University of Alberta

Synthesis and Immunological Profiling of Mycobacterial
Phenolic Glycolipids Analogs

by

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Dedicated to my beloved family, beautiful wife

and my princess daughter Hana

Abstract

Mycobacterium tuberculosis, *M. leprae* and *M. kansasii* are three members of the mycobacteria family that cause serious bacterial infections in humans. Another member of the mycobacteria family is *M*. bovis that affects a range of animals, mainly cattle, and humans. These four bacteria share a common characteristic, which is a complex cell wall that is important for survival of the bacteria, virulence and pathogenesis. Of particular interest to this project is a family of non-covalently bound cell surface antigens known as phenolic glycolipids (PGLs). Despite all the work done on PGLs from *Mtb* and *M. leprae*, their immunological profile is yet to be determined. In order to achieve this goal, a panel of all PGLs from the four mycobacteria is needed. The difficulty of getting these molecules from the bacteria made their chemical synthesis crucial. To this end, a panel of 27 synthetic analogs to all PGLs was synthesized with *p*-methoxyphenyl group at the reducing end. With these compounds in hand, they were tested for their ability to modulate cytokine as well as nitric oxide release by human macrophages. The results revealed PGLs have immunoinhibitory activity on the release of IL-6, TNF-α, IL-1β, MCP-1 and NO. The inhibition pattern was the same as the native PGL-I and is related to the polymethylation pattern of the molecule. In addition, all monosaccharides were inactive and disaccharide structure was the minimum needed to have activity. Furthermore, adding a simple lipid core increased the activity, but not to the level of the native PGLs. This suggested that the native lipid core is needed for the receptor recognition.

To extend the scope of this study, different cell stimulants were used, LPS (TLR2/4 agonist), ultra pure LPS (TLR4 agonist) and Pam3CSK4 (TLR2 agonist) were used. The results of this testing suggested that these analogs are targeting TLR2 and not TLR4 as the immunoinhibitory pattern of these molecules were maintained upon using LPS and Pam3CSK4 as a stimulant. However, no effect was obtained when ultra pure LPS was used. Finally, when these molecules were tested for apoptosis, none of them showed any activity to induce apoptosis.

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List of Abbreviations

[α]	specific rotation
μM	micromolar
Ac	acetyl
AcOH	acetic acid
All	allyl
appt	apparent triplet (NMR spectra)
Ac_2O	acetic anhydride
AG	arabinogalactan
AIBN	2,2'-azobis(2-methylpropionitrile)
AM	arabinomannan
aq.	aqueous
Ara*f*	arabinofuranose
Bn	benzyl
br	broad
Bu	butyl
BI_3	boron triiodide
Bz	benzoyl
n-Bu_4NI	tetra-*n*-butylammonium iodide
calcd	calculated
COSY	correlation spectroscopy
°C	degree Celsius
CSA	(±)-camphor-10-sulfonic acid

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
d	doublet (NMR spectra)
dd	doublet of doublet (NMR spectra)
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*'-dimethylformamide
DMSO	dimethylsulfoxide
DCs	dendritic cells
DMP	2,3-dimethoxypropane
equiv.	equivalent
Et	ethyl
Et_3N	triethylamine
Gal*f*	galactofuranose
h	hour(s)
HOAc	acetic acid
Hz	hertz
HD	Hansen's disease
INH	isoniazid
IFN-γ	interferon-γ
J	coupling constant
LAM	lipoarabinomannan
LPS	lipopolysaccharides
mAG	mycolyl–arabinogalactan

MAPc	mAG–peptidoglycan complex
m	multiplet (NMR spectra)
M	molar
MAS	mycocerosic acid synthase
m/z	mass to charge ratio (mass spectrometry)
MDR	multi-drug resistant
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	millilitre(s)
mM	millimole(s)
Milli-Q	(deionized) distilled water
NIS	*N*-iodosuccinimide
NTM	non-tuberculosis mycobacteria
NMR	nuclear magnetic resonance
Ph	phenyl
PMA	phorbol 12-myristate 13-acetate
PKS	polyketide synthase
PI	phosphatidyl-*myo*-inositol
ppm	parts per million (NMR spectra)
p-TsOH	*p*-toluenesulfonic acid
Py	pyridine

PMPOH	*p*-methoxyphenol
*p*HBA	*p*-hydroxybenzoic acid
PGL(s)	phenolic glycolipid(s)
q	quartet (NMR spectra)
R_f	retention factor
RMP	rifampin
PIMs	phosphatidyl-*myo*-inositol mannosides
PDIMs	phthiocerol dimycocerosates
PMP	*p*-methoxyphenyl
rt	room temperature
SMAS	short-chain mycocerosic acid synthase
s	singlet (NMR spectra)
satd.	saturated
TB	tuberculosis
TMSOTf	trimethylsilyltrifluoromethanesulfonate
Tol	tolyl
t	triplet (in NMR)
TBAF	tetra-*n*-butylammonium fluoride
TEA	triethylamine
$(CH_3)_3SiH$	trimethylsilane
OTf	trifluoromethanesulfonyl
THF	tetrahydrofuran
TLC	thin layer chromatography

TMS	trimethylsilyl
Ts	tosyl, *p*-toluenesulfonyl
TBDPS	*tert*-butyldiphenylsilyl
WHO	World Health Organization
XDR	extreme-drug resistant

Chapter 1

Introduction to mycobacterial infections and cell wall structure

1.1. General aspects about mycobacterial infections

1.1.1. Tuberculosis

Tuberculosis (TB), also known as Koch's disease, remains one of the most contagious and deadly bacterial infections that affect many parts of the world. *Mycobacterium tuberculosis* is the causative organism of TB, which is a member of the mycobacteria complex family. However, in less frequent cases, TB can be caused by other mycobacterial species, such as *M. bovis, M. africanum*, and *M. microti.*[1] With about fifty million new cases every year and claiming about 2–3 million lives, including 456,000 HIV – positive cases, TB remains one of the top ten global leading causes of death, more than any other bacterial pathogen.[2-5]

Tuberculosis is a highly infectious disease that is transmitted via aerosols that contain pathogenic bacteria through coughing, sneezing, or spitting. However, not all infected persons will develop active disease. Only 10% of infected individuals develop active TB, while about 90% are able to contain the infection due to an adequate immune response.[6] This immune response will lead to formation of granulomas containing mainly infected macrophages and lymphocytes. This leads to a state of dormancy where the infected individual will show no symptoms of TB, but is still infectious to others. This state of dormancy will remain as long as the balance between the host immune system and the bacterial burden is maintained. Once this balance is shifted to the pathogen,

especially in the case of immunocompromised patients (*e.g.,* patients with AIDS), the bacilli will be reactivated leading to an active disease.[7]

Tuberculosis can be classified into two main categories: pulmonary TB and extrapulmonary TB. In the case of pulmonary TB, *M. tuberculosis* attacks the respiratory system and the clinical manifestation includes prolonged and productive cough for more than three weeks. The patient coughs up blood and has chest pain. Also, some other symptoms will develop including fatigue, appetite and weight loss, night sweats, fever, chills, and pallor. In case of extrapulmonary TB, *M. tuberculosis* affects the central nervous system, genitourinary system, lymphatic system, and other major organs.[8]

In addition to the highly infectious nature of TB and co-infections with HIV, there are other characteristics that make TB even more deadly and that complicate its treatment. Amongst these characteristics is the emergence of multidrug-resistant strains (MDR-TB) and extremely-drug resistant strains (XDR-TB) of *M. tuberculosis*. MDR-TB are resistant to at least isoniazid (INH) and rifampin (RMP), the most potent first-line TB drugs,[7c] while XDR-TB is additionally resistant to fluoroquinolones and one of the injectable second line drugs including amikacin, kanamycin, and capreomycin.[9] The emergence of MDR-TB and XDR-TB has been attributed to either the improper use of antibiotics or incomplete treatment regimens, which requires a long time, usually about six months.[7c,7d,9] This drug resistance can also be due to spontaneous and random mutations of the bacterial genome, which lead to reduced susceptibility to certain drugs.[10]

Another characteristic is the ability of *M. tuberculosis* to invade and neutralize the sophisticated antimicrobial arsenal of alveolar macrophages that represent the first line of defense of the immune system.[11] This ability is believed to be due, in part, to unique cell wall components that help *M. tuberculosis* to invade macrophages via reorganization of membrane lipid, and control the phagosomal pH rendering macrophages as a protective niche for the bacilli.[12] In addition to this mechanism, there are other cell wall components that help *M. tuberculosis* suppress cytokine-induced apoptosis of the macrophage, as well as the action of other macrophage-mediated antimicrobial mechanisms. These cell wall components also form a shield that protects the bacilli against antimicrobial agents, which will be discussed in detail later in this chapter.

1.1.2. Leprosy

Leprosy, also known as Hansen's disease (HD), is another example of a deadly bacterial infection caused by a different member of the mycobacteria family, *M. leprae.*[13] The main site of attack by the organism is the skin and the peripheral nervous system; however, the clinical manifestations of HD depend on which form of the disease the patient has. Patients with the tuberculoid from of HD are relatively resistant to the pathogen and the infection is localized.[14,15] On the other hand, for patients with the lepromatous form of HD, the disease is aggressive and may lead to diffuse dermal lesions, neurologic disabilities, and nerve deformities.[1,13,16] HD is one of the leading causes of non-traumatic peripheral neuropathies causing about 500,000 new cases every year worldwide

and there are about 2–3 million current cases of individuals suffering from neurological disabilities.[17] The difficulty of treating leprosy is, again, mainly attributed to the complex nature of the cell envelope that acts as a protective shield preventing antimicrobial agents from affecting the bacilli.

1.1.3. Infection caused by *Mycobacterium kansasii.*

M. kansasii is a member of non-tuberculosis mycobacteria (NTM)[18] and is isolated mainly from tap water.[19] *M. kansasii* can cause pulmonary disease in immunocompetent patients, and pulmonary and extrapulmonary disease in immunocompromised patients.[20] *M. kansasii*-induced pneumonia shares the same clinical manifestations with lung tuberculosis, which has led to the suggestion that both illnesses share similarities in pathogenesis, and may be caused by similar virulence factors.[20] Again, the same difficulties are faced upon treatment.

I have mentioned three different bacterial infections caused by three different causative organisms belonging to the mycobacteria complex family. All share the same difficulties in their eradication, mainly due to the complex architecture of the cell wall of the bacilli. This complexity provides a protective shield that protects the bacilli from the reach of antimicrobial agents. As well, some components of the cell wall help the bacteria to modulate the immune response of the host, this allowing its survival. For this reason, I will be discussing the composition of the cell wall in the next section.

1.2. Mycobacterial cell wall composition

The complex architecture of the cell envelope of mycobacteria, which is highly abundant in lipids and constitutes about 40% of the dry weight of the bacteria,[21] makes it a major determinant of virulence and an important player in the process of pathogenesis.[22] This remarkably unique architecture provides the bacteria with an impermeable and protective barrier against any hostile environments, antimicrobial agents, as well as against the host immune system.[23] Moreover, this complexity is also the reason the treatment of drug-susceptible TB takes a long period of time and requires combination antibiotic therapy.[24] In addition, it has been shown that the outcome of mycobacterial infection is greatly dependent on the invasion, internalization and colonization of macrophages upon the initial contact of the bacilli with the alveolar macrophages. The internalization process of the bacilli inside macrophages is dependant on recognition of certain components of the cell wall through specific receptors on macrophage surfaces.[25,26]

The cell envelope is composed of two major components; the mycolyl–arabinogalactan (mAG) complex and lipoarabinomannan (LAM) (Figure 1-1).[27-29] The mAG is the largest structural component of the cell envelope and is covalently linked to peptidoglycan forming the mAG–peptidoglycan complex (MAPc), which is considered to be the cell-wall core.[30] The impermeability of mycobacteria to antibiotics has largely been attributed to the MAPc.[27-29] The second largest component is LAM, which has been shown to be highly antigenic and plays an important immunomodulatory role that suppresses the immune

response against the bacteria.[31,32] In this chapter, the structures of both the mAG complex and LAM will be discussed briefly; however, the main focus will be on one family of glycolipids known as phenolic glycolipids (PGLs) which are intercalated at the periphery of the cell wall and is one of the important cell surface antigens.

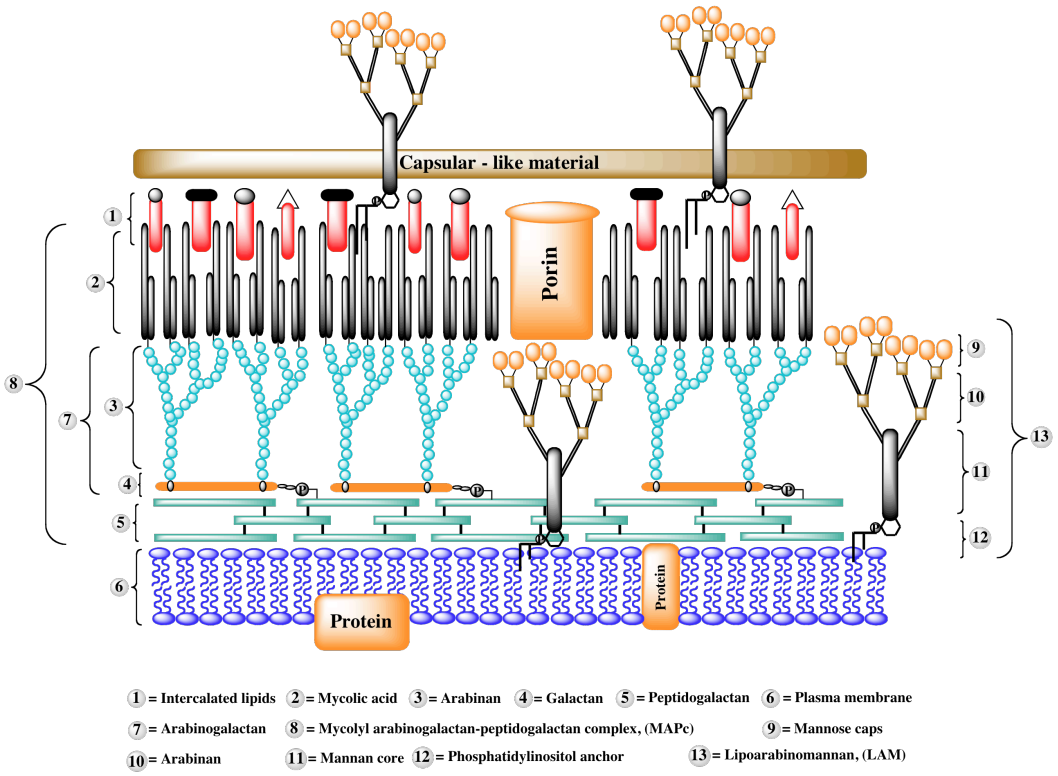


Figure 1-1. Schematic representation of the mycobacterial cell envelope.

1.2.1. Mycolyl–Arabinogalactan Complex (mAG)

Because the mycobacterial cell wall provides an important target for the development of new antimicrobial agents, work to define the structure of the cell wall started in the 1960s and 1970s.[32] The extensive work in this area revealed that the mycolyl–arabinogalactan (mAG) glycoconjugate is the largest structural

component of the cell envelope constituting about 35% of the total weight of the cell wall. The core structure of the mAG is composed of sugars in the five-membered furanose ring configuration, unlike most other bacterial polysaccharides. D-Arabinofuransoe (Ara*f*) and D-galactofuranose (Gal*f*) residues constitute the arabinan and galactan subunits, respectively (Figure 1-2).[33]

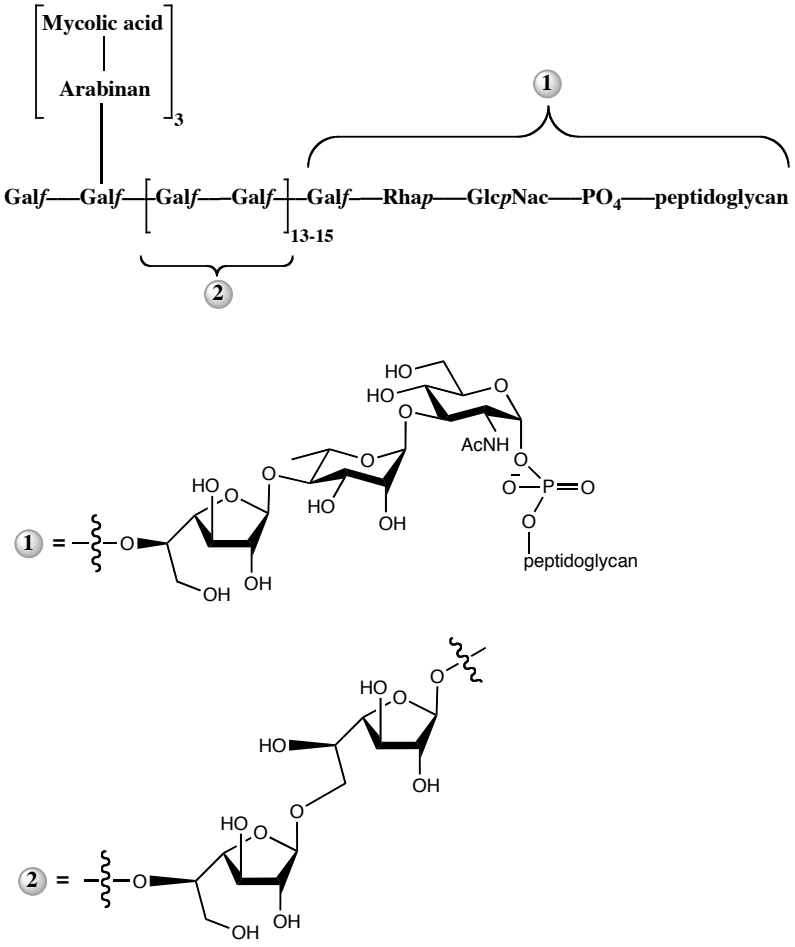


Figure 1-2. Structure of the mAG complex.

The peptidoglycan complex is linked to the galactan polymer through a rhamnose–*N*-acetylglucosamine disaccharide phosphate bridge. The arabinan motifs are esterified with mycolic acids at their non-reducing ends and the

mycolyl-arabinan domains are attached to Gal*f* residues 8, 10, and 12 of the galactan.

As shown in Figure 1-2, the linear galactan polymer consists of 30 to 35 alternating β-(1→5) and β-(1→6) linked D-Gal*f* residues. The galactan is covalently attached to the peptidoglycan polymer through a disaccharide phosphate linker, (α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-P).[33,34] This linear polymer is further branched at O-5 of Gal*f* residues 8, 10, and 12 with three identical arabinan units (averaging 31 Ara*f* residues each).[35,36]

The arabinan moiety of both AG and LAM is composed of α-(1→5)-linked Ara*f* residues with several α-(1→3)-linked Ara*f* motifs as branching points, as well as β-(1→2)–Ara*f* motifs that terminate the arabinan chains (Figure 1-3).[37] The non-reducing termini of the mAG-arabinan is composed of Ara6 (a branched hexasaccahride) domain,[38] which are the esterified with mycolic acids. Approximately two-thirds of the Ara6 motifs are mycolated while the remaining one-third is unmodified.

In the mycobacterial cell wall, three distinct classes of mycolic acids are found. They are the α-, methoxy- and ketomycolic acid (Figure 1-4).[39] α-Mycolic acids constitutes more than 70% of the mycolic acids content. Structural variations among the different mycolic acids is strain-dependent. These structural variations include the length of the terminal alkyl chain, the number of methylene groups between the cyclopropyl rings and the carboxyl group and presence of *cis*- or *trans*-cyclopropyl ring.[40]

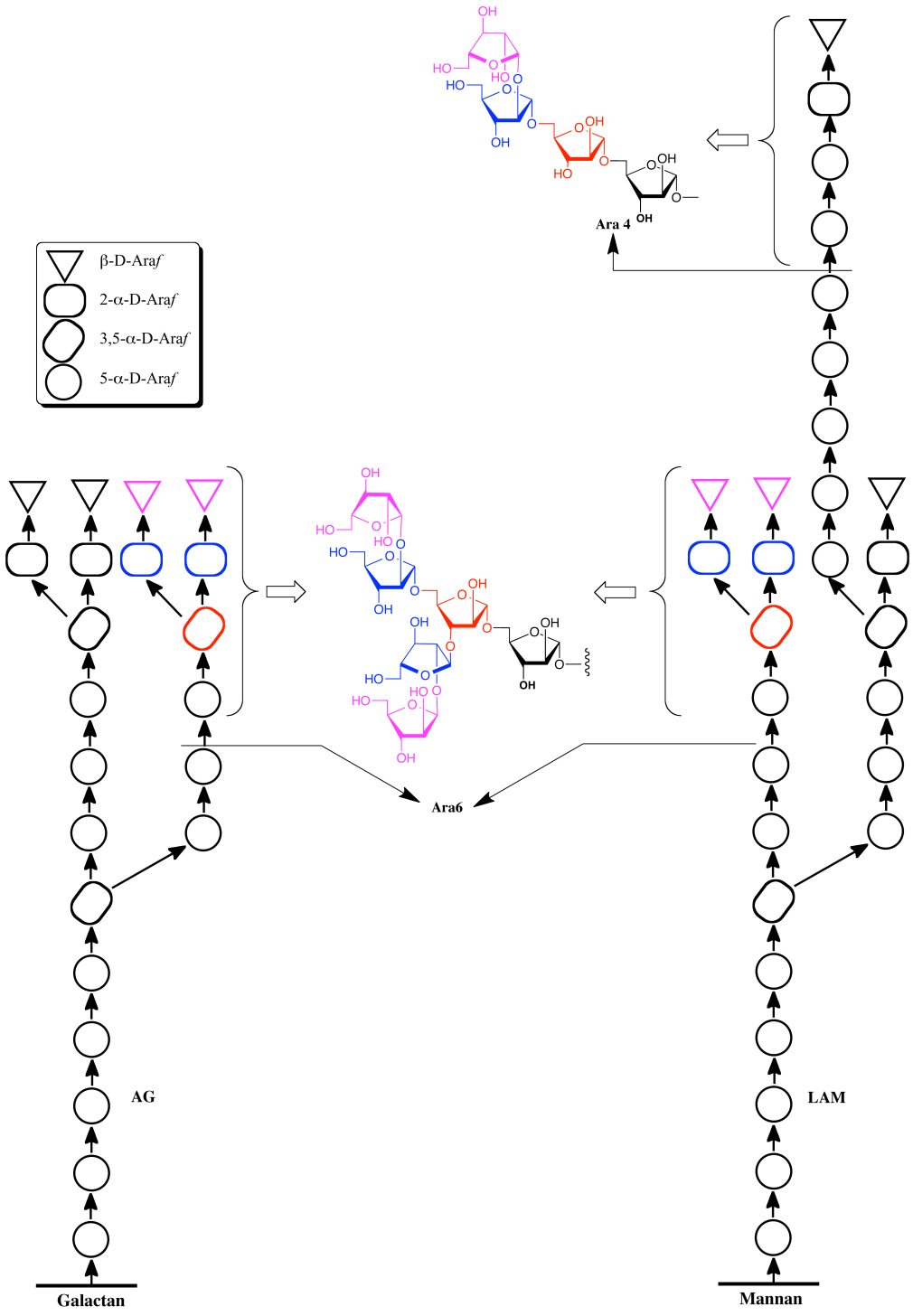


Figure 1-3. Structure of arabinans in AG and LAM.

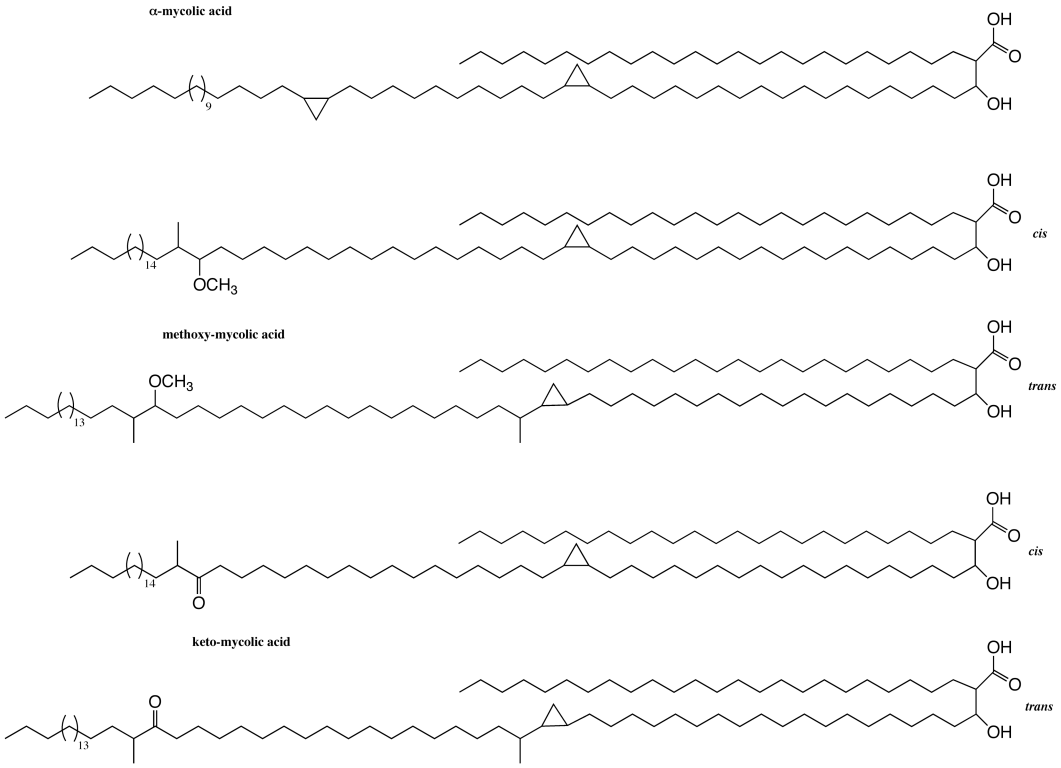


Figure 1-4. Chemical structures of mycolic acids.

1.2.2. Lipoarabinomannan (LAM)

The mycobacterial surface glycolipid LAM contains four main structural components (Figure 1-5): a phosphatidyl-*myo*-inositol (PI) anchor, a core mannan domain (composed of an α-(1→6)-linked main chain with α-(1→2) or sometimes α-(1→3)-linked side chain), a branched arabinan domain and a capping motif at the non-reducing end of the molecule. Unlike the mAG, the linear tetrasaccharide Ara4 is found at the non-reducing end. Also, instead of having mycolic acid as capping motifs, in LAM, α-Man*p* residues, inositol phosphate motifs and 5-thiomethyl-xylofuranose residues may be found. The nature of the capping motifs is species-dependent.[41] The major lipoglycans of the mycobacterial cell wall are

composed of the LAM together with its truncated analogues, lipomannan (LM) the phosphatidyl-*myo*-inositol mannosides (PIMs).[27,42a-d]

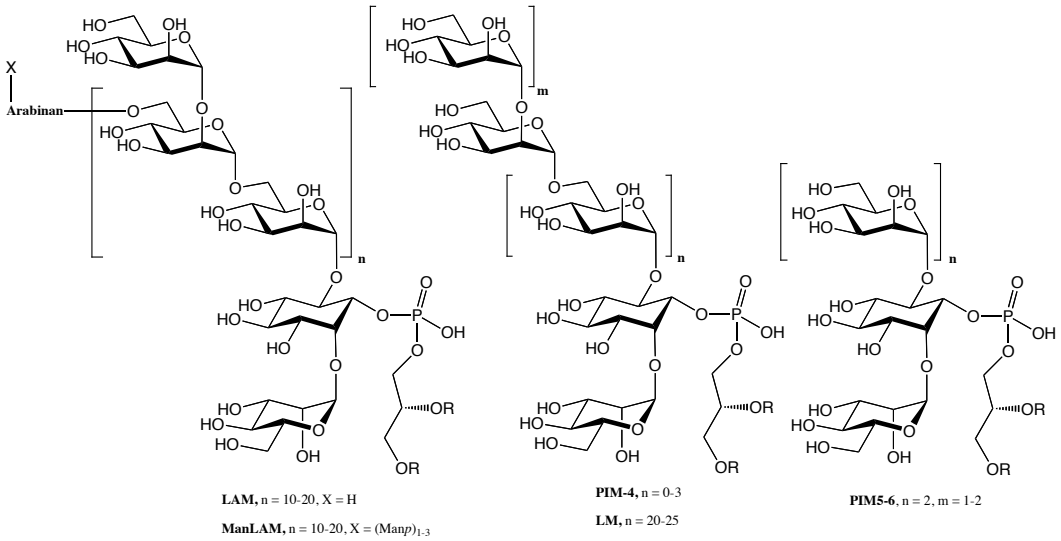


Figure 1-5. Structures of lipoarabinomannan (LAM), lipomannan (LM) and phosphatidyl-*myo*-inositol mannosides (PIMs). R = stearate, palmitate and/or tuberculostearate.

1.2.3. Peptidoglycan

The peptidoglycan is found just outside the plasma membrane. It is linked to the mAG through a phosphate disaccharide bridge (α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-P). This structural component is composed of chains of polysaccharides, which are made of alternating units of *N*-acetylglucosamine and *N*-glycolylmumaric acid residues. A tetrapeptide motif, composed of diaminopimelic acid, D-glutamic acid, L-alanine and D-alanine, esterify the O-3 position of the *N*-glycolylmumaric acid. These peptides on every two chains of the polysaccharide are cross-linked.[43-46]

1.2.4. Cell-surface extractable lipids

In addition to the large structural components of the cell wall, there are other smaller surface glycolipids intercalated between the mycolic acid chains, forming an asymmetric lipid bilayer. These compounds have diverse structures that are often species-specific. Examples of these compounds are glycopeptidolipids (GPLs), lipooligosaccharides (LOS) like 2,3-di-*O*-acyltrehalose and phenolic glycolipids (PGLs). Because GPLs and LOS are not relevant to this thesis, they will not be addressed any further; however, interesting reviews about these structures have been published[47,48] as well as the aforementioned three components.[49] The main focus of this chapter will be on one class of the cell-surface extractable glycolipids, the PGLs, as this class of compounds is the main target of my thesis.

1.3. Phenolic Glycolipids (PGLs)

1.3.1 Discovery of PGLs

In 1954, when using IR spectroscopy to study the lipid extracts of about 70 mycobacterial strains, Smith and coworkers placed the first brick in the history of PGLs, which are polyketide synthase-derived virulence factors.[50] It was discovered that these extracts contained lipids that have a phenolic moiety and were unique to a given mycobacterial species. These lipids were named "mycosides" and were thought to be limited to one species; however, after a short period of time, mycosides were also isolated from *M. bovis, M. kansasii*[51] and *M. marinum*.[52] After a period of negligence, PGLs were brought under the spot light

again in 1981 when Brennan and coworkers were able to isolate PGL-I from the *M. leprae*-infected liver tissue of armadillos (Figure 1-6).[53-57]

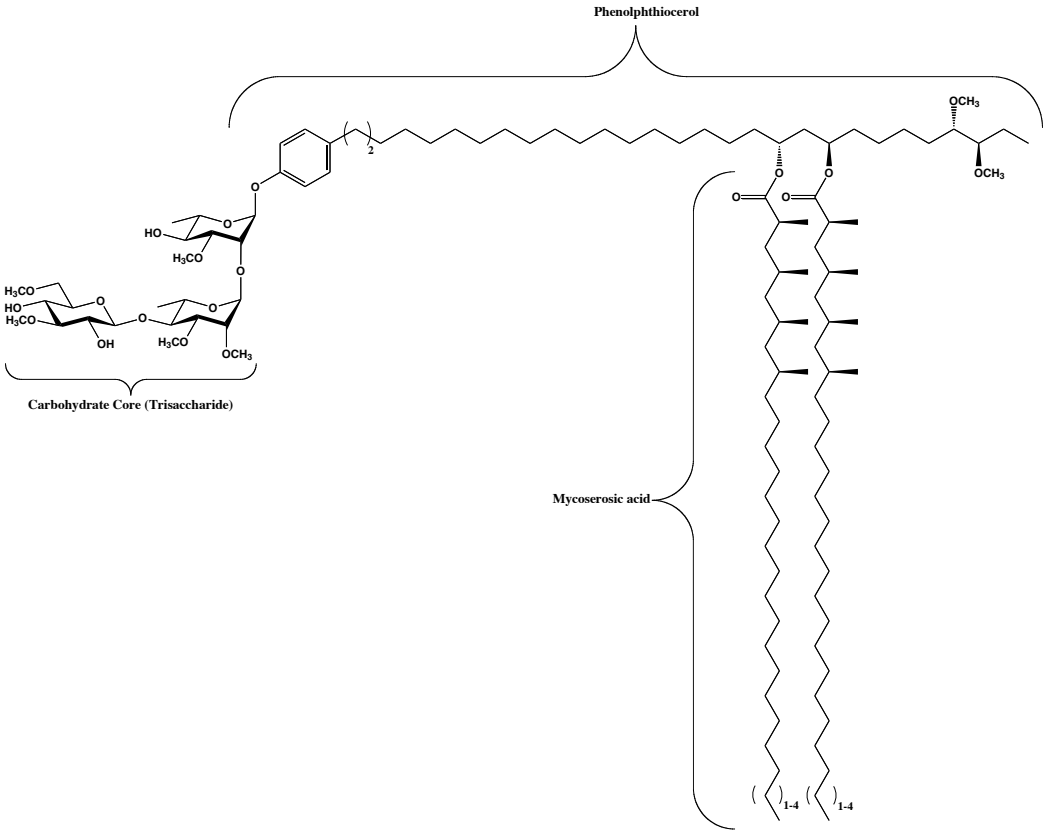


Figure 1-6. Structure of PGL-I from *M. leprae.*

After this discovery, and due to the advances in analytical methods such as NMR spectroscopy and mass spectrometry, the structures of PGLs from *M. bovis,*[58] *M. kansasii*[59] and *M. tuberculosis*[60] were elucidated. This combined structural work showed that the carbohydrate domain of PGLs is species-specific while the lipid core is conserved between species.

1.3.2. Structure and distribution of PGLs

The term PGL refers to molecules containing the phenol phthiocerol and related compounds collectively. These compounds have two main structural components: a long chain β-diol, terminated with a phenol ring, esterified on both aliphatic hydroxyl groups with mycocerosic acid (Figure 1-7), and a carbohydrate moiety. The phenol ring is glycosylated with either a mono-, di-, tri- or tetrasaccharide, depending on the species of origin. The presence of the glycosylated phenol ring differentiate PGLs from another class of compounds know as phthiocerol dimycocerosates (PDIMs) (Figure 1-7).

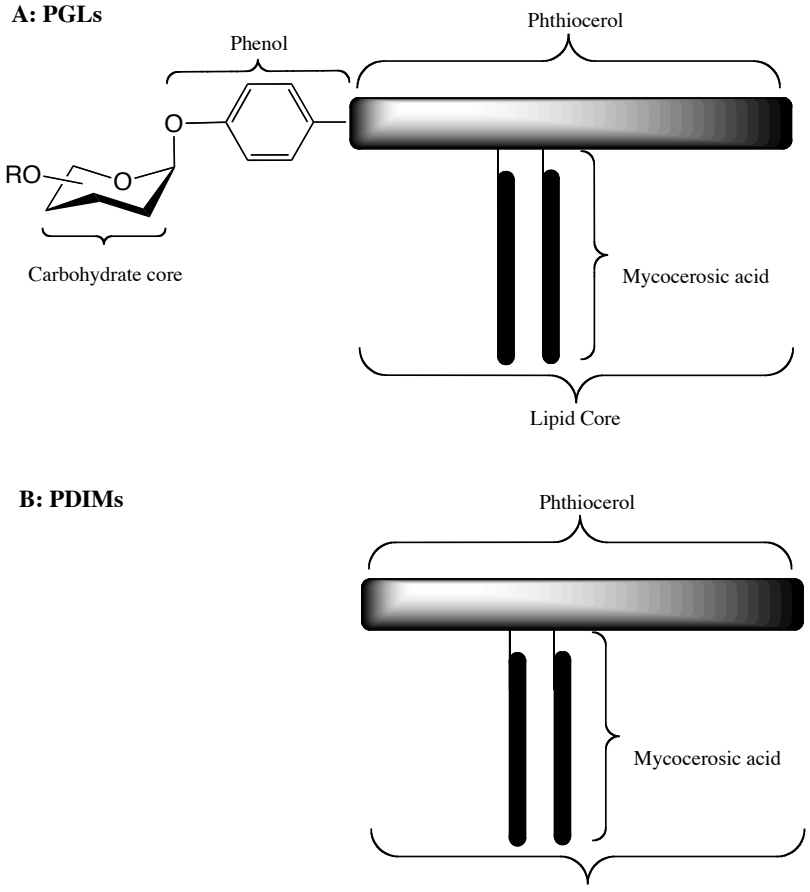
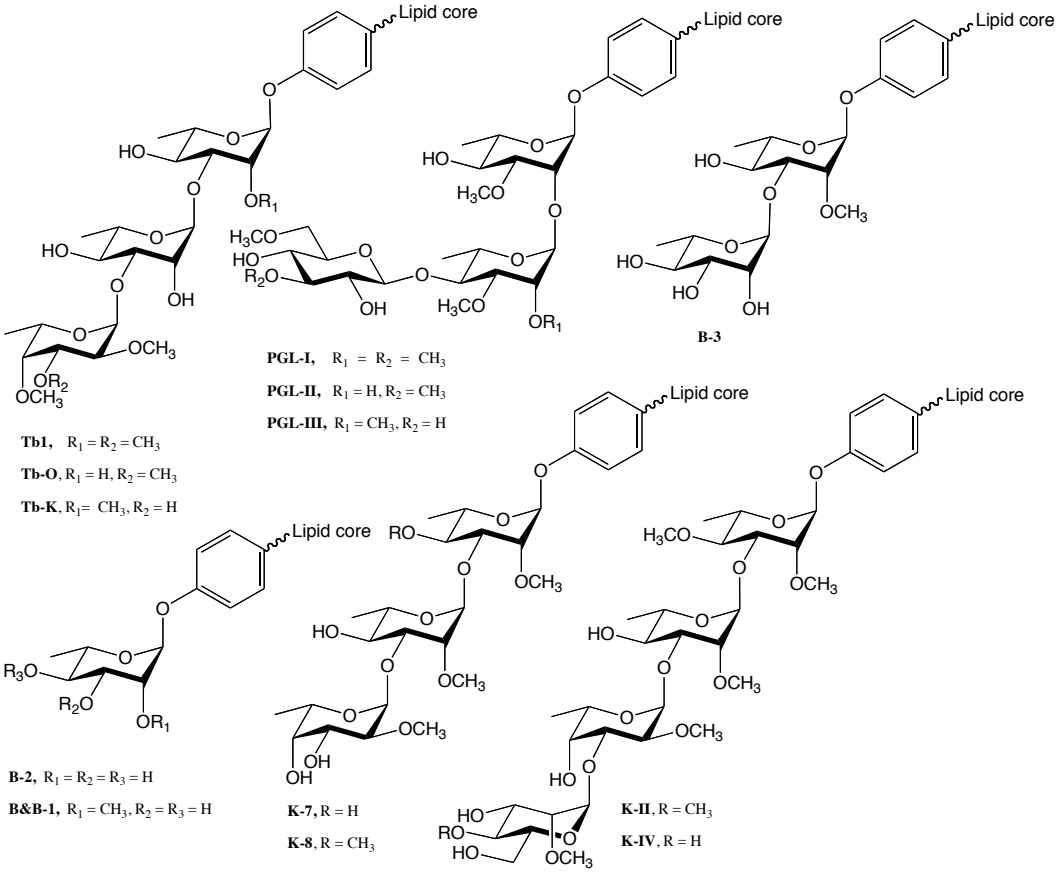


Figure 1-7. Schematic representation of PGLs and PDIMs.

As mentioned above (section 1.3.1.), the length and structure of the carbohydrate moiety is species-specific; however, multiple different PGLs can be found in a single strain. In *M. tuberculosis,* there are three PGLs isolated: Tb-1, Tb-O and Tb-K,[60-62] whereas another three PGLs have been isolated from *M. leprae*: PGL-I, PGL-II, and PGL-III.[61] Moreover, seven PGLs have been isolated from *M. kansasii*: K-I, K-II, K-IV, K-5, K-6, K-7 and K-8;[59] another four from *M. bovis:* B, B-1, B-2 and B-3 (Figure 1-8).[58]



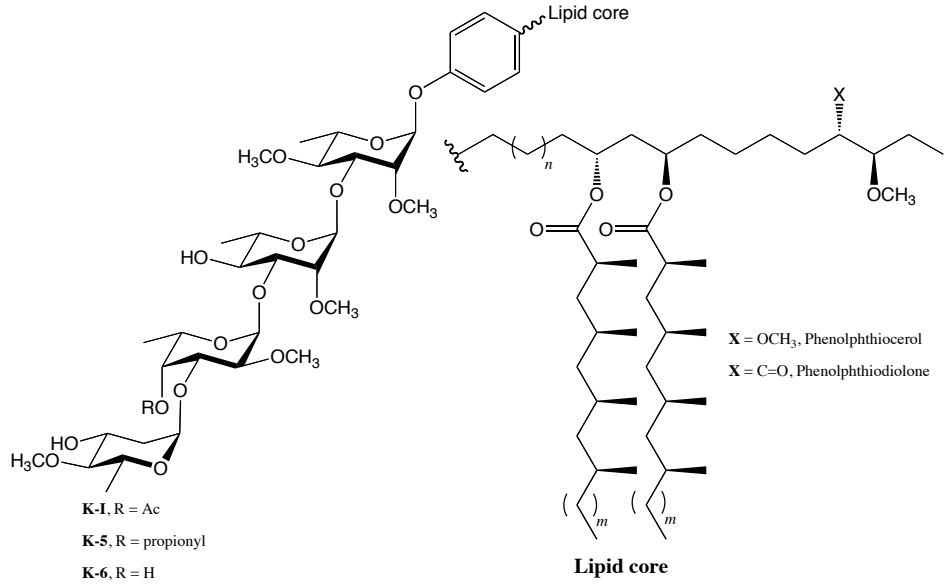


Figure 1-8. Structures of PGLs isolated from *M. tuberculosis* (n = 12–16 and m = 16–20)*, M. leprae* (n = 16 and m = 16–20)*, M. bovis leprae* (n = 12–16 and m = 16–20) and *M. kansasii leprae* (n = 14–18 and m = 16–18)*.* In case of B-1 and B-2, the lipid core is phenolphthiodiolone.[83]

It is worth mentioning that in case of *M. bovis,* the PGLs are not only different in the carbohydrate core, but also in the lipid domain. In case of B-1 and B-2, the lipid domain is phenolphthiodiolone, whereas in B and B-3, the lipid core is phenolphthiocerol.[63] Phenolthiodiolone is similar in structure to phenolphthiocerol, however, it has a carbonyl group at C4 instead of a OCH_3 group.

Considering the species distribution of PGLs and PDIM, it has been shown that both are unique to pathogenic mycobacteria.[64] However, is has also

been demonstrated that unlike PDIMs, PGLs are not found in all strains of *M. tuberculosis* complex; in fact, many strains of *M. tuberculosis* produce no PGLs.[65] Unlike *M. tuberculosis,* all strains of *M. leprae* produce PGLs.[54,55] Despite this difference, both species share the same features where multiple different PGLs can be found within a single strain. For example, Reed and coworkers recently showed that only hypervirulent strains of *M. tuberculosis* produce PGLs; be vide infra.[66]

1.3.3. Biosynthesis of PGLs

1.3.3.1. Biosynthesis of the lipid core

The lipid core of PGLs has two main components: mycocerosic acid and phenolphthiocerol. The biosynthesis of these species will be discussed in the following section.

1.3.3.1.1. Biosynthesis of mycocerosic acid

Unlike the biosynthesis of *n*-fatty acids, which utilizes malonyl–CoA, the biosynthesis of multiple methyl-branched fatty acids such as mycocerosic acid depends on the use of methylmalonyl–CoA as the precursor. In vertebrates, fatty acid synthase I (FAS I) is capable of synthesizing of 2,4,6,8-tetramethyl fatty acids from methylmalonyl–CoA by elongation of a shorter primer (C_2 or C_3).[67] This ability is due to the presence of a malonyl–CoA decarboxylase, which ensures that only methylmalonyl–CoA is available to FAS I, and hence prevents the formation of mixed products. However, the structures of the multi-methyl-

branched fatty acids in mycobacteria suggest another system is present that is specialized for their biosynthesis.

A leap forward in elucidating the biosynthetic pathway was achieved when Rainwater and Kolattukudy completed a cell free synthesis of mycocerosic acid from C_{20}-CoA and methylmalonyl–CoA, and were able to purify the enzyme that catalyzes this process from *M. bovis*.[67,68] This unique polyketide synthase (PKS) termed mycocerosic acid synthase (MAS) was found to be a multifunctional synthase, like FAS I, capable of producing C_{27}–C_{29} mycocerosic acids (Figure 1-9).

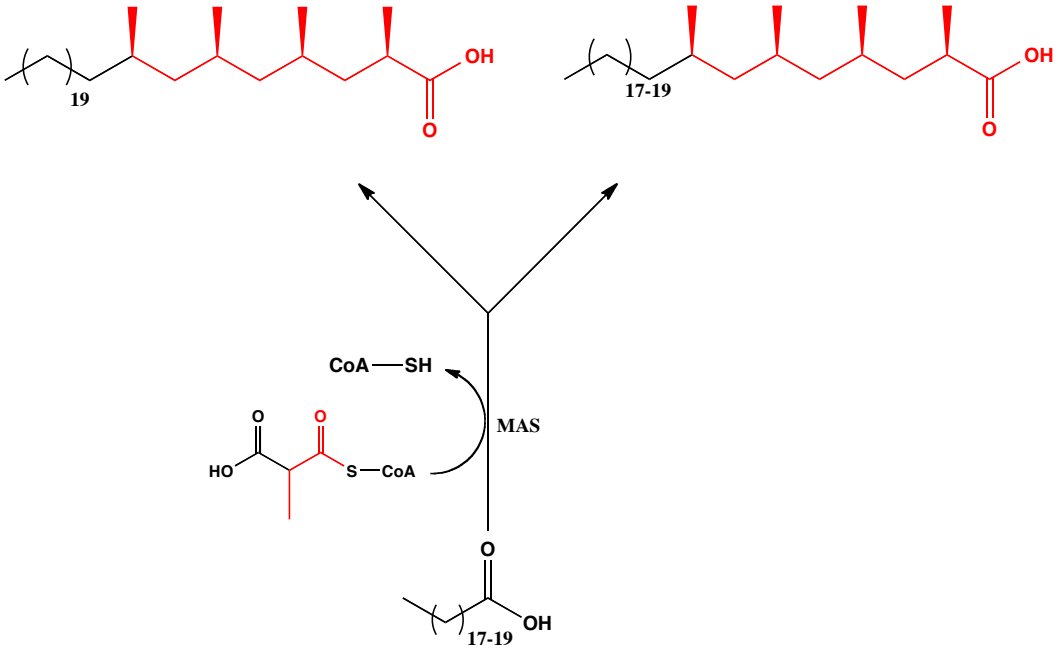


Figure 1-9. Postulated pathway for the synthesis of mycocerosic acid using MAS.

Subsequently, sequencing of the gene encoding for MAS was completed and the domain organization was found to be similar to that used for other polyketide natural products with KS–AT–DH–ER–KR–ACP sequence (KS =

ketosynthase, AT = acyltransferase, DH = dehydratase, ER = Enoylreductase, KR = ketoreductase, ACP = acyl carrier protein).[69] Interestingly, when both the AT and KS domains were overexpressed in *E. coli* and *M. smegmatis*, both naturally lacking the presence of MAS, they were only able to produce multiple methyl-branched fatty acids, and no linear fatty acids. This finding indicates that the two domains are selective for methylmalonyl-CoA.[70] Another unique characteristic of MAS is that it lacks a thioesterase domain, which means that the synthesized mycocerosic acids are not released from the enzyme. A number of recent studies have established the involvement of another acyl transferase (FadD28), which transfers the mycocerosic acid to phthiocerol or phenolphthiocerol (Figure 1-10).[71] As shown in Figure 1-10, there is another gene cluster found downstream to *mas* and known as *ppsA-E*, which is responsible for the synthesis of the phthiocerol and phenolthiocerol moiety and will be discussed later. Also, it shows other genes that are responsible for important proteins that are involved in the biosynthesis of phthiocerol and phenolphthiocerol, such as *fadD28* and *mmpL7* (see below).

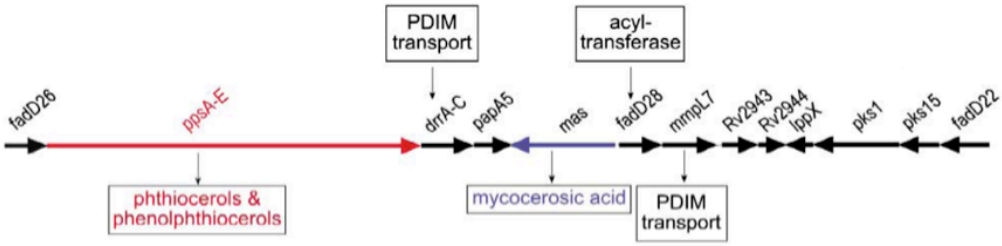


Figure 1-10. The genetic organization of the open reading frames responsible for the production and transportation of PGLs and PDIMS.

An additional form of MAS is present in *M. bovis*, and termed a short-chain mycocerosic acid synthase (SMAS), which is capable of synthesizing truncated forms of mycocerosic acid and releases the free products without the need of an acyl transferase.[72] In another study, Azad and coworkers demonstrated that *M. bovis* mutant strains, in which the *mas* gene is disrupted, loses the ability to synthesize either mycocerosic acid or mycosides.[73]

1.3.3.1.2. Biosynthesis of phthiocerol and phenolphthiocerol

Metabolic labelling studies using ^{14}C-labeled acetate and propionate demonstrated that both substrates are incorporated into the phthiocerol and phenolthiocerol structures.[74] This finding suggested that malonyl-CoA and methylmalonyl-CoA are both involved in the synthesis of the long aliphatic chain; however, at that time the enzymes that catalyze this process were not known. Fortunately, gene knockout experiments by Azad and coworkers allowed them to identify a gene cluster (*pps*) (Figure 1-10), which encodes a group of multifunctional polyketide synthase enzymes that catalyze the synthesis of phthiocerol and phenolphthiocerol from C22–C24 fatty acids and *p*-hydroxyphenylalkanoic acids, respectively.[67,74-77]

As described in Figure 1-11, the biosynthesis catalyzed by these Pps enzymes starts with three rounds of elongation with malonyl-CoA followed by reduction. Subsequently, there are two rounds of elongation with methylmalonyl-CoA followed by reduction. Finally, three tailoring steps of decarboxylation,

reduction and methylation complete the biosynthesis of phthiocerol or phenol-phthiocerol. The products of this biosynthesis are then diesterified with two molecules of mycocerosic acid through the action of the acyl transferase FadD28. The resulting PDIMS and PGLs are transported to the cell envelope via a transmembrane protein mmpL7.

This group of Pps enzymes were termed Pps1–5 in *M. bovis* and PpsA–E in *M. tuberculosis*. In the same study, they were able to establish that this *pps* gene cluster was conserved in the two most pathogenic species of mycobacteria: *M. tuberculosis* and *M. leprae.* Moreover, they demonstrated that disruption of this gene cluster abolished the synthesis of both phthiocerol and phenolphthiocerol related compounds. In the case of phenolphthiocerol, the biosynthesis of the precursor *p*-hydroxyphenylalkanoic acid involves a series of eight/nine successive elongation of *p*-hydroxybenzoic acid (*p*HBA) with malonyl-CoA by the Pks 15/1 enzyme.[65] To confirm the source of the *p*-hydroxyphenyl moiety in phenolphthiocerol is *p*-hydroxybenzoic acid, a metabolic feeding experiment with ^{14}C-labeled *p*-HBA was conducted resulting in PGLs that were radioactive, which confirmed this hypothesis.[78] While there is debate over the source of *p*HBA, Kolattukudy and coworkers had suggested *p*-hydroxybenzoyl-CoA as the source.[75,79]

In addition to the hypothesized biosynthetic pathway, in a recent study, Gokhale and coworkers discovered a new enzyme known as fatty acyl-AMP ligase (FAAL), which is involved in mycocerosic acid preactivation in *M. tuberculosis*.[80] This enzyme catalyzes the activation of fatty acid precursors via

conversion to the corresponding adenylate intermediates. Subsequently, the FAAL-produced adenylates are used by the polyketide synthase enzyme systems, MAS or Pps, in the synthesis of complex lipids such as PDIMS, sulfolipids and mycolic acids. However, they did not provide a hypothesis for how this enzymatic system evolved and whether or not this type of enzymatic activation is a general mechanism in the mycobacterial family.

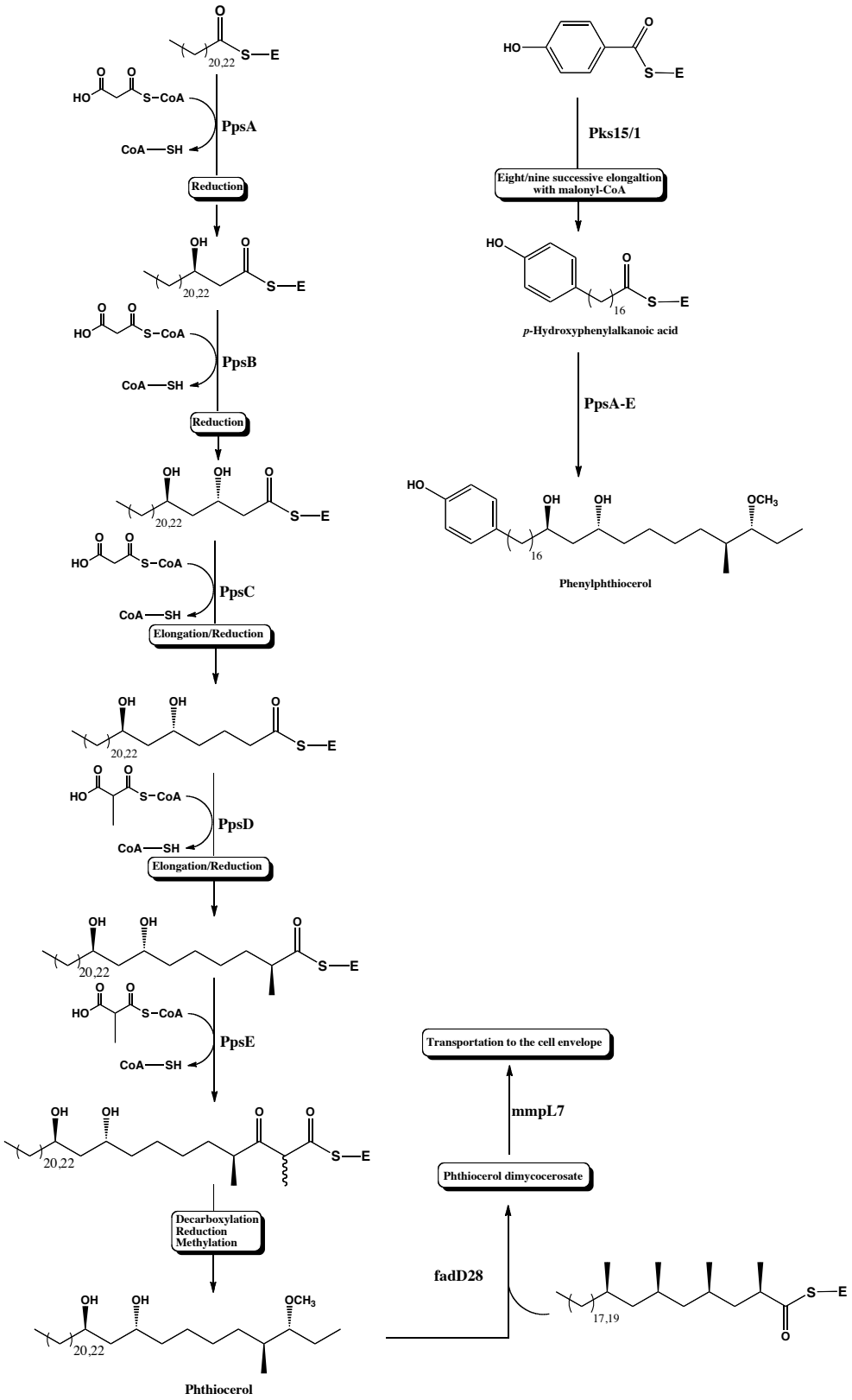


Figure 1-11. Postulated biosynthesis pathway of phthiocerol and phenolphthiocerol.

1.3.3.1.3. Biosynthesis of the carbohydrate core of PGLs

Despite the progress in elucidation of the biosynthesis of the lipid core of PGLs, the biosynthesis of the carbohydrate core structures still remain largely unknown. Guilhot and coworkers were able to identify a 70 kb chromosomal fragment that encodes for three putative glycosyltransferases, Rv2957, Rv2958c and Rv2962c, involved in the biosynthesis of PGL-tb and *p*-HBAD in *M. tuberculosis* (Figure 1-12).[81] To study the functions of these three gene products, they constructed mutant strains in which each of the three genes were inactivated one at a time. The results of this study established that Rv2962c catalyzes the transfer of the first rhamnosyl moiety to either *p*-hydroxybenzoic acid methyl ester or to phenolphthiocerol, while Rv2958c and Rv2957 catalyze the transfer of the second rhamnosyl and the fucosyl moieties, respectively.

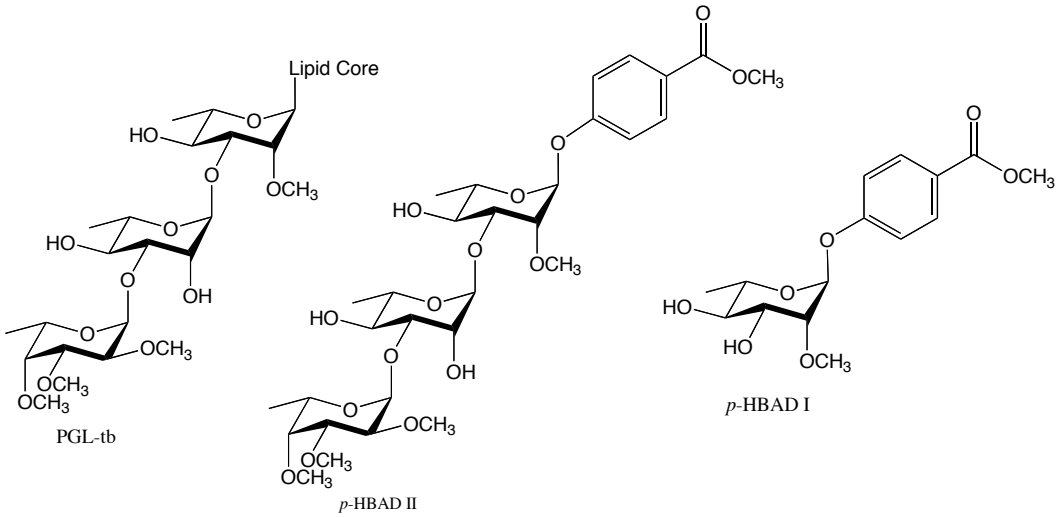


Figure 1-12. Structures of PGL-tb, *p*-HBAD I and *p*-HBAD II.

The timing of glycosylation during the assembly of PGLs is still not clear. Draper and coworkers have shown that glycosylation can take place on

phenolphthiocerol. This suggests that the glycosylation takes place as a final step in PGL biosynthesis.[82] On the other hand, Guilhot and coworkers have suggested that the glycosylation occurs at an earlier stage of the biosynthesis, taking the presence of glycosylated *p*-HBAD in the medium as an evidence of this hypothesis.[65] It is worth mentioning that the presentation of PGLs on the cell envelope was completely blocked in mutant strains that have the *mmpL7* gene, which encodes a transmembrane protein that transfers the phthiocerol and phenolphthiocerol to the cell envelope, inactivated. However, the synthesis of PGLs in the same strains was not affected.[64a,66] This finding indicates that the glycosylation step in PGL assembly takes place inside the bacterial cell before transportation to the cell envelope.

Because the carbohydrate domain seems to be essential to the biological properties of PGLs, as it is the variable domain, the lipid core will not be discussed any further. However, reviews about the lipid core and its biological importance have been published.[49,83]

1.3.4. Role of PGLs in Disease prognosis and pathogenesis

1.3.4.1. Role of PGLs in Leprosy

M. leprae is the causative organism of leprosy, which affects mainly the peripheral nervous system exhibiting contrasting manifestation amongst different patients.[87] Among all the pathogenic mycobacterial species, *M. leprae* was the first pathogen in which the role of PGL in disease progression was recognized.[54] The unique tropism of *M. leprae* to the neural tissue makes Schwann cells, the

myelinated glial cells of the peripheral nervous system, its main target.[88,89,79] This characteristic allows the pathogen to pass across the basal lamina and invade the Schwann cells (Figure 1-13).[90] To enter the Schwann cells, *M. leprae* first bind to laminin-2, a heterotrimeric glycoprotein consisting of α, β, and γ domains. After binding, laminin-2 acts as a bridge between the bacilli and the laminin receptor on the surface of the nerve cells, which then facilitates the invasion of the organism to the nerve cells.[91]

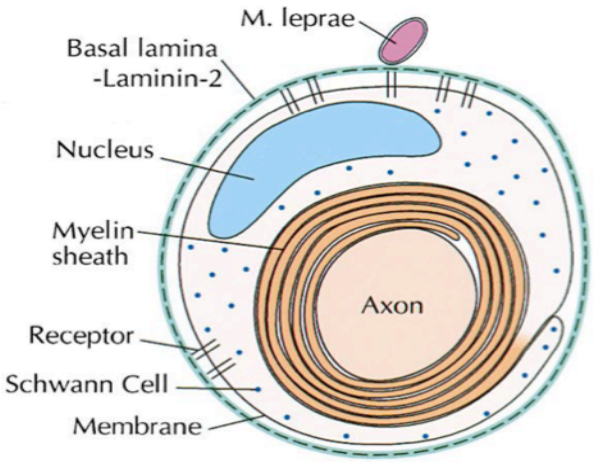


Figure 1-13. Schematic presentation of the interaction between *M. leprae* and Schwann cell. (Taken from: Ng, V.; Zanazzi, G.; Timpl, R.; Talts, J. F.; Salzer, J. L.; Brennan, P. J.; Rambukkana, A. *Cell* **2000**, *103*, 511–524.)

Recently, Rambukkana and coworkers provided an unambiguous demonstration that the binding motif in *M. leprae* responsible for this interaction is the trisaccharide moiety of its major PGL, PGL-I.[92] They also showed that any truncated forms of the trisaccharide results in a loss of binding with the nerve cell.

This finding explains why only *M. leprae* binds to nerve cells, as it is the only mycobacterial species to produce PGL-I.

The main function of the immune system is to combat infections; however, in case of leprosy, the immune response defines the broad spectrum of immunological and clinical manifestations of the disease.[93] A strong humoral immune response to the infection and weak cellular response gives rise to the lepromatous form of leprosy resulting in large amount of the bacilli to be found in both macrophages and Schwann cells of the peripheral nervous system. On the other end of the spectrum, when the antibody response is poor with a strong cellular response, tuberculoid leprosy occurs resulting in proliferation of lymphocytes as well as granuloma formation with small number of the bacilli present.

Many contradicting reports have been published about the role of PGLs in defining the spectrum of leprosy and, in particular, regarding whether or not PGL-I inhibits lymphocyte proliferation only in case of lepromatous leprosy or in the tuberculoid form as well.[94-96] The presence of PGL-I in all strains of *M. leprae* prompted Mehra and coworkers to suggest that specific T-lymphocyte suppressor cells were present only in patients with lepromatous leprosy and that these cells are activated by PGL-I.[94] In this study, they demonstrated that PGL-I displayed an inhibitory effect on ConA-induced T-lymphocyte proliferation, and that this inhibitory effect was abolished in the presence of a monoclonal antibody towards PGL-I. Moreover, the depletion of the T8 suppressor lymphocytes, a subpopulation of regulatory T-lymphocytes, abolished the *in vitro* inhibitory

effect of PGL-I. It was found that only the intact trisaccharide motif could mediate this effect, suggesting that this carbohydrate is the recognition domain for this interaction. Further support for this proposal came from studies in which the removal of mycocerosic acid did not affect this inhibitory response, whereas the monodeglycosylation or 3′-demethylation abolished the inhibitory effect of PGL-I. In a similar experiment, Prasad and coworkers showed that PGL-I had the same inhibitory effect in both lepromatous and tuberculoid patients.[97]

Another study by Makino and coworkers showed that dendritic cells (DCs) infected with *M. leprae* are less efficient at producing a proliferative response on T-cells. Moreover, DCs showed enhanced proliferative responses on T-cells when the effect of PGL-I, which is presented on the surface of DC, was masked with a monoclonal antibody.[98] The conclusion from these studies is that *M. leprae* has a suppressor effect on the proliferation of T-cells and this effect requires the recognition of the *M. leprae*-specific trisaccharide domain of PGL-I through a yet unknown receptor.

Another characteristic of pathogenic mycobacteria is their ability to invade and survive inside macrophages. These phagocytic cells use both oxygen-dependent and oxygen-independent antimicrobial systems. The oxygen dependent system includes the formation of superoxide, hydrogen peroxide and hydroxy radicals. A study by Klebanoff showed that PGL-I from *M. leprae* has a scavenging effect on these oxygen species.[99] In this study, they coated *Staphylococcus aureus* particles with PGL-I through a linking antibody and then used it to infect macrophages. They found that coating of the bacterial particles

with PGL-I completely blocked the staphylocidal effect of both control and INF-γ-activated macrophages. This experiment illustrated the effect of PGL-I on neutralizing the antimicrobial mechanism of macrophages.

Cytokines are differentially expressed in leprosy.[100] In tuberculoid leprosy, there is an INF-γ induced upregulation of IL-12 and downregulation of IL-10. This cytokine response directs the immune response in a T_H1-dependent manner, leading to a cellular immune response. On the other hand, the lepromatous form of leprosy is characterized by a reduced INF-γ response, which leads to downregulation of IL-12 and upregulation of IL-10. This directs a T_H2-dependent response, leading to humoral immune response. As mentioned earlier, PGL-I shows a cytokine induction profile similar effect to lepromatous leprosy. However, because PGL-I was found in all strains of *M. leprae*, and is found in both forms of leprosy, it does not appear to play a distinct role in defining the type of leprosy that will develop. This suggests that there are other strain-related factors or a polymorphism in the host immune genes that defines the form of leprosy that develops.

In addition to the role of PGL-I in pathogenesis, the high antigenicity of PGL-I has allowed it to be used for serological diagnosis of leprosy.[103] To this end, neoglycoproteins of PGL-I were synthesized and used to detect circulating antibodies against the trisaccharide. This represents a leap forward in the field of leprosy diagnosis. With the aid of these glycoproteins, antibodies against PGL-I can now be detected in patient sera with high sensitivity and high specificity.[104]

1.3.4.2. Role of PGLs in Tuberculosis

Among all *M. tuberculosis* strains, the Canetti strain was the first in which PGLs (Tb-1) were discovered.[60] Using ELISA and the monoclonal antibody MabIII604, which was raised against Tb-1, Kim and coworkers showed that 69% of all *M. tuberculosis* strains produce Tb-1 as compared to only 16% estimated using a TLC method.[105] The discrepancy between these two estimates is due partially to the fact the interaction between MabIII604 and PGLs, the basis of the ELISA assay, can result in false positives due to the recognition of *p*-HBAD II (Figure 1-12). When this mycobacterial PGL was used to detect the presence of anti-PGL antibodies from tuberculosis patients, the results showed either a low seroactivity, where only 24 out of 119 sera showed positive results, or a wide variation of the response was obtained ranging from 20% to 95%.[106] These findings clearly demonstrated that unlike *M. leprae*, PGLs cannot reliably be used as a diagnostic tool for TB. As a result of this failure, and because not all strains of tuberculosis are capable of producing PGL, it was thought that the PGLs in TB have no significant role in pathogenesis.

However, recent studies have changed this view. It has been shown that most of the *M. tuberculosis* strains that produce PGLs belong to Beijing genotype strains.[107] This genotype family, first identified by Van Soolingen and coworkers in 1995, was found to be very prevalent in Asia,[107a,107d] and is also responsible for other major outbreaks in different areas of the world.[107b] Guilhot and coworkers showed that only Beijing *M. tuberculosis* isolates produce phthiocerol dimycocerosates and phenolic glycolipids, whereas the non-Beijing isolates are

not capable of producing such lipids.[108] Moreover, stains of this genotype have also been associated with multidrug resistance in tuberculous meningitis especially in HIV-positive cases.[109] Another study has demonstrated that the Beijing genotype strains are hypervirulent in a murine disease model.[110] Genetic sequencing of these organisms showed that these strains possess an intact open reading frame (ORF) for the *pks15/1* gene, which confirms these strains have the ability to produce PGL-Tb.[66,107c] In two different studies conducted by van Spoolingen[110b] and by Kaplan[111], it was demonstrated that infection with the Beijing strain or exposure to its lipid extract elicited a T_H2-dependent immune response mediated by inhibition of TNF-α, IL-10 and IL-12 in experimental models. The non-Beijing strains, conversely, induced a T_H1-dependent response in the same models.

In a study by Barry and coworkers, using a Beijing HN 878 knock-out strain, in which the *pks15/1* gene was disrupted, it was shown that this mutant strain had lost the ability to produce PGLs. Moreover, when this mutant strain was used to infect mice, the animals survived longer than those infected with the wild-type strain. In the same study, it was found that the bone marrow-derived macrophages infected with the wild strains showed decreased secretion of the proinflammatory cytokines, TNF-α, IL-6 and IL-12, when compared to those infected with the mutant strain. However, when the mutant strain was complemented with an intact *pks15/1* gene, proinflammatory cytokine release decreased by 2–2.5 times.[66] It was also demonstrated that this effect by PGL-Tb on the level of several cytokines is dose-dependent. The results of this study

clearly implicate the role of PGL-tb as a virulence factor that modulates the host innate immunity, at least in the Beijing family.

A non-Beijing strain, NHN 5, which also has an intact *pks15/1* gene cluster and ORF, was unable to produce PGLs.[111] This finding established that not only the previously reported seven base pair mutation is responsible for the lack of PGL synthesis, but also that it might be a polymorphism of a single nucleotide that led to the disruption of the ORF of *pks15/1*, which requires further investigation.

Using a rabbit model of tuberculous meningitis, Kaplan and coworkers monitored disease prognosis as well as the host response to the infection with CDC1551, a PGL-deficient strain, compared to a W-Beijing strain HN878.[112] The purpose of this study was to evaluate the severity of the disease and to further investigate the role of PGLs in pathogenesis and hypervirulence.

It has been clearly established that TNF-α has a protective role against tuberculosis.[113-115] The clinically relevant example of this role is the reactivation of latent TB in rheumatoid arthritis patients due to the neutralization of TNF-α by an anti-TNF-α monoclonal antibody.[116] However, the persistent exposure to TNF-α can cause brain tissue damage as a result of induction of coagulation, which leads to vascular occlusion and necrosis.[117] Kaplan's study revealed that the infection of a rabbit model with either the CDC1551 strain or a PGL-deficient mutant HN878 strain showed an accelerated clearance of the bacilli. These infections also failed to elicit the production of TNF-α in cerebrospinal fluid (CSF) and caused very limited signs of the disease. On the other hand, infection

with HN878 resulted in severe progressive meningitis and induced greater TNF-α production in the CSF when compared to the mutant strain of HN878. This finding clearly established that PGL-tb is involved in the TNF-α induced inflammation of CNS and significantly contributes to virulence. The results of these studies, as well as others, suggest that the virulence of *M. tuberculosis* is not determined by a single microbial component or gene, but it is the result of several complex and dynamic interactions between the host and the pathogen.[118]

Despite the results demonstrating a role for PGL-tb in determining the virulence of *M. tuberculosis*, a recent study by Guilhot and Kaplan denies this role.[119] It was shown that when the PGL-deficient strain H36Rv was complemented with an intact *pks15/1* gene the bacilli were able to modulate the host immune response by affecting the level of several pro- and anti-inflammatory cytokines. However, this mutant did not increase virulence in either a mouse or a rabbit model. These findings led to the proposal that PGL-tb plays a role in the immunogenicity of the organism and acts in harmony with other bacterial factors, which are unique to a particular strain, to define the virulence of the organism.

Although the role of PGLs in *M. leprae* and *M. tuberculosis* infections has been extensively studied, similar studies of PGLs from *M. bovis* and *M. kansasii* have not yet investigated.

1.4. Overview of Thesis Research

As described earlier in section (1.3.3.), PGL-I was shown to be an important virulence factor that influences the progression of leprosy. This species

results in the tropism of the bacilli that gives it the ability to target the peripheral nervous system. Moreover, it was demonstrated that PGL-I may be acting with other microbial and host related factors to define the type of leprosy a patient develops. In addition, PGL-I is used as a diagnostic tool for detection of anti-PGL antibodies, with high sensitivity and specificity, as an indication of leprosy. In case of tuberculosis, although the role of PGL-tb in virulence is debatable, it was clearly established that PGL-tb plays an important role in the immunogenicity of organisms producing this molecule.

At the outset of this project, despite all that was known about PGLs in *M. tuberculosis* and *M. leprae,* their immunological profiles remained unknown. To provide a better understanding of how these molecules affect the host immune response and regulating the levels of different cytokines and/or interfering with apoptosis, we envisioned preparing a series of synthetic analogs of the carbohydrate cores of all PGLs isolated from *M. leprae, M. tuberculosis, M. bovis* and *M. kansasii*. These analogs were subsequently used to treat a THP-1 cell line, a model for the activated alveolar macrophages, to investigate their effect on the production of different cytokines. Details of the synthetic work used for the preparation of these analogs will be described in Chapters 2–5, while Chapter 6 will discuss the biological results.

This project is focused on better understanding the immunomodulatory properties of PGLs from pathogenic mycobacterial species as a step toward developing new antibacterials and vaccines against tuberculosis and leprosy as well as developing new diagnostic tools for their detection. Moreover, the

outcome of this project will provide a wealth of fundamental biochemical knowledge on these molecules. These compounds will also be of interest to other researchers working on mycobacterial disease. In addition, the synthetic chemistry developed en route to the targets will be useful to others in the field who may want to make related analogs in the future.

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Chapter 2

Synthesis of Carbohydrate Cores of Phenolic Glycolipids from *Mycobacterium leprae* and *Mycobacterium bovis*

2.1. Introduction

Leprosy, or Hansen's disease (HD), is a serious chronic bacterial infection of the skin and the peripheral nervous system caused by the bacterium *Mycobacterium leprae,* a member of the mycobacteria genus.[1] Advances in the use of multidrug therapies allow leprosy to be successfully controlled. However, there are still about 500,000 new cases reported every year and more than three million people infected with leprosy have suffered permanent neurologic disability.[2-5] Moreover, the emergence of the multidrug resistant strains makes the treatment of leprosy even more complicated.[1d,6,7] As previously detailed in Chapter 1, many of the difficulties of treating leprosy can be attributed mainly to the complex architecture of the cell envelope that acts as a protective shield preventing antimicrobial agents from affecting the bacilli.[8,9]

After the discovery of PGLs,[10-12] research into these molecules was neglected until Brennan and coworkers were able to isolate a *M. leprae* specific antigen (PGL-I, Figure 2-1), from *M. leprae*-infected liver tissue of armadillos. This PGL constituted 2% of the dry weight of the organism,[13,14] and it was found that all *M. leprae* strains produce PGLs. Brennan and coworkers[14] have shown that PGL-I has a structure typical of the PGL family, which is composed of two main parts: a carbohydrate domain and a lipid core. In the case of PGL-I, the

carbohydrate core is composed of a trisaccharide, consisting of a 3,6-dimethylated glucose unit at the non-reducing end, which is β-(1→4) linked to a 2,3-di-*O*-methylated rhamnose, which in turn is α-(1→2) linked to a 3-*O*-methylated rhamnose at the reducing end. The reducing end rhamnose is linked to the phenolphthiocerol, through an α linkage, which is diesterified with two mycoserosic acid units to complete the structure of the lipid core.

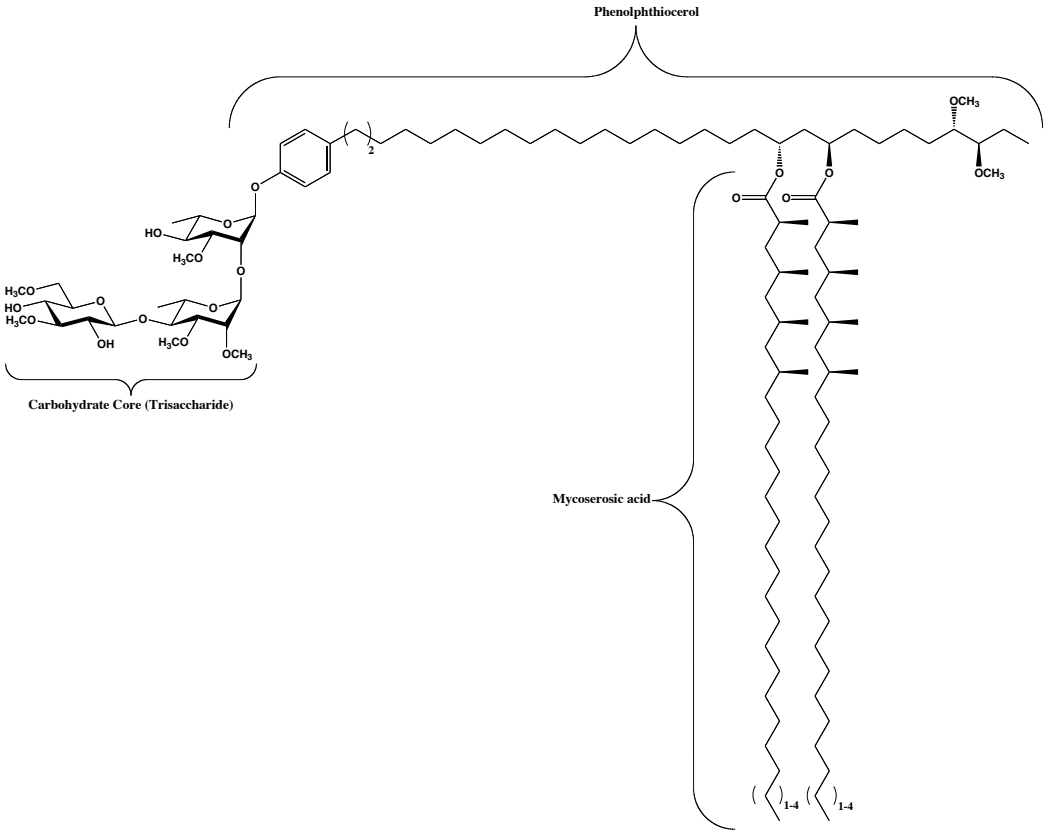


Figure 2-1. Structure of PGL-I isolated from *M. leprae.*

Since the discovery of PGL-I, it has been the subject of extensive research by many groups trying to elucidate its biosynthesis as well as its functions. Details

on the different properties and functions of PGLs *from M. leprae* were mentioned in Chapter 1.[15-22]

Mycobacterium bovis is another member of the mycobacteria family that causes tuberculosis in a range of animals, mainly cattle, and humans.[23] *M. bovis* can causes serious illness in cattle, with an estimated worldwide loss to agriculture of about $3 billion US annually.[24] *M. bovis* bacillus Calmette–Guérin is a strain of *M. bovis* that was attenuated over 13 years and is used as a vaccine for tuberculosis. This vaccine is known as the BCG vaccine. In 1989, *M. bovis*-specific PGLs named, B, B-1, B-2, and B-3 were isolated from *M. bovis* BCG.[25] Since the discovery of PGLs in *M. bovis,* no work has been done to identify their functions.

Despite all of the information known about PGL-I from *M. leprae,* and the very little is known about PGLs from *M. bovis*, and an understanding of their immunomodulatory activity remains unclear.[26] For that reason, we were interested in testing the effect of these PGLs on cytokine production by activated macrophages. However, the low native PGL-I bioavailability in leprosy patients` blood, the high hydrophobicity of the compounds, which complicates the use of ELISA (Enzyme-linked immunosorbent assay), and difficulty of growing mycobacteria make the use of native PGLs for assessing their immunomodulatory activity a tedious task.[22,27] To simplify the immunological profiling of PGLs from *M. leprae* and *M. bovis*, we decided to synthesize a panel of the carbohydrate cores of all PGLs bearing a *p*-methoxyphenol group at the reducing end to mimic the native PGLs lipid core. Once in hand we endeavoured to use these molecules

to test their effect on cytokine production and apoptosis. In this chapter, I will present the synthetic routes used to make synthetic analogues of the PGLs from *M. leprae* (Figure 2-2) and *M. bovis* (Figure 2-3).

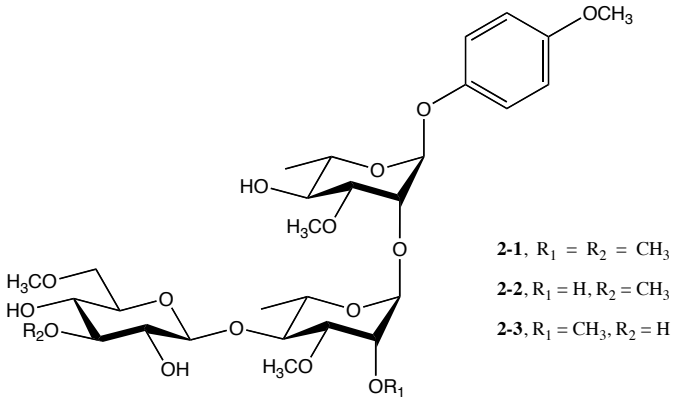


Figure 2-2: Structure of synthetic analogues of *M. leprae* PGLs.

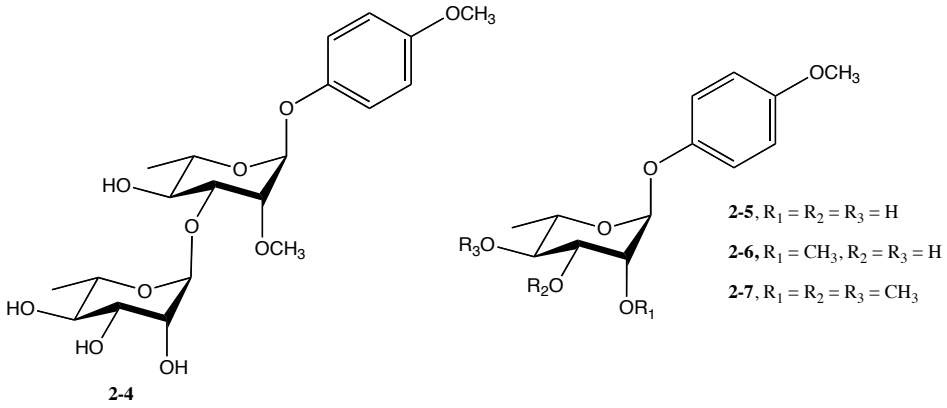


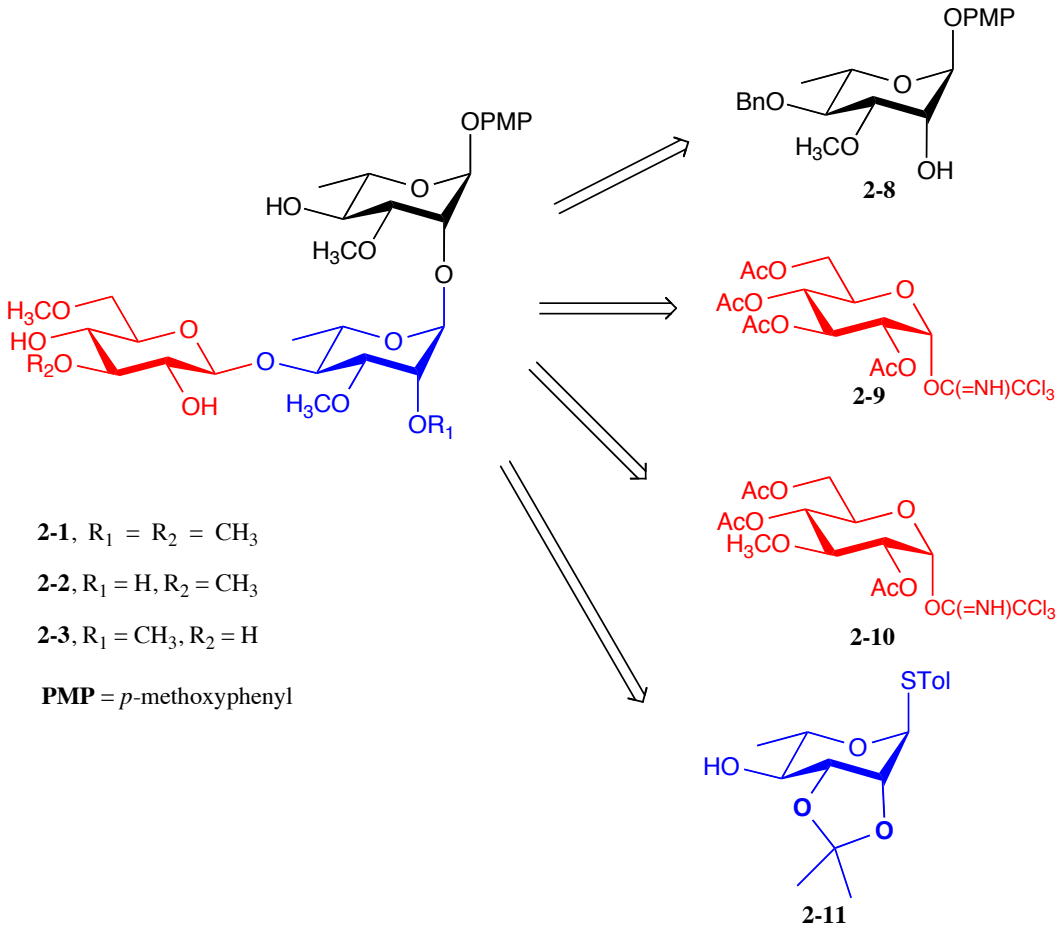
Figure 2-3: Structure of synthetic analogues of *M. bovis* PGLs.

2.2. Results and Discussion

2.2.1. Synthesis of analogues of PGLs from *M. leprae*, 2-1, 2-2, and 2-3

Retrosynthetic analysis of **2-1**–**2-3** showed that these molecules could be synthesized from four main building blocks: **2-8**, **2-9**, **2-10** and **2-11** (Figure 2-4). Compounds **2-8** and **2-11** are rhamnose based. Building block **2-8** is the reducing

end saccharide residue in this series and therefore it has a *p*-methoxyphenyl moiety, while building block **2-11** is a rhamnosyl thioglycoside in which O-2 and O-3 are protected with an isopropylidene acetal. Both **2-9** and **2-10** are glycosyl imidates; **2-9** is per-*O*-acetylated and **2-10** has a methyl group at O-3, and is acetylated at O-2, O-4 and O-6. With these four building blocks in hand, subsequent coupling and further protecting group manipulation was expected to provide the desired analogues in good yields.

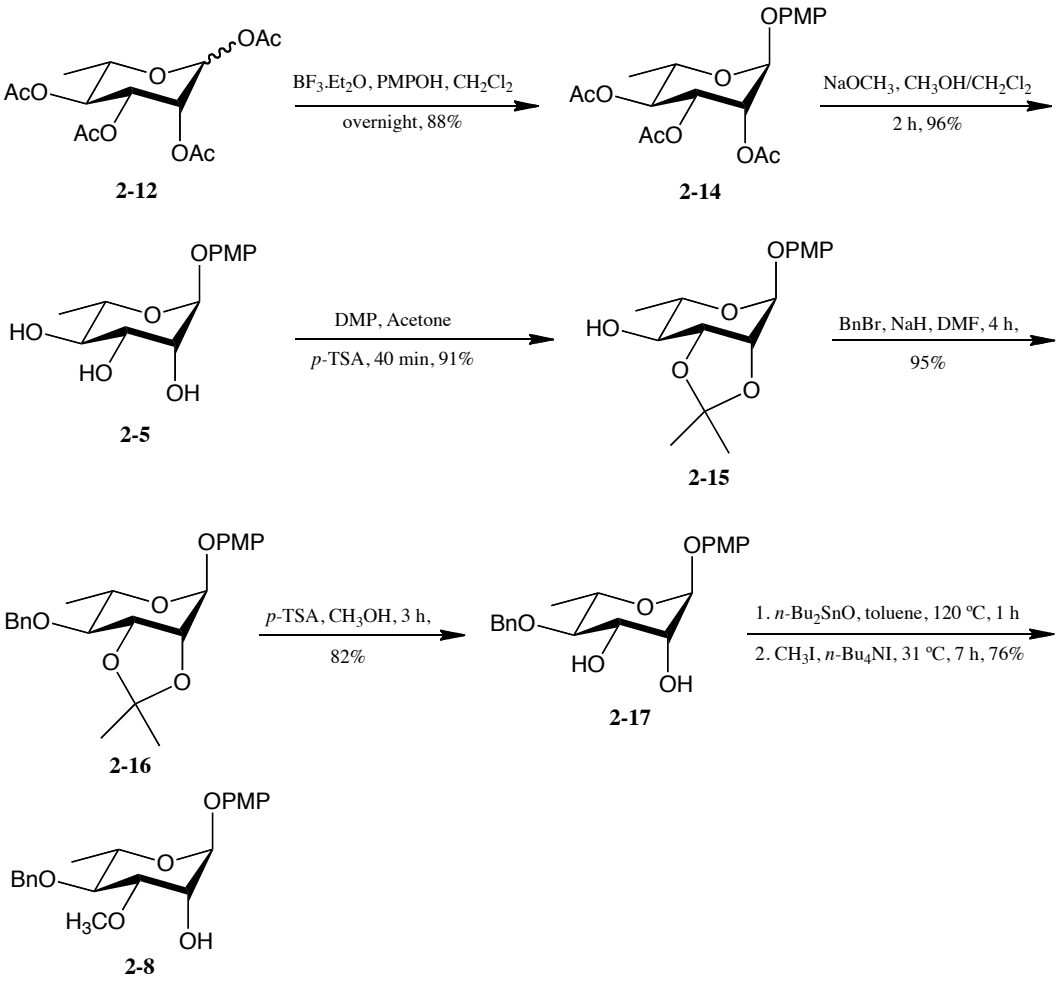


Scheme 2-1: Retrosynthetic analysis of building blocks **2-1**, **2-2**, **2-3**.

2.2.1.1. Synthesis of *p*-methoxyphenyl glycoside 2-8.

As illustrated in Scheme 2-2, the preparation of **2-8** began by glycosylation of donor **2-12** with *p*-methoxyphenol (PMPOH) using boron trifluoride etherate as the promoter[31] to give **2-14** in 88% yield. Subsequently, deacylation using sodium methoxide in methanol provided a 96% yield of **2-5**, which was then protected at O-2 and O-3 using 2,3-dimethoxypropane (DMP)[30] to give isopropylidene acetal **2-15** in 91% yield. The remaining hydroxyl group, at O-4, was protected by benzylation[30] using benzyl bromide and sodium hydride yielding **2-16** in 95% yield.

Subsequently, cleavage of the isopropylidene acetal[32] using a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) in methanol gave **2-17** in 82% yield. Finally, selective methylation of O-2 was achieved by heating diol **2-17** in toluene at reflux with di-*n*-butyltin oxide[33] to form the corresponding stannylidene acetal intermediate. After cooling the reaction mixture to 31 ºC, 10 equivalents of methyl iodide and tetra-*n*-butylammonium iodide (*n*-Bu_4NI) were added to this solution and the reaction was stirred for additional 7 h, which led to selective methylation of O-3 affording **2-8** in 76% yield.



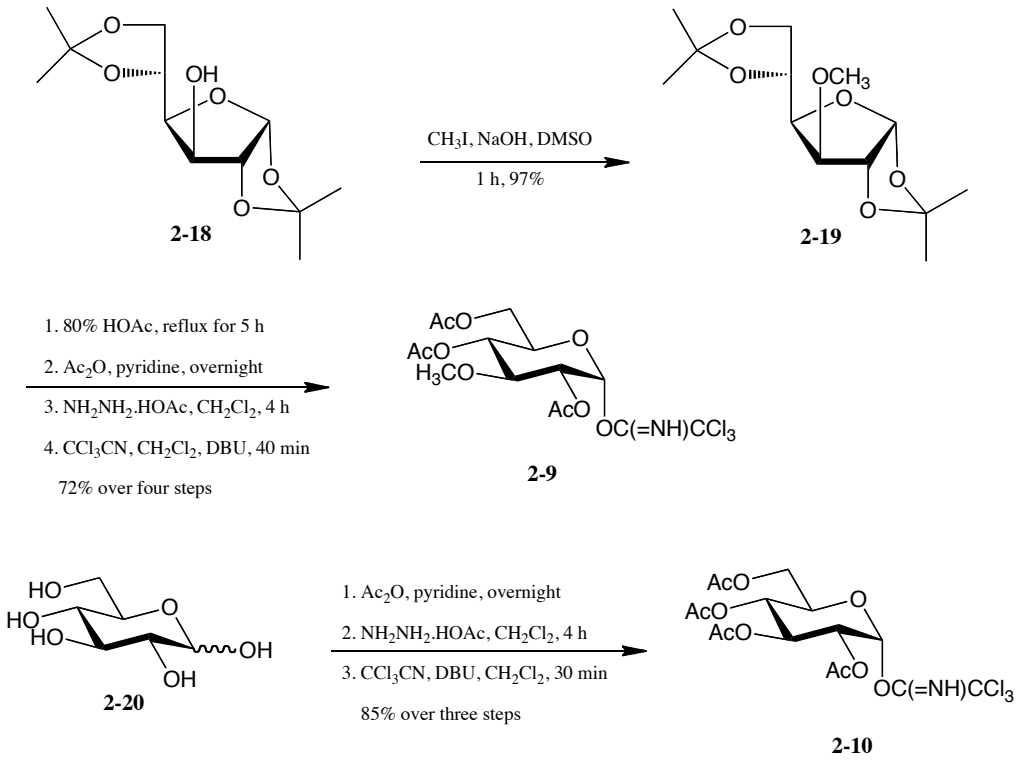
Scheme 2-2. Synthesis of *p*-methoxyphenyl glycoside **2-8**.

The regioselectivity of the final methylation step was established by ^{1}H NMR and ^{13}C NMR spectroscopy. The location of the methyl group in **2-8** was identified by the chemical shift of both H-3 (δ_H = 4.14 ppm) and C-3 (δ_C = 76.8 ppm) in the ^{1}H and ^{13}C NMR spectra. In the starting material, **2-17**, these resonances appear at 3.77 and 71.2 ppm, respectively, and the upfield chemical shift of these signals in the product is indicative of O-3 methylation. It is worth mentioning that when the reaction was promoted with cesium fluoride[34] instead of

tetra-*n*-butylammonium iodide, the methylation was not selective; a mixture of O-3 and O-2 methylated products was obtained.

2.2.1.2. Synthesis of imidates 2-9 and 2-10

As shown in Scheme 2-3, the synthesis of **2-9** started with methylation of commercially available 1,2:5,6-di-*O*-isoproylidene-D-glucofuranose (**2-18**). The reaction was carried out using methyl iodide[34] and sodium hydroxide in DMSO and furnished a 97% yield of **2-19**, in which O-3 was methylated. The next step was conversion of glucose from the furanose to pyranose form, which was achieved by heating **2-19** in 80% acetic acid[35] at reflux to give 3-*O*-methyl-α/β-D-glucopyranose. This intermediate was then acetylated using acetic anhydride and pyridine giving the corresponding derivative with an acetyl group at the anomeric position. The anomeric acetyl group was then removed selectively using hydrazine acetate[36] resulting in the expected reducing sugar. Finally, the corresponding glycosyl imidate derivative was prepared upon treatment with trichloroacetonitrile[37] and a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), to afford **2-9**, as the α-anomer, in 72% yield over four steps. The anomeric stereochemistry in **2-9** was confirmed by measuring $^{3}J_{1,2}$, which had a value of 3.7 Hz, clearly indicating the α-stereochemistry.



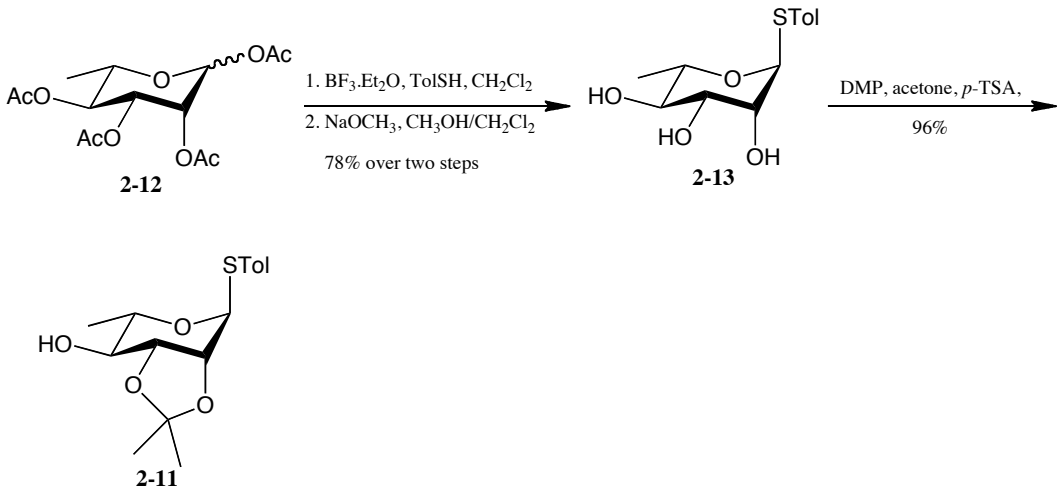
Scheme 2-3. Synthesis of imidates **2-9** and **2-10**.

Access to imidate **2-10** started with peracetylation of the commercially available D-glucopyranose, **2-20**, and the imidate was prepared using the same procedure and reaction conditions as those used for the synthesis of **2-9** (Scheme 2-3). The product was obtained in 85% yield over the three steps.

2.2.1.3. Synthesis of Thioglycoside 2-11

The synthesis of **2-11** is outlined in Scheme 2-4. Glycosylation[28,29] of **2-12** with *p*-thiocresol (*p*-toluenethiol, TolSH), using boron trifluoride etherate as the promoter and subsequent deacylation using Zemplén deacylation (sodium methoxide in methanol) afforded a 78% yield of **2-13** over the two steps. The next

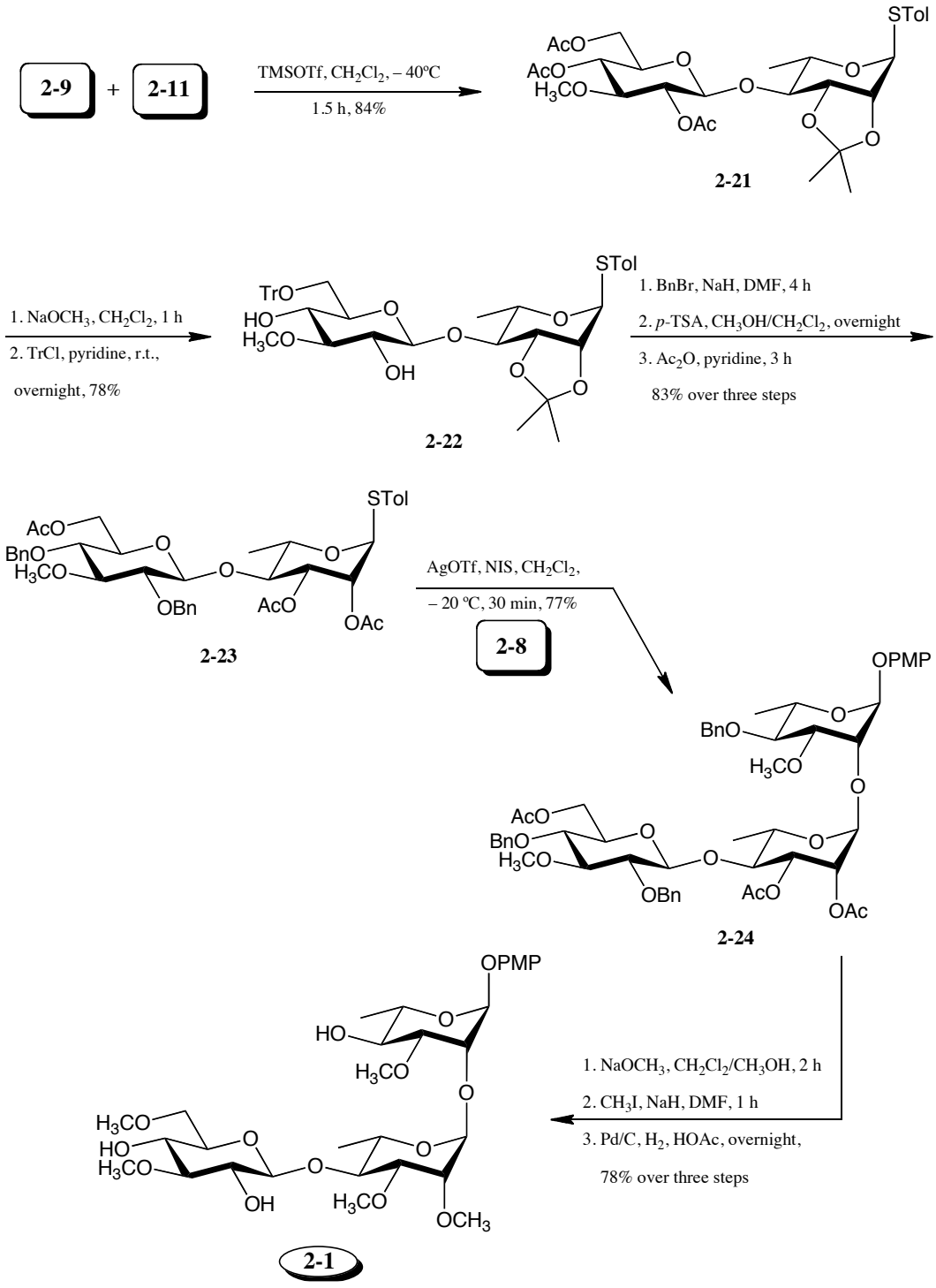
step was to protect both O-2 and O-3 in **2-13**, which was accomplished using (DMP) in acetone[30] in the presence of a catalytic amount of *p*-TSA. This reaction furnished the isopropylidene acetal-protected intermediate **2-11** in 96% yield (Scheme 2-4).



Scheme 2-4. Synthesis of thioglycoside building block **2-11**.

2.2.1.4. Synthesis of trisaccharide 2-1

With these four building blocks in hand, the assembly of the oligosaccharides could be performed starting with target **2-1** (Scheme 2-5). First, glycosylation of imidate **2-9** and alcohol **2-11** was achieved after activation of **2-9** using standard TMSOTf[37] conditions to give exclusively the β-anomer of **2-21** in 84% yield.



Scheme 2-5. Synthesis of trisaccharide **2-1**.

The stereoselectivity of the glycosylation reaction was controlled by neighbouring group participation of the acetyl group at O-2 and was confirmed by measuring the $^{1}J_{C\text{-}1',H\text{-}1'}$ in the product which was 160.4 Hz.[45] Moreover, another confirmation of the stereochemistry is the value of $^{3}J_{1',2'}$ which was found to be 8.0 Hz. Both values are consistent with β-glucosides.

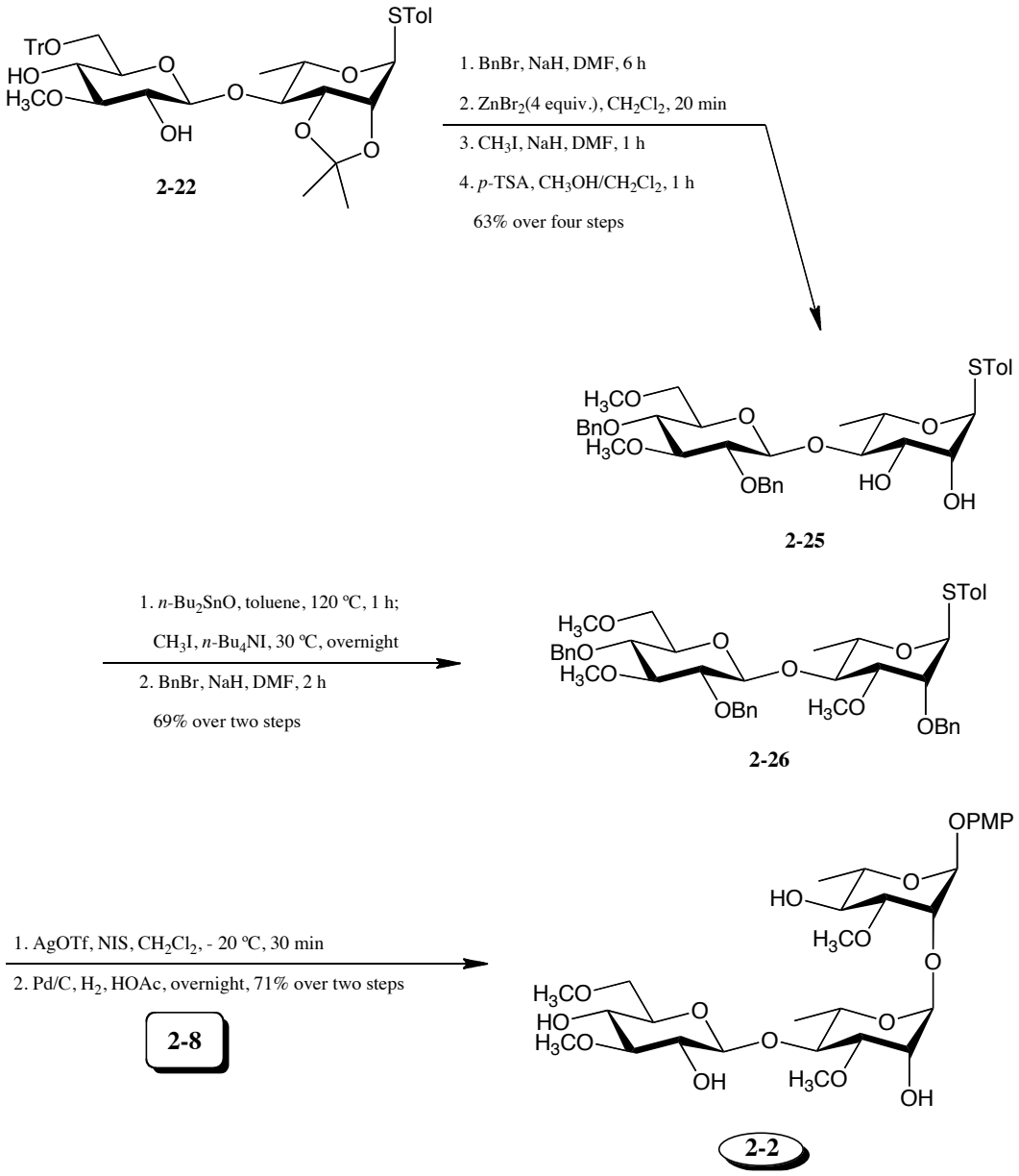
The product disaccharide was subjected to Zemplén deacylation yielding a triol, which was selectively protected at the 6′-OH using trityl chloride[38] and pyridine resulting in tritylated intermediate **2-22**. The remaining 2′-OH and 4′-OH groups were protected as benzyl ethers[30] via reaction with benzyl bromide and sodium hydride in DMF and the isopropylidene acetal was then removed using a catalytic amount of *p*-TSA[32] leading to the formation of a triol intermediate. Treatment of this triol with acetic anhydride[39] and pyridine afforded **2-23** in 83% yield over three steps.

The resulting disaccharide was activated using NIS and AgOTf[28] and coupled with alcohol **2-8** to yield the α-anomer of trisaccharide **2-24** in 77% yield. Only the α-anomer was obtained due to neighbouring group participation of the acetate on O-2 in the donor. The stereochemistry of the newly formed glycosidic linkage was established by a coupled HSQC experiment, which enabled the determination of the $^{1}J_{C\text{-}1',H\text{-}1'}$ (170 Hz).[45] Deprotection of the acetyl groups via Zemplén deacylation resulted in a diol, which was methylated using methyl iodide and sodium hydride. Finally, global debenzylation[32] by hydrogenolysis over palladium on charcoal resulted in the formation of trisaccharide **2-1** in 78% overall yield from trisaccharide **2-24**.

2.2.1.5. Synthesis of trisaccharide 2-2

Diol intermediate **2-22** (see Scheme 2-5 for its preparation) was used as the starting material for completing the synthesis of **2-2**. As detailed in Scheme 2-6, the synthesis began by protecting **2-22** via benzylation using benzyl bromide and sodium hydride. The trityl group was then removed selectively in presence of the 2,3-isopropylidene acetal using $ZnBr_2$[40] resulting in the formation of a triol intermediate. Subsequent, methylation using methyl iodide and sodium hydride followed by removal of the isopropylidene acetal using a catalytic amount of *p*-TSA[30] gave diol **2-25** in 63% yield over the four steps. This diol was then selectively methylated at the 3-OH group using the same reaction conditions as those used for making **2-8** (see Scheme 2-8). Next, the remaining hydroxyl group was benzylated to give **2-26** in 69% yield from **2-25**.

Glycosylation between thioglycoside **2-26** and alcohol **2-8** was achieved via activation of the thioglycoside with NIS–AgOTf[28] to afford a trisaccharide intermediate, which then gave a yield of 71% of the target trisaccharide, **2-2**, after debenzylation[32] using palladium on charcoal under a hydrogen atmosphere. The stereochemistry of the rhamnopyranosyl–rhamnopyranosyl linkage was established by measurement of the $^1J_{C\text{-}1,H\text{-}1}$ using a coupled HSQC experiment ($^1J_{C\text{-}1',H\text{-}1'}$ = 169 Hz), which is the value expected for an α-glycoside.[45a]

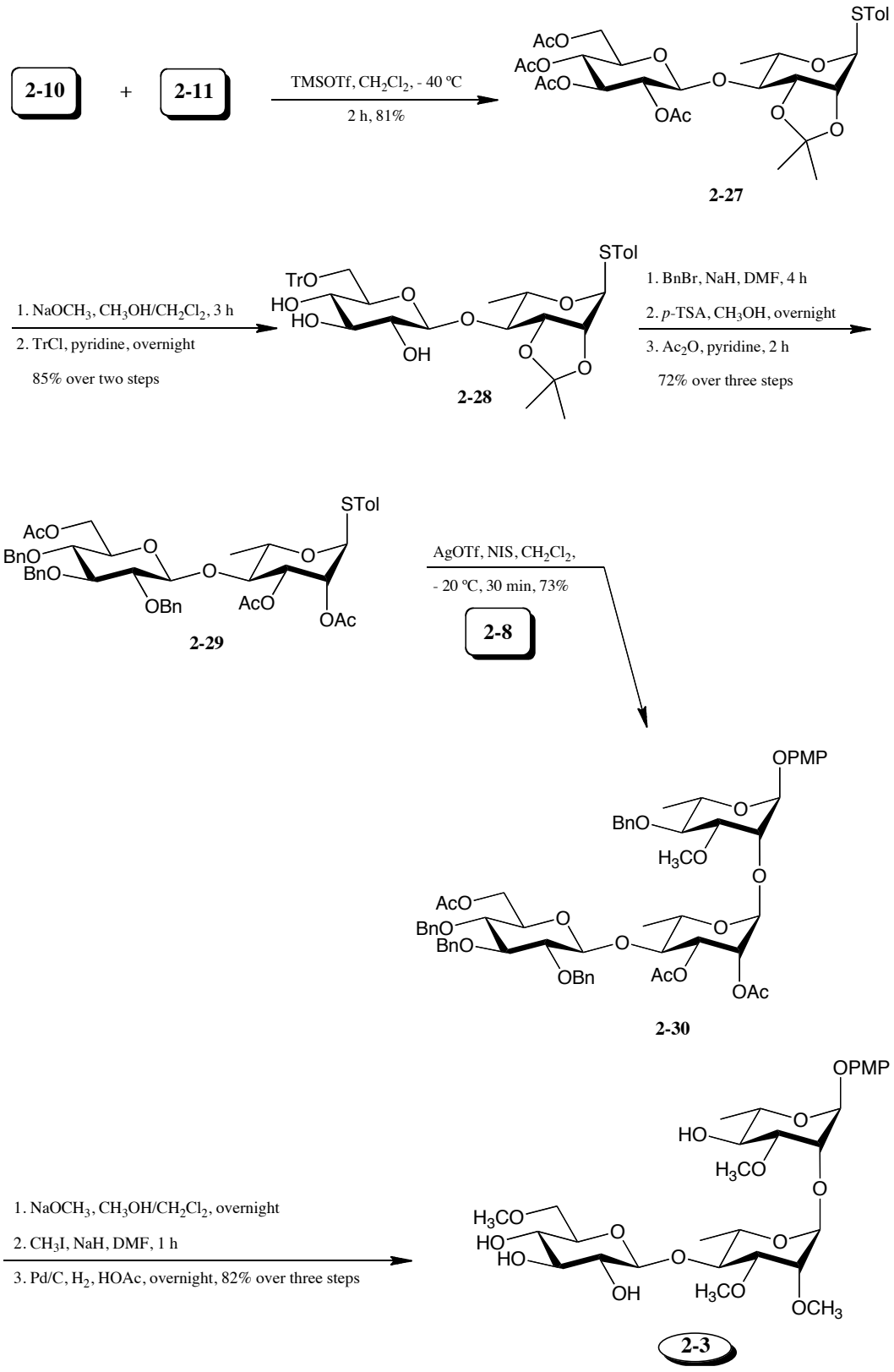


Scheme 2-6. Synthesis of trisaccharide **2-2**.

2.2.1.6. Synthesis of trisaccharide 2-3

The synthesis of trisaccharide **2-3** is shown in Scheme 2-7. First, disaccharide **2-27** was obtained in 81% yield after glycosylation of acceptor **2-11** with donor **2-10**, which was activated using TMSOTf.[41] That the disaccharide had

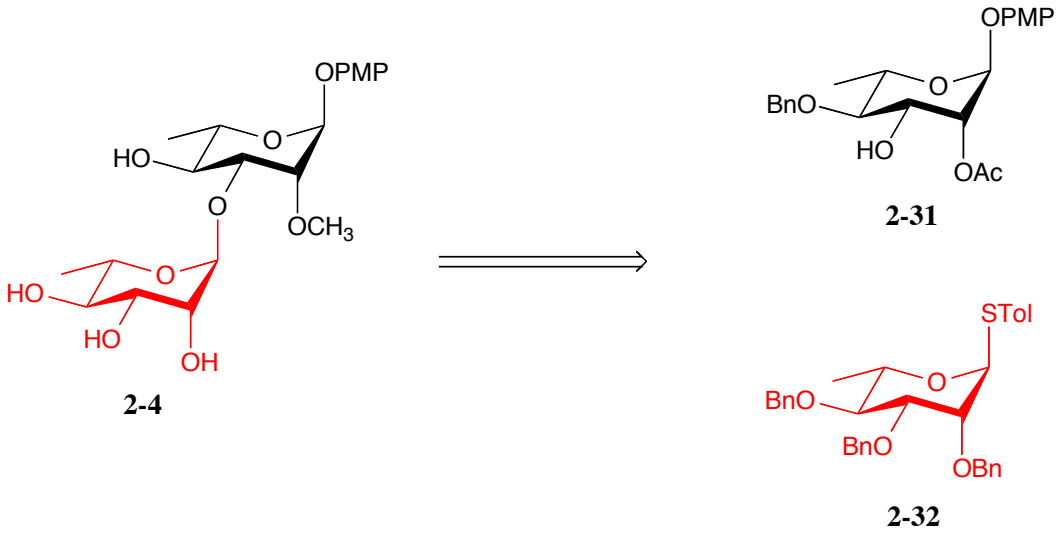
been obtained exclusively as the β-anomer was confirmed from the value of $^{1}J_{C\text{-}1',H\text{-}1'}$, which was found to be 160 Hz. Zemplén deacylation was performed to remove the acetyl groups and then the 6′-OH group was selectively protected by trityl group to afford **2-28** in 85% yield over two steps. The remaining hydroxyl groups, OH-2′, OH-3′, and OH-4′ were protected via benzylation. Next, acid-catalyzed hydrolysis of the 2,3-isopropylidene acetal using catalytic amount of *p*-TSA furnished a diol intermediate, which was acetylated (acetic anhydride and pyridine) to give the fully functionalized thioglycoside **2-29** in 72% yield over three steps. Acceptor **2-8** was glycosylated with donor **2-29** using NIS–AgOTf[28] activation, which furnished trisaccharide **2-30** in 73% yield. The α-stereochemistry of the newly formed glycosidic linkage was confirmed by a coupled HSQC experiment, from which the $^{1}J_{C\text{-}1',H\text{-}1'}$ was shown to be 171 Hz. The acetyl groups were then removed via Zemplén deacylation and the resulting hydroxyl groups were methylated using methyl iodide and sodium hydride. Finally, global debenzylation[39] gave trisaccharide **2-3** in 82% yield over two steps.



Scheme 2-7. Synthesis of trisaccharide **2-3**

2.2.2. Synthesis of analogues of PGLs from *M. bovis*, 2-4, 2-5, 2-6 and 2-7

M. bovis produces four different PGLs, and therefore I synthesized four carbohydrate cores bearing a *p*-methoxyphenol moiety at the reducing end (Figure 2-3). Analogue **2-4** is a disaccharide and can be obtained from monosaccharide building blocks **2-31**, and **2-32** (Scheme 2-8). On the other hand, analogues **2-5** to **2-7** are monosaccharides with a *p*-methoxyphenyl moiety at the reducing end.

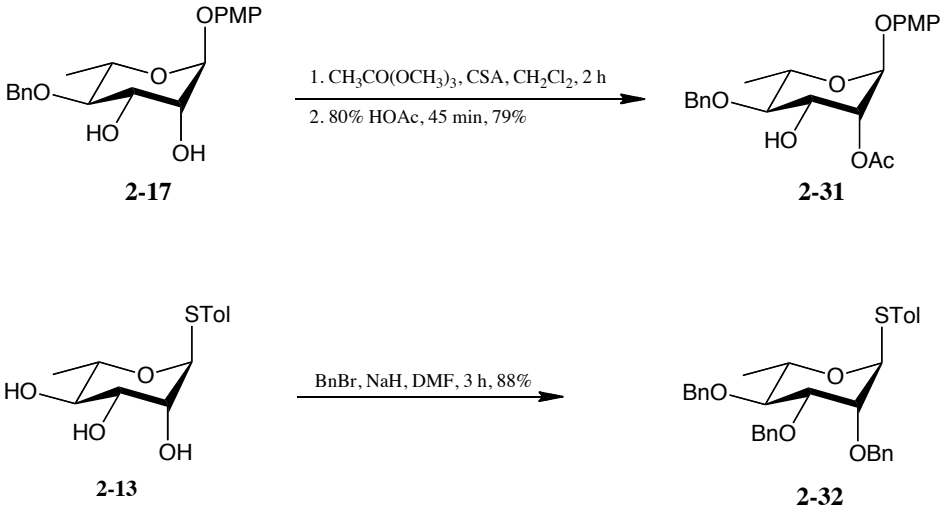


Scheme 2-8. Retrosynthetic analysis of disaccharide **2-4**.

2.2.2.1. Synthesis of disaccharide 2-4

As shown in Scheme 2-9, the synthesis of **2-4** started by preparing building blocks **2-31** and **2-32**. The route to **2-31** started from the advanced intermediate **2-17** (see Scheme 2-3 for its preparation). Selective acetylation of the axial hydroxyl group was achieved upon treatment of the diol with trimethyl orthoacetate[42] and (±)-camphor-10-sulfonic acid (CSA) to form an orthoester intermediate, which was then hydrolyzed with 80% aqueous acetic acid affording **2-31** in 79% yield over two steps. The regiochemistry was established by ^{1}H NMR and ^{13}C NMR

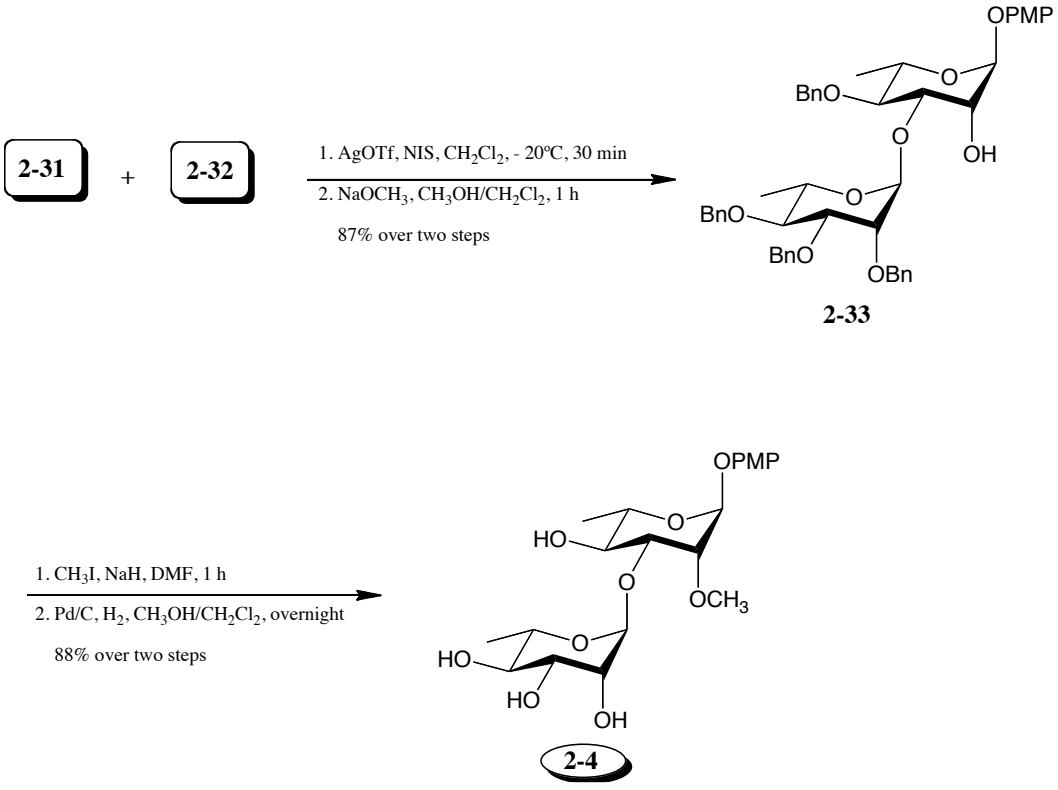
spectroscopy.



2-17 **2-31**

2-13 **2-32**

A: Synthesis of the building blocks **2-31** and **2-32**.



2-33

B. Assembly of disaccharide **2-4.**

Scheme 2-9. Synthesis of disaccharide **2-4**.

The location of acetyl group in **2-31** was identified from the ^{1}H and ^{13}C NMR spectra of the product. The chemical shift of both H-2 (δ_H = 5.29 ppm) and C-2 (δ_C = 72.4 ppm), which were shifted downfield compared to those signals in **2-17** (δ_H = 4.14-4.01 ppm and δ_C = 71.2 ppm). The other required monosaccharide precursor, **2-32**, was synthesized from the advanced intermediate **2-13** (see Scheme 2-2 for preparation of this compound). Sodium hydride-promoted[39] perbenzylation of **2-13** using 1.2 equivalent of benzyl bromide for each hydroxyl group gave a yield of 88% of **2-32**.

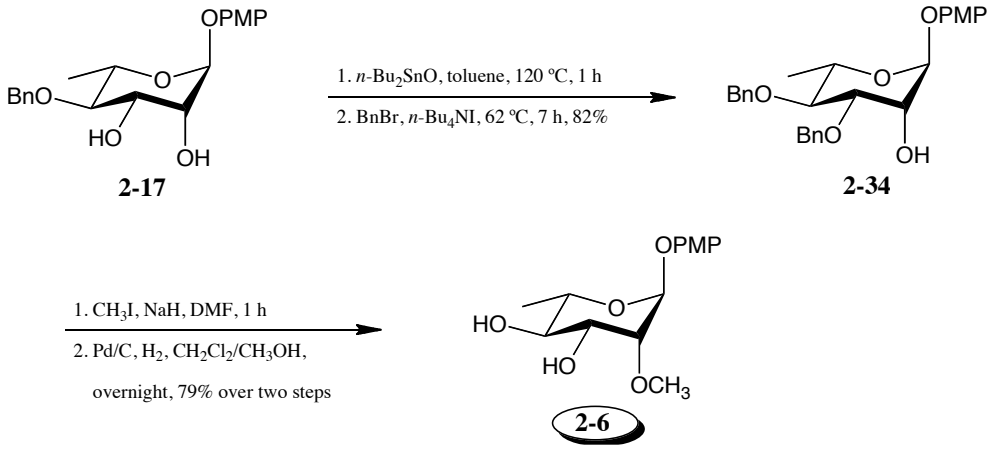
With these two building blocks in hand, acceptor **2-31** was glycosylated with donor **2-32** using NIS–AgOTf[29] activation. After the glycosylation, the acetyl group was removed with sodium methoxide to give disaccharide **2-33** as the α-anomer. The stereochemistry of the glycosidic linkage between the carbohydrate residues was confirmed by measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$, which had a value of 171 Hz, consistent with the α-sterochemistry.[45] The next step was methylation at O-2 using methyl iodide and sodium hydride. Subsequent debenzylation using palladium on charcoal under a hydrogen atmosphere gave **2-4** in 88% yield over two steps.

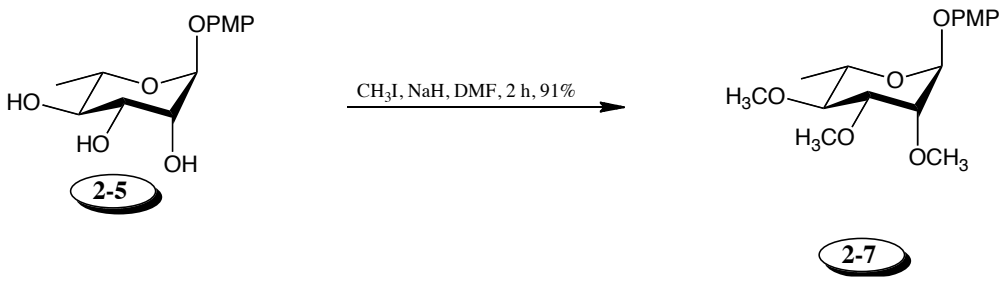
2.2.2.2. Synthesis of monosaccharides 2-5, 2-6 and 2-7

The synthesis of target **2-6** started from advanced intermediate **2-17** (see Scheme 2-3 for the preparation of this compound). The first step was the selective protection of 3-OH of **2-17**, which was accomplished by heating with dibutyltin oxide in toluene at reflux to form a stannylidene acetal intermediate. After

cooling, this intermediate was treated with benzyl bromide and tetra-*n*-butylammonium iodide (*n*-Bu_4NI), which resulted in the regioselective benzylation on the equatorial hydroxyl group giving **2-34** in 82% yield over the two steps.[43] ^1H NMR and ^{13}C NMR spectroscopy were used to establish the location of the benzyl group. For **2-34**, the chemical shift of H-3 in the ^1H NMR spectrum was at 4.04 ppm (compared to 4.14-4.01 ppm in the ^1H NMR spectrum of **2-17**). Similarly, in the ^{13}C NMR spectrum of **2-34**, the resonance for C-3 was present at 79.8 ppm, while in **2-17** this residue was found at 71.2 ppm. Finally, methylation of O-2 using methyl iodide and sodium hydride followed by hydrogenolysis over palladium on charcoal afforded **2-6** in 79% yield over two steps.

Target **2-7** was prepared from **2-5** via permethylation using methyl iodide and sodium hydride in a yield of 91% (Scheme 2-10).





Scheme 2-10. Synthesis of **2-5**, **2-6**, and **2-7**

2.3. Summary.

A panel of seven synthetic analogues of PGLs from *M. leprae* and *M. bovis*, **2-1–2-7** were synthesized using efficient linear synthetic routes in good overall yields. During the course of the synthesis of target compounds, use was made of thioglycoside donors and all newly formed glycosidic linkages were confirmed via measurement of $^{1}J_{C1,H1}$ values via coupled HSQC experiments. Another key feature of the routes were a series of regioselective alkylation and acylation reactions, which were achieved via the formation of tin and orthoester intermediates, respectively. Having access these compounds, the next step was to test their effect on cytokine production by activated macrophages as well as their ability to induce or inhibit apoptosis, which is described in Chapter 6.

2.4. Experimental

2.4.1. General Methods

Solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere before use. All reagents used in reactions were purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV_{254} (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by charring with a solution of anisaldehyde in ethanol, HOAc, and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). Organic solutions were concentrated under vacuum at < 50 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 or 500 MHz. ^{1}H NMR chemical shifts are referenced to TMS (0.0, $CDCl_3$) or CD_3OD (4.78, CD_3OD). ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.23, $CDCl_3$) or CD_3OD (48.9, CD_3OD). ^{1}H NMR data are reported as though they are first order and the peak assignments were made on the basis of 2D-NMR (^{1}H–^{1}H COSY and HMQC) experiments. The monosaccharide residues in the disaccharide and trisaccharides are labelled by no prime, prime, and double prime and these labels are maintained in the assignment of NMR spectra of all compounds (see Figure 2-4). Optical rotations were measured at 21 ± 2 °C at the sodium D line (589 nm) and are in

units of deg•mL(dm•g)$^{-1}$. ESI-MS spectra were carried out on samples suspended in THF or CH_3OH and added NaCl.

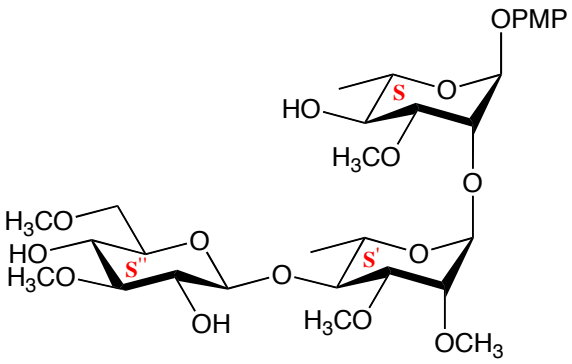
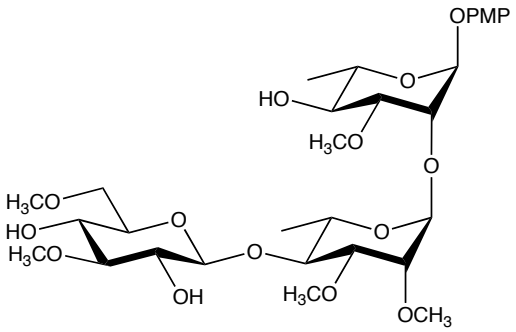


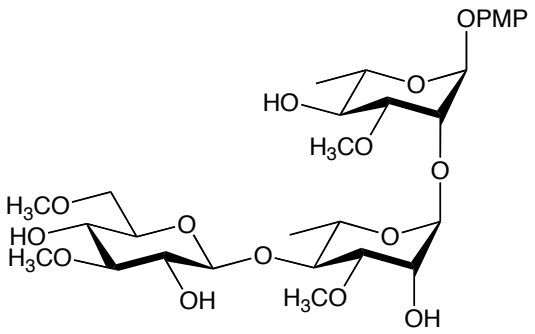
Figure 2-4. Numbering system used to label data.



***p*-Methoxyphenyl 3′′,6′′-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-2′,3′-di-*O*-methyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside (2-1)**

To a solution of **2-24** (30 mg, 0.03 mmol) in 1:1 CH_2Cl_2–CH_3OH (5 mL), 1M $NaOCH_3$ in CH_3OH (0.25 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. To a solution of the crude product and CH_3I (0.1 mL) in DMF (2 mL), NaH (60% in mineral oil, 8 mg, 0.05 mmol) was added at 0 ºC. Water (5

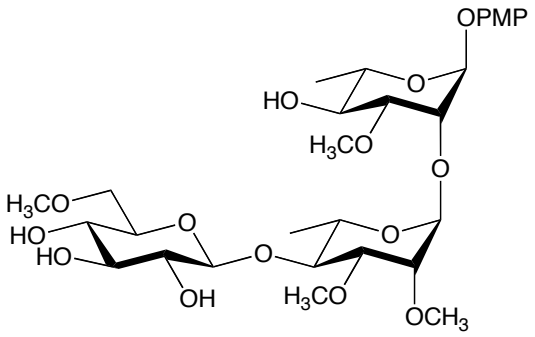
mL) was then added and the mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. The oil was dissolved in HOAc (5 mL) and Pd–C (6 mg) was added and the solution was stirred overnight under a hydrogen atmosphere. The solution was diluted with then CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting crude product was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **2-1** (23 mg, 78%) as an amorphous solid: R_f 0.55 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –30.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.98–6.94 (m, 2H, Ar), 6.84–6.81 (m, 2H, Ar), 5.36 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.09 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1′), 4.04 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.23–4.21 (m, 1H, H-2), 3.77 (s, 3H OCH_3), 3.75–3.73 (m, 3H, H-5, H-5′, H-2′), 3.67 (s, 3H, OCH_3), 3.65–3.54 (m, 7H, H-3, H-3′, H-4, H-4′, H-4″, H-6″ x 2), 3.52 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.42–3.39 (m, 2H, H-2″, H-5″), 3.37 (s, 3H, OCH_3), 3.15 (app t, 1H, $J_{2'',3''} = J_{3'',4''}$ = 9.0 Hz, H-3″), 1.33 (d, 3H, $J_{5',6'}$ = 6.3 Hz, H-6′), 1.27 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.1 (Ar), 117.5 (Ar x 2), 114.7 (Ar x 2), 105.6 (C-1″), 98.6 (C-1′), 98.0 (C-1), 85.5 (C-3″), 81.5 (C-4″), 81.4 (C-4), 80.2 (C-3′), 75.8 (C-3), 75.0 (C-4′), 74.4 (C-2′), 72.5 (C-6″), 72.3 (C-5″), 71.7 (C-2), 70.7 (C-2″), 70.0 (C-5), 68.3 (C-5′), 60.5 (O*C*H$_3$), 59.6 (O*C*H$_3$), 59.0 (O*C*H$_3$), 57.6 (O*C*H$_3$), 56.5 (O*C*H$_3$), 55.6 (O*C*H$_3$), 17.8 (C-6′), 17.6 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{30}H_{48}O_{15}Na$: 671.2885. Found 671.2880.



***p*-Methoxyphenyl 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside (2-2)**

To a solution of **2-26** (25 mg, 0.03 mmol) and **2-8** (15 mg, 0.04 mmol) in CH_2Cl_2 (10 mL) was added crushed 4 Å molecular sieves (100 mg). After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (10 mg, 0.04 mmol) and AgOTf (2.5 mg, 0.01 mmol) were added and the reaction mixture was stirred for another 30 min before it was neutralized with Et_3N (0.5 mL). The solution was filtered and concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil (25 mg). The oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (20 mL) and Pd–C (4.5 mg, 20% w/w) was added and the solution was stirred overnight under a hydrogen atmosphere. The solution was filtered, concentrated and the resulting crude product was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **2-2** (15 mg, 71%) as an amorphous solid: R_f 0.40 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –12.8 (c 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.96–6.90 (m, 2H, Ar-2,6), 6.81–6.78 (m, 2H, Ar-3,5), 5.34 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.03 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.48 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.17–4.16 (m, 1H, H-2), 3.92 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.72 (s, 3H, ArOCH_3), 3.69–3.63 (m, 3H, H-2′, H-5, H-5′),

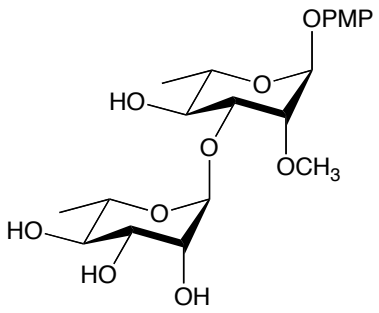
3.59 (s, 3H, OCH_3), 3.57–3.55 (m, 3H, H-4, H-4′, H-4″), 3.52 (s, 3H, OCH_3), 3.50–3.47 (m, 2H, H-6″ x 2), 3.44 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.34–3.28 (m, 3H, H-2″, H-3, H-5″), 3.06 (app t, 1H, $J_{2'',3''} = J_{3'',4''}$ = 11.0 Hz, H-3″), 1.26 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.20 (d, 3H, $J_{5',6'}$ = 6.5 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 156.0 (Ar), 151.4 (Ar), 118.5 (Ar x 2), 115.5 (Ar x 2), 105.5 (C-1″), 99.6 (C-1′), 99.2 (C-1), 87.4 (C-3″), 83.9 (C-3′), 81.8 (C-4), 81.3 (C-4″), 76.5 (C-3), 75.3 (C-4′), 74.9 (C-2′), 72.9 (C-5″), 72.7 (C-6″), 71.8 (C-2), 70.8 (C-2″), 70.4 (C-5), 68.8 (C-5′), 61.0 (O*C*H_3), 60.0 (O*C*H_3), 59.5 (O*C*H_3), 58.6 (O*C*H_3), 56.3 (O*C*H_3), 18.2 (C-6′), 18.1 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{29}H_{46}O_{15}Na$: 657.2729. Found: 657.2723.



***p*-Methoxyphenyl 6-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside (2-3)**

To a solution of **2-30** (25 mg, 0.03 mmol) in 1:1 CH_2Cl_2–CH_3OH (5 mL), 1M $NaOCH_3$ in CH_3OH (0.1 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. To a solution of the crude product and CH_3I (0.1 mL) in DMF (2 mL) NaH (60% in mineral oil, 8 mg, 0.45 mmol) was added at 0 ºC. After stirring

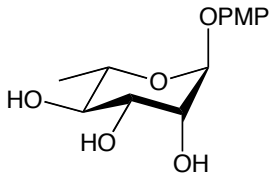
the solution for 1 h, water (5 mL) was added and the mixture was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil, which was dissolved in HOAc (5 mL). Pd–C (5 mg) was added and the solution was stirred overnight under a hydrogen atmosphere. The solution was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting crude product was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **2-30** (15 mg, 82%) as an amorphous solid: R_f 0.50 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –3.9 (c 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.98–6.95 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.36 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.10 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.44 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.23–4.22 (m, 1H, H-2), 3.78 (s, 3H, OCH_3), 3.77–3.73 (m, 3H, H-5, H-5′, H-5″), 3.68–3.54 (m, 8H, H-3, H-3′, H-3″, H-4, H-4′, H-4″, H-6″ x 2), 3.53 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.46–3.41 (m, 1H, H-2″), 3.39 (s, 3H, OCH_3), 3.37–3.33 (m, 1H, H-2′), 1.32 (d, 3H, $J_{5',6'}$ = 6.0 Hz, H-6′), 1.27 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.1 (Ar), 117.5 (Ar x 2), 114.7 (Ar x 2), 105.1 (C-1″), 98.4 (C-1′), 98.0 (C-1), 81.4 (C-4″), 81.2 (C-4), 80.2 (C-3″), 76.5 (C-3′), 75.8 (C-3), 74.7 (C-4′), 74.0 (C-2′), 72.8 (C-6″), 72.2 (C-5″), 71.8 (C-2), 71.5 (C-2″), 68.9 (C-5), 68.2 (C-5′), 59.6 (OCH_3), 59.0 (OCH_3), 57.7 (OCH_3), 56.7 (OCH_3), 55.7 (OCH_3), 17.7 (C-6), 17.6 (C-6′). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{29}H_{46}O_{15}Na$: 657.2729. Found: 657.2723.



***p*-Methoxyphenyl α-L-rhamnopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranoside (2-4)**

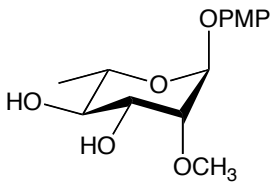
To a solution of **2-33** (25 mg, 0.03 mmol) and CH_3I (40 μL) in DMF (2 mL), was added NaH (60% in mineral oil, 5 mg) after the solution was cooled to 0 ºC. The solution was stirred for 1 h before water (5 mL) was added. The mixture was then diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (6:1 hexane–EtOAc) to give a colorless oil. The oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (5 mL) and Pd–C (4 mg) was added and the solution was stirred overnight under a hydrogen atmosphere. The solution was filtered, concentrated and the resulting crude product was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to give **2-4** (11.4 mg, 88%) as a sticky semi solid material: R_f 0.44 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –159.2 (*c* 1.3, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 6.99–6.93 (m, 2H, Ar-2,6), 6.83–6.77 (m, 2H, Ar-3,5), 5.37 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 5.04 (d, 1H, $J_{1',2'}$ = 1.4 Hz, H-1′), 3.99–3.97 (m, 2H, H-2′, H-3), 3.78–3.66 (m, 4H, H-2, H-3′, H-5, H-5′), 3.73 (s, 3H, ArOCH_3), 3.39 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.49 (s, 3H, OCH_3), 3.29 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4′), 1.29 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6), 1.21 (d,

3H, $J_{5',6'}$ = 6.5 Hz, H-6′); ^{13}C NMR (125.7 MHz, CD_3OD, δ_C) 156.4 (Ar), 151.8 (Ar), 119.0 (Ar x 2), 115.9 (Ar x 2), 104.0 (C-1′), 97.5 (C-1), 81.7 (C-2′), 79.5 (C-3), 74.1 (C-3′), 73.5 (C-2), 72.5 (C-4), 72.0 (C-4′), 70.9 (C-5′), 70.3 (C-5), 60.0 (O*C*H$_3$), 56.6 (ArO*C*H$_3$), 18.6 (C-6′), 18.5 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{20}H_{30}O_{10}Na$: 453.1731. Found: 453.1723.



***p*-Methoxyphenyl α-L-rhamnopyranoside (2-5)**

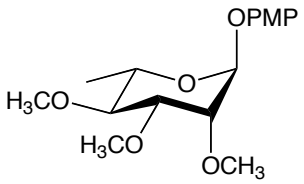
To a solution of **2-14** (5 g, 12.6 mmol) in 1:1 CH_2Cl_2–CH_3OH (100 mL) and 1M $NaOCH_3$ in CH_3OH (1 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting oil was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to afford **2-5** (3.27 g, 96%) as a white amorphous solid: R_f 0.32 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +67.5 (*c*, 0.1 CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 6.99–6.95 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.26 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 3.98–3.97 (m, 1H, H-3), 3.83 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.74 (s, 3H, ArOC*H*$_3$), 3.48–3.40 (m, 1H, H-5), 3.29–3.28 (m, 1H, H-4), 1.23 (d, 3H, $J_{6,5}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 156.3 (Ar), 151.7 (Ar), 118.7 (Ar x 2), 115.5 (Ar x 2), 100.6 (C-1), 73.8 (C-4), 72.1 (C-2), 72.0 (C-3), 70.3 (C-5), 55.9 (ArO*C*H$_3$), 17.8 (C-6). HRMS (ESI) calcd (M + Na)$^+$ $C_{13}H_{18}O_6Na$: 293.1001. Found: 293.1001.



***p*-Methoxyphenyl 2-*O*-methyl-α-L-rhamnopyranoside (2-6)**

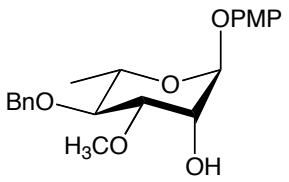
To a solution of **2-34** (171 mg, 0.38 mmol) and CH_3I (40 μL, 0.57 mmol) in DMF (2mL) was added NaH (60% in mineral oil, 14.6 mg, 0.68 mmol) after the solution was cooled to 0 ºC. The solution was stirred for 1 h before water (5 mL) was added and then the solution was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (6:1 hexane–EtOAc) to give a colorless oil (160 mg). The oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (5 mL) and Pd–C (32 mg) was added and the solution was stirred overnight under an atmosphere of hydrogen. The solution was filtered, concentrated and the resulting crude product was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to give **2-6** (95 mg, 89%) as an amorphous solid: R_f 0.60 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –56.5 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.01–6.97 (m, 2H, Ar-2,6), 6.85–6.81 (m, 2H, Ar-3,5), 5.42 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 3.86 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.73 (s, 3H, ArOCH_3), 3.64 (dd, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.59 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 3.49 (s, 3H, OCH_3-2), 3.37 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 1.20 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.2 (Ar), 150.7 (Ar), 117.8 (Ar x 2), 114.9 (Ar x 2), 95.6 (C-1), 80.4 (C-4), 74.1 (C-3), 71.7

(C-5), 68.7 (C-2), 59.2 (ArO*C*H$_3$), 55.9 (O*C*H$_3$), 17.8 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{14}H_{20}O_6Na$: 307.1155. Found: 307.1152.



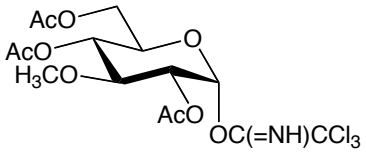
***p*-Methoxyphenyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside (2-7)**

To a solution of **2-5** (500 mg, 1.85 mmol) and CH_3I (0.42 mL, 6.66 mmol) in DMF (5 mL), was added NaH (60% in mineral oil, 213.12 mg, 8.88 mmol) at 0 ºC. The solution was stirred for 2 h before water (10 mL) was added and the solution was then diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (5:1 hexane–EtOAc) to give **2-7** (525 mg, 91%) as an amorphous solid: R_f 0.56 (5:1 hexane–EtOAc) $[\alpha]_D$ –146.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.03–6.99 (m, 2H, Ar-2,6), 6.86–6.82 (m, 2H, Ar-3,5), 5.43 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 3.79 (s, 3H, ArOC*H*$_3$), 3.77–3.76 (m, 1H, H-5), 3.71–3.67 (m, 2H, H-2, H-3), 3.59 (s, 3H, OC*H*$_3$), 3.58 (s, 3H, OC*H*$_3$), 3.56 (s, 3H, OC*H*$_3$), 3.21 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 1.28 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.1 (Ar), 150.7 (Ar), 117. 8 (Ar x 2), 114.9 (Ar x 2), 96.2 (C-1), 82.3 (C-3), 81.2 (C-4), 77.7 (C-2), 68.7 (C-5), 61.2 (O*C*H$_3$), 59.4 (O*C*H$_3$), 58.1 (O*C*H$_3$), 55.9 (ArO*C*H$_3$), 18.1 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{16}H_{24}O_6Na$: 335.1464. Found: 335.1465.



***p*-Methoxyphenyl 4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (2-8)**

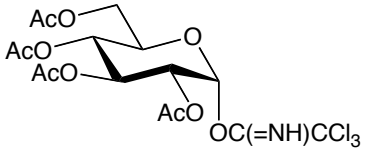
Diol **2-17** (2g, 5.54 mmol) was dissolved in toluene (60 mL) and *n*-Bu_2SnO (1.38 g, 5.54 mmol) was added. The reaction mixture was heated to 120 °C and stirred for 1 h, then cooled to 31 ºC before CH_3I (3.71 mL, 55.45 mmol) and *n*-Bu_4NI (2.40 g, 6.52 mmol) was added. The reaction mixture was stirred at 31 ºC for 16 h and then concentrated. The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **2-8** (1.57 g, 76%) as an amorphous solid: R_f 0.49 (2:1 hexane–EtOAc); $[\alpha]_D$ –72.8 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.36–7.26 (m, 5H, Ar), 6.99–6.97 (m, 2H, Ar-2,6), 6.83–6.81 (m, 2H, Ar-3,5), 5.45 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.87, 4.65 (ABq, 2H, J = 10.8 Hz, ArCH_2), 4.24–4.23 (ddd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.6 Hz, $J_{2,OH\text{-}2}$ = 2.6 Hz, H-2), 3.86–3.82 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.77 (s, 3H, ArOCH_3), 3.76–3.74 (m, 1H, H-3), 3.56 (s, 3H, OCH_3), 3.43 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.81 (d, 1H, J = 2.6 Hz, OH-2), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.8 (Ar), 150.1 (Ar), 138.4 (Ar), 128.3 (Ar x 2), 127.8 (Ar x 2), 127.6 (Ar), 117.5 (Ar x 2), 114.5 (Ar x 2), 97.8 (C-1), 81.4 (C-4), 79.7 (C-2), 75.2 (ArCH_2), 67.8 (C-3), 67.7 (C-5), 57.4 (OCH_3), 55.5 (ArOCH_3), 17.8 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{21}H_{26}O_6Na$: 397.1626. Found: 397.1623.



2,4,6-Tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate (2-9)

A solution of **2-19** (1 g, 3.65 mmol) in 80% aqueous HOAc (10 mL) was heated at reflux for 5 h before the solution was cooled and water (20 mL) was added. The solution was diluted with CH_2Cl_2 (40 mL), and washed with water (2 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was carried to the next step without further purification. The crude product was dissolved in pyridine (8 mL) and Ac_2O (2 mL) was added and the reaction mixture was stirred overnight before water (20 mL) was added. The mixture was extracted with CH_2Cl_2 (25 mL), and the organic layer was concentrated. To a solution of this crude product in CH_2Cl_2 (20 mL) hydrazine acetate (0.49 g, 5.47 mmol) was added and the reaction mixture was stirred at rt for 4 h before water (20 mL) was added. The solution was diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting crude product was carried to the next step without further purification. To a solution of the crude product and CCl_3CN (1 mL) in CH_2Cl_2 (20 mL), DBU (25 μL) was added and the reaction mixture was stirred for 30 min. Then, the reaction mixture was concentrated and the resulting crude product was rapidly purified via column chromatography (3:1 hexane–EtOAc, 1% Et_3N) to give **2-9** (2.11 g, 97%) as a colorless oil: R_f 0.38 (3:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.66 (s, 1H, N*H*), 6.51 (d, 1H,

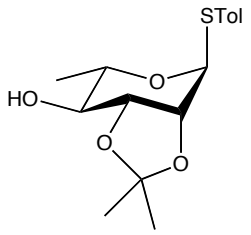
$J_{1,2}$ = 3.7 Hz, H-1), 5.12 (app t, 1H, $J_{3,4} = J_{4.5}$ = 10.0 Hz, H-4), 5.02 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.20 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.15 (ddd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 4.8 Hz, H-5), 4.12 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6b), 3.81 (app t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 3.49 (s, 3H, OCH_3), 2.11 (s, 3H, CH_3CO), 2.06 (s, 6H, CH_3CO x 2); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.6 (*C*=O), 170.0 (*C*=O), 169.3 (*C*=O), 160.7 (*C*=N), 93.3 (C-1), 78.3 (C-3), 71.7 (C-5), 70.5 (C-2), 68.8 (C-4), 61.7 (C-6), 20.7 (*C*H_3CO), 20.6(2) (*C*H_3CO), 20.5(4) (*C*H_3CO). HRMS (ESI) calcd $(M + Na)^+$ $C_{15}H_{20}Cl_3NO_9Na$: 486.0101. Found: 486.0101.



2,4,5,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (2-10)

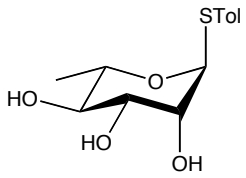
To a solution of D-glucose, (**2-20**, 1 g, 5.53 mmol) in pyridine (10 mL) Ac_2O (5 mL) was added at 0 ºC and the reaction mixture was stirred at rt overnight before water (30 mL) was added. The solution was diluted with CH_2Cl_2 (50 mL), and washed with water (2 × 40 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was carried to the next step without further purification. To a solution of the crude product in CH_2Cl_2 (20 mL) hydrazine acetate (0.75 g, 8.3 mmol) was added and the reaction mixture was stirred at rt for 4 h before water (20 mL) was added. The solution was diluted with CH_2Cl_2 (30 mL), and washed with water (2 × 40 mL). The organic layer was dried

(Na_2SO_4), filtered and concentrated. To a solution of the crude product and trichloroacetonitrile, (2 mL) in CH_2Cl_2 (10 mL) DBU (25 μL) was added and the reaction mixture was stirred for 30 min. Then, the reaction was concentrated and the resulting crude product was rapidly purified via column chromatography (3:1 hexane–EtOAc, 1% Et_3N) to give **2-10** (2.3 g, 85%) as a colorless oil: R_f 0.45 (3:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.69 (s, 1H, N*H*), 6.56 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 5.65 (app t, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 5.19 (app t, 1H, $J_{3,4}$ = 10.0 = $J_{4,5}$ = 10.0 Hz, H-4), 5.13 (dd, 1H, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.26 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6a), 4.18 (ddd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 4.1 Hz, H-5), 4.11 (dd, 1H, $J_{5,6b}$ = 4.1 Hz, $J_{6b,6a}$ = 12.6 Hz, H-6b), 2.07 (s, 3H, C*H*$_3$CO), 2.04 (s, 3H, C*H*$_3$CO), 2.02 (s, 3H, C*H*$_3$CO), 2.01 (s, 3H, C*H*$_3$CO); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.5 (*C*=O), 170.0 (*C*=O), 169.8 (*C*=O), 169.6 (*C*=O), 160.8 (*C*=N), 93.0 (C-1), 70.0(2) (C-5), 69.8(8) (C-3), 69.7(3) (C-2), 67.8 (C-4), 61.4 (C-6), 20.7 (*C*H$_3$CO x 2), 20.6 (*C*H$_3$CO), 20.4 (*C*H$_3$CO). HRMS (ESI) calcd $(M + Na)^+$ $C_{16}H_{20}Cl_3NO_{10}Na$: 514.0045. Found: 514.0047.



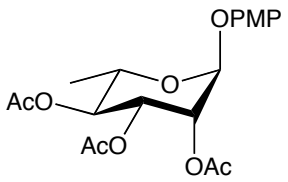
***p*-Tolyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (2-11)**

To a solution of **2-13** (2.5 g, 9.25 mmol) and 2,2-dimethoxypropane (2.09 mL, 17.05 mmol) in acetone (30 mL) was added *p*-TSA (0.13 g, 0.9 mmol). The reaction mixture was stirred for 30 min at rt, diluted with CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ soln (60 mL) and brine (60 mL). The organic layer was separated, dried with Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to afford **2-11** (2.75 g, 96%) as a white amorphous solid: R_f 0.41 (2:1 hexane–EtOAc); $[\alpha]_D$ +74 (*c*, 1.8 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.35 (m, 2H, Ar-2,6), 7.14–7.12 (d, 2H, J = 7.9 Hz, Ar-3,5), 5.67 (d, 1H, $J_{1,2}$ = 0.4 Hz, H-1), 4.35–4.34 (dd, 1H, $J_{1,2}$ = 0.4 Hz, $J_{2,3}$ = 5.6 Hz, H-2), 4.12–4.06 (m, 2H, H-3, H-4), 3.45 (dq, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 2.34 (s, 3H, ArCH_3), 1.53 (s, 3H, (CH_3)$_2$C), 1.37 (s, 3H, (CH_3)$_2$C), 1.25 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 137.8 (Ar), 132.4 (Ar x 2), 129.7 (Ar x 2), 129.4 (Ar), 109.6 ((CH$_3$)C), 84.0 (C-1), 78.3 (C-4), 76.4 (C-3), 75.1 (C-2), 66.8 (C-5), 28.1 (CH$_3$), 26.3 (CH$_3$), 21.0 (ArCH$_3$), 17.0 (C-6). HRMS (ESI) calcd (M + Na)$^+$ $C_{16}H_{22}O_4SNa$: 333.1137. Found: 333.1136.



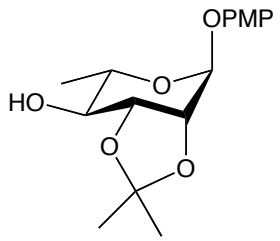
***p*-Tolyl 1-thio-α-L-rhamnopyranoside (2-13)**

To a solution of 1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose, (**2-12**, 5 g, 15.05 mmol) and thiocresol (2.24 g, 18.05 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added BF_3•OEt_2 (2.3 mL, 18.05 mmol) dropwise over 10 min. The reaction mixture was stirred for 7 h at 0 °C, diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ soln (100 mL) and brine (100 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated. The resulting residue was dissolved in 1:1 CH_2Cl_2–CH_3OH (100 mL) and 1M $NaOCH_3$ in CH_3OH (5 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting oil was purified by chromatography (10:1 CH_2Cl_2–CH_3OH) to afford **2-13** (3.2 g, 78%) as a white amorphous solid: R_f 0.45 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +41.3 (*c*, 0.2 CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 7.35–7.31 (m, 2H, Ar-2,6), 7.13–7.09 (m, 2H, Ar-3,5), 5.28 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 4.05–4.01 (m, 2H, H-2, H-5), 3.63 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.43 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 2.30 (s, 3H, ArCH_3), 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 138.5 (Ar), 133.1 (Ar x 2), 132.1 (Ar), 130.6 (Ar x 2), 90.4 (C-1), 74.0 (C-3), 73.6 (C-4), 72.8 (C-5), 70.7 (C-2), 20.9 (Ar*C*H_3), 17.7 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{13}H_{18}O_4SNa$: 293.0818. Found: 293.0818.



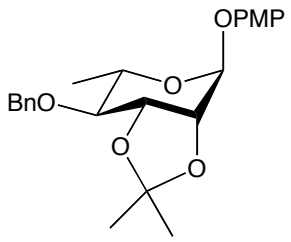
_p_-Methoxyphenyl 2,3,4-tri-_O_-acetyl-α-L-rhamnopyranoside (2-14)

To a solution of **2-12** (5 g, 15.05 mmol) and *p*-methoxyphenol (2.4 g, 18.05 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added BF_3**.**OEt_2 (2.3 mL, 18.05 mmol) dropwise over 10 min. The reaction mixture was stirred for 6 h at 0 °C, diluted with CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ soln (100 mL) and brine (100 mL). The organic layer was separated, dried with Na_2SO_4, filtered and the resulting oil was purified by chromatography (3:1 hexane–EtOAc) to afford **2-14** (5.24 g, 88%) as a colorless oil: R_f 0.30 (3:1 hexane–EtOAc); $[\alpha]_D$ +112.4 (*c*, 0.3 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.00 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.83 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.52–5.49 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, H-3), 5.43–5.42 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 5.34 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.15 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, H-4), 4.07–4.00 (dq, 1H, $J_{4,5}$ = 10 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 3.77 (s, 3H, ArOCH_3), 2.18 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.22 (d, 3H, $J_{6,5}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.1 (*C*=O), 170.0 (*C*=O), 169.98 (*C*=O), 117.6 (Ar x 2), 115.8 (Ar), 114.6 (Ar), 114.5 (Ar x 2), 96.4 (C-1), 70.9 (C-4), 69.7 (C-2), 68.9 (C-3), 66.9 (C-5), 55.5 (ArO*C*H_3), 20.7 (*C*H_3CO), 20.6(3) (*C*H_3CO), 20.5(8) (*C*H_3CO), 17.3 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{19}H_{24}O_9Na$: 419.1318. Found: 419.1317.



***p*-Methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (2-15)**

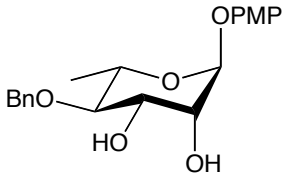
To a solution of **2-5** (3 g, 11.11 mmol) and 2,2-dimethoxypropane (2.72 mL, 22.22 mmol) in acetone (40 mL) was added *p*-TSA (0.07 g, 0.52 mmol). The reaction mixture was stirred for 40 min at rt, diluted with CH_2Cl_2 (80 mL) and washed with satd aq $NaHCO_3$ soln (60 mL) and brine (60 mL). The organic layer was dried with Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to afford **2-15** (3.13 g, 91%) as a white amorphous solid: R_f 0.58 (2:1 hexane–EtOAc); $[\alpha]_D$ +127.0 (*c*, 0.3 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 6.98 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.84 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.26 (br s, 1H, H-1), 4.27 (d, 1H, $J_{2,3}$ = 5.9 Hz, H-2), 4.02–3.99 (dd, 1H, $J_{2,3}$ = 5.9 Hz, $J_{3,4}$ = 7.2 Hz, H-3), 3.68 (s, 3H, ArOCH_3), 3.62–3.53 (dd, 1H, $J_{4,5}$ = 9.1 Hz, $J_{5.6}$ = 6.0 Hz, H-5), 3.15–3.09 (dt, 1H, $J_{3,4}$ = 7.2 Hz, $J_{4,5}$ = 9.1 Hz, H-4), 1.41 (s, 3H, (CH_3)$_2$C), 1.29 (s, 3H, (CH_3)$_2$C), 1.04 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.3 (Ar), 149.3 (Ar), 118.1 (Ar x 2), 114.3 (Ar x 2), 108.2 ((CH$_3$)*C*), 95.7 (C-1), 77.8 (C-4), 75.0 (C-2), 73.0 (C-3), 66.4 (C-5), 55.1 (ArO*C*H$_3$), 27.7 ((*C*H$_3$)$_2$C), 26.1 ((*C*H$_3$)$_2$C), 17.1 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{16}H_{22}O_6Na$: 333.1314. Found: 333.1313.



***p*-Methoxyphenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (2-16)**

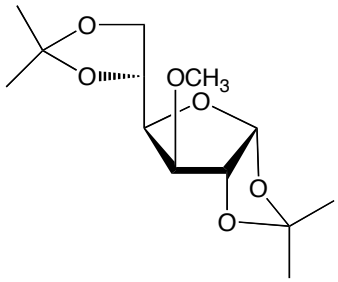
To a solution of **2-15** (2 g, 6.45 mmol) and BnBr (0.92 mL, 7.73 mmol) in DMF (15 mL) at 0 °C was added NaH (60% in mineral oil, 0.25 g, 10.3 mmol) portion-wise over 10 min. The reaction mixture was stirred for 4 h at rt before water (30 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (100 mL), and washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (5:1 hexane–EtOAc) to give **2-16** (2.45 g, 95%) as a colorless oil: R_f 0.50 (3:1 hexane–EtOAc); $[\alpha]_D$ –43.6 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.40–7.27 (m, 5H, Ar), 7.02–6.97 (m, 2H, Ar-2,6), 6.86–6.82 (m, 2H, Ar-3,5), 5.60 (d, 1H, $J_{1,2}$ = 0.6 Hz, H-1), 4.94, 4.66 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.45–4.42 (m, 1H, H-3), 4.37–4.36 (dd, 1H, $J_{1,2}$ = 0.6 Hz, $J_{2,3}$ = 5.8 Hz, H-2), 3.90–3.83 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 3.78 (s, 3H, ArOCH_3), 3.31–3.27 (dd, 1H, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 9.4 Hz H-4), 1.55 (s, 3H, (CH_3)$_2$C), 1.43 (s, 3H, (CH_3)$_2$C), 1.25 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.8 (Ar), 150.7 (Ar), 138.4 (Ar), 128.9 (Ar), 128.7 (Ar), 128.3 (Ar), 128.2 (Ar x 2), 127.9 (Ar x 2), 127.6 (Ar x 2), 109.4 ((CH$_3$)*C*), 96.1 (C-1), 81.0 (C-3), 78.5 (C-2), 76.0 (C-4), 72.9 (Ar*C*H$_2$), 65.3 (C-5), 55.5 (ArO*C*H$_3$), 27.9 ((*C*H$_3$)$_2$C),

26.3 ((*C*H$_3$)$_2$C), 17.7 (C-6). HRMS (ESI) calcd (M + Na)$^+$ $C_{23}H_{28}O_6Na$: 423.1784. Found: 423.1784.



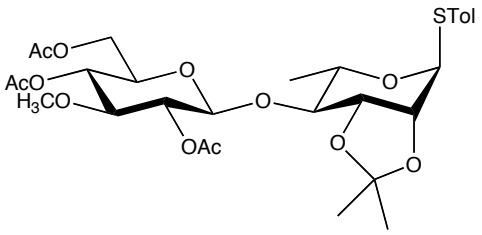
***p*-Methoxyphenyl 4-*O*-benzyl-α-L-rhamnopyranoside (2-17)**

To a solution of **2-16** (2 g, 5 mmol) in CH_3OH (40 mL) was added *p*-TSA (76 mg, 0.49 mmol). The reaction mixture was stirred for 3 h at rt before it was neutralized with Et_3N (1 mL) and concentrated. The crude product was purified by chromatography (2:1 EtOAc–hexane) to afford **2-17** (1.26 g, 82%) as an amorphous solid: R_f 0.55 (2:1 EtOAc–hexane); $[\alpha]_D$ –50.4 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.27 (m, 5H, Ar), 6.99–6.97 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.40 (s, 1H, H-1), 4.79, 4.74 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.14–4.10 (m, 2H, H-2, H-3), 3.90–3.86 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.78 (s, 3H, ArOCH_3), 3.43 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.50 (br s, 1H, OH-2), 2.38 (br s, 1H, OH-3), 1.33 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 154.8 (Ar), 150.1 (Ar), 138.1 (Ar), 128.6 (Ar x 2), 128.0 (Ar), 127.8 (Ar x 2), 117.5 (Ar x 2), 114.5 (Ar x 2), 98.0 (C-1), 81.5 (C-4), 71.2 (C-3), 71.0 (C-2), 67.8 (C-5), 55.6 (ArO*C*H$_3$), 18.0 (C-6). HRMS (ESI) calcd (M + Na)$^+$ $C_{20}H_{24}O_6Na$: 383.1471. Found: 383.1470.



1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-D-glucofuranose (2-19)

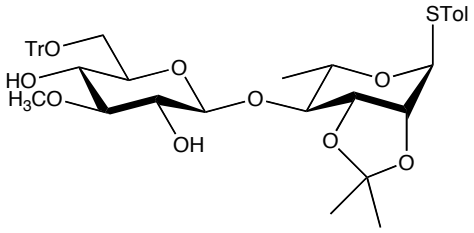
To a solution of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose, (**2-18**, 2 g, 7.29 mmol) and CH_3I (0.72 mL, 11.53 mmol) in DMSO (10 mL), NaOH (0.4 g, 9.21 mmol) dissolved in water (2 mL) was added portion-wise over 1 min at 0 ºC. The reaction mixture was stirred at rt for 30 min before water (50 mL) was added. The solution was diluted with CH_2Cl_2 (100 mL), and washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (4:1 hexane–EtOAc) to give **2-19** (1.94 g, 97%) as a colorless oil: R_f 0.56 (4:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 5.84 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.55 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-2), 4.31–3.98 (m, 4H, H-4, H-5, H-6 x 2), 3.77–3.75 (m, 1H, H-3), 3.97 (s, 3H, OCH_3), 1.49 (s, 3H, $(CH_3)_2C$), 1.42 (s, 3H, $(CH_3)_2C$), 1.35 (s, 3H, $(CH_3)_2C$), 1.31 (s, 3H, $(CH_3)_2C$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 111.6 ($(CH_3)_2C$), 108.9 ($(CH_3)_2C$), 105.1 (C-1), 83.6 (C-2), 81.8 (C-4), 80.9 (C-5), 72.3 (C-6), 67.1 (C-3), 58.1 (OCH_3), 26.8 ($(CH_3)_2C$), 26.7 ($(CH_3)_2C$), 26.1, ($(CH_3)_2C$), 25.3 ($(CH_3)_2C$). HRMS (ESI) calcd $(M + Na)^+$ $C_{13}H_{22}O_6Na$: 297.1314. Found: 297.1314.



***p*-Tolyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (2-21)**

To a solution of **2-9** (0.5 g, 1.07 mmol) and **2-11** (0.39 g, 1.29 mmol) in CH_2Cl_2 (30 mL) was added crushed 4 Å molecular sieves (200 mg). After the solution was stirred at rt for 30 min, the reaction mixture was cooled to –40 ºC and then TMSOTf (0.2 mL of 10% solution in CH_2Cl_2) was added and the mixture was stirred for another 1.5 h. After neutralization with Et_3N, the solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **2-21** (0.54 g, 84%) as a colorless oil. R_f 0.32 (2:1 hexane–EtOAc); $[\alpha]_D$ –33.1 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.35 (d, 2H, J = 7.9 Hz, Ar-2,6), 7.12 (d, 2H, J = 7.9 Hz, Ar-3,5), 5.63 (d, 1H, $J_{1,2}$ = 0.5 Hz, H-1), 5.01 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4′), 4.59 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 9.5 Hz, H-2′), 4.85 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.29 (dd, 1H, $J_{1,2}$ = 0.5 Hz, $J_{2,3}$ = 5.5 Hz, H-2), 4.17–4.05 (m, 4H, H-3, H-5′, H-6′ x 2), 3.60–3.55 (m, 2H, H-3′, H-5), 3.53 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.41 (s, 3H, OCH_3), 2.33 (s, 3H, ArCH_3), 2.15 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 1.52 (s, 3H, (CH_3)$_2$C), 1.36 (s, 3H, (CH_3)$_2$C), 1.19 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.7 (*C*=O), 169.5(2) (*C*=O), 169.4(7) (*C*=O), 138.1 (Ar), 133.6 (Ar x 2), 129.9 (Ar x 2), 129.3 (Ar), 109.4 ((CH$_3$)$_2$*C*), 100.1 (C-1), 84.0 (C-

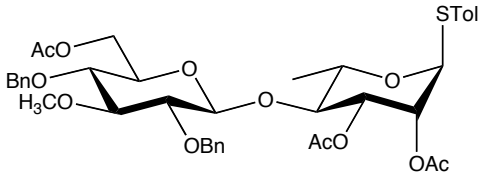
1′), 81.4 (C-3′), 80.1 (C-4′), 78.0 (C-5), 76.8 (C-5′), 72.3 (C-3), 72.1 (C-2), 69.4 (C-4), 65.6 (C-2′), 62.7 (C-6′), 58.7 (O*C*H$_3$), 28.1 ((*C*H$_3$)$_2$C), 26.6 ((*C*H$_3$)$_2$C), 21.2 (*C*H$_3$CO), 21.0 (*C*H$_3$CO), 20.7 (*C*H$_3$CO), 20.9 (Ar*C*H$_3$), 17.4 (C-6). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{29}H_{40}O_{12}SNa$: 635.2133. Found 635.2128.



***p*-Tolyl 3-*O*-methyl-6-*O*-trityl-β-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (2-22)**

To a solution of **2-21** (0.5 g, 0.82 mmol) in 1:1 CH_2Cl_2–CH_3OH (10 mL), 1M $NaOCH_3$ in CH_3OH (1 mL) was added. After stirring for 1 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. To a solution of the resulting oil in pyridine (10 mL), TrCl (0.25 g, 0.89 mmol) was added and the solution was stirred overnight at rt before water (30 mL) was added. The solution was diluted with CH_2Cl_2 (50 mL) and washed with water (2 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **2-22** (0.47 g, 77%) as a colorless oil: R_f 0.38 (2:1 hexane–EtOAc); $[\alpha]_D$ –105.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45–7.42 (m, 5H, Ar), 7.38–7.35 (m, 2H, Ar), 7.31–7.21 (m, 10H, Ar), 7.14–7.12 (m, 2H, Ar), 5.67 (d, 1H, $J_{1,2}$ = 0.5 Hz, H-1), 4.65 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′),

4.35 (dd, 1H, $J_{1,2}$ = 0.5 Hz, $J_{2,3}$ = 5.2 Hz, H-2), 4.28 (dd, 1H, $J_{2,3}$ = 5.2 Hz, $J_{3,4}$ = 8.9 Hz, H-3), 4.16–4.14 (m, 1H, H-5), 3.69–3.64 (m, 1H, H-3′), 3.68 (s, 3H, OCH_3), 3.61 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, H-4), 3.47–3.32 (m, 4H, H-2′, H-5′, H-6′ x 2), 3.19 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.0 Hz, H-4′), 2.34 (s, 3H, ArCH_3), 1.51 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.28 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 143.6 (Ar), 138.0 (Ar), 132.7 (Ar x 2), 129.9 (Ar x 2), 129.4 (Ar), 128.6 (Ar x 6), 127.9 (Ar x 6), 127.1 (Ar x 5), 109.8 ($(CH_3)_2C$), 103.3 (C-1′), 87.0 (Ph_3C), 85.5 (C-1), 84.0 (C-4), 81.4 (C-3), 77.7 (C-2′), 76.3 (C-5), 74.7 (C-4′), 74.5 (C-3′), 71.5 (C-2), 66.0 (C-5′), 63.9 (C-6′), 60.7 (OCH_3), 28.0 ($(CH_3)_2$C), 26.5 ($(CH_3)_2$C), 21.2 (ArCH_3), 17.3 (C-6). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{42}H_{48}O_9NaS$: 751.2911. Found 751.2913.

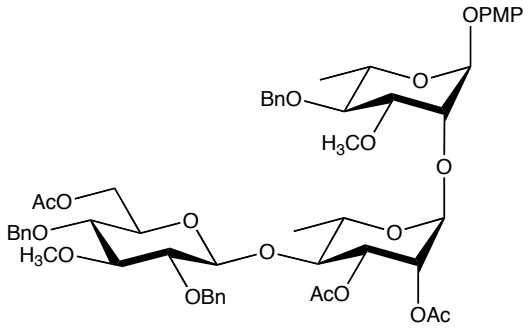


***p*-Tolyl 6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (2-23)**

To a solution of **2-22** (0.45 g, 0.59 mmol) and BnBr (0.2 mL, 1.41 mmol) in DMF (4 mL) at 0 °C was added NaH (60% in mineral oil, 46 mg, 1.9 mmol) portion-wise over 2 min. The reaction mixture was stirred for 4 h at rt and then water (10 mL) was added. The reaction mixture was diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The resulting crude product was dissolved in 1:1 CH_2Cl_2–

CH_3OH (10 mL), *p*-TSA (50 mg, 0.298 mmol) was added and the reaction mixture was stirred overnight before it was neutralized with Et_3N (2 mL). The solution was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. To a solution of the resulting crude product in pyridine (2 mL), Ac_2O (2 mL) was added and the solution was stirred for 3 h before water (10 mL) was added. The solution was diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **2-23** (380 mg, 83%) as a colorless oil: R_f 0.55 (2:1 hexane–EtOAc); $[\alpha]_D$ –49.9 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.27 (m, 12H, Ar), 7.14–7.12 (m, 2H, Ar), 5.48 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.30 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 5.27 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.87, 4.59 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.78, 4.64 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.51 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.37 (dd, 1H, $J_{5',6a'}$ = 2.3 Hz, $J_{6a',6b'}$ = 11.8 Hz, H-6a′), 4.27 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 4.17 (dd, 1H, $J_{5',6b'}$ = 5.3 Hz, $J_{6a',6b'}$ = 11.8 Hz, H-6b′), 3.72 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.64 (s, 3H, OCH_3), 3.49 (ddd, 1H, $J_{4',5'}$ = 9.3 Hz, $J_{5',6a'}$ = 2.3 Hz, $J_{5',6b'}$ = 5.3 Hz, H-5′), 3.39 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.3 Hz, H-4′), 3.33–3.25 (m, 2H, H-2′, H-3′), 2.33 (s, 3H, ArCH_3), 2.17 (s, 3H, CH_3CO), 2.12 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.32 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.6 (*C*=O), 169.9(0) (*C*=O), 169.8(5) (*C*=O), 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 132.6 (Ar x 2), 129.9 (Ar x 2), 129.7 (Ar), 128.5 (Ar x 2), 128.3 (Ar x 2), 128.2(2) (Ar x 2), 128.1(5) (Ar x 2), 128.0

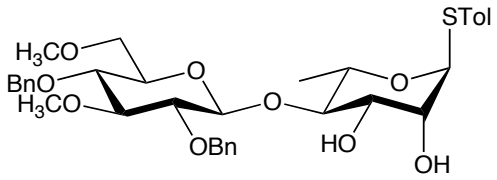
(Ar), 127.6 (Ar), 103.6 (C-1′), 86.9 (C-1), 85.9 (C-2′), 82.0 (C-4′), 77.6 (C-4), 76.8 (C-5), 75.1 (Ar*C*H_2), 74.7 (Ar*C*H_2), 72.4 (C-3′), 71.9 (C-2), 71.7 (C-3), 68.7 (C-5′), 63.1 (C-6′), 61.3 (ArO*C*H_3), 21.1 (*C*H_3CO), 21.0 (*C*H_3CO), 20.8 (*C*H_3CO), 20.7 (Ar*C*H_3), 17.7 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{40}H_{48}O_{12}NaS$: 775.2759. Found 775.2769.



***p*-Methoxyphenyl 6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (2-24)**

To a solution of **2-23** (0.1 g, 0.13 mmol) and **2-8** (0.06 g, 0.16 mmol) in CH_2Cl_2 (10 mL) was added crushed 4 Å molecular sieves (100 mg). After the mixture was stirred at rt for 30 min, it was cooled to –20 °C and then NIS (35.55 mg, 0.14 mmol) and AgOTf (9 mg, 0.04 mmol) were added and the reaction mixture was stirred for another 30 min before it was neutralized with Et_3N (0.5 mL). The solution was concentrated to a crude residue that was purified by chromatography (1:1 hexane–EtOAc) to give **2-24** (0.11 g, 77%) as an amorphous solid: R_f 0.30 (1:1 hexane–EtOAc); $[\alpha]_D$ –58.7 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.26 (m, 15H, Ar), 6.99–6.96 (m, 2H, Ar-2,6), 6.85–6.81 (m, 2H, Ar-3,5),

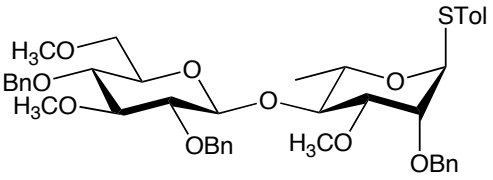
5.47 (dd, 1H, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.4 Hz, H-2′), 5.39 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.35 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.1 Hz, H-3′), 5.05 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.92, 4.67 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.86, 4.50 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.78, 4.62 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.50 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.38 (dd, 1H, $J_{5'',6a''}$ = 2.3 Hz, $J_{6a'',6b''}$ = 11.8 Hz, H-6a″), 4.22–4.21 (m, 1H, H-2), 4.16 (dd, 1H, $J_{5'',6b''}$ = 5.3 Hz, $J_{6a'',6b''}$ = 11.8 Hz, H-6b″), 3.93–3.87 (m, 1H, H-5′), 3.85–3.77 (m, 2H, H-3, H-5), 3.78 (s, 3H, OCH_3), 3.69–3.65 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.1 Hz, H-4′), 3.63 (s, 3H, OCH_3), 3.67 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.52 (s, 3H, OCH_3), 3.50–3.47 (m, 1H, H-5″), 3.30 (app t, 1H, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.7 Hz, H-4″), 3.30 (app t, 1H, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.7 Hz, H-3″), 3.26–3.22 (m, 1H, H-2″), 2.17 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 1.89 (s, 3H, CH_3CO), 1.29 (d, 3H, $J_{6',5'}$ = 6.2 Hz, H-6′), 1.28 (d, 3H, $J_{6,5}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.6 (*C*=O), 169.9 (*C*=O), 169.8 (*C*=O), 154.9 (Ar), 150.2 (Ar), 138.39 (Ar x 2), 137.9 (Ar), 128.5 (Ar x 2), 128.4 (Ar x 2), 128.3 (Ar x 2), 128.2 (Ar x 2), 128.1(3) (Ar x 2), 128.0(6) (Ar x 2), 127.9(6) (Ar), 127.6 (Ar x 2), 117.7 (Ar x 2), 114.6 (Ar x 2), 103.4 (C-1″), 99.0 (C-1), 97.9 (C-1′), 87.0 (C-3″), 81.9 (C-5), 81.4 (C-5′), 80.2 (C-4′), 77.5 (C-4″), 76.7 (C-4), 75.3 (Ar*C*H_2), 75.0 (Ar*C*H_2), 74.7 (Ar*C*H_2), 74.3 (C-2), 72.4 (C-3′), 71.5 (C-2″), 70.2 (C-2′), 68.8 (C-5″), 67.9 (C-3), 63.1 (*C*-6″), 61.3 (O*C*H_3), 58.2 (O*C*H_3), 55.7 (O*C*H_3), 21.1 (*C*H_3CO), 20.7(7) (*C*H_3CO), 20.7(5) (*C*H_3CO), 18.0 (C-6), 17.9 (C-6′). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{54}H_{66}O_{18}Na$: 1025.4142. Found 1025.4141.



***p*-Tolyl 2,4-di-*O*-benzyl-3,6-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-1-thio-α-L-rhamnopyranoside (2-25)**

To a solution of **2-22** (250 mg, 0.34 mmol) and BnBr (0.16 mL, 0.87 mmol) in DMF (3 mL), NaH (60% in mineral oil, 40 mg, 1.3 mmol) was added after the solution was cooled to 0 ºC. After 6 h, water (7 mL) was added and the mixture was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was dissolved in CH_2Cl_2 (10 mL). Anhydrous $ZnBr_2$ (200 mg, 0.88 mmol) was added and the solution was stirred for 20 min before it was filtered and concentrated. To a solution of the resulting crude product and CH_3I (0.15 mL) in DMF (3 mL), NaH (60% in mineral oil, 20 mg) was added and the reaction mixture was stirred for 1 h before water (5 mL) was added. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was dissolved in 1:1 CH_2Cl_2-CH_3OH (10 mL) and *p*-TSA (20 mg) was added. The solution was stirred for 1 h before it was neutralized with Et_3N (0.1 mL). The solution was concentrated and the resulting crude product was purified by chromatography (2:1 EtOAc–hexane) to give **2-25 (**180 mg, 63%) a syrup: R_f 0.4 (2:1 EtOAc–hexane); $[\alpha]_D$ –34.4 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.41 (d, 2H, J = 8.2 Hz, Ar-2,6), 7.39–7.30 (m, 10H, Ar), 7.15 (d, 2H, J = 8.2 Hz, Ar-3,5), 5.29 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-

1), 4.86, 4.64 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.78, 4.64 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.51 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.3 (dq, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.81 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 3.72 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.66 (dd, 1H, $J_{5',6a'}$ = 2.4 Hz, $J_{6a',6b'}$ = 10.9 Hz, H-6a′), 3.63 (s, 3H, OCH_3), 3.60 (dd, 1H, $J_{5',6b'}$ = 4.6 Hz, $J_{6b',6a'}$ = 10.9 Hz, H-6b′), 3.55 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.53–3.50 (m, 1H, H-5′), 3.41 (s, 3H, OCH_3), 3.40–3.38 (m, 1H, H-4′), 3.32–3.26 (m, 2H, H-2′, H-3′), 2.35 (s, 3H, ArCH_3), 1.40 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.5 (Ar), 138.4 (Ar), 138.1 (Ar), 132.6 (Ar x 2), 129.9 (Ar x 2), 129.8 (Ar), 128.4(5) (Ar x 2), 128.3(6) (Ar x 2), 128.2 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar), 127.6 (Ar), 103.7 (C-1′), 86.9 (C-1), 86.0 (C-5), 81.9 (C-4), 77.7 (C-2′), 76.7 (C-3′), 75.1 (ArCH$_2$), 74.8(1) (ArCH$_2$), 74.7(4) (C-2), 72.0 (C-5′), 71.7 (C-3), 71.6 (C-6′), 68.8 (C-4′), 61.3 (OCH$_3$), 59.6 (OCH$_3$), 20.7 (ArCH$_3$), 17.8 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{35}H_{44}O_9SNa$: 663.2604. Found: 663.2603.

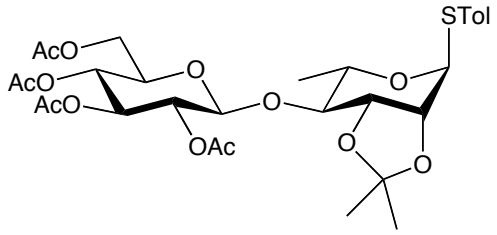


***p*-Tolyl 2,4-di-*O*-benzyl-3′,6′-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-2-*O*-benzyl-3-*O*-methyl-1-thio-α-L- rhamnopyranoside (2-26)**

Diol **2-25** (75 mg, 0.22 mmol) was dissolved in toluene (20 mL) and n-Bu_2SnO (89.4 mg, 0.24 mmol) was added. The reaction mixture was heated to 120 °C and stirred for 1 h, then cooled to 31 ºC before CH_3I (0.16 mL, 2.2 mmol) and n-

Bu_4NI (0.08 g, 0.26 mmol) was added. The reaction mixture was stirred at 31 ºC overnight and then concentrated. The crude product was dissolved in DMF (3 mL) and BnBr (50 μL, 0.28 mmol) was added. To this solution, NaH (60% in mineral oil, 10 mg, 0.28 mmol) was added and the reaction mixture was stirred for 2 h at rt before water (5 mL) was added. The solution was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 10 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated and the resulting crude product was purified by chromatography (4:1 hexane–EtOAc) to give **2-26** (63 mg, 71%) as a colorless oil: R_f 0.35 (3:1 hexane–EtOAc); $[\alpha]_D$ –5.3 (*c*, 1.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45 (d, 2H, J = 8.0 Hz, Ar-2,6), 7.38–7.25 (m, 15H, Ar), 7.13 (d, 2H, J = 8.0 Hz, Ar-3,5), 5.47 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.97, 4.64 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.86–4.82 (m, 3H, H-1′, ArCH_2), 4.52, 4.39 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.18–4.10 (m, 1H, H-5), 3.85 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.75 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.68 (s, 3H, OCH_3), 3.65 (dd, 1H, $J_{3',4'}$ = 3.6 Hz, $J_{4',5'}$ = 11.3 Hz, H-4′), 3.62 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 3.55–3.51 (m, 2H, H-6′ x 2), 3.37 (s, 6H, OCH_3 x 2), 3.33–3.27 (m, 3H, H-2′, H-3′, H-5′), 2.34 (s, 3H, ArCH_3), 1.36 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 141.0 (Ar), 139.0 (Ar), 138.5 (Ar), 138.1 (Ar), 137.5 (Ar), 131.7 (Ar x 2), 130.9 (Ar), 129.9 (Ar x 2), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar x 2), 128.1 (Ar x 2), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.0 (Ar), 102.7 (C-1′), 86.9 (C-1), 85.1 (C-5), 82.7 (C-4), 80.4 (C-2′), 79.4 (C-3), 77.7 (C-3′), 76.7 (C-2), 74.9 (Ar*C*H_2), 74.5 (C-5), 72.6 (Ar*C*H_2), 71.1 (Ar*C*H_2), 68.6 (C-4′), 65.3 (*C*H_2-6′), 61.2 (O*C*H_3-2′), 59.6

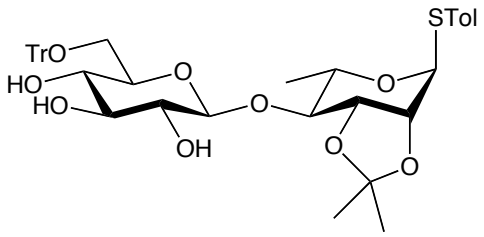
(O*C*H$_3$), 58.3 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 17.9 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{43}H_{52}O_9SNa$: 767.3224. Found: 767.3214.



***p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (2-27)**

To a solution of **2-10** (0.37 g, 1.07 mmol) and **2-11** (0.63 g, 1.29 mmol) in CH_2Cl_2 (30 mL) was added crushed 4 Å molecular sieves (200 mg). After the mixture was stirred at rt for 30 min, the solution was cooled to –40 ºC and then TMSOTf (0.2 mL of 10% solution in CH_2Cl_2) was added and the mixture was stirred for another 1.5 h. After neutralization with Et_3N (1 mL), the solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **2-27** (0.50 g, 81%) as an amorphous solid: R_f 0.38 (3:1–EtOAc); $[\alpha]_D$ –116.4 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.34 (d, 2H, J = 8.0 Hz, Ar-2,6), 7.12 (d, 2H, J = 8.0 Hz, Ar-3,5), 5.63 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 5.24 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 9.2 Hz, H-2′), 5.04 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 10.0 Hz, H-4′), 4.98 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.96 (dd, $J_{2',3'}$ = 9.2 Hz, $J_{3',4'}$ = 10.0 Hz, H-3′), 4.30 (dd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 5.5 Hz, H-2), 4.19 (dd, 1H, $J_{4,5}$ = 10.0 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 4.15 (dd, 1H, $J_{2,3}$ = 5.5 Hz, $J_{3,4}$ = 7.6 Hz, H-3), 4.15–4.06 (m, 2H, H-6′ x 2), 3.67 (ddd, 1H, $J_{4',5'}$ = 10.0 Hz, $J_{5',6a'}$ = 2.5 Hz, $J_{5',6b'}$ = 5.6 Hz, H-5′), 3.60 (dd, 1H, $J_{3,4}$ =

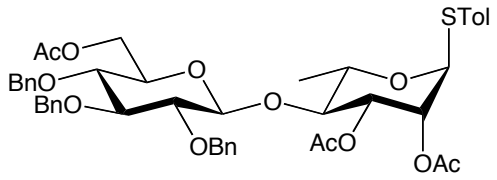
7.6 Hz, $J_{4,5}$ = 10.0 Hz, H-4), 2.33 (s, 3H, ArCH_3), 2.09 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.54 (s, 3H, (CH_3)$_2$C), 1.36 (s, 3H, (CH_3)$_2$C), 1.20 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.5 (*C*=O), 170.3 (*C*=O), 169.6 (*C*=O), 169.5 (*C*=O), 138.0 (Ar), 132.7 (Ar x 2), 129.8 (Ar x 2), 129.2 (Ar), 109.4 ((CH$_3$)$_2$*C*), 99.7 (C-1′), 84.0 (C-1), 80.2 (C-4), 77.8 (C-2), 76.4 (C-5), 72.9 (C-2′), 71.8 (C-3′), 71.5 (C-5′), 68.8 (C-4′), 65.5 (C-3), 62.3 (C-6′), 28.0 ((*C*H$_3$)$_2$C), 26.5 ((*C*H$_3$)$_2$C), 21.1 (*C*H$_3$CO), 20.8 (*C*H$_3$CO), 20.6(4) (*C*H$_3$CO), 20.6(1) (*C*H$_3$CO), 20.5(8) (Ar*C*H$_3$), 17.3 (C-6). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{30}H_{40}O_{13}SNa$: 663.2082. Found: 663.2080.



***p*-Tolyl 6-*O*-trityl-β-D-glucopyranosyl-(1→4)-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside (2-28)**

To a solution of **2-27** (0.26 g, 0.41 mmol) in 1:1 CH_2Cl_2–CH_3OH (10 mL), 1M $NaOCH_3$ in CH_3OH (0.25 mL) was added. After stirring for 1 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. To a solution of the resulting oil in pyridine (10 mL) TrCl (0.13 g, 0.45 mmol) was added and the solution was stirred overnight at rt before water (20 mL) was added. The solution was diluted with CH_2Cl_2 (30 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered,

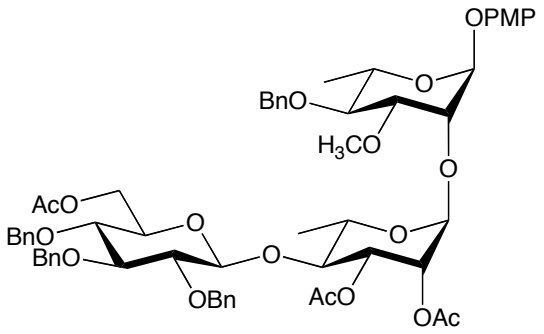
concentrated, and the resulting crude product was purified by chromatography (1:2 hexane–EtOAc) to give **2-28** (0.23 g, 85%) as a colorless oil: R_f 0.30 (1:2 hexane–EtOAc); $[\alpha]_D$ –120.9 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.48–7.46 (m, 5H, Ar), 7.39 (d, 2H, J = 8.0 Hz, Ar-2,6), 7.32–7.22 (m, 10H, Ar), 7.15 (d, 2H, J = 8.0 Hz, Ar-3,5), 5.69 (d,1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.70 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.36 (app t, 1H, $J_{2,3} = J_{3,4}$ = 8.7 Hz, H-3), 4.33 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 8.7 Hz, H-2), 4.19 (dq, 1H, $J_{4,5}$ = 11.0 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.71 (dd, 1H, $J_{3,4}$ = 8.7 Hz, $J_{4,5}$ = 11.0 Hz, H-4), 3.59–3.57 (m, 2H, H-6′ x 2), 3.45–3.34 (m, 4H, H-2′, H-3′, H-4′, H-5′), 2.36 (s, 3H, ArCH_3), 1.52 (s, 3H, (CH_3)$_2$C), 1.38 (s, 3H, (CH_3)$_2$C), 1.32 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 143.7 (Ar x 3), 138.0 (Ar), 132.7 (Ar x 3), 129.9 (Ar x 3), 129.4 (Ar), 128.6 (Ar x 4), 127.9 (Ar x 5), 127.3 (Ar), 127.1 (Ar x 3), 109.9 ((CH$_3$)*C*), 102.7 (C-1′), 86.8 (Ph$_3$*C*), 84.0 (C-1), 80.9 (C-5′), 76.8 (C-4), 76.7 (C-2), 76.4 (C-3), 74.7 (C-2′), 74.4 (C-3′), 71.4 (C-5), 66.0 (C-4′), 63.7 (C-6′), 28.0 ((*C*H$_3$)$_2$C), 26.5 ((*C*H$_3$)$_2$C), 21.2 (Ar*C*H$_3$), 17.3 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{41}H_{46}O_9SNa$: 737.2755. Found: 737.2756.



***p*-Tolyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (2-29)**

To a solution of **2-28** (0.21 g, 0.29 mmol) and BnBr (0.15 mL, 1.05 mmol) in DMF (3 mL) at 0 °C was added NaH (60% in mineral oil, 34 mg, 1.4 mmol). The reaction mixture was stirred for 4 h at rt before water (10 mL) was added. The solution was diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated. The resulting crude product was dissolved in 1:1 CH_2Cl_2–CH_3OH (10 mL) and *p*-TSA (20 mg) was added and the reaction mixture was stirred overnight before it was neutralized with Et_3N (1 mL). The solution was diluted with CH_2Cl_2 (10 mL), and washed with water (2 × 15 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. To a solution of the resulting crude product in pyridine (5 mL) Ac_2O (2 mL) was added and the solution was stirred for 3 h before (10 mL) was added. The solution was diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **2-29** (175 mg, 72%) as a colorless oil: R_f 0.41 (3:1 hexane–EtOAc); $[\alpha]_D$ –38.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.41 (d, 2H, J = 8.0 Hz, Ar-2,6), 7.37–7.27 (m, 15H, Ar), 7.15 (d, 2H, J = 8.0 Hz, Ar), 5.53 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.33–5.30 (m, 2H, H-1, H-3), 4.96, 4.82 (ABq, 2H, J = 10.0 Hz, ArCH_2), 4.89, 4.61 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.83, 4.68 (ABq, J = 11.2 Hz, ArCH_2), 4.59 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.43 (dd, 1H, $J_{5',6a'}$ = 1.6 Hz, $J_{6a',6b'}$ = 11.7 Hz, H-6a′), 4.31 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5.6}$ = 6.1 Hz, H-5), 4.21 (dd, 1H, $J_{5',6b'}$ = 4.6 Hz, $J_{6a',6b'}$ = 11.7 Hz, H-6b′), 3.78 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.67–3.63 (m, 1H, H-5′), 3.59–3.51 (m, 2H, H-3′, H-4′), 3.43 (dd, 1H, $J_{1',2'}$ =

8.0 Hz, $J_{2',3'}$ = 8.9 Hz, H-2′), 2.36 (s, 3H, ArCH_3), 2.15 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.35 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.8 (*C*=O), 170.5 (*C*=O), 170.1 (*C*=O), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 138.0 (Ar), 132.8 (Ar x 2), 130.2 (Ar x 2), 129.9 (Ar), 128.7 (Ar x 2), 128.6(1) (Ar x 2), 128.5(8) (Ar x 2), 128.5 (Ar x 2), 128.3 (Ar x 2), 128.2 (Ar), 127.9(3) (Ar x 3), 127.8(7) (Ar), 103.8 (C-1′), 86.2 (C-1), 85.1 (C-2′), 82.4 (C-4′), 77.9 (C-4), 77.6 (C-5), 75.6 (Ar*C*H_2), 75.4 (Ar*C*H_2), 75.2 (Ar*C*H_2), 72.8 (C-3′), 72.2 (C-2), 71.9 (C-3), 68.9 (C-5′), 63.3 (C-6′), 21.4 (*C*H_3CO), 21.3 (*C*H_3CO), 21.0 (*C*H_3CO), 21.9 (Ar*C*H_3), 17.9 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{46}H_{52}O_{12}NaS$: 851.3072. Found: 851.3059.

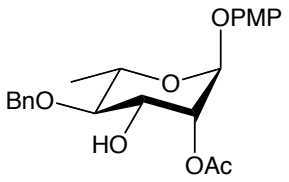


***p*-Methoxyphenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzyl-3-*O*-methyl-α-L-rhamnopyranoside (2-30)**

To a solution of **2-29** (55 mg, 0.06 mmol) and **2-8** (30 mg, 0.08 mmol) in CH_2Cl_2 (20 mL) was added crushed 4 Å molecular sieves (100 mg). After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (20.05 mg, 0.08 mmol) and AgOTf (5 mg, 0.02 mmol) were added and the reaction mixture was

stirred for another 30 min before the reaction was neutralized with Et_3N (1 mL). The solution was filtered and concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **2-30** (52 mg, 73%) as a colorless oil: R_f 0.39 (2:1 hexane–EtOAc); $[\alpha]_D$ –11.1 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.42–7.29 (m, 20H, Ar), 7.01 (d, 2H, J = 9.2 Hz, Ar-2,6), 6.86 (d, 2H, J = 9.2 Hz, Ar-3,5), 5.53–5.52 (m, 1H, H-2′), 5.43–5.38 (m, 2H, H-1, H-3′), 5.09 (m, 1H, H-1′), 4.98–4.58 (m, 8H, ArCH_2 x 4), 4.59 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1″), 4.44 (dd, 1H, $J_{5'',6a''}$ = 1.8 Hz, $J_{6a'',6b''}$ = 11.7 Hz, H-6a″) 4.26–4.25 (m, 1H, H-2), 4.20 (dd, 1H, $J_{5'',6b''}$ = 4.3 Hz, $J_{6b'',6a''}$ = 11.7 Hz, H-6b″), 3.95 (dd, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.88–3.82 (m, 2H, H-3, H-5), 3.74 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, H-4′), 3.80 (s, 3H, ArOCH_3), 3.76–3.58 (m, 3H, H-4, H-4″, H-5″), 3.55 (s, 3H, OCH_3), 3.47–3.39 (m, 2H, H-2″, H-3″), 2.20 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 1.96 (s, 3H,CH_3CO), 1.33 (d, 3H, $J_{5,6}$ = 6.8 Hz, H-6), 1.31 (d, 3H, $J_{5',6'}$ = 6.3 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.5 (*C*=O), 170.2 (*C*=O), 170.0 (*C*=O), 155.2 (Ar), 150.5 (Ar), 138.9 (Ar), 138.6 (Ar), 138.5 (Ar), 138.0 (Ar), 128.7 (Ar x 2), 128.6 (Ar x 2), 128.5 (Ar x 2), 128.3(2) (Ar x 2), 128.2(7) (Ar x 2), 128.2 (Ar x 2), 128.0 (Ar x 2), 127.9 (Ar x 2), 127.8 (Ar x 2), 117.9 (Ar x 2), 117.8 (Ar), 114.8(9) (Ar x 2), 114.8(7) (Ar), 103.9 (C-1″), 99.3 (C-1), 98.2 (C-1′), 82.3 (C-3″), 81.8 (C-5), 81.7 (C-5′), 80.5 (C-4′), 80.1 (C-4), 77.9 (C-4″), 76.8 (C-2″), 75.9 (Ar*C*H_2), 75.6 (Ar*C*H_2), 75.3 (Ar*C*H_2), 75.2 (Ar*C*H_2), 74.6 (C-2′), 72.8 (C-3′), 71.9 (C-2), 70.4 (C-2′), 69.0 (C-5″), 68.1 (C-3), 63.3 (C-6″), 58.4 (O*C*H_3), 55.9 (O*C*H_3), 21.3 (CO*C*H_3), 21.0(5) (CO*C*H_3), 21.0(3) (CO*C*H_3), 18.2

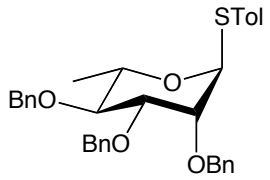
(C-6′), 18.1 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{60}H_{70}O_{18}Na$: 1101.4457. Found: 1101.4454.



***p*-Methoxyphenyl 2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranoside (2-31)**

To a solution of **2-17** (1 g, 2.77 mmol) and trimethyl orthoacetate, (0.71 mL, 5.55 mmol) in CH_2Cl_2 (15 mL) was added CSA (128 mg, 0.55 mmol). The reaction mixture was stirred for 2 h at rt before it was concentrated. The residue was dissolved in 80% aqueous HOAc and stirred for additional 45 min at rt. Water (10 mL) was added, and then the solution was concentrated, diluted with CH_2Cl_2 (20 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give **2-31** (1.04 g, 89%) as a colorless oil: R_f 0.39 (1:1 hexane–EtOAc); $[\alpha]_D$ –70.8 (*c* 1.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.29 (m, 5H, Ar), 6.97–6.94 (m, 2H, Ar-2,6), 6.84–6.80 (m, 2H, Ar-3,5), 5.35 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.29 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 4.86, 4.74 (ABq, 2H, J = 11.3 Hz, ArCH_2), 4.31 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.91 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.76 (s, 3H, ArOCH_3), 3.43 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.19 (s, 3H, CH_3CO), 1.33 (d, 1H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.8 (*C*=O), 155.1 (Ar), 150.1 (Ar), 138.2 (Ar), 128.5 (Ar x 2), 128.0 (Ar), 127.9 (Ar x 2), 117.7 (Ar

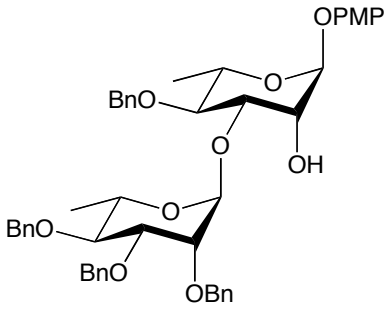
x 2), 114.6 (Ar x 2), 96.4 (C-1), 81.6 (C-2), 75.3 (Ar*C*H_2), 72.6 (C-4), 70.0 (C-3), 68.1 (C-5), 55.6 (ArO*C*H_3), 21.0 (Ar*C*H_3), 18.0 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{22}H_{26}NaO_7$: 425.1571. Found: 425.1571.



***p*-Tolyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (2-32)**

To a solution of **2-13** (2 g, 7.40 mmol) and BnBr (3.17 mL, 26.71 mmol) in DMF (15 mL) at 0 °C was added NaH (60% in mineral oil, 0.84 g, 35.52 mmol) portion-wise over 10 min. The solution was stirred for 4 h at rt before water (10 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (50 mL), and washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting crude product was purified by chromatography (5:1 hexane–EtOAc) to give **2-32** (3.66 g, 88%) as a colorless oil: R_f 0.62 (10:1 hexane–EtOAc); $[\alpha]_D$ –90.0 (*c* 3.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.28 (m, 17H, Ar), 7.11 (d, 2H, J = 8.0 Hz, Ar), 5.44 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.98, 4.68 (ABq, 2H, J = 10.8 Hz, ArCH_2), 5.75–4.62 (m, 4H, ArCH_2 x 4), 4.16 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 4.00 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 3.86 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.70 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.35 (s, 3H, ArCH_3), 1.36 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.8 (Ar), 138.5 (Ar), 138.2 (Ar), 138.0 (Ar), 137.7 (Ar), 132.2 (Ar x 2), 131.1 (Ar), 130.0 (Ar x 3), 128.6(3) (Ar), 128.6(1) (Ar

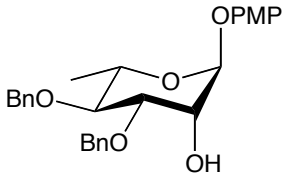
x 2), 128.5(9) (Ar x 3), 128.2 (Ar x 3), 128.0(4) (Ar), 127.9(5) (Ar), 127.9 (Ar), 127.8 (Ar), 86.4 (C-1), 80.8 (C-3), 80.3 (C-4), 76.7 (C-2), 75.7 (Ar*C*H_2), 72.3 (Ar*C*H_2 x 2), 69.5 (C-5), 21.3 (Ar*C*H_3), 18.2 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{34}H_{36}NaO_4S$: 563.2227. Found: 563.2225.



***p*-Methoxyphenyl 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside (2-33)**

To a solution of **2-32** (75 mg, 0.13 mmol) and **2-31** (60 mg, 0.16 mmol) in CH_2Cl_2 (10 mL), was added crushed 4 Å molecular sieves (50 mg). After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (41 mg, 0.16 mmol) and AgOTf (9.7 mg, 0.08 mmol) were added. The reaction mixture was stirred for another 30 min before it was neutralized with Et_3N (1 mL). The solution was filtered and concentrated to a crude residue that was dissolved in 1:1 CH_2Cl_2–CH_3OH (5 mL) and 1M $NaOCH_3$ in CH_3OH (0.2 mL) was added. After stirring for 1 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated (2:1 hexane–EtOAc) to give **2-33** (87.5 mg, 87%) as a colorless oil: R_f 0.45 (2:1 hexane–EtOAc); $[\alpha]_D$ –15.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.33–7.24 (m, 20H, Ar), 6.98–6.96 (m, 2H, Ar-

2,6), 6.83–6.81 (m, 2H, Ar-3,5), 5.36 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1), 5.17 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1′), 4.93, 4.67 (ABq, 2H, J = 10.9 Hz, ArCH_2), 4.66–4.54 (m, 6H, ArCH_2 x 3) 4.16–4.14 (m, 2H, H-2, H-3), 3.88–3.82 (m, 3H, H-3′, H-5, H-5′), 3.78–3.76 (m, 1H, H-2′), 3.77 (s, 3H, ArOCH_3), 3.66 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.46 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.2 Hz, H-4′), 2.27 (br s, 1H, OH-2), 1.37 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.22 (d, 3H, $J_{5',6'}$ = 6 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.2 (Ar), 138.4(1) (Ar x 2), 138.3(5) (Ar x 2), 138.2 (Ar x 2), 128.5 (Ar x 2), 128.4(1) (Ar x 2), 128.3(6) (Ar x 2), 128.1 (Ar x 2), 127.8 (Ar x 2), 127.6(2) (Ar x 4), 127.5(9) (Ar x 2), 127.3 (Ar x 2), 117.6 (Ar x 2), 114.6 (Ar x 2), 100.1 (C-1′), 98.0 (C-1), 80.3 (C-4′), 80.0 (C-4), 79.6(1) (C-5′), 79.5(8) (C-5), 75.8 (C-2), 75.4 (ArCH$_2$), 75.0 (ArCH$_2$), 72.7 (ArCH$_2$), 72.3 (ArCH$_2$), 70.9 (C-3), 69.2 (C-2′), 68.2 (C-3′), 55.6 (ArOCH$_3$), 18.1 (C-6′), 17.9 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{47}H_{52}O_{10}Na$: 799.3453. Found: 799.3447.



***p*-Methoxyphenyl 3,4-*O*-dibenzyl-α-L-rhamnopyranoside (2-34)**

Diol **2-17 (**2 g, 5.54 mmol) was dissolved in toluene (60 mL) and *n*-Bu_2SnO (1.38 g, 5.54 mmol) was added. The reaction mixture was heated to 120 °C and stirred for 1 h, then cooled to 62 ºC before BnBr (0.73 mL, 6.07 mmol) and *n*-Bu_4NI (2.40 g, 6.52 mmol) was added. The reaction mixture was stirred at 62 ºC for 7 h

and then cooled and concentrated. The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **2-34** (2.04 g, 82%) as a colorless oil: R_f 0.39 (2:1 hexane–EtOAc); $[\alpha]_D$ –127.0 (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.41–7.31 (m, 10H, Ar), 6.99–6.95 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.44 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.91, 4.67 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.77 (s, 2H, ArCH_2), 4.22–4.21 (m, 1H, H-2), 4.04 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.87 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.78 (s, 3H, ArOCH_3), 3.53 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 1.28 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.8 (Ar), 150.1 (Ar), 138.2 (Ar), 137.8 (Ar), 128.5 (Ar x 2), 128.3 (Ar x 2), 127.9 (Ar), 127.9 (Ar x 2), 127.8 (Ar x 2), 127.7 (Ar), 97.8 (C-1), 79.8(2) (C-3), 79.7(6) (C-2), 75.4 (ArCH$_2$), 72.1 (ArCH$_2$), 68.5 (C-4), 67.9 (C-5), 55.5 (ArOCH$_3$), 17.8 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{27}H_{30}O_6Na$: 473.1940. Found: 473.1941.

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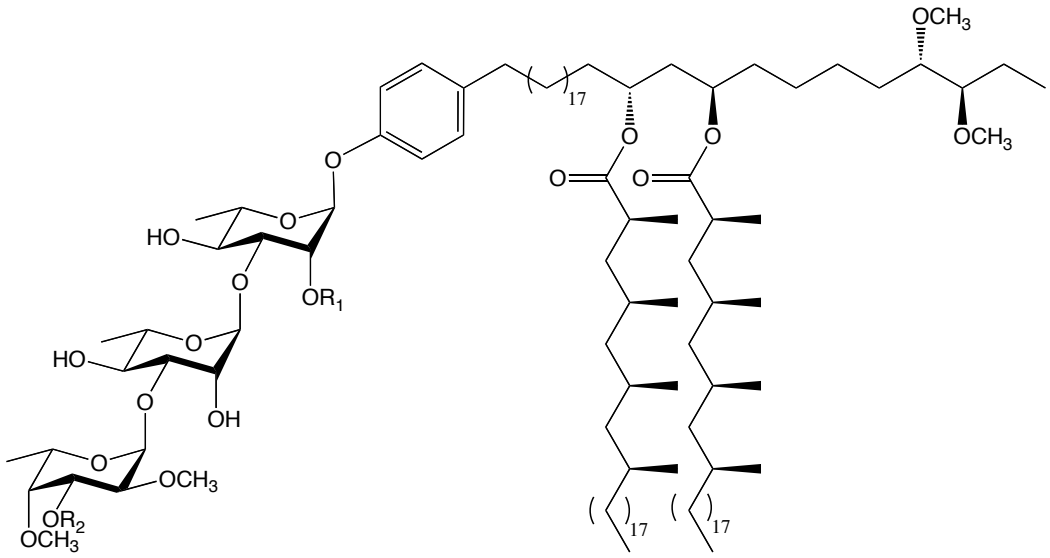
Chapter 3

Synthesis of Carbohydrate Cores of Phenolic Glycolipids from *Mycobacterium tuberculosis*

3.1. Introduction

Tuberculosis (TB) continues to be a serious bacterial infection with severe impacts on patients as well as the economy.[2,3] As mentioned in the introduction, (Chapter 1) the severity and complications of TB as well as the difficulties in its treatment are due in part to the complex nature of the cell wall.[4-6] Moreover, the emergence of multidrug resistant strains (MDR) and the rise in HIV co-infections pose a new worldwide threat and increased interest in the development of new anti-TB drugs.[7,8]

As mentioned in Chapter 2 (Section 2.1), we are interested in one of the structural components of the cell wall known as phenolic glycolipids (PGLs), polyketide synthase-derived virulence factors, which was first discovered in *Mycobacterium tuberculosis*.[9–11] It was shown that some strains of *M. tuberculosis* can produce three PGLs named as Tb1, Tb-O, and Tb-K, which all have the same lipid core and differ only in the carbohydrate domain (Figure 3-1).[12-14]



Tb1 = $R_1 = R_2 = CH_3$

Tb-O = $R_1 = H, R_2 = CH_3$

Tb-K = $R_1 = CH_3, R_2 = H$

Figure 3-1. Structures of PGLs from *M. tuberculosis.*

As in the case of PGLs from *M. leprae* (Chapter 2, Section 2.1.), despite all that is known about PGLs from *M. tuberculosis*, their immunomodulatory properties still remain to be investigated. We therefore chose to synthesize a panel of synthetic analogs of the carbohydrate cores of the three PGLs from *M. tuberculosis*, Tb1, Tb-O, and Tb-K, bearing *p*-methoxyphenyl moiety at the reducing end to mimic the native PGLs. Once in hand, these molecules were used to study their effects on cytokine production by the activated macrophages as well as the ability of these compounds to induce apoptosis. In this chapter, the synthetic routes used to make these analogs will be described (Figure 3-2).

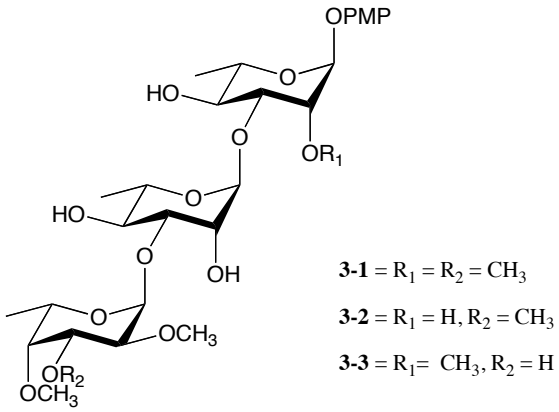


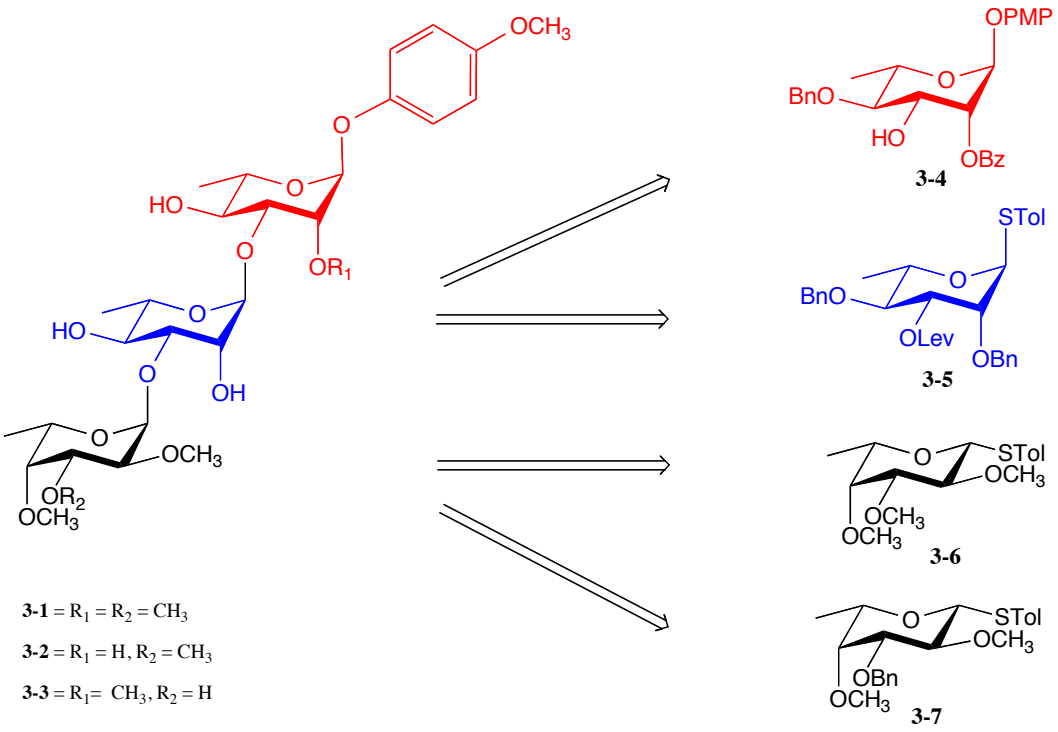
Figure 3-2. Structures of synthetic analogues of PGLs from *M. tuberculosis*.

3.2. Results and Discussions

3.2.1. Retrosynthetic analysis of 3-1–3-3

As illustrated in Scheme 3-1, the retrosynthetic analysis of the targets showed that **3-1**–**3-3** could be synthesized from four building blocks: **3-4**–**3-7**. Compounds **3-4** and **3-5** are rhamnose based. Building block **3-4** has a *p*-methoxyphenyl moiety at C-1 as it represents the "reducing" end of this series. Furthermore, in this compound O-2 is protected as benzoate ester, O-4 as a benzyl ether, and O-3 is unprotected and is ready for coupling with another sugar unit. The second rhamnose-based building block, compound **3-5**, is a thioglycoside that has both O-2 and O-4 protected as benzyl ethers while O-3 is protected as a levulinate ester, which can be selectively removed in the presence of other esters.[22] Finally, the other two building blocks, **3-6** and **3-7**, are fucose based and have different methylation patterns. Compound **3-6** is a tri-*O*-methylated fucose thioglycoside while **3-7** is methylated at both O-2 and O-4, whereas O-3 is protected as a benzyl ether. Having access to these four building blocks,

completing the synthesis of the target compounds, **3-1**, **3-2**, and **3-3** was achieved via assembly of them, as well as further protecting group manipulation.



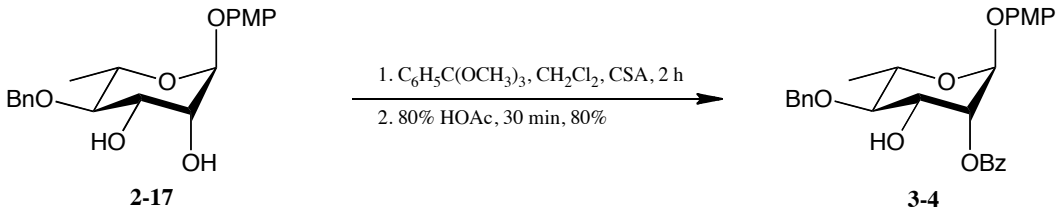
Scheme 3-1. Retrosynthetic analysis of trisaccharides **3-1**, **3-2**, and **3-3**.

3.2.2. Synthesis of Building blocks 3-4, 3-5, 3-6, and 3-7

3.2.2.1 Synthesis of building block 3-4

Advanced intermediate **2-17** (see Scheme 2-3 for the preparation of this compound), was used as the starting material for the synthesis of **3-4**. As described in Scheme 3-2, the synthesis started with the selective protection of O-2 of **2-17** using an orthoester formation–hydrolysis sequence.[15] Thus, glycoside **2-17** was treated with trimethyl orthobenzoate and CSA to form an orthoester

intermediate between O-2 and O-3. Subsequent acid-catalyzed hydrolysis of this intermediate using 80% aqueous acetic acid furnished an 80% overall yield of **3-4**. The regioselectivity of the process was established by ^{1}H NMR and ^{13}C NMR spectroscopy. The location of the benzoyl group in **3-4** was identified by the downfield chemical shift of both H-2 (δ_H = 4.46 ppm) and C-2 (δ_C = 81.6 ppm) in the ^{1}H and ^{13}C NMR spectra, respectively. For the starting material, **2-17**, these resonances appear at δ_H = (4.14–4.10 ppm, overlapped with H-3), and δ_C = 71.0 ppm in the ^{1}H NMR spectrum and ^{13}C NMR spectrum, respectively.



2-17 **3-4**

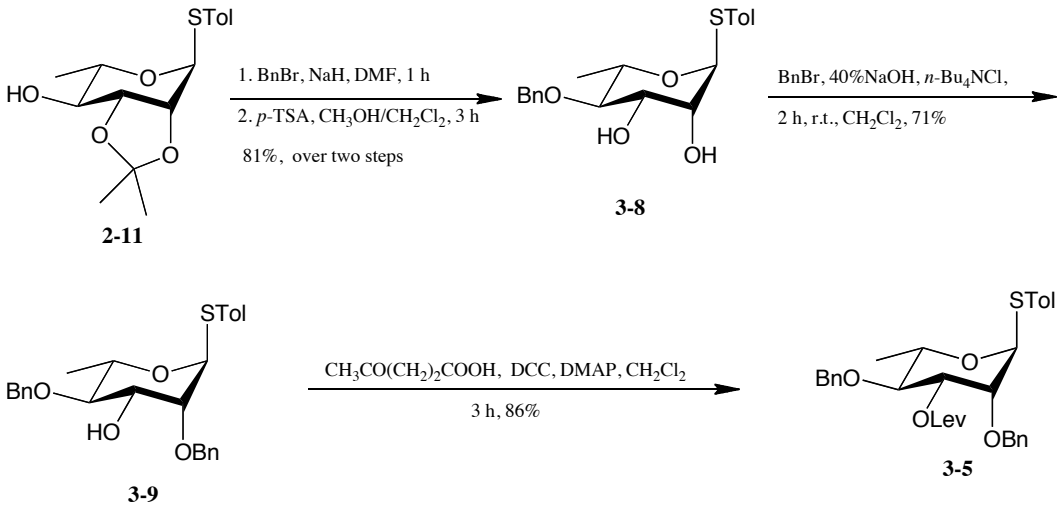
Scheme 3-2. Synthesis of compound **3-4**.

3.2.2.2 Synthesis of 3-5.

As illustrated in Scheme 3-3, to access compound **3-5**, the synthesis started from the advanced intermediate **2-11**, which was prepared as described in Chapter 2 (see Scheme 2-2). First, O-4 was protected as a benzyl ether upon treatment of **2-11** with benzyl bromide and sodium hydride.[16] Subsequent hydrolysis of the isopropylidene acetal using a catalytic amount of *p*-TSA provided intermediate **3-8** in 81% yield over two steps.

The next step was to regioselectively benzylate O-2. This goal was accomplished in one step using phase transfer alkylation,[17] which afforded a 71%

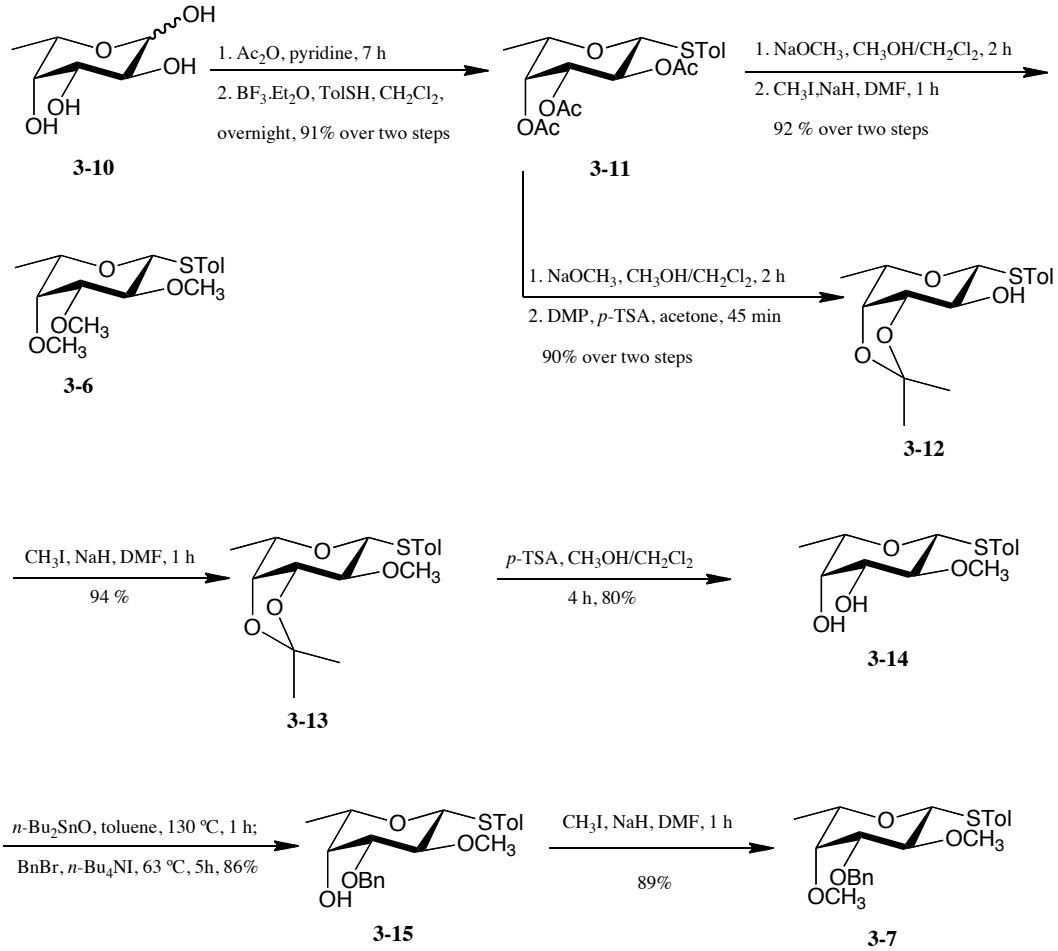
yield of the target **3-9**. This transformation was achieved via treatment of compound **3-8** in CH_2Cl_2 with tetra-*n*-butylammonium chloride (*n*-Bu_4NCl), a phase transfer agent, and 40% aqueous sodium hydroxide. Subsequently, benzyl bromide was added while the solution was being vigorously stirred and the reaction progress monitored by TLC. The regioselectivity of the reaction was confirmed from the chemical shifts of H-2 (δ_H = 4.06–4.01 ppm, overlapped with H-3) and C-2 (δ_C = 68.6 ppm) in the ^1H and ^{13}C NMR spectra, respectively. The upfield shift of these values from the original values of compound **3-8**, H-2 (δ_H = 4.2 ppm) and C-2 (δ_C = 72.8 ppm), confirmed the position of the benzyl group to be at O-2. Further support of the regioselectivity was obtained from the downfield chemical shift of H-3 (δ_H = 5.26 ppm) of compound **3-8** after performing an acetylation experiment. Finally, treatment of compound **3-9** with levulinic acid[18] and *N,N′*-dicyclohexylcarbodiimide (DCC) in the presence of catalytic amount of 4-(dimethylamino)pyridine (DMAP) afforded the target **3-5** in 86% yield.



Scheme 3-3. Synthesis of thioglycoside **3-5**.

3.2.2.3 Synthesis of building blocks 3-6 and 3-7

Scheme 3-4 details the construction of building blocks **3-6** and **3-7**. The synthesis started by peracylation of commercially available L-fucose, **3-10**, by treatment with acetic anhydride and pyridine. The peracylated intermediate was subsequently subjected to $BF_3.Et_2O$-promoted glycosylation[19] with (TolSH) affording a 91% yield of thioglycoside **3-11** over the two steps. Zemplén deacylation (sodium methoxide in methanol) followed by methylation of the resulting three hydroxyl groups using methyl iodide and sodium hydride in DMF provided the target **3-6** in a total yield of 92% over two steps.



Scheme 3-4. Synthesis of building blocks **3-6** and **3-7.**

To complete the synthesis of **3-7**, the triol resulting after Zemplén deacylation of intermediate **3-11** was treated with DMP in acetone and catalytic amount of *p*-TSA. This two-step sequence provided the isopropylidene acetal-protected intermediate **3-12** in 90% yield over two steps. Subsequently, the remaining hydroxyl group was protected via treatment with methyl iodide and sodium hydride affording the O-2 methyl protected intermediate **3-13** in 94% yield. Then, the isopropylidene acetal group was removed upon treatment of **3-13** with a catalytic amount of *p*-TSA to furnish diol **3-14** in 80% yield. The next step was the regioselective benzylation of the resulting diol at O-3, via formation of an organotin intermediate.[20] First, compound **3-14** was heated with dibutyltin oxide in toluene at reflux for 1 h to furnish the stannylidene acetal intermediate. Subsequent treatment of this intermediate with benzyl bromide and *n*-Bu_4NI provided an 86% yield of **3-15**.

The regioselectivity of benzylation was confirmed by ^1H NMR and ^{13}C NMR spectroscopy of the product. That the benzylation had occurred at O-2 was apparent from the chemical shifts of both H-3 (δ_H = 3.48 ppm) and C-3 (δ_C = 82.9 ppm). These resonances are further upfield shifted from where they occur in the ^1H and ^{13}C NMR spectra of compound **3-14**, H-3 (δ_H = 3.61–3.57 ppm, overlapped with H-5) and C-3 (δ_C = 86.0 ppm). Finally, an 89% yield of the target **3-7** was obtained after the sodium hydride-mediated methylation of **3-15** was performed.

3.2.3. Assembly of building blocks to synthesis 3-1, 3-2 and 3-3

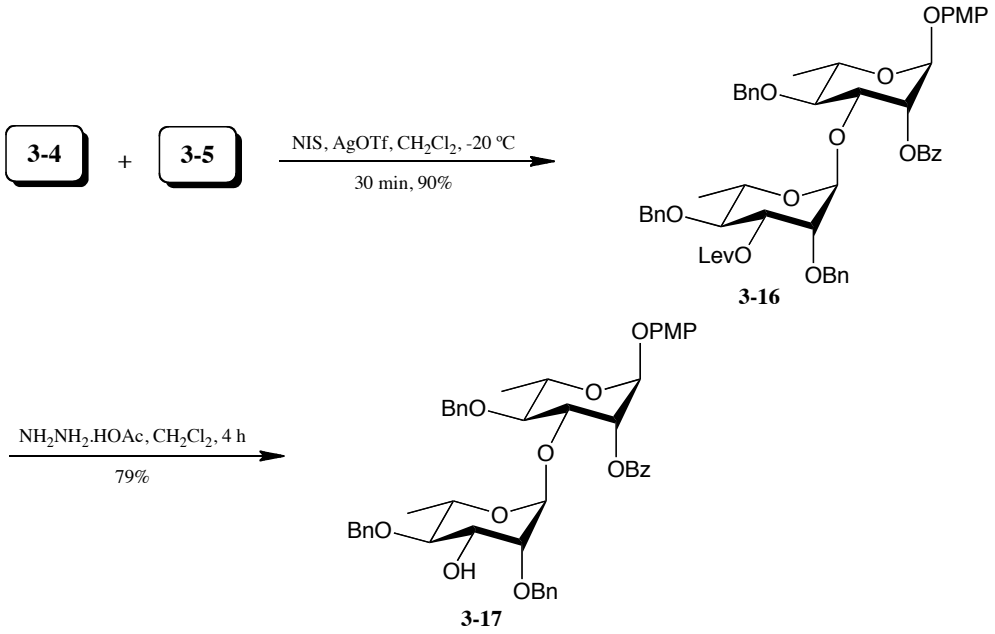
With building blocks **3-4**–**3-7** in hand, the synthesis of the target trisaccharides could, in turn, be completed via their coupling and further protecting group manipulations.

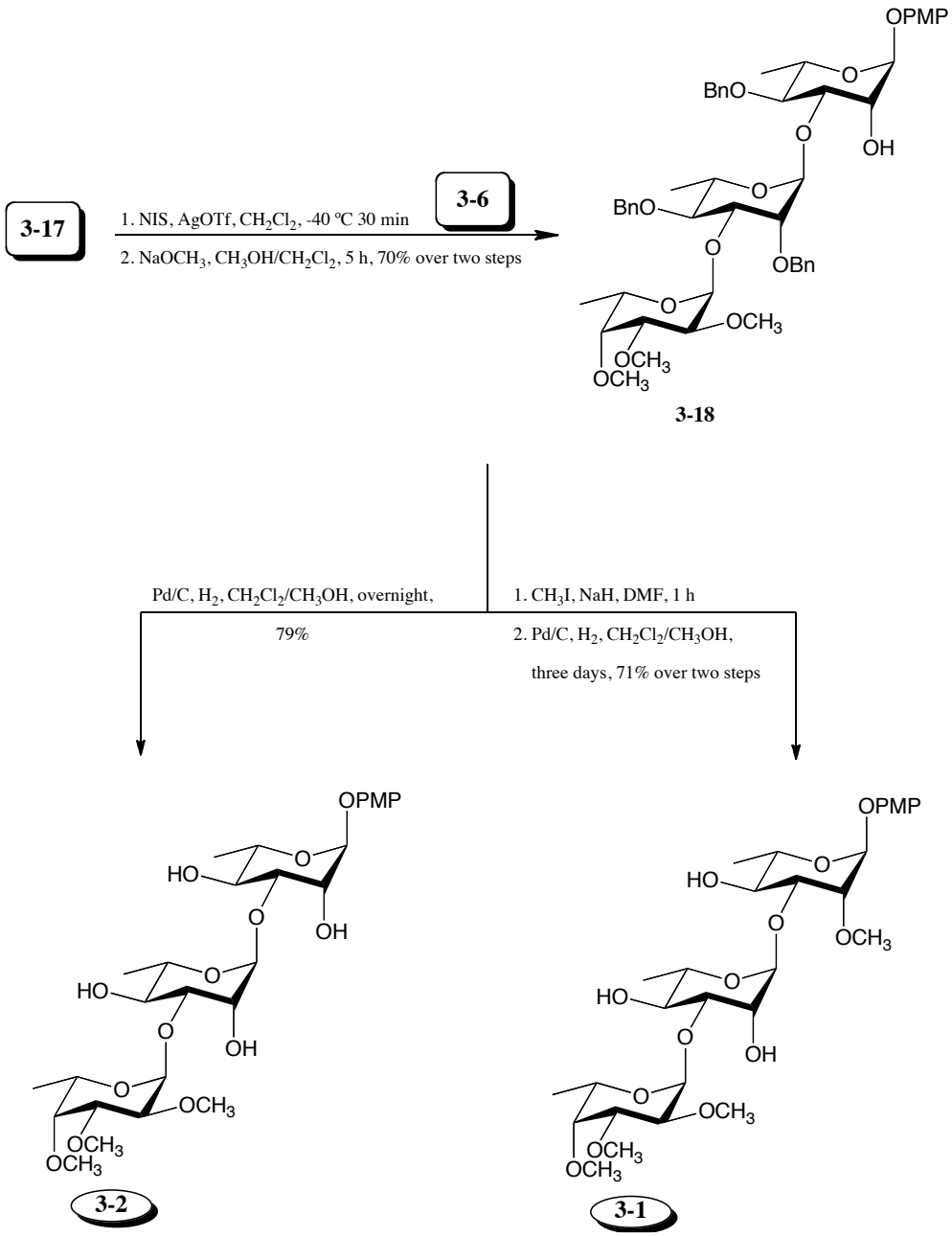
3.2.3.1. Synthesis of trisaccharides 3-1 and 3-2

As described in Scheme 3-5, the synthesis of **3-1** started by the construction of disaccharide **3-16**. To achieve this goal, donor **3-5** and acceptor **3-4** were coupled through NIS–AgOTf[21] promoted glycosylation to afford disaccharide **3-16** in 90% yield. The newly formed glycosidic linkage in disaccharide **3-16** was exclusively an α-(1→3) linkage, which was confirmed by a coupled HSQC experiment. From this experiment, $^{1}J_{C\text{-}1',H\text{-}1'}$ was found to have a value of 169 Hz, which is consistent with the α-stereochemistry.[24] Subsequently, treatment of disaccharide **3-16** with hydrazine acetate[22] resulted in selective removal of the levulinoyl group at O-3′ in the presence of the benzoyl group at O-2 affording **3-17** in 79% yield.

The next step was the construction of trisaccharide **3-18**, which was achieved via NIS–AgOTf mediated coupling of thioglycoside **3-6** and acceptor disaccharide **3-17**. Unexpectedly, this reaction failed to give the desired α-glycoside; instead, a 1:1 α/β mixture was obtained, which was not separable by chromatography. To overcome this problem, different reaction temperatures were evaluated ranging from rt to –78 ºC; however, there was no significant improvement of the α/β ratio. Fortunately, the use of an "inverse" glycosylation

procedure[23] circumvented this problem. In this procedure, a solution of the donor was added slowly to the acceptor–promotor solution over 10 minutes at –40 ºC. This addition sequence provided the α-anomer exclusively in 70% yield and we were not able to detect any β-anomer by TLC or NMR spectroscopy. The stereochemistry of the newly formed glycosidic linkage was confirmed by measuring the $^{1}J_{C\text{-}1'',H\text{-}1''}$ from a coupled HSQC experiment. The value, 170.0 Hz, was consistent with the α-stereochemistry.[24] Subsequently, Zemplén deacylation[21] of **3-18** gave alcohol **3-19** in 78% yield. The resulting hydroxyl group was protected as methyl ether using methyl iodide and sodium hydride.[16] Finally, debenzylation[19] using palladium on charcoal under a hydrogen atmosphere afforded the target compound **3-1** in 71% yield over two steps. With the advanced trisaccharide intermediate **3-19** in hand, completing the synthesis of **3-2** was achieved in 79% yield over one step via debenzylation[19] by hydrogenolysis over palladium on charcoal overnight (Scheme 3-5).

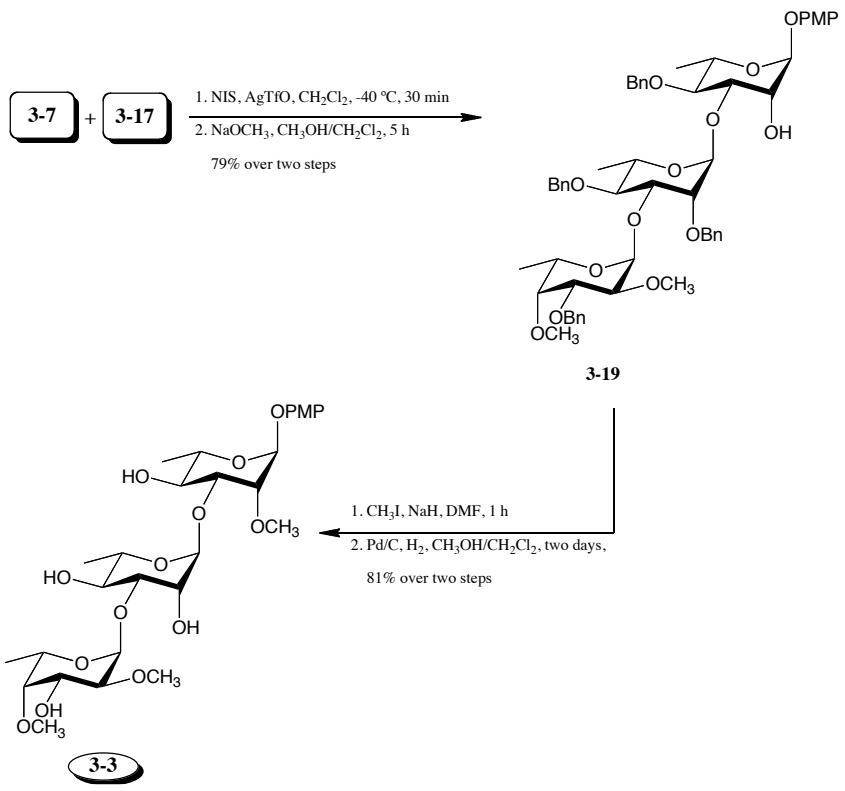




Scheme 3-5. Synthesis of trisaccharides **3-1** and **3-2**.

3.2.3.2. Synthesis of trisaccharide 3-3

Intermediate **3-17**, which was used in the assembly of compound **3-1**, was also used a starting material in the synthesis of the target trisaccharide **3-3**. As illustrated in Scheme 3-6, the synthesis started by fucosylation of acceptor **3-17** with donor **3-7** using NIS–AgOTf mediated inverse glycosylation[23] as previously described for the preparation of **3-18** (see Scheme 3-5). Subsequent Zemplén deacylation furnished trisaccharide **3-19** in 79% yield over two steps. That the required α linkage had obtained was clearly evident from the value of $^{1}J_{C\text{-}1'',H\text{-}1''}$ which was found to be 169.8 Hz. The synthesis was completed by methylation of O-2 and then hydrogenolysis over palladium on charcoal to afford an 81% yield of the target trisaccharide **3-3** over two steps (Scheme 3-7).



Scheme 3-6. Synthesis of trisaccharide **3-3**.

3.3. Summary

We completed the synthesis of a panel of three synthetic analogs of the PGLs from *M. tuberculosis* (**3-1, 3-2** and **3-3**) using efficient linear synthetic routes in very good overall yields. In the synthesis of the rhamnose-based building blocks (**3-5**) regioselective benzylation of the axial hydroxyl group (compound **3-9**, Scheme 3-3) was achieved using phase transfer alkylation, whereas the regioselective benzylation of the equatorial hydroxyl group (compound **3-15**, Scheme 3-4) was achieved via a stannylidene acetal. In both cases, the location of the newly installed alkyl group was confirmed by the chemical shifts of the appropriate resonances in the ^{1}H and ^{13}C NMR spectra. The trisaccharides were obtained as α-anomers only via fucosylation of a disaccharide acceptor, **3-17**, using a glycosylation procedure in which the sequence of the addition of donor and acceptor of a NIS–AgOTf mediated glycosylation was inversed compared to the normal method. Use of the more traditional method for the glycosylation resulted in a mixture of glycosides being formed. The correct stereochemistry of all glycosidic linkages were confirmed by performing coupled HSQC experiments, and measuring the $^{1}J_{C\text{-}1,H\text{-}1}$ of the newly introduced glycosdic residue.

3.4. Experimental

3.4.1. General Methods

Solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere before use. All reagents used in reactions were purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV_{254} (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by charring with a solution of anisaldehyde in ethanol, acetic acid, and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). The ratio between silica gel and residue ranged from 100:1 to 20:1 (w/w). Organic solutions were concentrated under vacuum at < 50 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 or 500 MHz. ^{1}H NMR chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.23, $CDCl_3$). ^{1}H NMR data are reported as though they are first order and the peak assignments were made on the basis of 2D-NMR (^{1}H–^{1}H COSY and HMQC) experiments. The monosaccharide residues in the disaccharide and trisaccharides are labelled by no prime, prime, and double prime as shown in Figure 3-3 and these labels are maintained in the assignment of NMR spectra of all compounds. Optical rotations were measured at 21 ± 2 °C at the sodium D line (589 nm) and are in units of deg•mL(dm•g)$^{-1}$. ESI-MS spectra were carried out on samples suspended in THF or CH_3OH and added NaCl.

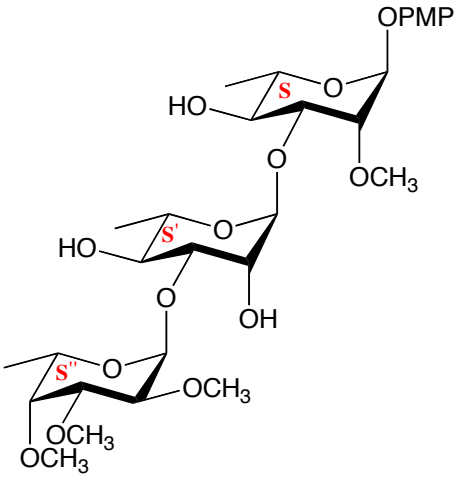
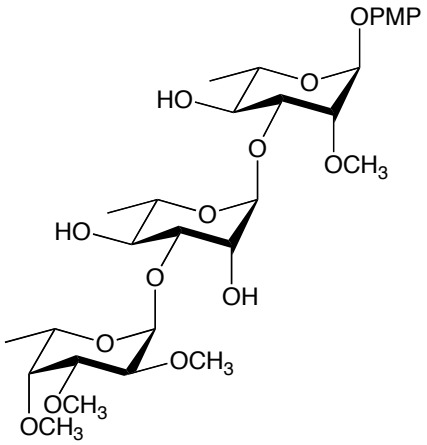


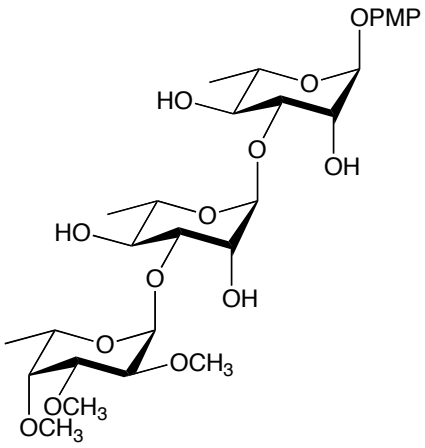
Figure 3-3. Numbering system used to label data.



***p*-Methoxyphenyl 2,3,4-tri-*O*-methyl-α-L-fucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranoside (3-1)**

To a solution of **3-19** (12 mg, 0.01 mmol) and CH_3I (20 μL) in DMF (2 mL) at 0 ºC, NaH (60% in mineral oil, 4 mg) was added. The reaction mixture was stirred for 1 h before water (5 mL) was added. The solution was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4),

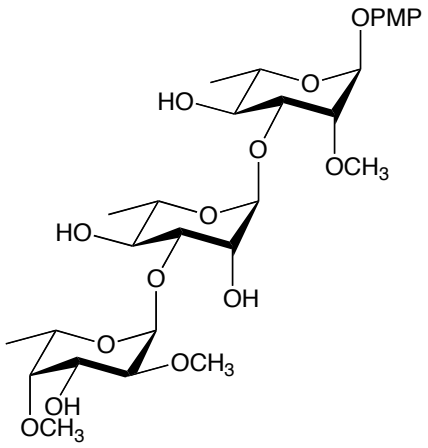
filtered, concentrated and the resulting oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (20 mL) and Pd–C (2.5 mg, 20% w/w) was added. The solution was stirred for 2 days under a hydrogen atmosphere before it was filtered, concentrated and purified by chromatography (15:1 CH_2Cl_2–CH_3OH) to give **3-1**(6 mg, 71%) as an amorphous solid: R_f 0.55 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –14.8 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.02–6.99 (m, 2H, Ar-2,6), 6.84–6.81 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.14–5.13 (m, 2H, H-1′, H-1″), 4.11 (dd, 1H, $J_{1',2'}$ = 1.8 Hz, $J_{2',3'}$ = 3.4 Hz, H-2′), 4.05 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.0 Hz, H-5), 4.03 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.93 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.79 (dd. 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.78–3.76 (m, 1H, H-5″), 3.77 (s, 3H, ArOC*H*$_3$), 3.74 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.69–3.61 (m, 4H, H-2″, H-4, H-4′, H-4″), 3.58 (s, 3H, OC*H*$_3$), 3.57 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.49 (s, 3H, OC*H*$_3$), 3.47–3.46 (m, 1H, H-3″), 1.36 (d, 3H, $J_{5'',6''}$ = 6.0 Hz, H-6″), 1.29 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6), 1.26 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.5 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 102.1 (C-1′), 100.8 (C-1″), 95.6 (C-1), 83.1 (C-3′), 81.0 (C-3), 80.0 (C-3″), 79.8 (C-4″), 79.0 (C-4), 78.8 (C-4′), 71.8 (C-2′), 71.6 (C-2″), 71.2 (C-2), 69.0 (C-5′), 68.7 (C-5), 67.5 (C-5″), 61.8 (O*C*H$_3$), 60.2 (O*C*H$_3$), 58.6 (O*C*H$_3$), 57.7 (O*C*H$_3$), 55.7 (O*C*H$_3$), 17.9 (C-6), 17.8 (C-6′), 16.7 (C-6″). (ESI) Calcd. for $(M + Na)^+$ $C_{29}H_{46}O_{14}Na$: 641.2785. Found 641.2784.



***p*-Methoxyphenyl 2,3,4-tri-*O*-methyl-α-L-fucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranoside (3-2)**

To a solution of **3-19** (15 mg, 0.02 mmol) in 1:1 CH_2Cl_2–CH_3OH (20 mL), Pd–C (3 mg, 20% w/w) was added and the reaction mixture was stirred for 3 days under a hydrogen atmosphere. The solution was then filtered, concentrated and the resulting residue was purified by chromatography (15:1 CH_2Cl_2–CH_3OH) to give **3-2** (8.5 mg, 71%) as an amorphous solid: R_f 0.45 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –12.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.84 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.37 (br s, 1H, H-1), 5.19 (br s, 1H, H-1′), 5.16 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1″), 4.16 (d, 1H, $J_{2',3'}$ = 3.3 Hz, H-2′), 4.07 (dq,1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 4.07 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 4.03 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.87 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.83–3.78 (m, 2H, H-2, H-5″), 3.77 (s, 3H, ArO*C*H$_3$), 3.69–3.62 (m, 4H, H-2″, H-4, H-4′, H-4″), 3.59 (s, 3H, O*C*H$_3$), 3.57 (s, 3H, O*C*H$_3$), 3.51 (s, 3H, O*C*H$_3$), 3.47–3.46 (m, 1H, H-3″), 1.35 (d, 3H, $J_{5'',6''}$ = 6.0 Hz, H-6″), 1.28 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.26 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); ^{13}C NMR (125.7 MHz,

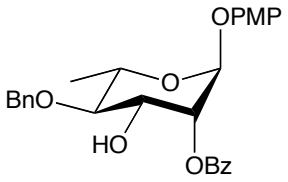
$CDCl_3$, δ_C) 155.0 (Ar), 150.2 (Ar), 117.6 (Ar x 2), 114.6 (Ar x 2), 101.8 (C-1″), 100.7 (C-1′), 98.4 (C-1), 82.8 (C-3′), 80.8 (C-3), 78.9(1) (C-3″), 78.9(0) (C-4″), 78.7 (C-4), 72.1 (C-2′), 71.5 (C-2), 71.0 (C-4′), 70.8 (C-2″), 69.0 (C-5′), 68.8 (C-5), 67.5 (C-5″), 61.9 (O*C*H_3), 60.2 (O*C*H_3), 57.7 (O*C*H_3), 55.7 (O*C*H_3), 17.7 (C-6), 17.6 (C-6′), 16.7 (C-6″). (ESI) Calcd. for $(M + Na)^+$ $C_{28}H_{44}O_{14}Na$: 627.2629. Found 627.2628.



***p*-Methoxyphenyl 2,4-di-*O*-methyl-α-L-fucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranoside (3-3)**

To a solution of **3-20** (15 mg, 0.02 mmol) and CH_3I (20 μL, 0.27 mmol) in DMF (2 mL) at 0 ºC, NaH (60% in mineral oil, 5 mg) was added. The solution was stirred for 1 h before water (5 mL) was added. The mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (20 mL) and Pd–C (3 mg) was added. The solution was stirred for 2 days under a hydrogen atmosphere before it was filtered. The filtrate was

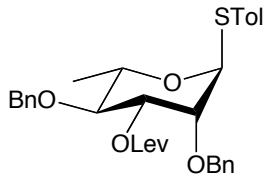
concentrated and the resulting residue was purified by chromatography (15:1 CH_2Cl_2–CH_3OH) to give **3-3** (7.7 mg, 81%) as an amorphous solid: R_f 0.39 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –18.4 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00–6.97 (m, 2H, Ar-2,6), 6.84–6.81 (m, 2H, Ar-3,5), 5.41 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.26 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 5.02 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.15 (dq,1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 4.05 (dd,1H, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.2 Hz, H-2′), 3.98–3.96 (m, 2H, H-3, H-3″), 3.81 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.76 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 3.69 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.72 (s, 3H, ArOC*H*$_3$), 3.68–3.63 (m, 1H, H-4′), 3.61–3.56 (m, 1H, H-5″), 3.55 (s, 3H, OC*H*$_3$), 3.52–3.48 (m, 1H, H-4″), 3.51 (s, 3H, OC*H*$_3$), 3.50 (s, 3H, OC*H*$_3$) , 3.45–3.42 (m, 1H, H-2″), 3.33–3.32 (m, 1H, H-4), 1.29 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″), 1.20 (d, 3H, $J_{5',6'}$ = 6.4 Hz, H-6′), 1.19 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 156.6 (Ar), 151.7 (Ar), 119.0 (Ar x 2), 115.7 (Ar x 2), 104.0 (C-1″), 100.0 (C-1′), 97.5 (C-1), 84.5 (C-3″), 81.6 (C-3), 80.6 (C-3′), 80.2 (C-4′), 79.3 (C-4), 73.5 (C-2), 73.1 (C-2′), 72.1 (C-2″), 71.1 (C-5), 70.9 (C-4″), 70.5 (C-5′), 68.0 (C-5″), 62.7 (O*C*H$_3$), 59.5 (O*C*H$_3$), 58.7 (O*C*H$_3$), 56.2 (O*C*H$_3$), 18.2 (C-6), 18.0 (C-6″), 16.8 (C-6′). (ESI) Calcd. for $(M + Na)^+$ $C_{28}H_{44}O_{14}Na$: 627.2623. Found 627.2629.



_p_-Methoxyphenyl 4-_O_-benzyl-2-_O_-benzoyl-α-L-rhamnopyranoside (3-4)

To a solution of *p*-methoxyphenyl 4-*O*-benzyl-α-L-rhamnopyranoside, (**2-17**, 1 g, 2.77 mmol) and trimethyl orthobenzoate, (0.95 mL, 5.54 mmol) in CH_2Cl_2 (15 mL), CSA (128 mg, 0.55 mmol) was added. The reaction mixture was stirred for 2 h at rt before it was concentrated and dissolved in 80% aqueous HOAc (10 mL) and stirred for additional 30 min at rt. Water (20 mL), was added and the reaction mixture was concentrated, diluted with CH_2Cl_2 (30 mL), and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **3-4** (1.02 g, 80%) as a colorless oil: R_f 0.42 (2:1 EtOAc-hexane); $[\alpha]_D$ –30.4 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.09–8.04 (m, 2H, Ar), 7.64–7.27 (m, 8H, Ar), 6.99 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.82 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.53 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 5.49 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.88, 4.79 (ABq, 2H, J = 11.1 Hz, ArCH_2), 4.43 (ddd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.2 Hz, $J_{3,OH\text{-}3}$ = 5.0 Hz, H-3), 3.98 (dq, 1H, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.78 (s, 3H, ArOCH_3), 3.56 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 2.22 (d, 1H, $J_{3,OH\text{-}3}$ = 5.0 Hz, OH-3), 1.38 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (*C*=O), 155.0 (Ar), 150.1 (Ar), 138.0 (Ar), 133.4 (Ar), 129.8 (Ar x 2), 129.5 (Ar), 128.5 (Ar x 2), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.9 (Ar), 117.6 (Ar x 2), 114.5 (Ar x 2), 96.4 (C-1), 81.6 (C-2), 75.1 (Ar*C*H_2), 73.0 (C-4), 70.3 (C-3), 68.1 (C-5),

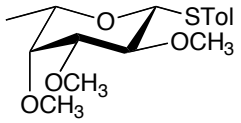
55.6 (ArO*C*H_3), 18.1 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{27}H_{28}O_7Na$: 487.1733. Found: 487.1729.



***p*-Tolyl 2,4-di-*O*-benzyl-3-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (3-5)**

To a solution of **3-9** (1 g, 2.22 mmol) and levulinic acid (380 mg, 3.33 mmol) in CH_2Cl_2 (20 mL) was added DCC (0.65 g, 3.33 mmol) and DMAP (135 mg, 1.11 mmol). The reaction mixture was stirred for 3 h before it was filtered. The filtrate was concentrated and the resulting residue was purified by chromatography (4:1 hexane–EtOAc) to afford **3-5** (1.32 g, 86%) as a colorless oil: R_f 0.6 (4:1 hexane–EtOAc); $[\alpha]_D$ –73.7 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.28 (m, 12H, Ar), 7.13 (d, 2H, J = 8.0 Hz, Ar), 5.40 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.23 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 4.78, 4.68 (ABq, 2H, J = 11.3 Hz, ArCH_2), 4.69, 4.58 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.26 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 4.13 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.73 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.76–2.44 (m, 4H, CH_2CH_2), 2.35 (s, 3H, CH_3CO), 2.18 (s, 3H, ArCH_3), 1.38 (d, 3H, $J_{6,5}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 206.4 (*C*=O), 172.3 (*C*=O), 138.5 (Ar), 138.0 (Ar), 137.8 (Ar), 132.4 (Ar x 2), 130.8 (Ar), 130.1 (Ar x 2), 128.7 (Ar x 3), 128.2 (Ar x 2), 128.1 (Ar x 2), 128.0 (Ar x 3), 86.2 (C-1), 79.4 (C-3), 77.8 (C-4), 72.9 (C-5), 69.3 (C-2), 38.1

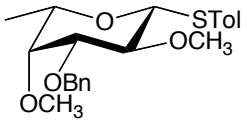
(CH_3CH_2), 30.1 (CH_3CO), 28.3 (CH_2CO), 21.4 ($ArCH_3$), 18.2 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{32}H_{36}O_6SNa$: 571.2125. Found 571.2125.



***p*-Tolyl 2,3,4-tri-*O*-methyl-1-thio-β-L-fucopyranoside (3-6)**

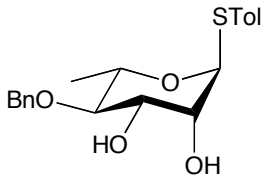
To a solution of **3-11** (2 g, 5.05 mmol) in 1:1 CH_2Cl_2–CH_3OH (30 mL), 1M $NaOCH_3$ in CH_3OH (3 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. To the solution of the resulting oil and CH_3I (1.1 mL, 17.53 mmol) in DMF (10 mL), NaH (60% in mineral oil, 0.56 g, 23.37 mmol) was added over 5 min at 0 °C. The reaction mixture was stirred for 1 h at rt before it was water (20 mL) was added. The solution was diluted with CH_2Cl_2 (30 mL) and washed with 1M HCl soln (20 mL) and brine (30 mL). The organic layer was dried with Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (3:1 hexane-EtOAc) to afford **3-6** (1.44 g, 92%) as an amorphous solid: R_f 0.42 (3:1 hexane–EtOAc); $[\alpha]_D$ –41.9 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45 (d, 2H, J = 8.1 Hz, Ar-2,6), 7.07 (d, 2H, J = 8.1 Hz, Ar-3,5), 4.38 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 3.57 (s, 6H, OCH_3 x 2), 3.51 (s, 3H, OCH_3), 3.46 (dq, 1H, $J_{4,5}$ = 2.7 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.38–3.33 (m, 2H, H-2, H-4), 3.18 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 2.30 (s, 3H, ArCH_3), 1.29 (d, 3H, $J_{6,5}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 137.2 (Ar), 132.4 (Ar), 131.8 (Ar), 130.6 (Ar), 129.7 (Ar), 129.4 (Ar), 88.0 (C-1), 86.2 (C-3), 79.1 (C-2), 78.6

(C-4), 74.1 (C-5), 61.8 (O*C*H$_3$), 61.0 (O*C*H$_3$), 58.3 (O*C*H$_3$), 19.0 (Ar*C*H$_3$), 14.6 (C-6). (ESI) Calcd. for (M + Na)$^+$ $C_{16}H_{24}O_4SNa$: 335.1293. Found 335.1293.



***p*-Tolyl 3-*O*-benzyl-2,4-di-*O*-methyl-1-thio-β-L-fucopyranoside (3-7)**

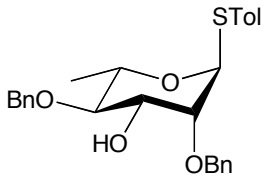
To a solution of **3-15** (0.8 g, 2.13 mmol) and CH_3I (0.2 mL, 2.55 mmol) in DMF (10 mL) at 0 °C was added NaH (60% in mineral oil, 82 mg, 3.39 mmol). The reaction mixture was stirred for 1 h at rt before water (20 mL) was added. The solution was diluted with CH_2Cl_2 (30 mL) and washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **3-7** (0.74 g, 89%) as a colorless oil: R_f 0.46 (5:1 hexane–EtOAc); $[\alpha]_D$ –36.0 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.48 (d, 2H, *J* = 8.1 Hz, Ar-2,6), 7.41–7.26 (m, 5H, Ar), 7.10 (d, 2H, *J* = 8.1 Hz, Ar-3,5), 4.78, 4.72 (ABq, 2H, *J* = 11.9 Hz, ArC*H*$_2$), 4.42 (d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 3.63 (s, 3H, OC*H*$_3$), 3.63 (s, 3H, OC*H*$_3$), 3.51–3.42 (m, 3H, H-2, H-3, H-5), 3.33–3.31 (m, 1H, H-4), 1.30 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6), ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.5 (Ar), 137.2 (Ar), 132.4 (Ar x 2), 130.7 (Ar), 129.5 (Ar x 2), 128.4 (Ar x 2), 127.7 (Ar), 127.6 (Ar x 2), 88.2 (C-1), 84.0 (C-2), 79.7 (C-4), 79.4 (C-3), 74.5 (C-5), 72.7 (Ar*C*H$_2$), 61.8 (O*C*H$_3$), 61.2 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 17.0 (C-6). (ESI) Calcd. for (M + Na)$^+$ $C_{22}H_{28}O_4SNa$: 411.1601. Found 411.1604.



***p*-Tolyl 4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (3-8)**

To a solution of **2-11** (2 g, 6.45 mmol) and BnBr (0.92 mL, 7.73 mmol) in DMF (15 mL), NaH (60% in mineral oil, 0.25 g, 10.3 mmol) was added portion-wise at 0 °C over 2 min. The reaction mixture was stirred for 1 h at rt before water (30 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (60 mL) and washed with water (2 × 50 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated and the resulting residue was carried to the next step without further purification. To a solution of the residue in 3:1 CH_3OH–CH_2Cl_2 (20 mL) was added *p*-TSA (40 mg, 20% w/w) and the reaction mixture was stirred for an additional 3 h. The reaction mixture was then neutralized with Et_3N (1 mL). The solution was concentrated, and the resulting residue was purified by chromatography (2:1 EtOAc–hexane) to give **3-8** (1.88 g, 81%) as an amorphous solid: R_f 0.36 (2:1 EtOAc–hexane); $[\alpha]_D$ –152.7 (*c*, 1.3 CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.41–7.30 (m, 7H, Ar), 7.12 (d, 2H, J = 7.9 Hz, Ar), 5.40 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.78, 4.75 (ABq, 2H, J = 11.0 Hz, ArC*H*$_2$), 4.25 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 4.18 (ddd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.4 Hz, $J_{2,OH\text{-}2}$ = 3.9 Hz, H-2), 3.96 (ddd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.4 Hz, $J_{3,OH\text{-}3}$ = 5.3 Hz, H-3), 3.44 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.87 (d, 1H, $J_{2,OH\text{-}2}$ = 3.9 Hz, OH-2), 2.63 (d, 1H, $J_{3,OH\text{-}3}$ = 5.3 Hz, OH-3), 2.33 (s, 3H, ArC*H*$_3$), 1.36 (d, 1H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.1 (Ar), 137.6 (Ar), 132.0 (Ar x 2),

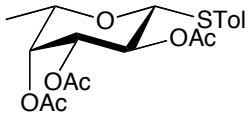
130.1 (Ar), 129.8 (Ar), 128.6 (Ar x 2), 128.0 (Ar x 2), 127.9 (Ar x 2), 87.7 (C-1), 81.8 (C-4), 75.0 (Ar*C*H_2), 72.5 (C-2), 71.8 (C-3), 68.5 (C-5), 21.0 (Ar*C*H_3), 17.8 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{20}H_{24}O_4SNa$: 383.1288. Found: 383.1291.



***p*-Tolyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside (3-9)**

To a solution of **3-8** (1.5 g, 4.16 mmol), BnBr (0.58 mL, 4.16 mmol) and *n*-Bu_4NCl (1.15 g, 4.16 mmol) in CH_2Cl_2 (20 mL), 40% NaOH (1 mL) was added and the reaction mixture was stirred vigorously for 2 h at rt. The solution was then neutralized with 1M HCl (2 mL), diluted with CH_2Cl_2 (40 mL) and washed with satd aq $NaHCO_3$ soln (50 mL) and brine (50 mL). The organic layer was dried with Na_2SO_4, filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to afford **3-9** (1.32 g, 71%) as an amorphous solid: R_f 0.31 (2:1 hexane–EtOAc); $[\alpha]_D$ –81.7 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.44–7.32 (m, 12H, Ar), 7.16 (d, 2H, J = 8.0 Hz, Ar), 5.55 (br s, 1H, H-1), 4.97, 4.72 (ABq, J = 11.0 Hz, ArCH_2), 4.77, 4.57 (ABq, J = 11.0 Hz, ArCH_2), 4.24 (dq, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 4.06–4.01 (m, 2H, H-2, H-3), 3.46 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 2.49 (d, 1H, J = 9.0 Hz, OH-3), 2.38 (s, 3H, ArCH_3), 1.40 (d, 1H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.6 (Ar), 137.7 (Ar), 137.47 (Ar), 132.2 (Ar x 2), 130.6 (Ar), 129.9 (Ar x 2), 128.7 (Ar x 2), 128.5 (Ar x 2), 128.2 (Ar), 128.1 (Ar x 2), 128.0

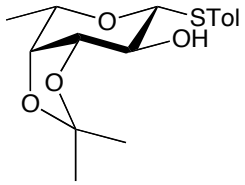
(Ar x 2), 127.8 (Ar), 85.9 (C-1), 82.5 (C-4), 80.1 (C-5), 75.2 (Ar*C*H_2), 72.4 (Ar*C*H_2), 72.1 (C-3), 68.6 (C-2), 21.2 (Ar*C*H_3), 18.0 (C-6). (ESI) Calcd. for (M + Na) $^+$ $C_{27}H_{30}O_4SNa$: 473.1757. Found 473.1756.



***p*-Tolyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranoside (3-11)**

To a solution of L-fucose, **3-10** (5 g, 30.48 mmol) in pyridine (25 mL) at 0 °C was added Ac_2O (20 mL). The reaction mixture was stirred for 7 h at rt before water (100 mL) was added. The solution was diluted with CH_2Cl_2 (150 mL) and washed with 1M HCl soln (100 mL x 2), satd aq $NaHCO_3$ soln (100 mL), water (100 mL x 2), and brine (100 mL). The organic layer was dried with Na_2SO_4, filtered and the resulting oil was purified by chromatography (3:1 hexane–EtOAc) to afford **3-10** (10.98 g, 91%) as colorless oil: R_f 0.55 (3:1 hexane–EtOAc); $[\alpha]_D$ –113.7 (*c* 0.6, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.40 (d, 2H, *J* = 8.2 Hz, Ar-2,6), 7.11 (d, 2H, *J* = 8.2 Hz, Ar-3,5), 5.23 (dd, 1H, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 2.8 Hz, H-4), 5.19 (app t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.9 Hz, H-2), 5.02 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 4.63 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 3.79 (dq, 1H, $J_{4,5}$ = 2.8 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 2.33 (s, 3H, ArCH_3), 2.13 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.22 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.6 (*C*=O), 170.1 (*C*=O), 169.5 (*C*=O), 138.2 (Ar), 132.9 (Ar x 2), 129.6 (Ar x 2), 129.1 (Ar), 86.9 (C-1), 73.1 (C-4), 72.5 (C-3), 70.4 (C-2), 67.4 (C-5), 21.2

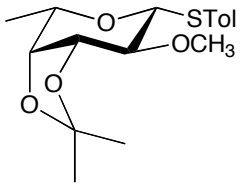
(CH$_3$CO), 20.9 (CH$_3$CO), 20.7 (CH$_3$CO), 20.6 (ArCH$_3$), 16.5 (C-6). HRMS (ESI) calcd $(M + Na)^+$ $C_{19}H_{24}O_7SNa$: 419.1141. Found: 419.1139.



***p*-Tolyl 3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (3-12)**

To a solution of **3-11** (5 g, 12.62 mmol) in 1:1 CH_2Cl_2–CH_3OH (50 mL), 1M $NaOCH_3$ in CH_3OH (5 mL) was added. After stirring for 2 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin and filtered. The filtrate was concentrated, and the resulting oil and was dissolved in acetone (50 mL) to which 2,2-dimethoxypropane (4.52 mL, 36.92 mmol) and *p*-TSA (200 mg) were added. The reaction mixture was stirred for 40 min at rt, neutralized with Et_3N (3 mL), diluted with CH_2Cl_2 (100 mL) and washed with satd. aq. $NaHCO_3$ soln. (100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (1:1 hexane–EtOAc) to afford **3-12** (3.5 g, 90%) as an amorphous solid: R_f 0.32 (1:1 hexane–EtOAc); $[\alpha]_D$ –81.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.46–7.44 (m, 2H, Ar-2,6), 7.14–7.12 (m, 2H, Ar-3,5), 4.36 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 4.06–4.02 (m, 2H, H-3, H-4), 3.85 (dq, 1H, $J_{4,5}$ = 2.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.51 (ddd, 1H, $J_{1,2}$ = 10.2 Hz, $J_{2,3}$ = 8.4 Hz, $J_{2,OH\text{-}2}$ = 2.2 Hz, H-2), 2.45 (d, 1H, $J_{OH\text{-}2,2}$ = 2.2 Hz, OH-2), 2.32 (s, 3H, ArCH_3), 1.44 (s, 3H, $(CH_3)_2$C), 1.42 (s, 3H, $(CH_3)_2$C), 1.35 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.3

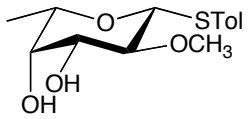
(Ar), 133.2 (Ar x 2), 129.7 (Ar x 2), 128.3 (Ar), 109.8 ($(CH_3)_2C$), 88.2 (C-1), 79.1 (C-3), 76.4 (C-4), 72.8 (C-5), 71.3 (C-2), 28.1 ($(CH_3)_2C$), 26.4 ($(CH_3)_2C$), 21.1 ($ArCH_3$), 17.0 (C-6). HRMS (ESI) Calcd. for (M + Na) $C_{16}H_{22}O_4NaS$: 333.1131. Found 333.1135.



***p*-Tolyl 3,4-*O*-isopropylidene-2-*O*-methyl-1-thio-β-L-fucopyranoside (3-13)**

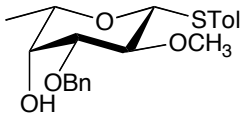
To a solution of **3-12** (2 g, 6.45 mmol) and CH_3I (0.5 mL, 7.73 mmol) in DMF (15 mL), at 0 °C, NaH (60% in mineral oil, 0.25 g, 10.3 mmol) was added portion-wise over 10 min. The reaction mixture was stirred for 4 h at rt before it water (30 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (100 mL), and washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting residue was purified by chromatography (3:1 hexane–EtOAc) to give **3-13** (1.96 g, 94%) as a colorless oil: R_f 0.42 (3:1 hexane–EtOAc); $[\alpha]_D$ –41.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.45–7.43 (m, 2H, Ar-2,6), 7.10–7.08 (m, 2H, Ar-3,5), 4.41 (d, 1H, J = 9.7 Hz, H-1), 4.09 (app t, 1H, $J_{1,2} = J_{2,3}$ = 9.7 Hz, H-2), 4.00 (dd, 1H, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 2.1 Hz, H-4), 3.76 (dq, 1H, $J_{4,5}$ = 2.1 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.52 (s, 3H, ArOCH_3), 3.20 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 2.31 (s, 3H, ArCH_3), 1.46 (s, 3H, $(CH_3)_2C$), 1.37 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.33 (s, 3H, $(CH_3)_2C$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 137.6 (Ar), 132.9 (Ar x 2), 129.6

(Ar), 129.5 (Ar x 2), 109.6 ($(CH_3)_2C$), 86.3 (C-1), 80.4 (C-3), 79.7 (C-2), 76.4 (C-5), 72.4 (C-4), 59.6 ($ArOCH_3$), 28.0 ($(CH_3)_2C$), 26.4 ($(CH_3)_2C$), 21.1 ($ArCH_3$), 21.08 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{17}H_{24}O_4SNa$: 347.1293. Found 347.1289.



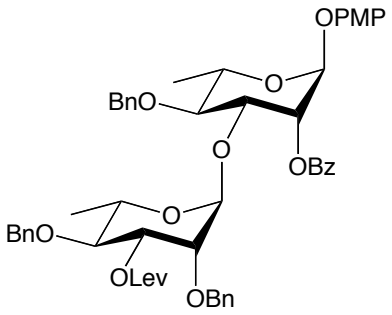
***p*-Tolyl 2-*O*-methyl-1-thio-β-L-fucopyranoside (3-14)**

To a solution of **3-13** (1.5 g, 4.62 mmol) in 3:1 CH_3OH–CH_2Cl_2 (20 mL) was added *p*-TSA (300 mg, 20% w/w) and the reaction mixture was stirred for 4 h, and then neutralized with Et_3N (2 mL). The solution was concentrated and the resulting residue was purified by chromatography (1:2 hexane–EtOAc) to give **3-14** (1.1 g, 80%) as an amorphous solid: R_f 0.3 (1:2 hexane–EtOAc); $[\alpha]_D$ –25.9 (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.44 (d, 2H, J = 8.1 Hz, Ar-2,6), 7.10 (d, 2H, J = 8.1 Hz, Ar-3,5), 4.43 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 3.74–3.73 (m, 1H, H-4), 3.64 (s, 3H, OCH_3), 3.61–3.57 (m, 2H, H-3, H-5), 3.22 (app t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.6 Hz, H-2), 2.78 (br s, 1H, OH), 2.31 (s, 3H, $ArCH_3$), 2.20 (br s, 1H, OH), 1.32 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 137.8 (Ar), 132.5 (Ar x 2), 129.8 (Ar), 129.6 (Ar x 2), 87.5 (C-1), 80.0 (C-3), 75.5 (C-2), 74.4 (C-5), 71.9 (C-4), 61.2 (OCH_3), 21.1 ($ArCH_3$), 16.6 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{14}H_{20}O_4SNa$: 307.0980. Found 307.0980.



***p*-Tolyl 3-*O*-benzyl-2-*O*-methyl-1-thio-β-L-fucopyranoside (3-15)**

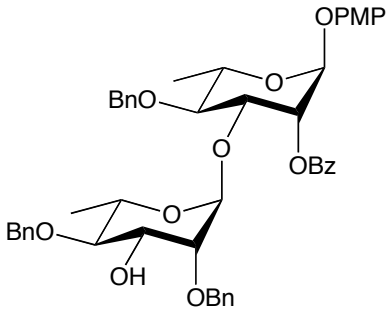
Diol **3-14** (1g, 3.5 mmol) was dissolved in toluene (30 mL) and *n*-Bu_2SnO (0.96 g, 3.85 mmol) was added. The reaction mixture was stirred for 1 h at 120 °C, then cooled to 62 ºC before BnBr (0.5 mL, 3.85 mmol) and *n*-Bu_4NI (1.42 g, 3.85 mmol) were added. The reaction mixture was stirred at 62 ºC for 7 h and then cooled and concentrated. The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **3-15** (0.85 g, 86%) as a colorless oil: R_f 0.35 (2:1 hexane–EtOAc); $[\alpha]_D$ +6.6 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.49 (d, 2H, *J* = 8.2 Hz, Ar-2,6), 7.39–7.32 (m, 5H, Ar), 7.12 (d, 2H, *J* = 8.2 Hz, Ar-3,5), 4.76, 4.70 (ABq, 2H, *J* = 11.9 Hz, ArC*H*$_2$), 4.44 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 3.81–3.97 (m, 1H, H-4), 3.63 (s, 3H, OC*H*$_3$), 3.54 (dq, 1H, $J_{4,5}$ = 1.9 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.48 (dd, 1H, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.39–3.34 (m, 1H, H-2), 2.35 (s, 3H, ArC*H*$_3$), 2.25 (d, 1H, $J_{4,OH\text{-}4}$ = 3.5 Hz, OH-4), 1.34 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, CDCl3, δ_C) 137.9 (Ar), 137.6 (Ar), 132.7 (Ar x 2), 129.9 (Ar), 129.6 (Ar x 2), 128.5 (Ar x 2), 128.0 (Ar), 127.8 (Ar x 2), 87.6 (C-1), 82.9 (C-2), 78.5 (C-3), 74.2 (C-5), 72.1 (Ar*C*H$_2$), 69.5 (C-4), 61.2 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 16.7 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{21}H_{26}O_4SNa$: 397.1448. Found 397.1449.



***p*-Methoxyphenyl 2,4-di-*O*-benzyl-3-*O*-levulinyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-benzoyl-α-L-rhamnopyranoside (3-16)**

To a solution of **3-4** (0.5 g, 1.07 mmol) and **3-5** (0.65 g, 1.2 mmol) in CH_2Cl_2 (30 mL), crushed 4 Å molecular sieves (300 mg) was added. After the reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (292 mg, 1.3 mmol) and AgOTf (62 mg, 0.24 mmol) were added and the solution was stirred for another 30 min. The reaction mixture was then neutralized with Et_3N (1 mL) and the solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **3-16** (0.85 g, 90%) as a syrup: R_f 0.29 (2:1 hexane–EtOAc); $[\alpha]_D$ –19.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.08 (m, 2H, Ar), 7.61–7.58 (m, 1H, Ar), 7.50–7.47 (m, 2H, Ar), 7.46–7.22 (m, 13H, Ar), 7.22–7.11 (m, 2H, Ar), 6.99 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.83 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.55 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 5.51 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.19 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 5.10 (d, 1H, $J_{1',2'}$ = 2.2 Hz, H-1′), 4.92, 4.72 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.58, 4.51 (ABq, 2H, J = 11.7 Hz, ArCH_2), 4.48, 4.42 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.39 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.97 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.91 (dd, 1H, $J_{1',2'}$ = 2.2 Hz, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.81 (dq, 1H, $J_{4',5'}$

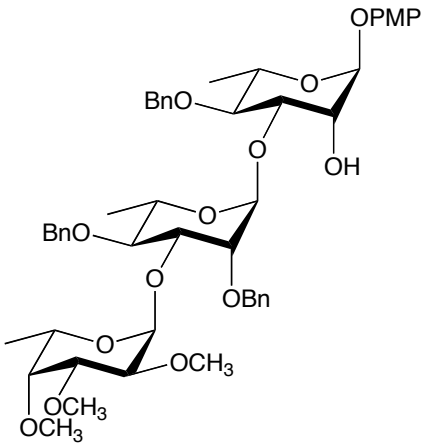
= 9.4 Hz, $J_{5',6'}$ = 6.3 Hz, H-5`), 3.66 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.58 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.4 Hz, H-4′), 2.65–2.35 (m, 4H, CH_2CH_2), 2.11 (s, 3H, CH_3-CH_2), 1.32 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6), 1.16 (d, 3H, $J_{5',6'}$ = 6.3 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 206.2 (*C*=O), 172.0 (*C*=O), 166.0 (Ar), 155.1 (Ar), 150.2 (Ar), 138.4 (Ar), 138.3 (Ar), 138.0 (Ar), 133.3 (Ar), 129.9 (Ar x 2), 129.7 (Ar), 128.5 (Ar x 2), 128.4 (Ar x 2), 128.3 (Ar x 2), 128.2 (Ar x 2), 127.7 (Ar x 2), 127.6(7) (Ar x 2), 127.6(5) (Ar), 127.4 (Ar), 127.3 (Ar x 2), 117.8 (Ar x 2), 114.6 (Ar x 2), 100.4 (C-1), 96.1 (C-1′), 80.0 (C-5′), 78.9 (C-5), 78.6 (C-4′), 76.7 (C-4), 75.4 (Ar*C*H_2), 73.8 (Ar*C*H_2), 73.5 (C-3′), 73.0 (Ar*C*H_2), 72.8 (C-2), 68.6 (C-2′), 68.4 (C-3), 55.7 (ArO*C*H_3), 37.8 (CH_2*C*H_2), 29.8 (*C*$H_2$$CH_2$), 28.0 (*C*$H_3$CO), 18.2 (C-6′), 17.8 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{52}H_{56}O_{13}Na$: 911.3613. Found 911.3613.



***p*-Methoxyphenyl 2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-benzoyl-α-L-rhamnopyranoside (3-17)**

To a solution of **3-16** (0.5 g, 0.56 mmol) in CH_2Cl_2 (30 mL), NH_2NH_2·HOAc (77 mg, 0.84 mmol) was added. The reaction mixture was stirred for 4 h before it was filtered. The filtrate was concentrated and the resulting residue was purified by

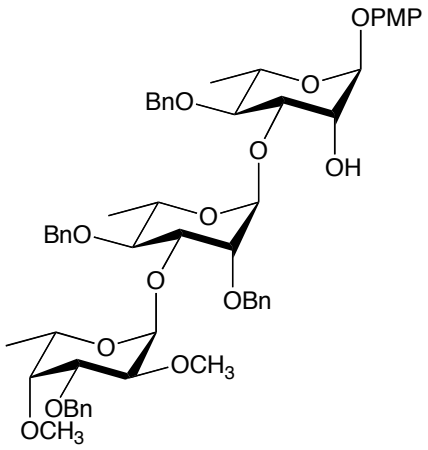
chromatography (1:1 hexane–EtOAc) to give **3-17** (350 mg, 79%) as a colorless oil: R_f 0.41 (1:1 hexane–EtOAc); $[\alpha]_D$ –20.6 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.08 (m, 2H, Ar), 7.63–7.60 (m, 1H, Ar), 7.51–7.48 (m, 2H, Ar), 7.36–7.26 (m, 12H, Ar), 7.23–7.21 (m, 3H, Ar), 7.02 (d, 2H, J = 9.1 Hz, Ar-2,6), 6.84 (d, 2H, J = 9.1 Hz, Ar-3,5), 5.56 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.52 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.21 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.85, 4.75 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.73, 4.57 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.50, 4.30 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.44 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.00 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.88 (ddd, 1H, $J_{2',3'}$ = 3.6 Hz, $J_{3',4'}$ = 9.5 Hz, $J_{3',OH\text{-}3'}$ = 8.9 Hz, H-3′), 3.79–3.76 (m, 1H, H-5′), 3.78 (s, 3H, ArOCH_3), 3.71 (dd,1H, $J_{1',2'}$ = 1.6 Hz, $J_{2',3'}$ = 3.6 Hz, H-2′), 3.67 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.31 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4′), 2.20 (d, 1H, $J_{3',OH\text{-}3'}$ = 8.9 Hz, OH-3′), 1.33 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.24 (d, 3H, $J_{5',6'}$ = 6.5 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 171.4 (*C*=O), 165.8 (Ar), 155.1 (Ar), 150.2 (Ar), 138.6 (Ar), 138.2 (Ar), 137.7 (Ar), 133.4 (Ar), 129.8 (Ar x 2), 129.7 (Ar), 128.6 (Ar x 2), 128.5(2) (Ar), 128.4(8) (Ar), 128.4(5) (Ar), 128.3 (Ar x 2), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar x 2), 127.6 (Ar x 2), 127.5 (Ar), 127.3 (Ar x 2), 117.8 (Ar x 2), 114.6 (Ar x 2), 99.5 (C-1′), 96.2 (C-1), 81.7 (C-4′), 80.5 (C-4), 79.8 (C-2′), 77.7 (C-3), 75.2 (Ar*C*H$_2$), 73.9 (Ar*C*H$_2$), 72.8 (C-2), 72.8 (Ar*C*H$_2$), 71.0 (C-3′), 68.5 (C-5), 68.1 (C-5′), 55.7 (Ar*C*H$_3$), 18.2 (C-6′), 17.9 (C-6). (ESI) Calcd. for $(M + Na)^+$ $C_{47}H_{50}O_{11}Na$: 813.3245. Found 813.3246.



***p*-Methoxyphenyl 2,3,4-tri-*O*-methyl-α-L-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside (3-18)**

Two solutions were prepared. Solution A was prepared by dissolving acceptor **3-17** (0.11 g, 0.14 mmol) in CH_2Cl_2 (15 mL); containing crushed 4 Å molecular sieves (100 mg). Solution B was prepared by dissolving donor **3-6** (53 mg, 0.17 mmol) in CH_2Cl_2 (10 mL); containing crushed 4 Å molecular sieves (100 mg). Both solutions A and B were stirred for 30 min at rt and then solution A was cooled to – 40 ºC before NIS (36 mg, 0.17 mmol) and AgOTf (10 mg, 0.04 mmol) were added. Solution B was then added dropwise to solution A over 10 min while stirring and then the reaction mixture was stirred for additional 30 min at –40 ºC before it was neutralized with Et_3N (2 mL). The solution was then filtered, the filtrate concentrated and the resulting residue was dissolved in 1:1 CH_2Cl_2–CH_3OH (10 mL) and 1M $NaOCH_3$ in CH_3OH (0.5 mL) was added. After stirring for 5 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting oil was purified by chromatography (1:1

hexane–EtOAc) to give **3-18** (67 mg, 70%) as a colorless liquid: R_f 0.55 (1:1 hexane–EtOAc); $[\alpha]_D$ –106.8 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.35 (m, 15 H, Ar), 7.00–6.97 (m, 2H, Ar-2,6), 6.84–6.81 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.27 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.20 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 5.17, 4.83 (ABq, 2H, *J* = 11.3 Hz, ArC*H*$_2$), 4.69, 4.60 (ABq, 2H, *J* = 11.2 Hz, ArC*H*$_2$), 4.56, 4.26 (ABq, 2H, *J* = 12.3 Hz, ArC*H*$_2$), 4.22 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.05 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.1 Hz, H-3) 3.92–3.84 (m, 2H, H-5, H-3′), 3.81 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, $J_{2',3'}$ = 3.1 Hz, H-2′), 3.77 (s, 3H, ArOC*H*$_3$), 3.69 (dq, 1H, $J_{4',5'}$ = 9.3 Hz, $J_{5',6'}$ = 6.5 Hz, H-5′), 3.65–3.59 (m, 2H, H-2″, H-3″), 3.57–3.53 (m, 3H, H-4, H-4′, H-5″), 3.55 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.37 (s, 3H, OC*H*$_3$), 3.22 (dd, 1H, $J_{3'',4''}$ = 2.9 Hz, $J_{4'',5''}$ = 1.9 Hz, H-4″), 1.35 (d, 3H, $J_{5',6'}$ = 6.5 Hz, H-6′), 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.01 (d, 3H, $J_{5'',6''}$ = 6.3 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.2 (Ar), 138.8(3) (Ar), 138.8(1) (Ar), 138.3 (Ar), 128.5 (Ar x 2), 128.2(8) (Ar x 2), 128.2(7) (Ar x 2), 127.6 (Ar x 2), 127.4(9) (Ar), 127.4(5) (Ar), 127.4(4) (Ar x 2), 127.2 (Ar x 2), 127.1 (Ar x 2), 117.6 (Ar x 2), 114.6 (Ar), 99.6 (C-1″), 99.3 (C-1′), 98.0 (C-1), 80.4 (C-4), 80.1 (C-2), 79.9 (C-3), 79.7(1) (C-3′), 79.6(5) (C-3″), 79.2 (C-2′), 78.7 (C-2″), 77.8 (C-4′), 75.0 (Ar*C*H$_2$), 74.8 (Ar*C*H$_2$), 71.6 (Ar*C*H$_2$), 71.1 (C-4″), 69.2 (C-5′), 68.3 (C-5), 66.4 (C-5″), 61.7 (O*C*H$_3$), 59.2 (O*C*H$_3$), 58.0 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.2 (C-6′), 17.9 (C-6), 16.6 (C-6″). ESI) Calcd. for $(M + Na)^+$ $C_{49}H_{62}O_{14}Na$: 897.4032. Found 897.4034.



***p*-Methoxyphenyl 3-*O*-benzyl-2,4-di-*O*-methyl-α-L-fucopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside (3-19)**

Two solutions were prepared. Solution A was prepared by dissolving **3-17** (0.11 g, 0.14 mmol) in CH_2Cl_2 (10 mL) containing crushed 4 Å molecular sieves (100 mg). Solution B was prepared by dissolving **3-7** (60 mg, 0.15 mmol) in CH_2Cl_2 (10 mL), containing crushed 4 Å molecular sieves (100 mg). Both solutions A and B were stirred for 30 min at rt and then solution A was cooled to – 40 ºC before NIS (43.3 mg, 0.16 mmol) and AgOTf (10 mg, 0.04 mmol) were added. Solution B was then added dropwise to Solution A over 10 min while stirring and then the reaction mixture was stirred for additional 30 min at –40 ºC before it was neutralized with Et_3N (2 mL). The reaction mixture was filtered, concentrated and the resulting residue was dissolved in 1:1 CH_3OH–CH_2Cl_2 (30 mL) and a catalytic amount of 1M $NaOCH_3$ in CH_3OH (0.25 mL) was added. After stirring for 5 h at rt, the reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting oil was purified by chromatography (2:1 hexane–

EtOAc) to give **3-20** (105.1 mg, 79%) as a colorless oil: R_f 0.45 (2:1 hexane–EtOAc); $[\alpha]_D$ –88.1 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.41–7.21 (m, 20H, Ar), 7.01 (d, 2H, *J* = 9.1 Hz, Ar-2,6), 6.84 (d, 2H, *J* = 9.1 Hz, Ar-3,5), 5.40 (br s, 1H, H-1), 5.26 (br s, 1H, H-1′), 5.24 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1″), 5.19, 4.61 (ABq, 2H, *J* = 11.1 Hz, ArCH_2), 4.83, 4.72 (ABq, 2H, *J* = 11.8 Hz, ArCH_2), 4.80, 4.69 (ABq, 2H, *J* = 11.2 Hz, ArCH_2), 4.54, 4.29 (ABq, 2H, *J* = 11.2 Hz, ArCH_2), 4.26–4.21 (m, 2H, H-2, H-3), 4.08 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 3.93–3.87 (m, 2H, H-5, H-5′), 3.84–3.82 (m, 2H, H-2′, H-3″), 3.78 (s, 3H, ArOCH_3), 3.73–3.63 (m, 3H, H-2″, H-4′, H-5″), 3.59 (s, 3H, OCH_3), 3.58–3.54 (m, 1H, H-4), 3.41 (s, 3H, OCH_3), 3.56–3.54 (m, 1H, H-4″), 2.44 (br s, 1H, OH-2), 1.37 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.01 (d, 3H, $J_{5'',6''}$ = 6.3 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.2 (Ar), 138.9 (Ar x 2), 138.3 (Ar x 2), 128.5(1) (Ar), 128.4(6) (Ar x 2), 128.7 (Ar x 2), 128.4 (Ar x 2), 128.3(1) (Ar x 2), 128.2(5) (Ar x 2), 127.7 (Ar), 127.5 (Ar), 127.5 (Ar), 127.4 (Ar x 2), 127.2 (Ar x 2), 127.1 (Ar x 2), 117.6 (Ar x 2), 114.6 (Ar x 2), 99.7 (C-1′), 99.3 (C-1″), 98.0 (C-1), 80.5 (C-4), 80.2 (C-4′), 79.9 (C-2), 79.7 (C-3), 79.1 (C-3″), 78.6 (C-3′), 78.5 (C-2′), 78.4 (C-2″), 75.0 (Ar*C*H_2), 74.8 (Ar*C*H_2), 72.6 (Ar*C*H_2), 71.6 (Ar*C*H_2), 71.1 (C-4″), 69.2 (C-5′), 68.3 (C-5), 66.6 (C-5″), 61.8 (O*C*H_3), 59.6 (O*C*H_3), 55.7 (O*C*H_3), 18.1 (C-6), 17.9 (C-6″), 16.5 (C-6′). (ESI) Calcd. for $(M + Na)^+$ $C_{55}H_{66}O_{14}Na$: 973.4342. Found 973.4345.

3.5. Bibliography

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Chapter 4

Synthesis of Carbohydrate Cores of Phenolic Glycolipids from *Mycobacterium kansasii*

4.1. Introduction

Mycobacterium kansasii is another member of mycobacteria complex family. It is the second most pathogenic species of non-tuberculous mycobacteria infecting humans, after *Mycobacterium avium,* and is a particular concern for those with AIDS.[1-4] *M. kansasii* can cause either a chronic and progressive pulmonary disease that is similar to tuberculosis or other disseminated infections in patients with a compromised immune system.[5-8] The cell wall of *M. kansasii* has the same complex architecture as that present in *M. tuberculosis*, which gives the organism the same unique abilities of survival and resistance. Not long after the discovery of PGLs in *M. tuberculosis,* seven glycolipids belong to the PGL family were isolated from *M. kansasii* (Figure 4-1).[9,10] Since their discovery, no work has been done to investigate their function; therefore, we synthesized a panel of 17 synthetic analogs to test their effect on cytokine production in the activated macrophages (Figure 4-2). Because there are only seven native PGLs isolated from *M. kansasii*, the other 10 of the 17 compounds were made to explore the effect of different alkylation patterns on the immunomodulatory activity of these molecules. The synthetic analogs were categorized into three series. Series I consists of four trisaccharides with an L-fucose capping motif, whereas series II consists of six tetrasaccharides with 6-deoxy-D-mannose capping motif. On the

other hand, Series III consists of seven tetrasaccharides with a D-mannose capping motif. In this chapter, the synthetic work used for the preparation of these analogs will be described.

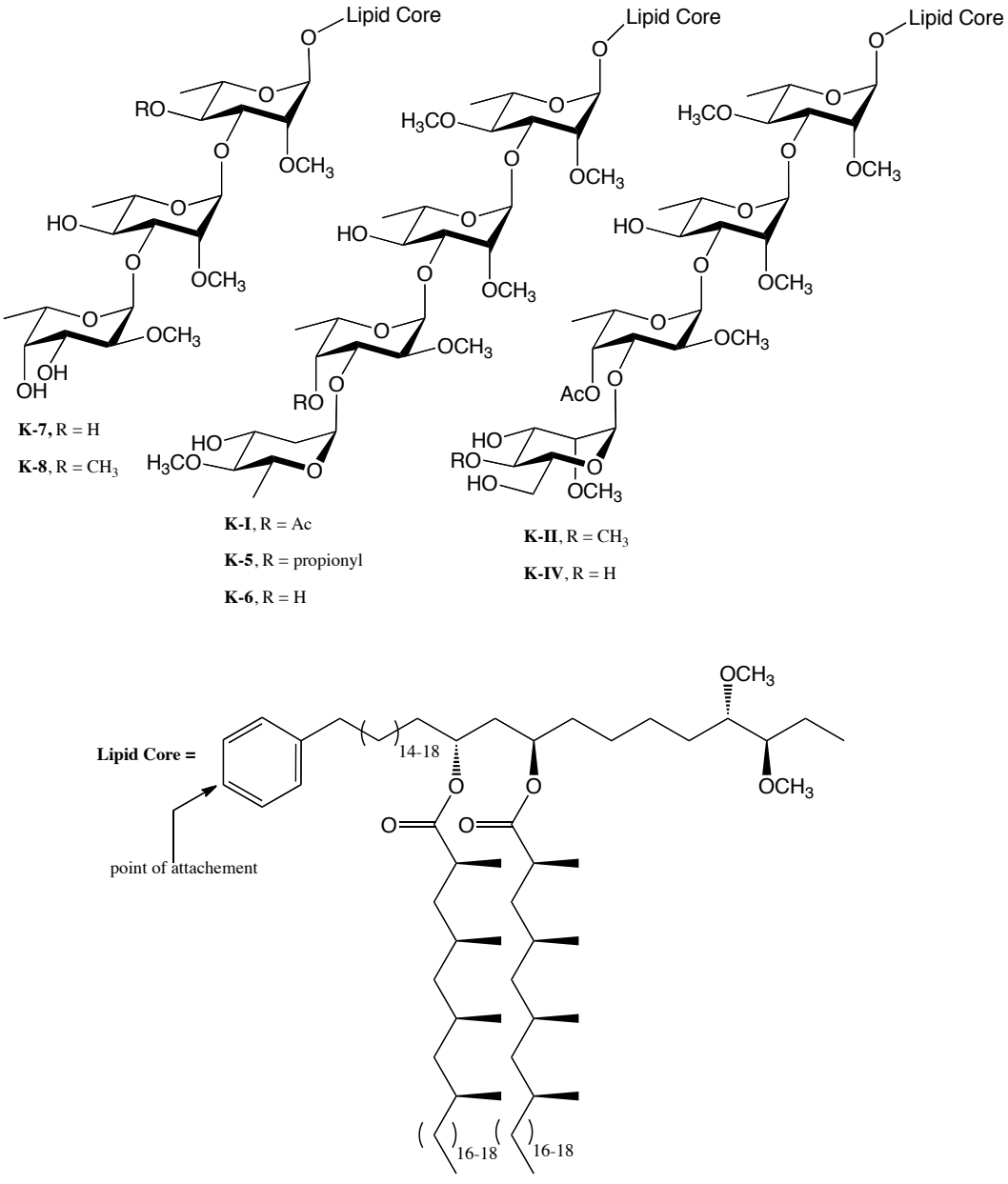


Figure 4-1. Structures of native PGLs from *M. kansasii*.

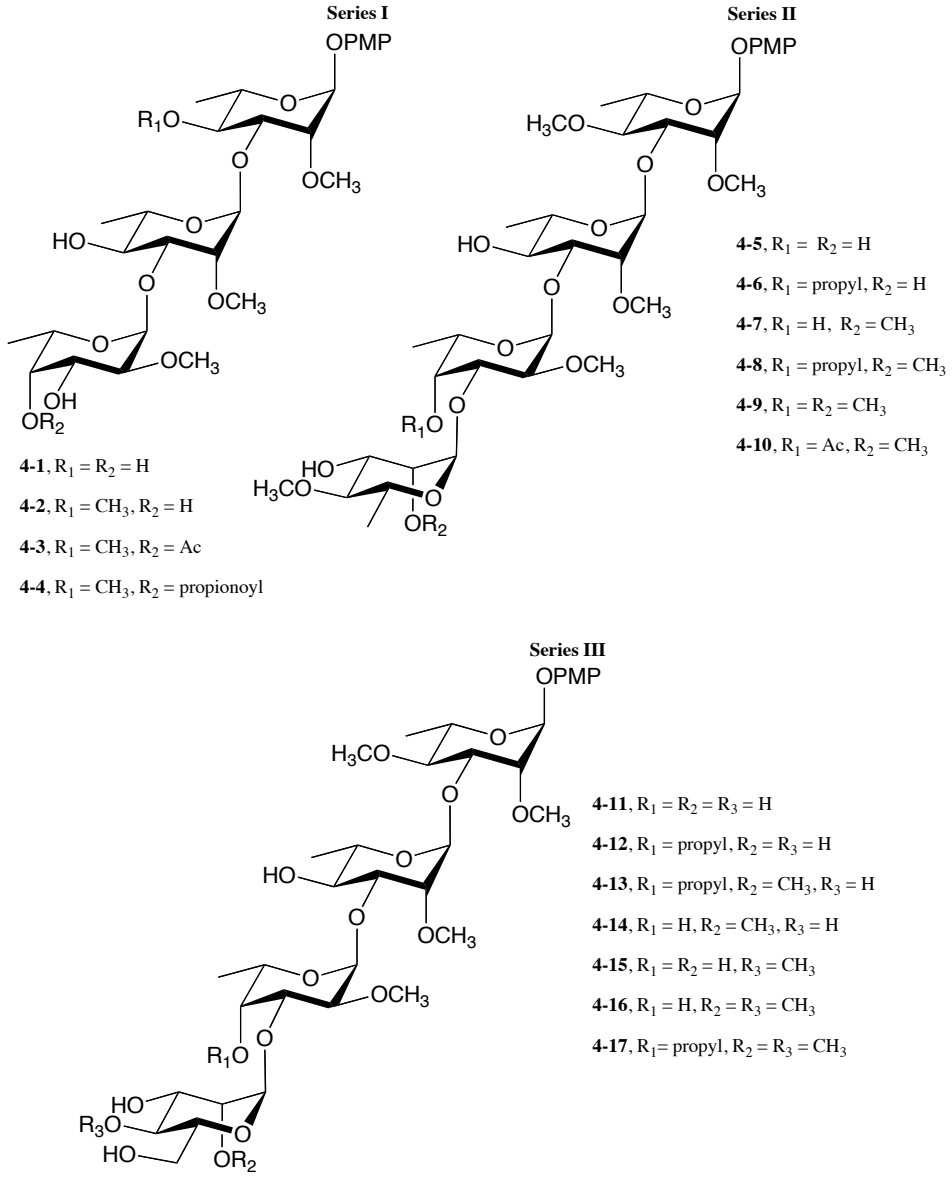


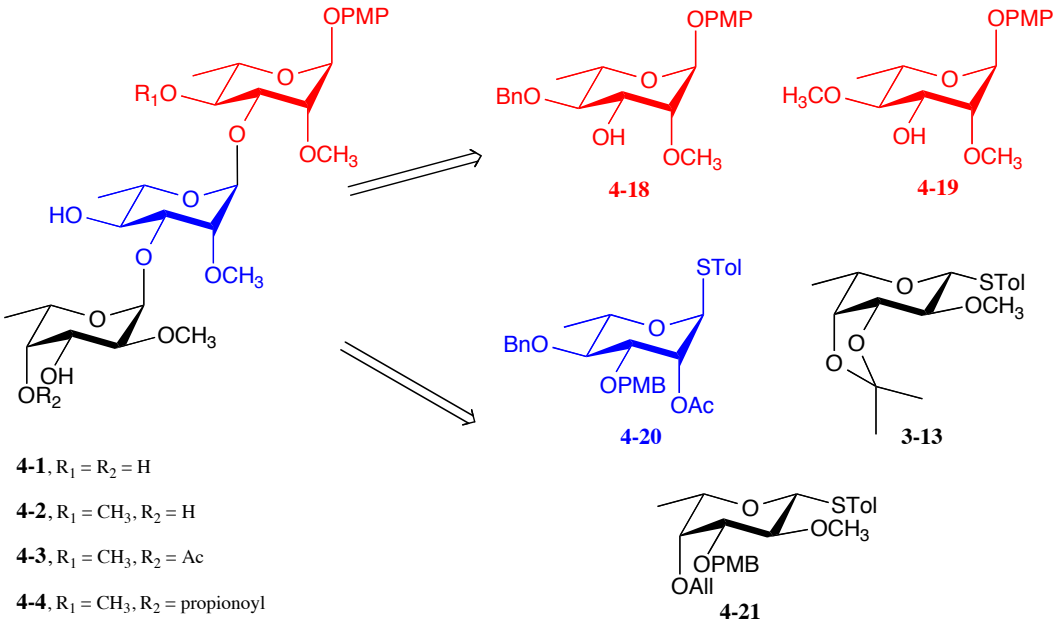
Figure 4-2. Structures of synthetic analogs.

4.2. Results and Discussion

4.2.1. Retrosynthetic analysis of series I targets 4-1–4-4

As illustrated in Scheme 4-1, we envisioned that the targets in series I could be obtained from five main building blocks, **4-19**–**4-22** and **3-13**. Both

building blocks **4-19** and **4-20** are functionalized rhamnose derviatives with a *p*-methoxyphenyl moiety at the reducing end; **4-21** is a rhamnose derivative with a *p*-thiocresol at the reducing end instead. Monosaccharide **3-13** is an intermediate that was previously prepared during the synthesis of compounds **3-6** and **3-7** (see Scheme 3-4 for its synthesis). Finally, building blocks **4-21** and **4-22** are functionalized fucose thioglycoside derivatives with a *p*-thiocresol moiety at the reducing end.



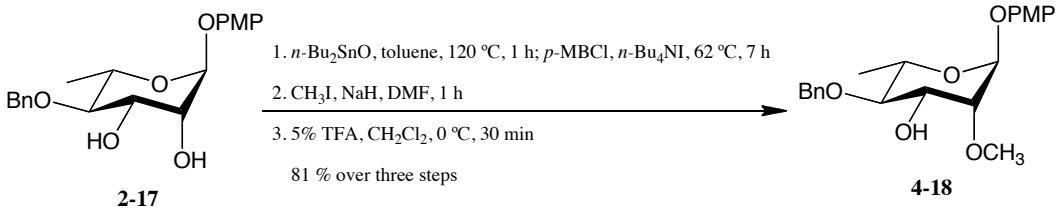
Scheme 4-1. Retrosynthetic analysis of series I targets.

4.2.2. Synthesis of building blocks 4-18–4-21

4.2.2.1. Synthesis of 4-18

As shown in Scheme 4-2, the synthesis of **4-18** was achieved in three steps starting from the previously synthesized advanced intermediate **2-17** (see Scheme 2-2 for its preparation). First, the installation of a methyl group at O-2 required

selective protection of O-3. This was accomplished by heating **2-17** with dibutyltin oxide[11] in toluene at reflux to form a stannylidene acetal intermediate. After the solution was cooled to 62 ºC, this intermediate was then allowed to react with *p*-methoxybenzyl chloride (PMBCl) in the presence of *n*-Bu_4NI affording O-3 benzylated intermediate. Subsequently, this intermediate was purified by column chromatography before O-2 was methylated[12] using methyl iodide and sodium hydride. Finally, acid-catalyzed[13] removal of the *p*-methoxybenzyl group afforded **4-18** in 81% yield over three steps. The upfield chemical shift of H-2 (δ_H = 3.68 ppm) and C-2 (δ_C = 68.2 ppm) in the ^1H and ^{13}C NMR spectra of the product, respectively, confirmed the regioselectivity of this process. In the starting material, **2-17**, these resonances appeared at 4.14–4.10 (H-2) and 71.0 ppm (C-2).

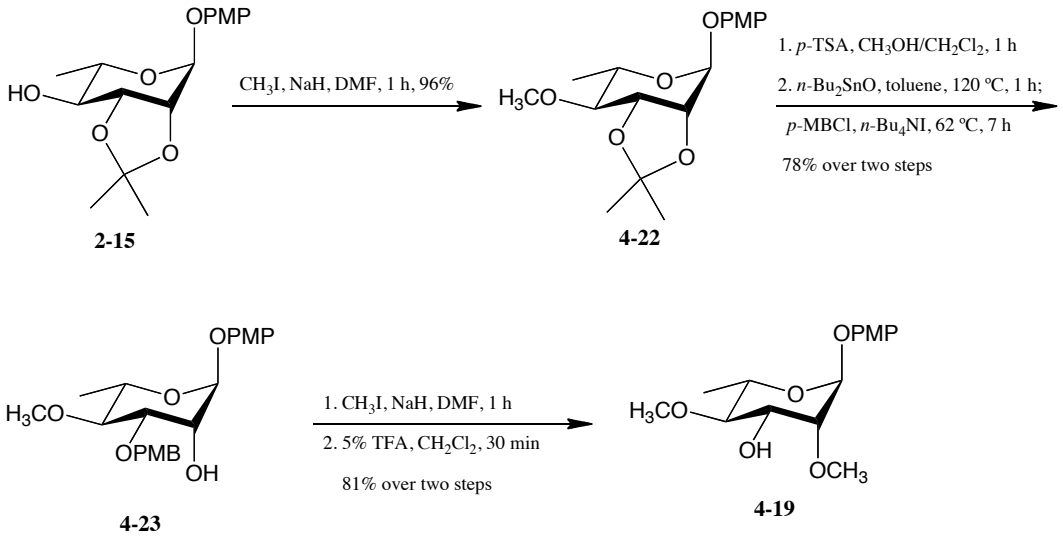


Scheme 4-2. Synthesis of building block **4-18**.

It is worth noting that phase transfer alkylation[24] was attempted for direct installation of the methyl group at O-2; however, instead of obtaining the correct regioisomer, a mixture of O-2 and O-3 methylated products was obtained. This mixture was not separable by column chromatography, and therefore the longer process outlined above was used.

4.2.2.2. Synthesis of building block 4-19

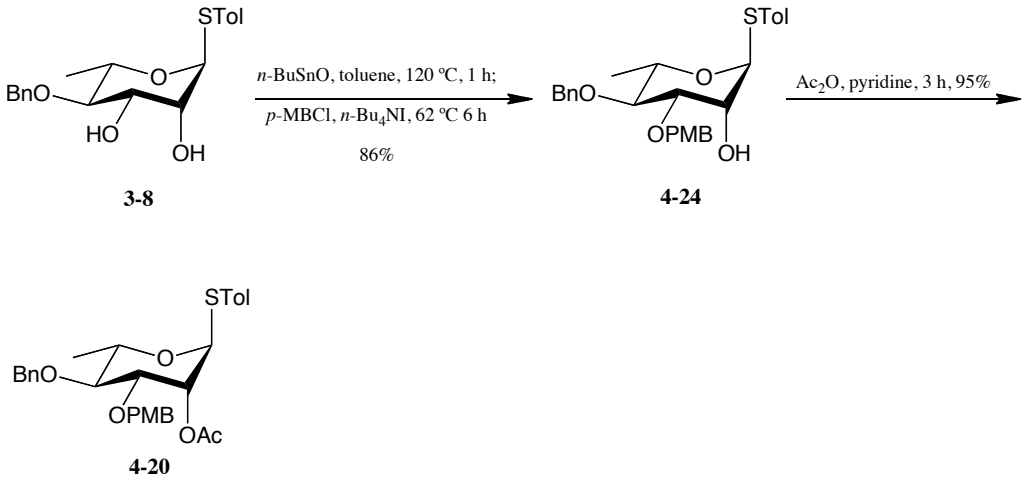
As illustrated in Scheme 4-2, to access target **4-19**, we utilized intermediate **2-15**, which was prepared as described in Chapter 2 (see Scheme 2-2). The first step was a sodium[12] hydride-mediated methylation of O-4 in **2-15** affording a 96% yield of **4-22**. Subsequent acid-catalyzed hydrolysis of the isopropylidene acetal gave a diol intermediate. As previously described for the preparation of **4-18**, a similar series of reactions and conditions were used to selectively benzylate this diol intermediate at O-3. This reaction provided compound **4-23** in 78% yield. Finally, methylation of O-2[14] followed by selective acid-catalyzed removal of the *p*-methoxybenzyl group at O-3 afforded an 81% yield of **4-19**. The upfield chemical shift of both H-2 (δ_H = 3.68 ppm) and C-2 (δ_C = 71.4 ppm) in the ^{1}H and ^{13}C NMR spectra, respectively, is confirmation the regioselectivity of tin-mediated benzylation at O-3.



Scheme 4-3. Synthesis of building block **4-19**.

4.2.2.3. Synthesis of thioglycoside 4-20

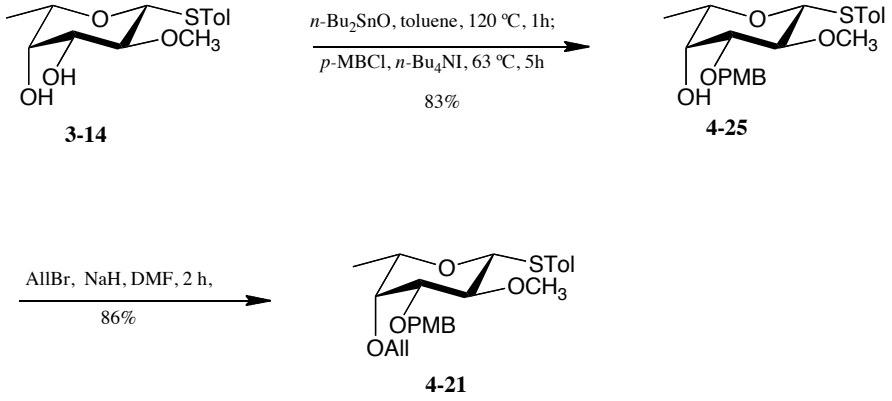
Scheme 4-4 details the synthesis of **4-20**. Intermediate **3-8**, which was used to synthesize **3-5** (see Scheme 3-3 for its preparation), was also used as a starting material for the preparation of **4-20**. Selective benzylation of O-4 was achieved using stannylidene acetal–mediated alkylation,[11] as described for compound **4-19**, affording a 85% yield of **4-24**. That the correct regioisomer had obtained was apparent from the upfield chemical shift of both H-3 (δ_H = 3.85 ppm) and C-3 (δ_C = 70.1 ppm) in the ^{1}H and ^{13}C NMR spectra, respectively. The remaining hydroxyl group, O-2, was acylated using acetic anhydride[15] and pyridine to afford **4-20** in a 95% yield. The strong downfield chemical shift of H-2 (δ_H = 5.61 ppm) after the acylation reaction further confirmed the regioselectivity of the tin-mediated alkylation step.



Scheme 4-4. Synthesis of building block **4-20**.

4.2.2.3. Synthesis of thiofucoside 4-21

The synthesis of **4-21** is described in Scheme 4-5. As illustrated, we started the synthesis by selective benzylation of O-3 of compound **3-14** (see Scheme 3-4 for the synthesis of this molecule) using stannylidene acetal–mediated alkylation[16] to afford **4-25** in 83% yield. As mentioned previously, the upfield chemical shifts of both H-3 (δ_H = 3.44 ppm) and C-3 (δ_C = 74.1 ppm) in the ^{1}H and ^{13}C NMR spectra, respectively, were used to confirm the regioselectivity of the process. Those resonances appeared at H-3 (δ_H = 3.61–3.57 ppm) and C-3 (δ_C = 80 ppm) in the ^{1}H and ^{13}C NMR spectra of the starting material, respectively. Subsequently, allylation of O-4 of **4-25** using allyl bromide[17] and sodium hydride afforded **4-21** in 85% yield.



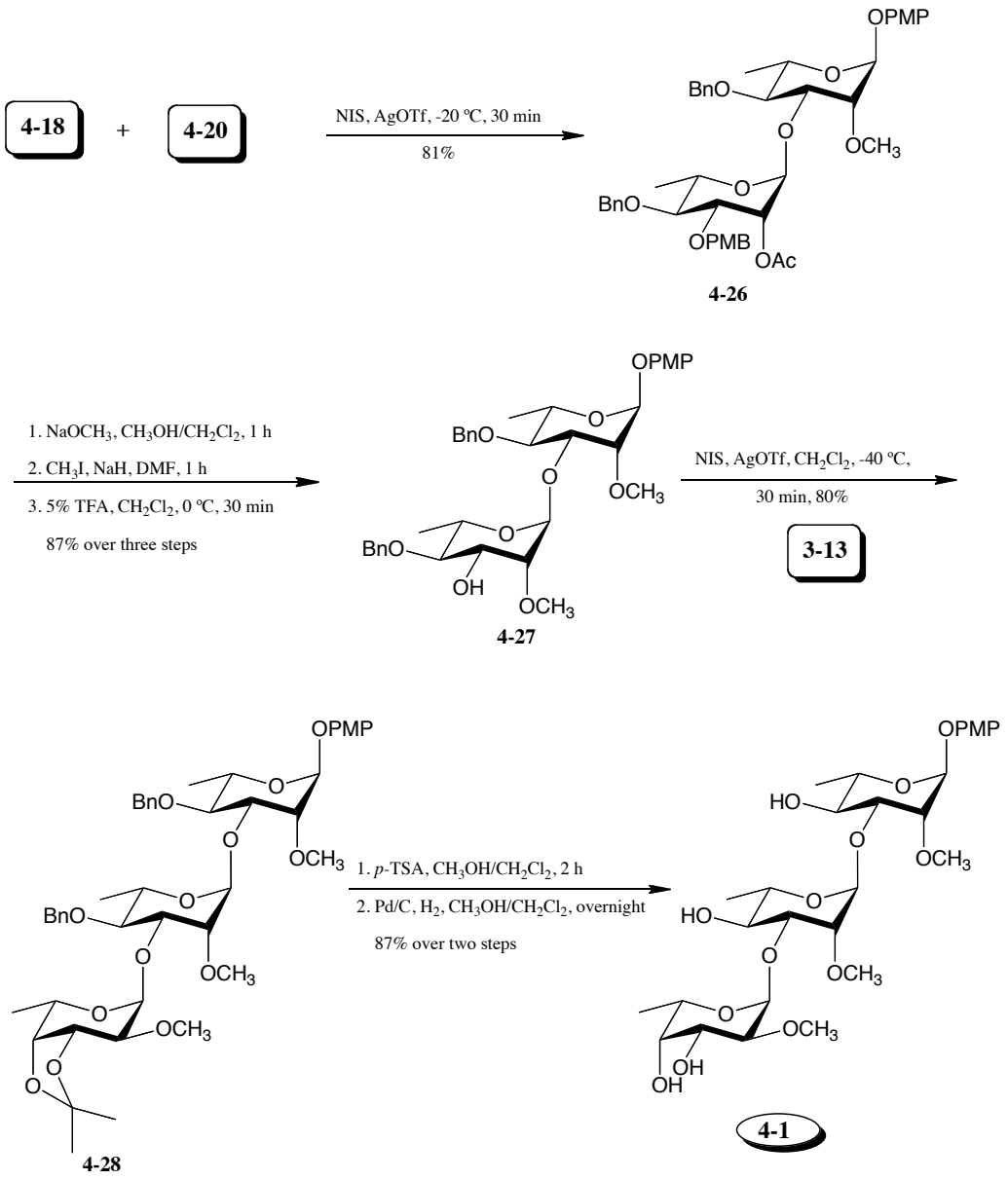
Scheme 4-5. Synthesis of building block **4-21**.

4.2.3. Assembly of series I (4-1–4-4)

4.2.3.1 Assembly of 4-1

With the four building blocks in hand, the assembly of the oligosaccharides was performed, starting with **4-1** (Scheme 4-6). The coupling of

thioglycoside donor **4-18** and acceptor **4-20** using NIS–AgOTf-mediated[16] activation afforded **4-26** in 81% yield. The magnitude of the $^{1}J_{C\text{-}1,H\text{-}1}$ (172 Hz) was used to confirm the α-stereochemistry of the newly formed glycosidic linkage.[25] Subsequent removal of the acetyl group using sodium methoxide in methanol afforded an alcohol intermediate, which was then methylated using sodium hydride and methyl iodide. After purification of this intermediate by column chromatography, the *p*-methoxybenzyl group was removed selectively via an acid-catalyzed hydrolysis affording disaccharide alcohol **4-27** in 87% yield over three steps. Disaccharide **4-27** was then fucosylated with donor **3-13** using an inverse glycosylation[18] procedure, as previously described for the synthesis of **3-18** (see Scheme 3-5), affording the corresponding trisaccharide **4-28** in 80% yield. As previously mentioned, the α stereochemistry of the formed glycosidic linkage was confirmed by the 170 Hz value of $^{1}J_{C\text{-}1,H\text{-}1}$, which was calculated from a coupled HSQC experiment.[25] To complete the synthesis of **4-1**, the isopropylidene acetal was removed using *p*-TSA in methanol and dichloromethane and then all benzyl groups were deprotected by hydrogenolysis providing **4-1** in 92% combined yield.



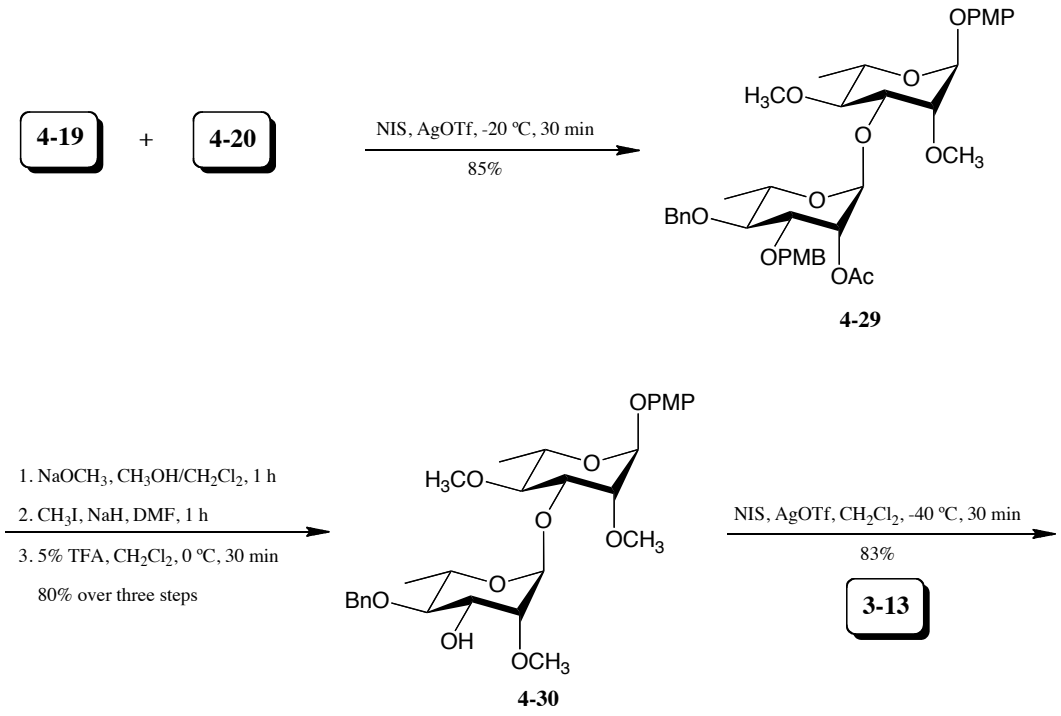
Scheme 4-6. Assembly of trisaccharide **4-1**.

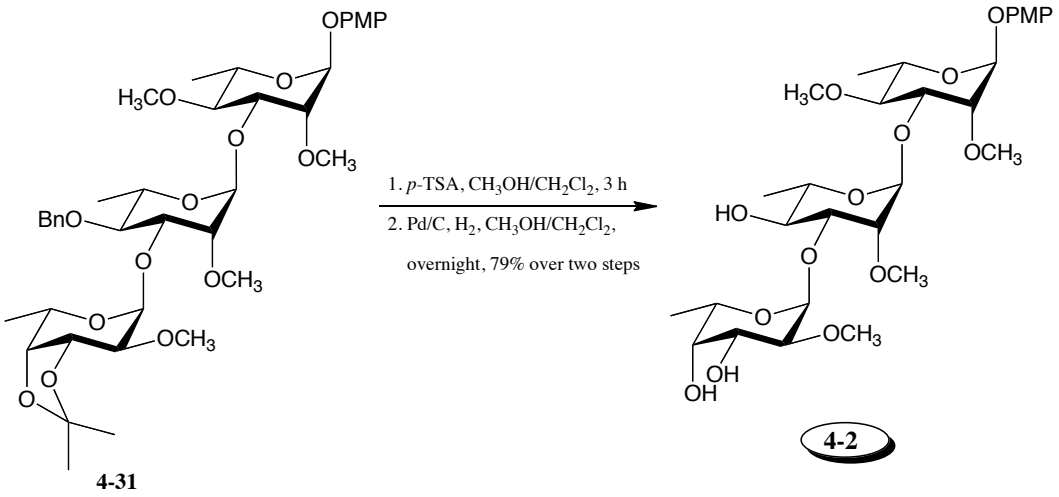
4.2.3.2 Assembly of target 4-2

As described in Scheme 4-7, the synthesis of **4-2** started by the coupling of donor **4-19** and acceptor **4-20** via NIS–AgOTf[19]-mediated activation affording the corresponding disaccharide **4-29** in 85% yield. Zemplén deacylation was used to remove the O-2 acetyl group and the resulting alcohol was methylated. This two-

step reaction provided a methylated intermediate that was purified by column chromatography before it was subjected to an acid-catalyzed hydrolysis to remove the *p*-methoxybenzyl group affording 83% yield of **4-30**.

Next, another NIS–AgOTf-mediated inverse[18] fucosylation of disaccharide alcohol **4-30** with donor **3-13** provided 83% yield of the corresponding trisaccharide **4-31**. Finally, removal of isopropylidene acetal with *p*-TSA[12] gave a diol intermediate, which was then debenzylated[13] via hydrogenolysis to afford **4-2** in 79% yield over two steps. In the glycosylation reactions described above, the α-stereochemistry of the glycosidic linkages was confirmed by the one-bond $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants for the anomeric carbon atoms. For all products, this value was between 169 and 170 Hz, clearly indicating the α-stereochemistry.[25]

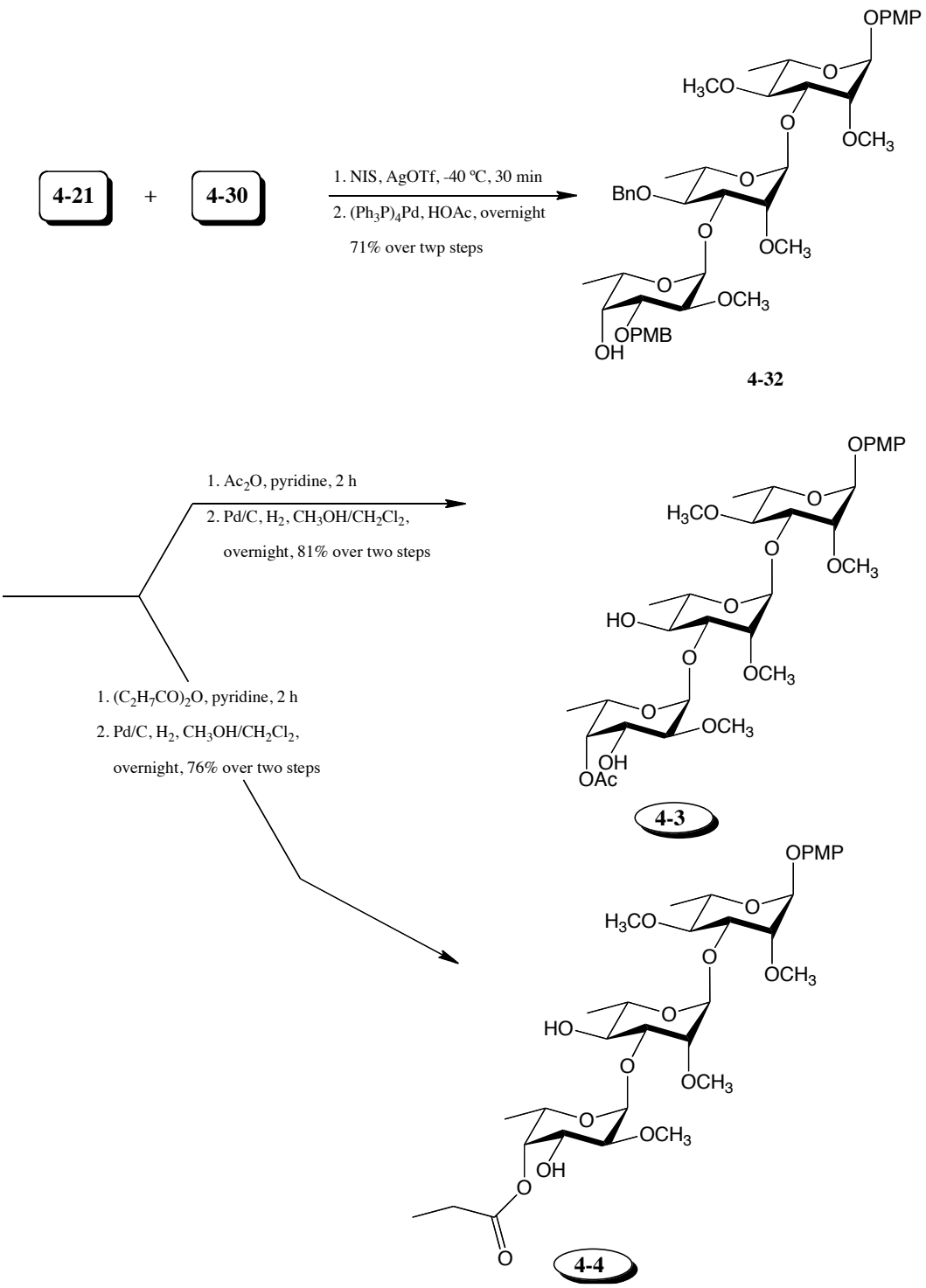




Scheme 4-7. Assembly of trisaccharide **4-2**.

4.2.3.3 Assembly of trisaccharides 4-3 and 4-4

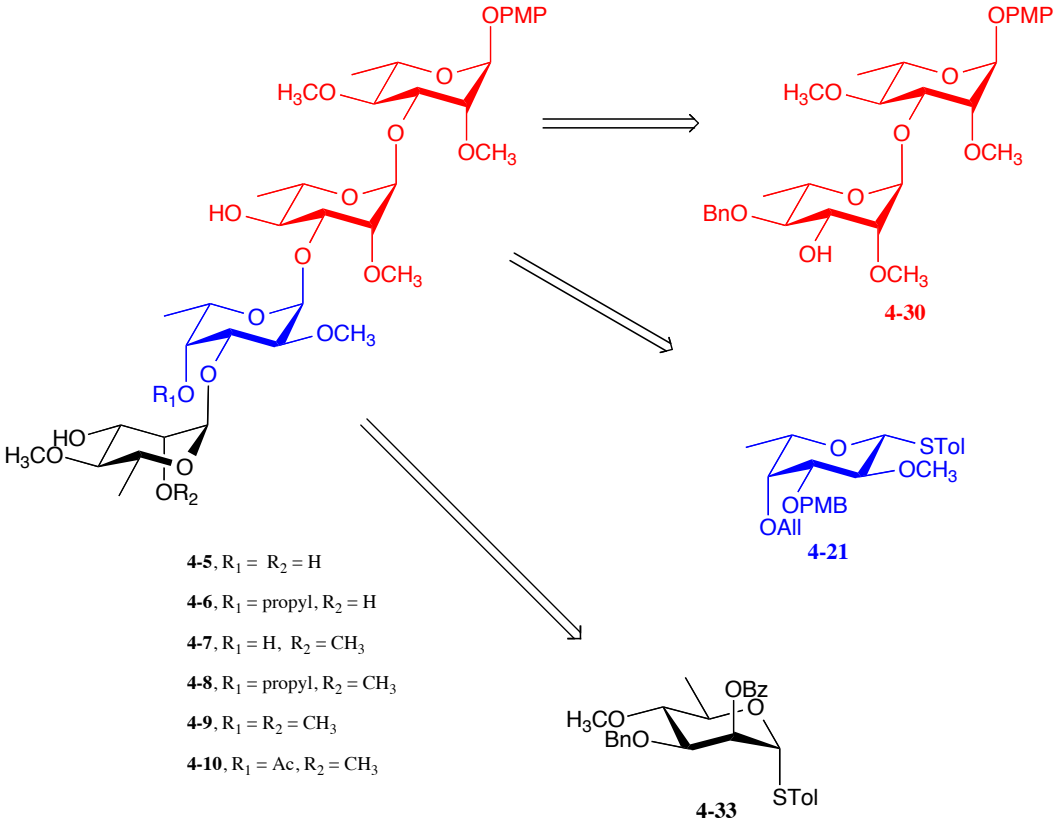
The synthesis of **4-3** and **4-4** is illustrated in Scheme 4-8. First, the previously employed inverse[18] fucosylation of acceptor **4-30** with donor **4-21** afforded a trisaccharide intermediate. This trisaccharide was purified by column chromatography and then subjected to selective removal of the allyl group at O-4′ using $(Ph_3P)_4Pd$[20] in acetic acid. This reaction afforded the corresponding trisaccharide **4-32** in 71% yield over two steps. The target, **4-3**, was obtained in 81% combined yield after the protection of O-3′ of a portion of **4-32** as an acetyl ester and hydrogenolysis to remove all benzyl groups. Using the remaining amount of **4-32**, acylation of O-3′ with propionic anhydride[12] in pyridine followed by debenzylation using palladium[13] on charcoal under a hydrogen atmosphere afforded **4-4** in 76% yield over two steps.



Scheme 4-8. Assembly of trisaccharides **4-3** and **4-4**.

4.2.4. Retrosynthetic analysis of series II targets 4-5–4-10

As illustrated in Scheme 4-9, we envisioned that the series II targets could be obtained from three primary building blocks: **4-21**, **4-30**, and **4-33**. Both building blocks **4-21** and **4-30** were previously synthesized in this chapter (see Scheme 4-5 and Scheme 4-7). Building block **4-33** is a fully protected 6-deoxy mannose species, with a *p*-thiocresol moiety at the reducing end. This building block will be added at the non-reducing end of the tetrasaccharides of the series II targets.



Scheme 4-9. Retrosynthetic analysis of series II targets.

4.2.4.1. Synthesis of building block 4-33

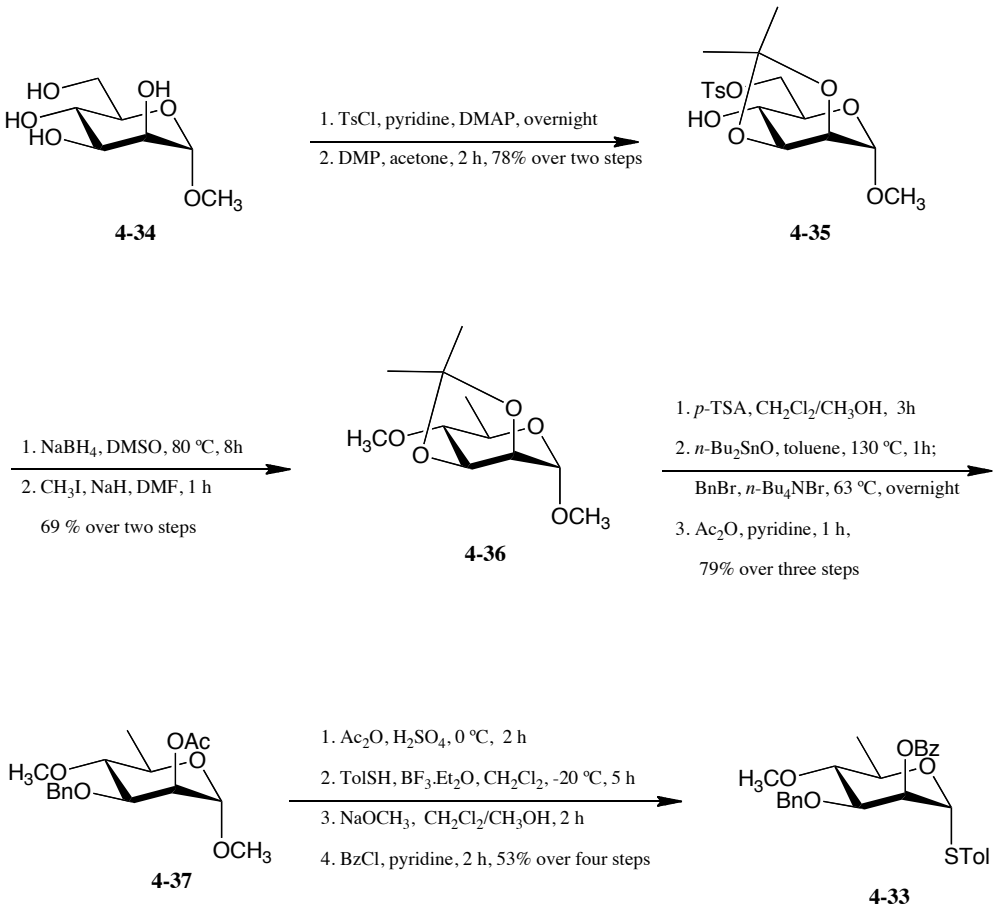
The synthesis of **4-33** started from the commercially available methyl α-D-mannopyranoside, **4-34**. As illustrated in Scheme 4-10, its preparation commenced with the selective protection of O-6 in **4-34** with tosyl chloride[21] and a catalytic amount of 4-dimethylaminopyridine (DMAP). Subsequently, O-2 and O-3 were protected as an isopropylidene acetal upon treatment with DMP and *p*-TSA in acetone to afford a 78% yield of target **4-35**. To access the 6-deoxy compound, **4-36**, tosylate **4-35** was treated with sodium borohydride ($NaBH_4$)[22] in DMSO to deoxygenate at O-6. After working up the deoxygenation reaction, the C-4 hydroxyl group was methylated to furnish **4-36** in 69% yield over the two steps.

Next, acid-catalyzed removal of the isopropylidene acetal afforded a diol intermediate, which was selectively benzylated at O-3 via stannylidene acetal–mediated alkylation[16] using similar conditions as those described for preparing **4-18** (Scheme 4-2). After the purification of the product, O-2 was protected as acetate ester affording **4-37** in 79% combined yield. The regioselectivity of the alkylation was confirmed by the large downfield chemical shift of H-2 (δ_H = 5.33 ppm) in the ^1H NMR spectrum of **4-37** after the acetylation step.

Finally, methyl glycoside **4-37** was converted into thioglycoside **4-33** upon treatment with acetic anhydride and catalytic amount of sulfuric acid to remove the methyl group at the anomeric centre, affording the corresponding glycosyl acetate. $BF_3.Et_2O$-promoted coupling of the resulting intermediate with *p*-thiocresol afforded a thioglycoside intermediate. The acetyl group at O-2 was

then removed via Zemplén deacylation and replaced with a benzoyl group under standard benzoylation conditions to afford the target thioglycoside **4-33** in 53% yield.

The purpose of replacing the acetyl group with the benzoyl group was to improve the yield of the α anomer upon coupling thioglycoside donor **4-33** with other acceptors.[23] The use of the O-2 acetyl derivative gave a mixture of α/β glycosides in a 1:1 ratio.



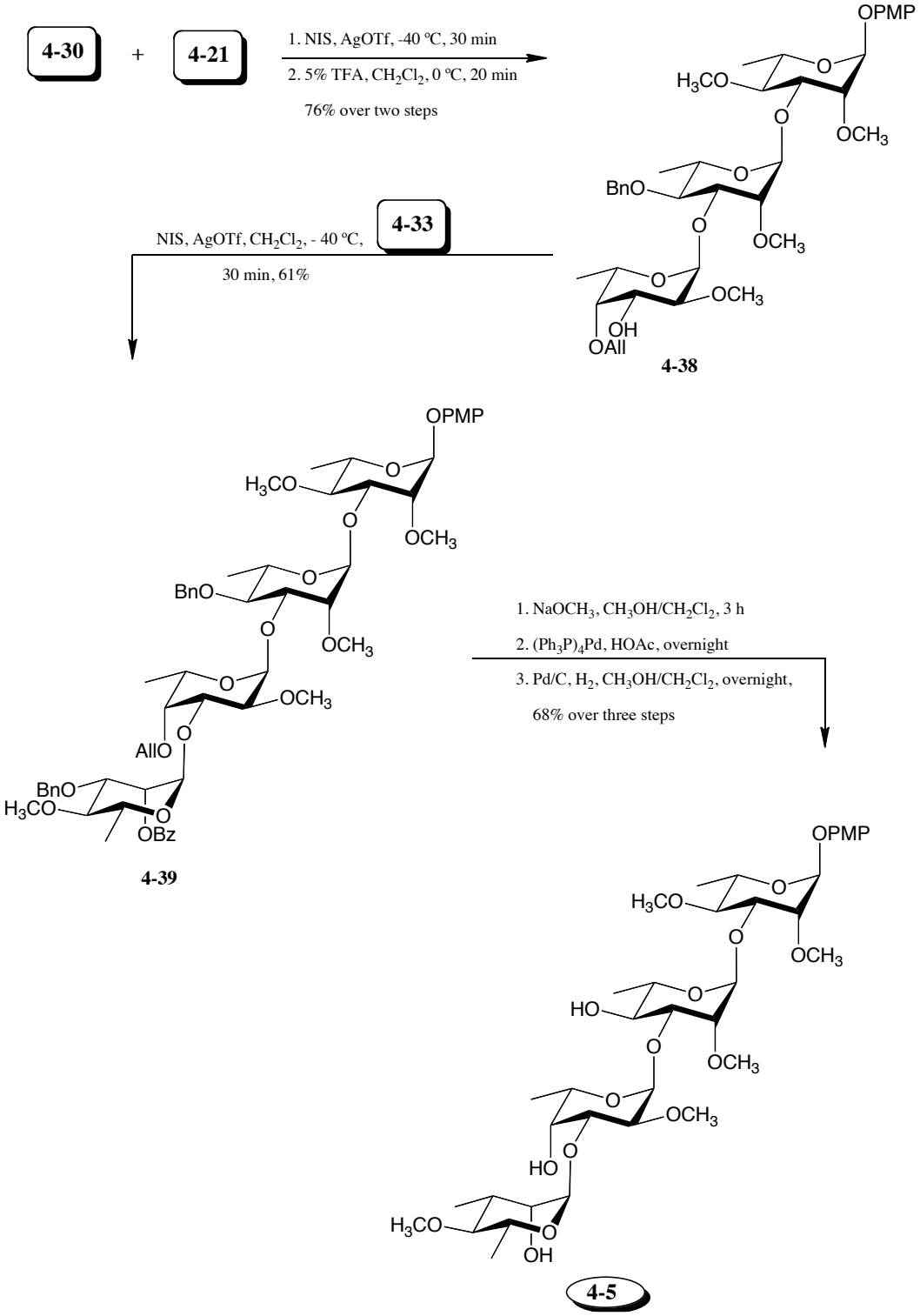
Scheme 4-10. Synthesis of building block **4-33**.

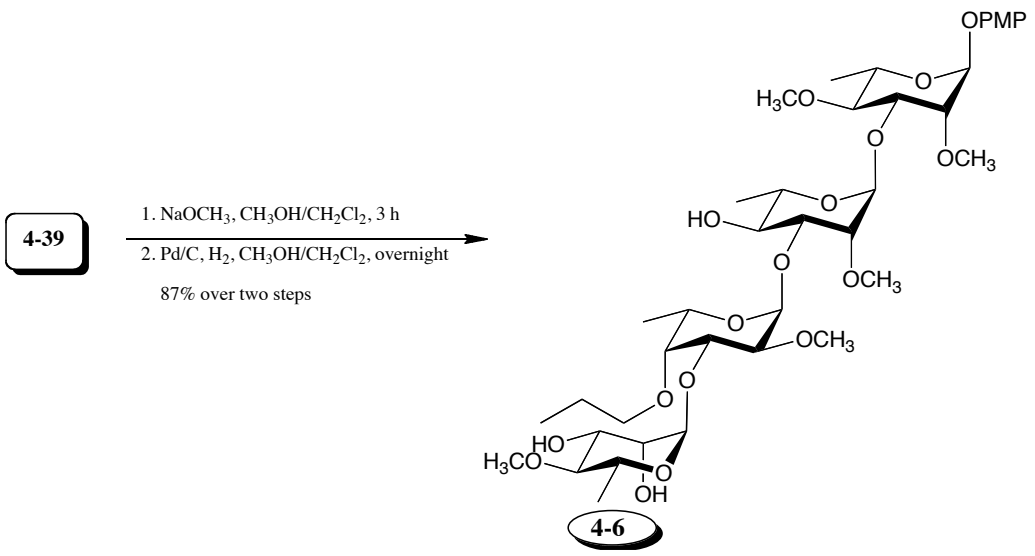
4.2.4.2. Assembly of tetrasaccharides 4-5 and 4-6

As illustrated in Scheme 4-11, glycosyl donor **4-30** and glycosyl acceptor **4-21** were coupled through the same inverse NIS–AgOTf[18] glycosylation procedure, previously described for the synthesis of **4-28**. The alcohol acceptor **4-38** was obtained in 75% yield upon treatment of the glycosylation product with 5% trifluoroacetic acid (TFA) to selectively remove the *p*-methoxybenzyl group at O-3′′. The removal of the *p*-methoxybenzyl group was done to facilitate the purification and characterization of the product. The direct purification of the glycosylation product was not possible.

With alcohol **4-38** in hand, it was coupled with donor thioglycoside **4-33** again using NIS–AgOTf[19] activation, which afforded, in 69% yield, the expected tetrasaccharide **4-39**. In the two glycosylation reactions described above, that the α-glycoside had been obtained was established by measuring the $^{1}J_{C\text{-}1,H\text{-}1}$ from a coupled HSQC experiment. For the two products, this value was 170 and 171 Hz, clearly indicating the α-stereochemistry.[25]

Having access to tetrasaccharide **4-39**, it was subjected to two different sets of deprotection reactions to complete the synthesis of targets **4-5** and **4-6**. Tetrasaccharide **4-5** was obtained by first deacylation using sodium methoxide in methanol and subsequent removal of the allyl group using $(Ph_3P)_4Pd$[20] in acetic acid to give an alcohol intermediate. After purification of this alcohol, hydrogenolysis over palladium on charcoal afforded target **4-5** in an overall yield of 68%. For tetrasaccharide **4-6** the target was obtained in 87% yield over two steps after Zemplén deacylation, purification of the resulting alcohol and a subsequent hydrogenolysis.

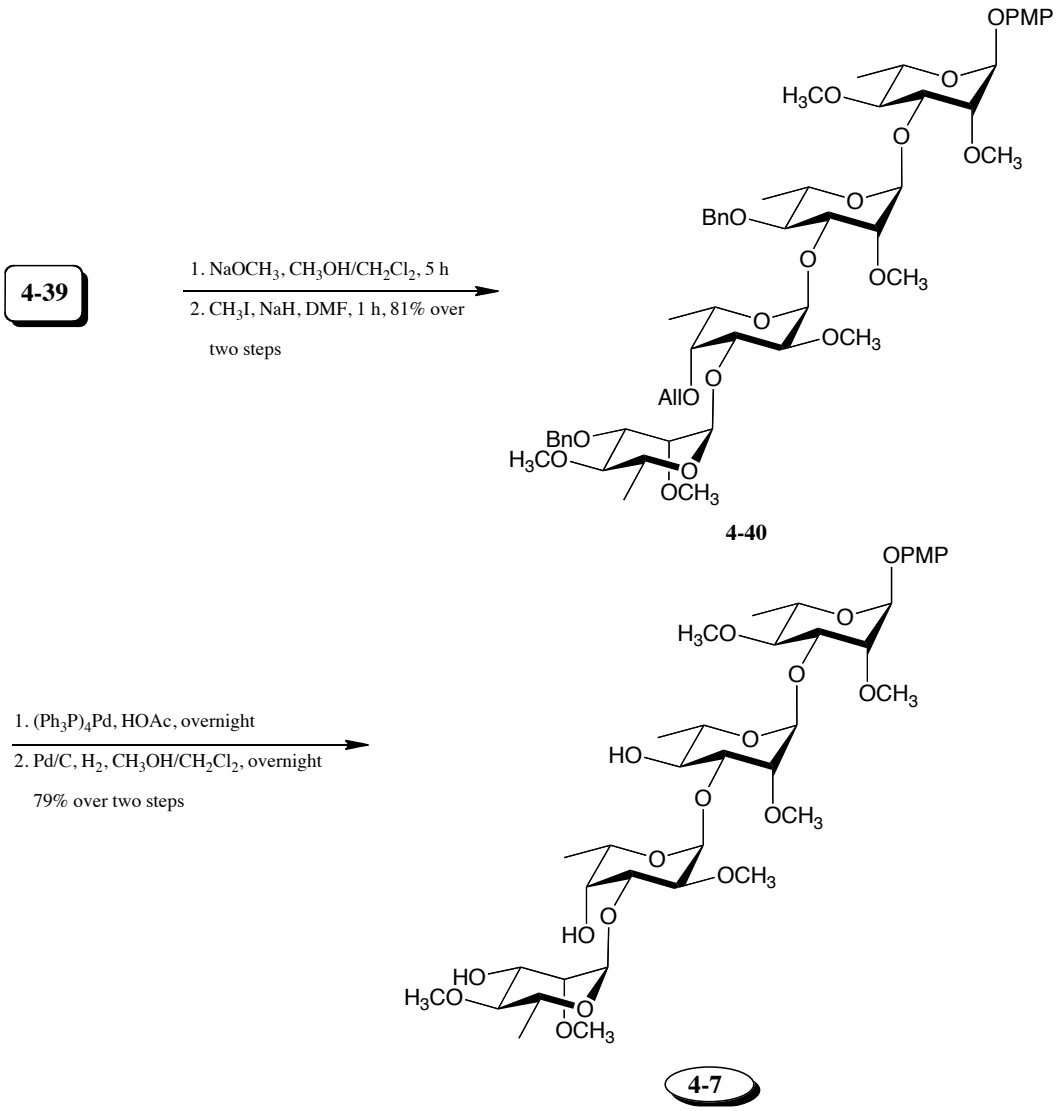

4-30
+
4-21
1. NIS, AgOTf, -40 ºC, 30 min
2. 5% TFA, CH2Cl2, 0 ºC, 20 min
76% over two steps
4-33
NIS, AgOTf, CH2Cl2, - 40 ºC,
30 min, 61%
4-38
1. NaOCH3, CH3OH/CH2Cl2, 3 h
2. (Ph3P)4Pd, HOAc, overnight
3. Pd/C, H2, CH3OH/CH2Cl2, overnight,
68% over three steps
4-39
4-5



Scheme 4-11. Synthesis of tetrasaccharides **4-5** and **4-6**.

4.2.4.3. Synthesis of tetrasaccharide 4-7

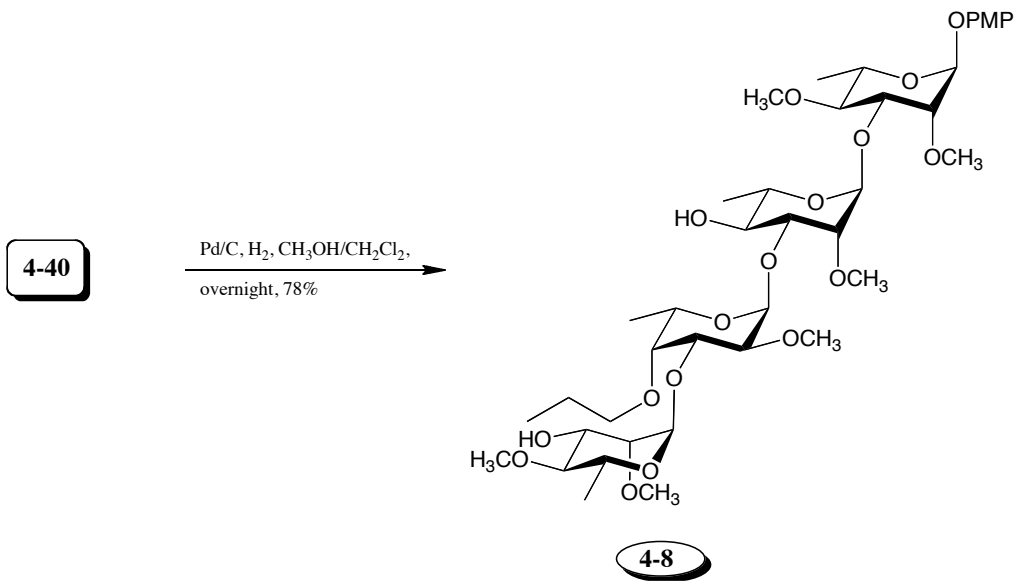
Target molecule **4-7** was also obtained from tetrasaccharide **4-39** described in the previous section (Scheme 4-12). First, treatment with sodium methoxide in methanol afforded an alcohol, which was then methylated[21] to furnish **4-40** in 81% yield. Finally, a 79% yield of target **4-7** was obtained after removal of allyl group using $(Ph_3P)_4Pd$[20] in acetic acid, purification of the resulting alcohol and then hydrogenolysis to deprotect all benzyl groups.



Scheme 4-12. Synthesis of tetrasaccharide **4-7**.

4.2.4.4. Synthesis of 4-8

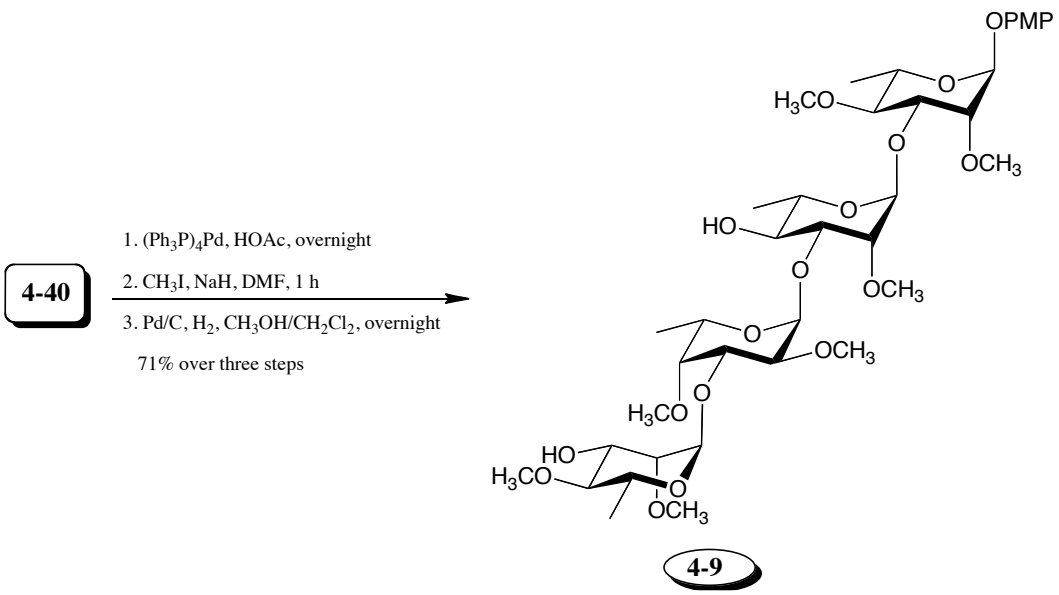
As shown in Scheme 4-13, target **4-8** was obtained in 78% yield via hydrogenolysis of the advanced tetrasaccharide **4-40** over palladium on charcoal.



Scheme 4-13. Synthesis of compound **4-8**.

4.2.4.5. Synthesis of tetrasaccharide 4-9

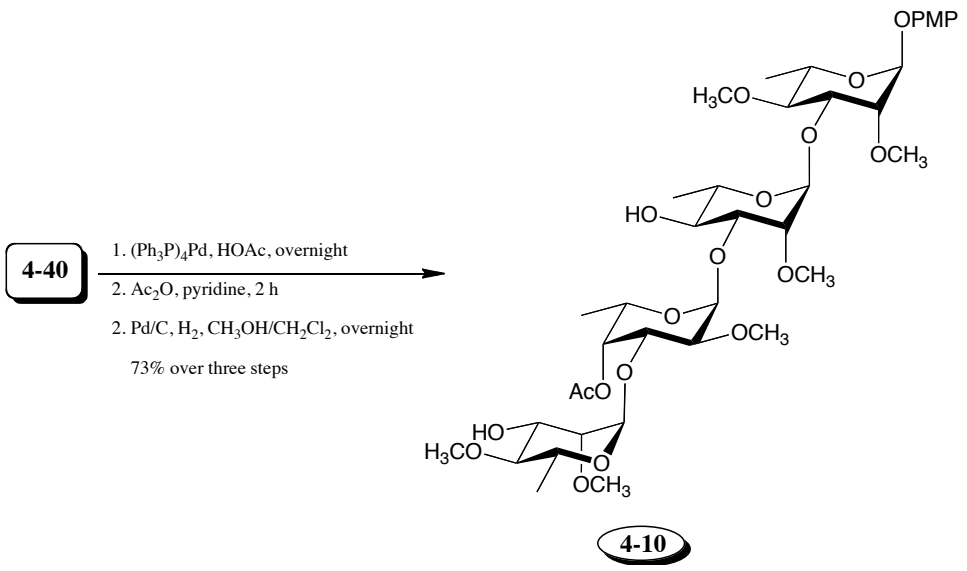
Starting from **4-40**, another set of protection and deprotection reactions were used to access tetrasaccharide **4-9** (Scheme 4-14). First, **4-40** was treated with $(Ph_3P)_4Pd$[20] in acetic acid to remove the allyl group followed by methylation of the resulting alcohol using methyl iodide and sodium hydride. After purification, subsequent hydrogenolysis to remove all benzyl groups afforded the target compound in 71% overall yield from **4-40**.



Scheme 4-14. Synthesis of compound **4-9**.

4.2.4.6. Synthesis of 4-10

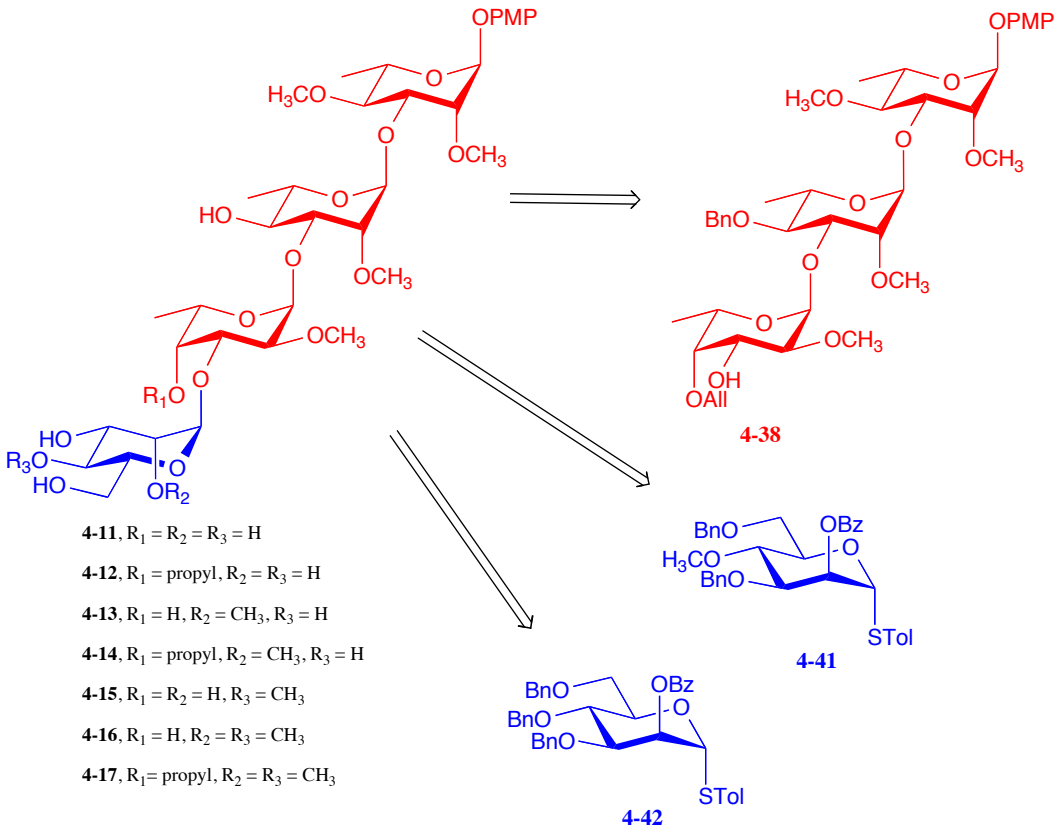
The synthesis of **4-10** is illustrated in Scheme 4-15. As described for some of the other target compounds, the preparation of the compound started by treatment of tetrasaccharide **4-40** with $(Ph_3P)_4Pd$[20] in acetic acid to remove the allyl group. Subsequent acetylation of the resulting alcohol with acetic anhydride and pyridine, purification of the resulting intermediate and then hydrogenolysis afforded **4-10** in 73% yield over three steps.



Scheme 4-15. Synthesis of tetrasaccharide **4-10**.

4.2.5. Retrosynthetic analysis of series III targets 4-11–4-18

As depicted in Scheme 4-16, to access series III targets building blocks **4-38**, **4-41**, and **4-42** needed to be prepared. Building block **4-38** is an advanced trisaccharide intermediate that was prepared during the assembly of the series II targets (see Scheme 4-11). Building blocks **4-41** and **4-42** are fully protected mannose thioglycoside derivatives that have a *p*-thiotoluyl moiety at the anomeric carbon.



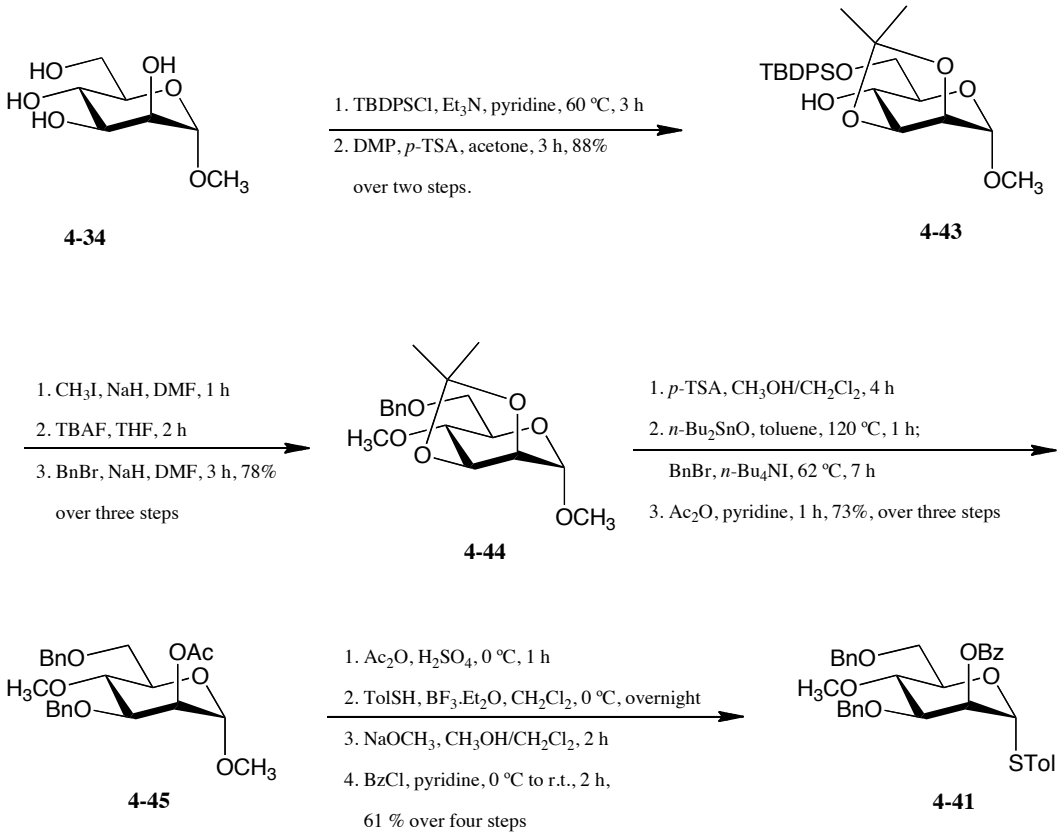
Scheme 4-16. Retrosynthetic analysis of series III targets.

4.2.5.1. Synthesis of building block 4-41

Scheme 4-17 details the construction of compound **4-41**. Similar to the synthesis of **4-33** (illustrated in Scheme 4-10), we started from the commercially available methyl α-D-mannopyranoside **4-34**. First, O-6 was protected as a silyl ether upon treatment with *tert*-butyl(chloro)diphenylsilane (TBDPSCl)[21] in pyridine and triethylamine (Et_3N). The resulting product was then treated with DMP in presence of *p*-TSA to afford **4-43** in 88% yield over two steps. Next, methyl iodide and sodium hydride were used to methylate the remaining hydroxyl group at O-4. Subsequent desilylation using *tert*-butylammonium fluoride

(TBAF)[21] provided a free hydroxyl group at O-6. After purification, the resulting alcohol was protected as a benzyl ether affording the fully protected methyl glycoside **4-44** in 78% yield over three steps.

Using the same reaction conditions and sequence employed to convert compound **4-36** into compound **4-33** (see Scheme 4-10 for the details), intermediate **4-44** was converted into building block **4-41** in good overall yield. The regioselectivity of the tin-mediated benzylation of **4-44** was established by the strong downfield chemical shift of H-2 (δ_H = 5.33 ppm) in the ^{1}H NMR spectrum of compound **4-45**.

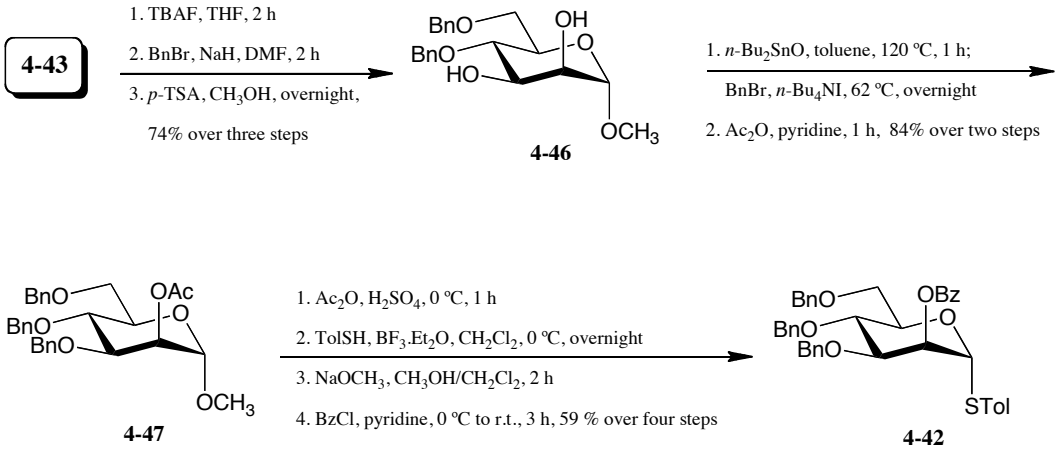


4-34 **4-43** **4-44** **4-45** **4-41**

Scheme 4-17. Synthesis of building block **4-41**.

4.2.5.2. Synthesis of building block 4-42

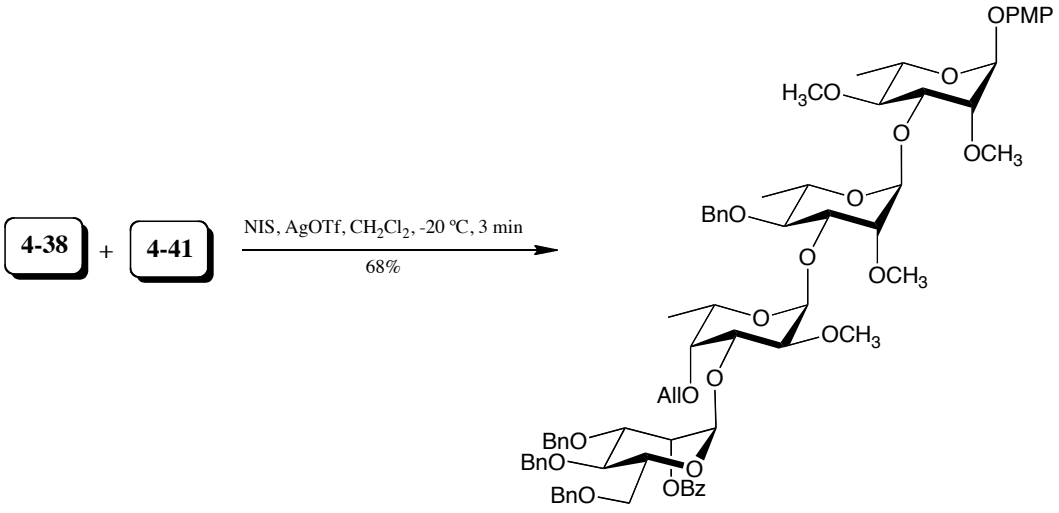
The advanced intermediate **4-43** was used as a starting for synthesis of building block **4-42** as shown in Scheme 4-18. Desilylation of **4-43** upon treatment with *tert*-butylammonium fluoride (TBAF)[21] in anhydrous THF afforded an alcohol intermediate. After purification of this intermediate, it was subsequently benzylated by treatment with benzyl bromide and sodium hydride. Finally, acid-catalyzed removal of the isopropylidene acetal afforded a 74% yield of diol **4-46**. Similarly, the same set of reaction conditions and sequence used for the conversion of **4-36** to **4-33** (see Scheme 4-10 for the details) was used to convert diol **4-46** to the fully functionalized building block **4-42** in good overall yield. As mentioned previously, the strong downfield chemical shift of H-2 (δ_H = 5.39 ppm) in the ^{1}H NMR spectrum of compound **4-47**, which bears an acyl group on O-2 confirmed the regioselectivity of the tin-mediated alkylation of compound **4-46**.



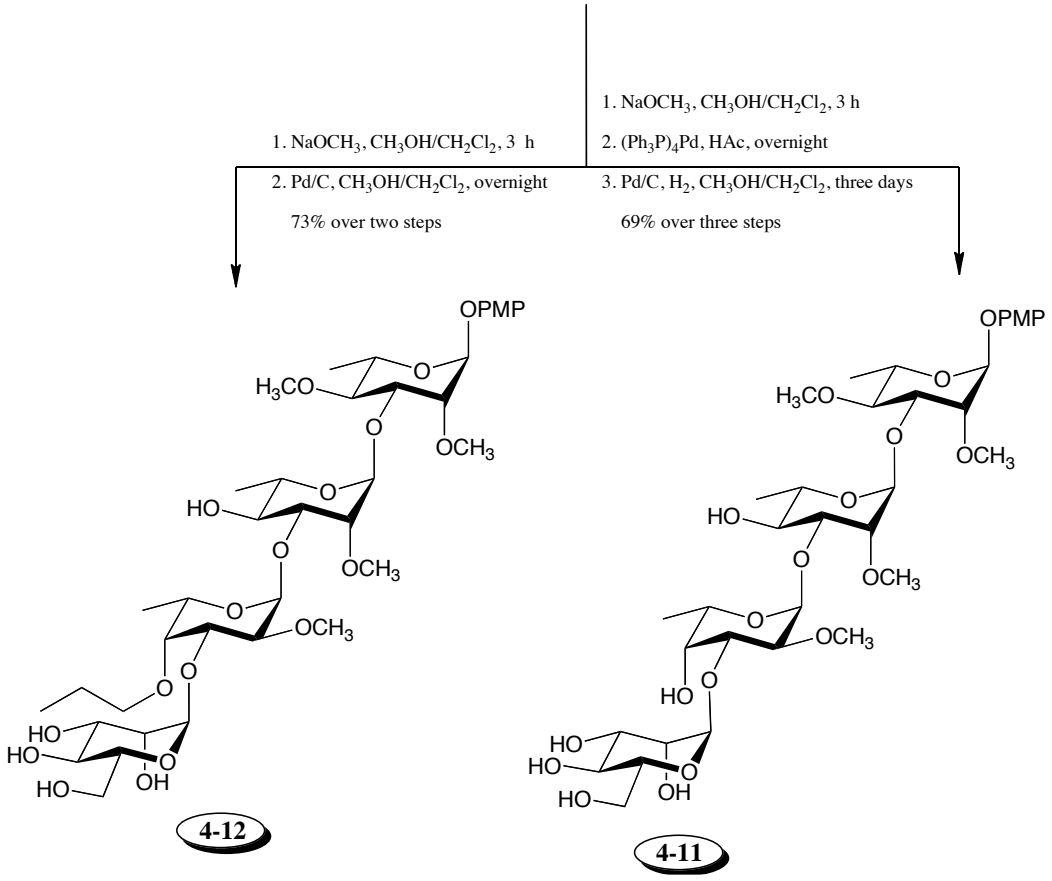
Scheme 4-18. Synthesis of building block **4-42**.

4.2.5.3. Synthesis of tetrasaccharides 4-11 and 4-12

With all building blocks in place, coupling of monosaccharide thioglycoside **4-42** and trisaccharide acceptor **4-38** using NIS–AgOTf[19] activation afforded tetrasaccharide **4-48** in 68% yield (Scheme 4-19). As mentioned previously, the stereochemistry of the glycosidic linkage was established by measuring the one-bond $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constant for the anomeric carbon of the newly produced bond. This value was found to be 172 Hz, which clearly indicated α-stereochemistry.[25] Both compounds **4-11** and **4-12** were obtained by subjecting **4-48** to two different sets of deprotection reactions. First, treatment of **4-48** with sodium methoxide in methanol followed by treatment with $(Ph_3P)_4Pd$ in acetic acid[20] furnished a diol intermediate. Subsequent hydrogenolysis to remove all benzyl groups afforded a 69% yield of target **4-11** over three steps. Alternatively, when **4-48** was subjected to Zemplén deacylation followed by hydrogenolysis, target **4-12**, which contains a propyl group at O-4 of the terminal fucose moiety, was obtained in 73% yield over two steps.



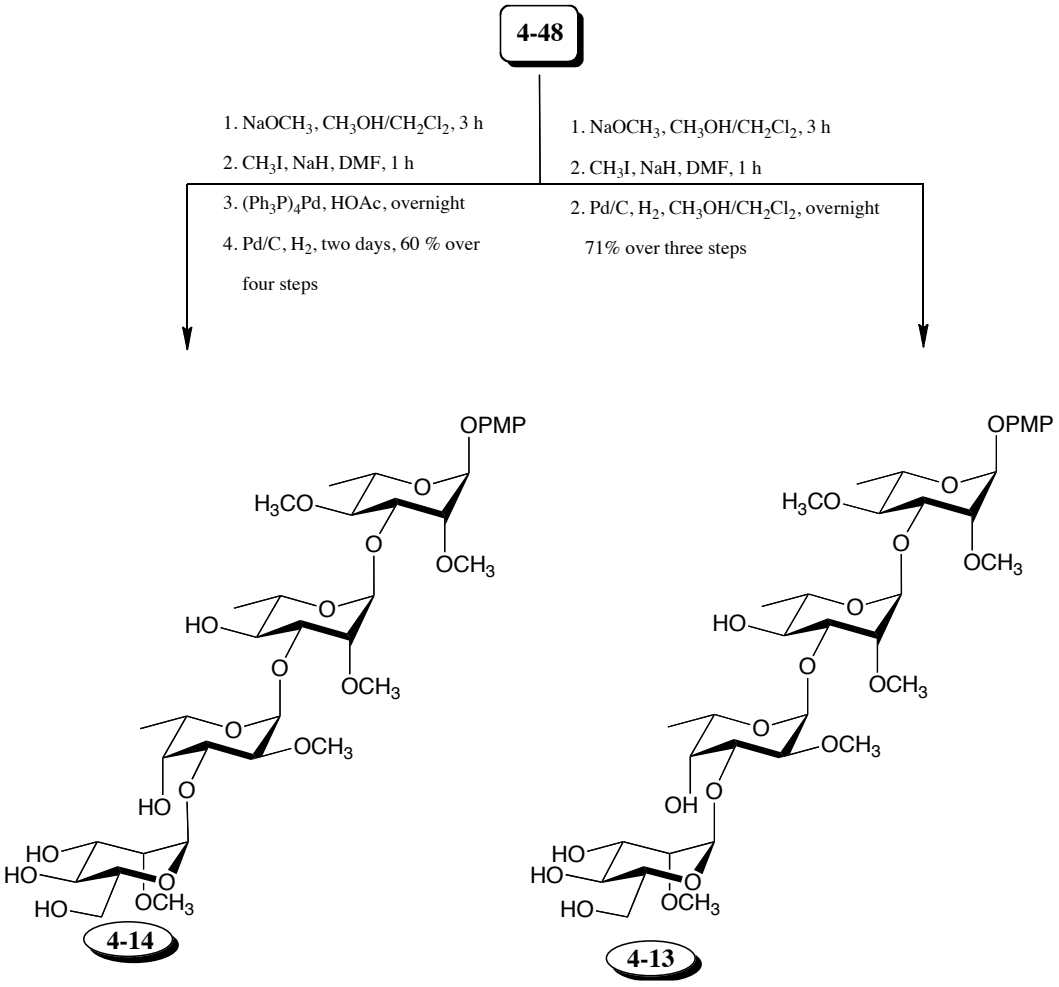
4-48



Scheme 4-19. Synthesis of tetrasaccharides **4-11** and **4-12**.

4.2.5.3. Synthesis of tetrasaccharides 4-13 and 4-14

With the fully functionalized tetrasaccharide **4-48** in hand, access to targets **4-13** and **4-14** was possible through a series of deprotection reactions as described in Scheme 4-20. First, the target **4-13** was obtained in a 71% yield after Zemplén deacylation, subsequent methylation of the resulting alcohol and finally, a hydrogenolysis to remove all benzyl protecting groups and reduce the allyl ether to a propyl ether. On the other hand, the selective removal of the allyl group using $(Ph_3P)_4Pd$ in acetic acid[20] before the final hydrogenolysis afforded target **4-14** in 60% combined yield.

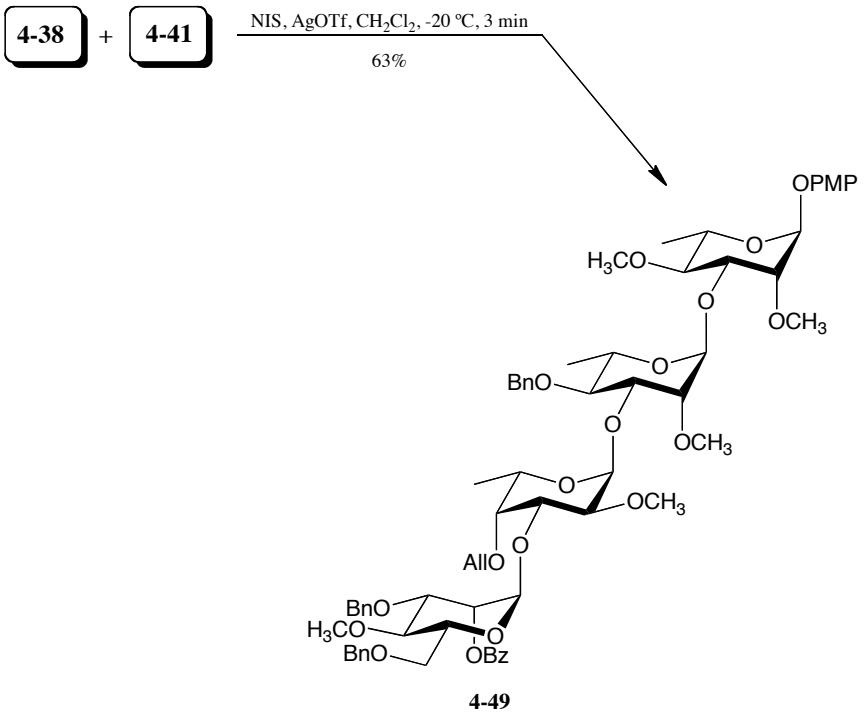


Scheme 4-20. Synthesis of tetrasaccharides **4-11** and **4-12**.

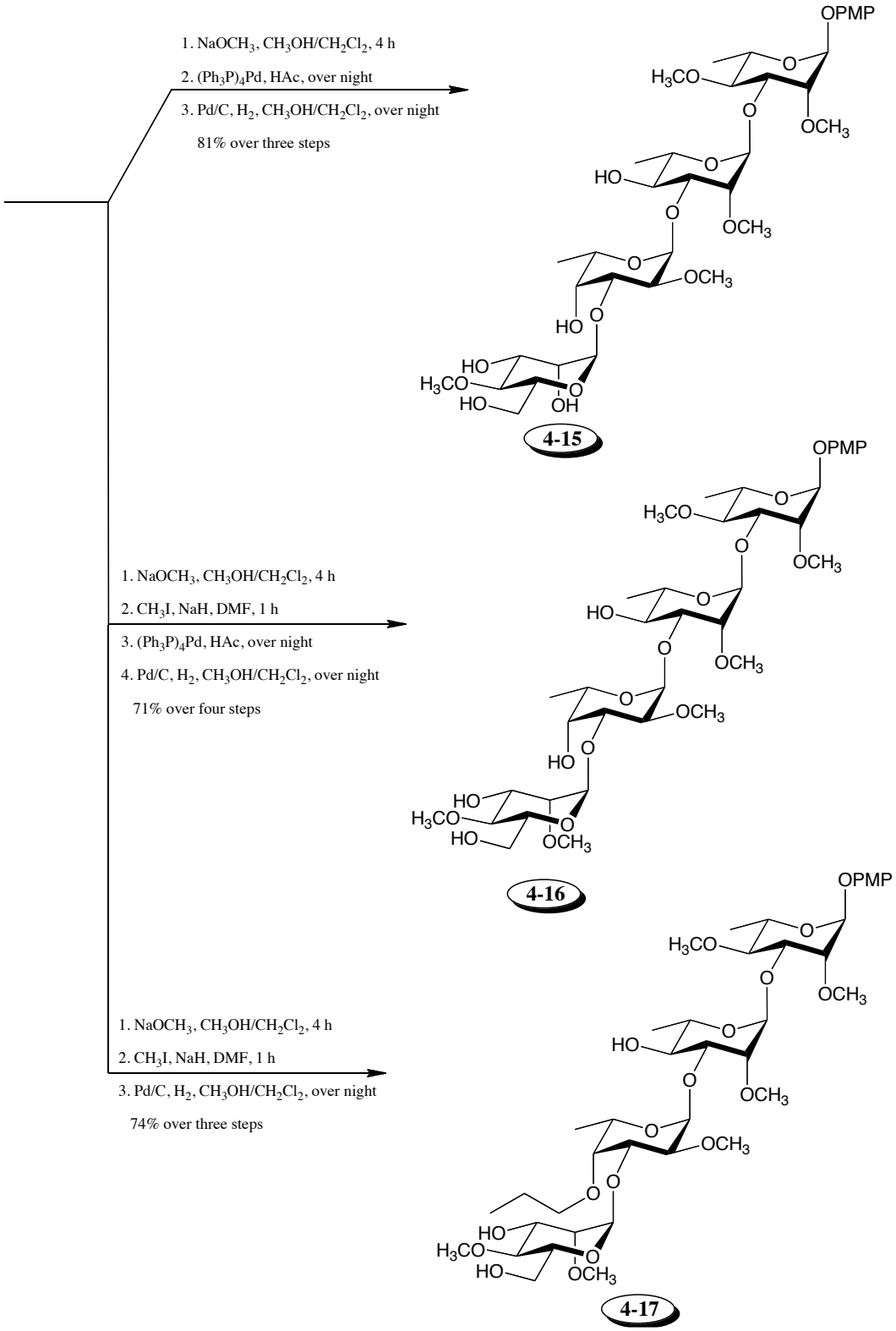
4.2.5.3. Assembly of 4-15–4-17.

With donor **4-41** and acceptor **4-38** in hand, the synthesis of these oligosaccharides could be performed, starting by making the tetrasaccharide intermediate **4-49** as described in Scheme 4-21. The coupling of **4-41** and **4-38** via NIS–AgOTf[19] activation afforded tetrasaccharide **4-49** in 63% yield. This compound was then used to complete the synthesis of the final compounds via various deprotection reactions. To access target **4-15**, Zemplén deacylation[12] was

performed followed by selective removal of the allyl protecting group using $(Ph_3P)_4Pd$[20] in acetic acid affording a diol intermediate. Subsequently, after the diol intermediate was purified, all benzyl groups were removed via hydrogenolysis[13] to furnish **4-15** in 81% yield over three steps. Similarly, the target tetrasaccharide **4-16** was obtained in 71% combined yield starting from **4-49**. First, Zemplén deacylation[12] was performed and the resulting alcohol was methylated with methyl iodide and sodium hydride. Subsequently, the allyl group was removed selectively under the same conditions as those used for the synthesis of **4-15**. Hydrogenolysis when then used to remove all of the benzyl groups thus affording the expected tetrasaccharide. Finally, compound **4-17** was obtained in 74% yield after Zemplén deacylation and subsequent methylation of the resulting alcohol followed by hydrogenolysis over palladium on charcoal for global debenzylation.[13]



4-49



Scheme 4-21. Synthesis of tetrasaccharides **4-15**–**4-17**.

4.3. Summary.

In this chapter, the synthesis of a panel of 17 synthetic analogs, **4-1** to **4-17**, of PGLs from *M. kansasii* using efficient linear routes were described. The stereochemistry of all synthesized glycosidic linkages was established using coupled HSQC experiments. The values of $^{1}J_{C\text{-}1,H\text{-}1}$ were in the range of 169–171 Hz for all products, which confirmed the α-stereochemistry. As the main goal of this project was to investigate the immunomodulatory effect of PGLs on the activated macrophages, these synthetic analogs were used for this purpose and the results will be discussed in Chapter 6.

4.3. Experimental

4.3.1. General Methods

Solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere before use. All reagents used in reactions were purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV_{254} (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by charring with a solution of anisaldehyde in ethanol, acetic acid, and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). The ratio between silica gel and residue ranged from 100:1 to 20:1 (w/w). Organic solutions were concentrated under vacuum at < 50 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 or 500 MHz. ^{1}H NMR chemical shifts are referenced to TMS (0.0, $CDCl_3$) or CD_3OD (4.78, CD_3OD). ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.23, $CDCl_3$). ^{1}H NMR data are reported as though they are first order and the peak assignments were made on the basis of 2D-NMR (^{1}H–^{1}H COSY and HMQC) experiments. The monosaccharide residues in the disaccharide and trisaccharides are labelled by no prime, prime, double, and tri–prime as shown in Figure 4-3 and these labels are maintained in the assignment of NMR spectra of all compounds. Optical rotations were measured at 21 ± 2 °C at the sodium D line (589 nm) and are in units of

deg•mL(dm•g)$^{-1}$. ESI-MS spectra were carried out on samples suspended in THF or CH_3OH and added NaCl.

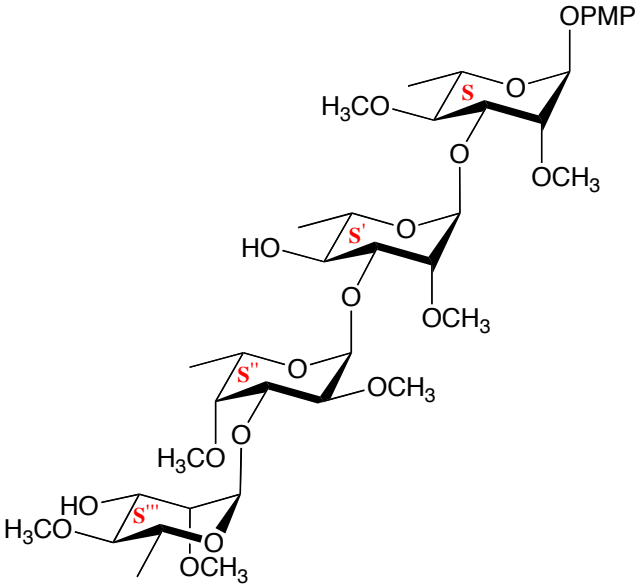
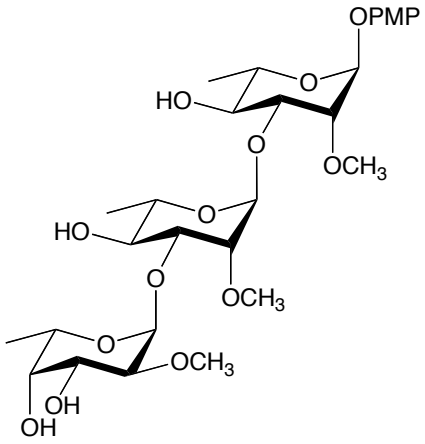


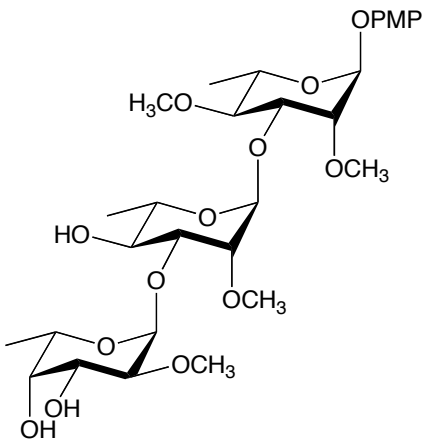
Figure 4-3. Numbering system for labelling data.



***p*-Methoxyphenyl 2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranoside (4-1)**

To a solution of **4-28** (16.5 mg, 0.02 mmol) in 1:1 CH_3OH–CH_3Cl_2 (10 mL), *p*-TSA (3 mg) was added and the solution was stirred for 3 h at rt. The reaction mixture was neutralized with Et_3N (50 μL), concentrated and the resulting residue was purified by chromatography (1:2 hexane–EtOAc) to give a syrup. The syrup was dissolved in 1:1 CH_3OH–CH_3Cl_2 (15 mL), Pd–C (3 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere at rt. The reaction mixture was then filtered, concentrated and the resulting residue was purified by chromatography (15:1 CH_2Cl_2–CH_3OH) to give **4-1** (10.5 mg, 87 %) as a colorless thick syrup: R_f 0.45 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +67.8 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.02–6.99 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.44 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.20 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1″), 4.25–4.21 (m, 1H, H-5″), 4.07–4.03 (m, 2H, H-3, H-3′), 3.89 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.84–3.83 (m, 1H, H-5), 3.80–3.74 (m, 3H, H-4, H-4′, H-4″), 3.78 (s, 3H, OC*H*$_3$), 3.72–3.68 (m, 1H, H-

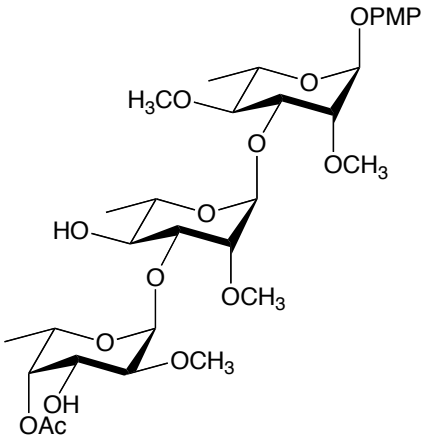
3″), 3.66 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.64–3.61 (m, 1H, H-2′) 3.54 (s, 3H, OC*H*$_3$), 3.52–3.51 (m, 1H, H-2″), 3.50 (s, 3H, OC*H*$_3$), 3.46 (s, 3H, OC*H*$_3$), 2.52 (br s, 1H, OH), 2.31 (br s, 2H, OH x 2), 1.35 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″), 1.31 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6), 1.29 (d, 3H, $J_{5',6'}$ = 6.3 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.5 (Ar), 117.5 (Ar x 2), 114.7 (Ar x 2), 99.8 (C-1″), 99.2 (C-1′), 95.5 (C-1), 83.0 (C-2″), 80.2(2) (C-2), 80.1(4) (C-2′), 80.1 (C-3″), 79.5 (C-4″), 71.9 (C-4′), 71.7 (C-4), 71.5 (C-5′), 69.9 (C-5), 69.0(1) (C-3′), 68.9(9) (C-3), 66.5 (C-5″), 59.3 (O*C*H$_3$), 58.7 (O*C*H$_3$), 58.6 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.0 (C-6), 17.8 (C-6′), 16.4 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{28}H_{44}NaO_{14}$: 627.2623. Found 627.2621.



***p*-Methoxyphenyl 2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-2)**

To a solution of **4-31** (10 mg, 0.012 mmol) in 1:1 CH_3OH–CH_3Cl_2 (10 mL), *p*-TSA (2 mg) was added. The reaction mixture was stirred for 3 h at rt before the addition of Et_3N (50 μL) and concentration. The resulting residue was purified by

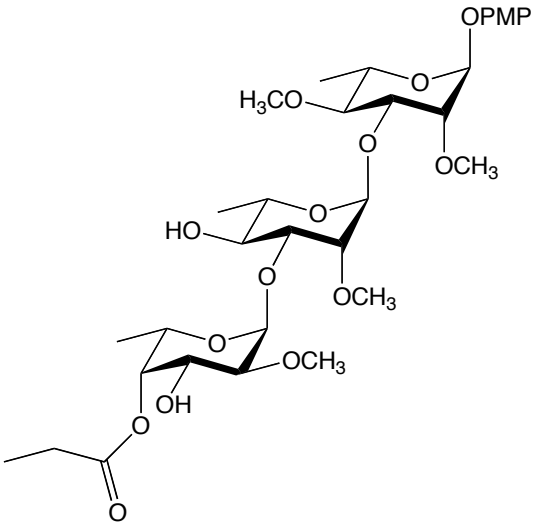
chromatography (1:2 hexane–EtOAc) to give a colorless oil (8 mg). The oil was dissolved in 1:1 CH_3OH–CH_3Cl_2 (20 mL), Pd–C (2 mg, 20% w/w) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere at rt. The reaction mixture was filtered, concentrated and the resulting crude product was purified by chromatography (15:1 CH_2Cl_2–CH_3OH) to give **4-2** (6.7 mg, 90%) as a thick syrup: R_f 0.55 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –19.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.02–6.99 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.44 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.20 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1″), 4.24 (dq, 1H, $J_{4'',5''}$ = 2.7 Hz, $J_{5'',6''}$ = 6.5 Hz, H-5″), 4.10 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.05 (dd, 1H, $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.87 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.84–3.83 (m, 1H, H-3″), 3.77–3.74 (m, 1H, H-5), 3.78 (s, 3H, OC*H*$_3$), 3.71–3.68 (m, 2H, H-2″, H-4″), 3.67 (dd, 1H, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.0 Hz, H-2′), 3.60 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 3.55 (s, 3H, OC*H*$_3$), 3.53 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.51–3.49 (m, 1H, H-2), 3.46 (s, 3H, OC*H*$_3$), 3.22 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 2.80 (br s, 1H, OH), 2.43 (br s, 1H, OH), 1.68 (br s, 1H, OH), 1.35 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.31 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.29 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.0 (Ar), 150.5 (Ar), 117.5 (Ar x 2), 114.7 (Ar x 2), 99.8 (C-1″), 99.2 (C-1′), 95.5 (C-1), 83.0 (C-4), 80.2(0) (C-2), 80.1(7) (C-2′), 80.1 (C-4′), 79.5 (C-4″), 71.9 (C-2″), 71.7 (C-5), 71.5 (C-3″), 69.9 (C-5′), 68.9 (C-3), 68.8 (C-3′), 66.5 (C-5″), 61.1 (O*C*H$_3$), 59.3 (O*C*H$_3$), 58.7 (O*C*H$_3$), 58.6 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.0 (C-6), 17.8 (C-6`), 16.4 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{29}H_{46}NaO_{14}$: 641.2780. Found 641.2775.



***p*-Methoxyphenyl 4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-3)**

To a solution of **4-32** (20 mg, 0.03 mmol) in pyridine (2 mL), Ac_2O (1 mL) was added and the reaction mixture was stirred for 2 h at rt. Water (5 mL) was then added and the solution was diluted with CH_2Cl_2 (10 mL), washed with water (2 x 8 mL), 1M HCl soln (2 x 8 mL) and brine. The organic layer was dried ($NaSO_4$), concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in the 1:1 CH_2Cl_2–CH_3OH (20 mL), Pd–C (4 mg) was added and the reaction mixture was stirred under a hydrogen atmosphere overnight. The reaction mixture was then filtered, concentrated and the resulting syrup was purified by chromatography (10:0.25 CH_2Cl_2–CH_3OH) to give **4-3** (12 mg, 81%) as a thick syrup: R_f 0.45 (10:0.25 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –32.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.97 (m, 2H, Ar-2,6), 6.82–6.81 (m, 2H, Ar-3,5), 5.39 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.23–5.22 (m, 1H, H-4″), 5.17 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.14 (d, 1H,

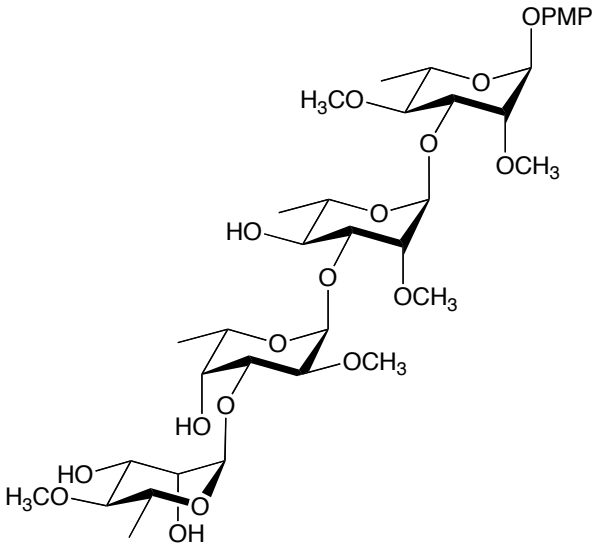
$J_{1'',2''}$ = 3.4 Hz, H-1″), 4.30 (dq, 1H, $J_{4'',5''}$ = 2.1 Hz, $J_{5'',6''}$ = 6.4 Hz, H-5″), 4.23 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.8 Hz, H-3′), 4.09 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 3.86 (dq, 1H, $J_{4',5'}$ = 9.8 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.76 (s, 3H, OC*H*$_3$), 3.71–3.68 (m, 2H, H-3″, H-5), 3.65–3.63 (m, 2H, H-2, H-2′), 3.55 (s, 3H, OC*H*$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.49–3.47 (m, 2H, H-2″, H-4′), 3.45 (s, 3H, OC*H*$_3$), 3.22 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 2.18 (s, 3H, C*H*$_3$CO), 1.36 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.28 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.12 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 171.3 (*C*=O), 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.0 (C-1″), 99.2 (C-1′), 95.6 (C-1), 83.0 (C-4), 82.3 (C-4′), 80.5 (C-2′), 80.3 (C-2), 79.4 (C-2″), 79.0 (C-3″), 73.4 (C-5), 71.5 (C-5′), 68.9 (C-3), 68.7 (C-3′), 68.6 (C-5″), 65.5 (C-4″), 61.0 (O*C*H$_3$), 59.4 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.5 (O*C*H$_3$), 55.7 (O*C*H$_3$), 20.8 (*C*H$_3$CO), 17.8(8) (C-6′), 17.8(4) (C-6), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{31}H_{48}NaO_{15}$: 683.2885. Found 683.2880.



***p*-Methoxyphenyl 2-*O*-methyl-4-*O*-propionyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-4)**

To a solution of **4-32** (10 mg, 0.012 mmol) in pyridine (2 mL), propanoic anhydride (1 mL) was added and the reaction mixture was stirred for 2 h at rt. After 2 h, water (5 mL) was added and the solution was diluted with CH_2Cl_2 (10 mL), washed with water (2 x 8 mL), 1M HCl soln (2 x 8 mL) and brine (8 mL). The organic layer was separated, concentrated and the resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in the 1:1 CH_2Cl_2–CH_3OH (20 mL), Pd–C (2 mg) was added and the reaction mixture was stirred overnight under hydrogen. The reaction mixture was then filtered, concentrated and the resulting residue was purified by chromatography (10:0.25 CH_2Cl_2–CH_3OH) to give **4-4** (7.2 mg, 88%) as a thick syrup: R_f 0.60 (20:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ –21.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00–6.97 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.39 (d,

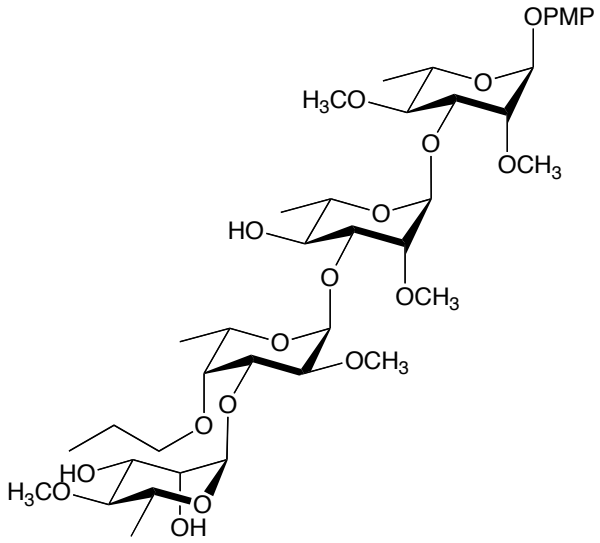
1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.23 (m, 1H, H-4″), 5.17 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 4.31 (dq, 1H, $J_{4'',5''}$ = 2.1 Hz, $J_{5'',6''}$ = 6.5 Hz, H-5″), 4.23 (dd, 1H, $J_{2',3'}$ = 3.5 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 4.10 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.86 (dq, 1H, $J_{4',5'}$ = 9.7 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.78–3.76 (m, 1H, H-5), 3.75 (s, 3H, OCH_3), 3.71–3.68 (m, 2H, H-2′, H-3″), 3.65–3.63 (m, 2H, H-2, H-4′), 3.55 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3), 3,52 (s, 3H, OCH_3), 3.48 (dd, 1H, $J_{1'',2''}$ = 3.3 Hz, $J_{2'',3''}$ = 9.8 Hz, H-2″), 3.46 (s, 3H, OCH_3), 3.22 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 2.47 (q, 2H, J = 7.6 Hz, CH_2CH$_3$), 1.35 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.19 (t, 3H, J = 7.6 Hz, CH_3CH$_2$), 1.12 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 174.8 (*C*=O), 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.0 (C-1″), 99.2 (C-1′), 95.6 (C-1), 83.0 (C-4), 82.3 (C-4′), 80.5 (C-2′), 80.3 (C-2), 79.4 (C-2″), 79.0 (C-3″), 73.2 (C-5), 71.5 (C-5′), 69.0 (C-3), 68.7 (C-3′), 68.7 (C-5″), 65.6 (C-4″), 61.0 (OCH_3), 59.3 (OCH_3), 59.0 (OCH_3), 58.5 (OCH_3), 55.7 (OCH_3), 27.5 (*C*H$_2$CH$_3$), 17.8(9) (C-6′), 17.8(4) (C-6), 16.3 (C-6″), 9.3 (*C*H$_3$CH$_2$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{32}H_{50}NaO_{15}$: 697.3042. Found 697.3037.



***p*-Methoxyphenyl 6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-5)**

To a solution of **4-39** (20 mg, 0.02 mmol) in 1:1 CH_3OH–CH_2Cl_2 (10 mL), 1M $NaOCH_3$ (0.1 mL) was added and the reaction mixture was stirred for 3 h at rt. The reaction mixture was then neutralized by the addition of Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in AcOH (3 mL) and then $(Ph_3P)_4Pd$ (4 mg) was added. The reaction mixture was stirred overnight and then filtered and water (5 mL) was added. The solution was diluted with CH_2Cl_2 (10 mL), washed with water (2 x 8 mL) and brine (10 mL). The organic layer was separated, dried ($NaSO_4$), concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. To the solution of the oil in 1:1 CH_3OH–CH_2Cl_2 (15 mL), Pd–C (5 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere before it was filtered and concentrated. The resulting crude product was purified

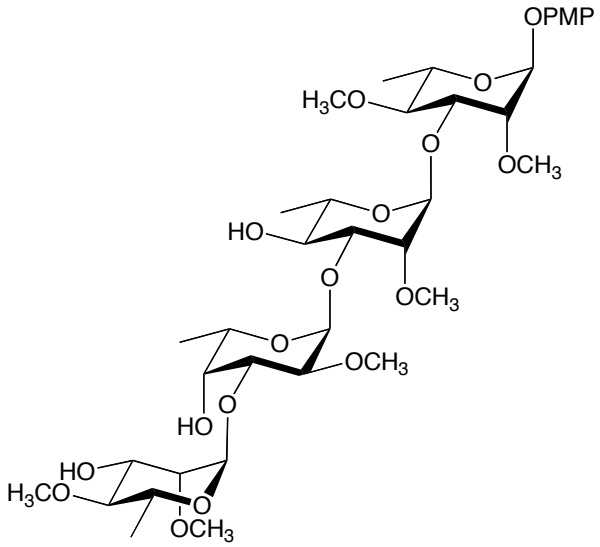
by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-5** (9.6 mg, 68%) as a colorless oil: R_f 0.30 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –36.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.96 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.38 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 5.17 (br s, 1H, H-1′), 5.08 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 5.05 (d, 1H, $J_{1''',2'''}$ = 1.6 Hz, H-1‴), 4.19 (dq, 1H, $J_{4'',5''}$ = 3.0 Hz, $J_{5'',6''}$ = 6.3 Hz, H-5″), 4.08 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, $J_{3',4'}$ = 9.4 Hz, H-3′), 4.05 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3) 3.99–3.98 (m, 1H, H-3″), 3.87–3.84 (m, 1H, H-5′, H-5‴), 3.79–3.78 (m, 1H, H-5), 3.79 (s, 3H, OC*H*$_3$), 3.73–3.65 (m, 3H, H-2, H-2′, H-2‴), 3.66–3.62 (m, 2H, H-2″, H-3‴), 3.61–3.57 (m, 2H, H-4′, H-4″), 3.57 (s, 3H, OC*H*$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.49 (s, 3H, OC*H*$_3$), 3.46 (s, 3H, OC*H*$_3$), 3.22 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.11 (app t, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 9.6 Hz, H-4‴), 2.90 (br s, 1H, OH), 2.30 (br s, 1H, OH), 1.77 (br s, 2H, OH x 2), 1.37–1.32 (m, 6H, H-6′, H-6″), 1.27–1.24 (m, 6H, H-6 , H-6‴); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 101.2 (C-1‴), 100.3 (C-1′), 98.9 (C-1″), 95.6 (C-1), 83.1(0) (C-4‴), 83.0(4) (C-4), 82.3 (C-4′), 80.5 (C-4″), 80.3 (C-3‴), 79.1 (C-2′), 78.6 (C-2″), 77.5 (C-2), 72.0 (C-2‴), 71.4 (C-5), 71.3 (C-5‴), 71.1 (C-5′), 69.0 (C-3), 68.7 (C-3″), 68.4 (C-3′), 66.2 (C-5″), 61.1 (O*C*H$_3$), 61.0 (O*C*H$_3$), 59.6 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.4 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.0 (C-6′), 17.9 (C-6), 17.8 (C-6‴), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{36}H_{58}NaO_{18}$: 801.3515. Found 801.3505.



***p*-Methoxyphenyl 6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl -4-*O*-propyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-6)**

To a solution of **4-39** (12 mg, 0.01 mmol) in 1:1 CH_3OH–CH_2Cl_2 (10 mL), 1M $NaOCH_3$ (0.1 mL) was added and the reaction mixture was stirred for 3 h at rt. The reaction mixture was neutralized by the addition of Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was purified by chromatography (1:1 hexane–EtOAc) to give an oil. This oil was dissolved in 1:1 CH_3OH–CH_2Cl_2 (15 mL), Pd–C (3 mg) was added and the reaction mixture was stirred overnight under hydrogen before it was filtered and concentrated. The resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-6** (7.8 mg, 87%) as a colorless oil: R_f 0.38 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –28.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.96 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.39 (br s, 1H, H-1), 5.16 (br s, 1H, H-1′), 5.07 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1″), 5.03 (br s, 1H, H-1‴), 4.14 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.5 Hz,

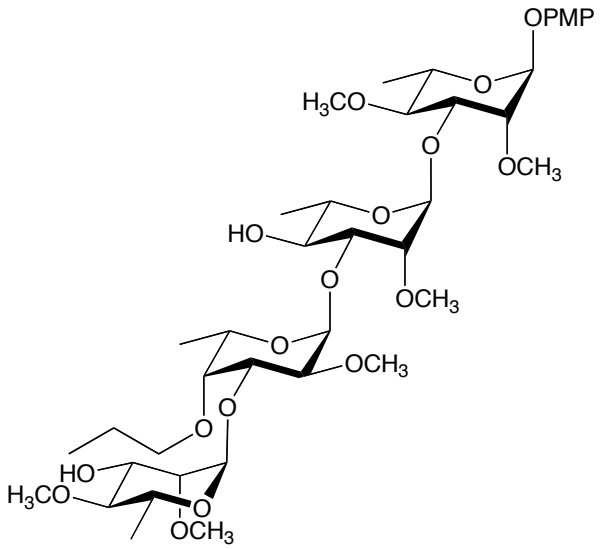
H-3′), 4.10 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 4.07 (dq, 1H, $J_{4'',5''}$ = 2.9 Hz, $J_{5'',6''}$ = 6.5 Hz, H-5″), 4.00–3.98 (m, 1H, H-3″), 3.86 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.82–3.78 (m, 2H, H-5, H-5‴), 3.76 (s, 3H, OCH_3), 3.70–3.65 (m, 5H, H-2, H-2′, H-2‴, CH_2O x 2), 3.64–3.60 (m, 3H, H-2″, H-3‴, H-4′), 3.57 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.40–3.37 (m, 1H, H-4‴), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.10–2.98 (m, 1H, H-4″), 1.65–1.58 (m, 2H, CH_2CH$_3$), 1.33 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.27–1.24 (m, 6H, H-6, H-6′), 1.21 (d, 3H, $J_{5''',6'''}$ = 6.5 Hz, H-6‴), 0.93 (t, 3H, J = 7.5 Hz, CH$_2$CH_3); ^{13}C NMR (125.7 MHz, CDCl$_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 101.2 (C-1‴), 100.2 (C-1′), 99.3 (C-1″), 95.6 (C-1), 83.4 (C-4″), 83.0 (C-4‴), 82.2 (C-4), 80.8 (C-4′), 80.4 (C-3‴), 80.3 (C-2′), 80.0 (C-2″), 79.4 (C-2), 76.4 (C-2‴), 75.8 (*C*H$_2$O), 71.4 (C-5), 71.3(3) (C-5‴), 71.3(0) (C-5′), 69.0 (C-3), 68.7 (C-3″), 68.2 (C-5″), 67.5 (C-3′), 61.1 (O*C*H$_3$), 60.9 (O*C*H$_3$), 59.4 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.4 (O*C*H$_3$), 55.7 (O*C*H$_3$), 23.5 (*C*H$_2$CH$_3$), 18.0 (C-6‴), 17.9 (C-6″), 17.8 (C-6′), 16.6 (C-6), 10.8 (CH$_2$*C*H$_3$). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{39}H_{64}NaO_{18}$: 843.3985. Found 843.3978.



***p*-Methoxyphenyl 6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-7)**

To a solution of **4-40** (15 mg, 0.014 mmol) in HOAc (3 mL), $(Ph_3P)_4Pd$ (3 mg) was added and the reaction mixture was stirred overnight at rt. The reaction mixture was then filtered, water (8 mL) was added and then it was diluted with CH_2Cl_2 (15 mL), washed with water (2 x 10 mL) and brine (10 mL). The organic layer was separated, dried (Na_2SO_4) and concentrated. The resulting residue was purified by chromatography (1:1 hexane–EtOAc) to give a colorless oil. The oil was dissolved in 1:1 CH_2Cl_2–CH_3OH (20 mL) and Pd–C (4 mg) was added. The reaction mixture was stirred overnight under hydrogen before it was filtered, concentrated and the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-7** (9.3 mg, 79%) as a thick syrup: R_f 0.44 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –23.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00–6.97 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.39 (d, 1H, $J_{1,2}$ = 1.8 Hz,

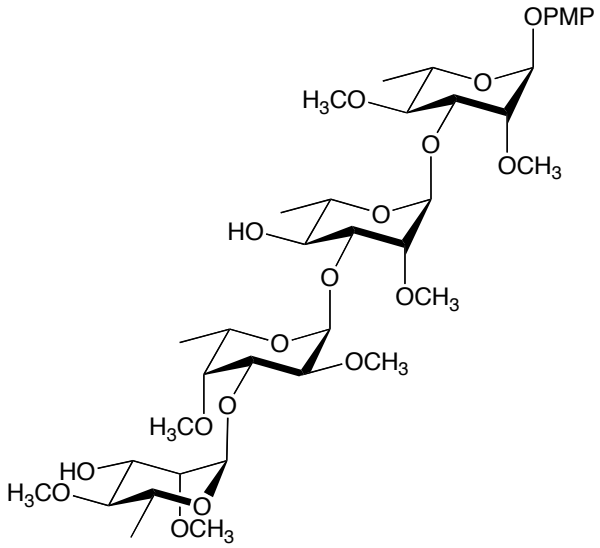
H-1), 5.17–5.16 (m, 2H, H-1′, H-1‴), 5.07 (d, 1H, $J_{1'',2''}$ = 3.1 Hz, H-1″), 4.20 (dq, 1H, $J_{4'',5''}$ = 2.7 Hz, $J_{5'',6''}$ = 6.5 Hz, H-5″), 4.11–4.06 (m, 2H, H-3, H-3′), 3.89–3.81 (m, 2H, H-3″, H-5′), 3.80–3.78 (m, 1H, H-5‴), 3.76 (s, 3H, OCH_3), 3.71–3.67 (m, 4H, H-2, H-2′, H-2″, H-5), 3.66–3.63 (m, 3H, H-3‴, H-4′, H-4″), 3.60 (dd, 1H, $J_{1''',2'''}$ = 1.8 Hz, $J_{2''',3'''}$ = 3.3 Hz, H-2‴), 3.58 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 3.22 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.00 (app t, 1H, $J_{3''',4'''} = J_{4''',5'''}$ = 9.7 Hz, H-4‴), 2.43 (d, 1H, J = 9.0 Hz, OH), 2.12 (br s, 1H, OH), 1.65 (br s, 1H, OH), 1.35 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.30 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6), 1.27–1.25 (m, 6H, H-6″, H-6‴); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.6 (C-1‴), 99.4 (C-1′), 97.9 (C-1″), 95.6 (C-1), 83.6 (C-4‴), 83.3 (C-4), 82.3 (C-4′), 80.7 (C-4″), 80.6 (C-3‴), 80.3 (C-2‴), 78.9(3) (C-2′), 78.8(7) (C-2″), 78.6 (C-2), 71.6(3) (C-5), 71.5(9) (C-5‴), 71.2 (C-5′), 68.9 (C-3), 68.7 (C-3″), 68.2 (C-3′), 66.3 (C-5″), 61.1 (O*C*H_3), 61.0 (O*C*H_3), 59.7 (O*C*H_3), 59.0 (O*C*H_3), 58.8 (O*C*H_3), 58.6 (O*C*H_3), 55.7 (O*C*H_3), 17.9(6) (C-6′), 17.9(4) (C-6), 17.9 (C-6‴), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{37}H_{60}NaO_{18}$: 815.3672. Found 815.3663.



***p*-Methoxyphenyl 6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-4-*O*-propyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-8)**

To a solution of **4-40** (10 mg, 0.01 mmol) in 1:1 CH_2Cl_2–CH_3OH (10 mL), Pd–C (4 mg) was added and the reaction mixture was stirred overnight under hydrogen at rt before it was filtered and concentrated. The resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-8** (6.4 mg, 78%) as a colorless oil: R_f 0.51 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –25.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.95 (m, 2H, Ar-2,6), 6.82–6.79 (m, 2H, Ar-3,5), 5.38 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.16-5.14 (m, 2H, H-1′, H-1‴), 5.05 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1″), 4.15–4.06 (m, 3H, H-3, H-3′, H-5″), 3.86 (dq, 1H, $J_{4',5'}$ = 9.6, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.87–3.77 (m, 2H, H-5, H-5‴), 3.75 (s, 3H, OCH_3), 3.73–3.59 (m, 8H, H-2, H-2′, H-2″, H-4′, H-3″, H-3‴, CH_2O x 2), 3.57 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 3.52–3.51 (m, 1H, H-2‴), 3.50 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.45 (s, 3H, OCH_3), 3.38–3.36 (m, 1H, H-4), 3.20 (app t, 1H,

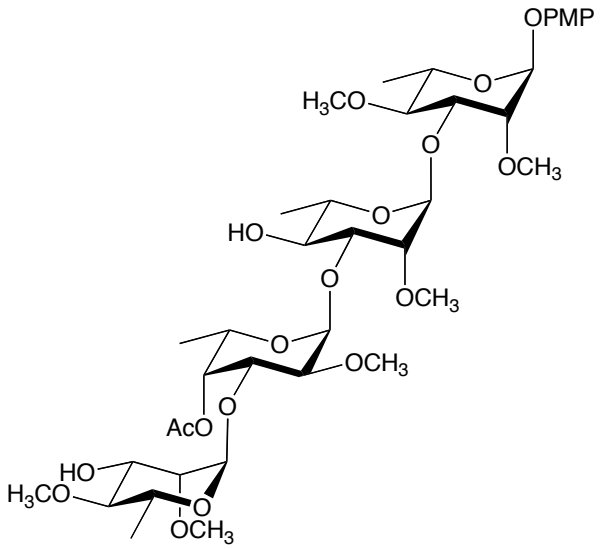
$J_{3''',4'''}$ = $J_{4''',5'''}$ 9.5 Hz, H-4‴), 3.10–2.96 (m, 1H, H-4″), 1.63–1.56 (m, 2H, CH_2CH$_3$), 1.34 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.31 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.26 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.20 (d, 3H, $J_{5''',6'''}$ = 6.3 Hz, H-6‴), 0.92 (t, 3H, J = 7.5 Hz, CH$_2$CH_3); ^{13}C NMR (125.7 MHz, CDCl$_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.5 (C-1‴), 99.5 (C-1′), 97.5 (C-1″), 95.6 (C-1), 83.8 (C-4″), 83.2 (C-4‴), 82.2 (C-4), 80.8 (C-4′), 80.7 (C-3‴), 80.5 (C-2‴), 80.3 (C-2′), 80.3 (C-2″), 79.3 (C-2), 76.2 (C-5′), 75.8 (*C*H$_2$O), 71.5 (C-5‴), 71.2 (C-5), 69.0 (C-3), 68.7 (C-3″), 68.0 (C-5″), 67.6 (C-3′), 61.1 (O*C*H$_3$), 61.0 (O*C*H$_3$), 59.3 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.7 (O*C*H$_3$), 58.5 (O*C*H$_3$), 55.7 (O*C*H$_3$), 23.5 (*C*H$_2$CH$_3$), 18.0 (C-6′), 17.8(9) (C-6), 17.8(4) (C-6‴), 16.6 (C-6″), 10.80 (*C*H$_3$CH$_2$). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{40}H_{66}NaO_{18}$: 857.4141. Found 857.4134.



***p*-Methoxyphenyl 6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2,4-*O*-dimethyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-9)**

To a solution of **4-40** (15 mg, 0.02 mmol) in AcOH (5 mL), $(Ph_3P)_4Pd$ (4 mg, 20% w/w) was added and the reaction mixture was stirred overnight at rt. The reaction mixture was filtered and water (10 mL) was added. The solution was diluted with CH_2Cl_2 (15 mL), washed with water (2 x 10 mL) and brine (10 mL). The organic layer was separated, dried ($NaSO_4$), concentrated and the resulting residue was dissolved in DMF (2 mL) and CH_3I (0.1 mL) was added. To this solution, NaH (5 mg) was added at 0 ºC and it was stirred for 1 h at rt and then chilled water (5 mL) was added before being diluted with CH_2Cl_2 (10 mL) and washed with water (2 x 10 mL). The organic layer was concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. To a solution of the oil in 1:1 CH_3OH–CH_2Cl_2 (10 mL), Pd–C (4 mg, 20% w/w) was added and the reaction mixture was stirred overnight under hydrogen before it was filtered and concentrated. The resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to **4-9** (11 mg, 71%) as a thick syrup: R_f 0.56 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –41.9 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.98–6.97 (m, 2H, Ar-2,6), 6.82–6.80 (m, 2H, Ar-3,5), 5.38 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.15–5.13 (m, 2H, H-1′, H-1″), 5.05 (d, 1H, $J_{1''',2'''}$ = 1.6 Hz, H-1‴), 4.19–4.10 (m, 3H, H-3, H-3′, H-5″), 3.93–3.84 (m, 3H, H-3″, H-5, H-5′), 3.76 (s, 3H, OCH_3), 3.77–3.63 (m, 6H, H-2, H-2′, H-2″, H-3‴, H-4′, H-5‴), 3.58 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 5.21–5.10 (m, 1H, H-2‴), 3.50 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.42 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.32–3.31 (m, 1H, H-4″), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 2.98 (app t, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 9.5 Hz, H-4‴), 1.37 (d, 3H, $J_{6',5'}$ = 6.2 Hz, H-6′),

1.35 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6), 1.29 (d, 3H, $J_{6''',5'''}$ = 6.2 Hz, H-6‴), 1.25 (d, 3H, $J_{6'',5''}$ = 6.5 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.5 (C-1‴), 99.6 (C-1′), 97.7 (C-1″), 95.5 (C-1), 83.8 (C-4‴), 83.2 (C-4), 82.3 (C-4″), 82.2 (C-4′), 80.8 (C-3‴), 80.5 (C-2‴), 80.3 (C-2′), 80.1 (C-2″), 79.2 (C-2), 76.4 (C-5′), 71.5 (C-5‴), 71.2 (C-5), 69.0 (C-3), 68.7 (C-3″), 68.0 (C-5″), 67.3 (C-3′), 62.0 (O*C*H$_3$), 61.0(7) (O*C*H$_3$), 61.0(6) (O*C*H$_3$), 59.4 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.6 (O*C*H$_3$), 58.6 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.0 (C-6′), 17.8(7) (C-6), 17.8(4) (C-6‴), 16.4 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{38}H_{62}NaO_{18}$: 829.3828. Found 829.3828.

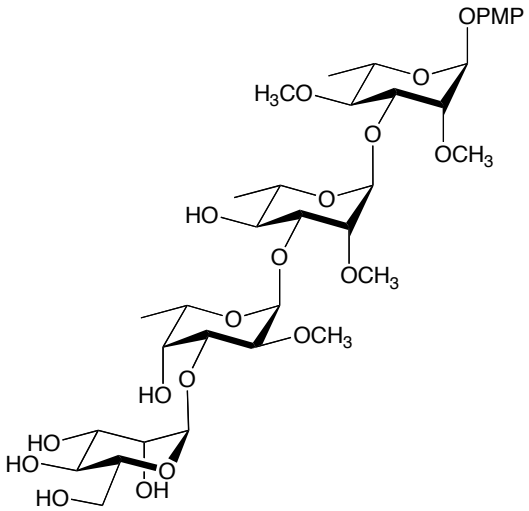


***p*-Methoxyphenyl 6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-10)**

To a solution of **4-40** (10 mg, 0.01 mmol) in AcOH (5 mL), $(Ph_3P)_4Pd$ (2 mg, 10% w/w) was added and the reaction mixture was stirred overnight at rt before it

was filtered water (10 mL) was added. The solution was diluted with CH_2Cl_2 (20 mL), washed with water (2 x 10 mL) and brine (10 mL). The organic layer was separated, dried ($NaSO_4$) and concentrated. The resulting residue was dissolved in pyridine (2 mL), Ac_2O (0.25 mL) was added and the reaction mixture was stirred for 2 h at rt. Water (5 mL) was added then then the solution was diluted with CH_2Cl_2 (10 mL) and washed with water (2 x 10 mL). The organic layer was concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup (39 mg). To the solution of the syrup in 1:1 CH_3OH–CH_2Cl_2 (15 mL), Pd–C (3 mg) was added and the reaction mixture was stirred overnight under hydrogen before it was filtered, concentrated and the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to **4-40** (6.3 mg, 79%) as a colorless oil: R_f 0.48 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –27.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.00–6.98 (m, 2H, Ar-2,6), 6.83–6.81 (m, 2H, Ar-3,5), 5.40 (br s, 1H, H-1), 5.22–5.21 (d, 1H, $J_{1'',2''}$ = 3.1 Hz, H-1″), 5.18 (br s, 1H, H-1′), 5.12–5.09 (m, 2H, H-1‴, H-4″), 4.29 (dq, 1H, $J_{4'',5''}$ = 2.9 Hz, $J_{5'',6''}$ = 6.5 H, H-5″), 4.18 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.6 Hz, H-3′), 4.10 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.89–3.84 (m, 1H, H-3″), 3.77 (s, 3H, OC*H*$_3$), 3.74–3.56 (m, 8H, H-2, H-2′, H-2″, H-3‴, H-4′, H-5, H-5′, H-5‴), 3.54 (s, 6H, OC*H*$_3$ x 2), 3.52 (s, 6H, OC*H*$_3$ x 2), 3.46–3.44 (m, 7H, OC*H*$_3$ x 2, H-2‴), 3.22 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 2.97 (app t, 1H, $J_{3''',4'''} = J_{4''',5'''}$ = 9.5 Hz, H-4‴), 1.80 (br s, 1H, OH), 1.66 (br s, 1H, OH), 2.14 (s, 3H, C*H*$_3$CO), 1.34 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.30 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.27 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6), 1.10 (d, 3H, $J_{5''',6'''}$ = 6.4 Hz, H-6‴); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C)

170.3 (*C*=O), 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.5 (C-1‴), 99.3 (C-1′), 98.1 (C-1″), 95.6 (C-1), 83.4 (C-4‴) , 83.3 (C-4), 82.3 (C-4′), 80.8 (C-3‴), 80.5 (C-2‴), 80.4 (C-2″), 79.7 (C-2′), 79.0 (C-2), 74.2 (C-5′), 73.1 (C-5‴), 71.6 (C-5), 70.7 (C-3), 68.9 (C-3′), 68.7 (C-3″), 67.9 (C-5″), 65.9 (C-4″), 61.0 (O*C*H_3), 60.3 (O*C*H_3), 59.7 (O*C*H_3), 59.0 (O*C*H_3), 58.7 (O*C*H_3), 58.6 (O*C*H_3), 55.7 (O*C*H_3), 20.8 (*C*H_3CO), 17.9 (C-6, C-6′, C-6‴), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{39}H_{62}NaO_{19}$: 857.3783. Found 857.3782.

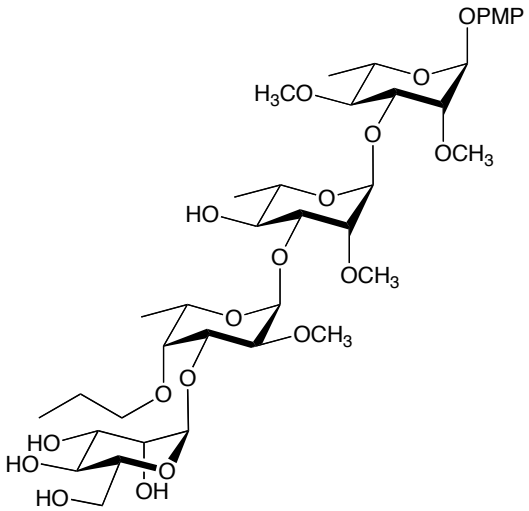


***p*-Methoxyphenyl α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl -α-L-rhamnopyranoside (4-11)**

To a solution of **4-48** (15 mg, 0.012 mmol) in 1:1 CH_3OH–CH_2Cl_2 (10 mL), 1M $NaOCH_3$ (0.5 mL) was added. The reaction mixture was stirred for 3 h at rt before it was neutralized by the addition of Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in AcOH (5 mL), $(Ph_3P)_4Pd$ (4

mg, 10% w/w) was added and the reaction mixture was stirred overnight at rt and then it was filtered. The filtrate was dilited with water (10 mL) and CH_2Cl_2 (20 mL), being being washed with water (2 x 10 mL), and brine (10 mL). The organic layer was dried ($NaSO_4$), concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. To the solution of the oil in 1:1 CH_3OH–CH_2Cl_2 (15 mL), Pd–C (3 mg) was added and the reaction mixture was stirred overnight under hydrogen. The reaction mixture was then filtered, concentrated and the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-11** (7.3 mg, 81%) as an amorphous solid: R_f 0.4 (10:0.75 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –45.3 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.01–6.98 (m, 2H, Ar-2,6), 6.68–6.82 (m, 2H, Ar-3,5), 5.42 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.20 (d, 1H, $J_{1'',2''}$ = 3.2 Hz, H-1″), 5.13 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.04 (d, 1H, $J_{1''',2'''}$ = 1.6 Hz, H-1‴), 4.12–4.04 (m, 2H, H-3′, H-5), 4.00 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.90–3.88 (m, 2H, H-3″, H-5′), 3.86–3.81 (m, 4H, H-5″, H-5‴, H-6‴ x 2), 3.78 (dd, 1H, $J_{1''',2'''}$ = 1.6 Hz, $J_{2''',3'''}$ = 3.1 Hz, H-2‴), 3.74 (s, 3H, OC*H*$_3$), 3.72–3.72 (m, 1H, H-2), 3.68–3.62 (m, 4H, H-2′, H-2″, H-3‴, H-4′), 3.58–3.56 (m, 2H, H-4″, H-4‴), 3.54 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.50 (s, 3H, OC*H*$_3$), 3.47 (s, 3H, OC*H*$_3$), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.29 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.22 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.20 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 101.8 (C-1‴), 100.1 (C-1″), 98.9 (C-1′), 95.56 (C-1), 82.4(9) (C-4), 82.4(8) (C-4‴), 82.2 (C-3‴), 80.7 (C-4″), 80.3 (C-4′), 79.2 (C-2″), 78.4 (C-2′), 78.3 (C-2), 73.8 (C-2‴), 71.3 (C-5), 71.3 (C-5‴),

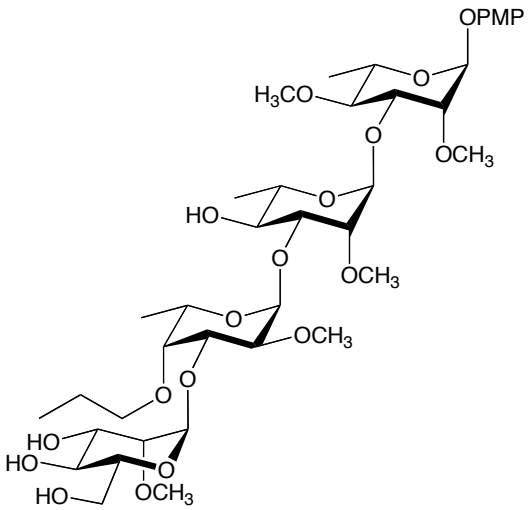
71.0 (C-5′), 69.0 (C-3″), 68.7 (C-3), 66.9 (C-3′), 66.5 (C-5″), 61.3 (C-6‴), 61.1 (O*C*H$_3$), 59.2 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.3 (O*C*H$_3$), 55.6 (O*C*H$_3$), 18.0 (C-6′), 17.8 (C-6), 16.4 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{35}H_{56}NaO_{19}$: 803.3308. Found 803.3303.



***p*-Methoxyphenyl α-D-mannopyranosyl-(1→3)-2-*O*-methyl-4-*O*-propyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-12)**

To a solution of **4-48** (15 mg, 0.012 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.15 mL) was added and the reaction mixture was stirred for 4 h at rt. It was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. To the solution of this syrup in 1:1 CH_3OH–CH_2Cl_2 (20 mL), Pd–C (4 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere. The reaction mixture was then filtered, concentrated and

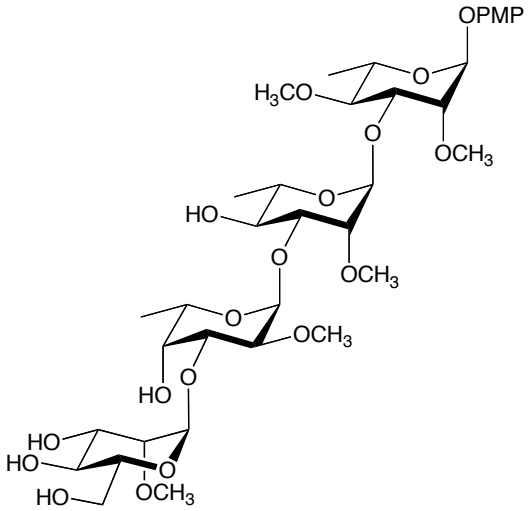
the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-12** (8.3 mg, 87%) as an amorphous solid: R_f 0.55 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –33.2 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.01–6.98 (m, 2H, Ar), 6.84–6.82 (m, 2H, Ar), 5.40 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.21 (br s, 1H, H-1′), 5.16 (br s, 1H, H-1‴), 5.12 (d, 1H, $J_{1'',2''}$ = 3.1 Hz, H-1″), 4.16–4.09 (m, 2H, H-5″, H-3′), 4.06 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.99–3.80 (m, 5H, H-3″, H-5′, H-5‴, H-6‴ x 2), 3.79 (s, 3H, OC*H*$_3$), 3.75–3.68 (m, 5H, H-2, H-2‴, H-5, C*H*$_2$O x 2), 3.64–3.59 (m, 4H, H-2′, H-2″, H-3‴, H-4′), 3.56–3.54 (m, 1H, H-4‴), 3.54 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.45 (s, 3H, OC*H*$_3$) 3.44–3.42 (m, 1H, H-4), 3.24–3.20 (m, 1H, H-4″), 1.66–1.59 (m, 2H, C*H*$_2$CH$_3$), 1.33 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.26 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.25 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 0.95 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 101.6 (C-1‴), 100.1 (C-1″), 98.9 (C-1′), 95.6 (C-1), 82.4 (C-4″), 82.1 (C-4), 80.7 (C-3‴), 80.5 (C-4‴), 80.3 (C-4′), 79.8 (C-2′), 79.6 (C-2″), 75.8 (*C*H$_2$O), 73.2 (C-2), 71.4 (C-2‴), 71.1 (C-5), 70.9 (C-5‴), 69.1 (C-5′), 68.7 (C-3″), 67.4 (C-3), 66.5 (C-3′), 61.2 (*C*-6‴), 61.0 (C-5″), 59.0 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.2 (O*C*H$_3$), 55.7 (O*C*H$_3$), 55.7 (O*C*H$_3$), 23.5 (*C*H$_2$CH$_3$), 18.0 (C-6′), 17.8 (C-6), 16.6 (C-6″), 10.8 (CH$_2$*C*H$_3$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{38}H_{62}NaO_{19}$: 845.3778. Found 845.3776.



***p*-Methoxyphenyl 2-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-4-*O*-propyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-13)**

To a solution of **4-48** (15 mg, 0.012 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.15 mL) was added and the reaction mixture was stirred for 3 h at rt. It was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in DMF (1 mL), CH_3I (0.1 mL) and NaH (60% in mineral oil, 0.5 mg, 0.022 mmol) were added at 0 ºC. The reaction mixture was stirred for 1 h at rt before the addition of chilled water (5 mL), and CH_2Cl_2 (10 mL). The organic layer was washed with water (2 x 10 mL) and brine (10 mL) and then separated, concentrated and the resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. To the solution of the syrup in 1:1 CH_3OH–CH_2Cl_2 (10 mL), Pd–C (4 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere. The solution was then filtered, concentrated and the resulting residue was purified by

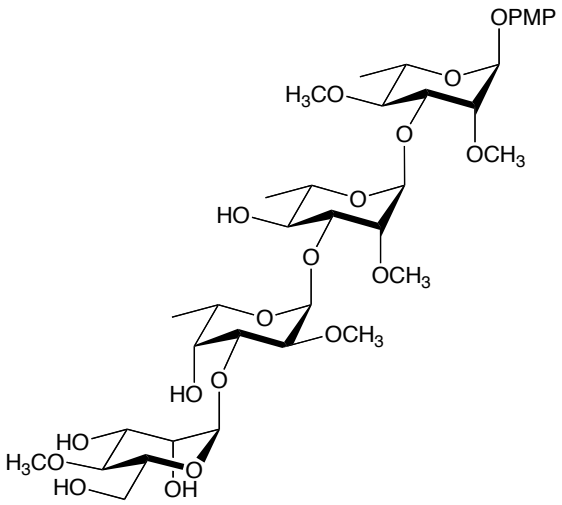
chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-13** (7.4 mg, 76%) as a thick syrup: R_f 0.45 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –38.1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.96 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.39 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.28 (br s, 1H, H-1′), 5.16 (br s, 1H, H-1‴), 5.09 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 4.16–4.17 (m, 2H, H-3′, H-5″), 4.08 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.89–3.85 (m, 3H, H-3″, H-6‴ x 2), 3.82–3.77 (m, 2H, H-5′, H-5‴), 3.76 (s, 3H, OC*H*$_3$), 3.74–3.67 (m, 5H, C*H*$_2$O x 2, H-2, H-2′, H-5), 3.65–3.62 (m, 2H, H-2″, H-2‴), 3.56–3.55 (m, 1H, H-3‴), 3.53 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.50 (s, 3H, OC*H*$_3$), 3.49–3.47 (m, 2H, H-4′, H-4‴), 3.46 (s, 3H, OC*H*$_3$), 3.43–3.42 (m, 1H, H-4″), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.65–1.58 (m, 2H, C*H*$_2$CH$_3$), 1.35 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.26 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.21 (d, 1H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 0.94 (t, 3H, *J* = 7.7 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.3 (C-1‴), 99.3 (C-1″), 97.8 (C-1′), 95.6 (C-1), 82.9 (C-4″), 82.2 (C-4), 80.6 (C-3‴), 80.5 (C-4‴), 80.3 (C-4′), 80.2 (C-2‴), 79.3 (C-2″), 75.9 (*C*H$_2$O), 75.8 (C-2′), 72.6 (C-2), 71.5 (C-5), 71.4 (C-5‴), 69.0 (C-5′), 68.8 (C-3″), 68.7 (C-3), 67.6 (C-3′), 62.5 (C-6‴), 61.1 (C-5″), 59.1 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.7 (O*C*H$_3$), 58.5 (O*C*H$_3$), 55.7 (O*C*H$_3$ x 2), 23.5 (*C*H$_2$CH$_3$), 17.9 (C-6′), 17.8 (C-6), 16.5 (C-6″), 10.8 (CH$_2$*C*H$_3$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{39}H_{64}NaO_{19}$: 859.3934. Found 859.3925.



***p*-Methoxyphenyl 2-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-14)**

To a solution of **4-48** (20 mg, 0.016 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.15 mL) was added and the reaction mixture was stirred for 3 h at rt. It was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in DMF (2 mL), CH_3I (0.1 mL) and NaH (60% in mineral oil, 0.7 mg, 0.027 mmol) were added at 0 ºC. The reaction mixture was stirred for 1 h at rt the addition of chilled water (5 mL) and CH_2Cl_2 (10 mL). The organic layer was washed with water (2 x 10 mL), and brine (10 mL) and then separated and concentrated. The resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. To the solution of the syrup in AcOH (5 mL), $(Ph_3P)_4Pd$ (2 mg, 10% w/w) was added and the reaction mixture was stirred overnight at rt before it was filtered. The filtrate was diluted with water (10 mL) and CH_2Cl_2 (20 mL), and then the organic layer was washed with

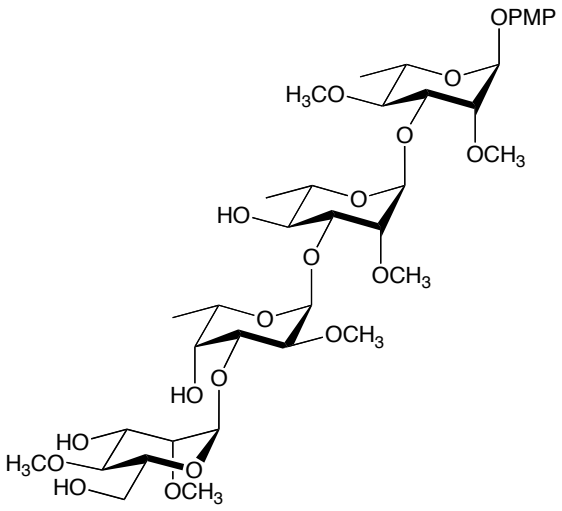
water (2 x 10 mL) and brine (10 mL) before it was dried ($NaSO_4$) and concentrated. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. To the solution of the oil in 1:1 CH_3OH–CH_2Cl_2 (15 mL), Pd–C (2 mg) was added and the reaction mixture was stirred for two days under a hydrogen atmosphere. It was then filtered, concentrated and the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-14** (8.8 mg, 71%) as an amorphous solid: R_f 0.50 (10:0.75 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –19.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.02–6.98 (m, 2H, Ar-2,6), 6.85–6.82 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.21 (br s, 1H, H-1′), 5.15 (br s, 1H, H-1‴), 5.10 (d, 1H, $J_{1″,2″}$ = 3.2 Hz, H-1″), 4.17 (dq, 1H, $J_{4″,5″}$ = 2.6 Hz, $J_{5″,6″}$ = 6.5 H, H-5″), 4.12–4.08 (m, 2H, H-3, H-3′), 3.97–3.80 (m, 7H, H-2, H-3″, H-5, H-5′, H-5‴, H-6‴ x 2), 3.79 (s, 3H, OC*H*$_3$), 3.72–3.70 (m, 3H, H-2′, H-2″, H-2‴), 3.69–3.65 (m, 2H, H-3‴, H-4″), 3.64–3.59 (m, 2H, H-4′, H-4‴), 3.56 (s, 3H, OC*H*$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.53 (s, 3H, OC*H*$_3$), 3.50 (s, 3H, OC*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.24 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 1.36 (d, 3H, $J_{5″,6″}$ = 6.5 Hz, H-6″), 1.29–1.26 (m, 6H, H-6, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.7 (C-1‴), 99.3 (C-1″), 97.8 (C-1′), 95.6 (C-1), 83.1 (C-4), 82.3 (C-4‴), 80.6 (C-3‴), 80.4 (C-4″), 80.2 (C-4′), 78.9 (C-2‴), 78.5(3) (C-2″), 78.4(4) (C-2′), 72.5 (C-2), 71.7 (C-5), 71.6 (C-5‴), 71.5 (C-5′), 69.2 (C-3″), 69.0 (C-3), 68.7 (C-3′), 66.5 (C-5″), 62.6 (C-6‴), 61.1 (O*C*H$_3$), 59.9 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.8 (O*C*H$_3$), 58.6 (O*C*H$_3$), 55.7 (O*C*H$_3$), 17.8(6) (C-6′), 17.8(5) (C-6), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{36}H_{58}NaO_{19}$: 817.3465. Found 817.3462.



***p*-Methoxyphenyl 4-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-15)**

To a solution of **4-49** (20 mg, 0.02 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.15 mL) was added and the reaction mixture was stirred for 4 h at rt. The reaction mixture was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give a syrup. To the solution of this syrup in AcOH (3 mL), $(Ph_3P)_4Pd$ (4 mg, 20 % w/w) was added and the reaction mixture was stirred overnight and then filtered. The filtrate was diluted with water (10 mL), and CH_2Cl_2 (20 mL) and then washed with water (2 x 10 mL) and brine (10 mL). The organic layer was separated, dried ($NaSO_4$), concentrated and the resulting oil was dissolved in 1:1 CH_3OH–CH_2Cl_2 (20 mL). To this solution, Pd–C (4 mg, 20% w/w) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere before it was filtered and concentrated. The

resulting crude product was purified by chromatography (10:0.75, CH_2Cl_2–CH_3OH) to give **4-15** (9.3 mg, 71%) as a thick syrup: R_f 0.50 (10:0.75, CH_2Cl_2–CH_3OH); $[\alpha]_D$ –41.7 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.01–6.98 (m, 2H, Ar-2,6), 6.85–6.81 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.19–5.18 (m, 2H, H-1′, H-1‴), 5.11 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 4.20–4.16 (m, 1H, H-5″), 4.11–4.08 (m, 2H, H-3′, H-3), 4.02–4.01 (m, 1H, H-3″), 3.95–3.85 (m, 4H, H-5, H-5′, H-6‴ x 2), 3.81–3.79 (m, 3H, H-2, H-2‴, H-5‴), 3.78 (s, 3H, OC*H*$_3$), 3.72–3.68 (m, 2H, H-2′, H-2″), 3.67–3.62 (m, 3H, H-3‴, H-4′, H-4″), 3.59 (s, 3H, OC*H*$_3$), 3.55 (s, 3H, OC*H*$_3$), 3.52 (s, 6H, OC*H*$_3$ x 2), 3.47 (s, 3H, OC*H*$_3$), 3.46–3.43 (m, 1H, H-4‴), 3.23 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.37 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″), 1.28–1.26 (m, 6H, H-6, H-6′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 101.0 (C-1‴), 100.1 (C-1″), 98.9 (C-1′), 95.6 (C-1), 82.8 (C-4‴), 82.2 (C-4), 80.5 (C-3‴), 80.3 (C-4″), 79.2 (C-4′), 78.8 (C-2″), 78.7 (C-2′), 78.4 (C-2), 78.2 (C-2‴), 71.3 (C-5), 71.1 (C-5‴), 69.0 (C-5′), 68.7 (C-3″), 68.6 (C-3), 66.5 (C-3′), 66.3 (C-5″), 62.0 (C-6‴), 61.1 (O*C*H$_3$), 60.8 (O*C*H$_3$), 59.5 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.2 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.0 (C-6``), 17.8 (C-6`), 16.3 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{36}H_{58}NaO_{19}$: 817.3465. Found 817.3459.

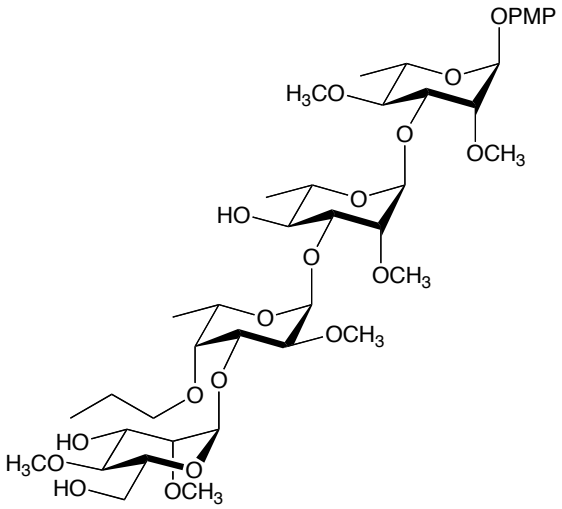


***p*-Methoxyphenyl 2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-16)**

To a solution of **4-49** (15 mg, 0.01 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL) was added 1M $NaOCH_3$ (0.15 mL) and the reaction mixture was stirred for 4 h at rt. The reaction mixture was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting crude product was dissolved in DMF (4 mL), CH_3I (0.1 mL) and NaH (60% in mineral oil, 6 mg) were added at 0 ºC. The reaction mixture was stirred for 1 h at rt before the addition of water (8 mL), and CH_2Cl_2 (10 mL). The organic layer was washed with water (2 x 10 mL) and brine (10 mL) before being concentrated. The resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in AcOH (3 mL), $(Ph_3P)_4Pd$ (3 mg, 20% w/w) was added and the reaction mixture was stirred overnight. The reaction mixture was then filtered, and the filtrate was dilted with water (10 mL), and CH_2Cl_2 (20 mL). The organic layer was washed

with water (2 x 10 mL) and brine (10 mL), and then separated, dried ($NaSO_4$), and concentrated to give an oil that was dissolved in 1:1 CH_3OH–CH_2Cl_2 (20 mL). To this solution, Pd–C (4 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere. The reaction mixture was then filtered, concentrated and the resulting crude product was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **4-16** (7.4 mg, 74%) as a thick syrup: R_f 0.39 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –39.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 6.99–6.96 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.21 (br s, 1H, H-1′), 5.18 (br s, 1H, H-1‴), 5.09 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 4.15–4.07 (dq, 1H, $J_{4'',5''}$ = 2.8 Hz, $J_{5'',6''}$ = 6.4 H, H-5″), 4.11 (dd, 1H, $J_{2',3'}$ = 3.0 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 4.07 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.91–3.86 (m, 4H, H-3″, H-5′, H-6‴ x 2), 3.80–3.76 (m, 3H, H-2, H-5, H-5‴), 3.78 (s, 3H, OC*H*$_3$), 3.74–3.69 (m, 3H, H-2′, H-2″, H-2‴), 3.66–3.62 (m, 3H, H-3‴, H-4′, H-4″), 3.58 (s, 3H, OC*H*$_3$), 3.56 (s, 3H, OC*H*$_3$), 3.53 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.30–3.23 (m, 2H, H-4, H-4‴), 2.70 (br s, 1H, OH), 2.51 (d, 1H, *J* = 8.5 Hz, OH), 1.72 (br s, 2H, OH x 2), 1.36 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.28 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.26 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.7 (C-1‴), 99.3 (C-1″), 97.7 (C-1′), 95.6 (C-1), 83.2 (C-4‴), 82.3 (C-4), 80.6(2) (C-3‴), 80.6(0) (C-4″), 80.3 (C-4′), 78.9 (C-2‴), 78.5 (C-2), 78.4 (C-2″), 78.3 (C-2′), 72.3 (C-5), 71.7 (C-5‴), 71.6 (C-5′), 71.4 (C-3″), 69.0 (C-3), 68.7 (C-3′), 66.4 (C-5″), 62.4 (C-6‴), 61.1 (O*C*H$_3$), 60.8 (O*C*H$_3$), 60.0 (O*C*H$_3$), 59.0 (O*C*H$_3$), 58.8 (O*C*H$_3$), 58.6 (O*C*H$_3$), 55.7 (O*C*H$_3$), 17.8(6) (C-6′),

17.8(4) (C-6), 16.3 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{37}H_{60}NaO_{19}$: 831.3621. Found 831.3613.

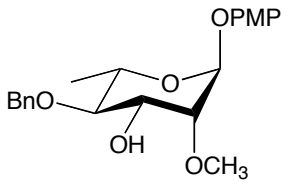


***p*-Methoxyphenyl 2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2-*O*-methyl-4-*O*-isopropyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-17)**

To a solution of **4-49** (15 mg, 0.01 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.15 mL) was added and the reaction mixture was stirred for 4 h at rt. The reaction mixture was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting crude product was dissolved in DMF (4 mL), CH_3I (0.1 mL) and NaH (60% in mineral oil, 4 mg) were added at 0 ºC. The reaction mixture was stirred for 1 h at rt before the addition of water (8 mL). The mixture was diluted with CH_2Cl_2 (15 mL), washed with water (2 x 10 mL) and brine (20 mL). The organic layer was separated, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. To

the solution of this syrup in dissolved in 1:1 CH_3OH–CH_2Cl_2 (20 mL), Pd–C (4 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere. The reaction mixture was then filtered, concentrated and the resulting crude product was purified by chromatography (10:0.75, CH_2Cl_2–CH_3OH) to give **4-17** (7.5 mg, 71%) as a thick syrup: R_f 0.44 (10:0.75, CH_2Cl_2–CH_3OH); $[\alpha]_D$ –27.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.01–6.98 (m, 2H, Ar-2,6), 6.85–6.81 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.20 (br s, 1H, H-1′), 5.18 (br s, 1H, H-1‴), 5.08 (d, 1H, $J_{1'',2''}$ = 3.2 Hz, H-1″), 4.21–4.06 (m, 3H, H-3, H-3′, H-5″), 3.91–3.86 (m, 3H, H-3″, H-5′, H-6‴ x 2), 3.80–3.76 (m, 3H, H-2, H-5, H-5‴), 3.78 (s, 3H, OC*H*$_3$), 3.74–3.71 (m, 4H, H-2′, H-2″, C*H*$_2$O), 3.70–3.62 (m, 3H, H-2‴, H-3‴, H-4′), 3.57 (s, 3H, OC*H*$_3$), 3.55 (s, 3H, OC*H*$_3$), 3.53 (s, 3H, OC*H*$_3$), 3.52 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.40–3.38 (m, 1H, H-4″), 3.30 (app t, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 9.8 Hz, H-4‴), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.61 (q, 1H, *J* = 7.5 Hz, C*H*$_2$CH$_2$O), 1.35 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.20 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 0.94 (t, 1H, *J* = 7.5 Hz, C*H*$_3$CH$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 100.4 (C-1‴), 99.4 (C-1″), 97.5 (C-1′), 95.6 (C-1), 83.1 (C-4‴), 82.2 (C-4″), 80.6(0) (C-4), 80.5(6) (C-3‴), 80.5 (C-4′), 80.4 (C-2‴), 80.3 (C-2′), 79.3 (C-2″), 78.3 (C-2), 75.8 (*C*H$_2$O), 72.7 (C-5), 72.1 (C-5‴), 71.6 (C-5′), 69.4 (C-3″), 69.0 (C-3), 68.7 (C-3′), 67.7 (C-5″), 62.5 (C-6‴), 61.1 (O*C*H$_3$), 60.9 (O*C*H$_3$), 59.3 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.6(1) (O*C*H$_3$), 58.5(8) (O*C*H$_3$), 55.7 (O*C*H$_3$), 23.52 (*C*H$_2$CH$_3$), 17.8(6) (C-6′), 17.8(4) (C-6), 16.3 (C-

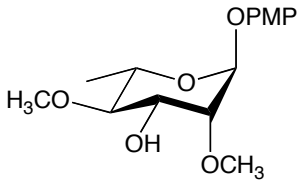
6″), 10.8 (*C*H_3CH_2). HRMS (ESI) Calcd. for (M + Na) $C_{40}H_{66}NaO_{19}$: 873.4091. Found 873.4083.



***p*-Methoxyphenyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (4-18)**

Diol **2-17** (1 g, 2.77 mmol) was dissolved in toluene (40 mL) and *n*-Bu_2SnO (0.7 g, 2.77 mmol) was added. The reaction mixture was stirred at 120 °C for 1 h and then it was cooled to 62 ºC before PMBCl (0.5 g, 3.05 mmol) and *n*-Bu_4NI (1.02 g, 2.77 mmol) were added. The reaction mixture was stirred at 62 ºC for additional 7 h and then concentrated. The resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give a colorless syrup. This syrup (1.05 g) was dissolved in DMF (10 mL) and CH_3I (0.2 mL, 3.32 mmol) was added. The reaction mixture was cooled to 0 ºC before NaH (60% in mineral oil, 106 mg, 4.43 mmol) was added and then the solution was stirred for additional 1 h at rt. Chilled water (20 mL) was added and the solution was diluted with CH_2Cl_2 (50 mL). The organic layer was separated, washed with water (2 x 40 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in CH_2Cl_2 (30 mL) and TFA (1.5 mL) was added dropwise over 2 min at 0 ºC. The reaction mixture was stirred for additional 30 min at 0 ºC before Et_3N (3 mL) was added. The mixture was concentrated and the resulting residue was purified by chromatography (1:1 hexane–EtOAc) to give **4-18** (0.84 g, 81%) as an

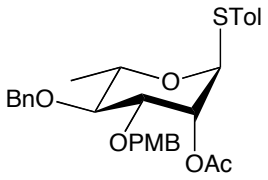
amorphous solid: R_f 0.55 (1:1 hexane–EtOAc); $[\alpha]_D$ –29.5 (*c* 0.4, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.39–7.27 (m, 5H, Ar), 7.01–6.98 (m, 2H, Ar-2,6), 6.85–6.81 (m, 2H, Ar-3,5), 5.46 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.93, 4.70 (ABq, 2H, J = 11.3 Hz, ArCH_2), 4.15–4.13 (m, 1H, H-3), 3.81 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.78 (s, 3H, OCH_3), 3.68 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 3.55 (s, 3H, OCH_3), 3.34 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.44 (br s, 1H, OH-3), 1.29 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 138.4 (Ar), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 95.2 (C-1), 82.1 (C-4), 80.6 (C-2), 75.1 (ArCH_2), 71.5 (C-3), 67.8 (C-5), 59.1 (OCH_3), 55.7 (OCH_3), 18.0 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{21}H_{26}NaO_6$: 397.1622. Found 397.1629.



***p*-Methoxyphenyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-19)**

To a solution of **4-23** (1.3 g, 3.22 mmol) and CH_3I (0.25 mL, 3.86 mmol) in DMF (10 mL), NaH (60% in mineral oil, 0.13 g, 5.15 mmol) was added at 0 °C. The reaction mixture was stirred for 4 h at rt before chilled water (30 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (100 mL) and washed with water (2 × 100 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated. The resulting residue was dissolved in CH_2Cl_2 (30 mL) and TFA (1.5 mL, 5% v/v) was added at 0 ºC. The solution was stirred for additional 30

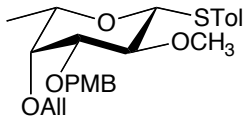
min and then Et_3N (3 mL) was added. After concentration of the solution, the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **4-19** (0.72 g, 81%) as a colorless oil: R_f 0.36 (2:1 hexane–EtOAc); $[\alpha]_D$ –57.7 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.03–6.99 (m, 2H, Ar-2,6), 6.87–6.81 (m, 2H, Ar-3,5), 5.45 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.05 (ddd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.3 Hz, $J_{3,OH-3}$ = 8.9 Hz, H-3), 3.80 (s, 3H, OC*H*$_3$), 3.74 (dq, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.68 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.62 (s, 3H, OC*H*$_3$), 3.56 (s, 3H, OC*H*$_3$), 3.07 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 2.45 (d, 1H, $J_{3,OH-3}$ = 8.9 Hz, OH-3), 1.30 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.2 (Ar), 150.7 (Ar), 117.8 (Ar x 2), 114.9 (Ar x 2), 95.5 (C-1), 84.0 (C-4), 80.8 (C-3), 71.4 (C-2), 68.2 (C-5), 61.2 (O*C*H$_3$), 59.3 (O*C*H$_3$), 55.9 (O*C*H$_3$), 18.2 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{15}H_{22}NaO_6$: 321.1314. Found 321.1313.



***p*-Tolyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (4-20)**

To a solution of **4-24** (2.2 g, 4.58 mmol) in pyridine (5 mL) at 0 °C, Ac_2O (3 mL) was added. The reaction mixture was stirred for 3 h at rt before water (10 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (30 mL) and washed with water (2 × 30 mL). The organic layer was separated, dried (Na_2SO_4),

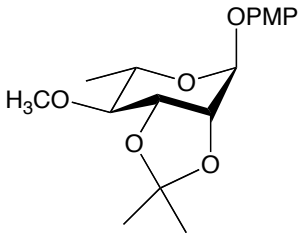
filtered, concentrated and the resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **4-20** (2.27 g, 95%) as an amorphous solid: R_f 0.52 (5:1 hexane–EtOAc); $[\alpha]_D$ –73.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.40–7.27 (m, 9H, Ar-H), 7.14 (d, 2H, J = 8.0 Hz, Ar-H), 6.90–6.86 (m, 2H, Ar-H), 5.61 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.35 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.95, 4.64 (ABq, 2H, J = 10.7 Hz, ArCH_2), 4.67, 4.51 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.25 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.92 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.82 (s, 3H, OCH_3), 3.50 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.35 (s, 3H, CH_3CO), 2.16 (s, 3H, ArCH_3), 1.36 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.5 (*C*=O), 159.6 (Ar), 138.7 (Ar), 138.1 (Ar), 132.6 (Ar x 2), 130.4 (Ar x 2), 130.1 (Ar x 4), 128.6 (Ar x 2), 128.2 (Ar x 2), 128.0 (Ar), 114.1 (Ar x 2), 86.8 (C-1), 80.4 (C-4), 78.1 (C-3), 75.7 (Ar*C*H_2), 71.7 (Ar*C*H_2), 70.9 (C-5), 69.3 (C-2), 55.5 (O*C*H_3), 21.4 (Ar*C*H_3), 18.1 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{30}H_{34}NaO_6S$: 545.1968. Found 545.1967.



***p*-Tolyl 4-*O*-allyl-3-*O*-*p*-methoxybenzyl-2-*O*-methyl-1-thio-β-L-fucopyranoside (4-21)**

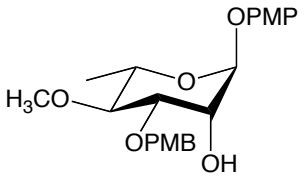
To a solution of compound **4-25** (1 g, 2.47 mmol) and AllBr (0.2 mL, 2.29 mmol) in DMF (10 mL) at 0 °C was added NaH (60% in mineral oil, 95 mg, 3.95 mmol). The reaction mixture was stirred for 2 h at rt before the addition of chilled water (30 mL). The solution was concentrated, diluted with CH_2Cl_2 (50 mL) and

washed with water (2 × 50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **4-21** (0.95 g, 86%) as a colorless oil: R_f 0.61 (5:1 hexane–EtOAc); $[\alpha]_D$ –14.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.49–7.46 (m, 2H, Ar), 7.31–7.27 (m, 2H, Ar), 7.09–7.07 (m, 2H, Ar), 6.90–6.87 (m, 2H, Ar), 5.98–5.88 (m, 1H, CH_2=C*H*), 5.28–5.27 (m, 1H, *C*H_2=CH), 5.17–5.14 (m, 1H, *C*H_2=CH), 4.66, 4.62 (ABq, 2H, *J* = 11.5 Hz, ArC*H*$_2$), 4.43–4.37 (m, 2H, *C*H_2O, H-1), 4.13–4.08 (m, 1H, *C*H_2O), 3.81 (s, 3H, OC*H*$_3$), 3.60 (s, 3H, OC*H*$_3$), 3.52–3.45 (m, 3H, H-2, H-4, H-5), 3.41 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 2.32 (s, 3H, ArC*H*$_3$), 1.28 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.2 (=*C*H), 137.1 (Ar), 135.6 (*C*H_2=), 132.2 (Ar x 2), 130.5 (Ar), 129.4 (Ar x 2), 129.2 (Ar x 2), 116.2 (Ar x 2), 113.8 (Ar x 2), 87.9 (C-1), 83.8 (C-3), 79.0 (C-2), 76.4 (Ar*C*H$_2$), 74.5 (*C*H_2O), 73.9 (C-4), 72.4 (C-5), 61.1 (O*C*H$_3$), 55.3 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 17.2 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{25}H_{32}NaO_5S$: 467.1863. Found 467.1855.



***p*-Methoxyphenyl 2,3-*O*-isopropylidene-4-*O*-methyl-α-L-rhamnopyranoside (4-22)**

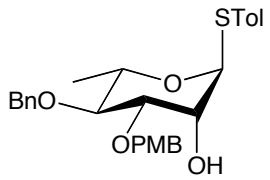
To a solution of compound **2-15** (2 g, 6.45 mmol) and CH_3I (0.49 mL, 7.73 mmol) in DMF (15 mL) was added NaH (60% in mineral oil, 0.25 g, 10.3 mmol) at 0 °C. The reaction mixture was then stirred for 4 h at rt before chilled water (30 mL) was added. The solution was concentrated, diluted with CH_2Cl_2 (100 mL) and washed with water (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **4-22** (2.01 g, 96%) as a colorless oil: R_f 0.56 (5:1 hexane–EtOAc); $[\alpha]_D$ –77.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.02–6.98 (m, 2H, Ar-2,6), 6.87–6.82 (m, 2H, Ar-3,5), 5.60 (br s, 1H, H-1), 4.35–4.29 (m, 2H, H-2, H-3), 3.81–3.74 (m, 1H, H-5), 3.79 (s, 3H, OC*H*$_3$), 3.57 (s, 3H, OC*H*$_3$), 3.07 (dd, 1H, $J_{3,4}$ = 8.9 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 1.60 (s, 3H, (C*H*$_3$)$_2$C), 1.42 (s, 3H, (C*H*$_3$)$_2$C), 1.25 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 155.2 (Ar), 150.5 (Ar), 118.0 (Ar x 2), 114.9 (Ar x 2), 109.6 ((CH$_3$)*C*), 96.5 (C-1), 83.8 (C-4), 78.5 (C-3), 76.3 (C-2), 65.7 (C-5), 59.7 (O*C*H$_3$), 55.9 (O*C*H$_3$), 28.3 ((*C*H$_3$)$_2$C), 26.6 ((*C*H$_3$)$_2$C), 18.0 (C-6). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{17}H_{24}NaO_6$: 347.1465. Found 347.1468.



***p*-Methoxyphenyl 3-*O*-*p*-methoxybenzyl-4-*O*-methyl-α-L-rhamnopyranoside (4-23)**

To a solution of **4-23** (2.4 g, 7.40 mmol) in 1:1 CH_3OH–CH_2Cl_2 (30 mL), *p*-TSA (100 mg) was added and the reaction mixture was stirred for 1 h at rt. The solution was then neutralized with Et_3N (2 mL) and concentrated. The resulting diol (2 g, 7.01 mmol) was dissolved in toluene (60 mL) and *n*-Bu_2SnO (1.75 g, 7.03 mmol) was added. The reaction mixture was stirred for 1 h at 120 °C, then cooled to 62 ºC before PMBCl (1.20 g, 7.73 mmol) and *n*-Bu_4NI (3.04 g, 8.28 mmol) were added. The reaction mixture was then stirred for additional 6 h at 62 ºC and then concentrated. The resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give **4-23** (2.33 g, 78%) as a colorless oil: R_f 0.46 (1:1 hexane–EtOAc); $[\alpha]_D$ –97.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.28 (m, 2H, Ar), 7.01–7.97 (m, 2H, Ar), 6.95–6.90 (m, 2H, Ar), 6.86–6.82 (m, 2H, Ar), 5.42 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.73, 4.69 (ABq, 2H, J = 11.1 Hz, ArCH_2), 4.16 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 3.91 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.78–3.74 (m, 1H, H-5), 3.60 (s, 3H, OCH_3), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, H-4), 2.60 (br s, 1H, OH-2), 1.28 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.7 (Ar), 155.1 (Ar), 150.5 (Ar), 130.4 (Ar), 129.7 (Ar x 2), 117.9 (Ar x 2), 114.9 (Ar x 2), 114.2 (Ar x 2), 98.2 (C-1), 82.1 (C-4), 79.5 (C-2), 72.3 (ArCH_2), 68.9 (C-3), 68.2

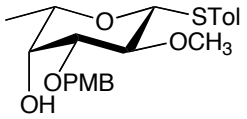
(C-5), 61.3 (OCH_3), 55.6 (OCH_3), 55.5 (OCH_3), 18.0 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{22}H_{28}NaO_7$: 427.1727. Found 427.1726.



***p*-Tolyl 4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-1-thio-α-L-rhamnopyranoside (4-24)**

Diol **3-8** (1 g, 2.76 mmol) was dissolved in toluene (60 mL) and *n*-Bu_2SnO (0.7 g, 2.76 mmol) was added. The reaction mixture was stirred at 120 °C for 1 h and then it was cooled to 62 ºC before PMBCl (0.49 g, 3.05 mmol) and *n*-Bu_4NI (2.40 g, 6.52 mmol) were added. The reaction mixture was stirred at 62 ºC for additional 6 h and then concentrated. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **4-24** (1.14 g, 86%) as an amorphous solid: R_f 0.42 (2:1 hexane–EtOAc); $[\alpha]_D$ –173.1 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.37–7.28 (m, 9H, Ar), 7.12–7.01 (m, 2H, Ar-2,6), 6.90–6.87 (m, 2H, Ar-3,5), 5.45 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.89, 4.64 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.65, 4.63 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.23–4.17 (m, 2H, H-2, H-5), 3.85 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.82 (s, 3H, OCH_3), 3.50 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.67 (br s, 1H, OH-2), 2.33 (s, 3H, ArCH_3), 1.30 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.5 (Ar), 138.4 (Ar), 137.6 (Ar), 132.1 (Ar x 2), 130.3 (Ar), 129.8 (Ar x 2), 129.8 (Ar), 129.7 (Ar x 2), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar), 114.0 (Ar x 2), 87.3 (C-

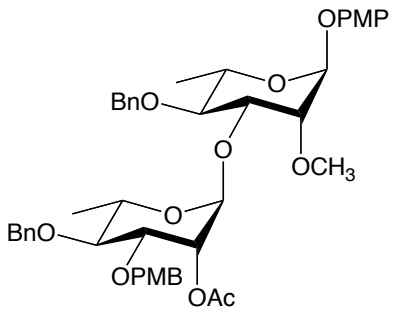
1), 80.1 (C-4), 79.8 (C-2), 75.4 (Ar*C*H_2), 71.9 (Ar*C*H_2), 70.1 (C-3), 68.7 (C-5), 55.3 (O*C*H_3), 21.1 (Ar*C*H_3), 17.8 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{28}H_{32}NaO_5S$: 503.1863. Found 503.1865.



***p*-Tolyl 3-*O*-*p*-methoxybenzyl-2-*O*-methyl-1-thio-β-L-fucopyranoside (4-25)**

Diol **3-14** (1 g, 3.50 mmol) was dissolved in toluene (30 mL) and *n*-Bu_2SnO (0.87 g, 3.51 mmol) was added. The reaction mixture was stirred for 1 h at 120 ºC and then it was cooled to 62 ºC before PMBCl (0.60 g, 3.86 mmol) and *n*-Bu_4NI (1.43 g, 3.86 mmol) were added. The reaction mixture was stirred at 62 ºC for additional 5 h and then concentrated. The resulting was purified by chromatography (2:1 hexane–EtOAc) to give **4-25** (1.17 g, 83%) as an amorphous solid: R_f 0.35 (2:1 hexane–EtOAc); $[\alpha]_D$ +1.6 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.48–7.45 (m, 2H, Ar), 7.30–7.27 (m, 2H, Ar), 7.11–7.09 (m, 2H, Ar), 6.90–6.86 (m, 2H, Ar), 4.64 (br s, 2H, ArC*H*$_2$), 4.41 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 3.80 (s, 3H, OC*H*$_3$), 3.75–3.74 (m, 1H, H-4), 3.60 (s, 3H, OC*H*$_3$), 3.51 (dq, 1H, $J_{4,5}$ = 2.7 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.44 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 3.32 (dd, 1H, $J_{1,2}$ = 9.8 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 2.32 (s, 3H, ArC*H*$_3$), 2.23 (s, 1H, OH-4), 1.34 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.4 (Ar), 137.6 (Ar), 132.7 (Ar x 2), 129.9 (Ar), 129.8 (Ar), 129.4(7) (Ar x 2), 129.4(5) (Ar x 2), 113.9 (Ar x 2), 87.5 (C-1), 82.5 (C-2), 78.4 (C-3), 74.1 (C-5),

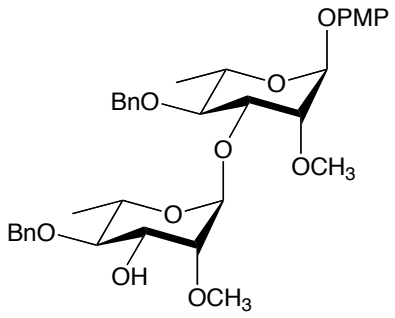
71.8 (Ar*C*H$_2$), 69.5 (C-4), 61.2 (O*C*H$_3$), 55.3 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 16.7 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{22}H_{28}NaO_5S$: 427.1555. Found 427.1549.



***p*-Methoxyphenyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (4-26)**

To a solution of donor **4-20** (0.95 g, 1.82 mmol) and acceptor **4-18** (0.82 g, 2.2 mmol) in CH_2Cl_2 (30 mL) was added crushed 4 Å molecular sieves (300 mg). After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC, and then NIS (416 mg, 1.85 mmol) and AgOTf (123 mg, 0.48 mmol) were added. The reaction mixture was stirred for an additional 30 min at –20 ºC before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was purified by chromatography (3:1 hexane–EtOAc) to give **4-26** (1.13 g, 81%) as an amorphous solid: R_f 0.50 (2:1 hexane–EtOAc); $[\alpha]_D$ –25.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.36–7.27 (m, 10 H, Ar), 7.21–7.19 (m, 2H, Ar), 6.97–6.96 (m, 2H, Ar), 6.83–6.78 (m, 4H, Ar), 5.52 (dd, 1H, $J_{1',2'}$ = 1.8 Hz, $J_{2',3'}$ = 3.3 Hz, H-2′), 5.40 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.09 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.92, 4.40 (ABq, 2H, *J* = 11.0 Hz, ArC*H*$_2$), 4.80, 4.61 (ABq, 2H, *J* = 11.0 Hz,

ArC*H*$_2$), 4.59 (ABq, 2H, *J* = 10.5 Hz, ArC*H*$_2$), 4.22 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.00–3.95 (m, 2H, H-3′, H-5′), 3.81–3.78 (m, 1H, H-5), 3.76 (s, 3H, OC*H*$_3$), 3.74 (s, 3H, OC*H*$_3$), 3.71 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.52 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.49 (s, 3H, OC*H*$_3$), 3.44 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.4 Hz, H-4′), 2.11 (s, 3H, C*H*$_3$CO), 1.35 (d, 3H, $J_{5',6'}$ = 6.0 Hz, H-6′), 1.24 (d, 1H, $J_{5,6}$ = 6.0 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.1 (*C*=O), 159.3 (Ar), 154.9 (Ar x 2), 150.4 (Ar x 2), 138.6 (Ar), 138.1 (Ar), 130.1 (Ar), 129.7 (Ar x 2), 128.5 (Ar x 2), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar), 127.7 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 113.8 (Ar x 2), 99.8 (C-1′), 95.6 (C-1), 80.3 (C-4′), 80.1 (C-4), 80.0 (C-3′), 78.8 (C-2), 77.6 (C-3), 75.4 (Ar*C*H$_2$), 75.4 (Ar*C*H$_2$), 71.4 (Ar*C*H$_2$), 69.2 (C-2′), 68.7 (C-5′), 68.5 (C-5), 58.9 (O*C*H$_3$), 55.7 (O*C*H$_3$), 55.2 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 18.2 (C-6), 18.0 (C-6`). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{44}H_{52}NaO_{12}$: 795.3351. Found 795.3343.

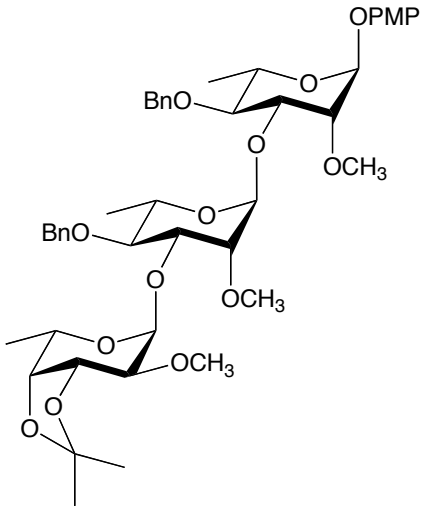


***p*-Methoxyphenyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (4-27)**

To a solution of **4-26** (0.5 g, 0.65 mmol) in 1:1 CH_3OH–CH_2Cl_2 (15 mL), 1M $NaOCH_3$ (0.5 mL) was added and the reaction mixture was stirred for 1 h at rt. The solution was then neutralized with Amberlite IR-120 H^+ resin, filtered and

concentrated. The resulting residue was dissolved in DMF (5 mL) and CH_3I (0.1 mL, 0.9 mmol) was added and the solution was cooled to 0 °C. To this solution, NaH (60% in mineral oil, 30 mg, 1.18 mmol) was added and then the reaction mixture was stirred for 1 h at rt. Chilled water (20 mL) was then added and the mixture was diluted with CH_2Cl_2 (40 mL), washed with water (2 x 30 mL), 1M HCl soln (2 x 30 mL) and brine (30 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (4:1 hexane–EtOAc) to give a syrup. The syrup was dissolved in CH_2Cl_2 (20 mL) and TFA (1 mL, 5% v/v) was added dropwise over 2 min after the solution was cooled to 0 ºC. The reaction mixture was stirred for additional 30 min at 0 ºC, before the addition of Et_3N (3 mL) and concentration. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **4-27** (0.4 g, 87%) as a colorless oil: R_f 0.35 (3:1 hexane–EtOAc); $[\alpha]_D$ –6.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.41–7.26 (m, 10H, Ar), 7.03–7.00 (m, 2H, Ar-2,6), 6.86–6.83 (m, 2H, Ar-3,5), 5.43 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.18 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.93, 4.82 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.71, 4.71 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.26 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.03 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.91–3.84 (m, 2H, H-5, H-5′), 3.78 (s, 3H, OCH_3), 3.73 (dd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 3.58 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.54 (s, 3H, OCH_3), 3.49 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, $J_{2',3'}$ = 3.7 Hz, H-2′), 3.31 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4′), 3.23 (s, 3H, OCH_3), 1.37 (d, 3H, $J_{5',6'}$ = 6.4 Hz, H-6′), 1.29 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 138.6 (Ar), 138.4 (Ar),

128.4 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar x 2), 127.7 (Ar x 2), 127.1 (Ar x 2), 117.6 (Ar x 2), 114.7 (Ar x 2), 98.7 (C-1′), 95.8 (C-1), 82.1 (C-4′), 81.0 (C-4), 80.6 (C-2′), 80.4 (C-2), 78.7 (C-3′), 75.1 (Ar*C*H_2), 71.6 (C-5′), 68.8 (C-5), 67.9 (C-3), 59.1 (O*C*H_3), 58.6 (O*C*H_3), 55.7 (O*C*H_3), 18.2 (C-6), 18.0 (C-6′). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{35}H_{44}NaO_{10}$: 647.2827. Found 647.2823.

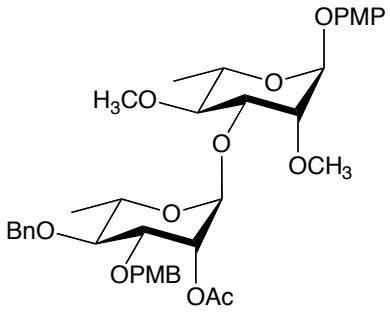


***p*-Methoxyphenyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (4-28)**

Two solutions were prepared. Solution A was prepared by dissolving donor **3-13** (50 mg, 0.15 mmol) in CH_2Cl_2 (10 mL) containing crushed 4 Å molecular sieves (50 mg). Solution B was prepared by dissolving acceptor **4-27** (80 mg, 0.13 mmol) in CH_2Cl_2 (15 mL) and crushed 4 Å molecular sieves (100 mg) was added. Both solutions A and B were stirred for 30 min at rt and then solution B was cooled to –40 ºC before NIS (36 mg, 0.16 mmol) and AgOTf (10.3 mg, 0.04

mmol) were added. Solution A was then added dropwise over 5 min to solution B while stirring. The reaction mixture was stirred for additional 30 min at –40 ºC before Et_3N (1 mL) was added. The solution was filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **4-28** (86 mg, 80%) as a colorless oil: R_f 0.40 (2:1 hexane–EtOAc); $[\alpha]_D$ +0.8 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.55 (m, 10H, Ar), 7.00–6.89 (m, 2H, Ar-2,6), 6.83–6.81 (m, 2H, Ar-3,5), 5.42 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.17 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''}$ = 3.2 Hz, H-1″), 5.15, 4.53 (ABq, 2H, *J* = 10.75 Hz, ArCH_2), 4.84, 4.64 (ABq, 2H, *J* = 11.0 Hz, ArCH_2), 4.39 (dq, 1H, $J_{4'',5''}$ = 2.7 Hz, $J_{5'',6''}$ = 6.3 Hz, H-5″), 4.32 (dd, 1H, $J_{2'',3''}$ = 8.9 Hz, $J_{3'',4''}$ = 2.8 Hz, H-3″), 4.21 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.06 (dd, 1H, $J_{3'',4''}$ = 2.8 Hz, $J_{4'',5''}$ = 2.7 Hz, H-4″), 4.02 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.6 Hz, H-3′), 3.95 (dq, 1H, $J_{4',5'}$ = 9.6 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.82 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.77 (s, 3H, OCH_3), 3.75–3.74 (m, 1H, H-2), 3.72–3.71 (m, 1H, H-2′), 3.56–3.52 (m, 1H, H-4), 3.51 (s, 3H, OCH_3), 3.56–3.45 (m, 1H, H-4′), 3.37 (s, 3H, OCH_3), 3.33 (dd, 1H, $J_{1'',2''}$ = 3.2 Hz, $J_{2'',3''}$ = 8.9 Hz, H-2″), 3.24 (s, 3H, OCH_3), 1.54 (s, 3H, (CH_3)$_2$C), 1.35–1.34 (m, 6H, (CH_3)$_2$C, H-6′), 1.29 (d, 3H, $J_{5'',6''}$ = 6.3 Hz, H-6″), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.5 (Ar), 139.0 (Ar), 138.3 (Ar), 128.4 (Ar x 2), 128.2 (Ar x 2), 127.9 (Ar x 2), 127.7 (Ar), 127.4 (Ar), 127.3 (Ar x 2), 117.5 (Ar x 2), 114.6 (Ar x 2), 108.8 ((CH$_3$)$_2$*C*), 99.3 (C-1″), 98.7 (C-1′), 95.4 (C-1), 81.8 (C-2″), 80.5 (C-4′), 80.3 (C-4), 80.1 (C-2′), 79.9 (C-2), 79.5 (C-5), 79.4 (C-5′), 76.1 (C-3′), 75.6 (C-3) 75.2 (Ar*C*H$_2$), 75.2 (Ar*C*H$_2$), 68.7 (C-4″), 68.5 (C-3″), 63.6 (C-5″),

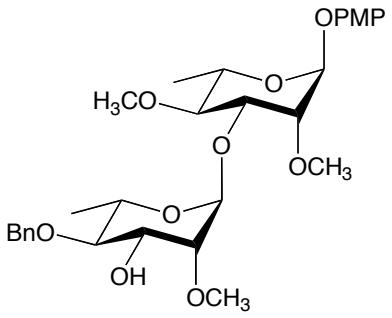
58.8 (O*C*H$_3$), 58.4 (O*C*H$_3$), 57.6 (O*C*H$_3$), 55.6 (O*C*H$_3$), 28.4 ((*C*H$_3$)$_2$C), 26.4 ((*C*H$_3$)$_2$C), 18.2 (C-6′), 18.0 (C-6), 16.6 (C-6″). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{45}H_{60}NaO_{14}$: 847.3875. Found 847.3867.



***p*-Methoxyphenyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-29)**

To a solution of donor **4-20** (0.47 g, 0.91 mmol) and acceptor **4-19** (0.31 g, 1.10 mmol) in CH_2Cl_2 (30 mL) was added crushed 4 Å molecular sieves (200 mg). After the reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC, and then NIS (209 mg, 0.93 mmol) and AgOTf (61.6 mg, 0.24 mmol) were added. The reaction mixture was stirred for additional 30 min before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was purified by chromatography (3:1 hexane–EtOAc) to give **4-29** (0.53 g, 85%) as an amorphous solid: R_f 0.29 (3:1 hexane–EtOAc); R_f 0.31 (3:1 hexane–EtOAc); $[\alpha]_D$ –6.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.25 (m, 5H, Ar), 7.27–7.25 (m, 2H, Ar), 7.00–6.96 (m, 2H, Ar), 6.85–6.81 (m, 4H, Ar), 5.46 (dd, 1H, $J_{1',2'}$ = 1.8 Hz, $J_{2',3'}$ = 3.3 Hz, H-2′), 5.37 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.08 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.94, 4.50 (ABq, 2H, *J* = 11.0 Hz, ArC*H*$_2$), 4.65, 4.63 (ABq, 2H, *J* =

11.2 Hz, Ar-CH_2), 4.10 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.99–3.93 (m, 2H, H-3′, H-5′), 3.79 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.68 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.65 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.52 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.47–3.45 (m, 1H, H-4′), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 2.17 (s, 3H, CH_3CO), 1.36 (d, 3H, $J_{5',6'}$ = 6.3 Hz, H-6′), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.3 (*C*=O), 159.3 (Ar), 154.9 (Ar), 150.4 (Ar), 138.6 (Ar), 130.1 (Ar), 129.7 (Ar x 2), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.7 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 113.8 (Ar x 2), 99.7 (C-1′), 95.9 (C-1), 82.4 (C-4), 80.2 (C-4′), 80.1 (C-2), 78.3 (C-5), 77.4 (C-5′), 75.4 (ArCH_2), 71.5 (ArCH_2), 69.4 (C-3), 68.8 (C-3′), 68.4 (C-2′), 61.1 (O*C*H_3), 59.1 (O*C*H_3), 55.7 (O*C*H_3), 55.3 (O*C*H_3), 21.1 (*C*H_3CO), 18.2 (C-6), 17.8 (C-6′). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{38}H_{48}NaO_{12}$: 719.3038. Found 719.3030.

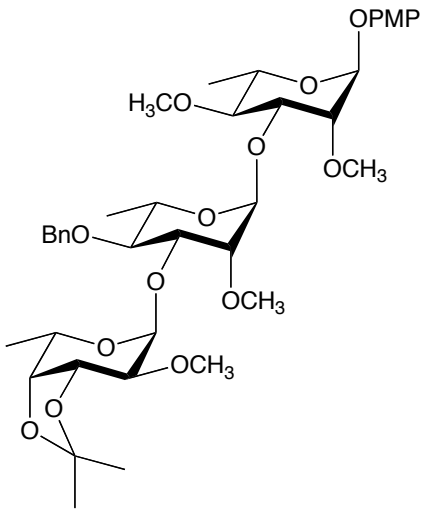


***p*-Methoxyphenyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-30)**

To a solution of **4-30** (0.5 g, 0.72 mmol) in 1:1 CH_3OH–CH_2Cl_2 (30 mL), 1M $NaOCH_3$ (0.5 mL) was added. The reaction mixture was stirred for 1 h at rt and then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The

resulting residue was dissolved in DMF (5 mL) and CH_3I (0.1 mL, 0.86 mmol) was added. To this solution, cooled to 0 °C, NaH (60% in mineral oil, 27.50 mg, 1.16 mmol) was added and then the reaction mixture was stirred for additional 1 h at rt. After 1 h, chilled water (10 mL) was added and the solution was diluted with CH_2Cl_2 (30 mL), washed with water (2 x 20 mL), 1M HCl soln (2 x 20 mL) and brine (20 mL). The organic layer was separated, dried (Na_2SO_4), filtered, concentrated and the resulting purified by chromatography (4:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in CH_2Cl_2 (20 mL) and TFA (1 mL, 5% v/v) was added dropwise over 2 min at 0 ºC. The reaction mixture was stirred for additional 30 min at 0 ºC before the addition of Et_3N (3 mL) and concentration. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give **4-30** (0.31 g, 80%) as a colorless oil: R_f 0.40 (2:1 hexane–EtOAc); $[\alpha]_D$ – 46.9 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.34 (m, 5H, Ar), 7.01–6.97 (m, 2H, Ar-2,6), 6.84–6.81 (m, 2H, Ar-3,5), 5.38 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.22 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.92, 4.70 (ABq, 2H, J = 11.0 Hz, ArC*H*$_2$), 4.13 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.02 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.6 Hz, H-3′), 3.87 (dq, 1H, $J_{4',5'}$ = 9.6 Hz, $J_{5',6'}$ = 6.5 Hz, H-5′), 3.77 (s, 3H, OC*H*$_3$), 3.70 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.67 (dd, 1H, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.62 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 3.55 (s, 3H, OC*H*$_3$), 3.53 (s, 3H, OC*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.31 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.6 Hz, H-4′), 3.25 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 1.35 (d, 1H, $J_{5',6'}$ = 6.5 Hz, H-6′), 1.27 (d, 1H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.4 (Ar), 138.5 (Ar), 128.4 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar),

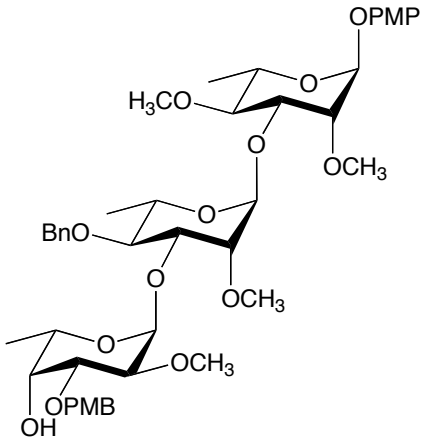
117.5 (Ar x 2), 114.6 (Ar x 2), 98.3 (C-1′), 95.9 (C-1), 82.6 (C-4), 82.1 (C-4′), 81.2 (C-2), 80.3 (C-2′), 78.3 (C-5), 75.1 ($ArCH_2$), 71.5 (C-5′), 68.8 (C-3′), 67.8 (C-3), 61.0 (OCH_3), 59.2 (OCH_3), 58.7 (OCH_3), 55.7 (OCH_3), 18.1 (C-6), 17.8 (C-6′). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{29}H_{40}NaO_{10}$: 571.2514. Found 571.2509.



***p*-Methoxyphenyl 3,4-*O*-isopropylidene-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-31)**

Two solutions were prepared. Solution A was prepared by dissolving donor **3-13** (25 mg, 0.08 mmol) in CH_2Cl_2 (10 mL); containing crushed 4 Å molecular sieves (100 mg). Solution B was prepared by dissolving acceptor **4-30** (38 mg, 0.06 mmol) in CH_2Cl_2 (10 mL); containing crushed 4 Å molecular sieves (100 mg). Both solutions A and B were stirred for 30 min at rt and then Solution B was cooled to –40 ºC before NIS (18 mg, 0.08 mmol) and AgOTf (5 mg, 0.02 mmol)

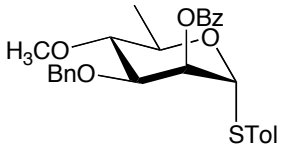
were added. Solution A was then added to Solution B dropwise over 5 min while stirring. The reaction mixture was stirred for additional 30 min at –40 ºC before it was neutralized by the addition of Et_3N (1 mL). The solution was filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **4-31** (37.3 mg, 83%) as a colorless oil: R_f 0.36 (2:1 hexane–EtOAc); $[\alpha]_D$ +89.0 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.39–7.55 (m, 5H, Ar), 7.00–6.89 (m, 2H, Ar), 6.83–6.81 (m, 2H, Ar), 5.43–5.42 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.17 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.14 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1″), 5.15, 4.53 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.45 (dq, 1H, $J_{4'',5''}$ = 2.8 Hz, $J_{5'',6''}$ = 6.4 Hz, H-5″), 4.34 (dd, 1H, $J_{2'',3''}$ = 9.8 Hz, $J_{3'',4''}$ = 2.8 Hz, H-3″), 4.47–4.44 (m, 2H, H-3, H-4″), 4.01 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.93 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.4 Hz, H-5′), 3.84–3.79 (m, 1H, H-5), 3.77 (s, 3H, OCH_3), 3.71–3.66 (m, 2H, H-2, H-2′), 3.55 (s, 3H, OCH_3), 3.51–3.47 (m, 7H, H-4′, OCH_3 x 2), 3.38 (s, 3H, OCH_3), 3.35 (dd, 1H, $J_{1'',2''}$ = 3.1 Hz, $J_{2'',3''}$ = 9.8 Hz, H-2″), 3.22 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 1.54 (s, 3H, (CH_3)$_2$C), 1.36–1.34 (m, 9H, (CH_3)$_2$C, H-6′, H-6″), 1.26 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.5 (Ar), 139.0 (Ar), 128.2 (Ar x 2), 127.9 (Ar x 2), 127.4 (Ar), 117.5 (Ar x 2), 114.6 (Ar x 2), 108.8 ((CH_3)$_2$*C*), 99.3 (C-1″), 98.7 (C-1′), 95.6 (C-1), 82.1 (C-4), 80.5 (C-2″), 80.3 (C-4′), 80.1 (C-2), 79.9 (C-2′), 79.5 (C-5), 79.4 (C-5′), 76.1 (C-3″), 75.6 (C-4″), 75.2 (Ar*C*H$_2$), 68.7 (C-3), 68.5 (C-3′), 63.6 (C-5″), 61.3 (O*C*H$_3$), 58.8 (O*C*H$_3$), 58.4 (O*C*H$_3$), 57.6 (O*C*H$_3$), 55.6 (O*C*H$_3$), 28.4 ((CH$_3$)$_2$C), 26.4 ((CH$_3$)$_2$C), 18.2 (C-6′), 18.0 (C-6″), 16.6 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{39}H_{56}NaO_{14}$: 771.3562. Found 771.3562.



***p*-Methoxyphenyl 3-*O*-*p*-methoxybenzyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2′-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-32)**

To a solution of donor **4-21** (70 mg, 0.16 mmol) in CH_2Cl_2 (10 mL), crushed 4 Å molecular sieves (100 mg) was added (Solution A). To another solution, containing acceptor **4-30** (72 mg, 0.13 mmol) in CH_2Cl_2 (10 mL) was added crushed 4 Å molecular sieves (100 mg) (Solution B). Both solutions were then stirred for 30 min at rt. After 30 min, Solution A was cooled to –40 ºC. To solution B was added NIS (36 mg, 0.16 mmol) and AgOTf (10 mg, 0.04 mmol) and it was then added dropwise to Solution A over 5 min while stirring. The reaction mixture was stirred for additional 30 min at –40 ºC before Et_3N (1 mL) was added. The solution was filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in AcOH (4 mL) and $(Ph_3P)_4Pd$ (18 mg, 20% w/w) was added. The reaction mixture was stirred overnight at rt before it was filtered, concentrated and the resulting residue was purified by chromatography (1:1 hexane–EtOAc) to give

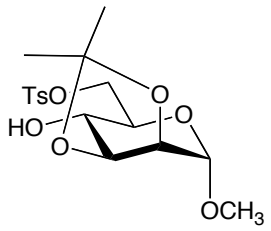
4-32 (76.4 mg, 71%) as colorless oil: R_f 0.52 (1:1 hexane–EtOAc); $[\alpha]_D$ +0.3 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.26 (m, 7H, Ar), 7.00–6.97 (m, 2H, Ar), 6.86–6.81 (m, 4H, Ar), 5.39 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.19, 4.55 (ABq, 2H, J = 11.0 Hz, ArCH_2), 5.20–5.17 (m, 2H, H-1′, H-1″), 4.72, 4.64 (ABq, 2H, J = 11.0 Hz, ArCH_2), 4.18 (dq, 1H, $J_{4'',5''}$ = 2.7 Hz, $J_{5'',6''}$ = 6.3 Hz, H-5″), 4.20–4.06 (m, 2H, H-3′, H-4″), 3.96–3.90 (m, 2H, H-3, H-5′), 3.86–3.84 (m, 1H, H-4′), 3.77 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.75–3.67 (m, 4H, H-2, H-2′, H-3″, H-5), 3.59 (dd, 1H, $J_{1'',2''}$ = 3.3 Hz, $J_{2'',3''}$ = 9.7 Hz, H-2″), 3.44 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.37 (s, 3H, OCH_3), 3.23 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.75 (br s, 1H, OH-3″), 1.33 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.29 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.27 (d, 1H, $J_{5'',6''}$ = 6.3 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.3 (Ar), 154.9 (Ar), 150.5 (Ar), 139.1 (Ar), 130.3 (Ar x 2), 129.3 (Ar x 2), 128.2 (Ar x 2), 127.8 (Ar x 2), 117.5 (Ar x 2), 114.6 (Ar x 2), 113.9 (Ar x 2), 99.8 (C-1″), 98.5 (C-1′), 95.6 (C-1), 82.0 (C-4), 80.7 (C-2″), 80.2 (C-2), 79.9 (C-2′), 79.6 (C-3″), 77.7 (C-5), 77.1 (C-4′), 75.1 (ArCH_2), 72.0 (ArCH_2), 70.1 (C-5′), 68.8 (C-3), 68.6 (C-4″), 65.7 (C-3′), 61.2 (C-5″), 59.2 (OCH_3), 59.1 (OCH_3), 58.9 (OCH_3), 57.8 (OCH_3), 55.7 (OCH_3), 55.2 (OCH_3), 18.2 (C-6″), 17.9 (C-6′), 16.4 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{44}H_{60}NaO_{15}$: 851.3824. Found 851.3809.



***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-deoxy-4-*O*-methyl-1-thio-α-D-mannopyranoside (4-33)**

To a solution of compound **4-37** (0.5 g, 1.54 mmol) in Ac_2O (10 mL) was added H_2SO_4 (0.1 mL) after the solution was cooled to 0 ºC. The reaction mixture was stirred for additional 2 h at 0 ºC before the addition of satd. aq. $NaHCO_3$ soln (10 mL), water (10 mL) and dilution with CH_2Cl_2 (40 mL). The organic layer was separated, washed with satd. aq. $NaHCO_3$ soln (2 x 30 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in CH_2Cl_2 (25 mL) and *p*-thiocresol (0.25 g, 2 mmol) was added. To this solution, $BF_3.Et_2O$ (0.25 mL, 2 mmol) was added at 0 ºC and the reaction mixture was stirred overnight at rt. Then, satd. aq. $NaHCO_3$ soln (25 mL) was added and the mixture was diluted with CH_2Cl_2 (30 mL). The organic layer was separated, washed with water (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in 1:1 CH_3OH–CH_2Cl_2 (20 mL), 1M $NaOCH_3$ (0.25 mL) was added and the reaction mixture was stirred for 2 h at rt. The solution was then neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolve in pyridine (5 mL) and BzCl, benzoyl chloride (0.21 mL, 2 mmol) was added at 0 ºC. The reaction mixture was stirred for additional 2 h at rt before the addition of water (20 mL) and dilution with CH_2Cl_2 (40 mL). The organic layer washed with satd. aq. $NaHCO_3$ soln (2 x

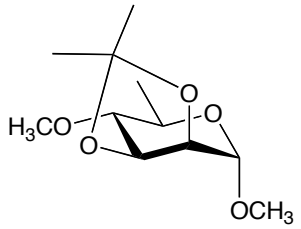
30 mL), brine (30 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (7:1 hexane–EtOAc) to give **4-33** (0.39 g, 53%) as a colorless oil: R_f 0.70 (7:1 hexane–EtOAc); $[\alpha]_D$ +91.3 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.70–7.67 (m, 1H, Ar), 7.59–7.53 (m, 3H, Ar), 7.47–7.44 (m, 2H, Ar), 7.38–7.26 (m, 6H, Ar), 7.11 (d, 2H, J = 8.0 Hz, Ar), 5.80 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 5.46 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.78, 4.61 (ABq, 2H, J = 11.5 Hz, ArC*H*$_2$), 4.21 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.91 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.61 (s, 3H, OC*H*$_3$), 3.33 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.32 (s, 3H, ArC*H*$_3$), 1.40 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.7 (*C*=O), 137.9 (Ar x 2), 134.5 (Ar), 133.2 (Ar), 132.3 (Ar), 130.6 (Ar), 130.1 (Ar), 129.9(3) (Ar), 129.8(7) (Ar x 2), 128.8(8) (Ar), 128.8(7) (Ar), 128.39 (Ar x 2), 128.33 (Ar x 2), 127.92 (Ar), 127.67 (Ar), 86.5 (C-1), 82.4 (C-4), 78.1 (C-3), 71.7 (Ar*C*H$_2$), 71.1 (C-2), 69.1 (C-5), 61.2 (O*C*H$_3$), 21.1 (Ar*C*H$_3$), 17.9 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{28}H_{30}NaO_5S$: 501.1712. Found 501.1710.



Methyl 2,3-*O*-isopropylidene-6-*O*-tosyl-α-D-mannopyranoside (4-35)

To a solution of methyl α-D-mannopyranoside (**4-34**, 2 g, 10.3 mmol) in pyridine (15 mL), DMAP (0.19 g, 0.1% w/w) and TsCl (2.04 g, 10.92 mmol) were added

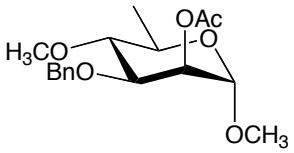
and the reaction mixture was stirred overnight at rt. Water (50 mL) was then added and the mixture was diluted with CH_2Cl_2 (60 mL), washed with water (2 × 60 mL), 1M HCl soln (2 x 60 mL), and finally brine (2 x 60 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in acetone (30 mL) and DMP (2 mL) and *p*-TSA (20 mg) were added. The reaction mixture was stirred for 2 h at rt and then Et_3N (1 mL) was added. The solution was concentrated and the resulting residue was purified by chromatography (4:1 hexane–EtOAc) to give **4-35** (3.12 g, 78%) as a colorless oil: R_f 0.49 (3:1 hexane–EtOAc); $[\alpha]_D$ –11.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.81–7.97 (m, 2H, Ar), 7.35–7.33 (m, 2H, Ar), 4.83 (br s, 1H, H-1), 4.28 (app d, 2H, $J_{5,6}$ = 4.0 Hz, H-6), 4.10–4.09 (m, 2H, H-2, H-3), 3.74–3.70 (m, H, H-5), 3.62–3.55 (m, 1H, H-4), 3.33 (s, 3H, OC*H*$_3$), 2.83 (d, 1H, $J_{4,OH-4}$ = 5.0 Hz, OH-4), 2.44 (s, 3H, ArC*H*$_3$), 1.47 (s, 3H, (C*H*$_3$)$_2$C), 1.32 (s, 3H, (C*H*$_3$)$_2$C); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 144.9 (Ar), 132.9 (Ar), 129.8 (Ar x 2), 128.0 (Ar x 2), 109.8 ((CH$_3$)$_2$*C*), 98.3 (C-1), 78.0 (C-3), 75.4 (C-2), 69.2 (C-6), 68.6 (C-4), 68.3 (C-5), 55.2 (O*C*H$_3$), 27.8 ((*C*H$_3$)$_2$C), 26.0 ((*C*H$_3$)$_2$C), 21.7 (Ar*C*H$_3$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{17}H_{24}NaO_8S$: 411.1084. Found 411.1086.



Methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methyl-α-D-mannopyranoside (4-36)

To a solution of **4-35** (1.5 g, 3.86 mmol) in DMSO (30 mL) was added $NaBH_4$ (0.18 g, 4.63 mmol) and the reaction mixture was stirred for 8 h at 80 ºC. The solution was cooled to 0 ºC before the addition of water (30 mL). The mixture was diluted with CH_2Cl_2 (50 mL), washed with water (2 x 50 mL), 1M HCl soln (2 x 50 mL) and brine (50 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in DMF (10 mL) and CH_3I (0.30 mL, 4.63 mmol) was added. To this solution, NaH (60% in mineral oil, 0.18 g, 6.2 mmol) was added at 0 ºC and then the reaction mixture was stirred for 1 h at rt. Then, chilled water (20 mL) was added and then mixture was diluted with CH_2Cl_2 (40 mL), washed with water (2 x 30 mL), 1M HCl soln (2 x 30 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **4-36** (0.62 g, 69%) as a colorless oil: R_f 0.65 (5:1 hexane–EtOAc); $[\alpha]_D$ +29.1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.84 (br s, 1H, H-1), 4.14–4.09 (m, 2H, H-2, H-3), 3.57 (dq, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.53 (s, 3H, OC*H*$_3$), 3.36 (s, 3H, OC*H*$_3$), 2.97 (dd, 1H, $J_{3,4}$ = 6.5 Hz, $J_{4,5}$ = 9.8 Hz, H-4), 1.55 (s, 3H, (C*H*$_3$)$_2$C), 1.35 (s, 3H, (C*H*$_3$)$_2$C), 1.28 (d, 3H, $J_{5,6}$ = 6.4

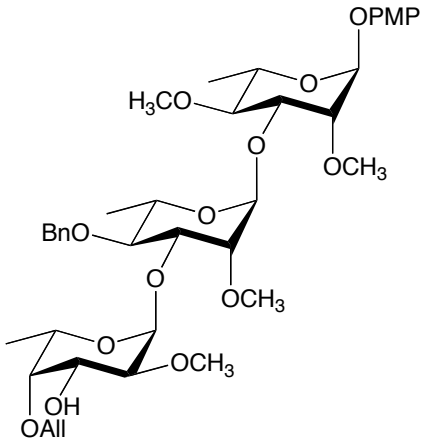
Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 109.3 ($(CH_3)_2C$), 98.3 (C-1), 83.8 (C-4), 78.5 (C-5), 76.2 (C-3), 64.7 (C-2), 59.6 (OCH_3), 55.0 (OCH_3), 28.3 ($(CH_3)C$), 26.5 ($(CH_3)C$), 17.9 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{11}H_{20}NaO_5$: 255.1203. Found 255.1206.



Methyl 2-*O*-acetyl-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranoside (4-37)

To a solution of **4-36** (0.5 g, 2.16 mmol) in 3:1 CH_3OH–CH_2Cl_2 (20 mL), *p*-TSA (10 mg) was added. The reaction mixture was stirred for 4 h at rt before the addition of Et_3N (1 mL). The reaction mixture was then concentrated and the resulting residue was dissolved in toluene (30 mL) and *n*-Bu_2SnO (0.53 g, 2.16 mmol) was added. The reaction mixture was heated at 120 ºC for 1 h. Then, it was cooled to 62 ºC, and *n*-Bu_4NI (0.6 g, 2.4 mmol) and BnBr (0.28 mL, 2.4 mmol) were added. The reaction mixture was stirred for additional 7 h at 62 ºC and then cooled and concentrated. The resulting residue was purified by chromatography (3:1 hexane–EtOAc) to give an oil. This oil was dissolved in pyridine (5 mL), before Ac_2O (2 mL) was added. The reaction mixture was stirred for 1 h at rt before the addition of water (20 mL). Dilution of the mixture with CH_2Cl_2 (40 mL) was followed by separation of the organic layer, which was washed with water (2 x 30 mL), 1M HCl soln (2 x 30 mL), brine (30 mL), dried (Na_2SO_4),

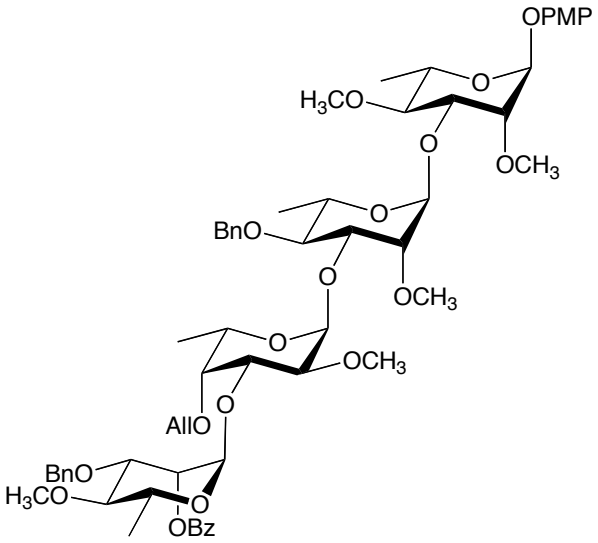
filtered and concentrated. The resulting residue was purified by chromatography (6:1 hexane–EtOAc) to give **4-37** (0.55 g, 79%) as a colorless oil: R_f 0.39 (6:1 hexane–EtOAc); $[\alpha]_D$ +22.5 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.38–7.28 (m, 5H, Ar-H), 5.33 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 4.68, 4.54 (ABq, 2H, *J* = 11.4 Hz, ArC*H*$_2$), 4.61 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 3.80 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.63 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.58 (s, 3H, OC*H*$_3$), 3.35 (s, 3H, OC*H*$_3$), 3.15 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.14 (s, 3H, C*H*$_3$CO), 1.34 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.6 (*C*=O), 138.4 (Ar), 128.6 (Ar x 2), 128.1 (Ar x 2), 127.9 (Ar), 98.9 (C-1), 82.1 (C-4), 78.0 (C-3), 71.9 (Ar*C*H$_2$), 69.2 (C-2), 67.8 (C-5), 61.3 (O*C*H$_3$), 55.1 (O*C*H$_3$), 21.3 (*C*H$_3$CO), 18.1 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{17}H_{24}NaO_6$: 347.1465. Found 347.1469.



***p*-Methoxyphenyl 4-*O*-allyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-38)**

Two solutions were prepared. Solution A was prepared by dissolving donor **4-21** (0.29 g, 0.64 mmol) in CH_2Cl_2 (15 mL); containing crushed 4 Å molecular sieves (50 mg). Solution B was prepared by dissolving acceptor **4-30** (300 mg, 0.53 mmol) in CH_2Cl_2 (15 mL); containing crushed 4 Å molecular sieves (100 mg). Both solutions were then stirred for 30 min at rt before solution B solution was cooled to –40 ºC, NIS (144 mg, 0.64 mmol) and AgOTf (39 mg, 0.15 mmol) were added. Solution A was then added to solution B dropwise over 5 min while stirring. Then, the reaction mixture was stirred for additional 30 min at –40 ºC before it was neutralized by the addition of Et_3N (1 mL). The solution was filtered, concentrated and the resulting residue was dissolved in CH_2Cl_2 (15 mL). To this solution, TFA (0.75 mL, 5% v/v) was added dropwise over 1 min at 0 ºC and reaction mixture was then stirred for additional 30 min at 0 ºC before it was neutralized by the addition of Et_3N (2 mL). The solution was concentrated and the resulting crude product was purified by chromatography (1:1 hexanes–EtOAc) to give **4-38** (302 mg, 76%) as a colorless oil: R_f 0.54 (1:1 hexanes–EtOAc); $[\alpha]_D$ +25.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.37–7.23 (m, 5H, Ar), 7.00–6.96 (m, 2H, Ar-2,6), 6.83–6.80 (m, 2H, Ar-3,5), 6.01–5.93 (m, 1H, CH_2=C*H*), 5.39 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.31–5.27 (m, 1H, CH_2=CH), 5.23 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1″), 5.20–5.18 (m, 1H, CH_2=CH), 5.16 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′) 5.13, 4.58 (ABq, 2H, *J* = 11.5 Hz, ArCH_2), 4.35–4.31 (m, 1H, CH_2O), 4.22–4.17 (m, 2H, CH_2O, H-5″), 4.14–4.08 (m, 2H, H-4″, H-3″), 4.06 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.02 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′) 3.95 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.76 (s, 3H, OCH_3), 3.74–3.72 (m,

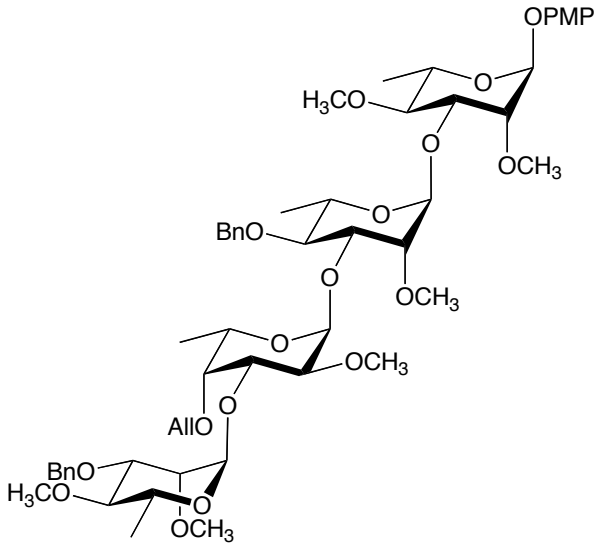
2H, H-2, H-2′), 3.68 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.59–3.58 (m, 1H, H-2″), 3.54 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.49–3.46 (m, 1H, H-4′), 3.29 (s, 3H, OCH_3), 3.21 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 2.34 (d, 1H, $J_{3'',OH\text{-}3''}$ = 2.5 Hz, OH-3″), 1.30 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.27 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.25 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.5 (Ar), 139.1 (Ar), 135.0 (=*C*H), 128.2 (Ar x 2), 127.4 (Ar x 2), 127.3 (Ar), 117.5 (Ar x 2), 117.4 (*C*H_2=CH), 114.6 (Ar x 2), 99.1 (C-1″), 98.6 (C-1′), 95.5 (C-1), 81.9 (C-4), 80.7 (C-4′), 80.2 (C-2″), 80.1 (C-5′), 79.5 (C-2), 79.4 (C-2′), 78.8 (C-5), 74.9 (ArCH_2), 74.8 (–*C*H_2O), 70.2 (C-4″), 68.7(0) (C-3′), 68.6(8) (C-3), 66.4 (C-3″), 61.2 (C-5″), 58.8 (O*C*H_3), 58.1 (O*C*H_3), 57.7(1) (O*C*H_3), 55.6(5) (O*C*H_3 x 2), 18.3 (C-6′), 17.9 (C-6), 16.9 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{39}H_{56}NaO_{14}$: 771.3562. Found 771.3560.



***p*-Methoxyphenyl 2-*O*-benzoyl-3-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→3)-4-*O*-allyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-39)**

To a solution of donor **4-33** (131 mg, 0.28 mmol) and acceptor **4-38** (120 mg, 0.16 mmol) in CH_2Cl_2 (20 mL), crushed 4 Å molecular sieves (200 mg) were added. After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC, NIS (67.4 mg, 0.3 mmol) and AgOTf (15.4 mg, 0.06 mmol) were added and the reaction mixture was stirred for additional 30 min at –20 ºC before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **4-39** (121.7 mg, 69%) as an amorphous solid: R_f 0.38 (2:1 hexane–EtOAc); $[\alpha]_D$ +28.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.09–8.07 (m, 2H, Ar), 7.58–7.55 (m, 1H, Ar), 7.47–7.44 (m, 2H, Ar), 7.30–7.09 (m, 10H, Ar), 7.01–6.95 (m, 2H, Ar), 6.85–6.80 (m, 2H, Ar), 5.94–5.86 (m, 1H, CH_2=C*H*), 5.67 (dd, 1H, $J_{1''',2'''}$ = 1.8 Hz, $J_{2''',3'''}$ =

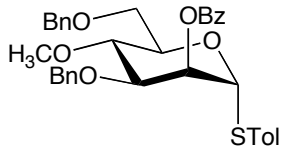
3.1 Hz, H-2‴), 5.39 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.28–5.27 (m, 1H, C*H*$_2$=CH), 5.25–5.24 (m, 1H, C*H*$_2$=CH), 5.17–5.14 (m, 3H, H-1′, H-1″, H-1‴), 5.13, 4.52 (ABq, 2H, J = 11.3 Hz, ArC*H*$_2$), 4.78.459 (ABq, 2H, J = 11.5 Hz, ArC*H*$_2$), 4.34–4.30 (m, 1H, –C*H*$_2$O), 4.23–4.20 (m, 2H, H-3″, H-5″), 4.10–4.06 (m, 2H, H-3′, C*H*$_2$O), 4.00 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.94 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.90 (dd, 1H, $J_{2''',3'''}$ = 3.1 Hz, $J_{3''',4'''}$ = 9.6 Hz, H-3‴), 3.84–3.78 (m, 1H, H-2), 3.76 (s, 3H, OC*H*$_3$), 3.75–3.68 (m, 4H, H-2′, H-2″, H-5, H-5‴), 3.59 (s, 3H, OC*H*$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.59 (s, 3H, OC*H*$_3$), 3.54 (s, 3H, OC*H*$_3$), 3.53–3.46 (m, 2H, H-4′, H-4″), 3.50 (s, 3H, OC*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.31 (s, 3H, OC*H*$_3$), 3.26 (app t, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 9.6 Hz, H-4‴), 3.22 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 1.38 (d, 3H, $J_{5''',6'''}$ = 6.3 Hz, H-6‴), 1.33 (d, 3H, $J_{5'',6''}$ = 6.5 Hz, H-6″), 1.27 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.24 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.6 (*C*=O), 154.9 (Ar), 150.5 (Ar), 139.1 (Ar), 138.1 (Ar), 135.1 (=*C*H), 133.1 (Ar), 130.1 (Ar x 2), 129.9 (Ar x 2), 128.4 (Ar x 2), 128.3 (Ar x 2), 128.1 (Ar x 2), 127.9 (Ar x 2), 127.6 (Ar), 127.5 (Ar), 127.1 (Ar), 117.5 (Ar x 2), 117.3 (*C*H$_2$=), 114.6 (Ar x 2), 99.7 (C-1‴), 99.1 (C-1′), 98.5 (C-1″), 95.6 (C-1), 82.4 (C-4), 82.0 (C-4‴), 81.5 (C-4″), 80.7 (C-4′), 80.2 (C-5‴), 80.0 (C-5), 79.5 (C-2′), 79.4 (C-2″), 79.0 (C-2), 75.5 (C-3‴), 75.1 (Ar*C*H$_2$), 74.4 (Ar*C*H$_2$), 71.3 (–*C*H$_2$O), 69.3 (C-5′), 68.7(3) (C-3), 68.6(5) (C-3′), 68.5 (C-3″), 66.9 (C-5″), 61.3 (C-2‴), 61.2 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.6 (O*C*H$_3$), 57.8 (O*C*H$_3$), 55.7 (O*C*H$_3$), 55.6 (O*C*H$_3$), 18.2 (C-6′), 18.2 (C-6), 17.9 (C-6‴), 16.8 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{60}H_{78}NaO_{19}$: 1125.5030. Found 1125.5020.



***p*-Methoxyphenyl 3-benzyl-6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-4-*O*-allyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-40)**

To a solution of **4-39** (100 mg, 0.09 mmol) in 1:1 CH_3OH–CH_2Cl_2 (20 mL), 1M $NaOCH_3$ (0.2 mL) was added and the reaction mixture was stirred for 5 h at rt. The reaction mixture was then neutralized by the addition of Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in DMF (3 mL), and then CH_3I (0.1 mL) and NaH (60% in mineral oil, 10 mg) were added. The reaction mixture was stirred for 1 h at rt before chilled water (8 mL) was added. The solution was diluted with CH_2Cl_2 (15 mL), washed with water (2 x 10 mL) and finally brine (10 mL). The organic layer was separated, concentrated and the resulting residue was purified by chromatography (3:1 hexane–EtOAc) to give **4-40** (83.6 mg, 91%) as a colorless oil: R_f 0.45 (3:1 hexane–EtOAc); $[\alpha]_D$ –28.1 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.22 (m, 10H, Ar), 7.00–6.96

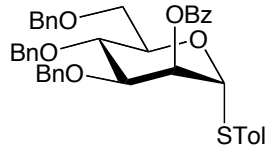
(m, 2H, Ar), 6.83–6.81 (m, 2H, Ar), 5.92–5.84 (m, 1H, =C*H*), 5.39 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.26–5.22 (m, 1H, CH_2=CH), 5.17–5.14 (m, 5H, H-1′, H-1″, H-1‴, CH_2=CH, ArCH_2), 4.72, 4.68 (ABq, 2H, J = 12.5 Hz, ArCH_2), 4.56 (d, 1H, J = 11.5 Hz, ArCH_2), 4.31–4.27 (m, 1H, CH_2O), 4.21–4.14 (m, 2H, H-3″, H-5″), 4.07–4.02 (m, 2H, H-3′, CH_2O), 3.99 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.94 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.2 Hz, H-5′), 3.76 (s, 3H, OCH_3), 3.75–3.72 (m, 2H, H-3‴, H-2), 3.70–3.67 (m, 2H, H-2′, H-2″), 3.66–3.60 (m, 2H, H-5, H-5‴), 3.58 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.48–3.46 (m, 3H, H-2‴, H-4′, H-4″), 3.47 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 3.22–3.18 (m, 2H, H-4, H-4‴), 3.17 (s, 3H, OCH_3), 1.33–1.31 (m, 6H, H-6, H-6′), 1.26 (d, 3H, $J_{5''',6'''}$ = 6.2 Hz, H-6‴), 1.21 (d, 3H, $J_{5'',6''}$ = 6.7 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.9 (Ar), 150.5 (Ar), 139.3 (Ar), 138.5 (Ar), 135.1 (=*C*H), 128.3 (Ar x 2), 128.1 (Ar x 2), 127.9 (Ar x 2), 127.6 (Ar), 127.3 (Ar x 2), 127.2 (Ar), 117.5 (Ar x 2), 117.3 (*C*H_2=), 114.6 (Ar x 2), 99.6 (C-1‴), 98.5 (C-1′), 98.4 (C-1″), 95.5 (C-1), 82.2 (C-4), 82.0 (C-4‴), 80.7 (C-4″), 80.2 (C-4′), 80.1 (C-2‴), 79.6 (C-5‴), 79.2 (C-5′), 79.0 (C-2′), 78.9 (C-2″), 78.4 (C-2), 75.9 (C-3‴), 75.0 (Ar*C*H_2), 74.3 (Ar*C*H_2), 72.2 (*C*H_2O), 68.7(0) (C-3), 68.6(5) (C-3′), 66.9 (C-5′), 61.2 (C-3″), 61.1 (C-5″), 58.8 (O*C*H_3), 58.7 (O*C*H_3), 58.1 (O*C*H_3), 57.6 (O*C*H_3), 57.6 (O*C*H_3), 55.7 (O*C*H_3), 55.6 (O*C*H_3), 18.2 (C-6′), 17.9 (C-6), 17.8 (C-6‴), 16.8 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{54}H_{76}NaO_{18}$: 1035.4924. Found 1035.4920.



***p*-Tolyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-1-thio-α-D-mannopyranoside (4-41)**

To a solution of compound **4-45** (1.04 g, 2.42 mmol) in Ac_2O (15 mL), H_2SO_4 (0.15 mL) was added at 0 ºC and the reaction mixture was stirred for additional 1 h at 0 ºC. A solution of satd. aq. $NaHCO_3$ soln (25 mL) was added followed by water (25 mL) and CH_2Cl_2 (60 mL). The organic layer was separated, washed with satd. aq. $NaHCO_3$ soln (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in CH_2Cl_2 (30 mL) and *p*-thiocresol (0.4 g, 3.13 mmol) was added. To this solution, $BF_3{\cdot}Et_2O$ (0.4 mL, 3.13 mmol) was added at 0 ºC and the mixture was stirred overnight at rt. The reaction A solution of satd. aq. $NaHCO_3$ (50 mL) was added and then resulting mixture was diluted with CH_2Cl_2 (30 mL). The organic layer was separated, washed with water (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by chromatography (6:1 hexane–EtOAc) to give a thick syrup. This syrup was dissolved in 1:1 CH_3OH–CH_2Cl_2 (20 mL) and catalytic amount of 1M $NaOCH_3$ (0.2 mL) was added. The reaction mixture was stirred for 2 h at rt and then it was neutralized by Amberlite IR-120 H^+ resin, filtered and concentrated. To the resulting residue in pyridine (10 mL), BzCl (0.3 mL, 3.13 mmol) was added at 0 ºC. The reaction mixture was stirred for additional 2 h at rt before the addition of water (30 mL). The mixture was diluted

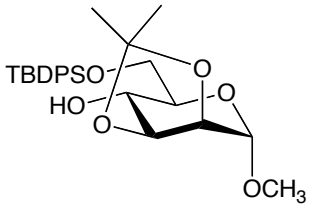
with CH_2Cl_2 (50 mL), washed with satd. aq. $NaHCO_3$ soln (2 x 40 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (6:1 hexane–EtOAc) to give **4-41** (0.96 g, 68%) as a colorless oil: R_f 0.61 (6:1 hexane–EtOAc); $[\alpha]_D$ +41.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.06–8.04 (m, 2H, Ar), 7.55–7.52 (m, 1H, Ar), 7.41–7.26 (m, 14H, Ar), 7.08–7.06 (d, 2H, J = 8.0 Hz, Ar), 5.83 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 5.57 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.81, 4.63 (ABq, 2H, J = 12.0 Hz, ArC*H*$_2$), 4.74, 4.56 (ABq, 2H, J = 11.5 Hz, ArC*H*$_2$), 4.32 (ddd, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 1.8 Hz, H-5), 3.98–3.93 (m, 2H, H-3, H-6a), 3.87 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.81 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.57 (s, 3H, OC*H*$_3$), 2.31 (s, 3H, ArC*H*$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.6 (*C*=O), 138.5 (Ar), 137.9 (Ar x 2), 137.8 (Ar), 133.2 (Ar), 132.4 (Ar), 130.6 (Ar), 130.0 (Ar x 2), 129.9 (Ar x 2), 128.9 (Ar), 128.3(8) (Ar x 2), 128.3(6) (Ar x 2), 128.3 (Ar x 2), 128.0 (Ar x 2), 127.7 (Ar), 127.4(7) (Ar x 2), 127.4(4) (Ar), 86.9 (C-1), 78.4 (C-4), 76.3 (C-3), 73.4 (Ar*C*H$_2$), 72.6 (C-5), 70.7 (C-2), 69.2 (C-6), 61.1 (O*C*H$_3$), 21.1 (Ar*C*H$_3$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{35}H_{36}NaO_6S$: 607.2130. Found 607.2131.



***p*-Tolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4-42)**

To a solution of compound **4-47** (1.22 g, 2.41 mmol) in Ac_2O (20 mL), H_2SO_4 (0.2 mL) was added at 0 ºC. The reaction mixture was stirred for additional 1 h at 0 ºC before the addition of satd. aq. $NaHCO_3$ soln (15 mL), water (15 mL) and CH_2Cl_2 (50 mL). The organic layer was separated, washed with satd. aq. $NaHCO_3$ soln (2 x 40 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in CH_2Cl_2 (20 mL) and *p*-thiocresol (0.38 g, 3.13 mmol) was added. To this solution, $BF_3{\cdot}Et_2O$ (0.4 mL, 3.13 mmol) was added at 0 ºC and the mixture was stirred overnight at rt. Then, satd. aq. $NaHCO_3$ soln (20 mL) and CH_2Cl_2 (20 mL) were added. The organic layer was separated, washed with water (2 x 30 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by chromatography (6:1 hexane–EtOAc) to give an oil. To the soultion of this oil in 1:1 CH_3OH–CH_2Cl_2 (20 mL), 1M $NaOCH_3$ (0.2 mL) was added and the reaction mixture was stirred for 2 h at rt. The solution was then neutralized by the addition of Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolve in pyridine (8 mL) and BzCl (0.3 mL, 3.13 mmol) was added at 0 ºC. The reaction mixture was stirred for additional 2 h at rt before the addition of water (30 mL). The solution was diluted with CH_2Cl_2 (50 mL), washed with satd. aq. $NaHCO_3$ soln (2 x 40 mL), brine (40 mL), dried (Na_2SO_4), filtered and concentrated. The resulting

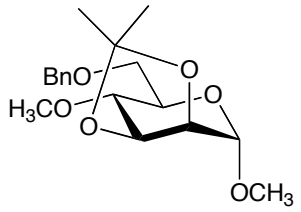
residue was purified by chromatography (6:1 hexane–EtOAc) to give **4-42** (0.94 g, 59%) as a colorless oil: R_f 0.65 (6:1 hexane–EtOAc); $[\alpha]_D$ +36.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.19–8.17 (m, 3H, Ar), 8.08–8.06 (m, 1H, Ar), 7.70–7.67 (m, 2H, Ar), 7.57–7.52 (m, 4H, Ar), 7.47–724 (m, 13H, Ar), 7.08–7.06 (m, 1H, Ar), 5.88 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 5.59 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.92, 4.59 (ABq, 2H, J = 10.8 Hz, ArCH_2), 4.82, 4.62 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.71, 4.52 (ABq, 2H, J = 11.8 Hz, ArCH_2), 4.43 (ddd, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 4.1 Hz, H-5), 4.18 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 4.09 (dd, 1H, $J_{5,6a}$ = 4.1 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.96 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.7 Hz, H-3), , 3.81 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 2.31 (s, 3H, ArCH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.6 (*C*=O), 138.4 (Ar), 138.3 (Ar), 137.9(6) (Ar), 137.9(3) (Ar), 134.5 (Ar), 133.2 (Ar), 133.0 (Ar), 132.4 (Ar), 130.6 (Ar x 3), 129.9(5) (Ar), 129.9(0) (Ar), 129.9 (Ar), 129.7 (Ar x 2), 128.8(9) (Ar x 3), 128.8(8) (Ar), 128.4(1) (Ar), 128.3(9) (Ar), 128.3(5) (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5(3) (Ar), 127.4(6) (Ar), 99.0 (C-1), 78.4 (C-3), 75.4 (Ar*C*H_2), 74.6 (C-4), 73.7 (Ar*C*H_2), 72.0 (Ar*C*H_2), 71.5 (C-5), 69.2 (C-6), 68.9 (C-2), 21.1 (Ar*C*H_3). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{41}H_{40}NaO_6S$: 683.2443. Found 683.2444.



Methyl 2,3-*O*-isopropylidene-6-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (4-43)

To a solution of methyl α-D-mannopyranoside, (**4-34**, 3 g, 15.05 mmol) in pyridine (20 mL) and Et_3N (2.5 mL), TBDPSCl (4.96 g, 18.06 mmol) was added and the reaction mixture was heated at 60 ºC for 3 h. Water (50 mL) was added and then solution was diluted with CH_2Cl_2 (80 mL), washed with water (2 × 60 mL), 1M soln HCl (2 x 60 mL) and brine (2 x 60 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in acetone (30 mL) before DMP (4 mL) and *p*-TSA (100 mg) were added. The reaction mixture was stirred for 3 h at rt before Et_3N (1 mL) was added. The reaction mixture was concentrated and the resulting crude product was purified by chromatography (4:1 hexane–EtOAc) to give **4-43** (6.25 g, 88%) as a colorless oil: R_f 0.78 (4:1 hexane–EtOAc); $[\alpha]_D$ –0.13 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.75–7.73 (m, 4H, Ar), 7.48–7.41 (m, 6H, Ar), 4.91 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1), 4.19–4.13 (m, 2H, H-2, H-3), 3.98–3.90 (m, 2H, H-6 x 2), 3.85–3.81 (m, 1H, H-4), 3.68–3.64 (m, 1H, H-5), 3.37 (s, 3H, OCH_3), 2.82 (s, 1H, OH-4), 1.53 (s, 3H, (CH_3)$_2$C), 1.37 (s, 3H, (CH_3)$_2$C), 1.10 (s, 9H, (CH_3)$_3$C); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 135.7 (Ar x 2), 135.6 (Ar x 2), 133.1 (Ar), 133.0 (Ar), 129.8(3) (Ar), 129.8(1) (Ar), 127.7(8) (Ar x 2), 127.7(4) (Ar x 2), 109.5

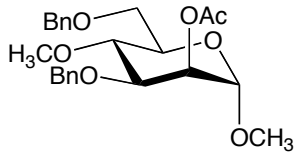
(($CH_3)_2C$), 98.3 (C-1), 78.2 (C-3), 75.3 (C-2), 70.6 (C-4), 69.5 (C-5), 64.7 (C-6), 54.9 (OCH_3), 27.9 (($CH_3)_2C$), 26.1 (($CH_3)_2C$), 26.8 (($CH_3)_3C$ x 3), 19.2 (($CH_3)_3C$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{26}H_{36}NaO_6Si$: 495.2173. Found 495.2171.



Methyl 6-*O*-benzyl-2,3-*O*-isopropylidene-4-*O*-methyl-α-D-mannopyranoside (4-44)

To a solution of compound **4-43** (3.05 g, 6.45 mmol) and CH_3I (0.6 mL, 7.75 mmol) in DMF (20 mL), NaH (60% in mineral oil, 0.25 g, 10.3 mmol) was added at 0 ºC portionwise over 2 min. The reaction mixture was stirred for 1 h at rt before the addition of water (30 mL). The solution was concentrated, diluted with CH_2Cl_2 (60 mL) and washed with water (2 × 50 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in THF (20 mL), TBAF (7.75 mL of 1M soln in THF, 7.75 mmol) was added and the reaction mixture was stirred for 2 h at rt. Water (50 mL) was added and the solution was diluted with CH_2Cl_2 (60 mL) and washed with 1M soln HCl (2 x 50 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in DMF (20 mL) and BnBr (1.9 mL, 15.48 mmol) was added. To this solution, NaH (60% in mineral

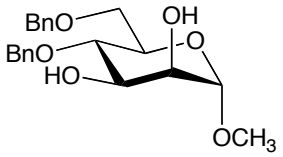
oil, 0.5 g, 20.65 mmol) was added portionwise over 2 min at 0 ºC and the reaction mixture was stirred for additional 3 h at rt before chilled water (40 mL) and CH_2Cl_2 (60 mL) were added. The organic layer was washed with water (2 x 50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (5:1 hexane–EtOAc) to give **4-45** (1.7 g, 78%) as a colorless oil: R_f 0.54 (5:1 hexane–EtOAc); $[\alpha]_D$ +30.6 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.39–7.27 (m, 5H, Ar), 4.95 (br s, 1H, H-1), 4.68, 4.58 (ABq, 2H, J = 11.5 Hz, ArCH_2), 4.22–4.18 (m, 1H, H-3), 4.13 (d, 1H, $J_{2,3}$ = 3.5 Hz, H-2), 3.77–3.64 (m, 3H, H-5, H-6 x 2), 3.41 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.34 (dd, 1H, $J_{3,4}$ = 9.7 Hz, $J_{4,5}$ = 8.9 Hz, H-4), 1.56 (s, 3H, (CH_3)$_2$C), 1.37 (s, 3H, (CH_3)$_2$C); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.7 (Ar), 128.5 (Ar x 2), 127.8 (Ar x 2), 127.7 (Ar), 109.5 ((CH$_3$)$_2$*C*), 98.6 (C-1), 78.9 (C-4), 78.1 (C-3), 76.1 (C-2), 73.7 (Ar*C*H$_2$), 69.6 (C-6), 68.6 (C-5), 59.4 (O*C*H$_3$), 55.1 (O*C*H$_3$), 28.2 ((*C*H$_3$)$_2$C), 26.5 ((*C*H$_3$)$_2$C). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{18}H_{26}NaO_6$: 361.1622. Found 361.1621.



Methyl 2-*O*-acetyl-3,6-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside (4-45)

To a solution of **4-44** (3 g, 8.9 mmol) in 3:1 CH_3OH–CH_2Cl_2 (50 mL), *p*-TSA (100 mg) was added and the mixture was stirred for 4 h at rt before Et_3N (2 mL) was added. The solution was concentrated and the resulting residue was dissolved

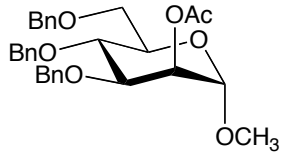
in toluene (70 mL) and *n*-Bu_2SnO (2.19 g, 8.87 mmol) was added. The reaction mixture was heated at 120 ºC for 1 h. Then, it was cooled to 62 ºC before *n*-Bu_4NI (3.59 g, 9.76 mmol) and BnBr (1.15 mL, 9.76 mmol) were added and the reaction mixture was stirred for additional 7 h at 62 ºC. The solution was then concentrated and the resulting residue was purified by chromatography (3:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in pyridine (10 mL), Ac_2O (5 mL) was added and the reaction mixture was stirred for 1 h at rt before water (40 mL) and and CH_2Cl_2 (60 mL) were added. The organic layer was separated, washed with water (2 x 50 mL), 1M HCl soln (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by chromatography (6:1 hexane–EtOAc) to give **4-45** (2.78 g, 73%) as a colorless oil: R_f 0.45 (6:1 hexane–EtOAc); $[\alpha]_D$ +29.8 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40–7.27 (m, 10H, Ar), 5.33 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 4.73, 4.68 (ABq, 2H, J = 10.1 Hz, ArCH_2), 4.72 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.58, 4.51 (ABq, 2H, J = 10.1 Hz, ArCH_2), 3.86 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.82–3.78 (m, 1H, H-5), 3.74–3.36 (m, 2H, H-6 x 2), 3.59 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 3.51 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.7 (*C*=O), 138.6 (Ar), 138.4 (Ar), 128.6 (Ar x 2), 128.5 (Ar x 2), 128.1 (Ar x 2), 127.9 (Ar x 2), 127.8 (Ar x 2), 99.0 (C-1), 78.2 (C-4), 76.7 (C-3), 73.7 (Ar*C*H_2), 72.0 (Ar*C*H_2), 71.6 (C-5), 69.3 (C-6), 69.0 (C-2), 61.1 (O*C*H_3 x 2), 21.4 (*C*H_3CO). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{24}H_{30}NaO_7$: 453.1884. Found 453.1885.



Methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (4-46)

To a solution of **4-43** (3 g, 6.35 mmol) in THF (40 mL), TBAF (7.75 mL of 1M soln in THF, 7.75 mmol) was added and the reaction mixture was stirred for 2 h at rt before water (60 mL) was added. The solution was diluted with CH_2Cl_2 (100 mL) and washed with 1M soln HCl (2 x 80 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The resulting residue was dissolved in DMF (20 mL) and BnBr (2 mL, 15.5 mmol) was added. To this solution, NaH (60% in mineral oil, 0.6 g, 20.64 mmol) was added portionwise over 2 min at 0 ºC and it was then stirred for additional 2 h at rt before chilled water (30 mL) and CH_2Cl_2 (60 mL) were added. The organic layer was washed with water (2 x 50 mL), satd. aq. $NaHCO_3$ soln (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by chromatography (4:1 hexane–EtOAc) to give a syrup. To the solution of this syrup in 2:1 CH_3OH–CH_2Cl_2 (30 mL), *p*-TSA (100 mg) was added. The reaction mixture was stirred overnight at rt before Et_3N (1 mL) was added. The solution was then concentrated and the resulting residue was purified by chromatography (1:1 hexane–EtOAc) to give **4-46** (1.76 g, 74%) as a colorless oil: *Rf* 0.45 (1:1 hexane–EtOAc); $[\alpha]_D$ +39.0 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.46–7.25 (m, 10H, Ar), 4.75, 4.57 (ABq, 2H, J = 12.1 Hz, ArCH_2), 4.76 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.69, 4.59 (ABq, 2H, J = 12.2 Hz, ArCH_2), 3.95–3.89 (m, 2H, H-2, H-3), 3.83–3.73 (m,

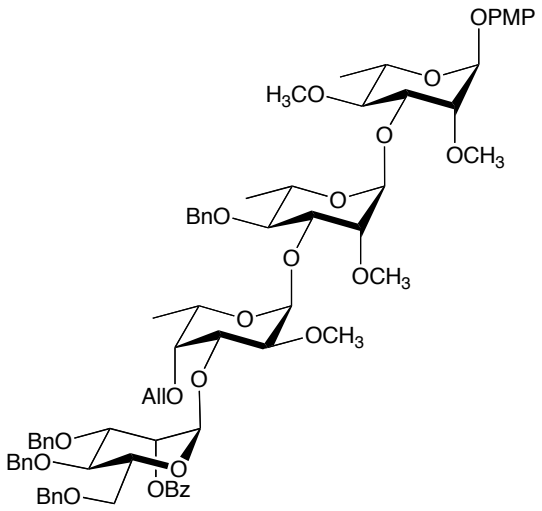
4H, H-4, H-5, H-6 x 2), 3.39 (s, 3H, OC*H*$_3$), 2.66 (d, 1H, $J_{2,OH-2}$ = 5.2 Hz, OH-2), 2.49 (d, 1H, $J_{3,OH-3}$ = 6.0 Hz, OH-3); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 138.6 (Ar), 138.2 (Ar), 135.8 (Ar), 135.0 (Ar), 129.8 (Ar), 128.8 (Ar), 128.6 (Ar), 18.2(2) (Ar), 128.1(6) (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 100.9 (C-1), 77.0 (C-4), 74.9 (Ar*C*H$_2$), 73.8 (Ar*C*H$_2$), 72.1 (C-3), 71.2 (C-2), 70.9 (C-5), 69.1 (C-6), 55.2 (O*C*H$_3$). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{21}H_{26}NaO_6$: 397.1622. Found 397.1625.



Methyl 2-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (4-47)

To a solution of **4-46** (1.6 g, 4.27 mmol) in toluene (40 mL), *n*-Bu_2SnO (1.17 g, 4.70 mmol) was added and the reaction mixture was heated at 120 ºC for 1 h. The solution was then cooled to 62 ºC, *n*-Bu_4NI (1.9 g, 5.19 mmol) and BnBr (0.62 mL, 5.14 mmol) were added and the reaction mixture was stirred overnight at 62 ºC. The reaction mixture was then concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. This syrup was dissolved in pyridine (10 mL) and Ac_2O (2 mL) was added. The reaction mixture was stirred for 1 h at rt before the addition of water (30 mL) and CH_2Cl_2 (50 mL). The organic layer was separated, washed with water (2 x 50 mL), 1M HCl soln (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated. The resulting crude product was purified by chromatography (6:1 hexane–EtOAc) to give **4-47** (1.81 g, 84%) as a colorless oil: R_f 0.52 (6:1 hexane–EtOAc); $[\alpha]_D$ +24.3 (*c* 1.3,

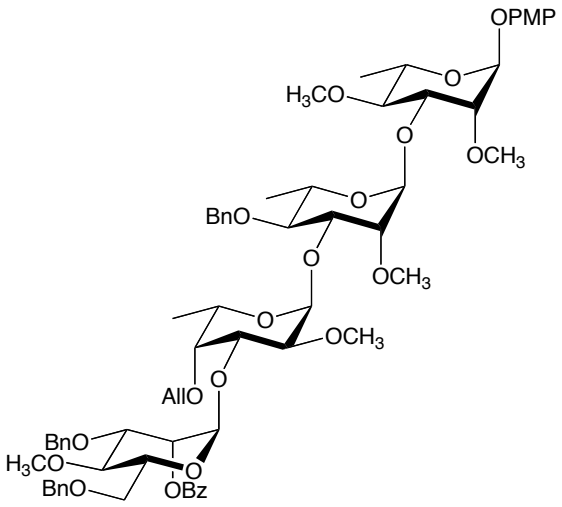
$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.40–7.28 (m, 13H, Ar), 7.21–7.17 (m, 2H, Ar), 5.39 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.89, 4.51 (ABq, 2H, J = 10.5 Hz, ArCH_2), 4.76 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.74, 4.55 (ABq, 2H, J = 10.5 Hz, ArCH_2), 4.72, 4.57 (ABq, 2H, J = 10.5 Hz, ArCH_2), 3.99 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 3.90 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.88–3.73 (m, 3H, H-5, H-6 x 2), 3.38 (s, 3H, OCH_3), 2.17 (s, 3H, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.7 (*C*=O), 138.7 (Ar), 138.5 (Ar), 138.2 (Ar), 128.6 (Ar x 2), 128.6 (Ar x 2), 128.3 (Ar x 2), 128.1 (Ar x 2), 128.0(0) (Ar x 2), 127.9(6) (Ar), 127.8(2) (Ar x 2), 127.8(1) (Ar x 2), 99.0 (C-1), 78.4 (C-5), 75.4 (Ar*C*H_2), 74.6 (C-4), 73.7 (Ar*C*H_2), 72.0 (C-6), 71.5 (C-3), 69.17 (Ar*C*H_2), 68.9 (C-2), 55.2 (O*C*H_3), 21.4 (*C*H_3CO). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{30}H_{34}NaO_7$: 529.2197. Found 529.2189.



***p*-Methoxyphenyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4-*O*-allyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-48)**

To a solution of donor **4-42** (360 mg, 0.55 mmol) and acceptor **4-38** (240 mg, 0.32 mmol) in CH_2Cl_2 (30 mL), crushed 4 Å molecular sieves (300 mg) were added. After the reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC, NIS (103.5 mg, 0.46 mmol) and AgOTf (30.8 mg, 0.12 mmol) were added. The reaction mixture was stirred for additional 30 min at –20 ºC before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **4-48** (259 mg, 63%) as a colorless oil: R_f 0.55 (2:1 hexane–EtOAc); $[\alpha]_D$ +0.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–8.06 (m, 2H, Ar), 7.57–7.53 (m, 1H, Ar), 7.50–7.711 (m, 22H, Ar), 7.00–6.97 (m, 2H, Ar), 6.84–6.81 (m, 2H, Ar), 5.93–5.86 (m, 1H, =C*H*), 5.73–5.72 (m, 1H, H-2‴), 5.39 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.36 (d, 1H, $J_{1',2'}$

= 1.8 Hz, H-1′), 5.27–5.23 (m, 1H, CH_2=), 5.18 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1″), 5.16–5.13 (m, 3H, H-1‴, =CH_2, ArCH_2), 4.92, 4.52 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.80, 4.55 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.70, 4.53 (ABq, 2H, J = 10.8 Hz, ArCH_2), 4.59 (d, 1H, J = 11.3 Hz, ArCH_2), 4.34–4.30 (m, 1H, CH_2O), 4.25 (dd, 1H, $J_{2'',3''}$ = 9.8 Hz, $J_{3'',4''}$ = 2.8 Hz, H-3″), 4.18 (dq, 1H, $J_{4'',5''}$ = 2.7 Hz, $J_{5'',6''}$ = 6.4 H, H-5″), 4.12–4.03 (m, 4H, CH_2O, H-6‴ x 2, H-5′), 4.01–3.92 (m, 3H, H-5‴, H-3, H-5), 3.87 (dd, 1H, $J_{2',3'}$ = 3.3 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.81–3.77 (m, 4H, OCH_3, H-2), 3.74–3.67 (m, 4H, H-2′, H-2″, H-3‴, H-4″), 3.57–3.54 (m, 4H, H-4‴, OCH_3), 3.50–3.46 (m, 4H, H-4′, OCH_3), 3.44 (s, 3H, OCH_3), 3.32 (s, 3H, OCH_3), 3.22 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 1.33 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′), 1.27 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6), 1.20 (d, 3H, $J_{5'',6''}$ = 6.4 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.5 (*C*=O), 154.9 (Ar), 150.5 (Ar), 139.1 (Ar), 138.6 (Ar), 138.6 (Ar), 137.9 (Ar), 135.2 (=*C*H), 133.0(2) (Ar), 130.0(3) (Ar), 130.0 (Ar), 129.1 (Ar), 128.4 (Ar x 2), 128.4 (Ar x 2), 128.3 (Ar x 2), 128.3 (Ar x 2), 128.1 (Ar x 2), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4(3) (Ar), 127.3(8) (Ar), 127.1 (Ar), 126.3 (Ar), 117.5 (Ar), 117.4 (*C*H_2=), 114.6 (Ar x 2), 99.6 (C-1‴), 99.3 (C-1″), 98.5 (C-1′), 95.6 (C-1), 82.0 (C-4), 81.4 (C-4′), 80.6 (C-4″), 80.0 (C-4‴), 79.4 (C-3‴), 79.3 (C-2″), 79.3 (C-2′), 78.9 (C-2), 77.8 (C-5‴), 75.9 (C-5), 75.1 (Ar*C*H_2), 75.1 (Ar*C*H_2), 74.3(9) (C-5′), 74.3(6) (Ar*C*H_2), 73.5 (Ar*C*H_2), 72.6 (C-3″), 71.4 (*C*H_2O), 69.4 (C-6‴), 68.9 (C-3), 68.7(3) (C-3′), 68.6(7) (C-5″), 66.9 (C-2‴), 61.2 (O*C*H_3), 58.9 (O*C*H_3), 38.6 (O*C*H_3), 57.8 (O*C*H_3), 55.7 (O*C*H_3), 18.2(1) (C-6′), 18.1(7) (C-6), 16.8 (C-6″). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{73}H_{88}NaO_{20}$: 1307.5761. Found 1307.5746.



4-49

***p*-Methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranosyl-(1→3)-4-*O*-allyl-2-*O*-methyl-α-L-fucopyranosyl-(1→3)-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (4-49)**

To a solution of donor **4-41** (158 mg, 0.27 mmol) and acceptor **4-38** (120 mg, 0.16 mmol) in CH_2Cl_2 (30 mL), crushed 4 Å molecular sieves (200 mg) were added. After the mixture was stirred at rt for 30 min, it was cooled to –20 ºC, NIS (52 mg, 0.23 mmol) and AgOTf (15.4 mg, 0.06 mmol) were added and the reaction mixture was stirred for additional 30 min at –20 ºC before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **4-49** (122 mg, 63%) a colorless oil: R_f 0.49 (4:1 hexane–EtOAc); $[\alpha]_D$ –23.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.11–8.03 (m, 3H, Ar), 7.59–7.10 (m, 17H, Ar), 6.99–6.96 (m, 2H, Ar), 6.83–6.80 (m, 2H, Ar), 5.93–5.85 (m, 1H, =C*H*), 5.67–5.66 (m, 1H, H-2‴), 5.38 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.32 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 5.27–5.23 (m,

1H, =CH_2), 5.16–5.14 (m, 3H, H-1″, H-1‴, =CH_2), 5.11, 4.66 (ABq, 2H, J = 11.8 Hz, ArCH_2), 4.78, 4.50 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.73, 4.53 (ABq, 2H, J = 11.75 Hz, ArCH_2), 4.68–4.61 (m, 1H, H-5″), 4.59 (dd, 1H, $J_{2'',3''}$ = 9.7 Hz, $J_{3'',4''}$ = 2.7 Hz, H-3″), 4.33–4.29 (m, 1H, CH_2O), 4.23–4.18 (m, 2H, H-5′, CH_2O), 4.09–4.04 (m, 2H, H-6‴ x 2), 3.99–3.94 (m, 2H, H-3, H-5), 3.88–3.84 (m, 2H, H-3′, H-5‴), 3.76 (s, 3H, OCH_3), 3.73–3.67 (m, 4H, H-2, H-2′, H-2″, H-3‴), 3.54 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.43 (s, 3H, OCH_3), 3.37–3.32 (m, 2H, H-4′, H-4″), 3.30 (s, 3H, OCH_3), 3.24–3.16 (m, 2H, H-4, H-4‴), 1.31 (d, 3H, $J_{6',5'}$ = 6.2 Hz, H-6′), 1.26 (d, 3H, $J_{6,5}$ = 6.2 Hz, H-6), 1.19 (d, 3H, $J_{6'',5''}$ = 6.5 Hz, H-6″); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 165.5 (*C*=O), 154.9 (Ar), 150.5 (Ar), 139.1 (Ar), 138.6 (Ar), 138.0 (Ar), 135.1 (=*C*H), 133.3 (Ar), 130.0 (Ar), 129.9 (Ar), 128.4 (Ar x 2), 128.3 (Ar), 128.2(9) (Ar), 128.2(6) (Ar), 128.1 (Ar x 2), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.4 (Ar x 2), 127.4 (Ar x 2), 127.3 (Ar x 2), 127.1 (Ar), 126.3 (Ar), 117.4(9) (Ar x 2), 117.4(8) (=*C*H$_2$), 114.6 (Ar x 2), 99.7 (C-1‴), 99.3 (C-1″), 98.5 (C-1′), 95.6 (C-1), 82.0 (C-4‴), 80.7 (C-4), 80.2 (C-4′), 80.0 (C-4″), 79.8 (C-2″), 79.5 (C-2′), 79.4 (C-2) , 78.9 (C-3‴), 77.6 (C-5‴), 76.3 (C-5), 75.9 (C-5′), 74.4 (Ar*C*H$_2$), 74.1 (C-3″), 73.9 (Ar*C*H$_2$), 73.5 (Ar*C*H$_2$), 71.7 (C-3), 71.4 (*C*H$_2$O), 71.1 (C-3′), 69.5 (C-6‴), 68.9 (C-5″), 68.7 (C-2‴), 61.2 (O*C*H$_3$), 58.9 (O*C*H$_3$), 58.6 (O*C*H$_3$), 57.7 (O*C*H$_3$), 57.4 (O*C*H$_3$), 55.7 (O*C*H$_3$), 18.1(9) (C-6′), 18.1(6) (C-6), 16.7 (C-6″). HRMS (ESI) Calcd. for (M + Na)$^+$ $C_{67}H_{84}NaO_{20}$: 1231.5448. Found 1231.5437.

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Chapter 5

Synthesis of Glycolipid Analogs of Phenolic Glycolipids from *Mycobacterium leprae* and *Mycobacterium kansasii*

5.1. Introduction

After completing the successful synthesis of a panel of all carbohydrate cores of PGLs bearing a *p*-methoxyphenyl moiety at the reducing end, ELISA assays were used to investigate the effect of these molecules on cytokine production by activated macrophages (Chapter 6). Encouraged by the biological testing results of these molecules, which revealed that compounds **2-1** and **4-9** (Figure 5-1) showed the highest activity as immunosuppressant, we envisioned expanding the scope of the project by studying the effect of adding a lipid core to these molecules.

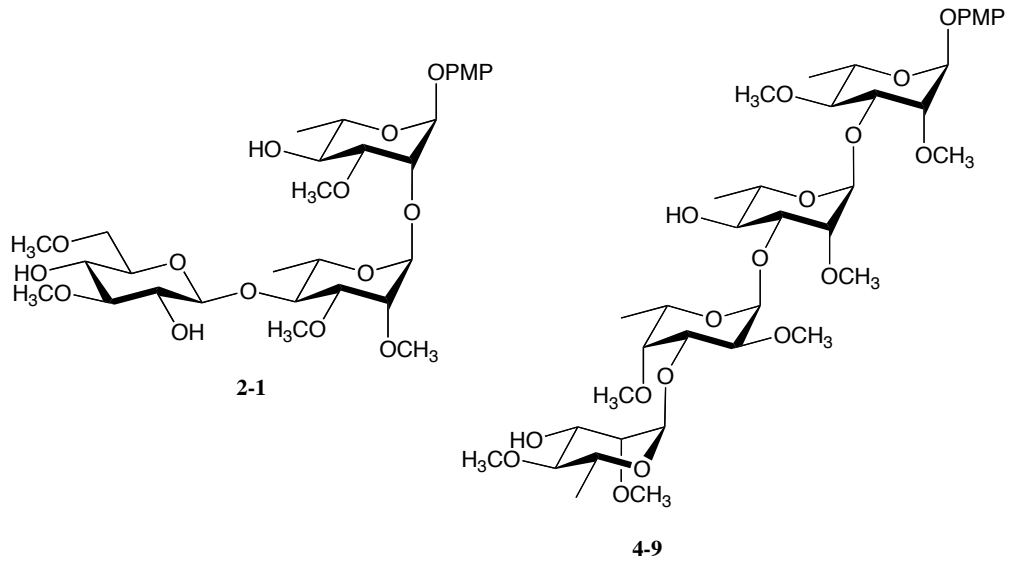


Figure 5-1. Structures of compounds **2-1** and **4-9**

However, the native lipid core is very complicated and its synthesis would be a substantial undertaking[1]. We postulated that the structure of the complex lipid could be replaced with a simpler group. Therefore, we chose to prepare glycolipids having the same carbohydrate cores as **2-1** and **4-9**, but in which the *p*-methoxyphenyl moiety at the reducing end, was replaced with a *p*-nonadecylphenyl group, a phenolic ring substituted at the para position with a C_{19} alkyl chain, compound **5-1** and **5-2** (Figure 5-2).

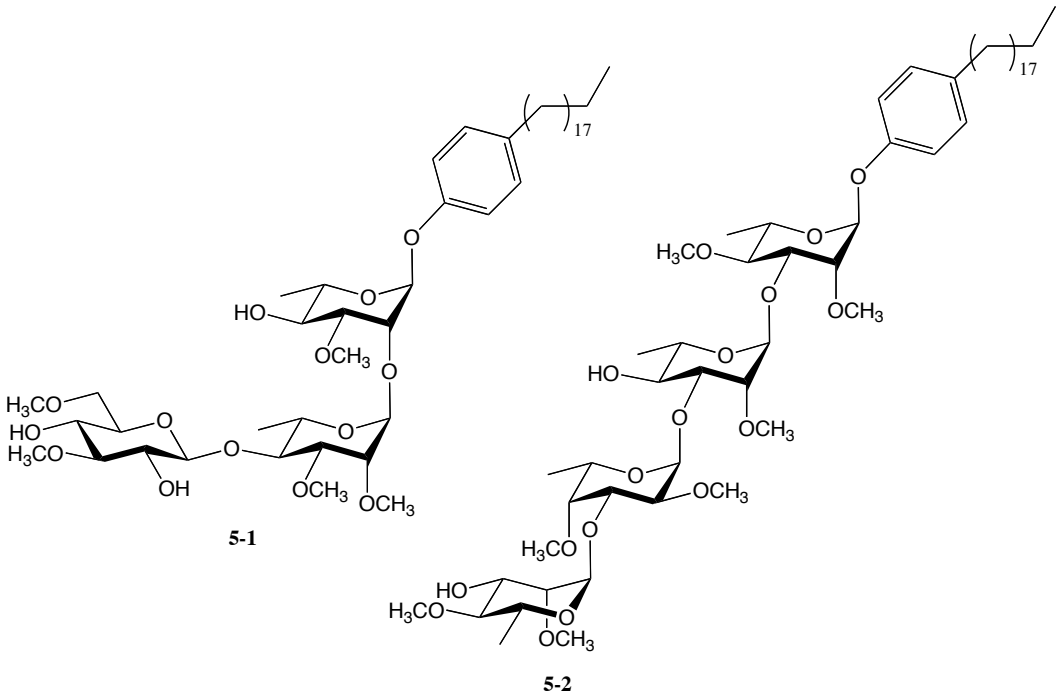


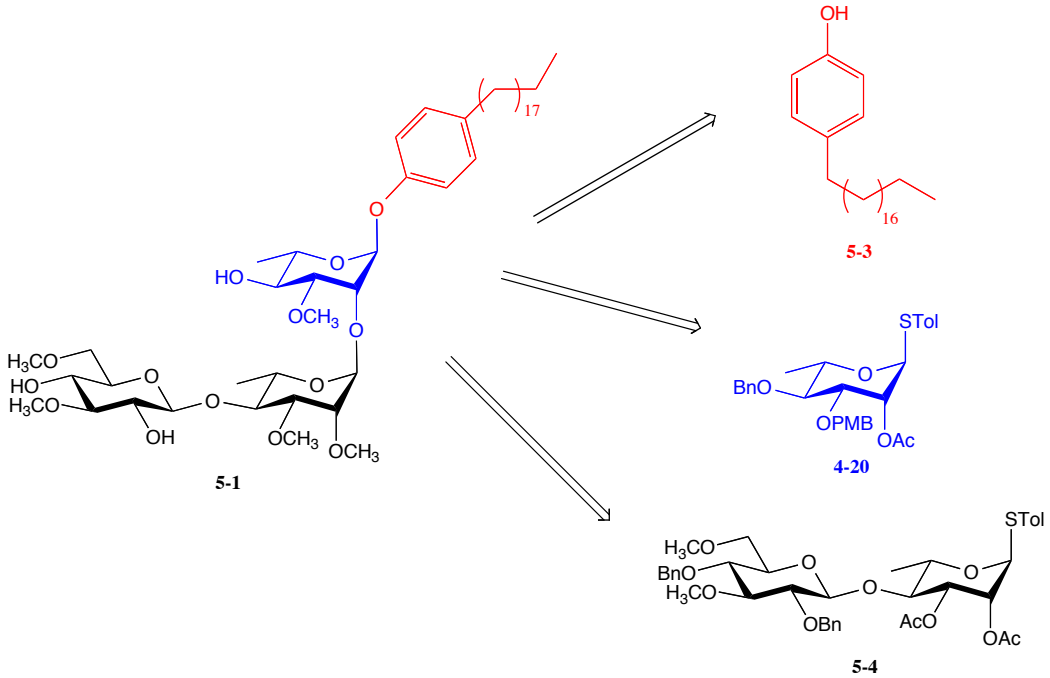
Figure 5-2. Structures of glycolipids **5-1** and **5-2**.

Once the synthesis of these two analogs was completed, their immunomodulatory properties were tested using the same assay used for the carbohydrate core analogs, the synthesis of which was described in Chapters 2–4. The results of their biological testing will be presented in Chapter 6.

5.2. Results and Discussion

5.2.1. Retrosynthetic analysis of glycolipid 5-1

As illustrated in Scheme 5-1, glycolipid **5-1** could be obtained from three main building blocks: **5-3**, **5-4**, and **4-20**. Building block **5-3** is a para-substituted phenol with an alkyl chain that is 19 carbons long. Building block **5-4** is a disaccharide composed of a fully functionalized glucopyranose that is β-(1→4) linked to a diacetylated rhamnose thioglycoside. Finally, building block **4-20** is a fully protected rhamnose thioglycoside that was prepared as described in Chapter 4 (see Scheme 4-4 for its preparation).



Scheme 5-1. Retrosynthetic analysis of glycolipid **5-1**.

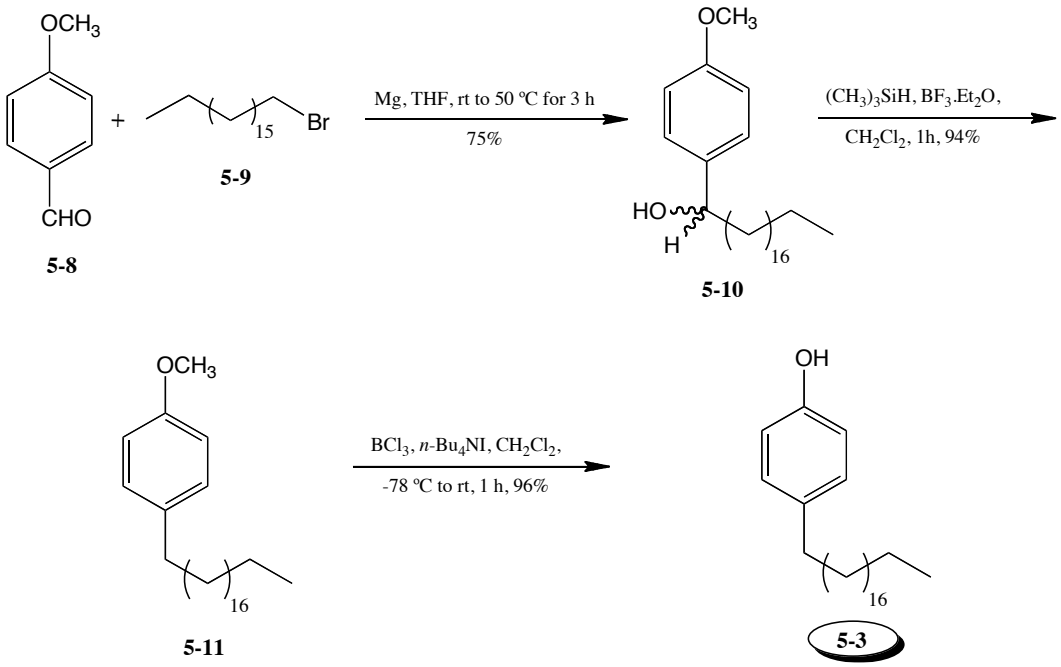
5.2.2. Synthesis of compound 5-1

The synthesis of glycolipid **5-1** started by making building blocks **5-3** and **5-4** and then through coupling reactions and further protecting group manipulations.

5.2.2.1. Synthesis of 5-3

Scheme 5-2 describes the synthesis of target **5-3**, which started with making the corresponding Grignard[1] reagent of stearyl bromide (**5-9**). This was achieved by the slow addition of a solution of **5-9** in THF to a mechanically stirred solution of activated magnesium. In order to start the reaction, it was necessary to warm it; however, after it began, it continued without the need for external heating. After the addition of **5-9** was complete, the solution was heated at reflux for an additional hour to ensure completion. The formed Grignard reagent was then reacted with *p*-methoxybenzaldehyde (**5-8**) affording the corresponding alcohol, **5-10**, in 75% yield over two steps.

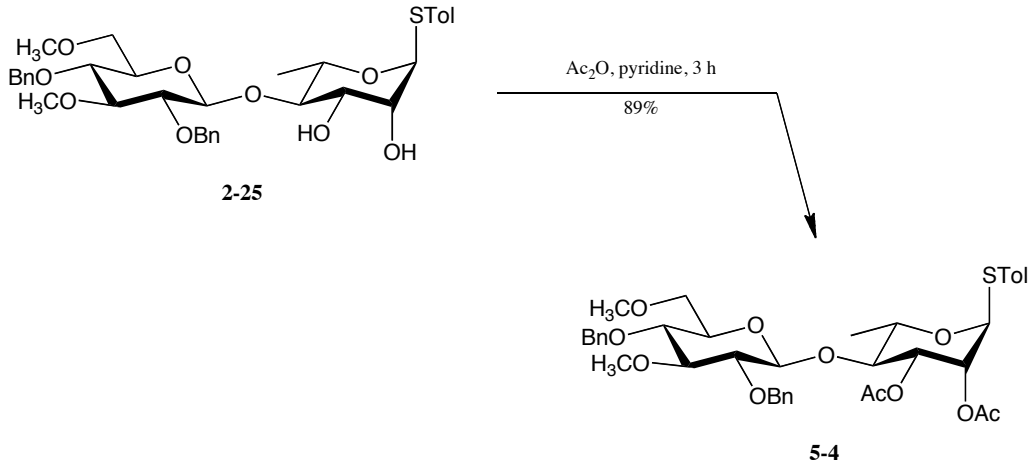
The next step was the deoxygenation of alcohol **5-10**, which was accomplished upon treatment with trimethylsilane ($(CH_3)_3SiH$) and $BF_3 \cdot Et_2O$ to furnish **5-11** in 95% yield.[3] Other methods were explored for the deoxygenation, such as the Barton–McCombie reaction,[4] and in situ prepared $(CH_3)_3SiI$.[5] However, only the use of $(CH_3)_3SiH$ and $BF_3 \cdot Et_2O$ was successful.[6,7] Finally, the target **5-3** was obtained in 95% yield after demethylation using boron triiodide (BI_3).[8] This reagent was prepared in situ from the reaction of BCl_3 and *n*-Bu_4NI after the solution was cooled to –78 ºC and then the reaction was allowed to continue for further 1 h at room temprature.



Scheme 5-2. Synthesis of building block **5-3**.

5.2.2.2. Synthesis of building block 5-4

As illustrated in Scheme 5-3, intermediate **2-25** (see Scheme 2-6 for the synthesis of this compound) afforded target **5-4** in 89% yield upon acetylation using acetic anhydride and pyridine (Scheme 5-3).

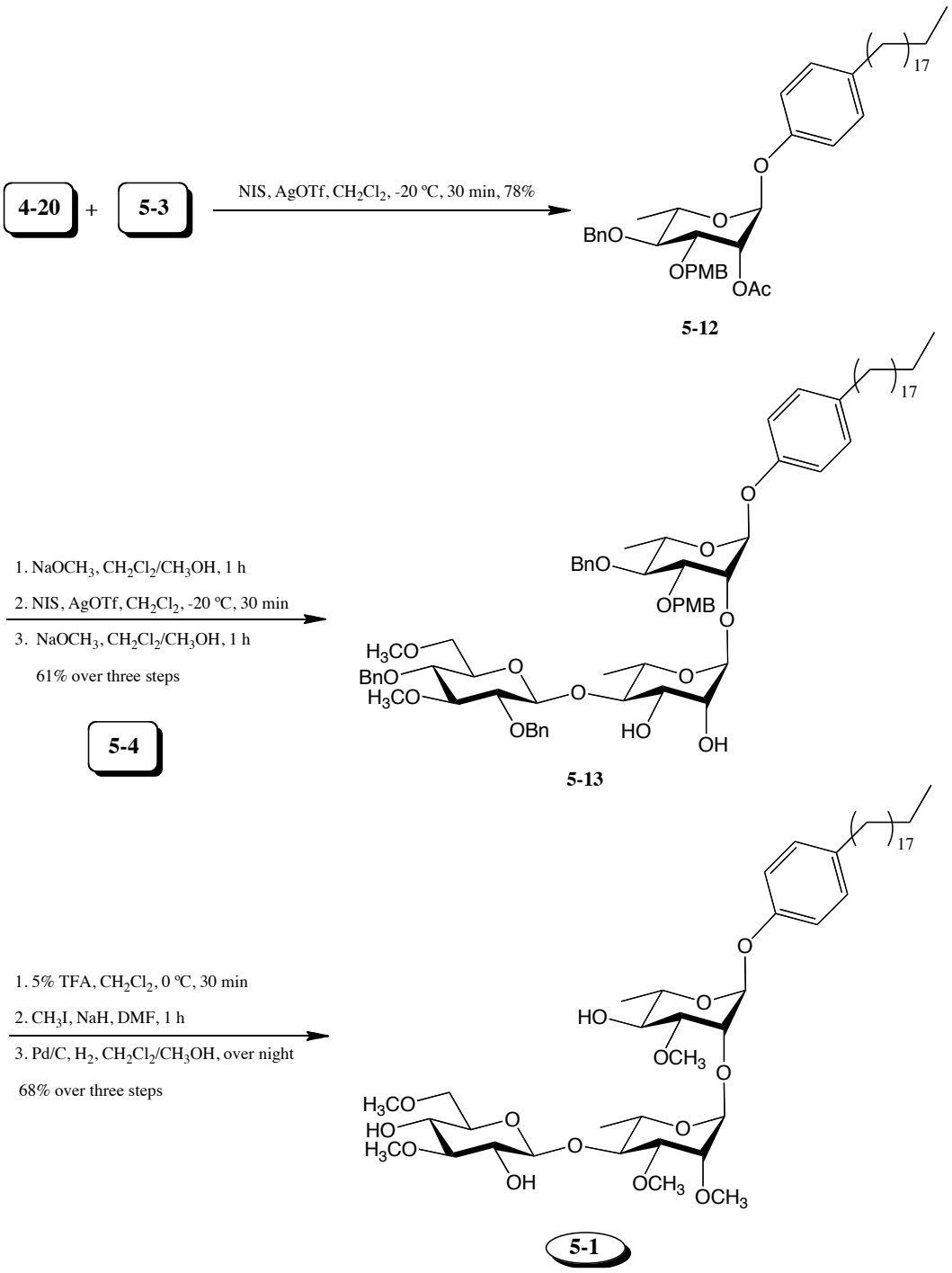


Scheme 5-3. Synthesis of building block **5-4**.

5.2.2.2. Assembly of glycolipid 5-1

The assembly of target **5-1** from the three building blocks is illustrated in Scheme 5-4. After successfully synthesizing donor **4-20** and acceptor **5-3**, glycoside **5-12** was obtained in 78% after NIS–AgOTf-promoted[9] coupling. Subsequently, Zemplén deacylation of **5-12** afforded the expected disaccharide. This alcohol was then coupled with donor **5-4**, again using NIS–AgOTf activation, to give a product that was deacylated to afford trisaccharide alcohol **5-13** in 61% yield over three steps. Treatment of **5-13** with 5% TFA provided the corresponding triol as a result of the selective removal of the *p*-methoxybenzyl group at O-3.[10] All the three hydroxyl groups of the triol were then methylated. Finally, hydrogenolysis over palladium on charcoal afforded 68% yield of target **5-1** over three steps (Scheme 5-4).

In the glycosylation reactions described above, leading to products **5-12** and **5-13**, the α-stereochemistry of the glycosidic linkages was confirmed by the one-bond $^{1}J_{\text{C-1,H-1}}$ heteronuclear coupling constants for the anomeric carbon atoms of the newly formed glycosidic linkages.[11] The magnitude of these coupling constants was 169 and 171 Hz, for **5-12** and **5-13**, respectively, clearly indicating the α-stereochemistry.

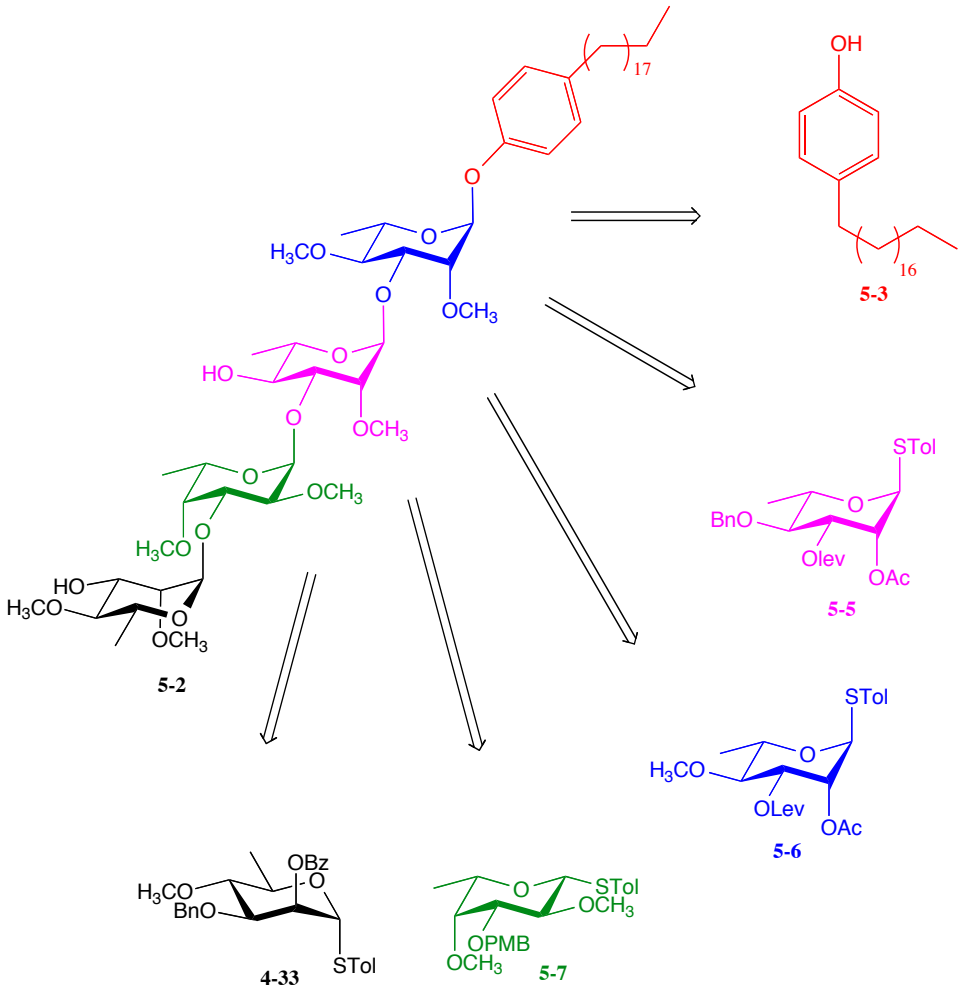


Scheme 5-4. Assembly of compound **5-1**.

5.2.3. Synthesis of compound 5-2

5.2.3.1. Retrosynthetic analysis of compound 5-2

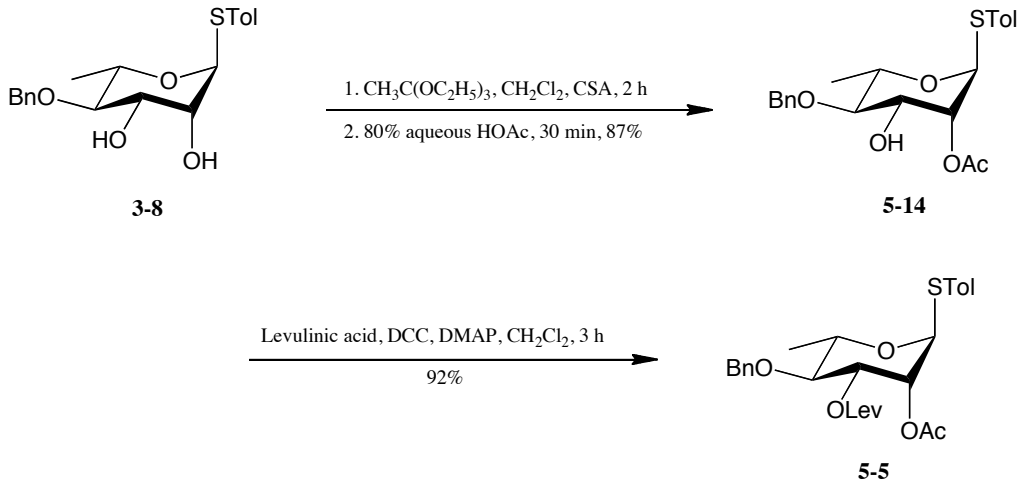
Retrosynthetic analysis of compound **5-2** suggested that it could be obtained from the five building blocks shown in Scheme 5-5. Building blocks **4-33** and **5-3** had been previously synthesized in Chapter 4 (Scheme 4-10) and Chapter 5 (Scheme 5-2), respectively. Building blocks **5-5** and **5-6** are fully functionalized rhamnose thioglycoside derivatives while building block **5-7** is a fully protected fucose thioglycoside derivative.



Scheme 5-5. Retrosynthetic analysis of glycolipid **5-2**.

5.2.3.2. Synthesis of building block 5-5

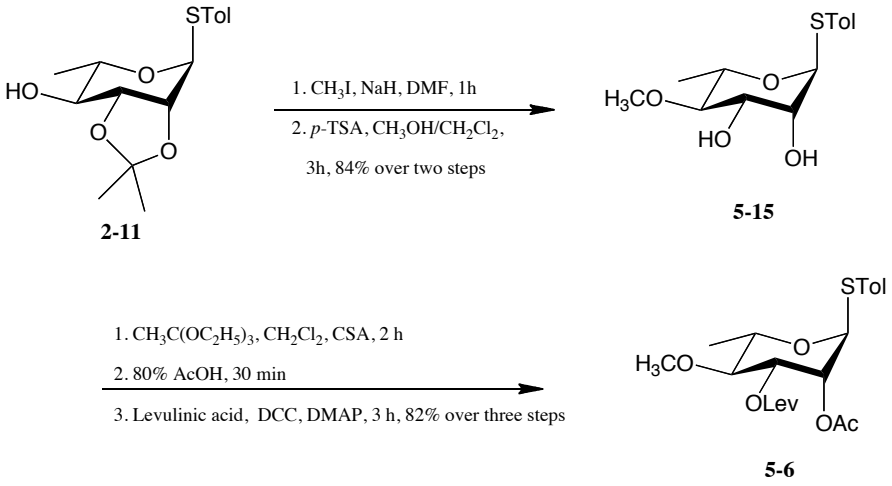
As illustrated in Scheme 5-6, the synthesis of **5-5** started from **3-8**, which was prepared as described in Chapter 3 (Scheme 3-3). To access compound **5-5**, O-2 was selectively acetylated via reaction with triethyl orthoacetate[12] in presence of a catalytic amount of CSA to form an orthoester intermediate, which, upon acid-catalyzed hydrolysis, afforded 87% yield the O-2 acetylated product, **5-14**, as a single regioisomer. The location of the acetyl group was confirmed by the strong downfield chemical shift of H-2 (δ_H = 5.39–5.37 ppm, overlapped with H-1). The remaining hydroxyl group at O-3 was subsequently protected as levulinoyl ester upon treatment with levulinic acid[13] and DCC, in the presence of DMAP to afford **5-5** in 92% yield.



Scheme 5-6. Synthesis of building block **5-5**.

5.2.3.3. Synthesis of building block 5-6

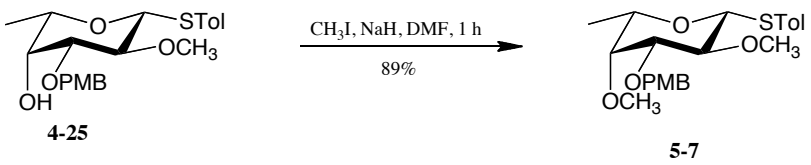
The synthesis of **5-6** is shown in Scheme 5-7, starting from **2-11** (see Scheme 2-4 for its preparation). First, methylation of O-4 using methyl iodide and sodium hydride followed by acid-catalyzed hydrolysis of the isopropylidene acetal afforded **5-15** in 84% yield. Having developed a route to **5-15** a similar series of reactions as those described for the preparation of **5-5**, afforded an 82% yield of **5-6** over the three-step sequence.



Scheme 5-7. Synthesis of building block **5-6**

5.2.3.4. Synthesis of building block 5-7

As illustrated in Scheme 5-8, to access building block **5-7** we used a previously prepared advanced intermediate **4-25**, which was synthesized as described in Chapter 4 (Scheme 4-5). Compound **5-7** was obtained in 89% yield after reaction of sodium hydride and methyl iodide.



4-25 **5-7**

Scheme 5-8. Synthesis of building block **5-7**.

5.2.3.4. Assembly of glycolipid 5-2

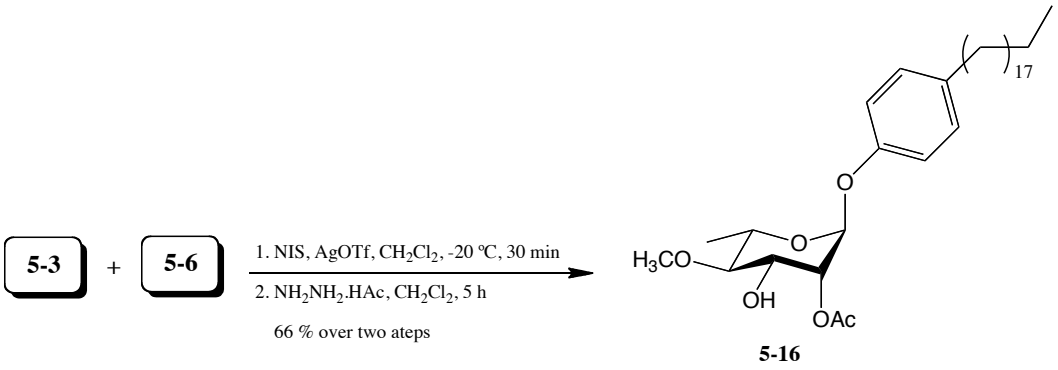
With all five building blocks in hand, coupling and deprotection reactions were successfully carried out to complete the synthesis of compound **5-2** as shown in Scheme 5-9. First, thioglycoside **5-6** was coupled with alcohol **5-3** using NIS–AgOTf[9] activation. Subsequent selective removal of the levulinoyl group was achieved by treatment with hydrazine acetate[11] providing alcohol **5-16** in 66% yield.

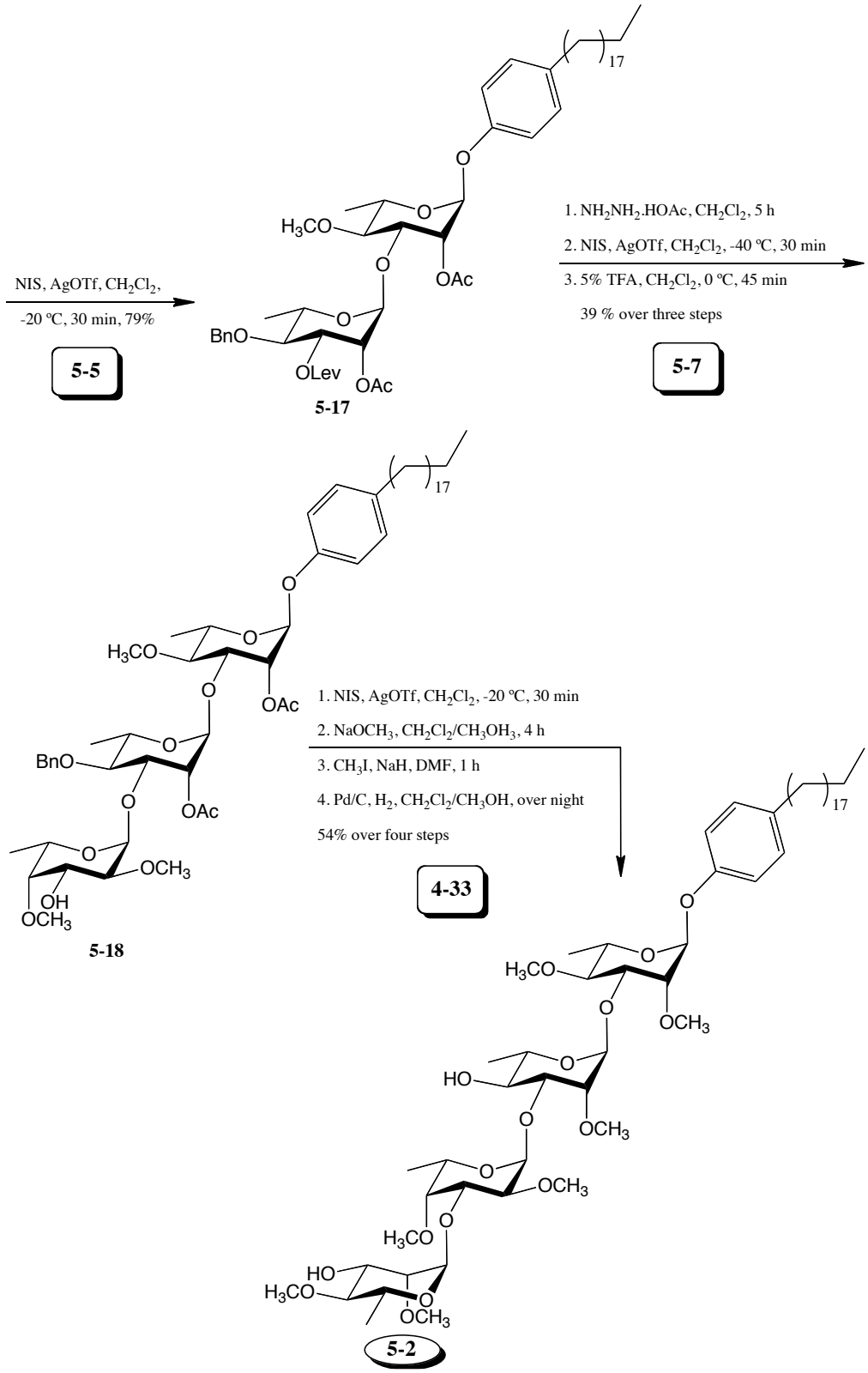
Disaccharide **5-17** was then obtained in 79% yield after another NIS–AgOTf-promoted coupling of donor **5-5** and acceptor **5-15**. Subsequently, the selective removal of levulinoyl group at O-3′ provided an alcohol acceptor. This acceptor was then directly fucosylated with donor **5-7** using the inverse glycosylation conditions[14] previously described for the preparation of compound **3-18** (Scheme 3-5). This glycosylation yielded trisaccharide alcohol **5-18** in 39% yield over three steps after removal of *p*-methoxybenzyl group using 5% trifluoroacetic acid (TFA).[10]

Finally, a tetrasaccharide intermediate was obtained after coupling of donor **4-33** and acceptor **5-18**. After the glycosylation, the benzoyl group was removed using sodium methoxide and then the resulting alcohol was methylated.

The target tetrasaccharide **5-2** was obtained in 54% yield over four steps after final hydrogenolysis over palladium on charcoal to remove all benzyl groups (Scheme 5-9).

All new glycosidic linkages produced in this synthetic route were α, which were obtained through neighbouring group participation except for compound **5-18**, which was due to the inverse glycosylation method.[14] That the α-stereochemistry of the new glycosidic linkages in all coupling reaction described above had been obtained, coupled HSQC experiments were performed and $^{1}J_{H\text{-}1,C\text{-}1}$ was calculated. For all products, this value was between 170 and 171 Hz, which clearly indicated the α-stereochemistry.





Scheme 5-9. Assembly of Target **5-2**

5.3. Summary

In this chapter, we completed the synthesis of two synthetic glycolipid analogs, **5-1** and **5-2**, through efficient linear synthetic routes in good overall yield. All glycosidic linkages were obtained with the α-stereochemistry either due to the neighbouring group participation from the acyl group at O-2 in the donor, or due to the use of an inverse glycosylation procedure. With these compounds in hand, the next step was to investigate their effect on cytokine production by activated macrophages and then compare their immunological profiles with the synthetic analogs that have a *p*-methoxyphenyl group at the reducing end, as well as native PGL-I. This comparative study will allow us to investigate the effect of the lipid core on the immunological properties of these compounds. The biological testing will be presented in Chapter 6.

5.4. Experimental

5.4.1. General Methods

Solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere before use. All reagents used in reactions were purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV_{254} (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by charring with a solution of anisaldehyde in ethanol, acetic acid, and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). Organic solutions were concentrated under vacuum at < 50 °C. ^1H NMR and spectra were recorded at 400 or 500 MHz. ^1H NMR chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 125.7 MHz and ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.23, $CDCl_3$). ^1H NMR data are reported as though they are first order and the peak assignments were made on the basis of 2D-NMR (^1H–^1H COSY and HMQC) experiments. The monosaccharide residues in the disaccharide and trisaccharides are labelled by no prime, prime, and double prime as shown in Figure 5-3 and these labels are maintained in the assignment of NMR spectra of all compounds. Optical rotations were measured at 21 ± 2 °C at the sodium D line (589 nm) and are in units of deg•mL(dm•g)$^{-1}$. ESI-MS spectra were carried out on samples suspended in CH_2Cl_2 and added NaCl.

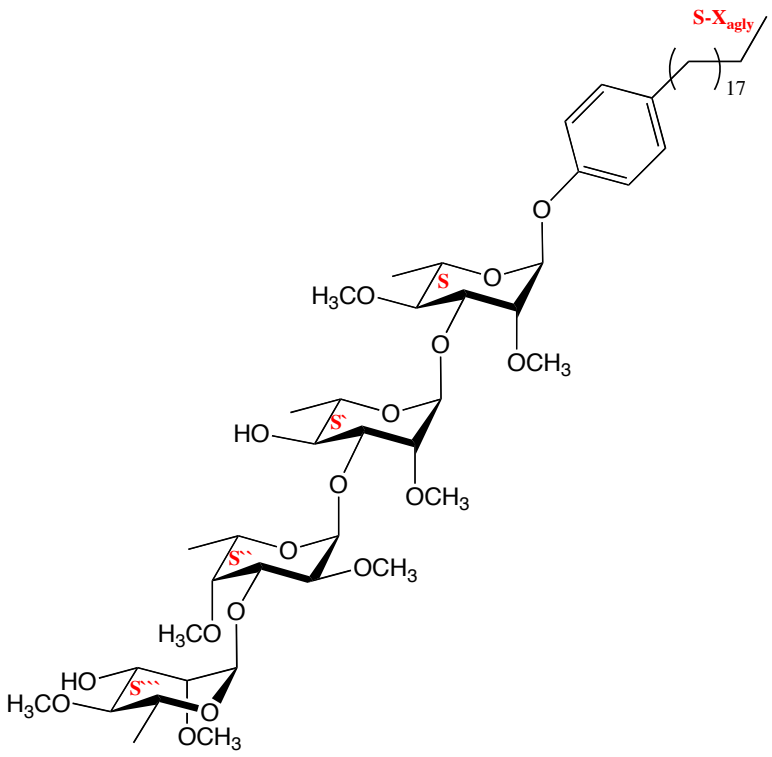
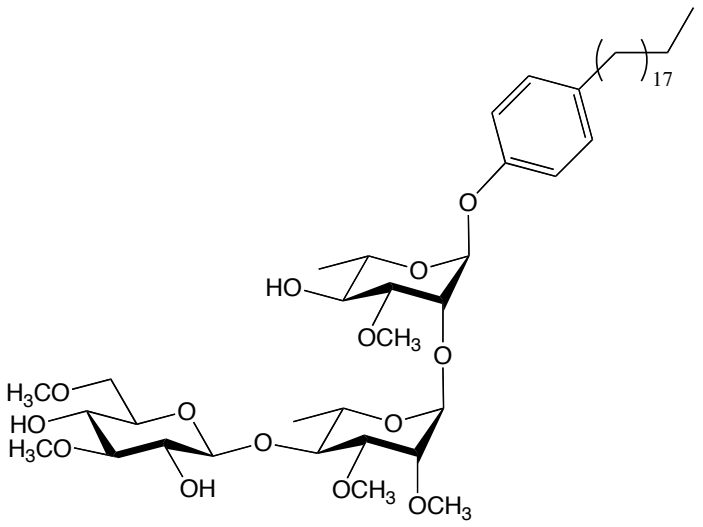


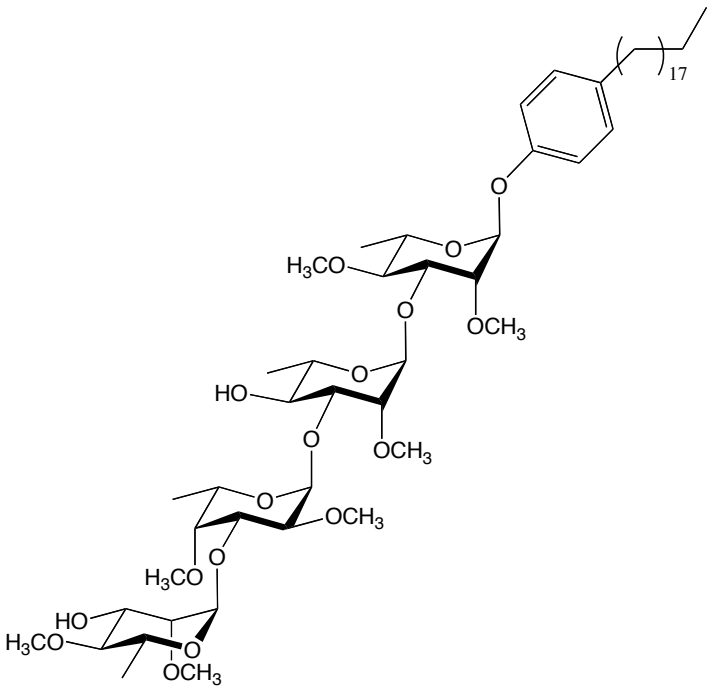
Figure 5-3. Numbering system used to label data.



***p*-Nonadecylphenyl 3,6-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-methyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-methyl-α-L-rhamnopyranoside (5-1)**

To a solution of **5-13** (25 mg, 0.02 mmol) in CH_2Cl_2 (10 mL) at 0 ºC, TFA (0.5 mL, 5% v/v) was added dropwise over 2 min. The reaction mixture was stirred for additional 30 min before Et_3N (1 mL) was added. The solution was concentrated and the resulting residue was dissolved in DMF (2 mL) and then CH_3I (0.1 mL) and NaH (60% in mineral oil, 5 mg) were added. After stirring for 1 h at rt, the reaction mixture was diluted with water (7 mL), and CH_2Cl_2 (10 mL). The organic layer was then washed with water (2 x 8 mL) and brine (8 mL) and then dried ($NaSO_4$), filtered, concentrated and the resulting oil was purified by chromatography (2:1 hexane–EtOAc) to give an oil. To the solution of the oil in 1:1 CH_3OH–CH_2Cl_2 (20 mL) was added Pd–C (5 mg, 20 % w/w) and the reaction mixture was stirred overnight under an atmosphere of hydrogen. The reaction

mixture was then filtered, concentrated and the resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **5-1** (12 mg, 68%) as a thick syrup: R_f 0.45 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –67.5 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.10–7.07 (m, 2H, Ar-2,6), 6.94–6.92 (m, 2H, Ar-3,5), 5.42 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 5.08 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.40 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1″), 4.21 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 3.84 (app t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.1 Hz, H-4′), 3.76–3.72 (m, 3H, H-2″, H-5, H-5′), 3.67 (s, 3H, OCH_3), 3.65–3.54 (m, 6H, H-3, H-3′, H-4, H-5″, H-6″), 3.52 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.43–3.39 (m, 2H, H-2′, H-3″), 3.37 (s, 3H, OCH_3), 3.15 (app t, 1H, $J_{3'',4''}$ = $J_{4'',5''}$ = 9.0 Hz, H-4″), 2.82 (br s, 1H, OH), 2.53 (t, 2H, $J_{1,2}$ = 7.5 Hz, CH_2-1$_{agly}$), 2.33 (br s, 1H, OH), 1.60–1.54 (m, 2H, CH_2-2$_{agly}$), 1.33–1.24 (m, 38H, H-6 x 3, H-6′ x 3, CH_2 x 16), 0.87 (t, 3H, $J_{18,19}$ = 7.0 Hz, CH_3-19$_{agly}$); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.1 (Ar), 136.9 (Ar), 129.3 (Ar x 2), 116.0 (Ar x 2), 105.6 (C-1″), 98.4 (C-1′), 97.4 (C-1), 85.5 (C-3″), 81.6 (C-4″), 81.4 (C-4), 80.2 (C-3′), 75.8 (C-3), 75.0 (C-4′), 74.1 (C-2′), 72.8 (C-6″), 72.1 (C-5″), 71.8 (C-2), 71.2 (C-2″), 70.0 (C-5), 68.3 (C-5′), 60.5 (O*C*H$_3$), 59.6 (O*C*H$_3$), 59.0 (O*C*H$_3$), 57.6 (O*C*H$_3$), 56.5 (O*C*H$_3$), 35.1 (*C*H$_2$), 31.9 (*C*H$_2$), 31.7 (*C*H$_2$), 29.7 (*C*H$_2$ x 10), 29.7 (*C*H$_2$), 29.6 (*C*H$_2$), 29.5 (*C*H$_2$), 29.4 (*C*H$_2$), 22.7 (*C*H$_2$), 17.8 (C-6′), 17.6 (C-6), 14.1 (C-19$_{agly}$). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{48}H_{84}O_{14}Na$: 907.5759. Found 907.5757.

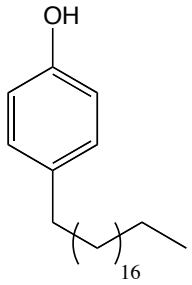


***p*-Nonadecylphenyl 6-deoxy-2,4-di-*O*-methyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-methyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (5-2)**

To a solution of **5-18** (20 mg, 0.02 mmol) and **4-33** (13.4 mg, 0.03 mmol) in CH_2Cl_2 (10 mL) was added crushed 4 Å molecular sieves (50 mg). After stirring for 30 min at rt, the reaction mixture cooled to –20 ºC, and then NIS (4.5 mg, 0.02 mmol) and AgOTf (1.6 mg, 0.006 mmol) were added. The reaction was stirred for additional 30 min before the addition of Et_3N (0.25 mL) concentration. The resulting crude residue was dissolved in 1:1 CH_3OH–CH_2Cl_2 (10 mL), and then 1M $NaOCH_3$ (0.1 mL) was added and the reaction mixture was stirred for 4 h at rt before being neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated. The resulting residue was dissolved in DMF (2 mL), CH_3I (0.2 mL) and NaH (60% in mineral oil, 5 mg) were added and the reaction mixture was

stirred for 1 h at rt before the addition of chilled water (5 mL) and CH_2Cl_2 (10 mL). The organic layer was washed with water (2 x 8 mL) and brine (8 mL), and then separated, dried ($NaSO_4$), filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a syrup. To the solution of the syrup in CH_2Cl_2 (10 mL), Pd–C (4 mg) was added and the reaction mixture was stirred overnight under a hydrogen atmosphere before it was filtered and concentrated. The resulting residue was purified by chromatography (20:1 CH_2Cl_2–CH_3OH) to give **5-2** (12 mg, 59%) as a thick syrup: R_f 0.55 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –48.8 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.11–7.09 (m, 2H, Ar-2,6), 7.00–6.97 (m, 2H, Ar-3,5), 5.47 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.24 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.20 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1″), 5.15 (d, 1H, $J_{1''',2'''}$ = 1.7 Hz, H-1‴), 4.17–4 .14 (m, 2H, H-2‴, H-3′), 4.04–3.97 (m, 2H, H-3, H-5‴), 3.87–3.81 (m, 2H, H-3″, H-5), 3.73–3.69 (m, 3H, H-2′, H-5′, H-5″), 3.66–3.62 (m, 2H, H-2, H-2″), 3.60 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 3.58–3.57 (m, 1H, H-3‴), 3.56 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.53–3.51 (m, 1H, H-4′), 3.50 (s, 3H, OCH_3), 3.47 (s, 3H, OCH_3), 3.46 (s, 3H, OCH_3), 3.29–3.28 (m, 1H, H-4‴), 3.26 (app t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 9.6 Hz, H-4″), 3.00 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.56 (t, 1H, $J_{1,2}$ = 7.8 Hz, CH_2-1_{agly}), 2.42 (br s, 1H, OH), 1.80 (br s, 1H, OH), 1.62–1.56 (m, 2H, CH_2-2_{agly}), 1.40 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6), 1.34 (d, 3H, $J_{5''',6'''}$ = 6.5 Hz, H-6‴), 1.31–1.27 (m, 35H, H-6″, CH_2 x 16), 1.24 (d, 3H, $J_{5',6'}$ = 6.5 Hz, H-6′), 0.89 (t, 3H, $J_{18,19}$ = 7.0 Hz, CH_3-19_{agly}); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 154.5 (Ar), 136.8 (Ar), 129.3 (Ar x 2), 116.1 (Ar x 2), 101.4 (C-1‴), 97.7 (C-1″), 97.6 (C-1′), 95.4 (C-1), 84.0 (C-4), 82.7 (C-4″), 82.2 (C-4‴), 81.3

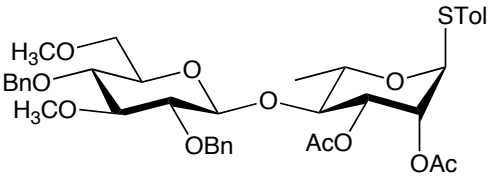
(C-4′), 80.7 (C-2″), 80.5 (C-3‴), 78.8 (C-2), 77.5 (C-5″), 75.7 (C-5′), 72.6 (C-2′), 71.6 (C-5), 71.3 (C-3″), 69.1 (C-5‴), 68.8 (C-3), 67.1 (C-3′), 66.8 (C-2‴), 62.0 (O*C*H$_3$), 61.0 (O*C*H$_3$), 61.1 (O*C*H$_3$), 59.3 (O*C*H$_3$), 58.6 (O*C*H$_3$), 57.3 (O*C*H$_3$), 57.1 (O*C*H$_3$), 35.1 (*C*H$_2$), 32.0 (*C*H$_2$), 31.7 (*C*H$_2$), 29.7 (*C*H$_2$ x 10), 29.6 (*C*H$_2$), 29.6 (*C*H$_2$), 29.4 (*C*H$_2$), 29.3 (*C*H$_2$), 22.7 (*C*H$_2$), 18.1 (C-6‴), 17.8(7) (C-6′), 17.8(4) (C-6), 16.2 (C-6″), 14.1 (C-19$_{agly}$). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{56}H_{98}O_{17}Na$: 1065.6696. Found 1065.6691.



4-Nonadecylphenol (5-3)

To a solution of **5-11** (0.5 g, 1.33 mmol) and *n*-Bu$_4$NI (1.23 g, 3.33 mmol) in CH_2Cl_2 (20 mL) at –78 ºC, BCl_3 (3.3 mL of 1M soln in heptanol, 3.33 mmol) was added dropwise over 5 min and then the reaction mixture was stirred for 30 min. After warming to rt, water (30 mL) was added and the solution was diluted with CH_2Cl_2 (50 mL), and then washed with water (2 x 30 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (7:1 hexane–EtOAc) to afford **5-3** (460 mg, 96%) as an amorphous solid: R_f 0.46 (7:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.06–7.02 (m, 2H, Ar-2,6), 6.76–6.73 (m, 2H, Ar-3,5), 2.53 (t, 2H,

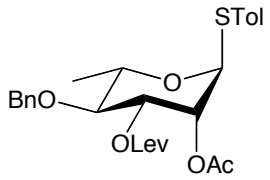
$J_{1',2'}$ = 7.6 Hz, CH_2-1′), 1.61–1.55 (m, 2H, CH_2-2′), 1.32–1.26 (m, 32 H, CH_2 x 16), 0.89 (t, 3H, $J_{18',19'}$ = 7.4 Hz, CH_3-19′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 153.5 (Ar), 135.4 (Ar), 129.6 (Ar x 2), 115.2 (Ar x 2), 35.2 (*C*H_2), 32.3 (*C*H_2), 31.9 (*C*H_2), 29.9 (*C*H_2 x 11), 29.8 (*C*H_2), 29.7 (*C*H_2), 29.5 (*C*H_2), 29.4 (*C*H_2), 14.3 (*C*H_3-19′). HRMS (ESI) Calcd for $(M)^+$ $C_{25}H_{44}O$: 360.3392. Found 360.3390.



***p*-Tolyl 2,4-di-*O*-benzyl-3,6-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (5-4)**

To a solution of **2-25** (250 mg, 0.39 mmol) in pyridine (4 mL) was added acetic anhydride (1 mL) and the reaction mixture was stirred for 1 h before the addition of water (10 mL) and CH_2Cl_2 (20 mL). The organic layer was washed with water (2 × 10 mL), dried (Na_2SO_4), filtered, concentrated and the resulting oil was purified by chromatography (2:1 hexane–EtOAc) to give **5-4** (251 mg, 89%) as a syrup: R_f 0.4 (2:1 hexane–EtOAc); $[\alpha]_D$ –34.4 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.43–7.41 (m, 2H, Ar-2,6), 7.40–7.30 (m, 10H, Ar), 7.17–7.15 (m, 2H, Ar-3,5), 5.51 (dd, 1H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.32 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 5.30 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 4.87, 4.65 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.78, 4.64 (ABq, 2H, J = 11.2 Hz, ArCH_2), 4.52 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.31 (dq, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.82 (app t, $J_{3,4}$ =

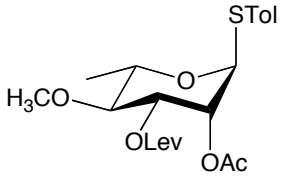
$J_{4,5}$ = 9.7 Hz, H-4), 3.67 (dd, 1H, $J_{5',6a'}$ = 2.4 Hz, $J_{6a',6b'}$ = 10.9 Hz, H-6a′), 3.63 (s, 3H, OCH_3), 3.60 (dd, 1H, $J_{5',6b'}$ = 4.6 Hz, $J_{6a',6b'}$ = 10.9 Hz, H-6b′), 3.50–3.54 (m, 1H, H-5′), 3.41 (s, 3H, OCH_3), 3.41–3.38 (m, 1H, H-4′), 3.32–3.26 (m, 2H, H-2′, H-3′), 2.35 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 1.89 (s, 3H, ArCH_3),1.40 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.0 (*C*=O), 169.9 (*C*=O), 138.5 (Ar), 138.4 (Ar), 1381 (Ar), 132.6 (Ar x 2), 129.9 (Ar x 2), 129.8 (Ar), 128.4(5) (Ar x 2), 128.3(6) (Ar x 2), 128.2 (Ar x 2), 128.0 (Ar x 2), 127.8 (Ar), 127.6 (Ar), 103.7 (C-1′), 86.9 (C-1), 86.0 (C-2), 81.9 (C-3), 77.7 (C-2′), 76.7 (C-4), 75.1 (Ar*C*H_2), 74.8(1) (Ar*C*H_2), 74.7(4) (C-5), 72.0 (C-3′), 71.7 (C-5′), 71.6 (C-6′), 68.8 (C-4′), 61.3 (O*C*H_3), 59.6 (O*C*H_3), 20.7 (Ar*C*H_3), 17.8 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{39}H_{48}O_{11}NaS$: 747.2810. Found 747.2803.



***p*-Tolyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (5-5)**

To a solution of compound **5-14** (0.9 g, 2.23 mmol) in CH_2Cl_2 (20 mL), levulinic acid (0.4 mL, 3.60 mmol), DCC (0.74 g, 3.60 mmol) and DMAP (67 mg, 0.55 mmol) were added and the reaction mixture was stirred for 3 h at rt. The solution was filtered, concentrated, and the resulting residue was purified by chromatography (4:1 hexane–EtOAc) to give **5-5** (1.03 g, 92%) as a colorless oil: R_f 0.42 (4:1 hexane–EtOAc); $[\alpha]_D$ –96.6 (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz,

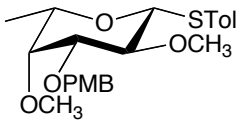
$CDCl_3$, δ_H) 7.39–7.28 (m, 7H, Ar), 7.12 (d, 2H, J = 8.4 Hz, Ar-2,6), 5.51 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.34–5.31 (m, 2H, H-1, H-3), 4.79, 4.68 (ABq, J = 11.2 Hz, ArCH_2), 4.38 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.60 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.80–2.66 (m, 2H, COCH_2), 2.55–2.49 (m, 2H, CH_2CO), 2.34 (s, 3H, ArCH_3), 2.19 (s, 3H, CH_3COCH_2), 2.15 (s, 3H, CH_3CO), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 206.4 (C=O), 172.0 (C=O), 170.2 (C=O), 138.3 (Ar), 138.2 (Ar), 132.7 (Ar x 2), 130.1 (Ar x 2), 130.0 (Ar), 128.7 (Ar x 2), 128.1 (Ar), 128.0 (Ar x 2), 86.3 (C-1), 79.1 (C-4), 77.0 (ArCH_2), 72.6 (C-3), 72.0 (C-5), 69.2 (C-2), 38.1 (CH_2CO), 30.1 (CH_3CO), 28.2 (CH_2COO), 21.4 (CH_3CO), 21.2 (ArCH_3), 18.1 (C-6). HRMS (ESI) Calcd for (M + Na)$^+$ $C_{27}H_{32}O_7NaS$: 523.1761. Found 523.1755.



***p*-Tolyl 2-*O*-acetyl-3-*O*-levulinoyl-4-*O*-methyl-1-thio-α-L-rhamnopyranoside (5-6)**

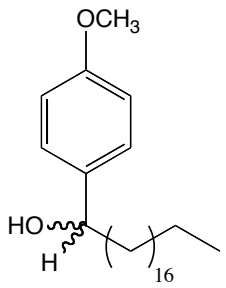
To a solution of **5-15** (0.78 g, 2.77 mmol) and triethyl orthoacetate (1.0 mL, 5.55 mmol) in CH_2Cl_2 (20 mL) was added CSA (128 mg, 0.55 mmol). The reaction mixture was stirred for 2 h at rt before it was concentrated and dissolved in 80% aqueous HOAc. After stirring for additional 30 min at rt. water (10 mL) was added to the solution and it was concentrated. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 20 mL). The organic layer was dried

(Na_2SO_4), filtered, concentrated, and the resulting syrup was carried to the next step without further purification. To a solution of the syrup in CH_2Cl_2 (20 mL) were added levulinic acid (0.4 mL, 3.60 mmol), DCC (0.74g, 3.60 mmol) and DMAP (67 mg, 0.55 mmol) and the reaction mixture was stirred for 3 h at rt. The solution was filtered, concentrated, and the resulting residue was purified by chromatography (4:1 hexane–EtOAc) to give **5-6** (0.96 g, 8 %) as a colorless oil: R_f 0.46 (4:1 hexane–EtOAc); $[\alpha]_D$ –76.4 (c 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δH) 7.35–7.32 (m, 2H, Ar-2,6), 7.12–7.08 (m, 2H, Ar-3,5), 5.46 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 5.29 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.27 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.22 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.53 (s, 3H, OC*H*$_3$), 3.29 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.89–2.49 (m, 4H, C*H*$_2$CO, C*H*$_2$COO), 2.33 (s, 3H, ArC*H*$_3$), 2.21 (s, 3H, C*H*$_3$CO), 2.13 (s, 3H, C*H*$_3$CO), 1.36 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 206.7 (*C*=O), 172.0 (*C*=O), 170.2 (*C*=O), 138.2 (Ar), 132.7 (Ar), 132.7 (Ar x 2), 130.1 (Ar x 2), 86.2 (C-1), 80.6 (C-4), 72.3 (C-3), 71.9 (C-5), 69.2 (C-2), 60.9 (O*C*H$_3$), 38.1 (*C*H$_2$CO), 30.1 (*C*H$_3$CO), 28.2 (*C*H$_2$COO), 21.4 (*C*H$_3$CO), 21.2 (Ar*C*H$_3$), 17.9 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{21}H_{28}O_7NaS$: 447.1448. Found 447.1446.



***p*-Tolyl 2,4-di-*O*-methyl-3-*O*-*p*-methoxybenzyl-1-thio-β-L-fucopyranoside (5-7)**

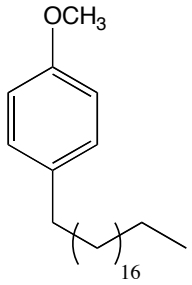
To a solution of **4-25** (0.89 g, 2.21 mmol) and CH_3I (0.21 mL, 3.32 mmol) in DMF (10 mL) at 0 °C, NaH (60% in mineral oil, 110 mg, 4.43 mmol) was added after. The reaction mixture was stirred for 1 h at rt and then diluted with chilled water (30 mL), and CH_2Cl_2 (30 mL). The organic layer was washed with water (2 x 20 mL), brine (20 mL) and then dried ($NaSO_4$), filtered, concentrated and the resulting oil was purified by chromatography (4:1 hexanes–EtOAc) to yield **5-7** (0.82 g, 89%) as a colorless oil: R_f 0.49 (4:1 hexane–EtOAc); $[\alpha]_D$ –36.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.46–7.44 (m, 2H, Ar), 7.31–7.25 (m, 2H, Ar), 7.10–7.05 (m, 2H, Ar), 6.90–6.86 (m, 2H, Ar), 4.68, 4.64 (ABq, 2H, J = 12.6 Hz, ArCH_2), 4.41 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 3.8 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3) , 3.58 (s, 3H, OCH_3), 3.49–3.39 (m, 3H, H-2, H-3, H-5), 3.27–3.26 (m, 1H, H-4), 2.39 (s, 3H, ArCH_3) 1.27 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 137.4 (Ar), 132.2 (Ar), 130.7 (Ar x 2), 130.3 (Ar), 129. (Ar), 129.4 (Ar x 2), 129.3 (Ar x 2), 113.8 (Ar x 2), 88.1 (C-1), 83.6 (C-4), 79.7 (C-3), 79.3 (C-2), 74.5 (C-5), 72.4 (ArCH$_2$), 61.8 (OCH$_3$), 61.2 (OCH$_3$), 55.3 (OCH$_3$), 21.1 (ArCH$_3$), 16.9 (C-6). HRMS (ESI) Calcd. for $(M + Na)^+$ $C_{23}H_{30}NaO_5S$: 441.1712. Found 441.1701.



4-(1-Hydroxynonadecyl) anisole (5-10)

To a solution of mechanically activated magnesium turnings (1.07 g, 44.98 mmol) in anhydrous THF (2 mL) in a three-necked rounded bottom flask was added 1-bromooctadecane (1 mL from a solution of 5 g in 20 mL anhydrous THF, 14.99 mmol) and the solution was gently warmed until it started to reflux by itself. The remained of 1-bromooctadecane solution (19 mL) was added dropwise over 20 min while the reaction mixture continued to reflux. After the addition was complete, the reaction mixture was heated at reflux for an 1 h. After 1 h, the reaction mixture was cooled to rt and *p*-anisaldehyde, **5-8**, (2.75 mL, 22.48 mmol) was added dropwise over 10 min. After the addition was complete, the reaction mixture was heated at reflux for 1 h. Then, the solution was cooled to rt and the excess Grignard reactant was quenched carefully by the addition of ice-cold water (50 mL). The solution was then diluted with CH_2Cl_2 (100 mL), washed with 1M HCl soln (2 x 50 mL), water (2 x 50 mL) and brine (75 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (7:1 hexane–EtOAc) to afford **5-10** (4.38 g, 75%) as an amorphous solid: R_f 0.48 (7:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.30–7.27 (m, 2H, Ar-2,6), 6.92–6.89 (m, 2H, Ar-3,5), 4.63 (dt, 1H, J = 7.1 Hz, J

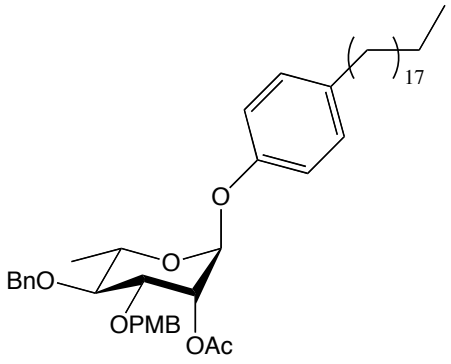
= 3.1 Hz, H-1′), 3.83 (s, 3H, OC*H*$_3$), 1.85–1.79 (m, 1H, H-2′a), 1.78 (d, 1H, *J* = 2.6 Hz, OH), 1.73–1.76 (m, 1H, H-2′b), 1.43–1.27 (m, 32H, C*H*$_2$ x 16), 0.91 (t, 3H, $J_{18',19'}$ = 7.0 Hz, C*H*$_3$-19′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.0 (Ar), 137.1 (Ar), 127.2 (Ar x 2), 113.8 (Ar x 2), 74.3 (C-1′), 55.3 (O*C*H$_3$), 39.0 (CH_2), 32.0 (CH_2), 29.7(2) (CH_2 x 8), 29.6(8) (CH_2), 29.6(2) (CH_2), 29.5(8) (CH_2), 29.5(6) (CH_2), 29.4 (CH_2), 25.9 (CH_2), 22.7 (CH_2), 14.2 (CH_3-19′). HRMS (ESI) Calcd for $(M–H_2O)^+$ $C_{26}H_{44}O$: 372.3392. Found 372.3393



4-Nonadecylanisole (5-11)

To a solution of **5-10** (0.78 g, 2 mmol) and Me_3SiH (0.5 mL, 4 mmol) in dry CH_2Cl_2 (4 mL) at 0°C, $BF_3{\cdot}Et_2O$ (0.5 mL, 4 mmol) was added and the reaction mixture was stirred for 1 h. The Lewis acid was quenched by the addition of sat. aq. $NaHCO_3$ soln (10 mL), and the mixture was diluted with CH_2Cl_2 (20 mL), and then washed with water (2 x 10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography (10:1 hexane–EtOAc) to afford **5-11** (0.71 g, 94%) as an amorphous solid: R_f 0.51 (10:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.11–7.07 (m, 2H, Ar-3,5), 6.84–6.80 (m, 2H, Ar-2,6), 3.79 (s, 3H, OC*H*$_3$), 2.54 (t, 2H, $J_{1',2'}$ = 7.6 Hz, C*H*$_2$-1′), 1.59–1.54 (m, 2H, C*H*$_2$-2′), 1.31–1.26 (m, 32H,

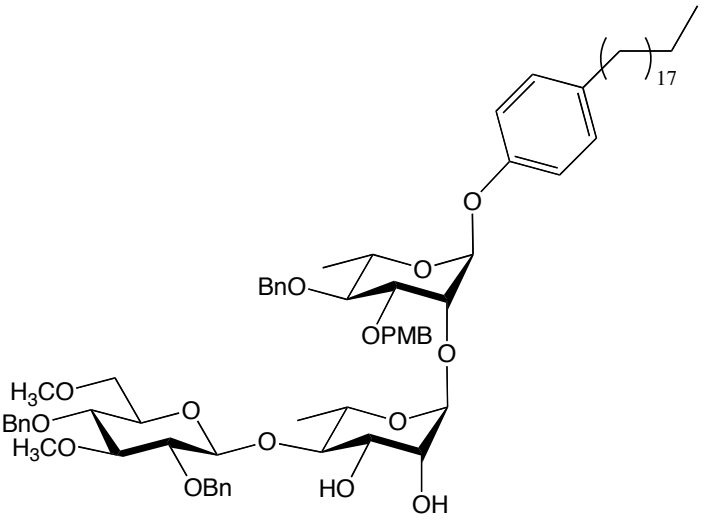
CH_2 x 16), 0.89 (t, 3H, $J_{18',19'}$ = 7.0 Hz, CH_3-19′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 157.2 (Ar), 134.7 (Ar), 128.8 (Ar x 2), 113.2 (Ar x 2), 54.9 (OCH_3), 34.7 (CH_2), 31.5 (CH_2), 31.4 (CH_2), 29.3 (CH_2 x 11), 29.2 (CH_2), 20.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 22.3 (CH_3-19′). HRMS Calcd for (ESI) $(M)^+$ $C_{26}H_{46}O$: 374.3549. Found 374.3546.



4-Nonadecylphenyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranoside (5-12)

To a solution of **4-20** (475 mg, 0.91 mmol) and **5-3** (396 mg, 1.1 mmol) in CH_2Cl_2 (20 mL) were added crushed 4 Å molecular sieves (200 mg). After the reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (207 mg, 0.92 mmol) and AgOTf (61.7 mg, 0.24 mmol) were added. The reaction mixture was stirred for another 30 min before the addition of Et_3N (1 mL). The solution was filtered and concentrated to a crude residue that was purified by chromatography (3:1 hexane–EtOAc) to give **5-12** (493 mg, 78%) as a colorless oil: R_f 0.46 (3:1 hexane–EtOAc); $[\alpha]_D$ –21.5 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.38–7.26 (m, 7H, Ar), 7.10–7.06 (m, 2H, Ar), 6.95–6.92 (m, 2H, Ar),

6.88–6.85 (m, 2H, Ar), 5.53 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 5.41 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.93, 4.53 (ABq, J = 10.9 Hz, ArCH_2), 4.70, 4.63 (ABq, J = 10.9 Hz, ArCH_2), 4.13 (dd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 3.90 (dq, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.81 (s, 3H, OCH_3), 3.49 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 2.54 (t, 2H, $J_{1,2}$ = 7.8 Hz, CH_2-1_{agly}), 2.19 (s, 3H, CH_3CO), 1.60–1.56 (m, 2H, CH_2-2_{agly}), 1.31 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.30–12.6 (m, 32H, CH_2 x 16), 0.89 (t, 1H, $J_{18,19}$ = 6.8 Hz, CH_3-19_{agly}); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.0 (*C*=O), 158.9 (Ar), 153.7 (Ar), 138.1 (Ar), 136.6 (Ar), 129.7 (Ar), 129.4 (Ar x 2), 128.9 (Ar x 2), 128.0 (Ar x 2), 127.5 (Ar x 2), 127.3 (Ar), 115.9 (Ar x 2), 113.4 (Ar x 2), 95.8 (C-1), 79.6 (C-3), 77.2 (C-4), 75.0 (Ar*C*H_2), 71.2 (Ar*C*H_2), 68.5 (C-2), 68.0 (C-5), 54.9 (O*C*H_3), 34.8 (*C*H_2), 31.6 (*C*H_2), 31.3 (*C*H_2), 29.3 (*C*H_2 x 10), 29.2 (*C*H_2), 29.2 (*C*H_2), 29.0 (*C*H_2), 28.9 (*C*H_2), 22.3 (*C*H_2), 20.7 (*C*H_3CO), 17.6 (C-6), 13.8 (*C*H_3-19_{agly}). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{48}H_{70}O_7Na$: 781.5014. Found 781.5006.

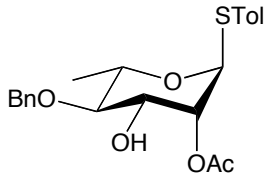


***p*-Nonadecylphenyl 2,4-di-*O*-benzyl-3,6-di-*O*-methyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-L-rhamnopyranoside (5-13)**

To a solution of **5-12** (75 mg, 0.09 mmol) in 1:1 CH_3OH–CH_2Cl_2 (10 mL), 0.1 M $NaOCH_3$ (0.25 mL) was added and the solution was stirred for 1 h at rt before it was neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil (69 mg). To a solution of this compound (69 mg, 0.09 mmol) and **5-4** (79.7 mg, 0.11 mmol) in CH_2Cl_2 (15 mL) were added crushed 4 Å molecular sieves (100 mg). After the reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC and then NIS (20.3 mg, 0.09 mmol) and AgOTf (7.7 mg, 0.03 mmol) were added. The reaction mixture was then stirred for additional 30 min before the addition of Et_3N (0.25 mL). The solution was concentrated to a crude residue that was dissolved in 1:1 CH_3OH–CH_2Cl_2 (10 mL) and 1M $NaOCH_3$ (0.1 mL) was added. The reaction mixture was stirred for 1 h at rt before it was

neutralized with Amberlite IR-120 H^+ resin, filtered, and concentrated. The resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **5-13** (67.6 mg, 61%) as a colorless oil: R_f 0.32 (2:1 hexane–EtOAc); $[\alpha]_D$ –6.4 (c 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 7.43–7.25 (m, 17H, Ar), 7.10–7.08 (m, 2H, Ar), 6.93–6.91 (m, 2H, Ar-2,6), 6.87–6.85 (m, 2H, Ar-3,5), 5.45 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 5.06 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.94–4.82 (m, 4H, ArCH_2 x 2), 4.66–4.62 (m, 4H, ArCH_2 x 2), 4.54 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 4.15–4.14 (m, 1H, H-2), 4.09–4.05 (m, 2H, H-3′, H-2′), 3.87–3.80 (m, 3H, H-5, H-5′, H-5″), 3.79 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.62–3.49 (m, 5H, H-3″, H-4′, H-4″, H-6″ x 2), 3.40–3.33 (m, 3H, H-2″, H-3, H-4), 3.38 (s, 3H, OCH_3), 2.55 (t, 2H, $J_{1,2}$ = 8.0 Hz, CH_2-1_{agly}), 2.39 (br s, 1H, OH-2′), 1.68–1.56 (m, 2H, CH_2-2_{agly}), 1.33 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.30 (d, 3H, $J_{5',6'}$ = 6.4 Hz, H-6′) 1.34–1.27 (m, 32H, CH_2 x 16), 0.89 (t, 3H, $J_{18,19}$ = 7.0 Hz, CH_3-19_{agly}); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 159.3 (Ar), 154.3 (Ar), 138.5 (Ar), 138.2 (Ar), 137.5 (Ar), 136.7 (Ar), 130.4 (Ar), 129.4 (Ar x 2), 129.3 (Ar x 2), 128.5 (Ar x 2), 128.5 (Ar x 2), 128.4 (Ar x 2), 128.2 (Ar x 2), 128.1 (Ar), 128.0 (Ar x 2), 127.9 (Ar x 2), 127.9 (Ar), 127.7 (Ar), 116.1 (Ar x 2), 113.9 (Ar x 2), 104.1 (C-1″), 100.8 (C-1′), 97.4 (C-1), 87.5 (C-3″), 83.9 (C-3), 82.1 (C-2″), 80.3 (C-3′), 79.2 (C-2′), 77.8 (C-4″), 75.5 (ArCH_2), 75.3 (ArCH_2), 74.9 (ArCH_2), 74.9 (C-2), 74.4 (C-5), 72.1 (ArCH_2), 71.2 (C-4), 71.1 (C-6″), 70.8 (C-5′), 68.6 (C-5″), 67.0 (C-4′), 61.2 (OCH_3), 59.5 (OCH_3), 55.3 (OCH_3), 35.2 (CH_2), 32.0 (CH_2), 31.7 (CH_2), 29.7(1) (CH_2 x 10), 29.6(7) (CH_2), 29.6(4) (CH_2), 29.6 (CH_2), 29.4 (CH_2), 22.7 (CH_2), 18.2 (C-6′), 17.6 (C-6), 14.2

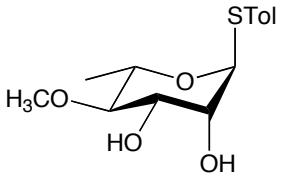
(C-19$_{agly}$). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{74}H_{104}O_{15}Na$: 1255.7273. Found 1255.7271.



***p*-Tolyl 2-*O*-acetyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (5-14)**

To a solution of **3-8** (0.99 g, 2.75 mmol) and triethyl orthoacetate (1.02 mL, 5.55 mmol) in CH_2Cl_2 (20 mL) was added CSA (128 mg, 0.55 mmol). The reaction mixture was stirred for 2 h at rt before it was concentrated and dissolved in 80% HOAc and stirred for additional 30 min at rt. Water (10 mL) was added and the mixture was concentrated, diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 20 mL). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated to a syrup, which was purified by chromatography (4:1 hexane–EtOAc) to give **5-14** (0.96 g, 87%) as a colorless oil: R_f 0.31 (1:2 hexane–EtOAc); $[\alpha]_D$ –133.4 (c 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.39–7.28 (m, 7H, Ar), 7.13 (d, 2H, J = 8.1 Hz, Ar-2,6), 5.40–5.37 (m, 2H, H-1, H-2), 4.79, 4.68 (ABq, J = 11.2 Hz, ArCH_2), 4.26 (dq, 1H, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 4.16–4.10 (m, 1H, H-3), 3.46 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 2.36 (s, 3H, ArCH_3), 2.28 (d, 1H, $J_{3,OH-3}$ = 5.1 Hz, OH-3), 2.16 (s, 3H, CH_3CO), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.9 (*C*=O), 138.4 (Ar), 138.2 (Ar), 132.6 (Ar x 2), 130.3 (Ar), 130.1 (Ar), 128.8 (Ar x 2), 128.3 (Ar), 128.2(2) (Ar), 128.1(4) (Ar x 2), 86.5 (C-1), 82.2 (C-4), 75.5 (Ar*C*H$_2$), 74.6 (C-3), 71.0 (C-

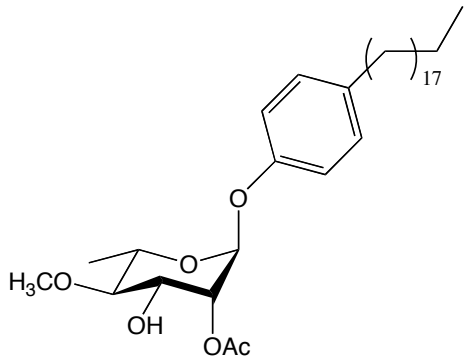
5), 68.9 (C-2), 21.4 (*C*H_3CO), 21.3 (Ar*C*H_3), 18.1 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{22}H_{26}O_5NaS$: 425.1393. Found 425.1405.



***p*-Tolyl 4-*O*-methyl-1-thio-α-L-rhamnopyranoside (5-15)**

To a solution of **2-11** (0.64 g, 2.1 mmol) in DMF (10 mL) and CH_3I (0.21 mL, 3.32 mmol), NaH (60% in mineral oil, 106 mg, 4.43 mmol) was added at 0 ºC and then the reaction mixture was stirred for additional 1 h at rt. The mixture was diluted with chilled water (30 mL) and CH_2Cl_2 (30 mL) and the organic layer was washed with water (2 x 20 mL) and brine (20 mL) before being dried ($NaSO_4$), filtered and concentrated. The resulting oil was dissolved 1:1 CH_3OH–CH_2Cl_2 (10 mL) and *p*-TSA (20 mg) was added. The reaction mixture was stirred for 3 h at rt before Et_3N (0.5 mL) was added. The solution was concentrated, and the resulting residue was purified by chromatography (1:1 hexane–EtOAc) to afford **5-14** (0.53 g, 84%) as an amorphous solid: R_f 0.29 (1:1 hexane–EtOAc); $[\alpha]_D$ –28.9 (c 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.37–7.34 (m, 2H, Ar-2,6), 7.13–7.11 (m, 2H, Ar-3,5), 5.41 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.20 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.15 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.89 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.59 (s, 3H, OCH_3), 3.18 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.34 (s, 3H, ArCH_3), 1.34 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 137.9 (Ar), 132.3 (Ar x 2), 130.5 (Ar), 130.07 (Ar x 2), 88.1 (C-

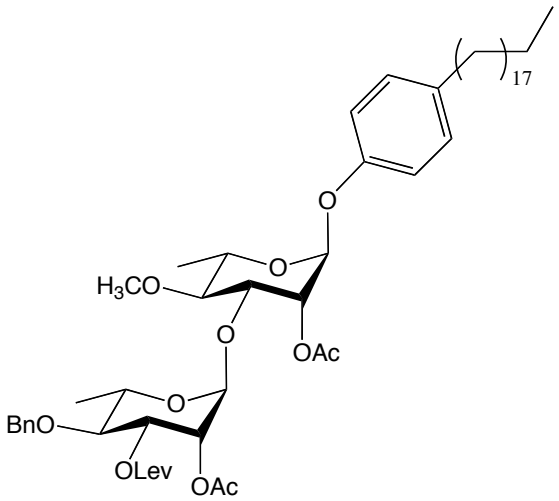
1), 83.7 (C-4), 72.8 (C-3), 71.9 (C-2), 68.8 (C-5), 61.0 (O*C*H$_3$), 21.4 (Ar*C*H$_3$), 18.1 (C-6). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{14}H_{20}O_4NaS$: 307.0975. Found 307.0977.



4-Nonadecylphenyl 2-*O*-acetyl-4-*O*-methyl-α-L-rhamnopyranoside (5-16)

To a solution of **5-6** (385 mg, 0.91 mmol) and **5-3** (396 mg, 1.1 mmol) in CH_2Cl_2 (20 mL) were added crushed 4 Å molecular sieves (200 mg). After stirring at rt for 30 min, the solution was cooled to –20 ºC and then NIS (206 mg, 0.92 mmol) and AgOTf (62 mg, 0.24 mmol) were added. The reaction mixture was then stirred for another 30 min before the addition of Et_3N (1 mL). The solution was concentrated to a crude residue that was dissolved in CH_2Cl_2 (20 mL) and NH_2NH_2·HOAc (126 mg, 1.36 mmol) was added. The reaction mixture was stirred for 5h at rt before it was concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give **5-16** (337 mg, 66%) as a colorless oil: R_f 0.28 (2:1 hexanes–EtOAc); $[\alpha]_D$ –3.6 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.08–7.05 (m, 2H, Ar-2,6), 6.94–6.91 (m, 2H, Ar-3,5), 5.40 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.26 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 4.22 (dd, 1H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.79 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ =

6.4 Hz, H-5), 3.59 (s, 3H, OCH_3), 3.13 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.53 (t, 2H, $J_{1',2'}$ = 7.6 Hz, CH_2-1′), 2.18 (s, 3H, CH_3CO), 1.59–1.53 (m, 2H, CH_2-2′), 1.26 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6), 1.29–1.24 (m, 32H, CH_2 x 16), 0.87 (t, 3H, $J_{18',19'}$ = 6.6 Hz, CH_3-19′); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 170.7 (*C*=O), 154.2 (Ar), 137.0 (Ar), 129.3 (Ar x 2), 116.3 (Ar x 2), 95.8 (C-1), 83.3 (C-4), 72.5 (C-3), 69.8 (C-5), 68.1 (C-2), 60.9 (O*C*H$_3$), 35.1 (*C*H$_2$), 31.9 (*C*H$_2$), 31.6 (*C*H$_2$), 29.7 (*C*H$_2$ x 10), 29.6 (*C*H$_2$), 29.5 (*C*H$_2$), 29.4 (*C*H$_2$), 29.3 (*C*H$_2$), 22.7 (*C*H$_2$), 21.0 (*C*H$_3$CO), 18.0 (C-6), 14.1 (*C*H$_3$-19′). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{34}H_{58}O_6Na$: 585.4126. Found 585.4122.

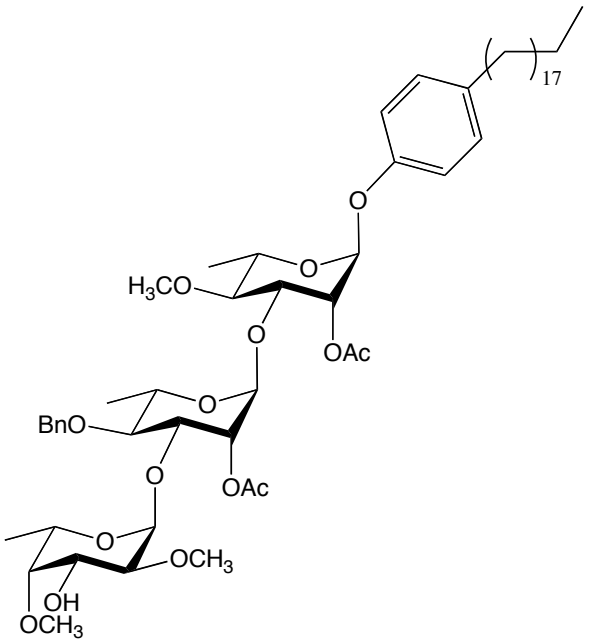


***p*-Nonadecylphenyl 2-*O*-acetyl-4-*O*-benzyl-3-*O*-levulinoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-methyl-α-L-rhamnopyranoside(5-17)**

To a solution of **5-5** (225 mg, 0.45 mmol) and **5-16** (309.1 mg, 0.55 mmol) in CH_2Cl_2 (20 mL), crushed 4Å molecular sieves (200 mg) were added. After the

reaction mixture was stirred at rt for 30 min, it was cooled to –20 ºC, and then NIS (103.5 mg, 0.46 mmol) and AgOTf (30.8 mg, 0.12 mmol) were added. The reaction mixture was stirred for 30 min before the addition of Et_3N (1 mL). The solution was filtered and concentrated to a crude residue that was purified by chromatography (2:1 hexane–EtOAc) to give **5-17** (333 mg, 79%) as a colorless oil: R_f 0.45 (2:1 hexane–EtOAc); $[\alpha]_D$ –39.7 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.36–7.25 (m, 5H, Ar), 7.07–7.04 (m, 2H, Ar-2,6), 6.92–6.90 (m, 2H, Ar-3,5), 5.36 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.30–5.26 (m, 3H, H-2, H-2′, H-3′), 5.02 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.72, 4.63 (ABq, 2H, J = 11.4 Hz, ArCH_2), 4.16 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.87 (dq, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.75 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.56 (s, 3H, OCH_3), 3.51 (app t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 3.21 (app t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.76–2.63 (m, 2H, CH_2-1″), 2.54–2.44 (m, 4H, CH_2CO, CH_2COO), 2.17 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 2.14 (s, 3H, CH_3CO), 1.57–1.54 (m, 2H, CH_2-2_{agly}), 1.31–1.24 (m, 38H, H-6 x 3, H-6′ x 3, CH_2 x 16), 0.87 (t, 3H, $J_{18,19}$ = 7.0 Hz, CH_3-19_{agly}); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 206.1 (*C*=O), 171.8 (*C*=O), 170.4 (*C*=O), 170.0 (*C*=O), 154.1 (Ar), 138.2 (Ar), 137.0 (Ar), 129.3 (Ar x 2), 128.4 (Ar x 2), 127.8 (Ar x 2), 127.7 (Ar), 116.3 (Ar x 2), 99.5 (C-1′), 95.6 (C-1), 82.1 (C-4), 78.4 (C-4′), 77.3 (C-3), 74.6 (Ar*C*H_2), 71.9 (C-3′), 71.8 (C-2), 70.4 (C-2′), 68.5 (C-5′), 68.5 (C-5), 37.9 (*C*H_2CO), 35.1 (*C*H_2COO), 31.9 (*C*H_2), 31.6 (*C*H_2), 29.8 (*C*H_3CO), 29.7 (*C*H_2 x 10), 29.6 (*C*H_2), 29.5 (*C*H_2), 29.4 (*C*H_2), 29.3 (*C*H_2), 28.0 (*C*H_2), 22.7 (*C*H_2), 21.0 (*C*H_3CO), 20.9 (*C*H_3CO), 17.9 (C-6′), 17.8

(C-6), 14.1 (C-19_{agly}). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{54}H_{82}O_{13}Na$: 961.5640. Found 961.5648.



***p*-Nonadecylphenyl 2,4-di-*O*-methyl-α-L-fucopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-methyl-α-L-rhamnopyranoside (5-18)**

To a solution of **5-17** (150 mg, 0.16 mmol) in CH_2Cl_2 (20 mL) was added $NH_2NH_2{\cdot}HOAc$ (22 mg, 0.24 mmol) and the reaction mixture was stirred for 5 h at rt. The solution was filtered, concentrated and the resulting residue was purified by chromatography (2:1 hexane–EtOAc) to give a colorless oil. Next, two solutions were prepared. Solution A was prepared by dissolving the product of the $NH_2NH_2{\cdot}HOAc$ reaction in CH_2Cl_2 (10 mL) and crushed 4Å molecular sieves (100 mg) was added. Solution B was prepared by dissolving **5-7** (87 mg, 0.21 mmol) in CH_2Cl_2 (10 mL) containing crushed 4Å molecular sieves (100 mg).

Both solutions A and B were stirred for 30 min at rt and then solution A was cooled to –40 ºC before NIS (51.8 mg, 0.23 mmol) and AgOTf (15.4 mg, 0.06 mmol) were added. Solution B was then added dropwise to solution A over 10 min while stirring. The reaction mixture was stirred for additional 30 min at –40 ºC before Et_3N (0.25 mL) was added. The solution was filtered, concentrated and the resulting residue was dissolved in CH_2Cl_2 (20 mL). To this solution at 0 ºC, TFA (1 mL, 5 % v/v) was added and the reaction mixture was stirred for 45 min. To this solution was added Et_3N (2 mL) and the mixture was then concentrated, and the resulting crude product was purified by chromatography (1:1 hexane–EtOAc) to give **5-18** (63 mg, 39 %) as a colorless oil: R_f 0.49 (1:1 hexane–EtOAc); $[\alpha]_D$ –29.8 (c 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.34–7.25 (m, 5H, Ar), 7.06 (d, 2H, J = 8.6 Hz, Ar-2,6), 6.91 (d, 2H, J = 8.6 Hz, Ar-3,5), 5.36 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.27 (dd, 1H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 5.19 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, $J_{3',4'}$ = 1.9 Hz, H-2′), 5.10 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.06 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1″), 4.67, 4.64 (ABq, 2H, J = 12.0 Hz, ArCH_2), 4.17 (dd,1H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 4.07–4.06 (m, 1H, H-3′), 4.02–3.99 (m, 2H, H-3″, H-5″), 3.84 (dq, 1H, $J_{4',5'}$ = 9.4 Hz, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.75 (dq, 1H, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.63–3.59 (m, 1H, H-2″), 3.62 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.49–3.46 (m, 1H, H-4′), 3.47 (s, 3H, OCH_3), 3.99–3.98 (m, 1H, H-4″), 3.21 (app t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 2.52 (t, 2H, $J_{1,2}$ = 8.0 Hz, CH_2-1_{agly}), 2.17 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.57–1.54 (m, 2H, CH_2-2‴), 1.29–1.23 (m, 41H, H-6 x 3, H-6′ x 3, H-6″ x 3, CH_2 x 16), 0.87 (t, 3H, $J_{18,19}$ = 6.8 Hz, CH_3-19_{agly}); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ_C) 167.9 (*C*=O),

167.7 (*C*=O), 151.6 (Ar), 135.9 (Ar), 134.5 (Ar), 126.7 (Ar x 2), 125.8 (Ar x 2), 125.1 (Ar x 3), 113.8 (Ar x 2), 98.0 (C-1″), 95.4 (C-1′), 93.2 (C-1), 80.0 (C-4), 79.4 (C-4″), 76.2 (C-4′), 75.9 (C-2″), 74.4 (C-5′), 73.8 (C-5), 72.2 (Ar*C*H_2), 70.8 (C-3″), 69.3 (C-5″), 67.3 (C-3), 66.2 (C-2′), 66.0 (C-3′), 64.3 (C-2), 59.7(4) (O*C*H_3), 58.7(9) (O*C*H_3), 54.7 (O*C*H_3), 32.6 (*C*H_2), 29.4 (*C*H_2), 29.1 (*C*H_2), 27.2 (*C*H_2 x 10), 27.1 (*C*H_2), 27.0 (*C*H_2), 26.7(8) (*C*H_2), 26.7(4) (*C*H_2), 22.2 (*C*H_2), 21.0 (*C*H_3CO), 20.8 (*C*H_3CO), 17.9 (C-6′), 17.7 (C-6), 16.5 (C-6″), 13.9 (*C*H_3-19_{agly}). HRMS (ESI) Calcd for $(M + Na)^+$ $C_{57}H_{90}O_{15}Na$: 1037.6178. Found 1037.6177.

5.5. Bibliography

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Chapter 6

Immunological Profiling of Mycobacterial PGL Synthetic Analogs

6.1. Overview of the role of cytokines in mycobacterial infections

Cytokines are immunomodulatory proteins that help orchestrate the immune response in many inflammatory conditions, such as bacterial infections. Cytokines can be classified as T_H1-type or T_H2-type cytokines.[1a,b] T_H1-type cytokines produce proinflammatory responses, which are responsible for killing of intracellular parasites and also involved in the autoimmune responses. However, T_H2-type cytokines produce anti-inflammatory responses and are associated with IgE and eosinophilic responses in atopy.[1b] It is worth noting that the production of T_H2-type cytokines requires the local presence of another cytokine known as IL-4 (Figure 6-1).

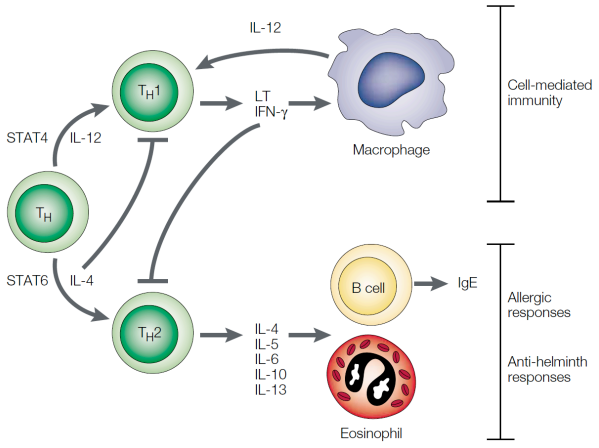


Figure 6-1. Cytokines and helper T-cell differentiation. [Adapted from: O`Shea, J. J.; Ma, A.; Lipsky, P. *Nat. Rev. Immunol.* **2002**, *2*, 37–45]

The interactions between mycobacteria and host defense mechanisms are complex and poorly understood. However, cytokines have been unequivocally established to play an important role in determining the disease outcome of infections by these pathogens.[2] Evidence for the relationship between cytokines and infection with mycobacteria have been obtained from studies on both experimental models as well as observations on patients with either genetic or drug-induced deficiencies of particular cytokines, such as Tumor Necrosis Factor-α (TNF-α).[2,3a,b]

A number of cytokines have been reported to be involved in regulating the innate immune response against mycobacterial infection. For full details about these cytokines, a review by Cooper *et al.*[4] provides an overview of the roles of different cytokines in the innate immune response as well as the mechanisms against mycobacterial infections. However, for my project, we were particularly interested in studying the effect of PGLs on the following cytokines: TNF-α, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), chemokine Monocyte chemotactic protein-1 (MCP-1), as well as nitric oxide (NO), as they have been shown to play an important role in mycobacterial infections. These roles will be explained in the next sections.

6.1.1. Role of TNF-α in mycobacterial infections

Tumor necrosis factor (TNF-α), a macrophage-derived cytokine, is one of the proinflammatory cytokines produced early after bacterial infections. TNF-α was always thought to be responsible for most of the pathology of mycobacterial

infections.[5] Despite the ability of *Mycobacterium tuberculosis* to weaken the host immune system response, it nevertheless attempts to fight infection by releasing proinflammatory cytokines of which TNF-α is a main player.[6]

The important role of TNF-α in mycobacterial infections was established through neutralization or deletion experiments on mice. Sugawara *et al.* showed that mice with a disrupted gene for TNF-α or its receptor, or which were treated with a TNF-α neutralizing antibody, were not able to control mycobacterial infection.[7] Instead, the mice suffered very low survival rates, impaired granuloma formation, as well as increased necrosis and bacteremia.[7] Further studies revealed that a deficiency of TNF-α production leads to rapid growth of the pathogen, impaired phagocyte activation, deficient chemokine expression and impaired influx of the pathogen into granulomas.[8] The protective mechanism of TNF-α was shown to be through promoting macrophage apoptosis, which deprives the pathogen of its preferred growth niche.[8-10] This, in turn, decreases pathogen viability and makes it more susceptible to killing by freshly recruited macrophages and facilitates the processing and presentation of the pathogen antigens.[8-10]

It is well documented that mycobacterial cell wall glycans can influence the production of TNF-α. For example, several components, such as lipomannan (LM) and phosphatidyl-*myo*-inositol LAM (PILAM) were reported to induce high levels of TNF-α via Toll-like Receptor 2 (TLR-2) activation.[11-13a] In contrast to PILAM, another related glycans to LAM known as ManLAMs, mannosylated

LAMs, were shown to inhibit the LPS-induced production of TNF-α by the human mononuclear phagocyte through binding to its mannose receptor.[13a-c] Moreover, it was shown that the release of TNF-α from human peripheral blood monocytes (PBMC) and alveolar macrophages can be induced upon exposure to mycobacterial preparations or purified LAM from *M. tuberculosis*.[14] On the other hand, Reed *et al.* showed that PGLs from *M. tuberculosis* inhibit the release of TNF-α in a dose-dependent manner from the bone marrow-derived macrophages (BMMs).[15]

6.1.2. Role of IL-6 in mycobacterial infections

IL-6 is a potent multifunctional cytokine, which regulates hematopoiesis, the acute phase response and inflammation.[16] It is a pleiotropic molecule, with both proinflammatory and anti-inflammatory properties,[17] and was first identified as a differentiation factor for B-cells.[18] In the context of mycobacterial infections, it was shown that the optimal development of T-cell-mediated protective effects against mycobacterial infections is dependent on IL-6.[19,20] In addition, the development of T-cell-mediated protective effects during vaccination against TB is also found to be IL-6–dependent.[19,20] The use of knockout or neutralization experiments in mice models revealed that infection with *M. tuberculosis* is lethal in IL-6 deficient mice, but can be controlled in IL-6 competent mice.[21,22] Another study by Tsao *et al.* showed, using bronchoalveolar lavage fluid analysis, increased levels of IL-6, TNF-α and IL-1β upon mycobacterial infection.[23]

Moreover, Reed *et al.* showed that, similar to TNF-α, treatment of BMMs with PGLs inhibited the release of IL-6.[15]

6.1.3. Role of IL-1β in mycobacterial infections

IL-1β is a proinflammatory cytokine, which is produced mainly by monocytes, macrophages and dendritic cells during the immune response to *M. tuberculosis* infection.[23-25] Raja *et al.* showed that IL-1β plays a central role in the acute phase response to mycobacterial infection by inducing fever and cachexia.[26] Another study showed that IL-1β is found in excess in tuberculosis patients.[17] Two knockout studies have revealed that mice deficient in IL-1β, or the receptor for IL-1 (IL-1R1), showed an increased susceptibility to mycobacterial growth and defective granuloma formation.[27a,b]

6.1.4. Role of IL-10 in mycobacterial infections

IL-10 is an anti-inflammatory cytokine produced by macrophages and T-cells and is found at elevated levels during *M. tuberculosis* infection. It has an antagonizing effect on proinflammatory responses through downregulation of TNF-α, IL-12 and INF-γ.[16b,28,29] The IL-10-mediated downregulation of the proinflammatory cytokines results in impaired $CD4^+$ T-cell responses, as well as impaired functions of the *M. tuberculosis*-infected antigen presenting cells *i.e.,* it has a protective role for *M. tuberculosis*.[16b] Furthermore, it was shown recently by O'Leary *et al.* that IL-10 blocks phagosomal maturation of the *M. tuberculosis*-infected human alveolar macrophages.[30]

6.1.5. Role of MCP-1 in mycobacterial infections

Chemokines (chemotactic cytokines) are of paramount importance in the recruitment of inflammatory cells to the site of infection. Many chemokines (40 to date) and chemokine receptors (16 to date) have been identified,[31] and a number of these proteins have been investigated with regard to *M. tuberculosis* infection. In this context, monocyte chemoattractant protein 1 (MCP-1) is a major chemokine that is produced by and acts on monocytes and macrophages. MCP-1 has been shown to be preferentially induced by *M. tuberculosis,*[32] and it was demonstrated that MCP-1 deficiency inhibited granuloma formation in a murine model.[33] Moreover, MCP-1 receptor deficient mice displayed reduced granuloma formation, suppressed Th1-mediated cytokine production and they died early after infection with *M. tuberculosis*.[34,35] In addition, the concentration of MCP-1 in alveolar lavage fluid,[36] serum[37] and pleural fluid[38] from tuberculosis patients was found to be elevated. The release of MCP-1 was inhibited in a dose-dependent manner from BMMs when they were treated with PGLs from *M. tuberculosis.*[15]

6.1.6. Role of Nitric oxide (NO) in mycobacterial infections

Nitric oxide is a nonspecific chemically reactive molecule, which is recognized as an important element in host defense against a large number of microbial infections. During bacterial infections, NO is produced in excess amounts due to induction of inducible nitric oxide synthase (iNOS), which in many cases orchestrates the innate immune resistance to the infection.[39] In the case of mycobacterial infections, studies on iNOS knockout and immunodeficient

murine animal models showed higher risk of dissemination and mortality when they were infected with *M. tuberculosis*.[40,41] Another study showed that a murine macrophage producing the natural resistance-associated protein-1 inhibited *M. tuberculosis* survival through the production of NO.[42,43] Furthermore, several studies revealed that murine macrophages have the ability to inhibit the intracellular growth of *M. leprae, M. bovis* and *M. tuberculosis* H37Rv via release of NO.[44-48]

While the role of NO in fighting mycobacterial infections in murine models is well established, there is controversy about its role in inhibiting and killing *M. tuberculosis* in humans.[49] However, there is a growing body of evidence collected from several studies suggesting that NO production by different *M. tuberculosis*-infected human cell lines induces a mycobactericidal effect.[50-54] In addition, it was reported that several cytokines (such as TNF-α, IFN-γ and IL-1β; alone or in combination) as well as some components stimulate *M. tuberculosis*-infected epithelial cells to produce NO and other mycobactericidal effects.[55-57]

6.2. Macrophage apoptosis in mycobacterial infections

Apoptosis, programmed cell death, is a normal and important component of a properly functioning immune system. It is also a common host defense mechanism against intracellular infections.[58] After infection with *M. tuberculosis*, bacilli use macrophages as a reservoir for continuous replication as well as a carrier for dissemination. In this context, macrophage apoptosis provides a means

of killing the internalized bacilli, containing and limiting their growth, which not only prevents the dissemination of the disease but also deprives the bacilli of their favorite growth niches.[59] In addition, it has been reported that apoptosis promotes microbicidal activities in the macrophages[60] and enhances the presentation of the pathogen antigens by dendritic cells via formation of apoptotic vesicles that have the mycobacterial antigens packaged inside.[61]

Several studies have reported that exposure of macrophages to attenuated *M. tuberculosis* complex strains (H37Ra and *M. bovis* Calmette–Guerin) induced apoptosis.[62] On the other hand, the exposure to the virulent strains suppressed the process of apoptosis and induced another process of cell lysis and death, necrosis.[63] Necrosis, in contrast to apoptosis, releases viable bacilli, which leads to extracellular spread of the infection, induces inflammation and promotes the recruitment of naïve macrophages that will serve as new hosts for another cycle of infection.[64] Furthermore, it was shown that apoptosis in case of exposure to the attenuated strains of *M. tuberculosis* happens in a TNF-α dependent manner.[65]

6.3. Toll-like receptors and mycobacterial infection

Toll-like receptors (TLRs) are type I membrane proteins, which are composed of two parts: an extracellular domain and a cytoplasmic domain.[66] Mammalian TLRs are a family of structurally conserved membrane receptors that have homology to *Drosophila* TLRs.[66a] Different microbial products, including mycobacterial antigens, can be recognized by distinct TLRs, which results in activation of the innate immune system.[66] Different mycobacterial cell

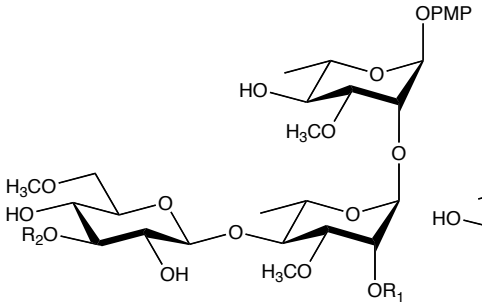
components have been shown to be recognized by TLRs. Bulut *et al.* and Hajar *et al.* showed that TLR2, TLR4 and TLR1/TLR6 are implicated in the recognition of mycobacterial antigens.[67]

TLR2-dependent activation of immune system was reported for lipoarabinomannan (LAM), lipomannan (LM), phosphatidyl-*myo*-inositol mannoside (PIM) and a 19kDa mycobacterial lipoprotein.[12,68] Moreover, the accumulation of results from different studies suggested that purified mycobacterial antigens as well as the whole bacterium preferentially recognized by TLR2 and TLR4, possibly in combination with other TLRs. This recognition results in the activation of MyD88, an adaptor protein that is crucial for the activity of TLRs, which in turn activates antibacterial effector pathways.[69] The role of TLRs in controlling the immune response against *M. tuberculosis* was confirmed in MyD88 deficient mice where these models were very sensitive to the infection and they developed a defect innate immune response.[69] MyD88 is an adaptor protein that promotes the signal transduction via binding to the TLR domain. Finally, Means *et al.* showed that exposure of primary macrophages to *M. tuberculosis* induced the production of NO in a TLR-2 dependent manner.[70]

As demonstrated above, the immune system uses different cytokines to orchestrate a defensive response against mycobacterial infection. To expand our knowledge of how mycobacteria can alter these cytokine-mediated immune response, a panel of synthetic analogs of all PGLs was designed and synthesized (Figure 6-2) as outlined in Chapters 2–5. With these compounds in hand, we

wanted to investigate their effect on the production of the cytokines that mediate the immune response against mycobacteria.

***M. leprae* analogs**

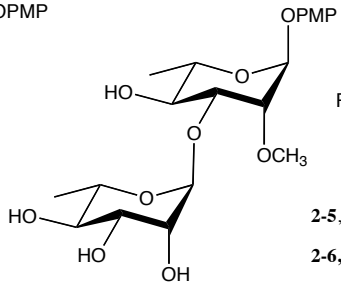


2-1, $R_1 = R_2 = CH_3$

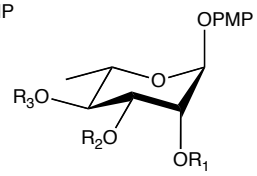
2-2, $R_1 = H, R_2 = CH_3$

2-3, $R_1 = CH_3, R_2 = H$

***M. bovis* analogs**



2-4

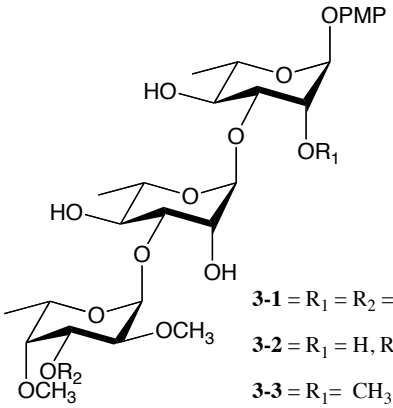


2-5, $R_1 = R_2 = R_3 = H$

2-6, $R_1 = CH_3, R_2 = R_3 = H$

2-7, $R_1 = R_2 = R_3 = CH_3$

***M. tuberculosis* analogs**

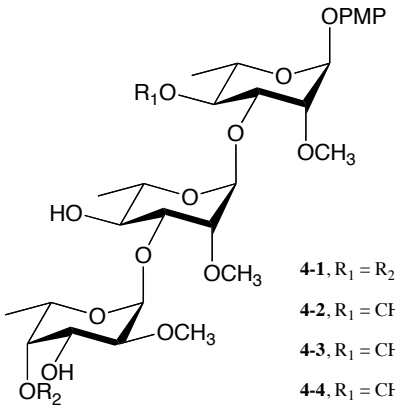


3-1 = $R_1 = R_2 = CH_3$

3-2 = $R_1 = H, R_2 = CH_3$

3-3 = $R_1 = CH_3, R_2 = H$

***M. kansasii*, series I analogs**



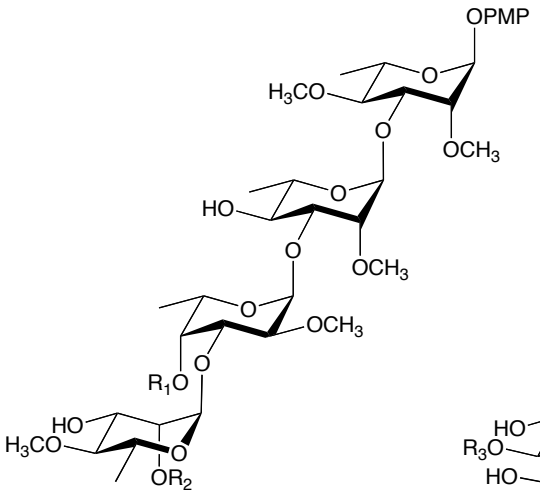
4-1, $R_1 = R_2 = H$

4-2, $R_1 = CH_3, R_2 = H$

4-3, $R_1 = CH_3, R_2 =$ Ac

4-4, $R_1 = CH_3, R_2 =$ propionoyl

***M. kansasii*, series II analogs**



4-5, $R_1 = R_2 = H$

4-6, R_1 = propyl, $R_2 = H$

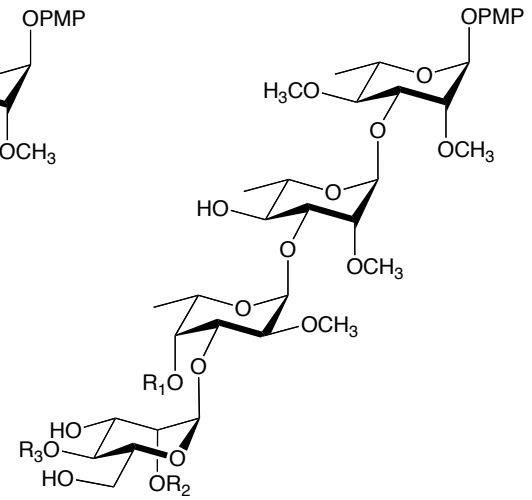
4-7, $R_1 = H$, $R_2 = CH_3$

4-8, R_1 = propyl, $R_2 = CH_3$

4-9, $R_1 = R_2 = CH_3$

4-10, R_1 = Ac, $R_2 = CH_3$

***M. kansasii*, series III analogs**



4-11, $R_1 = R_2 = R_3 = H$

4-12, R_1 = propyl, $R_2 = R_3 = H$

4-13, R_1 = propyl, $R_2 = CH_3$, $R_3 = H$

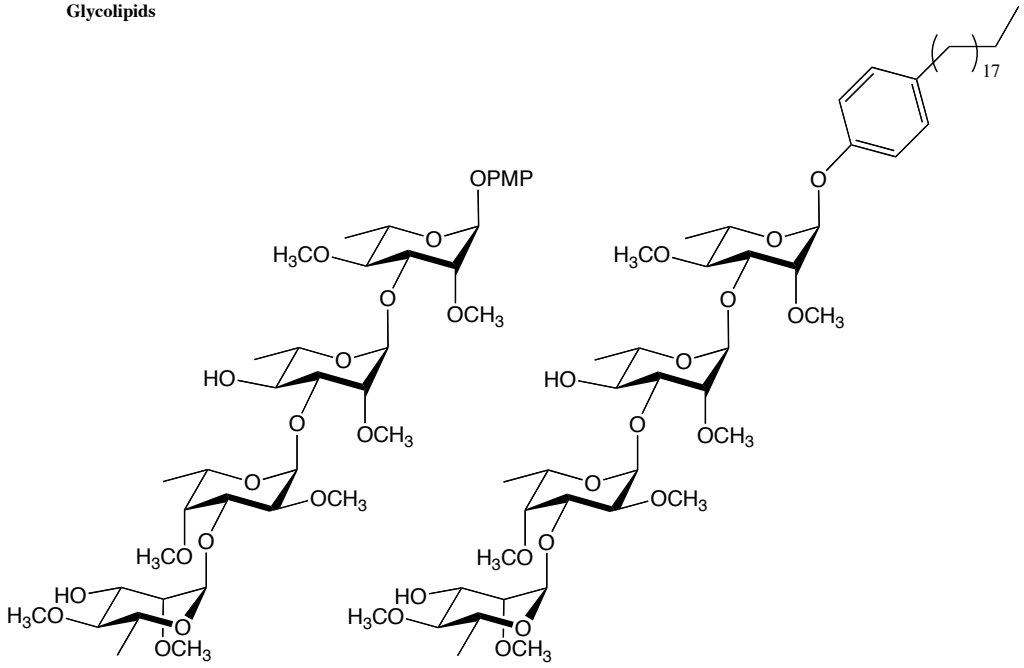
4-14, $R_1 = H$, $R_2 = CH_3$, $R_3 = H$

4-15, $R_1 = R_2 = H$, $R_3 = CH_3$

4-16, $R_1 = H$, $R_2 = R_3 = CH_3$

4-17, R_1= propyl, $R_2 = R_3 = CH_3$

Glycolipids



4-9 **5-2**

Figure 6-2. Structures of the synthetic PGL analogs.

6.4. Results and Discussion

The immunological profiling of the compounds shown in Figure 6-2 required an assay system that resembles the human alveolar macrophage. BMMs can be used for this purpose; however, a disadvantage of these cells is their donor variability. We chose instead to use the human acute leukemic monocyte cell line (THP-1). Unlike BMMs, THP-1 cells have the advantage of no donor variability of macrophage functions and they closely model the human alveolar macrophage. In addition, a large number of cells can be grown reproducibly and cells can be studied in two different stages; resting and activated. Finally, THP-1 cells have been often used in studies probing the effect of mycobacterial glycans on cytokine production.[71a-f]

We first tested if each compound can induce cytokine production in a cytokine stimulation assay. This was performed by first activating (differentiating) THP-1 monocytes into mature macrophages (A-THP-1) by treatment with Phorbol Myristate Acetate (PMA). The A-THP-1 macrophages in the unstimulated state were then treated with each compound and antibodies that recognize the cytokine of interest were used in ELISA to measure cytokine levels in the cell supernatants.

Each compound was also tested to determine if it could prevent the release of cytokines in a cytokine inhibition assay. In these assays, A-THP-1 macrophages were stimulated with LPS (a TLR2/TLR4 agonist), ultra pure LPS (a TLR4 agonist) or Pam3CSK4 (a TLR2 agonist) and then each compound was added. Cytokine levels were then measured as described above using ELISA.

Nitric oxide levels were determined using Griess reagent system.[72a,b] In this assay, the levels of nitrite (NO_2^-), which is a primary, stable and nonvolatile breakdown product of NO, are measured and a standard curve is constructed; the concentration of NO is determined from that curve. The assay relies on a diazo coupling reaction in which NO_2^- reacts with sulfanilamide to form a diazonium salt, which was then coupled with *N*-1-naphthylethylenediamine (NED) to give a colored product that can be measured spectrophotometrically (Figure 6-3).

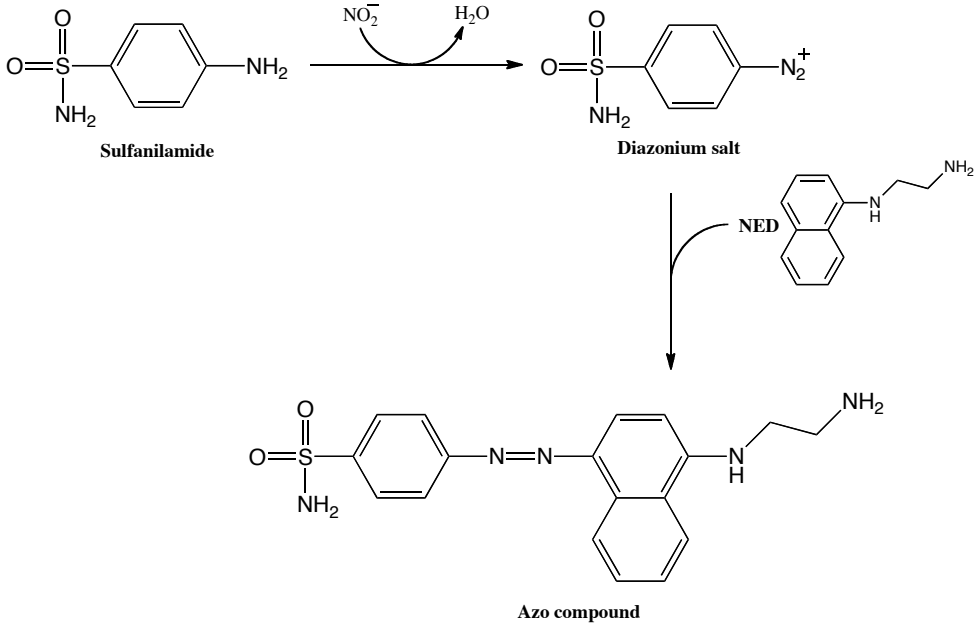


Figure 6-3. Chemical reactions involved in the measurement of NO_2^- using the Griess reagent system.

The ability of each compound to induce apoptosis was also evaluated. These assays were carried out using unstimulated A-THP-1 cells that were incubated with the test compounds for 24 h. Supernatants were removed and then the FLICA reagent as well as Hoechst and propidium iodide stains were added to

the remaining adherent cells for the specified time. A fluorescence microscope was then used to visualize the cells. Normal cells will be stained blue, apoptotic cells will be stained green, dying cells will be stained blue with bright white nuclei and necrotic cells will be stained red.[73a,b]

6.4.1. Cytokine stimulation assay results

The cytokine stimulation assays were carried out by treating the unstimulated activated THP-1 cells with each compound at a concentration of 50 μg/mL (0.1% DMSO/RPMI 1640). After 24 hours, ELISA was used to measure the level of each cytokine. As a positive control, LPS from *E. coli* (A.T.C.C. 12408) was used. We also evaluated the PGL-I from *M. leprae*, which was obtained from the Leprosy Research Materials Contract at Colorado State University. Each assay was carried out in triplicate and all compounds were evaluated twice. The data provided in the figures in this chapter are from a single triplicate assay.

As illustrated in Figure 6-4, in all cases the cytokine levels were comparable to those seen in the negative control (unstimulated A-THP-1 macrophages). Therefore the conclusion is that, unlike other mycobacterial cell wall glycans such as LAM, none of the synthetic analogs or the native PGL-I, stimulate the mature THP-1 cells to produce any of the tested proinflammatory cytokines. The results with PGL-I are in line with previous studies where the glycolipid was not shown to induce cytokine production.[15] It should be noted that in this assay, IL-10 was not produced at a detectable level. These results are in

agreement with IL-10 being a T_H2-type cytokine, which depends on the local presence of IL-4 and IL-13 to activate macrophages.[74]

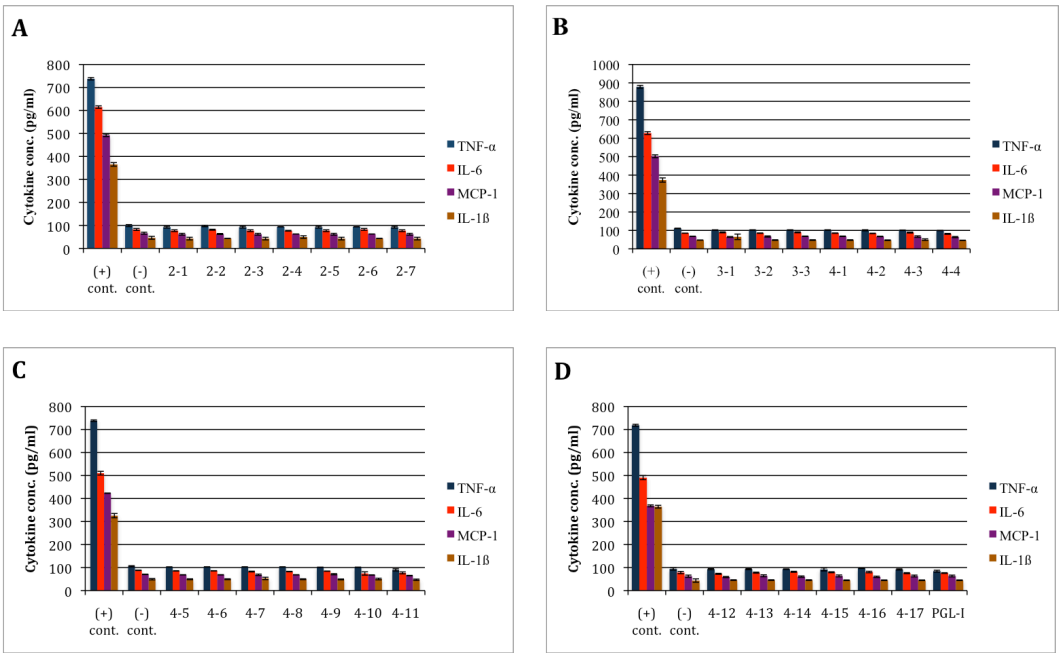


Figure 6-4. Concentration of cytokines from unstimulated A-THP-1 cells when treated with the each synthetic compound or PGL-I. Each compound at 50 µg/mL (dissolved in 0.1% DMSO in RPMI) was incubated with A-THP-1 cells for 24 h under the assay conditions described in the Section 6.6.4. Culture supernatants were then collected and stored at –80 ºC until being tested for cytokine levels using ELISA. *E. coli* (A.T.C.C. 12408) LPS-stimulated A-THP-1 was used as positive control and unstimulated A-THP-1 was used as negative control. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

6.4.2. Cytokine/NO inhibition assay results

After demonstrating that none of the compounds were able to stimulate the production of TNF-α, IL-6, MCP-1 or IL-1β in activated THP cells, we evaluated their ability to inhibit the formation of these immune signalling proteins. These experiments were carried out by first activating the THP-1 cells with PMA (5 ng/mL), and then stimulating the resulting A-THP-1 macrophages with 25 ng/mL of either LPS (a TLR2/TLR4 agonist)[75], ultra pure LPS (a TLR4 agonist)[76] or Pam3CSK4 (a TLR2 agonist).[77] Subsequently, each compound, at a concentration of 10 and 50 μg/mL, was added. After 24 hours, the cell supernatents were removed and cytokine levels were measured using ELISA.

In addition to evaluating the ability of each compound to reduce TNF-α, IL-6, MCP-1 and IL-1β levels, we also evaluated their effect upon NO production.

Carrying out these assays allowed us to both develop structure–activity relationships, and to determine the pathway (i.e., TLR2 vs TLR4) by which these compounds exert their effect.

6.4.2.1. Results from *M. leprae* synthetic PGL analogs

Figure 6-5 illustrates the concentration-dependent effect of **2-1**–**2-3**, synthetic analogs of PGLs from *M. leprae,* on TNF-α, IL-6, MCP-1, IL-1β and NO production by LPS-stimulated A-THP-1 macrophages. It is clear that **2-1**, **2-2** and **2-3** all inhibit the production of these proinflammatory molecules. As

expected, a larger effect was observed with when each compound was tested at 50 µg/mL as opposed to 10 µg/mL.

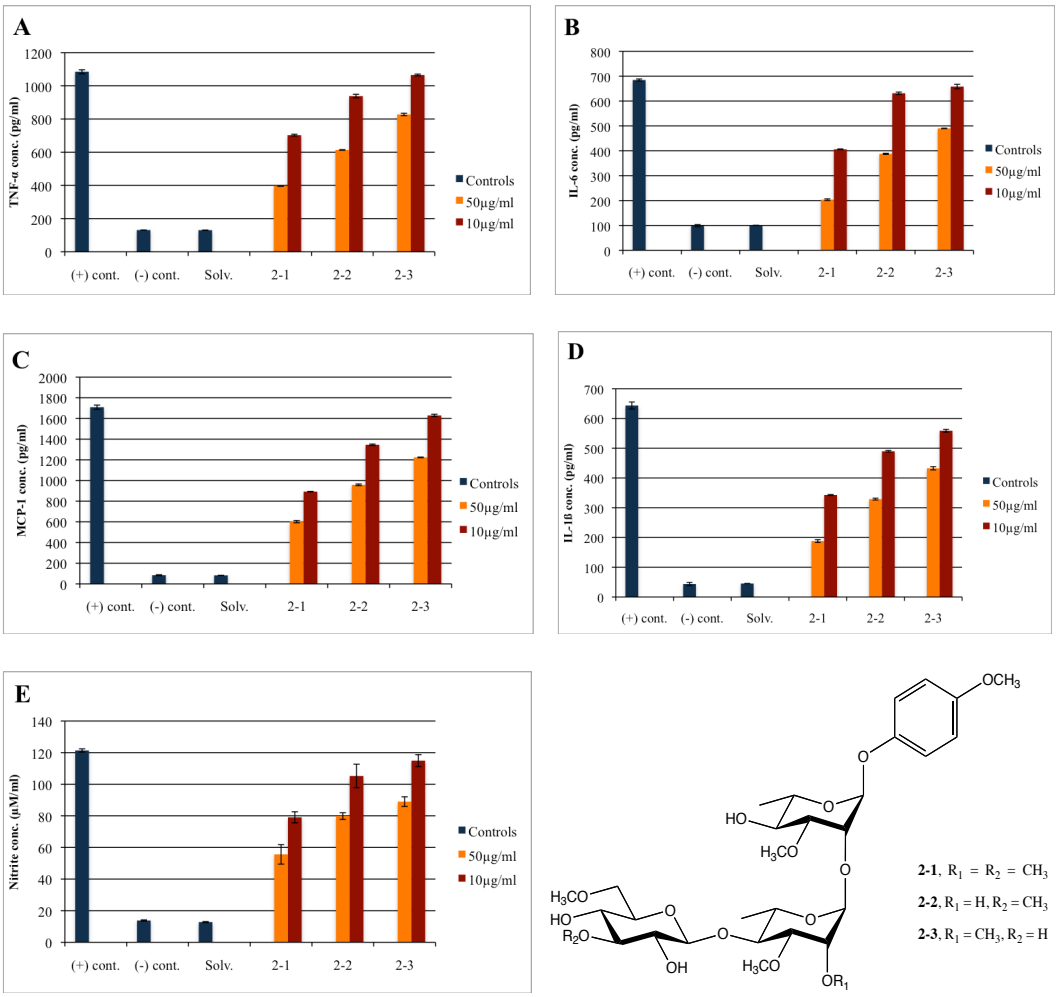


Figure 6-5. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with **2-1**–**2-3**. THP-1 cells were differentiated into mature macrophages by treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). The compounds, dissolved in 0.1% DMSO in RPMI, were added to the medium at concentrations of 10 and 50 µg/mL and were allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

These analyses also demonstrated that the inhibitory pattern was the same on all the tested cytokines and nitric oxide and that it is dependent on the methylation pattern of the molecule. Compound **2-1** showed the highest immunosuppressent activity and removal of O-2′ methyl group, compound **2-2**, resulted in loss of about 31–46% of activity on the tested cytokines. On the other hand, removal of the O-3″ methyl group, compound **2-3**, resulted in loss of 38%–60% of activity. This observation suggests that the O-3″ methyl group is more crucial for the inhibitory effect than the O-2′ methyl group, and that the presence of both is needed to obtain the highest effect.

In these experiments, *E. coli* LPS, a TLR2/TLR4 agonist, was used to stimulate the cells. The results presented above therefore suggest that **2.1**–**2.3** produce their inhibitory effect through blocking either the TLR2 and/or the TLR4 receptor. To further probe the mode of action, we repeated the same experiment by stimulating the cells with Pam3CSK4 (a TLR2 agonist).

As illustrated in Figure 6-6, **2-1**–**2-3** maintained the same concentration-dependent inhibitory pattern as when tested on the LPS-stimulated cells. Compound **2-1** showed the highest immunosuppressent activity causing about 63%–71% inhibition of the tested cytokines. Compound **2-2**, which lacks the O-2′ methyl group, showed about two-fold decrease in activity while compound **2-3**, lacking the O-3″ methyl group, showed about three-fold loss of activty. This result confirms the crucial role of the O-3″ methyl group for obtaining maximum immunosuppressant activity. The similar inhibitory pattern between the LPS-

stimulated and Pam3CSK4-stimulated cells, suggests that these compounds target the TLR2 receptor.

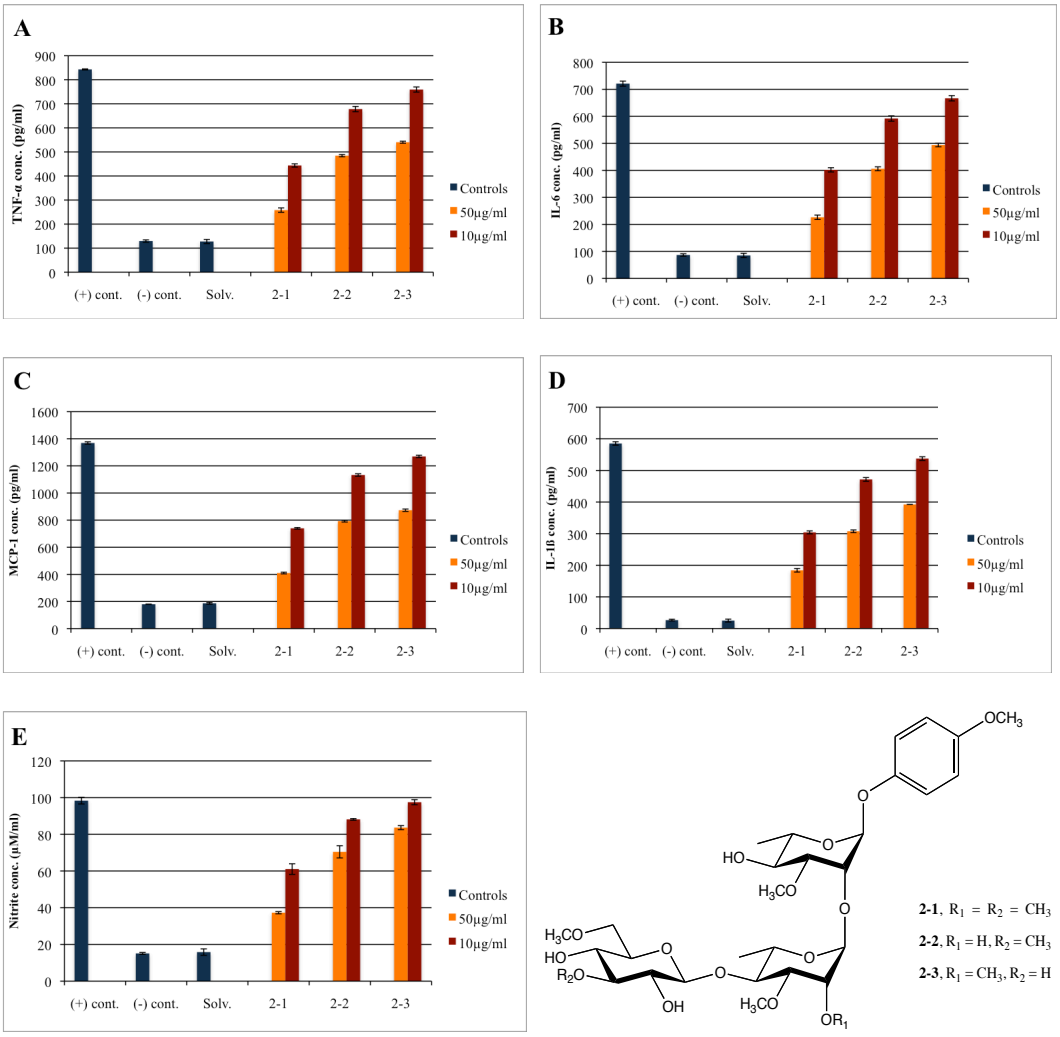


Figure 6-6. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with **2-1–2-3**. THP-1 cells were differentiated into mature macrophages by treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. The compounds, dissolved in 0.1% DMSO in RPMI, were added to the medium at concentrations of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

Further confirmation that **2.1**–**2.3** target TLR2 and not TLR4 was obtained by another experiment, in which the macrophages were stimulated with ultra-pure LPS from *E. coli* 0111:B4, which activates only TLR4.[76] The results of these assays (Figure 6-7) showed that **2-1**–**2-3** do not have any inhibitory activity on the release of cytokines by the ultra pure LPS-stimulated A-THP-1 cells. These results, together with the results from the previous two experiments, indicate that **2-1**–**2-3** appear to inhibit cytokine production through blocking TLR2.

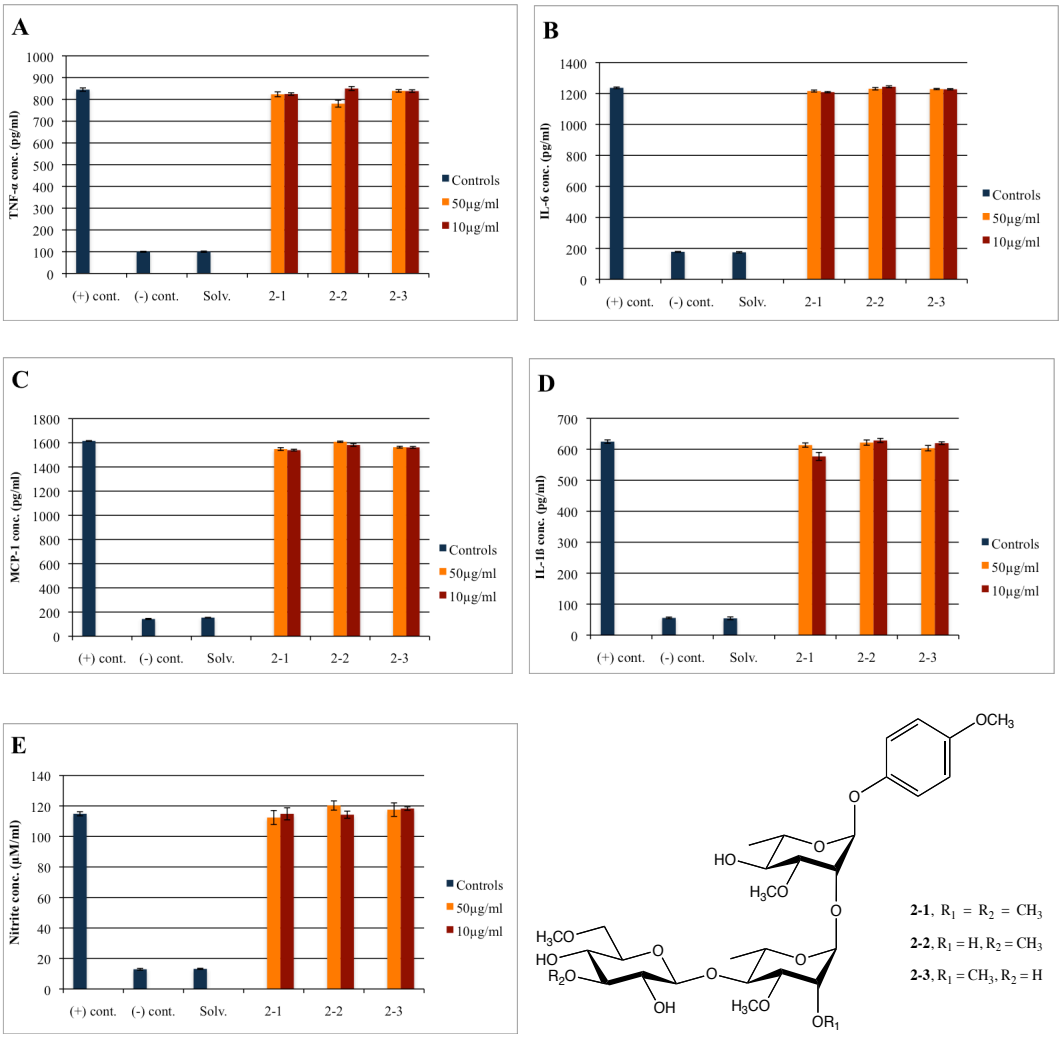


Figure 6-7. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with **2-1–2-3**. THP-1 cells were differentiated into mature macrophages by treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds,dissolved in 0.1% DMSO in RPMI, were added to the medium at concentrations of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

A graphical summary of the results of screening compounds **2-1–2-3** is presented in Figure 6-8.

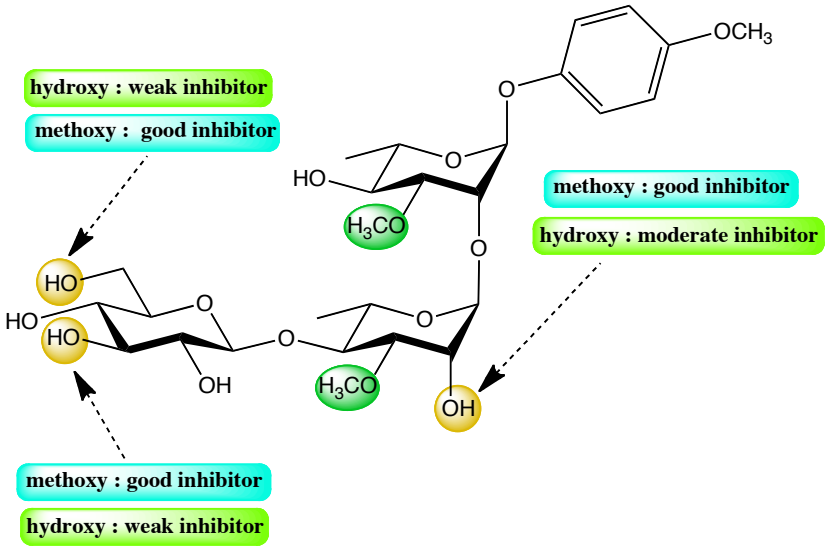


Figure 6-8. Summary of the cytokine inhibition activity of **2-1–2-3**. "Weak inhibitor" refers to inhibitory activity < 25% compared to positive control, "Moderate inhibitor" refers to inhibitory activity < 60% and > 25% compared to the positive control and "Good inhibitor" refers to inhibitory activity > 60% compared to the positive control and used at a concentration of 50 µg/mL of the tested compound. Groups in green color were kept as methyl groups as they are in

the native PGLs. When a group in yellow is hydroxyl, the other two groups in yellow are methoxy by default.

6.4.2.2. Results from *M. bovis* synthetic PGL analogs

In case of the synthetic analogs of *M. bovis* PGLs, compounds **2-4**–**2-7**, we used the same three experiments to determine their effect on cytokine release by macrophages and to obtain some insight on their mode of action. Illustrated in Figure 6-9 are the results from the experiment in which LPS was used as stimulus.

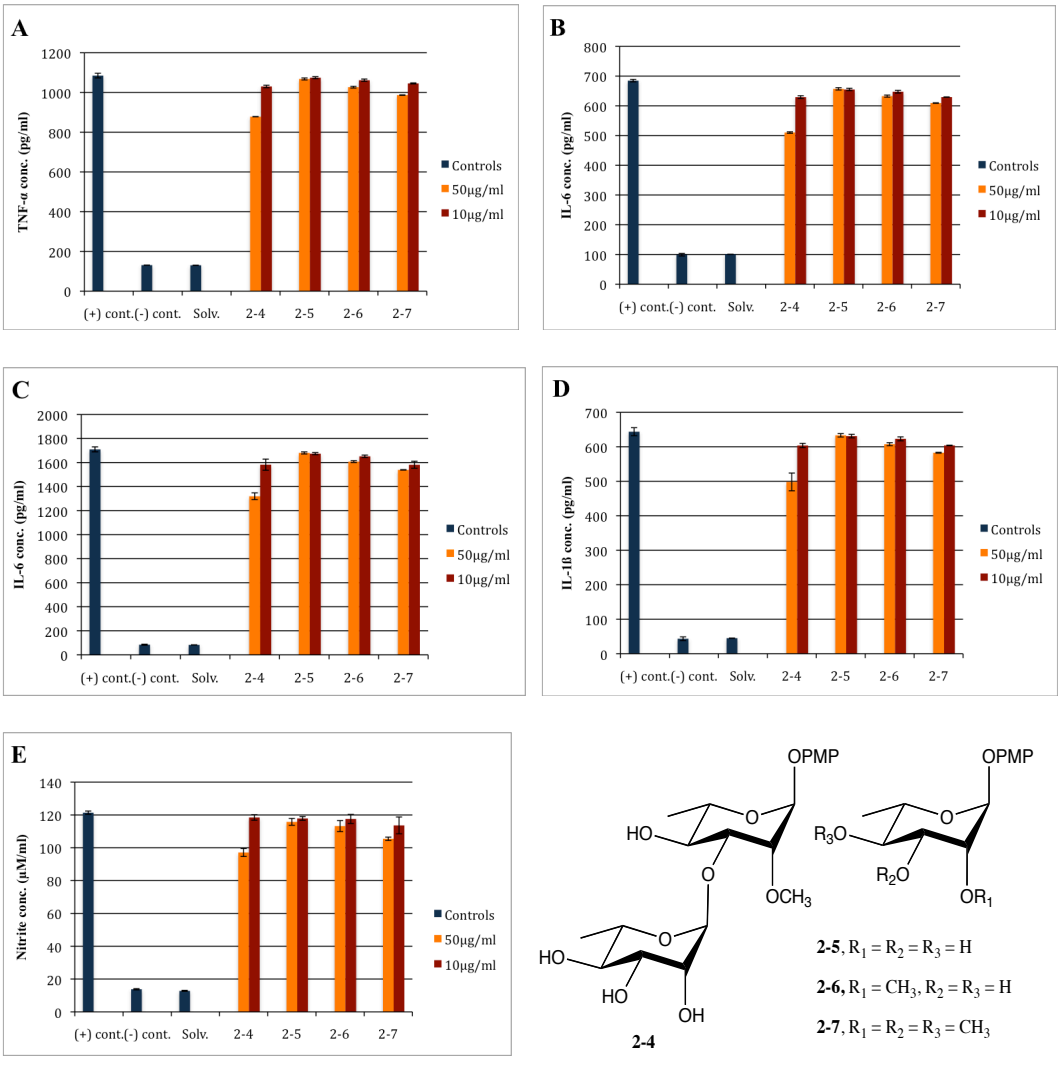
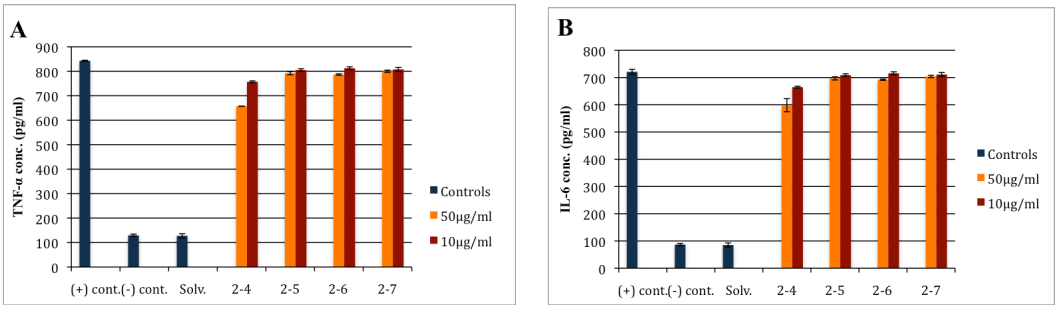


Figure 6-9. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with **2-4**–**2-7**. THP-1 cells were differentiated into mature macrophages by treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds, dissolved in 0.1% DMSO in RPMI, were added to the medium at concentrations of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

Compounds **2-5**–**2-7**, monosaccharides having a *p*-methoxybenzyl group at the reducing end, are virtually inactive. However, compound **2-4**, a disaccharide, showed weak inhibitory activity, 20–26%, on the release of the tested cytokines. This response is also concentration-dependent.

In a second experiment, where the TLR-2 agonist Pam3CSK4 was used as the stimulus, the same activity trend is observed (Figure 6-10). All the monosaccharides, **2-5**–**2-7**, showed no activity while disaccharide **2-4** showed weak inhibitory activity, 18–25%. When ultra pure LPS from *E. coli* (0111:B4) was used as the stimulus, none of the compounds showed any activity, not even disaccharide **2-4**, (Figure 6-11).



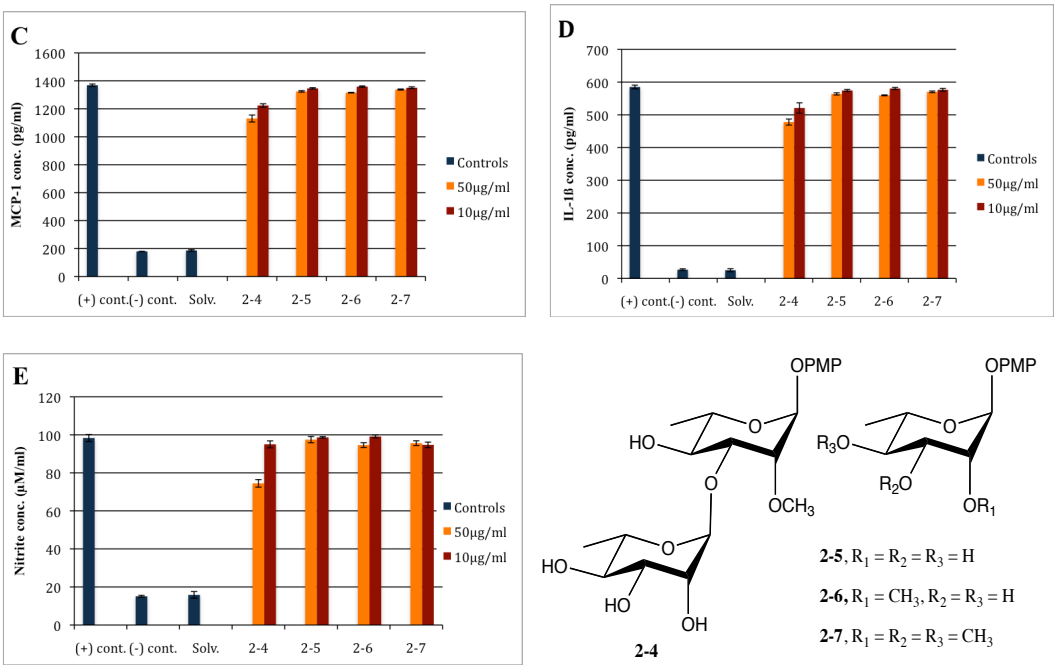


Figure 6-10. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **2-4**–**2-7**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

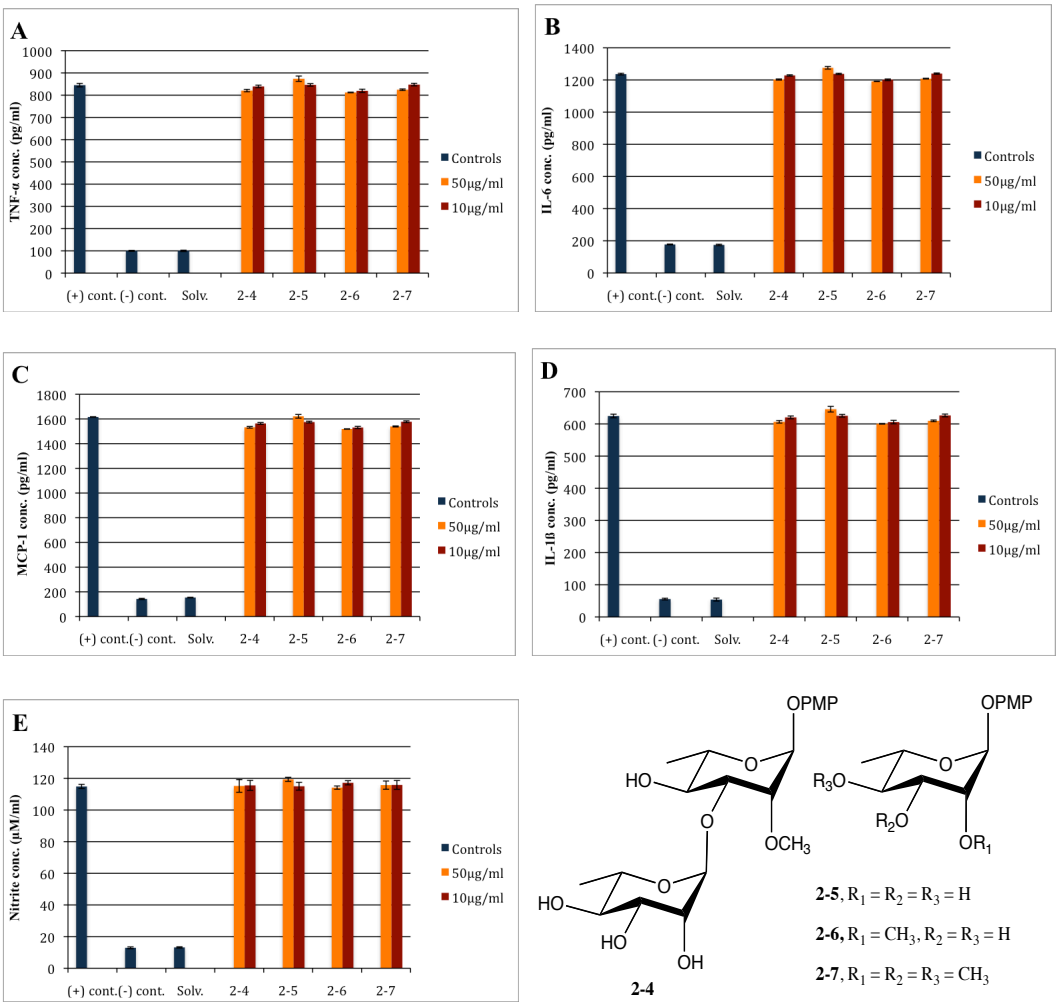


Figure 6-11. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **2-4–2-8**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

The results with **2-4**–**2-7** are in agreement with those from **2-1**–**2-3**, which confirmed that TLR2, not TLR4, is a possible target for these highly methylated compounds. Furthermore, it can be inferred that a disaccharide structure is the smallest structural motif that leads to any inhibitory activity, and that monosaccharides, at least those that have been tested here, are inactive. An even larger amount of inhibitory activity is observed with a trisaccharide structure.

6.4.2.3. Results from *M. tuberculosis* synthetic PGL analogs

Another series of synthetic analogs of PGLs from *M. tuberculosis*, compounds **3-1**–**3-3**, was tested using the same assay conditions as compounds **2-1**–**2-3**. First, LPS was used as a stimulus for the A-THP-1 cells and the results are shown in Figure 6-12.

As illustrated, a concentration-dependant inhibitory trend was observed. Among the tested trisaccharides, compound **3-1** showed the highest activity causing 52–60% inhibition of the released tested cytokines from the stimulated macrophages when used in a concentration of 50 μg/mL. Compound **3-2**, which lacks the O-2 methyl group, showed a considerable loss of activity of about 12–14% when compared to **3-1**. Trisaccharide **3-2** showed 38–48% inhibition of cytokine release. Removal of the O-3″ methyl group (compound **3-3**) resulted in even more loss of the inhibitory activity, 29–44% compared to **3-1**. Compound **3-3** was able to inhibit 16–28% of the released cytokines by the stimulated macrophages. These results indicated that methylation pattern is crucial for the

activity and both O-2 and O-3″ methyl groups are essential with the O-3″ methylation being most important.

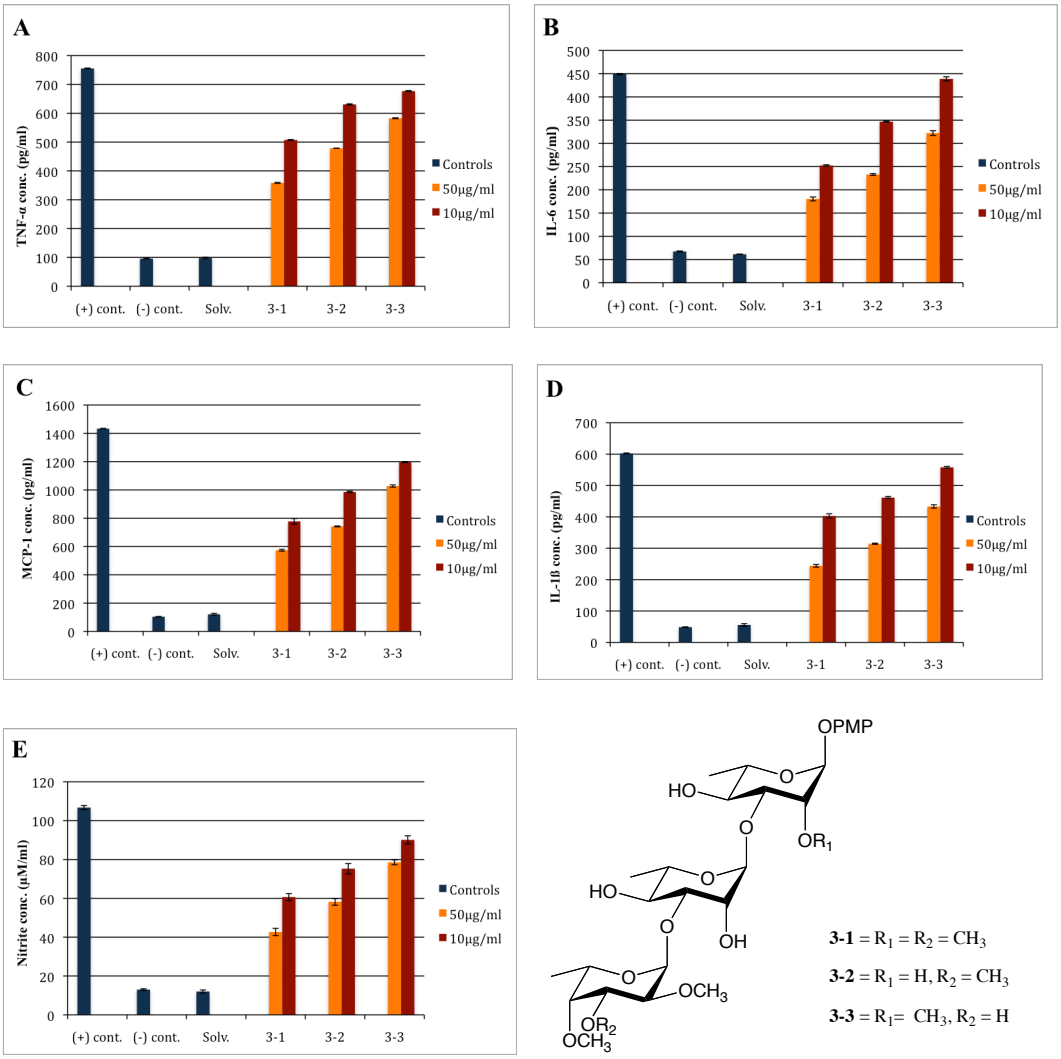


Figure 6-12. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **3-1–3-3**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine

levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

Gaining insight about the receptor these molecules are targeting was the next step. Determining if these compounds having TLR2 activity was achieved via using Pam3CSK4 as a stimulus instead of LPS and the results are shown in Figure 6-13. As expected, all tested trisaccharides retained the same concentration-dependent inhibitory pattern, which is also dependent on the methylation pattern of the molecule. As was observed using LPS as the stimulant, compound **3-1** proved to have the highest activity (57–63% inhibition of cytokine release) when used at concentration of 50 μg/mL. Trisaccharide **3-2**, which lacks the O-2 methyl group, inhibited 32–45% of the cytokine release, further demonstrating that the O-2 methyl group is needed for better activity. Trisaccharide **3-3**, lacking the O-3″ methyl group, showed more loss of activity (31–44%) when compared to analog **3-1** confirming the crucial role of this methyl group for obtaining the highest activity in this series. The conclusion is that for this series of trisaccharides, a permethylated fucose caping motif is essential to obtain the maximum inhibition of the released cytokines, and that the methyl group at O-2 is also essential.

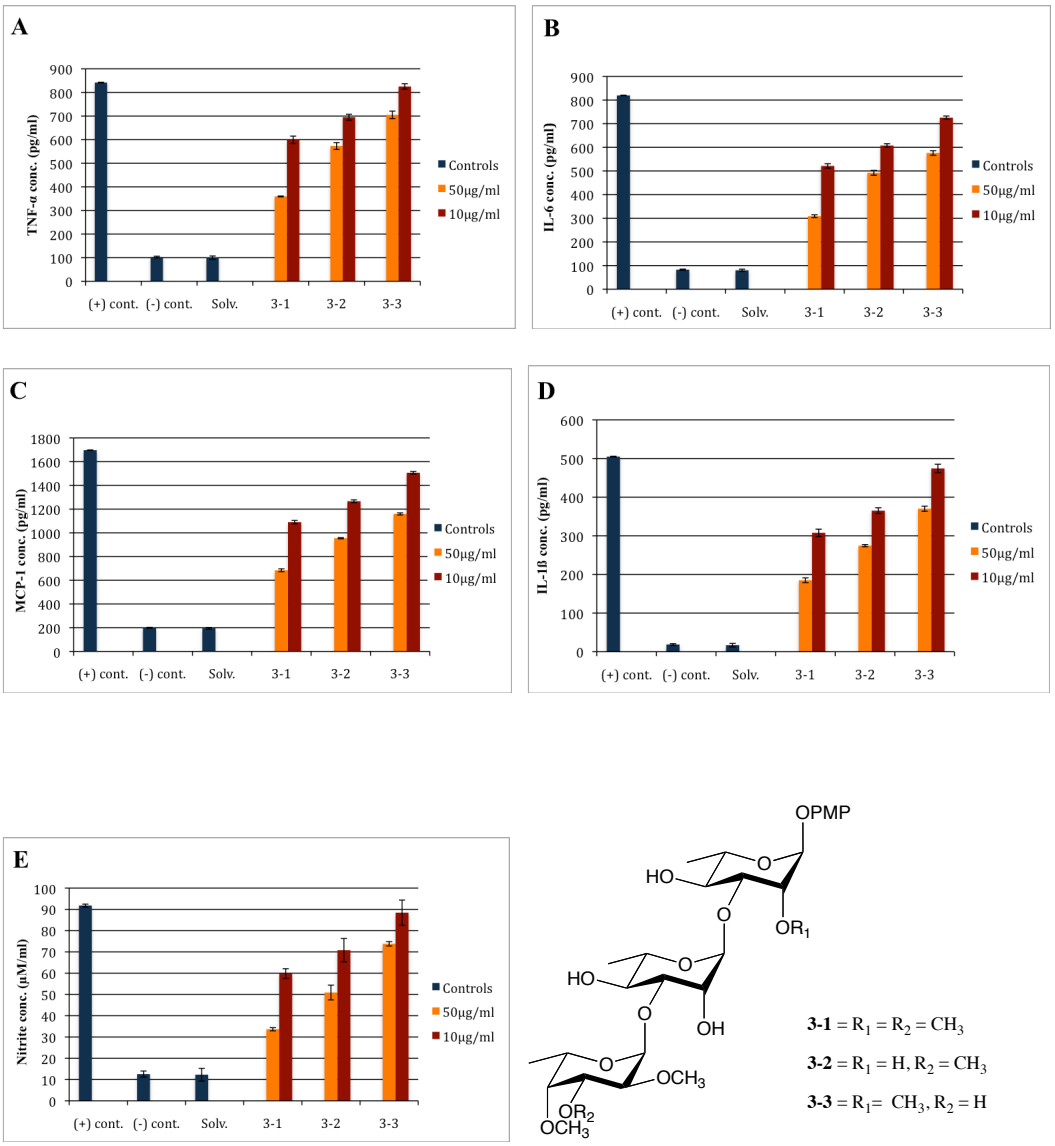


Figure 6-13. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **3-1**–**3-3**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

The possibility of targetting TLR4 was checked by using ultra pure LPS as the stimulus under the same assay conditions; the results are illustrated in Figure 6-14. As shown, none of the trisaccharides has any inhibitory activity under these conditions, which confirms our hypothesis that TLR2, not TLR4, is the target for these highly methylated glycans.

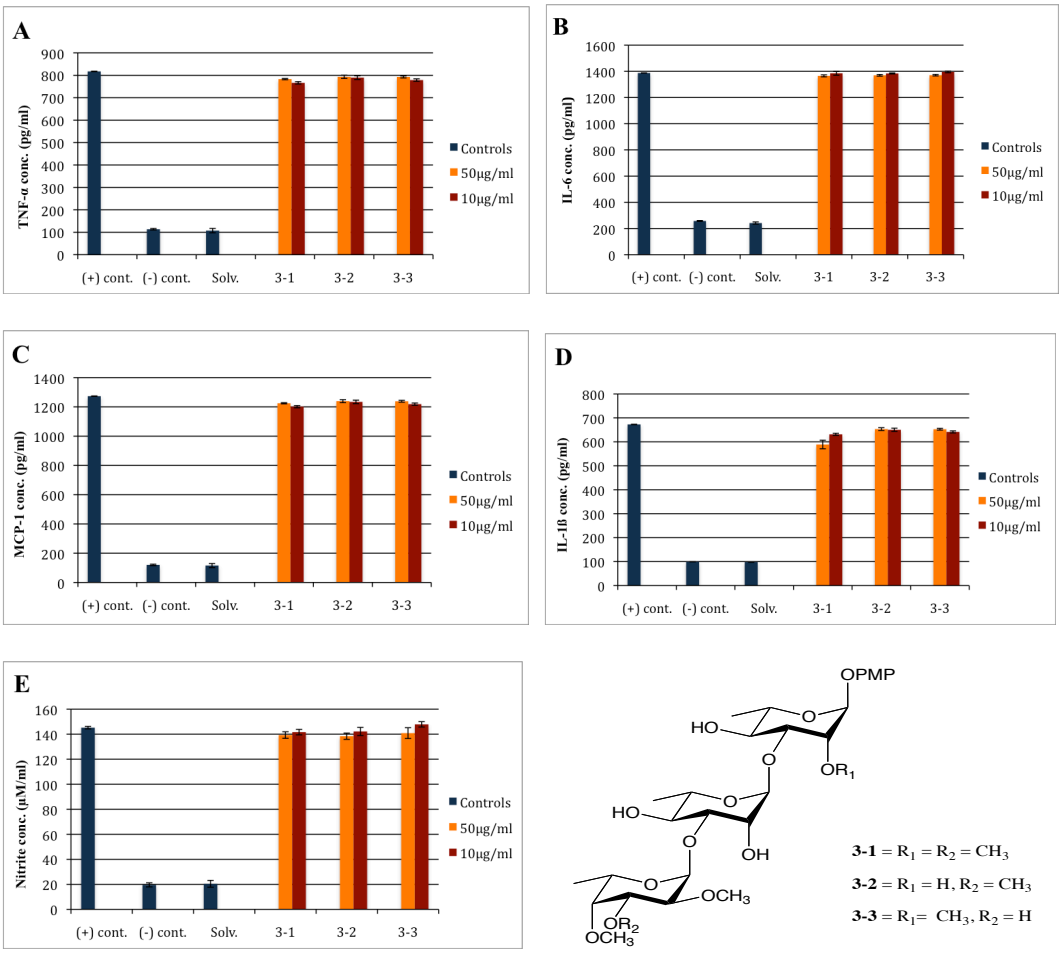


Figure 6-14. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon tretament with the test compounds, **3-1–3-3**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4).

Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

A graphical summary of the results of screening compounds **3-1–3-3** is presented in Figure 6-15.

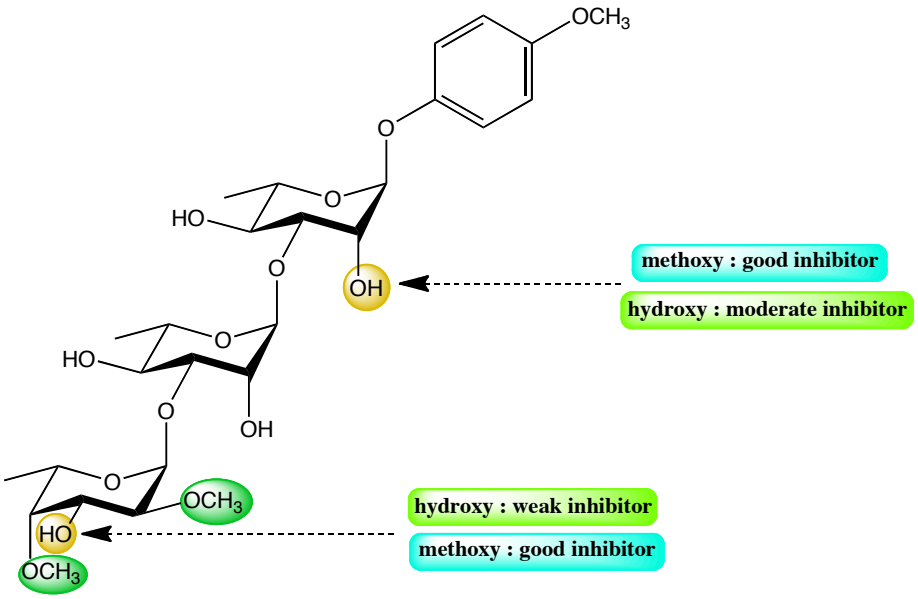


Figure 6-15. Summary of the cytokine release inhibition activity of trisaccharides **3-1–3-3**. "Weak inhibitor" refers to inhibitory activity < 30% compared to positive control, "Moderate inhibitor" refers to inhibitory activity < 50% compared to the positive control and "Good inhibitor" refers to inhibitory activity > 50% compared to the positive control and used at a concentration of 50 µg/mL of the tested compound. Groups in green color were kept as methyl groups as they are in the native PGLs. When a group in yellow is hydroxyl, the other group in yellow is methoxy by default.

6.4.2.4. Results from *M. kansasii* synthetic PGL analogs

Keeping in mind the main goal of this study, to immunologically profile PGLs, the Series I synthetic analogs of PGLs from *M. kansasii*, **4-1–4-4**, were tested using the same set of the three assays used before. The results from the first assay where LPS was used as a stimulus are shown in Figure 6-13.

Trisaccharides **4-1–4-4** are very similar in structure to the *M. tuberculosis* PGLs, differing only in methylation pattern, and, in two cases, possessing acylation on the terminal residue. All compounds in this series lack the methyl group at O-3 of the fucose moiety. Therefore, based on the results with **3-1**–**3-3** (Section 6.4.3.3), it was expected that the activity of these compounds would be dramatically lower than compound **3-1**, the most potent inhibitor of the *M. tuberculosis* PGL analogs.

This expectation was shown to be correct. Compounds **4-3** and **4-4** are virtually inactive while **4-1** and **4-2** possess low levels of inhibitory activity. Trisaccharide **4-1**, which lacks methyl group at both O-4 and O-4″ showed very weak inhibition. On the other hand, analog **4-2** showed a weak to moderate inhibition of the release cytokines. This compound still lacks O-4″ methyl, but has a methyl group at O-4. This suggests that adding a methyl group to this position on the reducing end residue increases the inhibitory activity. Compounds **4-3** and **4-4** differ from **4-2** by the presence of an ester group at O-4″ (acetate in **4-3** and propionyl in **4-4**). The relative lack of activity of these compounds could be due to the size of the acyl group, which prohibits the binding of the glycan to the receptor. If one postulates that the receptor has a hydrophobic pocket that binds

to the terminal fucose residue, it is possible that OCH_3 groups are tolerated, but that larger substituents are not.

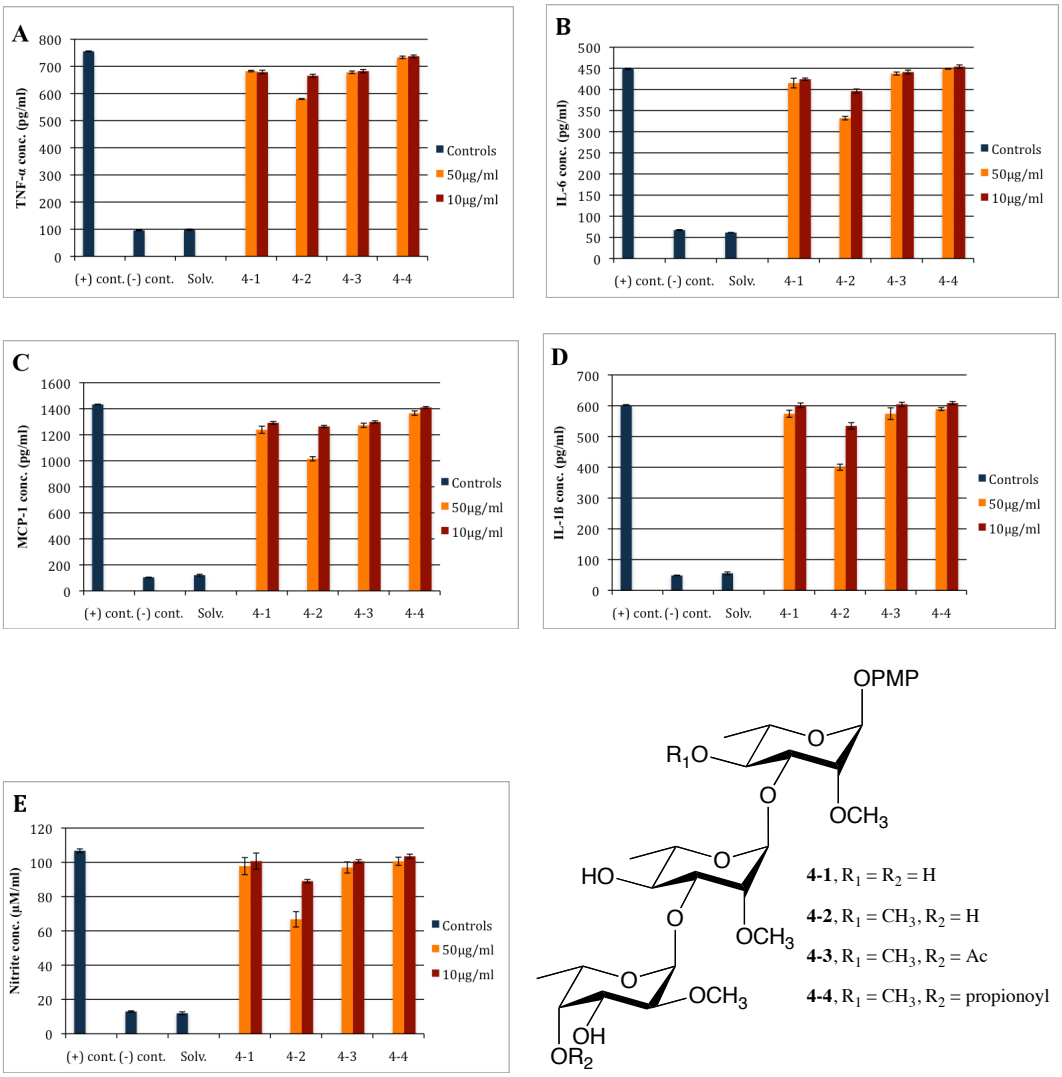


Figure 6-16. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-1**–**4-4**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine

levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

As illustrated in Figure 6-17, this series showed the same trend when they were tested using Pam3CSK4 as a stimulus. However, since trisaccharide **4-2** showed the same moderate inhibition activity, 33–39% inhibition at 50 µg/mL, this confirms that it exerted its action via TLR2.

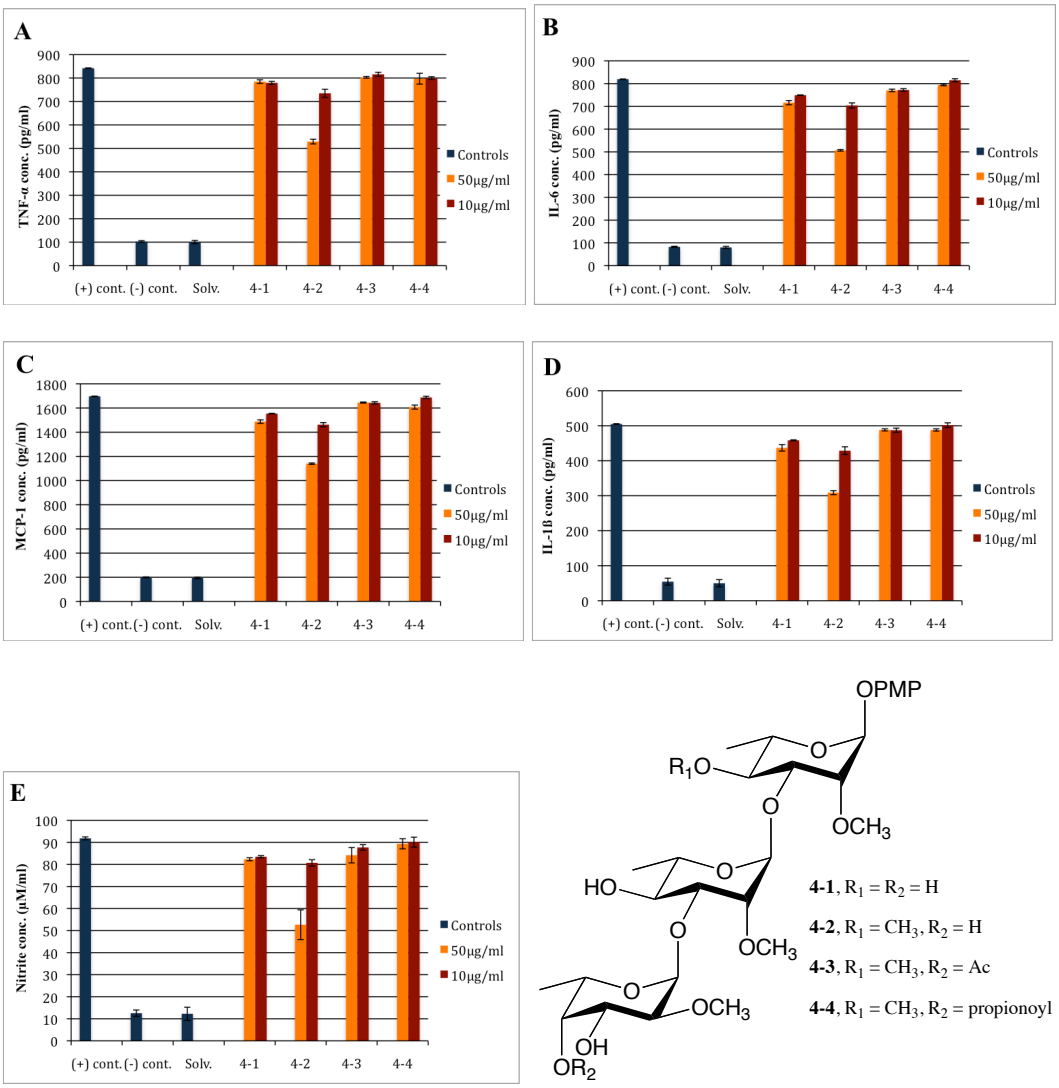
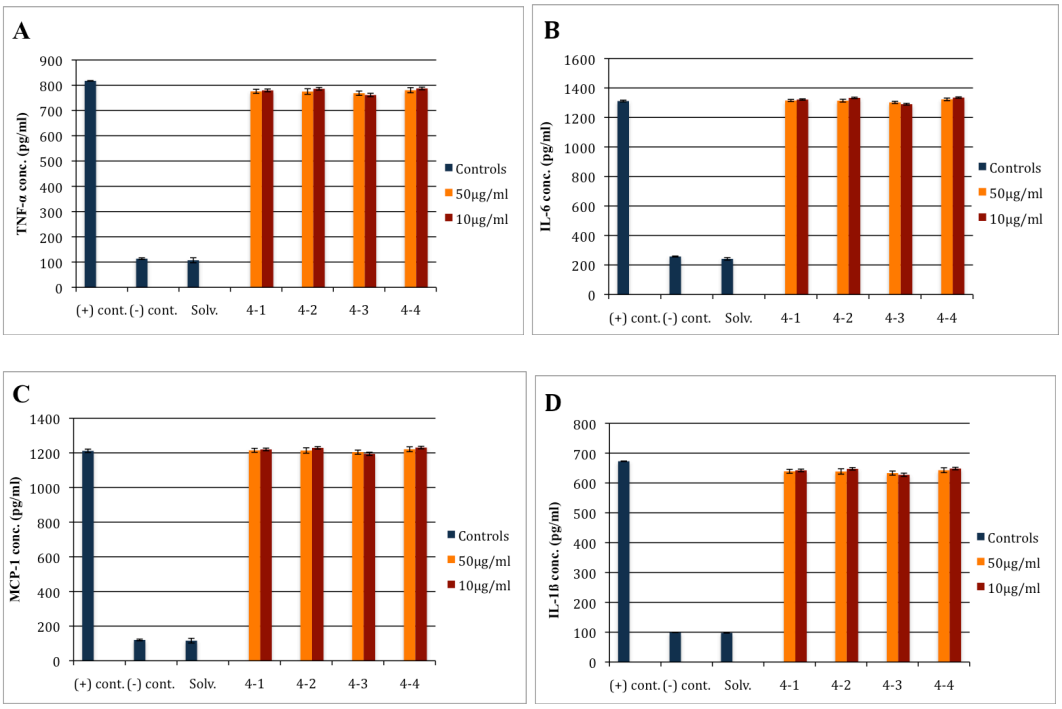


Figure 6-17. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **4-1**–**4-4**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

The final experiment was conducted using ultra pure LPS as a stimulus to check the activity on TLR4. As expected, none of the tested analogs even compound **4-2** showed any activity, which confirms our theory that TLR2 is the target receptor for these analogs and not TLR4. These results are illustrated in Figure 6-18.



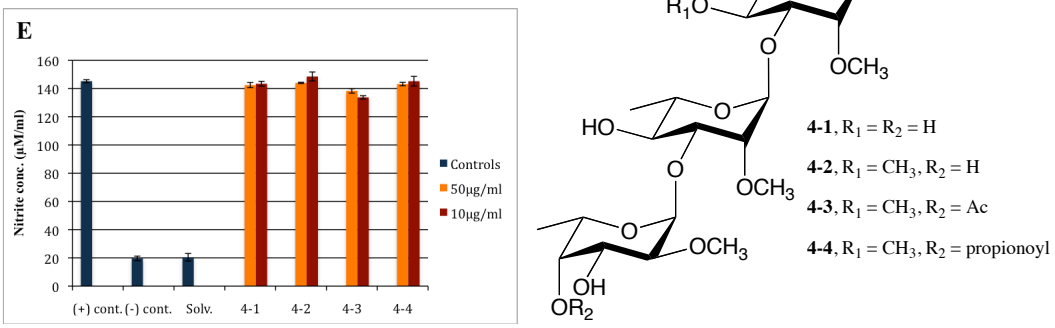


Figure 6-18. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-1**–**4-4**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

To sum up the results of these assays, a graphical summary of the results of screening compounds **4-1**–**4-4** is presented in Figure 6-19.

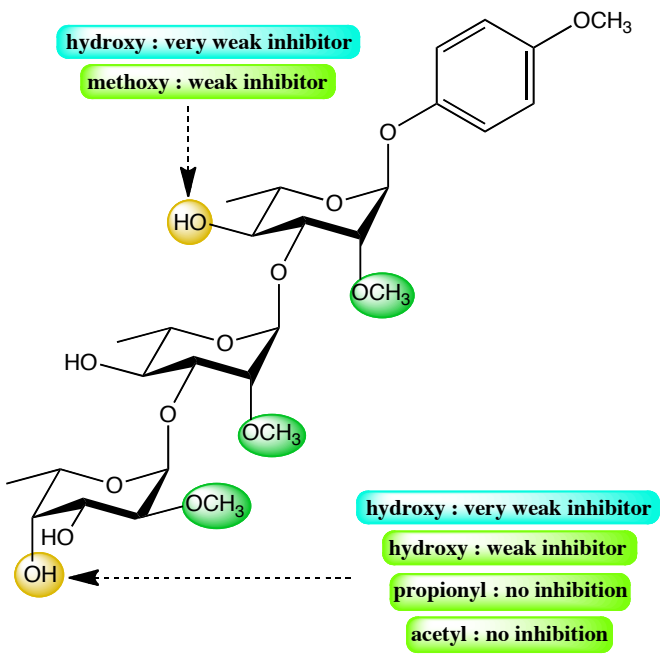


Figure 6-19. Summary of the cytokine release inhibition activity of trisaccharides **4-1**–**4-4**. Groups in green color were kept as methyl groups as they are in the native PGLs.

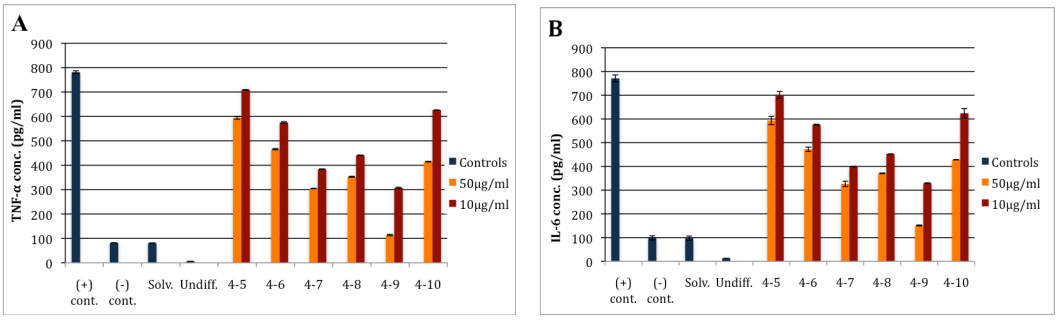
A second series of tetrasaccharides analogs, **4-5**–**4-10** (Series II compounds) were next evaluated. These tetrasaccharides are similar in structure to **3-1**–**3-3** and **4-1**–**4-4**, in that they all share an α-L-Fuc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p* core. However, they differ in methylation (alkylation) pattern and by the addition of a 6-deoxy-D-mannopyranose residue that is α-(1→3)-linked to the fucose residue. Compounds **4-6** and **4-8** are unnatural analogs, which contain a propyl group at O-4″. These analogs could be conveniently synthesized and were prepared to evaluate the importance of the substituent at this position.

The use of LPS as a stimulus (Figure 6.20) in the presence of the test compounds revealed that a concentration-dependent inhibitory pattern similar to those seen with the other compounds was obtained. The activity is also dependent

on the methylation pattern of the molecule. Tetrasaccharide **4-9** showed the highest activity as it inhibited 81–86% of the cytokine release from the stimulated macrophages. This molecule has all hydroxyl groups methylated except O-4′ and O-3‴.

Removal of the O-4″ methyl group in **4-9**, leading to analog **4-7**, resulted in a 23–40% loss of activity on the tested cytokines at 50 µg/mL concentration. This confirms the results obtained on **3-1**–**3-3** and **4-1**–**4-4** that the optimal substituent at O-4″ is a methyl group. Another confirmation of this proposal is that **4-8**, which has a propyl group at this position, is less active compared to **4-9** (52–55% inhibition of the cytokine release). Despite having an alkyl group at O-4″, the lower activity of tetrasaccharide **4-8** appears to be due to the larger size of the propyl group compared to a methyl group in **4-9**.

Analogs **4-6** and **4-10**, which differ in the substituents at O-4″ and O-2‴ are similarly potent inhibitors (~45% inhibition of cytokine release). It appears that these changes in substitution patterns exert a subtle effect upon their ability to inhibit cytokine release. Finally, compound **4-5**, which, compared to **4-9**, lacks methyl groups at O-4″ and O-2‴ has the lowest level of activity, indicating the importance of these two hydrophobic groups on inhibitory activity.



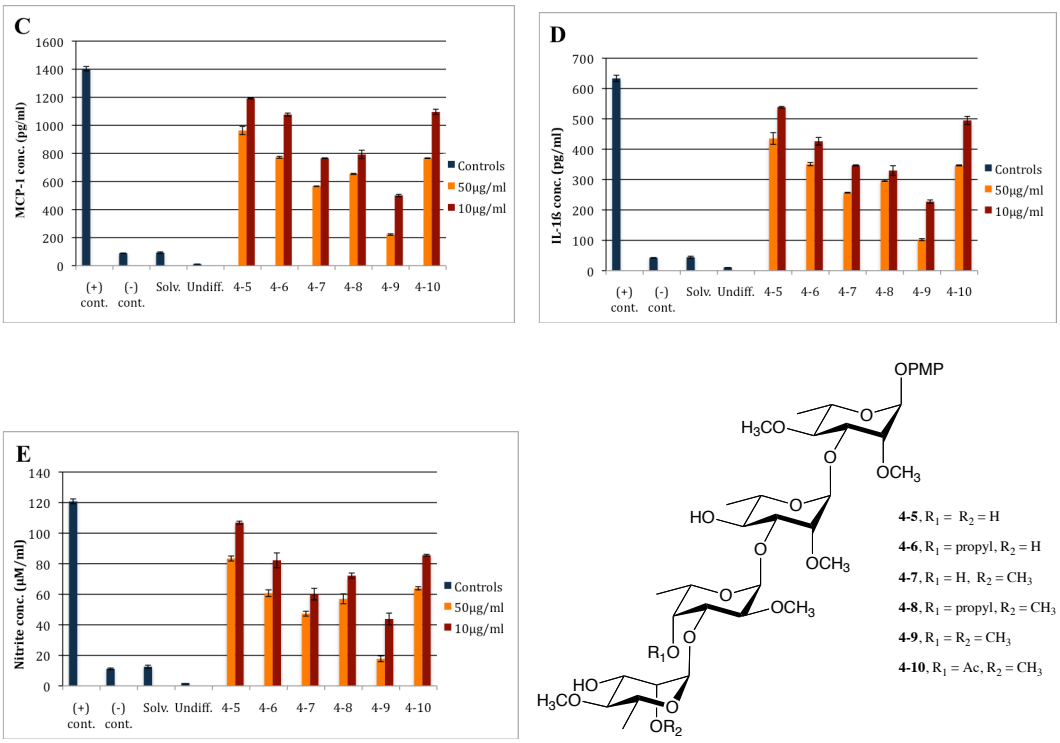


Figure 6-20. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-5**–**4-10**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

Following the same approach, another experiment was conducted using the same conditions using Pam3CSK4 as the stimulus to check the potential activity through TLR2. As shown in Figure 6-21 the same pattern of activity was obtained as in the previous experiment. Tetrasaccharide **4-9** is the most active,

resulting in up to 90% inhibition of cytokine release, and analog **4-5** is the least active. The same results suggest that these molecules target TLR2.

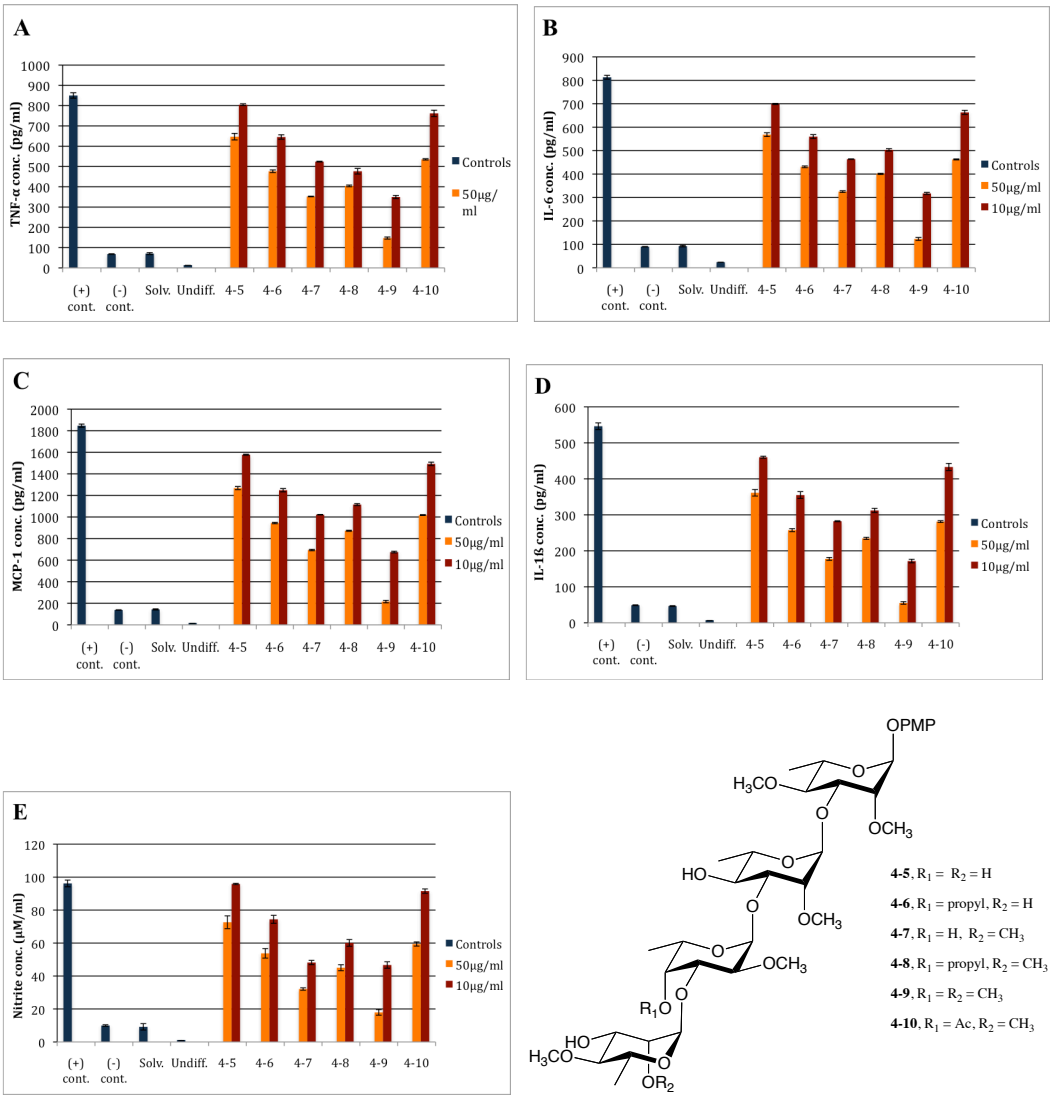


Figure 6-21. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **4-5**–**4-10**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to

incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

No activity was observed when ultra pure LPS was used as the stimulus, again indicating that these molecules do not produce their effect via TLR4 and suggesting that TLR2 is the site of action. The results of this experiment are shown in Figure 6-22.

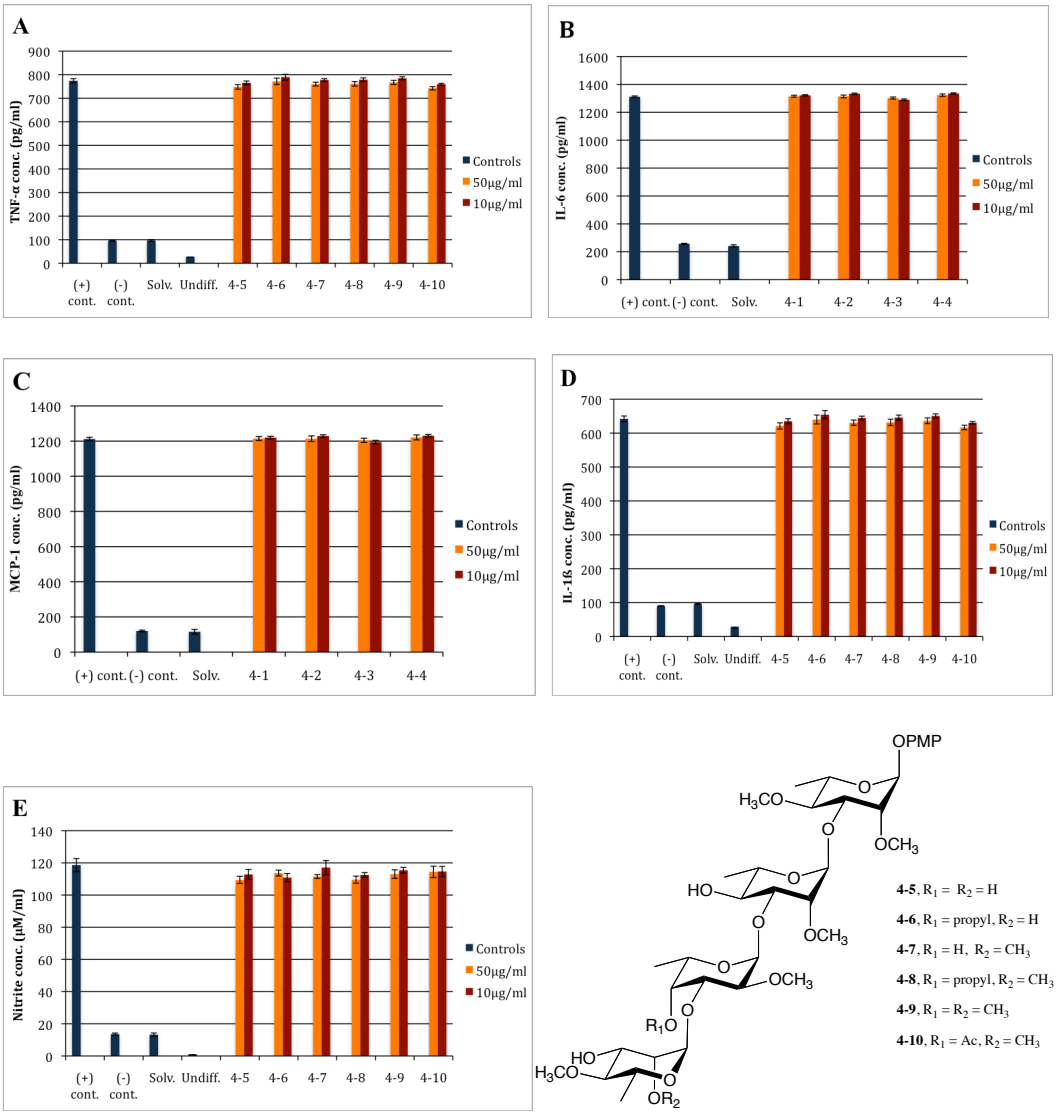


Figure 6-22. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-5–4-10**. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented. Non of the tested compounds showed any inhibition of the cytokine release from the stimulated macrophages.

Figure 6-23 is a graphical summary of the results of screening tetrasaccharides **4-5–4-10**.

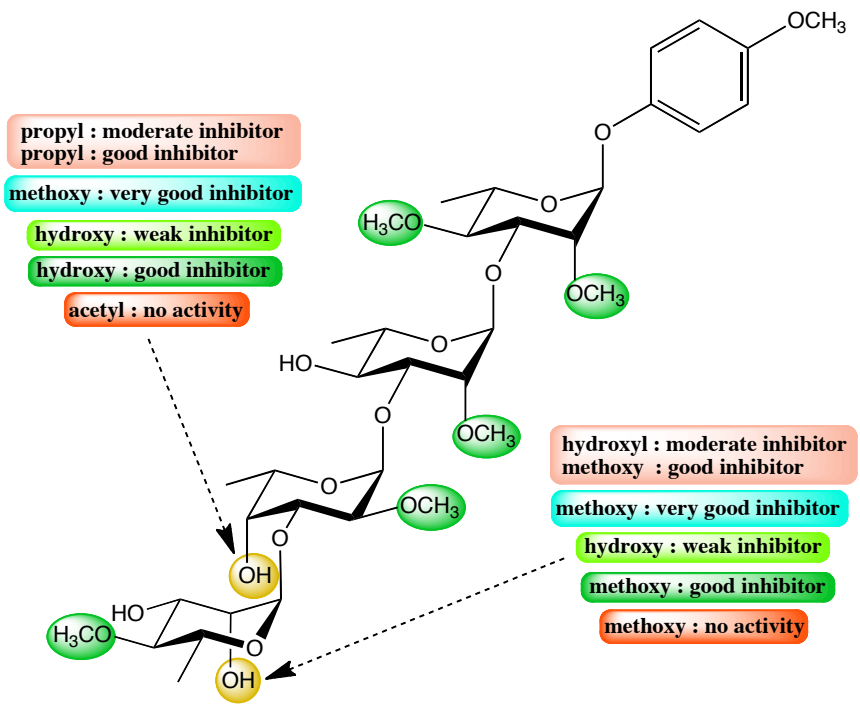


Figure 6-23. Summary of the cytokine release inhibition activity of tetrasaccharides **4-5–4-10**. "Weak inhibitor" refers to inhibitory activity < 25% compared to positive control, "Moderate inhibitor" refers to inhibitory activity < 60% and > 25% compared to the positive control and "Good inhibitor" refers to inhibitory activity > 60% compared to the positive control and used at a concentration of 50 μg/mL of the tested compound. Groups in green color were kept as methyl groups as they are in the native PGLs.

Another group of analogs, **4-11–4-17** (Series III targets), were next tested. These compounds differ from the Series II compounds **4-5**–**4-10** in that the 6-deoxy-α-D-mannopyranose residue is replaced with α-D-mannopyranose. Initial evaluation of these compounds using LPS stimulation revealed that compound **4-16,** showed the highest level of inhibition (42–50%). This compound lacks the O-4″ methyl group, but is methylated at both O-2‴ and O-4‴. Replacing the methyl group at O-4″ with a propyl group (compound **4-17**), resulted in a significant loss of activity. Compound **4-11**, which is not methylated at O-4″, O-2‴ or O-4‴ showed only weak inhibition activity. This result underscores the important role of methylation of these positions for the activity. Both compounds **4-12** and **4-13** have a propyl group at O-4″ and they showed weak activity, which agrees with the results obtained previously with compounds **4-6** and **4-8**. Moreover, the absence of O-4″ methyl and O-2‴ or O-4‴ methyl groups resulted in the same effect of lowering the activity as shown for compounds **4-14** and **4-15**. Finally, the very weak-to-moderate activity of this series indicated that dimethylated 6-deoxymannose unit is a better capping motif than a dimethylated mannose residue

when compared with the activities obtained from the Series II compounds (**4-5–4-10**).

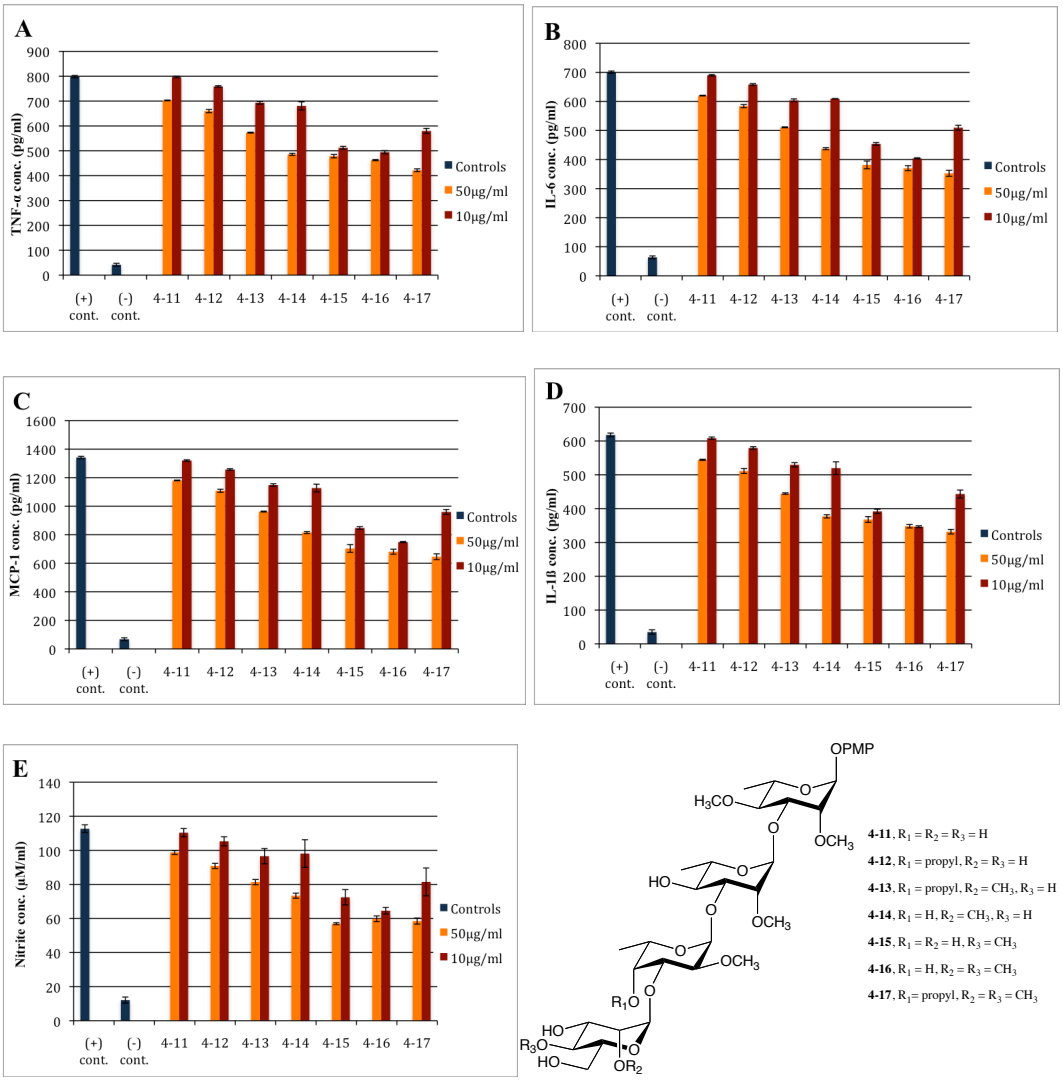


Figure 6-24. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-11–4-17**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed

twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

As we moved to the second assay where Pam3CSK4 was used as the stimulus, this series maintained the same trend of activity, which indicated that these compounds might be targeting TLR2. The results of this assay are shown in Figure 6-25.

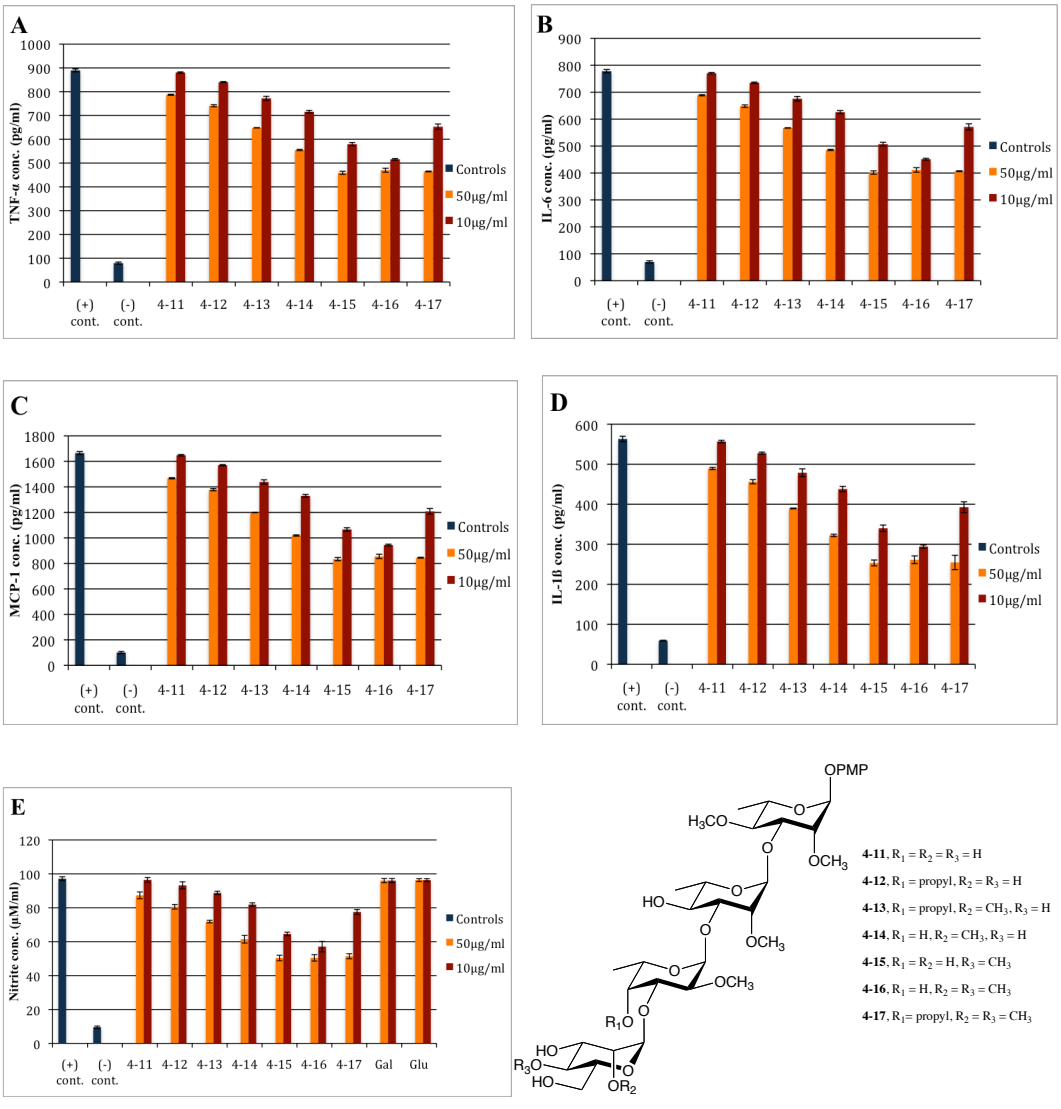
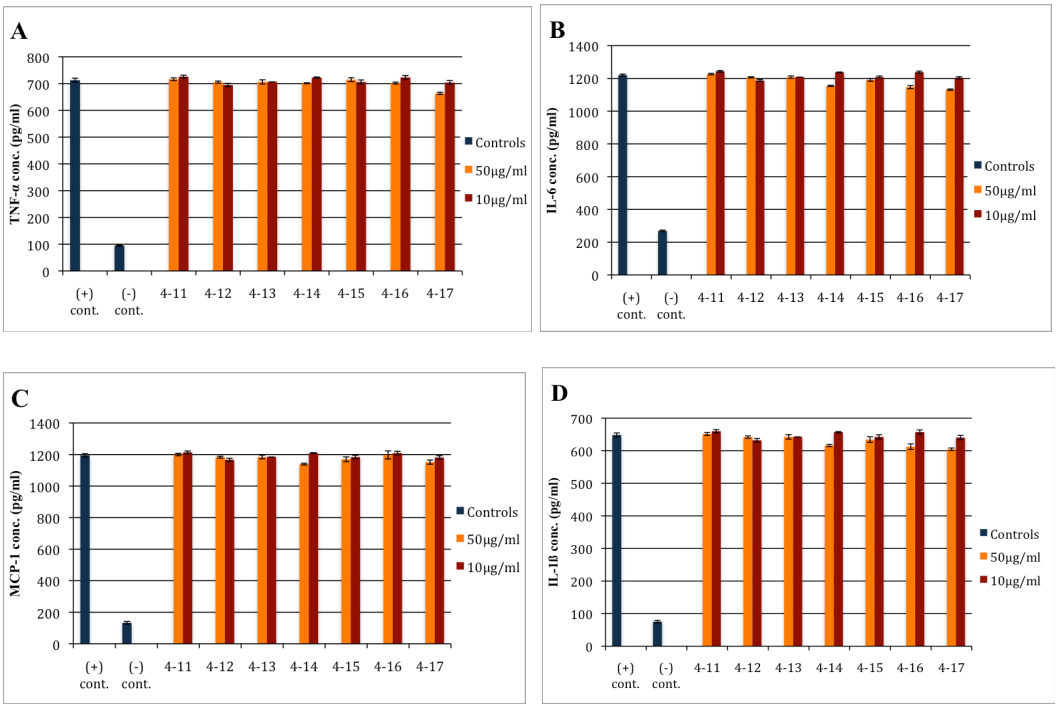


Figure 6-25. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **4-11–4-17**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

As illustrated in Figure 6-26, no activity was obtained from any of the tested analogs of this series in the third assay, ultra pure LPS was used as the stimulus, which indicated that none of them has an effect on TLR4.



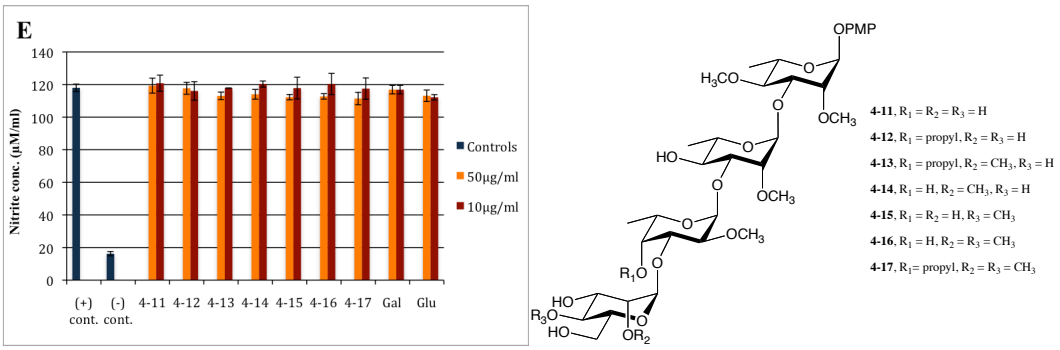


Figure 6-26. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **4-11–4-17**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate is presented.

Shown in Figure 6-27 a graphical summary of the testing results of compounds **4-11–4-17.**

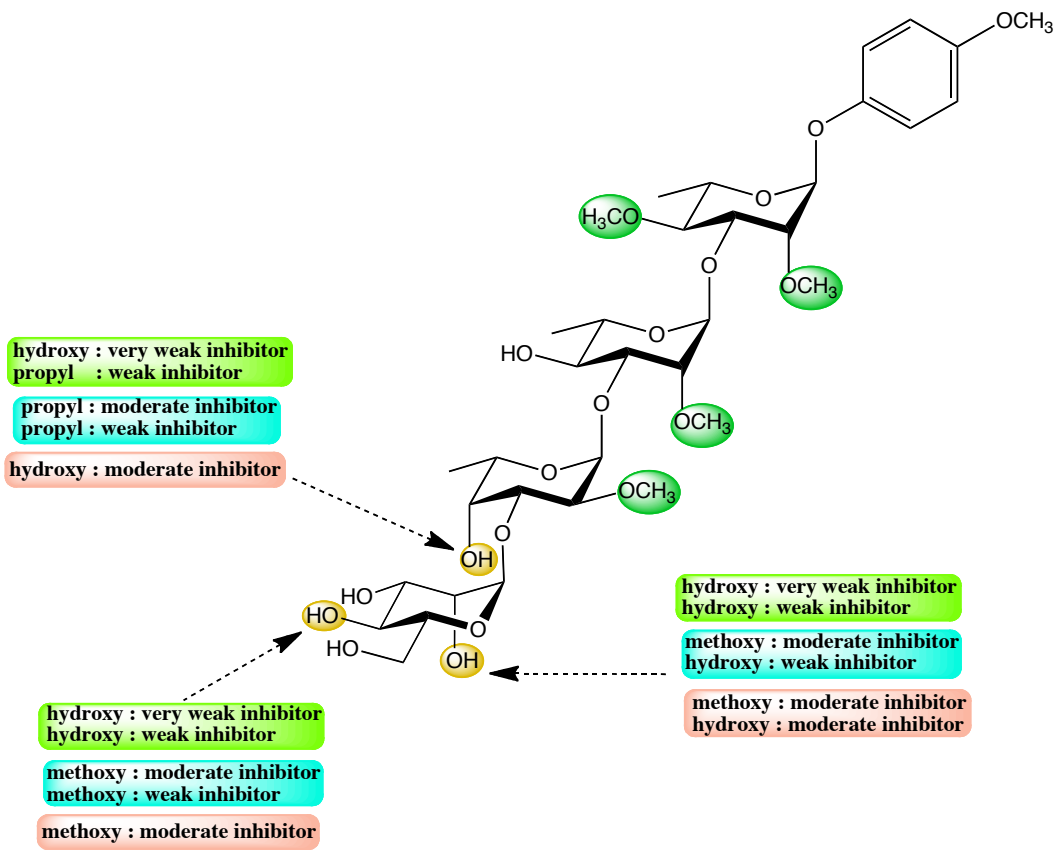


Figure 6-27. Summary of the cytokine release inhibition activity of tetrasaccharides **4-11–4-17**. "Very weak" refers to inhibitory activity < 12 % compared to the positive control, "Weak inhibitor" refers to inhibitory activity < 25% compared to positive control, "Moderate inhibitor" refers to inhibitory activity < 60% and > 25% compared to the positive control and used at a concentration of 50 μg/mL of the tested compound. Groups in green color were kept as methyl groups as they are in the native PGLs.

6.4.2.5. Results from gluco- and galactopyranoside standards.

We considered it important to probe the effect of the *p*-methoxyphenyl group on the observed activity. Therefore, two control compounds consisting of glucose (Glu) and galactose (Gal) linked to an α-linked *p*-methoxyphenyl moiety were synthesized. As shown in Figure 6-28–6-30, neither of these compounds

showed any inhibition activity with any of the three stimulants, which confirms that the inhibition effects we observe with the PGL analogs are not due the *p*-methoxyphenyl moiety. This result also agrees with the results obtained from testing of the analogs from *M. bovis*, which showed that all tested monosaccharides were inactive.

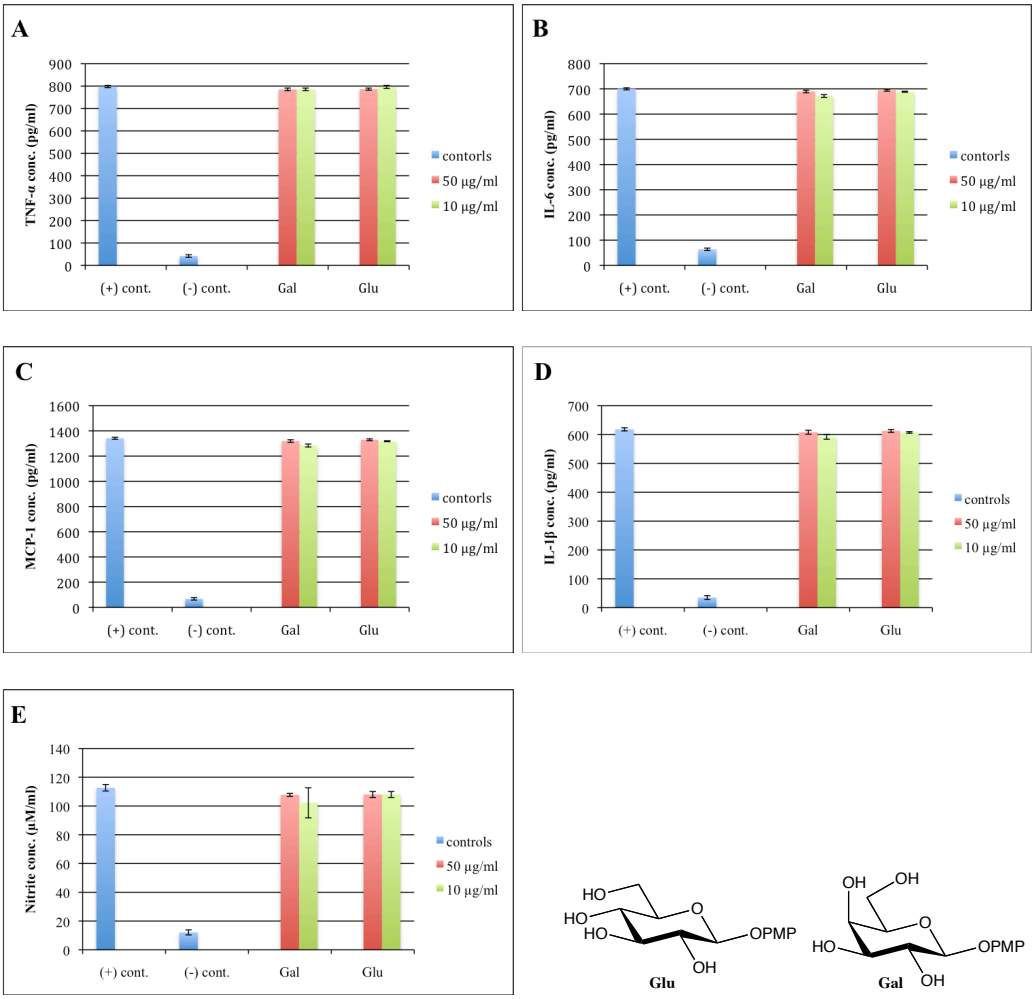


Figure 6-28. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the standard compounds, **Gal** and **Glu**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS

from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

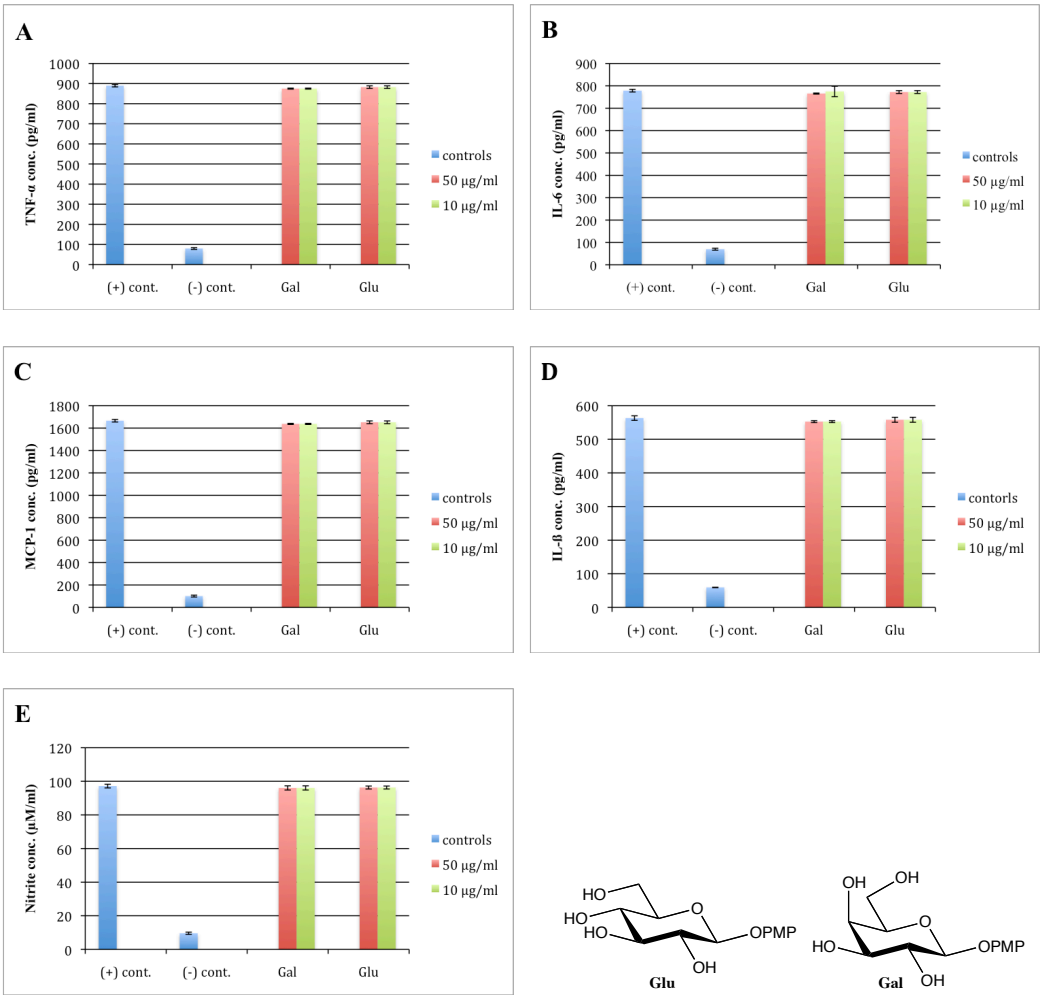


Figure 6-29. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the standards compounds, **Gal** and **Glu**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in

RPMI, at two concentraions of 10 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

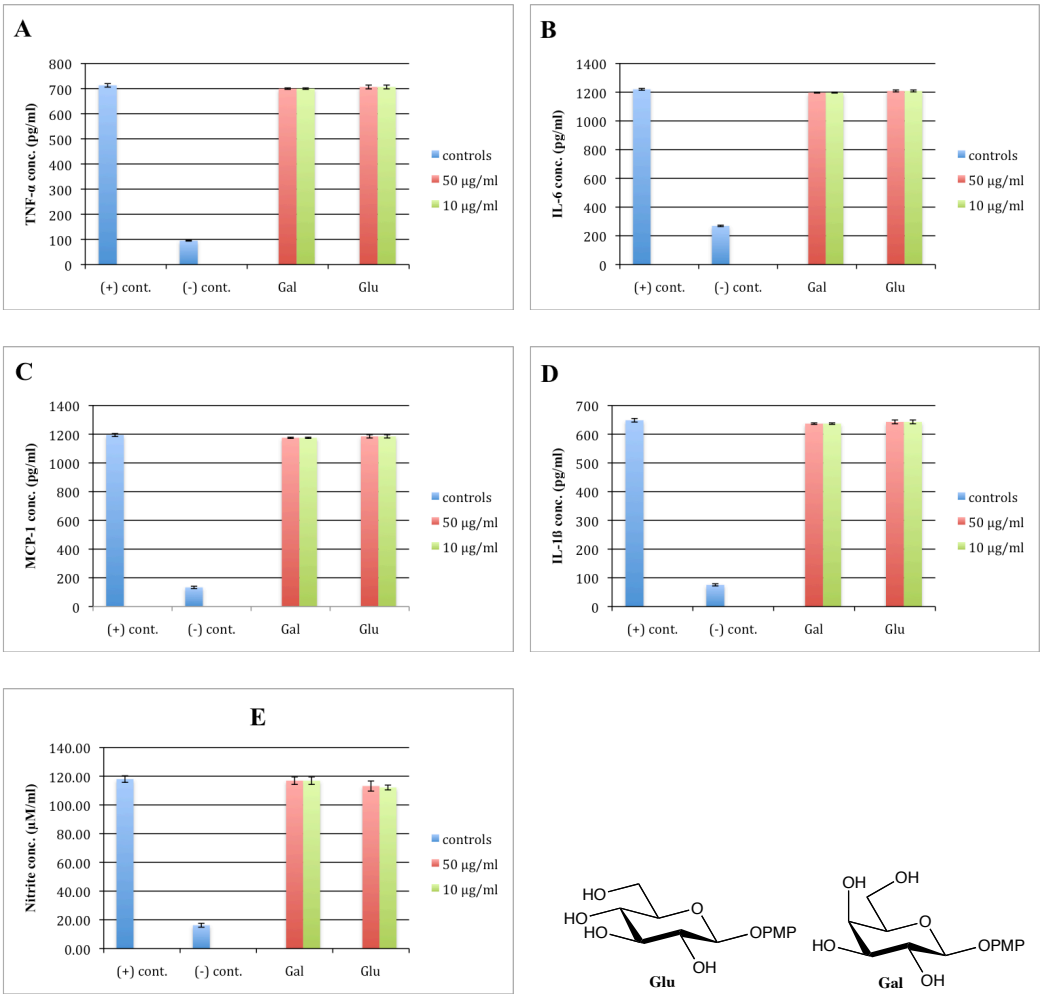
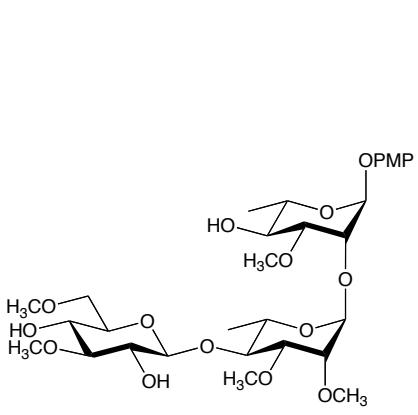


Figure 6-30. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the standard compounds, **Gal** and **Glu**. Gal refers to galactose and Glu refers to glucose. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at two concentraions of 10 and 50 µg/mL and

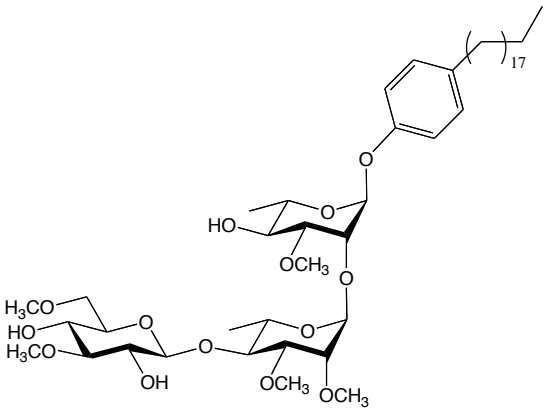
allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate is presented.

6.4.2.6. Results from glycolipids 5-1 and 5-2.

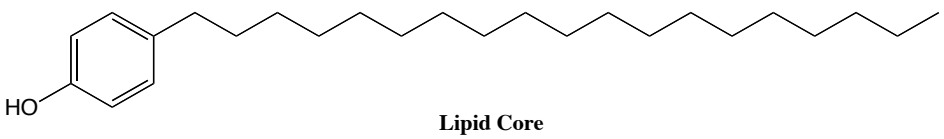
To further extend the scope of our study, we wanted to investigate the effect of the lipid core on the immunomodulatory activity of these analogs. Therefore, a simple lipid core was added to the two most active compounds **2-1** and **4-9** to generate glycolipids **5-1** and **5-2** (Figure 6-31, See Chapter 5 for the synthesis of these compounds). Then, the immunomodulatory activity of **5-1** and **5-2** was tested and compared to **2-1** and **4-9** as well as to the native PGL-I.



2-1



5-1



Lipid Core

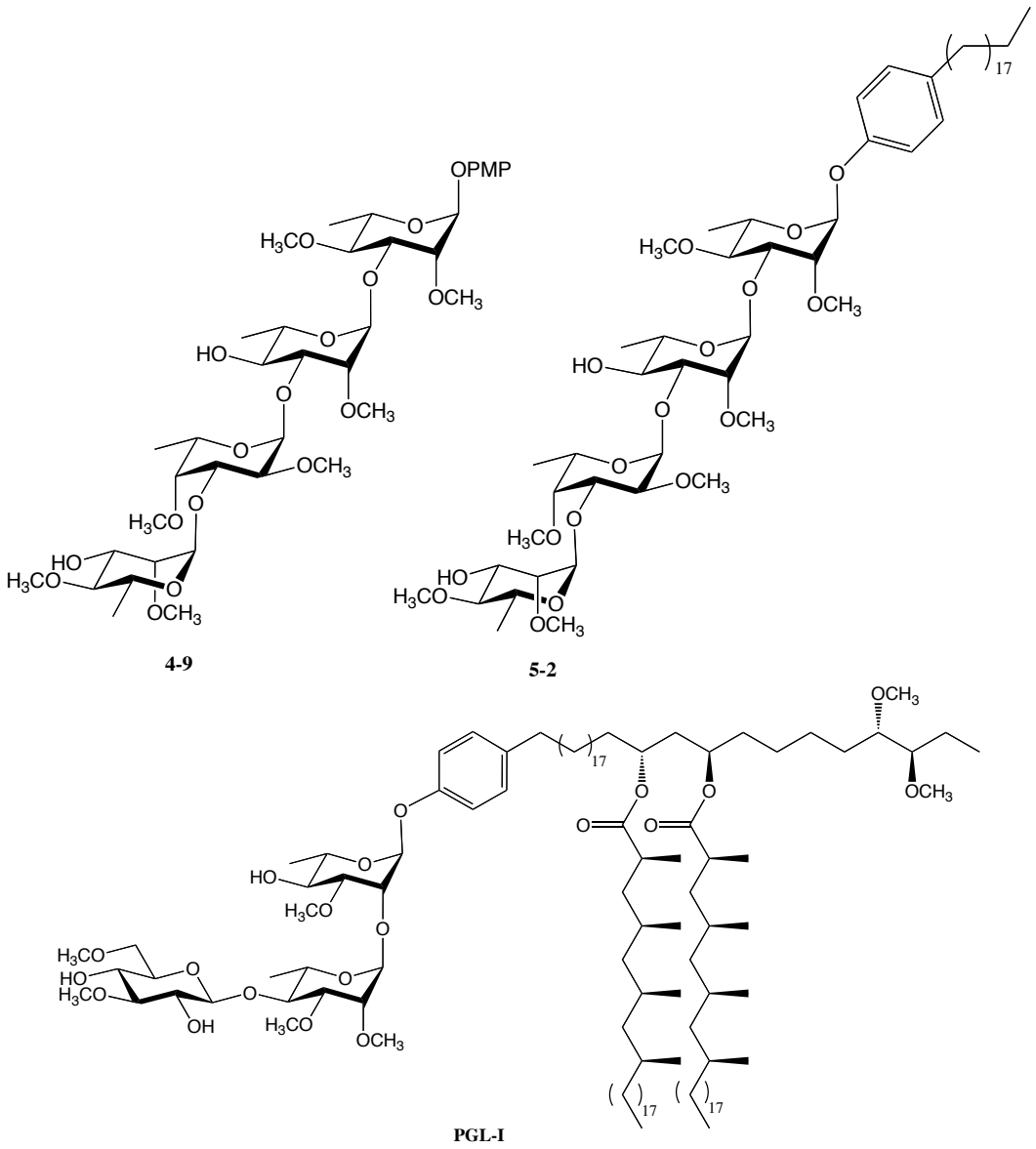


Figure 6-31. Structures of **2-1**, **4-9**, **5-1**, **5-2**, **PGL-I** and the lipid core.

Figure 6-32 illustrates the results obtained from the assay where LPS was used as the stimulus. As shown, the lipid core itself did not show any immunomodulatory activity. This indicated that the activities exerted by the synthetic PGLs require the presence of the carbohydrate moiety. The results

further demonstrated that all tested compounds showed a concentration-dependent inhibition properties when tested at concentrations of 10, 25 and 50 μg/mL. The addition of the lipid core to compound **2-1** to generate compound **5-1** resulted in an ~20% increase in activity. Compound **5-2** showed an ~15% increase in activity compared to **4-9**, which is due to the addition of the lipid core. Furthermore, PGL-I was found to be about 2.5 fold more active than glycolipid **5-1**, although the difference between the two molecules is only in the nature of the lipid core. Similar results were observed with compound **5-2**, the most active synthetic analog, which was found to be ~40% less active than the native PGL-I.

The conclusion of these results is that although the lipid core does not have any immunomodulatory activity by itself, its addition to the carbohydrate domain significantly increases the activity. Moreover, adding a simple lipid core did not increase the activity as much as the native one did. Because we found that TLR2 is a possible target to these molecules, these results could be explained by proposing that the addition of the lipid core makes these molecules better TLR2 ligands. This receptor is known to recognize glycolipids and there could be some recognition elements in the native lipid core that makes PGL-I better recognized than the analog with the simple lipid core, glycolipid **5-1**. Another conclusion is that the lipid core does not alter the pattern of activity, but rather affect the potency.

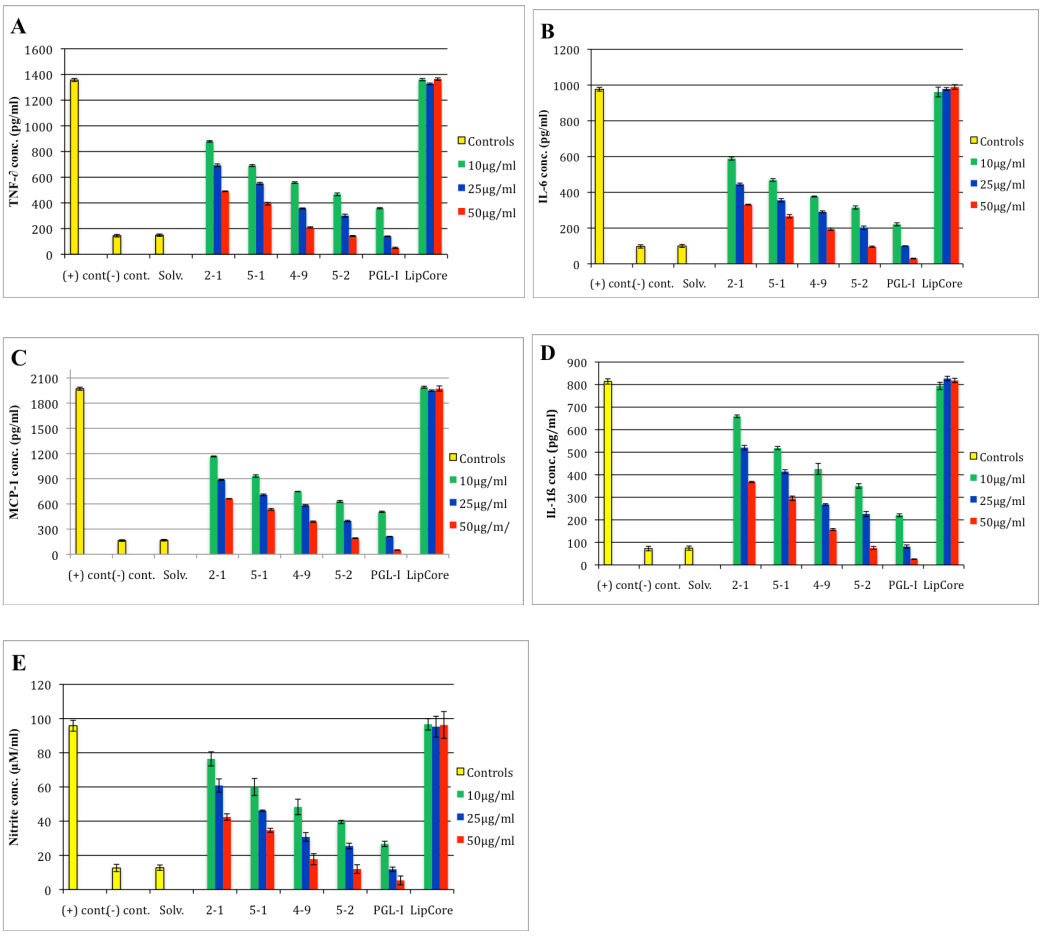


Figure 6-32. Concentration of cytokines produced from LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **2-1**, **4-9**, **5-1**, **5-2** and PGL-I. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of LPS from *E. coli* (A.T.C.C. 12408). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at three concentraions of 10, 25 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

The same pattern of immunomodulatory activity was obtained upon using Pam3CSK4 as the stimulus in another assay and the results are illustrated in

Figure 6-33. These results demonstrated that our hypothesis about TLR2 as a possible target for PGLs remains valid.

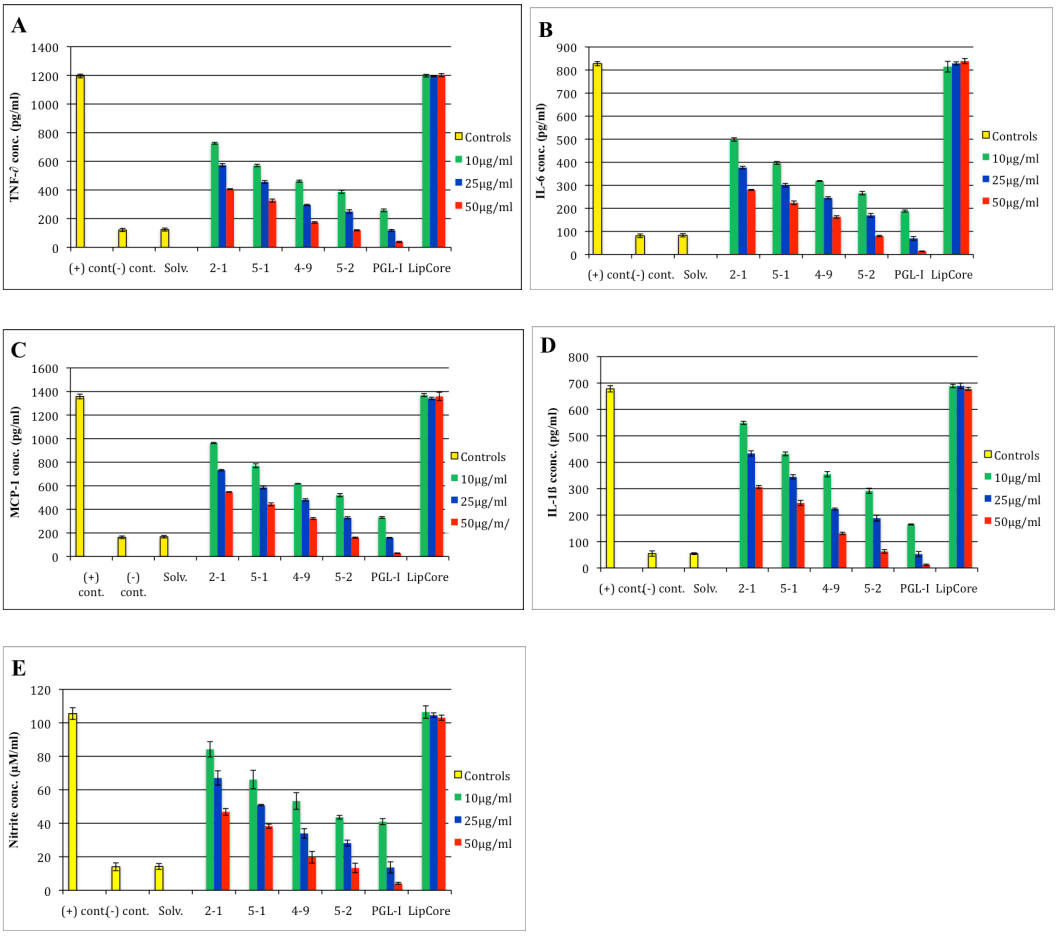


Figure 6-33. Concentration of cytokines produced from Pam3CSK4-stimulated A-THP-1 cells upon treatment with the test compounds, **2-1**, **4-9**, **5-1**, **5-2** and PGL-I. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of Pam3CSK4. Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at three concentraions of 10, 25 and 50 µg/mL and allowed to incubate for 24 h. Supernatants were collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented.

To add another confirmation that only TLR2 is the target for these molecules, a third assay was performed using ultra pure LPS as the stimulus and the results are shown in Figure 6-34. Having had no activity from any of the tested compounds indicated that TLR4 is not the receptor site for these molecules.

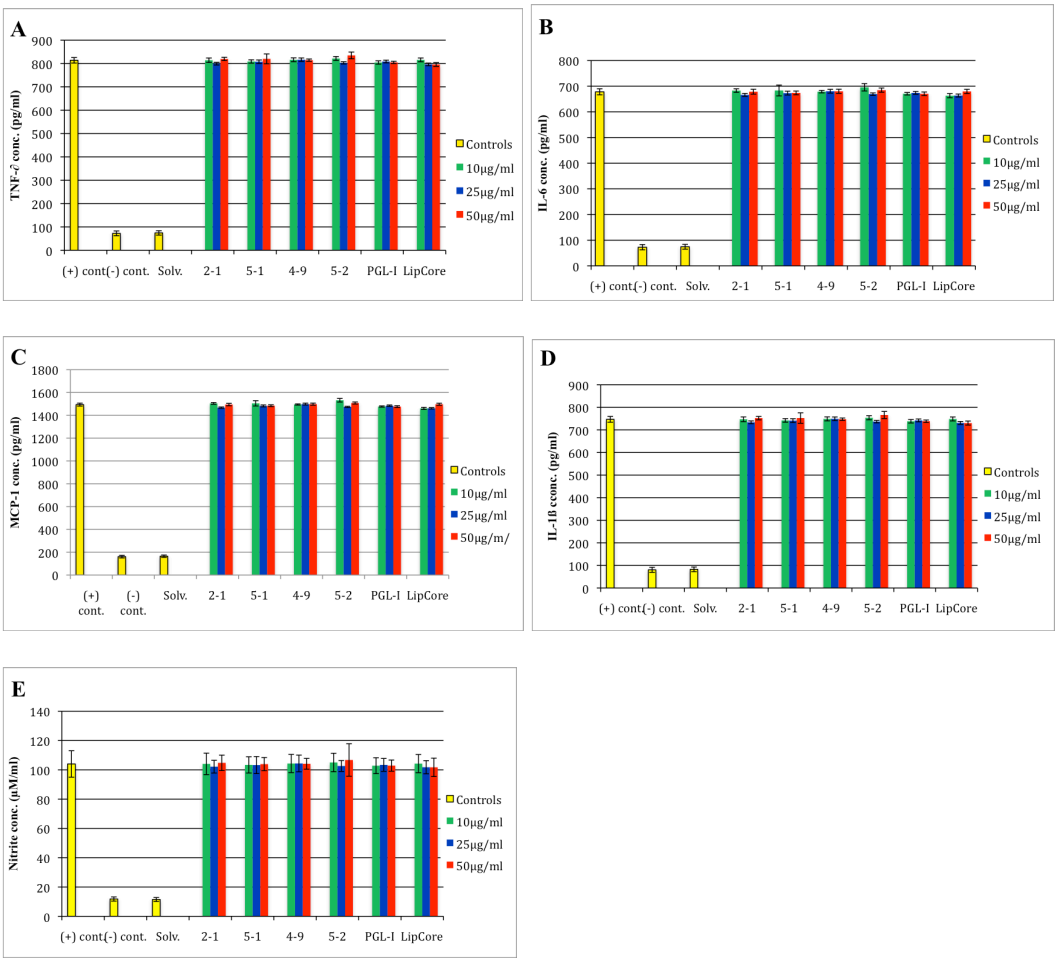


Figure 6-34. Concentration of cytokines produced from ultra pure LPS-stimulated A-THP-1 cells upon treatment with the test compounds, **2-1**, **4-9**, **5-1**, **5-2** and PGL-I. THP-1 cells were differentiated into mature macrophages, activated THP-1 (A-THP-1), via treatment with 5 ng/mL of PMA for 18 h and then stimulated with 25 ng/mL of ultra pure LPS from *E. coli* (0111:B4). Test compounds were added to the medium, dissolved in 0.1% DMSO in RPMI, at three concentraions of 10, 25 and 50 μg/mL and allowed to incubate for 24 h. Supernatants were

collected and assayed for cytokine levels using ELISA. Each experiment was performed twice and representative data (± S.D.) from a single experiment performed in triplicate are presented. None of the tested compounds showed any activity.

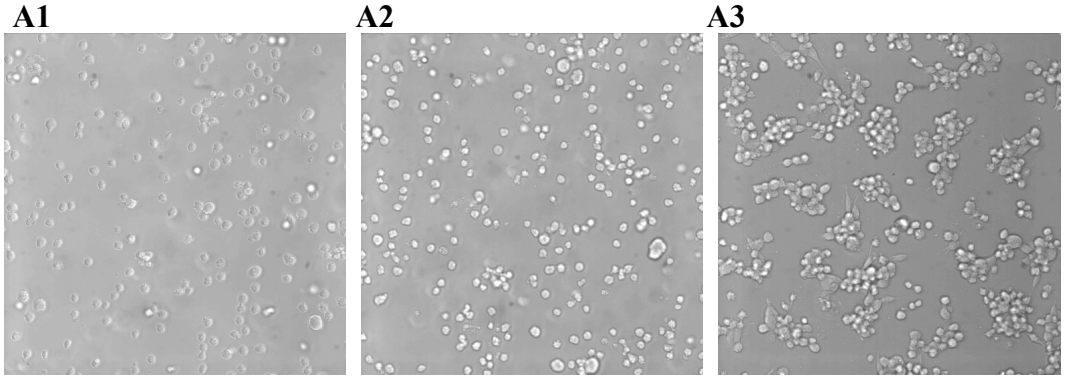
6.4.3. Potential apoptotic activity of the synthetic analogs and PGL-I

We also wanted to investigate if these molecules can stimulate apoptosis, using unstimulated A-THP-1 cells. The rational behind this is that we wanted to make sure that the effects we are seeing are due to the immunoinhibitory potential of the test compounds and not due to the induction of apoptosis. Since caspases are very important proteolytic enzymes that are closely associated with apoptosis, hence the choice of FLICA Apoptosis Detection Kit as a measure for apoptosis through the detection of many caspases activity (caspases-1, -3, -4, -5, -6, -7, -8, -9).[78] The poly-caspases FLICA apoptosis detection kit contains a green fluorescent-labeled inhibitor, which is a carboxyfluorescein derivative of valylalanylaspartic acid fluoromethyl ketone, a potent inhibitor of caspases activity.

In this assay, in addition to the polycaspases-labeling reagent, two other stains are used. One, the Hoechst stain, is used to label dying cells blue with bright white nuclei and the normal cells as blue. The other staining agent, propidium iodide, is used to label necrotic and dead cells red. Furthermore, the polycaspases-positive cells will be labeled green by FLICA reagent.

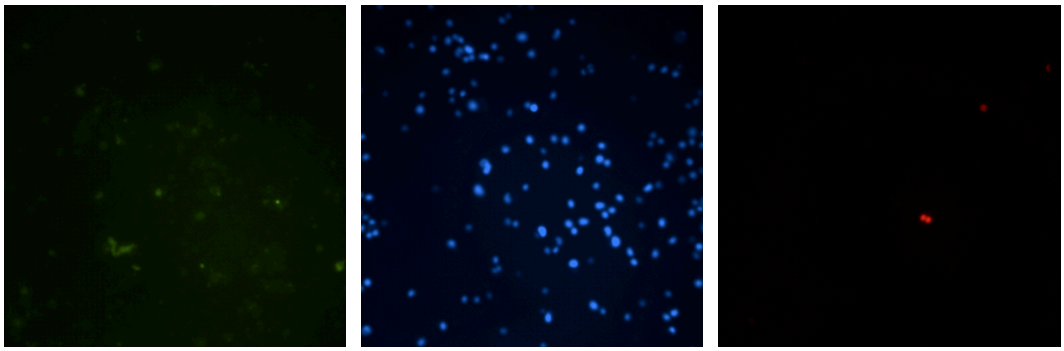
The results of this assay, illustrated in Figure 6-35, showed that none of the tested compounds induced apoptosis or necrosis of the A-THP-1 cells. The

positive control (LPS stimulated cells) showed green fluorescence in channel 1, which indicates apoptosis. The bandpass filter of channel 1 of the fluorescence microscope was adjusted to excitation of 490 nm and emission of >520 nm to view the green fluorescence of the caspases positive cells. In channel 2, a UV-filter with excitation at 365 nm and emission at 480 nm was used to view the dying cells stained blue with bright white nuclei, while normal cells stained only blue. All cells in this channel were stained blue with some having bright white nuclei indicating that these cells are dying. In channel 3, the bandpass filter was adjusted to excitation of 488-490 nm and emission at 635 nm to view the propidium iodide red-stained necrotic cells. Only a very few red cells, which is indicative of necrosis, are present. The negative control is the undifferentiated cells, which showed neither green nor red staining, indicating neither apoptosis nor necrosis. All examples of the tested compounds showed no green or red staining and all cells were stained blue without bright nuclei in channel 2.

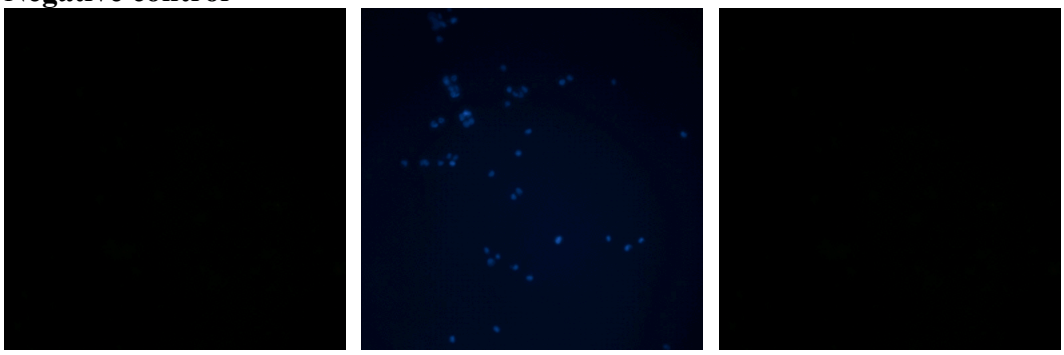


B1 **B2** **B3**

Positive control

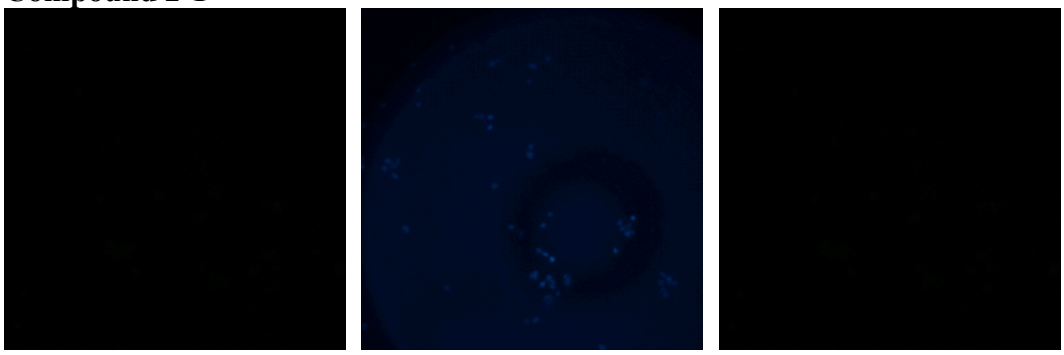


Negative control

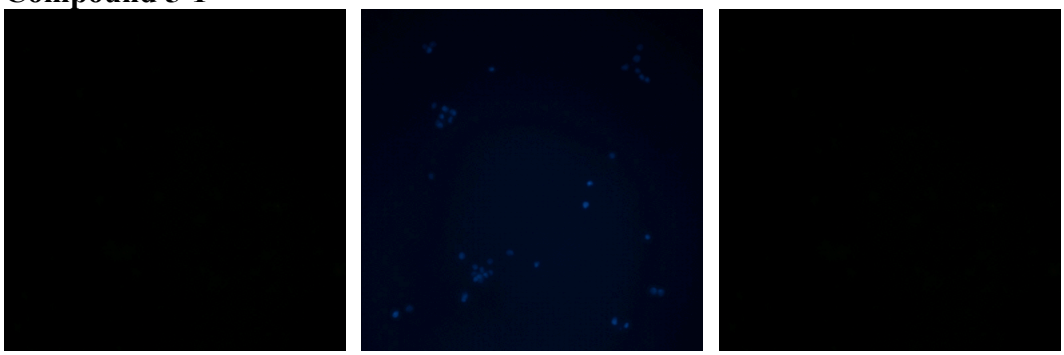


PGL-I

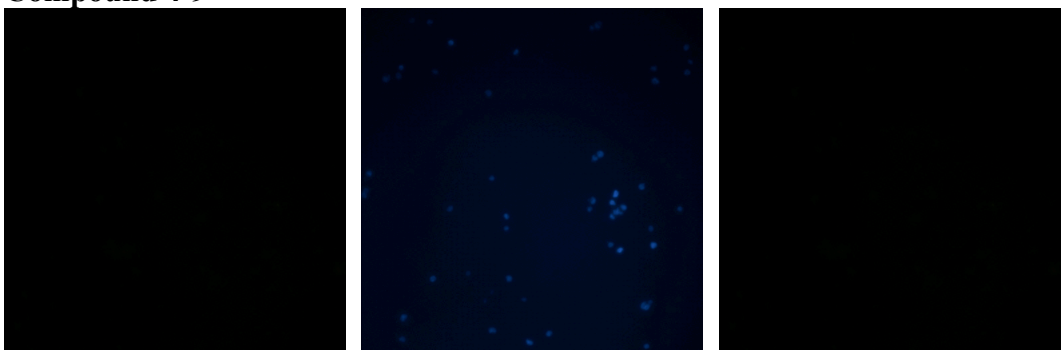
Compound 2-1



Compound 5-1



Compound 4-9



Compound 5-2

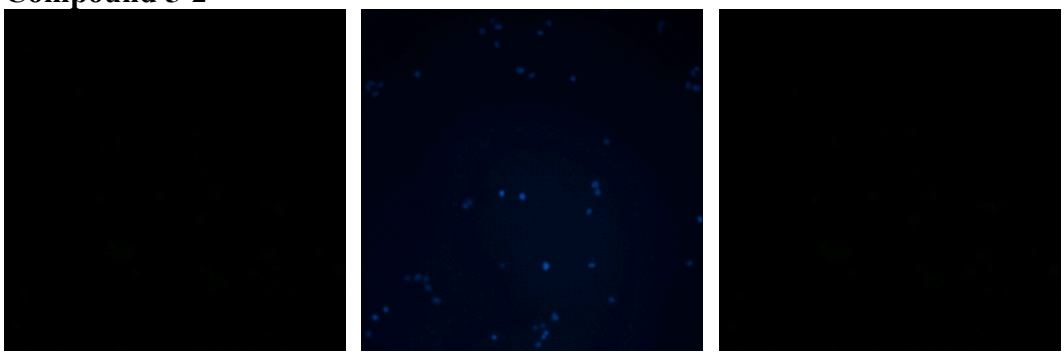


Figure 6-35. Representative examples apoptosis assay results. A1 is the undifferentiated suspension of THP-1 cells. A2 is THP-1 cells after 5 h treatment with PMA where cells have started to differentiate and convert into adherent cells. A3 is the activated (differentiated) THP-1 (A-THP-1) after 24 h treatment with PMA where all cells had converted into adherent mature macrophages. B1 is channel 1 of a fluorescence microscope where the UV filter was adjusted to capture green fluorescence from apoptotic cells (excitation at 488 nm and emission at 520 nm). B2

is channel 2 where the UV filter was adjusted to capture the blue fluorescence from the Hoechst stain (excitation at 365 nm and emission at 480 nm). B3 is channel 3 where the UV filter was adjusted to capture the red staining of propidium iodide (excitation at 490 nm and emission at 637 nm.

6.5. Conclusion

In this chapter, we performed immunological profiling of all the synthetic analogs as well as the native PGL-I. The results revealed that none of the tested synthetic analogs showed any immunostimulatory activity. However, most of the tested analogs as well as native PGL-I demonstrated a unique immunoinhibitory pattern, which is related to the methylation pattern of the molecule. Furthermore, these results revealed that monosaccharides are not active and at least a disaccharide structure is needed to obtain any activity. Compound **4-9**, which is a heavily methylated tetrasaccharide with a 6-deoxymannose capping motif showed the highest activity.

Another conclusion is that adding a simple lipid core to the most active two compounds to provide glycolipids **5-1** and **5-2** did not affect the pattern of activity, but increased the potency. However, the native PGL-I still shows higher activity than both **5-1** and **5-2**, which suggested that the native lipid core is better recognized by the receptor, and thus results in more activity than corresponding analogs with the simpler nonadecylphenyl or *p*-methoxyphenyl aglycones. Furthermore, the testing results showed that neither the synthetic analogs nor the native PGL-I binds to TLR4 and suggest instead that TLR2 is the receptor. Finally, the compounds were also tested for their ability to induce apoptosis. The

results revealed that neither the synthetic analogs nor the native PGL-I showed the ability to induce apoptosis.

6.6. Experimental

6.6.1. Chemicals, reagents and culture medium

Lipopolysaccharide (LPS) from *E. coli* (A.T.C.C. 12408), a TLR2/TLR4 agonist, and native PGL-I were generous gifts from Professor David R. Bundle at the University of Alberta, and the Leprosy Research Materials Contract at Colorado State University, respectively. Ultrapure LPS from *E.coli* 0111:B4, a TLR4 agonist, and the synthetic bacterial lipoprotein (Pam3CSK4), a TLR2/TLR1 agonist, were purchased from Invivogen (San Diego, USA). BD OptEIA human IL-1β, IL-6, MCP-1, TNF-α and IL-10 assay kits were purchased from BD Biosciences (Mississauga, Canada). The FLICA polycaspases kit for measuring apoptosis was purchased from ImmunoChemistry Technologies, LLC (Bloomington, USA). The Griess Reagent System was purchased from Promega (Madison, USA).

6.6.2. Cell culture

The human myeloid THP-1 monocyte/macrophage human cell line was a generous gift from Dr. Daniel R. Barreda and Dr. Christopher W. Cairo at the University of Alberta. The cells were maintained in continuous culture with RPMI 1640 with 10 mM L-glutamine (Gibco) supplemented with 10% fetal bovine serum (FBS) US source (Gibco), 100 units/mL penicillin and 100 μg/mL

streptomycin Pen Strep (Gibco) in an atmosphere of 5% CO_2 at 37 ºC, as a suspension of cells.

6.6.3 Cell inhibition assays

Cells were first activated into mature macrophages via treatment with PMA (Sigma) at a concentration of 5 ng/mL for 24 h[71f] and then washed three times with phosphate buffered saline (PBS). Cells were then rested for two days after the chemical differentiation to ensure that they came back to a resting phenotype before any treatment. Cells were then trypsinised, washed with and resuspended in serum-free culture medium at 5.4 x 10^5 cells/mL. After 2 h, 1 mL was transferred to a 24 well plate (BD Falcon), stimulators (LPS, Pam3CSK4, Ultra Pure LPS) and the test compounds were added in triplicate followed by incubation for 24 h at an atmosphere of 5% CO_2 at 37 °C. Unstimulated cells as well as the solvent used to dissolve the test compounds were used as negative controls while stimulated cells without the test compounds served as the positive controls. Stimulators were added at a concentration of 25 ng/mL and the test compounds were added at different concentrations of 10 or 50 µg/mL. Culture supernatants were harvested after 24 h and stored at –80 ºC for cytokine measurements using ELISA while the remaining cells were washed twice with PBS and then tested for apoptosis.

6.6.4. Cell stimulation assay

The assay was carried out exactly as the cell inhibition assay, except that cell stimulators were not added. Only the test compounds were added to the differentiated cells.

6.6.5. Cytokine production assay using ELISA

Cytokine concentrations in the culture supernatants were determined using ELISA according to the following procedure. First, microwells of the 96-well plate (BD) were coating of with 100 µL/well of the capture antibody diluted in the coating buffer (0.1 M Na_2CO_3, pH 9.5). After coating, the solution in the wells was aspirated and the wells were washed three times with ≥ 300 µL/well of the washing buffer (PBS with 0.05% Tween-20). Next, the wells were blocked using ≥ 200 µL/well of the assay diluent (PBS with 10 FBS (BD Pharmingen) pH 7.0.) and then incubated for 1 h at rt. After aspiration and washing three times as described for the coating step, samples and standard dilutions were prepared in the assay diluent. 100 µL of each sample, standard and control were then added to the appropriate well in triplicate, the plate was sealed and incubated for 2 h at rt. After samples were aspirated, wells were washed five times as described for the coating step. Then, 100 µL of the detector solution (detecting antibody conjugated to streptavidin–horseradish peroxidase reagent) was added to each well, the plate was sealed and incubated for 1 h at rt. All wells were then aspirated and washed as described for the coating step with seven total washes. Following the washing, a 100 µL of the substrate solution (tetramethylbenzidine (TMB) and hydrogen

peroxide) was added to each well, the plate was sealed and incubated for 30 min at rt in the dark. To stop the reaction after 30 min, 50 µL of the stop solution (1M H_3PO_4 or 2N H_2SO_4) was added to each well and a yellow color was produced whose intensity is dependent on the concentration. The absorbance of the resulting color was measured at 450 nm. To determine the concentrations of the samples, a standard curve was generated using the data from the standards and then the concentration of each sample was determined from the curve using the measured absorbance.

6.6.5. Nitric oxide (NO) assay

As mentioned before, nitric oxide concentration was determined in each sample via measuring the nitrite concentration in each sample using Griess reagent system. In this assay, a 50 µl of each sample as well as the nitrite standard solution was added to microwells of a 96-well plate (BD). Concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM of the nitrite standard solution were used to generate the standard curve. A 50 µl of sulfanilamide solution was added to each sample and standard well and the plate was incubated for 10 min at room temprature and protected from the light. After 10 min, another 50 µl of *N*-1-naphthylethylenediamine dihydrochloride (NED) was added to each well and the plate was incubated for another 10 min at room temprature and protected from the light. A purple/magenta color was developed and the absorbance was measured at a filter between 520 and 550 nm. The standard curve was generated from the data

of the standard solutions and then this curve was used to calculate the nitrite concentrations of the samples.

6.6.6. Apoptosis testing

After removal of the culture supernatants, the remaining adherent cells were washed three times with PBS and then 290 µL of fresh medium was added to each well and testing for apoptosis was done As follows. First, 10 µL of the FLICA reagent was added to each well, mixed well and then incubated at 37 °C under 5% CO_2 for 1 h. After 1 h, the medium was then removed and 1.5 µL of the Hoechst stain was added to 300 µL media (0.5 % v/v) and then added to each well. The plate was then incubated for 30 min at 37 °C under 5% CO_2. After incubation, cells were washed twice with 2 mL of the washing buffer and then 1.5 µL of propidium iodide stain dissolved in 300 µL media (0.5 % v/v) was then added to each well. After another incubation for 30 min at 37 °C under 5% CO_2, cells were washed twice with 2 mL of the washing buffer. Finally, cells were viewed immediately using fluorescent microscope with the specified UV filters.

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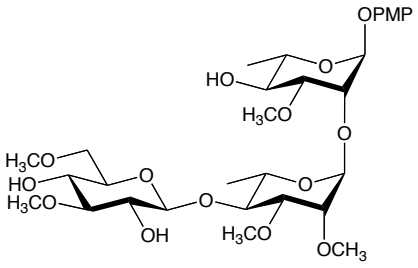
Chapter 7

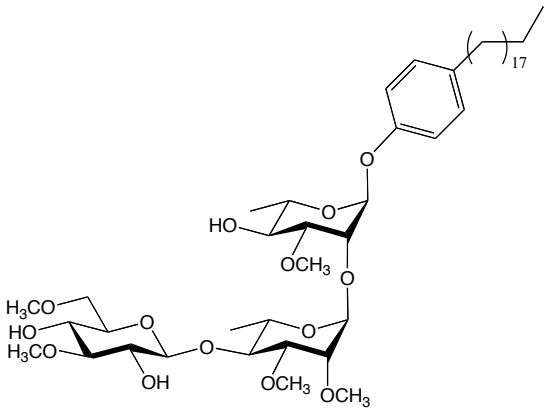
Conclusions and Future Work

7.1. Conclusions

The main goal of this study was to investigate the immunomodulatory properties of the carbohydrate domains of the mycobacterial PGLs and gain some insight into their receptor type. To fulfill this goal, a panel of synthetic analogs, **2-1–2-7, 3-1–3-3** and **4-1–4-17**, with glycan structures corresponding to all reported PGLs produced by *M. tuberculosis,*[1] *M. leprae,*[2] *M. bovis*[3] and *M. kansasii*[4] bearing a *p*-methoxyphenyl group at the reducing end were synthesized using efficient linear routes. In addition, another two glycolipids, **5-1** and **5-2**, were synthesized to study the effect of the lipid on the immunomodulatory properties (Figure 7-1).



2-1



5-1

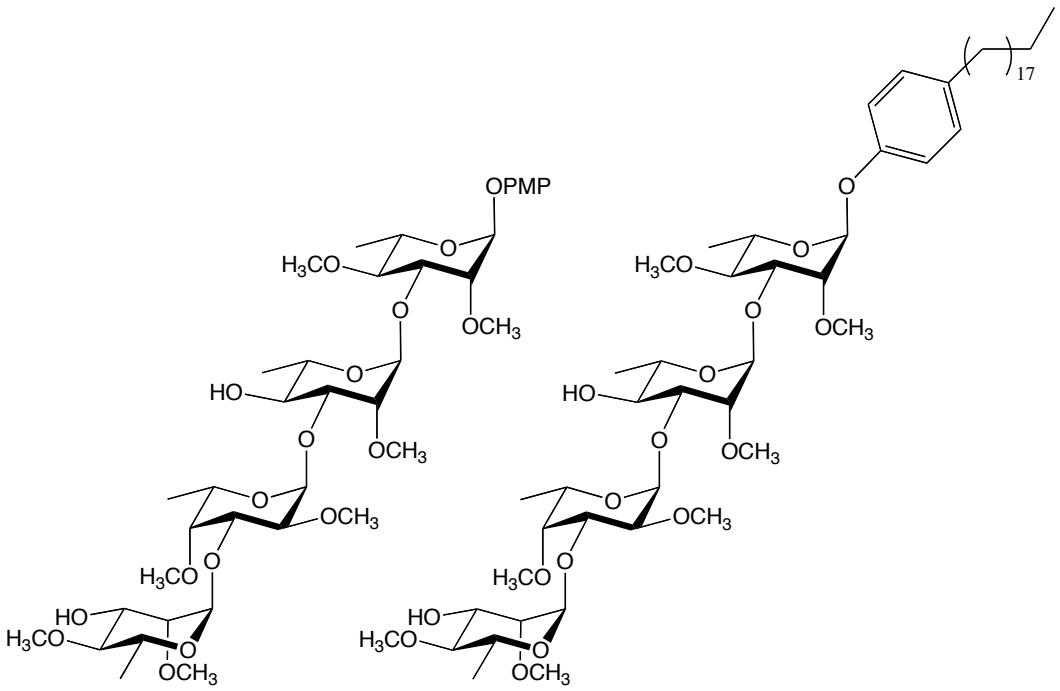


Figure 7-1. Structures of examples of the synthetic analogs.

IL-1β, IL-6, IL-10, TNF-α, MCP-1 were chosen as the cytokines to be tested as well as NO because they have a very important role in combating mycobacterial infections.[5-7] The biological testing of these molecules on the unstimulated A-THP1 macrophages showed that none of the synthetic analogs, nor the native PGL-I, stimulated the release of the tested cytokines. This result indicates that PGLs are not immunostimulants.

To investigate their immunoinhibitory properties, A-THP1 cells was stimulated with LPS, a TLR2/TLR4 agonist,[8] during the treatment with the tested compounds. This assay demonstrated that, with the exception of monosaccharides, these molecules showed a concentration-dependent immunoinhibitory pattern. The potency of this inhibitory activity is greatly

dependent on the methylation pattern of the compound. Furthermore, a disaccharide (e.g., **2-4**), appears to be the minimum structural motif required for immunoinhibitory activity. The best activity was obtained from the tetrasaccharide (**4-9**) that has dimethoxy-6-deoxy-D-mannopyranose capping motif at the non-reducing terminus.

The results also showed that replacement of some methyl groups with esters, acetyl or propionyl, results in a loss of activity, which is sometime dramatic. Moreover, when glucose and galactose, bearing a *p*-methoxyphenyl group at the reducing end, were tested, they showed no activity. This indicated that the observed effects appear due to the structure of carbohydrate moieties and not the *p*-methoxyphenyl group.

These findings raised a question related to receptor specificity and whether or not this immunoinhibitory pattern arises from the targeting of a specific TLR, or both TLR2 and TLR4. To test this hypothesis, Pam3CSK4, a TLR2 agonist,[9] was used as the stimulus. The results of this assay showed that the same inhibitory pattern was obtained as before, indicating that these molecules target TLR2. To further expand our understanding about the receptor site for these analogs, ultra pure LPS, a TLR4 agonist,[10] was used as the stimulus. Interestingly, none of the active compounds showed any inhibitory activity, thus indicating that TLR4 is not a target for these analogs.

To further expand the scope of this study, the effect of the lipid core on the immunomodulatory activity was investigated. This was achieved by adding a simple lipid core to the two most active compounds, **2-1** and **4-9**, to generate

glycolipids **5-1** and **5-2**. The activities of these compounds were then compared with those obtained with **2-1** and **4-9**, as well as the native PGL-I. The results showed that both glycolipids were more active than their carbohydrate counterparts, but they had the same inhibitory pattern. However, all compounds were less active than native PGL-I, which showed the same inhibitory patterns as the other active synthetic compounds. Moreover, PGL-I showed the same inhibitory pattern with the highest potency. As a control, it was shown that the lipid core itself showed no activity. These results lead to the conclusion that the native phenolthiocerol lipid core may have some structural elements that make it a better TLR2 ligand that the synthetic compounds and hence has higher potency.

Because apoptosis is very important in the immune response against mycobacterial infection, we wanted to investigate the ability of these analogs to induce apoptosis. The results showed that all the synthesized analogs were not able to induce apoptosis.

Finally, this work provides some evidence that PGLs counteract the immune response against the pathogen via inhibiting the release of important cytokines. This finding supports the notion that PGLs are important virulence factors.

7.2. Future work

In this study, we showed that PGLs synthetic analogs as well as PGLs inhibit the release of cytokines using ELISA. However, determining the levels of mRNA of the corresponding cytokine will quantify this inhibitory effect in an accurate way. Furthermore, we showed that the immunoinhibitory effect of PGLs is concentration-dependent. However, the factors that determine how much PGLs are produced by these organisms, whether it is host or pathogen related, is still a very interesting area of research.

We provided results suggesting that TLR2 as a possible receptor for PGLs. However, whether or not TLR2 is the only receptor or if there is another receptor site for these molecules remains an unanswered question. Moreover, the signaling pathway through which they produce their effect is another line of investigation that needs to be explored. To this end, testing the synthetic analogs of native PGLs in a knockout cell model that lack TLR2 will confirm that TLR2 is a receptor site for PGLs or not

Another area for the future would be to determine if there is species specificity in PGL recognition. For instance, PGLs from *M. leprae* have the same effect in humans as compared to cells from other origins. This might be tested by investigating the effect of PGLs or even our synthetic analogs on different cell lines to determine whether or not they will have the same effect.

We also showed that the lipid core does not affect the pattern of activity, but it increases the potency. To further confirm that, the native lipid core could be

added to one of the weakly active analogs, e.g. disaccharide **2-4**, and to see if this will increase the activity.

Coating of a non-pathogenic strain of mycobacteria with one of the synthetic glycolipid analog is another line of research. After coating, this strain will be used to infect macrophage and the effect on cytokine production will be determined. This will further confirm the immunoinhibitory activity of PGLs.[11]

7.2. Bibliography

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