**4.130**), mediated by CDI, led to the formation of protected glycosyl phospholipid, which was then deacetylated using sodium methoxide in a mixture dichloromethane in methanol. The desired product **4.52** was obtained in 55% yield over three steps.



Scheme 4-22: Synthesis of 27-mer **4.52**.

4.4 Summary

In summary, a chemical approach was developed to synthesize two lipid-linked O-PS (**4.51** and **4.52**) related to intermedites present in LPS O-chain biosynthesis in *E. coli* O9a. The first target, **4.51**, is made up of 11 monosaccharide residues, including ten mannose residues and one glucosamine residue. This structure contains two tetrasaccharide repeating units and one primer–adaptor trisaccharide, linked through a pyrophosphate moiety to farnesol. The second target, **4.52**, containing 27 monosaccharide residues, has a similar structure to **4.51**. However, instead of two repeating units, target **4.52** contains six repeating units.

The approach started with the chemical synthesis of mannose related building blocks (**4.66**–**4.72**) from D-mannose and another starting from D-glucosamine. Then, two synthetic routes were proposed and two repeating units (**4.61** and **4.64**) were synthesized using these building blocks using an iterative cycle of NIS/AgOTf-promoted glycosylations and hydrazine acetate-mediated levuninate ester cleavages. After comparing the synthetic efficiencies of routes using the two repeating units, we found that one, Route II, which involved tetrasacchariede **4.64**, was preferred. The same general approach, using NIS/AgOTf-promoted glycosylations and levulinate ester cleavage with hydrazine acetate, was employed to synthesize the primer–adaptor (**4.65**) and cap (**4.63**) motifs. Finally, a TBSOTf-promoted glycosylation method was developed for the glycosylations between oligosaccharide acceptors and an imidate donors generated from

the oligosaccharide building blocks. Excellent yields (82%–88%) were obtained for glycosylations with short oligosaccharide acceptors and donors. Following a 4 + 4 + 3 strategy, the 11-mer **4.122** was synthesized in good yield. After six additional steps, including Birch reduction to cleave the benzyl ether protecting group, the hemiacetal **4.127** was obtained and further coupled with farnesol phosphate to give **4.131**. The final deprotection of **4.131** afforded the target undecassaccharide **4.51**.

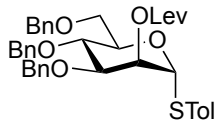
For the synthesis of 27-mer **4.52**, I adopted a 4 + 8 + 8 + 7 strategy. The yields for reactions between these large oligosaccharide motifs, such as the [12 + 8] and [20 + 7] glycosylations, were lower than for the shorter oligosaccharides, around 70%. For this target, the removal of the benzyl groups using Birch reduction was difficult. We found that the reaction temperature, reaction time and the concentration were important to prevent the formation of by-products. After several attempts, I discovered that low temperature (–80 °C), short reaction times (1–1.5 h) and low concentrations (0.0025 mmol/mL) were needed for this reaction to succeed. Following the same reaction sequence as that use for the 11-mer, the target 27-mer, **4.52**, was obtained.

4.5 Experimental section

General Methods: Reactions were carried out in oven-dried glassware. All reagents used were purchased from commercial sources and were used without further purification unless

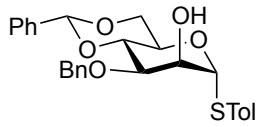
noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless stated otherwise, all reactions were carried out at r.t. under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with a solution of ammonium molybdate (12 g), ceric ammonium nitrate (0.42 g) and concentrated sulfuric acid (15 mL) in H_2O (235 mL). Unless otherwise indicated, all column chromatography was performed on silica gel 60 (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of deg·mL(dm·g)$^{-1}$. ^{1}H NMR spectra were recorded at 500 or 700 MHz, and chemical shifts are referenced to either TMS (0.0 ppm, $CDCl_3$) or HOD (4.78 ppm, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 150 or 175 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23 ppm, $CDCl_3$), external dioxane (67.40 ppm, D_2O) or CD_3OD (48.9 ppm, CD_3OD). The stereochemistry of the newly formed glycosidic linkages was confirmed by measuring $^1J_{C\text{-}1,\,H\text{-}1}$ values via an ^{1}H-coupled HSQC experiment. In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were concentrated under vacuum at < 40˚C (bath). Electrospray mass spectra (time-of-light analyzer) were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. MALDI mass spectrum was obtained in the linear positive mode of ionization on a MALDI TOF/TOF mass spectrometer using sinaoinic acid as the matrix.

General procedure A. Removal of PMP protecting group and formation of an trichloroacetimidate donors: CAN (5 equiv.) was added to a solution of compound **4.63**, **4.64**, **4.134** or **4.137** (1 equiv.) in 1:3:6 H_2O–CH_2Cl_2–CH_3CN (10 mL–300 mL, depending upon the amount of substrate) at 0 °C. The mixture was slowly warmed and vigorously stirred for 2 h at r.t.. The solution was then diluted with EtOAc and the organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography to afford the corresponding hemiacteal. Then, to a solution of the hemiacetal in dry CH_2Cl_2 (10 mL–200 mL) was added CCl_3CN (25 equiv.) and DBU (0.2 equiv.) at 0 °C and the mixture was stirred at r.t. for 1 h. The solution was then concentrated and the resulting residue was subjected to chromatography to afford the trichloroacetimidate intermediate for glycosylation reactions.



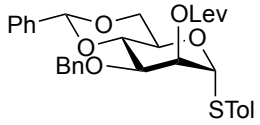
***p*-Tolyl 3,4,6-tri-*O*-Benzyl-2-*O*-levulinyl-1-thio-α-D-mannopyranoside (4.66):** To a solution of **4.80**[140] (200 mg, 0.36 mmol) in CH_2Cl_2 (15 mL) was added levulinic acid (83 mg, 0.72 mmol), EDC (137 mg, 0.72 mmol) and DMAP (4.4 mg, 0.036 mmol). The mixture was stirred at r.t. overnight and then water was added and the mixture was extracted with CH_2Cl_2. The organic phase was washed with a satd aq solution of $NaHCO_3$, brine,

dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 16→20% EtOAc in hexane) to afford **4.66** (222 mg, 95% yield) as a colorless oil. R_f 0.38 (2:1 hexane–EtOAc); $[\alpha]_D$ = +71.0 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.35–7.26 (m, 15 H, ArH), 7.20–7.19 (m, 2 H, ArH), 7.05–7.04 (m, 2 H, ArH), 5.58 (dd, 1 H, *J* = 3.0, 1.5 Hz, H-2), 5.43 (d, 1 H, *J* = 1.5 Hz, H-1), 4.87 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.70 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.63 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.54 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.53 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.46 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.33 (ddd, 1 H, *J* = 9.0, 5.0, 1.5 Hz, H-5), 3.93 (dd, 1 H, *J* = 9.0, 3.0 Hz, H-3), 3.90 (app t, 1 H, *J* = 9.0 Hz, H-4), 3.83 (dd, 1 H, *J* = 11.0, 5.0 Hz, H-6), 3.73 (dd, 1 H, *J* = 11.0, 2.0 Hz, H-6), 2.71–2.66 (m, 4 H, CH_3C=OC*H*$_2$, OC=OC*H*$_2CH_2$), 2.30 (s, 3 H, CH_3PhS), 2.11 (s, 3 H, C*H*$_3$C=OCH_2); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.2 (CH_3*C*=OCH_2), 171.9 (O*C*=OCH_2CH_2), 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 137.7 (Ar), 132.4 (Ar), 129.8 (Ar), 129.7 (Ar), 128.4 (Ar), 128.33 (Ar), 128.28 (Ar), 128.16 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.67 (Ar), 127.54 (Ar), 86.4 (C-1), 78.4 (C-3), 75.2 (Ph*C*H$_2$), 74.5 (C-4), 73.3 (Ph*C*H$_2$), 72.4 (C-5), 71.7 (Ph*C*H$_2$), 70.4 (C-2), 68.9 (C-6), 37.9 (CH_3C=O*C*H$_2$), 29.8 (*C*H$_3$C=OCH_2), 28.1 (CH_3C=OCH_2*C*H$_2$), 21.1 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{39}H_{42}NaO_7S$: 677.2543. Found: 677.2554.



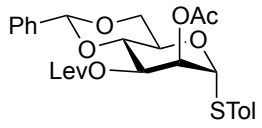
***p*-Tolyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-1-thio-α-D-mannopyranoside (4.79):** Compound **4.77**[141] (5.0 g, 13.3 mmol) was suspended in toluene (120 mL) treated with *n*-Bu_2SnO (4.0 g, 1.6 mmol) and heated at reflux for 6 h with a Dean–Stark trap. The reaction mixture was cooled to r.t. and then BnBr (3.18 g, 18.6 mmol), cesium fluoride (2.2 g, 14.6 mmol) and TBAI (5.38 g, 14.6 mmol) were added. The resulting mixture was stirred at 110 °C for 2 h. After cooling to r.t., the mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.79** (5.21 g, 85% yield) as a white foam; R_f 0.50 (2:1 hexane–EtOAc); $[\alpha]_D$ = +230.5 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.51–7.50 (m, 2 H, ArH), 7.39–7.31 (m, 10 H, ArH), 7.12–7.11 (m, 2 H, ArH), 5.61 (s, 1 H, PhC*H*$(O)_2$), 5.51 (d, 1 H, *J* = 1.0 Hz, H-1), 4.89 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.74 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.42 (app td, 1 H, *J* = 10.0, 5.0 Hz, H-5), 4.27 (app dt, 1 H, *J* = 3.5, 1.4 Hz, H-2), 4.20 (dd, 1 H, *J* = 10.3, 5.0 Hz, H-6), 4.16 (app t, 1 H, *J* = 10.5 Hz, H-4), 3.96 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.84 (app t, 1 H, *J* = 10.3 Hz, H-6), 2.81 (d, 1 H, *J* = 1.4 Hz, OH), 2.33 (s, 3 H, CH_3PhS); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.1 (Ar), 137.8 (Ar), 137.5 (Ar), 132.5 (Ar), 130.0 (Ar), 129.4 (Ar), 129.0 (Ar), 128.6 (Ar), 128.3 (Ar), 128.1 (Ar), 127.9 (Ar), 126.1 (Ar), 101.6 (PhCH$(O)_2$), 88.2 (C-1), 79.1

(C-4), 75.7 (C-3), 73.2 (Ph*C*H_2), 71.4 (C-2), 68.6 (C-6), 64.5 (C-5), 21.1 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{27}H_{28}NaO_5S$: 487.1550. Found: 487.1560.



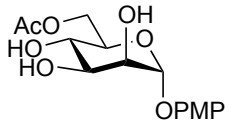
***p*-Tolyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-2-*O*-levulinyl-1-thio-α-D-mannopyranoside (4.67):** To a solution of **4.79** (1.2 g, 2.37 mmol) in CH_2Cl_2 (100 mL) was added levulinic acid (0.55 g, 4.74 mmol), EDC (0.91 g, 4.74 mmol) and DMAP (30 mg, 0.24 mmol). The mixture was stirred at r.t. overnight and then water was added and the mixture was extracted with EtOAc. The organic phase was washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.67** (1.24 g, 92% yield) as a white foam. R_f 0.16 (3:1 hexane–EtOAc); $[\alpha]_D$ = +91.0 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.52–7.51 (m, 2 H, ArH), 7.39–7.26 (m, 10 H, ArH), 7.12–7.11 (m, 2 H, ArH), 5.63 (s, 1 H, PhC*H*$(O)_2$), 5.59 (dd, 1 H, *J* = 3.3, 1.2 Hz, H-2), 5.38 (d, 1 H, *J* = 1.0 Hz, H-1), 4.70 (d, 1 H, *J* = 12.5 Hz, PhCH_2), 4.67 (d, 1 H, *J* = 12.5 Hz, PhCH_2), 4.36 (app td, 1 H, *J* = 10.0, 5.0 Hz, H-5), 4.22 (dd, 1 H, *J* = 10.3, 5.0 Hz, H-6), 4.09 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.00 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3), 3.84 (app t, 1 H, *J* = 10.3 Hz, H-6), 2.79–2.65 (m, 4 H, 2 x CH_2), 2.32 (s, 3 H, CH_3PhS), 2.16 (s, 3 H, CH_3C=O); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.1 (CH_3*C*=OCH_2), 171.9 (O*C*=OCH_2), 138.4 (Ar), 137.8 (Ar),

137.4 (Ar), 132.7 (Ar), 130.0 (Ar), 129.1 (Ar), 129.0 (Ar), 128.4 (Ar), 128.2 (Ar), 127.8 (Ar), 127.7 (Ar), 126.1 (Ar), 101.6 ($PhCH(O)_2$), 87.3 (C-1), 78.6 (C-4), 74.0 (C-3), 72.3 ($PhCH_2$), 71.5 (C-2), 68.5 (C-6), 65.0 (C-5), 38.0 ($CH_3C{=}OCH_2$), 29.8 ($CH_3C{=}OCH_2$), 28.0 ($CH_3C{=}OCH_2CH_2$), 21.1 (CH_3); HRMS (ESI) calcd for (M+Na) $C_{32}H_{34}NaO_7S$: 585.1920. Found: 585.1917.



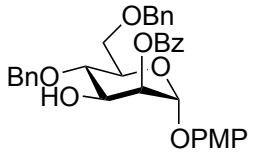
***p*-Tolyl 2-*O*-Acetyl-4,6-*O*-benzylidene-3-*O*-levulinyl-1-thio-α-D-mannopyranoside (4.68):** To a solution of **4.78**[148] (240 mg, 0.58 mmol) in CH_2Cl_2 (250 mL) was added levulinic acid (133 mg, 1.15 mmol), EDC (219 mg, 1.15 mmol) and DMAP (7.3 mg, 0.06 mmol). The mixture was stirred at r.t. overnight and then water was added and the mixture was extracted with EtOAc. The organic phase was washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 20→25% EtOAc in hexane) to afford **4.68** (266 mg, 90% yield) as a colorless oil. R_f 0.31 (3:2 hexane–EtOAc); $[\alpha]_D$ = +145.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.48–7.47 (m, 2 H, ArH), 7.38–7.35 (m, 5 H, ArH), 7.13–7.11 (m, 2 H, ArH), 5.59 (s, 1H, $PhCH(O)_2$), 5.58 (d, 1 H, *J* = 3.5 Hz, H-2), 5.38 (dd, 1 H, *J* = 10.0, 3.5 Hz, H-3), 5.35 (s, 1 H, H-1), 4.46 (app td, 1 H, *J* = 10.0, 5.0 Hz, H-5), 4.24 (dd, 1 H, *J* = 10.0, 5.0 Hz, H-6), 4.12 (app t, 1 H, *J* = 10.0 Hz, H-4), 3.85 (dd, 1 H, *J*

= 11.0, 1.5 Hz, H-6), 2.74–2.71 (app t, 2 H, J = 6.5 Hz, $CH_3C{=}OCH_2$), 2.60 (dt, 1 H, J = 17.0, 6.5 Hz, $OC{=}OCH_2CH_2$), 2.52 (dt, 1 H, J = 17.0, 6.5 Hz, $OC{=}OCH_2CH_2$), 2.32 (s, 3 H, CH_3PhS), 2.15 (s, 3 H, $CH_3C{=}OCH_2$), 2.14 (s, 3 H, $OC{=}OCH_3$); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.1 ($CH_3C{=}OCH_2$), 171.7 ($OC{=}OCH_2CH_2$), 169.8 ($OC{=}OCH_3$), 138.4 (Ar), 137.0 (Ar), 132.8 (Ar), 129.9 (Ar), 129.1 (Ar), 129.0 (Ar), 128.3 (Ar), 126.2 (Ar), 101.9 ($PhCH(O)_2$), 87.1 (C-1), 76.2 (C-4), 71.4 (C-2), 68.9 (C-3), 68.4 (C-6), 65.1 (C-5), 37.9 ($CH_3C{=}OCH_2$), 29.8 ($CH_3C{=}OCH_2$), 27.9 ($CH_3C{=}OCH_2CH_2$), 21.1 (CH_3), 20.8 ($OC{=}OCH_3$); HRMS (ESI) calcd for (M+Na) $C_{27}H_{30}NaO_8S$: 537.1554. Found: 537.1552.



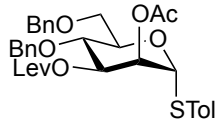
***p*-Methoxyphenyl 6-*O*-Acetyl-α-D-mannopyranoside (4.75):** *p*-Methoxyphenyl α-D-mannopyranoside **4.74**[138] (4.2 g, 14.7 mmol) was dissolved in sym-collidine (100 mL) and the solution was cooled to –35 °C. Acetyl chloride (2.3 g, 29.4 mmol) was then added dropwise over 30 min under vigorous stirring. After 2 h, CH_3OH (5 mL) was added, and the reaction was warmed to r.t. The crude mixture was concentrated and purified by chromatography (gradient 10→33% acetone in CH_2Cl_2) to afford **4.75** (3.7 g, 77% yield) as a white solid. R_f 0.14 (1:3 hexane–EtOAc); $[\alpha]_D$ = +63.9 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 6.96–6.95 (m, 2 H, ArH), 6.78–6.76 (m, 2 H, ArH), 5.43 (s, 1 H, H-1), 4.46 (dd, 1 H, J = 12.1, 5.5 Hz, H-6), 4.22 (dd, 1 H, J = 12.1, 2.0 Hz, H-6), 4.16 (dd, 1 H,

J = 3.4, 1.5 Hz, H-2), 4.04 (dd, 1 H, *J* = 9.4, 3.4 Hz, H-3), 3.86 (ddd, 1 H, *J* = 9.8, 5.5, 2.0 Hz, H-5), 3.74 (app t, 1 H, *J* = 9.8 Hz, H-4), 3.73 (s, 3 H, OCH_3), 2.01 (s, 3 H, OC=OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 172.2 (C=O), 155.1 (Ar), 150.0 (Ar), 117.8 (Ar), 114.6 (Ar), 98.8 (C-1), 71.4 (C-3), 71.0 (C-5), 70.5 (C-2), 67.6 (C-4), 63.5 (C-6), 55.6 (CH_3O), 20.9 (OC=OCH_3); HRMS (ESI) calcd for (M+Na) $C_{15}H_{20}NaO_8$: 351.1050. Found: 351.1045.



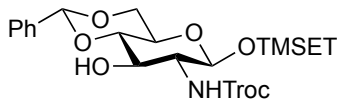
***p*-Methoxyphenyl 2-*O*-Benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.69):** *p*-Toluenesulfonic acid monohydrate (95 mg, 0.5 mmol) was added to a solution of **4.75** (0.73 g, 2.22 mmol) and trimethylorthobenzoate (3 mL) in DMF (0.7 mL) under N_2. After 1 h additional DMF (4.2 mL) was added and the suspension was cooled to 0 °C. NaH (60% in mineral oil, 528 mg, 13.2 mmol) was added and the mixture was stirred at 0 °C for 15 min. Then, benzyl bromide (1.14 g, 6.66 mmol) was added dropwise. After a further 1 h, ice water was added to the solution and the mixture was warmed to r.t. The organic phase was extracted with CH_2Cl_2. The organic extract was stirred vigorously in the presence of 1 M HCl for 1 h. At this time the mixture was diluted with CH_2Cl_2 and washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 14→25% EtOAc in hexane) to afford

4.69 (1.09 g, 86% yield) as a colorless oil; R_f 0.56 (3:2 hexane–EtOAc); $[\alpha]_D$ = +36.8 (*c* 1.9, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.05–8.03 (m, 2 H, ArH), 7.58–7.55 (m, 1 H, ArH), 7.40–7.26 (m, 12 H, ArH), 7.00–6.99 (m, 2 H, ArH), 6.80–6.78 (m, 2 H, ArH), 5.57 (d, 1 H, *J* = 1.9 Hz, H-1), 5.52 (dd, 1 H, *J* = 3.4, 1.9 Hz, H-2), 4.81 (d, 1 H, *J* = 11.1 Hz, PhC*H*$_2$), 4.71 (d, 1 H, *J* = 11.8 Hz, PhC*H*$_2$), 4.66 (d, 1 H, *J* = 11.1 Hz, PhC*H*$_2$), 4.50 (d, 1 H, *J* = 11.8 Hz, PhC*H*$_2$), 4.45 (ddd, 1 H, *J* = 9.6, 5.2, 3.4 Hz, H-3), 4.10 (app t, 1 H, *J* = 9.6 Hz, H-4), 3.98 (ddd, 1 H, *J* = 9.6, 3.7, 1.8 Hz, H-5), 3.90 (dd, 1 H, *J* = 11.0, 3.7 Hz, H-6), 3.75 (dd, 1 H, *J* = 11.0, 1.8 Hz, H-6), 3.74 (s, 3 H, OCH_3), 2.14 (d, 1 H, *J* = 5.2 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 155.1 (Ar), 150.0 (Ar), 138.3 (Ar), 138.1 (Ar), 133.3 (Ar), 129.9 (Ar), 129.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5 (Ar), 117.8 (Ar), 114.6 (Ar), 96.6 (C-1), 75.6 (C-4), 74.9 (Ph*C*H$_2$), 73.4 (Ph*C*H$_2$), 72.6 (C-2), 71.9 (C-5), 70.4 (C-3), 68.8 (C-6), 55.6 (CH_3O); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{C\text{-}1,\ H\text{-}1}$ = 174.6 Hz (C-1, H-1); HRMS (ESI) calcd for (M+Na) $C_{34}H_{34}NaO_8$: 593.2146. Found: 593.2154.



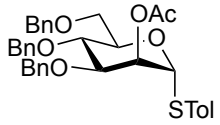
***p*-Tolyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-1-thio-α-D-mannopyranoside (4.70):** To a solution of **4.82**[139] (10.62 g, 21 mmol) in CH_2Cl_2 (250 mL) was added levulinic acid (4.85 g, 42 mmol), EDC (8.0 g, 42 mmol) and DMAP (256 mg, 2.1 mmol). The mixture

was stirred at r.t. overnight and then water was added and the mixture was extracted with EtOAc. The organic phase was washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 20→28% EtOAc in hexane) to afford **4.70** (12.1 g, 94% yield) as a colorless oil. R_f 0.35 (3:2 hexane–EtOAc); $[\alpha]_D$ = +97.9 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.40–7.23 (m, 12 H, ArH), 7.09–7.08 (m, 2 H, ArH), 5.51 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2), 5.45 (d, 1 H, *J* = 1.5 Hz, H-1), 5.34 (dd, 1 H, *J* = 10.0, 3.5 Hz, H-3), 4.72 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.71 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.56 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.50 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.42 (ddd, 1 H, *J* = 11.0, 10.0, 1.5 Hz, H-5), 4.07 (app t, 1 H, *J* = 10.0 Hz, H-4), 3.89 (dd, 1 H, *J* = 11.0, 4.5 Hz, H-6), 3.73 (dd, 1 H, *J* = 11.0, 1.5 Hz, H-6), 2.81–2.67 (m, 2 H, CH_3C=OC*H*$_2$), 2.58–2.47 (m, 2 H, OC=OC*H*$_2CH_2$), 2.33 (s, 3 H, CH_3PhS), 2.20 (s, 3 H, C*H*$_3$C=OCH_2), 2.16 (s, 3 H, OC=OC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.2 (CH_3*C*=OCH_2), 171.8 (O*C*=OCH_2CH_2), 170.0 (O*C*=OCH_3), 138.1 (Ar), 138.0 (Ar), 137.9 (Ar), 132.4 (Ar), 129.8 (Ar), 129.6 (Ar), 128.4 (Ar), 128.3 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 86.2 (C-1), 74.9 (Ph*C*H$_2$), 73.5 (Ph*C*H$_2$), 73.3 (C-4), 72.6 (C-3), 72.4 (C-5), 71.5 (C-2), 68.7 (C-6), 37.9 (CH_3C=O*C*H$_2$), 29.8 (*C*H$_3$C=OCH_2), 27.9 (CH_3C=OCH_2*C*H$_2$), 21.1 (CH_3), 21.0 (OC=O*C*H$_3$); HRMS (ESI) calcd for (M+Na) $C_{34}H_{38}NaO_8S$: 629.2180. Found: 629.2179.



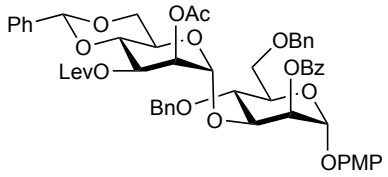
2-(Trimethylsilyl)ethyl 4,6-*O*-Benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl amino)-β-D-glucopyranoside (4.71): To a solution of guanidine chloride (0.4 g, 62.8 mmol) in CH_3OH (40 mL) was added 1 M of $NaOCH_3$ (2 mL). Then, this mixture was added to a solution of **4.85**[149] (6 g, 10.4 mmol) in CH_3OH (90 mL). The reaction mixture was stirred at r.t. for 20 min and then neutralized with Amberlite IR120 H^+ ion-exchange resin and concentrated to dryness. The resulting residue, benzaldehyde dimethyl acetal (1.89 g, 12.4 mmol) and CSA (0.58 g, 2.5 mmol) were dissolved in anhydrous CH_3CN (40 mL) and the mixture was stirred at r.t. for 4 h. After the addition of Et_3N, the mixture was diluted with EtOAc, washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 17→25% EtOAc in hexane) to afford **4.71** (4.85 g, 86% yield) as a white solid. R_f 0.53 (3:2 hexane–EtOAc); $[\alpha]_D$ = –30.5 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.49–7.47 (m, 2 H, ArH), 7.38–7.35 (m, 3 H, ArH), 5.54 (s, 1 H, PhC*H*$(O)_2$), 5.25 (br, 1 H, N*H*), 4.75 (d, 1 H, *J* = 12.0 Hz, C*H*$_2$$CCl_3$), 4.70 (d, 1 H, *J* = 8.0 Hz, H-1), 4.70 (d, 1 H, *J* = 12.0 Hz, C*H*$_2$$CCl_3$), 4.35 (dd, 1 H, *J* = 10.5, 5.0 Hz, H-6), 4.17 (br, 1 H, H-3), 3.96 (ddd, 1 H, *J* = 10.8, 9.7 5.5 Hz, TMSCH_2C*H*$_2$O), 3.78 (app t, 1 H, *J* = 10.5 Hz, H-6), 3.57 (ddd, 1 H, *J* = 11.0, 9.7 6.0 Hz, TMSCH_2C*H*$_2$O), 3.54 (app t, 1 H, *J* = 9.0 Hz, H-4), 3.49–3.45 (m, 1 H, H-5), 3.35–3.17 (m, 1 H, H-2), 3.04 (br, 1 H, OH), 0.97 (ddd, 1 H, *J* = 13.8, 11.2, 6.0 Hz, TMSC*H*$_2$$CH_2$O), 0.92 (ddd, 1 H, *J* = 13.8, 10.8, 5.5 Hz, TMSC*H*$_2$$CH_2$O), 0.01 (s, 9 H,

($C\mathit{H}_3$)$_3$Si); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.5 (NHC=O), 137.0 (Ar), 129.3 (Ar), 128.3 (Ar), 126.3 (Ar), 101.9 (PhCH(O)$_2$), 100.3 (C-1), 81.5 (C-4), 74.6 ($\mathit{C}H_2CCl_3$), 70.7 (C-3), 68.7 (C-6), 67.8 (TMSCH$_2\mathit{C}$H$_2$O), 66.0 (C-5), 59.1 (C-2), 18.2 (TMSCH$_2$CH$_2$O), −1.4 (CH$_3$)$_3$Si); HRMS (ESI) calcd for (M+NH$_4$) $C_{21}H_{34}Cl_3N_2O_7Si$: 559.1195. Found: 559.1183.



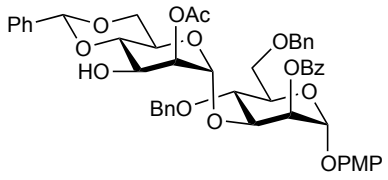
***p*-Tolyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4.72):** Compound **4.80**[140] (200 mg, 0.36 mmol) was dissolved in 3:2 pyridine–Ac_2O (5 mL) and the mixture was stirred at r.t. for 2 h. Then, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with 1M of HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 16→20% EtOAc in hexane) to afford **4.72** (222 mg, 96% yield) as a white solid; R_f 0.62 (2:1 hexane–EtOAc); $[\alpha]_D$ = +91.1 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.35–7.26 (m, 15 H, ArH), 7.20–7.18 (m, 2 H, ArH), 7.05–7.04 (m, 2 H, ArH), 5.59 (app t, 1 H, *J* = 2.0, H-2), 5.45 (d, 1 H, *J* = 2.0 Hz, H-1), 4.88 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.71 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.65 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.56 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.51 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.46 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.34–4.32 (m, 1 H, H-5), 3.95–3.93 (m, 2 H, H-3, H-4), 3.84 (dd, 1 H, *J* = 11.0, 4.5 Hz, H-6), 3.72 (dd, 1 H, *J* = 11.0, 1.5 Hz, H-6), 2.29 (s, 3 H, CH_3PhS), 2.13

(OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.4 (O*C*=OCH$_3$), 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 137.6 (Ar), 132.3 (Ar), 129.9 (Ar), 129.8 (Ar), 128.4 (Ar), 128.33 (Ar), 128.26 (Ar), 128.17 (Ar), 127.9 (Ar), 127.73 (Ar), 127.66 (Ar), 127.52 (Ar), 86.5 (C-1), 78.5 (C-3), 75.2 (Ph*C*H$_2$), 74.6 (C-4), 73.3 (Ph*C*H$_2$), 72.4 (C-5), 71.9 (Ph*C*H$_2$), 70.3 (C-2), 68.9 (C-6), 21.1 (OC=O*C*H$_3$); HRMS (ESI) calcd for (M+Na) $C_{36}H_{38}NaO_6S$: 621.2281. Found: 621.2283.



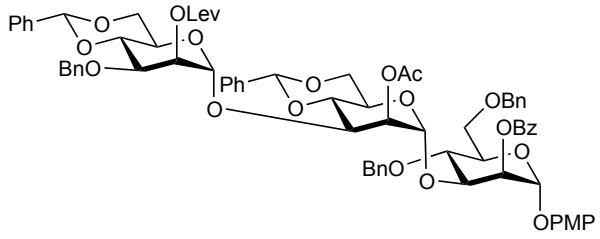
***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzylidene-3-*O*-levulinyl-α-D-manno pyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.86):** A mixture of donor **4.68** (160 mg, 0.31 mmol), acceptor **4.69** (161 mg, 0.28 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (20 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (94 mg, 0.42 mmol) and AgOTf (22 mg, 0.08 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h. Et_3N (0.1 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→33% EtOAc in hexane) to afford **4.86** (176 mg, 65% yield) as a white foam; R_f 0.24 (3:2 hexane−EtOAc); $[\alpha]_D$ = +31.6 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.12−8.11 (m, 2 H, ArH), 7.60−7.58 (m, 1 H, ArH), 7.42−7.26 (m, 15 H, ArH), 7.18−7.17 (m, 2 H,

ArH), 7.00–6.99 (m, 2 H, ArH), 6.79–6.78 (m, 2 H, ArH), 5.61 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.59 (d, 1 H, *J* = 2.0 Hz, H-1), 5.48 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2′), 5.43 (s, 1 H, PhC*H*$(O)_2$), 5.31 (dd, 1 H, *J* = 10.0, 3.0 Hz, H-3′), 5.15 (d, 1 H, *J* = 1.5 Hz, H-1′), 4.92 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.68 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.64 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.48 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.47 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3), 4.29 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.16 (dd, 1 H, *J* = 10.0, 5.0 Hz, H-6′), 4.00–3.97 (m, 2 H, H-5, H-4′), 3.93 (td, 2 H, *J* = 10.0, 5.0 Hz, H-5′), 3.89 (dd, 1 H, *J* = 11.0, 3.5 Hz, H-6), 3.75 (s, 3 H, OCH_3), 3.73–3.70 (m, 2 H, H-6, H-6′), 2.65 (t, 2 H, *J* = 7.0 Hz, CH_3C=OCH_2), 2.54 (dt, 1 H, *J* = 17.0, 7.0 Hz, OC=OCH_2CH_2), 2.46 (dt, 1 H, *J* = 17.0, 7.0 Hz, OC=OCH_2CH_2), 2.13 (s, 3 H, CH_3C=OCH_2), 2.09 (s, 3 H, OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.1 (CH_3C=OCH_2), 171.7 (O*C*=OCH_2), 169.7, (O*C*=OCH_3), 166.1 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.2 (Ar), 137.8 (Ar), 137.1 (Ar), 133.4 (Ar), 130.0 (Ar), 129.4 (Ar), 129.0 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 126.4 (Ar), 117.7 (Ar), 114.6 (Ar), 101.9 (Ph*C*H$(O)_2$), 100.8 (C-1′), 96.1 (C-1), 79.7 (C-3), 75.7 (Ph*C*H_2), 75.6 (C-4′), 73.9 (C-4), 73.4 (Ph*C*H_2), 72.1 (C-2), 72.0 (C-5), 69.9 (C-2′), 68.8 (C-3′), 68.7 (C-6), 68.5 (C-6′), 64.9 (C-5′), 55.6 (CH_3O), 37.8 (CH_3C=O*C*H_2), 29.7 (*C*H_3C=OCH_2), 27.9 (CH_3C=OCH_2*C*H_2), 20.7 (OC=O*C*H_3); HRMS (ESI) calcd for (M+NH_4) $C_{54}H_{60}NO_{16}$: 978.3907. Found: 978.3919.



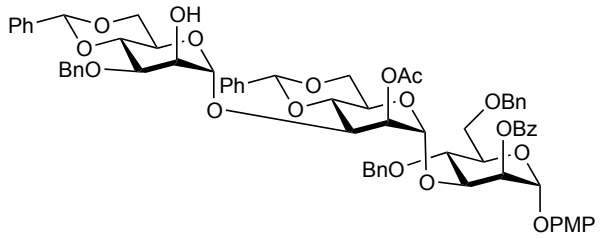
***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)- 2-*O*-benzoyl-4,6-di-*O*- benzyl-α-D-mannopyranoside (4.88):** A solution of **4.86** (190 mg, 0.2 mmol) and hydrazine acetate (37 mg, 0.4 mmol) in 9:1 CH_2Cl_2–CH_3OH (30 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was purified by chromatography (gradient 20→33% EtOAc in hexane) to afford **4.88** (156 mg, 93% yield) as a white foam; R_f 0.30 (3:2 hexane–EtOAc); $[\alpha]_D$ = +45.0 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.11–8.10 (m, 2 H, ArH), 7.62–7.60 (m, 1 H, ArH), 7.43–7.24 (m, 17 H, ArH), 7.01–7.00 (m, 2 H, ArH), 6.80–6.79 (m, 2 H, ArH), 6.00–5.59 (m, 2 H, H-2, H-1), 5.48 (s, 1 H, PhC*H*$(O)_2$), 5.28 (dd, 1 H, *J* = 3.5, 1.5 Hz, H″-2), 5.20 (s, 1 H, H-1′), 4.83 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.70 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.62 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.49 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.48 (dd, 1 H, *J* = 9.0, 3.0 Hz, H-3), 4.29 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.21 (dd, 1 H, *J* = 10.0, 4.0 Hz, H-6′), 4.11–4.09 (m, 1 H, H-3′), 4.00–3.98 (m, 1 H, H-5), 3.88 (dd, 1 H, *J* = 10.5, 3.5 Hz, H-6), 3.86–3.81 (m, 2 H, H-5′, H-4′), 3.75 (s, 3 H, OCH_3), 3.73–3.70 (m, 2 H, H-6′, H-6), 2.15 (d, 1 H, *J* = 4.0 Hz, OH), 2.13 (s, 3 H, OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.2, (O*C*=OCH_3), 165.9 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.2 (Ar), 137.8 (Ar), 137.1 (Ar), 133.4 (Ar), 129.9 (Ar), 129.5 (Ar), 129.2 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.17 (Ar), 128.14 (Ar), 127.9 (Ar), 127.6 (Ar), 126.4 (Ar), 117.8 (Ar), 114.6 (Ar), 102.2 (Ph*C*H$(O)_2$), 100.6

(C-1′), 96.2 (C-1), 78.6 (C-4′), 78.2 (C-3), 75.4 (Ph*C*H_2), 74.3 (C-4), 73.4 (Ph*C*H_2), 72.1 (C-2, C-5), 71.9 (C-2′), 68.6 (C-6), 68.4 (C-6′), 67.2 (C-3′), 64.2 (C-5′), 55.6 (CH_3O), 20.9 (OC=O*C*H_3); HRMS (ESI) calcd for (M+Na) $C_{49}H_{50}NaO_{14}$: 885.3093. Found: 885.3092.



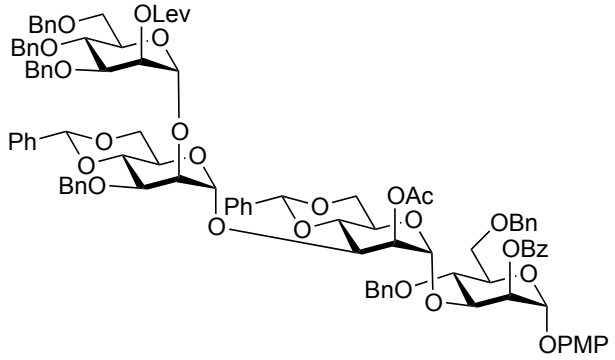
***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.89):** A mixture of donor **4.67** (118 mg, 0.21 mmol), acceptor **4.88** (140 mg, 0.16 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (15 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (61 mg, 0.27 mmol) and AgOTf (16 mg, 0.06 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (0.2 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.89** (141 mg, 67% yield) as a white foam; R_f 0.33 (3:2 hexane–EtOAc); $[\alpha]_D$ = +14.5 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09–8.08 (m, 2 H, ArH), 7.59–7.57 (m, 1 H, ArH), 7.46–7.22 (m, 27 H, ArH), 7.00–6.99 (m, 2 H, ArH), 6.80–6.78 (m, 2 H, ArH), 5.60 (app t, 1 H, *J* = 2.0 Hz, H-2), 5.56 (d, 1 H, *J* = 2.0 Hz, H-1), 5.52 (s, 1 H,

PhC*H*$(O)_2$), 5.51 (s, 1 H, PhC*H*$(O)_2$), 5.39 (dd, 1 H, *J* = 3.0, 1.5 Hz, H‴-2), 5.32 (dd, 1 H, *J* = 3.0, 1.0 Hz, H-2′), 5.20 (s, 1 H, H-1′), 5.07 (s, 1 H, H-1″), 4.82 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.70 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.64–4.58 (m, 3 H, PhCH_2), 4.51 (dd, 1 H, *J* = 9.0, 3.0 Hz, H-3), 4.48 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.29 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.22–4.19 (m, 2 H, H-3′, H-6′), 3.99–3.97 (m, 2 H, H-5, H-4′), 3.93–3.83 (m, 4 H), 3.80–3.70 (m, 4 H), 3.75 (s, 3 H, OCH_3), 3.58–3.55 (m, 1 H), 2.64–2.56 (m, 4 H, CH_3C=OCH_2, OC=OCH_2CH_2), 2.11 (s, 3 H, CH_3C=OCH_2), 2.00 (s, 3 H, OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.1 (CH_3C=OCH_2), 171.6 (O*C*=OCH_2), 170.2, (O*C*=OCH_3), 165.8 (PhC=O), 155.2 (Ar), 149.9 (Ar), 138.2 (Ar), 138.1 (Ar), 137.6 (Ar), 137.2 (Ar), 133.4 (Ar), 129.9 (Ar), 129.5 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.05 (Ar), 127.97 (Ar), 127.89 (Ar), 127.6 (Ar), 127.56 (Ar), 127.54 (Ar), 127.49 (Ar), 126.20 (Ar), 126.16 (Ar), 117.8 (Ar), 114.6 (Ar), 101.5 (Ph*C*H$(O)_2$), 101.3 (Ph*C*H$(O)_2$), 100.3 (C-1″), 99.5 (C-1′), 96.3 (C-1), 78.5, 78.4, 77.4 (C-3), 75.4 (Ph*C*H_2), 74.4 (C-4), 73.5 (Ph*C*H_2), 73.3, 72.1 (C-5), 71.96 (Ph*C*H_2), 71.9 (C-2), 71.6 (C-2′), 71.3 (C-3′), 69.6 (C-2″), 68.6, 68.5, 68.4, 64.5, 64.3, 55.6 (CH_3O), 38.0 (CH_3C=O*C*H_2), 29.7 (*C*H_3C=OCH_2), 28.0 (CH_3C=OCH_2*C*H_2), 20.7 (OC=O*C*H_3); HRMS (ESI) calcd for (M+NH_4) $C_{74}H_{80}NO_{21}$: 1318.5217. Found: 1318.5246.



***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)- 2-*O*-acetyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.90):** A solution of **4.89** (110 mg, 0.08 mmol) and hydrazine acetate (23 mg, 0.25 mmol) in 9:1 CH_2Cl_2–CH_3OH (30 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 20→33% EtOAc in hexane) to afford **4.90** (101 mg, 99% yield) as a white foam; R_f 0.44 (3:2 hexane–EtOAc); $[\alpha]_D$ = +34.7 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10–8.09 (m, 2 H, ArH), 7.59–7.57 (m, 1 H, ArH), 7.45–7.25 (m, 27 H, ArH), 7.01–6.99 (m, 2 H, ArH), 6.80–6.78 (m, 2 H, ArH), 5.61 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.51 (s, 1 H, PhC*H*$(O)_2$), 5.49 (s, 1 H, PhC*H*$(O)_2$), 5.35 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2′), 5.20 (d, 1 H, *J* = 1.5 Hz, H-1′), 5.10 (s, 1 H, H-1″), 4.86 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.80 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.70 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.65 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.61 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.51 (dd, 1 H, *J* = 9.0, 3.0 Hz, H-3), 4.49 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.29 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.22–4.19 (m, 2 H, H-3′, H-6′), 4.01–3.86 (m, 7 H), 3.79–3.70 (m, 4 H), 3.75 (s, 3 H, OCH_3), 3.58 (app t, 1 H, *J* = 10.5 Hz), 2.49 (d, 1 H, *J* = 1.0 Hz, OH), 2.07 (s, 3 H, OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 169.6 (O*C*=OCH_3), 165.8 (PhC=O),

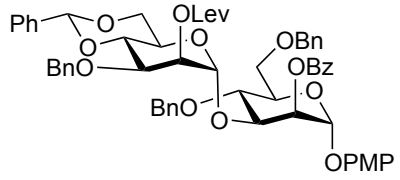
155.2 (Ar), 149.9 (Ar), 138.2 (Ar), 138.1 (Ar), 137.6 (Ar), 137.5 (Ar), 137.3 (Ar), 133.4 (Ar), 129.9 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.40 (Ar), 128.38 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.61 (Ar), 127.57 (Ar), 126.17 (Ar), 126.15 (Ar), 117.8 (Ar), 114.6 (Ar), 101.6 (Ph*C*H(O)$_2$), 101.5 (Ph*C*H(O)$_2$), 101.4 (C-1″), 100.3 (C-1′), 96.3 (C-1), 78.8, 78.2, 77.8 (C-3), 75.4 (Ph*C*H$_2$), 75.1, 74.3 (C-4), 73.5 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.3, 72.1, 71.94, 71.88, 69.9, 68.62, 68.60, 68.5, 64.6, 63.9, 55.6 (CH_3O), 20.8 (OC=O*C*H$_3$); HRMS (ESI) calcd for (M+Na) $C_{69}H_{70}NaO_{19}$: 1225.4404. Found: 1225.4413.



***p*-Methoxyphenyl 3,4,6-tri-*O*-Benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.91):** A mixture of donor **4.66** (38 mg, 0.058 mmol), acceptor **4.90** (54 mg, 0.045 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (5 mL) and stirred at r.t. for 10 min. The solution was then cooled to −5 °C, and then NIS (18 mg, 0.08 mmol) and AgOTf (5.8 mg, 0.022 mmol) were added. The solution

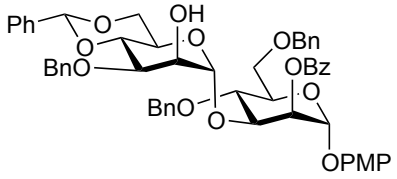
was slowly warmed to 0 °C and stirred for 1 h before Et_3N (0.2 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.91** (48 mg, 63% yield) as a white foam; R_f 0.41 (3:2 hexane–EtOAc); $[\alpha]_D$ = +20.8 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.11–8.09 (m, 2 H, ArH), 7.58–7.55 (m, 1 H, ArH), 7.48–7.11 (m, 42 H, ArH), 7.02–7.00 (m, 2 H, ArH), 6.81–6.79 (m, 2 H, ArH), 5.61 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.57 (d, 1 H, *J* = 2.0 Hz), 5.54 (s, 1 H), 5.51 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.40 (s, 1 H), 5.34 (dd, 1 H, *J* = 3.5, 1.5 Hz), 5.20 (d, 1 H, *J* = 1.5 Hz), 5.11 (d, 1 H, *J* = 1.5 Hz), 5.08 (d, 1 H, *J* = 1.5 Hz), 4.87–4.80 (m, 3 H), 4.73–4.70 (m, 2 H), 4.63 (d, 1 H, *J* = 11.5 Hz), 4.60 (d, 1 H, *J* = 12.5 Hz, PhC*H*$_2$), 4.54–4.50 (m, 2 H), 4.43 (d, 1 H, *J* = 11.5 Hz), 4.41 (d, 1 H, *J* = 10.5 Hz), 4.30 (app t, 1 H, *J* = 9.5 Hz), 4.24–4.17 (m, 3 H), 4.01–3.86 (m, 7 H), 3.79–3.70 (m, 4 H), 4.02–3.85 (m, 8 H), 3.81 (dd, 1 H, *J* = 10.5, 3.5 Hz), 3.76 (s, 3 H), 3.75–3.68 (m, 4 H), 3.56–3.51 (m, 2 H), 3.25 (dd, 1 H, *J* = 11.0, 3.0 Hz), 3.03 (dd, 1 H, *J* = 11.0, 1.5 Hz), 2.67–2.61 (m, 4 H), 2.08 (s, 3 H), 2.03 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.2, 171.6, 169.5, 165.8, 155.2, 149.9, 138.6, 138.5, 138.2, 138.1, 137.7, 137.6, 137.3, 133.4, 130.0, 129.4, 128.9, 128.8, 128.6, 128.46, 128.37, 128.29, 128.25, 128.13, 128.11, 128.07, 127.9, 127.7, 127.6, 127.56, 127.48, 127.41, 127.3, 126.24, 126.15, 117.8, 114.6, 101.7, 101.5, 100.6, 100.3, 99.4, 96.4, 79.1, 78.5, 77.8, 77.2, 75.6, 75.5, 75.3, 75.2, 74.5, 73.9, 73.5, 73.2, 73.0, 72.2, 72.0, 71.9, 71.65, 71.64, 70.6, 68.63, 68.59, 68.52,

68.45, 68.0, 64.6, 64.5, 55.6, 30.0, 29.7, 28.2, 20.7; HRMS (ESI) calcd for ($M+NH_4$) $C_{101}H_{108}NO_{26}$:1750.7154. Found: 1750.7189.



***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.92):** A mixture of donor **4.67** (6.40 g, 11.4 mmol), acceptor **4.69** (5.0 g, 8.77 mmol) and powdered 4Å molecular sieves was suspended in anhydrous CH_2Cl_2 (480 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (3.52 g, 15.8 mmol) and AgOTf (673 mg, 2.63 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (2.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.92** (6.4 g, 72% yield) as a white foam; R_f 0.26 (2:1 hexane–EtOAc); $[\alpha]_D$ = +31.7 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.06–8.05 (m, 2 H, ArH), 7.58–7.56 (m, 1 H, ArH), 7.38–7.15 (m, 22 H, ArH), 7.00–6.99 (m, 2 H, ArH), 6.79–6.78 (m, 2 H, ArH), 5.58 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.56 (d, 1 H, *J* = 2.0 Hz, H-1), 5.50 (s, 1 H, PhC*H*$(O)_2$), 5.38 (dd, 1 H, *J* = 3.5, 1.5 Hz, H-2′), 5.18 (d, 1 H, *J* = 1.5 Hz, H-1′), 4.74 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.60 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.52 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.50–4.45 (m, 4 H, PhCH_2, H-3), 4.22 (app t, 1 H, *J* = 10.0

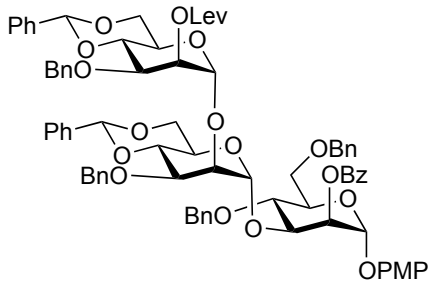
Hz, H-4′), 4.16 (dd, 1 H, J = 10.5, 4.5 Hz, H-6′), 3.98–3.96 (m, 1 H, H-5′), 3.96 (app t, 1 H, J = 9.5 Hz, H-4), 3.88–3.84 (m, 3 H, H-3′, H-5, H-6), 3.74 (s, 3 H, OCH_3), 3.71 (app t, 1 H, J = 10.5 Hz, H-6′), 3.66 (dd, 1 H, J = 11.0, 1.6 Hz, H-6), 2.74–2.60 (m, 4 H, $CH_3C{=}OCH_2$, $CH_3C{=}OCH_2CH_2$), 2.14 (s, 3 H, $CH_3C{=}OCH_2$,); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.1 ($CH_3C{=}OCH_2$), 171.7 ($OC{=}OCH_2$), 165.8 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.3 (Ar), 137.9 (Ar), 137.8 (Ar), 137.5 (Ar), 133.3 (Ar), 129.9 (Ar), 129.6 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 126.3 (Ar), 117.8 (Ar), 114.6 (Ar), 101.6 ($PhCH(O)_2$), 100.6 (C-1′), 96.2 (C-1), 78.2 (C-4), 78.0 (C-3), 75.4 ($PhCH_2$), 74.4 (C-4′), 73.6 (C-3′), 73.4 ($PhCH_2$), 72.1 ($PhCH_2$), 72.0 (C-5), 71.9 (C-2), 70.2 (C-2′), 68.6 (C-6), 68.5 (C-6′), 64.7 (C-5′), 55.6 (CH_3O), 38.0 ($CH_3C{=}OCH_2$), 29.8 ($CH_3C{=}OCH_2$), 28.0 ($CH_3C{=}OCH_2CH_2$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{\text{C-1, H-1}}$ = 171.5 Hz (C-1, H-1), $^1J_{\text{C-1′, H-1′}}$ = 171.5 Hz (C-1′, H-1′); HRMS (ESI) calcd for ($M+NH_4$) $C_{59}H_{64}NO_{15}$: 1026.4270. Found: 1026.4257.



***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)- 2-*O*-benzoyl-4,6-di-*O*- benzyl-α-D-mannopyranoside (4.93):** A solution of **4.92** (6.40 g, 6.35 mmol) and hydrazine acetate (1.05 g, 11.4 mmol) in 9:1 CH_2Cl_2–CH_3OH (300 mL)

was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 16→25% EtOAc in hexane) to afford **4.93** (5.6 g, 92% yield) as a white foam; R_f 0.28 (2:1 hexane–EtOAc); $[\alpha]_D$ = +40.1 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10–8.09 (m, 2 H, ArH), 7.59–7.57 (m, 1 H, ArH), 7.41–7.20 (m, 22 H, ArH), 7.02–7.00 (m, 2 H, ArH), 6.80–6.79 (m, 2 H, ArH), 5.61 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.49 (s, 1 H, PhC*H*$(O)_2$), 5.24 (d, 1 H, *J* = 1.0 Hz, H-1′), 4.74 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.71 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.68 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.57 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.53 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.48 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.47 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3), 4.24 (app t, 1 H, *J* = 10.0 Hz, H-4′), 4.19 (dd, 1 H, *J* = 10.0, 4.5 Hz, H-6′), 4.03 (app t, 1 H, *J* = 9.5 Hz, H-4), 3.99 (ddd, *J* = 10.0, 3.0, 1.5 Hz, H-5), 3.95 (dd, *J* = 3.0, 1.5 Hz, H-2′), 3.87 (dd, 1 H, *J* = 11.0, 3.0 Hz, H-6), 3.84 (td, 1 H, *J* = 10.0, 4.5 Hz, H-5′), 3.78 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3′), 3.75 (s, 3 H, OCH_3), 3.73 (app t, 1 H, *J* = 10.0 Hz, H-6′), 3.72 (dd, 1 H, *J* = 11.0, 1.5 Hz, H-6), 2.54 (s, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.8 (PhC=O), 155.2 (Ar), 150.0 (Ar), 138.3 (Ar), 138.0 (Ar), 137.9 (Ar), 137.7 (Ar), 133.3 (Ar), 129.9 (Ar), 129.8 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 126.3 (Ar), 117.9 (Ar), 114.6 (Ar), 102.3 (C-1′), 101.6 (Ph*C*H$(O)_2$), 96.4 (C-1), 78.6 (C-4′), 78.1 (C-3), 75.5 (C-3′), 75.4 (Ph*C*H_2), 74.5 (C-4), 73.5 (Ph*C*H_2), 73.1 (Ph*C*H_2), 72.2 (C-2), 72.1 (C-5), 70.4 (C-2′), 68.8 (C-6),

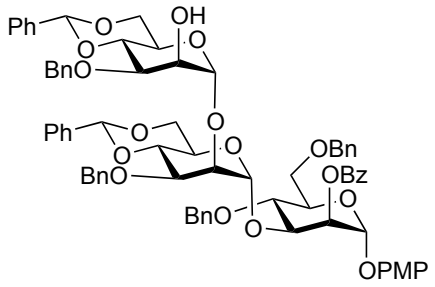
68.7 (C-6′), 64.2 (C-5′), 55.6 (CH_3O); HRMS (ESI) calcd for ($M+NH_4$) $C_{54}H_{58}NO_{13}$: 928.3903. Found: 928.3899.



***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-2-*O*-levulinyl-α-D-manno pyranosyl-(1→2)-3-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.94):** A mixture of donor **4.93** (4.70 g, 8.35 mmol), acceptor **4.67** (5.24 g, 5.76 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (480 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (2.58 g, 11.52 mmol) and AgOTf (442 mg, 1.73 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (2.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.94** (5.82 g, 73% yield) as a white foam; R_f 0.27 (2:1 hexane–EtOAc); $[\alpha]_D$ = +5.9 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10–8.09 (m, 2 H, ArH), 7.61–7.58 (m, 1 H, ArH), 7.51–7.50 (m, 1 H, ArH), 7.42–7.20 (m, 31 H, ArH), 7.03–7.02 (m, 2 H, ArH), 6.79–6.78 (m, 2 H, ArH), 5.60 (s, 1 H, PhC*H*$(O)_2$), 5.59–5.57 (m, 2 H, H-1, H-2), 5.56 (dd,

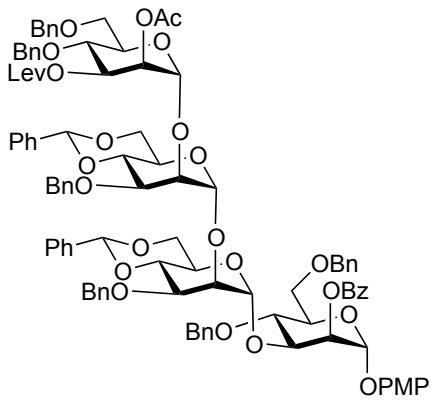
1 H, *J* = 3.5, 1.5 Hz, H-2″), 5.48 (s, 1 H, PhC*H*(O)$_2$), 5.11 (d, 1 H, *J* = 1.5 Hz, H-1′), 4.98 (d, 1 H, *J* = 1.5 Hz, H-1″), 4.72–4.66 (m, 4 H, 4 X PhC*H*$_2$), 4.62 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$), 4.51 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$), 4.45 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$), 4.43 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$), 4.40 (dd, 1 H, *J* = 9.5, 2.5 Hz, H-3), 4.19 (app t, 1 H, *J* = 10.0 Hz, H-4), 4.14 (dd, 1 H, *J* = 10.0, 4.5 Hz, H-6′), 4.07 (dd, 1 H, *J* = 10.0, 5.0 Hz, H-6″), 4.03 (dd, 1 H, *J* = 10.0, 3.0 Hz, H-3″), 4.00 (app t, 1 H, *J* = 10.0 Hz, H-4″), 3.98 (ddd, 1 H, *J* = 10.0, 3.5, 1.5 Hz, H-5), 3.94 (app t, 1 H, *J* = 9.5 Hz, H-4′), 3.89 (td, 1 H, *J* = 10.0, 5.0 Hz, H-5″), 3.86 (dd, 1 H, *J* = 3.0, 1.5 Hz, H-2′), 3.83 (dd, 1 H, *J* = 11.0, 3.5 Hz, H-6), 3.81 (dd, 1 H, *J* = 10.0, 3.0 Hz, H-3′), 3.77–3.74 (m, 1 H, H-5′), 3.75 (s, 3 H, OCH$_3$), 3.72–3.65 (m, 3 H, H-6, H-6′, H-6′), 2.79–2.63 (m, 4 H, CH$_3$C=OC*H*$_2$, CH$_3$C=OCH$_2$C*H*$_2$), 2.16 (s, 3 H, C*H*$_3$C=OCH$_2$,); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.1 (CH$_3$*C*=OCH$_2$), 171.6 (O*C*=OCH$_2$), 165.8 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 137.7 (Ar), 137.4 (Ar), 133.3 (Ar), 129.9 (Ar), 129.7 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.28 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.76 (Ar), 127.7 (Ar), 127.6 (Ar), 127.56 (Ar), 127.54 (Ar), 127.52 (Ar), 126.4 (Ar), 126.1 (Ar), 117.8 (Ar), 114.6 (Ar), 102.3 (C-1′), 101.6 (Ph*C*H(O)$_2$), 101.5 (Ph*C*H(O)$_2$), 100.7 (C-1″), 96.2 (C-1), 79.2 (C-3), 78.7 (C-4′), 78.5 (C-4″), 77.2 (C-2′), 75.4 (Ph*C*H$_2$), 75.2 (C-3′), 74.2 (C-4), 73.7 (C-3″), 73.4 (Ph*C*H$_2$), 73.2 (Ph*C*H$_2$), 72.3 (Ph*C*H$_2$), 72.2 (C-2), 72.1 (C-5), 69.7 (C-2″), 68.7 (C-6), 68.5 (C-6′, C-6″), 64.9 (C-5′), 64.5 (C-5″), 55.6 (CH$_3$O), 38.1 (CH$_3$C=O*C*H$_2$), 29.8 (*C*H$_3$C=OCH$_2$), 28.1

($CH_3C{=}OCH_2CH_2$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{C\text{-}1,\ H\text{-}1}$ = 174.8 Hz (C-1, H-1), $^1J_{C\text{-}1',\ H\text{-}1'}$ = 170.2 Hz (C-1′, H-1′), $^1J_{C\text{-}1'',\ H\text{-}1''}$ = 171.9 Hz (C-1″, H-1″); HRMS (ESI) calcd for ($M+NH_4$) $C_{79}H_{84}NO_{20}$: 1366.5581. Found: 1366.5570.



***p*-Methoxyphenyl 3-*O*-Benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.95):** A solution of **4.94** (5.53 g, 4.10 mmol) and hydrazine acetate (676 mg, 7.34 mmol) in 9:1 CH_2Cl_2–CH_3OH (300 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 16→25% EtOAc in hexane) to afford **4.95** (4.9 g, 97% yield) as a white foam; R_f 0.33 (2:1 hexane–EtOAc); $[\alpha]_D$ = +30.7 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10–8.09 (m, 2 H, ArH), 7.61–7.59 (m, 1 H, ArH), 7.51–7.49 (m, 2 H, ArH), 7.42–7.16 (m, 30 H, ArH), 7.04–7.03 (m, 2 H, ArH), 6.80–6.78 (m, 2 H, ArH), 5.60 (s, 1 H, PhC*H*$(O)_2$), 5.59–5.58 (m, 2 H, H-1, H-2), 5.47 (s, 1 H, PhC*H*$(O)_2$), 5.14 (d, 1 H, *J* = 1.5 Hz, H-1′), 5.12 (d, 1 H, *J* = 1.0 Hz, H-1″), 4.89 (d, 1 H, *J* = 11.7 Hz, PhCH_2), 4.74 (d, 1 H, *J* = 11.7 Hz, PhCH_2), 4.69 (d, 1 H, *J* = 12.2 Hz, PhCH_2), 4.68 (d, 1 H, *J* = 11.6

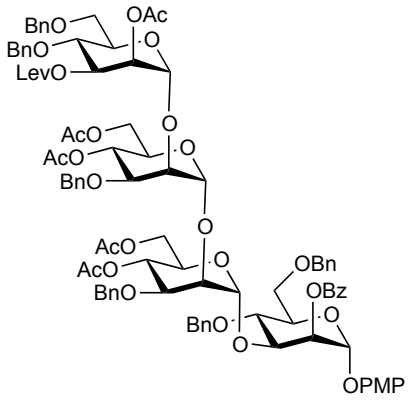
Hz, PhCH_2), 4.63 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.51 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.46 (d, 1 H, J = 12.2 Hz, PhCH_2), 4.46 (d, 1 H, J = 11.6 Hz, PhCH_2), 4.41 (dd, 1 H, J = 9.5, 2.5 Hz, H-3), 4.22–4.21 (m, 1 H, H-2″), 4.20 (app t, 1 H, J = 10.0 Hz, H-4), 4.15 (dd, 1 H, J = 10.0, 4.5 Hz, H-6′), 4.11 (app t, 1 H, J = 9.5 Hz, H-4″), 4.07 (dd, 1 H, J = 10.5, 5.0 Hz, H-6″), 3.98 (ddd, 1 H, J = 10.0, 3.5, 1.5 Hz, H-5), 3.97 (dd, 1 H, J = 9.5, 3.5 Hz, H-3″), 3.94–3.93 (m, 1 H, H-2′), 3.94 (app t, 1 H, J = 9.5 Hz, H-4′), 3.89 (td, 1 H, J = 10.0, 5.0 Hz, H-5″), 3.84 (dd, 1 H, J = 11.0, 3.5 Hz, H-6), 3.82 (dd, 1 H, J = 10.0, 3.0 Hz, H-3′), 3.76 (td, 1 H, J = 10.0, 5.0 Hz, H-5′), 3.75 (s, 3 H, OCH_3), 3.74–3.67 (m, 3 H, H-6, H-6′, H-6′), 2.58 (d, 1 H, J = 1.3 Hz, OH); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.9 (PhC=O), 155.2 (Ar), 149.9 (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 137.9 (Ar), 137.7 (Ar), 137.5 (Ar), 133.3 (Ar), 129.9 (Ar), 129.7 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.95 (Ar), 127.92 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 126.4 (Ar), 126.1 (Ar), 117.8 (Ar), 114.6 (Ar), 102.6 (C-1′), 102.1 (C-1″), 101.6 (Ph*C*H$(O)_2$), 101.5 (Ph*C*H$(O)_2$), 96.2 (C-1), 79.3 (C-3), 78.9 (C-4″), 78.7 (C-4′), 77.2 (C-2′), 75.5 (Ph*C*H_2), 75.4 (C-3′, C-3″), 74.2 (C-4), 73.4 (Ph*C*H_2), 73.3 (Ph*C*H_2), 73.2 (Ph*C*H_2), 72.3 (C-2), 72.1 (C-5), 69.9 (C-2″), 68.7, 68.6, 68.5 (C-6, C-6′, C-6″), 64.9 (C-5′), 64.0 (C-5″), 55.6 (CH_3O); HRMS (ESI) calcd for (M+Na) $C_{74}H_{74}NaO_{18}$: 1273.4767. Found: 1273.4772.



***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.96):** A mixture of acceptor **4.95** (4.60 g, 3.72 mmol), donor **4.70** (2.92 g, 4.83 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (400 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (1.58 g, 7.07 mmol) and AgOTf (285 mg, 1.11 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (2.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→33% EtOAc in hexane) to afford **4.96** (5.53 g, 86% yield) as a white solid; R_f 0.14 (2:1 hexane–EtOAc); $[\alpha]_D$ = +27.6 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.08–8.06 (m, 2 H, ArH), 7.59–7.57 (m, 1 H, ArH), 7.50–7.49 (m, 2 H, ArH), 7.39–7.09 (m, 40 H, ArH), 7.06–7.01 (m, 3 H, ArH), 6.79–6.77 (m, 2 H, ArH), 5.65 (s, 1 H, PhC*H*$(O)_2$), 5.57–5.55 (m, 2 H, H-1, H-2), 5.45–5.44 (m, 2 H, H′′′′-2, PhC*H*$(O)_2$), 5.40 (dd, 1 H, *J* = 10.0, 3.5 Hz, H′′′′-3), 5.17 (d, 1 H, *J* = 1.5 Hz, H-1′′′), 5.12

(d, 1 H, J = 1.5 Hz, H-1″), 5.10 (d, 1 H, J = 1.5 Hz, H-1′), 4.89 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.66 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.63 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.61 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.57 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.55 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.54 (d, 1 H, J = 12.5 Hz, PhCH_2), 4.46–4.39 (m, 5 H, PhCH_2, H-3), 4.22 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.19 (dd, 1 H, J = 1.5 Hz, H-2″), 4.16 (app t, 1 H, J = 9.5 Hz, H-4), 4.13 (dd, 1 H, J = 10.0, 4.5 Hz, H-6′), 3.99 (dd, 1 H, J = 10.5, 5.0 Hz, H-6″), 4.00–3.95 (m, 4 H), 3.93 (dd, 1 H, J = 3.0, 1.5 Hz, H-2′), 3.88 (app t, 1 H, J = 9.5 Hz, H-4′), 3.83–3.76 (m, 4 H), 3.74 (s, 3 H, OCH_3), 3.74–3.70 (m, 2 H), 3.67 (dd, 1 H, J = 11.0, 2.0 Hz), 3.65 (app t, 1 H, J = 10.0 Hz), 3.55 (dd, 1 H, J = 11.0, 3.0 Hz), 3.39 (dd, 1 H, J = 11.0, 2.0 Hz), 2.78 (dt, 1 H, J = 18.0, 7.5 Hz, CH_3C=OCH_2), 2.65 (dt, 1 H, J = 18.0, 6.5 Hz, CH_3C=OCH_2), 2.53 (dt, 1 H, J = 17.0, 7.0 Hz, OC=OCH_2CH_2), 2.44 (dt, 1 H, J = 17.0, 6.5 Hz, OC=OCH_2CH_2), 2.16 (s, 3 H, CH_3C=OCH_2), 2.07 (s, 3 H, OC=OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.3 (CH_3C=OCH_2), 171.8 (OC=OCH_2), 169.8 (CH_3C=O),165.8 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 137.8 (Ar), 137.7 (Ar), 137.6 (Ar), 133.3 (Ar), 129.9 (Ar), 129.7 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.33 (Ar), 128.32 (Ar), 128.28 (Ar), 128.22 (Ar), 128.1 (Ar), 128.0 (Ar), 127.99 (Ar), 127.92 (Ar), 127.89 (Ar), 127.87 (Ar), 127.7 (Ar), 127.69 (Ar), 127.63 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 126.4 (Ar), 126.1 (Ar), 117.8 (Ar), 114.6 (Ar), 102.5 (C-1′), 101.6 (C-1″), 101.5 (PhCH(O)$_2$), 101.3 (PhCH(O)$_2$), 99.4 (C-1‴), 96.2 (C-1), 79.2 (C-3), 78.8, 77.2, 76.5, 75.8, 75.5, 75.4 (PhCH$_2$), 75.3, 74.9 (PhCH$_2$), 74.1,

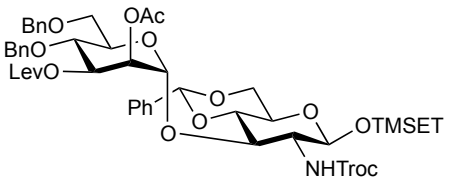
73.5 (Ph*C*H_2), 73.4 (Ph*C*H_2), 73.3 (Ph*C*H_2), 72.9 (Ph*C*H_2), 72.8, 72.3, 72.0, 71.8, 69.7, 68.7, 68.5, 68.4, 68.1, 64.9, 64.8, 55.6 (CH_3O), 37.9 (CH_3C=O*C*H_2), 29.8 (*C*H_3C=OCH_2), 28.0 (CH_3C=OCH_2*C*H_2), 20.9 (OC=O*C*H_3); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{C\text{-}1,\ H\text{-}1}$ = 175.4 Hz (C-1, H-1), $^1J_{C\text{-}1',\ H\text{-}1'}$ = 170.1 Hz (C-1′, H-1′), $^1J_{C\text{-}1'',\ H\text{-}1''}$ = 173.1 Hz (C-1″, H-1″), $^1J_{C\text{-}1''',\ H\text{-}1'''}$ = 173.3 Hz (C-1‴, H-1‴); HRMS (ESI) calcd for (M+Na) $C_{101}H_{104}NaO_{26}$: 1755.6708. Found: 1755.6690.



***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.64):** Tetrasaccharide **4.96** (1.40 g 0.81 mmol) was dissolved in a 1% solution of I_2 in CH_3OH (w/v, 120 mL) and the solution was heated at reflux for 6 h. The solution was cooled, a few crystals of $Na_2S_2O_3$ were added, and the suspension was stirred until the dark red solution went colorless. Then, the mixture was filtered and water was added. The mixture was extracted with EtOAc. The organic phase was washed with brine,

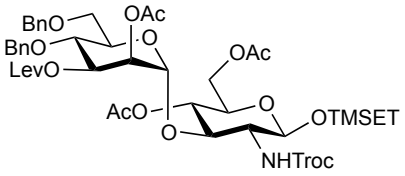
dried over Na_2SO_4, filtered and concentrated to dryness. The resulting residue was dissolved in 5:4 pyridine–Ac_2O (90 mL) and the mixture was stirred at r.t. for 2 h. Then, the solution was concentrated to dryness and the residue was dissolved in a 2% solution of HCl in acetone (30 mL). After 0.5 h, CH_2Cl_2 (150 mL) was added and the mixture was washed with 1M of HCl, a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by chromatography (gradient 33→66% EtOAc in hexane) to afford **4.64** (1.22 g, 88% yield) as a white solid. R_f 0.18 (1:1 hexane–EtOAc); $[\alpha]_D$ = +50.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.08–8.07 (m, 2 H, ArH), 7.62–7.60 (m, 1 H, ArH), 7.43–7.41 (m, 2 H, ArH), 7.36–7.01 (m, 30 H, ArH), 6.98–6.96 (m, 3 H, ArH), 6.79–6.77 (m, 2 H, ArH), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.54 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.39 (dd, 1 H, *J* = 9.5, 3.5 Hz, H-3‴), 5.31 (dd, 1 H, *J* = 3.5, 2.0 Hz, H-2‴), 5.30 (s, 1 H, H-1′), 5.25 (app t, 1 H, *J* = 10.0 Hz, H-4″), 5.16 (app t, 1 H, *J* = 10.0 Hz, H-4′), 4.94 (d, 1 H, *J* = 2.0 Hz, H-1″), 4.88 (d, 1 H, *J* = 1.5 Hz, H-1‴), 4.67 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.62–4.52 (m, 5 H, PhCH_2), 4.46–4.44 (m, 2 H, PhCH_2), 4.42 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3), 4.36 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.28–4.23 (m, 3 H, PhCH_2), 4.16 (app t, 1 H, *J* = 9.5 Hz, H-4), 4.08 (dd, 1 H, *J* = 12.0, 2.5 Hz), 4.04 (dd, 1 H, *J* = 12.0, 6.0 Hz), 4.02 (app t, 1 H, *J* = 2.5 Hz, H-2″), 3.96–3.79 (m, 9 H), 3.74 (s, 3 H, OCH_3), 3.69 (app t, 1 H, *J* = 2.0 Hz), 3.66–3.64 (m, 2 H), 3.57 (dd, 1 H, *J* = 10.5, 3.5 Hz), 3.42 (dd, 1 H, *J* = 11.0, 1.5 Hz), 2.77 (dt, 1 H, *J* = 18.0, 7.0 Hz, CH_3C=OCH_2), 2.63 (dt, 1 H, *J* = 18.0, 6.5 Hz, CH_3C=OCH_2), 2.52 (dt, 1 H, *J* = 17.0, 7.0 Hz, OC=OCH_2CH_2),

2.42 (dt, 1 H, J = 17.0, 6.5 Hz, OC=OCH_2CH$_2$), 2.14 (s, 3 H, CH_3C=OCH$_2$), 2.07 (s, 3 H, OC=OCH_3), 2.01 (s, 3 H, OC=OCH_3), 1.98 (s, 3 H, OC=OCH_3), 1.94 (s, 3 H, OC=OCH_3), 1.90 (s, 3 H, OC=OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.4 (CH$_3$*C*=OCH$_2$), 171.2 (O*C*=OCH$_2$), 170.8 (CH$_3$*C*=O), 170.7 (CH$_3$*C*=O), 169.7 (CH$_3$*C*=O), 169.6 (CH$_3$*C*=O), 169.3 (CH$_3$*C*=O), 165.6 (PhC=O), 155.3 (Ar), 149.7 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 137.8 (Ar), 137.7 (Ar), 137.5 (Ar), 133.5 (Ar), 129.8 (Ar), 129.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.32 (Ar), 128.31 (Ar), 128.25 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.77 (Ar), 127.73 (Ar), 127.68 (Ar), 127.64 (Ar), 127.57 (Ar), 127.54 (Ar), 127.51 (Ar), 125.49 (Ar), 117.7 (Ar), 114.6 (Ar), 100.9 (C-1′, C-1″), 98.6 (C-1‴), 96.4 (C-1), 77.4 (C-3), 76.9, 75.7, 75.3 (Ph*C*H$_2$), 74.7 (Ph*C*H$_2$), 74.5, 73.5, 73.40 (Ph*C*H$_2$), 73.39 (Ph*C*H$_2$), 72.9, 72.3, 72.2 (Ph*C*H$_2$), 72.1, 71.9 (Ph*C*H$_2$), 71.8, 71.7, 69.9, 69.75, 69.74, 68.6, 68.5, 67.4, 67.3, 62.8 , 62.6, 55.6 (CH$_3$O), 37.9 (CH$_3$C=O*C*H$_2$), 29.8 (*C*H$_3$C=OCH$_2$), 27.9 (CH$_3$C=OCH$_2$*C*H$_2$), 20.9 (OC=O*C*H$_3$), 20.8 (OC=O*C*H$_3$), 20.79 (OC=O*C*H$_3$), 20.7 (OC=O*C*H$_3$), 20.6 (OC=O*C*H$_3$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{\text{C-1, H-1}}$ = 173.3 Hz (C-1, H-1), $^1J_{\text{C-1′, H-1′}}$ = 174.4 Hz (C-1′, H-1′), $^1J_{\text{C-1″, H-1″}}$ = 172.8 Hz (C-1″, H-1″), $^1J_{\text{C-1‴, H-1‴}}$ = 175.0 Hz (C-1‴, H-1‴); HRMS (ESI) calcd for (M+NH$_4$) $C_{95}H_{108}NO_{30}$: 1742.6951. Found: 1742.6827.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-manno-pyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino) -β-D-glucopyranoside (4.107): A mixture of thioglycoside **4.70** (2 g, 3.68 mmol), acceptor **4.71** (2.45 g, 4.05 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (70 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (1.21 g, 5.43 mmol and AgOTf (282 mg, 1.1 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (1.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 25→33% EtOAc in hexane) to afford **4.107** (3.21 g, 85% yield) as a white solid; R_f 0.72 (1:1 hexane–EtOAc); $[\alpha]_D$ = +1.5 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.39–7.26 (m, 13 H, ArH), 7.20–7.19 (m, 2 H, ArH), 5.55 (d, 1 H, *J* = 8.8 Hz, N*H*), 5.52 (s, 1 H, PhC*H*$(O)_2$), 5.37 (app t, 1 H, *J* = 2.1, H-2′), 5.24 (dd, 1 H, *J* = 10.3, 2.1 Hz, H-3′), 5.22 (s, 1 H, H-1′), 4.65 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.64 (d, 1 H, *J* = 11.2 Hz, PhCH_2), 4.64 (d, 1 H, *J* = 12.0 Hz, CH_2CCl_3), 4.50 (d, 1 H, *J* = 12.0 Hz, CH_2CCl_3), 4.50 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.47 (d, 1 H, *J* = 11.2 Hz, PhCH_2), 4.34–4.32 (m, 2 H, H-1, H-6), 4.09 (app t, 1 H, *J* = 10.0 Hz, H-3), 4.06–4.04 (m, 1 H, H-5′), 3.88–3.84 (m, 1 H, TMSCH_2CH_2O), 3.79 (app t, 1 H, *J* = 9.0 Hz, H-4′), 3.76 (app t, 1 H, *J* = 10.0 Hz, H-6), 3.71–3.63 (m, 2 H, H-6′), 3.67 (app t, 1 H, *J* = 10.0 Hz, H-4), 3.44–3.40 (m, 2 H, H-

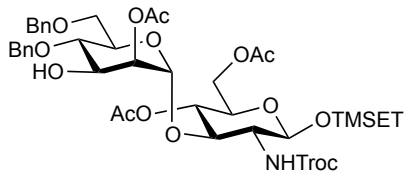
2, TMSCH$_2$C*H*$_2$O), 3.35 (app td, 1 H, *J* = 10.0, 5.0 Hz, H-5), 2.71 (dt, 1 H, *J* = 18.3, 7.0 Hz, CH$_3$C=OC*H*$_2$), 2.64 (dt, 1 H, *J* = 18.3, 6.5 Hz, CH$_3$C=OC*H*$_2$), 2.48 (dt, 1 H, *J* = 17.2, 7.0 Hz, OC=OC*H*$_2$CH$_2$), 2.43 (dt, 1 H, *J* = 17.2, 6.5 Hz, OC=OC*H*$_2$CH$_2$), 2.14 (s, 3 H, C*H*$_3$C=OCH$_2$), 2.04 (s, 3 H, OC=OC*H*$_3$), 0.90–0.86 (m, 2 H, TMSC*H*$_2$CH$_2$O), 0.00 (s, 9 H, (C*H*$_3$)$_3$Si); ^{13}C NMR (175 MHz, CDCl$_3$, δ_C) 206.2 (CH$_3$*C*=OCH$_2$), 171.6 (O*C*=OCH$_2$CH$_2$), 169.6 (O*C*=OCH$_3$), 153.9 (NHC=O), 138.0 (Ar), 137.8 (Ar), 137.0 (Ar), 133.8 (Ar), 130.0 (Ar), 128.8 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 125.9 (Ar), 101.2 (C-1), 100.9 (Ph*C*H(O)$_2$), 98.3 (C-1′), 81.9 (C-4), 74.6 (Ph*C*H$_2$), 74.5 (C-3), 74.4 (*C*H$_2$CCl$_3$), 73.7 (Ph*C*H$_2$), 73.3 (C-4′), 72.1 (C-3′), 71.3 (C-5′), 69.8 (C-2′), 69.1 (C-6′), 68.6 (C-6), 67.7 (TMSCH$_2$*C*H$_2$O), 65.8 (C-5), 56.8 (C-2), 37.8 (CH$_3$C=O*C*H$_2$), 29.8 (*C*H$_3$C=OCH$_2$), 27.9 (CH$_3$C=OCH$_2$*C*H$_2$), 20.7 (OC=O*C*H$_3$), 18.1 (TMS*C*H$_2$CH$_2$O), −1.4 (*C*H$_3$)$_3$Si); HRMS (ESI) calcd for (M+NH$_4$) C$_{48}$H$_{64}$Cl$_3$N$_2$O$_{15}$Si: 1041.3136. Found: 1041.3120.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.108): Disaccharide **4.107** (3.15 g, 3.08 mmol) was dissolved in 4:1 AcOH–H$_2$O (50 mL) and the solution was heated at 60 °C for 6 h. After cooling to r.t., the

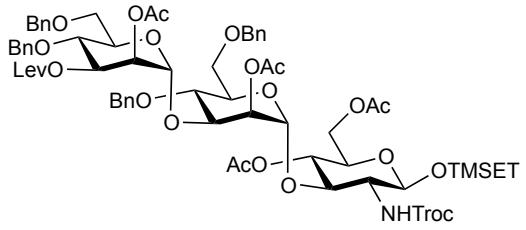
solvent was evaporated, the residue was dissolved with EtOAc, washed with a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. Then, the residue was dissolved in 2:3 Ac_2O–pyridine (25 mL) and stirred at r.t. for 2 h. The solvent was evaporated under high vacuum and the residue was diluted with CH_2Cl_2, washed with 1M HCl, a satd aq solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography (gradient 33→50% EtOAc in hexane) to afford **4.108** (2.95 g, 94% yield) as a white foam; R_f 0.26 (1:1 hexane–EtOAc); $[\alpha]_D$ = +19.2 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.39–7.25 (m, 8 H, ArH), 7.17–7.16 (m, 2 H, ArH), 5.90 (d, 1 H, *J* = 6.9 Hz, N*H*), 5.23 (dd, 1 H, *J* = 8.5, 3.0 Hz, H-3′), 5.04 (app t, 1 H, *J* = 9.5 Hz, H-4), 5.01 (app t, 1 H, *J* = 3.0 Hz, H-2′), 4.90 (s, 1 H, H-1′), 4.70 (d, 1 H, *J* = 11.8 Hz, C*H*$_2$CCl$_3$), 4.64 (d, 1 H, *J* = 11.2 Hz, PhC*H*$_2$), 4.60 (d, 1 H, *J* = 11.8 Hz, PhC*H*$_2$), 4.59 (d, 1 H, *J* = 8.5 Hz, H-1), 4.51 (d, 1 H, *J* = 11.8 Hz, PhC*H*$_2$), 4.46 (d, 1 H, *J* = 11.8 Hz, C*H*$_2$CCl$_3$), 4.44 (d, 1 H, *J* = 11.2 Hz, PhC*H*$_2$), 4.19 (dd, 1 H, *J* = 12.2, 5.0 Hz, H-6), 4.15 (app t, 1 H, *J* = 9.5 Hz, H-3), 4.05 (dd, 1 H, *J* = 12.2, 2.5 Hz, H-6), 4.02–4.00 (m, 1 H, H-5′), 3.86 (app td, 1 H, *J* = 10.0, 6.2 Hz, TMSCH$_2$C*H*$_2$O), 3.78 (app t, 1 H, *J* = 8.5 Hz, H-4′), 3.66–3.59 (m, 2 H, H-6′), 3.55–3.52 (m, 1 H, H-5), 3.44 (app td, 1 H, *J* = 10.0, 6.2 Hz, TMSCH$_2$C*H*$_2$O), 3.22–3.21 (m, 1 H, H-2), 2.68 (t, 2 H, *J* = 6.8 Hz, CH$_3$C=OC*H*$_2$), 2.45 (t, 2 H, *J* = 6.8 Hz, CH$_3$C=OC*H*$_2$), 2.13 (s, 3 H, C*H*$_3$C=OCH$_2$), 2.09 (s, 3 H, OC=OC*H*$_3$), 2.07 (s, 3 H, OC=OC*H*$_3$), 2.05 (s, 3 H, OC=OC*H*$_3$), 0.90–0.83 (m, 2 H, TMSC*H*$_2$CH$_2$O), –0.03 (s, 9 H, (C*H*$_3$)$_3$Si); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.1

(CH_3C=OCH_2), 171.4 (O*C*=OCH_2CH_2), 170.8 (O*C*=OCH_3), 169.9 (O*C*=OCH_3), 169.5 (O*C*=OCH_3), 154.1 (NHC=O), 137.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 99.7 (C-1), 98.8 (C-1′), 78.0 (C-3), 74.4 (CH_2CCl_3), 74.3 (PhCH_2), 73.7 (PhCH_2), 73.5 (C-4′), 71.5 (C-3′, C-5′, C-5), 70.7 (C-4), 70.5 (C-2′), 69.1 (C-6′), 67.5 ($TMSCH_2CH_2O$), 62.3 (C-6), 57.6 (C-2), 37.9 (CH_3C=OCH_2), 29.8 (CH_3C=OCH_2), 27.9 (CH_3C=OCH_2CH_2), 20.9 (OC=OCH_3), 20.8 (OC=OCH_3), 20.7 (OC=OCH_3), 18.1 ($TMSCH_2CH_2O$), −1.4 ($(CH_3)_3Si$); HRMS (ESI) calcd for ($M+NH_4$) $C_{45}H_{64}Cl_3N_2O_{17}Si$: 1037.3034. Found: 1037.3014.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6 -di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.109): A solution of **4.108** (2.86 g, 2.8 mmol) and hydrazine acetate (515 mg, 5.6 mmol) in 9:1 CH_2Cl_2–CH_3OH (100 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 33→50% EtOAc in hexane) to afford **4.109** (2.52 g, 98% yield) as a white foam; R_f 0.30 (1:1 hexane–EtOAc); $[\alpha]_D$ = +10.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.37–7.26 (m, 8 H, ArH), 7.22–7.21 (m, 2 H, ArH), 5.94 (d, 1 H, *J* = 7.3 Hz, N*H*), 5.02 (dd, 1 H, *J* = 9.9, 9.2 Hz, H-4), 4.92 (s, 1 H, H-1′), 4.86 (dd, 1 H, *J* = 3.5, 1.8 Hz, H-2′), 4.74 (d, 1 H, *J*

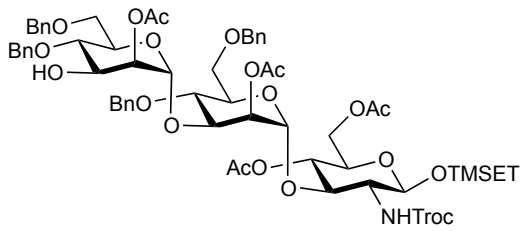
= 10.8 Hz, PhCH_2), 4.68 (d, 1 H, J = 11.8 Hz, CH_2CCl$_3$), 4.62 (d, 1 H, J = 11.4 Hz, PhCH_2), 4.58–4.51 (m, 4 H, H-1, CH_2CCl$_3$, PhCH_2), 4.21 (dd, 1 H, J = 12.3, 5.0 Hz, H-6), 4.12–4.09 (m, 1 H, H-3), 4.06 (dd, 1 H, J = 12.3, 2.6 Hz, H-6), 4.03 (dd, 1 H, J = 8.8, 3.5 Hz, H-3′), 3.96–3.94 (m, 1 H, H-5′), 3.87 (td, 1 H, J = 10.0, 6.5 Hz, TMSCH$_2$CH_2O), 3.72 (dd, 1 H, J = 10.2, 1.7 Hz, H-6′), 3.66–3.64 (m, 1 H, H-4′, H-6′), 3.55–3.54 (m, 1 H, H-5), 3.48–3.44 (m 1 H, TMSCH$_2$CH_2O), 3.26–3.23 (m, 1 H, H-2), 2.11 (s, 3 H, OC=OCH_3), 2.10 (s, 3 H, OC=OCH_3), 2.05 (s, 3 H, OC=OCH_3), 0.90–0.86 (m, 2 H, TMSCH_2CH$_2$O), –0.01 (s, 9 H, (CH_3)$_3$Si); ^{13}C NMR (125 MHz, CDCl$_3$, δ_C) 170.8 (O*C*=OCH$_3$), 170.4 (O*C*=OCH$_3$), 169.9 (O*C*=OCH$_3$), 154.1 (NHC=O), 138.0 (Ar), 137.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 99.8 (C-1), 98.9 (C-1′), 78.5 (C-3), 76.0 (C-4′), 74.9 (Ph*C*H$_2$), 74.4 (*C*H$_2$CCl$_3$), 73.6 (Ph*C*H$_2$), 72.9 (C-2′), 71.6 (C-5′), 71.5 (C-5), 70.8 (C-4), 70.0(C-3′), 69.2 (C-6′), 67.5 (TMSCH$_2$*C*H$_2$O), 62.3 (C-6), 57.6 (C-2), 21.0 (OC=O*C*H$_3$), 20.9 (OC=O*C*H$_3$), 20.8 (OC=O*C*H$_3$), 18.1 (TMS*C*H$_2$CH$_2$O), –1.4 (*C*H$_3$)$_3$Si); HRMS (ESI) calcd for (M+NH$_4$) C$_{40}$H$_{58}$Cl$_3$N$_2$O$_{15}$Si: 939.2667. Found: 939.2649.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-

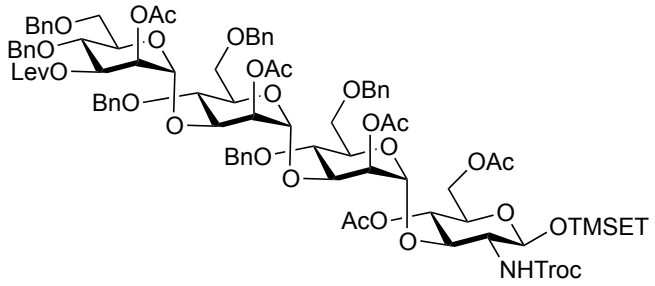
2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.110): A mixture of thioglycoside **4.70** (1.44 g, 2.38 mmol), acceptor **4.109** (2.0 g, 2.12 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (50 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (0.73 g, 3.25 mmol and AgOTf (166 mg, 0.65 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (1.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 25→40% EtOAc in hexane) to afford **4.110** (2.75 g, 91% yield) as a white foam; R_f 0.46 (1:1 hexane–EtOAc); $[\alpha]_D$ = +33.4 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.37–7.22 (m, 14 H, ArH), 7.18–7.16 (m, 6 H, ArH), 5.94 (d, 1 H, *J* = 7.5 Hz, N*H*), 5.30 (dd, 1 H, *J* = 3.3, 1.9 Hz, H-2″), 5.26 (dd, 1 H, *J* = 9.6, 3.2 Hz, H-3″), 5.06 (d, 1 H, *J* = 1.7 Hz, H-1″), 5.00 (dd, 1 H, *J* = 9.9, 9.2 Hz, H-4′), 4.91 (dd, 1 H, *J* = 3.0, 1.8 Hz, H-2′), 4.89 (s, 1 H, H-1′), 4.76 (d, 1 H, *J* = 11.9 Hz, PhC*H*$_2$), 4.75 (d, 1 H, *J* = 10.0 Hz, PhC*H*$_2$), 4.68 (d, 1 H, *J* = 11.8 Hz, C*H*$_2$CCl$_3$), 4.61 (d, 1 H, *J* = 11.3 Hz, PhC*H*$_2$), 4.60–4.49 (m, 5 H, H-1, C*H*$_2$CCl$_3$, PhC*H*$_2$), 4.47 (d, 1 H, *J* = 12.4 Hz, PhC*H*$_2$), 4.43 (d, 1 H, *J* = 10.0 Hz, PhC*H*$_2$), 4.21 (dd, 1 H, *J* = 12.5, 5.5 Hz, H-6), 4.12–4.09 (m, 1 H, H-3), 4.06 (dd, 1 H, *J* = 6.3, 3.3 Hz, H-3′), 4.05–4.03 (m, 2 H, H-6, H-4″), 3.94–3.92 (m, 1 H, H-5′), 3.88 (td, 1 H, *J* = 10.0, 6.0 Hz, TMSCH$_2$C*H*$_2$O), 3.82 (dd, 1 H, *J* = 11.0, 2.5 Hz, H-6″), 3.76 (app t, 1 H, *J* = 10.0 Hz, H-4′), 3.72 (app dt, 1 H, *J* = 9.7, 2.0 Hz, H-5″), 3.68 (dd, 1 H, *J* = 11.0, 1.8 Hz, H-6″), 3.66 (dd, 1 H, *J* = 10.0, 1.5 Hz, H-6′), 3.61–3.59 (m, 1 H, H-6′), 3.55–3.54 (m, 1 H, H-5),

3.48–3.44 (m 1 H, $TMSCH_2CH_2O$), 3.22–3.19 (m, 1 H, H-2), 2.69 (dt, 1 H, J = 18.4, 7.0 Hz, $CH_3C{=}OCH_2$), 2.61 (dt, 1 H, J = 18.4, 6.5 Hz, $CH_3C{=}OCH_2$), 2.46 (dt, 1 H, J = 17.3, 7.0 Hz, $OC{=}OCH_2CH_2$), 2.41 (dt, 1 H, J = 17.3, 6.5 Hz, $OC{=}OCH_2CH_2$), 2.13 (s, 3 H, $CH_3C{=}OCH_2$), 2.08 (s, 6 H, 2 x $OC{=}OCH_3$), 2.06 (s, 3 H, $OC{=}OCH_3$), 2.05 (s, 3 H, $OC{=}OCH_3$), 0.90–0.86 (m, 2 H, $TMSCH_2CH_2O$), –0.01 (s, 9 H, $(CH_3)_3Si$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.1 ($CH_3C{=}OCH_2$), 171.8 ($OC{=}OCH_2CH_2$), 170.7 ($OC{=}OCH_3$), 170.4 ($OC{=}OCH_3$), 169.9 ($OC{=}OCH_3$), 169.6 ($OC{=}OCH_3$), 154.1 (NHC=O), 138.4 (Ar), 138.3 (Ar), 137.7 (Ar), 128.4 (Ar), 128.3 (Ar), 128.28 (Ar), 128.25 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 99.7 (C-1), 99.6 (C-1″), 98.7 (C-1′), 78.6 (C-3), 77.3(C-3′), 75.2 ($PhCH_2$), 74.6 (C-4′), 74.5 ($PhCH_2$), 74.4 (CH_2CCl_3), 73.6 ($PhCH_2$), 72.5 (C-4″, C-5″), 72.2 (C-2′), 72.0 (C-3″), 71.9 (C-5′), 71.5 (C-5), 70.6 (C-4), 69.9 (C-2″), 68.9 (C-6′), 68.1 (C-6″), 67.6 ($TMSCH_2CH_2O$), 62.4 (C-6), 57.6 (C-2), 37.8 ($CH_3C{=}OCH_2$), 29.8 ($CH_3C{=}OCH_2$), 27.9 ($CH_3C{=}OCH_2CH_2$), 21.3 ($OC{=}OCH_3$), 20.9 ($OC{=}OCH_3$), 20.8 ($OC{=}OCH_3$), 18.1 ($TMSCH_2CH_2O$), –1.4 $(CH_3)_3Si$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{\text{C-1, H-1}}$ = 165.3 Hz (C-1, H-1), $^1J_{\text{C-1′, H-1′}}$ = 174.8 Hz (C-1′, H-1′), $^1J_{\text{C-1″, H-1″}}$ = 174.9 Hz (C-1″, H-1″); HRMS (ESI) calcd for ($M+NH_4$) $C_{67}H_{88}Cl_3N_2O_{23}Si$: 1421.4607. Found: 1421.4579.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.111): A solution of **4.110** (2.73 g, 1.9 mmol) and hydrazine acetate (320 mg, 3.5 mmol) in 9:1 CH_2Cl_2–CH_3OH (100 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 33→50% EtOAc in hexane) to afford **4.111** (2.35 g, 93% yield) as a white foam; R_f 0.53 (1:1 hexane–EtOAc); $[\alpha]_D$ = +33.2 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.38–7.25 (m, 16 H, ArH), 7.21–7.17 (m, 4 H, ArH), 5.88 (d, 1 H, *J* = 6.7 Hz, N*H*), 5.15 (dd, 1 H, *J* = 3.0, 1.5 Hz, H-2″), 5.10 (s, 1 H, H-1″), 5.00 (app t, 1 H, *J* = 9.5 Hz, H-4′), 4.88 (s, 1 H, H-1′), 4.88 (s, 1 H, H-2′), 4.77 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.73 (d, 1 H, *J* = 11.2 Hz, PhCH_2), 4.68 (d, 1 H, *J* = 11.2 Hz, CH_2CCl$_3$), 4.65 (d, 1 H, *J* = 11.3 Hz, PhCH_2), 4.61–4.48 (m, 5 H, H-1, CH_2CCl$_3$, PhCH_2), 4.45 (d, 1 H, *J* = 12.3 Hz, PhCH_2), 4.43 (d, 1 H, *J* = 10.8 Hz, PhCH_2), 4.21 (dd, 1 H, *J* = 12.1, 5.0 Hz, H-6), 4.14–4.01 (m, 1 H, H-3), 4.07 (dd, 1 H, *J* = 7.0, 2.7 Hz, H-3′), 4.05 (dd, 1 H, *J* = 12.1, 2.3 Hz, H-6), 3.98 (dd, 1 H, *J* = 9.5, 3.3 Hz, H-3″), 3.93–3.90 (m, 1 H, H-5′), 3.90–3.87 (m, 1 H, TMSCH$_2$CH_2O), 3.86 (app t, 1 H, *J* = 9.5 Hz, H-4″), 3.82 (dd, 1 H, *J* = 11.3, 3.0 Hz, H-6″), 3.79 (app t, 1 H, *J* = 9.0 Hz, H-4′), 3.71 (dd, 1 H, *J* = 11.3, 1.5 Hz, H-6″),

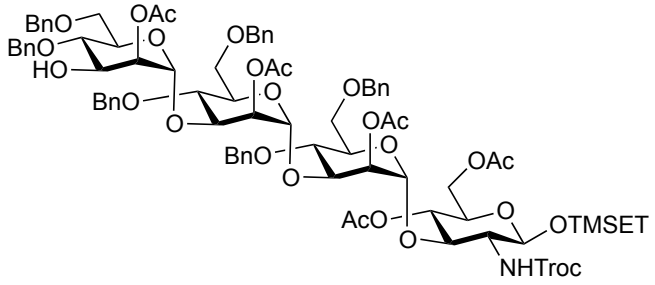
3.63–3.57 (m, 3 H, H-5″, H-6′), 3.55–3.53 (m, 1 H, H-5), 3.48–3.44 (m 1 H, $TMSCH_2CH_2O$), 3.22–3.19 (m, 1 H, H-2), 2.08 (s, 3 H, $OC=OCH_3$), 2.07 (s, 3 H, $OC=OCH_3$), 2.06 (s, 3 H, $OC=OCH_3$), 2.05 (s, 3 H, $OC=OCH_3$), 0.90–0.86 (m, 2 H, $TMSCH_2CH_2O$), –0.02 (s, 9 H, $(CH_3)_3Si$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.8 ($OC=OCH_3$), 170.6 ($OC=OCH_3$), 170.1 ($OC=OCH_3$), 169.6 ($OC=OCH_3$), 154.1 (NHC=O), 138.3 (Ar), 137.8 (Ar), 137.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.02 (Ar), 127.99 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 99.7 (C-1), 99.5 (C-1″), 98.8 (C-1′), 78.6 (C-3), 76.5(C-3′), 75.3 (C-4″), 75.1 ($PhCH_2$), 74.8 (C-4′), 74.6 ($PhCH_2$), 74.4 (CH_2CCl_3), 73.7 ($PhCH_2$), 73.6 ($PhCH_2$), 72.3 (C-2′), 72.2 (C-2″), 72.0 (C-5″, C-5′), 71.5 (C-5), 70.7 (C-4), 70.1 (C-1″), 68.9 (C-6′), 68.2 (C-6″), 67.6 ($TMSCH_2CH_2O$), 62.4 (C-6), 57.6 (C-2), 21.02 ($OC=OCH_3$), 20.99 ($OC=OCH_3$), 20.84 ($OC=OCH_3$), 20.82 ($OC=OCH_3$), 18.1 ($TMSCH_2CH_2O$), –1.4 $(CH_3)_3Si$); HRMS (ESI) calcd for $(M+NH_4)$ $C_{62}H_{82}Cl_3N_2O_{21}Si$: 1323.4239. Found: 1323.4213.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-

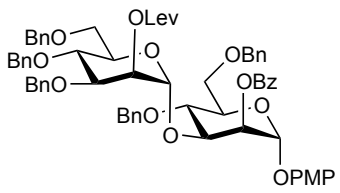
***O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroeth-oxycarbonylamino)-β-D-glucopyranoside (4.112):** A mixture of thioglycoside **4.70** (1.25 g, 2.06 mmol), acceptor **4.111** (2.43 g, 1.87 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (50 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (0.63 g, 2.81 mmol and AgOTf (143 mg, 0.56 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 25→40% EtOAc in hexane) to afford **4.112** (2.75 g, 83% yield) as a white solid; R_f 0.42 (1:1 hexane–EtOAc); $[\alpha]_D$ = +36.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.37–7.30 (m, 8 H), 7.28–7.18 (m, 18 H), 7.16–7.12 (m, 4 H), 5.85 (d, 1 H, *J* = 7.1 Hz), 5.30 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.28–5.27 (m, 1 H), 5.26 (dd, 1 H, *J* = 9.5, 3.0 Hz), 5.10 (s, 1 H), 5.08 (d, 1 H, *J* = 1.5 Hz), 4.99 (app t, 1 H, *J* = 9.5 Hz), 4.90 (s, 1 H), 4.87 (app t, 1 H, *J* = 2.5 Hz), 4.78 (d, 1 H, *J* = 10.5 Hz), 4.73 (d, 1 H, *J* = 12.1 Hz), 4.71 (d, 1 H, *J* = 10.9 Hz), 4.65 (d, 1 H, *J* = 11.5 Hz), 4.62–4.54 (m, 3 H), 4.53–4.43 (m, 5 H), 4.34 (d, 1 H, *J* = 10.5 Hz), 4.21–4.16 (m, 2 H), 4.13–4.02 (m, 5 H), 3.97 (app t, 1 H, *J* = 10.0 Hz), 3.90–3.85 (m, 2 H), 3.81–3.73 (m, 3 H), 3.65 (dd, 1 H, *J* = 11.0, 2.0 Hz), 3.63 (dd, 1 H, *J* = 11.0, 2.5 Hz), 3.59–3.52 (m, 4 H), 3.47–3.43 (m, 1 H), 3.40 (dd, 1 H, *J* = 11.0, 1.5 Hz), 3.19–3.16 (m, 1 H), 2.68 (dt, 1 H, *J* = 18.5, 7.0 Hz), 2.61 (dt, 1 H, *J* = 18.5, 6.5 Hz), 2.46 (dt, 1 H, *J* = 17.5, 7.0 Hz), 2.41 (dt, 1 H, *J* = 17.5, 6.5 Hz), 2.12 (s, 3 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H),

0.90–0.86 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.2, 171.8, 170.8, 170.4, 170.3, 169.8, 169.6, 154.0, 138.3, 138.2, 138.1, 138.0, 137.8, 137.6, 128.5, 128.4, 128.3, 128.27, 128.25, 128.22, 127.9, 127.86, 127.83, 127.64, 127.60, 127.5, 100.0, 99.7, 98.6, 95.6, 78.6, 77.6, 75.3, 75.1, 74.5, 74.4, 74.3, 74.2, 73.6, 73.5, 73.3, 72.6, 72.5, 72.4, 72.1, 72.0, 71.8, 71.5, 70.7, 70.0, 68.9, 68.1, 68.0, 67.5, 62.4, 60.4, 57.6, 37.9, 29.8, 27.9, 21.1, 21.0, 20.84, 20.82, 18.1, –1.4; ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{C\text{-}1,\,H\text{-}1}$ = 173.6, 170.8, 170.8, 161.0 Hz; HRMS (ESI) calcd for (M+NH_4) $C_{89}H_{112}Cl_3N_2O_{29}Si$: 1805.6180. Found: 1805.6149.



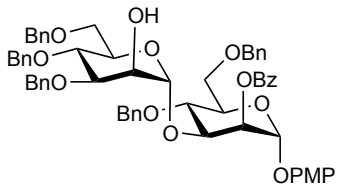
2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl-amino)-β-D-glucopyranoside (4.65): A solution of **4.112** (2.70 g, 1.5 mmol) and hydrazine acetate (250 mg, 2.7 mmol) in 9:1 CH_2Cl_2–CH_3OH (100 mL) was stirred at r.t. for 3 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 33→40% EtOAc in hexane) to afford **4.65** (2.39 g, 94% yield) as a white solid; R_f 0.53 (1:1 hexane–EtOAc); $[\alpha]_D$ = +38.6 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700

MHz, $CDCl_3$, δ_H) 7.36–7.19 (m, 24 H), 7.18–7.15 (m, 6 H), 5.85 (d, 1 H, *J* = 6.7 Hz), 5.23 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.14 (dd, 1 H, *J* = 3.3, 1.7 Hz), 5.10 (d, 1 H, *J* = 1.5 Hz), 5.09 (d, 1 H, *J* = 1.5 Hz), 4.99 (app t, 1 H, *J* = 9.5 Hz), 4.90 (s, 1 H), 4.89 (app t, 1 H, *J* = 2.5 Hz), 4.79 (d, 1 H, *J* = 10.0 Hz), 4.72 (d, 1 H, *J* = 11.7 Hz), 4.69 (d, 1 H, *J* = 10.9 Hz), 4.65 (d, 1 H, *J* = 11.9 Hz), 4.62–4.54 (m, 3 H), 4.51–4.43 (m, 6 H), 4.34 (d, 1 H, *J* = 10.5 Hz), 4.21–4.16 (m, 2 H), 4.13–3.98 (m, 6 H), 3.90–3.85 (m, 2 H), 3.81–3.74 (m, 3 H), 3.69 (app dt, 1 H, *J* = 9.5, 2.0 Hz), 3.65 (dd, 1 H, *J* = 11.0, 2.0 Hz), 3.62 (dd, 1 H, *J* = 11.0, 3.0 Hz), 3.59–3.52 (m, 4 H), 3.47–3.43 (m, 1 H), 3.40 (dd, 1 H, *J* = 11.0, 1.5 Hz), 3.19–3.16 (m, 1 H), 2.08 (s, 6 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 0.90–0.86 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.8, 170.4, 170.3, 170.1, 169.6, 154.0, 138.4, 138.3, 138.1, 138.0, 137.8, 137.6, 128.6, 128.5, 128.4, 128.3, 128.28, 128.27, 128.0, 127.96, 127.94, 127.86, 127.84, 127.81, 127.6, 127.5, 99.9, 99.7, 99.6, 98.6, 78.5, 77.8, 77.1, 75.3, 75.2, 75.0, 74.5, 74.4, 74.3, 73.5, 73.4, 72.6, 72.5, 72.3, 71.9, 71.8, 71.5, 70.7, 70.0, 68.9, 68.3, 68.1, 67.5, 62.4, 57.6, 21.1, 21.0, 20.9, 20.84, 20.82, 18.1, –1.4; ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{C\text{-}1,\ H\text{-}1}$ = 176.8, 172.3, 172.3, 162.2 Hz HRMS (ESI) calcd for (M+NH_4) $C_{84}H_{106}Cl_3N_2O_{27}Si$: 1707.5812. Found: 1707.5801.



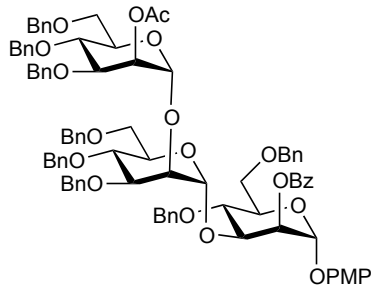
***p*-Methoxyphenyl 3,4,6-tri-*O*-Benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.113):** A mixture of acceptor **4.69** (1.08 g, 1.90 mmol), donor **4.66** (1.37 g, 2.09 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (100 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (702 mg, 3.13 mmol) and AgOTf (146 mg, 0.57 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h before Et_3N (1.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→33% EtOAc in hexane) to afford **4.113** (1.96 g, 94% yield) as a white foam; R_f 0.24 (2:1 hexane–EtOAc); $[\alpha]_D$ = +32.9 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.03–8.02 (m, 2 H, ArH), 7.56–7.54 (m, 1 H, ArH), 7.36–7.16 (m, 25 H, ArH), 7.07–7.05 (m, 2 H, ArH), 6.97–6.96 (m, 2 H, ArH), 6.77–6.76 (m, 2 H, ArH), 5.59 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.36 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2′), 5.23 (d, 1 H, *J* = 2.0 Hz, H-1′), 4.77 (d, 1 H, *J* = 10.5 Hz, PhCH_2), 4.75 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.67 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.65 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.54 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.52 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3), 4.47 (d, 1 H, *J* = 11.5 Hz, PhCH_2), 4.45 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.43 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.42 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.32 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.22 (app t, 1 H, *J* = 9.5 Hz, H-4), 3.96 (ddd, 1 H, *J* = 10.0, 3.0,

1.5 Hz, H-5), 3.88 (app t, 1 H, J = 9.5 Hz, H-4′), 3.86–3.83 (m, 3 H, H-3′, H-5′, H-6), 3.74 (s, 3 H, OCH_3), 3.68 (dd, 1 H, J = 11.0, 2.0 Hz, H-6), 3.63 (dd, 1 H, J = 11.0, 3.0 Hz, H-6′), 3.59 (dd, 1 H, J = 11.0, 1.5 Hz, H-6′), 2.65–2.61 (m, 4 H, $CH_3C{=}OCH_2$, $CH_3C{=}OCH_2CH_2$), 2.08 (s, 3 H, $CH_3C{=}OCH_2$,); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.1 ($CH_3C{=}OCH_2$), 171.8 ($OC{=}OCH_2$), 165.7 (PhC=O), 155.1 (Ar), 149.9 (Ar), 138.6 (Ar), 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 137.8 (Ar), 133.3 (Ar), 129.9 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.22 (Ar), 128.20 (Ar), 128.1 (Ar), 127.92 (Ar), 127.90 (Ar), 127.8 (Ar), 127.7 (Ar), 127.55 (Ar), 127.53 (Ar), 127.52 (Ar), 127.4 (Ar), 127.3 (Ar), 117.8 (Ar), 114.6 (Ar), 99.7 (C-1′), 96.2 (C-1), 77.7 (C-3′), 77.0 (C-3), 75.3 ($PhCH_2$), 74.54 (C-4), 74.53 ($PhCH_2$), 73.9 (C-4′), 73.4 ($PhCH_2$), 72.3 (C-5′), 72.1 (C-5), 72.0 (C-2), 71.7 ($PhCH_2$), 69.2 (C-2′), 68.7 (C-6), 68.4 (C-6′), 55.6 (CH_3O), 38.0 ($CH_3C{=}OCH_2$), 29.7 ($CH_3C{=}OCH_2$), 28.2 ($CH_3C{=}OCH_2CH_2$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 174.5 Hz (C-1, H-1), $^{1}J_{C\text{-}1',\ H\text{-}1'}$ = 174.5 Hz (C-1′, H-1′); HRMS (ESI) calcd for (M+Na) $C_{66}H_{68}NaO_{15}$: 1123.4450. Found: 1123.4434.



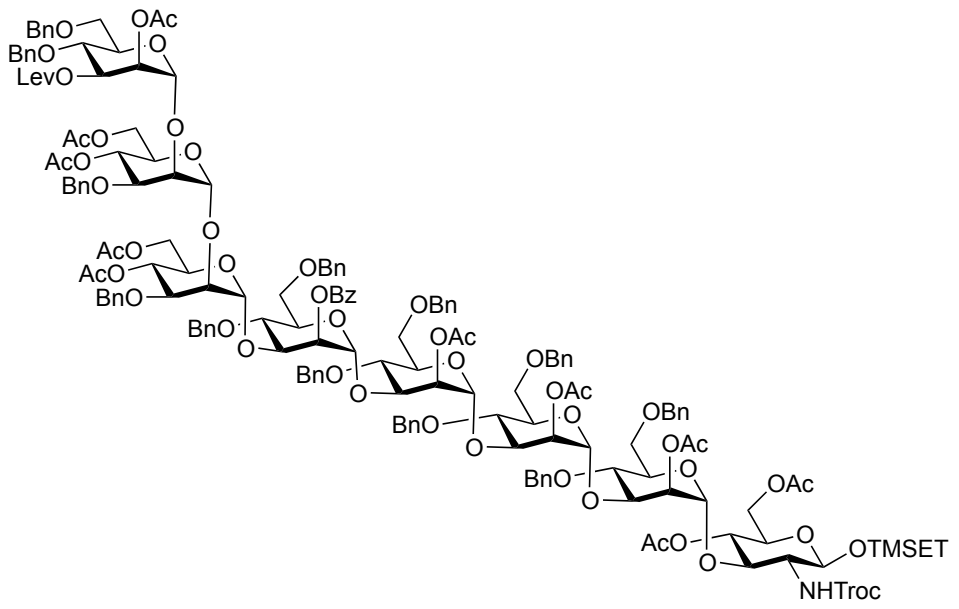
***p*-Methoxyphenyl 3,4,6-tri-*O*-Benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.114):** A solution of **4.113** (1.88 g, 1.71 mmol) and hydrazine acetate (282 mg, 3.07 mmol) in 9:1 CH_2Cl_2–CH_3OH (100 mL) was stirred at r.t.

for 2 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 33→40% EtOAc in hexane) to afford **4.114** (1.60 g, 93% yield) as a white foam; R_f 0.24 (2:1 hexane–EtOAc); $[\alpha]_D$ = +53.1 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.06–8.04 (m, 2 H, ArH), 7.57–7.55 (m, 1 H, ArH), 7.38–7.19 (m, 25 H, ArH), 7.09–7.07 (m, 2 H, ArH), 6.98–6.97 (m, 2 H, ArH), 6.78–6.77 (m, 2 H, ArH), 5.61 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.27 (d, 1 H, *J* = 2.0 Hz, H-1′), 4.72 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.70–4.68 (m, 2 H, PhC*H*$_2$), 4.65 (d, 1 H, *J* = 12.0 Hz, PhC*H*$_2$), 4.55 (d, 1 H, *J* = 11.0 Hz, PhC*H*$_2$), 4.53 (d, 1 H, *J* = 11.5 Hz, PhC*H*$_2$), 4.51 (dd, 1 H, *J* = 9.5, 3.0 Hz, H-3), 4.48–4.43 (m, 4 H, PhC*H*$_2$), 4.21 (app t, 1 H, *J* = 9.5 Hz, H-4), 3.98 (ddd, 1 H, *J* = 10.0, 3.0, 1.5 Hz, H-5), 3.90–3.84 (m, 4 H, H-2′, H-5′, H-6, H-4′), 3.74 (s, 3 H, OCH_3), 3.72 (dd, 1 H, *J* = 9.0, 3.0 Hz, H-3′), 3.69 (dd, 1 H, *J* = 11.0, 2.0 Hz, H-6), 3.63–3.60 (m, 2 H, H-6′), 3.59 (dd, 1 H, *J* = 11.0, 1.5 Hz, H-6′), 2.35 (d, 1 H, *J* = 3.0 Hz, OH); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 165.7 (PhC=O), 155.1 (Ar), 150.0 (Ar), 138.5 (Ar), 138.3 (Ar), 138.2 (Ar), 137.93 (Ar), 137.91 (Ar), 133.2 (Ar), 129.9 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.86 (Ar), 127.84 (Ar), 127.82 (Ar), 127.80 (Ar), 127.77 (Ar), 127.57 (Ar), 127.55 (Ar), 127.53 (Ar), 127.4 (Ar), 127.3 (Ar), 117.8 (Ar), 114.6 (Ar), 101.6 (C-1′), 96.3 (C-1), 79.7 (C-3′), 77.4 (C-3), 75.2 (Ph*C*H_2), 74.6 (C-4), 74.5 (Ph*C*H_2), 73.9 (C-4′), 73.4 (Ph*C*H_2), 72.3 (C-2), 72.15 (Ph*C*H_2), 72.11 (C-5), 71.9 (C-5′), 69.0 (C-2′), 68.7 (C-6), 68.4 (C-6′), 55.6 (CH_3O); HRMS (ESI) calcd for (M+Na) $C_{61}H_{62}NaO_{13}$: 1025.4083. Found: 1025.4066.



***p*-Methoxyphenyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)- 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.63):** A mixture of acceptor **4.114** (1.57 g, 1.56 mmol), donor **4.72** (1.08 g, 1.80 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (100 mL) and stirred at r.t. for 10 min. The solution was then cooled to −15 °C, and then NIS (602 mg, 2.69 mmol) and AgOTf (119 mg, 0.49 mmol) were added. The solution was slowly warmed to 0 °C and stirred for 1 h. Et_3N (1.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→25% EtOAc in hexane) to afford **4.63** (2.0 g, 86% yield) as a white foam; R_f 0.44 (2:1 hexane–EtOAc); $[\alpha]_D$ = +37.6 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.06–8.04 (m, 2 H, ArH), 7.57–7.55 (m, 1 H, ArH), 7.38–7.06 (m, 42 H, ArH), 6.95–6.94 (m, 2 H, ArH), 6.75–6.74 (m, 2 H, ArH), 5.62 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2), 5.57 (d, 1 H, *J* = 2.0 Hz, H-1), 5.48 (dd, 1 H, *J* = 3.0, 2.0 Hz, H-2″), 5.31 (d, 1 H, *J* = 1.5 Hz, H-1′), 5.02 (d, 1 H, *J* = 1.5 Hz, H-1″), 4.79 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.75 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.74 (d, 1 H, *J* = 11.0 Hz, PhCH_2), 4.66 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.62 (d, 1 H, *J* = 12.0 Hz, PhCH_2), 4.58 (d, 1 H, *J* = 12.5 Hz, PhCH_2), 4.57

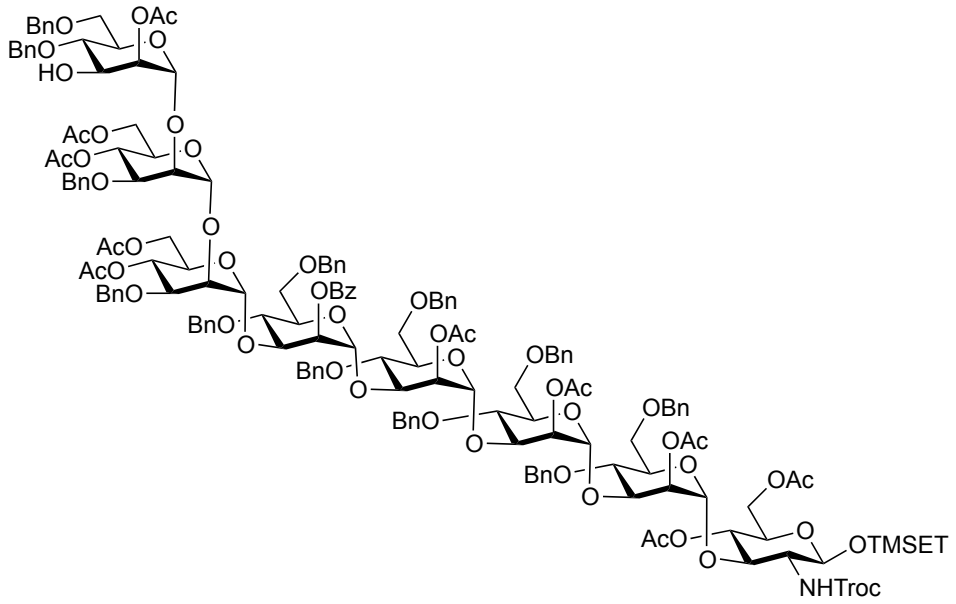
(d, 1 H, J = 11.5 Hz, PhCH_2), 4.52–4.49 (m, 3 H, PhCH_2), 4.48 (dd, 1 H, J = 9.5, 3.0 Hz, H-3), 4.42–4.39 (m, 4 H, PhCH_2), 4.36 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.28 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.17 (app t, 1 H, J = 9.5 Hz, H-4), 3.95–3.90 (m, 5 H, H-2′, H-4′, H-5, H-3″, H-5″), 3.85 (app t, 1 H, J = 9.5 Hz, H-4″), 3.81–3.77 (m, 3 H, H-6, H-3′, H-5′), 3.74 (s, 3 H, OCH_3), 3.67–3.63 (m, 2 H, H-6, H-6″), 3.57 (dd, 1 H, J = 11.0, 3.5 Hz, H-6′), 3.54 (dd, 1 H, J = 11.0, 1.5 Hz, H-6′), 3.69 (dd, 1 H, J = 10.5, 1.0 Hz, H-6″), 2.10 (s, 3 H, OC=OCH_3); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.1 (OC=OCH_3), 165.7 (PhC=O), 155.1 (Ar), 150.0 (Ar), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.3 (Ar), 138.28 (Ar), 138.26 (Ar), 138.19 (Ar), 138.0 (Ar), 133.2 (Ar), 129.9 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.17 (Ar), 128.12 (Ar), 128.11 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.56 (Ar), 127.54 (Ar), 127.48 (Ar), 127.46 (Ar), 127.44 (Ar), 127.39 (Ar), 127.37 (Ar), 127.26 (Ar), 127.21 (Ar), 117.9 (Ar), 114.5 (Ar), 101.0 (C-1′), 99.4 (C-1″), 96.3 (C-1), 79.1 (C-3′), 78.4 (C-3), 78.2 (C-3′), 75.3, 75.1 (PhCH$_2$), 74.9 (PhCH$_2$), 74.4 (PhCH$_2$), 74.3 (C-4), 74.1 (C-4″), 73.34 (PhCH$_2$), 73.3 (PhCH$_2$), 73.2 (PhCH$_2$), 72.8, 72.2, 72.1 (PhCH$_2$), 72.0, 71.97, 71.9 (PhCH$_2$), 68.8, 68.7, 68.6 68.5, 55.6 (CH_3O), 21.1 (OC=OCH$_3$); ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^1J_{\text{C-1, H-1}}$ = 176.2 Hz (C-1, H-1), $^1J_{\text{C-1′, H-1′}}$ = 170.5 Hz (C-1′, H-1′), $^1J_{\text{C-1″, H-1″}}$ = 171.9 Hz (C-1″, H-1″); HRMS (ESI) calcd for (M+Na) $C_{90}H_{92}NaO_{19}$: 1499.6125. Found: 1499.6120.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.120): Trichloroacetimidate **4.117** (780 mg) was prepared from tetrasaccharide **4.64** (1.19 g) in 65% yield following general procedure A described above. A mixture of tetrasaccharide acceptor **4.65** (532 mg, 0.31 mmol), trichloroacetimidate donor **4.117** (460 mg, 0.29 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (100 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C, and then TBSOTf (20 μL) was added. The solution was stirred for 1 h before Et_3N (0.2 mL) was added and the mixture was filtered. The filtrate was concentrated and the

resulting residue was purified by chromatography (gradient 30→60% EtOAc in hexane) to afford **4.120** (750 mg, 86%) yield as a white solid; R_f 0.09 (1:1 hexane–EtOAc); $[\alpha]_D$ = +27.9 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.99–7.98 (m, 2 H), 7.58–7.56 (m, 1 H), 7.37–7.06 (m, 60 H), 6.99–6.97 (m, 2 H), 5.84 (d, 1 H, *J* = 6.5 Hz), 5.47 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.37 (dd, 1 H, *J* = 9.5, 3.5 Hz), 5.30 (dd, 1 H, *J* = 3.5, 2.0 Hz), 5.28 (dd, 1 H, *J* = 3.0, 1.5 Hz), 5.26–5.21 (m, 3 H), 5.18–5.15 (m, 2 H), 5.12 (d, 1 H, *J* = 1.5 Hz), 5.09 (s, 1 H), 4.98 (dd, 1 H, *J* = 10.0, 9.0 Hz), 4.91 (d, 1 H, *J* = 1.5 Hz), 4.89 (s, 1 H), 4.86 (d, 1 H, *J* = 1.5 Hz), 4.84 (app t, 1 H, *J* = 2.5 Hz), 4.80 (d, 1 H, *J* = 10.5 Hz), 4.74 (d, 1 H, *J* = 10.5 Hz), 4.71 (d, 1 H, *J* = 10.5 Hz), 4.70 (d, 1 H, *J* = 12.0 Hz), 4.65–4.48 (m, 9 H), 4.44–4.42 (m, 5 H), 4.38–4.32 (m, 4 H), 4.25–4.06 (m, 11 H), 4.04–3.96 (m, 5 H), 3.92–3.72 (m, 14 H), 3.64–3.51 (m, 11 H), 3.46–3.34 (m, 4 H), 3.18–3.15 (m, 1 H), 2.77 (dt, 1 H, *J* = 18.5, 7.0 Hz), 2.63 (dt, 1 H, *J* = 18.5, 6.5 Hz), 2.51 (dt, 1 H, *J* = 17.0, 7.0 Hz), 2.41 (dt, 1 H, *J* = 17.0, 6.5 Hz), 2.14 (s, 3 H), 2.05–2.04 (m, 18 H), 1.93 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.88 (s, 3 H), 0.89–0.82 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 206.5, 171.3, 171.0, 170.8, 170.6, 170.3, 170.2, 170.0, 169.7, 169.6, 169.5, 169.3, 165.2, 154.0, 138.4, 138.3, 138.0, 137.9, 137.87, 137.84, 137.77, 137.73, 137.6, 137.5, 133.4, 129.8, 129.7, 128.5, 128.4, 128.3, 128.29, 128.28, 128.27, 128.24, 128.2, 128.1, 128.0, 127.9, 127.86, 127.84, 127.80, 127.78, 127.72, 127.70, 127.65, 127.62, 127.58, 127.54, 127.48, 127.47, 127.43, 127.37, 100.7, 99.8, 99.6, 98.7, 98.5, 78.2, 77.2, 76.7, 75.9, 75.8, 75.3, 75.1, 74.9, 74.7, 74.3, 74.1, 74.0, 73.5, 73.46, 73.39, 73.37, 73.33,

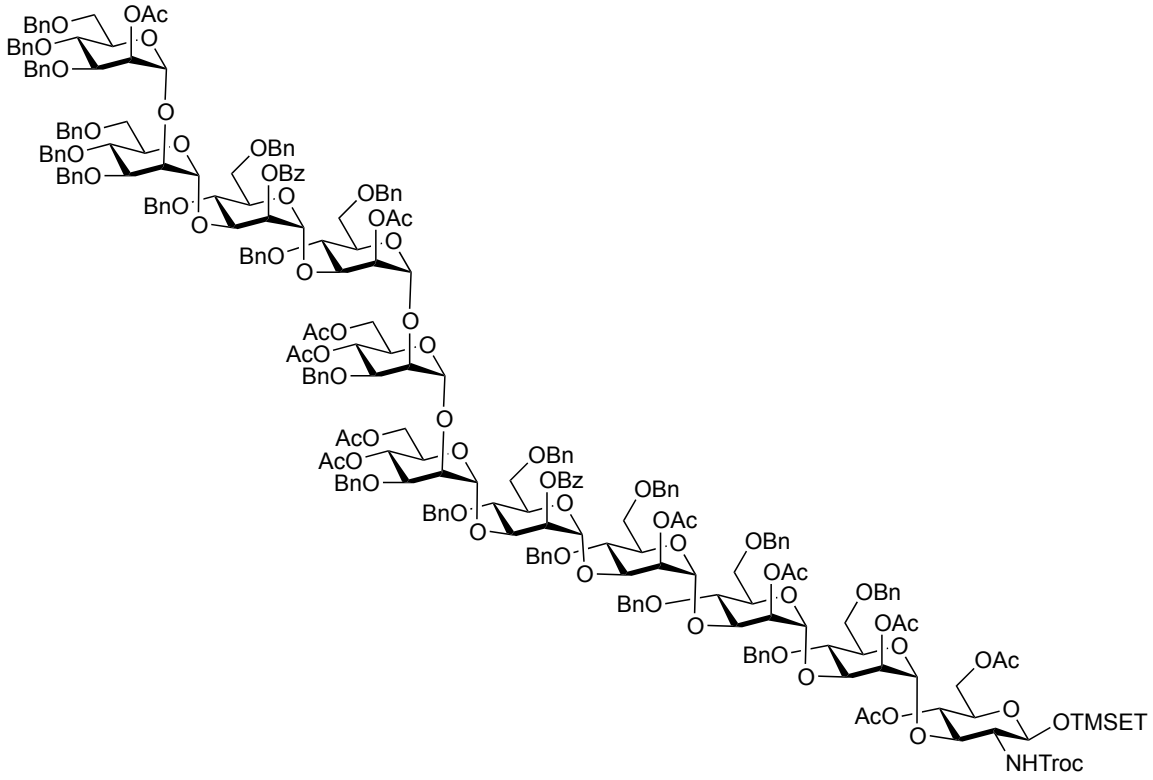
73.2, 72.9, 72.5, 72.4, 72.3, 72.2, 72.0, 71.9, 71.8, 71.76, 71.4, 70.7, 69.8, 69.6, 68.9, 68.4, 68.3, 68.0, 67.5, 67.2, 67.1, 62.5, 62.4, 62.3, 57.6, 37.9, 29.8, 27.9, 21.1, 21.0, 20.97, 20.92, 20.80, 20.78, 20.75, 20.67, 20.61, 18.0, −1.4; ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 175.0, 175.0, 174.3, 174.3, 174.3, 173.6, 172.2, 161.7 Hz; HRMS (ESI) calcd for $(M+3Na)^{+3}$ $C_{172}H_{198}Cl_3NNa_3O_{55}Si$: 1119.7080. Found: 1119.7119.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)- 4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyrano- side (4.121): A solution of **4.120** (0.95 g, 0.29 mmol) and hydrazine acetate

(53 mg, 0.58 mmol) in 9:1 CH_2Cl_2–CH_3OH (50 mL) was stirred at r.t. for 1 h. Then, the solution was concentrated at 40 °C for 0.5 h to achieve complete deproection of the levulinyl group. The resulting residue was subjected to chromatography (gradient 16→33% EtOAc in hexane) to afford **4.121** (0.86 g, 94% yield) as a white solid; R_f 0.13 (1:1 hexane–EtOAc); $[\alpha]_D$ = +29.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.00–7.99 (m, 2 H), 7.59–7.56 (m, 1 H), 7.37–7.07 (m, 60 H), 7.02–7.00 (m, 2 H), 5.83 (d, 1 H, *J* = 7.0 Hz), 5.47 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.28 (dd, 1 H, *J* = 3.0, 1.5 Hz), 5.25 (d, 1 H, *J* = 1.5 Hz), 5.22 (app t, 1 H, *J* = 2.0 Hz), 5.19–5.16 (m, 4 H), 5.11 (d, 1 H, *J* = 1.5 Hz), 5.08 (s, 1 H), 5.01 (d, 1 H, *J* = 1.5 Hz), 4.98 (app t, 1 H, *J* = 9.5 Hz), 4.88 (s, 1 H), 4.84 (app t, 1 H, *J* = 2.5 Hz), 4.79 (d, 1 H, *J* = 10.5 Hz), 4.78–4.70 (m, 5 H), 4.64 (d, 1 H, *J* = 11.5 Hz), 4.59–4.42 (m, 13 H), 4.37 (d, 1 H, *J* = 10.5 Hz), 4.34 (d, 1 H, *J* = 11.0 Hz), 4.33 (d, 1 H, *J* = 10.5 Hz), 4.28 (d, 1 H, *J* = 12.0 Hz), 4.23–4.06 (m, 11 H), 4.04–3.94 (m, 5 H), 3.91–3.81 (m, 9 H), 3.76–3.71 (m, 5 H), 3.65–3.34 (m, 15 H), 3.18–3.14 (m, 1 H), 2.08 (s, 3 H), 2.05–2.03 (m, 15 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.84 (s, 3 H), 0.88–0.84 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.8, 170.6, 170.5, 170.3, 170.2, 170.0, 169.6, 169.5, 165.2, 154.0, 138.4, 138.3, 138.28, 138.1, 137.9, 137.83, 137.78, 137.75, 137.6, 137.5, 133.4, 129.8, 129.7, 128.5, 128.47, 128.41, 128.38, 128.32, 128.29, 128.27, 128.20, 128.1, 128.0, 127.93, 127.91, 127.80, 127.78, 127.74, 127.72, 127.69, 127.62, 127.57, 127.50, 127.49, 127.43, 127.35, 100.8, 100.7, 99.8, 99.6, 99.2, 98.5, 78.2, 75.8, 75.5, 75.4, 75.3, 75.1, 75.04, 74.99, 74.94, 74.4, 74.3, 74.1, 74.0, 73.5, 73.4, 73.33,

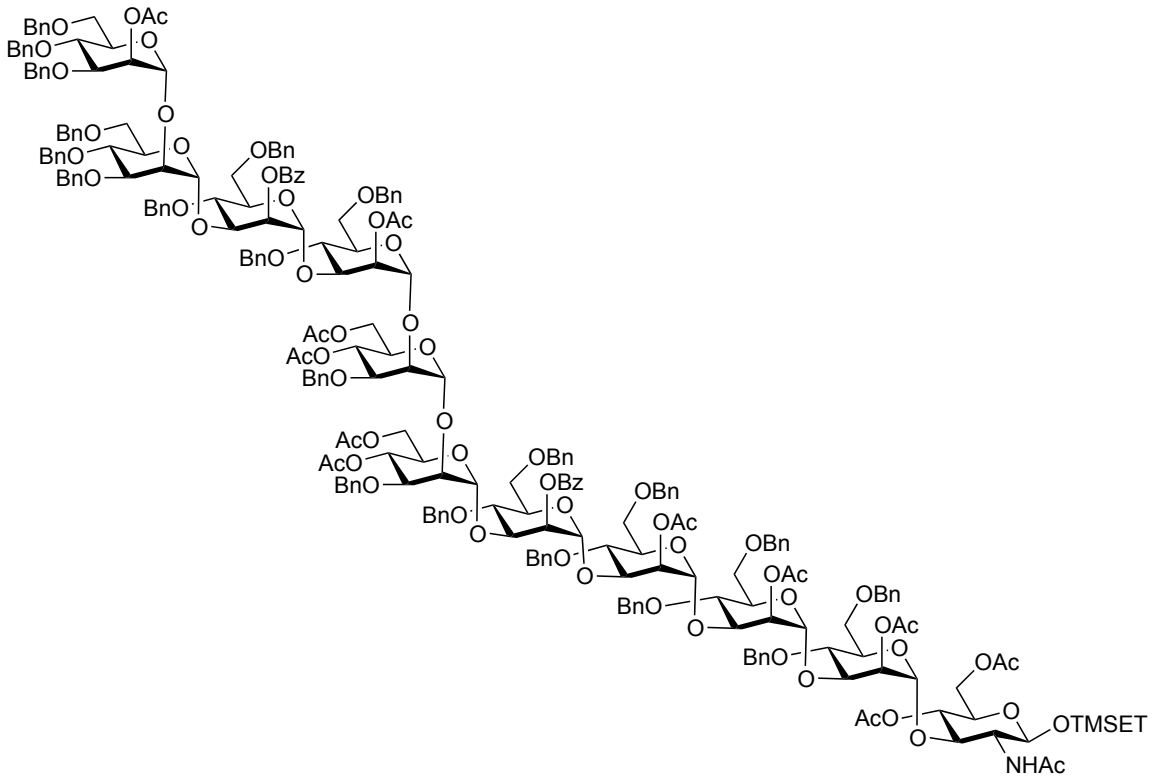
73.28, 73.24, 72.5, 72.4, 72.26, 72.25, 72.18, 72.0, 71.9, 71.8, 71.6, 71.4, 70.7, 70.6, 69.6, 69.4, 68.8, 68.3, 68.0, 67.5, 67.2, 67.1, 62.5, 62.3, 57.6, 21.1, 21.0, 20.92, 20.82, 20.79, 20.78, 20.76, 20.65, 20.61, 18.0, −1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{167}H_{200}Cl_3N_3O_{53}Si$: 1614.0935. Found: 1614.0952.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-

benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyrano- side (4.122): 742 mg of trichloroacetimidate donor **4.116** (742 mg) was formed from trisaccharide **4.63** (1.1 g) in 65% yield following general procedure A described above. Then, a mixture of acceptor **4.121** (650 mg, 0.20 mmol), trichloroacetimidate donor **4.116** (370 mg, 0.24 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (30 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C, and then TBSOTf (20 μL) was added. The solution was stirred for 1 h at r.t. before Et_3N (0.2 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 25→50% EtOAc in hexane) to afford **4.122** (800 mg, 86% yield) as a white solid; R_f 0.53 (1:1 hexane–EtOAc); $[\alpha]_D$ = +16.3 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.00–7.97 (m, 4 H), 7.58–7.56 (m, 1 H), 7.53–7.51 (m, 1 H), 7.36–6.98 (m, 104 H), 5.83 (d, 1 H, *J* = 6.5 Hz), 5.57 (s, 1 H), 5.47 (s, 1 H), 5.46 (s, 1 H), 5.30 (s, 1 H), 5.27 (s, 1 H), 5.25 (s, 1 H), 5.22–5.14 (m, 6 H), 5.11 (s, 1 H), 5.08 (s, 1 H), 5.02 (s, 1 H), 4.99–4.96 (m, 2 H), 4.89 (s, 1 H), 4.84–4.79 (m, 4 H), 4.76–4.64 (m, 8 H), 4.58–4.41 (m, 18 H), 4.38–4.32 (m, 7 H), 4.26–3.95 (m, 22 H), 3.93–3.69 (m, 24 H), 3.64–3.32 (m, 19 H), 3.18–3.14 (m, 1 H), 2.07 (s, 3 H), 2.06–2.04 (m, 12 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.82 (s, 3 H), 1.77 (s, 3 H), 0.88–0.84 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.8, 170.7, 170.5, 170.3, 170.2, 170.03,

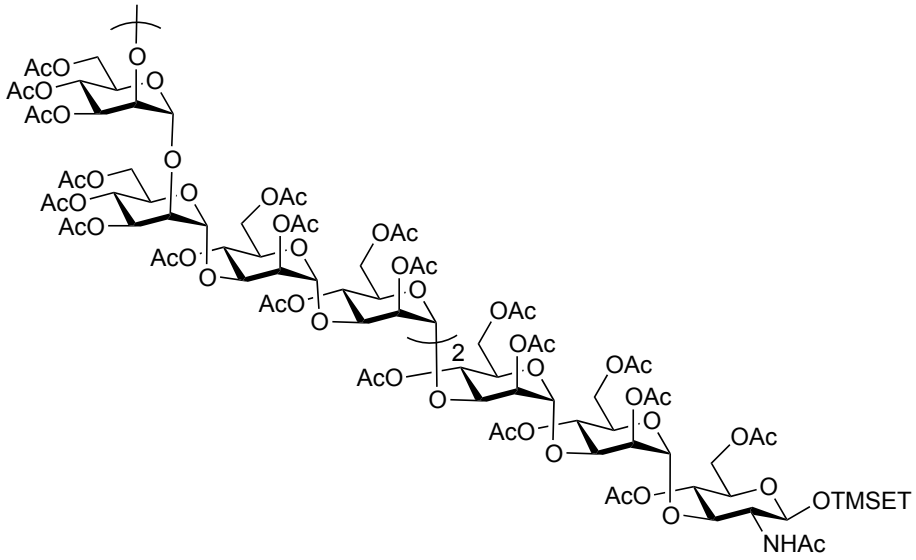
169.98, 169.90, 169.6, 169.5, 169.0, 165.4, 165.2, 154.0, 139.1, 138.8, 138.7, 138.6, 138.5, 138.4, 138.32, 138.30, 138.29, 138.1, 138.07, 137.97, 137.94, 137.85, 137.81, 137.78, 137.6, 137.5, 133.4, 133.0, 129.9, 129.8, 129.7, 128.6, 128.5, 128.42, 128.38, 128.36, 128.32, 128.27, 128.23, 128.19, 128.14, 128.12, 128.10, 128.0, 127.90, 127.88, 127.81, 127.77, 127.75, 127.71, 127.69, 127.66, 127.63, 127.59, 127.55, 127.50, 127.47, 127.40, 127.38, 127.35, 127.31, 127.18, 127.12, 101.1, 100.8, 99.8, 99.6, 99.4, 99.1, 99.0, 98.5, 79.3, 78.3, 78.2, 77.7, 76.5, 75.7, 75.5, 75.4, 75.3, 75.1, 75.0, 74.9, 74.87, 74.7, 74.6, 74.3, 74.2, 74.18, 74.14, 74.0, 73.9, 73.5, 73.4, 73.32, 73.29, 73.23, 73.18, 73.09, 72.7, 72.6, 72.48, 72.46, 72.43, 72.3, 72.19, 72.13, 72.0, 71.95, 71.89, 71.83, 71.7, 71.4, 70.7, 69.6, 69.5, 68.63, 68.58, 68.33, 68.27, 68.20, 68.0, 67.4, 67.23, 67.18, 62.6, 62.5, 62.3, 57.6, 21.1, 21.05, 21.04, 21.0, 20.9, 20.78, 20.76, 20.70, 20.6, 20.5, 18.0, −1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{250}H_{284}Cl_3N_3O_{70}Si$: 2290.3790. Found: 2290.3814.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4.124): To a solution of substrate **4.122** (800 mg, 0.18 mmol) in 3:1 THF–AcOH (84 mL) was added freshly activated zinc dust (2 g). After stirring for 3 h at r.t., the mixture was filtered and the filtrate was concentrated. The resulting residue was dissolved in 3:2 pyridine–Ac_2O

(25 mL) and the mixture was stirred at r.t. for 2 h. Then, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with 1M HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 40→66% EtOAc in hexane) to afford **4.124** (698 mg, 90% yield) as a white solid; R_f 0.19 (1:1 hexane–EtOAc); $[\alpha]_D$ = +9.8 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.00–7.98 (m, 4 H), 7.59–7.57 (m, 1 H), 7.54–7.52 (m, 1 H), 7.37–6.99 (m, 104 H), 6.49 (d, 1 H, *J* = 7.0 Hz), 5.58 (s, 1 H), 5.48 (app t, 1 H *J* = 2.0 Hz), 5.46 (app t, 1 H *J* = 2.0 Hz), 5.31 (d, 1 H *J* = 1.5 Hz), 5.28 (dd, 1 H *J* = 2.5, 1.5 Hz), 5.25 (d, 1 H *J* = 1.5 Hz), 5.23–5.16 (m, 6 H), 5.12 (s, 1 H), 5.10 (s, 1 H), 5.07 (d, 1 H *J* = 8.0 Hz), 5.03 (d, 1 H *J* = 1.5 Hz), 4.98 (s, 1 H), 4.90 (dd, 1 H *J* = 10.0, 9,0 Hz), 4.85–4.81 (m, 4 H), 4.77–4.67 (m, 8 H), 4.59–4.30 (m, 24 H), 4.27–4.12 (m, 14 H), 4.09–4.01 (m, 5 H), 3.98–3.70 (m, 25 H), 3.65–3.34 (m, 20 H), 2.96–2.93 (m, 1 H), 2.07 (s, 3 H), 2.06–2.04 (m, 12 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 1.92 (s, 3 H), 1.89 (s, 3 H), 1.83 (s, 3 H), 1.79 (s, 3 H), 1.77 (s, 3 H), 0.96–0.82 (m, 2 H), –0.01 (s, 9 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 171.4, 170.83, 170.81, 170.5, 170.22, 170.20, 170.04, 169.97, 169.91, 169.6, 169.5, 169.0, 165.4, 165.2, 154.0, 139.1, 138.8, 138.7, 138.6, 138.5, 138.4, 138.33, 138.30, 138.10, 138.08, 138.00, 137.98, 137.88, 137.82, 137.78, 137.6, 137.5, 137.2, 133.4, 133.0, 129.9, 129.8, 129.7, 128.6, 128.56, 128.53, 128.51, 128.43, 128.39, 128.37, 128.33, 128.31, 128.28, 128.24, 128.20, 128.16, 128.14, 128.12, 128.10, 128.04, 128.00, 127.97, 127.89, 127.83, 127.77, 127.74, 127.71, 127.67, 127.64, 127.61, 127.56,

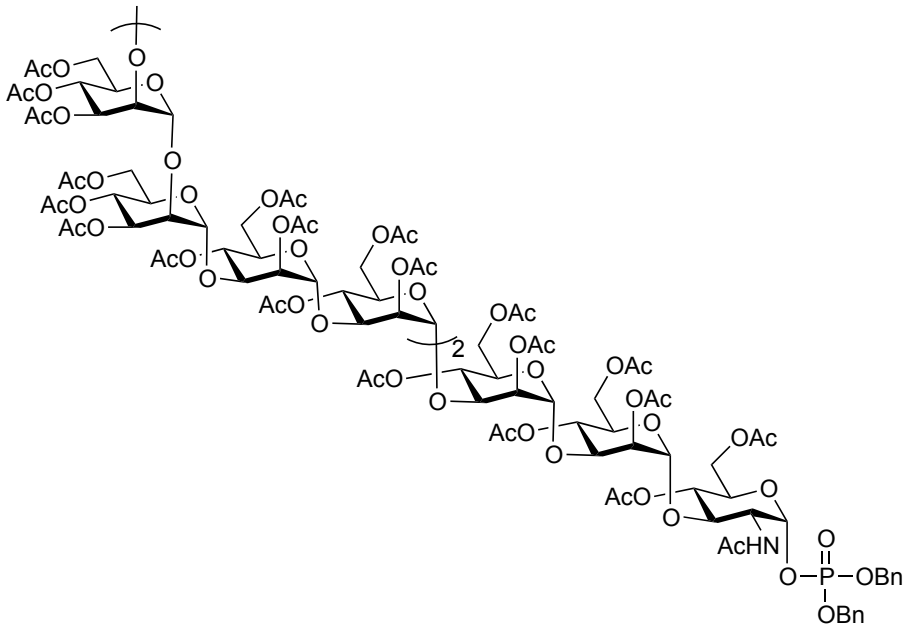
127.55, 127.51, 127.48, 127.44, 127.43, 127.41, 127.39, 127.35, 127.34, 127.32, 127.19, 127.14, 101.2, 100.8, 99.8, 99.6, 99.5, 99.4, 99.1, 99.0, 98.7, 80.3, 79.3, 78.3, 78.0, 77.7, 77.6, 77.3, 76.5, 75.7, 75.5, 75.49, 75.33, 75.27, 75.16, 75.04, 74.94, 74.89, 74.85, 74.7, 74.6, 74.3, 74.2, 74.19, 74.16, 74.07, 73.97, 73.6, 73.5, 73.32, 73.31, 73.24, 73.19, 73.09, 72.7, 72.6, 72.47, 72.44, 72.3, 72.19, 72.14, 71.99, 71.97, 71.89, 71.85, 71.7, 71.2, 70.3, 69.6, 69.5, 69.4, 68.4, 68.35, 68.28, 68.21, 68.12, 68.06, 67.28, 67.25, 67.19, 62.6, 62.51, 62.49, 58.7, 23.4, 21.1, 21.06, 21.05, 21.0, 20.9, 20.79, 20.75, 20.71, 20.6, 20.5, 18.0, –1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{249}H_{285}N_3O_{69}Si$: 2224.4321. Found: 2224.4363.



2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-Acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyran-osyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-

acetyl-α-D- mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*- acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4.56): Compound **4.124** (120 mg, 27.1 μmol) was dissolved in CH_3OH (10 mL), treated with $NaOCH_3$ (0.5 M solution in CH_3OH, 124 μL) and stirred at r.t. for 5 h. Water (0.10 mL) and then Amberlite IR120 H^+ ion-exchange resin was added. The mixture was then filtered and concentrated to provide crude compound **4.125**. Next, ammonia was condensed at −78 °C into 50 mL round-bottom flask equipped with a Dewar condenser and a magnetic stir bar (total volume 15 mL). Freshly cut sodium metal (60 mg) was added and the mixture was stirred at −78 °C for 10 min. A solution of crude compound **4.125** in THF (0.50 mL) was introduced via syringe and the mixture was stirred at −78 °C for 1 h before CH_3OH (2 mL) was added. The colorless solution was warmed to r.t. and then concentrated. The residue was dissolved in water (4 mL) and neutralized with Amberlite IR120 H^+ ion-exchange resin, filtered and concentrated to afford crude **4.126**. This compound was then suspended in 3:2 pyridine−Ac_2O (25 mL) and the mixture was stirred at r.t. for 1 day. Then, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with 1M of HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 66→95% EtOAc in hexane) to afford **4.56** (59 mg, 65% yield) as a white solid; R_f 0.57 (EtOAc); $[\alpha]_D$ = +14.8 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 6.25 (d, 1 H, *J* = 7.0 Hz), 5.58

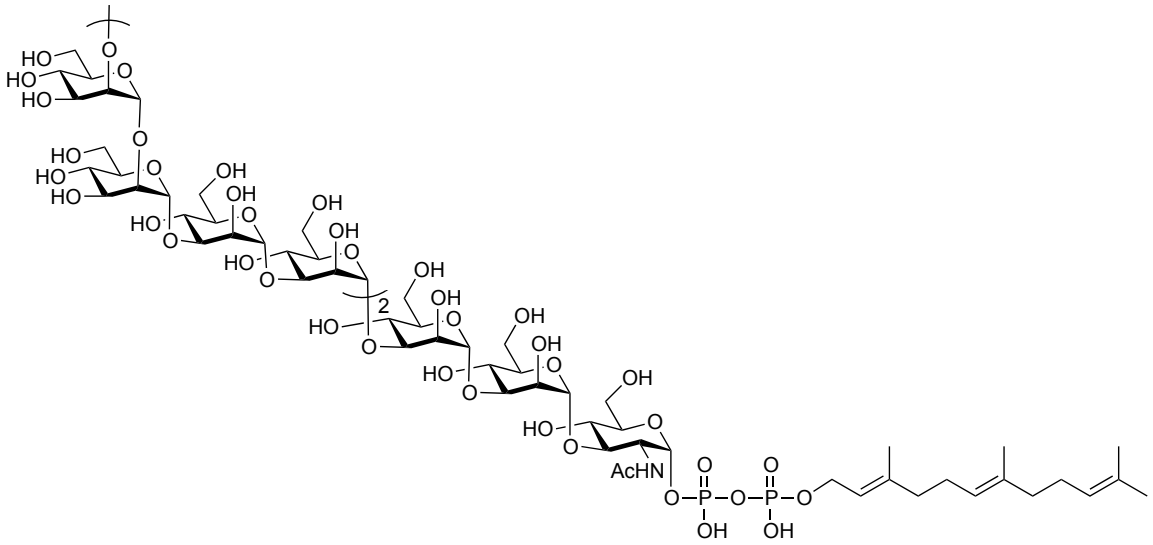
(s, 1 H), 5.36 (dd, 1 H J = 10.0, 3.0 Hz), 5.32 (app t, 1 H J = 10.0 Hz), 5.30–5.16 (m, 12 H), 5.12–5.09 (m, 2 H), 5.06–4.94 (m, 9 H), 4.91–4.89 (m, 5 H), 4.84 (d, 1 H J = 1.5 Hz), 4.82 (s, 1 H), 4.50 (app t, 1 H J = 9.5 Hz), 4.26–4.19 (m, 9 H), 4.17–3.85 (m, 29 H), 3.84–3.77 (m, 5 H), 3.64 (ddd, 1 H, J = 10.0, 5.0, 2.5 Hz), 3.54 (td, 1 H, J = 10.0, 6.5 Hz), 3.09–3.05 (m, 1 H), 2.15–2.07 (m, 69 H), 2.05–2.02 (m, 15 H), 2.00–1.97 (m, 18 H), 0.96–0.84 (m, 2 H), –0.01 (s, 9 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 171.1, 170.8, 170.69, 170.66, 170.63, 170.59, 170.56, 170.41, 170.38, 170.32, 170.22, 170.18, 170.16, 170.0, 169.85, 169.82, 169.79, 169.71, 169.67, 169.50, 169.45, 169.37, 169.35, 169.2, 99.9, 99.8, 99.49, 99.44, 99.40, 99.34, 99.25, 99.1, 98.8, 98.6, 80.6, 77.6, 77.4, 75.6, 75.4, 75.1, 75.0, 74.0, 73.7, 71.25, 71.23, 71.16, 71.13, 70.9, 70.7, 70.4, 69.9, 69.7, 69.63, 69.61, 69.56, 69.52, 69.48, 68.9, 68.6, 68.3, 67.31, 67.29, 67.23, 66.9, 66.73, 66.68, 66.1, 65.9, 65.3, 63.7, 62.4, 62.3, 62.23, 62.18, 61.96, 61.94, 61.8, 61.7, 61.6, 61.5, 58.3, 45.8, 23.6, 20.93, 20.90, 20.83, 20.81, 20.76, 20.73, 20.71, 20.68, 20.65, 20.64, 20.62, 20.58, 20.56, 20.53, 17.9, –1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{139}H_{201}N_3O_{89}Si$: 1682.0526. Found: 1682.0530.



2,3,4,6-tetra-*O*-Acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (4.128): To a solution of **4.56** (58 mg, 17.4 μmol) in dry CH_2Cl_2 (3 mL) was added TFA (1 mL) dropwise at 0 °C. After stirring for 3 h at r.t., the solution was concentrated, dissolved in CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography to afford hemiacital **4.127** (47 mg, 83% yield) as a white solid. Then, **4.127** (47 mg, 14.4 μmol) was dissolved in dry

CH_2Cl_2 (5 mL) before tetraazole (20.2 mg, 288 μmol) was added and the reaction mixture was cooled to 0 °C. After 10 min, dibenzyl *N*,*N*-diisopropylphosphoramidite (50 mg, 144 μmol) was added dropwise and the mixture was stirred at r.t. for 4 h. The mixture was cooled to −78 °C and *m*-CPBA (37 mg, 216 μmol) was added in one portion. The reaction mixture was warmed to r.t. and after stirring at r.t. for 2 h, CH_2Cl_2 was added. The mixture was washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 50→95% EtOAc in hexane) to afford phosphate **4.128** (38 mg, 75% yield) as a white solid; R_f 0.19 (1:10 hexane–EtOAc); $[\alpha]_D$ = +21.1 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.36–7.30 (m, 10 H), 5.85 (d, 1 H, *J* = 9.5 Hz), 5.58 (dd, 1 H, *J* = 6.0, 3.0 Hz), 5.36 (dd, 1 H *J* = 10.0, 3.0 Hz), 5.31 (app t, 1 H *J* = 10.0 Hz), 5.29–5.25 (m, 4 H), 5.22–5.15 (m, 8 H), 5.11–4.88 (m, 20 H), 4.86 (dd, 1 H, *J* = 3.0, 2.0 Hz), 4.83 (d, 1 H *J* = 2.0 Hz), 4.33–4.18 (m, 8 H), 4.16–3.92 (m, 25 H), 3.89–3.72 (m, 10 H), 3.12–3.09 (m, 1 H), 2.14–2.12 (m, 24 H), 2.10–2.02 (m, 60 H), 1.99–1.97 (m, 18 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.9, 170.75, 170.69, 170.66, 170.59, 170.54, 170.45, 170.41, 170.36, 170.27, 170.25, 170.22, 170.21, 170.14, 170.07, 170.03, 169.88, 169.86, 169.83, 169.73, 169.71, 169.53, 169.48, 169.42, 169.40, 169.3, 135.3, 135.2 (d, J_{PC} = 6.4 Hz), 135.1(d, J_{PC} = 6.4 Hz), 129.1, 128.8, 128.24, 128.20, 99.9, 99.8, 99.6, 99.5, 99.3, 99.2, 99.1, 98.4, 96.7 (d, $J_{PC\text{-}1}$ = 6.5 Hz), 77.7, 77.5, 76.5, 75.6, 75.5, 75.1, 75.0, 74.7, 74.0, 71.3, 71.0, 70.9, 70.13, 70.10, 70.09, 70.06, 69.85, 69.80, 69.72, 69.68, 69.62, 69.58, 68.7, 68.3, 67.4, 67.4, 67.0,

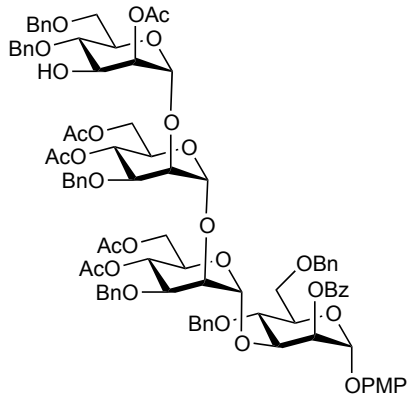
66.9, 66.7, 66.1, 66.0, 65.4, 62.7, 62.5, 62.4, 62.3, 62.0, 61.9, 61.8, 61.7, 61.6, 61.2, 60.4, 51.8 (d, J_{PC-2} = 7.3 Hz), 22.9, 21.1, 20.96, 20.92, 20.87, 20.83, 20.79, 20.71, 20.69, 20.62, 20.59; ^{31}P NMR (200 MHz, $CDCl_3$, δ_C) 2.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{148}H_{202}N_3O_{92}P$: 1762.0473. Found: 1762.0501.



α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl farnesyl diphosphate diammonium salt (4.51): To a solution of **4.128** (38 mg, 0.011 mmol) in THF (5 mL) was added palladium on charcoal (10%, 30 mg). The mixture was subjected to a hydrogen atmosphere for 4 h before being filtered through Celite and concentrated. The residue **4.129** was used in the next step without further purification. To

a solution of the crude phosphate **4.129** in dry CH_2Cl_2 (3 mL) was added 1,1'-carbonyldiimidazole (26 mg, 0.16 mmol). After stirring at r.t. for 2 h, a solution of 5% (v/v) anhydrous CH_3OH in CH_2Cl_2 (0.16 mL) was added to quench unreacted 1,1'-carbonyldiimidazole and the mixture stirred for 30 min. The solvent was concentrated and the residue was dissolved in DMF (0.6 mL). Farnesol phosphate **4.130** (53 mg, 0.16 mmol) was added and the reaction mixture stirred at r.t. for 7 days. The solvent was removed in vacuo and the residue was purified by Sephadex LH-20 (1:1 CH_3OH–CH_2Cl_2) to afford **4.131** as a crude product. To a solution of crude phosphate **4.131** in CH_3OH (5 mL) was added freshly prepared $NaOCH_3$ (1M solution in CH_3OH, 0.5 mL). The reaction mixture was stirred at r.t. for 3 h, and then the $NaOCH_3$ was quenched by addition of Amberlite IR120 (NH_4^+ form). The mixture was filtered, concentrated in vacuo and the residue purified by C_{18} chromatography (gradient 0→50% CH_3OH in H_2O) to afford **4.51** (13 mg, 56% yield) as a white solid. R_f 0.36 (2:3 H_2O–CH_3OH); $[\alpha]_D$ = +100.4 (*c* 0.1, CH_2Cl_2); ^1H NMR (700 MHz, D_2O, δ_H) 5.50 (dd, 1 H, *J* = 7.0, 3.0 Hz), 5.46 (app t, 1 H *J* = 7.5 Hz), 5.39 (s, 1 H), 5.38 (s, 1 H), 5.31 (s, 1 H), 5.26 (s, 1 H), 5.23–5.19 (m, 2 H), 5.13–5.10 (m, 4 H), 5.06 (s, 1 H), 5.05 (s, 1 H), 4.53–4.47 (m, 2 H), 4.25–4.19 (m, 6 H), 4.12–4.08 (m, 5 H), 4.03–3.66 (m, 53 H), 3.61–3.59 (m, 1 H), 3.22–3.18 (m, 1 H), 2.19–2.10 (m, 6 H), 2.08 (s, 3 H), 2.04 (t, 1 H *J* = 7.5 Hz), 1.73 (s, 3 H), 1.70 (s, 3 H), 1.64 (s, 6 H); ^{13}C NMR (125 MHz, D_2O, δ_C) 175.3, 144.0, 137.7, 134.5, 125.4, 125.2, 120.4 (d, J_{PC} = 8.0 Hz), 103.25, 103.23, 103.18, 103.07, 103.05, 101.9, 101.68, 101.64, 101.61, 95.6 (d, J_{PC} = 6.3 Hz),

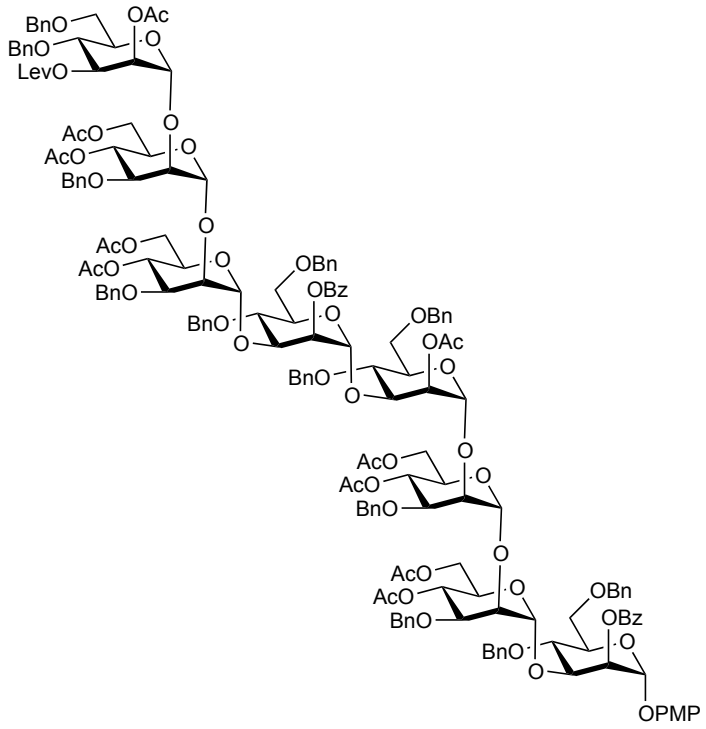
79.6, 79.5, 79.4, 79.3, 79.24, 79.20, 79.0, 78.5, 74.5, 74.4, 74.35, 74.30, 74.28, 74.24, 74.20, 73.9, 71.30, 71.28, 71.06, 70.99, 70.94, 70.68, 70.65, 70.57, 68.0, 67.8, 67.7, 67.17, 67.13, 67.10, 66.5, 64.0 (d, J_{PC} = 5.3 Hz), 62.1, 62.0, 61.94, 61.90, 61.5, 61.1, 53.2 (d, J_{PC} = 8.3 Hz), 39.7, 26.7, 26.5, 25.8, 23.3, 18.0, 16.6, 16.3; ^{31}P NMR (200 MHz, D_2O, δ_C) −10.6, −13.3; HRMS (ESI) calcd for $(M-2H)^{-2}$ $C_{83}H_{141}NO_{62}P_2$: 1101.8620. Found: 1101.8628.



***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.133):** A solution of **4.64** (1.04 g, 0.60 mmol) and hydrazine acetate (100 mg, 1.08 mmol) in 9:1 CH_2Cl_2–CH_3OH (150 mL) was stirred at r.t. for 2 h. Then, the solution was concentrated and the resulting residue was subjected to chromatography (gradient 33→50% EtOAc in hexane) to afford **4.133** (0.91 g, 93% yield) as a white solid; R_f 0.18 (1:1 hexane–EtOAc); $[\alpha]_D$ = +50.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10–8.09 (m, 2 H, ArH), 7.64–7.61 (m, 1 H, ArH), 7.44–7.42 (m, 2 H, ArH), 7.39–7.06 (m, 30 H, ArH), 6.99–6.97

(m, 3 H, ArH), 6.80–6.78 (m, 2 H, ArH), 5.58 (d, 1 H, J = 2.0 Hz, H-1), 5.56 (dd, 1 H, J = 3.0, 2.0 Hz, H-2), 5.30 (d, 1 H, J = 1.5 Hz, H-1′), 5.22–5.18 (m, 3 H, H-2‴, H-4′, H-4″), 5.06 (d, 1 H, J = 2.0 Hz, H-1″), 4.81 (d, 1 H, J = 1.5 Hz, H-1‴), 4.78 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.68 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.60 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.56 (d, 1 H, J = 11.0 Hz, PhCH_2), 4.54 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.49–4.43 (m, 5 H, PhCH_2, H-3), 4.33 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.27 (d, 1 H, J = 11.5 Hz, PhCH_2), 4.25 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.21 (app dt, 1 H, J = 9.0, 4.0 Hz, H-3‴), 4.17 (app t, 1 H, J = 9.5 Hz, H-4), 4.09 (dd, 1 H, J = 12.0, 2.5 Hz), 4.06 (dd, 1 H, J = 12.0, 5.6 Hz), 3.99 (ddd, 1 H, J = 10.0, 5.5, 2.5 Hz), 3.96–3.94 (m, 2 H), 3.91–3.80 (m, 6 H), 3.75 (s, 3 H, OCH_3), 3.74–3.70 (m, 2 H), 3.68–3.65 (m, 2 H), 3.59 (dd, 1 H, J = 11.0, 4.0 Hz), 3.45 (dd, 1 H, J = 10.5, 1.5 Hz), 2.11 (d, J = 4.0 Hz, OH), 2.10 (s, 3 H, OC=OCH_3), 2.02 (s, 3 H, OC=OCH_3), 1.96 (s, 3 H, OC=OCH_3), 1.95 (s, 3 H, OC=OCH_3), 1.90 (s, 3 H, OC=OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.8 (OC=OCH_3), 170.7 (OC=OCH_3), 170.6 (OC=OCH_3), 169.7 (OC=OCH_3), 169.6 (OC=OCH_3), 165.6 (PhC=O), 155.3 (Ar), 149.7 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.8 (Ar), 137.5 (Ar), 133.5 (Ar), 129.9 (Ar), 129.7 (Ar), 128.6 (Ar), 128.52 (Ar), 128.51 (Ar), 128.37 (Ar), 128.35 (Ar), 128.33 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.75 (Ar), 127.72 (Ar), 127.64 (Ar), 127.62 (Ar), 127.53 (Ar), 127.51 (Ar), 117.8 (Ar), 114.7 (Ar), 101.0 (C-1″), 100.9 (C-1′), 99.2 (C-1‴), 96.4 (C-1), 77.4 (C-3), 75.8, 75.6, 75.5, 75.4 (PhCH$_2$), 75.3, 75.0 (PhCH$_2$), 74.6 (C-4), 73.5 (PhCH$_2$), 73.4 (PhCH$_2$), 72.5, 72.3, 72.28 (PhCH$_2$), 72.2, 72.0

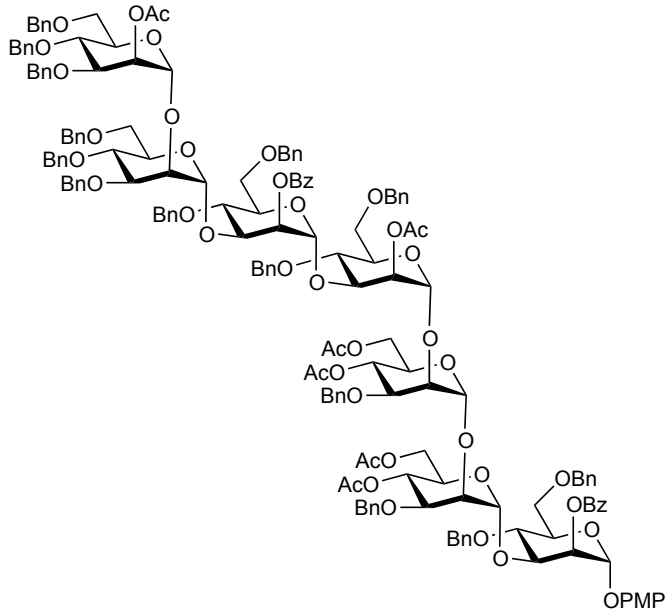
(Ph*C*H$_2$), 71.6, 70.6, 69.8, 69.7, 68.9, 68.4, 67.5, 67.2, 55.6 (CH$_3$O), 21.9 (OC=O*C*H$_3$), 20.9 (OC=O*C*H$_3$), 20.8 (OC=O*C*H$_3$), 20.71 (OC=O*C*H$_3$), 20.70 (OC=O*C*H$_3$); HRMS (ESI) calcd for $(M+NH_4)^+$ $C_{90}H_{102}NO_{28}$: 1644.6583. Found: 1644.6555.



***p*-Methoxyphenyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.134):** A mixture of tetrasaccharide acceptor **4.133** (2.30 g, 1.42 mmol), trichloroacetimidate donor **4.117**

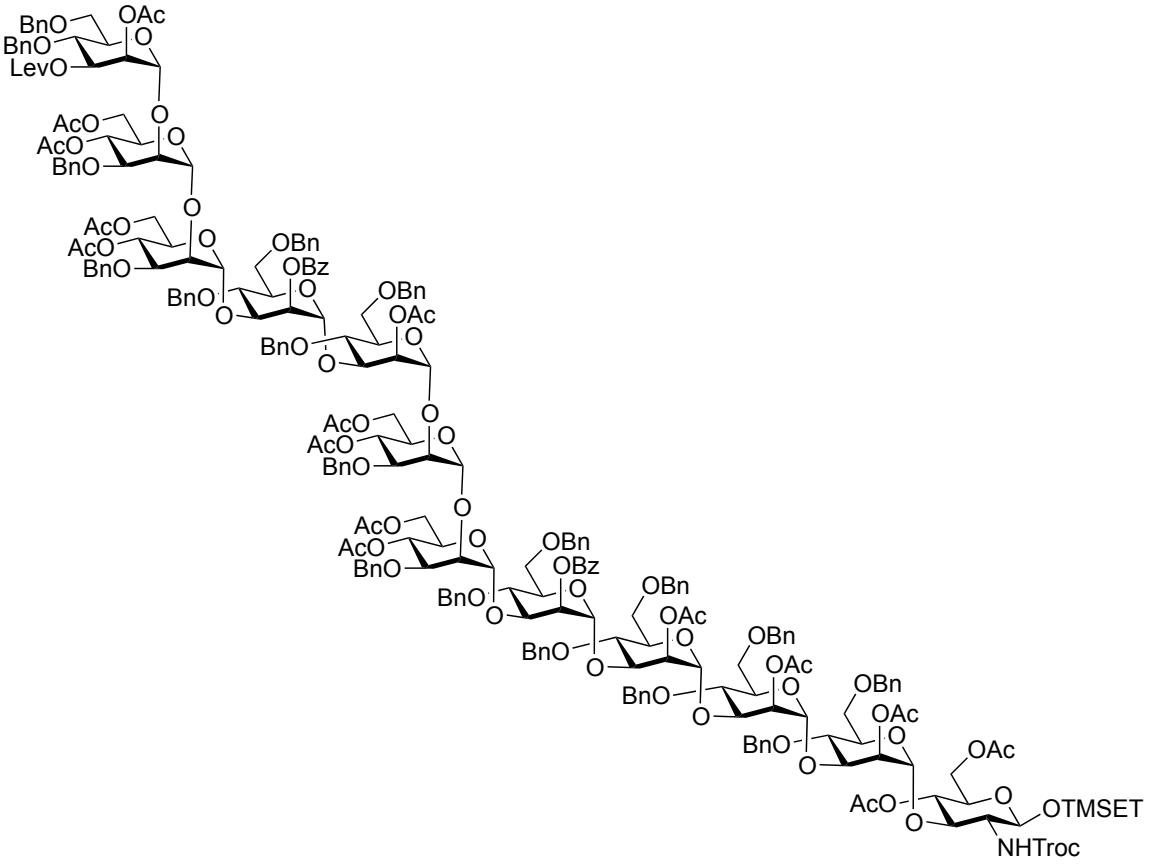
(2.73 g, 1.57 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (100 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C and then TBSOTf (50 μL) was added. The solution was stirred for 2 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 33→66% EtOAc in hexane) to afford **4.134** (4.05 g, 88%) yield as a white solid; R_f 0.42 (2:3 hexane–EtOAc); $[\alpha]_D$ = +30.4 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.08–8.06 (m, 2 H), 8.01–7.99 (m, 2 H), 7.62–7.59 (m, 1 H), 7.58–7.55 (m, 1 H), 7.42–6.99 (m, 66 H), 6.78–6.76 (m, 2 H), 5.56 (d, 1 H, *J* = 2.0 Hz), 5.54 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.50 (app t, 1 H, *J* = 2.0 Hz), 5.37 (dd, 1 H, *J* = 9.5, 3.5 Hz), 5.31 (d, 1 H, *J* = 1.5 Hz), 5.30 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.29 (s, 1 H), 5.25 (app t, 1 H, *J* = 10.0 Hz), 5.22 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.20–5.15 (m, 4 H), 5.00 (d, 1 H, *J* = 2.0 Hz), 4.92 (d, 1 H, *J* = 2.0 Hz), 4.85 (d, 1 H, *J* = 2.0 Hz), 4.79 (d, 1 H, *J* = 2.0 Hz), 4.76 (d, 1 H, *J* = 12.0 Hz), 4.75 (d, 1 H, *J* = 10.5 Hz), 4.67 (d, 1 H, *J* = 12.0 Hz),4.60–4.37 (m, 17 H), 4.30–4.20 (m, 6 H), 4.18–4.13 (m, 4 H), 4.08–4.01 (m, 3 H), 3.96–3.78 (m, 21 H), 3.72–3.70 (m, 1 H), 3.65–3.60 (m, 4 H), 4.57–4.53 (m, 2 H), 3.40 (dd, 1 H, *J* = 11.0, 1.5 Hz), 3.35 (d, 1 H, *J* = 11.0 Hz), 2.76 (dt, 1 H, *J* = 18.5, 7.5 Hz), 2.62 (dt, 1 H, *J* = 18.5, 6.5 Hz), 2.50 (dt, 1 H, *J* = 17.0, 7.0 Hz), 2.41 (dt, 1 H, *J* = 17.0, 6.5 Hz), 2.13 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.00 (s, 3 H), 1.94 (s, 3 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.88 (s, 3 H), 1.87 (s, 3 H), 1.82 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.5, 171.3, 171.1, 170.8, 170.7, 170.6, 169.8, 169.69, 169.68, 169.6, 169.3,

169.2, 165.6, 165.4, 155.3, 149.7, 139.1, 138.3, 138.1, 138.02, 138.00, 137.94, 137.8, 137.6, 137.5, 133.6, 133.3, 129.9, 129.8, 129.7, 128.7, 128.55, 128.50, 128.46, 128.39, 128.36, 128.34, 128.28, 128.25, 128.1, 128.0, 127.88, 127.87, 127.85, 127.76, 127.73, 127.62, 127.59, 127.53, 127.3, 117.8, 114.7, 101.01, 100.97, 100.87, 100.7, 99.3, 99.2, 98.8, 96.4, 77.5, 77.2, 76.6, 76.1, 75.93, 75.88, 75.76, 75.48, 75.44, 75.39, 74.97, 74.94, 74.8, 74.6, 74.5, 73.7, 73.47, 73.44, 73.41, 73.37, 72.94, 72.88, 72.4, 72.30, 72.27, 72.23, 72.19, 72.02, 71.96, 71.93, 71.91, 71.83, 71.79, 69.9, 69.8, 69.71, 69.69, 69.62, 68.5, 68.4, 68.3, 67.5, 67.3, 62.91, 62.88, 62.55, 62.44, 55.6, 37.9, 29.9, 28.0, 21.09, 21.08, 21.0, 20.9, 20.82, 20.81, 20.76, 20.72, 20.69, 20.63; ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 176.0, 176.0, 175.6, 175.6, 175.5, 173.3, 173.2, 173.1 Hz HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{178}H_{202}N_2O_{56}$: 1631.6505. Found: 1631.6521.



***p*-Methoxyphenyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-manno-pyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (4.137):** A mixture of tetrasaccharide acceptor **4.133** (941 mg, 0.56 mmol), trisaccharide trichloroacetimidate **4.116** (1070 mg, 0.70 mmol) and powdered 4Å molecular sieves was suspended in anhydrous CH_2Cl_2 (100 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C and then TBSOTf (40 μL) was added. The solution was stirred for 2 h at r.t. before Et_3N (0.2 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 16→33% EtOAc in hexane) to afford **4.137** (1.48 g, 87% yield) as a white solid; R_f 0.67 (1:1 hexane–EtOAc); $[\alpha]_D$ = +22.8 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.08–8.06 (m, 2 H), 7.99–7.98 (m, 2 H), 7.62–7.60 (m, 1 H), 7.54–7.52 (m, 1 H), 7.43–7.40 (m, 2 H), 7.36–7.25 (m, 72 H), 6.99–6.96 (m, 2 H), 6.78–6.77 (m, 2 H), 5.58 (app t, 1 H, *J* = 2.0 Hz), 5.56 (d, 1 H, *J* = 2.0 Hz), 5.54 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.48 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.31 (d, 1 H, *J* = 1.5 Hz), 5.29 (s, 1 H), 5.24 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.20–5.15 (m, 3 H), 5.03 (d, 1 H, *J* = 1.5 Hz), 5.00 (d, 1 H, *J* = 2.0 Hz), 4.85–4.84 (m, 2 H), 4.75 (d, 1 H, *J* = 11.0 Hz), 4.73–4.66 (m, 4 H), 4.59–4.53 (m, 5 H), 5.00–4.40 (m, 8 H), 4.38–4.32 (m, 4 H), 4.30–4.18 (m, 9 H), 4.15 (app t, 1 H, *J* = 9.5 Hz), 4.07 (dd, 1 H, *J* = 12.5, 2.5 Hz),

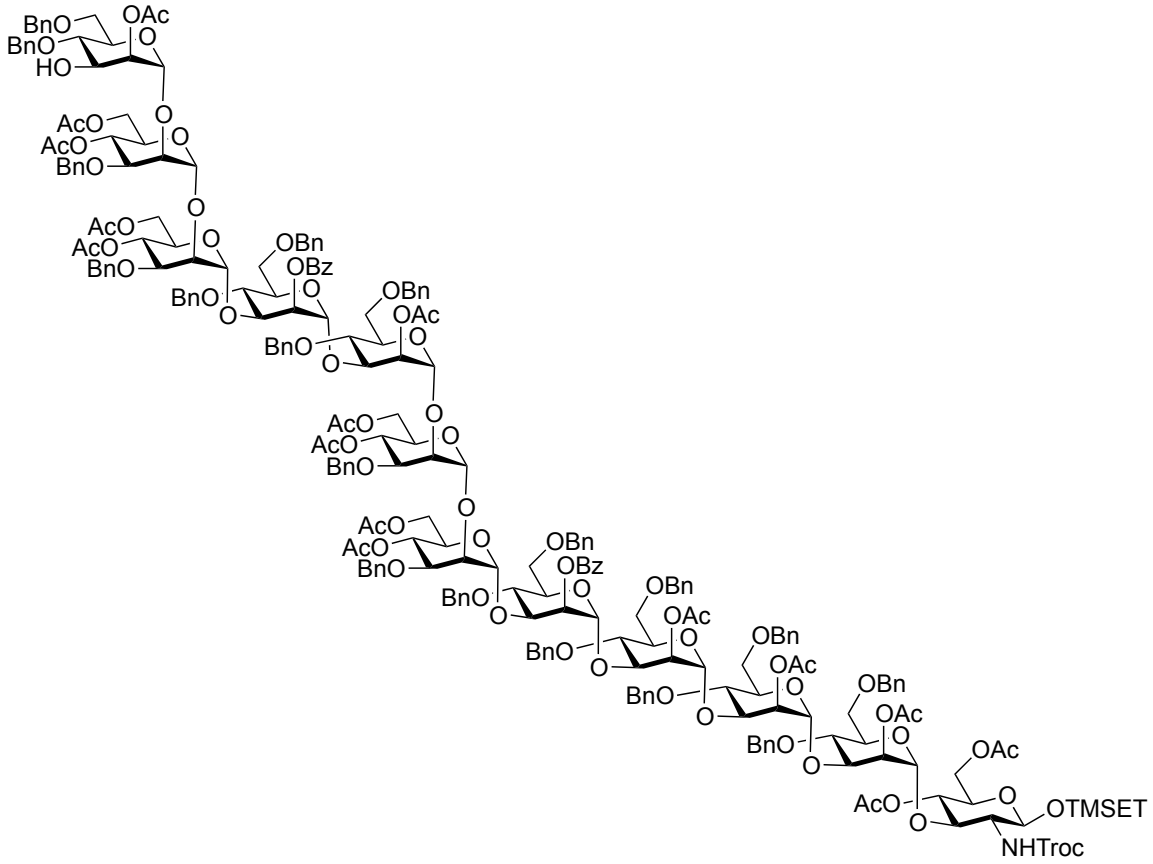
4.04 (dd, 1 H, J = 12.5, 5.0 Hz), 3.96–3.78 (m, 17 H), 3.75–3.70 (m, 4 H), 3.74 (s, 3 H), 3.65–3.63 (m, 2 H), 3.59 (dd, 1 H, J = 11.0, 3.5 Hz), 3.53–3.49 (m, 2 H), 3.41–3.34 (m, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.91 (s, 3 H), 1.89 (s, 3 H), 1.83 (s, 3 H); ^{13}C NMR (175 MHz, $CDCl_3$, δ_C) 170.8, 170.7, 170.0, 169.9, 169.6, 169.1, 165.6, 165.4, 155.2, 149.7, 139.1, 138.8, 138.7, 138.6, 138.5, 138.32, 138.29, 138.11, 138.07, 137.9, 137.8, 137.4, 133.5, 133.0, 129.9, 129.8, 129.6, 128.62, 128.59, 128.46, 128.44, 128.39, 128.33, 128.29, 128.25, 128.23, 128.20, 128.19, 128.15, 128.14, 128.13, 128.11, 128.0, 127.9, 127.8, 127.70, 127.66, 127.59, 127.57, 127.51, 127.47, 127.44, 127.41, 127.38, 127.33, 127.31, 127.18, 127.14, 127.13, 117.7, 114.7, 101.2, 100.98, 100.94, 99.4, 99.1, 98.9, 96.4, 79.2, 78.3, 77.7, 77.3, 77.2, 76.5, 75.6, 75.55, 75.4, 75.2, 74.94, 74.88, 74.80, 74.6, 74.5, 74.24, 74.18, 73.9, 73.4, 73.3 73.24, 73.19, 73.09, 72.8, 72.6, 72.4, 72.27, 72.19, 72.16, 72.12, 72.0, 71.9, 71.86, 71.83, 69.72, 69.67, 68.7, 68.6, 68.3, 68.2, 67.4, 67.3, 62.84, 62.80, 60.4, 55.6, 21.13, 21.06, 20.80, 20.75, 20.65, 20.57; ^{1}H-coupled HSQC (700 MHz, $CDCl_3$) $^{1}J_{C\text{-}1,\ H\text{-}1}$ = 176.4, 175.7, 175.7, 175.0, 175.0, 174.3, 172.9 Hz; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{173}H_{190}N_2O_{45}$: 1507.6315. Found: 1507.6323.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.140): The formation of octasaccharide trichloroacetimidate **4.136** (2.5 g) was

achieved from octasaccharide **4.134** (4.0 g) in 63% yield following general procedure A described above. A mixture of adaptor **4.65** (460 mg, 0.50 mmol), octasaccharide trichloroacetimidate **4.136** (740 mg, 0.23 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (40 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C and then TBSOTf (20 μL) was added. The solution was stirred at r.t. for 2 h before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 50→60% EtOAc in hexane) to afford **4.140** (950 mg, 86%) yield as a white solid; R_f 0.48 (2:3 hexane–EtOAc); $[\alpha]_D$ = +15.1 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.01–7.99 (m, 4 H), 7.58–7.55 (m, 2 H), 7.38–6.99 (m, 94 H), 5.83 (d, 1 H, *J* = 6.5 Hz), 5.49 (s, 1 H), 5.46 (s, 1 H), 5.37 (dd, 1 H, *J* = 9.5, 3.5 Hz), 5.31–5.15 (m, 13 H), 5.11 (s, 1 H), 5.08 (s, 1 H), 4.99–4.97 (m, 2 H), 4.93 (s, 1 H), 4.88 (s, 1 H), 4.85–4.70 (m, 9 H), 4.65–4.32 (m, 25 H), 4.27–3.70 (m, 50 H), 3.64–3.52 (m, 14 H), 3.46–3.34 (m, 5 H), 3.18–3.15 (m, 1 H), 2.76 (dt, 1 H, *J* = 18.0, 7.0 Hz), 2.63 (dt, 1 H, *J* = 18.0, 6.5 Hz), 2.50 (dt, 1 H, *J* = 17.0, 7.0 Hz), 2.41 (dt, 1 H, *J* = 17.0, 6.5 Hz), 2.14 (s, 3 H), 2.06–2.04 (m, 18 H), 2.02 (s, 3 H), 1.94 (s, 3 H), 1.92 (s, 3 H), 1.91 (s, 3 H), 1.90 (s, 3 H), 1.89 (s, 3 H), 1.88 (s, 3 H), 1.83 (s, 3 H), 1.77 (s, 3 H), 0.88–0.79 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.5, 171.3, 171.1, 170.8, 170.77, 170.5, 170.3, 170.2, 170.0, 169.8, 169.66, 169.61, 169.57, 169.3, 169.1, 165.4, 165.2, 154.0, 139.1, 138.5, 138.3, 138.15, 138.14, 137.98, 137.96, 137.93, 137.89, 137.8, 137.63, 137.61, 133.4, 133.3, 129.9, 129.83, 129.79, 129.7,

128.53, 128.47, 128.43, 128.38, 128.32, 128.30, 128.26, 128.22, 128.14, 127.93, 127.89, 127.85, 127.82, 127.73, 127.69, 127.62, 127.59, 127.57, 127.52, 127.45, 127.38, 127.3, 100.84, 100.79, 100.64, 99.9, 99.7, 99.3, 99.2, 98.8, 98.5, 95.5, 78.2, 76.5, 76.1, 75.9, 75.83, 75.79, 75.6, 75.48, 75.43, 75.3, 75.1, 74.92, 74.88, 74.75, 74.45, 74.39, 74.2, 74.1, 73.6, 73.54, 73.51, 73.42, 73.37, 73.33, 73.29, 72.92, 72.85, 72.51, 72.47, 72.38, 72.32, 72.26, 72.17, 72.04, 72.02, 71.93, 71.88, 71.81, 71.78, 71.75, 71.5, 70.7, 69.9, 69.7, 69.65, 69.60, 69.5, 68.5, 68.4, 68.3, 68.1, 67.5, 67.2, 62.7, 62.5, 62.41, 62.38, 57.6, 37.9, 29.9, 28.0, 21.08, 21.06, 21.04, 20.97, 20.95, 20.83, 20.81, 20.79, 20.76, 20.73, 20.67, 20.66, 20.59, 18.1, −1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{255}H_{296}Cl_3N_3O_{81}Si$: 2414.3980. Found: 2414.3971.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.141): A solution of **4.140** (0.9 g, 0.19 mmol) and hydrazine acetate

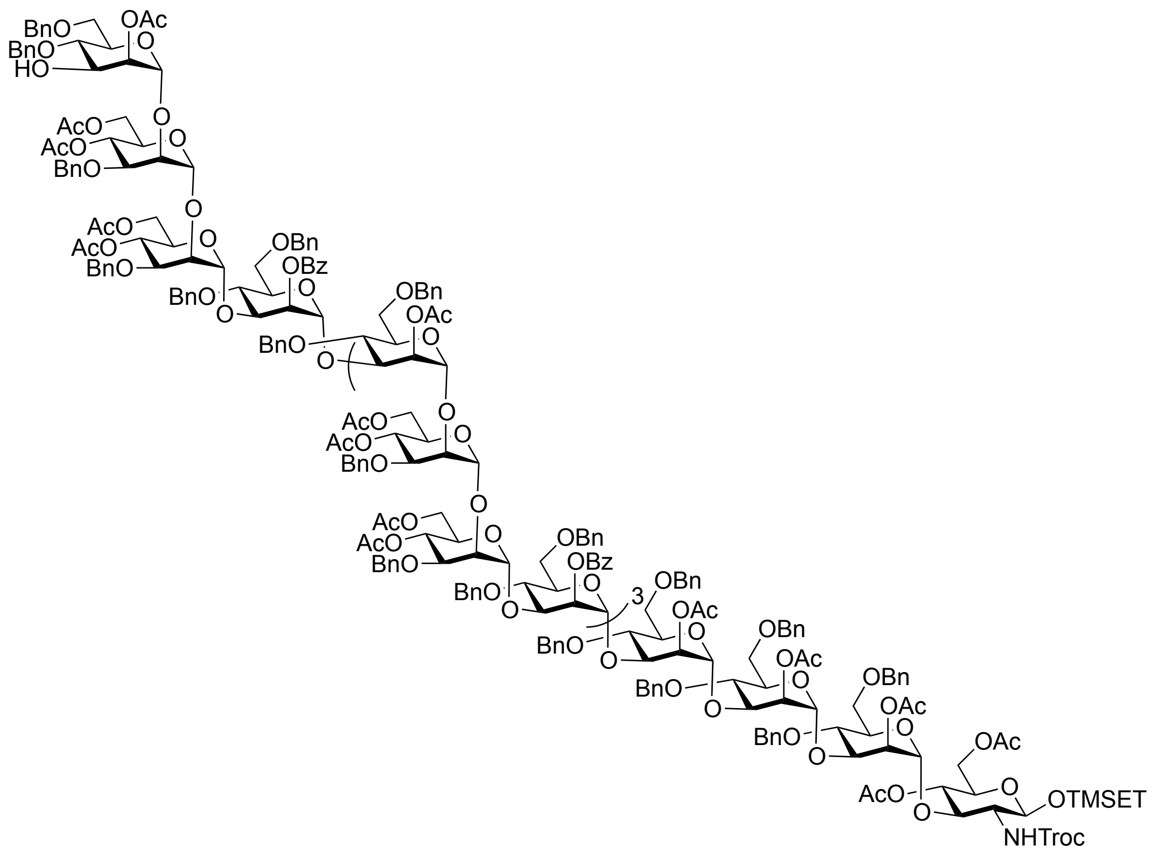
(86 mg, 0.93 mmol) in 9:1 CH_2Cl_2–CH_3OH (30 mL) was stirred at r.t. for 1 h. Then, the solution was concentrated at 40 °C for 0.5 h to achieve complete deproection of the levulinyl group. The resulting residue was subjected to chromatography (gradient 50→60% EtOAc in hexane) to afford **4.141** (0.77 g, 87% yield) as a white solid; R_f 0.47 (2:3 hexane–EtOAc); $[\alpha]_D$ = +20.0 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.02–7.98 (m, 4 H), 7.59–7.57 (m, 2 H), 7.38–6.99 (m, 94 H), 5.83 (d, 1 H, *J* = 7.0 Hz), 5.50 (s, 1 H), 5.46 (dd, 1 H, *J* = 3.0, 2.0 Hz), 5.31 (d, 1 H, *J* = 1.5 Hz), 5.28 (dd, 1 H, *J* = 3.0, 1.5 Hz), 5.25 (d, 1 H, *J* = 1.5 Hz), 5.22–5.15 (m, 9 H), 5.11 (s, 1 H), 5.09 (s, 1 H), 4.99–4.97 (m, 2 H), 4.89 (s, 1 H), 4.88 (s, 1 H), 4.84 (dd, 1 H, *J* = 3.0, 2.0 Hz), 4.80–4.70 (m, 8 H), 4.65–4.12 (m, 20 H), 4.39–4.32 (m, 4 H), 4.28–3.70 (m, 51 H), 3.67–3.51 (m, 14 H), 3.46–3.34 (m, 4 H), 3.18–3.15 (m, 1 H), 2.09 (s, 3 H), 2.06–2.04 (m, 15 H), 2.02 (s, 3 H), 1.93 (s, 3 H), 1.92 (s, 3 H), 1.905 (s, 3 H), 1.902 (s, 3 H), 1.899 (s, 3 H), 1.85 (s, 3 H), 1.83 (s, 3 H), 1.77 (s, 3 H), 0.88–0.79 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.9, 170.80, 170.77, 170.6, 170.5, 170.26, 170.20, 170.0, 169.7, 169.62, 169.57, 169.50, 169.1, 165.4, 165.3, 154.0, 139.1, 138.5, 138.4, 138.3, 138.2, 137.98, 137.95, 137.89, 137.85, 137.79, 137.78, 137.6, 133.4, 133.3, 129.9, 129.83, 129.79, 129.7, 128.53, 128.49, 128.46, 128.44, 128.38, 128.31, 128.29, 128.23, 128.14, 127.98, 127.95, 127.93, 127.87, 127.80, 127.78, 127.73, 127.69, 127.62, 127.58, 127.56, 127.52, 127.4, 127.3, 127.2, 100.8, 100.7, 99.9, 99.7, 99.3, 99.28, 99.21, 98.5, 78.2, 77.2, 76.0, 75.8, 75.7, 75.6, 75.5, 75.4, 75.3, 75.1, 74.95, 74.88, 74.5, 74.4, 74.2, 74.1, 73.53, 73.51, 73.37, 73.33, 73.28, 72.83,

72.51, 72.46, 72.36, 72.32, 72.27, 72.24, 72.03, 71.93, 71.90, 71.77, 71.74, 71.6, 71.5, 70.7, 70.6, 69.59, 69.52, 69.49, 68.8, 68.3, 68.1, 67.5, 67.3, 67.2, 62.7, 62.5, 62.4, 57.6, 21.08, 21.05, 20.97, 20.85, 20.83, 20.81, 20.76, 20.71, 20.67, 20.65, 20.59, 18.1, −1.4; HRMS (ESI) calcd for $(M+2(NH_4))^{+2}$ $C_{250}H_{290}Cl_3N_3O_{79}Si$: 2365.3796. Found: 2365.3757.

2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-3-*O*-levulinyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-manno-

pyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.142): A mixture of acceptor **4.141** (800 mg, 0.17 mmol), octasaccharide trichloroacetimidate **4.136** (772 mg, 0.24 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (40 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C and then TBSOTf (20 μL) was added. The solution was stirred at r.t. overnight before Et_3N (0.5 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 50→66% EtOAc in hexane) to afford **4.142** (944 mg, 71% yield) as a white solid; R_f 0.25 (2:3 hexane–EtOAc); $[\alpha]_D = +18.0$ (*c* 0.4, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.01–7.99 (m, 8 H), 7.58–7.56 (m, 4 H), 7.39–6.97 (m, 158 H), 5.83 (d, 1 H, *J* = 7.0 Hz), 5.50–5.47 (m, 4 H), 5.38 (dd, 1 H, *J* = 9.5, 3.5 Hz), 5.31–5.15 (m, 21 H), 5.11 (s, 1 H), 5.09 (s, 1 H), 5.00–4.97 (m, 4 H), 4.94 (s, 1 H), 4.89 (s, 1 H),

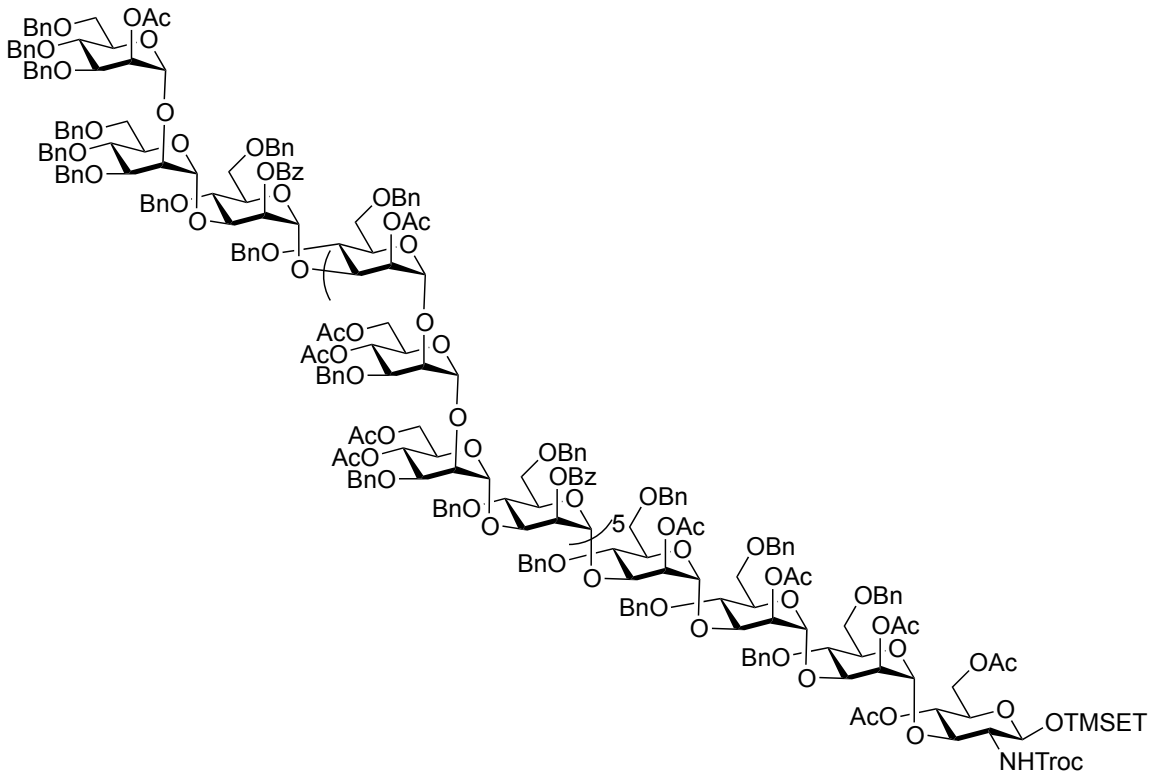
4.86–4.70 (m, 14 H), 4.65–4.32 (m, 39 H), 4.26–4.01 (m, 34 H), 3.99–3.71 (m, 56 H), 3.66–3.51 (m, 20 H), 3.46–3.34 (m, 6 H), 3.18–3.15 (m, 1 H), 2.77 (dt, 1 H, J = 18.0, 7.0 Hz), 2.63 (dt, 1 H, J = 18.0, 6.5 Hz), 2.51 (dt, 1 H, J = 17.0, 7.0 Hz), 2.42 (dt, 1 H, J = 17.0, 6.5 Hz), 2.14 (s, 3 H), 2.07–2.03 (m, 24 H), 1.94–1.92 (m, 15 H), 1.90–1.89 (m, 15 H), 1.849 (s, 3 H), 1.846 (s, 3 H), 1.835 (s, 3 H), 1.78 (s, 9 H), 0.88–0.79 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 206.5, 171.2, 171.1, 170.9, 170.80, 170.77, 170.55, 170.53, 170.26, 170.21, 170.0, 169.76, 169.72, 169.66, 169.59, 169.3, 169.1, 165.3, 165.2, 154.0, 139.14, 139.10, 138.5, 138.3, 138.16, 138.14, 138.07, 138.01, 137.99, 137.95, 137.89, 137.89, 137.81, 137.65, 137.61, 133.4, 133.3, 129.9, 129.8, 129.7, 128.53, 128.44, 128.38, 128.34, 128.31, 128.26, 128.24, 128.22, 128.14, 127.94, 127.87, 127.85, 127.83, 127.80, 127.79, 127.76, 127.74, 127.73, 127.67, 127.61, 127.59, 127.56, 127.4, 100.8, 100.6, 99.9, 99.7, 99.3, 99.2, 98.8, 98.6, 78.2, 77.9, 77.2, 76.6, 76.1, 75.96, 75.84, 75.68, 75.61, 75.51, 75.40, 75.31, 75.07, 75.01, 74.88, 74.75, 74.48, 74.39, 74.2, 74.1, 73.61, 73.54, 73.51, 73.42, 73.38, 73.33, 73.29, 72.92, 72.84, 72.51, 72.47, 72.36, 72.27, 72.22, 72.18, 72.03, 71.93, 71.88, 71.81, 71.78, 71.74, 71.5, 69.9, 69.70, 69.65, 69.60, 69.52, 68.5, 68.4, 68.3, 68.0, 67.5, 67.3, 67.2, 62.7, 62.56, 62.53, 62.41, 62.37, 57.6, 37.9, 29.8, 27.9, 21.08, 21.07, 21.04, 20.97, 20.95, 20.84, 20.81, 20.79, 20.76, 20.73, 20.67, 20.61, 20.59, 18.1, –1.4; HRMS (ESI) calcd for $(M+3(NH_4))^{+3}$ $C_{421}H_{480}Cl_3N_4O_{133}Si$: 2617.3251. Found: 2617.3245.



2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-

acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)- 2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.143): A solution of **4.142** (0.94 g, 0.12 mmol) and hydrazine acetate (222 mg, 2.4 mmol) in 9:1 CH_2Cl_2–CH_3OH (40 mL) was stirred at r.t. for 1 h. Then, the solution was concentrated at 40 °C for 1.5 h to achieve complete deproection of the levulinyl group. The resulting residue was subjected to chromatography (gradient 50→66% EtOAc in hexane) to afford **4.143** (806 mg, 87% yield) as a white solid; R_f 0.30 (2:3 hexane–EtOAc); $[\alpha]_D$ = +13.0 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.03–7.99 (m, 8 H), 7.59–7.56 (m, 4 H), 7.39–6.97 (m, 158 H), 5.83 (d, 1 H, *J* = 6.0 Hz), 5.51–5.47 (m, 4 H), 5.32–5.27 (m, 4 H), 5.25 (s, 1 H), 5.22–5.15 (m, 16 H), 5.11 (s, 1 H), 5.09 (s, 1 H), 5.04 (d, 1 H, *J* = 1.5 Hz), 5.00–4.97 (m, 3 H), 4.89 (s, 1 H), 4.85 (app t, 1 H, *J* = 2.0 Hz), 4.86–4.70 (m, 14 H), 4.65–4.32 (m, 39 H), 4.26–4.01 (m, 34 H), 3.99–3.71 (m, 57 H), 3.66–3.51 (m, 20 H), 3.46–3.34 (m, 6 H), 3.18–3.15 (m, 1 H), 2.09 (s, 3 H), 2.06–2.03 (m, 24 H), 1.94–1.92 (m, 12 H), 1.91–1.89 (m, 15 H), 1.855 (s, 3 H), 1.852 (s, 3 H), 1.845 (s, 3 H), 1.835 (s, 3 H), 1.78–1.77 (m, 9 H), 0.88–0.79 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.94, 170.90, 170.80, 170.77, 170.62, 170.53, 170.26, 170.21, 170.0, 169.73, 169.64, 169.52, 169.1, 165.3, 165.2, 154.0, 139.14, 139.13, 139.10, 138.5, 138.4, 138.3, 138.16, 138.01, 137.99, 137.97, 137.95, 137.89, 137.86,

137.81, 137.78, 137.61, 133.4, 133.3, 129.9, 129.8, 129.7, 128.53, 128.49, 128.45, 128.37, 128.31, 128.24, 128.22, 128.14, 127.99, 127.96, 127.94, 127.87, 127.80, 127.79, 127.76, 127.73, 127.70, 127.67, 127.63, 127.61, 127.58, 127.56, 127.4, 127.3, 100.77, 100.74, 100.66, 99.9, 99.7, 99.3, 99.2, 98.5, 78.2, 77.2, 76.0, 75.96, 75.84, 75.69, 75.63, 75.51, 75.40, 75.31, 75.07, 75.01, 74.96, 74.89, 74.55, 74.50, 74.39, 74.35, 74.2, 74.1, 73.54, 73.51, 73.38, 73.37, 73.33, 73.29, 72.84, 72.51, 72.47, 72.36, 72.26, 72.22, 72.03, 71.93, 71.90, 71.78, 71.74, 71.63, 71.48, 70.7, 70.6, 69.65, 69.59, 69.52, 68.8, 68.5, 68.3, 68.0, 67.5, 67.3, 67.27, 67.20, 62.7, 62.56, 62.53, 62.37, 57.6, 21.09, 21.04, 20.97, 20.87, 20.84, 20.81, 20.77, 20.71, 20.68, 20.61, 20.59, 18.1, −1.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{416}H_{462}Cl_3NNaO_{131}Si$: 7722.8. Found: 7722.5.

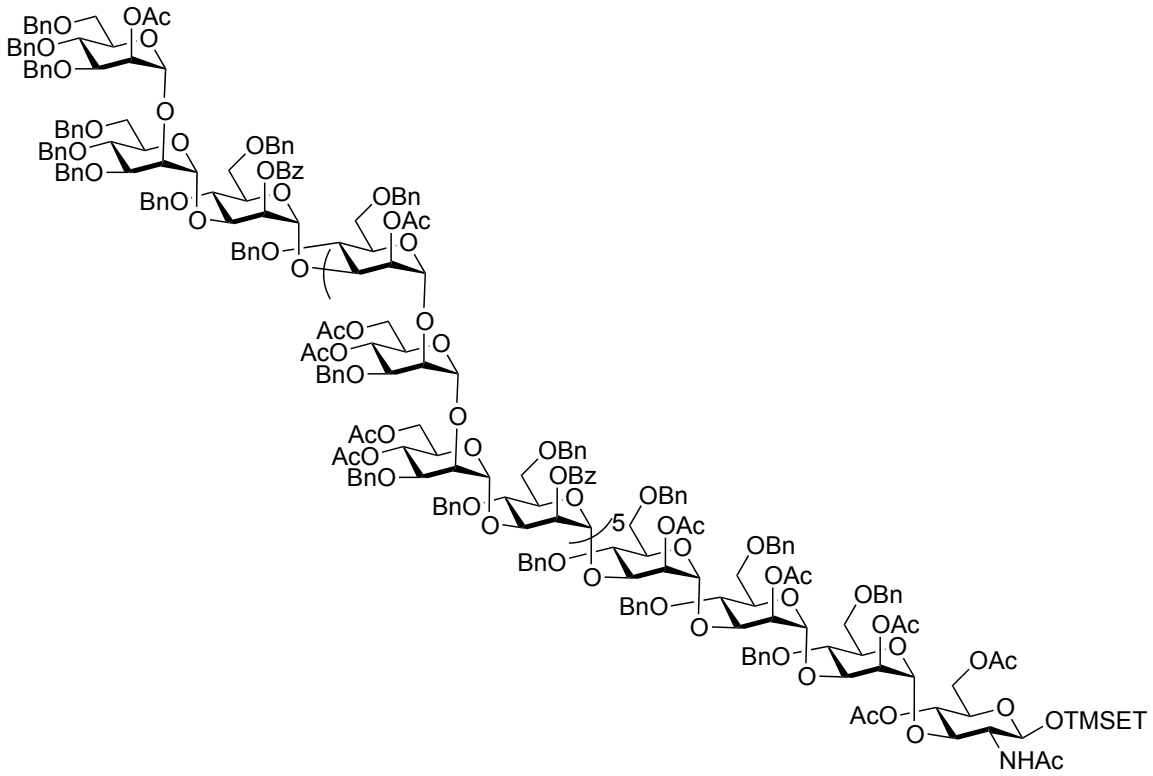


2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-ben-

zoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*- benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyran- osyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6- di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-manno- pyranosyl-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (4.144): Trichloroacetimidate **4.139** (0.94 g) was synthesized from heptasaccharide **4.137** (1.49 g) in 63% yield. following general procedure A described above. A mixture of acceptor **4.143** (963 mg, 0.125 mmol), heptasaccharide trichloroacetimidate **4.139** (530 mg, 0.175 mmol) and powdered 4 Å molecular sieves was suspended in anhydrous CH_2Cl_2 (80 mL) and stirred at r.t. for 10 min. The solution was then cooled to 0 °C and then TBSOTf (50 μL) was added. The solution was stirred at r.t. overnight before Et_3N (1.0 mL) was added and the mixture was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (gradient 50→63% EtOAc in hexane) to afford **4.144** (963 mg, 73%) yield as a white solid; R_f 0.53 (2:3 hexane–EtOAc); $[\alpha]_D$ = +6.1 (*c* 0.3, CH_2Cl_2); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.01–7.98 (m, 12 H), 7.58–7.52 (m, 6 H), 7.39–6.98 (m, 232

H), 5.83 (d, 1 H, J = 6.0 Hz), 5.58 (s, 1 H), 5.50–5.47 (m, 6 H), 5.31–5.28 (m, 6 H), 5.25 (s, 1 H), 5.23–5.16 (m, 21 H), 5.11 (s, 1 H), 5.09 (s, 1 H), 5.03 (d, 1 H, J = 1.5 Hz), 5.00–4.97 (m, 5 H), 4.89 (s, 1 H), 4.85–4.64 (m, 24 H), 4.59–4.33 (m, 54 H), 4.27–4.13 (m, 37 H), 4.12–4.00 (m, 8 H), 3.98–3.71 (m, 79 H), 3.66–3.50 (m, 25 H), 3.47–3.34 (m, 9 H), 3.18–3.15 (m, 1 H), 2.07 (s, 3 H), 2.06–2.04 (m, 24 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 1.93–1.92 (m, 15 H), 1.90–1.89 (m, 15 H), 1.85–1.84 (m, 15 H), 1.78–1.77 (m, 15 H), 0.88–0.79 (m, 2 H), –0.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.99, 170.94, 170.81, 170.78, 170.53, 170.26, 170.21, 170.07, 170.00, 169.93, 169.7, 169.6, 169.1, 169.0, 165.4, 165.3, 165.2, 154.0, 139.16, 139.13, 139.10, 138.8, 137.72, 138.68, 138.53, 138.49, 138.37, 138.34, 138.33, 138.16, 138.11, 138.08, 138.01, 137.99, 137.95, 137.89, 137.86, 137.81, 137.78, 137.61, 133.4, 133.3, 133.2, 129.9, 129.8, 129.7, 128.6, 128.53, 128.44, 128.41, 128.38, 128.34, 128.30, 128.26, 128.24, 128.22, 128.18, 128.14, 128.06, 127.94, 127.85, 127.80, 127.76, 127.73, 127.67, 127.63, 127.61, 127.56, 127.54, 127.51, 127.46, 127.44, 127.41, 127.38, 127.36, 127.25, 127.21, 127.15, 101.2, 100.78, 100.65, 99.9, 99.7, 99.6, 99.4, 99.27, 99.17, 99.09, 98.5, 79.2, 78.4, 78.2, 77.2, 76.9, 76.4, 75.96, 75.84, 75.64, 75.51, 75.39, 75.31, 75.07, 75.00, 74.89, 74.79, 74.65, 74.50, 74.39, 74.30, 74.2, 74.1, 74.0, 73.54, 73.52, 73.38, 73.33, 73.29, 73.22, 73.13, 72.84, 72.63, 72.51, 72.47, 72.36, 72.27, 72.22, 72.15, 72.03, 71.93, 71.90, 71.78, 71.74, 71.48, 70.7, 69.65, 69.58, 69.52, 68.65, 68.62, 68.49, 68.39, 68.25, 68.14, 68.06, 67.5, 67.3, 67.2, 62.7, 62.53, 62.37, 57.6, 21.18, 21.11,

21.09, 21.04, 20.97, 20.87, 20.84, 20.81, 20.77, 20.68, 20.61, 18.1, −1.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{582}H_{636}Cl_3NNaO_{174}Si$: 10578. Found: 10578.

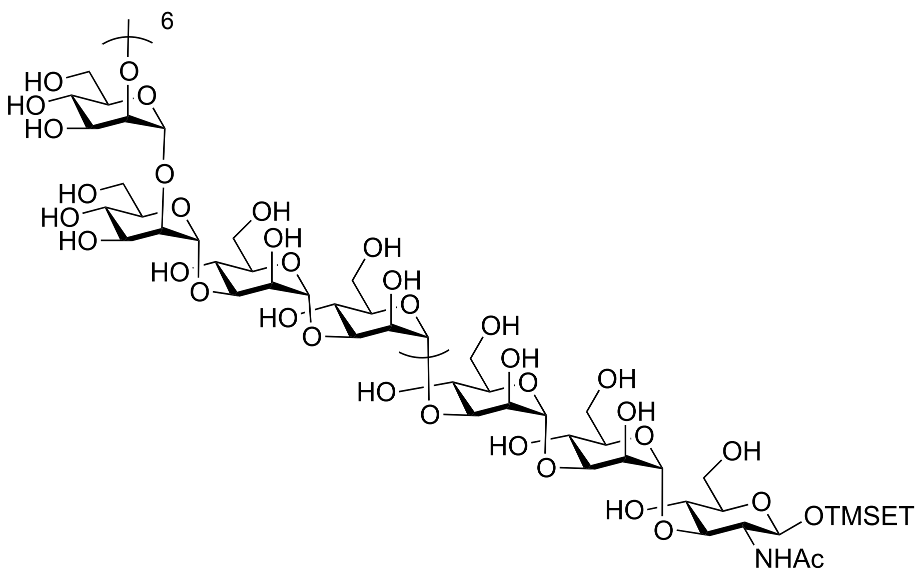


2-(Trimethylsilyl)ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-Acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-

di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-manno-pyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benz-oyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyrano-syl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-manno-pyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-ace-tyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4.145): To a solution of substrate **4.144** (940 mg, 0.09 mmol) in 3:1 THF–AcOH (40 mL) was added freshly activated zinc dust (2 g). After stirring for 3 h at r.t., the mixture was filtered and the filtrate was concentrated. The resulting residue was dissolved in 3:2 pyridine–Ac_2O (25 mL) and the mixture was stirred at r.t. for 2 h. Then, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with 1M HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 50→60% EtOAc in hexane) to afford **4.145** (742 mg, 80% yield) as a white solid; R_f 0.17 (2:3 hexane–EtOAc); $[\alpha]_D$ = +6.4 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.01–7.98 (m, 12 H),

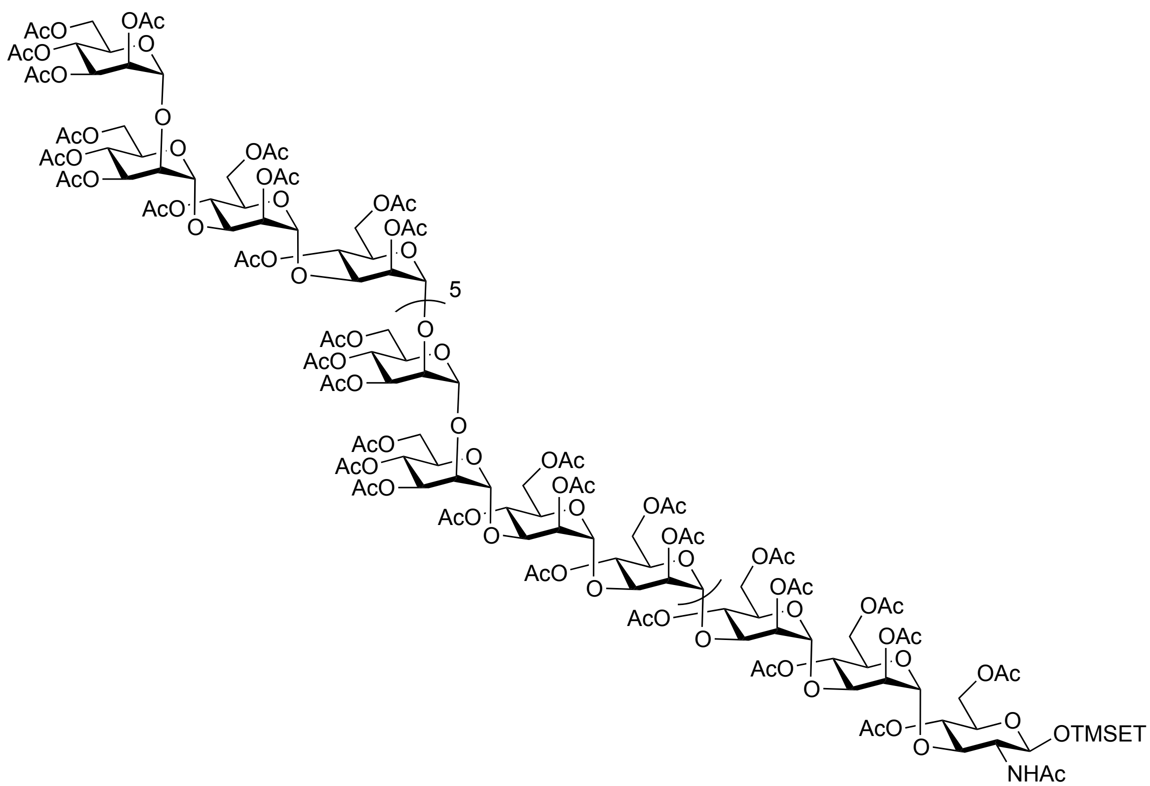
7.58–7.51 (m, 6 H), 7.39–6.98 (m, 232 H), 6.48 (d, 1 H, J = 7.0 Hz), 5.57 (s, 1 H), 5.50–5.47 (m, 6 H), 5.30–5.28 (m, 6 H), 5.25 (s, 1 H), 5.22–5.15 (m, 21 H), 5.11 (s, 1 H), 5.09 (s, 1 H), 5.06 (d, 1 H, J = 8.5 Hz), 5.02 (d, 1 H, J = 1.5 Hz), 4.99–4.98 (m, 5 H), 4.93 (dd, 1 H, J = 10.0, 9.0 Hz), 4.84–4.67 (m, 23 H), 4.58–4.30 (m, 53 H), 4.27–4.12 (m, 38 H), 4.09–4.00 (m, 6 H), 3.98–3.70 (m, 78 H), 3.66–3.48 (m, 26 H), 3.45–3.34 (m, 9 H), 2.95–2.92 (m, 1 H), 2.07 (s, 3 H), 2.05–2.03 (m, 24 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93–1.92 (m, 15 H), 1.90–1.89 (m, 15 H), 1.84–1.83 (m, 15 H), 1.78–1.77 (m, 18 H), 0.94–0.80 (m, 2 H), –0.02 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.4, 170.99, 170.94, 170.83, 170.80, 170.53, 170.24, 170.21, 170.07, 169.98, 169.93, 169.7, 169.6, 169.1, 169.0, 165.4, 165.3, 165.2, 139.16, 139.13, 139.10, 138.8, 137.72, 138.68, 138.53, 138.48, 138.37, 138.34, 138.32, 138.16, 138.11, 138.08, 138.01, 137.99, 137.95, 137.90, 137.86, 137.81, 137.78, 137.61, 137.5, 137.2, 133.4, 133.3, 133.1, 129.9, 129.8, 129.7, 128.62, 128.59, 128.53, 128.44, 128.38, 128.34, 128.30, 128.26, 128.23, 128.21, 128.18, 128.14, 128.11, 128.06, 128.02, 129.99, 127.85, 127.80, 127.76, 127.73, 127.70, 127.63, 127.61, 127.56, 127.54, 127.51, 127.46, 127.44, 127.41, 127.38, 127.36, 127.25, 127.21, 127.15, 101.2, 100.77, 100.64, 99.8, 99.66, 99.58, 99.42, 99.28, 99.18, 99.08, 98.8, 80.4, 79.2, 78.4, 78.1, 77.7, 77.6, 77.2, 76.9, 76.4, 75.96, 75.84, 75.64, 75.53, 75.39, 75.30, 75.07, 75.00, 74.89, 74.79, 74.65, 74.50, 74.39, 74.30, 74.2, 74.1, 74.0, 73.63, 73.53, 73.38, 73.35, 73.33, 73.28, 73.22, 73.13, 72.83, 72.64, 72.51, 72.47, 72.36, 72.27, 72.22, 72.15, 72.03, 71.93, 71.88, 71.78, 71.74, 71.2, 70.4, 69.65, 69.62, 69.52, 68.65, 68.50, 68.39, 68.25, 68.17, 68.09, 67.3,

67.2, 62.7, 62.53, 57.8, 23.4, 21.18, 21.10, 21.09, 21.04, 20.97, 20.87, 20.84, 20.78, 20.76, 20.67, 20.61, 20.59, 18.0, −1.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{581}H_{637}NNaO_{173}Si$: 10446. Found: 10446.



2-(Trimethylsilyl)ethyl α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)- α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranos-yl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-

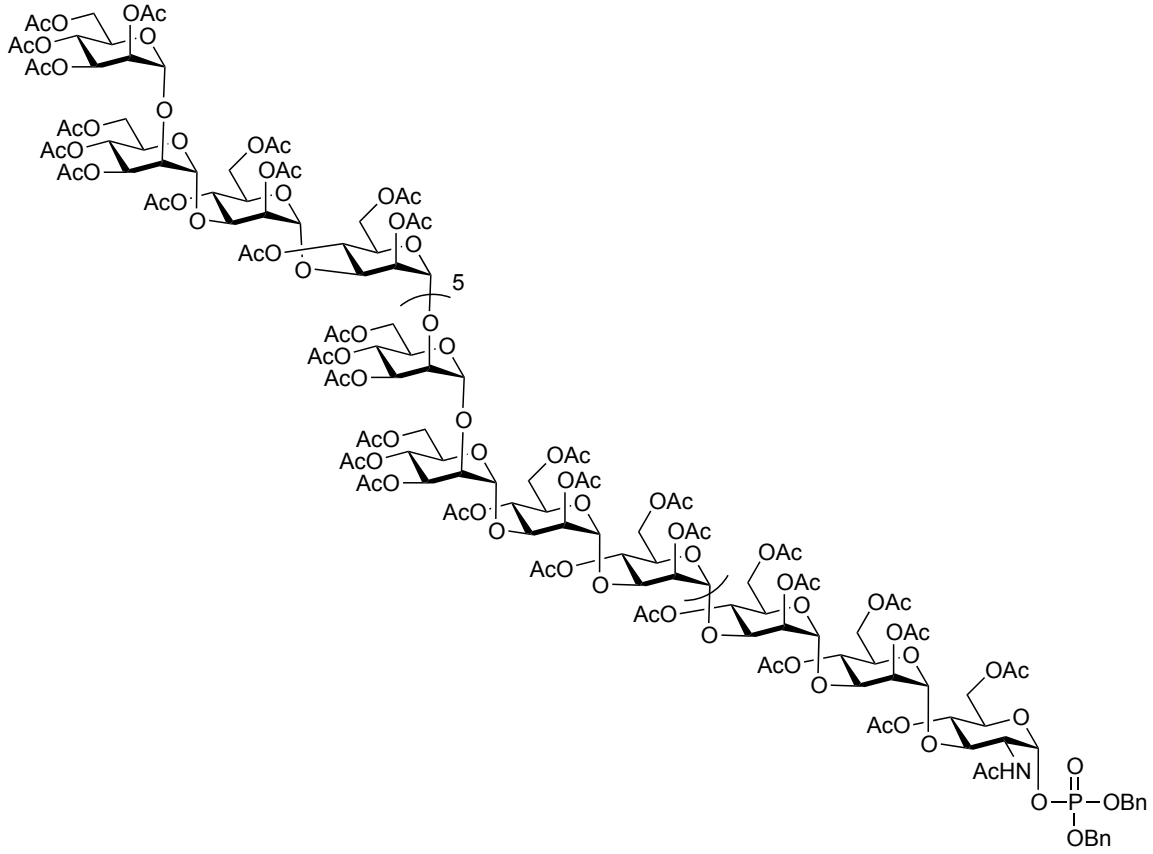
α-D-manno- pyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopy- ranoside (4.146): Ammonia was condensed at −78 °C into 50 mL round bottom flask equipped with a Dewar condenser and a magnetic stir bar (total volume 14 mL). Freshly cut sodium metal (70 mg) was added and the mixture was stirred at −78 °C for 5 min. A solution of compound **4.145** (25 mg, 2.4 μmol) in THF (0.40 mL) was introduced via syringe and the mixture was stirred at −80 °C for 1.5 h before CH_3OH (2 mL) was added. The colorless solution was warmed to r.t. and then concentrated. The residue was dissolved in water (4 mL) and the solution was neutralized with Amberlite IR120 H^+ ion-exchange resin, filtered, concentrated. The residue purified by C_{18} chromatography (gradient 0→30% CH_3OH in H_2O) to afford **4.146** (6.6 mg, 61% yield) as a white solid. $[\alpha]_D$ = +88.8 (*c* 0.2, H_2O); ^1H NMR (700 MHz, D_2O, δ_H) 5.39−5.38 (m, 6 H), 5.31−5.28 (m, 6 H), 5.15−5.13 (m, 7 H), 5.09 (s, 1 H), 5.06−5.05 (m, 6 H), 4.58 (d, 1 H, *J* = 8.5 Hz), 4.23−4.18 (m, 13 H), 4.12−4.05 (m, 13 H), 4.03−3.99 (m, 13 H), 3.97−3.64 (m, 119 H), 3.63−3.58 (m, 3 H), 2.95−2.92 (m, 1 H), 2.04 (s, 3 H), 1.02−0.86 (m, 2 H), 0.02 (s, 9 H); ^{13}C NMR (125 MHz, D_2O, δ_C) 174.9, 103.25, 103.22, 103.17, 103.07, 101.71, 101.68, 101.63, 101.1, 80.7, 79.6, 79.5, 79.39, 79.36, 79.31, 79.2, 76.6, 74.50, 74.44, 74.37, 74.29, 74.26, 74.21, 71.8, 71.3, 71.05, 71.01, 70.96, 70.91, 70.64, 70.57, 69.3, 68.0, 67.9, 67.8, 67.14, 67.10, 66.5, 62.07, 61.99, 61.96, 61.91, 61.62, 61.54, 55.1, 23.5, 18.1, −1.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{169}H_{287}NNaO_{136}Si$: 4557.6. Found: 4558.0.



2-(Trimethylsilyl)ethyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-manno-

pyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4.147): Compound **4.146** (64 mg, 14.1 μmol) was suspended in 3:2 pyridine–Ac_2O (5 mL) and the mixture was stirred at 45 °C overnight. Then, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with 1M HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography (gradient 60→75% acetone in hexane) to afford **4.147** (86 mg, 77% yield) as a white solid; R_f 0.24 (4:7 hexane-acetone); $[\alpha]_D$ = +18.9 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 6.25 (d, 1 H, *J* = 7.0 Hz), 5.37 (dd, 1 H, *J* = 10.0, 3.5 Hz), 5.34–5.17 (m, 32 H), 5.31–5.28 (m, 6 H), 5.14–5.10 (m, 6 H), 5.06–4.90 (m, 31 H), 4.84 (s, 1 H), 4.83 (s, 1 H), 4.50 (app t, 1 H, *J* = 9.0 Hz), 4.27–4.21 (m, 17 H), 4.16–3.89 (m, 79 H), 3.85–3.78 (m, 9 H), 3.72 (s, 2 H), 3.66–3.64 (m, 1 H), 3.54 (td, 1 H, *J* = 10.0, 6.5 Hz), 3.09–3.05 (m, 1 H), 2.16–2.12 (m, 69 H), 2.11–2.08 (m, 87 H), 2.05–2.03 (m, 39 H), 2.00–1.98 (m, 51 H), 0.97–0.83 (m, 2 H), 0.00 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.1, 170.9, 170.72, 170.68, 170.61, 170.59, 170.44, 170.40, 170.36, 170.26, 170.21, 170.19, 170.07, 169.85, 169.82, 169.71, 169.69, 169.52, 169.47, 169.43, 169.41, 169.39, 169.37, 169.28, 99.95, 99.83, 99.59, 99.54, 99.50, 99.42, 99.30, 99.15, 98.8, 98.6, 80.7,

77.7, 77.5, 77.2, 77.1, 75.6, 75.4, 75.2, 75.1, 74.3, 74.0, 73.7, 71.3, 71.2, 70.94, 70.89, 70.79, 70.5, 70.0, 69.8, 69.63, 69.58, 69.51, 69.0, 68.7, 68.4, 67.38, 67.31, 67.22, 67.0, 66.8, 66.7, 66.1, 66.0, 65.4, 63.8, 62.5, 62.37, 62.28, 62.23, 62.0, 61.87, 61.80, 61.76, 61.71, 61.6, 58.4, 23.7, 20.98, 20.95, 20.88, 20.86, 20.82, 20.79, 20.76, 20.72, 20.68, 20.62, 20.59, 20.56, 18.0, −1.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{331}H_{449}NNaO_{217}Si$: 7960.4. Found: 7960.6.

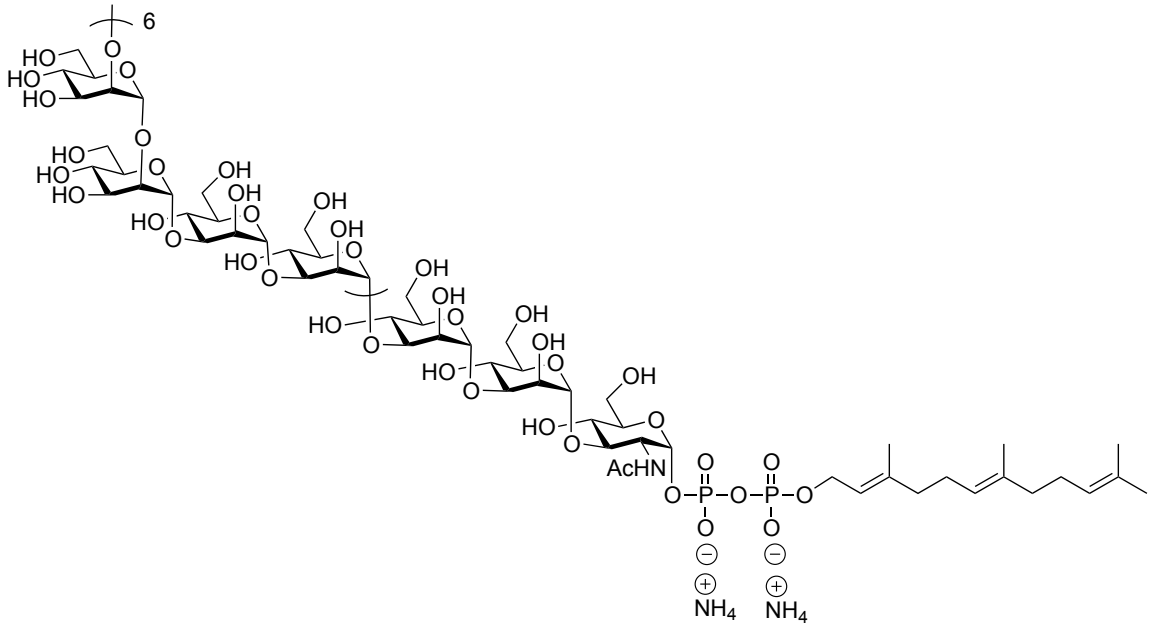


2,3,4,6-tetra-*O*-Acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-

***O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-acet-amido-4,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate (4.149):** To a solution of **4.147** (81 mg, 10.2 μmol) in dry CH_2Cl_2 (3 mL) was added TFA (1 mL) dropwise at 0 °C. After stirring for 3 h at r.t., the solution was concentrated, dissolved in CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified by chromatography (gradient 60→95% acetone in hexane) to afford hemiacital **4.148** (62 mg, 78% yield) as a white solid. Compound **4.148** (62 mg, 7.9 μmol) was dissolved in dry CH_2Cl_2 (5 mL), tetraazole (28 mg, 395 μmol) was added and the reaction mixture was

cooled to 0 °C. After 10 min, dibenzyl *N*,*N*-diisopropylphosphoramidite (82 mg, 237 μmol) was added dropwise and the mixture was stirred at r.t. for 4 h. The mixture was cooled to −78 °C and *m*-CPBA (61 mg, 355 μmol) was added in one portion and then the solition was warmed to r.t. After stirring at r.t. for 2 h, CH_2Cl_2 was added and the mixture was washed with saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by Sephadex LH-20 (1:1CH_3OH–CH_2Cl_2) to afford phosphate **4.149** (82 mg, 92% yield) as a white solid; R_f 0.12 (4:7 hexane–acetone); $[\alpha]_D$ = +17.3 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.37–7.31 (m, 10 H), 5.86 (d, 1 H, *J* = 9.5 Hz), 5.60 (dd, 1 H, *J* = 6.0, 3.0 Hz), 5.37 (dd, 1 H, *J* = 10.0, 3.0 Hz), 5.34 (app t, 1 H, *J* = 10.0 Hz), 5.30–5.25 (m, 13 H), 5.23–5.19 (m, 13 H), 5.18–5.09 (m, 10 H), 5.08–5.01 (m, 9 H), 5.00–4.97 (m, 14 H), 4.91–4.87 (m, 9 H), 4.84 (d, 1 H, *J* = 1.5 Hz), 4.34–4.19 (m, 16 H), 4.17–4.11 (m, 23 H), 4.08–3.93 (m, 46 H), 3.90–3.73 (m, 22 H), 2.17–2.13 (m, 51 H), 2.12–2.06 (m, 99 H), 2.05–2.03 (m, 42 H), 2.00–1.98 (m, 54 H); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.9, 170.75, 170.68, 170.59, 170.54, 170.44, 170.40, 170.37, 170.26, 170.21, 170.14, 170.07, 170.02, 169.86, 169.82, 169.72, 169.70, 169.52, 169.47, 169.43, 169.41, 169.40, 169.39, 169.28, 135.2 (d, J_{PC} = 6.1 Hz), 135.1(d, J_{PC} = 6.1 Hz), 129.8, 129.10, 129.08, 128.85, 128.24, 128.20, 99.95, 99.83, 99.59, 99.56, 99.50, 99.30, 99.18, 99.10, 98.4, 96.7 (d, $J_{PC\text{-}1}$ = 6.4 Hz), 77.7, 77.5, 77.2, 77.1, 76.5, 75.6, 75.4, 75.1, 75.0, 74.7, 74.3, 74.0, 71.3, 70.94, 70.89, 70.77, 70.13, 70.10, 70.04, 69.86, 69.80, 69.67, 69.63, 69.58, 69.49, 68.7, 68.4, 67.37, 67.31, 67.22, 67.02,

66.96, 66.7, 66.1, 66.0, 65.4, 62.7, 62.5, 62.37, 62.28, 62.03, 61.88, 61.81, 61.75, 61.71, 61.6, 61.2, 51.8 (d, J_{PC-2} = 7.3 Hz), 22.9, 20.95, 20.91, 20.86, 20.82, 20.79, 20.72, 20.68, 20.66, 20.62, 20.59, 20.56; ^{31}P NMR (160 MHz, $CDCl_3$, δ_C) −2.4; MALDI-TOF calcd for $(M+Na)^+$ $C_{340}H_{450}NNaO_{220}P$: 8125.0. Found: 8124.8.



α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-

(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl farnesyl diphosphate diammonium salt (4.52): To a solution of **4.149** (20 mg, 2.5 μmol) in THF (5 mL) was added palladium on charcoal (10%, 10 mg) and the solution was subjected to hydrogen atmosphere for 4 h. The mixture was filtered through Celite and the filtrate was concentrated. The residue **4.150** was used in the next step without further purification. To a solution of the crude phosphate **4.150** in dry CH_2Cl_2 (3 mL) was added 1,1'-carbonyldiimidazole (16 mg, 0.1 mmol). After stirring at r.t. for 2 h, a solution of 5% (v/v) solution of anhydrous CH_3OH in CH_2Cl_2 (0.10 mL) was added to quench the unreacted 1,1'-carbonyldiimidazole and the mixture was stirred for 30 min. The solvent was concentrated and the residue dissolved in DMF-d_7 (0.6 mL). Farnesol phosphate **4.130** (41 mg, 0.125 mmol) was added and the reaction mixture stirred at r.t. for 7 days. ^{31}P NMR spectroscopy showed that at this point all of the activated intermediate was consumed. The solvent was removed in vacuo and the residue purified by Sephadex LH-20 (1:1 CH_3OH-CH_2Cl_2) to afford **4.151** as a crude product. To a solution of crude phosphate **4.151** in CH_3OH–CH_2Cl_2 (5 mL, 4:1) was added freshly prepared $NaOCH_3$ (1M solution in CH_3OH, 1.0 mL). The reaction mixture was stirred at r.t. for 6 h, and the $NaOCH_3$ was quenched by addition of Amberlite IR120 (NH_4^+ form). The mixture was filtered, concentrated in vacuo and the residue purified by C_{18} chromatography (gradient 0→15% CH_3OH in H_2O) to

afford **4.52** (6.5 mg, 55% yield) as a white solid. $[\alpha]_D$ = +40.6 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (700 MHz, D_2O, δ_H) 5.50 (dd, 1 H, *J* = 7.0, 3.0 Hz), 5.46 (app t, 1 H *J* = 8.0 Hz), 5.39–5.38 (m, 6 H), 5.31 (s, 5 H), 5.26 (s, 1 H), 5.23–5.19 (m, 2 H), 5.13 (s, 7 H), 5.10 (s, 1 H), 5.06 (s, 1 H), 5.05 (s, 5 H), 4.53–4.47 (m, 2 H), 4.22–4.19 (m, 14 H), 4.12–4.08 (m, 13 H), 4.03–3.73 (m, 126 H), 3.72–3.66 (m, 13 H), 3.61–3.59 (m, 1 H), 2.19–2.10 (m, 6 H), 2.08 (s, 3 H), 2.04 (t, 1 H *J* = 7.5 Hz), 1.73 (s, 3 H), 1.70 (s, 3 H), 1.64 (s, 6 H); ^{13}C NMR (125 MHz, D_2O, δ_C) 175.3, 144.0, 137.7, 134.5, 125.4, 125.2, 120.4 (d, J_{PC} = 8.5 Hz), 103.25, 103.23, 103.18, 103.06, 101.98, 101.68, 101.63, 95.6 (d, J_{PC} = 6.5 Hz), 79.6, 79.5, 79.35, 79.30, 79.25, 79.22, 79.0, 78.5, 74.5, 74.4, 74.35, 74.30, 74.28, 74.24, 74.25, 74.20, 73.9, 71.30, 71.04, 71.00, 70.95, 70.63, 70.57, 68.0, 67.8, 67.7, 67.14, 67.10, 66.5, 64.0 (d, J_{PC} = 5.8 Hz), 62.06, 61.95, 61.90, 61.5, 61.1, 53.2 (d, J_{PC} = 7.5 Hz), 39.7, 26.7, 26.5, 25.8, 23.3, 18.0, 16.6, 16.2; ^{31}P NMR (200 MHz, D_2O, δ_C) −10.6 (d, J_{PP} = 20.0 Hz), −13.3 (d, J_{PP} = 20.0 Hz); HRMS (ESI) calcd for $(M-3H)^{-3}$ $C_{179}H_{298}NO_{142}P_2$: 1598.5201. Found: 1598.5180.

Chapter 5: Summary and future work

5.1 Summary and future work

In my research, I have investigated the synthesis of three different types of bacterial glycans. This thesis includes the synthesis of rhamnolipid analogs (Chapter 2), the synthesis of the *N*-acylated aminosugar motif of *M. marinum* lipooligosaccharide IV (Chapter 3), and the synthesis of lipid-linked oligosaccharides as probes of lipopolysaccharide biosynthesis (Chapter 4).

5.1.1 Synthesis of rhamnolipid analogs

In Chapter 2, I described the synthesis of four rhamnolipid analogs, in which the ester linkage connecting the two lipid chains in the natural compound is replaced with an amide, ether, hydrocarbon, or ketone group. The key step in the synthesis of the amide analog was an enantioselective 1,4-addition to an α:β unsaturated ester, which provided the (*R*)-β-amino ester. Further elaboration (amidation, glycosylation and deprotection) gave the amide analog. The key step in the synthesis of the ether analog was a silver oxide-assisted O-alkylation of a β-hydroxy-ester. Subsequent glycosylation and deprotection gave the ether analog. The synthesis of the hydrocarbon and ketone analogs share the same key alkene intermediate, which could be generated via a Wittig reaction using an aldehyde and an iodide as the substrates. Glycosylation of the resulting hydroxylated alkene intermediate and hydrogenation led to the hydrocarbon analog. For the ketone analog, the key

transformations were a four-step reaction sequence (silyl ether formation, hydrosilylation, oxidative cleavage and lactone formation) to obtain isomeric lactones bearing a hydroxyl group. Then, glycosylation, lactone ring opening and a late stage Ley–Griffith oxidation led to the target.

With these four rhamnolipid analogs in hand, the next step is to test their immunomodulatory activities against human mononuclear cells to determine their ability to induce the secretion of the cytokine TNF-α using ELISA. The immunomodulatory activities towards the pro-inflammatory cytokines IL-6 and IL-12 could also be tested. These cytokine induction assays will be done in collaboration with the group of Dr. Christopher Cairo in the University of Alberta. The results of these studies will inform the synthesis of additional analogs in the future.

5.1.2 Synthesis of *N*-acylated aminosugar fragments of *Mycobacterium marinum* lipooligosaccharide IV

In Chapter 3, I developed a convergent route to synthesize four unusual *N*-acylated monosaccharide fragments present in the LOS-IV from *M. marinum*. The general approach involved the synthesis of four lactam acid intermediates, which were coupled to a protected 4-amino-4,6-dideoxygalactose derivative. The monosaccharide residue was prepared via a conventional route. The lactam moieties were synthesized via two different approaches. The first approach was used to prepare the three targets with a methoxy group at the carbon

adjacent to the lactam carbonyl. The key step was the construction of highly substituted oxazolidine–pyrrolinone bicyclic ring system through a substrate controlled stereoselective cyclization reaction. This reaction produced all three stereocenters of the lactam moiety in a single step. Another route was developed to synthesize the lactam needed for the preparation of target lacking the methoxy group. An oxidation–cyclization–oxidation sequence of a Boc-protected amino alcohol was used to construct the core lactam intermediate. Further elaboration (*N*-methylation, amidation and deprotection) gave the target without the methoxy group.

The synthetic routes developed in Chapter 3 will be used for synthesis of terminal *N*-acylated monosaccharide fragments, which are needed for the preparation of the complete LOS-IV molecule. These *N*-acylated monosaccharide targets were synthesized bearing an aminooctyl aglycone. The amino group in the aglycone chains provides a convenient handle for conjugation to proteins for the generation of monoclonal antibodies against these motifs. In addition, once the complete LOS-IV molecules are synthesized, the immunomodulatory activities of these *N*-acylated monosaccharide fragments and the entire LOS-IV molecules will be evaluated.

5.1.3 Synthesis of *E. coli* O9a O-polysaccharide derivatives as probes of *E. coli* O9a lipopolysaccharide biosynthesis

In Chapter 4, I developed a route to prepare a series of *E. coli* O9a O-polysaccharide biosynthetic intermediates, which contain two and six tetrasaccharide repeating units connected via the primer–adaptor trisaccharide to farnesyl pyrophosphate. The approach started with the chemical synthesis of D-mannose and D-glucosamine building blocks. Then, using an iterative cycle of NIS/AgOTf-promoted glycosylations and hydrazine acetate-mediated levuninate ester cleavages, repeating units (**4.91** and **4.96**) involved in two synthetic routes were synthesized. After comparing the synthetic efficiency of the two routes, we chose the one that involved **4.96** to synthesize the target compounds. Then, a TBSOTf-promoted glycosylation method was developed for the glycosylations between oligosaccharide acceptors and an imidate donors generated from the oligosaccharide building blocks. Modest to excellent yields (71%–88%) were obtained for glycosylations with large oligosaccharide acceptors and donors.

Following a 4 + 4 + 3 strategy, the protected undecassaccharide was synthesized in good yield. After nine additional steps, involving *N*-protecting group transformation, Birch reduction, acetylation, removal of a TMSET group, phosphorylation, coupling with farnesol phosphate and final deprotection, the target 11-mer was synthesized. Then, I adopted a 4 + 8 + 8 + 7 strategy to synthesize the 27-mer target. For this target, using Birch reduction to remove all of the benzyl groups was difficult. After several attempts, I found

that three key factors, low temperature (–80 °C), short reaction times (1–1.5 h) and low concentrations (0.0025 mmol/mL), were critical for this reaction to succeed. Finally, following the same reaction sequence used for the 11-mer, the target 27-mer was obtained.

In the future, three larger targets with 10, 14 and 18 repeating units will be synthesized using the synthetic route developed in Chapter 4. Then, these molecules will be used as probes of LPS biosynthesis in collaboration with Dr. Chris Whitfield at the University of Guelph. At first, these O-PS fragments could be used as ligands in crystallization trials with WbdD to probe the chain termination mechanism. In addition, they could be used as substrates for WbdA to determine if there are changes in the recognition of O-PS fragments as the substrates gets larger. More interestingly, they could be used as substrate for for ABC transporter to understand the 'flipping' process, which is responsible for the transportation of the final lipid-linked O-PS across the inner membrane to the periplasm.

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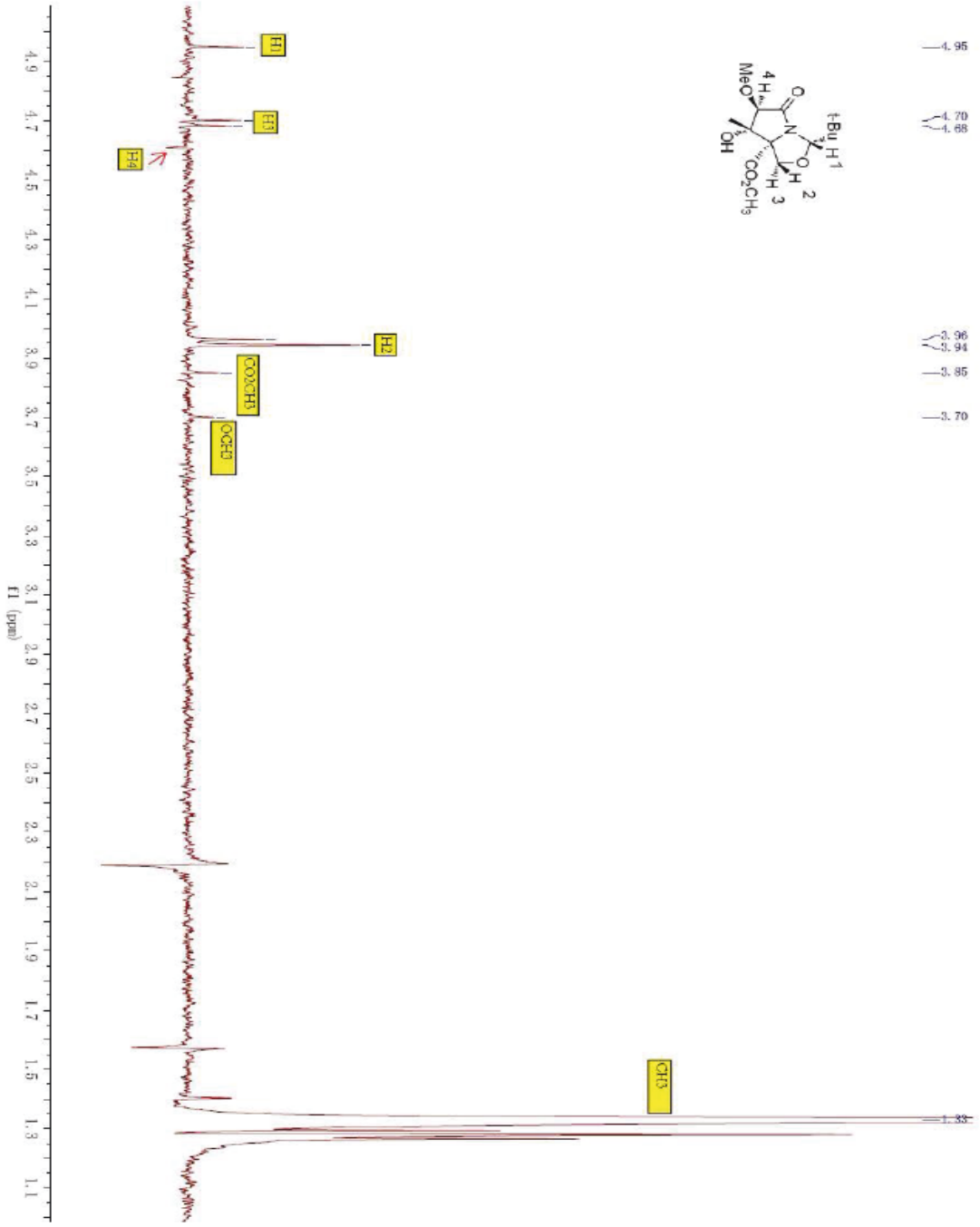
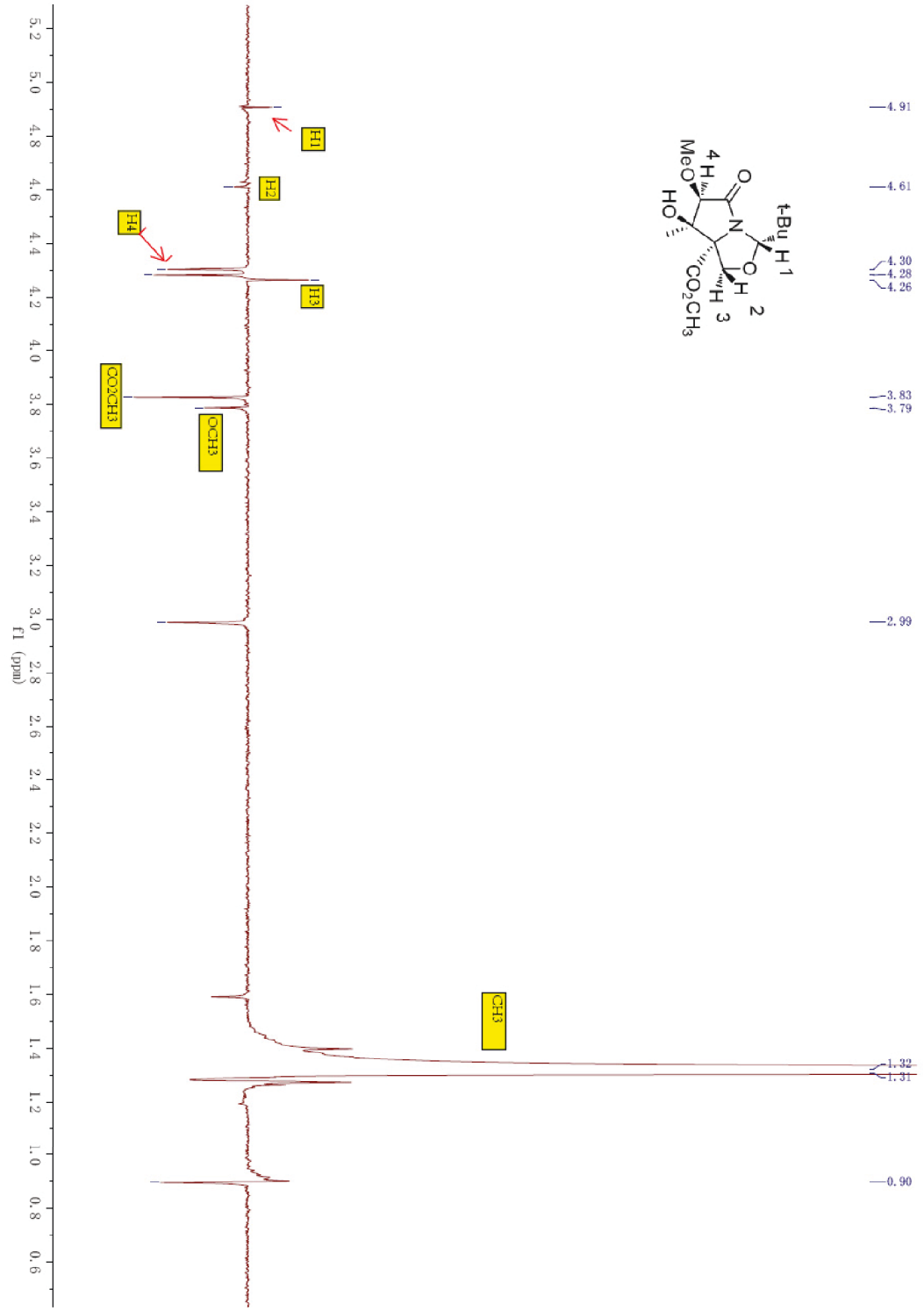
Appendix

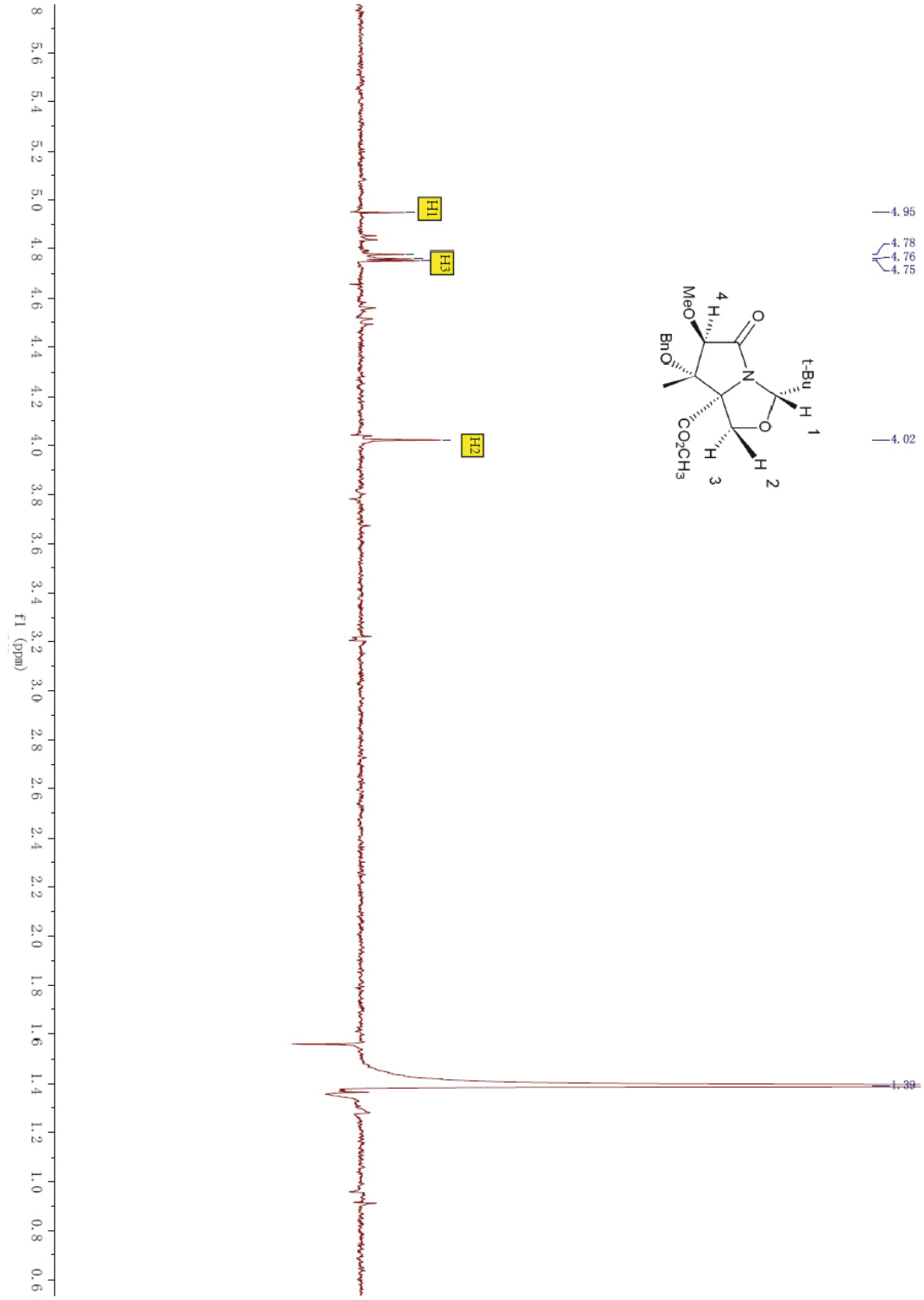
1. NOE spectrum of **3.47**



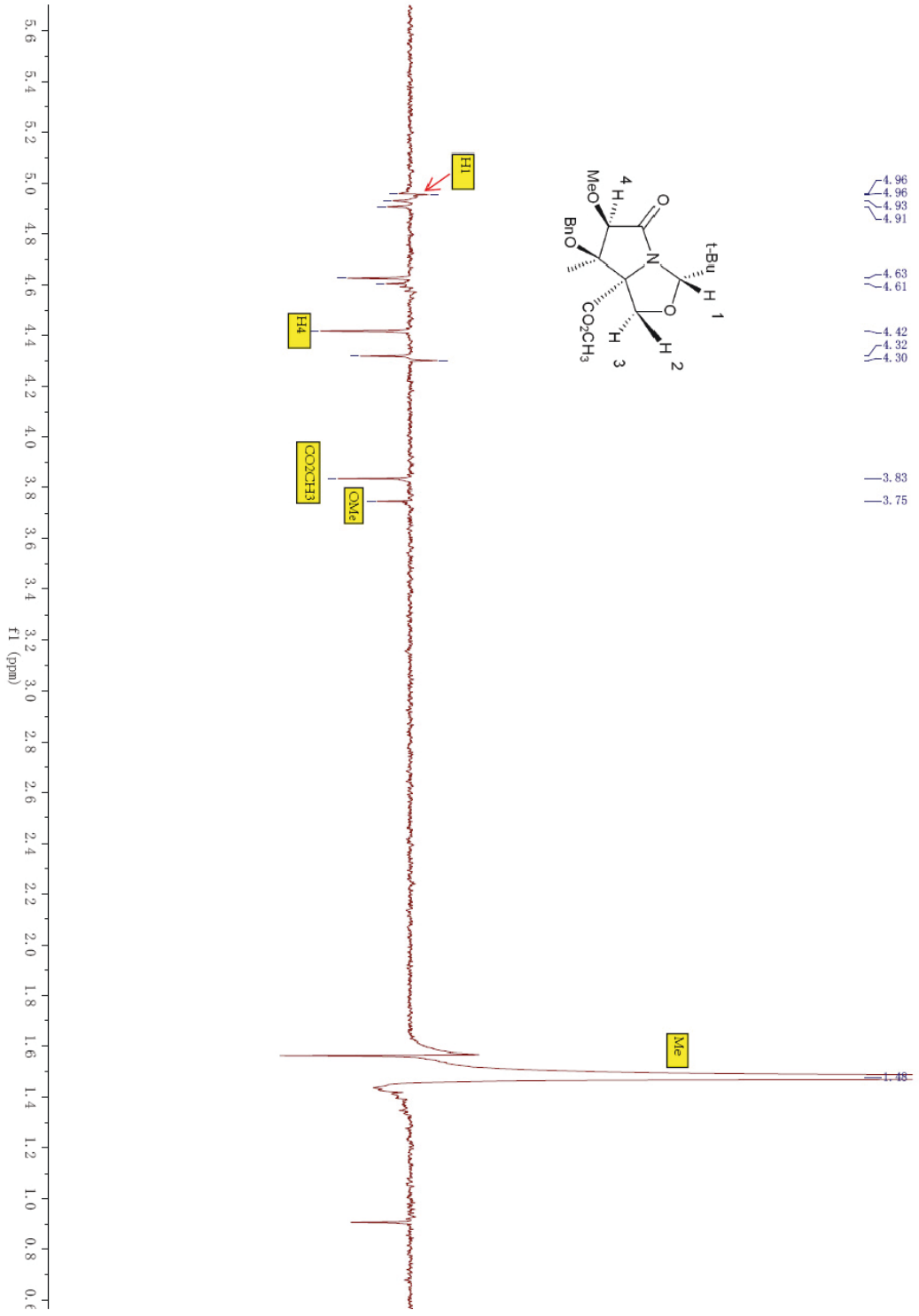
2. NOE spectrum of **3.65**



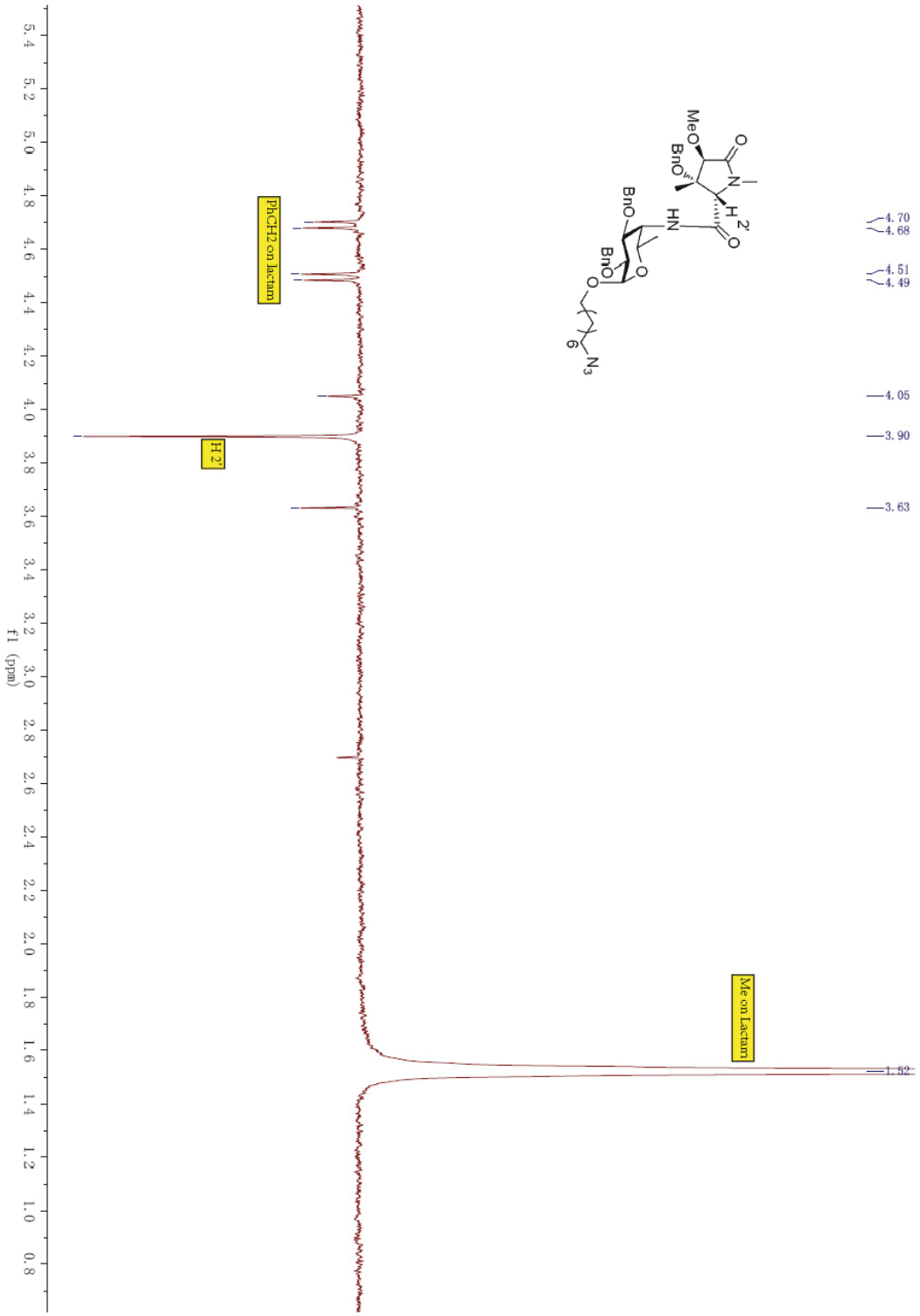
3. NOE spectrum of **3.69**



4. NOE spectrum of **3.70**



5. NOE spectrum of **3.86**



6. NOE spectrum of **3.87**