University of Alberta

Preparation and enzymatic recognition of α-D-mannopyranosyl-1-phosphate analogs

by

Lu Zou

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Chemistry

Fall 2011
Edmonton, Alberta

Abstract

Tuberculosis (TB), a contagious disease that currently affects one third of the entire world's population, is caused by *Mycobacterium tuberculosis* (MTB). To treat TB, multiple antibiotics must be used in combination for long drug regimens. These harsh requirements result from the structure of the hydrophobic mycobacterial cell wall, which serves as a permeability barrier and protects MTB from both antibiotics and the immune system of host cells.

Lipoarabinomannan (LAM), an important component and a major antigen of the mycobacterial cell wall, is able to modulate the host immune response, which contributes to the difficulties in treating TB. Given its important role in the persistent nature of MTB, the glycosyltransferases (GTs) involved in LAM biosynthesis are attracting more and more interest as potential targets for the action of novel anti-TB drugs. Among these GTs, a PPM-dependent α-(1→6)-ManT that is responsible for transferring the mannose residue from a polyprenolphosphomannose (PPM) donor to an oligosaccharide acceptor to give an elongated product has been a research focus in our group. The PPM donor used by this enzyme is biosynthesized from mannose-1-phosphate by the action of two enzymes, a nucleotidyltransferase (GDP-ManPP) and a PPM synthase. To better understand how these enzymes interact with the mannose substrates, a series of methoxy and deoxy derivatives of mannose-1-phosphate were chemically synthesized, then converted into the corresponding GDP-Man analogs catalyzed by GDP-ManPP. Steric interactions and hydrogen-bonding in the active site of GDP-ManPP with these analogs was mapped by carrying out the kinetic analysis

using an established colorimetric assay. Additionally, a PPM analog was synthesized and then shown to be substrate for PPM synthase.

Acknowledgement

First and foremost, I would like to give my genuine gratitude and appreciation to my advisor, Professor Todd L. Lowary for his constant supports and valuable guidance in the last three years of my graduate study. No words can exactly express my heartfelt thanks to him. He is the most amazing person I've ever met and will be the role model for the rest of my life. I feel extremely fortunate to have had the opportunity to join his group and learn from him.

I am heartily thankful to Myles Poulin and Li Xia for their helpful advice, endless patience and continued encouragements, which means a lot to me. I thank Ruixiang Blake Zheng for his professional knowledge in preparing GDP-ManPP and PPM synthase. This thesis cannot be done without him and Myles who contributed a lot to the kinetics analysis. I am very grateful to all my fellow labmates in the Lowary group, past and present, for being so nice and helpful at all time. Special thanks go to Dr. Wenjie Peng, Dr. Maju Joe, Dr. Akihiro Imamura, Dr. Cunsheng Zhu, Dr. Pui-Huang Tam, Dr. Peter Meloncelli, Dr. Patrice Plaza-Alexander, Yu Bai, Michelle Richards and Bing Bai.

I give sincere thanks to Yen-Ku Wu, Lu Deng, Zedu Huang, and my other friends for all their help.

Finally, I would like to thank my parents and my sister for always being by my side.

Table of Contents

List of Schemes

List of Tables

List of Figures

List of Abbreviations

[α]	specific rotation
Ac	acetyl
AG	arabinogalactan
AgOTf	silver trifluoromethane sulfonate
AIBN	2,2'-azo-*bis*-isobutyronitrile
Bn	benzyl
Bu	butyl
Bz	benzoyl
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*'-dimethylformamide
Et	ethyl
GDP	guanosine diphosphate
GT	glycosyltransferase
LAM	lipoarabinomannan
LM	lipomannan

ManLAM	mannosylated lipoarabinomannan
Man*p*	mannopyranose
ManT	mannosyltransgerase
MDR-TB	multidrug-resistant tuberculosis
Me	methyl
NIS	*N*-iodosuccinimide
Ph	phenyl
PI	phosphatidyl-*myo*-inositol
PIM	phosphatidyl-*myo*-inositol mannoside
PPM	polyprenylphospho-mannopyranose
Py	pyridine
R_f	retention factor
STD	standard
TB	tuberculosis
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran

TLC	thin layer chromatography
WHO	World Health Organization
XDR-TB	extensively drug-resistant tuberculosis

Chapter 1

Introduction

1.1. Tuberculosis (TB)

Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis*, is a contagious disease that currently affects one third of the entire world population.[1,2,3] According to the data reported by the World Health Organization (WHO), 1.7 million people died from TB in 2009, and 380,000 of those people were also HIV-positive. The largest number of deaths was in the African continent where four fifths of the countries with the highest estimated TB incidence rates in 2006 are located (**Figure 1.1)**. The estimated TB incidence rate in sub-Saharan Africa is almost twice that of the South-East Asian region where the highest number of new TB cases appeared in 2008.[4,5]

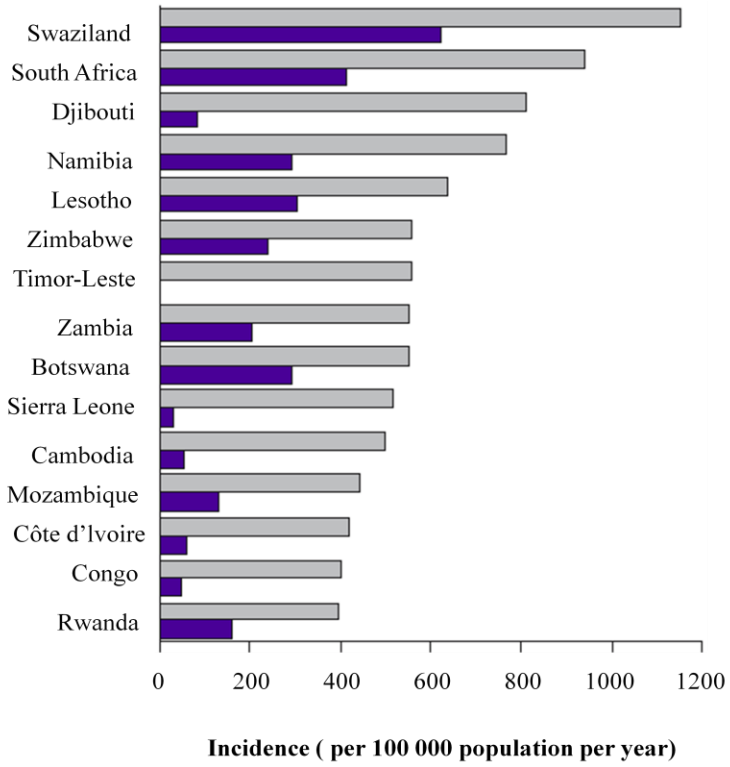


Figure 1.1. Fifteen countries with the highest estimated TB incidence rates per capita (all forms, grey bars) and corresponding incidence rates of HIV-positive TB cases (purple bars), 2006

A person can become infected with TB when he/she inhales a small amount of TB germs that are expelled by infected patients when they cough, spit, sneeze or speak. If not treated, each active TB disease carrier will infect 10–15 people on average every year. Not all those individuals infected with TB will develop the disease. Most people have no symptoms when they are infected with *Mycobacterium tuberculosis*. This is known as latent TB. Usually, the progression of latent TB to active TB will happen in 5–10% of all the cases during an infected person's lifetime.[6] However, people with suppressed immune systems (*e.g.*, those with HIV/AIDS[7] or transplant recipients) are more susceptible to TB and other mycobacterial diseases.[8]

Global population and demographic increases, increasing HIV infections, as well as increasing numbers of people in poverty combined together have led to world-wide growth in TB.[2] Moreover, the emergence of multidrug-resistant tuberculosis (MDR-TB)[3] and extensively drug-resistant tuberculosis (XDR-TB) increases the difficulty in treating those affected by the disease.

There has been a slow decrease, or a stabilization in TB incidence rates per capita in all six WHO regions; however, this is offset by the increase of the world population.[9] Additionally, TB is known to be a significant health concern in many developing countries; however, immigration, to some extent, has allowed the disease to disseminate widely across the world.[10] As a result, in 1993 the WHO declared tuberculosis to be a global emergency.[11] In addition to these population and demographic effects, the superimposition of HIV upon latent TB has given rise to a dramatic increase in TB incidence rates.[1] HIV infection

increases the risk of TB infection developing into disease from 10% per lifetime to 10% per year.[2] Among people who died from AIDS, TB is the most common cause of death in parts of Africa.[2] Synergy between HIV and TB speeds up the spread of both diseases.[2,12,13] The fact that more and more people are living in poverty also accelerates the progression of TB infection due to the poor nutrition, crowded conditions, and unsatisfactory and improper medical treatments. These conditions, and improper treatments, partially result in the increased occurrence of MDR-TB and XDR-TB.[14,15] Other than the ineffective treatment regimens, the failure of patient compliance (*e.g.*, inconsistent, insufficient use or overuse of antibiotics) is another main cause for the emergence of MDR-TB and XDR-TB. Multidrug-resistant tuberculosis is resistant to *isoniazid* (INH) and *rifampcin* (RMP), which are the two most powerful first-line anti-TB drugs used today.[16] The structures of these anti-TB therapeutics are shown in **Figure 1.2**.[17] MDR-TB can be treated with second-line drugs; however, the therapy is more time-consuming (up to two years), and more expensive, and results in severe side effects. Extensively drug-resistant tuberculosis is a form of MDR-TB that is also resistant to three or more of the six classes of second-line drugs (**Figure 1.3**),[17] which makes its treatment extremely difficult.[2]

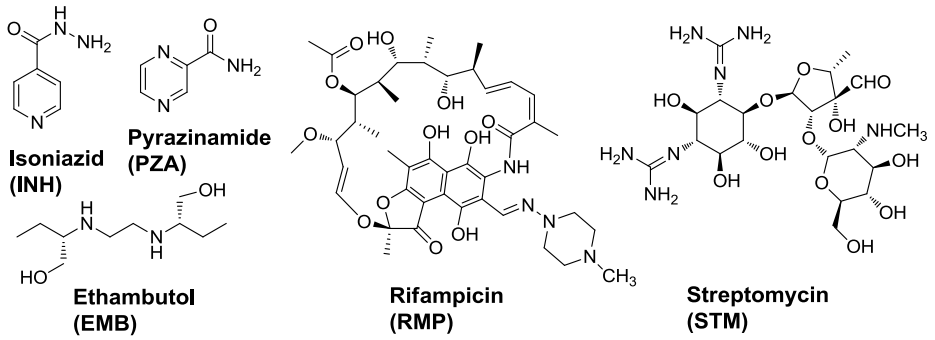


Figure 1.2. Structures of INH, RMP, PZA, EMB, and STM

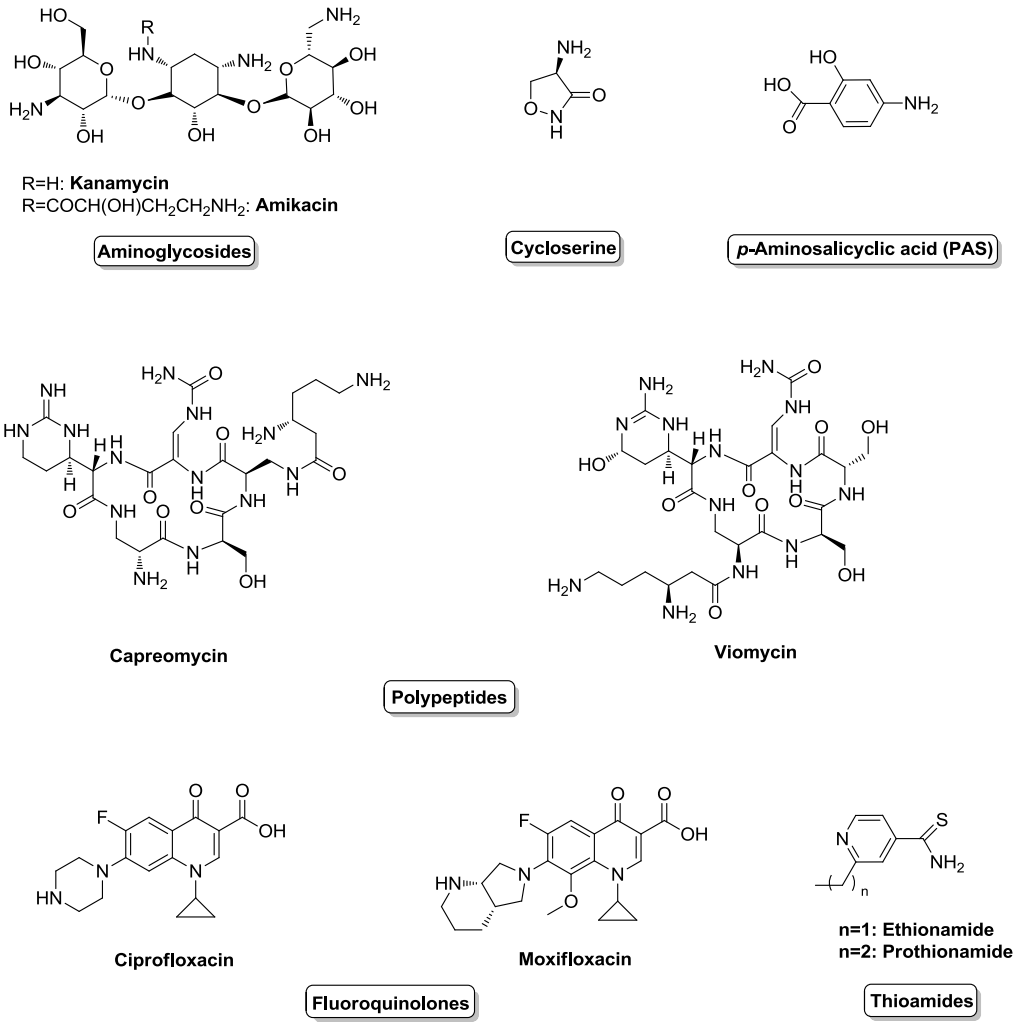


Figure 1.3. Six classes of second-line drugs used for the treatment of tuberculosis

1.2. General information about mycobacterium tuberculosis

Mycobacteria, rod-shaped bacteria with a hydrophobic cell wall, are a diverse group that includes more than 70 different species and can be found all over the world. They can be well protected from disinfectants and other water treatment methods because of their lipid-rich cell wall. *Mycobacterium tuberculosis* (MTB) and *Mycobacterium leprae,* two of the most well-known species, are the ethiological agents of tuberculosis (TB) and leprosy, respectively.

MTB (**Figure 1.4**) was first discovered and isolated by Robert Koch, a German physician, who was awarded the Nobel Prize in Physiology or Medicine in 1905 for his investigations and discoveries related to tuberculosis.[18] MTB complexes can usually be found in the upper lobes of lungs because MTB is an aerobe and therefore needs oxygen to survive. The pathogen can cause both latent and active TB infection.[6] After inhalation, the TB bacilli enter and replicate within pulmonary macrophages.[19] If an individual's immune system is not strong enough, the pathogen will not be destroyed by phagosome–lysosome fusion, and as a result, the bacteria will adapt to and survive in the hostile environment of macrophages. The persistent and resistant nature of MTB makes it an excellent parasites, and hard to eradicate.[20, 21,22,6,23] Persistence means the survivability of *M. tuberculosis* despite the treatment of antibiotics; resistance is used to describe genetic mutations of organisms, which enable them to lose susceptibility to antibiotics.

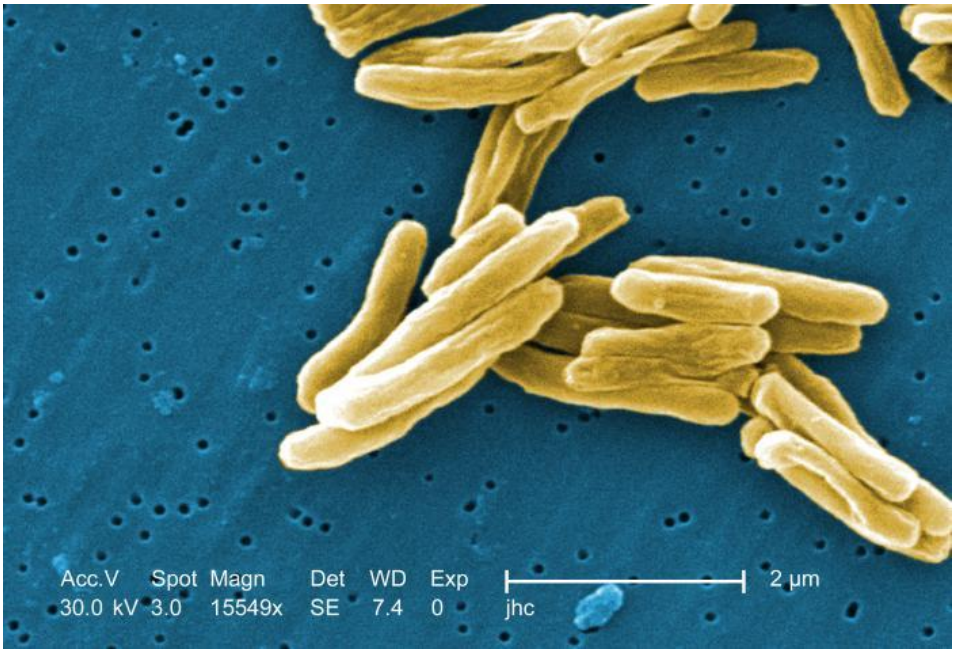


Figure 1.4. *Mycobacterium tuberculosis* scanning electron micrograph (http://www.textbookofbacteriology.net/tuberculosis.html)

1.3. Structure of the mycobacterial cell wall

As discussed in the first section, the standard therapy for TB takes patients at least six months. Additionally, treatment with a single antibiotic (anti-TB drug) is ineffective by itself; multiple antibiotics must be used in combination.[24] Five first-line drugs, *isoniazid* (INH), *rifampicin* (RMP), *pyrazinamide* (PZA), *ethambutol* (EMB), and *streptomycin* (STM), are usually used together in the standard regimen for treating tuberculosis.[25] The structures of the antibiotics are shown in **Figure 1.2**.[17] Moreover, the treatments can be prolonged for up to two years to cure drug resistant TB.[26,7]

The harsh requirements to treat TB are closely related to the structure of the mycobacterial cell wall,[27,28] which consequently has become the target of

many antituberculosis drugs.[29,30,31] This hydrophobic cell wall, serving as a permeability barrier, protects MTB from both antibiotics and the immune system of host cells.[32] The mycobacterial cell wall is comprised of four major components. They are peptidoglycan, the mycolyl–arabinogalactan (mAG) complex, lipoarabinomannan (LAM) and extractable lipids. A schematic structure of mycobacterial cell wall is shown in **Figure 1.5**.[33,32]

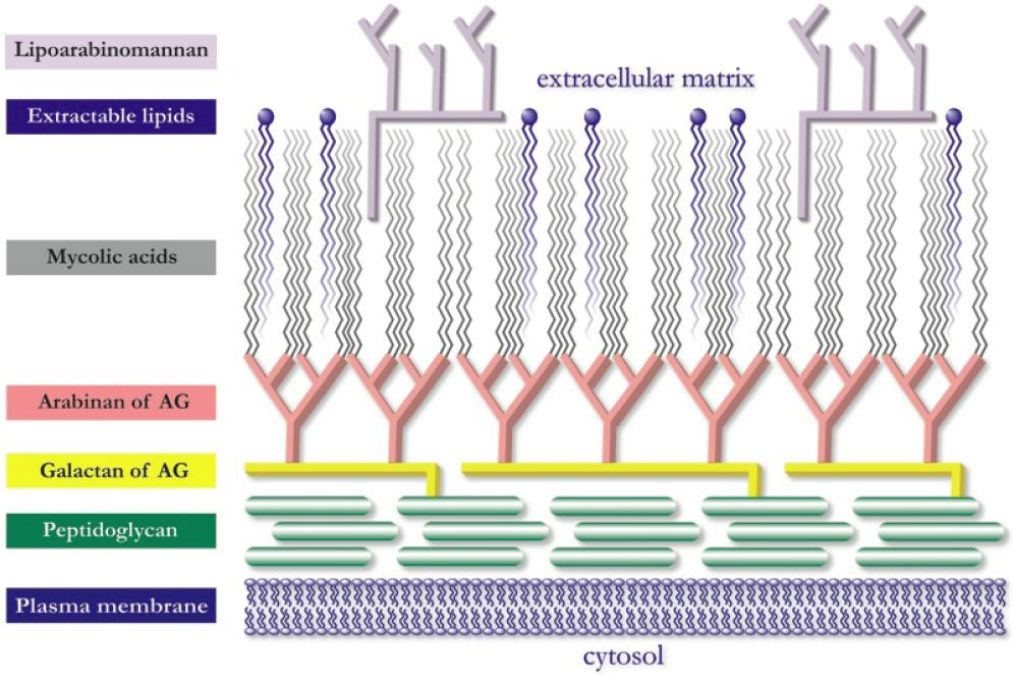


Figure 1.5. Model of the mycobacterial cell wall drawn by Michele R. Richards (University of Alberta)

1.3.1. Peptidoglycan

Peptidoglycan is found just outside the plasma membrane. As the backbone of the cell wall skeleton, it consists of cross-linked polysaccharide chains. The polysaccharide chains are formed from two sugar residues, *N*-

acetylglucosamine and *N*-glycolylmuramic acid connected alternately. A tetrapeptide chain, composed of L-alanyl-D-isoglutaminyl-*meso*-diaminopimelyl-D-alanine, is attached to O-3 of the *N*-glycolylmuramic acid residues (**Figure 1.6**).[34] A peptide bridge will be formed between two diaminopimelic acid residues or between D-alanine and diaminopimelic acid to cross-link the peptidoglycan chains.[34,35] Peptidoglycan can help secure other components of the cell wall and maintain the shape of the cell.

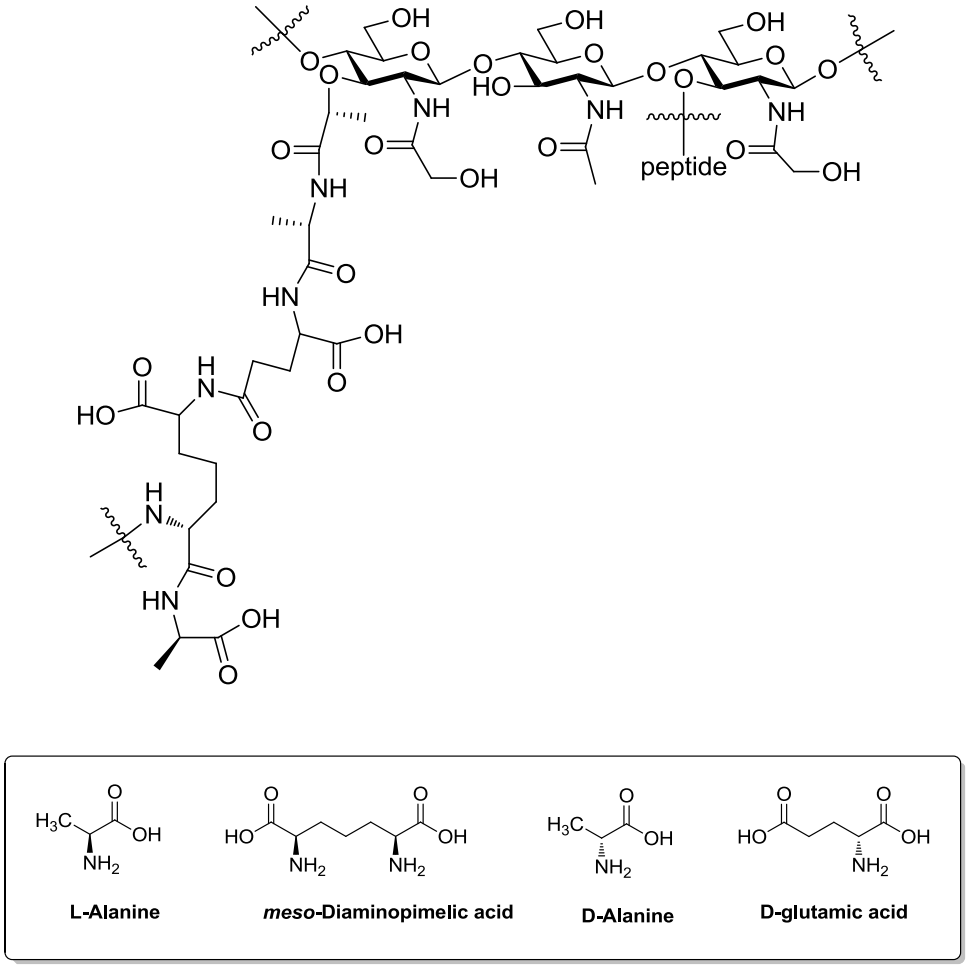


Figure 1.6. *N*-acetylglucosamine and *N*-glycolylmuramic acid linkages in the peptidoglycan

1.3.2. The mAG complex

The mAG complex, which is the largest structural component of the mycobacterial cell wall, is a lipidated polysaccharide composed of arabinogalactan (AG) and mycolic acids. The AG is composed of D-arabinofuranosyl (Ara*f*) and D-galactofuranosyl (Gal*f*) residues, which are linked to peptidoglycan covalently via a disaccharide phosphate linker unit, α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-PO_4 (**Figure 1.7**).[36,37]

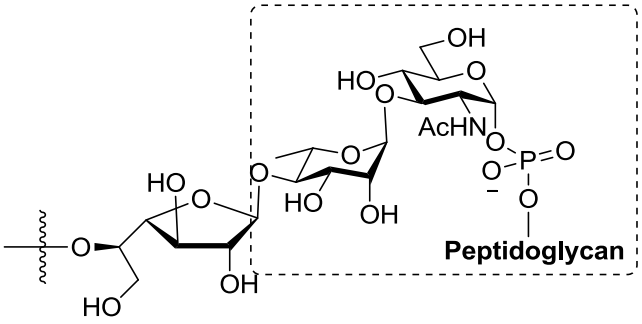


Figure 1.7. The disaccharide phosphate linker unit: α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-PO_4

As shown in **Figure 1.8**,[37] approximately 30 alternating β-(1→6) and β-(1→5) linked Gal*f* residues form the linear galactan.[38] Attached to the galactan are three identical branched arabinan chains, containing around 90 Ara*f* residues in total, connected through three different linkages, α-(1→5), α-(1→3), and β-(1→2).[39] These domains are attached to O-5 of the eighth, tenth, and twelfth Gal*f* residues in the galactan backbone.[40] The first-line anti TB drug ethambutol (EMB) inhibits the biosynthesis of these arabinan domains.[41]

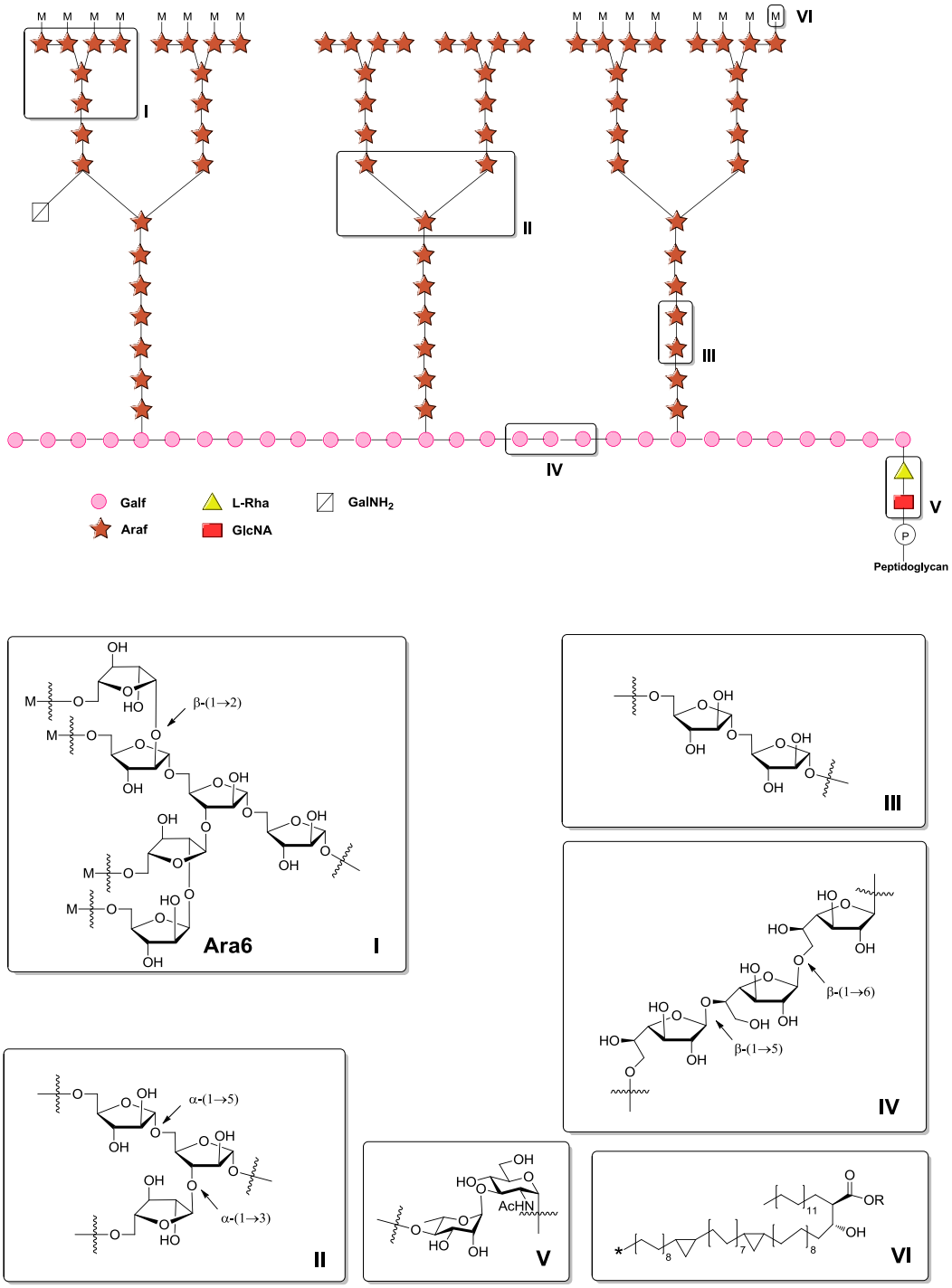


Figure 1.8. Structure of mycolyl–AG complex

The non-reducing ends of the mAG arabinan chains are composed of a branched hexasaccharide unit (Ara6, **Figure 1.8**). The primary hydroxyl groups of around two-thirds of the Ara6 motifs are esterified with mycolic acids. Mycolic acids are β-hydroxy fatty acids with a long α-alkyl side chain, which usually contains 70–90 carbons.[42] Three kinds of mycolic acids, α-mycolates, ketomycolates, and methoxymycolates (**Figure 1.9**), are found in the *M. tuberculosis* cell wall. α-Mycolate is a *cis*, *cis*-dicyclopropyl fatty acid, which is also the most abundant form (>70%) in *M. tuberculosis*. Ketomycolates and methoxymycolates contain either *cis*- or *trans*-cyclopropane rings. They each contribute a minor amount (10–15%) in *M. tuberculosis*.[43] The presence of these tightly packed lipids in mycobacteria gives the cell wall a low permeability to the environment, which helps to prevent the efficient passage of antibiotics into the organism.[44,45,46] The first-line anti TB drug isoniazid (INH) inhibits the biosynthesis of mycolic acids.

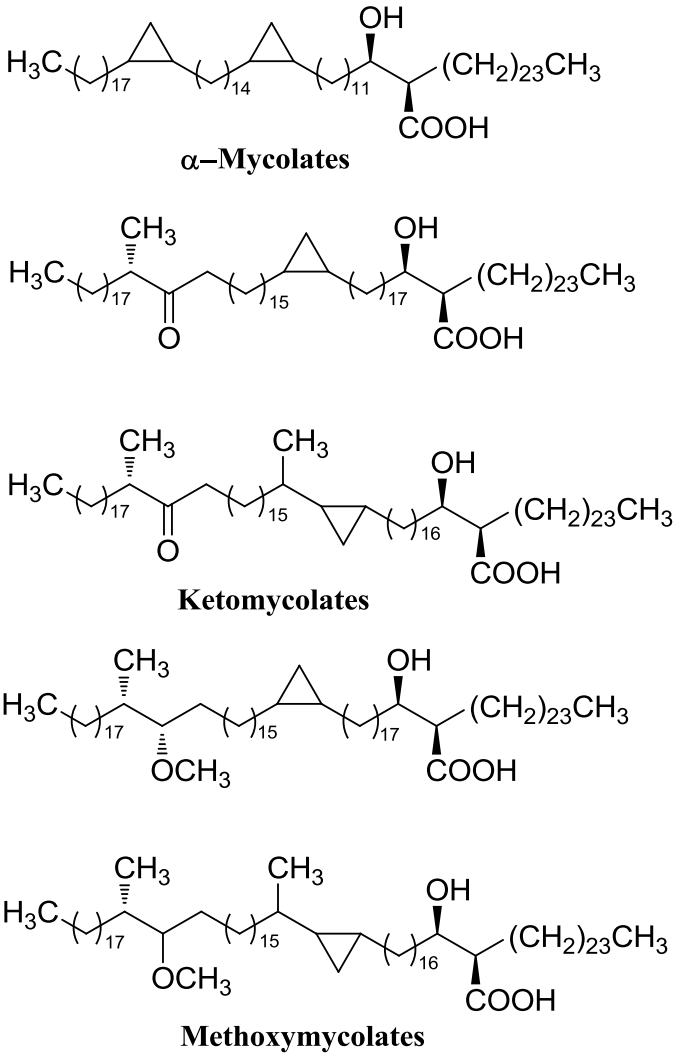


Figure 1.9. Representative structures of mycolic acids in mAG complex of *M. tuberculosis*

1.3.3. Lipoarabinomannan (LAM)

Lipoarabinomannan (LAM) and lipomannan (LM), two major glycolipids present in the mycobacterial cell wall, consist mainly of three components, a phosphatidyl-*myo*-inositol (PI) anchor, a polysaccharide core, and capping motifs at the non-reducing end of the molecule. The polysaccharide core of LAM

contains both mannan and arabinan domains (**Figure 1.10)**. LM, which can be considered as an analog of LAM, contains only the mannan domain.[47,48]

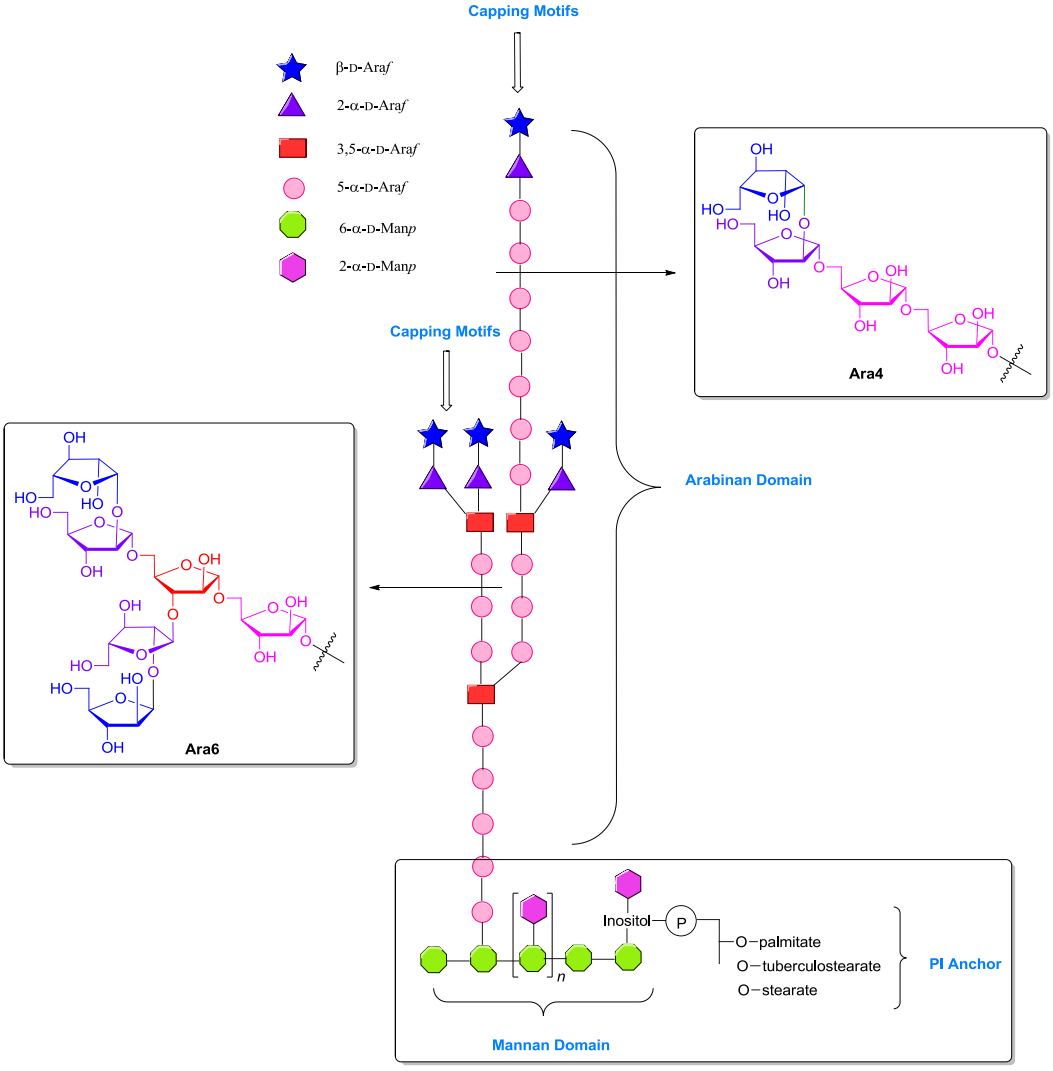
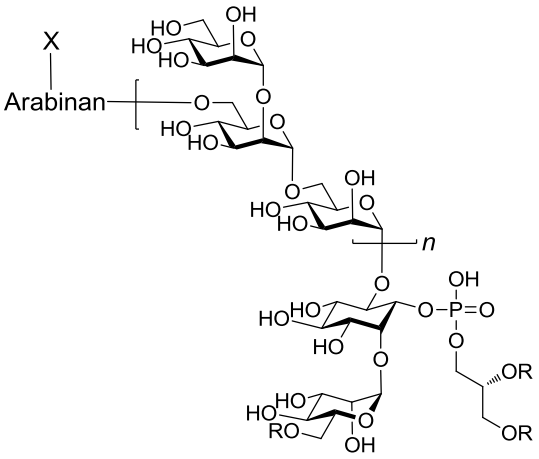


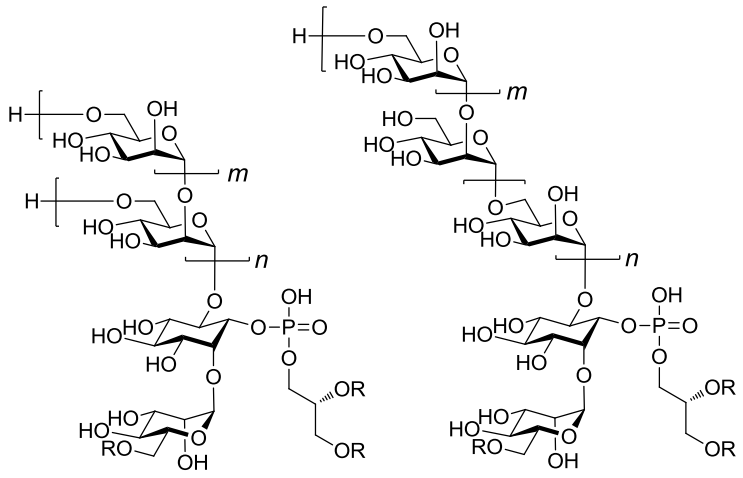
Figure 1.10. Schematic depiction of LAM

Structurally, LM, and LAM are more highly glycosylated relatives of the phosphatidyl-*myo*-inositol mannosides (PIMs), which are also found in the M. tuberculosis cell wall. The PIMs and LAM/LM all share a common phosphatidyl inositol (PI) core moiety mannosylated at the C-2 and C-6 positions (**Figure**

1.11[49]).[50,51] These structurally related molecules constitute the major lipoglycans of *Mycobacterium tuberculosis* cell wall. They are attached non-covalently to the plasma membrane via the fatty acid chains (**Figure 1.12**) of the PI anchor.



LAM, *n*=10-20, X=H
ManLAM, *n*=10-20, X=(Man*p*)$_{1-3}$



LM, *n*=20-25, *m*=0 or 1
PIM1-4, *n*=0-3

PIM5-6, *n*=2, *m*=1-2

Figure 1.11. Structures of lipoarabinomannan (LAM), ManLAM, lipomannan (LM), and phosphatidyl-*myo*-inositol mannosides (PIMs). R = palmitate, stearate and/or tuberculostearate (see also **Figure 1.12**)

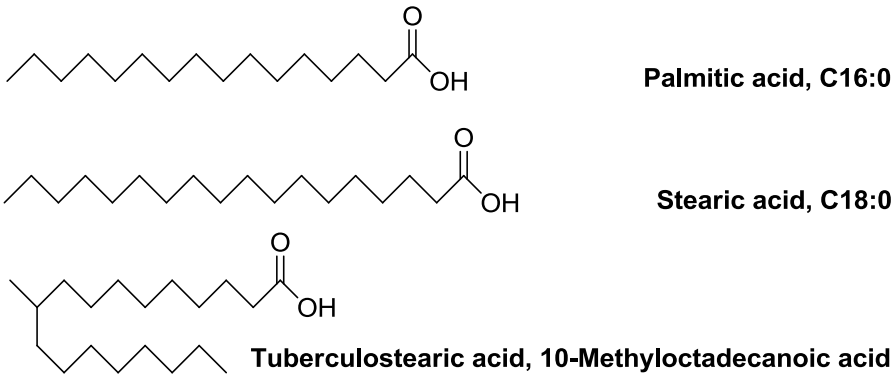


Figure 1.12. Structures of palmitic acid, stearic acid, and tuberculostearic acid. The salts and esters of palmitic acids, stearic acids, and tuberculosteric acids are called palmitates, stearates, and tuberculostearates, respectively.

The structural similarity of these lipoglycans diverges after AcPIM4, a common precursor of both the AcPIM6, as well as the LM and LAM structures. The addition of two consecutive α-(1→2)-linked Man*p* residues attached to the non-reducing end of AcPIM4 leads to AcPIM6, which is believed to be the dead-end product in the PIM biosynthetic pathway and not involved in the further biosynthesis of LM/LAM mannan core. (**Figure 1.13**) On the other hand, the addition of ~20 α-(1→6)-linked Man*p* residues to the non-reducing end of AcPIM4 will result in the linear mannan backbone of LM/LAM.[52,48,53]

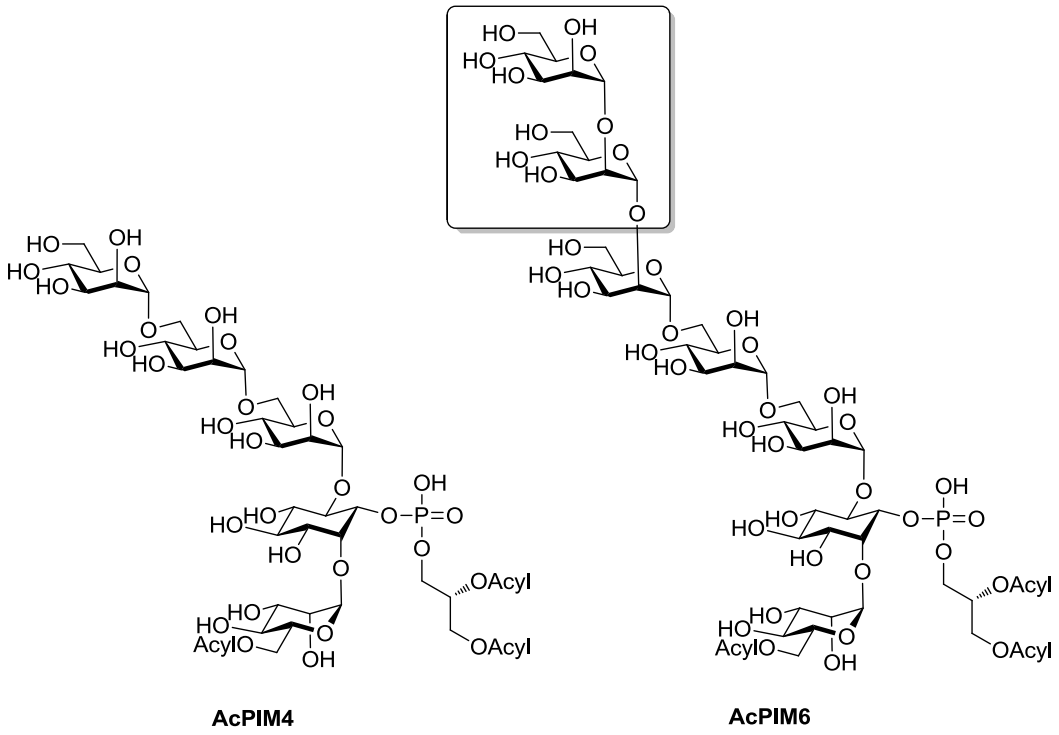


Figure 1.13. Structures of AcPIM4 and AcPIM6

As mentioned earlier, the polysaccharide portion of LAM contains a core mannan chain and an arabinan domain. The backbone mannan chain of LAM, contains a linear polymer of α-(1→6) linked mannopyranosyl (α-Man*p*) residues, and approximately half of these units are further modified with an additional α-Man*p* unit attached at the O-2 position. At the non-reducing end of this core glycan is an arabinan domain, which usually contains 50–80 Ara*f* residues.[54] Similar to the mAG complex (**Figure 1.8**), the arabinan moiety in LAM are also comprised of α-(1→5), α-(1→3), and β-(1→2) linked Ara*f* units. One of the differences between them lies in the terminal structures of the arabinan domains. In LAM, the terminal structures are either a biantennary hexa-arabinofuranoside (Ara6)[39] or linear tetra-arabinofuranoside (Ara4), while only the Ara6 motif is

present in the mAG complex. The structures of both Ara6 and Ara4 are shown in **Figure 1.14**. LAM also contains species-specific capping motifs attached to the arabinofuranosyl side chains. These capping motifs can contain Man*p* residues (*M. tuberculosis*, *M. leprae*, and *M. bovis*),[55,56] inositol phosphate motifs (*M. smegmatis*),[57,58] and 5-thiomethyl-xylofuranose residues (*M. tuberculosis* and *M. kansasii*).[59,60] When the arabinan side chains are capped with single α-Man*p* residue or short oligomannopyranosides (**Figure 1.11**), it is referred to as ManLAM.

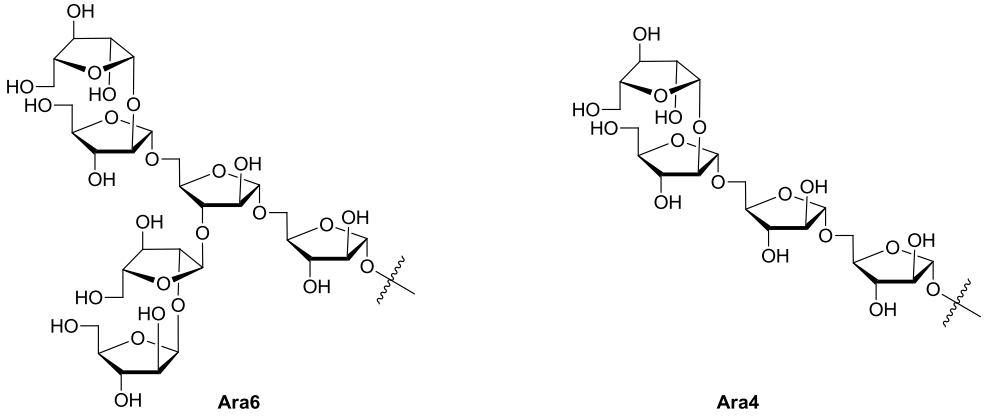


Figure 1.14. Structures of biantennary hexa-arabinofuranosides (Ara6) and linear tetra-arabinofuranosides (Ara4).

An important component and a major antigen of the mycobacterial cell wall, LAM is also able to modulate the host immune response through its immunoregulatory and anti-inflammatory functions.[59] It has been shown to be able to help suppress the proliferation of T lymphocytes, inhibit the activation of macrophages by interferon-γ, and neutralize oxygen-derived free radicals.[52,61-63]

1.3.4. Extractable glycolipids

Other than the large mycobacterial cell wall components discussed earlier, smaller lipids and glycolipids, known as extractable lipids, are associated noncovalently with the mycolic acids of the mAG to form an unsymmetrical bilayer (See **Figure 1.5**, page 8). It is believed that the lipid portion of the glycolipids is intercalated into the mycolic acids, and the oligosaccharide residues are left at the outer surface of the cell wall to interact with the environment.[33,42] The structures of these surface glycolipids are species specific, and include phenolic glycolipids, glycopeptidolipids, and lipooligosaccharides. Some examples of these structures are shown in **Figure 1.15**.[64,65]

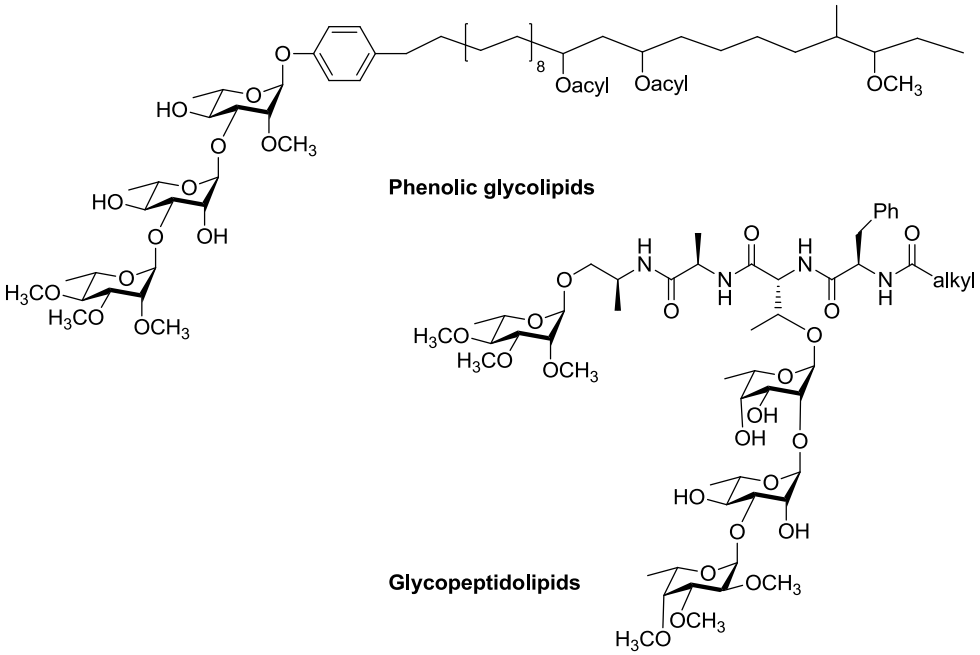


Figure 1.15. Examples of extractable glycolipids

1.4. Enzymes involved in LAM biosynthesis

The glycoconjugates that are the main components of mycobacterial cell wall, are assembled by a number of enzymes named glycosyltransferases (GTs). As discussed above, LAM, as a major antigenic component of the cell wall, serves as an indispensable modulator of the host immune response. Therefore, the glycosyltransferases involved in LAM biosynthesis are attracting more and more interest as potential targets for the action of novel anti-TB drugs.

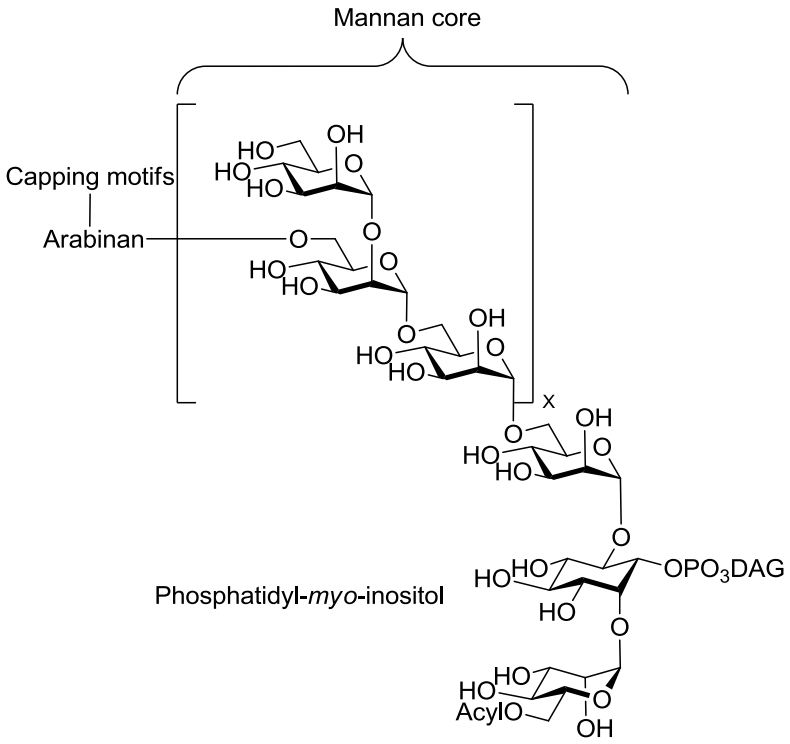


Figure 1.16. Four structural components of LAM

The chemical composition of the four structural components (the PI anchor, the core mannan chain, the branched arabinan domain, and the terminal capping motifs, **Figure 1.16**)[66] of LAM have already been studied; however, each individual step of the biosynthetic pathways and the GTs involved in LAM

biosynthesis are not well understood yet. This is due, at least partially, to difficulties in the isolation and characterization of the GTs, as well as the limited availability and difficulties in the synthesis of potential substrates.

1.4.1. Assembly of PIMs

Ten years ago, Brennan and co-workers proposed a pathway for mycobacterial LAM biosynthesis (**Figure 1.17**).[48] Since then, many steps in this pathway have been validated by biochemical and genetic approaches.[67-80] The reducing end of LAM and LM share structural similarities with PIMs (**Figure 1.11**), as discussed previously, which suggests the initial steps involved in their biosynthesis should be the same.[50]

As outlined in **Figure 1.17**, PIM1 is biosynthesized from PI[81] by the addition of a Man*p* residue to the C-2 position of the inositol moiety catalyzed by PimA using GDP-Man*p* as the donor substrate.[69] A crystal structure of PimA complexed with GDP-Man was the first crystal structure determined of a GT involved in mycobacterial cell wall biosynthesis.[82] A recent investigation found evidence to suggest that PimA binds the donor substrate GDP-Man*p* first and then allows for a conformational change to provide the binding site to the acceptor substrate (PI).[83] An acyltransferase Rv2611c (AcylT), the enzyme that catalyzes the subsequent acylation of PIM1, is able to attach the fatty acyl groups (shown in **Figure 1.12**) to the C-6 position of the newly added Man*p* residue.[68] The enzyme can also catalyze the same transformation at the PIM2 substrate stage to form AcPIM2.

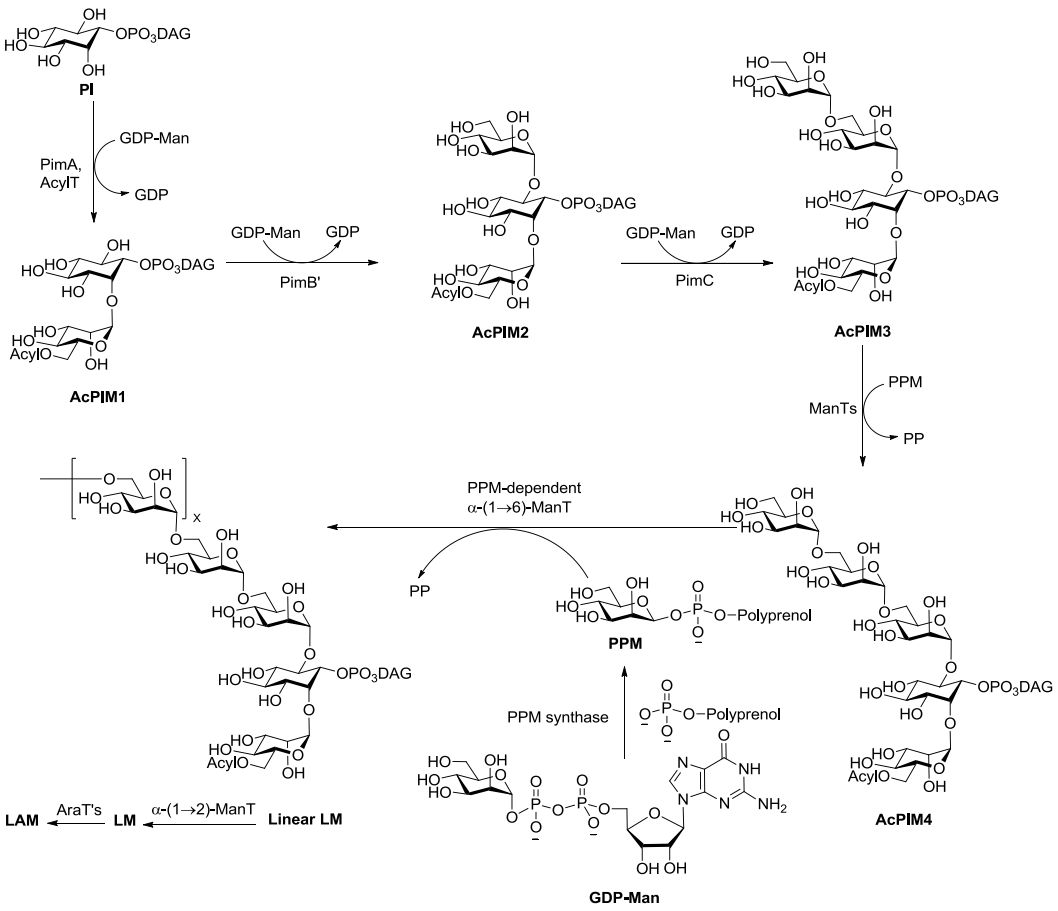


Figure 1.17. Proposed biosynthetic pathway for mycobacterial LM and LAM. DAG, diacylglycerol; AcylT, acyltransferase; PP, polyprenolphosphate; PPM, polyprenylphosphomannopyranose; AraT's, arabinosyltransferases

PimB', the gene product of *Rv2188c*, is responsible for the biosynthesis of PIM2 from PIM1. The second Man*p* residue is installed to the C-6 position of the inositol moiety of PIM1 (or AcPIM1) catalyzed by PimB' also using GDP-Man*p* as the donor substrate.[84,85] Nevertheless, a recent study points out that a PimB' knock-out mutant was still able to produce LM and LAM although in diminished amounts. This suggests that PimB' is not essential for LM and LAM biosynthesis or there is an additional enzyme also capable of adding the C-6 Man*p* to PIM1.[86]

A third mannosyltransferase, PimC, is the enzyme that catalyzes the biosynthesis of AcPIM3 from AcPIM2 in *M. tuberculosis* CDC1551; however, a PimC homolog is absent in *M. tuberculosis* H37Rv, *M. leprae*, and *M. smegmatis*. The above evidence suggests that redundancy exists in the PIMs/LM/LAM biosynthetic pathway.[71]

The PimA, PimB' and PimC mannosyltransferases all require GDP-Man*p* as the donor species.[40,71,70] The ManTs involved in the biosynthesis of more polar PIMs (PIM4–PIM6) and LM/LAM, on the other hand, require a C_{35}/C_{50}-polyprenylphosphomannopyranose (PPM) donor.[49] As shown in **Figure 1.17**, the addition of a Man*p* residue to the polyprenolphosphate from GDP-Man*p* catalyzed by PPM synthase yields this PPM donor species.[76]

More than one PPM-dependent α-(1→6)-ManT is needed for the biosynthesis of full length LM/LAM, which was shown by Brennan and coworkers in 2007.[53] Unfortunately, the ManT involved in AcPIM4 (the precursor of APIM6 and LM/LAM) biosynthesis from AcPIM3 remains to be discovered. So far, three PPM-dependent α-(1→2)-ManTs have been identified.[78,79,80] One of them, PimE, is believed to be responsible for the formation of AcPIM5. Whether it is the same enzyme to catalyze the transfer of the sixth Man*p* to AcPIM5 to form AcPIM6 or not is still unknown.[80]

1.4.2. Assembly of the mannan core

An α-(1→6)-ManT, encoded by the MSMEG4245 gene in *M. smegmatis*, and the corresponding gene in *M. tuberculosis* strain H37Rv (Rv2174) were

identified by Brennan and coworkers in 2007.[53] The deletion of the *Rv2174* gene led to the generation of truncated LMs, which suggests that it is responsible for mannan elongation in the biosynthesis of LM. Another gene homologous to Rv2174, NCgI2093 (MptA), has also been identified by Besra and coworkers in *Corynebacterium glutamicum*, a bacterium closely related to mycobacteria.[87] An additional mannosyltransferase, MptB (Rv1459/NCgI1505) was later identified, again by Besra and coworkers.[88] The functions of both enzymes are closely related to the assembly of the α-(1→6) mannan core. However, Besra and coworkers found that removal of an MptB homolog gene, *MSMEG3120*, from *M. smegmatis* did not have a great impact on LM/LAM biosynthesis. This indicates the existence of redundancy in the ManTs involved in mycobacterial core mannan biosynthesis.

As outlined above, the linear α-(1→6)-linked mannan core is further elaborated by the addition of single α-(1→2)-linked Man*p* units to about half of the core Man*p* residues.[52] Brennan and coworkers have suggested that these α-(1→2)-linked Man*p* units are not introduced simultaneously with the formation of the α-(1→6)-linked mannan backbone, but after the complete synthesis of the linear α-(1→6)-linked mannan core.[48,66] MSMEG4250 in *M. smegmatis* and Rv2181 in *M. tuberculosis* have already been identified as the genes encoding for the α-(1→2) ManTs involved in the α-(1→2)-linked Man*p* side-chain biosynthesis for LM/LAM.[79]

1.4.3. Synthesis of the arabinan domain and capping motifs

Further arabinosylation of LM is essential for the formation of LAM. Unfortunately, so far, only one arabinosyltransferase (EmbC) involved in the LAM arabinan domain biosynthesis has been identified.[74] EmbA, EmbB, and EmbC, the targets of ethambutol (EMB, **Figure 1.2**),[89,75] are three large homologous membrane proteins which are the products of the genes *embA*, *embB*, and *embC*, respectively.[90] EmbA and EmbB are known to be dedicated to the formation of arabinogalactan (AG).[73] EmbC has been shown to play a critical role in LAM arabinan synthesis.[91] Inactivation of the *embC* gene in *M. smegmatis* leads to stopping of LAM biosynthesis, although LM synthesis is unaffected.[74] LAM production can be restored when the *embC* gene is reintroduced on a plasmid.[74] The viability of the nonpathogenic species *M. smegmatis* was not threatened by the absence of *embC* gene; however, the *embC* gene was shown to be indispensable in the pathogenic *M. tuberculosis* under normal growth conditions.[74, 92]

All three Emb proteins share structural similarities even though they contribute to the synthesis of different cell wall components. They each contain about 13-transmembrane helices in the N-terminal domain and a globular C-terminal domain.[74,92] In EmbC the N-terminal domain is believed to be responsible for binding the acceptor substrates (LM), with the C-terminal domain responsible for the occurrence of arabinosylation. Shorter arabinans deficient in the linear Ara4 moiety (**Figure 1.14**) are produced by an EmbC mutant where the C-terminus is cleaved from the protein.[74] It has, therefore, been suggested that the

C-terminal domain acts as an α-(1→5)-AraT and catalyzes the synthesis of linear arabinan chain at the nonreducing end of LAM arabinan domain.[93]

As discussed in **Section 1.3.3**, different capping motifs, such as Man*p* residues, inositol phosphate motifs, and 5-thiomethyl-xylofuranose residues can be attached to the termini of the arabinosyl side chains. The enzymes involved in the biosynthesis of these capping motifs have not yet been well studied; however, two ManTs responsible for the assembly of ManLAM have been identified and their activities probed. One of them, Rv1635c, is a PPM-dependent α-(1→2)-ManT. It catalyzes the transfer of the first Man*p* residue of Man-capping motifs to the non-reducing end of the LAM arabinan domain.[78] The other enzyme (a ManT encoded by *Rv2181*) is the same enzyme that catalyzes the introduction of α-(1→2)-Man*p* branches to mannan core. This, consequently, strongly suggests that this ManT recognizes a single Man*p* unit as the acceptor. It is responsible for the further addition of α-(1→2)-Man*p* residues in Man-capping of ManLAM.[94]

1.5. Endeavours studying a PPM-dependent α-(1→6)-mannosyltransferase in our group

As discussed in **Section 1.4**, a number of GTs are involved in the LAM biosynthetic pathway. Mannosyltransferases (ManTs) that use PPM as the donor substrates, especially the PPM-dependent α-(1→6)-ManTs are less well studied than ManTs related to the initial biosynthetic steps that use GDP-Man*p* as the donor species (*e.g.*, PimA, PimB' and PimC).[66] Therefore, our interest has been focused on understanding the substrate specificity of a PPM-dependent α-(1→6)-

ManT that is responsible for the synthesis of the α-(1→6)-linked mannan core of LM and LAM.[95,96]

1.5.1. Previous work

The specificity of the PPM-dependent α-(1→6)-ManT with regard to the mannose acceptor was reported by a previous group member (Dr. Pui-Hang Tam) as part of his Ph.D-thesis work.[66,97] (**Figure 1.18**[98]) By synthesizing and screening a synthetically homologous series of mono- through tetrasaccharides (**1.1–1.4, Figure 1.19**[66]) against this PPM-dependent ManT, the effect of acceptor length on ManT activity was probed. This study demonstrated that an octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1.2, Figure 1.19**) is the minimum structural element recognized by ManT and serves as a good acceptor for the enzyme.[66,99] Consequently, a panel of monomethoxy and monodeoxy analogs (**1.5–1.18** in **Figure 1.19**) of this disaccharide substrate[100] were chemically synthesized to explore specific carbohydrate–protein interactions occurring in the active site of this ManT.

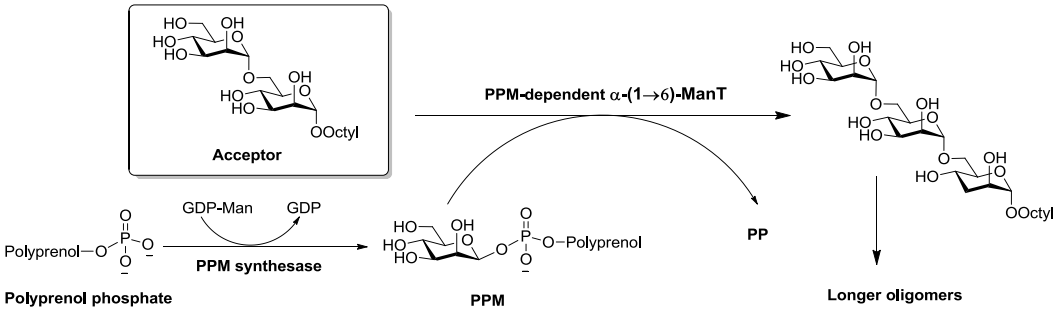


Figure 1.18. Reported assay for testing PPM-dependent α-(1→6)-ManT activity in mycobacterial membrane preparations[98]

The results of these studies indicate that the α-(1→2)-linked Man*p* units in the mannan core of LM/LAM are introduced after the assembly of a large linear α-(1→6)-linked mannan backbone, because methylation of either hydroxyl group at C-2' or C-2 results in complete loss of activity (**1.5** and **1.13**, **Figure 1.19**). Moreover, Dr. Tam's work identified that numerous important hydrogen-bonding interactions occur between the enzyme and the acceptor substrate based on the fact that five of the seven monodeoxy analogs tested lost activity. Of these analogs, only the 2'- and 4-deoxy analogs (**1.6** and **1.18**, **Figure 1.19**) are able to act as acceptor substrates for the enzyme. This also implies that 2'- and 4-hydroxyl groups are not essential for acceptor substrate recognition in the active site of this ManT.[66]

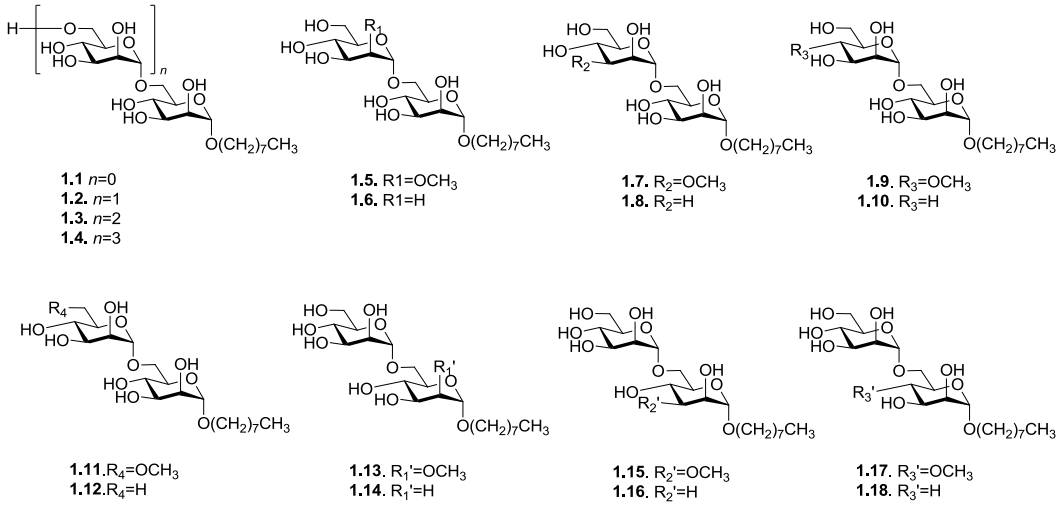


Figure 1.19. Acceptor analogues synthesized by Dr. Pui-Hang Tam

Additionally, in order to further extend the knowledge of the specificity of this enzyme in respect of acceptor substrates, Dr. Tam synthesized five epimeric and three amino analogs (**Figure 1.20**) of **1.2**.[98] Their subsequent biological

evaluations against the α-(1→6)-ManT demonstrate the significance of C-2 and C-4' hydroxyl groups in **1.2** for ManT catalysis according to the poor substrate activity of **1.21** and lack of activity of **1.22**. Amino analogs **1.24–1.26** turn out to be weak inhibitors of ManT.[98]

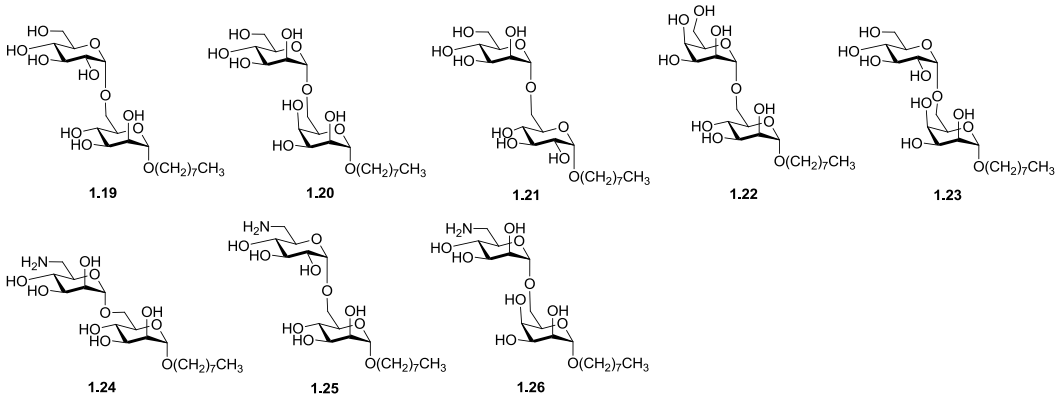


Figure 1.20. Structures of five epimeric and three amino analogs of **1.2**

1.5.2. Overview of this thesis

The PPM-dependent α-(1→6)-ManT studied in our group transfers a mannose residue from a polyprenolphosphomannose donor (**1.30**, **Figure 1.21**) to an oligosaccharide acceptor (**1.31**, **Figure 1.21**) to give an elongated product (**1.32**, **Figure 1.21**) containing one additional Man*p* residue. The previous studies by our group have focused on the specificity with regard to the acceptor substrate;[49,66,97] however, the specificity with regard to the mannose donor substrate is little known. The PPM donor used by this enzyme is biosynthesized from mannose-1-phosphate by the action of two enzymes: a nucleotidyltransferase (GDP-ManPP) and a PPM synthase.[76]

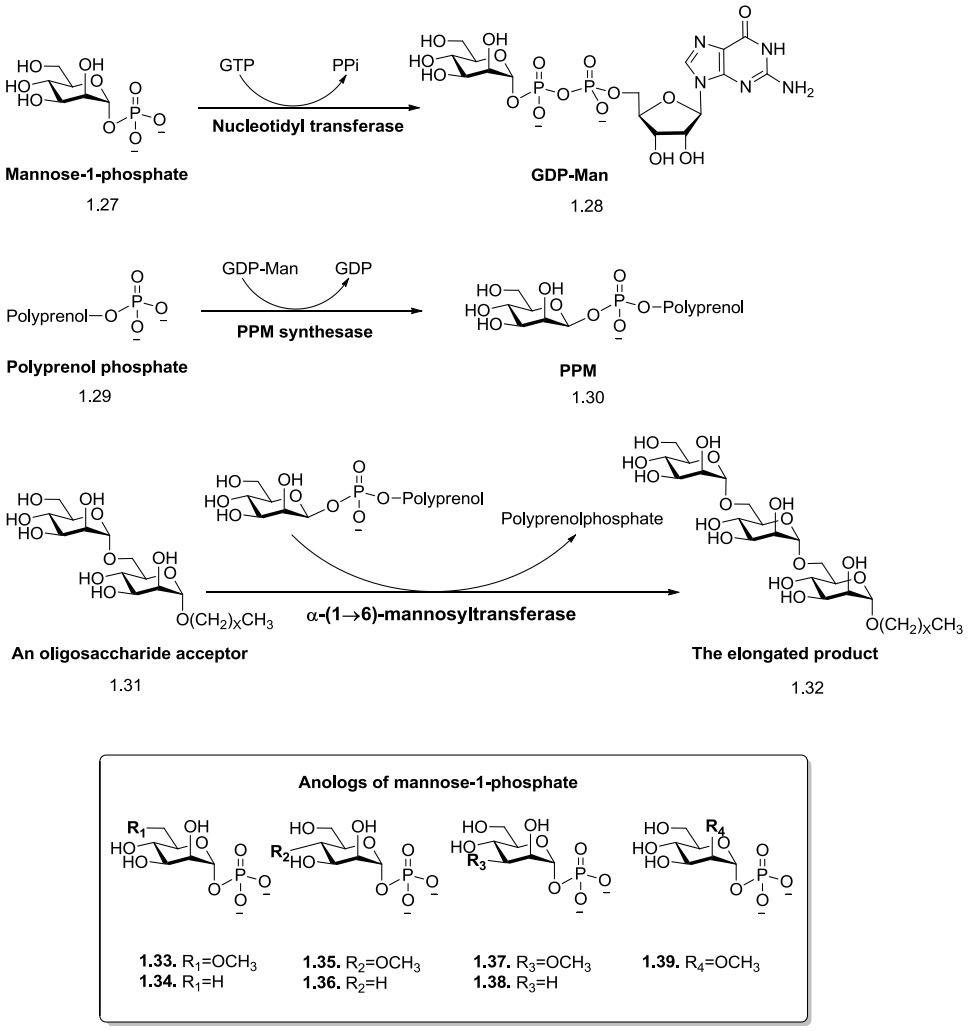


Figure 1.21. Schematic overview of targets for this thesis

We believed that a better understanding of how these enzymes interact with the donor substrate could be achieved by preparing a series analogs of mannose-1-phosphate, and testing them as substrates for these enzymes. First, a series of methoxy and deoxy derivatives of mannose-1-phosphate (**1.33–1.39, Figure 1.21**) were chemically synthesized, then, they were converted into the corresponding GDP-Man analogs enzymatically, catalyzed by GDP-ManPP. After that, a polyprenolphosphate analog[101] (**Figure 1.22**) was synthesized in order to

test the activity of PPM synthase, and to obtain the corresponding PPM donor analogs. Previous work has shown that these lipids are substrates for this enzyme. The final goal, if time permitted, was to explore the donor substrate binding interactions in the active site of PPM-dependent α-(1→6)-ManT using the PPM analogs prepared using the PPM synthase. The schematic overview of my project is shown in **Figure 1.21**.

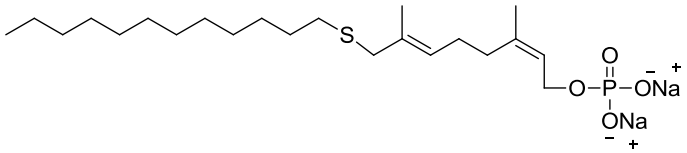


Figure 1.22. Structure of the polyprenolphosphate analog

Details of the synthetic work towards mannose-1-phosphate derivatives and the polyprenolphosphate analog are presented in Chapter 2. The biological evaluation of these compounds is illustrated in Chapter 3. The studies reported in these two chapters have enabled us to map important hydrogen bonding and steric interactions occurring in GDP-ManPP's active site. Because the required nucleotidyl transferease from mycobacteria has not been unequivocally identified, we used instead a related enzyme from *Salmonella enterica.*[102] In addition, preliminary data on the use of the synthesized GDP-Man analogs by the mycobacterial PPM synthase is reported in Chapter 3.

1.6. Bibliography

(1) Paolo, W. F.; Nosanchuk, J. D. *Lancet Infect. Dis* **2004**, *4*, 287-293.

(2) Davies, P. D. *Ann. Med.* **2003**, *35*, 235-243.

(3) Coker, R. J. *Trop. Med. Int. Health* **2004**, *9*, 25-40.

(4) World Health Organization. *Global tuberculosis control: surveillance, planning, financing*; WHO: Geneva, **2008**.

(5) World Health Organization. *Global tuberculosis control 2009: epidemiology, strategy, financing*; WHO: Geneva, **2009**.

(6) Parrish, N. M.; Dick, J. D.; Bishai, W. R. *Trends Microbiol.* **1998**, *6*, 107-112.

(7) Nachega, J. B.; Chaisson, R. E. *Clin. Infect. Dis.* **2003**, *36*, S24-S30.

(8) van Crevel, R.; Ottenhoff, T. H. M.; van der Meer, J. W. M. *Clin. Microbiol. Rev.* **2002**, *15*, 294-309.

(9) World Health Organization. *Tuberculosis Fact sheet number 104-Tuberculosis*; WHO: Geneva, **2010**.

(10) McKenna, M. T.; McCray, E.; Onorato, I. *New Engl. J. Med.* **1995**, *332*, 1071-1076.

(11) World Health Organization. *TB, a global emergency*; WHO: Geneva, **1994**.

(12) Badri, M.; Wilson, D.; Wood, R. *The Lancet* **2002**, *359*, 2059-2064.

(13) Freedberg, K. A.; Losina, E.; Weinstein, M. C.; Paltiel, A. D.; Cohen, C. J.; Seage, G. R.; Craven, D. E.; Zhang, H.; Kimmel, A. D.; Goldie, S. J. *New Engl. J. Med.* **2001**, *344*, 824-831.

(14) Tocque, K.; Bellis, M. A.; Beeching, N. J.; Syed, Q.; Remmington, T.; Davies, P. D. O. *Eur. Respir. J.* **2001**, *18*, 959-964.

(15) World Health Organization. *Emergence of XDR-TB*; WHO: Geneva, **2006**.

(16) World Health Organization. *Anti-tuberculosis drug resistance in the world: the WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance 1994-7*; WHO: Geneva, **1997**.

(17) Huebner, R. E.; Castro, K. G. *Annu. Rev. Med.* **1995**, *46*, 47-55.

(18) Jay, V. *Arch. Pathol. Lab. Med.* **2001**, *125*, 1148-1149.

(19) Fenton, M.; Vermeulen, M. *Infect. Immun.* **1996**, *64*, 683-690.

(20) Noss, E. H.; Pai, R. K.; Sellati, T. J.; Radolf, J. D.; Belisle, J.; Golenbock, D. T.; Boom, W. H.; Harding, C. V. *J. Immunol* **2001**, *167*, 910-918.

(21) Russell, D. G. *Nat Rev Mol Cell Biol* **2001**, *2*, 569-586.

(22) Malik, Z. A.; Denning, G. M.; Kusner, D. J. *J. Exp. Med* **2000**, *191*, 287-302.

(23) Zahrt, Thomas C. *Microb. Infect.* **2003**, *5*, 159-167.

(24) Bass, J., Jr; Farer, L.; Hopewell, P.; O'Brien, R.; Jacobs, R.; Ruben, F.; Snider, D., Jr; Thornton, G. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, 1359-1374.

(25) Janin, Y. L. *Biorg. Med. Chem.* **2007**, *15*, 2479-2513.

(26) Gandhi, N. R.; Moll, A.; Sturm, A. W.; Pawinski, R.; Govender, T.; Lalloo, U.; Zeller, K.; Andrews, J.; Friedland, G. *The Lancet* **2006**, *368*, 1575-1580.

(27) Mills, J. A.; Motichka, K.; Jucker, M.; Wu, H. P.; Uhlik, B. C.; Stern, R. J.; Scherman, M. S.; Vissa, V. D.; Pan, F.; Kundu, M.; Ma, Y. F.; McNeil, M. *J. Biol. Chem.* **2004**, *279*, 43540-43546.

(28) Vilcheze, C.; Morbidoni, H. R.; Weisbrod, T. R.; Iwamoto, H.; Kuo, M.; Sacchettini, J. C.; Jacobs, W. R., Jr. *J. Bacteriol.* **2000**, *182*, 4059-4067.

(29) Jarlier, V.; Nikaido, H. *J. Bacteriol.* **1990**, *172*, 1418-1423.

(30) Trias, J.; Benz, R. *Mol. Microbiol.* **1994**, *14*, 283-290.

(31) Barry, C. E.; Mdluli, K. *Trends Microbiol.* **1996**, *4*, 275-281.

(32) Brennan, P. J. *Tuberculosis (Edinburgh, Scotland)* **2003**, *83*, 91-97.

(33) Brennan, P. J.; Nikaido, H. *Annu. Rev. Biochem* **1995**, *64*, 29-63.

(34) Crick, D. C.; Mahapatra, S.; Brennan, P. J. *Glycobiology* **2001**, *11*, 107R-118R.

(35) Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199-215.

(36) McNeil, M.; Wallner, S. J.; Hunter, S. W.; Brennan, P. J. *Carbohydr. Res.* **1987**, *166*, 299-308.

(37) Daffe, M.; McNeil, M.; Brennan, P. J. *Carbohydr. Res.* **1993**, *249*, 383-398.

(38) Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734-6743.

(39) McNeil, M. R.; Robuck, K. G.; Harter, M.; Brennan, P. J. *Glycobiology* **1994**, *4*, 165-174.

(40) Alderwick, L. J.; Radmacher, E.; Seidel, M.; Gande, R.; Hitchen, P. G.; Morris, H. R.; Dell, A.; Sahm, H.; Eggeling, L.; Besra, G. S. *J. Biol. Chem.* **2005**, *280*, 32362-32371.

(41) Blanchard, J. S. *Annu. Rev. Biochem* **1996**, *65*, 215-239.

(42) McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1991**, *266*, 13217-13223.

(43) Qureshi, N.; Takayama, K.; Jordi, H. C.; Schnoes, H. K. *J. Biol. Chem.* **1978**, *253*, 5411-5417.

(44) Nikaido, H. *Science* **1994**, *264*, 382-388.

(45) Liu, J.; Barry, C. E.; Besra, G. S.; Nikaido, H. *J. Biol. Chem.* **1996**, *271*, 29545-29551.

(46) Yuan, Y.; Crane, D. C.; Musser, J. M.; Sreevatsan, S.; Barry, C. E. *J. Biol. Chem.* **1997**, *272*, 10041-10049.

(47) Khoo, K.-H.; Dell, A.; Morris, H. R.; Breman, P. J.; Chatterjee, D. *Glycobiology* **1995**, *5*, 117-127.

(48) Besra, G. S.; Morehouse, C. B.; Rittner, C. M.; Waechter, C. J.; Brennan, P. J. *J. Biol. Chem.* **1997**, *272*, 18460-18466.

(49) Tam, P.-H.; Lowary, T. L. *Curr. Opin. Chem. Biol.* **2009**, *13*, 618-625.

(50) Chatterjee, D.; Hunter, S. W.; McNeil, M.; Brennan, P. J. *J. Biol. Chem.* **1992**, *267*, 6228-6233.

(51) Venisse, A.; Rivière, M.; Vercauteren, J.; Puzo, G. *J. Biol. Chem.* **1995**, *270*, 15012-15021.

(52) Briken, V.; Porcelli, S. A.; Besra, G. S.; Kremer, L. *Mol. Microbiol.* **2004**, *53*, 391-403.

(53) Kaur, D.; McNeil, M. R.; Khoo, K.-H.; Chatterjee, D.; Crick, D. C.; Jackson, M.; Brennan, P. J. *J. Biol. Chem.* **2007**, *282*, 27133-27140.

(54) Khoo, K.-H.; Douglas, E.; Azadi, P.; Inamine, J. M.; Besra, G. S.; Mikušová, K.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1996**, *271*, 28682-28690.

(55) Fontecave, M.; Eliasson, R.; Reichard, P. *J. Biol. Chem.* **1987**, *262*, 12325-12331.

(56) Nigou, J.; Vercellone, A.; Puzo, G. *J. Mol. Biol.* **2000**, *299*, 1353-1362.

(57) Khoo, K.-H.; Dell, A.; Morris, H. R.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1995**, *270*, 12380-12389.

(58) Chatterjee, D.; Khoo, K.-H. *Glycobiology* **1998**, *8*, 113-120.

(59) Guérardel, Y.; Maes, E.; Elass, E.; Leroy, Y.; Timmerman, P.; Besra, G. S.; Locht, C.; Strecker, G.; Kremer, L. *J. Biol. Chem.* **2002**, *277*, 30635-30648.

(60) Joe, M.; Sun, D.; Taha, H.; Completo, G. C.; Croudace, J. E.; Lammas, D. A.; Besra, G. S.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 5059-5072.

(61) Schlesinger, L.; Hull, S.; Kaufman, T. *J. Immunol* **1994**, *152*, 4070-4079.

(62) Koppel, E. A.; Ludwig, I. S.; Sanchez Hernandez, M.; Lowary, T. L.; Gadikota, R. R.; Tuzikov, A. B.; Vandenbroucke-Grauls, C. M. J. E.; van Kooyk, Y.; Appelmelk, B. J.; Geijtenbeek, T. B. H. *Immunobiology* **2004**, *209*, 117-127.

(63) Geijtenbeek, T. B. H.; van Vliet, S. J.; Koppel, E. A.; Sanchez-Hernandez, M.; Vandenbroucke-Grauls, C. M. J. E.; Appelmelk, B.; van Kooyk, Y. *J. Exp. Med* **2003**, *197*, 7-17.

(64) Malaga, W.; Constant, P.; Euphrasie, D.; Cataldi, A.; Daffé, M.; Reyrat, J.-M.; Guilhot, C. *J. Biol. Chem.* **2008**, *283*, 15177-15184.

(65) Chatterjee, D.; Khoo, K. H. *Cell. Mol. Life Sci.* **2001**, *58*, 2018-2042.

(66) Tam, P.-H.; Besra, G. S.; Lowary, T. L. *ChemBioChem* **2008**, *9*, 267-278.

(67) Jackson, M.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 30092-30099.

(68) Korduláková, J.; Gilleron, M.; Puzo, G.; Brennan, P. J.; Gicquel, B.; Mikušová, K.; Jackson, M. *J. Biol. Chem.* **2003**, *278*, 36285-36295.

(69) Korduláková, J.; Gilleron, M.; Mikušová, K.; Puzo, G.; Brennan, P. J.; Gicquel, B.; Jackson, M. *J. Biol. Chem.* **2002**, *277*, 31335-31344.

(70) Schaeffer, M. L.; Khoo, K.-H.; Besra, G. S.; Chatterjee, D.; Brennan, P. J.; Belisle, J. T.; Inamine, J. M. *J. Biol. Chem.* **1999**, *274*, 31625-31631.

(71) Kremer, L.; Gurcha, S. S.; Bifani, P.; Hitchen, P. G.; Baulard, A.; Morris, H. R.; Dell, A.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *363*, 437-447.

(72) Alexander, D. C.; Jones, J. R. W.; Tan, T.; Chen, J. M.; Liu, J. *J. Biol. Chem.* **2004**, *279*, 18824-18833.

(73) Belanger, A. E.; Besra, G. S.; Ford, M. E.; Mikusová, K.; Belisle, J. T.; Brennan, P. J.; Inamine, J. M. *Proc. Natl. Acad. Sci. U. S. A* **1996**, *93*, 11919-11924.

(74) Zhang, N.; Torrelles, J. B.; McNeil, M. R.; Escuyer, V. E.; Khoo, K.-H.; Brennan, P. J.; Chatterjee, D. *Mol. Microbiol.* **2003**, *50*, 69-76.

(75) Berg, S.; Starbuck, J.; Torrelles, J. B.; Vissa, V. D.; Crick, D. C.; Chatterjee, D.; Brennan, P. J. *J. Biol. Chem.* **2005**, *280*, 5651-5663.

(76) Gurcha, S. S.; Baulard, A. R.; Kremer, L.; Locht, C.; Moody, D. B.; Muhlecker, W.; Costello, C. E.; Crick, D. C.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *365*, 441-450.

(77) Berg, S.; Kaur, D.; Jackson, M.; Brennan, P. J. *Glycobiology* **2007**, *17*, 35R-56R.

(78) Dinadayala, P.; Kaur, D.; Berg, S.; Amin, A. G.; Vissa, V. D.; Chatterjee, D.; Brennan, P. J.; Crick, D. C. *J. Biol. Chem.* **2006**, *281*, 20027-20035.

(79) Kaur, D.; Berg, S.; Dinadayala, P.; Gicquel, B.; Chatterjee, D.; McNeil, M. R.; Vissa, V. D.; Crick, D. C.; Jackson, M.; Brennan, P. J. *Proc. Natl. Acad. Sci. U. S. A* **2006**, *103*, 13664-13669.

(80) Morita, Y. S.; Sena, C. B. C.; Waller, R. F.; Kurokawa, K.; Sernee, M. F.; Nakatani, F.; Haites, R. E.; Billman-Jacobe, H.; McConville, M. J.; Maeda, Y.; Kinoshita, T. *J. Biol. Chem.* **2006**, *281*, 25143-25155.

(81) Brennan, P.; Ballou, C. E. *J. Biol. Chem.* **1967**, *242*, 3046-3056.

(82) Guerin, M. E.; Kordulakova, J.; Schaeffer, F.; Svetlikova, Z.; Buschiazzo, A.; Giganti, D.; Gicquel, B.; Mikusova, K.; Jackson, M.; Alzari, P. M. *J. Biol. Chem.* **2007**, *282*, 20705-20714.

(83) Guerin, M. E.; Schaeffer, F.; Chaffotte, A.; Gest, P.; Giganti, D.; Korduláková, J.; van der Woerd, M.; Jackson, M.; Alzari, P. M. *J. Biol. Chem.* **2009**, *284*, 21613-21625.

(84) Lea-Smith, D. J.; Martin, K. L.; Pyke, J. S.; Tull, D.; McConville, M. J.; Coppel, R. L.; Crellin, P. K. *J. Biol. Chem.* **2008**, *283*, 6773-6782.

(85) Mishra, A. K.; Batt, S.; Krumbach, K.; Eggeling, L.; Besra, G. S. *J. Bacteriol.* **2009**, *191*, 4465-4472.

(86) Torrelles, J. B.; DesJardin, L. E.; MacNeil, J.; Kaufman, T. M.; Kutzbach, B.; Knaup, R.; McCarthy, T. R.; Gurcha, S. S.; Besra, G. S.; Clegg, S.; Schlesinger, L. S. *Glycobiology* **2009**, *19*, 743-755.

(87) Mishra, A. K.; Alderwick, L. J.; Rittmann, D.; Tatituri, R. V. V.; Nigou, J.; Gilleron, M.; Eggeling, L.; Besra, G. S. *Mol. Microbiol.* **2007**, *65*, 1503-1517.

(88) Mishra, A. K.; Alderwick, L. J.; Rittmann, D.; Wang, C.; Bhatt, A.; Jacobs Jr, W. R.; Takayama, K.; Eggeling, L.; Besra, G. S. *Mol. Microbiol.* **2008**, *68*, 1595-1613.

(89) Ramaswamy, S. V.; Amin, A. G.; Goksel, S.; Stager, C. E.; Dou, S.-J.; El Sahly, H.; Moghazeh, S. L.; Kreiswirth, B. N.; Musser, J. M. *Antimicrob. Agents Chemother.* **2000**, *44*, 326-336.

(90) Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.;

Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M. A.; Rajandream, M. A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J. E.; Taylor, K.; Whitehead, S.; Barrell, B. G. *Nature* **1998**, *393*, 537-544.

(91) Escuyer, V. E.; Lety, M.-A.; Torrelles, J. B.; Khoo, K.-H.; Tang, J.-B.; Rithner, C. D.; Frehel, C.; McNeil, M. R.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **2001**, *276*, 48854-48862.

(92) Goude, R.; Amin, A. G.; Chatterjee, D.; Parish, T. *J. Bacteriol.* **2008**, *190*, 4335-4341.

(93) Shi, L.; Berg, S.; Lee, A.; Spencer, J. S.; Zhang, J.; Vissa, V.; McNeil, M. R.; Khoo, K.-H.; Chatterjee, D. *J. Biol. Chem.* **2006**, *281*, 19512-19526.

(94) Kaur, D.; Obregón-Henao, A.; Pham, H.; Chatterjee, D.; Brennan, P. J.; Jackson, M. *Proc. Natl. Acad. Sci. U. S. A* **2008**, *105*, 17973-17977.

(95) Subramaniam, V.; Gurcha, S. S.; Besra, G. S.; Lowary, T. L. *Biorg. Med. Chem.* **2005**, *13*, 1083-1094.

(96) Subramaniam, V.; Gurcha, S. S.; Besra, G. S.; Lowary, T. L. *Tetrahedron: Asymmetry* **2005**, *16*, 553-567.

(97) Tam, P.-H.; Lowary, T. L. *Carbohydr. Res.* **2007**, *342*, 1741-1772.

(98) Tam, P. H.; Lowary, T. L. *Org. Biomol. Chemistry* **2010**, *8*, 181-192.

(99) Yokoyama, K.; Ballou, C. E. *J. Biol. Chem.* **1989**, *264*, 21621-21628.

(100) Brown, J. R.; Field, R. A.; Barker, A.; Guy, M.; Grewal, R.; Khoo, K.-H.; Brennan, P. J.; Besra, G. S.; Chatterjee, D. *Biorg. Med. Chem.* **2001**, *9*, 815-824.

(101) Guy, M. R.; Illarionov, P. A.; Gurcha, S. S.; Dover, L. G.; Gibson, K. J. C.; Smith, P. W.; Minnikin, D. E.; Besra, G. S. *Biochem. J.* **2004**, *382*, 905-912.

(102) Elling, L.; Ritter, J. E.; Verseck, S. *Glycobiology* **1996**, *6*, 591-597.

Chapter 2

Synthesis of substrate analogs for probing the substrate specificity of GDP-mannose pyrophosphorylase, polyprenol phosphomannose synthase and α-(1→6)-mannosyltransferase

2.1. Introduction

Glycosyltransferases (GTs), enzymes responsible for biosynthesis of complex carbohydrates, are able to transfer glycosyl residues from sugar nucleotides or sugar lipid phosphates to an oligosaccharide acceptor to give an elongated product.[1] Specific inhibitors of these GTs, or sugar lipid phosphate substrates, would provide useful tools to study the biological role of these cell surface carbohydrates, and potentially lead to the development of new chemothereputics. To facilitate the development of these potential inhibitors, an understanding of the specific carbohydrate–protein interactions occurring in the active site of these enzymes is desired.

The identification of specific molecular recognition elements in the active site of a protein can be obtained by modifying single hydroxyl groups on the donor or acceptor substrate structures.[1-4] The specific parts of the substrate that are necessary for the recognition process, or for substrate turnover, can be determined using this method. Hydrogen-bonding and steric interactions in the active site of the enzyme can be mapped through the use of deoxy and methoxy substrate analogs. The deoxygenated derivatives are used to probe the hydrogen-bonding interactions between the polar groups in the active site and the hydroxyl groups on the sugar residue. The reason to choose hydrogen to replace a hydroxyl group is because it is non-polar, small and not capable of forming hydrogen bonds. The steric requirements of the active site are tested by replacing single hydroxyl groups with a methoxy group, which will add additional steric bulk to the

substrate while still being able to act as a hydrogen bond acceptor, although not a hydrogen-bond donor.[5]

Therefore, to carry out these studies, a panel of methoxy and deoxy analogs (**Figure 2.1**) of the natural α-D-mannopyranosyl-1-phosphate (Man*p*-1P, **2.1**) substrates of the LAM biosynthetic pathway were chemically synthesized. This chapter will focus on the chemical synthesis of these analogs from commercially available D-mannose. These synthetic Man*p*-1P analogs could then be used as probes to explore the carbohydrate–protein binding interactions of the mycobacterial LM and LAM biosynthetic enzymes. To date, these analogs have been screened against a nucleotidyltransferase (GDP-ManPP) from *Salmonella enterica*, to explore the substrate specificities of this enzyme. As will be discussed in Chapter 3, this enzyme was used to produce the corresponding GDP-Man*p* analogs.

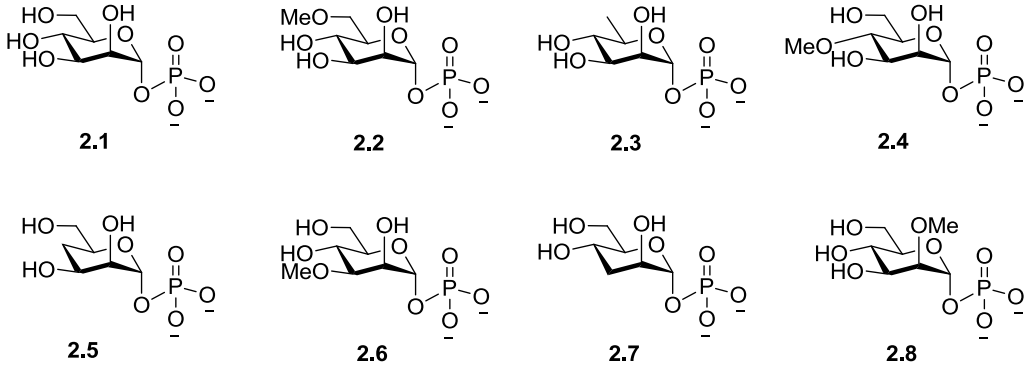
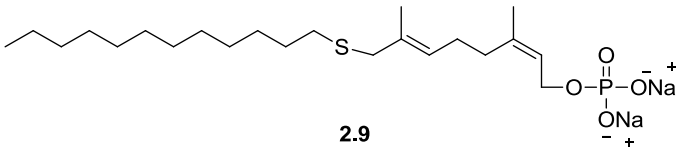


Figure 2.1. Synthetic methoxy and deoxy analogs used as probes of GDP-ManPP

Additionally, a polyprenol phosphate analog **2.9** was also synthesized to test the activity of PPM synthase with the GDP-Man analogs prepared using the GDP-ManPP enzyme, as will be described in Chapter 3.



2.9

Figure 2.2. The structure of the polyprenol phosphate analog

2.2. Results and discussion

The methoxy and deoxy analogs of Man*p*-1P were prepared using established protection–deprotection strategies to give selectively protected mannose residues with a single free hydroxyl group. Triphenylmethyl (Trityl, Tr) and *t*-butyldiphenylsilyl (TBDPS) groups were used to protect the primary hydroxyl group at C-6 position, benzylidene groups were used to protect C-4 and C-6 hydroxyl groups, and isopropylidene groups were used to protect C-2 and C-3 hydroxyl groups. Treating the free hydroxyl groups with methyl iodide (CH_3I) under basic conditions yielded the corresponding methoxy analogs. Substitution with iodine and reduction, or Barton–McCombie radical deoxygenation, were used to prepare the deoxy analogs.[5] All of the anomeric phosphates were prepared by the reaction of a thioglycoside donor and dibenzyl phosphate acceptor. The protecting groups, *e.g.*, benzyl (Bn), benzoyl (Bz), and acetyl (Ac), were then cleaved by catalytic hydrogenolysis and deacylation under mild basic conditions.

The magnitudes of $^{3}J_{\text{H-1,H-2}}$ are usually used to determine glycoside stereochemistry, when O-2 is equatorial, using the Karplus relationship. However, $^{3}J_{\text{H-1,H-2}}$ in mannose, which has axial O-2, are of similar magnitude in both α and β configurations. Consequently, one bond $^{13}C–^{1}H$ coupling constants are required to unambiguously determine mannose anomeric stereochemistry. Bock and

coworkers have shown that a $^{1}J_{C\text{-}1,H\text{-}1}$ ~ 170 Hz for D sugars in the $^{4}C_{1}$ conformation indicates an α-anomeric sugar configuration, while $^{1}J_{C\text{-}1,H\text{-}1}$ ~ 160 Hz suggests a β-anomeric sugar configuration. Additionally, the $^{1}J_{C\text{-}1,H\text{-}1}$ value (170 Hz) has been used previously by Jakeman and coworkers to confirm the formation α-L-rhamnopyranose-1-phosphates.[6] Therefore, the α-stereochemistry of the glycosidic linkages in this chapter was confirmed by measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants using coupled HSQC experiments.[6-8] The $^{1}J_{C\text{-}1,H\text{-}1}$ data of eight Man*p*-1P analog final products are listed in **Table 2.1**.

Table 2.1. $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants of eight Man*p*-1P analogs using coupled HSQC experiments

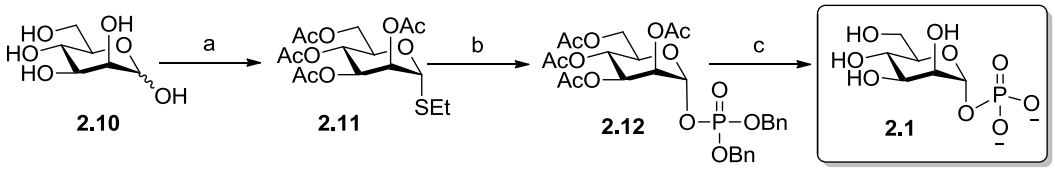
Product	$^{1}J_{C\text{-}1,H\text{-}1}$ (Hz)
α-D-Mannopyranosyl phosphate (**2.1**)	172.8
6-*O*-Methyl-α-D-mannopyranosyl phosphate (**2.2**)	170.9
6-Deoxy-α-D-mannopyranosyl phosphate (**2.3**)	171.2
4-*O*-Methyl-α-D-mannopyranosyl phosphate (**2.4**)	177.3
4-Deoxy-α-D-*lyxo*-hexopyranosyl phosphate (**2.5**)	169.9
3-*O*-Methyl-α-D-mannopyranosyl phosphate (**2.6**)	178.5
3-Deoxy-α-D-*arabino*-hexopyranosyl phosphate (**2.7**)	173.2
2-*O*-Methyl-α-D-mannopyranosyl phosphate (**2.8**)	177.9

As for the polyprenol phosphate analog, the desired product **2.9** was synthesized by using previously established strategies[9,10] starting from commercially available neryl acetate.

2.2.1. Synthesis of mannopyranosyl phosphate

To prepare the natural substrate mannose-1-phosphate, commercially available D-mannose **2.10** was first peracetylated, then converted to thioglycoside donor **2.11** with ethanethiol in the presence of BF_3•OEt_2 (Scheme 2.1). The product was obtained in 62% yield over the two steps. The fully protected mannosyl phosphate **2.12** was produced in 69% yield by coupling the thioglycoside donor and dibenzyl phosphate under standard *N*-iodosuccinimide–

silver triflate (NIS–AgOTf) activating conditions. Hydrogenolysis in the presence of palladium hydroxide under basic conditions was used for debenzylation. Finally, the weakly basic mixture of 5:2:1 CH_3OH–H_2O–Et_3N was used to cleave the acetyl groups to give the deprotected Man*p*-1P **2.1** in 85% yield from **2.12**.



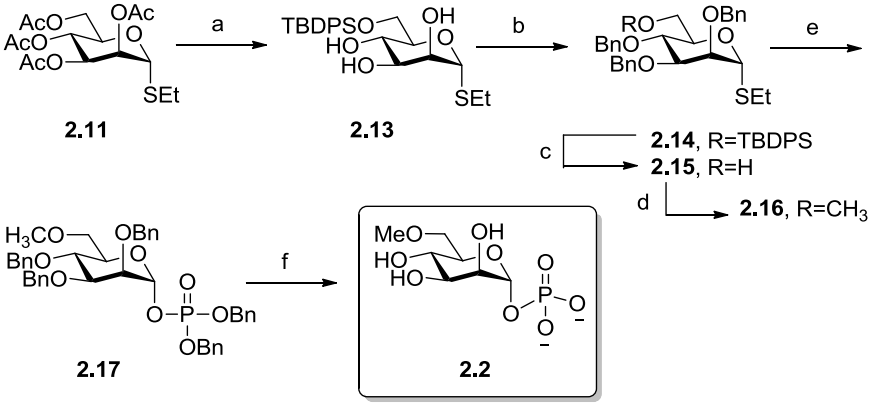
Scheme 2.1. Reagents and conditions: (a) i) Ac_2O, Pyridine; ii) EtSH, BF_3•OEt_2, 62%; (b) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 69%; (c) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 85%.

2.2.2. Synthesis of 6-methoxy mannopyranosyl phosphate

Two synthetic pathways were used to produce the 6-methoxy Man*p*-1P derivative (Schemes 2.2 and 2.3). The main difference between the two approaches lies in the different protecting groups used on the dibenzyl phosphate intermediates. In one route the C-2, C-3, and C-4 hydroxyl groups of the mannose residues were protected with benzyl ether groups and in the other they were protected with benzoyl ester groups. The overall yields of these two methods were 30% and 17%, respectively.

In the first method (Scheme 2.2), the initial step was the conversion of the fully acetylated thiglycoside **2.11** into the deprotected intermediate by treatment with sodium methoxide. Next a TBDPS group was used to protect the primary hydroxyl group of this deprotected derivative, and the intermediate product was

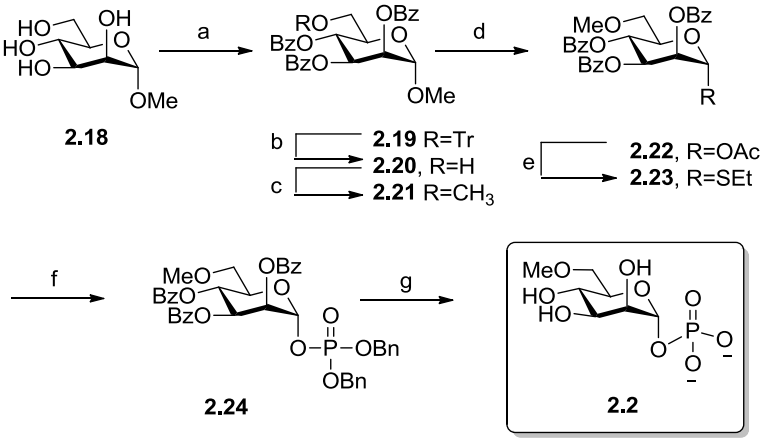
benzylated using benzyl bromide and sodium hydride. The TBDPS group was then cleaved and replaced with a methyl group to give the 6-methoxy compound **2.16** in 72% yield over two steps. The protected dibenzyl phosphate **2.17** was formed in 70% yield by phosphorylation with dibenzyl phosphate using NIS–AgOTf activation. Catalytic hydrogenolysis in the presence of $NaHCO_3$ was used to cleave all the benzyl groups in 91% yield.



Scheme 2.2. Reagents and conditions: (a) i) $NaOCH_3$, CH_3OH; ii) TBDPSCl, imidazole, 78%; (b) BnBr, NaH, TBAI, 84%; (c) TBAF, THF, 83%; (d) CH_3I, NaH, DMF, 87%; (e) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 70%; (f) H_2, $Pd(OH)_2$–C, $NaHCO_3$, CH_3OH, 91%.

For the second pathway (Scheme 2.3), the trityl group was used to protect C-6 hydroxyl group of commercially available methyl α-D-mannopyranoside (**2.18**), and the remaining hydroxyl groups were benzoylated providing **2.19** in a yield of 72% over the two steps. The trityl group was then removed using 10% HCl in methanol–dichloromethane and the resulting alcohol was substituted with a methyl group. Unfortunately, acyl group migration occurred during the

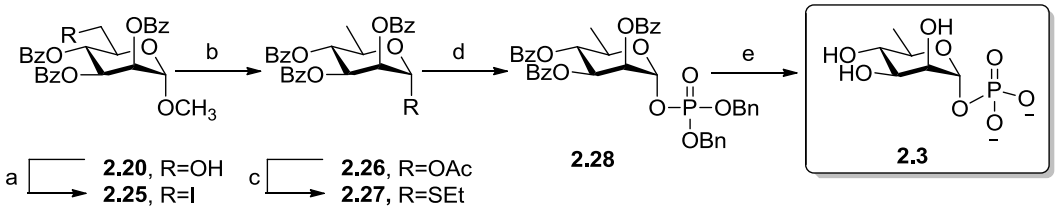
methylation step, even when mildly basic conditions (*e.g.*, Ag_2O–$CaSO_4$) were used, which led to the relatively low yield 52% of this step. Acetolysis conditions were used to replace the methyl group at the anomeric centre with an acetyl group, in 96% yield. Thioglycosylation, followed by coupling of the thioglycoside donor **2.23** with dibenzyl phosphate, gave phosphate **2.24** in a yield of 67% over the two steps. The 6-methoxy analog **2.2** was obtained using catalytic hydrogenolysis of the benzyl ethers **2.24** followed by treatment with CH_3OH–H_2O–Et_3N 5:2:1 in 85% yield over two steps.



Scheme 2.3. Reagents and conditions: (a) i) TrCl, DMAP, pyridine; ii) BzCl, 72%; (b) HCl, CH_2Cl_2, CH_3OH, 81%; (c) Ag_2O, $CaSO_4$, CH_3I, 52%; (d) Ac_2O–HOAc–H_2SO_4, 70:30:1, 96%; (e) EtSH, BF_3•OEt_2, CH_2Cl_2, 75%; (f) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 89%; (g) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 85%.

2.2.3. Synthesis of 6-deoxy mannopyranosyl phosphate

The synthesis of the 6-deoxy Man*p*-1P analog is shown in Scheme 2.4. This route used an intermediate (**2.20**) prepared in the course of the synthesis of the 6-methoxy analog. First, the free hydroxyl group of **2.20** was substituted by iodide in 65% yield using triphenylphospine and iodine. The methyl group at the anomeric centre of the product, **2.25**, was then replaced with acetyl group by acetolysis. This iodo-substituted derivative was then reduced using catalytic hydrogenation to yield 6-deoxy compound **2.26** in 72% yield from **2.25**. The subsequent thioglycosylation, phosphorylation and deprotection steps were the same as the steps described in the second method to synthesize the 6-methoxy analog. Using this approach, the 6-deoxy analog **2.3** was prepared in 43% yield over four steps.

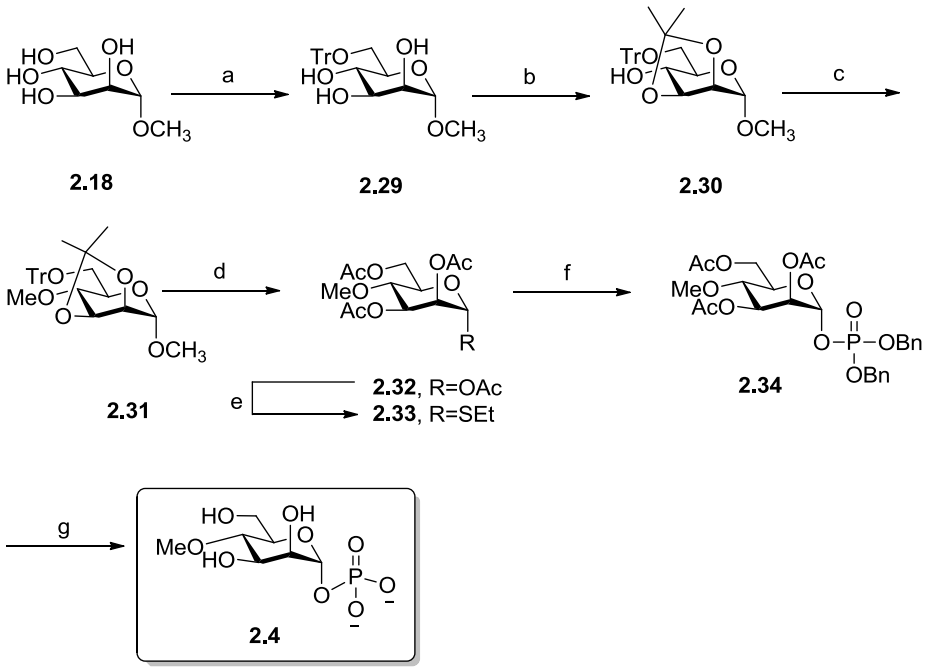


Scheme 2.4. Reagents and conditions: (a) PPh_3, imidazole, I_2, 65%; (b) i) Ac_2O–HOAc–H_2SO_4, 35:15:1; ii) Pd–C, H_2, Et_3N, EtOAc, CH_3OH, 72%; (c) EtSH, BF_3•OEt_2, CH_2Cl_2, 89%, α/β 4:1; (d) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 67%; (e) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 72%.

2.2.4. Synthesis of 4-methoxy mannopyranosyl phosphate

Shown in Scheme 2.5 is the synthesis of the 4-methoxy Man*p*-1P analog. Trityl chloride was first used to protect the C-6 hydroxyl group of commercially

available methyl α-D-mannopyranoside **2.18**. Next, the C-2 and C-3 hydroxyl groups were then protected with an isopropylidene group to give **2.30** with a free C-4 hydroxyl group in an overall yield of 65% from **2.18**. The hydroxyl group in **2.30** was methylated under standard conditions (CH_3I, NaH) to give the 4-methoxy analog **2.31** in 91% yield. Acetolysis of the methyl glycoside **2.31** to the corresponding glycosyl acetate **2.32**, followed by reaction with ethanethiol and BF_3 OEt_2, yielded thioglycoside **2.33**, in 39% yield from **2.31**. This compound was used as a donor in a reaction with dibenzyl phosphate under NIS–AgOTf activating conditions to produce protected phosphate **2.34** in 80% yield. Hydrogen and palladium hydroxide on carbon were used under basic conditions to cleave benzyl groups in the phosphate moiety. Finally, deacetylation was achieved using the weakly basic mixture of methanol–water–triethylamine, which provided **2.4** in an overall yield of 70% from **2.34**.

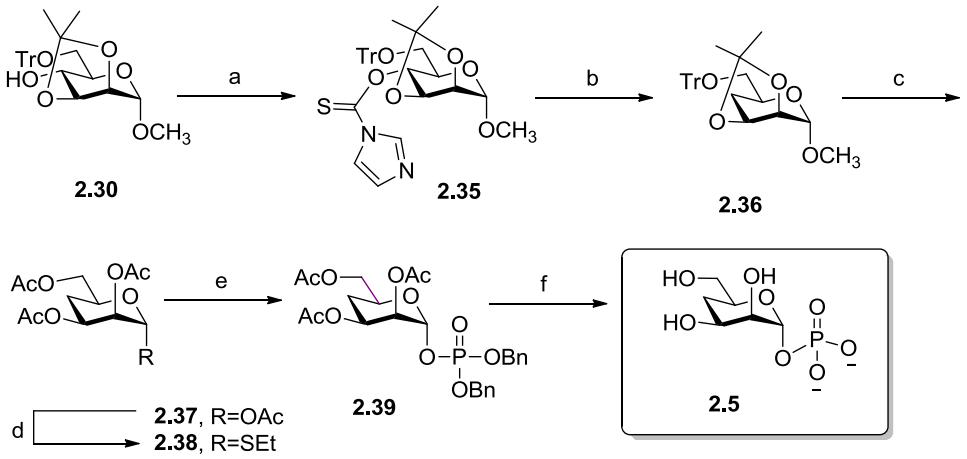


Scheme 2.5. Reagents and conditions: (a) TrCl, DMAP, pyridine, 85%; (b) DMP, *p*-TsOH, 76%; (c) CH_3I, NaH, DMF, 91%; (d) Ac_2O–HOAc–H_2SO_4, 35:15:1, 55%; (e) EtSH, BF_3•OEt_2, CH_2Cl_2, 70%; (f) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 80%; (g) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 70%.

2.2.5. Synthesis of 4-deoxy mannopyranosyl phosphate

In preparing the 4-deoxy Man*p*-1P analog, alcohol **2.30**, prepared in the course of synthesizing the 4-methoxy analog **2.3,** was used as an intermediate (Scheme 2.6). First, **2.30** was converted to the corresponding thiocarbamate **2.35** by reaction with 1,1'-thiocarbonyldiimidazole, and this product was reduced under Barton–McCombie deoxygenation conditions to give **2.36** in 90% yield over the two steps. The peracetylated compound **2.37** was obtained in one step by acetolysis in a modest (53%) yield. A number of other byproducts were observed

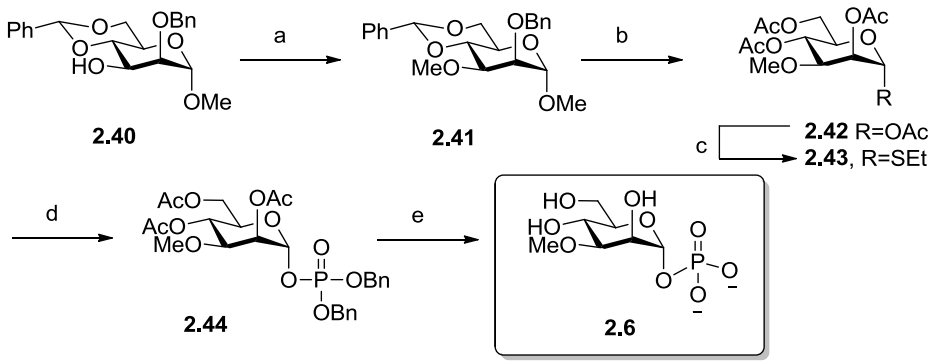
by TLC; however they were not isolated or characterized. Treatment of **2.37** with ethanethiol in the presence of BF_3•OEt_2 generated 32% yield of thioglycoside **2.38**. In addition, a 50% yield of the starting material was recovered after chromatography. The coupling of the thioglycoside donor with dibenzyl phosphate acceptor was achieved using standard NIS–AgOTf activating conditions to give the protected phosphate **2.39** in 81% yield. Deprotection of benzyl and acetyl groups as described previously for the other compounds, gave the 4-deoxy analog **2.5** in 62% yield over two steps.



Scheme 2.6. Reagents and conditions: (a) 1,1'-thiocarbonyldiimidazole, toluene, reflux, 98%; (b) AIBN, Bu_3SnH, toluene, reflux, 90%; (c) Ac_2O–HOAc–H_2SO_4, 35:15:1, 53%, α/β 6:1; (d) EtSH, BF_3•OEt_2, CH_2Cl_2, 32%; (e) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 81%; (f) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 62%.

2.2.6. Synthesis of 3-methoxy mannopyranosyl phosphate

The preparation of the 3-methoxy Man*p*-1P analog involved the use of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **2.40** (Scheme 2.7), which has been synthesized by a previous member of the group, Dr. Pui-Hang Tam. This compound was methylated to yield the 3-methoxy derivative **2.41** as shown in Scheme 2.7. The tetra-*O*-acetylated compound **2.42** was obtained in a single step from **2.41** after acetolysis in 65% yield, and subsequent thioglycosylation as described for the other compounds above gave thioglycoside donor **2.43**. The protected dibenzyl phosphate **2.44** was formed by glycosylation between the thioglycoside donor and dibenzyl phosphate under NIS–AgOTf activating condition. The yield of these two steps was 52% and 75%, respectively. A 5:2:1 mixture of CH_3OH–H_2O–Et_3N was used after catalytic hydrogenlysis to cleave both the acetyl and benzyl groups to give the deprotected 3-methoxy analog **2.6** in an overall yield of 67%.

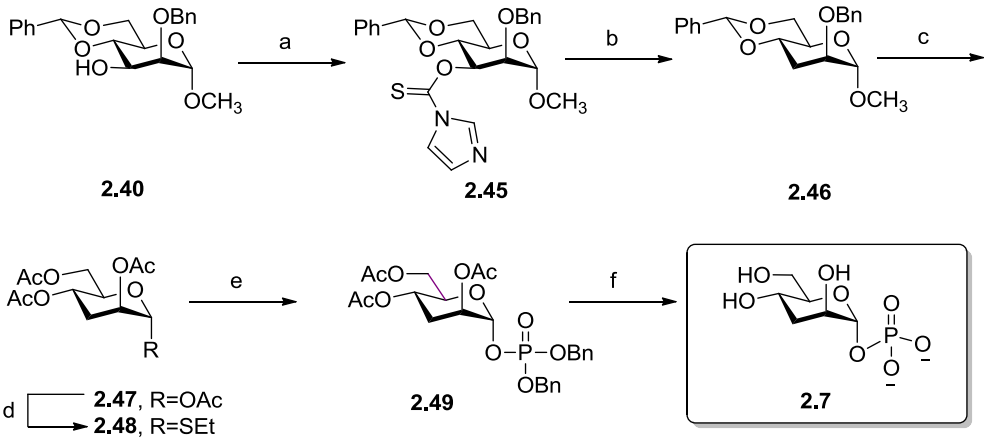


Scheme 2.7. Reagents and conditions: (a) CH_3I, NaH, DMF, 76%; (b) Ac_2O–HOAc–H_2SO_4, 35:15:1, 65%; (c) EtSH, BF_3•OEt_2, CH_2Cl_2, 52%; (d) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 75%; (e) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 67%.

2.2.7. Synthesis of 3-deoxy mannopyranosyl phosphate

To prepare the 3-deoxy Man*p*-1P analog, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **2.40** was first converted to **2.45** in 82% yield by reaction with 1,1'-thiocarbonyldiimidazole. This thiocarbamate derivative was then treated with azobisiobutyronitrile (AIBN) and tri-*n*-butyltin hydride to give the deoxygenated compound **2.46** in 57% yield. Acetolysis allowed for the formation of the peracetylated compound **2.47** in a single step although in a relatively low yield of 36% due to the low stability of the deoxy compound. A number of other byproducts were observed on TLC plate; however they were not isolated and characterized. Thioglycoside **2.48** was first coupled with dibenzyl phosphate to yield the protected 3-deoxy mannopyranosyl phosphate **2.49** in 45% overall yield from **2.47**. The benzyl groups were removed after catalytic

hydrogenlysis. Deacetylation was then performed in a mixture of CH_3OH–H_2O–Et_3N 5:2:1. The 3-deoxy analog **2.7** was obtained in 71% yield over two steps.

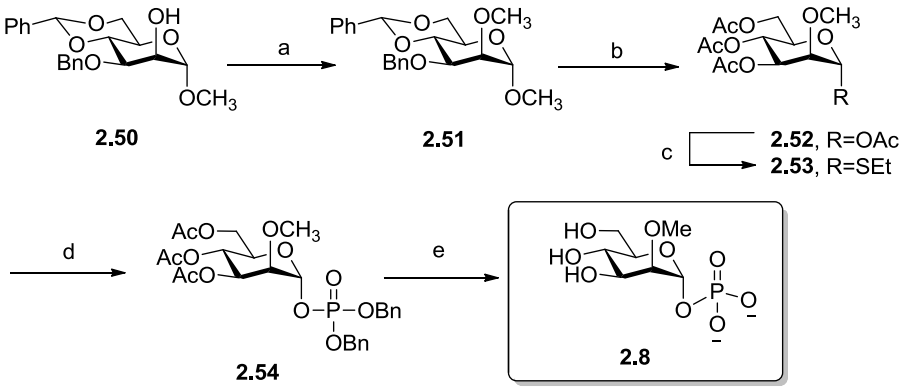


Scheme 2.8. Reagents and conditions: (a) 1,1'-thiocarbonyldiimidazole, toluene, 82%; (b) AIBN, *n*-Bu_3SnH, toluene, reflux, 57%; (c) Ac_2O–HOAc–H_2SO_4, 35:15:1, 36%; (d) EtSH, BF_3•OEt_2, CH_2Cl_2, 56%; (e) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 80%; (f) i) H_2, $Pd(OH)_2$–C, toluene, Et_3N, pyridine; ii) CH_3OH–H_2O–Et_3N, 5:2:1, 71%.

2.2.8. Synthesis of 2-methoxy mannopyranosyl phosphate

In order to synthesize the 2-methoxy Man*p*-1P analog, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **2.50**, which has been prepared by a previous member of the group (Dr. Pui-Hang Tam), was first methylated under standard conditions to give the 2-methoxy compound **2.51** as shown in Scheme 2.9. Acetolysis allowed for protecting group exchange to give the tetra-*O*-acetylated compound **2.52** in 81% yield in a single step. The mannosylated dibenzyl phosphate **2.54** was formed after thioglycosylation and phosphorylation steps, which were the same as those described for the synthesis of 3-methoxy

Man*p*-1P analog (Section 2.2.6) in 55% yield over two steps from **2.52**. After catalytic hydrogenolysis under basic conditions, without further purification, a mixture of $CH_3OH–H_2O–Et_3N$ 5:2:1 was used to deacetylate the compound to give the deprotected 2-methoxy analog **2.8** in 92% overall yield.



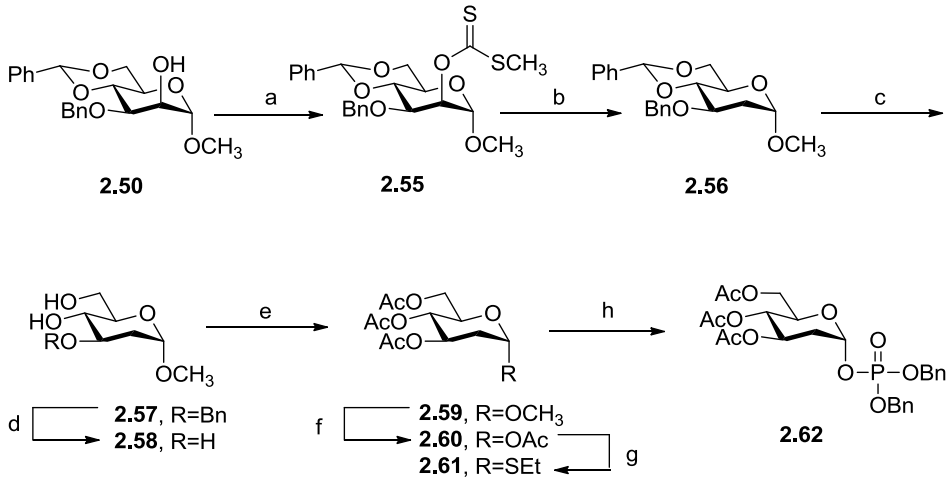
Scheme 2.9. Reagents and conditions: (a) CH_3I, NaH, DMF, 80%; (b) $Ac_2O–HOAc–H_2SO_4$, 35:15:1, 81%; (c) EtSH, $BF_3•OEt_2$, CH_2Cl_2, 65%; (d) HO-$P(O)(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 84%; (e) i) H_2, $Pd(OH)_2–C$, toluene, Et_3N, pyridine; ii) $CH_3OH–H_2O–Et_3N$, 5:2:1, 92%.

2.2.9. Attempts to synthesize 2-deoxy mannopyranosyl phosphate

The attempts to synthesize 2-deoxy Man*p*-1P analog are illustrated in Scheme 2.10. Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **2.50** was first treated with sodium hydride, carbon disulphide and methyl iodide to give xanthate **2.55**, which was then reduced, under Barton–McCombie deoxygenation conditions, to give the deoxy compound **2.56** in 66% yield over two steps.

When **2.56** was treated under acetolysis conditions, $Ac_2O–HOAc–H_2SO_4$ 35:15:1 mixture, only decomposition products were observed; therefore, an

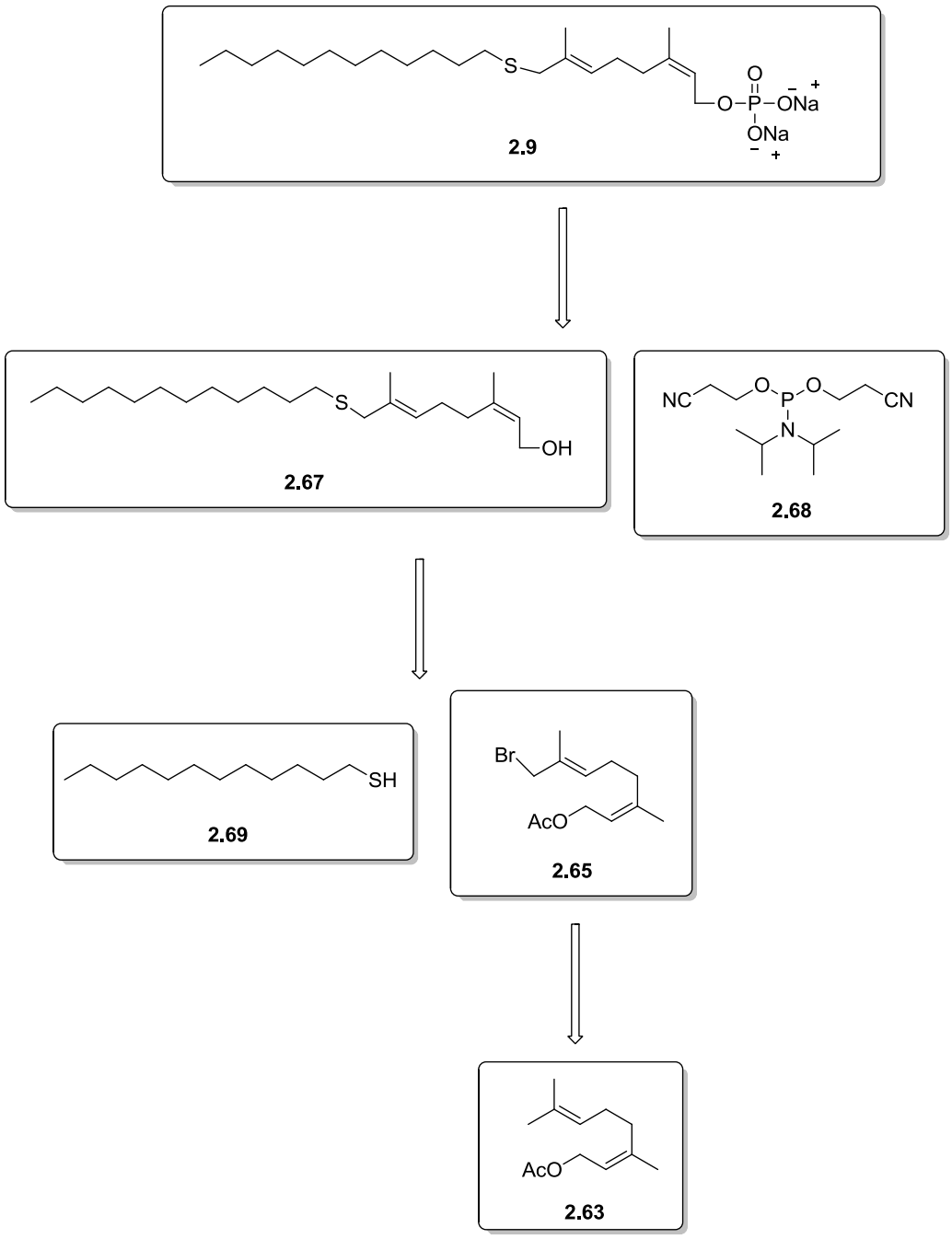
alternative route was required. The benzylidene and benzyl groups of deoxy compound **2.56** were cleaved under mild acidic conditions with I_2 in CH_3OH, and hydrogenolysis with $Pd(OH)_2$–C under a hydrogen atmosphere, respectively. Only around half of **2.56** reacted with I_2 to give **2.57** in a moderate yield (56%). The subsequent debenzylation proceeded in a good yield to give the triol **2.58**. The three free hydroxyl groups were protected with acetyl groups and the product **2.59** was treated under acetolysis conditions to give the peracetate **2.60**. Thioglycosylation and phosphorylation were carried out under the same conditions as the steps described earlier to produce other analogs to yield dibenzyl phosphate **2.62**. The occurrence of hydrolysis contributed to the low yields (52% and 34%) of these two reactions. The protected dibenzyl phosphate **2.62** was then treated with a weakly basic mixture of CH_3OH–H_2O–Et_3N after catalytic hydrogenlysis. Unfortunately, the deprotected 2-deoxy Man*p*-1P was only obtained in a very low yield and was not isolated and purified.



Scheme 2.10. Reagents and conditions: (a) THF, NaH, imidazole, carbon disulfide, CH_3I, 74%; (b) AIBN, *n*-Bu_3SnH, toluene, reflux, 89%; (c) I_2, CH_3OH, reflux, 56%; (d) H_2, $Pd(OH)_2$–C, CH_3OH, EtOAc, 91%; (e) Ac_2O, pyridine, 80%; (f) Ac_2O–HOAc–H_2SO_4, 35:15:1, 91%; (g) EtSH, BF_3•OEt_2, CH_2Cl_2, 52%; (h) HO-P(O)$(OBn)_2$, NIS, AgOTf, CH_2Cl_2, 34%.

2.2.10. Synthesis of the polyprenol phosphate analog

From a retrosynthetic analysis (Scheme 2.11) we envisioned the desired polyprenol phosphate analog **2.9** coming from the coupling of alcohol **2.67** with commercially available bis(2-cyanoethyl)-*N*,*N*-di-isopropyl phosphoramidite **2.68**. Alcohol **2.67** could, in turn, be generated through the formation of a thioether between 1-dodecanthiol **2.69** and a bromide precursor **2.65**. Finally, the bromide **2.65** could be synthesized from another commercially available reagent, neryl acetate **2.63**.

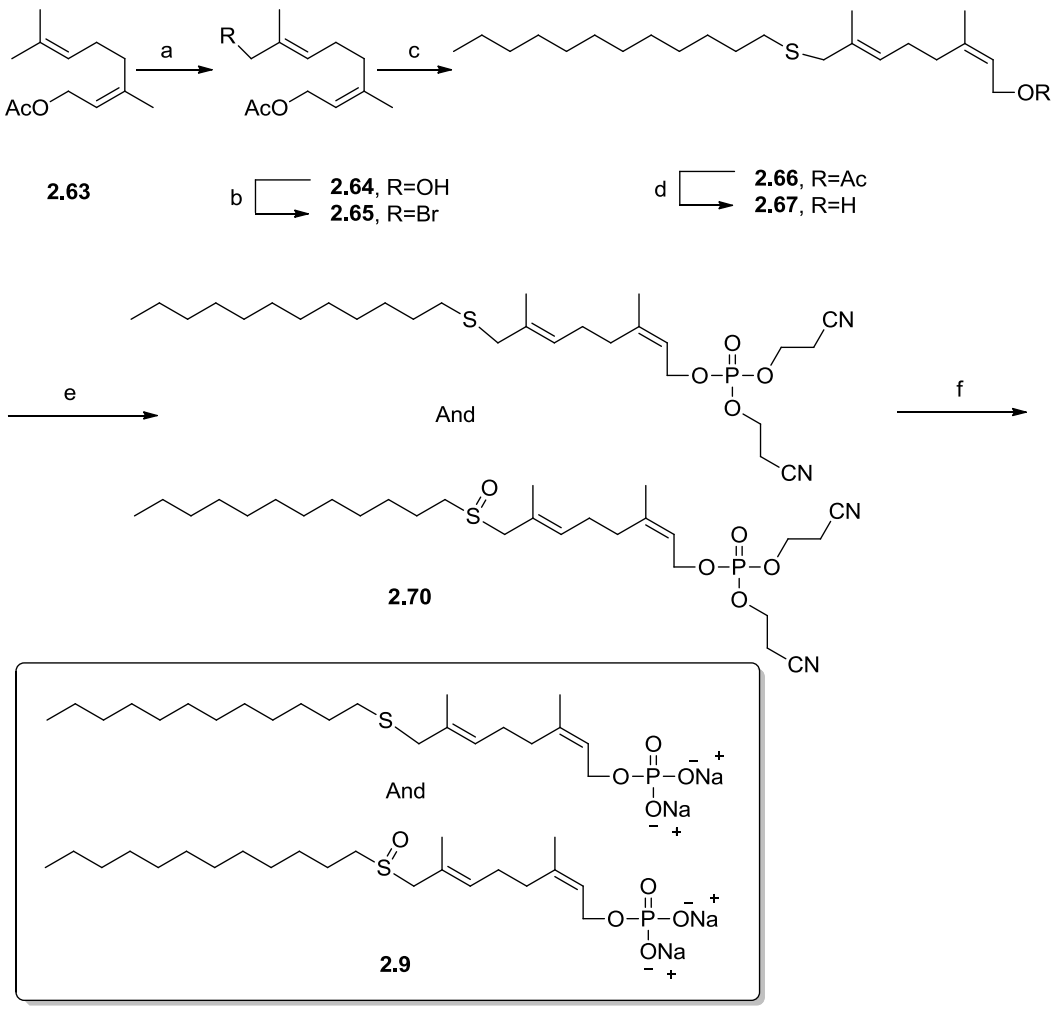


Scheme 2.11. Retrosynthetic analysis of the polyprenol phosphate analog

With this route in mind, the bromide precursor **2.65** was synthesized in two steps in 51% overall yield from neryl acetate **2.63** as shown in Scheme 2.12. First, the starting neryl acetate **2.63** was oxidized to alcohol **2.64** by treatment

with selenium dioxide, salicylic acid and *t*-butyl hydroperoxide. The resulting alcohol was then treated with tetrabromomethane and triphenylphosphine under basic conditions to give the required bromide precursor **2.65**.

With the bromide in hand, polyprenol **2.67** was synthesized through an S_N2 displacement reaction of the bromide with 1-dodecanthiol (**2.69**) under basic conditions followed by deprotection of the acetyl group under standard conditions to give the free alcohol. The polyprenol **2.67** was converted to the corresponding phosphite triester using the bis(2-cyanoethyl)-*N*,*N*-di-isopropyl phosphoramidite **2.68**.[11] The desired phosphate triester (**2.70**) was then formed by oxidation with hydrogen peroxide. Unfortunately, this reaction also resulted in partial oxidation of the thioether. However, this partial oxidation was not expected to interfere with the ability of the lipid to act as a substrate for PPM synthase (Chapter 3). Therefore, the protected polyprenol phosphate analog **2.70** was carried forward as a mixture of the thioether and sulfoxide. Deprotection was achieved by the use of sodium methoxide to yield the polyprenol phosphate analog **2.9** in 76% yield.



Scheme 2.12. Reagents and conditions: (a) SeO_2, *t*-BuOOH, salicylic acid, CH_2Cl_2, 61%; (b) CBr_4, PPh_3, 2,6-lutidine, CH_2Cl_2, 83%; (c) 1-Dodecanethiol, NaH, THF, 58%; (d) $NaOCH_3$, CH_3OH, 76%; (e) i) bis(2-cyanoethyl)-*N*,*N*-di-isopropyl phosphoramidite, tetrazole, THF, CH_3CN; ii) H_2O_2, 71%; (f) $NaOCH_3$, CH_3OH, 76%.

2.3. A summary of results

In conclusion, seven methoxy and deoxy analogs (**2.2**–**2.8**) of α-D-mannopyranosyl phosphate (**2.1**), as well as a polyprenol phosphate analog **2.9**

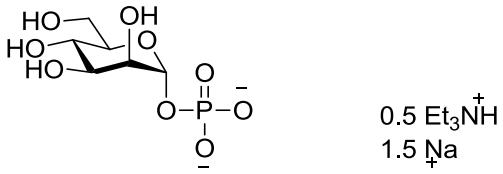
were synthesized chemically. A series of protecting group manipulations were used to give selectively protected mannose derivatives possessing a single free hydroxyl group. The single hydroxyl groups were then either reduced by Barton–McCombie deoxygenation, and reduction of the primary halide, or converted to the methyl ether. Acetolysis was used to generate the peracetylated mannose analogs. Even though only moderate yields could be achieved, this strategy afforded the peracetylated analogs in a single step. All glycosylation reactions involved the use of thioglycoside donors with a dibenzyl phosphate acceptor and the stereochemistry of the newly formed mannopyranoside bonds at the anomeric centre were determined by measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constants using coupled HSQC experiments.[7] This evaluation of these compounds as substrates for a GDP-Mannose pyrophosphorylase (GDP-ManPP, nucleotidyltransferase) and PPM synthase are described in Chapter 3.

2.4. Experimental section

General methods

All reagents used were purchased from commercial sources and were used without further purification unless noted. The solvents used were purified by successive passage through columns of alumina and copper under an argon atmosphere. All reactions were carried out under a positive pressure of argon at room temperature unless indicated otherwise. The reactions were monitored by analytical TLC on silica get 60-F_{254} (0.25mm, silicycle, Quebec, Canada), and the spots were visualized under UV light (254 nm) or stained by charring with

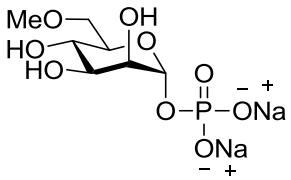
acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure and the products were purified by column chromatography on silica gel (230–400 mesh, Silicycle, Quebec, Canada) or SepPak C_{18} reverse phase cartridges (Waters). Before use, the cartridges were prewashed with 10 mL of MeOH followed by 20 mL of H_2O. Optical rotations were measured on Perkin Elmer 241 polarimeter at ambient temperature in units of degree•mL/(g•dm). ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz and chemical shifts were referenced to $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm), or D_2O (4.79 ppm). ^{13}C NMR spectra were recorded at 100 or 125 MHz and chemical shifts were referenced to $CDCl_3$ (77.1 ppm) or CD_3OD (49.0 ppm). Assignments of NMR spectra were based on two-dimensional experiments (^{1}H–^{1}H COSY or HSQC). The stereochemistry of the anomeric centres of the pyranose rings were confirmed by measuring the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant of coupled HSQC spectra.[7] Electrospray mass spectra were recorded on an Agilent Technologies 6220 TOF.



α-D-Mannopyranosyl phosphate mixed-triethylammonium-sodium salt (2.1)

The protected phosphate **2.12** (74 mg, 0.12 mmol) was dissolved in a mixture of toluene–Et_3N–pyridine (15:2:3, 6 mL). To the solution was added 20% $Pd(OH)_2$–C. The reaction mixture was stirred for 4 days under a hydrogen atmosphere. Then the catalyst was removed by filtration through Celite and the filtrate was

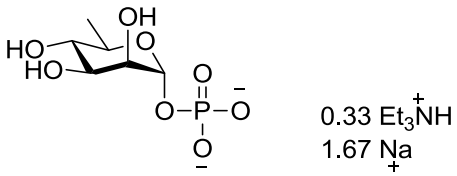
concentrated to afford a pale yellow syrup. The crude compound was dissolved in MeOH–H_2O–Et_3N (5:2:1, 4 mL) and the mixture was stirred for 2 days. Organic impurities were removed using a SepPak C_{18} cartridge eluting with H_2O. The H_2O fraction was lyophilized to afford **2.1** (35 mg, 85%) as a white solid $[\alpha]_D$ = +30.4 (*c* 0.3, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.34 (dd, *J* = 8.1, 1.8 Hz, 1H, H-1), 3.96–3.92 (m, 1H, H-2), 3.89 (dd, *J* = 9.6, 3.5 Hz, 1H, H-3), 3.87–3.79 (m, 2H, H-6a, H-5), 3.71 (dd, *J* = 11.9, 5.6 Hz, 1H, H-6b), 3.61 (app. t, *J* = 9.8 Hz, 1H, H-4), 3.17 (q, *J* = 7.3 Hz, 3H, 0.5 × N(C*H*$_2$CH$_3$)$_3$), 1.24 (t, *J* = 7.3 Hz, 4.5H, 0.5 × N(CH$_2$C*H*$_3$)$_3$). ^{13}C NMR (126 MHz, D_2O) δ 96.5 (d, $^2J_{C,P}$ = 5.2 Hz, 1C, C-1, $^1J_{C,H}$ = 172.8 Hz), 74.2 (C-5), 71.7 (d, $^3J_{C,P}$ = 8.1 Hz, 1C, C-2), 71.0 (C-3), 67.7 (C-4), 62.0 (C-6), 47.7 (N(*C*H$_2$CH$_3$)$_3$), 9.2 (N(CH$_2$*C*H$_3$)$_3$). ^{31}P NMR (161.90 MHz, D_2O) δ −0.51. The ^{1}H and ^{13}C NMR spectral data were consistent with that reported.[12] HRMS (ESI) *m/z* Calcd for $C_6H_{12}O_9P$ $[M–H]^-$: 259.0224. Found: 259.0223.



6-*O*-Methyl-α-D-mannopyranosyl phosphate disodium salt (2.2)

The protected α-D-mannopyranosyl phosphate **2.17** (181 mg, 0.25 mmol) was dissolved in MeOH (7.5 mL). A 1 M $NaHCO_3$ solution (0.75 mL) and 20% $Pd(OH)_2$–C (80 mg) were added to the mixture. The mixture was stirred for 6 days under a hydrogen atmosphere. Then the catalyst was removed by filtration through Celite and the filtrate was concentrated. Organic impurities were removed

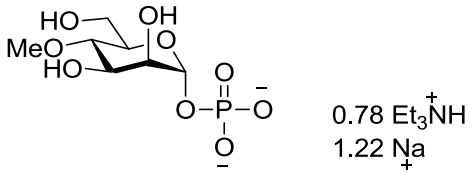
using a SepPak C_{18} cartridge eluting with H_2O. The filtrate was lyophilized to afford **2.2** (72 mg, 91%) as a white amorphous solid $[\alpha]_D$ = +30.3 (*c* 1.1, H_2O); ^1H NMR (400 MHz, D_2O) δ 5.30 (dd, *J* = 8.6, 1.1 Hz, 1H, H-1), 3.99–3.91 (m, 3H, H-2, H-3, H-5), 3.75 (dd, *J* = 10.9, 2.2 Hz, 1H, H-6a), 3.64 (dd, *J* = 10.9, 6.3 Hz, 1H, H-6b), 3.59 (dd, *J* = 10.0, 9.4 Hz, 1H, H-4), 3.40 (s, 3H, OC*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 95.4 (d, $^2J_{C,P}$ = 4.1 Hz, 1C, C-1, $^1J_{C,H}$ = 170.9 Hz), 72.1 (C-6), 71.9 (C-5), 71.5 (d, $^3J_{C,P}$ = 6.4 Hz, 1C, C-2), 70.5 (C-3), 67.5 (C-4), 56.0 (O*C*H$_3$). ^{31}P NMR (161.90 MHz, D_2O) δ 2.00. HRMS (ESI) *m/z* Calcd for $C_7H_{14}O_9P$ $[M–H]^-$: 273.0381. Found: 273.0380.



6-Deoxy-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (2.3)

The protected phosphate **2.28** (59 mg, 0.08 mmol) was dissolved in a mixture of toluene–Et_3N–pyridine (15:2:3, 2.8 mL). To the solution was added 20% $Pd(OH)_2$–C (12 mg). The reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford pale yellow syrup. Without further purification, the crude compound was dissolved in MeOH–H_2O–Et_3N (5:2:1, 8 mL) and the mixture was stirred for 6 days. The organic impurities were removed using a SepPak C_{18} cartridge eluting with H_2O. The H_2O fraction was lyophilized to afford **2.3** (18 mg, 72%) as a white solid $[\alpha]_D$ = +18.1 (*c* 0.2, H_2O); ^1H NMR

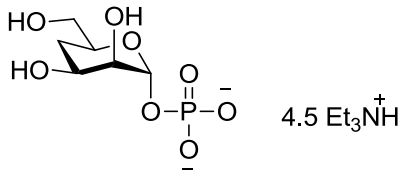
(500 MHz, D_2O) δ 5.38 (d, *J* = 7.8 Hz, 1H, H-1), 4.04 (br. s, 1H, H-2), 4.01–3.92 (m, 2H, H-5, H-3), 3.50 (app t, *J* = 9.8 Hz, 1H, H-4), 3.27 (q, *J* = 7.3 Hz, 2H, 0.33 × $N(CH_2CH_3)_3$), 1.38–1.32 (m, 6H, H-6, 0.33 × $N(CH_2CH_3)_3$). ^{13}C NMR (126 MHz, D_2O) δ 96.4 (d, $^2J_{C,P}$ = 5.4 Hz, 1C, C-1, $^1J_{C,H}$ = 171.2 Hz), 73.2 (C-4), 71.8 (d, $^3J_{C,P}$ = 8.3 Hz, 1C, C-2), 70.7 (C-3), 70.1 (C-5), 47.7 ($N(CH_2CH_3)_3$), 17.8 (C-6), 9.2 ($N(CH_2CH_3)_3$). ^{31}P NMR (161.90 MHz, D_2O) δ −0.71. HRMS (ESI) *m/z* Calcd for $C_6H_{12}O_8P$ $[M–H]^-$: 243.0275. Found: 243.0275.



4-*O*-Methyl-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (2.4)

The protected phosphate **2.34** (220 mg, 0.38 mmol) was dissolved in a mixture of toluene–Et_3N–pyridine (15:2:3, 4 mL). To the solution was added $Pd(OH)_2$–C (40 mg). The reaction mixture was stirred for 3 days under a hydrogen atmosphere. Then the catalyst was removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. Without purification the crude compound was dissolved in MeOH–H_2O–Et_3N (5:2:1, 8 mL) and the mixture was stirred for 2 days. Organic impurities were removed using a SepPak C_{18} cartridge eluting with H_2O. The H_2O fraction was lyophilized to yield **2.4** (101 mg, 70%) as a white solid. $[\alpha]_D$ = +28.8 (*c* 0.3, H_2O); ^1H NMR (500 MHz, D_2O) δ 5.33 (d, *J* = 8.1 Hz, 1H, H-1), 3.97 (dd, *J* = 9.6, 3.2 Hz, 1H, H-3), 3.94 (br. s, 1H, H-2), 3.85 (d, *J* = 12.0 Hz, 1H, H-6a), 3.83–3.78 (m, 1H, H-5), 3.73 (dd, *J* = 12.0, 5.2 Hz, 1H,

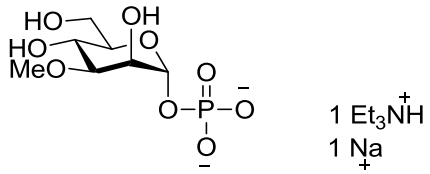
H-6b), 3.53 (s, 3H, OCH_3), 3.42 (app t, J = 9.8 Hz, 1H, H-4), 3.18 (q, J = 7.3 Hz, 4.7H, 0.78 × N(CH_2CH$_3$)$_3$), 1.26 (t, J = 7.3 Hz, 7H, 0.78 × N(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, D_2O) δ 96.5 (d, $^2J_{C,P}$ = 5.2 Hz, 1C, C-1, $^1J_{C,H}$ = 177.3 Hz), 77.7 (C-4), 73.2 (C-5), 72.0 (d, $^3J_{C,P}$ = 8.0 Hz, 1C, C-2), 70.8 (C-3), 61.7 (C-6), 61.1 (OCH_3), 47.7 (N(CH_2CH$_3$)$_3$), 9.2 (N(CH$_2$$CH_3$)$_3$). ^{31}P NMR (161.84 MHz, D_2O) δ − 0.29. HRMS (ESI) *m/z* Calcd for $C_7H_{14}O_9P$ $[M–H]^-$: 273.0381. Found: 273.0377.



4-Deoxy-α-D-*lyxo*-hexopyranosyl phosphate triethylammonium salt (2.5)

The protected phosphate **2.39** (45 mg, 0.08 mmol) was dissolved in a mixture of toluene–Et_3N–pyridine (15:2:3, 2.8 mL). To the solution was added 20% $Pd(OH)_2$–C (19 mg). The reaction mixture was stirred for 2 days under a hydrogen gas atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to give a pale yellow syrup. Without any further purification, the crude syrup was dissolved in MeOH–H_2O–Et_3N (5:2:1, 4 mL) and the mixture was stirred for 3 days. Any remaining organic impurities were removed using a SepPak C_{18} cartridge eluting with H_2O and the H_2O fraction was lyophilized to afford **2.5** (34 mg, 62%) as a white solid. $[\alpha]_D$ = +7.3 (*c* 0.2, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.41 (dd, J = 8.2, 1.5 Hz, 1H, H-1), 4.17 (ddd, J = 11.4, 4.8, 3.4 Hz, 1H, H-3), 4.14–4.06 (m, 2H, H-5), 3.81 (br. s, 1H,

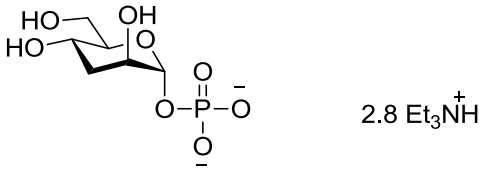
H-2), 3.65 (dd, J = 12.0, 3.4 Hz, 1H, H-6a), 3.60 (dd, J = 12.1, 6.6 Hz, 1H, H-6b), 3.19 (q, J = 7.3 Hz, 27H, 4.5 × N(CH_2CH$_3$)$_3$), 1.69–1.58 (m, 2H, H-4ax, H-4eq), 1.27 (t, J = 7.3 Hz, 40.5H, 4.5 × N(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, D$_2$O) δ 97.1 (d, $^2J_{C,P}$ = 5.2 Hz, 1C, C-1, $^1J_{C,H}$ = 169.9 Hz), 70.8 (C-5), 69.7 (d, $^3J_{C,P}$ = 7.5 Hz, 1C, C-2), 65.5 (C-3), 65.2 (C-6), 47.7 (N(CH$_2$CH$_3$)$_3$), 29.7 (C-4), 9.2 (N(CH$_2$$CH_3$)$_3$). ^{31}P NMR (161.90 MHz, D$_2$O) δ −0.43. HRMS (ESI) *m/z* Calcd for C$_6$H$_{12}$O$_8$P [M–H]$^-$: 243.0275. Found: 243.0271.



3-*O*-Methyl-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (2.6)

The protected phosphate **2.44** (33 mg, 0.06 mmol) was dissolved in a mixture of toluene–Et$_3$N–pyridine (15:2:3, 2.8 mL). To this solution was added 20% Pd(OH)$_2$–C (4 mg). The reaction mixture was stirred for 4 days under an atmosphere of hydrogen. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. The syrup was dissolved in MeOH–H$_2$O–Et$_3$N (5:2:1, 4 mL) and the mixture was stirred for 3 days. Any organic impurities were removed using a SepPak C$_{18}$ cartridge eluting with H$_2$O and the filtrate was lyophilized to afford **2.6** (16 mg, 67%) as a white solid. [α]$_D$ = +16.5 (*c* 0.1, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 5.42 (d, J = 7.8 Hz, 1H, H-1), 4.21 (br. s, 1H, H-2), 3.87-3.82 (m, 2H, H-5, H-6a), 3.74 (dd, J

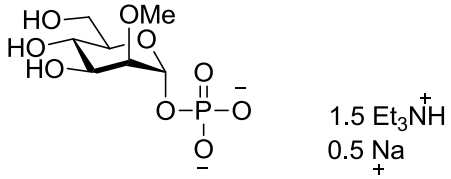
= 11.8, 5.0 Hz, 1H, H-6b), 3.69 (app t, *J* = 9.8 Hz, 1H, H-4), 3.59 (dd, *J* = 9.6, 2.0 Hz, 1H, H-3), 3.44 (s, 3H, OC*H*$_3$), 3.18 (q, *J* = 7.3 Hz, 6H, N(C*H*$_2$CH$_3$)$_3$), 1.26 (t, *J* = 7.2 Hz, 9H, N(CH$_2$C*H*$_3$)$_3$). ^{13}C NMR (126 MHz, D$_2$O) δ 96.8 (d, $^2J_{C,P}$ = 5.2 Hz, 1C, C-1, $^1J_{C,H}$ = 178.5 Hz), 80.27 (C-3), 74.4 (C-5), 67.3 (d, $^3J_{C,P}$ = 8.2 Hz, 1C, C-2), 66.5 (C-4), 61.8 (C-6), 57.2 (O*C*H$_3$), 47.7 (N(*C*H$_2$CH$_3$)$_3$), 9.2 (N(CH$_2$*C*H$_3$)$_3$). ^{31}P NMR (161.90 MHz, D$_2$O) δ −1.82. HRMS (ESI) *m/z* Calcd for C$_7$H$_{14}$O$_9$P [M–H]$^-$: 273.0381. Found: 273.0371.



3-Deoxy-α-D-*arabino*-hexopyranosyl phosphate triethylammonium salt (2.7)

The protected phosphate **2.49** (40 mg, 0.07 mmol) was dissolved in a mixture of toluene–Et$_3$N–pyridine (15:2:3, 2 mL). To the solution was added 20% Pd(OH)$_2$–C (10 mg). The reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate concentrated to afford a pale yellow syrup. This crude syrup was dissolved in MeOH–H$_2$O–Et$_3$N (5:2:1, 4 mL) and the mixture was stirred for 3 days. The organic impurities were removed using a SepPak C$_{18}$ cartridge eluting with water. The filtrate was then lyophilized to give **2.7** (26 mg, 71%) as a white solid. [α]$_D$ = +25.0 (*c* 0.3, H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 5.15 (d, *J* = 8.0 Hz, 1H, H-1), 3.86 (br. s, 1H, H-2), 3.81–3.68 (m, 3H, H-4, H-5, H-6a), 3.62 (dd, *J* = 12.0, 5.7 Hz, 1H, H-6b), 3.11 (q, *J* = 7.3 Hz, 17H, 2.8 × N(C*H*$_2$CH$_3$)$_3$), 2.02–1.96 (ddd, 1H, *J* = 13.5, 3.5, 3.5 Hz, 1H, H-3eq), 1.87 (ddd, 1H, *J* = 13.5, 11.0, 2.5 Hz, 1H, H-

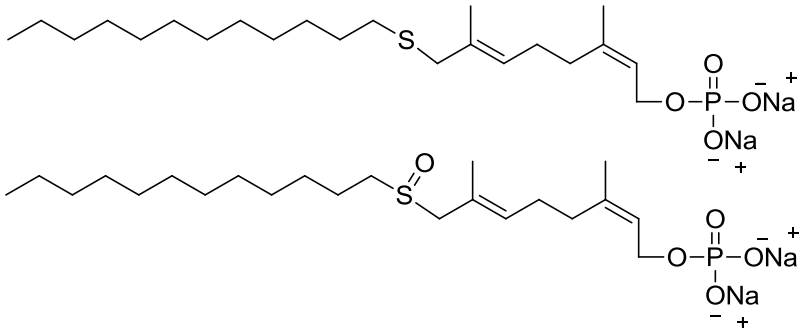
3ax), 1.18 (t, J = 7.3 Hz, 26H, 2.8 × N(CH_2CH_3)$_3$). ^{13}C NMR (126 MHz, D_2O) δ 95.1 (d, $^2J_{C,P}$ = 4.9 Hz, 1C, C-1, $^1J_{C,H}$ = 173.2 Hz), 75.1 (C-5), 69.0 (d, $^3J_{C,P}$ = 8.2 Hz, 1C, C-2), 62.3 (C-4), 62.2 (C-6), 47.7 (N(CH_2CH_3)$_3$), 33.6 (C-3), 9.2 (N(CH_2CH_3)$_3$). ^{31}P NMR (161.84 MHz, D_2O) δ −0.60. HRMS (ESI) calculated for $C_6H_{12}O_8P$ $[M–H]^-$: 243.0275. Found: 243.0273.



2-*O*-Methyl-α-D-mannopyranosyl phosphate mixed-triethylammonium-sodium salt (2.8)

The protected phosphate **2.54** (118 mg, 0.2 mmol) was dissolved in a mixture of toluene–Et_3N–pyridine (15:2:3, 2 mL). To this solution was added 20% $Pd(OH)_2$–C (11 mg) and the reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated to afford a pale yellow syrup. Without further purification, this crude compound was dissolved in MeOH–H_2O–Et_3N (5:2:1, 4 mL) and the mixture was stirred for 2 days. The organic impurities were removed using a SepPak C_{18} cartridge eluting with H_2O. The H_2O fraction was then lyophilized to afford **2.8** (80.2 mg, 92%) as a white solid. $[\alpha]_D$ = +24.8 (*c* 0.3, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.53 (dd, J = 8.1, 1.7 Hz, 1H, H-1), 3.93 (dd, J = 9.9, 3.5 Hz, 1H, H-3), 3.86 (dd, J = 12.2, 2.2 Hz, 1H, H-6a), 3.80 (ddd, J = 9.9, 6.0, 2.0 Hz, 1H, H-5), 3.71 (dd, J = 12.2, 6.0 Hz, 1H, H-6b), 3.61 (dd, J = 3.5, 1.9

Hz, 1H, H-2), 3.56 (app t, J = 10.0 Hz, 1H, H-4), 3.48 (s, 3H, OCH_3), 3.19 (q, J = 7.3 Hz, 9H, 1.5 × N(CH_2CH$_3$)$_3$), 1.26 (t, J = 7.3 Hz, 13.5H, 1.5 × N(CH$_2$CH_3)$_3$). ^{13}C NMR (126 MHz, D_2O) δ 93.4 (d, $^2J_{C,P}$ = 5.2 Hz, 1C, C-1, $^1J_{C,H}$ = 177.9 Hz), 81.6 (d, $^3J_{C,P}$ = 7.9 Hz, 1C, C-2), 74.3 (C-5), 70.7 (C-3), 67.9 (C-4), 61.9 (C-6), 59.8 (OCH$_3$), 47.7 (N(CH$_2$CH$_3$)$_3$), 9.2 (N(CH$_2$$CH_3$)$_3$). ^{31}P NMR (161.84 MHz, CD_3OD) δ 0.60. HRMS (ESI) *m/z* Calcd for $C_7H_{14}O_9P$ $[M–H]^-$: 273.0381. Found: 273.0381.

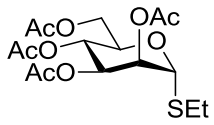


(2Z,6E)-8-(Dodecylthio)-3,7-dimethylocta-2,6-dienyl phosphate disodium salt

(2Z,6E)-8-(Dodecylsulfinyl)-3,7-dimethylocta-2,6-dienyl phosphate disodium salt (2.9)

The protected polyprenyl phosphate **2.70** (51 mg, 0.09 mmol) was stirred with NaOMe (9.9 mg, 0.18 mmol) in MeOH (2 mL) overnight. The desired product was purified using a SepPak C_{18} cartridge eluting with H_2O. The H_2O fraction was lyophilized to afford **2.9** (76%, 34 mg) as a white solid. Data for thioether: ^{1}H NMR (600 MHz, CD_3OD) δ 5.43 (t, J = 6.7 Hz, 1H, OCH$_2$-CH=C), 5.26 (br. s, 1H, C=CH-CH$_2$CH$_2$), 4.39 (t, J = 6.2 Hz, 2H, OCH_2), 3.06 (s, 2H, SCH_2), 2.34 (t, J = 7.3 Hz, 2H, SCH_2), 2.14 (d, J = 3.3 Hz, 4H, CH_2 × 2), 1.73 (s, 3H, CH_3), 1.70

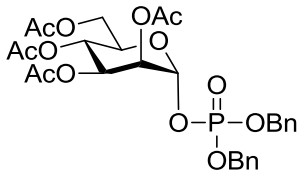
(s, 3H, CH_3), 1.52 (dt, J = 14.9, 7.4 Hz, 2H, SCH$_2$CH_2), 1.28 (s, 18H, CH_2 × 9), 0.89 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD$_3$OD) δ 138.8 (CH=*C*), 132.9 (CH=*C*), 128.5 (*C*H=C), 125.0 (d, $^3J_{C,P}$ = 8.9 Hz, 1C, PO-CH$_2$-*C*H=C), 62.3 (d, $^2J_{C,P}$ = 4.4 Hz, 1C, PO-*C*H$_2$), 42.1 (S*C*H$_2$), 33.1 (S*C*H$_2$), 33.0 (*C*H$_2$), 31.5 (*C*H$_2$), 30.7(6) (*C*H$_2$), 30.7(4) (*C*H$_2$), 30.6(9) (*C*H$_2$), 30.4(7) (*C*H$_2$), 30.4(4) (*C*H$_2$), 30.3(7) (*C*H$_2$), 29.9 (*C*H$_2$), 27.9 (*C*H$_2$), 23.7(4) (*C*H$_2$), 23.6(6) (*C*H$_3$), 15.0 (*C*H$_3$), 14.5 (*C*H$_3$). ^{31}P NMR (161.84 MHz, CD$_3$OD) δ 3.34. HRMS (ESI) *m/z* Calcd for $C_{22}H_{42}O_4PS$ $[M–H]^–$: 433.2547. Found: 433.2556. HRMS (ESI) *m/z* Calcd for $C_{22}H_{42}O_5PS$ $[M–H]^–$: 449.2496. Found: 449.2501.



Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (2.11)

Commercially available D-mannose **2.10** (50 g, 0.28 mol) was dissolved in pyridine (225 mL) and cooled to 0 ℃ before Ac_2O (197 mL, 2.09 mol) was added. The reaction mixture was stirred overnight. The solvent was then removed by evaporation and the resulting mixture was diluted with CH_2Cl_2, washed with distilled water, 1 M HCl, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, and concentrated to give a clear syrup. Without further purification, half of the crude residue (54 g, 0.14 mol) and ethanethiol (15 mL, 0.21 mol) were dissolved in dry CH_2Cl_2 (300 mL). After the addition of BF_3•OEt_2 (35 mL, 0.28 mol), the reaction mixture was stirred overnight. The solution was then diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and

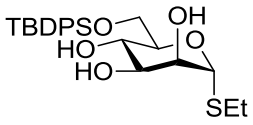
brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:1) to afford **2.11** (33.7 g, 62%) as a pale yellow syrup. $[\alpha]_D$ = +69.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41–5.22 (m, 4H, H-1, H-2, H-3, H-4), 4.44–4.38 (m, 1H, H-5), 4.33 (dd, *J* = 12.2, 5.3 Hz, 1H, H-6a), 4.12 (dd, *J* = 12.2, 2.1 Hz, 1H, H-6b), 2.65–2.45 (m, 2H, SC*H*$_2$CH$_3$), 2.18 (s, 3H, C(O)C*H*$_3$), 2.11 (s, 3H, C(O)C*H*$_3$), 2.07 (s, 3H, C(O)C*H*$_3$), 2.01 (s, 3H, C(O)C*H*$_3$), 1.33 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.0 (*C*=O), 169.8 (*C*=O), 169.8 (*C*=O), 82.3 (C-1), 71.2, 69.5, 68.9, 66.4 (C-2, C-3, C-4, C-5), 62.5 (C-6), 25.5 (S*C*H$_2$CH$_3$), 20.9 (C(O)*C*H$_3$), 20.7(3) (C(O)*C*H$_3$), 20.7(1) (C(O)*C*H$_3$), 20.6 (C(O)*C*H$_3$), 14.8 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{16}H_{24}O_9SNa$ $[M+Na]^+$: 415.1033. Found: 415.1033.



Dibenzyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl phosphate (2.12)

Thioglycoside **2.11** (275 mg, 0.7 mmol), dibenzyl phosphate (331 mg, 1.19 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (3 mL) and stirred for 30 min. Then, *N*-iodosuccinimide (236 mg, 1.05 mmol) and silver trifluoromethanesulfonate (54 mg, 0.21 mmol) were added at −55 ℃. The reaction mixture was stirred for 3 h at −55 ℃ to −15 ℃, and was then filtered through Celite. The filtrate was diluted with CH_2Cl_2, and washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic later

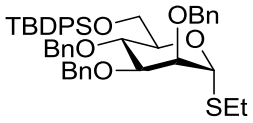
was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to afford **2.12** (293 mg, 69%) as a colorless oil $[\alpha]_D$ = +34.4 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43–7.29 (m, 10H, ArH), 5.60 (dd, *J* = 6.5, 1.8 Hz, 1H, H-1), 5.31–5.24 (m, 2H, H-3, H-4), 5.22 (app t, *J* = 2.2 Hz, 1H, H-2), 5.12–5.03 (m, 4H, C*H*$_2$Ph), 4.17 (dd, *J* = 12.4, 4.7 Hz, 1H, H-6a), 4.07–3.98 (m, 1H, H-5), 3.92 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6b), 2.13 (s, 3H, C(O)C*H*$_3$), 2.02 (s, 3H, C(O)C*H*$_3$), 1.99 (s, 3H, C(O)C*H*$_3$), 1.98 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5 (*C*=O), 169.7(*C*=O), 169.5(0) (*C*=O), 169.4(8) (*C*=O), 135.3 (d, $^3J_{C,P}$ = 6.6 Hz, 1C, Ar), 135.2 (d, $^3J_{C,P}$ = 6.6 Hz, 1C, Ar), 128.8(0) (Ar), 128.7(7) (Ar), 128.7(0) (Ar), 128.6(9) (Ar), 128.2 (Ar), 128.0 (Ar), 95.1 (d, $^2J_{C,P}$ = 5.7 Hz, 1C, C-1, $^1J_{C,H}$ = 182.1 Hz), 70.3 (C-5), 70.0 (d, $^2J_{C,P}$ = 5.7 Hz, 1C, *C*H$_2$Ph), 69.8 (d, $^2J_{C,P}$ = 5.4 Hz, 1C, *C*H$_2$Ph), 68.8 (d, $^3J_{C,P}$ = 11.3 Hz, 1C, C-2), 68.2 (C-3), 65.3 (C-4), 61.8 (C-6), 20.7 (C(O)*C*H$_3$), 20.6(3) (C(O)*C*H$_3$), 20.6(0) (C(O)*C*H$_3$), 20.5(9) (C(O)*C*H$_3$). ^{31}P NMR (202.33 MHz, $CDCl_3$) δ −3.24. HRMS (ESI) *m/z* Calcd for $C_{28}H_{33}O_{13}PNa$ $[M+Na]^+$: 631.1551. Found: 631.15485.



Ethyl 6-*O*-*t*-butyldiphenylsilyl-1-thio-α-D-mannopyranoside (2.13)

Thioglycoside **2.11** (5 g, 12.7 mmol) was dissolved in methanol (100 mL) and 1.0 M NaOMe (2 mL) was added dropwise. After stirring overnight, the reaction mixture was neutralized with Amberlite IR120 H^+ ion exchange resin and then concentrated. *t*-Butylchlorodiphenylsilane (5.6 mL, 21.7 mmol) was added to a

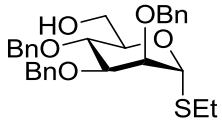
solution of the crude residue and imidazole (3.9 g, 57.3 mmol) in dry DMF (24 mL). The reaction mixture was stirred overnight and then quenched by the addition of MeOH (5 mL). The solvent was then evaporated and the resulting residue purified by chromatography (hexane–EtOAc 3:1 → 1:1) to provide **2.13** (4.6 g, 78%) as a colorless syrup. $[\alpha]_D$ = +88.0 (*c* 1.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ 7.77–7.67 (m, 4H, ArH), 7.47–7.33 (m, 6H, ArH), 5.30 (d, *J* = 1.0 Hz, 1H, H-1), 4.10–4.04 (m, 1H, H-5), 4.02 (dd, *J* = 10.9, 2.0 Hz, 1H, H-6a), 3.90 (dd, *J* = 3.4, 1.4 Hz, 1H, H-2), 3.84 (dd, *J* = 10.9, 7.0 Hz, 1H, H-6b), 3.68 (dd, *J* = 9.4, 3.4 Hz, 1H, H-3), 3.59 (app t, *J* = 9.6 Hz, 1H, H-4), 2.78–2.50 (m, 2H, SCH_2CH_3), 1.28 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3), 1.03 (s, 9H, $Si(Ph)_2C(CH_3)_3 \times 3$). ^{13}C NMR (125 MHz, CD_3OD) δ 136.8(4) (Ar), 136.8(0) (Ar), 134.9 (Ar), 134.8 (Ar), 130.7(9) (Ar), 130.7(5) (Ar), 128.7(3) (Ar), 128.7(0) (Ar), 85.4 (C-1), 75.4 (C-5), 73.5 (C-2), 73.4 (C-3), 69.2 (C-4), 65.3 (C-6), 27.3 ($Si(Ph)_2C(CH_3)_3$), 25.4 (SCH_2CH_3), 20.1 ($Si(Ph)_2C(CH_3)_3$), 15.2 (SCH_2CH_3). HRMS (ESI) *m/z* Calcd for $C_{24}H_{34}O_5SSiNa$ $[M+Na]^+$: 485.1788. Found: 485.1780.



Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-(*t*-butyldiphenylsilyl)-1-thio-α-D-mannopyranoside (2.14)

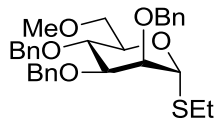
Thioglycoside **2.13** (4.6 g, 9.9 mmol) was dissolved in dry THF (40 mL) and cooled to 0 ℃. Then NaH (60% NaH in mineral oil, 2.3 g, 59.4 mmol) was added. The reaction mixture was stirred for 0.5 h before the addition of BnBr (5.3 mL,

44.6 mmol) and tetra-*n*-butylammonium iodide (0.37 g, 1 mmol). The reaction mixture was stirred overnight and then was quenched by the addition of MeOH. The solution was then diluted with CH_2Cl_2, washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, and concentrated and the crude residue purified by chromatography (hexane–EtOAc 18:1) to provide **2.14** (6.1 g, 84%) as a pale yellow syrup. $[\alpha]_D$ = +33.8 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87–7.12 (m, 25H, ArH), 5.39 (d, *J* = 1.3 Hz, 1H, H-1), 4.93–4.47 (m, 6H, C*H*$_2$Ph), 4.13–3.97 (m, 3H, H-4, H-5, H-6a), 3.90 (m, 2H, H-6b, H-3), 3.86–3.83 (m, 1H, H-2), 2.68–2.49 (m, 2H, SC*H*$_2$CH$_3$), 1.24 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$), 1.07 (s, 9H, Si(Ph)$_2$C(C*H*$_3$)$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.6 (Ar), 138.4(0) (Ar), 138.3(8) (Ar), 136.0 (Ar), 135.7 (Ar), 133.9 (Ar), 133.4 (Ar), 129.5(1) (Ar), 129.4(9) (Ar), 128.3(9) (Ar), 128.3(5) (Ar), 128.3(1) (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(4) (Ar), 127.6(6) (Ar), 127.6(5) (Ar), 127.6(3) (Ar), 127.5(7) (Ar), 127.5(2) (Ar), 81.4 (C-1), 80.6 (C-3), 77.0 (C-2), 75.2 (*C*H$_2$Ph), 75.0 (C-4), 73.4 (C-5), 72.2(4) (*C*H$_2$Ph), 72.2(0) (Ar*C*H$_2$), 63.3 (C-6), 26.8 (Si(Ph)$_2$C(*C*H$_3$)$_3$), 25.0 (S*C*H$_2$), 19.3 (Si(Ph)$_2$*C*(CH$_3$)$_3$), 14.8 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{45}H_{52}O_5SSiNa$ $[M+Na]^+$: 755.3197. Found: 755.3196.



Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (2.15)

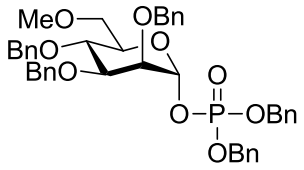
A 1 M solution of tetra-*n*-butylammonium fluoride in THF (13.4 mL, 13.4 mmol) was added dropwise to a solution of **2.14** (6.07 g, 8.3 mmol) in THF (29 mL), and the mixture was stirred overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 7:2) of the crude residue provided the alcohol **2.15** (3.4 g, 83%) as colorless oil. $[\alpha]_D$ = +81.0 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.27 (m, 15H, ArH), 5.31 (d, *J* = 1.2 Hz, 1H, H-1), 4.95–4.57 (m, 6H, ArC*H*$_2$), 4.04–3.98 (m, 2H, H-4, H-5), 3.89–3.79 (m, 4H, H-2, H-3, H-6a, H-6b), 2.67–2.48 (m, 2H, SC*H*$_2$CH$_3$), 1.93 (app t, *J* = 6.5 Hz, 1H, OH), 1.24 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.4 (Ar), 138.2 (Ar), 138.1 (Ar), 128.4 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(2) (Ar), 127.6(8) (Ar), 82.3 (C-1), 80.4 (C-3), 76.6 (C-2), 75.2 (*C*H$_2$Ph), 75.0 (C-4), 72.4(1) (C-5), 72.3(9) (*C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 62.4 (C-6), 25.4 (S*C*H$_2$), 14.9 (SCH$_2$*C*H$_3$). The ^{1}H and ^{13}C NMR spectral data were consistent with that previously reported.[13-14] HRMS (ESI) *m/z* Calcd for $C_{29}H_{34}O_5SNa$ $[M+Na]^+$: 517.2019. Found: 517.2012.



Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (2.16)

Monosaccharide **2.15** (148.4 mg, 0.3 mmol) was dissolved in DMF (3 mL) and the solution was cooled to 0 ℃. After the addition of NaH (60% NaH in mineral oil, 20.4 mg, 5.1 mmol), the mixture was stirred for 10 min before the addition of CH_3I (26 μL, 0.42 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with

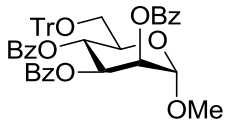
CH_2Cl_2, washed with distilled water and brine. The organic layer was dried over Na_2SO_4. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded **2.16** (133 mg, 87%) as a colorless syrup $[\alpha]_D$ = +82.1 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.26 (m, 15H, ArH), 5.39 (s, 1H, H-1), 4.94–4.50 (m, 6H, *C*H_2Ph), 4.09 (ddd, *J* = 9.8, 4.7, 1.8 Hz, 1H, H-5), 4.01 (app t, *J* = 9.3 Hz, 1H, H-4), 3.86–3.81 (m, 2H, H-2, H-3), 3.70 (dd, *J* = 10.6, 4.8 Hz, 1H, H-6), 3.60 (dd, *J* = 10.6, 2.0 Hz, 1H, H-6), 3.39 (s, 3H, OC*H*$_3$), 2.69–2.51 (m, 2H, S*C*$H_2$$CH_3$), 1.25 (t, *J* = 7.4 Hz, 1H, SCH_2*C*H_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6 (Ar), 138.3 (Ar), 138.1 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7(2) (Ar), 127.6(5) (Ar), 127.6 (Ar), 82.0 (C-1), 80.3 (C-3), 76.3 (C-2), 75.1(4) (*C*H_2Ph), 75.0(5) (C-4), 72.0(1) (*C*H_2Ph), 71.9(9) (CH_2Ph), 71.7 (C-5), 71.6 (C-6), 59.2 (O*C*H_3), 25.3 (S*C*$H_2$$CH_3$), 14.9 (S$CH_2$*C*$H_3$). HRMS (ESI) *m/z* Calcd for $C_{30}H_{36}O_5SNa$ $[M+Na]^+$: 531.2176. Found: 531.2169.



Dibenzyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranosyl phosphate (2.17)

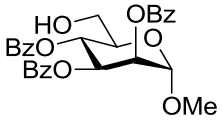
Thioglycoside **2.16** (50 mg, 0.1 mmol), dibenzyl phosphate (47.3 mg, 0.17 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (3 mL) and stirred for 40 min. Then, *N*-iodosuccinimide (34 mg, 0.15 mmol) and silver trifluoromethanesulfonate (8 mg, 0.03 mmol) were added at −30 ℃. The reaction mixture was stirred for 40 min from −30 ℃ to −20 ℃, and then was filtered

through Celite and diluted with CH_2Cl_2. The solution was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, and concentrated. The crude residue was purified by chromatography (hexane–EtOAc 5:2) to afford **2.17** (50.4 mg, 70%) as a colorless oil. $[\alpha]_D$ = +24.8 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48–7.27 (m, 25H, ArH), 5.86 (dd, *J* = 6.1, 1.9 Hz, 1H, H-1), 5.21–4.47 (m, 10H, C*H*$_2$Ph × 5), 4.11 (app t, *J* = 9.7 Hz, 1H, H-4), 3.95 (ddd, *J* = 10.0, 4.3, 1.5 Hz, 1H, H-5), 3.90 (dd, *J* = 9.5, 3.0 Hz, 1H, H-3), 3.80 (app t, *J* = 2.5 Hz, 1H, H-2), 3.68 (dd, *J* = 10.9, 4.5 Hz, 1H, H-6a), 3.55 (dd, *J* = 10.8, 1.7 Hz, 1H, H-6b), 3.39 (s, 3H, OC*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5 (Ar), 138.3 (Ar), 137.6, (Ar), 137.8 (d, $^3J_{C,P}$ = 6.9 Hz, 1C, Ar), 135.7 (d, $^3J_{C,P}$ = 7.0 Hz, 1C, Ar), 128.6(2) (Ar), 128.5(9) (Ar), 128.4(1) (Ar), 128.3(9) (Ar), 128.0(1) (Ar), 127.9(9) (Ar), 127.9 (Ar), 127.8 (Ar), 127.7(3) (Ar), 127.7(1) (Ar), 127.6(5) (Ar), 127.6(2) (Ar), 96.5 (d, $^2J_{C,P}$ = 6.3 Hz, 1C, C-1, $^1J_{C,H}$ = 176.4 Hz), 78.9 (C-3), 75.2 (*C*H$_2$Ph), 74.4 (d, $^3J_{C,P}$ = 9.0 Hz, 1C, C-2), 74.1 (C-4), 73.7 (C-5), 72.8 (*C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 71.1 (C-6), 69.5 (*C*H$_2$Ph), 69.4 (*C*H$_2$Ph), 59.3 (O*C*H$_3$). ^{31}P NMR (201.64 MHz, $CDCl_3$) δ −1.65. HRMS (ESI) *m/z* Calcd for $C_{42}H_{45}O_9PNa$ $[M+Na]^+$: 747.2693. Found: 747.2685.



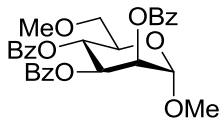
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-α-D-mannopyranoside (2.19)

To a solution of methyl α-D-mannopyranoside **2.18** (5.8 g, 0.03 mol) in dry pyridine (150 mL) was added trityl chloride (10 g, 0.036 mol) and DMAP (0.7 g, 6 mmol). The reaction mixture was stirred at 40 ℃ overnight and the cooled to 0 ℃ before the addition of benzoyl chloride (13.5 mL, 0.11 mol). The solution was stirred overnight and then quenched by the addition of MeOH. The solvent was evaporated and the crude residue diluted with CH_2Cl_2, washed with a 1 M HCl solution, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the residue was purified by chromatography (hexane–EtOAc 7:1 → 4:1) to provide **2.19** (16.2 g, 72%) as a pale yellow syrup. $[\alpha]_D$ = −115.6 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28–7.02 (m, 30H, ArH), 6.04 (app t, *J* = 10.1 Hz, 1H, H-4), 5.79 (dd, *J* = 10.1, 3.4 Hz, 1H, H-3), 5.69 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2), 5.05 (d, *J* = 1.7 Hz, 1H, H-1), 4.19 (ddd, *J* = 10.2, 4.7, 2.2 Hz, 1H, H-5), 3.57 (s, 3H, OC*H*$_3$), 3.42 (dd, *J* = 10.5, 2.3 Hz, 1H, H-6a), 3.31 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6b). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7 (*C*=O), 165.5 (*C*=O), 165.1 (*C*=O), 143.8 (Ar), 133.4 (Ar), 133.0(4) (Ar), 133.0(1) (Ar), 130.0 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.4 (Ar), 129.3 (Ar), 128.7 (Ar), 128.3 (Ar), 128.2 (Ar), 127.7 (Ar), 126.9 (Ar), 98.6 (C-1), 86.7 (O*C*(Ph)$_3$), 70.8 (C-5), 70.6 (C-2), 70.3 (C-3), 67.0 (C-4), 62.4 (C-6), 55.3 (O*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{47}H_{40}O_9Na$ $[M+Na]^+$: 771.2565. Found: 771.2563.



Methyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (2.20)

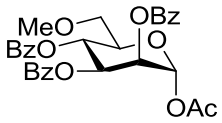
Monosaccharide **2.19** (749 mg, 1 mmol) was dissolved in 1:1 CH_2Cl_2–MeOH (10 mL). To this solution was added 10% HCl in MeOH (2 mL, HCl: MeOH v/v = 1:9). The solution was stirred overnight and then the solvent was evaporated. The residue was purified by chromatography (hexane–EtOAc 2:1 → 1:2) to provide **2.20** (410 mg, 81%) as a colorless syrup $[\alpha]_D = -156.7$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17–7.22 (m, 15H, ArH), 5.98 (dd, *J* = 10.1, 3.4 Hz, 1H, H-3), 5.86 (app t, *J* = 10.1 Hz, 1H, H-4), 5.69 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2), 5.02 (d, *J* = 1.5 Hz, 1H, H-1), 4.09 (ddd, *J* = 10.0, 3.9, 2.2 Hz, 1H, H-5), 3.86 (dd, *J* = 12.7, 2.2 Hz, 1H, H-6a), 3.80 (dd, *J* = 12.7, 4.0 Hz, 1H, H-6b), 3.53 (s, 3H, OC*H*$_3$), 2.62 (dd, *J* = 8.4, 5.8 Hz, 1H, O*H*). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5 (*C*=O), 165.6 (*C*=O), 165.5 (*C*=O), 133.7 (Ar), 133.6 (Ar), 133.2 (Ar), 129.9(4) (Ar), 129.9(2) (Ar), 129.7(2) (Ar), 129.3(3) (Ar), 129.2 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.3 (Ar), 98.8 (C-1), 70.9 (C-5), 70.6 (C-2), 69.6 (C-3), 67.3 (C-4), 61.4 (C-6), 55.5 (O*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{28}H_{26}O_9Na$ $[M+Na]^+$: 529.1469. Found: 529.1461.



Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-methyl-α-D-mannopyranoside (2.21)

Monosaccharide **2.20** (112.8 mg, 0.22 mmol) was dissolved in CH_3I (2.5 mL). To this solution was added Ag_2O (155 mg, 0.67 mmol) and $CaSO_4$ (121 mg, 0.89 mmol). The reaction mixture was stirred for 3 days, and then filtered through Celite. The filtrate was concentrated and the crude residue was purified by

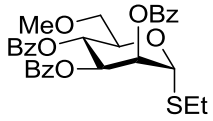
chromatography (hexane–EtOAc 4:1) to afford **2.21** (60 mg, 52%) as a colorless syrup as well as the side product methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methyl-α-D-mannopyranoside (35 mg, 31%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15–7.20 (m, 15H, ArH), 5.91–5.85 (m, 2H, H-4, H-3), 5.66 (dd, *J* = 2.8, 1.8 Hz, 1H, H-2), 5.00 (d, *J* = 1.7 Hz, 1H, H-1), 4.24-4.23 (m, 1H, H-5), 3.64 (m, 2H, H-6a, H-6b), 3.54 (s, 3H, OC*H*$_3$), 3.40 (s, 3H, OC*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5(7) (*C*=O), 165.5(6) (*C*=O), 165.4 (*C*=O), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 98.6 (C-1), 71.6 (C-6), 70.5 (C-2), 70.0 (C-5), 69.9, 67.4 (C-4, C-3), 59.6 (O*C*H$_3$), 55.5 (O*C*H$_3$). The ^{1}H and ^{13}C NMR spectral data were consistent with that previously reported.[15] HRMS (ESI) *m/z* Calcd for $C_{29}H_{28}O_9Na$ $[M+Na]^+$: 543.1626. Found: 543.1618.



1-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-6-*O*-methyl-α-D-mannopyranose (2.22)

Monosaccharide **2.21** (286 mg, 0.55 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (70:30:1 v/v/v, 5.05 mL). After stirring overnight, the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1) to afford **2.22** (289 mg, 96%) as a pale yellow syrup $[\alpha]_D$ = −130.2 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24–7.15 (m, 15H, ArH), 6.39 (d, *J* = 2.0 Hz, 1H, H-1), 5.98 (app t, *J* = 10.0 Hz,

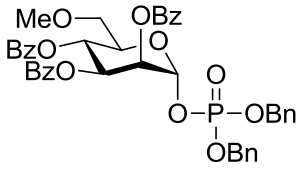
1H, H-4), 5.89 (dd, *J* = 10.2, 3.3 Hz, 1H, H-3), 5.71 (dd, *J* = 3.3, 2.0 Hz, 1H, H-2), 4.31 (app dt, *J* = 9.9, 3.9 Hz, 1H, H-5), 3.65 (d, *J* = 4.0 Hz, 2H, H-6a, H-6b), 3.38 (s, 3H, OC*H*$_3$), 2.26 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2 (*C*=O), 165.6 (*C*=O), 165.4 (*C*=O), 165.3 (*C*=O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0 (Ar), 129.7(8) (Ar), 129.7(6) (Ar), 129.0(8) (Ar), 129.0(6) (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 90.9 (C-1, $^1J_{C,H}$ = 178.5 Hz), 72.4 (C-5), 71.4 (C-6), 69.8 (C-3), 69.3 (C-2), 66.9 (C-4), 59.7 (O*C*H$_3$), 21.0 (C(O)*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{30}H_{28}O_{10}Na$ $[M+Na]^+$: 571.1575. Found: 571.1579.



Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-methyl-1-thio-α-D-mannopyranoside (2.23)

Monosaccharide **2.22** (280 mg, 0.51 mmol) and ethanethiol (57 μL, 0.77 mmol) were dissolved in CH_2Cl_2 (4 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (128 μL, 1.02 mmol). The reaction mixture was stirred overnight and then diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water, and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue purified by chromatography (hexane–EtOAc 5:1 → 4:1) to afford **2.23** (211 mg, 75%) as a pale yellow syrup $[\alpha]_D = -49.3$ (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12–8.10(m, 2H, ArH), 8.00–7.98 (m, 2H, ArH), 7.84–7.82 (m, 2H, ArH), 7.61–7.57 (m, 1H, ArH), 7.53–7.45 (m, 3H, ArH), 7.43–7.36 (m, 3H, ArH), 7.27–7.23 (m, 2H, ArH), 5.96 (app. t, *J* = 10.0 Hz, 1H, H-4), 5.81 (dd, *J* = 9.9, 3.3 Hz, 1H, H-3), 5.78 (dd, *J* = 3.3, 1.6 Hz, 1H, H-2), 5.58 (d, *J* = 1.4 Hz, 1H,

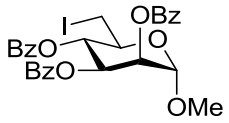
H-1), 4.62 (ddd, *J* = 9.9, 4.8, 3.0 Hz, 1H, H-5), 3.69–3.61 (m, 2H, H-6a, H-6b), 3.39 (s, 3H, OC*H*$_3$), 2.83–2.68 (m, 2H, SC*H*$_2$CH$_3$), 1.37 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.5(3) (*C*=O), 165.5(1) (*C*=O), 165.4 (*C*=O), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 82.2 (C-1), 72.3 (C-2), 71.4 (C-6), 70.6 (C-3), 70.4 (C-5), 67.6 (C-4), 59.6 (O*C*H$_3$), 25.5 (S*C*H$_2$CH$_3$), 14.9 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{30}H_{30}O_8SNa$ $[M+Na]^+$: 573.1554. Found: 573.1544.



Dibenzyl 2,3,4-tri-*O*-benzoyl-6-*O*-methyl-α-D-mannopyranosyl phosphate (2.24)

Thioglycoside **2.23** (103 mg, 0.18 mmol), dibenzyl phosphate (85 mg, 0.31 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 and stirred for 30 min. Then, *N*-iodosuccinimide (61 mg, 0.27 mmol) and silver trifluoromethanesulfonate (14 mg, 0.054 mmol) were added at –30 ℃. The reaction mixture was stirred for 2 h, and then was filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:2) to afford **2.24** (123 mg, 89%) as a colorless oil $[\alpha]_D$ = −87.4 (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23–7.18 (m, 25H, ArH), 5.97 (app t, *J* = 10.2 Hz, 1H, H-4), 5.95 (dd, *J* = 6.2, 2.3 Hz, 1H, H-1), 5.86 (dd, *J* = 10.1, 3.3 Hz,

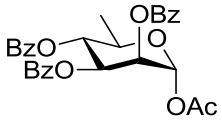
1H, H-3), 5.69 (dd, *J* = 3.2, 2.1 Hz, 1H, H-2), 5.24–5.17 (m, 4H, C*H*$_2$Ph × 2), 4.37 (app dt, *J* = 10.0, 3.6 Hz, 1H, H-5), 3.57–3.53 (m, 2H, H-6a, H-6b), 3.34 (s, 3H, OC*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.3(5) (*C*=O), 165.3(2) (*C*=O), 165.1 (*C*=O), 135.6 (d, $^3J_{C,P}$ = 7.1 Hz, 1C, Ar), 135.4 (d, $^3J_{C,P}$ = 6.8 Hz, 1C, Ar), 133.6 (Ar), 133.4 (Ar), 133.2 (Ar), 130.0 (Ar), 129.8(1) (Ar), 129.7(6) (Ar), 129.1 (Ar), 129.0 (Ar), 128.7(4) (Ar), 128.7(0) (Ar), 128.6(6) (Ar), 128.6 (Ar), 128.5 (Ar), 128.3(2) (Ar), 128.2(7) (Ar), 128.1 (Ar), 127.9 (Ar), 95.4 (d, $^2J_{C,P}$ = 5.5 Hz, 1C, C-1, $^1J_{C,H}$ = 179.9 Hz), 71.9 (C-5), 70.8 (C-6), 70.0 (d, $^2J_{C,P}$ = 5.3 Hz, 1C, *C*H$_2$Ph), 69.9 (d, $^2J_{C,P}$ = 5.4 Hz, 1C, *C*H$_2$Ph), 69.8 (d, $^3J_{C,P}$ = 11.3 Hz, 1C, C-2), 69.3 (C-3), 66.6 (C-4), 59.5 (O*C*H$_3$). ^{31}P NMR (161.84 MHz, $CDCl_3$) δ −2.97. HRMS (ESI) *m/z* Calcd for $C_{42}H_{39}O_{12}PNa$ $[M+Na]^+$: 789.2071. Found: 789.2063.



Methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-iodo-α-D-mannopyranoside (2.25)

To a solution of **2.20** (3.25 g, 6.4 mmol) in dry CH_2Cl_2 (34 mL) was added PPh_3 (3.37 g, 12.8 mmol), imidazole (874 mg, 12.8 mmol) and I_2 (3.26 g, 12.8 mmol) at 0 ℃. The solution was stirred overnight, diluted with CH_2Cl_2, and then washed with $Na_2S_2O_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the residue was purified by chromatography (hexane–EtOAc 4:1) to provide **2.25** (2.56 g, 65%) as a pale yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20–7.20 (m, 15H, ArH), 5.89 (dd, *J* = 10.0, 3.4 Hz, 1H, H-3), 5.77 (app t, *J* = 9.8 Hz, 1H, H-4), 5.68 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2), 5.02 (d, *J* = 1.7 Hz, 1H, H-

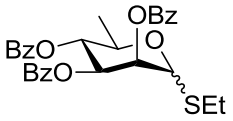
1), 4.11–4.05 (m, 1H, H-5), 3.60 (s, 3H, OCH_3), 3.49 (dd, J = 10.9, 2.6 Hz, 1H, H-6a), 3.38 (dd, J = 10.9, 8.1 Hz, 1H, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.6 (*C*=O), 165.5 (*C*=O), 165.4 (*C*=O), 133.6(1) (Ar), 133.5(5) (Ar), 133.2 (Ar), 132.3 (Ar), 132.2 (Ar), 131.6 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 98.8 (C-1), 70.7, 70.5, 70.2, 69.6 (C-2, C-3, C-4, C-5), 55.8 (O*C*H_3), 4.7 (C-6). The ^{1}H and ^{13}C NMR spectral data were consistent with that previously reported.[16] HRMS (ESI) *m/z* Calcd for $C_{28}H_{25}IO_8Na$ $[M+Na]^+$: 639.0486. Found: 639.0492.



1-*O*-Acetyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranose (2.26)

Monosaccharide **2.25** (867 mg, 1.4 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 9.2 mL). After stirring overnight the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and then dissolved in EtOAc (10 mL). To this solution was added 10% Pd–C (100 mg) and Et_3N (1.5 mL). The reaction mixture was stirred for 2 days under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated. The residue was purified by chromatography (hexane–EtOAc 4:1 → 3:1) to afford **2.26** (525 mg, 72%) as a pale yellow syrup. $[\alpha]_D$ = −133.5 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14–7.24 (m, 15H, ArH), 6.32 (d, J = 1.6 Hz, 1H, H-1), 5.86 (dd, J = 10.2, 3.4 Hz, 1H, H-3), 5.76–5.70 (m, 2H, H-2, H-4), 4.28 (dq, J

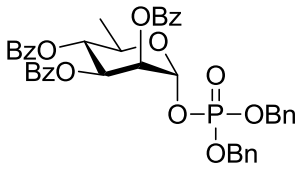
= 10.0, 6.0 Hz, 1H, H-5), 2.26 (s, 3H, C(O)CH_3), 1.40 (d, J = 6.2 Hz, 3H, H-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4 (*C*=O), 165.6 (*C*=O), 165.3 (*C*=O) 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 130.0 (Ar), 129.7(4) (Ar), 129.7(1) (Ar), 129.1(4) (Ar), 129.0(9) (Ar), 128.9(6) (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 90.8 (C-1, $^1J_{C,H}$ = 177.2 Hz), 71.2 (C-4), 69.7 (C-2), 69.6 (C-3), 69.1 (C-5), 21.0 (C(O)*C*H_3), 17.7 (C-6). HRMS (ESI) *m/z* Calcd for $C_{29}H_{26}O_9Na$ $[M+Na]^+$: 541.1469. Found: 541.1465.



Ethyl 2,3,4-tri-*O*-benzoyl-6-deoxy-1-thio-α/β-D-mannopyranoside (2.27)

Monosaccharide **2.26** (361 mg, 0.70 mmol) and ethanethiol (78 μL, 1.05 mmol) were dissolved in CH_2Cl_2 (6 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (176 μL, 1.40 mmol). The reaction mixture was stirred overnight and then was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 6:1 → 5:1) to afford **2.27** (324 mg, 89%, α/β 4:1) as a pale yellow syrup. Data for α isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26–7.17 (m, 15H, ArH), 5.85–5.78 (m, 2H, H-2, H-3), 5.78–5.71 (m, 1H, H-4), 5.52 (s, 1H, H-1), 4.59 (app dq, J = 9.5, 6.2 Hz, 1H, H-5), 2.87–2.59 (m, 2H, SC$H_2$$CH_3$), 1.41 (d, J = 6.3 Hz, 3H, H-6), 1.38 (t, J = 7.4 Hz, 3H, $SCH_2C$$H_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8 (C=O), 165.6 (C=O), 165.4 (C=O), 133.5 (Ar), 133.4 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7

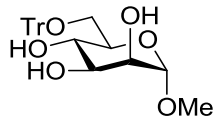
(Ar), 129.5 (Ar), 129.3 (Ar), 129.1 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 82.2 (C-1), 72.7, 72.1, 70.5 (C-2, C-3, C-4), 67.4 (C-5), 25.7 (S*C*$H_2$$CH_3$), 17.6 (C-6), 15.0 (S$CH_2$*C*$H_3$). ^{1}H and ^{13}C NMR spectral data were consistent with that previously reported.[17] HRMS (ESI) *m/z* Calcd for $C_{29}H_{28}O_7SNa$ $[M+Na]^+$: 543.1448. Found: 543.1448.



Dibenzyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranosyl phosphate (2.28)

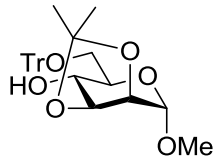
Thioglycoside **2.27** (64 mg, 0.12 mmol), dibenzyl phosphate (58 mg, 0.21 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (2 mL) and stirred at room temperature for 30 min. *N*–iodosuccinimide (42 mg, 0.19 mmol) and silver trifluoromethanesulfonate (10 mg, 0.039 mmol) were then added at −30 ℃. The reaction mixture was stirred for 2 h at −30 ℃, and then filtered through Celite and diluted with CH_2Cl_2. The solution was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1) to afford **2.28** (59 mg, 67%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (m, 2H, ArH), 7.97 (m, 2H, ArH), 7.83 (m, 2H, ArH), 7.66–7.25 (m, 19H, ArH), 5.85 (dd, *J* = 6.3, 1.9 Hz, 1H, H-1), 5.80 (dd, *J* = 10.2, 3.4 Hz, 1H, H-3), 5.69–5.64 (m, 2H, H-2, H-4), 5.23–5.14 (m, 4H, C*H*$_2$Ph), 4.29 (dq, *J* = 9.9, 6.2 Hz, 1H, H-5), 1.28 (d, *J* = 6.2 Hz, 3H, H-6). ^{31}P

NMR (201.64 MHz, CD_3Cl_3) δ −1.76. HRMS (ESI) *m/z* Calcd for $C_{41}H_{37}O_{11}PNa$ $[M+Na]^+$: 759.1966. Found: 759.1960.



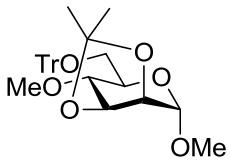
Methyl 6-*O*-trityl-α-D-mannopyranoside (2.29)

To a solution of methyl α-D-mannopyranoside **2.18** (3.9 g, 0.02 mol) in dry pyridine (100 mL) was added trityl chloride (6.7 g, 0.024 mol) and DMAP (0.47 g, 4 mmol). The solution was stirred at 40 ℃ overnight and then cooled. Evaporation of the solvent, followed by chromatography of the residue (hexane–EtOAc 1:1 → 1:3) afforded **2.29** (7.4 g, 85%) as a colorless syrup $[\alpha]_D$ = +21.9 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71–7.08 (m, 15H, ArH), 4.72 (d, *J* = 1.3 Hz, 1H, H-1), 3.96–3.87 (m, 1H, H-2), 3.79 (dd, *J* = 8.9, 3.4 Hz, 1H, H-3), 3.73 (app t, *J* = 9.3 Hz, 1H, H-4), 3.69–3.62 (m, 1H, H-5), 3.46 (d, *J* = 4.8 Hz, 1H, H-6a), 3.42 (dd, *J* = 9.8, 5.3 Hz, 1H, H-6b), 3.38 (s, 3H, OC*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.6 (Ar), 128.6 (Ar), 128.0 (Ar), 127.2 (Ar), 100.6 (C-1), 87.4 (O*C*(Ph)$_3$), 71.6 (C-3), 70.4 70.3, 69.8 (C-2, C-4, C-5), 64.9 (C-6), 55.0 (O*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{26}H_{28}O_6Na$ $[M+Na]^+$: 459.1778. Found: 459.1782.



Methyl 2,3-*O*-isopropylidene-6-*O*-trityl-α-D-mannopyranoside (2.30)

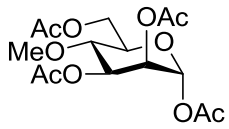
Monosaccharide **2.29** (4.2 g, 9.6 mmol) was dissolved in 2,2-dimethoxypropane (20 mL). To this solution was added *p*-TsOH (183 mg, 0.96 mmol) and the reaction mixture was stirred overnight. Then, the solution was quenched by the addition of $NaHCO_3$ (satd aq soln). The solvent was evaporated and the residue was diluted with CH_2Cl_2, washed with distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the residue was purified by chromatography (hexane–EtOAc 4:1) to give **2.30** (3.47 g, 76%) as a colorless oil. $[\alpha]_D = -1.3$ (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49–7.22 (m, 15H, ArH), 4.92 (s, 1H, H-1), 4.16–4.08 (m, 2H, H-2, H-3), 3.76–3.63 (m, 1H, H-4), 3.71–3.67 (m, 1H, H-5), 3.45–3.41 (m, 4H, H-6a, OCH_3), 3.38 (dd, *J* = 10.0, 5.2 Hz, 1H, H-6b), 2.45 (d, *J* = 3.8 Hz, 1H, O*H*), 1.50 (s, 3H, CH_3), 1.35 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.7 (Ar), 128.7 (Ar), 127.9 (Ar), 127.2 (Ar), 109.5 ($CO_2(CH_3)_2$), 98.3 (C-1), 87.1 (OC(Ph)$_3$), 78.0 (C-3), 75.3 (C-2), 70.7 (C-4), 68.8 (C-5), 64.3 (C-6), 55.0 (OCH$_3$), 27.9 (CH$_3$), 26.1 (CH$_3$). HRMS (ESI) *m/z* Calcd for $C_{29}H_{32}O_6Na$ $[M+Na]^+$: 499.2091. Found: 499.2095.



Methyl 2,3-*O*-isopropylidene-4-*O*-methyl-6-*O*-trityl-α-D-mannopyranoside (2.31)

Monosaccharide **2.30** (272 mg, 0.57 mmol) was dissolved in DMF (6 mL) and the solution was cooled to 0 ℃. After the addition of NaH (60% NaH in mineral oil, 41 mg, 1.03 mmol), the mixture was stirred for 10 min before the addition of

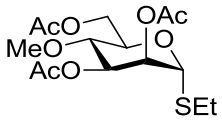
CH_3I (53 μL, 0.86 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with CH_2Cl_2 and washed with distilled water and brine. The organic layer was dried over Na_2SO_4. Evaporation of the solvent and chromatography (hexane–EtOAc 10:1) of the residue afforded **2.31** (253 mg, 91%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54–7.20 (m, 15H, ArH), 4.99 (s, 1H, H-1), 4.19–4.10 (m, 2H, H-3, H-2), 3.67-3.63 (m, 1H, H-5), 3.47 (s, 3H, OCH_3), 3.41 (dd, J = 9.9, 2.0 Hz, 1H, H-6a), 3.38 (dd, J = 10.2, 6.5 Hz, 1H, H-4), 3.32 (s, 1H, OCH_3), 3.20 (dd, J = 9.9, 5.4 Hz, 1H, H-6b), 1.58 (s, 1H, CH_3), 1.37 (s, 1H, CH_3). The ^{1}H spectral data was consistent with that previously reported.[18] HRMS (ESI) *m/z* Calcd for $C_{30}H_{34}O_6Na$ $[M+Na]^+$: 512.2248. Found: 513.2254.



1,2,3,6-Tetra-*O*-acetyl-4-*O*-methyl-α-D-mannopyranose (2.32)

Monosaccharide **2.31** (247 mg, 0.5 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 3.06 mL). After stirring overnight, the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. After drying over Na_2SO_4, the organic layer was concentrated and the crude residue purified by chromatography (hexane–EtOAc 2:1 → 1:1) to afford **2.32** (96 mg, 55%) as a pale yellow syrup. $[\alpha]_D$ = +42.5 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.03 (d, *J* = 1.8 Hz, 1H, H-1), 5.24 (dd, *J* = 9.7, 3.5 Hz, 1H, H-3), 5.22 (dd, *J* = 3.5, 2.0 Hz, 1H, H-2), 4.36–4.28 (m, 2H, H-6a, H-6b), 3.91-3.87 (m, 1H,

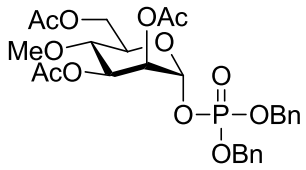
H-5), 3.56 (app t, J = 9.7 Hz, 1H, H-4), 3.47 (s, 3H, OCH_3), 2.16 (s, 3H, C(O)CH_3), 2.15 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.08 (s, 3H, C(O)CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7 (*C*=O), 169.8 (*C*=O), 169. 7 (*C*=O), 168.3 (*C*=O), 90. 7 (C-1, $^1J_{C,H}$ = 177.6 Hz), 74.3 (C-4), 71.8 (C-5), 71.3 (C-3), 68.7 (C-2), 62.9 (C-6), 60.7 (O*C*H_3), 20.9 (C(O)*C*H_3 × 2), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{15}H_{22}O_{10}Na$ $[M+Na]^+$: 385.1105. Found: 385.1104.



Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-methyl-1-thio-α-D-mannopyranoside (2.33)

Tetraacetate **2.32** (259 mg, 0.72 mmol) and ethanethiol (80 μL, 1.08 mmol) were dissolved in CH_2Cl_2 (4 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (180 μL, 1.44 mmol). The reaction mixture was stirred overnight and then was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1 → 1:1) to afford **2.33** (183 mg, 70%) as a pale yellow syrup. $[\alpha]_D$ = +75.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (dd, J = 3.4, 1.6 Hz, 1H, H-2), 5.23 (d, J = 1.5 Hz, 1H, H-1), 5.19 (dd, J = 9.6, 3.4 Hz, 1H, H-3), 4.41–4.30 (m, 2H, H-6a, H-6b), 4.25 (ddd, J = 9.9, 5.1, 2.3 Hz, 1H, H-5), 3.54 (app t, J = 9.7 Hz, 1H, H-4), 3.45 (s, 3H, OCH_3), 2.77–2.53 (m, 2H, SCH_2CH$_3$), 2.15 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.06 (s, 3H, C(O)CH_3), 1.29 (t, J = 7.4 Hz, 3H, SCH$_2$CH_3). ^{13}C NMR

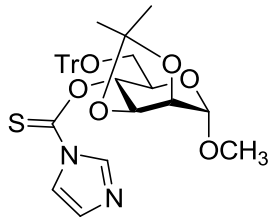
(125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 169.9 (*C*=O), 169.6 (*C*=O), 82.1 (C-1), 74.9 (C-4), 71.9 (C-3), 71.6 (C-2), 70.0 (C-5), 63.2 (C-6), 60.4 (O*C*H_3), 25.4 (S*C*H_2CH$_3$), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 14.8 (SCH$_2$*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{15}H_{24}O_8SNa$ $[M+Na]^+$: 387.1084. Found: 387.1084.



Dibenzyl 2,3,6-tri-*O*-acetyl-4-*O*-methyl-α-D-mannopyranosyl phosphate (2.34)

Thioglycoside **2.33** (178 mg, 0.49 mmol), dibenzyl phosphate (231 mg, 0.83 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 and stirred for 30 min. Then, *N*–iodosuccinimide (165 mg, 0.74 mmol) and silver trifluoromethanesulfonate (38 mg, 0.15 mmol) were added at −40 ℃. The reaction mixture was stirred for 3 h at −40 ℃ to −30 ℃, and was then filtered through Celite and diluted with CH_2Cl_2. The solution was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to give **2.34** (226 mg, 80%) as a colorless oil. $[\alpha]_D$ = +37.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.35 (m, 10H, ArH), 5.63 (dd, *J* = 6.6, 1.6 Hz, 1H, H-1), 5.29–5.26 (m, 2H, H-2, H-3), 5.14–5.06 (m, 4H, C*H*$_2$Ph), 4.26 (dd, *J* = 12.1, 4.8 Hz, 1H, H-6a), 4.21 (dd, *J* = 12.1, 2.3 Hz, 1H, H-6b), 3.98 (ddd, *J* = 9.6, 4.6, 2.3 Hz, 1H, H-5), 3.58–3.51 (m, 1H, H-4), 3.47 (s, 3H, OC*H*$_3$), 2.16 (s, 3H, C(O)C*H*$_3$), 2.09 (s, 3H, C(O)C*H*$_3$), 2.03 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.6 (*C*=O),

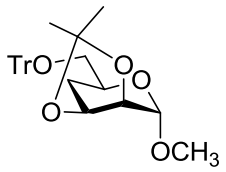
169.5 (*C*=O), 135.4 (d, $^{3}J_{C,P}$ = 6.9 Hz, 1C, Ar), 135.3 (d, $^{3}J_{C,P}$ = 6.8 Hz, 1C, Ar), 128.7(3) (Ar), 128.7(1) (Ar), 128.6(8) (Ar), 128.2 (Ar), 128.0 (Ar), 95.3 (d, $^{2}J_{C,P}$ = 5.3 Hz, 1C, C-1, $^{1}J_{C,H}$ = 178.7 Hz), 74.0 (C-4), 71.4 (C-5), 70.7 (C-3), 69.9 (d, $^{2}J_{C,P}$ = 5.5 Hz, 1C, *C*H_2Ph), 69.7(d, $^{2}J_{C,P}$ = 5.5 Hz, 1C, *C*H_2Ph), 69.2 (d, $^{3}J_{C,P}$ = 11.2 Hz, 1C, C-2), 62.7 (C-6), 60.6 (O*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{27}H_{33}O_{12}PNa$ $[M+Na]^+$: 603.1602. Found: 603.1603.



Methyl 4-*O*-imidazolylthiocarbonyl-2,3-*O*-isopropylidene-6-*O*-trityl-α-D-mannopyranoside (2.35)

To a solution of **2.30** (1 g, 2.1 mmol) in dry toluene (21 mL) was added 1,1'-thiocarbonyldiimidazole (1.5 g, 8.4 mmol) and the reaction mixture was heated at reflux overnight. After cooling, evaporation of the solvent and chromatography (hexane–EtOAc 1:1) of the residue yielded **2.35** (1.21 g, 98%) as a colorless syrup. $[\alpha]_D$ = +13.7 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (t, *J* = 0.9 Hz, 1H, imidazolyl), 7.42 (t, *J* = 1.5 Hz, 1H, imidazolyl), 7.41–7.37 (m, 6H, ArH), 7.23–7.12 (m, 9H, ArH), 6.99 (dd, *J* = 1.7, 0.8 Hz, 1H, imidazolyl), 5.82 (dd, *J* = 10.1, 7.4 Hz, 1H, H-4), 5.06 (s, 1H, H-1), 4.37 (dd, *J* = 7.3, 5.6 Hz, 1H, H-3), 4.23 (d, *J* = 5.6 Hz, 1H, H-2), 3.98–3.94 (m, 1H, H-5), 3.53 (s, 3H, OC*H*$_3$), 3.25 (dd, *J* = 11.0, 5.9 Hz, 1H, H-6a), 3.15 (dd, *J* = 11.0, 3.0 Hz, 1H, H-6b), 1.60 (s, 3H,

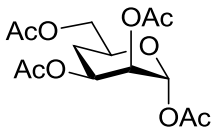
CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.3 (*C*=S), 143.5 (Ar), 130.7 (imidazolyl), 128.5 (Ar), 127.7 (Ar), 127.0 (Ar), 118.0 (imidazolyl), 110.5 ($CO_2(CH_3)_2$), 98.0 (C-1), 87.0 (O*C*(Ph)$_3$), 78.9 (C-4), 75.8 (C-2), 75.4 (C-3), 67.7 (C-5), 63.1 (C-6), 55.1 (O*C*H$_3$), 27.6 (*C*H$_3$), 26.3 (*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{33}H_{34}N_2O_6SNa$ $[M+Na]^+$: 609.2030. Found: 609.2029.



Methyl 4-deoxy-2,3-*O*-isopropylidene-6-*O*-trityl-α-D-*lyxo*-hexopyranoside (2.36)

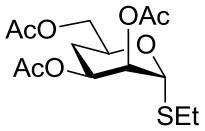
Thiocarbamate **2.35** (282 mg, 0.48 mmol) was dissolved in dry toluene (8 mL). Before the addition of AIBN (21 mg, 0.13 mmol) and tri-*n*-butylstannane (420 μL, 1.56 mmol), the solution was sparged with argon. The mixture was then heated at reflux overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded **2.36** (199 mg, 90%) as a pale yellow syrup. $[\alpha]_D$ = +6.4 (*c* 0.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53–7.22 (m, 15H, ArH), 4.98 (s, 1H, H-1), 4.37–4.27 (m, 1H, H-3), 3.96 (d, *J* = 5.5 Hz, 1H, H-2), 3.86 (dddd, *J* = 11.1, 6.6, 4.2, 2.5 Hz, 1H, H-5), 3.48 (s, 3H, OC*H*$_3$), 3.32 (dd, *J* = 9.6, 6.6 Hz, 1H, H-6a), 3.03 (dd, *J* = 9.6, 4.3 Hz, 1H, H-6b), 1.84 (ddd, *J* = 13.1, 6.7, 2.4 Hz, 1H, H-4eq), 1.53–1.48 (m, 1H, H-4ax), 1.47 (s, 3H, C*H*$_3$), 1.34 (s, 3H, C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.5 (Ar), 144.2 (Ar), 144.1 (Ar), 128.8(3) (Ar), 128.7(5) (Ar), 127.8 (Ar), 127.7 (Ar), 127.0 (Ar), 126.9 (Ar), 108.8 ($CO_2(CH_3)_2$), 98.7 (C-1), 86.5 (O*C*(Ph)$_3$), 73.2 (C-2), 70.9 (C-3), 66.6 (C-6), 65.9

(C-5), 55.0 (O*C*H$_3$), 31.1 (C-4), 28.1 (CH$_3$), 26.3 (CH$_3$). HRMS (ESI) *m/z* Calcd for $C_{29}H_{32}O_5Na$ $[M+Na]^+$: 483.2142. Found: 483.2137.



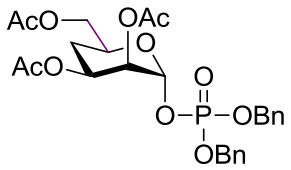
1,2,3,6-Tetra-*O*-acetyl-4-deoxy-α-D-*lyxo*-hexopyranose (2.37)

Monosaccharide **2.36** (3.3 g, 7.17 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 43.88 mL). After stirring overnight, the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1 → 3:2) to give **2.37** (1.26 g, 53%, α/β 6:1) as a pale yellow syrup. Data for α isomer: $[\alpha]_D$ = +55.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (d, *J* = 1.9 Hz, 1H, H-1), 5.30 (ddd, *J* = 11.9, 5.2, 3.2 Hz, 1H, H-3), 5.10 (app t, *J* = 2.1 Hz, 1H, H-2), 4.20–4.10 (m, 3H, H-5, H-6a, H-6b), 2.15 (s, 3H, C(O)C*H*$_3$), 2.14 (s, 3H, C(O)C*H*$_3$), 2.09 (s, 3H, C(O)C*H*$_3$), 2.03 (s, 3H, C(O)C*H*$_3$), 1.94–1.82 (m, 2H, H-4ax, H-4eq). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.0 (*C*=O), 169.8 (*C*=O), 168.3 (*C*=O), 91.6 (C-1, $^1J_{C,H}$ = 180.9 Hz), 68.4 (C-5), 66.5 (C-3), 66.0 (C-2), 65.6 (C-6), 27.8 (C-4), 20.9(0) (C(O)*C*H$_3$), 20.8(7) (C(O)*C*H$_3$), 20.7(7) (C(O)*C*H$_3$), 20.7(5) (C(O)*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{14}H_{20}O_9Na$ $[M+Na]^+$: 355.1000. Found: 355.0996.



Ethyl 2,3,6-tri-*O*-acetyl-4-deoxy-1-thio-α-D-*lyxo*-hexopyranoside (2.38)

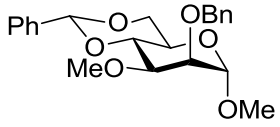
The tetraacetate **2.37** (15 mg, 0.045 mmol) and ethanethiol (4 μL, 0.054 mmol) were dissolved in dry CH_2Cl_2 (2 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (7 μL, 0.056 mmol). The reaction mixture was stirred overnight and was then diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 4:1 → 2:1) to afford **2.38** (6 mg, 32%) as a pale yellow syrup. $[\alpha]_D$ = +119.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.32 (s, 1H, H-1), 5.25–5.17 (m, 2H, H-3, H-2), 4.46 (ddd, *J* = 14.5, 6.4, 3.2 Hz, 1H, H-5), 4.21 (dd, *J* = 11.7, 6.4 Hz, 1H, H-6a), 4.13 (dd, *J* = 11.7, 3.7 Hz, 1H, H-6b), 2.74–2.56 (m, 2H, SCH_2CH$_3$), 2.15 (s, 3H, C(O)CH_3), 2.09 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.95–1.77 (m, 2H, H-4ax, H-4eq), 1.30 (t, *J* = 7.4 Hz, 3H, SCH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.1 (*C*=O), 169.8 (*C*=O), 82.8 (C-1, $^1J_{C,H}$ = 168.0 Hz), 69.4 (C-2), 66.9 (C-3), 66.5 (C-5), 65.9 (C-6), 28.3 (C-4), 25.4 (S*C*H$_2$CH$_3$), 20.9(6) (C(O)*C*H$_3$), 20.9(0) (C(O)*C*H$_3$), 20.8 (C(O)*C*H$_3$), 14.9 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{14}H_{22}O_7SNa$ $[M+Na]^+$: 357.0978. Found: 357.0980.



Dibenzyl 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*lyxo*-hexopyranosyl phosphate (2.39)

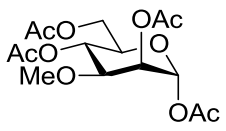
Thioglycoside **2.38** (33.4 mg, 0.1 mmol), dibenzyl phosphate (47.3 mg, 0.17 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (2 mL)

and stirred at room temperature for 30 min. *N*–iodosuccinimide (33.7 mg, 0.15 mmol) and silver trifluoromethanesulfonate (7.7 mg, 0.03 mmol) were then added at −40 ℃. The reaction mixture was stirred for 1 h at −40 ℃ to −30 ℃, and was then filtered through Celite and diluted with CH_2Cl_2. The solution was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to afford **2.39** (45 mg, 81%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.31 (m, 10H, ArH), 5.66 (dd, *J* = 6.3, 1.8 Hz, 1H, H-1), 5.22 (ddd, *J* = 12.0, 5.0, 3.0 Hz, 1H, H-3), 5.09–5.08 (m, 5H, H-2, C*H*$_2$Ph), 4.19–4.17 (m, 1H, H-5), 4.08 (dd, *J* = 11.8, 5.8 Hz, 1H, H-6a), 4.02 (dd, *J* = 11.8, 4.3 Hz, 1H, H-6b), 2.12 (s, 3H, C(O)C*H*$_3$), 2.01 (s, 3H, C(O)C*H*$_3$), 1.97 (s, 3H, C(O)C*H*$_3$), 1.89–1.77 (m, 2H, H-4ax, H-4eq). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (*C*=O), 169.7 (*C*=O), 169. 6 (*C*=O), 135.5 (d, $^3J_{C,P}$ = 6.9 Hz, 1C, Ar), 135.4 (d, $^3J_{C,P}$ = 6.8 Hz, 1C, Ar), 128.7 (Ar), 128.4 (Ar), 128.6 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 96.1 (d, $^2J_{C,P}$ = 5.4 Hz, 1C, C-1), 69.8 (d, $^2J_{C,P}$ = 5.5 Hz, 1C, *C*H$_2$Ph), 69.6 (d, $^2J_{C,P}$ = 5.5 Hz, 1C, *C*H$_2$Ph), 68.2 (C-5), 66.8 (d, $^3J_{C,P}$ = 11.3 Hz, 1C, C-2), 65.5 (C-3), 65.5 (C-6), 27.6 (C-4), 20.9 (C(O)*C*H$_3$), 20.8 (C(O)*C*H$_3$), 20.6 (C(O)*C*H$_3$). ^{31}P NMR (202.33 MHz, $CDCl_3$) δ −3.10. HRMS (ESI) *m/z* Calcd for $C_{26}H_{31}O_{11}PNa$ $[M+Na]^+$: 573.1496. Found: 573.1493.



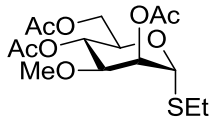
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (2.41)

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[19] **2.40** (1.29 g, 3.48 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 ℃. After the addition of NaH (60% NaH in mineral oil, 250 mg, 6.26 mmol), the mixture was stirred for 10 min before the addition of CH_3I (0.33 mL, 5.29 mmol). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL). The solution was then diluted with CH_2Cl_2, washed with distilled water and brine, and the organic layer was dried over Na_2SO_4. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded **2.41** (1.025 g, 76%) as a colorless syrup. $[\alpha]_D$ = +30.9 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54–7.26 (m, 10H, ArH), 5.64 (s, 1H, O_2CHPh), 4.84 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.75–4.72 (m, 2H, H-1, CH_2Ph), 4.28 (dd, *J* = 10.1, 4.7 Hz, 1H, H-6a), 4.18 (app t, *J* = 9.6 Hz, 1H, H-4), 3.93–3.86 (m, 2H, H-6b, H-2), 3.83–3.80 (m, 1H, H-5), 3.74 (dd, *J* = 10.0, 3.2 Hz, 1H, H-3), 3.50 (s, 1H, OCH_3), 3.37 (s, 1H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1 (Ar), 137.7 (Ar), 128.9 (Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.8 (Ar), 126.2 (Ar), 101.7 (O_2CHPh), 100.3 (C-1), 79.0 (C-4), 78.1 (C-3), 75.6 (C-2), 73.6 (CH_2Ph), 68.9 (C-6), 64.0 (C-5), 58.8 (OCH_3), 54.9 (OCH_3). HRMS (ESI) *m/z* Calcd for $C_{22}H_{26}O_6Na$ $[M+Na]^+$: 409.1622. Found: 409.1613.



1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-D-mannopyranose (2.42)

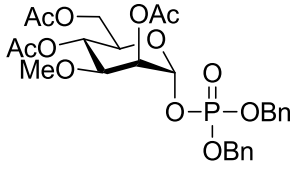
Monosaccharide **2.41** (1 g, 2.59 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 13.26 mL). After stirring for 2 days, the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:2 → 1:1) to afford **2.42** (610 mg, 65%) as a pale yellow syrup. $[\alpha]_D$ = +16.7 (*c* 1.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.06 (d, *J* = 2.0 Hz, 1H, H-1), 5.30 (dd, *J* = 3.3, 2.1 Hz, 1H, H-2), 5.20 (app t, *J* = 10.0 Hz, 1H, H-4), 4.21 (dd, *J* = 12.4, 5.2 Hz, 1H, H-6a), 4.12–4.06 (m, 1H, H-6b), 3.93 (ddd, *J* = 10.1, 5.2, 2.4 Hz, 1H, H-5), 3.63 (dd, *J* = 9.8, 3.4 Hz, 1H, H-3), 3.34 (s, 3H, OC*H*$_3$), 2.13 (s, 3H, C(O)C*H*$_3$), 2.12 (s, 3H, C(O)C*H*$_3$), 2.06 (s, 3H, C(O)C*H*$_3$), 2.05 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.0 (*C*=O), 169.7 (*C*=O), 168.1 (*C*=O), 91.0 (C-1, $^1J_{C,H}$ = 177.9 Hz), 76.8 (C-3), 70.8 (C-5), 67.0 (C-4), 66.6 (C-2), 62.4 (C-6), 57.9 (O*C*H$_3$), 20.8(8) (C(O)*C*H$_3$), 20.8(6) (C(O)*C*H$_3$), 20.8(3) (C(O)*C*H$_3$), 20.7(5) (C(O)*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{15}H_{22}O_{10}Na$ $[M+Na]^+$: 385.1105. Found: 385.1104.



Ethyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-1-thio-α-D-mannopyranoside (2.43)

Tetraacetate **2.42** (592 mg, 1.63 mmol) and ethanethiol (182 μL, 2.45 mmol) were dissolved in dry CH_2Cl_2 (8 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (411 μL, 3.27 mmol). The reaction mixture was stirred overnight and

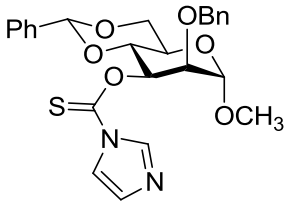
then was diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:1 → 2:1) to afford **2.43** (307 mg, 52%) as a pale yellow syrup. $[\alpha]_D$ = +71.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 (dd, *J* = 3.3, 1.8 Hz, 1H, H-2), 5.29 (d, *J* = 2.0 Hz, 1H, H-1), 5.18 (app t, *J* = 9.8 Hz, 1H, H-4), 4.30–4.25 (m, 2H, H-5, H-6a), 4.10–4.07 (m, 1H, H-6b), 3.56 (dd, *J* = 9.8, 3.2 Hz, 1H, H-3), 3.32 (s, 3H, OC*H*$_3$), 2.69–2.58 (m, 2H, SC*H*$_2$CH$_3$), 2.14 (s, 3H, C(O)C*H*$_3$), 2.08 (s, 3H, C(O)C*H*$_3$), 2.07 (s, 3H, C(O)C*H*$_3$), 1.30 (t, *J* = 7.5 Hz, 3H, SCH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.3 (*C*=O), 169.8 (*C*=O), 82.6 (C-1), 77.6 (C-3), 69.5 (C-2), 69.1 (C-5), 67.8 (C-4), 62.7 (C-6), 57.8 (O*C*H$_3$), 25.7 (S*C*H$_2$CH$_3$), 21.0 (C(O)*C*H$_3$), 20.9 (C(O)*C*H$_3$), 20.8 (C(O)*C*H$_3$), 14.9 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{15}H_{24}O_8SNa$ $[M+Na]^+$: 387.1084. Found: 387.1084.



Dibenzyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-mannopyranosyl phosphate (2.44)

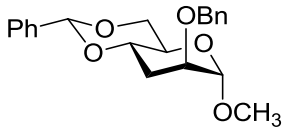
Thioglycoside **2.43** (292 mg, 0.8 mmol), dibenzyl phosphate (378 mg, 1.36 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (8 mL) and stirred at room temperature for 30 min. *N*–iodosuccinimide (270 mg, 1.2 mmol) and silver trifluoromethanesulfonate (62 mg, 0.24 mmol) were then added at −30 ℃. The reaction mixture was stirred for 2 h between −30 ℃ to −20 ℃, and then was filtered through Celite and diluted with CH_2Cl_2. The resulting solution

was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to give **2.44** (346 mg, 75%) as a colorless syrup. $[\alpha]_D$ = +21.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (m, 10H, ArH), 5.59 (dd, *J* = 6.5, 2.0 Hz, 1H, H-1), 5.21 (dd, *J* = 3.3, 2.3 Hz, 1H, H-2), 5.13 (app t, *J* = 9.9 Hz, 1H, H-4), 5.11–5.02 (m, 4H, C*H*$_2$Ph), 4.13 (dd, *J* = 12.5, 5.0 Hz, 1H, H-6a), 3.97–3.94 (m, 1H, H-5), 3.92 (dd, *J* = 12.5, 2.5 Hz, 1H, H-6b), 3.50 (dd, *J* = 10.0, 3.5 Hz, 1H, H-3), 3.24 (s, 3H, OC*H*$_3$), 2.08 (s, 3H, C(O)C*H*$_3$), 2.03 (s, 3H, C(O)C*H*$_3$), 1.94 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.7 (*C*=O), 169.6 (*C*=O), 135.4 (Ar), 135.3 (Ar), 128.8 (Ar), 128.8 (Ar), 128.7 (Ar), 128.2 (Ar), 128.1 (Ar), 95.6 (d, $^2J_{C,P}$ = 5.7 Hz, 1C, C-1, $^1J_{C,H}$ = 183.1 Hz), 76.1 (C-3), 70.5 (C-5), 69.9 (d, $^2J_{C,P}$ = 5.1 Hz, 1C, *C*H$_2$Ph), 69.8 (d, $^2J_{C,P}$ = 5.5 Hz, 1C, *C*H$_2$Ph), 67.0 (d, $^3J_{C,P}$ = 11.2 Hz, 1C, C-2), 66.6 (C-4), 62.1 (C-6), 57.9 (O*C*H$_3$), 20.8(1) (C(O)*C*H$_3$), 20.8 (C(O)*C*H$_3$), 20.6 (C(O)*C*H$_3$). ^{31}P NMR (201.64 MHz, $CDCl_3$) δ −1.91. HRMS (ESI) *m/z* Calcd for $C_{27}H_{33}O_{12}PNa$ $[M+Na]^+$: 603.1602. Found: 603.1595.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-imidazolylthiocarbonyl-α-D-mannopyranoside (2.45)

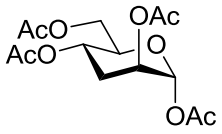
To a solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[19] **2.40** (225 mg, 0.6 mmol) in dry toluene (6 mL) was added 1,1'-thiocarbonyldiimidazole (431 mg, 2.4 mmol) and the reaction mixture was heated at reflux overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 1:1) of the resulting residue afforded **2.39** (237 mg, 82%) as a colorless syrup. $[\alpha]_D = -48.4$ (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (t, *J* = 1.0 Hz, 1H, imidazolyl), 7.57 (t, *J* = 1.5 Hz, 1H, imidazolyl), 7.45–7.17 (m, 10H, ArH), 7.03 (dd, *J* = 2.0, 1.0 Hz, 1H, imidazolyl), 5.85 (dd, *J* = 10.4, 3.6 Hz, 1H, H-3), 5.64 (s, 1H, O_2CHPh), 4.83 (d, *J* = 1.6 Hz, 1H, H-1), 4.69 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.48–4.40 (m, 2H, CH_2Ph, H-4), 4.36–4.31 (m, 2H, H-2, H-6a), 4.00–3.91 (m, 2H, H-5, H-6b), 3.45 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.8 (*C*=S), 136.9 (Ar), 136.8 (Ar), 130.7 (imidazolyl), 129.2 (Ar), 128.5 (Ar), 128.2(9) (Ar), 128.2(6) (Ar), 128.2 (Ar), 126.1 (Ar), 118.0 (imidazolyl), 102.0 (O_2CHPh), 99.7 (C-1), 78.9 (C-3), 75.9 (C-4), 74.4 (C-2), 73.5 (CH_2Ph), 68.8 (C-6), 63.9 (C-5), 55.3 (OCH_3). HRMS (ESI) *m/z* Calcd for $C_{25}H_{27}N_2O_6SNa$ $[M+Na]^+$: 483.1584. Found: 483.1584.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (2.46)

Thiocarbamate **2.45** (1.56 g, 3.24 mmol) was dissolved in toluene (35 mL). Before the addition of AIBN (185 mg, 1.13 mmol) and tri-*n*-butylstannane (3.04

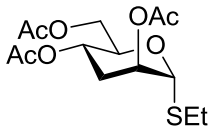
mL, 11.3 mmol), the solution was sparged with argon. The mixture was then heated at reflux overnight. After cooling, evaporation of the solvent and chromatography (hexane–EtOAc 8:1) of the residue afforded **2.46** (658 mg, 57%) as a pale yellow syrup. $[\alpha]_D$ = +56.0 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.29 (m, 10H, ArH), 5.58 (s, 1H, O_2C*H*Ph), 4.66 (s, 1H, H-1), 4.65 (d, *J* = 12.0 Hz, 1H, C*H*$_2$Ph), 4.61 (d, *J* = 12.1 Hz, 1H, C*H*$_2$Ph), 4.24 (dd, *J* = 11.0, 10.5 Hz, 1H, H-6a), 4.05–3.98 (m, 1H, H-4), 3.86–3.79 (m, 2H, H-5, H-6b), 3.69 (app dt *J* = 2.9, 1.2 Hz, 1H, H-2), 3.40 (s, 3H, OC*H*$_3$), 2.28–2.20 (app dt, *J* = 13.0, 3.5 Hz, 1H, H-3eq), 1.99 (ddd, *J* = 13.0, 12.0, 3.0 Hz, 1H, H-3ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.9 (Ar), 137.7 (Ar), 129.0 (Ar), 128.5 (Ar), 128.3 (Ar), 127.8 (Ar), 127.7 (Ar), 126.2 (Ar), 102.0 (O_2CHPh), 98.9 (C-1), 75.0 (C-2), 74.1 (C-4), 71.3 (C*H*$_2$Ph), 69.4 (C-6), 65.0 (C-5), 54.9 (O*C*H$_3$), 29.3 (C-3). HRMS (ESI) *m/z* Calcd for $C_{21}H_{24}O_5Na$ $[M+Na]^+$: 379.1516. Found: 379.1514.



1,2,4,6-Tetra-*O*-acetyl-3-deoxy-α-D-*arabino*-hexopyranose (2.47)

Monosaccharide **2.46** (1.78 g, 5 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 30.6 mL). After stirring overnight, the mixture was diluted with CH_2Cl_2 and washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1 → 3:2) to afford **2.47** (600 mg, 36%) as a pale yellow syrup. $[\alpha]_D$ = +24.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz,

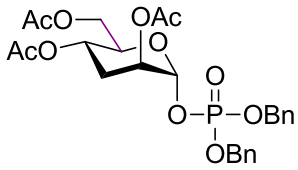
$CDCl_3$) δ 5.98 (s, 1H, H-1), 5.05 (app td, *J* = 11.0, 4.5 Hz, 1H, H-4), 4.92 (app dt, *J* = 3.2, 1.8 Hz, 1H, H-2), 4.24 (dd, *J* = 12.2, 5.3 Hz, 1H, H-6a), 4.12 (dd, *J* = 12.1, 2.4 Hz, 1H, H-6b), 3.98 (ddd, *J* = 10.1, 5.3, 2.4 Hz, 1H, H-5), 2.29 (dddd, *J* = 13.7, 4.5, 3.2, 1.0 Hz, 1H, H-3eq), 2.12 (s, 6H, C(O)CH_3 × 2), 2.07 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 2.01-1.96 (m, 1H, H-3ax). The ^{1}H spectral data was consistent with that previously reported.[20-21] HRMS (ESI) *m/z* Calcd for $C_{14}H_{20}O_9Na$ $[M+Na]^+$: 355.1000. Found: 355.0998.



Ethyl 2,4,6-tri-*O*-acetyl-3-deoxy-1-thio-α-D-*arabino*-hexopyranoside (2.48)

Tetraacetate **2.47** (25 mg, 0.08 mmol) and ethanethiol (7 μL, 0.096 mmol) were dissolved in CH_2Cl_2 (2 mL) and cooled to 0 ℃ before BF_3•OEt_2 (12 μL, 0.096 mmol) was added. The reaction mixture was stirred overnight and then diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 4:1 → 1:1) to afford **2.48** (15 mg, 56%) as a pale yellow syrup. $[\alpha]_D$ = +129.4 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.25 (s, 1H, H-1), 5.11–4.98 (m, 2H, H-2, H-4), 4.36 (ddd, *J* = 9.9, 5.6, 1.8 Hz, 1H, H-5), 4.30 (dd, *J* = 12.0, 6.0 Hz, 1H, H-6a), 4.16 (dd, *J* = 11.7, 1.7 Hz, 1H, H-6b), 2.74–2.58 (m, 2H, SC$H_2$$CH_3$), 2.25 (dddd, *J* = 13.5, 5.6, 1.8 Hz, 1H, H-3eq), 2.14 (s, 3H, C(O)CH_3), 2.10 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 1.95 (ddd, *J* = 13.9, 5.0, 3.2, 1.7 Hz, 1H, H-3ax), 1.32 (t, *J* = 7.4 Hz,

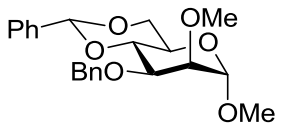
3H, SCH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.1 (*C*=O), 169.8 (*C*=O), 82.2 (C-1), 71.0 (C-2), 68.7 (C-4), 64.7 (C-5), 63.0 (C-6), 30.3 (C-3), 25.2 (SCH_2CH_3), 21.2 ($C(O)CH_3$), 21.0 ($C(O)CH_3$), 20.8 ($C(O)CH_3$), 15.0 (SCH_2CH_3). HRMS (ESI) *m/z* Calcd for $C_{14}H_{22}O_7SNa$ $[M+Na]^+$: 357.0978. Found: 357.098.



Dibenzyl 2,4,6-tri-*O*-acetyl-3-deoxy-α-D-*arabino*-hexopyranosyl phosphate (2.49)

Thioglycoside **2.48** (95 mg, 0.28 mmol), dibenzyl phosphate (132 mg, 0.47 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (3 mL) and stirred for 30 min. To this was added *N*–iodosuccinimide (94.5 mg, 0.42 mmol) and silver trifluoromethanesulfonate (22 mg, 0.08 mmol) at −40 ℃. The reaction mixture was stirred for an additional 3 h at −40 ℃ to −30 ℃, and then filtered through Celite and diluted with CH_2Cl_2. The resulting solution was washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to yield **2.49** (123 mg, 80%) as a colorless syrup. $[\alpha]_D$ = +37.8 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.30 (m, 10H, ArH), 5.55 (d, *J* = 6.3 Hz, 1H, H-1), 5.13–5.03 (m, 4H, CH_2Ph), 5.03–4.96 (m, 1H, H-4), 4.85–4.83 (m, 1H, H-2), 4.18 (dd, *J* = 12.1, 4.9 Hz, 1H, H-6a), 4.03 (ddd, *J* = 10.2, 4.9, 2.3 Hz, 1H, H-5), 3.99 (dd, *J* = 12.1, 2.3 Hz, 1H, H-6b), 2.20 (app dt, *J* = 13.8, 3.6 Hz, 1H, H-3eq), 2.10 (s, 3H, $C(O)CH_3$),

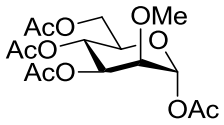
2.04 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.89 (ddd, J = 14.1, 11.4, 3.0, Hz, 1H, H-3ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.6 (*C*=O), 169.6 (*C*=O), 135.5 (d, $^3J_{C,P}$ = 3.9 Hz, 1C, Ar), 135.4 (d, $^3J_{C,P}$ = 3.4 Hz, 1C, Ar), 128.7(3) (Ar), 128.7(1) (Ar), 128.6(5) (Ar), 128.1 (Ar), 128.0 (Ar), 94.2 (d, $^2J_{C,P}$ = 5.8 Hz, 1C, C-1, $^1J_{C,H}$ = 182.7 Hz), 70.2 (C-5), 69.8 (d, $^2J_{C,P}$ = 5.3 Hz, 1C, *C*H_2Ph), 69.7 (d, $^2J_{C,P}$ = 5.3 Hz, 1C, *C*H_2Ph), 68.7 (d, $^3J_{C,P}$ = 11.4 Hz, 1C, C-2), 63.5 (C-4), 62.4 (C-6), 28.4 (C-3), 20.9 (C(O)*C*H_3), 20.7 (C(O)*C*H_3). ^{31}P NMR (201.64 MHz, $CDCl_3$) δ −1.88. HRMS (ESI) *m/z* Calcd for $C_{26}H_{31}O_{11}PNa$ $[M+Na]^+$: 573.1496. Found: 573.1499.



Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranoside (2.51)

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside **2.50**[19] (1.43 g, 3.8 mmol) was dissolved in DMF (15 mL) and the solution was cooled to 0 ℃. After adding NaH (60% NaH in mineral oil, 274 mg, 6.9 mmol), the mixture was stirred for 10 min before the addition of CH_3I (0.36 mL, 5.76 mmol). The reaction mixture was stirred overnight and then quenched by the addition of MeOH (1 mL). The solution was diluted with CH_2Cl_2, washed with distilled water and brine, and the organic layer was dried over Na_2SO_4. Evaporation of the solvent and chromatography (hexane–EtOAc 5:1) of the resulting residue gave **2.51** (1.17 g, 80%) as a colorless syrup. $[\alpha]_D$ = +55.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz,

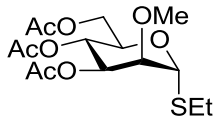
$CDCl_3$) δ 7.60–7.26 (m, 10H, ArH), 5.67 (s, 1H, O_2CHPh), 4.92 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.80 (br. s, 1H, H-1), 4.77 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.31 (dd, *J* = 10.0, 4.3 Hz, 1H, H-6a), 4.22 (app t, *J* = 9.5 Hz, 1H, H-4), 4.00 (dd, *J* = 9.9, 2.7 Hz, 1H, H-3), 3.92 (app t, *J* = 10.5 Hz, 1H, H-6b), 3.85–3.81 (m, 1H, H-5), 3.65–3.64 (m, 1H, H-2), 3.61 (s, 1H, OCH_3), 3.39 (s, 1H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7 (Ar), 137.7 (Ar), 128.8 (Ar), 128.3 (Ar), 128.2 (Ar), 127.6 (Ar), 127.5 (Ar), 126.1 (Ar), 101.5 (O_2CHPh), 100.1 (C-1), 79.5 (C-2), 79.2 (C-4), 76.2 (C-3), 73.1 (CH_2Ph), 68.9 (C-6), 64.2 (C-5), 60.1 (OCH_3), 54.9 (OCH3). HRMS (ESI) *m/z* Calcd for $C_{22}H_{26}O_6Na$ $[M+Na]^+$: 409.1622. Found: 409.1616.



1,3,4,6-Tetra-*O*-acetyl-2-*O*-methyl-α-D-mannopyranose (2.52)

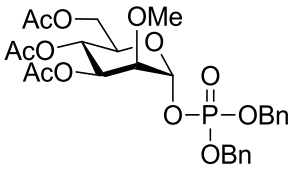
Monosaccharide **2.51** (1.17 g, 3 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 18.36 mL). After stirring overnight the mixture was diluted with CH_2Cl_2, washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue purified by chromatography (hexane–EtOAc 3:2) to afford **2.52** (894 mg, 81%) as a pale yellow syrup. $[\alpha]_D$ = +56.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.04 (d, *J* = 1.9 Hz, 1H, H-1), 5.23 (app t, *J* = 10.1 Hz, 1H, H-4), 5.08 (dd, *J* = 10.1, 3.3 Hz, 1H, H-3), 4.12 (dd, *J* = 12.4, 4.7 Hz, 1H, H-6a), 3.92 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6b), 3.87 (ddd, *J* = 10.0, 4.6, 2.3 Hz, 1H, H-5), 3.50 (dd, *J* = 3.2, 2.1 Hz, 1H, H-2), 3.36 (s, 3H, OCH_3), 2.01 (s, 3H, $C(O)CH_3$), 1.95 (s, 3H, $C(O)CH_3$),

1.93 (s, 3H, C(O)CH_3), 1.90 (s, 3H, C(O)CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 90.5 (C-1), 76.8 (C-2), 70.7 (C-3, C-5), 65.6 (C-4), 62.0 (C-6), 59.3 (O*C*H_3), 20.7 (C(O)*C*H_3), 20.6 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 20.4 (C(O)*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{15}H_{22}O_{10}Na$ $[M+Na]^+$: 385.1105. Found: 385.1099.



Ethyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl-1-thio-α-D-mannopyranoside (2.53)

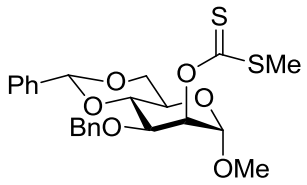
Tetraacetate **2.52** (888 mg, 2.45 mmol) and ethanethiol (272 μL, 3.68 mmol) were dissolved in CH_2Cl_2 (12 mL) and cooled to 0 ℃ before BF_3•OEt_2 (615 μL, 4.9 mmol) was added. The reaction mixture was stirred for 18 h and then diluted with CH_2Cl_2, washed with $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:1 → 2:1) to give **2.53** (583 mg, 65%) a pale yellow syrup. $[\alpha]_D$ = +85.7 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1H, H-1), 5.29–5.18 (m, 1H, H-4), 5.07 (dd, *J* = 9.8, 3.1 Hz, 1H, H-3), 4.29–4.14 (m, 2H, H-5, H-6a), 3.98 (d, *J* = 10.3 Hz, 1H, H-6b), 3.65 (d, *J* = 1.2 Hz, 1H, H-2), 3.37 (s, 3H, OCH_3), 2.69–2.45 (m, 2H, SC$H_2$$CH_3$), 1.97 (d, *J* = 13.8 Hz, 9H, C(O)CH_3 × 3), 1.24 (t, *J* = 7.4 Hz, 3H, SCH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 81.3 (C-1), 79.3 (C-2), 71.6 (C-3), 68.8 (C-5), 66.5 (C-4), 62.4 (C-6), 58.6 (O*C*H_3), 25.3 (S*C*$H_2$$CH_3$), 20.7 (C(O)*C*$H_3$), 20.6 (C(O)*C*$H_3$), 20.6 (C(O)*C*$H_3$), 14.8 ($SCH_2$*C*$H_3$). HRMS (ESI) *m/z* Calcd for $C_{15}H_{24}O_8SNa$ $[M+Na]^+$: 387.1084. Found: 387.1081.



Dibenzyl 3,4,6-tri-*O*-acetyl-2-*O*-methyl-α-D-mannopyranosyl phosphate (2.54)

Thioglycoside **2.53** (547 mg, 1.5 mmol), dibenzyl phosphate (709 mg, 2.55 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (14 mL) and stirred for 40 min. *N*–iodosuccinimide (507 mg, 2.25 mmol) and silver trifluoromethanesulfonate (116 mg, 0.45 mmol) were then added at −30 ℃. The reaction mixture was stirred for 40 min from −30 ℃ to −20 ℃, and then filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4 then concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1) to yield **2.54** (729 mg, 84%) as a colorless syrup. $[\alpha]_D$ = +38.4 (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.31 (m, 10H, ArH), 5.68 (dd, *J* = 6.4, 2.0 Hz, 1H, H-1), 5.31 (app t, *J* = 10.2 Hz, 1H, H-4), 5.18 (dd, *J* = 10.1, 3.2 Hz, 1H, H-3), 5.13–5.03 (m, 4H, C*H*$_2$Ph), 4.16 (dd, *J* = 12.4, 4.6 Hz, 1H, H-6a), 3.96 (ddd, *J* = 10.1, 4.6, 2.1 Hz, 1H, H-5), 3.90 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6b), 3.50 (dd, *J* = 3.1, 2.2 Hz, 1H, H-2), 3.37 (s, 3H, OC*H*$_3$), 2.06 (s, 3H, C(O)C*H*$_3$), 2.00 (s, 3H, C(O)C*H*$_3$), 1.96 (s, 3H, C(O)C*H*$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.0 (*C*=O), 169.4 (*C*=O), 135.4(1) (d, $^3J_{C,P}$ = 6.6 Hz, 1C, Ar), 135.3(5) (d, $^3J_{C,P}$ = 6.6 Hz, 1C, Ar), 128.8 (Ar), 128.6(9) (Ar), 128.6(8) (Ar), 128.2 (Ar), 128.0 (Ar), 95.2 (d, $^2J_{C,P}$ = 6.0 Hz, 1C, C-1, $^1J_{C,H}$ = 177.7 Hz), 77.5 (d, $^3J_{C,P}$ = 9.5 Hz, 1C, C-2), 70.4(0) (C-5), 70.3(7) (C-3), 69.8 (d,

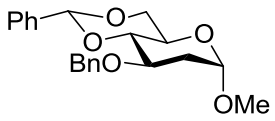
$^{2}J_{C,P}$ = 5.3 Hz, 1C, *C*H_2Ph), 69.7 (d, $^{2}J_{C,P}$ = 5.3 Hz, 1C, *C*H_2Ph), 65.5 (C-4), 61.9 (C-6), 59.7 (O*C*H_3), 20.8 (C(O)*C*H_3), 20.6 (C(O)*C*H_3). ^{31}P NMR (201.64 MHz, $CDCl_3$) δ −1.80. HRMS (ESI) *m/z* Calcd for $C_{27}H_{33}O_{12}PNa$ $[M+Na]^+$: 603.16019. Found: 603.16003.



Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(methylthio)thiocarbonyl-α-D-mannopyranoside (2.55)

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[19] **2.50** (372 mg, 1.0 mmol) was dissolved in dry THF (10 mL), and then NaH (60% NaH in mineral oil, 120 mg, 3 mmol) and imidazole (34 mg, 0.5 mmol) were added to the solution. The mixture was stirred for 1 h before the addition of carbon disulfide (0.6 mL). After the reaction mixture was stirred for 1 h, methyl iodide (0.31 mL, 5 mmol) was added and the mixture was stirred overnight. The solvent was evaporated and the residue was diluted with CH_2Cl_2, before being washed with distilled water. The organic layer was dried over Na_2SO_4. Concentration of the organic layer and chromatography (hexane–EtOAc 7:1) of the residue afforded **2.55** (344 mg, 74%) as a yellow syrup. $[\alpha]_D$ = −6.7 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53–7.23 (m, 10H, ArH), 6.16–6.15 (br s, 1H, H-2), 5.66 (s, 1H, PhC*H*O_2), 4.85 (d, *J* = 1.5 Hz, 1H, H-1), 4.71 (d, *J* = 12.5 Hz, 1H, C*H*$_2$Ph), 4.68 (d, *J* = 12.5 Hz, 1H, C*H*$_2$Ph), 4.29 (dd, *J* = 11.0, 10.5 Hz, 1H, H-6a), 4.15–4.06 (m, 2H, H-3, H-4),

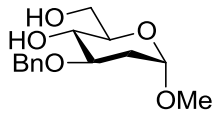
3.92–3.84 (m, 2H, H-5, H-6b), 3.39 (s, 3H, OCH_3), 2.62 (s, 3H, SCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.2 (C=S), 137.9 (Ar), 137.4 (Ar), 128.9 (Ar), 128.3 (Ar), 128.2 (Ar), 127.6 (Ar), 126.1 (Ar), 101.79 (Ph*C*HO_2), 98.8 (C-1), 78.8 (C-4), 78.0 (C-2), 73.9 (C-3), 72.3 (*C*H_2Ph), 68.8 (C-6), 63.8 (C-5), 55.2 (OCH_3), 19.2 (S*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{23}H_{26}O_6S_2Na$ $[M+Na]^+$: 485.1063. Found: 485.1057.



Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-*arabino*-hexopyranoside (2.56)

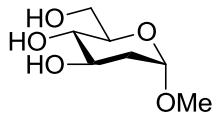
Xanthate **2.55** (172 mg, 0.37 mmol) was dissolved in toluene (10 mL). Before the addition of AIBN (36.5 mg, 0.22 mmol) and tri-*n*-butylstannane (0.3 mL, 1.1 mmol), the solution was sparged with argon. The mixture was then heated at reflux overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 8:1 → 6:1) of the residue afforded **2.56** (118 mg, 89%) as a pale yellow syrup. $[\alpha]_D$ = +67.9 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, cdcl$_3$) δ 7.55–7.25 (m, 10H, ArH), 5.64 (s, 1H, O_2C*H*Ph), 4.85 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.82 (d, *J* = 3.2 Hz, 1H, H-1), 4.70 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.31–4.24 (m, 1H, H-6a), 4.04 (ddd, *J* = 11.2, 9.0, 5.2 Hz, 1H, H-3), 3.86–3.77 (m, 2H, H-5, H-6b), 3.71 (app t, *J* = 9.0 Hz, 1H, H-4), 3.35 (s, 3H, OCH_3), 2.29 (ddd, *J* = 13.4, 5.2, 1.0 Hz, 1H, H-2eq), 1.82 (ddd, *J* = 13.4, 11.2, 3.8 Hz, 1H, H-2ax). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.8 (Ar), 137.7 (Ar), 128.9 (Ar), 128.4 (Ar), 128.2 (Ar), 127.7 (Ar), 127.5

(Ar), 126.1 (Ar), 101.4 (Ph*C*HO_2), 99.1 (C-1), 83.9 (C-4), 72.9(1) (*C*H_2Ph), 72.8(9) (C-3), 69.2 (C-6), 62.9 (C-5), 54.7 (O*C*H_3), 36.5 (C-2). HRMS (ESI) *m/z* Calcd for $C_{21}H_{24}O_5Na$ $[M+Na]^+$: 379.1516. Found: 379.1509.



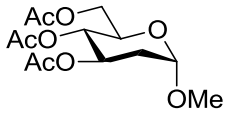
Methyl 3-*O*-benzyl-2-deoxy-α-D-*arabino*-hexopyranoside (2.57)

Monosaccharide **2.56** (48.4 mg, 0.1 mmol) was dissolved in CH_3OH (6 mL) before I_2 (25 mg, 0.1 mmol) was added. The reaction mixture was heated at reflux overnight. Evaporation of the solvent and chromatography (hexane–EtOAc 1:1 → 2:3) of the residue afforded **2.57** (15 mg, 56%) as a colorless syrup. $[\alpha]_D$ = +51.3 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.28 (m, 10H, ArH), 4.83 (d, *J* = 2.7 Hz, 1H, H-1), 4.67 (d, *J* = 11.6 Hz, 1H, *C*H_2Ph), 4.50 (d, *J* = 11.6 Hz, 1H, *C*H_2Ph), 3.87 (dd, *J* = 11.6, 3.7 Hz, 1H, H-6a), 3.81 (dd, *J* = 11.6, 4.7 Hz, 1H, H-6b), 3.79 (dd, *J* = 11.4, 4.9 Hz, 1H, H-3), 3.66–3.61 (m, 1H, H-5), 3.59–3.53 (app t, *J* = 9.3 Hz, 1H, H-4), 3.34 (s, 3H, OC*H*$_3$), 2.29 (ddd, *J* = 12.9, 4.9, 1.3 Hz, 1H, H-2eq), 1.61 (ddd, *J* = 12.9, 11.4, 3.6 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3 (Ar), 128.6 (Ar), 127.9 (Ar), 127.7 (Ar), 98.7 (C-1), 76.8 (C-3), 71.6 (C-4), 71.2(3) (*C*H_2Ph), 71.1(7) (C-5), 62.9 (C-6), 54.7 (O*C*H_3), 34.6 (C-2). HRMS (ESI) *m/z* Calcd for $C_{14}H_{20}O_5Na$ $[M+Na]^+$: 291.1203. Found: 291.1200.



Methyl 2-deoxy-α-D-*arabino*-hexopyranoside (2.58)

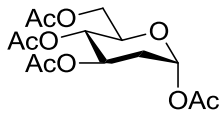
Monosaccharide **2.57** (15 mg, 0.06 mmol) was dissolved in CH_3OH–EtOAc (1:1 v/v, 2 mL) before 20% $Pd(OH)_2$–C (8 mg) was added. The mixture was stirred overnight under a hydrogen atmosphere. The catalyst was then removed by filtration through Celite and the filtrate was concentrated. The crude residue was purified by chromatography (CH_2Cl_2–CH_3OH 5:1) to afford **2.58** (9 mg, 91%) as a colorless syrup. $[\alpha]_D$ = +96.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.78 (d, *J* = 2.8 Hz, 1H, H-1), 4.29 (br. s, 3H, O*H* × 3), 3.96–3.84 (m, 2H, H-3, H-6a), 3.79 (d, *J* = 11.9 Hz, 1H, H-6b), 3.55–3.42 (m, 2H, H-4, H-5), 3.31 (s, 3H, OC*H*$_3$), 2.12 (app dd, *J* = 13.0, 5.0 Hz, 1H, H-2eq), 1.67 (ddd, *J* = 13.4, 11.5, 4.0 Hz, 1H, H-2ax). ^{13}C NMR (126 MHz, $CDCl_3$) δ 98.7 (C-1), 71.9, 71.5 (C-4, C-5), 68.2 (C-3), 61.8 (C-6), 54.8 (O*C*H$_3$), 37.3 (C-2). HRMS (ESI) *m/z* Calcd for $C_7H_{14}O_5Na$ $[M+Na]^+$: 201.0733. Found: 201.0734.



Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (2.59)

Monosaccharide **2.58** (101 mg, 0.57 mmol) was dissolved in pyridine (5 mL) before Ac_2O (0.3 mL, 3.17 mmol) was added dropwise. The reaction mixture was stirred overnight. The solvent was then removed by evaporation and the resulting mixture was diluted with CH_2Cl_2, washed with distilled water, 1 M HCl, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 3:2) to

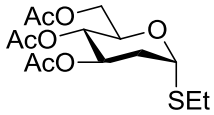
give **2.59** (137 mg, 80%) as a clear syrup. $[\alpha]_D$ = +115.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.30 (ddd, *J* = 11.6, 9.5, 5.4 Hz, 1H, H-3), 5.00 (app t, *J* = 9.8 Hz, 1H, H-4), 4.84 (d, *J* = 3.3 Hz, 1H, H-1), 4.30 (dd, *J* = 12.2, 4.7 Hz, 1H, H-6a), 4.07 (dd, *J* = 12.2, 2.3 Hz, 1H, H-6b), 3.94 (ddd, *J* = 10.1, 4.7, 2.3 Hz, 1H, H-5), 3.35 (s, 3H, OC*H*$_3$), 2.25 (ddd, *J* = 12.8, 5.4, 1.0 Hz, 1H, H-2eq), 2.09 (s, 3H, C(O)C*H*$_3$), 2.04 (s, 3H, C(O)C*H*$_3$), 2.01 (s, 3H, C(O)C*H*$_3$), 1.82 (ddd, *J* = 12.5, 11.9, 3.6 Hz, 1H, H-2ax). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2 (*C*=O), 169.9 (*C*=O), 169.5 (*C*=O), 98.1 (C-1), 69.3 (C-4), 69.1 (C-3), 67.7 (C-5), 62.4 (C-6), 54.9 (O*C*H$_3$), 34.9 (C-2), 21.0 (C(O)*C*H$_3$), 20.8 (C(O)*C*H$_3$), 20.7 (C(O)*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{13}H_{20}O_8Na$ $[M+Na]^+$: 327.1050. Found: 327.1047.



1,3,4,6-Tetra-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranose (2.60)

Monosaccharide **2.59** (238 mg, 0.78 mmol) was dissolved in Ac_2O–HOAc–H_2SO_4 (35:15:1 v/v/v, 4 mL). After stirring overnight, the mixture was diluted with CH_2Cl_2, washed with distilled water, $NaHCO_3$ (satd aq soln) and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue purified by chromatography (hexane–EtOAc 1:1) to afford **2.60** (236 mg, 91%) as a clear syrup. $[\alpha]_D$ = +90.4 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.25 (dd, *J* = 3.5, 1.3 Hz, 1H, H-1), 5.31 (ddd, *J* = 11.7, 9.6, 5.3 Hz, 1H, H-3), 5.07 (app t, *J* = 9.7 Hz, 1H, H-4), 4.30 (dd, *J* = 12.9, 4.7 Hz, 1H, H-6a), 4.07–4.02 (m, 2H, H-6b, H-5), 2.26 (ddd, *J* = 13.5, 5.3, 1.5 Hz, 1H, H-2eq), 2.12 (s, 3H, C(O)C*H*$_3$), 2.07 (s,

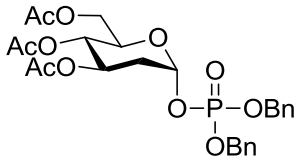
3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.95 (ddd, *J* = 13.6, 11.7, 3.7 Hz, 1H, H-2ax). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.2 (*C*=O), 169.7 (*C*=O), 168.9 (*C*=O), 90.9 (C-1, $^1J_{C,H}$ = 178.3 Hz), 70.2 (C-5), 68.7 (C-4), 68.4 (C-3), 61.9 (C-6), 33.9 (C-2), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.7(1) (C(O)*C*H_3), 20.6(6) (C(O)*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{14}H_{20}O_9Na$ $[M+Na]^+$: 355.1000. Found: 355.0994.



Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-*arabino*-hexopyranoside (2.61)

Monosaccharide **2.60** (35 mg, 0.1 mmol), ethanethiol (15 μL, 0.12 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (2 mL) and cooled to 0 ℃ before the addition of BF_3•OEt_2 (9 μL, 0.12 mmol). The reaction mixture was stirred for 3 h, then filtered through Celite and diluted with CH_2Cl_2. The resulting solution was washed with $NaHCO_3$ (satd aq soln), distilled water, and brine. The organic layer was dried over Na_2SO_4, concentrated and the crude residue was purified by chromatography (hexane–EtOAc 2:1) to afford **2.61** (18 mg, 52%) as a pale yellow syrup. $[\alpha]_D$ = +166.4 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (d, *J* = 5.5 Hz, 1H, H-1), 5.24 (ddd, *J* = 11.6, 9.3, 5.4 Hz, 1H, H-3), 4.98 (app t, *J* = 9.6 Hz, 1H, H-4), 4.39 (ddd, *J* = 9.9, 4.8, 2.2 Hz, 1H, H-5), 4.34 (dd, *J* = 12.2, 4.8 Hz, 1H, H-6a), 4.04 (dd, *J* = 12.2, 2.2 Hz, 1H, H-6b), 2.68–2.48 (m, 2H, SCH_2), 2.27 (ddd, *J* = 13.3, 5.4, 1.2 Hz, 1H, H-2eq), 2.16 (ddd, *J* = 13.4, 11.7, 5.9 Hz, 1H, H-2ax), 2.08 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3),

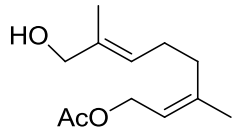
2.00 (s, 3H, C(O)CH_3), 1.28 (t, J = 7.4 Hz, 3H, SCH$_2$CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.0 (*C*=O), 169.7 (*C*=O), 79.63 (C-1), 69.6, 69.5 (C-3, C-4), 68.0 (C-5), 62.4 (C-6), 35.2 (C-2), 24.9 (S*C*H$_2$), 20.9 (C(O)*C*H$_3$), 20.7(9) (C(O)*C*H$_3$), 20.7(8) (C(O)*C*H$_3$), 14.7 (SCH$_2$*C*H$_3$). HRMS (ESI) *m/z* Calcd for $C_{14}H_{22}O_7SNa$ [M+Na]$^+$: 357.0978. Found: 357.0972.



Dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranosyl phosphate (2.62)

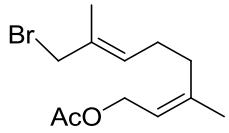
Thioglycoside **2.61** (78 mg, 0.23 mmol), dibenzyl phosphate (110 mg, 0.4 mmol), and powdered 4 Å molecular sieves were dissolved in dry CH_2Cl_2 (6 mL) and stirred for 40 min. *N*–iodosuccinimide (78 mg, 0.35 mmol) and silver trifluoromethanesulfonate (18 mg, 0.07 mmol) were then added at −40 ℃. The reaction mixture was stirred for 1 h at −40 ℃ to −30 ℃, and then filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with $Na_2S_2O_3$ (satd aq soln), $NaHCO_3$ (satd aq soln), distilled water and brine. The organic layer was dried over Na_2SO_4 then concentrated and the crude residue was purified by chromatography (hexane–EtOAc 1:1 with 1% Et_3N) to yield **2.62** (43 mg, 34%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.28 (m, 10H, ArH), 5.84–5.81 (m, 1H, H-1), 5.27 (ddd, J = 11.6, 9.6, 5.3 Hz, 1H, H-3), 5.13–5.04 (m, 4H, CH_2Ph × 2), 5.02 (app t, J = 9.9 Hz, 1H, H-4), 4.20 (dd, J = 12.5, 4.0 Hz, 1H, H-6a), 4.04 (ddd, J = 10.2, 3.8, 2.1 Hz, 1H, H-5), 3.86 (dd, J = 12.5, 2.2 Hz, 1H,

H-6b), 2.23 (ddd, *J* = 13.5, 5.3, 1.4 Hz, 1H, H-2eq), 2.03 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 2.00 (s, 3H, C(O)CH_3), 1.84 (ddd, *J* = 13.5, 11.6, 3.4 Hz, 1H, H-2ax). ^{31}P NMR (201.64 MHz, $CDCl_3$) δ −2.78. HRMS (ESI) *m/z* Calcd for $C_{26}H_{31}O_{11}PNa$ $[M+Na]^+$: 573.1496. Found: 573.1493.



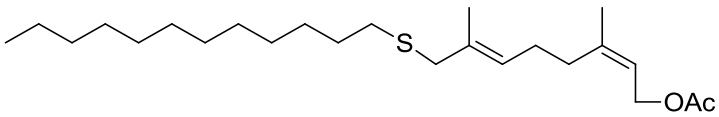
Acetic acid (2*Z*,6*E*)-8-hydroxy-3,7-dimethyl-octa-2,6-dienyl ester (2.64)

To a solution of SeO_2 (160 mg, 1.39 mmol) and salicylic acid (192 mg, 1.39 mmol) in dry CH_2Cl_2 (24 mL) was added anhydrous *t*-butyl hydroperoxide (6.25 mL, 50 mmol) and neryl acetate **2.63** (3 mL, 13.9 mmol). The reaction mixture was stirred overnight and then was quenched by the addition of $Na_2S_2O_3$ (satd aq soln), and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated to give a residue that was purified by chromatography (hexane–EtOAc 6:1) to provide **2.64** (1.8 g, 61%) as a pale yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (br. s, 1H, C=C*H*CH_2CH_2), 5.36 (t, *J* = 7.2 Hz, 1H, $AcOCH_2CH$=C), 4.57 (d, *J* = 7.2 Hz, 2H, AcOCH_2), 3.98 (s, 2H, HOCH_2), 2.16 (s, 2H, CH_2), 2.15 (s, 2H, CH_2), 2.05 (s, 3H, C(O)CH_3), 1.77 (s, 3H, CH_3), 1.66 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2 (*C*=O), 141.9 (CH=*C*), 135.6 (CH=*C*), 124.8 (*C*H=C), 119.6 ($AcOCH_2$*C*H=C), 68.7 (HO*C*H_2), 61.2 (AcO*C*H_2), 31.8 (*C*H_2), 26.0 (*C*H_2), 23.4 (C(O)*C*H_3), 21.0 (*C*H_3), 13.6 (*C*H_3). HRMS (ESI) *m/z* Calcd for $C_{24}H_{41}O_6$ $[2M+H]^+$: 425.2898. Found: 425.2898. The ^{1}H and ^{13}C NMR spectral data were consistent with those previously reported.[9]



Acetic acid (2*Z*,6*E*)-8-bromo-3,7-dimethyl-octa-2,6-dienyl ester (2.65)

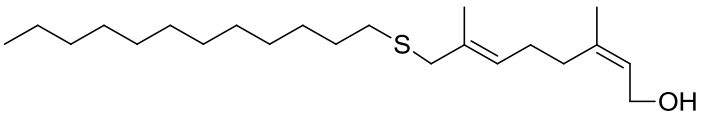
To a solution of **2.64** (165 mg, 0.77 mmol) in dry CH_2Cl_2 (20 mL) at 0 ℃ was added 2,6-lutidine (0.09 mL, 0.77 mmol), tetrabromomethane (510 mg, 1.54 mmol) and triphenylphosphine (404 mg, 1.54 mmol). The mixture was stirred at 0 ℃ for 2 h. The solvent was then evaporated and the residue was purified by chromatography (hexane–EtOAc 9:1 → 6:1) to provide **2.65** (176 mg, 83%) as a a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.58 (br. s, 1H, C=C*H*CH_2CH_2), 5.38 (t, *J* = 7.8 Hz, 1H, AcOCH_2C*H*=C), 4.56 (d, *J* = 8.0 Hz, 2H, AcO*C*H_2), 3.96 (s, 2H, Br*C*H_2), 2.16 (br. s, 4H, CH_2 × 2), 2.05 (s, 3H, C(O)CH_3), 1.76 (s, 6H, CH_3 × 2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0 (*C*=O), 141.7 (CH=*C*), 132.7 (CH=*C*), 130.2 (*C*H=C), 119.8 (AcOCH_2*C*H=C), 60.9 (AcO*C*H_2), 41.4 (Br*C*H_2), 31.3 (*C*H_2), 26.8 (*C*H_2), 23.4 (C(O)*C*H_3), 21.1 (*C*H_3), 14.7 (*C*H_3). The ^{1}H and ^{13}C NMR spectral data were consistent with those previously reported.[9]



(2Z,6E)-8-(Dodecylthio)-3,7-dimethylocta-2,6-dien-1-yl acetate (2.66)

To a solution of bromide **2.65** (74 mg, 0.27 mmol) in dry THF (3 mL) was added NaH (60% NaH in mineral oil, 21 mg, 0.53 mmol) and 1-Dodecanethiol **2.69** (0.1 mL, 0.42 mmol) at 0 ℃. The reaction mixture was stirred overnight and then

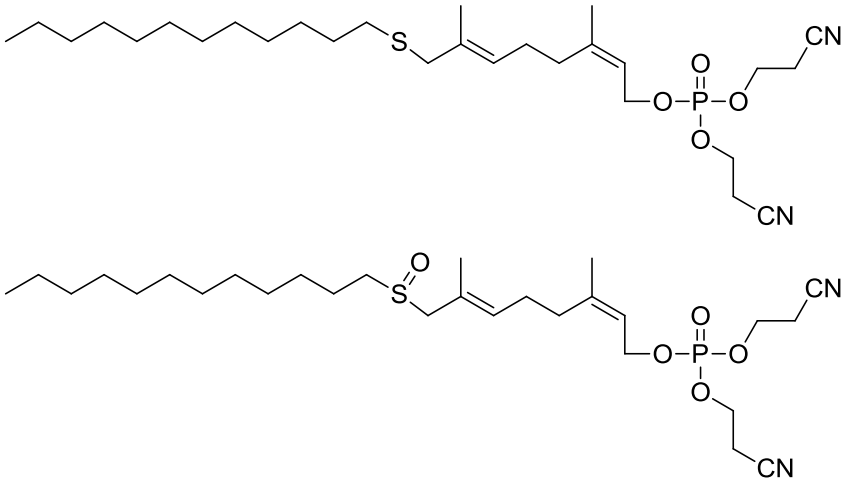
MeOH (1 mL) was added. The solution was diluted with CH_2Cl_2, washed with distilled water and brine. The organic layer was dried over Na_2SO_4, concentrated and the residue was purified by chromatography (hexane–EtOAc 10:1 → 8:1) to provide **2.66** (62 mg, 58%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (t, *J* = 7.2 Hz, 1H, AcOCH$_2$C*H*=C), 5.24 (br. s, 1H, C=C*H*CH$_2$CH$_2$), 4.58 (d, *J* = 7.2 Hz, 2H, AcOC*H*$_2$), 3.09 (s, 2H, SC*H*$_2$), 2.38 (t, *J* = 7.3 Hz, 2H, SC*H*$_2$), 2.16 (d, *J* = 3.2 Hz, 4H, C*H*$_2$ × 2), 2.06 (s, 3H, C(O)C*H*$_3$), 1.78 (s, 3H, C*H*$_3$), 1.72 (s, 3H, C*H*$_3$), 1.55 (dt, *J* = 14.5, 7.2 Hz, 2H, SCH$_2$C*H*$_2$), 1.27 (s, 18H, C*H*$_2$ × 9), 0.89 (t, *J* = 7.0 Hz, 3H, C*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0 (*C*=O), 142.2 (CH=*C*), 132.0 (CH=*C*), 126.9 (*C*H=C), 119.4 (AcOCH$_2$*C*H=C), 61.0 (AcO*C*H$_2$), 41.4 (S*C*H$_2$), 31.9 (S*C*H$_2$), 30.9 (*C*H$_2$), 29.6(8) (*C*H$_2$), 29.6(5) (*C*H$_2$), 29.5(8) (*C*H$_2$), 29.3(9) (*C*H$_2$), 29.3(6) (*C*H$_2$), 29.3(2) (*C*H$_2$), 29.2(7) (*C*H$_2$), 29.0 (*C*H$_2$), 26.7 (*C*H$_2$), 23.4 (*C*H$_3$), 22.7 (*C*H$_2$), 21.1 (C(O)*C*H$_3$), 14.9 (*C*H$_3$), 14.1 (*C*H$_3$).



(2Z,6E)-8-(Dodecylthio)-3,7-dimethylocta-2,6-dien-1-ol (2.67)

Acetate **2.66** (62 mg, 0.156 mmol) was dissolved in methanol. 1.0 M NaOMe was added to the solution until a pH of 9 was achieved. After stirring for 1 h, the reaction was neutralized with Amberlite IR120 H^+ ion exchange resin and then concentrated. The crude residue was purified by chromatography (hexane–EtOAc 8:1) to afford **2.67** (42 mg, 76%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.45 (t, *J* = 6.9 Hz, 1H, HOCH$_2$C*H*=C), 5.25 (br. s, 1H, C=C*H*CH$_2$CH$_2$), 4.12 (d,

J = 6.8 Hz, 2H, HOCH_2), 3.09 (s, 2H, SCH_2), 2.39 (t, *J* = 7.3 Hz, 2H, SCH_2), 2.14 (s, 4H, CH_2 × 2), 1.76 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 1.55 (dt, *J* = 14.5, 7.3 Hz, 2H, SCH_2CH_2), 1.41–1.33 (m, 2H, CH_2), 1.27 (s, 16H, CH_2 × 8), 0.89 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.3 (CH=*C*), 132.0 (CH=*C*), 127.0 (*C*H=C), 124.8 ($HOCH_2$*C*H=C), 59.0 (HOCH_2), 41.3 (SCH_2), 31.9 (SCH_2), 31.8 (*C*H_2), 31.0 (*C*H_2), 29.7 (*C*H_2), 29.6(8) (*C*H_2), 29.6(5) (*C*H_2), 29.5(7) (*C*H_2), 29.4(0) (*C*H_2), 29.3(6) (*C*H_2), 29.3 (*C*H_2), 29.0 (*C*H_2), 26.6 (*C*H_2), 23.4 (*C*H_3), 22.7 (*C*H_2), 15.0 (*C*H_3), 14.1 (*C*H_3).



Bis(2-cyanoethyl) ((2Z,6E)-8-(dodecylsulfinyl)-3,7-dimethylocta-2,6-dien-1-yl) phosphate

Bis(2-cyanoethyl) ((2Z,6E)-8-(dodecylthio)-3,7-dimethylocta-2,6-dien-1-yl) phosphate (2.70)

1-*H*-Tetrazole (58.6 mg, 0.84 mmol) was added to a stirred solution of prenol **2.67** (81.6 mg, 0.23 mmol) and bis(2-cyanoethyl)-*N*,*N*-di-isopropyl phosphoramidite **2.68** (75.6 mg, 0.28 mmol) in dry THF (3 mL) under argon. The reaction mixture

was stirred for 100 min before dry acetonitrile (3 mL) was added. The mixture was stirred an additional 100 min and then cooled to 0 ℃. H_2O_2 (39 μL, 0.29 mmol) was added to the solution at 0 ℃ and after 40 min the mixture was diluted with EtOAc, washed with a 10% Na_2SO_3 solution, a 5% $NaHCO_3$ solution, distilled water and brine. The organic layer was dried over Na_2SO_4. Concentration of the organic layer afforded a mixture of the thioether and sulfoxide **2.70** (88 mg, 71%) as a pale yellow oil. Data for thioether: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.45 (t, *J* = 7.3 Hz, 1H, $POCH_2CH$=C), 5.24 (br. s, 1H, C=CHCH_2CH_2), 4.65 (t, *J* = 8.1 Hz, 2H, $POCH_2CH$=C), 4.31–4.25 (m, 4H, OCH_2CH_2 × 2), 3.09 (s, 2H, SCH_2), 2.79 (t, *J* = 6.1 Hz, 4H, CH_2CN × 2), 2.38 (t, *J* = 7.3 Hz, 2H, SCH_2), 2.17 (br. s, 4H, CH_2 × 2), 1.81 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 1.63–1.46 (m, 2H, SCH_2CH_2), 1.27 (s, 18H, CH_2 × 9), 0.89 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.1 (CH=*C*), 132.4 (CH=*C*), 126.5 (*C*H=C), 119.1 (d, $^3J_{C,P}$ = 6.2 Hz, 1C, $POCH_2CH$=C), 116.4 (*C*N), 65.0 (d, $^2J_{C,P}$ = 6.0 Hz, 1C, $POCH_2CH$=C), 62.1 (d, $^2J_{C,P}$ = 5.3 Hz, 2C, $POCH_2$ × 2), 41.4 (CH_2), 32.0 (CH_2), 31.1 (CH_2), 29.6(8) (CH_2), 29.6(4) (CH_2), 29.5(7) (CH_2), 29.4(0) (CH_2), 29.3(6) (CH_2), 29.3 (CH_2), 29.0 (CH_2), 26.7 (CH_2), 23.5 (CH_3), 22.7 (CH_2), 19.7 (d, $^3J_{C,P}$ = 7.0 Hz, 2C, CH_2CN × 2), 15.1 (CH_3), 14.1 (CH_3). ^{31}P NMR (161.84 MHz, CD_3Cl_3) δ −1.87. HRMS (ESI) *m/z* Calcd for $C_{28}H_{49}N_2O_4PSNa$ $[M+Na]^+$: 563.3043. Found: 563.3045. HRMS (ESI) *m/z* Calcd for $C_{28}H_{49}N_2O_5PSNa$ $[M+Na]^+$: 579.2992. Found: 579.2985.

2.5. Bibliography

(1) Hindsgaul, O.; Kaur, K. J.; Srivastava, G.; Blaszczyk-Thurin, M.; Crawley, S. C.; Heerze, L. D.; Palcic, M. M. *J. Biol. Chem.* **1991**, *266*, 17858-17862.

(2) Spohr, U.; Hindsgaul, O.; Lemieux, R. U. *Can. J. Chem.* **1985**, *63*, 2644-2652.

(3) Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *249*, 163-195.

(4) Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1994**, *251*, 33-67.

(5) Mukherjee, A.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2000**, *326*, 1-21.

(6) Timmons, S. C.; Jakeman, D. L. *Carbohydr. Res.* **2008**, *343*, 865-874.

(7) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293-297.

(8) Duus, J. Ø.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589-4614.

(9) Tomooka, K.; Komine, N.; Fujiki, D.; Nakai, T.; Yanagitsuru, S.-i. *J. Am. Chem. Soc.* **2005**, *127*, 12182-12183.

(10) Crich, D.; Dudkin, V. *Org. Lett.* **2000**, *2*, 3941-3943.

(11) Elworthy, T. R.; Dunn, J. P.; Hogg, J. H.; Lam, G.; Saito, Y. D.; Silva, T. M. P. C.; Stefanidis, D.; Woroniecki, W.; Zhornisky, E.; Zhou, A. S.; Klumpp, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6344-6347.

(12) Polákov á, M.; Roslund, M. U.; Ekholm, F. S.; Saloranta, T.; Leino, R. *Eur. J. Org. Chem.* **2009**, *2009*, 870-888.

(13) Lemanski, G.; Ziegler, T. *Tetrahedron* **2000**, *56*, 563-579.

(14) Ottosson, H. *Carbohydr. Res.* **1990**, *197*, 101-107.

(15) Rozanas, C. R.; Gray, G. R. *Carbohydr. Res.* **1997**, *298*, 243-249.

(16) Haskins, W. T.; Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1946**, *68*, 628-632.

(17) Sandström, C.; Hakkarainen, B.; Matei, E.; Glinchert, A.; Lahmann, M.; Oscarson, S.; Kenne, L.; Gronenborn, A. M. *Biochemistry (Mosc).* **2008**, *47*, 3625-3635.

(18) Smith, F. *Journal of the Chemical Society (Resumed)* **1951**, 2646-2652.

(19) Tam, P.H.; Lowary, T. L. *Carbohydr. Res.* **2007**, *342*, 1741-1772.

(20) Watt, G. M.; Flitsch, S. L.; Fey, S.; Elling, L.; Kragl, U. *Tetrahedron: Asymmetry* **2000**, *11*, 621-628.

(21) Christian, R.; Schreiner, E.; Zbiral, E.; Schulz, G. *Carbohydr. Res.* **1989**, *194*, 49-61.

Chapter 3

Exploring the substrate specificity of a nucleotidyl transferase and mycobacterial PPM synthase

3.1. Introduction

GDP-Man, the product of GTP and mannose-1-phosphate (Man*p*-1P) catalysed by GDP-mannose pyrophosphorylase (GDP-ManPP)[1], is the donor substrate for many mannosyltransferases (ManTs).[2] AcPIM1–AcPIM3, as well as polyprenol monophosphomannose synthase (PPM synthase), which are indispensable to LAM biosynthesis, all utilize GDP-Man as donor species. A better understanding of how Man*p*-1P interacts with the GDP-ManPP active site can be achieved using chemically synthesized Man*p*-1P derivatives as substrates for GDP-ManPP to test the enzyme's activity.[2]

As discussed in Chapter 1, GDP-Man is used as donor species for PimA, PimB', and PimC mannosyltransferases.[3,4,5] The ManTs involved in the biosynthesis of the more polar PIMs (PIM4–PIM6) and the LM/LAM mannan, require, additionally, a polyprenylphosphomannopyranose (PPM) donor.[6] The addition of a Man*p* residue to the polyprenolphosphate from GDP-Man catalyzed by PPM synthase yields the PPM donor species.[7] Given the important role that the PPM donors play in the LAM biosynthesis, the study of PPM synthase is of significant interest nowadays.

Monosaccharides **2.1**–**2.8** (**Figure 2.1**) were used to map out the steric requirements and hydrogen-bonding interactions in the active site of GDP-ManPP. Details of the synthetic work towards mannose-1-phosphate derivatives have been presented in Chapter 2. Herein, the biochemical evaluation of these analogs as substrates for GDP-ManPP using an established colorimetric assay is discussed.

Additionally, the usage of the synthetic polyprenolphosphate analog **2.9** as substrate for PPM synthase is also reported.

3.2. Results and discussion

A panel of Man*p*-1P analogs (**2.2**–**2.8**), as well as Man*p*-1P (**2.1**) were screened to probe the GDP-ManPP substrate specificity. The GDP-ManPP we used was a related enzyme from *Salmonella enterica*, because the required nucleotidyl transferase from mycobacteria has not been unequivocally identified. In **2.2**–**2.8**, one of the hydroxyl groups of the parent monosaccharide has either been methylated or deoxygenated. After incubation with GDP-ManPP and GTP overnight, the corresponding GDP-Man and GDP-Man analogs were obtained as shown in **Figure 3.1**.

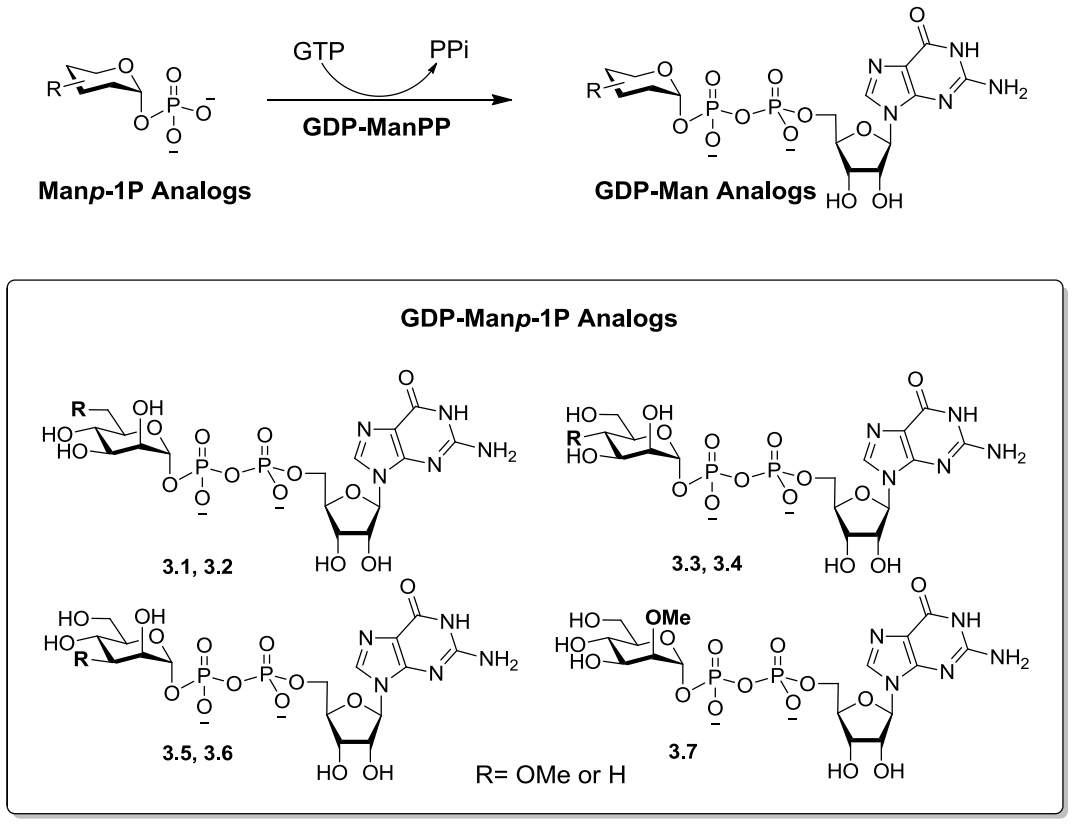


Figure 3.1. Reaction catalyzed by GDP-ManPP using Man*p*-1P analogs

An incubation of **2.1** with GDP-ManPP was used to confirm the activity of the protein. This enzymatic reaction was monitored by HPLC as seen in **Figure 3.2**. The reactions were stopped when the complete consumption of peak at 15 min, corresponding to GTP, was observed. During this time a new peak for GDP-Man*p* was formed at a retention time of ~8.00 min, which was found to elute at the same time as an authentic sample of GDP-Man*p*. The same incubations were carried out for **2.2**–**2.8** and in all cases the corresponding GDP-Man*p* analog peaks could be observed as seen in **Figure 3.2**. However, in the case of **2.4**, **2.5**,

2.7, and **2.8**, a peak corresponding to GDP, resulting from hydrolysis, was also observed, and in the case of **2.8** a much smaller amount of the GDP-Man*p* analog was produced. To confirm the identity of each GDP-Man*p* analog, the product peaks were isolated and analysed by mass spectrometry. For each of the seven analogs, a peak at *m/z* = 618 (methoxy analogs) or *m/z* = 588 (deoxy analogs) corresponding to the $[M–H]^-$ peaks was seen.

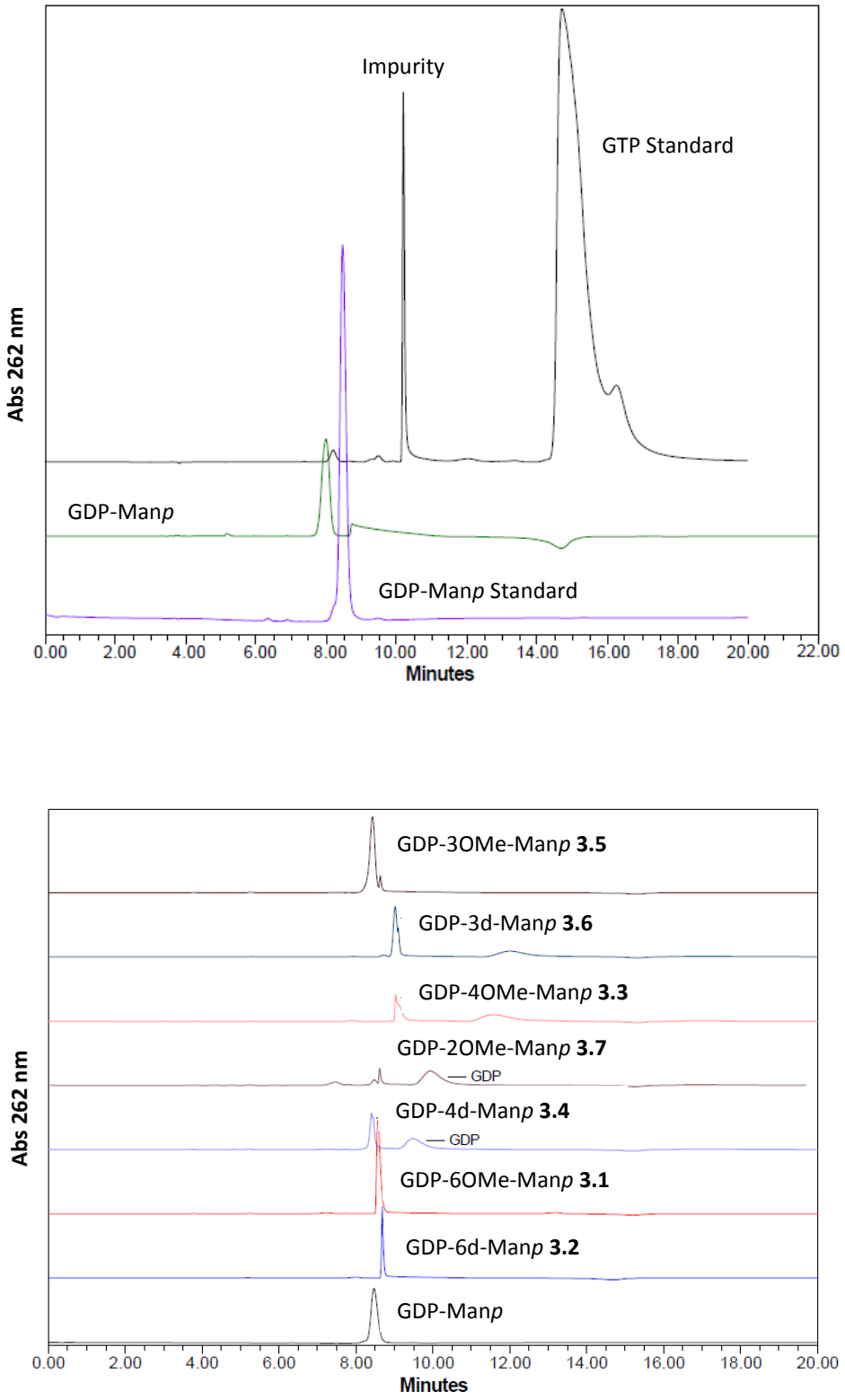


Figure 3.2. GDP-Man analogs HPLC traces

3.2.1. Relative activity of Manp-1P analogs for nucleotidyl transferase

After establishing that all seven Man*p*-1P analogs could serve as substrates for GDP-ManPP, the relative activity with each analog was assessed. To evaluate the relative activities of these Man*p*-1P analogs, an established colorimetric activity assay was used. The basic principle of this assay relies on the detection of the pyrophosphate (PPi, **Figure 3.1**) that is formed as byproduct of the GDP-ManPP catalyzed synthesis of GDP-Man using Man*p*-1P and GTP.[1] This pyrophosphate is hydrolyzed enzymatically to inorganic phosphate through the addition of inorganic pyrophosphatase. The resulting phosphate, ammonium molybdate, and malachite green dye interact to form a complex that shifts the absorbance maximum of the dye from 616.5 nm to 650 nm, which can be detected by UV–Vis spectroscopy (**Figure 3.3**).[8]

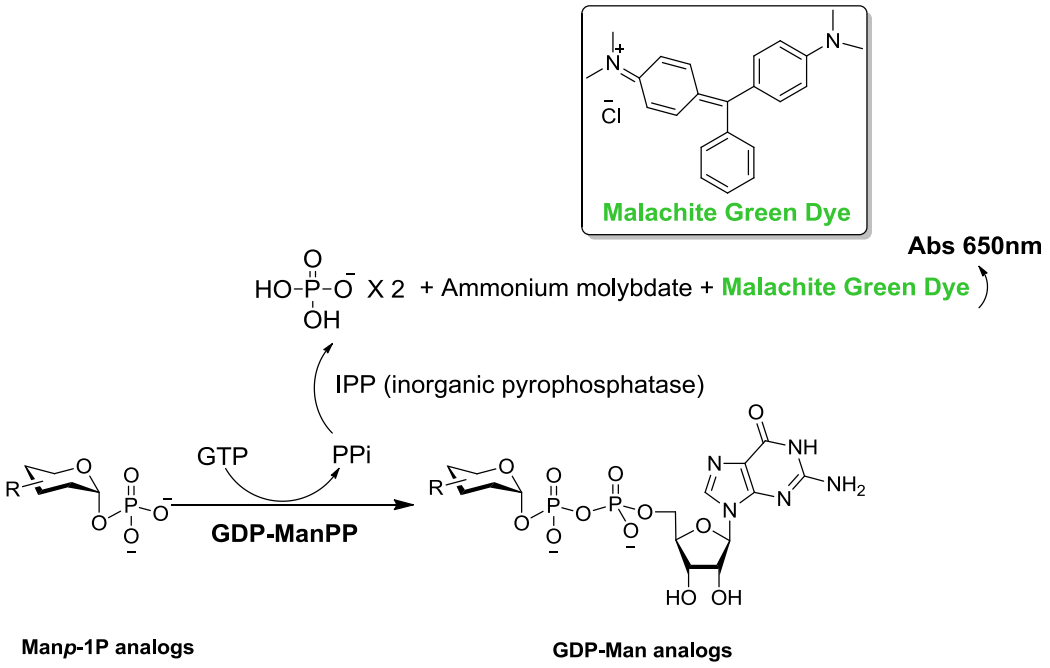


Figure 3.3. Colorimetric activity assay used to compare the enzymatic activity of **2.2**-**2.8** with the natural substrate Man*p*-1P **2.1**.

Using this assay, we compared the ability of **2.2**–**2.8** to act as substrates for GDP-ManPP compared to the parent compound (**2.1**), as illustrated in Figure **3.4**. All of the seven derivatives showed to be active in this assay, although with lower activities than **2.1**. In all cases, the deoxy analogs were found to have higher activity than the corresponding methoxy compounds. The 6-deoxy (**2.3**), 6-methoxy (**2.2**), and 4-deoxy (**2.5**) analogs demonstrated moderate to good relatively activities, while the 2-methoxy (**2.8**), 3-methoxy (**2.6**), and 4-methoxy (**2.4**) compounds showed a much lower activity compared with other analogs. For example, the 3-deoxy analog (**2.7**) showed a four-fold decrease in activity and the 2-methoxy, 3-methoxy, and 4-methoxy analogs displayed a six-, fourteen-, and

seventeen-fold decrease, respectively. Because both the 6-deoxy and 6-methoxy analogs (**2.3** and **2.2**) showed relatively good activity it is likely that this hydroxyl group does not interact significantly with GDP-ManPP. As the 2-methoxy, 3-methoxy, and 4-methoxy compounds all showed a large decrease in activity it is likely that these positions are bound tightly in the active site of GDP-ManPP. A graphical summary of the substrate specificity for GDP-ManPP is shown in **Figure 3.5**.[9]

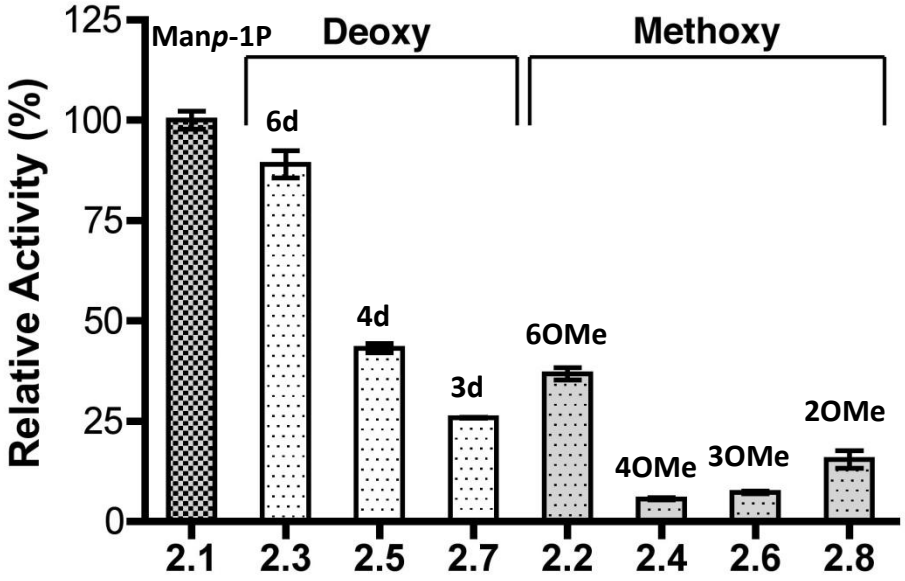


Figure 3.4. Substrate specificity of GDP-ManPP with **2.1** and derivatives **2.2**–**2.8**. Error bars refer to the standard deviations of duplicate reactions.

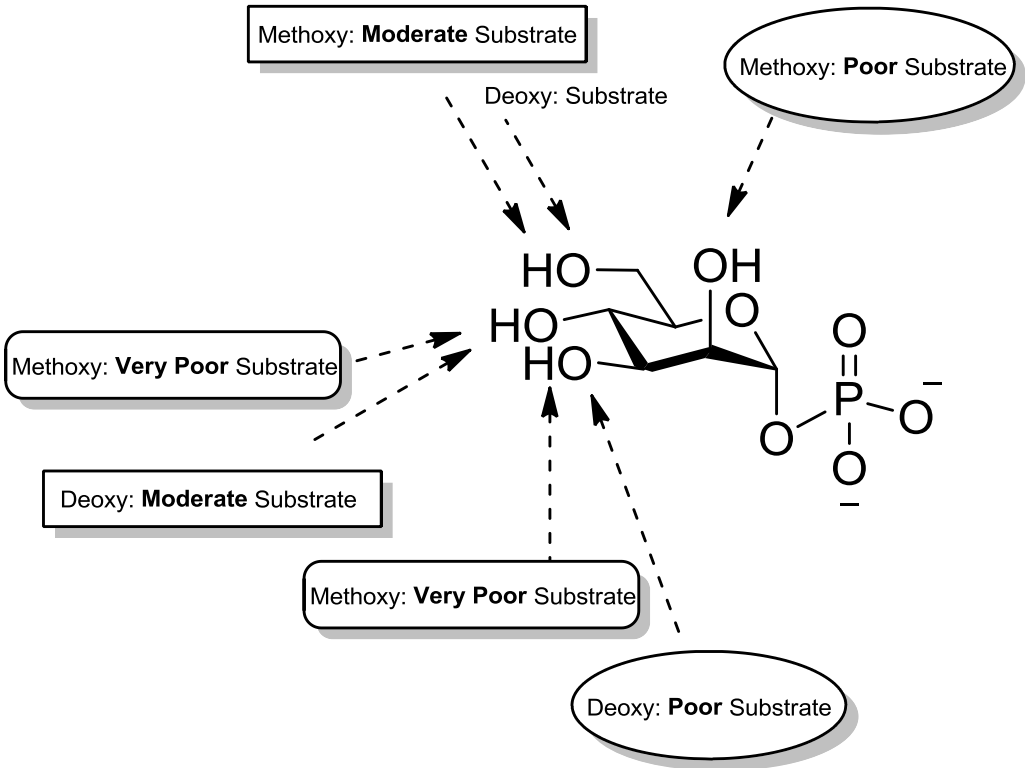


Figure 3.5. Summary of the substrate specificity of GDP-ManPP. "Very poor substrate" refers to a relative GDP-ManPP activity ≤ 10%; "Poor substrate" refers to a relative GDP-ManPP activity ≤ 30%, but ≥ 10%; "Moderate substrate" refers to a relative GDP-ManPP activity ≤ 50%, but ≥ 30%; "Substrate" refers to a relative GDP-ManPP activity ≥ 50% of that for the natural substrate **2.1**.

3.2.2. Kinetic analysis of Manp-1P analogs for nucleotidyl transferase

To better understand how these Man*p*-1P analogs interact with GDP-ManPP, kinetic analysis were performed using the same colorimetric activity assay with varying concentrations of the Man*p*-1P analog substrate. A summary of the results of these kinetics studies is provided in **Figure 3.6**, **Table 3.1**. As shown, both 6-deoxy Man*p*-1P and 6-methoxy Man*p*-1P bind relatively well in the active site of GDP-ManPP, showing only a two- or four-fold increase in K_M respectively. However, the turnover rate of 6-methoxy Man*p*-1P is much lower than 6-deoxy Man*p*-1P and the natural substrate, as observed from a greater than ten-fold decrease in k_{cat}. This result indicates that no critical hydrogen-bonding interactions are formed with this position, and, a bulky substituent at this position appears to interfere with the rate of substrate turnover. It is possible that this bulky substituent destabilizes binding with the enzyme in the correct conformation for turnover. The 4-deoxy analog **2.5** shows both a ten-fold increase in K_M and a eight-fold decrease in k_{cat}, which suggests that hydrogen-bonding to this position is important in substrate binding and turnover.

In the case of the 4-methoxy and 2-methoxy analogs, interactions between them and the protein are very weak, as seen by the greater then 100-fold increase in K_M, and consequently the turnover rates are also low. The binding between 3-methoxy analog is moderate, only a six-fold increase in the observed K_M, but it shows an extremely low turnover rate. These results all suggest that GDP-ManPP is not tolerant of bulky substituent at C-2, C-3, and C-4 positions, which is consistent with the results obtained from their relative activity.

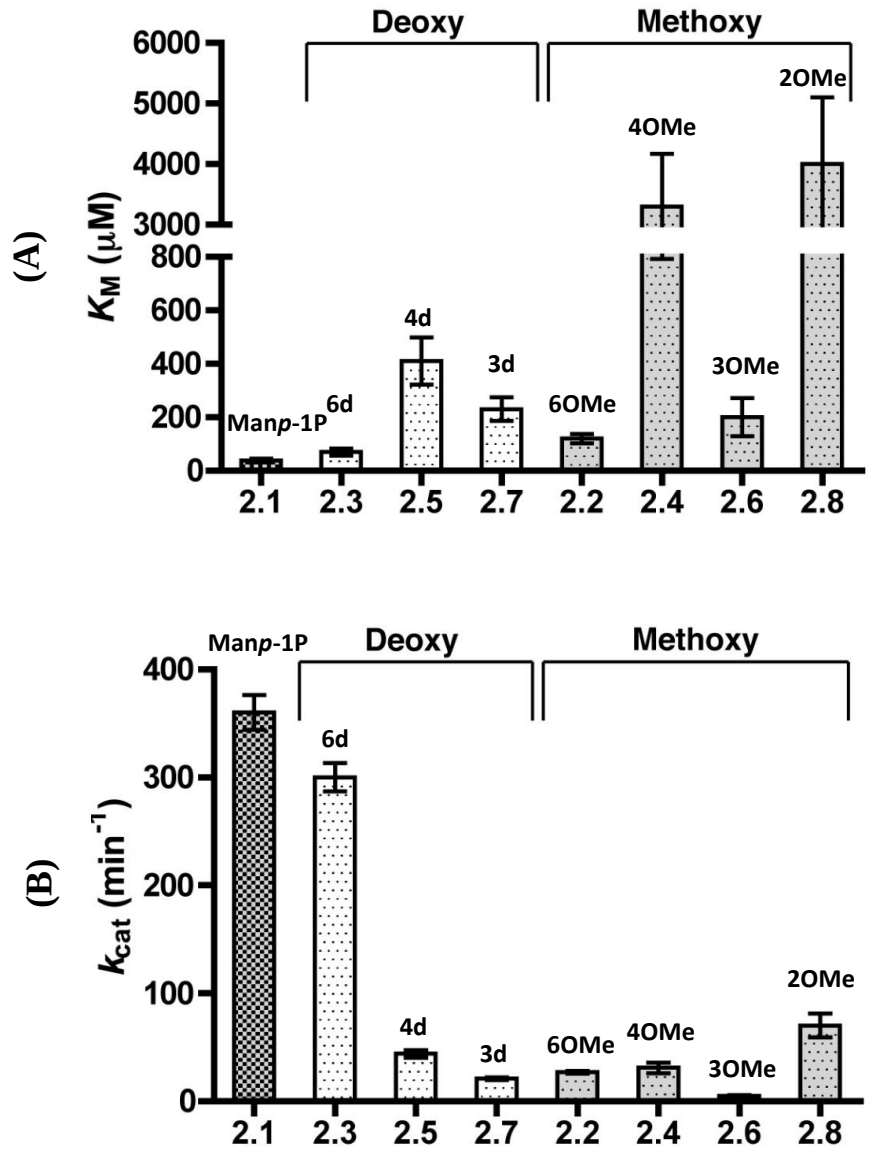


Figure 3.6. Summary of GDP-ManPP kinetics

(A). Comparison of K_M between Man*p*-1P analogs and Man*p*-1P as substrates for GDP-ManPP; (B). Comparison of k_{cat} between Man*p*-1P analogs and Man*p*-1P as substrates for GDP-ManPP

Table 3.1. K_M, k_{cat}, and k_{cat}/K_M of GDP-ManPP kinetic studies

	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (min^{-1} μM^{-1})
2.1 Man*p*-1P	40±6	360±16	9±3
2.3 6d-Man*p*-1P	70±13	300±13	4±1
2.5 4d-Man*p*-1P	410±88	44±3	0.11±0.03
2.7 3d-Man*p*-1P	230±44	21±1	0.09±0.02
2.2 6OMe-Man*p*-1P	120±18	27±1	0.23±0.06
2.4 4OMe-Man*p*-1P	3400±870	31±4.7	$(9\pm5) \times 10^{-3}$
2.6 3OMe-Man*p*-1P	200±72	5.2±0.7	$(2.6 \pm 0.1) \times 10^{-2}$
2.8 2OMe-Man*p*-1P	4000±1100	70±11	$(2 \pm 1) \times 10^{-2}$

3.2.3. Preliminary study on the use of a synthetic polyprenol phosphate analog by mycobacterial PPM synthase

The transfer of mannose residues from GDP-Man*p* to polyprenyl phosphates is catalyzed by PPM synthase as shown in **Figure 3.7**. The resulting PPM is known as a donor substrate for PPM-dependent α-(1→6)-ManT in the biosynthesis of LAM.[10] In order to expand our knowledge to the substrate specificity of PPM synthase and obtain the corresponding PPM analogs using GDP-Man analogs, a synthetic polyprenyl phosphate analog **2.9** was used to test the activity of a PPM synthase, termed as *Mt*-Ppm1/D2.[7,10] A structurally related analog, which is shown in **Figure 3.8**, has been reported to be a substrate for this enzyme.[10] The expected product, PPM analog **3.8**, was confirmed by LRMS.

Expression and purification of *Mt*-Ppm1/D2 was carried out by Ruixiang Blake Zheng.

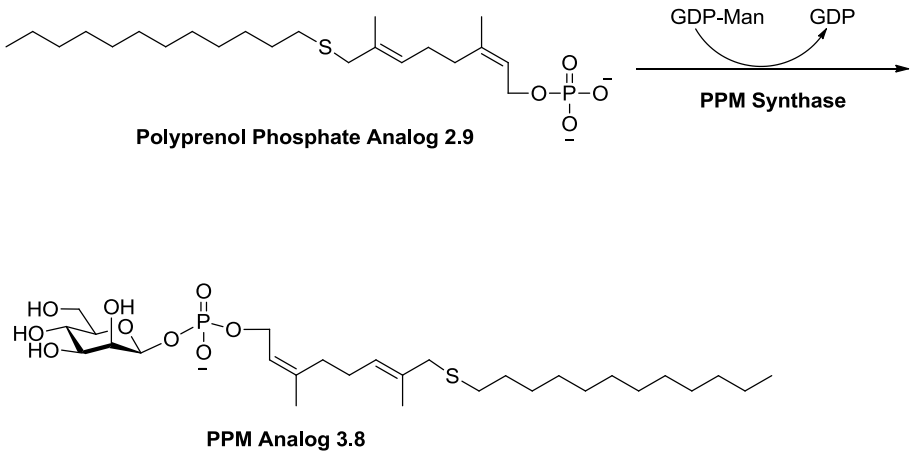
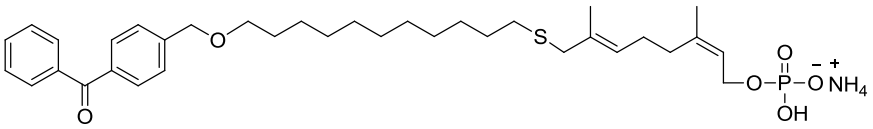


Figure 3.7. Reaction catalyzed by PPM synthase using the synthetic polyprenolphosphate analog **2.9**

The formation of the desired PPM analog indicates that PPM synthase only requires two prenol repeats for the effective binding, which is consistent with previous studies.[10] The synthesis of this lipid analog is far simpler and less expensive than that of natural substrate decaprenyl (C_{50})/undecaprenyl (C_{55}), which will allow us to produce sufficient quantities of this lipid to test the relative activity of PPM synthase with the GDP-Man*p* analogs. However, the activity of the PPM synthase is very low. The expression of PPM synthase needs to be optimized before any further studies can be done. Relative activity analyses can be performed when the direct quantification of the polyprenolphosphate analog activity is achievable.



Synthetic benzophenone-containing polyprenol phosphate-based compound

Figure 3.8. A structurally related polyprenol phosphate analog

3.3. Conclusion

In this chapter, we presented studies on the substrate specificity of a GDP-ManPP from *Salmonella enterica*, which is involved in GDP-Man biosynthesis, utilizing an established colorimetric activity assay.[8] Key interactions (*e.g.*, steric and hydrogen-bonding requirements) between the modified substrates and GDP-ManPP were determined.

We found that methylation of C-3 and C-4 hydroxyl groups on the natural substrate **2.1** led to almost a complete loss of activity. Moreover, methylation of the hydroxyl group at C-2 of **2.1** also contributed to a dramatic decrease on the substrate activity. These results demonstrate that GDP-ManPP is not tolerant of bulky substituent at C-2, C-3, and C-4 positions, which, in turn, suggests that the C-2, C-3, and C-4 hydroxyl are bound deeply inside the enzyme active site.

Another important conclusion was that a critical hydrogen-bonding interaction appeared to be formed between the protein and the C-3 hydroxyl group of mannose-1-phsophate **2.1**, as the activity of the 3-deoxy Man*p*-1P is much lower than the other deoxy derivatives. Additionally, both 6-methoxy and 6-deoxy

Man*p*-1P are good or moderate substrates for GDP-ManPP, which indicates that C-6 hydroxyl group of Man*p*-1P is not required for binding.

We also report that a synthetic polyprenolphosphate analog **2.9** could be converted to the corresponding PPM analog by the action of PPM synthase, which could be used as donor substrate for the PPM-dependent α-(1→6)-ManT in the mannan core biosynthesis.[10]

3.4. Experimental section

3.4.1. Enzymatic synthesis of GDP-ManPP analogs

GDP-α-D-mannose pyrophosphorylase from *Salmonella enterica* was cloned, expressed in *E.coli* BL21(DE3)plySs, and purified by Ruixiang Blake Zheng. The enzymatic synthesis of GDP-Man and GDP-Man analogs were carried out in NH_4HCO_3 buffer (10 mM, pH 8.0) containing $MgCl_2$ (5 mM), DTT (1 mM), GTP (5 mM), Man*p*-1P or its analogs (5 mM), GDP-ManPP (200 μg), and inorganic pyrophosphatase (1.25 U) in a total reaction volume of 100 μL. The mixture was incubated at room temperature overnight with shaking and the reactions were stopped by removing the extra enzymes though centrifugal filtration when HPLC analysis of the spin–filtered (Amicon YM10) solution indicated that the reaction was complete.

3.4.2. Characterization of GDP-ManPP analogs and PPM analog

HPLC analysis was carried out using a Waters 600E HPLC equipped with a photodiode array (PDA) detector, with monitoring at 262 nm; the system was controlled via Empower chromatography software. Monitoring the conversion of Man*p*-1P analogs into corresponding GDP-Man analogs was done by analytical HPLC on a Phenomenex C18 column (4.6 × 250 mm). A gradient elution using two buffers: buffer A (200 mM Et_3N–HOAc, pH 6.6) and buffer B (200 mM Et_3N–HOAc pH 6.6 containing 5% CH_3CN) was used. The gradient conditions employed used 96% buffer A and 4% buffer B for 10 min (isocratic), followed by a gradient of 4→100% buffer B over 15 min, 100% buffer B for 10 min. The column was re-equilibrated with a gradient of 100→4% buffer B for 15 min, followed by 96% buffer A and 4% buffer B for 10 min. The retention time of the product and starting material peaks is shown in **Table 3.2**. At the end of the reaction, the enzymes were removed by passing the incubation mixture through an empty 10 mL BioRad cartridge, equipped with a filter. The filtrate was then collected by passage through Amicon Ultra–15 centrifugal filter with a molecular weight cut off of 10,000 Daltons; this filtrate was centrifuged to remove soluble proteins. Following centrifugation, the filtrate was collected and was applied to a Sephadex G–15 gel filtration column to remove salts. The products were lyophilized and analyzed by MS. Confirmation of the GDP-Man and GDP-Man analogs by HRMS are listed in **Table 3.3**.

Table 3.2. Retention time of GTP, GDP-Man, and GDP-Man analogs

Product	**Retention Time (min)**
GTP	15.00
GDP-Man	8.00
6-deoxy GDP-Man	8.75
4-deoxy GDP-Man	8.50
3-deoxy GDP-Man	9.00
6-methoxy GDP-Man	8.75
4-methoxy GDP-Man	9.25
3-methoxy GDP-Man	8.50
2-methoxy GDP-Man	8.75

Table 3.3. MS spectra peak list of GDP-Man and GDP-Man analogs

Product	Formula	Calcd	Found	Ion
GDP-Man	$C_{16}H_{24}N_5O_{16}P_2$	604.0699	604.0691	$[M–H]^-$
6-deoxy GDP-Man	$C_{16}H_{24}N_5O_{15}P_2$	588.0750	588.0746	$[M–H]^-$
4-deoxy GDP-Man	$C_{16}H_{24}N_5O_{15}P_2$	588.0750	588.0740	$[M–H]^-$
3-deoxy GDP-Man	$C_{16}H_{24}N_5O_{15}P_2$	588.0750	588.0746	$[M–H]^-$
6-methoxy GDP-Man	$C_{17}H_{26}N_5O_{16}P_2$	618.0855	618.0852	$[M–H]^-$
4-methoxy GDP-Man	$C_{17}H_{26}N_5O_{16}P_2$	618.0855	618.0848	$[M–H]^-$
3-methoxy GDP-Man	$C_{17}H_{26}N_5O_{16}P_2$	618.0855	618.0849	$[M–H]^-$
2-methoxy GDP-Man	$C_{17}H_{26}N_5O_{16}P_2$	618.0855	618.0848	$[M–H]^-$

The PPM analog **3.8** was prepared using polyprenolphosphate analog **2.9** by Ruixiang Blake Zheng enzymatically, and confirmed by LRMS shown in **Appendix**.

3.4.3. GDP-ManPP activity assay

The malachite green dye reagent was prepared as previously reported by MacMillan and coworkers.[8] Briefly, to a 100 mL volumetric flask were added: 34.0 mg of malachite green oxalate salt, 1.236 g of ammonium molybdate, 3.4 mL of absolute ethanol, 80 mL of deionised water, 8.6 mL of concentrated HCl (37%), 1 mL of Tween 20 and deionised water up to 100 mL total volume. The resulting

mixture was stirred for 1 h, then was filtered (0.2 μm) and stored at 4 ℃ for 7 days.[8] The mixture was filtered again and calibrated by using a standard series of KH_2PO_4 solution at variable concentrations prior to use. On a 96–well plate, 25 μL of each standard was added to a well before the addition of 100 μL malachite reagent. The plate was incubated at 37 ℃ with mixing, and the absorbance was read at 650 nm over 10 minutes. The absorbance values at 10 minutes were plotted against the phosphate concentration, and from linear fitting an equation was obtained, where the slope gave the conversion factor.[8]

Assays for GDP-ManPP activity were prepared containing Man*p*-1P or Man*p*-1P analogs (500 μM), GTP (2 mM), GDP-ManPP (50 ng), iPPase (0.5 U), $MgCl_2$ (8 mM), DTT (1 mM), in 25 μL of TRIS 50 mM pH 7.6. The reactions were incubated for 10 minutes at 37 ℃ in a 96–well plate, after which time the reactions were quenched by the addition of 100 μL of malachite reagent. The colorimetric activity assay was carried out in a plate reader at 37 ℃, with mixing, for 10 minutes and the absorbance at 650 nm was then read.[8]

Kinetic analysises were carried out utilizing the same conditions as the activity assay, expect a variable concentrations of the Man*p*-1P or Man*p*-1P analog substrates was used as listed in **Table 3.4**.

Table 3.4. Variable concentrations of eight substrates used for kinetics analysis

Man*p*-1P (μM)	**6-deoxy (μM)**	**4-deoxy (μM)**	**3-deoxy (μM)**	**6-OMe (μM)**	**4-OMe (μM)**	**3-OMe (μM)**	**2-OMe (μM)**
900.0	2000.0	2000.0	2000.0	1000.0	4000.0	4000.0	4000.0
450.0	1000.0	1000.0	1000.0	500.0	2000.0	2000.0	2000.0
225.0	500.0	500.0	500.0	250.0	1000.0	1000.0	1000.0
112.5	250.0	250.0	250.0	125.0	500.0	500.0	500.0
56.3	125.0	125.0	125.0	62.5	250.0	250.0	250.0
28.1	62.5	62.5	62.5	31.3	125.0	125.0	125.0
14.1	31.3	31.3	31.3	15.6	62.5	62.5	62.5
7.0	15.6	15.6	15.6	7.8	31.3	31.3	31.3
3.5	7.8				15.6	15.6	15.6

3.5. Bibliography

(1) Davis, A. J.; Perugini, M. A.; Smith, B. J.; Stewart, J. D.; Ilg, T.; Hodder, A. N.; Handman, E. *J. Biol. Chem.* **2004**, *279*, 12462-12468.

(2) Elling, L.; Ritter, J. E.; Verseck, S. *Glycobiology* **1996**, *6*, 591-597.

(3) Alderwick, L. J.; Radmacher, E.; Seidel, M.; Gande, R.; Hitchen, P. G.; Morris, H. R.; Dell, A.; Sahm, H.; Eggeling, L.; Besra, G. S. *J. Biol. Chem.* **2005**, *280*, 32362-32371.

(4) Kremer, L.; Gurcha, S. S.; Bifani, P.; Hitchen, P. G.; Baulard, A.; Morris, H. R.; Dell, A.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *363*, 437-447.

(5) Schaeffer, M. L.; Khoo, K.-H.; Besra, G. S.; Chatterjee, D.; Brennan, P. J.; Belisle, J. T.; Inamine, J. M. *J. Biol. Chem.* **1999**, *274*, 31625-31631.

(6) Tam, P.-H.; Lowary, T. L. *Curr. Opin. Chem. Biol.* **2009**, *13*, 618-625.

(7) Gurcha, S. S.; Baulard, A. R.; Kremer, L.; Locht, C.; Moody, D. B.; Muhlecker, W.; Costello, C. E.; Crick, D. C.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *365*, 441-450.

(8) Marchesan, S.; Macmillan, D. *Chem. Commun.* **2008**, 4321-4323.

(9) Tam, P.-H.; Besra, G. S.; Lowary, T. L. *ChemBioChem* **2008**, *9*, 267-278.

(10) Guy, M. R.; Illarionov, P. A.; Gurcha, S. S.; Dover, L. G.; Gibson, K. J. C.; Smith, P. W.; Minnikin, D. E.; Besra, G. S. *Biochem. J.* **2004**, *382*, 905-912.

Chapter 4

Summary and future work

4.1. Summary

Lipoarabinomannan (LAM) is an essential cell wall component and immunologically active glycan found in mycobacteria, including the most well-known human pathogen *Mycobacterium tuberculosis*, which is the causative agent of tuberculosis (TB).[1] Therefore, the enzymes involved in the biosynthesis of LAM have undoubtedly become the targets for drug action.

One of these enzymes, a PPM-dependent α-(1→6)-ManT responsible for assembling the α-(1→6)-linked mannan core has been the focus of previous studies in our group. These studies have focused on understanding of the acceptor substrate specificity of this enzyme,[1-2] but little is known with regards to the polyprenol-phosphomannose (PPM) donor specificity. My research efforts have focused on probing the specificity of the enzymes involved in PPM biosynthesis, the donor substrate for the PPM-dependent α-(1→6)-ManT; specifically the nucleotidyltransferase (GDP-ManPP) and the PPM synthase. The donor substrate for this PPM-dependent α-(1→6)-ManT is assembled from mannose-1-phosphate catalyzed by these two enzymes (**Figure 4.2**). Therefore, a panel of methoxy and deoxy analogs of mannose-1-phosphate were prepared chemically in order to probe the specificity of GDP-ManPP. Seven analogs were generated by having one of their hydroxyl groups methylated or deoxygenated (**Figure 4.1**). These were then converted to the corresponding GDP-Man analogs catalyzed by GDP-ManPP, which will be tested as substrates for PPM synthase.

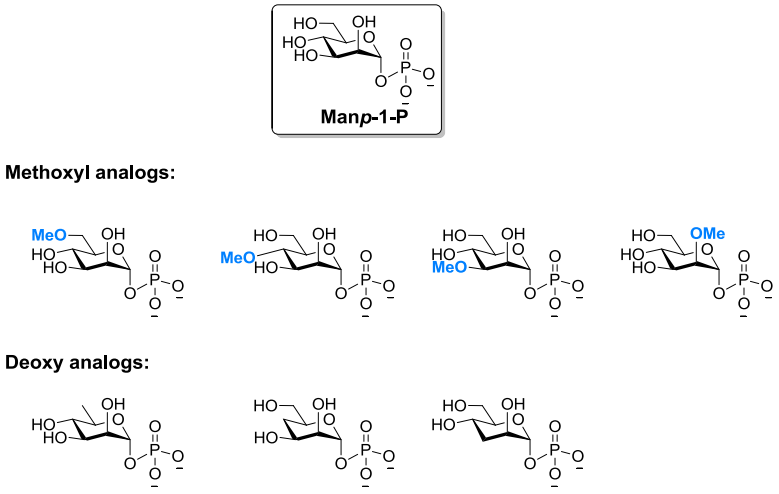


Figure 4.1. Synthetic Man*p*-1P and Man*p*-1P analogs

The biochemical evaluation of these derivatives using an established colorimetric assay[3] allowed us to explore the importance of specific steric and hydrogen-bonding interactions[4] occurring in the active site of GDP-ManPP from *Salmonella enterica* upon substrate binding and turnover. We discovered that all the single modified mannose-1-phosphate derivatives tested proved to act as substrates for GDP-ManPP, however with lower activities compared with the natural substrate. From this study we found that the C-2, C-3, and C-4 hydroxyl groups of mannose-1-phosphate are bound within the active site of GDP-ManPP and the addition of a methyl group at these positions is not tolerated. Conversely, the addition of a methyl group at the C-6 had a much smaller effect, suggesting that this position protrudes from the active site. The C-6 and C-4 hydroxyl groups do not appear to form critical hydrogen-bond with GDP-ManPP, while the

interaction between C-3 hydroxyl group and the enzyme appears to be critical for efficient substrate turnover.

A polyprenolphosphate analog[5] was then synthesized and confirmed to be substrate for PPM synthase by mass spectral data. The recombinant PPM synthase enzyme showed poor activity and so no further testing was done until more active enzyme could be produced.

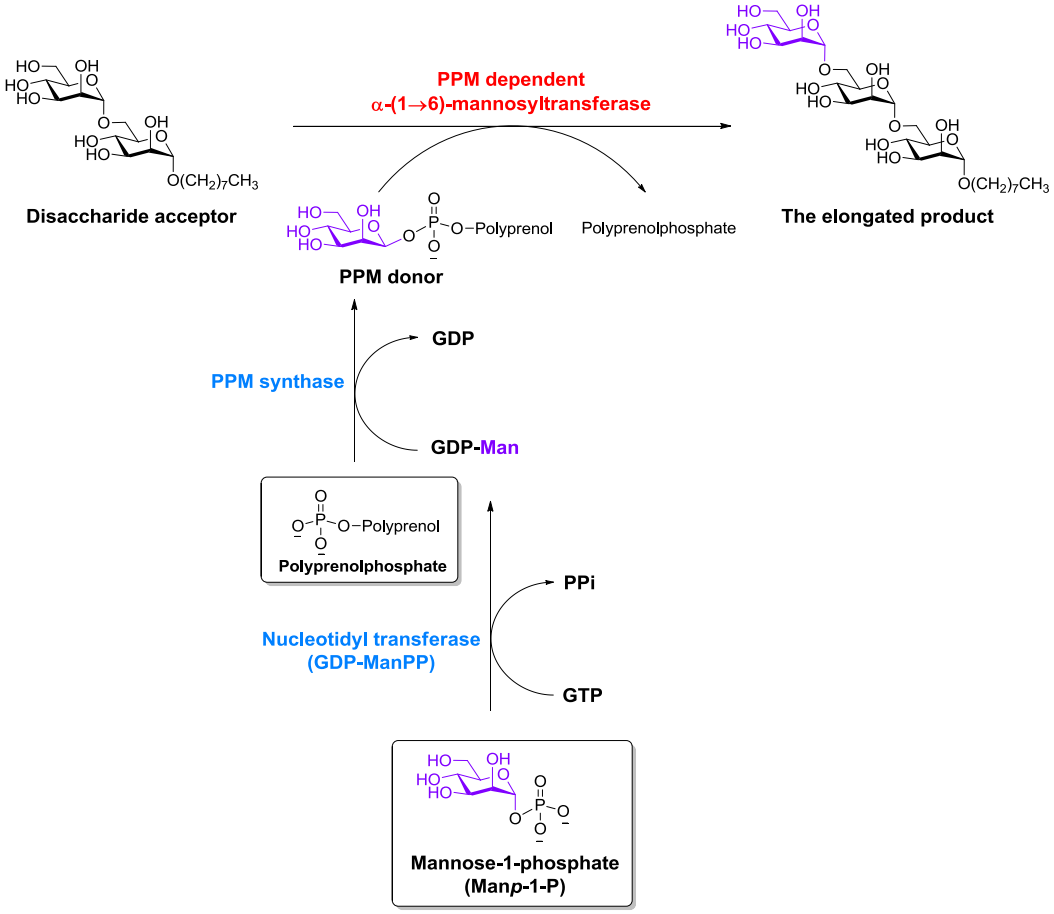


Figure 4.2. Relationship between GDP-ManPP, PPM synthase, and PPM-dependent α-(1→6)-ManT

4.2. Future work

With the GDP-Man analogs and polyprenol phosphate analogs in hand, our future work will focus on preparing the corresponding PPM analogs, which will be obtained using PPM synthase enzyme. If some of the GDP-Man derivatives cannot be converted to the corresponding PPM analogs enzymatically, they will be synthesized chemically. Additionally, we will probe the steric and hydrogen-bonding requirements for substrate recognition in the active site of PPM synthase. This will be done using our previously prepared GDP-Man analogs using the same strategy employed to study GDP-ManPP. In addition, we plan to use Man*p*-1P and Man*p*-1P derivatives to study mycobacterial GDP-ManPP, once it is unequivocally identified. Ultimately, it is our goal to use the PPM analogs, prepared with PPM synthase, to explore the donor substrate binding interactions in the active site of PPM-dependent α-(1→6)-ManT. Understanding these specific carbohydrate–protein interactions for the enzyme involved in mycobacterial mannan biosynthesis will facilitate the development of specific inhibitors for this process.

4.3. Bibliography

(1) Tam, P. H.; Lowary, T. L. *Org. Biomol. Chem* **2010**, *8*, 181-192.

(2) Tam, P. H.; Besra, G. S.; Lowary, T. L. *ChemBioChem* **2008**, *9*, 267-278.

(3) Marchesan, S.; Macmillan, D. *Chem. Commun.* **2008**, 4321-4323.

(4) Mukherjee, A.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2000**, *326*, 1-21.

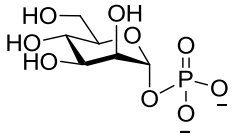
(5) Guy, M. R.; Illarionov, P. A.; Gurcha, S. S.; Dover, L. G.; Gibson, K. J. C.; Smith, P. W.; Minnikin, D. E.; Besra, G. S. *Biochem. J.* **2004**, *382*, 905-912.

Appendix

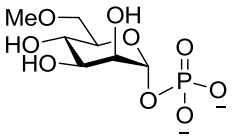
Kinetic plots for Man*p*-1P and Man*p*-1P derivatives and LRMS spectrum of PPM analog

(HPLC and MS data were required by Ruixiang Blake Zheng)

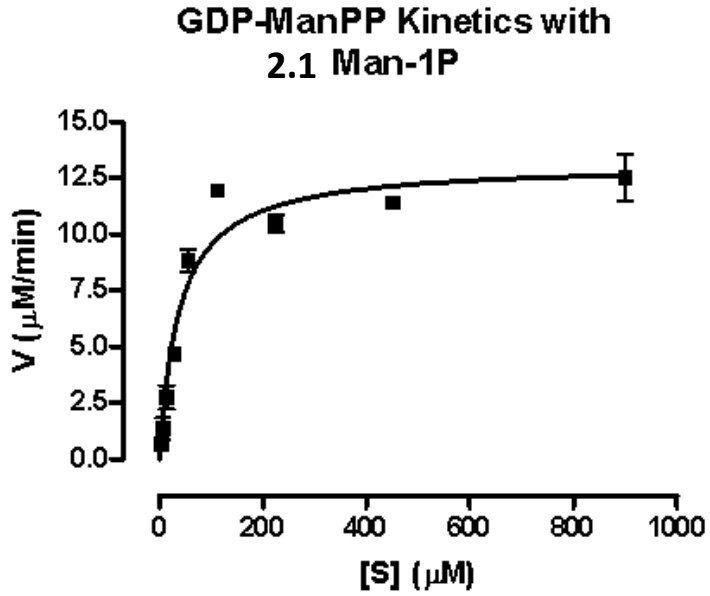
Kinetic plots for compounds 2.1 and 2.2

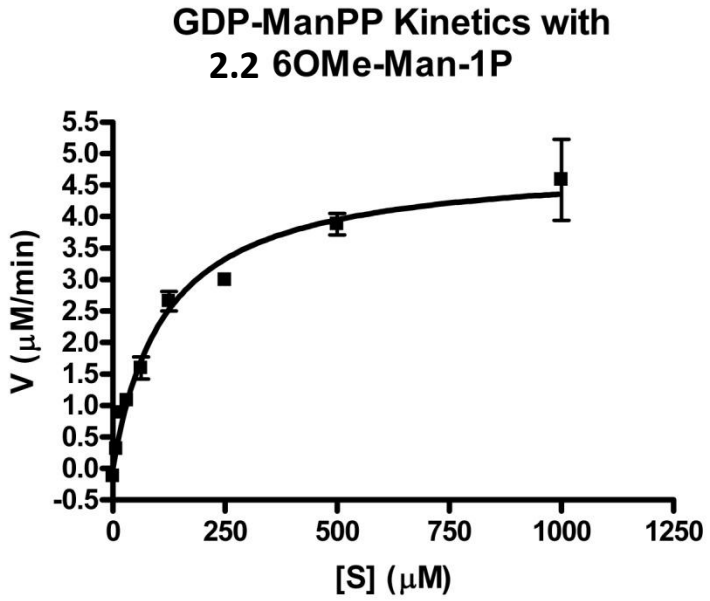


2.1 Man-1P

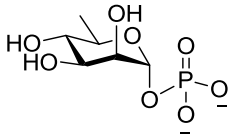


2.2 6OMe-Man-1P

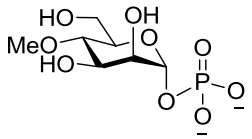




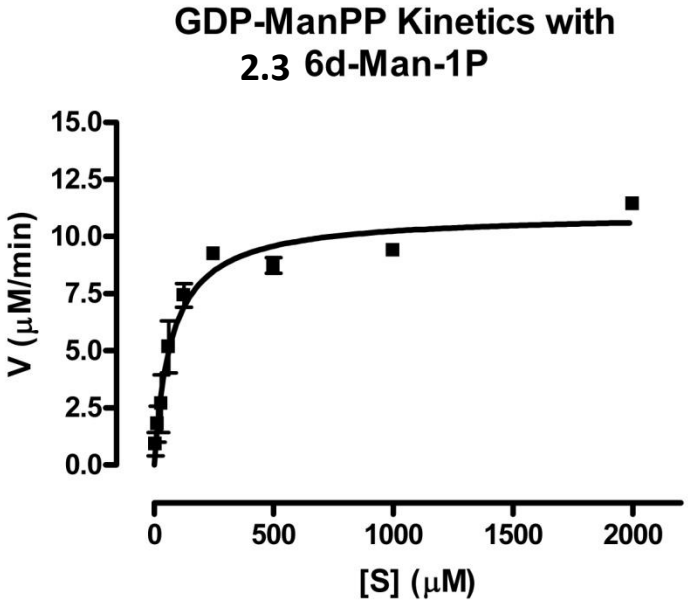
Kinetic plots for compounds 2.3 and 2.4

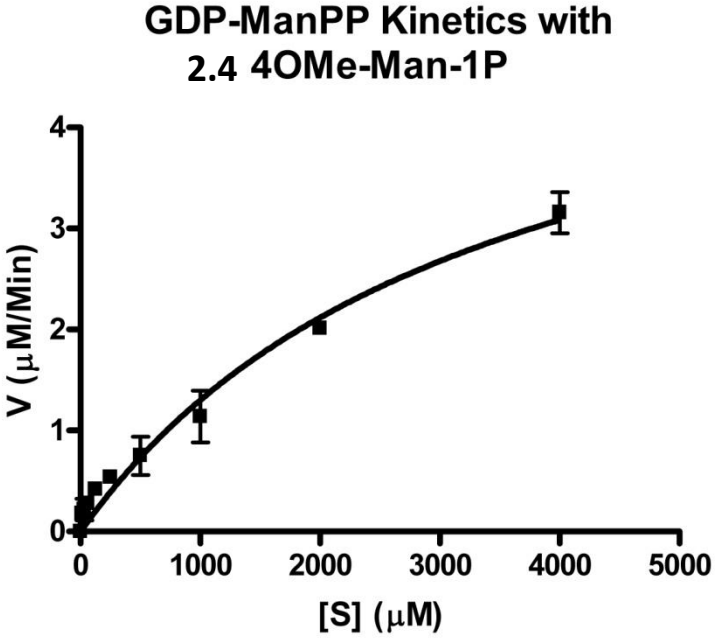


2.3 6d-Man-1P

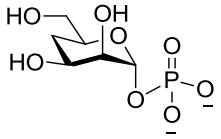


2.4 4OMe-Man-1P

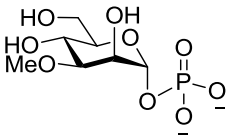




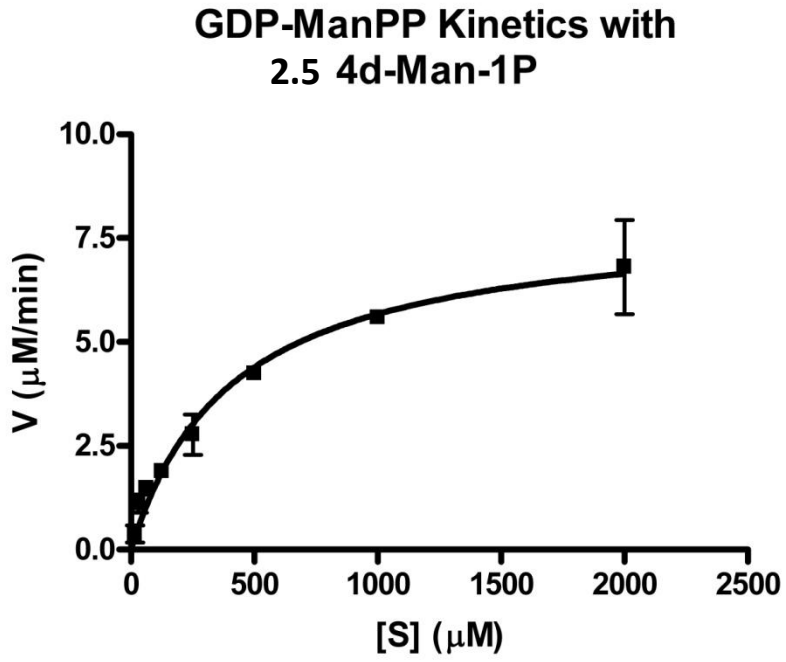
Kinetic plots for compounds 2.5 and 2.6

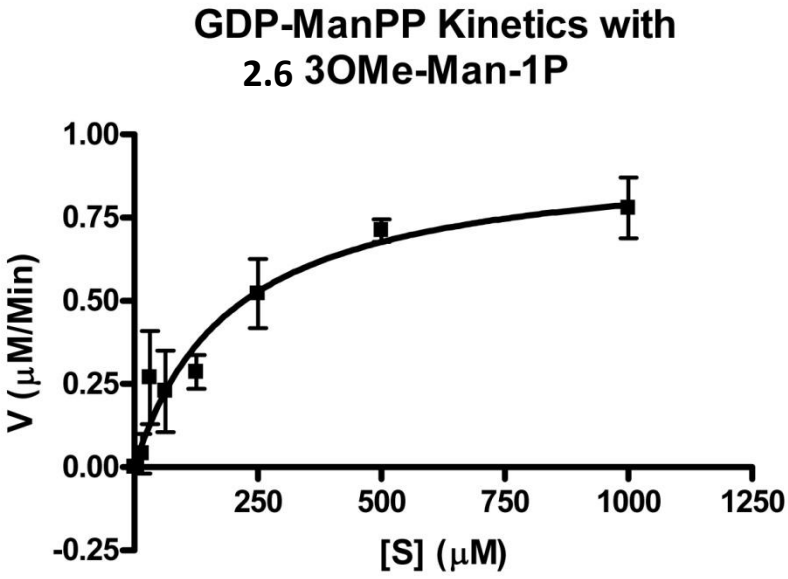


2.5 4d-Man-1P

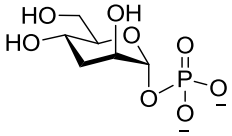


2.6 3OMe-Man-1P

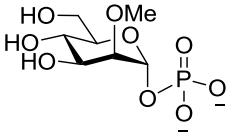




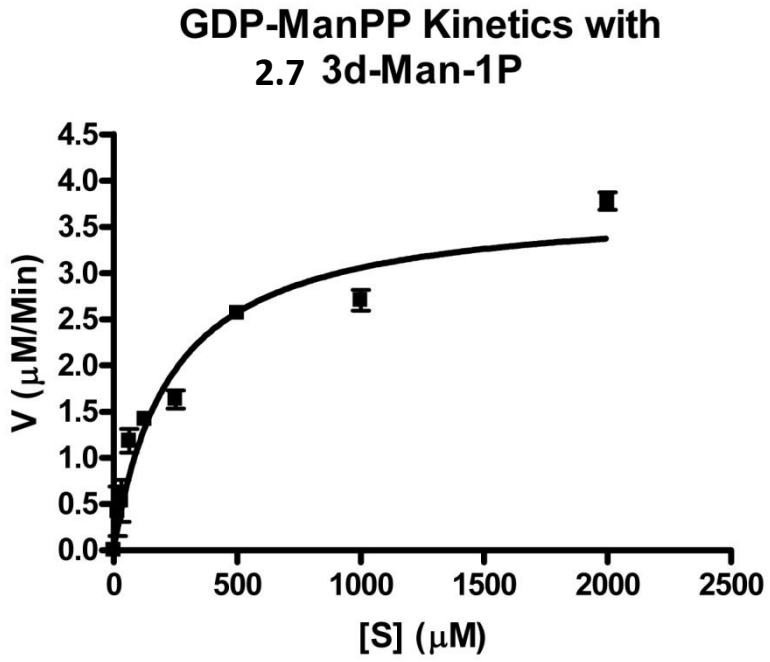
Kinetic plots for compounds 2.7 and 2.8

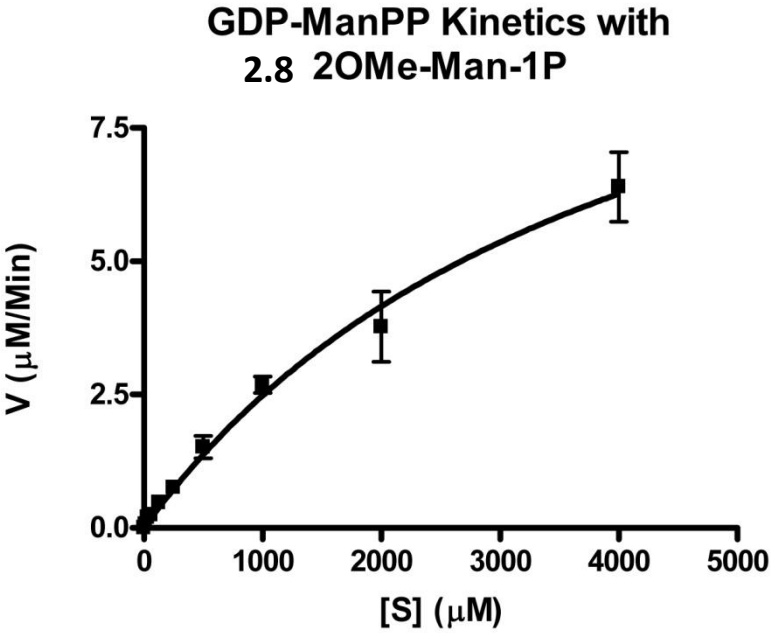


2.7 3d-Man-1P



2.8 2OMe-Man-1P





LRMS spectrum of enzymatic product using polyprenolphosphate analog 2.9 catalyzed by PPM synthase