Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 1083–1094
Bioorganic & Medicinal Chemistry

Modified mannose disaccharides as substrates and inhibitors of a polyprenol monophosphomannose-dependent α-(1→6)-mannosyltransferase involved in mycobacterial lipoarabinomannan biosynthesis

Vinodhkumar Subramaniam,[a,†] Sudagar S. Gurcha,[b,†] Gurdyal S. Besra[b] and Todd L. Lowary[a,*]

[a]*Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA*
[b]*School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

Received 6 October 2004; revised 15 November 2004; accepted 15 November 2004
Available online 9 December 2004

Abstract—A panel of α-(1→6)-linked mannose disaccharides (**5**–**8**) in which the 2′-OH group has been replaced, independently, by deoxy, fluoro, amino, and methoxy functionalities has been synthesized. Evaluation of these compounds as potential substrates or inhibitors of a polyprenol monophosphomannose-dependent α-(1→6)-mannosyltransferase involved in mycobacterial LAM biosynthesis demonstrated that the enzyme is somewhat tolerant substitution at this site. The enzyme recognizes the disaccharides with groups similar or smaller in size than the native hydroxyl (**6**–**8**), but not the disaccharide with the more sterically demanding methoxy group (**5**). The 2′-OH appears not form a critical hydrogen bonding interaction with the protein as the 2′-deoxy analog is a substrate for the enzyme.

1. Introduction

Tuberculosis has attracted increasing attention in recent years given its resurgence in the industrialized world and the emergence of drug resistant stains of the organism that causes this disease, *Mycobacterium tuberculosis*.[1–3] The treatment of tuberculosis, like other mycobacterial diseases (e.g., leprosy and AIDS-associated *M. avium* infections), is difficult and the standard treatment requires multiple antibiotics that are administered over a number of months.[4] The difficulty in treating these diseases arises from the unusual structure of the mycobacterial cell wall, which not only serves as a formidable barrier to the passage antibiotics but also enables the bacteria to resist the immune system of the host.[5,6] The major structural component of the mycobacterial cell wall is the mycolyl-arabinogalactan complex, which, together with another lipopolysaccharide, lipoarabinomannan (LAM), comprise the bulk of the structure. LAM is the major antigenic component of the cell wall and has been implicated in a large, and increasing, number of important immunological events.[7,8]

The structures of LAM's from a number of different actinomycetes have been reported in recent years and these investigations have revealed an impressive array of structural diversity between organisms.[9–17] The most studied of these is the mycobacterial lipoglycan, which, like all LAM molecules is built upon a phosphatidylinositol (PI) moiety that is noncovalently associated with the plasma membrane through its lipid portion. The inositol residue of the PI serves as the attachment point for a polysaccharide composed of mannopyranose and arabinofuranose. The mannan component is a polymer of α-(1→6)-linked mannopyranose residues that is further elaborated by additional mannopyranose units. Most commonly (e.g., in *M. tuberculosis*, *M. bovis*, and *M. smegmatis*), 50–70% of the mannose residues in the α-(1→6)-linked backbone are further glycosylated

Keywords: Mycobacteria; Oligosaccharides; Inhibitors; Mannosyltransferase; Synthesis.
* Corresponding author at present address: Alberta Ingenuity Centre for Carbohydrate Science and Department of Chemistry, The University of Alberta, Gunning-Lemieux Chemistry Centre, Edmonton, AB, Canada T6G 2G2. Tel.: +1 7804921861; fax: +1 7804927705; e-mail: tlowary@ualberta.ca
† Both authors contributed equally to this work.

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.11.027

at O-2 with single α-mannopyranose residues (**1**, Fig. 1). In contrast, in LAM from *M. chelonae*, these branching mannopyranose residues are attached α-(1→3).[18] To this mannan is attached a highly branched arabinan, which is substituted at its distal ends with a range of motifs including additional mannopyranose residues,[19,20] inositol phosphates moieties,[21] or 5-thiomethylxylofuranose.[22,23] A recent discovery is that LAM from *M. chelonae* lacks capping groups altogether.[18]

The biosynthesis of mycobacterial LAM is a topic of increasing interest.[7,8,24] To date, most of the focus has been on the steps involved in the assembly of the early mannosylated PI intermediates, and the related phosphadtidylinositol mannosides (PIMs).[25] In 1997, Besra et al.[24] proposed a pathway by which mycobacteria biosynthesize LAM (Fig. 2) and many of the steps in this pathway are now supported by experimental evidence. The donor substrates for the biosynthetic ManT's are

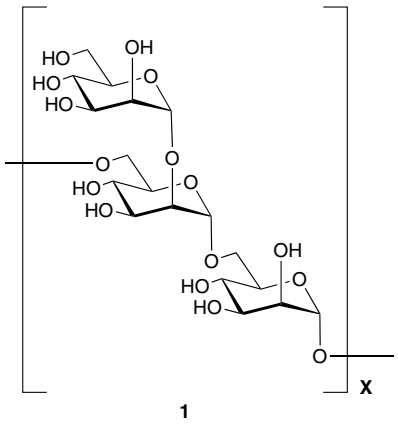
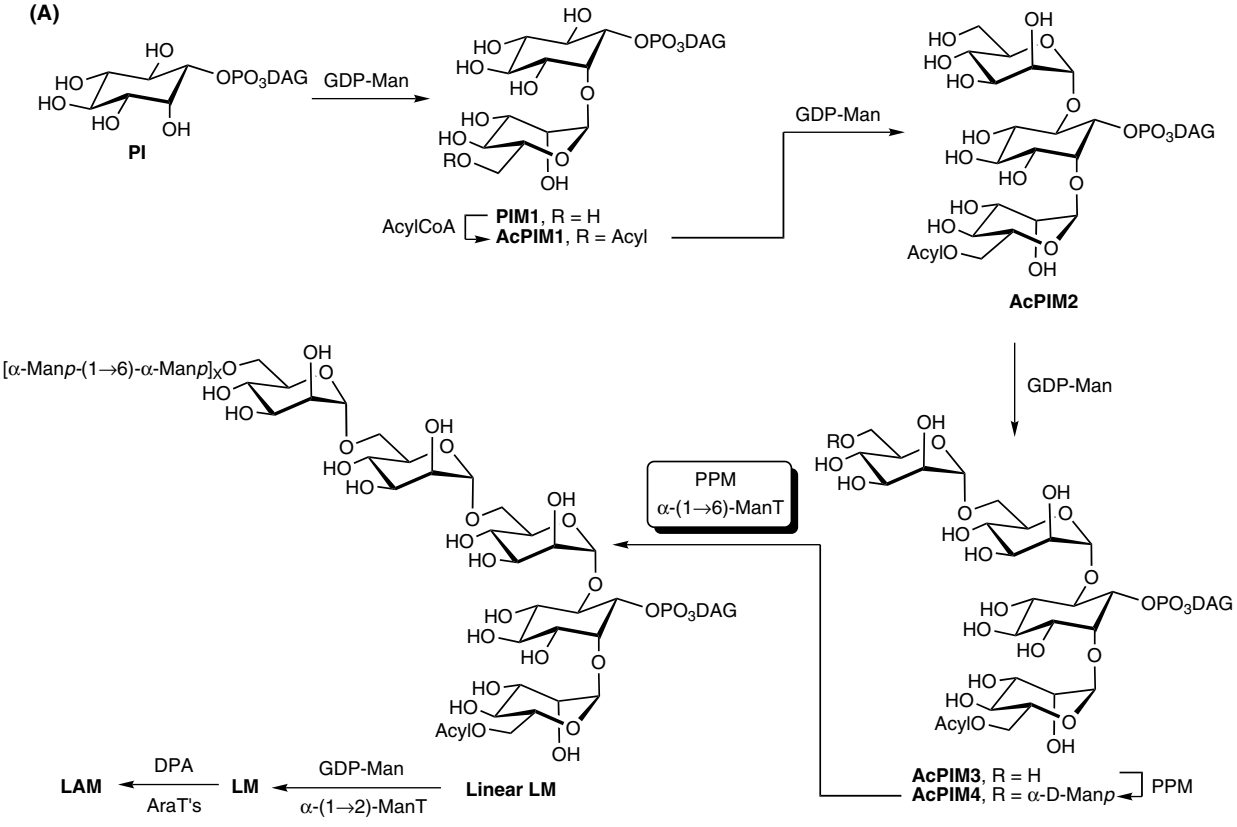
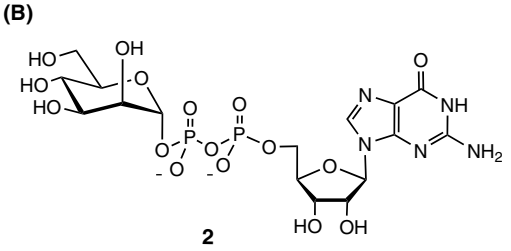


Figure 1. Common mannan core structure of mycobacterial LAM.

either GDP-mannose (GDP-Man, **2**) or polyprenol phosphomannose (PPM, **3**), a mixture of glycolipids





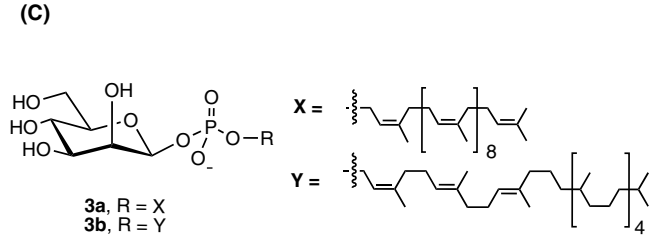
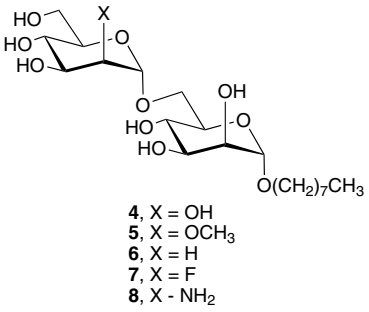


Figure 2. (A) Biosynthesis of mycobacterial lipoarabinomannan, DAG = diacylglycerol, DPA = decaprenolphosphoarabinose, AraT's = arabinosyltransferases. The ManT of interest in this paper is indicated by the shadowed box. (B) Structure of GDP-mannose (**2**). (C) The polyprenolphosphomannose derivatives (PPM, **3**) that serve as substrates for the PPM-dependent mycobacterial α-(1→6)-ManT.

differing in the length of the lipid chain, which are synthesized from **2** by the enzyme polyprenol monophosphomannose synthase.[26] The enzymes involved in the assembly of the α-(1→6)-linked backbone use PPM as the donor, while the α-(1→2)-branching residues are believed to be incorporated from GDP-Man.[24] A number of recent papers report the identification and characterization of mannosyltransferases (ManT's) involved in these processes.[27–30] However, to date, the PPM-dependent α-(1→6)-ManT involved in assembly of the mannan core of LAM (linear LM in Fig. 2) has not been identified. Nevertheless, a cell-free assay for this enzyme has been developed[24] and has been used to screen potential substrates of the enzyme.[31–33]

An understanding of the substrate specificities of the ManT's involved in LAM biosynthesis is important given the role of this polysaccharide in the progression of mycobacterial disease. Furthermore, potential inhibitors of these glycosyltransferases are of interest both as biochemical tools and as potential lead compounds for new antimycobacterial agents. We report here the synthesis of a panel of mannose disaccharide analogs and their subsequent screening against the PPM-dependent ManT responsible for the synthesis of the α-(1→6)-linked mannan core of LAM. The targets chosen (**5**–**8**) are derivatives of the α-D-Man*p*-(1→6)-α-D-Man*p*-$O(CH_2)_7CH_3$ disaccharide (**4**), a known substrate for this enzyme.[32] Singly modified oligosaccharide analogs such as **5**–**8** have been used previously and with great success to determine the steric and hydrogen bonding requirements of glycosyltransferases and some have of these compounds been shown to be potent inhibitors of the targeted enzymes.[34–40] Oligosaccharides **5**–**8** were chosen as probes of the substrate specificity of the enzyme with regard to the substituent at C-2′. The targets were synthesized as octyl glycosides based on previous investigations demonstrating that among a series of long-chain alkyl disaccharide derivatives, the octyl glycosides were the best substrates for this enzyme.[32] We further envisioned that these compounds would be useful in future investigations as potential inhibitors of the (to date unidentified) mannosyltransferase responsible for the addition of the α-(1→2) mannopyranosyl branches in the core of mature LAM.



4, X = OH
5, X = OCH_3
6, X = H
7, X = F
8, X - NH_2

2. Results and discussion

2.1. Synthesis of 4–8

For the preparation of **4**–**6** a convergent approach was chosen (Fig. 3) that involved the synthesis of the protected disaccharide, **15**, which in turn was obtained from monosaccharides **12** and **13**.[41]

The synthesis commenced (Scheme 1) with octyl α-D-mannopyranoside, **9**,[42] which was reacted with triphenylmethyl chloride and pyridine to provide the corresponding 6-*O*-triphenylmethyl ether, **10**, in 80% yield. The remaining hydroxyl groups were protected as benzyl ethers (benzyl bromide, NaH) affording **11** in 79% yield. Removal of the triphenylmethyl group was

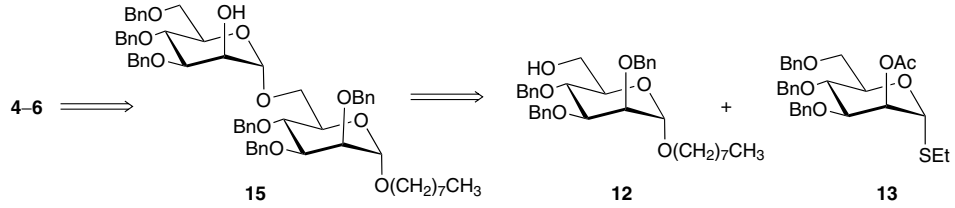
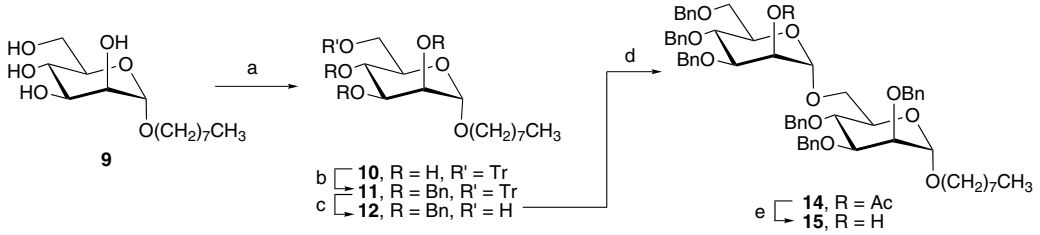
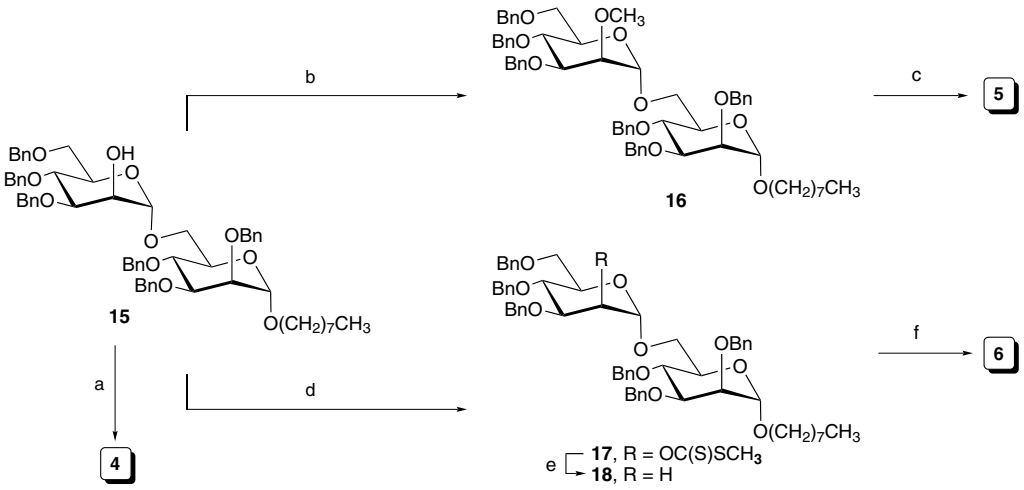


Figure 3. Retrosynthesis of **4**–**6**.



Scheme 1. Reagents and conditions: (a) TrCl, pyridine, 50 °C, 80%; (b) BnBr, NaH, DMF, 0 °C → rt, 79%; (c) *p*-TsOH, CH_2Cl_2, CH_3OH, 92%; (d) **13**, NIS, AgOTf, CH_2Cl_2, −40 → 0 °C, 86%; (e) $NaOCH_3$, CH_3OH, rt, 96%.

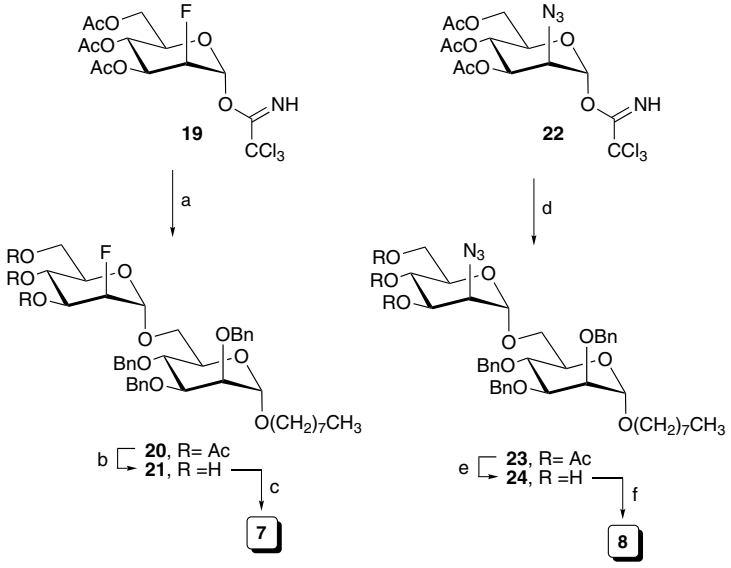


Scheme 2. Reagents and conditions: (a) H_2, Pd/C, CH_3OH, rt, 91%; (b) CH_3I, NaH, DMF, rt, 87%; (c) H_2, Pd/C, CH_3OH, rt, 87%; (d) NaH, CS_2, CH_3I, imidazole, THF, rt, 99%; (e) *n*-Bu_3SnH, AIBN, toluene, reflux, 61%; (f) H_2, Pd/C, CH_3OH, rt, 90%.

then achieved under standard conditions to give alcohol **12** (92% yield). Reaction[43] of **12** with thioglycoside **13**[41] in the presence of *N*-iodosuccinimide and silver triflate yielded disaccharide **14** in 86% yield. Subsequent removal of the acetyl group with sodium methoxide provided a 96% yield of **15**.

With disaccharide **15** in hand, it was converted to targets **4**–**6** as illustrated in Scheme 2. First, global deprotection of **15** with hydrogen and palladium and carbon yielded **4** in 91% yield. Alternatively, methylation of **15** with methyl iodide and sodium hydride yielded disaccharide **16**, which was then fully deprotected by hydrogenolysis to give **5** in 76% overall yield. The presence of the methyl group was confirmed by a singlet at 3.34 ppm in the ^1H NMR spectrum of **5**. To access the 2′-deoxy analog, **6**, disaccharide **15** was first converted to the corresponding xanthate derivative, **17**, and was then reduced with tri-*n*-butylstannane in the presence of AIBN yielding **18**. The conversion of **15** into **18** proceeded in 61% yield over the two steps. The target compound **6** was obtained in 90% yield from **18** by removal of the benzyl protecting groups under standard conditions. That the deoxygenation had taken place was clearly evident from the ^{13}C NMR spectrum of **6**, which showed the resonance for C-2′ at 37.2 ppm. Furthermore, in the ^1H NMR spectrum of **6**, the resonances for H-$2'_{eq}$ and H-$2'_{ax}$ appeared 2.03 and 1.59 ppm, respectively, as would be expected for a 2-deoxy sugar. Further proof of the structures of **4**–**6**, was obtained by measuring the $^1J_{C1,H1}$ of the terminal mannopyranose residue. The magnitudes of this coupling constant were between 169.1 and 170.1 Hz, thus confirming the stereochemistry of this residue as α.[44]

The remaining two disaccharides, **7** and **8**, were obtained as illustrated in Scheme 3. Reaction of **12** with the known glycosyl trichloroacetimidate **19**[45] in the presence of trimethylsilyl trifluoromethanesulfonate yielded



Scheme 3. Reagents and conditions: (a) **12**, TMSOTf, CH_2Cl_2, −10 → 0 °C, 89%; (b) $NaOCH_3$, CH_3OH, rt, 86%; (c) H_2, Pd/C, CH_3OH, 97%; (d) **12**, TMSOTf, CH_2Cl_2, −10 → 0 °C, 70%; (e) $NaOCH_3$, CH_3OH, rt, 95%; (f) H_2, Pd/C, CH_3OH, 76%.

20 in 89% yield. Although the use of the 3,4,5-tri-*O*-acetyl glycosyl imidate **19** required a two-step deprotection procedure on the product disaccharide, we nevertheless chose to use this donor based on previous reports[45] demonstrating that the acetylated species gives better α-selectivity in glycosylation reactions than the corresponding 3,4,5-tri-*O*-benzyl glycosyl imidate. The product was then deacetylated upon treatment with sodium methoxide in methanol to give **21**, which was next hydrogenated affording **7** in 83% overall yield. In a similar manner, the reaction of **12** with imidate **22**[46] provided a 70% yield of **23**. Disaccharide **8** was obtained in two steps and 72% overall yield by deacetylation to **24** followed by hydrogenolysis of the benzyl ethers. The $^{1}J_{C1,H1}$ of **7** and **8** were 169.5 and 168.4 Hz, respectively, as would be expected for an α-mannopyranoside.[44]

The presence of the fluorine atom in **7** and amino group in **8** were readily apparent by NMR spectroscopy. In the ^{13}C NMR spectrum of **7**, the resonances for C-1′, C-2′, and C-3′ were all split into doublets, with $J_{C,F}$ = 29.7, 172.6, and 17.2 Hz, respectively. In addition, a single fluorine signal was observed in the ^{1}H-coupled ^{19}F spectrum of **7**; this signal displayed the expected[47] ^{19}F–^{1}H coupling constants with H-1′ (7.2 Hz), H-2′ (48.7 Hz), and H-3′ (29.7 Hz). For **8**, the resonance of C-2′ appeared at 53.6 ppm in the ^{13}C NMR spectrum and the H-2′ resonance at 3.17 ppm in the ^{1}H NMR spectrum.

2.2. Screening of 4–8 as substrates for the PPM-dependent α-(1→6)-ManT from *M. smegmatis*

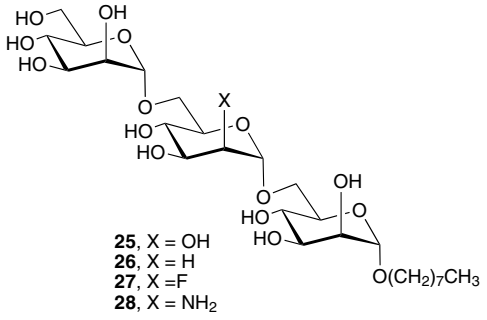
Once disaccharides **4**–**8** were synthesized, they were tested for activity as a substrate for the PPM-dependent α-(1→6)-ManT using the previously developed assay.[24] Briefly, each disaccharide at 2.0 mM was incubated with $^{14}C_1$-labeled β-D-mannopyranosyl phosphodecaprenol **3b** (synthesized in situ from $^{14}C_1$-labeled GDP-Man and decaprenolphosphate) and membrane extracts from *M. smegmatis*.[48] After 1 h, residual **3b** was removed by passage of the incubation mixture through an ion-exchange cartridge and the radioactivity in the effluent was measured by scintillation counting.

Under these conditions, disaccharides **4** and **6**–**8** all served as substrates for the enzyme as evidenced by the transfer of radioactivity from **3b** to the oligosaccharide. Only the 2′-methoxy analog, **5**, was inactive. Additional studies were then carried out to determine the K_m and V_{max} values for **4** and **6**–**8**, which are presented in Table 1. Interestingly, all of these oligosaccharides have similar K_m values, indicating that the enzyme binds each of the four compounds with comparable affinity. The V_{max} values for **4**, **6**, and **7** are also very similar, while the amino disaccharide, **8**, turns over slightly more slowly. However, the rate differences are not dramatic and it is therefore difficult to draw significant conclusions from this observation. To establish the identity of the products as trisaccharides, larger scale incubations with cold donor were carried out and the products were isolated and characterized by high-resolution mass spectrometry. The masses obtained for the products, as well all as the calculated values are presented in Table 1. These experiments enabled us to establish that trisaccharides were formed in these reactions. Previous studies[32] with disaccharide **4** clearly demonstrated that the product formed from these reactions is the linear trisaccharide **25**. Given the close structural similarity between **4** and the other oligosaccharides, we propose that products formed are also the corresponding linear trisaccharides (e.g., **26**–**28**).

Table 1. Screening of **4**–**8** as substrates of the PPM-dependent α-(1→6)-ManT from *M. smegmatis*

Compound	K_m (mM)	V_{max} (pmol/mg/min)	Mass of trisaccharide product	
			Calculated	Found[a]
4	1.19	4.76	616.2942	616.2920
5	—[b]	—[b]	—[b]	—[b]
6	1.63	4.44	600.2993	600.3056
7	1.76	4.17	618.2899	618.2855
8	1.33	2.63	615.3102	615.3076

[a] Determined by HR-ESI-MS.
[b] Disaccharide **5** is not a substrate.



To determine if any of these oligosaccharides were competitive inhibitors of the enzymes mixing experiments were carried out. In these studies, disaccharide **4** was used as a substrate at 0.2 mM and one of the other oligosaccharides **5**–**8** was added at concentrations of 1.0 and 2.0 mM. Under these conditions, no inhibition of transfer of radioactivity to **4** was observed, thus indicating that none of the compounds **5**–**8** sufficiently inhibit the enzyme in the presence of a 'native' acceptor.

Taken together, these results suggest that although the enzyme will tolerate groups of similar or smaller than a hydroxyl at the C-2′ position, it is not permissive of groups that are significantly larger. Furthermore, it appears that the 2′-OH group is not a 'key polar' group[49] of the carbohydrate epitope as the 2′-deoxy analog is a substrate for the enzyme.

In summary, we have described the synthesis of a panel of mannose-containing disaccharides in which the 2′-OH group has been replaced, independently, by deoxy, fluoro, amino, and methoxy functionalities. Evaluation of these compounds as potential substrates or inhibitors

of the PPM-dependent α-(1→6)-ManT involved in mycobacterial LAM biosynthesis revealed that the enzyme is somewhat tolerant substitution at this site. The enzyme recognizes groups equivalent or smaller in size than the native hydroxyl, but not the more sterically demanding methoxy derivative. In addition, it appears that protein does not form a critical hydrogen bonding interaction with the C-2′ hydroxyl group as the 2′-deoxy analog is a substrate for the enzyme. Future studies in the design of inhibitors based upon these oligosaccharides will involve replacement of the hydroxyl group at C-6′ with other functionalities, leading to compounds that are modified either at C-6′ only, or at both C-2′ and C-6′. For the latter case, we have demonstrated that when the C-6′ hydroxyl group in **8** is replaced by either an amino or fluoro substituent, the resulting compound is an inhibitor of the enzyme.[50]

3. Experimental

3.1. General methods

Unless otherwise indicated, all reactions were carried out at room temperature and under a positive pressure of argon. Solvents were evaporated under reduced pressure and below 40 °C. Analytical TLC was performed on silica gel 60-F_{254} (0.25 mm, Merck). Spots were detected under UV light or by charring with 10% H_2SO_4 in ethanol. Column chromatography was performed on Iatrobeads or silica gel. Iatrobeads refers to a beaded silica gel 6RS–8060, which is manufactured by Iatron Laboratories (Tokyo). The ratio between silica gel and compound ranged from 100 to 50:1 (w/w). 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 800 MHz, and first order proton chemical shifts, δ_H, are referenced to either to TMS (δ_H 0.0, $CDCl_3$) or HOD (δ_H 4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 125.8 or 150.9 MHz and ^{13}C chemical shifts, δ_C, are referenced to either to TMS (δ_C 0.0, $CDCl_3$), dioxane (δ_C 67.4, D_2O) or CD_3OD (δ_C 48.9). ^{19}F NMR spectra were recorded at 235.4 MHz and ^{19}F chemical shifts, δ_F, are referenced to (5%) $CFCl_3$ in absolute ethanol as the external standard. The assignment of resonances in all the final deprotected compounds **4**–**8** were made by two-dimensional homonuclear and heteronuclear shift correlation experiments. For **4**–**8**, the stereochemistry of both mannopyranose residues was proven through measurement of the $^1J_{C1-H1}$.[44] Fast atom bombardment mass spectra were recorded on samples suspended in thioglyceride matrix with a cesium gun. MALDI mass spectra were recorded on samples in an α-cyano-4-hydroxycinnamic acid matrix. Electrospray mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl.

3.2. Octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (4)

Disaccharide **15** (100 mg, 0.10 mmol) was dissolved in CH_3OH (15 mL), and 10% Pd/C (30 mg) was added. The solution was stirred overnight under a H_2 atmosphere and then the catalyst was separated by filtration and washed with CH_3OH (10 mL). After concentrating the filtrate and the washings, the product was purified by chromatography (4:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **4** (41 mg, 91%) as a foam. R_f 0.23 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +27.3 (*c* 0.7, H_2O); ^{1}H NMR (800 MHz, D_2O): δ_H 4.91 (br s, 1H, H-1′), 4.74 (br s, 1H, H-1), 3.91–3.93 (m, 1H, H-6a), 3.90 (br s, 1H, H-2′), 3.81 (br s, 1H, H-2), 3.78 (d, 1H, $J_{6a',6b'}$ = 11.9 Hz, H-6a′), 3.75 (dd, 1H, $J_{2',3'}$ = 2.0 Hz, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.69–3.71 (m, 2H, H-4, H-6b′), 3.65 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.5 Hz, H-3), 3.55–3.60 (m, 5H, H-5, H-6b, H-4′, H-5′, octyl OCH_2), 3.39 (dt, 1H, *J* = 6.4, 9.3 Hz, octyl OCH_2), 1.50–1.52 (m, 2H, octyl CH_2), 1.19–1.26 (m, 10H, octyl CH_2), 0.79 (t, 3H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, D_2O): δ_C 100.5 (C-1, $^1J_{C,H}$ = 171.3 Hz), 100.0 (C-1′, $^1J_{C,H}$ = 170.1 Hz), 73.0 (C-5′), 71.6 (C-3), 71.4 (C-5), 71.1 (C-3′), 70.7 (C-2), 70.4 (C-2′), 68.2 (octyl OCH_2), 67.0 (C-4), 66.8 (C-4′), 65.8 (C-6), 61.3 (C-6′), 32.0, 29.5, 29.5, 29.4, 26.3, 22.8 (octyl CH_2), 14.1 (octyl CH_3). HR-FAB-MS calcd for $C_{20}H_{38}O_{11}$ $[M+H]^+$ 455.2492, found 455.2479.

3.3. Octyl 2-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (5)

Disaccharide **16** (90 mg, 0.09 mmol) was hydrogenated with 10% Pd/C (23 mg) in CH_3OH (12 mL), as described for the preparation of **4**. The product was purified by chromatography (4:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **5** (36 mg, 87%) as a foam. R_f 0.57 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +123 (*c* 1.1, H_2O); ^{1}H NMR (800 MHz, D_2O): δ_H 4.90 (br s, 1H, H-1′), 4.70 (br s, 1H, H-1), 3.84 (dd, 1H, $J_{5,6b}$ = 3.8 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.78 (br s, 1H, H-2), 3.74 (dd, 1H, $J_{5',6a'}$ = 2.5 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6a′), 3.71–3.72 (m, 1H, H-3′), 3.59–3.63 (m, 5H, H-3, H-4, H-5, H-6a, H-6b′), 3.57 (dt, 1H, *J* = 6.9, 9.5 Hz, octyl OCH_2), 3.52 (ddd, 1H, $J_{5',6a'}$ = 2.5 Hz, $J_{5',6b'}$ = 4.1 Hz, $J_{4',5'}$ = 9.6 Hz, H-5′), 3.47 (d, 1H, $J_{2',3'}$ = 2.4 Hz, H-2′), 3.44 (t, 1H, $J_{3',4'} = J_{4',5}$ = 9.6 Hz, H-4′), 3.36 (dt, 1H, *J* = 6.9, 9.5 Hz, octyl OCH_2), 3.34 (s, 3H, OCH_3), 1.46–1.48 (m, 2H, octyl CH_2), 1.16–1.23 (m, 10H, octyl CH_2), 0.75 (t, 3H, *J* = 7.1 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, D_2O): δ_C 100.4 (C-1, $^1J_{C,H}$ = 170.0 Hz), 96.8 (C-1′, $^1J_{C,H}$ = 169.1 Hz), 80.5 (C-2′), 73.0 (C-5′), 71.5 (C-5), 71.4 (C-3), 70.8 (C-3′), 70.6 (C-2), 68.2 (octyl OCH_2), 67.5 (C-4′), 66.9 (C-4), 66.1 (C-6), 61.4 (C-6′), 59.3 (OCH_3), 31.9, 29.3, 29.3, 29.3, 26.2, 22.7 (octyl CH_2), 14.1 (octyl CH_3). HR-FAB-MS calcd for $C_{21}H_{40}O_{11}$ $[M+H]^+$ 469.2648, found 469.2672.

3.4. Octyl 2-deoxy-α-D-arabino-hexopyranosyl-(1→6)-α-D-mannopyranoside (6)

Disaccharide **18** (100 mg, 0.10 mmol) was hydrogenated with 10% Pd/C (20 mg) in CH_3OH (8 mL), as described for the preparation of **4**. The product was purified by chromatography (4:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **6** (40 mg, 90%) as a foam. R_f 0.53 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +44.0 (*c* 1.0, H_2O); ^{1}H NMR

(800 MHz, D_2O): δ_H 4.88 (d, 1H, $J_{2ax',1'}$ = 3.2 Hz, H-1′), 4.70 (br s, 1H, H-1), 3.79–3.82 (m, 2H, H-6b, H-3′), 3.77 (br s, 1H, H-2), 3.71 (d, 1H, $J_{6a',6b'}$ = 12.3 Hz, H-6a′), 3.64 (dd, 1H, $J_{5',6b'}$ = 4.9 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6b′), 3.59–3.63 (m, 3H, H-3, H-4, H-5), 3.55–3.57 (m, 2H, H-5′, octyl OCH_2), 3.51 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 10.7 Hz, H-6a), 3.34–3.36 (m, 1H, octyl OCH_2), 3.25 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 2.03 (dd, 1H, $J_{2'eq,3'}$ = 5.1 Hz, $J_{2'eq,2'ax}$ = 12.7 Hz, H-2′eq), 1.59 (ddd, 1H, $J_{2'ax,3'} = J_{2'eq,2'ax}$ = 12.7 Hz, $J_{2'ax,1'}$ = 3.2 Hz, H-2′ax), 1.45–1.48 (m, 2H, octyl CH_2), 1.15–1.22 (m, 10H, octyl CH_2), 0.74 (t, 3H, J = 7.2 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, D_2O): δ_C 100.5 (C-1, $^1J_{C,H}$ = 168.9 Hz), 97.4 (C-1′, $^1J_{C,H}$ = 169.5 Hz), 72.6 (C-5′), 71.6 (C-4′), 71.4 (C-5), 71.3 (C-3), 70.7 (C-2), 68.7 (C-3′), 68.2 (octyl OCH_2), 66.9 (C-4), 65.6 (C-6), 61.1 (C-6′), 37.2 (C-2′), 32.0, 29.5, 29.4, 29.3, 26.2, 22.8 (octyl CH_2), 14.1 (octyl CH_3). HR-FAB-MS calcd for $C_{20}H_{38}O_{10}$ $[M+H]^+$ 439.2543, found 439.2526.

3.5. Octyl 2-deoxy-2-fluoro-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (7)

Disaccharide **21** (120 mg, 0.17 mmol) was hydrogenated with 10% Pd/C (40 mg) in CH_3OH (12 mL), as described for the preparation of **4**. The product was purified by chromatography (4:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **7** (75 mg, 97%) as a foam. R_f 0.60 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +187 (*c* 0.7, H_2O); ^1H NMR (800 MHz, D_2O): δ_H 4.98 (d, 1H, $J_{H1',F}$ = 7.2 Hz, H-1′), 4.72 (d, 1H, $J_{H2',F}$ = 48.7 Hz, H-2′), 4.73 (br s, 1H, H-1), 3.96–3.98 (m, 1H, H-6b), 3.85 (dd, 1H, $J_{3',4'}$ = 8.1 Hz, $J_{H3',F}$ = 29.7 Hz, H-3′), 3.82 (br s, 1H, H-2), 3.79 (d, 1H, $J_{6a',6b'}$ = 12.0 Hz, H-6a′), 3.71 (dd, 1H, $J_{5,6b'}$ = 4.0 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6b′), 3.70 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.64–3.66 (m, 3H, H-3, H-4′, H-5), 3.55–3.61 (m, 3H, H-5, H-6a, octyl OCH_2), 3.36 (dt, 1H, J = 6.5, 9.4 Hz, octyl OCH_2), 1.49–1.51 (m, 2H, octyl CH_2), 1.20–1.26 (m, 10H, octyl CH_2), 0.80 (t, 3H, J = 7.1 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, D_2O): δ_C 100.6 (C-1, $^1J_{C,H}$ = 169.6 Hz), 97.3 (C-1′, $^1J_{C,H}$ = 169.5 Hz, $J_{C-1',F}$ = 29.7 Hz), 90.0 (C-2′, $J_{C-2',F}$ = 172.6 Hz), 73.1 (C-5′), 71.6 (C-4′), 71.4 (C-5), 70.7 (C-2), 70.2 (C-3′, $J_{C-3',F}$ = 17.2 Hz), 68.2 (octyl OCH_2), 67.2 (C-3), 66.7 (C-4), 66.2 (C-6), 61.0 (C-6′), 32.2, 29.8, 29.7, 29.6, 29.4, 22.8 (octyl CH_2), 14.2 (octyl CH_3). ^{19}F NMR (235.4 MHz, D_2O): δ_F −204.7 (ddd, 1F, $J_{H1',F}$ = 7.2 Hz, $J_{H2',F}$ = 48.7 Hz, $J_{H3',F}$ = 29.7 Hz, F-2′). HR-FAB-MS calcd for $C_{20}H_{37}O_{10}F$ $[M+Na]^+$ 479.2268, found 479.2234.

3.6. Octyl 2-amino-2-deoxy-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (8)

To a solution of **24** (70 mg, 0.09 mmol) in HOAc (5 mL), was added 10% Pd/C (25 mg). The solution was stirred overnight under an H_2 atmosphere and then the catalyst was separated by filtration and washed with CH_3OH (10 mL). After concentrating the filtrate and the washings, the product was purified by chromatography (10:2:0.5 $CHCl_3$–CH_3OH–5 N aq NH_4OH) on Iatrobeads to give **8** (32 mg, 76%) as a colorless foam. R_f 0.20 (10:4:1 $CHCl_3$–CH_3OH–(5 N) aq NH_4OH); $[\alpha]_D$ +35.5 (*c* 1.5, H_2O); ^1H NMR (800 MHz, D_2O): δ_H 4.77 (br s, 1H, H-1′), 4.70 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 3.88 (dd, 1H, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.84 (dd, 1H, $J_{2',3'}$ = 4.3 Hz, $J_{3',4'}$ = 9.6 Hz, H-3′), 3.80 (dd, 1H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 3.74 (dd, 1H, $J_{5',6a'}$ = 1.7 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a′), 3.69 (dd, 1H, $J_{5',6b'}$ = 4.9 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6b′), 3.67 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.63 (dd, 1H, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.56–3.61 (m, 4H, H-5, H-5′, H-6a, octyl OCH_2), 3.55 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, H-4′), 3.38 (dt, 1H, J = 6.4, 9.7 Hz, octyl OCH_2), 3.17 (d, 1H, $J_{2',3'}$ = 4.3 Hz, H-2′), 1.47–1.51 (m, 2H, octyl CH_2), 1.17–1.24 (m, 10H, octyl CH_2), 0.76 (t, 3H, J = 6.8 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, D_2O): δ_C 100.0 (C-1, $^1J_{C,H}$ = 169.7 Hz), 99.7 (C-1′, $^1J_{C,H}$ = 168.4 Hz), 72.5, 71.1, 71.0 (C-3, C-5, C-5′), 70.2 (C-2), 70.0 (C-3′), 67.9 (octyl OCH_2), 66.4 (C-4), 66.1 (C-4′), 65.6 (C-6), 60.6 (C-6′), 53.6 (C-2′), 31.5, 28.9, 28.9, 28.8, 25.7, 22.3 (octyl CH_2), 13.6 (octyl CH_3). HR-FAB-MS calcd for $C_{20}H_{39}O_{10}N$ $[M+H]^+$ 454.2652, found 454.2667.

3.7. Octyl 6-*O*-triphenylmethyl-α-D-mannopyranoside (10)

Octyl α-D-mannopyranoside **9**[42] (4.4 g, 15.2 mmol) was dissolved in pyridine (50 mL), and triphenylmethyl chloride (6.4 g, 22.8 mmol) was added. The solution was stirred overnight at 50 °C, then cooled and the solvent was evaporated. Traces of pyridine were coevaporated with toluene (3 × 50 mL). The crude brown residue was purified by chromatography (1:1 toluene–EtOAc) on silica gel to give **10** (6.4 g, 80%) as light yellow solid. R_f 0.42 (1:1 toluene–EtOAc); $[\alpha]_D$ +16.9 (*c* 1.3, $CHCl_3$); ^1H NMR (500 MHz, CD_3OD): δ_H 7.50–7.51 (m, 5H), 7.21–7.31 (m, 10H), 4.83 (br s, 1H), 3.98 (dt, 1H, J = 7.0, 9.4 Hz), 3.83–3.86 (m, 2H), 3.70 (dd, 1H, J = 3.4, 9.4 Hz), 3.52 (dt, 1H, J = 6.4, 9.5 Hz), 3.49 (dd, 1H, J = 9.7, 9.7 Hz), 3.33 (br s, 1H), 3.28 (dd, 1H, J = 1.7, 9.7 Hz), 1.68–1.75 (m, 2H), 1.45–1.50 (m, 2H), 1.27–1.39 (m, 8H), 0.88 (t, 3H, J = 6.9 Hz); ^{13}C NMR (125.8 MHz, CD_3OD): δ_C 147.8, 144.6, 129.0, 128.3, 127.7, 127.7, 127.0, 100.4, 86.7, 72.8, 72.0, 71.2, 68.2, 67.5, 64.4, 32.0, 29.7, 29.6, 29.5, 26.5, 22.8, 13.5. HR-MALDI-MS calcd for $C_{32}H_{42}O_6$ $[M+Na]^+$ 557.2879, found 557.2923.

3.8. Octyl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (11)

Monosaccharide **10** (1.26 g, 2.36 mmol) was dissolved in DMF (10 mL), the solution was cooled to 0 °C, and sodium hydride (0.28 g, 11.8 mmol) was added with stirring. Benzyl bromide (1.1 mL, 9.2 mmol) was added after allowing the solution to warm to room temperature. The reaction mixture was stirred overnight and then CH_3OH (5 mL) was added, followed by stirring for 3 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (30 mL). The organic layer was washed with water (2 × 25 mL), dried (Na_2SO_4), filtered, and concentrated to a yellow oil, which was purified by chromatography (toluene) on silica gel to give **11** (1.5 g, 79%) as a colorless oil. R_f 0.37 (toluene); $[\alpha]_D$

+18.7 (*c* 0.3, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.21–7.52 (m, 28H), 6.89 (d, 2H, J = 7.0 Hz), 4.91 (br s, 1H), 4.84 (d, 1H, J = 12.5 Hz), 4.62–4.64 (m, 4H), 4.27 (d, 1H, J = 10.4 Hz), 4.00 (dd, 1H, J = 9.6, 9.6 Hz), 3.90 (dd, 1H, J = 2.9, 9.7 Hz), 3.81–3.82 (m, 2H), 3.76 (ddd, 1H, J = 7.0, 7.0, 9.6 Hz), 3.50 (d, 1H, J = 9.6 Hz), 3.41 (dt, 1H, J = 6.4, 9.6 Hz), 3.27 (dd, 1H, J = 5.5, 9.7 Hz), 1.52–1.57 (m, 2H), 1.26–1.28 (m, 10H), 0.86 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 144.7, 139.2, 139.2, 138.7, 129.4, 128.8, 128.7, 128.7, 128.2, 128.2, 128.0, 128.0, 127.9, 127.3, 98.1, 86.7, 80.9, 76.2, 75.7, 75.6, 73.2, 72.8, 72.4, 72.4, 67.9, 63.6, 32.3, 30.0, 29.9, 29.8, 26.7, 23.2, 14.6. Anal. Calcd for $C_{53}H_{60}O_6$ (793.05): C, 80.56; H, 7.51, found: C, 80.34; H, 7.51.

3.9. Octyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (12)

Triphenylmethyl ether **11** (4.71 g, 5.86 mmol) was dissolved in CH_2Cl_2–CH_3OH (2:1, 60 mL), *p*-toluenesulfonic acid (1.0 g, 5.26 mmol) was added and the mixture was stirred for 45 min. The reaction mixture was then diluted with CH_2Cl_2 (40 mL), washed with a saturated $NaHCO_3$ solution (2 × 30 mL) and water (50 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated to a light yellow oil, which was purified by chromatography (4:1 toluene–EtOAc) on silica gel to give **12** (2.8 g, 92%) as a colorless oil. R_f 0.51 (1:1 toluene–EtOAc); $[\alpha]_D$ +27.6 (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.24–7.36 (m, 15H), 4.93 (d, 1H, J = 10.9 Hz), 4.77 (d, 1H, J = 12.4 Hz), 4.61–4.69 (m, 4H), 3.98 (dd, 1H, J = 9.5, 9.5 Hz), 3.92 (dd, 1H, J = 2.8, 9.4 Hz), 3.76–3.82 (m, 3H), 3.65 (dd, 1H, J = 4.1, 9.4 Hz), 3.61 (dt, 1H, J = 6.8, 9.5 Hz), 3.30 (dt, 1H, J = 6.5, 9.5 Hz), 1.48–1.51 (m, 2H), 1.26–1.31 (m, 10H), 0.88 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 139.1, 139.0, 138.9, 128.9, 128.9, 128.6, 128.3, 128.2, 128.2, 128.1, 128.0, 98.7, 80.8, 75.7, 75.6, 75.5, 73.4, 72.8, 72.7, 68.2, 62.9, 32.3, 29.9, 29.9, 29.7, 26.6, 23.2, 14.6. Anal. Calcd for $C_{35}H_{46}O_6$ (562.40): C, 74.70; H, 8.24, found: C, 74.65; H, 8.33.

3.10. Octyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (14)

Thioglycoside **13**[41] (337 mg, 0.63 mmol) and alcohol **12** (272 mg, 0.49 mmol) were dried under vacuum with powdered 4 Å molecular sieves (300 mg) overnight. Dry CH_2Cl_2 (12 mL) was added and the mixture was cooled to −40 °C and stirred for 10 min. *N*-Iodosuccinimide (176 mg, 0.79 mmol) was added and the solution was stirred for 20 min before silver triflate (40 mg, 0.16 mmol) was added. The reaction mixture was warmed to 0 °C over 30 min and then triethylamine (0.5 mL) was added. The yellow solution was filtered, diluted with CH_2Cl_2 (25 mL), washed with a saturated $Na_2S_2O_3$ solution (2 × 20 mL), followed by brine (20 mL) and water (20 mL). After drying (Na_2SO_4), the organic layer was concentrated to a brown syrup, which was purified by chromatography (4:1 hexane–EtOAc) on silica gel to give **14** (430 mg, 86%) as a colorless syrup. R_f 0.38 (4:1 hexane–EtOAc); $[\alpha]_D$ +40.5 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.12–7.39 (m, 30H), 5.50 (d, 1H, J = 2.0 Hz), 4.97 (br s, 1H), 4.91 (d, 1H, J = 11.2 Hz), 4.85 (d, 1H, J = 10.9 Hz), 4.81 (br s, 1H), 4.72 (s, 2H), 4.61–4.66 (m, 4H), 4.49 (d, 2H, J = 11.1 Hz), 4.44 (dd, 1H, J = 9.5, 9.5 Hz), 4.42–4.46 (m, 2H), 3.97 (dd, 1H, J = 2.9, 9.3 Hz), 3.88–3.91 (m, 4H), 3.80 (dd, 1H, J = 1.7, 9.7 Hz), 3.77–3.78 (m, 1H), 3.69–3.72 (m, 2H), 3.57–3.68 (m, 2H), 3.32 (dt, 1H, J = 6.5, 9.8 Hz), 2.13 (s, 3H), 1.48–1.51 (m, 2H), 1.26–1.29 (m, 10H), 0.87 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 170.7, 139.2, 139.0, 139.0, 138.8, 138.4, 128.9, 128.8, 128.8, 128.7, 128.7, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 98.5, 98.1, 80.9, 78.3, 75.5, 75.5, 75.4, 75.2, 74.7, 73.8, 73.1, 72.5, 71.9, 71.9, 71.6, 69.3, 69.0, 68.1, 67.2, 32.3, 29.9, 29.9, 29.7, 26.7, 23.1, 21.6, 14.6. HR-MALDI-MS calcd for $C_{64}H_{76}O_{12}$ $[M+Na]^+$ 1059.5234, found 1059.5286.

3.11. Octyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (15)

A solution of **14** (270 mg, 0.27 mmol) in methanol (20 mL), was treated with two drops of 1 M $NaOCH_3$. After stirring overnight, the solution was neutralized with a minimum amount of prewashed Amberlite 118 H^+ resin and concentrated to a syrup, which was purified by chromatography (2:1 hexane–EtOAc) on silica gel to give **15** (246 mg, 96%) as a colorless syrup. R_f 0.32 (2:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.21–7.38 (m, 28H), 7.13–7.15 (m, 2H), 5.09 (d, 1H, J = 1.1 Hz), 4.91 (d, 1H, J = 11.0 Hz), 4.85 (d, 1H, J = 10.9 Hz), 4.81 (d, 1H, J = 10.9 Hz), 4.80 (d, 1H, J = 1.5 Hz), 4.68 (ABq, 2H, J = 12.2 Hz, $\Delta\upsilon$ = 30.2 Hz), 4.44–4.64 (m, 7H), 3.16–4.13 (m, 13H), 3.32 (dt, 1H, J = 6.6, 9.7 Hz), 2.36 (d, 1H, J = 2.7 Hz), 1.48–1.51 (m, 2H), 1.26–1.30 (m, 10H), 0.87 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 139.0, 138.9, 138.8, 138.3, 129.0, 128.8, 128.8, 128.7, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 100.1, 98.1, 80.8, 80.1, 75.6, 75.6, 75.5, 75.1, 74.7, 73.8, 73.2, 72.6, 72.0, 71.9, 71.5, 69.3, 68.5, 68.1, 66.7, 32.3, 30.0, 30.0, 29.7, 26.7, 23.2, 14.6. HR-ESI-MS calcd for $C_{62}H_{74}O_{11}$ $[M+Na]^+$ 1017.5123, found 1017.5122.

3.12. Octyl 2-*O*-methyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (16)

Disaccharide **15** (126 mg, 0.14 mmol) was dissolved in DMF (5 mL), sodium hydride (33 mg, 11.8 mmol) was added and the mixture was stirred for 15 min. Methyl iodide (80 mL, 1.4 mmol) was added and the reaction mixture was stirred overnight before CH_3OH (1.0 mL) was added, followed by stirring for 1 h. The reaction mixture was concentrated and the residue was dissolved in CH_2Cl_2 (20 mL), and then washed with water (2 × 15 mL), dried (Na_2SO_4). The organic layer was filtered and concentrated to a light yellow syrup, which was purified by chromatography (4:1 hexane–EtOAc) on silica gel to give **16** (120 mg, 87%) as a colorless syr-

up. R_f 0.41 (4:1 hexane–EtOAc); $[\alpha]_D$ +47.7 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.11–7.37 (m, 30H), 5.14 (d, 1H, $J = 1.4$ Hz), 4.86 (d, 2H, $J = 10.9$ Hz), 4.80 (d, 1H, $J = 1.4$ Hz), 4.73 (ABq, 2H, $J = 12.3$ Hz, $\Delta\upsilon = 28.7$ Hz), 4.55–4.68 (m, 6H), 4.46 (dd, 1H, $J = 1.4$, 2.0 Hz), 4.43–4.44 (m, 2H), 3.59–3.94 (m, 12H), 3.42 (s, 3H), 3.32 (dt, 1H, $J = 6.5$, 9.6 Hz), 1.48–1.49 (m, 2H), 1.26–1.29 (m, 10H), 0.88 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 139.2, 139.0, 139.0, 138.9, 138.9, 138.7, 128.8, 128.8, 128.7, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.8, 98.3, 98.0, 80.9, 79.6, 75.7, 75.6, 75.5, 75.2, 75.0, 73.8, 73.4, 72.7, 72.2, 72.1, 72.0, 69.6, 68.0, 66.5, 59.3, 32.3, 29.9, 29.9, 29.7, 29.6, 23.1, 14.6. HR-MALDI-MS calcd for $C_{63}H_{76}O_{11}$ $[M+Na]^+$ 1031.5285, found 1031.5265.

3.13. Octyl 3,4,6-tri-*O*-benzyl-2-*O*-(methylthio)thiocarbonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (17)

Disaccharide **15** (300 mg, 0.30 mmol) was dissolved in THF (20 mL), and then sodium hydride (44 mg, 1.83 mmol) was added along with a catalytic amount of imidazole (10 mg). The mixture was stirred for 1 h, before carbon disulfide (230 mL, 3.9 mmol) was added and the stirring continued for an additional hour. Methyl iodide (132 mL, 2.12 mmol) was then added and the reaction mixture was stirred for 12 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (25 mL), and washed with water (20 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to a yellow syrup, which was purified by chromatography (4:1 hexane–EtOAc) on silica gel to give **17** (324 mg, 99%) as a light yellow syrup. R_f 0.46 (4:1 hexane–EtOAc); $[\alpha]_D$ +26.7 (*c* 1.7, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.16–7.38 (m, 30H), 6.26 (dd, 1H, $J = 2.0$, 2.0 Hz), 5.11 (d, 1H, $J = 2.0$ Hz), 4.91 (d, 1H, $J = 11.1$ Hz), 4.87 (d, 1H, $J = 10.9$ Hz), 4.80 (d, 1H, $J = 1.5$ Hz), 4.61–4.71 (m, 6H), 4.42–4.52 (m, 4H), 4.09 (dd, 1H, $J = 3.0$, 9.3 Hz), 3.98 (dd, 1H, $J = 9.6$, 9.6 Hz), 3.86–3.91 (m, 3H), 3.83 (dd, 1H, $J = 1.5$, 1.5 Hz), 3.73–3.77 (m, 4H), 3.65 (dd, 1H, $J = 1.4$, 9.2 Hz), 3.60 (dt, 1H, $J = 6.8$, 9.7 Hz), 3.31 (dt, 1H, $J = 6.8$, 9.7 Hz), 2.48 (s, 3H), 1.47–1.50 (m, 2H), 1.26–1.29 (m, 10H), 0.88 (t, 3H, $J = 6.4$ Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 215.5, 139.0, 139.0, 138.8, 128.8, 128.8, 128.7, 128.6, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 98.1, 97.3, 80.9, 78.3, 77.2, 75.6, 75.5, 75.4, 75.2, 75.0, 73.9, 73.1, 72.5, 72.1, 72.0, 71.5, 69.3, 68.2, 67.1, 32.3, 29.9, 29.9, 29.8, 26.7, 23.2, 19.1, 14.6. HR-MALDI-MS calcd for $C_{64}H_{76}O_{11}S_2$ $[M+Na]^+$ 1107.4727, found 1107.4630.

3.14. Octyl 3,4,6-tri-*O*-benzyl-2-deoxy-α-D-arabino-hexopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (18)

Xanthate **17** (296 mg, 0.27 mmol) was dissolved in dry toluene (50 mL), and tri-*n*-butylstannane (1.1 mL, 4.05 mmol) and AIBN (36 mg, 0.22 mmol) were added. The reaction mixture was heated at reflux for 2 h and the solvent was evaporated. The crude syrup was purified chromatography (4:1 hexane–EtOAc) on Iatrobeads to give **18** (160 mg, 61%). R_f 0.31 (4:1 hexane–EtOAc); $[\alpha]_D$ +52.2 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.14–7.37 (m, 30H), 5.12 (d, 1H, $J = 3.4$ Hz), 4.93 (d, 1H, $J = 11.2$ Hz), 4.88 (d, 1H, $J = 11.0$ Hz,), 4.81 (d, 1H, $J = 1.2$ Hz), 4.71 (ABq, 2H, $J = 12.4$ Hz, $\Delta\upsilon = 31.9$ Hz), 4.53–4.68 (m, 6H), 4.48 (d, 1H, $J = 11.0$ Hz), 4.43 (d, 1H, $J = 12.1$ Hz), 3.90–4.00 (m, 3H), 3.87 (dd, 1H, $J = 4.6$, 11.3 Hz), 3.76–3.78 (m, 1H), 3.74–3.75 (m, 1H), 3.70 (dd, 1H, $J = 4.4$, 10.4 Hz), 3.57–3.67 (m, 4H), 3.55 (dd, 1H, $J = 1.6$, 10.4 Hz), 3.32 (dt, 1H, $J = 6.5$, 9.7 Hz), 2.37 (dd, 1H, $J = 4.9$, 10.4 Hz), 1.68 (ddd, 1H, $J = 12.7$, 12.7, 3.4 Hz), 1.49–1.51 (m, 2H), 1.26–1.30 (m, 10H), 0.87 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 139.2, 139.1, 139.0, 138.6, 128.8, 128.8, 128.7, 128.4, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 98.2, 98.1, 80.9, 78.7, 75.6, 75.5, 75.4, 75.3, 75.3, 73.9, 73.1, 72.6, 72.0, 72.0, 71.3, 69.3, 68.1, 66.4, 35.7, 32.3, 29.9, 29.9, 29.7, 26.6, 23.1, 14.6. HR-MALDI-MS calcd for $C_{62}H_{74}O_{10}$ $[M+Na]^+$ 1001.5180, found 1001.5177.

3.15. Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (20)

Trichloroacetimidate **19**[45] (216 mg, 0.48 mmol) and alcohol **12** (223 mg, 0.40 mmol) were dried in vacuo with powdered 4 Å molecular sieves (300 mg) overnight. Dry CH_2Cl_2 (8 mL) was added and the mixture was cooled to −10 °C with stirring. A solution of TMSOTf (200 μL) in CH_2Cl_2 (1.25 mL) was added dropwise to the reaction mixture and the stirring was continued for 2 h, while warming to 0 °C. The solution was neutralized by the addition of a saturated $NaHCO_3$ solution (3 mL) and then, CH_2Cl_2 (30 mL) was added. The organic layer was washed with water (20 mL), dried (Na_2SO_4), filtered, and concentrated to a colorless syrup, which was purified by chromatography (4:1 hexane–EtOAc) on silica gel to give **20** (302 mg, 89%) as a colorless syrup. R_f 0.44 (4:1 hexane–EtOAc); $[\alpha]_D$ +58.6 (*c* 1.3, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.25–7.36 (m, 15H), 5.31 (dd, 1H, $J = 9.9$, 9.9 Hz), 5.27 (ddd, 1H, $J = 2.0$, 29.5, 9.9 Hz), 5.22 (dd, 1H, $J = 2.0$, 7.5 Hz), 4.98 (d, 1H, $J = 11.2$ Hz), 4.76 (ddd, 1H, $J = 2.0$, 2.0, 49.7 Hz), 4.75–4.79 (m, 2H), 4.60–4.66 (m, 4H), 4.19 (dd, 1H, $J = 4.6$, 12.3 Hz), 4.11 (dd, 1H, $J = 2.1$, 12.3 Hz), 4.01–4.03 (m, 1H), 3.90–3.92 (m, 3H), 3.88 (dd, 1H, $J = 4.7$, 11.4 Hz), 3.79 (dd, 1H, $J = 1.0$, 11.4 Hz), 3.69–3.71 (m, 1H), 3.60 (dt, 1H, $J = 6.7$, 9.7 Hz), 3.33 (dt, 1H, $J = 6.5$, 9.7 Hz), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.50–1.53 (m, 2H), 1.27–1.32 (m, 10H), 0.88 (t, 3H, $J = 7.0$ Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 171.2, 170.2, 170.0, 138.9, 138.8, 138.8, 128.8, 128.8, 128.8, 128.3, 128.1, 128.1, 128.1, 128.0, 98.2, 97.8 (d, $J = 29.0$ Hz), 87.4 (d, $J = 179.5$ Hz), 80.8, 75.5, 74.8, 73.3, 72.6, 72.2, 70.3, 70.1 (d, $J = 16.4$ Hz), 68.9, 68.2, 67.2, 66.4, 62.5, 32.3, 29.8, 29.8, 29.7, 26.6, 23.1, 21.2, 21.1, 21.1, 14.5; ^{19}F NMR (235.4 MHz, $CDCl_3$): δ_F −203.2 (ddd, 1F, $J = 7.5$, 49.7, 29.5 Hz). HR-MALDI-MS calcd for $C_{47}H_{61}O_{13}F$ $[M+Na]^+$ 875.3994, found 875.4004.

3.16. Octyl 2-deoxy-2-fluoro-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (21)

Disaccharide **20** (245 mg, 0.29 mmol) was deacylated in CH_3OH (15 mL), with five drops of 1 M $NaOCH_3$ as described for the preparation of **15**. The product was purified by chromatography (9:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **21** (180 mg, 86%) as a colorless syrup. R_f 0.42 (9:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +56.3 (*c* 0.4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.24–7.25 (m, 15H), 5.10 (dd, 1H, J = 0.8, 7.4 Hz), 4.94 (d, 1H, J = 11.2 Hz), 4.76 (d, 1H, J = 1.2 Hz), 4.71 (d, 1H, J = 12.3 Hz), 4.65 (d, 1H, J = 12.2 Hz), 4.60–4.64 (m, 3H), 3.62–3.94 (m, 12H), 3.56 (dt, 1H, J = 6.5, 9.6 Hz), 3.30 (dt, 1H, J = 6.5, 9.6 Hz), 1.47–1.49 (m, 2H), 1.26–1.31 (m, 10H), 0.88 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 138.8, 138.8, 138.6, 128.9, 128.8, 128.3, 128.2, 128.1, 128.1, 98.4 (d, J = 29.4 Hz), 98.0, 90.0 (d, J = 173.9 Hz), 80.5, 75.5, 74.8, 73.1, 72.6, 72.5, 72.5, 71.9, 71.1 (d, J = 17.7 Hz), 68.2, 67.9, 67.1, 62.1, 32.3, 29.8, 29.8, 29.7, 26.6, 23.1, 14.6; ^{19}F NMR (235.4 MHz, $CDCl_3$): δ_F −206.0 (ddd, 1F, J = 7.4, 49.5 Hz, 33.0 Hz). HR-MALDI-MS calcd for $C_{41}H_{55}O_{10}F$ $[M+Na]^+$ 749.3677, found 749.3646.

3.17. Octyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (23)

Trichloroacetimidate **22**[46] (335 mg, 0.71 mmol) and alcohol **12** (335 mg, 0.60 mmol) were dried in vacuo with powdered 4 Å molecular sieves (300 mg) overnight. Dry CH_2Cl_2 (8 mL) was added and the mixture was cooled to −10 °C with stirring. A solution of TMSOTf (350 μL) in CH_2Cl_2 (1.25 mL) was added dropwise to the reaction mixture and the stirring was continued for 2 h, while warming to 0 °C. The solution was neutralized by the addition of a saturated $NaHCO_3$ solution (0.5 mL) and CH_2Cl_2 (40 mL) was added. The organic layer was washed with water (20 mL), dried (Na_2SO_4), filtered, and concentrated to a colorless syrup. The crude syrup was purified by chromatography (3:1 hexane–EtOAc) on silica gel to give **23** (365 mg, 70%) as a colorless syrup. R_f 0.43 (3:1 hexane–EtOAc); $[\alpha]_D$ +42.1 (*c* 1.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.31–7.41 (m, 15H), 5.43 (dd, 1H, J = 3.8, 9.8 Hz), 5.34–5.35 (m, 1H), 5.33 (dd, 1H, J = 9.8, 9.8 Hz), 5.10 (d, 1H, J = 1.7 Hz), 5.05 (d, 1H, J = 11.2 Hz), 4.81 (d, 1H, J = 12.2 Hz), 4.80 (d, 1H, J = 1.7 Hz), 4.73 (d, 1H, J = 12.4 Hz), 4.68–4.71 (m, 3H), 4.21 (dd, 1H, J = 4.7, 12.3 Hz), 4.14 (dd, 1H, J = 2.3, 12.3 Hz), 4.10 (dd, 1H, J = 1.7, 3.8 Hz), 3.96–4.03 (m, 2H), 3.92 (dd, 1H, J = 4.7, 11.6 Hz), 3.82–3.83 (m, 1H), 3.81 (dd, 1H, J = 1.5, 11.6 Hz), 3.75–3.78 (m, 1H), 3.66 (dt, 1H, J = 6.8, 9.6 Hz), 3.39 (dt, 1H, J = 6.5, 9.7 Hz), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.54–1.58 (m, 2H), 1.29–1.37 (m, 10H), 0.94 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 171.2, 170.1, 170.0, 138.9, 138.8, 138.8, 128.8, 128.8, 128.3, 128.1, 128.1, 128.0, 98.6, 98.3, 80.8, 75.5, 75.4, 74.8, 73.3, 73.3, 72.6, 72.2, 71.2, 68.9, 67.2, 66.5, 62.5, 62.0, 32.2, 29.8, 29.8, 29.7, 26.6, 23.1, 21.2, 21.1, 20.9, 14.5. HR-ESI-MS calcd for $C_{47}H_{61}O_{13}N_3$ $[M+Na]^+$ 898.4097, found 898.4090.

3.18. Octyl 2-azido-2-deoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (24)

Disaccharide **23** (380 mg, 0.43 mmol) was deacylated in CH_3OH (8 mL), with four drops of 1 M $NaOCH_3$ as described for the preparation of **15**. The product was purified by chromatography (1:1 hexane–EtOAc) on silica gel to give **24** (310 mg, 95%) as a colorless syrup. R_f 0.54 (1:2 hexane–EtOAc); $[\alpha]_D$ +59.1 (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.13–7.29 (m, 15H), 4.90 (d, 1H, J = 0.6 Hz), 4.87 (d, 1H, J = 11.1 Hz), 4.70 (d, 1H, J = 1.6 Hz), 4.62 (ABq, 2H, J = 12.3 Hz, $\Delta\upsilon$ = 30.4 Hz), 4.52–4.53 (m, 2H), 4.51 (d, 1H, J = 11.4 Hz), 3.48–4.03 (m, 12H), 3.41 (dt, 1H, J = 6.5, 9.7 Hz), 3.23 (dt, 1H, J = 6.5, 9.7 Hz), 1.40–1.42 (m, 2H), 1.15–1.25 (m, 10H), 0.79 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$): δ_C 138.9, 138.8, 138.6, 128.9, 128.9, 128.8, 128.5, 128.3, 128.3, 128.2, 128.1, 99.3, 98.2, 80.6, 75.6, 75.2, 74.9, 73.2, 72.7, 72.6, 72.1, 71.7, 68.3, 68.1, 67.2, 64.2, 62.3, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.6. HR-ESI-MS calcd for $C_{41}H_{55}O_{10}N_3$ $[M+Na]^+$ 772.3780, found 772.3788.

3.19. Bacterial strains and growth conditions

M. smegmatis mc^2155 was a generous gift from W. R. Jacobs, Albert Einstein College of Medicine, Bronx, New York. Liquid cultures of *M. smegmatis* were grown at 37 °C in Luria Bertoni (LB) broth medium (Difco) supplemented with 0.05% Tween 80, biomass harvested, washed with phosphate buffered saline (PBS) and stored at −20 °C until further use.

3.20. Preparation of membrane fractions from *M.smegmatis*

M. smegmatis cells (10 g wet weight) were washed and re-suspended in 30 mL of buffer A, containing 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol and 10 mM $MgCl_2$ at 4 °C and subjected to probe sonication (Soniprep 150, MSE Sanyo Gallenkamp, Crawley, Sussex, UK; 1 cm probe) for a total time of 10 min in 60 s pulses and 90 s cooling intervals between pulses. The sonicate was centrifuged at 27,000*g* for 20 min at 4 °C. Membrane fractions were obtained by centrifugation of the clarified lysate at 100,000*g* for 1 h at 4 °C. The supernatant was carefully removed and the membranes gently re-suspended in buffer A at a protein concentration of 20 mg/mL. Protein concentrations were determined using the BCA Protein Assay Reagent kit (Pierce Europe, Oud-Beijerland, The Netherlands).

3.21. Evaluation of acceptor/inhibitor activity of compounds 4–8 in the PPM-dependent α-(1→6)-ManT assay

Compounds **4**–**8** at a concentration of 2.0 mM were dried under a stream of argon in a microcentrifuge tube (1.5 mL) and placed in a vacuum desiccator for 15 min. This was followed by the addition of 2.4 μM GDP-[U-^{14}C]mannose (321 mCi/mmol, 0.25 μCi; Dupont-New England Nuclear), 62.5 μM ATP, 10 μm $MgCl_2$,

62.5 μM DTT, 12.5 μM NaF, 0.25 mM decaprenolphosphate (in 1% CHAPS) and membrane fractions corresponding to 500 g. The final volume of the assays was adjusted to 80 μL with 50 mM MOPS (pH 8.0). The reaction mixtures were then incubated at 37 °C for 1 h. A $CHCl_3$–CH_3OH (1:1, 533 μL) solution was added to the incubation tubes and the entire contents centrifuged at 18,000*g*. The supernatant was recovered and dried under a stream of argon and re-suspended in C_2H_5OH–H_2O (1:1, 1 mL) and loaded onto a pre-equilibrated [C_2H_5OH–H_2O (1:1)] 1 mL Whatmann strong anion-exchange (SAX) cartridge, after which was washed with 3 mL of ethanol. The eluate was dried and the resulting products partitioned between the two phases arising from a mixture of *n*-butanol (3 mL) and H_2O (3 mL). The resulting organic phase was recovered following centrifugation at 3500*g* and the aqueous phase was again extracted twice with 3 mL of water saturated *n*-butanol, the pooled extracts were back-washed twice with water saturated with *n*-butanol (3 mL). The water saturated *n*-butanol fraction was dried and re-suspended in 200 μL of *n*-butanol. The total counts per minute of radiolabeled material extractable into the *n*-butanol phase was measured by scintillation counting using 10% of the labeled material and 10 mL of EcoScintA (National Diagnostics, Atlanta, GA, USA). The incorporation of [^{14}C]Man was determined by subtracting counts present in control assays (incubation of the reaction components in the absence of the compounds). The remainder of the labeled material was subjected to thin-layer chromatography in $CHCl_3$–CH_3OH–1 M $CH_3COO{\cdot}NH_4$–14.8 M NH_4OH–H_2O (180:140:9:9:23, v/v/v/v/v) on aluminum backed Silica Gel 60-F_{254} plates (E. Merck, Darmstadt, Germany). Autoradiograms were obtained by exposing TLCs to X-ray film (Kodak X-Omat) for three days to determine the extent of product formation.

Acknowledgements

The National Institutes of Health (AI44045–01) supported this work. GSB, Lister-Jenner Research Fellow, acknowledges support from The Medical Research Council, The Wellcome Trust and the Lister Institute for Preventive Medicine.

References and notes

1. Paolo, W. F., Jr.; Nosanchuk, J. D. *Lancet Infect. Dis.* **2004**, *4*, 287–293.
2. Kremer, L.; Besra, G. S. *Exp. Opin. Inv. Drugs* **2002**, *11*, 153–157.
3. Coker, R. J. *Trop. Med. Int. Health* **2004**, *9*, 25–40.
4. Bass, J. B., Jr.; Farer, L. S.; Hopewell, P. C.; Obrien, R.; Jacobs, R. F.; Ruben, F.; Snider, D. E.; Thornton, G. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, 1359–1374.
5. Brennan, P. J. *Tuberculosis* **2003**, *83*, 91–97.
6. Lowary, T. L. Mycobacterial Cell Wall Components. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; pp 2005–2080.
7. Nigou, J.; Gilleron, M.; Puzo, G. *Biochemie* **2003**, *85*, 153–166.
8. Briken, V.; Porcelli, S. A.; Besra, G. S.; Kremer, L. *Mol. Microbiol.* **2004**, *53*, 391–403.
9. Gibson, K. J. C.; Gilleron, M.; Constant, P.; Puzo, G.; Nigou, J.; Besra, G. S. *Biochem. J.* **2003**, *372*, 821–829.
10. Gibson, K. J. C.; Gilleron, M.; Constant, P.; Brando, T.; Puzo, G.; Besra, G. S.; Nigou, J. *J. Biol. Chem.* **2004**, *279*, 22973–22982.
11. Gibson, K. J. C.; Gilleron, M.; Constant, P.; Puzo, G.; Nigou, J.; Besra, G. S. *Microbiology* **2003**, *149*, 1437–1445.
12. Garton, N. J.; Gilleron, M.; Brando, T.; Dan, H.-H.; Giguère, S.; Puzo, G.; Prescott, J. F.; Sutcliffe, I. C. *J. Biol. Chem.* **2002**, *277*, 31722–31733.
13. Sutcliffe, I. C. *Arch. Oral Biol.* **1995**, *40*, 1119–1124.
14. Sutcliffe, I. C. *Anton. Leeuw.* **2000**, *78*, 195–201.
15. Flaherty, C.; Sutcliffe, I. C. *Syst. Appl. Microbiol.* **1999**, *22*, 530–533.
16. Flaherty, C.; Minnikin, D. E.; Sutcliffe, I. C. *Zentralbl. Bakteriol.* **1996**, *285*, 11–19.
17. Guérardel, Y.; Maes, E.; Briken, V.; Chirat, F.; Leroy, Y.; Locht, C.; Strecker, G.; Kremer, L. *J. Biol. Chem.* **2003**, *278*, 36637–36651.
18. Guérardel, Y.; Maes, E.; Elass, E.; Leroy, Y.; Timmerman, P.; Besra, G. S.; Locht, C.; Strecker, G.; Kremer, L. *J. Biol. Chem.* **2002**, *277*, 30635–30648.
19. Nigou, J.; Gilleron, M.; Cahuzac, B.; Bournery, J. D.; Herold, M.; Thurnher, M.; Puzo, G. *J. Biol. Chem.* **1997**, *272*, 23094–23103.
20. Khoo, K.-H.; Tang, J. B.; Chatterjee, D. *J. Biol. Chem.* **2001**, *276*, 3863–3871.
21. Khoo, K.-H.; Dell, A.; Morris, H. R.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1995**, *270*, 12380–12389.
22. Turnbull, W. B.; Shimizu, K. H.; Chatterjee, D.; Homans, S. W.; Treumann, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 3918–3922.
23. Treumann, A.; Feng, X. D.; McDonnell, L.; Derrick, P. J.; Ashcroft, A. E.; Chatterjee, D.; Homans, S. W. *J. Mol. Biol.* **2002**, *316*, 89–100.
24. Besra, G. S.; Morehouse, C. B.; Rittner, C. M.; Waechter, C. J.; Brennan, P. J. *J. Biol. Chem.* **1997**, *272*, 18460–18466.
25. Morita, Y. S.; Patterson, J. H.; Billman-Jacobe, H.; McConville, M. J. *Biochem. J.* **2004**, *378*, 589–597.
26. Gurcha, S. S.; Baulard, A. R.; Kremer, L.; Locht, C.; Moody, D. B.; Muhlecker, W.; Costello, C. E.; Crick, D. C.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *365*, 441–450.
27. Schaeffer, M. L.; Khoo, K.-H.; Besra, G. S.; Chatterjee, D.; Brennan, P. J.; Belisle, J. T.; Inamine, J. M. *J. Biol. Chem.* **1999**, *274*, 31625–31631.
28. Korduláková, J.; Gilleron, M.; Mikusová, K.; Puzo, G.; Brennan, P. J.; Gicquel, B.; Jackson, M. *J. Biol. Chem.* **2002**, *277*, 31335–31344.
29. Kremer, L.; Gurcha, S. S.; Bifani, P.; Hitchen, P. G.; Baulard, A.; Morris, H. R.; Dell, A.; Brennan, P. J.; Besra, G. S. *Biochem. J.* **2002**, *363*, 437–447.
30. Alexander, D. C.; Jones, J. R. W.; Tan, T.; Chen, J. M.; Liu, J. *J. Biol. Chem.* **2004**, *278*, 18824–18833.
31. Pathak, A. K.; Pathak, V.; Riordan, J. M.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Carbohydr. Res.* **2004**, *339*, 683–691.
32. Brown, J. R.; Field, R. A.; Barker, A.; Guy, M.; Grewal, R.; Khoo, K.-H.; Brennan, P. J.; Besra, G. S.; Chatterjee, D. *Bioorg. Med. Chem.* **2001**, *9*, 815–824.
33. Guy, M. R.; Illarionov, P. A.; Gurcha, S. S.; Dover, L. G.; Gibson, K. J. C.; Smith, P. W.; Minnikin, D. E.; Besra, G. S. *Biochem. J.* **2004**, *382*, 905–912.
34. Spohr, U.; Hindsgaul, O.; Lemieux, R. U. *Can. J. Chem.* **1985**, *63*, 2644–2652.

35. Mukherjee, A.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **2000**, *326*, 1–21.
36. Hindsgaul, O.; Kaur, K. J.; Srivastava, G.; Blaszczyk-Thurin, M.; Crawley, S.; Heerze, L. D.; Palcic, M. M. *J. Biol. Chem.* **1991**, *266*, 17858–17862.
37. Glaudemans, C. P. J.; Kovac, P.; Nashed, E. M. *Methods Enzymol.* **1994**, *247*, 305–322.
38. Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *249*, 163–195.
39. Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1994**, *251*, 33–67.
40. Laferté, S.; Chan, N. W. C.; Sujino, K.; Lowary, T. L.; Palcic, M. M. *Eur. J. Biochem.* **2000**, *267*, 4840–4849.
41. Callam, C. S.; Lowary, T. L. *J. Org. Chem.* **2001**, *66*, 8961–8972.
42. Ziegler, T.; Dettmann, R.; Duszenko, M.; Kolb, V. *Carbohydr. Res.* **1996**, *295*, 7–23.
43. Subramanim, V.; Lowary, T. L. *Tetrahedron* **1999**, *55*, 5965–5976.
44. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–299.
45. Spijker, N. M.; Slief, J. W.; van Boeckel, C. A. *J. Carbohydr. Chem.* **1993**, *12*, 1017–1041.
46. Briner, K.; Vasella, A. *Helv. Chim. Acta* **1987**, *70*, 1341–1356.
47. Csuk, R.; Glanzer, B. I. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 73–177.
48. The core of LAM from *M. tuberculosis* and *M. smegmatis* are identical and thus both organisms possess similar mannosyltransferase activities; the latter has been used as a model for *M. tuberculosis* because it is 'fast growing' and is not pathogenic to humans.
49. Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347–374.
50. Subramanian, V.; Gurcha, S. S.; Besra, G. S.; Lowary, T. L. *Tetrahedron: Asymmetry*, accepted for publication.