Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 923–928

BIOORGANIC & MEDICINAL CHEMISTRY

Studies on *n*-Octyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranosides for Mycobacterial Glycosyltransferase Activity

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Received 12 September 2001; accepted 24 September 2001

Abstract—The mycobacterial cell wall is a potential target for new drug development. Herein we report the preparation and activity of several *n*-octyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranoside derivatives. A cell-free assay system has been utilized for determination of the ability of disaccharide analogues to act as arabinosyltransferase acceptors using [^{14}C]-DPA as the glycosyl donor. In addition, in vitro inhibitory activity has been determined in a colorimetric broth microdilution assay system against MTB H37Ra and three clinical isolates of *Mycobacterium avium* complex (MAC). One of these disaccharides showed moderate activity against MTB. The biological evaluation of these disaccharides suggests that more hydrophobic analogues with a blocked reducing end showed better activity as compared to a totally deprotected disaccharide that more closely resembles the natural substrates in cell wall biosynthesis.

Introduction

Tuberculosis (TB) has long been a cause of morbidity and mortality worldwide. Over the past two decades, there has been an increased interest in developing new drugs to fight this deadly disease.[1] This resurgence in interest in fighting tuberculosis has resulted from several factors including the growing incidence of multi-drug resistant (MDR) forms of *Mycobacterium tuberculosis* (MTB) as well as the prevalence of tuberculosis in the AIDS community.[2] The development of MDR forms of tuberculosis and the publication of the TB genome has focused attention on alternative potential targets in TB that are unique to the bacterium. In particular, the putative mechanisms of action of the existing drugs isoniazid (INH), ethambutol (EMB) and ethionamide has focused target discovery efforts on new proteins involved in the biogenesis of the mycobacterial cell wall.[3] In that regard, the unique polysaccharides of the mycobacterial cell wall and their attendant preparative enzymes offer targets for the development of new drugs that will be highly active and selective for the tuberculosis bacillus.[4]

M. tuberculosis is primarily an intracellular pathogen which resides within the phagolysosomes of alveolar macrophages. Perhaps the highly intricate features of the mycobacterial cell wall have been developed as an adaptation to the harsh environment and the requirements for existence within the intracellular milieu. The polysaccharide structure of the MTB cell wall has been extensively studied.[5] These studies have shown the presence of arabinofuranose, galactofuranose, mannose and rhamnose in specific linkages that make up the critical polysaccharide underpinning for the mycolate fatty acids, all critical components for cell wall infrastructure and integrity.[5,6] The arabinogalactan (AG, Fig. 1) of the mycobacterial cell wall consists of an Ara*f* α(1→5) Gal*f* linkage that anchors the arabinan component to the galactan substructure.

In continuation of our ongoing search for anti-mycobacterial agents targeting biogenesis of the

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0968-0896/02/$ - see front matter
PII: S0968-0896(01)00343-1

mycobacterial cell wall polysaccharides,[7–9] we report herein the synthesis and biological evaluation of some *n*-octyl arabinofuranosyl-α(1→5)galactofuranosyl disaccharides.

Results and Discussion

Synthesis

The glycosyl acceptors octyl 2,3,6-tri-*O*-benzyl-β-D-galactofuranoside (**1**) and octyl 2,3,6-tri-*O*-methyl-β-D-galactofuranoside (**2**) were prepared as described earlier from β-D-galactofuranose pentaacetate.[8] 2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranosyl trichloroacetimidate **3** as a glycosyl donor was prepared as described earlier.[9] All acceptors and donor were purified by flash chromatography and characterized by spectral and elemental analysis. The stereoselective synthesis of disaccharides **4** and **5** (Fig. 2) were carried out by glycosylation in the presence of a Lewis acid. The trichloroacetimidate donor (**3**) and the acceptor were reacted for 2–4 h using the Lewis acid BF_3Et_2O as promoter at 0 °C under an inert atmosphere in dry CH_2Cl_2 at room temperature over powdered 4 Å molecular sieves. After standard workup and column chromatography on silica gel, the pure disaccharides **4** and **5** were obtained in high yields (86 and 79%, respectively). Further, disaccharides **4** and **5** were debenzoylated using 7 N NH_3/MeOH giving excellent yields of disaccharides **6** and **7**, respectively. The totally deblocked disaccharide **8** was obtained by the hydrogenation of disaccharide **6** over Pd/C at room temperature. The NMR, FABMS and, whenever necessary, APT and 2-D NMR experiments were performed to characterize all disaccharides. Based on NMR spectral studies of the disaccharides, the α-interglycosidic linkages were determined by coupling constants between H-1′ and H-2′ and the coupling constants ($J_{1',2'}$) were found to be ~1 Hz as expected for this configuration.[10] Similarly, in the ^{13}C NMR spectra the signals for C-1′ were observed in the range of 105–106 ppm, which also supported the α-linkage.

Biological activity

In vitro assay using whole cells. In vitro assays[11] of disaccharides were done with *Mycobacterium tuberculosis* H37Ra (ATCC 25177) and *M. avium* (NJ 168, NJ 211 and NJ 3404). The results are reported in Table 1. Ethambutol was used as a positive control. The disaccharides **4**, **5**, **7**, and **8** had MIC's >128 μg/mL, the highest concentration tested, whereas the disaccharide **6** had a MIC of 32 μg/mL for MTB H37Ra. Therefore, only modest activity was seen against MTB for the blocked disaccharide **6**. None of the compounds were active against *M. avium*.

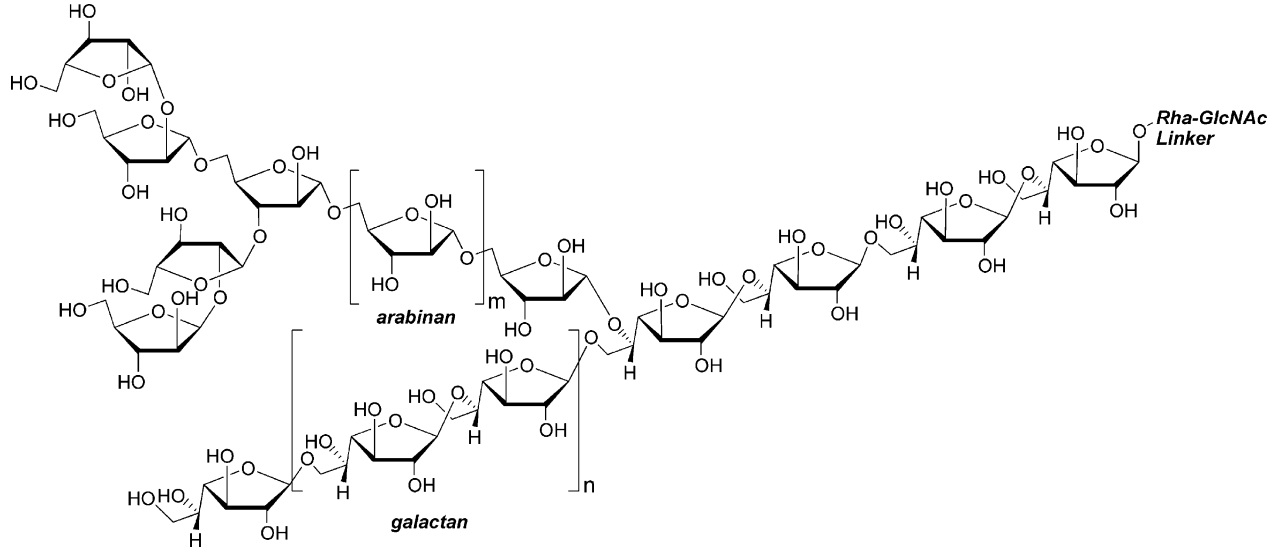

Figure 1. Structure of arabinogalactan of *Mycobacterium tuberculosis*.

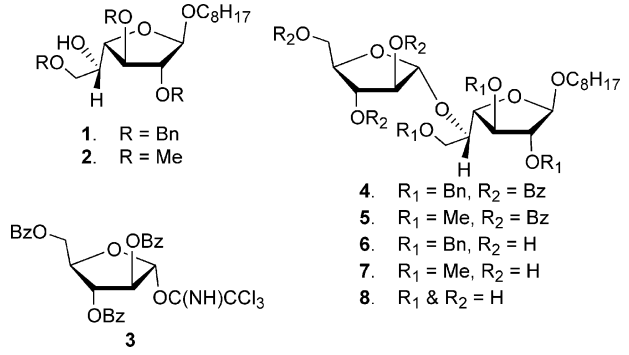

Figure 2. Structure of acceptors, donor sugars and disaccharides.

Table 1. Activity of octyl Ara*f* α(1→5) Gal*f* disaccharides against *Mycobacterium tuberculosis* (MTB) H37Ra and *Mycobacterium avium* complex (MAC)

Compd	MIC (μg/mL)			
	MTB H37Ra	MAC NJ168	MAC NJ211	MAC NJ3404
4	>128	Not done	>128	Not done
5	>128	>128	>128	>128
6	32	>128	>128	>128
7	>128	>128	>128	>128
8	>128	>128	>128	>128
EMB	4	8	4	8

EMB, ethambutol.

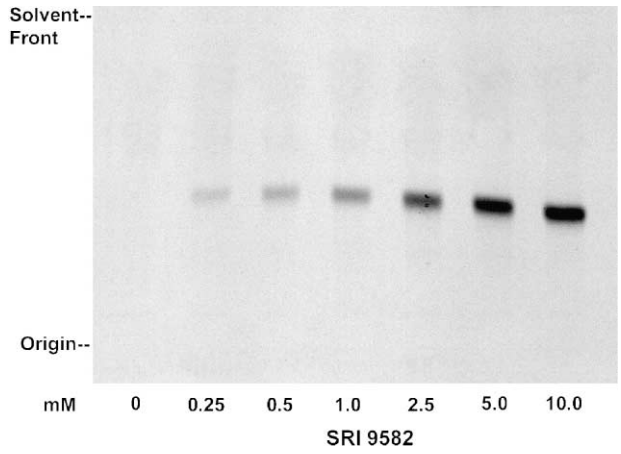

Figure. 3. An autoradiogram of reactions products produced through the inclusion of **SRI-9582**, mycobacterial membranes and [^{14}C]-DPA. Lane 1, no acceptor; lane 2, 0.25 mM; lane 3, 0.5 mM; lane 4, 1.0 mM; lane 5, 2.5 mM; lane 6, 5.0 mM; and lane 7, 10 mM. TLC/autoradiography was performed using 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.

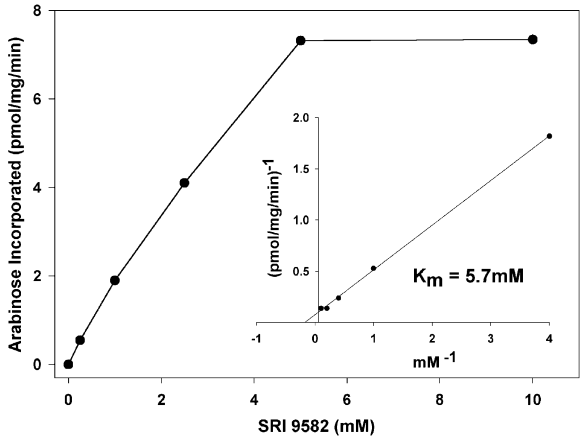

Figure 4. Kinetic analysis of acceptor SRI-9582. The inset illustrates the double reciprocal plot for SRI-9582 as a substrate for the mycobacterial arabinosyltransferase.

Arabinosyl transferase acceptor activity. Based on the previous use of specific arabinose-based neoglycolipid acceptors,[12] compounds **6** (SRI-20249), **7** (SRI-20305) and **8** (SRI-9582) were synthesized and compared as potential acceptors of [^{14}C]Ara*f* from [^{14}C]-DPA within an arabinosyltransferase assay. Assays performed in the presence of membranes resulted in [^{14}C]Ara*f* incorporation from [^{14}C]-DPA for only the fully de-blocked Ara-Gal acceptor **8**, related blocked-compounds **6** and **7** possessed no detectable acceptor activity. TLC/autoradiography (Fig. 3) demonstrated the enzymatic conversion of the Ara-Gal disaccharide **8** to its corresponding trisaccharide product, formed by the attachment of a [^{14}C]Ara*f* unit to the 5′-OH of disaccharide **8** as reported previously for the mycobacterial rabinosyltransferase assay.[12] The compounds **6** and **7** were not recognized as substrates for the arabinosyltransferase enzyme(s), presumably due to the presence of the methyl and benzyl ether protecting groups on the reducing sugar. The calculation of kinetic constants (Fig. 4) revealed that disaccharide **8** possessed a K_m value of 5.7 mM and V_{max} of 8.0 pmol/mg/min. Further competition-based experiments established that disaccharides **6** and **7** were effective inhibitors of their native acceptor **8** in the arabinosyltransferase assay resulting in IC_{50} values of 1.16 mM and 3.20 mM for disaccharides **6** and **7**, respectively. Compound **6** was also active against MTB H37Ra in vitro.

Experimental

Synthesis

General procedures. All reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents from Aldrich were used in the reactions without further processing. Whenever necessary, compounds and starting materials were dried by azeotropic removal of water with toluene under reduced pressure. The 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 workup and chromatography were reagent grade from Fisher Scientific. Flash chromatography was carried out on Fisher silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 and 75 MHz, respectively. The ^{1}H NMR spectra at 600 MHz was recorded on a Bruker Advance 600 System. Coupling constants (*J*) are reported in Hz and chemical shifts are in ppm (δ) relative to residual solvent peak or internal standard. Microanalyses were performed on a Perkin Elmer 2400 CHN analyzer, and FABMS data were recorded on a Varian/MAT 311A double-focusing mass spectrometer by adding NBA with or without LiCl.

Octyl-2,3,6-tri-*O*-benzyl-5-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl) - β - D - galactofuranoside (4). The trichloroacetimidate donor **3** (647 mg, 1.07 mmol) and the acceptor **1** (500 mg, 0.89 mmol) were dissolved in dry CH_2Cl_2 (25 mL) and dry powdered 4Å molecular seives were added. The mixture was stirred for 15 min and cooled to −20 °C. The promoter BF_3Et_2O (0.14 mL, 1.07 mmol) was dissolved in 1.0 mL of dry CH_2Cl_2, and this solution was added to reaction mixture dropwise. The reaction mixture was then stirred at room temperature for 30 min and filtered. A cold, saturated $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with chloroform (20 mL). The $CHCl_3$ layer was washed with deionized water followed by a brine solution, dried over Na_2SO_4 and concentrated. The resulting syrup was subjected to column chromatography on silica gel (cyclohexane/EtOAc, 5:1) afforded the pure disaccharide **4** (769 mg, 86%) as a colorless syrup. FABMS (LiCl) *m*/*e* 1013.6 $[M+Li]^+$. $C_{61}H_{66}O_{13}$:1/2 H_2O (found: C, 72.27; H, 6.71. requires C, 72.12; H, 6.70). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07–7.96, 7.57–7.19 (25H, each m, Aromatic), 5.54 (1H, s, H-2′), 5.53 (1H, m, H-3′), 5.46 (1H, s, H-1′), 5.01 (1H, s, H-1), 4.69 (1H, m, H-5_a′), 4.66 (1H, m, H-4′), 4.60–4.37

(5H, m, 2×CH_2–Aromatic, H-5$_b$′), 4.20 (1H, m, H-4), 4.15 (1H, dd, J = 5.8, 11.3 Hz, H-5), 4.02 (1H, m, H-3), 4.00 (1H, d, J = 0.9 Hz, H-2), 3.67 (1H, m, OCH_2), 3.63 (2H, d, J = 5.4 Hz, H_2-6), 3.34 (1H, m, OCH_2), 1.55 (2H, m, CH_2), 1.25 (10H, m, CH_2), 0.87 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.16, 165.77, 165.18 (3×C=O), 138.11, 137.76, 137.60, 133.36, 133.32, 132.92, 129.98, 129.85, 129.73, 129.26,128.42, 128.39, 128.38, 128.28, 128.24, 127.95, 127.92, 127.79, 127.65, 127.48, 127.44 (Aromatic), 105.76 (C-1′), 105.68 (C-1), 88.41 (C-2), 83.26 (C-3), 82.32 (C-2′), 80.62 (C-4, C-4′), 77.84 (C-3′), 75.40 (C-5), 73.20, 71.90 (2CH_2–Aromatic), 70.02 (C-6), 67.57 (OCH_2), 63.60 (C-5′), 31.82, 29.50, 29.34, 29.28, 26.14, 22.66 (6×CH_2), 14.11 (CH_3).

Octyl-2,3,6-tri-*O*-methyl-5-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl) - β - D - galactofuranoside (5). The trichloroacetimidate donor **3** (327 mg, 0.54 mmol) and the acceptor **2** (150 mg, 0.45 mmol) were dissolved in dry CH_2Cl_2 (15 mL), and dry powdered 4 Å molecular sieves were added under Ar atmosphere. The mixture was stirred for 15 min and cooled to 0 °C. The promoter BF_3Et_2O (68 μL, 0.54 mmol) was dissolved in 1.0 mL of dry CH_2Cl_2 and added to the reaction mixture dropwise. The reaction mixture was then stirred at room temperature for 30 min and filtered. A cold, saturated $NaHCO_3$ solution (10 mL) was added, and the mixture was extracted with chloroform (20 mL). The $CHCl_3$ layer was washed with deionized water followed by a brine solution, dried over Na_2SO_4 and concentrated. The resulting syrup was subjected to column chromatography on silica gel (cyclohexane/EtOAc, 6:1) that afforded the pure disaccharide **5** (276 mg, 79%) as a colorless syrup. FABMS (LiCl) m/e 785.5 $[M+Li]^+$. $C_{43}H_{54}O_{13}$:0.5 H_2O (found: C, 65.32; H, 6.82. requires C, 65.55; H, 7.03). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12–7.99, 7.61–7.28 (m, Aromatic), 5.58 (1H, s, H-1′, H-2′, H-3′), 4.96 (1H, s, H-1), 4.81 (1H, dd, J = 3.0, 11.2 Hz, H-5$_a$′), 4.73 (1H, m, H-4′), 4.67 (1H, dd, J = 4.7, 11.2 Hz, H-5$_b$′), 4.08 (1H, m, H-4, H-5), 3.72 (1H, m, H-2, H-3), 3.66 (1H, m, OCH_2), 3.57 (2H, m, H_2-6), 3.41, 3.39, 3.32 (3×OCH_3), 3.36 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.25 (10H, m, CH_2), 0.86 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.22, 165.84, 165.26 (3×C=O), 133.62, 133.39, 133.34, 129.96, 129.83, 129.77, 129.24, 128.43, 128.39, 128.39, 128.25 (aromatic), 105.89 (C-1′), 105.03 (C-1), 89.98 (C-2), 85.36 (C-3), 82.24 (C-2′), 80.88 (C-4), 80.74 (C-4′), 77.84 (C-3′), 75.33 (C-5), 72.37 (C-6), 67.50 (OCH_2), 63.83 (C-5′), 58.92, 57.83, 57.38 (3OCH_3), 31.80, 29.44, 29.35, 29.24, 26.07, 22.63 (6CH_2), 14.08 (CH_3).

Octyl-2,3,6-tri-*O*-benzyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranoside (6). To a solution of disaccharide **4** (437 mg, 0.43 mmol) in dry methanol (15 mL) was added 7 N NH_3/MeOH (8 mL) dropwise, and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was concentrated in vacuo to give a syrup that was subjected to flash column chromatography on silica gel G ($CHCl_3$/MeOH 95:5), giving **6** as a colorless syrup (264 mg, 88%). FABMS (LiCl) m/e 701.6 $[M+Li]^+$. $C_{40}H_{54}O_{10}$:0.5 H_2O (found: C, 68.27; H, 7.78 requires C, 68.25; H, 7.73). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.19 (m, Aromatic), 4.59 (1H, s, H-1′), 4.55 (1H, s, H-1), 4.59–4.32 (4H, m, 2×CH_2–romatic), 4.17 (1H, ddd, J = 2.0, 2.1, 3.4 Hz, H-4′), 4.12 (1H, m, H-5), 4.02 (1H, m, H-4), 4.01 (1H, d, J = 0.2 Hz, H-2′), 3.96 (1H, dd, J = 1.0, 3.0 Hz, H-2), 3.94 (1H, t, J = 0.9 Hz, H-3′), 3.82 (1H, dd, J = 2.4, 11.6 Hz, H-5$_a$′), 3.77 (1H, m, H-3), 3.73 (1H, dd, J = 2.1, 11.6 Hz, H-5$_b$′), 3.65 (1H, m, OCH_2), 3.46 (1H, dd, J = 8.1, 10.2 Hz, H-6$_a$), 3.41 (1H, dd, J = 3.9, 10.2 Hz, H-6$_b$), 3.35 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.27 (10H, m, CH_2), 0.88 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 137.34, 137.29, 137.19, 128.42, 128.34, 128.32, 128.03, 128.01, 127.96, 127.87, 127.81, 127.77 (aromatic), 107.01 (C-1′), 105.61 (C-1), 87.89 (C-2), 87.32 (C-4′), 83.92 (C-3), 80.83 (C-4), 78.59 (C-2′), 78.05 (C-3′), 73.21 (CH_2–aromatic), 73.06 (C-5), 72.03, 71.90 (2×CH_2–aromatic), 69.15 (C-6), 67.58 (OCH_2), 61.70 (C-5′), 31.77, 29.40, 29.31, 29.21, 26.08, 22.61 (6×CH_2), 14.06 (CH_3).

Octyl-2,3,6-tri-*O*-methyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranoside (7). The disaccharide **5** (100 mg, 0.13 mmol) was stirred with 10 mL of 7 N NH_3/MeOH at room temperature overnight. The reaction mixture was concentrated in vacuo to give a syrup that was subjected to flash column chromatography on silica gel G ($CHCl_3$/MeOH 7:1) to give **7** as a colorless syrup (50 mg, 83%). FABMS (LiCl) m/e 473.6 $[M+Li]^+$. $C_{22}H_{42}O_{10}$:1/3 H_2O (found: C, 55.93; H, 8.81. requires C, 55.99; H, 9.09). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.82 (1H, s, H-1′), 4.74 (1H, s, H-1), 4.59 (1H, m, 2′-OH), 4.23 (1H, d, J = 1.6 Hz, H-4′), 4.13 (1H, m, H-5), 4.04 (4H, m, H-2′, H-3′, 3′-OH, 5′-OH), 3.96 (1H, t, J = 6.6 Hz, H-4), 3.86 (1H, dd, J = 1.8, 11.4 Hz, H-5$_a$′), 3.78 (1H, dd, J = 0.9, 11.4 Hz, H-5$_b$′), 3.68 (1H, dd, J = 0.7, 2.3 Hz, H-2), 3.65 (1H, m, OCH_2), 3.52 (1H, dd, J = 2.2, 6.8 Hz, H-3), 3.40 (3H, m, H_2-6, OCH_2), 3.39 (6H, s, OCH_3), 3.36 (3H, s, OCH_3), 1.55 (2H, m, CH_2), 1.27 (10H, m, CH_2), 0.88 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 106.95 (C-1′), 105.20 (C-1), 89.41 (C-2), 87.39 (C-4′), 85.98 (C-3), 81.09 (C-4), 78.76 (C-2′), 77.98 (C-3′), 72.52 (C-5), 71.77 (C-6), 67.47 (OCH_2), 61.61 (C-5′), 31.67, 29.29, 29.21, 29.10, 25.95, 22.51 (6×CH_2), 13.97 (CH_3).

Octyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranoside (8). To a methanol solution (5 mL) of disaccharide **6** (100 mg, 0.14 mmol) was added Pd/C (10%, 25 mg), and the mixture was stirred at room temperature under hydrogen (30 mL, 24 h). Filtration through a Celite pad and concentration gave a viscous, colorless oil. Flash column chromatography on silica gel ($CHCl_3$/MeOH 3:1) afforded the pure disaccharide **8** (54 mg, 90%) as a colorless syrup. FABMS (LiCl) m/e 431.3 $[M+Li]^+$. $C_{19}H_{36}O_{10}$:H_2O (found: C, 51.84; H, 8.45. requires C, 51.58; H, 8.59). ^{1}H NMR (600 MHz, D_2O) δ 5.33 (1H, s, H-1′), 5.09 (1H, s, H-1), 4.30 (1H, dd, J = 1.8, 3.6 Hz, H-2′), 4.26 (1H, ddd, J = 3.6, 5.4, 6.0 Hz, H-4′), 4.18 (3H, br m, H-2, H-3, H-4), 4.08 (1H, dd, J = 3.6, 6.0 Hz, H-3′), 4.03 (1H, m, H-5), 3.95 (1H, dd, J = 3.6, 12.1 Hz, H-5$_a$′), 3.91 (1H, dd, J = 6.6, 12.0 Hz, H-6a), 3.87 (1H, dd, J = 4.8, 12.0 Hz, H-6b), 3.85 (1H, m, OCH_2), 3.84 (1H, dd, J = 6.0, 12.1 Hz, H-5$_b$′), 3.64 (1H, m, OCH_2), 1.73 (2H, m, CH_2), 1.43 (10H, m, CH_2), 1.01 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 108.73 (C-1′), 107.29 (C-1),

84.15 (C-4′), 82.18, 81.54, 81.42 (C-2, C-4, C-2′), 78.23 (C-3), 76.80 (C-3′), 76.69 (C-5), 68.71 (OCH_2), 61.85 (C-6), 61.52 (C-5′), 31.56, 29.16, 28.97, 28.90, 25.69, 22.41 ($6\times CH_2$), 13.76 (CH_3).

Arabinosyltransferase assay

Bacterial strains and growth conditions. *M. smegmatis* mc^2155 was a generous gift from W. R. Jacobs, Albert Einstein College of Medicine, Bronx, New York.[13] 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.

Preparation of membrane and cell wall enzyme fractions. *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 60 min at 4 °C and the resulting mycobacterial cell wall pellets were re-suspended in buffer A. Percoll (Pharmacia, Sweden) was added to yield a 60% suspension and centrifuged at 27,000*g* for 1 h at 4 °C. The upper, particulate diffuse and enzymatically active cell wall (P60) band was collected and washed three times with buffer A and re-suspended in buffer A at a final protein concentration of 10 mg/mL. 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 re-suspended in buffer A at a protein concentration of 20 mg/mL. Protein concentrations were determined using the BCA Protein Assay Reagent kit (Pierce Europe, Oud-Beijerland, The Netherlands).

Transferase assay. Compounds **6**, **7**, and **8** at a range of concentrations from 0.25 to 10.0 mM (prepared from stored 100 mM ethanol stocks) and [^{14}C]-DPA (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 and 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 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 *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, 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). Another 10% 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 aluminium 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. Competition based experiments were performed by mixing compounds together at various concentrations (**8**, 0.4 mM with **7** or **6** at 1.0, 2.0 and 3.6 mM) followed by thin-layer chromatography/autoradiography as described earlier to determine the extent of product formation.

Acknowledgements

The authors are thankful to Dr. J. M. Riordan for NMR and Mr. M. D. Richardson for FABMS. The 600 MHz NMR work was supported by NCI grant CA-13148 (the University of Alabama at Birmingham UAB shared NMR Core Facility). This work was supported by NIH/NIAID grant R01AI45317. G.S.B., who is currently a Lister Institute-Jenner Research Fellow, acknowledges support from The Medical Research Council (49343 and 49342). The *M. avium* strains were kindly provided by Dr. Leonid Heifets, National Jewish Center for Immunology and Respiratory Diseases, Denver, CO, USA.

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