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Tetrahedron 59 (2003) 10239–10248

TETRAHEDRON

Arabinofuranose disaccharide analogs as inhibitors of *Mycobacterium tuberculosis*

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Received 10 September 2003; revised 16 October 2003; accepted 16 October 2003

Abstract—Several octyl 5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside disaccharide analogs substituted at the 5-position of the non-reducing end sugar were synthesized and tested in vitro against *Mycobacterium tuberculosis* (*M.tb.*), *Mycobacterium avium* complex (MAC) as well as in a cell free assay system for arabinosyltransferase acceptor/inhibitor activity. A few compounds showed interesting inhibitory activity in the cell free assay as well as against the whole microorganism in vitro.
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1. Introduction

Tuberculosis (TB) is one of the primary killers worldwide, especially in developing nations, in spite of the availability of several potent antimycobacterial agents.[1] Annually, millions die worldwide due to this epidemic disease, and the problem is amplified by the apparent synergism with HIV. Furthermore, the appearance of multiple drug resistant (MDR-TB) forms of TB throughout the world hinders its control, and has raised the concern that this disease may once again resurface as an incurable disease even in developed nations.[2] MDR-TB is not only fatal; it is very expensive to treat and has become a significant economic burden for developing countries. The development of newer, faster acting and safer anti-tubercular agents as well as more effective vaccines are urgently needed to fight this devastating disease.[3] In particular, there is a critical awareness that new classes of drugs with mechanisms of action that are unique from the presently used anti-TB agents will be needed to treat drug resistant forms of the disease.[4] In fact, there are few treatment options for patients infected with the highly intractable forms of MDR-TB.[5] TB chemotherapy relies largely on mycobacterial-specific drugs inhibiting bacterial metabolism and the biosynthesis of the cell envelope.[6] Presently, the recommended therapy is six months treatment with the combination of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB). The prolonged therapy times and drug side effects have increased treatment noncompliance leading to the generation of drug resistant strains of *M.tb.*[7]

The bacterial cell wall has been a robust target for development of antimycobacterial drugs. For example, the two clinical antitubercular drugs INH and EMB act primarily through inhibition of cell wall biosynthesis, specifically targeting mycolic acid and AG synthesis, respectively.[8,9] The mycobacterial cell wall possesses a series of complex polysaccharides containing several unique monosaccharides in well defined linkages.[10] Predominantly, the sugars are mannopyranose (Man*p*), rhamnopyranose (Rha*p*), galactofuranose (Gal*f*) and arabinofuranose (Ara*f*) containing several different linkages built into four basic superstructural motifs, lipoarabinomannan (LAM), arabinomannan (AM), arabinogalactan (AG) and linker disaccharide.[10] The arabinan portion present in AM and AG is shown in Figure 1. These important carbohydrate components are critical for the cell wall integrity, and alteration at any site can lead to disturbance in cell wall biosynthesis. On the basis of the fact that Rha*p*, Gal*f* and Ara*f* are not found in mammalian cells, derivatives of structurally relevant saccharides containing these sugars might be acceptors/inhibitors that could hamper mycobacterial cell wall biosynthesis.

Recently, we have reported the syntheses and antimycobacterial activities of several 6,6′-dideoxytrehalose *N*,*N*′-dialkylamino- and 6,6′-bis(sulfonamido) analogs that might possibly interfere with mycolylation pathways in the mycobacterial cell wall.[11] With the goal of developing novel and selective antitubercular agents with mechanisms unique from current clinical agents such as EMB that target

Keywords: *Mycobacterium tuberculosis*; glycosyltransferase; arabinofuranose; inhibitors.
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0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.10.054

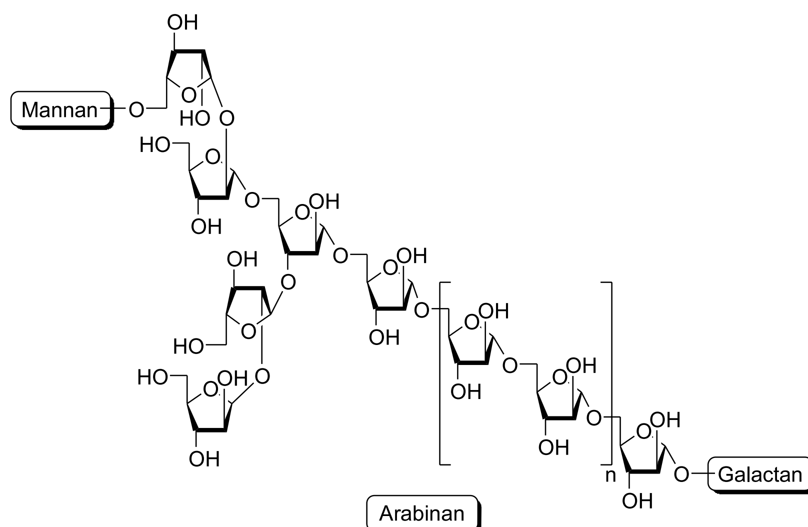


Figure 1. Arabinan motif present in *M. tb.* cell wall polysaccharides.

the AG,[9] we have also prepared several different disaccharides containing Rha*p*, Gal*f* and Ara*f* that possess acceptor/inhibitor capability.[12–17] Specifically, octyl disaccharides of Gal*f* and Ara*f* containing free hydroxyl groups, similar to natural substrates, have shown acceptor capability with inhibitor activity, albeit modest, in a cell free assay system. Partially blocked disaccharides at the non-reducing end have also shown modest inhibitory activity.[12–15] Herein we have synthesized a small number of disaccharides possessing substitutions at the 5-position of the reducing sugar of octyl Ara*f*(α1→5)Ara*f* disaccharide template (Fig. 2) with the intention of probing the active, catalytic site-binding region of the mycobacterial arabinosyltransferase(s). This approach is based on the fact that enzymes that synthesize and utilize Ara*f* are essential to mycobacteria and the premise that agents specifically designed against these targets will result in highly selective and potent anti-tubercular drugs.

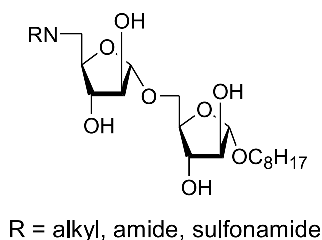


Figure 2. General structure of octyl Ara*f*(α 1→5)Ara*f* disaccharide derivatives.

2. Result and discussion

Utilization of the α(1→5)-linked Ara*f*–Ara*f* disaccharide substrates by the appropriate mycobacterial arabinosyltransferase would involve the addition of an Ara*f* unit at the 5-position of the non-reducing end of the disaccharide. Herein, we have prepared 1-*O*-octyl-Ara*f*(α1→5)Ara*f* disaccharides (**12**–**25**) possessing the general structure as shown in Figure 2 in order to probe the effects of modification in the catalytic site-binding region of a typical substrate disaccharide.

2.1. Approach

In designing a synthetic route for the disaccharide derivatives with the general structure depicted in Figure 2, we endeavored to develop a reasonably adaptable and efficient approach. We have previously synthesized the disaccharide octyl 5-(α-D-arabinofuranosyl)-α-D-arabinofuranose **1**[14] (Fig. 3), and we attempted to introduce a leaving group (tosyl or triflate) at the 5-position of the reducing end (5′-position) to afford structure **2**. The amino group could then be introduced via azide displacement and reduction. Unfortunately, tosylation resulted in little or no substitution, and under harsher conditions di- and tri-substituted products were formed. As a result of the poor results with tosylation of the disaccharide as an approach for introduction of the amine function, we decided to incorporate the incipient amine function as an azido group into the glycosyl donor first. Hence, the building blocks **8** (the glycosyl donor possessing an azido group at the

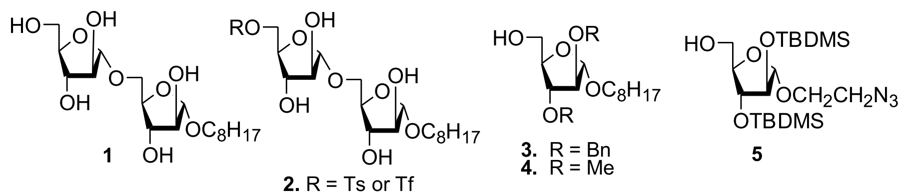
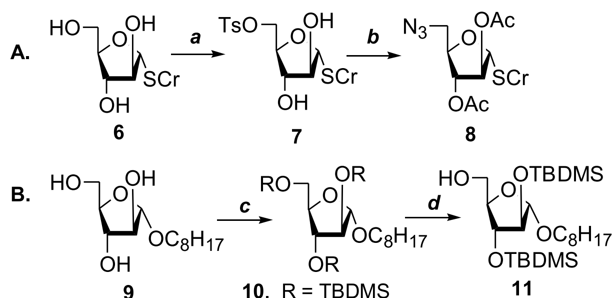


Figure 3.

5-position) and **11** (the acceptor) were utilized to prepare disaccharide **12**.

2.1.1. Synthesis of building blocks. We have extensively used thiocresyl glycosides possessing ester-protecting groups as glycosyl donors for 1,2-*trans* glycosylation in both the galactofuranose and arabinofuranose disaccharide series.[13–15] Utilizing a similar approach we synthesized the donor sugar **8**. The synthesis of both **8** and **11** are outlined in Scheme 1.

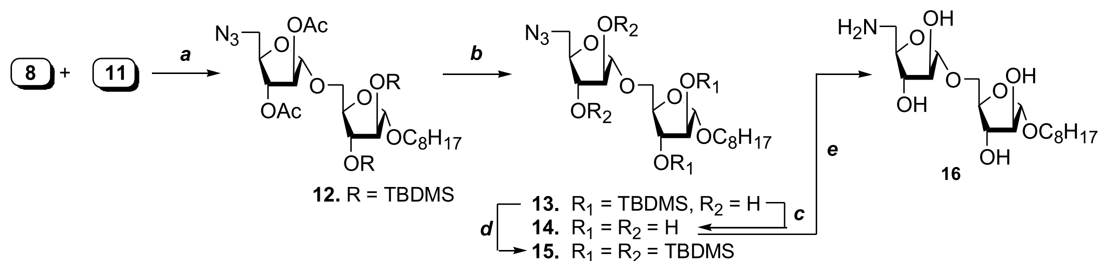


Scheme 1. Synthesis of building blocks **8** and **11**. *Reagents and conditions.* (a) TsCl, Py, 0°C, 4 h, 49%; (b) NaN_3, DMF, 50°C, overnight, Ac_2O, Py, overnight, 69%; (c) TBDMSCl, imidazole, DMF, rt, 6 h, 96%; (d) TFA–water (1:1), THF, −4°C, 4 h, 74%.

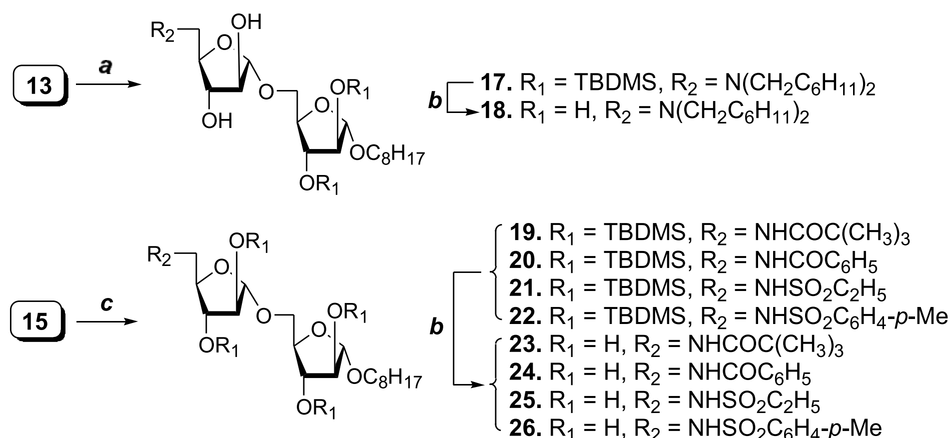
2.1.1.1. Building block 8. To access **8** we started with 1-thiocresyl-α-D-arabinofuranose **6** as reported,[18] and in a simple reaction sequence the 5-hydroxyl group was selectively tosylated with tosyl chloride in pyridine at 0°C to afford **7** in 49% yield. In this reaction, unreacted starting material **6** was recovered during purification by column chromatography. The compound **7** was then heated overnight at 50°C with sodium azide in DMF and, after concentration in vacuo, the crude material was dissolved in dry pyridine and treated with acetic anhydride at room temperature. After standard workup and purification by column chromatography, pure donor **8** was obtained in 69% yield. The ^{1}H NMR and ^{13}C NMR spectral data showed the anomeric proton at 5.33 ppm as a doublet possessing $J_{1,2}$=1.8 Hz, and the anomeric carbon appeared at 91.2 ppm respectively supporting the 1,2-*trans* configuration as compared with sugar derivative **6** reported in literatue.[18]

2.1.1.2. Building block 11. In a previous communication, we described the synthesis of similar arabinofuranosyl acceptors **3** and **4** used in the synthesis of disaccharide **1** (Fig. 3).[14] During the synthesis of arabinofuranose disaccharides as photoaffinity probes, however, we have demonstrated an effective route to prepare acceptor **5** (Fig. 3) possessing a free hydroxyl group at the 5-position, available to undergo glycosylation.[15] Utilizing similar methodology, we prepared acceptor **11** starting from the earlier synthesized octyl α-D-arabinofuranose **9**[14] as shown in Scheme 1. First, all the three hydroxyl groups present were protected with a *tert*-butyldimethylsilyl (TBDMS) functionality via the reaction with TBDMSCl in dimethylformamide in the presence of imidazole at room temperature to afford **10** in 96% yield. The selective deblocking of the TBDMS group at the 5-position was successfully achieved in high yields using trifluoroacetic acid in water (1:1) at −4°C. After 4 h of reaction followed by column chromatographic purification, acceptor **11** was obtained in 74% yield. The α-configuration at the anomeric center of **11** was assigned on the basis of the ^{1}H NMR spectrum that showed the anomeric proton at 4.79 ppm as a doublet with a $J_{1,2}$ coupling constant value of 1.4 Hz.

2.1.1.3. Glycosylation reaction and analog preparation. As outlined in Scheme 2, 1,2-*trans* glycosylation was carried out by the reaction between alcohol **11** and glycosyl donor **8** in presence of *N*-iodosuccinimide and the Lewis acid promoter tin(II) triflate in dry dichloromethane. The reagents were added at 0°C, and the reaction was carried out at rt for 45 min. After column chromatographic purification, pure disaccharide **12** was obtained in 69% yield. ^{1}H NMR spectra confirmed the α-glycosylation as H-1′ was observed at 5.14 ppm as a singlet ($J_{1',2'}$=0 Hz), whereas the signal for H-1 was seen at 4.77 ppm with $J_{1,2}$=1.9 Hz. Disaccharide **12** was deacetylated using 7N NH_3/MeOH at room temperature to afford partially blocked disaccharide **13**. The totally deblocked disaccharide **14** possessing an azido group at the 5-position of the non-reducing end sugar was obtained by desilylating **13** with tetraethylammonium fluoride in THF in excellent yield. The ^{1}H NMR spectrum showed the H-1 proton as a doublet at 4.83 ppm with $J_{1,2}$=1.7 Hz, whereas the H-1′ proton was observed as a doublet at 4.95 ppm with $J_{1,2}$=1.6 Hz, confirming the α-anomeric configuration in disaccharide **14**. The azido group in disaccharide **14** was reduced to an amino functionality by reduction with ammonium formate over 10% Pd/C in methanol at room temperature. Purification on a short Bio-Beads™ SM-4 (20–50 mesh) column using 5% methanol in water gave the amino disaccharide **16** in 64% yield. Disaccharide analogs **19–22** (Scheme 3), on the other hand, were prepared via intermediate disaccharide **15**, synthesized from **13**. The disaccharide **13** was treated with TBDMSCl in the presence of imidazole using DMF as the solvent at 50°C to block both the 2-OH and 3-OH at the reducing end, which was utilized further for derivatization at the 5′-position of this disaccharide.



Scheme 2. Glycosylation reaction. *Reagents and conditions.* (a) NIS, $Sn(OTf)_2$, CH_2Cl_2, 0°C–rt, 45 min, 69%; (b) 7N NH_3/MeOH, MeOH, rt, 6 h, 74%; (c) $Et_4N^+F^-$, THF, rt, overnight, 96%; (d) TBDMSCl, imidazole, DMF, 50°C, overnight, 79%; (e) HCO_2NH_4, 10% Pd/C, MeOH, rt, 4 h, 64%.



Scheme 3. 5′-substututed Ara*f*(α1→5)Ara*f* disaccharides. *Reagents and conditions.*(a) $C_6H_{11}CHO$, 10% Pd/C, MeOH, rt, 1 h, 87%; (b) $Et_4N^+F^-$, THF, rt, overnight, **18**: 96%, **23**: 77%, **24**: 91%, **25**: 95%, **26**: 88%; (c) HCO_2NH_4, 10% Pd/C, MeOH, rt, 2 h, RCOCl/RSO_2Cl, *N*-methyl imidazole, MeOH, 0°C, 8–12 h, **19**: 86%, **20**: 77%, **21**: 72%, **22**: 73%.

The disaccharide analog **17** was prepared by reductive amination. Compound **13** was reacted with cyclohexanecarboxyaldehyde in DMF over 10% Pd/C under an H_2 atmosphere to afford analog **17** possessing dicyclohexyl groups attached at the 5′-amino group. The desilylation of **17** gave analog **18** in excellent yield. The ^1H NMR and ESI-MS supported the expected structure as the anomeric protons H-1 and H-1′ were observed at 4.76 ppm as a doublet $J_{1,2}$=1.4 Hz and at 5.01 ppm as a singlet, respectively. For the preparation of disaccharide analogs **19–22**, the 5′-azido group in **15** was reduced by reacting with ammonium formate over 10% Pd/C in methanol. The resulting amino analog was used in the ensuing coupling reactions without further purification. In a typical reaction sequence, the reduced disaccharide was reacted with 1.5 equiv. of the appropriate acid chloride or sulfonyl chloride in methanol for 2 h. After the usual workup and purification by column chromatography, pure analogs **19–22** were obtained in good overall yields. The fully deblocked disaccharide analogs **23–26** were obtained by desilylating with $Et_4N^+F^-$ in dry THF followed by column chromatographic purification.

Table 1. Biological data on 5′-substituted Ara*f*(α1→5)Ara*f* disaccharides

Compounds	In vitro against *M. tb.* MIC in μg/mL	Cell Free assay IC_{50} in mM
14	>12.8	2.93
15	>12.8	–
16[a]	>12.8	–
17	>12.8	–
18	8	1.56
19	>12.8	–
20	>12.8	–
21	>12.8	–
22	>12.8	–
23	>12.8	2.49
24	>12.8	2.25
25	>12.8	3.55
26	>12.8	1.82
Ethambutol	2–4	–

[a] In intial screening (3.6 mM with control acceptor at 0.4 mM), no inhibition was observed hence no IC_{50} was measured.

All new compounds were characterized using ^1H NMR and ESI-MS spectral techniques. The NMR values of mono- and di-saccharides were compared with the values reported in the literature.[14–19] Whenever needed, NOE, decoupling and D_2O exchange experiments were performed in order to confirm NMR assignments.

2.2. Biological activity

2.2.1. In vitro activity. The disaccharide analogs were screened in an in vitro bacterial growth inhibition assay against *M.tb.* strain H37Ra.[20] The results are tabulated in Table 1. Compound **18**, possessing dicyclohexylamino substitution at the 5′-position of the disaccharide, gave good inhibition with an MIC of 8 μg/mL. This MIC compares favorably with that of EMB (MIC 2–4 μg/mL), the frontline antitubercular drug targeting the mycobacterial arabinan. Interestingly, compound **18** also showed inhibitor activity against *M. avium* complex (MAC) NJ211 with an MIC of 16 μg/mL.

2.2.2. Cell free assay. The fully deblocked disaccharide analogs were tested in the cell-free enzymatic arabinosyltransferase acceptor assay. Based on the previous use of specific arabinose-based neoglycolipid acceptors[14] compounds **14**, **16**, **18**, **23**, **24–26** were synthesized and compared as potential acceptors of [^{14}C]Ara*f* from DP-[^{14}C]A in the transferase assay.[21] Assays performed in the presence of membranes resulted in [^{14}C]Ara*f* incorporation from DP-[^{14}C]A only for the control α(1→5)-linked octyl arabinofuranosyl disaccharide,[14] and as predicted compounds blocked at the accepting hydroxyl group on the non-reducing end of the disaccharide did not show any detectable acceptor activity. They did, however, have promising inhibitory activity at a concentration of 3.6 mM (with control acceptor at 0.4 mM). Further competition-based experiments established that several compounds were effective as inhibitors. Specific IC_{50} values were determined and are shown in Table 1.

3. Conclusion

Within this small set were included basic (amine), poorly

acidic (carboxamide), and relatively acidic (sulfonamide) groups that may interact with acidic/basic residues within the catalytic glycosyltransferase active site pocket. Compound **18**, possessing a sterically large dicyclohexylamino substitution at the 5′-position of the inhibitor disaccharide, showed the most promising activity. It is also notable that there are few reports[22–24] showing that substitution of this site within the catalytic pocket of glycosyltransferases is tolerated and can lead to inhibitors. Our results verify earlier data, and suggest that there may be significant steric tolerance for substitution of the acceptor portion of these compounds within the catalytic active site. Furthermore, although it has been hypothesized that substitution at this position with basic amino groups may be ideal to interact with COOH functions in the catalytic active site,[23] our data with this particular transferase do not necessarily bear that out. For example, the amino-substituted disaccharide **16** showed no inhibition in the initial transferase assay. Although the dicyclohexylmethylamino derivative **18**, a basic substitution, was the most active with an IC_{50} of 1.56 mM, other analogs (e.g. **26**—IC_{50} 1.82 mM) that contain carboxamides or sulfonamides are comparably active. Hydrophobicity may play a more important role for this particular glycosyltransferase in inhibition of these types of disaccharides than specific interactions within the catalytic active site. The present study will be useful for the preparation of second-generation disaccharide analogs as inhibitors of *M.tb.*that may better utilize the active site cavity and take advantage of specific chemistry therein.

4. Experimental

4.1. Synthesis

All reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents from Aldrich were used in the reactions as such. Whenever necessary, compounds and starting materials were dried by azeotropic removal of water with toluene under reduced pressure. In the case of $Et_4N^+F^-$, however, this material was purchased from Aldrich as the hydrated form and was used as such. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spray with $(NH_4)_2SO_4$ solution (150 g ammonium sulfate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for workup and chromatography were reagent grade from Fisher Scientific. Flash chromatography was carried out on Fischer silica gel 60 (230–400 mesh). Melting points were determined with a Mel-Temp II capillary melting points apparatus and is uncorrected. ^1H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 MHz and 75 MHz respectively. Coupling constants (J) are reported in Hz and chemical shifts are in ppm (δ) relative to residual solvent peak or internal standard. ESI-MS was recorded on a BioTof-2 time-of-flight mass spectrometer.

4.1.1. 1-Deoxy-1-thiocresyl-5-tosyl-α-D-arabinofuranoside (7). 1-Deoxy-1-thiocresyl-α-D-arabinofuranoside **6**[18] (5.00 g, 19.53 mmol) was dissolved in dry pyridine (20 mL), cooled to 0°C under argon, and tosyl chloride (4.47 g, 23.44 mmol) was added. The reaction mixture was stirred overnight at 0°C and 4 h at rt. The reaction mixture was poured into an ice-water mixture and extracted with $CHCl_3$ (2×75 mL). The organic layer was dried over Na_2SO_4 and concentrated to a syrup. Column chromatography using $CHCl_3$/MeOH (9:1) as the eluant of the crude product gave purified compound **7** (3.92 g, 49%) as a colorless oil. MS-ESI (LiCl): m/z 417 $[M+Li]^+$. ^1H NMR ($CDCl_3$): δ 7.77 (2H, dd, J=1.8, 6.6 Hz, Ar), 7.35–7.29 (4H, m, Ar), 7.09 (2H, d, J=7.9 Hz), 5.20 (1H, d, J=2.6 Hz, H-1), 4.23 (2H, d, J=3.1 Hz, H_2-5), 4.13–4.10 (3H, m, H-2, H-3, H-4), 2.42 (3H, s, CH_3), 2.32 (3H, s, CH_3). ^{13}C NMR ($CDCl_3$): δ 145.09, 137.69 (C), 132.24 (2×CH), 132.18 (C), 129.86, 129.68 (2×CH), 129.59 (C), 127.91 (2×CH), 91.14 (C-1), 81.21 (C-2), 79.54 (C-3), 76.46 (C-4), 68.62 (C-5), 21.57 (CH_3), 21.04 (CH_3).

4.1.2. 1,5-Dideoxy-2,3-di-*O*-acetyl-5-azido-1-thiocresyl-α-D-arabinofuranoside (8). Compound **7** (3.83 g, 9.34 mmol) was dissolved in dry DMF (20 mL) and NaN_3 (911 mg, 14.01 mmol) was added. The reaction mixture was heated at 50°C overnight, and was then concentrated in vacuo to a syrup. The syrup was dissolved in dry pyridine (15 mL) and Ac_2O (1.98 mL, 21.00 mmol) was added. The reaction mixture was stirred overnight at rt and poured into an ice-water mixture. It was extracted with $CHCl_3$ (2×75 mL), the organic layer was dried over Na_2SO_4 and concentrated to a syrup. Column chromatography on silica gel using $CHCl_3$/MeOH (99:1) gave pure compound **8** as a colorless oil (2.15 g, 69%). MS-ESI: m/z 388.21 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ 7.42–7.39 (2H, m, Ar), 7.14 (2H, d, J=7.9 Hz), 5.33 (1H, d, $J_{1,2}$=1.8 Hz, H-1), 5.28 (1H, t, $J_{1,2}$=$J_{2,3}$=1.8 Hz, H-2), 5.07–5.04 (1H, m, H-3), 4.46–4.42 (1H, m, H-4), 3.67 (1H, dd, $J_{4,5a}$=3.0 Hz, $J_{5a,5b}$=13.3 Hz, H-5a), 3.49 (1H, dd, $J_{4,5b}$=4.9 Hz, $J_{5a,5b}$=13.3 Hz, H-5b), 2.34 (3H, s, CH_3), 2.13, 2.12 (3H each, s, 2×OCH_3). ^{13}C NMR ($CDCl_3$): δ 170.16, 169.67 (2×C=O), 138.12 (C), 132.67, 129.84 (4×CH), 129.48 (C), 91.20 (C-1), 81.65 (C-4), 81.48 (C-2), 77.80 (C-3), 51.12 (C-5), 21.11 (CH_3), 20.72 (2×OCH_3).

4.1.3. Octyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (10). Octyl α-D-arabinofuranoside **9**[14] (2.00 g, 7.63 mmol) was dissolved in dry DMF (10 mL) and TBDMSCl (4.01 g, 26.71 mmol) was added followed by imidazole (1.56 g, 22.89 mmol). The reaction mixture was stirred at rt for 6 h and was poured into an ice-water mixture. Extraction with $CHCl_3$ (3×50 mL) followed by drying on Na_2SO_4 and concentration gave the crude product as a syrup. Column chromatography on silica gel using cyclohexane/EtOAc (96:4) gave pure compound **10** as a colorless oil (4.43 g, 96%). MS-ESI: m/z 627.38 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ 4.74 (1H, d, $J_{1,2}$=1.6 Hz, H-1), 4.00 (1H, dd, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.5 Hz, H-2), 3.96 (1H, dd, $J_{2,3}$=3.5 Hz, $J_{3,4}$=5.7 Hz, H-3), 3.91–3.86 (1H, m, H-4), 3.77–3.63 (3H, m, H_2-5, OCH_2), 3.37–3.30 (1H, m, OCH_2), 1.56–1.51 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.90–0.86 (30H, m, 10×CH_3), 0.082, 0.077, 0.072, 0.064 (s, 6×CH_3).

4.1.4. Octyl 2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (11). Compound **10** (4.00 g, 6.62 mmol) was dissolved in dry THF (20 mL) and cooled to −4°C. Ten

(10) mL of a TFA–water mixture (1:1) was added, and the reaction mixture was stirred at −4°C for 4 h. The reaction mixture was then poured into an ice-water mixture and extracted with $CHCl_3$ (2×50 mL). The organic layer was dried over Na_2SO_4 and concentrated to a syrup. Column chromatography on silica gel using cyclohexane/EtOAc (95:5) gave pure compound **11** as a colorless oil (2.65 g, 74%). MS-ESI: *m/z* 514.34 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ 4.79 (1H, d, $J_{1,2}$=1.4 Hz, H-1), 4.06–3.94 (3H, m, H-2, H-3, H-4), 3.82 (1H, ddd, $J_{4,5a}$=2.3 Hz, $J_{5a,5\text{-}OH}$=4.5 Hz, $J_{5a,5b}$=12.1 Hz, H-5a), 3.73–3.60 (2H, m, H-5b, OC*H*$_2$), 3.39–3.31 (1H, m, OC*H*$_2$), 2.01 (1H, dd, $J_{5a,5\text{-}OH}$=4.5 Hz, $J_{5b,5\text{-}OH}$=7.5 Hz, 5-OH), 1.59–1.52 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.91–0.87 (21H, m, 7×CH_3), 0.102, 0.092, 0.087, 0.069 (each 3H, s, 4×CH_3).

4.1.5. Octyl 5-*O*-(5-deoxy-5-azido-2,3-di-*O*-acetyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (12). Compound **11** (2.24 g, 4.57 mmol), activated powdered 4 Å molecular sieves (800 mg), and glycosylation donor **9** (2.00 g, 5.48 mmol) in dry CH_2Cl_2 (25 mL) were cooled at 0°C under an Argon atmosphere. The mixture was stirred for 15 min, and NIS (1.23 g, 5.48 mmol) followed by $Sn(OTf)_2$ (287 mg, 0.69 mmol) were added to initiate coupling. The reaction mixture was allowed to stir for 30 min at rt, and the reaction was quenched by addition of Et_3N (1.5 mL), diluted with CH_2Cl_2 (50 mL) and filtered through a celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (2×15 mL), followed by washing with saturated aqueous $NaHCO_3$ (25 mL). The organic layer was dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by column chromatography (cyclohexane/EtOAc 3:1) to give disaccharide **12** as a colorless oil (2.31 g, 69%). MS-ESI: *m/z* 754.32 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ 5.17 (1H, d, $J_{2',3'}$=1.3 Hz, H-2′), 5.14 (1H, s, H-1′), 4.95 (1H, dd, $J_{2',3'}$=1.3 Hz, $J_{3',4'}$=4.8 Hz, H-3′), 4.77 (1H, d, $J_{1,2}$=1.9 Hz, H-1), 4.22 (1H, ddd, $J_{4',5'a}$=3.0 Hz, $J_{3',4'}$=4.8 Hz, $J_{4',5'b}$=4.9 Hz, H-4′), 4.01 (1H, dd, $J_{1,2}$=1.9 Hz, $J_{2,3}$= 3.7 Hz, H-2), 3.99–3.92 (2H, m, H-3, H-4), 3.80 (1H, dd, $J_{4,5a}$=4.1 Hz, $J_{5a,5b}$=11.0 Hz, H-5a), 3.72–3.60 (3H, m, H-5b, H-5′a, OC*H*$_2$), 3.44 (1H, dd, $J_{4',5'b}$=4.9 Hz, $J_{5'a,5'b}$= 13.2 Hz, H-5′b), 3.37–3.30 (1H, m, OC*H*$_2$), 4.77, 3.96 (each 3H, s, 2×OCH_3), 2.09 (2H, m, CH_2), 2.08 (10H, br s, 5×CH_2), 1.69–1.27 (21H, m, 7×CH_3), 0.897, 0.879, 0.857 (each s, 4×CH_3).

4.1.6. Octyl 5-*O*-(5-deoxy-5-azido-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (13). To a solution of compound **12** (2.00 g, 2.73 mmol) in dry methanol (20 mL) was added 7N NH_3/MeOH (50 mL). The reaction mixture was stirred at room temperature for 6 h and concentrated in vacuo to a syrup. Chromatography on silica gel using cyclohexane/EtOAc (9:1) gave pure compound **13** as a colorless oil (1.31 g, 74%). MS-ESI: *m/z* 670.44 $[M+Na]^+$. ^1H NMR ($CDCl_3$, D_2O exchanged): δ 5.08 (1H, s, H-1′), 4.76 (1H, d, $J_{1,2}$= 1.3 Hz, H-1), 4.17 (1H, ddd, $J_{4',5'a}$=2.8 Hz, $J_{3',4'}$=3.7 Hz, $J_{4',5'b}$=3.8 Hz, H-4′), 4.08 (1H, br s, H-2′), 4.03–3.97 (2H, m, H-2, H-4), 3.87–3.78 (3H, m, H-3, H-5a, H-3′), 3.67–3.59 (4H, m, H-5b, H_2-5′, OC*H*$_2$), 3.53–3.28 (1H, m, OC*H*$_2$), 1.60–1.50 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.90–0.86 (21H, m, 7×CH_3), 0.093, 0.081, 0.058 (each s, 4×CH_3).

4.1.7. Octyl 5-*O*-(5-deoxy-5-azido-α-D-arabinofuranosyl)-α-D-arabinofuranoside (14). Compound **13** (50 mg, 0.06 mmol) was dissolved in dry THF (4 mL) and $Et_4N^+F^-$ (52 mg, 0.34 mmol) was added. The reaction mixture was stirred overnight and concentrated to a syrup. Column chromatography ($CHCl_3$/MeOH, 8:1) gave compound **14** as a colorless oil (23 mg, 96%). MS-ESI: *m/z* Found 442.2176 $[M+Na]^+$, calcd 442.2159 for $C_{18}H_{33}N_3O_8$. ^1H NMR (CD_3OD): δ 4.95 (1H, dd, $J_{1',2'}$=1.6 Hz, H-1′), 4.83 (1H, d, $J_{1,2}$=1.7 Hz, H-1), 4.04 (1H, ddd, $J_{4',5'a}$=3.5 Hz, $J_{4',5'b}$= 6.2 Hz, $J_{3',4'}$=6.6 Hz, H-4′), 4.00 (1H, dd, $J_{1',2'}$=1.6 Hz, $J_{2',3'}$=3.8 Hz, H-2′), 4.03–3.99 (1H, m, H-4), 3.94 (1H, dd, $J_{1,2}$=1.7 Hz, $J_{2,3}$=3.9 Hz, H-2), 3.87 (1H, dd, $J_{2',3'}$=3.8 Hz, $J_{3',4'}$=6.6 Hz, H-3′), 3.86–3.79 (2H, m, H-3, H-5a), 3.73–3.65 (1H, m, OCH_2), 3.65 (1H, dd, $J_{4,5b}$=3.6 Hz, $J_{5a,5b}$= 11.1 Hz, H-5b), 3.50 (1H, dd, $J_{4',5'a}$=3.3 Hz, $J_{5'a,5'b}$=13.3 Hz, H-5′a), 3.44–3.36 (2H, m, OCH_2), 3.37 (1H, dd, $J_{4',5'b}$=6.2 Hz, $J_{5'a,5'b}$=13.3 Hz, H-5′b), 1.62–1.53 (2H, m, CH_2), 1.30 (10H, br s, 5×CH_2), 0.92–0.87 (3H, m, CH_3).

4.1.8. Octyl 5-*O*-(5-deoxy-5-azido-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (15). Compound **13** (670 mg, 1.03 mmol) was dissolved in dry DMF (10 mL) and TBDMSCl (390 mg, 2.60 mmol) was added followed by imidazole (280 mg, 4.12 mmol). The reaction mixture was stirred overnight at 50°C and was then poured into an ice-water mixture. Extraction with $CHCl_3$ (3×15 mL) followed by drying on Na_2SO_4 and concentration gave the crude product as a syrup. Column chromatography on silica gel using cyclohexane/EtOAc (95:5) gave pure compound **15** as a colorless oil (637 mg, 79%). MS-ESI: *m/z* Found 898.5594 $[M+Na]^+$, calcd 898.5618 for $C_{42}H_{89}N_3O_8Si_4$. ^1H NMR ($CDCl_3$): δ 4.86 (1H, dd, $J_{1',2'}$=1.8 Hz, H-1′), 4.74 (1H, d, $J_{1,2}$=1.9 Hz, H-1), 4.08 (1H, dd, $J_{1',2'}$=1.8 Hz, $J_{2',3'}$=4.0 Hz, H-2′), 4.05–3.99 (1H, m, H-4′), 4.00 (1H, dd, $J_{1,2}$=1.9 Hz, $J_{2,3}$=4.0 Hz, H-2), 3.98–3.90 (3H, m, H-3, H-4, H-3′), 3.79 (1H, dd, $J_{4,5a}$=4.5 Hz, $J_{5a,5b}$=11.0 Hz, H-5a), 3.72–3.65 (1H, m, OC*H*$_2$), 3.56 (1H, dd, $J_{4,5b}$= 3.1 Hz, $J_{5a,5b}$=11.0 Hz, H-5b), 3.48 (1H, dd, $J_{4',5'a}$=3.3 Hz, $J_{5'a,5'b}$=13.2 Hz, H-5′a), 3.36–3.26 (2H, m, H-5′b, OC*H*$_2$), 1.60–1.50 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.91–0.86 (39H, m, 13×CH_3), 0.097, 0.093, 0.083, 0.077, 0.074, 0.064 (each s, 8×CH_3).

4.1.9. Octyl 5-*O*-(5-deoxy-5-amino-α-D-arabinofuranosyl)-α-D-arabinofuranoside (16). Compound **15** (30 mg, 0.07 mmol) was dissolved in MeOH (5 mL) and 10% Pd/C (15 mg) was added followed by HCO_2NH_4 (18 mg, 0.29 mol). The reaction mixture was stirred 4 h at rt and filtered through celite. The solvent was evaporated and the resulting syrup was purified by passing the aqueous solution (syrup in 5 mL) through a small column of Bio-Beads™ SM-4 (20–50 mesh) and eluting with H_2O–MeOH (5%) to yield compound **16** as a colorless oil (18 mg, 64%). MS-ESI: *m/z* Found 416.2234 $[M+Na]^+$, calcd 416.2254 for $C_{18}H_{35}NO_8$. ^1H NMR (MeOH-d$_4$): δ 4.98 (1H, s, H-1′), 4.83 (1H, d, $J_{1,2}$=1.7 Hz, H-1), 4.06–3.98 (3H, m, H-4, H-2′ H-4′), 3.95 (1H, dd, $J_{1,2}$=1.7 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.89–3.80 (2H, m, H-3, H-5a), 3.76–72 (1H, m, H-3′), 3.72–3.64 (2H, m, H-5b, OC*H*$_2$), 3.44–3.36 (2H, m, OC*H*$_2$), 3.12–3.06 (1H, m, H-5′a), 2.94 (1H, dd, $J_{4',5'b}$=7.7 Hz,

$J_{5'a,5'b}$=13.1 Hz, H-5′b), 1.62–1.53 (2H, m, CH_2), 1.30 (10H, br s, 5×CH_2), 0.92–0.87 (3H, m, CH_3).

4.1.10. Octyl 5-(5-deoxy-5-*N*-dicylohexyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (17). Compound **13** (50 mg, 0.07 mmol) was dissolved in MeOH (3 mL) and 10% Pd/C (20 mg) was added. Cyclohexane carboxyaldehyde (6 μL, 0.07 mmol) was added, and the reaction mixture was stirred at rt under H_2 atmosphere for 1 h. Filtration through a celite pad and concentration gave a viscous oil. Purification by column chromatography on silica gel (cyclohexane/EtOAc, 3:1) afforded **17** as a colorless oil (55 mg, 87%). MS-ESI: *m/z* Found 814.6022 $[M+H]^+$, calcd 814.6042 for $C_{44}H_{87}NO_8Si_2$. ^{1}H NMR ($CDCl_3$): δ 5.01 (1H, s, H-1′), 4.76 (1H, d, $J_{1,2}$=1.4 Hz, H-1), 4.17 (1H, br s, H-4′), 4.03–3.97 (3H, m, H-2, H-4, H-2′), 3.83–3.76 (3H, m, H-3, H-5a, H-3′), 3.68–3.58 (1H, m, OCH_2), 3.59 (1H, dd, $J_{4,5b}$=3.2 Hz, $J_{5a,5b}$=10.4 Hz, H-5b), 3.35–3.28 (1H, m, OCH_2), 2.71–2.60 (2H, m, H_2-5′), 2.44 (2H, dd, *J*=7.7, 12.6 Hz, NCH_2), 2.16 (2H, dd, *J*=5.2, 12.6 Hz, NCH_2), 1.87–1.60 (4H, m, cyclohexyl CH_2′s), 1.57–1.50 (10H, m, cyclohexyl), 1.46–1.41 (2H, m, CH_2), 1.37 (10H, br s, 5×CH_2), 1.20–1.08 (8H, m, cyclohexyl), 0.90–0.86 (21H, m, 7×CH_3), 0.09, 0.07, 0.06 (each s, 4×CH_3). ^{13}C NMR ($CDCl_3$): δ 108.20 (C-1), 107.69 (C-1′), 87.17 (C-4′), 84.27 (C-2), 81.72 (C-4), 79.70, 79.66 (C-3, C-3′), 78.00 (C-2′), 67.67 (OCH_2), 65.74 (C-5), 64.79 (2×CH_2N), 58.25 (C-5′), 35.70 (CH), 32.34, 32.04 (4×CH_2), 31.80, 29.622, 29.35, 29.22 (4×CH_2), 26.45, 26.17, 26.15, 26.05 (7×CH_2), 25.81, 25.67 (6×CH_3), 22.62 (CH_2), 17.86, 17.79 (2×C), 14.06 (CH_3), −4.28, −4.59, −4.70, −4.86 (4×CH_3).

4.1.11. Octyl 5-(5-deoxy-5-*N*-dicylohexyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (18). Compound **17** (50 mg, 0.06 mmol) was treated with $Et_4N^+F^-$ (28 mg, 0.18 mmol) in dry THF (3 mL) as described for the preparation of **13**. Purification by column chromatography ($CHCl_3$/MeOH, 9:1) yielded **18** (20 mg, 96%) as an oil. MS-ESI: *m/z* Found 586.4305 $[M+Na]^+$, calcd 586.4313 for $C_{32}H_{59}NO_8$. ^{1}H NMR ($CDCl_3$): δ 4.99 (2H, s, H-1, H-1′), 4.20–4.14 (2H, m, H-4, H-4′), 4.00–3.94 (4H, m, H-2, H-3, H-3′, H-5a), 3.88 (1H, br s, H-2), 3.76–3.67 (1H, m, OCH_2), 3.69 (1H, dd, $J_{4,5b}$=3.3 Hz, $J_{5a,5b}$=10.0 Hz, H-5b), 3.47–3.39 (1H, m, OCH_2), 2.66 (2H, d, *J*=3.5 Hz, H_2-5′), 2.39 (2H, dd, *J*=7.2, 12.7 Hz, NCH_2), 2.19 (2H, dd, *J*=5.7, 12.7 Hz, NCH_2), 1.83–1.63 (14H, m, cyclohexyl), 1.60–1.54 (2H, m, CH_2), 1.28 (10H, br s, 5×CH_2), 1.20–1.11 (8H, m, cyclohexyl), 0.90–0.86 (3H, m, CH_3). ^{13}C NMR (CD_3OD): δ 109.52 (C-1, C-1′), 83.72, 83.66, 83.47, 83.19 (C-2, C-4, C-2′, C-3′), 81.42 (C-4′), 79.48 (C-3), 68.95 (OCH_2), 68.17 (C-5), 64.44 (2×CH_2N), 59.28 (C-5′), 37.57 (2×CH), 33.08 (4×CH_2), 33.02 (CH_2), 30.72 (CH_2), 30.51 (CH_2), 30.43 (CH_2), 28.07 (2×CH_2), 27.33 (4×CH_2), 27.29 (CH_2), 23.72 (CH_2), 14.43 (CH_3).

4.1.12. Octyl 5-(5-deoxy-5-*tert*-butylamido-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (19). Compound **14** (60 mg, 0.07 mmol) was dissolved in MeOH (5 mL) and 10% Pd/C (40 mg) was added followed by HCO_2NH_4 (17 mg, 0.27 mmol). The reaction mixture was stirred 2 h at rt and filtered through celite. The solvent was evaporated, and the resulting syrup was used as such for further reaction. This crude amino disaccharide was dissolved in 2 mL of dry dichloromethane and cooled to 0°C. To it was added pivaloyl chloride (10 μL, 0.09 mmol), *N*-methylimidazole (10 μL, 0.12 mmol), and the reaction mixture was stirred for 8 h at 0°C. The reaction mixture was poured into an ice-water mixture and extracted with $CHCl_3$ (2×10 mL). The organic layer was dried over Na_2SO_4 and concentrated to syrup. Column chromatography using cyclohexane/EtOAc (98:2) afforded purified **19** (55 mg, 96%) as an oil. MS-ESI: *m/z* Found 956.2284 $[M+Na]^+$, calcd 956.6289 for $C_{47}H_{99}NO_9Si_4$. ^{1}H NMR ($CDCl_3$): δ 5.95 (1H, t, *J*=5.3 Hz, NH), 4.84 (1H, s, H-1′), 4.74 (1H, d, $J_{1,2}$=1.8 Hz, H-1), 4.08 (1H, dd, $J_{1',2'}$=1.1 Hz, $J_{2',3'}$=2.9 Hz, H-2′), 4.01–3.95 (4H, m, H-2, H-3, H-4, H-4′), 3.76 (1H, dd, $J_{2',3'}$=2.9 Hz, $J_{3',4'}$=6.6 Hz, H-3′), 3.74 (1H, dd, $J_{4,5a}$=3.7 Hz, $J_{5a,5b}$=11.3 Hz, H-5a), 3.73–3.64 (1H, m, OCH_2), 3.58–3.53 (1H, m, H-5b), 3.53–3.49 (2H, m, H_2-5′), 1.55 (1H, m, OCH_2), 1.60–1.52 (2H, m, CH_2), 1.30–1.25 (10H, m, 5×CH_2), 1.20 (9H, s, 3×CH_3), 0.89–0.87 (39H, m, 13×CH_3), 0.093, 0.084, 0.076, 0.066 (each s, 8×CH_3).

4.1.13. Octyl 5-(5-deoxy-5-phenylamido-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (20). Compound **14** (60 mg, 0.07 mmol) was treated with HCO_2NH_4 (17 mg, 0.27 mmol) in MeOH (5 mL) over 10% Pd/C (40 mg) followed by reaction with benzoyl chloride (10 μL, 0.09 mmol), *N*-methylimidazole (10 μL, 0.12 mmol) in CH_2Cl_2 (4 mL) as described earlier for the preparation of **19**. Purification by column chromatography (cyclohexane/EtOAc, 98:2) yielded **20** (50 mg, 77%) as an oil. MS-ESI: *m/z* Found 976.5971 $[M+Na]^+$, calcd 976.5976 for $C_{49}H_{95}NO_9Si_4$. ^{1}H NMR ($CDCl_3$): δ 8.07–8.05, 7.78–7.75, 7.51–7.38 (each m, Ar), 6.44 (1H, t, *J*=5.3 Hz, NH), 4.91 (1H, s, H-1′), 4.73 (1H, d, $J_{1,2}$=1.9 Hz, H-1), 4.15–4.11 (1H, m, H-4′), 4.09 (1H, dd, $J_{1',2'}$=0.9 Hz, $J_{2',3'}$=2.6 Hz, H-2′), 4.01 (1H, dd, $J_{1,2}$=1.9 Hz, $J_{2,3}$=4.2 Hz, H-2), 3.99–3.95 (2H, m, H-3, H-4), 3.87 (1H, dd, $J_{2',3'}$=2.6 Hz, $J_{3',4'}$=5.7 Hz, H-3′), 3.79–3.64 (3H, m, H-5a, H_2-5′, OCH_2), 3.50 (1H, dd, $J_{4,5b}$=3.2 Hz, $J_{5a,5b}$=11.2 Hz, H-5b), 3.34–3.27 (1H, m, OCH_2), 1.55–1.50 (2H, m, CH_2), 1.27 (10H, m, 5×CH_2), 0.89–0.84 (39H, m, 13×CH_3), 0.106, 0.084, 0.077, 0.072, 0.064, 0.052 (each s, 8×CH_3).

4.1.14. Octyl 5-(5-deoxy-5-ethylsulfonamido-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranosyl)-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (21). Compound **14** (60 mg, 0.07 mmol) was treated with HCO_2NH_4 (17 mg, 0.27 mmol) in MeOH (5 mL) over 10% Pd/C (40 mg) followed by reaction with ethanesulfonyl chloride (8 μL, 0.09 mmol), *N*-methylimidazole (10 μL, 0.12 mmol) in CH_2Cl_2 (4 mL) as described earlier for the preparation of **19**. Purification by column chromatography ($CHCl_3$/MeOH, 9:1) afforded **21** (50 mg, 77%) as an oil. MS-ESI: *m/z* Found 964.5646 $[M+Na]^+$, calcd 964.5645 for $C_{44}H_{95}NO_{10}SSi_4$. ^{1}H NMR ($CDCl_3$): δ 4.87 (1H, s, H-1′), 4.74 (1H, d, $J_{1,2}$=1.9 Hz, H-1), 4.59 (1H, t, *J*=5.5 Hz, NH), 4.08–4.03 (1H, m, H-4′), 4.07 (1H, dd, $J_{1',2'}$=1.2 Hz, $J_{2',3'}$=2.7 Hz, H-2′), 4.00 (1H, dd, $J_{1,2}$=1.9 Hz, $J_{2,3}$=3.5 Hz, H-2), 3.97–3.91 (2H, m, H-3, H-4), 3.90 (1H, dd, $J_{2',3'}$=2.7 Hz, $J_{3',4'}$=5.5 Hz, H-3′), 3.73 (1H, dd, $J_{4,5a}$=4.3 Hz, $J_{5a,5b}$=11.2 Hz, H-5a), 3.72–3.64 (1H, m, OCH_2), 3.57 (1H,

dd, $J_{4,5b}$=3.2 Hz, $J_{5a,5b}$=11.2 Hz, H-5b), 3.42–3.35 (1H, m, H-5′a), 3.35–3.27 (1H, m, OCH_2), 3.24 (1H, dd, $J_{4',5'b}$=5.7 Hz, $J_{5'a,5'b}$=13.0 Hz, H-5′b), 3.05 (2H, dd, J=7.4, 14.7 Hz, CH_2), 1.56–1.50 (2H, m, CH_2), 1.37 (3H, t, J=7.4 Hz, CH_3), 1.27 (10H, m, 5×CH_2), 0.90–0.86 (39H, m, 13×CH_3), 0.113, 0.105, 0.092, 0.083, 0.075, 0.071 (each s, 8×CH_3).

4.1.15. Octyl 5-[5-deoxy-5-(*p*-methylphenyl)sulfonamido-2,3-di-*O-tert*-butyldimethylsilyl-α-D-arabinofuranosyl]-2,3-di-*O-tert*-butyldimethylsilyl-α-D-arabinofuranoside (22). Compound **14** (60 mg, 0.07 mmol) was treated with HCO_2NH_4 (17 mg, 0.27 mmol) in MeOH (5 mL) over 10% Pd/C (40 mg) followed by reaction with tosyl chloride (17 mg, 0.09 mmol), *N*-methylimidazole (10 μL, 0.12 mmol) in CH_2Cl_2 (4 mL) as described earlier for the preparation of **19**. Purification by column chromatography (cyclohexane/EtOAc, 95:5) yielded **22** (50 mg, 73%) as an oil. MS-ESI: *m/z* Found 1026.5787 [M+Na]$^+$, calcd 1026.5802 for $C_{49}H_{97}NO_{10}SSi_4$. ^{1}H NMR ($CDCl_3$): δ 7.73, 7.30 (each 2H, d, J=8.2 Hz, Ar), 4.78 (1H, s, H-1′), 4.76 (1H, t, J=5.7 Hz, NH), 4.72 (1H, d, $J_{1,2}$=1.8 Hz, H-1), 4.01 (1H, dd, $J_{1',2'}$=1.3 Hz, $J_{2',3'}$=3.0 Hz, H-2′), 3.99 (1H, dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.96–3.90 (3H, m, H-3, H-4, H-4′), 3.84 (1H, dd, $J_{2',3'}$=3.0 Hz, $J_{3',4'}$=5.8 Hz, H-3′), 3.71–3.64 (1H, m, OCH_2), 3.65 (1H, dd, $J_{4,5a}$=3.4 Hz, $J_{5a,5b}$=11.4 Hz, H-5a), 3.50 (1H, dd, $J_{4,5b}$=3.1 Hz, $J_{5a,5b}$=11.4 Hz, H-5b), 3.34–3.27 (1H, m, OCH_2), 3.20–3.13 (1H, m, H-5′a), 3.10–3.02 (1H, m, H-5′b), 1.60–1.51 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.90–0.84 (39H, m, 13×CH_3), 0.094, 0.082, 0.076, 0.067, 0.062, 0.045, 0.035, 0.023 (each 3H, m, CH_3).

4.1.16. Octyl 5-(5-deoxy-5-*tert*-butylamido-α-D-arabinofuranosyl)-α-D-arabinofuranoside (23). Compound **19** (45 mg, 0.05 mmol) was treated with $Et_4N^+F^-$ (43 mg, 0.30 mmol) in dry THF (4 mL) as described for the preparation of **13**. Purification by column chromatography ($CHCl_3$/MeOH, 8:1) afforded **23** (21 mg, 91%) as an oil. MS-ESI: *m/z* Found 500.2817 [M+Na]$^+$, calcd 500.2830 for $C_{23}H_{43}NO_9$. ^{1}H NMR (CD_3OD): δ 4.94 (1H, s, H-1′), 4.83 (1H, d, $J_{1,2}$=1.8 Hz, H-1), 4.06 (1H, ddd, $J_{4',5'a}$=$J_{4',5'b}$=4.8 Hz, $J_{3',4'}$=5.3 Hz, H-4′), 4.00 (1H, dd, $J_{1',2'}$=1.2 Hz, $J_{2',3'}$=2.8 Hz, H-2′), 4.02–3.97 (1H, m, H-4), 3.94 (1H, dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.87 (1H, dd, $J_{2,3}$=3.7 Hz, $J_{3,4}$=6.5 Hz, H-3), 3.81 (1H, dd, $J_{4,5a}$=5.1 Hz, $J_{5a,5b}$=11.0 Hz, H-5a), 3.72 (1H, dd, $J_{2',3'}$=2.8 Hz, $J_{3',4'}$=5.3 Hz, H-3′), 3.69–3.62 (1H, m, OCH_2), 3.63 (1H, dd, $J_{4,5b}$=3.7 Hz, $J_{5a,5b}$=11.0 Hz, H-5b), 3.44–3.36 (3H, m, H_2-5′, OCH_2), 1.59–1.53 (2H, m, CH_2), 1.30 (10H, m, 5×CH_2), 1.18 (9H, s, 3×CH_3), 0.92–0.87 (3H, m, CH_3).

4.1.17. Octyl 5-(5-deoxy-5-phenylamido-α-D-arabinofuranosyl)-α-D-arabinofuranoside (24). Compound **20** (50 mg, 0.05 mmol) was treated with $Et_4N^+F^-$ (47 mg, 0.31 mmol) in dry THF (4 mL) as described for the preparation of **13**. Purification by column chromatography ($CHCl_3$/MeOH, 9:1) yielded **24** (20 mg, 77%) as an oil. MS-ESI: *m/z* Found 520.2508 [M+Na]$^+$, calcd 520.2517 for $C_{25}H_{39}NO_9$. ^{1}H NMR (CD_3OD): δ 7.84–7.81, 7.55–7.49, 7.47–7.41 (each m, Ar), 4.99 (1H, s, H-1′), 4.82 (1H, d, $J_{1,2}$=1.7 Hz, H-1), 4.20–4.15 (1H, m, H-4′), 4.03 (1H, dd, $J_{1',2'}$=1.2 Hz, $J_{2',3'}$=2.9 Hz, H-2′), 4.02–3.98 (1H, m, H-4), 3.94 (1H, dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.88 (1H, dd, $J_{2,3}$=3.7 Hz, $J_{3,4}$=5.7 Hz, H-3), 3.85–3.81 (2H, m, H-3′, H-5′a), 3.71–3.63 (4H, m, H-5′b, H_2-5, OCH_2), 3.42–3.35 (1H, m, OCH_2), 1.59–1.54 (2H, m, CH_2), 1.29 (10H, m, 5×CH_2), 0.91–0.87 (3H, m, CH_3).

4.1.18. Octyl 5-(5-deoxy-5-ethylsulfonamido-α-D-arabinofuranosyl)-α-D-arabinofuranoside (25). Compound **21** (40 mg, 0.04 mmol) treated with $Et_4N^+F^-$ (38 mg, 0.35 mmol) in dry THF (4 mL) as described for the preparation of **13**. Purification by column chromatography ($CHCl_3$/MeOH, 9:1) yielded **25** (20 mg, 95%) as an oil. MS-ESI: *m/z* Found 508.2192 [M+Na]$^+$, calcd 508.2186 for $C_{20}H_{39}NO_{10}S$. ^{1}H NMR (CD_3OD): δ 4.93 (1H, d, $J_{1',2'}$=1.2 Hz, H-1′), 4.83 (1H, d, $J_{1,2}$=1.7 Hz, H-1), 4.02–3.96 (2H, m, H-4, H-4′), 4.00 (1H, dd, $J_{1',2'}$=1.5 Hz, $J_{2',3'}$=3.4 Hz, H-2′), 3.94 (1H, dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.87 (1H, dd, $J_{2,3}$=3.7 Hz, $J_{3,4}$=6.4 Hz, H-3), 3.82 (1H, dd, $J_{4,5a}$=5.4 Hz, $J_{5a,5b}$=11.3 Hz, H-5a), 3.78 (1H, dd, $J_{2',3'}$=3.4 Hz, $J_{3',4'}$=5.8 Hz, H-3′), 3.72–3.65 (1H, m, OCH_2), 3.64 (1H, dd, $J_{4,5b}$=3.5 Hz, $J_{5a,5b}$=11.3 Hz, H-5b), 3.44–3.36 (1H, m, OCH_2), 3.37 (1H, dd, $J_{4',5'a}$=3.7 Hz, $J_{5'a,5'b}$=13.7 Hz, H-5′a), 3.23 (1H, dd, $J_{4',5'b}$=6.6 Hz, $J_{5'a,5'b}$=13.7 Hz, H-5′b), 3.13–3.05 (2H, m, CH_2), 1.62–1.50 (2H, m, CH_2), 1.35–1.26 (13H, m, 5×CH_2, CH_3), 0.92–0.87 (3H, m, CH_3).

4.1.19. Octyl 5-[5-deoxy-5-(*p*-methylphenyl)sulfonamido-α-D-arabinofuranosyl]-α-D-arabinofuranoside (26). Compound **22** (45 mg, 0.04 mmol) treated with $Et_4N^+F^-$ (41 mg, 0.27 mmol) in dry THF (4 mL) as described for the preparation of **13**. Purification by column chromatography ($CHCl_3$/MeOH, 8:1) afforded **26** (21 mg, 88%) as an oil. MS-ESI: *m/z* Found 570.2327 [M+Na]$^+$, calcd 570.2343 for $C_{25}H_{41}NO_{10}S$. ^{1}H NMR (CD_3OD): δ 7.73 (2H, d, J=8.2 Hz, Ar), 7.37 (1H, dd, J=0.6, 8.2 Hz, Ar), 4.85 (1H, s, H-1′), 4.82 (1H, d, $J_{1,2}$=1.7 Hz, H-1), 3.99–3.88 (2H, m, H-4, H-4′), 3.95 (1H, dd, $J_{1',2'}$=1.4 Hz, $J_{2',3'}$=3.4 Hz, H-2′), 3.93 (1H, dd, $J_{1,2}$=1.7 Hz, $J_{2,3}$=3.7 Hz, H-2), 3.85 (1H, dd, $J_{2,3}$=3.7 Hz, $J_{3,4}$=6.6 Hz, H-3), 3.74 (1H, dd, $J_{4,5a}$=5.4 Hz, $J_{5a,5b}$=11.1 Hz, H-5a), 3.73 (1H, dd, $J_{2',3'}$=3.4 Hz, $J_{3',4'}$=5.4 Hz, H-3′), 3.71–3.64 (1H, m, OCH_2), 3.57 (1H, dd, $J_{4,5b}$=3.6 Hz, $J_{5a,5b}$=11.1 Hz, H-5b), 3.43–3.36 (1H, m, OCH_2), 3.14 (1H, dd, $J_{4',5'a}$=4.1 Hz, $J_{5'a,5'b}$=13.4 Hz, H-5′a), 3.01 (1H, dd, $J_{4',5'b}$=6.5 Hz, $J_{5'a,5'b}$=13.4 Hz, H-5′b), 1.60–1.54 (2H, m, CH_2), 1.29 (10H, m, 5×CH_2), 0.91–0.87 (3H, m, CH_3).

4.2. Biological

4.2.1. In vitro assay. In vitro inhibition assays[20] of the arabinofuranosyl disaccharide analogs were performed on *Mycobacterium tuberculosis* (H37Ra, ATCC 25177) and *Mycobacterium avium* (NJ 211).

4.2.2. Arabinosyltransferase assay.[21] Compounds **14**, **16**, **18**, **23**, **24**–**26** at a range of concentrations from 0.25 to 6.0 mM (which were stored as 100 mM ethanol stocks) and DP[^{14}C]A (20,000 cpm, 9 mM, 10 μL [stored in chloroform/methanol, 2:1]), were dried under a stream of argon in a microcentrifuge tube (1.5 mL) and placed in a vacuum desiccator for 15 min to remove any residual solvent. The dried constituents of the assay were then resuspended in 8 μL of a 1% aqueous solution of Igepal. The remaining

constituents of the arabinosyltransferase assay containing 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM ATP, membranes (250 μg) were added to a final reaction volume of 80 μL. The reaction mixtures were then incubated at 37°C for 1 h. A $CHCl_3/CH_3OH$ (1:1, 533 μL) solution was then added to the incubation tubes and the entire contents centrifuged at 18,000*g*. The supernatant was recovered and dried under a stream of argon and re-suspended in C_2H_5OH/H_2O (1:1, 1 mL) and loaded onto a pre-equilibrated (C_2H_5OH/H_2O [1:1]) 1 mL Whatmann strong anion exchange (SAX) cartridge which was washed with 3 mL of ethanol. The eluate was dried and the resulting products partitioned between the two phases arising from a mixture of *n*-butanol (3 mL) and H_2O (3 mL). The resulting organic phase was recovered following centrifugation at 3,500*g* and the aqueous phase was again extracted twice with 3 ml of *n*-butanol saturated water, the pooled extracts were back-washed twice with water saturated with *n*-butanol (3 mL). The *n*-butanol-saturated water fraction was dried and re-suspended in 200 μL of *n*-butanol. The total cpm of radiolabeled material extractable into the *n*-butanol phase was measured by scintillation counting using 10% of the labeled material and 10 ml of EcoScintA (National Diagnostics, Atlanta). The incorporation of [^{14}C]Ara*f* was determined by subtracting counts present in control assays (incubation of the reaction components in the absence of the compounds). Another 10% of the labeled material was subjected to thin-layer chromatography (TLC) in $CHCl_3/CH_3OH/NH_4OH/H_2O$ (65:25:0.5:3.6) on aluminium backed Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). Autoradiograms were obtained by exposing TLCs to X-ray film (Kodak X-Omat) for 3 days. Competition based experiments were performed by mixing compounds together at various concentrations, α(1→5)-linked octyl arabinofuranosyl disaccharide[13] at 0.4 mM with disaccharide analogs at 0.5, 1.0, 2.0, 3.0 and 6.0 mM followed by thin-layer chromatography/autoradiography as described earlier to determine the extent of product formation and IC_{50}.[13]

Acknowledgements

The authors are thankful to Dr J. M. Riordan for NMR spectra and Mr M. D. Richardson for ESI-MS data. This work was supported by NIH/NIAID grant R01AI45317. GSB who is currently a Lister Institute-Jenner Research Fellow acknowledges support from The Medical Research Council (49343 and 49342). The *M. avium* strain (NJ211) was kindly provided by Dr Leonid Heifets, National Jewish Center for Immunology and Respiratory Diseases, Denver, CO, USA.

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