An Efficient Multigram-Scale Preparation of Dihydroxyacetone Phosphate†

Sang-Hun Jung,‡ Jin-Hyun Jeong,‡ Paula Miller, and Chi-Huey Wong*,‡

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, and Monsanto Agricultural Company, 800 N. Lindbergh Boulevard, St. Louis, Missouri 63167

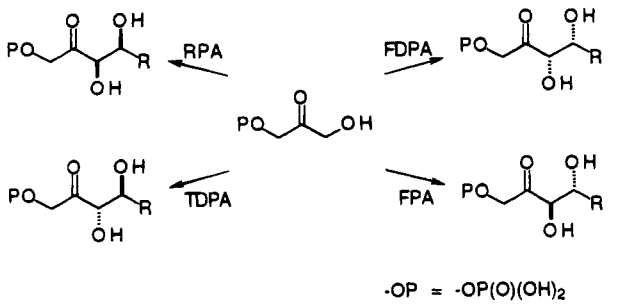
Received June 16, 1994

Introduction

Enzyme-catalyzed aldol addition reactions have proven synthetically useful.[1] Of the more than 20 aldolases known, the enzymes D-fructose-1,6-diphosphate aldolase (EC 4.1.2.13, EDPA), L-fuculose-1-phosphate aldolase (EC 4.1.2.17, FPA), L-rhamnulose-1-phosphate aldolase (EC 4.1.2.19, RPA), and D-tagatose-1,6-diphosphate aldolase (EC 4.1.2.-, TDPA) are of particular interest because of their ability to generate four possible stereoisomers at the two formed stereogenic centers[1b,2] (Scheme 1). These four enzymes require dihydroxyacetone phosphate (DHAP) as donor while accepting a broad spectrum of aldehyde substrates.[1j,3] Commercially available DHAP is, however, expensive and unstable, and therefore improvement of its synthesis and manipulation has been a subject of interest.

Currently, there are five different methods for the preparation of DHAP: (1) generation of DHAP *in situ* from fructose-1,6-diphosphate using FDPA and triosephosphate isomerase,[3] (2) enzymatic phosphorylation of dihydroxyacetone (DHA) using adenosine-5′-triphosphate (ATP) and glycerol kinase with regeneration of ATP,[3-5] (3) generation of DHAP *in situ* from L-glycerol-3-phosphate using flavine-dependent glycerol phosphate oxidase (GPO),[6] (4) chemical phosphorylation of protected DHA dimer using phosphorus oxychloride,[7] diphenyl phosphorochloridate (DPPC),[8] or dibenzyl *N,N*-diethylphosphoramidite (DDP) followed by oxidation,[9] (5) phosphorylation of protected dihydroxyacetone with DPPC.[10] Generation of DHAP *in situ* from FDP is convenient, but the presence of FDP complicates product isolation.[1b,j] Moreover, the overall equilibrium may be unfavorable for the synthesis. The enzymatic phosphorylation of DHAP may be too expensive for a large-scale process, though DHAP can be generated *in situ* and the reaction can be coupled with other enzymatic reactions, especially with those requiring ATP regeneration. The generation of DHAP *in situ* from glycerophosphate oxidase-catalyzed oxidation of L-glycerol-3-phosphate coupled with catalase (EC 1.11.1.6) could be generally applicable. The oxidase, however, only accepts the L substrate, and this strategy suffers from the high cost of L-glycerol-3-phosphate and the formation of oxidandt (H_2O_2) during the reaction. On the basis of our experience, we prefer to prepare DHAP separately as the enzymatic aldol reaction can be performed with fewer reactants and the products are easier to isolate. The known chemical procedures for the preparation of DHAP are, however, low yielding and complicated by multistep purification procedures and in certain cases involve formation of unstable intermediates. We report here a practical procedure suitable for a multigram-scale synthesis of DHAP from DHA dimer (Scheme 2).

Scheme 1.[a] Enzymatic Aldol Reactions



[a] RPA: L-rhamnulose-1-phosphate aldolase; FDPA: D-fructose-1,6-diphosphate aldolase; TDPA: D-tagatose-1,6-diphosphate aldolase; FPA: L-fuculose-1-phosphate aldolase.

Results and Discussion

The hydroxyl group at the acetal position of dihydroxyacetone dimer **1** was protected with triethyl orthoformate in ethanol[11] as described previously.[9] In order to have a clean reaction, DHA is added slowly[12] so that the concentration of DHA will not exceed 0.5 M. As aqueous workup and recrystallization[7-9] gave a low yield of **2**, which is sensitive to acid and heat, dry workup conditions were applied, and residual sulfuric acid was removed by addition of anhydrous sodium bicarbonate followed by filtration. The filtrate was concentrated to a syrup under reduced pressure and trituated with hexane to produce a white solid which was filtered and dried in vacuum desiccator at room temperature. This product was highly

† Supported by the NIH (GM44154).

‡ Scripps Research Institute.

§ Visiting scientist from Chung-Nam National University of Korea.

|| Monsato Agricultural Co.

(1) (a) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F., III; Gautheron, C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499 references therein. (b) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. references therein. (c) Takaoka, Y.; Kajimoto, T.; Wong, C.-H. *J. Org. Chem.* **1993**, *58*, 4809. (d) Lin, C. H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (e) Kajimoto, T.; Liu, K. L.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187. (f) Narvor, C. G.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 7816. (g) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280. (h) Turner, N. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 624. (i) Pederson, R. L.; Kim, M.-J.; Wong, C.-H. *Tetrahedron Lett.* **1988**, *29*, 4645. (j) Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 627.

(2) Henderson, I.; Sharpless, K. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 558.

(3) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199.

(4) Simon, E. S.; Grabowski, S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 8920.

(5) Crans, D. C.; Whitesides, G. M. *J. Am. Chem. Soc.* **1985**, *107*, 7019.

(6) Fessner, W.-D.; Sinerius, G. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 209.

(7) Effenberger, F.; Straub, A. *Tetrahedron Lett.* **1987**, *28*, 1641.

(8) Colbran, R. L.; Jones, J. K. N.; Matheson, N. K.; Rozema, I. *Carbohydr. Res.* **1967**, *4*, 355. For the phosphorylation, 3.5 equiv of DPPC were used and the purification of product **4** required recrystallization.

(9) Pederson, R. L.; Esker, J.; Wong, C.-H. *Tetrahedron Lett.* **1991**, *47*, 2643.

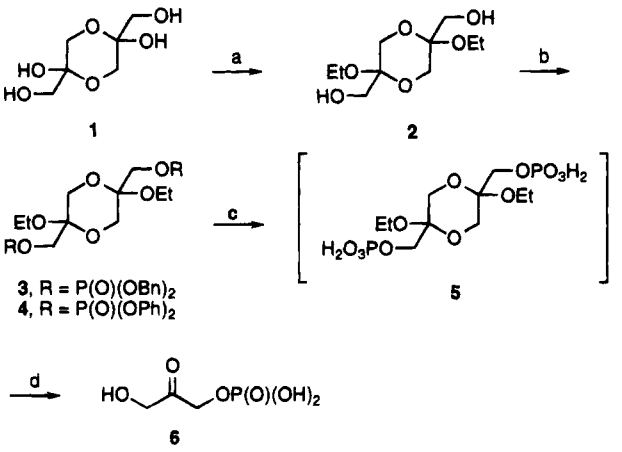
(10) Ballou, C. E.; Fisher, H. O. L. *J. Am. Chem. Soc.* **1956**, *78*, 1659.

(11) When 98% ethanol is used, the amount of triethyl orthoformate should be increased as much as the equivalents of water calculated.

(12) Addition of DHA in one time gave the product in more than 80% yield and higher than 90% purity. If the initial DHA concentration is lower than 0.5 M, the reaction gave the product with higher purity.

0022-3263/94/1959-7182$04.50/0 © 1994 American Chemical Society

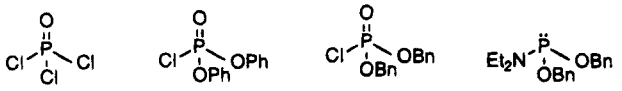
Scheme 2. Synthesis of Dihydroxyacetone Phosphate[a]



[a] (a) $HC(OEt)_3$, cat. H_2SO_4, EtOH (97%); (b) for **3**, dibenzyl *N,N*-diethylphosphoramidite, 1,2,4-triazole followed by H_2O_2 or dibenzyl phosphorochloridate, Pyr; for **4**, $ClP(O)(OPh)_2$, Pyr (96%); (c) H_2, 50 psi, Pd/C (4 h from **3**, 84%) of PtO_2 (24 h from **4**); (d) H_2O, 65 °C (66% from **4**).

pure based on TLC and NMR analyses and used without further purification.

The phosphorylation of **2** can be accomplished by reaction with $POCl_3$, with DDP followed by oxidation, with dibenzyl phosphorochloridate (DBPC),[13] or with DPPC; however, the reaction is often incomplete when a



stoichiometric amount of the phosphorylation reagent is used. An excess amount of the phosphorylating agent, therefore, was used in all cases,[7-9,14] which led to contamination of the phosphorylation reagent. A tedious purification procedure is therefore required for the method using $POCl_3$,[7] and recrystallization of **3** is inevitable in the method using DDP[9] or DBPC.[14] This recrystallization usually results in a significant loss of product **3** due to its relative instability to heat and acid. In addition, the phosphorylation with DDP requires 1,2,4-triazole, and DBPC is relatively expensive. Despite the easy removal by hydrogenolysis of the benzyl groups of **3**, the preparation of DHAP on large scales using DDP or DBPC as phosphorylation reagent was often inefficient. The phosphorylation of **2** with a stoichiometric amount of DPPC was therefore examined in the presence of different solvents and bases under various conditions: pyridine, pyridine (2.5 equiv) in ethyl acetate or dichloromethane, triethylamine, or triethylamine (2.5 equiv) in ethyl acetate or dichloromethane from 0 °C to room temperature. Using pyridine in different organic solvents, the reaction was not complete even after two days at room temperature.[15] The reactions in triethylamine or triethylamine in different solvents were incomplete and gave a mixture of products. The phosphorylation of **2** was, however, successfully accomplished with a stoichiometric amount of DPPC in pyridine, which eliminates contamination of the product so that no purification procedure was needed after simple workup. The NMR spectrum and TLC of **4** indicated only a mixture of *cis* and *trans* isomers (approximately 1:1 ratio). These isomers, although separable by silica gel column chromatography, were used directly in the next step.

Removal of the phenyl group from compound **4** was accomplished in 24 h at room temperature by hydrogenolysis (50 psi H_2) using platinum oxide (5% by weight) in ethanol. To ensure completion of the reaction, a small aliquot was withdrawn, evaporated, and examined by NMR. If the reaction is incomplete, the catalyst was removed by filtration and another new catalyst added for further hydrogenolysis. After completion of the reaction, the catalyst was removed by filtration and the solvent evaporated under reduced pressure. At this stage, intermediate **5** can be isolated as trisodium salt according to the procedure described previously.[9] To simplify the procedure, however, we dissolved it in distilled water[16] (1 g of phosphate **4** in 3 mL) and the solution was heated to 55–65 °C for 5–7 h. After hydrolysis, the solution was cooled to room temperature and adjusted to pH 3.7 and the concentration of DHAP was determined by enzymatic assay (65.5% yield).[17] The resulting DHAP was frozen and found to be stable for up to 5 months as determined by enzymatic assay.

This procedure for the preparation of DHAP is marked with high overall yield (61%) and easy operation without the need for further purification during the synthesis. DHAP prepared from this procedure has been successfully applide to the synthesis of sugar derivatives in our laboratory.

Experimental Section

Glycerol-3-phosphate dehydrogenase and NADH for enzyme assay were purchased from Sigma. Thin layer chromatography was performed on Silica gel GF_{254} plates from Merck and visualized with cerium sulfate–ammonium molybdate stain.[1j] NMR spectra were recorded in the presence of TMS (in $CDCl_3$) or acetone (in D_2O) as internal standard.

2,5-Diethoxy-*p*-dioxane-2,5-dimethanol (2). To an oven-dry 1 L round-bottom flask were added anhydrous ethanol (500 mL), concentrated sulfuric acid (3 mL, 56 mmol), and triethyl orthoformate (105 mL, 631 mmol). This solution was refluxed for 30 min under nitrogen. The solution was then cooled to 0–4 °C and dihydroxyacetone dimer (4.23 g, 23.5 mmol) was added every 12 h over 3 days (total 25.4 g, 141 mmol).[12] After the final addition of dihydroxyacetone dimer, the solution was stirred for an additional 1 day and anhydrous sodium bicarbonate (19.0 g, 226 mmol) was added. After stirring for 30 min at 0–4 °C, the reaction mixture was warmed to room temperature and filtered through a 5 cm pad of an equal mixture of Celite and silica gel (230–400 mesh), and then the filter cake was washed with ethyl acetate[18] (100 mL). The filtrate was concentrated. The residue was mixed with ethyl acetate (100 mL) and concentrated. Hexane (400 mL) was then added and the white solid formed was filtered, washed with hexane (100 mL), and dried in vacuum desiccator to afford **3** (32.3 g, 97.0%) as a white solid, which was identical to an authentic sample by NMR and TLC.[9]

For further characterization, compound **2** (0.5 g, 2.12 mmol) was stirred with acetic anhydride (1.7 g, 16.67 mmol) in pyridine

(13) Kenner, G. W.; Todd, A. R.; Weymouth, F. J. *J. Chem. Soc.* **1952**, 3675.

(14) Three equivalents of DBPC are needed for the phosphorylation of **2** to complete the reaction, presumably due to the lower reactivity of DBPC compared to DPPC.

(15) The phosphorylation reactions can be completed using 3 equiv of DPPC and 4 equiv of base, but the product was always impure after workup.

(16) Less than 50 mL of water should be used for the hydrolysis of **5** obtained from 10 g of **4**. Further dilution may prolong the hydrolysis and thus result in a lower yield of DHAP.

(17) Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 3rd ed.; Verlag Chemie: Deerfield, FL, 1984; Vol. 2, pp 146–7.

(18) Ethyl acetate should be dried over anhydrous sodium sulfate before use.

(10 mL) for 3 h at room temperature and then diluted with ethyl acetate (50 mL). The resulting solution was washed with water, cold aqueous hydrochloride, and saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and evaporated under vacuum. TLC (ethyl acetate: hexane = 1:1) and the NMR spectrum of the residue indicated a mixture of *cis* and *trans* isomers which were separated by column chromatography (R_f 0.43, 208 mg, R_f 0.59, 256 mg; overall yield 68.4%) and recovered as white solid. NMR ($CDCl_3$) for the *trans* isomer (R_f = 0.43) δ 1.24 (t, J = 7.1 Hz, 6H), 2.09 (s, 6H), 3.58 (m, 4H), 3.60 (d, J = 11.6 Hz, 2H), 3.82 (d, J = 11.6 Hz, 2H), 4.00 (d, J = 12.1 Hz, 2H), 4.25 (d, J = 12.1 Hz, 2H); HRMS calcd for $C_{14}H_{24}O_8$ + Na 343.1369, found 343.1359. NMR ($CDCl_3$) for the *cis* isomer (R_f = 0.59) δ 1.17 (t, 7.0 Hz, 6H), 2.09 (s, 6H), 3.47 (q, J = 7.0 Hz, 1H), 3.49 (q, J = 7.0 Hz, 1H), 3.59 (dd, J = 1.8, 12.4 Hz, 2H), 3.67 (q, J = 7.1 Hz, 1H), 3.69 (q, J = 7.1 Hz, 1H), 3.74 (d, J = 12.4 Hz, 2H), 4.00 (d, J = 12.1 Hz, 2H), 4.28 (d, J = 1.9, 12.1, Hz, 2H); HRMS calcd for $C_{14}H_{24}O_8$ + Na 343.1369, found 343.1360.

2,5-Diethoxy-*p*-dioxane-2,5-dimethanol *O*-2^1,*O*-5^1-Bis-(diphenyl phosphate) (4). Compound **2** (10 g, 42.4 mmol) obtained from the previous step was dissolved in anhydrous pyridine (45 mL). The resulting solution was cooled to 0–5 °C on an ice bath and diphenyl phosphorochloridate (22.8 g, 84.8 mmol) was added dropwise over 1 h. After stirring for additional 10 min, the reaction mixture was poured into ethyl ether (250 mL) and washed sucessively with water (200 mL), cold 1 N aqueous hydrochloric acid (200 mL), water, saturated sodium bicarbonate aqueous solution, and water. After being dried with anhydrous sodium sulfate, the ether layer was evaporated under reduced pressure and the residue was dried under high vacuum. NMR spectrum and TLC of the residue showed only product **4** (28.3 g, 95.6% yield), which existed as a mixture of *trans* and *cis* isomers with R_f 0.47 and 0.53 (SiO_2, hexane:ethyl acetate = 1:1), respectively. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.36–7.32 (m, 8H), 7.23–7.18 (m, 12H), 4.32 (d, J = 11 Hz, 1H), 4.27 (dd, J = 11.2 Hz, 1H), 4.09 and 4.06 (dd, J = 11.6 Hz, 2H), 3.75 and 3.59 (J_{AB} = 12 Hz, 2H), 3.73 and 3.52 (J_{AB} = 12 Hz, 2H), 3.64–3.47 (J_{AB} = 9, 7 Hz, 4H), 1.17 and 1.12 (t, J = 7 Hz, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 150.4, 129.8, 125.5, 120.0, 97.0, 93.6, 66.5, 64.7, 63.5, 61.2, 58.2, 15.2; m.p. 73–77 °C (lit.[8] 78 °C); IR (neat) 3069, 2978, 2930, 2896, 1590, 1489, 1456, 1289, 1189, 1164, 1054; HRMS calcd for $C_{34}H_{38}O_{12}P_2$, 723.1736 (M + Na$^+$), found 723.1749; MS (*m/e*, M + Na$^+$), 723 (100, 719 (6), 702 (6), 701 (11).

Dihydroxyacetone Phosphate (6). Compound **4** (22.4 g, 32.0 mmol) was dissolved in ethanol (150 mL), and platinum oxide (1 g) was added. The reaction mixture was shaken under hydrogen gas (50 psi) for 1 day at room temperature. During the initial 3 h, hydrogen uptake was very fast as indicated by the pressure drop. To ensure completion of the hydrogenolysis, a small aliquot was withdrawn and evaporated, and the NMR spectrum examined in D_2O until the signals of the aromatic shifts at δ 7.23 and 7.32 from **4** disappeared. After removal of the catalyst by filtration through Celite, the solvent was evaporated under reduced pressure below 35 °C. The residual solvent was further removed under high vacuum for 2 h at room temperature. The residue was dissolved in distilled water (70 mL)[16] and the resulting solution (pH 0.8) was heated at 65 °C for 5 h. After cooling to room temperature, the mixture was adjusted with 3 N NaOH and water to pH 3.7 and to a total volume of 100 mL. This final solution contained 42 mmol of DHAP based on enzyme assay[17] (65.6%), with an overall yield of 60.9% based on dihydroxyacetone dimer. ^{1}H-NMR (500 MHz, D_2O) δ 4.46 (s), 4.44 (s), 4.38 (s), 4.27 (s), 3.71 (s), 3.70 (s), 3.42 (s), 2.13 (s), 1.22 (s). This solution was then frozen and stored for over 5 months without loss of DHAP as determined by enzymatic assay.

Additions and Corrections

Vol. 58, 1993

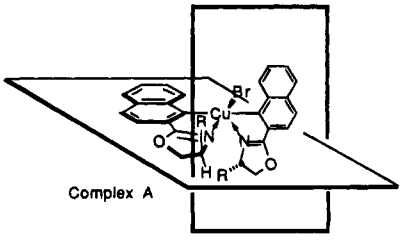
Mikael Bergdahl, Magnus Eriksson, Martin Nilsson,* and Thomas Olsson*. Iodotrimethylsilane-Promoted Additions of Monoorganocopper Compounds to α,β-Unsaturated Ketones, Esters, and Lactones .

Page 7242, column 1, line 53, should read "of copper(I) iodide (6.5–7.0 mmol, 1.3–1.4 equiv) in dry solvent (15 mL) at −78".

Vol. 59, 1994

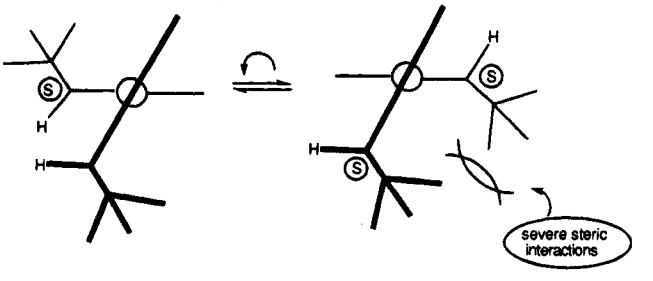
Todd D. Nelson and A. I. Meyers*. The Asymmetric Ullmann Reaction. 2. The Synthesis of Enantiomerically Pure C_2-Symmetric Binaphthyls.

Page 2657. Complex A should be drawn as follows:



Complex A

A more simplified view of the two complexes (A, B) may be drawn as follows:



Elimelech Rochlin and Zvi Rappoport*. Mapping the Enantiomerization Routes of Triarylvinyl Propellers. Barriers for the Three-Ring Flip and the Three Different Two-Ring Flips of *m*-Methoxy-Substituted Trimesitylvinyl Isopropyl Ethers.

Page 3864, Figure 6. Drawings G and I should be interchanged.