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Synthesis of Ethyl 5-*O*-(α-D-arabinofuranosyl)-6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside Present in Motif E of the *Mycobacterium tuberculosis* Cell Wall

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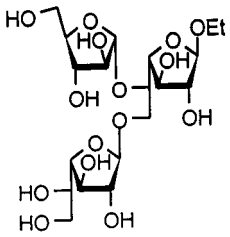
ABSTRACT



The stereocontrolled synthesis of the trisaccharide ethyl 5-*O*-(α-D-arabinofuranosyl)-6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside present in motif E of the *Mycobacterium tuberculosis* cell wall is described.

Three million deaths occur every year due to tuberculosis, and perhaps it constitutes the single largest lethal disease of the world.[1] The mycobacterium species, namely *Mycobacterium tuberculosis* and *M. avium* and to lesser extent *M. kansasii*, are associated with AIDS, and therefore, in recent years, they have attracted unprecedented attention. The impact of tuberculosis, as an opportunistic infection, on developing countries has been phenomenal because of increasing incidences of AIDS in these countries.[2−4]

The unique cell wall structure of *M. tuberculosis* contains two major polysaccharides, lipoarabinomannan (LAM) and arabinogalactan (AG), in which both arabinose and galactose residues are found in the furanose form.[5]

The five major motifs of AG, namely motifs A−E (**1** − **5**), have been isolated and characterized[6] as shown in Figure 1. Synthesis of these motifs provides opportunities to investigate, in detail, their role in immunological response arising from mycobacterial infection. The structural analysis revealed that motifs A,[7a,8a] B, and C[8c] contain only arabinofuranose residues, whereas motif D has exclusively galactofuranoses. Motif E is novel and unique because of the presence of the both arabinofuranose and galactofuranose residues in its structural framework. In addition, the oligosaccharide structure of motif E is sterically complicated because both the 5- and 6- positions of the reducing

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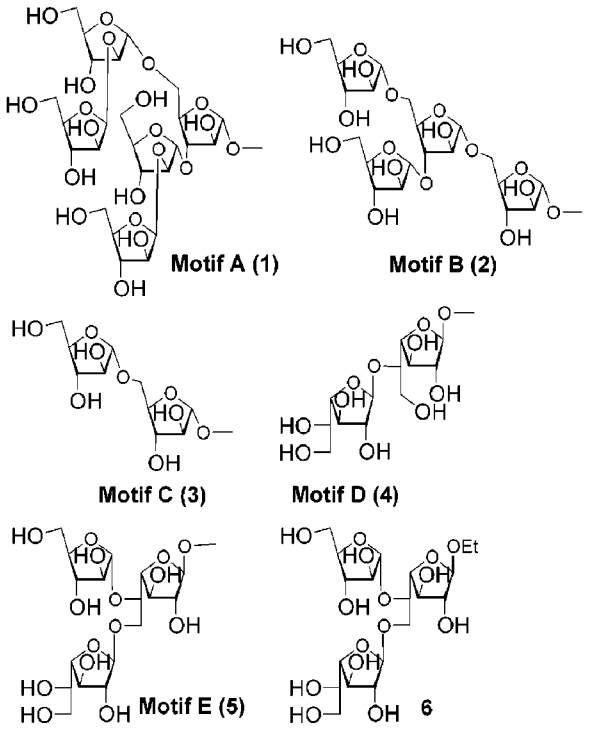
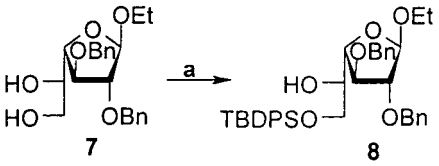


Figure 1.

galactofuranose component are linked with arabinofuranosyl and galactofuranosyl residues, respectively. The synthesis of the pentaarabinofuranoside of motif A (**1**) was first described from our laboratories. This communication reports the first stereocontrolled synthesis of 5-*O*-(α-D-arabinofuranosyl)-6-*O*-(*β*-D-galactofuranosyl)-*β*-D-galactofuranose as ethyl glycoside **6** (Figure 1) belonging to motif E.

Our first concern was the preparation of the aglycone (**8**) in which the 2- and 3-positions are blocked with permanent protecting benzyl ethers while the 6-position is blocked with a temporary protecting TBDPS ether. The known ethyl 2,3-di-*O*-benzyl-*β*-D-galactofuranose **7**,[9] obtained from D-galactose in 5 steps, was treated with TBDPS-chloride in the presence of imidazole to give the 6-*O*-TBDPS derivative **8** (Scheme 1).

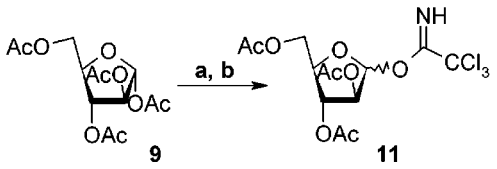
Scheme 1



(a) TBDPS-Cl, Im., CH_2Cl_2, rt, 3 h, 90%.

2,3,5-Tri-*O*-acetyl-D-arabinofuranosyl trichloroimidate (**11**), as the glycosylating agent, was obtained by selective deprotection of the 1-*O*-acetyl group of 1,2,3,5-tetra-*O*-acetyl-α-D-arabinofuranose (**9**)[10] with tri-*n*-butyltin ethoxide in refluxing $ClCH_2CH_2Cl$ and subsequent exposure of **10** with $CCl_3CN-DBU-CH_2Cl_2$ at 0 °C (Scheme 2).

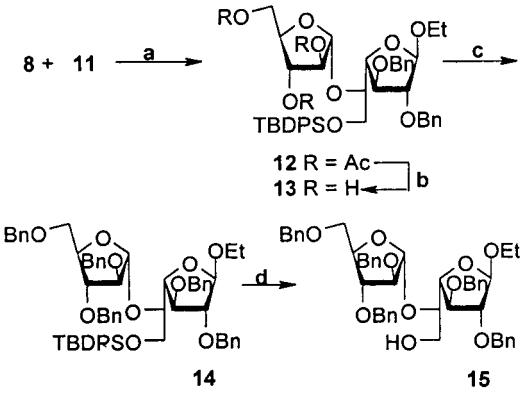
Scheme 2



(a) Bu_3SnOEt, $ClCH_2CH_2Cl$, reflux, 3 h, 70%
(b) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 65%

A coupling reaction[11] between **8** and **11** was carried out, with BF_3:OEt_2 as an activator, to give the disaccharide **12** which was isolated in 70% yield and characterized after Zemplén deacetylation[12] (Scheme 3). The ^1H NMR and ^{13}C

Scheme 3



(a) BF_3:OEt_2(cat.), CH_2Cl_2, MS 4 A° powder, -20 °C-rt, 2 h, 70%; (b)NaOMe, MeOH, rt, 2 h, 80 %; (c) NaH, BnBr, DMF, 3 h, 0 °C-rt, 87%;(d) n-Bu_4N F, THF, rt, 0.5 h, 92%.

NMR spectra of **13** were in complete agreement with the assigned structure.[13] The location of the signal due to C-1′ at δ 104.5 in the ^{13}C NMR spectrum of **13** confirmed the α-linkage at the newly formed glycosidic bond.[13] Compound **13** was transformed into the 2,2′,3,3′,5′-penta-*O*-benzyl

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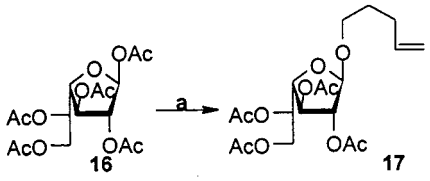
derivative (**14**) by using NaH and benzyl bromide in DMF. Removal of the temporary TBDPS protecting group with 1 M Bu_4NF solution in THF gave the aglycone (**15**) containing the 6-hydroxyl (Scheme 3).

The glycosidation reaction of **15**, with substituted galactofuranosyl derivatives, turned out to be a difficult proposition because trichloroacetaimidate methodology,[11] SEt promoted glycosidation,[14] and Helfrich reaction[14] failed in our hands. Finally, Fraser-Ried's *n*-pentenyl glycosidation technique[15] was successful. Thus, 1,2,3,5,6-penta-*O*-acetyl-β-D-galactofuranose **16**[16] and 4- pentene-1-ol in the presence of a catalytic amount of BF_3:OEt_2 in CH_2Cl_2 at 0 °C gave the β-pentenyl glycoside derivative **17** (Scheme 4).

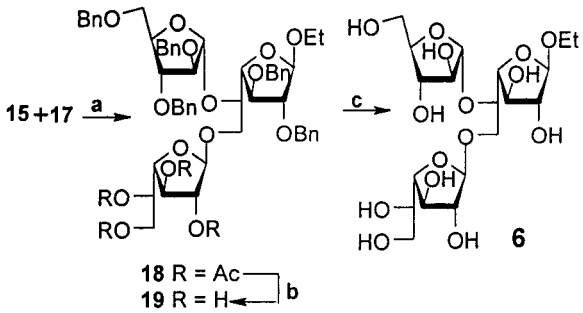
Scheme 4



(a) 4-pentene-1-ol, BF_3:OEt_2, 4 A° MS powder, CH_2Cl_2, 0 °C-rt, 2 h, 85%.

The coupling reaction between **15** and **17** in the presence of *N*-iodosuccinamide and triflic acid (cat.) in CH_2Cl_2 at ambient temperature for 48 h gave the trisaccharide **18**. The Zemplen deacetylation of **18** provided the penta-*O*-benzylated derivative **19** which was analyzed by the ^{1}H NMR and ^{13}C NMR spectral data. For example, in the ^{1}H NMR spectrum of **19,** signals due to three anomeric protons were observed at δ 4.85, 4.95, and 5.17 as singlets. The ^{13}C NMR spectrum showed anomeric carbons at δ 105.3, 107.0, and 107.8. Finally, compound **19** was deprotected by hydrogenolysis over 10% $Pd(OH)_2$/C in methanol to give the target molecule **6** (Scheme 5).

Scheme 5



(a) NIS, TfOH (Cat.), CH_2Cl_2, 4 A° MS powder, rt, 48 h, 65 %; (b) NaOMe, MeOH, rt, 2 h, 72 %; (c) 10% $Pd(OH)_2$/C, MeOH, H_2, rt, 8 h, 90%.

The high resolution ^{1}H NMR spectrum of **6** showed resonances due to anomeric protons at δ 4.89, 4.93, and 5.10 as singlets. The chemical shifts of anomeric carbons were revealed at δ 106.5, 107.5, and 108.2 in the ^{13}C NMR spectrum. In addition, the FAB−MS and elemental analysis of **6** were in complete agreement with the assigned structure.

In conclusion, this communication reports the first synthesis of a novel trisaccharide present in motif E of *Mycobacterium tuberculosis.* The arabinogalactan caused profound interest for two fundamental reasons, (1) it appears to be essential for viability and (2) three out of the four sugars, namely *araf*, *galf*, and *rhamp*, are not found in humans. Only recently it has been established that ethambutol, the drug of choice for TB since 35 years, is involved in the inhibition of biosynthetic pathway of arabinan, thus establishing it to be a valuable target for the discovery of new drugs.[6e,17]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **6**, **13**, **14**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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