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A Simple Route for Synthesis of 4-Phospho-D-Erythronate

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Abstract

4-Phospho-D-erythronate is an intermediate in synthesis of pyridoxal 5'-phosphate in some bacteria and an inhibitor of ribose 5-phosphate isomerase. Previous synthetic schemes for the preparation of 4-phospho-D-erythronate required expensive precursors and typically gave low yields. We report a straightforward synthesis of 4-phospho-D-erythronate from the inexpensive precursor D-erythronolactone in 5 steps with a preparatively useful yield of 22%.

Keywords

4-phospho-D-erythronate; D-erythronolactone; phenyldiazomethane; benzylation

4-Phospho-D-erythronate (4PE, **1**) is an intermediate in the synthesis of pyridoxal 5'-phosphate (PLP) in some bacteria. In gamma proteobacteria such as *Escherichia coli*, 4PE dehydrogenase (PdxB) converts 4PE to 2-oxo-3-hydroxy-4-phospho-butanoate (**2**) with concomitant reduction of NAD⁺ to NADH. Efforts to study this enzyme have been frustrated by the puzzling finding that multiple turnovers of the substrate could not be achieved *in vitro*.¹ 4PE is also oxidized to **2** by alpha proteobacteria such as *Sinorhizobium meliloti*, but the reaction is catalyzed by a flavoenzyme called PdxR that is not homologous to PdxB.² Studies of these enzymes have been hampered by the inavailability of synthetic 4PE. Synthesis of 4PE by the strategy described herein enabled mechanistic studies of *E. coli* PdxB, leading to the discovery that the nicotinamide cofactor is not released from the enzyme after substrate turnover. The tightly bound NADH formed after oxidation of 4PE must be regenerated by reduction of an α -keto acid such as α -ketoglutarate, oxaloacetate, or pyruvate before a second turnover can occur (see Scheme 1).³ The availability of 4PE will facilitate further studies of the mechanism and structure of this unusual enzyme.

4PE is also of interest because it is an inhibitor of ribose 5-phosphate isomerase (Rpi).⁴ Rpi is required for synthesis of ribose and therefore for synthesis of ribonucleotides, deoxyribonucleotides, and histidine. In plants, Rpi is also involved in the Calvin cycle carbon fixation pathway. Two unrelated families of Rpi's have been recognized. RpiA is found in all three domains of life, while RpiB is found only in some bacteria and protozoa, including pathogens such as *Yersinia pestis*, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, *Rickettsia rickettsii* and *proWazekii*, *Trypanosoma cruzi*

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and *brucei*, *Leishmania major*, *Entamoeba histolytica* and *Giardia lamblia*. Rpis are potential targets for drugs and herbicides, and analogs of 4PE have been synthesized and evaluated as potential inhibitors of these enzymes.^{5,6}

Most published syntheses of 4PE have involved oxidation of a phosphorylated sugar precursor. The earliest reported synthesis used lead tetraacetate to oxidize glucose-6-phosphate.⁷ However, 4PE was neither purified from by-products nor adequately analyzed to assure the purity of 4PE. Later, traces of 4PE were obtained from fructose 1,6-diphosphate employing a multi-step procedure followed by a complicated purification.⁸ Woodruff and Wolfenden prepared 4PE by oxidizing erythrose-4-phosphate with bromine,⁴ as previously reported by Horecker.⁹ However, the reaction yield and purification procedure were not described, and characterization of the product was limited to a comparison of its R_f with that of the product reported in reference vii. The reference for the Horecker paper provided by Woodruff and Wolfenden appears to be erroneous since we were unable to locate the original paper. Gupta et al. explored the oxidation of erythrose 4-phosphate by gold(III) salts, but the product of the reaction was neither purified nor characterized.¹⁰ Although direct oxidation of 4-erythrose 4-phosphate to 4PE is appealing, we chose not to pursue this route because the starting material, D-erythrose 4-phosphate, is available only as an impure preparation at a current cost of >\$6500 per gram. Thus, preparation of the gram quantities of 4PE needed for kinetic and structural studies would be prohibitively expensive.

An interesting alternative strategy for synthesis of 4PE employs selective phosphorylation of the 4-hydroxyl of a protected erythronic acid.¹¹ The methyl ester of erythronic acid was converted to methyl 2,3-*O*-dibenzoyl 4-*O*-trityl D-erythronic acid. After removal of the trityl group, the 4-hydroxyl was phosphorylated with diphenyl phosphorochloridate. After deprotection, 4PE was isolated by fractional crystallization as the bis(dicyclohexyl ammonium) salt in 19% yield from methyl 2,3-*O*-dibenzoyl 4-*O*-trityl D-erythronic acid. The product was characterized only by elemental analysis. We were able to reproduce the reported synthesis of methyl erythronate. However, purification of the product by silica gel chromatography was unsuccessful because methyl erythronate underwent spontaneous cyclization, regenerating erythrone. Preparation of methyl 2,3-*O*-dibenzoyl 4-*O*-trityl D-erythronate directly from the impure methyl erythronate was successful, but proceeded in less than 10% yield.

We sought a more robust method for synthesis of 4PE from an inexpensive precursor to supply the gram quantities required for kinetic and structural studies of enzymes such as PdxB, PdxR and Rpi. The strategy we report here requires five steps, most of which are accomplished with good to high yield, beginning from the inexpensive precursor D-erythrone (lactone) (\$30 per gram, TCI America).

Our synthetic strategy is shown in Scheme 2. The hydroxyl groups of commercially available D-erythrone (3) were protected using benzyl bromide in the presence of freshly prepared Ag_2O ¹² (as described by Marshall et al).¹³ The yellowish oil was purified on a silica column (ca. 50 g silica) using toluene:ethylacetate 8:2 mixture as the eluent. Compound 4 was obtained as a white semi-solid (712 mg, R_f = 0.35). Recrystallization from boiling heptanes gave white crystals (34% yield, >99% pure, melting point 87 – 89 °C). It should be noted that we did not attempt to optimize the yield of this step because the starting material is inexpensive. A variation of this procedure was reported to give a 91% yield, although in our hands the two procedures gave similar yields.¹⁴

The lactone ring of 4 was opened using LiOH (1.1 eq) in water at 4 °C for 48 h with stirring. The reaction mixture was neutralized with Dowex 50W-X12 (H^+ -form). After addition of 20

mL of methanol, the slurry was stirred for 20 min at 4 °C. The suspension was filtered and the filtrate containing **5** (ca. 95%, by ¹H NMR) was used for the next step without isolation.

Compound **5** was converted to the corresponding methyl ester by treatment with diazomethane. However, this compound underwent cyclization upon purification by silica gel chromatography. Furthermore, hydrolysis of the methyl ester after phosphitylation and oxidation of the phosphite (as shown in Scheme 2 for the corresponding benzyl ester) proved problematic due to extensive β-elimination. Consequently, we explored the possibility of generating the benzyl ester of **5** using phenyldiazomethane. Use of phenyldiazomethane for this purpose is uncommon, but has been reported.¹⁵ Formation of **6** using phenyldiazomethane generated as described by Dudman and Reeze¹⁶ proved successful. Gratifyingly, in contrast to the corresponding methyl ester, **6** was stable under the conditions required for silica gel chromatography and was purified as a clear oil (84% yield).

Compound **6** was converted to **7** (76% yield) by phosphitylation with dibenzylphosphoramidite followed by oxidation of the phosphite with *tert*-butyl peroxide in the same vessel. Removal of the benzyl protecting groups by hydrogenation over Pd/C did not proceed to completion in H₂O even after 24 h at 60 psi H₂. However, complete deprotection was achieved in methanol: H₂O (95:5) after 24 h using 20 – 60 psi H₂, yielding 4PE in 22% overall yield from D-erythrone (3).¹⁷

It is now possible to synthesize ample quantities of 4PE in excellent yield in 5 steps from an inexpensive precursor. Utilization of a benzyl ester as opposed to a methyl ester was the key to the success of this synthetic strategy. There are numerous examples where synthesis of carbohydrate derivatives could benefit from a strategy employing complete benzyl protection followed by hydrogenolysis. This strategy will be of particular use in the case of C-1 carboxylates given the tendency of methyl esters of such compounds to cyclize to the corresponding lactone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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17. ^1H NMR (500 MHz, D_2O , free acid) δ 3.75–3.87 (m, 2H), 3.94 (distorted dd, $J_1 = 9.4$ Hz, $J_2 = 4.6$ Hz, 1H), 4.20 (d, $J = 4.6$ Hz, 1H). ^{13}C APT NMR (100.6 MHz, CDCl_3) δ 65.7 (2, poorly resolved d), 71.0 (1), 71.5 (1, d, $J_{\text{C-P}} = 8.4$ Hz), 175.5(0). ^{31}P NMR (161.9 MHz, D_2O) δ 1.1. Predicted exact mass for $\text{C}_4\text{H}_8\text{O}_8\text{P}^-$ 214.995130; observed mass 214.996458.

Scheme 1.

Oxidation of 4PE by PdxB generates NADH at the active site, which must be re-oxidized by reaction with an α -keto acid such as pyruvate, oxaloacetate or α -ketoglutarate.

Scheme 2.

Conditions and yields: (a) i. Ag_2O (4 eq), BnBr (2.7 eq), ether, 12 h, 25 °C; ii. silica gel chromatography (toluene: ethyl acetate, 8:2, $R_f = 0.35$); iii. crystallization (boiling heptanes), 34%; (b) i. LiOH (1.1 eq), H_2O , 48 h, 4 °C; ii. neutralization with Dowex 50W-X12 (H^+ -form, 2.2 eq) in 50% MeOH; iii. removal of Dowex 50W-X12 by filtration; (c) i. slight excess benzyldiazomethane in CH_2Cl_2 ; ii. silica gel chromatography (pentane: ethyl acetate, 4:1, $R_f = 0.3$), clear oil, 84%; (d) i. dibenzylphosphoramidite (1.3 eq), CH_3CN , tetrazole (1.3 eq), 1 h, 4 °C; ii. tBuOOH (1.7 eq), 2 h, 4 °C; iii. silica gel chromatography (ethyl acetate: CH_2Cl_2 , 1: 9, $R_f = 0.40$), 76%; (e) H_2 , 60 psi, Pd/C , MeOH, 12 h, 4 °C, quantitative yield.