Synthesis of pentaarabinofuranosyl structure motif A of *Mycobacterium tuberculosis*

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The first synthesis of motif A, the branched chain arabinofuranosyl pentasaccharide [t-β-Ara*f*-(1 → 2)-α-D-Ara*f*]$_2$-3,5-α-D-Ara*f*-(1 → 5) which constitutes the major humoral immunological epitope in the arabinogalactan cell wall of *Mycobacterium tuberculosis* is described.

Tuberculosis (TB) continues to affect developing countries as 8 000 000 new cases and 3 000 000 deaths occur every year.[1] As a consequence of the HIV epidemic, the occurrence of TB particularly in developed countries has risen sharply. The etiological agent, *Mycobacterium* (*M.*) *tuberculosis* has been extensively investigated and Fig. 1 represents a schematic diagram of macro structural motifs (A–E) of cell wall arabinogalactan.[2a] The fine structure of the cell wall of mycobacteria allows us to understand drug and solute impenetrability, antigen processing and presentation by accessory cells, and aspects of immunopathogenesis. The structurally unusual and biologically significant arabinofuranosyl residue of motif A (**1**, Fig. 1) is responsible for the antigenicity of arabinogalactan.[2a] It is speculated that, in part or complete, structural motif A is the major humoral immunological epitope of arabinogalactan *vis a vis* whole mycobacteria.[2d] Our interest in the chemistry of compounds derived from *M. tuberculosis* has previously resulted in the synthesis[3] of oligosaccharide fragments of glycolipids and glycopeptide cell wall segments. In this report, we communicate the first synthesis of the branched chain arabinofuranosyl pentasaccharide [t-β-Ara*f*-(1 → 2)-α-D-Ara*f*]$_2$-3,5-α-D-Ara*f*-(1 → 5) which forms the crucial part of structural motif A of the *M. tuberculosis* cell wall.

Synthesis of **1a** was initiated from D-arabinose which was transformed into 5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-(propane-2,2-diyl)-β-D-arabinofuranose (**2**) in two steps.[4] Subsequent desilylation using Bu$^n_4$NF in THF at ambient temperature gave 1,2-*O*-(propane-2,2-diyl)-β-D-arabinofuranose (**3**).[5] Reaction of **3** with NaH–BnBr in DMF protected both the hydroxy groups to afford the 3,5-di-*O*-benzyl derivative **4**. Conversion of **4** into the *n*-pentenyl glycoside was effected with pent-4-en-1-ol in the presence of TsOH to obtain a 1 : 1 anomeric mixture of α,β-*n*-pentenyl glycosides (**5** and **6**) which were separated by silica gel column chromatography (Scheme 1). In another sequence,

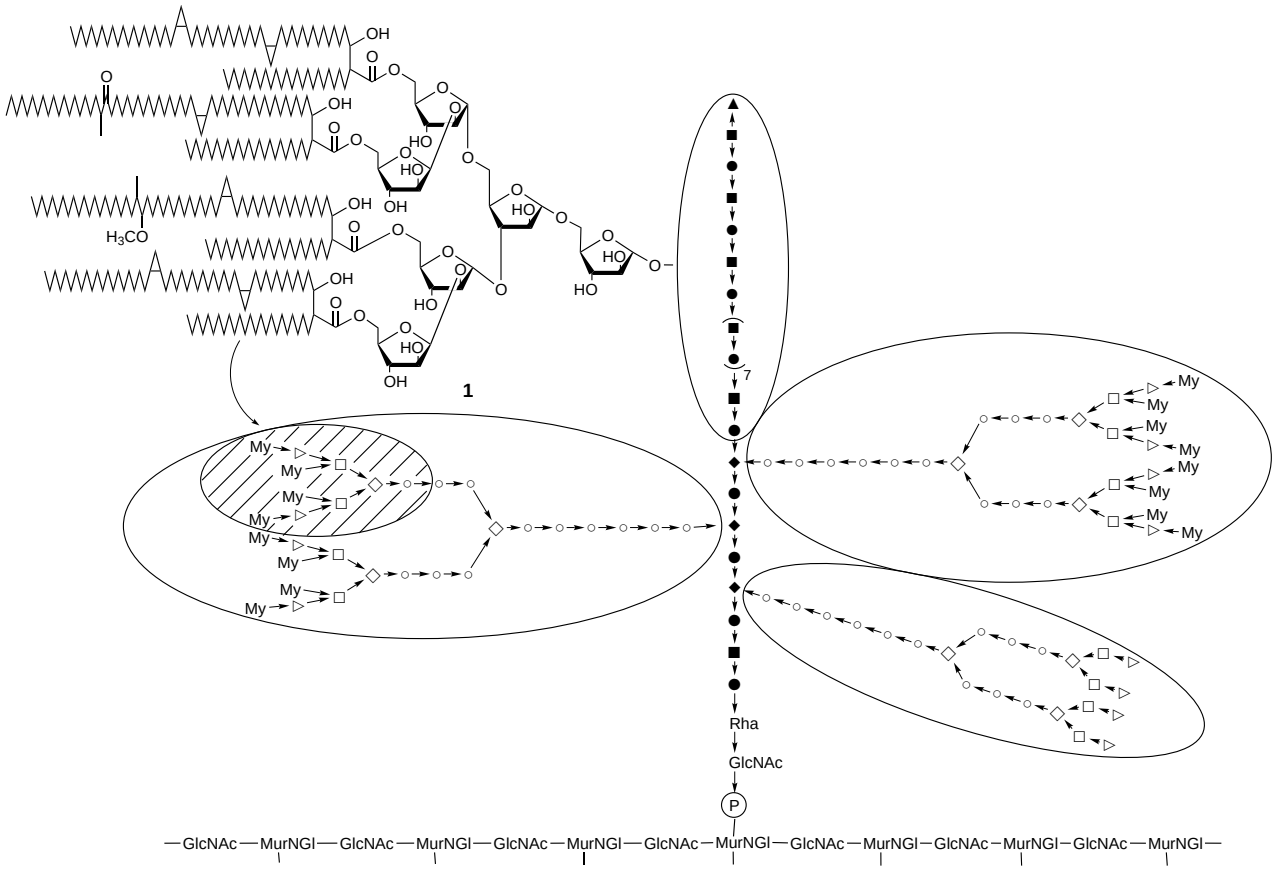
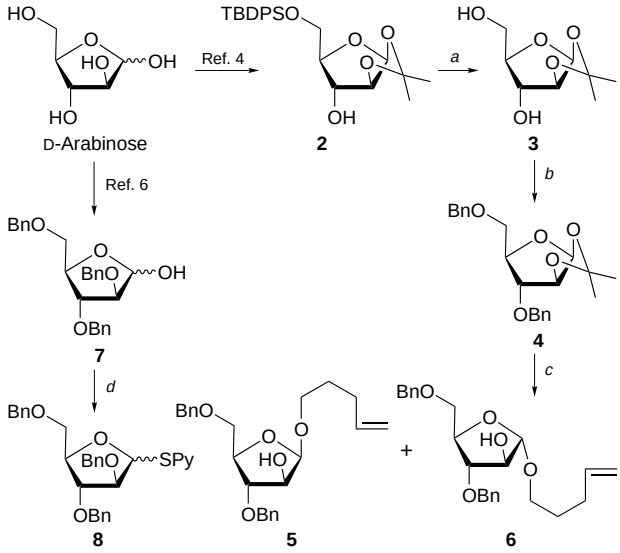


Fig. 1 Schematic diagram of the proposed illustration of the macro structural motifs of the cell wall arabinogalactan. My, Mycolic acid; (▼) t-β-D-Ara*f*; (□) 2-α-D-Ara*f*; (◇) 3, 5-α-D-Ara*f*; (▲) t-β-D-Gal*f*; (■) 6-β-D-Gal*f*; (●) 5-β-D-Gal*f*; (◆) 5,6-β-D-Gal*f*; GlcNAc, *N*-acetylglucosamine; Rha, rhamnose; MurNGl, *N*-glycolylmuramic acid.

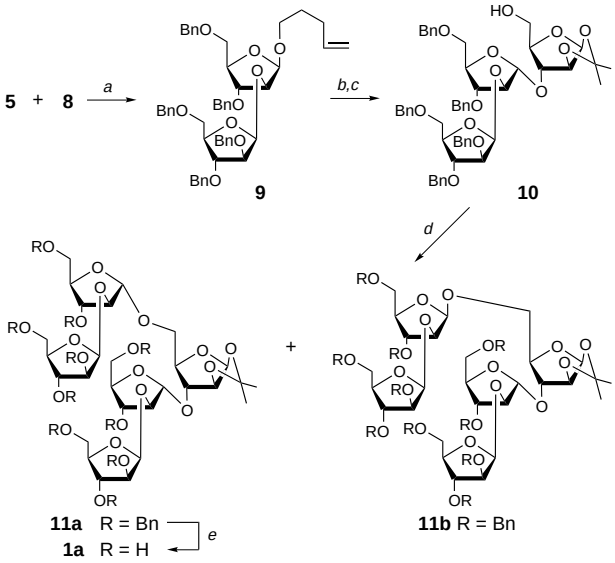


Scheme 1 *Reagents and conditons*: (*a*) Bu^n_4NF, THF, room temp., 2 h, 96%; (*b*) NaH,BnBr, DMF, 0 °C–room temp., 83%; (*c*) Pent-4-en-1-ol, TsOH, CH_2Cl_2, 60 °C, 2 h, 85%; (*d*) PySSPy, Bu^n_3P, CH_2Cl_2, room temp., 30 min, 98%

2,3,5-tri-*O*-benzyl-α,β-D-arabinofuranose (**7**)[6] was transformed into the corresponding *S*-(2-pyridyl)-1-thiofuranoside **8** by reacting with 2,2′-dithiodipyridyl and Bu^n_3P in CH_2Cl_2.[7]

The coupling reaction of **5** with **8** was promoted[8] by the protocol developed in our laboratory, according to which 5% MeI in dry CH_2Cl_2 was used as an activator to give the β-disaccharide **9**. Its structure was confirmed by ^1H and ^{13}C NMR spectroscopy (Scheme 2).

The *O*-glycosylation of **2**[4] with the above formed *n*-pentenyl disaccharide **9** was induced in the presence of iodonium dicollidine perchlorate (IDCP)[9] in CH_2Cl_2, followed by desilylation of the coupled product with Bu^n_4NF in THF, resulted in the isolation of the trisaccharide **10** whose newly formed glycosidic linkage was confirmed as having an α-configuration by the ^1H NMR spectrum. For example, the characteristic resonances due to H-1′ was located at δ 5.05 as a singlet, whereas H-1 and H-1″ protons appeared as doublets at δ 4.90 and 5.75, respectively, as expected for β-anomeric configurations. In addition, the ^{13}C NMR spectrum of **10** showed resonances due to anomeric carbons at $\delta_{C\text{-}1}$ 100.1, $\delta_{C\text{-}1'}$ 105.3 and $\delta_{C\text{-}1''}$ 105.5.



Scheme 2 *Reagents and conditions*: (*a*) 5% MeI in CH_2Cl_2, 57 °C, 4 Å MS powder, 15 h, 69%; (*b*) IDCP CH_2Cl_2, 4 Å MS powder, 24 h, 62%; (*c*) Bu^n_4NF, THF, room temp., 3 h, 95%; (*d*) I(*s*-Collidine)$_2ClO_4$, CH_2Cl_2, 4 Å MS powder, 12 h, 70%; (*e*) $Pd(OH)_2$/C, MeOH,H_2, room temp., 12 h, 97%

The OH group at C-5 of compound **10** was glycosylated again with donor **9** under the conditions reported above. However, in this reaction, a 3 : 2 mixture of α- and β-pentasaccharides (**11a** and **11b**) was formed. The major α-anomeric product (**11a**) was isolated by silica gel column chromatography, hydrogenolysis of which over $Pd(OH)_2$/C at normal temperature and pressure for 12 h gave the required pentasaccharide **1a**. The structure of **1a** was fully characterised by ^1H, ^{13}C NMR and FABMS analysis.[9]

In conclusion it is pertinent to mention that resistance to the current regime of anti-TB drugs is developing rapidly and therefore there is a constant need to discover new drugs. It is reported that (*S*,*S*)-ethambutol inhibits arabinan biosynthesis and therefore the arabinan segment of the cell wall provides an attractive target for development of new drugs because of the xenobiotic status of the human host. The present synthesis of the pentaarabinofuranoside of structure motif A of *M. tuberculosis* cell wall opens a new vista in this direction.

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Notes and References

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9 *Selected data* for **9**: δ_H(200 MHz, $CDCl_3$) for anomeric protons: 5.05 (d, *J* 4.6), 5.15 (d, *J* 4.1); δ_C(50 MHz, $CDCl_3$) for anomeric carbons: 98.5, 100.2; FABMS: 823 (M + Na)$^+$. For **10**: δ_H(200 MHz, $CDCl_3$) for anomeric protons: 4.90 (d, *J* 4.6), 5.05 (s), 5.76 (d, *J* 4.6); δ_C(50 MHz, $CDCl_3$) for anomeric carbons: 100.1, 105.3, 105.5; FABMS: 928 (M + Na)$^+$. For **11a**: δ_H(200 MHz, $CDCl_3$) for anomeric protons: 4.92 (d, *J* 4.6), 5.04 (s), 5.18 (d, *J* 4.7), 5.29 (s), 5.70 (d, *J* 4.8); δ_C(50 MHz, $CDCl_3$) for anomeric carbons: 100.1, 100.4, 105.2, 105.5, 106.0; FABMS: 1643 (M + Na)$^+$. For **11b**: δ_H(200 MHz, $CDCl_3$) for anomeric protons: 4.90 (d, *J* 4.70), 5.02 (s), 5.09 (d, *J* 4.65), 5.29 (d, *J* 4.8), 5.70 (d, *J* 4.7); δ_C (50 MHz, $CDCl_3$) for anomeric carbons: 98.3, 100.2, 100.6, 105.1, 105.5; FABMS: 1643 (M + Na)$^+$. For **1a**: δ_H(400 MHz, D_2O) for anomeric protons: 4.90 (d, *J* 4.20), 5.10 (d, *J* 4.65), 5.15 (s), 5.28 (s), 6.00 (d, *J* 4.8); δ_C(50 MHz, D_2O) for anomeric carbons: 102.0, 102.1, 106.3, 106.5, 106.9; FABMS: 741 (M + Na)$^+$.

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