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Oligosaccharides as inhibitors of mycobacterial arabinosyltransferases. Di- and trisaccharides containing C-3 modified arabinofuranosyl residues

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Abstract—The assembly of the arabinan portions of cell wall polysaccharides in mycobacteria involves a family of arabinosyltransferases (AraT's) that promote the polymerization of decaprenolphosphoarabinose. Mycobacterial viability depends upon the ability of the organism to synthesize an intact arabinan and thus compounds that inhibit these AraT's are both useful biochemical tools as well as potential lead compounds for new anti-tuberculosis agents. We describe here the preparation of oligosaccharide fragments of mycobacterial arabinan that contain arabinofuranosyl residues modified at C-3 by the replacement of the hydroxyl group with an amino, azido or methoxy functionality. Subsequent testing of these oligosaccharides as inhibitors of mycobacterial AraT's revealed that all inhibited the enzymes, but to varying degrees. In further studies, each compound was shown to have only low activity as an inhibitor of mycobacterial growth.

1. Introduction

The reemergence of mycobacterial diseases such as tuberculosis and AIDS-associated *M. avium* as human health threats has spurred increasing interest in the development of new antibiotics for treating these infections.[1,2] The urgency of this task has been heightened by the emergence of drug resistant mycobacterial strains[3,4] and difficulties in treating these diseases in AIDS-patients.[5] Among the arsenal of front-line anti-mycobacterial agents used for the treatment of these diseases are drugs, for example, isoniazid and ethambutol, that prevent the assembly of the mycobacterial cell wall, a structure that is critical for the viability of these organisms.[6–8] Therefore, significant effort in the quest for new anti-mycobacterial agents has been expended on identifying compounds that inhibit enzymes involved in cell wall biosynthesis.[9–11]

In earlier papers, we described syntheses of arabinofuranosyl-containing oligosaccharides that were designed to be substrates[12–14] or inhibitors[15,16] of mycobacterial arabinosyltransferases (AraT's), enzymes that are responsible for assembling the arabinan portions of two key cell-wall polysaccharides, lipoarabinomannan (LAM) and arabinogalactan (AG). The arabinan of AG and LAM consists of a linear chain of α-(1→5)-linked D-arabinofuranose (Ara*f*) residues, with periodic α-(1→3)-linked branch points to which additional α-(1→5)-linked arabinan chains are connected.[7,8] At the nonreducing ends of each arabinan chain in AG is attached a hexasaccharide motif, **1**, (Chart 1) while in LAM, the terminal motifs are either **1**, or tetrasaccharide **2**.[7,8,17] Thus, three types of arabinofuranosyl linkages, α-(1→5), α-(1→3), and β-(1→2), are present in these polysaccharides. The glycosyltransferases responsible for the assembly of these glycans catalyze the condensation of decaprenolphosphoarabinose (DPA, **3**) with an acceptor species (e.g., **4**) ultimately yielding the polysaccharide (Fig. 1).[18,19] Mycobacterial AraT's have been validated as targets for drug action through the demonstration that ethambutol, an anti-tuberculosis

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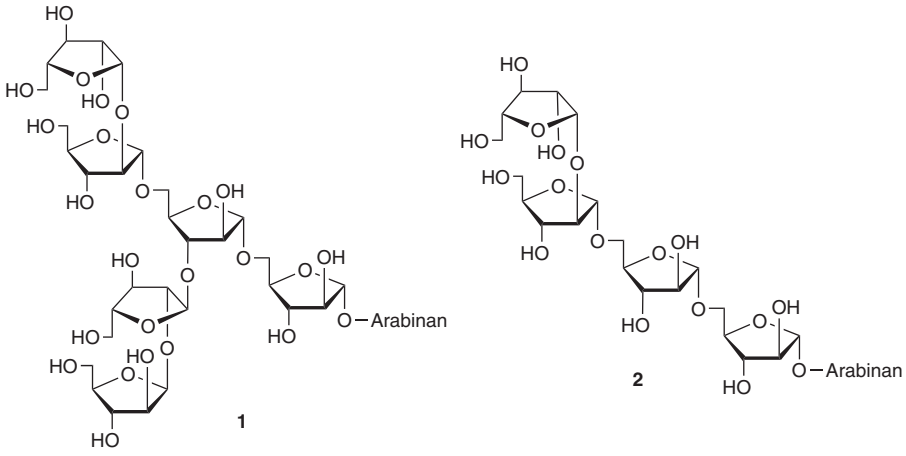


Chart 1.

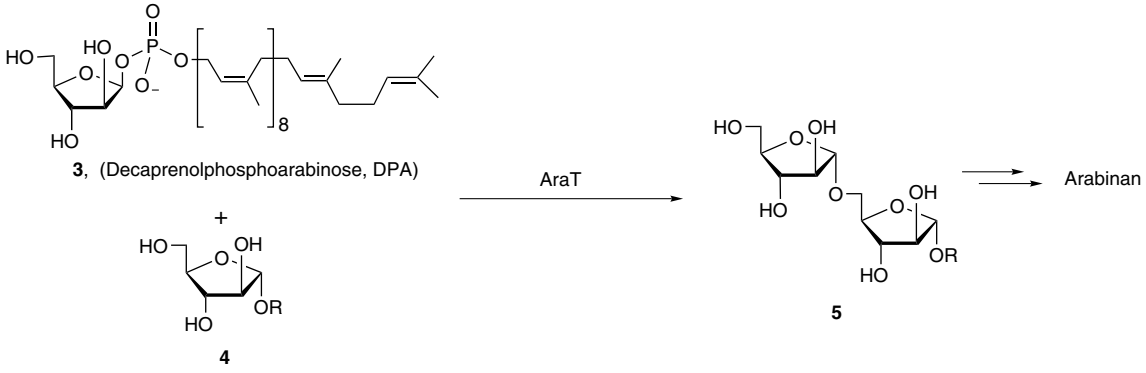
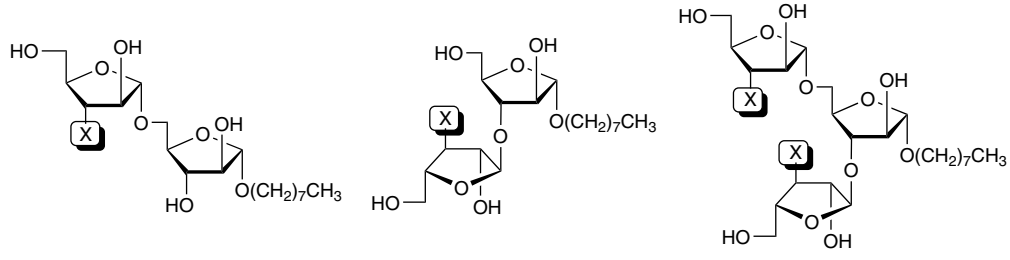


Figure 1. Prototypical arabinosyltransferase-catalyzed reaction.

drug, inhibits at least one of these enzymes, likely the α-(1→3) AraT.[18,20–22] Thus, other compounds that prevent arabinan assembly will not only be useful biochemical tools, but also potential lead compounds for new anti-tuberculosis agents.

It has previously been demonstrated that di- and triarabinofuranoside fragments of AG and LAM (e.g., **6**–**8**) are substrates for mycobacterial AraT's.[18,23] We describe here the synthesis of analogs of these oligosaccharides and their subsequent evaluation as inhibitors of mycobacterial AraT's. In addition, these compounds have been screened for their ability to inhibit mycobacterial growth. It is well-established[24–30] that oligosaccharide analogs in which one or more hydroxyl groups are replaced by other functionalities frequently inhibit the enzymes that normally bind their unmodified parent structures. Thus, we prepared and evaluated derivatives of **6**–**8** in which the hydroxyl group at C-3′ and/or C-3″ has been substituted with methoxy, azido, or amino groups (oligosaccharides **9**–**17**, Chart 2).



6, X = OH
9, X = OCH_3
12, X = N_3
15, X = NH_2

7, X = OH
10, X = OCH_3
13, X = N_3
16, X = NH_2

8, X = OH
11, X = OCH_3
14, X = N_3
17, X = NH_2

Chart 2.

2. Results and discussion

2.1. Synthesis of 9–17

The preparation of **9**–**17** required first the synthesis of the monosaccharide building blocks **18**–**22** (Chart 3). Acceptors **18**–**20** are known and were prepared as described earlier.[15] The general strategy used to access **21** and **22** involved the introduction of the appropriate functionality via the nucleophilic opening of methyl 2,3-anhydro-α-D-*xylo*furanoside (**23**, Scheme 1).[31] The addition of nucleophiles to 2,3-anhydrofuranosides possessing the α-D-*xylo* stereochemistry proceeds in a highly regioselective manner, with attack at C-3 being preferred, presumably due to a combination of steric and electronic factors.[32] As outlined below, this approach was successful and in all cases the regioselectivity of the epoxide ring opening reactions could be established by NMR spectroscopy. In the ^{1}H NMR spectra of the products, the anomeric hydrogen appeared as a singlet or doublet with $^3J_{H1,H2} < 2$ Hz; in the ^{13}C NMR spectra, the anomeric carbon resonated between 105 and 110 ppm. These data clearly demonstrate that the products formed have the D-*arabino* stereochemistry.[33] Had the epoxide opened with the opposite regioselectivity, providing a product with the D-*xylo* stereochemistry, the $^3J_{H1,H2}$ value would be 4–5 Hz and the anomeric carbons would have appeared between 100 and 105 ppm.

The 3-*O*-methylated thioglycoside **21** was obtained (Scheme 1) in four steps from **23**. First, reaction with sodium methoxide in methanol at reflux afforded diol **24** in 57% yield. The remainder of the mass balance in this reaction was unreacted **23**. Although allowing the reaction to continue for a longer period of time resulted in total conversion of the starting material, prolonging the reaction also led to additional decomposition products. We therefore found it more efficient to stop the reaction prior to the complete consumption of **23**. Diol **24** could be readily separated from epoxide **23**, and the latter could then be re-subjected to the opening reaction. With **24** in hand, it was then acetylated (giving **25**) and converted to triacetate **26** upon acetolysis with acetic anhydride and sulfuric acid. This two-step sequence gave **26** in 75% overall yield as an inseparable 20:1 α/β mixture of anomers. Reaction of **26** with *p*-thiocresol and boron-trifluoride etherate yielded **21** (80%) again as an inseparable mixture of anomers in which the α-glycoside predominated (20:1 α/β). An analogous series of transformations was used to convert **23** into the 3-azido thioglycoside **22**. Opening of the epoxide ring with sodium azide afforded **27** and acetylation of this diol gave **28** in 63% overall yield from **23**. Acetolysis of the methyl glycoside **28** provided peracetate **29**, which, without further purification, was reacted with *p*-thiocresol and boron-trifluoride etherate yielding **22** in 83% yield, as a 5:1 α/β mixture of anomers that could be separated by chromatography.

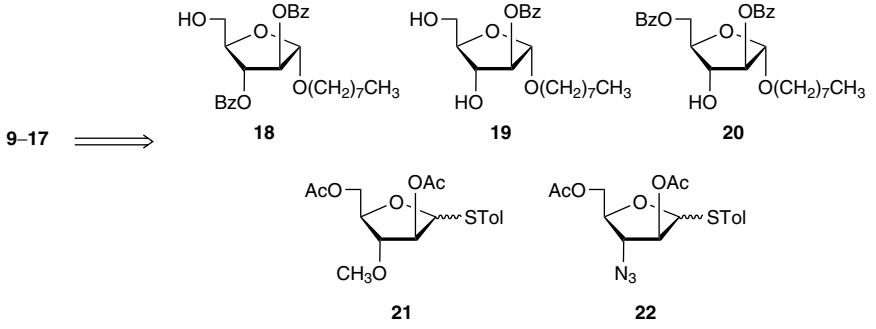
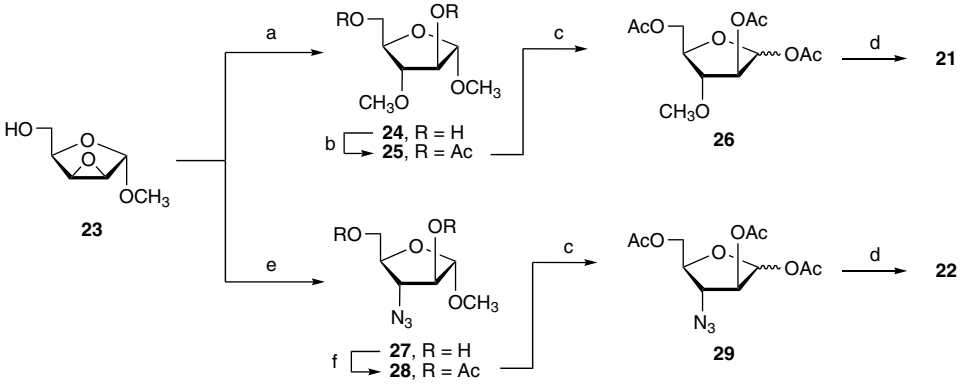
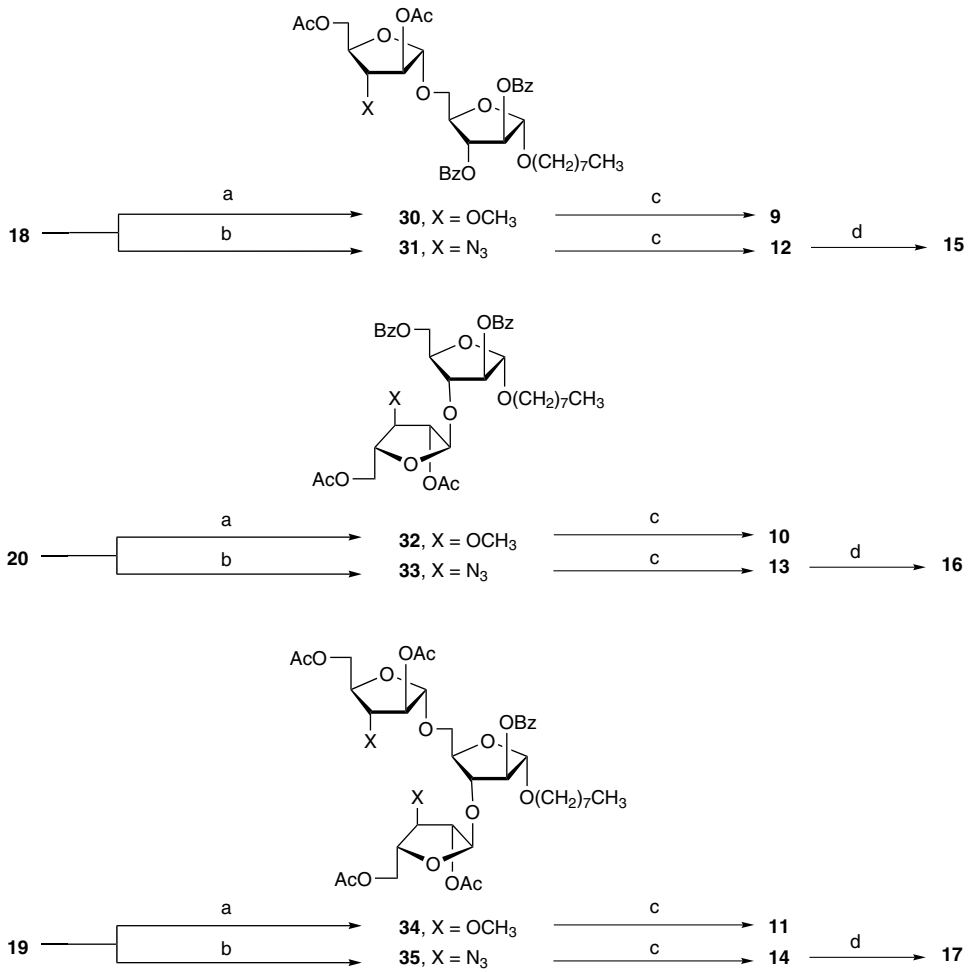


Chart 3.



Scheme 1. Reagents and conditions: (a) $NaOCH_3$, CH_3OH, reflux, 57%. (b) Ac_2O, pyridine, 0 °C → rt, 83%. (c) Ac_2O, H_2SO_4, 0 °C, 91% (for **25**), 99% (for **28**). (d) *p*-Thiocresol, BF_3–OEt_2, CH_2Cl_2, 0 °C, 80% (for **26**), 83% (for **29**). (e) NaN_3, NH_4Cl, EtOH, reflux. (f) Ac_2O, pyridine, rt, 63% (from **23**).



Scheme 2. Reagents and conditions: (a) **21**, *N*-iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, 0 °C, 71% (for **18**), 76% (for **20**), 78% (for **19**). (b) **22**, *N*-iodosuccinimide, $AgOSO_2CF_3$, CH_2Cl_2, 0 °C, 82% (for **18**), 90% (for **20**), 78% (for **19**). (c) $NaOCH_3$, CH_3OH, rt, 92% (for **30**), 96% (for **31**), 70% (for **32**), 95% (for **33**), 97% (for **34**), 84% (for **35**). (d) Ph_3P, H_2O, THF, rt, 32% (for **12**), 73% (for **13**), 80% (for **14**).

Having successfully synthesized thioglycosides **21** and **22**, these donors were then coupled with acceptors **18**–**20** to provide the required oligosaccharides (Scheme 2). The glycosylations were all carried out using *N*-iodosuccinimide and silver triflate as the promoters and the reactions afforded the protected oligosaccharides **30**–**35** in 71–90% yield. The stereochemistry of the glycosylations was established by NMR spectroscopy. In all cases, the $^3J_{H1,H2}$ of the newly introduced arabinofuranosyl residue was 0–2 Hz, indicative of the α-stereochemistry of this residue. Similarly, the chemical shifts of the anomeric carbons were between 105 and 110 ppm, which is expected for the α-arabinofuranosides.[33] Had the β-arabinofuranosides been produced, the $^3J_{H1,\ H2}$ values would have been larger (4–5 Hz) and the chemical shifts of the anomeric carbons at higher field (100–105 ppm).[33] The protected derivatives were then deacylated with sodium methoxide to give **9**–**14** in 70–97% yields. The aminosugar-containing oligosaccharides were obtained upon reduction of **12**–**14** with triphenylphosphine in wet THF to give the products **15**, **16**, and **17** in 32%, 73%, and 80% yields, respectively. The reduction of **12** was very slow and the reaction appeared to stop after stirring for 24 h. Therefore, **12** and **15** were separated by chromatography and the re-isolated starting material was treated again with triphenylphosphine and water.

2.2. Screening of compounds as substrates and inhibitors of mycobacterial arabinosyltransferases

With the oligosaccharides in hand, they were first evaluated in the mycobacterial AraT assay to determine their activity as substrates. The available assay uses a membrane fraction from mycobacteria as the enzyme source. Previous studies have demonstrated that this membrane fraction possesses both α-(1→5) and β-(1→2) AraT activities, but appears to lack the enzyme responsible for the installation of the α-(1→3)-linked Ara*f* residues in mycobacterial arabinan.[18] As expected,[18,23] the parent oligosaccharides **6**–**8**,[12] showed activity as substrates for these enzymes. More interesting, however, was that none of the modified analogs were substrates when screened at a concentration of 3.6 mM.

These oligosaccharides were therefore next tested as inhibitors of the enzymes. For a given compound, the corresponding parent oligosaccharide **6**, **7**, or **8** was used as the substrate at 0.4 mM, while each potential

Table 1. Activity of oligosaccharide analogs against mycobacterial AraT's[a] and *M. tuberculosis* strain $H_{37}Rv$[b]

Compound	% Inhibition of AraT activity	% Inhibition of mycobacterial growth
9	34	8
10	59	14
11	37	20
12	36	13
13	75	17
15	23	4
16	49	10
17	25	12

[a] All compounds were screened at a concentration 3.6 mM as outlined in Section 3.27.

[b] All compounds were screened at a concentration of 6.25 μg/mL using the Alamar Blue assay.[34]

inhibitor was added at a concentration of 3.6 mM. All of the modified oligosaccharides are inhibitors of the AraT's, but to varying degrees (Table 1). The most potent inhibitor was azido disaccharide **13** (75% inhibition), while the weakest was the amino disaccharide **15** (23% inhibition). Because all of the compounds tested were AraT inhibitors, it is difficult to draw conclusions about the origin of the inhibition. The analysis of the data is further complicated by the presence of two AraT activities, α-(1→5) and β-(1→2), in the incubation mixture. We note, however, that the analogs of the α-(1→3)-linked disaccharide **7**, compounds **10**, **13**, and **16**, were the most potent inhibitors. Although the significance of this observation is unclear, these results suggest that, in future analog synthesis to identify inhibitors of these enzymes, the focus should be on using this disaccharide scaffold, rather than the α-(1→5)-linked disaccharide **6** or trisaccharide **8**.

2.3. Screening of compounds as inhibitors of *M. tuberculosis* $H_{37}Rv$ growth

The oligosaccharides that were tested as inhibitors of mycobacterial AraT's were next screened for their ability to prevent the growth of *M. tuberculosis* strain $H_{37}Rv$. To assess inhibitory potency, each compound was evaluated at a concentration of 6.25 μg/mL in the fluorescence-based Alamar Blue microplate assay,[34] which makes use of a dye that changes from blue (and nonfluorescent) to pink (and fluorescent) in the presence of the growing bacteria. When a compound that inhibits bacterial growth is added, a decrease in fluorescence results, which can be quantitated as a percent inhibition of growth (Table 1).

From the data presented in Table 1 it is clear that although all of the oligosaccharides screened do inhibit mycobacterial growth, they do so only to a minimal degree. The most potent compound is the 3′,3″-dimethoxy trisaccharide, **11**, but only a 20% inhibition of growth is observed.[35] In addition, there is no apparent correlation between the potency of a given compound as an inhibitor of mycobacterial AraT's and its activity against bacterial growth. For example, the most potent AraT inhibitor, disaccharide **13**, does not inhibit bacterial growth to any more appreciable level than does disaccharide **12**, which is a substantially weaker inhibitor of the enzymes.

The lack of activity of these compounds against the bacteria could be attributed to the relatively hydrophilic character of the oligosaccharides, which would prevent them from entering the organism by diffusion through the highly lipophilic outer layer of the mycobacterial cell wall. Similarly, these compounds would be expected to be too large to enter the organism through cell wall porins, which serve as the entry point for some antituberculosis drugs.[36] The results here parallel those obtained with other oligosaccharide fragments of mycobacterial cell wall polysaccharides. To date, in general only low levels of activity have been observed and efforts to increase the activity of these compounds by making them more lipophilic through the addition of hydrophobic groups, for example, as in **36** and **37** (Chart 4), have, for the most part, resulted in only marginal improvements in activity.[37–39] A notable exception is disaccharide **38**, which has been shown to have an MIC only 2–4 fold higher than ethambutol.[40] However, it remains to be demonstrated that the potency **38** is a consequence its ability to inhibit mycobacterial AraT's. The detergent nature of this compound, or the ones whose synthesis is described here, could result in an inhibition of bacterial growth or enzymatic activity.

2.4. Conclusions

In summary, we describe here the synthesis of a panel of oligosaccharide fragments of mycobacterial arabinan and their subsequent testing as inhibitors of mycobacterial AraT's and mycobacterial growth. All the compounds do, to some degree, inhibit the AraT's, with those based upon the α-(1→3)-linked disaccharide **7**

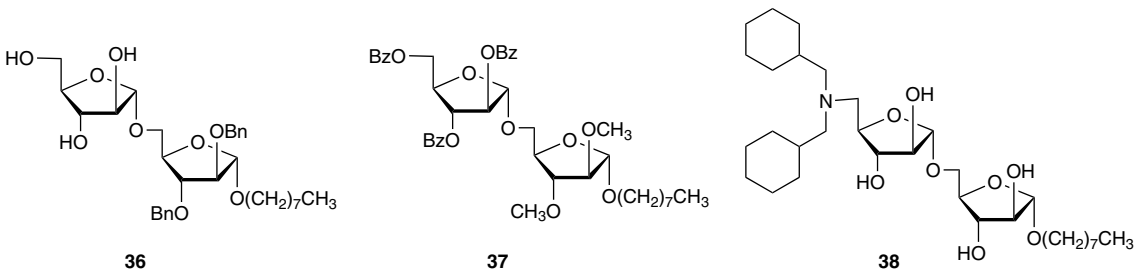


Chart 4.

being the most potent. Subsequent testing of these compounds as inhibitors of mycobacterial growth revealed only poor levels of activity.

3. Experimental

3.1. General methods

Reactions were carried out in oven-dried glassware. Solvents were distilled from appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254}. Spots were detected under UV light or by charring with 10% H_2SO_4, in EtOH. Unless otherwise indicated, all column chromatography was performed on silica gel 60 (40–60 μM). Iatrobeads refers to a beaded silica gel 6RS–8060, which is manufactured by Iatron Laboratories (Tokyo). The ratio between silica gel and crude product 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 250, 400, or 500 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$) or HOD (4.78, D_2O and CD_3OD). ^{13}C NMR spectra were recorded at 63, 100, or 125 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23, $CDCl_3$), external dioxane (67.40, D_2O) or CD_3OD (48.9, CD_3OD). Electrospray mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. Oligosaccharides **6**–**8** were prepared as previously described.[12]

3.2. Octyl 5-*O*-(3-*O*-methyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (9)

To a solution of **30** (75 mg, 0.12 mmol) in dry CH_3OH (10 mL), was added dropwise $NaOCH_3$ (0.1 M in CH_3OH) until the pH of the reaction mixture was 11. After stirring overnight, the reaction mixture was neutralized with few drops of acetic acid and then concentrated. The resulting residue was purified by chromatography (hexanes/EtOAc, 1:2) to give **9** (45 mg, 92%) as a syrup: R_f 0.51 (hexanes/EtOAc, 1:2); $[\alpha]_D$ +102.7 (*c* 0.9, CH_3OH); ^{1}H NMR (400 MHz, D_2O, δ_H) 5.12 (s, 1H), 4.98 (s, 1H), 4.27 (s, 1H), 4.16–4.12 (m, 2H), 4.09–4.08 (m, 1H), 4.04–4.01 (m, 1H), 3.93–3.87 (m, 2H), 3.80–3.70 (m, 4H), 3.53–3.49 (m, 1H), 3.48 (s, 3H), 1.65 (m, 2H), 1.36–1.34 (m, 10H), 0.93–0.91 (m, 3H); ^{13}C NMR (100 MHz, D_2O, δ_C) 108.4, 107.9, 87.4, 83. 7, 82. 2, 81.8, 78.7, 77.3, 68.6, 66.7, 62.1, 58.0, 33.2, 29.8 (2), 29.7, 26.3, 22.9, 14.2; HR-ESI-MS Calcd for $[C_{19}H_{36}O_9]Na^+$ 431.2257, found 431.2271.

3.3. Octyl 3-*O*-(3-*O*-methyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (10)

Disaccharide **32** (100 mg, 0.14 mmol) was debenzoylated in dry CH_3 OH (10 mL), as described for the preparation of **9**. The product was purified by chromatography (CH_2Cl_2/CH_3OH, 6:1) to give **10** (40 mg, 70%) as a syrup: R_f 0.57 (CH_2Cl_2/CH_3OH, 6:1); $[\alpha]_D$ +116.8 (*c* 1.4, CH_3OH); ^{1}H NMR (400 MHz, D_2O, δ_H) 5.13 (s, 1H), 4.92 (s, 1H), 4.15 (s, 2H), 4.03–3.96 (m, 3H), 3.80–3.76 (m, 2H), 3.73–3.62 (m, 3H), 3.59 (d, 1H, J = 5.8 Hz), 3.45–3.39 (m, 1H), 3.38 (s, 3H), 1.55–1.54 (m, 2H), 1.27–1.25 (m, 10H), 0.88–0.82 (m, 3H); ^{13}C NMR (100 MHz, D_2O, δ_C) 107.9 (2), 87.3, 83.5, 82.9, 82.2, 80.6, 79.3, 68.0, 61.9, 61.0, 58.8, 32.2, 29.8, 29.8, 29.6, 26.3, 22.9, 14.2; HR-ESI-MS Calcd for $[C_{19}H_{36}O_9]Na^+$ 431.2257, found 431.2248.

3.4. Octyl 3,5-di-*O*-(3-*O*-methyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (11)

Trisaccharide **34** (90 mg, 0.1 mmol) was debenzoylated in dry CH_3OH (10 mL), as described for the preparation of **9**. The product was purified by chromatography (hexanes/EtOAc, 1:4) to give **11** (54 mg, 97%) as a syrup: R_f 0.30 (hexanes/EtOAc, 1:4); $[\alpha]_D$ +114.4 (*c* 1.2, CH_3OH); ^{1}H NMR (400 MHz, D_2O, δ_H) 5.19 (s, 1H), 5.11 (s, 1H), 5.00 (s, 1H), 4.26 (s, 1H), 4.23–4.17 (m, 4H), 4.12–4.09 (m, 1H), 4.06–4.05 (m, 1H), 3.96–3.94 (m, 1H), 3.87–3.84 (m, 2H), 3.78–3.68 (m, 6H), 3.52–3.51 (m, 1H), 3.47 (s, 3H), 3.46 (s, 3H), 1.63 (m, 2H), 1.34 (m, 10H), 0.93–0.91 (m, 3H); ^{13}C NMR (100 MHz, D_2O, δ_C) 108.2, 108.0, 108.0, 87.4, 87.3, 83.6, 83.5, 82.7, 81.7, 80.4, 79.4, 78.8, 68.1, 66.1, 62.1, 61.9, 58.0, 57.9, 32.2, 29.7, 29.6, 26.3, 22.9, 21.0, 14.2; HR-ESI-MS Calcd for $[C_{25}H_{46}O_{13}]Na^+$ 577.2836, found 577.2847.

3.5. Octyl 5-*O*-(3-azido-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (12)

Disaccharide **31** (50 mg, 0.08 mmol) was debenzoylated in dry CH_3OH (10 mL), as described for the preparation of **9**. The product was purified by chromatography (CH_2Cl_2/CH_3OH, 10:1) to give **12** (32 mg, 96%) as a syrup: R_f 0.21 ($CHCl_3$/CH_3OH, 10:1); $[\alpha]_D$ +98.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 5.06 (s, 1H), 4.99 (s, 1H), 4.16–4.15 (m, 2H), 4.10–4.08 (m, 1H), 4.01 (s, 1H), 3.99 (d, 1H, J = 1.9 Hz), 3.95 (dd, 1H, J = 2.5, 11.1 Hz), 3.92–3.88 (m, 2H), 3.74–3.68 (m, 3H), 3.46–3.41 (m, 1H), 1.57–1.55 (m, 2H), 1.28–1.27 (m, 10H), 0.89–0.86 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 108.6, 108.0, 85.4, 83.1, 79.8, 79.6, 78.2, 68.1, 67.4, 66.5, 61.9, 32.0, 29.7, 29.5, 29.4, 26.3, 22.8, 14.3; HR-ESI-MS Calcd for $[C_{18}H_{33}O_8N_3]Na^+$ 442.2165, found 442.2169.

3.6. Octyl 3-*O*-(3-azido-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (13)

Disaccharide **33** (110 mg, 0.15 mmol) was debenzoylated in dry CH_3OH (15 mL), as described for the preparation of **9**. The product was purified by chromatography (CH_2Cl_2/CH_3OH, 10:1) to give **13** (60 mg, 95%) as a syrup: R_f 0.54 (CH_2Cl_2/CH_3OH, 6:1); $[\alpha]_D$ +98.7 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 5.21 (s, 1H), 4.95 (s, 1H), 4.23 (s, 2H) 4.14–4.12 (m, 1H), 4.06–4.04 (m, 1H), 4.01–3.99 (m, 1H), 3.89 (d, 2H, J = 12.2 Hz), 3.77 (dd, 2H, J = 3.2, 6.1 Hz), 3.69–3.65 (m, 2H), 3.45–3.41 (m, 1H), 1.61–1.57 (m, 2H), 1.29–1.28 (m, 10H), 0.91–0.87 (m, 3H); ^{13}C NMR

(100 MHz, CD_3OD, δ_C) 107.80, 107.67, 82.36, 82.37, 82.14, 80.56, 80.40, 67.96, 65.97, 61.61, 60.87, 32.05, 29.69, 29.60, 29.48, 26.25, 22.86, 14.28; HR-ESI-MS Calcd for $[C_{18}H_{33}O_8N_3]Na^+$ 442.2165, found 442.2149.

3.7. Octyl 3,5-di-*O*-(3-azido-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (14)

Trisaccharide **35** (50 mg, 0.06 mmol) was debenzoylated in dry CH_3OH (5 mL), as described for the preparation of **9**. The product was purified by chromatography on Iatrobeads ($CHCl_3/CH_3OH$, 10:1) to give **14** (29 mg, 84%) as a colorless oil: R_f 0.46 ($CHCl_3/CH_3OH$, 6:1); $[\alpha]_D$ +78.9 (*c* 0.8, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 5.10 (s, 1H), 5.03 (s, 1H), 4.88 (s, 1H), 4.18–4.17 (m, 2H), 4.12–4.11 (m, 1H), 4.07–4.05 (m, 4H), 3.96–3.95 (m, 1H), 3.83–3.79 (m, 4H), 3.67–3.59 (m, 4H), 3.37–3.35 (m, 1H), 1.53–1.51 (m, 2H), 1.22–1.20 (m, 10H), 0.83–0.79 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 109.5, 109.0, 108.9, 84.0, 83.7, 81.9, 81.6, 81.4, 78.7, 69.3, 67.8, 67.5 (2), 63.1 (2), 33.3, 31.2, 31.0, 30.9, 30.8, 27.5, 24.1, 15.6; HR-ESI-MS Calcd for $[C_{23}H_{40}O_{11}N_6]Na^+$ 599.2652, found 599.2653.

3.8. Octyl 5-*O*-(3-amino-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (15)

A solution of **12** (41 mg, 0.09 mmol) and Ph_3P (40 mg, 0.15 mmol) in THF (5 mL) containing a few drops of water was stirred for 24 h. The solution was then concentrated and the product was purified by chromatography on Iatrobeads ($CHCl_3/CH_3OH$, 2:1 with 2% Et_3N) to give **15** (11 mg, 32%): R_f 0.14 ($CHCl_3/CH_3OH$, 4:1); $[\alpha]_D$ +76.8 (*c* 0.9, CH_3OH); ^1H NMR (400 MHz, D_2O, δ_H) 5.24 (s, 1H), 5.13 (s, 1H), 4.16–4.12 (m, 3H), 4.04–4.03 (m, 2H), 3.87–3.82 (m, 2H), 3.77–3.74 (m, 3H), 3.59–3.54 (m, 1H), 3.22–3.20 (m, 1H), 1.64–1.62 (m, 2H), 1.31 (m, 10H), 0.90–0.88 (m, 3H); ^{13}C NMR (100 MHz, D_2O, δ_C) 107.8, 106.4, 87.9, 84.9, 83.0, 81.0, 75.3, 68.7, 61.8, 60.0, 58.3, 31.8, 29.3, 29.2, 29.1, 25.9, 22.6, 14.0; HR-ESI-MS Calcd for $[C_{18}H_{35}O_8N]Na^+$ 416.2260, found 416.2265.

3.9. Octyl 3-*O*-(3-amino-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (16)

A solution of **13** (30 mg, 0.07 mmol) and Ph_3P (21 mg, 0.08 mmol) in THF (5 mL) containing a few drops of water was stirred for 24 h. The solution was then concentrated and the product was purified by chromatography on Iatrobeads ($CHCl_3/CH_3OH$, 2:1 with 2% Et_3N) to give **16** (20 mg, 73%): R_f 0.48 ($CHCl_3/CH_3OH$, 2:1); $[\alpha]_D$ +79.6 (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 5.07 (s, 1H), 4.84 (s, 1H), 4.04 (dd, 1H, $J = 1.3$, 2.7 Hz), 4.01–3.97 (m, 1H), 3.93 (dd, 1H, $J = 2.7$, 5.9 Hz), 3.88 (dd, 1H, $J = 1.2$, 2.9 Hz), 3.86–3.85 (m, 1H), 3.75 (dd, 1H, $J = 3.4$, 11.8 Hz), 3.70–3.61 (m, 4H), 3.41–3.37 (m, 1H), 2.98 (dd, 1H, $J = 2.9$, 5.2 Hz), 1.58–1.51 (m, 2H), 1.35–1.12 (m, 10H), 0.89–0.86 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 109.7, 109.6, 87.7, 84.5, 83.6, 83.3, 82.2, 69.9, 63.7, 63.1, 60.8, 33.2, 30.8, 30.6, 30.6, 27.4, 23.9, 14.6; HR-ESI-MS Calcd for $[C_{18}H_{35}O_8N]Na^+$ 416.2260, found 416.2259.

3.10. Octyl 3,5-di-*O*-(3-amino-3-deoxy-α-D-arabinofuranosyl)-α-D-arabinofuranoside (17)

A solution of **14** (28 mg, 0.05 mmol) and Ph_3P (26 mg, 0.1 mmol) in THF (5 mL) containing a few drops of water was stirred for 24 h. The solution was then concentrated and the product was purified by chromatography on Iatrobeads ($CHCl_3/CH_3OH$, 2:1 with 2% Et_3N) to give **17** (21 mg, 80%): R_f 0.11 ($CHCl_3/CH_3OH$, 4:1); $[\alpha]_D$ +109.7 (*c* 0.7, CH_3OH); ^1H NMR (400 MHz, D_2O, δ_H) 5.10 (s, 1H), 5.04 (s, 1H), 4.99 (s, 1H), 4.19–4.16 (m, 1H), 4.15–4.14 (m, 1H), 4.01–3.98 (m, 1H), 3.97–3.96 (m, 3H), 3.89–3.86 (m, 2H), 3.79–3.74 (m, 3H), 3.70–3.62 (m, 3H), 3.55–3.49 (m, 1H), 3.05 (dd, 1H, $J = 3.0$, 5.9 Hz), 3.01 (dd, 1H, $J = 3.1$, 6.1 Hz), 1.56–1.52 (m, 2H), 1.26–1.17 (m, 10H), 0.82–0.79 (m, 3H); ^{13}C NMR (100 MHz, D_2O, δ_C) 107.9, 107.8, 107.7, 85.7, 85.2, 83.0, 82.1, 81.8, 81.4, 79.4, 68.4, 66.6, 62.0, 62.0, 58.6, 58.6, 31.5, 29.0, 28.8, 28.6, 25.6, 22.4, 13.8; HR-ESI-MS Calcd for $[C_{23}H_{44}O_{11}N_2]Na^+$ 547.2843, found 547.2856.

3.11. *p*-Toluyl 2,5-di-*O*-acetyl-3-O-methyl-1-thio-α-D-arabinofuranoside (21)

To a solution of **26** (1.1 g, 3.8 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C was added *p*-thiocresol (564 mg, 4.5 mmol). After stirring for 10 min at 0 °C, BF_3–OEt_2 (0.72 mL, 5.7 mmol) was added dropwise and then the reaction mixture was warmed slowly to room temperature. After 90 min, Et_3N (0.2 mL) was added and the reaction mixture was concentrated. The residue was purified by chromatography (hexanes/EtOAc, 3:1) to provide **21** (1.07 g, 80%) as a syrup (α/β, 20:1): R_f 0.7 (hexanes/EtOAc, 2:1); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.41 (d, 2H, $J = 8.1$ Hz), 7.12 (d, 2H, $J = 8.1$ Hz), 5.47 (s, 1H), 5.26 (dd, 1H, $J = 1.5$, 1.5 Hz), 4.48–4.44 (m, 1H), 4.36–4.24 (m, 2H), 3.67 (d, 1H, $J = 5.1$ Hz), 3.46 (s, 3H), 2.33 (s, 3H), 2.11 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.9, 169.8, 138.0, 132.7, 130.4, 123.0, 91.8, 86.3, 81.3, 80.7, 63.6, 58.6, 21.3, 21.1, 21.0; HR-ESI-MS Calcd for $[C_{17}H_{22}O_6S]Na^+$ 377.1035, found 377.1021.

3.12. *p*-Toluyl 2,5-di-*O*-acetyl-3-azido-3-deoxy-1-thio-D-arabinofuranoside (22)

Glycosyl acetate **29** (350 mg, 1.16 mmol) was converted into **22** with *p*-thiocresol (173 mg, 1.39 mmol) and BF_3–OEt_2 (0.21 mL, 1.7 mmol) in dry CH_2Cl_2 (15 mL) as described for the preparation of **21**. The product was purified by chromatography (hexanes/EtOAc, 6:1) to provide **22** (351 mg, 83%) as two separable anomers in a 5:1 α/β ratio. α-Anomer: R_f 0.66 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +120.0 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.38 (d, 2H, $J = 8.1$ Hz), 7.12 (d, 2H, $J = 8.1$ Hz), 5.45 (d, 1H, $J = 2.3$ Hz), 5.17 (dd, 1H, $J = 2.3$, 2.7 Hz), 4.32–4.27 (m, 3H), 3.87 (dd, 1H, $J = 3.4$, 6.9 Hz), 2.32 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.4, 169.7, 138.3,

134.0, 133.8, 129.9, 129.1, 90.4, 82.3, 79.0, 66.8, 62.7, 21.2, 20.7 (2); HR-ESI-MS Calcd for $[C_{16}H_{19}O_5N_3S]Na^+$ 388.0943, found 388.0940. β-Anomer: R_f 0.72 (hexanes/EtOAc, 2:1); $[\alpha]_D$ −89.7 (*c* 0.7, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 7.37 (d, 2H, *J* = 8.1 Hz), 7.16 (d, 2H, *J* = 8.1 Hz), 5.63 (d, 1H, *J* = 5.7 Hz), 5.22 (dd, 1H, *J* = 5.7, 6.6 Hz), 4.41 (dd, 1H, *J* = 5.9, 11.6 Hz), 4.31 (dd, 1H, *J* = 5.2, 11.6 Hz), 4.17 (dd, 1H, *J* = 6.6, 6.7 Hz), 4.01–3.96 (m, 1H), 2.33 (s, 3H), 2.20 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.7, 170.1, 138.4, 133.1, 130.1, 129.4, 89.3, 78.9, 78.0, 65.6, 63.9, 21.3, 21.0, 20.9; HR-ESI-MS Calcd for $[C_{16}H_{19}O_5N_3S]Na^+$ 388.0943, found 388.0931.

3.13. Methyl 3-*O*-methyl-α-D-arabinofuranoside (24)

To a solution of **23** (200 mg, 1.37 mmol) in dry CH_3OH (10 mL), was added a 1 M solution of $NaOCH_3$ in CH_3OH (10 mL, 10 mmol). The reaction mixture was heated at reflux for 24 h before being cooled and neutralized with acetic acid. The solution was concentrated and purified by chromatography (hexanes/EtOAc, 1:2) to give **24** (140 mg, 57%) as a colorless oil: R_f 0.4 (hexanes/EtOAc, 1:2). The NMR data for this compound were identical to those reported previously.[41]

3.14. Methyl 2,5-di-*O*-acetyl-3-*O*-methyl-α-D-arabinofuranoside (25)

To a solution of **24** (900 mg, 5.05 mmol) in dry pyridine (10 mL) at 0 °C was added dropwise Ac_2O (4.7 mL, 50 mmol). The reaction mixture was stirred at rt overnight, then diluted with CH_2Cl_2 and washed successively with 0.1 M HCl, water, and brine. After drying (Na_2SO_4), the solution was filtered and concentrated and the residue purified by chromatography (hexanes/EtOAc, 2:1) to give **25** (1.1 g, 83%) as a syrup: R_f 0.7 (hexanes/EtOAc, 1:2); $[\alpha]_D$ +101.3 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 5.05 (d, 1H, *J* = 1.2 Hz), 4.92 (s, 1H), 4.36–4.32 (m, 1H), 4.24–4.19 (m, 2H), 3.58 (m, 1H), 3.44 (s, 3H), 3.41 (m, 3H), 2.11 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 169.9, 107.4, 85.4, 81.0, 80.6, 64.1, 58.7, 55.3, 21.1, 21.0; HR-ESI-MS Calcd for $[C_{11}H_{18}O_7]Na^+$ 285.0950, found 285.0933.

3.15. 1,2,5-Tri-*O*-acetyl-3-*O*-methyl-α-D-arabinofuranose (26)

A solution of **25** (1.09 g, 4.2 mmol) in Ac_2O (20 mL) was cooled to 0 °C and a H_2SO_4/Ac_2O solution (0.3:2, 1 mL) was added. The reaction was stirred at 0 °C for 60 min then cooled and a saturated aqueous solution of $NaHCO_3$ (20 mL) was added. After stirring for 10 min, CH_2Cl_2 (40 mL) was added and the organic layer was washed successively with a saturated aqueous solution of $NaHCO_3$, water, and brine. After drying (Na_2SO_4), the organic phase was filtered and concentrated and the residue was purified by chromatography (hexanes/EtOAc, 2:1) to give **26** (1.1 g, 91%) as a syrup (α/β, 20:1): R_f 0.33 (hexanes/EtOAc, 1:1); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 6.17 (s, 1H), 5.17 (d, 1H, *J* = 1.4 Hz), 4.35–4.25 (m, 3H), 4.22–4.17 (m, 1H), 3.45 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.8, 169.7, 169.7, 100.0, 85.9, 82.7, 80.4, 63.8, 58.7, 21.3, 21.0 (2); HR-ESI-MS Calcd for $[C_{12}H_{18}O_8]Na^+$ 313.0899, found 313.0884.

3.16. Methyl 3-azido-3-deoxy-α-D-arabinofuranoside (27)

A solution of **23** (1.0 g, 6.85 mmol), NaN_3 (884 mg, 13.7 mmol) and NH_4Cl (800 mg, 15.1 mmol) in ethanol (20 mL) and water (8 mL) was heated at reflux for 24 h. The reaction mixture was cooled and concentrated to give crude **27** (1.3 g) as a yellow-white solid that was used crude in the next reaction: R_f 0.21 (toluene/EtOAc, 2:1). The NMR data for this compound were identical to those reported previously.[42]

3.17. Methyl 2,5-di-*O*-acetyl-3-azido-3-deoxy-α-D-arabinofuranoside (28)

To a solution of **27** (1.3 g crude, 6.85 mmol) in dry pyridine (25 mL) was added dropwise Ac_2O (6.4 mL, 68 mmol). The reaction mixture was stirred at rt overnight, then diluted with ether and washed successively with 0.1 M HCl, water, and brine. After drying (Na_2SO_4), the solution was filtered and concentrated. The residue was purified by chromatography (hexanes/EtOAc, 6:1) to give **28** (1.17 g, 63% from **23**) as a syrup: R_f 0.51 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +84.5 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 4.99 (d, 1H, *J* = 2.0 Hz), 4.93 (s, 1H), 4.32–4.21 (m, 2H), 4.11–4.07 (m, 1H), 3.70 (dd, 1H, *J* = 2.0, 6.3 Hz), 3.37 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.7, 168.0, 106.6, 82.4, 79.5, 66.6, 63.3, 55.1, 20.9; HR-ESI-MS Calcd for $[C_{10}H_{15}O_6N_3]Na^+$ 296.0858, found 296.0845.

3.18. 1,2,5-Tri-*O*-acetyl-3-azido-3-deoxy-D-arabinofuranose (29)

A solution of **28** (340 mg, 1.24 mmol) in Ac_2O (10 mL) was cooled to 0 °C for as a mixture of H_2SO_4/Ac_2O (0.3:2, 0.4 mL) was added slowly. The reaction mixture was stirred at 0 °C for 1 h before a saturated aqueous solution of $NaHCO_3$ was added until no more evolution of gas was observed. The mixture was diluted with CH_2Cl_2 (10 mL) and the organic layer was separated and washed successively with a saturated solution of $NaHCO_3$, water, and brine. After drying (Na_2SO_4), the solution was filtered and concentrated to give **29** (370 mg, 99%) as a syrup that was used crude in the preparation of **22**: R_f 0.60 (hexanes/EtOAc, 2:1).

3.19. Octyl 5-*O*-(2,3-di-*O*-acetyl-3-*O*-methyl-α-D-arabinofuranosyl)-2-*O*-benzoyl-α-D-arabinofuranoside (30)

A solution of alcohol **18**[15] (150 mg, 0.42 mmol), thioglycoside **21** (171 mg, 0.48 mmol) and powdered molecular sieves (4 Å, 250 mg) in dry CH_2Cl_2 (15 mL) was cooled to 0 °C and then *N*-iodosuccinimide (135 mg, 0.6 mmol) and silver triflate (20 mg, 0.08 mmol) were added. After stirring for 5 min at 0 °C, Et_3N (0.1 mL) was added and the reaction mixture was then diluted with CH_2Cl_2

(10 mL) and filtered through Celite. The filtrate was washed successively with a saturated aqueous solution of $Na_2S_2O_3$, water, and brine. After drying (Na_2SO_4), the organic phase was filtered and concentrated and the residue was purified by chromatography (hexanes/EtOAc, 6:1 → 2:1) to give **30** (178 mg, 71%) as a syrup: R_f 0.35 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +89.0 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.05–8.01 (m, 2H), 7.61–7.56 (m, 1H), 7.47–7.44 (m, 2H), 5.20 (s, 1H), 5.12 (s, 1H), 5.11 (m, 1H), 5.08 (d, 1H, $J = 0.9$ Hz), 4.32–4.16 (m, 4H), 3.95 (dd, 1H, $J = 4.2$, 11.1 Hz), 3.77–3.73 (m, 2H), 3.54 (d, 1H, $J = 4.8$ Hz), 3.50–3.46 (m, 1H), 3.30–3.28 (m, 4H), 2.09 (s, 3H), 2.08 (s, 3H), 1.63–1.56 (m, 2H), 1.27 (m, 10H), 0.89–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.9, 169.7, 166.8, 133.7, 130.0, 129.5, 128.7, 106.3, 105.5, 86.3, 85.9, 82.3, 81.1, 80.4, 77.3, 68.1, 66.4, 64.0, 58.4, 32.0, 29.7, 29.5, 29.4, 26.3, 22.8, 21.0 (2), 14.3; HR-ESI-MS Calcd for $[C_{30}H_{44}O_{12}]Na^+$ 619.2731, found 619.2715.

3.20. Octyl 5-*O*-(2,3-di-*O*-acetyl-3-azido-3-deoxy-α-D-arabinofuranosyl)-2-*O*-benzoyl-α-D-arabinofuranoside (31)

Alcohol **18**[15] (35 mg, 0.09 mmol) was glycosylated with thioglycoside **22** (52 mg, 0.14 mmol) in dry CH_2Cl_2 (10 mL) with *N*-iodosuccinimide (60 mg, 0.26 mmol), silver triflate (5 mg, 0.02 mmol), and powdered molecular sieves (4 Å, 100 mg) as described for the preparation of **30**. Purification of the product by chromatography (hexanes/EtOAc, 3:1) gave **31** (45 mg, 82%) as a syrup: R_f 0.64 (hexanes/EtOAc, 1:1); $[\alpha]_D$ +99.7 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.04–8.02 (m, 2H), 7.61–7.57 (m, 1H), 7.48–7.44 (m, 2H), 5.23 (s, 1H), 5.15 (s, 1H), 5.07 (d, 1H, $J = 2.7$ Hz), 5.05 (d, 1H, $J = 2.2$ Hz), 4.32–4.12 (m, 4H), 3.91 (dd, 1H, $J = 4.7$, 11.2 Hz), 3.77–3.74 (m, 2H), 3.68 (dd, 1H, $J = 2.1$, 6.4 Hz), 3.49–3.45 (m, 1H), 3.32 (d, 1H, $J = 5.4$ Hz), 2.1 (s, 6H), 1.63 (m, 2H), 1.34–1.27 (m, 10H), 0.89–0.85 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.7, 169.9, 167.0, 133.8, 130.0, 129.3, 128.7, 105.7, 105.4, 86.1, 82.5, 82.3, 79.7, 77.0, 68.2, 66.7, 66.4, 63.2, 32.0, 29.6, 29.5, 29.4, 26.2, 22.8, 20.9, 14.3; HR-ESI-MS Calcd for $[C_{29}H_{41}O_{11}N_3]Na^+$ 630.2639, found 630.2652.

3.21. Octyl 3-*O*-(2,5-di-*O*-acetyl-3-*O*-methyl-α-D-arabinofuranosyl)-2,5-di-*O*-benzoyl-α-D-arabinofuranoside (32)

Alcohol **20**[15] (171 mg, 0.36 mmol) was glycosylated with thioglycoside **21** (155 mg, 0.43 mmol) in dry CH_2Cl_2 (15 mL) with *N*-iodosuccinimide (122 mg, 0.54 mmol), silver triflate (19 mg, 0.07 mmol), and powdered molecular sieves (4 Å, 250 mg) as described for the preparation of **30**. Purification of the product by chromatography (hexanes/EtOAc, 5:1 → 2:1) gave **32** (190 mg, 76%) as a syrup: R_f 0.47 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +89.7 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.01–7.99 (m 4H), 7.58–7.56 (m, 1H), 7.49–7.47 (m, 1H), 7.43–7.39 (m, 2H), 7.28–7.24 (m, 2H), 5.41 (s, 1H), 5.31 (s, 1H), 5.22 (s, 1H), 5.19 (s, 1H), 4.68 (dd, 1H, $J = 2.8$, 12.1 Hz), 4.53 (dd, 1H, $J = 4.4$, 12.1 Hz), 4.44–4.41 (m, 1H), 4.38–4.36 (m, 1H), 4.27–4.20 (m, 1H), 4.18–4.13 (m, 2H), 3.77–3.73 (m, 1H), 3.56–3.48 (m, 2H), 3.43 (s, 3H), 2.11 (s, 3H), 2.02 (s, 3H), 1.66–1.62 (m, 2H), 1.29 (m, 10H), 0.88–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.8, 169.6, 166.3, 165.6, 133.5, 133.1, 130.0, 129.8, 129.5, 128.6, 128.4, 105.9, 105.6, 86.4, 82.9, 81.0, 80.8, 80.7, 80.6, 67.7, 63.8, 63.3, 58.5, 32.0, 29.5, 29.5, 29.3 26.2, 22.8, 21.1, 20.8, 14.2; HR-ESI-MS Calcd for $[C_{37}H_{48}O_{13}]Na^+$ 723.2992, found 723.3003.

3.22. Octyl 3-*O*-(2,5-di-*O*-acetyl-3-azido-3-deoxy-α-D-arabinofuranosyl)-2,5-*O*-benzoyl-α-D-arabinofuranoside (33)

Alcohol **20**[15] (120 mg, 0.25 mmol) was glycosylated with thioglycoside **22** (112 mg, 0.30 mmol) in dry CH_2Cl_2 (15 mL) with *N*-iodosuccinimide (67 mg, 0.30 mmol), silver triflate (12 mg, 0.04 mmol), and powdered molecular sieves (4 Å, 250 mg) as described for the preparation of **30**. Purification of the product by chromatography (hexanes/EtOAc, 4:1) gave **33** (160 mg, 90%) as a syrup: R_f 0.61 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +123.7 (*c* 0.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.00 (d, 4 H, $J = 7.4$ Hz), 7.60–7.57 (m, 1H), 7.51–7.47 (m, 1H), 7.43–7.39 (m, 2H), 7.28–7.24 (m, 2H), 5.51 (s, 1H), 5.29 (s, 1H), 5.25 (s, 1H), 5.16 (d, 1H, $J = 2.2$ Hz), 4.66 (dd, 1H, $J = 3.0$, 12.1 Hz), 4.53 (dd, 1H, $J = 4.3$, 12.1 Hz), 4.43–4.40 (m, 1H), 4.33 (d, 1H, $J = 5.4$ Hz), 4.25–4.21 (m, 1H), 4.18–4.15 (m, 1H), 4.11–4.07 (m, 1H), 3.79–3.73 (m, 1H), 3.69 (dd, 1H, $J = 2.6$, 6.5 Hz), 3.54–3.48 (m, 1H), 2.13 (s, 3H), 2.03 (s, 3H), 1.64–1.62 (m, 2H), 1.31–1.28 (m, 10H), 0.90–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.5, 169.8, 166.3, 165.6, 133.6 (2), 129.9, 129.8 (2), 129.4, 128.6, 128.4, 105.8, 104.9, 82.7, 82.5, 81.1, 80.6, 80.0, 67.7 (2), 63.2, 63.1, 32.0, 29.5, 29.4, 29.3, 26.2, 22.7, 20.8, 20.7, 14.2; HR-ESI-MS Calcd for $[C_{36}H_{45}O_{12}N_3]Na^+$ 734.2901, found 734.2915.

3.23. Octyl 3,5-di-*O*-(2,5-di-*O*-acetyl-3-*O*-methyl-α-D-arabinofuranosyl)-2-*O*-benzoyl-α-D-arabinofuranoside (34)

Alcohol **19**[15] (150 mg, 0.41 mmol) glycosylated with thioglycoside **21** (387 mg, 1.09 mmol) in dry CH_2Cl_2 (15 mL) with *N*-iodosuccinimide (236 mg, 1.06 mmol) silver triflate (55 mg, 0.21 mmol), and powdered molecular sieves (4 Å, 500 mg) as described for the preparation of **30**. Purification of the product by chromatography (hexanes/EtOAc, 6:1 → 2:1) gave **34** (270 mg, 78%) as a syrup: R_f 0.27 (hexanes/EtOAc, 2:1); $[\alpha]_D$ +104.2 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.07–8.04 (m, 2H), 7.59–7.55 (m, 1 H), 7.46–7.43 (m, 2H), 5.34 (s, 1H), 5.27 (d, 1H, $J = 1.4$ Hz), 5.16 (d, 1H, $J = 1.3$ Hz), 5.15 (s, 1H), 5.13 (s, 1H), 5.08 (d, 1H, $J = 0.9$ Hz), 4.33–4.15 (m, 8 H), 3.94 (dd, 1H, $J = 3.9$, 11.3 Hz), 3.76–3.69 (m, 2H), 3.56 (d, 1H, $J = 5.2$ Hz), 3.50–3.44 (m, 2H), 3.42 (s, 3H), 3.22 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.66–1.59 (m, 2H), 1.29–1.27 (m, 10H), 0.89–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.9 (2), 169.7, 169.6, 165.8, 133.5, 130.0, 129.8, 128.6, 106.1, 105.9, 105.6, 86.5, 86.5, 83.0, 81.4, 81.0, 81.0, 80.7, 80.5 (2), 67.7, 65.1, 64.0, 63.7, 58.5, 58.3, 32.0, 29.6, 29.4, 26.2, 22.8,

21.1 (2), 20.9, 14.3; HR-ESI-MS Calcd for $[C_{40}H_{58}O_{18}]Na^+$ 849.3521, found 849.3506.

3.24. Octyl 3,5-di-*O*-(2,5-di-*O*-acetyl-3-azido-3-deoxy-α-D-arabinofuranosyl)-2-*O*-benzoyl-α-D-arabinofuranoside (35)

Alcohol **19**[15] (193 mg, 0.53 mmol) was glycosylated with thioglycoside **22** (481 mg, 1.32 mmol) in dry CH_2Cl_2 (10 mL) with *N*-iodosuccinimide (297 mg, 1.32 mmol) silver triflate (85 mg, 0.33 mmol), and powdered molecular sieves (4 Å, 500 mg) as described for the preparation of **30**. Purification of the product by chromatography (hexanes/EtOAc, 6:1 → 2:1) to give **35** (350 mg, 78%) as a syrup: R_f 0.71 (hexanes/EtOAc, 1:1); $[\alpha]_D$ +98.7 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.06–8.04 (m, 2H), 7.61–7.57 (m, 1H), 7.48–7.44 (m, 2H), 5.45 (s, 1H), 5.25 (d, 1H, $J = 1.2$ Hz), 5.16 (d, 2H, $J = 2.3$ Hz), 5.13 (d, 1H, $J = 2.3$ Hz), 5.03 (d, 1H, $J = 1.9$ Hz), 4.31–4.20 (m, 6H), 4.13–4.07 (m, 2H), 3.91 (dd, 1H, $J = 3.7$, 11.1 Hz), 3.73–3.68 (m, 4H), 3.49–3.43 (m, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 1.65–1.58 (m, 2H), 1.43–1.26 (m, 10H), 0.93–0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.6 (2), 169.9, 169.8, 165.8, 133.7, 130.0, 129.6, 128.7, 105.9, 105.6, 105.0, 82.8, 82.5 (2), 81.1, 80.8, 80.0 (2), 67.7, 66.9, 65.5, 63.2, 32.0, 29.6, 29.5, 29.4, 26.2, 22.8, 20.9 (3), 20.9, 14.3; HR-ESI-MS Calcd for $[C_{38}H_{52}O_{16}N_6]Na^+$ 871.3337, found 871.3348.

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

M. smegmatis cells (10 g wet weight) were washed and re-suspended at 4 °C in 30 mL of buffer A, which contained 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol, and 10 mM $MgCl_2$, 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 27,000*g* supernatant 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. Arabinosyltransferase assay

Compounds **9**, **10**, **11**, **12**, **13**, **15**, **16**, and **17** at a concentration of 3.6 mM (previously shown to have no acceptor activity at this concentration) and [^{14}C]-DPA (**3**, 40,000 cpm, 18 mM, 20 μL [stored in chloroform/methanol, 2:1]), were dried under a stream of argon in a microcentrifuge tube (1.5 mL) and placed in a vacuum desiccator for 15 min to remove any residual solvent together with the appropriate acceptor compounds (**6**, **7**, or **8**) to determine any potential inhibitory properties of the modified compounds. The dried constituents of the assay were then resuspended in 8 mL of a 1% aqueous solution of Igepal CA-630 (Sigma). The remaining constituents of the arabinosyltransferase assay containing 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM ATP, and membranes (1 mg) were added to a final reaction volume of 80 μL. The reaction mixtures were then incubated at 37 °C for 1 h. A $CHCl_3/CH_3OH$ (1:1, 533 μL) solution was then 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, 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 cpm 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]Ara*f* 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 $CHCl_3/CH_3OH/NH_4OH/H_2O$ (65:25:0.5:3.6) 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. Inhibition of product formation using compounds **6**, **7**, and **8** at 0.4 mM as acceptors was determined by exposure of phosphorimaging screens (Kodak) for 24 h. The detected radiation was quantified using a Molecular Imager FX (BIO RAD) and Quantity One software. Inhibition was then calculated by expressing the radioactive product of each acceptor as a percentage of the total radiation in all acceptor/inhibitor derived products.

3.28. Measurement of anti-tuberculosis activity of the oligosaccharides

Measurement of the anti-tuberculosis activity of the target compounds was carried out as previously reported using the fluorescence-based Alamar Blue microplate assay.[34] All compounds were tested against *M. tuberculosis*

strain $H_{37}Rv$ (ATCC 27294) at a concentration 6.25 μg/mL. The results of these assays, expressed as a percent inhibition of growth of the bacteria, are provided in Table 1.

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