Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 8781–8783

Stereoselectivity in the synthesis of polyprenylphosphoryl β-D-ribofuranoses

Avraham Liav,[a,*] Ewa Swiezewska,[b] Ewa Ciepichal[b] and Patrick J. Brennan[a]

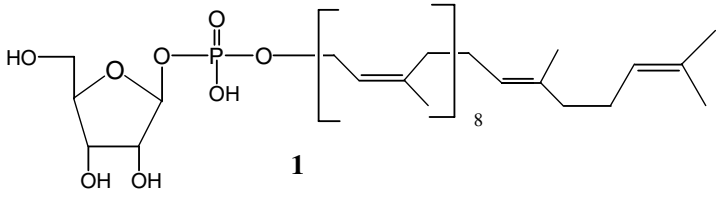
[a]*Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523, USA*
[b]*Polish Academy of Sciences, Institute of Biochemistry and Biophysics, ul. Pawninskiego 5a, 02-106 Warsaw, Poland*

Received 4 August 2006; revised 28 September 2006; accepted 29 September 2006
Available online 20 October 2006

Abstract—Decaprenylphosphoryl β-D-arabinofuranose (DPA) is a key arabinose donor in mycobacteria. The *ribo* analog of DPA (DPR) has also been found in mycobacteria. It has recently been confirmed that DPA is formed via a two-step epimerization of DPR. The stereoselective synthesis of DPR as well as two shorter analogs of DPR is described.
© 2006 Elsevier Ltd. All rights reserved.

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] A key mycobacterial arabinose donor, decaprenylphosphoryl β-D-arabinofuranose (DPA), has been found in the lipid extracts of *Mycobacterium smegmatis* and implicated in the biogenesis of the two major cell wall polysaccharides, arabinogalactan, and lipoarabinomannan.[4] The *ribo* analog of DPA (DPR, **1**, Fig. 1) has also been found in mycobacteria[5] and it has been recently found that DPR is the precursor of DPA in *M. smegmatis*.[6] In order to study the enzymatic conversion of DPR to DPA in a more detailed manner, it was necessary to develop a synthesis of DPR that will yield sufficient amount of the product. Accordingly, the stereoselective synthesis of DPR has been accomplished. Also described in this manuscript is the stereoselective synthesis of the analogous neryl and farnesyl products (having C_{10} and C_{15} lipid chains, respectively).

Our first approach to the synthesis of DPR was based on the scheme described for the analogous DPA product.[7,8] According to this scheme, 2,3,5-tri-O-TBDMS-D-ribose (**2**, Scheme 1) was first synthesized from D-ribose as described for the *arabino* analog. Conversion of **2** into the corresponding dibenzyl phosphate intermediate, using a bromide as a donor, gave mainly the undesired α-1-phosphate. However, when the trichloroacetimidate derivative of **2** was employed a 2:1 β/α anomeric mixture was obtained (as judged by TLC). Separation of the two anomeric 1-phosphates was achieved by column chromatography and the β-anomer was isolated in 20% yield (based on **2**).[9] Catalytic hydrogenolysis of the β-anomer in ethanol in the presence of Pd/C 10% catalyst gave phosphate intermediate **3**. The next step in the synthesis,

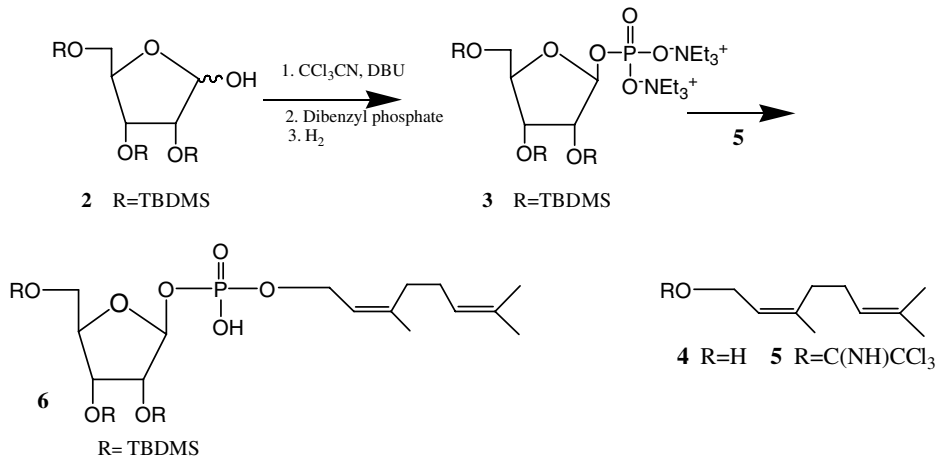


1

Figure 1. The structure of DPR.

Keywords: Decaprenylphosphoryl β-D-arabinofuranose; Decaprenylphosphoryl β-D-ribofuranose; Mycobacteria.
* Corresponding author. Tel.: +1 970 491 5537; fax: +1 970 491 1815; e-mail: avraham.liav@colostate.edu

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.09.163

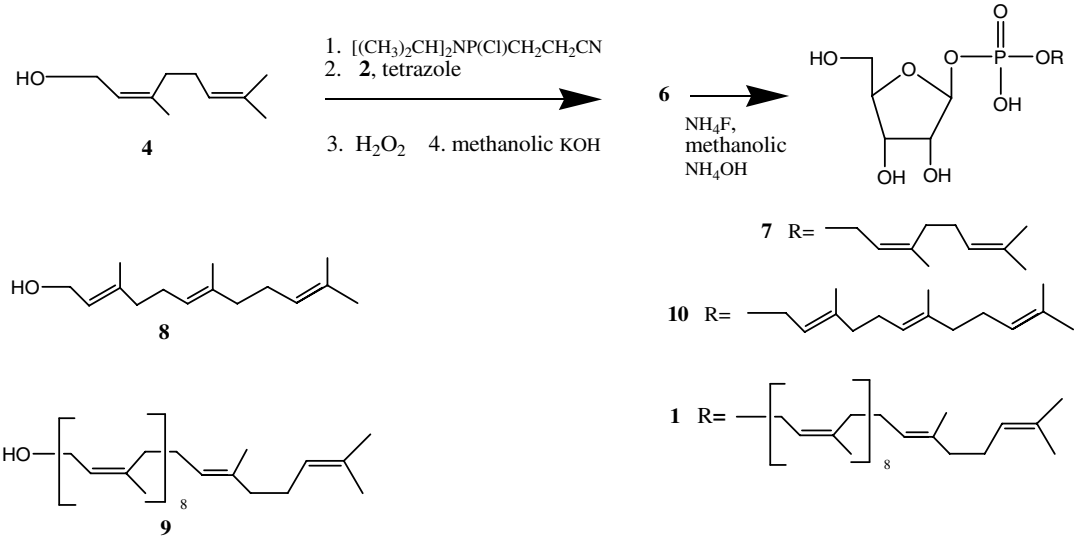


Scheme 1. Synthesis of nerylphosphoryl-2,3,5-tri-O-TBDMS β-D-ribofuranose from a 1-phosphate intermediate.

the coupling of **3** to a polyprenol, was studied by utilizing the more readily available C_{10} nerol (**4**). Thus, treatment of **3** with the trichloroacetimidate derivative (**5**) of nerol (**4**) as described before[8] gave the tri-O-TBDMS product **6** (in 28%).

The overall poor yield in the synthesis of **6** by Scheme 1 prompted us to seek a more straightforward and stereoselective approach to the synthesis of DPR and its analogs. An alternative method, based on a phophoramidite coupling, has already been applied to the synthesis of DPA.[2] However, in that instance the inactive α-anomer (with the 1,2-*trans* configuration) was obtained as the major product (favored by a 5:1 ratio).[2] It was reasonable to assume that in the case of DPR, the β-anomer (which also has the 1,2-*trans* configuration) would be the favored product. Following this prediction, the coupling of nerol (**4**) and 2,3,5-tri-O-TBDMS-D-ribose (**2**) was then attempted. Nerol(**4**) was first treated with 2-cyanoethyl *N*,*N* diisopropylchlorophosphoramidite and the resulting phosphoramidite intermediate was coupled with **2** in the presence of tetrazole. Subsequent oxidation with hydrogen peroxide, followed by treatment with methanolic KOH, gave nerylphophoryl-2,3,5-tri-O-TBDMS β-D-ribofuranose. After purification by column chromatography, the product was found to be identical (^{1}H NMR, TLC) with **6** obtained by Scheme 1. As predicted, the phosphoramidite scheme was found to be highly stereoselective, and only the β-anomer could be isolated. Removal of the TBDMS groups by treatment with ammonium fluoride in methanolic ammonia[8] gave nerylphosphoryl β-D-ribofuranose (**7**).[10]

In a similar manner, phosphoramidite coupling of the C_{15} *trans, trans*-farnesol (**8**) or decaprenol (**9**) with **2**, and subsequent deprotection produced the farnesyl analog (**10**) and DPR (**1**),[11] respectively. TLC examination of **1** was consistent with the previous finding.[12] The ^{1}H NMR spectrum of synthetic **1** was found to be in agreement with the structure and very similar to the spectrum of the natural product.[5] The anomeric proton signal appeared as a doublet (δ 5.43, $J = 4.8$ Hz) as expected. A full characterization of **1** is given in note[13] (see Scheme 2).



Scheme 2. Synthesis of DPR and analogs by the phosphoramidite method.

The synthesis of shorter chain analogs (such as **7** and **10**) is important since the use of these products in enzymatic assays will alleviate the solubility problem associated with the highly hydrophobic DPR. Moreover, the shorter polyprenols are significantly less expensive than decaprenol. In regard to the activity of the shorter chain analogs, it has already been observed that polyprenyl derivatives of mannosyl-1-phosphate in yeast showed no selectivity for the polyprenyl chain length in the transfer of mannose from polyprenylphosphoryl-mannose to an insoluble polymer.[14] More relevantly, the C_{10}, citronellylphosphoryl β-D-arabinofuranose exhibited some donor activity in mycobacteria.[15]

Acknowledgement

This work was supported by NIH grant No. 4-R37 AI 18357.

References and notes

1. Hueber, R. E.; Castro, K. G. *Annu. Rev. Med.* **1995**, *46*, 47–55.
2. Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 11829–11832.
3. Brennan, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29–63.
4. Wolucka, B. A.; McNeil, M. R.; de Hoffman, E.; Chojnacki, T.; Brennan, P. J. *J. Biol. Chem.* **1994**, *269*, 23325–23328.
5. Wolucka, B. A.; de Hoffmann, E. *J. Biol. Chem.* **1995**, *270*, 20151–20155.
6. Mikusova, K.; Huang, H.; Yagi, T.; Holsters, M.; D'Haeze, W.; Scherman, M.; Brennan, P. J.; McNeil, M. R.; Crick, D. C. *J. Bacteriol.* **2005**, *187*, 8020–8025.
7. Liav, A.; Brennan, P. J. *Tetrahedron Lett.* **2005**, *46*, 2937–2939.
8. Liav, A.; Huang, H.; Ceipichal, E.; Brennan, P. J.; McNeil, M. R. *Tetrahedron Lett.* **2006**, *47*, 545–547.
9. A mixture of 10:1 petroleum ether–ethyl acetate was used for elution and triethyl amine (1%) was added to the mixture in order to minimize the decomposition of the unstable intermediates, but there was still a significant loss of the products during the chromatography. The *ribo*-phosphates were found to be less stable than the corresponding arabinose products.[7,8]
   The signal for the anomeric proton in the ^{1}H NMR spectrum of the β-1-phosphate appeared as a doublet (δ 5.06, $J_{H\text{-}1,P} = 4.8$ Hz), in agreement with the structure.
10. Physical data for **9**. ^{1}H NMR (300 MHz, CD_3OD): $\delta = 5.47$ (d, $J = 4.8$ Hz, 1H, H-1), 5.41 (t, $J = 6.9$ Hz, 1H), 5.15–5.13 (m, 1H), 4.40 (t, $J = 6.9$ Hz, 2H), 4.29 (dd, $J = 4.8$, 7.8 Hz, 1H), 3.98–3.93 (m, 2H), 3.81 (dd, $J = 3.0$, 12.0 Hz, 1H), 3.62 (dd, $J = 5.4$, 12.0 Hz, 1H), 2.12 (s, 4H), 1.75, 1.69, 1.62 (3s, 9H). Mass spectroscopy in the negative ion electrospray mode gave the ion 365.1 (M−1). Optical rotation: $[\alpha]_D$ −15.5 (*c* 0.27, methanol). The ^{1}H NMR spectrum of **10** was very similar to that of **9**.
11. Synthesis of **1** by the phosphoramidite method. A solution of decaprenol (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 tetrazole (20 mg) and a solution of the tri-O-TBDMS derivative (**2**, 32 mg) 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 1% ammonium hydroxide solution) 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 at a ratio of 4:1. The filtrate was dried and the residue was chromatographed on silica gel. Elution with dichloromethane–methanol–ammonium hydroxide at a ratio of 65:25:4 removed fast moving byproducts. Continued elution with the same solvent system, followed by dichloromethane–methanol–ammonium hydroxide at a ratio of 65:125:4 gave DPR (**1**). Yield: 7.5 mg (78%).
12. TLC comparison of **1** with DPA (in chloroform–methanol–water-*M*-ammonium acetate–ammonium hydroxide 180:140:23:9:9) showed DPR to move slightly slower than DPA, as observed before.[8]
13. Physical data for **1**. ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 5.43 (d, $J = 4.8$ Hz, 1H), 5.37 (t, $J = 7.2$ Hz, 1H), 5.10 (m, 7H), 4.36 (t, $J = 6.4$ Hz, 2H), 4.26 (dd, $J = 4.8$, 7.6 Hz, 1H), 3.92 (m, 2H), 3.77 (dd, $J = 2.4$, 12.0 Hz, 1H), 3.58 (dd, $J =$ 4.8, 12.0 Hz, 1H), 2.06–1.93 (m, 33H), 1.70 (s, 3H), 1.65 (s, 15H), 1.62 (s, 3H), 1.60 (s, 9H), 1.35–1.30 (s, 6H). Two additional low-field protons are apparently obscured by the large solvent signal (HOD, after exchange with the hydroxyl protons, at δ 4.87). High resolution mass-spectrometry in the negative ion electrospray mode produced the M−1 ion 909.67; calcd for $C_{55}H_{91}O_8P$: 909.68 (the calculated value was based on the most abundant carbon atom, 12.00 Da). Optical rotation: $[\alpha]_D$ −6.4 (*c* 0.5, methanol).
14. Pless, D. D.; Palamarczyk, G. *Biochim. Biophys. Acta* **1978**, *529*, 21–28.
15. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 951–954.