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Studies on β-D-Gal$_f$-(1→4)-α-L-Rha$_p$ Octyl Analogues as Substrates for Mycobacterial Galactosyl Transferase Activity

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Abstract—The biochemically unique structures of sugar residues in the outer cell wall of *Mycobacterium tuberculosis* (MTB) make the pathways for their biosynthesis and utilization attractive targets for the development of new and selective anti-tubercular agents. A cell-free assay system for galactosyltransferase activity using UDP[^{14}C]Gal as the glycosyl donor, as well as an in vitro colorimetric broth micro-dilution assay system, were used to determine the activities of three β-D-gal$_f$(1→4)-α-L-rham$_p$ octyl disaccharides as substrates and antimycobacterial agents respectively. The cell-free enzymatic studies using compounds **8** and **10** suggested that these disaccharides bind to and are effective substrates for a putative mycobacterial galactosyltransferase. The modified acceptor **8** was found to be a slower but prolonged binder as compared to the less substituted analogue **10** as evidenced by their K_m and V_{max} values. Moderate antimycobacterial activity was observed with compounds **8** and **9** against MTB H37Ra and three clinical isolates of *Mycobacterium avium* complex (MAC).

Introduction

The specter of a worldwide resurgence of mycobacterial diseases, particularly tuberculosis (TB) and its drug resistant forms,[1–3] has generated an intense effort to characterize the molecular genetics[4,5] and attendant biochemical pathways in these bacteria with the goal of identifying novel targets for the development of new and selective agents.[6] In particular, recent advances in the characterization of the mycobacterial cell wall have led to the identification of a vast array of highly unique biochemical targets that could lead to a new generation of potent and very selective anti-tubercular agents. The biochemically unique structures of sugar residues in the outer cell wall of the TB bacillus make the pathways for their biosynthesis and utilization attractive targets for the development of such agents. The cell wall itself is an established target for drugs against TB as is evidenced by the presently accepted mechanisms of action of the clinically effective agents isoniazid (INH), ethambutol (EMB), and ethionamide (ETH).[7–10] Furthermore, the waxy exterior of the mycobacterial cell wall acts as a protective barrier against both host cellular immune response and synthetic antibacterial agents. Thus, new drugs acting against selective targets in the cell wall should, in combination, enhance activity of other established antimycobacterial agents and possibly other standard antibacterials. In this report, we focused specifically on inhibitors of the biosynthesis and utilization of the galactan portion of the arabinogalactan and its critical underpinning disaccharide, L-rha$_p$-(α1→3) -D-glcNAc (Fig. 1).

Rationally designed agents based on the mechanism of synthesis and utilization of gal$_f$- and/or rha$_p$-containing polysaccharides, structures that are not present in mammalian cells, have the potential to overcome the lack of specificity and resistance problems encountered with currently used agents such as EMB and the mycolylation inhibitor INH.

Herein we report the synthesis and screening of β-D-gal$_f$(1→4)-α-L-rham$_p$ octyl disaccharides as probes and potential inhibitors of mycobacterial galactan synthetic enzymes, the galactosyltransferases. The *n*-octyl group has been shown to be suitable for studies of mycobacterial

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arabinosyltransferases and other glycosyltransferases.[11–17] The enzymatic studies for galactosyltransferase activity were carried out using a cell-free assay system with UDP[^{14}C]Gal as the glycosyl donor. Compounds were also evaluated as inhibitors of whole bacteria in vitro against *Mycobacterium tuberculosis* MTB H37Ra and *Mycobacterium avium* strains MAC NJ168, NJ211, NJ3404 using a colorimetric broth micro-dilution assay.

Results and Discussion

Synthesis of disaccharides

Several approaches[18–21] have been attempted, but our successful synthesis is presented in Scheme 1. Commercially available L-rhamnose monohydrate **1** was acetylated quantitatively with Ac_2O/pyridine after coevaporation with toluene. Tetraacetylated rhamnose **2** was obtained as an α:β mixture in a ratio of 5:2. Lewis acid catalyzed acylation of **2** (α/β mixture) using $SnCl_4$ with *n*-octanol gave pure α-isomer **3** in 83% yield.[22] Removal of the acetyl groups with NaOMe in MeOH followed by protecting the 2,3-positions with an isopropylidene group gave compound **5**. Several conditions for coupling of aglycon **5** with penta-*O*-acetyl-β-D-galactofuranoside,[23] or tetra-*O*-acetyl-β-D-galactofuranosyl chloride[24] **6** using various Lewis acids such as $SnCl_4$, TMSOTf, NIS/TMSOTf, NIS/TESOTf failed to produce the desired disaccharide. The low reactivity of the 4-hydroxyl group in rhamnose may be the cause for these failures. Finally, using Koenigs–Knorr coupling conditions, treatment of aglycon **5** with tetra-*O*-acetyl-β-D-galactofuranosyl chloride **6** in the presence of $Hg(CN)_2$ and $HgBr_2$, we

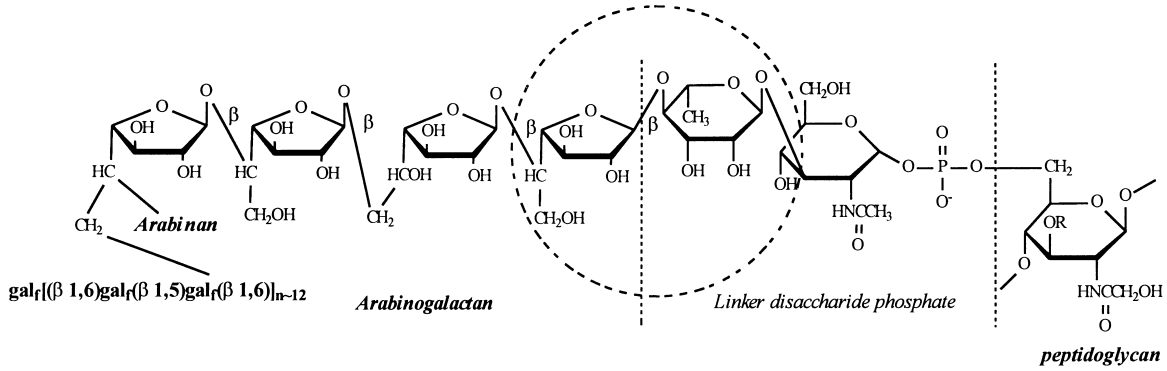
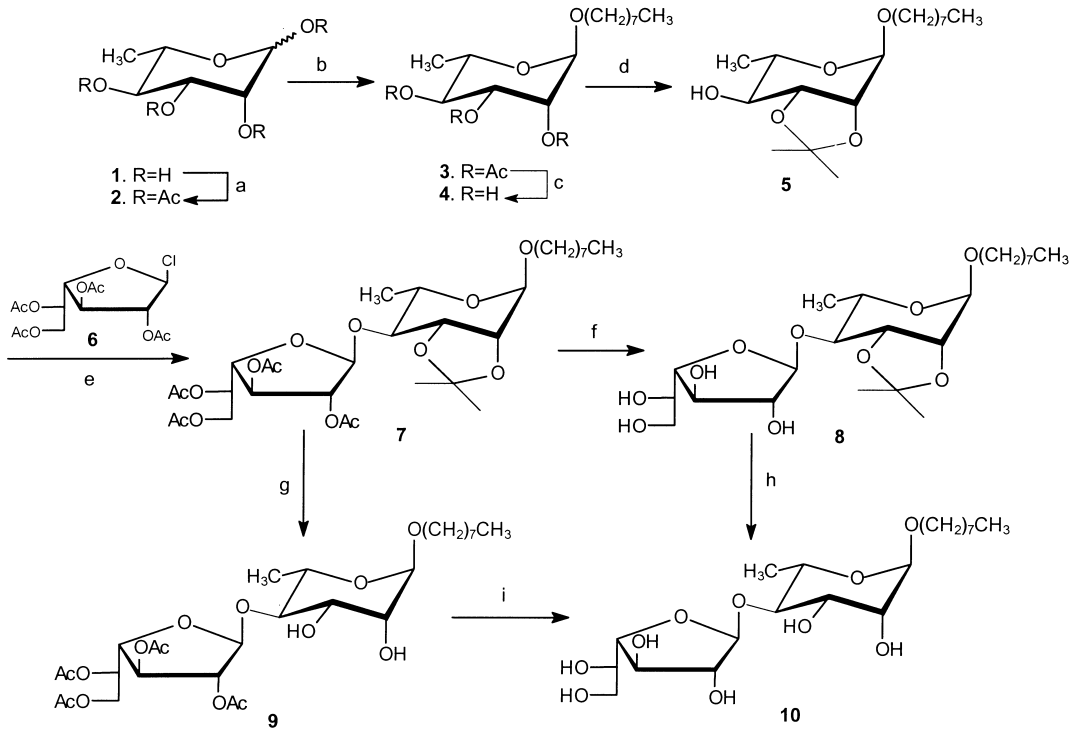


Figure 1. Structure of arabinogalactan, linker disaccharide phosphate and peptidoglycan part of cell wall polysaccharide of *Mycobacerium tuberculosis*.



Scheme 1. Synthesis of disaccharides. Reagents and conditions: (a) Ac_2O, Pyridine, rt, 4 h, 98%; (b) $SnCl_4$, $CH_3(CH_2)_7OH$, CH_3CN, 0°C–rt, 83%; (c) NaOMe, MeOH, rt, 2 h, 99%; (d) 2,2′-dimethoxypropane, (1S)-(+)-10-camphorsulphonic acid, acetone, rt, 2 h, 96%; (e) $Hg(CN)_2$, $HgBr_2$, CH_3CN, 2 h, 79%; (f) NaOMe, MeOH, rt, 1 h, 90%; (g) TFA containing 1% H_2O, $CHCl_3$, rt, 3 h, 91%; (h) TFA containg 1% H_2O, $CHCl_3$ rt, 3 h, 85%; (i) NaOMe, MeOH, rt, 1 h, 99%.

obtained the desired disaccharide **7** in 79% yield. The isopropylidene and acetate groups were removed selectively to obtain disaccharide **8** and **9** using NaOMe/MeOH and trifluoroacetic acid (TFA) containing 1% H_2O in $CHCl_3$, respectively. The totally deprotected *n*-octyl β (1→4) disaccharide **10** was obtained from both compounds **8** and **9** as a colorless solid that was fully characterized by high resolution NMR spectral studies.

Biological studies

Enzymatic studies. The acceptor activity of the synthetic disaccharide derivatives described within this communication were determined by a radioactive assay which quantified the rate of transfer of [^{14}C]Gal from UDP-[^{14}C]Gal to the 5′-OH of the hydrophobic acceptors **8** (SRI-9542) and **10** (SRI-9551). Initial screening demonstrated that both potential acceptors were recognized as substrates for the mycobacterial galactosyltransferase(s), resulting in the formation of the corresponding trisaccharide product, as determined by autoradiography/thin-layer chromatography (Fig. 2). We

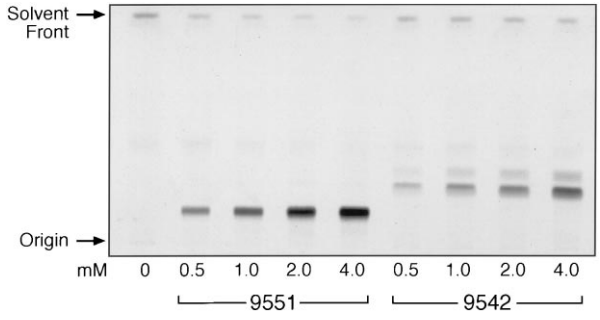


Figure 2. An autoradiogram of reaction products produced through the inclusion of **8** (SRI-9542) and **10** (SRI-9551) at various concentrations, mycobacterial membranes and UDP-[^{14}C]Gal. TLC/autoradiography was performed using chloroform:methanol:ammonium hydroxide:water (65:25:0.5:3.6) and products revealed through exposure to Kodak X-Omat film at −70°C for 48 h.

presume that the primary glycosyltransferase activity will involve a β(1→5)galactosyltransferase since the next expected attachment in the cell wall would be a β(1→5) linkage. In the absence of clear structural proof for the trisaccharide products in Figure 2, however, we cannot definitively state that only a β(1→5)galactosyl transferase was involved. Interestingly, there is evidence of another product forming in the transferase assay which may indicate other glycosyltransferase activity that appears to recognize the more substituted and hydrophobic disaccharide substrate SRI 9542. This other product is not evident in the glycosylation of SRI 9551. These results also indicated that in the case of **8** (SRI-9542) the mycobacterial galactosyltransferase(s) tolerate modifications at the 2- and 3-OH groups of rhamnopyranoside without significant loss of acceptor recognition. Calculation of kinetic constants (Fig. 3) revealed that both **8** (SRI-9542) and **10** (SRI-9551) possessed similar K_m values (**8**, SRI-9542 = 4.0 mM; **10**, SRI-9551 = 4.4 mM), however, the modified acceptor **8** (SRI-9542) possessed a lower $V_{max} = 0.07$ pmol/mg/min in comparison to the native acceptor **10** (SRI-9551) $V_{max} = 0.33$ pmol/mg/min. Further competition based experiments (Fig. 4) established a pattern that was consistent with a non-competitive mode of action for **8** (SRI-9542) yielding a K_i value of 1.7 mM, less than half that of the corresponding acceptor K_m. The above study has clearly allowed an investigation into the active site of the mycobacterial galactosyltransferase(s), and provided for the first time an inhibitor of this enzyme that possesses a K_i significantly lower than the K_m of the corresponding acceptor.

Antimycobacterial activity. The activities of compounds **8**, **9** and **10** against MTB and MAC are presented in Table 1. Compounds **8** and **9** inhibited the growth of both MTB H37Ra and MAC NJ211 with a MIC of $>12.8 \leq 128$ μg/mL. The MIC of **9** against MAC NJ3404, and **10** against MTB and all three MAC strains, was >128 μg/mL.

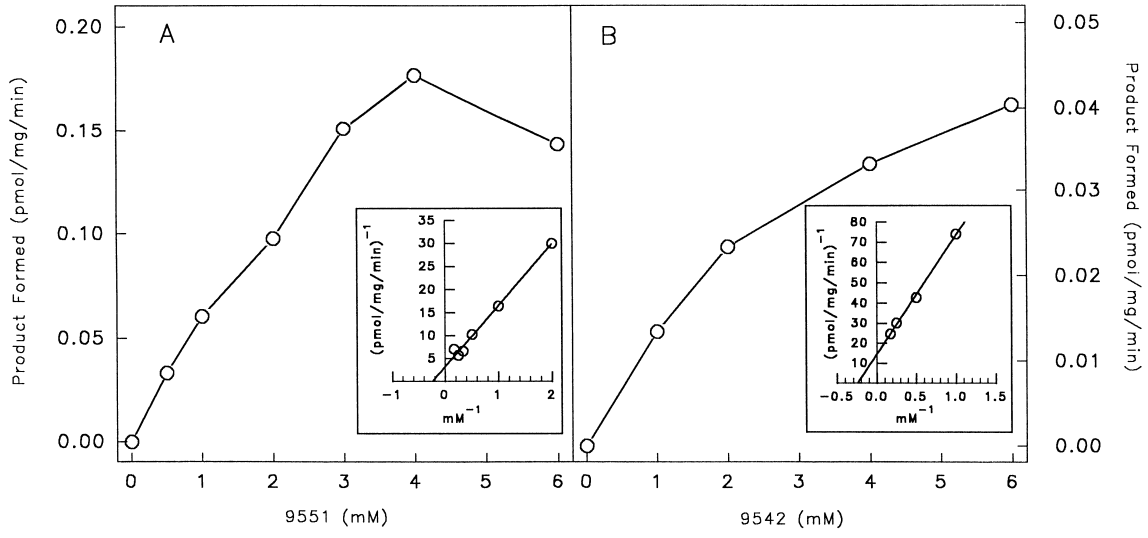


Figure 3. Kinetic analysis of acceptors **8** (SRI-9542) and **10** (SRI-9551). The inset illustrates the double reciprocal plot for both **8** (SRI-9542) and **10** (SRI-9551) as substrates for the β(1→5)galactosyltransferase.

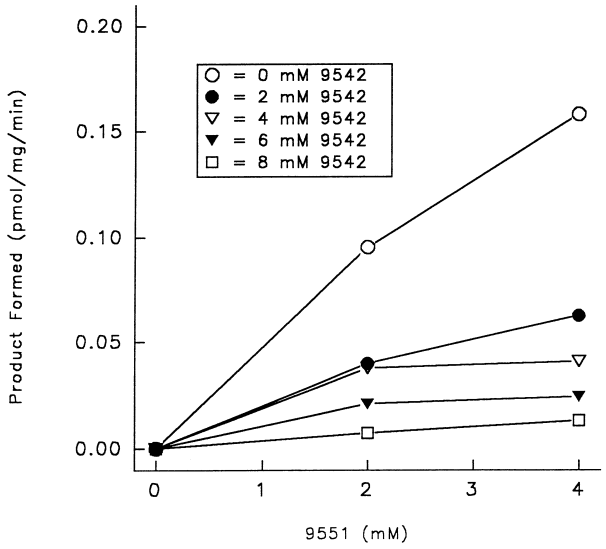


Figure 4. Kinetic analysis of the inhibitory properties of acceptor **8** (SRI-9542).

Table 1. Inhibitor activities of disaccharides against *Mycobacterium tuberculosis* (MTB) and *Mycobacterium avium* (MAC) in vitro

Compd no.	MIC[a] (μg/mL)			
	MTB H37Ra	MAC NJ168	MAC NJ211	MAC NJ3404
8	>12.8≤128	ND[b]	>12.8≤128	>12.8≤128
9	>12.8≤128	ND	>12.8≤128	>128
10	>128	>128	>128	>128

[a] MIC: minimum inhibitory concentration.
[b] ND: not determined.

We concluded based on the acceptor experiments in the cell free-assay system that disaccharides **8** and **10** are acceptors for the mycobacterial glycosyltransferases. Furthermore, the analogue having a more substituted reducing end (2,3-*O*-isopropylidene (**8**)) showed better enzyme binding than the unsubstituted counterpart (2,3-dihydroxyl (**10**)). In vitro assays of **8** and **9** showed that they are moderately active as growth inhibitors of MTB and MAC while **10** was not active at the concentrations screened. Hydrophobicity of the substrate clearly plays a part in access to and binding of the glycosyl processing enzymes.

Experimental

General procedure

All manipulations were conducted under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents from Aldrich were used in the reactions. L-Rhamnose monohydrate and D-(+)-galactose were purchased from Pfanstiehl Labs Inc. and Acros, respectively. When 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, chromatography and recrystallizations 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 point apparatus, and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Nicolet NT 300NB instrument at 300 and 75 MHz, respectively. The ^1H NMR spectra of compound **10** was performed on a Bruker AM600 at 600 MHz. 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. FAB mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer either by adding NBA (3-nitrobenzyl alcohol) or LiCl.

L-Rhamnopyranose pentaacetate (2). L-Rhamnopyranose monohydrate **1** (20.0 g, 0.12 mol) was coevaporated with toluene (3×50 mL) and dried overnight in vacuo over P_2O_5. It was dissolved in dry pyridine (60 mL), acetic anhydride (58 mL, 0.61 mol) was added, and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was poured into an ice–water mixture and extracted with EtOAc (3×100 mL), and the combined organic layer was dried over Na_2SO_4 and concentrated to give pure compound **2** (α:β, 5:2 mixture) as a light-yellow viscous oil (39.82 g, 98%). R_f 0.44 (cyclohexane:EtOAc, 2:1). ^1H NMR (300 MHz, $CDCl_3$; α:β, 5:2 mixture) δ 6.02 (1H, d, J = 1.8 Hz, H-1α), 5.84 (1H, d, J = 1.1 Hz, H-1β), 5.47 (1H, br s, H-2, β-isomer), 5.31 (1H, dd, J = 3.4, 10.0 Hz, H-3, α-isomer), 5.25 (1H, dd, J = 2.0, 3.7 Hz, H-2, α-isomer), 5.11 (3H, m, H-3, H-4, β-isomer; H-4, α-isomer), 3.94 (1H, m, H-5, α-isomer), 3.67 (1H, m, H-5, β-isomer), 2.21, 2.17, 2.16, 2.10, 2.00 (each 3H, s, $OCOCH_3$), 2.07 (6H, s, 2×$OCOCH_3$), 1.29 (3H, d, J = 6.2 Hz, H_3-6, β-isomer), 1.24 (3H, d, J = 6.4 Hz, H_3-6, α-isomer). ^{13}C NMR (75 MHz, $CDCl_3$; α:β, 5:2 mixture): δ 170.0, 169.88, 169.69, 169.63 (4×$OCOCH_3$), 168.29 ($OCOCH_3$ at C-1, β-anomer), 168.19 ($OCOCH_3$ at C-1, α-anomer), 90.44 (C-1, $^1J_{CH}$ = 177.0 Hz, α-anomer), 90.13 (C-1, $^1J_{CH}$ = 163.1 Hz, β-anomer), 71.26, 70.52, 70.26, 70.07, 68.60, 68.52, 68.45, 68.35 (C-2α,β, C-3α,β, C-4α,β, C-5α,β), 20.71, 20.60, 20.57, 20.49, 20.38 (8×$OCOCH_3$), 17.26 (C-6, α-anomer), 17.20 (C-6, β-anomer). FABMS (NBA) m/z 332 $[M]^+$, 273 $[M+H\text{-}HOAc]^+$, FABMS (LiCl) m/z 339 $[M+Li]^+$. (Found: C, 46.85; H, 5.79. $C_{14}H_{20}O_9.1.5H_2O$ requires C, 46.79; H, 5.61).

Octyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (3). To a solution of compound **2** (10.0 g, 30.12 mmol) in dry CH_3CN (120 mL) was added $SnCl_4$ (4.23 mL, 36.14 mmol) at 0°C and the mixture was stirred for 30 min. To the cold solution, *n*-octanol (4.74 mL, 30.12 mmol) was added dropwise over a period of 30 min. The reaction was stirred for an additional 30 min while warming to room temperature. The reaction was quenched with water (50 mL) and extracted with $CHCl_3$ (2×100 mL). The chloroform layer was washed with saturated aqueous $NaHCO_3$ solution (3×20 mL), brine (2×20 mL),

dried over Na_2SO_4, and concentrated in vacuo. The resulting crude oil was purified via flash chromatography (cyclohexane:EtOAc, 6:1) to yield **3** as a colorless oil (10.04 g, 83%). R_f 0.53 (cyclohexane:EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.30 (1H, dd, J = 3.5, 9.9 Hz, H-3), 5.23 (1H, dd, J = 1.8, 3.5 Hz, H-2), 5.06 (1H, t, J = 9.9 Hz, H-4), 4.71 (1H, d, J = 1.5 Hz, H-1), 3.86 (1H, m, H-5), 3.66 (1H, m, OCH_2), 3.41(1H, m, OCH_2), 2.15, 2.05 1.99 (each 3H, s, 3×$OCOCH_3$), 1.57 (2H, m, CH_2), 1.29 (10H, brs, 5×CH_2), 1.22 (3H, d, J = 6.1 Hz, H_3-6), 0.89 (3H, br t, J = 6.1, 7.0 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.90, 169.77, 169.71 (4×$OCOCH_3$), 97.21 (C-1, $^1J_{CH}$ = 170.8 Hz), 71.03 (C-4), 69.82 (C-2), 69.01 (C-3), 68.00 (OCH_2), 65.99 (C-5), 31.61, 29.12, 28.99, 25.86, 22.44 (5×CH_2), 20.69, 20.56, 20.50 (3×$OCOCH_3$), 17.20 (C-6), 13.88 (CH_3). FABMS (NBA) m/z 402 $[M]^+$, 343 $[M+H\text{-}HOAc]^+$, 273 $[M+H\text{-}(CH_3)_7CH_3]^+$, FABMS (LiCl) m/z 409 $[M+Li]^+$. (Found: C, 59.56; H, 8.63. $C_{20}H_{34}O_8$ requires C, 59.68; H, 8.51).

Octyl α-L-rhamnopyranoside (4). To a dry methanolic solution (25 mL) of compound **3** (10.0 g, 24.87 mmol), a NaOMe solution in methanol (25% w/v, 27 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, and concentrated in vacuo to give a syrup which after flash chromatography ($CHCl_3$:MeOH, 7:1) gave **4** as a colorless oil (6.90 g, 99%). R_f 0.34 ($CHCl_3$:MeOH, 7:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.69 (2H, br s, 2×OH, D_2O exchanged), 4.51 (2H, d, J = 1.5 Hz, H-1, 1×OH, D_2O exchanged), 3.56 (1H, dd, J = 1.5, 3.3 Hz, H-2), 3.49 (1H, m, OCH_2), 3.34 (3H, br m, H-3, H-5, OCH_2), 3.15 (1H, t, J = 9.2 Hz, H-4), 1.48 (2H, br d, CH_2), 1.25 (10H, br s, 5×CH_2), 1.12 (3H, d, J = 6.2 Hz, H_3-6), 0.85 (3H, t, J = 7.0 Hz, CH_3). FABMS (NBA) m/z 277 $[M+H]^+$, FABMS (LiCl) m/z 283 $[M+Li]^+$. (Found: C, 60.77; H, 10.19. $C_{14}H_{28}O_5$ requires C, 60.84; H, 10.21).

Octyl 2,3-isopropylidene-α-L-rhamnopyranoside (5). To a solution of compound **4** (6.80 g, 24.6 mmol) in dry acetone (50 mL) was added 2,2′-dimethoxypropane (7.69 mL, 62.5 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (580 mg, 2.5 mmol) at room temperature. After 2 h stirring, Et_3N was added dropwise to attain pH 7.0. The solution was concentrated in vacuo to give a crude oil that after flash chromatography (cyclohexane:EtOAc, 10:1) gave **5** as a colorless oil (7.57 g, 96%). R_f 0.70 ($CHCl_3$:MeOH, 9:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (1H, s, H-1), 4.14 (1H, d, J = 5.7 Hz, H-3), 4.09 (1H, t, J = 6.3 Hz, H-2), 3.69 (2H, m, H-5, OCH_2), 3.42 (2H, m, H-4, OCH_2), 2.33 (1H, d, J = 4.2 Hz, 4-OH, D_2O exchanged), 1.58 (2H, m, CH_2), 1.53 (3H, s, CH_3), 1.31 (16H, m, H_3-6, CH_3, 5×CH_2), 0.88 (3H, t, J = 6.7 Hz, CH_3). FABMS (NBA) m/z 317 $[M+H]^+$, 187 $[M+H\text{-}HO(CH_3)_7CH_3]^+$, FABMS (LiCl) m/z 323 $[M+Li]^+$. (Found: C, 64.28; H, 10.04. $C_{17}H_{32}O_5$ requires C, 64.53; H, 10.19).

Octyl 4-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (7). A mixture of aglycon **5** (1.21 gm, 3.83 mmol), $Hg(CN)_2$ (1.42 g, 5.63 mmol) and $HgBr_2$ (2.03 g, 5.63 mmol) was coevaporated with CH_3CN (3×10 mL) and redissolved in dry CH_3CN (15 mL). Tetra-*O*-acetyl-β-D-galactofuranosyl chloride[24] **6** (1.40 g, 3.83 mmol) in dry CH_3CN (5 mL) was added dropwise, and the reaction mixture was stirred at room temperature. The mixture turned yellow immediately, but the color dissipated in 2 h. Evaporation of solvent gave a residue which was redissolved in $CHCl_3$ (50 mL), extracted with 1 M KBr aqueous solution (2×25 mL) and saturated aqueous solution of $NaHCO_3$ (1×25 mL), dried over Na_2SO_4, and concentrated. Column chromatography of the crude oil (cyclohexane:EtOAc, 2:1) gave compound **7** as colorless oil (1.59 g, 79%). R_f 0.60 (ether:-toluene, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.56 (1H, s, H-1′), 5.38 (1H, m, H-5′), 5.09 (1H, d, J = 1.3 Hz, H-2′), 4.99 (1H, dd, J = 1.5,5.2 Hz, H-3′), 4.95 (1H, s, H-1), 4.31 (1H, m, H-6′), 4.20 (3H, m, H-3, H-4′, H-6′), 4.08 (1H, d, J = 5.5 Hz, H-2), 3.66 (2H, m, H-5, OCH_2), 3.52 (1H, m, H-4), 3.40 (1H, m, OCH_2), 2.12 (6H, s, 2×$OCOCH_3$), 2.08, 2.02 (each 3H, s, $OCOCH_3$), 1.57 (5H, m, CH_2, CH_3), 1.33 (3H, br s, CH_3), 1.27 (10H, m, 5×CH_2), 1.24 (3H, d, J = 6.1 Hz, H_3-6), 0.89 (3H, br t, J = 7.0 Hz, CH_3). FABMS (NBA) m/z 646 $[M]^+$, 517 $[M+H\text{-}HO(CH_3)_7CH_3]^+$, FABMS (LiCl) m/z 653 $[M+Li]^+$.

Octyl 4-*O*-(β-D-galactofuranosyl)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (8). To a dry methanolic solution (20 mL) of disaccharide **7** (500 mg, 0.77 mmol), a NaOMe solution in methanol (25% w/v, 5 mL) was added dropwise. The reaction mixture was allowed to stir at room temperature for 1 h. Dowex H^+ was added to the reaction mixture and the pH was adjusted to neutral. The solution was filtered and concentrated in vacuo to give a syrup which was purified via flash chromatography ($CHCl_3$:MeOH, 7:1) to give **8** as a solid foam (333 mg, 90%). R_f 0.51 ($CHCl_3$:MeOH, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.37 (1H, s, H-1′), 5.02 (1H, d, J = 5.3 Hz, OH, D_2O exchanged), 4.93 (1H, s, H-1), 4.69 (1H, br s, OH, D_2O exchanged), 4.31 (1H, br d, J = 5.5 Hz, OH, D_2O exchanged), 4.21 (1H, t, J = 5.9 Hz, H-3), 4.01 (5H, m, H-2, H-2′, H-3′, H-4′, H-5′), 3.67 (4H, m, H-4, H-5, H-6′, OCH_2), 3.43 (3H, m, H-6′, OCH_2, OH, D_2O exchanged), 1.55 (5H, br s, CH_2, CH_3), 1.35 (3H, br s, CH_3), 1.27 (10H, br s, 5×CH_2), 1.23 (3H, d, J = 5.9 Hz, H_3-6), 0.88 (3H, br t, J = 6.4, 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 109.50 (C), 107.55 (C-1′), 96.74 (C-1), 85.01 (C-4′), 81.04 (C-2′), 78.25, 78.07, 77.94 (C-2, C-3, C-3′), 76.21 (C-4), 70.88 (C-5′), 67.71 (OCH_2), 64.20 (C-6′), 63.97 (C-5), 31.80, 29.34, 29.19 (3×CH_2), 27.93, 6.30 (2×CH_3), 26.09, 22.62 (2×CH_2), 17.74 (C-6), 13.63 (CH_3). FABMS (NBA) m/z 501 $[M+Na]^+$, 349 $[M+H\text{-}HO(CH_2)_7CH_3]^+$, FABMS (LiCl) m/z 485 $[M+Li]^+$. (Found: C, 56.94; H, 8.76. $C_{23}H_{42}O_{10}.1/2H_2O$ requires C, 56.65; H, 8.88).

Octyl 4-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-α-L-rhamnopyranoside (9). To a solution of disaccharide **7** (500 mg, 0.77 mmol) in $CHCl_3$ (10 mL) was added trifluoroacetic acid (TFA) containing 1% H_2O (2 mL), and the reaction mixture was stirred for 3 h. The solution was concentrated in vacuo and coevaporated with toluene (2×10 mL) to remove traces of TFA. Flash chromatography (cyclohexane:EtOAc, 1:1) gave compound **9** as colorless oil (426 mg, 91%). R_f 0.41 ($CHCl_3$:MeOH, 98:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (2H, m, H-1′, H-5′),

5.11 (1H, dd, $J=2.6$, 6.8 Hz, H-3′), 4.96 (1H, d, $J=2.6$ Hz, H-2′), 4.75 (1H, s, H-1), 4.30 (2H, m, H-4′, H-6′), 4.18 (1H, dd, $J=7.0$, 11.6 Hz, H-6′), 3.96 (2H, m, H-2, H-3), 3.66 (2H, m, H-5, OCH_2), 3.52 (1H, t, $J=9.1$ Hz, H-4), 3.41 (1H, m, OCH_2), 3.33 (1H, d, $J=4.4$ Hz, OH, D_2O exchange), 2.35 (1H, d, $J=2.9$ Hz, OH, D_2O exchange), 2.14 (6H, s, $2\times OCOCH_3$), 2.10, 2.05 (each 3H, s, $OCOCH_3$), 1.56 (2H, m, CH_2), 1.28 (13H, br d, $J=5.9$ Hz, H_3-6, $5\times CH_2$), 0.89 (3H, t, $J=6.1$, 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.34, 170.40, 169.99, 169.84 ($4\times OCOCH_3$), 107.24 (C-1′), 99.19 (C-1), 83.50 (C-4), 80.82 (C-4′), 79.32 (C-2′), 75.72 (C-3′), 71.62, 71.39 (C-2, C-3), 68.91 (C-5′), 67.78 (OCH_2), 65.96 (C-5), 62.35 (C-6′), 31.82, 29.44, 29.34, 29.21, 26.11, 22.63 ($6\times CH_2$), 20.84, 20.77, 20.63 ($3\times OCOCH_3$), 17.64 (C-6), 14.07 (CH_3). FABMS (LiCl) m/z 613 $[M+Li]^+$. (Found: C, 54.54; H, 7.51. $C_{28}H_{46}O_{14}.1/2H_2O$ requires C, 54.62; H, 7.58).

Octyl 4-*O*-(β-D-galactofuranosyl)-α-L-rhamnopyranoside (10). From compound **8.** To a solution of disaccharide **8** (250 mg, 0.52 mmol) in $CHCl_3$ (10 mL) was added trifluoroacetic acid (TFA) containing 1% H_2O (1 mL) and reaction mixture was stirred for 2 h. The reaction was concentrated in vacuo, and coevaporated with toluene (2×10 mL) to remove traces of TFA. Flash chromatography ($CHCl_3$:MeOH, 2:1) gave compound **10** as colorless solid (195 mg, 85%).

From compound **9.** To a solution of disaccharide **9** (250 mg, 0.41 mmol) in dry methanol (10 mL) was added methanolic ammonia (2 N, 3 mL) dropwise, and the reaction mixture was allowed to stir at room temperature for 30 min. Dowex H^+ was added to the reaction mixture and the pH was adjusted to neutral. The resulting solution was filtered and concentrated in vacuo to give a syrup, which on flash chromatography ($CHCl_3$:MeOH 2:1) gave **10** as colorless solid (179 mg, 99%). Mp 86–90°C. R_f 0.59 ($CHCl_3$:MeOH, 1:1), ^1H NMR (600 MHz, D_2O) δ 5.40 (1H, d, $J=2.0$ Hz, H-1′), 4.87 (1H, d, $J=1.0$ Hz, H-1), 4.25 (1H, m, $J_{1',2'}=2.0$ Hz, $J_{2',3'}=4.7$ Hz, H-2′), 4.23 (1H, t, $J_{2',3'}=4.7$ Hz, $J_{3',4'}=7.0$ Hz, H-3′), 4.07 (1H, dd, $J_{4',5'}=3.3$ Hz, $J_{3',4'}=7.0$ Hz, H-4′), 4.03 (1H, d, $J_{1,2}=1$Hz, $J_{2,3}=1.5$ Hz, H-2), 4.00–3.97 (2H, m, H-5, H-5′), 3.84–3.76 (4H, m, H-3, H_2-6′, OCH_2), 3.69 (1H, t, $J_{3,4}=J_{4,5}=9.4$ Hz, H-4), 3.57 (1H, m, OCH_2), 1.74 (2H, m, CH_2), 1.48–1.43 (13H, br s, H_3-6, $5\times CH_2$), 1.03 (3H, br d, $J=6.8$ Hz, CH_3). ^{13}C NMR (75 MHz, D_2O) δ 99.89 (C-1′), 96.33 (C-1), 75.03, 72.67, 71.89, 71.75, 70.50, 70.31 (C-2, C-3, C-4, C-2′, C-3′, C-4′), 68.62, 68.43 (C-5, C-5′), 67.47 (C-6′), 60.85 (OCH_2), 31.67, 29.19, 29.13, 29.08, 25.85, 22.40, ($6\times CH_2$), 16.94 (C-6), 13.63 (CH_3). FABMS (LiCl) m/z 445 $[M+Li]^+$. (Found: C, 52.41; H, 8.64. $C_{28}H_{46}O_{14}.H_2O$ requires C, 52.63; H, 8.83).

Enzyme based acceptor studies

Compound **8** (SRI-9542) and compound **10** (SRI-9551) (at a range of concentrations from 0.5 to 6.0 mM) were incubated with 0.5 μCi of UDP-[^{14}C]Gal (Amersham, 257 mCi/mmol) in a buffer containing 50 mM MOPS, pH 7.9, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 62.5 μM ATP and 50 μL of a membrane preparation from *M. smegmatis* (0.5 mg of protein) in a total volume of 160 μL for 1 h at 37°C. A chloroform:methanol (1:1, 1.07 mL) solution was then added to the incubation tubes and the entire contents centrifuged at 14,000 rpm. The supernatant was recovered, dried under a stream of argon, resuspended in ethanol:water (1:1, 1 mL) and loaded onto a 1 mL Whatman strong anion exchange (SAX) cartridge. The cartridge was then washed with ethanol (3 mL). The eluants were combined, dried, and the resulting products partitioned between the two phases arising from *n*-butanol (3 mL) and water (3 mL). The organic phase was recovered and back washed with water saturated with *n*-butanol twice (3 mL each). The *n*-butanol fraction was dried, resuspended in 200 μL of *n*-butanol saturated with water. Fifty microliters of this solution were subjected to scintillation counting, and another 50 μL were applied to an analytical thin-layer chromatogram developed in chloroform: methanol:ammonium hydroxide:water (65:25:0.5:3.6) and subjected to autoradiography. The incorporation of [^{14}C]Gal was determined by subtracting counts present in control assays (incubation of the reaction components in the absence of the acceptors). Competition based experiments were performed by mixing the two acceptors together at various concentrations (0–8 mM) followed by thin-layer chromatography/autoradiography as described earlier to determine the extent of product formation.

In vitro activity against MAC and MTB

Assays were performed using a colorimetric microdilution broth assay which has been described elsewhere.[25] Each compound was assayed in duplicate at 4-log_{10} dilutions consisting of 0.128, 1.28, 12.8 and 128 μg/mL. The minimum inhibitory concentration (MIC) was recorded as the lowest drug concentration that inhibited the growth completely.

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