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A convenient synthesis of dihydroxyacetone phosphate from acetone

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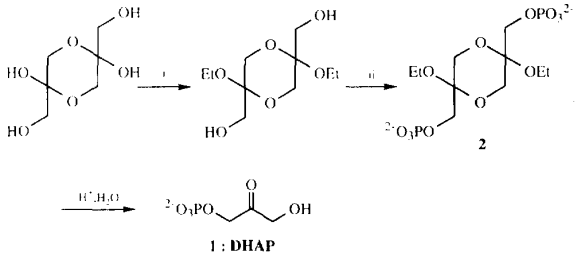
Summary – A new chemical method for preparation of dihydroxyacetone phosphate (DHAP) from acetone is described. This method improves the synthesis of a stable precursor of DHAP, which is the necessary substrate of several aldolases. Thus, monosaccharides and their derivatives could be obtained from this compound by aldolase-catalyzed condensations.

dihydroxyacetone phosphate (DHAP) / aldolase / monosaccharide

Dihydroxyacetone phosphate (DHAP, 3-hydroxy-2-oxopropyl phosphate) **1** is an intermediate in glycolysis obtained by the aldolase-catalyzed splitting of fructose-1,6-bisphosphate. The increasing number of applications of enzymatic aldol reactions in organic syntheses [1] make this compound an important starting material, since DHAP is the necessary substrate of the most commonly used enzyme, fructose-1,6-bisphosphate aldolase, and of two other promising enzymes, rhamnulose-1-phosphate and fuculose-1-phosphate aldolases [2].

DHAP has been synthesized by enzymatic methods. 1,3-Dihydroxyacetone can be phosphorylated using glycerolkinase and adenosine triphosphate (ATP) with *in situ* regeneration of the latter [3]. Alternatively, L-glycerol-1-phosphate can be oxidized by NAD^+ in the presence of glycerol-phosphate dehydrogenase [3a], or, as recently described by Fessner and Sinerius, by O_2 in the presence of glycerol oxidase [4]. Although these methods can offer some advantages, especially the latter which appears to be promising for large-scale applications, they suffer from the need to provide an additional phosphate, namely phosphoenol pyruvate or acetylphosphate for ATP regeneration, or L-glycerol-1-phosphate in the oxidation methods. Thus chemical synthesis may be an easier method for DHAP production.

The most recent published syntheses of DHAP are based on the method of Colbran *et al* [5], in which protected dihydroxyacetone is phosphorylated by diphenylphosphorochloridate, leading, after hydrolysis, to the precursor **2** (fig 1). This synthesis has recently been improved by Wong and coworkers giving **2** in 78% yield [6]. In Effenberger's synthesis [7], which remains the shorter method, phosphorylation is achieved by $POCl_3$ in pyridine, and **2** is isolated as its barium salt. In the Pederson synthesis [8], the phosphite method used in polynucleotide syntheses is employed. All these methods lead to the same intermediate **2**, which is also the stock material, because DHAP is too unstable to be stored. Consequently, the hydrolysis of **2** is the crucial step, since no further purification is possible. However, due to the stability of the dioxane ring, the yield is never higher than 74%.



i) ethylorthoformate, H^+; ii) diphenylchlorophosphate, then H_2/Pd (Colbran method) or $POCl_3$ in pyridine (Effenberger method), or dibenzyl-*N*,*N*-diethylphosphoramidite, triazole, then H_2O_2 and Pd/H_2 (Wong method).

Fig 1. Chemical synthesis of DHAP.

A more suitable precursor of DHAP is acetyl dihydroxyacetone dimethyl acetal **3b** (3-acetoxy-2,2-dimethoxypropan-1-ol), described by Ballou and Fischer [9] and which leads to DHAP in 95% yield in the last step. However, this synthesis starts from 3-chloropropane-1,2-diol and eight steps are necessary to obtain DHAP with an overall yield of 13%.

We describe here a more convenient synthesis of **3** and DHAP from acetone (in four steps from commercially available 1,3-dibromoacetone), the salient feature of which is the partial hydrolysis of diacetate **4** by pig

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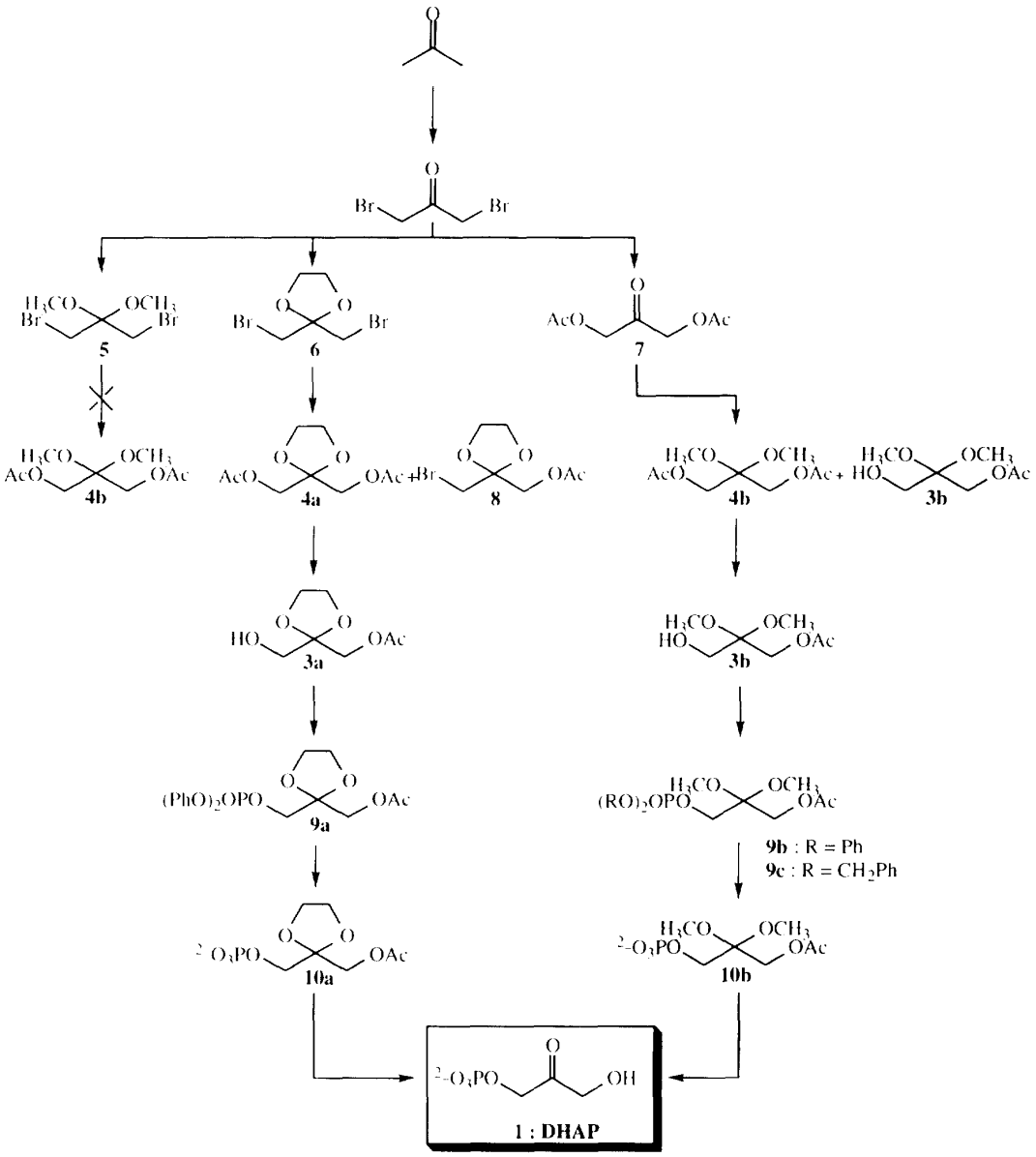

Fig 2. Synthesis of dihydroxyacetone phosphate (DHAP) from acetone.

pancreatic lipase (fig 2). Compound **3** was then phosphorylated using Ballou's procedure or Todd's method, leading to a stable precursor of DHAP.

Results and discussion

Starting from dibromoacetone, our synthesis involves the following steps: protection of the ketone group; substitution of bromide by acetate ions; partial hydrolysis of the diacetate obtained; and phosphorylation (*Method A*). Alternatively, the substitution reaction can be done before the ketone protection (*Method B*).

Method A

1,3-Dibromo-2,2-dimethoxypropane **5**, which is easily prepared from acetone, was treated with potassium acetate in refluxing benzene in the presence of tetrabutylammonium bromide as phase transfer catalyst. In these conditions, however, the acetal protection was inefficient and diacetyl-dihydroxyacetone (1,3-diacetoxypropan-2-one) was not isolated. Accordingly we decided to use the more stable cyclic acetal **6**.

Dibromoacetone reacted with ethane-1,2-diol in acidic medium to give **6** in 92% yield. Substitution of

bromide by acetate was first carried out in boiling benzene in the presence of tetrabutylammonium bromide. After 2 d, starting material still remained and monoacetate **8** was isolated in 40% yield; only traces of the expected diacetate **4a** were present. We therefore used the method described by Barry *et al* [10]: a mixture of potassium acetate, dibromide **6** and a catalytic amount of methyltrioctyl ammonium chloride without solvent was heated at 120°C for 2 d. The reaction did not go to completion, but we isolated **4a** in 44% yield along with **8** in 10% yield. Prolongation of reaction time or increase of the temperature failed to improve the yield. Hydrolysis of **4a** by pig pancreatic lipase at pH 7 led to monoacetate **3a** in 85% yield. Compound **3a** could also be hydrolyzed by lipase but at a very slow rate and so it was easy to stop the reaction after the first hydrolysis. The final yield of **3a** was 37% from dibromoacetone.

The phosphorylation was performed by diphenylphosphorochloridate as described previously [9] giving **9a** (86%). This compound led to DHAP after hydrogenolysis on PtO_2 and acid hydrolysis. Hydrogenolysis occurred in practically quantitative yield, but the final acid hydrolysis was difficult; we had to heat the mixture for 4 h at 80°C and the yield of DHAP measured by enzymatic assay was only 50%.

Method B

Since it was impossible to improve the yield of the substitution reaction by acetate, and since the hydrolysis of the dioxolane ring was difficult, we decided to perform the substitution reaction on the unprotected dibromoacetone and to use dimethyl acetal as a protective group. In this case, diacetate **7** was readily obtained in 92% yield. Alternatively, **7** could be synthesized from dihydroxyacetone by reaction with acetic anhydride in 98% yield [11]. Acetal formation was more difficult since the operating conditions favored the transesterification of the acetates. Various conditions were tested. The best results were obtained using trimethyl orthoformate in methanol with zinc chloride as a catalyst. The expected compound **4b** was isolated in only 39% yield. However, the transesterification reaction affected only one acetate group and afforded **3b** in 26% yield. Compound **4b** was hydrolyzed by lipase in the same conditions as **4a** giving **3b** in 85% yield. Hence the final yield of **3b** was 61% from **7** and 56% from dibromoacetone (60% from dihydroxyacetone).

The phosphorylation was performed as above, giving **9b** in 90% yield. This compound led to DHAP after hydrogenolysis and acid hydrolysis with a yield of 95%.

Hydrogenolysis of **9** required PtO_2 as a catalyst. To reduce the cost of the synthesis, we tried to use the dibenzylester as a protective group for phosphate. No reaction was observed when dibenzyl phosphorochloridate was used for phosphorylation of **3b**. Phosphorylation was carried out in 51% yield by action of dibenzyl phosphorobromidate in pyridine. This compound was prepared *in situ* from dibenzyl phosphite and bromotrichloromethane [12]. Hydrogenolysis of **9c** on Pd afforded **10b** in more than 85% yield.

This new synthesis of DHAP (using the optimal *Method B*, phosphorylation by diphenyl phosphorochloridate) occurs in six steps and with an overall yield of 47.8% from dibromoacetone or 51.3% from dihydroxyacetone. These yields are in the same range or better than those for the most recent syntheses (57.7% in Wong's synthesis and 34.6% in Effenberger's synthesis). However, our method offers an important advantage related to the last step. To be used in enzyme-catalyzed synthesis, DHAP must be produced at the last moment from a stable precursor. No purification is possible at that stage. It is important to obtain a concentrated solution, which is as pure as possible and contains no inorganic phosphate. The hydrolysis of our precursor is performed with a 95% yield. In the other syntheses, a prolonged heating in acidic conditions is necessary for deprotection of **2** and so a maximum yield of 74% is obtained together with a significant amount of inorganic phosphates resulting from the hydrolysis of DHAP under these conditions.

Another feature of this synthesis is the possibility of obtaining labeled DHAP, since the starting material, acetone, is available in a labeled form.

Conclusion

We synthesized DHAP with an overall yield of 50% from commercial dibromoacetone. Compounds **9b** and **10b** can act as precursors of DHAP. They are obtained in four or five steps from inexpensive materials, and only one purification is necessary. They lead to DHAP with high yields. The dioxolane protecting group should be avoided for the protection of ketone in DHAP since its hydrolysis is difficult.

Experimental section

Materials and methods

Chemicals were purchased from Aldrich and Lancaster, and were reagent grade. Dowex resin, enzymes and biochemicals were obtained from Sigma. Merck silica gel (70–230 mesh) was used for column chromatography. UV spectra were recorded with a Secomam S 1000G spectrophotometer. IR spectra were determined on a Perkin Elmer 881 spectrophotometer. NMR specta were recorded on a Bruker AC 400 (400 MHz ^1H NMR and 100 MHz ^{13}C NMR) spectrophotometer.

1,3-Dibromoacetone

This substance was prepared according to published procedures of Fredga and Myrbäck [13]. Repeated distillations of 1,3-dibromoacetone gave bp 79.5–80.5°C/9 mmHg and mp 25.5–27.0°C (49% yield).

^1H NMR (400 MHz, $CDCl_3$) δ: 4.25.

^{13}C NMR (100 MHz, $CDCl_3$) δ: 31.5 (CH_2Br), 193.5 (C=O).

2,2-Bis(bromomethyl)-1,3-dioxolane **6**

In a flask equipped with a Dean–Stark trap (for azeotropic removal of water) and a reflux condenser, dibromoacetone (4.04 g; 18.7 mmol) and 2.08 mL of ethylene glycol (2.32 g; 37.4 mmol) in benzene (70 mL) with a small quantity of *para*-toluenesulfonic acid were refluxed for 12 h. The mixture was evaporated. and washed with water (4 × 15 mL) after addition of Et_2O (30 mL). The organic layer was dried ($MgSO_4$) and evaporated to yield 4.47 g (92% yield) of a colorless liquid which crystallized at room temperature.

^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.75 (s, 4H, CH_2CH_2), 4.10 (s, 4H, CH_2Br).

^{13}C NMR (100 MHz, $CDCl_3$), δ: 33.8 (CH_2Br), 66.7 (CH_2CH_2), 106.7 (C quat ketal).

1,3-Diacetoxypropan-2-one **7**

Freshly dried and crushed potassium acetate (15 g; 153 mmol) and tetrabutylammonium bromide (1.25 g, 3.87 mmol) were added to dibromoacetone (5 g; 23.1 mmol) in benzene (50 mL). The mixture was stirred for 24 h at room temperature, filtered *in vacuo* with a Büchner funnel, and washed several times with AcOEt. The organic layer was washed with water and this aqueous layer was continuously extracted with AcOEt for 12 h. The organic layers were collected, dried ($MgSO_4$) and evaporated *in vacuo* to give a crude product which was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate 1:1), to yield 3.65 g (92% yield) of a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.12 (s, 6H, CH_3), 4.72 (s, 4H, CH_2).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 20 (CH_3), 66 (CH_2), 170 (C=O, acetate), 198 (C=O, ketone).

2,2-Bis(acetoxymethyl)-1,3-dioxolane **4a**

Compound **6** (3.84 g; 14.8 mmol) was placed in a flask equipped with a reflux condenser and a calcium chloride tube. Methyltrioctylammonium chloride (Aliquat 336®, 1.17 g; 2.89 mmol) and freshly dried and crushed potassium acetate (5.72 g; 58.4 mmol) were added to protected dibromoacetone **6**. The mixture was heated at 120°C for 2 d. The Aliquat 336® was adsorbed by stirring for 20 min with silica gel (20 g) after addition of Et_2O (3 vol). The solid was filtered off. The mixture was washed with water, the aqueous layer was extracted with ether, the organic layers were collected and dried ($MgSO_4$). After removal of the solvent, the crude product was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate 1:1) to afford the pure diacetate **4a** (1.15 g,44%) and the monoacetate **8** (0.29 g, 10%) as oily liquids.

^{1}H NMR (400 MHz, $CDCl_3$) δ: **8**: 2.15 (s, 3H, CH_3), 3.45 (s, 2H, CH_2Br), 4.10 (m, 4H, CH_2CH_2), 4.20 (s, 2H, CH_2OAc). **4a**: 2.15 (s, 6H, CH_3), 4.00 (s, 4H, CH_2CH_2), 4.15 (s, 4H, CH_2 OAc).

1,3-Diacetoxy-2,2-dimethoxypropane **4b** *and 3-acetoxy-2,2-dimethoxypropan-1-ol* **3b**

Freshly dried $ZnCl_2$ (417 mg, 3.06 mmol) and freshly distilled trimethyl orthoformate (1.25 mL, 11.4 mmol) were added to anhydrous methanol (5 mL). After addition of diacetate **7** (1 g; 5.75 mmol), the mixture was heated at 65°C for 2 d. Methanol and trimethyl orthoformate were removed by evaporation *in vacuo* ($T < 30$°C). The residue was washed with ammonium hydroxide 0.2 N (5 mL). The aqueous layer was continuously extracted with ethylacetate. The collected organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate 1:1) to afford protected diacetate **4b** (0.49 g, 39%) and protected hydroxylated monoacetate **3b** (0.27 g, 26%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: **4b**: 2.12 (s, 6H, CH_3), 3.27 (s, 6H, OCH_3), 4.16 (s, 4H, CH_2). **3b**: 2.1 (s, 3H, CH_3), 2.5 (s, 1H, OH), 3.2 (s, 6H, OCH_3), 3.55 (d, 2H, CH_2OH), 4.15 (s, 2H, CH_2OAc).

^{13}C NMR (100 MHz, $CDCl_3$) δ: **3b**: 20 (CH_3 acetate), 48 (OCH_3), 60 (CH_2OAc), 99 (C quat ketal), 170 (C=O). **4b:** 20.6 (CH_3 acetate), 48.4 (OCH_3), 59.8 (CH_2OH), 60.2 (CH_2OAc), 99.8 (C quat ketal), 171 (C=O).

2-Acetoxymethyl-2-(hydroxymethyl)-1,3-dioxolane **3a**

Lipase (1800 U) was added to a stirred suspension of diacetate **4a** (1 g; 4.58 mmol) in methanol (2 mL) and phosphate buffer (pH 7.0; 0.02 M; 30 mL). NaOH (0.5 M) was gradually added to maintain stable pH 7. This aqueous solution was continuously extracted overnight with ether. The organic layer was dried and concentrated to give **3a** as a liquid (0.68 g, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.15 (s, 3H, CH_3), 2.70 (s, 1H, OH), 3.60 (s, 2H, CH_2OH), 4.00 (m, 4H, CH_2CH_2), 4.10 (s, 2H, CH_2OAc).

3-Acetoxy-2,2-dimethoxypropan-1-ol **3b**

Compound **4b** was hydrolyzed to **3b** (85%) as described for compound **3a**.

[2-(Acetoxymethyl)-1,3-dioxolan-2-yl]methyl diphenyl phosphate **9a**

Diphenyl phosphorochloridate (2.12 g; 1.64 mL, 7.89 mmol) was added dropwise to the stirred solution of **3a** (1 g, 5.68 mmol) in anhydrous pyridine (3.75 mL) at −10°C. The reaction was left overnight at 4°C. Water (a few drops) was added to destroy excess phosphorylating reagent; the syrup was dissolved in benzene (26 mL) and washed successively with 15 mL each of water, cold hydrochloric acid (1 N), cold potassium carbonate (1 M) and water. The organic layer was dried ($MgSO_4$) and concentrated to a syrup. The crude product was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate 1:1) to afford 2 g of **9a** (86% yield).

^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.00 (s, 3H, CH_3), 3.30 (m, 4H, CH_2CH_2), 4.05 (s, 2H, CH_2OAc), 4.15 (d, 2H, CH_2OP, J_{POCH} = 7 Hz), 7.25 (m, 10H, Ph).

^{13}C NMR (100 MHz, $CDCl_3$), δ: 20.8 (CH_3), 63.2 (CH_2OAc), 65.9 (CH_2CH_2), 67.7 (CH_2OP), 106.0 (C quat ketal), 120.1, 125.5, 129.9, 150.6 (aromatic), 170.3 (C=O).

Anal calc for $C_{19}H_{21}O_8P$ (408.34): C, 55.89; H, 5.18; O, 31.34; P, 7.58. Found : C, 55.65; H, 5.21; O, 31.64; P, 7.59.

3-Acetoxy-2,2-dimethoxypropyl diphenyl phosphate **9b**

Compound **3b** gave **9b** (90%) as described for compound **9a**.

IR (neat): 2 970, 2 930, 2 840, 1 755, 1 590, 1 480, 1 455, 1 290, 1 240, 1 190, 1 070, 960, 690 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.85 (s, 3H, CH_3), 3.05 (s, 6H, OCH_3), 3.95 (s, 2H, CH_2OAc), 4.05 (d, 2H, CH_2OP, J_{POCH} = 6.5 Hz), 7.1 (m, 10H, Ph).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.8 (CH_3), 48.5 (OCH_3), 60 (CH_2OAc), 64 (CH_2OP), 99 (C quat ketal), 120.1, 125.5, 129.8, 150.5 (aromatic), 170 (C=O).

Anal calc for $C_{19}H_{23}O_8P$ (410.36): C, 55.61; H, 5.65; O, 31.19; P, 7.55. Found: C, 55.49; H, 5.47; O, 31.61; P, 7.43.

3-Acetoxy-2,2-dimethoxypropyl dibenzyl phosphate **9c**

Compound **3b** (1 g; 5.62 mmol) was dissolved in anhydrous pyridine (3.5 mL). A solution of $CBrCl_3$ (2.9 mL, 5.83 g, 29.4 mmol) and dibenzyl phosphite (3.2 mL, 3.80 g, 14.4 mmol) was added dropwise at −10°C. The mixture was stirred for 3 h at room temperature. Water (a few drops) was added to destroy the excess phosphorylating reagent;

the syrup was dissolved in ethyl acetate (26 mL) and washed successively with 15 mL each of water, cold hydrochloric acid (1 N), cold potassium carbonate (1 M) and water. The organic layer was dried ($MgSO_4$) and concentrated to a syrup. The crude product was purified by column chromatography on silica gel (eluent: cyclohexane/ethyl acetate 1:1) to afford **9c** (1.25 g, 51%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.0 (s, 3H, CH_3), 3.2 (s, 6H, OCH_3), 3.9 (d, 2H, CH_2OP, J_{POCH} = 7 Hz), 4.15 (s, 2H, CH_2OAc), 5.05 (dd, 4H, CH_2Ph), 7.35 (s, 10H, Ph).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 21 (CH_3), 48.4 (OCH_3), 59 (CH_2OAc), 62.7, 63.5 (CH_2Ph) 69.5 (CH_2OP), 82 (C quat ketal), 128, 128.5, 136 (aromatic), 170 (C=O).

[2-(Acetoxymethyl)-1,3-dioxolan-2-yl]methyl phosphate **10a**

Compound **9a** (1 g; 2.44 mmol) was dissolved in pure methanol (100 mL), platinum oxide (200 mg) was added, and the solution was hydrogenolyzed under 25 psi of H_2 for 2 d at room temperature. The mixture was filtered and the catalyst was rinsed with methanol. The methanol solution was concentrated under reduced pressure to yield a syrup. Water (4 mL) was added, the pH was adjusted to 7 with lithium hydroxide (1 N), and the solution was lyophilized to yield **10a** (0.63 g, 98%) as a white powder.

^{1}H NMR (400 MHz, D_2O) δ: 2.2 (s, 3H, CH_3), 3.85 (d, 2H, CH_2OP, J_{POCH} = 5.5 Hz), 4.15 (m, 4H, CH_2CH_2), 4.35 (s, 2H, CH_2OAc).

^{13}C NMR (100 MHz, D_2O) δ: 21.2 (CH_3), 64.8 (CH_2OAc), 66.1 (CH_2OP), 67 (CH_2CH_2), 108 (C quat ketal), 175 (C=O acetate).

3-Acetoxy-2,2-dimethoxypropyl phosphate **10b**

Compound **9b** was hydrogenolyzed to **10b** (99%) as described for compound **10a**.

^{1}H NMR (400 MHz, D_2O, pH 7.5) δ: 2.25 (s, 3H, CH_3), 3.43 (s, 6H, OCH_3), 3.93 (d, 2H, CH_2OP, J_{POCH} = 5.5 Hz), 4.30 (s, 2H, CH_2OAc).

^{13}C NMR (100 MHz, D_2O, pH 7.5) δ: 21 (CH_3), 49 (OCH_3), 61 (CH_2OAc), 63 (CH_2OP), 101 (C quat ketal), 167 (C=O, Ac).

Compound **10b** could also be obtained from **9c** by hydrogenation on Pd/C for 2 d under 50 psi at room temperature (86% yield).

DHAP **1** *from [2-(acetoxymethyl)-1,3-dioxolan-2-yl] methyl phosphate* **10a**

Compound **10a** (100 mg; 0.37 mmol) was dissolved in water (7.5 mL). The solution was adjusted to pH 1.5 by addition of H_2SO_4 (50 μL). The mixture was heated at 70°C for 4 h, and assayed with glycerophosphate deshydrogenase to yield DHAP (50%) [14].

DHAP **1** *from 3-acetoxy-2,2-dimethoxypropyl phosphate* **10b**

To a solution of **10b** (100 mg; 0.37 mmol) in water (7.5 mL) was added Dowex 50 (H^+) resin until the pH was 1.5. The mixture was heated at 65°C for 4 h. The resin was filtered and washed with water. The enzymatic assay for DHAP yielded 0.352 mmol (95%).

^{1}H NMR (400 MHz, D_2O, pH 7.5, ketone/hydrate 9:8) δ: ketone: 4.55 (d, 2H, CH_2OP, J_{POCH} = 6.5 Hz), 4.60 (s, 2H, CH_2OH); hydrate: 3.65 (s, 2H, CH_2OH), 3.90 (d, 2H, CH_2OP, J_{POCH} = 7.5 Hz).

^{13}C NMR (100 MHz, D_2O, pH 7.5) δ: 65.9–66.4–67.0–68.4 (CH_2); 96.0 (C quat hydrate), 212.5 (C=O ketone).

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