Published in final edited form as:
Bioorg Med Chem. 2009 January 15; 17(2): 872–881. doi:10.1016/j.bmc.2008.11.027.

Synthesis of Deoxygenated (α 1→5)-Linked Arabinofuranose Disaccharides as Substrates and Inhibitors of Arabinosyltransferases of *Mycobacterium tuberculosis*

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Abstract

Arabinosyltransferases (AraTs) play a critical role in mycobacterial cell wall biosynthesis and are potential drug targets for the treatment of tuberculosis, especially multi-drug resistant forms of *M. tuberculosis* (MTB). Herein, we report the synthesis and acceptor/inhibitory activity of Ara*f* α(1→5) Ara*f* disaccharides possessing deoxygenation at the reducing sugar of the disaccharide. Deoxygenation at either the C-2 or C-3 position of Ara*f* was achieved via a free radical procedure using xanthate derivatives of the hydroxyl group. The α(1→5)-linked disaccharides were produced by coupling *n*-octyl α-Ara*f* 2-/3-deoxy, 2-fluoro glycosyl acceptors with an Ara*f* thioglycosyl donor. The target disaccharides were tested in a cell free mycobacterial AraTs assay as well as an *in vitro* assay against MTB H_{37}Ra and *M. avium* complex strains.

Introduction

Tuberculosis (TB) is one of the primary killers worldwide in spite of the availability of several potent anti-mycobacterial agents.[1] Millions die annually from this disease, and the problem is amplified by the apparent synergism with HIV.[2,3] Mycobacterial diseases have attracted renewed attention in recent years because of their increased incidence worldwide and the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains.[4] MDR-TB infections are significantly more difficult to treat with second-line therapies that are typically more expensive and have considerable side-effects. XDR-TB[5] develops when these second-line drugs are also misused or mismanaged and therefore become ineffective. Because XDR-TB is resistant to first- and second-line drugs, treatment options are seriously limited.

During the last two decades, new programs have been initiated to elucidate the systems biology of the tubercle bacillus with a focus on new, valid targets for novel anti-tubercular drug discovery. Many unique metabolic processes occur during the biosynthesis of cell wall components, including arabinogalactan and mycolic acids.[6] Among the front line drugs for treatment of TB, two drugs isoniazid (INH) and ethambutol (EMB) target the mycobacterial cell wall that is essential for the survival of pathogen.[7] The structure of the cell wall has now been systematically elucidated in terms of its component complex polysaccharides, the specific

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chemical linkages therein, and the macromolecular structure of the mycolylarabinogalactan complex.[8] The two major oligosaccharide portions, lipoarabinomannan (LAM) and arabinogalactan (AG), contain arabinofuranose (Ara*f*) units.

Chemical analysis of the fragments of LAM and AG have revealed that both are composed of linear arabinan consisting of α (1→5) linked Ara*f* units, and a branched Ara*f* hexasaccharide at the terminus with α (1→3) and β(1→2) linked Ara*f* units. The assembly of the arabinan portions of cell wall polysaccharides in mycobacteria involves a family of AraTs[9] that promote the polymerization of Ara*f* units using decaprenolphosphoarabinofuranose (DPA) as the sugar donor. Mycobacterial viability requires an intact arabinan, and thus compounds that inhibit these glycosyltransferases (GTs) are both useful biochemical tools as well as potential lead compounds for new selective anti-tubercular agents as Ara*f* is not present in mammals.

At the inception of the mycobacterial GTs program, our intention was to prepare prototype disaccharides that would be substrates for assay development and could probe the acceptor activity of the various cell wall GTs.[10,11] Neoglycosides **1a**, **1b** and **1c** (Chart 1) were previously synthesized and evaluated for their potential as acceptors/inhibitors.[11] As those efforts advanced, our work turned to examining the various substitution patterns of the acceptor disaccharides to study the acceptor tolerance for various alterations, and the ability of these substitutions to affect inhibition relative to the standard acceptor disaccharides for each transferase. Based on the substrate activity of a control acceptor, *n*-octyl Ara*f* α(1→5) Ara*f* (**1a**), several analogs possessing *N*-alkyl, amide and sulphonamide functionalities at the C-5 position of the non-reducing sugar were prepared and screened as competitive inhibitors.[12] Among a small set of disaccharides **2**, *N*,*N*-dicyclohexylamino disaccharide analog **3** showed superior activity as an inhibitor.[12]

Ideally, and with the growing body of SAR information, we could begin to move from what might be considered routine acceptor-like and non-drug-like disaccharides to compounds that would more closely fit drug-like molecules. Next, we prepared symmetrical *C*-linked and pseudo-symmetrical *O*-linked disaccharides **4** and **5** possessing *N*,*N*-dicyclohexylamino moieties. Both *C*-sugars and *C*-linked disaccharides may offer advantages as enzyme probes and inhibitors, and most importantly, this linkage may prevent glycosidase-mediated cleavage. However, both **4** and **5** showed only modest inhibitory activity in the cell free assay system as well as *in vitro* against MTB H_{37}Ra and *M. avium* strains.[13] In a parallel study, the Lowary group has also synthesized Ara*f* di- and trisaccharide analogs possessing substitution at the C-5 position(s) of the non-reducing sugars; activity was not reported for these compounds.[14]

Our eventual goal was to move from prototype acceptor disaccharides to potent drug-like GTs inhibitors. In this work, our goal was to assess the requirement for typical saccharide-like OH substitutions (e.g. hydroxy to deoxy sugars), and some of these substitutions are reported herein. Secondly, the 2-deoxy-2-fluoro-Ara*f* substitution is known to stabilize glycosidic linkages, and might improve drug-like properties of even more saccharide-like inhibitors.

Deoxy sugars as well as their fluoro counterparts are present in many natural products and are a medicinally useful group of compounds.[15] Deoxy derivatives have been prepared as inhibitors of glycosidases,[16] GTs,[17] and also to establish which hydroxyl groups are involved in interaction with lectins.[18] The preparation and biological activity of deoxy sugars and deoxy sugar oligosaccharides have been reviewed.[19] Some of the general methods for the preparation of deoxy sugars are reductive methods, using such starting materials as epoxides, thio sugars, *C*-halosugars, carboxylate esters, sulfonates, or even by direct reduction of hydroxyl groups. Unsaturated sugars are also excellent sources for deoxygenated derivatives via electrophilic addition of hydrogen halides, and glycals through acid catalyzed addition of water or alcohols.[20] Other more direct, approaches have also been developed recently[21] and the syntheses of a

small number of deoxy furanose derivatives, especially 2-deoxy-glycosides, have also been reported.[22] Specifically, 2-deoxy Ara*f* di- and trisaccharides were recently reported through reductive desulfonylation.[23]

The most common preparative method used is radical chain chemistry for the transformation of a secondary alcohol to the corresponding deoxy derivative. Firstly, alcohols are converted to a thiocarbonyl derivative (thioxobenzoates, xanthates, or thiocarbonylimidazolides), and, on reduction with tributyltin hydride, these derivatives afford deoxy compounds in good yields.[24] Utilizing this approach, we report the synthesis of 1-*O*-octyl-Ara*f* α(1→5) Ara*f* disaccharides **6** and **7** possessing deoxygenation at the 2-and 3-position of the reducing end respectively as shown in Figure 1. Also, disaccharide **8** was synthesized possessing 2-deoxy-2-fluoro at the reducing end of the disaccharide (Figure 1) starting from 2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose. These analogs were prepared in order to further define the growing SAR profile of the mycobacterial AraTs relative to the simple Ara*f* α(1→5) Ara*f* template acceptor.

Result and Discussion

The synthesis of target 2-deoxy and 3-deoxy Ara*f* disaccharides **6** and **7** were initiated from a common known Ara*f* glycoside precursor **11**[25] (Schemes 1 and 2). The synthesis of precursor **11** began with commercially available D-arabinose that efficiently transformed to 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose **9**.[26] Glycoside **9** was subsequently converted to *n*-octyl α-D-arabinofuranoside (**10**) as reported earlier.[11] Reaction of **10** with 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane ($TIPDSCl_2$) in pyridine and purification by column chromatography on SiO_2 afforded the 3,5-silyl blocked glycoside **11** (Scheme 1).

The synthesis of *n*-octyl 2-deoxy glycosyl acceptor **14** was achieved in three steps starting from precursor glycoside **11**. The deoxygenation at the 2-position in glycoside **11** was carried out in one pot by converting **11** to the xanthate intermediate through reaction with CS_2, MeI in the presence of NaH followed by radical reduction with Bu_3SnH and AIBN. After workup and purification, 2-deoxy glycoside **12** was obtained in 86% overall yield. Glycoside **12** was desilylated using $Et_4N^+F^-$ in THF to give **13** in 89% yield. The final step in the synthesis of glycosyl acceptor **14** was carried out without purification of the intermediates. The 5-OH group in **13** was selectively tritylated, and the product was subsequently benzoylated and detritylated to give **14** in 65% overall yield after purification. The ^{1}H NMR spectrum of glycosyl acceptor **14** in $CDCl_3$ showed the anomeric proton at δ 5.27 ppm as a doublet of doublets ($J_{1,2eq}$ = 0.9 Hz, $J_{1,2ax}$ = 5.2 Hz). The H-2 axial and equatorial protons in **14** were observed as *ddd* at δ 2.43 ppm and δ 2.21 ppm respectively. These assignments were made on the basis of coupling constant correlation and nOe experiments. The decoupled and DEPT ^{13}C NMR spectra of **14** were also supportive of the structure, and showed the anomeric carbon and C-2 at δ 103.64 and δ 39.31 ppm respectively.

The synthesis of 3-deoxy acceptor glycoside **19** began from glycoside **11** (Scheme 2). The 2-hydroxyl group in **11** was protected by a pivaloyl group to give **15** that, on desilylation using $Et_4N^+F^-$ in THF, produced **16** in excellent yield. The 5-OH group of compound **16** was then selectively tritylated to produce glycoside **17**. Compound **17** was deoxygenated via a free radical pathway in two steps as described for **12** to give the 3-deoxy glycoside **18** in 83% yield after purification. To access the desired 3- deoxy glycosyl acceptor **19**, compound **18** was detritylated at −20 °C using TFA in $CHCl_3$. The structure of 3-deoxy glycoside **19** was supported by the ^{1}H NMR spectrum that showed the anomeric proton at δ 4.99 ppm as a singlet and signals from axial and equatorial protons at C-3 were observed as *ddd* at δ 1.72 and δ 2.47 ppm respectively. The ^{13}C NMR spectra of **19** showed the anomeric carbon and C-3 at δ 105.97 and δ 31.51 ppm respectively.

Both 2-deoxy and 3-deoxy Ara*f* glycosyl acceptors **14** and **19** were subjected to glycosylation using the known thioglycoside donor **20**[27] as represented in Scheme 3. Glycosyl acceptor **14** on reaction with thioglycoside **20** in the presence of activator NIS and Lewis acid Sn $(OTf)_2$ gave disaccharide **21** in 85% yield after purification. Disaccharide **21** was fully characterized by the ^{1}H NMR spectrum and anomeric proton signals were evident as a doublet at δ 5.28 ppm (H-1, $J_{1,2ax}$ = 4.7 Hz) and as a singlet at δ 5.13 ppm (H-1′), suggesting 1,2-*trans* glycosylation. The assigned structure was further supported by the ^{13}C NMR spectrum in which the signals from the anomeric carbons C-1 and C-1′ were observed at δ 104.02 ppm and δ 105.68 ppm respectively. Disaccharide **21** was deprotected using 7N NH_3/MeOH to produce the 2-deoxygenated disaccharide **6**. The ^{1}H NMR spectrum of **6** in CD_3OD at 600 MHz showed the anomeric proton H-1 as a doublet of doublets at δ 5.11 ppm ($J_{1,2eq}$ = 1.9 Hz, $J_{1,2ax}$ = 5.5 Hz) and the H-1′ proton at δ 4.89 ppm as a doublet ($J_{1',2'}$= 1.2 Hz). The ^{13}C NMR spectrum of **6** showed anomeric carbons C-1′ and C-1 at δ 109.68 and 105.24 ppm respectively.

The synthesis of 3-deoxygenated disaccharide **22** was accomplished by the reaction of glycosyl acceptor **19** and glycosyl donor **20** similar to the synthesis of **21**. The α-glycosylation was supported by the ^{1}H NMR spectrum of **22** that showed the anomeric H-1′ signal at 5.09 ppm, but the H-1 signal was unresolved from the H-3′ proton signal. The ^{13}C NMR spectrum of **22** showed anomeric carbons C-1 and C-1′ signals at 106.04 and 105.65 ppm respectively. Global de-protection of disaccharide **22** with 25% NaOMe in MeOH solution resulted in disaccharide **7**. The ^{1}H NMR spectrum of **7** at 600 MHz in CD_3OD showed the anomeric protons H-1 and H-1′ at δ 4.87 ppm and δ 4.92 ppm as a singlet and doublet ($J_{1',2'}$ = 1.2 Hz) respectively. The ^{13}C NMR spectrum of **7** showed anomeric carbons at δ 110.09 and δ 109.56 ppm assigned to C-1 and C-1′ respectively.

The synthesis of 2-fluoro disaccharide **8** was achieved starting from known glycoside 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose **23**[28] as presented in Scheme 4.

The 2-fluoro Ara*f* **23** was treated with *n*-octanol in CH_3CN in presence of Lewis acid $SnCl_4$ similar to a reported glycosylation method[29] and resulted in a mixture of α- and β-octyl glycosides. After chromatographic purification on SiO_2, the desired α-octyl Ara*f* glycoside **24** was obtained in 54% yield. The α-configuration in **24** was supported by the ^{1}H NMR spectrum that showed the anomeric proton as a doublet at δ 5.30 ppm ($J_{1,F}$ = 10.3 Hz) and the H-2 proton at δ 5.10 as a doublet of doublets ($J_{2,3}$ = 0.7 Hz, $J_{2,F}$ = 49.66 Hz). The ^{13}C NMR decoupled spectrum of **24** showed C-1 and C-2 as doublets at δ 105.16 ($J_{1,F}$ = 34.8 Hz) and δ 98.25 ppm ($J_{2,F}$ = 181.9 Hz) respectively. The synthesis of glycoside **25** was achieved in an overall yield of 81% by a three step reaction sequence in one reaction vessel. In the first step, glycoside **24** was debenzoylated by NH_3/MeOH. After completion of the reaction, it was concentrated under vacuum to syrup that was utilized without purification. In the second step, the 5-hydroxyl group was selectively tritylated using TrCl in pyridine at 50 °C. In the third step, the tritylated glycoside was benzoylated by adding BzCl in the same reaction vessel to give glycoside **25**. Purified **25** was treated with 5% TFA in $CHCl_3$ at −20 °C to remove the trityl group to give acceptor glycoside **26** in 81% yield. The structure of glycoside **26** was characterized by ^{1}H NMR, ^{13}C NMR and HR-ESIMS spectra.

Coupling of the glycosyl acceptor **26** and thioglycosyl donor **20** was carried out in the presence of activator NIS and the Lewis acid $Sn(OTf)_2$ at 0 °C to yield disaccharide **27** in 88% yield. The 1,2-*trans* glycosylation was supported by the ^{1}H NMR spectrum that displayed anomeric signals as a doublet at δ 5.25 ppm ($J_{1,F}$ = 10.4 Hz, H-1) and a signlet at δ 5.17 ppm (H-1′). The ^{13}C NMR spectrum **27** showed anomeric carbons at δ 105.51 as a singlet and δ 105.01 ppm as a doublet ($^2J_{1,F}$ = 34.8 Hz). Finally, removal of all acyl protecting groups in **27** by NH_3/MeOH produced the final 2-deoxy-2-fluoro glycoside **8** in 88% yield. The ^{1}H NMR spectrum in CD_3OD of disaccharide **8** showed anomeric signals at δ 5.06 (d, $J_{1,F}$ = 12.2 Hz)

and δ 4.94 (d, $J_{1',2'}$;= 1.2 Hz) whereas the ^{13}C NMR spectrum showed anomeric carbons at δ 109.68 (C-1′) and δ 106.5 (d, $^{2}J_{F,C-1}$ = 127.6 Hz).

All new compounds were characterized using ^{1}H NMR, ^{13}C NMR and HR-ESIMS. The NMR shifts of mono and disaccharides were compared with literature results.[10,12,15,30] nOe, decoupling and D_2O exchange experiments were performed to confirm NMR assignments as needed.

Biological activity

***In vitro* activity—**The arabinofuranosyl disaccharides were screened against *M. tuberculosis* (MTB H_{37}Ra) and *M. avium* (NJ 211) *in vitro* as reported.[31] Disaccharides **6**, **7** and **8** were active in the concentration range >12.8≤128 μg/mL for both strains.

Cell free assay—Based on the previous use of specific Ara*f*-based neoglycolipid acceptors,[32] compounds **6**–**8** were assessed as acceptors and competitive inhibitors. Assays were performed in the presence of membranes, resulting in [^{14}C]Ara*f* incorporation from the established enzymatic Ara*f* donor DP-[^{14}C]Ara*f* by compounds **6**, **7** and **8**. TLC autoradiography (Figure 2) demonstrated the enzymatic conversion of **6**, **7** and **8** to their corresponding homologated products. The enzymatic kinetic analysis for compounds **6**, **7** and **8** (Figure 3) gave K_m values of 0.15 mM, 0.11 mM and 0.56 mM respectively. Further competition-based, experiments established that **6**, **7** and **8** were weak competitive inhibitors of their native acceptor **1a** [Ara*f*(1→5)Ara*f*-*O*-C_8H_{17}] in the AraTs assay resulting in an IC_{50} values of 2.45 mM, 2.15 mM, 3.58 mM, respectively.

Conclusion

In conclusion, we have efficiently prepared three new α (1→5)–linked Ara*f* disaccharides **6**, **7** and **8** possessing deoxygenation at the 2- or 3-position of the reducing sugar as biochemical probes to study substrate specificity of AraTs in MTB. Deoxygenation removes a potential metabolic site, but, additionally, the added hydrophobicity may play an important role for inhibiting AraTs as has been noted in reports by us and others.[11–14] The activity of these disaccharides as acceptors and competitive inhibitors was evaluated, and only moderate activities were seen against the AraTs in a cell free assay system as well as in the *invitro* antibacterial assay against MTB H_{37}Ra and MAC strain NJ211. Clearly, the deoxygenated disaccharides can act as modest acceptors, and can affect bacterial growth. In a previous study, compounds **1b** and **1c** (possessing methyl and benzyl protecting groups at the reducing end of an Ara*f* disaccharide), the reported IC_{50} values were 3.70 and 1.12 mM respectively.[11] The IC_{50} values found for three new deoxy disaccharides **6**, **7** and **8** were comparable to values obtained for compounds **1b** and **1c**. Deoxygenation, i.e. alteration in hydrophilicity, at the reducing end in the disaccharides **6**, **7**, and **8** did not significantly alter AraTs substrate or competitive inhibitory activity when compared to other disaccharides bearing substitution at the reducing and non-reducing ends.[11–14] Currently, bis(cyclohexylmethyl)amino substituted Ara*f* disaccharide **3** is the only reported disaccharide that has shown both enzyme inhibition and prevention of mycobacterial growth.[12] The results from this investigation also clearly indicate that the reducing end sugar can be altered to other similar pentose ring systems to effectively bind in the catalytic region of AraTs enzymes.

Experimental

Synthesis

All reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. All chemicals and anhydrous solvents from Aldrich were used as is in

the reactions. Whenever necessary, compounds and starting materials were dried by azeotropic removal of water with toluene under reduced pressure. Reactions were monitored by thin-layer chromatography (TLC) on precoated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spray with $(NH_4)_2SO_4$ solution (150 g ammonium sulfate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for work-up and chromatography were reagent grade from Fisher Scientific. Flash chromatography was carried out on Fischer silica gel 60 (230–400 Mesh). The ^1H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 MHz and 75 MHz respectively. Coupling constants (*J*) values are reported in Hz and chemical shifts are in ppm (δ) relative to residual solvent peak or internal standard (TMS). The peak assignments in NMR were done on the basis of coupling constant values and whenever needed nOe experiments were performed. The HR-ESIMS were recorded on a BioTof-2 time-of-flight mass spectrometer.

Octyl 3,5-*O*-1,1,3,3-tetra-*iso*-propyldisilyl-α-D-arabinofuranoside (11)[25]

Octyl α-D-arabinofuranoside **10**[11] (3.00 g, 11.5 mmol) was dissolved in dry pyridine (100 mL) under argon atmosphere and cooled to 0 °C. $TIPSCl_2$ (4.0 mL, 12.6 mmol) was added and the reaction mixture was stirred at 0 °C for 4 hr. The solvent was evaporated to dryness and the remaining oil was dissolved in 150 mL $CHCl_3$. The $CHCl_3$ solution was washed with deionized water (2×50 mL) and dried over Na_2SO_4. It was concentrated to a syrup that on purification by column chromatography over silica gel using $CHCl_3$–MeOH (95:5) gave compound **11** (4.44 g, 77% yield). HR-ESIMS: *m/z* Found 527.3193 $[M+Na]^+$, calcd 527.3200 for $C_{25}H_{52}O_6Si_2Na$. ^1H NMR ($CDCl_3$): δ 4.87 (1H, dd, $J_{1,2}$ = 0.7 Hz, $J_{1,2\text{-}OH}$ = 2.5 Hz, H-1), 4.18 (1H, dd, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 6.3 Hz H-3), 4.14 (1H, ddd, $J_{1,2}$ = 0.7 Hz, $J_{2,2\text{-}OH}$ = 2.5 Hz, $J_{2,3}$ = 6.3 Hz, H-2), 3.99 (1H, dd, $J_{4,5a}$ = 3.1 Hz, $J_{5a,5b}$ = 12.5 Hz, H-5a), 3.93 (1H, dd, $J_{4,5b}$ = 3.7 Hz, $J_{5a,5b}$ = 12.5 Hz, H-5b), 3.88 (1H, ddd, $J_{3,4}$ = 3.0 Hz, $J_{4,5a}$ = 3.1 Hz, $J_{4,5b}$ = 3.7 Hz, H-4), 3.70 (1H, m, OCH_2), 3.41 (1H, m, OCH_2), 2.00 (1H, d, $J_{1,2\text{-}OH}$ = $J_{2,2\text{-}OH}$ = 2.5 Hz, 2-OH), 1.58 (2H, m, CH_2), 1.29 (10H, m, 5xCH_2), 1.05 (28H, m, 4xCH, 8xCH_3), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 106.75 (C-1), 82.64 (C-4), 80.71 (C-2), 76.94 (C-3), 68.36 (OCH_2), 61.55 (C-5), 31.82, 29.63, 29.36, 29.24, 26.07, 22.65 (CH_2), 17.46, 17.33, 17.26, 17.19, 17.14, 17.09, 17.05, 16.99, 14.09 (CH_3), 13.50, 13.15, 12.81, 12.56 (CH).

Octyl 2-deoxy-3,5-di-*O*-1,1,3,3-tetra-*iso*-propyldisilyl-α-D-arabinofuranoside (12)

A solution of compound **11** (1.60 g, 3.17 mmol) was dissolved in dry DMF (25 mL) under argon atmosphere and cooled to 0 °C. CS_2 (1.1 mL, 18.38 mmol) was added and the reaction mixture was stirred at 0 °C for 15 minutes followed by addition of NaH (380 mg, 9.51 mmol). The reaction mixture was further stirred for 30 minutes and MeI (2.0 mL, 31.70 mmol) was added dropwise. The solution was stirred for 20 minutes at 0 °C and allowed to warm to room temperature over 30 minutes. Deionized water (50 mL) was added to the reaction mixture followed by extraction with EtOAc (3×100 mL). The combined EtOAc extract was washed with deionized water (50 mL), brine solution (50 mL), dried over Na_2SO_4 and concentrated to yellow oil. The oil was dried under vacuum at room temperature for 1 hr and was used further without purification. The crude thiocarbonyl intermediate product was dissolved in dry toluene (30 mL) and Bu_3SnH (3.2 mL, 12.01 mmol) followed by AIBN (113 mg, 0.69 mmol) were added. The reaction mixture was refluxed for 3 hrs, cooled to room temperature and concentrated under vacuum. The crude product was purified over silica gel column using cyclohexane–EtOAc (9:1) as the eluent to produce compound 12 (1.33 g, yield 86%) with slight impurities of tributyltin salts and was used as such for next step. ESIMS: *m/z* Found 511.3249 $[M+Na]^+$, calcd 511.3251 for $C_{25}H_{52}O_5Si_2Na$. ^1H NMR ($CDCl_3$): δ 5.04 (1H, dd, $J_{1,2eq}$ = 3.5 Hz, $J_{1,2ax}$ = 5.7 Hz, H-1), 4.29 (1H, m, $J_{3,4}$ = 6.8 Hz, $J_{2eq,3}$ = 7.0 Hz, $J_{2ax,3}$ = 8.5 Hz, H-3), 4.02 (1H, m, H-5a), 3.85 (1H, m, H-4, H-5b), 3.70 (1H, m, OCH_2), 3.39 (1H, m, OCH_2), 2.45 (1H, ddd, $J_{1,2ax}$ = 5.7 Hz, $J_{2ax,3}$ = 8.5 Hz, $J_{2ax,2eq}$ = 13.3 Hz, H-2_{ax}), 1.94 (1H, ddd, $J_{1,2eq}$ =

3.5 Hz, $J_{2eq,3}$ = 7.0 Hz, $J_{2ax,2eq}$ = 13.3 Hz, H-2_{eq}), 1.55 (2H, m, CH_2), 1.27 (10H, m, 5xCH_2), 1.05 (28H, m, 4xCH, 8xCH_3), 0.88 (3H, m, CH_3).

Octyl 2-deoxy-α-D-arabinofuranoside (13)

Slightly impure compound **12** (1.20 g, 2.66 mmol) was dissolved in a mixture of dry THF (20 mL) and CH_3CN (20 mL) under argon atmosphere, and $Et_4N^+F^-$ (834 mg, 5.59 mmol) was added. The reaction mixture was stirred at room temperature for 3 hrs and concentrated to syrup. Purification over silica gel column using $CHCl_3$–MeOH (9:1) gave pure compound **13** (538 mg, 89%). HR-ESIMS: *m/z* Found 269.1727 $[M+Na]^+$, calcd 269.1729 for $C_{13}H_{26}O_4Na$. ^1H NMR (DMSO-d_6): δ 4.98 (1H, dd, $J_{1,2eq}$ = 2.6 Hz, $J_{1,2ax}$ = 5.8 Hz, H-1), 4.81 (1H, d, $J_{3,3\text{-}OH}$ = 5.3 Hz, 3-OH), 4.62 (1H, t, $J_{5a,5\text{-}OH}$ = $J_{5b,5\text{-}OH}$ = 5.7 Hz, 5-OH), 3.90 (1H, dddd, $J_{2eq,3}$ = 4.9 Hz, $J_{3,4}$ = 5.2 Hz, $J_{3,3\text{-}OH}$ = 5.3 Hz, $J_{2ax,3}$ = 8.4 Hz, H-3), 3.67 (1H, ddd, $J_{4,5a}$ = 3.5 Hz, $J_{3,4}$ = 5.2 Hz, $J_{4,5b}$ = 5.7 Hz, H-4), 3.57 (1H, m, OCH_2), 3.49 (1H, ddd, $J_{4,5a}$ = 3.5 Hz, $J_{5a,5\text{-}OH}$ = 11.4 Hz, H-5a), 3.35 (1H, dd, $J_{4,5b}$ = $J_{5,5\text{-}OH}$ = 5.7 Hz, $J_{5a,5b}$ = 11.4 Hz, H-5b), 3.27 (1H, m, OCH_2), 2.27 (1H, ddd, $J_{1,2ax}$ = 5.8 Hz, $J_{2ax,3}$ = 8.4 Hz, $J_{2ax,2eq}$ = 13.4 Hz, H-2_{ax}), 1.62 (1H, dddd, $J_{1,2eq}$ = 2.6 Hz, $J_{2eq,3}$ = 4.9 Hz, J = 7.6 Hz, $J_{2ax,2eq}$ = 13.4 Hz, H-2_{eq}), 1.47 (2H, m, CH_2), 1.27 (10H, m, 5xCH_2), 0.87 (3H, m, CH_3).

Octyl 3-*O*-benzoyl-2-deoxy-α-D-arabinofuranoside (14)

To a dry pyridine (10 mL) solution of compound **13** (497 mg, 2.01 mmol) were added TrCl (672 mg, 2.40 mmol) and DMAP (25 mg, 0.20 mmol) at room temperature. The reaction mixture was heated at 45 °C overnight and TLC confirmed the completion of the reaction. It was cooled to room temperature and BzCl (0.36 mL, 3.07 mmol) was added dropwise. The reaction mixture was heated at 50 °C overnight, cooled to room temperature and concentrated under vacuum to give a syrup. The syrup was dissolved in $CHCl_3$ (30 mL) and cooled to −20 °C, and 5% TFA in $CHCl_3$ (11 mL) was added dropwise. The reaction mixture was stirred for 4 hrs at the same temperature, co-evaporated with ethanol (2×20 mL), and concentrated to syrup. Column chromatography using cyclohexane–EtOAc (1:1) on the oil gave glycoside **14** (396 mg, overall yield in three steps 65%) as a colorless syrup. HR-ESIMS: *m/z* Found 373.1996 $[M+Na]^+$, calcd 373.1985 for $C_{20}H_{30}O_5Na$. ^1H NMR ($CDCl_3$): δ 8.06 (2H, m, Ar), 7.57 (1H, m, Ar), 5.33 (1H, ddd, $J_{2eq,3}$ = 2.2 Hz, $J_{3,4}$ = 3.8 Hz, $J_{2ax,3}$ = 5.9 Hz, H-3), 5.27 (1H, dd, $J_{1,2eq}$ = 0.9 Hz, $J_{1,2ax}$ = 5.2 Hz, H-1), 4.25 (1H, dd, $J_{3.4}$ = $J_{4,5a}$ = 3.8 Hz, $J_{4,5b}$ = 4.0 Hz, H-4), 3.88 (1H, dd, $J_{4,5a}$ = 3.8 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 3.83 (1H, dd, $J_{4,5b}$ = 4.0 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5b), 3.72 (1H, m, OCH_2), 3.43 (1H, m, OCH_2), 2.43 (1H, ddd, $J_{1,2ax}$ = 5.2 Hz, $J_{2ax,3}$ = 5.9 Hz, $J_{2ax,2eq}$ = 13.4 Hz, H-2_{ax}), 2.21 (1H, ddd, $J_{1,2eq}$ = 0.9 Hz, $J_{2eq,3}$ = 2.2 Hz, $J_{2ax,2eq}$ = 13.4 Hz, H-2_{eq}), 1.59 2H, (m, CH_2), 1.30 (10H, m, 5xCH_2), 0.85 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 166.69 (C=O), 133.15 (CH, Ar), 129.86 (C, Ar), 129.70, 128.32 (each CH, Ar), 103.64 (C-1), 83.55 (C-4), 74.86 (C-3), 67.46 (OCH_2), 62.74 (C-5), 39.31 (C-2), 31.79, 29.34, 29.39, 29.25, 26.24, 22.62 (each CH_2), 14.04 (CH_3).

Octyl 2-*O*-pivaloyl-3,5-*O*-1,1,3,3-tetra-*iso*-propyldisilyl- α-D-arabinofuranoside (15)

Compound **11** (2 g, 3.96 mmol) was dissolved in dry pyridine (10 mL) and pivaloyl chloride (0.6 mL, 4.75 mmol) and DMAP (48 mg, 0.40 mmol) were added. The reaction mixture was stirred overnight at room temperature and TLC showed completion of the reaction. It was concentrated to a syrup, and purification by column chromatography over silica gel using cyclohexane–EtOAc (95:5) gave compound **15** (2.26 g) in 97% yield. HR-ESIMS: *m/z* Found 611.3760 $[M+Na]^+$, calcd 611.3775 for $C_{30}H_{60}O_7Si_2Na$. ^1H NMR ($CDCl_3$): δ 5.10 (1H, dd, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 4.4 Hz, H-2), 4.78 (1H, d, $J_{1,2}$ = 1.1 Hz, H-1), 4.32 (1H, dd, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 7.0 Hz, H-3), 3.98 (3H, m, H-4, H_2-5), 3.64 (1H, m, OCH_2), 3.41 (1H, m, OCH_2), 1.58 (2H, m, CH_2), 1.27 (10H, m, 5xCH_2), 1.20 (9H, s, 3xCH_3), 1.05 (28H, m, 4xCH, 8xCH_3), 0.87 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 177.42 (C=O), 105.48 (C-1), 84.16 (C-2), 81.43 (C-4),

76.55 (C-3), 67.79 (OCH_2), 62.20 (C-5), 38.61 (C), 31.83, 29.50, 29.34, 29.24 (CH_2), 27.03 (CH_3), 26.04, 22.65 (CH_2), 17.42, 17.32, 17.29, 17.13, 17.05, 16.98, 16.94, 14.06 (CH_3), 13.58, 13.22, 12.78, 12.53 (CH_2).

Octyl 2-*O*-pivaloyl-α-D-arabinofuranoside (16)

Compound **15** (2.20 g, 3.74 mmol) was treated with $Et_4N^+F^-$ (1.00 g, 6.73 mmol) as described in the synthesis of compound **13**. Purification over silica gel column using $CHCl_3$–MeOH (95:5) gave pure compound **16** (1.18 g, 91%). HR-ESIMS: *m/z* Found 369.2249 $[M+Na]^+$, calcd 369.2253 for $C_{18}H_{34}O_6Na$. ^1H NMR ($CDCl_3$): δ 5.06 (1H, s, H-1), 4.81 (1H, dd, $J_{1,2}$ = 0.9 Hz, $J_{2,3}$ = 2.5 Hz, H-2), 4.13 (1H, ddd, $J_{4,5b}$ = 3.3 Hz, $J_{4,5a}$ = 4.6 Hz, $J_{3,4}$ = 5.8 Hz, H-4), 3.96 (1H, ddd, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = $J_{3,3\text{-}OH}$ = 5.8 Hz, H-3), 3.89 (1H, ddd, $J_{4,5a}$ = 4.6 Hz, $J_{5a,5\text{-}OH}$ = 7.9 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 3.73 (2H, m, H-5b, OCH_2), 3.44 (1H, m, OCH_2), 3.16 (1H, d, $J_{3,3\text{-}OH}$ = 5.8 Hz, 3-OH), 1.82 (1H, dd, $J_{5b,5\text{-}OH}$ = 5.2 Hz, $J_{5a,5\text{-}OH}$ = 7.9 Hz, 5-OH), 1.59 (2H, m, CH_2), 1.27 (10H, m, 5xCH_2), 1.22 (9H, s, 3xCH_3), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 178.91 (C=O), 105.19 (C-1), 85.32 (C-2), 84.15 (C-4), 76.44 (C-3), 67.90 (OCH_2), 62.02 (C-5), 38.71 (C), 31.79, 29.45, 29.31, 29.21 (CH_2), 27.04 (CH_3), 26.06, 22.63 (CH_2), 14.07 (CH_3).

Octyl 2-*O*-pivaloyl-5-*O*-trityl-α-D-arabinofuranoside (17)

To a dry pyridine (20 mL) solution of compound **16** (1.18 g, 3.41 mmol) were added trityl chloride (1.16 g, 4.16 mmol) and DMAP (42 mg, 0.34 mmol), and the reaction mixture was stirred at 50 °C overnight. Co-evaporation with toluene (2×50 mL) resulted in an oil that was dissolved in $CHCl_3$ (100 mL), washed with water (2×20 mL), dried over Na_2SO_4 and concentrated. Column chromatography (cyclohexane–EtOAc 95:5) gave compound **17** as an oil (1.70 g, 85%). HR-ESIMS: *m/z* Found 611.3339 $[M+Na]^+$, calcd 611.3349 for $C_{37}H_{48}O_6Na$. ^1H NMR ($CDCl_3$): δ 7.46 (6H, m, Ar), 7.37 (9H, m, Ar), 5.05 (1H, s, H-1), 4.81 (1H, dd, $J_{1,2}$ = 0.7 Hz, $J_{2,3}$ = 2.2 Hz, H-2), 4.28 (1H, dd, $J_{3,4}$ = 4.7 Hz, $J_{4,5b}$ = 5.3 Hz, $J_{4,5a}$ = 5.8 Hz, H-4), 3.96 (1H, ddd, $J_{2,3}$ = 2.2 Hz, $J_{3,4}$ = 4.7 Hz, $J_{3,3\text{-}OH}$ = 7.1 Hz, H-3), 3.77 (1H, m, OCH_2), 3.29 (1H, dd, $J_{4,5a}$ = 5.8 Hz, $J_{5a,5b}$ = 9.7 Hz, H-5a), 3.19 (1H, dd, $J_{4,5b}$ = 5.3 Hz, $J_{5a,5b}$ = 9.7 Hz, H-5b), 3.04 (1H, d, $J_{3,3\text{-}OH}$ = 7.1 Hz, 3-OH), 1.60 (2H, m, CH_2), 1.31 (10H, m, 5xCH_2), 1.08 (9H, s, 3xCH_3), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 178.43 (C=O), 143.81 (C), 128.66, 127.79, 126.98 (CH), 105.10 (C-1), 86.58 (C), 83.95 (C-2), 83.86 (C-4), 77.22 (C-3), 67.80 (OCH_2), 63.82 (C-5), 38.53 (C), 31.78, 29.51, 29.31, 29.20 (CH_2), 26.94 (CH_3), 26.08, 22.61 (CH_2), 14.06 (CH_3).

Octyl 3-deoxy-2-*O*-pivaloyl-5-*O*-trityl-α-D-arabinofuranoside (18)

A solution of compound **17** (1.70 g, 2.89 mmol) was dissolved in dry DMF (25 mL) under argon atmosphere and cooled to 0 °C. CS_2 (1.01 mL, 16.75 mmol) was added and reaction mixture was stirred at 0 °C for 15 minutes followed by addition of NaH (347 mg, 8.67 mmol). Reaction mixture was further stirred for 30 min, MeI (1.8 mL, 28.9 mmol) was added dropwise. The reaction was stirred for 20 min at 0 °C and allowed to warm to room temperature in 30 min. Deionized water (50 mL) was added to reaction mixture and extracted with EtOAc (3×100 mL). The combined EtOAc extract was washed with deionized water (50 mL), aq. satd. NaCl solution (50 mL), dried over Na_2SO_4 and concentrated to a yellow oil. It was dried under vacuum at room temperature for 1 hr and was used further without purification. The crude intermediate product was dissolved in dry toluene (30 mL) and Bu_3SnH (2.0 mL, 7.35 mmol) followed by AIBN (69 mg, 0.42 mmol) were added. The reaction mixture was refluxed for 3 hrs, cooled to room temperature and concentrated under vacuum. The crude product was purified over silica gel column using cyclohexane–EtOAc (98:2) as the eluent to produce compound **18** (1.37 g, yield 83%). HR-ESIMS: *m/z* Found 595.3405 $[M+Na]^+$, calcd 595.3394 for $C_{37}H_{48}O_5Na$. ^1H NMR ($CDCl_3$): δ 7.45 (6H, m, Ar), 7.26 (9H, m, Ar), 4.98 (1H, dd,

$J_{2,3ax}$ = 1.3 Hz, $J_{2,3eq}$ = 6.2 Hz, H-2), 4.96 (1H, s, H-1), 4.51 (1H, dddd, $J_{3ax,4}$ = 4.3 Hz, $J_{4,5b}$ = 5.8 Hz, $J_{4,5a}$ = 6.4 Hz, $J_{3eq,4}$ = 8.5 Hz, H-4), 3.68 (1H, m, OCH_2), 3.41 (1H, m, OCH_2), 3.31 (1H, dd, $J_{4,5a}$ = 6.4 Hz, $J_{5a,5b}$ = 9.2 Hz, H-5a), 3.00 (1H, dd, $J_{4,5b}$ = 5.8 Hz, $J_{5a,5b}$ = 9.2 Hz, H-5b), 2.48 (1H, ddd, $J_{2,3eq}$ = 6.2 Hz, $J_{3eq,4}$ = 8.5 Hz, $J_{3ax,3eq}$ = 14.2 Hz, H-3_{eq}), 1.70 (1H, ddd, $J_{2,3ax}$ = 1.3 Hz, $J_{3ax,4}$ = 4.3 Hz, $J_{3ax,3eq}$ = 14.2 Hz, H-3_{ax}), 1.54 (2H, m, CH_2), 1.30 (10H, m, 5xCH_2), 1.02 (9H, s, 3xCH_3), 0.88 (3H, m, CH_3).

Octyl 3-deoxy-2-*O*-pivaloyl-α-D-arabinofuranoside (19)

Compound **18** (1.00 g, 1.75 mmol) was dissolved in $CHCl_3$ (30 mL) and cooled to −20 °C, and 5% TFA in $CHCl_3$ (11 mL) was added dropwise. The reaction mixture was stirred for 4 hr, co-evaporated with ethanol (2×20 mL), and concentrated to syrup. Column chromatography (cyclohexane–EtOAc, 3:1) yielded compound **19** (479 mg, yield 83%) as a colorless syrup. HR-ESIMS: *m/z* Found 353.2296 $[M+Na]^+$, calcd 353.2304 for $C_{18}H_{34}O_5Na$. ^1H NMR ($CDCl_3$): δ 5.05 (1H, dd, $J_{2,3ax}$ = 1.3 Hz, $J_{2,3eq}$ = 6.4 Hz, H-2), 4.99 (1H, s, H-1), 4.31 (1H, m, H-4), 3.77 (1H, ddd, $J_{4,5a}$ = 3.1 Hz, $J_{5a,OH}$ = 5.7 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 3.66 (1H, m, OCH_2), 3.60 (1H, ddd, $J_{4,5b}$ = 5.5 Hz, $J_{5b,OH}$ = 6.7 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5b), 3.41 (1H, m, OCH_2), 2.47 (1H, ddd, $J_{2,3eq}$ = 6.4 Hz, $J_{3eq,4}$ = 8.8 Hz, $J_{3ax,3eq}$ = 14.4 Hz, H-3_{eq}), 1.87 (1H, dd, $J_{5a,OH}$ = 5.7 Hz, $J_{5b,OH}$ = 6.7 Hz, 5- OH), 1.72 (1H, ddd, $J_{2,3ax}$ = 1.3 Hz, $J_{3ax,5a}$ = 5.7 Hz, $J_{3ax,5b}$ = 6.7 Hz, H-3_{ax}), 1.58 (2H, m, CH_2), 1.28 (10H, m, 5xCH_2), 1.19 (9H, s, 3xCH_3), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 177.60 (C=O), 105.97 (C-1), 78.34 (C-4), 77.44 (C-2), 67.39, (OCH_2), 64.76 (C-5), 38.57 (C), 31.80 (CH_2), 31.51 (C-3), 29.51, 29.33, 29.21 (CH_2), 27.03 (CH_3), 26.07, 22.62 (CH_2), 14.07 (CH_3).

Octyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)-2- deoxy-3-*O*-benzoyl-α-D-arbinofuranoside (21)

Donor glycoside **20** (695 mg, 1.82 mmol), acceptor glycoside **14** (425 mg, 1.21 mmol) and activated, powdered 4Å molecular sieves (200 mg) in dry CH_2Cl_2 (15 mL) were cooled at 0 ° C under argon atmosphere. The mixture was stirred for 15 min, and NIS (327 mg, 1.45 mmol) followed by $Sn(OTf)_2$ (51 mg, 0.12 mmol) was added to initiate coupling. It was stirred for 30 min at rt, and the reaction was quenched by addition of Et_3N (1 mL). The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (20 mL), followed by washing with saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, the solvent was removed under vacuum, and the residue was purified by column chromatography (cyclohexane–EtOAc, 3:1) to give pure disaccharide **21** as a colorless oil (627 mg, 85%). HR-ESIMS: *m/z* Found 631.2741 $[M+Na]^+$, calcd 631.2725 for $C_{31}H_{44}O_{12}Na$. ^1H NMR ($CDCl_3$): δ 8.05 (2H, m, Ar), 7.57 (1H, m, Ar), 7.43 (2H, m, Ar), 5.43 (1H, ddd, $J_{3,4}$ = 3.0 Hz, $J_{2eq,3}$ = 4.6 Hz, $J_{2ax,3}$ = 7.7 Hz, H-3), 5.28 (1H, d, $J_{1,2ax}$ = 4.7 Hz, H-1), 5.13 (1H, s, H-1′), 5.11 (1H, d, $J_{2',3'}$ = 1.3 Hz, H-2′), 4.97 (1H, dd, $J_{2',,3',}$ = 1.3 Hz, $J_{3',,4',}$ = 4.6 Hz, H-3′), 4.43 (1H, dd, $J_{4',5a',}$ = 3.0 Hz, $J_{5a',,5b',}$ = 11.3 Hz, H-5a′), 4.36 (1H, dd, $J_{3,4}$ = $J_{4,5a}$ = 3.0 Hz, $J_{4,5b}$ = 5.7 Hz, H-4), 4.27 (1H, ddd, $J_{4',5a',}$ = 3.0 Hz, $J_{3',,4',}$ = 4.6 Hz, $J_{4',5b',}$ = 5.7 Hz, H-4′), 4.21 (1H, dd, $J_{4',5b',}$ = 5.7 Hz, $J_{5a',5b',}$ = 11.3 Hz, H-5b′), 4.00 (1H, dd, $J_{4.5a}$ = 3.0 Hz, J_{5a5b} = 11.1 Hz, H-5a), 3.71 (2H, m, H-5b, OCH_2), 3.42 (1H, m, OCH_2), 2.46 (1H, ddd, $J_{1,2ax}$ = 4.7 Hz, $J_{2ax,3}$ = 7.7 Hz, $J_{2ax,2eq}$ = 13.1 Hz, H-2_{ax}), 2.10 (1H, m, H-2_{eq}), 2.09, 2.10, 2.11 (each 3H, s, 3xCH_3), 1.58 (2H, m, CH_2), 1.31 (10H, m, 5xCH_2), 0.86 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.52, 170.07, 169.42, 166.36 (4xC=O), 133.04 (CH, Ar), 130.00 (C, Ar), 129.63, 128.28 (each CH_2, Ar), 105.68 (C-1′), 104.02 (C-1), 82.50 (C-4), 80.90 (C-4′), 80.85 (C-2′), 77.06 (C-3′), 74.83 (C-3), 67.42 (OCH_2), 66.67 (C-5), 63.26 (C-5′), 39.25 (C-2), 31.77, 29.76, 29.37, 29.23, 26.23, 22.58 (6xCH_2), 20.71, 20.68, 20.64, 14.02 (4xCH_3).

Octyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)-3- deoxy-2-*O*-pivaloyl-α-D-arbinofuranoside (22)

Donor glycoside **20** (503 mg, 1.32 mmol), acceptor glycoside **19** (290 mg, 0.90 mmol) were coupled as described for the synthesis of disaccharide **21**. The crude disaccharide was purified by column chromatography (cyclohexane–EtOAc, 3:1) to give pure disaccharide **22** (449 mg, 87%) as a colorless oil. HR-ESIMS: *m/z* Found 611.3038 $[M+Na]^+$, calcd 611.3001 for $C_{29}H_{48}O_{12}Na$. ^1H NMR ($CDCl_3$): δ 5.13 (1H, d, J = 1.8 Hz, H-2′), 5.09 (1H, s, H-1′), 5.03 (1H, dd, $J_{2,3ax}$ = 1.7 Hz, $J_{2,3eq}$ = 6.4 Hz, H-2), 4.98 (2H, m, H-1, H-3′), 4.42 (1H, dd, $J_{4'5a'}$= 2.1 Hz, $J_{5a',5b'}$= 10.6 Hz, H-5a′), 4.36 (1H, m, H-4), 4.24 (2H, m, H-4′, H-5b′), 3.82 (1H, dd, $J_{4,5a}$ = 6.2 Hz, $J_{5a,5b}$ = 10.3 Hz, H-5a), 3.65 (1H, m, OCH_2), 3.51 (1H, dd, $J_{4,5b}$ = 5.5 Hz, $J_{5a,5b}$ = 10.3 Hz, H-5b), 3.40 (1H, m, OCH_2), 2.47 (1H, ddd, $J_{2,3eq}$ = 6.4 Hz, $J_{3eq,4}$ = 8.2 Hz, $J_{3ax,3eq}$ = 14.2 Hz, H-3_{eq}), 2.10 (6H, s, 2xCH_3), 2.09 (3H, s, CH_3), 1.71 (1H, ddd, $J_{2,3ax}$ = 1.7 Hz, J = 5.0 Hz, $J_{3ax,3eq}$ = 14.2 Hz, H-3_{ax}), 1.55 (2H, m, CH_2), 1.28 (10H, m, 5xCH_2), 1.19 (9H, s, 3xCH_3), 0.88 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 177.69, 170.56, 170.12, 169.54 (C=O), 106.04 (C-1), 105.65 (C-1′), 81.19 (C-2′), 80.22 (C-4′), 77.32 (C-2), 77.10 (C-3′), 76.12 (C-4), 69.66 (OCH_2), 67.41 (C-5), 63.18 (C-5′), 38.53 (C), 32.51 (C-3), 31.79, 29.50, 29.33, 29.21 (CH_2), 27.02 (CH_3), 26.05 (CH_2), 22.61 (CH_2), 20.76, 20.72 (CH_3), 14.06 (CH_3).

Octyl 2-deoxy-2-fluoro-3,5-*O*-dibenzoyl-α-D-arabinofuranoside (24)

Compound **23**[28] (900 mg, 1.94 mmol) was dissolved in dry CH_3CN (200 mL) was added $SnCl_4$ (0.27 mL, 2.33 mmol) at room temperature and the mixture was stirred for 15 min. To this solution, *n*-octanol (0.37 mL, 2.33 mmol) was added dropwise over a period of 30 min. It was again stirred for 1 hr at room temperature. Celite (2.0 g) was added cooled in ice-water bath and a saturated aqueous $NaHCO_3$ solution was added dropwise to precipitate tin salts. After complete precipitation, the mixture was filtered through celite and washed with chloroform (2×10 mL), concentrated to a syrup, and re-dissolved in $CHCl_3$ (100 mL). The solution was next washed with water (2×25 mL) and brine (2×25 mL), dried over Na_2SO_4 and concentrated under vacuum to give a crude oil. Column chromatography (cyclohexane–EtOAc, 7:1) gave the desired α-isomer **24** as a colorless oil (403 mg, 54% yield). HR-ESIMS: *m/z* Found 495.2153 $[M+Na]^+$, calcd 495.2154 for $C_{27}H_{33}FO_6Na$. ^1H NMR ($CDCl_3$): δ 8.05 (4H, m, Ar), 7.57 (2H, m, Ar), 7.43 (4H, m, Ar), 5.48 (1H, ddd, $J_{2,3}$ = 0.7 Hz, $J_{3,4}$ = 4.7 Hz, $J_{3,F}$ = 22.4 Hz, H-3), 5.30 (1H, d, $J_{1,2}$ = 0 Hz, $J_{1,F}$ = 10.3 Hz, H-1), 5.10 (1H, dd, $J_{1,2}$ = 0.0 Hz, $J_{2,3}$ = 0.7 Hz, $J_{2,F}$ = 49.66 Hz, H-2), 4.74 (1H, dd, $J_{4,5a}$ = 3.5 Hz, $J_{5a,5b}$ = 11.9 Hz, H_{5a}), 4.62 (1H, dd, $J_{4,5b}$ = 4.6 Hz, $J_{5a,5b}$ = 11.9 Hz, H_{5b}), 4.50 (1H, dd, $J_{4,5a}$ = 3.5 Hz, $J_{4,5b}$ = 4.6 Hz, H-4), 3.76 (1H, m, OCH_2), 3.49 (1H, m, OCH_2), 1.59 (2H, m, CH_2), 1.30 (10H, m, 5xCH_2), 0.86 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 166.37, 165.77 (2xC=O), 133.68, 133.18, 129.97, 129.90, 128.59, 128.44 (Ar), 105.16 ($J_{1,F}$ = 34.8 Hz, C-1), 98.25 ($J_{2,F}$ = 181.9 Hz, C-2), 81.00 ($J_{4,F}$ = 1.8 Hz, C-4), 77.63 ($J_{3,F}$ = 30.5 Hz, C-3), 67.61 (OCH_2), 63.83 (C-5), 31.90, 29.58, 29.45, 29.35, 26.23, 22.73 (CH_2), 14.16 (CH_3).

Octyl 2-deoxy-2-fluoro-3-*O*-benzoyl-5-*O*-trityl-α-D-arabinofuranoside (25)

To a solution of saccharide **24** (260 mg, 0.55 mmol) in dry methanol (5 mL) was added 7N NH_3/MeOH (10 mL) and the reaction mixture was stirred overnight at room temperature. TLC showed no starting material and it was concentration under vacuum to an oil. The crude product was dissolved in dry pyridine (4 mL) and trityl chloride (108 mg, 0.39 mmol) and DMAP (4 mg, 0.03 mmol) were added. The reaction mixture was stirred at 50 °C overnight, cooled to room temperature and BzCl (0.05 mL, 0.44 mmol) was added. The reaction mixture was heated overnight at 50 °C. Co-evaporation with toluene (2×25 mL) resulted in an oil that was dissolved in $CHCl_3$ (100 mL), washed with water (2×20 mL), dried over Na_2SO_4 and concentrated. Column chromatography (cyclohexane– EtOAc, 95:5) gave compound **25** as oil (272 mg, overall yield 81%). HR-ESIMS: *m/z* Found 633.2997 $[M+Na]^+$, calcd 633.2987 for

$C_{39}H_{43}FO_5Na$. ^1H NMR ($CDCl_3$): δ 8.03 (2H, m, Ar), 7.51 (9H, m, Ar), 7.20 (9H, m, Ar), 5.41 (1H, ddd, $J_{2,3}$ = 1.0 Hz, $J_{3,4}$ = 5.1 Hz, $J_{3,F}$ = 22.6 Hz, H-3), 5.25 (1H, d, $J_{1,F}$ = 10.6 Hz, H-1), 4.99 (1H, dd, $J_{2,3}$ = 1.0 Hz, $J_{2,F}$ = 49.7 Hz, H-2), 4.32 (1H, dd, $J_{4,5a}$ = 2.5 Hz, J = 9.5 Hz, H-5a), 3.75 (1H, ddd, $J_{4,5b}$ = 6.6 Hz, J = 9.5 Hz, H-5b), 3.49 (1H, dd, $J_{4,5a}$ = 2.5 Hz, $J_{4,5b}$ = 6.6 Hz, H-4), 3.41 (2H, m, OCH_2), 1.59 (2H, m, CH_2), 1.30 (10H, m, CH_2), 0.86 (3H, m, CH_3).

Octyl 2-deoxy-2-fluoro-3-*O*-benzoyl-α-D-arabinofuranoside (26)

Compound **25** (80 mg, 0.13 mmol) was dissolved in $CHCl_3$ (3 mL) and cooled to −20 °C, and 5% TFA in $CHCl_3$ (1.3 mL) was added dropwise. The reaction mixture was stirred for 1 hr and cold aqueous satd. $NaHCO_3$ solution (10 mL) was added. It was extracted with $CHCl_3$ (2×15 mL), washed with deionized water (2×10 mL) and dried over Na_2SO_4. After concentration and column chromatography (cyclohexane–EtOAc, 5:1) compound **26** (38 mg, yield 81%) was obtained. HR-ESIMS: *m/z* Found 391.1891 $[M+Na]^+$, calcd 391.1891 for $C_{20}H_{29}FO_5Na$. ^1H NMR ($CDCl_3$): δ 8.04 (2H, m, Ar), 7.60 (1H, m, Ar), 7.46 (2H, m, Ar), 5.38 (1H, ddd, $J_{1,3}$ = 0.9 Hz, $J_{3,4}$ = 4.8 Hz, $J_{3,F}$ = 23.0 Hz, H-3), 5.25 (1H, d, $J_{1,2}$ = 0 Hz, $J_{1,F}$ = 10.4 Hz, H-1), 5.10 (1H, dd, $J_{1,4}$ = 1.1 Hz, $J_{2,F}$ = 49.7 Hz, H-2), 4.24 (1H, dd, J = 4.3 Hz, 8.4 Hz, H-4), 3.93 (2H, m, H_2-5), 3.74 (1H, m, OCH_2), 3.48 (1H, m, OCH_2), 2.15 (1H, dd, J = 5.3, 7.9 Hz, 5-OH), 1.60 (2H, m, CH_2), 1.29 (10H, m, 5xCH_2), 0.86 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 166.01 (C=O), 133.63, 129.89 (CH), 129.81 (C), 128.52 (CH), 104.84 (d, $^2J_{1,F}$ = 34.8 Hz, C-1), 98.43 (d, $1J_{2,F}$ = 181.9 Hz, C-2), 83.29 (d, $3J_{4,F}$ = 1.8 Hz, C-4), 77.30 (d, $^2J_{3,F}$ = 29.0 Hz, C-3), 67.44 (OCH_2), 62.32 (C-5), 31.81, 29.49, 29.37, 29.26 (CH_2), 26.14 (CH_2), 22.65 (CH_2), 14.07 (CH_3).

Octyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl-(1→5)-2- deoxy-2-fluoro-3-*O*-benzoyl-α-D-arbinofuranoside (27)

Glycosyl donor **20** (47 mg, 0.12 mmol), glycosyl acceptor **26** (30 mg, 0.08 mmol) were coupled as described for the synthesis of disaccharide **21**. The crude disaccharide was purified by column chromatography (cyclohexane–EtOAc, 3:1) to give pure disaccharide **27** (45 mg, 88%) as a colorless oil. HR-ESIMS: *m/z* Found 649.2620 $[M+Na]^+$, calcd 649.2631 for $C_{31}H_{43}FO_{12}Na$. ^1H NMR ($CDCl_3$): δ 8.03 (2H, m, Ar), 7.59 (1H, m, Ar), 7.45 (2H, m, Ar), 5.51 (1H, ddd, $J_{2,3}$ = 0.9 Hz, $J_{3,4}$ = 5.1 Hz, $J_{3,F}$ = 22.3 Hz, H-3), 5.25 (1H, d, $J_{1,F}$ = 10.6 Hz, H-1), 5.18 (1H, d, $J_{2',,3',}$ = 1.3 Hz, H-2′), 5.17 (1H, s, H-1′), 5.04 (1H, dd, $J_{2,3}$ = 0.9 Hz, $J_{2,F}$ = 43.8 Hz, H-2), 4.95 (1H, m, H-3′), 4.44 (1H, dd, $J_{4',,5',a}$ = 3.4 Hz, $J_{5',a,5',b}$ = 11.6 Hz, H-5′a), 4.33 (2H, m, H-4, H-4′), 4.21 (1H, dd, $J_{4',,5',b}$ = 5.6 Hz, $J_{5',a,5',b}$ = 11.6 Hz, H-5′b), 4.03 (1H, dd, $J_{4,5a}$ = 3.8 Hz, $J_{5a,5b}$ = 11.3 Hz, H-5a), 3.83 (1H, dd, $J_{4,5b}$ = 3.1 Hz, $J_{5a,5b}$ = 11.3 Hz, H-5b), 3.74 (1H, m, OCH_2), 3.47 (1H, m, OCH_2), 2.09 (6H, s, 2xOCH_3), 2.08 (3H, s, OCH_3), 1.58 (2H, m, CH_2), 1.28 (10H, m, 5xCH_2), 0.86 (3H, m, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.62, 170.43, 169.38 ($COCH_3$), 165.58 (COC_6H_5), 133.53, 129.82 (CH), 129.17 (C), 128.49 (CH), 105.51 (C-1′), 105.01 (d, $^2J_{1,F}$ = 34.8 Hz, C-1), 98.70 (d, $^2J_{2,F}$ = 182.5 Hz, C-2), 81.38 (d, $3J_{4,F}$ = 1.2 Hz, C-4), 81.04 (C-4′), 80.74 (C-2′), 77.00 (C-3′), 76.61 (d, $^2J_{3,F}$ = 18.9 Hz, C-3), 67.47 (OCH_2), 65.49 (C-5), 63.38 (C-5′), 31.80, 29.46, 29.36, 29.26, 26.13, 22.63 (CH_2), 20.78, 20.74, 20.62 ($COCH_3$), 14.06 (CH_3).

Octyl α-D-arabinofuranosyl-(1→5)-2-deoxy-α-D-arbinofuranoside (6)

To a solution of disaccharide **21** (200 mg, 0.33 mmol) in dry methanol (5 mL) was added 7N NH_3/MeOH (5 mL) and the reaction mixture was stirred overnight at room temperature. Concentration in vacuum, and column chromatography ($CHCl_3$–MeOH, 9:1) gave the de-blocked disaccharide containing a minor impurity. For the final purification, an aqueous solution (5 mL) of the disaccharide was passed through a small column packed with Bio-Beads™ SM-4 (20–50 mesh) and lyophilized to afford **6** as a hygroscopic solid (105 mg, 85%).

HR-ESIMS: *m/z* Found 401.2156 $[M+Na]^+$ calcd 401.2164 for $C_{18}H_{34}O_8Na$. ^1H NMR (CD_3OD): δ 5.11 (1H, dd, $J_{1,2eq}$ = 1.9 Hz, $J_{1,2ax}$ = 5.5 Hz, H-1), 4.89 (1H, d, $J_{1',,2',}$ = 1.2 Hz, H-1′), 4.15 (1H, ddd, $J_{2eq,3}$ = 5.6 Hz, $J_{2ax,3}$ = 7.9 Hz, $J_{3,4}$ = 8.6 Hz, H-3), 4.00 (1H, ddd, $J_{4,5a}$ = $J_{4,5b}$ = 4.6 Hz, $J_{3,4}$ = 8.6 Hz, H-4), 3.95 (1H, dd, $J_{1',,2',}$ = 1.2 Hz, $J_{2',,3',}$ = 3.3 Hz, H-2′), 3.94 (1H, ddd, $J_{4',,5',a}$ = 3.3 Hz, $J_{4',,5',b}$ = 5.6 Hz, $J_{3',,4',}$ = 6.3 Hz, H-4′), 3.81 (1H, dd, $J_{2',,3',}$ = 3.3 Hz, $J_{3',,4',}$ = 6.3 Hz, H-3′), 3.78 (1H, dd, $J_{4,5a}$ = 4.6 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5a), 3.73 (1H, dd, $J_{4',,5',a}$ = 3.3 Hz, $J_{5',a,5',b}$ = 11.8 Hz, H-5a′), 3.68 (1H, m, OCH_2), 3.62 (1H, dd, $J_{4',,5',b}$ = 5.6 Hz, $J_{5',a,5',b}$ = 11.8 Hz, H-5b′), 3.54 (1H, dd, $J_{4,5b}$ = 4.6 Hz, $J_{5a,5b}$ = 11.8 Hz, H-5b), 3.38 (1H, m, OCH_2), 2.35 (1H, ddd, $J_{1,2ax}$ = 5.5 Hz, $J_{2ax,3}$ = 7.9 Hz, $J_{2ax,2eq}$ = 13.6 Hz, H-2_{ax}), 1.82 (1H, dddd, $J_{1,2eq}$ = 1.9 Hz, J = 3.7 Hz, $J_{2eq,3}$ = 5.6 Hz, $J_{2ax,2eq}$ = 13.6 Hz, H-2_{eq}), 1.55 (2H, m, CH_2), 1.32 (10H, m, 5xCH_2), 0.89 (3H, m, CH_3). ^{13}C NMR (CD_3OD): δ 109.68 (C-1′), 105.24 (C-1), 85.79 (C-4′), 84.89 (C-4), 83.31 (C-2′), 78.84 (C-3′), 72.82 (C-3), 68.88 (OCH_2), 68.31 (C-5), 63.08 (C-5′), 42.10 (C-2), 33.00, 30.75, 30.49, 30.41, 27.31, 23.70 (6xCH_2), 14.42 (CH_3).

Octyl α-D-arabinofuranosyl-(1→5)-3-deoxy-α-D-arbinofuranoside (7)

To a solution of disaccharide **22** (160 mg, 0.35 mmol) in dry methanol (6 mL) was added 25% w/v NaOMe/MeOH (0.15 mL), and the reaction mixture was stirred overnight at room temperature. Concentration under vacuum and column chromatography ($CHCl_3$–MeOH, 9:1) gave the deblocked disaccharide. For the final purification, an aqueous solution (5 mL) of the disaccharide was passed through a small column packed with Bio-Beads™ SM-4 (20–50 mesh), and the eluted fractions were lyophilized to afford **7** as a hygroscopic solid (77 mg, 75%). HR-ESIMS: *m/z* Found 401.2108 $[M+Na]^+$, calcd 401.2146 for $C_{18}H_{34}O_8Na$. ^1H NMR (CD_3OD): δ 4.92 (1H, d, $J_{1',,2',}$ = 1.2 Hz, H-1′), 4.87 (1H, s, H-1), 4.29 (1H, m, H-4), 4.08 (1H, dd, $J_{2,3ax}$ = 1.8 Hz, $J_{2,3eq}$ = 3.7 Hz, H-2), 3.96 (1H, m, H-4′), 3.97 (1H, dd, $J_{1',,2',}$ = 1.2 Hz, $J_{2',,3',}$ = 3.3 Hz, H-2′), 3.82 (1H, dd, $J_{2',,3',}$ = 3.3 Hz, $J_{3',,4',}$ = 5.8 Hz, H-3′), 3.80 (1H, dd, $J_{4,5a}$ = 5.3 Hz, $J_{5a,5b}$ = 10.5 Hz, H-5a), 3.73 (1H, dd, $J_{4',,5',a}$ = 3.4 Hz, $J_{5',a,5',b}$ = 11.8 Hz, H-5′ a), 3.67 (1H, m, OCH_2), 3.62 (1H, dd, $J_{4',,5',b}$ = 5.3 Hz, $J_{5',a,5',b}$ = 11.8 Hz, H-5′b), 3.55 (1H, dd, $J_{4,5b}$ = 6.5 Hz, $J_{5a,5b}$ = 10.5 Hz, H-5b), 3.37 (1H, m, OCH_2), 2.34 (1H, ddd, $J_{2,3eq}$ = 3.7 Hz, $J_{3eq,4}$ = 8.7 Hz, $J_{3ax,3eq}$ = 13.5 Hz, H-3_{eq}), 1.67 (1H, ddd, $J_{2,3ax}$ = 1.8 Hz, $J_{3ax,4}$ = 4.7 Hz, J = 13.5 Hz, H-3_{ax}), 1.52 (2H, m, CH_2), 1.30 (10H, m, 5xCH_2), 0.89 (3H, m, CH_3). ^{13}C NMR (CD_3OD): δ 110.09 (C-1), 109.56 (C-1′), 86.06 (C-4′), 83.08 (C-2′), 78.72 (C-3′), 78.29 (C-4), 76.11 (C-2), 70.66 (C-5), 68.21 (OCH_2), 63.07 (C-5), 35.61 (C-3), 33.01, 30.70, 30.41, 27.28, 23.71 (CH_2), 14.43 (CH_3).

Octyl α-D-arabinofuranosyl-(1→5)-2-deoxy-2-fluoro-α- D-arbinofuranoside (8)

To a solution of disaccharide **27** (40 mg, 0.06 mmol) in dry methanol (3 mL) was added 7N NH_3/MeOH (5 mL), and the reaction mixture was stirred overnight at room temperature. Concentration under vacuum and column chromatography ($CHCl_3$–MeOH 9:1) gave disaccharide **8** as an oil (22 mg, 88%). HR-ESIMS: *m/z* Found 419.2051 $[M+Na]^+$, calcd 419.2055 for $C_{18}H_{33}FO_8Na$. ^1H NMR (300 MHz, CD_3OD): 5.06 (1H, d, $^3J_{1,F}$ = 12.2 Hz, H-1), 4.94 (1H, d, $J_{1',,2',}$ = 1.2 Hz, H-1′), 4.75 (1H, dd, $J_{2,3}$ = 2.0 Hz, $^2J_{2,F}$ = 52.2 Hz, H-2), 4.14 (1H, ddd, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 6.7 Hz, $^3J_{3,F}$ = 26.3 Hz, H-3), 4.03 (1H, ddd, $J_{3,4}$ = 6.7 Hz, $J_{4,5b}$ = 3.4 Hz, $J_{4,5a}$ = 5.9 Hz, H-4), 4.00 (1H, dd, $J_{1',,2',}$ = 3.3 Hz, $J_{2',,3',}$ = 3.3 Hz, H-2′), 3.97 (1H, ddd, $J_{4',,5a',}$ = 3.4 Hz, $J_{4',,5b',}$ = 4.9 Hz, $J_{3',,4',}$ = 6.1 Hz, H-4′), 3.86 (1H, dd, $J_{4',5',a}$ = 4.9 Hz, $J_{5',a5',b}$ = 11.3 Hz, H-5′a), 3.83 (1H, dd, $J_{2',,3',}$ = 3.3 Hz, $J_{3',,4',}$ = 6.1 Hz, H-3′), 3.75 (1H, dd, $J_{4,5a}$ = 3.4 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 3.70 (1H, m, OCH_2), 3.67 (1H, dd, $J_{4',5b'}$= 3.4 Hz, $J_{5a\ ',5b\ '}$= 11.3 Hz, H-5b′), 3.64 (1H, dd, $J_{4,5b}$ = 5.9 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5b), 3.43 (1H, m, OCH_2), 1.58 (2H, m, CH_2), 1.34 (10H, m, 5xCH_2), 0.90 (3H, m, CH_3). ^{13}C NMR (CD_3OD): δ 109.68 (C-1′), 106.5 (d, $^2J_{F,C-1}$ = 127.6 Hz, C-1), 103.37 (d, $1J_{F,C-2}$ = 181.3 Hz, C-2), 85.80 (C-4′), 83.25 (d, $3J_{F,C-4}$ = 4.9 Hz, C-4), 83.24 (C-2′), 78.84 (C-3′), 77.21 (d, $^2J_{F,C-3}$ = 26.3 Hz,

C-3), 68.69 (OCH_2), 67.48 (C-5), 63.06 (C-5′), 33.00, 30.56, 30.46, 30.40, 27.21, 23.70 ($6xCH_2$), 14.43 (CH_3).

Biological

In vitro assay—*In-vitro* inhibition assays[31] of the Ara*f* disaccharides **6**, **7** and **8** were performed on *Mycobacterium tuberculosis* (MTB H_{37}Ra, ATCC 25177) and *Mycobacterium avium* (NJ 211).

Arabinosyltransferase assay[32]—Compounds **6**, **7** and **8** at a range of concentrations from 0.1 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 AraTs 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 resuspended 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 labelled 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 labelled 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. IC_{50} values for **6**, **7** and **8** were evaluated by competition-based experiments performed by mixing either **6**, **7** or **8** at various concentrations 0.5, 0.75, 1.0, 2.0, 4.0 and 6.0 mM with Ara*f* α(1→5)Ara*f*-*O*-C_8H_{17} at 0.4 mM concentration followed by thin-layer chromatography/autoradiography (TLC not shown) as described earlier, and the extent of product formation was determined. The R_f on TLC of product formed by native acceptor **1a** was found to be 0.33 and readily distinguishable from other product bands; the R_f values of products formed by **6**, **7** and **8** were 0.40, 0.42 and 0.45 respectively.

Acknowledgments

This work was supported by NIH/NIAID grant R01AI45317. We acknowledge Dr. Kamal Tiwari for providing precursor compound **23**. GSB acknowledges support in the form of a Personal Research Chair from Mr. James Bardrick, a Royal Society Wolfson Research Merit Award, as a former Lister Institute-Jenner Research Fellow, the Medical Research Council (G9901077 and G0500590) and The Welcome Trust (081569/2/06/2). 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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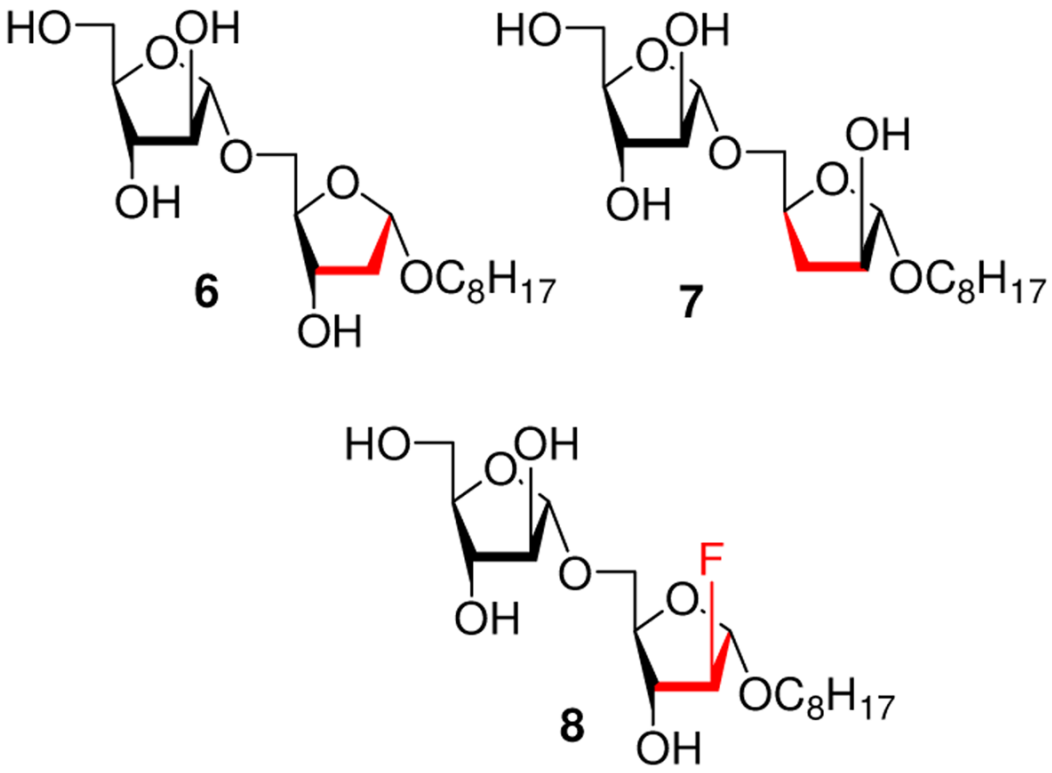
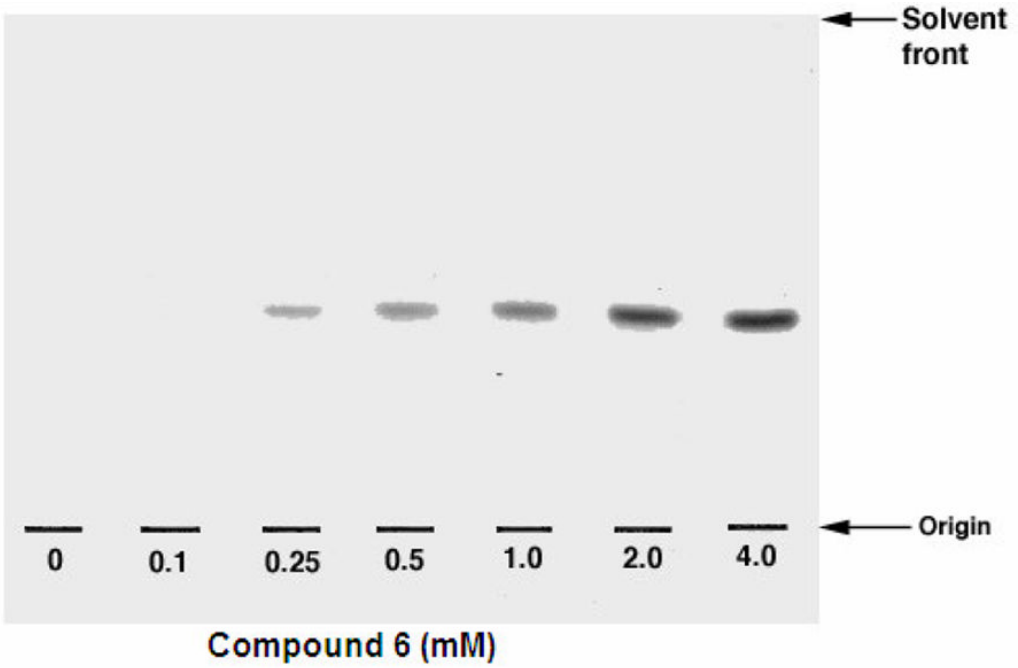


Figure 1.
Target Ara*f* α(1→5)Ara*f* disaccharides



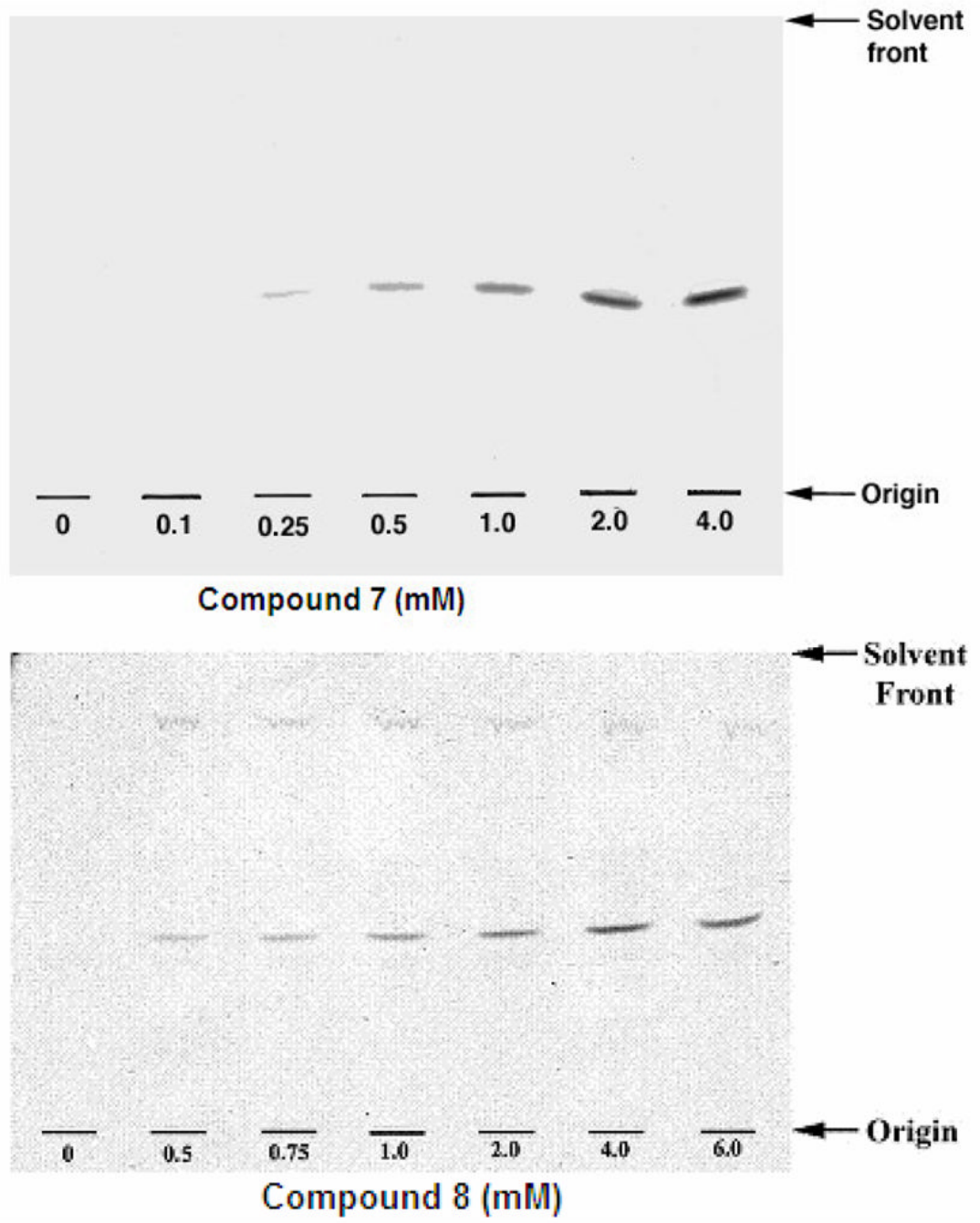


Figure 2.

TLC autoradiograms of reaction products produced through the inclusion of **6**, **7** and **8**, mycobacterial membranes and DP[^{14}C]A. The TLC/autoradiography performed using $CHCl_3$:MeOH:NH_4OH:H_2O (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 3 days.

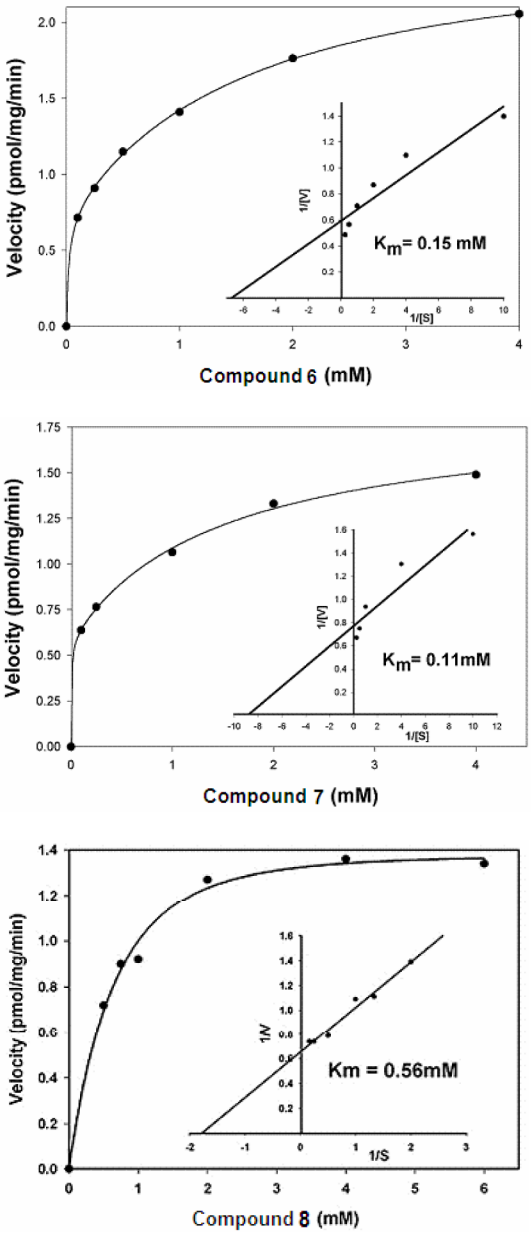
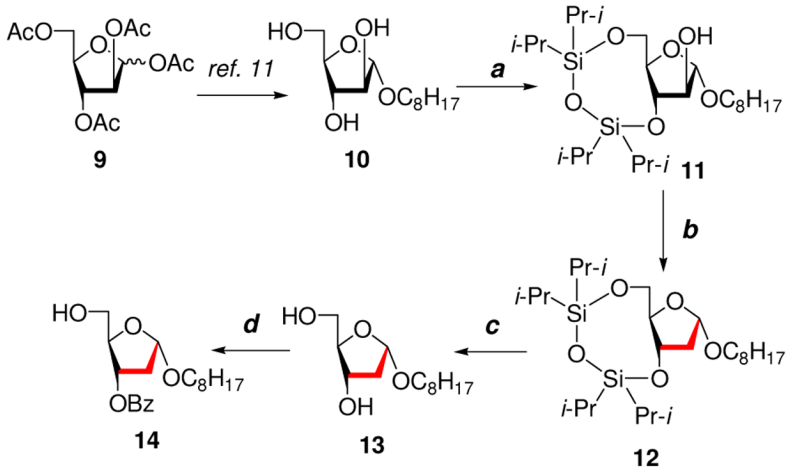


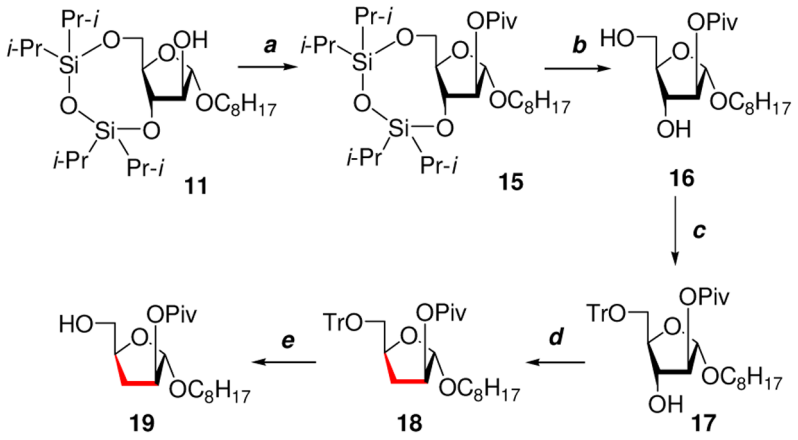
Figure 3.

Kinetic analysis of acceptors **6**, **7** and **8**. The inset illustrates the double reciprocal plot for substrates for the mycobacterial AraTs.



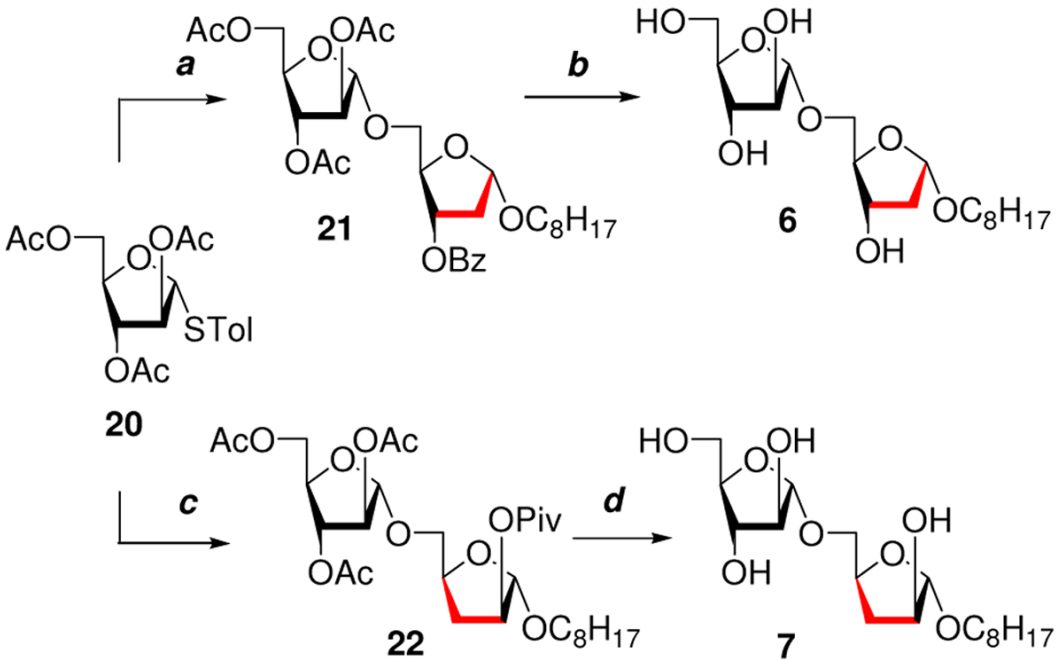
Scheme 1.

Reagents and conditions: (a) $TIPSCl_2$, pyridine, 0 °C, 4 h, 87%; (b) (i) CS_2, NaH, MeI, DMF, 0 °C–rt, 30 min, (ii) Bu_3SnH, AIBN, rt, 3 h, overall yield 86%; (c) $Et_4N^+F^-$, THF, rt, 3 h, 89%; (d) (i) TrCl, DMAP, pyridine, 45 °C, overnight, (ii) BzCl, pyridine, 50 °C, overnight; (iii) 5% TFA/$CHCl_3$, −20 °C, 4 h, overall yield 65%.



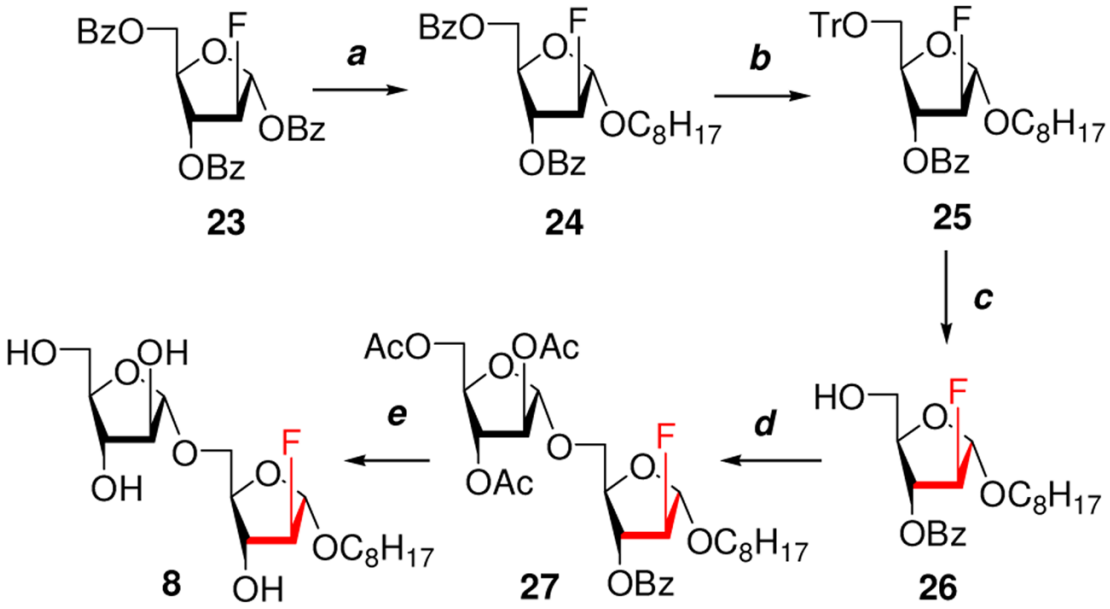
Scheme 2.

(a) $(CH_3)_3CCOCl$, pyridine, rt, overnight, 97%; (b) $Et_4N^+F^-$, THF, rt, 3 h, 91%; (c) TrCl, DMAP, Pyridine, 45 °C, 3 h, 85%; (d) (i) CS_2, NaH, MeI, DMF, 0 °C–rt, 30 min (ii) Bu_3SnH, AIBN, rt, 3 h, overall yield 83%; (e) 5% TFA/$CHCl_3$, −20 °C, 4 h, 83%.



Scheme 3.

Reagents and conditions. (a) **14**, NIS, $Sn(OTf)_2$, CH_2Cl_2, 0 °C, 30 min, 85%; (b) 7N NH_3/MeOH, rt, overnight, 85%; (c) **19**, NIS, $Sn(OTf)_2$, CH_2Cl_2, 0 °C, 30 min, 87%; (d) 7N NH_3/MeOH, rt, overnight, 87%; (e) NaOMe, MeOH, rt, overnight, 75%.



Scheme 4.

Reagents and conditions. (a) $C_8H_{17}OH$, $SnCl_4$, CH_3CN, rt, 30 min, 54%; (b) (i) 7N NH_3/MeOH, rt, overnight, (ii) TrCl, DMAP, pyridine, 45 °C, 48 h, (iii) BzCl, pyridine, rt, overnight, overall yield 81%; (c) 5% TFA/$CHCl_3$, −20 °C, 4 h, 81%; (d) **20**, NIS, $Sn(OTf)_2$, CH_2Cl_2, 0 °C, 30 min, 88%; (e) 7N NH_3/MeOH, rt, overnight, 88%.

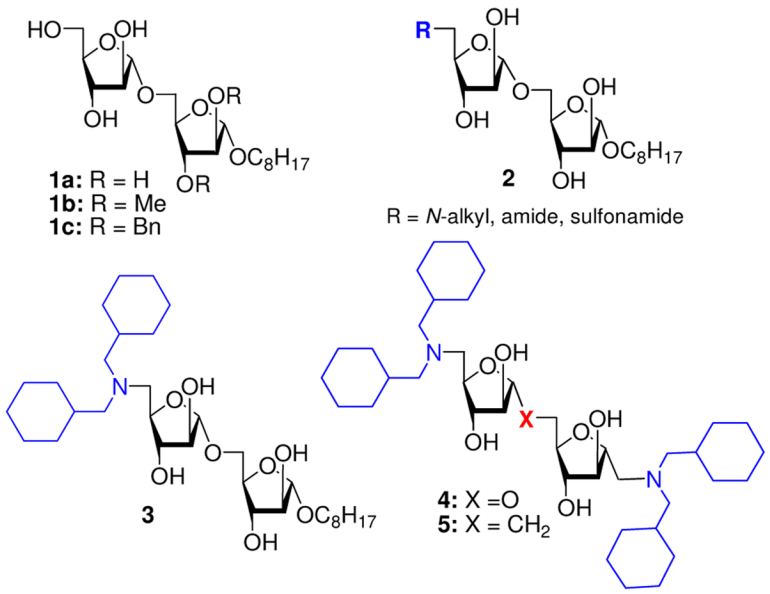


Chart 1.
Previously synthesized Ara*f* α(1→5)Ara*f* disaccharide analogs