Pergamon

Tetrahedron Letters 42 (2001) 2231–2234

TETRAHEDRON LETTERS

Stereospecific synthesis of 5-phospho-α-D-arabinosyl-*C*-phosphonophosphate (pA*C*pp): a stable analogue of the putative mycobacterial cell wall biosynthetic intermediate 5-phospho-D-arabinosyl pyrophosphate (pApp)

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Received 4 December 2000; revised 8 January 2001; accepted 17 January 2001

Abstract—The stereospecific synthesis of 5-phospho-α-D-arabinosyl-*C*-phosphonophosphate (pA*C*pp) from D-glucosamine is described. This compound was evaluated for its ability to serve as a stable analogue of the putative mycobacterial cell wall biosynthetic intermediate 5-phospho-D-arabinosyl pyrophosphate (pApp). The phosphonophosphate proved incapable of interfering with formation of the mycobacterial arabinan precursor decaprenylphospho-arabinose (DpA) in vitro. © 2001 Elsevier Science Ltd. All rights reserved.

Since the mid 1980s there has been a disturbing resurgence in the incidence of tuberculosis world-wide, resulting in the highest ever annual mortality rate from the disease in 1997. There are 8 million new infections and 3 million deaths from TB every year; it is expected that in the next decade 90 million new cases will occur, resulting in 30 million deaths.[1] There is a real need to identify new targets and develop new classes of drugs for the treatment of TB. To date, several front-line anti-tubercular agents have targeted steps in mycobacterial cell wall biosynthesis, including isoniazid, ethionamide and ethambutol.[2] It is the complex combination of mycolylated arabinogalactan, lipoarabinomannan and other components that contribute to the impregnable nature of the cell wall. It has been established that most of the D-arabinofuranosyl residues in cell wall arabinan-containing structures are derived from decaprenylphospho-arabinose (DpA);[3–5] it follows that inhibition of the biosynthesis of this molecule should prove deleterious to the organism.

The biosynthesis of DpA originates from 5-phosphoribosyl pyrophosphate (pRpp), which is formed by the pentose shunt pathway, and might occur via one of two routes (Scheme 1).[6] Route **A** would involve epimerisation of the C-2 position of pRpp to give the *arabino*-configured pApp, followed by conversion to 5-phospho decaprenylphospho-arabinose (5p-DpA) and dephosphorylation to DpA. Alternatively, attachment of the lipid tail might precede C-2 epimerisation; in route **B**, pRpp might first be converted to 5-phospho decaprenylphospho-ribose (5p-DpR), followed by C-2 epimerisation to *arabino*-configured 5p-DpA and dephosphorylation to give DpA. Intermediates 5p-DpA and 5p-DpR have been identified in cell-free incubations by HPLC and by TLC/autoradiography, as have products DpA and DpR. However, experiments using radioactive 5p-DpR and *Mycobacterium smegmatis* cell wall preparations failed to produce either 5p-DpA or DpA, instead producing DpR as the sole product.[6] This suggests the absence of 5p-DpR and DpR 2-epimerase activities in such preparations, hinting at a role for pApp as a biosynthetic intermediate in DpA formation.

In order to assess whether or not pApp does indeed serve as an intermediate in the biosynthesis of DpA, its chemical synthesis was considered. However, in light of

Keywords: D-arabinose; 2,5-anhydro-D-mannitol; phosphonophosphate; mycobacteria; cell wall biosynthesis.

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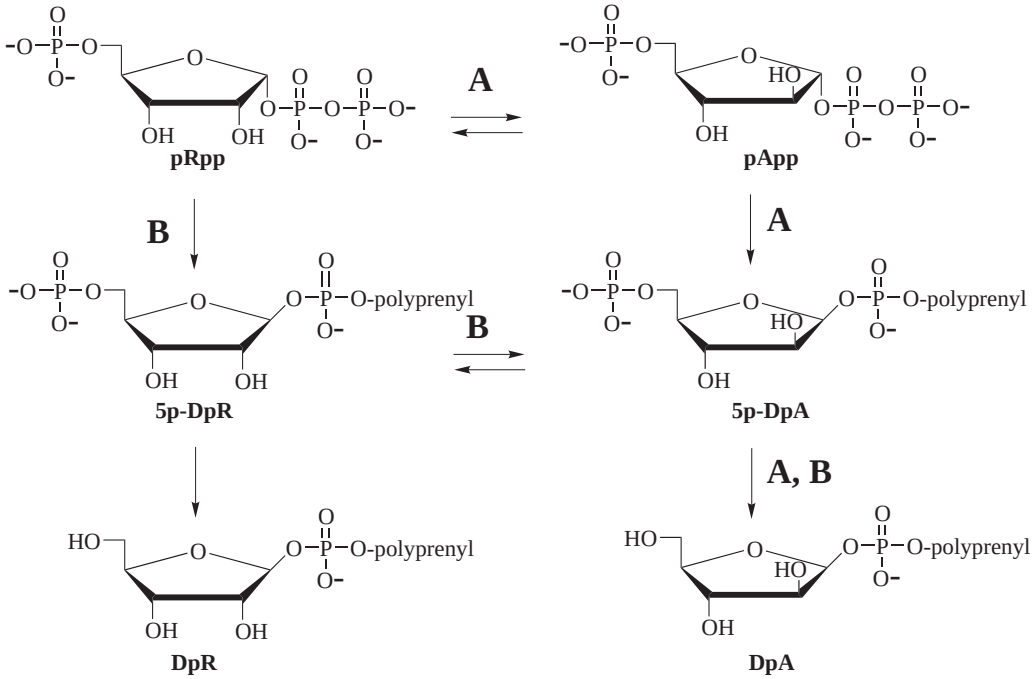
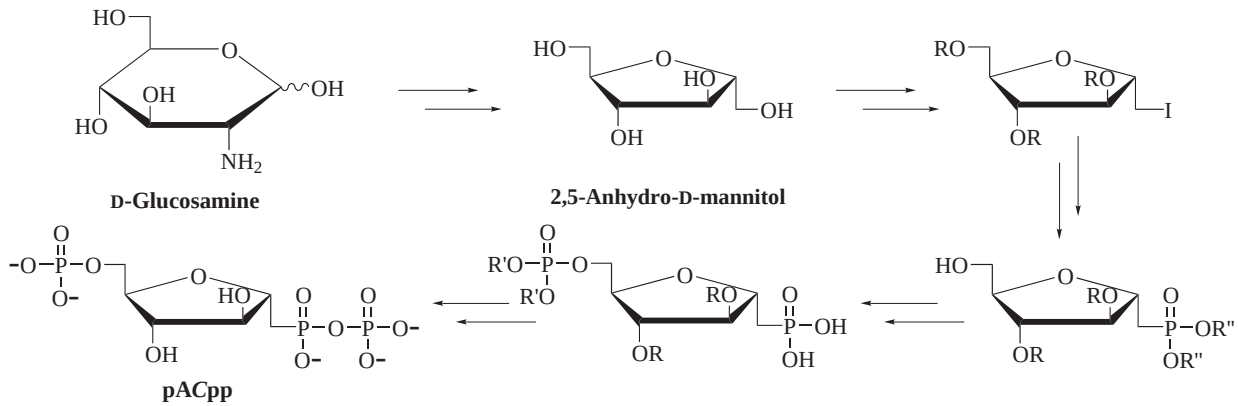
PII: S0040-4039(01)00118-6



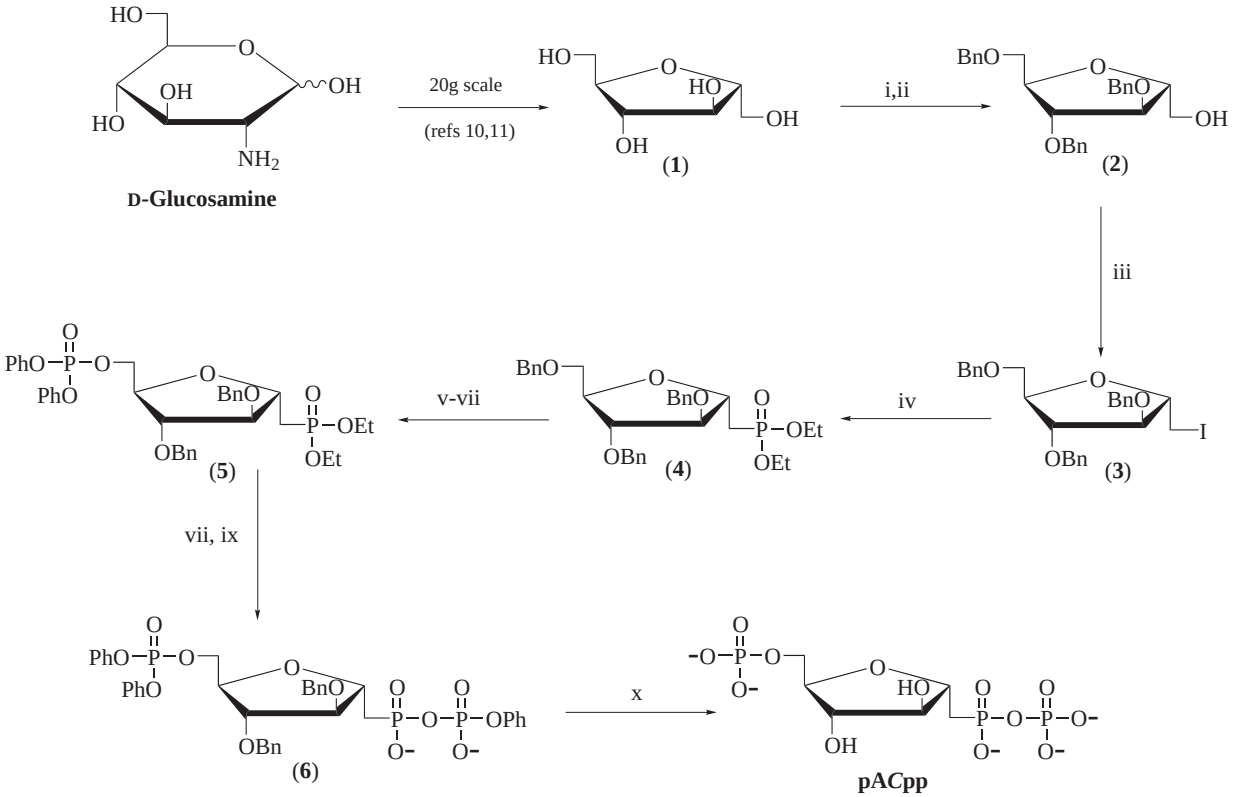
Scheme 1. Prospective biosynthetic routes (**A** and **B**) from 5-phosphoribosyl pyrophosphate (pRpp) to decaprenylphosphoarabinose (DpA).



Scheme 2. Synthetic approach to 5-phospho-D-arabinosyl-α-*C*-phosphonophosphate (pA*C*pp).

difficulties encountered in the attempted synthesis and deprotection of this compound,[7] an alternative approach involving synthesis of a stable analogue, namely 5-phospho-α-D-arabinosyl-*C*-phosphonophosphate (pA*C*pp), was investigated.[8] An earlier synthesis of pA*C*pp by McClard and co-workers[9] employed addition of dimethylmethylphosphonate to a protected D-arabinofuranose, giving an inseparable diastereomeric mixture of *gluco*- and *manno*-configured phosphonate intermediates. This prevented detailed analysis of all but the final synthetic compounds, which themselves proved difficult to separate. We favoured a stereospecific route to pA*C*pp, where the potentially problematic formation of the required *manno*-configured anhydrosugar was in place at an early stage in the synthesis. This led us to consider a suitably protected 2,5-anhydro-D-mannitol derivative as a synthetic intermediate, since 2,5-anhydro-D-mannitol itself is readily accessible from D-glucosamine via a well established stereospecific diazonium ion-mediated ring contraction reaction[10,11] (Scheme 2).

Synthesis (Scheme 3): 2,5-Anhydro-D-mannitol **1** was prepared from glucosamine hydrochloride on a 20 g scale using established literature procedures.[10,11] Selective mono-*O*-tritylation, subsequent benzylation and de-tritylation, without purification of the intermediate, gave tri-*O*-benzylated primary alcohol **2**[12] in good overall yield. Introduction of the phosphonate functionality was achieved in a straightforward manner, using Arbuzov chemistry, from the corresponding primary iodide **3**, which was prepared from primary alcohol **2** using PPh_3, NIS and DMF.[13] The large up field shift of the ^{13}C NMR signal attributed to C-1 (from 62.7ppm in **2** to 6.9ppm in **3**) reflected the success of the reaction.



Scheme 3. Synthesis of 5-phospho-α-D-arabinosyl-*C*-phosphonophosphate (pA*C*pp). (i) TrCl, pyr (41%); (ii) NaH, BnBr, DMF; TsOH, CH_2Cl_2/MeOH, 5:1 (82%, two steps); (iii) NIS, Ph_3P, DMF (76%); (iv) $P(OEt)_3$, Δ (83%); (v) conc. H_2SO_4, Ac_2O; Na, MeOH (81%, two steps); (vi) diphenylphosphoryl chloride, pyr (98%); (vii) TMS–Br, CH_2Cl_2 (quant.); (viii) carbonyldiimidazole, DMF, phenylphosphate (44%); (ix) H_2, Pd–C, AcOH; H_2, PtO_2, Pt–C, AcOH (46%, two steps).

Unfortunately a first attempt at the Arbuzov reaction, using refluxing trimethyl phosphite, gave no conversion of iodide **3** to the corresponding dimethylphosphonate, ruling out direct comparison of subsequent synthetic intermediates with McClard's data.[8,9†] On switching to the higher boiling triethyl phosphite, the Arbuzov chemistry proceeded in good yield. The identity of the diethylphosphonate **4** was supported by the appearance of a characteristic signal at 28.2 ppm in the ^{31}P NMR spectrum and the observed couplings of H-1 and H-1′ (18.9 and 18.4 Hz), C-1 (137.5 Hz) and C-2 (7.5 Hz) with phosphorus. Subsequent steps in the synthesis followed the route described by McClard.[9] Selective acetolysis of the 6-*O*-benzyl group of **4** followed by deacetylation resulted in the unmasking of the primary alcohol, which was phosphorylated with diphenylphosphoryl chloride to give **5**. In addition to ^1H and ^{13}C NMR data, the appearance of a new signal at −13.0 ppm in the ^{31}P NMR spectrum, characteristic of a phosphate ester,[14] confirmed the identity of the product. Dealkylation of phosphonate **5** with bromotrimethylsilane and subsequent formation of the phosphonophosphate, via activation with carbonyldiimidazole and coupling to phenyl phosphate, also proceeded satisfactorily. The resulting protected phosphonophosphate **6** gave rise to doublets in its ^{31}P NMR spectrum at 13.4 and −16.1 ppm, both with a 24.5 Hz P–P coupling, confirming formation of the phosphonophosphate moiety. The phenyl and benzyl groups in **6** were removed by catalytic hydrogenation, which for no obvious reason required the sequential use of palladium and platinum catalysts for maximum efficiency.

The stereospecific synthesis of the pA*C*pp was successfully completed in 11 steps from readily available 2,5-anhydro-D-mannitol in 3.4% overall yield. For comparison, McClard's synthesis[9] required only seven steps, but necessitated a difficult separation of three polar compounds at the final stage.

† Selected data: 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-diethylphosphono-6-*O*-diphenylphosphono-D-mannitol (**5**).
Amorphous solid (calcd for $C_{36}H_{42}O_{10}P_2$: C, 62.07; H, 6.08; found C, 61.80; H, 6.35); δ_H ($CDCl_3$), 1.27 (6H, dt, *J* 7.06, *J* 1.54, 2×C$\underline{H}_3$CH$_2$OP), 2.15 (1H, dd, $J_{H,P}$ 18.9, $J_{1,2}$ 6.4, H-1), 2.20 (1H, dd, $J_{H,P}$ 18.4, $J_{1',2}$ 7.85, H-1′), 3.97–4.61 (14H, m, 2×CH$_3$C$\underline{H}_2$OP, 2×PhC$\underline{H}_2$, H-2,3,4,5,6,6′), 7.12–7.38 (20H, Ar); δ_C 16.3 (C$\underline{H}_3$CH$_2$OP), 16.4 (C$\underline{H}_3$CH$_2$OP), 61.6 (CH$_3$C$\underline{H}_2$OP, $J_{C,P}$ 6.54), 61.9 (C$\underline{H}_3$CH$_2$OP, $J_{C,P}$ 6.54), 68.1 (C-6, $J_{C,P}$ 6.60), 71.6 (PhC$\underline{H}_2$), 71.8 (PhC$\underline{H}_2$), 79.0 (C-3 or 4), 81.7 (C-5, $J_{C,P}$ 8.40), 84.3 (C-3 or 4), 85.8 (C-2, $J_{C,P}$ 7.95), 120.0–129.7 (Ar), 137.3 (quat. Ar), 137.5 (quat. Ar), 150.4 (quat. Ar), 150.5 (quat. Ar); δ_P (decoupled) 26.1 (phosphonyl), −13.0 (phosphoryl). Lit. for dimethylphosphonate,[9] δ_P 30.22, −11.63.
5-Phospho-α-D-arabinosyl-*C*-phosphonophosphate (**pA*C*pp**)
δ_H (D_2O) 2.25 (2H, dd, $J_{1\&1',P}$ 18.7, $J_{1,1'}$ 7.0, H-1,1′), 3.98 (2H, m, H-6,6′), 4.00–4.21 (4H, m, H-2,3,4,5); δ_C 28.9 (C1), 60.9 (C6), 74.0 (C4), 75.6 (C2), 77.3 (C3 or C5), 78.1 (C3 or C5); δ_P (decoupled) 14.3 (phosphonyl), 0.7 (phosphoryl), −10.6 (phosphoryl anhydride). NMR data in accord with literature values.[9] ES-MS: calcd for $C_6H_{15}O_{13}P_3$: 387.9725, found $[M-H]^-$ 386.9650.

Biological assays: Phosphonophosphate pA*C*pp was tested for its ability to inhibit incorporation of radiolabel from pRpp into organic soluble material (i.e. 5-P-DpA/5-P-DpR and DpA/DpR) by membrane preparations of both *M. bovis* (BCG strain) and *M. smegmatis* using standard protocols.[6] Results showed less than 20% inhibition at 5 mM concentration, in both the presence and absence of detergent.

In summary, we have developed a stereospecific synthesis of 5-phospho-D-arabinosyl-α-*C*-phosphonophosphate (pA*C*pp), a stable analogue of the putative mycobacterial cell wall biosynthetic intermediate 5-phospho-D-arabinosylpyrophosphate (pApp), and shown that it is incapable of interrupting key steps in mycobacterial arabinan formation in vitro. One might choose to interpret these results as indicating that pApp is not in fact an intermediate in mycobacterial cell wall biosynthesis. However, the acidity of a phosphonophosphate does not necessarily accurately reflect that of the pyrophosphate,[15] which might contribute to the lack of activity observed. Alternatively, the anomeric oxygen atom of pApp might be important for epimerase recognition. The enzymes responsible for conversion of pRpp to DpA are membrane bound;[6] substrate channelling arising from protein complex formation might also account for the inability of pA*C*pp to interfere with DpA biosynthesis.

Acknowledgements

This work was supported by US NIH/NIAID AIDS-FIRCA grant R03 TW00938-01 and NIH grant AI 33706, the UK BBSRC (studentship to P.McG.) and the Ohio State University. We thank the EPSRC Mass Spectrometry Service at the University of Wales, Swansea for invaluable support.

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