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Synthesis of Phosphono Analogues of Dihydroxyacetone Phosphate and Glyceraldehyde 3-Phosphate

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Abstract—The present paper describes the synthetic routes of six phosphono analogues of dihydroxyacetone phosphate and five phosphono analogues of glyceraldehyde 3-phosphate through α -, β - and γ -hydroxyphosphonate esters precursors containing a protected carbonyl group. In some situations, depending on the sequence used for the deprotection of the phosphonate and carbonyl groups, the aldol/ketol rearrangement allowed the synthesis of either dihydroxyacetone phosphate or glyceraldehyde 3-phosphate analogues from the same precursors. All these analogues are of interest both as active-site probes and as potential substrates for glycolytic enzymes such as fructose 1,6-diphosphate aldolases (EC 4.1.2.13). © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Glycolytic enzymes fructose-1,6-diphosphate aldolases reversibly catalyze the production of D-fructose-1,6diphosphate (FDP) from D-glyceraldehyde 3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP) (Scheme 1).^{1,2} The enzyme from rabbit muscle (EC 4.1.2.13), currently in use for carbohydrate synthesis,³ accepts a large variety of aldehydes as substrates, but is rather selective toward DHAP since only limited structural modifications are accepted.⁴ In addition to the synthetic purposes in organic chemistry, aldolases are also considered as targets for the development of new antiparasitic drugs since glycolysis is the only source of energy for parasites such as trypanosomes (bloodstream form).⁵ Among aldolase inhibitors,⁶ those corresponding to substrate analogues are of interest as active-site probes of the enzyme and for the design of a new class of inhibitors.7-10

Phosphono analogues of natural phosphates display modified chemical and biological properties, and are of high interest to biology and medicine.^{11,12} Along these lines, the synthesis of six phosphono analogues of DHAP and five phosphono analogues of G-3-P can be reported. The two key reactions consist of a regiospecific ring opening of two appropriate epoxides by

phosphorus-containing nucleophiles in presence of $BF_3 \cdot OEt_2$,^{13,14} or the addition of the latter to an aldehyde derivative, allowing the formation of a carbon–phosphorus bond of the α -, β - and γ -hydroxy phosphonate ester precursors. Furthermore, considering that DHAP and G-3-P are isomeric, their phosphono analogues are quantitatively prepared from the same precursor by taking advantage of the α -ketol rearrangement.¹⁵ Besides their specific interest for the study of aldolases and other glycolytic enzymes (triose phosphate isomerase, glycerol 3-phosphate dehydrogenase...), these analogues are potential substrates of aldolases, hence leading, in addition, to other analogues.^{16–18}

Results

Syntheses of DHAP analogues (7a,b and 9a,b) from phosphonate esters 4a and 4b

As shown in Scheme 2, ketalization,^{19,20} dehydrohalogenation,^{20,21} followed by quantitative epoxidation²² of the starting 2-chlorobutanone gave epoxide **3**. The ring opening of the latter compound by phosphite or phosphonate anion in presence of BF₃·OEt₂ yielded β - and γ -hydroxyphosphonate esters **4a** and **4b**. This reaction also produced the formation of the by-products **4'a** or **4'b**, resulting from solvent (THF) insertion,^{13,14} more especially with the phosphite anion, where **4'a** represents about 50% of the products.²³ Oxidation of **4a** and **4b** according to the Pfitzner–Moffat method²⁴ produced ketones **5a** and **5b**.²⁵ Catalytic hydrogenation

Key words: Phosphonic acids and derivs; isosteres; substituent effects; enzymes and enzyme reactions.

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Scheme 2. Synthetic scheme for the synthesis of compounds **9a,b** and **7a,b**. (a) HOCH₂CH₂OH, TsOH, benzene, 82%; (b) KOH, HOCH₂CH₂OH, 130 °C (82%); (c) *m*-CPBA, CH₂Cl₂ (98%); (d) (BnO)₂P(O)H or (BnO)₂P(O)CH₃, *n*-BuLi, Et₂O·BF₃, THF, -80 °C (*n*=1: 40%, *n*=2: 75%); (e) DCC, pyridine, DMSO, TFA (*n*=1: 79%, *n*=2: 85%); (f) 1. H₂, Pd/C, MeOH; 2. Ba(OH)₂ (*n*=1: 92%, *n*=2: 97%); (g) DOWEX 50WX8 (H⁺), H₂O; (h) 1. DOWEX 50WX8 (H⁺), H₂O; 2. Ba(OH)₂ (*n*=1: 97%, *n*=2: 97%).

removed the benzyl protecting group to give the corresponding phosphonic acids **6a**, **6b**, **8a** and **8b**, which were isolated as barium salts. Subsequently, deketalization led to α -hydroxyketone phosphonates **7a** and **7b**, and α -diketophosphonic acids **9a** and **9b**. The attempts to isolate the two latter compounds as barium or sodium salts failed, and gave decomposition products due likely to possible reaction of the formed phosphonate anion on one of the two dicarbonyl groups. However, **9a** and **9b** could be stored without decomposition in an aqueous acidic medium.

Analogues of G-3-P (16a–c and 18a,b) and of DHAP (19a,b) from phosphonate esters 13a–d

Several methods were examined to prepare key epoxide (\pm) glycidaldehyde diethyl acetal **11** (vide infra) from acroleine diethyl acetal (see Scheme 3): (a) oxidation by *m*-chloroperbenzoic acid²⁴ was not quantitative, due likely to the poor stability of epoxide **11** in acidic medium, (b) in contrast, the reaction carried out in neutral conditions with hydrogen peroxide in presence of benzonitrile²⁶ gave a fair yield epoxide, (c) this epoxide could also be obtained in two steps with diol **10** as an intermediate,²⁷ (d) through 3-*O*-mesylate²⁷ or **10**, or (e) using the Mitsunobu reaction.^{18,28}

As shown in Scheme 4, the β - and γ -hydroxyphosphonate esters 13a and 13b were obtained by ring opening of the epoxide 11, respectively, by phosphite and phosphonate anion catalyzed by BF₃·OEt₂.^{13,14} As for compound 4a, the 13a yield was significantly lowered because of a side reaction with the solvent. Nevertheless, its parent phosphonate ester 13c could be quantitatively obtained in another way using aldehyde 12 (easily formed from glyoxal²⁹) and lithium diethyl methylphosphonate carbanion. Additionally, this aldehyde reacted also with diethylphosphite anion to give the α -hydroxyphosphonate ester **13d** also in high yield. Oxidation (step d), deprotection of the phosphonate moiety with trimethylsilvl bromide (step f),³⁰ followed by deketalization (step h) of phosphonate esters 13a and 13b yielded G-3-P analogues 18a and 18b.³¹ Deprotection of the phosphonate group of compounds 13b-d (step e) in basic conditions³² gave the corresponding phosphonic acids 15a-c as lithium salts; subsequently

$(EtO)_{2}CH \xrightarrow{a/b} (EtO)_{2}CH \xrightarrow{O} (E$

Scheme 3. Synthetic scheme for the synthesis of glycidaldehyde diethyl acetal 11. (a) *m*-CPBA, CH₂Cl₂, 0 °C to rt (5–10%); (b) H₂O₂, PhCN, KHCO₃, MeOH (70%); (c) KMnO₄, H₂O (54%); (d) 1. CH₃SO₂Cl, pyridine; 2. NaOH, H₂O (56%); (e) DEAD, Ph₃P, benzene (77%).

deketalization (step g) yielded α -hydroxyaldehyde phosphonates **16a–c**. In contrast, the deprotection of the carbonyl group of **13b** and **13c** carried out in more acidic conditions (step i) gave α -hydroxyketonephosphonate esters **19a** and **19b** analogues of DHAP.^{33–35} The reversibility of this reaction has been previously demonstrated³³ and can be rationalized in terms of an α -ketol rearrangement.¹⁵ In its turn, deprotection of the phosphonate esters **19a** and **19b** provided the corresponding phosphonic acid analogues of DHAP.³³

Relative reactivities of G-3-P and DHAP analogues with DHAP and G-3-P, respectively

Phosphonic analogues of G-3-P (16a–c and 18a,b) and DHAP (7a,b and 19a,b) have been tested as substrates with rabbit muscle aldolase. The progress of the aldose-catalyzed condensation was followed as previously described^{4,18} and the initial relative rates (V_{rel}) for the different reactions expressed as a percentage of the initial relative velocity with the natural substrates G-3-P or DHAP, are given in Table 1.

Acceptable relative rates are obtained with the aldehyde derivatives and these substrates are applicable to a large-scale synthesis. In contrast, the data obtained with the DHAP analogues show that only the phosphonate isostere of DHAP (**19b**) is substrate for aldolase.¹⁷ These results represent new examples that confirm the high selectivity of muscle aldolase toward the DHAP structure for the enzyme-catalyzed aldol condensation as previously suggested by other works.^{4,9,16}

Discussion

Whereas the access to DHAP analogues through diazoketones is well documented,^{8,9,27} the syntheses of phosphonate analogues is more limited. The present work supplements this route with, as a key step, the formation of α -, β - and γ -hydroxyphosphonate ester intermediates (**4a,b** and **13a–d**), which allow both DHAP and G-3-P analogues.

Regiospecific ring opening of epoxides 3 and 11 by phosphite or methane phosphonate anions in presence of $BF_3 \cdot OEt_2$ furnished most of the intermediates. However, we observed solvent participation in the progress of the reaction, more especially when phosphite anion was used, and consequently 4a and 13a yields were lowered. Conversely, we found the use of aldehyde derivative 12 to be superior to that of epoxide 11 as a nucleophile acceptor leading to the formation of intermediates 13c and 13d, quantitatively.

The scope of these syntheses is enlarged by the opportunity given with the α -ketol rearrangement that allows, depending on the deprotection conditions, access either to DHAP or to G-3-P analogues from the same **13b** and **13c** intermediates. Moreover, some DHAP (**7a**, **9a** and **19a**) and G-3-P (**16a**, **16c** and **18a**) analogues described here are only possible or stable as phosphonate and have no equivalent in the phosphate series (e.g. **7a**,



Scheme 4. Synthetic scheme for the synthesis of compounds **16a–c**, **18a,b** and **19a,b**. (a) (EtO)₂P(O)H or (EtO)₂P(O)CH₃, *n*-BuLi, Et₂O·BF₃, THF, -80 °C (*n*=1: 46%, *n*=2: 80%); (b) *n*-BuOH, hexane, DOWEX 50WX8 (H⁺), reflux (62%); (c) (EtO)₂P(O)CH₃ or (EtO)₂P(O)H, *n*-BuLi, THF, -80 °C (*n*=1: 93%, *n*=0: 77%); (d) DCC, pyridine, DMSO, TFA, benzene (*n*=1: 76%, *n*=2: 89%); (e) LiOH, NaBH₄, H₂O, 120 °C, 2 Bar (*n*=1: 90%, *n*=2: 96%, *n*=0: 92.5%); (f) 1. Me₃SiBr, 24 h, -30 °C; 2. H₂O, Ba(OH)₂ (*n*=1: 92%, *n*=2: 96%); (g) 1. DOWEX 50WX8 (H⁺), H₂O; 2. NaOH or LiOH (*n*=1: 96%, *n*=2: 96%, *n*=0: 91%); (h) DOWEX 50WX8 (H⁺), H₂O; (i) HCl 0.1 M, 45 °C (*n*=1: 54%, *n*=2: 70%).

which presents a specific interest by its reversed structure, compared to that of DHAP). This is of particular significance for synthetic purposes, as well as for mechanistic studies of FDP-aldolases and possibly other glycolytic enzymes that operate with DHAP or G-3-P as substrates. The same routes can be used to prepare chiral compounds starting from chiral epoxides such as D-glycidaldehyde and diethylacetal obtained from fructose^{18,26} or D-3-chloro-2-hydroxypropanal.³⁶

Experimental

Materials and methods

Chemicals and solvents were reagent grade. The NMR spectra were recorded in CDCl₃ or D₂O on a Bruker AC80 (80-MHz ¹H NMR) or a Bruker AC200 (200-MHz ¹H NMR, 50-MHz ¹³C NMR and 81-MHZ ³¹P NMR) spectrometer. All chemical shifts are reported in

Table 1. Relative reactivities of G-3-P and DHAP analogues with DHAP and G-3-P, respectively, in rabbit muscle aldolase-catalyzed aldol condensations

Compounds	$V_{\rm rel}{}^{\rm a}$	Substrate class ^b
G-3-P	100 ^c	+ + +
16a	36	+ + +
16b	41	+ + +
16c	7	+
18a	26	+ + +
18b	28	+ + +
DHAP	100	+ + +
7a	< 0.01	
7b	< 0.01	
19a	< 0.01	
19b	6.1 (10) ^d	+

^a Reactivities were measured in 0.1 M triethanolamine buffer (pH 7.0, 25 °C) containing both substrates at 50 mM concentration.

^b Substrate class according to the scale previously used: $(+ + +) V_{rel} > 25; (+ +) 25 > V_{rel} > 1; (+) 10 > V_{rel} > 1; (-) V_{rel} < 1.$

 $^{\circ}V_{\text{rel}}$ for the D stereoisomer.

^d Value from literature.^{4,16}

parts per million with respect to TMS for ¹H and ¹³C spectra and H_2PO_4 for ³¹P as internal standards. Elemental analyses were performed by the Ecole Nationale Supérieure de Chimie de Toulouse. Flash chromatography was performed on a Merck Geduran SI 60 (0.040–0.063 mM).

Enzymatic aldol condensations and kinetic measurements

Enzymatic assays and kinetic measurements were carried out by the procedure previously described.^{4,18}

Chemical syntheses

 (\pm) -2-(1-Chloro-ethyl)-2-methyl-1,3-dioxolane (1). To a round-bottomed flask equiped with a Dean-Stark system and containing 30 g (0.24 mol) of 3-chloro-2-butanone in 400 mL of benzene, were added 15 g (0.325 mol) of ethylene glycol and 1 g (5.74 mmol) of *p*-toluene sulfonic acid monohydrate. The mixture was refluxed and stirred for 20 h and the solvent removed under reduced pressure. The residue was dissolved in CH₂Cl₂ (250 mL) and the solution washed with saturated NaHCO₃ and brine. The organic layer was dried (Na_2SO_4) and concentrated. The remaining oil was distilled at 43 °C (1 mm Hg) to give 1 as a colourless oil (30.1 g, 83%). ¹H NMR $(CDCl_3) \delta 1.39 (s, 3H), 1.48 (d, J = 6.8 Hz, 3H), 3.94 (q, J = 6.8 Hz), 3.94 (q, J$ J = 6.8 Hz, 1H) 3.98 (s, 4H); ¹³C NMR (CDCl₃) δ 20.0, 20.1, 60.9, 65.4, 65.6, 110.1. Anal. calcd for C₆H₁₁O₂Cl: C, 47.8; H, 7.36; O, 21.2. Found: C, 47.6; H, 7.4; O, 20.9.

2-Methyl-2-vinyl-1,3-dioxolane (2). Compound **1** (5 g, 33.2 mmol) was added dropwise to a stirred solution of KOH (12 g, 0.214 mol) in ethylene glycol (24 mL) at 125–130 °C. After 5 h of warming, the mixture was purified by distillation at 110–112 °C (760 mm Hg) to yield **2** as a colourless oil (3.1 g, 82%). IR (film) n(C=C) cm⁻¹: 1675. ¹H NMR (CDCl₃) δ 1.44 (s, 3H), 3.9–4.0 (m, 4H), 5.12 (dd, J=1.7 Hz, J=10.5 Hz, 1H), 5.45 (dd, J=1.7 Hz, J=17.2 Hz, 1H), 5.8 (dd, J=10.5 Hz, J=

17.2 Hz, 1H); ¹³C NMR (CDCl₃) δ 24.6, 64.5, 107.4, 114.8, 136.4. Anal. calcd for C₆H₁₀O₂: C, 63.1; H, 8.83; O, 28.1. Found: C, 62.9; H, 8.88; O, 28.5.

 (\pm) -2-Methyl-2-oxiranyl-1,3-dioxolane (3). A mixture of 2 (3.1 g, 0.27 mmol) and *m*-chloroperbenzoic acid (25 g, 0.144 mmol) in 200 mL of CH₂Cl₂ was stirred at room temperature for 5 days. Saturated NaHCO₃ (100 mL) was added to the mixture which was cooled to 0 °C. The aqueous layer was separated and extracted with CH₂Cl₂ $(3 \times 200 \text{ mL})$. The organic fractions were combined, washed with 3% Na₂S₂O₃, saturated NaHCO₃ and water, dried over anhydrous MgSO4, and evaporated under reduced pressure. The remaining residue was purified by flash distillation (80 °C, 5 mm Hg) to yield 3 as a colourless oil (3.45 g, 98%). ¹H NMR (CDCl₃) δ 1.29 (s, 3H), 2.59 (d, J=3.4 Hz, 2H), 2.93 (t, J=3.4 Hz, 1H), 3.8–3.9 (m, 4H); ¹³C NMR (CDCl₃) (21.8, 43.75, 54.9, 65.5, 66.0, 106.6. Anal. calcd for $C_6H_{10}O_3$: C, 55.3; H, 7.74; O, 36.9. Found: C, 55.3; H, 7.6; O, 36.7.

(±)-Dibenzyl [2-(2-methyl-1,3-dioxolan-2-yl)-2-hydroxyethyll-phosphonate (4a). A 1.6 M solution of *n*-BuLi in hexane (19 mL, 30.4 mmol) was added dropwise to a stirred solution of dibenzylphosphite (6.0 g, 23 mmol) in dry THF (60 mL) at -80 °C. After 30 min of stirring, the mixture was added dropwise to a stirred solution of 3 (1 g, 7.68 mmol) in dry THF (40 mL) at -80 °C. After 15 min of stirring, BF₃·OEt₂ (3.8 mL, 30.9 mmol) was slowly introduced. The reaction mixture was stirred for 2 h at -70 °C, then overnight at room temperature. Saturated NH₄Cl (20 mL) was added, the solvents were removed under reduced pressure and the remaining residue dissolved in 100 mL of ethyl acetate. The organic solution was washed with brine, dried over MgSO₄, then concentrated and purified by flash chromatography (CH₂Cl₂:MeOH, 98:2 to 96:4) to provide 4a as a colourless oil (1.2 g, 40%). ³¹P NMR (CDCl₃) δ 32.0; ¹H NMR (CDCl₃) δ 1.27 (s, 3H), 1.5–1.9 (m, 2H), 3.1 (br s, 1H, D₂O exchangeable), 3.8–4.0 (m, 5H), 4.9– 5.2 (m, 4H), 7.33 (s, 10H); ¹³C NMR (CDCl₃) δ 19.7, 28.7 (d, ¹*J*_{CP} = 142 Hz), 65.1, 65.5, 67.4, 67.6, 70.2, 110.8 (d, ${}^{3}J_{CP} = 19.2 \text{ Hz}$), 128.4, 128.7, 136.3. Anal. calcd for C₂₀H₂₅O₆P: C, 61.2; H, 6.38. Found: C, 61.4; H, 6.3.

(±)-Dibenzyl [3-(2-methyl-1,3-dioxolan-2-yl)-3-hydroxypropyl]-phosphonate (4b). Compound 4b was prepared from 3 (1 g, 7.68 mmol) in 75% yield (2.34 g) by following the same procedure described for 4a, using dibenzylmethylphosphonate (6.4 g, 23 mmol) in place of dibenzylphosphite. ³¹P NMR (CDCl₃) (34.1; ¹H NMR (CDCl₃) δ 1.24 (s, 3H), 1.5–2.3 (m, 4H), 2.5 (m, 1H, D₂O exchangeable), 3.5 (m, 1H), 3.92 (m, 4H), 4.9–5.2 (m, 4H), 7.33 (s, 10H); ¹³C NMR (CDCl₃) δ 19.58, 22.7 (d, ¹J_{CP}=142 Hz), 24.2, 65.1, 65.3, 67.2, 74.9 (d, ³J_{CP}=14.3 Hz), 110.2, 127.6, 128.2, 136.5. Anal. calcd for C₂₁H₂₇O₆P: C, 62.06; H, 6.65; O, 23.6. Found: C, 62.4; H, 6.62; O, 23.8.

Dibenzyl [2-(2-methyl-1,3-dioxolan-2-yl)-2-oxo-ethyl]phosphonate (5a). To a stirred mixture of dicyclohexylcarbodiimide (0.95 g, 4.59 mmol) and dry pyridine

(0.18 mL, 2.22 mmol) in dry benzene (20 mL) was added dropwise 4a (0.40 g, 1.02 mmol) in dry DMSO (3 mL) under nitrogen atmosphere. Trifluoroacetic acid (0.090 mL, 1.1 mmol) was added and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the remaining residue dissoved in 60 mL of ether. The suspension (dicyclohexylurea) was filtered off and the filtrate washed with brine $(3 \times 30 \text{ mL})$. The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The residue was chromatographed (CH₂Cl₂: MeOH, 95:5) to yield 5a as a colourless oil (0.30 g, We OI1, 95.5) to yield 5a as a colourless on (0.55 g, 75%). ³¹P NMR (CDCl₃) δ 21.3; ¹H NMR (CDCl₃) δ 1.41 (s, 3H), 3.26 (d, ²J_{HP}=22 Hz, 2H), 3.8–4.0 (m, 4H), 5.0–5.1 (m, 4H), 7.34 (s, 10H); ¹³C NMR (CDCl₃) δ 20.32, 35.88 (d, ¹J_{CP}=135 Hz) 65.62, 68.0, 107.6 (d, 20.32, 20.32, 100.6 (m, 120.6 120.6 120.6 120.6 (m, 120.6 120.6 120.6 120.6 (m, 120.6 120.6 120.6 (m, 120 ${}^{3}J_{CP} = 5$ Hz), 128.4, 128.6, 136.0, 199.6. Anal. calcd for C₂₀H₂₃O₆P: C, 61.5; H, 5.89; O, 24.6. Found: C, 61.7; H, 5.8; O, 24.8.

Dibenzyl [3-(2-methyl-1,3-dioxolan-2-yl)-3-oxo-propyl]phosphonate (5b). Compound 5b was prepared from 4b (0.90 g, 2.2 mmol) in 78% yield (0.70 g) by following the same procedure described as for 5a, using 1.37 g (6.66 mmol) of dicyclohexylcarbodiimide and 4 mL of dry DMSO. ³¹P NMR (CDCl₃) δ 32.7; ¹H NMR (CDCl₃) δ 1.28 (s, 3H), 1.8–2.1 (m, 2H), 2.6–2.8 (m, 2H) 3.7–4.0 (m, 4H), 4.8–5.1 (m, 4H) 7.25 (s, 10H); ¹³C NMR (CDCl₃) δ 19.37 (d, ¹J_{CP}=144 Hz), 20.6, 29.5, 65.5, 67.4, 107.5, 127.9, 128.5, 136.2, 205.2 (d, ³J_{CP}= 14.3 Hz). Anal. calcd for C₂₁H₂₅O₆P: C, 62.37; H, 6.19; O, 23.76. Found: C, 62.39; H, 6.25; O, 23.9.

 (\pm) -[2-(2-Methyl-1,3-dioxolan-2-yl)-2-hydroxy-ethyl]-phosphonic acid, barium salt (6a). To a suspension of Pd:C (10%, 50 mg) in a solution of water: EtOH (1:1, 20 mL), was added 4a (0.10 g, 0.255 mmol). The mixture was degassed and hydrogenated for 12h at atmospheric pressure. The catalyst was filtered off and the pH was adjusted to 7.6 with saturated $Ba(OH)_2$. The solution was then freeze-dried, and the residue dissolved in 8 mL of distilled water. The suspension was discarded by centrifugation, barium salt 6a was precipitated by addition of ethanol (24 mL) and the resulting mixture was kept at 0°C for 3h. The salt was collected by centrifugation, washed twice with ethanol (80%, then absolute), ethyl ether and dried in vacuo to yield 6a (0.082 g, 92%). ³¹P NMR (D₂O) δ 21.2; ¹H NMR (D₂O) δ 1.31 (s, 3H), 1.5–1.9 (m, 2H), 3.8–4.0 (m, 1H), 4.02 (s, 4H); ¹³C NMR (D₂O) δ 21.9, 34.0 (d, ¹J_{CP} = 135 Hz), 67.6, 67.9, 73.7, 109.1. Anal. calcd for C₆H₁₁O₆PBa: C, 20.75; H, 3.17; O, 27.66. Found: C, 20.6; H, 3.26; O, 27.75.

(±)-[3-(2-Methyl-1,3-dioxolan-2-yl)-3-hydroxy-propyl]phosphonic acid, barium salt (6b). Compound 6b was prepared from 4b (0.15 g, 3.69 mmol) in 90% yield (0.120 g) by following the same procedure described for 6a. ³¹P NMR (D₂O) δ 23.5; ¹H NMR (D₂O) δ 1.30 (s, 3H), 1.35–2.0 (m, 4H), 3.5 (m, 1H), 4.0 (s, 4H); ¹³C NMR (D₂O) δ 21.75, 28.58 (d, ¹J_{CP}=131 Hz), 28.68, 67.4, 77.9 (d, ³J_{CP}=14.9 Hz), 113.3. Anal. calcd for C₇H₁₃O₆PBa: C, 23.37; H, 3.6; O, 26.6. Found: C, 23.4; H, 3.55; O, 26.4. (\pm) -2-Hydroxy-3-oxo-butyl-1-phosphonic acid, barium salt (7a). Salt 6a (0.050 g, 0.144 mmol) was treated with 0.5 mL of DOWEX 50 WX8 (H⁺) in 0.5 mL of water for 10 min. The resin was discarded by centrifugation and washed with water $(3 \times 0.25 \text{ mL})$. The aqueous solutions were combined and incubated for 4 days at room temperature. The progress of hydrolysis was monitored by ³¹P NMR. The pH was adjusted to 7.4 with saturated Ba(OH)₂ and the solution was freezedried. The resulting powder was washed with ether and dried in vacuo to give 7a as a white powder (0.045 g, 97%). ³¹P NMR (D_2O) δ 18.2; ¹H NMR (D_2O) δ 1.5– 1.9 (m, 2H), 2.1 (s, 3H), 3.3–3.5 (m, 1H); ¹³C NMR $(D_2O) \delta 30.9, 34.3 \text{ (d, } {}^{1}J_{CP} = 131 \text{ Hz}), 76.8, 209.0 \text{ (d,}$ ${}^{3}J_{CP}$ = 14.9 Hz). Anal. calcd for C₄H₇O₅PBa: C, 15.8; H, 2.3; O, 26.4. Found: C, 15.6; H, 2.2; O, 26.8.

(±)-3-Hydroxy-4-oxo-pentyl-1-phosphonic acid, barium salt (7b). Compound 7b was obtained from 6b (0.100 g, 0.277 mmol) in 97% yield (0.090 g) by following the same procedure described for 7a. ³¹P NMR (D₂O) δ 24.45; ¹H NMR (D₂O) δ 1.5–2.0 (m, 4H), 2.08 (s, 3H), 3.2–3.5 (m, 1H); ¹³C NMR (D₂O) δ 26.31 (d, ¹*J*_{CP}= 133 Hz), 28.2, 29.9, 79.9 (d, ³*J*_{CP}=15.2 Hz), 217.5 Anal. calcd for C₅H₉O₅PBa: C, 18.9; H, 2.8; O, 25.2. Found: C, 18.7; H, 2.78; O, 25.4.

[2-(2-Methyl-1,3-dioxolan-2-yl)-2-oxo-ethyl]-phosphonic acid, barium salt (8a). Compound 8a was obtained from 5a (0.200 g, 0.513 mmol) in 79% yield by following the same procedure as described for 6a. ³¹P NMR (D₂O) δ 12.0; ¹H NMR (D₂O) δ 1.46 (s, 3H), 3.1 (d, ²J_{PH}=20 Hz, 2H) 3.8–4.1 (m, 4H); ¹³C NMR (D₂O) δ 22.6, 39.7 (d, ¹J_{CP}=134 Hz), 67.6, 111.0 (d, ³J_{CP}= 10.0 Hz), 201.4. Anal. calcd for C₆H₉O₆PBa: C, 20.87; H, 2.6; O, 27.82. Found: C, 21.0; H, 2.62; O, 27.65.

[3-(2-Methyl-1,3-dioxolan-2-yl)-3-oxo-propyl]-phosphonic acid, barium salt (8b). Compound 8b was obtained from 5b (0.190 g, 0.47 mmol) in 85% yield (0.150 g) by following the same procedure described for 6a. ³¹P NMR (D₂O) δ 23.1; ¹H NMR (D₂O) δ 1.48 (s, 3H), 1.5–1.7 (m, 2H), 2.65 (m, 2H), 3.85–4.1 (m, 4H); ¹³C NMR (D₂O) δ 22.9, 24.4 (d, ¹J_{CP} = 132 Hz), 34.6, 68.0, 110.6, 212.0 (d, ³J_{CP} = 14.0 Hz). Anal. calcd for C₇H₁₁O₆PBa: C, 23.4; H, 3.06; O, 26.76. Found: C, 23.6; H, 3.12; O, 26.9.

2,3-Dioxo-butyl-1-phosphonic acid (9a). Salt **8a** (0.140 g, 0.405 mmol) was treated with 1 mL of DOWEX 50 WX8 (H+) in 1 mL of water for 10 min. The resin was discarded and washed three times with water (0.50 mL). The aqueous solutions were combined and incubated for 7 days at 100 °C. The progress of the hydrolysis was monitored by ³¹P and ¹³C NMR. The title compound, did not show decomposition, was used for enzymatic studies without further purification. ³¹P NMR (D₂O+H₂O) δ 16.1; ¹³C NMR (D₂O+H₂O) δ 25.8, 38.5 (d, ¹*J*_{CP}=129 Hz, CH₂-P), 90.6 (C2, hydrated form), 208.2 (d, ³*J*_{CP}=23.0 Hz).

3,4-Dioxo-pentyl-1-phosphonic acid (9b). Compound **9b** was prepared from **8b** by following the same procedure as described for **9a**. ³¹P NMR ($D_2O + H_2O$) δ 26.5; ¹³C

NMR (D₂O + H₂O) δ 22.7 (d, ¹*J*_{CP} = 139 Hz), 26.3, 32.9, 98.9 (d, ³*J*_{CP} = 21.0 Hz, C3, hydrated form), 209.0.

 (\pm) -Glyceraldehyde diethyl acetal (10). To a stirred solution of acroleine diethylacetal (10g, 77 mmol) in 85 mL of distilled water was added dropwise 13 g (84.6 mmol) of KMnO₄ in 300 mL of water over 1.5 h. The reaction mixture was stirred for 3h at room temperature then refluxed for 1 h. After cooling, the precipitate was filtered off and K₂CO₃ (170 g) was added to the filtrate and the aqueous solution was extracted with ethyl acetate $(3 \times 100 \text{ mL})$. The organic layer was dried over MgSO₄ and evaporated under reduced pressure. The remaining residue was purified by flash chromatography (CH₂Cl₂:MeOH, 95:5) to give 10 as a colourless oil (6.9 g, 54%). ¹H NMR (CDCl₃) δ 1.17 (s, 6H), 2.6 (m, 2H, D₂O exchangeable), 3.4-3.9 (m, 7H, CH-O, CH₂O), 4.44 (d, ${}^{3}J_{HH} = 7.0 \text{ Hz}$, 1H, O-CH-O); ${}^{13}C$ NMR (CDCl₃) δ 15.3, 62.5, 63.6, 64.2, 71.8, 103.4. Anal. calcd for C₇H₁₆O₄: C, 51.21; H, 9.82; O, 39.02. Found: C, 51.17; H, 10.01; O, 39.20.

(\pm) -Glycidaldehyde diethyl acetal (11). Three different methods were used to obtain the title compound.

Method A: To a stirred solution of acetal 10 (2.25 g, 13.7 mmol) in dry pyridine (10 mL) cooled at -10° C was added dropwise methane sulfonyl chloride (1.72 g, 15 mmol) over 20 min. The reaction mixture was kept at 0°C for 2h, then at room temperature overnight. The solvent was removed under reduced pressure and the remaining residue dissolved in 20 mL of CH₂Cl₂. The organic solution was washed with saturated NaHCO₃ $(3 \times 50 \text{ mL})$, brine (50 mL), dried over MgSO₄, and concentrated under reduced pressure to yield the 3-Omesylate of 10 as a colourless oil (3.0 g, 90%), which was used in the next step without further purification; the ¹H and ¹³C NMR spectrum were consistent with those reported in the literature.²⁷ To a stirred solution of the 3-O-mesylate of 10 (2.9 g, 12 mmol) in 20 mL of water at 30 C containing phenolphtalein was added a solution of 1 N NaOH over 90 mN to keep th pH of the mixture slightly basic. The progress of the reaction was monitored by TLC (CH₂Cl₂:MeOH, 98:2). The reaction mixture was extracted with ethylacetate $(3 \times 20 \text{ mL})$. The organic layer was washed with brine, dried over MgSO₄ and concentrated under reduce pressure. The resulting residue was purified by flash distillation at 70°C (15-16 mm Hg) to yield 11 as a colourless oil (1.10 g, 56%) based on glyceraldehyde diethyl acetal).

Method B: Diethyl azido dicarboxylate (2.40 g, 13.8 mmol) was added dropwise to a stirred mixture of triphenylphosphine (3.11 g, 11.82 mmol) and acetal **10** (1.80 g, 11 mmol) in dry benzene (40 mL) under nitrogen atmosphere. An exothermic reaction was observed. The reaction mixture was stirred overnight at room temperature. The benzene was removed under reduced pressure and the remaining residue purified by flash distillation to yield **11** (1.26 g, 78%).

Method C: To a stirred solution of acrolein diethyl acetal (25 g, 192 mmol) in 100 mL of methanol was

added KHCO₃ (3 g, 30 mmol), benzonitrile (20.4 g, 200 mmol) then 30% hydrogen peroxide (23.4 g, 206 mmol). The reaction mixture was stirred for 15 h at room temperature then 4 h at 40 °C. Water (75 mL) was added and most of the methanol was removed under reduced pressure. The aqueous mixture was extracted with CH_2Cl_2 (4×100 mL), the combined organic layers dried over MgSO₄ and concentrated under reduced pressure. The resulting residue was extracted with 60 mL of hexane, the insoluble product (benzamide) filtered out and the solvent concentrated. The residue was purified by flash distillation to give 11 (19.6 g, 70%). 1 H NMR (CDCl₃) δ 1.29 and 1.33 (2t, J = 7 Hz, 2 CH₃), 2.53 (m, 2H, CHCH₂), 2.86 (m, 1H, CH-O), 3.2-3.6 (m, 4H, CH₂CH₃) 4.1 (d, J=4.3 Hz, 1H, CH); ¹³C NMR (CDCl₃) δ 15.0, 43.5, 51.6, 62.0, 62.6, 101.4. Anal. calcd for C₇H₁₄O₃: C, 57.52; H, 9.65; O, 32.87. Found: C, 57.1; H, 9.82; O, 33.24.

Di *n*-butoxy-2,2 ethanal (12). A mixture of 40% glyoxal (36.25 g, 0.25 mol), 230 mL of butanol, 325 mL of hexane and 25 g of DOWEX 50 WX8 (H+) was refluxed for 2h in a round-bottomed flask equipped with a Dean-Stark system. The mixture reaction was cooled to room temperature, the resin filtered off and NaHCO₃ (12.5 g) added. After standing for 30 min, the precipitate was filtered off and the solvent removed under reduced pressure. The resulting residue was purified by flash distillation at 70 °C (2 mm Hg) to yield 12 as a colourless oil (29.1 g, 62%). ¹H NMR (CDCl₃) δ 0.89 (t, J=7 Hz, 2 CH₃), 1.5 (m, 8H, CH₂), 3.6 (m, 4H, CH₂O), 4.5 (d, J=2.1 Hz, 1H, O-CH-O), 9.4 (d, J=2.1 Hz, 1H, CHO); ¹³C NMR (CDCl₃) δ 13.8, 19.2, 31.8, 67.3, 101.2, 196.9. Anal. calcd for C₁₀H₂₀O₃: C, 63.83; H, 10.64; O, 25.53. Found: C, 64.0; H, 10.72; O, 25.41.

(±)-Diethyl (3,3-diethoxy-2-hydroxy-propyl)-1-phosphonate (13a). Compound 13a was prepared from the acetal 11 (0.80 g, 5.48 mmol) in 46% yield (0.720 g) by following the same procedure as described for 4a, using diethyl phosphite in place of dibenzyl phosphite. ³¹P NMR (CDCl₃) δ 30.8; ¹H NMR (CDCl₃) δ 1.18 (t, 6H, CH₃ acetal), 1.31 (t, 6H, CH₃ ester), 1.9–2.1 (m, 2H, CH₂–P), 3.15 (s, 1H D₂O exchangeable), 3.5–3.8 (m, 5H, CH–O, OCH₂ acetal), 4.0–4.15 (m, 4H, CH₂O ester), 4.37 (d, 1H, CH acetal); ¹³C NMR (CDCl₃) δ 15.3, 16.4, 28.0 (d, ¹J_{CP}=141 Hz, CH₂–P), 61.8, 63.5, 64.0, 67.7, 104.1 (d, ³J_{CP}=17.2 Hz, CH acetal). Anal. calcd for C₁₁H₂₅O₆P: C, 46.5; H, 8.80; O, 33.8. Found: C, 46.6; H, 8.75; O, 33.6.

(±)-Diethyl (4,4-diethoxy-3-hydroxy-butyl)-1-phosphonate (13b). Compound 13b was prepared from acetal 11 (3 g, 20 mmol) in 76% yield (4.5 g) by following the same procedure as described for 4a, using diethylmethyl phosphonate (9.3 g, 61 mmol) instead dibenzyl phosphite. ³¹P NMR (CDCl₃) δ 30.8; ¹H NMR (CDCl₃) δ 1.15 and 1.17 (2t, 6H, CH₃ acetal), 1.27 (t, 6H, CH₃ ester), 1.4–2.1 (m, 4H, CH₂–CH₂–P), 2.5 (m, 1H D₂O exchangeable), 3.3–3.8 (m, 5H, CH–O, OCH₂ acetal), 4.0–4.15 (m, 4H, CH₂O ester), 4.21 (d, 1H, CH acetal); ¹³C NMR (CDCl₃) δ 15.4, 16.5, 21.8 (d, ¹J_{CP}=142 Hz, CH₂–P), 24.9, 61.6, 63.6, 71.5 (d, ³J_{CP}=15.2 Hz, CH–O), 104.8. Anal. calcd for $C_{12}H_{27}O_6P$: C, 48.3; H, 9.0; O, 32.2. Found: C, 47.9; H, 9.1; O, 31.6.

 (\pm) -Diethyl (3,3-dibutoxy-2-hydroxy-propyl)-1-phos**phonate** (13c). A 1.6 M solution of *n*-BuLi in hexane (20.6 mL, 33 mmol) was added dropwise to a stirred solution of diethylmethyl phosphonate (5.0 g, 33 mmol) in 25 mL of dry THF at -70 °C under nitrogen atmosphere. After 30 min of stirring at -70 °C, acetal 12 (5.64 g, 30 mmol) in 50 mL of dry THF was added dropwise. The reaction mixture was stirred for 1h at -20 °C then overnight at room temperature and quenched with saturated NH₄Cl (20 mL). The solvent was removed under reduced pressure and the residue dissolved in 100 mL of ethyl acetate. The organic solution was washed with brine, dried over MgSO₄ and concentrated. The resulting residue was purified by flash chromatography (CH₂Cl₂:MeOH, 96:4) to yield 13c as a colourless oil (9.5 g, 93%). ³¹P NMR (CDCl₃) δ 30.8; ¹H NMR (CDCl₃) δ 0.84 (t, 6H, CH₃ acetal), 1.25 (t, 6H, CH3 ester), 1.3-1.6 (m, 8H, CH₂), 1.65-1.95 (m, 2H, CH₂-P), 3.12 (s, 1H D₂O exchangeable), 3.4-3.7 (m, 4H, OCH₂ acetal), 3.95 (m, 1H, CH-O), 3.97-4.1 (m, 4H, CH₂O ester), 4.29 (d, 1H, CH acetal); ¹³C NMR (CDCl₃) δ 13.8, 16.4, 19.3, 28.0 (d, ${}^{1}J_{CP} = 142$ Hz, CH_2-P), 31.9, 61.7, 67.6, 67.7, 68.2, 104.3 (d, ${}^{3}J_{CP}=$ 17.4 Hz, CH acetal). Anal. calcd for C₁₅H₃₃O₆P: C₂ 52.9; H, 9.70; O, 28.2. Found: C, 52.8; H, 9.75; O, 28.3.

 (\pm) -Diethyl (2,2-dibutoxy-1-hydroxy-ethyl)-1-phosphonate (13d). Compound 13d was prepared from the acetal 12 (3.1 g, 22 mmol) in 77% yield (4.0 g) by following the same procedure as described for 13c, except that diethylphosphite was used in place of diethylmethylphosphonate. ³¹P NMR (CDCl₃) δ 21.3; ¹H NMR (CDCl₃) δ 0.9 (t, 6H, CH₃ acetal), 1.31 (t, 6H, CH₃ ester), 1.3-1.5 (m, 4H, CH₂), 1.5–1.7 (m, 4H, CH₂), 2.9 (dd, ${}^{3}J_{HH} =$ 5.5 Hz, ${}^{3}J_{\text{HP}} = 14.5$ Hz, 1H D₂O exchangeable), 3.45–3.8 (m, 4H, OCH₂ acetal), 3.85-4.0 (td, ${}^{3}J_{HH} = 5.0$ Hz, $^{2}J_{HP} = 5.0 \text{ Hz}, 1 \text{H}, \text{CH} = -0, 4.05 - 4.3 \text{ (m}, 4 \text{H}, \text{CH}_{2} \text{O}$ ester), 4.73 (dd, ${}^{3}J_{HH} = 5.1 \text{ Hz}$, ${}^{3}J_{HP} = 14.5 \text{ Hz}$, 1H, CH acetal); ¹³C NMR (CDCl₃) δ 13.9, 16.8, 19.3, 31.8, 63.0, 63.7, 67.4, 68.4, 69.0 (d, ${}^{1}J_{CP} = 157$ Hz, CH–P), 101.1 $(d, {}^{2}J_{CP} = 7.2 \text{ Hz}, \text{CH acetal})$. Anal. calcd for $C_{14}H_{31}O_{6}P$: C, 51.53; H, 9.51; O, 29.45. Found: C, 50.98; H, 9.82; O, 30.14.

Diethyl (3,3-diethoxy-2-oxo-propyl)-1-phosphonate (14a). Compound **14a** was prepared from **13a** (0.25 g, 0.88 mmol) in 76% yield (0.190 g) by following the same procedure as described for **5a**, using 0.18 g (2.22 mmol) of dicyclohexylcarbodiimide and 3.5 mL of dry DMSO. ³¹P NMR (CDCl₃) δ 20.2; ¹H NMR (CDCl₃) δ 1.22 (t, 6H, CH₃ acetal), 1.31 (t, 6H, CH₃ ester), 3.25 (d, ²J_{HP}=21.8 Hz, 2H, CH₂-P), 3.5–3.7 (m, 4H, OCH₂ acetal), 4.0–4.2 (m, 4H, CH₂O ester), 4.71 (s, 1H, O–CH–O); ¹³C NMR (CDCl₃) δ 13.6, 16.3, 35.7 (d, ¹J_{CP}=132 Hz, CH₂-P), 62.5, 63.6, 101.7 (d, ³J_{CP}= 9.8 Hz, CH acetal), 201.6. Anal. calcd for C₁₁H₂₃O₆P: C, 46.8; H, 8.15; O, 34.0. Found: C, 46.5; H, 8.27; O, 34.2.

Diethyl (4,4-diethoxy-3-oxo-butyl)-1-phosphonate (14b). Compound **14b** was prepared from **13b** (0.35 g, 1.17 mmol) in 89% yield (0.310 g) by following the same procedure described as for **5a**. ³¹P NMR (CDCl₃) δ 31.6; ¹H NMR (CDCl₃) δ 1.23 (t, 6H, CH₃ acetal), 1.32 (t, 6H, CH₃ ester), 1.8–2.2 (m, 2H, CH₂–P), 2.8–3.0 (m, 2H, CH₂C=O), 3.5–3.75 (m, 4H, OCH₂ acetal), 4.0–4.15 (m, 4H, CH₂O ester), 4.60 (s, 1H, CH acetal); ¹³C NMR (CDCl₃) δ 15.1, 16.4, 18.9 (d, ¹J_{CP}=145 Hz, CH₂–P), 30.2, 61.6, 63.5, 102.4, 202.4 (d, ³J_{CP}=15.4 Hz, C=O). Anal. calcd for C₁₂H₂₅O₆P: C, 48.65; H, 8.44; O, 32.4. Found: C, 48.8; H, 8.2; O, 32.6.

 (\pm) -3,3-Dibutoxy-2-hydroxy-propyl-1-phosphonic acid, dilithium salt (15a). A mixture of acetal 13c (2g, 5.88 mmol), LiOH monohydrate (1.72 g, 41 mmol) and NaBH₄ (0.2 g, 5.1 mmol) in 30 mL of distilled water was warmed for 12h under pressure (2 Bar). Lithium salt 15a was precipitated by addition of ethanol (90 mL) and the resulting mixture was kept at 0 °C for 3 h. The salt was collected by centrifugation, washed twice with ethanol (80%, then absolute), ethyl ether and dried in vacuo to yield **15a** as a white powder (1.56 g, 90%). ³¹P NMR (D₂O) δ 20.0; ¹H NMR (D₂O) δ 0.89 (t, 6H, CH₃ acetal), 1.25-2.0 (m, 10H, CH₂, CH₂-P), 3.6-3.8 (m, 4H, OCH₂ acetal), 3.8–4.0 (m, 1H, CH–O), 4.38 (d, 1H, O-CH-O); ¹³C NMR (D₂O) δ 15.8, 21.4, 33.2 (d, ${}^{1}J_{CP} = 127 \text{ Hz}, \text{ CH}_{2} - \text{P}), 33.8, 70.7, 71.1, 71.2, 108.0 (d,)$ ${}^{3}J_{CP} = 18.0 \text{ Hz}$, CH acetal). Anal. calcd for C₁₁H₂₃ O₆PLi₂: C, 44.59; H, 7.77; O, 32.4. Found: C, 44.82; H, 7.9; O, 32.72.

(±)-4,4-Diethoxy-3-hydroxy-butyl-1-phosphonic acid, dilithium salt (15b). Compound 15b was prepared from 13b (1.5 g, 5.06 mmol) in 96% yield (1.23 g) by following the same procedure as described for 15a. ³¹P NMR (D₂O) δ 22.9; ¹H NMR (D₂O) δ 1.22 (t, 6H, CH₃ acetal), 1.3–2.0 (m, 4H, CH₂–CH₂–P), 3.5–3.9 (m, 5H, CH–O, OCH₂ acetal), 4.46 (d, ³J_{HH}=4.5 Hz, 1H, O–OH–O); ¹³C NMR (D₂O) δ 17.1, 27.9 (d, ¹J_{CP}= 121 Hz, CH₂–P), 29.2, 66.7, 67.2, 75.3 (d, ³J_{CP}=14.0 Hz, CH–O) 107.3. Anal. calcd for C₈H₁₇O₆PLi₂: C, 37.8; H, 6.7; O, 37.8. Found: C, 37.65; H, 6.9; O, 38.1.

(±)-2,2-Dibutoxy-1-hydroxy-ethyl-1-phosphonic acid, dilithium salt (15c). Compound 15c was prepared from 13d (1.4 g, 4.6 mmol) in 92.5% yield by following the same procedure described as for 15a. ³¹P NMR (D₂O) δ 3.97; ¹H NMR (D₂O) δ 0.92 (t, 6H, CH₃ acetal), 1.25– 1.5 (m, 4H, CH₂), 1.5–1.7 (m, 4H, CH₂), 3.5–3.85 (m, 4H, OCH₂ acetal), 3.85–4.0 (m, 1H, CH–O), 4.70 (m, 1H, O–CH–O); ¹³C NMR (D₂O) δ 15.9, 21.5, 33.9, 70.5, 72.8 (d, ¹J_{CP}=154 Hz, CH–P), 102.4. Anal. calcd for C₁₀H₂₁O₆PLi₂: C, 42.55; H, 7.44; O, 34.04. Found: C, 42.32; H, 7.66; O, 34.47.

(±)-2-Hydroxy-3-oxo-propyl-1-phosphonic acid, disodium salt (16a). Compound 16a was prepared from 15a (0.050 g, 0.17 mmol) in 96% yield by following the same procedure described as for 7a, except that the pH was adjusted to 7.4 with 1 N NaOH, then freeze-dried. ³¹P NMR (D₂O) δ 20.6; ¹H NMR (D₂O) δ 1.6–1.95 (m, 2H, CH₂–P), 3.8–3.95 (m, 1H, CH–O), 4.88 (d, ³J_{HH}=4.4 Hz, 1H, HC=O, hydrated form); ¹³C NMR (D₂O) δ 32.3 (d, ¹J_{CP}=136 Hz, CH₂–P), 72.4, 94.4 (d, ${}^{3}J_{CP}$ = 16.4 Hz, C=O, hydrated form). Anal. calcd for C₃H₅O₅PNa₂, H₂O: C, 16.67; H, 3.24; O, 44.43. Found: C, 16.99; H, 3.45; O, 44.82.

(±)-3-Hydroxy-4-oxo-butyl-1-phosphonic acid, disodium salt (16b). Compound 16b was prepared from 15b (0.060 g, 0.235 mmol) in 96% yield (1.20 g) by following the same procedure described as for 16a. ³¹P NMR (D₂O) δ 23.7; ¹H NMR (D₂O) δ 1.4–1.9 (m, 4H, CH₂–CH₂–P), 3.75–3.85 (m, 1H, CH–O), 4.92 (d, ³J_{HH}=5.1 Hz, 1H, HC–O, hydrated form); ¹³C NMR (D₂O) δ 25.75 (d, ¹J_{CP}=135 Hz, CH₂–P), 27.65, 76.5 (d, ³J_{CP}=16.0 Hz, CH–O) 94.2 (C=O, hydrated form. Anal. calcd for C₄H₇O₅PNa₂, H₂O: C, 20.87; H, 3.91; O, 41.74. Found: C, 21.62; H, 4.09; O, 42.26.

(±)-1-Hydroxy-2-oxo-ethyl-1-phosphonic acid, dilithium salt (16c). Compound 16c was prepared from 15c (0.100 g, 0.35 mmol) in 91% yield (0.055 g) by following the same procedure described as for 16a except that 1 M LiOH was used to adjust the pH to 7.4. ³¹P NMR (D₂O) δ 6.6; ¹H NMR (D₂O) δ 3.8–4.0 (m, 1H, CH–O), HC=O (hydrated form) mixed up with H₂O; ¹³C NMR (D₂O) δ 71.5 (d, ¹J_{CP}=146 Hz, CH–P), 93.4 (C=O, hydrated form). Anal. calcd for C₂H₃O₅PLi₂, H₂O: C, 14.12; H, 2.94; O, 56.47. Found: C, 14.58; H, 3.26; O, 57.2.

3,3-Diethoxy-2-oxo-propyl-1-phosphonic acid, barium salt (17a). Freshly distilled trimethylsilyl bromide (1.4 mL, 10.6 mmol) was added slowly with stirring to the acetal 14a (0.110 g, 0.39 mmol) at -20 °C under nitrogen atmosphere. The reaction mixture was stirred for 24 h at room temperature. Excess trimethylsilyl bromide was removed under reduced pressure and water (5 mL) added to the remaining residue at -30 °C. The aqueous solution was washed with ether, the pH adjusted to 7.6 with saturated $Ba(OH)_2$ then freeze-dried. The remaining residue was dissolved in a minimum volume of water and the barium salt was precipitated by addition of three volumes of absolute ethanol. The resulting mixture was kept at 0 °C for 3 h, the salt collected by centrifugation, washed twice with ethanol (80%, then absolute), ethyl ether and dried in vacuo to yield 18a as a white powder (0.130 g, 92%): 31 P NMR (D₂O) δ 11.4; ¹H NMR (D₂O) δ 1.20 (t, 6H, CH₃), 3.10 (d, ²J_{HP} = 19.5 Hz, 2H, CH₂-P), 3.5–3.65 (m, 4H, CH₂-O), 5.25 (s, 1H, O-CH-O); ¹³C NMR (D₂O) δ 15.45, 32.2 (d, ${}^{1}J_{CP} = 131 \text{ Hz}, \text{ CH}_2 - \text{P}), 63.1, 101.3 \text{ (d, } {}^{3}J_{CP} = 8.9 \text{ Hz},$ CH acetal), 200.9. Anal. calcd for C₇H₁₃O₆PBa: C, 23.27; H, 3.6; O, 26.6. Found: C, 23.7; H, 3.97; O, 27.28.

4,4-Diethoxy-3-oxo-butyl-1-phosphonic acid, barium salt (17b). Compound 17b was prepared from acetal 14b (0.280 g, 0.945 mmol) in 93% yield (0.330 g) by following the same procedure as described for 18a. ³¹P NMR (D₂O) δ 22.2; ¹H NMR (D₂O) δ 1.22 (t, 6H, CH₃), 1.7–2.1 (m, 2H, CH₂–P), 2.8–2.95 (m, 2H, CH₂C=O), 3.45–3.65 (m, 4H, OCH₂), 5.17 (s, 1H, O–CH–O); ¹³C NMR (D₂O) δ 14.9, 19.35 (d, ¹J_{CP}=139 Hz, CH₂–P), 33.6, 63.4, 103, 201 (d, ³J_{CP}=12.9 Hz, C=O). Anal. calcd for C₈H₁₅O₆PBa: C, 25.6; H, 4.05; O, 25.5. Found: C, 26.2; H, 4.2; O, 26.3.

2,3-Dioxo-propyl-1-phosphonic acid (18a). Compound **18a** was prepared from acetal **17a** (0.110 g, 0.30 mmol) by following the same procedure described for **9a**, except that the incubation was carried out for 24 h at room temperature. ³¹P NMR (D₂O+H₂O) δ 14.6; ¹³C NMR (D₂O+H₂O) δ 34.9 (d, ¹*J*_{CP}=127 Hz, CH₂-P), 92.9 (d, ³*J*_{CP}=14.9 Hz, HC=O, hydrated form), 200.5 (C=O ketone).

3,5-Dioxo-butyl-1-phosphonic acid (18b). Compound **18b** was prepared from acetal **17b** (0.280 g, 0.75 mmol) by following the same procedure as described for **18a**. ³¹P NMR (D₂O + H₂O) δ 25.2; ¹³C NMR (D₂O + H₂O) δ 18.9 (d, ¹*J*_{CP}=125 Hz, CH₂-P), 33.6, 92.3 (HC=O, hydrated form), 201.2 (d, ³*J*_{CP}=13.6 Hz, C=O ketone).

Diethyl (2-oxo-3-hydroxy-propyl)-1-phosphonate (19a). To a 100-mL round-bottomed flask containing 13c (1.5 g, 5.03 mmol) in 10 mL of demineralized water were added 0.100 mL of concentrated HCl (35%). The mixture was warmed to 45°C for 2h and monitored by TLC (CH₂Cl₂:MeOH, 9:1); the solution was freezedried. The resulting residue was purified by flash chromatography (CH₂Cl₂:MeOH, 9:1) to yield 19a as a colourless oil (0.502 mg, 54%). ³¹P NMR (CDCl₃) δ 19.4; ¹H NMR (CDCl₃) δ 1.28 (t, 3H, CH₃), 3.14 (d, $^{2}J_{\text{HP}} = 26.9 \text{ Hz}, \text{ CH}_{2} - \text{P}$), 3.51 (br, 1H, D₂O exchangeable), 4.10 (m, 4H, CH₂O) 4.27 (s, 2H, CH₂O); ¹³C NMR (CDCl₃) δ 18.4, 38.7 (d, ¹*J*_{CP} = 127 Hz, CH₂-P), 62.1, 63.0, 69.0, 202.6 (d, ${}^{2}J_{CP} = 6.5$ Hz, C=O). Anal. calcd for C₇H₁₅O₅P: C, 40.0; H, 7.14; O, 38.1. Found: C, 40.06; H, 7.21; O, 38.24.

Diethyl (3-oxo-4-hydroxy-butyl)-1-phosphonate (19b). Compound 19b was prepared from 13b (1.5 g, 4.41 mmol) in 70% yield by following the same procedure as described for 19a. ³¹P NMR (CDCl₃) δ 30.7; ¹H NMR (CDCl₃) δ 1.26 (t, 3H, CH₃), 1.8–2.2 (m, 2H, CH₂–P), 2.6–2.8 (m, 2H, CH₂–CH₂C=O), 4.04 (m, 4H, CH₂O–P), 4.2 (s, 2H, CH₂O); ¹³C NMR (CDCl₃) δ 16.4, 19.1 (d, ¹*J*_{CP}=145 Hz, CH₂–P), 31.3, 61.8, 66.0, 208.1 (d, ³*J*_{CP}=13.4 Hz, C=O). Anal. calcd for C₈H₁₇ O₅P: C, 42.8; H, 7.6; O, 35.7. Found: C, 43.1; H, 7.7; O, 35.4.

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