A Three-Step Preparation of Dihydroxyacetone Phosphate Dimethyl Acetal†

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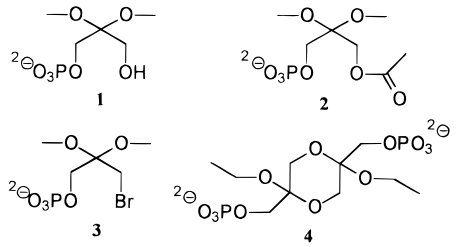
Introduction

Dihydroxyacetone phosphate (DHAP) is a biochemical that acts as a substrate for a number of enzymes, including triose phosphate isomerase,[1] α-glycerol phosphate dehydrogenase,[1] and several types of aldolases.[1] Because the aldolases can be used in the synthesis of a variety of carbohydrates on a preparative scale,[2] DHAP has taken on a new importance as a precursor molecule in organic synthesis. As a result, there is interest in developing new ways of producing this compound.

Because DHAP itself is unstable, there have been two general strategies for its synthesis: (1) enzymatic preparation of DHAP solutions for immediate use and (2) chemical synthesis of stable DHAP precursors (containing a protected ketone group) that may be stored indefinitely. The limitations of enzymatic preparations have been discussed elsewhere[3] and will not be addressed herein. Several stable DHAP precursors have been reported in the literature: dihydroxyacetone phosphate dimethyl acetal[4] (or diethyl acetal[4,5]) (**1**), 3-acetoxy hydoxyacetone phosphate dimethyl acetal[6] (**2**), 3-bromo hydroxyacetone phosphate dimethyl acetal[7] (**3**), and 2,5-diethoxy-*p*-dioxane-2,5-dimethanol O-2[1],O-5[1]-bis-phosphate (**4**).[8]



Compound **1** was synthesized by Ballou and Fischer[4] in 1956, the first time a stable precursor of DHAP was prepared. This synthesis was later modified by Weiling and E[5] who made the diethyl acetal. Because **1** is converted nearly quantitatively[4] to DHAP by acid hydrolysis, it is an excellent precursor for the production of this biochemical. However, the preparation of **1** is somewhat lengthy, requiring six steps from a commercially available material. Similarly, **2** is converted in >95% yield to DHAP by acid hydrolysis, but its synthesis requires six steps from acetone (or five steps from 1,3-dibromoacetone).[6] Compounds **3** and **4** are synthetically more attractive because they are made easily and efficiently in only three steps from 1,3-dibromoacetone[7] and the dihydroxyacetone dimer,[8] respectively. Effenberger and Straub[9] originally synthesized **4** from the dihydroxyacetone dimer in 1987. This preparation was later modified by Pederson et al.[3a] and then by Jung et al.,[8] significantly improving the overall yield. However, **4** has one significant drawback—it is converted to DHAP with only a 66% yield.[8] Similarly, **3** is converted in <73% yield[7] to DHAP. It must be noted, however, that these impure DHAP preparations made from **3** and **4** have been shown to be useful for at least some enzymatic syntheses.[7,8]

The ideal precursor would combine the benefits outlined above. It would be efficiently converted into DHAP (like **1** and **2**), and itself be synthesized easily in just a few steps from a cheap, commercially available compound (like **3** and **4**). Furthermore, the synthesis of the ideal precursor molecule would not involve the use of expensive reagents, such as Pt or Pd, metals that are used in the synthesis of all four of the precursors shown above. In this regard, we report herein the synthesis of **1** by a three-step method that is inexpensive, is easily carried out, and can be accomplished on a gram scale.

Results and Discussion

Dihyrdoxyacetone dimethyl acetal (**6**) (see Scheme 1) was made according to a slight modification of a previously published method,[10] the only difference being the manner of product isolation. Cesarotti et al.,[10] who reported a yield of 75.7%, purified **6** by column chromatography. In this study, distillation was used because it was cheaper and more convenient. Three fractions were obtained, with fraction 2 found to be compound **6**, isolated in 41% yield. Although the data are not presented herein, both NMR and TLC analyses of fractions 1 and 3 indicated the presence of **6**, though these fractions were impure. However, no attempt was made to isolate **6** from these fractions and thus improve the yield of the reaction.

The cyclic phosphate triester **7** was made according to the general procedure reported by Penny and Belleau[11]

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(2) For example: (a) Henderson, I.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 558. (b) Valentin, M.-L.; Bolte, J. *Tetrahedron Lett.* **1993**, *34*, 8103. (c) Wong, C.-H.; Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Dumas, D. P.; Liu, L.-C.; Ichikawa, Y.; Shen, G.-J. *Ind. J. Chem.* **1993**, *32B*, 135. (d) Fessner, W.-D.; Badia, J.; Eyrisch, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, *33*, 5231. (e) Schmid, W.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 9670. (f) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (g) Lehninger, A. L.; Sice, J. *J. Am. Chem. Soc.* **1955**, *77*, 5343.

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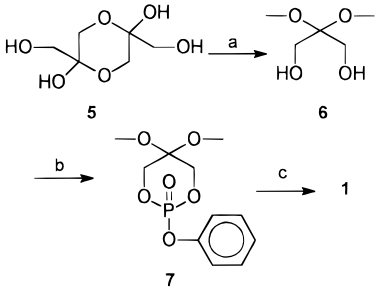
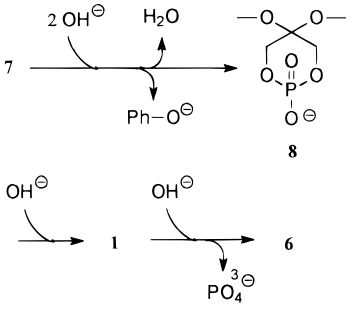
Scheme 1. Synthesis of Dihydroxyacetone Phosphate Dimethyl Acetal[a]



[a] (a) $HC(OMe)_3$, MeOH, cat. TsOH; (b) $PhOP(O)Cl_2$, C_6H_5N; (c) 0.1 M $Ba(OH)_2$, 96 °C.

Scheme 2



for the reaction of phenyl dichlorophosphate with diols. As evidenced by NMR spectra and the results of elemental analyses, **7** was found to be pure, and so it was not recrystallized. In their work, Penny and Belleau[11] prepared a number of cyclic phosphate triesters and obtained yields ranging from 47 to 78%. The 72% yield reported herein for the preparation of **7** falls within this range.

The hydrolysis of **7** was carried out at 96 ± 2 °C in 0.1 M barium hydroxide. When **7**, which is insoluble in water, was added to the hot barium hydroxide solution, a two-phase mixture resulted. Within 20 min, a homogeneous solution was observed as **7** was (presumably) converted to **8** (Scheme 2), an anion soluble in water. As evidenced by enzyme assays, **8** was hydrolyzed to **1** in 16 h, under the conditions of the reaction. The conversion of **7** to **1** (66% yield) took advantage of the very large difference in the rate of basic hydrolysis of phosphate di- and monoesters. It is known that phosphate diester monoanions react with hydroxide rapidly as compared with the rate of reaction of hydroxide with phosphate monoester dianions.[12] Thus, the cleavage of **1** to **6** (Scheme **2**) was slow enough to allow **1** to be isolated from the reaction solution.

The three-step synthesis of **1** presented herein is straightforward and relatively inexpensive. The overall yield of 20% was disappointing, but since the reactions can be carried out on a multigram scale, it is a convenient method for the preparation of an excellent precursor of dihydroxyacetone phosphate.

Experimental Section

General. All reagents and solvents (including anhydrous solvents) were purchased from either the Sigma or Aldrich Chemical Co. and were used without further purification. Dicyclohexylammonium sulfate was synthesized according to the procedure of Ballou and Fischer.[4] NMR spectra were recorded on a 300 MHz instrument.[13] Chemical shifts were determined relative to TMS in $CDCl_3$ or to HOD (4.8 ppm) in D_2O. A calibrated thermometer was used for measuring melting points, but no stem correction was made.

2,2-Dimethoxy-1,3-propanediol (6). This compound was synthesized by a slight modification of the method of Cesarotti et al.[10] Dihydroxyacetone dimer (**5**) (75 g, 0.42 mol) was stirred overnight in a solution containing 90 mL of trimethyl orthoformate, 1000 mL of anhydrous methanol, and 300 mg of *p*-toluene sulfonic acid monohydrate. The reaction was halted by adding 900 mg of anhydrous sodium carbonate and by stirring the mixture overnight, during which time most of the salt dissolved. The solvent plus unreacted trimethylorthoformate was removed by evaporation under reduced pressure, and the resulting oil was distilled in vacuo at 2 Torr. Three fractions (fraction 1, bp 105−115 °C; fraction 2, bp 115−125 °C; fraction 3, bp. 125−130 °C), which solidified upon cooling, were obtained and recrystallized from toluene. Recrystallized fraction 2, 46.7 g (0.34 mol, 41%), was found to be pure by elemental analysis.

Anal. Calcd. for $C_5H_{12}O_4$: C, 44.11; H, 8.88. Found: C, 44.05; H, 9.08.

Compound **6** was very hygroscopic and was stored at room temperature in a desiccator protected from moisture.

5,5-Dimethoxy-2-phenoxy-2-oxo-1,3,2-dioxaphosphorinane (7). Compound **6** (15.0 g) was dissolved in 65 mL of anhydrous pyridine in a two-neck 100 mL round bottom flask. The solution was cooled in an ice/salt bath, and 15 mL of phenyl dichlorophosphate was added dropwise stirring over the course of 1 h, keeping the temperature of the reaction mixture below 10 °C. The mixture was stirred for an additional 20 h at room temperature. Solid pyridinium hydrochloride was removed by filtration of the mixture on a Buchner funnel, the solids were washed with benzene, and the combined filtrate plus washes were evaporated to dryness under reduced pressure (T < 40 °C). The residue was triturated with water, and the resulting solid was isolated by centrifugation at 9000*g* for 10 min. The solid was washed consecutively with 50 mL each of water, saturated sodium bicarbonate, and water. The crude product was dissolved in 300 mL of warm benzene after drying the solid overnight on a watch glass (or in a vacuum desiccator for several hours). The resulting cloudy mixture was stirred while heating gently for 20 min, cooled for 10 min, and then dried by the addition of anhydrous sodium sulfate. Filtration of the mixture followed by evaporation of the solvent under reduced pressure yielded 21.9 g (72%) of a white, fluffy solid: mp 100−101 °C; ^{1}H NMR ($CDCl_3$) δ 3.27, (s, 3 H), 3.36, (s, 3 H), 4.25−4.43 (m, 4 H), 7.16−7.4 (m, 4.6 H). Anal. Calcd for $C_{11}H_{15}O_6P$: C, 48.2; H, 5.51; P, 11.3. Found: C, 48.2; H, 5.63; P, 11.4.

Compound **7** was stored at −18 °C, but it could be kept at room temperature for several months without significant decomposition as evidenced by NMR.

2,2-Dimethoxy-1,3-propanediol Phosphate (1). Compound **7** (2.5 g) was added in one portion to 250 mL of a stirred 0.10 M solution of barium hydroxide that had been preheated to 94−98 °C in an oil bath. The compound melted, and the two phases that resulted disappeared within 20 min as the phenyl ester was hydrolyzed. The solution, protected from the atmosphere by the use of a soda lime tube, was heated with stirring for 16 h (the extent of the reaction was determined enzymatically as described below), at which time the reaction mixture was removed from the oil bath and allowed to cool for 1.5 h. A solution of 8.1 g of dicyclohexylammonium sulfate (27.3 mmol, a 10% excess) in 50 mL of water was added, and the resulting mixture stirred for 15 min. The barium sulfate precipitate was removed by centrifugation at 10000*g* for 10 min and washed three times with 50 mL of water. The combined supernatant and washings were evaporated to dryness under reduced pressure (T < 40 °C). The residue remaining was dissolved in boiling hot absolute ethanol, and the mixture was vacuum filtered on a Buchner funnel using filter paper in order to remove the excess dicyclohexylammonium sulfate and any salts of inorganic phosphate that may have been present. If the filtrate was still cloudy, then it was passed

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(13) The authors wish to thank Dr. Karol Bruzik, Dr. David Rausch, and William Lambert for obtaining NMR spectra.

through a 0.45 μm nylon filter. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in about 30 mL of water. The mixture was filtered through a 0.45 μm nylon filter, and the filtrate was evaporated to dryness under reduced pressure. The solid obtained was dissolved in 8 mL of water, the pH of the solution was adjusted to 10.1 with cyclohexylamine, and 35 mL of acetone was added. The mixture was allowed to stand overnight at 4 °C, and the white solid, which crystallized, was collected by filtration on a Buchner funnel, containing a medium porosity glass frit. The solid was washed with several portions of acetone and allowed to air-dry on the funnel for several hours. Yield = 1.74 g. For NMR analysis, 30−50 mg of the solid was dissolved in 1 mL of water, and the solution was stirred with about 0.5 g of Dowex-50(H^+) for 1 min to remove the cyclohexylammonium groups. After filtration to remove the Dowex polymer, solid $NaHCO_3$ was added in portions until the pH was about 7. The solution was freeze-dried, and the resulting solid was dissolved in D_2O. ^1H NMR (D_2O) δ 3.28 (s, 6 H), 3.63 (s, 2 H), 3.76 (d, J = 5.4 Hz, 2.1 H). Anal. Calcd for the dicyclohexylammonium salt $C_{17}H_{39}N_2O_7P\cdot H_2O$: C, 47.2; H, 9.56; N, 6.48. Found: C, 47.4; H, 9.64; N, 6.53.

The filtrate and washes from above were combined and placed overnight at 4 °C in order to obtain a second crop. Yield = 0.87 g. The dicyclohexylammonium salt was converted into the sodium salt for NMR analysis as described above. ^1H NMR (D_2O) δ 3.28 (s, 6 H), 3.63 (s, 2 H), 3.76 (d, J = 5.5 Hz, 2 H). Anal. Calcd for the dicyclohexylammonium salt $C_{17}H_{39}N_2O_7P\cdot H_2O$: C, 47.2; H, 9.56; N, 6.48. Found: C, 46.9; H, 9.56; N, 6.48. The combined yield for the two crops was 2.61 g (66%).

The rate of ring opening of **7** to produce **1** was monitored enzymatically. A 0.5 mL aliquot of the barium hydroxide hydrolysis reaction solution was removed at various times and mixed with 0.5 g of Dowex-50(H^+) for 60 s. The resulting mixture was filtered, and the Dowex was washed twice with 0.5 mL of water. The combined filtrate plus washings (pH ≅ 2) was incubated at 65 °C for 25 min to hydrolyze the dimethyl acetal of dihydroxyacetone phosphate. This solution was transferred quantitatively to a 10.00 mL volumetric flask and diluted to the mark with a 0.05 M Tris buffer, pH 7.5. An aliquot of this buffered solution was then assayed enzymatically for dihydroxyacetone phosphate.[14] Note that although this method was adequate to measure the extent of reaction, it appeared that the hydrolysis of the acetal at 65 °C for 25 min was sensitive to pH. Preliminary observations seemed to indicate that if the pH of the combined filtrate plus washings was above 2, then incomplete hydrolysis of the acetal occurred. When the pH was lower, then quantitative hydrolysis seemed to occur. More work must be done to produce a reproducible acetal hydrolysis procedure at 65 °C.

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