A Novel Efficient Synthesis of Dihydroxyacetone Phosphate and Bromoacetol Phosphate for Use in Enzymatic Aldol Syntheses

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Dihydroxyacetone phosphate (DHAP, **7**) and bromoacetol phosphate (BAP, **6**) were synthesized in four and five steps, respectively, starting from 1,3-dibromoacetone (**2**). The key step involves desymetrization and ketone protection of **2** to prepare alcohol **3**. Phosphorylation of **3** followed by hydrogenolysis and then deprotection of the ketal function afforded **6**. A solution of **7** was prepared after treatment of **6** with NaOH. This original route allows a short and convenient preparation of DHAP in large scale and high purity for application to the synthesis of sugar derivatives and preparation of BAP for triosephosphate isomerase inhibition.

Introduction

Aldolases have emerged from the enzyme revolution in asymmetric synthesis as major tools for *de novo* synthesis of monosaccharides and other chiral compounds of related structures.[1] Four known aldolases catalyze the condensation of dihydroxyacetone phosphate (DHAP, **7**) for a large variety of aldehydes. Among them, the glycolytic enzyme, fructose-1,6-diphosphate aldolase, has been the most widely used for synthetic applications over the last 15 years.[2] Rhamnulose-1-phosphate aldolase and fuculose-1-phosphate aldolase also found interesting applications for the synthesis of unusual monosaccharides like L-fructose[3] and L-fuculose.[4] Although commercially available, DHAP is too expensive for large scale synthesis and its preparation for synthetic purposes remains a subject of interest.

Current preparations of DHAP involve both enzymatic and chemical methods. The easiest synthesis incorporates the formation of DHAP *in situ* from fructose 1,6-diphosphate (FDP) in the presence of triosephosphate isomerase (TPI).[1,5] The aldolase-catalyzed retroaldolization of FDP generates DHAP and glyceraldehyde-3-phosphate (GAP) which undergoes concomitant isomerization into the desired product by TPI. This method, while restricted to FDP-aldolase, is convenient and commonly used. However, GAP competes with the unnatural aldehyde and low yields ensue. Furthermore, the presence of FDP can complicate product isolation.[2d,6]

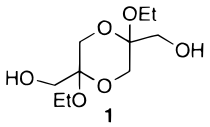

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Figure 1.

Two other enzymatic methods have been developed: phosphorylation of dihydroxyacetone (DHA) using glycerol kinase and ATP[5b,7] and oxidation of L-glycerol 1-phosphate by molecular oxygen in the presence of glycerophosphate-oxidase.[8] In the first method, the necessary ATP regeneration requires the previous synthesis of phosphoenolpyruvate or acetylphosphate. In the second one, L-glycerol 1-phosphate has to be provided. Both methods are expensive for a large scale preparation of DHAP (**7**), and chemical preparation is thus preferred.

The most efficient syntheses of DHAP are based on the original method of Colbran *et al.*[9] in which protected DHA dimer **1** (Figure 1) is phosphorylated by diphenyl phosphorochloridate (DPPC). Hydrogenolysis of the phenyl groups over PtO_2 followed by ketal hydrolysis then leads to DHAP. In 1987, Effenberger reported the shortest synthesis to date, in which phosphorylation is achieved by reaction with $POCl_3$ and isolation of the free phosphate as its barium salt.[10] Phosphitylation with dibenzyl *N,N*-diethylphosphoramidite followed by oxidation has also been used in order to obtain a phosphate protected with the easily removable benzyl groups.[11] Recently, Colbran's synthesis using DPPC has been optimized by Wong and co-workers to produce DHAP in 61% overall yield.[12] However, the limiting step of this synthesis remains the final hydrolysis of the protected dimer which gives DHAP in just 66% yield.

Likewise, DHAP was proved to be formed by basic hydrolysis of bromoacetol phosphate (BAP).[13] This close

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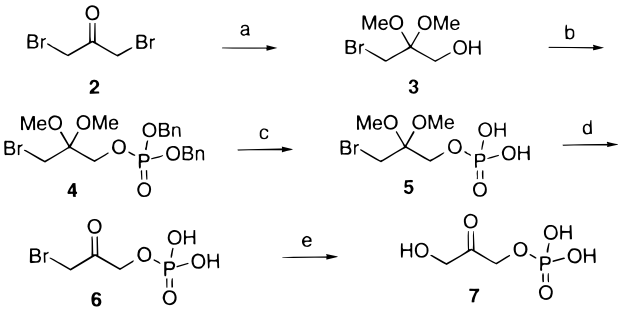
Scheme 1. Synthesis of Bromoacetol Phosphate and Dihydroxyacetone Phosphate[a]



[a] MeONa, MeOH (85%); (b) $P(OBn)_3$, I_2, Pyr, CH_2Cl_2 (91%); (c) H_2, Pd/C, MeOH (99%); (d) H_2O, 65 °C; (e) NaOH (73% from **4**).

analogue of DHAP, also a molecule of interest for aldolase-catalyzed syntheses, behaves as a strong irreversible inhibitor of TPI,[13,14] an enzyme often present in aldolase preparations. Due to the high specific activity of TPI, even traces of this enzyme are responsible for partial isomerization of DHAP into GAP, thus lowering the yield of the enzymatic synthesis. As a very poor competitive inhibitor of aldolase, BAP has already been incorporated in synthetic pathways by several groups to circumvent this problem.[15] Two syntheses of BAP have been described. The first preparation involves the reaction of 1-bromo-3-diazoacetone with an excess of phosphoric acid.[14] Despite its brevity, this synthesis exploits the use of hazardous diazomethane and BAP is not isolated from the reaction mixture. In the second case, BAP has been prepared from 3-bromopropane-1,2-diol in six steps and in an overall yield of approximately 10%.[13]

In the course of our research directed toward sugar analogues via enzymatic reactions, we recently described an original approach to DHAP from 1,3-dibromoacetone.[16] DHAP was obtained in 95% yield from a dimethyl ketal precursor in a similar fashion to the original synthesis of DHAP by Ballou and Fischer.[17] We report here a fundamental improvement of our procedure giving access to both BAP and DHAP on a gram scale (Scheme 1).

Results and Discussion

While the starting 1,3-dibromoacetone (**2**) is commercially available, it is also easily synthesized on a large scale by acidic bromination of acetone followed by two fractional distillations.[18]

In a rapid reaction, **2** is then converted to ketal **3** (85% isolated yield) by treatment with sodium methylate in methanol at −10 °C. This unusual ketalization in basic conditions is thought to proceed through a nucleophilic addition of the methoxide ion into the carbonyl group followed by an opening of the resulting epoxide *via* a sn1 process. This key reaction avoids the previous two-step (70% overall yield) synthesis starting from expensive 3-bromopyruvic acid[19] and brings both protection of the carbonyl group and desymmetrization of the substrate into a single step.

As phosphorylation of **3** using $POCl_3$ was not convenient because of resultant mixtures of organic and inorganic phosphates and as the phosphotriester prepared in modest yield (60%) from 1 equiv of diphenyl phosphorochloridate (DPPC) was debrominated when submitted to hydrogenation over PtO_2, we took advantage of the phosphorylation reagents dibenzyl phosphorochloridate and bromidate which contain more labile benzyl substituents. In both cases **4** was obtained in very poor yield (≤20%). Phosphorylation of **3** was finally realized using dibenzyl phosphoroiodidate (DBPI) generated from tribenzyl phosphite and iodine.[20] Quantitative phosphorylation of cholesterol has been reported with a stoichiometric amount of DBPI−CH_2Cl_2 as solvent and 4 equiv of pyridine. However, the reaction of **3** was incomplete even after long reaction times, which we attribute to the neopentyl nature of the primary alcool. Attempts with different solvents and bases gave no significant improvement in the outcome of the phosphorylation reaction. However, when 2 equiv of DBPI was used, **4** was obtained in 91% yield after purification by flash chromatography. Tribenzyl phosphite is easily prepared from PCl_3 and benzyl alcohol, and this procedure was found more convenient than the phosphitylation/oxidation process using phosphoramidites.

Removal of the benzyl groups was easily achieved by hydrogenolysis under atmospheric pressure using Pd on charcoal (5%) in MeOH. The reaction was complete within 30 min, and **5** was obtained in quantitative yield after filtration and evaporation of the solvent. No halogen substitution was observed under these mild conditions, as shown by NMR analysis of the dicyclohexylammonium salt of **5**.

After addition of distilled water (4 mL/g of **5**), the solution was heated to 65 °C. ^1H NMR measurements indicated a $t_{1/2}$ of 12 min for the hydrolysis and a maximum yield of 90% after 1.5 h. The slower phosphate hydrolysis also observed was responsible for lowering the yield under longer reaction time. BAP (**6**) solution can be stored frozen after adjusting the pH to 4.5 with sodium bicarbonate.

DHAP (**7**) was finally generated by addition of 1 M sodium hydroxide (3 equiv) to the aqueous solution of **6**. The pH of the reaction was kept under 10.5 during the addition to minimize phosphate hydrolysis. When debromination was complete, the pH of the solution was then rapidly dropped to 3.7 with Dowex 50 (H^+). After filtration, the concentration of DHAP (**7**) was determined by enzymatic assay[21] (73% from **4**). Residual bromide ions have no influence on the assay.

This stock solution of DHAP was stored frozen for months without noticeable decomposition.

This short and facile procedure represents a completely new route for the preparation of BAP and DHAP from acetone or 1,3-dibromoacetone and inexpensive reagents. Furthermore, this synthesis provides an alternative and efficient use of dibenzyl phosphoroiodidate in the phosphorylation of alcohols. The procedure adapted to large scale preparation was found particularly suitable for obtaining sensitive compounds of biological interest like DHAP.

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BAP and DHAP obtained with 69 and 56.5% overall yields, respectively, were successfully applied to aldolase-catalyzed syntheses in our laboratory.

Experimental Section[22]

Tribenzyl Phosphite. To a vigorously stirred solution of PCl_3 (17.5 mL, 0.2 mol) in anhydrous Et_2O (1 L) at −10 °C were successively added triethylamine (86.4 mL, 0.62 mol) in Et_2O (100 mL) and benzyl alcohol (62.1 mL, 0.6 mol) in Et_2O (100 mL). The resultant suspension was stirred for 30 min at −10 °C and 24 h at rt. The chlorohydrate was filtered and washed with Et_2O (4 × 100 mL). The combined filtrate and washings were concentrated under reduced pressure to give 70 g of crude material. After dilution with cyclohexane-Et_2O-Et_3N (49.5:49.5:1) (50 mL), the product was purified on a silica gel column (400 g, 5 cm x 40 cm) and eluted with the same solvent. Tribenzylphosphite was isolated as a colorless liquid (53 g, 75%) and must be stored in the absence of oxygen to avoid its oxidation to tribenzyl phosphate: ^{1}H NMR (400 MHz) δ 7.40 (15H, m), 4.98 (6H, d).

3-Bromo-2,2-dimethoxy-1-propanol (3). A solution of dibromoacetone (**2**) (10.8 g, 50 mmol) in methanol (100 mL) was added dropwise to a solution of sodium methoxide (4.05 g, 75 mmol) in methanol (200 mL) at −15 °C. The solution was then allowed to warm to 25 °C. After neutralization with 1M $KHSO_4$, and evaporation of methanol under reduced pressure ($T \leq 30$ °C), the residue was diluted with water (25 mL) and extracted with Et_2O (2 × 50 mL). The ethereal solution was washed with brine (25 mL) and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure ($T \leq 30$°C) yielded 9.2 g of crude **3**. If necessary, **3** can be purified by flash-chromatography (eluent, cyclohexane−Et_2O, 5:5, v/v) to give **3** as a pale yellow oil (85%). Although **3** is unstable when exposed to air and light, it can be conveniently stored for a long period under Ar at −18 °C: ^{1}H NMR (400 MHz) δ 3.74 (2H, d), 3.48 (2H, s), 3.28 (6H, s), 2.03 (1H, t); ^{13}C NMR (100 MHz) δ 100.0, 59.2, 48.7, 30.0.

3-Bromo-2,2-dimethoxypropyl Dibenzyl Phosphate (4). I_2 (12.7 g, 50 mmol) was added to a solution of tribenzyl phosphite (18.5 g, 52.5 mmol) in EtOH-free CH_2Cl_2 (100 mL) at −20 °C. After 20 min, the clear, colorless solution was allowed to warm to 25 °C and was subsequently added dropwise over a period of 1 h to a solution of **3** (5 g, 25 mmol) and pyridine (8 mL, 100 mmol) in CH_2Cl_2 (100 mL) at −30 °C. The solution was then allowed to warm to 25 °C, filtered, and concentrated under reduced pressure. The residue was diluted with Et_2O (200 mL) and water (50 mL). The organic layer was washed with aqueous $KHSO_4$ 0.3 M (3 × 50 mL), aqueous saturated $NaHCO_3$ (50 mL), and brine (50 mL) and dried over $MgSO_4$. After concentration, the crude compound (14.5 g) was chromatographed over silica gel (400 g) (eluent, CH_2Cl_2−acetone, 97:3,) to give **4** (10.4 g, 91%) as a colorless liquid: IR (neat film) 1450, 1280, 1079, 1022, 738, 697 cm^{-1}; ^{1}H NMR (400 MHz) δ 7.35 (10H, m), 5.08 (4H, d), 4.12 (2H, d), 3.43 (2H, s), 3.25 (6H, s); ^{13}C NMR (100 MHz) δ 135.7, 128.6, 128.0, 99.4, 69.6, 62.6, 48.8, 29.6. Anal. Calcd for $C_{19}H_{24}BrO_6P$ (459): C, 49.69; H, 5.27; P, 6.74. Found: C, 49.63; H, 5.33; P, 6.68.

3-Bromo-2,2-dimethoxypropyl DihydrogenPhosphate (5). To a solution of **4** (9.2 g, 20 mmol) in methanol (100 mL) was added 5% Pd/C (400 mg). Hydrogenolysis was performed at 25 °C using a balloon. The reaction was complete after 30 min. The catalyst was removed by filtration and the solvent evaporated under reduced pressure and under high vacuum to give **5** as a colorless syrup (5.5 g, 99%). Analytical data were obtained from the dicyclohexylammonium salt of **5** formed by reaction of **5** with 2 equiv of cyclohexylamine: ^{1}H NMR (400 MHz, D_2O) δ 4.80 (6H, s large), 3.73 (2H, d), 3.48 (2H, s), 3.20 (6H, s), 3.01 (2H, m), 1.85 (4H, m), 1.67 (4H, m), 1.52 (2H, m), 1.23 (8H, m), 1.05 (2H, m); ^{13}C NMR (100 MHz, D_2O) δ 102.6, 62.2, 53.0, 52.4, 51.0, 32.6, 32.1, 26.5, 26.0.

3-Bromo-2-oxopropyl Dihydrogen Phosphate (6) (BAP). A solution of **5** (5.5 g, 20 mmol) in distilled water (40 mL) was heated at 65 °C for 1.5 h. BAP (**6**) was obtained in 90% yield as measured by ^{1}H NMR. This solution can be stored frozen after adjusting the pH to 4.5 with solid bicarbonate. **6** exists in solution free or hydrated: ^{1}H NMR (400 MHz, D_2O) δ 4.82 (2H, d), 4.32 (2H, s), 4.02 (2H, d), 3.58 (2H, s); ^{13}C NMR (100 MHz, D_2O) δ 204.0, 95.8, 71.1, 70.7, 38.5, 34.6.

Dihydroxyacetone Phosphate (7) (DHAP). To the solution of **6** (from 20 mmol of **5**) was added dropwise a 1 M NaOH solution (≤60 mL) over a period of about 20 min: during addition of the third equivalent, the bromine substitution is evidenced by rapid pH fluctuations and the addition was regulated to keep the pH between 10 and 10.5. The addition was stopped when the pH quickly raised to 11 (57 mL < v < 60 mL). Dowex 50 (H^+) was then rapidly added until the pH reached 3.7. The final solution contained 14.6 mmol of DHAP, based on enzymatic assay (73% from **4**). This solution can be stored frozen for a few months. For the enzymatic assay, the decrease in optical density (ΔOD) at 340 nm is measured after addition of 10 μL of a DHAP solution (in the range of 1−4 mM) to 1 mL of triethanolamine−HCl buffer (0.1 M, pH 7.5) containing glycerol-3-phosphate dehydrogenase (10 μg/mL) and NADH (O.25 mM). After correction for dilution, the concentration of DHAP in the assay is ΔOD/6220 in mol·L^{-1}.

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(22) **General.** IR spectra were determined on a Perkin-Elmer 881 spectrometer, and the resonances are expressed in frequency units (v, cm^{-1}). NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer. $CHCl_3$ (δ = 7.27), $CDCl_3$ (δ = 77.1) was used as the respective internal standard expressed in ppm. Column chromatography was performed on Merck kieselgel 60 (0.040−0.063 mm), and commercial kieselgel 60 F254 plates were used for thin layer chromatography. All solvents were distilled before use following usual procedures. Satisfactory analytical data (±0.3%) were obtained for all new compounds at theService Central d'Analyse du CNRS, Solaize, France.