Synthesis and mycobacterial growth inhibition activities of bivalent and monovalent arabinofuranoside containing alkyl glycosides

Kottari Naresh,[a] Binod Kumar Bharati,[b] Narayanaswamy Jayaraman*[a] and Dipankar Chatterji[b]

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Arabinofuranosides constitute one of the important components of cell wall structures of mycobacteria. With this importance of arabinofuranosides in mind, alkyl glycosides bearing arabinofuranoside trisaccharides were prepared, wherein the sugars were presented either in the monovalent or bivalent forms. Following the synthesis, the monovalent and bivalent alkyl glycosides were tested for their activities in a mycobacterial growth assay. The growth of the mycobacterial strain *M. smegmatis* was assessed in the presence of the alkyl glycosides and it was realized that the alkyl glycosides acted as inhibitors of the mycobacterial growth. The inhibition of the growth, caused by the above alkyl glycosides, was not observed for the arabinofuranose trisaccharide alone, without the alkyl groups, and for an alkyl glycoside bearing maltose as the sugar component.

Introduction

Alkyl glycosides comprising arabinofuranosyl residues have immense significance as they are major components of mycobacterial cell walls. Seminal works of Brennan, Chatterjee and coworkers have established that arabinofuranose residues are present prominently in the arabinogalactan and lipoarabinomannan components of the mycobacterial cell wall structures.[1] For example, it was shown that the lipoarabinomannans from *Mycobacterium smegmatis* are comprised of 40–70 arabinofuranosyl residues, in addition to 11 mannopyranosyl residues, phosphatidyl inositols and lipidic chains. The non-reducing end of the arabinofuranosyl oligomer portion requires the presence of a few mannopyranosyl oligosaccharides that act as caps and the caps are important in order to activate virulence to the mycobacteria. There are no structural homologues of arabinofuranoside containing alkyl glycosides in mammalian systems and, in this context, the mycobacterial originated alkyl glycosides are inherently foreign to the mammalian immune systems.[2] The importance associated with the arabinan structures in mycobacterial cell walls led to the investigations, aimed at, for example, (i) to delineate the functions of arabinofuranosyl transferase enzymes that are responsible for the construction of arabinans;[3] (ii) to construct structural analogues that act as arabinofuranosyl transferase inhibitors that could be useful to prevent the growth of the mycobacterium[4] and (iii) to evolve highly immunogenic lipoarabinomannan oligosaccharides protein conjugates as potential TB vaccines.[5] Studies previously had shown that individual mannose, arabinose and lipid residues are essential as components in order to engage a preferential binding to, for example, pulmonary surfactant proteins present in the alveolar macrophages.[6] The requirement of the lipid component in the alkyl glycosides denotes the essential role of forming alkyl glycoside–protein aggregates necessary for the biological functions.[7] In this respect, the presence of multivalent effects, seen commonly in carbohydrate–protein interactions,[8] are apparent in arabinomannan bearing alkyl glycoside–protein interactions. Multivalent effects provide significantly enhanced binding affinities, often through cross-linking processes.[9] Studies of the multivalent effects in alkyl glycosides containing arabinofuranosyl residues were thus considered appropriate. Accordingly, the synthesis of bivalent and monovalent arabinofuranosyl trisaccharides containing alkyl glycosides was accomplished. In an earlier study, the ability of the synthetic alkyl glycosides was assessed in a mycobacterial growth assay. This report provides details of the synthesis of arabinofuranosyl trisaccharide containing alkyl glycosides and the influence of these ligands on the growth profile of the *M. smegmatis*.

[a]*Department of Organic Chemistry, Indian Institute of Science, Bangalore, 560 012, India. E-mail: jayaraman@orgchem.iisc.ernet.in; Fax: +91-80-2360-0529*
[b]*Molecular Biophysics Unit, Indian Institute of Science, Bangalore, 560 012, India*

Results and discussion

Syntheses of monovalent and bivalent alkyl glycosides incorporated with the arabinofuranose trisaccharide were targeted. Incorporation of linkers between the branch points and the sugar moieties was desired in the case of the bivalent ligand. It was shown previously that through bond distances of 15 Å between the sugars were required for their effective lectin binding, from studies of the binding α-D-mannopyranoside containing bivalent ligands binding to lectin concanavalin A.[10] Further, in order to achieve the hydrophobic–hydrophilic balance in the alkyl glycosides, the presence of double alkyl chains were deemed necessary. Arabinofuranosyl trisaccharide containing glycosides **1** and **2** (Fig. 1), were thus planned. In addition to the alkyl glycosides **1** and **2**, a maltose based alkyl glycoside was also considered to be useful for a comparison purpose in the biochemical studies.

Synthesis

The target alkyl glycoside structures have three distinct structural components, namely, (i) the sugar component; (ii) the ethylene glycol functionalized glycerol moiety and (iii) the long alkyl lyophilic component.

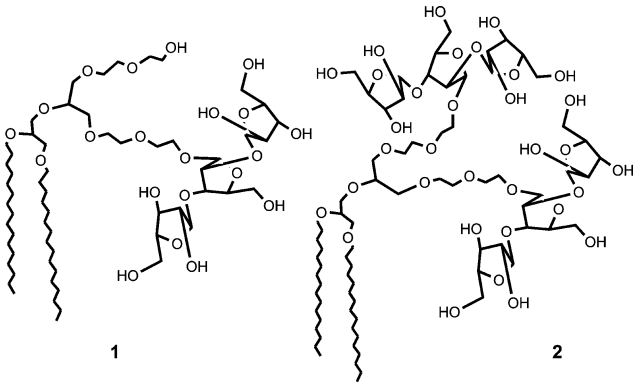
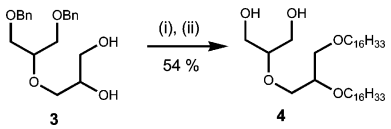
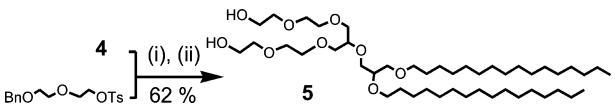


Fig. 1 Molecular structures of monovalent (**1**) and bivalent alkyl glycosides (**2**).

Diethylene glycol and long alkyl group functionalized glycerol. The synthesis of this derivative was planned through *O*-alkylations of long alkyl group functionalized glycerol with an appropriately derivatized diethylene glycol. The long alkyl group functionalized glycerol was planned, in turn, through bis-*O*-alkylation of diol **3**[11] with the long alkyl group moiety. Diol **3** was subjected to *O*-alkylation with 1-bromohexadecane, followed by de-*O*-benzylation (H_2 and Pd–C (10%)), to afford *O*-alkylated derivative **4** (Scheme 1). Derivative **4** was subjected to bis-*O*-alkylation with a protected diethylene glycol tosylate[12] and a further de-*O*-benzylation afforded the diethylene glycol and long chain alkyl group functionalized diol **5** (Scheme 2).



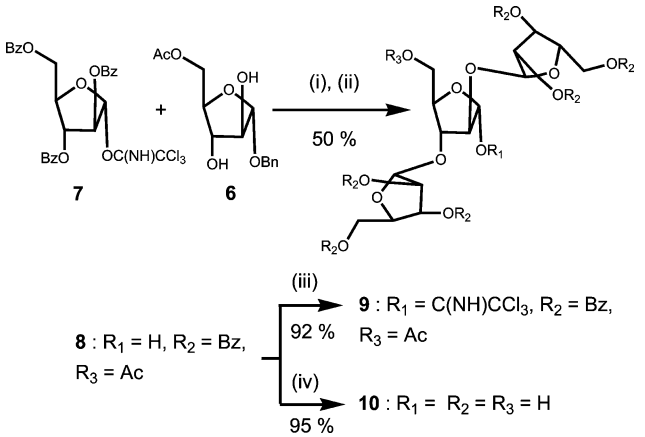
Scheme 1 *Reagents and conditions*: (i) $C_{16}H_{33}Br$, NaH, THF, 36 h, reflux; (ii) H_2, Pd–C, EtOAc–MeOH (1 : 1), rt, 24 h.



Scheme 2 *Reagents and conditions*: (i) NaH, THF, reflux, 36 h; (ii). H_2, Pd–C, EtOAc–MeOH (1 : 1), rt, 18 h.

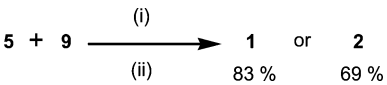
Synthesis of arabinofuranoside trisaccharide. The required glycosyl acceptor benzyl-6-*O*-acetyl-α-D-arabinofuranoside **6** was prepared by the reaction of benzyl-α-D-arabinofuranoside[13] with Ac_2O in the presence of Bu_2SnO. The arabinofuranoside **6** served as the acceptor for a glycosylation with 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl trichloroacetimidate (**7**)[13] and the glycosylation was conducted in the presence of TMSOTf, to afford the protected trisaccharide (Scheme 3). De-*O*-benzylation at the reducing end of the trisaccharide afforded the lactol **8**, which was converted subsequently to an activated trichloroacetimidate glycosyl donor **9**. The complete deprotection of the protecting groups in **8** afforded free hydroxyl group containing trisaccharide **10**.

The preparation of monovalent (**1**) and bivalent (**2**) alkyl arabinofuranosides was performed with the acceptor **5** and controlled molar equivalents of the trisaccharide donor **9**. Thus, the



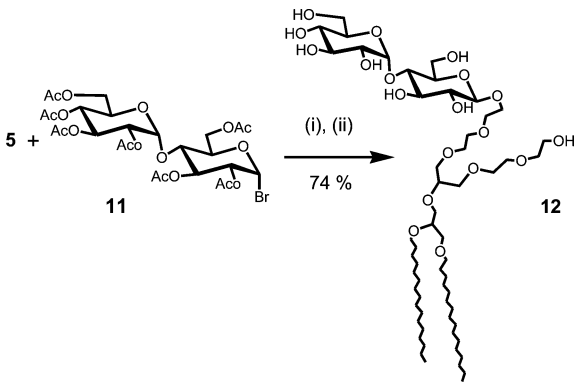
Scheme 3 *Reagents and conditions*: (i). TMSOTf, rt, 2 h, CH_2Cl_2, MS (4 Å); (ii). H_2, Pd–C, EtOAc–MeOH (1 : 1), rt, 24 h; (iii). CCl_3CN, DBU, CH_2Cl_2, −10 °C, 20 min; (iv). NaOMe–MeOH : THF, rt, 24 h.

reaction of one and two molar equivalents of trichloroacetimidate **9** with the acceptor diol **5**, in the presence of TMSOTf, followed by the deprotection led to the formation of monovalent alkyl arabinofuranoside **1** and bivalent alkyl arabinofuranoside **2**, respectively, in good yields (Scheme 4).



Scheme 4 *Reagents and conditions*: (i) TMSOTf, CH_2Cl_2, MS (4 Å), −10 °C, 2 h; (ii). NaOMe–MeOH : THF, rt, 24 h.

Synthesis of the monovalent alkyl glycoside bearing the disaccharide maltose **12** was also accomplished. Accordingly, the glycosylation of diol **5** with the acetobromo maltose **11**,[14] using Ag_2CO_3 as the activator, followed by a deprotection, afforded the monovalent alkyl disaccharide **12** (Scheme 5).



Scheme 5 *Reagents and conditions*: (i). Ag_2CO_3, $AgClO_4$, CH_2Cl_2, rt, 36 h, 78%; (ii). NaoMe–MeOH, rt, 18 h.

The identities and structural homogeneities of the alkyl glycosides **1**, **2**, **10** and **12** were established by NMR spectroscopy and mass spectrometry. In the ES-MS spectrum of **1**, the molecular ion peak was identified as the base peak. For **2**, mass spectral peaks corresponding to the consecutive loss of arabinofuranosyl oxonium ions were observed, in addition to the presence of the molecular ion peak. The molecular structures were further confirmed by ^1H and ^{13}C NMR spectroscopy.

The critical micellar concentrations (CMCs) at which the alkyl arabinofuranosides **1** and **2** undergo transformation into micelles were assessed, using a fluorescence probe 8-anilino-1-naphthalene sulfonic acid (ANSA).[15] The measured CMC of each alkyl glycoside was **1**: 3.7 μM and **2**: 5.2 μM. The increase in the hydrophilic component in **2** was found to increase the CMC value, in comparison to **1**.

Effect of monovalent (1) and bivalent (2) alkyl arabinofuranosides on the growth profile of *M. smegmatis*. The newly synthesized alkyl arabinofuranosides **1** and **2** were studied in a mycobacterial growth assay, in order to identify their effects on the mycobacterial growth pattern. In addition to **1** and **2**, the arabinofuranose trisaccharide **10** and the alkyl glycoside bearing the maltosyl sugar unit **12** were also taken up for the studies. The growth of the wild type *M. smegmatis,* which is a rapidly growing mycobacterium, was examined in Middlebrook broth. The synthetic alkyl glycosides **1**, **2** and **12** and the arabinofuranose trisaccharide **10** were incorporated into the growth medium as a solution in aq. methanol (50%). It was observed that alkyl arabinofuranosides **1** and **2** reduced the growth rate of the mycobacterium significantly. Fig. 2 shows the growth profiles for solutions having differing amounts of the alkyl glycosides **1** and **2**. Arabinofuranose trisaccharide **10** and the alkyl glycoside **12** did not reduce the growth rate of mycobacterium and the growth was comparable to that of the wild type. A maximum retardation of 62% was observed for the monovalent alkyl glycoside **1** in the solution containing 200 μg mL^{-1} of the ligand **1** and this maximum retardation of growth required ~ 60 h. On the other hand, it was observed that the bivalent sugar ligand **2** exhibited a maximum inhibition of 35% in the solution containing 100 μg mL^{-1} of the ligand and the time required for this maximum inhibition was ~ 30 h. In comparison to the inactivity of the arabinofuranosyl trisaccharide **10** and the alkyl glycoside **12** in the mycobacterial growth pattern, the above results showed that **1** and **2** inhibited the growth. Interestingly, the maximum inhibition did not persist throughout the duration of the analysis. Specifically, it was observed that the bivalent ligand **2** showed a reduction in inhibition after ~ 40 h and the mycobacterium continued to grow further after the initial retardation in the exponential phase. The recovery of growth after a retardation in the presence of the alkyl glycoside is unlikely to result from a substrate-like activity of the alkyl glycoside beyond a period in the exponential phase of the growth. The fact that the arabinofuranose trisaccharide **10**, constituting the sugar component of the ligands **1** and **2**, did not lead an effect in the growth rate augment the above inference on the activity profile of the ligands **1** and **2**. A possible reason for the retardation in the mycobacterial growth in the presence of the ligands **1** and **2** could be due to a slow diffusion of the nutrients mediated by porins penetrating the cell wall. An important controlling factor influencing the growth is the thick hydrophobic cell wall. Diffusion of nutrients through the cell wall is promoted by porins, which are channels formed from stable oligomeric protein, localized in the cell wall.[16] Studies previously have shown that the mycobacterial porins are the major determinants of permeability to hydrophilic molecules and nutrients.[17] The incorporation of ligands **1** and **2** in the cell wall, in some form, might thus affect the diffusion of the nutrients through the cell wall. The ligands **1** and **2** may incorporate at different rates, which could depend on the extent of

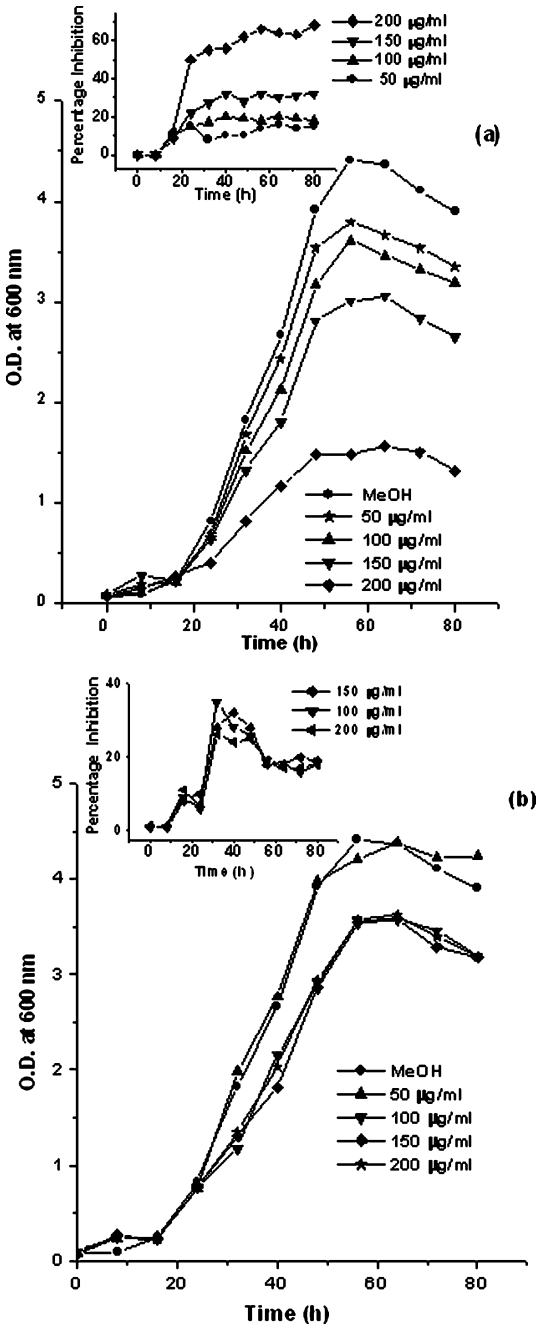


Fig. 2 Effect of (a) monovalent alkyl glycoside **1** and (b) bivalent alkyl glycoside **2** on the growth profile of *M. smegmatis*. Inset in each figure shows the percentage inhibition of the growth of *M. smegmatis* in the presence of alkyl glycosides **1** and **2** relative to the profile for the growth in methanol alone.

the hydrophilicities and hydrophobicities. The more hydrophilic **2** might thus incorporate into the cell wall in a process analogous to faster association and dissociation, thereby leading to the inhibition at an early period of the growth. On the other hand, the relatively more hydrophobic **1** requires higher concentrations and also inhibition of growth occurs at the later period of the growth. Alternatively, the inhibition of growth by ligands **1** and **2** could be due to some other factors. Clearly, it is difficult to ascertain the origin of the inhibitory effect by these ligands at present.

The fact that the arabinofuranose trisaccharide **10** alone and the alkyl glycoside **12** did not have an effect on the growth imply that both the nature of the sugar ligand and the long alkyl groups are essential for the functional nature of the alkyl arabinofuranosides **1** and **2**.

Conclusion

Arabinofuranosyl trisaccharides containing alkyl glycosides, relevant to the class of mycobacterial cell wall components, were designed. A monovalent alkyl glycoside (**1**), with one trisaccharide moiety, and a bivalent alkyl glycoside (**2**), bearing two trisaccharide moieties, were synthesized and their CMCs assessed. The alkyl glycosides were tested in a mycobacterial growth assay and it was found that the alkyl glycosides were able to inhibit the mycobacterial growth to varying degrees. The observations of this report open up a possibility that arabinofuranoside containing alkyl glycosides may form a new class of inhibitors of mycobacterial growth.

Experimental section

General information

Solvents were dried and distilled according to literature procedures. All chemicals were purchased from commercial sources and were used without further purification. Silica gel (100–200 mesh) was used for column chromatography and TLC analysis was performed on commercial plates coated with silica gel 60 F_{254}. Visualization of the spots on TLC plates was achieved by UV radiation or spraying 5% sulfuric acid in ethanol. High-resolution mass spectra were obtained from Q-TOF instrument by electrospray ionization (ESI). ^{1}H and ^{13}C NMR spectral analyses were performed on a spectrometer operating at 300 MHz, 400 MHz, and 75 MHz, 100 MHz, respectively in $CDCl_3$ solutions unless otherwise stated. Chemical shifts are reported with respect to tetramethylsilane (TMS) for ^{1}H NMR and the central line (77.0 ppm) of $CDCl_3$ for ^{13}C NMR. Coupling constants (J) are reported in Hz. Standard abbreviations s, d, t, dd, br s, m refer to singlet, doublet, triplet, doublet of doublet, broad singlet, multiplet.

5-*O*-Hexadecyl-2-hydroxymethyl-3,7-dioxatricosane-1,5-diol (4)

Diol **3**[11] (1.32 g, 3.82 mmol) in THF (5 mL) was added to a suspension of NaH (0.3 g, 7.5 mmol, 60% in mineral oil) in THF (25 mL). After stirring for 30 min. at 60 °C, 1-bromohexadecane (3 mL, 10 mmol) in THF (5 mL) was added slowly, stirred at the same temperature for 36 h, cooled, filtered and filtrate was concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with water (3×20 mL). The organic phase was dried (Na_2SO_4), filtered and the filtrate was concentrated *in vacuo*. A solution of the crude reaction mixture in EtOAc–MeOH (1 : 1) (15 mL) was treated with Pd–C (10%) (0.25 g) and stirred under H_2 (1 bar) for 18 h. Filtration of the reaction mixture, followed by purification (pet. ether–EtOAc 1 : 4) afforded **4**, as a white powder. Yield: 1.29 g (54%); R_f (pet. ether–EtOAc 1 : 4) 0.4; mp 48–50 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, J = 6.6 Hz, 6 H), 1.17–1.22 (br s, 52 H), 1.51–1.56 (m, 4 H), 3.36–3.74 (m, 16 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 20.9, 22.6, 25.1, 26.0, 29.3, 29.5, 31.8, 60.3, 62.1, 62.3, 69.8, 70.1, 71.8, 77.9, 81.4; ES-MS Calcd. for $C_{38}H_{78}O_5Na$ [M + Na]: 637.5747, found 637.5745.

10-*O*-Hexadecyl-8-(7′-hydroxy-2′,5′-dioxaheptyl)-3,6,9,13-tetraoxanonacosane-1,10-diol (5)

Derivative **4** (1.29 g, 2.1 mmol) in THF (5 mL) was added to a suspension of NaH (0.11 g, 60% in mineral oil, 2.75 mmol) in THF (20 mL). After refluxing for 30 min, 1-*O*-benzyl-5-*O*-tosyl-3-oxa-pentane-1,5-diol[12] (1.7 g, 5 mmol) in THF (5 mL) was added slowly, stirred at reflux for 36 h, cooled, filtered and filtrate was concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (50 mL), washed with water (3×30 mL). The organic phase was dried, filtered and the filtrate was concentrated *in vacuo*. A solution of the crude residue in EtOAc–MeOH (1 : 1) (30 mL) was treated with Pd–C (10%) (0.15 g), stirred under H_2 (1 bar) for 18 h. Filtration of the reaction mixture, followed by purification (EtOAc–MeOH, 19 : 1), afforded **5**, as a white solid. Yield: 1.02 g (62%); R_f (EtOAc) 0.3; mp 36–38 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J = 6.6 Hz, 6 H), 1.91–1.22 (br s, 52 H), 1.51–1.56 (m, 4 H), 3.38–3.71 (m, 32 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.8, 21.2, 22.9, 25.5, 26.0, 29.4, 29.6, 31.6, 60.9, 61.1, 66.2, 68.2, 69.0, 69.9, 70.4, 71.2, 71.6, 72.3, 76.6, 77.6, 78.0; ES-MS Calcd. for $C_{46}H_{94}O_9Na$ [M + Na]: 813.6796, found 813.6835; Anal. Calcd. for $C_{46}H_{94}O_9$: C 69.83, H 11.97; found: C 69.52, H 11.79.

Benzyl 5-*O*-acetyl-α-D-arabinofuranoside (6)

To a solution of benzyl-α-D-arabinofuranoside[13] (0.12 g, 0.5 mmol) in PhMe–C_6H_6 (9 : 1) (20 mL), Bu_2SnO (0.16 g, 0.64 mmol) was added. The solution was refluxed for 18 h, after which half the amount of the solvents was removed. Ac_2O (0.06 mL, 0.64 mmol) was then added to the reaction mixture at room temperature and the solution was stirred for 2 h, concentrated *in vacuo* and purified (pet. ether–EtOAc 2 : 3) to afford **6**, as a gum. Yield: 0.09 g (71%); R_f (pet. ether–EtOAc 1 : 3) 0.33; $[\alpha]_D = -33$ (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.0 (s, 3 H), 3.7 (br s, 2 H), 4.0–4.23 (m, 5 H), 4.42 (d, J = 11.7 Hz, 1 H), 4.67 (d, J = 11.7 Hz, 1 H), 4.95 (br s, 1 H), 7.0–7.4 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.8, 64.1, 69.2, 77.8, 80.8, 82.3, 106.6, 128.0, 128.4, 136.9, 171.3; ES-MS Calcd. for $C_{14}H_{18}O_6Na$ [M + Na]: 305.1002, found 305.1001.

5-*O*-Acetyl-[2,3,5-tri-*O*-benzoyl-α-D-(1→2)-arabinofuranosyl]-[2,3,5-tri-*O*-benzoyl-α-D-(1→3)-arabinofuranosyl]-D-arabinofuranose (8)

A solution of **6** (0.24 g, 0.85 mmol), **7**[13] (1.24 g, 2.04 mmol) and MS 4 Å, (0.3 g) in CH_2Cl_2 (30 mL) was stirred for 15 min. TMSOTf (0.032 mL in 2 mL CH_2Cl_2, 0.17 mmol) was then added dropwise at −10 °C under N_2 atmosphere, the reaction mixture was stirred for 2 h at room temperature, neutralized with Et_3N, filtered and filtrate concentrated *in vacuo* and purified (pet. ether–EtOAc 2 : 1) to afford the protected derivative of **8**, as a foamy solid. Yield: 0.52 g (54%); R_f (pet. ether–EtOAc 6.5 : 3.5) 0.5; $[\alpha]_D = +17.4$ (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.01 (s, 3 H), 4.36 (s, 2 H), 4.46–4.57 (m, 2 H), 4.64–4.82 (m, 8 H), 5.24 (s, 1 H), 5.51 (s, 1 H), 5.55 (br s, 1 H), 5.6–5.64 (m, 5 H), 7.1–8.0 (m, 35 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.5, 60.3, 62.1, 63.5, 69.2, 78.8, 81.3, 81.6, 82.1, 82.4, 86.9, 105.5, 105.8, 127.6–129.7, 132.0, 133.3,

137.2, 165.3–166.1, 170.6; ES-MS Calcd. for $C_{66}H_{58}O_{20}Na$ [M + Na]: 1193.3, found 1193.4.

A solution of above foamy solid (0.54 g, 0.45 mmol) in EtOAc–MeOH (1 : 1) (50 mL) was treated with Pd–C (10%) (0.05 g), stirred under H_2 (1 bar) for 18 h. Filtration of the reaction mixture, followed by purification (pet. ether–EtOAc 2 : 1) afforded **8**, as a white glassy solid. Yield: 0.49 g (94%); R_f (pet. ether–EtOAc 1 : 1): 0.50; $[\alpha]_D = +8.6$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.03 (s, 3 H), 3.1 (br s, 1 H), 4.1–4.3 (m, 1 H), 4.37–4.53 (m, 4 H), 4.62–4.79 (m, 6 H), 5.44–5.53 (m, 4 H), 5.60–5.66 (m, 3 H), 7.26–8.06 (m, 30 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 60.3, 63.5, 63.7, 64.9, 77.5, 78.2, 79.3, 80.4, 80.8, 81.4, 81.6, 81.9, 82.1, 85.6, 96.5, 101.6, 105.3, 105.7, 128.3–129.9, 133.0, 133.4, 133.6, 165.3–166.1, 170.6; ES-MS Calcd. for $C_{59}H_{52}O_{20}Na$ [M + Na]: 1103.3, found 1103.3.

[α-D-(1→2)-Arabinofuranosyl]-[α-D-(1→3)-arabinofuranosyl]-D-arabinofuranose (10)

To a solution of **8** (0.10 g, 0.091 mmol) in THF–MeOH (1 : 1) (5 mL), NaOMe in MeOH (0.01 mL) was added and stirred at room temperature for 18 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and concentrated *in vacuo* to afford **10**, as a foamy solid. Yield: 0.04 g (95%); ^{1}H NMR (D_2O, 300 MHz) δ 3.1–4.04 (m, 15 H), 4.7–5.1 (m, 3 H); ^{13}C NMR (D_2O, 75 MHz) δ 62.1, 62.3, 63.4, 66.1, 67.4, 68.4, 69.5, 69.7, 70.5, 72.1, 72.8, 73.4, 77.3, 81.0, 82.1, 84.9, 93.6, 97.7, 110.2; ES-MS Calcd. for $C_{15}H_{26}O_{13}Na$ [M + Na]: 437.1, found 437.1.

[11-*O*-Hexadecyl-8-(7′-hydroxy-2′,5′-dioxaheptyl)-3,6,9,13-tetraoxanonacosyl]-[α-D-(1→2)-arabinofuranosyl]-[α-D-(1→3)-arabinofuranosyl]-α-D-arabinofuranoside (1)

To a stirred solution of trisaccharide **8** (0.05 g, 0.05 mmol) and CCl_3CN (0.17 mL, 0.17 mmol) in CH_2Cl_2 (10 mL), DBU (7.6 µL, 5 µmol) (0.075 mL from a stock solution of 0.1 mmol in CH_2Cl_2) as added at −10 °C and stirred for 20 min. The crude reaction mixture was separated using pet. ether–EtOAc (7 : 3) to afford the trichloroacetimidate **9**, as a foamy solid. Yield: 0.06 g (92%). A solution of **9** (0.06 g, 0.047 mmol), **5** (0.03 g, 0.04 mmol) and MS 4 Å (0.1 g) in CH_2Cl_2 (10 mL) was stirred for 15 min. TMSOTf (5.7 µL, 5 µmol) (0.057 mL from a stock solution of 0.1 mmol in CH_2Cl_2) was added dropwise at −10 °C, under N_2 atmosphere. The reaction mixture was stirred at room temperature for 2 h, neutralized with Et_3N, filtered and filtrate concentrated *in vacuo* and purified (pet. ether–EtOAc 1 : 1) to afford the protected derivative of **1**. Yield: 0.05 g (88%); R_f (pet. ether–EtOAc 1 : 1) 0.3; $[\alpha]_D = +14.2$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (t, J = 6.6 Hz, 6 H), 1.17–1.21 (br s, 52 H), 1.51–1.56 (m, 4 H), 2.02 (s, 3 H), 3.24–3.8 (m, 31 H), 4.3–4.8 (m, 10 H), 5.21 (s, 1 H), 5.50–5.82 (m, 7 H), 7.2–8.3 (m, 30 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 20.1, 22.5, 26.0, 28.9, 29.1, 29.6, 30.0, 31.8, 61.6, 63.0, 63.5, 69.3, 70.2, 70.4, 70.8, 71.6, 74.6, 77.4, 78.3, 78.8, 80.9, 81.2, 81.4, 82.5, 86.9, 105.6, 105.9, 106.2, 127.2–129.8, 133.0, 133.6, 165.4–165.8, 170.7; MALDI-TOF-MS Calcd. for $C_{105}H_{144}O_{28}Na$ [M + Na]: 1876.0, found 1875.9.

To a solution of the protected derivative of **1** (0.04 g, 0.02 mmol) in THF–MeOH (1 : 1) (10 mL), NaOMe in MeOH (0.010 mL) was added at room temperature and stirred for 24 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and filtrate concentrated to afford **1**, as a foamy solid. Yield: 0.02 g, (95%); $[\alpha]_D = +47.3$ (*c* 1, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ 0.86 (t, J = 6.4 Hz, 6 H), 1.2–1.39 (m, 52 H), 1.51–1.56 (m, 4 H), 3.3–4.2 (m, 45 H), 5.04–5.2 (m, 3 H); ^{13}C NMR (CD_3OD, 75 MHz) δ 14.4, 23.7, 27.2, 30.4, 30.7, 33.1, 62.2, 62.6, 62.8, 63.0, 71.4, 71.9, 72.0, 73.7, 78.6, 79.5, 79.8, 82.0, 83.6, 85.6, 85.8, 87.8, 107.9, 108.7, 109.3; ES-MS Calcd. for $C_{61}H_{118}O_{21}Na$ [M + Na]: 1209.8, found 1209.9.

[11-*O*-Hexadecyl-8-({7′-[α-D-(1→2)-arabinofuranosyl]-[α-D-(1→3)-arabinofuranosyl]-α-D-arabinofuranosyl}-2′,5′-dioxaheptyl)-3,6,9,13-tetraoxanonacosyl]-[α-D-(1→2)-arabinofuranosyl]-[α-D-(1→3)-arabinofuranosyl]-α-D-arabinofuranoside (2)

A solution of **9** (0.06 g, 0.04 mmol), **5** (0.016 g, 0.02 mmol) and MS 4 Å, (0.1 g) in CH_2Cl_2 was stirred for 15 min. TMSOTf (5.7 µL, 5 µmol) (0.057 mL from a stock solution of 0.1 mmol in CH_2Cl_2) was added drop-wise at −10 °C, under N_2 atmosphere. The reaction mixture was stirred at room temperature for 2 h, neutralized with Et_3N, filtered and filtrate concentrated *in vacuo* and purified (pet. ether–EtOAc, 2 : 1) to afford the protected derivative of **2**. Yield: 0.07 g (74%); R_f (pet. ether–EtOAc 1 : 1): 0.7; $[\alpha]_D = +8.7$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 6.6 Hz, 6 H), 1.17–1.22 (br s, 52 H), 1.51–1.55 (m, 4 H), 2.02 (s, 6 H), 3.2–3.8 (m, 30 H), 4.3–4.8 (m, 20 H), 5.17 (s, 2 H), 5.52–5.82 (m, 14 H), 7.2–8.3 (m, 60 H); ^{13}C NMR ($CDCl_3$, 75 MHz), δ 14.0, 20.2, 22.5, 25.6, 25.9, 29.2, 29.5, 29.9, 31.7, 61.5, 63.5, 69.1, 69.3, 70.1, 70.5, 70.7, 70.9, 71.5, 72.5, 77.8, 80.9, 81.3, 82.2, 86.9, 105.3, 105.6, 106.2, 128.2–129.8, 132.4, 132.6, 132.9, 133.4, 165.1–166.1, 170.6; MALDI-TOF-MS Calcd. for $C_{164}H_{194}O_{47}Na_2$ [M + 2Na]: 2961.3, found 2961.7.

To a solution of the protected derivative of **2** (0.03 g, 0.01 mmol) in THF–MeOH (1 : 1) (10 mL), NaOMe in MeOH (0.01 mL) was added at room temperature and stirred for 18 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and concentrated *in vacuo* to afford **2**, as a foamy solid. Yield: 0.015 g (94%); $[\alpha]_D$ = +45.4 (*c* 1, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ 0.86 (t, J = 6.4 Hz, 6 H), 1.2–1.49 (m, 52 H), 1.51–1.56 (m, 4 H), 3.3–4.2 (m, 60 H), 5.05–5.19 (m, 6 H); ^{13}C NMR (CD_3OD, 75 MHz), δ 14.4, 23.7, 27.2, 30.5, 30.8, 31.1, 33.0, 62.2, 62.7, 63.9, 71.4, 71.9, 72.1, 72.5, 73.4, 73.7, 78.6, 79.5, 79.8, 82.0, 83.6, 85.4, 85.7, 87.8, 108.0, 108.7, 109.1. MALDI-TOF-MS Calcd. for $C_{76}H_{142}O_{33}Na$ [M + Na]: 1605.9, found 1605.9, 1341.0 [M − 2 ($C_5H_9O_3$) + Na + 2 H^+], 1208 [M − 3 ($C_5H_9O_3$) + Na + 2 H^+].

[11-*O*-Hexadecyl-8-(7′-hydroxy-2′,5′-dioxaheptyl)-3,6,9,13-tetraoxanonacosyl]-[α-D-(1→4)-glucopyranosyl]-β-D-glucopyranoside (12)

Hepta-*O*-acetyl maltosyl bromide **11**[13] (0.1 g, 0.143 mmol) and **5** (0.12 g, 0.143 mmol) were dissolved in CH_2Cl_2 (20 mL), Ag_2CO_3 (0.04 g, 0.143 mmol) and $AgClO_4$ (4 mg, 0.0143 mmol) were added, stirred for 18 h at room temperature, filtered and the filtrate concentrated *in vacuo* and purified (pet. ether–EtOAc, 1 : 3). The resulting product (0.16 g) in THF–MeOH (1 : 1) (8 mL), NaOMe in MeOH (0.01 mL) was added and stirred at room temperature for

18 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and concentrated *in vacuo* to afford **12**, as a foamy solid. Yield: (0.13 g, 74%); $[\alpha]_D$ = +9 (*c* 1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (t, J = 6.9 Hz, 6 H), 1.29 (br s, 52 H), 1.53–1.58 (m, 4 H), 3.21–3.38 (m, 4 H), 3.39–3.64 (m, 35 H), 3.82–3.88 (m, 3 H), 4.19 (d, J = 7.6 Hz, 1 H), 5.15 (d, J = 4 Hz, 1 H). ^{13}C NMR (CD_3OD, 100 MHz) δ 14.4, 21.3, 23.7, 27.3, 30.5, 30.7, 33.0, 62.1, 62.7, 71.1, 71.5, 71.9, 72.5, 73.7, 74.1, 74.7, 75.0, 76.6, 77.8, 79.5, 79.8, 81.3, 102.9, 105.3. ES-MS Calcd. for $C_{58}H_{114}O_{19}Na$ [M + Na]: 1137.8, found 1137.8.

Mycobacterial growth assay

Microbial growth is a global indicator for the metabolic regulation occurring inside the cell. The growth of the bacteria was quantified as a function of the increase in the cell density by a spectrophotometric measurement. The wild type strain of *M. smegmatis* used in this study was mc^{2}155. *M. smegmatis* was grown in Middlebrook 7H9 broth (Difco) supplemented with 0.05% Tween 80 and 2% glucose. After having a sufficient growth in the primary culture tubes, the secondary culture tubes were incubated and the synthetic alkyl glycosides **1**, **2** and **12** and the arabinofuranose trisaccharide **10** were incorporated into the medium after a period of 8 h, as a solution in aq. methanol (50%), in varying concentrations. *M. smegmatis* culture without the inhibitors was used as a negative control. The growth rates were determined in triplicate by measuring at OD_{600}.

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