University of Alberta

Studies of Glycosyltransferases Involved in Mycobacterial Cell Wall Biosynthesis

by

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Dedication

To my family

Abstract

Lipoarabinomannan (LAM) and the mycolyl-arabinogalactan (mAG) complex are two major entities found in the cell wall of *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis in humans. Given their important roles in the viability and virulence of the pathogen, enzymes involved in these pathways represent a rich source of potential therapeutic drug targets. As fundamental understanding of substrate–enzyme interactions is often essential in the drug discovery process, the purpose of this study was to investigate the substrate specificities of an α-(1→6)-mannosyltransferase (ManT) and a β-(1→5,6)-galactofuranosyltransferase (GlfT2), two key enzymes involved in the biosynthesis of LAM and mAG, respectively.

Although the ManT activity had been detected using an established radioactive assay, its substrate specificity remained poorly defined. The current study focused on the design, synthesis and evaluation of acceptor substrate analogs of ManT. Among those analogs prepared were those containing methoxy-, hydrogen-, and amino-substituted carbohydrate residues as well as epimeric derivatives. A homologous series of oxygen- and sulfur-linked mannosides were also prepared. Evaluation of these analogs revealed the steric requirements and hydrogen bonding interactions of the enzyme, and the effect of acceptor length on mannosyltransferase activity. Also, these results provided additional

insight into the role of ManTs and allowed the current proposed pathway of LAM to be further revised.

Another objective of the current study was to understand how GlfT2 catalyzes the alternating β-(1→5) and β-(1→6)-galactofuranosyl transfers in a single active site. A panel of mono- and dideoxy trisaccharide derivatives was synthesized, in which hydroxyl groups at either or both C-5 and C-6 positions on the sugar residues at the reducing ends were selectively removed. Biological evaluation of these analogs using a spectrophotometric assay, and structural analysis of some of the enzymatic products, showed that the removal of the hydroxyl group(s) in the acceptors appeared to have no dramatic effect on either GlfT2 activity or the regioselectivity of its galactosylation. These results suggest that groups other than the C-5 and C-6 hydroxyl groups of the acceptors are more critical for the enzyme catalysis. The identification of these key elements would be the further objective of this project.

The results from these fundamental studies provide important information about how these enzymes interact with their substrates at the molecular level. More importantly, this work will serve as the basis for the further design of potential inhibitors, which are potential lead compounds for novel therapeutic agents that are active against tuberculosis.

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List of Abbreviations

AG	arabinogalactan
AIBN	2,2′-azo-*bis*-isobutyronitrile
Ara*f*	arabinofuranose
AraT	arabinosyltransferases
AS	*Aspergillus saitoi*
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
DCC	dicyclohexylcarbodiimide
DPA	decaprenol phosphoarabinose
EMB	ethambutol
Fuc*p*	fucopyranose
Gal*f*	galactofuranose
GDP	guanosine diphosphate
Glc*p*	glucopyranose
Glc*p*NAc	*N*-acetylglucosamine
GlfT	galactofuranosyltransferase
GPL	glycopeptidolipids
GT	glycosyltransferase
INH	isoniazid
JB	jack bean
LAM	lipoarabinomannan
LB	Luria Bertoni

LM	lipomannan
MAC	*M. avium–intracellulare* complex
mAG	mycolyl–arabinogalactan complex
$ManGlcAGroAc_2$	1,2-di-*O*-C_{16}/$C_{18:1}$-(α-D-mannopyranosyl)-(1→4)-(α-D-glucopyranosyluronic acid)-(1→3)-glycerol
ManLAM	mannosylated lipoarabinomannan
Man*p*	mannopyranose
ManT	mannosyltransferase
MDR-TB	multidrug-resistant TB
MGLPs	6-*O*-methylglucosyl-containing lipopolysaccharides
MMP	*O*-methyl-mannose polysaccharides
MOPS	3-(*N*-morpholino)propanesulfonic acid
NADH	nitcotinamide adenine dinucleotide, reduced form
NIS	*N*-iodosuccinimide
PAS	*p*-aminosalicyclic acid
PBS	phosphate buffered saline
PGL	phenolic glycolipids
PI	phosphatidyl-*myo*-inositol
PIM	phosphatidyl-*myo*-inositol mannoside
PPM	polyprenylphospho-mannopyranose
Rha*p*	rhamnopyranose
RMP	Rifampin
STD	saturation transfer difference

TB	tuberculosis
TLC	thin layer chromatography
TMSOTf	trimethylsilyl triflate
WHO	World Health Organization
XDR-TB	extremely-drug resistant
XM	*Xanthomonas manihotis*

Chapter 1

Introduction to the Mycobacterial Cell Wall

1.1. General Aspects of *Mycobacterium tuberculosis*

Tuberculosis (TB), caused by the pathogenic bacterial species *Mycobacterium tuberculosis*, is a deadly infectious disease that infects one-third of the world's population and kills nearly two million people worldwide each year.[1-3] Recent surveillance and survey data reported by the World Health Organization (WHO) in 2007 estimates that annually there are 9.27 million new cases of TB and an estimated 1.3 million deaths from the disease, including 456,000 who were also HIV-positive.[4] The rise in HIV infections,[5] and the recent emergence of drug-resistant strains of *M. tuberculosis*[6] have made the treatment of TB more difficult and have contributed to the global TB epidemic.

Tuberculosis mainly attacks the respiratory system, and in these cases it is known as pulmonary TB. However, it can also affect the central nervous system, lymphatic system, the genitourinary system, and other major systems, which are collectively referred to as extrapulmonary tuberculosis. The disease is primarily spread through aerosol droplets that are expelled when an individual with active TB coughs, sneezes or speaks. Statistically, each year this individual can infect 10 to 15 other people with whom they have prolonged and frequent contacts.[4] *M.*

tuberculosis is carried on droplets through the air and enters the body via the airway. Mycobacteria invade and replicate within macrophages after reaching pulmonary alveoli. The establishment of the infection by the organism depends on their ability to persist within the hostile environment of macrophages. In this regard, mycobacteria are quite successful parasites and manage to avoid destruction by the macrophage and survive, and even replicate, within these white blood cells.[7]

Although the progression from TB infection to the active disease occurs in less than 5% of cases, the pathogen is almost never eradicated from the host. Instead, it maintains latency, a state in which the infected individual has no obvious symptoms. The latent infection however can be reactivated and developed to TB disease even many years after the initial infection. In particular, individuals with compromised immune systems (*e.g.,* those with HIV/AIDS[5]) are subject to a higher risk of reactivation. In fact, countries with higher prevalence of HIV in general populations, such as in Africa and South-East Asia, have higher numbers of HIV-positive TB cases and deaths.[4]

Besides co-infection with HIV, the emergence of multidrug-resistant TB (MDR-TB) and extremely-drug resistant TB (XDR-TB) has been the subject of increasing recent concern around the globe. The development of these drug resistant TB strains is due, in part, to the improper use of antibiotics in chemotherapy of drug-susceptible TB patients. This may result from ineffective treatment regimens prescribed by the health care

workers and the failure by patients to follow the prescribed medications. MDR-TB is a strain that has resistance to isoniazid (INH) and rifampin (RMP), two of the most potent first-line TB drugs (see section 1.4.1). On the other hand, XDR-TB is one with resistance to the fluoroquinolones and one of the three injectable second-line drugs including amikacin, kanamycin and capreomycin. In particular, XDR-TB has been the subject of substantial recent media coverage[8] stemming in part from an incident[8c] in which an individual thought to be infected with an extreme drug-resistant strain travelled between Europe and North America potentially infecting many people. Although it was later discovered that this individual had the less serious MDR-TB,[8d] not XDR-TB, this incident underscored the contagious nature of the disease and the need for new anti-TB agents.

1.2. Structural Components of the Mycobacterial Cell Wall

Treatments of drug-susceptible TB are available but normally require a long drug regimen involving multiple antibiotics.[9] These intensive treatments are required due to the unusual structure of the mycobacterial cell wall, which both protects the organism from the immune system of the host (e.g., by allowing it to survive in macrophages), and also serves as a formidable barrier to the passage of therapeutic agents.[10] The two major entities of the mycobacterial cell wall are the mycolyl–arabinogalactan (mAG) complex and lipoarabinomannan (LAM) (Figure 1-1).[10-12] The mAG is the largest structural component of the cell wall and

acts as a permeability barrier to the passage of antibiotics.[10-12] LAM is a major cell wall antigen and a number of recent studies have demonstrated that this glycoconjugate is an important modulator of the immune response arising from mycobacterial infections.[13,14]

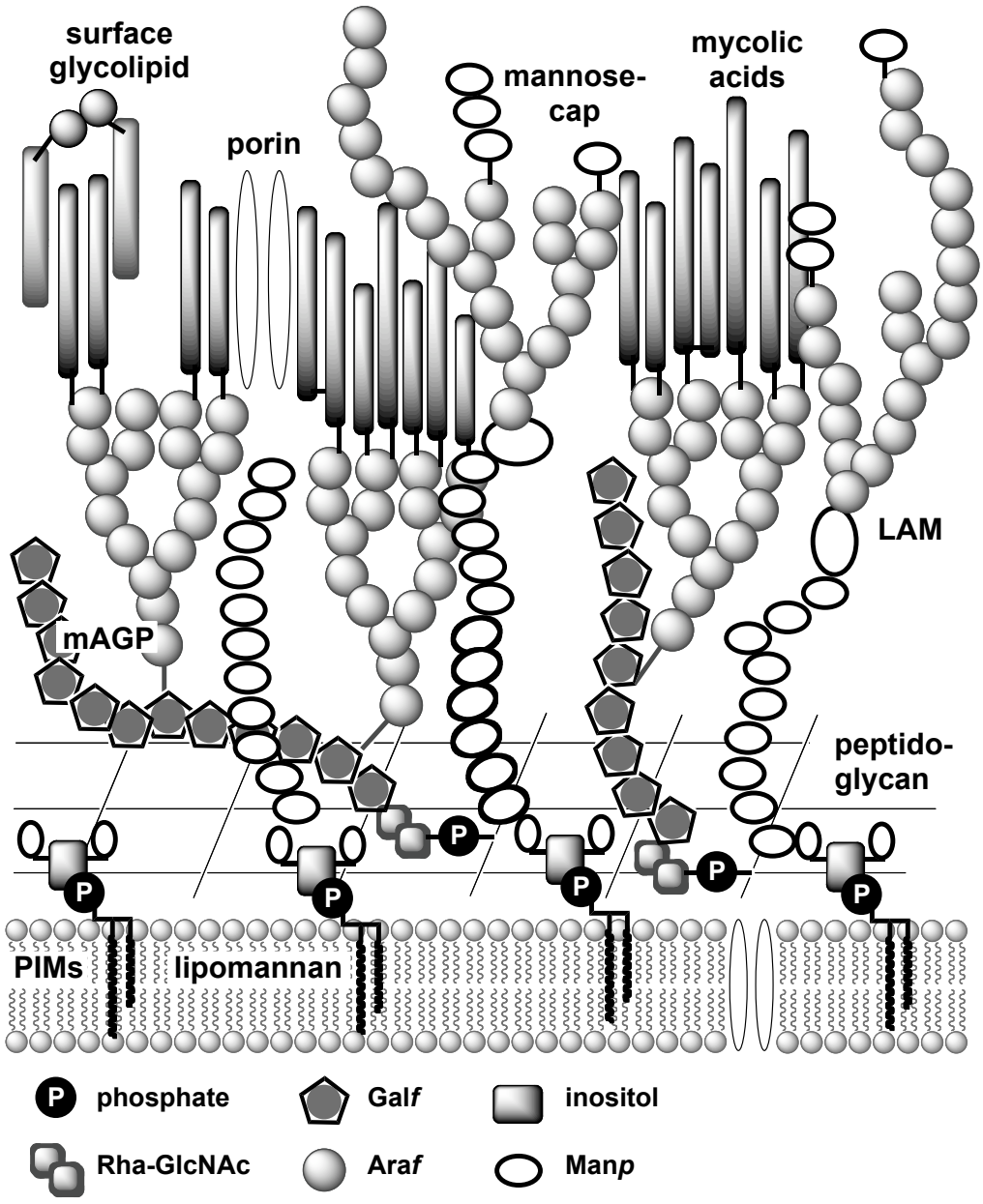
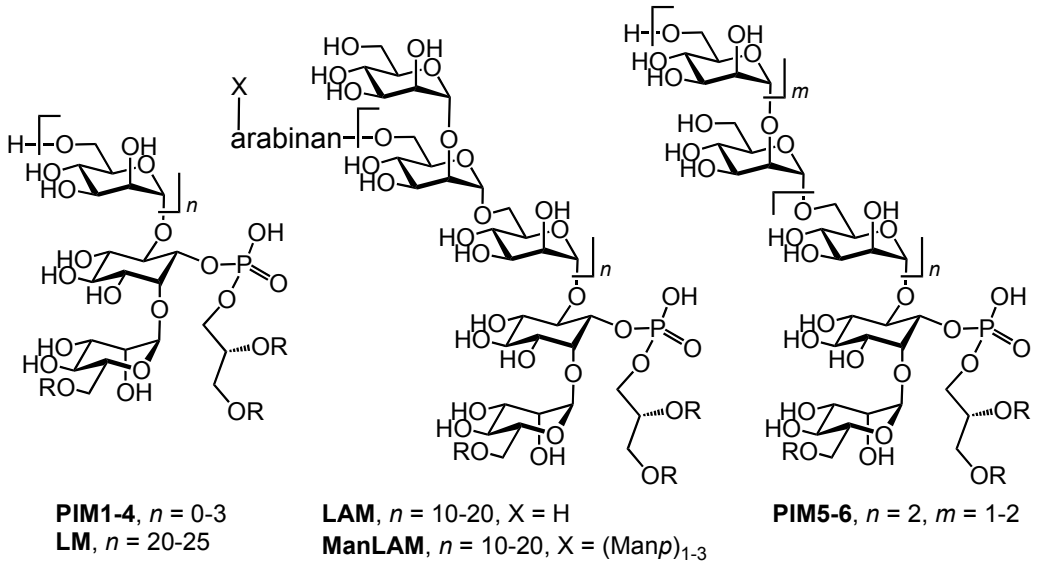


Figure 1-1. Model of the mycobacterial cell wall.

1.2.1. Lipoarabinomannan (LAM)

LAM contains four major structural features (Figure 1-2): a phosphatidyl-*myo*-inositol (PI) anchor, a core mannan chain, an arabinan domain and terminal capping motifs found at the non-reducing end of the molecule. The polysaccharide, together with its truncated analogues, lipomannan (LM) and the phosphatidyl-*myo*-inositol mannosides (PIMs), constitute the major lipoglycans of the mycobacterial cell wall.[10-13]



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

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

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

Figure 1-2. Structures of phosphatidyl-*myo*-inositol mannosides (PIMs), lipomannan (LM) and lipoarabinomannan (LAM). R = palmitate, stearate and/or tuberculostearate.

As shown in Figure 1-2, the reducing end of LAM shares structural similarities with the PIMs and LM, in that the inositol residues of all of these lipoglycans are mannosylated at the C-2 and C-6 positions.[15] The predominant fatty acyl chains attached to the glycerol moiety of the PIs are palmitate (C16:0), octadecanoic acid (stearate, C18:0) and/or 10-

methyloctadecanoate (tuberculostearate, C19). The presence of these fatty acid chains on the anchors allow the non-covalent attachment of these lipoglycans to the plasma membrane.

AcPIM4 appears to be the branch point between the PIM and LM/LAM pathways. The addition of two α-(1→2)-linked mannopyranosyl (Man*p*) residues to AcPIM4 leads to AcPIM6. It is believed that AcPIM6 is an end product in the PIM biosynthetic pathway as it is not found in the mannan backbone of LM and LAM. On the other hand, PIM4 can be extended to a linear α-(1→6)-linked mannan with a chain length of 20–25 α-Man*p* residues.[13,16,17] This linear structure is further elaborated by additional α-Man*p* units on approximately half of these residues. In species such as *M. tuberculosis*, *M. leprae*, *M. kansasii* and *M. smegmatis*[13] these side chains are attached to O-2 of the α-(1→6) linked Man*p* residues, while in *M. chelonae* they are attached to O-3.[18]

The arabinan domain of LAM is a highly branched motif. The exact structure and complexity of which depends on the identity of the mycobacterial species.[19,20] In general, each LAM carries about 50 to 80 arabinofuranosyl (Ara*f*) residues.[21] The arabinan moieties in both LAM and mAG share a common feature in which the core structures are built of α-(1→5)-linked Ara*f* with several α-(1→3)-linked branch points; the non-reducing ends are further terminated by β-(1→2)-Ara*f* motifs. Recently, a full range of arabinan chains as large as 18 to 22 residues have been released intact from *M. smegmatis* LAM by cleavage with an *endo*-

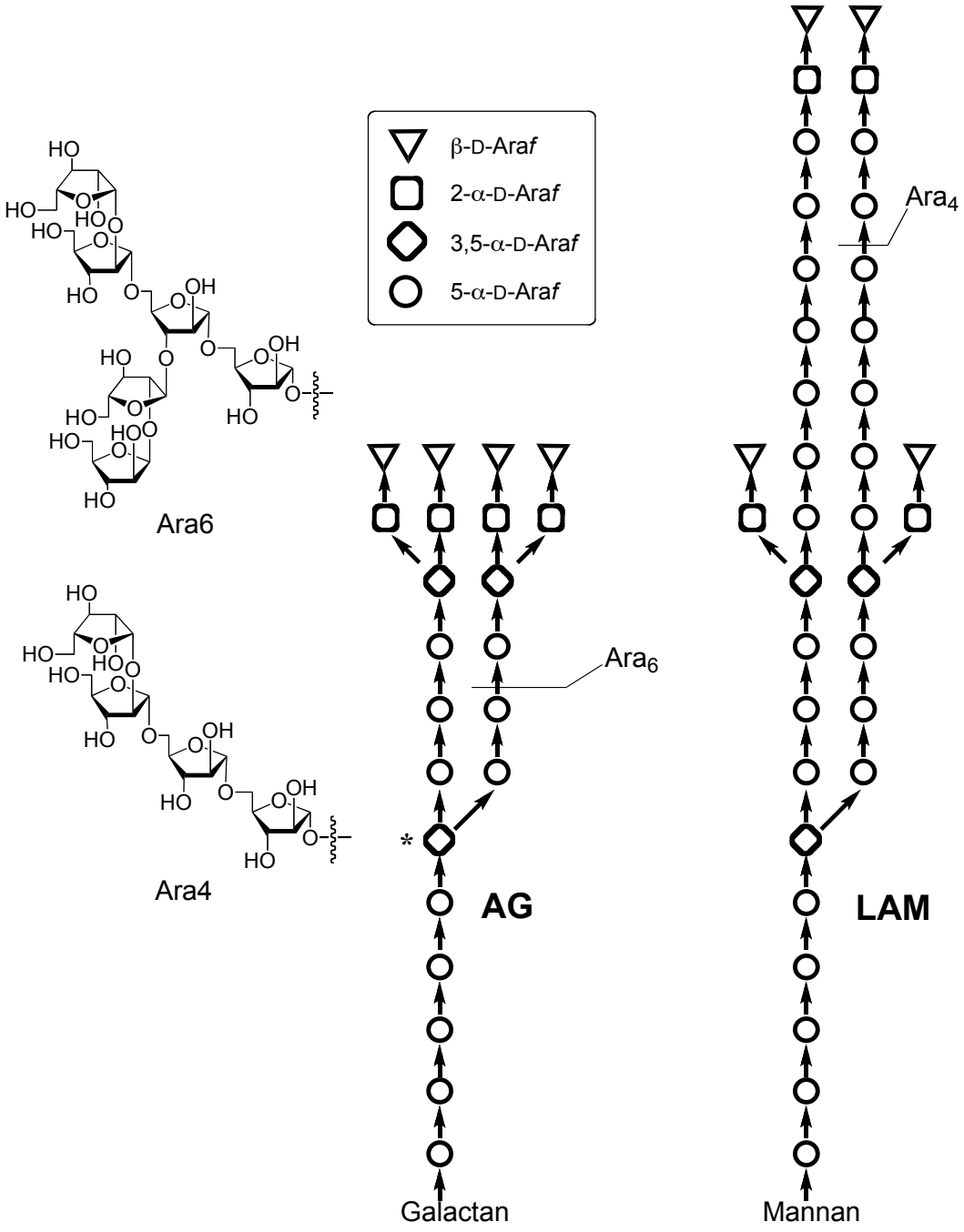


Figure 1-3. Structures of arabinans in arabinogalactan (AG) and lipoarabinomann (LAM). Hallmarks of AG-arabinan and LAM-arabinan are the presences of Ara_6 and Ara_4 termini, respectively. The asterisk indicates the attachment of either a succinyl ester or Gal*p*NH_2 at O2 of the inner-branched Ara*f* unit.

arabinase.[20] Subsequent structural elucidation by NMR spectroscopy and tandem mass spectroscopy provided data that are consistent with a structural model as shown in Figure 1-3. Unlike mAG-arabinan (see discussion below), the number of arabinose residues attached to the termini in LAM-arabinan appears to vary from species to species.

The nature of the LAM capping motifs is also species specific and these groups include short oligomannopyranosides (*M. tuberculosis, M. leprae, M. bovis* and *M. avium*),[22] inositol phosphate motifs (*M. smegmatis*)[23] and 5-thiomethyl-xylofuranose residues (*M. tuberculosis*).[24] The attachment of single α-Man*p* residues or short oligomanno-pyranosides to the non-reducing end of arabinan has been suggested to be an important feature of LAM (referred as ManLAM) found in pathogenic species of mycobacteria such as *M. tuberculosis, M. leprae,* and *M. avium*. The mannose capping motifs were shown to promote the binding and entry of these pathogens into antigen-presenting cells.[14] However a recent paper has questioned the role of these capping motifs in virulence.[25]

1.2.2. Mycolyl-Arabinogalactan Complex (mAG)

In addition to lipoarabinomannan (LAM), the other major entity of the mycobacterial cell wall is the mycolyl-arabinogalactan (mAG) complex. This glycoconjugate is the largest structural component of the cell wall and contributes ~35% of the cell wall mass. Interestingly, unlike LAM, the core structure of this mAG moiety is mainly made up of five membered ring

sugar residues, D-galactofuranose (Gal*f*) and D-Ara*f*, which constitute the galactan and arabinan subcomponents, respectively.[26]

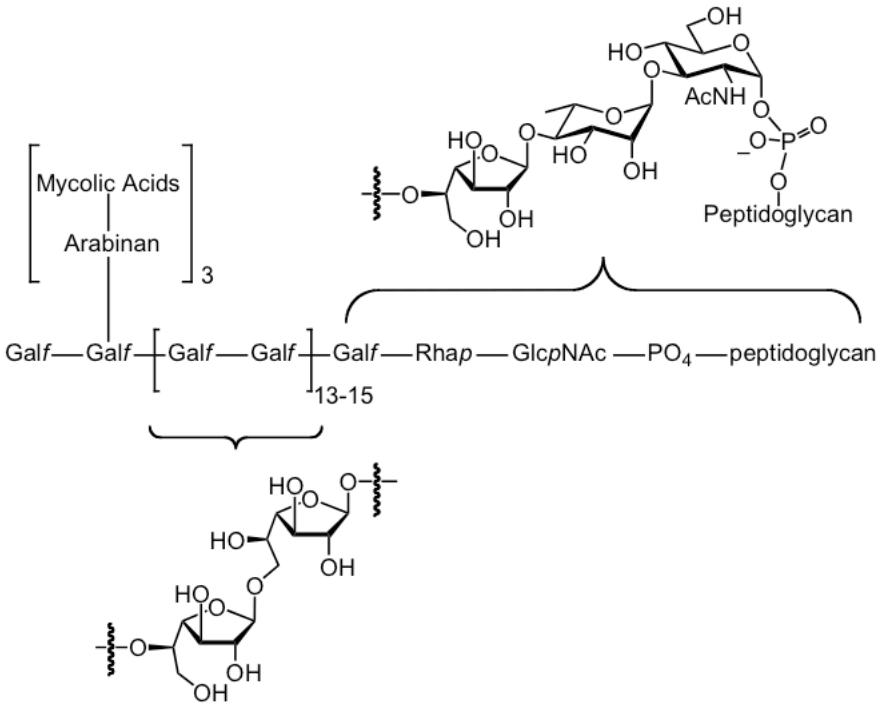


Figure 1-4. Structure of the mycobacterial mAGP complex in which the galactan and disaccharide linkage to peptidoglycan are shown. The arabinan motifs, with numbers of mycolic acids attached at the non-reducing ends, are added to the eighth, tenth and twelfth Gal*f* residues. See Figure 1-3 for the structure of the arabinan.

As shown in Figure 1-4, the linear galactan, composed of 30 to 35 alternating β-(1→5) and β-(1→6) linked Gal*f* residues,[26,27] is covalently attached to the cell wall peptidoglycan via a disaccharide linker consisting of an α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucosamine-

phosphate unit (α-L-Rha*p*-(1→3)-Glc*p*NAc-P).[28] The linear polymer is further extended with three identical arabinan units (on average, 31 Ara*f* residues each unit).[29a] Recent model studies by the Besra group using *Corynebacterium glutamicum* revealed that these three arabinan chains are linked to O-5 of the eighth, tenth and twelfth Gal*f* residues of the galactan backbone.[30] Considering the high degree of similarity of the mAG complexes between mycobacteria and corynebacteria, it is believed that similar attachments of the arabinan domains are present in mycobacterial galactan.[29a]

The non-reducing end of mAG-arabinan is quite different from that of LAM-arabinan (Figure 1-3). Instead of the linear Ara4,[20] the characteristic branched hexasaccharide units (Ara6)[31] can be found at the termini of arabinans in mAG. Also, unlike the LAM-arabinan, which is substituted with α-Man*p* residues or other capping motifs, the nonreducing termini of these arabinan moieties in the mAG are esterified with mycolic acids, which are long chain branched lipids containing 70–90 carbon atoms.[32] Approximately two-thirds of the Ara6 motifs are mycolated while the remaining one-third of these domains are unmodified.

Three distinct classes of mycolic acids are found in the mycobacterial cell wall. They are the α-, methoxy- and keto-mycolic acids (Figure 1-5).[33] α-Mycolic acids, the most abundant form (>70%), is a *cis*, *cis*-dicyclopropyl fatty acid. Depending on the strains, the length of the terminal alkyl chain and the number of methylene groups between the

cyclopropyl rings and carboxyl group can be varied. In addition, structural variations can also be found from both methoxy- and keto-mycolic acids, which contain either *cis*- or *trans*- cylcopropane rings.[34]

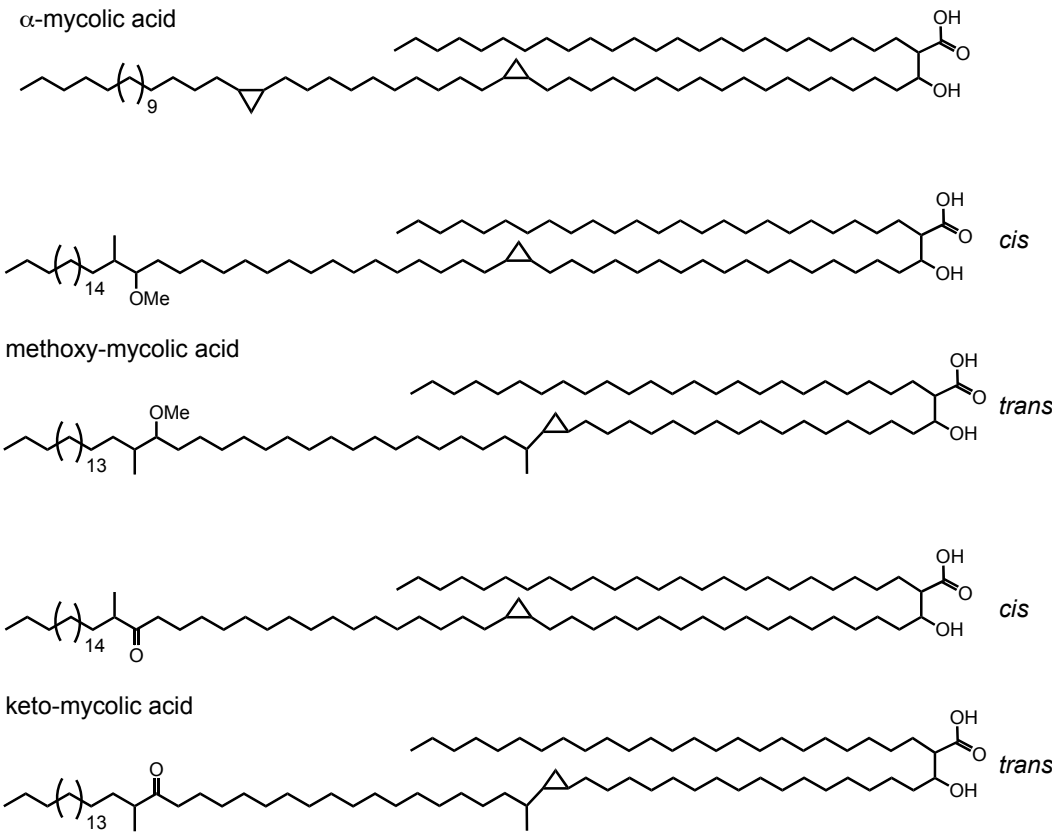


Figure 1-5. Chemical structures of mycolic acids from *M. tuberculosis*.

Early structural characterization of the arabinan domain in the mAG provided evidence that a galactosamine unit (Gal*p*NH_2) is attached to C-2 position of the internal 3,5-branched arabinosyl residue of AG (indicated by the asterisk in Figure 1-3) isolated from *M. tuberculosis* but not *M.*

smegmatis.[29a,b] A recent study in which the structure of the mAG arabinan was revisited by mass spectrometry and NMR spectroscopy by Khoo and co-workers[29c] led to further revision of the earlier proposed model of the mAG. Although the Gal*p*NH_2 units were only found in the mycolylated arabinan moiety from *M. tuberculosis*, succinyl ester groups were found in the non-mycolated arabinan chains isolated from both *M. tuberculosis* and *M. smegmatis*. However, the roles of the succinyl ester and galactosamine substituents on the arabinan moiety remain uncertain. As hypothesized by the authors, it is possible that succinylation of the inner-branched arabinose units may prevent subsequent mycolylation at the termini of AG, but this proposal remains to be investigated.

1.2.3. Other cell wall components.

In addition to the mAG and LAM, the mycobacterial cell wall contains different classes of surface glycolipids, the structures of which are species specific (Figure 1-6). The lipid portion of these molecules is thought to be bound between the mycolic acid residues at the outer surface of the cell wall. Examples of these compounds are glycopeptidolipids (GPLs) (found only in *Mycobacterium avium* and *Mycobacterium intracellulare*),[35] phenolic glycolipids (PGLs)[36] and lipooligosaccharides like 2,3-di-*O*-acyltrehalose.[37] As these species are not relevant to my thesis, they will not be addressed any further here, but a number of reviews on these species have been published.[37-40]

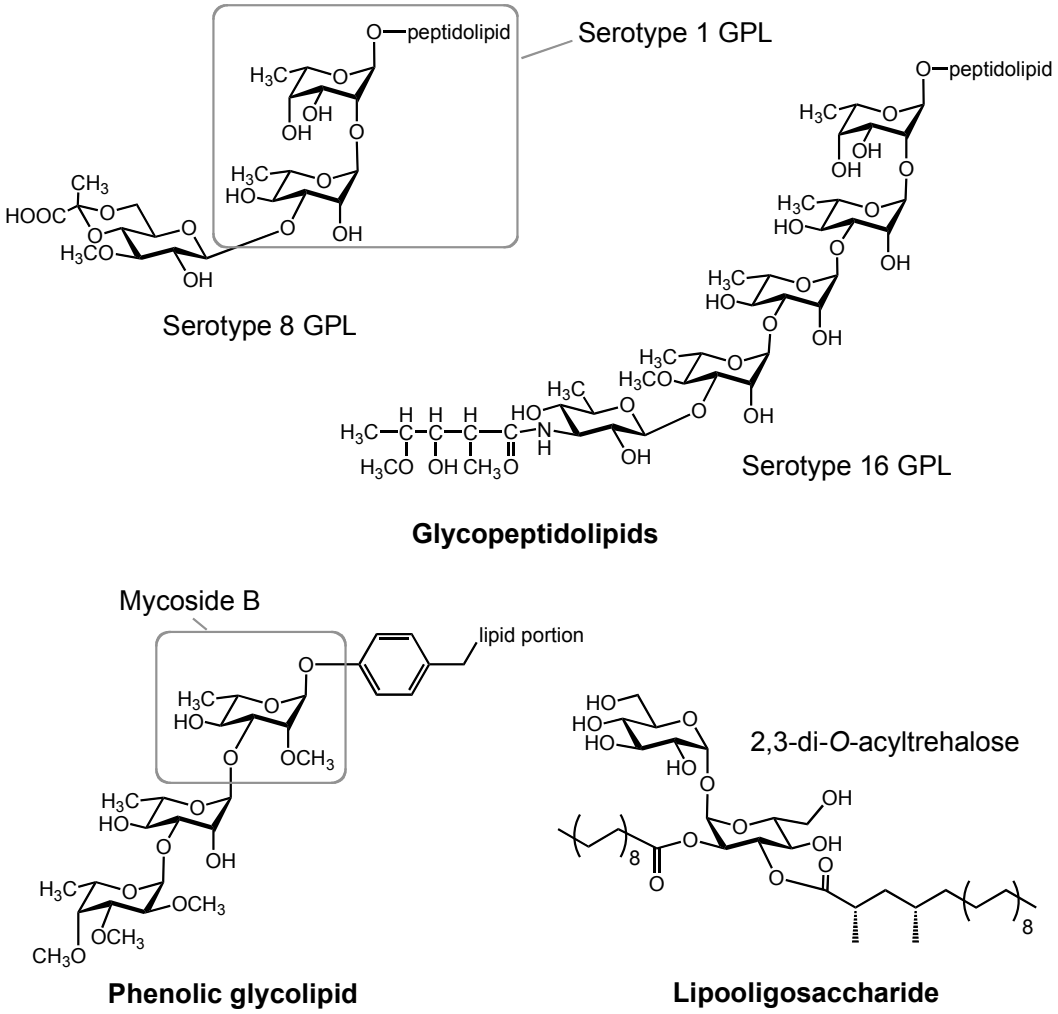


Figure 1-6. Examples of surface glycoplipids.

1.3. Glycosyltransferases Involved in Cell Wall Biosynthesis

As described in section 1.2, the mycobacterial cell wall is made up of arrays of carbohydrate-containing molecules. The biosynthesis of

complex glycans such as the mAG complex and LAM require numbers of enzymes, namely glycosyltransferases (GTs). Given the important roles of LAM and the mAG complex in the progression of mycobacterial disease, inhibitions of GTs involved in these biosynthetic pathways could provide a new avenue for drug development against TB.

Although the chemical composition of the mycobacterial cell wall and its individual components are now well understood, unfortunately, the individual steps in the biosynthetic pathways remain poorly defined. This is mainly due to difficulties in the identification and subsequent isolation of the corresponding GTs. Also, the synthesis of potential substrates and intermediates with such complexity is another daunting task in studying these enzymes. In fact, the GTs that participate in these biosynthetic pathways are just beginning to be unravelled, but only with the aid of the complete mycobacterial genomes[41] and considerable effort in related genetic and biochemical studies.[40] The following sections provide a brief overview of current knowledge and recent findings of GTs involved in mAG and LAM biosynthesis.

1.3.1. Biosynthesis of LAM

1.3.1.1. Assembly of PIMs

The common core structure among the PIMs, LM and LAM suggest that the early steps in their biosynthesis are the same.[15a] That the biosynthesis of the PIMs originated from the PI anchor was first

demonstrated by Brennan and Ballou as early as four decades ago.[42] As shown in Figure 1-7, PI is synthesized by PI synthase (PgsA1) through the condensation of *myo*-inositol and the diglyceride of CDP-diacylglycerol.[43] The LM/LAM pathway is initiated by the transfer of an α-Man*p* residue from GDP-mannose to the O-2 position of the inositol moiety to form PIM1 (Figure 1-6). This mannosylation is catalyzed by the α-mannosyltransferase PimA.[44] *In vivo* studies have demonstrated that the formation of PIM1 and higher derived PIMs is required for proper cell growth and provided evidence that the presence of *pimA* gene, just like *pgsA1*, the gene encoding for PI synthase, is essential in mycobacteria.[44] Recently, the crystal structure of PimA in complex with GDP-Man has been determined.[45] Along with other extensive substrate binding and catalysis studies, the obtained crystal structure allowed the authors to propose a model of interfacial catalysis. It was proposed that the association of PimA with anionic membrane lipids stimulates enzyme catalysis by facilitating substrate diffusion from the lipid bilayer to the catalytic site or perhaps by inducing an allosteric changes in the enzyme structure. A more recent investigation, suggests that PimA first binds GDP-Man*p*, and then undergoes a conformational change, which provides the binding site for the acceptor substrate, PI.[46]

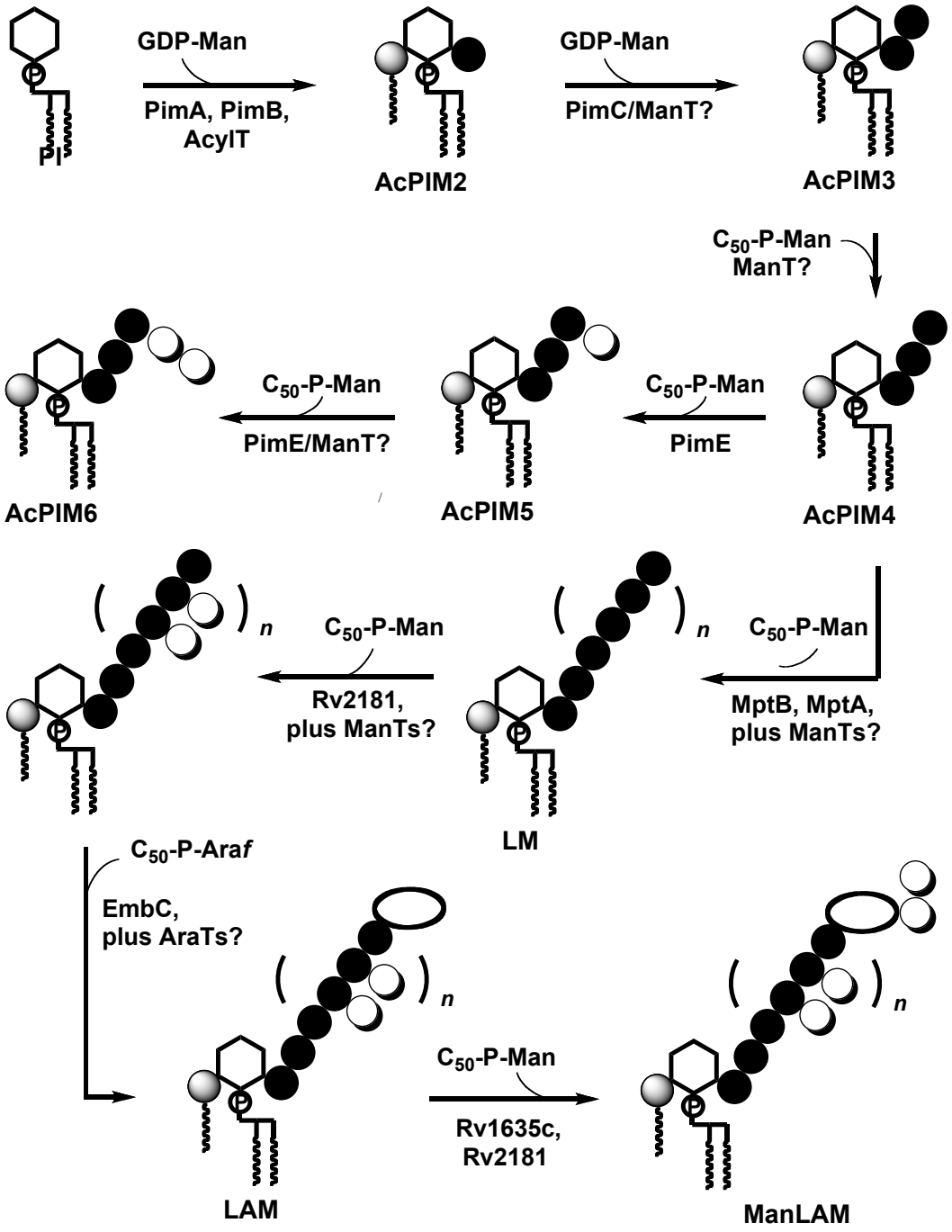


Figure 1-7. Current knowledge of GTs involved in the LAM biosynthetic pathway.

The subsequent acylation of PIM1 is catalyzed by an acyltransferase Rv2611c (AcylT). This protein has been shown to install fatty acyl groups such as palmitate and tuberculostearate at the C-6

position of the Man*p* residue linked to the C-2 position of the inositol moiety.[47] Although PIM1 is the main lipid acceptor, this enzyme also catalyzes the acyl transfer to PIM2 as demonstrated by cell-free assays conducted in the same study. In fact, the sequence of the acylation and the subsequent mannosylation catalyzed by PimB is unclear and two possible models for the formation of AcPIM2 from PIM1 have been proposed.[42,48]

The use of gene knock-out mutants in probing the role of various mycobacterial GTs is complicated by the fact that many of these mutants are not viable. To circumvent this problem, Besra and coworkers have pioneered the use of disruptive mutants in *Corynebacterium glutamicum,* which possesses similar cell wall glycoconjugates, but for which these mutations are generally not lethal.[30] This approach has been used to probe the biosynthesis of PIM2 from PIM1. Based on initial studies, it had been believed that the second α-Man*p* transfer to the 6-position of the *myo*-inositol of PIM1 (or AcPIM1) was catalyzed by the gene product of Rv0557.[49] For almost a decade this protein was referred as PimB and was proposed to catalyze the formation of PIM2 (or AcPIM2). However, a recent model study in *Corynebacterium glutamicum* by Besra and coworkers demonstrated that this protein is involved in the biosynthesis of 1,2-di-*O*-C_{16}/$C_{18:1}$-(α-D-mannopyranosyl)-(1→4)-(α-D-glucopyranosyluronic acid)-(1→3)-glycerol (ManGlcAGroAc_2).[50] As a result of this study, this protein has been reassigned as an α-mannopyranosyl-

glucopyranosyluronic acid-transferase (MgtA). Through a combination of bioinformatic, genetic and biochemical analyses, a new gene, Rv2188c has recently been identified and the corresponding gene product (PimB') has been shown to be involved in AcPIM2 biosynthesis.[51,52] Very recently, a PimB knock-out mutant was shown to produce LM and LAM still, albeit in reduced amounts. This finding supports the notion that there is redundancy built into the biochemical pathways that assemble the PIMs, LM and LAM.[53]

AcPIM2 is converted to AcPIM by α-(1→6)-mannosyl transfer catalyzed by PimC, which has been identified from *M. tuberculosis* CDC1551, a clinical isolate. However, this mannosyltransferase is dispensable as disruption of *pimC* gene in *M. bovis* BCG neither affected *in vivo* growth nor altered the composition of PIMs, LM and LAM of the mutant strain.[54] As mentioned above for PimB', the absence of a PimC homolog in *M. tuberculosis* H37Rv, *M. leprae and M. smegmatis* underscores that there appears to be redundancy in the genes that are involved in the biosynthetic pathway leading to the PIMs and also LM/LAM. Although accumulated evidence suggests that further mannosylation leads to AcPIM4,[55] a precursor of both AcPIM6 and LM/LAM, enzymes involved in its biosynthesis have yet been discovered.

It is worth mentioning that the mannosyltransferases such as PimA, PimB' and PimC, which are involved in the initial steps of the PIMs (AcPIM2 or AcPIM3 in *M. tuberculosis* CDC1551) and LM/LAM

biosynthetic pathways require GDP-mannose as sugar donor.[30,49,54] On the other hand, the syntheses of more polar AcPIMs (AcPIM4 to AcPIM6) and lipomannan are C_{35}/C_{50}-polyprenylphosphomannose (PPM) dependent.[16,55-60] The C_{35}/C_{50}-PPM, in turn, is synthesized from GDP-Man*p* and polyprenols by the polyprenol monophosphomannose synthase.[61] One of the examples of PPM-dependent enzymes is PimE, an α-(1→2)-mannosyltransferase.[56] Genetic knockout and *in vivo* metabolic labelling experiments clearly demonstrated that this enzyme catalyzes the α-(1→2)-mannosyl transfer required for AcPIM5 synthesis. At this point, whether PimE also mediates the synthesis of AcPIM6 is uncertain, although previous *in vivo* studies have shown that the conversion of AcPIM4 into AcPIM6 was quite efficient in a cell-free system.[55a]

1.3.1.2. Assembly of α-(1→6)-mannan core

As described earlier, AcPIM4 is also the precursor for LM/LAM biosynthesis. The conversion of AcPIM4 into the linear α-(1→6)-linked mannan core requires another PPM-dependent α-(1→6)-mannosyltransferase (ManT). The isolation of the enzyme involved in this polymerization step has been problematic as has the determination of the gene encoding for it. However, a cell-free assay for α-(1→6)-ManT activity has been developed and used to screen potential substrates and inhibitors of the enzyme.[57,58,61-65] Furthermore, it is unclear whether a single ManT is

responsible for the installation of all the α-(1→6)-linked residues of the core mannan.

Recently, through genetic knockout and complementation studies, Brennan and coworkers have provided evidence suggesting that more than one α-(1→6)-ManT may be involved in full length LM/LAM biosynthesis.[17] They identified an α-(1→6)-ManT (MSMEG4245) in *M. smegmatis*, as well as its corresponding ortholog Rv2174 in the pathogenic *M. tuberculosis* strain H37Rv, which is responsible for the latter elongation steps of LM biosynthesis. Accordingly, disruption of this gene resulted in the formation of truncated LMs with sizes ranging from 5 to 20 Man*p* residues.

The involvement of a novel ManT in the latter stages of LM biosynthesis has also been suggested by Besra and co-workers. Their model study also demonstrated that NCgl2093 from *C. glutamicum* (MptA), an ortholog of Rv2174, is involved in the latter stages of biosynthesis of the α-(1→6)-mannan core of LM.[60a] Following similar approaches, this research group has further identified another mannosyltransferase MptB (Rv1459/NCgl1505) involved in the core α-(1→6)-mannan biosythesis.[60b] However deletion of the homologous gene in *M. smegmatis* (MSMEG3120) had no dramatic effect on LM/LAM synthesis, again showing the functional redundancy of mycobacterial mannosyltransferases.

1.3.1.3. Introduction of α-(1→2)-Man*p* branching in mannan core

The α-(1→6)-mannan core of LM/LAM is further functionalized with single α-(1→2)-linked Man*p* units, which are added to approximately 50% of the core residues. The enzyme responsible for the introduction of these branching unit is encoded in *M. tuberculosis* by the Rv2181 gene (MSMEG4250 in *M. smegmatis*) and employs PPM as the donor.[59] In the initial model for LAM/LM biosynthesis suggested by Brennan and coworkers, the α-(1→2)-branching was proposed to follow the complete synthesis of the α-(1→6)-linked mannan.[16] However, recent findings by the Brennan group suggest an alternate pathway, in which the α-(1→6) and α-(1→2) branching ManTs may work in a concerted manner. In their investigations it was demonstrated that deletion of MSMEG4245 coding sequence led to the formation of homologous series of Man*p* polymers (22 to 34 sugar residues) varying by just one mannosyl unit in the wild type LM.

While it remains unclear whether the α-(1→2)-linked residues are introduced after, or simultaneously with, the formation of the α-(1→6)-linked backbone, the α-(1→2)-mannosyltransferases, MSMEG4250 in *M. smegmatis* and Rv2181 in *M. tuberculosis*, have been identified and their abilities to catalyze the addition of α-(1→2) branches to the mannan core of LM/LAM have been demonstrated.[59]

1.3.1.4. Biosynthesis of the arabinan domain

As revealed in Figure 1-2, the branched mannan core of LM is further arabinosylated to form LAM. Unfortunately, little is known about the biogenesis of arabinan domains of LAM, and about the number of arabinosyltransferases involved in the pathway. To date, only one arabinosyltransferase, named EmbC, has been identified to be involved, through genetic knockout and complementation studies.[66a] EmbC, together with the EmbA and EmbB proteins, is encoded by the genes in the *embCAB* operon.[41] It was shown that interruption of *embC* gene in *M. smegmatis* resulted in defects in LAM arabinan biosynthesis, although it had no effect on bacterial viability.[66a] However, a more recent study by Goude and coworkers demonstrated that the expression of the *embC* gene is essential under normal growth conditions for the pathogenic strain *M. tuberculosis*.[67] On the other hand, the synthesis of the arabinan domain of mAG is only affected by *embA* or *embB* disruption.[68] A more detailed discussion of the EmbA and EmbB proteins involved in the mAG complex biosynthesis can be found in section 1.3.2.

Despite their different roles in the cell wall biosynthesis, these three Emb proteins are predicted to share a common structural feature, which contains a domain with 13 membrane-spanning regions in the N-terminus and a globular C-terminal domain.[66a] Previous site-directed mutagenesis studies have confirmed the significance of a proline-rich motif, and an aspartic acid residue within EmbC; substitutions of these residues led to

the reduction of LAM biosynthesis.[66b] It has been shown that the globular C-terminal domain is essential for the arabinosyltransferase activity of EmbC.[20] Cleavage of the C-terminus of the protein resulted in the formation of smaller arabinans lacking the linear Ara4 moiety. Moreover, the proline-rich motif, conserved in all three Emb paralogs, has been previously characterized in polysaccharide co-polymerases, which are involved in polymerization or chain length determination of polysaccharides in other bacterial species.[66] On balance, available data suggests that EmbC, as well as EmbA and EmbB, are likely involved in Ara*f* transfer and chain length regulation for the formation of the unbranched α-(1→5) arabinan backbone (Figure 1-3).

Recently, the Chatterjee group has discovered a novel α-(1→5) arabinosyltransferase activity in membrane preparations isolated from *M. smegmatis*.[69] Although the corresponding gene has yet to be identified, the authors demonstrated that this enzyme, distinct from the Emb proteins, introduced up to five α-(1→5)-linked Ara*f* residues to synthetic arabinose-based acceptors. Structural analysis further revealed that such initiation and elongation could occur at either O-3 or O-5 position of the branched α-3,5 Ara*f* (Figure 1-8). At this point, it remains unclear whether this enzyme activity is involved in LAM or AG biosynthesis, or both.

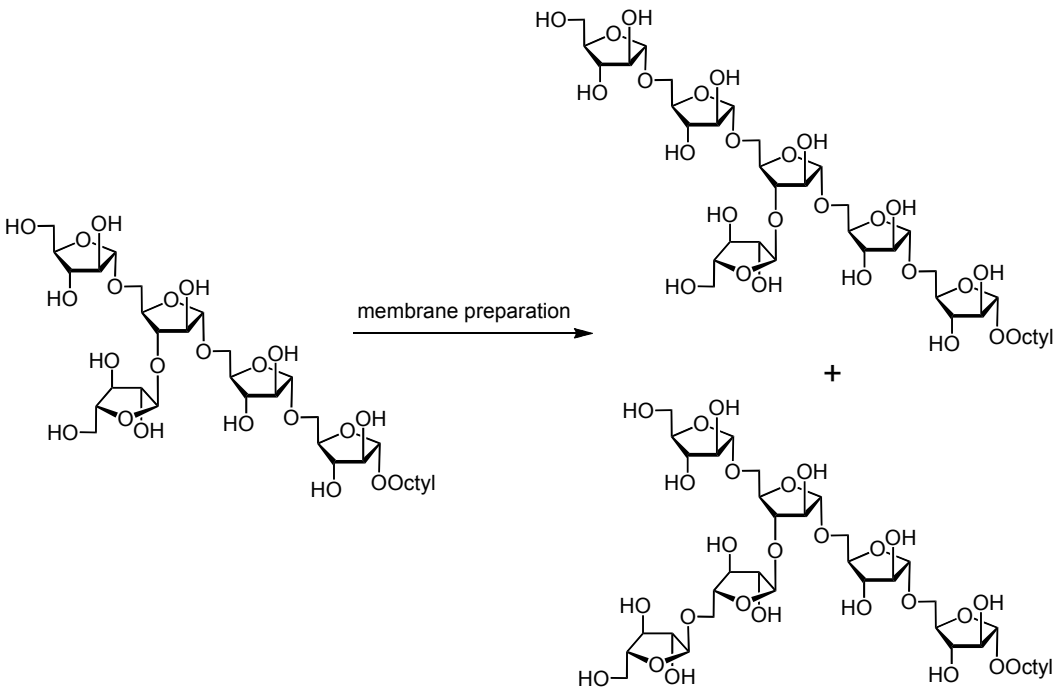


Figure 1-8. Reaction catalyzed by a novel α-(1→5) arabinosyltransferase activity in membrane preparations.[69]

1.3.1.5. Synthesis of capping motifs

The enzymes involved in the addition of the capping motifs leading to ManLAM, PI-LAM or the species capped with 5-thiomethylxylofuranose residues have not been widely studied. However, a PPM-dependent α-(1→2)-ManT involved in LAM capping to generate ManLAM has been identified.[70] Accordingly, the mannosyltransferase MT1671/Rv1635c catalyzes the first α-(1→2) mannosylation at the non-reducing arabinan termini of LAM. Interestingly, a more recent study showed that the further addition of α-(1→2) Man*p* residues are catalyzed by the GT encoded by Rv2181, the same enzyme that is responsible for the mannan core

branching.[71] This latter finding thus suggests that the Rv2181-encoded GT recognizes a single Man*p* residue as the acceptor, rather than larger more structurally-unique motifs.

1.3.2. Biosynthesis of mAG Complex

1.3.2.1 Assembly of linker disaccharide

As illustrated in Figure 1-9, the biosynthetic pathway of mAG complex initiates from the sequential addition of *N*-acetylglucosaminyl phosphate and rhamnose to the lipid carrier decaprenyl monophosphate to form the polyprenol bound "linker disaccharide" α-Rha*p*-(1→3)-Glc*p*NAc-P-P-Polyprenyl (Rha*p*-Glc*p*NAc-P-P-C_{50}). These steps are catalyzed by a Glc*p*NAc-phosphotransferase (Rfe, Rv1302, a homolog of WecA from *E. coli*) and a rhamnosyltransferase (WbbL, Rv3265c), respectively.[72] Both enzymes use sugar nucleotide donors, i.e., UDP-Glc*p*NAc or TDP-Rha*p*.

1.3.2.2 Assembly of galactan core

The subsequent formation of the galactan portion, which consists of alternating β-(1→5) and β-(1→6)-linked Gal*f* residues, is then synthesized by the action of only two bifunctional galactofuranosyltransferases. It was initially shown that Rv3782 from *M. tuberculosis*, later referred to as GlfT1, is involved in the initial stages of the galactan biosynthesis. A cell-free assay using membrane and cell wall fractions from *M. smegmatis*

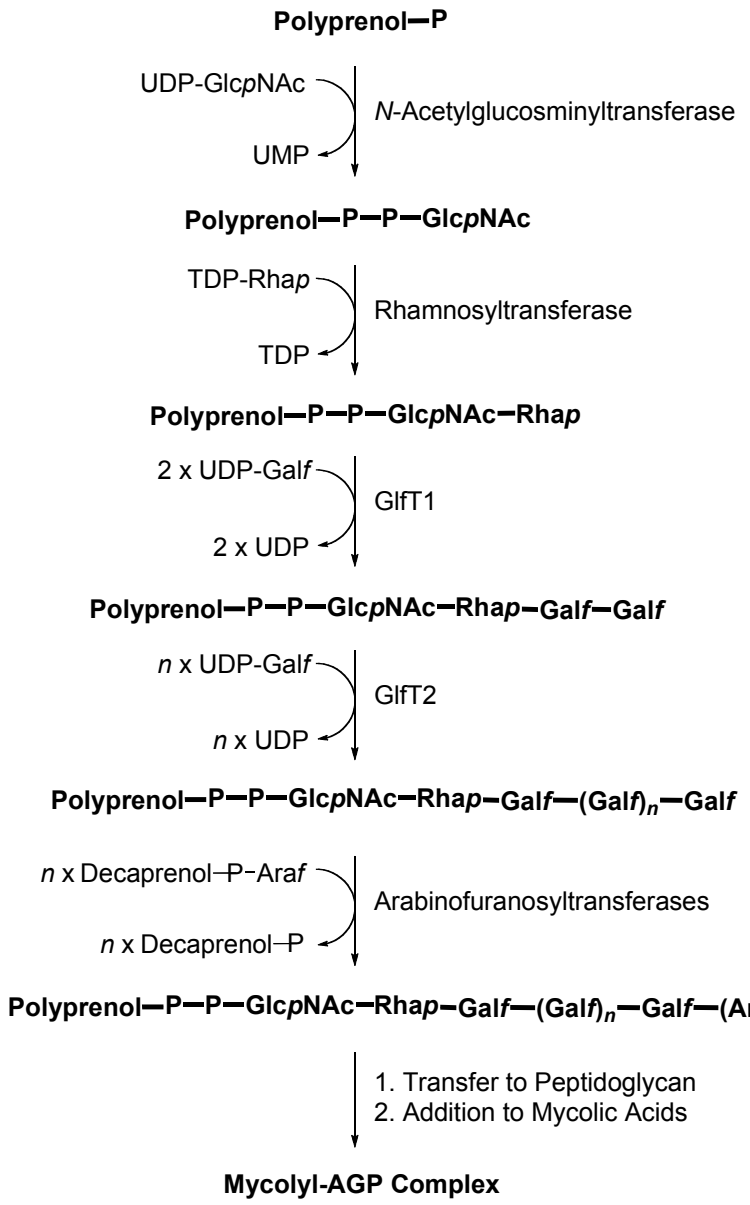


Figure 1-9. Current knowledge of GTs involved in the mAG biosynthetic pathway.

containing overexpressed GlfT1 led to the formation of Gal*f*-Rha*p*-GlcNAc*p*-P-P-C_{50} and Gal*f*-Gal*f*-Rha*p*-GlcNAc*p*-P-P-C_{50}, suggesting the enzyme catalyzes both β-(1→4) and β-(1→5) Gal*f* transfers.[73a] To further confirm the observed bifunctionality of GlfT1, the gene MSMEG_6367, an *M. smegmatis* ortholog of Rv3782, was cloned and overexpressed as a

soluble protein in *E. coli* and its dual transferase activities were demonstrated.[73b]

While GlfT1 catalyzes the additions of the first two Gal*f* units, another galactofuranosyltransferase Rv3808c in *M. tuberculosis*, now known as GlfT2, is responsible for formation of the bulk of the galactan.[74] The overexpression of GlfT2 in the membrane fraction of *E. coli* subsequently allowed for the development of a cell-free enzyme assay using disaccharide neoglycolipid acceptors with either a β-(1→5) or β-(1→6) linkage.[75] This early study unambiguously demonstrated the dual functionality of GlfT2, which is capable of catalyzing both (1→5) and (1→6) galactofuranosylations. Recently, the Lowary and Besra groups separately reported improvements in the overexpression of GlfT2 using the pET expression system in *E. coli* cells[76] in the absence and presence of chaperone proteins, respectively.[77]

The availability of GlfT2 in its soluble form should provide opportunities for other biochemical experiments such as protein crystallization and kinetic studies. For example, previous evaluation of a panel of synthetic mono- and oligosaccharide analogs have shown that trisaccharides are better substrates than disaccharides for GlfT2.[76] In addition, access to the protein will allow more detailed studies focusing on the bifunctional nature of the enzyme. One issue is to determine whether there are one or two active sites. In this regard, a recent paper has reported the use of competition saturation transfer difference NMR

spectroscopy on GlfT2, which suggested that the enzyme has a single active site.[78]

As indicated in Figure 1-9, both GlfT1 and GlfT2 use the sugar nucleotide UDP-Gal*f* as the donor species. In turn, UDP-Gal*f* is synthesized from UDP-Gal*p* by the enzyme UDP-Gal*p* mutase (Rv3809c, Glf).[79] On the other hand, the subsequent arabinosylations catalyzed by GTs such as the EmbCAB and Aft proteins (see sections 1.3.1.4 and 1.3.2.3), require β-D-arabinofuranosyl-1-monophosphoryl-decaprenol (C_{50}-P-Ara*f*) as the only arabinose donor.[30] It has been shown that C_{50}-P-Ara*f* is derived from 5-phosphoribosyl-pyrophosphate (pRpp), and ultimately from glucose by a number of enzymes.[80]

1.3.2.3 Assembly of arabinan domain

It has been known for some time that both EmbA and EmbB are involved in the mAG arabinan biosynthesis, and that these proteins are the target of the antituberculosis drug ethambutol (EMB).[68a] More recently another family of AraT's, the Aft enzymes, have been identified. Similar to the Emb proteins, the Aft enzymes use DPA as the donor source; however, in contrast to these more well-studied AraT, they are not inhibited by EMB.[81-84]

In the search for the function of the Emb and Emb proteins, it was shown that disruption of either *embA* or *embB* in *M. smegmatis* led to the formation of linear Ara4 motif (β-Ara*f*-(1→2)-α-Ara*f*-(1→5)-α-Ara*f*-(1→5)-α-

Ara*f*) instead of the characteristic terminal Ara6 motif ([β-Ara*f*-(1→2)-α-Ara*f*]$_2$-3,5-α-Ara*f*-(1→5)-α-Ara*f*) as shown in Figure 1-3.[68b] Recently, Chatterjee and coworkers have developed a cell-free assay using pR[^{14}C]pp as the indirect donor.[85] They demonstrated that the simultaneous expression of both EmbA and EmbB is required for the transfer of two Ara*f* residues, β-Ara*f*-(1→2)-α-Ara*f*, to the synthetic linear Ara4.

One of the hypotheses suggested was that these two proteins act in concert to install the α-(1→3)-branched Ara*f* residue to the Ara4 motif, and that additional enzymes install the second Ara*f* residue. In fact, the discovery and characterization of a novel AraT, termed AftB (Rv3805c in *M. tuberculosis* and its orthologue NCgl2780 in *C. glutamicum*), is consistent with such a hypothesis. Deletion of the *aftB* gene in *C. glutamicum* resulted in the complete absence of terminal β-(1→2)-Ara*f* residues in the AG. In addition, this mutant strain was fully complemented by the expression of either Rv3805c or NCgl2780.[81] At this point it is uncertain whether AftB is solely responsible for the successive addition of two terminal arabinosyl units at a 3,5-branched Ara*f* residue leading to the Ara6 motif (See Figure 1-3) or if an additional AraT is required for the second β-(1→2)-Ara*f* addition.

In addition to the above hypothesis, it is also possible that the EmbA and/or the EmbB protein may act as an α-(1→5) AraT that is responsible for the synthesis of the entire linear interior arabinan portion of

AG. Indirect support for this hypothesis, comes from the recent identification of an α-(1→3)-arabinosyltransferase AftC (Rv2673 in *M. tuberculosis* and MSMEG2785 in *M. smegmatis*) by Besra and coworkers.[82] Deletion of the *M. smegmatis aftC* gene led to the absence of α-(1→3)-Ara*f* residues on AG. On the other hand, this mutant strain was fully complemented by the expression of either MSMEG2785 or Rv2673. Interestingly, *in vitro* cell-free assay with membrane preparation in the presence of synthetic Ara2 acceptor showed no α-(1→3)-linked Ara*f* products, but detectable levels of α-(1→5)-AraT activity; this observed α-(1→5) transfer was inhibited by the presence of EMB. On the contrary, the α-(1→3)-transferase activity of AftC was revealed using linear α-(1→5)-Ara5 acceptor. More importantly, unlike Emb proteins, the observed AftC activity was unaffected by the presence of EMB. These results taken together are consistent with an arabinan biosynthetic pathway in which the 3,5-branched point(s) are introduced by AftC and the linear Ara*f* portions by α-(1→5)-GT(s), with EmbA and EmbB as the possible candidates.

In search for the orthologous *embA* and *embB* genes within *Corynebacterianeae*, it was found that *C. glutamicum* possesses only a single *emb* gene.[30] Deletion of this particular gene led to a truncated AG with only three terminal Ara*f* residues attached to O-5 of the eighth, tenth and twelfth Gal*f* residues of the galactan backbone. The significance of this study is that it led to the subsequent identification of the arabinosyltransferase AftA (Rv3792), which catalyzes the first Ara*f*

addition to the galactan chain.[83] The homolog of Rv3792 in *M. smegmatis*, MEMEG_6386, has also been identified recently.[84] Based on current published data, Besra and coworkers have proposed a model for the mAG arabinan biosynthesis, which is shown in Figure 1-8.[82] However, the full pathway remains, at present, far from fully elucidated.

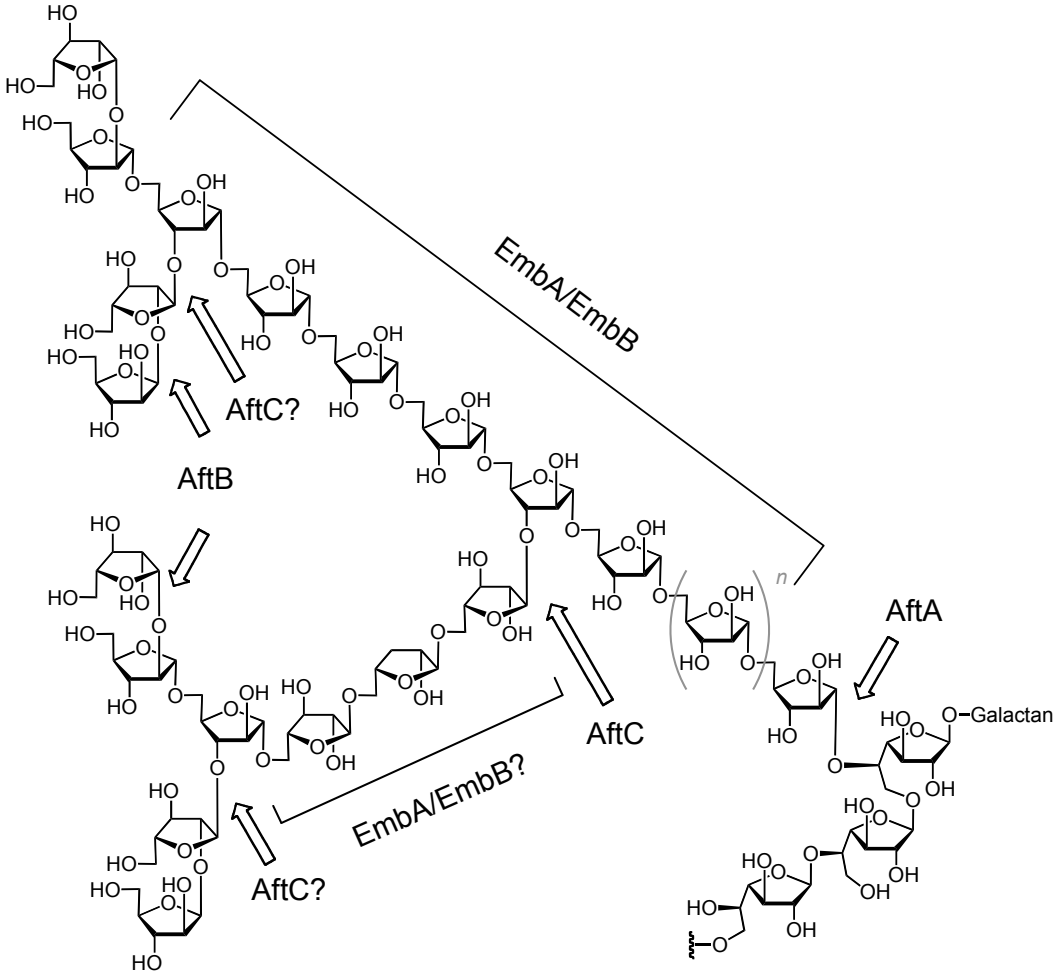


Figure 1-10. Proposed model of arabinan biosynthesis.

1.4. The Mycobacterial Cell Wall As a Potential Drug Target

1.4.1. Current Antituberculosis Drugs

Successful treatment of TB and other mycobacterial diseases requires a long period of drug treatment involving a number of antibiotics.[9] The standard regimens require two months treatment with ethambutol (EMB), isoniazid (INH), pyrazinamide and rifampin (RMP) followed by four months of INH and RMP treatment (Figure 1-11).[86]

In addition to these front-line drugs, there are six classes of drugs also commonly used for the treatment of TB. They are the aminoglycosides amikacin and kanamycin, polypeptides such as capreomycin and viomycin, fluoroquinolones (e.g., ciprofloxacin and moxifloxacin), thioamides including ethionamide and prothionamide, cycloserine, and *p*-aminosalicyclic acid (PAS) (Figure 1-11). These classes of compounds are considered as second-line treatments due to the fact that they are less effective, more toxic or not available everywhere due to the high cost.

Other compounds (e.g., moxifloxacin, Figure 1-11 and SQ109, Figure 1-12) are promising candidates for the treatment of TB, and are currently in clinical trials.[87] The biochemical processes targeted by these compounds include the biosynthesis of fatty acid, RNA, DNA, protein, cell wall biosynthesis, etc. The mechanisms of action of these antituberculosis drugs or antimycobacterials have been discussed and are summarized in recent review articles.[86a,87,88]

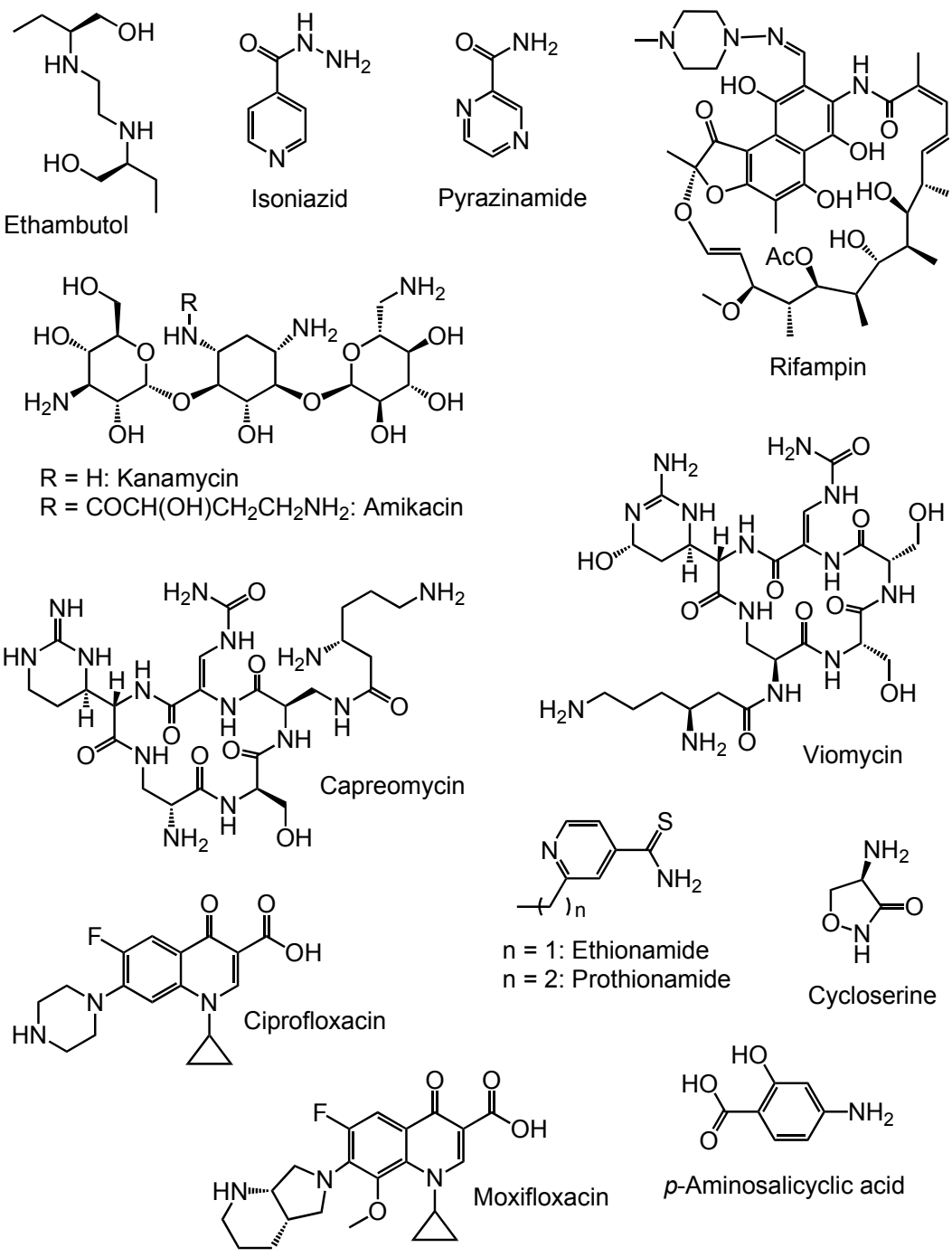


Figure 1-11. First and second line drugs used for the treatment of tuberculosis.

Of particular relevance to the research described in this thesis are the classes of molecules that specifically inhibit mycobacterial cell wall biosynthesis. One example is INH, which interferes with mycolic acid biosynthesis.[89] An even more relevant drug is EMB. Although its precise biochemical target is still uncertain, an accumulating body of evidence has demonstrated that its inhibitory effect is focused on arabinan biosynthesis.[21,66b,90] In fact, the effect on AraTs involved in LAM and mAG biosynthetic pathways (e.g., the Emb proteins) have been reported previously.[68,69,82,90a] Consistent with its role as an inhibitor of arabinan biosynthesis, treatment of mycobacteria with EMB leads to a reduction in the mycolic acids being expressed in the cell wall (by reducing the number of sites for mycolation) and consequently improves the efficiency of other antibiotics by making the cell wall more permeable.[91]

Although EMB continues to be one of the front-line agents in tuberculosis chemotherapy, its relatively modest minimum inhibitory concentration (10 μM) has prompted efforts to optimize the drug further. Thus, recent combinatorial chemistry approaches have generated over 10,000 compounds carrying the ethylenediamine scaffold of EMB.[92] Twenty-seven compounds were found to be as active as EMB based on a sequential set of *in vitro* and *in vivo* tests. Within the series, compound SQ109 (Figure 1-12) was identified as the most potent and is currently in phase I clinical trials.[92b] Interestingly, unlike EMB, SQ109 and two other drug leads (SQ775 and SQ786) seem to exert their inhibitory effects on

different biochemical targets and/or pathways than cell wall biosynthesis.[88a,93]

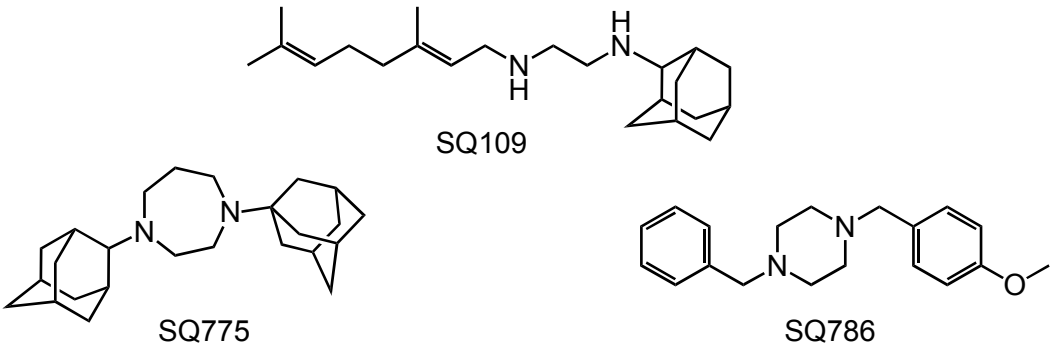


Figure 1-12. Second generation of 1,2-ethylenediamine based lead compounds with activity against *Mycobacterium tuberculosis*.

1.4.2. Cell Wall Components As New Drug Targets

As discussed in sections 1.2 and 1.3, the structural composition of LAM and the mAG complex have been well defined and many of the enzymes (particularly the GTs) that participate in the biosynthesis have been identified and characterized in recent genetic and biochemical studies.[40] Although the enzymes in these pathways appear as attractive pharmaceutical targets, a better understanding of their mechanisms and the substrate specificities is essential.

One of the common approaches for studying substrate–protein interactions involves the chemical synthesis of analogs that closely mimic the natural substrate. The biochemical screening of such compounds often reveals important information about how these enzymes interact with their

substrates at the molecular level. Such fundamental studies should provide general guidance for the design of potential inhibitors, which are potential lead compounds for novel therapeutic agents that are active against tuberculosis.

1.5. Overview of Thesis Research

Among the many GTs that catalyze polysaccharide assembly during the biosynthesis of LAM and the mAG complex (section 1.3), a PPM-dependent α-(1→6)-mannosyltransferase (ManT) and the β-(1→5, 6)-galactofuranosyltransferase (GlfT2) responsible for the polymerization of the linear portions of lipomannan and galactan, respectively, are the main proteins of my research interest.

At the outset of this project, although ManT activity had been detected using an established radiochemical assay *in vitro*,[58] its substrate specificity was poorly defined. To provide a better understanding of how the enzyme interacts with the acceptor, my initial efforts were focused on synthesizing substrate analogs including methoxy (OCH_3) and hydrogen (H)-substituted disaccharides, as well as α-(1→6)-linked Man*p* oligomers. These analogs were subsequently evaluated as potential substrates and/or inhibitors of ManT. Details of the synthetic work towards the desired analogs are presented in Chapter 2. The biological evaluation of these compounds is reported in Chapter 3. The work described in these two chapters enabled us to probe the steric and hydrogen-bonding

requirements of the enzyme and the effect of acceptor length on enzymatic activity.

On the basis of the biochemical results presented in Chapter 3, a panel of epimeric and amino disaccharide derivatives was synthesized and evaluated (Chapter 4). In addition, to explore the effect of acceptor length on ManT further, a homologous series of linear sulfur-linked oligomannosides was also synthesized and screened against the enzyme. (Chapter 5). As described in these two chapters, screening of these analogs allowed us to acquire additional information regarding the substrate preference of ManT.

In addition to probing the substrate specificity of ManT, a substantial amount of my research effort was focused on GlfT2. We were interested in synthesizing a panel of mono- and dideoxy trisaccharides, which could be evaluated as potential substrates and/or inhibitors of the enzyme. In addition to mapping the hydrogen-bonding network in the active site of GlfT2, these compounds would be useful in addressing whether some of the hydroxyl groups on the substrates are essential for the proper binding to the enzyme and control the alternating (β-(1→5) vs β-(1→6)) glycosidic bond formation. Details of the synthetic work and discussion of the biological results will be described in Chapter 6.

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Chapter 2

Synthesis of Substrate Analogs for Probing the Substrate Specificity of α-(1→6)-Mannosyltransferase

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2.1. Introduction

A model for the biosynthesis of mycobacterial LAM was proposed ten years ago by Brennan and co-workers (Figure 2-1)[1] and since that time many of the steps in this process have been supported by biochemical and genetic evidence.[2-10] Of particular relevance to the work described here is the biosynthesis of the mannan core of LAM, which has received significant scrutiny. Current evidence points to a process in which a number of mannosyltransferases (ManT's),[4,7-10] and acyltransferases[3] act in concert by adding single monosaccharide residues and acyl groups to PI leading initially to AcPIM2 and then AcPIM4, which serves as a key branch point between PIM and LM/LAM biosynthesis. From AcPIM4, the α-(1→6)-linked backbone of the mannan core is assembled and the α-(1→2)-linked mannopyranosyl side chains are introduced. Whether the α-(1→2)-linked residues are introduced after, or simultaneously with, the formation of the α-(1→6)-linked backbone is unknown. However, in the initial model suggested by Brennan and co-workers, the α-(1→2)-branching was proposed to follow the complete synthesis of the α-(1→6)-linked mannan.[1]

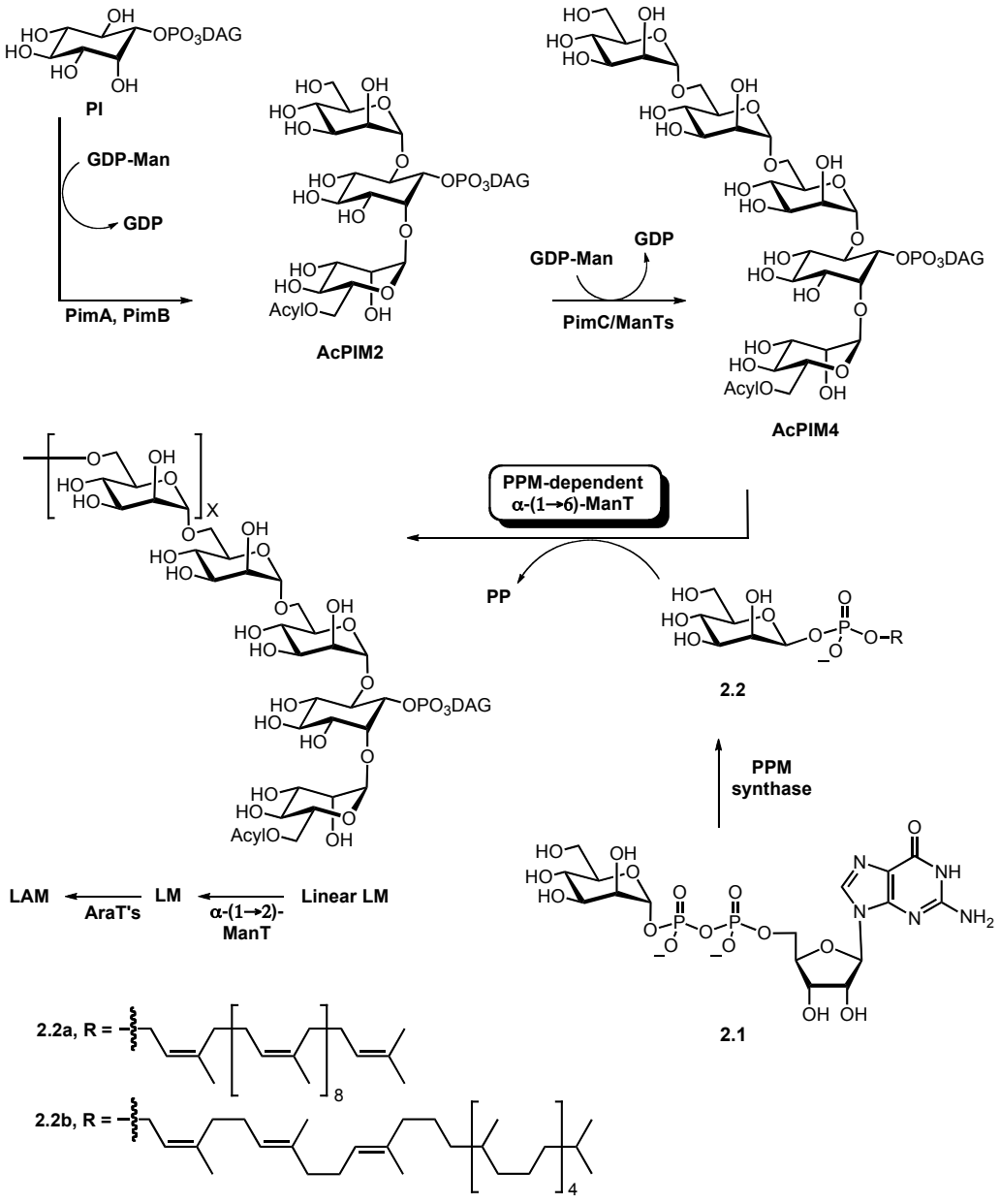


Figure 2-1. Proposed biosynthetic pathway for mycobacterial LM and LAM. DAG, diacylglycerol; PP, polyprenolphosphate; AraT's, arabinosyltransferases.

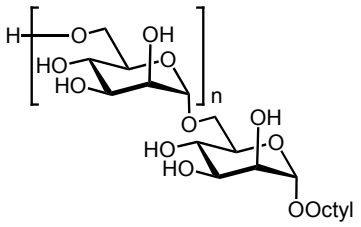
The donor substrates for these ManT's are either GDP-mannose (GDP-Man, **2.1**) or polyprenolphosphomannose (PPM, **2.2**), which is synthesized from **2.1** by the enzyme polyprenol monophosphomannose synthase.[6] The ManT's involved in the initial biosynthetic steps leading to AcPIM2 and AcPIM4, including PimA, PimB and PimC (only found in *M. tuberculosis* CDC1551), which use GDP-Man as the donor species have received the most attention.[4] Among the achievements in this area is the recent report of a crystal structure of PimA in complex with GDP-Man.[11]

In contrast, the enzymes that utilize PPM as the donor species have been less well studied, but three PPM-dependent α-(1→2)-ManT's, which are involved in LM/LAM core branching,[8] arabinan motif capping,[9] and AcPIM6 biosynthesis[10] have been identified. To date, the PPM-dependent α-(1→6)-ManT involved in LM/LAM assembly has remained elusive, but a cell-free assay for its activity has been developed and has been used to screen the potential substrates and inhibitors of the enzyme.[6,12-18] It is, at this point, unclear whether a single ManT is responsible for the installation of all the α-(1→6)-linked residues of the core mannan. Indeed, very recently, through genetic knockout and complementation studies, Brennan and coworkers have provided evidence that suggests that more than one α-(1→6)-ManT may be involved in full length LM/LAM biosynthesis.[19]

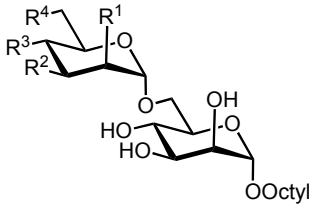
Given the important roles of LM and LAM in the progression of mycobacterial disease, a better understanding of their biosynthesis is of

interest. Our efforts in this area have been focused on probing the substrate specificity of the PPM-dependent ManT responsible for the synthesis of the α-(1→6)-linked mannan core of LM and LAM.[17,18] The specificity of this enzyme remains poorly understood and knowledge of the substrate requirements in the active site of the enzyme would facilitate design of potent and specific inhibitors. Herein, a panel of octyl mannopyranoside analogs (Figure 2-2) were synthesized and screened against this PPM-dependent mannosyltransferase (Chapter 3).

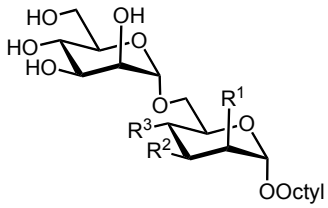
A homologous series of mono- through tetrasaccharides (**2.3**–**2.6**) was used to probe the effect of acceptor length on activity. In addition, a panel of methoxy and deoxy analogs (**2.7**–**2.20**) of the known[15] disaccharide substrate α-D-Man*p*-(1→6)-α-D-Man*p*-O$(CH_2)_7CH_3$ (**2.4**), was evaluated to explore the acceptor specificity of the enzyme further. Singly modified oligosaccharide analogs such as **2.7**–**2.20** have been of great utility in probing carbohydrate-protein interactions,[20] and these studies have provided compounds that are not only useful biochemical tools but also have led to the identification of potent glycosyltransferase inhibitors. In the present case, compounds with the latter activity are potential lead compounds for new classes of anti-mycobacterial agents.



2.3, n = 0
2.4, n = 1
2.5, n = 2
2.6, n = 3



2.7, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$
2.8, $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$
2.9, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$, $R^4 = OH$
2.10, $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$
2.11, $R^1 = OH$, $R^2 = OH$, $R^3 = OCH_3$, $R^4 = OH$
2.12, $R^1 = OH$, $R^2 = OH$, $R^3 = H$, $R^4 = OH$
2.13, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OCH_3$
2.14, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$



2.15, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = OH$
2.16, $R^1 = H$, $R^2 = OH$, $R^3 = OH$
2.17, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$
2.18, $R^1 = OH$, $R^2 = H$, $R^3 = OH$
2.19, $R^1 = OH$, $R^2 = OH$, $R^3 = OCH_3$
2.20, $R^1 = OH$, $R^2 = OH$, $R^3 = H$

Figure 2-2. Synthetic acceptor analogs used as probes of the PPM-dependent ManT involved in the synthesis of the α-(1$\rightarrow$6)-linked mannan core of LM and LAM**.**

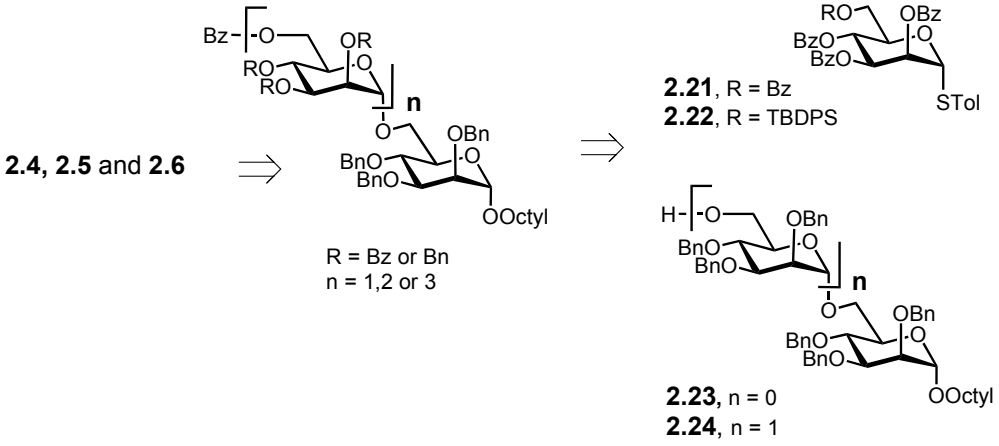
2.2. Results and Discussion

The activity of a PPM-dependent α-(1→6)-mannosyltransferase in a membrane preparation from *M. smegmatis* was first demonstrated by Yokoyama and Ballou[12] and a cell-free assay was later developed by Brown *et al.*[15] Both studies showed that the ManT utilizes β-D-mannopyranosyl phosphodecaprenol (**2.2**) (synthesized in situ from **2.1** and decaprenol phosphate) as the donor and catalyzes the α-(1→6)-mannosylation of oligomannopyranoside acceptors. In particular, the latter studies[15] demonstrated that octyl dimannopyranoside **2.4** (Figure 2-2) is a good acceptor for the enzyme. The hydrophobic nature of the octyl agylcone allows convenient product isolation and characterization after the assays,[21] and a small panel of analogs of **2.4** was recently screened as potential substrates and inhibitors of the enzyme.[17,18] In our current study, a larger series of synthetic octyl mannopyranoside acceptors was synthesized and tested as substrates for the ManT under the established assay conditions[12-18] to probe the specificity of the enzyme further (see Chapter 3).

2.2.1. Synthesis of Octyl Mannopyranosides 2.4–2.6

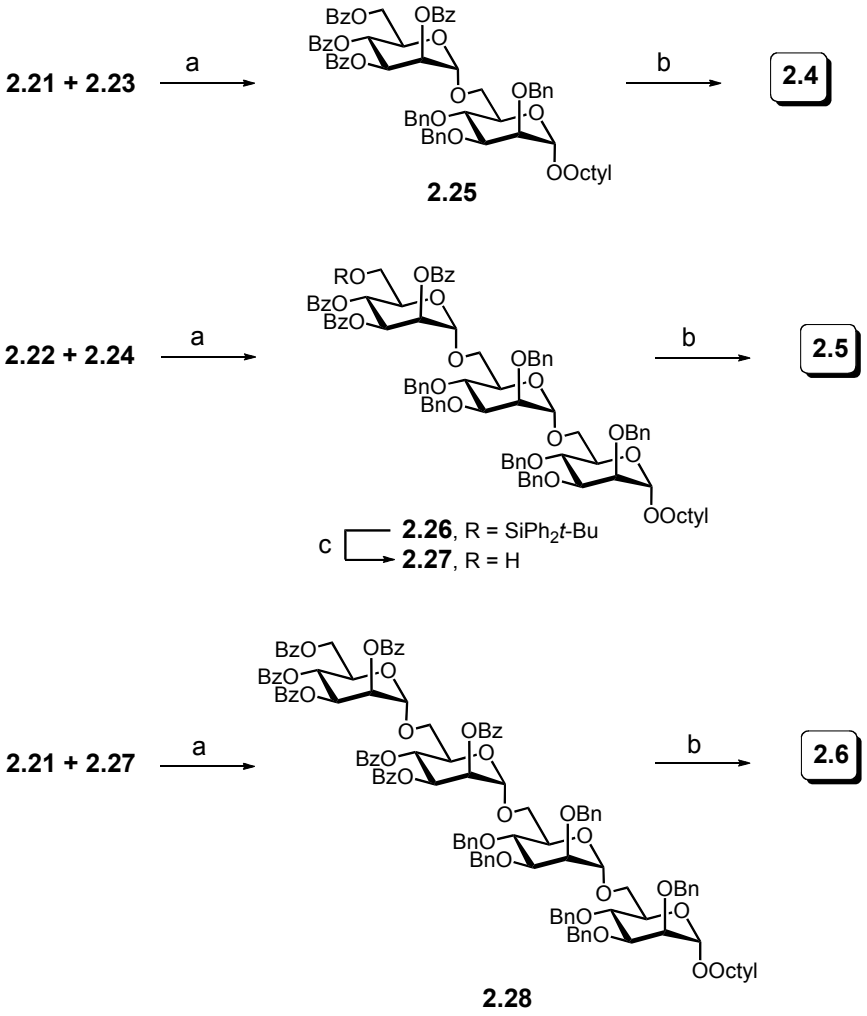
Considering that AcPIM4, the proposed initial substrate for the PPM-dependent α-(1→6)-ManT, consists of a linear α-(1→6)-trimannopyranoside, oligomannosides longer than two residues may be better acceptors. Indeed, the early studies of Yokoyama and Ballou

demonstrated that longer methyl oligomannosides could act as substrates for this α-(1→6)-ManT with improving K_M values.[12] To determine the effect of the acceptor length of the octyl glycoside counterparts on ManT catalysis, oligosaccharides **2.4**–**2.6** were synthesized from the known thioglycoside **2.22**[22] and acceptors **2.23** and **2.24** as shown in Scheme 2-1 using the overall general strategy developed by Watt and Williams.[22] The donor **2.21** was synthesized in 64% yield over two steps from D-mannose, by first benzoylation and, without purification, reaction with *p*-thiocresol and boron trifluoride etherate (as described in the experimental section). The synthesis of **2.23** and **2.24** are described in Schemes 2-5 and 2-7, respectively.



Scheme 2-1. Retrosynthetic analysis of **2.4**, **2.5** and **2.6**.

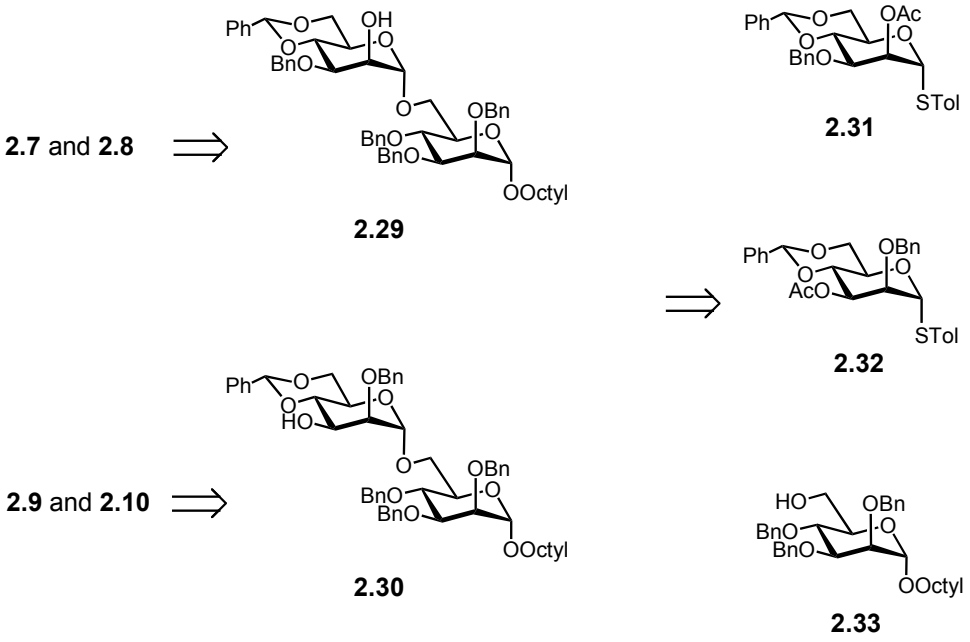
As illustrated in Scheme 2-2, the coupling of thioglycoside **2.21** with alcohol **2.23** using *N*-iodosuccinimide–trimethylsilyl triflate (NIS–TMSOTf) activation[23] afforded the corresponding disaccharide **2.25** in 96% yield. Subsequent debenzoylation of **2.25** using sodium methoxide followed by hydrogenolysis afforded the desired disaccharide **2.4**[17] in 87% over two steps. Under the same glycosylation conditions, the coupling of donor **2.22** and acceptor **2.24** afforded trisaccharide **2.26** in excellent yield (98%). To avoid possible acyl migration and debenzoylation using tetra-*n*-butylammonium fluoride, intermediate **2.26** was desilyated using hydrogen fluoride in pyridine[24] to give **2.27** in 90% yield. Subsequent removal of the benzoyl and benzyl protecting groups under standard conditions afforded the target trimannoside **2.5**[22] in 95% overall yield. With alcohol **2.27** in hand, coupling with thioglycoside **2.21** under NIS–TMSOTf activation provided the corresponding protected tetrasaccharide **2.28** in good yield (90%). Final deprotection furnished the desired oligomannoside **2.6**[22] in 62% yield over two steps. In the glycosylation reactions described above, the α-stereochemistry of the glycosidic linkages was confirmed by the one-bond $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants for the anomeric carbon atoms.[25] For all products, this value was between 167 and 174 Hz, clearly indicating the α-stereochemistry.



Scheme 2-2. Reagents and conditions: a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 96% for **2.25**, 98% for **2.26,** 90% for **2.28**; b) i: $NaOCH_3$, MeOH or MeOH/CH_2Cl_2 (8:1); ii: H_2, 20% $Pd(OH)_2$, MeOH; over two steps: 87% for **2.4**, 95% for **2.5,** 62% for **2.6**; c) HF·pyridine/ pyridine/ THF (1: 2: 5), 90%.

2.2.2. Synthesis of Deoxy And Methoxy Analogs

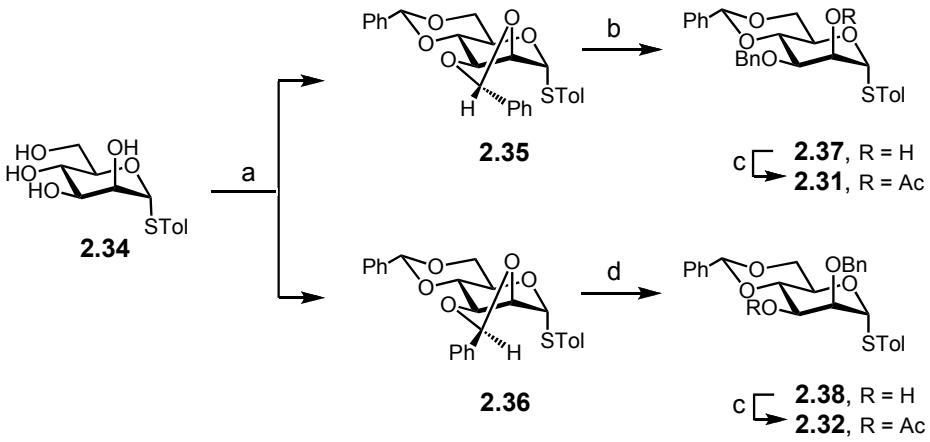
While octyl mannopyranosides **2.4**–**2.6** were used to evaluate the effect of acceptor length on ManT activity, a panel of disaccharide analogs **2.7**–**2.20** was synthesized and used to explore the enzyme substrate specificity further. In **2.7**–**2.20**, one of the hydroxyl groups of the parent disaccharide has been either been replaced with a methoxy group, or deoxygenated. The synthesis of this panel of disaccharides is described below.



Scheme 2-3. Retrosynthetic analysis of **2.7**–**2.10**.

2.2.2.1. Synthesis of 2'- and 3'-Deoxy and Methoxy Disaccharides, 2.7–2.10

For the preparation of the 2'-methoxy (**2.7**),[17] 2'-deoxy (**2.8**),[17] 3'-methoxy (**2.9**) and 3'-deoxy (**2.10**) analogs, a divergent approach was chosen (Scheme 2-3); it involved the protected disaccharides **2.29** and **2.30** as intermediates. These oligosaccharides could, in turn, be obtained from thioglycosides **2.31** and **2.32** and glycosyl acceptor **2.33**.[17]



Scheme 2-4. Reagents and conditions: (a) $PhCH(OCH_3)_2$, *p*-TsOH, DMF, 60 °C, 72% (1:3 *exo:endo* ratio); (b) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 87%; (c) Ac_2O, pyridine, CH_2Cl_2, rt, 79% for **2.31**, 94% for **2.32**; (d) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 0 °C, 66%.

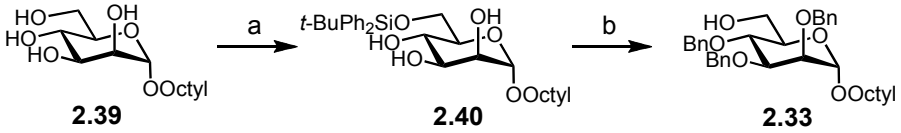
As illustrated in Scheme 2-4, the donors required for the synthesis of **2.7**–**2.10**, thioglycosides **2.29** and **2.30**, were obtained from the known thioglycoside **2.34**,[22] which was first protected as the di-benzylidene acetal

using benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid.[26] This reaction afforded a 1:3 mixture of the *exo*-isomer **2.35** and *endo*-isomer **2.36** in 72% combined yield. The diastereoisomers were discriminated by 2D NOESY experiments, in which the dioxolane acetal hydrogen showed an NOE to H-2 for compound **2.36** and to H-4 for compound **2.35**. The more reactive dioxolane ring of diastereoisomer **2.35** was regio- and chemoselectively opened with lithium aluminum hydride and aluminum trichloride at room temperature to give alcohol **2.37** in 87% yield.[26] The regioselectivity was confirmed by the subsequent acetylation (vide infra) in which the chemical shift of H-2 moved from 4.28 to 5.63 ppm.

Under the same reaction conditions, the dioxolane ring of the *endo*-isomer **2.36** was opened to give, in addition to the expected alcohol **2.38**, three major byproducts as indicated by TLC. Although the structures of these byproducts were not unequivocally established, they are presumably produced as the result of non-specific opening or hydrolysis of the dioxolane and dioxane rings. The formation of these side products could be minimized by carrying out the reaction at lower temperature (0 °C), which provided alcohol **2.38** as the major product in 66% yield. The regioselectivity was established by the downfield shift of the H-3 hydrogen (5.34 ppm) after acetylation. This and the previously mentioned acetylation reactions were carried out by treatment of both **2.37** and **2.38**

with acetic anhydride and pyridine to give glycosyl donors **2.29** and **2.30** in 79% and 94% yields, respectively.

The glycosyl acceptor needed for the synthesis of **2.7**–**2.10**, octyl glycoside **2.33**,[17] was synthesized from known alcohol **2.39**[17] as illustrated in Scheme 2-5. Reaction of **2.39** with *tert*-butylchlorodiphenylsilane and imidazole provided silyl ether **2.40** in 94% yield. The remaining hydroxyl groups were protected as benzyl ethers by reaction with sodium hydride and benzyl bromide and the silyl group was removed upon treatment with tetra-*n*-butylammonium fluoride, which furnished glycosyl acceptor **2.33** in 91% yield over the two steps.

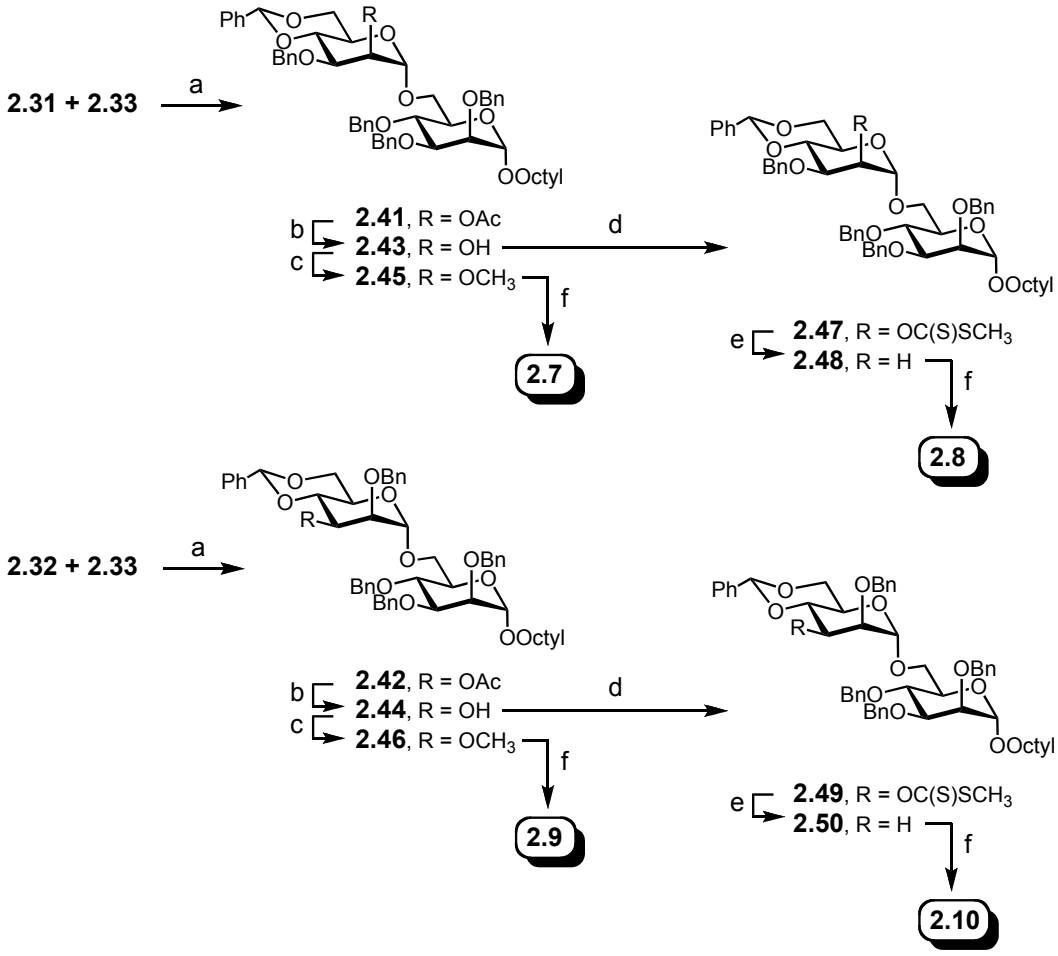


2.39 **2.40** **2.33**

Scheme 2-5. Reagents and conditions: (a) *t*-$BuPh_2SiCl$, imidazole, DMF, 45 °C, 94%; (b) i) NaH, BnBr, DMF; ii) *n*-Bu_4NF, THF, rt, 91% over 2 steps.

With these three monosaccharides in hand, coupling of thioglycosides **2.31** or **2.32** with alcohol **2.33** using NIS/AgOTf activation[23] afforded the corresponding disaccharides **2.41** or **2.42** in 89% and 83% yields, respectively (Scheme 2-6). Consistent with earlier work from the Crich group,[27] thioglycoside **2.32** was a highly α-selective donor and we could isolate none of the β-mannopyranoside. As mentioned previously,

the α-stereochemistry of the glycosidic linkages was confirmed by the one-bond $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants for the anomeric carbon atoms.[25] In all cases, this value was between 168 and 176 Hz, clearly indicating the α-stereochemistry.



Scheme 2-6. Reagents and conditions: (a) NIS, AgOTf, 4 Å MS, CH_2Cl_2, 0 °C, 89% for **2.41**, 83% for **2.42**; (b) $NaOCH_3$, CH_3OH, rt, 94% for **2.43**, 89% for **2.44**; (c) NaH, CH_3I, DMF, rt, 91% for **2.45**, 76% for **2.46**; (d) NaH, CS_2, THF, then CH_3I, rt, 96% for **2.47**, 94% for **2.49**; e) *n*-Bu_3SnH, AIBN, toluene, 41% for **2.48**, 37% for **2.50**; (f) H_2, $Pd(OH)_2$, CH_3OH, 90% for **2.7**, 96% for **2.8**, 94% for **2.9**, 91% for **2.10**.

Subsequent removal of the acetyl groups from these fully protected disaccharides using sodium methoxide gave **2.43** (94% yield) and **2.44** (89% yield). The target disaccharides **2.7**[17] and **2.9** were prepared by methylation of **2.43** and **2.44** with methyl iodide and sodium hydride to give **2.45** and **2.46** (91% and 76%, respectively), followed by full deprotection by hydrogenolysis. This two-step sequence provided **2.7** in 90% yield from **2.45** and **2.9** in 94% yield from **2.46**.

To access the deoxy analog **2.8**,[17] disaccharide **2.43** was converted to the corresponding xanthate **2.47** and then reduced[28] with tri-*n*-butylstannane in the presence of AIBN (2,2′-azo *bis*isobutyronitrile) yielding **2.48** in 41% yield. A number of other byproducts were observed on TLC, but these were not isolated and characterized. That the deoxygenation had occurred was clearly evident from the ^{1}H and ^{13}C NMR spectra for **2.48**. In the ^{1}H NMR spectrum, resonances for H-2'$_{ax}$ and H-2'$_{eq}$ of the expected multiplicity (ddd) were present at 1.78 and 2.35 ppm, respectively. In addition, in the ^{13}C NMR spectrum a resonance at 36.4 ppm could be assigned to C-2'. The target compound **2.8**[17] was obtained in 96% yield after deprotection with hydrogen and palladium hydroxide.

As described for the preparation of **2.8**, a similar series of reactions starting with **2.44** gave first the xanthate **2.49** and then the deoxy analog **2.50** in 37% overall yield. Similar to the case of **2.49**, this reaction was accompanied by the formation of a number of byproducts as detected by TLC but which were not structurally characterized. These byproducts

presumably result, at least in part, from radical abstraction of the benzylidene hydrogen in **2.47** and **2.49**. Removal of the protecting groups in **2.50** by hydrogenolysis provided a 91% yield of **2.10**.

2.2.2.2. Synthesis of 4' and 6' Deoxy and Methoxy Disaccharides, 2.11–2.14

An intermediate used in the assembly of **2.7**–**2.10**, disaccharide **2.43**, was used in the synthesis of the 4'- and 6'-deoxy and methoxy disaccharides, **2.11**–**2.14**, as illustrated in Scheme 2-7. The hydroxyl group of **2.43** was first protected as benzyl ether affording **2.51** in good (94%) yield. Treatment of **2.51** with excess triethylsilane and trifluoromethanesulfonic acid[29] at –78 °C afforded alcohol **2.52** in 61% yield. Alternatively, reaction of **2.51** with lithium aluminum hydride and aluminum trichloride gave the expected alcohol **2.53** in 85% yield. The regioselectivities of both 4,6-*O*-benzylidene openings were excellent with less than 4% of the undesired regioisomers. The regioselectivities of both reactions were based on the ^{13}C NMR spectra of the products, in which the chemical shift of C-6' of **2.52** (70.4 ppm) is more downfield than that of **2.53** (62.3 ppm).

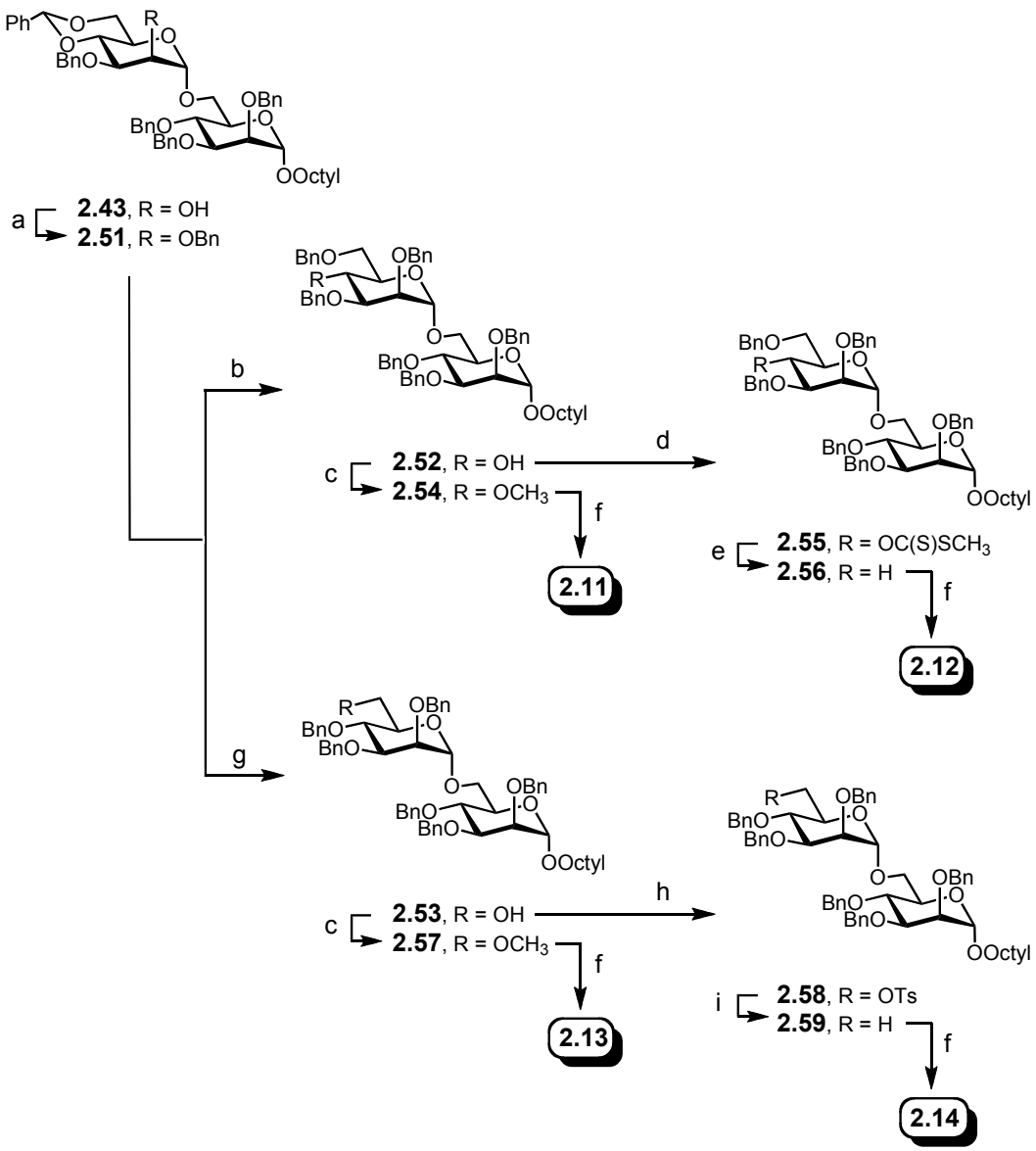
With **2.52** in hand, disaccharides **2.11** and **2.12** were prepared via routes similar to **2.7**–**2.10**. Methylation of **2.52** yielded disaccharide **2.54**, which was deprotected to furnish the 4′-methoxy analog **2.11** in 84% yield. Alternatively, **2.52** was converted to the corresponding xanthate **2.55** (96%

yield) followed by reduction with tri-*n*-butylstannane in the presence of AIBN to give a 64% yield of **2.56**. The target compound **2.12** was then obtained in 87% yield after hydrogenation. To synthesize **2.13**, alcohol **2.53** was methylated affording a 96% yield of **2.57**, which was then deprotected by hydrogenolysis providing the target in quantitative yield.

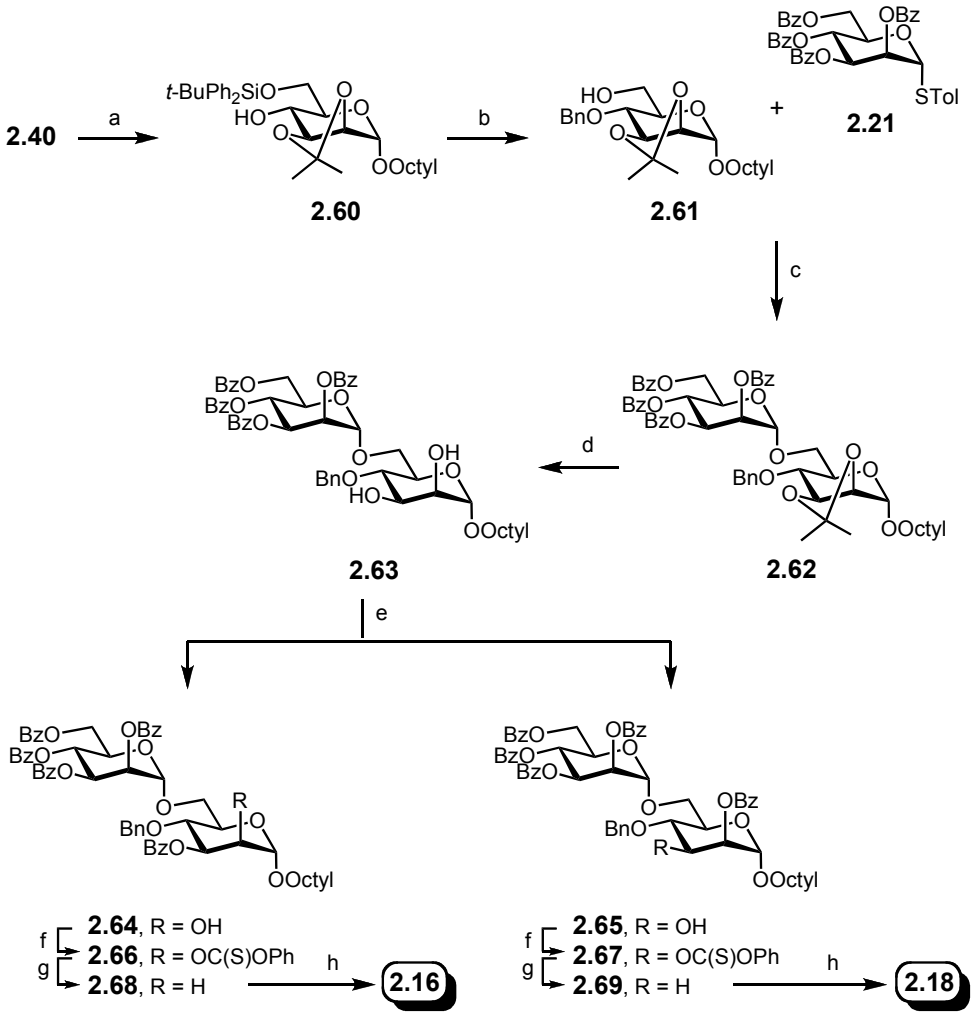
Target **2.14** was also obtained from **2.53**. This disaccharide alcohol was first reacted with *p*-toluenesulfonyl chloride and pyridine to afford the tosylate **2.58** (90% yield), which was subsequently converted to **2.59** in 62% yield upon reaction with lithium aluminum hydride. In addition to the expected product **2.59**, alcohol **2.53**, the product of O–S bond cleavage by the reducing agent, was isolated in 33% yield. Final deprotection of **2.59** furnished the desired deoxy analog **2.14** (96% yield).

2.2.2.3. Synthesis of 2 and 3-Deoxy Disaccharides 2.16 and 2.18

As illustrated in Scheme 2-8, a divergent approach was used for the preparation of the 2- and 3-deoxy disaccharide analogs **2.16** and **2.18**. Reaction of silyl ether **2.40** with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-TsOH gave a 96% yield of alcohol **2.60**. Benzylation of the hydroxyl group at C-4 and subsequent removal of the silyl protecting group provided the expected acceptor **2.61** in 76% yield over the two steps.



Scheme 2-7. Reagents and conditions: (a) NaH, BnBr, DMF, rt, 94%; (b) Et_3SiH, TfOH, CH_2Cl_2, rt, 61%; (c) NaH, CH_3I, DMF, rt, 77% for **2.54**, 96% for **2.57**; (d) NaH, CS_2, THF, then CH_3I, rt, 96%; e) *n*-Bu_3SnH, AIBN, toluene, 64%; (f) H_2, $Pd(OH)_2$, CH_3OH, 84% for **2.11**, 87% for **2.12**, 98% for **2.13**, 96% for **2.14**; (g) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 85%; (h) TsCl, pyridine, rt, 90%; (i) $LiAlH_4$, ether, rt, 62%.



Scheme 2-8. Reagents and conditions: (a) *t*-$BuPh_2SiCl$, imidazole, DMF, 45 °C, 96%; (b) i) $(OCH_3)_2CH(CH_3)_2$, *p*-TsOH, acetone; ii) *n*-Bu_4NF, THF, rt, 76% over 2 steps; (c) **2.21**, NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 92%; (d) 80% $AcOH/H_2O$, 50 °C, 88%; (e) i) *n*-Bu_2SnO, toluene, 110 °C; ii) BzCl, rt, 63% of **2.64** and 32% of **2.65**; (f) PhO(C=S)Cl, imidazole, CH_3CN, 74% for **2.66**, 70% for **2.67**; (g) *n*-Bu_3SnH, AIBN, toluene, 84% for **2.68**, 32% for **2.69**; (h) i) H_2, $Pd(OH)_2$, CH_3OH, rt; ii) $NaOCH_3$, CH_3OH, rt, 78% for **2.16** (over 2 steps), 93% for **2.18** (over 2 steps).

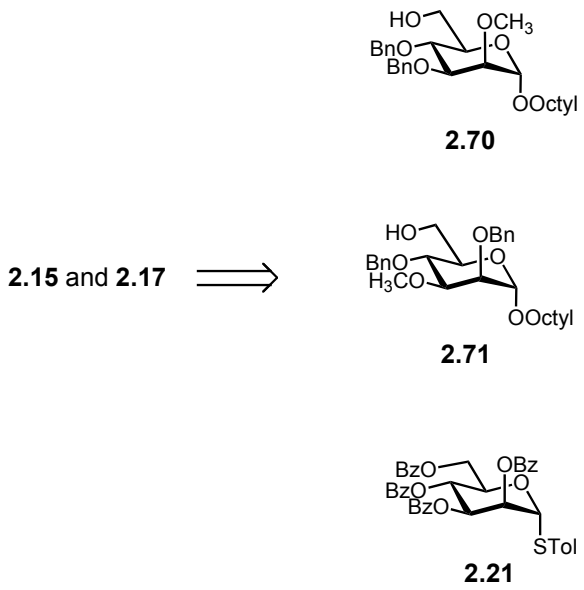
With these building blocks in place, coupling of acceptor **2.61** with the glycosyl donor **2.21** in a NIS/TMSOTf-promoted reaction gave disaccharide **2.62** in 92% yield. Subsequent hydrolysis of the isopropylidene acetal with 80% aqueous AcOH provided diol **2.63** (88%), which was converted into a 2:1 mixture of **2.64** and **2.65** (63% and 32% yield, respectively) in a two-step sequence. The significant amount of benzoylation on O-2 was somewhat surprising as the tin-mediated benzoylation was envisioned to take place nearly exclusively at the equatorial 3-hydroxyl group.[30] Nevertheless, these compounds could be separated by chromatography and this approach provided rapid access to both regioisomers **2.64** and **2.65**, which led to the target compounds in few more steps.

Treatment of both **2.64** and **2.65** with phenyl chlorothionoformate and DMAP gave the corresponding thiocarbonyl derivative **2.66** and **2.67** in good yields (74% and 70%, respectively). These thiocarbonyl derivatives[31] were employed, as opposed to the xanthates we used in earlier deoxygenations, due to concerns about acyl migration occurring upon treatment with sodium hydride, as required for xanthate generation. Reaction of both derivatives with tri-*n*-butylstannane and AIBN provided the deoxy intermediates **2.68** and **2.69**, in 84% and 32% yield, respectively. We are unsure as to the origin of the poor product yield from the deoxygenation of **2.67** and similar results were observed from repeated experiments. Subsequent hydrogenolysis of **2.68** and **2.69**

followed by debenzoylation afforded the corresponding target deoxy analogs **2.16** and **2.18** in 78% and 93% overall yield, respectively.

2.2.2.4. Synthesis of 2 and 3-Methoxy Disaccharides 2.15 and 2.17

We envisioned that the synthesis of disaccharides **2.15** and **2.17** could be achieved from the coupling of either octyl glycoside **2.70** or **2.71** with thioglycoside **2.21** (Scheme 2-9). The preparation of **2.21** was discussed above and the synthesis of **2.70** and **2.71** is presented in Scheme 2-10.

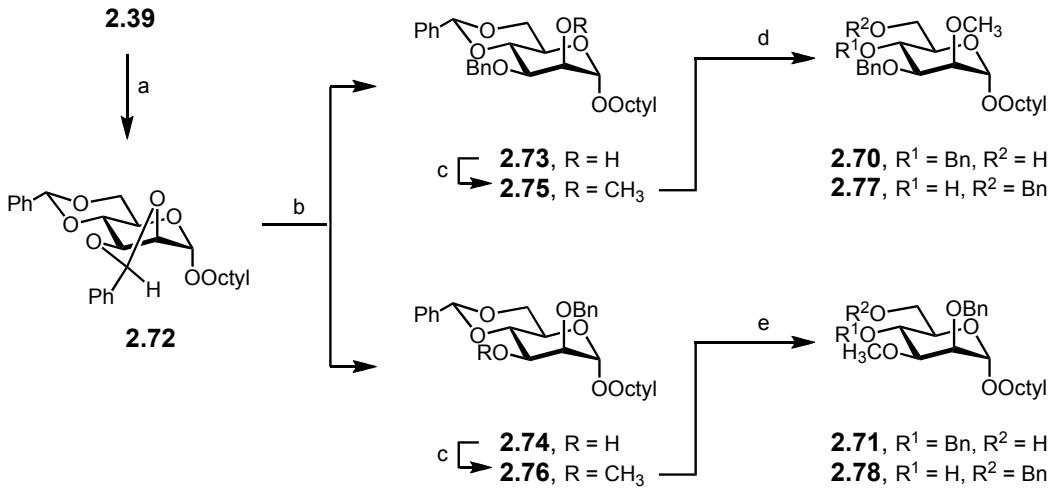


Scheme 2-9. Retrosynthetic analysis of **2.15** and **2.17**.

The preparation of **2.70** and **2.71** started with tetrol **2.39**,[17] which was converted to the 2,3:4,6-di-*O*-benzylidene derivative **2.72** upon

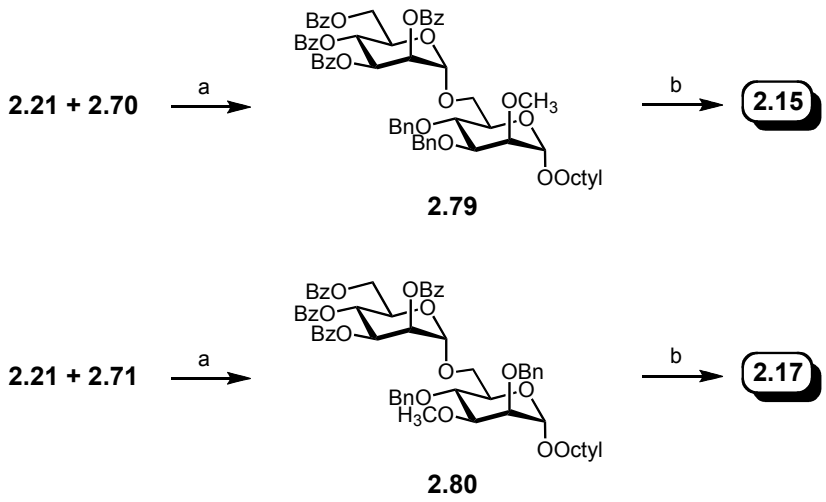
reaction with benzaldehyde dimethyl acetal and *p*-TsOH. The product was obtained as a ~1:1 mixture of *exo*- and *endo*-diastereomers in 86% combined yield. Without separation, this mixture was subjected to reductive ring opening with lithium aluminum hydride and aluminum trichloride at 0 °C to afford chromatographically separable alcohols **2.73** and **2.74** (32% and 36% yield). These compounds were each independently methylated to furnish compounds **2.75** and **2.76** in good yield.

Benzylidene ring opening of **2.75** and **2.76** with lithium aluminum hydride and aluminum trichloride (allowing the reaction to proceed overnight at room temperature as opposed to one hour at 0 °C as for the opening of the dioxolane ring in **2.72**) provided the expected glycosyl acceptors **2.70** and **2.71** in 66% and 57%, respectively, together with the corresponding 6-*O*-benzyl regioisomers (**2.77** and **2.78**). Compared with the dioxane ring opening of **2.75**, the reaction with **2.76** was less regioselective and a significant amount (22%) of regioisomer **2.78** was also obtained in addition to the desired product **2.71**. These results are consistent with those previously reported, in which the high regioselectivity of the 4,6-*O*-benzylidene ring openings under these conditions requires the presence of a bulky substituent at the C-3 position.[26]



Scheme 2-10. Reagents and Conditions. (a) $PhCH(OCH_3)_2$, *p*-TsOH, acetone, rt, 86%; (b) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 32% of **2.73** and 36% of **2.74**; (c) NaH, CH_3I, DMF, 94% for **2.75**, 92% for **2.76**; (d) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 66% of **2.70** + 6% of **2.77**; (e) $AlCl_3$, $LiAlH_4$, CH_2Cl_2, Et_2O, rt, 57% of **2.71** and 22% of **2.78**.

Having in hand all of the monosaccharide building blocks, the assembly of disaccharides **2.15** and **2.17** was straightforward. Coupling of thioglycoside **2.21** with alcohols **2.70** and **2.71** under NIS/TMSOTf activation afforded the corresponding protected disaccharides **2.79** and **2.80** in 89% and 96% yields, respectively (Scheme 2-11). Subsequent removal of the benzoyl and benzyl protecting groups in **2.79** and **2.80** under standard conditions afforded the target deoxy analogs **2.15** and **2.17** in good yields over the two steps.



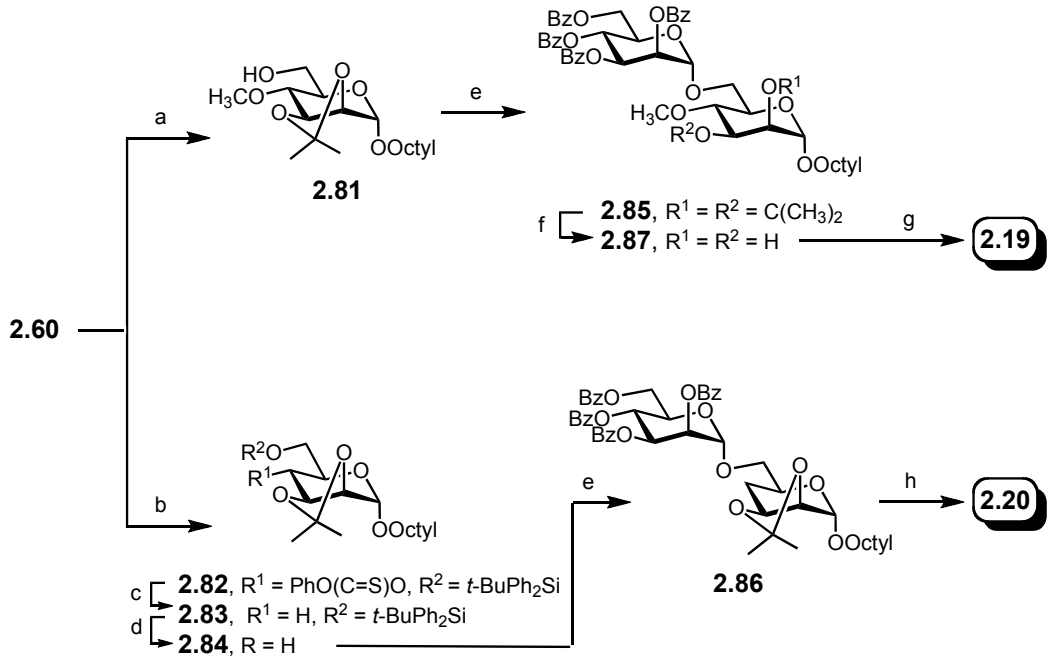
Scheme 2-11. Reagents and conditions: (a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 89% for **2.79**, 96% for **2.80**; (b) i) $NaOCH_3$, CH_3OH, rt; ii) H_2, $Pd(OH)_2$, CH_3OH, rt, 91% and 98% for **2.15**, 87% and 93% for **2.17**.

2.2.2.5. Synthesis of 4-Methoxy and 4-Deoxy Disaccharides 2.19 and 2.20

To prepare the 4-methoxy and 4-deoxy analogs, **2.19** and **2.20**, we started with the protected octyl glycoside **2.60**. As illustrated in Scheme 2-12, methylation of the hydroxyl group at C-4 proceed under standard conditions to provide an intermediate that was desilylated using tetra-*n*-butylammonium fluoride yielding **2.81** in 89% over two steps. To effect deoxygenation at C-4, **2.60** was reacted with phenyl chlorothionoformate and DMAP to give the corresponding thiocarbonyl derivative **2.82** (84% yield), which was in turn reduced with tri-*n*-butylstannane and AIBN providing **2.83** in 88% yield. This deoxygenated intermediate was

desilyated using tetra-*n*-butylammonium fluoride to give the desired monosaccharide **2.84** in 72% yield.

Glycosylation of alcohols **2.81** and **2.84** with thioglycoside **2.21** under the standard activation conditions (NIS/TMSOTf), afforded disaccharides **2.85** and **2.86** in 79% and 75% yields, respectively. Cleavage of the isopropylidene acetal in **2.85** yielded diol **2.87**, which was then treated with sodium methoxide in methanol giving **2.19** in 81% overall yield. Similar treatment of **2.86** provided disaccharide **2.20** in 94% yield over two steps.



Scheme 2-12. Reagents and conditions: (a) i) NaH, CH_3I, DMF; ii) *n*-Bu_4NF, THF, 2 steps, 89%; (b) PhO(C=S)Cl, imidazole, CH_3CN, 84%; (c) *n*-Bu_3SnH, AIBN, toluene, 88%; (d) *n*-Bu_4NF, THF, 72%; (e) **2.21**, NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 79% for **2.85**, 75% for **2.86**; (f) 80% AcOH/H_2O, 50 °C, 97%; (g) $NaOCH_3$, CH_3OH, rt, 86%; (h) i) 80% AcOH/H_2O, 50 °C; ii) $NaOCH_3$, CH_3OH, rt, 94%.

2.3. Conclusions

In conclusion, along with the homologous series of octyl mannopyranoside (**2.4–2.6**), 14 deoxy and methoxy analogs (**2.7–2.20**) of the α-D-Man*p*-(1→6)-α-D-Man*p*-OOctyl, disaccharide, **2.4**, were synthesized via routes in which the key methylation or deoxygenation reactions occurred at either the mono- or disaccharide level. All glycosylation reactions involved the use of octyl glycoside acceptors and thioglycoside donors and the stereochemistry of the mannopyranoside bond formed was established by measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$. This panel of compounds were tested as potential substrates and inhibitors for a polyprenol monophosphomannose-dependent α-(1→6)-mannosyltransferase involved in the biosynthesis of the α-(1→6)-linked mannan core of mycobacterial LAM (Chapter 3).

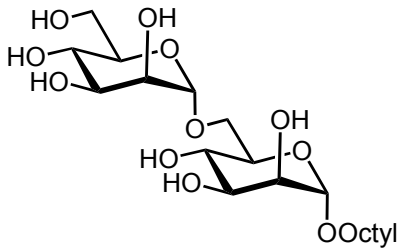
2.4. Experimental Section

General Methods for Chemical Synthesis

All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless indicated otherwise, all reactions were performed at room temperature and under a positive pressure of argon. The reactions were monitored by analytical TLC on silica gel 60-F_{254} (0.25 mm, Silicycle) and spots were detected under UV light or by charring with

acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure at <40 °C. Products were purified by column chromatography using silica gel (40–60 μM) or SepPak C_{18} reverse phase cartridges (Waters). Before use, the cartridges were prewashed with 10 mL of MeOH followed by 10 mL of H_2O. Optical rotations were measured at 22 ± 2 °C and are in units of degrees·mL/(g·dm). ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$), or HOD (4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz and chemical shifts are referenced to internal $CDCl_3$ (77.23, $CDCl_3$), or CD_3OD (48.9, CD_3OD). Assignments of NMR spectra were made based on two-dimensional (^{1}H–^{1}H COSY and HMQC) experiments. All ^{1}H and ^{13}C NMR spectra of synthesized compounds can be found in the Supporting Information. The stereochemistry at the anomeric centers of the pyranose rings were proven by measuring the $^1J_{C1\text{-}H1}$.[42] Electrospray mass spectra were recorded on samples suspended in mixtures of THF with MeOH and added NaCl.

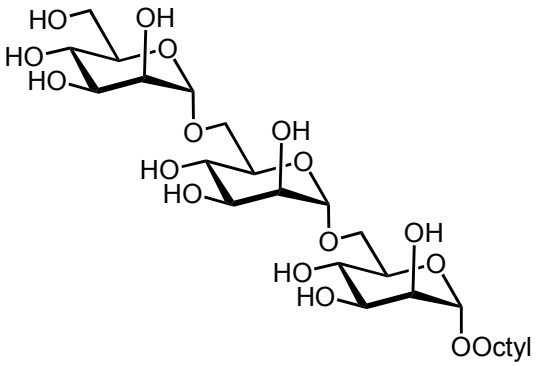
Octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.4).



Disaccharide **2.25** (359 mg, 0.31 mmol) was dissolved in MeOH (25 mL) and $NaOCH_3$ (135 mg) was added. After 2 h, the solution was neutralized with AcOH and the

debenzoylated intermediate was purified by chromatography (10:1 CH_2Cl_2–MeOH, R_f 0.32). The resulting colorless oil (200 mg, 0.28 mmol) was subsequently dissolved in MeOH (15 mL) and 20% $Pd(OH)_2$ (50 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated to give **2.4** (124 mg, 87% over two steps) as a foam. R_f 0.15 (4:1 CH_2Cl_2-MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.81 (d, 1H, *J* = 1.8 Hz, H-1′), 4.70 (d, 1H, *J* = 1.8 Hz, H-1), 3.89 (ddd, 1H, *J* = 10.8, 3.3, 2.0 Hz, H-6a), 3.84 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 3.82 (dd, 1H, *J* = 11.4, 1.8 Hz, H-6a′), 3.78 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2), 3.60–3.74 (m, 9H, H-3, H-4, H-5, H-6b, H-3′, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.0, 6.6 Hz, octyl OC*H*$_2$), 1.52–1.64 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.44 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1/C-1′),101.5 (C-1/C-1′), 74.3, 73.1, 72.9, 72.7 (4C, C-3, C-3′, C-5, C-5′), 72.2 (C-2/C-2′), 72.1 (C-2/C-2′), 68.7 (C-4), 68.6 (octyl O*C*H_2), 68.6 (C-4′), 67.4 (C-6), 62.9 (C-6′), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2306.

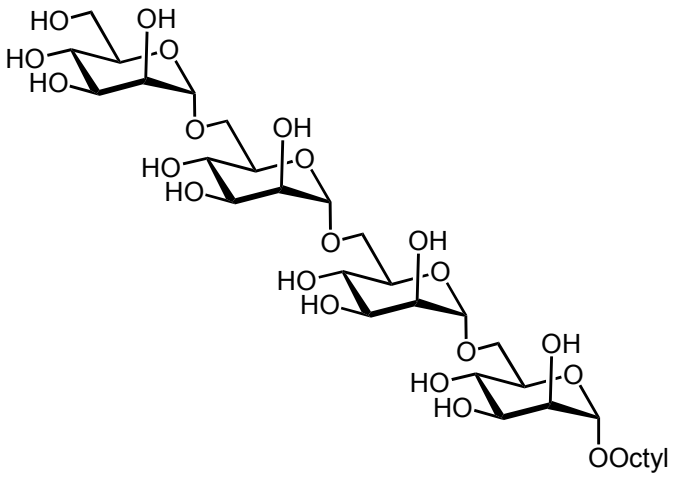
Octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.5)



Trisaccharide **2.27** (73 mg, 0.050 mmol) was dissolved in 8:1 MeOH–CH_2Cl_2 (6 mL) and $NaOCH_3$ (32 mg) was added. After stirring overnight, the solution was neutralized with AcOH and the crude product was purified by chromatography (15:1 CH_2Cl_2–MeOH) to give the partially deprotected trisaccharide as a colorless oil (15:1 CH_2Cl_2–MeOH, R_f 0.36). The partially deprotected compound was then dissolved in MeOH (4 mL) and 20% $Pd(OH)_2$ (23 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue was dissolved in H_2O (1 mL) and loaded onto a prewashed Sep-pak C_{18} reverse phase cartridge. The column was washed with H_2O (10 mL) and the desired product was eluted with MeOH (8 mL), concentrated, and lyophilized to give **2.5** (23 mg, 95%) as a foam. R_f 0.43 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.85 (d, 1H, J = 1.5 Hz, H-1′), 4.77 (d, 1H, J = 2.0 Hz, H-1′′), 4.72 (d, 1H, J = 2.0 Hz, H-1), 3.56–3.88 (m, 19H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2′′, H-3′′, H-4′′, H-5′′, H-6a′′, H-6b′′, octyl OCH_2), 3.40 (dt, 1H, J = 10.0, 6.3 Hz, octyl OCH_2), 1.53–1.62 (m, 2H, octyl OCH_2CH_2), 1.25–1.42

(m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.5 (C-1), 101.1 (C-1′′), 100.9 (C-1′), 74.5, 73.1, 72.9, 72.9, 72.5, 72.3, 72.3, 72.1, 72.0, 68.7, 68.7, 68.7 (12 C, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′), 68.6 (octyl O*C*H$_2$), 67.4 (C-6/C-6′), 67.2 (C-6/C-6′), 63.0 (C-6′′), 33.0 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.5 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{16}$: 639.2835. Found: 639.2835.

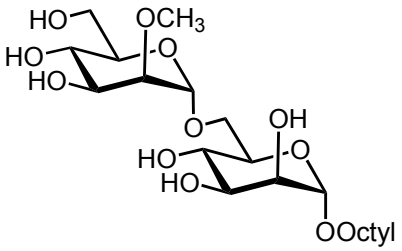
Octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.6)



Tetrasaccharide **2.28** (78 mg, 0.038 mmol) was dissolved in 8:1 MeOH–CH_2Cl_2 (9 mL) and $NaOCH_3$ (43 mg) was added. After overnight stirring, the solution was neutralized with AcOH and the crude product was purified by chromatography to give the partially deprotected tetrasaccharide as pale yellow oil (9:1 CH_2Cl_2–MeOH, R_f 0.30). The partially deprotected compound was dissolved in MeOH (6 mL) and 20% $Pd(OH)_2$ (25 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the

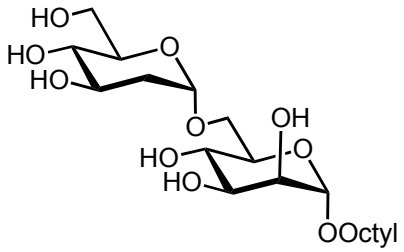
catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue was dissolved in H_2O (1 mL) and loaded on a prewashed Sep-pak C_{18} reverse phase cartridge. The column was washed with H_2O (10 mL) and the desired product was eluted with MeOH (8 mL), concentrated, and lyophilized to give **2.6** (18 mg, 62%) as a foam. R_f 0.27 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H NMR (600 MHz, D_2O) δ_H 4.91 (d, 1H, *J* = 1.8 Hz, H-1′), 4.90 (br s, 1H, H-1′′), 4.88 (br s, 1H, H-1′′′), 4.84 (br s, 1H, H-1), (m, 25H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6a′, H-6b′, H-2′′, H-3′′, H-4′′, H-5′′, H-6a′′, H-6b′′, H-2′′′, H-3′′′, H-4′′′, H-5′′′, H-6a′′′, H-6b′′′, octyl OC*H*$_2$), 3.52 (dt, 1H, *J* = 9.6, 6.0 Hz, octyl OC*H*$_2$), 1.54–1.66 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.23–1.42 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, D_2O) δ_C 100.8, 100.5, 100.2, 100.2 (4 C, C-1, C-1′, C-1′′, C-1′′′), 73.6, 71.9, 71.8, 71.8, 71.7, 71.7, 71.6, 71.5, 71.5, 71.1, 70.9, 70.9, 70.8, 67.6, 67.5, 67.5 (16 C, C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2′′, C-3′′, C-4′′, C-5′′, C-2′′′, C-3′′′, C-4′′′, C-5′′′), 68.8 (octyl O*C*H$_2$), 66.5, 66.5, 66.4 (3 C, C-6, C-6′, C-6′′), 61.8 (C-6′′′), 32.2 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 26.5 (octyl *C*H$_2$), 23.1 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{32}H_{58}O_{21}$: 801.3363. Found: 801.3363.

Octyl 2-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.7)



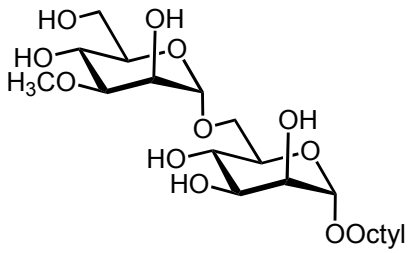
Disaccharide **2.45** (105 mg, 0.11 mmol) was dissolved in CH_3OH (8 mL) and 20% $Pd(OH)_2$ (50 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated to give **2.7** (50 mg, 90%) as a foam: R_f 0.52 (4:1, CH_2Cl_2–CH_3OH); ^{1}H NMR (600 MHz, D_2O) δ_H 5.03 (br s, 1H, H-1′), 4.82 (br s, 1H, H-1), 3.99 (dd, 1H, *J* = 11.1, 4.5 Hz, H-6a), 3.92 (br s, 1H, H-2), 3.84–3.94 (m, 2H, H-3′, H-6a′), 3.64–3.80 (m, 7H, H-3, H-4, H-5, H-6b, H-5′, H-6b′, octyl OC*H*$_2$), 3.77–3.64 (m, 2H, H-2′, H-4′), 3.44–3.54 (m, 4H, OC*H*$_3$, octyl OC*H*$_2$), 1.55–1.66 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.23–1.44 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, D_2O) δ_C 100.4 (C-1), 96.8 (C-1′), 80.4 (C-2′), 73.0 (C-5′), 71.4 (C-5), 71.3 (C-3), 70.8 (C-3′), 70.6 (C-2), 68.1 (octyl O*C*H$_2$), 67.4 (C-4′), 66.8 (C-4), 66.0 (C-6), 61.3 (C-6′), 59.3 (O*C*H$_3$), 31.9 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.0 (octyl *C*H$_3$). The ^{1}H and ^{13}C NMR spectral data were consistent with that reported.[17]

Octyl 2-*O*-deoxy-α-D-*arabino*-hexopyranosyl-(1→6)-α-D-mannopyranoside (2.8)



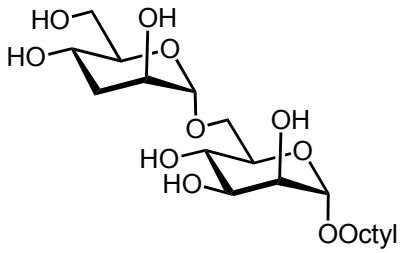
Prepared from disaccharide **2.48** (102 mg, 0.11 mmol) in CH_3OH (8 mL) and 20% $Pd(OH)_2$ (50 mg) as described for **2.7**, to give **2.8** (46 mg, 96%) as a foam: R_f 0.47 (4:1, CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, D_2O) δ_H 5.07 (d, 1H, J = 2.5 Hz, H-1′), 4.87 (br s, 1H, H-1), 3.93–4.04 (m, 3H, H-2, H-6a, H-3′), 3.90 (dd, 1H, J = 12.5, 2.3 Hz, H-6a′), 3.64–3.87 (m, 7H, H-3, H-4, H-5, H-6b, H-5′, H-6b′), 3.50–3.58 (m, 1H, octyl OCH_2), 3.43 (dd, 1H, J = 9.5, 9.5 Hz, H-4′), 2.22 (dd, 1H, J = 12.8, 5.3 Hz, H-2′$_{eq}$), 1.77 (ddd, 1H, J = 12.8, 12.8, 2.5 Hz, H-2′$_{ax}$), 1.60–1.72 (m, 2H, octyl OCH$_2$CH_2), 1.28–1.50 (m, 10H, octyl CH_2), 0.93 (t, 3H, J = 6.5 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O) δ_C 100.9 (C-1), 97.9 (C-1′), 73.1 (C-5′), 72.0, 71.9, 71.8, 71.2, (C-2, C-3, C-5, C-4′), 69.2 (C-3′), 68.8 (octyl OCH$_2$), 67.4 (C-4), 66.1 (C-6), 61.6 (C-6′), 37.7 (C-2′), 32.5 (octyl CH$_2$), 29.9 (octyl CH$_2$), 29.8 (octyl CH$_2$), 29.8 (octyl CH$_2$), 26.7 (octyl CH$_2$), 23.2 (octyl CH$_2$), 14.6 (octyl CH$_3$). The ^{1}H and ^{13}C NMR spectral data were consistent with that reported.[17]

Octyl 3-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.9)



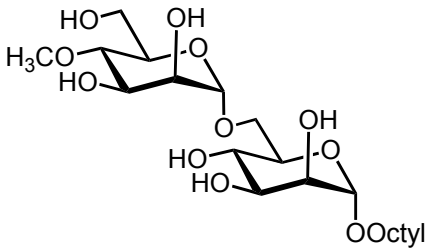
Prepared from disaccharide **2.46** (97 mg, 0.11 mmol) in CH_3OH (8 mL) and 20% $Pd(OH)_2$ (50 mg) as described for **2.7**, to give **2.9** (49 mg, 94%) as a foam: R_f 0.43 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +65.4 (*c* 0.6, CH_3OH); ^1H NMR (600 MHz, D_2O) δ_H 4.93 (br s, 1H, H-1′), 4.82 (br s, 1H, H-1), 4.19 (br s, 1H, H-2′), 3.98 (dd, 1H, *J* = 10.8, 4.8 Hz, H-6a), 3.92 (br, s, 1H, H-2), 3.87 (app d, 1H, *J* = 11.4 Hz, H-6a′), 3.66–3.80 (m, 8H, H-3, H-4, H-5, H-6b, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.46–3.54 (m, 2H, H-3′, octyl OC*H*$_2$), 3.48 (s, 3H, OC*H*$_3$,), 1.56–1.66 (m, 2H, octyl OCH_2CH_2), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, D_2O) δ_C 100.7 (C-1), 100.2 (C-1′), 80.9 (C-3′), 73.2 (C-5′), 71.8 (C-3/C-5), 71.8 (C-3/C-5), 70.9 (C-2), 68.4 (octyl O*C*H_2), 67.2 (C-2′), 66.6 (C-4), 66.4 (C-6), 66.2 (C-4′), 61.5 (C-6′), 57.0 (O*C*H_3), 32.2 (octyl *C*H_2), 29.7 (octyl *C*H_2), 29.7 (octyl *C*H_2), 29.6 (octyl *C*H_2), 26.5 (octyl *C*H_2), 23.0 (octyl *C*H_2), 14.4 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{21}H_{40}O_{11}]Na^+$: 491.2463. Found: 491.2465.

Octyl 3-*O*-deoxy-α-D-arabino-hexopyranosyl-(1→6)-α-D-mannopyranoside (2.10)



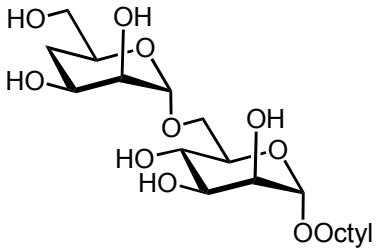
Prepared from disaccharide **2.50** (71 mg, 0.080 mmol) in CH_3OH (7 mL) and 20% $Pd(OH)_2$ (35 mg) as described for **2.7**, after chromatographic purification on Iatrobeads (4:1, CH_2Cl_2–CH_3OH) to give **2.10** (32 mg, 91%) as a foam: R_f 0.47 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +86.6 (*c* 0.7, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.70 (d, 1H, *J* = 1.2 Hz, H-1), 4.65 (br s, 1H, H-1′), 3.89–3.93 (m, 1H, H-6a), 3.62–3.82 (m, 11H, H-2, H-3, H-4, H-5, H-6b, H-2′, H-4′, H-5′, H-6a′, H-6b′, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 1.97 (ddd, 1H, *J* = 13.2, 3.9, 3.9 Hz, H-3′$_{eq}$), 1.85 (ddd, 1H, *J* = 13.2, 11.4, 3.0 Hz, H-3′$_{ax}$), 1.53–1.63 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1), 100.1 (C-1′), 75.3 (C-5′), 73.2 (C-5), 72.9, 72.2, 69.1, 69.1 (C-2, C-3, C-4, C-2′), 68.6 (octyl O*C*H$_2$), 67.3 (C-6), 63.1 (C-6′), 62.9 (C-4′), 35.9 (C-3′), 33.0 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{20}H_{38}O_{10}]Na^+$: 461.2357. Found: 461.2360.

Octyl 4-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.11)



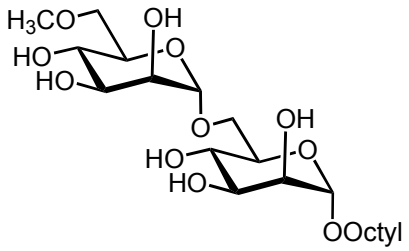
Prepared from disaccharide **2.54** (83 mg, 0.082 mmol) in CH_3OH (7 mL) and 20% $Pd(OH)_2$ (40 mg) as described for **2.7**, after chromatographic purification on Iatrobeads (4:1, CH_2Cl_2–CH_3OH) to give **2.11** (32 mg, 84%) as a foam: R_f 0.42 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +78.5 (*c* 0.9, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.79 (d, 1H, *J* = 1.2 Hz, H-1′), 4.69 (d, 1H, *J* = 1.2 Hz, H-1), 3.89 (dd, 1H, *J* = 10.8, 5.4 Hz, H-6a), 3.75–3.82 (m, 4H, H-2, H-2′, H-3′, H-6a′), 3.60–3.75 (m, 7H, H-3, H-4, H-5, H-6b, H-5′, H-6b′, octyl OC*H*$_2$), 3.53 (s, 3H, OC*H*$_3$), 3.35–3.44 (m, 2H, , H-4′, octyl OC*H*$_2$), 1.51–1.63 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.44 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1), 101.3 (C-1′), 78.4 (C-4′), 73.4, 73.4, 73.1, 72.9, 72.7, 72.5, 72.2, 68.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 68.6 (octyl O*C*H_2), 68.5, 67.5 (C-6), 62.5 (C-6′), 60.9 (O*C*H_3), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.5 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{21}H_{40}O_{11}]Na^+$: 491.2463. Found: 491.2463.

Octyl 4-deoxy-α-D-*lyxo*-hexopyranosyl-(1→6)-α-D-mannopyranoside (2.12)



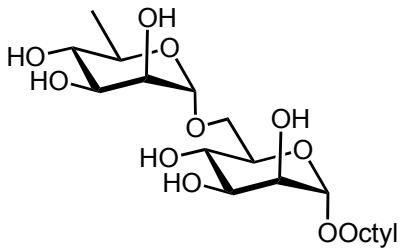
Prepared from disaccharide **2.56** (68 mg, 0.069 mmol) in CH_3OH (6 mL) and 20% $Pd(OH)_2$ (30 mg) as described for **2.7**, after chromatographic purification on Iatrobeads (4:1, CH_2Cl_2–CH_3OH) to give **2.12** (27 mg, 87%) as a foam: R_f 0.40 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +71.9 (*c* 0.7, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.85 (d, 1H, *J* = 1.0 Hz, H-1′), 4.70 (d, 1H, *J* = 1.5 Hz, H-1), 3.97 (ddd, 1H, *J* = 12.0, 5.0, 3.0 Hz, H-3′), 3.85–3.94 (m, 2H, H-6a, H-5′), 3.78 (dd, 1H, *J* = 3.0, 1.5 Hz, H-2), 3.61–3.72 (m, 6H, H-3, H-4, H-5, H-6b, H-2′, octyl OC*H*$_2$), 3.55 (d, 2H, *J* = 5.0 Hz, H-6a′, H-6b′), 3.40 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.68 (ddd, 1H, *J* = 12.0, 12.0, 12.0 Hz, H-4′$_{ax}$), 1.53–1.62 (m, 3H, H-4′$_{eq}$, octyl OCH$_2$C*H*$_2$), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.0 (C-1′), 101.6 (C-1), 73.2 (C-3/C-4), 72.9 (C-3/C-4), 72.2, (C-2), 70.4 (C-5′), 70.2 (C-2′), 68.6 (C-5), 68.6 (octyl O*C*H$_2$), 67.3 (C-6), 66.8 (C-3′), 66.0 (C-6′), 33.0 (octyl *C*H$_2$), 31.3 (C-4′), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{20}H_{38}O_{10}]Na^+$: 461.2357. Found: 461.2359.

Octyl 6-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.13)



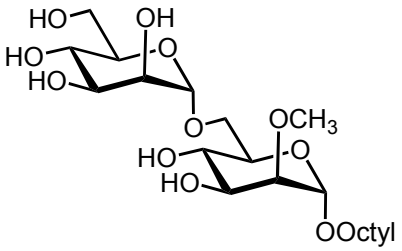
Prepared from disaccharide **2.57** (93 mg, 0.092 mmol) in CH_3OH (8 mL) and 20% $Pd(OH)_2$ (45 mg) as described for **2.7**, to give **2.13** (42 mg, 98%) as a foam: R_f 0.38 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +67.0 (*c* 2.1, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.79 (d, 1H, *J* = 1.5 Hz, H-1′), 4.69 (d, 1H, *J* = 1.2 Hz, H-1), 3.86–3.91 (m, 1H, H-6a), 3.83 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2′), 3.78 (dd, 1H, *J* = 3.0, 1.2 Hz, H-2), 3.75 (ddd, 1H, *J* = 9.6, 6.0, 2.1 Hz, H-5′), 3.56–3.75 (m, 9H, H-3, H-4, H-5, H-6b, H-3′, H-4′, H-6a′, H-6b′, octyl OC*H*$_2$), 3.37–3.42 (m, 4H, OC*H*$_3$, octyl OC*H*$_2$), 1.51–1.63 (m, 2H, octyl OCH_2CH_2), 1.24–1.44 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1′), 101.5 (C-1), 73.4 (C-6′), 73.1, 73.0 (C-3, C-3′), 72.9 (C-5), 72.7 (C-5′), 72.2 (C-2), 72.0 (C-2′), 68.8 (C-4/C-4′), 68.6 (octyl O*C*H$_2$), 68.5 (C-4/C-4′), 67.5 (C-6), 59.5 (O*C*H$_3$), 33.0 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{21}H_{40}O_{11}]Na^+$: 491.2463. Found: 491.2458.

Octyl 6-deoxy-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (2.14)



Prepared from disaccharide **2.59** (77 mg, 0.079 mmol) in CH_3OH (7 mL) and 20% $Pd(OH)_2$ (35 mg) as described for **2.7**, to give **2.14** (33 mg, 96%) as a foam: R_f 0.48 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +66.9 (*c* 1.2, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.73 (d, 1H, *J* = 2.0 Hz, H-1′), 4.70 (d, 1H, *J* = 1.5 Hz, H-1), 3.82–3.87 (m, 2H, H-6a, H-2′), 3.78 (dd, 1H, *J* = 3.0, 1.5 Hz, H-2), 3.60–3.73 (m, 7H, H-3, H-4, H-5, H-6b, H-3′, H-5′, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.6, 6.3, 6.3 Hz, octyl OC*H*$_2$), 3.36 (dd, 1H, J = 9.5, 9.5 Hz, H-4′), 1.51–1.63 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 1.25 (d, 3H, *J* = 6.5 Hz, H-6′), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1), 101.4 (C-1′), 74.1 (C-4′), 73.1 (C-3), 72.9 (C-3′), 72.5 (C-4), 72.2 (C-2, C-2′), 69.6 (C-5′), 68.6 (C-5), 68.6 (octyl O*C*H_2), 67.4 (C-6), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 18.0 (C-6′), 14.4 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{20}H_{38}O_{10}]Na^+$: 461.2357. Found: 461.2355.

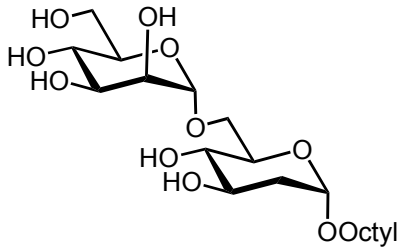
Octyl α-D-mannopyranosyl-(1→6)-2-*O*-methyl-α-D-mannopyranoside (2.15)



Disaccharide **2.79** (251 mg, 0.24 mmol) was dissolved in CH_3OH (20 mL) and $NaOCH_3$ (108 mg) was added. After 2 h, the solution was neutralized with AcOH and, following concentration, the crude product was purified by chromatography (10:1, CH_2Cl_2–CH_3OH) to give an intermediate as a pale yellow oil (140 mg, 91%). Debenzylation of this intermediate was acheived using CH_3OH (10 mL) and 20% $Pd(OH)_2$ (30 mg) as described for **2.7**, to give **2.15** (88 mg, 98%) as a foam: R_f 0.49 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +57.6 (*c* 2.1, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.84 (d, 1H, *J* = 1.8 Hz, H-1), 4.81 (d, 1H, *J* = 1.8 Hz, H-1′), 3.86–3.90 (m, 1H, H-6a), 3.84 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′), 3.81 (dd, 1H, *J* = 11.7, 2.1 Hz, H-6a′), 3.58–3.74 (m, 9H, H-3, H-4, H-5, H-6b, H-3′, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.44 (s, 3H, OC*H*$_3$), 3.43 (dt, 1H, *J* = 9.6, 6.0 Hz, octyl OC*H*$_2$), 3.39 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2), 1.53–1.63 (m, 2H, octyl OCH_2CH_2), 1.25–1.43 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.5 (C-1), 98.3 (C-1′), 82.2 (C-2), 74.4 (C-5′), 73.2 (C-5), 72.7 (C-3′), 72.7 (C-3), 72.1 (C-2′), 68.8 (C-4/C-4′), 68.7 (octyl O*C*H$_2$), 68.6 (C-4/C-4′), 67.4 (C-6), 62.9 (C-6′), 59.3 (O*C*H$_3$), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4

(octyl CH_2), 23.7 (octyl CH_2), 14.4 (octyl CH_3). ESIMS: *m/z* calcd for $[C_{21}H_{40}O_{11}]Na^+$: 491.2463. Found: 491.2462.

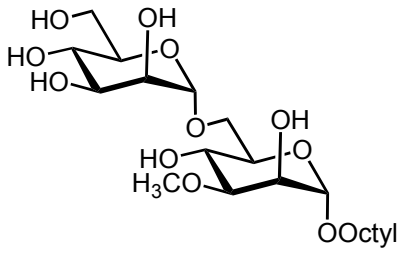
Octyl-α-D-mannopyranosyl-(1→6)-2-deoxy-α-D-*arabino*-hexopyranoside (2.16)



Debenzylation of disaccharide **2.68** (107 mg, 0.10 mmol) was acheived using 20% $Pd(OH)_2$ (30 mg) in CH_3OH (8 mL) as described for **2.7**, to obtain a white solid intermediate, which was dissolved in CH_3OH (10 mL) before $NaOCH_3$ (54 mg) was added. After stirring for 2 h, the solution was neutralized with AcOH, concentrated, and the crude product purified by chromatography on Iatrobeads (4:1, CH_2Cl_2–CH_3OH) to give **2.16** (35 mg, 78%) as a foam: R_f 0.43 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +90.4 (*c* 2.0, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.84 (d, 1H, *J* = 1.2 Hz, H-1), 4.80 (d, 1H, *J* = 1.8 Hz, H-1′), 3.91 (dd, 1H, *J* = 11.1, 5.1 Hz, H-6a), 3.78–3.84 (m, 3H, H-3, H-2′, H-6a′), 3.60–3.73 (m, 7H, H-5, H-6b, H-3′, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.35 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 3.28 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 2.04 (ddd, 1H, *J* = 13.2, 5.4, 1.2 Hz, H-2$_{eq}$), 1.51–1.62 (m, 3H, H-2$_{ax}$, octyl OCH_2CH_2), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.5 (C-1′), 98.7 (C-1), 74.4 (C-3′), 73.2 (C-4), 72.7 (C-4′/C-5), 72.7 (C-4′/C-5), 72.1 (C-2′), 70.2 (C-3), 68.6 (C-5′), 68.4 (octyl O*C*H_2), 67.3 (C-6), 62.9 (C-6′), 39.0 (C-2), 33.0

(octyl CH_2), 30.7 (octyl CH_2), 30.6 (octyl CH_2), 30.4 (octyl CH_2), 27.5 (octyl CH_2), 23.7 (octyl CH_2), 14.4 (octyl CH_3). ESIMS: *m/z* calcd for $[C_{20}H_{38}O_{10}]Na^+$: 461.2357. Found: 461.2359.

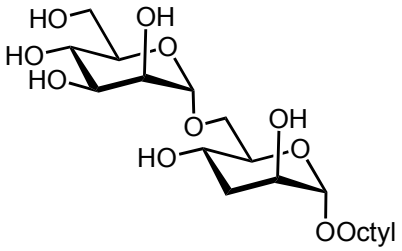
Octyl α-D-mannopyranosyl-(1→6)-3-*O*-methyl-α-D-mannopyranoside (2.17)



Debenzoylation of disaccharide **2.80** (252 mg, 0.28 mmol) using $NaOCH_3$ (108 mg) in CH_3OH (25 mL) as described for **2.15**, gave a pale yellow oil intermediate (133 mg, 87%). A portion of this intermediate (60 mg, 0.092 mmol) was debenzylated in CH_3OH (5 mL) and 20% $Pd(OH)_2$ (15 mg) to give **2.17** (41 mg, 93%) as a foam: R_f 0.31 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +70.4 (*c* 1.2, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.81 (d, 1H, *J* = 1.8 Hz, H-1′), 4.74 (d, 1H, *J* = 1.8 Hz, H-1), 3.99 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2), 3.89 (dd, 1H, *J* = 11.1, 5.8 Hz, H-6a), 3.83 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 3.81 (dd, 1H, *J* = 12.0, 1.8 Hz, H-6a′), 3.61–3.74 (m, 8H, H-4, H-5, H-6b, H-3′, H-4′, H-5′, H-6b′, octyl O*CH*$_2$), 3.44 (s, 3H, O*CH*$_3$), 3.41 (dt, 1H, *J* = 10.2, 6.3 Hz, octyl O*CH*$_2$), 3.33 (dd, 1H, *J* = 9.3, 3.2 Hz, H-3), 1.54–1.64 (m, 2H, octyl OCH_2CH_2), 1.25–1.43 (m, 10H, octyl CH_2), 0.90 (t, 3H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.6 (C-1), 101.4 (C-1′), 82.6 (C-3), 74.3 (C-5′), 73.1 (C-5), 72.7 (C-4), 72.1 (C-2′), 68.7 (octyl O*CH*$_2$), 68.6 (C-3′), 68.1 (C-2), 67.4 (C-4′), 67.3 (C-6), 62.9 (C-6′), 57.4 (O*CH*$_3$),

33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{21}H_{40}O_{11}]Na^+$: 491.2463. Found: 491.2464.

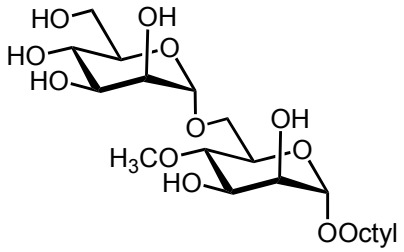
Octyl-α-D-mannopyranosyl-(1→6)-3-deoxy-α-D-*arabino*-hexopyranoside (2.18)



As described for **2.16**, disaccharide **2.69** (32 mg, 0.030 mmol) was debenzylated in CH_3OH (3 mL) and 20% $Pd(OH)_2$ (10 mg) to give a white solid intermediate, which was reacted with $NaOCH_3$ (22 mg) in CH_3OH (4 mL) to give **2.18** (12 mg, 93%) as a foam: R_f 0.45 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +65.2 (*c* 0.6, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.81 (d, 1H, *J* = 1.8 Hz, H-1′), 4.54 (br s, 1H, H-1), 3.86 (dd, 1H, *J* = 10.8, 6.0 Hz, H-6a), 3.84 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 3.77–3.83 (m, 2H, H-4, H-6a′), 3.74–3.76 (m, 1H, H-2), 3.69–3.74 (m, 4H, H-6b, H-3′, H-6b′, octyl OC*H*$_2$), 3.62–3.67 (m, 3H, H-5, H-4′, H-5′), 3.41 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 1.99 (ddd, 1H, *J* = 13.2, 3.0, 3.0 Hz, H-3_{eq}), 1.80 (ddd, 1H, *J* = 13.2, 11.4, 3.0 Hz, H-3_{ax}), 1.53–1.64 (m, 2H, octyl OCH_2CH_2), 1.25–1.44 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.4 (C-1′), 100.3 (C-1), 74.3 (C-4′/C-5), 74.1 (C-4′/C-5), 72.7 (C-3′), 72.2 (C-2′), 69.2 (C-2), 68.6 (C-5′), 68.4 (octyl O*C*H_2), 67.6 (C-6), 62.9 (C-4), 62.8 (C-6′), 36.3 (C-3), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.5 (octyl

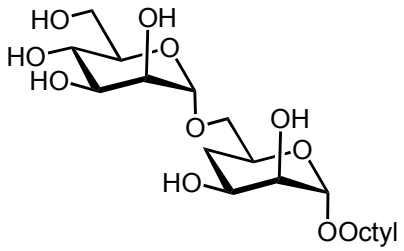
CH_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). ESIMS: *m/z* calcd for [$C_{20}H_{38}O_{10}$]Na^+: 461.2357. Found: 461.2360.

Octyl α-D-mannopyranosyl-(1→6)-4-*O*-methyl-α-D-mannopyranoside (2.19)



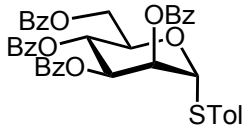
Prepared from diol **2.87** (69 mg, 0.078 mmol) in CH_3OH (7 mL) and $NaOCH_3$ (38 mg) as described for **2.15**, to give **2.19** (32 mg, 86%) as a foam: R_f 0.44 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +84.3 (*c* 0.6, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.85 (d, 1H, *J* = 1.8 Hz, H-1′), 4.67 (br s, 1H, H-1), 3.86–3.90 (m, 2H, H-6a, H-2′), 3.82 (dd, 1H, *J* = 11.7, 2.1 Hz, H-6a′), 3.60–3.76 (m, 8H, H-2, H-3, H-6b, H-3′, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.58 (ddd, 1H, *J* = 10.2, 5.4, 1.8 Hz, H-5), 3.54 (s, 3H, OC*H*$_3$), 3.34–3.41 (m, 2H, H-4, octyl OC*H*$_2$), 1.51–1.61 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.41 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.8 (C-1′), 101.5 (C-1), 78.7 (C-4), 74.6 (C-3′/C-5′), 72.9, 72.7, 72.5 (C-2, C-3, C-4′), 72.3 (C-5), 72.1 (C-2′), 68.6 (C-3′/C-5′), 68.6 (octyl O*C*H_2), 67.5 (C-6), 62.9 (C-6′), 61.1 (O*C*H_3), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.5 (octyl *C*H_3). ESIMS: *m/z* calcd for [$C_{21}H_{40}O_{11}$]Na^+: 491.2463. Found: 491.2466.

Octyl α-D-mannopyranosyl-(1→6)-4-deoxy-α-D-*lyxo*-hexopyranoside (2.20)



Disaccharide **2.86** (99 mg, 0.11 mmol) was dissolved in 80% AcOH/H_2O (2 mL) and heated at 50 °C overnight. The solution was then diluted with EtOAc (20 mL), washed with satd aq $NaHCO_3$ (2 x 5 mL), dried ($MgSO_4$), and concentrated to a colorless residue that was reacted with $NaOCH_3$ (54 mg) in CH_3OH (10 mL) as described for **2.15**, to give **2.20** (46 mg, 94% over two steps) as a foam: R_f 0.38 (4:1, CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +64.7 (*c* 2.3, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.78 (d, 1H, *J* = 2.0 Hz, H-1′), 4.75 (d, 1H, *J* = 1.5 Hz, H-1), 3.87–3.94 (m, 2H, H-3, H-5), 3.80–3.84 (m, 2H, H-2′, H-6a′), 3.76 (dd, 1H, *J* = 10.6, 6.5 Hz, H-6a), 3.56–3.73 (m, 6H, H-2, H-3′, H-4′, H-5′, H-6b′, octyl OC*H*$_2$), 3.46 (dd, 1H, *J* = 10.6, 3.8 Hz, H-6b), 3.38 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 1.73 (ddd, 1H, *J* = 12.0 Hz, H-4$_{ax}$), 1.52–1.62 (m, 3H, H-4$_{eq}$, octyl OCH$_2$C*H*$_2$), 1.24–1.41 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.2 (C-1), 101.4 (C-1′), 74.5 (C-3′), 72.7 (C-4′), 72.1 (C-2′), 70.7 (C-6), 70.3 (C-2), 68.7 (C-5/C-5′), 68.6 (C-5/C-5′), 68.5 (octyl O*C*H$_2$), 66.9 (C-3), 33.0 (octyl *C*H$_2$), 31.6 (C-4), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{20}H_{38}O_{10}]Na^+$: 461.2357. Found: 461.2357.

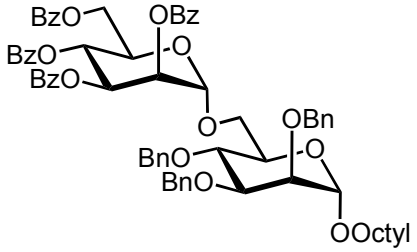
***p*-Tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (2.21)**



D-Mannose (2.74 g, 15.0 mmol) was dissolved in pyridine (19 mL) and was cooled at 0 °C. Benzoyl chloride (19.0 mL, 140 mmol) was added dropwise and the reaction mixture was stirred overnight. The reaction mixture was then diluted with CH_2Cl_2 (100 mL), washed with water (2 x 50 mL), 1 M HCl (3 x 50 mL), satd aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), and concentrated to yellow syrup. The crude (6.29 g, 8.9 mol) and *p*-thiocresol (1.3 g, 11.0 mol) were dissolved in CH_2Cl_2 (50 mL). After the addition of $BF_3.Et_2O$ (3.4 mL, 0.061 mol), the reaction mixture was stirred for 24 h. The solution was diluted with CH_2Cl_2 (100 mL), washed with satd aq $NaHCO_3$ (50 mL), water (2 x 50 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography (4:1, hexane–EtOAc) to give **2.21** (3.95 g, 64%) as a white foam: R_f 0.38 (4:1, hexane–EtOAc); $[\alpha]_D$ = +33.0 (*c* 2.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.04–8.10 (m, 4H, ArH), 8.01 (m, 2H, ArH), 7.87 (m, 2H, ArH), 7.59 (m, 3H, ArH), 7.36–7.48 (m, 9H, ArH), 7.25–7.32 (m, 2H, ArH), 7.01 (d, 2H, *J* = 8.0 Hz, ArH), 6.15 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 5.98 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2), 5.90 (dd, 1H, *J* = 10.0, 3.3 Hz, H-3), 5.72 (d, 1H, *J* = 1.5 Hz, H-1), 5.03 (ddd, 1H, *J* = 10.0, 5.0, 2.5 Hz, H-5), 4.67 (dd, 1H, *J* = 12.5, 2.5 Hz, H-6a), 4.57 (dd, 1H, *J* = 12.5, 5.0 Hz, H-6b), 2.30 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 138.4 (Ar), 133.5 (2 x Ar), 133.3 (Ar), 133.0 (Ar), 132.7 (2 x Ar), 130.0 (2 x Ar), 129.9 (2 x Ar), 129.9

(2 x Ar), 129.8 (2 x Ar), 129.8 (2 x Ar), 129.5 (Ar), 129.3 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (2 x Ar), 128.5 (2 x Ar), 128.4 (2 x Ar), 128.3 (2 x Ar), 86.3 (C-1), 71.9 (C-2), 70.4 (C-3), 69.8 (C-5), 67.2 (C-4), 63.1 (C-6), 21.1 (SPh*C*H$_3$). ESIMS: *m/z* calcd for $[C_{41}H_{34}O_9S]Na^+$: 725.1816. Found: 725.1813.

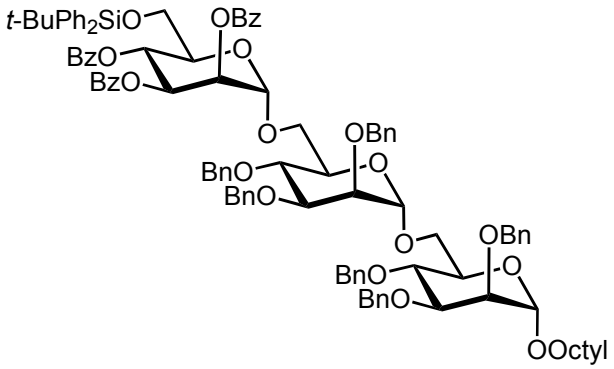
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.25)



Thioglycoside **2.21** (620 mg, 0.89 mmol), alcohol **2.23**[17] (369 mg, 0.66 mmol), and powdered 4 Å molecular sieves (625 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (25 mL) was added and the reaction mixture was cooled to 0 °C before the addition of *N*-iodosuccinimide (230 mg, 0.99 mmol) and TMSOTf (44 mg, 0.20 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude residue was purified by chromatography (4:1 hexane–EtOAc) to give **2.25** (724 mg, 96%) as a yellow oil. R_f 0.34 (4:1 hexane–EtOAc); $[\alpha]_D$ = +5.7 (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–8.15 (m, 2H, ArH), 8.05–8.09 (m, 2H, ArH), 7.91–7.94 (m, 2H, ArH), 7.81–7.85 (m, 2H, ArH), 7.49–7.62 (m, 3H, ArH), 7.20–7.45 (m, 24H, ArH), 6.12 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 5.95 (dd, 1H, *J* = 10.0, 3.0 Hz, H-3′), 5.77 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′), 5.22 (d, 1H, *J* = 1.8 Hz, H-1′),

5.04 (d, 1H, J = 11.5 Hz, PhCH_2), 4.84 (d, 1H, J = 1.0 Hz, H-1), 4.78 (d, 1H, J = 12.5 Hz, PhCH_2), 4.73 (d, 1H, J = 11.5 Hz, PhCH_2), 4.69 (d, 1H, J = 11.0 Hz, PhCH_2), 4.62–4.69 (m, 3H, PhCH_2, H-6a′), 4.53 (ddd, 1H, J = 10.0, 3.5, 3.5 Hz, H-5′), 4.45 (dd, 1H, J = 12.5, 3.5 Hz, H-6b′), 4.00 (dd, 1H, J = 12.0, 6.0 Hz, H-6a), 3.94–3.99 (m, 4H, H-3, H-4, H-5, H-6b), 3.82 (br s, 1H, H-2), 3.77 (dt, 1H, J = 9.5, 6.5 Hz, octyl OCH_2), 3.42 (dt, 1H, J = 9.5, 6.5 Hz, octyl OCH_2), 1.55–1.65 (m, 2H, octyl OCH_2CH_2), 1.20–1.42 (m, 10H, octyl CH_2), 0.85 (t, 3H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 138.5 (Ar), 138.5 (Ar), 133.3 (Ar), 133.0 (Ar), 132.9 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 129.3 (Ar), 129.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.9 (Ar), 127.7 (Ar), 127.7 (Ar), 127.5 (Ar), 97.8 (C-1′, $^1J_{C,H}$ = 172.9 Hz), 97.6 (C-1, $^1J_{C,H}$ = 167.0 Hz), 80.5 (C-3), 75.1 (PhCH_2), 74.9 (C-2/H-4), 74.9 (C-2/H-4), 72.7 (PhCH_2), 72.1 (PhCH_2), 71.4 (C-5), 70.5 (C-2′), 70.0 (C-3′), 68.7 (C-5′), 67.8 (octyl OCH_2), 67.1 (C-6), 67.1 (C-4′), 62.8 (C-6′), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.5 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{69}H_{72}O_{15}$: 1163.4763. Found: 1163.4763.

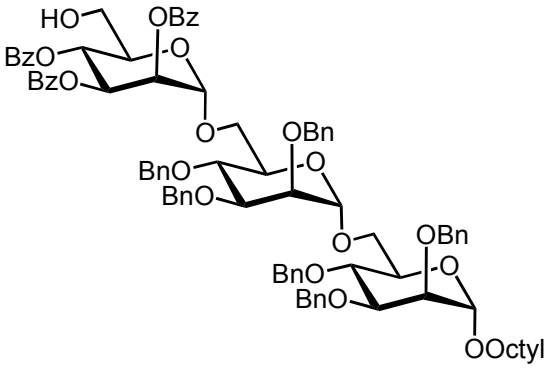
Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranosyl-(1→6)- 2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.26)



Thioglycoside **2.22**[22] (125 mg, 0.15 mmol), alcohol **2.24** (121 mg, 0.12 mmol), and powdered 4 Å molecular sieves (100 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (4 mL) was added and cooled to 0 °C before the addition of *N*-iodosuccinimide (43 mg, 0.18 mmol) and TMSOTf (7 μL, 0.036 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through a short pad of Celite and concentrated. The crude residue was purified by chromatography (4:1 hexane–EtOAc) to give **2.26** (204 mg, 98%) as a pale yellow oil. R_f 0.36 (4:1 hexane–EtOAc); $[\alpha]_D$ = -7.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–8.15 (m, 2H, ArH), 7.80–7.88 (m, 4H, ArH), 7.70–7.74 (m, 2H, ArH), 7.10–7.60 (m, 47H, ArH), 6.16 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′′), 5.87 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3′′), 5.76 (dd, 1H, *J* = 3.5, 2.0 Hz, H-2′′), 5.17 (d, 1H, *J* = 2.0 Hz, H-1′′), 5.14 (d, 1H, *J* = 1.5 Hz, H-1′), 5.04 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.91 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.80 (d, 1H, *J* = 1.5 Hz, H-1), 4.65–4.77 (m, 7H, PhCH_2), 4.57 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.52 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.49 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.11 (ddd, 1H, *J* =

10.0, 2.5, 2.5 Hz, H-5′′), 3.98–4.06 (m, 3H, H-4, H-4′, H-6a′), 3.90–3.96 (m, 3H, H-3, H-2′, H-3′), 3.87 (dd, 1H, *J* = 11.3, 4.8 Hz, H-6a), 3.70–3.81 (m, 7H, H-2, H-5, H-6b, H-5′, H-6b′, H-6a′′, H-6b′′), 3.59 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.29 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.41–1.49 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.30 (m, 10H, octyl C*H*$_2$), 1.05 (s, 9H, *tert*-butyl), 0.86 (t, 3H, *J* = 7.3 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.1 (C=O), 165.1 (C=O), 165.1 (C=O), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.3 (Ar), 138.2 (Ar), 135.7 (Ar), 135.7 (Ar), 135.4 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.8 (Ar), 132.7 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.4 (Ar), 129.4 (Ar), 129.3 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 128.2 (Ar), 128.2 (Ar), 128.1 (Ar), 128.1 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.5 (Ar), 127.4 (Ar), 127.4 (Ar), 127.4 (Ar), 127.4 (Ar), 127.2 (Ar), 127.1 (Ar), 98.0 (C-1′, $^1J_{C,H}$ = 171.4 Hz), 97.9 (C-1′′, $^1J_{C,H}$ = 174.3 Hz), 97.7 (C-1, $^1J_{C,H}$ = 167.0 Hz), 80.4 (C-3), 79.5 (C-3′), 75.0 (C-2), 74.9 (Ph*C*H$_2$), 74.8 (Ph*C*H$_2$), 74.7 (C-2′), 74.5 (C-4), 74.5 (C-4′), 72.6 (Ph*C*H$_2$), 72.3 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 71.7 (C-5′), 71.3 (C-5), 71.2 (Ph*C*H$_2$), 71.1 (C-5′′), 70.6 (C-2′′), 70.6 (C-3′′), 67.5 (octyl O*C*H$_2$), 66.6 (C-4′′), 66.6 (C-6), 65.9 (C-6′), 62.3 (C-6′′), 31.7 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.1 (octyl *C*H$_2$), 26.6 (C(*C*H$_3$)), 26.1 (octyl *C*H$_2$), 22.5 (octyl *C*H$_2$), 19.1 (*C*(CH$_3$)), 14.0 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{105}H_{114}O_{19}Si$: 1729.7616. Found: 1729.7620.

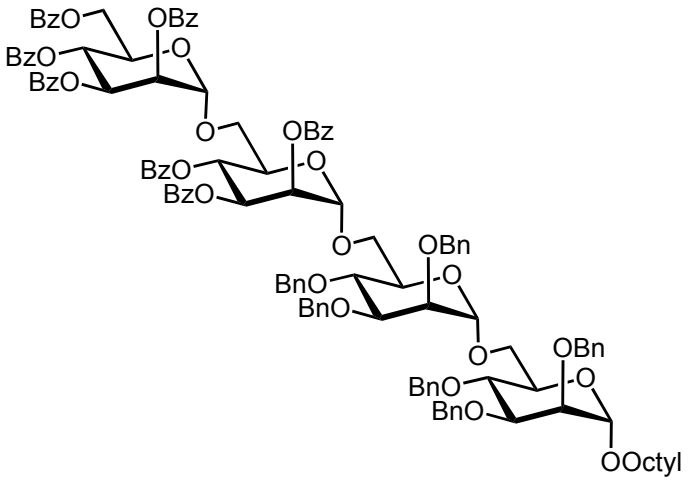
Octyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.27)



Trisaccharide **2.26** (229 mg, 0.13 mmol) was stirred in a solution of 70% HF·pyridine– pyridine–THF (3.2 mL, ratio of 1:2:5). Upon completion, the reaction mixture was diluted with EtOAc, washed twice with 0.5 M HCl, sat. aq. $CuSO_4$, and dried ($MgSO_4$). The solvent was evaporated and the crude residue was purified by chromatography (3:1 hexane–EtOAc) to give **2.27** (178 mg, 90%) as a colorless oil. R_f 0.18 (3:1 hexane–EtOAc); $[\alpha]_D$ = -3.6 (*c* 2.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.09–8.13 (m, 2H, ArH), 7.93–7.98 (m, 2H, ArH), 7.78–7.82 (m, 2H, ArH), 7.59–7.64 (m, 1H, ArH), 7.47–7.54 (m, 3H, ArH), 7.15–7.44 (m, 35H, ArH), 6.00 (dd, 1H, *J* = 10.3, 3.5 Hz, H-3′′), 5.77 (dd, 1H, *J* = 10.3, 10.3 Hz, H-4′′), 5.75 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2′′), 5.18 (d, 1H, *J* = 1.5 Hz, H-1′′), 5.13 (d, 1H, *J* = 1.0 Hz, H-1′), 5.09 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.93 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.82 (d, 1H, *J* = 1.5 Hz, H-1), 4.67-4.75 (m, 5H, PhCH_2), 4.64 (s, 2H, PhCH_2), 4.59 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.53 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.50 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.05 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.95–4.04 (m, 5H, H-4, H-6a/6a′, H-2′, H-3′, H-5′′), 3.93 (dd, 1H, *J* = 9.8, 3.3 Hz, H-3), 3.88 (dd, 1H, *J* = 11.5, 5.0 Hz, H-6a/6a′), 3.79–3.84 (m, 2H, H-2, H-5′),

3.66–3.78 (m, 4H, H-5, H-6b, H-6b′, H-6a′′), 3.55–3.64 (m, 2H, H-6b′′, octyl OC*H*$_2$), 3.33 (dt, 1H, *J* = 10.0, 6.8 Hz, octyl OC*H*$_2$), 2.57 (dd, 1H, *J* = 8.0, 6.0 Hz, O*H*), 1.45–1.54 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.33 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.5 (C=O), 165.2 (C=O), 165.2 (C=O), 138.8 (Ar), 138.7 (Ar), 138.6 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 133.5 (Ar), 133.3 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.6 (Ar), 129.4 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 98.2 (C-1′), 97.9 (C-1′′), 97.8 (C-1), 80.5 (C-3′), 79.5 (C-3), 75.1 (C-2), 75.0 (Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 74.8 (C-2′), 74.6 (C-4/C-4′), 74.5 (C-4/C-4′), 72.8 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.7 (C-5), 71.3 (Ph*C*H$_2$), 71.2 (C-5′), 70.8 (C-2′′), 70.6 (C-5′′), 69.6 (C-3′′), 67.6 (octyl O*C*H$_2$), 67.6 (C-4′′), 67.0 (C-6), 66.2 (C-6′), 61.2 (C-6′′), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{89}H_{96}O_{19}$: 1491.6438. Found: 1491.6437.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.28)

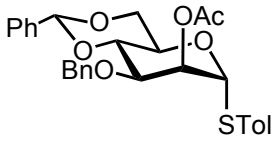


Thioglycoside **2.21** (45 mg, 0.064 mmol), alcohol **2.27** (86 mg, 0.050 mmol), and powdered 4 Å molecular sieves (50 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (2 mL) was added and cooled to 0 °C before the addition of *N*-iodosuccinimide (19 mg, 0.080 mmol) and TMSOTf (3 μL, 0.016 mmol). The mixture was stirred at 0 °C for 30 min and neutralized with triethylamine, before being filtered through a short of Celite and concentrated. The crude residue was purified by chromatography (3:1 hexane–EtOAc) to give **2.28** (93 mg, 90%) as a colorless oil. R_f 0.21 (3:1 hexane–EtOAc); $[\alpha]_D$ = +12.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.20–8.22 (m, 2H, ArH), 7.99–8.06 (m, 6H, ArH), 7.93–7.96 (m, 2H, ArH), 7.84–7.88 (m, 4H, ArH), 7.13–7.60 (m, 51H, ArH), 6.18 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′′′), 6.11 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′′), 6.06 (dd, 1H, *J* = 10.2, 3.3 Hz, H-3′′), 5.96 (dd, 1H, *J* = 10.2, 3.0 Hz, H-3′′′), 5.88 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′′), 5.84 (dd, 1H, *J* = 3.0, 1.8

Hz, H-2‴), 5.20 (d, 1H, *J* = 1.8 Hz, H-1‴), 5.17 (br s, 1H, H-1′), 5.13 (d, 1H, *J* = 1.8 Hz, H-1″), 5.09 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.92 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.82 (d, 1H, *J* = 1.8 Hz, H-1), 4.67–4.75 (m, 5H, PhC*H*$_2$), 4.64 (s, 2H, PhC*H*$_2$), 4.58 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.50 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.38 (dd, 1H, *J* = 12.0, 2.4 Hz, H-6a‴), 4.27–4.32 (m, 2H, H-5″, H-5‴), 4.20 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6b‴), 4.12 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4′), 4.00–4.05 (m, 2H, H-4, H-6a′), 3.91–3.96 (m, 5H, H-3, H-6a, H-2′, H-3′, H-6a″), 3.85 (ddd, 1H, *J* = 9.6, 4.8, 1.5 Hz, H-5′), 3.75–3.80 (m, 4H, H-2, H-5, H-6b, H-6b′), 3.66 (dd, 1H, *J* = 11.4, 2.1 Hz, H-6b″), 3.60 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.31 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.42–1.50 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.30 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 165.3 (C=O), 165.1 (C=O), 165.1 (C=O), 138.9 (Ar), 138.7 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 133.3 (Ar), 133.2 (Ar), 132.9 (Ar), 132.9 (Ar), 132.8 (Ar), 130.1 (Ar), 129.9 (Ar), 129.9 (Ar), 129.7 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.4 (Ar), 129.4 (Ar), 129.2 (Ar), 129.2 (Ar), 128.8 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 98.3 (C-1‴, $^1J_{C,H}$ = 174.0 Hz), 98.2 (C-1′, $^1J_{C,H}$ = 169.5 Hz), 98.1 (C-1″, $^1J_{C,H}$ = 172.3 Hz), 97.8 (C-1, $^1J_{C,H}$ = 167.0 Hz), 80.5 (C-3), 79.6 (C-3′), 75.1 (C-2), 75.0 (Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 74.7 (C-2′),

74.5 (C-4), 74.5 (C-4′), 72.7 (Ph*C*H_2), 72.4 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.8 (C-5), 71.5 (C-5′), 71.3 (Ph*C*H_2), 70.4, 70.3, 70.3, 70.2 (4C, C-2″, C-3″, C-2‴, C-3‴), 69.2 (C-5″), 68.8 (C-5‴), 67.6 (octyl O*C*H_2), 67.0 (C-6/C-6′), 66.9 (C-4″/C-4‴), 66.6 (C-4″/C-4‴), 66.3 (C-6/C-6′), 66.1 (C-6″), 62.4 (C-6‴), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESI calcd. for (M + Na) $C_{123}H_{122}O_{28}$: 2071.3. Found 2070.8.

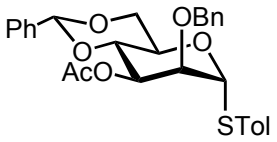
_p_-Tolyl 2-_O_-acetyl-3-_O_-benzyl-4,6-_O_-benzylidene-1-thio-α-D-mannopyranoside (2.31)



Monosaccharide **2.37** (2.2 g, 4.7 mmol) was dissolved in CH_2Cl_2 (50 mL) and pyridine (7.2 mL) and acetic anhydride (3.6 mL) were added. The reaction mixture was stirred overnight and then diluted with CH_2Cl_2 (150 mL), before being washed with 1 M HCl (3 x 50 mL), satd aq $NaHCO_3$ (50 mL), water (50 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography (6:1, hexane–EtOAc) to give **2.31** (1.89 g, 79%) as a foam: R_f 0.42 (6:1, hexane–EtOAc); $[\alpha]_D$ = +119.5 (*c* 7.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52–7.55 (m, 2H, ArH), 7.29–7.43 (m, 10H, ArH), 7.13 (d, 2H, *J* = 8.4 Hz, ArH), 5.64 (s, 1H, benzylidene H), 5.63 (dd, 1H, *J* = 3.2, 1.2 Hz, H-2), 5.40 (d, 1H, *J* = 1.2 Hz, H-1), 4.75 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.70 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.37 (ddd, 1H, *J* = 10.0, 9.6, 4.8 Hz, H-5), 4.25 (dd, 1H, *J* = 10.4, 4.8 Hz, H-6a), 4.15 (dd, 1H, *J* = 9.6,

9.6 Hz, H-4), 4.03 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3), 3.87 (dd, 1H, *J* = 10.4, 10.0 Hz, H-6b), 2.34 (s, 3H, SPhC*H*$_3$), 2.16 (s, 3H, (C(O)C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.9 (C=O), 138.3 (Ar), 137.6 (Ar), 137.3 (Ar), 132.6 (2 x Ar), 129.9 (2 x Ar), 129.1 (Ar), 128.8 (Ar), 128.3 (2 x Ar), 128.1 (2 x Ar), 127.7 (3 x Ar), 126.0 (2 x Ar), 101.5 (benzylidene *C*H), 87.4 (C-1), 78.4 (C-4), 74.0 (C-3), 72.2 (Ph*C*H$_2$), 71.2 (C-2), 68.3 (C-6), 65.0 (C-5), 21.1 (Ph*C*H$_3$), 20.9 (C(O)*C*H$_3$). ESIMS: *m/z* calcd for $[C_{29}H_{30}O_6S]Na^+$: 529.1655. Found: 529.1652.

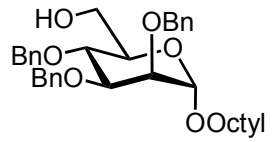
_p_-Tolyl 3-_O_-acetyl-2-_O_-benzyl-4,6-_O_-benzylidene-1-thio-α-D-mannopyranoside (2.32)



Prepared from monosaccharide **2.38** (1.8 g, 3.9 mmol), pyridine (7.0 mL) and acetic anhydride (3.5 mL) in CH_2Cl_2 (40 mL) as described for **2.31**, to give **2.32** (1.83 g, 94%) as a foam: R_f 0.45 (4:1, hexane–EtOAc); $[\alpha]_D$ = +76.3 (*c* 5.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.50–7.53 (m, 2H, ArH), 7.33–7.42 (m, 10H, ArH), 7.16 (d, 2H, *J* = 8.0 Hz, ArH), 5.61 (s, 1H, benzylidene H), 5.51 (d, 1H, *J* = 1.2 Hz, H-1), 5.34 (dd, 1H, *J* = 10.4, 3.2 Hz, H-3), 4.70 (d, 1H, *J* = 12.0Hz, PhC*H*$_2$), 4.63 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.44 (ddd, 1H, *J* = 10.0, 10.0, 4.8 Hz, H-5), 4.25–4.30 (m, 3H, H-2, H-4, H-6a), 3.90 (dd, 1H, *J* = 10.0, 10.0 Hz, H-6b), 2.37 (s, 3H, SPhC*H*$_3$), 2.06 (s, 3H, (C(O)C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.2 (C=O), 138.1 (Ar), 137.3 (Ar), 137.3 (Ar), 132.5 (2 x Ar), 130.0 (2 x Ar), 129.7 (Ar), 129.1 (Ar), 128.6 (2 x Ar) 128.3

(2 x Ar), 128.2 (Ar), 128.1 (2 x Ar), 126.3 (2 x Ar), 101.8 (benzylidene *C*H), 86.7 (C-1), 77.5 (C-2/C-4), 76.3 (C-2/C-4), 73.0 (Ph*C*H_2), 70.5 (C-3), 68.5 (C-6), 65.3 (C-5), 21.2 (SPh*C*H_3), 21.0 (C(O)*C*H_3). ESIMS: *m/z* calcd for $[C_{29}H_{30}O_6S]Na^+$: 529.1655. Found: 529.1652.

Octyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.33)



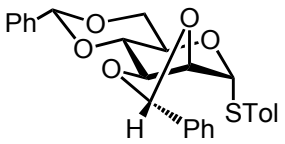
Triol **2.40** (4.18 g, 7.87 mmol) was dissolved in DMF (25 mL) and BnBr (6.0 mL, 48.0 mmol) was added. The solution was cooled in an ice bath and 60% NaH in mineral oil (2.05 g, 51.3 mmol) was added portion wise, and the mixture was warmed to room temperature. After 3 h, the reaction was quenched by the addition of CH_3OH (15 mL), diluted with EtOAc (90 mL), washed with water (3 x 40 mL), brine (40 mL) and dried ($MgSO_4$), filtered and concentrated to a pale yellow oil. The crude product was dissolved in THF (50 mL) and 1.0 M tetra-*n*-butylammonium fluoride in THF (16.0 mL, 16.0 mmol) was added and the solution was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography (4:1, hexane–EtOAc) to give **2.33** (4.03 g, 91% over two steps) as a colorless oil: R_f 0.24 (4:1, hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.27–7.44 (m, 15H, ArH), 4.96 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.81 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.81 (d, 1H, *J* = 1.5 Hz, H-1), 4.64–4.74 (m, 4H, PhCH_2), 4.00 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 3.95 (dd, 1H, *J* = 9.5, 3.0 Hz, H-3), 3.86 (ddd, 1H, *J* = 11.5, 3.0 Hz, H-6a), 3.76–3.83 (m, 2H, H-2, H-6b), 3.64–3.70

(m, 1H, H-5), 3.63 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.34 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.09 (dd, 1H, *J* = 7.5, 5.5 Hz, O*H*), 1.46–1.78 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.24–1.38 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.6 (Ar), 138.4 (Ar), 138.4 (Ar), 128.4 (3 x Ar), 128.4 (2 x Ar),127.8 (2 x Ar), 127.7 (2 x Ar), 127.7 (2 x Ar), 127.6 (2 x Ar), 127.6 (2 x Ar), 98.2 (C-1), 80.3 (C-3), 75.3 (Ph*C*H$_2$), 75.1 (C-2, C-4), 72.9 (Ph*C*H$_2$), 72.1 (C-5), 67.7 (octyl O*C*H$_2$), 62.5 (C-6), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ^{1}H and ^{13}C NMR spectral data were consistent with that reported.[17]

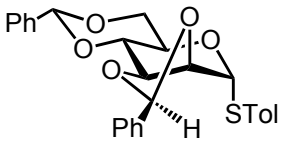
_p_-Tolyl _exo_(phenyl)-2,3:4,6-di-_O_-benzylidene-1-thio-α-D-mannopyranoside (2.35) and _p_-Tolyl _endo_(phenyl)-2,3:4,6-di-_O_-benzylidene-1-thio-α-D-mannopyranoside (2.36)

Tetraol **2.34**[22] (2.0 g, 7.0 mmol) was dissolved in DMF (5.0 mL) and benzylaldehyde dimethyl acetal (1.5 mL, 10.0 mmol) and *p*-TsOH (21 mg, 0.11 mmol) were added. The reaction mixture was rotated under reduced pressure at 60 °C on a rotary evaporator. After 2 h, additional DMF (50 mL), benzylaldehyde dimethyl acetal (1.5 mL, 10.0 mmol) and *p*-TsOH (21 mg, 0.11 mmol) were added and stirring was continued for 3 h. The reaction mixture was neutralized with satd aq $NaHCO_3$, diluted with CH_2Cl_2 (100 mL), washed with water (3 x 50 mL), dried ($MgSO_4$), and concentrated. Recrystalization of the crude product from 1:1, acetone–

EtOH (300 mL) gave a mixture of **2.35** and **2.36**. A second recrystallization of this material afforded a mixture of **2.35** and **2.36** in 23:1 ratio (584 mg, 18%). The mother liquor was concentrated and the resulting solid recrystalized from 1:1, acetone–EtOH (75 mL) to give crystalline of **2.36** and **2.35** in 20:1 ratio (964 mg, 30%). The mother liquor was concentrated and purified by chromatography (9:1, hexane–EtOAc) to give **2.36** and **2.35** in 12:1 ratio (773 mg, 24%).



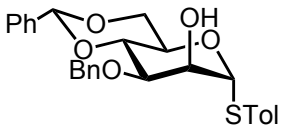
2.35: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.53–7.58 (m, 2H, ArH), 7.44–7.59 (m, 2H, ArH), 7.36–7.41 (m, 8H, ArH), 7.15 (d, 2H, *J* = 8.0 Hz, ArH), 6.31 (s, 1H, dioxolane H), 5.80 (s, 1H, H-1), 5.65 (s, 1H, dioxane H), 4.68 (dd, 1H, *J* = 9.0, 5.5 Hz, H-3), 4.38 (d, 1H, *J* = 5.5 Hz, H-2), 4.34 (ddd, 1H, *J* = 10.5, 9.0, 5.0 Hz, H-5), 4.25 (dd, 1H, *J* = 10.5, 5.0 Hz, H-6a), 3.98 (dd, 1H, *J* = 9.0, 9.0 Hz, H-4), 3.79 (dd, 1H, *J* = 10.5, 10.5 Hz, H-6b), 2.35 (s, 3H, PhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (Ar), 138.5 (Ar), 137.1 (Ar), 133.1 (2 x Ar), 130.0 (2 x Ar), 129.2 (Ar), 129.2 (Ar), 128.7 (Ar), 128.4 (2 x Ar), 128.3 (2 x Ar), 126.3 (2 x Ar), 126.0 (2 x Ar), 103.1 (dioxolane *C*H), 102.0 (dioxane *C*H), 85.0 (C-1), 77.8 (C-4), 75.9 (C-2), 75.3 (C-3), 68.6 (C-6), 61.6 (C-5), 21.2 (Ph*C*H$_3$). Anal. Calcd for $C_{27}H_{26}O_5S$ (462.56): C, 70.11; H, 5.61; S, 6.93. Found: C, 69.93; H, 5.70; S, 7.01. ESIMS: *m/z* calcd for $[C_{27}H_{26}O_5S]Na^+$: 485.1393. Found: 485.1391.



2.36: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.50–7.55 (m, 4H, ArH), 7.34–7.43 (m, 8H, ArH), 7.15 (d, 2H, *J* =

7.5 Hz, ArH), 5.99 (s, 1H, dioxolane H), 5.86 (s, 1H, H-1), 5.51 (s, 1H, dioxane H), 4.54 (dd, 1H, *J* = 8.0, 8.0 Hz, H-3), 4.49 (d, 1H, *J* = 8.0 Hz, H-2), 4.27 (ddd, 1H, *J* = 10.5, 10.0, 5.0 Hz, H-5), 4.19 (dd, 1H, *J* = 10.5, 5.0 Hz, H-6a), 3.81 (dd, 1H, *J* = 10.0, 8.0 Hz, H-4), 3.69 (dd, 1H, *J* = 10.5, 10.5 Hz, H-6b), 2.35 (s, 3H, PhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (Ar), 137.2 (Ar), 137.0 (Ar), 133.2 (2 x Ar), 130.0 (2 x Ar), 130.0 (Ar), 129.5 (Ar), 129.1 (Ar), 128.5 (2 x Ar), 128.2 (2 x Ar), 126.5 (2 x Ar), 126.2 (2 x Ar), 104.1 (dioxolane *C*H), 101.8 (dioxane *C*H), 84.5 (C-1), 80.8 (C-4), 78.6 (C-2), 74.0 (C-3), 68.6 (C-6), 61.7 (C-5), 21.2 (Ph*C*H$_3$). Anal. Calcd for $C_{27}H_{26}O_5S$ (462.56): C, 70.11; H, 5.61; S, 6.93. Found: C, 69.92; H, 5.72; S, 7.02. ESIMS: *m/z* calcd for $[C_{27}H_{26}O_5S]Na^+$: 485.1393. Found: 485.1395.

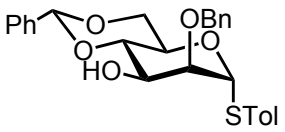
_p_-Tolyl 3-_O_-benzyl-4,6-_O_-benzylidene-1-thio-α-D-mannopyranoside (2.37)



$AlCl_3$ (0.16 g, 1.2 mmol) was dissolved in prechilled Et_2O (4 mL) and stirred for 10 min before $LiAlH_4$ (1.2 mL of 1.0 M solution in ether) was added. This mixture was stirred for an additional 10 min before addition to **2.35** (480 mg, 1.0 mmol) in 1:1, CH_2Cl_2–Et_2O (15 mL). The reaction mixture was stirred for 1 h and then quenched by the addition of EtOAc followed by water. The reaction mixture was diluted with EtOAc (60 mL), washed with water (3 x 20 mL), dried ($MgSO_4$), and concentrated to a light yellow oil, which was purified

by chromatography (4:1, hexane–EtOAc) to give **2.37** (419 mg, 87%) as a light yellow oil: R_f 0.21 (4:1, hexane–EtOAc); $[\alpha]_D$ = +222.2 (*c* 2.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.50–7.55 (m, 2H, ArH), 7.31–7.42 (m, 10H, ArH), 7.13 (d, 2H, *J* = 8.5 Hz, ArH), 5.63 (s, 1H, benzylidene H), 5.53 (d, 1H, *J* = 1.3 Hz, H-1), 4.90 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.75 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.36 (ddd, 1H, *J* = 10.4, 9.5, 5.0 Hz, H-5), 4.28 (dd, 1H, *J* = 3.4, 1.3 Hz, H-2), 4.22 (dd, 1H, *J* = 10.4, 5.0 Hz, H-6a), 4.19 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 3.98 (dd, 1H, *J* = 9.5, 3.4 Hz, H-3), 3.86 (dd, 1H, *J* = 10.4, 10.4 Hz, H-6b), 2.85 (br s, 1H, O*H*), 2.34 (s, 3H, PhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.0 (Ar), 137.8 (Ar), 137.5 (Ar), 132.4 (2 x Ar), 129.9 (2 x Ar), 129.4 (Ar), 129.0 (Ar), 128.5 (2 x Ar), 128.2 (2 x Ar), 128.0 (Ar), 127.9 (2 x Ar), 126.1 (2 x Ar), 101.6 (benzylidene *C*H), 88.2 (C-1), 79.1(C-4), 75.7 (C-3), 73.2 (Ph*C*H$_2$), 71.4 (C-2), 68.6 (C-6), 64.5 (C-5), 21.1 (Ph*C*H$_3$). ESIMS: *m/z* calcd for $[C_{27}H_{28}O_5S]Na^+$: 487.1550. Found: 487.1552.

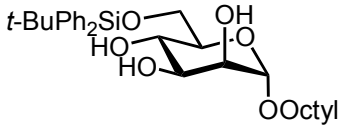
p-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (2.38)



Prepared from a solution of $AlCl_3$ (0.24 g, 1.8 mmol) and $LiAlH_4$ (1.8 mmol) in ether (7.8 mL) and **2.36** (655 mg, 1.4 mmol) in 1:1, CH_2Cl_2–ether (15 mL) at 0 °C as described for **2.37**, to give **2.38** (430 mg, 66%) as a light yellow oil: R_f 0.36 (4:1, hexane–EtOAc); $[\alpha]_D$ = +145.4 (*c* 3.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$)

δ_H 7.54–7.56 (m, 2H, ArH), 7.33–7.42 (m, 10H, ArH), 7.16 (d, 2H, *J* = 8.0 Hz, ArH), 5.60 (s, 1H, benzylidene H), 5.54 (d, 1H, *J* = 1.0 Hz, H-1), 4.75 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.66 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.35 (ddd, 1H, *J* = 10.0, 10.0, 5.0 Hz, H-5), 4.25 (dd, 1H, *J* = 10.0, 5.0 Hz, H-6a), 4.16 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3), 4.12 (dd, 1H, *J* = 3.5, 1.0 Hz, H-2), 4.02 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 3.86 (dd, 1H, *J* = 10.0, 10.0 Hz, H-6b), 2.54 (br s, 1H, O*H*), 2.39 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.1 (Ar), 137.4 (Ar), 137.3 (Ar), 132.5 (2 x Ar), 130.0 (2 x Ar), 129.8 (Ar), 129.2 (Ar), 128.7 (2 x Ar), 128.3 (2 x Ar), 128.2 (Ar), 128.1 (2 x Ar), 126.4 (2 x Ar), 102.2 (benzylidene *C*H), 86.7 (C-1), 80.1 (C-2), 79.7 (C-4), 73.2 (Ph*C*H$_2$), 69.1 (C-3), 68.5 (C-6), 64.7 (C-5), 21.2 (SPh*C*H$_3$). Anal. Calcd for $C_{27}H_{28}O_5S$ (464.57): C, 69.80; H, 6.07; S, 6.90. Found: C, 69.60; H, 6.15; S, 6.62. ESIMS: *m/z* calcd for $[C_{27}H_{28}O_5S]Na^+$: 487.1550. Found: 487.1555.

Octyl 6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranoside (2.40)

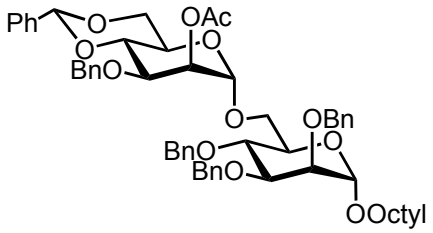


Tetraol **2.39**[17] (3.93 g, 13.4 mmol) and imidazole (2.29 g, 33.6 mmol) were dissolved in DMF (15 mL) and *tert*-butylchlorodiphenylsilane (4.2 mL, 16.1 mmol) was added. The reaction mixture was heated at 45 °C for 3 h and was quenched with water (2 mL). The mixture was then diluted with EtOAc (60 mL), washed with water (3 x 20 mL), 1M HCl (20 mL), satd aq $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated to colorless oil. The

crude product was purified by chromatography (1:1, hexane–EtOAc) to give **2.40** (6.69 g, 94%) as a colorless oil: R_f 0.35 (1:1, hexane–EtOAc); $[\alpha]_D$ = +21.1 (*c* 7.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68–7.71 (m, 4H, ArH), 7.37–7.45 (m, 6H, ArH), 4.77 (d, 1H, *J* = 1.6 Hz, H-1), 3.78–3.96 (m, 5H, H-2, H-3, H-4, H-6a, H-6b), 3.58–3.68 (m, 2H, H-5, octyl OC*H*$_2$), 3.35 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 2.06–3.00 (br s, O*H*), 1.51–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.37 (m, 10H, octyl C*H*$_2$), 1.07 (s, 9H, *tert*-butyl C*H*$_3$), 0.89 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 135.6 (4 x Ar), 132.9 (Ar), 132.8 (Ar), 129.9 (2 x Ar), 127.8 (4 x Ar), 99.4 (C-1), 71.8 (C-2), 70.7 (C-3/C-4/C-5), 70.6 (C-3/C-4/C-5), 70.5 (C-3/C-4/C-5), 67.7 (octyl O*C*H$_2$), 65.3 (C-6), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (*tert*-butyl *C*), 29.2 (octyl *C*H$_2$), 26.8 (*tert*-butyl *C*H$_3$), 26.1 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 19.2 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{30}H_{46}O_6Si]Na^+$: 553.2956. Found: 553.2956.

Octyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.41)

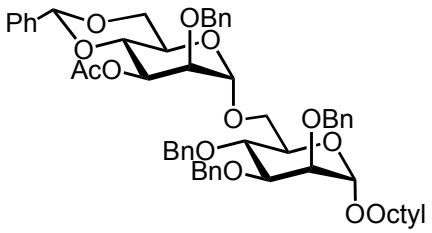
Thioglycoside **2.31** (460 mg, 0.91 mmol), alcohol **2.33** (378 mg, 0.67 mmol), and powdered 4 Å molecular sieves (350 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (20 mL) was added and the reaction was cooled to 0 °C before the addition of *N*-iodosuccinimide (240 mg, 1.1 mmol) and AgOTf



(52 mg, 0.20 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude residue was purified by chromatography (4:1, hexane–EtOAc) to give **2.41** (272 mg, 89%) as a yellow oil: R_f 0.34 (4:1, hexane–EtOAc); $[\alpha]_D$ = +33.4 (*c* 2.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.48–7.50 (m, 2H, ArH), 7.19–7.41 (m, 23H, Ar), 5.63 (s, 1H, benzylidene H), 5.52 (dd, 1H, *J* = 3.2, 1.5 Hz, H-2′), 4.93 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.91 (d, 1H, *J* = 1.5 Hz, H-1′), 4.82 (d, 1H, *J* = 2.0 Hz, H-1), 4.75 (s, 2H, PhCH_2), 4.66 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.63 (s, 2H, PhCH_2), 4.59 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.53 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.23 (dd, 1H, *J* = 10.0, 4.5 Hz, H-6a′), 4.06 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.99 (dd, 1H, *J* = 9.5, 3.2 Hz, H-3′), 3.90–3.97 (m, 2H, H-3, H-5′), 3.80–3.90 (m, 3H, H-4, H-6a, H-6b′), 3.79 (dd, 1H, *J* = 2.5, 2.0 Hz, H-2), 3.69–3.70 (m, 2H, H-5, H-6b), 3.60 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OCH_2), 3.34 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OCH_2), 2.16 (s, 3H, (CO)CH_3), 1.45–1.55 (m, 2H, octyl OCH_2CH_2), 1.20–1.35 (m, 10H, octyl CH_2), 0.89 (t, 3H, *J* = 7.5 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.0 (*C*=O), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.1 (Ar), 137.8 (Ar), 128.9 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 126.3 (Ar), 101.7 (benzylidene *C*H), 99.1 (C-1′, $^1J_{C,H}$ = 173.5 Hz), 97.8 (C-1, $^1J_{C,H}$ = 167.5 Hz), 80.5 (C-3), 78.5 (C-4′), 75.2 (Ph*C*H_2), 75.0 (C-2), 74.8 (C-4), 73.9 (C-3′), 72.8 (Ph*C*H_2), 72.2 (Ph*C*H_2), 72.0 (Ph*C*H_2), 71.3 (C-5), 69.7 (C-2′), 68.9 (C-6′), 67.8 (octyl

O*C*H$_2$), 67.3 (C-6), 64.1 (C-5′), 32.0 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 22.8 (octyl *C*H$_2$), 21.1 ((CO)*C*H$_3$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{57}H_{68}O_{12}$ (945.14): C, 72.43; H, 7.25. Found: C, 72.72; H, 7.31. ESIMS: *m/z* calcd for $[C_{57}H_{68}O_{12}]Na^+$: 967.4603. Found: 967.4606.

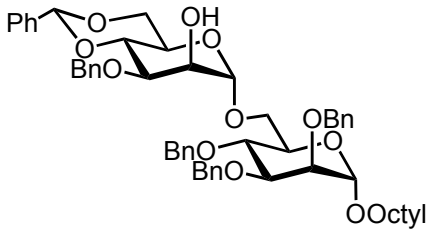
Octyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.42)



Prepared from thioglycoside **2.32** (353 mg, 0.70 mmol), alcohol **2.33** (299 mg, 0.53 mmol), powdered 4 Å molecular sieves (500 mg), *N*-iodosuccinimide (190 mg, 0.85 mmol) and AgOTf (41 mg, 0.16 mmol) in CH_2Cl_2 (20 mL) as described for **2.41**, to give **2.42** (417 mg, 83%) as a colorless oil: R_f 0.42 (6:1, hexane–EtOAc); $[\alpha]_D$ = +23.8 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.42–7.48 (m, 2H, ArH), 7.22–7.39 (m, 23H, ArH), 5.56 (s, 1H, benzylidene H), 5.33 (dd, 1H, *J* = 10.0, 3.3 Hz, H-3′), 5.14 (d, 1H, *J* = 1.5 Hz, H-1′), 4.98 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.77 (br s, 1H, H-1), 4.76 (s, 1H, *J* = 13.0 Hz, PhC*H*$_2$), 4.63–4.70 (m, 4H, PhC*H*$_2$), 4.57 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.44 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.26 (dd, 1H, *J* = 10.0, 5.0 Hz, H-6a′), 4.15 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 3.76–4.45 (m, 8H, H-2, H-3, H-4, H-6a, H-6b, H-2′, H-5′, H-6b′), 3.73 (m, 1H, H-5), 3.63 (dt, 1H, *J* = 10.0, 6.8 Hz, octyl OC*H*$_2$), 3.35

(dt, 1H, *J* = 10.0, 6.8 Hz, octyl OC*H*$_2$), 2.00 (s, 3H, (CO)C*H*$_3$), 1.47–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.35 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.0 (*C*=O), 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 138.0 (Ar), 137.5 (Ar), 128.9 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.8 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 126.2 (Ar), 101.7 (benzylidene *C*H), 98.7 (C-1′, $^1J_{C,H}$ = 175.8 Hz), 97.9 (C-1, $^1J_{C,H}$ = 175.8 Hz), 80.5 (C-3), 76.8 (C-2′), 76.4 (C-4′), 75.2 (Ph*C*H$_2$), 75.1 (C-2), 74.8 (C-4), 73.4 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 72.0 (C-5), 70.4 (C-3′), 68.9 (C-6′), 67.7 (octyl O*C*H$_2$), 66.2 (C-6), 64.0 (C-5′), 31.9 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 21.0 ((CO)*C*H$_3$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{57}H_{68}O_{12}$ (945.14): C, 72.43; H, 7.25. Found: C, 71.96; H, 7.17. ESIMS: *m/z* calcd for $[C_{57}H_{68}O_{12}]Na^+$: 967.4603. Found: 967.4605.

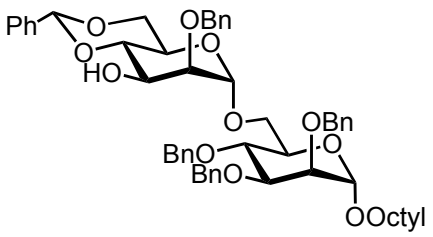
Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.43)



Disaccharide **2.41** (502 mg, 0.53 mmol) was dissolved in CH_3OH (25 mL) and $NaOCH_3$ (140 mg) was added. After 1 h, the solution was neutralized with AcOH and the crude product was purified by chromatography (3:1, hexane–EtOAc) to give **2.43** (450 mg, 94%) as a

colorless oil: R_f 0.23 (3:1, hexane–EtOAc); $[\alpha]_D$ = +49.7 (*c* 2.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.46–7.52 (m, 2H, ArH), 7.22–7.42 (m, 23H, ArH), 5.61 (s, 1H, benzylidene H), 5.06 (d, 1H, *J* = 1.5 Hz, H-1′), 4.93 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.83 (d, 1H, *J* = 2.0 Hz, H-1), 4.78 (d, 2H, *J* = 12.0 Hz, PhC*H*$_2$), 4.70 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.67 (s, 2H, PhC*H*$_2$), 4.66 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.56 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.25 (dd, 1H, *J* = 10.0, 4.5 Hz, H-6a′), 4.16 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2′), 4.11 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.82–3.97 (m, 6H, H-3, H-4, H-6a, H-3′, H-5′, H-6b′), 3.80 (dd, 1H, *J* = 2.0 Hz, H-2), 3.76 (dd, 2H, *J* = 11.5, 2.3 Hz, H-6b), 3.68–3.73 (m, 1H, H-5), 3.61 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.35 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 2.54 (br s, 1H, O*H*), 1.47–1.56 (m, 2H, octyl OCH_2CH_2), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.7 (Ar), 138.6 (Ar), 138.6 (Ar), 138.2 (Ar), 137.9 (Ar), 129.0 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.5 (Ar), 128.3 (Ar), 128.1 (Ar), 127.9 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 126.3 (Ar), 101.7 (benzylidene *C*H), 100.4 (C-1′), 97.8 (C-1), 80.3 (C-3), 78.9 (C-4′), 75.3 (C-3′), 75.2 (Ph*C*H_2), 75.1 (C-2), 74.6 (C-4), 72.7 (Ph*C*H_2), 72.7 (Ph*C*H_2), 72.1 (Ph*C*H_2), 71.6 (C-5), 69.8 (C-2′), 68.9 (C-6′), 67.8 (octyl O*C*H_2), 66.6 (C-6), 63.4 (C-5′), 32.0 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 26.3 (octyl *C*H_2), 22.8 (octyl *C*H_2), 14.2 (octyl *C*H_3). Anal. Calcd for $C_{55}H_{66}O_{11}$ (903.11): C, 73.15; H, 7.37. Found: C, 72.89; H, 7.40. ESIMS: *m/z* calcd for $[C_{55}H_{66}O_{11}]Na^+$: 925.4497. Found: 925.4498.

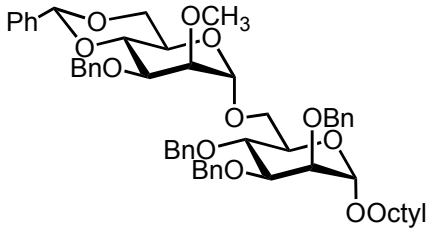
Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.44)



Prepared from disaccharide **2.42** (232 mg, 0.25 mmol) in CH_3OH (15 mL) and $NaOCH_3$ (81 mg) as described for **2.43**, to give **2.44** (197 mg, 89%) as a pale yellow oil: R_f 0.31 (4:1, hexane–EtOAc); $[\alpha]_D$ = +33.8 (*c* 3.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.46–7.54 (m, 2H, ArH), 7.24–7.44 (m, 23H, ArH), 5.58 (s, 1H, benzylidene H), 5.17 (d, 1H, *J* = 1.0 Hz, H-1′), 4.99 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 2.0 Hz, H-1), 4.78 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.63–4.71 (m, 5H, PhC*H*$_2$), 4.51 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.25 (dd, 1H, *J* = 10.3, 4.3 Hz, H-6a′), 4.10 (dd, 1H, *J* = 9.4, 3.8 Hz, H-3′), 4.00 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4′), 3.86–4.38 (m, 5H, H-3, H-4, H-6a, H-2′, H-5′), 3.74–3.86 (m, 3H, H-2, H-6b, H-6b′), 3.72 (m, 1H, H-5), 3.62 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.35 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.28 (br s, 1H, O*H*), 1.48–1.58 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 137.9 (Ar), 137.5 (Ar), 129.0 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 126.4 (Ar), 102.1 (benzylidene *C*H), 98.4 (C-1′), 98.0 (C-1), 80.4 (C-3), 79.6 (C-4′), 78.9 (C-2′), 75.2 (Ph*C*H$_2$), 75.0 (C-2), 74.6 (C-4), 73.3 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.9 (C-5), 68.9 (C-6′), 68.6 (C-3′),

67.7 (octyl O*C*H_2), 66.4 (C-6), 63.5 (C-5′), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{55}H_{66}O_{11}]Na^+$: 925.4497. Found: 925.4499.

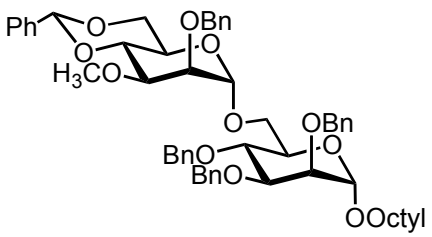
Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.45)



Disaccharide **2.43** (270 mg, 0.30 mmol) was dissolved in DMF (5 mL) and the solution was cooled in an ice bath. Then 60% NaH in mineral oil (18 mg, 0.45 mmol) was added and the mixture was stirred for 10 min before the addition of CH_3I (60 μL, 0.90 mmol). The reaction mixture was stirred for 1 h and quenched by the addition of CH_3OH (5 mL). The mixture was diluted with EtOAc (50 mL), washed with water (3 x 15 mL), and dried ($MgSO_4$), filtered and concentrated to a colorless oil. The crude product was purified by chromatography (4:1, hexane–EtOAc) to give **2.45** (251 mg, 91%) as a colorless oil: R_f 0.32 (4:1, hexane–EtOAc); $[\alpha]_D$ = +58.5 (*c* 1.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.46–7.51 (m, 2H, ArH), 7.24–7.42 (m, 22H, ArH), 7.16–7.20 (m, 1H, ArH), 5.61 (s, 1H, benzylidene H), 5.07 (d, 1H, *J* = 1.8 Hz, H-1′), 4.89 (d, 1H, *J* = 10.5 Hz, PhC*H*$_2$), 4.76–4.82 (m, 3H, H-1, PhC*H*$_2$), 4.71 (d, 2H, *J* = 13.0 Hz, PhC*H*$_2$), 4.67 (s, 2H, PhC*H*$_2$), 4.49 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$),

4.37–4.25 (m, 1H, H-6a′), 4.14 (dd, 1H, *J* = 9.3, 9.3 Hz, H-4′), 3.80–3.95 (m, 6H, H-3, H-4, H-6a, H-3′, H-5′, H-6b′), 3.80 (br s, 1H, H-2), 3.74 (dd, 1H, *J* = 12.0, 1.5 Hz, H-6b), 3.70 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′), 3.65–3.72 (m, 1H, H-5), 3.59 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 3.47 (s, 3H, OC*H*$_3$), 3.33 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 1.45–1.55 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.7 (Ar), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.3 (Ar), 101.6 (benzylidene *C*H), 99.2 (C-1′), 98.1 (C-1), 80.5 (C-3), 79.4 (C-2′), 79.3 (C-4′), 75.5 (C-2/C-3′), 75.3 (C-2/C-3′), 75.3 (Ph*C*H$_2$), 74.7 (C-4), 73.1 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 71.9 (C-5), 69.0 (C-6′), 67.9 (octyl O*C*H$_2$), 66.5 (C-6), 64.3 (C-5′), 59.9 (O*C*H$_3$), 32.0 (octyl *C*H$_2$), 29.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 22.8 (octyl *C*H$_2$), 14.2 (octyl *C*H$_3$). Anal. Calcd for $C_{56}H_{68}O_{11}$ (917.13): C, 73.34; H, 7.47. Found: C, 73.04; H, 7.44. ESIMS: *m/z* calcd for [$C_{56}H_{68}O_{11}$: 939.4654. Found: 939.4656.

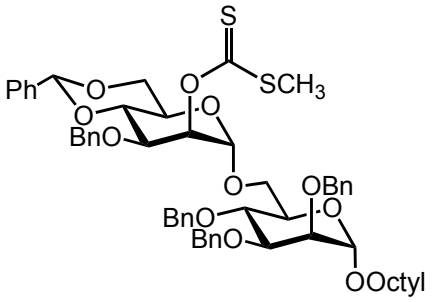
Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.46)



Prepared from disaccharide **2.44** (197 mg, 0.22 mmol), 60% NaH in mineral oil (13 mg, 0.33 mmol) and CH_3I (42 μL, 0.66

mmol) in DMF (5 mL) as described for **2.45**, to give **2.46** (154 mg, 76%) as a colorless oil: R_f 0.38 (6:1, hexane–EtOAc); $[\alpha]_D$ = +35.6 (*c* 4.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.46–7.52 (m, 2H, *J* = 7.8, 1.8 Hz, ArH), 7.24–7.42 (m, 23H, ArH), 5.62 (s, 1H, benzylidene H), 5.07 (d, 1H, *J* = 2.0 Hz, H-1′), 5.00 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 1.5 Hz, H-1), 4.75 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.72 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.63–4.71 (m, 5H, PhC*H*$_2$), 4.24 (dd, 1H, *J* = 10.0, 4.5 Hz, H-6a′), 4.16 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.90–4.00 (m, 5H, H-3, H-4, H-6a, H-2′, H-5′), 3.85 (dd, 1H, *J* = 10.0, 10.0 Hz, H-6b′), 3.80 (dd, 1H, *J* = 2.0, 1.8 Hz, H-2), 3.70–3.78 (m, 3H, H-5, H-6b, H-3′), 3.63 (dt, 1H, *J* = 10.0, 6.8 Hz, octyl OC*H*$_2$), 3.44 (s, 3H, OC*H*$_3$), 3.37 (dt, 1H, *J* = 10.0, 6.8 Hz, octyl OC*H*$_2$), 1.49–1.58 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 137.8 (Ar), 128.8 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 126.3 (Ar), 101.7 (benzylidene *C*H), 99.3 (C-1′), 97.9 (C-1), 80.4 (C-3), 79.0 (C-4′), 77.9 (C-3′), 76.1 (C-4), 75.1 (Ph*C*H$_2$), 75.0 (C-2′), 74.7 (C-2), 73.3 (Ph*C*H$_2$), 72.8 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.6 (C-5), 68.9 (C-6′), 67.8 (octyl O*C*H$_2$), 66.5 (C-6), 64.2 (C-5′), 58.5 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{56}H_{68}O_{11}]Na^+$: 939.4654. Found: 939.4657.

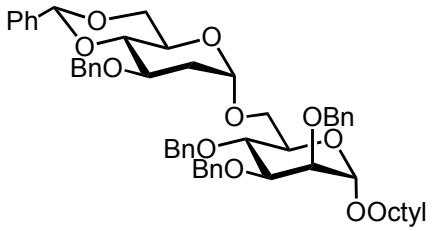
Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(methylthio)thiocarbonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.47)



Disaccharide **2.43** (293 mg, 0.32 mmol) was dissolved in THF (20 mL), and then 60% NaH in mineral oil (38 mg, 0.96 mmol) and imidazole (10 mg), were added. The mixture was stirred for 1 h before the addition of carbon disulfide (0.19 mL, 3.2 mmol) and stirring continued for 1 h. Methyl iodide (0.10 mL, 1.6 mmol) was added and the mixture was stirred overnight. The solvent was evaporated and the crude product was dissolved in CH_2Cl_2 (25 mL), washed with water (10 mL), dried ($MgSO_4$), and concentrated to a yellow oil. The crude product was then purified by chromatography (6:1, hexane–EtOAc) to give **2.47** (310 mg, 96%) as a yellow oil: R_f = 0.41 (6:1, hexane–EtOAc); $[\alpha]_D$ = +24.6 (*c* 2.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.47–7.52 (m, 2H, ArH), 7.18–7.42 (m, 23H, ArH), 6.26 (dd, 1H, *J* = 2.8, 1.5 Hz, H-2′), 5.65 (s, 1H, benzylidene H), 5.07 (d, 1H, *J* = 1.5 Hz, H-1′), 4.93 (d, 1H, *J* = 11.3 Hz, PhCH_2), 4.81 (d, 1H, *J* = 2.0 Hz, H-1), 4.74 (s, 2H, PhCH_2), 4.67 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.63 (s, 2H, PhCH_2), 4.62 (d, 1H, *J* = 12.5 Hz, PhCH_2), 4.55 (d, 1H, *J* = 11.3 Hz, PhCH_2), 4.26 (dd, 1H, *J* = 9.8, 4.8 Hz, H-6a′), 4.08–4.14 (m, 2H, H-3′, H-4′), 3.94–4.00 (m, 1H, H-5′), 3.91 (dd, 1H, *J* = 9.0, 3.0 Hz, H-3), 3.82–3.88 (m, 3H, H-4, H-6a, H-6b′), 3.76–3.81 (m, 2H,

H-2, H-6b), 3.72 (ddd, 1H, *J* = 9.5, 6.0, 1.5 Hz, H-5), 3.60 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.34 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.61 (s, 3H, SC*H*$_3$), 1.45–1.55 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.35 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 215.7 (*C*=S), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 137.9 (Ar), 137.6 (Ar), 128.9 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 126.2 (Ar), 101.7 (benzylidene *C*H), 97.6 (C-1), 97.6 (C-1′), 80.3 (C-3), 78.8 (C-4′), 77.9 (C-2′), 75.1 (Ph*C*H$_2$), 74.7 (C-2/C-4), 74.8 (C-2/C-4), 73.9 (C-3′), 72.7 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 72.0 (Ph*C*H$_2$), 71.0 (C-5), 68.8 (C-6′), 67.7 (octyl O*C*H$_2$), 66.9 (C-6), 63.9 (C-5′), 31.9 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 19.2 (S*C*H$_3$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{57}H_{68}O_{11}S_2]Na^+$: 1015.4095. Found: 1015.4096.

Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-arabinohexopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.48)

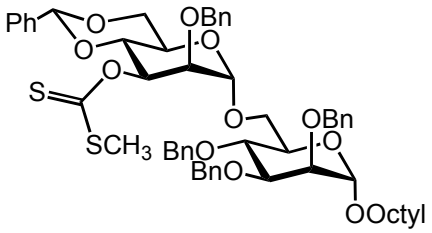


Xanthate **2.47** (310 mg, 0.31 mmol) was dissolved in toluene (15 mL) and tri-*n*-butylstannane (1.3 mL, 0.47 mmol) and AIBN (51 mg, 0.31 mmol) were added. The mixture was heated at reflux for 3 h and the solvent was evaporated.

The crude product was purified by chromatography (4:1, hexane–EtOAc) to give **2.48** (113 mg, 41%) as a colorless oil: R_f = 0.42 (4:1, hexane–EtOAc); $[\alpha]_D$ = +50.8 (*c* 3.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.46–7.54 (m, 2H, ArH), 7.20–7.42 (m, 23H, ArH), 5.62 (s, 1H, benzylidene H), 5.08 (br d, 1H, *J* = 3.5 Hz, H-1′), 4.95 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.83 (d, 1H, *J* = 1.5 Hz, H-1), 4.80 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.78 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.70 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.63–4.68 (m, 3H, PhC*H*$_2$), 4.60 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.23 (dd, 1H, *J* = 10.0, 5.0 Hz, H-6a′), 4.03 (ddd, 1H, *J* = 11.0, 11.0, 5.0 Hz, H-3′), 3.84–3.98 (m, 4H, H-3, H-4, H-6a, H-5′), 3.66–3.82 (m, 5H, H-2, H-5, H-6b, H-4′, H-6b′), 3.63 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.36 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.35 (dd, 1H, *J* = 13.3, 5.0 Hz, H-2′$_{eq}$), 1.78 (ddd, 1H, *J* = 13.3, 11.0, 3.5 Hz, H-2′$_{ax}$), 1.47–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.35 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.3 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.7 (Ar), 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 137.8 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.7 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 126.1 (Ar), 101.4 (benzylidene *C*H), 98.1 (C-1′), 97.7 (C-1), 84.0 (C-4′), 80.4 (C-3), 75.2 (Ph*C*H$_2$), 75.0 (C-2), 74.9 (C-4), 72.8 (C-3′), 72.8 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 71.5 (C-5), 69.2 (C-6′), 67.7 (octyl O*C*H$_2$), 66.1 (C-6), 63.1 (C-5′), 36.4 (C-2′), 31.9 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{55}H_{66}O_{10}$ (887.11): C, 74.47; H, 7.50.

Found: C, 74.10; H, 7.89. ESIMS: *m/z* calcd for $[C_{55}H_{66}O_{10}]Na^+$: 909.4548. Found: 909.4551.

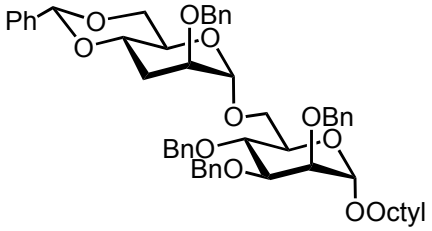
Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methylthio)thiocarbonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.49)



Prepared from disaccharide **2.44** (219 mg, 0.24 mmol), 60% NaH in mineral oil (29 mg, 0.72 mmol), imidazole (10 mg), carbon disulfide (0.15 mL, 2.4 mmol) and methyl iodide (0.15 mL, 2.4 mmol) in THF (13 mL) as described for **2.47**, to give **2.49** (227 mg, 94%) as a colorless oil: R_f = 0.31 (6:1, hexane–EtOAc); $[\alpha]_D$ = +5.2 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.44–7.50 (m, 2H, ArH), 7.21–7.40 (m, 23H, ArH), 6.15 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3′), 5.61 (s, 1H, benzylidene H), 5.14 (d, 1H, *J* = 1.5 Hz, H-1′), 4.99 (d, 1H, *J* = 11.0 Hz, PhCH_2), 4.77 (d, 1H, *J* = 1.5 Hz, H-1), 4.76 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.64–4.70 (m, 4H, PhCH_2), 4.51 (d, 1H, *J* = 11.5 Hz, PhCH_2), 4.48 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.40 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 4.36 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2′), 4.29 (dd, 1H, *J* = 10.5, 5.0 Hz, H-6a′), 4.09 (ddd, 1H, *J* = 10.0, 10.0, 5.0 Hz, H-5′), 3.99 (dd, 1H, *J* = 9.5, 9.5 Hz, H-6a), 3.85–4.02 (m, 3H, H-3, H-4, H-6b′), 3.72–3.81 (m, 3H, H-2, H-5, H-6b), 3.65 (dt, 1H, *J* = 9.8, 6.5 Hz, octyl OCH_2), 3.35 (dt, 1H, *J* = 9.8, 6.5 Hz, octyl OCH_2), 2.55 (s, 3H, SCH_3), 1.46–1.58 (m, 2H, octyl

OCH_2CH_2), 1.22–1.36 (m, 10H, octyl CH_2), 0.90 (t, 3H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 215.0 (*C*=S), 138.6 (Ar), 138.5 (Ar), 138.4 (Ar), 137.9 (Ar), 137.4 (Ar), 128.9 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 126.2 (Ar), 101.6 (benzylidene *C*H), 99.2 (C-1′), 97.9 (C-1), 80.5 (C-3), 79.1 (C-3′), 76.3 (C-4′), 75.8 (C-2′), 75.3 (Ph*C*H_2), 75.1 (C-2), 74.9 (C-4), 73.6 (Ph*C*H_2), 73.0 (Ph*C*H_2), 72.2 (Ph*C*H_2), 72.0 (C-5), 68.9 (C-6′), 67.6 (octyl O*C*H_2), 66.4 (C-6), 64.1 (C-5′), 31.9 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.3 (octyl *C*H_2), 22.7 (octyl *C*H_2), 19.1 (S*C*H_3), 14.1 (octyl *C*H_3). Anal. Calcd for $C_{57}H_{68}O_{11}S_2$ (993.27): C, 68.92; H, 6.90; S, 6.46. Found: C, 68.41; H, 6.86; S, 6.18. ESIMS: *m/z* calcd for $[C_{57}H_{68}O_{11}S_2]Na^+$: 1015.4095. Found: 1015.4094.

Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-deoxy-α-D-arabinohexopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.50)

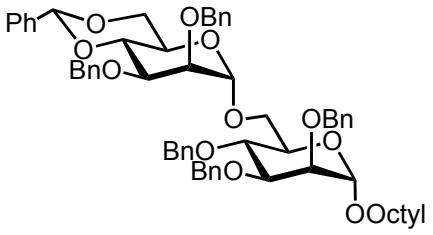


Xanthate **2.49** (206 mg, 0.21 mmol) was dissolved in toluene (10 mL) and added dropwise to a solution of tri-*n*-butylstannane (85 μL, 0.32 mmol) and AIBN (10 mg, 0.063 mmol) in toluene (15 mL) at reflux, over a period of 1 h. The mixture was heated under reflux for 1 h and tri-*n*-butylstannane (85 μL, 0.32 mmol) and AIBN (10 mg, 0.063 mmol) in toluene (5 mL) were

added and the reaction mixture was continually heated under reflux for 1 h. The solvent was evaporated and the crude product was purified by chromatography (6:1, hexane–EtOAc) to give **2.50** (69 mg, 37%) as a colorless oil: R_f = 0.41 (6:1, hexane–EtOAc); $[\alpha]_D$ = +52.1 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.45–7.50 (m, 2H, ArH), 7.23–7.39 (m, 23H, AHr), 5.57 (s, 1H, benzylidene H), 4.99 (br s, 1H, H-1′), 4.98 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 1.5 Hz, H-1), 4.76 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.64–4.69 (m, 4H, PhC*H*$_2$), 4.52 (s, 2H, PhC*H*$_2$), 4.21 (dd, 1H, *J* = 5.3, 4.8 Hz, H-6a′), 3.92–4.04 (m, 5H, H-3, H-4, H-6a, H-4′, H-5′), 3.76–3.83 (m, 4H, H-2, H-6b, H-2′, H-6b′), 3.70–3.75 (m, 1H, H-5), 3.62 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 3.35 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 2.19 (ddd, 1H, *J* = 12.5, 3.5, 3.5 Hz, H-3′$_{eq}$), 2.01 (ddd, 1H, *J* = 12.5, 12.5, 3.0 Hz, H-3′$_{ax}$), 1.47–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.35 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 138.2 (Ar), 137.8 (Ar), 128.9 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 126.2 (Ar), 102.0 (benzylidene *C*H), 97.9 (C-1′), 97.9 (C-1), 80.4 (C-3), 75.5 (C-2/C-2′), 75.2 (PhCH$_2$), 75.0 (C-2/C-2′), 74.7 (C-4/C-4′), 74.3 (C-4/C-4′), 72.8 (PhCH$_2$), 72.2 (PhCH$_2$), 71.8 (C-5), 71.3 (PhCH$_2$), 69.4 (C-6′), 67.7 (octyl OCH$_2$), 66.4 (C-6), 65.2 (C-5′), 31.8 (octyl CH$_2$), 29.5 (octyl CH$_2$), 29.4 (C-3′), 29.4 (octyl CH$_2$), 29.2 (octyl CH$_2$), 26.2 (octyl CH$_2$), 22.7 (octyl CH$_2$), 14.1 (octyl CH$_3$). Anal. Calcd for $C_{55}H_{66}O_{10}$ (887.11): C, 74.47; H, 7.50. Found: C,

74.39; H, 7.55. ESIMS: *m/z* calcd for $[C_{55}H_{66}O_{10}]Na^+$: 909.4548. Found: 909.4551.

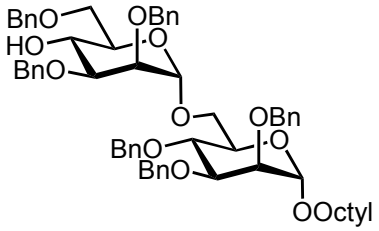
Octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.51)



Prepared from disaccharide **2.43** (1.57 g, 1.6 mmol), 60% NaH in mineral oil (0.10 g, 2.6 mmol) and BnBr (0.32 mL, 2.6 mmol) in DMF (15 mL) as described for **2.33**, to give **2.51** (1.62 g, 94%) as a colorless oil: R_f 0.28 (9:1, hexane–EtOAc); $[\alpha]_D$ = +35.6 (*c* 2.3, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.48–7.52 (m, 2H, ArH), 7.18–7.40 (m, 28H, ArH), 5.64 (s, 1H, benzylidene H), 5.09 (br s, 1H, H-1′), 4.90 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.80 (d, 1H, *J* = 1.8 Hz, H-1), 4.75 (d, 1H, *J* = 12.6 Hz, PhC*H*$_2$), 4.74 (d, 1H, *J* = 12.6 Hz, PhC*H*$_2$), 4.63–4.72 (m, 5H, PhC*H*$_2$), 4.63 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.52 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.20–4.27 (m, 2H, H-4′, H-6a′), 3.90–3.98 (m, 2H, H-2′, H-3′), 3.84–3.95 (m, 5H, H-3, H-4, H-6a, H-5′, H-6b′), 3.79 (dd, 1H, *J* = 1.8, 1.8 Hz, H-2), 3.73 (dd, 1H, *J* = 11.7, 1.5 Hz, H-6b), 3.66–3.71 (m, 1H, H-5), 3.59 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.34 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.46–1.54 (m, 2H, octyl OCH_2CH_2), 1.22–1.34 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.6 (Ar), 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 137.9 (Ar), 128.7 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3

(Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 101.5 (benzylidene *C*H), 99.5 (C-1′), 97.8 (C-1), 80.4 (C-3), 79.1 (C-4′), 76.9 (C-3′), 75.8 (C-2′), 75.2 (Ph*C*H_2), 75.0 (C-2), 74.6 (C-4), 73.4 (Ph*C*H_2), 72.8 (Ph*C*H_2), 72.6 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.7 (C-5), 68.9 (C-6′), 67.7 (octyl O*C*H_2), 66.4 (C-6), 64.3 (C-5′), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). Anal. Calcd for $C_{62}H_{72}O_{11}$ (993.23): C, 74.97; H, 7.31. Found: C, 74.63; H, 7.54. ESIMS: *m/z* calcd for $[C_{62}H_{72}O_{11}]Na^+$: 1015.4967. Found: 1015.4963.

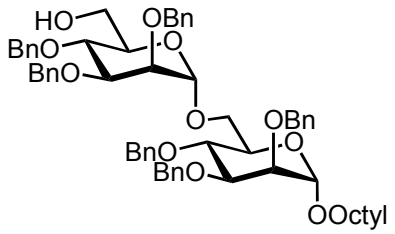
Octyl 2,3,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.52)



A solution of disaccharide **2.51** (450 mg, 0.45 mmol) and powdered 4 Å molecular sieves (1.8 g) in CH_2Cl_2 (6 mL) was stirred at room temperature for 1 h. The solution was cooled to –78 °C followed by the addition of Et_3SiH (0.22 mL, 1.4 mmol) and TfOH (0.14 mL, 1.5 mmol). After 1 h, the reaction was quenched with triethylamine and CH_3OH, diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$, dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography (4:1, hexane–EtOAc) to give **2.52** (276 mg, 61%) and **2.53** (17 mg, 4%) as colorless oils: R_f 0.33 (4:1, hexane–EtOAc); $[\alpha]_D$ = +7.9 (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.18–

7.42 (m, 30H, ArH), 5.16 (d, 1H, *J* = 1.5 Hz, H-1′), 4.91 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 1.8 Hz, H-1), 4.75 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.64–4.71 (m, 4H, PhC*H*$_2$), 4.61 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.60 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.55 (d, 2H, *J* = 11.5 Hz, PhC*H*$_2$), 4.51 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.40 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.07 (ddd, 1H, *J* = 10.0, 10.0, 1.3 Hz, H-4′), 3.91–4.00 (m, 4H, H-3, H-4, H-6a, H-2′), 3.81 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2), 3.67–3.81 (m, 6H, H-5, H-6b, H-3′, H-5′, H-6a′, H-6b′), 3.60 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.34 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.43 (d, 1H, *J* = 1.3 Hz, O*H*), 1.47–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.4 (Ar), 127.4 (Ar), 98.4 (C-1′), 97.9 (C-1), 80.4 (C-3), 78.7 (C-3′), 75.2 (C-2), 75.1 (Ph*C*H$_2$), 75.1 (C-2′), 74.2 (C-4), 73.5 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.7 (C-5), 71.5 (C-5′), 71.1 (Ph*C*H$_2$), 70.4 (C-6′), 67.7 (C-4′), 67.7 (octyl O*C*H$_2$), 66.2 (C-6), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{62}H_{74}O_{11}]Na^+$: 1017.5123. Found: 1017.5128.

Octyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.53)

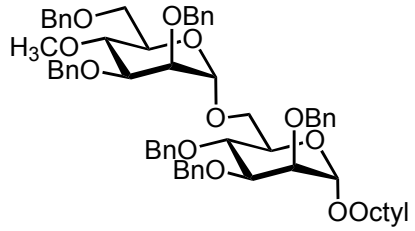


Prepared from a solution of $AlCl_3$ (62 mg, 0.46 mmol) and $LiAlH_4$ (0.46 mmol) in ether (3.5 mL) and disaccharide **2.51** (382 mg, 0.39 mmol) in 1:1, CH_2Cl_2–ether (15 mL) as described for **2.37** (except that the reaction was stirred overnight), to give **2.53** (324 mg, 85%) and **2.52** (11 mg, 3%) as colorless oils: R_f 0.24 (3:1, hexane–EtOAc); $[\alpha]_D$ = +33.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.18–7.40 (m, 30H, ArH), 5.09 (br s, 1H, H-1′), 4.94 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.91 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.80 (d, 1H, *J* = 1.5 Hz, H-1), 4.47–4.77 (m, 10H, PhC*H*$_2$), 3.87–4.00 (m, 6H, H-3, H-4, H-6a, H-2′, H-3′, H-4′), 3.64–3.81 (m, 6H, H-2, H-5, H-6b, H-5′, H-6a′, H-6b′), 3.59 (dt, 1H, *J* = 9.7, 6.4 Hz, octyl OC*H*$_2$), 3.33 (dt, 1H, *J* = 9.7, 6.4 Hz, octyl OC*H*$_2$), 1.78 (br s, 1H, O*H*), 1.45–1.55 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.35 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.6 (Ar), 138.6 (Ar), 138.5 (Ar), 138.5 (Ar), 138.3 (Ar), 128.8 (Ar), 128.8 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.5 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 127.9 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.5 (Ar), 127.5 (Ar), 98.3 (C-1′), 97.8 (C-1), 80.4 (C-3), 79.4 (C-3′), 79.4 (C-4′), 75.1 (C-2), 75.1 (Ph*C*H$_2$), 74.7 (C-2′), 74.6 (C-4), 72.8 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 72.2 (C-5′), 72.1 (Ph*C*H$_2$), 71.6 (Ph*C*H$_2$), 71.6 (C-5), 67.7 (octyl

O*C*H_2), 66.1 (C-6), 62.3 (C-6′), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{62}H_{74}O_{11}]Na^+$: 1017.5123. Found: 1017.5136.

Octyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside

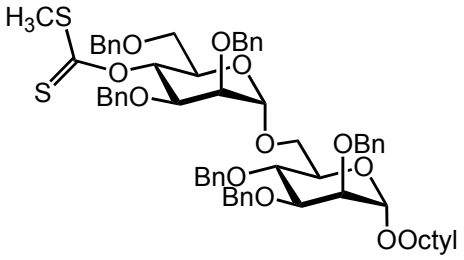
(2.54)



Prepared from disaccharide **2.52** (134 mg, 0.14 mmol), 60% NaH in mineral oil (8.1 mg, 0.20 mmol) and CH_3I (13 μL, 0.20 mmol) in DMF (3 mL) as described for **2.45**, to give **2.54** (105 mg, 77%) as a colorless oil: R_f 0.22 (4:1, hexane–EtOAc); $[\alpha]_D$ = +30.3 (*c* 1.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.19–7.40 (m, 30H, ArH), 5.12 (d, 1H, *J* = 2.1 Hz, H-1′), 4.89 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.78 (d, 1H, *J* = 2.1 Hz, H-1), 4.73 (d, 1H, *J* = 12.6 Hz, PhC*H*$_2$), 4.69 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.67 (d, 1H, *J* = 12.6 Hz, PhC*H*$_2$), 4.65 (s, 2H, PhC*H*$_2$), 4.65 (s, 2H, PhC*H*$_2$), 4.57 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.54 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.52 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.51 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 3.90–3.96 (m, 3H, H-3, H-4, H-6a), 3.90 (dd, 1H, *J* = 3.6, 2.1 Hz, H-2′), 3.81 (dd, 1H, *J* = 7.8, 3.6 Hz, H-3′), 3.78 (dd, 1H, *J* = 2.1, 2.1 Hz, H-2), 3.64–3.76 (m, 6H, H-5, H-6b, H-4′, H-5′, H-6a′, H-6b′), 3.58 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.49 (s, 3H, OC*H*$_3$), 3.32 (dt, 1H, *J* = 9.6, 6.6 Hz,

octyl OC*H*$_2$), 1.46–1.54 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.34 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.8 (Ar), 138.6 (Ar), 138.6 (Ar), 138.5 (Ar), 138.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 98.1 (C-1′), 97.8 (C-1), 80.4 (C-3), 79.4 (C-3′), 76.5 (C-4′), 75.1 (C-2), 75.1 (C-2′), 75.1 (PhCH$_2$), 74.7 (C-4), 73.3 (PhCH$_2$), 72.8 (PhCH$_2$), 72.4 (PhCH$_2$), 72.2 (PhCH$_2$), 71.9 (C-5′), 71.7 (C-5), 71.6 (PhCH$_2$), 69.4 (C-6′), 67.6 (octyl OCH$_2$), 66.1 (C-6), 60.6 (OCH$_3$), 31.8 (octyl CH$_2$), 29.4 (octyl CH$_2$), 29.4 (octyl CH$_2$), 29.2 (octyl CH$_2$), 26.2 (octyl CH$_2$), 22.7 (octyl CH$_2$), 14.1 (octyl CH$_3$). ESIMS: *m/z* calcd for $[C_{63}H_{76}O_{11}]Na^+$: 1031.5280. Found: 1031.5280.

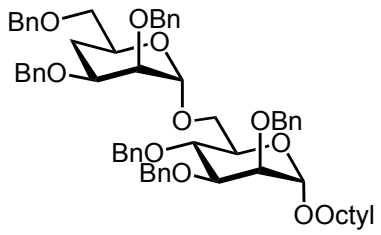
Octyl 2,3,6-tri-*O*-benzyl-4-*O*-(methylthio)thiocarbonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.55)



Prepared from disaccharide **2.52** (148 mg, 0.15 mmol), 60% NaH in mineral oil (18 mg, 0.45 mmol), carbon disulfide (0.092 mL, 1.5 mmol) and methyl iodide (0.094 mL, 1.5 mmol) in THF (9 mL) as described for **2.47**, to give **2.55** (154 mg, 96%) as a light yellow oil: R_f = 0.37 (6:1, hexane–EtOAc); $[\alpha]_D$ = +34.1 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.16–7.42 (m, 30H,

ArH), 6.36 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 5.14 (br s, 1H, H-1′), 4.88 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 1.9 Hz, H-1), 4.63–4.77 (m, 6H, PhC*H*$_2$), 4.53 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.49 (s, 2H, PhC*H*$_2$), 4.45 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.42 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 3.91–4.01 (m, 6H, H-3, H-4, H-6a, H-2′, H-3′, H-5′), 3.81 (dd, 1H, *J* = 1.9, 1.9 Hz, H-2), 3.72 (dd, 1H, *J* = 11.8, 1.3 Hz, H-6b), 3.66–3.71 (m, 1H, H-5), 3.54–3.63 (m, 3H, H-6a′, H-6b′, octyl OC*H*$_2$), 3.34 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.48 (s, 3H, SC*H*$_3$), 1.48–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 215.4 (*C*=S), 138.6 (Ar), 138.6 (Ar), 138.5 (Ar), 138.3 (Ar), 138.3 (Ar), 138.0 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.3 (Ar), 127.3 (Ar), 98.7 (C-1′), 97.8 (C-1), 80.4 (C-3), 77.7 (C-4′), 76.4 (C-3′), 75.2 (C-2), 75.1 (Ph*C*H$_2$), 75.0 (C-2′), 74.5 (C-4), 73.4 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.8 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.6 (C-5), 71.3 (Ph*C*H$_2$), 70.5 (C-5′), 69.7 (C-6′), 67.7 (octyl O*C*H$_2$), 66.5 (C-6), 31.9 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 19.2 (S*C*H$_3$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{64}H_{76}O_{11}S_2$ (1084.48): C, 70.82; H, 7.06; S, 5.91. Found: C, 71.04; H, 6.91; S, 5.93. ESIMS: *m/z* calcd for $[C_{64}H_{76}O_{11}S_2]Na^+$: 1107.4721. Found: 1107.4725.

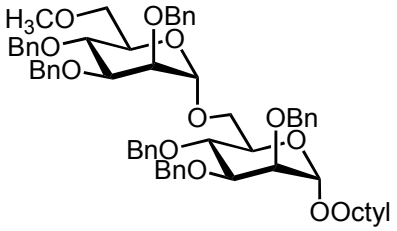
Octyl 2,3,6-tri-*O*-benzyl-4-deoxy-α-D-*lyxo*-hexopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.56)



Prepared from xanthate **2.55** (129 mg, 0.12 mmol) in toluene (2 mL) and tri-*n*-butylstannane (50 μL, 0.18 mmol) and AIBN (6 mg, 0.037 mmol) as described for **2.50**, to give **2.56** (76 mg, 64%) as a colorless oil: R_f = 0.31 (6:1, hexane–EtOAc); $[\alpha]_D$ = +20.8 (*c* 0.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.18–7.41 (m, 30H, ArH), 5.15 (d, 1H, *J* = 1.2 Hz, H-1′), 4.89 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.81 (d, 1H, *J* = 1.8 Hz, H-1), 4.75 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.74 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.70 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.70 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.69 (s, 2H, PhCH_2), 4.54 (s, 2H, PhCH_2), 4.49 (d, 2H, *J* = 12.0 Hz, PhCH_2), 4.45 (d, 1H, *J* = 12.0 Hz, PhCH_2), 3.91 (m, 4H, H-3, H-4, H-6a, H-5′), 3.84–3.91 (m, 2H, H-2′, H-3′), 3.80 (dd, 1H, *J* = 2.4, 1.8 Hz, H-2), 3.72 (dd, 1H, *J* = 12.0, 1.8 Hz, H-6b), 3.68–3.72 (m, 1H, H-5), 3.59 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OCH_2), 3.56 (dd, 1H, *J* = 10.2, 6.0 Hz, H-6a′), 3.45 (dd, 1H, *J* = 10.2, 4.2 Hz, H-6b′), 3.33 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OCH_2), 1.98 (ddd, 1H, *J* = 12.0, 12.0, 12.0 Hz, H-4′$_{ax}$), 1.73–1.78 (m, 1H, H-4′$_{eq}$), 1.47–1.55 (m, 2H, octyl OCH_2CH_2), 1.21–1.35 (m, 10H, octyl CH_2), 0.90 (t, 3H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 139.0 (Ar), 138.6 (Ar), 138.6 (Ar), 138.5 (Ar), 138.4 (Ar), 138.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.6 (Ar), 127.4 (Ar), 127.3

(Ar), 99.3 (C-1′), 97.8 (C-1), 80.4 (C-3), 75.1 (C-2), 75.0 (Ph*C*H_2), 74.8 (C-4), 73.6 (C-2′), 73.3 (Ph*C*H_2), 73.2 (C-3′), 73.0 (C-6′), 72.7 (Ph*C*H_2), 72.5 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.6 (C-5), 70.0 (Ph*C*H_2), 68.2 (C-5′), 67.6 (octyl O*C*H_2), 66.2 (C-6), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 29.2 (C-4′), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{62}H_{74}O_{10}]Na^+$: 1001.5174. Found: 1001.5174.

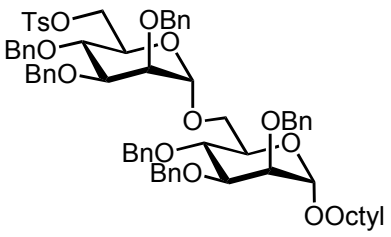
Octyl 2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.57)



Prepared from disaccharide **2.53** (109 mg, 0.11 mmol) in DMF (3 mL) and 60% NaH in mineral oil (7.2 mg, 0.18 mmol) and CH_3I (12 μL, 0.18 mmol) as described for **2.45**, to give **2.57** (107 mg, 96%) as a colorless oil: R_f 0.41 (4:1, hexane–EtOAc); $[\alpha]_D$ = +31.1 (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.18–7.40 (m, 30H, ArH), 5.15 (d, 1H, *J* = 1.5 Hz, H-1′), 4.94 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.90 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 1.5 Hz, H-1), 4.64–4.76 (m, 6H, PhC*H*$_2$), 4.59 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.57 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.90 (d, 2H, *J* = 12.0 Hz, PhC*H*$_2$), 3.89–3.99 (m, 6H, H-3, H-4, H-6a, H-2′, H-3′, H-4′), 3.66–3.81 (m, 4H, H-2, H-5, H-6b, H-5′), 3.51–3.62 (m, 3H, H-6a′, H-6b′, octyl OC*H*$_2$), 3.30–3.37 (m, 4H, OC*H*$_3$, octyl OC*H*$_2$), 1.46–1.55 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.35 (m, 10H, octyl

C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.9 (Ar), 138.7 (Ar), 138.6 (Ar), 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 98.3 (C-1′), 97.8 (C-1), 80.5 (C-3), 79.5 (C-3′), 75.1 (C-2), 75.1 (Ph*C*H$_2$), 75.0 (Ph*C*H$_2$), 75.0 (C-2′), 74.9 (C-4), 74.6 (C-4′), 72.8 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.7 (C-6′), 71.6 (C-5), 71.5 (C-5′), 71.5 (Ph*C*H$_2$), 67.6 (octyl O*C*H$_2$), 66.1 (C-6), 59.1 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{63}H_{76}O_{11}$ (1008.54): C, 74.97; H, 7.59. Found: C, 74.30; H, 7.71. ESIMS: *m/z* calcd for $[C_{63}H_{76}O_{11}]Na^+$: 1031.5280. Found: 1031.5282.

Octyl 2,3,4-tri-*O*-benzyl-6-*O*-*p*-tolylsulfonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.58)

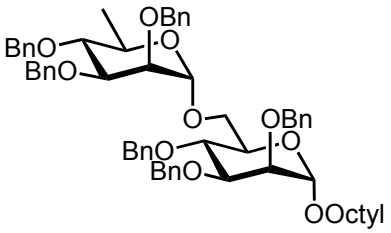


Disaccharide **2.53** (241 mg, 0.24 mmol) was dissolved in pyridine (1 mL) and the solution was cooled in an ice bath followed by the addition of *p*-toluenesulfonyl chloride (137 mg, 0.72 mmol). The reaction mixture was stirred overnight. The mixture was diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl (3 x 10 mL), satd aq $NaHCO_3$ (10 mL), water (10 mL), dried ($MgSO_4$), and concentrated to a colorless oil. The crude product was then purified by chromatography

(4:1, hexane–EtOAc) to give **2.58** (251 mg, 90%) as a colorless oil: R_f 0.37 (4:1, hexane–EtOAc); $[\alpha]_D$ = +27.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.74–7.78 (m, 2H, ArH), 7.14–7.39 (m, 32H, ArH), 5.04 (d, 1H, *J* = 1.8 Hz, H-1′), 4.88 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.87 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.78 (d, 1H, *J* = 1.8 Hz, H-1), 4.73 (d, 1H, *J* = 12.6 Hz, PhC*H*$_2$), 4.67 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.59–4.66 (m, 4H, PhC*H*$_2$), 4.54 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.46 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.45 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.42 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.15–4.21 (m, 2H, H-6a′, H-6b′), 3.81–3.93 (m, 6H, H-3, H-4, H-6a, H-2′, H-3′, H-4′), 3.78 (dd, 1H, *J* = 2.4, 1.8 Hz, H-2), 3.72–3.80 (m, 1H, H-5), 3.61–3.67 (m, 2H, H-5′, H-6b), 3.57 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.31 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 2.37 (s, 3H, SPhC*H*$_3$), 1.45–1.53 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.34 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 144.4 (Ar), 138.6 (Ar), 138.5 (Ar), 138.5 (Ar), 138.3 (Ar), 138.1 (Ar), 129.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.7 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.5 (Ar), 127.4 (Ar), 98.0 (C-1′), 97.8 (C-1), 80.4 (C-3), 79.2 (C-3′), 75.1 (C-2), 75.0 (Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 74.7 (C-4/C-2′/C-4′), 74.6 (C-4/C-2′/C-4′), 73.9 (C-4/C-2′/C-4′), 72.9 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.6 (C-5), 71.3 (Ph*C*H$_2$), 70.0 (C-5′), 68.9 (C-6′), 67.7 (octyl O*C*H$_2$), 66.2 (C-6), 59.1 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 21.6 (SPh*C*H$_3$), 14.1 (octyl *C*H$_3$). Anal. Calcd for

$C_{69}H_{80}O_{13}S$ (1149.43): C, 72.10; H, 7.02; S, 2.79. Found: C, 71.96; H, 6.93; S, 2.66. ESIMS: *m/z* calcd for $[C_{69}H_{80}O_{13}S]Na^+$: 1171.5212. Found: 1171.5211.

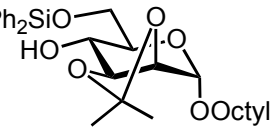
Octyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (2.59)



Disaccharide **2.58** (130 mg, 0.11 mmol) was dissolved in ether (5 mL) and 1 M $LiAlH_4$ (0.22 mL, 0.22 mmol) was added. The mixture was stirred overnight and quenched with EtOAc followed by water. The mixture was diluted with EtOAc (25 mL), washed with water (3 x 10 mL), and dried ($MgSO_4$), filtered and concentrated to a colorless oil. The crude product was purified by chromatography (9:1, hexane–EtOAc) to give **2.59** (69 mg, 62%) and **2.53** (37 mg, 33%) both as colorless oils. Compound **2.59**: R_f 0.44 (6:1, hexane–EtOAc); $[\alpha]_D$ = +25.6 (*c* 0.5, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ_H 7.18–7.40 (m, 30H, ArH), 5.03 (d, 1H, *J* = 1.8 Hz, H-1′), 4.94 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.90 (d, 1H, *J* = 10.8 Hz, PhCH_2), 4.79 (d, 1H, *J* = 1.8 Hz, H-1), 4.73 (d, 1H, *J* = 11.7 Hz, PhCH_2), 4.68 (s, 2H, PhCH_2), 4.68 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.65 (s, 2H, PhCH_2), 4.61 (d, 1H, *J* = 11.7 Hz, PhCH_2), 4.57 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.51 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.51 (d, 1H, *J* = 10.8 Hz, PhCH_2), 3.89–3.97 (m, 4H, H-3, H-4, H-6a, H-2′), 3.87 (dd, 1H, *J* = 9.0, 3.0 Hz, H-3′), 3.78 (dd, 1H, *J* = 2.7, 1.8

Hz, H-2), 3.70–3.76 (m, 1H, H-5′), 3.66–3.70 (m, 2H, H-5, H-6b), 3.55–3.62 (m, 2H, H-4′, octyl OC*H*$_2$), 3.32 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.46–1.53 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.21–1.33 (m, 13H, H-6′, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.8 (Ar), 138.7 (Ar), 138.5 (Ar), 138.5 (Ar), 138.5 (Ar), 138.4 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 98.1 (C-1′), 97.8 (C-1), 80.5 (C-3), 80.5 (C-4′), 79.5 (C-3′), 75.3 (C-2′), 75.2 (Ph*C*H$_2$), 75.1 (C-2), 75.1 (Ph*C*H$_2$), 74.7 (C-4), 72.8 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.6 (C-5), 71.5 (Ph*C*H$_2$), 68.1 (C-5′), 67.6 (octyl O*C*H$_2$), 66.0 (C-6), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 18.0 (C-6′), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{62}H_{74}O_{10}$ (979.24): C, 76.04; H, 7.62. Found: C, 75.97; H, 7.72. ESIMS: *m/z* calcd for $[C_{62}H_{74}O_{10}]Na^+$: 1001.5174. Found: 1001.5177.

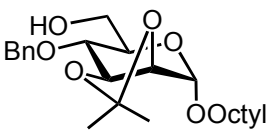
Octyl 2,3-*O*-isopropylidene-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranoside (2.60)



Triol **2.40** (595 mg, 1.12 mmol), 2,2-dimethoxypropane (1.2 mL, 9.0 mmol), and *p*-TsOH (4.3 mg) were dissolved in acetone (15 mL) and the mixture was stirred for 2 h. The reaction mixture was neutralized with triethylamine, concentrated, and purified by chromatography (6:1, hexane–EtOAc) to give **2.60** (615 mg, 96%) as a colorless oil: R_f 0.29 (6:1, hexane–EtOAc);

$[\alpha]_D$ = +1.0 (c 2.3, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.68–7.73 (m, 4H, ArH), 7.36–7.46 (m, 6H, ArH), 4.97 (s, 1H, H-1), 4.17 (dd, 1H, *J* = 6.5, 6.5 Hz, H-3), 4.13 (d, 1H, *J* = 6.0 Hz, H-2), 3.85–3.94 (m, 2H, H-6a, H-6b), 3.80 (dd, 1H, *J* = 9.3, 6.5 Hz, H-4), 3.63–3.70 (m, 2H, H-5, octyl OC*H*$_2$), 3.38 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.81 (br s, 1H, O*H*), 1.51–1.60 (m, 2H, octyl OCH_2CH_2), 1.50 (s, 3H, isopropylidene C*H*$_3$), 1.36 (s, 3H, isopropylidene C*H*$_3$), 1.20–1.38 (m, 10H, octyl C*H*$_2$), 1.07 (s, 9H, *tert*-butyl C*H*$_3$), 0.89 (t, 3H, *J* = 6.5 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 135.8 (4 x Ar), 133.3 (Ar), 133.2 (Ar), 129.9 (2 x Ar), 127.9 (4 x Ar), 109.6 (isopropylidene *C*), 97.2 (C-1), 78.3 (C-3), 75.6 (C-2), 70.7 (C-4), 69.8 (C-5), 67.9 (octyl O*C*H_2), 64.9 (C-6), 32.0 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 28.0 (isopropylidene *C*H_3), 27.0 (*tert*-butyl *C*H_3), 26.3 (*tert*-butyl *C*), 26.2 (isopropylidene *C*H_3), 22.8 (octyl *C*H_2), 19.3 (octyl *C*H_2), 14.2 (octyl *C*H_3). Anal. Calcd for $C_{33}H_{50}O_6Si$ (570.83): C, 69.43; H, 8.83. Found: C, 68.98; H, 8.75. ESIMS: *m/z* calcd for $[C_{33}H_{50}O_6Si]Na^+$: 593.3269. Found: 593.3270.

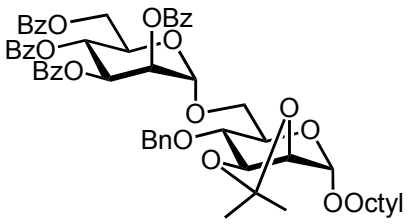
Octyl 2,3-*O*-isopropylidene-4-*O*-benzyl-α-D-mannopyranoside (2.61)



Benzylation of monosaccharide **2.60** (2.3 g, 4.0 mmol) using BnBr (0.73 mL, 6.0 mmol) and 60% NaH in mineral oil (0.24 g, 6.0 mmol) in DMF (10 mL) was performed as described for **2.33**, to obtain a residue as a yellow oil. Desilylation of the crude product was performed in THF (50 mL) and TBAF

(8.0 mL, 8.0 mmol) as described for **2.33**, to give **2.61** (1.29 g, 76% over two steps) as a crystalline solid. R_f 0.38 (4:1, hexane–EtOAc); $[\alpha]_D$ = +45.8 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.26–7.48 (m, 5H, ArH), 5.02 (s, 1H, H-1), 4.77 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.64 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.34 (dd, 1H, *J* = 6.4, 6.4 Hz, H-3), 4.15 (d, 1H, *J* = 6.4 Hz, H-2), 3.85 (dd, 1H, *J* = 11.6, 3.2 Hz, H-6a), 3.75 (dd, 1H, *J* = 11.6, 4.8 Hz, H-6b), 3.62–3.72 (m, 2H, H-5, octyl OC*H*$_2$), 3.54 (dd, 1H, *J* = 10.0, 6.4 Hz, H-4), 3.40 (dt, 1H, *J* = 9.6, 6.4 Hz, octyl OC*H*$_2$), 1.94 (br s, 1H, O*H*), 1.48–1.62 (m, 5H, isopropylidene C*H*$_3$, octyl OCH_2CH_2), 1.20–1.49 (m, 13H, isopropylidene C*H*$_3$, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.3 (Ar), 128.5 (2 x Ar), 128.2 (2 x Ar), 127.9 (Ar), 109.4 (isopropylidene *C*), 97.3 (C-1), 78.9 (C-3), 76.4 (C-2/H-4), 76.2 (C-2/H-4), 73.0 (Ph*C*H_2), 68.5 (C-5), 68.0 (octyl O*C*H_2), 62.8 (C-6), 31.9 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.3 (octyl *C*H_2), 28.1 (isopropylidene *C*H_3), 26.5 (isopropylidene *C*H_3), 26.3 (octyl *C*H_2), 22.8 (octyl *C*H_2), 14.1 (octyl *C*H_3). Anal. Calcd for $C_{24}H_{38}O_6$ (422.27): C, 68.22; H, 9.06. Found: C, 68.28; H, 9.06. ESIMS: *m/z* calcd for $[C_{24}H_{38}O_6]Na^+$: 445.2561. Found: 445.2559.

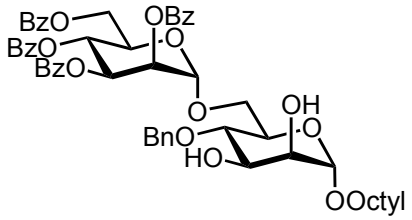
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranoside (2.62)



Prepared from thioglycoside **2.21** (498 mg, 0.71 mmol), alcohol **2.61** (250 mg, 0.59 mmol), powdered 4 Å molecular sieves (0.5 g), *N*-iodosuccinimide (210 mg, 0.89 mmol) and TMSOTf (32 μL, 0.18 mmol) as described for **2.41**, to give **2.62** (547 mg, 92%) as a yellow oil: R_f 0.36 (4:1, hexane–EtOAc); $[\alpha]_D$ = -1.1 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.10–8.14 (m, 2H, ArH), 8.03–8.07 (m, 2H, ArH), 7.88–7.92 (m, 2H, ArH), 7.81–7.85 (m, 2H, ArH), 7.55–7.61 (m, 2H, ArH), 7.49–7.54 (m, 1H, ArH), 7.23–7.45 (m, 14H, ArH), 6.13 (dd, 1H, *J* = 10.3, 10.3 Hz, H-4′), 5.92 (dd, 1H, *J* = 10.3, 3.3 Hz, H-3′), 5.72 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2′), 5.17 (d, 1H, *J* = 1.5 Hz, H-1′), 5.09 (br s, 1H, H-1), 4.99 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.66 (dd, 1H, *J* = 12.3, 2.5 Hz, H-6a′), 4.65 (d, 1H, *J* = 12.0 Hz, PhCH_2), 4.50 (ddd, 1H, *J* = 10.3, 2.5 Hz, H-5′), 4.35–4.42 (m, 2H, H-3, H-6b′), 4.21 (d, 1H, *J* = 6.0 Hz, H-2), 3.87–3.98 (m, 4H, H-5, H-6a, H-6b, octyl OCH_2), 3.50–3.58 (m, 2H, H-4, octyl OCH_2), 1.64–1.72 (m, 2H, octyl OCH_2CH_2), 1.58 (s, 3H, isopropylidene CH_3), 1.41 (s, 3H, isopropylidene CH_3), 1.16–1.42 (m, 10H, octyl CH_2), 0.83 (t, 3H, *J* = 6.8 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 138.1 (Ar), 133.3 (Ar), 133.0 (Ar), 132.9 (Ar), 130.0 (Ar), 129.8 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar),

128.4 (Ar), 128.3 (Ar), 127.7 (Ar), 127.7 (Ar), 109.4 (isopropylidene *C*), 97.4 (C-1′, $^1J_{C,H}$ = 172.9 Hz), 96.9 (C-1, $^1J_{C,H}$ = 169.9 Hz), 78.8 (C-3), 76.1 (C-2), 76.1 (C-4), 72.6 (Ph*C*H_2), 70.4 (C-2′), 70.0 (C-3′), 68.8 (C-5′), 67.8 (octyl O*C*H_2), 67.6 (C-4′), 67.1 (C-6), 66.9 (C-5), 62.7 (C-6′), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 28.1 (isopropylidene *C*H_3), 26.4 (isopropylidene *C*H_3), 26.3 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.0 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{58}H_{64}O_{15}]Na^+$: 1023.4137. Found: 1023.4133.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-*O*-benzyl-α-D-mannopyranoside (2.63)

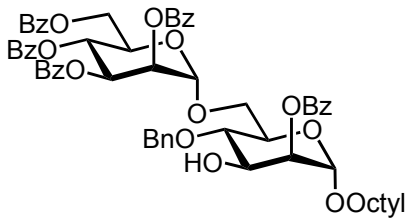


Disaccharide **2.62** (496 mg, 0.50 mmol) was dissolved in 80% AcOH/H_20 (10 mL) and heated at 50 °C overnight. The reaction mixture was then diluted with EtOAc (50 mL), washed with satd aq $NaHCO_3$ (2 x 25 mL), dried ($MgSO_4$), and concentrated. The crude residue was purified by chromatography (1:1, hexane–EtOAc) to give **2.63** (421 mg, 88%) as a colorless oil: R_f 0.28 (1:1, hexane–EtOAc); $[\alpha]_D$ = +4.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.09–8.12 (m, 2H, ArH), 8.05–8.08 (m, 2H, ArH), 7.93–7.96 (m, 2H, ArH), 7.84–7.88 (m, 2H, ArH), 7.55–7.62 (m, 2H, ArH), 7.49–7.54 (m, 1H, ArH), 7.35–7.46 (m, 12H, ArH), 7.26–7.33 (m, 2H, ArH), 6.14 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.93 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′), 5.75 (dd,

1H, *J* = 3.6, 1.8 Hz, H-2′), 5.38 (d, 1H, *J* = 1.8 Hz, H-1′), 4.98 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.85 (d, 1H, *J* = 1.2 Hz, H-1), 4.82 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.69 (dd, 1H, *J* = 12.2, 2.4 Hz, H-6a′), 4.51 (ddd, 1H, *J* = 10.2, 3.9, 2.4 Hz, H-5′), 4.44 (dd, 1H, *J* = 12.2, 3.9 Hz, H-6b′), 4.08 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6a), 4.02 (dd, 1H, *J* = 8.4, 3.6 Hz, H-3), 3.90–3.96 (m, 2H, H-2, H-6b), 3.77–3.85 (m, 2H, H-4, H-5), 3.72 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.44 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 2.65 (br s, 1H, O*H*), 2.48 (br s, 1H, O*H*), 1.56–1.63 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.40 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.6 (C=O), 165.5 (C=O), 165.5 (C=O), 138.4 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 129.1 (Ar), 128.6 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 99.4 (C-1), 97.8 (C-1′), 75.9 (C-4), 74.9 (Ph*C*H$_2$), 72.2 (C-3), 71.3 (C-2), 71.0 (C-5), 70.8 (C-2′), 69.9 (C-3′), 69.0 (C-5′), 68.0 (octyl O*C*H$_2$), 66.9 (C-4′), 66.8 (C-6), 62.8 (C-6′), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{50}H_{60}O_{15}$ (961.06): C, 68.74; H, 6.29. Found: C, 68.23; H, 6.23. ESIMS: *m/z* calcd for $[C_{55}H_{60}O_{15}]Na^+$: 983.3824. Found: 983.3823.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-α-D-mannopyranoside (2.64) and Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-benzoyl-4-*O*-benzyl-α-D-mannopyranoside (2.65)

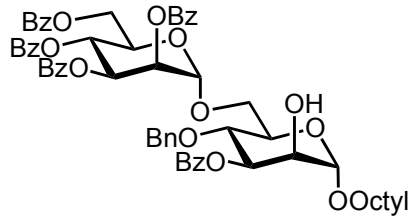
Disaccharide **2.63** (637 mg, 0.66 mmol) and Bu_2SnO (168 mg, 0.66 mmol) in toluene (15 mL) was heated under reflux for 3 h. The reaction mixture was cooled to room temperature and BzCl (0.10 mL, 0.83 mmol) was added and stirring was continued overnight. The solvent was then evaporated and the crude residue was purified by chromatography (3:1, hexane–EtOAc) to give **2.64** (442 mg, 63%) and **2.65** (227 mg, 32%) both as white foams.



Compound **2.65**: R_f 0.28 (3:1, hexane–EtOAc); $[\alpha]_D$ = -2.3 (*c* 1.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.08–8.13 (m, 4H, ArH), 7.99–8.04 (m, 2H, ArH), 7.88–7.92 (m, 2H, ArH), 7.83–7.87 (m, 2H, ArH), 7.53–7.61 (m, 2H, ArH), 7.25–7.53 (m, 18H, ArH), 6.14 (dd, 1H, *J* = 10.3, 10.3 Hz, H-4′), 5.96 (dd, 1H, *J* = 10.3, 3.3 Hz, H-3′), 5.81 (dd, 1H, *J* = 3.3, 2.0 Hz, H-2′), 5.41 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2), 5.29 (d, 1H, *J* = 2.0 Hz, H-1′), 5.05 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.96 (d, 1H, *J* = 1.5 Hz, H-1), 4.85 (d, 1H, *J* = 11.8 Hz, PhCH_2), 4.66 (dd, 1H, *J* = 12.0, 2.5 Hz, H-6a′), 4.44 (ddd, 1H, *J* = 10.0, 3.1, 2.5 Hz, H-5′), 4.37–4.40 (m, 2H, H-6b′, H-3), 4.15 (dd, 1H, *J* = 11.3, 4.3 Hz, H-6a), 3.92–4.02 (m, 3H, H-4, H-5, H-6b), 3.77 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OCH_2), 3.49 (dt, 1H, *J* = 9.5, 6.8

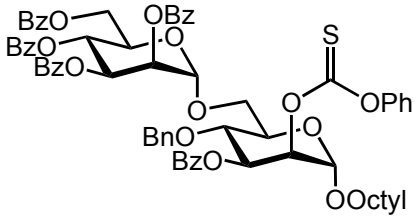
Hz, octyl OCH_2), 1.59–1.68 (m, 2H, octyl OCH$_2$CH_2), 1.20–1.43 (m, 10H, octyl CH_2), 0.87 (t, 3H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.5 (C=O), 166.1 (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 138.2 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.4 (Ar), 129.2 (Ar), 129.0 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.8 (Ar), 97.9 (C-1′), 97.5 (C-1), 75.9 (C-4), 75.0 (PhCH$_2$), 73.3 (C-2), 71.1 (C-3), 70.7 (C-5), 70.3 (C-2′), 70.2 (C-3′), 69.0 (C-5′), 68.2 (octyl OCH$_2$), 66.8 (C-4′), 66.5 (C-6), 62.7 (C-6′), 31.8 (octyl CH$_2$), 29.5 (octyl CH$_2$), 29.4 (octyl CH$_2$), 29.3 (octyl CH$_2$), 26.2 (octyl CH$_2$), 22.7 (octyl CH$_2$), 14.1 (octyl CH$_3$). ESIMS: *m/z* calcd for [$C_{62}H_{64}O_{16}$]Na$^+$: 1087.4087. Found: 1087.4086.

Compound **2.64**: R_f 0.16 (3:1, hexane–EtOAc); $[\alpha]_D$ = +11.1 (*c* 1.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.15–8.19 (m, 2H, ArH), 8.06–8.14 (m, 4H, ArH), 7.95–7.99 (m, 2H, ArH), 7.86–7.90 (m, 2H, ArH), 7.56–7.62 (m, 3H, ArH), 7.35–7.55 (m, 10H, ArH), 7.19–7.32 (m, 7H, ArH), 6.16 (dd, 1H, J = 10.0, 10.0 Hz, H-4′), 5.99 (dd, 1H, J = 10.0, 3.5 Hz, H-3′), 5.82 (dd, 1H, J = 3.5, 1.5 Hz, H-2′), 5.62 (dd, 1H, J = 9.8, 3.0 Hz, H-3), 5.45 (d, 1H, J = 1.5 Hz, H-1′), 4.88 (d, 1H, J = 1.8 Hz, H-1), 4.85 (d, 1H, J = 11.0 Hz, PhCH_2), 4.75 (d, 1H, J = 11.5 Hz, PhCH_2), 4.70 (dd, 1H, J = 11.5, 2.0 Hz, H-6a′), 4.44–4.52 (m, 2H, H-5′, H-6b′), 4.31 (dd, 1H, J = 9.8, 9.8 Hz, H-4), 4.23 (dd, 1H, J = 3.0, 1.8 Hz, H-2), 4.10 (dd, 1H, J =



12.0, 4.0 Hz, H-6a), 3.94–4.00 (m, 2H, H-5, H-6b), 3.74 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 3.48 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 1.59–1.67 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.42 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.6 (C=O), 165.6 (C=O), 165.6 (C=O), 165.5 (C=O), 137.8 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 99.7 (C-1), 98.0 (C-1′), 75.2 (C-3), 75.0 (Ph*C*H$_2$), 72.9 (C-4), 71.7 (C-5), 70.9 (C-2′), 69.9 (C-2/C-3′), 69.8 (C-2/C-3′), 69.0 (C-5′), 68.3 (octyl O*C*H$_2$), 67.0 (C-4′), 66.5 (C-6), 62.8 (C-6′), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for [$C_{62}H_{64}O_{16}$]Na$^+$: 1087.4087. Found: 1087.4083.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-*O*-phenoxythiocarbonyl-α-D-mannopyranoside (2.66)

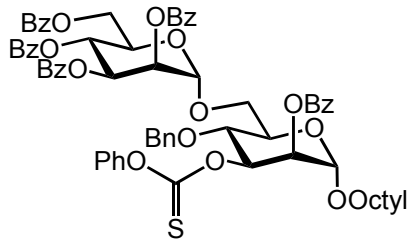


Disaccharide **2.64** (224 mg, 0.21 mmol) was dissolved in acetonitrile (2.2 mL) and DMAP (64 mg, 0.52 mmol) and phenyl chlorothionoformate (37 µL, 0.27 mmol) were added. The reaction mixture was stirred overnight and concentrated. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with water

(15 mL), brine (15 mL), dried ($MgSO_4$), and concentrated as a yellow crude. The crude product was purified by chromatography (3:1, hexane–EtOAc) to give **2.66** (186 mg, 74%) as a foam: R_f 0.40 (3:1, hexane–EtOAc); $[\alpha]_D$ = +11.2 (*c* 1.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–8.18 (m, 4H, ArH), 8.03–8.07 (m, 2H, ArH), 7.92–7.96 (m, 2H, ArH), 7.75–7.80 (m, 2H, ArH), 7.55–7.65 (m, 3H, ArH), 7.48–7.54 (m, 3H, ArH), 7.34–7.45 (m, 7H, ArH), 7.14–7.31 (m, 10H, ArH), 6.89–6.92 (m, 2H, ArH), 6.15 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 6.03 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3′), 5.89 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2), 5.88 (dd, 1H, *J* = 3.5, 1.5 Hz, H-2′), 5.84 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3), 5.34 (d, 1H, *J* = 1.5 Hz, H-1′), 5.16 (d, 1H, *J* = 1.5 Hz, H-1), 4.86 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.69–4.77 (m, 2H, PhC*H*$_2$, H-6a′), 4.46–4.52 (m, 2H, H-5′, H-6b′), 4.25 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 4.05–4.11 (m, 2H, H-5, H-6a), 3.95–4.01 (m, 1H, H-6b), 3.82 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.57 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.64–1.72 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.46 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 194.6 (C=S), 166.2 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 153.3 (Ar), 137.7 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.0 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.3 (Ar), 129.2 (Ar), 129.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 126.4 (Ar), 121.7 (Ar), 98.2 (C-1′), 96.4 (C-1), 79.6 (C-2), 75.2 (Ph*C*H$_2$), 73.5 (C-4), 72.4 (C-3), 71.4 (C-5), 70.6 (C-2′), 69.8 (C-3′), 69.0 (C-5′), 68.6 (octyl O*C*H$_2$), 67.1 (C-4′),

66.6 (C-6), 62.8 (C-6′), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{69}H_{68}O_{17}]Na^+$: 1223.4070. Found: 1223.4070.

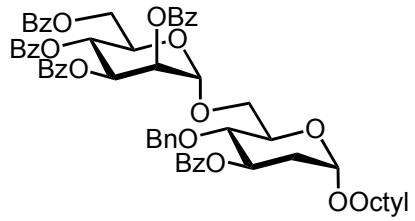
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-benzoyl-4-*O*-benzyl-3-*O*-phenoxythiocarbonyl-α-D-mannopyranoside (2.67)



Prepared from disaccharide **2.65** (227 mg, 0.21 mmol), DMAP (64 mg, 0.52 mmol) and phenyl chlorothionoformate (37 μL, 0.27 mmol) in acetonitrile (1.6 mL) as described for **2.66**, to give **2.67** (180 mg, 70%) as a foam: R_f 0.39 (3:1, hexane–EtOAc); $[\alpha]_D$ = -14.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.13–8.17 (m, 2H, ArH), 8.09–8.13 (m, 2H, ArH), 8.01–8.05 (m, 2H, ArH), 7.90–7.94 (m, 2H, ArH), 7.84–7.88 (m, 2H, ArH), 7.55–7.61 (m, 2H, ArH), 7.24–7.54 (m, 21H, ArH), 7.07–7.11 (m, 2H, ArH), 6.15 (dd, 1H, *J* = 10.3, 10.3 Hz, H-4′), 6.05 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3), 5.96 (dd, 1H, *J* = 10.3, 3.0 Hz, H-3′), 5.86 (dd, 1H, *J* = 3.5, 1.8 Hz, H-2), 5.83 (dd, 1H, *J* = 3.0, 2.0 Hz, H-2′), 5.30 (d, 1H, *J* = 2.0 Hz, H-1′), 5.02 (d, 1H, *J* = 1.8 Hz, H-1), 4.98 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.63–4.68 (m, 1H, H-6a′), 4.33–4.43 (m, 3H, H-5′, H-6b′, H-4), 4.16 (dd, 1H, *J* = 11.5, 4.0 Hz, H-6a), 4.10 (m, 1H, H-5), 3.95 (dd, 1H, *J* = 11.5, 1.5 Hz, H-6b), 3.79 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.54 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl

OCH_2), 1.64–1.71 (m, 2H, octyl OCH$_2$CH_2), 1.21–1.44 (m, 10H, octyl CH_2), 0.86 (t, 3H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 193.7 (C=S), 166.1 (C=O), 165.7 (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 153.4 (Ar), 137.8 (Ar), 133.4 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 129.4 (Ar), 129.2 (Ar), 129.0 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 127.9 (Ar), 127.7 (Ar), 126.5 (Ar), 121.9 (Ar), 98.1 (C-1′), 97.7 (C-1), 82.1 (C-3), 75.1 (PhCH$_2$), 73.1 (C-4), 71.0 (C-5), 70.2 (C-2′/C-3′), 70.1 (C-2′/C-3′), 69.7 (C-2), 69.0 (C-5′), 68.5 (octyl OCH$_2$), 66.8 (C-4′), 66.2 (C-6), 62.6 (C-6′), 31.8 (octyl CH$_2$), 29.4 (octyl CH$_2$), 29.4 (octyl CH$_2$), 29.2 (octyl CH$_2$), 26.1 (octyl CH$_2$), 22.6 (octyl CH$_2$), 14.1 (octyl CH$_3$). Anal. Calcd for $C_{69}H_{68}O_{17}$ (1200.42): C, 68.98; H, 5.71; S, 2.67. Found: C, 69.27; H, 5.71; S, 2.55. ESIMS: *m/z* calcd for $[C_{69}H_{68}O_{17}]Na^+$: 1223.4070. Found: 1223.4068.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-α-D-*arabino*-hexopyranoside (2.68)

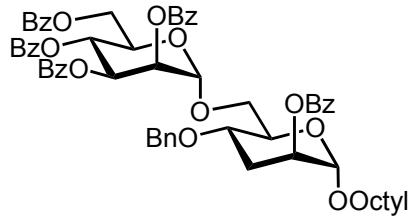


Prepared from phenoxy thiocarbonate **2.66** (154 mg, 0.13 mmol) in toluene (2.2 mL), tri-*n*-butylstannane (55 μL, 0.20 mmol) and AIBN (8 mg, 0.049 mmol) in toluene (9 mL) as described for **2.50**, to give **2.68** (113 mg, 84%) as a foam: R_f 0.30 (4:1, hexane–EtOAc); $[\alpha]_D$ = 13.9 (*c* 1.7, $CHCl_3$); ^{1}H NMR (500 MHz, CD_2Cl_2) δ_H

8.08–8.12 (m, 4H, ArH), 8.05–8.09 (m, 2H, ArH), 7.93–7.96 (m, 2H, ArH), 7.84–7.87 (m, 2H, ArH), 7.58–7.65 (m, 3H, ArH), 7.36–7.56 (m, 10H, ArH), 7.19–7.33 (m, 7H, ArH), 6.09 (dd, 1H, *J* = 10.5, 10.5 Hz, H-4′), 5.92 (dd, 1H, *J* = 10.5, 3.5 Hz, H-3′), 5.82 (dd, 1H, *J* = 3.5, 2.0 Hz, H-2′), 5.58 (ddd, 1H, *J* = 11.5, 9.5, 5.5 Hz, H-3), 5.28 (d, 1H, *J* = 2.0 Hz, H-1′), 4.97 (br d, 1H, *J* = 3.5 Hz, H-1), 4.86 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.76 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.66 (dd, 1H, *J* = 12.0, 2.5 Hz, H-6a′), 4.52 (ddd, 1H, *J* = 10.5, 4.3, 2.5 Hz, H-5′), 4.43 (dd, 1H, *J* = 12.0, 4.3 Hz, H-6b′), 4.11 (dd, 1H, *J* = 11.2, 4.5 Hz, H-6a), 4.00 (ddd, 1H, *J* = 9.5, 4.5, 1.8 Hz, H-5), 3.96 (dd, 1H, *J* = 11.2, 1.8 Hz, H-6b), 3.84 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 3.75 (ddd, 1H, *J* = 9.5, 6.5, 6.5 Hz, octyl OC*H*$_2$), 3.45 (ddd, 1H, *J* = 9.5, 6.5, 6.5 Hz, octyl OC*H*$_2$), 2.36 (dd, 1H, *J* = 13.0, 6.0, 1.0 Hz, H-2$_{eq}$), 1.87 (ddd, 1H, *J* = 13.0, 11.5, 3.5 Hz, H2$_{ax}$), 1.62–1.72 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.20–1.46 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_2Cl_2) δ_C 166.3 (C=O), 166.0 (C=O), 165.7 (C=O), 165.7 (C=O), 165.7 (C=O), 138.6 (Ar), 133.8 (Ar), 133.8 (Ar), 133.6 (Ar), 133.5 (Ar), 133.4 (Ar), 130.8 (Ar), 130.5 (Ar), 130.1 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 129.7 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.8 (Ar), 128.7 (Ar), 128.1 (Ar), 128.1 (Ar), 98.3 (C-1′), 97.3 (C-1), 77.3 (C-4), 75.1 (Ph*C*H$_2$), 73.2 (C-3), 71.0 (C-5), 70.8 (C-2′/C-3′), 70.7 (C-2′/C-3′), 69.4 (C-5′), 68.2 (octyl O*C*H$_2$), 67.3 (C-4′), 67.1 (C-6), 63.2 (C-6′), 35.8 (C-2), 32.3 (octyl *C*H$_2$), 30.0 (octyl *C*H$_2$), 29.9 (octyl *C*H$_2$), 29.7 (octyl *C*H$_2$), 26.7 (octyl *C*H$_2$), 23.1 (octyl *C*H$_2$), 14.2 (octyl *C*H$_3$). Anal.

Calcd for $C_{62}H_{64}O_{15}$ (1048.42): C, 70.98; H, 6.15. Found: C, 70.56; H, 6.27. ESIMS: *m/z* calcd for $[C_{62}H_{64}O_{15}]Na^+$: 1071.4137. Found: 1071.4138.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-benzoyl-4-*O*-benzyl-3-deoxy-α-D-*arabino*-hexopyranoside (2.69)



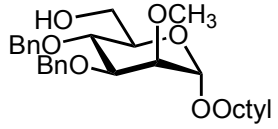
Prepared from phenoxy thiocarbonate **2.67** (154 mg, 0.13 mmol) in toluene (2.2 mL) and tri-*n*-butylstannane (55 μL, 0.20 mmol) and AIBN (8 mg, 0.049 mmol) in toluene (9 mL) at reflux as described for **2.50**, to give **2.69** (42 mg, 32%) as a colorless oil: R_f 0.32 (4:1, hexane–EtOAc); $[\alpha]_D$ = +5.6 (*c* 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.08–8.14 (m, 4H, ArH), 8.03–8.06 (m, 2H, ArH), 7.87–7.91 (m, 2H, ArH), 7.83–7.86 (m, 2H, ArH), 7.54–7.61 (m, 2H, ArH), 7.23–7.52 (m, 18H, ArH), 6.14 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.98 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′), 5.81 (dd, 1H, *J* = 3.6, 1.5 Hz, H-2′), 5.27 (d, 1H, *J* = 1.5 Hz, H-1′), 5.24–5.27 (m, 1H, H-2), 4.87 (br s, 1H, H-1), 4.76 (d, 1H, *J* = 11.4 Hz, PhCH_2), 4.65 (dd, 1H, *J* = 12.0, 2.4 Hz, H-6a′), 4.58 (d, 1H, *J* = 11.4 Hz, PhCH_2), 4.49 (ddd, 1H, *J* = 10.2, 4.2, 2.4 Hz, H-5′), 4.36 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6b′), 4.15 (dd, 1H, *J* = 10.9, 4.5 Hz, H-6a), 3.99–4.06 (m, 2H, H-4, H-5), 3.93 (dd, 1H, *J* = 10.9, 1.2 Hz, H-6b), 3.84 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OCH_2), 3.54 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OCH_2), 2.48 (ddd, 1H, *J* = 13.4,

3.6, 3.6 Hz, H-3_{eq}), 2.13 (ddd, 1H, *J* = 13.4, 13.4, 3.0 Hz, $H3_{ax}$), 1.58–1.72 (m, 2H, octyl OCH_2CH_2), 1.20–1.46 (m, 10H, octyl CH_2), 0.87 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.8 (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 137.9 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 127.0 (Ar), 97.7 (C-1′), 96.4 (C-1), 71.2 (C-4), 70.9 (PhCH_2), 70.6 (C-2), 70.4 (C-2′), 70.2 (C-3′), 69.4 (C-5), 68.8 (C-5′), 67.9 (octyl OCH_2), 66.9 (C-4′), 66.7 (C-6), 62.7 (C-6′), 31.8 (octyl CH_2), 29.6 (octyl CH_2), 29.5 (octyl CH_2), 29.3 (octyl CH_2), 26.3 (octyl CH_2), 22.7 (octyl CH_2), 22.7 (C-3), 14.1 (octyl CH_3). ESIMS: *m/z* calcd for $[C_{62}H_{64}O_{15}]Na^+$: 1071.4137. Found: 1071.4140.

Octyl 3,4-di-*O*-benzyl-2-*O*-methyl-α-D-mannopyranoside (2.70) and Octyl 3,6-di-*O*-benzyl-2-*O*-methyl-α-D-mannopyranoside (2.77)

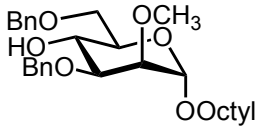
Prepared from a solution of $AlCl_3$ (150 mg, 1.1 mmol) and $LiAlH_4$ (1.1 mmol) in ether (4.1 mL) and **2.75** (317 mg, 0.63 mmol) in 1:1, CH_2Cl_2–ether (8 mL) as described for **2.37** (except that the reaction mixture was stirred overnight), to give **2.70** (194 mg, 66%) and its regioisomer **2.77** (19 mg, 6%) as colorless oils.

Compound **2.70**, R_f 0.27 (2:1, hexane–EtOAc); $[\alpha]_D$ = +41.9 (*c* 2.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H



7.26–7.42 (m, 10H, ArH), 4.93 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.85 (d, 1H, *J* = 1.9 Hz, H-1), 4.74 (s, 2H, PhC*H*$_2$), 4.64 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 3.93 (dd, 1H, *J* = 9.8, 3.2 Hz, H-3), 3.86 (dd, 1H, *J* = 9.8, 9.8 Hz, H4), 3.83 (dd, 1H, *J* = 11.5, 3.0 Hz, H-6a), 3.77 (dd, 1H, *J* = 11.5, 5.0 Hz, H-6b), 3.61–3.68 (m, 2H, H-5, octyl OC*H*$_2$), 3.54 (dd, 1H, *J* = 3.2, 1.9 Hz, H-2), 3.52 (s, 3H, OC*H*$_3$), 3.37 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.97 (br s, 1H, O*H*), 1.52–1.69 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.24–1.36 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.4 (Ar), 138.4 (Ar), 128.4 (2 x Ar), 128.4 (2 x Ar), 128.1 (2 x Ar), 127.8 (2 x Ar), 127.7 (Ar), 127.6 (Ar), 97.5 (C-1), 80.1 (C-3), 78.1 (C-2), 75.3 (Ph*C*H$_2$), 75.0 (C-4), 72.4 (Ph*C*H$_2$), 72.0 (C-5), 67.8 (octyl O*C*H$_2$), 62.5 (C-6), 59.4 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{29}H_{42}O_6$ (486.64): C, 71.57; H, 8.70. Found: C, 71.44; H, 8.78. ESIMS: *m/z* calcd for $[C_{29}H_{42}O_6]Na^+$: 509.2874. Found: 509.2877.

Compound **2.77**, R_f 0.44 (2:1, hexane–EtOAc); $[\alpha]_D$ = +22.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H



7.24–7.42 (m, 10H, ArH), 4.89 (d, 1H, *J* = 2.0 Hz, H-1), 4.74 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.65 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.57–4.62 (m, 2H, PhC*H*$_2$), 3.94 (dd, 1H, *J* = 9.5, 9.5 Hz, H4), 3.67–3.80 (m, 5H, H-3, H-5, H-6a, H-6b, octyl OC*H*$_2$), 3.53 (dd, 1H, *J* = 3.0, 2.0 Hz, H-2), 3.47 (s, 3H, OC*H*$_3$), 3.40 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.49 (br s, 1H, O*H*), 1.53–1.62 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.40 (m, 10H, octyl C*H*$_2$), 0.90 (t,

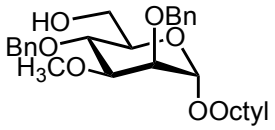
3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.3 (2 x Ar), 128.5 (2 x Ar), 128.3 (2 x Ar), 127.9 (2 x Ar), 127.6 (2 x Ar), 127.5 (2 x Ar), 97.5 (C-1), 79.7 (C-3), 77.3 (C-2), 73.5 (Ph*C*H$_2$), 72.0 (Ph*C*H$_2$), 71.3 (C-5), 70.5 (C-6), 68.0 (C-4), 67.8 (octyl O*C*H$_2$), 59.1 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{29}H_{42}O_6]Na^+$: 509.2874. Found: 509.2876.

Octyl 2,4-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (2.71) and

Octyl 2,6-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (2.78)

Prepared from a solution of $AlCl_3$ (150 mg, 1.1 mmol) and $LiAlH_4$ (1.1 mmol) in ether (4.1 mL) and disaccharide **2.76** (317 mg, 0.66 mmol) in 1:1, CH_2Cl_2–ether (8 mL) as described for **2.37** (except that the reaction mixture was stirred overnight) to give **2.71** (182 mg, 57%) and **2.78** (71 mg, 22%) as colorless oils.

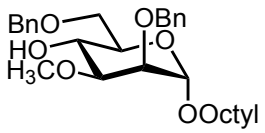
Compound **2.71**, R_f 0.33 (3:1, hexane–EtOAc); $[\alpha]_D$ = +42.8 (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H



7.26–7.42 (m, 10H, ArH), 4.93 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.80 (d, 1H, *J* = 1.8 Hz, H-1), 4.71 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.65 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 3.88 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 3.84 (dd, 1H, *J* = 11.5, 3.0 Hz, H-6a), 3.81 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2), 3.77 (dd, 1H, *J* = 11.5, 5.0 Hz, H-6b), 3.59–3.67 (m, 3H, H-3, H-5, octyl OC*H*$_2$), 3.47 (s, 3H, OC*H*$_3$), 3.34 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl

OC*H*$_2$), 2.00 (br s, 1H, O*H*), 1.48–1.56 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.34 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.6 (Ar), 138.4 (Ar), 128.4 (2 x Ar), 128.4 (2 x Ar), 128.0 (2 x Ar), 127.8 (2 x Ar), 127.7 (Ar), 127.7 (Ar), 98.1 (C-1), 82.1 (C-3), 75.1 (Ph*C*H$_2$), 75.1 (C-4), 74.4 (C-2), 72.9 (Ph*C*H$_2$), 71.9 (C-5), 67.8 (octyl O*C*H$_2$), 62.5 (C-6), 57.7 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{29}H_{42}O_6$ (486.64): C, 71.57; H, 8.70. Found: C, 71.40; H, 8.78. ESIMS: *m/z* calcd for $[C_{29}H_{42}O_6]Na^+$: 509.2874. Found: 509.2875.

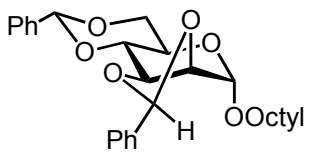
Compound **2.78**, R_f 0.26 (3:1, hexane–EtOAc); $[\alpha]_D$ = +3.7 (*c* 2.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H



7.24–7.40 (m, 10H, ArH), 4.89 (d, 1H, *J* = 1.5 Hz, H-1), 4.71 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.68 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.64 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.60 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 3.98 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4), 3.73–3.83 (m, 4H, H-2, H-5, H-6a, H-6b), 3.68 (dt, 1H, *J* = 9.5, 7.0 Hz, octyl OC*H*$_2$), 3.66 (dd, 1H, *J* = 9.4, 3.0 Hz, H-3), 3.36–3.43 (m, 4H, octyl OC*H*$_2$, OC*H*$_3$), 2.57 (br s, 1H, O*H*), 1.51–1.60 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.3 (Ar), 138.2 (Ar), 128.3 (2 x Ar), 128.3 (2 x Ar), 127.8 (2 x Ar), 127.7 (2 x Ar), 127.5 (Ar), 127.5 (Ar), 97.9 (C-1), 81.2 (C-3), 73.5 (Ph*C*H$_2$), 73.0 (C-2), 72.6 (Ph*C*H$_2$), 71.3 (C-5), 70.5 (C-6), 68.0 (C-4), 67.8 (octyl O*C*H$_2$), 57.2 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4

(octyl CH_2), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESIMS: *m/z* calcd for $[C_{29}H_{42}O_6]Na^+$: 509.2874. Found: 509.2877.

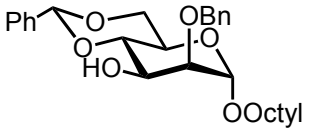
Octyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside (2.72)



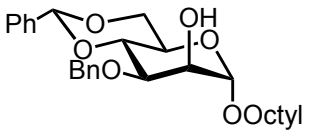
Prepared from tetraol **2.39**[17] (3.68 g, 12.6 mmol), benzylaldehyde dimethyl acetal (2.5 mL, 16.4 mmol) and *p*-TsOH (24 mg, 0.13 mmol) in DMF (5.0 mL) as described for **2.35** and **2.36**, to give an ~1:1 mixture of diastereoisomers **2.72** (5.10 g, 86%) as a colorless oil: R_f 0.35 (12:1, hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.23–7.67 (m, 20H, Ar), 6.30 (s, 1H), 5.98 (s, 1H), 5.66 (s, 1H), 5.54 (s, 1H), 5.12 (s, 1H), 5.19 (s, 1H), 4.67 (t, 1H, 6.5 Hz), 4.51 (t, 1H, 6.5 Hz), 4.24–4.38 (m, 4H), 4.16 (d, 1H, *J* = 6.6 Hz), 3.68–3.80 (m, 9H), 3.41–4.52 (m, 2H), 1.54–1.67 (m, 4H, octyl OCH_2CH_2), 1.23–1.46 (m, 20H, octyl CH_2), 0.86–0.96 (m, 6H, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.7, 137.3, 137.2, 137.1, 129.7, 129.4, 129.1, 129.0, 129.0, 128.5, 128.4, 128.3, 128.2, 128.2, 126.6, 126.3, 126.3, 126.1, 104.1, 103.0, 102.0, 101.7, 97.8, 97.6, 80.7, 78.6, 77.7, 77.3, 77.0, 76.8, 75.6, 75.5, 74.2, 69.0, 68.9, 68.2, 68.1, 60.4, 60.4, 31.8, 31.8, 29.4, 29.4, 29.4, 29.2, 29.2, 26.2, 26.1, 22.7, 22.7, 14.1.

Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (2.73) and Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (2.74)

Prepared from a solution of $AlCl_3$ (1.63 g, 12.2 mmol) and $LiAlH_4$ (12.2 mmol) in ether (47.2 mL) and **2.72** (4.77 g, 10.2 mmol) in 1:1, CH_2Cl_2–ether (70 mL) at 0 °C as described for **2.37**, to give **2.74** (1.72 g, 36%) and **2.73** (1.52 g, 32%) as a colorless oil.

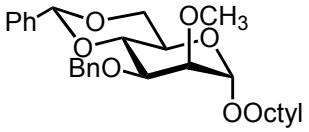


Compound **2.74**, R_f 0.40 (4:1, hexane–EtOAc); $[\alpha]_D$ = +14.2 (*c* 4.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.49–7.52 (m, 2H, ArH), 7.26–7.41 (m, 8H, ArH), 5.59 (s, 1H, benzylidene H), 4.84 (d, 1H, *J* = 1.2 Hz, H-1), 4.77 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.72 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.26 (dd, 1H, *J* = 9.0, 3.8 Hz H-6a), 4.11 (dd, 1H, *J* = 9.4, 3.6 Hz, H-3), 3.92 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4), 3.76–3.87 (m, 3H, H-2, H-5, H-6b), 3.67 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 3.38 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 2.30 (br s, 1H, O*H*), 1.54–1.59 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.24–1.40 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 137.7 (Ar), 137.4 (Ar), 129.0 (Ar), 128.6 (Ar), 128.2 (2 x Ar), 128.0 (2 x Ar), 127.9 (2 x Ar), 126.3 (2 x Ar), 102.1 (benzylidene *C*H), 98.3 (C-1), 79.6 (C-4), 78.7 (C-2), 73.7 (Ph*C*H$_2$), 68.9 (C-6), 68.8 (C-3), 68.0 (octyl O*C*H$_2$), 63.4 (C-5), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{28}H_{38}O_6$ (470.60): C, 71.46; H, 8.14. Found: C, 71.75; H, 8.34. ESIMS: *m/z* calcd for [$C_{28}H_{38}O_6$]:H$^+$ 471.2741. Found: 471.2742.

Compound **2.73**, R_f 0.23 (4:1, hexane–EtOAc); 

$[\alpha]_D$ = +40.8 (*c* 2.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.51–7.53 (m, 2H, ArH), 7.26–7.42 (m, 8H, ArH), 5.63 (s, 1H, benzylidene H), 4.88 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 4.87 (d, 1H, *J* = 1.4 Hz, H-1), 4.74 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 4.24–4.33 (m, 1H, H-6a), 4.11 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4), 4.07 (dd, 1H, *J* = 3.5, 1.4 Hz, H-2), 3.94 (dd, 1H, *J* = 9.4, 3.5 Hz, H-3), 3.81–3.91 (m, 2H, H-5, H-6b), 3.69 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 3.42 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 2.68 (br s, 1H, O*H*), 1.54–1.62 (m, 2H, octyl OCH_2C*H*$_2$), 1.23–1.40 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.1 (Ar), 137.6 (Ar), 129.4 (Ar), 128.4 (Ar), 128.9 (2 x Ar), 128.2 (2 x Ar), 127.8 (2 x Ar), 126.0 (2 x Ar), 101.5 (benzylidene *C*H), 99.9 (C-1), 79.0 (C-4), 75.8 (C-3), 73.1 (Ph*C*H$_2$), 70.1 (C-2), 68.9 (C-6), 68.0 (octyl O*C*H$_2$), 63.2 (C-5), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{28}H_{38}O_6$ (470.60): C, 71.46; H, 8.14. Found: C, 71.33; H, 8.55. ESIMS: *m/z* calcd for $[C_{28}H_{38}O_6]Na^+$: 493.2561. Found: 493.2561.

Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranoside (2.75)

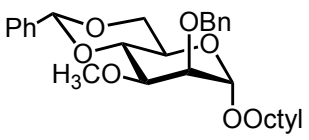
Prepared from monosaccharide **2.73** (461 mg, 

0.98 mmol), 60% NaH in mineral oil (60 mg, 1.5 mmol) and CH_3I (0.20 mL, 3.0 mmol) in DMF (15 mL) as described for

2.45, to give **2.75** (445 mg, 94%) as a colorless oil: R_f 0.48 (4:1, hexane–EtOAc); $[\alpha]_D$ = +56.15 (*c* 3.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.49–7.54 (m, 2H, ArH), 7.26–7.43 (m, 8H, ArH), 5.64 (s, 1H, benzylidene H), 4.91 (d, 1H, *J* = 12.4 Hz, PhC*H*$_2$), 4.85 (d, 1H, *J* = 1.6 Hz, H-1), 4.74 (d, 1H, *J* = 12.4 Hz, PhC*H*$_2$), 4.26 (dd, 1H, *J* = 9.8, 4.2 Hz, H-6a), 4.17 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 3.98 (dd, 1H, *J* = 9.8, 3.3 Hz, H-3), 3.87 (dd, 1H, *J* = 9.8, 9.8 Hz, H-6b), 3.80 (ddd, 1H, *J* = 9.8, 9.6, 4.2 Hz, H-5), 3.68 (dt, 1H, *J* = 9.6, 7.0 Hz, octyl OC*H*$_2$), 3.61 (dd, 1H, *J* = 3.3, 1.6 Hz, H-2), 3.58 (s, 3H, OC*H*$_3$), 3.41 (dt, 1H, *J* = 9.6, 7.0 Hz, octyl OC*H*$_2$), 1.54–1.62 (m, 2H, octyl OCH_2CH_2), 1.24–1.39 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.7 (Ar), 137.7 (Ar), 129.2 (Ar), 128.8 (Ar), 128.3 (2 x Ar), 128.2 (2 x Ar), 127.5 (2 x Ar), 126.0 (2 x Ar), 101.4 (benzylidene *C*H), 98.9 (C-1), 79.8 (C-2), 79.3 (C-4), 76.4 (C-3), 73.2 (Ph*C*H$_2$), 68.9 (C-6), 67.9 (octyl O*C*H$_2$), 64.1 (C-5), 60.1 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{29}H_{40}O_6$ (484.62): C, 71.87; H, 8.32. Found: C, 71.57; H, 8.37. ESIMS: *m/z* calcd for $[C_{29}H_{40}O_6]Na^+$: 507.2717. Found: 507.2716.

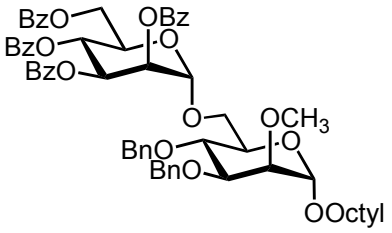
Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (2.76)

Prepared from monosaccharide **2.74** (461 mg, 0.98 mmol), 60% NaH in mineral oil (60 mg, 1.5



mmol) and CH_3I (0.20 mL, 3.0 mmol) in DMF (15 mL) as described for **2.45**, to give **2.76** (444 mg, 92%) as a colorless oil: R_f 0.41 (6:1, hexane–EtOAc); $[\alpha]_D$ = +39.9 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.49–7.55 (m, 2H, ArH), 7.27–7.44 (m, 8H, ArH), 5.63 (s, 1H, benzylidene), 4.84 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.80 (d, 1H, *J* = 1.6 Hz, H-1), 4.72 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.26 (dd, 1H, *J* = 9.8, 4.2 Hz, H-6a), 4.18 (dd, 1H, *J* = 9.8, 9.8 Hz, H-4), 3.88 (dd, 1H, *J* = 9.8, 9.8 Hz, H-6b), 3.87 (dd, 1H, *J* = 3.2, 1.6 Hz, H-2), 3.82 (ddd, 1H, *J* = 9.8, 9.8, 4.2 Hz, H-5), 3.75 (dd, 1H, *J* = 9.8, 3.2 Hz, H-3), 3.66 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 3.52 (s, 3H, OC*H*$_3$), 3.37 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OC*H*$_2$), 1.52–1.62 (m, 2H, octyl OCH_2CH_2), 1.24–1.38 (m, 10H, octyl C*H*$_2$), 0.91 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 138.2 (Ar), 137.7 (Ar), 128.8 (Ar), 128.4 (2 x Ar), 128.2 (2 x Ar), 128.0 (2 x Ar), 127.7 (Ar), 126.1 (2 x Ar), 101.6 (benzylidene *C*H), 99.2 (C-1), 79.1 (C-4), 78.1 (C-3), 75.9 (C-2), 73.5 (Ph*C*H_2), 68.9 (C-6), 67.9 (octyl O*C*H_2), 64.1 (C-5), 58.8 (O*C*H_3), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.1 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{29}H_{40}O_6]Na^+$: 507.2717. Found: 507.2715.

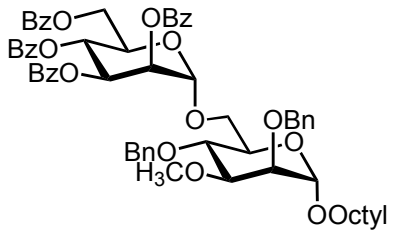
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-benzyl-2-*O*-methyl-α-D-mannopyranoside (2.79)



Prepared from thioglycoside **2.21** (252 mg, 0.36 mmol), alcohol **2.70** (145 mg, 0.29 mmol), powdered 4 Å molecular sieves (250 mg), *N*-iodosuccinimide (110 mg, 0.44 mmol) and TMSOTf (27 mg, 0.12 mmol) in CH_2Cl_2 (10 mL) as described for **2.41**, to give **2.79** (272 mg, 89%) as a yellow oil: R_f 0.37 (3:1, hexane–EtOAc); $[\alpha]_D$ = +5.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–8.14 (m, 2H, ArH), 8.04–8.08 (m, 2H, ArH), 7.90–7.94 (m, 2H, ArH), 7.81–7.85 (m, 2H, ArH), 7.55–7.61 (m, 2H, ArH), 7.48–7.53 (m, 1H, ArH), 7.23–7.46 (m, 19H, ArH), 6.11 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 5.94 (dd, 1H, *J* = 10.0, 3.3 Hz, H-3′), 5.75 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2′), 5.19 (d, 1H, *J* = 1.5 Hz, H-1′), 5.03 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.90 (d, 1H, *J* = 2.0 Hz, H-1), 4.78 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.74 (d, 1H, *J* = 11.5 Hz, PhC*H*$_2$), 4.61–4.69 (m, 2H, H-6a′, PhC*H*$_2$), 4.51 (ddd, 1H, *J* = 10.0, 4.3, 4.3 Hz, H-5′), 4.44 (dd, 1H, *J* = 12.3, 4.3 Hz, H-6b′), 3.94–4.00 (m, 2H, H-3, H-6a), 3.83–3.90 (m, 3H, H-4, H-5, H-6b), 3.80 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 3.58 (dd, 1H, *J* = 3.0, 2.0 Hz, H-2), 3.52 (s, 3H, OC*H*$_3$), 3.46 (dt, 1H, *J* = 10.0, 6.5 Hz, octyl OC*H*$_2$), 1.58–1.70 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.19–1.43 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 138.4 (Ar), 133.3 (Ar), 133.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar),

129.7 (Ar), 129.5 (Ar), 129.3 (Ar), 129.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.2 (Ar), 127.9 (Ar), 127.7 (Ar), 127.7 (Ar), 97.6 (C-1′, $^{1}J_{C,H}$ = 175.8 Hz), 97.1 (C-1, $^{1}J_{C,H}$ = 169.9 Hz), 80.4 (C-3), 77.9 (C-2), 75.2 (Ph*C*H_2), 74.8 (C-4), 72.3 (Ph*C*H_2), 71.3 (C-5), 70.5 (C-2′), 70.0 (C-3′), 68.7 (C-5′), 67.9 (octyl O*C*H_2), 67.1 (C-6), 67.1 (C-4′), 62.8 (C-6′), 59.2 (O*C*H_3), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.3 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{63}H_{68}O_{15}]Na^{+}$: 1087.4450. Found: 1087.4455.

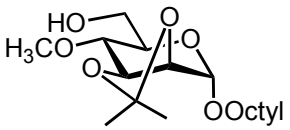
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-benzyl-3-*O*-methyl-α-D-mannopyranoside (2.80)



Prepared from thioglycoside **2.21** (225 mg, 0.32 mmol), alcohol **2.71** (130 mg, 0.27 mmol), powdered 4 Å molecular sieves (225 mg), *N*-iodosuccinimide (96 mg, 0.41 mmol) and TMSOTf (24 mg, 0.11 mmol) in CH_2Cl_2 (9 mL) as described for **2.41**, to give **2.80** (275 mg, 96%) as a yellow oil: R_f 0.26 (4:1, hexane–EtOAc); $[\alpha]_D$ = +2.1 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.10–8.14 (m, 2H, ArH), 8.04–8.08 (m, 2H, ArH), 7.90–7.94 (m, 2H, ArH), 7.80–7.84 (m, 2H, ArH), 7.48–7.62 (m, 3H, ArH), 7.20–7.44 (m, 19H, ArH), 6.11 (dd, 1H, *J* = 10.3, 10.3 Hz, H-4′), 5.94 (dd, 1H, *J* = 10.3, 3.3 Hz, H-3′), 5.75 (dd, 1H, *J* = 3.3, 2.0 Hz, H-2′), 5.20 (d, 1H, *J* = 2.0 Hz, H-1′), 5.02 (d, 1H, *J* = 12.3 Hz, PhC*H*$_2$), 4.84 (d, 1H, *J* = 1.5 Hz, H-1), 4.79 (d, 1H, *J* = 13.0 Hz,

PhC*H*$_2$), 4.73 (d, 1H, *J* = 13.0 Hz, PhC*H*$_2$), 4.67 (d, 1H, *J* = 12.3 Hz, PhC*H*$_2$), 4.63 (dd, 1H, *J* = 12.4, 2.5 Hz, H-6a′), 4.51 (ddd, 1H, *J* = 10.3, 3.8, 2.5 Hz, H-5′), 4.43 (dd, 1H, *J* = 12.4, 3.8 Hz, H-6b′), 3.99 (dd, 1H, *J* = 11.0, 5.5 Hz, H-6a), 3.80–3.92 (m, 4H, H-2, H-4, H-5, H-6b), 3.76 (dt, 1H, *J* = 9.5, 7.0 Hz, octyl OC*H*$_2$), 3.69 (dd, 1H, *J* = 9.0, 3.0 Hz, H-3), 3.39–3.48 (m, 4H, OC*H*$_3$, octyl OC*H*$_2$), 1.54–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.16–1.42 (m, 10H, octyl C*H*$_2$), 0.84 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 138.6 (Ar), 138.4 (Ar), 133.3 (Ar), 133.0 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 129.3 (Ar), 129.2 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.6 (Ar), 97.7 (C-1′, $^1J_{C,H}$ = 175.8 Hz), 96.9 (C-1, $^1J_{C,H}$ = 175.8 Hz), 82.2 (C-3), 75.0 (Ph*C*H$_2$), 74.9 (C-4), 74.1 (C-2), 72.7 (Ph*C*H$_2$), 71.1 (C-5), 70.5 (C-2′), 70.0 (C-3′), 68.7 (C-5′), 67.8 (octyl O*C*H$_2$), 67.2 (C-6), 67.1 (C-4′), 62.7 (C-6′), 57.5 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{63}H_{68}O_{15}]Na^+$: 1087.4450. Found: 1087.4451.

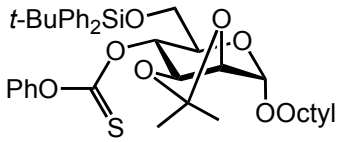
Octyl 2,3-*O*-isopropylidene-4-*O*-methyl-α-D-mannopyranoside (2.81)



Methylation of monosaccharide **2.60** (735 mg, 1.3 mmol) was prepared using CH_3I (0.25 mL, 3.9 mmol) and 60% NaH in mineral oil (77 mg, 1.9 mmol) in DMF (20 mL) as

described for **2.45**, to obtain a crude product that was desilylated in THF (8 mL) and TBAF (2.6 mL, 2.6 mmol) as described for **2.33**, to give **2.81** (395 mg, 89% over two steps) as a colorless oil: R_f 0.23 (4:1, hexane–EtOAc); $[\alpha]_D$ = +47.4 (c 2.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.00 (s, 1H, H-1), 4.20 (dd, 1H, *J* = 7.0, 7.0 Hz, H-3), 4.11 (d, 1H, *J* = 7.0 Hz, H-2), 3.91 (dd, 1H, *J* = 11.5, 3.5 Hz, H-6a), 3.74 (dd, 1H, *J* = 11.5, 4.5 Hz, H-6b), 3.67 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.52–3.58 (m, 4H, H-5, OC*H*$_3$), 3.40 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.31 (dd, 1H, *J* = 10.0, 7.0 Hz, H-4), 2.00 (br s, 1H, O*H*), 1.50–1.60 (m, 5H, isopropylidene C*H*$_3$, octyl OCH_2CH_2), 1.20–1.38 (m, 13H, isopropylidene C*H*$_3$, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 109.2 (isopropylidene *C*), 97.1 (C-1), 78.4 (C-3), 78.4 (C-4), 75.9 (C-2), 68.3 (C-5), 67.8 (octyl O*C*H$_2$), 62.6 (C-6), 59.2 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 28.0 (isopropylidene *C*H$_3$), 26.3 (isopropylidene *C*H$_3$), 26.1 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{18}H_{34}O_6$ (346.46): C, 62.40; H, 9.89. Found: C, 62.45; H, 10.01. ESIMS: *m/z* calcd for $[C_{18}H_{34}O_6]Na^+$: 369.2248. Found: 369.2248.

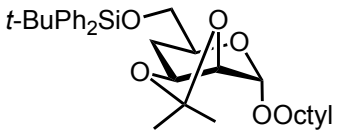
Octyl 2,3-*O*-isopropylidene-4-*O*-phenoxythiocarbonyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranoside (2.82)



Prepared from monosaccharide **2.60** (404 mg,

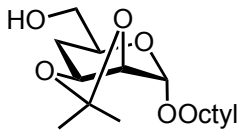
0.71 mmol), DMAP (220 mg, 1.78 mmol) and phenyl chlorothionoformate (0.13 mL, 0.92 mmol) in acetonitrile (2 mL) as described for **2.66**, to give **2.82** (419 mg, 84%) as a colorless oil: R_f 0.58 (9:1, hexane–EtOAc); $[\alpha]_D$ = -21.3 (c 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69–7.75 (m, 4H, ArH), 7.33–7.46 (m, 8H, ArH), 7.25–7.29 (m, 1H, ArH), 6.88–6.92 (m, 2H, ArH), 5.60 (dd, 1H, *J* = 10.3, 7.0 Hz, H-4), 5.11 (br s, 1H, H-1), 4.46 (dd, 1H, *J* = 7.0, 5.5 Hz, H-3), 4.23 (d, 1H, *J* = 5.5 Hz, H-2), 3.94–4.00 (m, 1H, H-5), 3.78–3.89 (m, 3H, H-6a, H-6b, octyl OC*H*$_2$), 3.46 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 2.09 (br s, 1H, O*H*), 1.56–1.64 (m, 2H, octyl OCH_2CH_2), 1.59 (s, 3H, isopropylidene C*H*$_3$), 1.39 (s, 3H, isopropylidene C*H*$_3$), 1.22–1.39 (m, 10H, octyl C*H*$_2$), 1.07 (s, 9H, *tert*-butyl C*H*$_3$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 194.8 (C=S), 153.5 (Ar), 135.8 (2 x Ar), 135.6 (2 x Ar), 133.3 (Ar), 133.2 (Ar), 129.6 (Ar), 129.6 (Ar), 129.4 (2 x Ar), 127.7 (2 x Ar), 127.7 (2 x Ar), 126.5 (Ar), 121.9 (2 x Ar), 110.1 (isopropylidene *C*), 96.6 (C-1), 79.9 (C-4), 76.1 (C-2), 75.7 (C-3), 69.2 (C-5), 67.6 (octyl O*C*H_2), 63.1 (C-6), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 27.6 (isopropylidene *C*H_3), 26.8 (*tert*-butyl *C*H_3), 26.5 (isopropylidene *C*H_3), 26.2 (*tert*-butyl *C*), 22.7 (octyl *C*H_2), 19.2 (octyl *C*H_2), 14.1 (octyl *C*H_3). Anal. Calcd for $C_{40}H_{54}O_7SSi$ (706.34): C, 67.95; H, 7.70; S, 4.54. Found: C, 67.86; H, 7.78; S, 4.37. ESIMS: *m/z* calcd for $[C_{40}H_{54}O_7SSi]Na^+$: 729.3250. Found: 729.3252.

Octyl 4-deoxy-2,3-*O*-isopropylidene-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-*lyxo*-hexopyranoside (2.83)



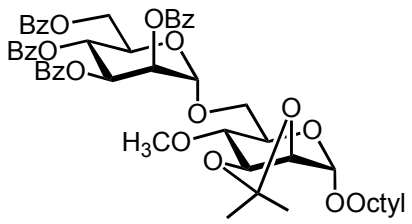
Prepared from phenoxy thiocarbonate **2.82** (398 mg, 0.56 mmol) in toluene (12 mL) and a solution of tri-*n*-butylstannane (290 μL, 1.1 mmol) and AIBN (35 mg, 0.21 mmol) in toluene (50 mL) at reflux as described for **2.50**, to give **2.83** (274 mg, 88%) as a colorless oil: R_f = 0.27 (15:1, hexane–EtOAc); $[\alpha]_D$ = +12.5 (*c* 2.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.67–7.72 (m, 4H, ArH), 7.36–7.45 (m, 6H, ArH), 5.04 (s, 1H, H-1), 4.37 (ddd, 1H, *J* = 9.5, 6.6, 6.3 Hz, H-3), 3.97 (d, 1H, *J* = 6.3 Hz, H-2), 3.78–3.84 (m, 1H, H-5), 3.70–3.78 (m, 2H, H-6a, octyl OC*H*$_2$), 3.62 (dd, 1H, *J* = 10.3, 4.8 Hz, H-6b), 3.41 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.90 (ddd, 1H, *J* = 13.5, 6.6, 2.3 Hz, H-4$_{eq}$), 1.50–1.62 (m, 3H, H-4$_{ax}$, octyl OCH$_2$C*H*$_2$), 1.49 (s, 3H, isopropylidene C*H*$_3$), 1.35 (s, 3H, isopropylidene C*H*$_3$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 1.07 (s, 9H, *tert*-butyl C*H*$_3$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 135.6 (4 x Ar), 133.5 (2 x Ar), 129.6 (Ar), 129.6 (Ar), 127.6 (4 x Ar), 108.7 (isopropylidene *C*), 97.4 (C-1), 73.4 (C-3), 71.0 (C-2), 67.4 (C-6), 67.0 (C-5), 66.7 (octyl O*C*H$_2$), 31.8 (octyl *C*H$_2$), 30.7 (C-4), 29.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 28.1 (isopropylidene *C*H$_3$), 26.8 (*tert*-butyl *C*H$_3$), 26.3 84(isopropylidene *C*H$_3$), 26.2 (*tert*-butyl, *C*), 22.6 (octyl *C*H$_2$), 19.2 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{33}H_{50}O_5Si$ (554.34): C, 71.44; H, 9.08. Found: C, 71.45; H, 9.19. ESIMS: *m/z* calcd for $[C_{33}H_{50}O_5Si]Na^+$: 577.3320. Found: 577.3321.

Octyl 4-deoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hexopyranoside (2.84)



Prepared from deoxy monosaccharide **2.83** (237 mg, 0.43 mmol) in THF (12 mL) and TBAF (2.2 mL, 2.2 mmol) as described for **2.33**, to give **2.84** (97 mg, 72%) as a colorless oil: R_f = 0.27 (3:1, hexane–EtOAc); $[\alpha]_D$ = +52.1 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.00 (br s, 1H, H-1), 4.39 (ddd, 1H, *J* = 8.5, 6.0, 6.0 Hz, H-3), 3.97 (d, 1H, *J* = 6.0 Hz, H-2), 3.80–3.86 (m, 1H, H-5), 3.71 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 3.65 (dd, 1H, *J* = 11.8, 3.5 Hz, H-6a), 3.61 (dd, 1H, *J* = 11.8, 6.8 Hz, H-6b), 3.43 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.88 (ddd, 1H, *J* = 13.5, 6.0, 3.8 Hz, H-4$_{eq}$), 1.54–1.66 (m, 3H, H-4$_{ax}$, octyl OCH$_2$C*H*$_2$), 1.52 (s, 3H, isopropylidene C*H*$_3$), 1.34 (s, 3H, isopropylidene C*H*$_3$), 1.22–1.36 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 109.0 (isopropylidene *C*), 97.7 (C-1), 73.4 (C-2), 70.4 (C-3), 67.9 (octyl O*C*H$_2$), 66.8 (C-5), 65.7 (C-6), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 29.0 (C-4), 27.9 (isopropylidene *C*H$_3$), 26.2 (octyl *C*H$_2$), 26.1 (isopropylidene *C*H$_3$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{17}H_{32}O_5$ (316.22): C, 64.53; H, 10.19. Found: C, 64.58; H, 10.12. ESIMS: *m/z* calcd for $[C_{17}H_{32}O_5]Na^+$: 339.2142. Found: 339.2142.

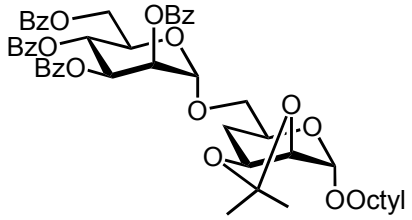
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3-*O*-isopropylidene-4-*O*-methyl-α-D-mannopyranoside (2.85)



Prepared from thioglycoside **2.21** (130 mg, 0.19 mmol), alcohol **2.81** (54 mg, 0.16 mmol), powdered 4 Å molecular sieves (125 mg), *N*-iodosuccinimide (54 mg, 0.24 mmol) and TMSOTf (14 mg, 0.064 mmol) in CH_2Cl_2 (5 mL) as described for **2.41**, to give **2.85** (117 mg, 79%) as a yellow oil: R_f 0.34 (4:1, hexane–EtOAc); $[\alpha]_D$ = -19.0 (*c* 2.7, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.11–8.15 (m, 2H, ArH), 8.04–8.08 (m, 2H, ArH), 7.90–7.94 (m, 2H, ArH), 7.81–7.84 (m, 2H, ArH), 7.56–7.62 (m, 2H, ArH), 7.49–7.53 (m, 1H, ArH), 7.34–7.45 (m, 7H, ArH), 7.24–7.29 (m, 2H, ArH), 6.14 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.94 (dd, 1H, *J* = 10.2, 3.3 Hz, H-3′), 5.73 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 5.19 (d, 1H, *J* = 1.8 Hz, H-1′), 5.08 (br s, 1H, H-1), 4.74 (dd, 1H, *J* = 12.3, 2.4 Hz, H-6a′), 4.56 (ddd, 1H, *J* = 10.2, 3.9, 2.4 Hz, H-5′), 4.49 (dd, 1H, *J* = 12.3, 3.9 Hz, H-6b′), 4.27 (dd, 1H, *J* = 6.9, 6.0 Hz, H-3), 4.18 (d, 1H, *J* = 6.0 Hz, H-2), 3.99 (dd, 1H, *J* = 10.8, 6.6 Hz, H-6a), 3.86–3.92 (m, 2H, H-6b, octyl OC*H*$_2$), 3.83 (ddd, 1H, *J* = 10.2, 6.6, 1.8 Hz, H-5), 3.56 (s, 3H, OC*H*$_3$), 3.53 (dt, 1H, *J* = 9.0, 6.6 Hz, octyl OC*H*$_2$), 3.32 (dd, 1H, *J* = 10.2, 6.9 Hz, H-4), 1.62–1.72 (m, 2H, octyl OCH_2CH_2), 1.60 (s, 3H, isopropylidene C*H*$_3$), 1.17–1.46 (m, 13H, octyl C*H*$_2$, isopropylidene C*H*$_3$), 0.83 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 165.2 (C=O), 165.2 (C=O), 133.4 (Ar), 133.0 (Ar),

133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 109.3 (isopropylidene *C*), 97.3 (C-1′, $^1J_{C,H}$ = 172.3 Hz), 96.9 (C-1, $^1J_{C,H}$ = 168.8 Hz), 78.6 (C-3), 78.2 (C-4), 76.0 (C-2), 70.4 (C-2′), 70.0 (C-3′), 68.8 (C-5′), 67.8 (octyl O*C*H$_2$), 67.6 (C-4′), 66.9 (C-5), 66.9 (C-6), 62.8 (C-6′), 59.0 (O*C*H$_3$), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 28.1 (isopropylidene *C*H$_3$), 26.4 (isopropylidene *C*H$_3$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.0 (octyl *C*H$_3$). Anal. Calcd for $C_{52}H_{60}O_{15}$ (925.02): C, 67.52; H, 6.54. Found: C, 67.20; H, 6.57. ESIMS: *m/z* calcd for $[C_{52}H_{60}O_{15}]Na^+$: 947.3824. Found: 947.3822.

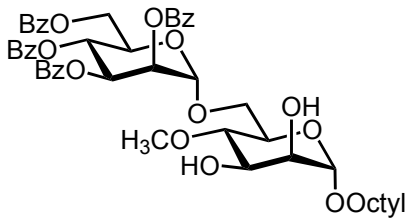
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-deoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hexopyranoside (2.86)



Prepared from thioglycoside **2.21** (95 mg, 0.14 mmol), alcohol **2.84** (36 mg, 0.11 mmol), powdered 4 Å molecular sieves (100 mg), *N*-iodosuccinimide (39 mg, 0.17 mmol) and TMSOTf (4 μL, 0.023 mmol) in CH_2Cl_2 (4 mL) as described for **2.41**, to give **2.86** (76 mg, 75%) as a colorless oil: R_f 0.27 (4:1, hexane–EtOAc); $[\alpha]_D$ = -16.7 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.10–8.14 (m, 2H, ArH), 8.04–8.08 (m, 2H, ArH), 7.91–7.95 (m, 2H, ArH), 7.81–7.85 (m, 2H, ArH), 7.55–7.62 (m, 2H, ArH), 7.49–7.54 (m, 1H, ArH), 7.34–7.45 (m, 7H, ArH), 7.24–7.30 (m, 2H, ArH), 6.14 (dd, 1H, *J* = 10.3, 10.3

Hz, H-4′), 5.92 (dd, 1H, *J* = 10.3, 3.0 Hz, H-3′), 5.71 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′), 5.14 (d, 1H, *J* = 1.8 Hz, H-1′), 5.09 (br s, 1H, H-1), 4.73 (dd, 1H, *J* = 12.3, 2.3 Hz, H-6a′), 4.56–4.61 (m, 1H, H-5′), 4.48 (dd, 1H, *J* = 12.3, 4.3 Hz, H-6b′), 4.43 (ddd, 1H, *J* = 9.0, 6.4 Hz, H-3), 4.01–4.07 (m, 1H, H-5), 4.02 (d, 1H, *J* = 6.0 Hz, H-2), 3.88–3.97 (m, 2H, H-6a, octyl OC*H*$_2$), 3.60 (dd, 1H, *J* = 10.0, 3.5 Hz, H-6b), 3.54 (dt, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 1.94 (ddd, 1H, *J* = 13.0, 6.4, 2.5 Hz, H-4$_{eq}$), 1.53–1.72 (m, 3H, H-4$_{ax}$, octyl OCH$_2$C*H*$_2$), 1.54 (s, 3H, isopropylidene C*H*$_3$), 1.16–1.44 (m, 10H, octyl C*H*$_2$), 1.37 (s, 3H, isopropylidene C*H*$_3$), 0.82 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 165.4 (C=O), 165.3 (C=O), 133.4 (Ar), 133.4 (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.7 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 128.6 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 109.0 (isopropylidene *C*), 97.5 (C-1, $^1J_{C,H}$ = 168.5 Hz), 97.1 (C-1′, $^1J_{C,H}$ = 172.9 Hz), 73.3 (C-2), 70.6 (C-3), 70.5 (C-2′), 70.4 (C-6), 70.0 (C-3′), 68.8 (C-5′), 67.8 (octyl O*C*H$_2$), 66.8 (C-4′), 65.0 (C-5), 62.8 (C-6′), 31.8 (octyl *C*H$_2$), 30.3 (C-4), 29.5 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 28.1 (isopropylidene *C*H$_3$), 26.3 (octyl *C*H$_2$), 26.2 (isopropylidene *C*H$_3$), 22.6 (octyl *C*H$_2$), 14.0 (octyl *C*H$_3$). ESIMS: *m/z* calcd for $[C_{51}H_{58}O_{14}]Na^+$: 917.3719. Found: 917.3716.

Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-*O*-methyl-α-D-mannopyranoside (2.87)



Prepared from disaccharide **2.85** (85 mg, 0.092 mmol) in 80% AcOH/H_2O (2 mL) as described for **2.63**, to give **2.87** (79 mg, 97%) as a yellow oil: R_f 0.26 (1:1, hexane–EtOAc); $[\alpha]_D$ = -12.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.10–8.13 (m, 2H, ArH), 8.06–8.09 (m, 2H, ArH), 7.93–7.97 (m, 2H, ArH), 7.84–7.87 (m, 2H, ArH), 7.55–7.62 (m, 2H, Ar), 7.49–7.53 (m, 1H, ArH), 7.34–7.45 (m, 7H, ArH), 7.24–7.29 (m, 2H, ArH), 6.15 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.94 (dd, 1H, *J* = 10.2, 3.0 Hz, H-3′), 5.74 (dd, 1H, *J* = 3.0, 1.8 Hz, H-2′), 5.41 (d, 1H, *J* = 1.8 Hz, H-1′), 4.84 (br s, 1H, H-1), 4.73 (dd, 1H, *J* = 12.0, 2.7 Hz, H-6a′), 4.58 (ddd, 1H, *J* = 10.2, 4.2, 2.7 Hz, H-5′), 4.51 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6b′), 4.08 (dd, 1H, *J* = 12.0, 4.8 Hz, H-6a), 3.92–3.96 (m, 3H, H-2, H-3, H-6b), 3.69–3.78 (m, 2H, H-5, octyl OC*H*$_2$), 3.67 (s, 3H, OC*H*$_3$), 3.49 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4), 3.44 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 2.55 (br s, 2H, O*H*), 1.55–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.19–1.41 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.7 (C=O), 165.5 (C=O), 165.5 (C=O), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.3 (Ar), 129.1 (Ar), 129.0 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 99.4 (C-1), 97.6 (C-1′), 77.6 (C-4), 72.1 (C-3), 71.3 (C-2), 70.9 (C-2′), 70.9 (C-5), 69.9 (C-3′), 69.0 (C-5′), 68.0 (octyl O*C*H$_2$), 66.9 (C-4′), 66.7 (C-6), 62.9 (C-6′), 60.9

(O*C*H_3), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). ESIMS: *m/z* calcd for $[C_{52}H_{60}O_{15}]Na^+$: 907.3511. Found: 907.3509.

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Chapter 3

Exploring Substrate Specificity of α-(1→6)-Mannosyltransferase

A version of this chapter has been published. P. H. Tam, G. S. Besra, T. L. Lowary. *ChemBioChem*, **2008**, *9*, 267-278.

3.1. Introduction

The activity of a PPM-dependent α-(1→6)-mannosyltransferase (ManT) in a membrane preparation from *M. smegmatis* was first demonstrated by Yokoyama and Ballou.[1] It was observed that the isolated cell extract catalyzed the transfer of a mannosyl residue from GDP-mannose (**3.1**, Figure 3-1) to mannose to form a homologous series of α-(1→6)-linked mannooligosaccharides with up to 12 sugar units. The authors further demonstrated that the sugar nucleotide **3.1** was initially converted to β-D-mannopyranosyl phosphodecaprenol (PPM, **3.2**), which served as the actual donor for the observed α-(1→6)-mannosylation in vitro (Figure 3-1).

Besra and co-workers later demonstrated that the synthesis of **3.2** was catalyzed by PPM synthase from *M. tuberculosis* and also identified its homologues from other mycobacterial species.[2] Recently, Brown *et al* have established a cell-free assay for ManT using radiolabeled GDP-mannose (**3.1**) as the indirect donor and have screened a series of synthetic mannosides with variable algycons.[3] Consistent with the previous studies, the observed ManT activity appeared to be PPM- but not

GDP-dependent as the presence of amphomycin and calcium essentially abolished the formation of mannosylated products. It has been shown that the antiobiotic amphomycin dramatically affected the synthesis of **3.2** and subsequently prevented the mannosyl transfer.[4]

In addition, Brown and co-workers also demonstrated that octyl dimannopyranoside **2.4** (Figure 3-2) is a good acceptor for ManT. The hydrophobic nature of the octyl agylcone allows convenient product isolation and characterization after the assays.[5] Based on these previous findings, a small panel of analogs of **2.4** was recently synthesized and screened as potential substrates and inhibitors of the enzyme,[6,7] although the specificity of ManT remains poorly understood.

To expand our knowledge of the substrate requirements in the active site of the enzyme, a larger panel of synthetic analogs of **2.4** was synthesized and their abilities to serve as substrates and/or inhibitors were evaluated using the aforementioned established radioactive assay. We hypothesized that octyl glycosides such as analogs **2.4**–**2.6** would be used to determine the effect of the acceptor length on ManT catalysis while disaccharides **2.7**–**2.20** would be used to map out the steric requirements and hydrogen bonding interactions in the active site of ManT (Figure 3-2). Details of the synthetic work towards the target analogs **2.3**–**2.20** has been described in the previous chapter. Herein, the evaluation of these analogs as substrates and/or inhibitors for ManT under the established

assay conditions[5] is reported. We envisioned this fundamental study would facilitate the design of potent and specific inhibitors of ManT in the future.

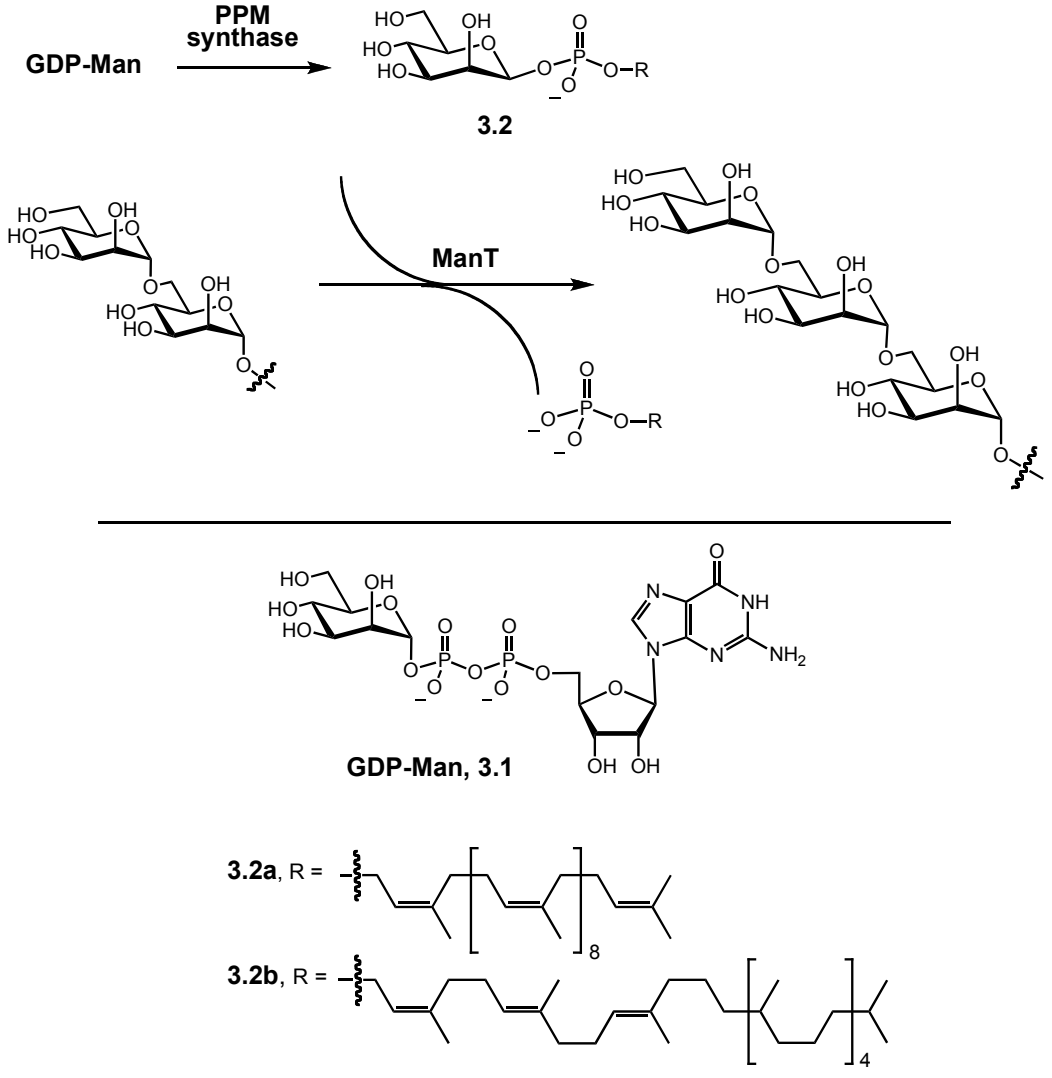
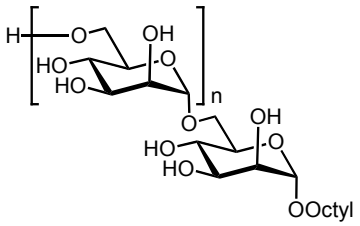
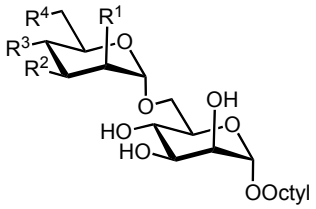


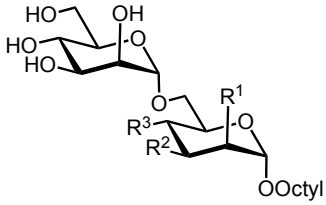
Figure 3-1. Reaction catalyzed by PPM-dependent α-(1→6)-mannosyltransferase (ManT).



2.3, n = 0
2.4, n = 1
2.5, n = 2
2.6, n = 3



2.7, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$
2.8, $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$
2.9, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$, $R^4 = OH$
2.10, $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$
2.11, $R^1 = OH$, $R^2 = OH$, $R^3 = OCH_3$, $R^4 = OH$
2.12, $R^1 = OH$, $R^2 = OH$, $R^3 = H$, $R^4 = OH$
2.13, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OCH_3$
2.14, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$



2.15, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = OH$
2.16, $R^1 = H$, $R^2 = OH$, $R^3 = OH$
2.17, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$
2.18, $R^1 = OH$, $R^2 = H$, $R^3 = OH$
2.19, $R^1 = OH$, $R^2 = OH$, $R^3 = OCH_3$
2.20, $R^1 = OH$, $R^2 = OH$, $R^3 = H$

Figure 3-2. Synthetic acceptor analogs used as probes of the PPM-dependent ManT involved in the synthesis of the α-(1→6)-linked mannan core of LM and LAM**.**

3.2. Results and Discussion

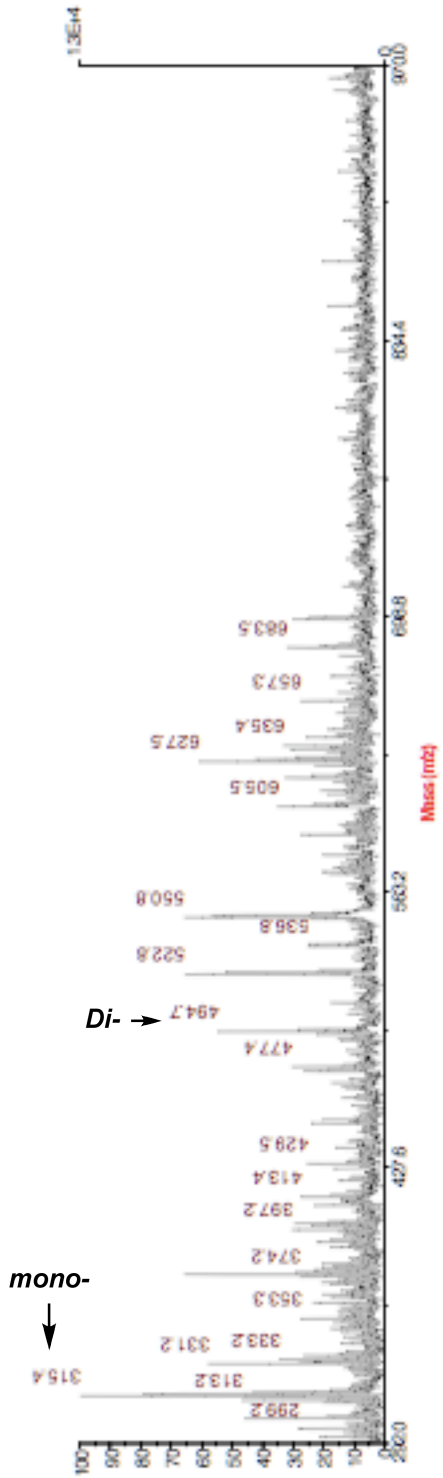
3.2.1. Optimal Acceptor Length For ManT Catalysis

To determine the optimal length of oligomannopyranosides for ManT catalysis, oligosaccharides **2.3**–**2.6** were screened as potential substrates for the enzyme in a cell-free assay using ^{3}H-labeled GDP-mannose (**3.1**, Figure 3-1), which is converted to PPM (**3.2**, Figure 3-1), by the membrane fraction.[3] The results are summarized in Table 3-1. At 2 mM concentration, disaccharide **2.4** and trisaccharide **2.5** were the best acceptor substrates for the enzyme, showing roughly comparable activities. On the other hand, monosaccharide **2.3** and tetrasaccharide **2.6** were relatively poor substrates for the ManT, possessing only 8% and 54% activity relative to **2.4**, respectively. Our findings are consistent with the previous report by Yokoyama and Ballou,[1] in which methyl α-D-mannopyranoside was shown to act as an acceptor with much lower efficiency than larger oligomers.

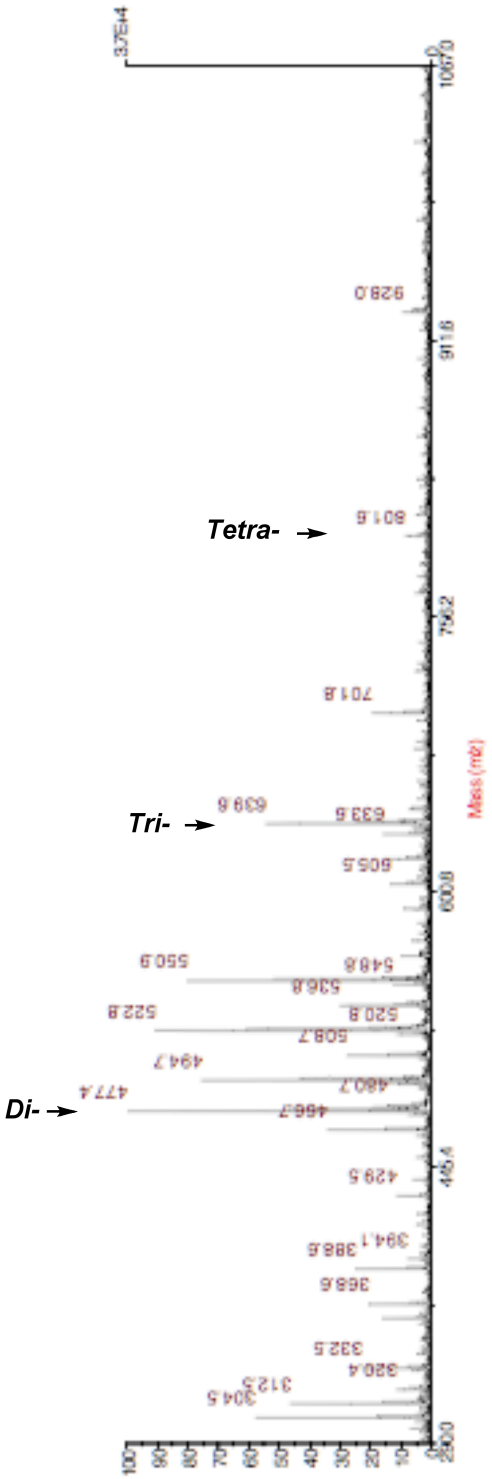
The lower efficiency of tetramannoside **2.6** to act as an acceptor substrate may result from the presence of an endogenous α-(1$\rightarrow$6)-*endo*-mannosidase, which catalyzes the removal of trisaccharide units from the nonreducing end of the pentasaccharide product, as has been previously observed.[1] The simultaneous synthesis and degradation of a pentasaccharide product from **2.6**, by ManT and mannosidase activities, respectively, would be expected to lead to the lower apparent acceptor efficiency of the tetrasaccharide.

It is also possible that the tetrasaccharide substrate is degraded by this α-(1→6)-*endo*-mannosidase, thus affording a trisaccharide lacking an octyl group (and which would therefore not be detected in the assay) and octyl α-D-mannopyranoside, **2.3**, a very poor substrate for the ManT. In the mass spectrum of the product obtained from the incubation mixtures with **2.6** (Figure 3-3D), a peak (m/z = 315.4) corresponding to **2.3** was detected, thus supporting this hypothesis. In addition, treatment of the tetrasaccharide under the assay conditions, both with and without the donor substrate, followed by TLC analysis showed the formation of mono-, di-, and trisaccharides (Figure 3-4).

To date, only limited information about the specificity this α-(1→6)-*endo*-mannosidase is available[1] and no structural information on this protein, or to the best of our knowledge any other *endo*-mannosidase, has been reported. This dearth of information makes it difficult to make a more definitive statement about the interference of this glycosidase with the assay. Nevertheless, based on these results, it is possible to conclude that the disaccharide unit appears to be the minimal acceptor length required for ManT catalysis and that significant increases in activity are not observed by increasing the size of the acceptor to a trisaccharide.



A)



B)

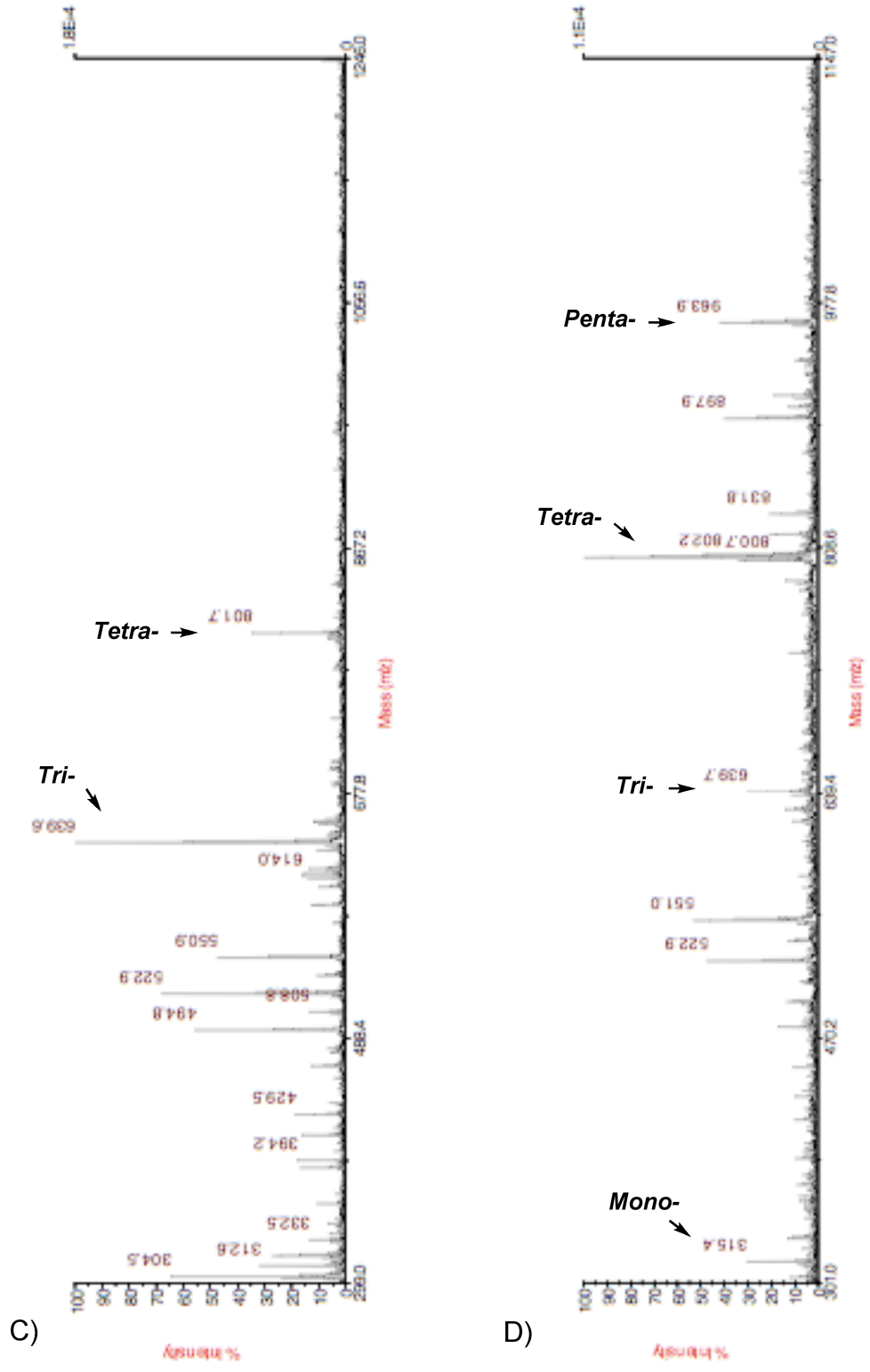


Figure 3-3. MALDI mass spectra of enzymatic products isolated from incubation mixtures using analogs **2.3** (A), **2.4** (B), **2.5** (C), and **2.6** (D) at 2 mM concentrations.

Table 3-1. Comparison of the ManT activities using potential acceptors **2.3**–**2.6**

Acceptor	Relative activity [%] [i]	Mass of oligosaccharide product [ii]		Glycosidic linkage formed by enzymatic reaction [iii]
		Calculated	Found	
2.3	8 ± 2	477.5	477.5	n.d.[iv]
2.4	100 ± 2	639.3	639.5	α-(1→6)
2.5	108 ± 4	801.4	801.7	α-(1→6)
2.6	54 ± 10	963.9	963.9	n.d.

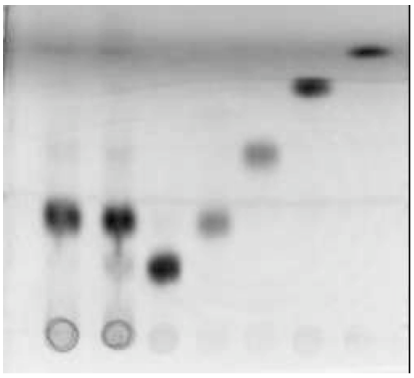
[i] Relative activities were measured at 2.0 mM acceptor concentration with 0.2 μCi of [^{3}H] GDP-Man and are expressed with respect to disaccharide **2.4**. 100% activity corresponds to 0.36 pmol mg^{-1} min^{-1}.

[ii] The enzymatic products were isolated from large-scale incubations and their masses were determined by MALDI mass spectrometry. The calculated and found values correspond to the sodium adducts.

[iii] The structure of the enzymatic products was elucidated by ^{1}H NMR spectroscopy. The chemical shifts of the anomeric protons were shown to be identical with those of the authentic tri- and tetrasaccharides obtained by chemical synthesis.

[iv] Not determined.

Lane 1: **2.6** + membrane prep.
Lane 2: **2.6** + membrane prep. + GDP-mannose
Lane 3: octyl pentasaccharide
Lane 4: octyl tetrasaccharide
Lane 5: octyl trisaccharide
Lane 6: octyl disaccharide
Lane 7: octyl monosaccharide



1 2 3 4 5 6 7

Figure 3-4. Large-scale (750 μL) ManT reactions using the octyl tetrasaccharide, compound **2.6**, in the presence or absence of GDP-mannose were performed as described in the paper. After 4 days of incubation at 37 °C, one-third of the reaction was subjected to organic extraction and C_{18} reverse phase column purification. The eluate was concentrated, redissolved in 25 μL water, and used for TLC analysis (7:2:1 EtOAc/MeOH/H_2O).

3.2.2. Characterization of Enzymatic Products

In addition to the PPM-dependent α-(1→6)-mannosyltransferase, the crude membrane extract of *M. smegmatis* used in these assays also contain α-(1→2)-ManT's. For example, recent studies have identified three PPM-dependent α-(1→2)-ManT's involved in LM branching, the capping of the arabinan domain, and the biosynthesis of AcPim6.[8–10] In addition, the earlier study by Yokoyama and Ballou detected trace amounts of products arising from α-(1→2)-ManT activity.[1]

To confirm that the observed addition of radiolabeled mannose to **2.3**–**2.6** arose from α-(1→6)-ManT activity, and not from α-(1→2)-ManT activity, more detailed structural characterization of the products was required. Therefore, in addition to the radiochemical assays described above, milligram-scale enzymatic incubations of **2.3**–**2.6** with unlabelled GDP-Man and the membrane fraction were carried out. After the incubations, the enzymatic products were purified using a C_{18} SepPak cartridge[5] and then analyzed by MALDI mass spectrometry (Figure 3-3).

As shown in Table 3-1, these analyses confirmed that a single mannopyranose unit was transferred to each of the acceptor substrates being examined. In addition, TLC analysis (data not shown) of the products clearly showed that the R_f values of the enzymatic products were identical to those of the authentic samples obtained by chemical synthesis (Chapter 2). Finally, the enzymatic products from the incubations of

acceptors **2.4** and **2.5** were purified by preparative TLC and the structures of the products were analyzed by ^{1}H NMR spectroscopy.

Based on the mass spectrometry results, the product of the reaction with disaccharide **2.4** would be **2.5** and trisaccharide **2.5** would yield tetrasaccharide **2.6**. Comparison of the anomeric region of the spectrum of the product obtained from incubation of **2.4** with the membrane preparation and GDP-Man revealed excellent agreement with the spectrum of authentic synthetic **2.5** (Figure 3-5). Similarly, the spectrum of the product obtained for the reaction with **2.5** was a match with an authentic sample of **2.6** (Figure 3-6).

As a further support of structure, the product resulting from tetrasacccharide **2.6**, a pentasaccharide, was treated with an α-(1→2)-specific mannosidase[10,11] and, as determined by TLC, no cleavage of the polysaccharide was observed as shown in Figure 3-7. To ensure that the mannosidase was active, an octyl α-(1→2)-dimannoside (Figure 3-7, far left) was included in this experiment. The digestion of this disaccharide was set up under similar conditions to that for the pentasaccharide except only 1.25 µL of enzyme (40 mU/mL) was used (one-third the amount of enzyme used for the digestion of pentasaccharide). For the disaccharide, the TLC plate clearly shows two new spots corresponding to octyl α-mannopyranoside (top) and mannose (bottom). When the pentasaccharide was used as the substrate (Figure 3-7, middle), no mannose fragment was observed, even though a higher concentration of

mannosidase was used. Attempted purification of the pentasaccharide product by preparative TLC gave a mixture of the tetra- and pentasaccharides (*right)* However, this TLC shows that there is no diminishment or disappearance of the pentasaccharide.

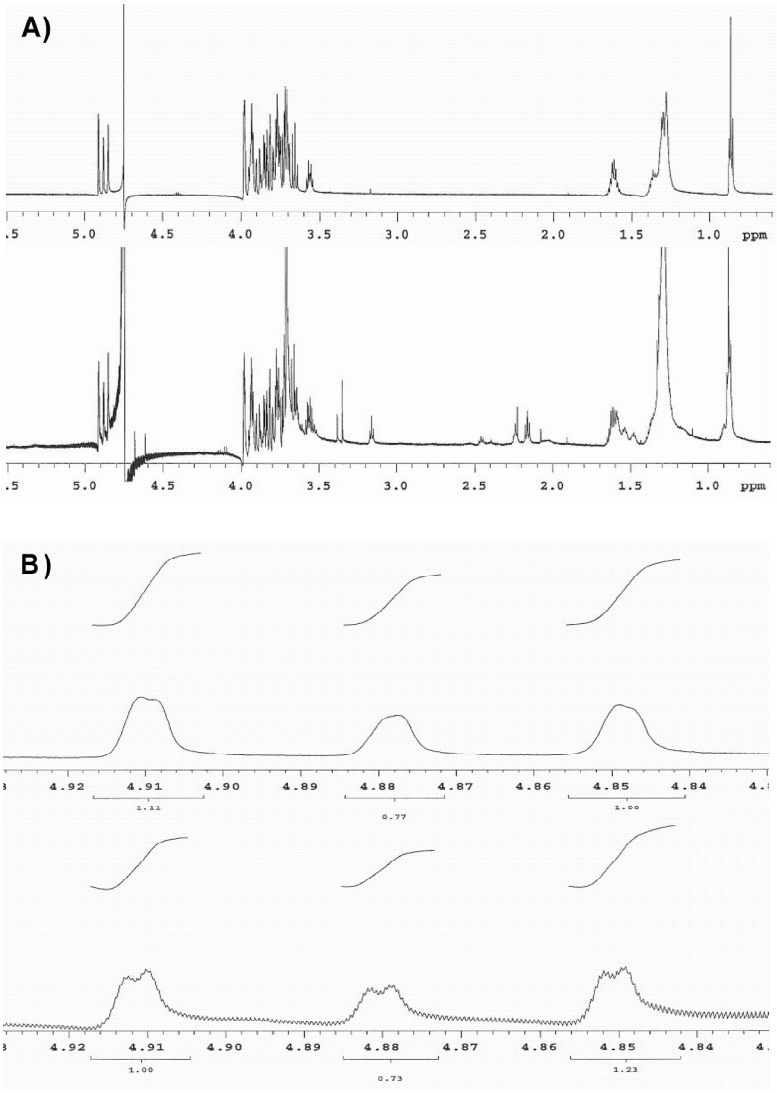


Figure 3-5. A) Full spectra of compound **2.5** (*top*) and trisaccharide purified from the milligram-scale reaction (*bottom*). B) Partial spectra are shown for the comparison of the anomeric protons between compound **2.5** (*top*) and purified trisaccharide from the reaction (*bottom*).

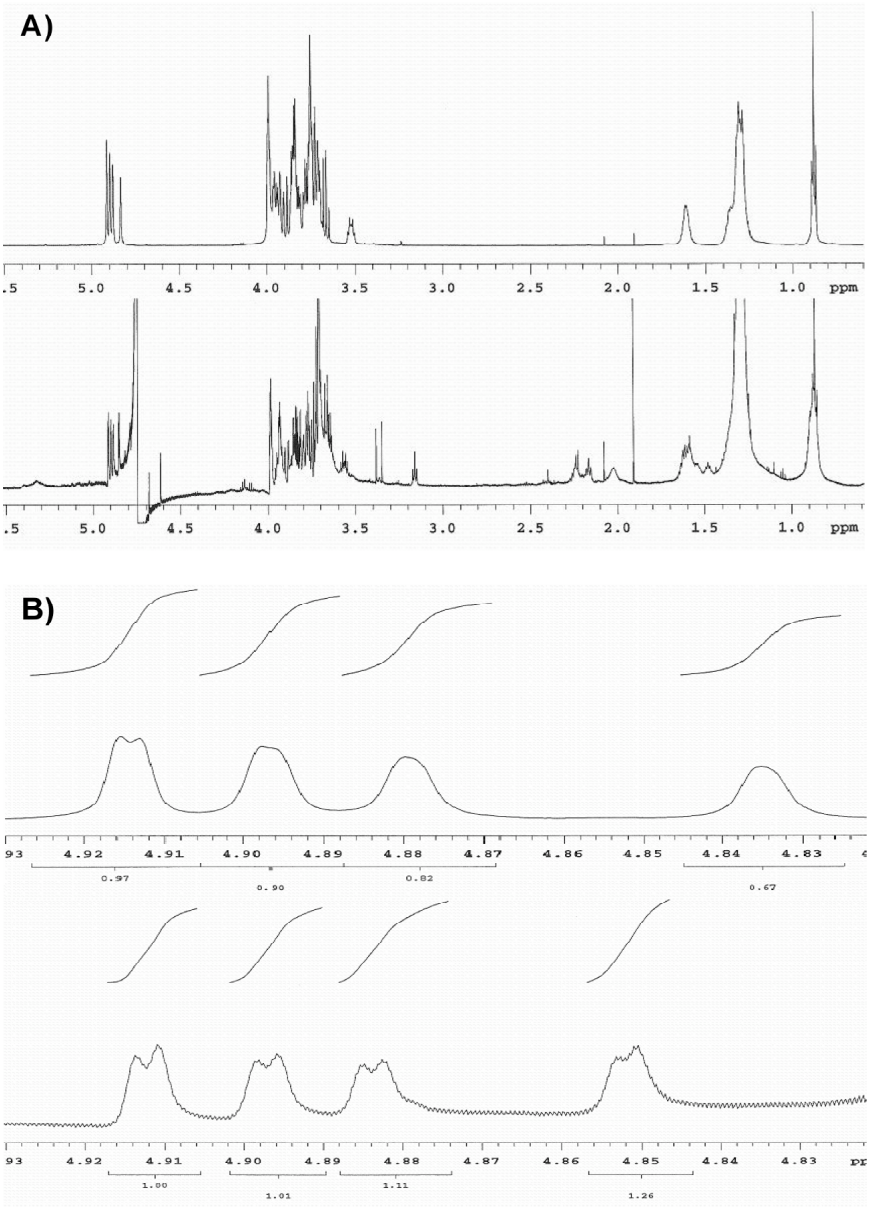


Figure 3-6. A) Full spectra of compound **2.6** (*top*) and tetrasaccharide purified from the milligram-scale reaction (*bottom*). B) Partial spectra are shown for the comparison of the anomeric protons between compound **2.6** (*top*) and purified tetrasaccharide from the reaction (*bottom*).

Given the proposed processive nature of this ManT, it would be expected that a homologous series of products would be observed in these incubations. In an earlier study,[3] by monitoring the transfer of ^{14}C-labelled mannose to the acceptor, disaccharide **2.4** was shown to give predominantly trisaccharide **2.5** and trace amounts of tetrasaccharide **2.6**. In our hands, the larger oligomers were not observed from TLC analyses. This is likely due to the poor sensitivity of this detection method, as compared to the radiochemical method used earlier,[3] as well as the existence of an extremely small amount of the longer oligosaccharides compared with the product resulting from the addition of a single mannose residue.

Further complicating the situation is the presence of the aforementioned (1→6)-*endo*-mannosidase, which would preferentially degrade the longer oligomers. In the MALDI-MS analyses as shown in Figure 3-3, although longer oligosaccharides were observed (e.g., disaccharide **2.4** going to trisaccharide **2.5** and tetrasaccharide **2.6** and similar observations when using **2.3** and **2.5** as the substrates), the peak intensities were comparable to background noise. While the lack of the longer oligomers could be the result of the cleavage of the products by the *endo*-mannosidase, it could also be that these oligosaccharides are generally poor substrates compared to those present in the natural system, which would include the AcPIM4 core. The relative importance of

these issues are impossible to resolve in the absence of a pure ManT, free from any endo-mannosidase activity.

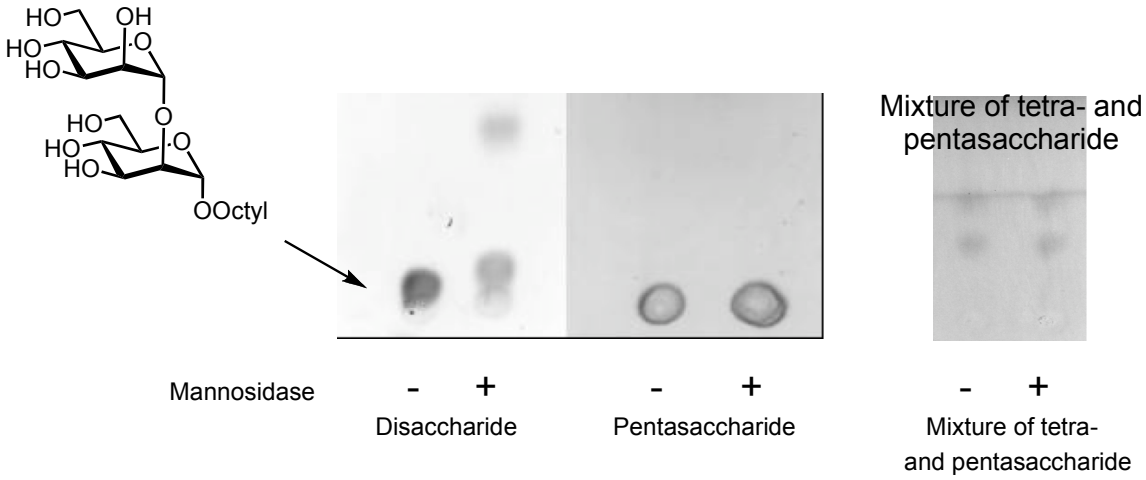


Figure 3-7. Another portion (250 µL) of the large-scale ManT reaction (in the presence of GDP-mannose) as described in Figure 3.4 was purified. The eluate was concentrated, redissolved in small amount of MeOH, and the pentasaccharide was further purified by preparative TLC as described in the experimental section. The purified product was dried under vacuum overnight and redissolved in 20 µL 1 x buffer (supplied by Glyko). To 7.5 µL of the redissolved product, 2.5 µL of *Aspergillus saitoi* α-(1→2)-mannosidase (40 mU/mL) was added and the reaction was incubated at 37 °C for 19 h. Another 1.0 µL of fresh enzyme (40 mU/mL) was added and the digestion was continued for a further 19 h. The reaction was then diluted with 40 µL water and extracted with 50 µL $CHCl_3$–MeOH (2:1); the aqueous layer was separated and used for the TLC analysis (7:1 CH_2Cl_2–MeOH).

3.2.3. Determination of Kinetic Parameters

Acceptors **2.4**, **2.5** and **2.6** were sufficiently active to allow further kinetic characterization and representative examples of the kinetic experiments are shown in Figure 3-8. First, to determine the quantity of the membrane preparation needed for the assays, we studied product formation from **2.4** as a function of mass of membrane fraction. As shown in Figure 3-8A, product formation was proportional to the amount of cell membrane preparation added to the assays up to ~100 μg, at which point product formation reached a plateau. Also, with acceptor **2.4**, the incorporation of the radiolabeled mannose was linear with time up to 60 minutes (Figure 3-8B). This linear correlation between the incorporation of radiolabeled product and time was also observed in the subsequent kinetic studies using other acceptor substrates. Finally, the reaction rate with **2.4** reached a plateau at acceptor concentrations above 2 mM. The Michaelis-Menten plot for **2.4** is given in Figure 3-8C and those for **2.5** and **2.6** can be found in Appendix.

As shown in Table 3-2, the apparent K_M values for these compounds were in the range of 147 to 234 μM. The V_{max} values for **2.4** and **2.5** were very similar, 0.28 and 0.25 pmol mg^{-1} min^{-1}, respectively. On the other hand, the V_{max} value for tetrasaccharide **2.6** was reduced by 2.2 fold (0.13 pmol mg^{-1} min^{-1}), compared to that of disaccharide **2.4**. However, as discussed above, it is plausible that the apparent slower turnover of acceptor **2.6** might be due to the simultaneous degradation of

the radiolabeled product and the acceptor substrate, which consequently affects the apparent kinetic parameters of **2.6**.

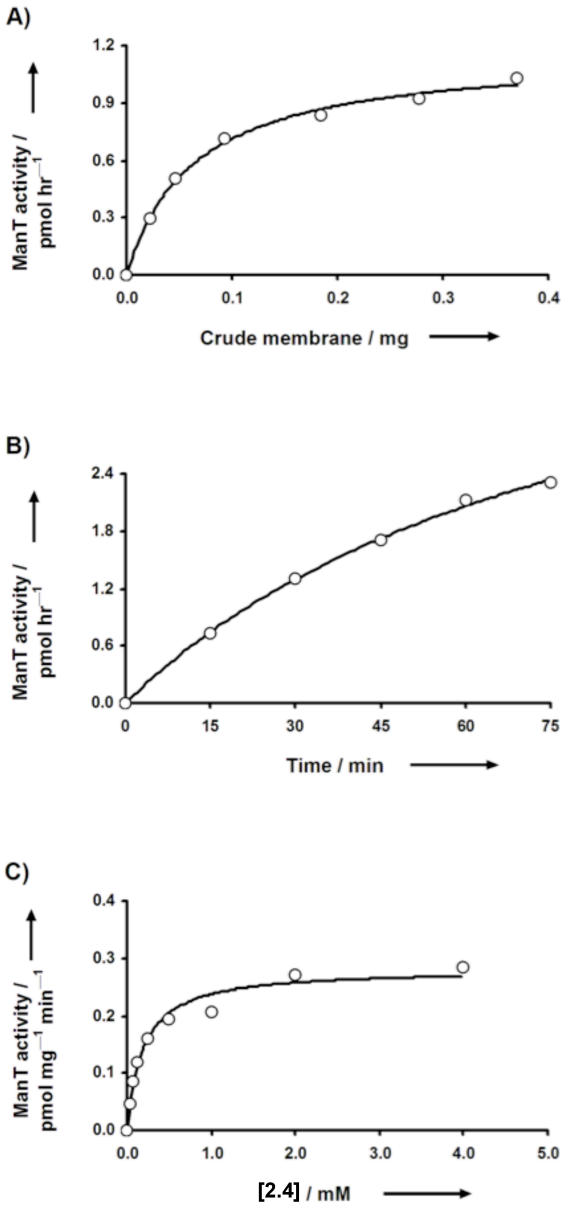


Figure 3-8. Representative graphs for ManT kinetics with acceptor **2.4**. (A) Incorporation of radiolabeled [^{3}H]-mannose into product relative to protein amount. The activity of the enzyme was determined using different protein amounts. All other reaction conditions were identical to those described in the cell-free assay as described in the experimental section. (B) Incorporation of radiolabeled [^{3}H]-mannose into **2.4** as a function of time. An acceptor concentration of 4 mM was used and the assays were terminated at the indicated time points. (C) Incorporation of radiolabeled [^{3}H]-mannose into **2.4** as a function of acceptor concentration. Assays were performed at different substrate concentrations (0.03, 0.06, 0.13, 0.25, 0.5, 1, 2, and 4 mM). Control experiments without the addition of acceptor were also performed in parallel. The data obtained were subjected to nonlinear regression analysis using GraphPad Prism 4.0.

Table 3-2. Kinetic parameters for oligosaccharide substrates of ManT [i]

Analog	K_M [μM]	V_{max} [pmol mg^{-1} min^{-1}]
2.4	188 ± 33	0.28 ± 0.013
2.5	234 ± 50	0.25 ± 0.015
2.6	147 ± 27	0.13 ± 0.001
2.9	201 ± 39	0.16 ± 0.010
2.11	437 ± 104	0.072 ± 0.010
2.17	216 ± 31	0.22 ± 0.010
2.19	272 ± 56	0.16 ± 0.010
2.8	307 ± 49	0.23 ± 0.010
2.20	111 ± 25	0.23 ± 0.012

[i] Kinetic parameters were determined using a range of acceptor concentrations (0.03 to 4.0 mM) by nonlinear regression analysis of the Michaelis-Menten equation with the GraphPad Prism 4.0 program.

3.2.4. Acceptor Specificity of ManT

As the data presented above demonstrated that the octyl dimannoside **2.4** appeared to be the minimal acceptor required for ManT catalysis, and that the trisaccharide did not lead to substantially better activity, a panel of disaccharide analogs **2.7–2.20** were screened to explore the enzyme substrate specificity further. In **2.7**–**2.20**, one of the hydroxyl groups of the parent disaccharide has been either been replaced with a methoxy group, or deoxygenated. Their ability to act as acceptor substrates for the ManT were compared with the parent compound **2.4** (Figure 3-9).

Among the disaccharides synthesized, only **2.7** and **2.8**, the C-2′-methoxy and C-2′-deoxy analogs of **2.4**, respectively, had been tested against the ManT enzyme previously.[6] Our findings here are consistent with that previous report, which showed that the C-2′-deoxy disaccharide **2.8** but not **2.7** served as a substrate for the ManT. Interestingly, the 2-methoxy analog, **2.15**, was also inactive and the 2-deoxy analog (**2.16**) was also a poor substrate for ManT with a relative mannosylation rate of 11%.

The results with the methoxy analogs **2.7** and **2.15** demonstrate that the enzyme is not tolerant of bulky substituent at the C-2′ and C-2 positions. These results have implications in the overall pathway for LAM biosynthesis as they suggest that all (or at least more than two) of the α-(1→6)-linked residues must be assembled before the α-(1→2)-mannopyranosyl branch points are attached. If the relatively small methyl group shuts down α-(1→6)-ManT activity, a much larger monosaccharide residue would be expected to do the same. While this hypothesis should be studied with longer oligomers, these results support the original model for the introduction of these branching residues into the polymer.[4] In addition, the data obtained with the deoxy analogs **2.8** and **2.16** show that while the C2′ hydroxyl group does not appear to form a critical hydrogen bond with the enzyme, the interaction between the C-2 hydroxyl group and the protein appears to be essential for activity.

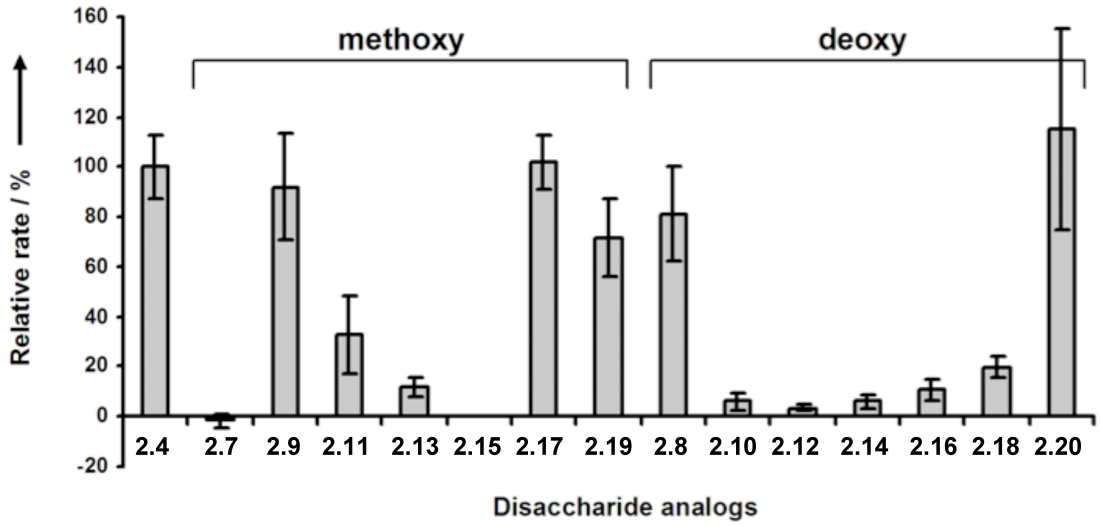


Figure 3-9. Acceptor specificity of the mycobacterial mannosyltransferase with disaccharide **2.4** and derivatives. Each acceptor was tested at 2 mM and was incubated with 0.05 μCi of [^{3}H] GDP-Man under the assay conditions as described in the experimental section. Relative activities for each acceptor are expressed as a percentage of the incorporation of [^{3}H]-mannose into the parent compound **2.4**. 100% activity corresponds to 2.67 pmol mg^{-1} h^{-1} α-(1→6)-ManT activity. Error bars refer to the standard deviations of duplicate reactions.

Although both the methoxy analogs at C2′ and C2 (**2.7** and **2.15**) are not substrates for the enzyme, methylation of O-3′/O-3 and O-4′/O-4 of disaccharide **2.4** resulted in compounds (**2.9**, **2.11**, **2.17** and **2.19**) that are accepted by the ManT, but a substantial decrease in activity is observed for the 4′ methoxy analog, **2.11**. In terms of the polar interactions with the

enzyme, the hydroxyl groups at C-3′, C-4′, C-2 and C-3 of **2.4** seem to be essential for recognition as deoxygenation of any of them produces compounds (**2.10**, **2.12**, **2.16** and **2.18**) that are essentially inactive. Similar to the 2′-deoxy derivative **2.8**, deoxygenation at C-4, leading to compound **2.20**, does not influence recognition by the enzyme.

Finally, although lacking a reactive C-6′ hydroxyl group, small amounts of [^{3}H]-mannosylated products are detected when the 6′-methoxy and deoxy analogs, **2.13** and **2.14**, respectively, are screened as acceptor substrates. These unexpected observations could be explained by the presence in the crude cell membrane preparation of an α-(1→2)-ManT, as was previously noted by Yokoyama and Ballou.[1]

The NMR spectra of the products obtained from the incubations of **2.4** and **2.5** demonstrated the formation of α-(1→6)-linkages, however, it is possible that some α-(1→2)-ManT activity is present in levels that cannot be detected by a relatively insensitive technique such as NMR spectroscopy. Although it is expected that this membrane fraction contains the ManT responsible for the addition of the α-(1→2)-linked branches in the core mannan, if one assumes that the enzyme normally recognizes a longer mannan substrate it is conceivable that disaccharide **2.4** is a relatively poor substrate for the α-(1→2)-ManT. Therefore, this activity can only be detected when using compounds (e.g., **2.13** and **2.14**) that cannot act as α-(1→6)-ManT substrates.

Unfortunately, the small turnover observed for these compounds precluded the isolation and characterization of the products. However, to test if the introduction of α-(1→2)-linked mannopyranose residues into **2.13** and **2.14** was responsible for the observed radioactivity transfer, the products of the reactions were treated with an α-(1→2)-specific mannosidase and then the amount of radioactivity quantitated again (Table 3.3). This treatment led, in the case of **2.13**, to no appreciable decrease in radioactivity and, in the case of **2.14**, only a marginal reduction, thus suggesting that the formation of (1→2)-linked mannopyranosyl linkages was not leading, at least exclusively, to the apparent substrate activity of these analogs.

We remain unsure as to the origin of radioactivity transferred to **2.13** and **2.14** but other possibilities include the ability of these compounds to serve as substrates for another mannosyltransferase in the membrane fraction (e.g., the α-(1→4)-ManT involved in the biosynthesis of 3-*O*-methyl-mannose polysaccharides[12]) or the cleavage of these substrates by an endogenous mannosidase to a monosaccharide that is then a substrate for the ManT. We note, however, that the latter possibility is problematic given the data provided in Table 3.1 and earlier studies[1] demonstrating that monosaccharides are poor substrates for the enzyme. The possibility that **2.13** and **2.14** were contaminated with small amounts of the parent disaccharide, **2.4**, was ruled out by MS and NMR spectral data obtained for these compounds.

Table 3-3. Effect of α-(1→2)-mannosidase treatment on products

	Before digestion (dpm)	After digestion (dpm)
control sample 1 (no acceptor)	570	604
control sample 2 (no acceptor)	718	689
2.13-sample 1 (6' methoxy)	5261	5431
2.13-sample 2 (6' methoxy)	5292	6139
2.14-sample 1 (6' deoxy)	1311	980
2.14-sample 2 (6' deoxy)	1333	800

A graphical summary of the results of screening disaccharides **2.7**–**2.20** against the enzyme is presented in Figure 3-10.

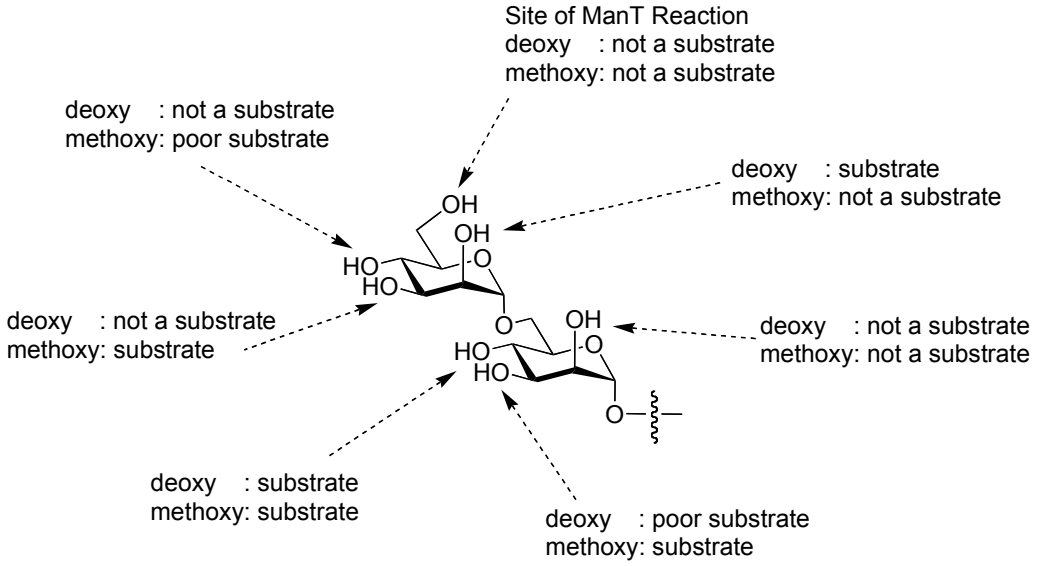


Figure 3-10. Summary of the substrate specificity of the ManT. "Not a substrate" refers to a relative ManT activity ≤12% of that for the natural substrate **2.4**; "poor substrate" refers to a relative ManT activity ≤33% of that for the natural substrate **2.4**.

3.2.5. Kinetic Characterization of Active Substrate Analogs

Among the disaccharide analogs being screened, compounds **2.8**, **2.9**, **2.11**, **2.17**, **2.19**, and **2.20** were the most efficient acceptor substrates for the ManT, with relative activities >20% of that for the natural substrates **2.4**. Full kinetic characterization of these six compounds was carried out and their kinetic constants are listed in Table 3-2; the Michaelis-Menten plots for these disaccharide analogs can be found in Appendix.

The kinetic constants for these analogs were generally similar to those observed with the parent compound **2.4**. For example, compounds **2.8**, **2.9**, **2.17**, **2.19** and **2.20** had K_M values ranging from 111–307 μM and V_{max} values of 0.16–0.23 pmol mg^{-1} min^{-1}, which are comparable to the values obtained for **2.4** (K_M = 188 μM and V_{max} = 0.28 pmol mg^{-1} min^{-1}. The only exception was the 4′-methoxy analog **2.11**, which has a K_M (437 μM) ~2.3-fold higher than that of **2.4** and also significantly larger than other disaccharide analogs evaluated (Table 3-2). In addition, **2.11** has a much smaller V_{max} (0.072 pmol mg^{-1} min^{-1}) indicating that this acceptor turns over significantly more slowly than the other derivatives.

It is possible that the steric bulk of the methyl group hinders mannosylation of the 6′-OH group by the enzyme, as has been observed previously in a substrate for *N*-acetylglucosaminyltransferase-V (GlcNAcT-V).[13] In this earlier example, the glycosylation of a mannose 6-OH group in a trisaccharide substrate for GlcNAcT-V, was inhibited by methylation at O-4 of the mannose residue undergoing glycosylation. Apparently, the C-

4′ hydroxyl group of the dimannoside acceptor not only plays an important role in enzyme catalysis but also substrate recognition as deoxygenation at this position resulted in no enzymatic activity.

3.3. Conclusions

In this chapter, we report studies on the substrate specificity of a PPM-dependent α-(1→6)-mannosyltransferase involved in the mycobacterial LAM biosynthesis. Screening of a homologous series of octyl glycoside oligomers ranging in size from monosacccharides to tetrasaccharides revealed that, in agreement with earlier studies,[1,3] a disaccharide motif is the minimum epitope recognized by the enzyme and that significant increases in activity were not gained by moving to larger substrates. Through the subsequent analysis of a panel of monomethoxy and monodeoxy analogs of disaccharide substrate **2.4**, key interactions with the protein were identified (Figures 3-9 and 3-10).

Among the most important findings is that methylation of the hydroxyl groups at C-2 of either mannopyranose residue in **2.4** leads to complete loss of activity. These results suggest that the attachment of the α-(1→2)-mannopyranosyl branches in the mannan core of LM/LAM must occur after a larger α-(1→6)-linked mannan is assembled. Further support for this hypothesis came from an experiment in which treatment of the pentasaccharide resulting from tetrasaccharide **2.6** with an α-(1→2)

specific mannosidase did not lead to cleavage of the product as detected by TLC.

Another important conclusion is that the enzyme appears to form critical hydrogen bonding interactions with a number of the hydroxyl groups on the substrate as deoxygenation leads, in all but two cases (C-2′ and C-4), to essentially total loss of activity.

3.4. Experimental Section

Bacterial strains and growth conditions

M. smegmatis mc^2155 was a generous gift from Professor William R. Jacobs, Jr. at the Albert Einstein College of Medicine. The bacteria were grown at 37 °C in 100 mL of Luria Bertoni (LB) broth medium containing 0.05% Tween 80 to an A_{600nm} of < 1.0 (~two days from a frozen bacterial stock). The 50 mL liquid cultures were then transferred to 2 × 1 L of fresh media and cultured further for 24 h at 37 °C. Cells were harvested by centrifugation, washed with phosphate buffered saline (PBS) and stored at –20 °C until use.

Preparation of membrane fractions from *M. smegmatis*

The *M. smegmatis* cell pellet (~10 g wet weight) was washed and resuspended in 100 mL of 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (adjusted to pH 7.9 with KOH) containing 5 mM β-mercaptoethanol and 10 mM $MgCl_2$ supplemented with Complete

Protease Inhibitor Cocktail Tablets (Roche) at 4 °C. The cells were subjected to two passes through a French Press cell (Thermo Spectronic) at 20,000 psi. The cell lysate was centrifuged at 600 × *g* for 15 min and then at 27,000 × *g* for 20 min. The resulting supernatant was centrifuged at 100,000 × *g* for 60 min. The supernatant was carefully removed and the membrane pellets were gently resuspended in 1 mL of 50 mM MOPS buffer, pH 7.9, containing 5 mM β-mercaptoethanol and 10 mM $MgCl_2$. Protein concentrations were determined by the BCA™ Protein Assay (Pierce) using bovine serum albumin as the standard.

Radiochemical activity assays

The ManT enzyme activity was determined using the previously established cell-free system.[3] Unless indicated otherwise, the synthetic acceptor analogs at a concentration of 2.0 mM were incubated with 0.20 μCi of guanosine diphosphate mannose, [mannose-2-^{3}H] (American Radiolabeled Chemicals, Inc., 20 Ci/mmol) in 50 mM MOPS buffer, pH 7.9, containing 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.25 mM decaprenol phosphate (in 0.25% CHAPS) (Larodan Fine Chemicals, Sweden) and membrane fraction (92.5 μg of protein) in a total volume of 80 μL. All assays were performed in duplicate and control assays without acceptor were also performed in parallel to correct for the presence of endogenous acceptor.

The enzymatic activities were determined using SepPak radiochemical C_{18} assays.[5] Briefly, after incubation at 37 °C for 1 h, the reactions were stopped by adding 120 μL of $CHCl_3$–MeOH (2:1, v/v) and the mixtures were centrifuged. The supernatants were recovered and further diluted with H_2O before loading onto SepPak C_{18} cartridges (Waters). The unreacted donor was removed by washing the cartridges with H_2O (50 mL) and the radiolabeled products were eluted with MeOH (4.0 mL). The isolated products in the eluants were quantified by liquid scintillation counting on a Beckman LS6500 Scintillation Counter using 10 mL of Ecolite cocktail. For kinetic analysis, the ManT activities were determined using a range of acceptor concentrations (0.03 to 4.0 mM). All other reaction conditions were identical to the cell-free assay as described above. Assays were performed under the conditions in which the formations of radiolabeled products were linear for both time and protein concentration. The kinetic parameters K_M and V_{max} were obtained by nonlinear regression analysis using the Michaelis-Menten equation with the GraphPad Prism 4.0 program (GraphPad Software, San Diego, CA).

Product characterizations from milligram-scale incubations

Large-scale ManT reactions were performed for the structural characterization using acceptor substrates **2.3–2.6**. A typical reaction containing 50 mM MOPS buffer, pH 7.9, 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 2 mM acceptor, 2 mM GDP-mannose, and the *M.*

smegmatis membrane preparation, was incubated at 37 °C with gentle rotation for 5 days. The reaction mixture was loaded directly on the C_{18} reverse-phase cartridge and the unreacted donor was washed away with H_2O (50 mL) and the product was eluted subsequently with MeOH (4 mL). The solvent was evaporated and the residue was redissolved in H_2O (50 μL).

The conversion of the acceptor substrate to the enzymatic product could be visualized by thin layer chromatography (TLC) on SIL G-25 silica gel plates (Macherey-Nagel) after developing with the following solvent systems: A) 17:2:1 EtOAc–MeOH–H_2O gave R_f values of 0.5 and 0.23 for mono- and dimannoside, respectively; B) 17:2:1 EtOAc–MeOH–H_2O, developed three times to give R_f values of 0.38 and 0.13 for the di- and trimannosides, respectively; 7:21 EtOAc–MeOH–H_2O and developed twice to give R_f values of 0.35 and 0.22 for the tetra- and pentamannosides, respectively. Visualization of compounds was achieved by heating the TLC plates after dipping them in a solution of 3% anisaldehyde in sulfuric acid. In addition, the mass of the product was characterized by MALDI mass spectrometry on a Voyager Elite time-of-flight spectrometer on sample suspended in 2,5-dihydroxy benzoic acid, using the delayed-extraction mode and positive-ion detection.

For characterization of products using ^1H NMR spectroscopy, the reaction products were purified by preparative TLC. The area corresponding to the product on the TLC plate was scraped and dissolved

in H_2O. The resulting solution was stirred for 15 min and centrifuged. The solution was filtered through a 0.2 μm nylon membrane filter (Pall Life Sciences) before being applied to the SepPak C_{18} cartridge. After washing the column with H_2O, the product was eluted with MeOH (4 mL). The solvent was evaporated and the product was lyophilized overnight and dissolved in D_2O. One-dimensional ^1H NMR spectra were recorded on a Varian i600 instrument.

Attempts to elucidate the origin of the unexpected apparent acceptor activity of 2.13 and 2.14

An enzymatic reaction (total volume of 80 μL) in the presence of 2.0 mM of either acceptor **2.13** or **2.14** was performed as described previously. After incubation at 37 °C for 1 h, the reaction was extracted with organic solvent and the aqueous layer was loaded on a prewashed C_{18} cartridge as usual. The radiolabeled product was eluted with 4 mL MeOH. A 1.5 mL portion of the eluant was quantified by liquid scintillation counting. Another 1.5 mL portion of the eluant was concentrated by evaporating the solvent. To set up the mannosidase digestion, the radiolabeled residue was redissolved in 5.0 μL 1 x buffer (Glyko) and 2.5 μL of *Aspergillus saitoi* α-(1→2)-mannosidase (40 mU/mL) was added. The reaction was incubated at 37 °C for 19 h. At this point, another 2.5 μL of fresh enzyme was added and the reaction was further incubated for an additional 19 h. The reaction was then diluted with water and loaded on a prewashed C_{18} column for

purification. The eluant (4 mL MeOH) was quantified by liquid scintillation counting.

3.5. Bibliography

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Chapter 4

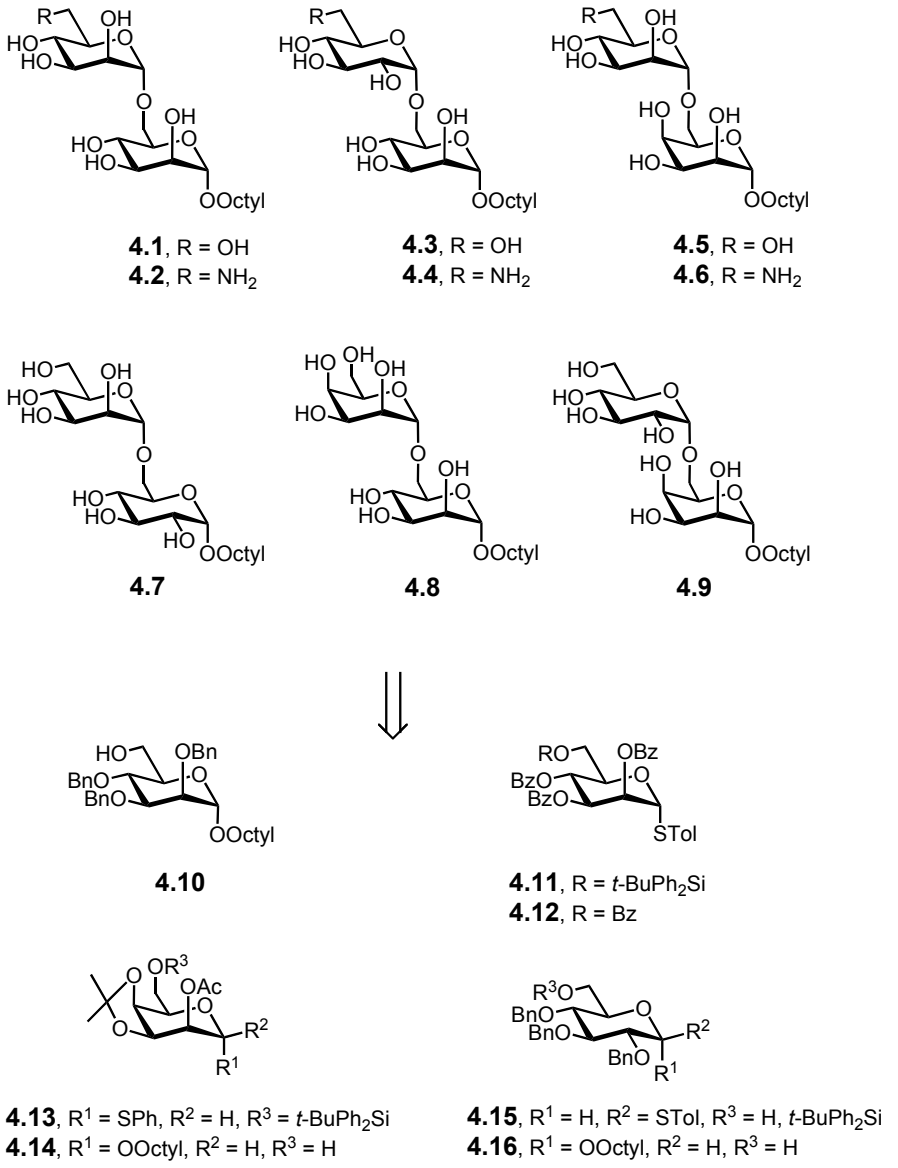
Synthesis and Evaluation of Epimeric and Amino Disaccharide Analogs as Potential Substrates and Inhibitors for α-(1→6)-Mannosyltransferase

4.1. Introduction

In the previous chapters, we reported the preparation and biochemical evaluation of a panel of monomethoxy and monodeoxy analogs of disaccharide acceptor **4.1** (Scheme 4-1) as substrates for ManT. Among the important findings were that the enzyme appears to form critical hydrogen bonding interactions with a number of hydroxyl groups on the substrate as deoxygenation leads, in all but two cases (C-2′ and C-4), to essentially a total loss of activity. To extend our understanding of the substrate specificity of ManT further, we synthesized and screened five epimeric (**4.3**, **4.5**, **4.7**–**4.9**) and three amino (**4.2**, **4.4** and **4.6**) analogs of **4.1** (Scheme 4-1).

The importance of the epimers was to determine if the *manno*-configuration was absolutely required by the enzyme, or if disaccharides with rings in the *gluco*- (**4.3**, **4.7**, **4.9**) or *talo*- (**4.5**, **4.8**, **4.9**) configuration were also recognized as substrates. The results obtained from these analogs would provide additional insight into the important steric and hydrogen bonding interactions in the active site. In addition, the amino analogs were synthesized and evaluated as potential inhibitors. We envisioned that the amino groups of **4.2**, **4.4** and **4.6** would be protonated

at physiological pH, and the resulting ammonium derivatives might serve as inhibitors via an ionic interaction with a negatively charged residue in the enzyme active site, resulting in the formation of a tight enzyme–inhibitor complex.[1] In this chapter, we report the synthesis of these modified disaccharide analogs and their subsequent biochemical evaluation with ManT.



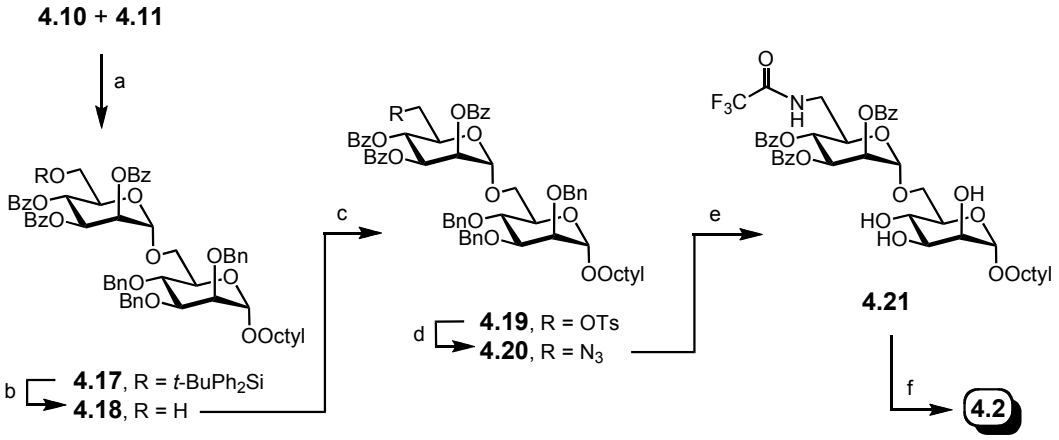
Scheme 4-1. Target synthetic analogs and the required building blocks.

4.2. Results and Discussion

4.2.1. Synthesis of Disaccharide Analogs

As shown in Scheme 4-1, octyl disaccharides **4.2–4.9** were assembled from the precursors **4.10–4.16**. It has been shown that the octyl agylcone is an efficient substrate of ManT and allows convenient product isolation and characterization in cell-free enzymatic assays using reverse phase chromatography.[2,3]

As illustrated in Scheme 4.2, amino derivative **4.2** was prepared from thioglycoside **4.11**[4] and alcohol **4.10**[5] using NIS–TMSOTf activation,[6] which afforded the corresponding disaccharide **4.17** in 85% yield. For all glycosylation reactions involving mannopyranoside and talopyranoside donors described in this chapter, the α-stereochemistry of the glycosidic linkages was confirmed by measurement of the one-bond heteronuclear



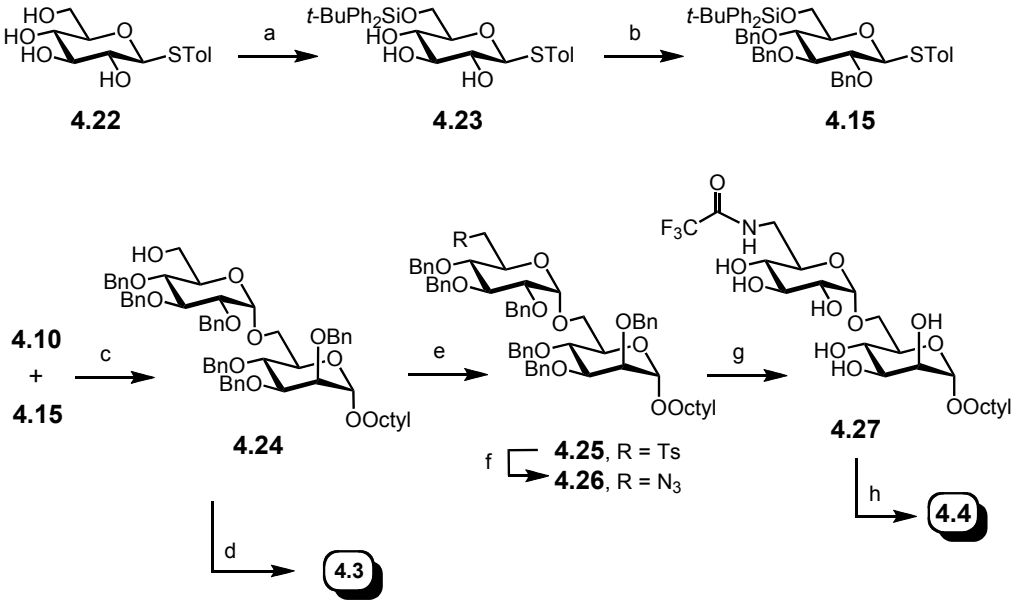
Scheme 4-2. Reagents and conditions: a) NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0 °C, 85%; b) HF·pyridine–pyridine–THF (1:2:20), 66%; c) TsCl, pyridine, 91%; d) NaN_3, DMF, reflux, 98%; e) i: $Pd(OH)_2$–C, pyridine, H_2; ii: $(CF_3CO)_2O$, pyridine, 0 ^oC to rt.; iii: $Pd(OH)_2$–C, MeOH, H_2, 68% over three steps; f) NaOMe, MeOH, 61%.

coupling constant for the anomeric carbon atom ($^1J_{C\text{-}1,H\text{-}1}$). In all cases, this value was between 167 and 176 Hz, clearly indicating the α-stereochemistry.[7]

The next step in the synthesis was the removal of the silyl group. To avoid possible acyl migration and debenzoylation using the usual method, treatment with tetra-*n*-butylammonium fluoride, intermediate **4.17** was desilyated using hydrogen fluoride in pyridine to give **4.18** in 66% yield.[8] The resulting primary alcohol in **4.18** was then tosylated using tosyl chloride in pyridine (91%). Treatment of **4.19** with sodium azide afforded the azido-disaccharide **4.20** in 98% yield. That the substitution had occurred was confirmed from the ^{1}H NMR spectrum of **4.20**, in which the C-6′ protons were in the region of 3.38–3.48 ppm, as would be expected for hydrogens adjacent to an azido functionality. In addition, in the ^{13}C NMR spectrum, a resonance at 51.2 ppm could be assigned to C-6′.

Similar to previous observations from the group,[9] reduction of the azide and deprotection of the benzyl groups in a single reaction using hydrogenation over a palladium catalyst resulted in failure. Thin layer chromatographic analysis of the reaction mixture showed several spots, presumably a series of partially-debenzylated derivtives of **4.20**. No further reduction was observed, even after prolonged reaction times. Instead, we adopted our previous published procedure in which the azido group of **4.20** was reduced to the amine by hydrogenation over $Pd(OH)_2$–C in pyridine, and the product was immediately converted to its *N*-

trifluoroacetamide using trifluoroacetic anhydride in pyridine.[9] Subsequent debenzylation using $Pd(OH)_2$–C catalyst was achieved without incident and provided triol **4.21** in 68% yield over three steps. Final deprotection of **4.21** furnished a 61% yield of the desired amino derivative **4.2**.



Scheme 4-3. Reagents and conditions: a) *t*-$BuPh_2SiCl$, imidazole, DMF, 45 °C; b) NaH, BnBr, DMF, 95%; c) i: NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0 °C, 5:1 of α:β isomers; ii: HF·pyridine–pyridine–THF (1:2:20), 50%; d) $Pd(OH)_2$–C, MeOH, H_2, 82%; e) TosCl, pyridine, 89%; f) NaN_3, DMF, reflux, 85%; g) i: $Pd(OH)_2$–C, pyridine, H_2; ii: $(CF_3CO)_2O$, pyridine, 0 °C to rt.; iii: $Pd(OH)_2$–C, MeOH, H_2, 72% over three steps; h) NaOMe, MeOH, 79%.

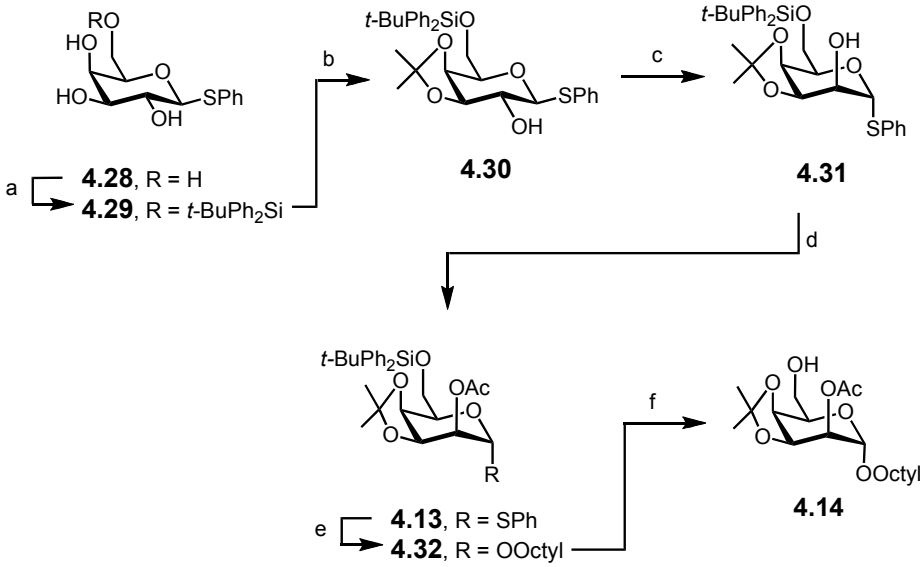
As illustrated in Scheme 4-3, thioglycoside **4.15**, the donor required for the synthesis of disaccharides **4.3** and **4.4**, was obtained from the known thioglucoside **4.22**.[10] Reaction of **4.22** with *tert*-butylchlorodiphenylsilane and imidazole provided silyl ether intermediate

4.23. Without purification, the remaining hydroxyl groups of **4.23** were protected as benzyl ethers by reaction with sodium hydride and benzyl bromide to afford **4.15** in 95% yield over the two steps. The coupling of **4.15** with known acceptor **4.10**,[5] under NIS–TMSOTf activation, afforded the inseparable diastereoisomers (~5:1 α:β ratio). Subsequent desilylation using hydrogen fluoride followed by column purification gave the α isomer **4.24** in 50% yield over two steps. Final deprotection of the benzyl ethers afforded analog **4.3** in 82% yield.

The amino derivative **4.4** was synthesized via a route analogous to **4.2**. Thus, the primary OH group in disaccharide **4.24** was tosylated to provide **4.25** (89%). Reaction with sodium azide under reflux gave azidosugar **4.26** in 85% yield. As was seen for compound **4.20**, the primary azido functionality in **4.26** was evident in the ^{13}C NMR spectrum, which showed a resonance at 51.2 ppm. The azido group of **4.26** was then converted to the corresponding *N*-trifluoroacetamide derivative in two steps (azide reduction and trifluoroacetylation of the product amine). Without purification, the crude intermediate was hydrogenated to provide **4.27** in 72% yield over three steps. Final deprotection by treatment with sodium methoxide furnished a 79% yield of the target analog **4.4**.

The glycosyl acceptor needed for the synthesis of **4.5** and **4.6**, octyl taloside **4.14**, was synthesized from a known thioglycoside **4.28**[11] as illustrated in Scheme 4-4. Reaction of **4.28** with *tert*-butylchlorodiphenylsilane and imidazole provided silyl ether **4.29** in 94%

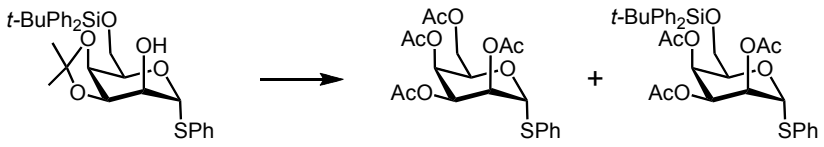
yield. Reaction of silyl ether **4.29** with 2,2-dimethoxypropane in the presence of a catalytic amount of *p*-TsOH gave a quantitative yield of alcohol **4.30**.



Scheme 4-4. Reagents and conditions: a) *t*-$BuPh_2SiCl$, imidazole, DMF, 45 °C, 94%; b) dimethoxypropane, *p*TsOH, acetone, quant.; c) i: oxalyl chloride, DMSO, –78 °C; then alcohol **4.30**, warm to –60 °C; then Et_3N, warm to rt.; ii: $NaBH_4$, MeOH, 62%; d) Ac_2O, CH_2Cl_2, pyridine (1:1), 89%; e) octanol, NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0 °C, 89%; f) TBAF, THF, 74%.

Oxidation of the C-2 hydroxyl group under Swern conditions and subsequent reduction with sodium borohydride gave an anomeric mixture of thioglycoside **4.31** (62% of a 6:1 α/β mixture of isomers). Notably, in the ^1H NMR spectrum of the major isomer, the H-1 proton was shifted to 5.33 ppm, which in good agreement with the α-configuration at the anomeric

center. Presumably, the basic conditions of the oxidation reaction resulted in the anomerization of the ketone product. However, the $J_{1,2}$ (7.4 Hz) of the product obtained after borohydride reduction did not allow us to distinguish between the *galacto*- and *talo*-configuration. The larger coupling constant value is probably due to the distortion of the chair conformation of the pyranose ring by the isopropylidene protecting group. Therefore, to confirm the stereochemistry at C-2, **4.31** was hydrolyzed with 80% aqueous AcOH and subsequently reacted with acetic anhydride in the presence of pyridine and DMAP to afford **4.33** and **4.34** (Scheme 4-5). The $J_{1,2}$ and $J_{2,3}$ (1.3 and 3.7 Hz) of the resulting derivative **4.33** are consistent with the *talo*-configuration, in turn indicating that **4.31** also possesses the *talo*- stereochemistry.



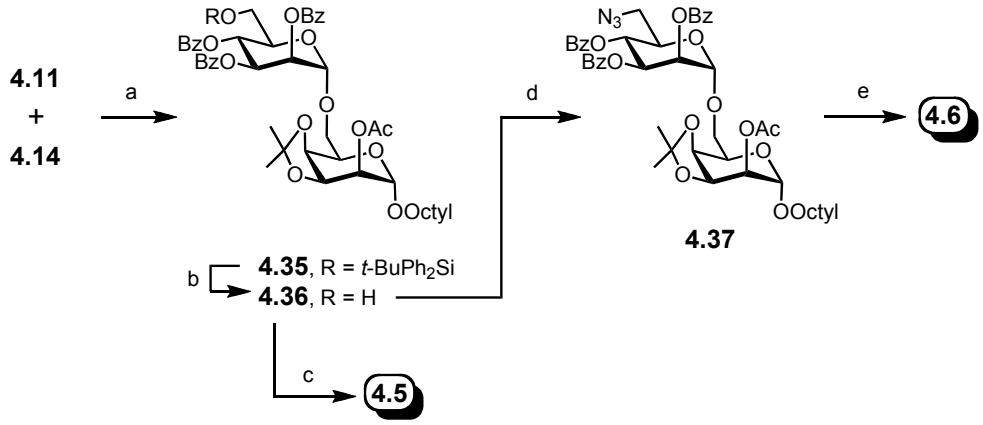
4.31 **4.33** **4.34**

Scheme 4-5. Reagents and conditions: i: 80% aq. AcOH, 50 °C; ii: Ac_2O, DMAP, CH_2Cl_2, pyridine, 65% for **4.33** and 26% for **4.34**.

Having determined that the oxidation–reduction sequence had provided the correct product, **4.31** was treated (Scheme 4-4) with acetic anhydride thus providing an 89% yield of **4.13**. The C-2 acetyl group in **4.13**, due to its ability to participate, provided high α-stereoselectivity in the subsequent glycosylation to give octyl talopyranoside **4.32** in 89% yield.

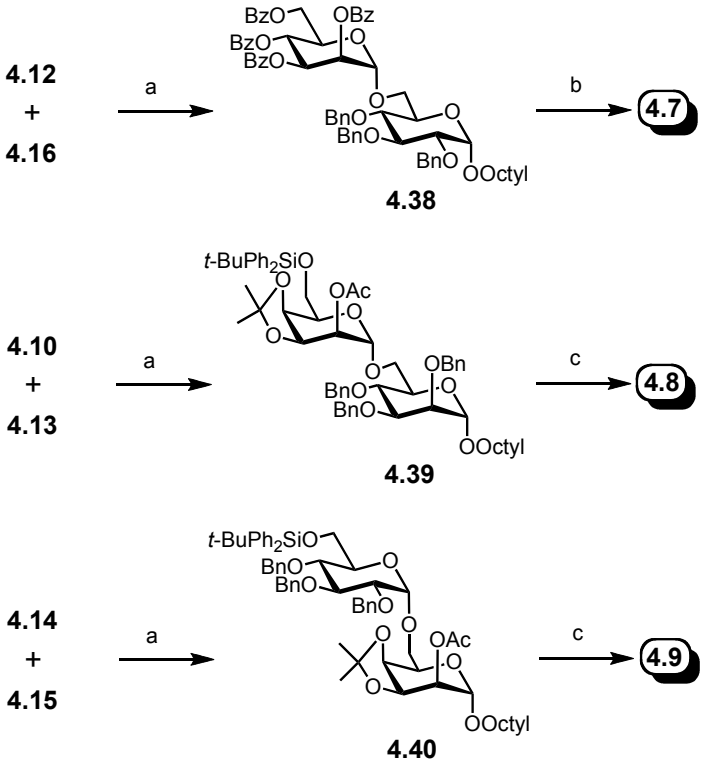
Finally, treatment of **4.32** with tetra-*n*-butylammonium fluoride provided a 74% yield of the expected alcohol **4.14**.

With talopyranosyl acceptor **4.14** in hand, coupling with thioglycoside **4.11** under NIS–TMSOTf activation afforded the corresponding protected disaccharide **4.35** in 90% yield (Scheme 4-6). Deprotection at C-6 proceeded under standard conditions to give a 77% yield of **4.36**. Cleavage of the isopropylidene acetal and treatment with sodium methoxide in methanol gave **4.5** in 91% overall yield. To prepare amino analog **4.6**, alcohol **4.36** was converted to azido disaccharide **4.37** via the two step sequence (91 and 84% yields, respectively) as described for the preparation of **4.20**. Reduction of azide **4.37** upon removal of the isopropylidene acetal and acyl protecting groups provided aminosugar **4.6** in 86% yield.



Scheme 4-6. Reagents and conditions: a) NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0 °C, 90%; b) HF·pyridine–pyridine–THF (1:2:20), 77%; c) i: 80% aq. AcOH, 50 °C; ii: NaOMe, MeOH, 91%; d) i: TosCl, pyridine, 91%; ii: NaN_3, DMF, reflux, 84%; e) i: 80% aq. AcOH, 50 °C; ii: NaOMe, MeOH; iii: $Pd(OH)_2$–C, MeOH, H_2, 86% over three steps.

The preparation of disaccharides **4.7**–**4.9** is illustrated in Scheme 4-7. Glycosylation of alcohols (**4.10**, **4.14**, **4.16**[12]) with thioglycosides (**4.12**[13], **4.13**, **4.15**) upon activation with NIS–TMSOTf, gave the corresponding disaccharides **4.38**, **4.39**, and **4.40** in good to excellent yields (92%, 81% and 88%, respectively). Debenzoylation and hydrogenolysis gave desired compound **4.7** (84% yield). To obtain **4.8** and **4.9**, disaccharides **4.39** and **4.40** were subjected to a four-step sequence (desilylation, deacetylation, acetal cleavage and hydrogenolysis) to give the expected products in overall yields of 45% and 62%, respectively.



Scheme 4-7. Reagents and conditions: a) NIS, TMSOTf, 4Å MS, CH_2Cl_2, 0 °C, 92% for **4.38**, 81% for **4.39**, 88% for **4.40** (5:1 of α:β isomers); b) i: NaOMe, MeOH; ii: $Pd(OH)_2$–C, MeOH, H_2, 84%; c) i: TBAF, THF; ii: NaOMe, MeOH; iii: 80% aq. AcOH, 50 °C; iv: $Pd(OH)_2$–C, MeOH, H_2, 45% for **4.8**; 62% for **4.9**.

4.2.2. Screening Analogs As Substrates And Inhibitors

Once target analogs **4.2**–**4.9** were synthesized, they were screened as potential substrates and inhibitors for the PPM-dependent α-(1→6)-ManT, which was partially purified from *M. smegmatis*.[2,14] Initially, each analog, at 2.0 mM concentration, was incubated with ^{3}H-labeled GDP-mannose **3.1** (Figure 3-1, Chapter 3), as the indirect donor, and membrane extracts. After incubation for one hour, the radiolabeled products were recovered by solvent extraction and reverse phase chromatography, and the radioactivity was measured by scintillation counting.

The ability of **4.2**–**4.9** to act as acceptor substrates for ManT were compared with the parent compound **4.1** and the results are summarized in Table 4-1. The initial screening revealed that analogs **4.3** (α-Glc*p*-(1→6)-α-Man*p*) and **4.5** (α-Man*p*-(1→6)-α-Tal*p*) are moderate substrates of ManT with 41% and 57% activity, relative to the rate of mannosylation using **4.1** (α-Man*p*-(1→6)-α-Man*p*). Our previous data suggested that the hydroxyl group at C-2′ and C-4 of **4.1** were not crucial for substrate–enzyme interaction, as the replacement with hydrogen had no dramatic effect on ManT catalysis (Chapter 3). However, it appears that epimerization at either position, leading to analog **4.3** or **4.5**, significantly affects the efficiency to serve as a ManT substrate. This finding is further indicated by the apparent kinetic parameters shown in Table 4-1. These acceptor analogs, have K_M values of 1.8 mM (**4.3**) and 1.2 mM (**4.5**),

which are 20- and 13-fold larger, respectively, than that of **4.1** (0.09 mM), The V_{max} values of **4.3** or **4.5** are comparable to that of the parent compound.

Table 4-1. Summary of ManT activities using analogs **4.1**–**4.9**

Analog	Relative activity [a] (%)	K_M [b] (mM)	V_{max} [b] (pmol/mg/min)	Inhibition [c] (%)	Mass of oligosaccharide product [d]
4.1	100	0.09 ± 0.01	0.37 ± 0.005	—	639.2, 801.3
4.2	4	— [e]	—	79	—
4.3	41	1.80 ± 0.71	0.34 ± 0.071	—	639.1, 801.1
4.4	0	—	—	91	—
4.5	57	1.20 ± 0.24	0.40 ± 0.031	—	639.2, 801.2
4.6	<1	—	—	70	—
4.7	10	3.04 ± 0.69	0.10 ± 0.014	—	639.3, 801.3, 963.3
4.8	<1	—	—	—	—
4.9	10	2.27 ± 0.12	0.11 ± 0.003	—	639.3, 801.3

[a] Relative activities were measured at 2.0 mM acceptor concentration with 0.2 μCi of [^{3}H] GDP-Man and are expressed with respect to disaccharide **4.1**. 100% activity corresponds to 29.8 pmol mg^{-1} hr^{-1}.
[b] Kinetic parameters were determined using a range of acceptor concentrations by nonlinear regression analysis of the Michaelis–Menten equation with the GraphPad Prism 4.0 program.
[c] Compounds were screened at a concentration of 2.0 mM with **4.1** as the substrate at 0.2 mM.
[d] The enzymatic products were isolated from large-scale incubations and their masses were determined by MALDI mass spectrometry. The found values correspond to the sodium adducts, which were in good agreement with the calculated values.
[e] Not determined.

The lower relative activity of **4.3** and **4.5** compared to **4.1** appears to be due to the steric requirements of the ManT. For both compounds, the

hydroxyl group is placed in the opposite orientation (changed from axial to equatorial in **4.3**, or *vice versa* in **4.5**). These changes may lead to potentially negative steric interactions with amino acid residues in the active site pocket thus resulting in an unfavorable enzyme–substrate interaction and a therefore a higher K_m. These observations suggest that the enzyme preferentially recognizes disaccharides in which both residues are in the *manno*-configuration, but will also accept analogs of different stereochemical configurations, but with lower affinity.

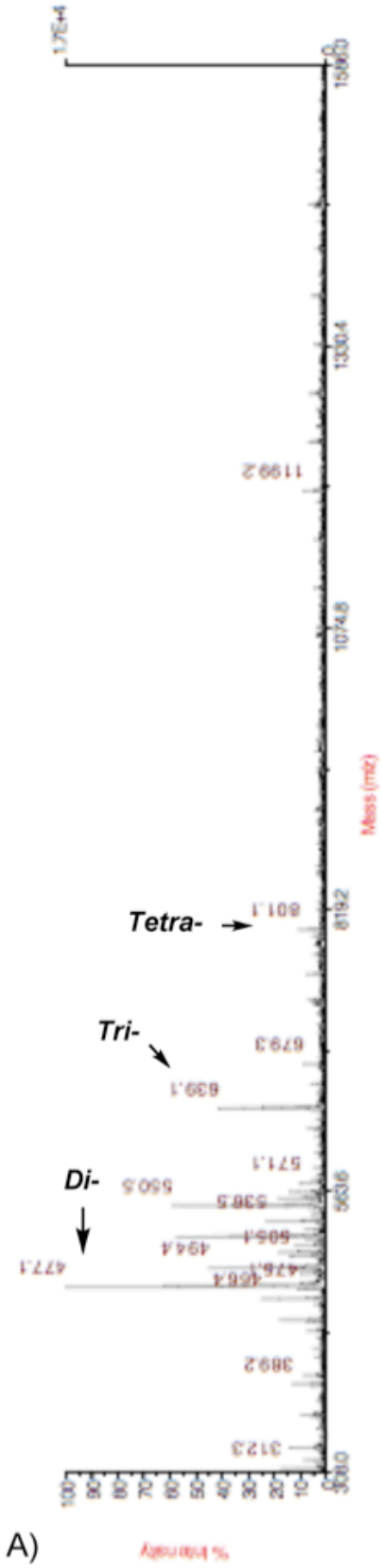
This finding is further supported by the results obtained from analogs **4.7**–**4.9**, in which α-Tal*p*-(1→6)-α-Man*p* analog **4.8** is not a substrate, and both the α-Man*p*-(1→6)-α-Glc*p* **4.7** and α-Glc*p*-(1→6)-α-Tal*p* analogs **4.9** are poor substrates with only 10% relative activity compared to **4.1**. Consistent with the results for **4.3** and **4.5**, subsequent kinetic analysis provided larger apparent K_M values for **4.7** and **4.9**, 3.04 mM and 2.27 mM, respectively. However, the V_{max} values for these substrates was only marginally less than the native substrate, **4.1**. The poor substrate activity of **4.7** and the lack of activity of **4.8**, is consistent with our previous work, which indicated that the C-4′ and C-2 hydroxyl groups are essential for ManT catalysis. In **4.3** and **4.5**, the orientations of the C-4′ and C-2 hydroxyl groups were the same as in the parent compound, while the stereochemistry at one other centre was inverted (C-2′ in **4.3** and C-4 in **4.5**), and these compounds were weak substrates for the enzyme. However, evaluation of an analog in which the

stereochemistry at both C-2′ and C-4 were inverted, the α-Glc*p*-(1→6)-α-Tal*p* isomer **4.9**, showed even weaker ManT substrate activity.

In summary, the change of the configuration of the disaccharide motif, regardless of whether the change was at the reducing or non-reducing end, did not enhance but rather reduced the ManT activity. This finding indicates that the enzyme requires a *manno*- configuration of both rings for optimal activity. These results thus elaborate our previous findings, which demonstrated that the hydroxyl groups on the dimannoside motif are not only important for hydrogen bonding interactions but their stereochemistries are also crucial for ManT catalysis.

4.2.3. Characterization of Enzymatic Products by Mass Spectrometry

In addition to the radiochemical assays, milligram-scale enzymatic incubations of **4.1**, **4.3**, **4.5**, **4.7** and **4.9** with unlabelled GDP-Man and the membrane fraction were carried out to determine the structure of the oligosaccharide products that were produced from these analogs. After the incubations, the enzymatic products were purified as described in the Experimental Section (4.4) and analyzed by MALDI mass spectrometry. As shown in Figure 4-1, incubations of ManT with these analogs all resulted in the formation of the corresponding trisaccharides and tetrasaccharides as the major and minor products, respectively. Contrary to the proposed processive nature of this enzyme,[14] a homologous series


Tetra-
Tri-
Di-
Mass (m/z)
Abundance %

A)


Tetra-
Tri-
Di-
Mass (m/z)
Abundance %

B)

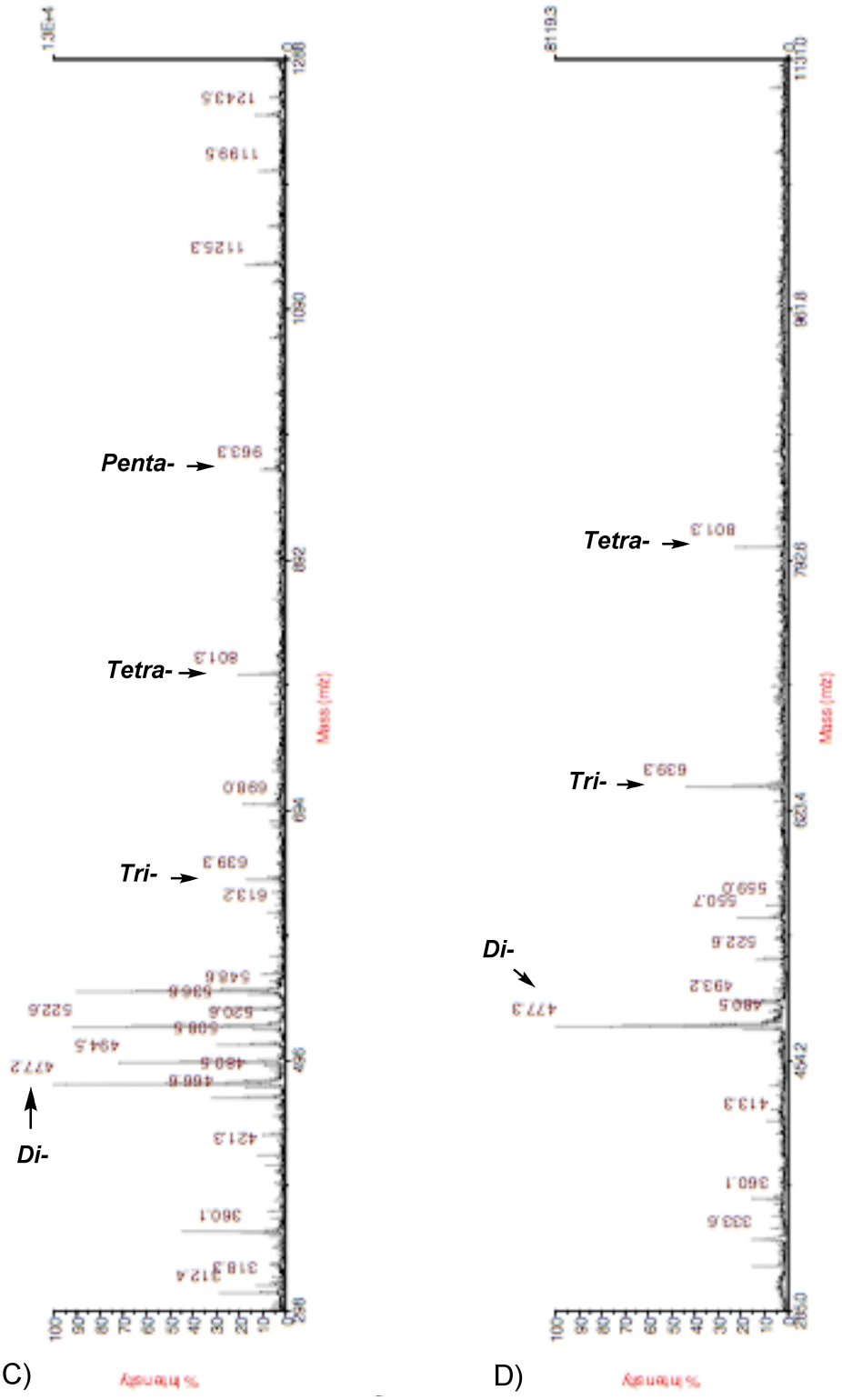


Figure 4-1. MALDI mass spectra of enzymatic products isolated from incubation mixtures using analogs **4.3** (A), **4.5** (B), **4.7** (C), and **4.9** (D) at 2 mM concentrations.

of products were not observed. The absence of these larger oligosaccharides is probably due to much lower acceptor concentrations of the resulting tri- and tetrasaccharide products, resulting in slower rates of the subsequent elongation reactions. It is also possible that the larger enzymatic products (e.g., tetra- and pentasaccharide) are degraded by the α-(1→6)-*endo*-mannosidase (Chapter 3).

4.2.4. Characterization of Enzymatic Products by Glycosidase Digestion

In addition to the PPM-dependent α-(1→6)-mannosyltransferases, the crude membrane extract of *M. smegmatis* used in these assays also contains α-(1→2)-ManT's involved in LM branching, the capping of the arabinan domain, and the biosynthesis of AcPim6.[14-17] In addition, the α-(1→4)-ManT involved in the biosynthesis of 3-*O*-methyl-mannose polysaccharides was reported previously by Ballou.[18,19] To confirm that the observed addition of radiolabeled mannose to **4.3**, **4.5**, **4.7** and **4.9** arose from α-(1→6)-ManT activity, the radiochemical assays were repeated on a larger scale with ^{3}H-labelled GDP-Man. After the reaction, the [^{3}H]Man-labeled enzymatic products were purified, divided evenly and subjected to enzymatic digestions.

As illustrated in Figure 4-2, there is no significant difference of the radioactivities between the control experiments and the samples that have undergone treatment with the α-Man-(1→2)-α-Man-specific *Aspergillus*

saitoi α-(1→2)-mannosidase (AS). These results demonstrate that none of the mannosylated products contained an α-Man-(1→2)-α-Man linkage. On the other hand, digestions of the radiolabeled products using α-mannosidases from jack bean (JB, α-(1→2,3,6)-specific) and *Xanthomonas manihotis* (XM, unbranched α-(1→6)-specific) removed essentially all the [^{3}H]-labeled mannose units. The combination of these mannosidases enabled the relative proportions of the α-(1→6)-and α-(1→3/4)-linkages to be determined. These results further suggested that the observed enzymatic activities in all cases, except analog **4.3**, resulted exclusively from an action by the α-(1→6)-specific ManT in the cell free assays.

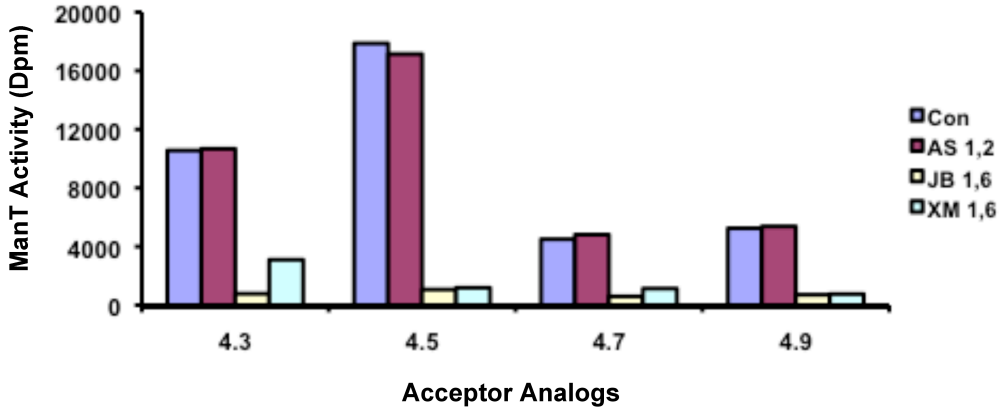


Figure 4-2. Mannosidase digestion of products formed from **4.3**, **4.5**, **4.7** and **4.9.** Each acceptor at 2 mM was incubated with [^{3}H] GDP-Man under the assay conditions as described in the experimental section. The radiolabeled enzymatic products were divided evenly after purification and treated with *exo*-mannosidases including *Aspergillus saitoi* α-(1→2)-mannosidase (AS), jack bean α-(1→2,3,6)-mannosidase (JB) and *Xanthomonas manihotis* α-(1→6)-mannosidase (XM). The mannosidase-digested samples were purified

using C_{18} reverse phase column and the radioactivities were compared with the controls (without any mannosidase treatment).

In the case of **4.3**, the small difference of the residual radioactivities between the initial product and that treated with mannosidases from jack bean and *X. manihotis* suggest that a small amount of the enzymatic products from **4.3** might contain α-(1→3/4)-linkages. Although the α-(1→4)-ManT involved in the biosynthesis of 3-*O*-methyl-mannose polysaccharides (MMPs), has been found in membrane extracts from *M. smegmatis* and might catalyze the observed α-(1→4)-mannosylation,[18,19] we consider this as unlikely because this particular enzyme requires larger oligosaccharides such as tetramannosides to be active as substrate acceptors. In fact, no formation of a pentasaccharide product was observed in the mass spectrum of the enzymatic products from the incubation of **4.3** (Figure 4-1A).

In addition to the α-(1→4)-ManT involved in MMP biosynthesis, Besra and co-workers have recently demonstrated that *M. tuberculosis* MgtA is able to utilize Glc*p*AGroAc_2 (1,2-di-*O*-C_{16}/$C_{18:1}$-α-D-glucopyranosyluronic acid-(1→3)-glycerol as a substrate to form Man*p*-α-(1→4)-Glc*p*AGroAc_2 (Figure 4-3).[20]

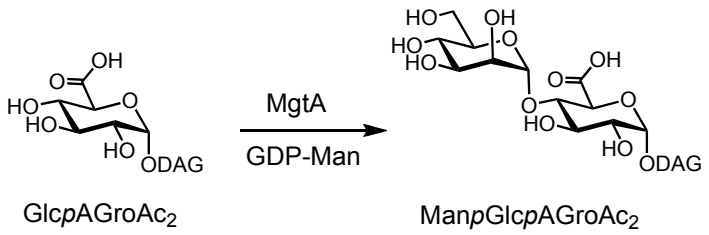


Figure 4-3. Reaction catalyzed by MgtA. DAG = diacylglycerol.

Perhaps the relaxed substrate specificity of this enzyme, which allows it to recognize both PIM1 and GlcAGroAc$_2$, also allows MgtA to recognize analog **4.3** as a substrate acceptor. With regard to this proposal, it is important to note that the non-reducing residue in **4.3**, is Glc*p* and this may be a reasonable substrate for this newly discovered mannosyltransferase activity. It is also important to consider that an MgtA-like enzymatic activity has not, to date, been identified in *M. smegmatis*, the source of the membrane fraction used in these investigations. Although disaccharide **4.9** also carries a Glc*p* residue at its non-reducing terminus, no α-(1→3/4)-linkage was detected from the *exo*-mannosidase treatments. Unfortunately, we were unable to carry out any further investigation due to the small turnover of **4.3**, and the negligible amount of the observed α-(1→3/4)-linked products obtained in the assay. Also, the absence of a pure α-(1→6)-ManT, or other mannosyltransferases in our laboratory makes it difficult to address this question.

4.2.5. Inhibition Effects of Amino Analogs

As expected, analogs **4.2**, **4.4** and **4.6**, which lack a hydroxyl group at C-6′, are not substrates of ManT; all compounds show relative activities below 5% (Table 4-1). These amino derivatives were next tested as inhibitors against ManT using **4.1** as the substrate. In these studies, analogs **4.2**, **4.4** and **4.6** were screened at a concentration of 2.0 mM with the parent compound **4.1** at 0.2 mM. As shown in Table 4-1, disaccharides **4.2**, **4.4** and **4.6** inhibit the mannosylation of **4.1** by 79, 91 and 70%, respectively. These results are consistent with the hypothesis that the resulting ammonium derivatives of **4.2**, **4.4** and **4.6** can interact with a negatively charged residue in the enzyme active site (e.g., an aspartate or glutamate) via an ionic interaction and lead to the observed inhibition. It should be pointed out, however, that these are very weak to modest inhibitors and not comparable to other aminosugar-containing acceptor analogs, which have been demonstrated to be very potent glycosyltransferase inhibitors.[21]

To examine if the inhibition was proportional to an increase in inhibitor concentration, we studied the mannosylation of **4.1** (fixed concentration of 0.2 mM) by ManT in the presence of these amino analogs at concentrations up to 4 mM. In all cases, we observed a 50% inhibition at about 1 mM under these conditions, as shown in Figure 4-4.

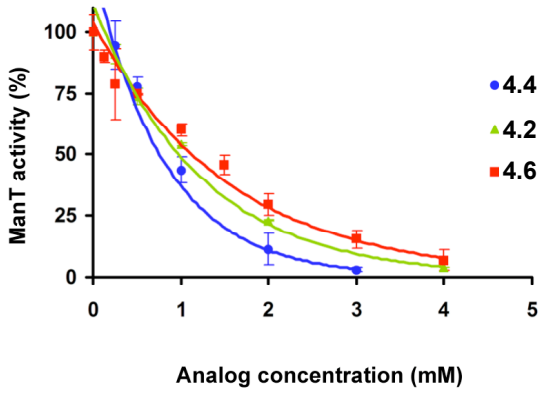


Figure 4-4. Inhibition effects of amino analogs **4.2**, **4.4** and **4.6** on ManT activity. The activities were determined using aminosugar analog concentrations up to 4.0 mM with **4.1** as the acceptor substrate at 0.2 mM. All other reaction conditions were identical to the cell-free assay as described in the experimental section.

Based on the data shown in Table 4.1, the substrate preference for ManT, based on the relative activity of the epimeric substrate analogs, seems to follow the order of α-Man*p*-(1→6)-α-Man*p* **4.1** (100%) > α-Man*p*-(1→6)-α-Tal*p* **4.5** (57%) > α-Glc*p*-(1→6)-α-Man*p* **4.3** (41%). We were therefore surprised to see that the 6-amino-α-Glc*p*-(1→6)-α-Man*p* analog **4.4**, rather than 6-amino-α-Man*p*-(1→6)- α-Man*p* analog **4.2** is a relatively better inhibitor. One explanation for this result is that these amino analogs may not be competing with **4.1** in the active site, but rather act in a noncompetitive or uncompetitive fashion. However, it is difficult to investigate the mode of inhibition due to the presence of the other aforementioned glycosyltransferases (e.g., α-(1→2)-ManT) in the cell extract. These enzymes might potentially interact with the amino derivatives and complicate the interpretation of the experimental data.

Moreover, given that these analogs are at best modest inhibitors of α-(1→6)-mannosylation, we chose not to probe further the mechanism by which they prevent glycosylation

4.3. Conclusions

In this chapter we have described the synthesis of a panel of epimeric and amino analogs of disaccharide **4.1** and their evaluation as potential substrates or inhibitors of a PPM-dependent α-(1→6)-mannosyltransferase involved in LAM/LM biosynthesis. The results presented here, in agreement with our previous findings that the hydroxyl groups at C-4′ and C-2 positions in **4.1**, are important for both the polar interactions and steric requirements. In particular, a change of the *manno*-configuration of the parent substrate **4.1,** at either the reducing or non-reducing end, to the *gluco*- or *talo*-configuration, results in poor interaction with ManT, with the most pronounced loss of activity being observed in a compound in which the stereochemistry at C-4′ and C-2 are both inverted. Analogs **4.2, 4.4** and **4.6**, in which the OH group at C-6′ has been substituted with an amino group, are weak inhibitors of ManT. The observed inhibition might result from an ionic interaction of the protonated amino groups of **4.2, 4.4** and **4.6** with a negatively charged residue in the enzyme active site, and lead to the formation of a competitive enzyme–inhibitor complex. However, the substrate specificity trends observed in the epimeric substrates are different from those seen with the aminosugar

analogs suggesting that the mode of inhibition may not be competitive. Regardless of the mode of action, the α-Man*p*-(1→6)-α-Man*p* (**4.2**), α-Glc*p*-(1→6)-α-Man*p* (**4.4**), and α-Man*p*-(1→6)-α-Tal*p* (**4.6**) analogs all show 50% inhibition at a concentration of ~1 mM.

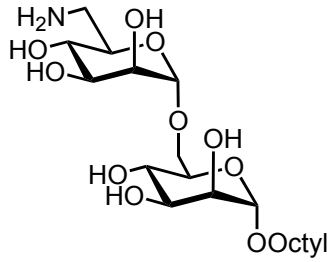
4.4. Experimental Section

General methods for chemical synthesis

All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless indicated otherwise, all reactions were performed at room temperature (rt) and under a positive pressure of argon. The reactions were monitored by analytical TLC on silica gel 60-F_{254} (0.25 mm, Silicycle) and spots were detected under UV light or by charring with acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure at <40 °C. Products were purified by chromatography using silica gel (40–60 μM), Iatrobeads (Iatron Laboratories, Tokyo) or SepPak C_{18} reverse phase cartridges (Waters). Before use, the SepPak cartridges were prewashed with 10 mL of MeOH followed by 10 mL of H_2O and the desired products were eluted with 4 mL of MeOH. Optical rotations were measured at 22 ± 2 °C and are in units of degrees·mL/(g·dm). ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$), or

HOD (4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz and chemical shifts are referenced to internal $CDCl_3$ (77.23, $CDCl_3$), or CD_3OD (48.9, CD_3OD). Assignments of NMR spectra were made based on two-dimensional (^1H–^1H COSY and HMQC) experiments. The stereochemistry at the anomeric centres of the mannopyranose and talopyranose rings were proven by measuring the $^1J_{C1\text{-}H1}$.[7] ^{19}F spectra were recorded at 376 MHz, and chemical shifts were referenced to external $CFCl_3$. Samples were prepared by the cast film method and infrared spectra were measured on a FT-IR spectrometer. Electrospray mass spectra were recorded on samples suspended in mixtures of THF with MeOH and added NaCl.

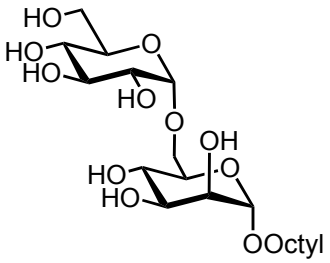
Octyl 6-amino-6-deoxy-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (4.2)



Disaccharide **4.21** (17 mg, 0.020 mmol) was dissolved in MeOH (4 mL) and 1 M NaOMe (1 mL) was added. After stirring overnight, the solution was neutralized with Amberlite 120 resin (H^+ form), filtered and concentrated. The crude was dissolved in satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (5 mL). The aqueous layer was purified by C_{18} reverse phase column to give **4.21** (6 mg, 61%), after lyophilization, as white solid; $[\alpha]_D$ = +75.0 (*c* 0.3, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ_H 4.78 (d, 1H, *J* = 1.7 Hz, H-1′), 4.69 (d, 1H, *J* = 1.7 Hz, H-

1), 3.87–3.93 (m, 1H, H-5′), 3.83 (dd, 1H, *J* = 3.5, 1.7 Hz, H-2′), 3.76 (dd, 1H, *J* = 2.9, 1.7 Hz, H-2), 3.58–3.74 (m, 6H, H-3′, H-4, H-5, H-6a, H-6b, octyl OC*H*$_2$), 3.57 (dd, 1H, *J* = 7.2, 2.9 Hz, H-3), 3.50 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.39 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 2.97 (dd, 1H, *J* = 13.4, 2.7 Hz, H-6a′), 2.74 (dd, 1H, *J* = 13.4, 7.2 Hz, H-6b′), 1.52–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.22–1.44 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD$_3$OD) δ_C 101.7 (C-1′), 101.4 (C-1), 73.1 (2C, C-5′, C-5), 72.9 (C-3), 72.5 (C-3′), 72.2 (C-2′/C-2), 72.1 (C-2′/C-2), 70.1 (C-4′), 68.7 (octyl O*C*H$_2$), 68.6 (C-4), 67.4 (C-6), 43.2 (C-6′), 33.0 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + H) $C_{20}H_{40}NO_{10}$: 454.2647. Found: 454.2645.

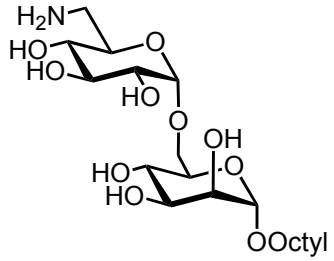
Octyl α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside (4.3)



Disaccharide **4.24** (37 mg, 0.036 mmol) was dissolved in CH$_3$OH (4 mL) and 20% Pd(OH)$_2$–C (15 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The crude product was purified by chromatography on Iatrobeads (4:1 CH$_2$Cl$_2$–CH$_3$OH) to give **4.3** (14 mg, 82%) as clear glass. R_f 0.54 (4:1 CH$_2$Cl$_2$-MeOH); $[\alpha]_D$ = +99.1 (*c* 0.3, CH$_3$OH); ^{1}H NMR (500 MHz, CD$_3$OD) δ_H 4.82 (d, 1H, *J* = 3.7 Hz, H-1′), 4.70 (d, 1H, *J* = 1.7 Hz, H-1), 4.00 (dd, 1H, *J* =

10.6, 4.0 Hz, H-6a), 3.61–3.82 (m, 9H, H-3′, H-5′, H-6a′, H-6b′, H-2, H-3, H-4, H-5, octyl OC*H*$_2$), 3.60 (dd, 1H, *J* = 10.6, 2.3 Hz, H-6b), 3.39 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 3.35 (dd, 1H, *J* = 9.6, 3.7 Hz, H-2′), 3.30 (overlap with residual CD_2HOD peak, 1H, H-4′), 1.52–1.62 (m, 2H, octyl OCH_2CH_2), 1.23–1.42 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.8 (C-1), 100.0 (C-1′), 74.3 (C-5′/C-5), 73.9 (C-2′), 73.5 (C-5′/C-5), 72.8 (C-3′, C-3), 72.3 (C-2), 71.8 (C-4′), 68.8 (octyl O*C*H$_2$), 68.4 (C-4), 67.3 (C-6), 62.6 (C-6′), 33.0 (octyl *C*H$_2$), 30.6(3) (octyl *C*H$_2$), 30.5(6) (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2305.

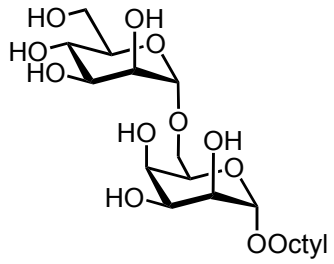
Octyl 6-amino-6-deoxy-α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside (4.4)



Disaccharide **4.27** (61 mg, 0.062 mmol) was dissolved in MeOH (4.5 mL) and 1 M NaOMe (1 mL) was added. After stirring overnight, the solution was neutralized with Amberlite 120 resin (H^+ form), filtered and concentrated. The crude was dissolved in satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (5 mL). The aqueous layer was purified by C18 reverse phase column to give **4.4** (14 mg, 79%), after lyophilization, as white solid; $[\alpha]_D$ = +149.2 (*c* 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ_H 4.81 (d, 1H, *J* = 3.8 Hz, H-1′), 4.71 (d, 1H, *J* = 1.8 Hz, H-

1), 4.01 (dd, 1H, *J* = 10.5, 3.9 Hz, H-6a), 3.77 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 3.77 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2), 3.58–3.74 (m, 6H, H-3′, H-5′, H-3, H-5, H-6b, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 3.35 (dd, 1H, *J* = 9.7, 3.8 Hz, H-2′), 3.13 (dd, 1H, *J* = 9.8, 8.9 Hz, H-4′), 3.00 (dd, 1H, *J* = 13.4, 2.8 Hz, H-6a′), 2.68 (dd, 1H, *J* = 13.4, 7.7 Hz, H-6b′), 1.52–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.23–1.42 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.9 (C-1), 99.8 (C-1′), 75.3 (C-5′/C-5), 73.9 (C-5′/C-5), 73.6 (C-2′), 73.5 (C-4′), 72.9, 72.8 (C-3′, C-3), 72.2 (C-2), 68.9 (octyl O*C*H$_2$), 68.2 (C-4), 67.1 (C-6), 43.8 (C-6′), 33.0 (octyl *C*H$_2$), 30.6(3) (octyl *C*H$_2$), 30.5(5) (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + H) $C_{20}H_{40}NO_{10}$: 454.2647. Found: 454.2649.

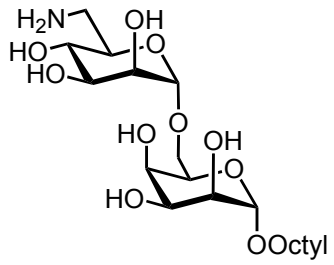
Octyl α-D-mannopyranosyl-(1→6)-α-D-talopyranoside (4.5)



Protected disaccharide **4.36** (84 mg, 0.077 mmol) was dissolved in THF (5 mL) and AcOH (12 μL, 0.46 mmol) and 1.0 M tetra-*n*-butylammonium fluoride in THF (0.15 mL, 0.15 mmol) were added. After stirring overnight, the mixture was filtered through a short pad of silica gel and concentrated. The crude product was redissolved in 4:1 AcOH–H_2O (5 mL) and heated at 50 °C for 3 h. The mixture was diluted with EtOAc (20 mL), washed with satd aq $NaHCO_3$ (5 mL), dried (Na_2SO_4), and concentrated. The resulting product was

subsequently dissolved in MeOH (5 mL) and NaOMe (54 mg) was added. After 2 h, the solution was neutralized with AcOH and, following concentration, the crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **4.5** (21 mg, 91% over three steps) as clear glass. R_f 0.54 (4:1 CH_2Cl_2-MeOH); $[\alpha]_D$ = +78.2 (*c* 0.4, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.80 (br s, 1H, H-1), 4.79 (d, 1H, *J* = 1.8 Hz, H-1′), 3.60–3.74 (m, 2H, H-5, H-6a), 3.82 (dd, 1H, *J* = 11.8, 2.3 Hz, H-6a′), 3.80 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 3.58–3.74 (m, 9H, H-3′, H-4′, H-5′, H-6b′, H-2, H-3, H-4, H-6b, octyl OC*H*$_2$), 3.43 (dt, 1H, *J* = 9.7, 6.3 Hz, octyl OC*H*$_2$), 1.52–1.62 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.41 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.2 (C-1), 101.6 (C-1′), 74.6 (C-5′), 72.7, 72.6 (C-3′, C-3), 72.2 (C-2), 71.7 (C-2′), 71.0 (C-5), 68.9 (octyl O*C*H_2), 68.5 (C-4′), 67.9 (C-6), 67.2 (C-4), 62.9 (C-6′), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2307.

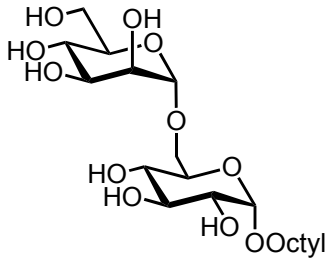
Octyl 6-amino-6-deoxy-α-D-mannopyranosyl-(1→6)-α-D-talopyranoside (4.6)



Disaccharide **4.37** (61 mg, 0.062 mmol) was dissolved in 4:1 AcOH–H_2O (5 mL) and heated at 50 °C for 6 h. The mixture was cooled, diluted with EtOAc (20 mL), washed with satd aq

$NaHCO_3$ (5 mL), dried (Na_2SO_4), and concentrated. The crude product was subsequently dissolved in MeOH (4 mL) and 1 M NaOMe (1 mL) was added. After stirring overnight, the solution was neutralized with Amberlite 120 resin (H^+ form), filtered and concentrated. The azidosugar intermediate was dissolved in CH_3OH (8 mL) and 20% $Pd(OH)_2$–C (50 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The eluant was concentrated, redissolved in satd aq $NaHCO_3$ (5 mL) and extracted with EtOAc (5 mL). The aqueous layer was purified by on a C_{18} reverse phase cartridge to give **4.6** (24 mg, 86%), after lyophilization, as white solid; $[\alpha]_D$ = +100.0 (*c* 0.2, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ_H 4.81 (br s, 1H, H-1), 4.79 (d, 1H, *J* = 1.6 Hz, H-1′), 3.86–3.94 (m, 2H, H-5, H-6a), 3.80 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 3.77–3.81 (m, 1H, H-2), 3.58–3.75 (m, 6H, H-3′, H-5′, H-3, H-4, H-6b, octyl OC*H*$_2$), 3.52 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4′), 3.42 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 3.05 (dd, 1H, *J* = 13.5, 2.9 Hz, H-6a′), 2.83 (dd, 1H, *J* = 13.5, 7.0 Hz, H-6b′), 1.52–1.64 (m, 2H, octyl OCH_2CH_2), 1.23–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.2 (C-1), 101.6 (C-1′), 73.7 (C-5′), 72.6, 72.5 (C-3′, C-3), 72.1 (C-2′), 71.8 (C-2), 71.1 (C-5), 69.8 (C-4′), 68.9 (octyl O*C*H_2), 68.2 (C-6), 67.2 (C-4), 43.4 (C-6′), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). HRMS (ESI) calcd. for (M + H) $C_{20}H_{40}NO_{10}$: 454.2647. Found: 454.2649.

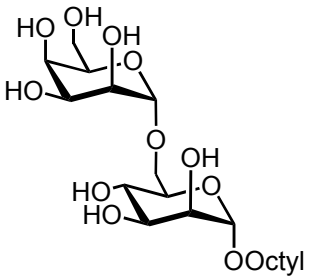
Octyl α-D-mannopyranosyl-(1→6)-α-D-glucopyranoside (4.7)



Disaccharide **4.38** (45 mg, 0.041 mmol) was dissolved in MeOH (6 mL) and NaOMe (32 mg) was added. After stirring overnight, the solution was neutralized with acetic acid, concentrated, and the residue was purified by chromatography (10:1 CH_2Cl_2–CH_3OH). The pure fractions were collected, concentrated, and redissolved in CH_3OH (8 mL) and 20% $Pd(OH)_2$–C (30 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated to give **4.7** (16 mg, 84%) as clear glass. R_f 0.36 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = +95.7 (*c* 0.5, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.80 (d, 1H, *J* = 1.7 Hz, H-1′), 4.74 (d, 1H, *J* = 3.9 Hz, H-1), 3.89 (dd, 1H, *J* = 11.1, 5.3 Hz, H-6a), 3.78–3.84 (m, 2H, H-2′, H-6a′), 3.65–3.74 (m, 5H, H-3′, H-6b′, H-5, H-6b, octyl OC*H*$_2$), 3.58–3.65 (m, 3H, H-4′, H-5′, H-3), 3.43 (dt, 1H, *J* = 9.6, 6.3 Hz, octyl OC*H*$_2$), 3.37 (dd, 1H, *J* = 9.7, 3.9 Hz, H-2), 3.33 (dd, 1H, *J* = 10.0, 9.0 Hz, H-4), 1.56–1.69 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.24–1.46 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 101.5 (C-1′), 100.2 (C-1), 75.3 (C-3), 74.4 (C-2), 73.6, 72.7, 72.2, 72.1 (4*C*, C-2′, C-3′, C-5′, C-5), 71.7 (C-4), 69.3 (octyl O*C*H$_2$), 68.6 (C-4′), 67.1 (C-6), 62.9 (C-6′), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl

CH_3). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2305.

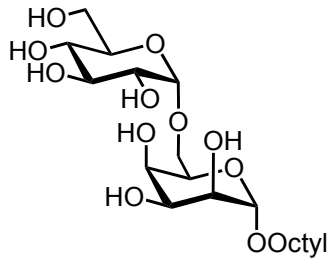
Octyl α-D-talopyranosyl-(1→6)-α-D-mannopyranoside (4.8)



Disaccharide **4.39** (39 mg, 0.037 mmol) was dissolved in THF (3 mL) and 1.0 M tetra-*n*-butylammonium fluoride in THF (0.15 mL, 0.15 mmol) was added and the solution was stirred at rt overnight. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with H_2O (5 mL), and the organic phase was dried (Na_2SO_4) and concentrated. The resulting crude product was redissolved in MeOH (4 mL) and NaOMe was added (22 mg). The reaction mixture was stirred for 3 h and neutralized with AcOH. The solution was then diluted with CH_2Cl_2 (15 mL), washed with H_2O (5 mL), and the organic phase was concentrated. The partially deprotected intermediate was dissolved and stirred in 4:1 HOAc–H_2O (5 mL) at 50 °C overnight. The mixture was diluted with CH_2Cl_2 (15 mL), washed with satd aq $NaHCO_3$ (5 mL x 2), and the organic phase was dried (Na_2SO_4) and concentrated. The resulting crude product was redissolved in CH_3OH (8 mL) and 20% $Pd(OH)_2$–C (15 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–MeOH) to give **4.8** (8 mg, 45%) as clear glass.

R_f 0.38 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = +83.5 (*c* 0.3, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.90 (d, 1H, *J* = 1.5 Hz, H-1′), 4.70 (d, 1H, *J* = 1.7 Hz, H-1), 3.80–3.93 (m, 3H, H-3′, H-4′, H-6a), 3.77 (dd, 1H, *J* = 3.2, 1.7 Hz, H-2), 3.71–3.77 (m, 5H, H-2′, H-5′, H-6a′, H-6b′, H-6b), 3.60–3.69 (m, 4H, H-3, H-4, H-5, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.6, 6.4 Hz, octyl OC*H*$_2$), 1.52–1.62 (m, 2H, octyl OCH_2C*H*$_2$), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.0 (C-1′), 101.7 (C-1), 73.1, 72.8, 72.5, 72.2 (5C, C-2′, C-3′, C-2, C-3, C-4), 71.8 (C-4′), 68.6 (octyl O*C*H_2), 68.6 (C-5), 67.5 (C-6), 67.3 (C-5′), 62.9 (C-6′), 33.0 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.4 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.4 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2303.

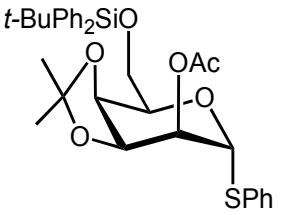
Octyl α-D-glucopyranosyl-(1→6)-α-D-talopyranoside (4.9)



Disaccharide **4.40** (85 mg, 0.081 mmol) was dissolved in THF (7 mL) and 1.0 M tetra-*n*-butylammonium fluoride in THF (0.32 mL, 0.32 mmol) was added and the solution was stirred at rt overnight. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with H_2O (5 mL), and the organic phase was dried (Na_2SO_4) and concentrated. The resulting crude product was redissolved in MeOH (8 mL) and NaOMe was added (43 mg). The solution was stirred for 2 h and neutralized with AcOH. The mixture was diluted with CH_2Cl_2 (15 mL),

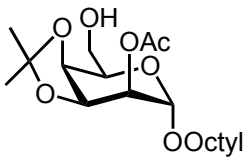
washed with H_2O (5 mL), and the organic phase was concentrated. The partially deprotected intermediate was dissolved and stirred in 4:1 HOAc–H_2O (5 mL) at 50 °C overnight. The mixture was diluted with CH_2Cl_2 (15 mL), washed with sat. aq. $NaHCO_3$ (5 mL x 2), and the organic phase was dried (Na_2SO_4) and concentrated. The resulting crude product was redissolved in CH_3OH (8 mL) and 20% $Pd(OH)_2$–C (20 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2-MeOH) to give **4.9** (23 mg, 62%) as clear glass. R_f 0.33 (4:1 CH_2Cl_2-MeOH); $[\alpha]_D$ = +102.9 (*c* 1.3, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.82 (d, 1H, *J* = 3.7 Hz, H-1′), 4.80 (d, 1H, *J* = 1.5 Hz, H-1), 3.93 (dd, 1H, *J* = 6.3, 6.3 Hz, H-5), 3.84–3.90 (m, 2H, H-4, H-6a), 3.79 (dd, 1H, *J* = 11.9, 2.4 Hz, H-6a′), 3.66–3.76 (m, 5H, H-6b′, H-2, H-3, H-6b, octyl OC*H*$_2$), 3.59–3.66 (m, 2H, H-3′, H-5′), 3.41 (dt, 1H, *J* = 9.7, 6.4 Hz, octyl OC*H*$_2$), 3.40 (dd, 1H, *J* = 9.8, 3.7 Hz, H-2′), 3.31 (dd, 1H, *J* = 9.8, 8.9 Hz, H-4′), 1.52–1.62 (m, 2H, octyl OCH_2CH_2), 1.24–1.42 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 102.3 (C-1), 100.2 (C-1′), 75.2 (C-3′), 73.8 (C-5′), 73.6 (C-2′), 72.7 (C-2), 71.7 (C-4′), 71.4 (C-3), 70.8 (C-5), 69.0 (octyl O*C*H$_2$), 68.0 (C-6), 67.1 (C-4), 62.6 (C-6′), 33.0 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.4 (2C, octyl *C*H$_2$), 27.4 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_{11}$: 477.2306. Found: 477.2299.

Phenyl 2-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-1-thio-α-D-talopyranoside (4.13)



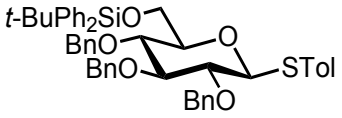
Monosaccharide **4.31** (102 mg, 0.086 mmol) was dissolved in 1:1 CH_2Cl_2–pyridine (4 mL) and acetic anhydride (0.16 mL) was added. The reaction mixture was stirred for 4 h and then diluted with CH_2Cl_2 (25 mL) before being washed with 1 M HCl (3 x 10 mL), satd aq $NaHCO_3$ (10 mL) and H_2O (10 mL). The organic layer was dried ($MgSO_4$), concentrated and the crude product was purified by chromatography (6:1 hexane–EtOAc) to give **4.13** (98 mg, 89%) as colorless oil: R_f 0.40 (6:1 hexane–EtOAc); $[\alpha]_D$ = +77.0 (*c* 2.8, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.68–7.73 (m, 4H, ArH), 7.50–7.54 (m, 2H, ArH), 7.35–7.45 (m, 6H, ArH), 7.21–7.26 (m, 3H, ArH), 5.45 (d, 1H, *J* = 8.6 Hz, H-1), 5.08 (dd, 1H, *J* = 8.6, 2.9 Hz, H-2), 4.60 (dd, 1H, *J* = 7.6, 2.9 Hz, H-3), 4.39 (dd, 1H, *J* = 7.6, 1.9 Hz, H-4), 3.93 (ddd, 1H, *J* = 6.5, 6.2, 1.9 Hz, H-5), 3.84 (dd, 1H, *J* = 10.4, 6.2 Hz, H-6a), 3.77 (dd, 1H, *J* = 10.4, 6.5 Hz, H-6b), 2.19 (s, 3H, C(O)C*H*$_3$), 1.44 (s, 3H, C(C*H*$_3$)$_2$), 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.05 (s, 9H, C(C*H*$_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 135.7 (2C, Ar), 135.6 (2C, Ar), 133.5 (Ar), 133.4 (2C, Ar), 132.7 (2C, Ar), 129.6 (2C, Ar), 128.8 (2C, Ar), 127.7 (2C, Ar), 127.6 (3C, Ar), 110.8 (*C*(CH$_3$)$_2$), 83.8 (C-1), 73.7 (C-4), 75.6 (C-3), 71.5 (C-5), 69.1 (C-2), 62.4 (C-6), 26.8 (C(*C*H$_3$)$_3$), 26.1 (C(*C*H$_3$)$_2$), 25.5 (C(*C*H$_3$)$_2$), 21.1 (C(O)*C*H$_3$), 19.2 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{33}H_{40}O_6SiS$: 615.2207. Found: 615.2207.

Octyl 2-*O*-acetyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.14)



Octyl talopyranoside **4.32** (112 mg, 0.18 mmol) was dissolved in THF (5 mL) and 1.0 M tetra-*n*-butylammonium fluoride in THF (0.9 mL, 0.90 mmol) was added and the solution was stirred at rt overnight. The solvent was evaporated and the residue was purified by chromatography (2:1 hexane–EtOAc) to give **4.14** (51 mg, 74%) as a colorless oil. R_f 0.11 (2:1 hexane–EtOAc); $[\alpha]_D$ = +87.8 (*c* 1.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.88–4.95 (m, 2H, H-1, H-2), 4.59 (dd, 1H, *J* = 7.5, 2.5 Hz, H-3), 4.35 (dd, 1H, *J* = 7.5, 1.8 Hz, H-4), 3.70–3.90 (m, 4H, H-5, H-6a, H-6b, octyl OC*H*$_2$), 3.45 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 2.16 (s, 3H, C(O)C*H*$_3$), 2.08 (br s, 1H, O*H*), 1.48–1.62 (m, 5H, C(C*H*$_3$)$_2$, octyl OCH$_2$C*H*$_2$), 1.20–1.39 (m, 13H, C(C*H*$_3$)$_2$, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.7 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 110.8 (*C*(CH$_3$)$_2$), 97.6 (C-1, $^1J_{C,H}$ = 171.9 Hz), 74.6 (C-4), 72.2 (C-3), 70.5 (C-2), 69.8 (C-5), 68.2 (octyl O*C*H$_2$), 62.2 (C-6), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.3(1) (octyl *C*H$_2$), 29.2(6) (octyl *C*H$_2$), 26.0(3) (C(*C*H$_3$)$_2$), 26.0(0) (octyl *C*H$_2$), 25.2 (C(*C*H$_3$)$_2$), 22.6 (octyl *C*H$_2$), 21.1 (C(O)*C*H$_3$), 14.1 (octyl *C*H$_3$). Anal. Calcd for $C_{19}H_{34}O_7$ (422.27): C, 68.22; H, 9.06. Found: C, 68.28; H, 9.06. HRMS (ESI) calcd. for (M + Na) $C_{19}H_{34}O_7$: 397.2197. Found: 397.2198.

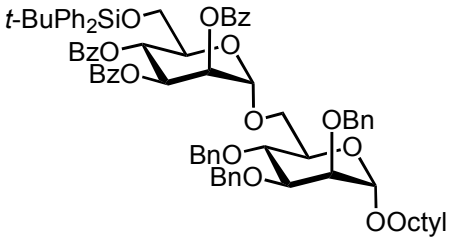
***p*-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (4.15)**

Tetraol **4.22**[10] (1.50 g, 5.24 mmol) and 

imidazole (0.90 g, 13.1 mmol) were dissolved in DMF (15 mL) and *tert*-butylchlorodiphenylsilane (1.7 mL, 6.55 mmol) was added. The reaction mixture was heated at 45 °C for 5 h and was quenched by the addition of H_2O (2 mL). The mixture was then diluted with EtOAc (60 mL), washed with H_2O (3 x 20 mL), 1M HCl (20 mL) and satd aq $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$), concentrated and the resulting oil was dissolved in DMF (4.5 mL) and BnBr (1.0 mL, 8.6 mmol) was added. The solution was cooled in an ice bath and 60% NaH in mineral oil (0.30 g, 7.64 mmol) was added portion wise, and the mixture was warmed to rt. After 3 h, the reaction was quenched by the addition of CH_3OH (15 mL), diluted with EtOAc (90 mL), washed with H_2O (3 x 40 mL), brine (40 mL) and dried ($MgSO_4$), filtered and concentrated to pale yellow oil, was purified by chromatography (9:1 hexane–EtOAc) to give **4.15** (1.43 g, 95%) as a colorless oil: R_f 0.33 (9:1 hexane–EtOAc); $[\alpha]_D$ = –14.4 (*c* 1.8, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.77–7.81 (m, 2H, ArH), 7.70–7.74 (m, 2H, ArH), 7.50–7.54 (m, 2H, ArH), 7.39–7.45 (m, 4H, ArH), 7.24–7.38 (m, 15H, ArH), 7.13–7.17 (m, 2H, ArH), 7.70–7.04 (m, 2H, ArH), 4.85–4.92 (m, 4H, PhCH_2), 4.74 (d, 1H, *J* = 10.2 Hz, PhCH_2), 4.70 (d, 1H, *J* = 10.8 Hz, PhCH_2), 4.63 (d, 1H, *J* = 9.8 Hz, H-1), 4.00 (dd, 1H, *J* = 11.4, 1.8 Hz, H-6a), 3.95 (dd, 1H, *J* = 11.4, 3.7 Hz, H-6b), 3.80 (dd, 1H, *J* = 8.9, 8.9 Hz, H-4), 3.72 (dd, 1H, *J* = 8.9, 8.9 Hz, H-3), 3.53 (dd, 1H, *J* = 9.8, 8.9 Hz, H-2), 3.38 (ddd, 1H, *J* = 8.9, 3.7, 1.8 Hz, H-5), 2.31 (s, 3H,

C*H*$_3$), 1.11 (s, 9H, C(C*H*$_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.5 (Ar), 135.9 (2C Ar), 135.7 (2C, Ar), 133.5 (Ar), 133.0 (Ar), 132.4 (2C, Ar), 130.2 (Ar), 129.7, 129.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.7(9), 127.7(5), 127.7(3), 127.6(8) (23 x Ar), 87.8 (C-1), 86.9 (C-3), 80.8 (C-2), 80.0 (C-5), 77.5 (C-4), 76.0 (Ph*C*H$_2$), 75.3 (Ph*C*H$_2$), 75.1 (Ph*C*H$_2$), 62.7 (C-6), 26.9 (C(*C*H$_3$)$_3$), 21.1 (*C*H$_3$), 19.3 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{50}H_{54}O_5SiS$: 817.3354. Found: 817.3356.

Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.17)

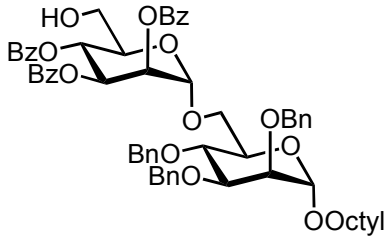


Thioglycoside **4.11**[4] (4.12 g, 4.9 mmol), alcohol **4.10**[5] (2.30 g, 4.1 mmol), and powdered 4 Å molecular sieves (2.5 g) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (100 mL) was added and the reaction mixture was cooled to 0 °C before the addition of *N*-iodosuccinimide (1.5 g, 6.2 mmol) and TMSOTf (0.22 mL, 1.2 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude residue was purified by chromatography (6:1 hexane–EtOAc) to give **4.17** (4.45 g, 85%) as a pale yellow oil. R_f 0.26 (6:1 hexane–EtOAc); $[\alpha]_D$ = –30.2 (*c* 3.0, CH_2Cl_2); ^{1}H NMR (600 MHz,

$CDCl_3$) δ_H 8.13–8.17 (m, 2H, ArH), 7.83–7.91 (m, 4H, ArH), 7.72–7.76 (m, 2H, ArH), 7.56–7.61 (m, 3H, ArH), 7.51–7.55 (m, 1H, ArH), 7.18–7.46 (m, 26H, ArH), 7.13–7.17 (m, 2H, ArH), 6.18 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.88 (dd, 1H, *J* = 10.2, 3.3 Hz, H-3′), 5.77 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 5.23 (d, 1H, *J* = 1.8 Hz, H-1′), 5.02 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.84 (d, 1H, *J* = 1.8 Hz, H-1), 4.75 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.71 (d, 1H, *J* = 12.0 Hz, PhC*H*$_2$), 4.69 (d, 1H, *J* = 11.4 Hz, PhC*H*$_2$), 4.65 (s, 2H, PhC*H*$_2$), 4.22 (ddd, 1H, *J* = 10.2, 3.0, 3.0 Hz, H-5′), 3.92–3.98 (m, 3H, H-3, H-4, H-6a), 3.83–3.89 (m, 2H, H-5, H-6b), 3.83 (d, 2H, *J* = 3.0 Hz, H-6a′, H-6b′), 3.80 (dd, 1H, *J* = 2.4, 1.8 Hz, H-2), 3.76 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.42 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.54–1.63 (m, 2H, octyl OCH_2CH_2), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 1.07 (s, 9H, $C(CH_3)_3$), 0.84 (t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.4 (*C*=O), 165.3 (*C*=O), 165.2 (*C*=O), 138.5 (Ar), 138.5 (Ar), 138.5 (Ar), 135.8 (2C, Ar), 135.8 (2C, Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 130.0 (Ar), 129.8, 129.7(0), 129.6(7), 129.6, 129.5(2), 129.4(9), 129.4(6), 128.5, 128.3(7), 128.3(3), 128.3(0), 128.2(6), 128.1(7), 127.8(4), 127.8(2), 127.7, 127.6, 127.5, 127.4(7) (37C, Ar), 97.7(1) (C-1′/C-1, $^1J_{C,H}$ = 172.3 Hz), 97.6(6) (C-1′/C-1, $^1J_{C,H}$ = 168.8 Hz), 80.6 (C-3), 75.0 (Ph*C*H_2), 74.9(4) (C-2), 74.9(0) (C-4), 72.6 (Ph*C*H_2), 72.1 (Ph*C*H_2), 71.4 (C-5), 71.2 (C-5′), 70.8 (C-2′), 70.7 (C-3′), 67.8 (octyl O*C*H_2), 66.8(7) (C-6), 66.8(1) (C-4′), 62.5 (C-6′), 31.8 (octyl *C*H_2), 29.5 (2C, octyl *C*H_2), 29.3 (octyl *C*H_2), 26.7 ($C(CH_3)_3$),

26.2 (octyl CH_2), 22.7 (octyl CH_2), 19.2 ($C(CH_3)_3$), 14.1 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{78}H_{86}O_{14}Si$: 1297.5679. Found: 1297.5683.

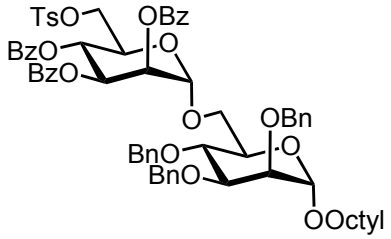
Octyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.18)



Silylated disaccharide **4.17** (4.0 g, 3.1 mmol) was dissolved in THF (25 mL) and 70% HF·pyridine (2 mL) and pyridine (5 mL) were added and the solution was stirred overnight. The crude product was then diluted with CH_2Cl_2 (150 mL), washed with H_2O (50 mL), 1 M HCl (2 x 50 mL), and satd aq $NaHCO_3$ (50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue was purified by chromatography to give **4.18** as a colorless oil (2.2 g, 66%): R_f 0.34 (3:1 hexane–EtOAc); $[\alpha]_D$ = –28.3 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.09–8.13 (m, 2H, ArH), 7.93–7.97 (m, 2H, ArH), 7.78–7.83 (m, 2H, ArH), 7.59–7.64 (m, 1H, ArH), 7.47–7.56 (m, 3H, ArH), 7.20–7.44 (m, 20H, ArH), 6.00 (dd, 1H, *J* = 10.2, 3.6 Hz, H-3′), 5.81 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.74 (dd, 1H, *J* = 3.6, 1.8 Hz, H-2′), 5.21 (d, 1H, *J* = 1.8 Hz, H-1′), 5.04 (d, 1H, *J* = 10.6 Hz, PhCH_2), 4.82 (d, 1H, *J* = 1.8 Hz, H-1), 4.77 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.72 (d, 1H, *J* = 12.6 Hz, PhCH_2), 4.69 (d, 1H, *J* = 11.6 Hz, PhCH_2), 4.65 (s, 2H, PhCH_2), 4.11 (ddd, 1H, *J* = 10.2, 3.6, 3.0 Hz, H-5′), 3.92–3.98 (m, 3H, H-3, H-4, H-6a), 3.82–3.89 (m, 2H, H-5, H-6b), 3.79 (dd, 1H, *J* = 2.4, 1.8 Hz, H-2), 3.71–3.80 (m,

2H, H-6a′, octyl OC*H*$_2$), 3.66 (ddd, 1H, *J* = 12.6, 6.0, 3.6 Hz, H-6b′), 3.40 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 2.59 (dd, 1H, *J* = 8.4, 6.0 Hz, O*H*), 1.52–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.5 (*C*=O), 165.3 (*C*=O), 165.2 (*C*=O), 138.4(9) (Ar), 138.4(8) (Ar), 138.4(2) (Ar), 133.6 (Ar), 133.4 (Ar), 133.0 (Ar), 129.9(3), 129.8(8), 129.7, 129.5, 129.3, 128.9, 128.6, 128.5, 128.4, 128.3(4), 128.2(9), 128.2, 127.8, 127.7, 127.6, 127.5 (30C, Ar), 97.8 (C-1′/C-1), 97.7 (C-1′/C-1), 80.5 (C-3), 75.0 (Ph*C*H$_2$), 74.9 (C-2/C-4), 74.8 (C-2/C-4), 72.7 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 71.2 (C-5), 70.8 (C-5′), 70.6 (C-2′), 69.6 (C-3′), 67.8 (octyl O*C*H$_2$), 67.5 (C-4′), 67.2 (C-6), 61.3 (C-6′), 31.8 (octyl *C*H$_2$), 29.5 (2C, octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{68}O_{14}$: 1059.4501. Found: 1059.4488.

Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-*p*-toluenesulfonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.19)

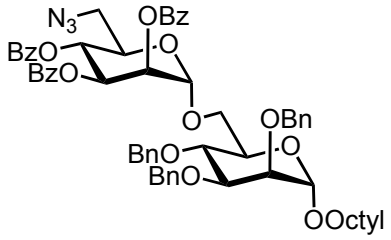


Disaccharide **4.18** (155 mg, 0.15 mmol) was dissolved in pyridine (2 mL) and the solution was cooled to 0 °C in an ice bath followed by the addition of *p*-toluenesulfonyl chloride (58 mg, 0.30 mmol). The reaction mixture was stirred overnight and then was diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl (3 x 10 mL), satd aq $NaHCO_3$ (10 mL) and H_2O (10 mL). The organic layer was dried ($MgSO_4$),

concentrated and the crude product was then purified by chromatography (3:1 hexane–EtOAc) to give **4.19** as a colorless oil (162 mg, 91%): R_f 0.36 (3:1 hexane–EtOAc); $[\alpha]_D$ = –22.9 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.06–8.09 (m, 2H, ArH), 7.82–7.86 (m, 2H, ArH), 7.77–7.81 (m, 2H, ArH), 7.72–7.76 (m, 2H, ArH), 7.59–7.64 (m, 1H, ArH), 7.46–7.54 (m, 3H, ArH), 7.18–7.44 (m, 20H, ArH), 7.13–7.17 (m, 2H, ArH), 5.84 (dd, 1H, *J* = 10.0, 2.9 Hz, H-3′), 5.81 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4′), 5.68 (dd, 1H, *J* = 2.9, 2.0 Hz, H-2′), 5.12 (d, 1H, *J* = 2.0 Hz, H-1′), 5.02 (d, 1H, *J* = 11.3 Hz, PhC*H*$_2$), 4.84 (d, 1H, *J* = 1.9 Hz, H-1), 4.77 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.73 (d, 1H, *J* = 12.5 Hz, PhC*H*$_2$), 4.68 (d, 1H, *J* = 11.3 Hz, PhC*H*$_2$), 4.65 (s, 2H, PhC*H*$_2$), 4.37 (ddd, 1H, *J* = 10.0, 4.9, 2.4 Hz, H-5′), 4.27 (dd, 1H, *J* = 11.0, 2.4 Hz, H-6a′), 4.16 (dd, 1H, *J* = 11.0, 4.9 Hz, H-6b′), 3.91–3.98 (m, 2H, H-3, H-4), 3.89 (dd, 1H, *J* = 11.1, 5.9 Hz, H-6a), 3.80–3.87 (m, 2H, H-5, H-6b), 3.80 (dd, 1H, *J* = 2.0, 1.9 Hz, H-2), 3.74 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.7, 6.5 Hz, octyl OC*H*$_2$), 2.30 (s, 3H, tosyl C*H*$_3$), 1.52–1.62 (m, 2H, octyl OCH_2C*H*$_2$), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 0.84 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.2 (*C*=O), 165.1(7) (*C*=O), 165.0(8) (*C*=O), 144.5 (Ar), 138.4(9) (Ar), 138.4(7) (Ar), 138.4(5) (Ar), 133.4 (Ar), 133.3 (Ar), 133.0 (Ar), 132.7 (Ar), 130.0, 129.7, 129.6, 129.4, 129.2, 129.0, 128.6, 128.4, 128.3(4), 128.2(8), 128.2(0), 128.1, 127.9(2), 127.8(6), 127.7, 127.5(3), 127.5(1) (34C, Ar), 97.7 (C-1), 97.5 (C-1′), 80.4 (C-3), 75.1 (Ph*C*H_2), 74.8 (2C, C-2, C-4), 72.7 (Ph*C*H_2), 72.1 (Ph*C*H_2), 71.3 (C-5), 70.3 (C-2′), 69.8

(C-3′), 68.6 (C-5′), 68.0 (C-6′), 67.8 (octyl O*C*H_2), 67.3 (C-6), 66.9 (C-4′), 31.8 (octyl *C*H_2), 29.5 (2C, octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 21.5 (tosyl *C*H_3), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{69}H_{74}O_{16}S$: 1213.4590. Found: 1213.4593.

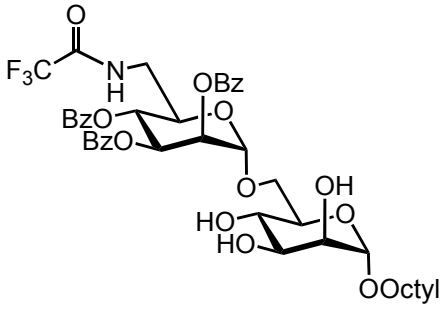
Octyl 6-azido-2,3,4-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.20)



Tosylated disaccharide **4.19** (136 mg, 0.12 mmol) was dissolved in DMF (2 mL) and sodium azide (68 mg, 1.0 mmol) was added and the solution was heated under reflux for 6 h. The crude solution was then diluted with EtOAc (25 mL) and washed with H_2O (10 mL). The organic phase was dried (Na_2SO_4), filtered, concentrated and the residue was purified by chromatography (6:1 hexane–EtOAc) to give **4.20** as a colorless oil (119 mg, 98%): R_f 0.31 (6:1 hexane–EtOAc); $[\alpha]_D$ = +3.5 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.10–8.14 (m, 2H, ArH), 7.90–7.95 (m, 2H, ArH), 7.78–7.83 (m, 2H, ArH), 7.60–7.65 (m, 1H, ArH), 7.48–7.55 (m, 3H, ArH), 7.18–7.45 (m, 20H, ArH), 5.89 (dd, 1H, *J* = 9.9, 3.2 Hz, H-3′), 5.84 (dd, 1H, *J* = 9.9, 9.9 Hz, H-4′), 5.73 (dd, 1H, *J* = 3.2, 1.8 Hz, H-2′), 5.20 (d, 1H, *J* = 1.8 Hz, H-1′), 5.03 (d, 1H, *J* = 11.3 Hz, PhC*H*$_2$), 4.83 (d, 1H, *J* = 1.7 Hz, H-1), 4.76 (d, 1H, *J* = 11.2 Hz, PhC*H*$_2$), 4.71 (d, 1H, *J* = 11.3 Hz, PhC*H*$_2$), 4.69 (d, 1H, *J* = 11.2 Hz, PhC*H*$_2$), 4.64 (s, 2H, PhC*H*$_2$), 4.33 (ddd,

1H, *J* = 9.9, 6.5, 3.2 Hz, H-5′), 3.95–4.04 (m, 3H, H-3, H-4, H-6a), 3.90 (dd, 1H, *J* = 11.1, 1.7 Hz, H-6b), 3.84–3.89 (m, 1H, H-5), 3.79 (dd, 1H, *J* = 2.2, 1.7 Hz, H-2), 3.74 (dt, 1H, *J* = 9.7, 6.9 Hz, octyl OC*H*$_2$), 3.38–3.48 (m, 3H, H-6a′, H-6b′, octyl OC*H*$_2$), 1.53–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.6 (*C*=O), 165.2 (*C*=O), 165.1 (*C*=O), 138.4(9) (2C, Ar), 138.4(5) (Ar), 133.4(4) (Ar), 133.4(1) (Ar), 133.0 (Ar), 129.9 (Ar), 129.8, 129.7, 129.5, 129.2, 129.0, 128.6, 128.4(4), 128.4(1), 128.3(5), 128.2(9), 128.2, 127.9, 127.8, 127.7, 127.6, 127.5(4), 127.5(3) (29C, Ar), 97.7 (C-1), 97.6 (C-1′), 80.5 (C-3), 75.1 (Ph*C*H$_2$), 74.9 (C-2/C-4), 74.8 (C-2/C-4), 72.7 (Ph*C*H$_2$), 72.1 (Ph*C*H$_2$), 71.3 (C-5), 70.4 (C-2′/C-5′), 70.1 (C-2′/C-5′), 69.7 (C-3′), 68.0 (C-4′), 67.8 (octyl O*C*H$_2$), 67.3 (C-6), 51.2 (C-6′), 31.8 (octyl *C*H$_2$), 29.5 (2C, octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{67}N_3O_{13}$: 1084.4566. Found: 1084.4569. FTIR: 2102.3 cm^{-1}.

Octyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-trifluoroacetamido-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (4.21)

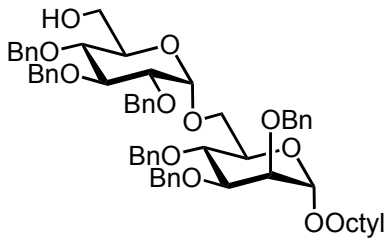


Azide **4.20** (50 mg, 0.047 mmol) was dissolved in pyridine (3 mL) and 20% $Pd(OH)_2$–C (10 mg) was added. The mixture was stirred for 4.5 h under a H_2 atmosphere and the catalyst was

separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue was redissolved in pyridine (2 mL) before trifluoroacetic anhydride (16 μL, 0.11 mmol) was added dropwise at 0 °C. The mixture was slowly warmed to rt and stirred overnight. The solution was then diluted with EtOAc (25 mL), washed with H_2O (10 mL) and satd aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4), concentrated and the resulting crude product was then redissolved in MeOH (6 mL) and 20% $Pd(OH)_2$–C (10 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue purified by chromatography (15:1 CH_2Cl_2–MeOH) to give **4.21** as a clear glass (28 mg, 68%): R_f 0.35 (15:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = –29.3 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.02–8.07 (m, 2H, ArH), 7.93–7.98 (m, 2H, ArH), 7.78–7.82 (m, 2H, ArH), 7.60–7.65 (m, 1H, ArH), 7.46–7.55 (m, 3H, ArH), 7.36–7.45 (m, 3H, ArH), 7.23–7.27 (m, 3H, ArH, C(O)N*H*), 5.96 (dd, 1H, *J* = 10.1, 3.4 Hz, H-3′), 5.75 (dd, 1H, *J* = 3.4, 1.8 Hz, H-2′), 5.70 (dd, 1H, *J* = 10.1, 10.1 Hz, H-4′), 5.25 (d, 1H, *J* = 1.8 Hz, H-1′), 4.87 (d, 1H, *J* = 1.3 Hz, H-1), 4.41 (ddd, 1H, *J* = 10.1, 4.9, 2.7 Hz, H-5′), 4.10 (dd, 1H, *J* = 11.4, 4.5 Hz, H-6a), 3.92–3.98 (m, 3H, H-6a′, H-2, H-4), 3.92 (dd, 1H, *J* = 11.4, 1.9 Hz, H-6b), 3.85 (dd, 1H, *J* = 9.9, 3.0 Hz, H-3), 3.79 (dd, 1H, *J* = 9.9, 3.0, 1.9 Hz, H-5), 3.73 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OC*H*$_2$), 3.41–3.50 (m, 2H, H-6b′, octyl OC*H*$_2$), 3.03 (br s, 2H, *OH*), 1.83 (br s, 1H, *OH*), 1.54–1.64 (m, 2H, octyl OCH_2CH_2), 1.18–1.42 (m,

10H, octyl CH_2), 0.86 (t, 3H, J = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.5 (*C*=O), 165.6 (*C*=O), 165.4 (*C*=O), 157.5 (CF_3*C*=O, $^2J_{C=O,F}$ = 37.1 Hz), 133.8 (Ar), 133.7(7) (Ar), 133.4 (Ar), 129.9 (2C, Ar), 129.8 (2C, Ar), 129.7 (2C, Ar), 129.0 (Ar), 128.8 (Ar), 128.7 (2C, Ar), 128.6 (2C, Ar), 128.4 (2C, Ar), 128.3(5) (Ar), 116.0 (*C*F_3, ^1J = 287.4 Hz), 99.9 (C-1), 97.7 (C-1′), 72.3 (C-3), 71.1 (C-5), 71.0 (C-2), 70.5 (C-2′), 69.4 (C-3′), 68.7 (C-4), 68.1 (octyl O*C*H_2), 67.8(3) (C-4′), 67.8(1) (C-5′), 67.8 (C-6), 39.7 (C-6′), 31.8 (octyl *C*H_2), 29.4 (2C, octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3); ^{19}F NMR (376.1 MHz, $CDCl_3$) δ_F –76.2 (s, 3F). HRMS (ESI) calcd. for (M + Na) $C_{43}H_{50}NO_{14}F_3$: 884.3076. Found: 884.3078.

Octyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.24)

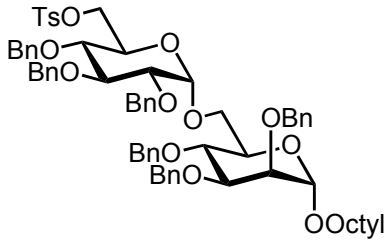


Thioglycoside **4.15** (110 mg, 0.14 mmol), alcohol **4.10**[5] (65 mg, 0.12 mmol), and powdered 4 Å molecular sieves (100 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (4 mL) was added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (41 mg, 0.17 mmol) and TMSOTf (6 µL, 0.035 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude product was partially purified by chromatography (9:1 hexane–

EtOAc) to give the desired disaccharide as a mixture of α and β isomers (5:1). The silylated disaccharide mixture was then dissolved in THF (2 mL) and and 1.0 M tetra-*n*-butylammonium fluoride in THF (0.31 mL, 0.31 mmol) was added and the solution was stirred at rt overnight. The solvent was evaporated and the residue was purified by chromatography (3:1 hexane–EtOAc) to give **4.24** (59 mg, 50%) as a colorless oil. R_f 0.32 (3:1 hexane–EtOAc); $[\alpha]_D$ = +33.5 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.18–7.40 (m, 30H, ArH), 5.11 (d, 1H, *J* = 3.5 Hz, H-1′), 4.92 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.88 (d, 1H, *J* = 11.1 Hz, PhC*H*$_2$), 4.87 (d, 1H, *J* = 11.2 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 1.7 Hz, H-1), 4.75 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.62–4.72 (m, 7H, PhC*H*$_2$), 4.55 (d, 1H, *J* = 11.6 Hz, PhC*H*$_2$), 4.05 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 4.00 (dd, 1H, *J* = 9.2, 9.2 Hz, H-3′), 3.92 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3), 3.75–3.89 (m, 6H, H-5′, H-6a′, H-2, H-5, H-6a, H-6b), 3.62–3.72 (m, 2H, H-6b′, octyl OC*H*$_2$), 3.51 (dd, 1H, *J* = 9.2, 9.2 Hz, H-4′), 3.47 (dd, 1H, *J* = 9.2, 3.5 Hz, H-2′), 3.33 (dt, 1H, *J* = 9.6, 6.7 Hz, octyl OC*H*$_2$), 1.72 (dd, 1H, *J* = 7.7, 4.9 Hz, O*H*), 1.46–1.54 (m, 2H, octyl OCH_2CH_2), 1.20–1.34 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.9 (Ar), 138.8 (Ar), 138.7 (Ar), 138.4(2) (Ar), 138.4(0) (Ar), 138.3 (Ar), 128.4, 128.3, 128.2(9), 128.2(8), 128.2(2), 128.1(9), 128.0, 127.9(2), 127.9(1), 127.7, 127.6(3), 127.6(1), 127.5(3), 127.5(1), 127.5(0), 127.4(7) (30C, Ar), 97.8 (C-1), 96.3 (C-1′), 81.6 (C-3′), 80.4 (C-2′), 80.3 (C-3), 77.5 (C-4′), 75.5 (Ph*C*H_2), 75.3 (C-2), 75.1(4) (Ph*C*H_2), 75.1(0) (C-4), 74.9 (Ph*C*H_2), 72.9 (Ph*C*H_2), 72.6

(Ph*C*H_2), 72.2 (Ph*C*H_2), 72.0 (C-5′/C-5), 70.8 (C-5′/C-5), 67.7 (octyl O*C*H_2), 65.8 (C-6), 62.0 (C-6′), 31.8 (octyl *C*H_2), 29.4 (2C, octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{74}O_{11}$: 1017.5123. Found: 1017.5126.

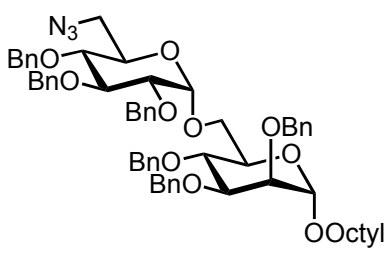
Octyl 2,3,4-tri-*O*-benzyl-6-*O*-*p*-toluenesulfonyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.25)



Disaccharide **4.24** (126 mg, 0.13 mmol) was dissolved in pyridine (3 mL) and the solution was cooled to 0°C in an ice bath followed by the addition of *p*-toluenesulfonyl chloride (49 mg, 0.25 mmol). The reaction mixture was stirred overnight and then the mixture was diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl (3 x 10 mL), satd aq $NaHCO_3$ (10 mL), and H_2O (10 mL). The organic layer was dried ($MgSO_4$), concentrated and the crude product was then purified by chromatography (6:1 hexane–EtOAc) to give **4.25** (129 mg, 89%) as a colorless oil: R_f 0.27 (6:1 hexane–EtOAc); $[\alpha]_D$ = +43.1 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.75–7.79 (m, 2H, ArH), 7.20–7.38 (m, 30H, ArH), 7.12–7.16 (m, 2H, ArH), 5.02 (d, 1H, *J* = 3.4 Hz, H-1′), 4.92 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.90 (d, 1H, *J* = 11.2 Hz, PhC*H*$_2$), 4.83 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 1.8 Hz, H-1), 4.70 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.61–4.69 (m, 6H, PhC*H*$_2$), 4.55 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.42 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.23 (dd, 1H, *J* = 10.6, 3.6 Hz, H-6a),

4.18 (dd, 1H, *J* = 10.6, 2.1 Hz, H-6b), 3.88–4.03 (m, 4H, H-3′, H-5′, H-3, H-5), 3.79 (dd, 1H, *J* = 3.1, 1.8 Hz, H-2), 3.74–3.80 (m, 2H, H-6a′, H-6b′, H-4), 3.64 (dt, 1H, *J* = 9.7, 6.8 Hz, octyl OC*H*$_2$), 3.48 (dd, 1H, *J* = 10.1, 9.0 Hz, H-4′), 3.45 (dd, 1H, *J* = 9.6, 3.4 Hz, H-2′), 3.32 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 2.38 (s, 3H, tosyl CH_3), 1.44–1.54 (m, 2H, octyl OCH_2CH_2), 1.20–1.34 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 144.7 (Ar), 138.7 (Ar), 138.6(9) (Ar), 138.6(4) (Ar), 138.3(4) (Ar), 138.2(7) (Ar), 138.1 (Ar), 133.0 (Ar), 129.8, 128.4, 128.3(1), 128.3(0), 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6(3), 127.6(0), 127.5(4), 127.5(1), 127.4(9) (34C, Ar), 97.8 (C-1), 96.4 (C-1′), 81.5 (C-3′), 80.5 (C-3), 79.9 (C-2′), 76.8 (C-4′), 75.4 (Ph*C*H$_2$), 75.3 (C-2), 75.0(8) (C-5′), 75.0(6) (Ph*C*H$_2$), 74.8 (Ph*C*H$_2$), 72.9 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 72.2 (Ph*C*H$_2$), 71.8 (C-4), 68.6(2) (C-6), 68.5(6) (C-5), 67.7 (octyl O*C*H$_2$), 66.1 (C-6′), 31.8 (octyl *C*H$_2$), 29.4(5) (octyl *C*H$_2$), 29.4(2) (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 21.6 (tosyl *C*H$_3$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{69}H_{80}O_{13}S$: 1171.5212. Found: 1171.5210.

Octyl 6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (4.26)

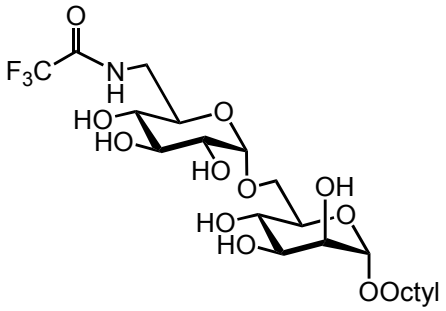


Tosylated disaccharide **4.25** (100 mg, 0.093 mmol) was dissolved in DMF (2 mL) and

sodium azide (54 mg, 0.84 mmol) was added and the solution was heated under reflux for 6 h. The crude product was then diluted with EtOAc (25 mL) and washed with H_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue was purified by chromatography (6:1 hexane–EtOAc) to give **4.26** as a colorless oil (76 mg, 85%): R_f 0.36 (6:1 hexane–EtOAc); $[\alpha]_D$ = +58.6 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.18–7.38 (m, 30H, ArH), 5.14 (d, 1H, *J* = 3.4 Hz, H-1′), 4.94 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.91 (d, 1H, *J* = 11.1 Hz, PhC*H*$_2$), 4.90 (d, 1H, *J* = 11.2 Hz, PhC*H*$_2$), 4.81 (d, 1H, *J* = 1.7 Hz, H-1), 4.73 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.70 (d, 1H, *J* = 10.9 Hz, PhC*H*$_2$), 4.62–4.70 (m, 5H, PhC*H*$_2$), 4.57 (d, 1H, *J* = 11.1 Hz, PhC*H*$_2$), 4.56 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.06 (dd, 1H, *J* = 9.6, 9.6 Hz, H-3), 3.98 (dd, 1H, *J* = 9.4, 9.4 Hz, H-3′), 3.86–3.95 (m, 3H, H-5′, H-4, H-6a), 3.84 (dd, 1H, *J* = 11.7, 1.5 Hz, H-6b), 3.77–3.82 (m, 2H, H-2, H-5), 3.65 (dt, 1H, *J* = 9.7, 6.8 Hz, octyl OC*H*$_2$), 3.51 (dd, 1H, *J* = 9.4, 3.4 Hz, H-2′), 3.42–3.48 (m, 2H, H-4′, H-6a′), 3.30–3.36 (m, 2H, H-6b′, octyl OC*H*$_2$), 1.45–1.53 (m, 2H, octyl OCH_2C*H*$_2$), 1.20–1.34 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.7(7) (Ar), 138.7(3) (Ar), 138.6(8) (Ar), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 128.4, 128.3(1), 128.2(6), 128.2(2), 128.1, 128.0, 127.9(7), 127.9(4), 127.8, 127.7, 127.6(1), 127.5(8), 127.5(1), 127.4(8) (30C, Ar), 97.8 (C-1), 96.3 (C-1′), 81.4 (C-3′), 80.4 (C-4), 80.2 (C-2′), 78.3 (C-4′), 75.5 (Ph*C*H_2), 75.3 (C-2), 75.1(4) (Ph*C*H_2), 75.1(2) (C-3), 75.0 (Ph*C*H_2), 72.8 (Ph*C*H_2), 72.5 (Ph*C*H_2), 72.2 (Ph*C*H_2), 71.9 (C-5), 69.9 (C-5′), 67.6

(octyl O*C*H_2), 66.0 (C-6), 51.4 (C-6′), 31.8 (octyl *C*H_2), 29.4 (2C, octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{73}N_3O_{10}$: 1042.5188. Found: 1042.5188. FTIR: 2099.9 cm^{-1}.

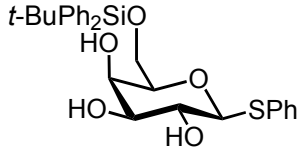
Octyl 6-deoxy-6-trifluoroacetamido-α-D-glucopyranosyl-(1→6)-α-D-mannopyranoside (4.27)



Azide **4.26** (67 mg, 0.066 mmol) was dissolved in pyridine (3 mL) and 20% $Pd(OH)_2$–C (17 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue was redissolved in pyridine (2 mL) before trifluoroacetic anhydride (20 µL, 0.13 mmol) was added dropwise at 0 °C. The mixture was slowly warmed to rt and stirred overnight. The solution was then diluted with EtOAc (25 mL), washed with H_2O (10 mL) and satd aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4), concentrated and the resulting crude product was then redissolved in MeOH (6 mL) and 20% $Pd(OH)_2$–C (15 mg) was added. The mixture was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the residue purified by chromatography (8:1 CH_2Cl_2–MeOH) to give **4.27** as a

clear glass (26 mg, 72%): R_f 0.24 (8:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = +74.4 (*c* 0.4, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 4.82 (d, 1H, *J* = 3.7 Hz, H-1′), 4.71 (d, 1H, *J* = 1.5, 1.8 Hz, H-1), 3.96 (dd, 1H, *J* = 10.7, 4.0 Hz, H-6a), 3.74–3.80 (m, 3H, H-5′, H-2, H-4), 3.60–3.73 (m, 5H, H-3′, H-6a′, H-3, H-5, octyl OCH_2), 3.58 (dd, 1H, *J* = 10.7, 2.2 Hz, H-6b), 3.48 (dd, 1H, *J* = 14.0, 7.8 Hz, H-6b′), 3.40 (dt, 1H, *J* = 9.7, 6.4 Hz, octyl OCH_2), 3.37 (dd, 1H, *J* = 9.7, 3.7 Hz, H-2′), 3.12 (dd, 1H, *J* = 9.9, 8.9 Hz, H-4′), 1.52–1.62 (m, 2H, octyl OCH_2CH_2), 1.24–1.42 (m, 10H, octyl CH_2), 0.89 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 159.3 (CF_3C=O, $^2J_{C=O,F}$ = 37.1 Hz), 117.6 (CF_3, ^1J = 285.9 Hz),101.8 (C-1), 99.7 (C-1′), 75.0 (C-3′), 73.8 (C-2′), 73.6 (C-4′), 72.8(3) (C-3), 72.8(2) (C-5), 72.2 (C-2), 71.1 (C-5′), 68.8 (octyl OCH_2), 68.3 (C-4), 67.1 (C-6), 42.1 (C-6′), 33.0 (octyl CH_2), 30.6 (octyl CH_2), 30.5 (octyl CH_2), 30.4 (octyl CH_2), 27.4 (octyl CH_2), 23.7 (octyl CH_2), 14.4 (octyl CH_3); ^{19}F NMR (376.1 MHz, $CDCl_3$) δ_F –77.5 (s, 3F). HRMS (ESI) calcd. for (M + Na) $C_{22}H_{38}NO_{11}F_3$: 572.2289. Found: 572.2286.

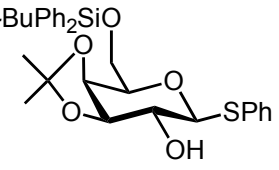
Phenyl 6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (4.29)



Tetraol **4.28**[11] (2.68 g, 9.85 mmol) and imidazole (1.68 g, 24.6 mmol) were dissolved in DMF (6 mL) and *tert*-butylchlorodiphenylsilane (3.2 mL, 12.3 mmol) was added. The reaction mixture was heated at 45 °C for 3 h and was quenched by the addition of H_2O (2 mL). The mixture was then diluted

with EtOAc (100 mL), washed with H_2O (3 x 30 mL), 1M HCl (30 mL) and satd aq $NaHCO_3$ (30 mL). The organic layer was dried ($MgSO_4$), concentrated, and the crude product was purified by chromatography (1:1 hexane–EtOAc) to give **4.29** (4.73 g, 94%) as a colorless oil: R_f 0.35 (1:1 hexane–EtOAc); $[\alpha]_D$ = –19.0 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.67–7.75 (m, 4H, ArH), 7.53–7.57 (m, 2H, ArH), 7.35–7.47 (m, 6H, ArH), 7.23–7.29 (m, 3H, ArH), 4.50 (d, 1H, *J* = 9.7 Hz, H-1), 4.09 (m, 1H, H-4), 3.92–4.00 (m, 2H, H-6a, H-6b), 3.68 (ddd, 1H, *J* = 9.7, 9.7, 1.7 Hz, H-2), 3.55–3.62 (m, 2H, H-3, H-5), 2.96 (d, 1H, *J* = 6.4 Hz, O*H*), 2.94 (d, 1H, *J* = 3.7 Hz, O*H*), 2.73 (d, 1H, *J* = 1.7 Hz, O*H*), 1.07 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 135.7 (2C, Ar), 135.6 (2C, Ar), 132.9 (Ar), 132.7 (Ar), 132.6 (Ar), 132.2 (2C, Ar), 129.9 (2C, Ar), 129.0 (2C, Ar), 127.8 (5C, Ar), 88.6 (C-1), 78.2 (C-5), 75.0 (C-3), 70.0 (C-2), 69.4 (C-4), 63.8 (C-6), 31.8 (octyl *C*H_2), 26.8 (C(*C*H_3)$_3$), 19.2 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{28}H_{34}O_5SiS$: 553.1789. Found: 553.1785.

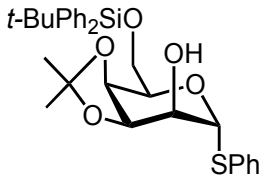
Phenyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (4.30)



Triol **4.29** (3.37 g, 6.60 mmol), 2,2-dimethoxypropane (6.5 mL, 52.8 mmol), and *p*-TsOH (25 mg) were dissolved in acetone (90 mL) and the mixture was stirred for 2 h. The reaction mixture was neutralized with triethylamine, concentrated, and purified by chromatography (4:1

hexane–EtOAc) to give **4.30** (3.70 g, quant.) as white foam: R_f 0.30 (4:1 hexane–EtOAc); $[\alpha]_D$ = –2.3 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69–7.74 (m, 4H, ArH), 7.52–7.56 (m, 2H, ArH), 7.35–7.46 (m, 6H, ArH), 7.24–7.29 (m, 3H, ArH), 4.43 (d, 1H, *J* = 10.3 Hz, H-1), 4.27 (dd, 1H, *J* = 5.5, 2.0 Hz, H-4), 4.07 (dd, 1H, *J* = 6.9, 5.5 Hz, H-3), 3.89–4.01 (m, 3H, H-5, H-6a, H-6b), 3.55 (dd, 1H, *J* = 10.3, 6.9, 2.3 Hz, H-2), 2.41 (d, 1H, *J* = 2.3 Hz, O*H*), 1.41 (s, 3H, C(CH_3)$_2$), 1.33 (s, 3H, C(CH_3)$_2$), 1.06 (s, 9H, C(CH_3)$_3$; ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 135.6(4) (2C, Ar), 135.6(2) (2C, Ar), 133.4 (Ar), 133.3 (Ar), 132.4 (2C, Ar), 132.3 (Ar), 129.7(1) (Ar), 129.7(0) (Ar), 129.0 (2C, Ar), 127.9 (Ar), 127.7 (2C, Ar), 127.6 (2C, Ar), 110.1 (*C*(CH$_3$)$_2$), 88.3 (C-1), 79.0 (C-3), 77.2 (C-5), 73.3 (C-4), 71.6 (C-2), 63.0 (C-6), 28.1 (C(*C*H$_3$)$_2$), 26.8 (C(*C*H$_3$)$_3$), 26.3 (C(*C*H$_3$)$_2$), 19.2 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{31}H_{38}O_5SiS$: 573.2102. Found: 573.2105.

Phenyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1-thio-α-D-talopyranoside (4.31)

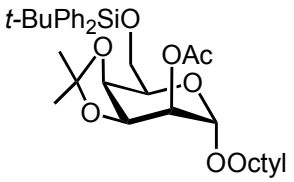


Oxalyl chloride (350 µL, 0.69 mmol) was dissolved in CH_2Cl_2 (3.5 mL) and DMSO (110 µL, 1.52 mmol) was added dropwise at –78 °C. After stirring for 30 min, alcohol **4.30** (253 mg, 0.46 mmol) in CH_2Cl_2 (3.5 mL) was added dropwise to the mixture over 10 min. After being stirred for 20 min, the solution was then warmed to –60 °C and triethylamine (0.43 mL, 3.1 mmol) was added

slowly as the solution warmed to rt over 40 min. The reaction was quenched by the addition H_2O and the organic layer was washed with H_2O (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), concentrated, and the crude ketone intermediate was redissolved in MeOH (17 mL). Sodium borohydride (35 mg, 0.92 mmol) was then added and the mixture was stirred for 20 min before being neutralized with AcOH. The solution was concentrated and the crude product was purified by chromatography (3:1 hexane–EtOAc) to give α-glycoside **4.31** (135 mg, 53%) and its β-glycoside isomer (23 mg, 9%) as a colorless oils; α-glycoside **4.31**, R_f 0.32; β-glycoside, R_f 0.46 (3:1 hexane–EtOAc); Only the α-glycoside was fully characterized: $[\alpha]_D$ = +107.9 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69–7.74 (m, 4H, ArH), 7.53–7.57 (m, 2H, ArH), 7.34–7.46 (m, 6H, ArH), 7.21–7.29 (m, 3H, ArH), 5.33 (d, 1H, *J* = 7.4 Hz, H-1), 4.55 (dd, 1H, *J* = 7.5, 3.4 Hz, H-3), 4.35 (dd, 1H, *J* = 7.5, 2.0 Hz, H-4), 3.97 (ddd, 1H, *J* = 6.6, 6.0, 2.0 Hz, H-5), 3.85 (dd, 1H, *J* = 10.3, 6.0 Hz, H-6a), 3.80 (dd, 1H, *J* = 10.3, 6.6 Hz, H-6b), 3.75 (ddd, 1H, *J* = 7.4, 7.2, 3.4 Hz, H-2), 2.50 (d, 1H, *J* = 7.2 Hz, O*H*), 1.45 (s, 3H, C(CH_3)$_2$), 1.35 (s, 3H, C(CH_3)$_2$), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 135.7 (2C, Ar), 135.6 (2C, Ar), 133.5 (Ar), 133.4 (2C, Ar), 132.6 (2C, Ar), 129.7 (2C, Ar), 128.9 (2C, Ar), 127.7 (Ar), 127.6(4) (2C, Ar), 127.6(0) (2C, Ar), 110.3 (*C*(CH$_3$)$_2$), 87.2 (C-1), 73.3 (2C, C-3, C-4), 70.8 (C-5), 68.3 (C-2), 62.7 (C-6), 26.8 (C(*C*H$_3$)$_3$), 26.0 (C(*C*H$_3$)$_2$), 25.3 (C(*C*H$_3$)$_2$), 19.2 (*C*(CH$_3$)$_3$).

HRMS (ESI) calcd. for (M + Na) $C_{31}H_{38}O_5SiS$: 573.2102. Found: 573.2107.

Octyl 2-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.32)

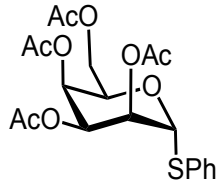


Thioglycoside **4.13** (31 mg, 0.052 mmol) and powdered 4 Å molecular sieves (50 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (2 mL) was added and the solution was cooled to 0 °C before the sequential addition of octanol (10 µL, 0.065 mmol), *N*-iodosuccinimide (16 mg, 0.065 mmol) and TMSOTf (3 µL, 0.016 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude residue was purified by chromatography (4:1 hexane–EtOAc) to give **4.32** (272 mg, 89%) as a colorless oil. R_f 0.38 (4:1 hexane–EtOAc); $[\alpha]_D$ = +35.6 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.68–7.73 (m, 4H, ArH), 7.35–7.45 (m, 6H, ArH), 4.92 (dd, 1H, *J* = 6.2, 2.9 Hz, H-2), 4.86 (d, 1H, *J* = 6.2 Hz, H-1), 4.56 (dd, 1H, *J* = 7.6, 2.9 Hz, H-3), 4.32 (dd, 1H, *J* = 7.6, 1.7 Hz, H-4), 3.79–3.89 (m, 3H, H-5, H-6a, H-6b), 3.75 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OCH_2), 3.39 (dt, 1H, *J* = 9.7, 6.9 Hz, octyl OCH_2), 2.16 (s, 3H, C(O)CH_3), 1.50–1.60 (m, 2H, octyl OCH_2CH_2), 1.44 (s, 3H, C(CH_3)$_2$), 1.20–1.34 (m, 13H, C(CH_3)$_2$, octyl CH_2), 1.06 (s, 9H, C(CH_3)$_3$), 0.88 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.3 (*C*=O), 135.7 (2 x Ar), 135.6 (2 x Ar), 135.5 (2 x Ar),

127.6(1) (2 x Ar), 127.5(8) (4 x Ar), 110.5 (isopropylidene *C*), 97.4 (C-1), 74.1 (C-4), 72.3 (C-3), 70.9 (C-2), 70.7 (C-5), 67.9 (octyl O*C*H_2), 62.6 (C-6), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.8 (C(*C*H_3)$_3$), 26.1 (C(*C*H_3)$_2$), 26.0 (octyl *C*H_2), 25.3 (C(*C*H_3)$_2$), 22.6 (octyl *C*H_2), 21.1 (C(O)*C*H_3), 19.2 (*C*(CH$_3$)$_3$), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{35}H_{52}O_7Si$: 635.3375. Found: 635.3374.

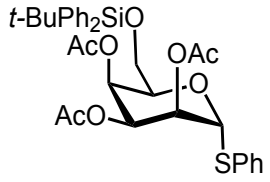
Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-talopyranoside (4.33) and phenyl 2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-talopyranoside (4.34)

Talopyranoside **4.31** (20 mg, 0.036 mmol) was dissolved in 4:1 AcOH–H_2O (2 mL) and heated at 50 °C for 1 h. The reaction mixture was then diluted with EtOAc (10 mL) and washed with satd aq $NaHCO_3$ (2 x 5 mL). The organic layer was dried ($MgSO_4$), and concentrated. The crude residue was then dissolved in pyridine (1.5 mL) and acetic anhydride (0.4 mL) and DMAP (small grain) were added. The reaction mixture was stirred for 1 day and then diluted with CH_2Cl_2 (10 mL), before being washed with 0.5 M HCl (3 x 5 mL), water (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), concentrated and the resulting crude mixture was purified by chromatography (2:1 hexane–EtOAc) to give **4.33** (10 mg, 65%) and **4.34** (6 mg, 26%) as colorless oils.



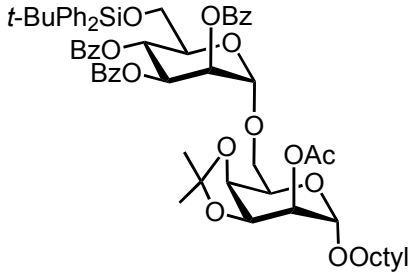
4.33: R_f 0.27 (2:1, hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.34–7.51 (m, 2H, ArH), 7.30–7.34 (m, 3H,

ArH), 5.57 (d, 1H, *J* = 1.2 Hz, H-1), 5.36 (m, 1H, H-4), 5.31 (ddd, 1H, *J* = 3.8, 1.2, 1.2 Hz, H-2), 5.25 (dd, 1H, *J* = 3.8, 3.8 Hz, H-3), 4.77 (app. td, 1H, *J* = 6.4, 1.6 Hz, H-5), 4.15–4.22 (m, 2H, H-6a, H-6b), 2.14 (s, 6H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 2.00 (s, 3H, C(O)CH_3).



4.34: R_f 0.52 (2:1, hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.61–7.64 (m, 4H, ArH), 7.34–7.44 (m, 8H, ArH), 7.20–7.24 (m, 3H, ArH), 5.51 (m, 1H, H-4), 5.48 (d, 1H, *J* = 1.3 Hz, H-1), 5.32 (ddd, 1H, *J* = 3.7, 1.3, 1.3 Hz, H-2), 5.27 (dd, 1H, *J* = 3.7, 3.7 Hz, H-3), 4.77 (app. td, 1H, *J* = 6.0, 1.4 Hz, H-5), 3.66–3.75 (m, 2H, H-6a, H-6b), 2.11 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.03 (s, 9H, C(CH_3)$_3$).

Octyl 2,3,4,-tri-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.35)

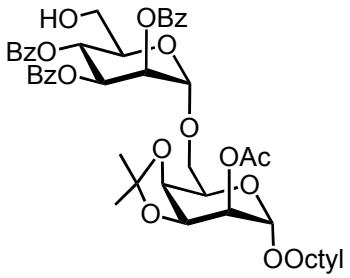


Thioglycoside **4.11**[4] (186 mg, 0.20 mmol), alcohol **4.14** (55 mg, 0.15 mmol), and powdered 4 Å molecular sieves (100 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (4 mL) was added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (53 mg, 0.23 mmol) and TMSOTf (8 µL, 0.045 mmol). The mixture was stirred for 1 h at 0 °C and neutralized

with triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (4:1 hexane–EtOAc) to give **4.35** as a colorless oil (144 mg, 90%): R_f 0.21 (4:1 hexane–EtOAc); $[\alpha]_D$ = –47.1 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.11–8.16 (m, 2H, ArH), 7.83–7.94 (m, 4H, ArH), 7.70–7.75 (m, 2H, ArH), 7.51–7.62 (m, 3H, ArH), 7.25–7.46 (m, 12H, ArH), 7.12–7.17 (m, 2H, ArH), 6.20 (dd, 1H, *J* = 10.2, 10.2 Hz, H-4′), 5.82 (dd, 1H, *J* = 10.2, 3.3 Hz, H-3′), 5.73 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 5.16 (d, 1H, *J* = 1.8 Hz, H-1′), 5.01 (dd, 1H, *J* = 5.7, 3.0 Hz, H-2), 4.91 (d, 1H, *J* = 5.7 Hz, H-1), 4.64 (dd, 1H, *J* = 7.8, 3.0 Hz, H-3), 4.41 (dd, 1H, *J* = 7.8, 1.8 Hz, H-4), 4.20–4.25 (m, 1H, H-5′), 4.02–4.07 (m, 1H, H-5), 3.82–3.97 (m, 4H, H-6a′, H-6b′, H-6a, octyl OC*H*$_2$), 3.76 (dd, 1H, *J* = 10.2, 6.0 Hz, H-6b), 3.46 (dd, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 2.05 (s, 3H, C(O)C*H*$_3$), 1.54–1.64 (m, 2H, octyl C*H*$_2$), 1.48 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 1.10–1.34 (m, 10H, octyl C*H*$_2$), 1.08 (s, 9H, C(C*H*$_3$)$_3$), 0.80 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 165.5 (*C*=O), 165.4 (*C*=O), 165.2 (*C*=O), 135.8 (2C, Ar), 135.5 (2C, Ar), 133.3 (Ar), 133.1(1) (Ar), 133.0(7) (Ar), 133.0(0) (Ar), 129.9(9) (2C, Ar), 129.8 (2C, Ar), 129.7(3) (2C, Ar), 129.7(0) (2C, Ar), 129.6 (Ar), 129.5 (Ar), 129.4 (Ar), 129.3 (Ar), 128.5 (2C, Ar), 128.3 (2C, Ar), 128.2 (2C, Ar), 127.6 (2C, Ar), 127.5 (2C, Ar), 110.8 (*C*(CH$_3$)$_2$), 97.5 (C-1, $^1J_{C,H}$ = 173.0 Hz), 97.3 (C-1′, $^1J_{C,H}$ = 172.2 Hz), 74.0 (C-4), 72.3 (C-3), 71.4 (C-5′), 70.7 (C-2, C-3′), 70.5 (C-2′), 68.3 (C-5), 68.1 (octyl O*C*H$_2$), 66.5 (C-4′), 66.0 (C-6), 62.4 (C-6′), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4

(octyl CH_2), 29.3 (octyl CH_2), 26.7 ($C(CH_3)_3$), 26.1(0) ($C(CH_3)_2$), 26.0(7) (octyl CH_2), 25.3 ($C(CH_3)_2$), 22.6 (octyl CH_2), 21.1 ($C(O)CH_3$), 19.2 ($C(CH_3)_3$), 14.0 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{74}O_{15}Si$: 1109.4689. Found: 1109.4695.

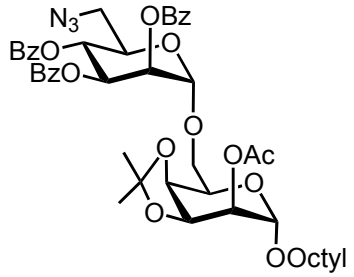
Octyl 2,3,4,-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.36)



Disaccharide **4.35** (137 mg, 0.13 mmol) was dissolved in THF (4 mL) and then 70% HF·pyridine (0.2 mL) and pyridine (1 mL) were added. After stirring overnight, the crude product was then diluted with CH_2Cl_2 (50 mL), washed with H_2O (15 mL), 1 M HCl (2 x 15 mL), and satd aq $NaHCO_3$ (15 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and the residue was purified by chromatography to give **4.36** as a colorless oil (82 mg, 77%): R_f 0.55 (1:1 hexane–EtOAc); $[\alpha]_D$ = –42.0 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.08–8.12 (m, 2H, ArH), 7.94–7.98 (m, 2H, ArH), 7.79–7.84 (m, 2H, ArH), 7.59–7.64 (m, 1H, ArH), 7.46–7.60 (m, 3H, ArH), 7.36–7.45 (m, 3H, ArH), 7.23–7.28 (m, 2H, ArH), 5.93 (dd, 1H, *J* = 10.1, 3.4 Hz, H-3′), 5.80 (dd, 1H, *J* = 10.1, 10.1 Hz, H-4′), 5.67 (dd, 1H, *J* = 3.4, 1.7 Hz, H-2′), 5.15 (d, 1H, *J* = 1.7 Hz, H-1′), 4.98 (dd, 1H, *J* = 6.2, 2.9 Hz, H-2), 4.90 (d, 1H, *J* = 6.2 Hz, H-1), 4.62 (dd, 1H, *J* = 7.6, 2.9 Hz, H-3), 4.39 (dd, 1H, *J* = 7.6, 1.8 Hz, H-4), 4.18 (ddd, 1H, *J* = 10.1, 3.9, 2.2 Hz, H-5′), 4.05 (ddd, 1H, *J* =

7.0, 4.9, 1.8 Hz, H-5), 3.92 (dd, 1H, *J* = 10.4, 7.0 Hz, H-6a), 3.74–3.91 (m, 4H, H-6a′, H-6b′, H-6b, octyl OC*H*$_2$), 3.46 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OC*H*$_2$), 2.75 (dd, 1H, *J* = 8.4, 5.7 Hz, O*H*), 2.16 (s, 3H, C(O)C*H*$_3$), 1.57–1.65 (m, 2H, octyl C*H*$_2$), 1.51 (s, 3H, C(C*H*$_3$)$_2$), 1.34 (s, 3H, C(C*H*$_3$)$_2$), 1.14–1.54 (m, 10H, octyl C*H*$_2$), 0.83 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 166.4 (*C*=O), 165.4 (*C*=O), 165.3 (*C*=O), 133.6 (Ar), 133.5 (Ar), 133.1 (Ar), 129.9(1) (2C, Ar), 129.8(6) (2C, Ar), 129.7 (2C, Ar), 129.3 (Ar), 129.2 (Ar), 128.8 (Ar), 128.6 (2C, Ar), 128.5 (2C, Ar), 128.3 (2C, Ar), 110.9 (*C*(CH$_3$)$_2$), 97.6 (C-1′/C-1), 97.5 (C-1′/C-1), 74.3 (C-4), 72.4 (C-3), 71.1 (C-5′), 70.6 (C-2′), 70.5 (C-2), 69.6 (C-3′), 68.7 (C-5), 68.3 (octyl O*C*H$_2$), 67.3 (C-4′), 66.7 (C-6), 61.5 (C-6′), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.0(9) (C(*C*H$_3$)$_2$), 26.0(7) (octyl *C*H$_2$), 25.3 (C(*C*H$_3$)$_2$), 22.6 (octyl *C*H$_2$), 21.1 (C(O)*C*H$_3$), 14.0 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{46}H_{56}O_{15}$: 871.3511. Found: 871.3508.

Octyl 6-azido-2,3,4,-tri-*O*-benzoyl-6-deoxy-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.37)

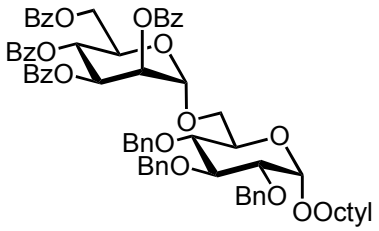


Disaccharide **4.36** (86 mg, 0.10 mmol) was dissolved in pyridine (1 mL) and the solution was cooled to 0 °C in an ice bath followed by the addition of *p*-toluenesulfonyl chloride (50 mg, 0.25 mmol). The reaction mixture was stirred overnight and then

diluted with CH_2Cl_2 (25 mL), washed with 1 M HCl (3 x 10 mL), satd aq $NaHCO_3$ (10 mL), and H_2O (10 mL). The organic layer was dried ($MgSO_4$) and concentrated to colorless oil. The crude intermediate was dissolved in DMF (2 mL) and sodium azide (65 mg, 1.0 mmol) was added and the solution was heated under reflux for 4 h. The crude product was then diluted with EtOAc (25 mL) and washed with H_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue purified by chromatography (3:1 hexane–EtOAc) to give **4.37** as a colorless oil (74 mg, 84%): R_f 0.27 (3:1 hexane–EtOAc); $[\alpha]_D$ = –11.3 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.08–8.14 (m, 2H, ArH), 7.91–7.96 (m, 2H, ArH), 7.78–7.83 (m, 2H, ArH), 7.59–7.65 (m, 1H, ArH), 7.47–7.55 (m, 3H, ArH), 7.35–7.45 (m, 3H, ArH), 7.23–7.29 (m, 2H, ArH), 5.82–5.88 (m, 2H, H-3′, H-4′), 5.68 (dd, 1H, *J* = 2.8, 1.7 Hz, H-2′), 5.12 (d, 1H, *J* = 1.7 Hz, H-1′), 4.99 (dd, 1H, *J* = 6.2, 2.8 Hz, H-2), 4.93 (d, 1H, *J* = 6.2 Hz, H-1), 4.64 (dd, 1H, *J* = 7.6, 2.8 Hz, H-3), 4.40 (dd, 1H, *J* = 7.6, 1.8 Hz, H-4), 4.36 (ddd, 1H, *J* = 10.1, 6.4, 2.6 Hz, H-5′), 4.06 (ddd, 1H, *J* = 7.6, 7.2, 5.1 Hz, H-5), 3.99 (dd, 1H, *J* = 10.2, 7.2 Hz, H-6a), 3.88 (dt, 1H, *J* = 9.8, 6.5 Hz, octyl OC*H*$_2$), 3.79 (dd, 1H, *J* = 10.2, 5.1 Hz, H-6b), 3.47–3.56 (m, 2H, H-6a′, octyl OC*H*$_2$), 3.44 (dd, 1H, *J* = 13.3, 2.6 Hz, H-6b′), 2.17 (s, 3H, C(O)C*H*$_3$), 1.57–1.66 (m, 2H, octyl C*H*$_2$), 1.54 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 1.12–1.38 (m, 10H, octyl C*H*$_2$), 0.82 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 165.6 (*C*=O), 165.4 (*C*=O), 165.2 (*C*=O), 133.5 (2C, Ar), 133.1 (Ar), 129.9 (2C, Ar), 129.8 (2C,

Ar), 129.7 (2C, Ar), 129.3 (Ar), 129.1 (Ar), 128.8 (Ar), 128.6 (2C, Ar), 128.5 (2C, Ar), 128.3 (2C, Ar), 111.0 (*C*(CH_3)$_2$), 97.6 (C-1), 97.2 (C-1′), 74.1 (C-4), 72.4 (C-3), 70.8 (C-2), 70.4 (C-5′), 70.2 (C-2′), 69.7 (C-5), 68.3 (C-3′), 68.2 (octyl O*C*H_2), 67.9 (C-4′), 66.4 (C-6), 51.3 (C-6′), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.1 (octyl *C*H_2), 26.0 (C(*C*H_3)$_2$), 25.4 (C(*C*H_3)$_2$), 22.6 (octyl *C*H_2), 21.1 (C(O)*C*H_3), 14.0 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{46}H_{55}N_3O_{14}$: 896.3576. Found: 896.3573. FTIR: 2102.6 cm^{-1}.

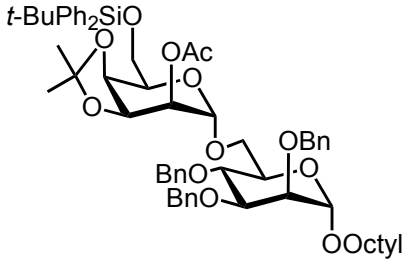
Octyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4.38)



Thioglycoside **4.12** (39 mg, 0.056 mmol), alcohol **4.16** (25 mg, 0.045 mmol), and powdered 4 Å molecular sieves (75 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (3 mL) was added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (16 mg, 0.068 mmol) and TMSOTf (2 μL, 0.014 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (4:1 hexane–EtOAc) to give **4.38** (47 mg, 92%) as a pale yellow oil. R_f 0.26 (4:1 hexane–EtOAc); $[\alpha]_D$ = +16.4 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.04–8.12 (m, 4H, ArH), 7.90–7.94 (m, 2H, ArH), 7.82–7.87 (m, 2H, ArH), 7.49–7.63 (m,

3H, ArH), 7.25–7.47 (m, 24H, ArH), 6.09 (dd, 1H, *J* = 10.1, 10.1 Hz, H-4′), 5.90 (dd, 1H, *J* = 10.1, 3.3 Hz, H-3′), 5.75 (dd, 1H, *J* = 3.3, 1.8 Hz, H-2′), 5.18 (d, 1H, *J* = 1.8 Hz, H-1′), 5.05 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 5.04 (d, 1H, *J* = 11.7 Hz, PhC*H*$_2$), 4.85 (d, 1H, *J* = 11.0 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 11.7 Hz, PhC*H*$_2$), 4.79 (d, 1H, *J* = 3.7 Hz, H-1), 4.72 (d, 1H, *J* = 11.7 Hz, PhC*H*$_2$), 4.69 (d, 1H, *J* = 11.7 Hz, PhC*H*$_2$), 4.65 (dd, 1H, *J* = 12.1, 2.4 Hz, H-6a′), 4.43 (ddd, 1H, *J* = 10.1, 4.4, 2.4 Hz, H-5′), 4.37 (dd, 1H, *J* = 12.1, 4.4 Hz, H-6b′), 4.07 (dd, 1H, *J* = 9.2, 9.2 Hz, H-3), 3.97 (dd, 1H, *J* = 11.0, 5.1 Hz, H-6a), 3.92 (ddd, 1H, *J* = 9.0, 5.1, 1.5 Hz, H-5), 3.83 (dd, 1H, *J* = 11.0, 1.5 Hz, H-6b), 3.73 (dt, 1H, *J* = 9.8, 7.0 Hz, octyl OC*H*$_2$), 3.59 (dd, 1H, *J* = 9.2, 3.7 Hz, H-2), 3.56 (dd, 1H, *J* = 9.2, 9.0 Hz, H-4), 3.47 (dt, 1H, *J* = 9.8, 6.6 Hz, octyl OC*H*$_2$), 1.64–1.76 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.46 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (Ar), 165.5 (Ar), 165.3 (Ar), 165.2 (Ar), 138.9 (Ar), 138.4 (Ar), 138.3 (Ar), 133.4, 133.1, 133.0(2), 130.0(0), 129.8(5), 129.8(0), 129.7(5), 129.7(3), 129.4, 129.2, 129.1, 128.6, 128.4(8), 128.4(3), 128.4(1), 128.3(6), 128.2(7), 127.9(8), 127.9(2), 127.8, 127.7(7), 127.7(0), 127.5 (35C, Ar), 97.8 (C-1′, $^1J_{C,H}$ = 174.6 Hz), 96.5 (C-1), 82.1 (C-3), 80.5 (C-2), 77.9 (C-4), 75.6 (Ph*C*H$_2$), 75.1 (Ph*C*H$_2$), 73.1 (Ph*C*H$_2$), 70.3, 70.0 (C-2′, C-3′), 69.9 (C-5), 68.9 (C-5′), 68.3 (octyl O*C*H$_2$), 66.9 (C-4′), 66.7 (C-6), 62.7 (C-6′), 31.9 (octyl *C*H$_2$), 29.5(2) (octyl *C*H$_2$), 29.4(8) (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{69}H_{72}O_{15}$: 1163.4763. Found: 1163.4766.

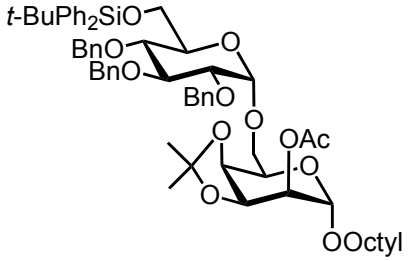
Octyl 2-*O*-acetyl-3,4-*O*-isopropylidene-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-mannopyranosyl-(1→6)-2,3,4,-tri-*O*-benzyl-α-D-talopyranoside (4.39)



Thioglycoside **4.13** (39 mg, 0.065 mmol), alcohol **4.10**[5] (33 mg, 0.057 mmol), and powdered 4 Å molecular sieves (75 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (3 mL) was added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (20 mg, 0.23 mmol) and TMSOTf (4 μL, 0.020 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (6:1 hexane–EtOAc) to give **4.39** as a colorless oil (54 mg, 81%): R_f 0.22 (6:1 hexane–EtOAc); $[\alpha]_D$ = +33.6 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.68–7.74 (m, 4H, ArH), 7.22–7.42 (m, 21H, ArH), 5.12 (d, 1H, *J* = 6.2, H-1′), 4.99 (dd, 1H, *J* = 6.2, 3.1 Hz, H-2′), 4.86 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.79 (d, 1H, *J* = 1.8 Hz, H-1), 4.72 (d, 1H, *J* = 12.4 Hz, PhCH_2), 4.68 (d, 1H, *J* = 12.4 Hz, PhCH_2), 4.63 (s, 2H, PhCH_2), 4.58 (dd, 1H, *J* = 7.6, 3.1 Hz, H-3′), 4.56 (d, 1H, *J* = 10.7 Hz, PhCH_2), 4.38 (dd, 1H, *J* = 7.6, 1.9 Hz, H-4′), 3.79–3.96 (m, 7H, H-5′, H-6a′, H6b′, H-3, H-4, H6a, H-6b), 3.75 (dd, 1H, *J* = 3.1, 1.8 Hz, H-2), 3.70 (ddd, 1H, *J* = 9.6, 5.8, 1.5 Hz, H-5), 3.60 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OCH_2), 3.31 (dt, 1H, *J* = 9.7, 6.5 Hz, octyl OCH_2), 2.04 (s, 3H, C(O)CH_3), 1.42–1.53 (m, 5H,

octyl CH_2, C(CH_3)$_2$), 1.21–1.34 (m, 13H, octyl CH_2, C(CH_3)$_2$), 1.06 (s, 9H, C(CH_3)$_3$), 0.89 (t, 3H, J = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.4 (*C*=O), 138.7 (Ar), 138.5 (Ar), 135.7 (2C, Ar), 135.6 (2C, Ar), 133.5(3) (Ar), 133.4(6) (Ar), 129.5(9) (Ar), 129.5(8) (Ar), 128.3(3), 128.2(9), 128.0, 127.8, 127.6, 127.5(9), 127.5(5), 127.5(1), 127.4 (20C, Ar), 110.4 (*C*(CH$_3$)$_2$), 97.6(2) (C-1, $^1J_{C,H}$ = 167.0 Hz), 97.6(0) (C-1′, $^1J_{C,H}$ = 175.8 Hz), 80.3 (C-3), 75.3 (C-2), 75.1(7) (C-4), 75.1(6) (Ph*C*H$_2$), 73.9 (C-4′), 72.7 (Ph*C*H$_2$), 72.2 (C-3′), 72.1 (Ph*C*H$_2$), 71.7 (C-5), 70.8 (C-2′), 70.3 (C-5′), 67.4 (octyl O*C*H$_2$), 66.3 (C-6), 62.4 (C-6′), 31.8 (octyl *C*H$_2$), 29.4 (2C, octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.8 (C(*C*H$_3$)$_3$), 26.2 (octyl *C*H$_2$), 26.1 (C(*C*H$_3$)$_2$), 25.3 (C(*C*H$_3$)$_2$), 22.7 (octyl *C*H$_2$), 20.9 (C(O)*C*H$_3$), 19.2 (*C*(CH$_3$)$_3$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{80}O_{12}Si$: 1067.5311. Found: 1067.5315.

Octyl 2,3,4-tri-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-α-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,4-*O*-isopropylidene-α-D-talopyranoside (4.40)



Thioglycoside **4.15** (143 mg, 0.18 mmol), alcohol **4.14** (53 mg, 0.14 mmol), and powdered 4 Å molecular sieves (150 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (5 mL) was added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (50 mg, 0.21 mmol) and TMSOTf

(8 μL, 0.042 mmol). The mixture was stirred for 1 h at 0 °C and neutralized with triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (4:1 hexane–EtOAc) to give the α glycoside (97 mg, 66%, R_f 0.31, 4:1 hexane–EtOAc) and β-glycoside (32 mg, 22%, R_f 0.38, 4:1 hexane–EtOAc) isomers, both as a colorless oils. Only the α isomer **4.40** was fully characterized. $[\alpha]_D$ = +40.1 (*c* 1.8, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.65–7.73 (m, 4H, ArH), 7.24–7.44 (m, 19H, ArH), 7.16–7.19 (m, 2H, ArH), 4.96 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.90–3.94 (m, 3H, PhC*H*$_2$, H-1′, H-2), 4.84 (d, 1H, *J* = 6.4 Hz, H-1), 4.81 (d, 1H, *J* = 10.8 Hz, PhC*H*$_2$), 4.78 (d, 1H, *J* = 11.9 Hz, PhC*H*$_2$), 4.74 (d, 1H, *J* = 11.9 Hz, PhC*H*$_2$), 4.66 (d, 1H, *J* = 11.1 Hz, PhC*H*$_2$), 4.57 (dd, 1H, *J* = 7.6, 2.8 Hz, H-3), 4.39 (dd, 1H, *J* = 7.6, 1.7 Hz, H-4), 4.01 (dd, 1H, *J* = 9.4, 9.4 Hz, H-3′), 3.90–3.98 (m, 2H, H-6a′, H-5), 3.87 (dd, 1H, *J* = 11.3, 1.6 Hz, H-6b′), 3.78–3.83 (m, 1H, H-5′), 3.69–3.78 (m, 3H, H-4′, H-6a, octyl OC*H*$_2$), 3.61 (dd, 1H, *J* = 10.0, 6.5 Hz, H-6b), 3.57 (dd, 1H, *J* = 9.4, 3.6 Hz, H-2′), 3.35 (dt, 1H, *J* = 9.8, 6.6 Hz, octyl OC*H*$_2$), 2.15 (s, 3H, C(O)C*H*$_3$), 1.42–1.52 (m, 5H, octyl C*H*$_2$, C(C*H*$_3$)$_2$), 1.31 (s, 3H, C(C*H*$_3$)$_2$), 1.15–1.30 (m, 10H, octyl C*H*$_2$), 1.06 (s, 9H, *t*-Bu), 0.85 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.2 (*C*=O), 138.8 (Ar), 138.6 (Ar), 138.5 (Ar), 135.8 (2C, Ar), 135.6 (2C, Ar), 133.7 (Ar), 133.3 (Ar), 129.5(5), 129.5(1), 128.4, 128.3, 128.1, 127.7, 127.6(3), 127.6(0), 127.5, 127.4 (21C, Ar), 110.6 (*C*(CH$_3$)$_2$), 97.4 (C-1, $^1J_{C,H}$ = 172.3 Hz), 96.9 (C-1′), 82.2 (C-3′), 80.4 (C-2′), 77.7 (C-4′), 75.9 (Ph*C*H$_2$), 74.9 (Ph*C*H$_2$), 74.2 (C-

4), 75.2 (C-4), 72.8 (Ph*C*H_2), 72.4 (C-3), 71.5 (C-5′), 71.0 (C-2), 68.5 (C-5), 68.0 (octyl O*C*H_2), 65.7 (C-6), 62.8 (C-6′), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.8 (C(*C*H_3)$_3$), 26.2 (C(*C*H_3)$_2$), 26.0 (octyl *C*H_2), 25.5 (C(*C*H_3)$_2$), 22.6 (octyl *C*H_2), 21.1 (C(O)*C*H_3), 19.3 (*C*(CH_3)$_3$), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{62}H_{80}O_{12}Si$: 1067.5311. Found: 1067.5312.

Bacterial strains and growth conditions

M. smegmatis mc^{2}155 was a generous gift from Professor William R. Jacobs, Jr. at the Albert Einstein College of Medicine. The bacteria were grown at 37 °C in 100 mL of Luria Bertoni (LB) broth medium containing 0.05% Tween 80 to an A_{600nm} of < 1.0 (~two days from a frozen bacterial stock). 50 mL liquid cultures were then transferred to 2 × 1 L of fresh media and cultured further for 24 h at 37 °C. Cells were harvested by centrifugation, washed with phosphate buffered saline (PBS) and stored at –20 °C until use.

Preparation of membrane fractions from *M. smegmatis*

The *M. smegmatis* cell pellet (~10 g wet weight) was washed and resuspended in 100 mL of 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (adjusted to pH 7.9 with KOH) containing 5 mM β-mercaptoethanol and 10 mM $MgCl_2$ supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche) at 4 °C. The cells were

subjected to two passes through a Thermo Spectronic French Pressure Cell Press at 20,000 psi. The cell lysate was centrifuged at 600 × *g* for 15 min and then at 27,000 × *g* for 20 min. The resulting supernatant was centrifuged at 100,000 × *g* for 60 min. The supernatant was carefully removed and the membrane pellets were gently resuspended in 1 mL of 50 mM MOPS buffer, pH 7.9, containing 5 mM β-mercaptoethanol and 10 mM $MgCl_2$. Protein concentrations were determined by the BCA™ Protein Assay (Pierce) using bovine serum albumin as the standard.

Radiochemical activity assays

The ManT enzyme activity was determined using the previously established cell-free system.[2] Unless indicated otherwise, the synthetic acceptor analogs at a concentration of 2.0 mM were incubated with 0.20 μCi of guanosine diphosphate mannopyranose (GDP-mannose), [mannose-2-^{3}H] (American Radiolabeled Chemicals, Inc., 20 Ci/mmol) in 50 mM MOPS buffer, pH 7.9, containing 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, and membrane fraction (94.6 μg of protein) in a total volume of 80 μL. All assays were performed in duplicate and control assays without acceptor were also performed in parallel to correct for the presence of endogenous acceptor. The enzymatic activities were determined using radiochemical SepPak C_{18} assays.[3] Briefly, after incubation at 37 °C for 1 h, the reactions were stopped by adding 100 μL of $CHCl_3$–MeOH (2:1 v/v) and the mixtures were centrifuged. The

supernatants were recovered and further diluted with H_2O before loading onto SepPak C_{18} cartridges (Waters). The unreacted donor was removed by washing the cartridges with H_2O (50 mL) and the radiolabeled products were eluted with MeOH (4.0 mL). The isolated products in the eluants were quantified by liquid scintillation counting on a Beckman LS6500 Scintillation Counter using 10 mL of Ecolite cocktail. For kinetic analysis, the ManT activities were determined using a range of acceptor concentrations. All other reaction conditions were identical to the cell-free assay as described above. Assays were performed under the conditions in which the formations of radiolabeled products were linear for both time and protein concentration. The kinetic parameters K_M and V_{max} were obtained by nonlinear regression analysis using the Michaelis-Menten equation with the GraphPad Prism 4.0 program (GraphPad Software, San Diego, CA).

Product characterizations from milligram-scale incubations

Large-scale ManT reactions were performed for the structural characterization using acceptor substrates **4.3**, **4.5**, **4.7** and **4.9**. A typical reaction containing 50 mM MOPS buffer, pH 7.9, 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 2 mM acceptor, 2 mM GDP-mannose, and the *M. smegmatis* membrane preparation, was incubated at 37 °C with gentle rotation for 2 days. The reaction was stopped by adding equal volume of $CHCl_3$–MeOH (2:1, v/v) and the mixtures were centrifuged. The

supernatant was recovered and further diluted with H_2O before loading onto the C_{18} reverse-phase cartridge and the unreacted donor was washed away with H_2O (50 mL) and the product was eluted subsequently with MeOH (4 mL). The solvent was evaporated and the residue was redissolved in H_2O (50 μL). The conversion of the acceptor substrate to the enzymatic product was analyzed by MALDI mass spectrometry on a Voyager Elite time-of-flight spectrometer on sample suspended in 2,5-dihydroxy benzoic acid, using the delayed-extraction mode and positive-ion detection.

Glycosidic linkage analysis by exo-mannosidases

Large-scale ManT reactions (160 μL) were performed using acceptor substrates **4.3**, **4.5**, **4.7** and **4.9**, and the radioactive enzymatic products were purified as usual. After removal of methanol, the residues were redissolved in 200 μL H_2O. Ten microliters of the suspension were digested with exo-mannosidases, *Aspergillus saitoi* α-(1→2)-mannosidase (AS) (Glyko), jack bean α-(1→2,3,6)-mannosidase (JB) (Glyko) and *Xanthomonas manihotis* α-(1→6)-mannosidase (XM) (New England Biolabs), according to the manufacturers' procedures. After incubation for one day, the reaction mixtures were purified using a C_{18} reverse-phase cartridge as described above.

4.5. Bibliography

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Chapter 5

Thiooligosaccharides as Tools for the Study of α-(1→6)-Mannosyltransferase-Catalyzed Mannan Formation

5.1. Introduction

As described in the previous chapters, a model for the biosynthesis of mycobacterial LM/LAM was proposed by Brennan and coworkers (Figure 5-1).[1] The biosynthetic pathway requires a number of mannosyltransferases (ManT's)[2-6] and acyltransferases,[7] which act in concert by adding single monosaccharide residues and acyl groups to PI leading initially to AcPIM2 and then AcPIM4, a precursor for both AcPIM6 and LM/LAM.[8-10] PimE, a polyprenol phosphomannose (PPM) dependent α-(1→2)-mannosyltransferase, has been recently identified and shown to catalyze the conversion of AcPIM4 to AcPIM5.[6] However, it remains unclear if the same enzyme also mediates the α-(1→2)-mannosylation of AcPIM6 from AcPIM5.

In comparison with the process by which the PIMs are assembled, the steps and enzymes involved in LM/LAM biosynthesis are less defined. In particular, the enzyme (or enzymes) required for the production of the core mannan domain have remained elusive. Although it is known that the transferase(s) use PPM as the donor source, the isolation and characterization of PPM-dependent α-(1→6)-mannosyltransferase(s) involved in LM/LAM assembly has to date not been successful. One

outstanding question is how many ManTs are involved in synthesizing the the α-(1→6)-linked residues of the core mannan.

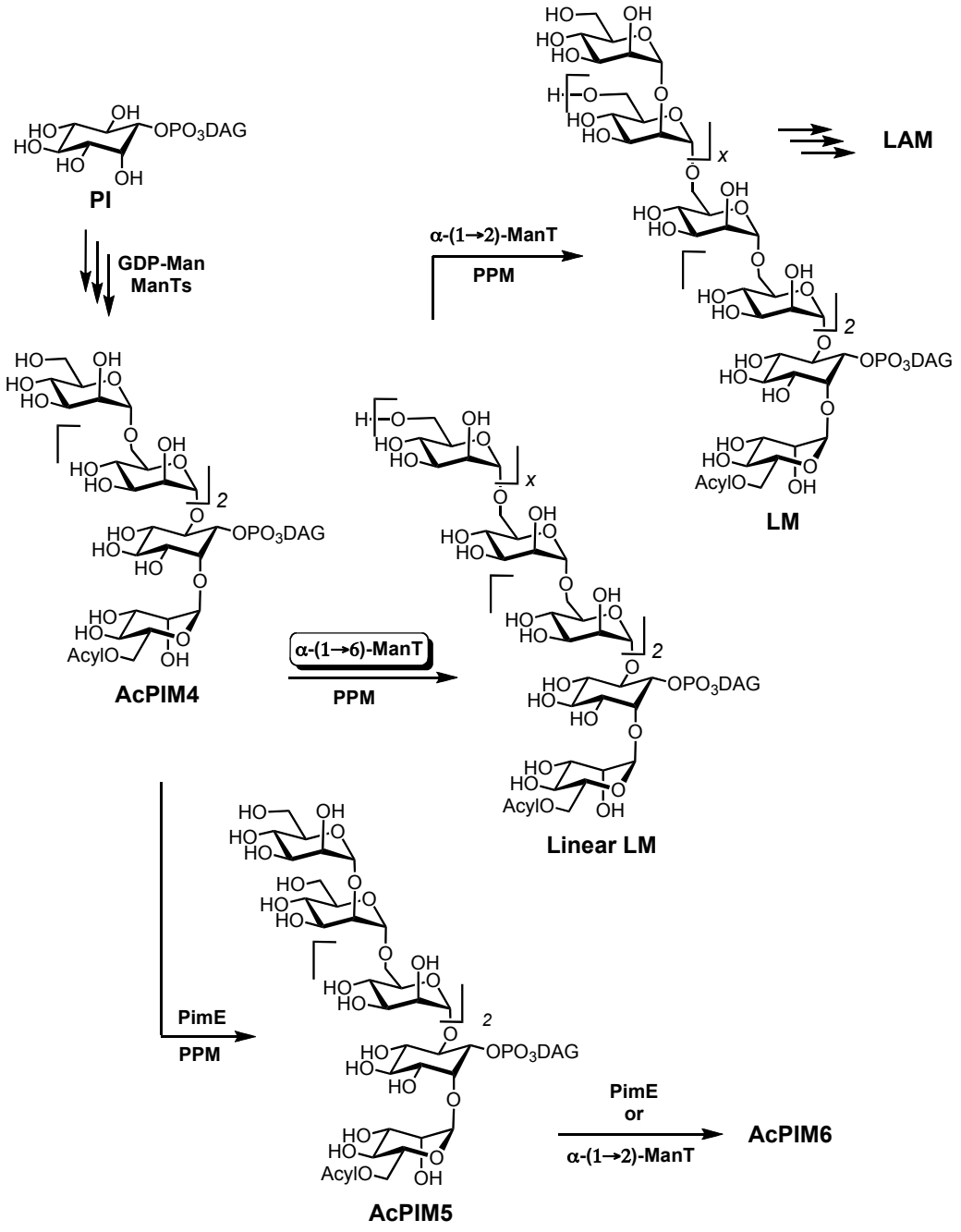
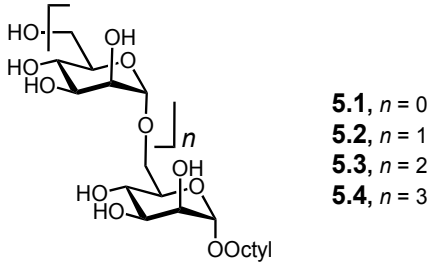


Figure 5-1. Proposed pathway for mycobacterial PIM, LM/LAM biosynthesis. DAG, diacylglycerol.

Recently, through genetic knockout and complementation studies, Brennan and coworkers have provided evidence suggesting that more than one α-(1→6)-ManT may be involved in full length LM/LAM biosynthesis.[11] In their investigation these authors identified a novel PPM-dependent α-(1→6)-ManT that is involved in the late stages of the polymer formation; deletion of the gene (Rv2174) encoding for the enzyme resulted in the formation of truncated form of LM. The α-(1→6)-mannosylation catalyzed by this novel ManT was also demonstrated by Besra and coworkers in a separate study.[12] A cell-free assay for the detection of α-(1→6)-ManT activity has been developed and has been used for nearly 10 years to screen potential substrates and inhibitors of this transferase activity.[13-19] However, it is currently unclear if this assay is measuring the activity of the protein encoded by Rv2174 or some other ManTs.

Our previous efforts in probing the substrate specificity of these α-(1→6)-ManTs using octyl mannosides **5.1**–**5.4** (Figure 5-2) revealed that, in agreement with earlier studies,[14,16] a disaccharide motif (**5.2**) is the



5.1, *n* = 0
5.2, *n* = 1
5.3, *n* = 2
5.4, *n* = 3

Figure 5-2. Structures of the synthetic linear octyl mannoside acceptors for mycobacterial mannosyltransferase(s).

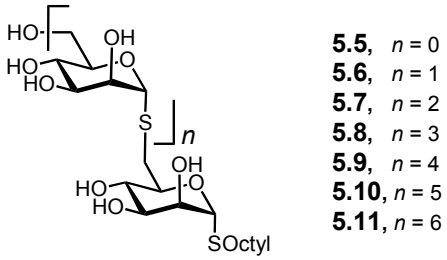
minimum substrate recognized by the enzymatic activity (Chapter 3). We hypothesized that larger oligosaccharides might be better mimics of the natural substrate, i.e., AcPIM4 or an elongated mannan fragment that is the substrate for a ManT acting late in the pathway, and thus show improved rates of product formation. However, tetrasaccharide **5.4** was shown to act as an acceptor of ManT with much lower efficiency than smaller oligomers. We proposed that this unexpected observation was due to the presence of an endogenous α-(1→6)-*endo*-mannosidase[14] in the crude membrane fraction used in the assay, which catalyzed the removal of trisaccharide units from the nonreducing end of both the tetrasaccharide substrate and the pentasaccharide product. Thus, we envisioned that the preparation of larger oligosaccharides would not provide us with significant additional insights into the specificity of these ManTs.

Challenged by this problem, we considered the preparation of larger oligosaccharide analogs that would be resistant to *endo*-mannosidase cleavage. Such compounds could also be used to address another unanswered question in LAM/LM biosynthesis: the point at which the α-(1→2)-Man*p* branching residues are added to the mannan. It had been believed that the α-(1→2)-branching only takes place upon the complete synthesis of the α-(1→6)-linked mannan.[1,14] However, in recent work from the Brennan group, which identified the PPM-dependent α-(1→2)-ManT involved in LM core branching, it was proposed that the α-(1→6) and α-(1→2) mannosyltransferases work in a concerted manner.[11]

Work presented in Chapter 3 of this thesis supports a pathway in which the branching residues are added after the chain is fully extended. First, we demonstrated that disaccharides possessing methyl groups at O-2 were shown not to be substrates for the enzyme. If a methyl group at this position prevents turnover by these ManTs, then the presence of an even larger monosaccharide residue would also stop further α-(1→6)-mannosylation. Second, treatment of the pentasaccharide product resulting from tetrasaccharide **5.4** with an α-(1→2) specific mannosidase did not lead to cleavage of the product. Based on this latter result, it is possible to suggest that the α-(1→2)-mannosylation may require longer chain lengths (greater than four α-(1→2)-mannopyranoside residues). One other possibility is that this enzymatic activity is not present or inactive under the assay conditions. However, as outlined above for the α-(1→6)-ManT, the interference of *endo*-mannosidase in the cell free assay, and the absence of a pure α-(1→2)-ManT enzyme prohibited us from further investigating the effect of the acceptor length on this enzymatic activity, and the spatial relationship of the α-(1→6) and α-(1→2) mannosylations in the pathway.

As a choice of *endo*-mannosidase stable oligosaccharide mimics, we selected *S*-linked oligosaccharides (thiooligosaccharides) **5.5**–**5.11** (Figure 5-3). It has been well documented that thiooligosaccharides are resistant to enzymatic hydrolysis.[20] In fact, these molecules are valuable tools for structural and mechanistic studies of glycosyl hydrolases. To

date, there are numerous reports in the literature using this class of carbohydrate mimetic as competitive inhibitors of glycosidases[21] and for the formation of stable complexes with glycosidases for X-ray crystallographyic analysis.[22] Conversely, only a few examples of the uses of thiooligosaccharide analogs as substrates for other classes of carbohydrate-active proteins have been published.



5.5, *n* = 0
5.6, *n* = 1
5.7, *n* = 2
5.8, *n* = 3
5.9, *n* = 4
5.10, *n* = 5
5.11, *n* = 6

Figure 5-3. Structures of the synthetic *S*-linked octyl mannoside acceptors **5.5**–**5.11**.

One example is work from Southwick and coworkers, which demonstrated that the NodA (acyltransferase), NodB (deacetylase) and NodH (sulfotransferase) from *Sinorhizobium meliloti* synthesize a Nod factor analog from a thiochitotetramer starting material (Figure 5-4).[23] This chitin thiotetrasaccharide has also been shown to be a potent inducer of the extracellular regulated kinases (EKR) pathway.[24] In addition, the branched *S*-linked oligosaccharides act as efficient elicitors to induce phytoalexin accumulation in soybean cotyledon tissue and bind to a glucan-binding protein of soybean.[25]

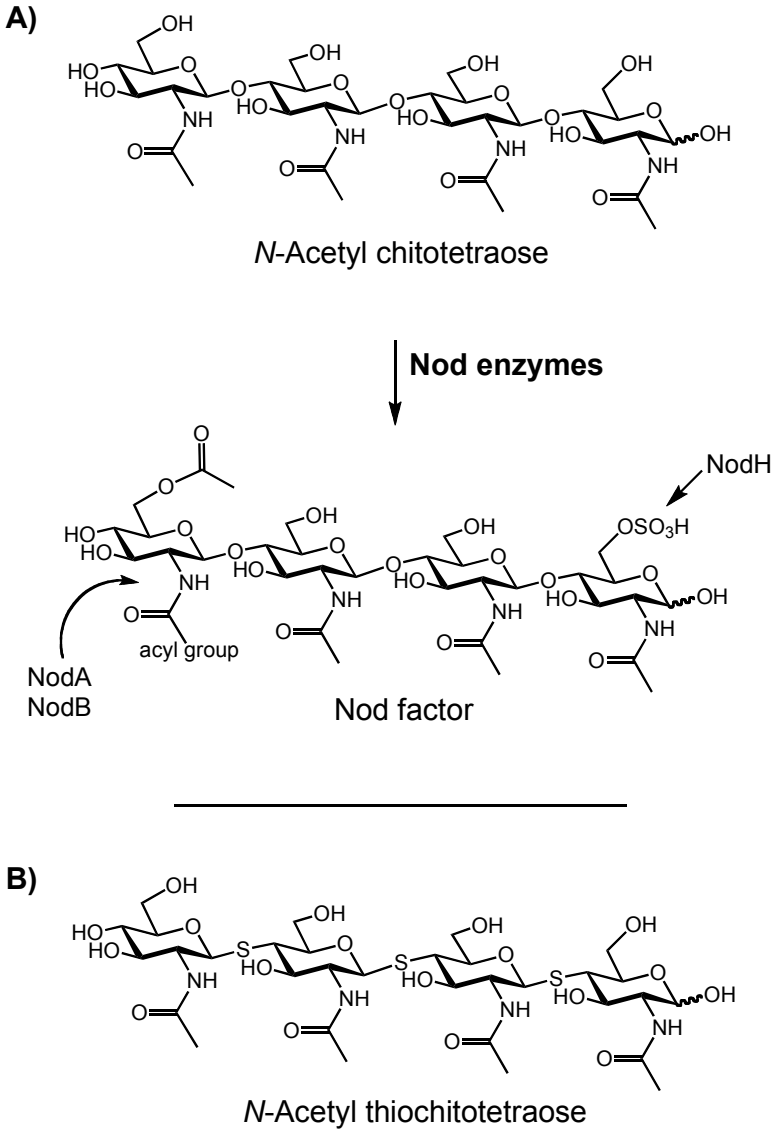


Figure 5-4. Activity of *Sinorhizobium meliloti* NodAB and NodH enzymes. A) Formation of the Nod factor, β-(1,4)-*N*-acetylglucosamine oligosaccharide, catalyzed by the Nod enzymes (NodA, acyltransferase, NodB, deacetylase and NodH, sulfotransferase. B) Structure of Nod factor mimic analog, *N*-acetyl thiochitotetrose.

In other work, Bundle and coworkers reported a chemoenzymatic approach for thiooligosaccharide synthesis using α-(1→3)-

galactosyltransferase from *Neisseria menigitidis* and observed an unexpected result, in which the enzymatic product is a tetrasaccharide instead of a trisaccharide (Figure 5-5A). It was shown that the enzyme catalyzes the glycosyl transfer to a trisaccharide containing a sulfur glycosidic linkage, although with much lower efficiency than the natural substrate.[26] Moreover, it has also been shown that the thiotrisaccharide analogs serve as acceptors for *N*-acetylglucosaminyltransferase-V with 2- to 3-fold increase in V_{max} but higher K_M values as compared with their parent compound.[27]

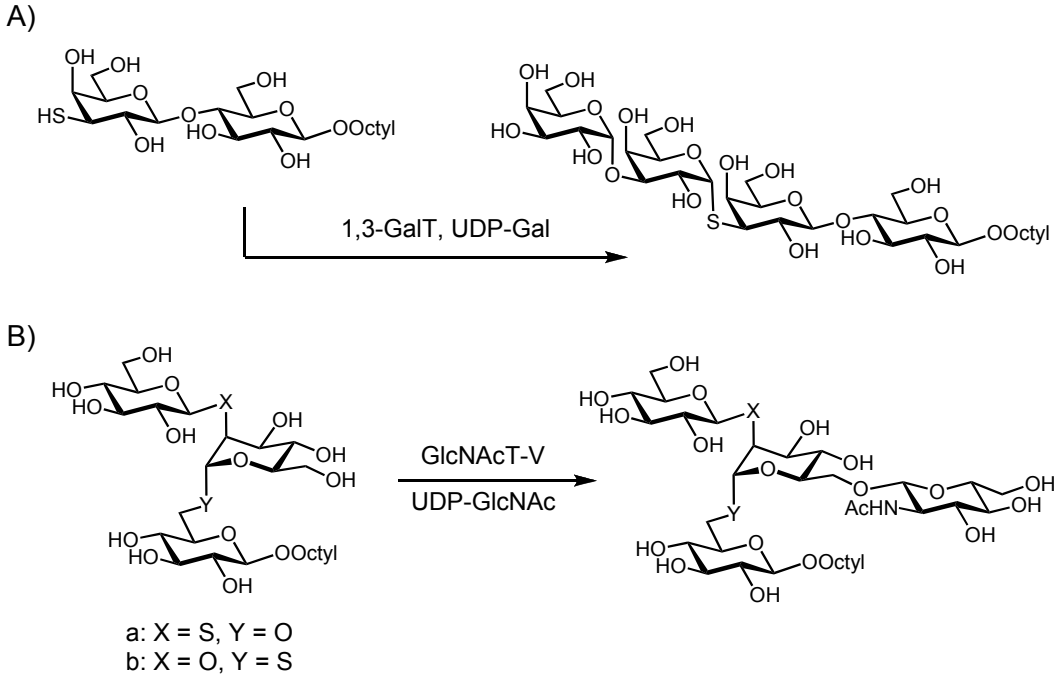
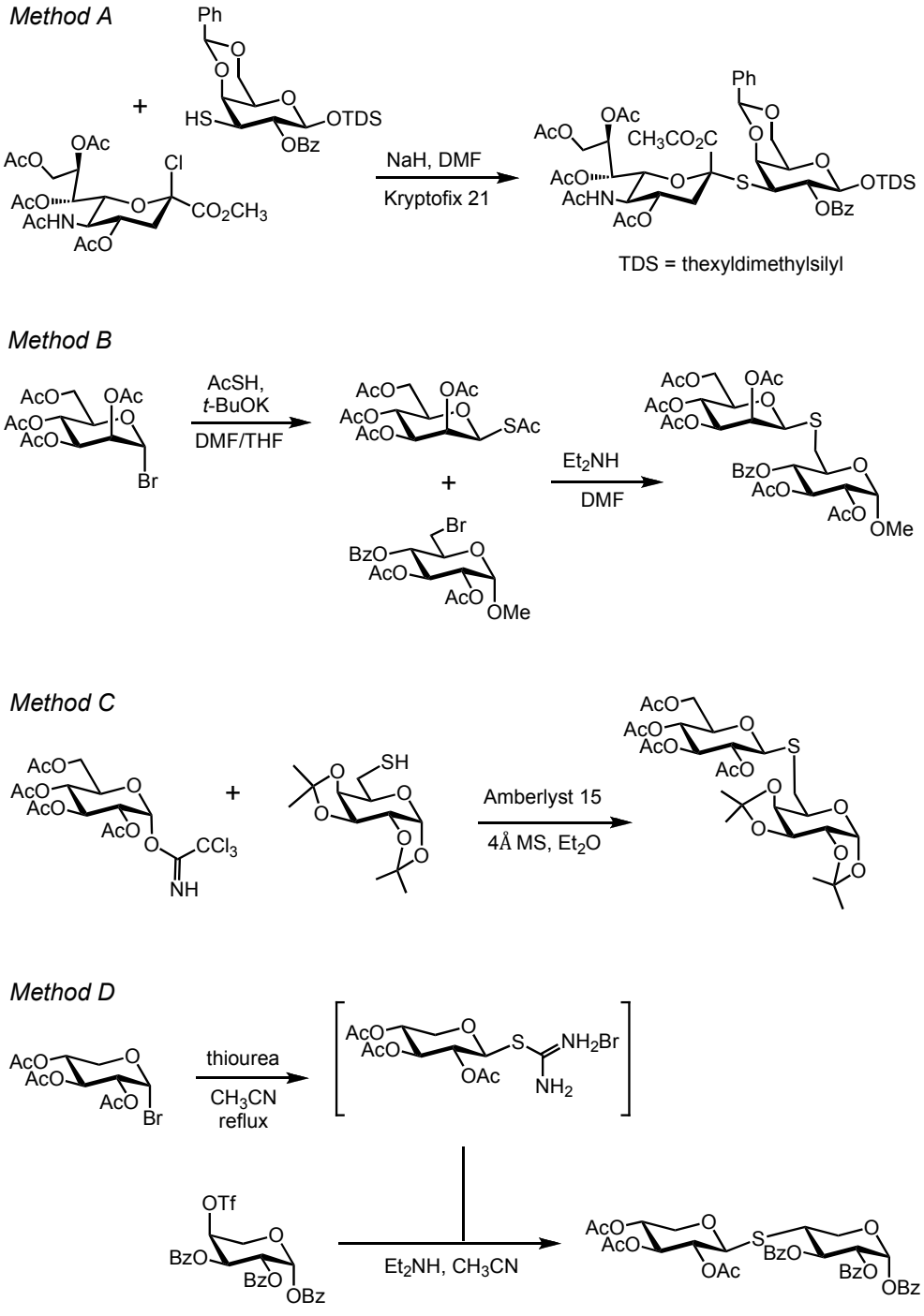


Figure 5-5. Enzymatic synthesis of thiooligosaccharides. A) Reaction catalyzed by α-(1→3)-galactosyltransferase (1,3-GalT). B) Reactions catalyzed by *N*-acetylglucosaminyltransferase-V (GlcNAcT-V).

Given this background, we speculated that larger thiooligomannoside acceptors may be resistant to *endo*-mannosidase cleavage, due to the presence of sulfur-glycosidic linkages and, more importantly, be recognized by the ManT of interest to us. Herein, we describe the synthesis of a homologous series of thiosaccharides **5.5**–**5.11** (Figure 5-3) and also the biochemical evaluation of these analogs using the ManT assay. We envisioned these analogs would be useful to address questions regarding the sequence of the α-(1→6)- and α-(1→2)-mannosylation reactions, and also the effect of acceptor length on the α-(1→6)-ManT catalysis.

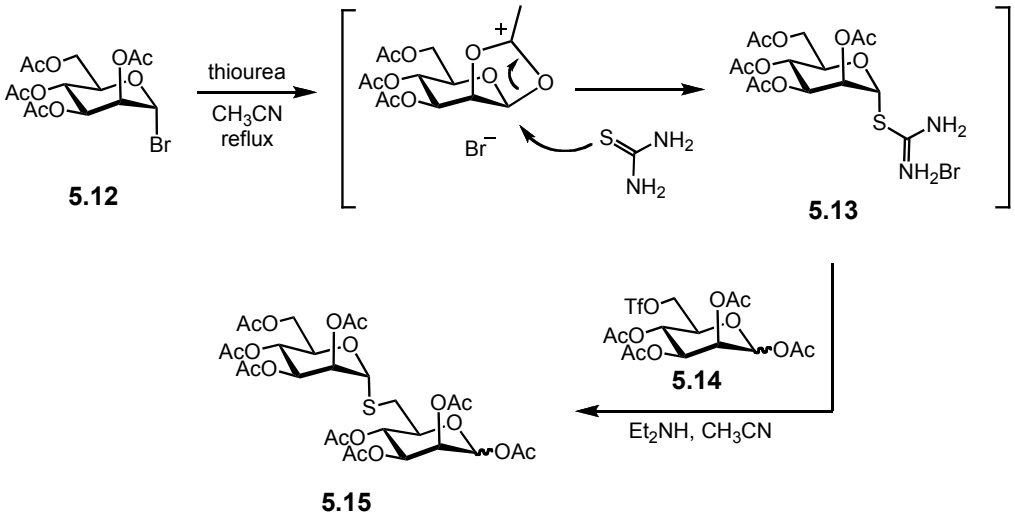
5.2. Results And Discussion

Many methods for the formation of thioglycosidic linkages have been reported in the literature and summarized in a recent review by Pachamuthu and Schmidt.[28] Some of these common approaches are illustrated in Scheme 5-1. Due to the high nucleophility of thiolate group, the thioglycosidic linkage is readily formed by the base-promoted S_N2 displacement of glycosyl halide (Method A),[29] or halo- or triflate-activated glycoside (Method B and D).[30,31] Alternatively, the thioglycosidic bond can also be formed by coupling a sugar donor, such as *O*-glycosyl trichloroacetimidate, with a thiol acceptor under acidic conditions (Method C).[32]



Scheme 5-1. Some common approaches for the synthesis of thioglycosidic linkages.

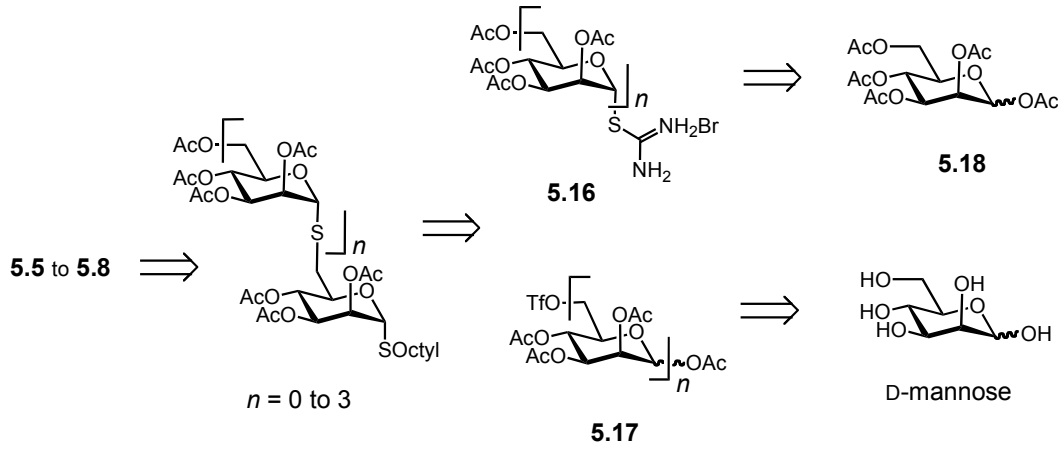
We envisioned that the α-(1→6)-linked thiomannopyranosides **5.5**–**5.11** could be obtained using an efficient approach (Scheme 5-1, Method D) that was recently developed by Ibatullin and coworkers.[31] As illustrated in Scheme 5-2, reaction of 2,3,4,6 tetra-*O*-acetyl-α-D-mannopyranosyl bromide[33] (**5.12**, Scheme 5-2) with thiourea under mild conditions should lead to the formation of isothiouronium α-mannoside **5.13**.[34] Notably, reactions of glycosyl halides with thiol acceptors by the base-promoted methods (Scheme 5-1, Methods A and B) would lead to the formation of thioglycosides with opposite stereochemistry at the anomeric centers to their precursors (e.g., reaction of **5.12** with potassium thioacetate (Method B) leads to the β-thiomannoside as reported by the Bundle group).[30] The conversion of the isothiourea group of **5.13** to thiolate sets up the stage for the S_N2 displacement of triflate **5.14** and results in the desired α-(1→6)-thioglycoside **5.15**.



Scheme 5-2. Formation of α-(1→6)-linked thiomannopyranoside.

5.2.1. Synthesis of sulfur-linked octyl mannopyranosides 5.5–5.11

Following the retrosynthetic analysis described in Scheme 5-2, we envisioned the synthesis of octyl mannopyranosides **5.5**–**5.8** could be achieved from the coupling of the key intermediates **5.16** with triflate **5.17** (or octyl bromide for **5.5**) (Scheme 5-3). In turn, both **5.16** and **5.17** could be easily prepared from mannosyl peracetate **5.18**[35] and D-mannose, respectively.

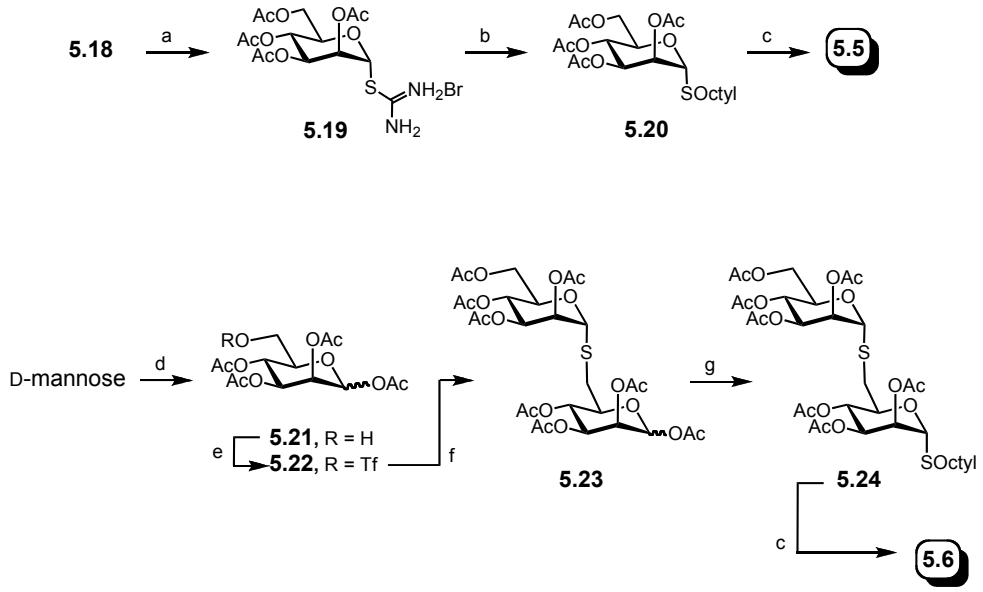


Scheme 5-3. Retrosynthetic analysis of **5.5–5.8**.

5.2.1.1. Synthesis of 5.5–5.8

As illustrated in Scheme 5-4, the precursor required for the synthesis of **5.5** and **5.6**, isothiouronium salt **5.19**, was obtained through a two-step synthetic sequence starting from mannosyl peracetate **5.18**. First, **5.18** was converted to glycosyl bromide by reaction with HBr in acetic acid;[35] subsequent reaction with thiourea under reflux in acetone

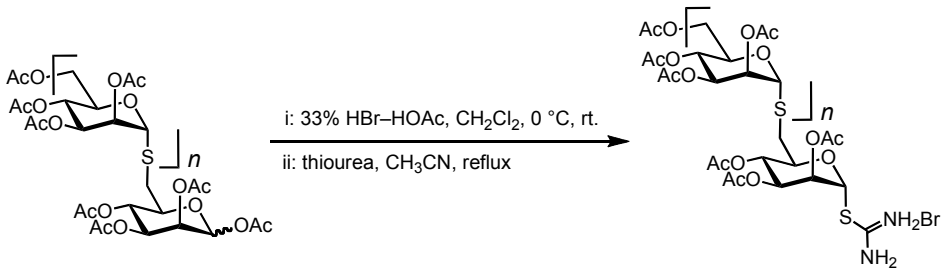
gave the desired product. This reaction afforded isothiouronium salt **5.19** in 45% yield after recrystallization from the aqueous solution.[34] Treatment of **5.19** with octyl bromide and triethylamine resulted in the formation of octyl mannopyranosides **5.20** in 78% yield. Subsequent removal of the acetyl groups from **5.20** using sodium methoxide gave the desired *S*-linked monosaccharide **5.5** (90%).



Scheme 5-4. Reagents and conditions: a) i: 33% HBr–HOAc, CH_2Cl_2, 0 °C→rt.; ii: thiourea, CH_3CN, reflux, 45%; b) $CH_3(CH_2)_7Br$, Et_3N, CH_3CN, 78%; c) NaOMe, MeOH, 90% for **5.5**, 89% for **5.6**; d) i: TrCl, DMAP, pyridine; then Ac_2O; ii: *p*-TSA, CH_2Cl_2–MeOH (2:1), 58%; e) Tf_2O, CH_2Cl_2–pyridine (20:1), –25 °C, 87%; f) **5.19**, Et_3N, CH_3CN, 79%; g) i: 33% HBr–HOAc, CH_2Cl_2, 0 °C→rt.; ii: thiourea, CH_3CN, reflux (in situ generation of **5.27**, Scheme 5-5); iii: $CH_3(CH_2)_7Br$, Et_3N, CH_3CN, 64%.

The triflate derivative (**5.22**) needed for the synthesis of **5.6** was synthesized from D-mannose in a three-step sequence as shown in Scheme 5-4. The primary hydroxyl group of the starting material was

protected with a trityl group, and the remaining hydroxyl groups were acetylated. The trityl group was then removed upon treatment with *p*-toluenesulfonic acid to provide the monosaccharide alcohol **5.21** (58% yield), which was reacted with triflic anhydride to furnish triflate derivative **5.22** in 87% yield as a 7:1 α:β mixture of anomers. Reaction of **5.22** with **5.19** in the presence of triethylamine provided a 79% yield of sulfur-linked disaccharide analog **5.23**. With **5.23** in hand, peracetylated octyl mannopyranoside **5.24** was synthesized via a route similar to that of **5.20**. The anomeric acetyl group in **5.23** was converted to the corresponding isothiouronium salt **5.27** in situ (Scheme 5-5) and subsequent treatment with octyl bromide and triethylamine resulted in the formation of octyl mannopyranosides **5.24** (64% yield). Final deprotection of **5.24** furnished the desired disaccharide **5.6** (89% yield). It should be noted that the larger isothiouronium salts **5.28** and **5.29** were also prepared from **5.25** and **5.26** by this approach.



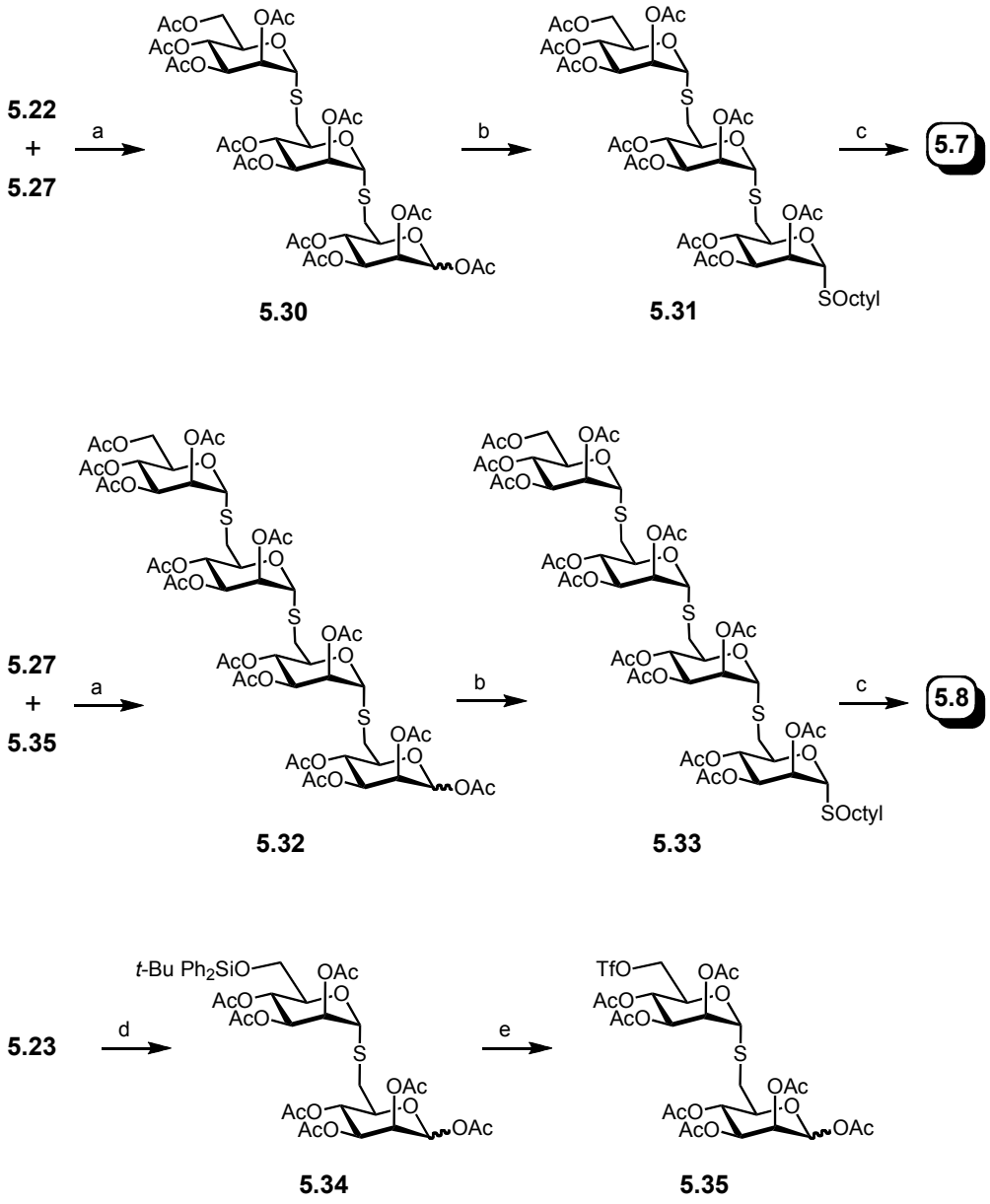
5.23, *n* = 1
5.25, *n* = 2
5.26, *n* = 3

5.27, *n* = 1
5.28, *n* = 2
5.29, *n* = 3

Scheme 5-5. Reagents and conditions: a) i: 33% HBr–HOAc, CH_2Cl_2, 0 °C→rt.; ii: thiourea, CH_3CN, reflux. The resulting isothioureas were generated in situ and used without purification.

The preparation of trimer **5.7** required the coupling of **5.27** (Scheme 5-5) with **5.22** as shown in Scheme 5-6. Upon the addition of triethylamine, thiourea intermediate **5.27** was converted to the corresponding thiolate, which then displaced the triflate group in **5.22** to afford trimannopyranose **5.30** in 45% overall yield. The moderate yield of the coupling step using this general strategy was probably due to the decomposition of reactive mannosyl bromides (the precursor for the formation of thiourea intermediate) and the elimination of the triflate group (E2 elimination instead of S_N2 displacement). Analogous to the synthesis of **5.24** in Scheme 5-4, the anomeric acetyl group in **5.30** at the reducing end was converted to the octyl aglycon to give **5.31** in 55% yield. The target *S*-linked analog **5.7** was obtained in good yield after Zemplén deacetylation (70%).

Similarly, the synthesis of tetrasaccharide **5.8** could be achieved from the coupling of **5.27** (Scheme 5-5) with **5.35** to provide **5.32** in 48% yield. To access the triflate **5.35** for the synthesis of the tetramer, thiodisaccharide **5.23** was first deacetylated and subsequently silylated with *tert*-butylchlorodiphenylsilane followed by acetylation, which provided **5.34** in 78% yield over the two steps. The silylated disaccharide **5.34** was treated with hydrogen fluoride in pyridine[37] and the resulting alcohol was then reacted with triflic anhydride to furnish triflate **5.35** in 84% yield over two steps. Intermediate **5.32** was converted to thiourea **5.29** in situ



Scheme 5-6. Reagents and conditions: a) **5.22** or **5.35**, Et_3N, CH_3CN, 45% for **5.30**, 48% for **5.32**; b) i: 33% HBr–HOAc, CH_2Cl_2, 0 °C→rt.; ii: thiourea, CH_3CN, reflux (in situ generation of **5.28** or **5.29**, Scheme5-5); iii: $CH_3(CH_2)_7Br$, Et_3N, CH_3CN, 55% for **5.31**; 28% for **5.33**; c) $NaOMe_3$, MeOH, 70% for **5.7**, 59% for **5.8**; d) i: NaOMe, MeOH; ii: *t*-$BuPh_2SiCl$, pyridine; then Ac_2O, 78% over two steps; e) i: HF·pyridine/ pyridine/ THF (1: 4: 20); ii: Tf_2O, CH_2Cl_2–pyridine (20:1), –25 °C, 84% over two steps.

(Scheme 5-5) and reacted with octyl bromide and triethylamine to provide **5.33** in a disappointing yield of 28%. Finally, deprotection using sodium methoxide gave the desired tetramer **5.8** in a yield of 59%. The relatively low yield of **5.33** (28%) is probably due to the formation and accumulation of other byproducts (e.g., decomposition of the glycosidic linkage under acidic conditions in the bromination step or hydrolysis of the reactive glycosyl bromide) over the three step synthetic sequence.

It is worth mentioning that the formation of sulfur-linked glycosidic linkages was evident from the ^{1}H and ^{13}C NMR spectra for all the thiomannoside derivatives synthesized in this study. For instance, in the ^{1}H NMR spectrum for **5.23**, the resonances for H-6a and H-6b of the expected multiplicity (dd) were present at 2.67 and 2.90 ppm, respectively. In addition, in the ^{13}C NMR spectrum, a resonance at 31.7 ppm could be assigned to C-6. Moreover, the α-stereochemistry of the glycosidic linkages of **5.20**, **5.24**, **5.31** and **5.33** was confirmed by the magnitude of the one-bond heteronuclear coupling constants for the anomeric carbon atoms ($^{1}J_{C\text{-}1,H\text{-}1}$).[36] In all cases, this value was between 167 and 172 Hz, clearly indicating the α-stereochemistry. On the other hand, the $^{1}J_{C\text{-}1,H\text{-}1}$ values of β-thiomannosides ranges from 148 to 160 Hz.[30,36]

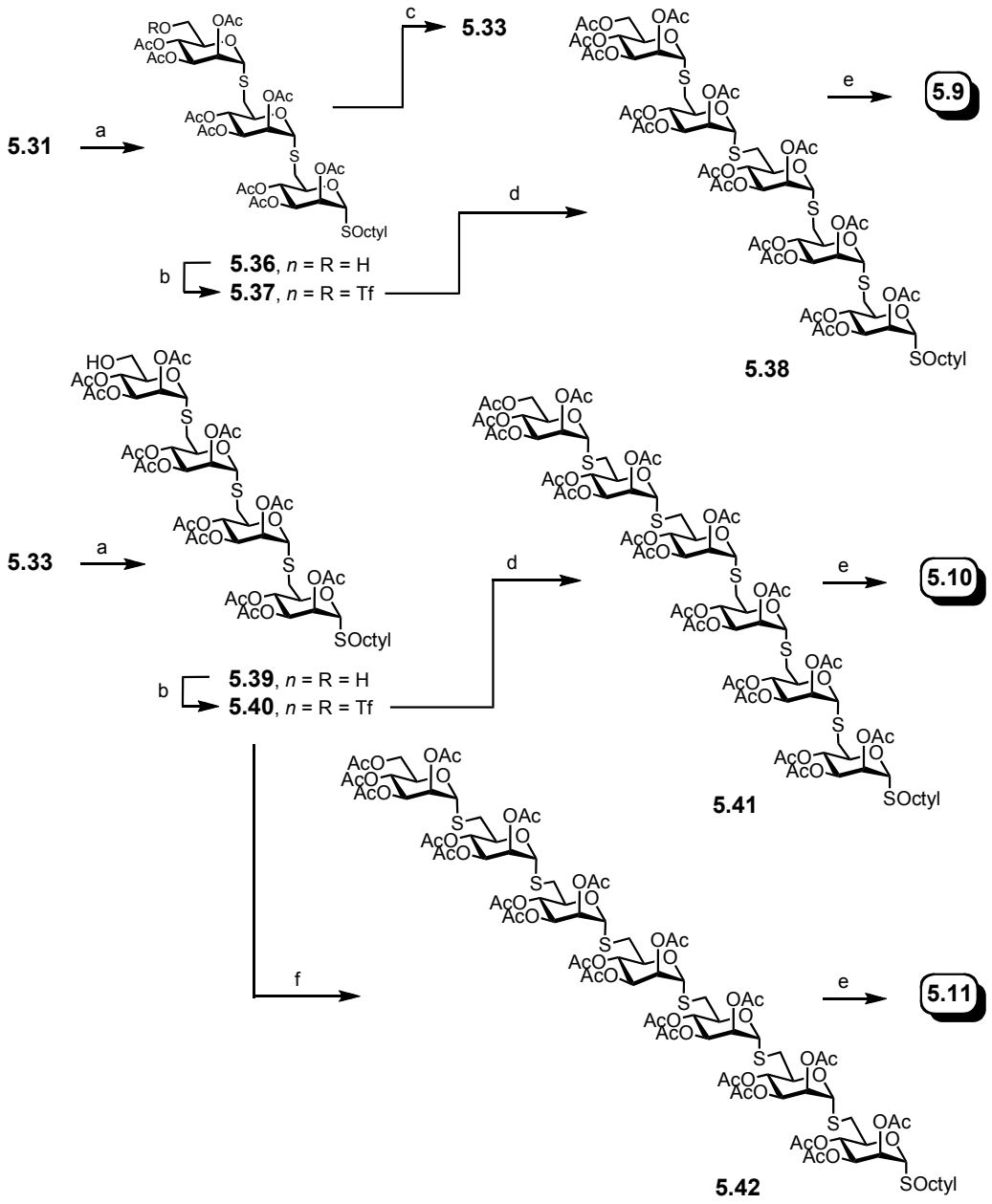
5.2.1.2. Synthesis of 5.9–5.11

We also attempted to prepare the octyl pentamannoside following the approach outlined in Scheme 5-6 by coupling the isothiouronium salt

5.29, derived from **5.26**, with monosaccharide triflate **5.22**. However, the formation of byproducts was more substantial than with the shorter oligomers, and low product yields were observed. Therefore, to access the longer oligosaccharides, the synthetic route was slightly modified.

As illustrated in Scheme 5-7, tri- and tetramannopyranosides **5.31** and **5.33** were first deacetylated, then silylated with *tert*-butylchlorodiphenylsilane followed by acetylation, and the silyl protective groups were subsequently removed using hydrogen fluoride in pyridine to give alcohols **5.36** and **5.39** in 69% and 85% yields, respectively, over three steps. To prepare pentamer **5.38**, alcohol **5.36** was first derivatized to triflate **5.37** (89% yield) and subsequently reacted with isothiouronium salt **5.27** (prepared from disaccharide **5.23** in situ). This coupling reaction provided thiosaccharide **5.38** in 63% yields. Remarkably, this alternative approach significantly improved the yield of the tetramer **5.31** by the coupling of **5.37** with **5.19** (97% yield as compared with the low product yield described in Scheme 5-5). Removal of the protective groups in **5.38** afforded **5.9** (63% yield).

Via the same approach, alcohol **5.39** was used for the synthesis of **5.10** and **5.11**. The triflate intermediate **5.40** derived from **5.39** (without column purification) was reacted with the isothiouronium salts **5.27** and **5.28** (prepared in situ from **5.23** and **5.25**, Scheme 5-5), to give protected saccharides **5.41** and **5.42** in 41% and 44% yields, respectively. Removal



Scheme 5-7. Reagents and conditions: a) i: NaOMe, MeOH; ii: *t*-BuPh_2SiCl, pyridine; then Ac_2O; iii: HF·pyridine, pyridine,THF (1: 4: 20), 69% for **5.36** and 85% for **5.39**; b) Tf_2O, CH_2Cl_2–pyridine (20:1), -25 °C, 89% for **5.37**; c) **5.19**, Et_3N, CH_3CN, 97% for **5.31**; d) **5.27** (generated in situ from **5.23**, Scheme 5-5), Et_3N, CH_3CN, 45% for **5.38** and 41% for **5.41**; e) NaOMe, MeOH, 63% for **5.9**, 40% for **5.10**, 22% for **5.11**; f) **5.28** (generated in situ from **5.25**, Scheme 5-5), Et_3N, CH_3CN, 44%.

of the protecting groups provided the target compounds **5.10** and **5.11** in 40% and 22% yields under unoptimized purification conditions. The low product yields are due to the unexpected poor solubilities of **5.10** and **5.11** in either methanol or water and loss of products during subsequent purification. We later found that both **5.10** and **5.11** could be dissolved in DMSO.

Due to heavy overlapping of resonances in the anomeric region of the ^{13}C NMR spectrum, the one-bond $^{1}J_{C\text{-}1,H\text{-}1}$ heteronuclear coupling constants of the protected oligomers **5.38**, **5.41** and **5.42** were not determined. However, the chemical shifts of the anomeric protons and carbons of the longer oligomers were in good agreement with those of **5.20**, **5.24**, **5.26** and **5.29**.

5.2.2. Screening As Substrate Acceptors For ManT

To determine whether the thiooligomannopyranosides act as active substrates for the α-(1$\rightarrow$6)-ManT(s), **5.5**–**5.11** were screened in the cell-free assay described earlier (Figure 3-1, Chapter 3).[16] By comparison with the known substrate, the *O*-linked disaccharide **5.2**, our initial analysis revealed that *S*-linked monomer **5.5** (~2%), was not an efficient substrate for the ManT (Figure 5-6). This observation is consistent with our previous results, in which the *O*-linked counterpart, monosaccharide **5.1**, was also inactive as an acceptor (Table 3-1, Chapter 3). On the other hand, thiomannoside analogs **5.6**–**5.11** are moderate substrates for ManT(s)

with relative mannosylation rates from 27% to 57% of that for the *O*-linked disaccharide **5.2**. Interestingly, a steady improvement of the relative enzyme activity was observed as the chain length increases from two to five Man*p* residues (from **5.6** to **5.9**). However, with the hexa- and heptamer (**5.10** and **5.11**) further improvement of the mannosyltransferase activity was not observed; instead with slightly lower activities than the pentamer **5.9** (48 and 33%) were detected.

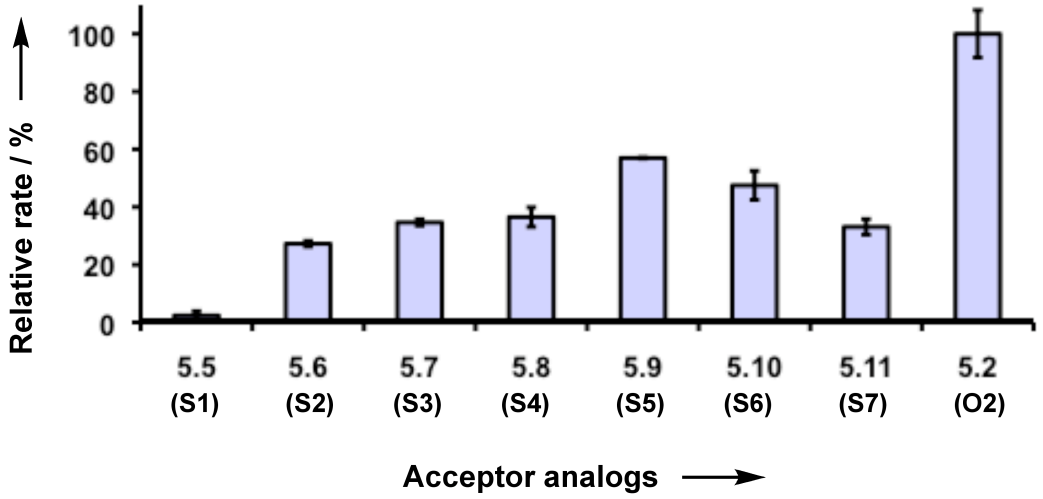


Figure 5-6. Acceptor specificity of the mycobacterial mannosyltransferase(s) with various thiosaccharide derivatives. Each acceptor at 2 mM was used and incubated with 0.2 μCi of [^{3}H] GDP-Man under the assay conditions as described in the experimental section. Relative activities for each acceptor are expressed as a percentage of the incorporation of [^{3}H]-mannose into the parent compound **5.2**. 100% activity corresponds to 0.35 pmol mg^{-1} min^{-1} for the mannosyltransferase activity. Abbreviations in brackets: S and O refer to sulfur- and oxygen-glycosidic linkages, respectively, and number refers to the length of acceptor analog. For example, S-2 refers to sulfur-linked octyl dimannoside.

5.2.3. Structural Analysis of Enzymatic Products

In addition to the PPM-dependent α-(1→6)-ManT(s), the crude membrane extract of *M. smegmatis* used in these assays also contains α-(1→2)-ManT's. For example, recent studies have identified three PPM-dependent α-(1→2)-ManT's involved in LM branching, the capping of the arabinan domain, and the biosynthesis of AcPim6.[4-6] An earlier study by Yokoyama and Ballou also detected trace amounts of products arising from α-(1→2)-ManT activity.[14] In addition, the α-(1→4)-ManT involved in the biosynthesis of 3-*O*-methyl-mannose polysaccharides were reported by Ballou to be present in cell membrane fractions from some mycobacteria.[38,39]

To determine if the observed incorporation of ^{3}H-labeled mannose into **5.6–5.11** arose solely from the action of the α-(1→6)-ManT(s), the glycosidic linkages of the products were characterized by *exo*-mannosidase digestions. The radiochemical assays were repeated using analogs **5.6–5.11** with ^{3}H-labelled GDP-Man and the radiolabeled enzymatic products were purified as usual. The [^{3}H]Man residues prepared from the corresponding *S*-linked acceptors were divided evenly and subjected to the enzymatic digestions. As illustrated in Figure 5-7, there is no significant difference of the radioactivities between the control experiments and the samples that had undergone treatment with α-Man-(1→2)-α-Man-specific *Aspergillus saitoi* α-(1→2)-mannosidase (AS).

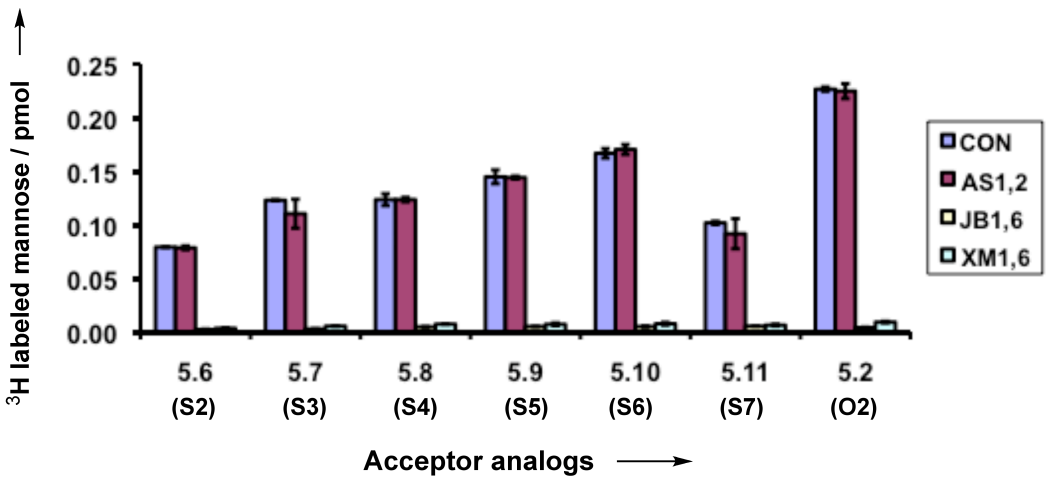


Figure 5-7. Enzymatic digestion of isolated ManT products. Each acceptor at 2 mM was used and incubated with [^{3}H] GDP-Man under the assay conditions as described in the experimental section. The radiolabeled enzymatic products were divided evenly after purification and treated with *exo*-mannosidases including *Aspergillus saitoi* α-(1→2)-mannosidase (AS), jack bean α-(1→2,3,6)-mannosidase (JB) and *Xanthomonas manihotis* α-(1→6)-mannosidase (XM). The mannosidase-digested samples were purified using a C_{18} reverse phase column and the radioactivities were compared with controls (CON) without any mannosidase treatment. Abbreviations in brackets: S and O refer to sulfur- and oxygen-glycosidic linkages, respectively, and number refers to the length of acceptor analog. For example, S-2 refers to sulfur-linked octyl dimannoside.

These results demonstrated that none of the mannosylated products contain α-(1→2)-glycosidic linkages. On the other hand, digestions of each radiolabeled product using α-mannosidases from jack bean (JB, α-(1→2,3,6)-specific) and *Xanthomonas manihotis* (XM, unbranched α-(1→6)-specific) essentially removed all the [^{3}H]-labeled mannose units. The combination of these last two mannosidases enabled the relative proportions of α-(1→6) and α-(1→3/4) linkages to be determined. Taken

together, these exo-mannosidase degradation experiments indicate that the observed Man*p* residues were added exclusively by α-(1→6)-specific mannosyltransferase(s) present in the cell free assay mixtures.

To gain additional insight into the structure of the products formed in these reactions, larger scale incubations of **5.6–5.11** with GDP-mannose and cell membrane crude fraction were performed. The enzymatic products, after purification using a C_{18} SepPak cartridge, were analyzed by MALDI mass spectrometry as shown in Figure 5.8. The mass spectrum of the product from the enzymatic reaction of **5.6** (Figure 5.8 A) indicated the formation of not only a trisaccharide product at *m/z* = 671 but also a homologous series of oligosaccharides at *m/z* = 833, 995, 1157, 1481, which corresponds to tetra-, penta-, hexa- and octasaccharide oligomers. The formation of longer oligosaccharides were also observed from the mass spectra of the isolated products from the reactions of **5.7–5.11** (Figure 5.8 B–F).

In all cases, no apparent degradation by α-(1→6)-*endo*-mannosidase were detected from the mass spectra of **5.6–5.11** and TLC of the product mixtures of **5.6** and **5.9** (data not shown). Furthermore, assuming that all of the oligosaccharides ionize to similar degrees, the relative abundance of the different oligosaccharides could be estimated from the mass spectrum of the product.

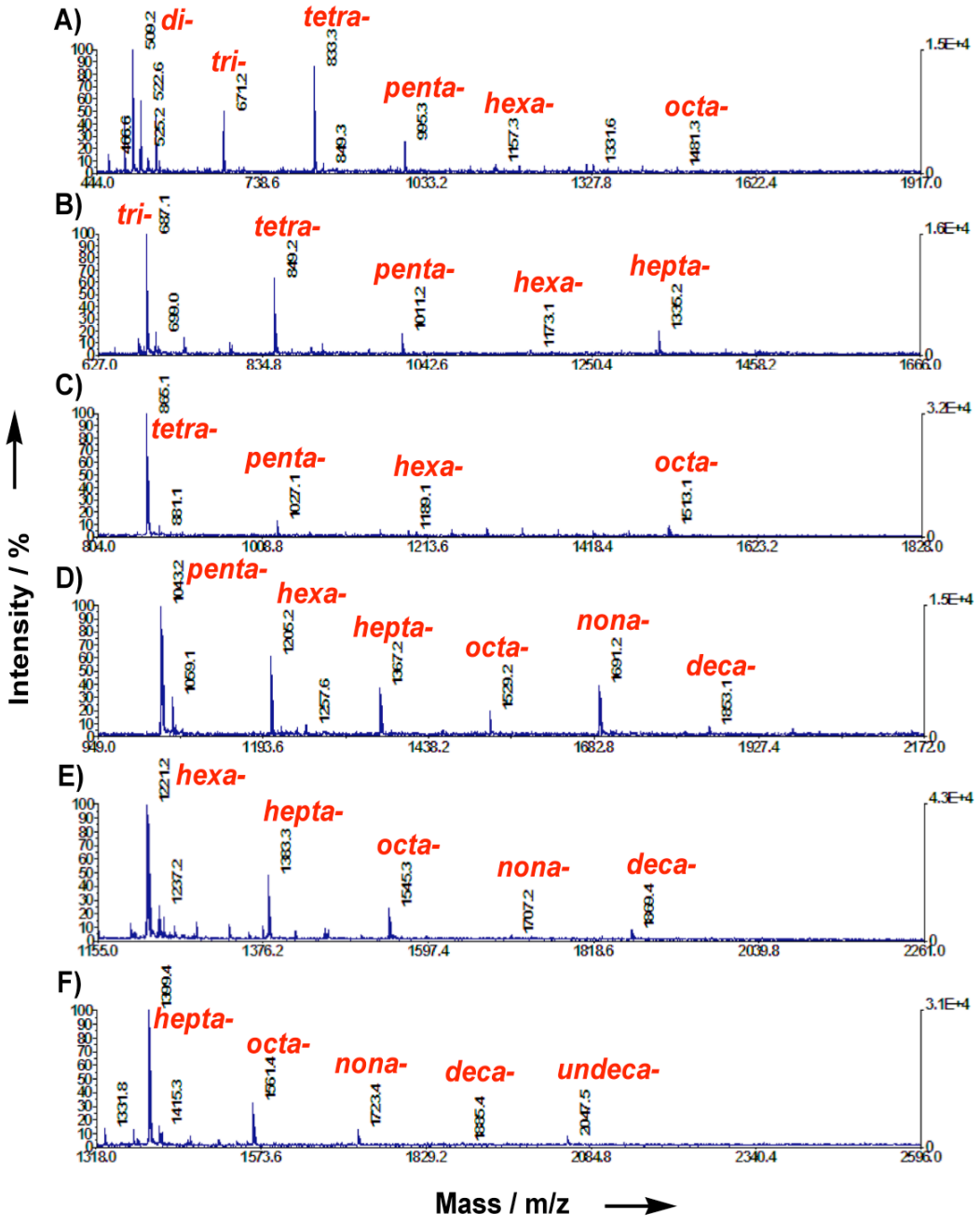


Figure 5-8. MALDI mass spectra of enzymatic products isolated from incubation mixtures using analogs **5.6** (A), **5.7** (B), **5.8** (C), **5.9** (D), **5.10** (E), and **5.11** (F) at 2 mM concentrations. The reactions were performed as described in the experimental section. The enzymatic products were isolated and the masses were determined by MALDI mass spectrometry. The found values correspond to the sodium adducts.

As revealed by Figure 5-8A, B and C, the enzyme(s) seemed to react more slowly with the tetrasaccharide acceptor. For example, in the case of **5.6**, upon the addition of two Man*p* residues, the resulting product, an *S*/*O*-linked tetramannoside, was a poor substrate for ManT(s) and resulted in the accumulation of this tetrasaccharide (*m/z* = 833, Figure 5-5A). This is further supported by the MS profile obtained from the enzymatic reaction using **5.7** where the ratio of peaks between the pentasaccharide and tetrasaccharide also suggests accumulation of the latter (Figure 5.8B). The same conclusion can be drawn from the data with **5.8**; only small amounts of enzymatic products, judged by the relative peak intensities for larger oligomers, were observed from Figure 5.8C. In contrast, reaction of **5.9–5.11** resulted in the formation of a series of longer polymers as shown in Figures 5-8D, E and F.

It is not clear why certain lengths of products such as tetrasaccharide accumulated in the enzymatic reactions. It is plausible that the formation of the observed products was catalyzed by a mixture of mannosyltransferases. For example, one enzyme could catalyze the formation of the oligosaccharide to a certain chain length such as a tetrasaccharide, which is then acted on by another ManT for longer polymer biosynthesis. Another possibility is that the elongated portions of the longer polymers, consisting of the *O*-glycosidic linkages, could potentially be degraded by *endo*-mannosidase[14] present in the membrane preparation. This would also be expected to lead to the accumulation of

certain products. However, at this point these hypotheses are pure speculation and further testing of them requires the identification of other ManT enzymes involved in LM/LAM biosynthesis. More importantly, the purification to homogeneity of these enzymes is essential to define their substrate specificities and mechanisms.

Recent studies by Kaur et al. have demonstrated that at least two α-(1→6)-ManT's are required for the proper elongation of lipomannan in *M. smegmatis*.[11] These workers identified a novel glycosyltransferase, which catalyzes the later stage of α-(1→6)-mannosylation of mature polymers (21–34 Man*p* residues) consisting of α-(1→6)-mannopyranosides with α-(1→2) Man*p* branching. Deletion of the encoding gene (Rv2174) resulted in a truncated form of lipomannan (<20 Man*p* units) containing both α-(1→6) and α-(1→2) linkages. The observations of the presence of a series of Man*p* polymers (21–34 Man*p*) varying by just one sugar unit in the wild type LM seem to support the hypothesis that α-(1→6) and α-(1→2) mannosyltransferases work in a concerted manner. However, in the absence of information regarding the branching positions of LM and the mechanistic studies of ManT(s) (processive vs nonprocessive), this hypothesis remains questionable.

In contrast, the data presented here demonstrated that no α-(1→2) linkage was detected, even using the longest oligomer (heptamer **5.11**) as the substrate acceptor. This in turn suggests that the α-(1→2) ManT catalyzes the addition of the branching Man*p* residues after the linear

oligosaccharide is synthesized. This is consistent with Yokoyama and Ballou's early work in this area, in which the formation of a series of oligosaccharides with up to 12 Man*p* units were isolated after the incubation of the cell membrane with GDP-mannose. These polymers were all shown to be hydrolyzed by *endo*-α-(1→6)-mannosidase but not by *exo*-α-(1→2) mannosidases.[14]

5.2.4. Kinetic characterization

After demonstrating that the thiooligosaccharides **5.6**–**5.11** are stable to α-(1→6)-*endo*-mannosidase and, at the same time, were able to act as ManT substrates, we investigated further the correlation between the acceptor length and rate of reaction. In particular, we hoped to determine if longer polysaccharides increase substrate affinity and turnover by the ManT. Considering that the observed α-(1→6)-mannosylation may be catalyzed by two or more mannosyltransferases, and that the multiple additions of Man*p* residues to the substrate acceptors will influence kinetic parameters, the values obtained can only be regarded as relative to each other, and useful only for illustrating the effect of acceptor length on ManT catalysis.

As shown in Figure 5-9, unlike the *O*-linked disaccharide **5.2**, the Michaelis–Menten curves for thiooligosaccharides **5.6**–**5.11** all display a pattern of "substrate inhibition". That is, a decrease in the incorporation of [^{3}H]Man into the radiolabled product was observed at higher substrate

concentrations (~0.5 mM for **5.6**–**5.9** and ~0.25 mM for **5.10** and **5.11**). We propose that the apparent substrate inhibition is due to the fact that the addition of [^{3}H]Man into the thiooligosaccharide-acceptor leads to the formation of the *O*/*S*-linked hybrid product, which is more potent substrate for the enzyme. This hypothesis is supported by the preliminary data shown in Figure 5-6, which indicated that the *O*-linked acceptor **5.2** was a better substrate than its *S*-linked counterpart **5.6** (with 27% the relative activity of **5.2**) (Figure 5-10).

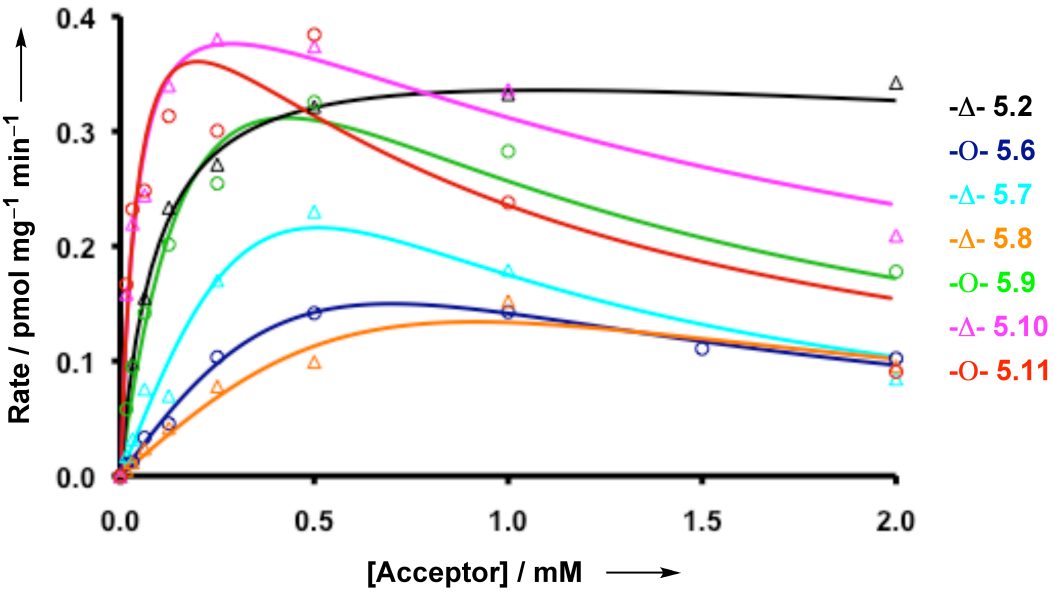


Figure 5-9. Incorporation of radiolabeled [^{3}H]-mannose into **5.2** and **5.6**–**5.11** as a function of acceptor concentration. Assays were performed at different substrate concentrations (0.016, 0.031, 0.063, 0.13, 0.25, 0.5, 1 and 2 mM).

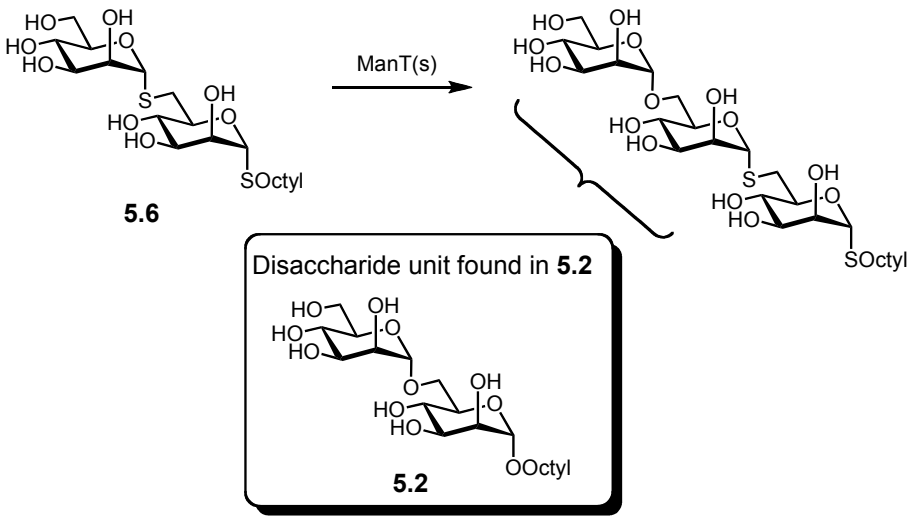


Figure 5-10. The *O*/*S*-linked hybrid product formed from the ManT reaction using acceptor **5.6**.

The kinetic properties observed from the thiooligosaccharides **5.6**–**5.11** may suggest the nonprocessive nature of the mannosyltransferase(s), in which the sequential additions of Man*p* residues require the release and binding of the elongated products. The dissociation of the enzyme–acceptor complex would allow the more potent *O*/*S*-linked hybrid acceptor to compete for the active site, and result in the apparent product inhibition. The nonprocessivity of the mannosyltransferase activity may also explain the results of Kaur and coworkers, in which a series of Man*p* polymers (21–34 Man*p*), varying by just one sugar unit in the wild type LM, were identified from the mass spectrum.[13]

The data shown in Figure 5-9 also gives some insight into the effect of chain-length on activity. For example, if the apparent inhibition effect

taking place past 0.25 mM concentration is not considered, the initial mannosylation velocity of thiopentamannoside **5.9** is comparable with that of the parent substrate **5.2**. Also, faster rates were even observed with the longer polymers **5.10** and **5.11** at 0.25 mM concentration. As illustrated in Figure 5-6, the slopes of the kinetic curves at the lower concentration range (0.016 to 0.25 mM) become steeper as the length of the thiooligosaccharide chain increases. Regardless of the aforementioned complexity of this cell free assay, our data strongly suggest that larger oligomers improve the efficiency of ManT catalysis.

In contradiction to this trend, tetrasaccharide **5.8** exhibited much slower kinetics even than that of **5.6**. From our previous study, we hypothesized that the lower ManT activity against *O*-tetrasaccharide **5.4** was due to the simultaneous degradation of the radiolabeled product and the acceptor substrate by α-(1$\rightarrow$6)-*endo*-mannosidase (Figure 3-4, Chapter 3). Although this does happen, there also appear to be other factors that contribute to the slower turnover of **5.4,** as a similar observation was also obtained for acceptor **5.8**, which is resistant to the *endo*-mannosidase degradation. As discussed previously, we are uncertain why tetrasaccharides **5.8** or **5.4** serve as poor substrates for ManT(s). However, it is impossible to address this issue in the absence of a pure ManT that is free from the interferences such as other mannosyltransferases and mannosidase.

To provide a more detailed comparison of the effect of the acceptor length on ManT catalysis, the kinetic parameters of analogs **5.6**–**5.11** were estimated using the Michaelis–Menten equation (Table 5-1). To avoid influence from the apparent substrate inhibition effect, the V_{max} and K_M values of the acceptors were estimated from the concentration ranges from 0.016 to 0.5 mM (**5.6**–**5.9**, **5.11**) and 0.016 to 0.25 mM (**5.2**, **5.10**). As presented in Table 5-1, a significant improvement of the apparent K_M values was observed for the larger polymers. For example, the Michaelis constant of thiopentasaccharide **5.9** (K_M = 106 μM) was comparable with that of the parent *O*-linked disaccharide **5.2** (K_M = 81 μM). Also, the K_M values of **5.10** and **5.11** increased by ~2.7- and 4.1-fold, respectively, as compared **5.2**. More interestingly, although tetramannoside **5.8** had the lowest V_{max} value among the acceptor analogs (0.18 pmol mg^{-1} min^{-1}), its K_M constant (K_M = 377 μM) fit between those of the tri- and pentasaccharides (**5.7**, K_M = 431 μM and **5.9**, K_M = 106 μM). These apparent kinetic values strongly suggest that the larger oligomers are indeed more efficiently mannosylated by ManT.

Table 5-1. Kinetic data of analogs as substrates of ManT [i]		
Analog	K_M [μM]	V_{max} [pmol mg^{-1} min^{-1}]
5.2	81 ± 11	0.37 ± 0.020
5.6	577 ± 182	0.31 ± 0.062
5.7	431 ± 180	0.43 ± 0.10
5.8	377 ± 78	0.18 ± 0.020
5.9	106 ± 12	0.38 ± 0.016
5.10	30 ± 6	0.40 ± 0.023
5.11	20 ± 6	0.35 ± 0.022
[i] Enzyme activities were determined using a range of acceptor concentrations (0.016 to 0.5 mM for **5.6**–**5.9**, **5.11** and 0.016 to 0.25 mM for **5.2**, **5.10**). Kinetic parameters were obtained by nonlinear regression analysis using the Michaelis-Menten equation with the GraphPad Prism 4.0 program (GraphPad Software, San Diego, CA).		

5.3. Conclusions

In this chapter, we report the synthesis of a homologous series of octyl thioglycoside oligomers (**5.5**–**5.11**), ranging in size from monosacccharide to heptasaccharide. The formation of the sulfur glycosidic linkages were obtained via the couplings of mannosyl isothiouronium salts and triflate derivatized mannosides.[24] Subsequently, these synthetic oligosaccharide analogs were tested against a PPM-dependent α-(1→6)-mannosyltransferase(s) involved in mycobacterial LM/LAM biosynthesis.

Our results demonstrate that all thiosaccharide acceptors **5.6**–**5.11**, except for monosaccharide **5.5,** are moderate substrates for ManT(s),

which is in agreement with our earlier report that the enzyme requires a disaccharide motif as the minimum epitope for activity. Importantly, without the interference of *endo*-mannosidase, the non-hydrolysable thiooligosaccharide analogs allowed us to investigate further the apparent effect of polymer length on ManT catalysis. The smaller apparent K_M values with larger polymers suggest that the ManT(s) catalyze the mannosylation more efficiently using longer oligosaccharide acceptors. In addition, the observed "substrate inhibition" suggests that the successive addition of Man*p* residues may require the dissociation and rebinding of the substrates in the active site. Moreover, through structural analysis by *exo*-mannosidases, no α-(1→2)-glycosidic linkages were detected, even from the enzymatic product using heptathiosaccharide **5.11** as a substrate. This result strongly suggests that the attachment of the α-(1→2)-mannopyranosyl branches in the mannan core of LM/LAM must occur after a larger α-(1→6)-linked mannan is assembled.

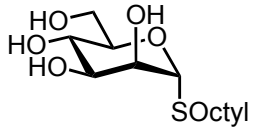
5.4. Experimental Section

General methods for chemical synthesis

All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless indicated otherwise, all reactions were performed at room temperature and under a positive pressure of argon. The reactions were monitored by analytical TLC on silica gel 60-F_{254} (0.25 mm, Silicycle) and spots were detected under UV light or by charring with acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure at <40 °C. Products were purified by column chromatography using silica gel (40–60 μM), Iatrobeads (Iatron Laboratories, Tokyo) or SepPak C_{18} reverse phase cartridges (Waters). Before use, the cartridges were prewashed with 10 mL of MeOH followed by 10 mL of H_2O. Optical rotations were measured at 22 ± 2 °C and are in units of degrees·mL/(g·dm). ^{1}H NMR spectra were recorded at 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$), or HOD (4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz and chemical shifts are referenced to internal $CDCl_3$ (77.23, $CDCl_3$), or CD_3OD (48.9, CD_3OD). Assignments of NMR spectra were made based on two-dimensional (^{1}H–^{1}H COSY and HMQC) experiments. The stereochemistry at the anomeric centers of the pyranose rings were proven by measuring the $^1J_{C1\text{-}H1}$.[36] Electrospray mass spectra were

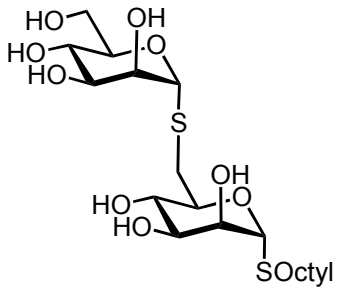
recorded on samples suspended in mixtures of THF with MeOH and added NaCl.

Octyl 1-thio-α-D-mannopyranoside[40] (5.5)



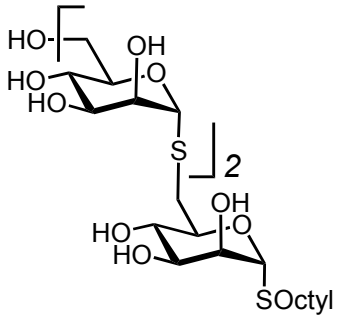
Monosaccharide **5.20** (2.25 g, 4.7 mmol) was dissolved in MeOH (45 mL) and 1 M NaOMe (5 mL) was added. After 2 h, the solution was neutralized with Amberlite IR120 resin (H^+ form), filtered and concentrated. The deprotected crude product (39 mg) was further purified by column chromatography on Iatrobeads (10:1 CH_2Cl_2–MeOH) to give **5.5** as a white foam (35 mg, 90%): R_f 0.20 (10:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = +166.6 (*c* 0.6, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 5.20 (d, 1H, *J* = 1.1 Hz, H-1), 3.89 (dd, 1H, *J* = 2.9, 1.1 Hz, H-2), 3.86–3.91 (m, 1H, H-5), 3.79 (dd, 1H, *J* = 11.9, 2.5 Hz, H-6a), 3.72 (dd, 1H, *J* = 11.9, 5.7 Hz, H-6b), 3.62–3.68 (m, 2H, H-3, H-4), 2.66 (ddd, 1H, *J* = 12.9, 7.6, 7.5 Hz, octyl SC*H*$_2$), 2.57 (ddd, 1H, *J* = 12.9, 7.4, 7.4 Hz, octyl SC*H*$_2$), 1.55–1.68 (m, 2H, octyl C*H*$_2$), 1.22–1.44 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 86.5 (C-1), 74.9, 73.8 (C-2, C-5), 73.2 (C-3), 68.9 (C-4), 62.8 (C-6), 33.0 (octyl *C*H$_2$), 31.9 (octyl S*C*H$_2$), 30.7 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 30.3 (octyl *C*H$_2$), 29.9 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{14}H_{28}O_5S$: 331.1550. Found: 331.1546.

Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.6)



Prepared from disaccharide **5.24** (1.63 g, 2.1 mmol) as described for the synthesis of **5.5**. The deprotected crude product (124 mg) was further purified by column chromatography on Iatrobeads (4:1 CH_2Cl_2–MeOH) to give **5.6** as a white foam (110 mg, 89%): R_f 0.31 (4:1 CH_2Cl_2–MeOH); $[\alpha]_D$ = +257.4 (*c* 1.5, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 5.28 (d, 1H, *J* = 1.1 Hz, H-1′), 5.17 (d, 1H, *J* = 1.2 Hz, H-1), 4.03 (ddd, *J* = 8.6, 8.6, 2.3 Hz, H-5), 3.88–3.92 (m, 3H, H-2′, H-5′, H-2), 3.83 (dd, 1H, *J* = 11.8, 2.3 Hz, H-6a′), 3.73 (dd, 1H, *J* = 11.8, 5.8 Hz, H-6b′), 3.60–3.68 (m, 4H, H-3′, H-4′, H-3, H-4), 3.14 (dd, 1H, *J* = 14.0, 2.4 Hz, H-6a), 2.74 (dd, 1H, *J* = 14.0, 8.1 Hz, H-6b), 2.67–2.72 (m, 1H, octyl SC*H*$_2$), 2.58 (ddd, 1H, *J* = 12.9, 7.5, 7.5 Hz, octyl SC*H*$_2$), 1.58–1.69 (m, 2H, octyl C*H*$_2$), 1.24–1.45 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 86.2 (C-1′, C-1), 75.0, 73.6(9), 73.6(6) (C-2′, C-5′, C-2), 73.4 (C-5), 73.2, 73.1 (C-3′, C-3), 71.5 (C-4), 68.9 (C-4′), 62.9 (C-6′), 33.0(1) (octyl *C*H$_2$), 32.9(8) (C-6, octyl *C*H$_2$), 31.7 (octyl S*C*H$_2$), 30.6 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 30.3 (octyl *C*H$_2$), 30.0 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{20}H_{38}O_9S_2$: 509.1850. Found: 509.1843.

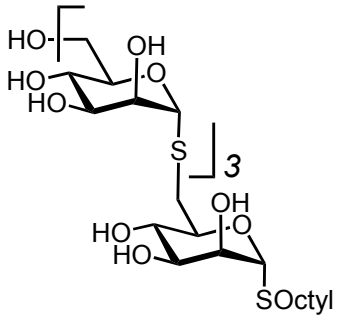
Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.7)



Prepared from trisaccharide **5.31** (0.79 g, 0.73 mmol) as described for the synthesis of **5.5**. An amount of the deprotected crude product (33 mg) was further purified by column chromatography on Iatrobeads (12:2:1 EtOAc–MeOH–H_2O) to give **5.7** as a white foam (23 mg, 70%): R_f 0.22 (12:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D$ = +286.8 (*c* 0.6, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 5.29 (d, 1H, *J* = 1.1 Hz, H-1′), 5.26 (d, 1H, *J* = 1.1 Hz, H-1′′), 5.18 (d, 1H, *J* = 1.1 Hz, H-1), 4.01–4.08 (m, 2H, H-5′, H-5), 3.86–3.96 (m, 4H, H-2′′, H-5′′, H-2′, H-2), 3.83 (dd, *J* = 11.8, 2.3 Hz, H-6a′′), 3.72 (dd, *J* = 11.8, 6.0 Hz, H-6b′′), 3.58–3.70 (m, 6H, H-3′′, H-4′′, H-3′, H-4′, H-3, H-4), 3.17 (dd, 2H, *J* = 13.7, 2.2 Hz, H-6a′, H-6a), 2.80 (dd, 1H, *J* = 13.7, 8.6 Hz, H-6b), 2.66–2.74 (m, 2H, H-6b′, octyl SCH_2), 2.58 (ddd, 1H, *J* = 12.8, 7.3, 7.3 Hz, octyl SCH_2), 1.58–1.70 (m, 2H, octyl CH_2), 1.22–1.46 (m, 10H, octyl CH_2), 0.93 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 86.1(4) (C-1′), 86.0(9) (C-1′′), 85.9 (C-1), 75.0 (C-5′′), 73.7, 73.5, 73.5 (C-2′′, C-2′, C-2), 73.2(5), 73.2(2), 73.1(9), 73.1(5), 73.0 (C-3′′, C-3′, C-5′, C-3, C-5), 71.8, 71.6 (C-4′, C-4), 69.0 (C-4′′), 62.9 (C-6′′), 33.0 (C-6′), 33.0 (octyl CH_2), 32.6 (C-6), 31.7 (octyl SCH_2), 30.6 (octyl CH_2), 30.4 (octyl CH_2), 30.3 (octyl CH_2), 30.0 (octyl CH_2), 23.7 (octyl CH_2), 14.5

(octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{13}S_3$: 687.2149. Found: 687.2151.

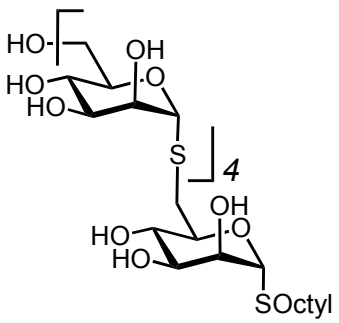
Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.8)



Prepared from tetrasaccharide **5.33** (63 mg, 0.045 mmol) as described for the synthesis of **5.5**. The crude product was purified by chromatography on Iatrobeads (8:2:1 EtOAc–MeOH–H_2O) to give **5.8** as a white foam (23 mg, 59%): R_f 0.35 (8:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D$ = +317.1 (*c* 0.7, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 5.30 (d, 1H, *J* = 1.2 Hz, H-1‴), 5.28 (br s, 2H, H-1″, H-1′), 5.18 (d, 1H, *J* = 1.2 Hz, H-1), 4.00–4.10 (m, 3H, H-5″, H-5′, H-5), 3.88–3.98 (m, 5H, H-2‴, H-5‴, H-2″, H-2′, H-2), 3.83 (dd, *J* = 11.9, 2.4 Hz, H-6a‴), 3.72 (dd, *J* = 11.9, 6.0 Hz, H-6b‴), 3.58–3.71 (m, 8H, H-3‴, H-4‴, H-3″, H-4″, H-3′, H-4′, H-3, H-4), 3.15–3.24 (m, 3H, H-6a″, H-6a′, H-6a), 2.67–2.84 (m, 4H, H-6b″, H-6b′, H-6b, octyl SCH_2), 2.58 (ddd, 1H, *J* = 12.8, 7.4, 7.4 Hz, octyl SCH_2), 1.57–1.70 (m, 2H, octyl CH_2), 1.24–1.46 (m, 10H, octyl CH_2), 0.89 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 86.0(7), 86.0(3), 85.9(5), 85.8 (C-1‴, C-1″, C-1′, C-1), 75.0 (C-5‴), 73.7, 73.6, 73.6, 73.4, 73.3(3), 73.2(7), 73.2(4), 73.2(1), 73.1, 73.1, 73.0 (C-2‴, C-3‴, C-2″, C-3″, C-5″, C-2′, C-3′, C-5′, C-2, C-3, C-5),

71.9, 71.8, 71.5 (C-4″, C-4′, C-4), 69.0 (C-4‴), 62.9 (C-6‴), 33.0 (octyl CH_2), 33.0, 32.7, 32.5 (C-6″, C-6′, C-6), 31.7 (octyl SCH_2), 30.6 (octyl CH_2), 30.4 (octyl CH_2), 30.3 (octyl CH_2), 30.0 (octyl CH_2), 23.7 (octyl CH_2), 14.5 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{32}H_{58}O_{17}S_4$: 865.2449. Found: 865.2446.

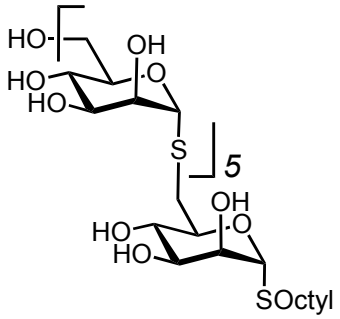
Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.9)



Prepared from pentassaccharide **5.38** (62 mg, 0.037 mmol) as described for for the synthesis of **5.5**. The crude product was purified by chromatography on Iatrobeads (7:2:1 EtOAc–MeOH–H_2O) to give **5.9** as a white foam (23 mg, 63%): R_f 0.37 (7:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D$ = +312.1 (*c* 0.5, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 5.33, 5.32, 5.31, 5.30 (4 x br s, 4H, H-1⁗, H-1‴, H-1″, H-1′), 5.20 (br s, 1H, H-1), 4.02–4.10 (m, 4H, H-5‴, H-5″, H-5′, H-5), 3.89–3.98 (m, 6H, H-2⁗, H-5⁗, H-2‴, H-2″, H-2′, H-2), 3.85 (dd, *J* = 12.0, 2.4 Hz, H-6a⁗), 3.75 (dd, *J* = 12.0, 6.0 Hz, H-6b⁗), 3.59–3.71 (m, 10H, H-3⁗, H-4⁗, H-3‴, H-4‴, H-3″, H-4″, H-3′, H-4′, H-3, H-4), 3.15–3.25 (m, 4H, H-6a‴, H-6a″, H-6a′, H-6a), 2.68–2.84 (m, 5H, H-6b‴, H-6b″, H-6b′, H-6b, octyl SCH_2), 2.60 (ddd, 1H, *J* = 13.2, 7.8, 7.8 Hz, octyl SCH_2), 1.58–1.70 (m, 2H, octyl CH_2), 1.24–1.45 (m, 10H, octyl CH_2), 0.90

(t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 86.0(7), 86.0(5), 85.9(4), 85.9(2), 85.6 (C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 75.0 (C-5′′′′), 73.7, 73.6, 73.6, 73.4, 73.4, 73.3, 73.2(8), 73.2(1), 73.21(7) 73.1, 73.1, 73.0(8), 73.0(3), 72.9(8) (C-2′′′′, C-3′′′′, C-2′′′, C-3′′′, C-5′′′, C-2′′, C-3′′, C-5′′, C-2′, C-3′, C-5′, C-2, C-3, C-5), 71.8(8), 71.8(1), 71.8(1), 71.5 (C-4′′′, C-4′′, C-4′, C-4), 69.0 (C-4′′′′), 62.9 (C-6′′′′), 33.0 (octyl *C*H_2), 33.0, 32.7, 32.7, 32.5 (C-6′′′, C-6′′, C-6′, C-6), 31.7 (octyl S*C*H_2), 30.6 (octyl *C*H_2), 30.4 (octyl *C*H_2), 30.3 (octyl *C*H_2), 30.0 (octyl *C*H_2), 23.7 (octyl *C*H_2), 14.5 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{38}H_{68}O_{21}S_5$: 1043.2749. Found: 1043.2743.

Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.10)



Prepared from protected oligosaccharide **5.41** (48 mg, 0.024 mmol) as described for the synthesis of **5.5**. The crude product was purified using a C_{18} reverse phase cartridge. The title compound **5.10** was eluted with MeOH and, after lyophilization, obtained as a white foam (12 mg, 40%): R_f 0.48 (5:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D$ = +420.1 (*c* 0.1, DMSO); ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ_H 5.04–5.10 (m, 6H, H-1′′′′′, H-1′′′′, H-1′′′, H-1′′, H-1′, H-1),

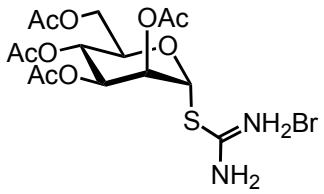
4.96–5.04 (m, 5H, O*H*), 4.88–4.94 (m, 6H, O*H*), 4.76 (d, 1H, *J* = 5.1 Hz, O*H*), 4.71 (d, 1H, *J* = 5.5 Hz, O*H*), 4.64–4.70 (m, 4H, O*H*), 4.61 (d, 1H, *J* = 5.3 Hz, O*H*), 4.46 (t, 1H, *J* = 5.8 Hz, O*H*), 3.76–3.86 (m, 5H), 3.76–3.86 (m, 5H), 3.61–3.74 (m, 8H, H-6a′′′′′), 3.34–3.51 (m, 13H, H-6b′′′′′), 2.94–3.02 (m, 5H, H-6a′′′′, H-6a′′′, H-6a′′, H-6a′, H-6a), 2.56–2.68 (m, 6H, H-6b′′′′, H-6b′′′, H-6b′′, H-6b′, H-6b, octyl SCH_2), 2.48–2.56 (m, 1H, octyl SCH_2), 1.50–1.59 (m, 2H, octyl CH_2), 1.18–1.36 (m, 10H, octyl CH_2), 0.85 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ_C 84.8(9), 84.8(9), 84.8(5), 84.7(5, 84.7(0), 84.4 (6C, C-1′′′′′, C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 74.2, 72.3, 72.1(3), 72.0*8), 71.7, 71.6(4), 71.5(9), 71.4, 71.3, 69.8, 69.7, 69.6(9), 69.4, 67.1 (C-4′′′′′), 60.9 (C-6′′′′′), 31.8, 31.7, 31.1 (C-6′′′′, C-6′′′, C-6′′, C-6′, C-6, octyl *C*H_2), 29.7 (octyl S*C*H_2), 28.8 (octyl *C*H_2), 28.5 (octyl *C*H_2), 28.4 (octyl *C*H_2), 28.2 (octyl *C*H_2), 21.9 (octyl *C*H_2), 13.8 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{44}H_{78}O_{25}S_6$: 1221.3049. Found: 1221.3037.

Octyl 1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranosyl-(1→6)-1-thio-α-D-mannopyranoside (5.11)

Prepared from protected oligosaccharide **5.42** (69 mg, 0.030 mmol) as described for the synthesis of **5.5.** The crude product was purified using a C_{18} reverse phase cartridge. The title compound **5.11** was eluted with

MeOH and, after lyophilization, obtained as a white foam (9 mg, 22%): R_f 0.31 (5:2:1 EtOAc–MeOH–H_2O); $[\alpha]_D$ = +460.6 (*c* 0.1, DMSO); ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ_H 5.05–5.10 (m, 7H, H-1′′′′′′, H-1′′′′′, H-1′′′′, H-1′′′, H-1′′, H-1′, H-1), 4.96–5.04 (m, 5H, O*H*), 4.98 (d, 1H, *J* = 4.5 Hz, O*H*), 4.88–4.95 (m, 7H, O*H*), 4.76 (d, 1H, *J* = 5.0 Hz, O*H*), 4.71 (d, 1H, *J* = 5.5 Hz, O*H*), 4.64–4.70 (m, 5H, O*H*), 4.60 (d, 1H, *J* = 5.5 Hz, O*H*), 4.46 (t, 1H, *J* = 6.0 Hz, O*H*), 3.61–3.86 (m, 18H), 3.28–3.52 (m, 19H, H-6a′′′′′), 2.94–3.03 (m, 6H, H-6a′′′′′, H-6a′′′′ ,H-6a′′′, H-6a′′, H-6a′, H-6a), 2.56–2.68 (m, 7H, H-6b′′′′′, H-6b′′′′, H-6b′′′, H-6b′′, H-6b′, H-6b, octyl SCH_2), 2.48–2.56 (m, 1H, octyl SCH_2), 1.50–1.59 (m, 2H, octyl CH_2), 1.18–1.36 (m, 10H, octyl CH_2), 0.85 (t, 3H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ_C 84.8(9), 84.8(5), 84.7(6), 84.7(0), 84.4 (7C, C-1′′′′′′, C-1′′′′′, C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 74.2, 72.3, 72.1(3), 72.0(9), 71.7, 71.6(4), 71.5(9), 71.3(8), 71.3(2), 71.2(6), 69.8, 69.7, 69.6(9), 69.4, 67.1 (C-4′′′′′′), 60.9 (C-6′′′′′′), 31.9, 31.8, 31.7(2), 31.7(0), 31.1 (C-6′′′′′, C-6′′′′, C-6′′′, C-6′′, C-6′, C-6, octyl *C*H_2), 29.7 (octyl S*C*H_2), 28.8 (octyl *C*H_2), 28.5 (octyl *C*H_2), 28.4 (octyl *C*H_2), 28.2 (octyl *C*H_2), 21.9 (octyl *C*H_2), 13.8 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{50}H_{88}O_{29}S_7$: 1399.3348. Found: 1399.3344.

2-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl)-2-thiopseudourea hydrobromide[34] (5.19)

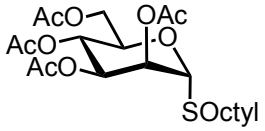


1,2,3,4,6-penta-*O*-acetyl-α-D-mannopyranose **5.18** (32.7 g, 83.8 mmol), prepared using a known procedure,[25] was dissolved in HOAc (60 mL) and 33% HBr–HOAc (70 mL) was added at 0 °C. The reaction mixture was stirred overnight, diluted with CH_2Cl_2 (150 mL) and then washed with cold H_2O (50 mL) and satd aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting product was dried under vacuum for 15 min before being dissolved in acetone (40 mL). To this solution was added thiourea (6.3 g, 83.2 mmol) and the mixture was heated at reflux for an 1 h. After cooling, the sovent was evaporated and the crude product was partitioned between CH_2Cl_2 (30 mL) and H_2O (40 mL), and the desired product was crystallized from the aqueous phase at 4 °C. After filtration, **5.19** was obtained as a white crystalline solid (18.4 g, 45%): ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 6.43 (dd, 1H, *J* = 1.8, 0.6 Hz, H-1), 5.46 (dd, 1H, *J* = 3.5, 1.8 Hz, H-2), 5.33 (dd, 1H, *J* = 9.9, 9.9 Hz, H-4), 5.15 (dd, 1H, *J* = 9.9, 3.5 Hz, H-3), 4.50 (dddd, 1H, *J* = 9.9, 5.9, 2.6, 0.6 Hz, H-5), 4.29 (dd, 1H, *J* = 12.5, 5.9 Hz, H-6a), 4.22 (dd, 1H, *J* = 12.5, 2.6 Hz, H-6b), 2.81 (d, 4H, *J* = 13.1 Hz, N*H*$_2$), 2.13 (s, 3H, C(O)C*H*$_3$), 2.05 (s, 3H, C(O)C*H*$_3$), 2.05 (s, 3H, C(O)C*H*$_3$), 1.96 (s, 3H, C(O)C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.6 (*C*=*O*), 170.2 (*C*=*O*), 170.0 (*C*=*O*), 169.9 (*C*=*O*), 82.9 (C-1), 72.3 (C-2), 69.7 (C-5), 69.5 (C-3), 66.0 (C-4), 62.4 (C-6), 20.7

(C(O)*C*H$_3$), 20.4(9) (C(O)*C*H$_3$), 20.4(7) (C(O)*C*H$_3$), 20.3(7) (C(O)*C*H$_3$). HRMS (ESI) calcd. for (M + H) $C_{15}H_{23}N_2O_9S$: 499.1972. Found: 499.1970.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[40] (5.20)

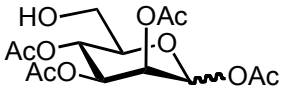
Isothiourea **5.19** (3.0 g, 6.2 mmol) was dissolved in CH_3CN (25 mL) and octyl bromide (1.1 mL, 6.2 mmol) and triethylamine (2.6 mL, 18.6 mmol) were added.



The reaction mixture was stirred for 1 h, and then diluted with EtOAc (25 mL) and washed with H_2O (15 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the resulting crude product was purified by chromatography (3:1 hexane–EtOAc) to give **5.20** as an amorphous white solid (2.28 g, 78%): R_f 0.38 (3:1 hexane–EtOAc); $[\alpha]_D$ = +87.4 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.32 (dd, 1H, *J* = 3.0, 1.3 Hz, H-2), 5.26–5.33 (m, 2H, H-3, H-4), 5.23 (d, 1H, *J* = 1.3 Hz, H-1), 4.36–4.41 (m, 1H, H-5), 4.30 (dd, 1H, *J* = 12.2, 5.3 Hz, H-6a), 4.07 (dd, 1H, *J* = 12.2, 2.3 Hz, H-6b), 2.63 (ddd, 1H, *J* = 12.9, 7.6, 7.1 Hz, octyl SC*H*$_2$), 2.55 (ddd, 1H, *J* = 12.9, 7.5, 7.5 Hz, octyl SC*H*$_2$), 2.15 (s, 3H, C(O)C*H*$_3$), 2.08 (s, 3H, C(O)C*H*$_3$), 2.03 (s, 3H, C(O)C*H*$_3$), 1.97 (s, 3H, C(O)C*H*$_3$), 1.57–1.66 (m, 2H, octyl C*H*$_2$), 1.20–1.42 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5 (*C*=*O*), 169.9 (*C*=*O*), 169.7(2) (*C*=*O*), 169.6(8) (*C*=*O*), 82.6 (C-1, $^1J_{C,H}$ = 167.0 Hz), 71.2 (C-2), 69.5 (C-5), 68.9 (C-3), 66.4 (C-4), 62.5 (C-6), 31.8 (octyl S*C*H$_2$), 31.4 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.1(3) (octyl *C*H$_2$), 29.0(6) (octyl *C*H$_2$), 28.8

(octyl *C*H_2), 22.6 (octyl *C*H_2), 20.9 (C(O)*C*H_3), 20.6(9) (C(O)*C*H_3), 20.6(7) (C(O)*C*H_3), 20.6(0) (C(O)*C*H_3), 14.0 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{22}H_{36}O_9S$: 499.1972. Found: 499.1970.

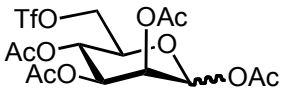
1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.21)



D-Mannose (6.0 g, 33.2 mmol) was dissolved in pyridine (90 mL) and trityl chloride (11.3 g, 39.9 mmol) and DMAP (0.82 g, 6.64 mmol) were added. The reaction mixture was stirred overnight and then acetic anhydride (30 mL) was added and the solution was stirred for another 5 h. The reaction mixture was then diluted in CH_2Cl_2 (150 mL), washed with 1 M HCl (3 x 50 mL), satd aq $NaHCO_3$ (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4), filtered concentrated and the resulting syrup was dissolved in 2:1 CH_2Cl_2–MeOH (90 mL) and *p*-toluenesulfonic acid (6.3 g, 33.2 mmol) was added. After the reaction was finished, the solvent was evaporated and the crude product was dissolved in CH_2Cl_2 (150 mL) and extracted with satd aq $NaHCO_3$ (50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by column chromatography to give an α/β mixture (2:1 ratio) of **5.21** as a colorless oil (6.7 g, 58%). Data for the major α isomer: R_f 0.28 (2:3 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.35 (d, 1H, *J* = 1.7 Hz, H-1), 5.36 (dd, 1H, *J* = 10.1, 3.5 Hz, H-3), 5.28 (dd, 1H, *J* = 10.1, 10.1 Hz, H-4), 5.23 (dd, 1H, *J* = 3.5, 1.7 Hz, H-2), 4.57 (ddd, 1H, *J* = 10.1, 4.4, 2.3 Hz, H-5), 3.68 (dd, 1H,

J = 12.8, 2.3 Hz, H-6a), 3.59 (dd, 1H, *J* = 12.8, 4.4 Hz, H-6b), 2.15 (s, 3H, C(O)C*H*$_3$), 2.15 (s, 3H, C(O)C*H*$_3$), 2.07 (s, 3H, C(O)C*H*$_3$), 2.00 (s, 3H, C(O)C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.3 (*C*=*O*), 169.9 (*C*=*O*), 169.7 (*C*=*O*), 168.2 (*C*=*O*), 90.7 (C-1), 77.9 (C-5), 70.6 (C-2), 69.6 (C-2), 69.4 (C-3), 65.8 (C-4), 61.1 (C-6), 20.8 (C(O)*C*H$_3$), 20.7 (C(O)*C*H$_3$), 20.6(6) (C(O)*C*H$_3$), 20.6(2) (C(O)*C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{14}H_{20}O_{10}$: 371.0949. Found: 371.0953.

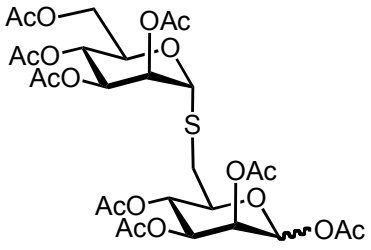
1,2,3,4-tetra-*O*-acetyl-6-*O*-trifluoromethanesulfonyl-D-mannopyranose (5.22)



Monosaccharide alcohol **5.21** (6.20 g, 17.8 mmol) was dissolved in CH_2Cl_2 (80 mL) and pyridine (6 mL, 71.2 mmol) and triflic anhydride (3.6 mL, 21.4 mmol) were added. The reaction mixture was stirred for 1 h at –25 °C, and then diluted with CH_2Cl_2 (70 mL). The solution was then washed with 1 M HCl (2 x 50 mL), satd aq $NaHCO_3$ (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue was purified by column chromatography to give an α/β mixture (7:1 ratio) of **5.22** as a pale brown oil (7.41 g, 87%). Data for the major α isomer: R_f 0.22 (2:1 hexane–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.11 (d, 1H, *J* = 2.0 Hz, H-1), 5.38 (dd, 1H, *J* = 9.6, 3.2 Hz, H-3), 5.32 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 5.26 (dd, 1H, *J* = 3.2, 2.0 Hz, H-2), 4.50–4.60 (m, 2H, H-6a, H-6b), 4.10–4.60 (m, 1H, H-5), 2.18 (s, 6H, C(O)C*H*$_3$), 2.10 (s, 3H, C(O)C*H*$_3$), 2.02 (s, 3H,

C(O)CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.8 (*C*=*O*), 169.5 (*C*=*O*), 169.4 (*C*=*O*), 167.6 (*C*=*O*), 80.1 (C-1), 73.2 (C-6), 70.1 (C-5), 68.2 (C-3), 68.0 (C-2), 65.3 (C-4), 20.6 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 20.4(7) (C(O)*C*H_3), 20.4(2) (C(O)*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{15}H_{19}O_{12}F_3S$: 503.0442. Found: 503.0444.

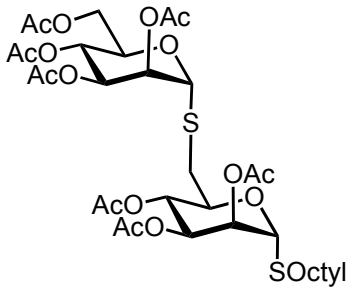
2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.23)



Triflate **5.22** (7.41 g, 15.4 mmol) was dissolved in CH_3CN (60 mL) and isothiouronium salt **5.19** (7.50, 15.4 mmol) and triethylamine (6.5 mL, 46.6 mmol) were added. The reaction mixture was stirred for 1 h and the crude mixture was the solution was diluted with EtOAc (100 mL) and washed with H_2O (25 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue was purified by chromatography (1:1 hexane–EtOAc) to give an α/β mixture (2:1 ratio) of **5.23** as a white foam (8.49 g, 79%); R_f 0.24 (1:1 hexane–EtOAc). Data for α isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.02 (d, 1H, *J* = 1.5 Hz, H-1), 5.20–5.42 (m, 7H, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 4.26–4.35 (m, 2H, H-5′, H-6a′), 4.40–4.10 (m, 2H, H-6b′, H-5), 2.90 (dd, 1H, *J* = 14.3, 3.0 Hz, H-6a), 2.67 (dd, 1H, *J* = 14.3, 3.5 Hz, H-6b), 1.95–2.20 (m, 24H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.6 (*C*=*O*), 169.9(8) (*C*=*O*), 169.9(5) (*C*=*O*), 169.8(5) (*C*=*O*), 169.8(4) (*C*=*O*),

169.7 (2C, *C*=*O*), 168.0 (*C*=*O*), 90.4 (C-1′), 82.8 (C-1), 71.9 (C-5), 70.9 (C-2′, C-3′, C-5′, C-2, C-3), 67.9 (C-4), 62.3(6) (C-4′), 62.2(7) (C-6′), 31.7 (C-6), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.6(9) (2C, C(O)*C*H_3), 20.7 (2C, C(O)*C*H_3), 20.6(6) (C(O)*C*H_3), 20.5 (C(O)*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{28}H_{38}O_{18}S$: 717.1671. Found: 717.1673.

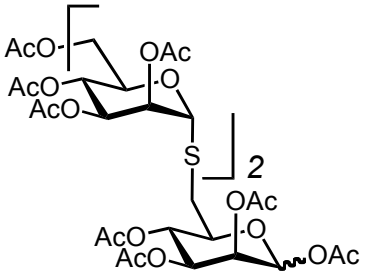
Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.24)



Disaccharide **5.23** (2.32 g, 3.33 mmol) was stirred in 33% HBr–AcOH (6 mL) until no starting material was detected by TLC (3 to 4 h). The mixture was diluted with cold CH_2Cl_2 (30 mL) and extracted with H_2O (10 mL) and satd aq $NaHCO_3$ (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to a pale yellow foam. After being dried under high vacuum for ~15 min, the crude glycosyl bromide was dissolved in CH_3CN (6 mL) and thiourea (0.38 g, 5.0 mmol) was added. The mixture was heated under reflux for 1 h. After the mixture was cooled to rt, octyl bromide (0.86 mL, 5.0 mmol) and triethylamine (1.4 mL, 10.0 mmol) were added, and the reaction mixture was stirred for another 1 h. The crude mixture was then diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, concentrated and the resulting residue was purified by column chromatography (3:2 hexane–EtOAc) to give **5.24**

as a colorless oil (1.67 g, 64%): R_f 0.21 (3:2 hexane–EtOAc); $[\alpha]_D$ = +134.3 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 5.33 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2′), 5.23–5.34 (m, 6H, H-1′, H-3′, H-4′, H-2, H-3, H-4), 5.18 (d, 1H, *J* = 1.2 Hz, H-1), 4.31–4.37 (m, 2H, H-5′, H-5), 4.28 (dd, 1H, *J* = 12.3, 4.9 Hz, H-6a′), 4.06 (dd, 1H, *J* = 12.3, 2.1 Hz, H-6b′), 2.86 (dd, 1H, *J* = 14.0, 3.0 Hz, H-6a), 2.72 (dd, 1H, *J* = 14.0, 7.0 Hz, H-6b), 2.62–2.70 (m, 1H, octyl SCH_2), 2.59 (ddd, 1H, *J* = 13.0, 7.5, 7.5 Hz, octyl SCH_2), 2.15 (s, 3H, C(O)CH_3), 2.15 (s, 3H, C(O)CH_3), 2.10 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 1.98 (s, 6H, C(O)CH_3), 1.56–1.66 (m, 2H, octyl CH_2), 1.20–1.44 (m, 10H, octyl CH_2), 0.87 (t, 3H, *J* = 6.5 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.6 (*C*=*O*), 170.0 (*C*=*O*), 169.9 (*C*=*O*), 169.7 (*C*=*O*), 169.6(4) (*C*=*O*), 169.6(1) (2C, *C*=*O*), 82.5 (C-1′, $^1J_{C,H}$ = 168.5 Hz), 82.1 (C-1, $^1J_{C,H}$ = 167.0 Hz), 71.1 (C-2), 70.8 (C-2′), 69.7, 69.4, 69.4, 69.2, 68.9 (C-3′, C-5′, C-3, C-4, C-5), 66.2 (C-4′), 62.3 (C-6′), 31.8(2) (octyl S*C*H_2), 31.7(6) (C-6), 31.2 (octyl *C*H_2), 29.3 (octyl *C*H_2), 29.1(3) (octyl *C*H_2), 29.1(0) (octyl *C*H_2), 28.7 (octyl *C*H_2), 22.6 (octyl *C*H_2), 20.9 (2C, C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.6(7) (C(O)*C*H_3), 20.6(5) (C(O)*C*H_3), 20.6(1) (C(O)*C*H_3), 20.5(6) (C(O)*C*H_3), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{34}H_{52}O_{16}S_2$: 803.2589. Found: 803.2592.

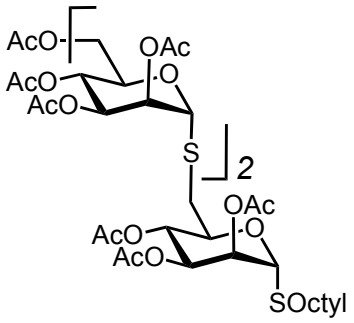
2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.30)



Disaccharide **5.23** (3.16 g, 4.55 mmol) was dissolved in 33% HBr–HOAc (8 mL) and stirred at 0 °C for 4 h. The solution was then diluted with CH_2Cl_2 (150 mL), washed with cold H_2O (50 mL) and satd aq $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated, and the product was dried under vacuum for 15 min before being dissolved in CH_3CN (8.5 mL). To the reaction mixture was added thiourea (0.36 g, 83.2 mmol) and the solution was heated at reflux for 1 h. After being cooled to rt, **5.22** (1.53 g, 3.19 mmol) and triethylamine (1.9 mL, 13.7 mmol) were added to the solution and stirring was continued for 1 h. The crude mixture was then diluted with EtOAc (100 mL) and washed with H_2O (25 mL), The organic layer was dried ($MgSO_4$), filtered, concentrated and the resulting residue was purified by chromatography (2:3 hexane–EtOAc) to give an α/β mixture (10:3 ratio) of **5.30** as a yellow foam (1.43 g, 45%). Data for α isomer: R_f 0.28 (2:3, hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.05 (d, 1H, *J* = 1.8 Hz, H-1), 5.44 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4), 5.21–5.36 (m, 10H, H-1″, H-2″, H-3″, H-4″, H-1′, H-2′, H-3′, H-4′, H-2, H-3), 4.33 (ddd, 1H, *J* = 10.2, 4.8, 2.4 Hz, H-5″), 4.26–4.32 (m, 1H, H-5′/H-5), 4.28 (dd, 1H, *J* = 12.0, 4.8 Hz, H-6a″), 4.05–4.10 (m, 2H, H-6b″, H-5′/H-5), 3.00 (dd, 1H, *J* = 14.7,

3.0 Hz, H-6a′/H-6a), 2.81 (dd, 1H, *J* = 14.7, 3.0 Hz, H-6a′/H-6a), 2.66–2.71 (m, 2H, H-6b′, H-6b), 1.96–2.18 (m, 33H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.6 (*C*=*O*), 170.0 (*C*=*O*), 169.9(7) (*C*=*O*), 169.9(2) (*C*=*O*), 169.8(5) (*C*=*O*), 169.7(9) (*C*=*O*), 169.6(9) (*C*=*O*), 169.6(5) (*C*=*O*), 169.6(2) (*C*=*O*), 169.6(0) (*C*=*O*), 168.1 (*C*=*O*), 90.3 (C-1), 82.4, 82.0 (C-1′′, C-1′), 71.8 (C-5′/C-5), 70.7(9), 70.7(6), 69.8, 69.4, 69.2, 69.1, 69.0, 68.6 (C-2′′, C-3′′, C-5′′, C-2′, C-3′, C-5′/C-5, C-2, C-3), 68.2 (C-4′), 67.7 (C-4), 66.2 (C-4′′), 62.3 (C-6′′), 31.4, 31.0 (C-6′, C-6), 20.9 (C(O)*C*H_3), 20.8(4) (C(O)*C*H_3), 20.8(2) (C(O)*C*H_3), 20.7(9) (C(O)*C*H_3), 20.7(4) (C(O)*C*H_3), 20.7(0) (2C, C(O)*C*H_3), 20.6(6) (C(O)*C*H_3), 20.5(8) (C(O)*C*H_3), 20.5(3) (2C, C(O)*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{40}H_{54}O_{25}S_2$: 1021.2288. Found: 1021.2293.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl -1-thio-α-D-mannopyranoside (5.31)



Prepared from trisaccharide **5.30** (1.34 g, 1.39 mmol) as described for the synthesis of **5.24**, to give **5.31** as a colorless oil (0.79 g, 55%): R_f 0.30 (1:1 hexane–EtOAc); $[\alpha]_D$ = +149.4 (*c* 1.6, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 5.23–5.34 (m, 11H, H-1′′, H-2′′, H-3′′, H-4′′, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 5.20 (d, 1H, *J* = 1.1 Hz, H-1), 4.28–4.36 (m, 3H, H-5′′, H-5′, H-5), 4.27

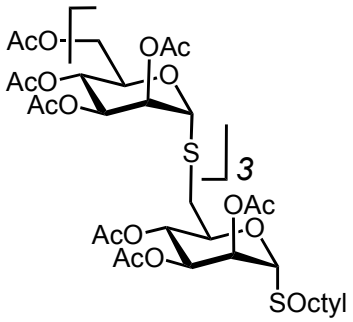
(dd, 1H, *J* = 12.3, 4.9 Hz, H-6a′′), 4.08 (dd, 1H, *J* = 12.3, 2.3 Hz, H-6b′′), 2.95 (dd, 1H, *J* = 14.5, 2.9 Hz, H-6a), 2.83 (dd, 1H, *J* = 14.2, 2.8 Hz, H-6a′), 2.74 (dd, 1H, *J* = 14.5, 7.0 Hz, H-6b), 2.64–2.72 (m, 2H, H-6b′, octyl SC*H*$_2$), 2.59 (ddd, 1H, *J* = 12.8, 7.4, 7.4 Hz, octyl SC*H*$_2$), 2.16, 2.14, 2.10, 2.07, 2.04, 2.04, 1.98, 1.97 (m, 30H, C(O)C*H*$_3$), 1.56–1.65 (m, 2H, octyl C*H*$_2$), 1.20–1.43 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5 (*C*=*O*), 170.1 (*C*=*O*), 169.9 (*C*=*O*), 169.7(5) (*C*=*O*), 169.7(3) (*C*=*O*), 169.6(5) (*C*=*O*), 169.6(2) (2C, *C*=*O*), 169.5(8) (2C, *C*=*O*), 82.0, 82.0 (C-1′′, C-1′, $^1J_{C,H}$ = 170.5 Hz), 81.9 (C-1, $^1J_{C,H}$ = 168.8 Hz), 71.2, 70.9, 70.7 (C-2′′, C-2′, C-2), 69.6, 69.4(2), 69.4(0), 69.2, 69.2(4), 69.2(0), 68.9, 68.8 (C-3′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.3 (C-4′′), 62.3 (C-6′′), 31.8 (octyl *C*H$_2$), 31.5 (C-6′), 31.3 (C-6), 31.1 (octyl S*C*H$_2$), 29.3 (octyl *C*H$_2$), 29.1(3) (octyl *C*H$_2$), 29.1(0) (octyl *C*H$_2$), 28.7 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 20.8(8) (C(O)*C*H$_3$), 20.8(7) (C(O)*C*H$_3$), 20.8(2) (2C, C(O)*C*H$_3$), 20.7 (C(O)*C*H$_3$), 20.6(6) (2C, C(O)*C*H$_3$), 20.6(2) (C(O)*C*H$_3$), 20.5(8) (C(O)*C*H$_3$), 20.5(6) (C(O)*C*H$_3$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{46}H_{68}O_{23}S_3$: 1107.3206. Found: 1107.3204.

2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.32)

Disaccharide triflate **5.35** (1.17 g, 1.49 mmol) was dissolved in CH_3CN (5 mL) and added to a mixture of isothiouronium salt **5.27** (prepared from **5.23** (1.80 g, 2.6 mmol) as described for the synthesis of **5.30**) and triethylamine (0.6 mL, 4.47 mmol) in CH_3CN (5 mL). The reaction mixture was stirred for 1 h, diluted with EtOAc (100 mL), and washed with H_2O (25 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue purified by chromatography (1:2 hexane–EtOAc) to give an α/β mixture (5:1 ratio) of **5.32** as a yellow foam (0.93 g, 48%); R_f 0.24 (1:2 hexane–EtOAc); for α isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.05 (d, 1H, *J* = 2.0 Hz, H-1), 5.44 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 5.21–5.40 (m, 14H, H-1‴, H-2‴, H-3‴, H-4‴, H-1″, H-2″, H-3″, H-4″, H-1′, H-2′, H-3′, H-4′, H-2, H-3), 4.03–4.10 (m, 6H, H-6a‴, H-6b‴, H-5‴, H-5″, H-5′, H-5), 3.03 (dd, 1H, *J* = 15.0, 3.0 Hz, H-6a″/H-6a′/H-6a), 2.93 (dd, 1H, *J* = 14.5, 2.0 Hz, H-6a″/H-6a′/H-6a), 2.82 (dd, 1H, *J* = 14.5, 3.0 Hz, H-6a″/H-6a′/H-6a), 2.64–2.80 (m, 3H, H-6a‴, H-6b′, H-6b), 1.93–2.20 (m, 42H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.6 (*C*=*O*), 170.5 (*C*=*O*), 170.0 (*C*=*O*), 169.9(8) (*C*=*O*), 169.9(5) (*C*=*O*), 169.9(2) (2C, *C*=*O*), 169.8(5) (*C*=*O*), 169.8(0) (*C*=*O*), 169.7(7) (*C*=*O*), 169.7(5) (2C, *C*=*O*), 169.6 (*C*=*O*), 168.1 (*C*=*O*), 90.3 (C-1), 82.2, 81.8, 81.1 (C-1‴, C-1″, C-1′), 71.8, 70.9, 70.8, 70.7, 69.5, 69.1, 69.0, 68.7, 68.6, 68.2, 67.7 (15C, C-2‴, C-3‴, C-5‴, C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4, C-5), 66.3 (C-4‴), 62.3 (C-6‴), 31.3, 30.7 (C-6″, C-6′, C-6),

20.8(9), 20.8(7), 20.8(2), 20.7(7), 20.7(4), 20.7(0), 20.6(6), 20.5(9) (14C, C(O)*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{52}H_{70}O_{32}S_3$: 1325.2905. Found: 1325.2925.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl -1-thio-α-D-mannopyranoside (5.33)

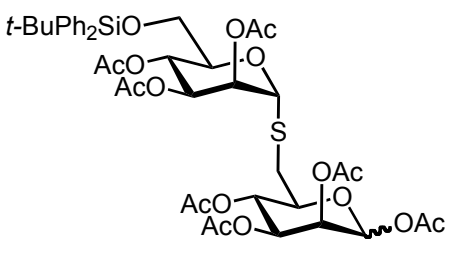


Method A: Prepared from tetrasaccharide **5.32** (123 mg, 0.12 mmol) as described for the synthesis of **5.24** to give **5.33** as a colorless oil (37 mg, 28%).

Method B: Triflate derivative **5.37** (32 mg, 0.027 mmol) was dissolved in CH_3CN (1 mL) and added to a mixture of **5.19** (15 mg, 0.031 mmol) and triethylamine (10 µL, 0.072 mmol) in CH_3CN (1 mL). The reaction mixture was stirred for 1 h and the crude mixture was then diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue was purified by chromatography (3:2 hexane–EtOAc) to give **5.33** as a colorless oil (37 mg, 97%): R_f 0.32 (2:3 hexane–EtOAc); $[\alpha]_D$ = +137.1 (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.18–5.35 (m, 15H, H-1′′′, H-2′′′, H-3′′′, H-4′′′, H-1′′, H-2′′, H-3′′, H-4′′, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 5.21 (br s, 1H, H-1), 4.23–4.36 (m, 5H, H-5′′′, H-6a′′′, H-5′′, H-5′, H-

5), 4.07–4.12 (m, 1H, H-6b′′′), 2.92–3.03 (m, 2H, H-6a′, H-6a), 2.81 (dd, 1H, *J* = 14.1, 1.5 Hz, H-6a′′), 2.56–2.78 (m, 5H, H-6b′′, H-6b′, H-6b, octyl SC*H*$_2$), 1.92–2.20 (m, 39H, C(O)C*H*$_3$), 1.52–1.66 (m, 2H, octyl C*H*$_2$), 1.20–1.42 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5 (*C*=*O*), 170.1 (*C*=*O*), 170.0 (*C*=*O*), 169.9 (*C*=*O*), 169.7(9) (*C*=*O*), 169.7(8) (*C*=*O*), 169.6(8) (*C*=*O*), 169.6(2) (3C, *C*=*O*), 169.6(0) (3, *C*=*O*), 81.9, 81.7, 81.6 (C-1′′′, C-1′′, C-1′, $^1J_{C,H}$ = 171.7, 168.7, 168.7 Hz), 81.0 (C-1, $^1J_{C,H}$ = 170.2 Hz), 71.2, 70.9(2), 70.9(0), 70.8 (C-2′′′, C-2′′, C-2′, C-2), 69.4(2), 69.4(2), 69.3(7), 69.2(3), 69.2(0), 69.1(9), 69.1(9), 69.0(4), 69.0(4), 68.9, 68.8 (C-3′′′, C-5′′′, C-3′′, C-4′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.3 (C-4′′′), 62.3 (C-6′′′), 31.8 (octyl *C*H$_2$), 31.2 (C-6′′), 31.0 (octyl S*C*H$_2$), 30.9, 30.7 (C-6′, C-6), 29.2 (octyl *C*H$_2$), 29.1 (octyl *C*H$_2$), 29.1 (octyl *C*H$_2$), 28.7 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 20.8(9), 20.8(7), 20.8(7), 20.8(3), 20.6(9), 20.6(6), 20.6(6), 20.6(2), 20.5(9), 20.5(7) (13C, C(O)*C*H$_3$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{58}H_{84}O_{30}S_4$: 1411.3823. Found: 1411.3836.

2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-α-D-mannopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.34)

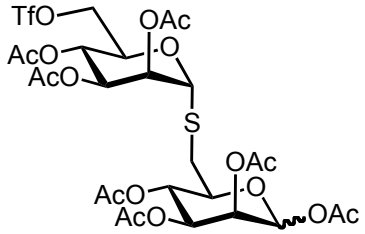


Disaccharide **5.23** (0.19 g, 0.28 mmol) was dissolved in MeOH (9 mL) and 1 M NaOMe (1 mL) was added. The reaction

mixture was stirred for 2 h and neutralized with Amerlite IR120 resin (H^+ form). The crude mixture was filtered, concentrated and dried under vacuum. The resulting oil was dissolved in pyridine (8 mL) and *tert*-butylchlorodiphenylsilane (75 µL, 0.29 mmol) was added at 0 °C and the mixture was stirred at rt overnight. Without purification, acetic anhydride (2 mL) was added, and the reaction mixture was stirred for another 5 h. The mixture was then diluted with CH_2Cl_2 (30 mL), washed with 1 M HCl (2 x 10 mL), satd aq $NaHCO_3$ (10 mL) and H_2O (10 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue was purified by chromatography (2:1 hexane–EtOAc) to give an α/β mixture (10:3 ratio) of **5.34** as a colorless oil (0.19 g, 78%): R_f 0.21 (2:1 hexane–EtOAc). Data for α isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62–7.72 (m, 4H, ArH), 7.34–7.45 (m, 6H, ArH), 6.05 (d, 1H, *J* = 2.0 Hz, H-1), 5.42 (dd, 1H, *J* = 9.9, 10.0 Hz, H-4′), 5.40 (dd, 1H, *J* = 9.9, 9.9 Hz, H-4), 5.30–5.37 (m, 3H, H-1′, H-2′, H-3), 5.25 (dd, 1H, *J* = 10.0, 3.2 Hz, H-3′), 5.22 (dd, 1H, *J* = 3.3, 2.0 Hz, H-2), 4.15 (ddd, 1H, *J* = 9.9, 4.7, 2.1 Hz, H-5′), 4.04 (ddd, 1H, *J* = 9.9, 5.3, 3.2 Hz, H-5), 3.77 (dd, 1H, *J* = 11.5, 4.7 Hz, H-6a′), 3.65 (dd, 1H, *J* = 11.5, 2.1 Hz, H-6b′), 2.94 (dd, 1H, *J* = 14.4, 3.2 Hz, H-6a), 2.65 (dd, 1H, *J* = 14.4, 5.3 Hz, H-6b), 2.16 (s, 6H, C(O)CH_3), 2.14 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.99 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.89 (s, 3H, C(O)CH_3), 1.07 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 169.9(4) (*C*=*O*), 169.9(1) (*C*=*O*), 169.8 (*C*=*O*), 169.5 (*C*=*O*), 169.4 (*C*=*O*), 168.0 (*C*=*O*), 135.8 (2C, Ar), 135.6 (2C, Ar), 133.3 (Ar), 133.1 (Ar), 129.7

(2C, Ar), 127.7 (2C, Ar), 127.6 (2C, Ar), 90.4 (C-1), 82.5 (C-1′), 72.0, 71.9 (C-5′, C-5), 71.2 (C-2′), 69.7 (C-3′), 68.7 (C-2), 68.2 (C-3), 67.8 (C-4), 66.4 (C-4′), 62.5 (C-6′), 31.5 (C-6), 26.7 ($C(CH_3)_3$), 20.9 ($C(O)CH_3$), 20.8 ($C(O)CH_3$), 20.6 (4C, $C(O)CH_3$), 19.3 ($C(CH_3)_3$). HRMS (ESI) calcd. for (M + Na) $C_{42}H_{54}O_{17}SiS$: 913.2743. Found: 913.2740.

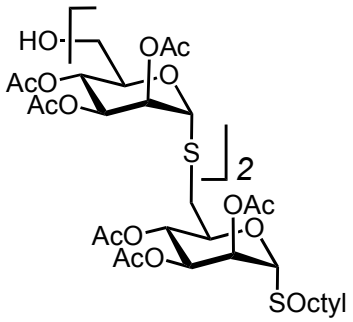
2,3,4-tri-*O*-acetyl-6-*O*-trifluoromethanesulfonyl-1-thio-α-D-mannopyranosyl-(1→6)-1,2,3,4-tetra-*O*-acetyl-D-mannopyranose (5.35)



Silylated disaccharide **5.34** (1.62 g, 1.82 mmol) was dissolved in THF (20 mL), 70% HF·pyridine (1 mL) and pyridine (4 mL) were added, and the reaction mixture was stirred overnight. The solution was then diluted in CH_2Cl_2 (100 mL), washed with H_2O (30 mL), 1 M HCl (2 x 30 mL) and satd aq $NaHCO_3$ (30 mL). The organic layer was dried (Na_2SO_4), filtered concentrated and the resulting crude product was dissolved in CH_2Cl_2 (12 mL). Pyridine (0.6 mL, 7.27 mmol) and triflic anhydride (0.37 mL, 2.2 mmol) were added to this solution and the reaction mixture was stirred for 1 h at –25 °C. The reaction mixture was then diluted in CH_2Cl_2 (100 mL), washed with 1 M HCl (2 x 30 mL), satd aq $NaHCO_3$ (30 mL) and H_2O (30 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the residue purified by chromatography to give an α/β mixture (6:1 ratio) of **5.35** as a pale

yellow oil (1.20 g, 84%), Data for α isomer: R_f 0.32 (1:1 hexane–EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.01 (d, 1H, *J* = 2.0 Hz, H-1), 5.37 (dd, 1H, *J* = 9.9, 9.9 Hz, H-4), 5.30–5.35 (m, 2H, H-1′, H-3′), 5.22–5.27 (m, 4H, H-2′, H-4′, H-2, H-3), 4.56 (dd, 1H, *J* = 11.3, 5.4 Hz, H-6a′), 4.50 (dd, 1H, *J* = 11.3, 2.6 Hz, H-6b′), 4.43–4.49 (m, 1H, H-5′), 4.01 (ddd, 1H, *J* = 9.9, 5.7, 3.0 Hz, H-5), 2.96 (dd, 1H, *J* = 14.4, 3.0 Hz, H-6a), 2.65 (dd, 1H, *J* = 14.4, 5.7 Hz, H-6b), 2.17 (s, 3H, C(O)CH_3), 2.17 (s, 3H, C(O)CH_3), 2.16 (s, 3H, C(O)CH_3), 2.08 (s, 6H, C(O)CH_3), 2.06 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.0 (*C*=*O*), 169.9 (*C*=*O*), 169.8 (*C*=*O*), 169.7(4) (*C*=*O*), 169.7(3) (*C*=*O*), 169.6 (*C*=*O*), 169.1 (*C*=*O*), 90.3 (C-1), 82.9 (C-1′), 73.9 (C-6′), 71.7 (C-5), 70.6, 68.9, 68.7, 68.6, 68.2 (C-2′, C-3′, C-5′, C-2, C-3), 67.8 (C-4), 66.1 (C-4′), 31.8 (C-6), 20.7(9) (C(O)*C*H_3), 20.7(5) (C(O)*C*H_3), 20.6(8) (C(O)*C*H_3), 20.6(4) (C(O)*C*H_3), 20.6(1) (C(O)*C*H_3), 20.5 (2C, C(O)*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{27}H_{35}O_{19}F_3S_2$: 807.1058. Found: 807.1057.

Octyl 2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.36)

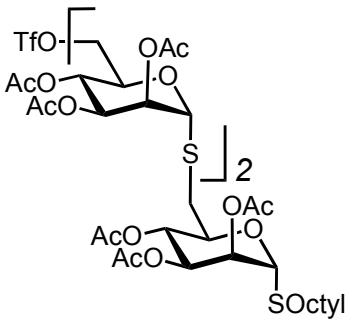


Prepared from trisaccharide **5.31** (0.79 g, 0.73 mmol) as described for the synthesis of **5.34** to give the silylated trisaccharide intermediate.

The crude product was dissolved in THF (10 mL) and 70% HF·pyridine (0.5 mL) and pyridine (2 mL) were added. The solution was then stirred overnight, diluted with CH_2Cl_2 (50 mL) and then washed with H_2O (15 mL), 1 M HCl (2 x 15 mL) and satd aq $NaHCO_3$ (15 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated and the resulting residue was purified by chromatography to give **5.36** as a colorless oil (0.45 g, 69%): R_f 0.33 (1:2 hexane–EtOAc); $[\alpha]_D$ = +162.0 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (600 MHz, CD_2Cl_2) δ_H 5.18–5.35 (m, 12H, H-1″, H-2″, H-3″, H-4″, H-1′, H-2′, H-3′, H-4′, H-1, H-2, H-3, H-4), 4.30–5.37 (m, 2H, H-5′, H-5), 4.06–4.51 (m, 1H, H-5″), 3.57–3.67 (m, 2H, H-6a″, H-6b″), 2.89–2.98 (m, 2H, H-6a′, H-6a), 2.66–2.78 (m, 3H, H-6b′, H-6b, octyl SC*H*$_2$), 2.62 (ddd, 1H, *J* = 12.8, 7.2, 7.2 Hz, octyl SC*H*$_2$), 2.48 (dd, 1H, *J* = 7.9, 6.2 Hz, OH), 2.13, 2.06, 2.06, 2.04, 1.99, 1.96 (m, 27H, C(O)C*H*$_3$), 1.56–1.67 (m, 2H, octyl C*H*$_2$), 1.20–1.44 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 171.0 (*C*=*O*), 170.4 (*C*=*O*), 170.3(7) (*C*=*O*), 170.3(3) (*C*=*O*), 170.1(8) (*C*=*O*), 170.1(5) (*C*=*O*), 170.0(7) (*C*=*O*), 170.0(6) (2C, *C*=*O*), 82.6, 82.5, 82.3 (C-1″, C-1′, C-1), 72.0, (C-5″), 71.4, 71.3, 71.1 (C-2″, C-2′, C-2), 70.4, 70.2 (C-5′, C-5), 69.9, 69.7, 69.6 (C-3″, C-3′, C-3), 69.0 (2C, C-4′, C-4), 66.8 (C-4″), 61.6 (C-6″), 32.2 (octyl *C*H$_2$), 32.1, 32.0 (C-6′, C-6), 31.5 (octyl S*C*H$_2$), 29.7 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 23.0 (octyl *C*H$_2$), 21.0 (2C, C(O)*C*H$_3$), 20.9(9) (2C, C(O)*C*H$_3$), 20.9(7) (C(O)*C*H$_3$), 20.9(3) (C(O)*C*H$_3$), 20.8(3) (C(O)*C*H$_3$),

20.7(9) (2C, C(O)*C*H_3), 14.2 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{44}H_{66}O_{22}S_3$: 1065.3100. Found: 1065.3103.

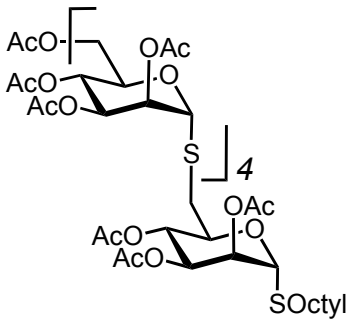
Octyl 2,3,4-tri-*O*-acetyl-6-*O*-trifluoromethanesulfonyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.37)



Prepared from **5.36** (96 mg, 0.092 mmol) as described for the synthesis of **5.22** to give **5.37** as a colorless oil (97 mg, 89%): R_f 0.55 (1:1 hexane–EtOAc); $[\alpha]_D$ = +126.1 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.23–5.36 (m, 11H, H-1″, H-2″, H-3″, H-4″, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 5.20 (d, 1H, *J* = 1.3 Hz, H-1), 4.56 (dd, 1H, *J* = 11.4, 5.5 Hz, H-6a″), 4.51 (dd, 1H, *J* = 11.4, 2.4 Hz, H-6b″), 4.44 (ddd, 1H, *J* = 8.8, 5.5, 2.4 Hz, H-5″), 4.30–4.37 (m, 2H, H-5′, H-5), 2.90–2.97 (m, 2H, H-6a′, H-6a), 2.65–2.79 (m, 3H, H-6b′, H-6b, octyl SCH_2), 2.59 (ddd, 1H, *J* = 12.4, 7.4, 7.4 Hz, octyl SCH_2), 2.17 (s, 3H, C(O)CH_3), 2.16 (s, 3H, C(O)CH_3), 2.15 (s, 3H, C(O)CH_3), 2.08 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 2.05 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.98 (s, 6H, C(O)CH_3), 1.57–1.67 (m, 2H, octyl CH_2), 1.20–1.45 (m, 10H, octyl CH_2), 0.88 (t, 3H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.1 (*C*=*O*), 169.9(6) (*C*=*O*), 169.9(5) (*C*=*O*), 169.8 (*C*=*O*), 169.7(4) (*C*=*O*), 169.7(1) (*C*=*O*), 169.6(0) (*C*=*O*), 169.5(7) (*C*=*O*),

169.5(4) (*C*=*O*), 82.2, 82.0, 81.9(7) (C-1′′, C-1′, C-1), 73.7 (C-6′′), 71.2, 70.7(9), 70.7(6) (C-2′′, C-2′, C-2), 69.4(1), 69.3(9), 69.3(2), 69.2(6), 68.8(5), 68.7(9), 68.7(5), 68.6 (C-3′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.1 (C-4′′), 31.8, 31.7, 31.4 (C-6′, C-6, octyl *C*H_2), 31.1 (octyl S*C*H_2), 29.2 (octyl *C*H_2), 29.1(4) (octyl *C*H_2), 29.1(1) (octyl *C*H_2), 28.7 (octyl *C*H_2), 22.6 (octyl *C*H_2), 20.9 (C(O)*C*H_3), 20.8(2) (2C, C(O)*C*H_3), 20.8(0) (C(O)*C*H_3), 20.6(2) (2C, C(O)*C*H_3), 20.5(7) (3C, C(O)*C*H_3), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{45}H_{65}O_{24}F_3S_4$: 1197.2593. Found: 1197.2587.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.38)

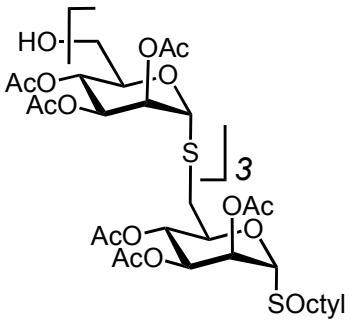


Triflate derivative **5.37** (32 mg, 0.027 mmol) was dissolved in CH_3CN (1 mL) and added to a mixture of isothiouronium salt **5.27** (prepared from **5.23** (34 mg, 0.049 mmol)) and triethylamine (20 µL, 0.15 mmol) in CH_3CN (1 mL). The reaction mixture was stirred for 1 h, diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue was purified by chromatography (2:3 hexane–EtOAc) to give **5.38** as an amorphous white solid (40 mg, 63%):

R_f 0.41 (1:2 hexane–EtOAc); $[\alpha]_D$ = +192.3 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 5.21–5.33 (m, 19H, H-1′′′′, H-2′′′′, H-3′′′′, H-4′′′′, H-1′′′, H-2′′′, H-3′′′, H-4′′′, H-1′′, H-2′′, H-3′′, H-4′′, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 5.20 (d, 1H, *J* = 1.3 Hz, H-1), 4.20–4.33 (m, 6H, H-5′′′′, H-6a′′′′, H-5′′′, H-5′′, H-5′, H-5), 4.09 (dd, 1H, *J* = 12.3, 2.3 Hz, H-6b′′′′), 3.02 (dd, 1H, *J* = 15.1, 2.5 Hz, H-6a′′/H-6a′/H-6a), 3.00 (dd, 1H, *J* = 14.9, 2.3 Hz, H-6a′′/H-6a′/H-6a), 2.93 (dd, 1H, *J* = 14.7, 2.3 Hz, H-6a′′/H-6a′/H-6a), 2.79 (dd, 1H, *J* = 14.4, 2.5 Hz, H-6a′′′), 2.73 (dd, 1H, *J* = 15.2, 7.4 Hz, H-6b′′/H-6b′/H-6b), 2.66–2.76 (m, 4H, H-6b′′′, H-6b′′/H-6b′/H-6b, octyl SC*H*$_2$), 2.59 (ddd, 1H, *J* = 12.8, 7.4, 7.4 Hz, octyl SC*H*$_2$), 1.94–1.98 (m, 48H, C(O)C*H*$_3$), 1.57–1.64 (m, 2H, octyl C*H*$_2$), 1.20–1.42 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5, 170.1, 170.0, 169.9(7), 169.9(6), 169.9(3), 169.8(5), 169.8(0), 169.7(8), 169.7(5), 169.6(6), 169.6(1), 169.5(7) (16C, *C*=*O*), 81.9, 81.6, 81.3, 80.6(4), 80.6(0) (C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 71.2, 70.9(6) (3C), 70.8(0) (C-2′′′′, C-2′′′, C-2′′, C-2′, C-2), 69.5, 69.4(5), 69.4(0), 69.3(8), 69.3(3), 69.3(0), 69.2(6), 69.2(1), 69.1(4), 69.0(8), 68.9(6), 69.9(6), 68.8(4), 68.7(9) (14C, C-3′′′′, C-5′′′′, C-3′′′, C-4′′′, C-5′′′, C-3′′, C-4′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.3 (C-4′′′′), 62.3 (C-6′′′′), 31.8 (octyl *C*H$_2$), 31.2 (C-6′′′), 30.9 (octyl S*C*H$_2$), 30.6, 30.4, 30.3 (C-6′′, C-6′, C-6), 29.2 (octyl *C*H$_2$), 29.1(3) (octyl *C*H$_2$), 29.1(0) (octyl *C*H$_2$), 28.7 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 20.9(6), 20.9(5), 20.8(9), 20.8(4), 20.8(2), 20.6(8), 20.6(5), 20.6(2),

20.6(0), 20.5(6) (16C, C(O)*C*H_3), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{70}H_{100}O_{37}S_5$: 1715.4439. Found: 1715.4441.

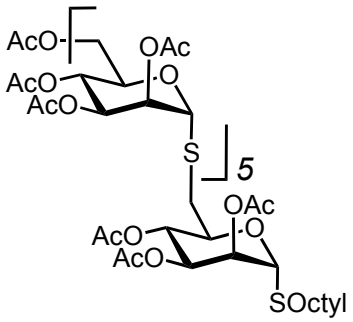
Octyl 2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.39)



Prepared from tetrasaccharide **5.33** (0.38 g, 0.28 mmol) as described for the synthesis of **5.34** to give **5.39** as an amorphous white solid (0.22 g, 85%): R_f 0.35 (1:2 hexane–EtOAc); $[\alpha]_D$ = +167.3 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, CD_2Cl_2) δ_H 5.22–5.36 (m, 15H, H-1‴, H-2‴, H-3‴, H-4‴, H-1″, H-2″, H-3″, H-4″, H-1′, H-2′, H-3′, H-4′, H-2, H-3, H-4), 5.21 (d, 1H, *J* = 1.2 Hz, H-1), 4.26–4.35 (m, 3H, H-5″, H-5′, H-5), 4.10 (ddd, 1H, *J* = 10.2, 3.6, 3.6 Hz, H-5‴), 3.62–3.68 (m, 2H, H-6a‴, H-6b‴), 2.98 (dd, 1H, *J* = 14.7, 2.8 Hz, H-6a″/H-6a′/H-6a), 2.92 (dd, 1H, *J* = 14.5, 2.8 Hz, H-6a″/H-6a′/H-6a), 2.88 (dd, 1H, *J* = 14.3, 3.1 Hz, H-6a″/H-6a′/H-6a), 2.65–2.77 (m, 4H, H-6b″, H-6b′, H-6b, octyl SCH_2), 2.60 (ddd, 1H, *J* = 12.8, 7.4, 7.4 Hz, octyl SCH_2), 2.15 (s, 3H, C(O)CH_3), 2.15 (s, 9H, C(O)CH_3),, 2.11 (s, 3H, C(O)CH_3), 2.09 (s, 3H, C(O)CH_3), 2.08 (s, 3H, C(O)CH_3), 2.05 (s, 3H, C(O)CH_3), 2.00 (s, 3H, C(O)CH_3), 1.99 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.56–1.65 (m, 2H, octyl CH_2), 1.20–1.44

(m, 10H, octyl CH_2), 0.87 (t, 3H, J = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.8 (*C*=*O*), 170.1(3) (*C*=*O*), 170.1(0) (*C*=*O*), 170.0(6) (*C*=*O*), 169.9 (*C*=*O*), 169.8(2) (*C*=*O*), 169.7(9) (2C, *C*=*O*), 169.7(5) (*C*=*O*), 169.6 (3C, *C*=*O*), 81.9 (C-1), 81.8, 81.6, 81.3 (C-1‴, C-1″, C-1′), 71.5, (C-5‴), 71.2, 71.0, 70.8(4), 70.7(9) (C-2‴, C-2″, C-2′, C-2), 69.5, 69.4 (2C), 69.2(5), 69.2(0), 69.1(0), 69.0(7), 69.0(3), 68.9(6), 68.8 (C-3‴, C-3″, C-4″, C-5″, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.7 (C-4‴), 61.3 (C-6‴), 31.8 (octyl *C*H_2), 31.5, 31.0(3), 30.9(9) (2C) (C-6″, C-6′, C-6, octyl S*C*H_2), 29.3 (octyl *C*H_2), 29.1(4) (octyl *C*H_2), 29.1(1) (octyl *C*H_2), 28.7 (octyl *C*H_2), 22.6 (octyl *C*H_2), 20.8(7), 20.8(4), 20.7(7), 20.7(2), 20.6(1), 20.6(0), 20.5(7) (12C, C(O)*C*H_3), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{56}H_{82}O_{29}S_4$: 1369.3717. Found: 1369.3714.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.41)

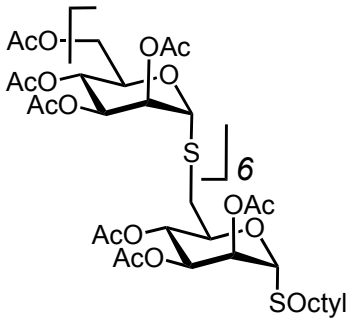


The triflate derivative of **5.39** (95 mg, 0.070 mmol) was prepared as described for the synthesis of **5.35**. After being dried under vacuum for 15 min, the product was added to a

mixture of **5.27** (prepared from **5.23** (76 mg, 0.11 mmol)) and triethylamine (45 μL, 0.33 mmol). The reaction mixture was stirred for 1 h, diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue was purified by chromatography (1:2 hexane–EtOAc) to give **5.41** as an amorphous white solid (58 mg, 41%): R_f 0.26 (1:2 hexane–EtOAc); $[\alpha]_D$ = +185.4 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 5.18–5.36 (m, 24H, H-1′′′′′, H-2′′′′′, H-3′′′′′, H-4′′′′′, H-1′′′′, H-2′′′′, H-3′′′′, H-4′′′′, H-1′′′, H-2′′′, H-3′′′, H-4′′′, H-1′′, H-2′′, H-3′′, H-4′′, H-1′, H-2′, H-3′, H-4′, H-1, H-2, H-3, H-4), 4.20–4.34 (m, 7H, H-5′′′′′, H-6a′′′′′, H-5′′′′, H-5′′′, H-5′′, H-5′, H-5), 4.09 (dd, 1H, *J* = 12.5, 2.0 Hz, H-6b′′′′′), 2.89–3.07 (m, 4H, H-6a′′′, H-6a′′, H-6a′, H-6a), 2.79 (dd, 1H, *J* = 14.5, 2.5 Hz, H-6a′′′′), 2.55–2.82 (m, 7H, H-6b′′′′, H-6b′′′, H-6b′′, H-6b′, H-6b, octyl SC*H*$_2$), 1.90–2.20 (m, 57H, C(O)C*H*$_3$), 1.57–1.65 (m, 2H, octyl C*H*$_2$), 1.18–1.43 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.5 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5, 170.1, 169.9(8), 169.9(6), 169.9(3), 169.8(8), 169.8(3), 169.8(1), 169.7(6), 169.6(5), 169.6(0), 169.5(8) (19C, *C*=*O*), 81.8, 81.5, 81.2, 80.5, 80.4, 80.2 (C-1′′′′′, C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 71.2, 71.0(4), 70.9(8) (3C), 70.8 (C-2′′′′′, C-2′′′′, C-2′′′, C-2′′, C-2′, C-2), 69.5, 69.2(8), 69.2(2), 69.1, 69.0, 68.9(9), 68.9(2), 68.8(7), 68.8(3), 68.7 (17C, C-3′′′′′, C-5′′′′′, C-3′′′′, C-4′′′′, C-5′′′′, C-3′′′, C-4′′′, C-5′′′, C-3′′, C-4′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.3 (C-4′′′′′), 62.3 (C-6′′′′′), 31.8 (octyl *C*H$_2$), 31.1 (C-6′′′′), 30.9 (octyl S*C*H$_2$), 30.5, 30.3, 30.0, 29.8 (C-6′′′, C-6′′, C-6′, C-6), 29.2 (octyl *C*H$_2$), 29.1(2) (octyl *C*H$_2$), 29.1(0) (octyl *C*H$_2$),

28.7 (octyl *C*H_2), 22.6 (octyl *C*H_2), 20.8(9), 20.8(4), 20.8(2), 20.7(8), 20.7(6), 20.7(3), 20.6(8), 20.6(5), 20.6(1), 20.5(6), (19C, C(O)*C*H_3), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{82}H_{116}O_{44}S_6$: 2019.5056. Found: 2019.5062.

Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (5.42)



The triflate derivative of **5.39** (104 mg, 0.077 mmol) was prepared as described for the synthesis of **5.35**. After being dried under vacuum for 15 min, the crude product was added to a mixture of isothiourea salt **5.28** (prepared in situ from **5.25** (140 mg, 0.14 mmol)) and triethylamine (60 µL, 0.42 mmol). The reaction mixture was stirred for 1 h, diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, concentrated and the resulting residue was purified by chromatography (1:2 hexane–EtOAc) to give **5.42** as an amorphous white solid (79 mg, 44%): R_f 0.30 (1:2 hexane–EtOAc); $[\alpha]_D$ = +195.2 (*c* 1.0, CH_2Cl_2); ^{1}H NMR

(500 MHz, $CDCl_3$) δ_H 5.18–5.36 (m, 28H, H-1′′′′′′, H-2′′′′′′, H-3′′′′′′, H-4′′′′′′, H-1′′′′′, H-2′′′′′, H-3′′′′′, H-4′′′′′, H-1′′′′, H-2′′′′, H-3′′′′, H-4′′′′, H-1′′′, H-2′′′, H-3′′′, H-4′′′, H-1′′, H-2′′, H-3′′, H-4′′, H-1′, H-2′, H-3′, H-4′, H-1, H-2, H-3, H-4), 4.19–4.34 (m, 8H, H-5′′′′′′, H-6a′′′′′′, H-5′′′′′, H-5′′′′, H-5′′′, H-5′′, H-5′, H-5), 4.10 (dd, 1H, *J* = 12.5, 2.0 Hz, H-6b′′′′′′), 2.90–3.09 (m, 5H, H-6a′′′′, H-6a′′′, H-6a′′, H-6a′, H-6a), 2.78 (dd, 1H, *J* = 14.5, 2.5 Hz, H-6a′′′′′), 2.56–2.77 (m, 8H, H-6b′′′′′, H-6b′′′′, H-6b′′′, H-6b′′, H-6b′, H-6b, octyl SCH_2), 1.90–2.20 (m, 66H, C(O)CH_3), 1.54–1.65 (m, 2H, octyl CH_2), 1.18–1.43 (m, 10H, octyl CH_2), 0.87 (t, 3H, *J* = 6.5 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.5, 170.2, 169.9(9), 169.9(6), 169.9(3), 169.8(4), 169.8(2), 169.8(0), 169.7(6), 169.6(6), 169.6(1) (22C, *C*=*O*), 81.8, 81.5, 81.1, 80.4, 80.3, 80.1, 80.0 (C-1′′′′′′, C-1′′′′′, C-1′′′′, C-1′′′, C-1′′, C-1′, C-1), 71.2, 71.1, 70.9(9), 70.9(2), 70.8 (7C, C-2′′′′′′, C-2′′′′′, C-2′′′′, C-2′′′, C-2′′, C-2′, C-2), 69.5(1), 69.4(7), 69.3, 69.2(9), 69.2(7), 69.2(3), 69.1, 69.0(5), 69.0(2), 68.9(9), 68.9(6), 68.9(1), 68.8, 68.7(1), 68.6(7), 68.6(1) (20C, C-3′′′′′′, C-5′′′′′′, C-3′′′′′, C-4′′′′′, C-5′′′′′, C-3′′′′, C-4′′′′, C-5′′′′, C-3′′′, C-4′′′, C-5′′′, C-3′′, C-4′′, C-5′′, C-3′, C-4′, C-5′, C-3, C-4, C-5), 66.3 (C-4′′′′′′), 62.3 (C-6′′′′′′), 31.8 (octyl *C*H_2), 31.1 (C-6′′′′′), 30.9 (octyl S*C*H_2), 30.4, 30.3, 29.9, 29.7(4), 29.6(6) (C-6′′′′, C-6′′′, C-6′′, C-6′, C-6), 29.2 (octyl *C*H_2), 29.1(3) (octyl *C*H_2), 29.1(0) (octyl *C*H_2), 28.7 (octyl *C*H_2), 22.6 (octyl *C*H_2), 20.9, 20.8(5), 20.8(2), 20.7(8), 20.6(8), 20.6(5), 20.6(1), 20.5(7) (22C, C(O)*C*H_3), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{94}H_{132}O_{51}S_7$: 2323.5673. Found: 2323.5635.

Bacterial strains and growth conditions

M. smegmatis mc^2155 was a generous gift from Professor William R. Jacobs, Jr. at the Albert Einstein College of Medicine. The bacteria were grown at 37 °C in 100 mL of Luria Bertoni (LB) broth medium containing 0.05% Tween 80 to an A_{600nm} of < 1.0 (~two days from a frozen bacterial stock). The 50 mL liquid cultures were then transferred to 2 × 1 L of fresh media and cultured further for 24 h at 37 °C. Cells were harvested by centrifugation, washed with phosphate buffered saline (PBS) and stored at –20 °C until use.

Preparation of membrane fractions from *M. smegmatis*

The *M. smegmatis* cell pellet (~10 g wet weight) was washed and resuspended in 100 mL of 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (adjusted to pH 7.9 with KOH) containing 5 mM β-mercaptoethanol and 10 mM $MgCl_2$ supplemented with Complete Protease Inhibitor Cocktail Tablets (Roche) at 4 °C. The cells were subjected to two passes through a Thermo Spectronic French Pressure Cell Press at 20,000 psi. The cell lysate was centrifuged at 600 × *g* for 15 min and then at 27,000 × *g* for 20 min. The resulting supernatant was centrifuged at 100,000 × *g* for 60 min. The supernatant was carefully removed and the membrane pellets were gently resuspended in 1 mL of 50 mM MOPS buffer, pH 7.9, containing 5 mM β-mercaptoethanol and 10

mM $MgCl_2$. Protein concentrations were determined by the BCA™ Protein Assay (Pierce) using bovine serum albumin as the standard.

Radiochemical activity assays

The ManT enzyme activity was determined using the previously established cell-free system.[9] Unless indicated otherwise, the synthetic acceptor analogs at a concentration of 2.0 mM were incubated with 0.20 μCi of guanosine diphosphate mannose, [mannose-2-^{3}H] (American Radiolabeled Chemicals, Inc., 20 Ci/mmol) in 50 mM MOPS buffer, pH 7.9, containing 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, and membrane fraction (94.6 μg of protein) in a total volume of 80 μL. All assays were performed in duplicate and control assays without acceptor were also performed in parallel to correct for the presence of endogenous acceptor. The enzymatic activities were determined using SepPak radiochemical C_{18} assays.[34] Briefly, after incubation at 37 °C for 1 h, the reactions were stopped by adding 100 μL of $CHCl_3$–MeOH (2:1 v/v) and the mixtures were centrifuged. The supernatants were recovered and further diluted with H_2O before loading onto SepPak C_{18} cartridges (Waters). The unreacted donor was removed by washing the cartridges with H_2O (50 mL) and the radiolabeled products were eluted with MeOH (4.0 mL). The isolated products in the eluants were quantified by liquid scintillation counting on a Beckman LS6500 Scintillation Counter using 10 mL of Ecolite cocktail. For kinetic analysis, the ManT activities were

determined using a range of acceptor concentrations (0.016 to 2.0 mM). All other reaction conditions were identical to the cell-free assay as described above. Assays were performed under the conditions in which the formations of radiolabeled products were linear for both time and protein concentration. The kinetic parameters K_M and V_{max} were obtained by nonlinear regression analysis using the Michaelis-Menten equation with the GraphPad Prism 4.0 program (GraphPad Software, San Diego, CA).

Product characterizations from milligram-scale incubations

Large-scale ManT reactions were performed for the structural characterization using acceptor substrates **9–14**. A typical reaction containing 50 mM MOPS buffer, pH 7.9, 1 mM ATP, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 2 mM acceptor, 2 mM GDP-mannose, and the *M. smegmatis* membrane preparation, was incubated at 37 °C with gentle rotation for 2 days. After organic wash, the aqueous phase of the reaction mixture was loaded directly on the C_{18} reverse-phase cartridge and the unreacted donor was washed away with H_2O (50 mL) and the product was eluted subsequently with MeOH (4 mL). The solvent was evaporated and the residue was redissolved in H_2O (50 μL). The conversion of the acceptor substrate to the enzymatic product was analyzed by MALDI mass spectrometry on a Voyager Elite time-of-flight spectrometer on sample suspended in 2,5-dihydroxy benzoic acid, using the delayed-extraction mode and positive-ion detection.

Glycosidic linkage analysis by *exo*-mannosidases

Large-scale ManT reactions (160 µL) were performed using acceptor substrates **9–14** and the radioactive enzymatic products were purified as usual. After removal of methanol, the residues were redissolved in 200 µL H_2O. Ten microliters of the suspension were digested with exo-mannosidases, *Aspergillus saitoi* α(1,2)-mannosidase (AS) (Glyko), jack bean α(1-2,3,6)-mannosidase (JB) (Glyko) and *Xanthomonas manihotis* α-(1,6)-mannosidase (XM) (New England Biolabs), according to the manufacturers' procedures. After one day incubation for a day, the reaction mixtures were purified by the C_{18} reverse-phase cartridge.

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Chapter 6

Synthesis and Evaluation of Mono- and Dideoxy-Galactofuranosides as Substrates for GlfT2, a β-(1→5, 6)-Galactofuranosyltransferase

6.1. Introduction

In addition to lipoarabinomannan (LAM), the other major entity of the mycobacterial cell wall is the mycolyl-arabinogalactan (mAG) complex. This glycan is the largest structural component of the cell wall and contributes ~35% of the cell wall mass. Interestingly, unlike LAM, the core structure of this mAG moiety is mainly made up of five membered ring sugar residues, galactofuranose (Gal*f*) and arabinofuranose (Ara*f*), which constitute the galactan and arabinan subcomponents, respectively. As shown in Figure 6-1, the linear galactan (~30 to 35 Gal*f* residues) is covalently attached to cell wall peptidoglycan via a disaccharide linker consisting of an α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucosamine-phosphate unit (α-L-Rha*p*-(1→3)-Glc*p*NAc-P). The linear polymer is further functionalized with three identical arabinan units (~90 arabinofuranose residues in total). In turn, the about two-thirds of the nonreducing termini of these arabinan moieties are esterified with mycolic acids, which are long chain branched lipids containing 70–90 carbon atoms.[1,2]

The biosynthesis of galactan has attracted much attention in recent years; in particular, the steps and enzymes involved in the initial stage of

the pathway have been well studied.[3-8] As illustrated in Figure 6-2, the pathway initiates from the sequential additions of *N*-acetylglucosaminyl phosphate and rhamnose to a lipid carrier decaprenyl monophosphate to forms the disaccharide linker α-Rha*p*-(1→3)-Glc*p*NAc*p*-P-P-polyprenyl. The subsequent formation of the galactan portion, which consists of alternating β-(1→5) and β-(1→6)-linked Gal*f* residues, is then synthesized by the action of only two bifunctional glycosyltransferases, GlfT1 and GlfT2. It has been shown that GlfT1 catalyzes the first two Gal*f* additions, formation of both β-Gal*f*-(1→4)-Rha*p* and β-Gal*f*-(1→5)-Gal*f* linkages, to the α-Rha*p*-(1→3)-Glc*p*NAc*p*-P-P-Polyprenyl acceptor.[6,8] On the other hand, GlfT2, a β-(1→5,6)-galactofuranosyltransferase, is responsible for formation of the bulk of galactan.[3-5,7]

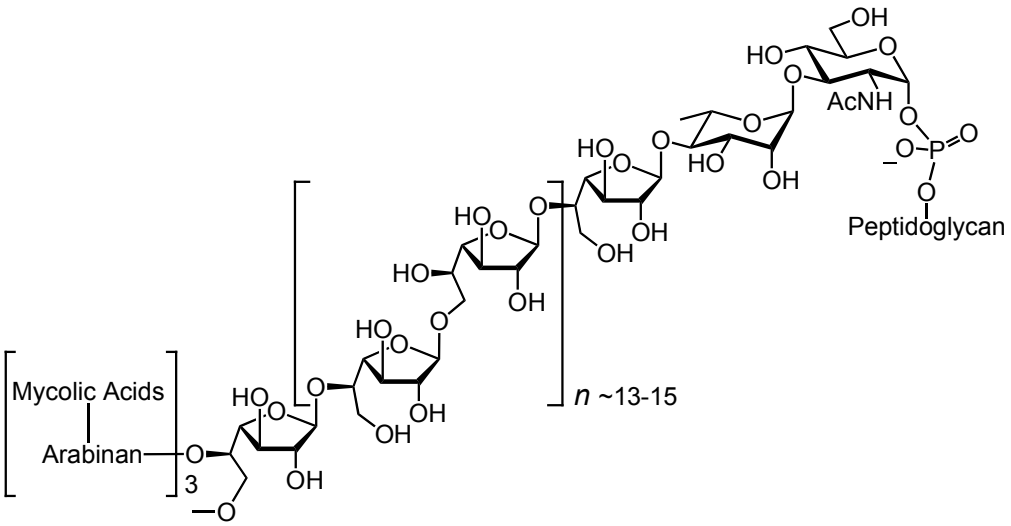


Figure 6-1. Structure of the mycobacterial mAGP complex in which the galactan and the disaccharide linkage to peptidoglycan are emphasized. The arabinan motifs, with numbers of mycolic acids attached at the non-reducing ends, are added to the eighth, tenth and twelfth Gal*f* residues.

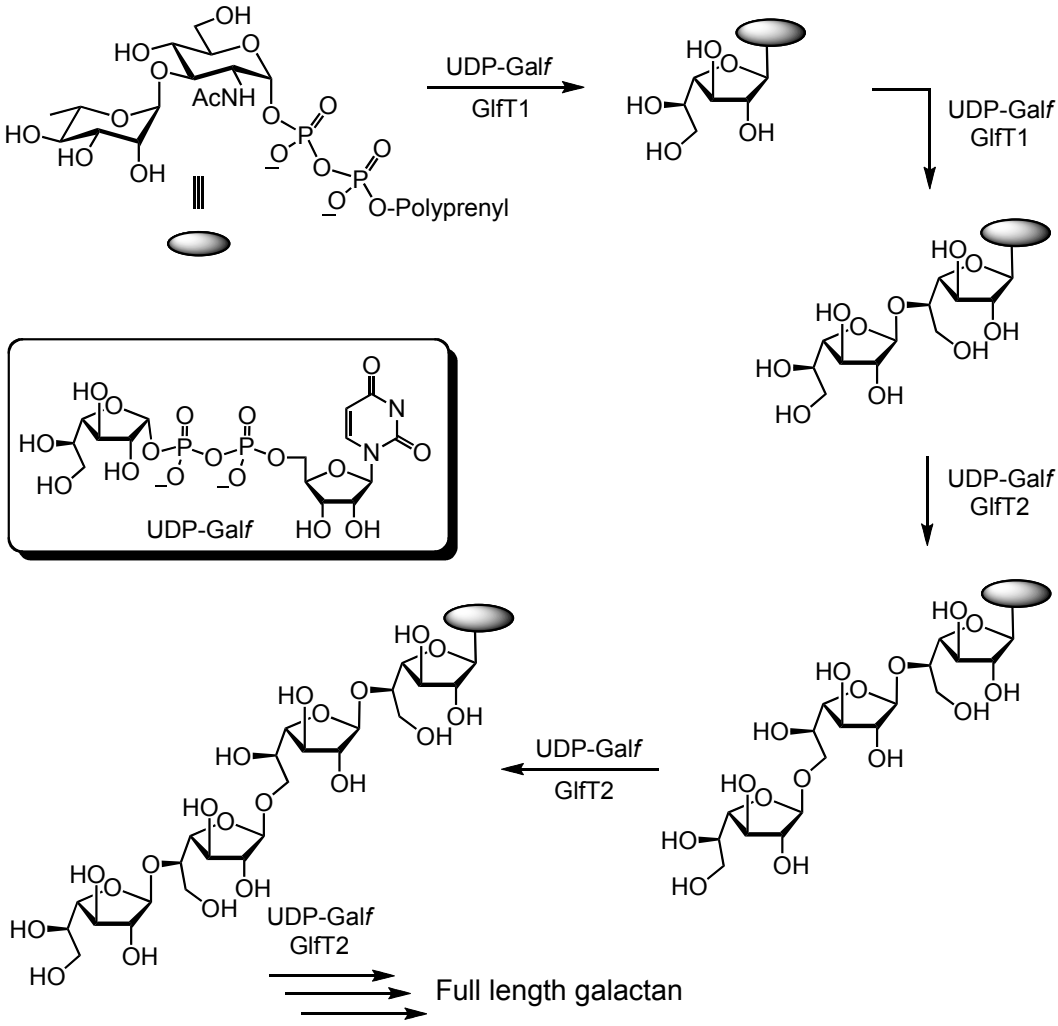


Figure 6-2. Current model for the biosynthesis of mycobacterial galactan.

Given the importance of the mAG complex to the viability of mycobacteria, key enzymes involved in the biosynthetic pathway represent a rich source of therapeutic drug targets. In fact, our research interest has been focused on the study of GlfT2 over the last few years. Our previous studies have demonstrated that octyl trisaccharides **6.1** and

6.2 (Figure 6-3) serve as effective substrates for the two glycosyl transfers catalyzed by GlfT2.[5] Accordingly, trisaccharide **6.1** is a substrate for the β-(1→6)-transferase activity while **6.2** is a substrate for the β-(1→5)-transferase activity. Due to its bifunctionality, the resulting enzymatic products from both **6.1** and **6.2** are also the substrates of GlfT2 and lead to the formation of a series of oligosaccharides, with alternating β-(1→5) and (1→6)-linkages, which can be observed in preparative-scale assays.

Recent work using saturation transfer difference **(**STD)-NMR, has shown that **6.1** and **6.2** compete for the same binding site on the protein, suggesting that the enzyme has a single active site.[9] To probe further the mechanism by which the enzyme catalyzes both glycosyl transfers, deoxygenated analogs **6.3**–**6.6** (Figure 6-3) have been synthesized and screened as inhibitors of GlfT2.[10] This study revealed that both the mono- (**6.3** and **6.4**) and di-deoxy analogs (**6.5** and **6.6**) were able to compete against their corresponding parental substrates. The conclusion of this work was that the removal of the C-5 and C-6 hydroxyl groups on the terminal residues of **6.1** and **6.2** has no effect on the recognition by the enzyme (although as they lack a reactive hydroxyl group they are inhibitors, rather than substrates). To investigate further if other C-5 and C-6 hydroxyl groups on **6.1** and **6.2** play important roles for the polar interaction with GlfT2, additional deoxygenated analogs **6.7**–**6.16** (Figure 6-4) were synthesized and their ability to serve as substrate acceptors for the enzyme was evaluated.

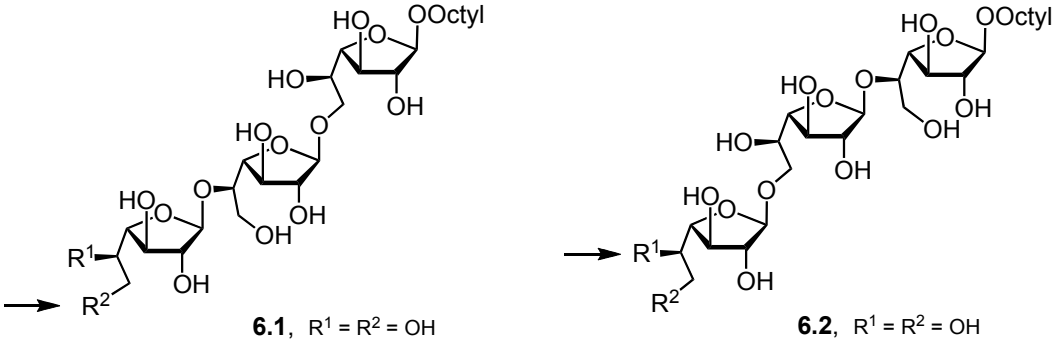


Figure 6-3. Demonstrated GlfT2 substrates (**6.1**, **6.2**) and deoxygenated target inhibitory analogs (**6.3**–**6.6**) from a previous study.[10] Arrows indicate the site of GlfT2-catalyzed glycosylation of **6.1** and **6.2**.

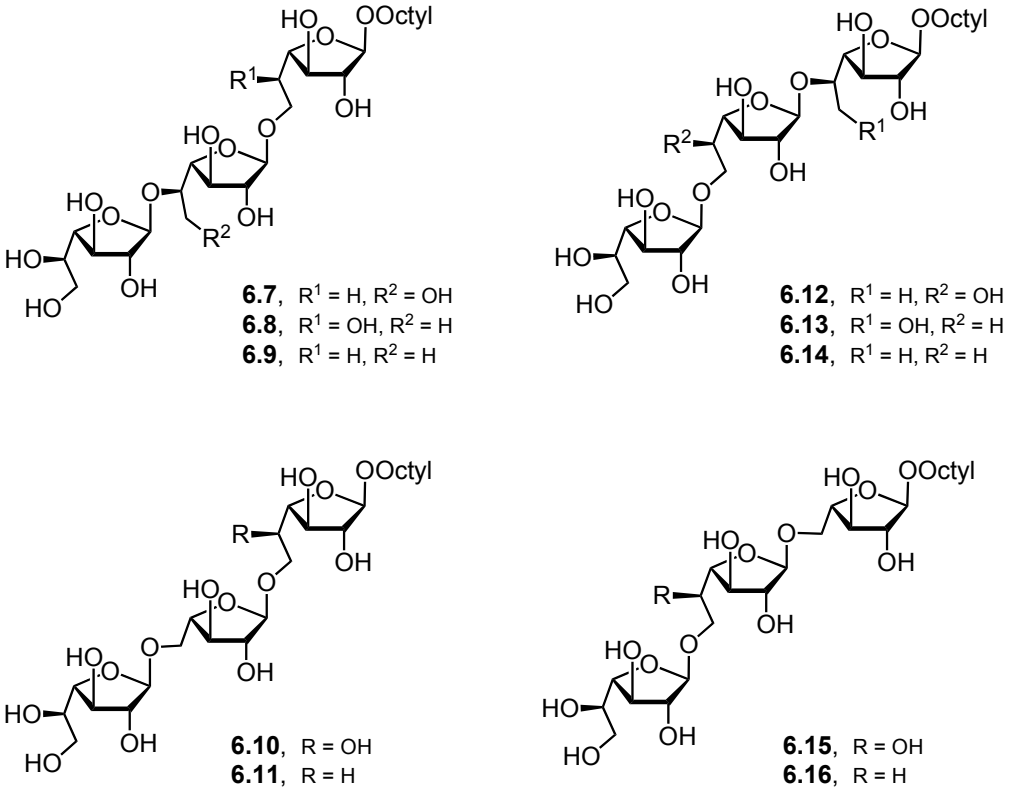
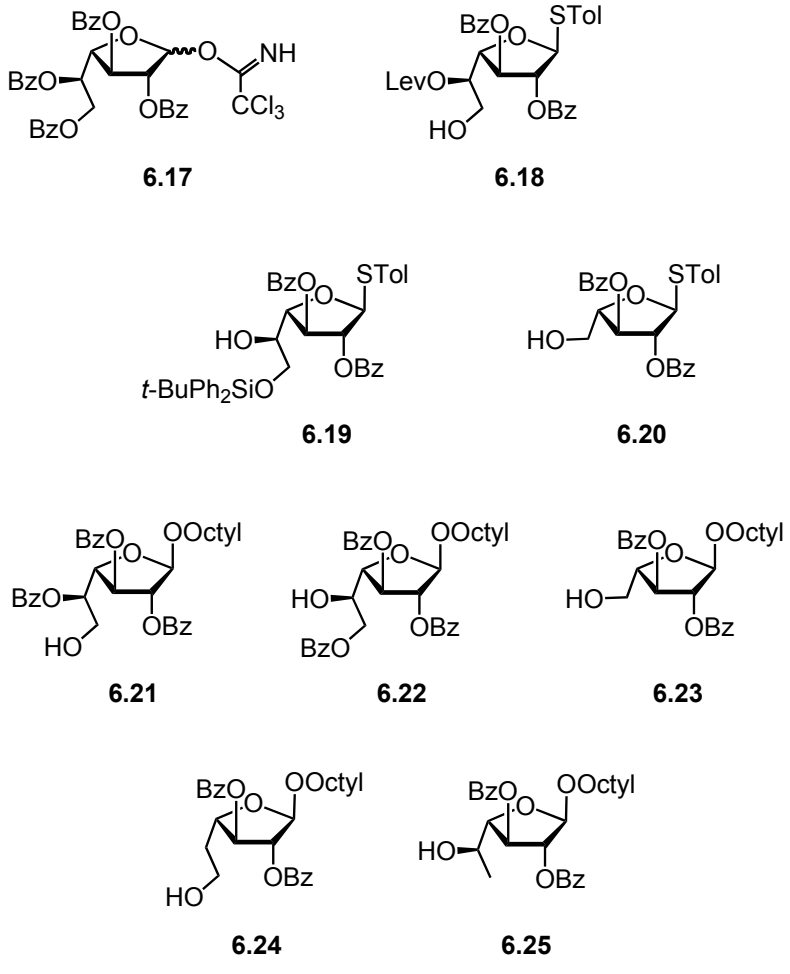


Figure 6-4. Deoxygenated target analogues (**6.7**–**6.16**).

6.2. Results And Discussion

6.2.1. Synthesis of Deoxygenated Trisaccharide Analogs

Our initial task was to synthesize the trisaccharide targets **6.7**–**6.16**. We envisioned that both mono- and dideoxy derivatives could be assembled from building blocks **6.17**–**6.25** (Figure 6-5). Coupling of the known imidate **6.17**[11] with thioglycoside alcohols **6.18**, **6.19** and **6.20** would generate disaccharide donors, which in turn could be reacted with the known octyl glycoside acceptors **6.21** and **6.22**,[12] and **6.23**–**6.25**.



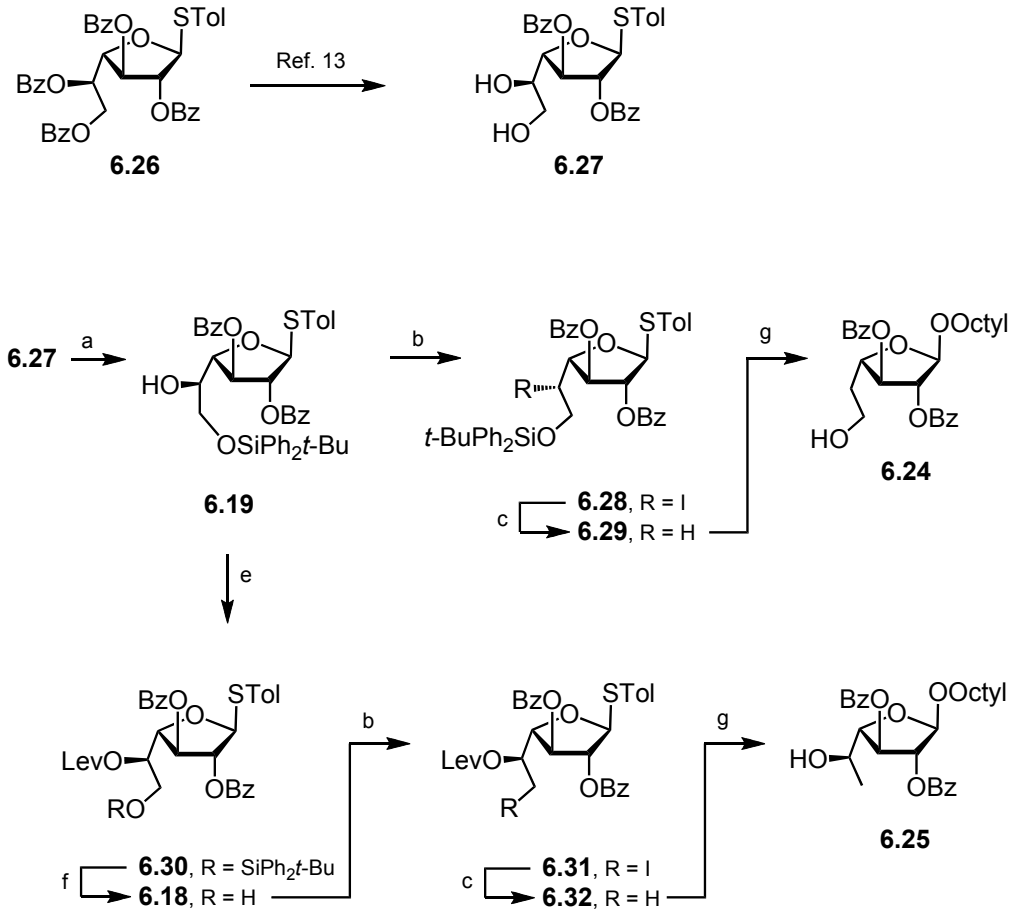
6.17 **6.18** **6.19** **6.20** **6.21** **6.22** **6.23** **6.24** **6.25**

Figure 6-5. Key building block for the synthesis of analogs **6.7**–**6.16**.

The preparation of octyl glycoside alcohols **6.24** and **6.25** is shown in Scheme 6-1. Reaction of diol **6.27**, which was derived from *p*-tolyl 2,3,5,6-tetra-*O*-benzoyl-galactofuranoside **6.26**,[13] with *tert*-butyldiphenylchlorosilane and imidazole provided silyl ether **6.19** in 96% yield. The secondary hydroxyl group of **6.19** was then treated with triphenylphosphine, imidazole and iodine to afford iodide **6.28** in excellent yield (97%). Consistent with the C-I bond formation, a resonance at 33.6 ppm in the ^{13}C NMR spectrum could be assigned to C-5 for **6.28**. Although the stereogenic center at C-5 in **6.28** would be removed in the subsequent reduction, we noticed that the $^{3}J_{H4,H5}$ coupling constant changed from 2.5 Hz (**6.19**) to 6.1 Hz (**6.28**) suggesting an inversion of configuration. A similar observation was also obtained from the iodination of **5.38** to give **5.39** (see below).

Subsequent treatment of **6.28** with a palladium catalyst under a hydrogen atmosphere yielded 5-deoxy thioglycoside **6.29** (85%). However, the presence of thioglycoside made the reaction somewhat sluggish, in which a typical reaction would require more catalyst (50% w/w) and longer reaction time (4 days). That the deoxygenation had occurred was clearly evident from the ^{1}H and ^{13}C NMR spectra for **6.29**. In the ^{1}H NMR spectrum, two multiplets corresponding to H-5a and H-5b were observed in the region of 2.04–2.25 ppm. In addition, in the ^{13}C NMR spectrum a resonance at 35.7 ppm could be assigned to C-5.

Reaction of **6.29** with octanol under NIS–TMSOTf activation[14] afforded the desired octyl glycoside. Subsequent desilylation using hydrogen fluoride followed by column purification gave **6.24** in 81% yield over two steps.

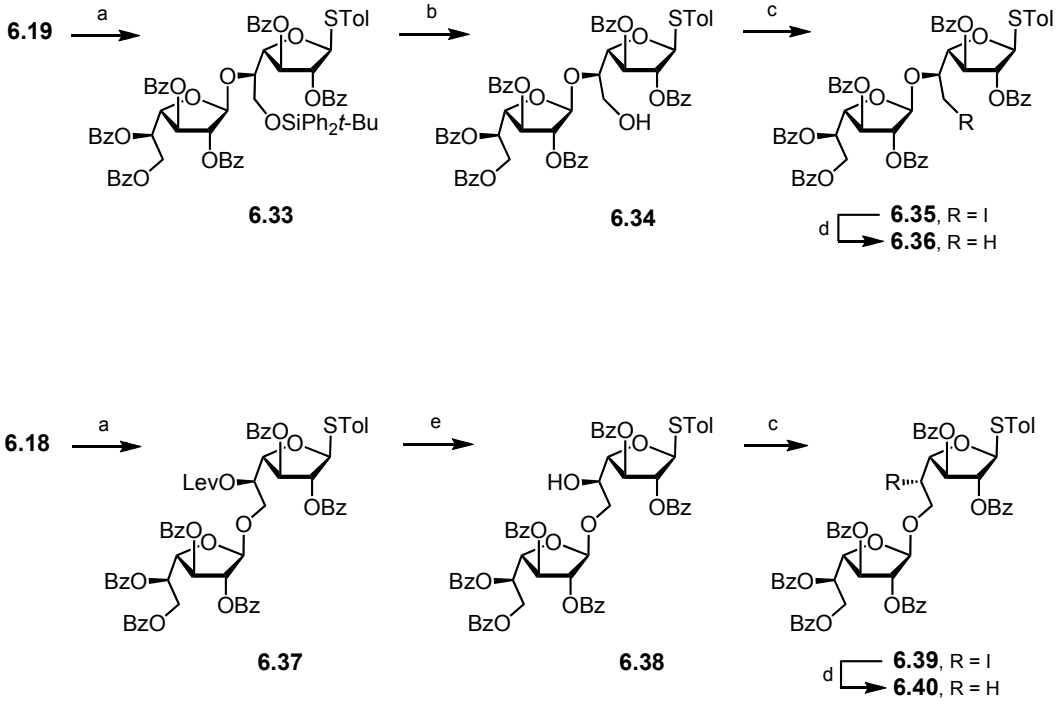


Scheme 6-1. Reagents and conditions: a) *t*-$BuPh_2SiCl$, imidazole, THF, 96%; b) PPh_3, imidazole, I_2, toluene, reflux, 93% for **6.28**, 76% for **6.31**; c) 20% $Pd(OH)_2$–C, *i*-Pr_2EtN, EtOAc, 85% for **6.29**, 86% for **6.32**; d) i: Octanol, NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C; ii: THF–pyridine–70% HF·pyridine (20:4:1), 81%; e) Levulinic acid, DCC, DMAP, CH_2Cl_2, 95%; f) THF–pyridine–70% HF·pyridine (20:4:1), 90%; g) i: Octanol, NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C; ii: hydrazine acetate, THF–MeOH (10:1), 76%.

For this, and all of the glycosylation reactions described in this chapter, the β-stereochemistry of the glycosidic linkages was confirmed by the ^{1}H and ^{13}C NMR spectra of the products. For example, in the ^{1}H NMR spectrum of **6.24**, the anomeric hydrogen resonances of the newly formed glycosidic linkages appeared as singlet at 5.22 ppm, and in the ^{13}C NMR spectrum, the anomeric carbon resonance appeared at 105.4 ppm. Both are consistent with the β-Gal*f* stereochemistry.[15]

Alternatively, to access **6.25**, the secondary OH group of **6.19** was protected with a levulinoyl group using levulinic acid and DCC (dicyclohexylcarbodiimide) to give galactofuranoside **6.30** in good yield (95%). Analogous to the synthesis of **6.24**, the primary hydroxyl group of **6.18**, which was obtained after removal of the silyl protective group from **6.30**, was subjected to iodination and palladium-catalyzed reduction to generate **6.31** and **6.32** in 76% and 86% yields, respectively. Formation of the carbon–halogen bond in **6.31** was revealed by the ^{13}C NMR spectrum, which showed a resonance at 0.9 ppm for C-6. On the other hand, the C-6 signal of **6.32** was shifted downfield (16.2 ppm) upon removal of the iodo substituent. The structure of **6.32** was further supported by the ^{1}H NMR spectrum, in which the C-6 methyl group appeared as a doublet at 1.41 ppm. Coupling of thioglycoside **6.32** with octanol under NIS–TMSOTf activation and subsequent removal of the levulinoyl group using hydrazine acetate afforded the corresponding monosaccharide **6.25** in 76% yield over two steps.

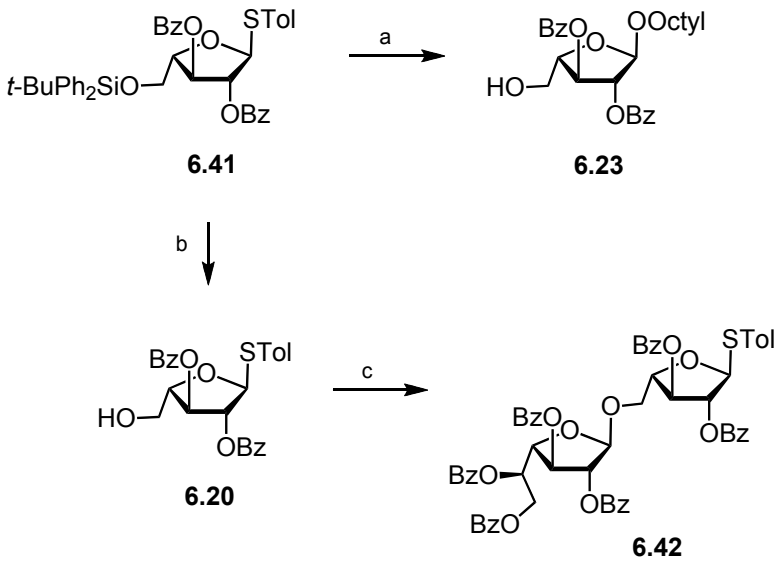
As the two-step deoxygenation described above worked equally well at both the primary and secondary positions, we envisioned the removal of these hydroxyl groups could be performed at the disaccharide level, which would consequently, shorten the synthetic routes of all the mono- and dideoxy target analogs. As illustrated in Scheme 6-2, the previously synthesized intermediates **6.18** and **6.19** could serve as acceptors for the coupling with imidate **6.17**[11] to afford disaccharides **6.33** and **6.37** in excellent yields. Removal of the silyl group in **6.33** and the levulinoyl group in **6.37** under the usual conditions provided alcohols **6.34** and **6.38** in yields of 87% and 93% respectively.



Scheme 6-2. Reagents and conditions: a) TMSOTf, 4 Å MS, CH_2Cl_2, –20 °C, quant. for **6.33**, 90% for **6.37**; b) THF–pyridine–70% HF·pyridine (20:4:1), 87%; c) PPh_3, imidazole, I_2, toluene, reflux, 97% for **6.35**, 94% for **6.39**; d) 20% $Pd(OH)_2$–C, *i*-Pr_2EtN, EtOAc, 89% for **36**, 83% for **40**; e) hydrazine acetate, THF–MeOH (10:1), 93%.

Treatment of **6.34** with triphenylphosphine, imidazole and iodine afforded iodide **6.35** in excellent yield (94%). The following reduction of **6.35** with hydrogen in the presence of a palladium catalyst also went smoothly to generate the 6-deoxy disaccharide **6.36** in 89% yield. Deoxygenation of **6.38** via the same synthetic steps used to prepare **6.36** was next examined. Gratifyingly, iodination of alcohol **6.38** and reduction of the resulting intermediate generated **6.39** and **6.40** in 94 and 83% yields, respectively. The formation of the desired halide and deoxygenated intermediates were evident from the NMR spectra, as described above for the preparation of **6.24** and **6.25**.

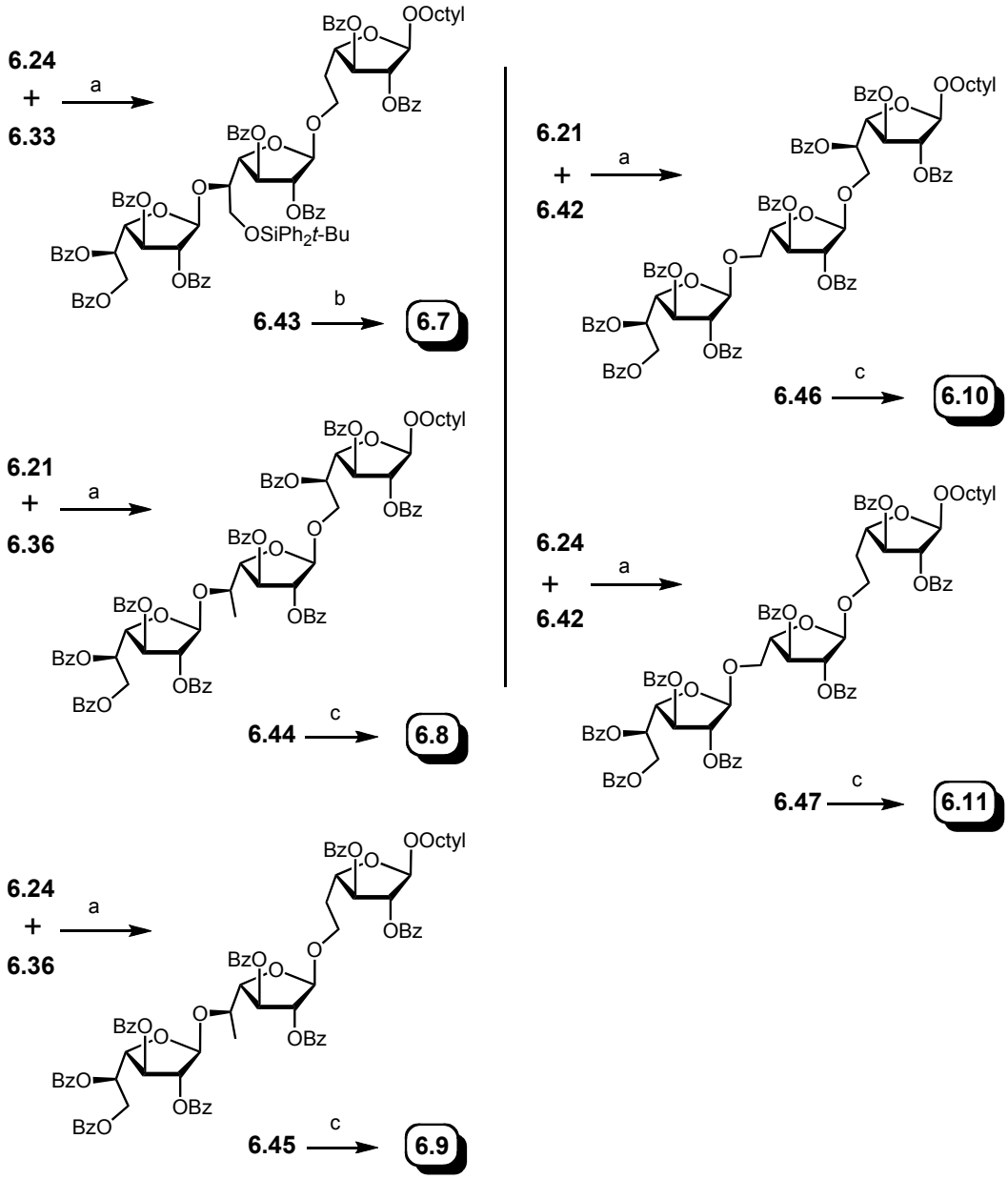
The above deoxy building blocks were needed for the assembly of trisaccharides **6.7**–**6.9** and **6.12**–**6.14**. In contrast, L-arabinofuranosides **6.20** and **6.23** were required for the synthesis of analogs **6.10**, **6.11**, **6.15** and **6.16**. Alcohols **6.20** and **6.23** could be prepared (Scheme 6.3) from silyl ether **6.41**, which was synthesized as described elsewhere.[16] Similar to the synthesis of octyl glycoside **6.24**, glycosylation between **6.41** and octanol using NIS and TMSOTf followed by HF treatment yielded **6.23** (81% over two steps). Alternatively, removal of the silyl group from **6.41** generated alcohol **6.20** (82%), which was coupled with imidate **6.17**,[11] in the presence of catalytic amount of TMSOTf, to afford disaccharide **6.42** in 69% yield.



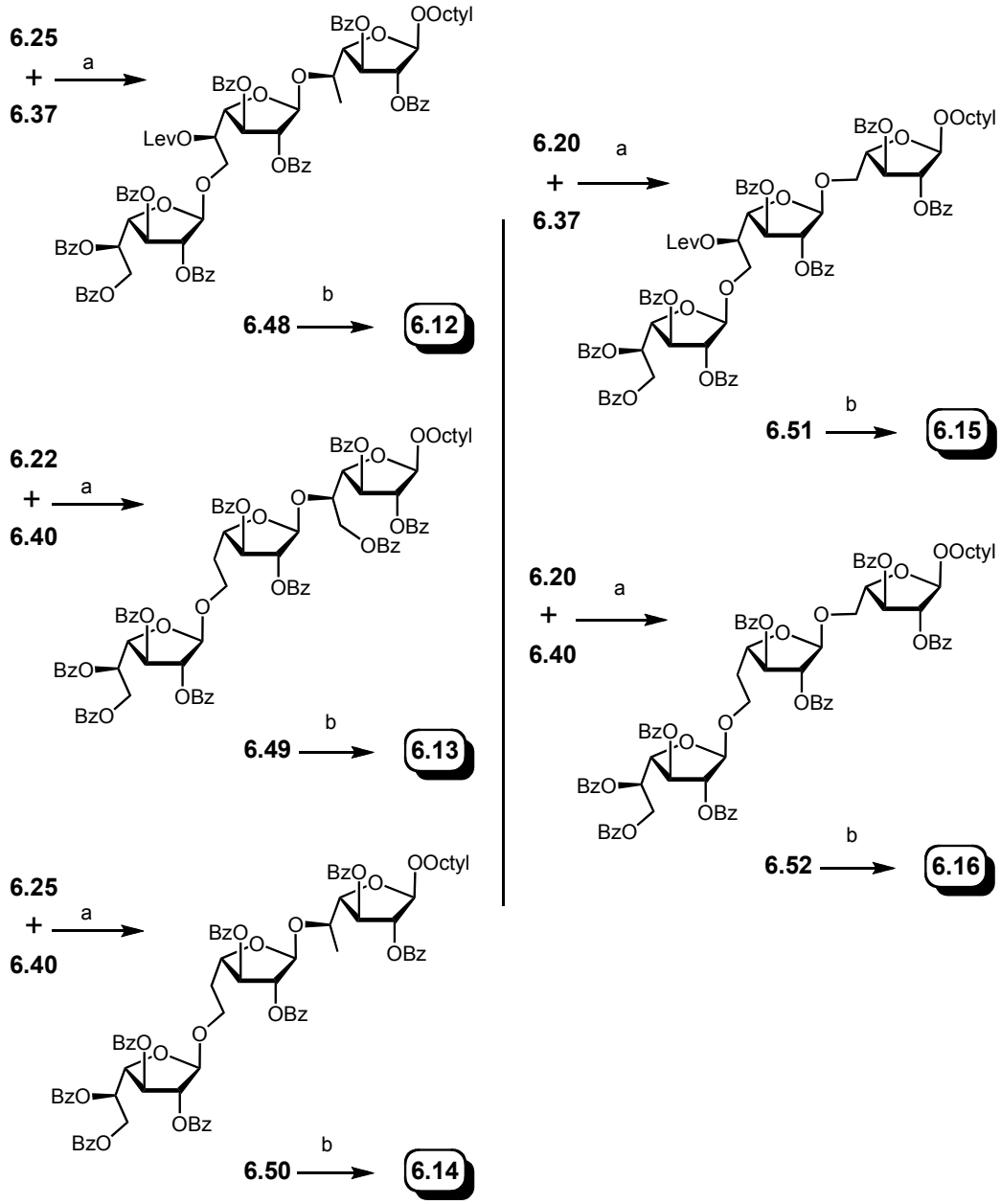
Scheme 6-3. Reagents and conditions: a) i: Octanol, NIS, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C; ii: THF–pyridine–70% HF·pyridine (20:4:1), 81%; b) THF–pyridine–70% HF·pyridine (20:4:1), 82%; c) TMSOTf, 4 Å MS, CH_2Cl_2, –20 °C**,** 69%.

With the key building blocks in hand, thioglycosides **6.33**, **6.36**, **6.37**, **6.40** and **6.42** and acceptors **6.21**–**6.25**, our final task was the assembly of the desired trisaccharide analogs **6.7**–**6.16**. As shown in Scheme 6-4, under the usual NIS–TMSOTf activation protocol, coupling of disaccharide donor **6.33** with acceptor **6.24** provided the protected trisaccharide **6.43** in quantitative yield. Desilylation using HF in pyridine followed by basic hydrolysis afforded target analog **6.7** in a yield of 77% over two steps. Moreover, coupling of donor **6.36** with acceptors **6.21** and **6.24** generated the corresponding trisaccharides **6.44** and **6.45** in 97% and 85% yields, respectively. Final deprotection using catalytic sodium methoxide yielded **6.8** and **6.9** in good yields (78% and 87%). To obtain the L-arabinofuranosyl-containing analogs, disaccharide **6.42** was reacted

with acceptors **6.21** and **6.24** under the usual glycosylation conditions to afford intermediates **6.46** and **6.47** (88% and 93%). Removal of all the benzoyl protective groups provided **6.10** and **6.11** in 89% and 82% yields.



Scheme 6-4. Reagents and conditions: a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, –15 °C, quant. for **6.43**, 97% for **6.44,** 85% for **6.45**, 88% for **6.46**, 93% for **6.47**; b) i: THF–pyridine–70% HF·pyridine (20:4:1); ii: NaOMe, MeOH, 77%; c) NaOMe, MeOH, 78% for **6.8**, 87% for **6.9,** 89% for **6.10**, 82% for **6.11**.



Scheme 6-5. Reagents and conditions: a) NIS, TMSOTf, 4 Å MS, CH_2Cl_2, –15 °C, 95% for **6.48**, 81% for **6.49**, 80% for **6.50**, 99% for **6.51**, 81% for **6.52**; b) NaOMe, MeOH, 90% for **6.12**, 72% for **6.13**, 75% for **6.14,** 88% for **6.15**, 86% for **6.16**.

Following the same approach used for the synthesis of **6.7**–**6.11**, mono- and dideoxy derivatives **6.12**–**6.16** were obtained as described in Scheme 6-5. Coupling of disaccharide donor **6.37** with acceptors **6.20** and **6.25** provided trisaccharides **6.48** and **6.51** in excellent yields (95% and 99%, respectively). Deacylation under basic conditions afforded target analogs **6.12** and **6.15** in yields of 90% and 88%, respectively. In addition, coupling of donor **6.40** with acceptors **6.22**, **6.25** and **6.20** generated the corresponding trisaccharides **6.49**, **6.50** and **6.52** in 81%, 80% and 81% yields, respectively. Final deprotection using catalytic sodium methoxide provided **6.13** (72% yield), **6.14** (75% yield), and **6.16** (86% yield).

6.2.2. Screening As Substrate Acceptors For GlfT2

Upon the completion of our synthetic work, the abilities of these deoxygenated analogs to serve as substrates for GlfT2 catalysis were first evaluated using a recently reported coupled spectrophotometric assay for the enzyme.[17] This work was carried out by Jean Pearcey, a technician in the group. In this assay, the formation of UDP, a by-product of the glycosyl transfer by GlfT2, is coupled to the oxidation of NADH to NAD^+, which can be monitored spectrophotometrically(Figure 6-6). The β-(1→6)-transferase activity of GlfT2 using **6.1** as the substrate was compared to analogs **6.7**–**6.11**. As shown in Figure 6-7A, all these analogs are active substrates for GlfT2 with comparable activities, ranging from 64 to 132%, of the known substrate **6.1**. Similarly, both mono- and di-deoxy analogs

6.12–**6.16** are also substrates for the enzyme, as indicated by their relative β-(1→5)-glycosyltransferase activities compared to that of the parent trisaccharide **6.2** (Figure 6-7B). In particular, the observed relative activities from **6.12** and **6.15** are roughly two and three fold more than that of the control trisaccharide, respectively.

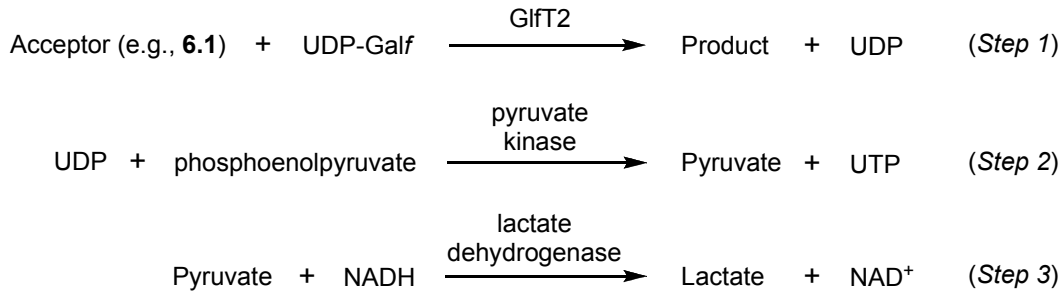


Figure 6-6. Coupled spectrophotometric assay for measuring GlfT2 activity. The decrease in absorption at 340 nm, resulting from oxidation of NADH, is proportional to GlfT2 activity. NADH: nitcotinamide adenine dinucleotide, reduced form).

The preliminary results shown in Figure 6-7 clearly demonstrated that removal of these C-5 and C-6 hydroxyl groups has no dramatic effect on how the enzyme interacts with these trisaccharides. In other words, neither the 5- nor the 6-OH groups of the substrates such as **6.1** and **6.2** are the key recognition elements by GlfT2. Indeed in some cases the compounds appear to be substantially better substrates.

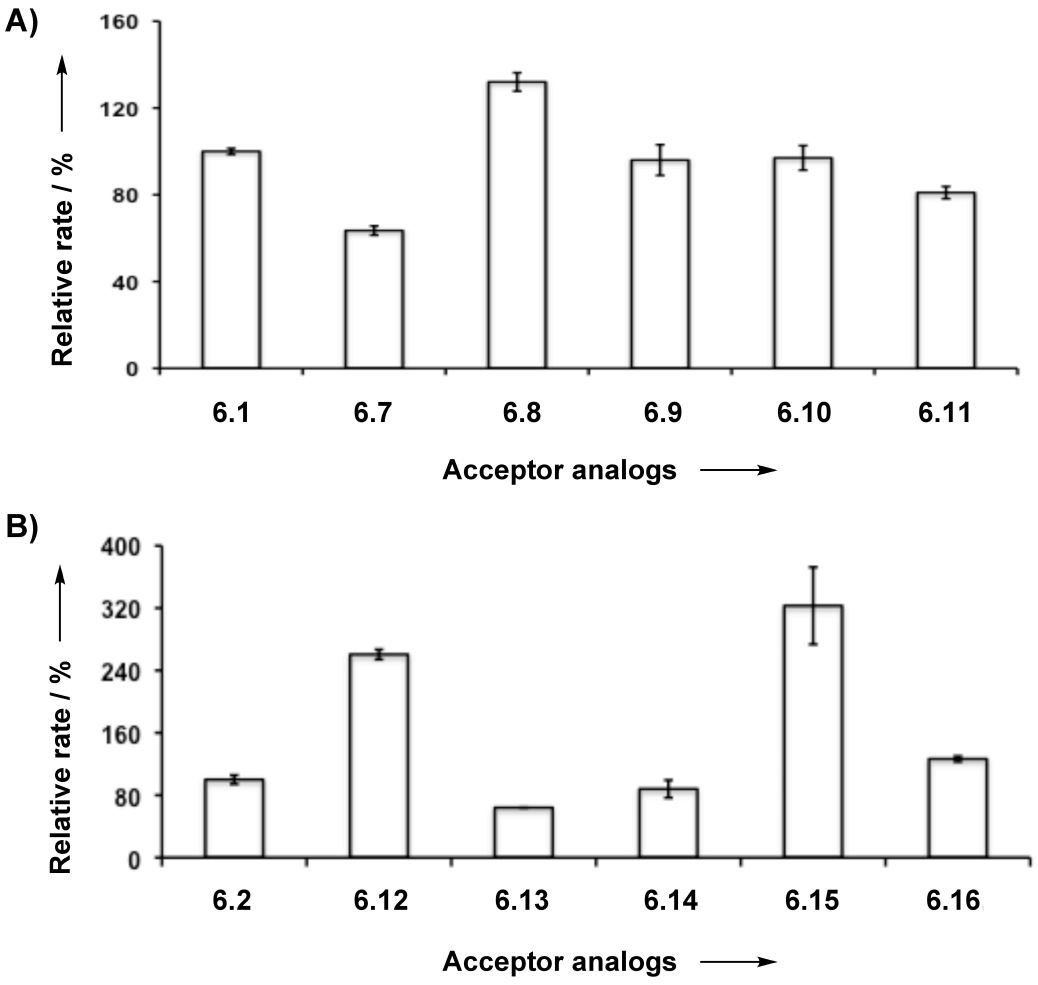


Figure 6-7. Preliminary screening of deoxygenated trisaccharide analogs as substrates for GlfT2. Each acceptor was tested at 0.5 mM and was incubated with UDP-Gal*f* under the reported assay conditions.[16] Relative activities for each acceptor are expressed as a percentage of the incorporation of galactofuranose into the parent compound **6.1** (A) and **6.2** (B). Charts are reproduced with the permission of J. Pearcey.

6.2.3. Structural Analysis of Enzymatic Products

Whether the ring hydroxyl groups at C-2 and C-3 are critical for the hydrogen-bonding interactions with the enzyme remains to be determined. However, the results presented in Figure 6-7 clearly show that the removal of the C-5 and the C-6 hydroxyl groups on the reducing and internal rings of the trisaccharide substrates (both **6.1** and **6.2**) has no apparent effect on GlfT2 catalysis. However, given that a number of the compounds appeared to be better substrates that the parent compounds, we considered one question: Would the removal of these hydroxyl groups influence how these substrates bind to the active site of GlfT2 and consequently affect the regiochemical outcome of the glycosyl transfers? To address this question, milligram-scale enzymatic incubations of **6.7**–**6.11** were carried out under conditions similar to those used in the spectrophotometric assay. After the incubations, the enzymatic products were purified by preparative TLC followed by reverse phase column chromatography[18] and the structures of the products were analyzed by ^{1}H NMR spectroscopy.

Comparison of the anomeric region of the spectrum of the tetrasaccharide product, obtained from incubation of **6.7** with GlfT2 and UDP-Gal*f*, with that of **6.7**, a new proton signal appeared at 4.92 ppm. The chemical shift of this proton is consistent with a β-(1→6)-linkage (Figure 6-8B), not a β-(1→5)-linkage.[5] The formation of the product from the reaction was also supported by MALDI-MS analysis, in which a peak (m/z = 785.3)

corresponding to the tetrasaccharide (sodium adduct) was detected. Formation of the β-(1→6)-linkages were also observed from the enzymatic products obtained from the incubation mixtures with **6.8**–**6.11**. In all cases, the new proton signals also appeared at ~4.92 ppm. Thus, it appears that these modified substrates are glycosylated with the same regioselectivity of the parent trisaccharide **6.1**.

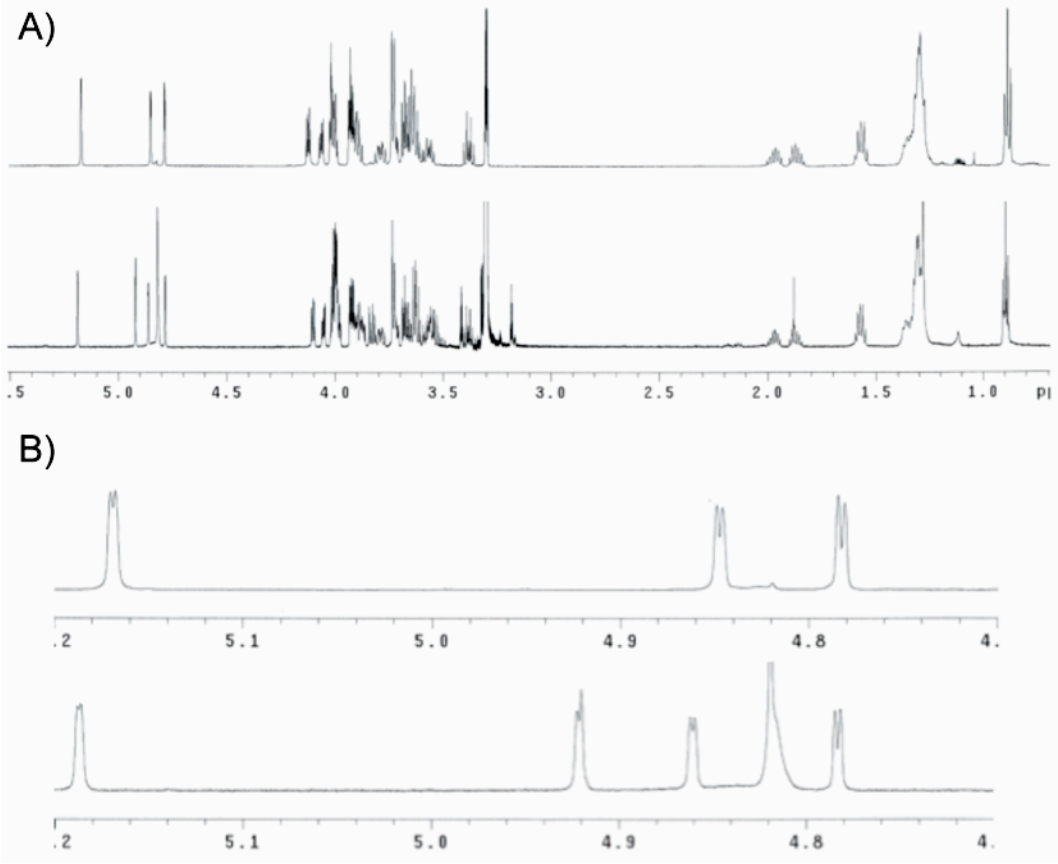


Figure 6-8. NMR spectra of tetrasaccharide from enzymatic reaction. A) Full spectra of compound **6.7** (*top*) and tetrasaccharide purified from the milligram-scale reaction (*bottom*). B) Partial spectra are shown for the comparison of the anomeric protons between compound **6.7** (*top*) and purified tetrasaccharide from the reaction (*bottom*).

On the other hand, attempts to obtain the corresponding tetrasaccharides from the incubations with β-(1→6), β-(1→5)-linked trisaccharide analogs (**6.12**–**6.16**) have yet to be successful. In all cases, incubations of these acceptors with GlfT2 and UDP-Gal*f* led to the formation of a homologous series of oligosaccharide products. This is due to the processive nature of GlfT2, and its faster rate for β-(1→6) glycosyl transfer compared to β-(1→5) transfer.[5] As a consequence of this rate difference, the initial tetrasaccharide formed from the slower β-(1→5) transfer, also serves as a substrate for the enzyme and immediately led to the formation of pentasaccharides in the faster β-(1→6) transfer step. Thus, TLC analysis showed the initial formation of the expected tetrasccaharides, which were gradually replaced by longer oligosaccharide products over time.

6.2.4. Kinetic characterization

To gain additional insight into the substrate preference of GlfT2, full kinetic characterizations of analogs **6.7**–**6.16** were carried out (Table 6-1). All of the enzyme kinetic work was performed by Jean Pearcey. The β-(1→5) and β-(1→6) glycosyltransferase activities with these deoxygenated analogs were compared with those of the parent trisaccharide substrates **6.1** and **6.2**.

Table 6-1. Summary of GlfT2 activities using analogs **6.1**, **6.2** and **6.7**–**6.12**[a,b]

Analog	K_M (mM)	V_{max} (pmol/min)	k_{cat} (min^{-1})	k_{cat} / K_M ($mM^{-1}min^{-1}$)	Rel. k_{cat} / K_M
6.1	0.566 ± 0.061	5733 ± 205	281	497	1.0
6.7	0.525 ± 0.068	4361 ± 158	214	408	0.8
6.8	0.328 ± 0.032	5166 ± 183	254	773	1.6
6.9	0.149 ± 0.019	3517 ± 127	124	830	1.7
6.10	0.631 ± 0.103	5759 ± 416	283	448	0.9
6.11	0.224 ± 0.026	3160 ± 117	155	691	1.4
6.2	0.502 ± 0.058	1580 ± 67	4.62	9.2	1.0
6.12	1.144 ± 0.334	3582 ± 383	10.47	9.1	1.0
6.13	1.331 ± 0.226	2465 ± 201	7.21	5.4	0.6
6.14	0.596 ± 0.091	3469 ± 219	17.0	28.6	3.1
6.15	0.101 ± 0.023	2363 ± 135	6.91	68.2	7.4
6.16	0.119 ± 0.015	3089 ± 105	9.03	76.2	8.3

[a] Kinetic parameters were determined using a range of acceptor concentrations by nonlinear regression analysis of the Michaelis-Menten equation with the GraphPad Prism 4.0 program.
[b] Table is reproduced with the permission of J. Pearcey.

6.2.4.1. Analogs of the β-(1→5), β-(1→6)-linked trisaccharide 6.1

As shown in Table 6-1, the kinetic constants for β-(1→5), β-(1→6)-linked trisaccharide analogs **6.7, 6.8** and **6.10** were generally similar to those observed with the parent compound **6.1** (K_M = 0.566 mM and k_{cat} = 281 min^{-1}). In contrast, **6.9** and **6.11** have a K_M (0.149 and 0.224 mM) 3.8 and 2.5-fold lower than that of **6.1**, respectively, thus indicating that these two deoxy analogs are better substrates for GlfT2 than the parent compound. This notion is further supported by the observed k_{cat}/K_M values of **6.9** and **6.11** (830 and 691 $mM^{-1}\cdot min^{-1}$, which are 1.7 and 1.4-fold larger than that of **6.1** (497 $mM^{-1}\cdot min^{-1}$). Interestingly, although both **6.9** and **6.11** appear to be better substrates for the enzyme, their k_{cat} values are reduced by 2.3 and 1.8-fold, respectively, compared with that of **6.1**. Presumably, the corresponding enzymatic products of analogs **6.9** and **6.11** also have high affinities in the active site of GlfT2. Perhaps the slow release of these products consequently leads to the smaller turnover rates of these analogs.

Correlation of the observed kinetic parameters with the structural modifications on analogs **6.7**–**6.11** revealed the general roles of 5- and 6′-OH groups on the β-(1→5), β-(1→6)-linked trisaccharide **6.1** interacting with the enzyme. First of all, removal of both exocylic hydroxyl groups leads to better interaction of the enzyme with its substrate, and perhaps with its product, as indicated by the smaller k_{cat} and larger k_{cat}/K_M values of **6.9** and **6.11**. Interestingly, the presence of hydroxyl groups at the C5

positions on the substrates such as **6.8** and **6.10** results in larger k_{cat} and smaller k_{cat}/K_M values than those of **6.9** and **6.11**. Hypothetically, this C5-hydroxyl group may prevent close contact between the substrate and GlfT2 and allow the release of the enzymatic product. As shown in Table 6-1, the k_{cat} values of **6.8** and **6.10** (254 and 283 min^{-1}, respectively), are comparable to that of **6.1** (281 min^{-1}). Secondly, **6.8** is a better GlfT2 substrate than **6.10** as indicated by their K_M and k_{cat}/K_M values of 0.328 mM and 773 $mM^{-1}\cdot min^{-1}$ (for **6.8**) and 0.631 mM and 448 $mM^{--1}\cdot min^{--1}$ (for **6.10**). It is possible that the presence of the C6′ methylene group provides an additional hydrophobic interaction with the enzyme in the active site; indeed , a similar trend is also observed from the comparison of the kinetic parameters of **6.9** and **6.11**.

6.2.4.2. Analogs of the β-(1→6), β-(1→5)-linked trisaccharide 6.2

Consistent with our previous reports,[5,17] although the K_M value of **6.2** (0.502 mM) is comparable with that of **6.1** (0.566 mM), the β-(1→5) glycosyl transfer requires much higher concentration of GlfT2 in the assay. As a result, the k_{cat} values of **6.2**, as well as its deoxygenated analogs **6.12**–**6.16**, are smaller than those of the β-(1→5), β-(1→6)-linked isomers **6.1** and **6.7**–**6.11**. As indicated in Table 6-1, the 6-deoxy **6.12** and 5′-deoxy analogs **6.13** have K_M's (1.144 and 1.331 mM) ~2-fold larger than that of **6.2**, indicating that these two mono-deoxy analogs bind poorly to the active site of GlfT2. On the other hand, the K_M values of **6.15** and **6.16**

(0.101 and 0.119 mM) are at least 4.2-fold smaller than that of **6.2**, which suggest that these analogs bind better to the enzyme.

Comparison the k_{cat}/K_M values of this set of β-(1→6), β-(1→5)-linked trisaccharide analogs, revealed that **6.15** and **6.16** exhibit 7.4- and 8.3-fold increases in enzyme turnover compared to the parent substrate **6.2**. Noticeably, removal of the hydroxymethoxy group in **6.2**, resulting in **6.15**, significantly improves its binding affinity to GlfT2, when its K_M and k_{cat}/K_M values are compared with those of **6.12** and **6.2** (Table 6-1). In fact, comparing the kinetic parameters of **6.16** with those of **6.14** and **6.13** further supports this trend. It is plausible that removal of the hydroxymethoxy groups on the reducing-end residues improves the substrate–enzyme interaction in the catalytic pocket.

In contrast, the role of the 5′-OH on **6.2** is difficult to rationalize. For instance, the absence of this hydroxyl group, as in **6.13**, seems to reduce the binding affinity of the trisaccharide to GlfT2; while the removal of 5′-OH in **6.12**, resulting in **6.14**, slightly improves the substrate specificity by 3 fold. On the other hand, there is only marginal difference on the kinetic values between **6.15** and **6.16** regardless of the presence or absence of the hydroxyl group at the C-5′ position. Perhaps deoxygenation at either or both the C-5′ and C-6 positions influence the overall conformation of the substrate and, consequently, affect how it interacts with the enzyme. Unfortunately, this proposal is pure speculation in the absence of information regarding the architecture of the enzyme active site. In

addition, the observed kinetics of analogs **6.12**–**6.16** might result from both β-(1→5) and β-(1→6) glycosyltransferase activities. Considering there is significant difference of the k_{cat} values between β-(1→5), β-(1→6)-linked trisaccharide (**6.1**, **6.7**–**6.11**) and their β-(1→6), β-(1→5)-linked isomers (**6.2**, **6.12**–**6.16**), the initially formed tetrasaccharide products, resulting from the β-(1→5) glycosyl transfer on analogs **6.2** and **6.12**–**6.16**, should immediately serve as substrates for the β-(1→6) glycosylation by GlfT2. Therefore, the observed kinetic values of the β-(1→6), β-(1→5)-linked trisaccharides are only relative.

6.3. Conclusions

In this chapter, we report the synthesis of the mono- and dideoxy trisaccharide analogs **6.7**–**6.16** and, subsequently, the evaluation of their abilities to serve as substrates for the β-(1→5) and β-(1→6) glycosyltransferase activities of GlfT2. Our preliminary results demonstrated that all of the deoxygenated analogs **6.7**–**6.11** are substrates for the β-(1→6) transfer of GlfT2. Likewise, the β-(1→6), β-(1→5)-linked isomers **6.12**–**6.16** are also the substrates for the β-(1→5) glycosyltransferase activities of the enzyme, though with much slower reaction rates. Our results suggest that the hydroxyl groups at the C5′ and C6 positions on **6.1** and **6.2** are not essential for the substrate recognition by the enzyme. In fact, kinetic analysis of these deoxygenated analogs further demonstrated that the removal of these hydroxyl groups leads to

better interactions of the substrates with the enzyme. In addition, through the subsequent structural analysis of the β-(1→5), β-(1→6)-linked trisaccharide analogs, the removal of the exocyclic groups, at the C6′ and C5 positions, appears to have no consequence on the regiochemistry of the β-(1→6) galactosylation by GlfT2. In summary, the study described here provides additional insight regarding the substrate preference of GlfT2. Recently, the crystal structure of the enzyme bound to UDP has solved in the group. Future work by other group members will involve the use of these, and other acceptor substrates, as ligand or future crystallization studies.

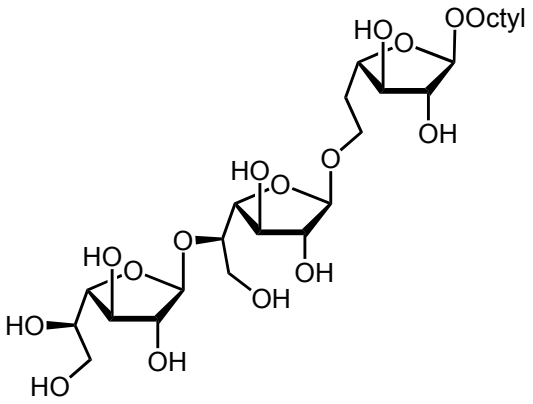
6.4. Experimental Section

General methods for chemical synthesis

All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless indicated otherwise, all reactions were performed at room temperature and under a positive pressure of argon. The reactions were monitored by analytical TLC on silica gel 60-F_{254} (0.25 mm, Silicycle) and spots were detected under UV light or by charring with acidified anisaldehyde solution in ethanol. Organic solvents were evaporated under reduced pressure at <40 °C. Products were purified by chromatography using silica gel (40–60 μM), Iatrobeads (Iatron

Laboratories, Tokyo) or SepPak C_{18} reverse phase cartridges (Waters). Before use, the SepPak C_{18} cartridges were prewashed with 10 mL of MeOH followed by 10 mL of H_2O. Optical rotations were measured at 22 ± 2 °C and are in units of degrees·mL/(g·dm). ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$), or HOD (4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz and chemical shifts are referenced to internal $CDCl_3$ (77.23, $CDCl_3$), or CD_3OD (48.9, CD_3OD). Assignments of NMR spectra were made based on two-dimensional (^{1}H–^{1}H COSY and HMQC) experiments. Electrospray mass spectra were recorded on samples suspended in mixtures of THF with MeOH and added NaCl. MALDI mass spectra were recorded using a Voyager Elite time-of-flight spectrometer on samples suspended in 2,5-dihydroxy benzoic acid, using the delayed-extraction mode and positive-ion detection.

Octyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-5-deoxy-α-L-*arabino*-hexofuranoside (6.7)

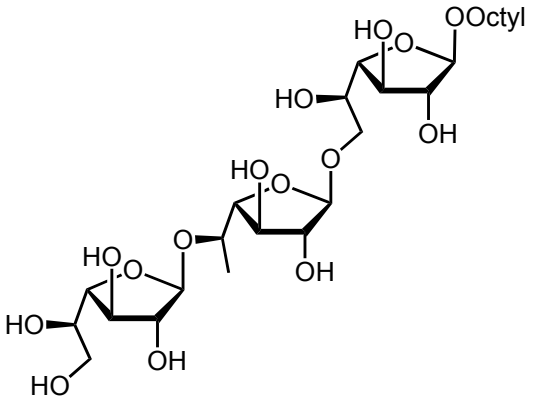


Silyl trisaccharide **6.43** (105 mg, 0.067 mmol) was dissolved in 20:4:1 THF–pyridine–70% HF·pyridine (6.25 mL) and the

reaction mixture was stirred overnight. The crude product was then diluted in EtOAc, washed with satd aq $NaHCO_3$ twice and dried (Na_2SO_4). The organic layer was filtered and concentrated. Without further purification, the resulting crude product was dissolved in MeOH (9 mL) and 1 M NaOMe was added dropwise until the pH of solution reached ~11. The reaction mixture was stirred for 2 h and was neutralized by the addition of Amerlite IR120 resin (H^+ form). The crude mixture was filtered, concentrated and purified by chromatography on Iatrobeads (3:1 CH_2Cl_2–CH_3OH) to give **6.7** as a clear glass (32 mg, 77% over two steps): R_f 0.44 (3:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –97.5 (*c* 0.5, CH_3OH); ^1H NMR (500 MHz, CD_3OD) δ_H 5.17 (d, 1H, *J* = 1.4 Hz, H-1″), 4.85 (d, 1H, *J* = 1.6 Hz, H-1′), 4.78 (d, 1H, *J* = 1.6 Hz, H-1), 4.12 (dd, 1H, *J* = 5.5, 3.1 Hz, H-4″), 4.06 (dd, 1H, *J* = 6.2, 3.3 Hz, H-3′), 3.98–4.03 (m, 3H, H-2″, H-3″, H-4′), 3.86–3.94 (m, 4H, H-2′, H-5′, H-2, H-4), 3.79 (ddd, 1H, *J* = 9.9, 7.6, 6.2 Hz, H-6a), 3.59–3.75 (m, 7H, H-6a″, H-6b″, H-6a′, H-6b′, H-3, H-5″, octyl OC*H*$_2$), 3.56 (ddd, 1H, *J* = 9.9, 6.3, 6.3 Hz, H-6b), 3.38 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.93–2.01 (m, 1H, H-5a), 1.82–1.91 (m, 1H, H-5b), 1.53–1.61 (m, 2H, octyl OCH_2C*H*$_2$), 1.22–1.41 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.3 (C-1″), 109.2 (C-1′), 109.0 (C-1), 85.0 (C-4″), 84.2, 84.1, 83.4, 83.0, 82.7 (C-2″, C-2′, C-4′, C-2, C-4), 81.5 (C-4), 78.8 (C-3″), 78.6 (C-3′), 77.3 (C-5′), 72.3 (C-5″), 68.7 (octyl O*C*H_2), 65.7 (C-6), 64.3 (C-6″), 62.8 (C-6′), 34.5 (C-5), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.3 (octyl

CH_2), 23.7 (octyl *C*H_2), 14.5 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{15}$: 623.2885. Found: 623.2882.

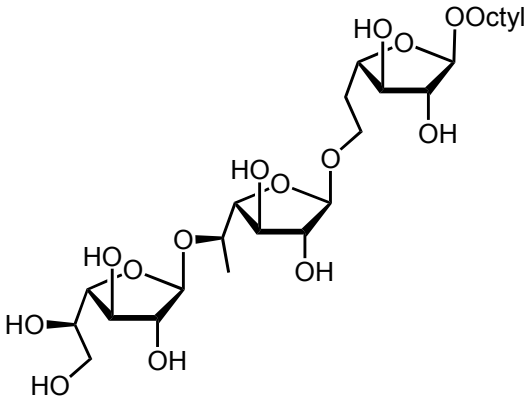
Octyl β-D-galactofuranosyl-(1→5)-6-deoxy-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (6.8)



Octyl trisaccharide **6.44** (106 mg, 0.069 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **6.8** as a clear glass (32 mg, 78%): R_f 0.58 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –121.7 (*c* 0.3, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 5.03 (br s, 1H, H-1″), 4.89 (d, 1H, *J* = 1.1 Hz, H-1′), 4.84 (d, 1H, *J* = 1.8 Hz, H-1), 4.08 (dd, 1H, *J* = 5.3, 3.2 Hz, H-4″), 3.94–4.02 (m, 5H, H-3″, H-2′, H-3′, H-5′, H-3), 3.94 (dd, 1H, *J* = 3.0, 1.3 Hz, H-2″), 3.92 (dd, 1H, *J* = 4.0, 1.8 Hz, H-2), 3.82–3.89 (m, 3H, H-4′, H-4, H-5), 3.78 (dd, 1H, *J* = 10.2, 4.8 Hz, H-6a), 3.70–3.75 (m, 1H, H-5″), 3.70 (dt, 1H, *J* = 9.7, 6.7 Hz, octyl OC*H*$_2$), 3.64 (dd, 1H, *J* = 11.2, 5.9 Hz, H-6a″), 3.61 (dd, 1H, *J* = 11.2, 6.8 Hz, H-6b″), 3.51 (dd, 1H, *J* = 10.2, 7.0 Hz, H-6b), 3.40 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 1.54–1.61 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.25–1.40 (m, 10H, octyl C*H*$_2$), 1.24 (d, 3H, *J* = 6.4 Hz, H-6′), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.7 (C-1′), 109.3 (C-1),

106.6 (C-1′′), 87.6 (C-4′), 85.3 (C-4′′), 84.5 (C-4), 83.5 (C-2′/C-2), 83.4 (C-2′/C-2), 82.6 (C-2′′), 79.1 (C-3′′/C-3′/C-3), 79.0 (C-3′′/C-3′/C-3), 78.9 (C-3′′/C-3′/C-3), 72.4 (C-5′′), 71.8 (C-5′), 71.0 (C-5), 70.5 (C-6), 68.9 (octyl O*C*H_2), 64.3 (C-6′′), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.6 (octyl *C*H_2), 30.5 (octyl *C*H_2), 27.3 (octyl *C*H_2), 23.7 (octyl *C*H_2), 15.6 (C-6′), 14.5 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{15}$: 623.2885. Found: 623.2889.

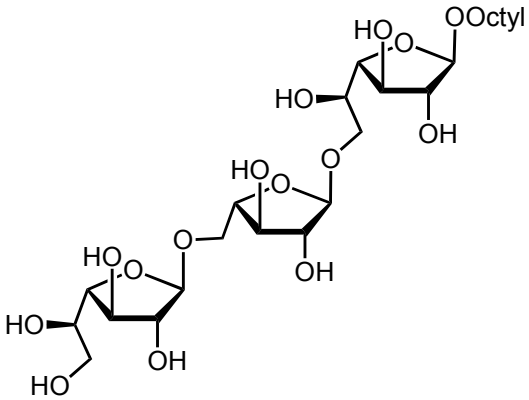
Octyl β-D-galactofuranosyl-(1→5)-6-deoxy-β-D-galactofuranosyl-(1→6)- 5-deoxy-α-L-*arabino*-hexofuranoside (6.9)



Octyl trisaccharide **6.45** (63 mg, 0.055 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (5:1 CH_2Cl_2–CH_3OH) to give **6.9** as a clear glass (23 mg, 87%): R_f 0.52 (5:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –159.2 (*c* 0.4, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 5.02 (br s, 1H, H-1′′), 4.85 (d, 1H, *J* = 1.2 Hz, H-1′), 4.78 (d, 1H, *J* = 1.8 Hz, H-1), 4.07 (dd, 1H, *J* = 5.1, 3.1 Hz, H-4′′), 3.87–4.03 (m, 7H, H-2′′, H-3′′, H-2′, H-3′, H-5′, H-2, H-4), 3.84 (dd, 1H, *J* = 5.7, 4.6 Hz, H-4′), 3.80 (ddd, 1H, *J* = 9.9, 7.9, 6.0 Hz, H-6a), 3.53–3.75 (m, 6H, H-5′′, H-6a′′, H-6b′′, H-3, H-6b, octyl O*CH*$_2$), 3.38 (dt, 1H, *J* = 9.6, 6.7 Hz, octyl

OCH_2), 1.93–2.02 (m, 1H, H-5a), 1.82–1.90 (m, 1H, H-5b), 1.53–1.61 (m, 2H, octyl CH_2), 1.24–1.41 (m, 10H, octyl CH_2), 1.24 (d, 3H, *J* = 6.4 Hz, H-6′), 0.90 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.3 (C-1′), 109.0 (C-1), 106.5 (C-1″), 87.4 (C-4′), 85.3 (C-4″), 84.2, 83.6, 83.0, 82.5 (C-2″, C-2′, C-2, C-3), 81.5 (C-4), 79.1 (C-3″), 79.0 (C-3′), 72.4 (C-5″), 71.8 (C-5′), 68.7 (octyl O*C*H_2), 65.6 (C-6), 64.3 (C-6″), 34.5 (C-5), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.3 (octyl *C*H_2), 23.7 (octyl *C*H_2), 15.6 (C-6′), 14.5 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{14}$: 607.2936. Found: 607.2934.

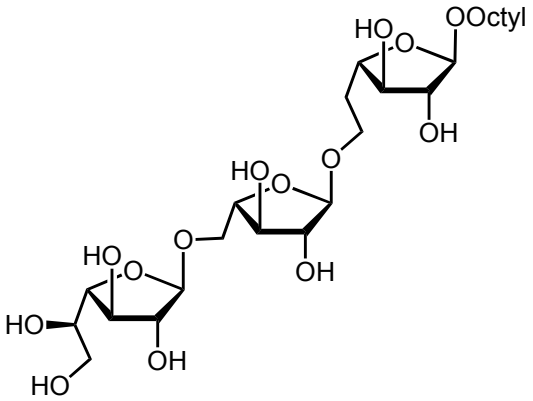
Octyl β-D-galactofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→6)-β-D-galactofuranoside (6.10)



Octyl trisaccharide **6.46** (63 mg, 0.041 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (3:1 CH_2Cl_2–CH_3OH) to give **6.10** as a clear glass (21 mg, 89%): R_f 0.50 (3:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –100.1 (*c* 0.3, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.93 (d, 1H, *J* = 1.4 Hz, H-1′), 4.91 (d, 1H, *J* = 0.9 Hz, H-1″), 4.84 (d, 1H, *J* = 1.6 Hz, H-1), 4.06 (dd, 1H, *J* = 9.5, 5.2 Hz, H-4), 3.96–4.02 (m, 5H, H-2″, H-3″, H-4″, H-2′, H-3′), 3.90–3.94 (m, 2H, H-2, H-3), 3.81–3.89 (m, 3H, H-4′, H-5, H-6a), 3.80 (dd,

1H, *J* = 10.3, 4.7 Hz, H-5a′), 3.58–3.74 (m, 5H, H-5′′, H-6a′′, H-6b′′, H-6b, octyl OC*H*$_2$), 3.52 (dd, 1H, *J* = 10.3, 6.8 Hz, H-5b′), 3.40 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 1.53–1.62 (m, 2H, octyl C*H*$_2$), 1.22–1.40 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 110.1 (C-1′′/C-1′), 109.6 (C-1′′/C-1′), 109.3 (C-1), 84.9, 84.6, 84.4, 83.5, 83.2, 82.9 (C-2′′, C-4′′, C-2′, C-4′, C-2, C-4), 79.1 (C-3), 78.9 (2C, C-3′′, C-3′), 72.5 (C-5′′), 71.0 (C-5), 70.6 (C-5′), 68.9 (octyl O*C*H$_2$), 68.2 (C-6), 64.4 (C-6′′), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.6 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.3 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{25}H_{46}O_{15}$: 609.2729. Found: 609.2733.

Octyl β-D-galactofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→6)-5-deoxy-α-L-*arabino*-hexofuranoside (6.11)

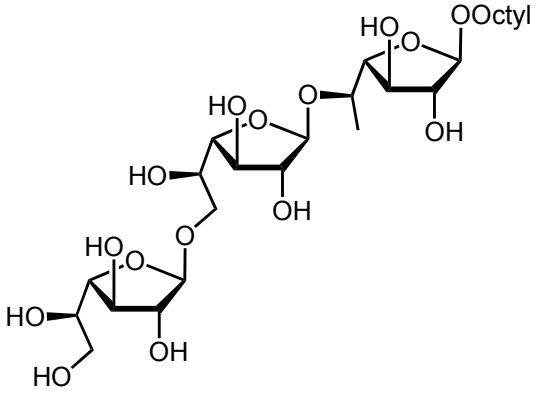


Octyl trisaccharide **6.47** (55 mg, 0.039 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **6.11** as a clear glass (19 mg, 82%): R_f 0.58 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –128.2 (*c* 0.3, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.93 (d, 1H, *J* = 1.5 Hz, H-1′′), 4.87 (d, 1H, *J* = 1.4 Hz, H-1′), 4.78 (d, 1H, *J* = 1.7 Hz, H-1), 3.97–4.07 (m, 4H, H-2′′, H-3′′, H-4′′, H-4′),

3.96 (dd, 1H, *J* = 3.3, 1.4 Hz, H-2′), 3.87–3.94 (m, 3H, H-3′, H-2, H-4), 3.78–3.86 (m, 2H, H-5a′, H-6a), 3.54–3.74 (m, 7H, H-5′′, H-6a′′, H-6b′′, H-5b′, H-3, H-6b, octyl OC*H*$_2$), 3.38 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.94–2.03 (m, 1H, H-5a), 1.82–1.91 (m, 1H, H-5b), 1.53–1.61 (m, 2H, octyl C*H*$_2$), 1.22–1.42 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.6 (C-1′′), 109.5 (C-1′), 109.0 (C-1), 84.8, 84.2, 84.1, 83.3, 83.0(5), 82.9(7) (C-2′′, C-4′′, C-2′, C-4′, C-2, C-3), 81.4 (C-4), 79.0 (C-3′′), 78.9 (C-3′), 72.5 (C-5′′), 68.7 (octyl O*C*H$_2$), 68.2 (C-5′), 65.7 (C-6), 64.4 (C-6′′), 34.5 (C-5), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.3 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.4 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{25}H_{46}O_{14}$: 593.2780. Found: 593.2782.

Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-6-deoxy-β-D-galactofuranoside

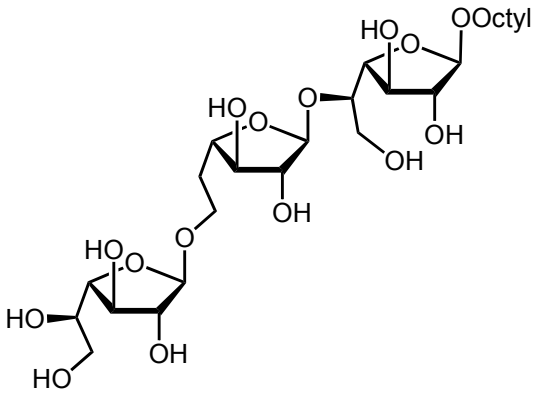
(6.12)



Octyl trisaccharide **6.48** (88 mg, 0.057 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **6.12** as a clear glass (31 mg, 90%): R_f 0.50 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –126.6 (*c*

0.6, CH_3OH); ^1H NMR (600 MHz, CD_3OD) δ_H 5.04 (br s, 1H, H-1′), 4.92 (br s, 1H, H-1′′), 4.81 (br s, 1H, H-1), 4.04 (dd, 1H, *J* = 4.7, 3.7 Hz, H-4′), 3.96–4.03 (m, 5H, H-2′′, H-3′′, H-4′′, H-3′, H-5), 3.91–3.95 (m, 3H, H-2′, H-2, H-3), 3.86–3.90 (m, 1H, H-5′), 3.82 (dd, 1H, *J* = 10.5, 5.1 Hz, H-6a′), 3.80 (dd, 1H, *J* = 6.0, 4.5 Hz, H-4), 3.72 (ddd, 1H, *J* = 6.9, 5.8, 3.3 Hz, H-5′′), 3.66 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 3.58–3.65 (m, 2H, H-6a′′, H-6b′′), 3.53 (dd, 1H, *J* = 10.5, 7.1 Hz, H-6b′), 3.41 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OC*H*$_2$), 1.54–1.60 (m, 2H, octyl C*H*$_2$), 1.25–1.40 (m, 10H, octyl C*H*$_2$), 1.23 (d, 3H, *J* = 6.5 Hz, H-6), 0.89 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.9 (C-1′′), 109.3 (C-1), 106.3 (C-1′), 87.1 (C-4), 86.2 (C-4′), 85.0 (C-4′′), 83.9 (C-2), 82.9 (C-2′′), 82.4 (C-2′), 79.2 (C-3′), 79.1 (C-3), 78.9 (C-3′′), 72.5 (C-5′′), 71.7 (C-5), 71.2 (C-5′), 70.4 (C-6′), 68.9 (octyl O*C*H$_2$), 64.5 (C-6′′), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.3 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 15.5 (C-6), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{15}$: 623.2885. Found: 623.2885.

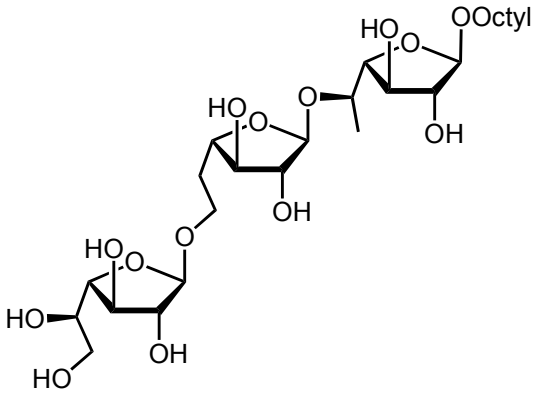
Octyl β-D-galactofuranosyl-(1→6)-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-β-D-galactofuranoside (6.13)



Octyl trisaccharide **6.49** (118 mg, 0.076 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **6.13** as a clear glass (33 mg, 72%): R_f 0.31 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –119.6 (*c* 0.4, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 5.15 (d, 1H, *J* = 1.1 Hz, H-1′), 4.89 (d, 1H, *J* = 1.5 Hz, H-1′′), 4.83 (d, 1H, *J* = 1.8 Hz, H-1), 4.10 (ddd, 1H, *J* = 7.6, 5.4, 5.4 Hz, H-4′), 4.05 (dd, 1H, *J* = 6.4, 3.5 Hz, H-3), 4.02 (dd, 1H, *J* = 3.3, 1.5 Hz, H-2′′), 3.95–4.02 (m, 4H, H-3′′, H-4′′, H-2′, H-4), 3.92 (dd, 1H, *J* = 3.5, 1.8 Hz, H-2), 3.82–3.90 (m, 2H, H-6a′, H-5), 3.56–3.74 (m, 8H, H-5′′, H-6a′′, H-6b′′, H-3′, H-6b′, H-6a, H-6b, octyl OC*H*$_2$), 3.40 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.95–2.03 (m, 1H, H-5a′), 1.83–1.92 (m, 1H, H-5b′), 1.53–1.61 (m, 2H, octyl C*H*$_2$), 1.22–1.40 (m, 10H, octyl C*H*$_2$), 0.89 (t, 3H, *J* = 6.9 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.4 (2C, C-1′, C-1), 108.5 (C-1′′), 84.8, 83.8, 83.7, 83.3, 83.1, 82.9, 82.6 (C-2′′, C-4′′, C-2′, C-4′, C-2, C-3, C-4), 78.9 (2C, C-3′′, C-3′), 76.9 (C-5), 72.6 (C-5′′), 68.9 (octyl O*C*H$_2$), 65.7 (C-6′), 64.5 (C-6′′), 62.7 (C-6), 34.8 (C-5′), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.3 (octyl *C*H$_2$), 23.7

(octyl CH_2), 14.5 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{15}$: 623.2885. Found: 623.2884.

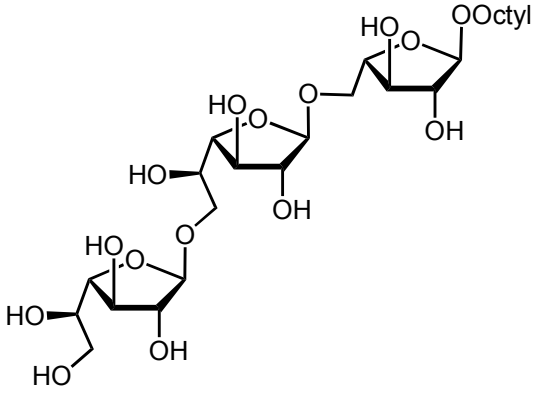
Octyl β-D-galactofuranosyl-(1→6)-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-6-deoxy-β-D-galactofuranoside (6.14)



Octyl trisaccharide **6.50** (66 mg, 0.046 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (5:1 CH_2Cl_2–CH_3OH) to give **6.14** as a clear glass (20 mg, 75%): R_f 0.31 (5:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –138.9 (*c* 0.3, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 5.00 (br s, 1H, H-1′), 4.88 (d, 1H, *J* = 1.5 Hz, H-1′′), 4.81 (d, 1H, *J* = 1.8 Hz, H-1**),** 4.05 (ddd, 1H, *J* = 7.6, 5.4, 5.4 Hz, H-4′), 3.93–4.01 (m, 6H, H-2′′, H-3′′, H-4′′, H-2′, H-3, H-5), 3.92 (dd, 1H, *J* = 3.4, 1.8 Hz, H-2), 3.85 (ddd, 1H, *J* = 10.0, 8.0, 5.7 Hz, H-6a′), 3.80 (dd, 1H, *J* = 6.2, 4.3 Hz, H-4), 3.55–3.73 (m, 6H, H-5′′, H-6a′′, H-6b′′, H-3′, H-6b′, octyl OCH_2), 3.41 (dt, 1H, *J* = 9.7, 6.6 Hz, octyl OCH_2), 1.94–2.02 (m, 1H, H-5a′), 1.84–1.92 (m, 1H, H-5b′), 1.54–1.61 (m, 2H, octyl CH_2), 1.24–1.40 (m, 10H, octyl CH_2), 1.23 (d, 3H, *J* = 6.5 Hz, H-6), 0.89 (t, 3H, *J* = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.4 (C-1′′/C-1), 109.3 (C-1′′/C-1), 105.9 (C-1′), 87.1 (C-4), 84.8 (C-2′), 83.9 (C-2), 83.4, 83.3, 83.0 (C-2′′, C-

4′′, C-4′), 82.8 (C-3′), 79.1 (C-3′′/C-3), 78.9 (C-3′′/C-3), 72.6 (C-5′′), 71.4 (C-5), 68.9 (octyl O*C*H_2), 65.7 (C-6′), 64.5 (C-6′′), 34.8 (C-5′), 33.0 (octyl *C*H_2), 30.7 (octyl *C*H_2), 30.5 (octyl *C*H_2), 30.4 (octyl *C*H_2), 27.3 (octyl *C*H_2), 23.7 (octyl *C*H_2), 15.5 (C-6), 14.5 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{48}O_{14}$: 607.2936. Found: 607.2931.

Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-α-L-arabinofuranoside (6.15)

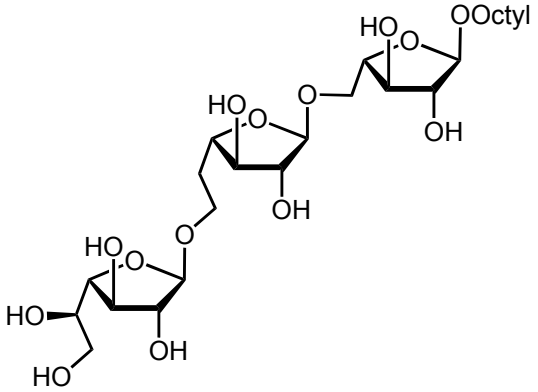


Octyl trisaccharide **6.51** (95 mg, 0.063 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (4:1 CH_2Cl_2–CH_3OH) to give **6.15** as a clear glass (32 mg, 88%): R_f 0.42 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –121.4 (*c* 0.4, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ_H 4.93 (d, 1H, *J* = 1.4 Hz, H-1′′), 4.92 (d, 1H, *J* = 1.1 Hz, H-1′), 4.84 (d, 1H, *J* = 1.6 Hz, H-1), 3.94–4.02 (m, 7H, H-2′′, H-3′′, H-4′′, H-2′, H-3′, H-4′, H-3), 3.94 (dd, 1H, *J* = 3.9, 1.9 Hz, H-2), 3.88 (dd, 1H, *J* = 6.5, 3.7 Hz, H-4), 3.84–3.88 (m, 1H, H-5′), 3.83 (dd, 1H, *J* = 11.1, 5.2 Hz, H-6a′), 3.82 (dd, 1H, *J* = 10.2, 5.0 Hz, H-5a), 3.70–3.74 (m, 1H, H-5′′), 3.68 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 3.58–3.67 (m, 3H, H-6a′′, H-6b′′, H-6b′), 3.53 (dd, 1H, *J* = 10.2, 6.9 Hz, H-5b), 3.40 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.54–1.61 (m, 2H, octyl C*H*$_2$),

1.24–1.40 (m, 10H, octyl CH_2), 0.89 (t, 3H, J = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.9 (C-1′), 109.5 (2C, C-1′′, C-1), 85.3 (C-4′′/C-4′), 85.0 (C-4′′/C-4′), 83.7 (C-3′/C-3/C-2), 83.5 (C-3′/C-3/C-2), 83.0(2) (C-2′′/C-2′), 82.9(7) (C-2′′/C-2′), 79.1 (C-4), 78.9 (2C, C-3′′, C-3′/C-3/C-2), 72.5 (C-5′′), 71.1 (C-5′), 70.4 (C-5), 69.0 (octyl OCH_2), 68.1 (C-6′), 64.5 (C-6′′), 33.0 (octyl CH_2), 30.7 (octyl CH_2), 30.5 (octyl CH_2), 30.4 (octyl CH_2), 27.3 (octyl CH_2), 23.7 (octyl CH_2), 14.5 (octyl CH_3). HRMS (ESI) calcd. for (M + Na) $C_{25}H_{46}O_{15}$: 609.2729. Found: 609.2735.

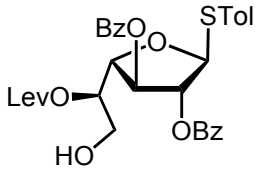
Octyl β-D-galactofuranosyl-(1→6)-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-α-L-arabinofuranoside (6.16)

Octyl trisaccharide **6.52** (88 mg, 0.063 mmol) was deprotected using catalytic NaOMe in MeOH (9 mL) as described for **6.7**. The crude product was purified by chromatography on Iatrobeads (5:1 CH_2Cl_2–CH_3OH) to give **6.16** as a clear glass (31 mg, 86%): R_f 0.17 (5:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = –128.9 (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ_H 4.89 (d, 1H, J = 1.6 Hz, H-1′), 4.88 (d, 1H, J = 1.7 Hz, H-1′′), 4.84 (d, 1H, J = 1.6 Hz, H-1), 3.93–4.02 (m, 7H, H-2′′, H-3′′, H-4′′, H-2′, H-4′, H-2, H-4), 3.89 (dd, 1H, J = 6.5, 3.8 Hz, H-3), 3.78–3.87 (m, 2H, H-6a′, H-5a), 3.55–3.74 (m, 7H, H-5b′′, H-6a′′, H-6b′′, H-3′, H-6b′, H-5b, octyl



OC*H*$_2$), 3.41 (dt, 1H, *J* = 9.6, 6.6 Hz, octyl OC*H*$_2$), 1.94–2.03 (m, 1H, H-5a′), 1.84–1.93 (m, 1H, H-5b′),1.54–1.62 (m, 2H, octyl C*H*$_2$), 1.22–1.40 (m, 10H, octyl C*H*$_2$), 0.90 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ_C 109.5 (C-1), 109.4 (C-1′′/C-1′), 109.2 (C-1′′/C-1′), 84.9, 83.8, 83.7, 83.5, 83.0, 82.9, 82.3 (C-2′′, C-4′′, C-2′, C-3′, C-4′, C-2, C-4), 79.1 (C-3), 78.9 (C-3′′), 72.6 (C-5′′), 69.0 (octyl O*C*H$_2$), 67.9 (C-5), 65.6 (C-6′), 64.5 (C-6′′), 34.6 (C-5′), 33.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 30.5 (octyl *C*H$_2$), 30.4 (octyl *C*H$_2$), 27.3 (octyl *C*H$_2$), 23.7 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{25}H_{46}O_{14}$: 593.2780. Found: 593.2784.

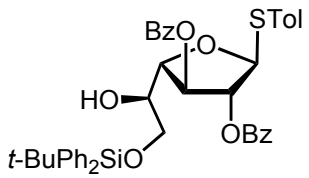
_p_-Tolyl 2,3-di-_O_-benzoyl-5-_O_-levulinoyl-1-thio-β-D-galactofuranoside (6.18)



The monosaccharide **6.30** (0.84 g, 1.0 mmol) was dissolved in 20:4:1 THF–pyridine–70% HF·pyridine (37.5 mL) and the reaction mixture was stirred overnight. The crude product was then diluted in EtOAc (25 mL) and washed with satd aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by chromatography (1:1 hexane–EtOAc) to give **6.18** (0.54 g, 90%) as a pale yellow oil: R_f 0.38 (1:1 hexane–EtOAc); $[\alpha]_D$ = –53.6 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.00–8.18 (m, 4H, ArH), 7.55–7.64 (m, 2H, ArH), 7.40–7.52 (m, 6H, ArH), 7.15 (app d, 2H, *J* = 7.6 Hz, ArH), 5.70 (d,

1H, *J* = 1.8 Hz, H-1), 5.67 (dd, 1H, *J* = 1.8, 1.8 Hz, H-2), 5.56 (dd, 1H, *J* = 4.8, 1.8 Hz, H-3), 5.40 (ddd, 1H, *J* = 4.8, 4.8, 4.6 Hz, H-5), 4.75 (dd, *J* = 4.8, 4.6 Hz, H-4), 3.87–3.98 (m, 2H, H-6a, H-6b), 2.47–2.83 (m, 4H, Lev CH_2), 2.33 (s, 3H, SPhCH_3), 2.13 (s, 3H, Lev CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 206.0 (Lev ketone C=O), 172.5 (Lev ester C=O), 165.6 (C=O), 165.3 (C=O), 138.0, 133.7, 133.6, 133.1, 130.0, 129.9(0), 129.8(6), 129.3, 129.0, 128.9, 128.5 (18C, Ar), 91.2 (C-1), 81.9 (C-2/C-4), 81.8 (C-2/C-4), 77.6 (C-3), 72.9 (C-5), 62.2 (C-6), 38.2 (Lev CH_2), 29.7 (Lev CH_3), 28.1 (Lev CH_2), 21.1 (SPhCH_3). HRMS (ESI) calcd. for (M + Na) $C_{32}H_{32}O_9S$: 615.1659. Found: 615.1652.

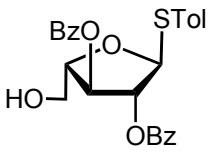
_p_-Tolyl 2,3-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-1-thio-β-D-galactofuranoside (6.19)



Diol **6.27** (1.1 g, 2.2 mmol) and imidazole (0.59 g, 8.6 mmol) were dissolved in THF (15 mL) and *tert*-butylchlorodiphenylsilane (0.92 mL, 3.6 mmol) was added at 0 °C. The solution was stirred overnight before being quenched by the addition of H_2O (2 mL). The mixture was then diluted with EtOAc (100 mL), washed with $NaHCO_3$ (25 mL) and H_2O (25 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The crude product was purified by chromatography (4:1 hexane-EtOAc) to give **6.19** (1.5 g, 96%) as a colorless oil: R_f 0.46 (4:1 hexane-EtOAc); $[\alpha]_D$ = –56.4 (*c* 2.8, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–8.15 (m, 2H, ArH), 8.03–8.07

(m, 2H, ArH), 7.55–7.69 (m, 6H, ArH), 7.32–7.53 (m, 12H, ArH), 7.06–7.11 (m, 2H, ArH), 5.74 (ddd, 1H, *J* = 5.1, 1.8, 0.8 Hz, H-3), 5.71 (dd, 1H, *J* = 1.8, 0.8 Hz, H-1), 5.69 (dd, 1H, *J* = 1.8, 1.8 Hz, H-2), 4.77 (dd, 1H, *J* = 5.1, 2.5 Hz, H-4), 4.20 (ddd, 1H, *J* = 7.4, 6.4, 2.5 Hz, H-5), 3.85 (dd, 1H, *J* = 10.2, 6.4 Hz, H-6a), 3.79 (dd, 1H, *J* = 10.2, 7.4 Hz, H-6b), 2.45 (d, 1H, *J* = 8.0 Hz, O*H*), 2.33 (s, 3H, SPhCH_3), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.7 (C=O), 165.3 (C=O), 138.0, 135.5, 133.5, 133.2, 133.1, 133.0, 130.0, 129.9, 129.7(9), 129.7(6), 129.2, 129.1, 128.5(3), 128.5(0), 127.8 (30C, Ar), 91.7 (C-1), 82.0 (2C, C-2, C-4), 78.1 (C-3), 70.8 (C-5), 64.7 (C-6), 26.8 (C(*C*H_3)$_3$), 21.2 (SPh*C*H_3), 19.2 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{43}H_{44}O_7SSi$: 755.2469. Found: 755.2461.

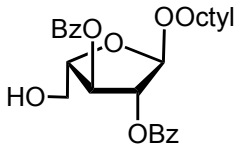
_p_-Tolyl 2,3-di-*O*-benzoyl-1-thio-α-L-arabinofuranoside (6.20)



The silyl group was removed from thioglycoside **6.41**[16] (1.1 g, 1.5 mmol) in 20:4:1 THF–pyridine–70% HF-pyridine (26 mL) as described for **6.18**. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.20** (0.58 g, 82%) as a colorless oil: R_f 0.39 (3:1 hexane–EtOAc); $[\alpha]_D$ = –68.4 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.09–8.16 (m, 2H, ArH), 8.02–8.08 (m, 2H, ArH), 7.40–7.66 (m, 8H, ArH), 7.15 (app d, 2H, *J* = 8.0 Hz, ArH), 5.70–5.75 (m, 2H, H-1, H-2), 5.55 (br d, 1H, *J* = 4.5 Hz, H-3), 4.59 (ddd, 1H, *J* = 4.5, 4.4, 4.4 Hz, H-4), 3.98–4.08 (m, 2H, H-5a, H-5b), 2.33 (s, 3H, SPhCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.2 (C=O),

138.2, 133.7, 133.6, 132.9, 130.0(6), 130.0(4), 129.9(7), 129.8(9), 129.8(7) 129.7(6), 129.0(4), 128.9(8), 128.5(9), 128.5(6) (18C, Ar), 91.8 (C-1), 83.7 (C-4), 82.2 (C-2), 77.9 (C-3), 62.0 (C-5), 21.2 (SPh*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{26}H_{24}O_6S$: 487.1186. Found: 487.1190.

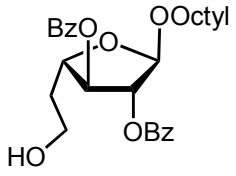
Octyl 2,3-di-*O*-benzoyl-α-L-arabinofuranoside (6.23)



Thioglycoside **6.41** (1.6 g, 2.3 mmol) and powdered 4 Å molecular sieves (1.5 g) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (40 mL) and octanol (0.40 mL, 2.5 mmol) were added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (0.68 g, 2.9 mmol) and TMSOTf (125 μL, 0.69 mmol). The mixture was stirred for 15 min and neutralized by the addition of triethylamine, before being filtered through Celite and concentrated. The crude residue was partially purified by chromatography (9:1 hexane-EtOAc) and then dissolved in 20:4:1 THF–pyridine–70% HF-pyridine (62.5 mL) and the reaction mixture was stirred overnight. The crude product was then diluted in EtOAc (50 mL), washed with satd aq $NaHCO_3$ (25 mL) and the organic layer dried (Na_2SO_4), filtered and concentrated. The crude product was purified by chromatography (4:1 hexane–EtOAc) to give **6.23** (0.87 g, 81%) as a colorless oil: R_f 0.29 (4:1 hexane–EtOAc); $[\alpha]_D$ = +16.8 (*c* 2.8, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.03–8.10 (m, 4H, ArH), 7.56–7.62 (m, 2H, ArH), 7.43–7.48 (m, 4H, ArH), 5.53 (d, 1H, *J* = 1.4 Hz, H-2), 5.43 (dd, 1H, *J* = 4.7, 1.4 Hz, H-3), 5.24 (s, 1H, H-1), 4.32 (ddd, 1H,

J = 3.8, 4.7, 4.1 Hz, H-4), 4.02 (dd, 1H, *J* = 12.0, 3.8 Hz, H-5a), 3.99 (dd, 1H, *J* = 12.0, 4.1 Hz, H-5b), 3.77 (dt, 1H, *J* = 9.4, 6.7 Hz, octyl OC*H*$_2$), 3.53 (dt, 1H, *J* = 9.4, 6.2 Hz, octyl OC*H*$_2$), 2.16 (br s, 1H, OH), 1.57–1.62 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.46 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 165.4 (C=O), 133.5, 129.9, 129.8, 129.2(4), 129.1(7), 128.5, 128.4 (12C, Ar), 105.5 (C-1), 83.7 (C-4), 81.8 (C-2), 77.9 (C-3), 67.5 (octyl O*C*H$_2$), 62.5 (C-5), 31.8 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{27}H_{34}O_7$: 493.2197. Found: 493.2197.

Octyl 2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranoside (6.24)



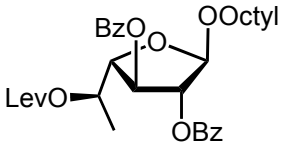
Thioglycoside **6.29** (153 mg, 0.21 mmol) and powdered 4 Å molecular sieves (300 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (12 mL) and octanol (100 μL, 0.64 mmol) were added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (58 mg, 0.25 mmol) and TMSOTf (12 μL, 0.064 mmol). The mixture was stirred for 30 min and neutralized by the addition of triethylamine, before being filtered through Celite and concentrated. The crude residue was partially purified by chromatography (9:1 hexane-EtOAc) and then dissolved in 20:4:1 THF–pyridine–70% HF-pyridine (6.25 mL) and the reaction mixture was stirred overnight. The crude product was then diluted in EtOAc (25 mL) and

washed with satd aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The product was purified by chromatography (3:1 hexane–EtOAc) to give **6.24** (0.87 g, 81%) as a colorless oil: R_f 0.32 (3:1 hexane–EtOAc); $[\alpha]_D$ = 0.0 (*c* 0.6, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.03–8.11 (m, 4H, ArH), 7.56–7.63 (m, 2H, ArH), 7.43–7.50 (m, 4H, ArH), 5.49 (d, 1H, *J* = 1.3 Hz, H-2), 5.32 (dd, 1H, *J* = 4.4, 1.3 Hz, H-3), 5.22 (s, 1H, H-1), 4.44 (ddd, 1H, *J* = 8.1, 4.4, 4.4 Hz, H-4), 3.90 (dd, 2H, *J* = 5.6 Hz, H-6a, H-6b), 3.75 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 3.52 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 2.05–2.26 (m, 2H, H-5a, H-5b), 1.55–1.70 (m, 2H, octyl OCH_2C*H*$_2$), 1.18–1.42 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.8 Hz, octyl C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.3 (C=O), 133.5, 133.4, 129.9, 129.8(3), 129.7(6), 129.3, 129.2, 128.5(2), 128.4(8), 128.4 (12C, Ar), 105.4 (C-1), 82.4 (C-4), 81.6 (C-2), 80.5 (C-3), 67.6 (octyl O*C*H_2), 60.5 (C-6), 35.7 (C-5), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{28}H_{36}O_7$: 507.2353. Found: 507.2357.

Octyl 2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranoside (6.25)

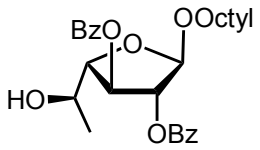
Octyl 2,3-di-*O*-benzoyl-6-deoxy-5-*O*-levulinoyl-β-D-galactofuranoside

Thioglycoside **6.32** (223 mg, 0.40 mmol) and powdered 4 Å molecular sieves (0.5 g) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (20



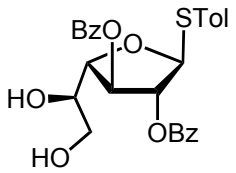
mL) and octanol (0.19 mL, 1.2 mmol) were added and the solution was cooled to 0 °C before the addition of *N*-iodosuccinimide (108 mg, 0.45 mmol) and TMSOTf (21 μL, 0.12 mmol). The mixture was stirred for 15 min and neutralized by the addition of triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give the desired intermediate as a pale yellow oil (232 mg, quant.): R_f 0.26 (3:1 hexane–EtOAc); $[\alpha]_D$ = +143.2 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.08 (app t, 4H, *J* = 7.0 Hz, ArH), 7.59 (app t, 2H, *J* = 7.5 Hz, ArH), 7.43–7.50 (m, 4H, ArH), 5.45 (br d, 1H, *J* = 5.0 Hz, H-3), 5.43 (br s, 1H, H-2), 5.34 (qd, 1H, *J* = 6.4, 5.0 Hz, H-5), 5.23 (s, 1H, H-1), 4.31 (dd, *J* = 5.0, 5.0 Hz, H-4), 3.77 (dt, 1H, *J* = 9.4, 6.6 Hz, octyl OC*H*$_2$), 3.54 (dt, 1H, *J* = 9.4, 6.5 Hz, octyl OC*H*$_2$), 2.49–2.88 (m, 4H, Lev CH_2), 2.13 (s, 3H, Lev CH_3), 1.58–1.60 (m, 2H, octyl OCH_2CH_2), 1.40 (d, 3H, *J* = 6.4 Hz, H-6), 1.20–1.46 (m, 10H, octyl C*H*$_2$), 0.88 (t, 3H, *J* = 6.7 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 206.3 (Lev ketone C=O), 172.0 (Lev ester C=O), 165.5 (C=O), 165.4 (C=O), 133.4(7), 133.4(2), 129.9(0), 129.8(5), 129.8(1), 129.7(7), 129.3(0), 129.2(9), 128.4(9), 128.4(4) (12C, Ar), 105.5 (C-1), 83.7 (C-4), 82.1 (C-2), 77.1 (C-3), 69.4 (C-5), 67.6 (octyl O*C*H_2), 38.0 (Lev CH_2), 31.8 (octyl *C*H_2), 29.7 (Lev CH_3), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 28.2 (Lev CH_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 16.1 (C-6), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{33}H_{42}O_9$: 605.2721. Found: 605.2724.

Octyl 2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranoside (6.25)



Octyl glycoside above (220 mg, 0.38 mmol) was dissolved in 10:1 THF–MeOH (20 mL) and $NH_2NH_2{\cdot}H_2O$ in AcOH (5 mL, 1:2 v/v) was added. After 30 min, the reaction mixture was diluted in EtOAc (10 mL), washed with satd aq $NaHCO_3$ (2 x 10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified by chromatography (4:1 hexane–EtOAc) to give **6.25** as a pale yellow oil (139 mg, 76%): R_f 0.28 (4:1 hexane–EtOAc); $[\alpha]_D$ = –12.7 (*c* 2.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.04–8.10 (m, 4H, ArH), 7.56–7.62 (m, 2H, ArH), 7.43–7.49 (m, 4H, ArH), 5.45–5.50 (m, 2H, H-2, H-3), 5.23 (s, 1H, H-1), 4.12–4.19 (m, 2H, H-4, H-5), 3.77 (dt, 1H, *J* = 9.4, 6.7 Hz, octyl OC*H*$_2$), 3.54 (dt, 1H, *J* = 9.4, 6.2 Hz, octyl OC*H*$_2$), 1.58–1.72 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.35 (d, 3H, *J* = 6.5 Hz, H-6), 1.20–1.46 (m, 10H, octyl C*H*$_2$), 0.87 (t, 3H, *J* = 6.5 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.9 (C=O), 165.3 (C=O), 133.4(9), 133.4(6), 129.9(1), 129.8(6), 129.3, 129.2, 128.5, 128.4(9), 128.4(5) (12C, Ar), 105.5 (C-1), 86.5 (C-4), 81.9 (C-2), 77.8 (C-3), 67.5(4) (C-5), 67.4(7) (octyl O*C*H$_2$), 31.8 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 19.5 (C-6), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{28}H_{36}O_7$: 507.2353. Found: 507.2354.

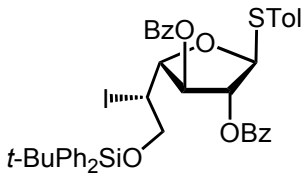
***p*-Tolyl 2,3-di-*O*-benzoyl-1-thio-β-D-galactofuranoside (6.27)**



p-Tolyl 2,3,5,6-tetra-*O*-benzoyl-galactofuranoside[13] (9.6 g, 16 mmol) was dissolved in MeOH (9 mL) and 1 M NaOMe was added dropwise until the pH of solution reached ~11. The reaction mixture was stirred for 2 h and was neutralized by the addition of Amerlite IR120 resin (H^+ form). The crude mixture was filtered and concentrated. The pale yellow solid product was redissolved in acetone (200 mL), and 2,2-dimethoxypropane (3.2 mL, 24 mmol), and *p*-TsOH·H_2O (300 mg) were added. The mixture was stirred for 30 min, neutralized by the addition of triethylamine and concentrated. The partially protected crude product was redissolved in 1:1 pyridine**–**CH_2Cl_2 (50 mL) and benzoyl chloride (5.2 mL, 41 mmol) was added at 0 °C and the reaction mixture was stirred overnight. The solution was diluted in CH_2Cl_2 (100 mL), washed with 1M HCl (3 x 50 mL), satd aq $NaHCO_3$ (50 mL), dried (Na_2SO_4), filtered and concentrated to a pale brown oil. The syrup was then redissolved in 80% aqueous AcOH (100 mL) and heated at 50 °C for 3 h. After cooling to rt, the mixture was diluted with CH_2Cl_2 (150 mL), washed with ice-cold satd aq $NaHCO_3$ (3 x 50 mL), dried ($MgSO_4$), filtered and concentrated. The desired diol was purified by chromatography (1:1 hexane–EtOAc) to give **6.27** as an amorphous white solid (4.4 g, 71% over four steps): R_f 0.30 (1:1 hexane–EtOAc); $[\alpha]_D$ = –83.3 (*c* 1.6, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.12–8.17 (m, 2H, ArH), 8.05–8.11 (m, 2H, ArH), 7.58–7.68 (m, 2H, ArH), 7.45–7.55 (m, 6H,

ArH), 7.18 (app d, 2H, *J* = 8.0 Hz, ArH), 5.68–5.74 (m, 3H, H-1, H-2, H-3), 4.61 (dd, 1H, *J* = 4.7, 3.4 Hz, H-4), 4.15–4.22 (m, 1H, H-5), 3.88 (dd, 1H, *J* = 11.6, 5.4 Hz, H-6a), 3.82 (dd, 1H, *J* = 11.6, 4.5 Hz, H-6b), 2.37 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.9 (C=O), 165.2 (C=O), 138.3, 133.7, 133.6, 133.1, 130.0, 129.9, 129.8, 129.3, 128.9, 128.5 (18C, Ar), 91.8 (C-1), 84.0 (C-4), 81.7 (C-2), 78.0 (C-3), 70.4 (C-5), 64.2 (C-6), 21.1 (SPh*C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{27}H_{26}O_7S$: 517.2915. Found: 517.2913.

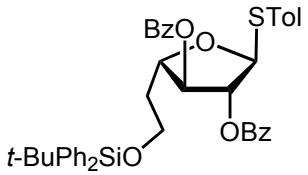
_p_-Tolyl 2,3-di-_O_-benzoyl-5-deoxy-5-iodo-6-_O-tert_-butyldiphenylsilyl- 1-thio-α-L-altrofuranoside (6.28)



Alcohol **6.19** (292 mg, 0.40 mmol) was dissolved in toluene (12 mL) and triphenylphosphine (315 mg, 1.2 mmol), imidazole (164 mg, 2.4 mmol) and iodine (280 mg, 1.1 mmol) were added. The mixture was heated under reflux for 2.5 h. After cooling to rt, the reaction was quenched by the addition of satd aq $NaHCO_3$ followed by satd aq NaH_2SO_3. The solution was extracted twice with EtOAc (2 x 25 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated. The desired compound was obtained after chromatography (6:1 hexane–EtOAc) to give **6.28** as a colorless oil (320 mg, 93%): R_f 0.46 (6:1 hexane–EtOAc); $[\alpha]_D$ = –34.7 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.16 (app d, 2H, *J* = 7.5 Hz, ArH), 8.04 (app d, 2H, *J* = 7.5 Hz, ArH), 7.55–7.68 (m, 6H,

ArH), 7.27–7.51 (m, 12H, ArH), 7.11 (app d, 2H, *J* = 7.5 Hz, ArH), 5.74 (d, 1H, *J* = 4.5 Hz, H-3), 5.70 (s, 1H, H-1), 5.58 (s, 1H, H-2), 4.96 (dd, 1H, *J* = 6.1, 4.5 Hz, H-4), 4.46 (app. q, 1H, *J* = 6.1 Hz, H-5), 4.04 (dd, 1H, *J* = 11.4, 6.1 Hz, H-6a), 3.99 (dd, 1H, *J* = 11.4, 6.1 Hz, H-6b), 2.33 (s, 3H, SPhCH_3), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.1(4) (C=O), 165.0(9) (C=O), 138.1, 135.6, 135.5, 134.8, 133.5, 133.2, 133.0, 132.8, 130.1, 130.0, 129.9, 129.8(1), 129.7(9), 129.6(5), 129.6(0), 129.2, 129.0, 128.5(1), 128.4(7), 127.8, 127.7 (30C, Ar), 91.9 (C-1), 82.5 (C-2/C-4), 82.4 (C-2/C-4), 80.3 (C-3), 65.8 (C-6), 33.6 (C-5), 26.8 (C(CH$_3$)$_3$), 21.2 (SPhCH$_3$), 19.3 (C(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{43}H_{43}IO_6SSi$: 865.1487. Found: 865.1490.

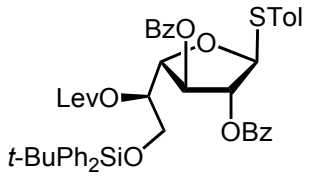
_p_-Tolyl 2,3-di-_O_-benzoyl-5-deoxy-6-_O-tert_-butyldiphenylsilyl-1-thio-α-L-_arabino_-hexofuranoside (6.29)



Monosaccharide **6.28** (230 mg, 0.27 mmol) was dissolved in EtOAc (4 mL) and 20% $Pd(OH)_2$ (100 mg) and *i*-Pr_2EtN (0.10 mL, 0.64 mmol) were added. The mixture was stirred under a H_2 atmosphere at 32 °C for 4 days. The catalyst was separated by filtration through a short pad of Celite. The filtrate was concentrated and the resulting crude product was purified by chromatography (6:1 hexane–EtOAc) to give **6.29** (166 mg, 85%) as a colorless oil: R_f 0.50 (6:1 hexane–EtOAc); $[\alpha]_D$ = –54.6 (*c* 2.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.13–8.16 (m, 2H, ArH), 8.02–8.05

(m, 2H, ArH), 7.56–7.70 (m, 6H, ArH), 7.30–7.52 (m, 12H, ArH), 7.11 (app d, 2H, *J* = 7.8 Hz, ArH), 5.66 (dd, 1H, *J* = 1.9, 1.9 Hz, H-2), 5.63 (dd, 1H, *J* = 1.9, 0.7 Hz, H-1), 5.42 (ddd, 1H, *J* = 5.1, 1.9, 0.7 Hz, H-3), 4.85 (ddd, 1H, *J* = 9.0, 5.1, 4.7 Hz, H-4), 3.84–3.93 (m, 2H, H-6a, H-6b), 2.34 (s, 3H, SPhCH_3), 2.18–2.25 (m, 1H, H-5a), 2.04–2.12 (m, 1H, H-5b), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.5 (C=O), 165.2 (C=O), 137.9, 135.5, 133.7(1), 133.6(7), 133.5, 133.4, 133.0, 130.2, 130.0, 129.9(7), 129.9(0), 129.8(6), 129.7, 129.5(6), 129.5(5), 129.4, 129.2, 128.4(8), 128.4(6), 127.6(4), 127.6(2) (30C, Ar), 91.3 (C-1), 82.6 (C-2), 80.6 (C-3), 79.4 (C-4), 60.3 (C-6), 35.7 (C-5), 26.8 (C(*C*H_3)$_3$), 21.2 (SPh*C*H_3), 19.2 (*C*(CH$_3$)$_3$). HRMS (ESI) calcd. for (M + Na) $C_{43}H_{44}O_6SSi$: 739.2520. Found: 739.2532.

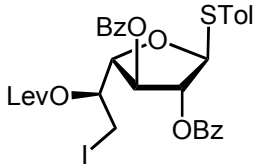
_p_-Tolyl 2,3-di-_O_-benzoyl-5-_O_-levulinoyl-6-_O_-_tert_-butyldiphenylsilyl-1-thio-β-D-galactofuranoside (6.30)



Alcohol **6.19** (0.89 g, 1.2 mmol) and DMAP (76 mg, 0.62 mmol) were dissolved in CH_2Cl_2 (40 mL). Levulinic acid (0.2 mL, 1.9 mmol) and DCC (0.38 g, 1.9 mmol) were added to the reaction at –10 °C and the solution was slowly warmed to rt over 1 h. Once the reaction was complete, the insoluble salt was removed by filtration and the filtrate was washed with H_2O (20 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated and the crude product was purified by

chromatography (3:1 hexane–EtOAc) to give **6.30** as a pale yellow oil (0.97 g, 95%): R_f 0.34 (3:1 hexane–EtOAc); $[\alpha]_D$ = –33.8 (*c* 2.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.11 (app d, 2H, *J* = 7.3 Hz, ArH), 8.05 (app d, 2H, *J* = 7.4 Hz, ArH), 7.25–7.70 (m, 18H, ArH), 7.08 (app d, 2H, *J* = 8.0 Hz, ArH), 5.68 (br s, 1H, H-1), 5.65 (br s, 1H, H-2), 5.49–5.52 (m, 2H, H-3, H-5), 4.92 (dd, 1H, *J* = 4.8, 4.8 Hz, H-4), 3.91 (d, 2H, *J* = 4.6 Hz, H-6a, H-6b), 2.52–2.70 (m, 4H, Lev CH_2), 2.33 (s, 3H, SPhCH_3), 2.01 (s, 3H, Lev CH_3), 1.02 (s, 9H, C(CH_3)$_3$),; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 206.0 (Lev ketone C=O), 171.9 (Lev ester C=O), 165.3(4) (C=O), 165.2(7) (C=O), 138.0, 135.5(6), 135.5(0), 133.5(0), 133.4(8), 133.0(4), 133.0(X), 133.0(0), 132.9(7), 130.0, 129.8(9), 129.8(3), 129.7(5), 129.7(2), 129.6(7), 129.6(3), 129.1(3), 129.0(9), 128.4(7), 128.4(0), 127.7 (30C, Ar), 91.2 (C-1), 82.1 (C-2), 80.4 (C-4), 77.4 (C-3), 72.4 (C-5), 62.0 (C-6), 38.0 (Lev CH_2), 29.7 (Lev CH_3), 28.0 (Lev CH_2), 26.7 (C(*C*H_3)$_3$), 21.1 (SPh*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{48}H_{50}O_9SSi$: 755.2469. Found: 755.2461.

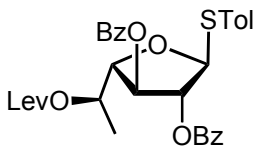
p-Tolyl 2,3-di-*O*-benzoyl-6-deoxy-6-iodo-5-*O*-levulinoyl-1-thio-β-D-galactofuranoside (6.31)



Alcohol **6.18** (417 mg, 0.70 mmol), triphenylphosphine (462 mg, 1.8 mmol), imidazole (235 mg, 3.5 mmol) and iodine (411 mg, 1.6 mmol) were dissolved in toluene (18 mL) and the reaction was carried out as described for **6.28**. The desired product was purified by chromatography (2:1 hexane–EtOAc)

to give **6.31** as a colorless oil (373 mg, 76%): R_f 0.38 (2:1 hexane–EtOAc); $[\alpha]_D$ = –56.7 (*c* 2.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.05–8.13 (m, 4H, ArH), 7.57–7.64 (m, 2H, ArH), 7.43–7.54 (m, 6H, ArH), 7.17 (app d, 2H, *J* = 8.0 Hz, ArH), 5.70 (d, 1H, *J* = 1.9 Hz, H-1), 5.66 (dd, 1H, *J* = 1.9, 1.9 Hz, H-2), 5.42–5.47 (m, 2H, H-3, H-5), 4.92 (dd, *J* = 5.0, 3.6 Hz, H-4), 3.44 (dd, *J* = 10.1, 7.5 Hz, H-6a), 3.40 (dd, *J* = 10.1, 6.4 Hz, H-6b), 2.49–2.79 (m, 4H, Lev CH_2), 2.35 (s, 3H, SPhC*H*$_3$), 2.12 (s, 3H, Lev CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 205.9 (Lev ketone C=O), 171.8 (Lev ester C=O), 165.5 (C=O), 165.3 (C=O), 138.5, 133.6(7), 133.6(5), 130.1, 129.8(8), 129.8(5), 129.1, 129.0, 128.9, 128.5 (18C, Ar), 91.3 (C-1), 82.1 (C-2), 81.6 (C-4), 77.6 (C-3), 71.9 (C-5), 38.0 (Lev CH_2), 29.7 (Lev CH_3), 28.0 (Lev CH_2), 21.2 (SPh*C*H_3), 0.9 (C-6). HRMS (ESI) calcd. for (M + Na) $C_{32}H_{31}IO_8S$: 725.0677. Found: 725.0674.

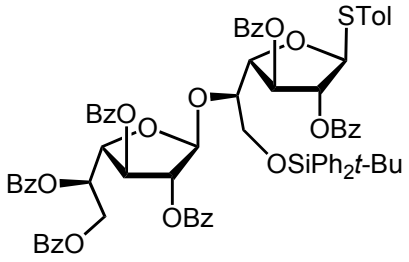
***p*-Tolyl 2,3-di-*O*-benzoyl-6-deoxy-5-*O*-levulinoyl-1-thio-β-D-galactofuranoside (6.32)**



Iodide **6.31** (348 mg, 0.50 mmol) was dissolved in EtOAc (7 mL) and 20% $Pd(OH)_2$ (150 mg) and *i*-Pr_2EtN (0.17 mL, 0.99 mmol) were added. The reduction was performed as described for **6.29**. The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.32** as a colorless oil (246 mg, 86%): R_f 0.42 (2:1 hexane–EtOAc); $[\alpha]_D$ = –59.7 (*c* 1.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.04–8.13 (m, 4H, ArH), 7.56–7.64 (m, 2H, ArH), 7.43–7.52 (m,

6H, ArH), 7.15 (app d, 2H, *J* = 8.0 Hz, ArH), 5.69 (d, 1H, *J* = 1.8 Hz, H-1), 5.62 (dd, 1H, *J* = 1.8, 1.8 Hz, H-2), 5.53 (dd, 1H, *J* = 4.8, 1.8 Hz, H-3), 5.38 (qd, 1H, *J* = 6.4, 4.8 Hz, H-5), 4.57 (dd, *J* = 4.8, 4.8 Hz, H-4), 2.47–2.78 (m, 4H, Lev CH_2), 2.34 (s, 3H, SPhCH_3), 2.12 (s, 3H, Lev CH_3), 1.41 (d, 3H, *J* = 6.4 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 206.2 (Lev ketone C=O), 172.0 (Lev ester C=O), 165.4 (C=O), 165.3 (C=O), 138.1, 133.6, 133.5, 133.0, 130.0, 129.9, 129.8, 129.6, 129.1, 128.7, 128.6, 128.5 (18C, Ar), 91.2 (C-1), 83.7 (C-4), 82.3 (C-2), 77.4 (C-3), 69.2 (C-5), 38.0 (Lev CH_2), 29.7 (Lev CH_3), 28.2 (Lev CH_2), 21.2 (SPhCH_3), 16.2 (C-6). HRMS (ESI) calcd. for (M + Na) $C_{32}H_{32}O_8S$: 599.1710. Found: 599.1711.

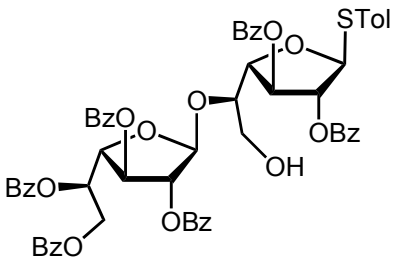
_p_-Tolyl 2,3,5,6-tetra-_O_-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-_O_-benzoyl-6-_O_-_tert_-butyldiphenylsilyl-1-thio-β-D-galactofuranoside (6.33)



Trichloroacetimidate **6.17**[11] (176 mg, 0.24 mmol), thioglycoside acceptor **6.19** (152 mg, 0.21 mmol) and powdered 4 Å molecular sieves (200 mg) were dried for 2 h under vacuum with P_2O_5. The mixture was dissolved in CH_2Cl_2 (8 mL) and the solution was cooled to ~20 °C before the addition of TMSOTf (4 μL, 0.021 mmol). The solution was stirred for 15 min and then neutralized by the addition of triethylamine before being filtered through Celite and concentrated. The crude product was purified by chromatography (3:1

hexane–EtOAc) to give **6.33** as a pale yellow amorphous solid (272 mg, quant.): R_f 0.31 (3:1 hexane–EtOAc); $[\alpha]_D$ = –33.5 (*c* 2.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01–8.06 (m, 4H, ArH), 7.84–7.94 (m, 8H, ArH), 7.62–7.67 (m, 4H, ArH), 7.16–7.48 (m, 26H, ArH), 7.01 (app d, 2H, *J* = 8.0 Hz, ArH), 6.01 (ddd, 1H, *J* = 6.5, 4.0, 4.0 Hz, H-5′), 5.83 (dd, 1H, *J* = 5.5, 1.0 Hz, H-3), 5.64–5.67 (m, 2H, H-1, H-2), 5.62 (dd, 1H, *J* = 5.0, 1.0 Hz, H-3′), 5.59 (d, 1H, *J* = 1.0 Hz, H-2′), 5.56 (s, 1H, H-1′), 4.98 (dd, 1H, *J* = 5.0, 4.0 Hz, H-4′), 4.74 (dd, 1H, *J* = 5.5, 3.5 Hz, H-4), 4.63–4.71 (m, 2H, H-6a′, H-6b′), 4.41 (ddd, 1H, *J* = 6.5, 6.0, 4.0 Hz, H-5), 4.01 (dd, 1H, *J* = 11.0, 6.5 Hz, H-6a), 3.98 (dd, 1H, *J* = 11.0, 6.0 Hz, H-6b), 2.27 (s, 3H, SPhCH_3), 1.00 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.7 (C=O), 165.5 (C=O), 165.4(5) (C=O), 165.3(6) (C=O), 165.1 (C=O), 137.9, 135.6(1), 135.5(9), 135.5(5), 133.4, 133.3, 133.2, 133.0, 132.9, 132.8(9), 132.8(4), 132.7, 130.0, 129.9, 129.8(3), 129.8(0), 129.7, 129.6(9), 129.6(5), 129.6(2), 129.1, 129.0, 128.9, 128.8, 128.5(6), 128.5(2), 128.4(5), 128.4(3), 128.3(7), 128.3(3), 128.3(0), 128.2(7), 128.1(9), 128.1(2), 127.7(7), 127.7(5) (54C, Ar), 105.2 (C-1′), 90.6 (C-1), 82.1 (C2′/C-4′), 82.0 (C2′/C-4′), 81.5 (C-4), 81.2 (C-2), 77.8 (C-3′), 77.2 (C-3), 75.7 (C-5), 70.5 (C-5′), 63.9 (C-6′), 63.3 (C-6), 26.7 (C(*C*H_3)$_3$), 21.1 (SPh*C*H_3), 19.1 (*C*(CH$_3$)$_3$). MALDI-TOFMS calcd. for (M + Na) $C_{77}H_{70}O_{16}SSi$: 1333.4046. Found: 1333.4037.

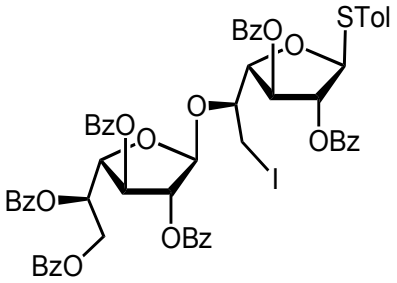
***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-1-thio-β-D-galactofuranoside (6.34)**



The silyl group was removed from disaccharide **6.33** (759 mg, 0.58 mmol) in 20:4:1 THF–pyridine–70% HF-pyridine (28 mL) as described for **6.18**. The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.34** (542 mg, 87%) as a colorless oil: R_f 0.32 (2:1 hexane–EtOAc); $[\alpha]_D$ = –48.2 (*c* 1.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.87–8.08 (m, 12H, ArH), 7.22–7.60 (m, 20H, ArH), 7.09 (app d, 2H, *J* = 8.0 Hz, ArH), 6.00 (ddd, 1H, *J* = 5.0, 5.0, 3.5 Hz, H-5′), 5.85 (dd, 1H, *J* = 4.7, 2.2 Hz, H-3), 5.72 (d, 1H, *J* = 2.2 Hz, H-1), 5.70 (s, 1H, H-1′), 5.69 (dd, 1H, *J* = 5.6, 1.6 Hz, H-3′), 5.66 (dd, 1H, *J* = 2.2, 2.2 Hz, H-2), 5.56 (d, 1H, *J* = 1.6 Hz, H-2′), 4.93 (dd, 1H, *J* = 5.6, 3.5 Hz, H-4′), 4.73 (dd, 1H, *J* = 4.7, 4.7 Hz, H-4), 4.60–4.67 (m, 2H, H-6a′, H-6b′), 4.37 (ddd, 1H, *J* = 4.7, 4.5, 4.5 Hz, H-5), 3.95–4.05 (m, 2H, H-6a, H-6b), 2.29 (s, 3H, SPhCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0(3) (C=O), 166.0(0) (C=O), 165.7 (C=O), 165.6 (C=O), 165.5 (C=O), 165.3 (C=O), 138.3, 133.6, 133.5(2), 133.5(0), 133.4, 133.2, 133.1, 133.0, 130.0(0), 129.9(7), 129.9(4), 129.8(8), 129.8(4), 129.8(0), 129.7(8), 129.6(9), 129.6(1), 129.5, 129.4, 128.9(1), 128.8(8), 128.7(6), 128.7, 128.6, 128.5, 128.4, 128.3(8), 128.3(1), 128.2(7) (42C, Ar), 105.6 (C-1), 91.2 (C-1′), 83.2 (C-2′), 82.8 (C-4), 81.7 (C-2), 81.5 (C-4′), 77.5 (C-3′), 77.4 (C-3), 76.1 (C-5), 70.3 (C-5′), 63.8 (C-6′), 62.3 (C-6), 21.1

(SPh*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{61}H_{52}O_{16}S$: 1095.2868. Found: 1095.2857.

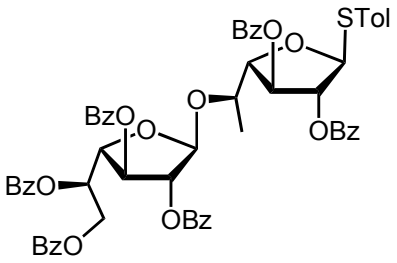
_p_-Tolyl 2,3,5,6-tetra-_O_-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-_O_-benzoyl-6-deoxy-6-iodo-1-thio-β-D-galactofuranoside (6.35)



Disaccharide alcohol **6.34** (253 mg, 0.24 mmol), triphenylphosphine (155 mg, 0.59 mmol), imidazole (79 mg, 1.2 mmol) and iodine (138 mg, 0.54 mmol) were dissolved in toluene (6 mL) and the reaction was performed as described for **6.28**. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.35** as a pale yellow amorphous solid (143 mg, 97%): R_f 0.28 (3:1 hexane–EtOAc); $[\alpha]_D$ = –62.1 (*c* 2.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.02–8.08 (m, 6H, ArH), 7.87–7.94 (m, 4H, ArH), 7.82 (app d, 2H, *J* = 7.0 Hz, ArH), 7.24–7.60 (m, 18H, ArH), 7.18 (app t, 2H, *J* = 7.5 Hz, ArH), 7.12 (app d, 2H, *J* = 8.5 Hz, ArH), 6.05 (ddd, 1H, *J* = 7.3, 4.0, 3.8 Hz, H-5′), 5.83 (dd, 1H, *J* = 5.1, 2.1 Hz, H-3), 5.64–5.69 (m, 4H, H-1′, H-2′, H-1, H-2), 5.63 (br d, 1H, *J* = 5.0 Hz, H-3′), 5.03 (dd, 1H, *J* = 5.0, 3.8 Hz, H-4′), 4.97 (dd, 1H, *J* = 5.1, 3.1 Hz, H-4), 4.76 (dd, 1H, *J* = 11.9, 4.0 Hz, H-6a′), 4.68 (dd, 1H, *J* = 11.9, 7.3 Hz, H-6b′), 4.55 (ddd, 1H, *J* = 8.6, 5.5, 3.1 Hz, H-5), 3.54 (dd, 1H, *J* = 10.2, 5.5 Hz, H-6a), 3.48 (dd, 1H, *J* = 10.2, 8.6 Hz, H-6b), 2.32 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.7 (C=O),

165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 138.3, 133.6, 133.5, 133.4, 133.3, 133.2, 132.9, 130.1, 130.0, 129.8(7), 129.8(2), 129.7(9), 129.7(3), 129.7(2), 129.6, 129.5, 129.2, 128.8, 128.7, 128.5(4), 128.5(0), 128.4(1), 128.3(8), 128.2(8), 128.2(3), 128.2(1), 128.1(6) (42C, Ar), 104.6 (C-1), 91.1 (C-1′), 82.4, 82.3, 82.2, 81.6 (C2′, C-4′, C-2, C-4), 77.8 (C-3′), 77.4 (C-3), 75.2 (C-5), 70.5 (C-5′), 63.7 (C-6′), 21.2 (SPh*C*H_3), 1.0 (C-6). HRMS (ESI) calcd. for (M + Na) $C_{61}H_{51}IO_{15}S$: 1205.1886. Found: 1205.1882.

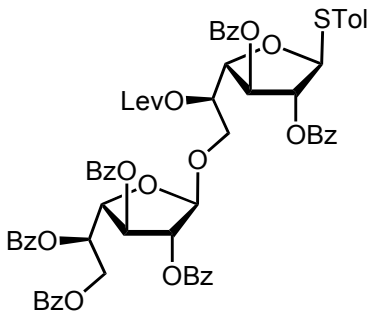
_p_-Tolyl 2,3,5,6-tetra-_O_-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-_O_-benzoyl-6-deoxy-1-thio-β-D-galactofuranoside (6.36)



Disaccharide **6.35** (270 mg, 0.23 mmol) was dissolved in EtOAc (4 mL) and 20% $Pd(OH)_2$ (135 mg) and *i*-Pr_2EtN (45 μL, 0.26 mmol) were added. The reduction was performed as described for the synthesis of **6.29**. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.36** as a colorless oil (214 mg, 89%): R_f 0.32 (3:1 hexane–EtOAc); $[\alpha]_D$ = –46.3 (*c* 3.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.03–8.11 (m, 4H, ArH), 7.84–7.98 (m, 8H, ArH), 7.25–7.61 (m, 18H, ArH), 7.18 (app t, 2H, *J* = 7.5 Hz, ArH), 7.07 (app d, 2H, *J* = 8.5 Hz, ArH), 6.05 (td, 1H, *J* = 5.7, 3.5 Hz, H-5′), 5.83 (dd, 1H, *J* = 5.0, 2.0 Hz, H-3), 5.69 (br s, 1H, H-1), 5.68 (dd, 1H, *J* = 2.0, 2.0 Hz, H-2), 5.62 (br d, 1H, *J* = 5.2 Hz, H-3′), 5.58 (br s, 1H, H-2′),

5.54 (br s, 1H, H-1′), 4.98 (dd, 1H, *J* = 5.2, 3.5 Hz, H-4′), 4.71 (d, 2H, *J* = 5.7 Hz, H-6a′, H-6b′), 4.55 (dd, 1H, *J* = 5.0, 5.0 Hz, H-4), 4.44 (qd, 1H, *J* = 6.5, 5.0 Hz, H-5), 2.29 (s, 3H, SPhCH_3), 1.43 (d, 3H, *J* = 6.5 Hz, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.8 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 137.9, 133.5, 133.4(7), 133.4(2), 133.3, 133.2, 133.1, 132.9, 132.5, 130.0, 129.9(4), 129.9(0), 129.8(6), 129.7(8), 129.7(5), 129.6(9), 129.6(3), 129.0(5), 129.0(2), 128.9, 128.8, 128.5(6), 128.5(2), 128.4(9), 128.4(4), 128.3(9), 128.3(6), 128.3(0), 128.2(8), 128.2(4), 128.2(0), 128.1 (42C, Ar), 103.0 (C-1′), 91.0 (C-1), 84.9 (C-4), 82.4 (C-4′), 81.9 (C-2), 81.6 (C-2′), 77.9 (C-3′), 77.2 (C-3), 70.4 (C-5′), 70.3 (C-5), 63.9 (C-6′), 21.1 (SPh*C*H_3), 15.0 (C-6). HRMS (ESI) calcd. for (M + Na) $C_{61}H_{52}O_{15}S$: 1079.2919. Found: 1079.2911.

_p_-Tolyl 2,3,5,6-tetra-_O_-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-_O_-benzoyl-5-_O_-levulinoyl-1-thio-β-D-galactofuranoside (6.37)



The reaction was performed as described for the synthesis of **6.33**. TMSOTf (8 μL, 0.042 mmol) was added to a solution of trichloroacetimidate **6.17**[11] (356 mg, 0.48 mmol), thioglycoside acceptor **6.18** (248 mg, 0.42 mmol) and powdered 4 Å molecular sieves (250 mg) in CH_2Cl_2 (10 mL). The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.37** as a colorless oil (441 mg, 90%): R_f 0.23 (2:1 hexane–

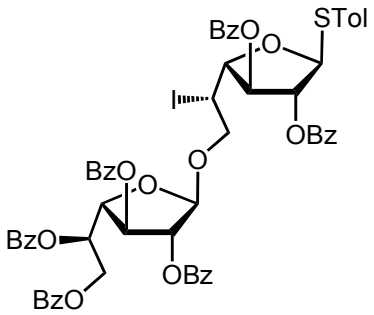
EtOAc); $[\alpha]_D$ = –37.0 (*c* 2.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00–8.12 (m, 8H, ArH), 7.97 (app d, 2H, *J* = 7.0 Hz, ArH), 7.82 (app d, 2H, *J* = 8.0 Hz, ArH), 7.22–7.60 (m, 20H, ArH), 7.10 (app d, 2H, *J* = 8.0 Hz, ArH), 6.02–6.10 (m, 1H, H-5′), 5.74 (s, 1H, H-1), 5.60–5.70 (m, 2H, H-2, H-5), 5.54–5.63 (m, 2H, H-3′, H-3), 5.44 (s, 1H, H-2′), 5.34 (s, 1H, H-1′), 4.61–4.82 (m, 4H, H-4′, H-6a′, H-6b′, H-4), 4.09 (dd, 1H, *J* = 10.0, 6.5 Hz, H-6a), 3.81 (dd, 1H, *J* = 10.0, 6.5 Hz, H-6b), 2.47–2.76 (m, 4H, Lev CH_2), 2.26 (s, 3H, SPhC*H*$_3$), 2.07 (s, 3H, Lev CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 206.0 (Lev ketone C=O), 172.0 (Lev ester C=O), 166.1 (C=O), 165.7 (C=O), 165.6 (C=O), 165.4 (C=O), 165.3 (2C, C=O), 138.0, 133.6, 133.5, 133.4, 133.3, 133.1, 132.9, 133.0, 132.3, 130.0(5), 130.9(5), 129.9(1), 129.8(6), 129.7(9), 129.7(2), 129.6, 129.5, 129.0(4), 129.0(1), 129.9(7), 128.8(7), 128.6, 128.5, 128.3(9), 128.3(5), 128.3(0) (42C, Ar), 105.9 (C-1′), 91.2 (C-1), 82.1 (C-2′), 82.0 (C-4′), 81.9 (C-2), 81.3 (C-4), 77.5 (C-3), 77.1 (C-3′), 70.5 (C-5′), 70.3 (C-5), 65.4 (C-6), 63.9 (C-6′), 38.0 (Lev CH_2), 28.0 (Lev CH_3), 28.0 (Lev CH_2), 21.1 (SPh*C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{66}H_{58}O_{18}S$: 1193.3236. Found: 1193.3241.

_p_-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-1-thio-β-D-galactofuranoside (6.38)

Disaccharide **6.37** (555 mg, 0.38 mmol) was dissolved in 10:1 THF–MeOH (22 mL) and $NH_2NH_2{\cdot}H_2O$ in AcOH (6 mL, 1:2 v/v) was added. The reaction was performed as described for the synthesis of **6.25** to yield the

crude product, which was purified by chromatography (2:1 hexane–EtOAc) to give **6.38** as a white amorphous solid (474 mg, 93%): R_f 0.35 (2:1 hexane–EtOAc); $[\alpha]_D$ = –4.9 (*c* 2.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00–8.10 (m, 8H, ArH), 7.94–7.98 (m, 2H, ArH), 7.83–7.88 (m, 2H, ArH), 7.24–7.62 (m, 20H, ArH), 7.11 (app d, 2H, *J* = 8.0 Hz, ArH), 6.03–6.08 (m, 1H, H-5′), 5.78 (dd, 1H, *J* = 4.9, 1.7 Hz, H-3), 5.74 (br s, 1H, H-1), 5.69 (dd, 1H, *J* = 1.7, 1.7 Hz, H-2), 5.62 (br d, 1H, *J* = 5.2 Hz, H-3′), 5.49 (d, 1H, *J* = 1.2 Hz, H-2′), 5.39 (s, 1H, H-1′), 4.70–4.76 (m, 2H, H-4′, H-6a′), 4.67 (dd, 1H, *J* = 11.9, 4.1 Hz, H-6b′), 4.63 (dd, 1H, *J* = 4.9, 2.6 Hz, H-4), 4.34–4.41 (m, 1H, H-5), 3.97 (dd, 1H, *J* = 10.1, 5.9 Hz, H-6a), 3.78 (dd, 1H, *J* = 10.1, 6.6 Hz, H-6b), 2.64 (br s, 1H, OH), 2.26 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.7(9) (C=O), 165.7(2) (C=O), 165.6(7) (C=O), 165.4 (C=O), 165.3 (C=O), 138.1, 133.5(9), 133.5(7), 133.4, 133.3, 133.2, 133.0, 132.4, 130.0, 129.9(7), 129.9(2), 129.8(9), 129.8(4), 129.7(8), 129.7(2), 129.5(8), 129.5(2), 129.0(5), 129.0(1), 128.9, 128.8, 128.6, 128.5, 128.4(1), 128.3(8), 128.3(2) (42C, Ar), 106.1 (C-1′), 91.7 (C-1), 83.0 (C-4), 82.2 (C-2′), 81.9 (C-2), 81.6 (C-4′), 77.8 (C-3), 77.6 (C-3′), 70.2 (C-5′), 69.2 (C-5), 68.5 (C-6), 63.7 (C-6′), 21.1 (SPh*C*H$_3$). MALDI-TOFMS calcd. for (M + Na) $C_{61}H_{52}O_{16}S$: 1095.2868. Found: 1095.2864.

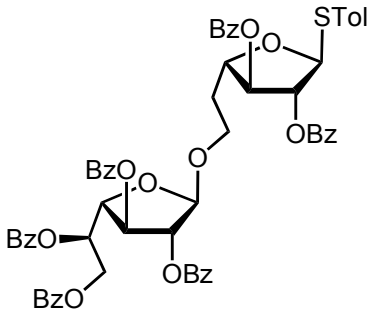
***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-5-iodo-1-thio-α-L-altrofuranoside (6.39)**



Disaccharide alcohol **6.38** (463 mg, 0.43 mmol), triphenylphosphine (282 mg, 1.1 mmol), imidazole (143 mg, 2.1 mmol) and iodine (251 mg, 0.98 mmol) were dissolved in toluene (12 mL) and the reaction mixture was performed as described for the synthesis of **6.28**. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.39** as a pale yellow amorphous solid (478 mg, 94%): R_f 0.26 (3:1 hexane–EtOAc); $[\alpha]_D$ = –33.0 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.03–8.12 (m, 8H, ArH), 7.96–8.00 (m, 2H, ArH), 7.83–7.87 (m, 2H, ArH), 7.25–7.60 (m, 20H, ArH), 7.11 (app d, 2H, *J* = 8.0 Hz, ArH), 6.10–6.15 (m, 1H, H-5′), 5.78 (ddd, 1H, *J* = 4.5, 1.0, 1.0 Hz, H-3), 5.76 (d, 1H, *J* = 1.0 Hz, H-1), 5.61 (dd, 1H, *J* = 1.0, 1.0 Hz, H-2), 5.61 (br d, 1H, *J* = 4.5 Hz, H-3′), 5.47 (d, 1H, *J* = 1.0 Hz, H-2′), 5.36 (s, 1H, H-1′), 4.91 (dd, 1H, *J* = 4.5, 3.5 Hz, H-4′), 4.84 (dd, 1H, *J* = 6.5, 4.5 Hz, H-4), 4.76 (dd, 1H, *J* = 11.5, 7.5 Hz, H-6a′), 4.65–4.72 (m, 2H, H-6b′, H-5), 4.19 (dd, 1H, *J* = 11.0, 6.0 Hz, H-6a), 4.08 (dd, 1H, *J* = 11.0, 6.0 Hz, H-6b), 2.27 (s, 3H, SPhC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.7(4) (C=O), 165.6(6) (C=O), 165.3 (C=O), 165.2 (2C, C=O), 138.3, 133.6, 133.5, 133.4, 133.3, 133.2, 133.0(3), 133.0(0), 129.9(6), 129.8(9), 129.8(2), 129.7(7), 129.5(7), 129.5(1), 128.9(5), 128.9(1), 128.8(8), 128.8(1), 128.6(4), 128.6(0), 128.4(7),

128.4(4), 128.3(8), 128.3(1) (42C, Ar), 105.7 (C-1′), 92.1 (C-1), 84.0 (C-4), 82.3 (C-2), 82.2 (C-2′), 81.9 (C-4′), 79.9 (C-3), 77.6 (C-3′), 70.3 (C-5′), 69.6 (C-6), 63.5 (C-6′), 28.7 (C-5), 21.1 (SPh*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{61}H_{51}IO_{15}S$: 1205.1886. Found: 1205.1905.

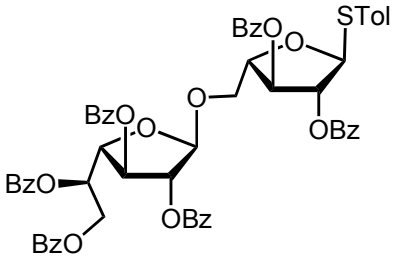
_p_-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-1-thio-α-L-*arabino*-hexofuranoside (6.40)



Disaccharide **6.39** (467 mg, 0.39 mmol) was dissolved in EtOAc (13 mL) and 20% $Pd(OH)_2$ (230 mg) and *i*-Pr_2EtN (135 μL, 0.78 mmol) were added. The reduction was performed as described for the synthesis of **6.29**. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.40** as a colorless oil (348 mg, 83%): R_f 0.27 (3:1 hexane–EtOAc); $[\alpha]_D$ = –48.9 (*c* 1.2, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.94–8.10 (m, 10H, ArH), 7.82–7.86 (m, 2H, ArH), 7.24–7.60 (m, 20H, ArH), 7.10 (app d, 2H, *J* = 7.8 Hz, ArH), 6.08 (ddd, 1H, *J* = 7.8, 4.2, 4.2 Hz, H-5′), 5.71 (br s, 1H, H-1), 5.65 (dd, 1H, *J* = 1.2, 1.2 Hz, H-2), 5.58 (br d, 1H, *J* = 4.8 Hz, H-3′), 5.48 (dd, 1H, *J* = 5.4, 1.2 Hz, H-3), 5.46 (d, 1H, *J* = 0.6 Hz, H-2′), 5.33 (br s, 1H, H-1′), 4.68–4.76 (m, 3H, H-4′, H-6a′, H-4), 4.60 (dd, 1H, *J* = 12.0, 4.2 Hz, H-6b′), 4.03 (dd, 1H, *J* = 10.5, 4.5, 4.5 Hz, H-6a), 3.76 (dd, 1H, *J* = 10.5, 5.0, 5.0 Hz, H-6b), 2.30–2.38 (m, 1H, H-5a), 2.24 (s, 3H, SPhCH_3), 2.14–2.22 (m, 1H, H-5b); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O),

165.8 (C=O), 165.7 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 137.8, 133.6, 133.4, 133.3(3), 133.3(1), 133.2(6), 133.2(3), 133.1, 133.0, 132.2, 130.3, 130.0(2), 130.9(8), 129.8(8), 129.8(5) 129.8(1), 129.7, 129.6, 129.2, 129.1, 128.9(9), 128.9(5), 128.6, 128.5(7), 128.5(2), 128.4(6), 128.3(6), 128.2(9) (42C, Ar), 105.6 (C-1′), 91.5 (C-1), 82.5 (C-2), 82.1 (C-2′), 81.6 (C-4′), 80.4 (C-4), 80.2 (C-3), 77.7 (C-3′), 70.3 (C-5′), 63.9 (C-6′), 63.4 (C-6), 32.8 (C-5), 21.0 (SPh*C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{61}H_{52}O_{15}S$: 1079.2919. Found: 1079.2914.

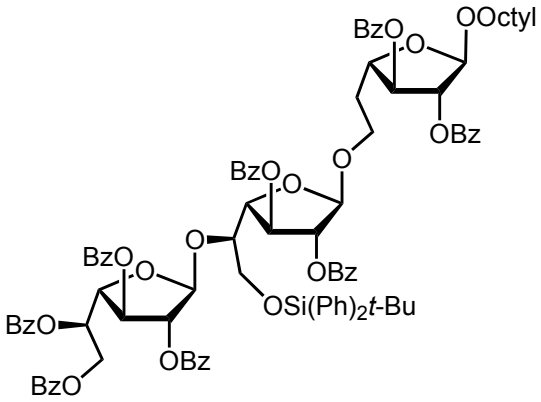
_p_-Tolyl 2,3,5,6-tetra-_O_-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-_O_-benzoyl-1-thio-α-L-arabinofuranoside (6.42)



The reaction was performed as described for the synthesis of **6.33**. TMSOTf (11 μL, 0.060 mmol) was added to a solution of trichloroacetimidate **6.17**[11] (455 mg, 0.60 mmol), thioglycoside acceptor **6.20** (260 mg, 0.56 mmol) and powdered 4 Å molecular sieves (437 mg) in CH_2Cl_2 (15 mL). The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.42** as a white amorphous solid (404 mg, 69%): R_f 0.27 (3:1 hexane–EtOAc); $[\alpha]_D$ = –42.6 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.06–8.14 (m, 4H, ArH), 7.88–8.02 (m, 8H, ArH), 7.22–7.64 (m, 20H, ArH), 7.10 (app d, 2H, *J* = 8.0 Hz, ArH), 6.08 (ddd, 1H, *J* = 6.8, 3.9, 3.6 Hz, H-5′), 5.73–5.77 (m, 3H, H-1, H-2, H-3), 5.63 (br d, 1H, *J* = 5.0 Hz, H-3′), 5.60 (d, 1H, *J* = 1.0

Hz, H-2′), 5.48 (s, 1H, H-1′), 4.89 (dd, 1H, *J* = 5.0, 3.6 Hz, H-4′), 4.70–4.80 (m, 3H, H-6a′, H-6b′, H-4), 4.28 (dd, 1H, *J* = 11.3, 4.5 Hz, H-5a), 4.01 (dd, 1H, *J* = 11.3, 2.7 Hz, H-5b), 2.30 (s, 3H, SPhCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.8 (C=O), 165.6 (2C, C=O), 165.3 (C=O), 165.2 (C=O), 138.0, 133.6, 133.5, 133.3(4), 133.2(8), 133.1(9), 133.0, 132.7, 130.0, 130.9(9), 129.9(3), 129.8(6), 129.8(3), 129.8(0), 129.7, 129.5(9), 129.5(7), 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3(8), 128.3(0), 128.2(9) (42C, Ar), 105.7 (C-1′), 91.5 (C-1), 82.0 (C-2′/C-2/C-4), 82.0 (C-2′/C-2/C-4), 81.9 (C-2′/C-2/C-4), 81.6 (C-4′), 77.6 (C-3′), 77.4 (C-3), 70.4 (C-5′), 65.8 (C-5), 63.8 (C-6′), 21.1 (SPh*C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{60}H_{50}O_{15}S$: 1065.2763. Found: 1065.2761.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranoside (6.43)

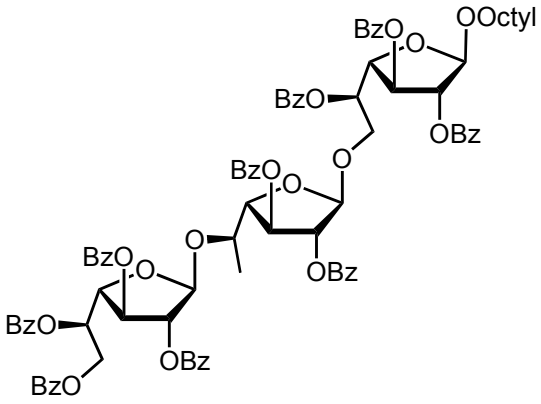


Disaccharide donor **6.33** (100 mg, 0.076 mmol), monosaccharide acceptor **6.24** (32 mg, 0.054 mmol) and powdered 4 Å molecular sieves (100 mg) were dried overnight under vacuum with P_2O_5. Dry CH_2Cl_2 (4 mL) and were added and the solution was cooled to –15 °C before the addition of *N*-iodosuccinimide (20 mg, 0.084 mmol) and

TMSOTf (4 μL, 0.020 mmol). The mixture was stirred for 30 min and neutralized by the addition of triethylamine, before being filtered through Celite and concentrated. The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.43** as a pale yellow oil (107 mg, quant.): R_f 0.26 (3:1 hexane–EtOAc); $[\alpha]_D$ = –14.8 (*c* 0.8, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.06–8.10 (m, 2H, ArH), 7.98–8.05 (m, 6H, ArH), 7.91–7.95 (m, 2H, ArH), 7.83–7.90 (m, 6H, ArH), 7.62–7.67 (m, 4H, ArH), 7.24–7.60 (m, 26H, ArH), 7.22 (app t, 2H, *J* = 7.8 Hz, ArH), 7.17 (app t, 2H, *J* = 7.8 Hz, ArH), 6.06 (ddd, 1H, *J* = 7.6, 3.7, 3.7 Hz, H-5′′), 5.78 (dd, 1H, *J* = 5.6, 1.8 Hz, H-3′), 5.70 (s, 1H, H-1′′), 5.65 (d, 1H, *J* = 1.3 Hz, H-2′′), 5.62 (br d, 1H, *J* = 5.3 Hz, H-3′′), 5.46 (d, 1H, *J* = 1.8 Hz, H-2′), 5.43 (d, 1H, *J* = 1.4 Hz, H-2), 5.34 (br d, 1H, *J* = 4.4 Hz, H-3), 5.20 (s, 1H, H-1′), 5.13 (s, 1H, H-1), 5.07 (dd, 1H, *J* = 5.3, 3.7 Hz, H-4′′), 4.81 (dd, 1H, *J* = 12.1, 3.7 Hz, H-6a′′), 4.72 (dd, 1H, *J* = 12.1, 7.6 Hz, H-6b′′), 4.55 (dd, 1H, *J* = 5.6, 3.7 Hz, H-4′), 4.42–4.45 (m, 1H, H-5′), 4.38 (ddd, 1H, *J* = 8.7, 4.6, 4.4 Hz, H-4), 4.08 (dd, 1H, *J* = 10.8, 6.6 Hz, H-6a′), 3.99 (dd, 1H, *J* = 10.8, 5.4 Hz, H-6b′), 3.92 (ddd, 1H, *J* = 10.0, 6.9, 6.9 Hz, H-6a), 3.71 (ddd, 1H, *J* = 10.0, 6.3, 6.3 Hz, H-6b), 3.67 (dt, 1H, *J* = 9.4, 6.6 Hz, octyl OCH_2), 3.41 (dt, 1H, *J* = 9.4, 6.5 Hz, octyl OCH_2), 2.19–2.27 (m, 1H, H-5a), 2.07–2.16 (m, 1H, H-5b), 1.50–1.60 (m, 2H, octyl OCH_2CH_2), 1.18–1.40 (m, 10H, octyl CH_2), 0.97 (s, 9H, $C(CH_3)_3$), 0.86 (t, 3H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.6(9) (C=O), 165.6(5) (C=O), 165.5(8) (C=O), 165.5(1) (C=O), 165.3(9) (C=O), 165.3(7) (C=O),

165.1 (C=O), 135.5(5), 135.5(2), 133.4, 133.2(3), 133.2(0), 133.1(6), 133.1(1), 133.0, 132.9, 132.8, 129.9(2), 129.9(1), 129.9(0), 129.8(5), 129.8(3), 129.8(0), 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.5, 128.3(9), 128.3(7), 128.3(3), 128.2(8), 128.2(5), 128.2(0), 128.1, 127.8, 127.7 (60C, Ar), 105.4(0) (C-1′/C-1), 105.3(8) (C-1′/C-1), 105.0 (C-1′′), 82.2 (C2′′/C-2′/C-2), 82.0 (2C, C2′′/C-2′/C-2), 81.7 (C-4′′), 81.6 (C-4′), 80.5 (C-3), 80.0 (C-4), 77.9 (C-3′′), 77.0 (C-3′), 74.9 (C-5′), 70.6 (C-5′′), 67.4 (octyl O*C*H_2), 63.9 (C-6′′), 63.8 (C-6), 63.7 (C-6′), 33.4 (C-5), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.7 (C(*C*H_3)$_3$), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 19.3 (*C*(CH$_3$)$_3$), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{98}H_{98}O_{23}Si$: 1693.6160. Found: 1693.6164.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (6.44)

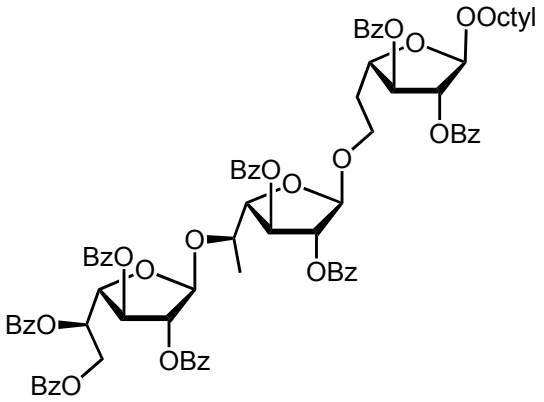


The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (26 mg, 0.11 mmol) and TMSOTf (5 μL, 0.026 mmol) were added to a solution of disaccharide donor **6.36** (107 mg, 0.10 mmol), monosaccharide acceptor **6.21** (53 mg, 0.088

mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.44** as a pale yellow oil (132 mg, 97%): R_f 0.23 (3:1 hexane–EtOAc); $[\alpha]_D$ = –17.6 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.76–8.10 (m, 18H, ArH), 7.22–7.56 (m, 25H, ArH), 7.12 (app t, 2H, *J* = 8.0 Hz, ArH), 6.07 (ddd, 1H, *J* = 7.6, 3.7, 3.4 Hz, H-5′′), 5.86–5.92 (m, 1H, H-5), 5.77 (br d, 1H, *J* = 4.7 Hz, H-3′), 5.57–5.62 (m, 3H, H-2′′, H-3′′, H-3), 5.54 (s, 1H, H-1′′), 5.44 (br s, 2H, H-2′, H-2), 5.28 (s, 1H, H-1), 5.26 (s, 1H, H-1′), 5.04 (dd, 1H, *J* = 5.6, 3.4 Hz, H-4′′), 4.82 (dd, 1H, *J* = 12.1, 3.7 Hz, H-6a′′), 4.75 (dd, 1H, *J* = 12.1, 7.6 Hz, H-6b′′), 4.68 (dd, 1H, *J* = 5.0, 3.9 Hz, H-4), 4.34–4.42 (m, 2H, H-4′, H-5′), 4.17 (dd, 1H, *J* = 10.8, 5.5 Hz, H-6a), 4.03 (dd, 1H, *J* = 10.8, 6.8 Hz, H-6b), 3.75 (dt, 1H, *J* = 9.7, 6.8 Hz, octyl OC*H*$_2$), 3.52 (dt, 1H, *J* = 9.7, 6.3 Hz, octyl OC*H*$_2$), 1.54–1.64 (m, 2H, octyl OCH_2CH_2), 1.38 (d, 3H, *J* = 6.0 Hz, H-6′), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.5 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.8(3) (C=O), 165.7(7) (C=O), 165.6(4) (C=O), 165.6(1) (C=O), 165.5(8) (C=O), 165.4(8) (C=O), 165.3 (C=O), 165.2 (C=O), 133.3, 133.2, 133.0(7), 133.0(1), 132.9, 129.9(7), 129.9(4), 129.9(3), 129.9(1), 129.8(5), 129.8(1), 129.7(6), 129.7(0), 129.6(9), 129.6(5), 129.2, 129.1, 128.9, 128.8, 128.3(9), 128.3(6), 128.3(2), 128.2, 128.1 (54C, Ar), 105.9 (C-1′/C-1), 105.7 (C-1′/C-1), 102.6 (C-1′′), 85.6 (C-4′), 82.3 (C2′′/C-2′/C-2), 82.2 (C2′′/C-2′/C-2), 81.8 (C2′′/C-2′/C-2), 81.5 (C-4′′), 81.2 (C-4), 78.0 (C-3′′/C-3), 77.6 (C-3′′/C-3), 76.9 (C-3′), 71.5 (C-5), 70.5 (C-5′′), 69.2 (C-5′), 67.8

(octyl O*C*H_2), 66.1 (C-6), 64.0 (C-6′′), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.1 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.6 (C-6′), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{89}H_{84}O_{24}$: 1559.5245. Found: 1559.5234.

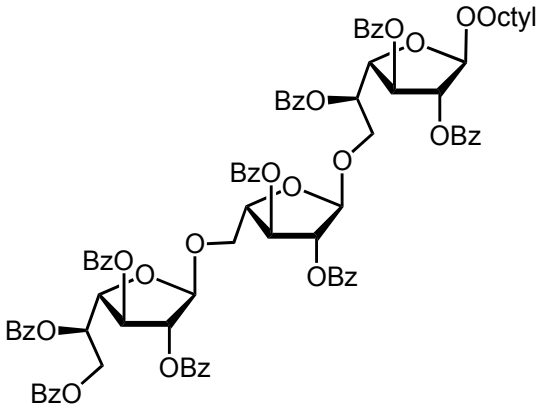
Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranoside (6.45)



The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (19 mg, 0.081 mmol) and TMSOTf (3.5 μL, 0.020 mmol) were added to a solution of the disaccharide donor **6.36** (79 mg, 0.075 mmol), monosaccharide acceptor **6.24** (32 mg, 0.065 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.45** as a pale yellow amorphous solid (79 mg, 85%): R_f 0.25 (3:1 hexane–EtOAc); $[\alpha]_D$ = –8.9 (*c* 1.7, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.05–8.10 (m, 4H, ArH), 7.97–8.03 (m, 4H, ArH), 7.89–7.96 (m, 6H, ArH), 7.81–7.85 (m, 2H, ArH), 7.25–7.60 (m, 22H, ArH), 7.15 (app t, 2H, *J* = 8.4 Hz, ArH), 6.09 (ddd, 1H, *J* = 7.6, 3.6, 3.4 Hz, H-5′′), 5.76 (dd, 1H, *J* = 5.2, 1.4 Hz, H-3′), 5.61 (d, 1H, *J* = 1.2 Hz, H-2′′), 5.60 (dd, 1H, *J* = 5.4, 1.2 Hz,

H-3′′), 5.56 (s, 1H, H-1′′), 5.48 (d, 1H, *J* = 1.4 Hz, H-2′), 5.45 (d, 1H, *J* = 1.5 Hz, H-2), 5.37 (dd, 1H, *J* = 4.7, 1.5 Hz, H-3), 5.21 (s, 1H, H-1′), 5.18 (s, 1H, H-1), 5.04 (dd, 1H, *J* = 5.4, 3.4 Hz, H-4′′), 4.82 (dd, 1H, *J* = 12.1, 3.6 Hz, H-6a′′), 4.75 (dd, 1H, *J* = 12.1, 7.6 Hz, H-6b′′), 4.38–4.47 (m, 2H, H-5′, H-4), 4.35 (dd, 1H, *J* = 5.2, 4.1 Hz, H-4′), 3.98 (ddd, 1H, *J* = 10.0, 7.3, 6.2 Hz, H-6a), 3.74 (ddd, 1H, *J* = 10.0, 5.9, 5.9 Hz, H-6b), 3.72 (dt, 1H, *J* = 9.6, 6.7 Hz, octyl OC*H*$_2$), 3.47 (dt, 1H, *J* = 9.6, 6.5 Hz, octyl OC*H*$_2$), 2.25–2.34 (m, 1H, H-5a), 2.11–2.19 (m, 1H, H-5b), 1.52–1.62 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.41 (d, 3H, *J* = 6.6 Hz, H-6′), 1.16–1.38 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 6.6 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.7(7) (C=O), 165.7(1) (C=O), 165.6(6) (C=O), 165.6(1) (C=O), 165.3(8) (C=O), 165.3(4) (C=O), 165.2(8) (C=O), 133.4, 133.2(8), 133.2(6), 133.2, 133.1, 132.9, 130.0, 129.8(8), 129.8(4), 129.7(9), 129.7(3), 129.7(2), 129.7(0), 129.6(5), 129.6(4), 129.4, 129.3, 129.2, 129.1, 128.8, 128.5, 128.4(3), 128.4(0), 128.3(6), 128.3(3), 128.2, 128.1 (48C, Ar), 105.4(4) (C-1′/C-1), 105.4(1) (C-1′/C-1), 102.6 (C-1′′), 85.2 (C-4′), 82.3 (C-2′′), 82.1 (C-2), 82.0 (C-2′), 81.5 (C-4′′), 80.6 (C-3), 80.0 (C-4), 78.1 (C-3′′), 77.0 (C-3′), 70.5 (C-5′′), 69.4 (C-5′), 67.5 (octyl O*C*H$_2$), 64.0 (C-6′′), 63.8 (C-6), 33.6 (C-5), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.2 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.7 (C-6′), 14.1 (octyl *C*H$_3$). MALDI-TOFMS calcd. for (M + Na) $C_{82}H_{80}O_{22}$: 1439.5034. Found: 1439.5031.

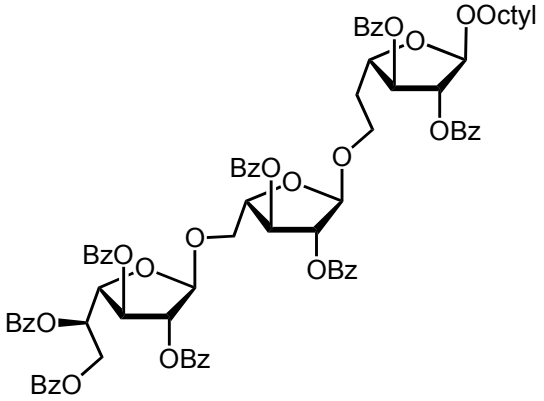
Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (6.46)



The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (20 mg, 0.085 mmol) and TMSOTf (4 μL, 0.020 mmol) were added to a solution of disaccharide donor **6.42** (82 mg, 0.076 mmol), monosaccharide acceptor **6.21** (41 mg, 0.066 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.46** as a pale amorphous solid (91 mg, 88%): R_f 0.46 (2:1 hexane–EtOAc); $[\alpha]_D$ = –14.5 (*c* 2.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.99–8.09 (m, 8H, ArH), 7.84–7.97 (m, 10H, ArH), 7.18–7.56 (m, 27H, ArH), 6.06 (ddd, 1H, *J* = 7.3, 3.9, 3.8 Hz, H-5′′), 5.84–5.89 (m, 1H, H-5), 5.64 (br d, 1H, *J* = 5.5 Hz, H-3′), 5.63 (br d, 1H, *J* = 4.9 Hz, H-3), 5.60 (br d, 1H, *J* = 5.1 Hz, H-3′′), 5.56 (d, 1H, *J* = 1.0 Hz, H-2′′), 5.48 (d, 1H, *J* = 1.3 Hz, H-2′), 5.42–5.45 (m, 2H, H-1′′, H-2), 5.30 (s, 1H, H-1′), 5.27 (s, 1H, H-1), 4.87 (dd, 1H, *J* = 5.1, 3.8 Hz, H-4′′), 4.81 (dd, 1H, *J* = 12.0, 3.9 Hz, H-6a′′), 4.74 (dd, 1H, *J* = 12.0, 7.3 Hz, H-6b′′), 4.68 (dd, 1H, *J* = 4.9, 4.9 Hz, H-4), 4.49–4.54 (m, 1H, H-4′), 4.21 (dd, 1H, *J* = 11.3, 5.3 Hz, H-6a), 4.18 (dd, 1H, *J* = 11.3, 4.0 Hz, H-5a′), 4.05 (dd, 1H, *J* = 11.3, 6.5 Hz, H-6b), 3.85

(dd, 1H, *J* = 11.3, 2.2 Hz, H-5b′), 3.74 (dt, 1H, *J* = 9.5, 6.6 Hz, octyl OC*H*$_2$), 3.52 (dt, 1H, *J* = 9.5, 6.4 Hz, octyl OC*H*$_2$), 1.54–1.64 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.42 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.8(4) (C=O), 165.7(6) (2C, C=O), 165.6 (C=O), 165.5(5) (C=O), 165.4(8) (C=O), 165.2 (2C, C=O), 133.3, 133.2, 133.0(5), 132.9(7), 129.9, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0(7), 129.0(0), 128.9, 128.8, 128.4, 128.3, 128.2 (54C, Ar), 106.2 (C-1′), 105.7 (2C, C-1′′, C-1), 82.3 (C-4′), 82.2 (C-2), 81.8 (C-2′′), 81.7 (C-2′), 81.5 (C-4′′), 81.3 (C-4), 77.6 (C-3′′/C-3′/C-3), 77.5 (C-3′′/C-3′/C-3), 77.0 (C-3′′/C-3′/C-3), 71.5 (C-5), 70.5 (C-5′′), 67.8 (octyl O*C*H$_2$), 66.1 (C-6), 65.5 (C-5′), 63.8 (C-6′′), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{88}H_{82}O_{24}$: 1545.5088. Found: 1545.5080.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranoside (6.47)



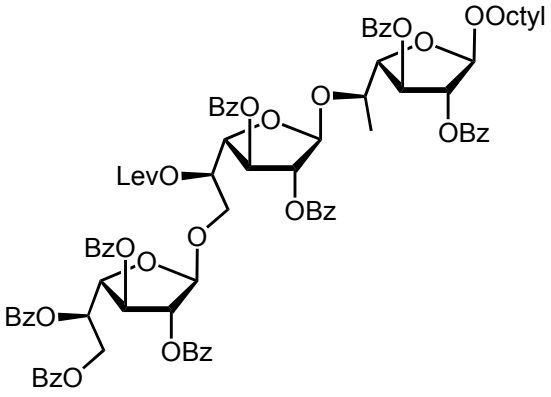
The reaction was performed as described for the synthesis of **6.43**, *N*-iodosuccinimide (16 mg, 0.067 mmol) and TMSOTf (3 μL, 0.016 mmol) were added to a

solution of disaccharide donor **6.42** (64 mg, 0.062 mmol), monosaccharide acceptor **6.24** (26 mg, 0.054 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.47** as a colorless oil (70 mg, 93%): R_f 0.30 (3:1 hexane–EtOAc); $[\alpha]_D$ = –5.6 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.87–8.11 (m, 16H, ArH), 7.25–7.60 (m, 22H, ArH), 7.22 (app t, 2H, *J* = 8.0 Hz, ArH), 6.08 (ddd, 1H, *J* = 7.3, 3.8, 3.8 Hz, H-5′′), 5.66 (br d, 1H, *J* = 5.1 Hz, H-3′), 5.62 (br d, 1H, *J* = 5.2 Hz, H-3′′), 5.60 (d, 1H, *J* = 0.6 Hz, H-2′′), 5.53 (d, 1H, *J* = 1.1 Hz, H-2′), 5.48 (s, 1H, H-1′′), 5.44 (d, 1H, *J* = 1.3 Hz, H-2), 5.38 (dd, 1H, *J* = 4.9, 1.3 Hz, H-3), 5.26 (s, 1H, H-1′), 5.18 (s, 1H, H-1), 4.90 (dd, 1H, *J* = 5.2, 3.8 Hz, H-4′′), 4.82 (dd, 1H, *J* = 12.0, 3.8 Hz, H-6a′′), 4.76 (dd, 1H, *J* = 12.0, 7.3 Hz, H-6b′′), 4.48–4.53 (m, 1H, H-4′), 4.45 (ddd, 1H, *J* = 8.8, 4.9, 4.7 Hz, H-4), 4.23 (dd, 1H, *J* = 11.2, 4.3 Hz, H-5a′), 4.01 (ddd, 1H, *J* = 9.9, 7.2, 7.2 Hz, H-6a), 3.95 (dd, 1H, *J* = 11.2, 2.4 Hz, H-5b′), 3.77 (ddd, 1H, *J* = 9.9, 6.0, 6.0 Hz, H-6b), 3.71 (dt, 1H, *J* = 9.5, 6.7 Hz, octyl OC*H*$_2$), 3.47 (dt, 1H, *J* = 9.5, 6.5 Hz, octyl OC*H*$_2$), 2.25–2.37 (m, 1H, H-5a), 2.12–2.23 (m, 1H, H-5b), 1.50–1.64 (m, 2H, octyl OCH_2CH_2), 1.16–1.40 (m, 10H, octyl C*H*$_2$), 0.85 (t, 3H, *J* = 7.0 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.8 (2C, C=O), 165.7 (C=O), 165.6 (C=O), 165.4 (C=O), 165.3 (C=O), 165.2 (C=O), 133.4, 133.3(4), 133.3(1), 133.2(8), 133.2(4), 133.1(9), 133.1(4), 133.0, 130.0, 129.8(5), 129.8(1), 129.7(6), 129.7(2), 129.7(0), 129.6, 129.5, 129.4, 129.3, 129.2, 129.0(2), 128.9(9), 128.8,

128.5, 128.4(3), 128.4(1), 128.3(6), 128.3(5), 128.2(7), 128.2(1) (48C, Ar), 105.6(7) (C-1′′/C-1′), 105.6(4) (C-1′′/C-1′), 105.4 (C-1), 82.1 (C-4′), 81.8(8) (C-2), 81.8(8) (C-2′′/C-2′), 81.8(2) (C-2′′/C-2′), 81.6 (C-4′′), 80.5 (C-3), 80.0 (C-4), 77.6 (C-3′′), 77.2 (C-3′), 70.5 (C-5′′), 67.5 (octyl O*C*H_2), 65.7 (C-5′), 63.8 (2C, C-6′′, C-6), 33.5 (C-5), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.2 (octyl *C*H_2), 26.1 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{81}H_{78}O_{22}$: 1425.4877. Found: 1425.4874.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-*O*-levulinoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranoside

(6.48)

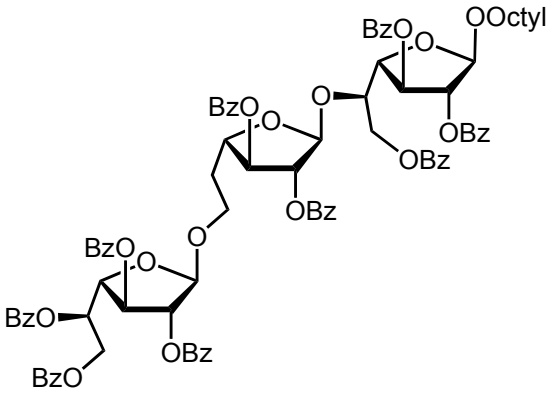


The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (21 mg, 0.091 mmol) and TMSOTf (4 μL, 0.023 mmol) were added to a solution of disaccharide donor **6.37** (95 mg, 0.081 mmol), monosaccharide acceptor **6.25** (37 mg, 0.076 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.48** as a pale yellow oil (111 mg, 95%): R_f 0.26 (2:1 hexane–EtOAc); $[\alpha]_D$ = –6.9 (*c* 2.2, CH_2Cl_2); ^{1}H

NMR (500 MHz, $CDCl_3$) δ_H 8.10–8.14 (m, 2H, ArH), 7.92–8.06 (m, 10H, ArH), 7.80–7.90 (m, 4H, ArH), 7.24–7.60 (m, 23H, ArH), 7.18 (app t, 1H, *J* = 7.5 Hz, ArH), 6.02–6.07 (m, 1H, H-5′′), 5.70–5.76 (m, 2H, H-5′, H-3), 5.61 (br d, 1H, *J* = 5.0 Hz, H-3′′), 5.59 (d, 1H, *J* = 1.4 Hz, H-2′), 5.52 (s, 1H, H-1′), 5.47 (dd, 1H, *J* = 5.0, 1.4 Hz, H-3′), 5.44 (d, 1H, *J* = 1.5 Hz, H-2), 5.43 (d, 1H, *J* = 1.2 Hz, H-2′′), 5.26 (s, 1H, H-1′′), 5.17 (s, 1H, H-1), 4.77 (dd, 1H, *J* = 5.0, 4.1 Hz, H-4′), 4.68–4.75 (m, 3H, H-4′′, H-6a′′, H-6b′′), 4.33–4.40 (m, 1H, H-5), 4.28 (dd, 1H, *J* = 5.1, 4.1 Hz, H-4), 4.11 (dd, 1H, *J* = 11.3, 3.8 Hz, H-6a′), 3.87 (dd, 1H, *J* = 11.3, 8.1 Hz, H-6b′), 3.71 (dt, 1H, *J* = 9.5, 6.7 Hz, octyl OC*H*$_2$), 3.48 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 2.44–2.68 (m, 4H, Lev CH_2), 1.99 (s, 3H, Lev CH_3), 1.54–1.65 (m, 2H, octyl OCH_2CH_2), 1.42 (d, 3H, *J* = 6.4 Hz, H-6), 1.18–1.42 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.1 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 205.1 (Lev ketone C=O), 172.1 (Lev ester C=O), 166.0 (C=O), 165.7 (C=O), 165.6(3) (C=O), 165.5(9) (C=O), 165.5 (C=O), 165.4 (C=O), 165.2(4) (C=O), 165.2(2) (C=O), 133.5, 133.3, 133.2(9), 133.2(5), 133.1, 132.9, 130.0, 129.9(4), 129.9(0), 129.8, 129.7(8), 129.7(6), 129.7(2), 129.6, 129.5, 129.4, 129.3, 129.1, 129.0, 128.9(8), 128.9(3), 128.5(2), 128.4(6), 128.3(9), 128.3(5), 128.3(2), 128.2(9), 128.2 (48C, Ar), 106.6 (C-1′′), 105.3 (C-1), 102.8 (C-1′), 85.0 (C-4), 82.0(3) (C-2′′), 81.9(7) (C-2′/C-2), 81.9(3) (C-2′/C-2), 81.6 (2C, C-4′′, C-4′), 77.7 (C-3′), 77.4 (2C, C-3′′, C-3), 71.0 (C-5′), 70.4 (C-5′′), 69.8 (C-5), 67.4 (octyl O*C*H_2), 67.3 (C-6′), 63.7 (C-6′′), 38.0 (2C, Lev CH_2), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.5

(Lev CH_3), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.6 (octyl CH_2), 14.9 (C-6), 14.1 (octyl CH_3). MALDI-TOFMS calcd. for (M + Na) $C_{87}H_{86}O_{25}$: 1553.5350. Found: 1553.5356.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (6.49)

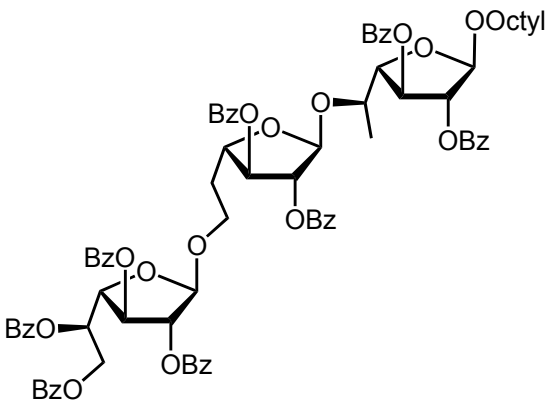


The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (34 mg, 0.14 mmol) and TMSOTf (6 μL, 0.033 mmol) were added to a solution of disaccharide donor **6.40** (139 mg, 0.13 mmol), monosaccharide acceptor **6.22** (69 mg, 0.11 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (3:1 hexane–EtOAc) to give **6.49** as a pale yellow amorphous solid (142 mg, 81%): R_f 0.20 (3:1 hexane–EtOAc); $[\alpha]_D$ = +11.7 (*c* 2.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.92–8.06 (m, 14H, ArH), 7.81–7.85 (m, 4H, ArH), 7.19–7.57 (m, 27H, ArH), 6.08 (ddd, 1H, *J* = 6.5, 5.1, 3.5 Hz, H-5″), 5.79 (dd, 1H, *J* = 5.4, 1.8 Hz, H-3), 5.68 (s, 1H, H-1′), 5.65 (dd, 1H, *J* = 1.6 Hz, H-2′), 5.58 (br d, 1H, *J* = 5.5 Hz, H-3″), 5.50 (dd, 1H, *J* = 2.6, 1.6 Hz, H-3′), 5.47 (dd, 1H, *J* = 1.8, 0.6 Hz, H-2), 5.41 (d, 1H, *J* = 1.2 Hz, H-2″), 5.22 (s, 1H, H-1″), 5.20

(s, 1H, H-1), 4.68–4.77 (m, 5H, H-4′′, H-6a′′, H-6b′′, H-6a, H-6b), 4.61–4.66 (m, 2H, H-4′, H-5), 4.53 (dd, 1H, *J* = 5.4, 3.4 Hz, H-4), 3.90 (ddd, 1H, *J* = 10.2, 6.6, 6.6 Hz, H-6a′), 3.78 (ddd, 1H, *J* = 10.2, 6.6, 6.6 Hz, H-6b′), 3.71 (dt, 1H, *J* = 9.6, 6.7 Hz, octyl OC*H*$_2$), 3.47 (dt, 1H, *J* = 9.6, 6.7 Hz, octyl OC*H*$_2$), 2.20 (ddd, 2H, *J* = 6.6, 6.6, 6.6 Hz, H-5a′, H-5b′), 1.54–1.65 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.40 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.2 (C=O), 166.1 (C=O), 165.7 (C=O), 165.6(2) (C=O), 165.5(9) (C=O), 165.5(6) (C=O), 165.4(9) (C=O), 165.3 (C=O), 165.1 (C=O), 133.4, 133.3, 133.2(5), 133.2(4), 133.1, 133.0(7), 132.9(6), 132.9, 129.9, 129.8(6), 129.8(3), 129.7(9), 129.7(2), 129.6(7), 129.5(8), 129.2, 129.1(5), 129.1(3), 129.0, 128.9, 128.5(0), 128.4(9), 128.3, 128.2(9), 128.2(3), 128.1 (54C, Ar), 106.0 (C-1′′), 105.6 (C-1′), 105.5 (C-1), 82.1 (C-2′′), 82.0 (C-2′), 81.9 (2C, C-2, C-4), 81.5 (C-4′), 81.4 (C-4′′), 80.0 (C-3′), 77.7 (C-3′′), 77.0 (C-3), 73.2 (C-5), 70.4 (C-5′′), 67.6 (octyl O*C*H$_2$), 64.8 (C-6′′/C-6′/C-6), 64.6 (C-6′′/C-6′/C-6), 63.8 (C-6′′/C-6′/C-6), 33.1 (C-5′), 31.8 (octyl *C*H$_2$), 29.5 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). MALDI-TOFMS calcd. for (M + Na) $C_{89}H_{84}O_{24}$: 1559.5245. Found: 1559.5236.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-2,3-di-*O*-benzoyl-6-deoxy-β-D-galactofuranoside (6.50)

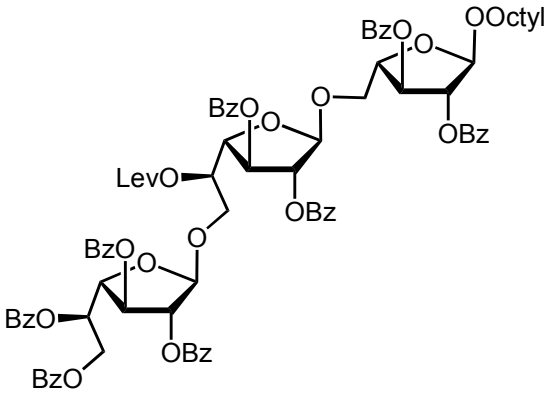
The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (22 mg, 0.094 mmol) and TMSOTf (4 μL, 0.023 mmol) were added to a solution of disaccharide donor



6.40 (92 mg, 0.087 mmol), monosaccharide acceptor **6.25** (39 mg, 0.075 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.50** as a pale yellow oil (90 mg, 80%): R_f 0.46 (2:1 hexane–EtOAc); $[\alpha]_D$ = +12.8 (*c* 2.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.93–8.13 (m, 12H, ArH), 7.81–7.86 (m, 4H, ArH), 7.24–7.60 (m, 23H, ArH), 7.19 (app t, 1H, *J* = 8.0 Hz, Ar-H), 6.08 (ddd, 1H, *J* = 5.8, 3.5, 3.5 Hz, H-5′′), 5.71 (br d, 1H, *J* = 5.0 Hz, H-3), 5.57–5.61 (m, 2H, H-3′′, H-2′), 5.22 (s, 1H, H-1′), 5.45 (br d, 1H, *J* = 6.5 Hz, H-3′), 5.44 (br s, 2H, H-2′′, H-2), 5.28 (s, 1H, H-1′′), 5.16 (s, 1H, H-1), 4.76 (dd, 1H, *J* = 5.1, 3.5 Hz, H-4′′), 4.74 (d, 2H, *J* = 5.8 Hz, H-6a′′, H-6b′′), 4.62 (app. q, 1H, *J* = 6.5 Hz, H-4′), 4.32–4.40 (m, 1H, H-5), 4.30 (dd, 1H, *J* = 5.0, 5.0 Hz, H-4), 3.95 (app. dt, 1H, *J* = 10.3, 6.5 Hz, H-6a′), 3.85 (app. dt, 1H, *J* = 10.3, 6.5 Hz, H-6b′), 3.71 (dt, 1H, *J* = 9.6, 6.8 Hz, octyl OCH_2), 3.47 (dt, 1H, *J* = 9.6, 6.4 Hz, octyl OCH_2), 2.25 app. q, 2H, *J* = 6.5 Hz, H-5a′, H-5b′), 1.52–1.64 (m, 2H, octyl OCH_2CH_2), 1.42 (d, 3H, *J* = 7.2 Hz, H-6), 1.18–1.42 (m, 10H, octyl CH_2), 0.85 (t, 3H, *J* = 6.5 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.7

(C=O), 165.6(9) (C=O), 165.6(3) (C=O), 165.5 (C=O), 165.4 (C=O), 165.2(7) (C=O), (X) 165.2(6) (C=O), 133.4, 133.3(0), 133.2(6), 133.2(3), 133.1, 133.0(0), 132.9(7), 132.9(5), 129.8(9), 129.8(3), 129.8(0), 129.7(5), 129.7(3), 129.6(4), 129.5(7), 129.4, 129.3, 129.2(2), 129.1(6), 128.9(9), 128.9(7), 128.5(3), 128.4(6), 128.3, 128.2(9), 128.2(3), 128.1 (48C, Ar), 106.0 (C-1′), 105.4 (C-1), 102.9 (C-1′′), 85.0 (C-4), 82.3 (C-2′), 82.1 (C-2′′/C-2), 82.0 (C-2′′/C-2), 81.4 (C-4′′), 80.9 (C-4′), 80.6 (C-3′), 77.7 (C-3′′), 77.0 (C-3), 70.4 (C-5′′), 69.9 (C-5), 67.4 (octyl O*C*H_2), 64.7 (C-6′), 63.7 (C-6′′), 33.2 (C-5′), 31.8 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 15.0 (C-6), 14.1 (octyl *C*H_3). HRMS (ESI) calcd. for (M + Na) $C_{82}H_{80}O_{22}$: 1439.5034. Found: 1439.5036.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-*O*-levulinoyl-β-D-galactofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (6.51)

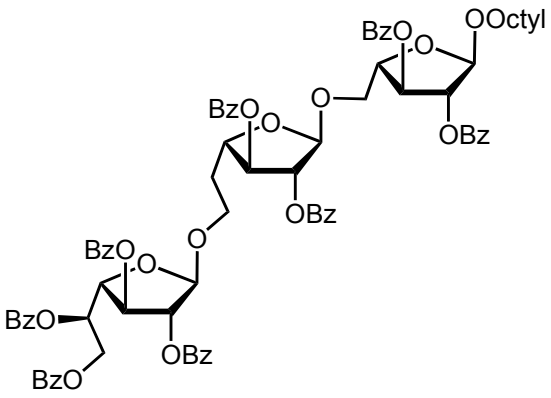


The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (22 mg, 0.091 mmol) and TMSOTf (4 μL, 0.022 mmol) were added to a solution of disaccharide donor **6.37** (99 mg, 0.084 mmol), monosaccharide acceptor **6.20** (35 mg, 0.073 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by

chromatography (2:1 hexane–EtOAc) to give **6.51** as a pale yellow amorphous solid (110 mg, 99%): R_f 0.23 (2:1 hexane–EtOAc); $[\alpha]_D$ = –5.0 (*c* 1.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.90–8.12 (m, 14H, ArH), 7.82 (app d, 2H, *J* = 8.0 Hz, ArH), 7.20–7.60 (m, 24H, ArH), 6.08 (ddd, 1H, *J* = 3.5, 3.5, 3.5 Hz, H-5′′), 5.69 (ddd, 1H, *J* = 7.4, 4.4, 4.3 Hz, H-5′), 5.57–5.64 (m, 3H, H-3′′, H-2′, H-3), 5.46–5.52 (m, 2H, H-2′′, H-3′), 5.45 (s, 1H, H-1′), 5.43 (s, 1H, H-2), 5.31 (s, 1H, H-1′′), 5.22 (s, 1H, H-1), 4.70–4.80 (m, 3H, H-4′′, H-6a′′, H-6b′′), 4.69 (dd, 1H, *J* = 4.3, 4.3 Hz, H-4′), 4.42 (dd, 1H, *J* = 4.6, 2.7 Hz, H-4), 4.18 (dd, 1H, *J* = 11.3, 4.6 Hz, H-5a), 4.13 (dd, 1H, *J* = 10.8, 4.4 Hz, H-6a′), 3.96 (dd, 1H, *J* = 11.3, 2.7 Hz, H-5b), 3.88 (dd, 1H, *J* = 10.8, 7.4 Hz, H-6b′), 3.74 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 3.49 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 2.44–2.72 (m, 4H, Lev CH_2), 2.02 (s, 3H, Lev CH_3), 1.54–1.68 (m, 2H, octyl OCH_2CH_2), 1.18–1.46 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 6.5 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 205.9 (Lev ketone C=O), 172.1 (Lev ester C=O), 166.0 (C=O), 165.7 (C=O), 165.6(5) (C=O), 165.6(1) (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 165.1 (C=O), 133.5, 133.3(6), 133.3(2), 133.3(0), 133.2, 133.1(7), 133.1(1), 132.9(5), 129.9(7), 129.9(5), 129.8(9), 129.8(7), 129.7(9), 129.7(6), 129.7(1), 129.6, 129.5, 129.4, 129.2, 129.1, 129.0, 128.9(7), 128.9(2), 128.5, 128.4, 128.3(8), 128.3(3), 128.2(8), 128.2(5) (48C, Ar), 106.3 (C-1′′), 105.9 (C-1), 105.6 (C-1′), 82.0, 81.8, 81.7(5), 81.6(9), 81.6(7), 81.5 (C2′′, C-4′′, C-2′, C-4′, C-2, C-4), 77.4 (3C, C-3′′, C-3′, C-3), 71.0 (C-5′), 70.4 (C-5′′), 67.5 (octyl O*C*H_2), 66.6 (C-6′), 66.2 (C-

5), 63.7 (C-6′′), 38.0 (2C, Lev CH_2), 31.8 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.4 (Lev CH_3), 29.3 (octyl *C*H_2), 28.1 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.6 (octyl *C*H_2), 14.1 (octyl *C*H_3). MALDI-TOFMS calcd. for (M + Na) $C_{86}H_{84}O_{25}$: 1539.5194. Found: 1539.5183.

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3-di-*O*-benzoyl-5-deoxy-α-L-*arabino*-hexofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (6.52)



The reaction was performed as described for the synthesis of **6.43**. *N*-iodosuccinimide (30 mg, 0.13 mmol) and TMSOTf (5 μL, 0.026 mmol) were added to a solution of disaccharide donor **6.40** (124 mg, 0.12 mmol), monosaccharide acceptor **6.20** (48 mg, 0.10 mmol) and powdered 4 Å molecular sieves (100 mg) in CH_2Cl_2 (4 mL). The crude product was purified by chromatography (2:1 hexane–EtOAc) to give **6.52** as a pale yellow oil (116 mg, 81%): R_f 0.50 (2:1 hexane–EtOAc); $[\alpha]_D$ = –8.5 (*c* 2.6, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 7.94–8.10 (m, 12H, ArH), 7.87–7.91 (m, 2H, ArH), 7.82–7.86 (m, 2H, ArH), 7.22–7.59 (m, 24H, ArH), 6.08–6.12 (m, 1H, H-5′′), 5.58–5.62 (m, 2H, H-3′′, H-2′, H-3), 5.50 (d, 1H, *J* = 1.3 Hz, H-2′′), 5.44 (d, 1H, *J* = 1.1 Hz, H-2), 5.42 (br d, 1H, *J* = 4.6 Hz, H-3′), 5.40 (s, 1H, H-1′), 5.33 (s, 1H, H-1′′), 5.21 (s, 1H, H-

1), 4.79 (dd, 1H, *J* = 5.2, 3.3 Hz, H-4′′), 4.72–4.79 (m, 2H, H-6a′′, H-6b′′), 4.55 (ddd, 1H, *J* = 7.8, 7.8, 4.6 Hz, H-4′), 4.42 (ddd, 1H, *J* = 4.6, 4.5, 3.0 Hz, H-4), 4.17 (dd, 1H, *J* = 11.2, 4.5 Hz, H-5a), 4.01 (ddd, 1H, *J* = 10.1, 6.6, 6.6 Hz, H-6a′), 3.94 (dd, 1H, *J* = 11.2, 3.0 Hz, H-5b), 3.84 (ddd, 1H, *J* = 10.1, 6.4, 6.4 Hz, H-6b′), 3.74 (dt, 1H, *J* = 9.5, 6.8 Hz, octyl OC*H*$_2$), 3.49 (dt, 1H, *J* = 9.5, 6.3 Hz, octyl OC*H*$_2$), 2.19–2.34 (m, 2H, H-5a′, H-5b′), 1.54–1.68 (m, 2H, octyl OCH$_2$C*H*$_2$), 1.18–1.44 (m, 10H, octyl C*H*$_2$), 0.86 (t, 3H, *J* = 7.2 Hz, octyl C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1 (C=O), 165.7 (C=O), 165.6(6) (C=O), 165.6(4) (C=O), 165.6(1) (C=O), 165.4 (C=O), 165.3 (C=O), 165.1 (C=O), 133.4, 133.3, 133.2(6), 133.2(3), 133.1(2), 133.0(7), 132.9(7), 130.0, 129.8(7), 129.8(2), 129.7, 129.6(2), 129.5(7), 129.4, 129.3, 129.2, 128.9(8), 128.9(7), 128.5, 128.4, 128.3(6), 128.3(3), 128.3(1), 128.2(8), 128.2(1), 128.1(6) (48C, Ar), 105.8(3) (C-1′′/C-1′), 105.8(0) (C-1′′/C-1′), 105.6 (C-1), 82.1 (C-2), 81.9 (C-2′′/C-2′/C-4), 81.8(2) (C-2′′/C-2′/C-4), 81.7(9) (C-2′′/C-2′/C-4), 81.4 (C-4′′), 80.9 (C-4′), 80.3 (C-3′), 77.6 (C-3′′/C-3), 77.4 (C-3′′/C-3), 70.4 (C-5′′), 67.4 (octyl O*C*H$_2$), 66.2 (C-5), 64.3 (C-6′), 63.7 (C-6′′), 33.3 (C-5′), 31.8 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.6 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). HRMS (ESI) calcd. for (M + Na) $C_{81}H_{78}O_{22}$: 1425.4877. Found: 1425.4873.

6.5. Bibliography

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Chapter 7

Conclusions and Future Work

7.1. Conclusions and Future Work

Tuberculosis is a deadly infectious disease caused by the human pathogen *Mycobacterium tuberculosis*. LAM and the mAG complex are two major entities found in the mycobacterial cell wall. LAM has been implicated in a number of immunological events[1] and the mAG serves as an impermeable barrier to the passage of antibiotics.[2] Because formation of an intact cell wall is essential for mycobacterial viability, the enzymes involved in these pathways represent a rich source of potential therapeutic drug targets. Inhibition of the key biosynthetic enzymes would subsequently disrupt the integrity of the bacterial cell wall.

Among the many GTs that catalyze the assembly of LAM and the mAG complex, my research efforts focused on two of these enzymes, a PPM-dependent α-(1→6)-mannosyltransferase (ManT) and a β-(1→5, 6)-galactofuranosyltransferase (GlfT2). As shown in Figure 7-1, ManT is proposed to catalyze the synthesis of the α-(1→6)-linked mannan core of LM/LAM.[3] Similarly, GlfT2 is a polymerising GT that catalyzes the assembly of the bulk of galactan chain, which consists of alternating β-(1→5) and β-(1→6)-linked Gal*f* residues.[4]

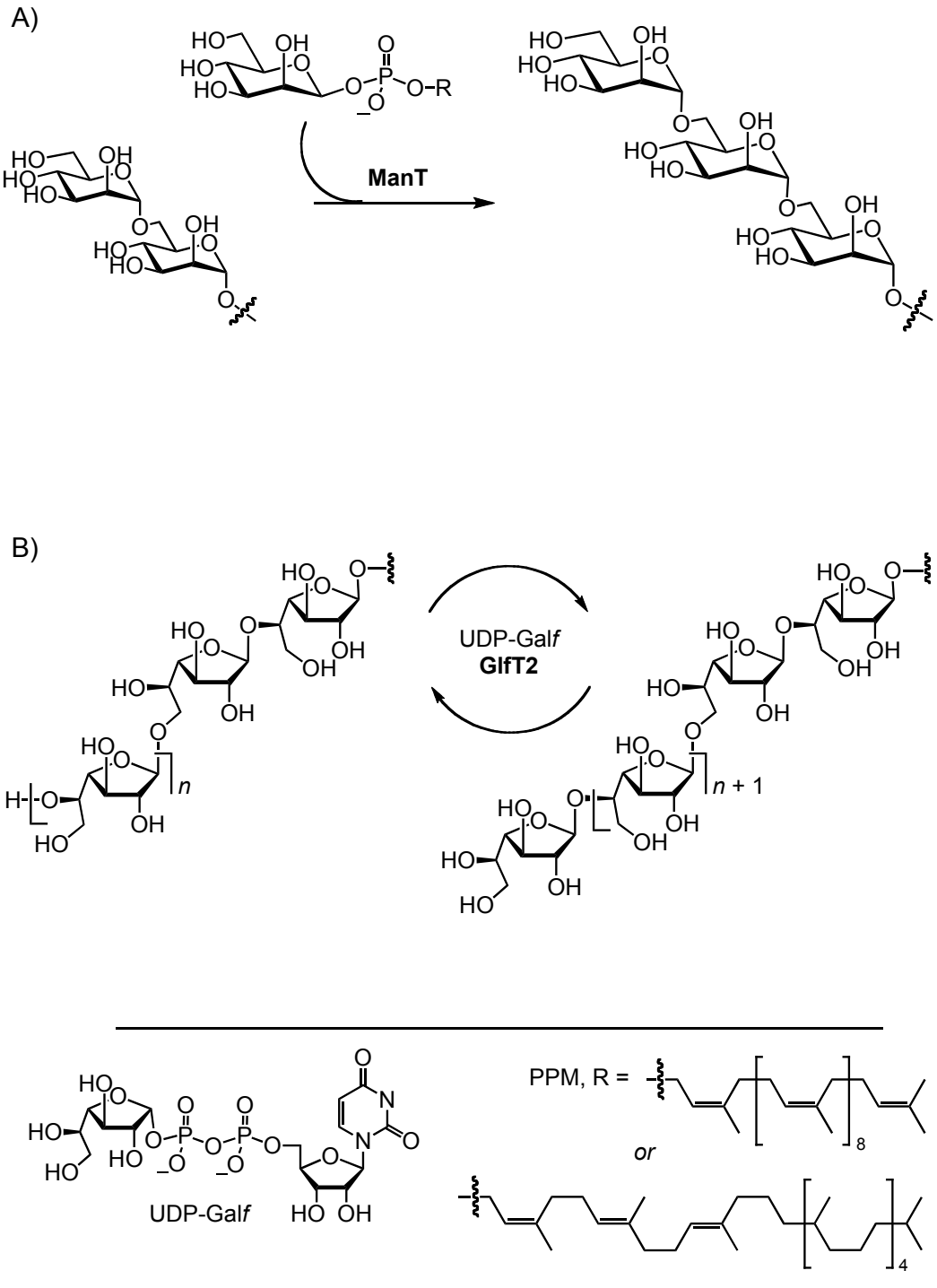

Figure 7-1. Reactions catalyzed by A) PPM-dependent α-(1→6)-mannosyltransferase (ManT) and B) β-(1→5, 6)-galactofuranosyltransferase (GlfT2).

7.1.1. ManT

Ever since Yokoyama and Ballou demonstrated the PPM-dependent α-(1→6)-mannosyltransferase activity in a membrane preparation from *M. smegmatis*,[3] a cell free assay has been used to screen synthetic glycosides for their ability to act as acceptor substrates and/or inhibitors.[5,6] Despite these efforts, the enzyme has yet to be identified, and its substrate specificity remains poorly defined. Therefore, our initial goal was to probe the substrate specificity of this enzyme, which is involved in the synthesis of the α-(1→6)-linked mannan core of LAM.

As discussed in Chapter 3, screening of a homologous series of synthetic octyl glycoside oligomers ranging in size from mono- to tetrasaccharides (**2.3**–**2.6**, Figure 7-2), revealed that a disaccharide motif is the minimum epitope recognized by ManT, and that significant increases in activity were not gained by moving to larger substrates. As a result, the subsequent investigation of the substrate specificity of the enzyme was based on the screening of a panel of monomethoxy and monodeoxy analogs of disaccharide **2.4**.

Biochemical evaluation of these disaccharide analogs by the established cell free assay allowed us to identify key interactions, such as steric and hydrogen-bonding requirements, between the protein and the acceptor substrate. Importantly, it was found that methylation of the

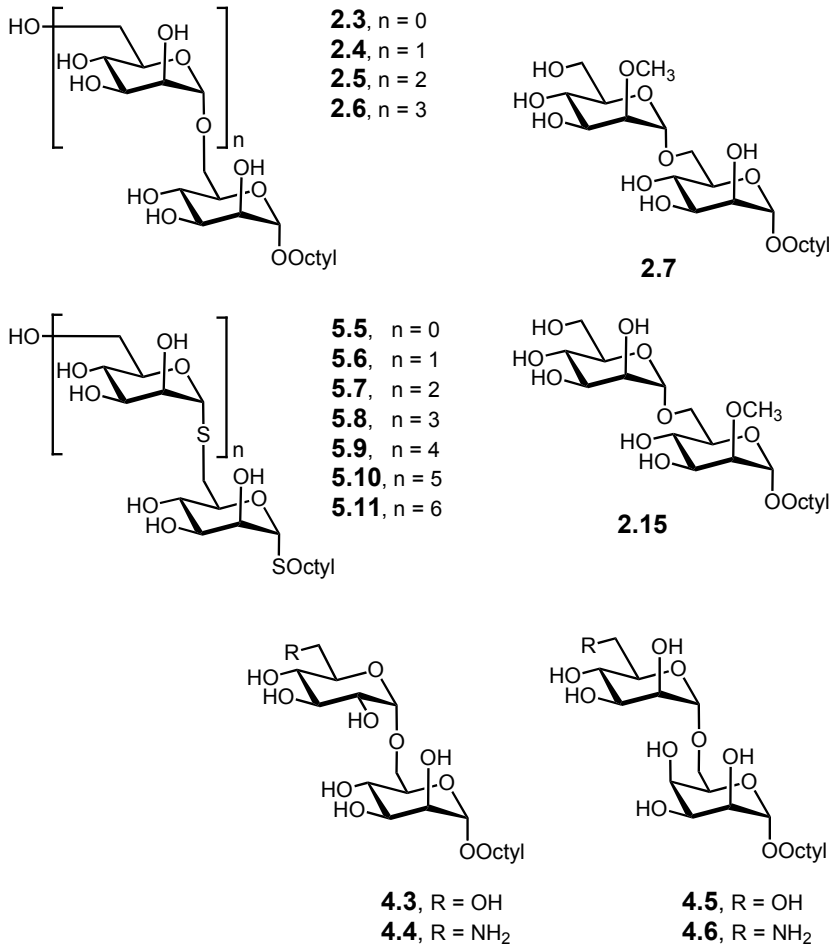

Figure 7-2. Representative examples of synthetic analogs used for probing the substrate specificity of ManT.

hydroxyl groups at C-2 of either mannopyranose residue in **2.4** (analogs **2.7** and **2.15**, Figure 7-2), led to complete loss of activity. Apparently, a methyl substituent at either position exerted enough steric influence to shut down ManT catalysis. Considering that a Man*p* residue is much more sterically demanding than a methyl group, an acceptor branched with an α-(1→2)-Man*p* unit would not be a substrate for ManTs catalysis. In

addition, structural characterization of enzymatic products from the incubations of **2.3**–**2.6** with unlabelled GDP-Man and the membrane fraction showed no detectable α-(1→2)-linkages. Therefore, the attachment of the α-(1→2)-Man*p* branches in the mannan core of LM/LAM must occur after a larger α-(1→6)-linked mannan is assembled.

However, this finding immediately brought up another related question regarding the substrate specificity of α-(1→2)-mannosyltransferase: What is the minimum length of α-(1→6)-linked oligomannoside required for α-(1→2)-Man*p* transfer? Unfortunately, the presence of endogenous α-(1→6)-*endo*-mannosidase,[3a] which catalyzes the removal of trisaccharide units from the nonreducing end of the enzymatic product, prevented us from screening larger oligosaccharides.

It is well known that thiooligosaccharides are resistant to enzymatic hydrolysis,[7] therefore a homologous series of thiosaccharides **5.5**–**5.11** (Figure 7-2) was synthesized and the numbers of that series were screened as ManT substrates (Chapter 5). Our results not only demonstrated that thiosaccharide acceptors **5.6**–**5.11** are modest substrates for ManT but also confirmed that all the corresponding enzymatic products contained solely α-(1→6)-glycosidic linkages by structural analysis using *exo*-mannosidases. Thus, this result provides another line of evidence in support of the hypothesis that the attachment of the α-(1→2)-mannopyranosyl branches in the mannan core of LM/LAM takes place only after a larger α-(1→6)-linked mannan is assembled.

After the completion of our study of ManT, two PPM-dependent α-(1→6)-mannosyltransferases were identified by other groups. It was shown that MptB and MptA (Figure 7-3) are involved in the initial and the latter stages of the biosynthesis of the α-(1→6)-mannan core of LM, respectively.[8,9] In addition, deletion of the homologous *mptB* gene in *M. smegmatis* had no dramatic effect on LM/LAM synthesis, indicating the functional redundancy for ManT in mycobacteria.

It is possible that the observed mannosyl transfer activities in our study were catalyzed by any or the combination of these ManT enzymes. Although the precise substrate specificities of these enzymes obviously needs to be further defined, our study along with the recent findings by others[8,9] allow refinement of the current proposed model of the LM/LAM biosynthesis. As the observed ManT activity requires acceptor substrate carrying the disaccharide motif, AcPIM3 can possibly be the precursor for the α-(1→6)-mannan in the pathway as shown in Figure 7-3.

The early stages of the α-(1→6)-mannosylation is then catalyzed by the PPM-dependent MptB and/or other yet to be identified ManT(s). As no α-(1→2)-linkage was even observed with the octa- and nonasaccharides, the largest enzymatic products obtained from the cell free assay using thiomannoside **5.11**, it is believed that the linear mannan core has to reach a certain (long) chain length before the α-(1→2) residues are introduced by the PPM-dependent α-(1→2)-ManT (Rv2181). This proposal is, in fact,

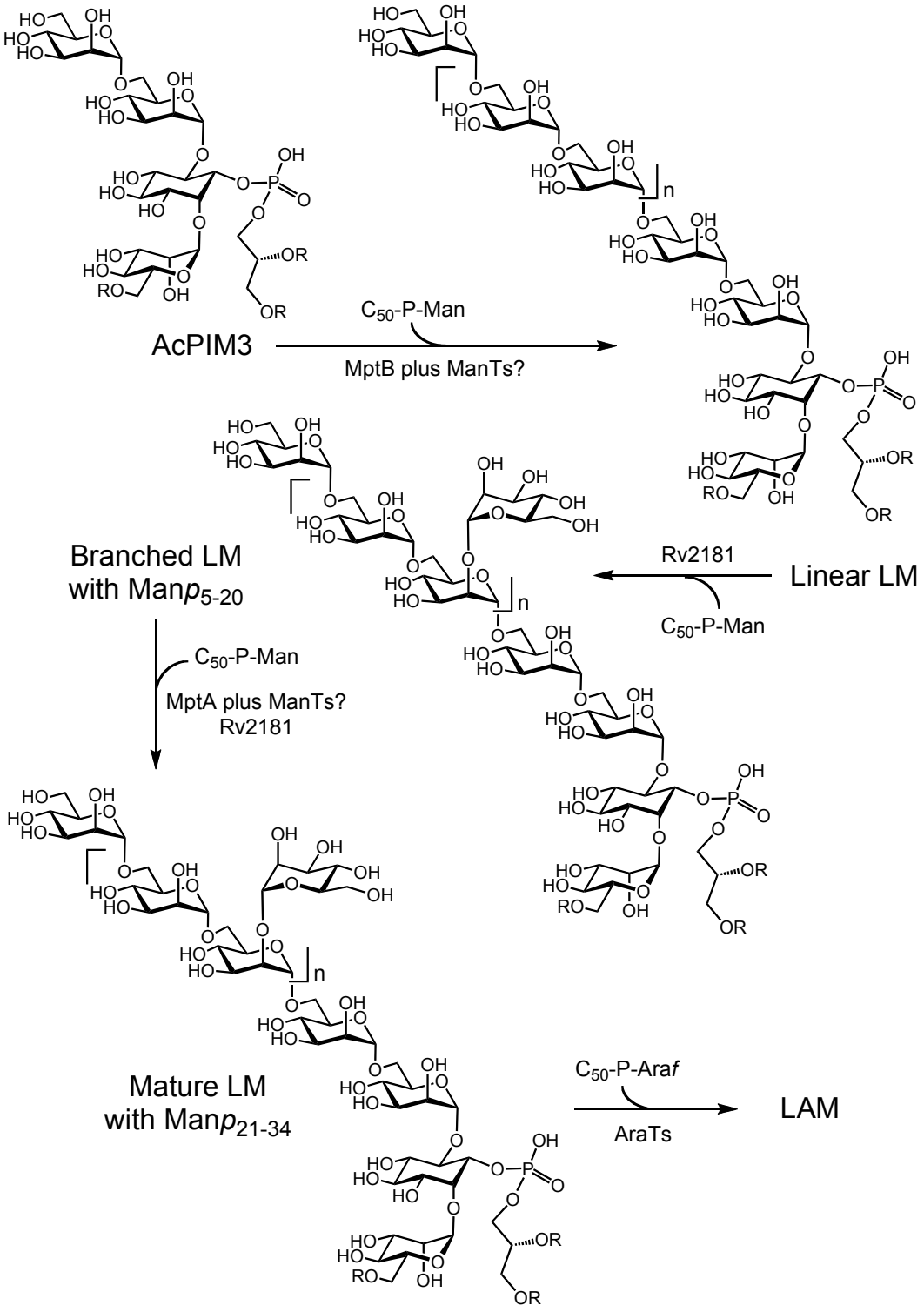

Figure 7-3. Revised model of LM/LAM biosynthesis.

in good agreement with the observation made by the Brennan group that the deletion of MptA encoding gene resulted in a truncated form of lipomannan (<20 Man*p* units) consisting of both α-(1→6) and α-(1→2) linkages.[8a] The addition of 12–15 mannosyl residues is then catalyzed by the action of MptA and the protein encoded by the Rv2181 gene. However, whether these enzymes work in a sequential or a concerted manner remains uncertain at this point.

Besides the additional insights regarding the steric requirements and hydrogen bonding interactions in the active site of ManT(s), the results obtained from the screening of monomethoxy and monodeoxy analogs (e.g., **2.7** and **2.15**) led to the synthesis and evaluation of a second panel of isomeric disaccharides (e.g., **4.3** and **4.5**, Figure 7-2). In these compounds the mannose residue at the reducing and/or non-reducing end of the parent substrate **2.4** was changed to the *gluco*- and/or *talo*-configuration. Comparison of the relative activities and the kinetic parameters (for those serving as substrates) of this panel of analogs to those of the known acceptor **2.4**, clearly showed that ManT(s) possesses a strong preference for substrates containing the α-(1→6)-dimannosyl motif.

Furthermore, we also observed very modest inhibition of enzyme activity by amino derivatives such as **4.4** and **4.6**. The observed inhibitory effect might result from the formation of a ionically-stabilized enzyme–inhibitor complex. As proposed by Lemieux,[9] the amino groups of these

analogs will be protonated at the physiological pH and the resulting ammonium ions form an ionic interaction with a negatively charged residue in the enzyme active site. However, as discussed in Chapter 4, we also consider other possibility that the amino derivatives (e.g., **4.4** and **4.6**, Figure 7-2) might inhibit the enzyme noncompetitively or uncompetitively.

With the recent identification of MptA and MptB, it appears that there are at least three α-(1→6)-mannosyltransferases involved in the LM/LAM biosynthetic pathway. Therefore, the results herein only represent the relative substrate preference of ManT(s). Determination of the precise specificities of these individual ManTs thus require the availabilities of pure enzymes; unfortunately the overexpression of these enzymes has met with very limited success so far.[8,9]

7.1.2. GlfT2

As shown in Figure 7-1B, GlfT2 is a bifunctional galactofuranosyltransferase that catalyzes both β-(1→5) and β-(1→6) Gal*f* transfers in an alternate manner for the formation of galactan.[4] In an attempt to address the question of whether the enzyme catalyzes both glycosyl transfers in single or separate active sites, previous work in the group involved the synthesis of a panel of mono- and di-deoxygenated trisaccharides. Briefly, removal of the C-5 and/or C-6 hydroxyl group on the non-reducing terminal residues of **6.1** and **6.2**, resulting in analogs **6.3**–**6.6**, had no effect on the substrate recognition by the enzyme (Figure

7-4).[12] Also, recent STD spectroscopy experiments showed that acceptors **6.1** and **6.2** bind competitively to GlfT2, suggesting the enzyme catalyzes both transfer reactions in a single active site.[13]

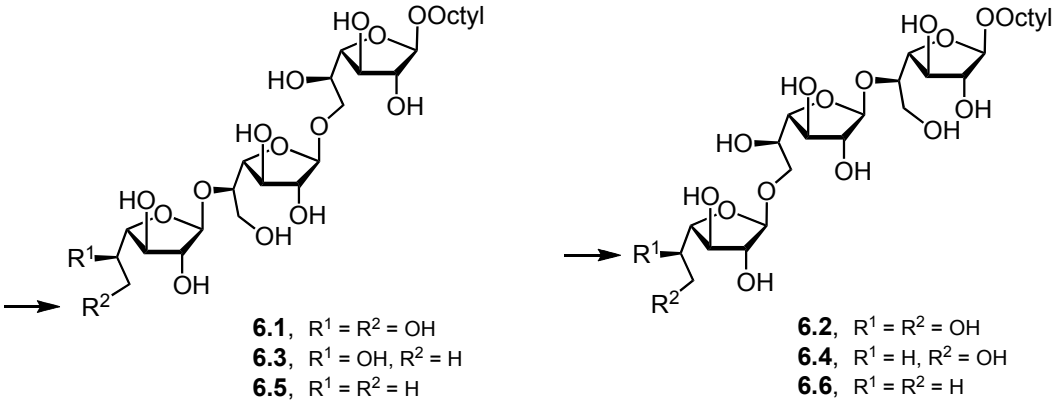

Figure 7-4. Demonstrated GlfT2 substrates (**6.1**, **6.2**) and deoxygenated target analogues (**6.3**–**6.6**) from previous study.[12]

To extend our study to investigate what the key elements are to control the two different modes of Gal*f* transfer further, additional deoxygenated analogs **6.7**–**6.16** (Figure 7-5) were synthesized and their ability to serve as substrate acceptors for the enzyme were evaluated. The purpose of this study was two fold. The first goal was to explore if any of the C-5 and C-6 hydroxyl groups on **6.1** and **6.2** play important roles for the polar interaction with GlfT2. The second purpose was to investigate if

removal of these hydroxyl groups influences the rate or the regioselectivity of galactosylation by GlfT2.

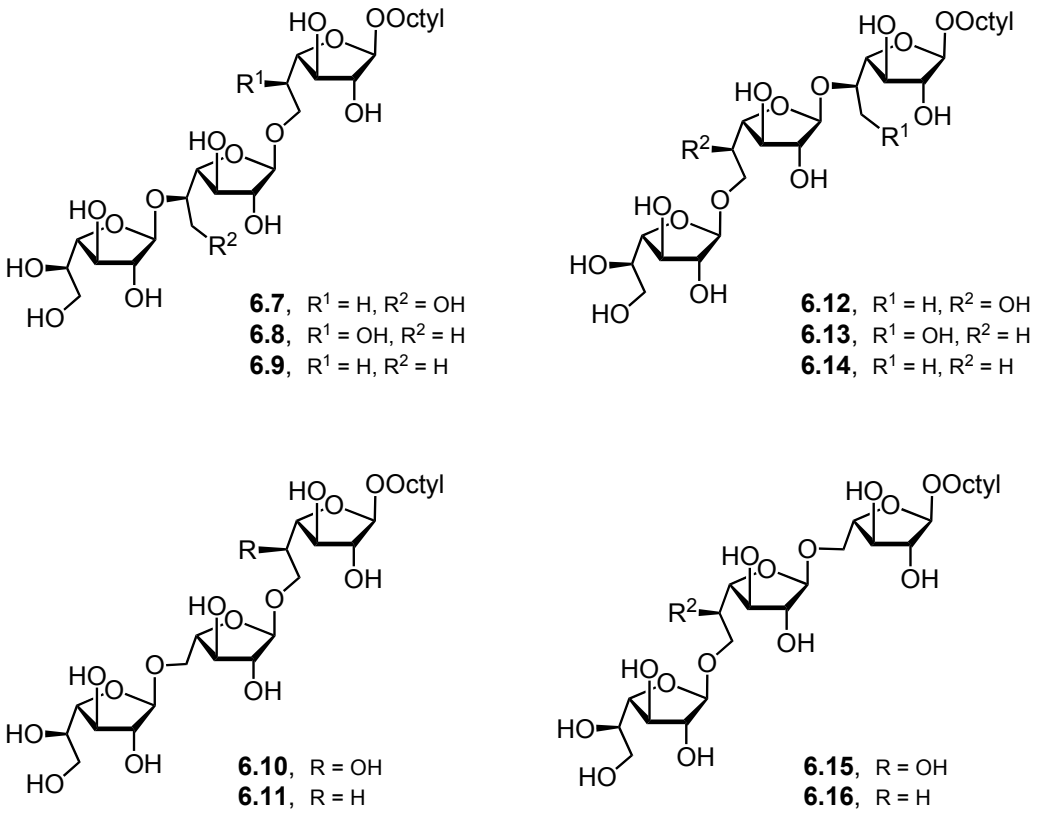

Figure 7-5. Demonstrated deoxygenated target analogues (**6.7**–**6.16**) from our current study.

As discussed in Chapter 6, deoxygenated analogs **6.7**–**6.16** are all substrates for the corresponding β-(1→5) and β-(1→6) transfers of GlfT2. Apparently, the hydroxyl groups at the C5′ and C6 positions on **6.1** and **6.2** are not essential for substrate recognition by the enzyme. This is further supported by our kinetic analysis, in which the removal of these hydroxyl groups in some, but not all cases, led to more efficient

glycosylation of the substrate by the enzyme. Obviously, more questions remain to be addressed based on these results. For instance, it is important to identify the key polar interactions of the substrate–enzyme complex (e.g., C-2 and C-3 hydroxyl groups on the known substrates **6.1** and **6.2**) and to determine the effect on substrate binding in the absence of such interactions.

In addition, structural analysis of the enzymatic products from the reactions of the β-(1→5), β-(1→6)-linked deoxygenated trisaccharide analogs (**6.7**–**6.11**) confirmed the expected β-(1→6)-linkages at the non-reducing end. In other words, the removal of the exocyclic OH group, at the C6′ and/or C5 position, has no consequence to the β-(1→6) galactosylation by GlfT2. However, the use of the analogous experiments for reactions of β-(1→6), β-(1→5)-linked analogs (**6.12**–**6.16**) proved to be problematic, as the formation of a homologous series of oligosaccharide products was observed. The difficulties in obtaining the enzymatic products as tetrasaccharides are due to the polymerizing nature of GlfT2 and its faster rate for the β-(1→6) glycosyl transfer. The initial tetrasaccharide products, formed from the slower β-(1→5) transfer to the trisaccharide, also served as substrates for the enzyme and immediately led to the formation of pentasaccharides in the faster β-(1→6) transfer step.[14]

Hypothetically, this problem could be overcome by the use of 6′′-methoxy or 6′′-deoxy UDP-Gal*f* (Figure 7-6) in the biological assay as the

installation of either analog at the non-reducing end would abolish further polymerization. An additional advantage of using these donor analogs is that they allow more precise measurement of the kinetic parameters of the corresponding Gal*f* transfer. As illustrated in Figure 7-6, instead of measuring activities resulting from multiple glycosylations, the observed data will represent only the β-(1→5) transferase activity of GlfT2. However, it is possible that such modified sugar nucleotide analogs will not be recognized by the enzyme.

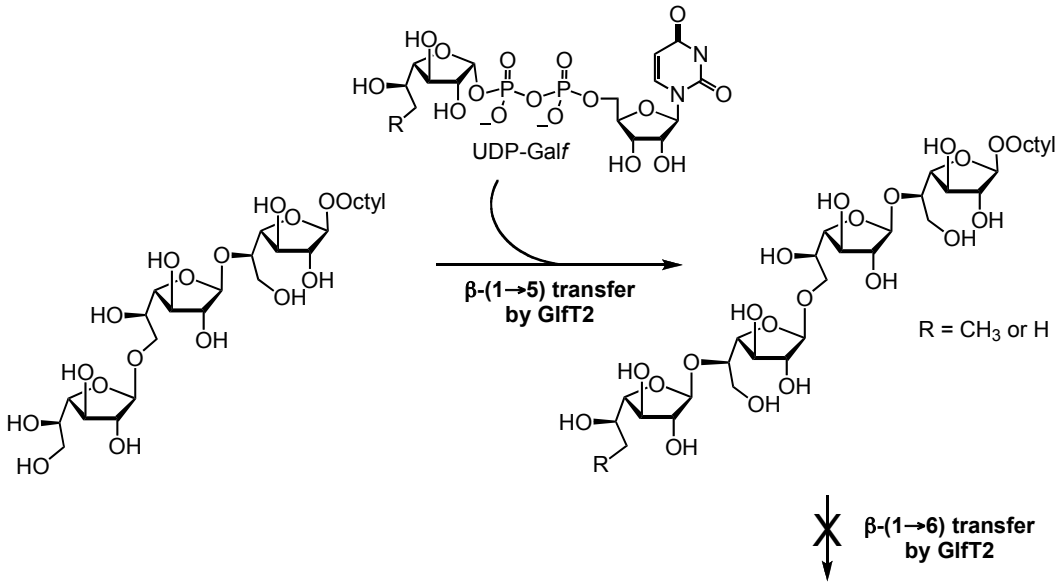

Figure 7-6. Proposed reaction of the use of methoxy and deoxy UDP-Gal*f* substrates in the GlfT2 assay.

The overexpression of GlfT2 as a soluble protein[14,15] and the development of spectrophotometric assay for its activity[16] have significantly advanced our study of the enzyme regarding its role in the

mAG synthetic pathway and its bifunctional catalysis. In particular, the soluble GlfT2 protein is now available for structural determination by X-ray crystallization and for substrate binding study by saturation transfer difference spectroscopy.[13] Indeed, Dr. Kenneth Ng at the University of Calgary has recently been successful in solving the crystal structure of the enzyme. As complementary to these methods, screening of substrate derivatives, as exemplified in our current study, identified some key interactions between the enzyme and its substrate. Therefore, studies of this type provide additional structural insight into the substrate specificity and activity of GlfT2. However, the panel of deoxygenated trisaccharide analogs used in our study only partially revealed the substrate preference of GlfT2, additional panels of analogs are required in future studies to fully understand the substrate–GlfT2 interaction and the origin of its regioselectivity in glycosylation.

In summary, I have carried out fundamental studies on α-(1→6)-ManT(s) and β-(1→5, 6)-GlfT2, which are involved in the biosynthesis of mycobacterial LAM and the mAG complex, respectively. The design, synthesis and biological evaluation of analogs that closely mimic the natural acceptor substrate provided important information about how these enzymes interact with their substrates at the molecular level and additional insights into their roles in the biosynthetic pathway. More significantly, our preliminary results will serve as general guidance for the further design and evaluation of potential substrates/inhibitors against ManT(s) and

GlfT2. We believe such approaches will eventually provide us with a comprehensive understanding of substrate–enzyme interactions and lead to the discovery of novel therapeutic agents that are active against tuberculosis.

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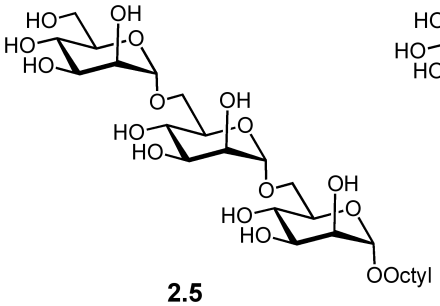
Appendix

Kinetic plots for compounds 2.5 and 2.6



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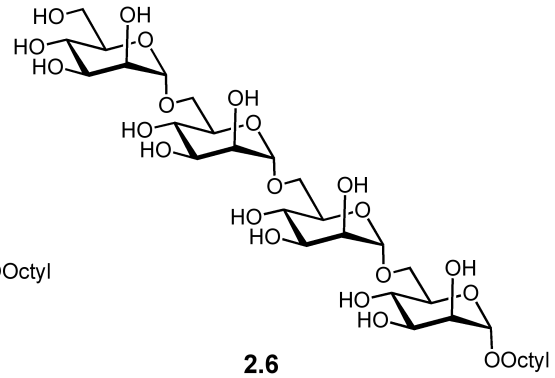
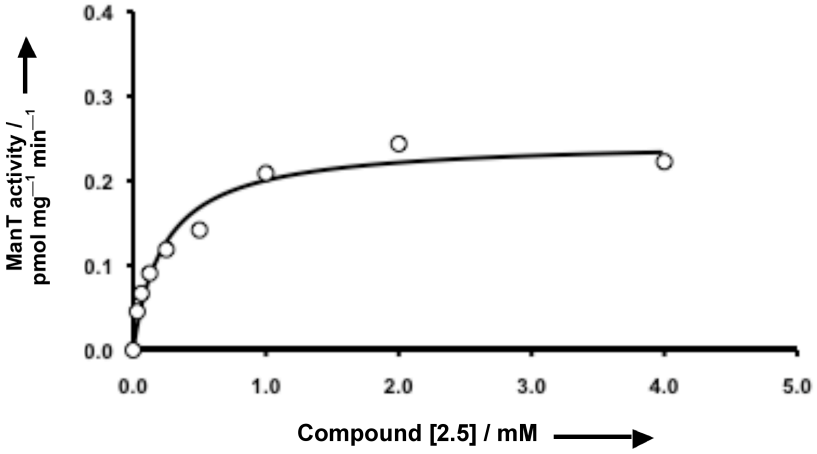
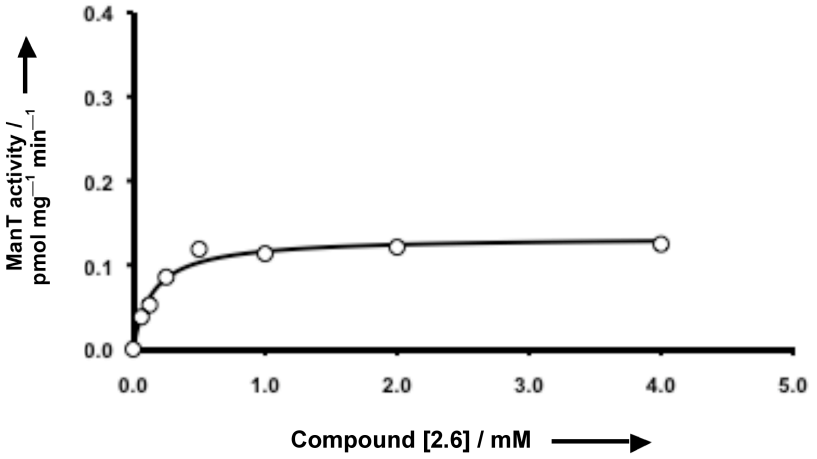
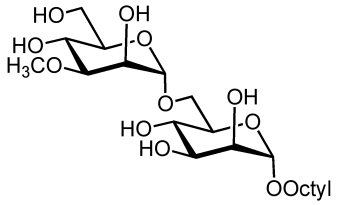


2.6





Kinetic plots for compounds 2.9 and 2.11



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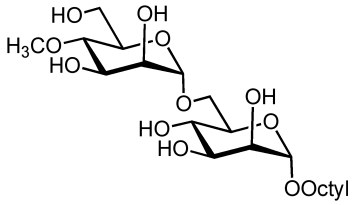
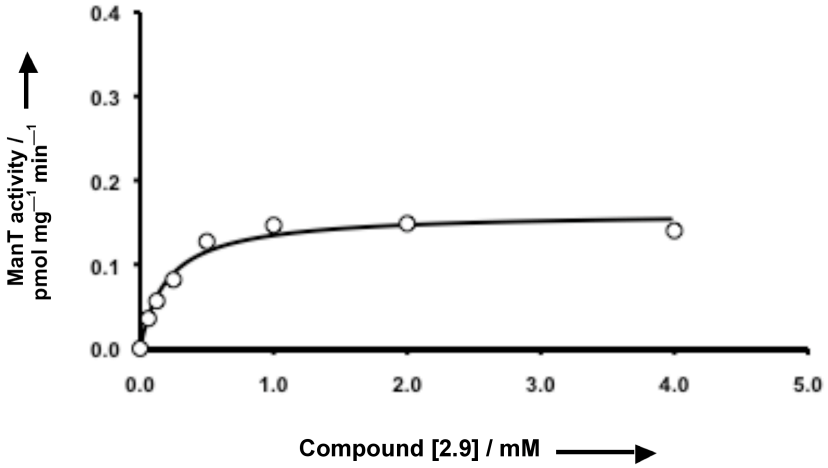
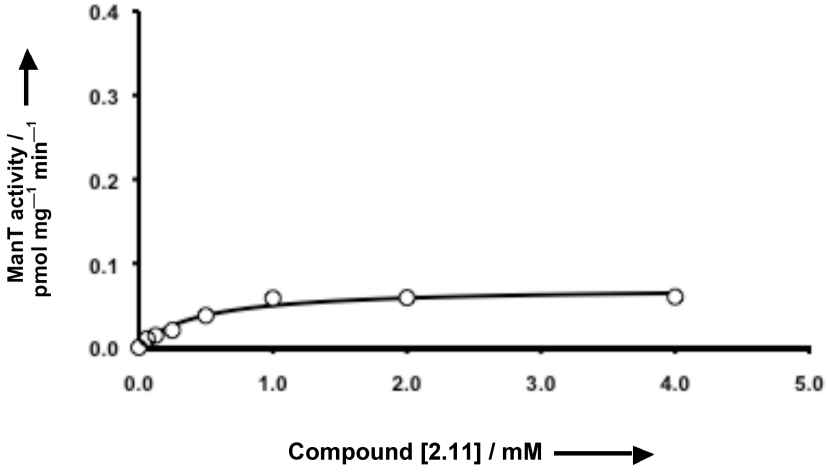
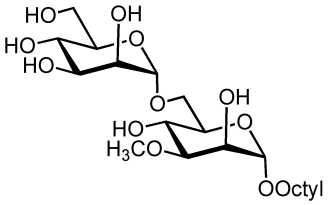


2.11

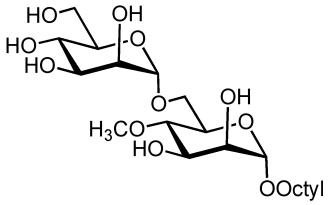




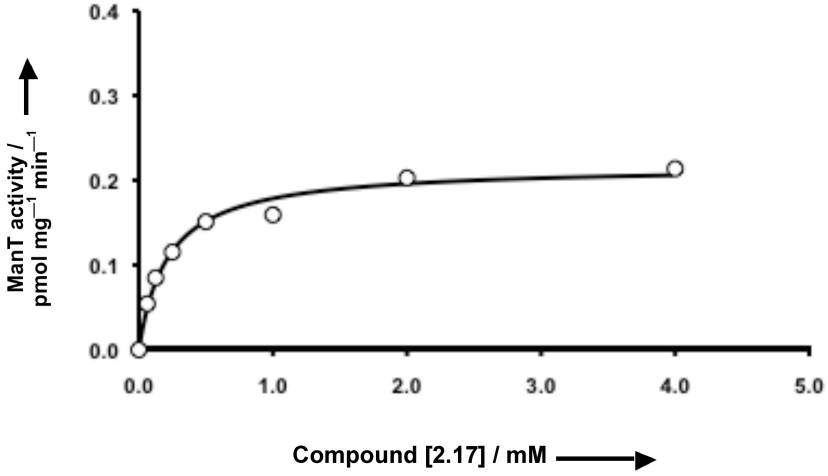
Kinetic plots for compounds 2.17 and 2.19

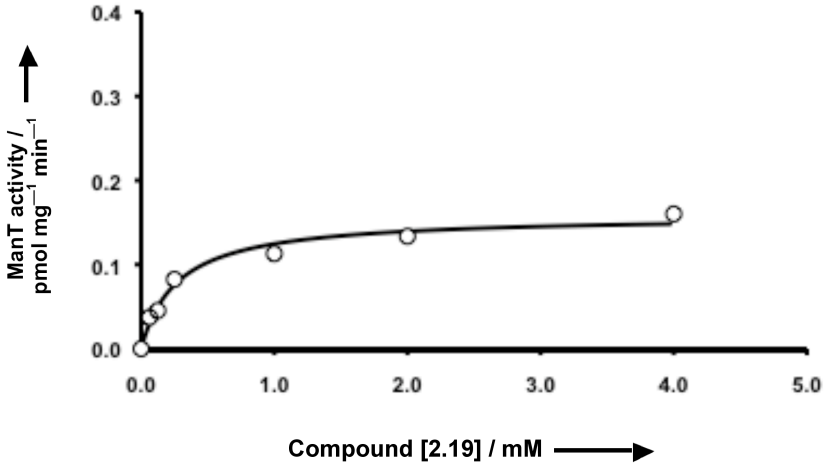


2.17

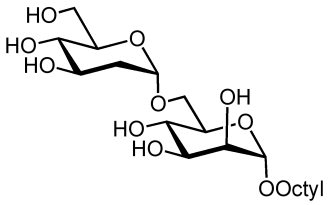


2.19

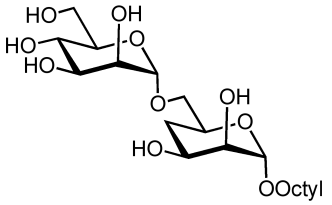




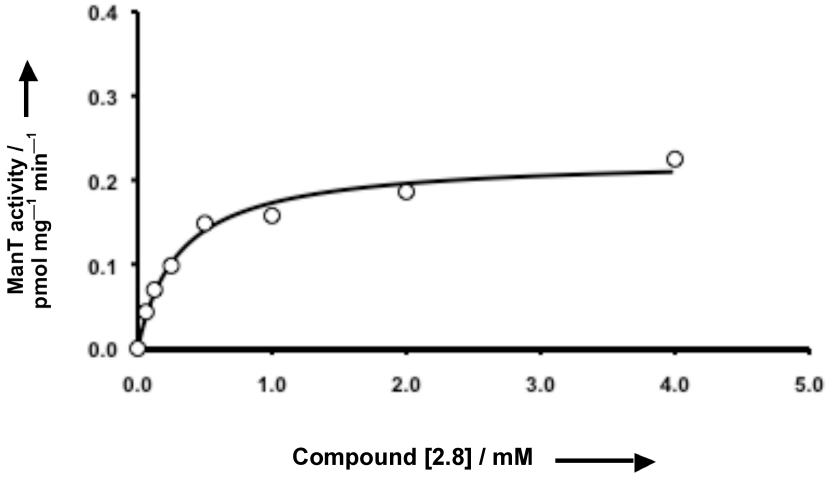
Kinetic plots for compounds 2.8 and 2.20

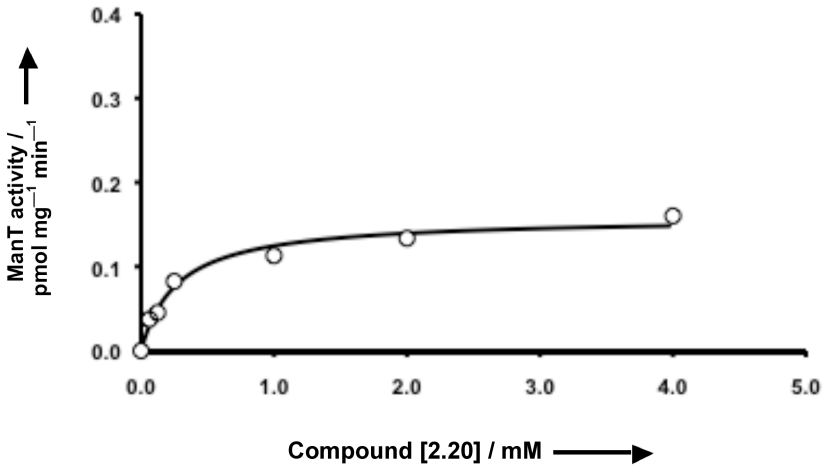


2.8

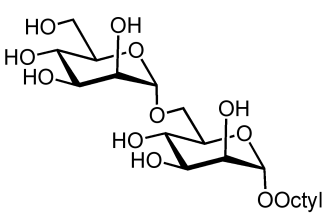


2.20

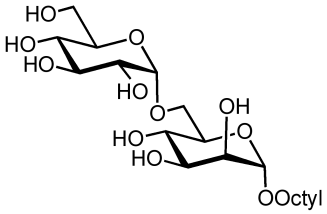




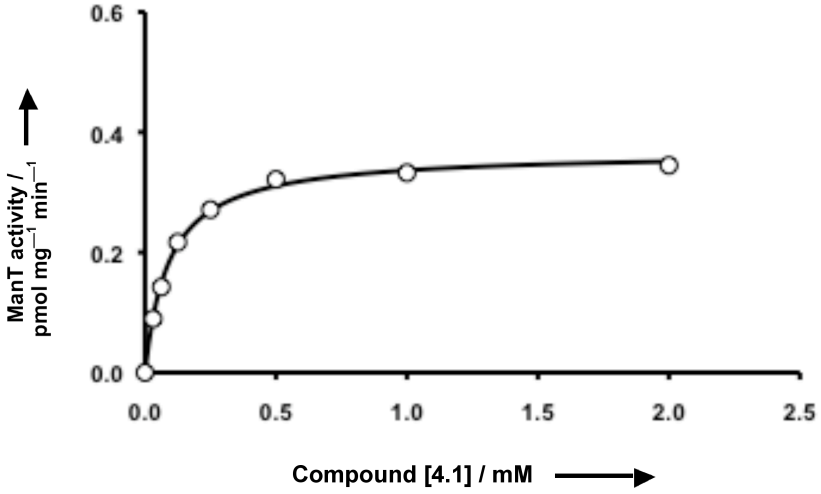
Kinetic plots for compounds 4.1 and 4.3

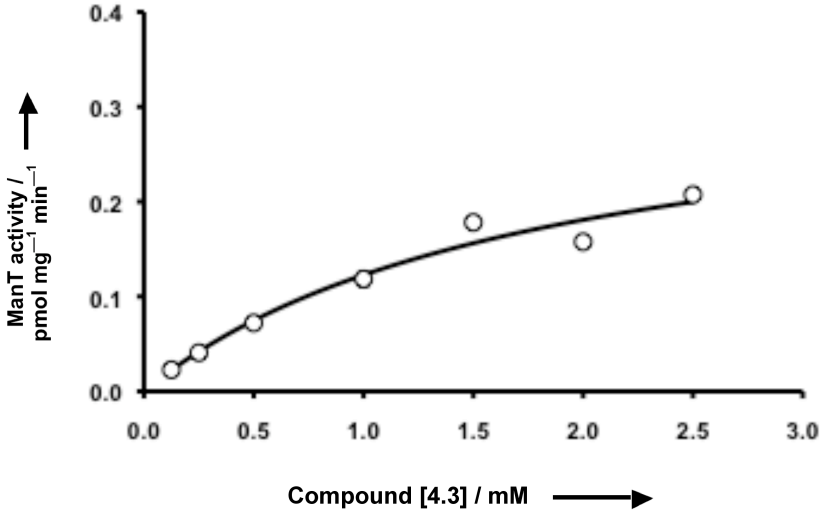


4.1

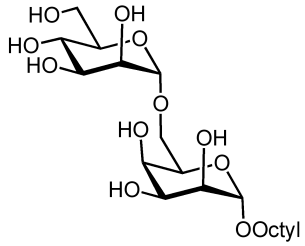


4.3

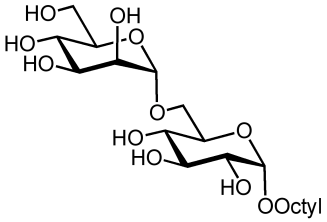




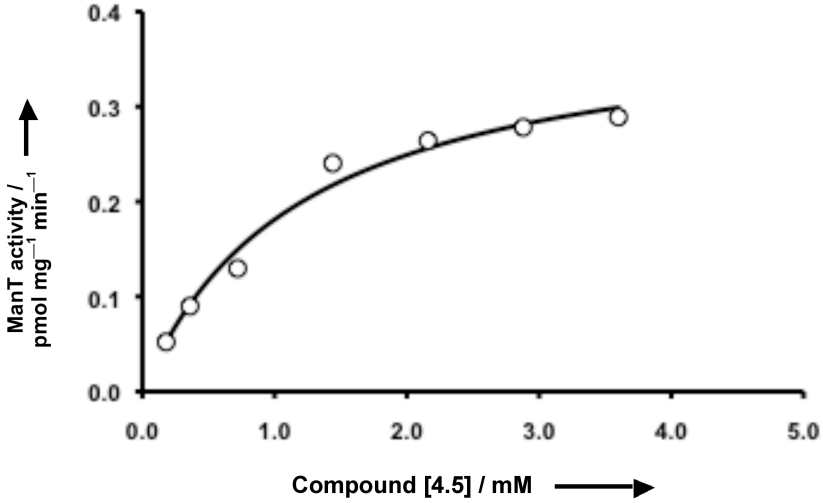
Kinetic plots for compounds 4.5 and 4.7

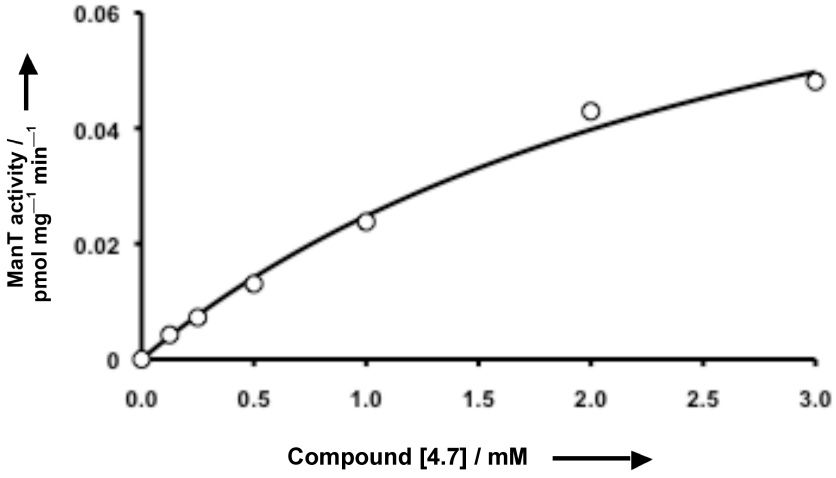


4.5

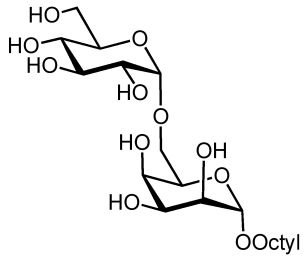


4.7

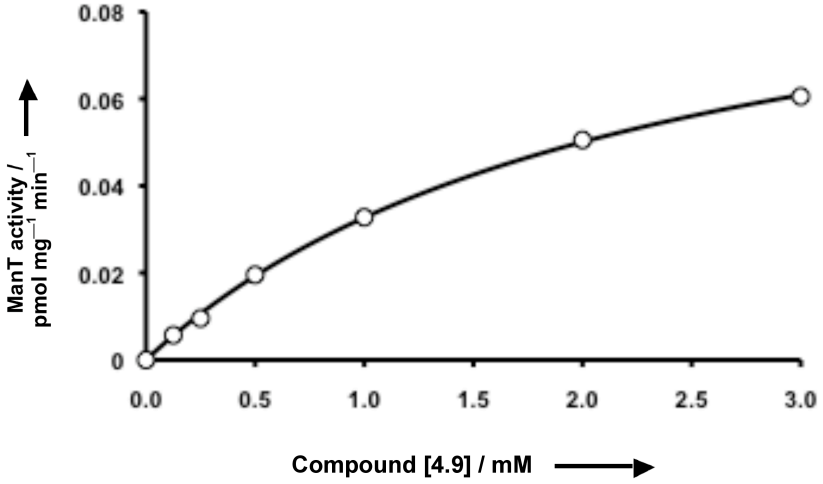




Kinetic plot for compound 4.9



4.9



Example of ManT activity calculation:

Amount of product formation per hour at a fixed substrate concentration:

= (observed radioactivity)* (1 μCi / 2.22 x 10^6 dpm)* (1 nmol / 20 μCi)

e.g., = (15291 dpm)* (1 μCi / 2.22 x 10^6 dpm)* (1 nmol / 20 μCi)

= 0.34 pmol

ManT activity:

= (product formation)* (1 / amount of enzyme)* (1 / 60 min)

e.g., = (0.34 pmol)* (1 / 0.095 mg)* (1 / 60 min)

= 0.060 pmol.mg^{-1}.min^{-1}