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Synthesis of β-arabinofuranoside glycolipids, studies of their binding to surfactant protein-A and effect on sliding motilities of *M. smegmatis*

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Abstract Surfactant protein A (SP-A), which is a lung innate immune system component, is known to bind glycolipids present at the cell surface of a mycobacterial pathogen. Lipoarabinomannan (LAM), a component of mycobacterial thick, waxy cell wall, is one of the glycolipid ligands for SP-A. In order to assess binding of synthetic glycolipids with SP-A and the glycosidic linkage preferences for the interaction, β-arabinofuranoside trisaccharide glycolipids constituted with β-(1→2), β-(1→3) and β-(1→2), β-(1→5) linkages relevant to LAM were synthesized through chemical glycosylations. The efficacies of synthetic glycolipids to interact with SP-A were assessed by using the surface plasmon resonance (SPR) technique, from which association-dissociation rate constants and equilibrium binding constants were derived. The equilibrium binding constants of the interaction of two constitutionally varying β-arabinofuranoside glycolipids with SP-A were found to be in the millimolar range. A comparison of the results with few α-anomeric arabinofuranoside glycolipids showed that glycolipids with β-anomeric linkages were having relatively lower equilibrium binding constants than those with α-anomeric linkages in binding to the protein, whereas oligosaccharides alone, without lipidic chains, exhibited higher equilibrium binding constants. Further, the synthetic compounds inhibited the growth of mycobacteria and affected sliding motilities of the bacteria, although to an extent relatively lesser than that of synthetic compounds constituted with α-anomeric linkages.

Keywords β-Arabinofuranosides · Glycolipids · Glycosylation · *M. smegmatis* · Sliding motility · Surface plasmon resonance · Surfactant protein-A

Abbreviations

EDC	*N*-Ethyl-*N*′-(dimethyl-aminopropyl) carbodiimide
NHS	*N*-Hydroxysuccinimide
CM	Carboxymethyl
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid
HMQC	Heteronuclear multiple quantum coherence
LAM	Lipoarabinomannan
SP-A	Surfactant protein-A
SP-D	Surfactant protein-D
SPR	Surface plasmon resonance

Introduction

The first line of defense to a host cell system against pathogenic invasion is the process of phagocytosis of pathogens by pulmonary surfactant proteins, such as, SP-A and SP-D. The thick, waxy cell-wall components of pathogens comprising lipopolysaccharides bind to pulmonary surfactant

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proteins in the initiation of host-defense mechanisms against pathogen infection [1]. LAM is a primary component of mycobacterial cell wall and is a ligand for binding to SP-A [2]. LAM is constituted with repeating units of arabinofuranosides, mannopyranosides and lipid portions linked through an inositol core fragment [3, 4]. Synthetic glycolipids constituted with the above sugars were shown previously to act as inhibitors of mycobacterial growth, through affecting mycobacterial biofilms and motilities [5, 6]. Synthetic glycolipids appeared to play a role in affecting the normal profiles of glycopeptidolipids and proteins involved in the biosynthesis of cell-wall components. Subsequent studies revealed that such synthetic glycolipids also bind to a surfactant protein, namely, SP-A, which is an innate, immune protein present at the host cells. Studies with the aid of SPR technique showed that the binding of synthetic glycolipids to SP-A occurred in millimolar to sub-millimolar concentrations [7]. Synthetic glycolipids were constituted with α-anomeric arabinofuranosides, both linear and branched oligosaccharides, in the studies conducted so far. Due to the fact that carbohydrate binding proteins show finely-tuned anomeric configurational preferences to a sugar ligand, in addition to well-preserved specificities [8], it was deemed necessary to study the efficacies of glycolipids constituted with β-anomeric arabinofuranosides binding to SP-A protein. Arabinofuranoside trisaccharide glycolipids having β-anomeric linkages at the non-reducing ends and double hexadecyloxy lipid moieties, interconnected to the sugar moiety through a glycerol core, were synthesized. The synthetic glycolipids were assessed for their binding efficacies to SP-A protein, by using the SPR technique. The synthetic glycolipids with β-anomeric linkages were also investigated for their effect in mycobacterial growth and motilities. Details of synthesis and studies are presented herein.

Results and discussion

Synthesis Repeating units of native lipoarabinomannan glycolipids consist of (1→2), (1→3) and (1→5) glycosidic linkages [3, 4]. Synthetic glycolipids presenting these linkages were thus considered appropriate towards studies to identify their protein binding efficiencies. Structures of chosen glycolipids **1** and **2**, constituted with β-(1→2), β-(1→3) and β-(1→2), β-(1→5) linkages, respectively, are shown in Fig. 1. A diethylene glycol moiety was incorporated as a spacer between the sugar groups and the branched glycerol moiety derivatised with double hexadecyl groups.

Synthesis of β-arabinofuranoside glycolipids **1** and **2** was initiated from arabinofuranosyl trichloroacetimidate **3** [9], which was subjected to (i) glycosylation with alcohol **4** [7] and (ii) deprotection of benzoate groups, to afford **5** (Scheme 1). In the ^{1}H NMR spectrum of **5**, an apparent singlet at 5.08 ppm, corresponded to H-1 nucleus of the α-anomer, whereas anomeric carbon in **5** appeared at 108.0 ppm in the ^{13}C NMR spectrum. Regioselective protection of *C*5 hydroxyl group in **5** was performed using Bu_2SnO mediated acetylation to afford **6**, which was used further as glycosyl acceptor for the synthesis of glycolipid **1**. The formation of **6** was confirmed by the appearance of a doublet at 5.07 ppm ($J_{H1,\ H2}$=2.4 Hz) in the ^{1}H NMR spectrum and a resonance at 108.1 ppm in the ^{13}C NMR spectrum. Further, *C*5 nucleus in **6** appeared at 64.2 ppm in the ^{13}C NMR spectrum, whereas the same in **5** appeared at 62.1 ppm. Towards incorporation β-Ara*f* linkages, a low temperature activation of thiocresyl donor **7** was adopted [10, 11]. Gycosylation of diol **6** with donor **7**, in the presence of NIS/AgOTf, at −40°C, afforded trisaccharide **8**, containing two β-Ara*f* linkages (Scheme 2). In the ^{1}H NMR spectrum of **8**, doublets at 5.01 and 5.04 ppm ($J_{H1,\ H2}$=5.2 Hz), corresponded to the newly generated β-arabinofuranoside linkages at *C*-2 and *C*-3 of the core furanoside ring. On the other hand, H-1 of the α-Ara*f* linkage, attached to lipid moiety, appeared as a singlet at 4.99 ppm. β-Arabinofuranoside linkages in **8** were also confirmed with the presence of peaks at 100.3 and 100.8 ppm in the ^{13}C NMR spectrum. Further confirmation was secured from HMQC analysis of **8**, which showed strong correlations between resonances for carbon and proton of β-Ara*f* linkages. Disilyl functionality in **8** was removed using TBAF in THF. Subsequent deprotection of benzyl groups and ester group, using Pd/C, H_2 and NaOMe/MeOH, respectively, afforded glycolipid **1**. The structure of **1** was confirmed by NMR spectroscopy and mass spectrometry. The H-1 nucleus for β-Ara*f* linkages was observed as doublets at 5.05 and 5.08 ppm ($J_{H1,\ H2}$=2.8 and 3.6 Hz), whereas the

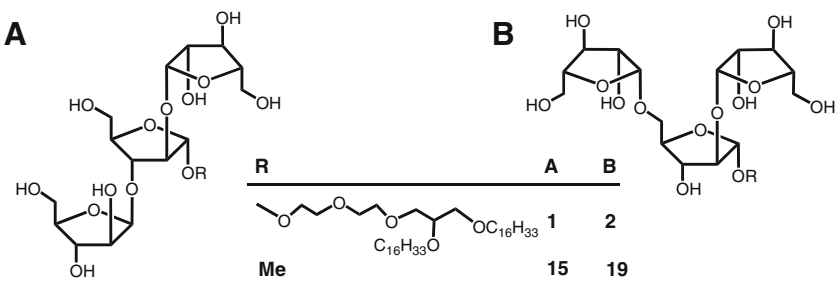
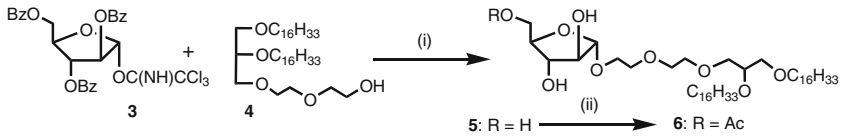


Fig. 1 Molecular structures of trisaccharides and glycolipids with β-arabinofuranoside linkages at the non-reducing ends



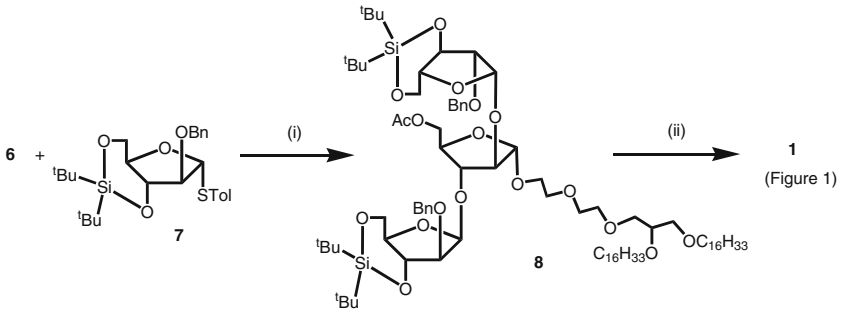
Scheme 1 *Reagents and conditions*: (i). (**a**) $BF_3.OEt_2$, MS 4 Å, CH_2Cl_2, rt, 30 min.; (**b**) NaOMe, MeOH/THF (1:1), rt, 6 h, 72%; (ii). (**a**) Bu_2SnO, PhMe, reflux, 18 h; (**b**) Ac_2O, PhMe, rt, 2 h, 52%

same for α-Ara*f* linkage resonated at 5.09 ppm as an apparent singlet. In ^{13}C NMR spectrum of **1**, peaks at 102.6 and 103.8 ppm, corresponded to β-arabinofuranoside linkages.

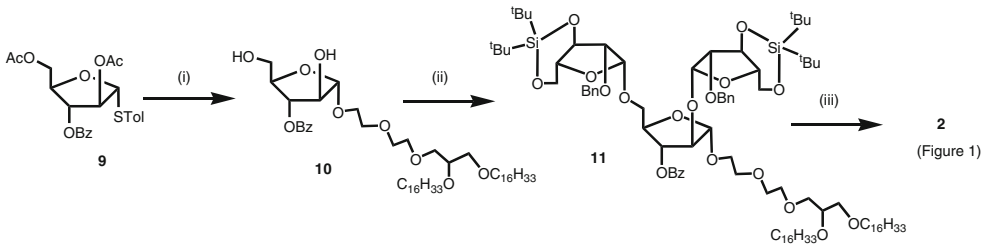
Towards synthesis of glycolipid **2**, glycosyl acceptor **10** was synthesized first, through a glycosylation of thioglycoside **9** with alcohol **4**, in the presence of NIS/AgOTf, followed by a deprotection of acetate groups, using AcCl/MeOH (Scheme 3). The α-linkage between the lipid moiety and furanose moiety in **10** was confirmed by a resonance at 108.4 ppm in ^{13}C NMR spectrum. Double glycosylation of **10** with donor **7**, in the presence of NIS/AgOTf, at −40°C, afforded trisaccharide **11** containing β-arabinofuranoside linkages (Scheme 3). In ^1H NMR spectrum of **11**, doublets at 5.02 and 5.10 ppm ($J_{H1,\ H2}$=5.2 Hz) were attributed to β-Ara*f* linkages at *C*-2 and *C*-5 of the core furanoside unit, whereas an apparent singlet at 5.13 ppm, corresponded to α-Ara*f* linkage between core furanoside and lipid moiety. The β-anomeric configurations in **11** were further confirmed by presence of a peak at 100.7 ppm in ^{13}C NMR spectrum. Deprotection of silyl, benzyl and acetate groups in **11** afforded the glycolipid **2**. The H-1 nucleus of β-Ara*f* linkages of **2** was observed as doublets at 4.91 and 5.00 ppm ($J_{H1,\ H2}$=4.4 Hz), whereas the same for α-Ara*f* linkage appeared as a doublet at 5.04 ppm ($J_{H1,\ H2}$=1.8 Hz). In the ^{13}C NMR spectrum of **2**, peaks at 102.4, 103.3 ppm corresponded to β-Ara*f* linkages and a resonance at 107.7 ppm to α-Ara*f* linkage.

In order to study the effect of lipid chain in biological and protein binding studies, oligosaccharides **15** and **19** (Fig. 1), containing β-(1→2), β-(1→3) and β-(1→2), β-(1→5) linkages between the furanoside units, were also synthesized. Synthesis of the oligosaccharides **15** and **19** was initiated from the methyl-α-D-arabinofuranoside **12**. Dibutyltin oxide mediated acetylation led to the formation of **13**, which was subjected to double glycosylation with donor **9**, in the presence of NIS/AgOTf, to afford trisaccharide **14**, in 52% yield (Scheme 4). The β-Ara*f* linkages in **14** were confirmed by the presence of two doublets at 5.00 and 5.15 ppm (*J*= 5.2 Hz) in the ^1H NMR spectrum. Deprotection of silyl, benzyl and acetate groups in **14** led to the formation of oligosaccharide **15**. Similarly, synthesis of oligosaccharide **19** was initiated with *O*-diacetylation of **12**, mediated by dibutyltin oxide. Benzoylation and subsequent *O*-deacetylation of **16**, furnished diol **17**, necessary to synthesise **19**. The β-Ara*f* linkages were installed on **17**, using **9** as the glycosyl donor (Scheme 5), to afford the protected trisaccharide **18**. In the ^1H NMR spectrum of **18**, doublets at 5.05 and 5.10 ppm (*J*=5.2 Hz) confirmed the formation of β-Ara*f* linkages at C-2 and C-5 position. Deprotection of ester and silyl protecting groups in **18** led to trisaccharide **19**, in 57% yield. The constitution of oligosaccharides **15** and **19** was also confirmed by mass spectrometry.

SPR studies Studies of β-arabinofuranoside trisaccharides and glycolipids were performed, in order to identify their interactions with a host protein, namely, pulmonary surfactant protein-A (SP-A) by using the SPR method. SP-A is an abundant surfactant protein secreted by alveolar macrophages and it mediates various immune cell responses. The immune responses of SP-A are reviewed extensively by Wright and co-workers [1, 12, 13]. SP-A has a bouquet structure of six trimers and possesses molecular weight in the range of 26–38 kDa [14]. SP-A is known to bind pathogens, such as, HIV and mycobacterium by interacting with oligosaccharide glycolipids present on the cell surfaces [15–17]. The binding studies of SP-A with the polysaccharide glycolipids extracted from mycobacterial cell wall components were studied in detail, with the aid of SPR technique



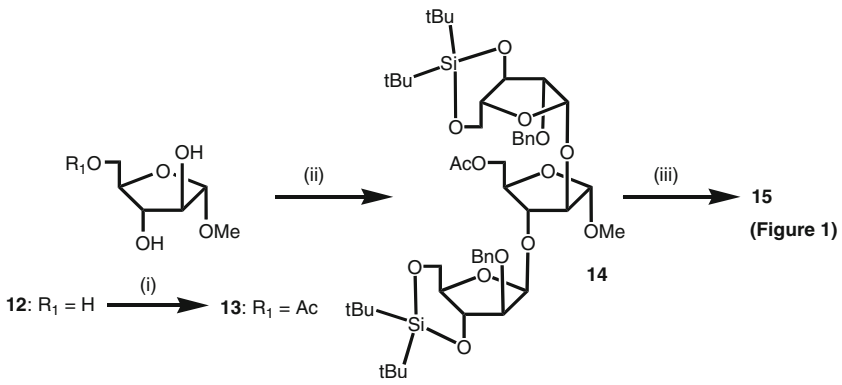
Scheme 2 *Reagents and conditions*: (i) AgOTf, NIS, MS 4 Å, CH_2Cl_2, −40°C, 30 min., 54%; (ii) (**a**) TBAF, THF, rt, 8 h; (**b**) Pd-C, H_2, THF, rt, 18 h; (**c**) NaOMe, MeOH, rt, 2 h, 61%



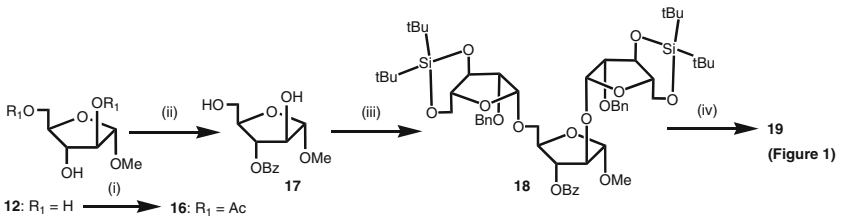
Scheme 3 *Reagents and conditions*: (i) (**a**) **4**, AgOTf, NIS, MS 4 Å, CH_2Cl_2, −40°C, 30 min.; (**b**) AcCl, MeOH/CH_2Cl_2 (1:1), rt, 6 h, 66%. (ii) **7**, AgOTf, NIS, MS 4 Å, CH_2Cl_2, −40°C, 30 min., 62%; (iii) (**a**) TBAF, THF, rt, 8 h; (**b**) Pd-C, H_2, THF, rt, 18 h; (**c**) NaOMe, MeOH, rt, 6 h, 55%

[2]. Crystallographic determinations of SP-A bound to mannose, α-D-methyl mannoside and glycerol were reported recently, from which significant conformational changes were seen to occur to the protein upon ligand binding [18]. It was surmised further that SP-A might bind to a range of ligands, as a result of its ability to undergo ligand-induced conformational changes, flexible structures and the ability to utilize varied cations for stabilization. We studied previously the interactions of SP-A with synthetic linear and branched oligosaccharides glycolipids constituted with α-arabinofuranosides. The evaluation of kinetic parameters showed millimolar to submillimolar range of binding constants, for the interaction of SP-A with synthetic glycolipids [7].

For the protein binding studies, SP-A, purified from the lung lavage surfactant pellets of patients with alveolar proteinosis, was immobilized onto the CM-5 sensor chip. The carboxylic acid groups of the sensor chip were activated through EDC/NHS coupling, followed by immobilizing SP-A over the surface, by injecting at a flow rate of 5 μL/min, to level of 3000 RU. In order to eliminate the non-specific interactions and the bulk change of RU, a surface immobilized with ethanolamine was used as a control. Varying concentrations of synthetic compounds **1**, **2**, **15** and **19** were dissolved in HEPES-buffer and injected over the surfaces of SP-A and ethanolamine. Response units of the sensor surface immobilized with SP-A were subtracted from the surface immobilized with ethanolamine, so as to eliminate non-specific interactions, as well as bulk change in RU due to variation in the refractive index of the medium. It was found that glycolipids **1** and **2** did not show binding to SP-A up to 50 μM concentration. Glycolipids **1** and **2** required a concentration of ~122 μM to exhibit a significant binding to SP-A. Injections of increasing concentrations of arabinan glycolipids **1** and **2** provided an increasing response (Fig. 2). Absence of mass transport was ascertained by passing through analyte of a fixed concentration and varying the flow rates. The sensorgrams did not differ significantly, indicating that the ligand-receptor interaction was specific. Global fitting of the sensorgrams of **1** and **2**, using BIA evaluation models led to poor agreement between experimental and fitting data. Due to this reason, kinetic parameters were obtained through separate fitting for the association and dissociation phases individually. The fitting curves are shown as superimposed lines in Fig. 2 and the association (k_a) and dissociation rate constants (k_d), for each concentration of **1** and **2** are given in Table 1. From the analysis, the association rate constants (k_a) were found to be in the range of 0.3 to 0.85 M^{-1} s^{-1}, whereas the dissociation rate constants (k_d) were varied between 2.21 and 3.2 × 10^{-3} s^{-1}. The equilibrium constants (K_a) values were in the range of 93 and 274 M^{-1}. The equilibrium constants for glycolipids with α-arabinofuranoside linkages were evaluated previously to be in the range of 490 to 47,200 M^{-1} [7]. Both association and dissociation rate constants were found to be lower by more than an order of magnitude for **1** and **2**, when compared to glycolipids with α-arabinofuranoside linkages. Consequently, the equilibrium binding constants of the interaction of **1** and **2** with SP-A were



Scheme 4 *Reagents and conditions*: (i) (**a**) Bu_2SnO, MeOH, reflux, 18 h; (**b**) Ac_2O, CH_2Cl_2, 35°C, 12 h, 56%. (ii) **9**, AgOTf, NIS, MS 4 Å, CH_2Cl_2, −40°C, 30 min., 52%; (iii) (**a**) TBAF, THF, rt, 8 h; (**b**) Pd-C, H_2, THF, rt, 18 h; (**c**) NaOMe, MeOH, rt, 2 h, 55%



Scheme 5 *Reagents and conditions*: (i) (**a**) Bu_2SnO, MeOH, reflux, 18 h; (**b**) Ac_2O, CH_2Cl_2, 35°C, 18 h, 69%; (ii) Py/BzCl, CH_2Cl_2, rt, 12 h; (**b**) AcCl, MeOH/CH_2Cl_2 (1:1), rt, 4 h, 66%. (iii) **9**, AgOTf, NIS, MS 4 Å, CH_2Cl_2, −40°C, 30 min., 49%; (iv) (**a**) TBAF, THF, rt, 8 h; (**b**) Pd-C, H_2, THF, rt, 18 h; (**c**) NaOMe, MeOH, rt, 12 h, 57%

found to be lower than that of α-arabinofuranoside glycolipids. This observation implies that α-anomers are preferable over the β-anomers in the ligand-receptor interactions concerning arabinofuranoside glycolipids binding to SP-A. The interaction of arabinofuranoside glycolipids with SP-A is un-known currently. In conjunction with our previous report, the present study shows that arabinofuranoside glycolipids, that are major constituents of LAMS, bind to SP-A to the extent of sub-millimolar concentrations in the case of α-anomers and the interaction is weaker with the β-anomeric arabinofuranoside glycolipids.

Trisaccharides **15** and **19** (Fig. 1), without lipidic chains, were also assessed for their efficacies to interact with SP-A. The corresponding sensorgrams for these trisaccharides are shown in Fig. 3. As in the case of glycolipids **1** and **2**, global fitting of data did not lead to low χ^2 values and thus data were fitted in separate k_{on}/k_{off} model. The binding constants for each concentration of **15** and **19** are given in Table 2. The association constants for **15** were found to be in the range of 2,470 to 9,090 M^{-1}, whereas for the derivative **19**, K_A values varied between 25,600 and 76,900 M^{-1}. The association and equilibrium binding constants for **15** and **19** were found to be significantly higher when compared to glycolipids **1** and **2**. The β-arabinan glycolipids showed relatively low binding constants, when compared to α-anomeric glycolipids. As observed with the interaction of α-arabinofuranoside glycolipids and the corresponding oligosaccharide with SP-A previously [7], higher association rate constants (k_{on}) for oligosaccharide interaction with the protein result in higher equilibrium binding constants (K_a).

Microbial pathogens are known to interact with pulmonary surfactant proteins in alveolar macrophages [1]. SP-A and SP-D recognizes the mycobacterial pathogens by interacting with oligosaccharides present at cell surfaces [2, 19]. Riviére and co-workers reported the interaction of LAM and lipomannans, extracted from mycobacterial cells, with SP-A and the equilibrium constants were found to be in the sub-micromolar concentrations [2]. In order to analyze binding efficiencies of arabinofuranosides, that constitute underlying scaffolds of LAMs, with SP-A, we considered structurally homogeneous synthetic glycolipids, containing α- and β-arabinofuranoside linkages, were necessary. We reported recently the binding affinities of synthetic α-arabinofuranoside containing glycolipids with SP-A [7]. From the kinetic analysis, 1:1 Langmuir model was found to provide best fitting for the sensorgrams, indicating the 1:1 stoichiometry between the protein and ligand. The binding constants of glycolipids containing β-arabinofuranoside linkages were found to be considerably weaker than glycolipids constituted with α-anomeric configuration, as identified in the present study. Whereas LAMs with mannose caps are known to bind SP-A, studies with synthetic arabinofuranoside glycolipids establish that arabinofuranoside is also a cognate ligand for SP-A, yet with a preference to the configuration of the glycosidic bond. Thus, the α-anomeric arabinofuranoside glycolipids are better ligands for SP-A than the β-anomeric

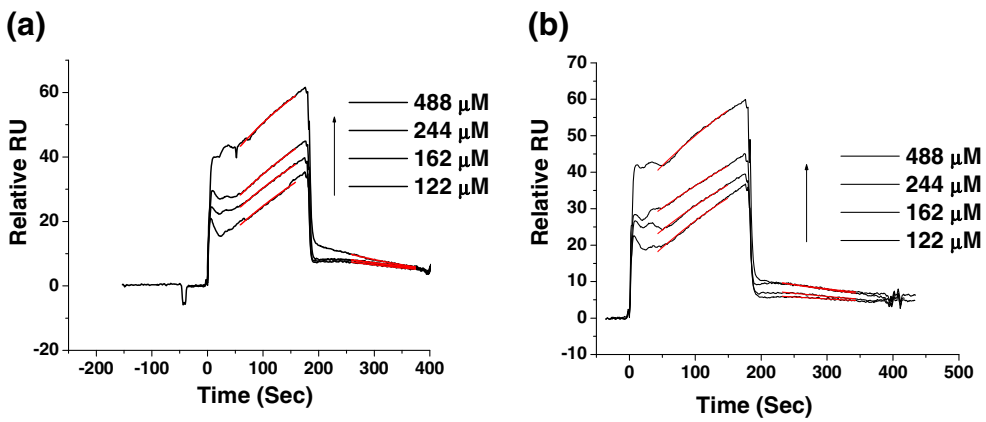


Fig. 2 SPR sensorgrams of (**a**) **1** and (**b**) **2** upon interaction with SP-A

Table 1 Kinetic data of the interactions of **1** and **2** with SP-A

Concentration (μM)	k_a (M^{-1} s^{-1})		k_d (s^{-1}) (10^3)		K_a (M^{-1}) (10^{-2})	
	1	2	1	2	1	2
122	0.36	0.62	2.56	3.2	1.42	1.95
162	0.49	0.54	2.76	2.95	1.78	1.85
244	0.50	0.54	3.04	2.21	1.67	2.46
488	0.29	0.85	3.14	3.10	0.93	2.74

The error values were found to be ±4 to 6%. χ^2 values were<1

glycolipids. In relation to the structural features of SP-A being closer to mannose binding lectins [1], the presence of mannose residues on to synthetic glycolipids is likely to improve the interactions stronger than arabinofuranoside glycolipids alone.

Effect of synthetic oligosaccharides and glycolipids on mycobacterial growth and sliding motility Subsequent to assessing the interactions of synthetic oligosaccharides and corresponding glycolipids with SP-A, their effects on growth and motility profiles of *M. smegmatis* were undertaken. These studies were followed by adopting procedures reported previously [5–7] to evaluate the effects of α-anomeric oligosaccharides and glycolipids. In mycobacterial growth assays, conducted in triplicates, glycolipids **1** and **2** showed inhibition at 50 μg/mL and 100 μg/mL, whereas trisaccharides **15** and **19**, without lipid portion, did not show significant changes (Figs. 4 and 5). Compounds **1**–**4** were found to be non-toxic, as bacterial growth occurred, although at reduced levels when compared to the wild type bacteria. The inhibitions of mycobacterial growth were in the range of 15–25% in both log and stationary phases. The extent of inhibition of mycobacterial growth was found to be lesser than to that of glycolipids with α-anomeric configurations (20–35%) [5–7], implying that the nature of anomeric configuration has an effect on the inhibition profile.

The compounds were tested further for their effects on spreading ability of mycobacteria. In this assay, we noticed that **1** and **2** showed significant inhibition at 50 μg/mL, whereas **15** and **19** did not. Upon increasing the concentration of **15** and **19** to 100 μg/mL, it was observed that trisaccharide **19** affected the sliding motility greatly, in contrast to **15** which remained to exhibit sliding motility nearly to the same extent as that of concentration 50 μg/mL (Fig. 6). The spreading zone was controlled, as opposed to the same in wild type, which was spreading towards the periphery of the plate. In this assay, we find that the effects of synthetic compounds are nearly the same as that of previously reported [5–7] glycolipids and oligosaccharides, constituted with α-anomeric linkages. The wild type bacteria without trisaccharides and glycolipids were used as control in these studies.

Conclusion

In an effort to verify the anomeric configurational preferences of synthetic arabinofuranoside glycolipids binding to SP-A, trisaccharide glycolipids with β-anomeric linkages, comprising β-(1→2), β-(1→3) and β-(1→5) linkages between the furanoside moieties were synthesized. Synthesis involved cyclic disilyl protected thioglycoside as the glycosyl donor in order to incorporate β-arabinofuranoside glycosidic linkages. The interactions of synthetic glycolipids with SP-A protein were assessed with the aid of surface plasmon resonance technique and the binding constants were seen to be in the millimolar range. In conjunction with studies on glycolipids having α-anomeric linkages, the studies showed that (i) arabinofuranosides are also cognate ligands for binding to SP-A and (ii) the α-anomers bind SP-A stronger than the β-anomers. Studies of trisaccharides and glycolipids in mycobacterial growth and sliding motility assays showed that the synthetic compounds affected both

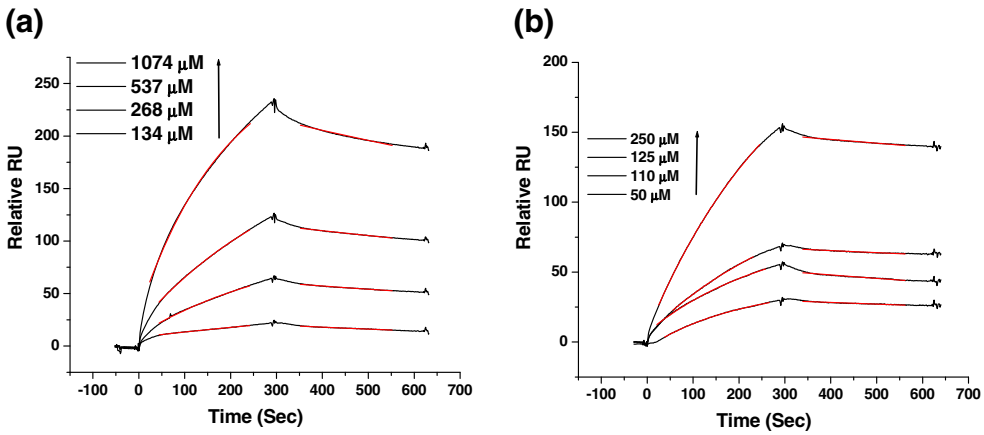


Fig. 3 SPR sensorgrams of (**a**) **15** and (**b**) **19** upon interaction with SP-A

Table 2 Kinetic data of the interactions of **15** and **19** with SP-A

Concentration (**15**) (μM)	k_a (M^{-1} s^{-1})	k_d (s^{-1}) (10^4)	K_a (M^{-1}) (10^{-3})	Concentration (**19**) (μM)	k_{on} (M^{-1} s^{-1})	k_d (s^{-1}) (10^4)	K_a (M^{-1}) (10^{-4})
134	2.20	8.92	2.47	50	16.2	5.05	3.22
268	4.62	5.65	8.14	110	13.8	1.89	7.69
537	4.88	4.49	9.43	125	12.2	2.29	4.34
1074	4.45	4.9	9.09	250	10.8	4.28	2.56

The error values were found to be ±4 to 6%. χ^2 values were<1

growth and motility and the extent was comparable to but lesser than that of α-anomeric glycosides and glycolipids. These observations imply that glycolipids with β-anomeric linkages exhibit relatively lesser effect than those constituted with α-anomeric linkages. As we surmised previously, it is likely that the synthetic glycolipids impair normal biosynthesis of cell wall structures necessary for maturation and growth of mycobacteria, leading to the observed inhibitory activities of synthetic glycolipids.

Material and methods

General information Solvents were dried and distilled according to literature procedures [20]. Chemicals were purchased from commercial sources and were used without further purifications. 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% sulphuric 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 $CDCl_3$ solution unless otherwise stated. Chemical shifts are reported with respect to tetramethyl silane (TMS) for ^{1}H NMR spectra and the central line (77.0 ppm) of $CDCl_3$ for ^{13}C NMR spectra. Coupling constants (*J*) are reported in Hz. Abbreviations s, d, t, dd, br s, app s, m refer to singlet, doublet, triplet, doublet of doublet, broad singlet, apparent singlet, multiplet, respectively.

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl α-D-arabinofuranoside (5) A solution of **3** [9] (3 g, 4.95 mmol), **4** [7] (3.1 g, 4.95 mmol) and MS 4 Å (2 g) in CH_2Cl_2 (60 mL) was stirred for 15 min. $BF_3.OEt_2$ (0.62 mL, 4.95 mmol) was added, stirred for 30 min. at rt, neutralized with Et_3N, filtered, filtrate concentrated *in vacuo*. NaOMe in MeOH (1 mL, 1 M) was added to a solution of the protected derivative of **5** in MeOH/THF (1:1) (50 mL), stirred for 6 h at rt, neutralized with Amberlite ion-exchange (H^+) resin, filtered, filtrate concentrated *in vacuo* and purified (SiO_2, EtOAc) to afford **5** (2.7 g, 72%). R_f (EtOAc) 0.65; $[\alpha]_D$ +15.5 (*c*=1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=7.2 Hz, 6 H), 1.25 (br s, 52 H), 1.53–1.55 (m, 4 H), 3.41–3.67 (m, 16 H), 3.79 (dd, *J*= 12 Hz, *J*=2.4 Hz, 1 H), 3.85–3.90 (m, 2 H), 4.00 (br s, 1 H), 4.06 (br s, 1 H), 4.19 (q, *J*=2.4 Hz, 1 H), 5.08 (app s, 1 H); ^{13}C

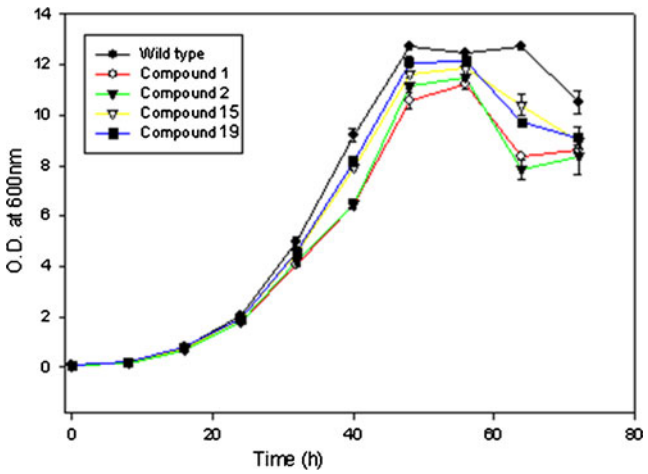


Fig. 4 Effects of **1, 2, 15** and **19** (50 μg/mL) on the growth profile of *M. smegmatis*

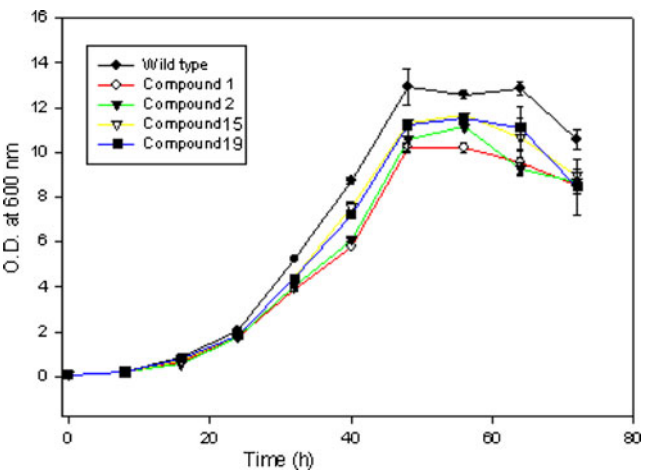


Fig. 5 Effects of **1, 2, 15** and **19** (100 μg/mL) on the growth profile of *M. smegmatis*

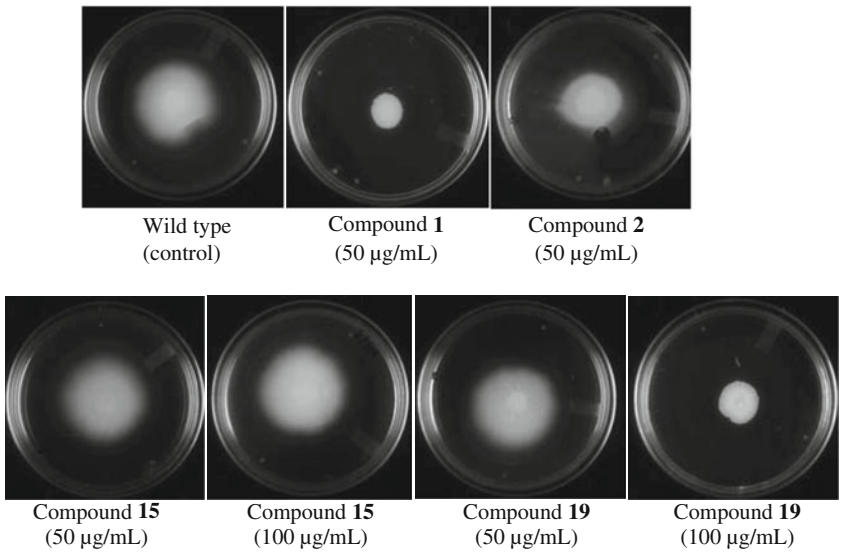


Fig. 6 Sliding motility assay showing the spreading ability of mycobacteria (3 days old), in the presence of different synthetic oligoarabinofuranosides and glycolipids **1, 2, 15** and **19**

NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 26.1, 29.3, 29.5, 29.6, 29.7, 30.0, 31.9, 62.1, 65.6, 70.3, 70.4, 70.6, 70.7, 71.3, 71.7, 77.9, 78.0, 79.2, 86.9, 108.0; ESI-MS Calcd. for $C_{44}H_{88}O_9Na$ [M + Na]: 783.6. Found 783.7.

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl 5-O-acetyl-α-D-arabinofuranoside (6) Bu_2SnO (0.89 g, 3.6 mmol) was added to a solution of **5** (2.6 g, 3.4 mmol) in PhMe (60 mL), the solution refluxed for 18 h, after which half the amount of solvents was removed *in vacuo*. Ac_2O (0.34 mL, 3.6 mmol) was added to the reaction mixture, stirred for 2 h at rt, concentrated *in vacuo* and purified (SiO_2, pet. ether: EtOAc = 1:3) to afford **6**, as a gum (1.42 g, 52%). R_f (pet. ether:EtOAc = 1:4) 0.6; $[\alpha]_D$ +24.6 (c = 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=7.2 Hz, 6 H), 1.25 (br s, 52 H), 1.53–1.55 (m, 4 H), 2.10 (br s, 3 H), 3.41–3.65 (m, 19 H), 3.89 (br s, 2 H), 4.11–4.13 (m, 1 H), 4.23–4.27 (m, 2 H), 5.07 (d, *J*=2.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 20.8, 22.7, 26.0, 26.1, 29.3, 29.5, 29.6, 29.7, 30.0, 31.9, 64.2, 66.3, 70.3, 70.4, 70.6, 70.7, 71.2, 71.7, 77.7, 77.9, 80.5, 83.2, 108.1, 170.7; ESI-MS Calcd. for $C_{46}H_{90}O_{10}Na$ [M + Na]: 825.6432. Found 825.6434.

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl-β-D-[2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-D-arabinofuranosyl]-(1→2)-[β-D-(2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-D-arabinofuranosyl)]-(1→3) -5-O-acetyl-α-D-arabinofuranoside (8) A solution of **6** (0.12 g, 0.15 mmol), **7** [11] (0.22 g, 0.45 mmol) and MS 4 Å (0.3 g) in CH_2Cl_2 (10 mL) was stirred for 15 min, NIS (0.13 g, 0.6 mmol) and AgOTf (0.015 g, 0.06 mmol) were added at −40°C, under N_2 atmosphere, the reaction mixture was stirred for 30 min., neutralized with Et_3N, filtered, filtrate diluted with CH_2Cl_2 (20 mL), washed with satd. aq. sodium thiosulphate (2× 10 mL) and water (1×10 mL). The organic phase was dried (Na_2SO_4), filtered, filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 4:1) to afford **8**, as a gum (0.12 g, 54%). R_f (pet. ether/EtOAc 5:1) 0.6; $[\alpha]_D$ -31.7 (*c*=1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=6.4 Hz, 6 H), 0.98 (br s, 18 H), 1.05 (br s, 9 H), 1.06 (br s, 9 H), 1.25 (br s, 52 H), 1.53–1.56 (m, 4 H), 2.03 (s, 3 H), 3.40–3.59 (m, 18 H), 3.74–3.80 (m, 1 H), 3.84–3.94 (m, 4 H), 4.00 (d, *J*=6 Hz, 1 H), 4.12 (dd, *J*=5.6 Hz, *J*=12 Hz, 1 H), 4.17–4.19 (m, 1 H), 4.21–4.32 (m, 6 H), 4.67–4.71 (m, 2 H), 4.75–4.80 (m, 2 H), 4.99 (app s, 1 H), 5.01 (d, *J*=5.2 Hz, 1 H), 5.04 (d, *J*=5.2 Hz, 1 H), 7.28–7.36 (m, 10 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 20.1, 20.8, 22.5, 22.7, 26.1. 27.1, 27.5, 29.3, 29.5, 29.6, 29.7, 30.1, 31.9, 63.7, 66.6, 68.3, 70.3, 70.5, 70.6, 70.8, 70.9, 71.4, 71.7, 71.8, 71.9, 73.8, 77.9, 78.2, 78.3, 79.5, 80.8, 80.9, 84.0, 86.7, 100.3, 100.8, 106.8, 127.7, 127.9, 128.0,128.3, 137.8, 138.9, 170.7; ESI-MS Calcd. for $C_{86}H_{150}O_{18}Si_2Na$ [M + Na]: 1550.0 (100%), 1551.0 (96%). Found 1550.0 (100%), 1551.0 (96%).

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl-β-D-arabinofuranosyl-(1→2)-[β-D-arabinofuranosyl]-(1→3)-α-D-arabinofuranoside (1) TBAF (0.2 mL, 1 M in THF) was added to a solution of **8** (0.10 g, 0.065 mmol) in THF stirred at rt for 8 h, concentrated *in vacuo* and purified (SiO_2, EtOAc) to remove polar impurities. A solution of the reaction mixture in THF (10 mL) was treated with Pd–C (10%) (0.03 g), stirred while bubbling H_2 (g) (1 bar) for 18 h, the reaction mixture filtered and solvent removed *in vacuo*. NaOMe in MeOH (0.25 mL, 1 M) was added to a solution of the crude residue in MeOH (5 mL) and stirred for 2 h at rt, neutralized with Amberlite ion-exchange (H^+) resin, filtered, filtrate concentrated *in vacuo* and purified (SiO_2, EtOAc:MeOH = 9:1) to afford **1** (0.04 g, 61%). $[\alpha]_D$ -14.6 (*c*=1, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 0.90 (t, *J*= 6.4 Hz, 6 H), 1.29 (br s, 52 H), 1.54–1.57 (m, 4 H), 3.45–

3.81 (m, 25 H), 3.93–3.96 (m, 4 H), 4.08–4.09 (m, 1 H), 4.19 (d, *J*=5.6 Hz, 1 H), 4.49 (s, 1 H), 5.05 (d, *J*=3.6 Hz, 1 H), 5.08 (d, *J*=2.8 Hz, 1 H), 5.09 (app s, 1 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.5, 23.8, 27.3, 30.5. 30.6, 30.8, 31.1, 33.1, 62.7, 64.3, 65.0, 67.6, 71.4, 71.5, 71.6, 71.9, 72.0, 72.2, 72.6, 75.5, 76.0, 78.6, 78.8, 78.9, 79.3, 79.6, 84.4, 84.7, 85.5, 87.6, 102.6, 103.8, 107.9; ESI-MS Calcd. for $C_{54}H_{104}O_{17}Na$ [M + Na]: 1047.7171. Found 1047.7106.

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl 3-O-benzoyl-α-D-arabinofuranoside (10) A solution of **9** [7] (0.46 g, 1.03 mmol), **4** (0.65 g, 1.03 mmol) and MS 4 Å (0.3 g) in CH_2Cl_2 (20 mL) was stirred for 15 min., NIS (0.33 g, 1.5 mmol) and AgOTf (0.04 g, 0.15 mmol) were added at −40°C, under N_2 atmosphere. The reaction mixture was stirred for 30 min., neutralized with Et_3N, filtered, filtrate diluted with CH_2Cl_2 (50 mL), washed with satd. aq. sodium thiosulphate (2×20 mL), water (1×20 mL), dried (Na_2SO_4), filtered, filtrate concentrated *in vacuo*. AcCl (0.5 mL) was added to a solution of crude residue in MeOH/CH_2Cl_2 (1:1) (40 mL), stirred for 6 h at rt, diluted with CH_2Cl_2 (100 mL), washed with the satd. aq. $NaHCO_3$ (2×20 mL) solution, brine (20 mL) and water (20 mL). The organic phase was dried (Na_2SO_4), filtered, filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 7:3) to afford **10**, as a foamy solid (0.59 g, 66%). R_f (pet. ether: EtOAc = 1:3) 0.6; $[\alpha]_D$ +20.9 (*c*=1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=6.4 Hz, 6 H), 1.25 (br s, 52 H), 1.53–1.55 (m, 4 H), 3.40–3.71 (m, 16 H), 3.84–3.92 (m, 4 H), 4.22 (app s, 1 H), 4.32–4.34 (m, 2 H), 5.11 (d, *J*=4 Hz, 1 H), 5.16 (app s, 1 H), 7.43 (t, *J*=7.6 Hz, 2 H), 7.57 (t, *J*=7.6 Hz, 1 H), 8.03 (d, *J*=7.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.6, 26.0, 26.1, 29.3, 29.5, 29.6, 29.7, 29.9, 31.9, 61.9, 66.6, 70.5, 70.6, 70.8, 71.2, 71.7, 77.8, 79.4, 80.3, 83.1, 83.3, 108.4, 128.4, 129.4, 129.8, 133.3, 166.7; ESI-MS Calcd. for $C_{51}H_{92}O_{10}Na$ [M + Na]: 887.6. Found 887.3.

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl-β-D-[2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-D-arabinofuranosyl]-(1→2)-[β-D-(2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-D-arabinofuranosyl)]-(1→5)-3-O-benzoyl-α-D-arabinofuranoside (11) A solution of **10** (0.15 g, 0.17 mmol), **7** (0.25 g, 0.51 mmol) and MS 4 Å (0.3 g) in CH_2Cl_2 (10 mL) was stirred for 15 min, NIS (0.17 g, 0.75 mmol) and AgOTf (0.02 g, 0.075 mmol) were added at −40°C, under N_2 atmosphere. The reaction mixture was stirred at same temperature for 30 min., neutralized with Et_3N, filtered, filtrate diluted with CH_2Cl_2 (20 mL), washed with satd. aq. sodium thiosulphate (2×10 mL) and water (1×10 mL). The organic phase was dried (Na_2SO_4), filtered, filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 9:1) to afford **11**, as a gum (0.17 g, 62%). R_f (pet. ether:EtOAc = 9:1) 0.5; $[\alpha]_D$ -35.6 (*c*=1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J*=6.4 Hz, 6 H), 0.96 (br s, 9 H), 0.98 (br s, 9 H), 1.04 (br s, 9 H), 1.06 (br s, 9 H), 1.25 (br s, 52 H), 1.52–1.55 (m, 4 H), 3.39–3.64 (m, 17 H), 3.70–3.74 (m, 1 H), 3.78–3.80 (m, 1 H), 3.84–3.94 (m, 6 H), 4.13 (dd, *J*=9.2 Hz, *J*= 4.8 Hz, 1 H), 4.19 (d, *J*=2 Hz, 1 H), 4.24 (dd, *J*=9.2 Hz, *J*= 4.8 Hz, 1 H), 4.31 (t, *J*=9.2 Hz, 2 H), 4.40 (dt, *J*=9.6 Hz, *J*= 4.8 Hz, 1 H), 4.68–4.80 (m, 4 H), 5.02 (d, *J*=5.2 Hz, 1 H), 5.10 (d, *J*=5.2 Hz, 1 H), 5.13 (app s, 1 H), 5.31 (dd, *J*= 5.2 Hz, *J*=2.8 Hz, 1 H), 7.24–7.44 (m, 11 H), 7.55 (t, *J*= 7.2 Hz, 1 H), 8.00 (d, *J*=7.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 20.0, 22.5, 22.7, 26.1, 27.1, 27.5, 29.3, 29.5, 29.7, 29.8, 30.1, 31.9, 66.6, 68.2, 68.4, 69.3, 70.3, 70.5, 70.6, 70.8, 71.4, 71.5, 71.6, 71.9, 73.6, 73.8, 77.2, 77.8, 78.1, 78.2, 78.4, 80.6, 80.8, 81.4, 86.7, 100.7, 106.3, 127.5, 127.7, 127.9, 128.0, 128.2, 128.3, 129.7, 133.1, 137.8, 138.0, 165.6; MALDI-TOF MS Calcd. for $C_{91}H_{152}O_{18}Si_2Na$ [M + Na]: 1612.0 (100%), 1613.0 (98.4%). Found 1611.8 (100%), 1612.8 (98.4%).

8-O-hexadecyl-3,6,10-trioxa-1,8-hexacosanedioyl-β-D-arabinofuranosyl-(1→2)-[β-D-arabinofuranosyl]-(1→5)-α-D-arabinofuranoside (2) TBAF (0.3 mL, 1 M in THF) was added to a solution of **11** (0.15 g, 0.094 mmol) in THF stirred for 8 h at rt, concentrated *in vacuo* and purified (SiO_2, EtOAc) to remove polar impurities. A solution of the reaction mixture in THF (15 mL) was treated with Pd–C (10%) (0.04 g) and bubbled with H_2 (g) (1 bar) for 18 h, after which the reaction mixture was filtered, solvent removed *in vacuo.* NaOMe in MeOH (0.25 mL, 1 M) was added to a solution of the residue in MeOH (5 mL), stirred for 6 h at rt, neutralized with Amberlite ion-exchange (H^+) resin, filtered, filtrate concentrated *in vacuo* and purified (EtOAc:MeOH = 9:1) to afford **2** (0.052 g, 55%). $[\alpha]_D$ -11.8 (*c* 0.5, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 0.90 (t, *J*=6.4 Hz, 6 H), 1.29 (br s, 52 H), 1.54–1.58 (m, 4 H), 3.43–3.79 (m, 24 H), 3.81–3.84 (m, 1 H), 3.92–4.11 (m, 7 H), 4.91 (d, *J*=4.4 Hz, 1 H), 5.00 (d, *J*=4.4 Hz, 1 H), 5.04 (d, *J*=1.8 Hz, 1 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.4, 23.7, 27.3, 30.4, 30.6, 30.7, 30.8, 31.2, 33.1, 64.4, 64.6, 68.4, 68.9, 71.5, 71.6, 71.9, 72.0, 72.2, 72.6, 75.8, 76.1, 76.9, 78.9, 79.3, 82.4, 84.4, 84.6, 88.9, 102.4, 103.3, 107.7; ESI-MS Calcd. for $C_{54}H_{104}O_{17}Na$ [M + Na]: 1047.7. Found 1047.8.

Methyl 5-O-acetyl-α-D-arabinofuranoside (13) Bu_2SnO (0.62 g, 2.5 mmol) was added to a solution of **12** (0.41 g, 2.5 mmol) in MeOH (25 mL). The solution was refluxed for 18 h and concentrated *in vacuo*. Ac_2O (0.24 mL, 2.5 mmol) was added to the solution of the crude residue in CH_2Cl_2 and the reaction mixture was stirred at 35°C for 12 h, concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 1:4) to afford **13**, as a gum. Yield: 0.28 g (56%); R_f (EtOAc) 0.5; ^{1}H

NMR (400 MHz, $CDCl_3$) δ 2.08 (s, 3 H), 3.04 (d, *J*=4.8 Hz, 1 H), 3.16 (d, *J*=8.9 Hz, 1 H), 3.38 (s, 3 H), 3.85 (s, 1 H), 4.05 (s, 1 H), 4.15 (q, *J*=4.2 Hz, 1 H), 4.22–4.32 (m, 2 H), 4.87 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 55.1, 64.1, 77.9, 80.4, 82.9, 108.8, 171.0; ESI-MS Calcd. for $C_8H_{14}O_6Na$ [M + Na]: 229.0688, found 229.0685.

Methyl 2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-β-D-arabinofuranosyl-(1→2)-[-2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-β-D-arabinofuranosyl]-(1→3)-5-O-acetyl-α-D-arabinofuranoside (14) A solution of **13** (0.14 g, 0.68 mmol), **9** (0.99 g, 2.04 mmol) and MS 4 Å (0.3 g) in CH_2Cl_2 (10 mL) was stirred for 15 min., NIS (0.55 g, 2.45 mmol) and AgOTf (0.104 g, 0.41 mmol) were added at −40°C, under N_2 atmosphere. The reaction mixture was stirred for 30 min, neutralized with Et_3N, filtered and filtrate diluted with CH_2Cl_2 (50 mL), washed with satd. aq. sodium thiosulphate (2×10 mL) and water (1×10 mL). The organic phase was dried (Na_2SO_4), filtered and filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 9:1) to afford **14**, as a gum. Yield: 0.33 g (52%); R_f (pet. ether/EtOAc 9:1) 0.19; ^1H NMR (400 MHz, $CDCl_3$) δ 0.96 (br s, 9 H), 0.97 (br s, 9 H), 1.05 (br s, 18 H), 2.07 (s, 3 H), 3.36 (br s, 3 H), 3.53–3.60 (m, 1 H), 3.85–3.95 (m, 5 H), 4.01–4.18 (m, 6 H), 4.29–4.36 (m, 3 H), 4.62–4.85 (m, 5 H), 5.00 (d, *J*=5.2 Hz, 1 H), 5.15 (d, *J*=5.2 Hz, 1 H), 7.28–7.37 (m, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1, 20.8, 27.1, 27.5, 55.0, 63.2, 67.4, 68.2, 71.7, 71.9, 73.8, 78.2, 78.3, 79.1, 80.7, 81.4, 81.5, 87.5, 87.7, 100.3, 107.4, 127.5, 127.6, 127.7, 127.8, 128.0, 128.3, 137.8, 137.9, 170.7. ESI-MS Calcd. for $C_{48}H_{74}O_{14}Si_2Na$ [M + Na]: 953.4515, found 953.4514.

Methyl β-D-arabinofuranosyl-(1→2)-[β-D-arabinofuranosyl]-(1→3)-α-D-arabinofuranoside (15) TBAF (0.15 mL, 1 M in THF) was added to a solution of **14** (0.074 g, 0.08 mmol) in THF stirred at rt for 8 h, concentrated *in vacuo* and purified (SiO_2, EtOAc) to remove polar impurities. A solution of the reaction mixture in THF (10 mL) was treated with Pd–C (10%) (0.04 g) and stirred in the presence of H_2 (g) (1 bar) for 18 h. The reaction mixture was filtered, solvent removed *in vacuo*. NaOMe in MeOH (0.2 mL, 1 M) was added to a solution of the crude residue in MeOH (5 mL) at room temperature and stirred for 2 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and filtrate concentrated *in vacuo* and purified (SiO_2, EtOAc: MeOH = 9:1) to afford **3**. Yield: 0.019 g (55%); R_f (EtOAc/MeOH 9:1) 0.14; $[\alpha]_D$ +22 (*c* 1, MeOH); ^1H NMR (400 MHz, D_2O) δ 3.31 (s, 3 H), 3.56–4.06 (m, 14 H), 4.16–4.19 (m, 1 H), 4.96 (s, 1 H), 5.00–5.09 (m, 2 H); ^{13}C NMR (100 MHz, D_2O) δ 54.6, 60.5, 61.1, 63.0, 74.0, 76.1, 76.6, 80.6, 81.1, 81.8, 81.9, 82.3, 82.6, 83.9, 84.0, 85.0, 85.6, 100.7, 107.9; ESI-MS Calcd. for $C_{16}H_{28}O_{13}Na$ [M + Na]: 451.1428, found 451.1426.

Methyl 2,5-di-O-acetyl-α-D-arabinofuranoside (16) Bu_2SnO (0.62 g, 2.5 mmol) was added to a solution of **12** (0.41 g, 2.5 mmol) in MeOH (25 mL). The solution was refluxed for 18 h and concentrated *in vacuo*. Ac_2O (0.47 mL, 5 mmol) was added to the solution of the crude residue in CH_2Cl_2 and the reaction mixture was stirred at 35°C for 18 h, concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 1:1) to afford **16**, as a gum. Yield: 0.43 g (69%); R_f (EtOAc) 0.73; ^1H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 3 H), 2.11 (s, 3 H), 3.23 (d, *J*=5.9 Hz, 1 H), 3.40 (s, 3 H), 3.92 (d, *J*=2.4 Hz, 1 H), 4.17–4.21 (m, 2 H), 4.32 (d, *J*=8.2 Hz, 1 H), 4.83 (d, *J*=2.1 Hz, 1 H), 4.97 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 55.1, 63.6, 76.9, 81.6, 84.7, 106.3, 170.8, 171.1; ESI-MS Calcd. for $C_{10}H_{16}O_7Na$ [M + Na]: 271.0794, found 271.0792.

Methyl 3-O-benzoyl-α-D-arabinofuranoside (17) Pyridine (0.42 mL, 5.19 mmol) and BzCl (0.48 mL, 4.1 mmol) were added to a solution of **16** (0.43 g, 1.73 mmol) in CH_2Cl_2 (25 mL) at 0°C. The reaction mixture was stirred for 12 h**,** diluted with CH_2Cl_2 (100 mL), washed with dil. aq. HCl (2×25 mL) solution, satd. aq. $NaHCO_3$ solution (2×25 mL), water (25 mL), dried (Na_2SO_4), filtered and filtrate concentrated *in vacuo*. AcCl (0.5 mL) was added to a solution of the crude product in CH_2Cl_2/MeOH (1:1) (50 mL) and stirred at room temperature for 4 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with satd. aq. $NaHCO_3$ (2×20 mL) solution, brine (20 mL), water (20 mL), dried (Na_2SO_4), filtered and filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 3:1), to afford **17**, as a solid. Yield: 0.32 g (66%). R_f (pet. ether/EtOAc 1:1) 0.47; ^1H NMR (400 MHz, $CDCl_3$) δ 2.88 (br s, 1 H), 3.41 (br s, 3 H), 3.92–3.99 (m, 3 H), 4.28–4.33 (m, 2 H), 5.00 (app s, 1 H), 5.09 (d, *J*=4.0 Hz, 1 H), 7.44 (t, *J*= 7.6 Hz, 2 H), 7.58 (t, *J*=7.3 Hz, 1 H), 8.02 (d, *J*=7.7 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.0, 61.8, 79.8, 80.6, 83.0, 109.4, 128.5, 129.2, 129.8, 133.5, 167.0. ESI-MS Calcd. for $C_{13}H_{16}O_6Na$ [M + Na]: 291.0845, found 291.0849.

Methyl 2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-β-D-arabinofuranosyl-(1→2)-[-2-O-benzyl-3,5-O-(di-tert-butylsilanediyl)-β-D-arabinofuranosyl]-(1→5)-3-O-benzoyl-α-D-arabinofuranoside (18) A solution of **17** (0.07 g, 0.26 mmol), **9** (0.38 g, 0.78 mmol) and MS 4 Å (0.3 g) in CH_2Cl_2 (10 mL) was stirred for 15 min, NIS (0.21 g, 0.94 mmol) and AgOTf (0.04 g, 0.16 mmol) were added at −40°C, under N_2 atmosphere. The reaction mixture was stirred for 30 min., neutralized with Et_3N, filtered and

filtrate diluted with CH_2Cl_2 (50 mL), washed with satd. aq. sodium thiosulphate (2×10 mL) and water (1×10 mL). The organic phase was dried (Na_2SO_4), filtered and filtrate concentrated *in vacuo* and purified (SiO_2, pet. ether:EtOAc = 12:1) to afford **18**, as a gum. Yield: 0.13 g (49%); R_f (pet. ether/EtOAc 9:1) 0.24; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (br s, 9 H), 0.97 (br s, 9 H), 1.04 (br s, 9 H), 1.05 (br s, 9 H), 3.34 (br s, 3 H), 3.55–3.63 (m, 2 H), 3.74 (dd, *J*=11.2 Hz, *J*=7.6 Hz, 1 H), 3.85–3.94 (m, 5 H), 4.11–4.13 (m, 2 H), 4.22–4.26 (m, 1 H), 4.32 (t, *J*=9.2 Hz, 2 H), 4.38–4.44 (m, 1 H), 4.68–4.81 (m, 4 H), 5.00 (app s, 1 H), 5.05 (d, *J*= 5.2 Hz, 1 H), 5.10 (d, *J*=5.2 Hz, 1 H), 5.27–5.29 (m, 1 H), 7.28–7.44 (m, 12 H), 7.56 (t, *J*=7.6 Hz, 1 H), 8.00 (d, *J*=7.6 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.1, 27.1, 27.5, 54.9, 68.1, 68.3, 69.3, 71.5, 71.9, 73.6, 73.8, 78.1, 78.3, 78.4, 80.6, 80.9, 81.1, 85.9, 86.9, 100.7, 107.4, 127.5, 127.7, 127.9, 128.0, 128.2, 128.3, 129.6, 129.7, 133.1, 137.8, 165.6. ESI-MS Calcd. for $C_{53}H_{76}O_{14}Si_2Na$ [M + Na]: 1015.4671, found 1015.4673.

Methyl β-D-arabinofuranosyl-(1→2)-[-β-D-arabinofuranosyl]-(1→5)-α-D-arabinofuranoside (19) TBAF (0.2 mL, 1 M in THF) was added to a solution of **18** (0.11 g, 0.11 mmol) in THF stirred at rt for 8 h, concentrated *in vacuo* and purified (SiO_2, pet. Ether/EtOAc 1:9) to remove polar impurities. A solution of the reaction mixture in THF (10 mL) was treated with Pd–C (10%) (0.05 g) and stirred in the presence of H_2 (g) (1 bar) for 18 h. The reaction mixture was filtered, solvent removed *in vacuo.* NaOMe in MeOH (0.25 mL, 1 M) was added to a solution of the crude residue in MeOH (5 mL) at room temperature and stirred for 12 h, neutralized with Amberlite ion-exchange (H^+) resin, filtered and filtrate concentrated *in vacuo* and purified (SiO_2, EtOAc:MeOH = 9:1) to afford **19**. Yield: 0.026 g (57%); R_f (EtOAc/MeOH 4:1) 0.19; $[\alpha]_D$ +1.5 (*c* 2, MeOH); ^{1}H NMR (400 MHz, D_2O) δ 3.30 (s, 3 H), 3.52–4.04 (m, 15 H), 4.80 (s, 1 H), 4.90–4.95 (m, 2 H); ^{13}C NMR (100 MHz, D_2O) δ 54.8, 61.1, 62.9, 74.4, 74.6, 76.2, 76.3, 76.5, 80.6, 81.9, 82.0, 82.8, 83.8, 86.1, 100.5, 101.5, 108.2; ESI-MS Calcd. for $C_{16}H_{28}O_{13}Na$ [M + Na]: 451.1428, found 451.1429.

SPR studies The studies were conducted using a Biacore 3000 SPR instrument. A continuous flow of HEPES-P buffer was maintained over the sensor surface at a flow rate of 10 μl/min. The CM5 sensor chip was activated with an injection of a solution containing *N*-ethyl-*N* -(3-diethylaminopropyl) carbodiimide (EDC) (0.2 M) and *N*-hydroxysuccinimide (NHS) (0.05 M). Surfactant protein A (SP-A), purified from the lung lavage surfactant pellets of patients with alveolar proteinosis [21], in NaOAc buffer (100 μg/mL) was injected over the activated flow cell. The immobilization procedure was completed by an injection of ethanolamine hydrochloride (1 M) (70 μL), followed by a flow of the buffer (100 μL/min.), in order to eliminate physically adsorbed compounds. Synthetic arabinofuranoside glycolipids were dissolved in HEPES buffer and passed over flow cells and binding studies were performed. Glycolipids in buffer were passed for 300 s for the association phase and buffer alone for 300 s for the dissociation phase. The sensor chip was regenerated between each cycle using EDTA solution (5 mM) for 60 s, followed by an injection of buffer alone for 60 s. Primary sensorgrams were analyzed by 1:1 Langmuir model, fitted to separate k_{on} and k_{off}, using the BIA evaluation software.

Mycobacterial growth assay Mycobacterial growth assay was performed to check the effect of synthesized compounds on the growth profile of *M. smegmatis*. In this study, wild type *M. smegmatis* strain mc^{2}155, a rapidly growing mycobacterium was used as a model organism. Wild type *M. smegmatis* was exposed to 50 μg/ml and 100 μg/ml concentration of newly synthesized drugs and their effects on mycobacterial growth were monitored. Wild type *M. smegmatis* was grown in Middlebrook 7H9 broth (Difco) supplemented with 2% glucose and 0.05% tween 80 in shaking incubator at 37°C. Synthetic compounds were dissolved in sterile Milli-Q water and added after 8 h of inoculation. Growth pattern was observed up to 72 h by measuring the optical density (O.D.) at 600 nm as described earlier [5].

Sliding motility studies Sliding motility assay was performed to study spreading ability of mycobacteria on the moist surface in the presence of synthesized compounds as described earlier (Naresh *et al*., 2011). In this study, Middlebrook 7H9 broth (Difco) supplemented with 2% glucose was solidified with 0.3% high grade agarose (Sigma-Aldrich Inc., St. Louis, USA) in petriplates. Synthetic compounds were added to the media (50 and 100 μg/mL) before they solidified. Culture (10 μL) adjusted to OD 0.5 was placed at the center of petriplate and allowed to dry in the biosafety hood. Once dried, petriplates were further incubated at 37°C in humidified incubator and motility was monitored for 4–7 days.

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