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Improved Straightforward Chemical Synthesis of Dihydroxyacetone Phosphate through Enzymatic Desymmetrization of 2,2-Dimethoxypropane-1,3-diol

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Received July 28, 2004

Abstract: Dihydroxyacetone phosphate (DHAP) was synthesized in high purity and yield in four steps starting from dihydroxyacetone dimer (DHA) (47% overall yield). DHA was converted into 2,2-dimethoxypropane-1,3-diol, which was desymmetrized by acetylation with lipase AK. The alcohol function was phosphorylated to give dibenzyl phosphate ester **4**. From **4**, two routes were investigated for large-scale synthesis of DHAP. First, acetate hydrolysis was performed prior to hydrogenolysis of the phosphate protective groups. The acetal hydrolysis was finally catalyzed by the phosphate group itself. Second, acetate and acetal hydrolysis were performed in one single step after hydrogenolysis.

Four dihydroxyacetone phosphate-dependent aldolases are known to catalyze the condensation of a variety of aldehydes with dihydroxyacetone phosphate (DHAP) to give monosaccharides and other chiral compounds of related structures.[1] These DHAP aldolases have shown complementary diastereoselectivity for the two stereocenters connected by the newly formed C−C bond, and three of them are useful catalysts for applications in organic synthesis. Of these, the glycolytic enzyme fructose-1,6-bisphosphate aldolase has been the most widely used over the last 20 years.[2] DHAP is the essential donor substrate, and although commercially available it is too expensive for large-scale synthesis. Large-scale DHAP production requires efficient and reliable syntheses. This is still a matter of research, as attested by the many enzymatic[3] or synthesis procedures[4] recently described. The main drawbacks to enzymatic reactions are often related to the high cost of enzymes, specific equipment,[3f] and difficult isolation of the final desired compound from excess DHAP precursors such as glycerol.[3e] Moreover, substrate inhibition phenomena often limit the final DHAP concentration to 100 mM.[3f] In contrast, chemical syntheses yield stable precursors (Figure 1) that can be stored in large quantities and converted into DHAP just before use in enzymatic aldol reactions.

Compound **1** is the most widely used and is readily prepared from dihydroxyacetone dimer (DHA).[4e] Compound **2** has been obtained from commercially available 1,3-dibromoacetone in a few steps.[4c] A three-step preparation of a cyclic phosphate **3** as an alternative DHAP precursor has recently been described.[4b] However, from **1**, **2**, and **3**, DHAP is formed in moderate yield with several impurities, mainly inorganic phosphate. Moreover, these syntheses are not highly reproducible. The dimethyl acetal precursor **4** used by Ballou et al.[5] and Valentin et al.[4d] appears the most suited for achieving the last steps in high yield. However, its preparation from dibromoacetone[4d] or 3-chloropropane-1,2-diol[5] involves too many steps for a large-scale application.

The present study describes a fundamental improvement in DHAP chemical synthesis via **4** in terms of time, yield, purity and reproducibility. The route for preparing DHAP is depicted in Scheme 1.

We report a three-step synthesis of compound **4** from DHA with only one short purification step and its one-pot conversion into DHAP on large scale.

Results and Discussion. Dihydroxyacetone dimethyl acetal **6** was prepared according to a slight modification of the previously described method.[4b,6] To use the crude diol **6** in the next step, neutralization and removal of TsOH was achieved with the basic Amberlyst A26 resin (OH^- form) instead of Na_2CO_3. In the second step, monofunctionalization of the diol was performed via a lipase-catalyzed transesterification with use of vinyl acetate as the acyl donor.[7] Of the lipases tested (Amano AK: *Pseudomonas fluorescens* lipase; Amano PS: *Burkholderia cepacia* lipase; CAL: *Candida antartica* lipase; CRL: *Candida rugosa* lipase; CCL: *Candida cylindracea* lipase; PPL: porcine pancreas lipase; and WGL: wheat germ lipase), Amano AK gave the best results: most of the diol **6** was promptly converted into monoacetate **7** before the slow appearance of the undesired diacetate.

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10.1021/jo048697k CCC: $27.50
Published on Web 11/12/2004

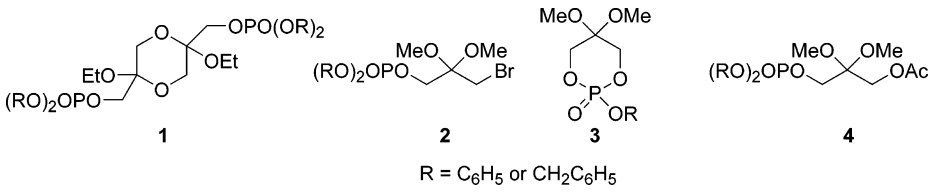
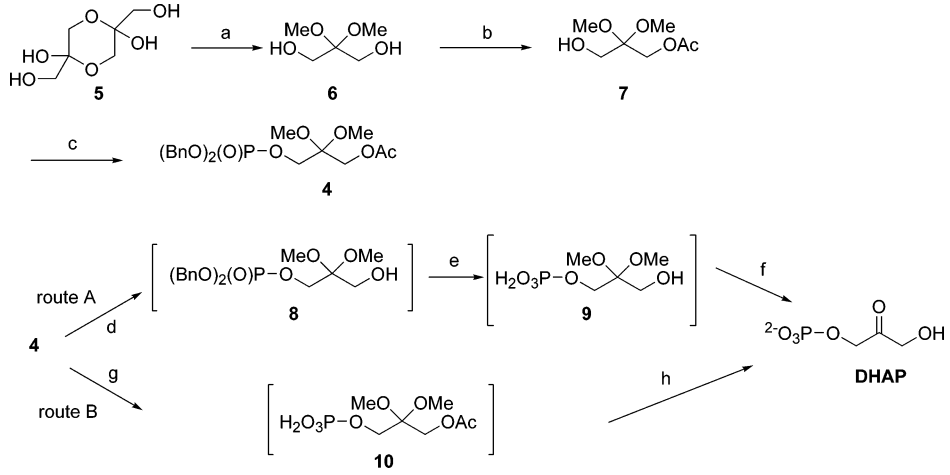


FIGURE 1. Structures of dihydroxyacetone phosphate (DHAP) precursors.

SCHEME 1. Dihydroxyacetone Phosphate Synthesis[a]



[a] Reagents and conditions: (a) $HC(OMe)_3$, MeOH, cat. TsOH, rt (ref 2b); (b) lipase AK (Amano), vinyl acetate, isopropyl ether, 30 °C (61% from **5**); (c) $P(OBn)_3$, I_2, Pyr, CH_2Cl_2, −30 °C (93%); (d) NaOH, MeOH, rt; (e) H_2, Pd/C, MeOH, rt; (f) H_2O, 45 °C (84% from **4**); (g) H_2, Pd/C, MeOH, rt; (h) H_2O, 65 °C (62% from **4**).

The monoester **7** was distilled under reduced pressure and isolated in 61% yield from **5**. Phosphorylation of compound **7** was performed following the procedure described by Gefflaut et al.[4c] The choice of benzyl preferred to phenyl for phosphate protecting groups was based on the facile hydrogenolysis of the former with no need for pressure and expensive PtO_2 catalyst. Two equivalents of the phosphorylating agent, dibenzyl phosphoroiodidate (DBPI) generated from $P(OBn)_3$ and I_2, were necessary to give the precursor **4** in 93% yield after short silica gel column purification.

From **4**, two routes to prepare DHAP were explored. Following pathway A, three reactions were consecutively performed without isolation of the intermediates. First, a basic hydrolysis of the ester group was carried out with a stoechiometric amount of NaOH, leading to the crude alcohol **8**. Sodium acetate was simply removed by an aqueous washing of a DCM solution of **8**. An analytical sample of **8** was also prepared by column chromatography. Next, the crude **8** was hydrogenated in the presence of Pd/C (10%) in MeOH to quantitatively remove the benzyl groups. Finally, we took advantage of the acidity of the free phosphate monoester to catalyze the hydrolysis of the dimethoxy acetal. This reaction was performed in distilled water at 45 °C. Following pathway B, two steps were consecutively performed. The hydrogenolysis was followed by the ester and acetal hydrolyses catalyzed by the acidic phosphate group at 65 °C. In both cases, the DHAP was assayed enzymatically.[8] The acid hydrolysis of intermediates **9** and **10** led to different results. Following route A, hydrolysis of **9** gave a 424 mM DHAP solution in 50 min (84% yield calculated from **4**). This chemical yield is in the same range as the one previously described from the same precursor by Valentin et al.[4d] From route B, a 62% yield from **4** was reached in 90 min. The higher temperature and reaction time required for acetate hydrolysis were responsible for DHAP decomposition, leading to a lower yield. These results are in agreement with data from the literature.[3f] As determined by ^{13}C and ^{31}P NMR, the DHAP sample from route A was free of inorganic phosphate, and methanol was the sole impurity detected. At pH 3.5, DHAP was present in its ketone and hydrate forms as already described in the literature.[4d] In conclusion, pathway **A** is clearly the best method for preparing DHAP from 4.

This short and facile procedure was performed with only one distillation and one short chromatography on silica gel with inexpensive reagents. DHAP overall yield from DHA was 47%. A DHAP solution of high purity and concentration can thus be obtained on several gram scale and stored at −18 °C for several months without noticeable decomposition. In the case of the use of commercially available diphenylphosphorochloridate to introduce the phosphate group, pathway B would be more suitable owing to the instability of this protection under the basic conditions necessary for acetate hydrolysis in route A.

Experimental Section

2,2-Dimethoxy-1,3-propanediol (6). This compound was synthesized by a slight modification of the method described by Ferroni et al.[4b] Starting with 20 g (111 mmol) of **5**, the reaction was stopped by adding 2.2 g of Amberlyst A 26 (OH^- form). After neutralization (15 min), the resin was removed by filtration and the filtrate was evaporated to dryness. Compound **6** was

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obtained as a crude, slightly yellow solid in quantitative yield (30 g; 220 mmol).

3-Hydroxy-2,2-dimethoxypropyl Acetate (7). Lipase Amano AK (600 mg, 12 000 U) was added to a solution of crude diol **6** (30 g, 220 mmol) dissolved in 450 mL of a mixture of vinyl acetate and isopropyl ether (2/1). The suspension was stirred for 18 h at 30 °C. The lipase was removed by filtration through a membrane (0.2 μm). The filtrate was evaporated to dryness. Compound **7** was purified by distillation under reduced pressure (0.1 mmHg, 72 °C) and was isolated as a colorless oil in 61% yield from **5** (23.88 g). Its spectral data are in accordance with the previously described ones.

3-Acetoxy-2,2-dimethoxypropyl Dibenzyl Phosphate (4). Iodine (11.37 g, 44.8 mmol) was added to a solution of tribenzyl phosphite[4c] (16.57 g, 47.04 mmol) in 100 mL of anhydrous EtOH-free CH_2Cl_2 at −30 °C. This solution was then added dropwise to a solution of **7** (4 g, 22.4 mmol) in a mixture of anhydrous pyridine (7.26 mL, 90 mmol) and anhydrous CH_2Cl_2 (120 mL) at −30 °C, under argon. When the addition was completed, the mixture was allowed to warm to rt for 30 min and then filtered on Celite, concentrated to dryness, and dissolved in 180 mL of an Et_2O/water (5/1) mixture. The organic layer was washed with 50 mL of $KHSO_4$ (0.3 M), 50 mL of saturated $NaHCO_3$, and 100 mL of brine, dried over $MgSO_4$, and evaporated to dryness. Compound **4** was purified on a short column (5 cm diameter; 300 mL of silica gel; eluant cyclohexane/AcOEt 7/3 then 4/6) and obtained as a colorless oil in 93% yield (9.13 g). Its spectral data are in accordance with the previously described ones.

Dihydroxyacetone Phosphate (DHAP). Route A: 3-Hydroxy-2,2-dimethoxypropyl Dibenzyl Phosphate (8). Aqueous NaOH (1 M; 6.84 mL) was added to a solution of **4** (3 g, 6.84 mmol) in 10 mL of methanol. The mixture was stirred at rt for 30 min. After dilution with water (15 mL), alcohol **8** was extracted with CH_2Cl_2 (2 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness to quantitatively give crude **8**.

Purification of Compound 8 for Full Characterization. **8** (200 mg crude) was purified by flash chromatography with cyclohexane/AcOEt (4/6) as eluant. **8** was isolated as a colorless oil in 93% yield (186 mg). IR (film) 3411,1497, 1455, 1381, 1258, 1090, 893 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 10H), 5.05 (m, 4H), 4.01 (d, 2H), 3.62 (s, 2H), 3.25 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 128.7−128.0, 100.2, 69.7, 62.4, 59.0, 48.4. ES^+ (MeOH), M + Na^+ calcd for $C_{19}H_{25}O_7NaP$ 419.1236, found 419.1236. Anal. Calcd for $C_{19}H_{25}O_7P$ (396.3): C, 57.57; H, 6.36; P, 7.81. Found: C, 57.52; H, 6.52; P, 7.80.

To the crude compound **8** in 30 mL of methanol was added 140 mg of 10% Pd/C. The mixture was stirred for 45 min at rt under hydrogen atmosphere, using a balloon. Pd/C was removed by filtration through a membrane (0.2 μm) and the filtrate was evaporated to dryness. Distilled water (13.2 mL) was added and the solution (theor. 518 mM of **9**) was stirred at 45 °C. The DHAP formation was followed by enzymatic assay. Once the maximum yield (84% in 50 min from **4**) was reached, the reaction was cooled in an ice bath and the pH was adjusted to 3.7 with NaOH (3N). At pH 3.7, the DHAP solution (424 mM) can be stored at −18 °C for several months without noticeable degradation.

Route B: To 3 g of compound **4** (6.84 mmol) in 30 mL of methanol was added 140 mg of 10% Pd/C. The mixture was stirred for 45 min at rt under hydrogen atmosphere, using a balloon. Pd/C was removed by filtration through a membrane (0.2 μm) and the filtrate was evaporated to dryness. Distilled water (13.2 mL) was added and the solution (theor. 518 mM of **10**) was stirred at 65 °C. The DHAP formation was followed by enzymatic assay. Once the maximum yield (62% in 90 min from **4**) was reached, the reaction was stopped as described in route A.

Supporting Information Available: General experimental information, Excel graphics for DHAP formation following route A and B and Wong's method, DHAP ^{13}C NMR and ^{31}P NMR spectra in H_2O + D_2O from route A and ^{13}C NMR spectrum from route B. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048697K