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Concise synthesis of an arabinofuranose hexasaccharide present in the cell wall of *Mycobacterium tuberculosis*

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ABSTRACT

Mycobacterium cell wall consists of three major polysaccharide portions and arabinofuranose (Ara*f*) is present in two of the major portions, arabinogalactan (AG) and lipoarabinomannan (LAM). A peculiar Ara*f* hexasaccharide possessing two β-linked Ara*f* units are present in both AG and LAM polysaccharides. Herein, we report an efficient and concise synthesis of this Ara*f* hexasaccharide using single starting 3,5-TIPDS protected Ara*f* thioglycoside precursor. Double β-glycosylation was achieved using strained cyclic 2-*p*-methoxybenzyl-3,5-TIPDS Ara*f* thioglycoside donor.

Mycobacterium tuberculosis has attracted renewed attention over the last three decades owing to increasing infections worldwide.[1] Additionally, AIDS patients and others with compromised immune systems are susceptible to opportunistic infections caused by *Mycobacterium avium* and *Mycobacterium kansaii*, resulting in a mortality rate of millions per year.[2] Drugs with novel targets are urgently needed especially with the emergence of multiple drug resistant (MDR-TB)[3] and extreme drug resistant (XDR-TB)[4] strains. Biosynthesis of the mycobacterial cell wall is one such vital drug target which protects the bacillus within the macrophage and also functions as an effective barrier to antibiotics.[5] The mycobacterial cell envelope is typically composed of complex polysaccharides bound to mycolic acids and the three major macromolecular components of the cell wall are lipoarabinomannan (LAM), arabinogalactan (AG), and peptidoglycan.[6] Arabinan portion in AG and LAM is a linear oligomer (α1→5) linked Ara*f* units and a branched arabinan pentasaccharide at the terminus that consists of (α1→5), (α1→3), and (β1→2) linked Ara*f* units.[7] The assembly of the arabinan portions of cell wall polysaccharides in mycobacteria involves a family of arabinosyltransferases (AraTs) that promote the polymerization of decaprenolphosphoarabinose (DPA) as the substrate, and each enzyme constructing different glycosidic linkages. These known AraTs include the Emb (EmbA, EmbB, and EmbC) proteins, AftA, AftB, and AftC enzymes, which are responsible for biosynthesis of arabinan with unique glycosyl linkages.[7] The ability of mycobacteria to construct cell wall AG and LAM are critical survival pathway and the inhibitors of the involved glycosyltransferase enzymes are good targets for drug discovery.[8]

A complete synthesis of the arabinan portion and its fragments has already been reported.[9] The key installation of β-Ara*f* units was achieved by several methods and initially it was achieved by the Intermolecular Aglycon Delivery (IAD) method.[10] Other methods employed for the synthesis of major fragments of the arabinan with β-Ara*f* units have successfully used *p*-tolyl 1-thio-2,3,5-tri-*O*-benzyl arabinofuranoside or conformationally anchored 2-benzyl thioarabinoglycoside.[11] A reliable and general direct method for the stereoselective β-arabinofuranosylation employing a 2′-carboxybenzyl arabinofuranoside as the glycosyl donor has also been reported.[12]

Here, we report an efficient synthesis route to synthesize neoglycolipid hexasaccharide **1** (Fig. 1) containing two β-Ara*f* units, which can be utilized to study biosynthesis of arabinan portion present in cell wall of TB. The ether protective group at C-2 position on arabinosyl donors is essential for the β-stereoselectivity.[10–13] The protective groups at 3- and 5-positions were also found to affect the stereoselectivity of arabinofuranosylation. Conformational restraint introduced by the eight-membered ring by tetraisopropyldisilylene at 3,5-O-protection can aid for the enhanced β-selectivity. The β-selectivity was drastically enhanced by using donors protected with 3,5-TIPDS, possibly due to conformational constraints on the furanose ring.[13] To simplify synthesis of **1**, we chose an approach in which it was assembled via protected hexasaccharide **2** utilizing donors **3**–**5** containing the suitable functional

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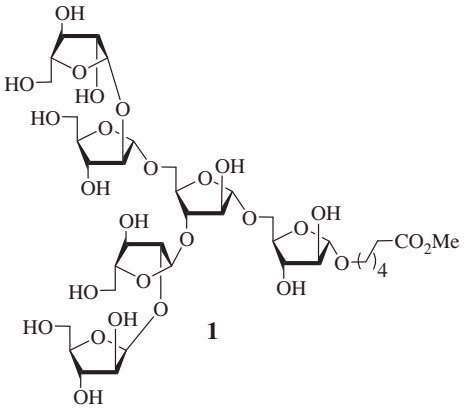
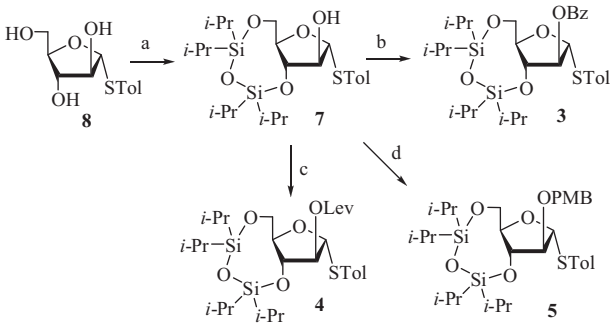


Figure 1. Structure of neo-glycolipid Ara*f* hexasaccharide similar to Ara*f* branched polysaccharide present in cell wall of *Mycobacterium tuberculosis*.



Scheme 1. Reagents and conditions: (a) Ref.[12]; (b) BzCl, dry Py, rt, 0 °C–rt, 3 h, 88%; (c) Levulinic acid, DCC, DMAP, CH_2Cl_2, 0 °C, 1 h, 90%; (d) entry 9 in Table 1.

groups at C-2 position for 1,2-*trans* and -*cis* regio- and chemoselective glycosylations (Fig. 2). The *p*-methoxybenzyl (PMB) protecting group at C-2 in the donor **5** was selected over benzyl or silyl ether because if the direct coupling reaction does not produce cleaner β-arabinosylation, then an indirect activation and coupling IAD approach[10] can be exploited to install desired β-Ara*f* units. The synthesis of all three donor precursors **3**–**5** can be achieved from one single 3,5-TIPDS protected Ara*f* thioglycoside **7**.

The monosaccharide building blocks **3**–**5** were synthesized starting from easily accessible *p*-tolyl 1-thio-α-D-arabinofuranoside **8**[14] as shown in Scheme 1. Thioglycoside **8** was treated with 1,3-dichloro-1,1;3,3-tetraisopropyldisiloxane (TIPDSCl_2) in pyridine for 2 h at 0 °C and after usual workup and purification by column chromatography provided the 3,5-disiloxane product **7**[13] in 66% yield. The donor thioglycosides **3** and **4** were conveniently synthesized in large scale from thioglycoside **7**. The 2-benzoylation of **7** with benzoyl chloride at room temperature gave thioglycoside donor **3** in 98% yield and the reaction of **7** with levulinic acid in the presence of DCC and DMAP produced 2-levulinoyl ester donor thioarabinoside **4** in 90% yield. The synthesis of donor thioglycoside **5**, for β-arabinosylation, seemed very straightforward but usual conditions did not produce the desired product. Several optimization reactions were performed to achieve of *p*-methoxybenzylation on thioglycoside **7** as shown in Table 1. Finally, donor thioglycoside **5** was obtained in 77% yield upon reaction of **7** with *p*-methoxybenzyl bromide and NaH in DMF at −70 °C after column chromatographic purification.

The next task was to synthesize the diol acceptor, methyl 6-hexanoate anchored glycoside **9**, which was achieved in a two step reaction sequence from thioglycoside **3** (Scheme 2). The glycosylation of **3** with methyl 6-hydroxyhexanoate at 0 °C with activator NIS and Lewis acid promoter AgOTf gave exclusive α-isomer of arabinofuranoside **6**. The cyclic siloxane protecting group in **6** was removed by $Et_4N^+F^-$ to obtain acceptor 3,5-diol glycoside **9** in quantitative yield.

With gram quantities of all the building arbinofuranosyl units in hand, we carried out assembly of hexaarabinofuranoside **1** (Scheme 3). In the first step, selective glycosylation of *n*-octyl Ara*f* 3,5-diol **9** at C-5 hydroxyl, to preclude several protection and deprotection steps, was undertaken with **3** using several Lewis acid activators at various temperatures. Coupling condition was found to be optimal at −20 °C in CH_2Cl_2 promoted by a stoichiometric amount of $Sn(OTf)_2$ in the presence of NIS. Under these conditions the coupling reaction gave desired disaccharide **10** with complete α-selectivity, aided by benzoyl participating group at C2, in 73% yield. To sustain further glycosylation reactions, the 3-OH group in **10** was firstly protected with benzoyl group and further treatment of crude product with $Et_4N^+F^-$ in THF at −20 °C unmasked the C3 and C4 hydroxyl groups on the non-reducing end terminus sugar of disaccharide. Purification of crude product from de-silylation reaction on Silica gel G column provided disaccharide **11** in 80% yield over two steps.

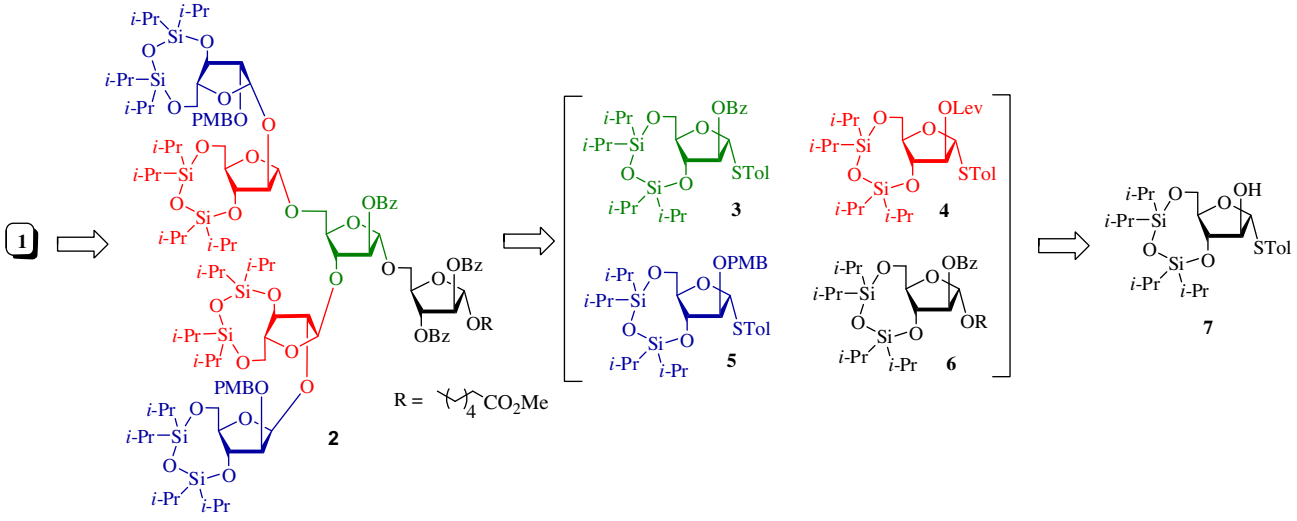
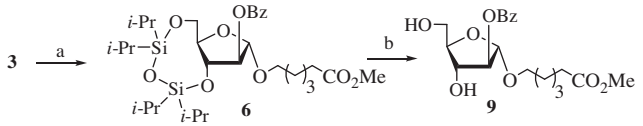


Figure 2. Synthetic plan for target hexasaccharide **1**.

Table 1
Reaction optimization of *p*-methoxybenzylation of thioglycoside **7**

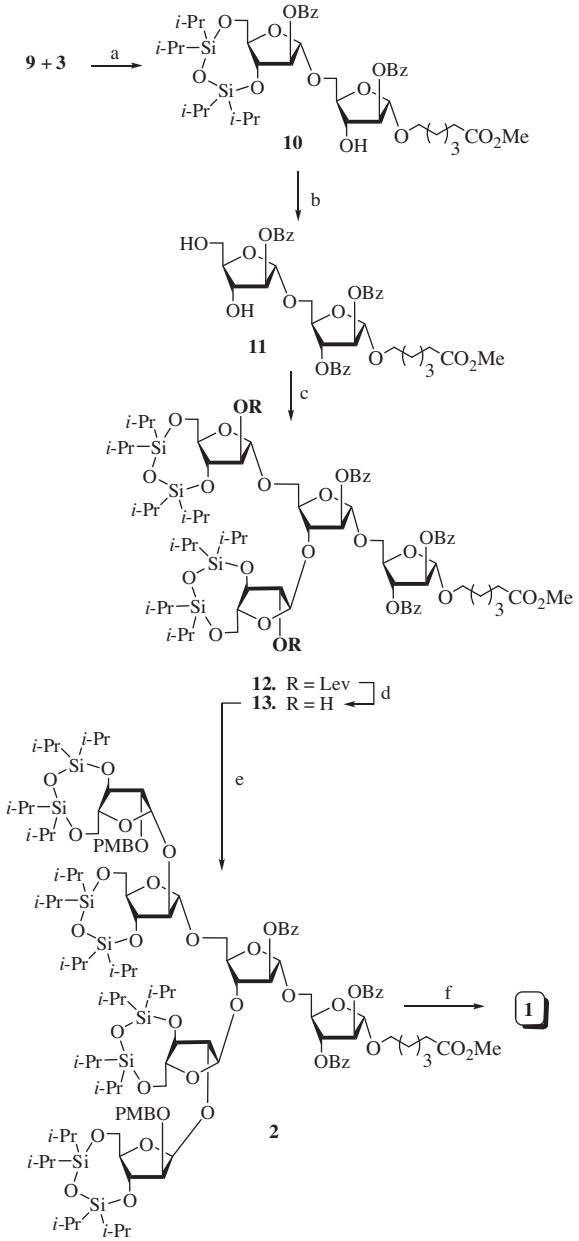
Entry	Reagent (1.1 equiv)	Base (1.0 equiv)	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	PMBCl	NaH	DMF	0–rt	0.5	0
2	PMBCl	NaH	THF	0	0.5	10
3	PMBCl	$CsCO_3$	DMF	0–50	12	12
4	PMBBr	NaH	DMF	0–rt	1	10
5	PMBBr	NaH	THF	0–rt	1	15
6	PMBBr	NaH	DMF	0	1	20
7	PMBBr	NaH	DMF	−20	1	25
8	PMBBr	NaH	DMF	−50	12	60
9	**PMBBr**	**NaH**	**DMF**	**−70**	**20**	**77**

[a] Isolated yield; purification was performed on neutral Alumina media.



Scheme 2. Reagents and conditions: (a) NIS, AgOTf, dry CH_2Cl_2, −20 °C, 15 min, 82%; (b) $Et_4N^+F^-$, dry THF, 0 °C, quant, 3 h.

To achieve the synthesis of tetrasaccharide **12**, double glycosylation with 2-levulinoyl containing Ara*f* donor **4** was carried out at 3- and 5-positions on the disaccharide **11**. Tetrasaccharide **12** was obtained in 81% yield after purification. Removal of levulinoyl groups on **12** with hydrazine acetate gave acceptor **13** which was then further used in double β-arabinofuranosylation with donor thioglycoside **5**. In a small scale trial reaction, tetrasaccharide **13** was reacted for 1.5 h with 2.5 equiv of donor thioglycoside **5** in the presence of activator NIS and TMSOTf at −78 °C. The reaction was monitored on TLC which showed complete consumption of donor glycoside **5** and formation of two products, one major hexasaccharide **2** and other a pentasaccharide (supported by MS analysis of reaction mixture). Thereafter, one more equivalent of donor thioglycoside **5** was added at −78 °C to the above reaction mixture and was further stirred for 30 min. The TLC of the reaction mixture showed the complete conversion of mixture to hexasaccharide **2**. Next, the above reaction was repeated again with 150 mg of tetrasaccharide **13** which was treated for 2 h with 3.5 equiv of donor thioglycoside **6** in the presence of activator NIS and TMSOTf at −78 °C and TLC showed the formation of one product. After usual workup and column chromatographic purification on silica gel, double β-arabinosylated hexasaccharide **2** was obtained in 79% yield as an oil with excellent stereoselectivity. In the ^{13}C NMR spectrum of **2**, the four anomeric carbon signals of α-linked Ara*f* units were seen at δ 106.08, 105.66, 105.63, and 105.29 ppm whereas, the signals from two anomeric carbons of β-linked Ara*f* units were observed at δ 98.95 and 98.77 ppm.[9] Cyclic siloxane TIPDS protecting groups in hexasaccharide **2** were removed by reacting overnight at −20 °C with $Et_4N^+F^-$ in THF. After removal of the reaction solvent, the crude product was purified by a quick column chromatography on Silica gel. The desilylated hexasaccharide product thus obtained was treated with ceric ammonium nitrate in CH_3CN–H_2O mixture to remove *p*-methoxybenzyl groups and after usual workup the product was used without purification in the final de-benzoylation step. The crude product obtained from the above reaction was treated overnight with 7 N NH_3 in MeOH solution and desired hexaarabinofuranoside **1** was obtained in 82% yield after column chromatographic purification. The ^1H and ^{13}C NMR spectra of **1** showed the signals corresponding to the desired product. In the ^{13}C NMR spectra of **1** in CD_3OD, the four anomeric carbon signals from α-Ara*f* units were observed at δ 108.19, 108.07, 106.12, and 105.83 ppm, and two anomeric carbon signals at δ 101.27 and 101.19 ppm were assigned to β-Ara*f* units.[9]



Scheme 3. Reagents and conditions: (a) NIS, $Sn(OTf)_2$, dry CH_2Cl_2, 10 min, −20 °C, 73%; (b) (i) BzCl, dry Py, rt, 4 h; (ii) $Et_4N^+F^-$, THF, −20 °C–rt, 3 h, overall 80% in two steps; (c) **4** (2.5 equiv), NIS, AgOTf, dry CH_2Cl_2, 0 °C, 2 h, 81%; (d) hydrazinium acetate in MeOH, CH_2Cl_2, rt, 4 h, 82%; (e) **5** (3.5 equiv), NIS, TMSOTf, dry CH_2Cl_2, −78 °C, 2 h, 79%; (f) (ii) $Et_4N^+F^-$, dry THF, rt, overnight; (ii) CAN, THF–H_2O, 0 °C, 6 h; (iii) 7 N NH_3/MeOH, rt, overnight, overall 82% in three steps.

We report an efficient and concice synthesis of neo-glycolipid hexaarabinofuranosides present in *M. tuberculosis* via routes that are modified compared to those used for the preparation of the corresponding methyl oligoarabinoses. A selective C5-glycosylation on Ara*f*-3,5-diols (**9**) was used to save protection deprotection manipulations and several reaction steps were combined together to save tedious column chromatographic purifications. The crucial stereoselective β-capping was successfully achieved using cyclic strained glycoside **5** promoted by NIS and TMSOTf at −78 °C.

Supplementary data

Supplementary data (experimental and analytical details of all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.016.

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