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Studies on α(1→5) Linked Octyl Arabinofuranosyl Disaccharides for Mycobacterial Arabinosyl Transferase Activity

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Abstract—The appearance multi-drug resistant *Mycobacterium tuberculosis* (MTB) throughout the world has prompted a search for new, safer and more active agents against tuberculosis. Based on studies of the biosynthesis of mycobacterial cell wall polysaccharides, octyl 5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside analogues were synthesized and evaluated as inhibitors for *M. tuberculosis* and *Mycobacterium avium*. A cell free assay system has been used for the evaluation of these disaccharides as substrates for mycobacterial arabinosyltransferase activity.

Introduction

In spite of the availability of highly active anti-tubercular agents, tuberculosis remains one of the primary causes of human death and suffering worldwide.[1] Approximately 2–3 million people die annually from the disease, and each year an additional 8 million people become ill with tuberculosis. It is estimated that one third of the world's population is infected with the bacteria that causes tuberculosis. Of even greater concern is the development of highly drug-resistant forms of the disease, multiple drug-resistant tuberculosis (MDR-TB), that raises the specter that tuberculosis may once again become an incurable disease. Treatment of MDR-TB is both difficult and expensive,[2,3] and for more intractable forms, few treatment options are available.[4] Public health officials worldwide are now calling for increased funding and research for the development of newer, safer drugs and vaccines to combat this deadly disease.[5]

The biosynthesis of the mycobacterial cell wall is an excellent target for future drug development based on recent structural studies as well as the fact that several of the more active anti-tubercular agents that are currently in use act on the cell wall of MTB.[6] For example, ethambutol (EMB) and isoniazid (INH) target two critical cell wall components, the arabinogalactan and the mycolic acids, respectively.[6] The arabinogalactan contains both arabinofuranose and galactofuranose moieties, and this cell wall component critically underpins the cell wall infrastructure, maintaining cell wall integrity. Neither of these sugar forms is found in humans, thus making the biochemistry and attendant enzyme pathways ideal targets for the development of new, selective anti-tubercular agents. The arabinan portion of the cell wall is composed of arabinofuranose homopolymers with different linkages, namely α(1→5), α(1→2) and α(1→3), and most likely requires several different sugar processing enzymes, or arabinosyltransferases, for its complete synthesis.[7]

A key mycobacterial arabinose donor β-D-arabinofuranosyl-1-monophosphoryldecaprenol has been identified within lipid extracts and is implicated in the biogenesis of the two major cell wall polysaccharides arabinogalactan (AG) and liporabinomannan (LAM).[8] Several synthetic *O*- and *S*-alkyl arabinofuranoside acceptors have been prepared as acceptors for the development of an arabinosyltransferase assay.[9] Recently, several workers have also synthesized the arabinosyl oligosaccharides present in the cell wall of

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mycobacteria for further biological and structural studies.[10,11] The highly branched arabinofuranosyl hexasaccharide (Fig. 1) found at the non-reducing termini of mycobacterial AG and LAM was prepared as its methyl glycoside via a route that is both highly efficient and stereocontrolled.[10] An alternative synthetic approach has been reported starting from 1,2,5-orthoester of D-arabinose to synthesize an octyl penta-arabinofuranoside component of the mycobacterial cell wall.[11] High-resolution NMR studies have been done on methyl arabinofuranose polysaccharides to determine the identity and equilibrium populations of preferred conformers for each furanose ring.[10d] Additionally, gas-phase computational methods have also been applied to synthetic arabinofuranose saccharides in order to determine the energy profiles and structural parameters as a function ring conformation.[12] How these results relate to the actual enzyme-bound intermediates or structure of the arabinofuranose ring in AG are unclear.

As a continuation of our work to design probes and inhibitors of mycobacterial glycosyltransferases,[13] we report herein the synthesis of 1-*O*-octyl-arabinofuranosyl disaccharide derivatives that are similar to the natural substrate (Fig. 2). Their anti-mycobacterial activity and activity as arabinosyltransferase acceptors are also reported.

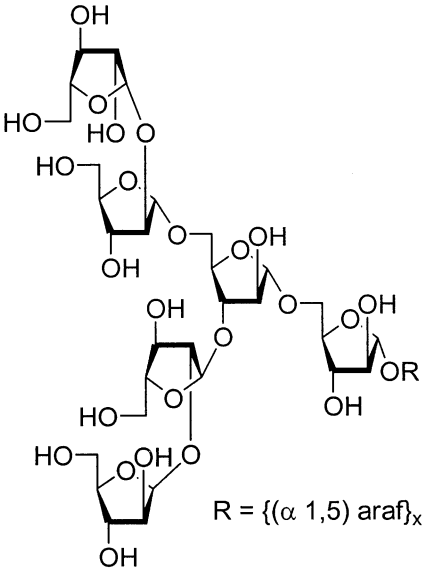


Figure 1. Hexarabinoside found in mycobacterial arabinogalactan (AG) and lipoarabinomannan (LAM).

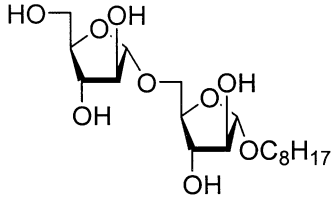


Figure 2. Structure of α(1→5) linked arabinofuranosyl disaccharide similar to natural substrate.

Result and discussion

Synthesis

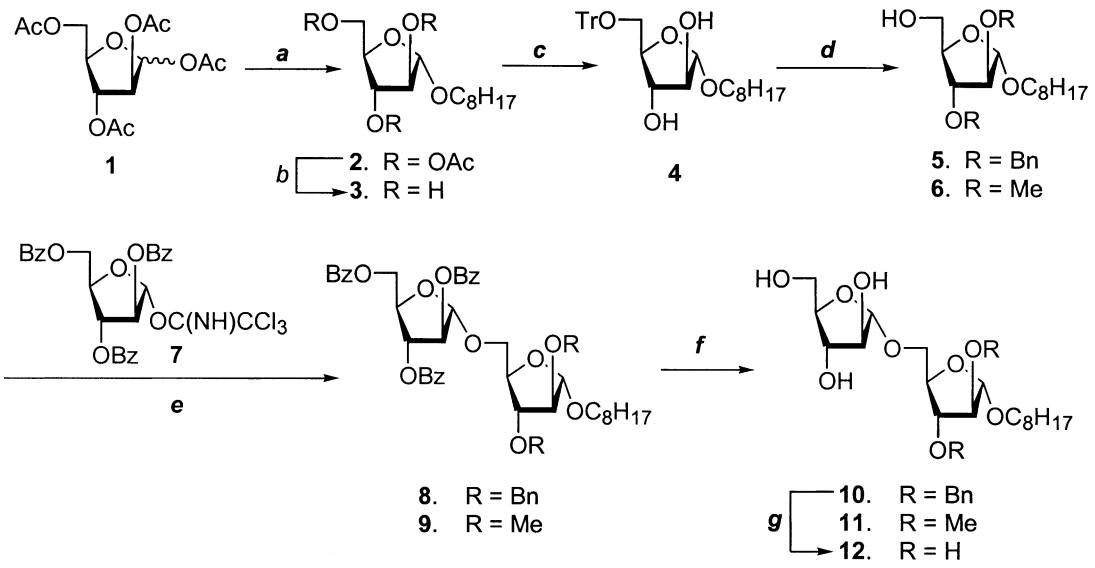
The synthesis of arabinosyl disaccharides is represented in Scheme 1. Several approaches were used for the synthesis of these arabinofuranosides[10,11] in order to prepare α(1→5) disaccharides having bulky and small protecting groups at the reducing end, and to study the effects of these substitutions on activity. The reaction of **1**, 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranoside,[14] with *n*-octanol in the presence of $SnCl_4$ as Lewis acid gave **2** as the pure α-isomer (75%) after column chromatography.[13a] The octyl compound **2** was then deacetyled with 7 N NH_3/MeOH to give **3** in an overall yield of 86% after purification. The 5-position of the sugar **3** was blocked with a trityl group to get compound **4** in 73% yield. Compound **4** was further blocked at both the 2- and 3-positions with either benzyl or methyl groups and then detritylated with 5% trifluoroacetic acid in chloroform at room temperature to give compound **5** or **6** as glycosyl acceptors for disaccharide synthesis. The trichloroacetimidate donor[15] and the acceptors **5** or **6** were reacted for 2 h in the presence of the promoter $BF_3.Et_2O$ at 0 °C under an inert atmosphere in dry CH_2Cl_2 at room temperature over powdered 4 Å molecular seives. After workup and column chromatography on silica gel G (70–230 mesh), the pure disaccharides **8** and **9** were obtained in 88 and 85% yield, respectively. The disaccharides were debenzoylated with 7 N NH_3/MeOH to yield the partially blocked disaccharides **10** and **11** in 98 and 90% yield, respectively. Lastly, the fully de-blocked disaccharide **12**, which more closely resembles the natural substrate for cell wall polysaccharide synthesis, was made by Pd/C catalyzed hydrogenation of the disaccharide **10**. All the new compounds were characterized by CHN analysis, FABMS and NMR spectroscopy. The anomeric carbons of α-D-arabinofuranosides resonate in the range of 108–105 ppm whereas in the proton NMR spectrum, the anomeric protons showed a coupling $^3J_{H1,H2}$ in the range of 0.8–1.2 Hz suggesting α-glycosylation.[16] Further, the NOE, decoupling, D_2O exchanged and APT experiments were performed as required in order to confirm NMR assignments and stereochemistry at the anomeric centers.

In-vitro assay

In-vitro assays[17] of arabinofuranosyl disaccharides were done on *Mycobacterium tuberculosis* (MTB H37Ra) and *Mycobacterium avium* (NJ 211). The disaccharides **8**, **9**, **11**, **12** have shown activity $>128\,\mu g/mL$ whereas the disaccharide **10** have shown $>12.8 \leq 128\,\mu g/mL$ for both strains.

Acceptor activity

Based on the previous use of specific arabinose based neoglycolipid acceptors,[9] compounds **10** and **11** were synthesized and compared with the fully de-blocked arabinose acceptor **12**. Assays performed in the presence of membranes resulted in [^{14}C]Ara*f* incorporation from DP-[^{14}C]A for both compounds **11** and **12** with no



Scheme 1. Synthesis of α(1→5) linked arabinofuranosyl disaccharides. Reagents & Conditions: *(a)* $C_8H_{17}OH$, $SnCl_4$, CH_3CN, 30 min, rt, 75%; *(b)* 7N NH_3/MeOH, rt, 5 h, 86%; *(c)* TrCl, Py, rt, overnight, 73%; *(d)* BnBr/Mel, NaH, THF, rt, 3 h, 5% TFA/$CHCl_3$, $CHCl_3$, 1 h, rt, **5**: 78%, **6**: 66%; *(e)* $BF_3.Et_2O$, CH_2Cl_2, 0°C, 30 min, **8**: 88%, **9**: 85%; *(f)* 7N NH_3/MeOH, rt, overnight, **10**: 98%, **11**: 90%; *(g)* Pd/C, H_2, MeOH, rt, overnight, 99%

detectable activity for **10** (see Fig. 3). TLC/autoradiography (Fig. 3) demonstrated the enzymatic conversion of the both disaccharide **11** and **12** to their corresponding trisaccharide products, [^{14}C]Ara*f* to the 5′-OH and 3′-OH of **12** as reported previously[9] and, presumably, a similar linkage profile for **11**. Compound **10** was not recognized as a substrate for the arabinosyltransferase enzyme(s), presumably due to the bulky benzyl ether protecting groups on the reducing sugar. In contrast, **11**, which possessed methyl ether protecting groups on the reducing sugar, was recognized as a substrate, however, with a significant loss of acceptor recognition (see Fig. 3). Further competition-based, experiments established that **10** and **11** were effective inhibitors of their native acceptors (**12**) in the arabinosyltransferase assay resulting in IC_{50} values of 1.12 and 3.70 mM for **10** and **11**, respectively.

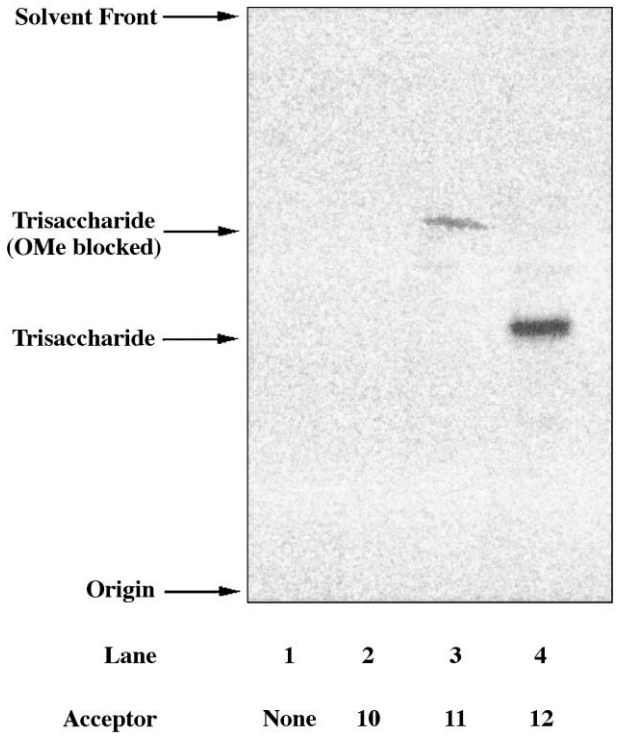


Figure 3. An autoradiogram of reactions products produced through the inclusion of **10**, **11,** and **12**, mycobacterial membranes and DP[^{14}C]A. Lane 1, no acceptor; lane 2, **10**; lane 3, **11**; and lane 4, **12**. The compounds were included at a final concentration of 2 mM and TLC/autoradiography performed using chloroform/methanol/ammonium hydroxide/water (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 3 days.

Experimental

Synthesis

General procedure. All reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents from Aldrich were used in the reactions as such. Whenever necessary, compounds and starting materials were dried by azeotropic removal of water with toluene under reduced pressure. Reactions were monitored by thin-layer chromatography (TLC) on precoated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spray with $(NH_4)_2SO_4$ solution (150 g ammonium sulfate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for work up and chromatography were reagent grade from Fisher Scientific. Flash chromatography was carried out on Fischer silica gel 60 (230–400 mesh). Melting points were determined with a Mel-Temp II capillary melting points apparatus and is uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 and

75 MHz, respectively. Coupling constants (*J*) are reported in Hz and chemical shifts are in ppm (δ) relative to residual solvent peak or internal standard. Microanalysis was performed on a Perkin–Elmer 2400 CHN analyzer. FABMS were recorded on a Varian/MAT 311A double-focusing mass spectrometer either by adding NBA/LiCl.

Octyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside (2). An anomeric mixture of D-arabinofuranose tetraacetate[14] **1** (10.0 g, 31.45 mmol) was dissolved in dry CH_3CN (150 mL) was added $SnCl_4$ (4.4 mL, 37.74 mmol) at 0 °C and the mixture was stirred for 30 min. To this cold solution, *n*-octanol (5.94 mL, 37.74 mmol) was added dropwise over a period of 30 min. It was again stirred for 1 h at room temperature. Celite (2.0 g) was added cooled in an ice-water bath and a saturated aqueous $NaHCO_3$ solution was added dropwise to precipitate tin salts. After complete precipitation, the mixture was filtered through Celite and washed with chloroform (2×10 mL), concentrated to syrup, and redissolved in $CHCl_3$ (400 mL). The organic solution was next washed with water (2×50 mL) and brine (2×50 mL), dried over Na_2SO_4 and concentrated in vacuo to give the crude oil. Flash chromatography (cyclohexane/EtOAc, 5:1) gave **2** as colorless oil (9.1 g, 75%). FAB-MS (LiCl) *m/e* 467 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 5.07 (1H, d, $J=0.4$, 1.5 Hz, H-2), 5.02 (1H, s, H-1), 4.96 (1H, ddd, $J=0.7$, 1.5, 4.9 Hz, H-3), 4.44 (1H, m, H-5_a), 4.22 (2H, m, H-4, H-5_b), 3.69 (1H, m, OCH_2), 3.45 (1H, m, OCH_2), 2.109, 2.106, 2.103 (each 3H, s, 3×OAc), 1.57 (2H, m, CH_2), 1.28 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3).

Octyl α-D-arabinofuranoside (3). To a solution of compound **2** (9.00 g, 23.19 mmol) in dry methanol (50 mL) was added a NaOMe solution in methanol (25% w/v, 9.0 mL) dropwise while cooling in an ice-bath. The reaction mixture was allowed to stir at room temperature for 5 h. It was concentrated in vacuo to give a syrup, which after flash chromatography ($CHCl_3$/MeOH 95:5) gave a viscous oil. Crystallization from diethylether gave pure **3** as solid (5.22 g, 86%). Mp 49 °C. FAB-MS (NBA) *m/e* 263 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 5.01 (1H, s, H-1), 4.18 (1H, ddd, $J=1.5$, 2.0, 2.5 Hz, H-4), 4.00 (2H, m, H-2, H-3), 3.90 (1H, dd, $J=2.5$, 11.7 Hz, H-5_a), 3.82 (1H, dd, $J=2.0$, 11.7 Hz, H-5_b), 3.69 (1H, m, OCH_2), 3.45 (1H, m, OCH_2), 2.99 (1H, d, $J=11.4$ Hz, 3-OH, D_2O exchangeable), 2.40 (1H, m, 2-OH, D_2O exchangeable), 1.58 (2H, m, CH_2), 1.26 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 107.95 (C-1), 86.70 (C-4), 79.02 (C-2), 77.88 (C-3), 67.80 (OCH_2), 61.78 (C-5), 31.77, 29.46, 29.29, 29.19, 26.08, 22.61 (6×CH_2), 14.06 (CH_3).

Octyl 5-*O*-trityl-α-D-arabinofuranoside (4). To a dry pyridine (60 mL) solution of compound **3** (5.1 g, 19.46 mmol) was added trityl chloride (8.14 g, 29.19 mmol), and the reaction mixture was stirred at 50 °C overnight. The reaction mixture was coevaporated with toluene (2×50 mL), redissolved in $CHCl_3$ (150 mL), washed with water (2×20 mL), dried over Na_2SO_4 and concentrated. Column chromatography ($CHCl_3$/MeOH 98:2) gave compound **4** as pale yellow oil (7.16 g, 73%). FAB-MS (LiCl) *m/e* 505 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.42–7.25 (15H, m, aromatic), 5.09 (1H, s, H-1), 4.10 (1H, dd, $J=1.2$, 2.0 Hz, H-4), 3.97 (1H, br d, $J=11.2$ Hz, H-2), 3.87 (1H, br d, $J=10.8$ Hz, H-3), 3.83 (1H, d, $J=11.2$ Hz, 2-OH, D_2O exchangeable), 3.73 (1H, m, OCH_2), 3.66 (1H, dd, $J=2.8$, 10.5 Hz, H-5_a), 3.46 (1H, m, OCH_2), 3.24 (1H, dd, $J=2.0$, 10.5 Hz, H-5_b), 2.92 (1H, d, $J=11.9$ Hz, 3-OH, D_2O exchangeable), 1.55 (2H, m, CH_2), 1.27 (10H, m, 5×CH_2), 0.87 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.80, 128.80, 128.04, 127.98, 127.44 (aromatic), 108.06 (C-1), 88.40 (C), 86.47 (C-4), 78.67 (C-2), 78.25 (C-3), 67.66 (OCH_2), 63.47 (C-5), 31.77, 29.52, 29.30, 29.19, 26.12, 22.62 (6×CH_2), 14.07 (CH_3).

Octyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside (5). Compound **4** (4.0 g, 7.94 mmol) was dissolved in dry THF (50 mL) and NaH (60% dispersion in mineral oil, 571 mg, 23.82 mmol) was added, and the mixture was stirred at room temperature for 15 min. The reaction was cooled to 0 °C and benzyl bromide (2.83 mL, 23.82 mmol) was added dropwise, and the resulting mixture was stirred for 3 h at room temperature. Methanol (20 mL) was added, and the reaction was concentrated to dryness, redissolved in $CHCl_3$ (250 mL), washed with water (2×50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated to a colorless oil. The oil was dissolved in $CHCl_3$ and cooled to 0 °C, and 5% trifluoroacetic acid in $CHCl_3$ (10 mL) was added dropwise. The reaction mixture was stirred for 1 h, coevaporated with ethanol (2×20 mL), and concentrated to syrup. Flash chromatography (cyclohexane/EtOAc 10:1) yielded compound **5** (2.25 g, 78%) as a colorless syrup. FAB-MS (LiCl) *m/e* 449 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.28 (10H, m, aromatic), 5.02 (1H, d, H-1), 4.62–4.49 (4H, 4×d, each $J=11.8$ Hz, 2×CH_2–aromatic), 4.15–4.11 (1H, m, H-4), 4.03 (1H, dd, $J=1.1$, 3.1 Hz, H-2), 3.98 (1H, dd, $J=3.1$, 6.6 Hz, H-3), 3.84 (1H, dd, $J=2.8$, 12.1 Hz, H-5_a), 3.70 (1H, m, OCH_2), 3.65 (1H, dd, $J=3.9$, 12.1 Hz, H-5_b), 3.39 (1H, m, OCH_2), 1.59 (2H, m, CH_2), 1.27 (10H, m, CH_2), 0.86 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.84, 137.40, 128.42, 128.36, 127.90, 127.83, 127.69 (aromatic), 106.21 (C-1), 88.10 (C-2), 82.66 (C-3), 81.68 (C-4), 72.24, 71.92 (CH_2-aromatic), 67.66 (OCH_2), 62.18 (C-5), 31.80, 29.49, 29.34, 29.25, 26.11, 22.63 (6×CH_2), 14.07 (CH_3).

Octyl 2,3-di-*O*-methyl-α-D-arabinofuranoside (6). Compound **4** (2.0 g, 3.97 mmol) was dissolved in dry THF (20 mL) and NaH (60% dispersion in mineral oil, 286 mg, 11.91 mmol) was added. The mixture was stirred at room temperature for 15 min, next, the reaction was cooled to 0 °C, and iodomethane (0.7 mL, 9.93 mmol) was added dropwise followed by stirring for 3 h at room temperature. Methanol (10 mL) was added, the solution was concentrated to dryness, the oil was redissolved in $CHCl_3$ (100 mL), and the organic solution was washed with water (2×20 mL) and brine (20 mL), and dried over Na_2SO_4. The dry organic solution was concentrated to a colorless oil that was dissolved in $CHCl_3$. The chloroform solution was cooled to 0 °C,

and 5% trifluoroacetic acid in $CHCl_3$ (5 mL) was added dropwise. The reaction mixture was then stirred for 30 min, coevaporated with ethanol (2×10 mL), and concentrated to syrup. After purification on silica gel column using cyclohexane/ethylacetate (5:1) as eluant, compound **6** was obtained as a colorless, viscous oil (66%). FABMS (LiCl) *m/e* 297 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 4.99 (1H, s, H-1), 4.06 (1H, m, H-4), 3.89 (1H, m, H-5_a), 3.73 (1H, dd, $J=1.1$, 2.6 Hz, H-2), 3.68 (3H, m, H-3, H-5_b, OCH_2), 3.43 (1H, m, OCH_2), 3.42, 3.41 (each 3H, s, 2×OCH_3), 1.87 (1H, dd, $J=4.4$, 8.1 Hz, 5-OH, D_2O exchangeable), 1.58 (2H, m, CH_2), 1.28 (10H, m, CH_2), 0.88 (3H, m, CH_3).

Octyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (8). The trichloroacetimidate donor **7** (2.46 g, 4.06 mmol) and the acceptor **5** (1.12 g, 2.54 mmol) were dissolved in dry CH_2Cl_2 (30 mL) and dry powdered 4 Å molecular sieves were added. The mixture was stirred for 15 min and cooled to 0 °C. The promoter $BF_3{\cdot}Et_2O$ (0.52 mL, 4.06 mmol) was dissolved in 2 mL of dry CH_2Cl_2 and added to the reaction mixture dropwise. The reaction mixture was stirred for an additional 30 min while warming to ambient temperature and filtered. The filtrate was washed with cold saturated anhydrous $NaHCO_3$ followed by distilled water, and the organic layer was dried over Na_2SO_4 and concentrated. Column chromatography of the resulting oil on silica gel (cyclohexane/EtOAc, 10:1) afforded the pure disaccharide **8** (1.96 g, 88%) as a colorless syrup. FAB-MS (LiCl) *m/e* 893 $[M+Li]^+$. $C_{53}H_{58}O_{12}$. 1.0 H_2O (found: C, 70.35; H, 7.72, requires C, 70.3; H, 6.68). ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–7.96, 7.57–7.19 (25H, each m, aromatic), 5.59 (1H, d, $J=1.0$ Hz, H-2′), 5.53 (1H, d, $J=4.7$ Hz, H-3′), 5.36 (1H, s, H-1′), 5.05 (1H, s, H-1), 4.78 (1H, dd, $J=3.2$, 12.0 Hz, H-$5'_a$), 4.62 (1H, dd, $J=4.8$, 12.0 Hz, H-$5'_b$), 4.50 (4H, m, 2×CH_2-aromatic), 4.38 (1H, m, H-4′), 4.20 (1H, m, H-4), 4.04 (1H, dd, $J=3.5$, 6.6 Hz, H-3), 4.03 (1H, d, $J=1.0$ Hz, H-2), 3.94 (1H, dd, $J=4.4$, 11.1 Hz, H-5_a), 3.75 (1H, dd, $J=3.4$, 11.1 Hz, H-5_b), 3.69 (1H, m, OCH_2), 3.37 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.26 (10H, m, CH_2), 0.87 (3H, m, CH_3).

Octyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-di-*O*-methyl-α-D-arabinofuranoside (9). The acceptor **6** (123 mg, 0.42 mmol) was reacted with donor **7** (305 mg, 0.50 mmol) in the presence of $BF_3{\cdot}Et_2O$ (0.06 mL, 0.5 mmol) as described for the preparation of **8**. After purification on a silica gel column (cyclohexane/EtOAc, 5:1) disaccharide **9** was obtained as a colorless syrup (264 mg, 85%). FAB-MS (LiCl) *m/e* 741 $[M+Li]^+$. $C_{41}H_{50}O_{12}$. 1.3 H_2O (Found: C, 64.87; H, 6.74. requires C, 64.95; H, 6.99). ^1H NMR (300 MHz, $CDCl_3$): δ 8.12–7.98, 7.60–7.24 (15H, each m, aromatic), 5.61 (1H, d, $J=1.1$ Hz, H-2′), 5.56 (1H, d, $J=4.6$ Hz, H-3′), 5.40 (1H, s, H-1′), 4.99 (1H, s, H-1), 4.84 (1H, dd, $J=3.3$, 11.9 Hz, H-$5'_a$), 4.68 (1H, dd, $J=4.9$, 11.9 Hz, H-$5'_b$), 4.60 (1H, ddd, $J=3.3$, 4.6, 4.9 Hz, H-4′), 4.15–4.11 (1H, m, H-4), 3.97 (1H, dd, $J=4.7$, 11.1 Hz, H-5_a), 3.79 (1H, dd, $J=3.7$, 11.1 Hz, H-5_b), 3.73 (1H, m, H-3), 3.72 (1H, d, $J=1.0$ Hz, H-2), 3.68 (1H, m, OCH_2), 3.39 (1H, m, OCH_2), 3.38, 3.36 (each 3H, s, OCH_3), 1.56 (2H, m, CH_2), 1.26 (10H, m, CH_2), 0.87 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.22, 165.80, 165.27 (3×C=O), 133.47, 133.02, 130.03, 129.86, 129.74, 129.71, 128.50, 128.45, 128.28 (aromatic), 105.84 (C-1′), 105.55 (C-1), 90.05 (C-2), 85.47 (C-3), 81.86 (C-2′), 81.45 (C-4), 80.04 (C-4′), 77.89 (C-3′), 67.71 (OCH_2), 66.71 (C-5′), 63.81 (C-5), 31.82, 29.45, 29.36, 29.25, 26.08, 22.65 (6×CH_2), 14.10 (CH_3).

Octyl 5-*O*-(α-D-arabinofuranosyl)-2,3-di-*O*-benzyl-α-D-arabinofuranoside (10). To a solution of disaccharide **8** (1.70 g, 1.92 mmol) in dry methanol (25 mL) was added 7 N NH_3/MeOH (5 mL) dropwise, and the reaction mixture was allowed to stir at room temperature overnight. Concentration in vacuo to give a syrup, followed by flash chromatography ($CHCl_3$/MeOH 96:4) gave **10** as colorless oil (1.08 g, 98%). FAB-MS (NBA) *m/e* 575 $[M+H]^+$. $C_{32}H_{46}O_9$. 0.7 H_2O (found: C, 65.30; H, 7.83. requires C, 65.40; H, 7.99). ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.26 (m, aromatic), 5.03 (1H, s, H-1′), 5.01 (1H, s, H-1), 4.61–4.37 (4H, m, OCH_2–aromatic), 4.16 (1H, ddd, $J=3.1$, 5.3, 5.9 Hz, H-4′), 4.02 (1H, d, $J=9.7$ Hz, H-3′), 3.98 (1H, dd, $J=0.9$, 2.4 Hz, H-2), 3.94 (1H, m, H-2′), 3.90 (1H, ddd, $J=1.9$, 2.3, 5.5 Hz, H-4), 3.84–3.76 (4H, m, H-3, H-5_a, H-5_b, H-$5'_a$), 3.70–3.62 (2H, m, H-$5'_b$, OCH_2), 3.56 (1H, d, $J=9.8$ Hz, 3′-OH, D_2O exchangeable), 3.38 (1H, m, OCH_2), 3.22 (1H, d, $J=11.9$ Hz, 2′-OH, D_2O exchangeable), 1.56 (2H, m, CH_2), 1.28 (10H, m, CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.57, 137.19 (C, aromatic), 128.49, 128.41, 128.14, 128.04, 127.79 (CH, aromatic), 107.46 (C-1′), 105.94 (C-1), 87.32 (C-2), 86.94 (C-4′), 83.60 (C-3), 80.38 (C-4), 78.88 (C-2′), 77.77 (C-3′), 72.04, 71.90 (CH_2–aromatic), 67.67 (OCH_2), 65.89 (C-5), 61.70 (C-5′), 31.82, 29.43, 29.36, 29.26, 26.11, 22.65 (6×CH_2), 14.11 (CH_3).

Octyl 5-*O*-(α-D-arabinofuranosyl)-2,3-di-*O*-methyl-α-D-arabinofuranoside (11). From disaccharide **9** (190 mg, 0.26 mmol) by the method reported for the preparation of **10**, compound **11** was obtained as a syrup (98 mg, 90%) after column chromatography ($CHCl_3$/MeOH, 95:5). FAB-MS (LiCl) *m/e* 429 $[M+Li]^+$. $C_{20}H_{38}O_9$. 0.2 H_2O (found: C, 56.32; H, 8.99, requires C, 56.38; H, 9.08). ^1H NMR (300 MHz, $CDCl_3$): δ 5.07 (1H, s, H-1′), 5.01 (1H, s, H-1), 4.19 (1H, ddd, $J=2.2$, 4.3, 4.4 Hz, H-4′), 4.11 (1H, ddd, $J=3.6$, 4.9, 5.8 Hz, H-4), 4.07 (1H, m, H-2′), 4.00 (1H, m, H-3′), 3.90 (1H, dd, $J=5.1$, 11.8 Hz, H-$5'_a$), 3.89 (1H, dd, $J=3.0$, 10.4 Hz, H-5_a), 3.81 (1H, dd, $J=2.1$, 11.8 Hz, H-$5'_b$), 3.73 (1H, dd, $J=3.6$, 10.4 Hz, H-5_b), 3.71 (1H, d, $J=1.3$ Hz, H-2), 3.67 (1H, m, OCH_2), 3.54 (1H, dd, $J=1.5$, 5.8 Hz, H-3), 3.41 (2H, m, OCH_2, 2′-OH, D_2O exchangeable), 3.40 (6H, s, 2×OCH_3), 1.63 (1H, s, 3′-OH, D_2O exchangeable), 1.57 (2H, m, CH_2), 1.29 (10H, m, CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 107.80 (C-1′), 105.48 (C-1), 89.37 (C-2), 87.11 (C-4′), 86.43 (C-3), 80.55 (C-4), 78.94 (C-3′), 77.81 (C-2′), 67.71 (OCH_2), 66.55 (C-5), 61.84 (C-5′), 57.99, 57.52 (2×OCH_3), 31.77, 29.37, 29.30, 29.20, 26.02, 22.61 (6×CH_2), 14.06 (CH_3).

Octyl 5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside (12). To a methanol solution (5 mL) of dis-

accharide **10** (107 mg, 0.87 mmol) was added Pd/C (10%, 50 mg), and the mixture was stirred at room temperature under hydrogen (30 mL, 24 h). Filtration through a Celite pad and concentration gave viscous, colorless oil. Flash chromatography ($CHCl_3$/MeOH 5:1) gave compound **12** as a colorless syrup (74 mg, 99%). FAB-MS (LiCl) *m/e* 401 $[M+Li]^+$. $C_{18}H_{34}O_9 \cdot 0.5\ H_2O$ (found: C, 53.56; H, 8.72, requires C, 53.58; H, 8.74). ^1H NMR (300 MHz, D_2O, acetone as reference): δ 4.96 (1H, d, $J=1.2$ Hz, H-1), 4.86 (1H, d, $J=1.5$ Hz, H-1′), 4.02 (1H, dd, $J=1.4$, 3.2 Hz, H-2), 3.94 (4H, m, H-4, H-2′, H-3′, H-4′), 3.85 (1H, dd, $J=3.1$, 5.9 Hz, H-3), 3.79 (1H, dd, $J=4.7$, 11.3 Hz, H-$5'_a$), 3.72 (1H, dd, $J=3.4$, 12.3 Hz, H-5_a), 3.66–3.58 (3H, m, H-5_b, H-$5'_b$, OCH_2), 3.38 (1H, m, OCH_2), 1.49 (2H, m, CH_2), 1.20 (10H, m, CH_2), 0.79 (3H, m, CH_3). ^{13}C NMR (75 MHz, D_2O, acetone as reference): δ 107.79 (C-1′), 107.64 (C-1), 84.27 (C-4′), 82.04 (C-4), 81.5 (C-2), 81.25 (C-2′), 76.94 (C-3), 76.89 (C-3′), 68.59 (OCH_2), 66.72 (C-5′), 61.46 (C-5), 31.86, 29.37, 29.34, 29.26, 25.95, 22.65 ($6\times CH_2$), 13.96 (CH_3).

Arabinosyltransferase assay

Compounds **10**–**12**, at a range of concentrations from 0.25 to 6.0 mM (which were stored as 100 mM ethanol stocks) and DP[^{14}C]A [20,000 cpm, 9 mM, 10 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. The dried constituents of the assay were then resuspended in 8 μL of a 1% aqueous solution of Igepal. 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, membranes (250 μg) 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 Whatman 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 *n*-butanol saturated water, the pooled extracts were back-washed twice with water saturated with *n*-butanol (3 mL). The *n*-butanol-saturated water 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, 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). Another 10% of the labeled material was subjected to thin-layer chromatography (TLC) 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 TLC's to X-ray film (Kodak X-Omat) for 3 days. Competition based experiments were performed by mixing compounds together at various concentrations (**12**, 0.4 mM with **10** or **11** at 0.5, 1.0, 2.0, 4.0 and 6.0 mM) followed by thin-layer chromatography/autoradiography as described earlier to determine the extent of product formation.

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References and Notes

1. (a) World Health Organization. In *Anti-tuberculosis Drug Resistance in the World. The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance*. World Health Organization (Publication # WHO/TB/97.229), Geneva, Switzerland 1997. (b) Cohn, D. L.; Bustreo, F.; Raviglione, M. C. *Clin. Infect. Dis.* **1997**, *24*, S121. (c) Douglas, J. G.; McLeod, M.-J. *Clin. Pharmacokinet.* **1999**, *37*, 127. (d) Basso, L. A.; Blanchard, J. S. *Adv. Exp. Med. Biol.* **1998**, *456*, 115. (e) Bastian, I.; Colebunders, R. *Drugs* **1999**, *58*, 633. (f) Bradford, W. Z.; Daley, C. L. *Infect. Dis. Clin. North Am.* **1998**, *12*, 157. (g) Rattan, A.; Kalia, A.; Ahmad, N. *Emerging Infect. Dis.* **1998**, *4*, 195. (h) Butler, D. *Nature* **2000**, *406*, 670.
2. (a) Bodiang, C. K. *Scot. Med. J.* **2000**, *45*, 25. (b) Van Scoy, R. E.; Wilkowske, C. J. *Mayo. Clin. Proc.* **1999**, *74*, 1038. (c) Sung, S.-W.; Kang, C. H.; Kim, Y. T.; Han, S. K.; Shim, Y.-S.; Kim, J. H. *Eur. J. Cardio-Thoracic Surg.* **1999**, *16*, 187.
3. (a) Ravilione, M. C. *Scot. Med. J.* **2000**, *45*, 52. (b) Long, R. *CMAJ.* **2000**, *163*, 425. (c) Walsh, C. *Nature* **2000**, *406*, 775.
4. (a) Smith, M. B.; Boyars, M. C.; Veasey, S.; Woods, G. L. *Arch. Pathol. Lab. Med.* **2000**, *124*, 1267. (b) Pozniak, A.; Int, J. *Tuberc. Lung Dis.* **2000**, *4*, 993. (c) Maddry, J. A.; Suling, W. J.; Reynolds, R. C. *Res. Microbiol.* **1996**, *147*, 106.
5. NIAID web site: http://www.niaid.nih.gov/factsheets/tb.htmhttp://www.niaid.nih.gov/factsheets/tbrsch.htm and http://www.who.int/gtb/publications/factsheet/index.htm.
6. (a) McNeil, M.; Wallner, S. J.; Hunter, S. W.; Brennan, P. J. *Carbohydr. Res.* **1987**, *166*, 299. (b) Brennen, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29. (c) Lee, R. E.; Brennan, P. J.; Besra, G. S. *Curr. Top. Microbiol. Immunol.* **1996**, *215*, 1. (d) Brennan, P. J.; Besra, G. S. *Biochem. Soc. Trans.* **1997**, *25*, 188. (e) Mikusova, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890.
7. (a) Chatterjee, D. *Curr. Opin. Chem. Biol.* **1997**, *1*, 579. (b) Daffe, M.; Draper, P. *Adv. Microb. Physiol.* **1998**, *39*, 131.
8. (a) Wolucka, B. A.; McNeil, M. R.; deHoffman, E.; Chojnacki, T.; Brennan, P. J. *J. Biol. Chem.* **1994**, *269*, 23328. (b) Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 11829. (c) Scherman, M. S.; Kalbe-Bournonville, L.; Bush, D.; Xin, Y.; Deng, L.; McNeil, M. *J. Biol. Chem.* **1996**, *271*, 29652.

9. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121.
10. (a) Ayers, J. D.; Lowary, T. L.; Morehouse, C. B.; Besra, G. S. *Biorg. Med. Chem. Lett.* **1998**, *8*, 437. (b) D'Souza, F. W.; Cheshev, P. E.; Ayers, J. D.; Lowary, T. L. *J. Org. Chem.* **1998**, *63*, 9037. (c) D'Souza, F. W.; Lowary, T. L. *Org. Lett.* **2000**, *2*, 1493. (d) D'Souza, F. D.; Ayers, J. D.; McCarren, P. R.; Lowary, T. L. *J. Am. Chem. Soc.* **2000**, *122*, 1251.
11. Sanchez, S.; Bamhaoud, T.; Prandi, J. *Tetrahedron Lett.* **2000**, *41*, 7447.
12. Gordon, M. T.; Lowary, T. L.; Hadad, C. M. *J. Org. Chem.* **2000**, *65*, 4954.
13. (a) Pathak, A. K.; El-Kattan, Y. A.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998**, *39*, 1497. (b) Reynolds, R. C.; Bansal, N.; Rose, J.; Friedrich, J.; Suling, W. J.; Maddry, J. A. *Carbohydr. Res.* **1999**, *317*, 164. (c) Pathak, A. K.; Besra, G. S.; Crick, D.; Maddry, J. A.; Morehouse, C. B.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **1999**, *7*, 2407. (d) Pathak, A. K.; Pathak, V.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **2001**, *42*, 979.
14. (a) Guthrie, R. D.; Smith, S. C. *Chem. Ind. (London)* **1968**, 547. (b) Kam, B. L.; Barascut, J.-L.; Imbach, J.-L. *Carbohydr. Res.* **1979**, *69*, 135.
15. (a) Preparation of the D-sugar: Pathak, A. K.; Pathak, V.; Khare, N. K.; Maddry, J. A.; Reynolds, R. C. *Carb. Lett.* **2001**, *4(2)*, in press. (b) For preparation of the L-sugar: Du, Y.; Pan, Q.; Kong, F. *Carbohydr. Res.* **2000**, *323*, 28. (c) Du, Y.; Pan, Q.; Kong, F. *Carbohydr. Res.* **2000**, *329*, 17.
16. Mizutani, K.; Kasai, R.; Nakamura, M.; Tanaka, O.; Matsuura, H. *Carbohydr. Res.* **1989**, *27*, 27.
17. Suling, W. J.; Reynolds, R. C.; Barrow, E. W.; Wilson, L. N.; Piper, J. R.; Barrow, W. W. *J. Antimicrob. Chemother.* **1998**, *42*, 811.