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Stereoselective syntheses of heptaprenylphosphoryl β-D-arabino-and β-D-ribo-furanoses

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ABSTRACT

The stereoselective syntheses of heptaprenylphosphoryl β-D-arabinofuranose and heptaprenylphosphoryl β-D-ribofuranose are described. In the synthesis of the D-arabino product, the stereoselectivity was achieved by the coupling of a suitably protected β-D-arabinofuranosyl phosphate intermediate with an activated form of heptaprenol and subsequent deprotection. In the case of the ribo-analog, the desired β-anomer could be obtained by the more convenient phosphoramidite method. The products were successfully employed in the mycobacterial epimerase assay.

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The global rise in tuberculosis and drug-resistant *Mycobacterium tuberculosis* still present a threat to human health[1] and require 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.[2,3] The presence of octahydroheptaprenylphoshoryl-β-D-arabinofuranose in *M. smegmatis* has been shown before.[4,5] However, this metabolite is not produced in *M. tuberculosis*[6] and its role in bacteria remains unknown. A key arabinose donor for arabinan biosynthesis in mycobacteria is decaprenylphosphoryl β-D-arabinofuranose (DPA),[4] which is formed from its ribo- analog (decaprenylphosphoryl β-D-ribofuranose; DPR) via a two-step epimerization catalyzed by the action of the Rv3790 and Rv3791 gene products from *M. tuberculosis*.[7] In order to investigate the activities of the enzymes involved in the epimerization reaction we decided to synthesize medium chain compounds—heptaprenyl (C_{35})-analogs of DPA (**1**) and DPR (**2**) in addition to their previously produced long chain—decaprenol (C_{50})-[8,9] and short chain—nerol (C_{10})-[9,10] containing counterparts. Our aim was to test these as possible substrates for the epimerase reaction and identify compounds that would be suitable for the further development of the epimerase assay. In an unrelated study a C_{35}-lipid II product was found to be a better substrate for bacterial transglycosylases.[11]

A. *The stereoselective synthesis of heptaprenylphosphoryl* β-D-*arabino-furanose* (**1**): In order to achieve stereoselectivity in the synthesis of the title product and to obtain the unfavored β-anomer (1,2-cis orientation) as the major product we have used the route

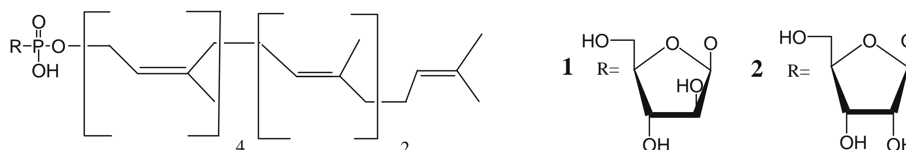

Figure 1. The structures of heptaprenylphosphoryl β-D-arabino-(**1**) and β-D-ribo-(**2**) furanoses.

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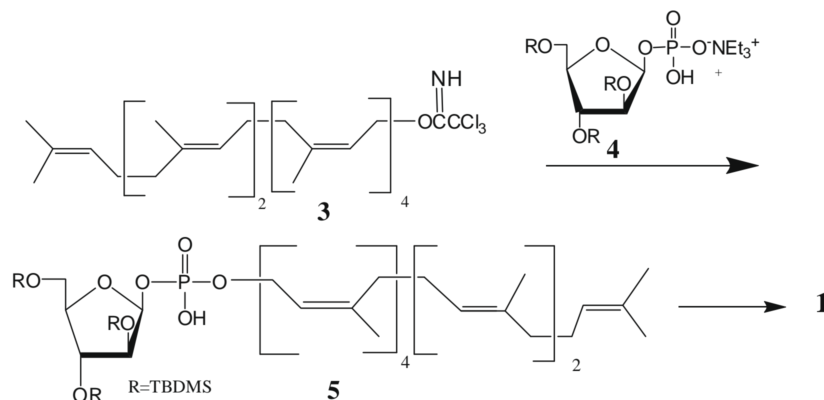
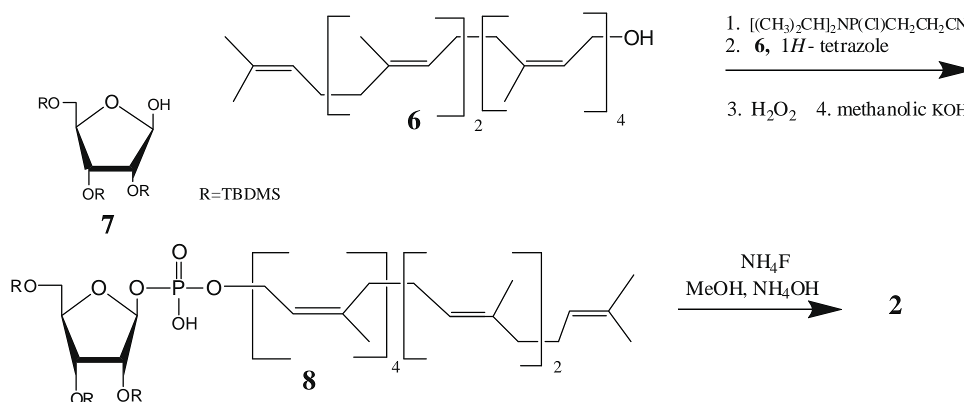


Scheme 1. The stereoselective synthesis of heptaprenylphosphoryl β-D-arabino-furanose (**1**).



Scheme 2. The stereoselective synthesis of heptaprenylphosphoryl β-D-ribo-furanose (**2**).

described for the shorter analogs[10] and DPA.[8] According to this scheme[12] heptaprenol was converted into the corresponding trichloroacetimidate intermediate (**3**, Scheme 1) which was then coupled to 2,3,5-tri-*O*-TBDMS-β-D-arabinofuranosyl phosphate (**4**)[10] to yield the protected product **5**. Deprotection with ammonium fluoride in methanolic ammonia produced the heptaprenyl analog **1**. In the ^{1}H NMR spectrum of **1**[13] the H-1 signal appears as a triplet ($J_{1,2} = J_{1,P} = 4.6$ Hz), indicating that the product exists in the β-configuration. The other features of the spectrum are in agreement with the structure. The mass spectrum of **1**(in the negative electrospray mode) produced the ion 705.452 (M−1) (see Fig. 1).

B. *The stereoselective synthesis of heptaprenylphosphoryl β-D-ribofuranose* (**2**): In contrast to the polyprenylphosphoryl-arabino products where the desired β-D-configuration is not the favored one, in the ribo-analogs (which possess the 1,2-trans configuration) the β-anomer is the predominant one. Consequently, the heptaprenyl analog can be made by the more convenient phophoramidite method, as already described for the synthesis of DPR and the shorter analogs.[9] Using this procedure[14] heptaprenol (**6**, Scheme 2) was first treated with 2-cyanoethyl *N*,*N*-diisopropylchloro-phosphoramidite in the presence of diisopropyl ethylamine, and the resulting phosphoramidite intermediate was coupled to 2,3,5-tri-*O*-TBDMS-ribofuranose (**7**)[9] in the presence of 1*H*-tetrazole. Subsequent oxidation with hydrogen peroxide followed by treatment with methanolic KOH gave heptaprenylphosphoryl-2,3,5-tri-*O*-TBDMS-β-D-furanose (**8**). Removal of the TBDMS groups as described above gave heptaprenylphosphoryl β-D-ribofuranose (**2**). The H-1 signal in the ^{1}H NMR spectrum of **2** (doublet, $J_{1,P} = 4.8$, Hz; $J_{1,2} = 0$ Hz) confirms that the product exists in the β-D-configuration The mass spectrum of **2** (in the negative electrospray mode) produced the ion 705.448 (M−1).

C. *Investigation of decaprenylphosphoryl β-D-ribofuranose (DPR), heptaprenylphosphoryl β-D-ribofuranose (HPR), and nerylphosphoryl β-D-ribofuranose (NPR) as possible substrates for the epimerization reaction*[15]: Due to the high hydrophobicity of the natural substrate for epimerization catalyzed by Rv3790/Rv3791, that is, DPR, alternative, more water-soluble substrates—HPR (**2**) and NPR were tested.[16] The compounds were used in 2 mM concentration and the reaction mixtures contained Rv3790 and Rv3791 gene products present in the soluble extracts of the *Escherichia coli* strains producing the respective enzymes. Conversion of the tested substrates to the corresponding Ara-containing compounds was examined by GC analysis of the alditol acetates prepared from the monosaccharides released from the reaction mixtures after their incubation: Increase in Ara content over the control reaction mixtures with inactivated enzymes reflected the activity of the Rv3790/91 enzymes. We found out that the best substrate in this assay was HPR (**2**), which was converted about four times more efficiently than DPR and about three times more efficiently than NPR. This compound will thus be used for further optimization of the enzymatic assay and subsequent testing of the putative inhibitors of the reaction.

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12. *The synthesis of* **1**. To an ice-cold solution of heptaprenol (16 mg) in methylene chloride (0.5 mL) were added trichloroacetonitrile (40 μL) and DBU (9 μL). The mixture was stirred at room temperature for 30 min, and dried under vacuum. Toluene (2 × 0.3 mL) was added to the residue, and the supernatant was added to the arabinosyl phosphate intermediate **4**[10] (40 mg). DMF (0.2 mL) was added, and the mixture was stirred at 70 °C for 4 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 tri-*O*-TBDMS derivative of **1** (**5**). This product was then treated with ammonium fluoride (90 mg) and 15% methanolic ammonium hydroxide solution (2 mL) in methanol (4 mL) at 65 °C for 14 h. The mixture was cooled and diluted with methylene chloride, and the insoluble material was filtered off and washed with methylene chloride–methanol 5:1. The filtrate was dried and the residue was chromatographed on silica gel. The product was eluted with methylene chloride–methanol–ammonium hydroxide 65:125:4 (12 mg, 53%).
13. *Physical data for* **1**. NMR data (500 MHz, CD_3OD): δ 5.47 (t, *J* = 4.6 Hz, 1H, H-1), 5.42 (t, *J* = 6.4 Hz, 1H), 5.13–5.07 (m, 4 H), 4.43 (t, 6.8 Hz, 2H), 4.06 (t, *J* = 7.6 Hz, 1H), 3.98 (m, 1H), 3.80–3.72 (m, 1H), 3.64 (dd, *J* = 5.0, 12.0 Hz, 1H), 2.14–1.94 (m, 20H), 1.74 (s, 3H), 1.68 (s, 9H), 1.64 (s, 3H), 1.61 (s, 3H), 1.60 (s, 6H), 1.34–1.26 (m, 6H). Mass spectrometry (negative ion-electrospray mode): 705.452 (M−1).
14. *The synthesis of* **2**. A solution of heptaprenol (23 mg) in dichloromethane (0.8 mL) was saturated with nitrogen. Diisopropylethyl amine (15 μL) was added and the mixture was cooled (ice bath). 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (20 μL) was added and the mixture was stirred at room temperature for 1 h. The mixture was cooled (ice-bath) and 1*H*-tetrazole (20 mg) and a solution of the tri-*O*-TBDMS derivative (**7**, 32 mg)[10] in dichloromethane (0.3 mL) were added. The mixture was then stirred at room temperature for 4 h. The mixture was dried and the residue was triturated with petroleum ether (3 × 3 mL). The organic solution was dried and the residue was dissolved in tetrahydrofuran (1.5 mL). Hydrogen peroxide solution (35%, 20 μL) was added and the mixture was stirred at room temperature for 30 s. It was then treated with methanolic potassium hydroxide solution (5%, 6 mL) at room temperature for 30 min. The mixture was partitioned between water (10 mL) and dichloromethane (10 mL). The organic phase was washed with a saturated sodium chloride solution and dried under vacuum. The residue was chromatographed on silica gel (60 Å, 70–230 mesh). Elution with dichloromethane–methanol 5:1 (containing concentrated ammonium hydroxide solution, 1% v/v) removed at first fast moving impurities. Continued elution with the same solvent system gave the pure product. Yield: 19 mg (46%). Finally, the product was deprotected by treatment with ammonium fluoride (150 mg) in methanolic ammonium hydroxide solution (5%, 9 mL) at 67 °C for 17 h. The mixture was cooled and diluted with dichloromethane. The insoluble crystalline material was filtered off and washed with dichloromethane–methanol 4:1. The filtrate was dried and the residue was chromatographed on silica gel. Elution with dichloromethane–methanol–ammonium hydroxide 65:25:4 removed fast moving byproducts. Continued elution with the same solvent system, followed by dichloromethane–methanol–ammonium hydroxide 65:125:4 gave the ribo-analog **2**. Yield: 7.5 mg (78%).
15. *Physical data for* **2**. NMR data (500 MHz, CD_3OD): δ 5.48 (d, *J* = 4.8 Hz, 1H, H-1), 5.42 (t, *J* = 6.8 Hz, 1H), 5.16–5.09 (m, 4 H), 4.41 (t, *J* = 6.6 Hz, 2H), 4.30 (dd, *J* = 4.4, 7.3 Hz, 1H), 3.99 (d, *J* = 4.4 Hz, 1H), 3.98–3.95 (m, 1H), 3.82 (dd, *J* = 2.9, 12.2 Hz, 1H), 3.63 9dd, *J* = 5.37, 12.2 Hz 1H), 2.14–1.97 (m, 20H), 1.76 (s, 3H), 1.70 (s, 9H), 1.69 (s, 3H), 1.63 (s, 3H), 1.61 (s, 6H), 1.37–1.31 (m, 6H). Mass spectrometry (negative ion-electrospray mode): 705.448 (M−1).
16. *E. coli* strain producing Rv3790 (*E. coli* BL21(DE3)/pET15b-Rv3790) was a kind gift from Professor Giovanna Riccardi (University of Pavia, Italy). *E. coli* strain producing Rv3791 (*E. coli* BL21(DE3) pLysS/pET28a-Rv3791) was prepared in our laboratory. The induced cells were resuspended in five times volume of Buffer A (50 mM MOPS, pH 7.9; 5 mM β-mercaptoethanol; 10 mM $MgCl_2$) and disintegrated by probe sonication. The cell lysates were centrifuged at 20,000*g* for 20 min and the supernatants served as sources of the enzymes. For the epimerization reaction, 15 μL of each supernatant, 2 mM substrate (DPR, HPR, or NPR), 0,125 mM NAD^+, and Buffer A were used in the final volume of 40 μL. After 1 h incubation at 37 °C the reactions were stopped by addition of 200 μL of 2 M TFA and alditol acetates were prepared and analyzed, as described.[17]
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