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Tetrahedron: *Asymmetry* 16 (2005) 553–567

Synthetic disaccharide analogs as potential substrates and inhibitors of a mycobacterial polyprenol monophosphomannose-dependent α-(1→6)-mannosyltransferase

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Received 16 November 2004; accepted 23 November 2004

Abstract—Analogs of the α-D-Man*p*-(1→6)-α-D-Man*p*-O$(CH_2)_7CH_3$ disaccharide **4**, a known substrate for a polyprenol monophosphomannose-dependent α-(1→6)-mannosyltransferase involved in mycobacterial LAM biosynthesis, have been synthesized and screened as potential substrates and inhibitors of the enzyme. In the disaccharides synthesized, the hydroxyl groups at C-2 and C-6 on the reducing end residue have been replaced by combinations of amino, fluoro, and methoxy functionalities **9**–**14**. In addition, a disaccharide in which the nonreducing mannopyranose residue was replaced with a 3,6-anhydromannopyranose residue **34** was synthesized from a byproduct formed during one of the reactions leading to **14**. When tested against the enzyme, none were active as substrates, as would be expected as all lack the C-6′ hydroxyl group to which an additional sugar residue would be transferred. Evaluation of these compounds as inhibitors of the enzyme revealed that only three, **11**, **12**, and **13**, all of which contain one or more amino groups, inhibited the enzyme. The most potent inhibitor was the diamino-disaccharide, **11**.

1. Introduction

Lipoarabinomannan (LAM) and lipomannan (LM) are structurally complex glycolipids that are major constituents of the cell wall complex in mycobacteria, including the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*.[1,2] These polysaccharides are key immunomodulatory molecules and play critical roles in the ability of these organisms to survive in the host.[3,4] The resurgence of tuberculosis as a health threat in the industrialized world and the appearance of drug resistant mycobacterial strains have prompted increased interest not only in understanding the role of LAM and LM in the progression of mycobacterial disease but also in the development of new antimycobacterial therapies that target the biosynthesis of these glycans.[5,6]

The structure of mycobacterial LM consists of a membrane associated phosphatidylinositol moiety elaborated with a polysaccharide that has, as a core, a polymer of α-(1→6)-linked mannopyranose residues. In 50–70% of the monosaccharide residues in this mannan core, are attached single α-mannopyranose capping units, most commonly at O-2[1,2] **1** (Fig. 1), but in some cases at

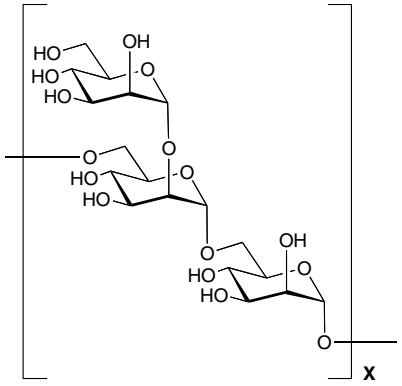

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Figure 1. Mannan core structure of mycobacterial LAM and LM.

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doi:10.1016/j.tetasy.2004.11.063

O-3.[7] Mycobacterial LAM shares the structural features of LM described above. However, attached to the non-reducing end of the mannan is a highly branched arabinan domain composed of α-(1→5)-, α-(1→3)-, and β-(1→2)-linked arabinofuranose residues. The terminal ends of this arabinan are further substituted with a diverse range of motifs including additional mannopyranose residues,[8,9] inositol phosphates moieties,[10] or 5-thiomethylxylofuranose.[11,12]

The biosynthesis of mycobacterial LAM, LM, and the related phosphadtidylinositol mannosides, has received increasing attention since a pathway for their assembly was first proposed.[13] Recent studies have supported this pathway (Fig. 2), and have led to the identification of the mannosyltransferases (ManT) involved in the early biosynthetic steps,[14–18] and an enzyme that catalyzes the formation of one of the donor species, polyprenol phosphomannose (PPM) **2**.[19] The donor substrates for the biosynthetic ManT's are either **2** or GDP-Man, **3**. The former serves as the substrate for the enzymes involved in the assembly of the α-(1→6)-linked backbone, while the latter is believed to be the substrate for the enzymes that introduce the α-(1→2)-branching residues.[13]

The enzyme of interest to the work described here is the PPM-dependent α-(1→6)-ManT that is responsible for the assembly of the mannan core of LAM and LM (linear LM in Fig. 2). Although this enzyme has not been isolated, a cell-free assay for its activity has been developed, which has been used to evaluate potential substrates and inhibitors.[20–22]

Because of the important role of these polysaccharides in the progression of mycobacterial disease, understanding the substrate specificities of the ManT's involved in LAM/LM biosynthesis is important. In addition, the identification of potent and specific inhibitors of these glycosyltransferases would be useful in fundamental biochemical studies of LAM pathogenesis and also would serve as lead structures for novel antimycobacterial drugs. In a previous report,[23] we described the synthesis of a panel of mono-functionalized mannose disaccharides **5**–**8** and their evaluation as substrates and inhibitors of the PPM-dependent ManT that assembles the α-(1→6)-linked mannan core of LAM and LM. Herein we describe further studies in this area, in which the α-D-Man*p*-(1→6)-α-D-Man*p*-O$(CH_2)_7CH_3$ disaccharide **4**, a known[20] substrate for this enzyme, has been modified

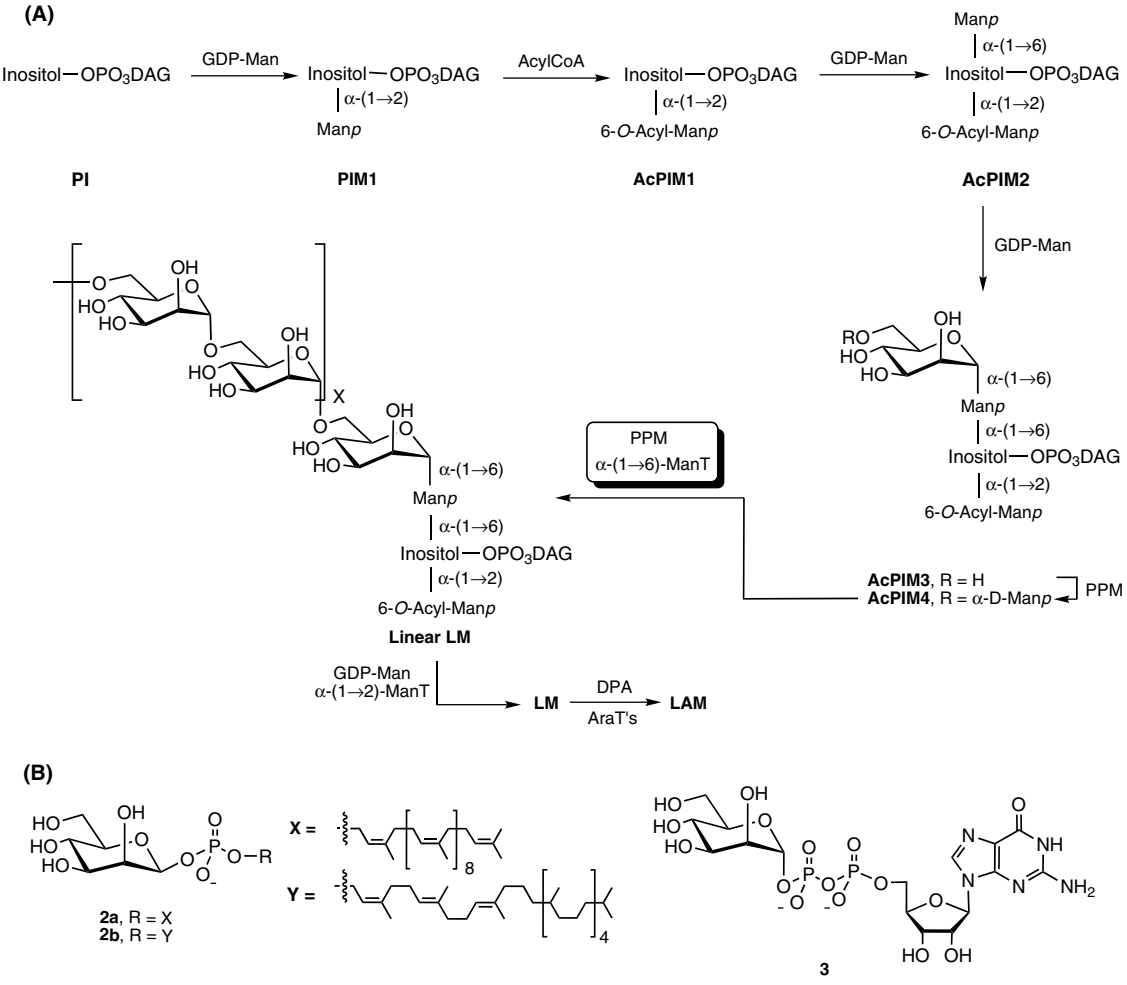
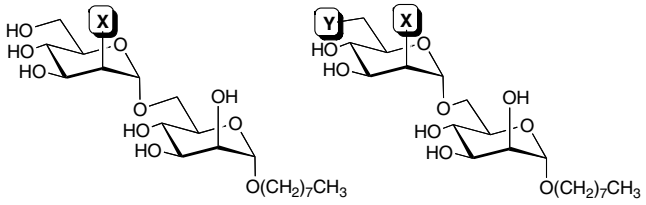


Figure 2. (A) Biosynthesis of mycobacterial lipomannan and lipoarabinomannan. DAG = diacylglycerol, DPA = decaprenolphosphoarabinose, AraT's = arabinosyltransferases. The ManT of interest is indicated by the shadowed box. (B) Structure of polyprenolphosphomannose derivatives (PPM) **2** and GDP-Mannose **3**.

by the replacement of two of the hydroxyl groups on the nonreducing residue with combinations of amino, fluoro, and methoxy functionalities **9**–**14** (Chart 1).



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

9, X = F, Y = NH_2
10, X = F, Y = F
11, X = NH_2, Y = NH_2
12, X = NH_2, Y = F
13, X = OCH_3, Y = NH_2
14, X = OCH_3, Y = F

Chart 1.

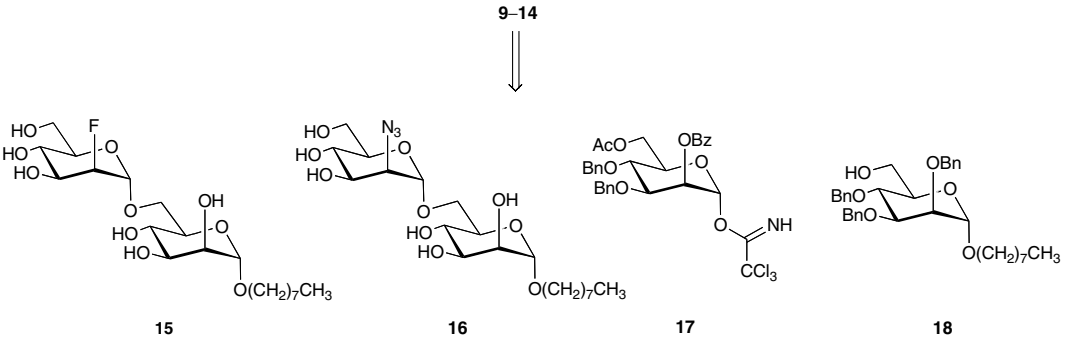
2. Results and discussion

2.1. Synthesis of disaccharides 9–14

The synthesis of **9**–**14** involved the use of four known building blocks (Chart 2), fluorodisaccharide **15**,[23] azidodisaccharide **16**,[23] imidate **17**,[24] and octyl glycoside **18**.[23] These oligosaccharides were synthesized as octyl glycosides given previous studies that have shown that among a series of long-chain alkyl disaccharide derivatives, the octyl glycosides were the best substrates for this enzyme.[20]

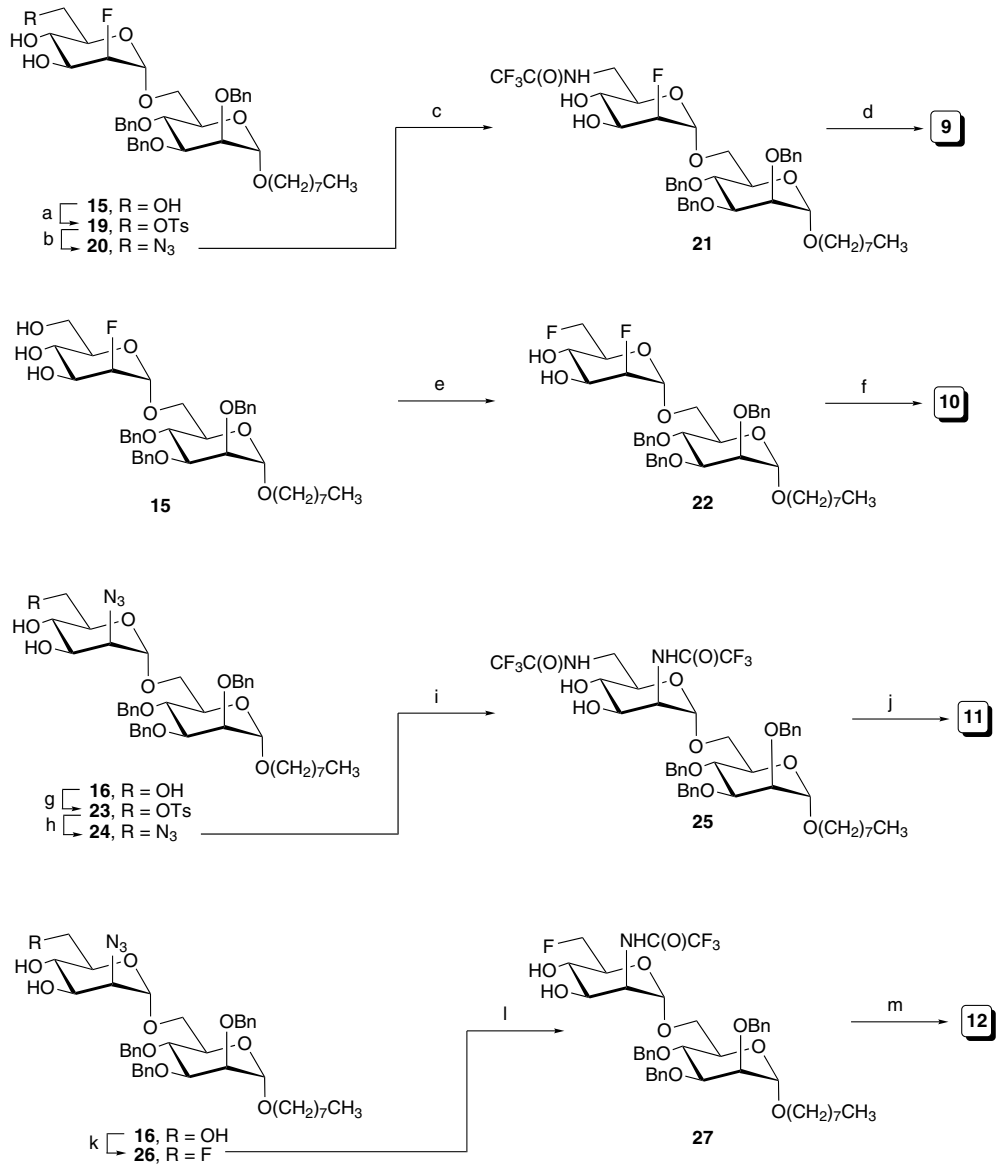
Disaccharides **15** and **16** were used in the synthesis of **9**–**12** as illustrated in Scheme 1, through selective substitution of the C-6 hydroxyl group on the nonreducing residue and then deprotection. The route to **9** involved first selective tosylation of the primary alcohol in **15** under standard conditions [tosyl chloride, pyridine, and 4-(*N*,*N*-dimethylamino)pyridine] to afford the expected product, **19**, in 68% yield. Treatment of **19** with sodium azide and 15-crown-5 at 60 °C gave the azide-substituted disaccharide **20** in 92% yield. That substitution had taken place was evident from the ^{1}H NMR spectrum of **20**, which showed the signals for the hydrogens attached to C-6′ as doublets of doublets at 3.39 and 3.32 ppm, consistent with an azide moiety on a primary carbon. Similarly, in the ^{13}C NMR spectrum the resonance of C-6′ appeared at 51.3 ppm. With this disaccharide in hand, we had hoped to reduce the azide and deprotect the benzyl ethers in a single step by reaction with hydrogen and palladium on carbon in acetic acid. However, we were unsuccessful in obtaining good yields of the desired product, **9**, under these conditions. Thin layer chromatographic analysis of the reaction mixture showed several spots even after 2 days, all of them giving the characteristic red color with the ninhydrin reagent suggesting the presence of an amine functional group in all compounds. We therefore chose an alternate approach in which **20** was first reacted with triphenylphosphine in a mixture of THF and water to afford the corresponding amine. After the reduction, the amine was immediately converted to its *N*-trifluoroacetamide, by reaction with trifluoroacetic anhydride in pyridine followed by stirring with methanol, to cleave any *O*-trifluoroacetate esters. The desired trifluoroacetamide product **21** was obtained in 83% yield over the two steps. Hydrogenation of **21** in acetic acid followed by treatment with aqueous sodium hydroxide afforded **9** in 47% yield over two steps. In addition to the desired compound **9**, an amino-disaccharide containing one benzyl group was obtained. The molecular weight was determined by high-resolution mass spectrometry, but it was not possible to identify the location of this benzyl group on the molecule from the NMR spectra. Regardless, when this byproduct was subjected again to the hydrogenation reaction, it was cleanly deprotected, affording additional **9**. In total, a 78% yield of **9** from **21** was obtained.

The synthesis of **10** was more straightforward. First, the primary alcohol in **15** was replaced with fluorine, upon reaction with an excess of diethaminosulfur trifluoride (DAST) at −40 °C in dichloromethane. A 55% yield of the product **22** was obtained. Both the ^{13}C and ^{1}H NMR spectra of **22** showed the expected couplings with fluorine, and in the ^{1}H-coupled ^{19}F spectrum, the fluorine signal appeared as a doublet of doublet of doublets ($^2J_{H6'a,F} = {}^2J_{H6'b,F} = 47.1$ Hz, $^3J_{H5',F} = 28.2$ Hz), as would be expected for a C-6 fluorinated pyranoside moiety.[25] Other products, presumably compounds containing more than one fluorine atom were observed by TLC, but these were not isolated nor further characterized. Hydrogenation of **22** in acetic acid proceeded efficiently providing a 90% yield of **10**.



15 **16** **17** **18**

Chart 2.



Scheme 1. Reagents and conditions: (a) TsCl, pyridine, DMAP, rt, 68%; (b) NaN_3, 15-crown-5, DMF, 60 °C, 92%; (c) Ph_3P, H_2O, THF, rt, then $(CF_3CO)_2O$, pyridine, CH_2Cl_2, rt, 83%; (d) H_2, Pd/C, HOAc, rt, then NaOH, CH_3OH, H_2O, rt, 78%; (e) DAST, CH_2Cl_2, −40 °C, 55%; (f) H_2, Pd/C, HOAc, rt, 90%; (g) TsCl, pyridine, rt, 70%; (h) NaN_3, 15-crown-5, DMF, 60 °C, 85%; (i) Ph_3P, H_2O, THF, rt, then $(CF_3CO)_2O$, pyridine, rt, 34%; (j) H_2, Pd/C, rt, then $NaOH_3$, CH_3OH, H_2O, rt, 61%; (k) DAST, CH_2Cl_2, −40 °C, 72%; (l) Ph_3P, H_2O, THF, rt, then $(CF_3CO)_2O$, pyridine, rt, 64%; (m) H_2, Pd/C, rt, then NaOH, CH_3OH, H_2O, rt, 64%.

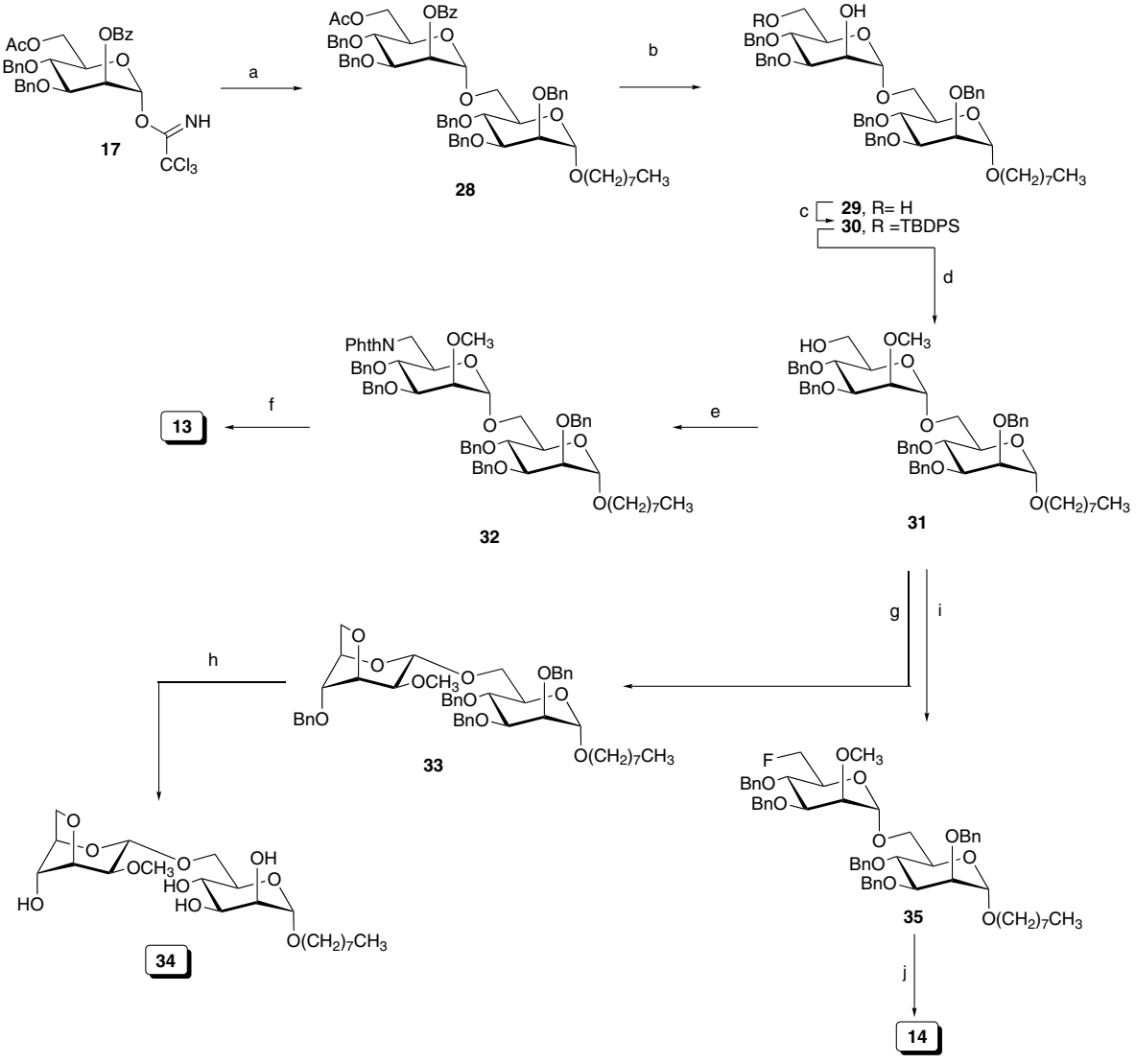
The diamino-disaccharide **11** was prepared via a route analogous to **9**. Thus, disaccharide **16** was selectively tosylated at the primary hydroxyl group providing **23** in 70% yield. Treatment of **23** with sodium azide and 15-crown-5 in DMF at 60 °C gave the diazide **24** in 85% yield. As was the case for **20**, the presence of a primary azide moiety could be confirmed by ^{13}C NMR spectroscopy, which showed the resonance for C-6′ at 51.3 ppm. Based on the poor results obtained with the attempted simultaneous azide reduction and benzyl deprotection of **21** via hydrogenation, we chose instead to reduce the azides in **23** with triphenylphosphine in THF and water. This reaction provided a crude diamine, which was immediately treated with trifluoroacetic anhydride in the presence of pyridine. Addition of methanol, to remove any *O*-trifluoroacetate esters, was followed by purification, which, in this case, provided a disappointing 34% yield of the expected di(trifluoroacetamide) **25**. Hydrogenation of **25** in methanol followed by treatment with aqueous sodium hydroxide afforded **11** in 61% yield over two steps. Unlike the hydrogenolysis of **21**, in the deprotection of **25**, the formation of a mono-*O*-benzylated amino-disaccharide byproduct was not observed.

Disaccharide **12** was obtained in two steps from **16**. First, reaction of **16** with excess DAST at −40 °C in dichloromethane afforded the expected mono-fluorinated adduct **26** in 72% yield, together with some minor byproducts (observable by TLC, but not isolated). Incorporation of the fluorine at C-6′ was evident from ^{1}H–^{19}F and ^{13}C–^{19}F coupling constants observed

in the ^{1}H and ^{13}C spectra, respectively. Similarly, in the ^{1}H-coupled ^{19}F spectrum, the signal for F-6′ appeared as the expected doublet of doublet of doublets ($^{2}J_{H6'a,F} = {}^{2}J_{H6'b,F} = 46.7$ Hz, $^{3}J_{H5',F} = 28.1$ Hz). As was done for the synthesis of the other amine-containing disaccharides, the azide in **26** was selectively reduced (triphenylphosine in THF/water) and the resulting amine was protected as the trifluoroacetamide. The product, **27**, was obtained in 64% yield over two steps. Hydrogenation of **27** in methanol followed by treatment with sodium hydroxide afforded **12** in 34% yield. As was true for **21**, the other major product of this deprotection sequence was a mono-*O*-benzylated amino-disaccharide of undetermined structure. Further hydrogenation of this byproduct provided additional product. In total, a 64% yield of **12** was obtained from **27**.

The preparation of disaccharides **13** and **14** is illustrated in Scheme 2. Coupling of the known imidate **17**[24] and the protected octyl glycoside **18**[23] was carried out using TMSOTf as the promoter, which afforded the protected disaccharide **28** in 91% yield. Excellent α-selectivity was achieved by starting the glycosylation reaction at −10 °C and allowing it to react as it warmed to 0 °C.[26] Deacetylation of **28** with sodium methoxide gave diol **29** in 96% yield. This diol was then reacted with TBDPSCl in the presence of imidazole, which yielded disaccharide **30** in 74% yield. Methylation of **30** (methyl iodide and sodium hydride) afforded a product that was, without purification, treated with tetrabutylammonium fluoride trihydrate, affording **31** in 67% yield over the two steps. Disaccharide **31** was the key intermediate in the preparation of **13** and **14**.

Amino-disaccharide **13** was synthesized first. In the preparation of the other amine-containing disaccharides **9**, **11**, and **12** we used the azide as a precursor to the amine. However, given the difficulties in cleanly



Scheme 2. Reagents and conditions: (a) **18**, CH_2Cl_2, −10 °C, 91%; (b) $NaOCH_3$, CH_3OH, rt, 96%; (c) TBDPSCl, DMF, imidazole, rt, 74%; (d) CH_3I, DMF, 0 °C→rt, then *n*-Bu_4NF–$3H_2O$, THF, rt, 67%; (e) PhthNH, diisopropylazodicarboxylate, Ph_3P, THF, rt, 85%; (f) $H_2NCH_2CH_2NH_2$, *n*-BuOH, 90 °C, then H_2, Pd/C, HOAc, rt, 84%; (g) DAST, CH_2Cl_2, −40 °C, 83%; (h) H_2, Pd/C, HOAC, rt, 65%; (i) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine, 0 °C, then *n*-Bu_4NF, THF, 50 °C, 78%; (j) H_2, Pd/C, HOAc, rt, 74%.

reducing the azide and removing the benzyl ether protecting groups, we chose another approach for the preparation of **13**. Instead, as a synthon for the amine we chose the phthalimido group, which could be readily incorporated into disaccharide **31** upon reaction with phthalimide in the presence of diisopropylazodicarboxylate and triphenylphosphine. The product, **32**, was obtained in 85% yield. The presence of the phthalimido group was apparent from the ^{1}H NMR spectrum of **32**, which showed, in the aromatic region, four protons (7.56–7.59 and 7.69–7.73 ppm, two hydrogens each) distinct from those arising from the benzyl protecting groups (7.15–7.37 ppm). In the ^{13}C NMR spectrum of **32**, an amide carbonyl carbon resonance was observed (168.6 ppm) and the resonance for C-6′ appeared at 59.1 ppm, as would be expected.[27] Removal of the phthalimido group in **32** was achieved by treatment with ethylenediamine in *n*-butanol at 80 °C.[28] At the end of the phthalimide cleavage reaction, the solution was concentrated and the resulting amine was, without further purification, hydrogenated in acetic acid yielding an 84% yield of **13** over two steps.

As described above, selective fluorination of the primary hydroxyl group in disaccharides **15** and **16** upon treatment with DAST in dichloromethane at −40 °C proceeded as expected, yielding the corresponding C-6′ fluorinated compounds. We therefore first explored this method for the incorporation of fluorine into **31**. However, when **31** was reacted with DAST under conditions identical to those used for the synthesis of **22** and **26**, none of the desired fluorinated compound was isolated. Instead, this reaction afforded, in 83% yield, the 3,6-anhydromannose-containing disaccharide **33**. The structure of **33** was supported by the magnitude of the $J_{1',2'}$, which was 6.5 Hz, indicating that this pyranose ring was in the 'inverted' (^{1}C$_4$) chair conformation. In addition, the ^{1}H NMR spectrum showed only eight benzylic hydrogens and **20** aryl hydrogens, as would be expected. Further support for the structure was obtained from two-dimensional NMR experiments and high-resolution mass spectrometry. The formation of 3,6-anhydrosugars with concomitant loss of benzyl groups during DAST-induced fluorination reactions is precedented[29] and a plausible pathway for the formation of **33** is shown in Figure 3. It is unclear why **31** readily undergoes anhydrosugar formation upon treatment with DAST, while **15** and **16** appear to not undergo this reaction to an appreciable degree. However, it should be mentioned that one of the uncharacterized byproducts from the fluorination reactions of **15** and **16**, could be the corresponding 3,6-anhydrosugar derivatives. To provide an anhydrosugar-containing substrate suitable for testing against the ManT, **33** was deprotected under standard conditions yielding disaccharide **34** in 65% yield.

Because direct fluorination of **31** was unsuccessful, we changed our approach to **14**. Reaction of **31** with trifluoromethanesulfonic acid anhydride in the presence of pyridine at 0 °C yielded the corresponding trifluoromethanesulfonate, which was immediately treated with tetrabutylammonium fluoride in THF at 50 °C to afford **35** in 78% yield over two steps. Incorporation of the fluorine was clearly apparent from the ^{1}H, ^{13}C, and ^{19}F NMR spectra, as described above for **22** and **26**. Hydrogenation of **35** in acetic acid afforded **14** in 74% yield.

2.2. Screening of 9–14 and 34 as substrates and inhibitors of the PPM-dependent α-(1→6)-ManT from *M. smegmatis*

With disaccharides **9**–**14** and **34** synthesized, each was screened as a potential substrate for the PPM-dependent α-(1→6)-ManT using the previously developed assay.[20] None of these oligosaccharides were shown to be substrates for this enzyme, as would be expected as they all lack a hydroxyl group at C-6′. The disaccharides were next tested as competitive inhibitors of the enzyme. In these studies, disaccharide **4** was used as the substrate at 0.2 mM and one of the other oligosaccharides was added at a concentration of 2.0 mM. The results of these inhibition studies are provided in Table 1. Oligosaccharides **9**, **10**, **14**, and **36** were shown not to inhibit the enzyme, while **11**, **12**, and **13** were weak to moderate inhibitors. At 2.0 mM, disaccharide **11** inhibited transfer of mannose to **4** by 57%; disaccharide **12**, 33%; and disaccharide **13**, 30%.

Table 1. Screening of **9**–**14** and **34** as inhibitors of the PPM-dependent α-(1→6)-ManT from *M. smegmatis*[a]

Compound	**9**	**10**	**11**	**12**	**13**	**14**	**34**
Inhibition (%)	0	0	57	33	30	0	0

[a] All compounds were screened at a concentration 2.0 mM with **4** as the substrate at 0.2 mM as described in Section 3.27.

With regard to the inhibition data, all three of the active compounds have an amino group, which will be protonated at physiological pH. These groups would therefore be expected to form tight-binding interactions with negatively charged groups in the active site, and this could lead to the observed inhibition.[27] In this regard, we note that the most potent compound, **11**, possesses two amino

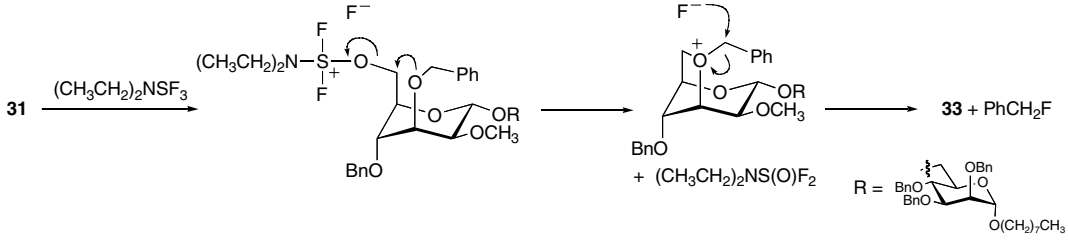


Figure 3. Proposed pathway for DAST-induced formation of **33** from **31**.

groups and might therefore form such interactions with two anionic groups in the active site of the enzyme. In our previous studies,[23] we demonstrated that the amino-disaccharide **8**, the singly labeled parent compound for **11** and **12**, is a substrate for the PPM-dependent ManT. It is therefore not surprising that replacement of the C-6′ hydroxyl group with another functionality produces an inhibitor. In contrast, the data with the 2′-methoxy-6′-amino disaccharide, **13**, is difficult to reconcile with our earlier investigations[23] in which it was demonstrated that the parent 2′-methoxy-disaccharide, **5**, was neither a substrate nor an inhibitor of the enzyme. Our previous results suggest that the enzyme will not tolerate sterically demanding groups at C-2′. That **13** inhibits the enzyme, albeit weakly, is curious and could be the result of the ionic interactions arising from the 6′-amino group overriding the steric congestion caused by the 2′-methoxy moiety. An alternate explanation is that the expected surfactant nature of **11**–**13** could nonspecifically inhibit the enzyme through a detergent effect. However, we view this as unlikely because disaccharide **9**, which would be expected to have detergent properties similar to those for **12**, did not inhibit the enzyme. It is also possible that **13** does not act as a competitive inhibitor of the enzyme, but rather acts in noncompetitive or uncompetitive fashion. Clearly, additional investigations with a larger panel of compounds, and preferably with purified enzymes are needed to obtain a more detailed picture of the mode by which these compounds inhibit this enzyme.

3. Experimental

3.1. General methods

Unless otherwise indicated, all reactions were carried out in freshly distilled solvents 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 silica gel or Iatrobeads, a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo). The ratio between adsorbent 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/gd m. ^{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 compounds **9**–**14** and **34** were made by two-dimensional homonuclear and heteronuclear shift correlation experiments. For **9**–**14** and **34** the stereochemistry of both pyranose residues was proven through measurement of the $^1J_{C1-H1}$.[30] Electrospray mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl.

3.2. Octyl 6-amino-2,6-dideoxy-2-fluoro-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 9

Disaccharide **21** (140 mg, 0.17 mmol) was dissolved in HOAc (10 mL) and 10% Pd/C (60 mg) was added. The solution was stirred overnight under a H_2 atmosphere and the catalyst was separated by filtration and washed with CH_3OH (2 × 20 mL). After concentrating the filtrate and the washings, the residue was redissolved in CH_3OH (10 mL), aqueous NaOH (1.5 mL, 1 M) was added and the reaction mixture stirred overnight. The solution was neutralized with pre-washed Amberlite 118 H^+ resin and concentrated. The product was purified by chromatography (10:2:0.5 $CHCl_3$–CH_3OH–5N aqueous NH_4OH) on Iatrobeads to give **9** (36 mg, 47%) as a colorless solid. A mono *O*-benzylated amino-disaccharide byproduct (40 mg) was also isolated and was then redissolved in HOAc (4 mL) and 10% Pd/C (20 mg) was added and the solution was stirred overnight under an H_2 atmosphere. Filtration of the solution, followed by concentration and purification as above gave additional product (24 mg, 31%). R_f 0.22 (10:4:1 $CHCl_3$–CH_3OH–5N aqueous NH_4OH); $[\alpha]_D$ = +62.4 (*c* 0.1, H_2O); ^{1}H NMR (800 MHz, CD_3OD) δ_H 5.00 (d, 1H, $J_{H1',F}$ = 7.5 Hz, H-1′), 4.75 (br s, 1H, H-1), 4.63 (dd, 1H, $J_{2',3'}$ = 2.6 Hz, $J_{H2',F}$ = 50.0 Hz, H-2′), 3.94 (dd, 1H, $J_{5,6b}$ = 5.2 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.79 (d, 1H, $J_{2,3}$ = 1.4 Hz, H-2), 3.76 (dd, 1H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.74 (ddd, 1H, $J_{2',3'}$ = 2.6 Hz, $J_{3',4'}$ = 9.7 Hz, $J_{H3',F}$ = 31.0 Hz, H-3′), 3.68 (ddd, 1H, *J* = 6.7, 6.7, 9.4 Hz, octyl OCH_2), 3.62–3.66 (m, 4H, H-3, H-4, H-5, H-5′), 3.49 (dd, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.7 Hz, H-4′), 3.41 (ddd, 1H, *J* = 6.7, 6.7, 9.4 Hz, octyl OCH_2), 3.07 (dd, 1H, $J_{5',6a'}$ = 1.6 Hz, $J_{6a',6b'}$ = 13.4 Hz, H-6a′), 3.12 (dd, 1H, $J_{5',6b'}$ = 7.8 Hz, $J_{6a',6b'}$ = 13.4 Hz, H-6b′), 1.55–1.61 (m, 2H), 1.29–1.40 (m, 10H), 0.89 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 101.7 (C-1, $^1J_{C,H}$ = 167.9 Hz), 98.5 (C-1′, $^1J_{C,H}$ = 171.6 Hz, $J_{C-1',F}$ = 29.7 Hz), 91.2 (C-2′, $J_{C-2',F}$ = 174.3 Hz), 74.0 (C-5′), 73.0 (C-5), 72.8 (C-3), 72.1 (C-2), 71.6 (C-3′, $J_{C-3',F}$ = 17.5 Hz), 70.3 (C-4′), 68.7 (octyl OCH_2), 68.4 (C-4), 67.5 (C-6), 43.5 (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 (235.4 MHz, CD_3OD) δ_F −207.3 (ddd, 1F, $J_{H1',F}$ = 7.5 Hz, $J_{H2',F}$ = 50.0 Hz, $J_{H3',F}$ = 31.0 Hz, F-2′). HR-ESI-MS calcd for $C_{20}H_{38}FNO_9$ $[M+H]^+$ 456.2609, found 456.2611.

3.3. Octyl 2,6-dideoxy-2,6-difluoro-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 10

Disaccharide **22** (80 mg, 0.11 mmol) was dissolved in HOAc (8 mL) and 10% Pd/C (35 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 (5:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **10** (45 mg, 90%) as a foam. R_f 0.65 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +63.7 (*c* 0.3, CH_3OH); ^1H NMR (800 MHz, CD_3OD) δ_H 5.01 (d, 1H, $J_{H1',F}$ = 7.1 Hz, H-1′), 4.70 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1), 4.60–4.67 (m, 2H, H-2′, H-6b′), 4.58 (dd, 1H, $J_{6a',6b'}$ = 12.0 Hz, $J_{H6b',F}$ = 47.1 Hz, H-6a′), 3.91 (dd, 1H, $J_{5,6b}$ = 4.1 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.80 (ddd, 1H, $J_{5',6b'}$ = 3.6 Hz, $J_{4',5'}$ = 9.7 Hz, $J_{H5',F}$ = 25.9 Hz, H-5′), 3.76–3.78 (m, 2H, H-2, H-3′), 3.75 (d, 1H, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.70 (dd, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.7 Hz, H-4′), 3.63–3.68 (m, 4H, H-3, H-4, H-5, octyl OCH_2), 3.40 (ddd, 1H, *J* = 6.2, 6.2, 9.6 Hz, octyl OCH_2), 1.55–1.61 (m, 2H), 1.29–1.40 (m, 10H), 0.90 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 101.7 (C-1, $^1J_{C,H}$ = 167.9 Hz), 98.8 (C-1′, $^1J_{C,H}$ = 170.8 Hz, $J_{C-1',F}$ = 29.6 Hz), 91.0 (C-2′, $J_{C-2',F}$ = 174.6 Hz), 82.0 (C-6, $J_{C-6',F}$ = 171.6 Hz), 73.2 (C-5′, $J_{C-5',F}$ = 17.8 Hz), 73.1, 72.8 (C-3, C-5), 72.1 (C-2), 71.7 (C-3′, $J_{C-3',F}$ = 17.4 Hz), 68.6 (C-4), 68.4 (octyl OCH_2), 67.8 (C-6), 67.4 (C-4′, $J_{C-4',F}$ = 6.2 Hz), 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 (235.4 MHz, CD_3OD) δ_F −207.6 (ddd, F, $J_{H1',F}$ = 7.1 Hz, $J_{H2',F}$ = 49.4 Hz, $J_{H3',F}$ = 33.0 Hz, F-2′), −236.8 (ddd, 1F, $J_{H6a',F}$ = $J_{H6b',F}$ = 47.1 Hz, $J_{H5',F}$ = 25.9 Hz, F-6′). HR-ESI-MS calcd for $C_{20}H_{36}F_2O_9$ $[M+Na]^+$ 481.2225, found 481.2220.

3.4. Octyl 2,6-diamino-2,6-dideoxy-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 11

Disaccharide **25** (102 mg, 0.11 mmol) was dissolved in HOAc (6 mL) and 10% Pd/C (35 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 residue was redissolved in CH_3OH (10 mL), aqueous NaOH (1 mL, 1 M) was added and the reaction mixture was stirred overnight. The solution was neutralized with pre-washed Amberlite 118 H^+ resin and concentrated. The product was purified by chromatography (10:2:0.5 $CHCl_3$–CH_3OH–5N aqueous NH_4OH) on Iatrobeads to give **11** (30 mg, 61%) as a colorless solid. R_f 0.47 (10:4:1 $CHCl_3$–CH_3OH–5N aqueous NH_4OH); $[\alpha]_D$ = +64.5 (*c* 0.2, CH_3OH); ^1H NMR (800 MHz, D_2O) δ_H 5.07 (br s, 1H, H-1′), 4.78 (br s, 1H, H-1), 4.07 (dd, 1H, $J_{2',3}$ = 4.7 Hz, $J_{3',4}$ = 9.7 Hz, H-3′), 3.92 (dd, 1H, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6b), 3.87–3.90 (m, 2H, H-2, H-5′), 3.76 (d, 1H, $J_{6a,6b}$ = 11.2 Hz, H-6a), 3.71–3.73 (m, 2H, H-3, H-5), 3.63–3.68 (m, 2H, H-4, octyl OCH_2), 3.63 (d, 1H, $J_{2',3}$ = 4.7 Hz, H-2′), 3.50 (ddd, 1H, *J* = 6.2, 6.2, 9.6 Hz, octyl OCH_2), 3.48 (dd, 1H, $J_{3',4'}$ = $J_{4,5'}$ = 9.7 Hz, H-4′), 3.42 (dd, 1H, $J_{5',6a'}$ = 2.3 Hz, $J_{6a',6b'}$ = 13.4 Hz, H-6a′), 3.12 (dd, 1H, $J_{5',6b'}$ = 9.6 Hz, $J_{6a',6b'}$ = 13.4 Hz, H-6b′), 1.52–1.56 (m, 2H), 1.20–1.30 (m, 10H), 0.78 (t, 3H, *J* = 6.6 Hz); ^{13}C NMR (150.9 MHz, D_2O) δ_C 101.4 (C-1, $^1J_{C,H}$ = 170.3 Hz), 98.2 (C-1′, $^1J_{C,H}$ = 171.8 Hz), 72.5, 72.1 (C-3, C-5), 71.4 (C-2), 69.8 (C-5′), 69.6 (octyl OCH_2), 69.3 (C-4′), 68.6 (C-3′), 68.0 (C-6), 67.9 (C-4), 55.1 (C-2′), 41.8 (C-6′), 32.5 (octyl CH_2), 29.9 (octyl CH_2), 29.9 (octyl CH_2), 29.8 (octyl CH_2), 26.8 (octyl CH_2), 23.4 (octyl CH_2), 14.8 (octyl CH_3). HR-ESI-MS calcd for $C_{20}H_{40}N_2O_9$ $[M+H]^+$ 453.2812, found 453.2793.

3.5. Octyl 2-amino-2,6-dideoxy-6-fluoro-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 12

Disaccharide **27** (92 mg, 0.11 mmol) in was dissolved in HOAc (7 mL) and 10% Pd/C (45 mg) was added. 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 residue was redissolved in CH_3OH (10 mL), aqueous NaOH (1 mL, 1 M) was added and the solution was stirred overnight. The reaction mixture was neutralized with pre-washed Amberlite 118 H^+ resin and concentrated. The product was purified by chromatography (10:2:0.5 $CHCl_3$–CH_3OH–5N aqueous NH_4OH) on Iatrobeads to give **12** (17 mg, 34%) as a foam. A mono *O*-benzylated amino-disaccharide byproduct (40 mg) was also isolated. This compound was dissolved in HOAc (6 mL) and 10% Pd/C (20 mg) was added and the solution was stirred overnight under a H_2 atmosphere. Filtration followed by concentration and purification as described above gave additional **12** (15 mg, 30%). R_f 0.62 (10:4:1 $CHCl_3$–CH_3OH–5N aqueous NH_4OH); $[\alpha]_D$ = +24.0 (*c* 0.2, CH_3OH); ^1H NMR (800 MHz, CD_3OD) 4.81 (br s, 1H, H-1′), 4.69 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.63 (ddd, 1H, $J_{5',6b'}$ = 4.4 Hz, $J_{6a',6b'}$ = 10.2 Hz, $J_{H6b',F}$ = 47.1 Hz, H-6b′), 4.57 (ddd, 1H, $J_{5',6a'}$ = 1.7 Hz, $J_{6a',6b'}$ = 10.2 Hz, $J_{H6a',F}$ = 47.1 Hz, H-6a′), 3.87 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.82 (dd, 1H, $J_{2',3'}$ = 4.2 Hz, $J_{3',4'}$ = 9.3 Hz, H-3′), 3.78 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 3.71 (d, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.69 (ddd, 1H, *J* = 6.7, 6.7, 9.6 Hz, octyl OCH_2), 3.66 (dd, 1H, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.8 Hz, H-3), 3.62–3.64 (m, 3H, H-4, H-5, H-5′), 3.58 (dd, 1H, $J_{3',4}$ = $J_{4',5'}$ = 9.7 Hz, H-4′), 3.40 (ddd, 1H, *J* = 6.7, 6.7, 9.6 Hz, octyl OCH_2), 3.11 (d, 1H, $J_{2',3'}$ = 4.2 Hz, H-2′), 1.55–1.60 (m, 2H, octyl CH_2), 1.28–1.40 (m, 10H, octyl CH_2), 0.90 (t, 3H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 101.8 (C-1′, $^1J_{C,H}$ = 168.6 Hz), 101.6 (C-1, $^1J_{C,H}$ = 167.9 Hz), 83.5 (C-6′, d, *J* = 170.9 Hz), 73.2 (C-3′), 72.9 (C-5′, d, *J* = 17.6 Hz), 72.8 (C-2), 72.2 (C-5), 71.9 (C-3′), 68.6 (C-4), 68.5 (octyl OCH_2), 66.7 (C-6), 67.1 (d, C-4′, $J_{C-4',F}$ = 6.8 Hz), 55.7 (C-2′), 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 (235.4 MHz, CD_3OD) δ_F −234.4 (ddd, 1F, $J_{H6a',F}$ = $J_{H6b',F}$ = 47.1 Hz, $J_{H5',F}$ = 25.9 Hz, F-6′). HR-ESI-MS calcd for $C_{20}H_{38}FNO_9$ $[M+H]^+$ 456.2609, found 456.2599.

3.6. Octyl 6-amino-6-deoxy-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 13

Disaccharide **32** (164 mg, 0.16 mmol) was dissolved in *n*-butanol (18 mL), and ethylenediamine (4 mL) was added. After stirring overnight at 90 °C, the solution was cooled to room temperature and concentrated under vacuum. The residue was coevaporated with toluene (2 × 15 mL) and ethanol (20 mL) to provide a yellow syr-

up. This crude amine was dissolved in HOAc (5 mL), and 10% Pd/C (35 mg) was added. The solution was stirred overnight under a H_2 atmosphere and 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–5N aqueous NH_4OH) on Iatrobeads to give **13** (34 mg, 84% over two steps) as a colorless solid. R_f 0.63 (10:4:1 $CHCl_3$–CH_3OH–5N aqueous NH_4OH); $[\alpha]_D$ = +49.4 (*c* 0.2, CH_3OH); ^1H NMR (800 MHz, CD_3OD) δ_H 5.05 (br s, 1H, H-1′), 4.75 (br s, 1H, H-1), 3.91 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.83–3.85 (m, 2H, H-2, H-5′), 3.82 (d, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.79 (dd, 1H, $J_{2',3}$ = 3.4 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.72–3.73 (m, 2H, H-5, octyl OCH_2), 3.70 (dd, 1H, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.66 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.54–3.55 (m, 1H, H-2′), 3.49 (dd, 1H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4′), 3.48 (s, 3H, OCH_3), 3.45 (ddd, 1H, *J* = 6.7, 6.7, 9.5 Hz, octyl OCH_2), 3.39 (dd, 1H, $J_{5',6a'}$ = 2.9 Hz, $J_{6a',6b'}$ = 13.0 Hz, H-6a′), 3.03 (dd, 1H, $J_{5,6b'}$ = 9.1 Hz, $J_{6a',6b'}$ = 13.0 Hz, H-6b′), 1.58–1.64 (m, 2H), 1.31–1.44 (m, 10H), 0.92 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 101.7 (C-1, $^1J_{C,H}$ = 167.9 Hz), 98.2 (C-1′, $^1J_{C,H}$ = 169.8 Hz), 81.8 (C-2′), 73.1 (C-3), 72.2 (C-5), 72.1 (C-3′, C-5′), 70.3 (C-4′), 70.2 (C-2), 68.7 (octyl OCH_2), 68.6 (C-4), 67.7 (C-6), 59.3 (OCH_3), 42.3 (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). HR-ESI-MS calcd for $C_{21}H_{41}NO_{10}$ $[M+H]^+$ 468.2808, found 468.2824.

3.7. Octyl 6-deoxy-6-fluoro-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 14

Disaccharide **35** (100 mg, 0.11 mmol) was dissolved in HOAc (6 mL), and 10% Pd/C (40 mg) was added. 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 (4:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **14** (40 mg, 74%) as a foam. R_f 0.74 (4:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +59.7 (*c* 0.2, CH_3OH); ^1H NMR (800 MHz, CD_3OD) δ_H 5.02 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.72 (br s, 1H, H-1), 4.63 (ddd, 1H, $J_{5',6b'}$ = 4.2 Hz, $J_{6a',6b'}$ = 10.1 Hz, $J_{H6b',F}$ = 46.7 Hz, H-6b′), 4.58 (ddd, 1H, $J_{5',6a'}$ = 1.1 Hz, $J_{6a',6b'}$ = 10.1 Hz, $J_{H6a',F}$ = 46.7 Hz, H-6a′), 3.91 (dd, 1H, $J_{5,6b}$ = 5.1 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.81 (br s, 1H, H-2), 3.79 (dd, 1H, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.7 Hz, H-3), 3.76 (d, 1H, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.73–3.74 (m, 1H, H-5), 3.72 (ddd, 1H, *J* = 6.6, 6.6, 9.7 Hz, octyl OCH_2), 3.65–3.69 (m, 3H, H-3, H-4, H-5), 3.58 (dd, 1H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4′), 3.52 (dd, 1H, $J_{1',2'}$ = 1.3 Hz, $J_{2',3'}$ = 3.4 Hz, H-2), 3.49 (s, 3H, OCH_3), 3.43 (ddd, 1H, *J* = 6.6, 6.6, 9.7 Hz, octyl OCH_2), 1.58–1.65 (m, 2H), 1.32–1.42 (m, 10H), 0.92 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 101.7 (C-1, $^1J_{C,H}$ = 167.9 Hz), 98.2 (C-1′, $^1J_{C,H}$ = 168.9 Hz), 83.4 (C-6′, $J_{C\text{-}6',F}$ = 170.9 Hz), 81.8 (C-2′), 73.2 (C-5), 73.1 (C-5′, $J_{C\text{-}5,F}$ = 18.5 Hz), 72.8 (C-3′), 72.4 (C-3), 72.1 (C-2), 68.6 (octyl OCH_2), 68.5 (C-4), 67.7 (C-4′, $J_{C\text{-}4',F}$ = 6.8 Hz), 67.6 (C-6), 59.3 (OCH_3), 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 (235.4 MHz, CD_3OD) δ_F −234.6 (ddd, 1F, $J_{H6a',F} = J_{H6b',F}$ = 46.7 Hz, $J_{H5',F}$ = 25.9 Hz, F-6′). HR-ESI-MS calcd for $C_{21}H_{39}FO_{10}$ $[M+H]^+$ 493.2425, found 493.2415.

3.8. Octyl 2-deoxy-2-fluoro-6-*O*-*p*-toluenesulfonyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 19

Disaccharide **15** (255 mg, 0.35 mmol) was dissolved in pyridine (8 mL), and *p*-toluenesulfonyl chloride (330 mg, 1.70 mmol) and DMAP (100 mg, 0.80 mmol) were added. After stirring for 18 h, the solution was concentrated under vacuum. The residue was purified by chromatography (1:1 hexane–EtOAc) to give **19** (209 mg, 68%) as a colorless syrup. R_f 0.64 (1:1 hexane–EtOAc); $[\alpha]_D$ = +34.1 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.84 (d, 2H, *J* = 8.3 Hz), 7.29–7.42 (m, 17H), 5.11 (dd, 1H, *J* = 1.8, 7.2 Hz), 4.99 (d, 1H, *J* = 11.2 Hz), 4.81 (d, 1H, *J* = 1.6 Hz), 4.79 (d, 1H, *J* = 12.2 Hz), 4.75 (ddd, 1H, *J* = 1.8, 3.8, 49.4 Hz), 4.69–4.71 (m, 3H), 4.61 (d, 1H, *J* = 11.2 Hz), 4.34 (dd, 1H, *J* = 2.9, 11.1 Hz), 4.18 (d, 1H, *J* = 11.1 Hz), 3.96–3.98 (m, 2H), 3.91 (ddd, 1H, *J* = 4.1, 4.1, 10.7 Hz), 3.83 (br s, 1H), 3.79–3.81 (m, 3H), 3.73 (dd, 1H, *J* = 1.5, 11.7 Hz), 3.69–3.70 (m, 1H), 3.61 (ddd, 1H, *J* = 6.7, 6.7, 9.6 Hz), 3.37 (ddd, 1H, *J* = 6.7, 6.7, 9.6 Hz), 2.48 (s, 3H), 1.54–1.57 (m, 2H), 1.32–1.38 (m, 10H), 0.94 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 145.3, 138.9, 138.8, 138.7, 133.3, 130.2, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 98.4 (d, *J* = 29.4 Hz), 98.1, 89.3 (d, *J* = 174.6 Hz), 80.6, 75.4, 75.3, 74.7, 73.2, 72.5, 72.0, 71.1 (d, *J* = 17.5 Hz), 70.6, 69.0, 68.2, 67.8, 67.3, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 22.1, 14.5; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −205.6 (ddd, 1F, *J* = 7.2, 49.4, 33.0 Hz). HR-ESI-MS calcd for $C_{48}H_{61}FO_{12}S$ $[M+Na]^+$ 903.3765, found 903.3757.

3.9. Octyl 6-azido-2,6-dideoxy-2-fluoro-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 20

Tosylate **19** (209 mg, 0.24 mmol) was dissolved in DMF (10 mL), and NaN_3 (85 mg, 1.3 mmol) and 15-crown-5 (230 μL, 1.3 mmol) were added. After stirring overnight at 60 °C, the solution was concentrated under vacuum. The residue was taken in CH_2Cl_2 (2 × 40 mL), washed with water (30 mL), dried (Na_2SO_4), filtered, and concentrated to a yellow syrup. The crude syrup was purified by chromatography (3:1 hexane–EtOAc) to give **20** (166 mg, 92%) as a light colorless syrup. R_f 0.72 (1:1 hexane–EtOAc); $[\alpha]_D$ = +37.2 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.24–7.37 (m, 15H), 5.14 (dd, 1H, *J* = 1.2, 7.2 Hz), 4.94 (d, 1H, *J* = 11.0 Hz), 4.78 (d, 1H, *J* = 1.4 Hz), 4.74 (d, 1H, *J* = 12.2 Hz), 4.71 (ddd, 1H, *J* = 1.2, 2.0, 49.4 Hz), 4.63–4.66 (m, 3H), 4.59 (d, 1H, *J* = 11.1 Hz), 3.98 (dd, 1H, *J* = 3.9, 11.4 Hz), 3.90–3.94 (m, 2H), 3.67–3.76 (m, 6 H), 3.57 (ddd, 1H, *J* = 6.7, 6.7, 9.6 Hz), 3.45 (dd, 1H, *J* = 2.5,

13.2 Hz), 3.39 (dd, 1H, J = 5.8, 13.2 Hz), 3.32 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 1.49–1.51 (m, 2H), 1.27–1.33 (m, 10H), 0.88 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.5, 138.4, 138.3, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 98.0 (d, J = 29.6 Hz), 97.7, 89.2 (d, J = 174.2 Hz), 80.1, 75.5, 75.4, 74.6, 73.2, 72.5, 72.0, 71.9, 71.3 (d, J = 17.7 Hz), 69.1, 68.3, 67.4, 51.3, 31.8, 29.5, 29.4, 29.2, 26.2, 22.7, 14.1; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −205.6 (ddd, 1F, J = 7.2, 49.4, 30.6 Hz). HR-ESI-MS calcd for $C_{41}H_{54}FN_3O_9$ $[M+Na]^+$ 774.3742, found 774.3787.

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

Azidodisaccharide **20** (166 mg, 0.22 mmol) was dissolved in THF (8 mL), and H_2O (2 mL) and PPh_3 (173 mg, 0.66 mmol) were added. After stirring overnight, the mixture was concentrated and dried under vacuum for 2 h. The crude amine was dissolved in pyridine (6 mL) and CH_2Cl_2 (6 mL), and trifluoroacetic anhydride (0.6 mL) was added dropwise. After stirring overnight, the reaction mixture was concentrated. The residue was taken in CH_2Cl_2 (30 mL), washed with ice-cold 1 M HCl solution (20 mL) and a saturated aqueous $NaHCO_3$ solution (10 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated to a brown syrup. The crude syrup was dissolved in CH_3OH (10 mL) and stirred for 4 h and then concentrated. The residue was purified by chromatography (1:1 hexane–EtOAc) to give **21** (150 mg, 83% over two steps) as a light yellow syrup. R_f 0.44 (1:1 hexane–EtOAc); $[\alpha]_D$ = +39.4 (c 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.27–7.43 (m, 15H), 6.57 (d, 1H, J = 6.7 Hz), 5.15 (dd, 1H, J = 2.1, 7.3 Hz), 5.03 (d, 1H, J = 11.2 Hz), 4.83 (d, 1H, J = 1.7 Hz), 4.81 (d, 1H, J = 12.2 Hz), 4.76 (ddd, 1H, J = 2.1, 2.1, 49.8 Hz), 4.71–4.73 (m, 3H), 4.65 (d, 1H, J = 11.2 Hz), 3.98–4.02 (m, 2H), 3.94 (dd, 1H, J = 4.5, 11.6 Hz), 3.85–3.89 (m, 2H), 3.74–3.81 (m, 4H), 3.65 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 3.58 (dd, 1H, J = 9.6, 9.6 Hz), 3.46 (ddd, 1H, J = 4.5, 4.5, 9.6 Hz), 3.38 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 1.56–1.59 (m, 2H), 1.29–1.41 (m, 10H), 0.96 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 158.5 (q, J = 37.2 Hz), 138.8, 138.7, 138.6, 130.0, 128.9, 128.7, 128.4, 128.3, 128.2, 128.1, 128.0, 116.3 (q, J = 288 Hz), 98.4 (d, J = 30.0 Hz), 98.2, 89.4 (d, J = 175.4 Hz), 80.6, 75.5, 75.4, 74.8, 73.3, 72.6, 72.0, 70.7 (d, J = 15.6 Hz), 70.6, 69.1, 68.3, 67.3, 40.9, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.5; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −74.6 (s, 3F), −205.6 (ddd, 1F, J = 7.3, 49.8, 30.0 Hz). HR-ESI-MS calcd for $C_{43}H_{55}F_4NO_{10}$ $[M+Na]^+$ 844.3660, found 844.3634.

3.11. Octyl 2,6-dideoxy-2,6-difluoro-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*- benzyl-α-D-mannopyranoside 22

Fluorodisaccharide **15** (180 mg, 0.25 mmol) was dissolved in CH_2Cl_2 (6 mL) cooled to −40 °C, and DAST (130 μL, 1.0 mmol) was added. After stirring for 2 h, CH_3OH (1 mL) was added and the solution was concentrated. The residue was purified by chromatography (3:1 hexane–EtOAc) on silica gel to give **22** (100 mg, 55%) as a colorless syrup. R_f 0.70 (1:1 hexane–EtOAc); $[\alpha]_D$ = +42.5 (c 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.22–7.40 (m, 15H), 5.15 (dd, 1H, J = 1.6, 8.9 Hz), 4.92 (d, 1H, J = 11.4 Hz), 4.77 (d, 1H, J = 1.5 Hz), 4.74 (d, 1H, J = 12.2 Hz), 4.61–4.67 (m, 4H′), 4.59 (d, 1H, J = 11.1 Hz), 4.58 (ddd, 1H, J = 3.6, 10.2, 47.1 Hz), 4.57 (ddd, 1H, J = 1.5, 10.2, 47.1 Hz), 3.91–3.99 (m, 3H), 3.66–3.76 (m, 6 H), 3.57 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 3.32 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 2.70 (br s, 1H), 2.31 (br s, 1H), 1.44–1.51 (m, 2H), 1.26–1.36 (m, 10H), 0.88 (t, 3H, J = 7.1 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C 138.4, 138.3, 138.2, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 98.0 (d, J = 29.7 Hz), 97.7, 89.1 (d, J = 174.3 Hz), 82.0 (d, J = 172.3 Hz), 80.1, 77.2, 75.0, 74.9, 74.2, 72.8, 72.1, 71.5, 71.1 (d, J = 17.7 Hz), 67.8, 67.1 (d, J = 6.8 Hz), 66.9, 31.8, 29.4, 29.5, 29.2, 26.1, 22.7, 14.1; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −205.6 (ddd, 1F, J = 8.9, 49.4, 32.9 Hz), −234.8 (ddd, 1F, J = 47.1, 47.1, 28.2 Hz). HR-ESI-MS calcd for $C_{41}H_{54}F_2O_9$ $[M+Na]^+$ 751.3634, found 751.3607.

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

Disaccharide **16** (450 mg, 0.60 mmol) was dissolved in CH_2Cl_2 (5 mL) and pyridine (5 mL), *p*-toluenesulfonyl chloride (172 mg, 0.90 mmol) was added. After stirring overnight, the solution was concentrated under vacuum. The residue was purified by chromatography (3:1 hexane–EtOAc) to give **23** (375 mg, 70%) as a colorless syrup. R_f 0.61 (1:1 hexane–EtOAc); $[\alpha]_D$ = +31.7 (c 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.84 (d, 2H, J = 8.0 Hz), 7.29–7.43 (m, 17H), 5.00 (d, 1H, J = 12.0 Hz), 4.99 (d, 1H, J = 1.4 Hz), 4.82 (d, 1H, J = 1.6 Hz), 4.76 (ABq, 2H, J = 12.1 Hz, $\Delta\nu$ = 41.3 Hz), 4.68–4.69 (m, 2H), 4.63 (d, 1H, J = 11.4 Hz), 4.31 (dd, 1H, J = 3.6, 11.5 Hz), 4.18 (dd, 1H, J = 1.0, 11.5 Hz), 4.01 (dd, 1H, J = 1.4, 4.2 Hz), 3.90–3.98 (m, 4H), 3.81–3.82 (m, 1H), 3.68–3.73 (m, 4H), 3.63 (ddd, 1H, J = 6.8, 6.8, 9.6 Hz), 3.38 (ddd, 1H, J = 6.8, 6.8, 9.6 Hz), 2.48 (s, 3H), 1.55–1.57 (m, 2H), 1.33–1.38 (m, 10H), 0.94 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 145.0, 138.9, 138.8, 138.7, 133.3, 130.2, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 99.4, 98.2, 80.6, 75.4, 75.3, 74.6, 73.3, 72.6, 72.1, 71.3, 70.8, 68.3, 68.1, 67.7, 67.5, 63.6, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 22.1, 14.5. HR-ESI-MS calcd for $C_{48}H_{61}N_3O_{12}S$ $[M+Na]^+$ 926.3868, found 926.3850.

3.13. Octyl 2,6-diazido-2,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*- benzyl-α-D-mannopyranoside 24

Tosylate **23** (78 mg, 0.09 mmol) was dissolved in DMF (2 mL), and NaN_3 (18 mg, 0.27 mmol) and 15-crown-5 (50 μL, 0.27 mmol) were added. After stirring overnight at 60 °C, the solution was concentrated under vacuum. The crude residue was purified by chromatography (4:1 hexane–EtOAc) to give **24** (60 mg, 85%) as a light yellow syrup. R_f 0.73 (1:1 hexane–EtOAc); $[\alpha]_D$ = +47.0 (c 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.24–7.40 (m, 15H), 5.02 (d, 1H,

J = 1.3 Hz), 4.95 (d, 1H, J = 11.1 Hz), 4.80 (d, 1H, J = 1.6 Hz), 4.72 (ABq, 2H, J = 12.1 Hz, $\Delta\nu$ = 31.6 Hz), 4.63–4.65 (m, 2H), 4.61 (d, 1H, J = 11.1 Hz), 3.98–4.00 (m, 3H), 3.96 (dd, 1H, J = 3.0, 9.4 Hz), 3.87 (dd, 1H, J = 5.0, 9.4 Hz), 3.79 (dd, 1H, J = 1.6, 2.5 Hz), 3.71 (dd, 1H, J = 1.7, 11.7 Hz), 3.66–3.69 (m, 2H), 3.58 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 3.57 (dd, 1H, J = 9.4, 9.4 Hz), 3.39 (dd, 1H, J = 2.6, 12.1 Hz), 3.37 (m, 2H), 1.49–1.52 (m, 2H), 1.27–1.32 (m, 10H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.4, 138.3, 138.1, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 99.0, 97.7, 80.2, 75.1, 74.9, 74.2, 72.9, 72.2, 71.6, 71.6, 71.2, 68.6, 67.9, 67.2, 63.4, 51.3, 31.8, 29.4, 29.3, 29.2, 26.2, 22.7, 14.1. HR-ESI-MS calcd for $C_{41}H_{54}N_6O_9$ $[M+Na]^+$ 797.3844, found 797.3842.

3.14. Octyl 2,6-dideoxy-2,6-di-(trifluoroacetamido)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 25

Diazide **24** (300 mg, 0.39 mmol) was dissolved in THF (25 mL), and H_2O (5 mL) and PPh_3 (720 mg, 2.7 mmol) were added. After stirring overnight, the reaction mixture was concentrated and dried under vacuum for 2 h. The crude amine was dissolved in pyridine (5 mL), and trifluoroacetic anhydride (5 mL) was added dropwise. After stirring overnight, the reaction mixture was concentrated. The residue was dissolved in CH_3OH (20 mL) and stirred for 4 h and then concentrated. The residue was taken in CH_2Cl_2 (50 mL), washed with ice-cold 1 M HCl solution (2 × 30 mL) and water (30 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated to a brown syrup. The residue was purified by chromatography (3:1 hexane–EtOAc) to give **25** (120 mg, 34% over two steps) as a light yellow syrup. R_f 0.73 (1:1 hexane–EtOAc); $[\alpha]_D$ = +14.6 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.31–7.42 (m, 15H), 6.80 (d, 1H, J = 7.0 Hz), 6.50 (d, 1H, J = 7.0 Hz,), 5.08 (br s, 1H), 5.05 (d, 1H, J = 11.3 Hz), 4.86 (d, 1H, J = 1.0 Hz), 4.75 (ABq, 2H, J = 12.1 Hz, $\Delta\nu$ = 19.4 Hz), 4.67–4.69 (m, 2H), 4.63 (d, 1H, J = 11.3 Hz), 4.41 (d, 1H, J = 5.6 Hz), 4.07–4.10 (m, 1H), 3.99 (dd, 1H, J = 2.8, 9.5 Hz), 3.93 (dd, 1H, J = 9.4, 9.4 Hz), 3.88 (dd, 1H, J = 4.7, 11.0 Hz), 3.83 (dd, 1H, J = 1.0, 2.8 Hz), 3.72–3.82 (m, 4H), 3.65 (ddd, 1H, J = 6.7, 6.7, 9.4 Hz), 3.39–3.41 (m, 4H), 3.32 (dd, 1H, J = 3.3, 9.5 Hz), 1.55–1.58 (m, 2H), 1.32–1.37 (m, 10H), 0.94 (t, 3H, J = 6.6 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 158.8 (q, J = 37.4 Hz), 158.3 (q, J = 37.4 Hz), 138.8, 138.7, 138.6, 129.0, 128.9, 128.8, 128.4, 128.3, 128.2, 128.1, 116.2 (q, J = 287.4 Hz), 116.1 (q, J = 288.2 Hz), 98.4, 98.2, 80.6, 75.4, 75.2, 74.9, 73.2, 72.5, 71.5, 70.1, 69.4, 69.1, 68.3, 67.8, 53.8, 40.8, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.5. HR-ESI-MS calcd for $C_{45}H_{56}F_6N_2O_{11}$ $[M+Na]^+$ 937.3681, found 937.3716.

3.15. Octyl 2-azido-2,6-dideoxy-6-fluoro-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 26

Disaccharide **16** (150 mg, 0.20 mmol) was dissolved in CH_2Cl_2 (3 mL) and cooled to −40 °C, and then DAST (1.40 μL, 1.1 mmol) was added. After stirring for 2 h, CH_3OH (1 mL) was added and the solution was concentrated. The residue was purified by chromatography (3:1 hexane–EtOAc) to give **26** (110 mg, 72%) as a colorless syrup. R_f 0.65 (1:1 hexane–EtOAc); $[\alpha]_D$ = +43.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.16–7.31 (m, 15H), 4.94 (br s, 1H), 4.88 (d, 1H, J = 11.1 Hz), 4.73 (br s, 1H), 4.64 (ABq, 2H, J = 12.1 Hz, $\Delta\nu$ = 30.4 Hz), 4.50–4.52 (m, 3H), 4.42 (ddd, 1H, J = 3.4, 10.1, 46.7 Hz), 4.36 (dd, 1H, J = 10.1, 46.7 Hz), 3.84–3.93 (m, 5 H), 3.71–3.72 (m, 1H), 3.53–3.62 (m, 4H), 3.51 (ddd, 1H, J = 6.7, 6.7, 9.4 Hz), 3.26 (ddd, 1H, J = 6.7, 6.7, 9.4 Hz), 3.04 (br s, 1H), 2.71 (br s, 1H), 1.39–1.42 (m, 2H), 1.15–1.25 (m, 10H), 0.81 (t, 3H, J = 7.0 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.8, 138.6, 138.4, 129.0, 129.0, 128.9, 128.6, 128.4, 128.3, 128.2, 99.4, 98.0, 82.4 (d, J = 172.3 Hz), 80.5, 75.4, 75.1, 74.5, 73.2, 72.6, 71.8, 71.7, 71.6 (d, J = 17.8 Hz), 68.4, 67.4, 67.1 (d, J = 6.8 Hz), 63.8, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.6; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −234.8 (ddd, 1F, J = 46.7, 46.7, 28.1 Hz). HR-ESI-MS calcd for $C_{41}H_{54}FN_3O_9$ $[M+Na]^+$ 774.3736, found 774.3725.

3.16. Octyl 2,6-dideoxy-6-fluoro-2-trifluoroacetamido-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 27

Disaccharide **26** (150 mg, 0.20 mmol) was dissolved in THF (8 mL), and H_2O (1 mL) and PPh_3 (155 mg, 0.59 mmol) were added. After stirring overnight, the reaction mixture was concentrated and dried under vacuum for 2 h. The crude amine was dissolved in pyridine (5 mL) and trifluoroacetic anhydride (3 mL) was added dropwise. The reaction mixture was stirred overnight and concentrated. The residue was dissolved in CH_2Cl_2 (25 mL), washed with ice-cold 1 M HCl solution (2 × 15 mL) and a saturated $NaHCO_3$ solution (15 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated to a brown syrup. The crude syrup was dissolved in CH_3OH (10 mL) and stirred for 4 h and then concentrated. The residue was purified by chromatography (3:1 hexane–EtOAc) to give **27** (105 mg, 64% over two steps) as a light yellow syrup. R_f 0.63 (1:1 hexane–EtOAc); $[\alpha]_D$ = +51.9 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.30–7.43 (m, 15H), 6.57 (d, 1H, J = 9.1 Hz), 5.03 (d, 1H, J = 11.2 Hz), 5.00 (br s, 1H), 4.86 (d, 1H, J = 1.2 Hz), 4.79–4.80 (m, 2H), 4.67–4.68 (m, 2H), 4.65 (d, 1H, J = 11.2 Hz), 4.47–4.59 (m, 3H), 4.12 (dd, 1H, J = 4.4, 9.8 Hz), 3.96–3.99 (m, 3H), 3.74–3.84 (m, 4H), 3.65 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 3.61 (dd, 1H, J = 9.8, 9.8 Hz), 3.38 (ddd, 1H, J = 6.7, 6.7, 9.6 Hz), 3.13 (br s, 1H), 3.03 (br s, 1H), 1.55–1.58 (m, 2H), 1.33–1.40 (m, 10H), 0.95 (t, 3H, J = 6.8 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 158.5 (q, J = 37.9 Hz), 138.8, 138.7, 138.6, 128.9, 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 116.2 (q, J = 288 Hz), 99.2, 98.1, 82.1 (d, J = 172.2 Hz), 80.6, 75.4, 75.3, 74.8, 73.2, 72.5, 71.5, 71.2 (d, J = 17.8 Hz), 70.3, 68.3, 67.9, 66.7 (d, J = 6.9 Hz), 53.8, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.5; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −74.6 (s, 3F), −234.8 (ddd,

1F, $J_{H6a',F} = J = 47.1, 47.1, 28.3$ Hz). HR-ESI-MS calcd for $C_{43}H_{55}F_4NO_{10}$ $[M+Na]^+$ 844.3654, found 844.3640.

3.17. Octyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 28

Trichloroacetimidate **17**[24] (1.67 g, 2.6 mmol) and alcohol **18**[23] (1.20 g, 2.1 mmol) were dried in vacuo with powdered 4 Å molecular sieves (1.0 g) overnight before CH_2Cl_2 (35 mL) was added and the mixture was cooled to −10 °C with stirring. A solution of TMSOTf (135 μL) in CH_2Cl_2 (400 μL) was added dropwise to the reaction mixture and the stirring was continued for 2 h. The solution was neutralized by the addition of a saturated aqueous $NaHCO_3$ solution (1.0 mL) and CH_2Cl_2 (2 × 50 mL) was added. The organic layer was washed with water (30 mL), dried (Na_2SO_4), filtered, and concentrated to a colorless syrup. The crude syrup was purified by chromatography (4:1 hexane–EtOAc) to give **28** (2.0 mg, 91%) as a colorless syrup. R_f 0.55 (4:1 hexane–EtOAc); $[\alpha]_D$ = +24.0 (*c* 0.4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 8.07 (dd, 2H, *J* = 1.1, 8.4 Hz), 7.59 (dd, 1H, *J* = 1.2, 7.7 Hz), 7.45 (dd, 2H, *J* = 8.1, 8.1 Hz), 7.16–7.40 (m, 25H), 5.74 (dd, 1H, *J* = 2.0, 3.0 Hz), 5.06 (d, 1H, *J* = 2.0 Hz), 4.93 (d, 1H, *J* = 11.1 Hz), 4.87 (d, 1H, *J* = 11.0 Hz), 4.82 (d, 1H, *J* = 2.0 Hz), 4.76–4.78 (m, 2H), 4.74 (d, 1H, *J* = 11.3 Hz), 4.62–4.63 (m, 2H), 4.57 (d, 1H, *J* = 11.0 Hz), 4.49 (d, 1H, *J* = 11.1 Hz), 4.47 (d, 1H, *J* = 11.3 Hz), 4.29 (dd, 1H, *J* = 2.1, 11.9 Hz), 4.24 (dd, 1H, *J* = 3.8, 11.9 Hz), 4.09 (dd, 1H, *J* = 3.0, 8.6 Hz), 3.89–3.95 (m, 4H), 3.86 (dd, 1H, *J* = 9.8, 9.8 Hz), 3.79 (dd, 1H, *J* = 2.0, 2.0 Hz), 3.70–3.74 (m, 2H), 3.59 (ddd, 1H, *J* = 6.8, 6.8, 9.7 Hz), 3.33 (ddd, 1H, *J* = 6.8, 6.8, 9.7 Hz), 2.00 (s, 3H), 1.47–1.50 (m, 2H), 1.24–1.29 (m, 10H), 0.87 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 170.7, 165.3, 138.5, 138.4, 138.3, 138.1, 137.7, 130.1, 129.9, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 97.9, 97.6, 80.4, 77.8, 75.1, 74.9, 74.8, 74.6, 73.4, 72.7, 72.0, 71.5, 71.5, 69.8, 68.5, 67.7, 66.8, 63.3, 31.9, 29.5, 29.4, 29.2, 26.2, 22.7, 20.8, 14.1. HR-ESI-MS calcd for $C_{64}H_{74}O_{13}$ $[M+Na]^+$ 1073.5027, found 1073.5011.

3.18. Octyl 3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 29

Disaccharide **28** (1.90 g, 1.8 mmol) was dissolved in CH_3OH (50 mL) and 10 drops of 1 M $NaOCH_3$ was added. After stirring overnight, the solution was neutralized with pre-washed Amberlite 118 H^+ resin and concentrated to a syrup, which was purified by chromatography (1:1 hexane–EtOAc) to give **29** (1.56 g, 96%) as a colorless syrup. R_f 0.44 (1:1 hexane–EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.20–7.48 (m, 25H), 5.06 (d, 1H, *J* = 1.7 Hz), 4.95 (d, 1H, *J* = 11.1 Hz), 4.86 (d, 1H, *J* = 11.1 Hz), 4.81 (d, 1H, *J* = 1.7 Hz), 4.72 (ABq, 2H, *J* = 12.3 Hz, Δ*v* = 27.8 Hz), 4.62–4.64 (m, 3H), 4.55 (ABq, 2H, *J* = 11.6 Hz, Δ*v* = 20.2 Hz), 4.52 (d, 1H, *J* = 12.0 Hz), 4.05–4.06 (m, 1H), 3.69–3.93 (m, 11H), 3.58 (ddd, 1H, *J* = 6.8, 6.8, 9.6 Hz), 3.32 (ddd, 1H, *J* = 6.8, 6.8, 9.7 Hz), 2.52 (d, 1H, *J* = 1.8 Hz), 2.05 (br s, 1H), 1.48–1.51 (m, 2H), 1.26–1.31 (m, 10H), 0.87 (t, 3H, *J* = 6.7 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.9, 138.8, 138.2, 129.0, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 99.9, 99.1, 80.7, 79.9, 75.6, 75.5, 75.4, 75.0, 74.4, 73.2, 72.5, 72.1, 72.0, 71.9, 68.5, 68.2, 66.7, 62.4, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.6.

3.19. Octyl 3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 30

Diol **29** (650 mg, 0.72 mmol) was dissolved in DMF (6 mL), and TBDPSCl (0.22 mL, 0.85 mmol) and imidazole (90 mg, 1.3 mmol) were added. After stirring overnight, the solution was concentrated under vacuum. The residue was taken in CH_2Cl_2 (2 × 30 mL), washed with water (25 mL), and dried (Na_2SO_4), filtered, and concentrated to a colorless syrup. The crude syrup was purified by chromatography (4:1 hexane–EtOAc) to give **30** (610 mg, 74%) as a colorless syrup. R_f 0.37 (4:1 hexane–EtOAc); $[\alpha]_D$ = +29.4 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.73 (dd, 2H, *J* = 1.0, 7.8 Hz), 7.68 (dd, 2H, *J* = 1.0, 7.8 Hz), 7.15–7.40 (m, 31H), 5.08 (br s, 1H), 4.88 (d, 1H, *J* = 11.0 Hz), 4.87 (d, 1H, *J* = 11.0 Hz), 4.81 (br s, 1H), 4.71 (ABq, 2H, *J* = 12.3 Hz, Δ*v* = 24.0 Hz), 4.60–4.64 (m, 4H), 4.56 (d, 1H, *J* = 10.9 Hz), 4.49 (d, 1H, *J* = 10.9 Hz), 4.14 (br s, 1H), 3.80–3.97 (m, 7 H), 3.77 (br s, 1H), 3.68–3.71 (m, 3H), 3.59 (ddd, 1H, *J* = 6.9, 6.9, 9.2 Hz), 3.32 (ddd, 1H, *J* = 6.9, 6.9, 9.2 Hz), 2.30 (br s, 1H), 1.48–1.51 (m, 2H), 1.19–1.31 (m, 10H), 1.04 (s, 9H), 0.87 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.5, 135.9, 135.7, 129.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 99.6, 97.6, 80.3, 79.8, 75.2, 75.1, 75.0, 74.6, 74.1, 72.6, 72.3, 72.1, 71.7, 71.6, 68.2, 67.6, 66.0, 62.9, 31.9, 29.5, 29.4, 29.3, 26.8, 26.2, 22.7, 19.3, 14.1, −1.5. HR-ESI-MS calcd for $C_{71}H_{86}O_{11}Si$ $[M+Na]^+$ 1165.5837, found 1165.5830.

3.20. Octyl 3,4-di-*O*-benzyl-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 31

Alcohol **30** (580 mg, 0.51 mmol) was dissolved in DMF (3 mL) the solution cooled to 0 °C and then NaH (60 mg, 2.5 mmol) was added. After stirring for 10 min, CH_3I (0.35 mL, 5.6 mmol) was added and the reaction mixture was stirred overnight, before CH_3OH (1 mL) was added, followed by stirring for 1 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (2 × 25 mL), washed with water (25 mL), dried (Na_2SO_4), filtered, and concentrated to a light yellow syrup, which was dried under vacuum for 4 h. The crude syrup was dissolved in THF (14 mL) and *n*-Bu_4NF–$3H_2O$ (786 mg, 2.5 mmol) was added. After stirring for 48 h, the reaction mixture was concentrated and the residue was purified by chromatography (3:1 hexane–EtOAc) to give **31** (310 mg, 67% over two steps) as a colorless syrup. R_f 0.41 (2:1 hexane–EtOAc); $[\alpha]_D$ = +28.5 (*c* 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ_H 7.17–7.39 (m, 25H), 5.09 (d, 1H, *J* = 1.6 Hz), 4.92 (d, 1H, *J* = 11.0 Hz), 4.89 (d, 1H, *J* = 11.0 Hz), 4.81 (d, 1H, *J* = 1.6 Hz), 4.73 (ABq, 2H, *J* = 12.2 Hz, Δ*v* = 36.0 Hz), 4.63–4.66 (m, 3H), 4.60 (d, 1H, *J* = 11.0 Hz), 4.58 (d,

1H, J = 11.9 Hz), 4.46 (d, 1H, J = 11.0 Hz), 3.87–3.94 (m, 4H), 3.83 (dd, 1H, J = 9.5, 9.5 Hz), 3.79 (dd, 1H, J = 1.6, 2.0 Hz), 3.73 (dd, 1H, J = 2.6, 11.7 Hz), 3.61–3.71 (m, 4H), 3.58 (ddd, 1H, J = 6.8, 6.8, 9.6 Hz), 3.41 (s, 3H), 3.33 (ddd, 1H, J = 6.8, 6.8, 9.6 Hz), 1.92 (br s, 1H), 1.49–1.51 (m, 2H), 1.26–1.32 (m, 10H), 0.88 (t, 3H, J = 6.8 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 139.0, 138.9, 138.8, 138.6, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 98.3, 98.1, 80.8, 79.4, 77.8, 75.6, 75.6, 75.6, 75.1, 75.0, 73.4, 72.6, 72.5, 72.1, 72.1, 68.1, 66.5, 62.7, 59.5, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.6. HR-ESI-MS calcd for $C_{56}H_{70}O_{11}$ $[M+Na]^+$ 941.4816, found 941.4791.

3.21. Octyl 3,4-di-*O*-benzyl-6-deoxy-2-*O*-methyl-6-phthalimido-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 32

Alcohol **31** (170 mg, 0.19 mmol), PPh_3 (100 mg, 0.38 mmol), and phthalimide (43 mg, 0.29 mmol) were dissolved in THF (8 mL) and diisopropylazodicarboxylate (75 μL, 0.38 mmol) was added. After stirring overnight, the solution was concentrated under vacuum and the residue was purified by chromatography (4:1 hexane–EtOAc) to give **32** (170 mg, 85%) as a colorless syrup. R_f 0.34 (3:1 hexane–EtOAc); $[\alpha]_D$ = +16.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.69–7.73 (m, 2H), 7.56–7.59 (m, 2H), 7.15–7.37 (m, 25H), 5.04 (br s, 1H), 5.03 (d, 1H, J = 11.0 Hz), 4.77 (d, 1H, J = 2.0 Hz), 4.75 (d, 1H, J = 10.8 Hz), 4.74 (d, 1H, J = 12.2 Hz), 4.71 (d, 1H, J = 11.4 Hz), 4.67 (d, 1H, J = 12.2 Hz), 4.62–4.64 (m, 2H), 4.52 (d, 1H, J = 12.0 Hz), 4.51 (d, 1H, J = 11.9 Hz), 4.38 (d, 1H, J = 10.8 Hz), 4.00–4.04 (m, 1H), 3.98 (dd, 1H, J = 7.1, 13.8 Hz), 3.90 (dd, 1H, J = 4.3, 13.8 Hz), 3.83–3.88 (m, 2H), 3.81 (dd, 1H, J = 9.5, 9.5 Hz), 3.76 (d, 1H, J = 2.3 Hz), 3.74 (dd, 1H, J = 2.0, 2.0 Hz), 3.72 (dd, 1H, J = 9.5, 9.5 Hz), 3.54–3.59 (m, 3H), 3.51 (ddd, 1H, J = 6.8, 6.8, 9.7 Hz), 3.33 (s, 3H), 3.29 (ddd, 1H, J = 6.8, 6.8, 9.7 Hz), 1.45–1.47 (m, 2H), 1.24–1.32 (m, 10H), 0.87 (t, 3H, J = 6.9 Hz); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 168.6, 139.3, 139.0, 138.9, 138.5, 134.0, 132.5, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 123.5, 98.2, 97.6, 80.7, 79.5, 77.5, 75.8, 75.6, 75.0, 74.9, 73.4, 73.4, 72.7, 72.4, 71.8, 71.8, 69.3, 68.0, 66.2, 59.1, 32.3, 29.9, 29.7, 29.3, 26.6, 23.1, 14.5. HR-ESI-MS calcd for $C_{64}H_{73}NO_{12}$ $[M+Na]^+$ 1070.5030, found 1070.4985.

3.22. Octyl 3,6-anhydro-4-*O*-benzyl-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 33

Disaccharide **31** (133 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (7 mL) and the solution was cooled to −40 °C and DAST (100 μL, 0.76 mmol) was added. After stirring for 6 h, CH_3OH was added (1 mL) and the solution was concentrated. The residue was taken in CH_2Cl_2 (2 × 20 mL), washed with water (20 mL) and dried (Na_2SO_4), filtered, and concentrated to a brown syrup. The crude syrup was purified by chromatography (3:1 hexane–EtOAc) to give **33** (100 mg, 83%) as a light yellow syrup. R_f 0.53 (2:1 hexane–EtOAc); $[\alpha]_D$ = +29.5 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.21–7.37 (m, 20H, Ph), 4.93 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-1′), 4.86 (d, 1H, J = 10.6 Hz, benzylic CH_2), 4.81 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.74 (d, 1H, J = 12.2 Hz, benzylic CH_2), 4.73 (d, 1H, J = 10.5 Hz, benzylic CH_2), 4.62–4.68 (m, 4H, benzylic CH_2), 4.47 (d, 1H, J = 11.8 Hz, benzylic CH_2), 4.36 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 2.0 Hz, H-2), 4.23–4.27 (m, 3H, H-3, H-4′, H-5′), 3.87–3.94 (m, 4H, H-3, H-4, H-5, H-6b′), 3.81 (dd, 1H, $J_{5',6a'}$ = 1.4 Hz, $J_{6a',6b'}$ = 11.0 Hz, H-6a′), 3.73–3.76 (m, 2H, H-6a, H-6b), 3.63 (ddd, 1H, J = 6.6, 6.6, 9.6 Hz, octyl OCH_2), 3.49 (dd, 1H, $J_{1',2'}$ = 6.5 Hz, $J_{2',3'}$ = 1.2 Hz, H-2′), 3.41 (s, 3H, OCH_3), 3.32 (ddd, 1H, J = 6.6, 6.6, 9.6 Hz, octyl OCH_2), 1.47–1.50 (m, 2H, octyl CH_2), 1.25–1.32 (m, 10H, octyl CH_2), 0.88 (t, 3H, J = 7.1 Hz, octyl CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ_C 138.8, 138.7, 138.5, 137.4, 128.5, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.5, 102.0 (C-1′), 97.6 (C-1), 80.1, 78.0, 77.3, 75.5, 75.2, 75.2, 74.9, 72.6, 72.6, 72.5, 71.7, 71.3, 69.7, 68.2, 67.6 (ring and benzylic C, octyl OCH_2), 59.0 (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). HR-ESI-MS calcd for $C_{49}H_{62}O_{10}$ $[M+Na]^+$ 833.4241, found 833.4232.

3.23. Octyl 3,6-anhydro-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 34

Anhydrosugar **33** (94 mg, 0.12 mmol) was dissolved in HOAc (8 mL), and 10% Pd/C (35 mg) was added. 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 (9:1 CH_2Cl_2–CH_3OH) on Iatrobeads to give **34** (34 mg, 65%) as a foam. R_f 0.36 (9:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ = +74.8 (*c* 0.2, CH_3OH); ^{1}H NMR (800 MHz, CD_3OD) δ_H 4.89 (d, 1H, $J_{1',2'}$ = 6.6 Hz, H-1′), 4.73 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.25 (dd, 1H, $J_{5',6b'}$ = $J_{4',5'}$ = 2.6 Hz, H-5′), 4.22 (d, 1H, $J_{3',4'}$ = 6.1 Hz, H-3′), 4.20 (dd, 1H, $J_{3',4'}$ = 6.1 Hz, $J_{4',5'}$ = 2.6 Hz, H-4′), 4.07 (dd, 1H, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6b), 4.03 (d, 1H, $J_{6a',6b'}$ = 10.5 Hz, H-6a′), 3.98 (dd, 1H, $J_{5',6b'}$ = 2.6 Hz, $J_{6a',6b'}$ = 10.5 Hz, H-6b′), 3.88 (d, 1H, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.80 (dd, 1H, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 3.74 (ddd, 1H, J = 6.6, 6.6, 9.7 Hz, octyl OCH_2), 3.68–3.71 (m, 3H, H-3, H-4, H-5), 3.46 (d, 1H, $J_{1',2'}$ = 6.6 Hz, H-2′), 3.49 (s, 3H, OCH_3), 3.42 (ddd, 1H, J = 6.6, 6.6, 9.7 Hz, octyl OCH_2), 1.58–1.62 (m, 2H), 1.32–1.41 (m, 10H), 0.92 (t, 3H, J = 7.2 Hz); ^{13}C NMR (150.9 MHz, CD_3OD) δ_C 102.8 (C-1, $^1J_{C,H}$ = 161.4 Hz), 101.6 (C-1, $^1J_{C,H}$ = 168.1 Hz), 79.5 (C-2′), 77.1 (C-4′), 76.9 (C-3′), 73.2 (C-5), 72.5 (C-3), 72.2 (C-2), 72.0 (C-5′), 70.4 (C-6′), 69.6 (C-6), 68.6 (octyl OCH_2), 68.6 (C-4), 58.8 (OCH_3), 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). HR-ESI-MS calcd for $C_{21}H_{38}O_{10}$ $[M+Na]^+$ 473.2363, found 473.2325.

3.24. Octyl 3,4-di-*O*-benzyl-6-deoxy-6-fluoro-2-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside 35

Alcohol **31** (147 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (4 mL) and pyridine (130 μL, 1.6 mmol), and the solution was cooled to 0 °C before trifluoromethanesulfonic acid anhydride (50 μL, 0.32 mmol) was added dropwise. After stirring for 1 h at 0 °C, the solution was diluted with CH_2Cl_2 (2 × 20 mL), washed with ice-cold aqueous 1 M HCl (20 mL) and then ice-cold water (20 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated to a yellow syrup. The crude trifluoromethanesulfonate ester was dissolved in *n*-Bu_4NF (1 M) in THF (5 mL) and stirred at 50 °C overnight. The solution was concentrated and the residue was purified by chromatography (2:1 hexane–EtOAc) to give **35** (115 mg, 78% over two steps) as a colorless syrup. R_f 0.71 (2:1 hexane–EtOAc); $[\alpha]_D$ = +36.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.17–7.39 (m, 25H), 5.12 (d, 1H, *J* = 2.0 Hz), 4.93 (d, 1H, *J* = 11.0 Hz), 4.89 (d, 1H, *J* = 11.0 Hz), 4.80 (d, 1H, *J* = 2.0 Hz), 4.76 (d, 1H, *J* = 12.2 Hz), 4.69 (d, 1H, *J* = 12.2 Hz), 4.64–4.67 (m, 4H), 4.57 (d, 1H, *J* = 12.2 Hz), 4.52 (ddd, 1H, *J* = 3.6, 10.1, 48.1 Hz), 4.47 (d, 1H, *J* = 11.0 Hz), 4.46 (ddd, 1H, *J* = 1.4, 10.1, 48.1 Hz), 3.85–3.93 (m, 7 H), 3.79 (dd, 1H, *J* = 2.0, 2.0 Hz), 3.70 (dd, 1H, *J* = 1.3, 11.6 Hz), 3.67 (dd, 1H, *J* = 2.0, 2.0 Hz), 3.58 (ddd, 1H, *J* = 6.8, 6.8, 9.6 Hz), 3.42 (s, 3H), 3.33 (ddd, 1H, *J* = 6.8, 6.8, 9.6 Hz), 1.48–1.51 (m, 2H), 1.26–1.31 (m, 10H), 0.88 (t, 3H, *J* = 6.7 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C 139.0, 138.9, 138.8, 138.6, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 98.4, 98.1, 82.7 (d, *J* = 172.7 Hz), 80.9, 79.4, 77.6, 75.7, 75.6, 75.6, 75.5, 74.1 (d, *J* = 6.4 Hz), 73.5, 72.7, 72.1, 72.0, 71.5 (d, *J* = 18.2 Hz), 68.1, 66.6, 59.4, 32.3, 29.9, 29.8, 29.7, 26.6, 23.1, 14.6; ^{19}F NMR (235.4 MHz, $CDCl_3$) δ_F −231.9 (ddd, 1F, *J* = 48.1, 48.1, 28.0 Hz). HR-ESI-MS calcd for $C_{56}H_{69}FO_{10}$ $[M+Na]^+$ 943.4772, found 943.4863.

3.25. Bacterial strains and growth conditions

M. smegmatis mc^{2}155 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.

3.26. 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 resuspended 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).

3.27. Evaluation of acceptor/inhibitor activity of compounds 9–14 and 34 in the PPM-dependent α-(1→6)-ManT assay

Compounds **9**–**14** and **34** 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 μg. 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 3 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.

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