

## Stereoselective synthesis of decaprenylphosphoryl $\beta$ -D-arabinofuranose

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**Abstract**—Decaprenylphosphoryl  $\beta$ -D-arabinofuranose (DPA) is known to be a key arabinose donor in mycobacteria. In order to study the biosynthesis of the major polysaccharides from the mycobacterial cell wall, it was necessary to develop a practical and stereoselective synthetic scheme for DPA. This goal was achieved by coupling of a suitably protected  $\beta$ -D-arabinofuranosyl phosphate intermediate with an activated form of decaprenol and subsequent deprotection.

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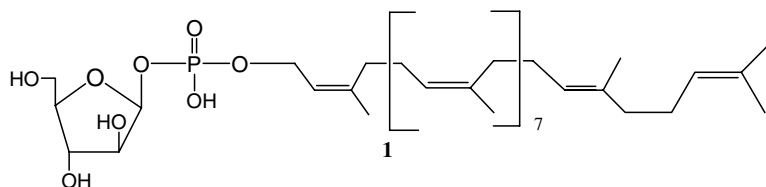
The global rise in tuberculosis and drug-resistant *Mycobacterium tuberculosis* still presents a threat to human health,<sup>1</sup> and requires the development of new drug targets and drugs. The D-arabinan segments of the mycobacterial cell wall are excellent targets for new drug development due to the xenobiotic status of D-arabinofuranose.<sup>2,3</sup> A key mycobacterial arabinose donor,  $\beta$ -D-arabinofuranosyl-1-monophosphoryldecaprenol (DPA, **1**, Fig. 1), has been found in the lipid extracts of *Mycobacterium smegmatis* and implicated in the biogenesis of the two major cell wall polysaccharides arabinogalactan (AG) and lipoarabinomannan (LAM).<sup>4</sup> Moreover, DPA is required for arabinosyl transferase assays in Actinomycetales and also in *Azorhizobium* where genes involved in DPA formation and utilization have been discovered.<sup>5–7</sup> In order to study the biosynthesis of AG and LAM in mycobacteria, it was necessary to develop a synthetic scheme that will provide sufficient amounts of this important donor, and such a synthesis was undertaken.<sup>3</sup> The published procedure was based on phosphorimidite coupling of decaprenol and 2,3,5-tri-O-TBDMS-arabinofuranose and it gave a product that consisted of a 5:1  $\alpha/\beta$  anomeric mixture. In a more recent publication<sup>8</sup> this method was modified, but once again, the same anomeric ratio was observed. In an

effort to establish a synthetic pathway that will yield the desired  $\beta$  anomer as the major product, we adopted an alternative synthetic scheme. Our approach to the synthesis of DPA is based on the coupling of a decaprenyl trichloroacetimidate intermediate (**2**, Scheme 1) and a suitably protected arabinofuranosyl 1-phosphate derivative (**3**, Scheme 1). Such an approach was employed for the first time in the synthesis of various polyprenylphosphoryl hexoses from sugar 1-phosphates and polyprenyl trichloroacetimidates.<sup>9,10</sup> Recently we used this scheme successfully in the synthesis of the C<sub>15</sub>-farnesylphosphoryl and the C<sub>10</sub>-nerylphosphoryl  $\beta$ -D-arabinofuranoses from the same  $\beta$ -D-arabinofuranose 1-phosphate derivative and the corresponding oligoprenyl trichloroacetimidate intermediates.<sup>11</sup> We now wish to describe the synthesis of DPA by the same approach.

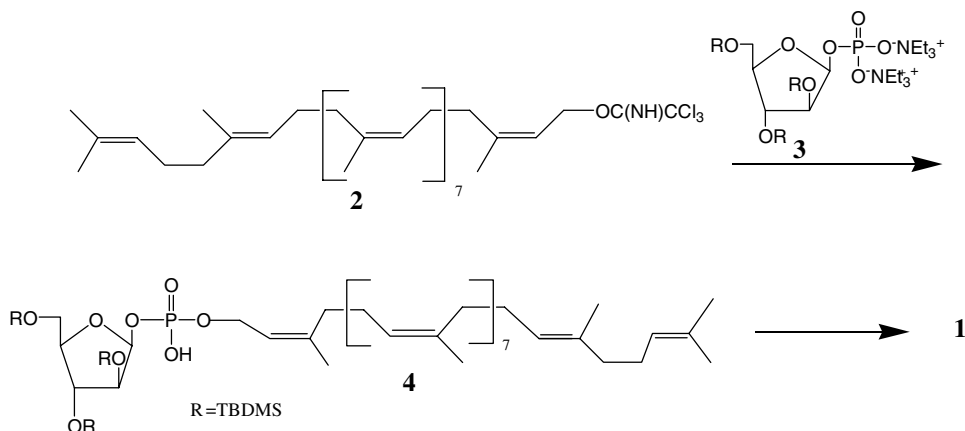
The stereoselective synthesis of  $\beta$ -D-arabinofuranosyl phosphate derivative **3** was accomplished by our published procedure,<sup>11</sup> but an effort was made to increase the proportion of the desired  $\beta$ -anomer. It was found that under strictly dry conditions (in particular, re-drying of the phosphorylating agent), the  $\beta$ -anomer predominated by a 9:1 ratio (as judged by <sup>1</sup>H NMR). Complete separation of the two anomers (by column chromatography) at that point was not feasible, but the earlier fractions were found to contain an even larger proportion (as much as 95%) of the  $\beta$ -anomer. The coupling of the decaprenyl intermediate **2** and the arabinose phosphate derivative **3** proceeded by a slightly modified

**Keywords:** DPA;  $\beta$ -D-Arabinofuranosyl phosphate; Mycobacteria.

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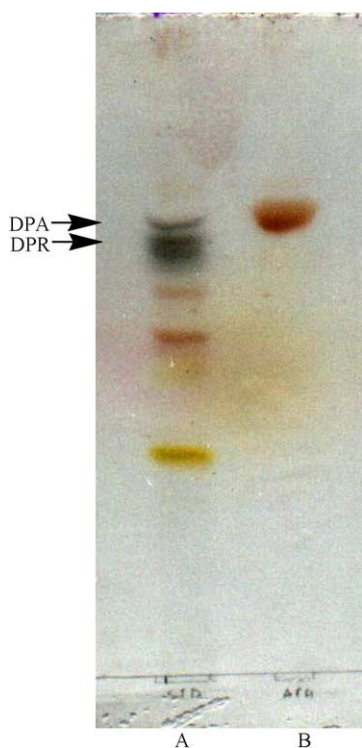
**Figure 1.** The structure of decaprenylphosphoryl  $\beta$ -D-arabinofuranose.



**Scheme 1.** The synthesis of decaprenylphosphoryl  $\beta$ -D-arabinofuranose.

procedure.<sup>12</sup> The rather sensitive trichloroacetimidate intermediate (**2**) was not purified by chromatography as was done in the case of the shorter analogs.<sup>11</sup> Instead, it was dissolved in toluene and the clear solution was separated by decantation. Also, the coupling of **2** and

**3** was carried out in a mixture of toluene–DMF in order to accommodate the polar phosphate derivative and the highly non-polar decaprenyl intermediate. Under these conditions, the product (**4**) was obtained in a relatively reasonable yield (43%). Deprotection of the coupled product (**4**) with ammonium fluoride in a 5% solution of ammonium hydroxide in methanol as described before<sup>11</sup> followed by chromatography on silica gel gave the pure DPA (**1**). The synthetic DPA was compared by TLC with the mixture of DPA and DPR (decaprenylphosphoryl  $\beta$ -D-ribofuranose) from *M. smegmatis*<sup>13</sup> and found to be identical with the natural product (see Fig. 2).<sup>14</sup> Mass spectroscopy and <sup>1</sup>H NMR data are in agreement with the structure.<sup>15</sup> The relatively large amount of the synthetic DPA allowed us to obtain a better-resolved spectrum than that reported for the natural product.<sup>4</sup>



**Figure 2.** TLC comparison of the autoradiogram of the DPA–DPR mixture from *M. smegmatis*<sup>13</sup> (A) and the synthetic DPA (B).

### Acknowledgement

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12. Synthesis of **4**. To an ice-cold solution of decaprenol (11.5 mg) in methylene chloride (0.5 mL) were added trichloroacetonitrile (20  $\mu$ L) and DBU (3  $\mu$ L). The mixture was stirred at room temperature for 20 min, and dried under vacuum. Toluene (2  $\times$  0.3 mL) was added to the residue, and the supernatant was added to the arabinosyl phosphate intermediate **3**<sup>11</sup> (27 mg). DMF (0.1 mL) was added, and the mixture was stirred at 67 °C for 6 h. The mixture was dried and the residue was chromatographed on silica gel (60 Å, 70–230 mesh). Elution with methylene chloride–methanol 5:1 (containing 0.5% of concentrated ammonium hydroxide solution) removed fast moving byproducts. Continued elution with the same solvent system gave the product (9 mg, 43%).
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15. Physical data for the synthetic DPA. FAB mass spectroscopy produced the 933.533 ion (M+Na). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  = 5.48 (t, *J* = 4.5 Hz, 1H), 5.44 (t, *J* = 6.5 Hz, 1H), 5.23–5.18 (m, 2H), 4.80–4.74 (m, 2H), 4.45 (t, *J* = 6.5 Hz, 1H), 4.30 (t, *J* = 6.5 Hz, 1H), 4.09 (t, *J* = 7.5 Hz, 1H), 4.01–3.98 (m, 1H), 3.82–3.75 (m, 2H), 3.69–3.62 (m, 2H), 2.14–1.98 (m, 28 H), 1.76 (s, 3H), 1.70 (s, 12H), 1.69 (s, 3H), 1.63 (s, 3H), 1.62 (s, 6H), 1.37–1.31 (m, partially obscured by the signal at 1.31, 3H), 1.31 (s, 6H), 0.94–0.91 (m, 6H). Two additional low-field protons are probably obscured by the large solvent signal (HOD, after exchange with the hydroxyl protons;  $\delta$  4.90). Optical rotation:  $[\alpha]_{\text{D}} -6.0$  (*c* 0.3, methanol).