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AN IMPROVED SYNTHESIS OF DIHYDROXYACETONE PHOSPHATE

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ABSTRACT

An improved procedure has been developed for the synthesis of dihydroxyacetone phosphate (DHAP). Reaction of 2,5-diethoxy-p-dioxane-2,5-dimethanol (**1**) and the trivalent phosphorylating reagent dibenzyl-N,N-diethylphosphoramidite (DDP) in the presence of 1,2,4-triazole or tetrazole followed by oxidation with H_2O_2 gave 2,5-diethoxy-p-dioxane-2,5-dimethanol-O-2^1-O-5^1-bis(phosphate) tetrabenzyl ester (**2**) in 98% yield. Compound **2** was then hydrogenated in the presence of H_2-Pd/C to give, after neutralization, 2,5-diethoxy-p-dioxane-2,5-dimethanol-O-2^1-O-5^1-bis(phosphate) as a stable trisodium salt (**3**) in 84% yield. Treatment of **3** with Dowex 50 (H^+) generates 2 equivalents of DHAP.

INTRODUCTION

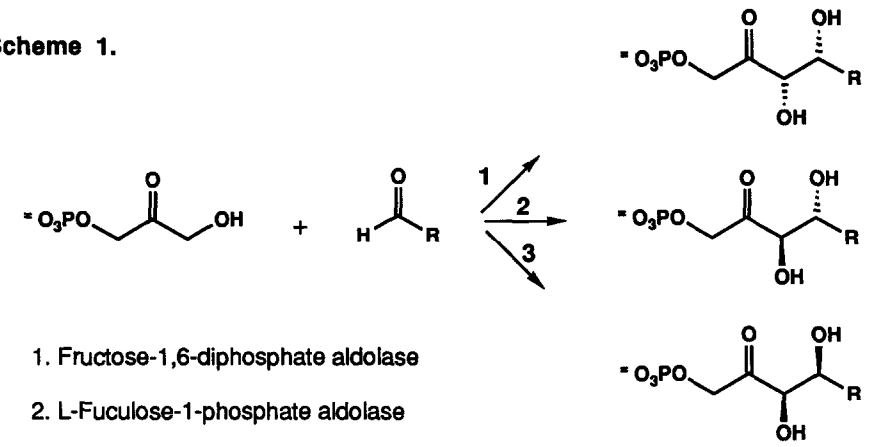
Enzymatic aldol reactions have demonstrated their synthetic usefulness in organic synthesis, particularly in the synthesis of common and uncommon sugars.[1-6] Three aldolases require dihydroxyacetone phosphate (DHAP) as the donor (Scheme 1).[4,7] Since DHAP is relatively unstable and not readily available, its availability will thus influence the practicality of DHAP-requiring aldolases as synthetic catalysts.

Three methods have been used for the synthesis of DHAP (1): by *in situ* generation from fructose-1,6-diphosphate (FDP) using FDP aldolase and triosephosphate isomerase,[2] (2) by enzymatic phosphorylation of dihydroxyacetone (DHA) using adenosine-5'-triphosphate (ATP) and glycerol kinase with *in situ* regeneration of ATP[8,9] (3) or by chemical phosphorylation of the protected DHA dimer[10] via $POCl_3$[5] or $(PhO)_2P(O)Cl$.[11] Alternatively, DHAP can be replaced with a mixture of dihydroxyacetone and a catalytic amount of arsenate.[12]

All of these strategies have certain advantages and disadvantages. The *in situ* generation of DHAP from FDP is the most common of the above methods; however, the overall equilibrium may not be favorable for the synthesis. Furthermore, if the reaction does not go to completion, the presence of FDP will complicate the isolation of the product. Enzymatic phosphorylation of DHAP from DHA is too expensive for large scale reactions, and the previously reported chemical synthesis of DHAP either required a tedious workup with toxic barium salts or the use of the expensive PtO_2 catalyst.

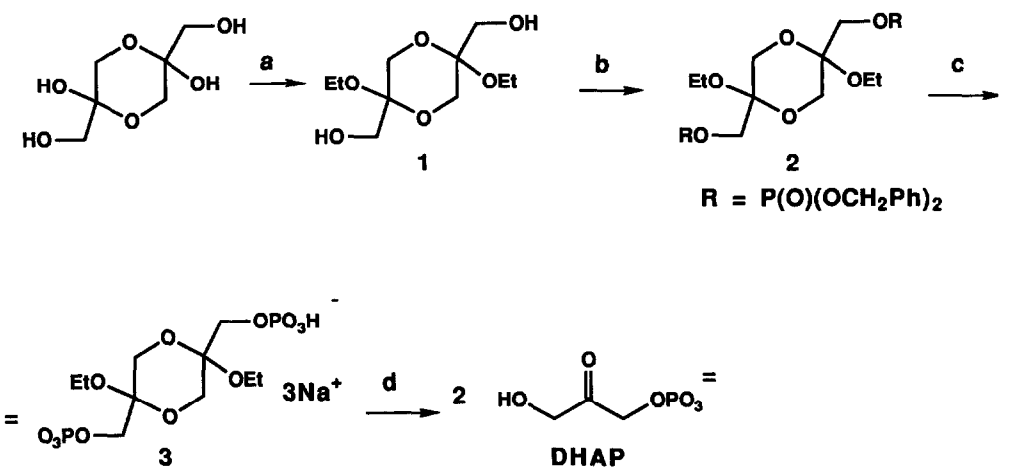
We report an improved synthesis using inexpensive starting materials. The overall yield of DHAP from DHA is 55% (Scheme 2) compared to 35% based on the method of Effenberger.[5] The

Scheme 1.



1. Fructose-1,6-diphosphate aldolase
2. L-Fuculose-1-phosphate aldolase
3. L-Rhamnulose-1-phosphate aldolase

Scheme 2.



a) EtOH , H_2SO_4 cat., $HC(OEt)_3$ (50-87% yield) b) i) $(PhCH_2O)_2PNEt_2$, Triazole, (4 eq.); ii) 30% Hydrogen Peroxide (98% yield) c) Pd/C H_2, EtOH (84% yield) d) H^+, H_2O, 65° C, 4 hr (74% yield).

trivalent phosphorylating reagent is prepared by a one pot reaction, in good yields and is stable for long periods of time. 1,2,4-Triazole or tetrazole was used to facilitate the phosphorylating reaction. Tetrazole may be used in a catalytic amount (10 molar percent); however, these reactions are 3-5 times slower compared to the corresponding reactions using stoichiometric amounts of tetrazole. The reaction proceeds in excellent yields, it doesn't require chromatography and this process is suitable for large scale reactions (Table 1). All the intermediates are crystalline and easy to handle. The product **3** obtained based on this method has high purity, is not hygroscopic and is stable at room temperature. It can be easily converted to DHAP by treatment with Dowex 50 (H^+).

EXPERIMENTAL

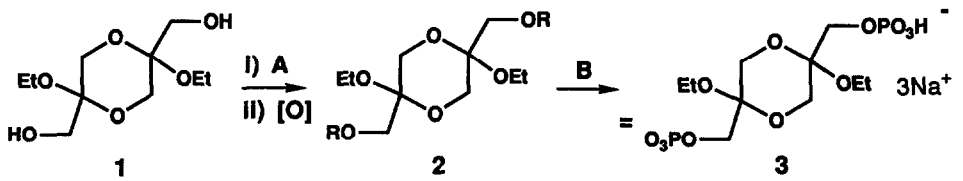
Reagents were purchased from Aldrich Chemical Co. or Fisher Scientific and were used without further purification. 1,2,4-Triazole and tetrazole were purchased from Fluka. The enzymes were purchased from Sigma. 200 MHz ^1H and 50 MHz ^{13}C NMR spectra were recorded on a Varian XL-200 instrument.

2,5-Diethoxy-p-Dioxane-2,5-Dimethanol (1). A modified procedure of Fischer and Mildbrandt[10] was used for the synthesis of 2,5-diethoxy-p-dioxane-2,5-dimethanol. To an oven dried 1-liter round-bottomed flask was added 400 mL of anhydrous ethanol, 1.84 g of conc. H_2SO_4 (18.8 mmol), and 31.12 g (210 mmol) of triethylorthoformate. This solution was refluxed for 30 min. under N_2. The solution was then cooled to 4° C and 1.41 g (7.8 mmol) of dry dihydroxyacetone dimer (DHA) was added every 12 h for 72 h. After the last addition, the solution was stirred for an additional 24 h at 4° C. Water (4 mL) was added and the mixture was stirred for 30 min. The pH of the solution was adjusted to 8.0 with 2 N NaOH and the excess ethanol was removed under reduced pressure. The white precipitate was triturated with 2 x 100 mL ether, the ether fractions were combined and extracted with 20 mL of satd. NaCl. The combined aqueous fraction was extracted with ether (3 x 40 mL) to recover more product. The combined ether fractions were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was recrystallized from ethyl acetate/heptane to produce 2.6 g of the title compound.

The white precipitate from the ether triturations was again triturated with 100 mL of ether until product could not be detected in the extract by TLC (about 500 mL) (4:1 EtOAc:Hex Rf 0.6 and 0.5). The ether fractions were combined, concentrated under reduced pressure, and the product was recrystallized from ethyl acetate/heptane to yield 7.1 g. Combining the two recrystallized precipitates yields 9.7 g (41.1 mmol, 87 %) **1**. Compound **1** was identical to reported values.[5,10,11]

Dibenzyl-N,N-Diethylphosphoramidite (DDP). To a dry 3-necked 5-liter round-bottomed flask equipped with an overhead stirrer was added 4.2 L of anhydrous ether and 137.33 g (1.0 mol) of phosphorus trichloride. The solution was cooled to 0° C, under dry N_2, 73.14 g (1.0 mol)

Table 1. Various Syntheses of 2,5-Diethoxy-p-Dioxane-2,5-Dimethanol-O-2[1], O-5[1]-bis (Phosphate) Trisodium Salt (**3**) as Shown in Scheme 1.



1 2 3

A	R	B	3 % yield
$(PhCH_2O)_2PNEt_2$/triazole[a]	$P(O)(OCH_2Ph)_2$	H_2 Pd/C	84%
$(PhCH_2O)_2PNEt_2$/tetrazole[a]	$P(O)(OCH_2Ph)_2$	H_2 Pd/C	85%
$(PhO)_2PNEt_2$[b]/PPTS[c]	$P(O)(OPh)_2$	H_2 PtO_2	80%
$(PhO)_2PCl$[d]	$P(O)(OPh)_2$	H_2 PtO_2	72%
$(PhO)_2P(O)Cl$[e]	$P(O)(OPh)_2$	H_2 PtO_2	92%
$(PhO)_2PNEt_2$/imidazole[f]	$P(O)(OPh)_2$	H_2 PtO_2	<10%
PCl_3	$P(O)O_3^{=}$	——	9%[g]
$POCl_3$	$P(O)O_3^{=}$	——	80%[g,h]

a) Used four equivalents of triazole or two equivalents of tetrazole for each equivalent of **1**. b) Prepared from $(PhO)_2PCl$ + 2 Et_2NH. c) PPTS (Pyridinium-p-Toluenesulfonate). d) Hewertson, W.; Smith, B. C.; Shaw, R. A. Inorganic Synthesis **1962**, 1, 68. e) Used four equivalents of $(PhO)_2P(O)Cl$ for each equivalent of **1** and oxidation wasn't required. See ref. 11. f) Used four equivalents of imidazole per equivalent of **1**, the reaction was very slow. g) Isolated as the Ba^{+2} salt. h) See ref. 5.

diethylamine and 101.19 g (1.0 mol) triethylamine were added dropwise. The mixture was stirred at room temp for 24 h (efficient stirring is required for good yields). The solution was again cooled to 0° C and 216.28 g (2.0 mol) of benzyl alcohol and 220.0 g (2.17 mol) of triethylamine were added dropwise. The solution was stirred at room temp for 36 h. The triethylamine hydrochloride salt was removed by filtration and the ether was removed under reduced pressure. The remaining residue was vacuum distilled, $Bpt_{0.3}$ 154-156° C, to yield 196.5 g (0.619 mol, 62%) DDP. This compound is stable and can be kept for several months without any decomposition or disproportionation. ^1H NMR (200 MHz, $CDCl_3$) δ 1.1 (2t, 6H, 2 CH_3, J 7.1 Hz), 3.1 (2q, 4H, 2 NCH_2, J 7.1 Hz), 4.75, 4.8 (2d, 4H, 2 $C\underline{H}_2Ph$), 7.3 (m, 10H, 2 Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.5 (CH_3), 38.7, 39.1 (NCH_2), 62.5, 62.9 ($\underline{C}H_2Ph$), 123.0, 123.2, 124.1 and 138.8 (aromatic). This compound was used for the phosphorylation of tyrosine, but no procedure was reported for the synthesis of DDP.[13,14]

2,5-Diethoxy-p-Dioxane-2,5-Dimethanol-O-2^1,O-5^1-bis(Phosphate) Tetrabenzyl Ester (2). To a 250 mL round-bottomed flask containing 100 mL of dry THF, 5.9 g (25.0 mmol) **1** and 6.9 g (100 mmol) 1,2,4-triazole were stirred under N_2. To the solution was added 33.33 g (105 mmol) DDP, and the solution was stirred at room temp for 24 h. The solution was cooled to -78° C in a dry ice/acetone bath and 17 mL of 30% hydrogen peroxide was added in a single portion. The solution was allowed to warm to room temp and stirred for 90 min. The THF was removed under reduced pressure, 300 mL of ether was added and the solution was extracted with 20 mL of 1 N Na_2SSO_3, 2 x 100 mL 1 N HCl, 100 mL satd. $NaHCO_3$, and 100 mL H_2O. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield 17.96 g (23.8 mmol, 98%) of **2** as a syrup. Compound **2** was recrystallized from ether and hexane. ^1H NMR (200 MHz, $CDCl_3$) δ 1.15, 1.20 (2t, 6H, J=7.1 Hz, eq/ax), 3.31-4.12 (m, 12H, 2 $CH_3C\underline{H}_2$, 4 CH_2O), 4.95, 5.05 (2s, 8H, 4 $PhC\underline{H}_2$), 7.31 (s, 20H, 4 Ph). ^{13}C NMR (50 MHz, $CDCl_3$) δ 15.32, 15.33, 56.57, 56.58, 63.46, 63.47, 65.46, 65.47, 65.57, 65.59, 69.42, 69.43, 69.49, 69. 53, 69. 54, 69.60, 93.65, 93. 69, 127.97, 128.61, 135.71. HRMS for $C_{38}H_{46}O_{12}P_2$ calculated 756.2465; observed 756.2486.

2,5-Diethoxy-p-Dioxane-2,5-Dimethanol-O-2^1,O-5^1-bis(Phosphate) Trisodium Salt (3). Compound **2** was dissolved into 200 mL ethanol, 5.0 g 10% Pd/C was added and the solution was hydrogenated under 50 psi of H_2 for 2 days. TLC indicated no starting material present. The mixture was filtered and the catalyst was rinsed with 200 mL ethanol. The ethanol solution was concentrated under reduced pressure to yield a syrup. Water (300 ml) was added, the pH was adjusted to 7.2 with 5 N NaOH and the solution was lyophilized to yield 10.98 g (20.9 mmol, 84%) **3** as a white powder. Compound **3** exists as a trihydrate. ^1H NMR (200 MHz, D_2O) δ 1.2, 1.25 (2t, 6H), 3.5-3.95 (m, 12H), 4.65 (s, 6H, 3 H_2O).

Dihydroxyacetone Phosphate (DHAP). To a 100 mL round-bottomed flask containing 1.0 g (1.9 mmol) and 50 mL of water was added Dowex 50 (H^+) resin (~ 20 mL) until the pH was 1.5. The mixture was heated at 65° C for 4 h. The resin was filtered and washed with water (2 x 20 mL). The aqueous fractions were combined and the pH was adjusted to 3.0 with 1 N NaOH. Enzymatic assay[15,16] for DHAP yielded 2.8 mmol (74%).

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