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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).

Introduction

Glycolytic enzymes fructose-1,6-diphosphate aldolases reversibly catalyze the production of D-fructose-1,6-diphosphate (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 epoxidation[22] of the starting 2-chlorobutanone gave epoxide **3**. The ring opening of the latter compound by phosphite or phosphonate anion in presence of $BF_3{\cdot}OEt_2$ 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 method[24] 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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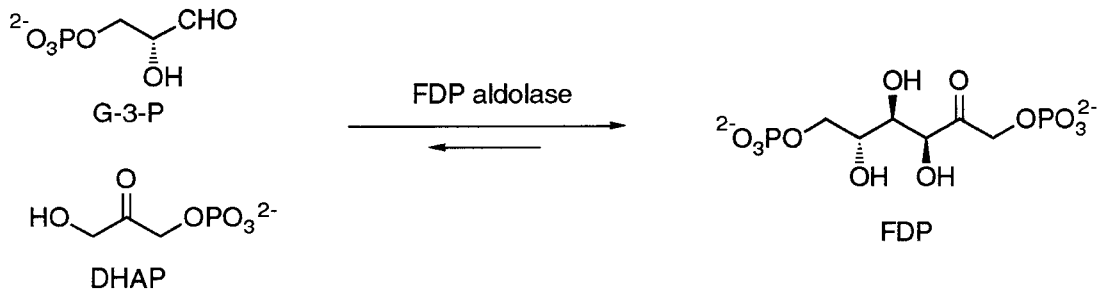
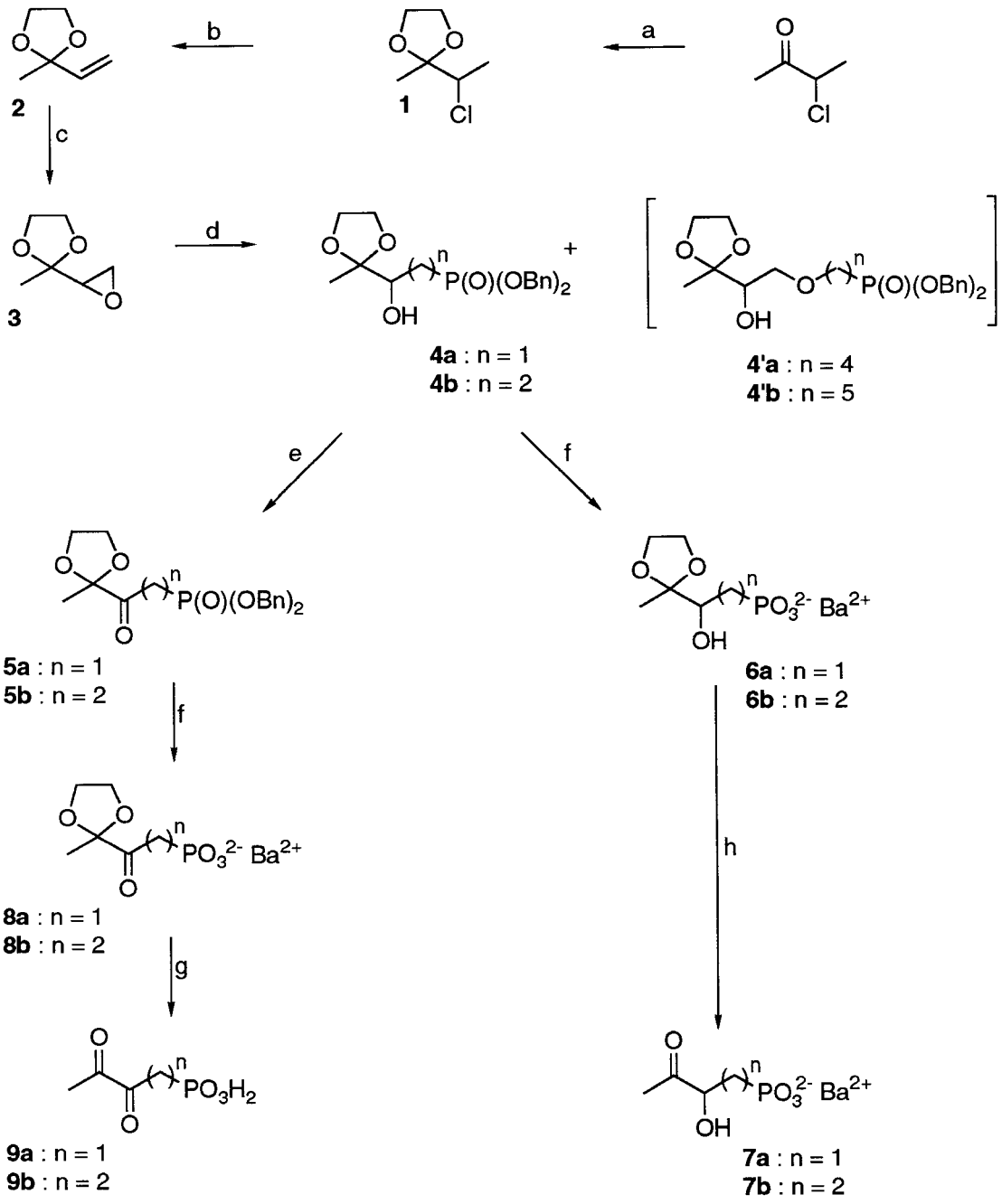


Scheme 1. Reaction catalyzed by aldolase.



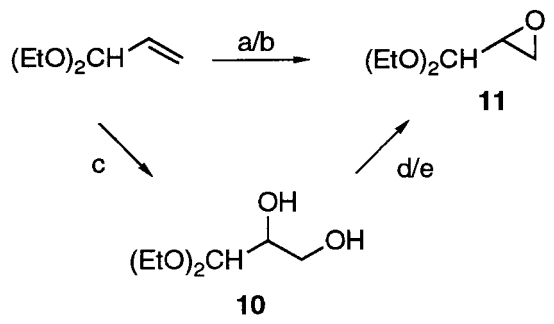
Scheme 2. Synthetic scheme for the synthesis of compounds **9a,b** and **7a,b**. (a) $HOCH_2CH_2OH$, TsOH,benzene, 82%; (b) KOH, $HOCH_2CH_2OH$, 130 °C (82%); (c) *m*-CPBA, CH_2Cl_2 (98%); (d) $(BnO)_2P(O)H$ or $(BnO)_2P(O)CH_3$, *n*-BuLi, $Et_2O{\cdot}BF_3$, THF, −80 °C ($n=1$: 40%, $n=2$: 75%); (e) DCC, pyridine, DMSO, TFA ($n=1$: 79%, $n=2$: 85%); (f) 1. H_2, Pd/C, MeOH; 2. $Ba(OH)_2$ ($n=1$: 92%, $n=2$: 97%); (g) DOWEX 50WX8 (H^+), H_2O; (h) 1. DOWEX 50WX8 (H^+), H_2O; 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 (±) glycidaldehyde diethyl acetal **11** (vide infra) from acroleine diethyl acetal (see Scheme 3): (a) oxidation by *m*-chloroperbenzoic acid[24] 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 benzonitrile[26] 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*-mesylate[27] 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 $BF_3{\cdot}OEt_2$.[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 glyoxal[29]) 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 trimethylsilyl 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 conditions[32] gave the corresponding phosphonic acids **15a–c** as lithium salts; subsequently 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 demonstrated[33] 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]



Scheme 3. Synthetic scheme for the synthesis of glycidaldehyde diethyl acetal **11**. (a) *m*-CPBA, CH_2Cl_2, 0 °C to rt (5–10%); (b) H_2O_2, PhCN, $KHCO_3$, MeOH (70%); (c) $KMnO_4$, H_2O (54%); (d) 1. CH_3SO_2Cl, pyridine; 2. NaOH, H_2O (56%); (e) DEAD, Ph_3P, benzene (77%).

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.

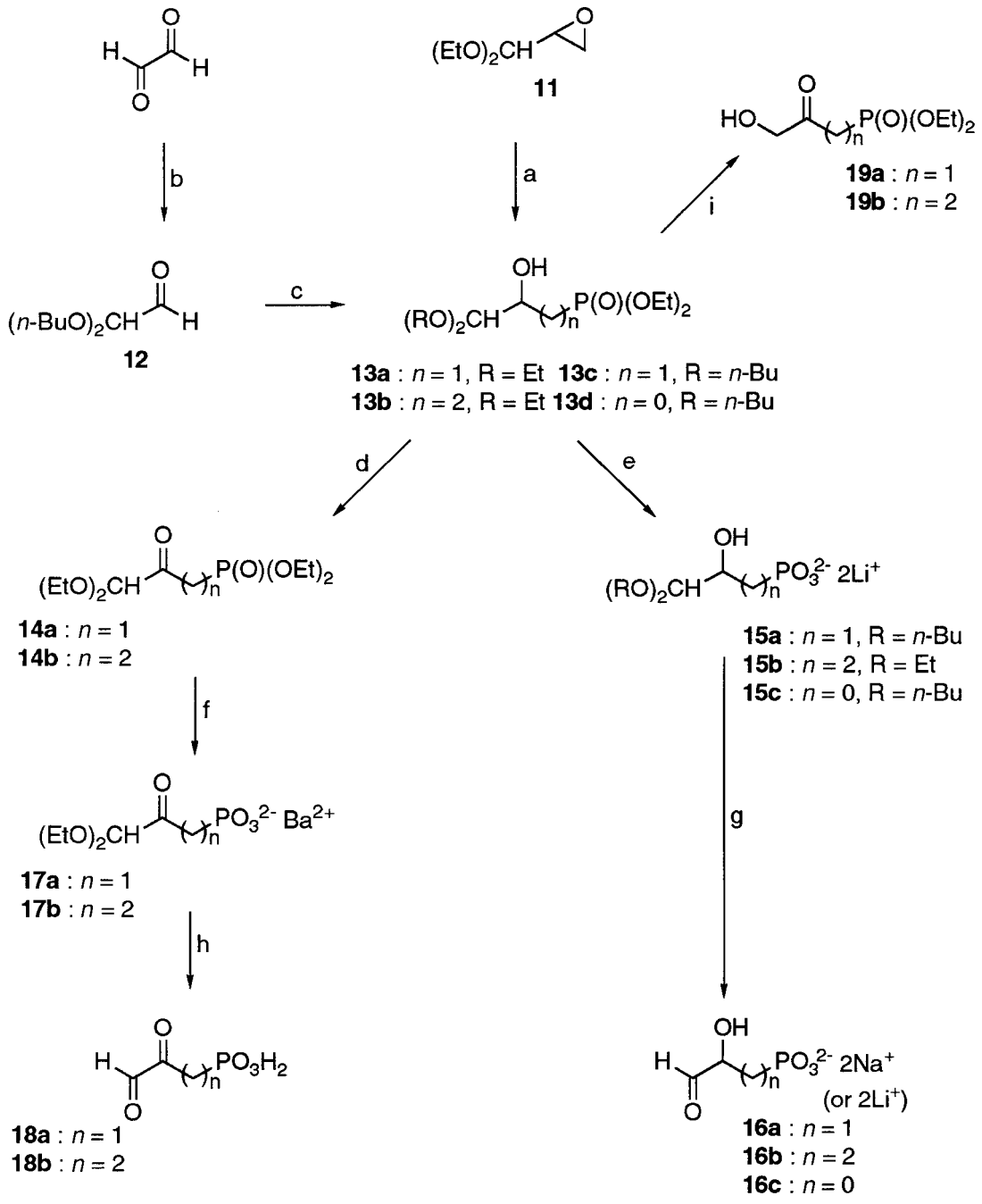
Acceptable relative rates are obtained with the aldehyde derivatives and these substrates are applicable to a large-scale synthesis. In contrast, the data obtained with the DHAP analogues show that only the phosphonate isostere of DHAP (**19b**) is substrate for aldolase.[17] These results represent 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 $(EtO)_2P(O)CH_3$, *n*-BuLi, $Et_2O{\cdot}BF_3$, THF, −80 °C (*n* = 1: 46%, *n* = 2: 80%); (b) *n*-BuOH, hexane, DOWEX 50WX8 (H^+), reflux (62%); (c) $(EtO)_2P(O)CH_3$ 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, $NaBH_4$, H_2O, 120 °C, 2 Bar (*n* = 1: 90%, *n* = 2: 96%, *n* = 0: 92.5%); (f) 1. Me_3SiBr, 24 h, −30 °C; 2. H_2O, $Ba(OH)_2$ (*n* = 1: 92%, *n* = 2: 96%); (g) 1. DOWEX 50WX8 (H^+), H_2O; 2. NaOH or LiOH (*n* = 1: 96%, *n* = 2: 96%, *n* = 0: 91%); (h) DOWEX 50WX8 (H^+), H_2O; (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 $CDCl_3$ or D_2O on a Bruker AC80 (80-MHz ^1H NMR) or a Bruker AC200 (200-MHz ^1H NMR, 50-MHz ^{13}C NMR and 81-MHZ ^{31}P 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_{rel}[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: (+ + +) $V_{rel} > 25$; (+ +) $25 > V_{rel} > 1$; (+) $10 > V_{rel} > 1$; (—) $V_{rel} < 1$.
[c] V_{rel} for the D stereoisomer.
[d] Value from literature.[4,16]

parts per million with respect to TMS for ^1H and ^{13}C spectra and H_2PO_4 for ^{31}P 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

(±)-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 CH_2Cl_2 (250 mL) and the solution washed with saturated $NaHCO_3$ 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 ($CDCl_3$) δ 1.39 (s, 3H), 1.48 (d, $J = 6.8$ Hz, 3H), 3.94 (q, $J = 6.8$ Hz, 1H) 3.98 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.0, 20.1, 60.9, 65.4, 65.6, 110.1. Anal. calcd for $C_6H_{11}O_2Cl$: 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 ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) δ 24.6, 64.5, 107.4, 114.8, 136.4. Anal. calcd for $C_6H_{10}O_2$: C, 63.1; H, 8.83; O, 28.1. Found: C, 62.9; H, 8.88; O, 28.5.

(±)-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 CH_2Cl_2 was stirred at room temperature for 5 days. Saturated $NaHCO_3$ (100 mL) was added to the mixture which was cooled to 0 °C. The aqueous layer was separated and extracted with CH_2Cl_2 (3×200 mL). The organic fractions were combined, washed with 3% $Na_2S_2O_3$, saturated $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, 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 ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) (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-hydroxy-ethyl]-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, $BF_3{\cdot}OEt_2$ (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 NH_4Cl (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 $MgSO_4$, then concentrated and purified by flash chromatography (CH_2Cl_2:MeOH, 98:2 to 96:4) to provide **4a** as a colourless oil (1.2 g, 40%). ^{31}P NMR ($CDCl_3$) δ 32.0; ^1H NMR ($CDCl_3$) δ 1.27 (s, 3H), 1.5–1.9 (m, 2H), 3.1 (br s, 1H, D_2O exchangeable), 3.8–4.0 (m, 5H), 4.9–5.2 (m, 4H), 7.33 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 19.7, 28.7 (d, $^1J_{CP} = 142$ Hz), 65.1, 65.5, 67.4, 67.6, 70.2, 110.8 (d, $^3J_{CP} = 19.2$ Hz), 128.4, 128.7, 136.3. Anal. calcd for $C_{20}H_{25}O_6P$: C, 61.2; H, 6.38. Found: C, 61.4; H, 6.3.

(±)-Dibenzyl [3-(2-methyl-1,3-dioxolan-2-yl)-3-hydroxy-propyl]-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. ^{31}P NMR ($CDCl_3$) (34.1; ^1H NMR ($CDCl_3$) δ 1.24 (s, 3H), 1.5–2.3 (m, 4H), 2.5 (m, 1H, D_2O exchangeable), 3.5 (m, 1H), 3.92 (m, 4H), 4.9–5.2 (m, 4H), 7.33 (s, 10H); ^{13}C NMR ($CDCl_3$) 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 $C_{21}H_{27}O_6P$: 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 (3×30 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed (CH_2Cl_2: MeOH, 95:5) to yield **5a** as a colourless oil (0.30 g, 75%). ^{31}P NMR ($CDCl_3$) δ 21.3; ^1H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) δ 20.32, 35.88 (d, $^1J_{CP}$ = 135 Hz) 65.62, 68.0, 107.6 (d, $^3J_{CP}$ = 5 Hz), 128.4, 128.6, 136.0, 199.6. Anal. calcd for $C_{20}H_{23}O_6P$: 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. ^{31}P NMR ($CDCl_3$) δ 32.7; ^1H NMR ($CDCl_3$) 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); ^{13}C NMR ($CDCl_3$) 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 $C_{21}H_{25}O_6P$: C, 62.37; H, 6.19; O, 23.76. Found: C, 62.39; H, 6.25; O, 23.9.

(±)-[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 12 h 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 3 h. 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%). ^{31}P NMR (D_2O) δ 21.2; ^1H NMR (D_2O) δ 1.31 (s, 3H), 1.5–1.9 (m, 2H), 3.8–4.0 (m, 1H), 4.02 (s, 4H); ^{13}C NMR (D_2O) δ 21.9, 34.0 (d, $^1J_{CP}$ = 135 Hz), 67.6, 67.9, 73.7, 109.1. Anal. calcd for $C_6H_{11}O_6PBa$: 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**. ^{31}P NMR (D_2O) δ 23.5; ^1H NMR (D_2O) δ 1.30 (s, 3H), 1.35–2.0 (m, 4H), 3.5 (m, 1H), 4.0 (s, 4H); ^{13}C NMR (D_2O) 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 $C_7H_{13}O_6PBa$: C, 23.37; H, 3.6; O, 26.6. Found: C, 23.4; H, 3.55; O, 26.4.

(±)-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 (3×0.25 mL). The aqueous solutions were combined and incubated for 4 days at room temperature. The progress of hydrolysis was monitored by ^{31}P NMR. The pH was adjusted to 7.4 with saturated $Ba(OH)_2$ and the solution was freeze-dried. The resulting powder was washed with ether and dried in vacuo to give **7a** as a white powder (0.045 g, 97%). ^{31}P 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); ^{13}C NMR (D_2O) δ 30.9, 34.3 (d, $^1J_{CP}$ = 131 Hz), 76.8, 209.0 (d, $^3J_{CP}$ = 14.9 Hz). Anal. calcd for $C_4H_7O_5PBa$: 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**. ^{31}P NMR (D_2O) δ 24.45; ^1H NMR (D_2O) δ 1.5–2.0 (m, 4H), 2.08 (s, 3H), 3.2–3.5 (m, 1H); ^{13}C NMR (D_2O) δ 26.31 (d, $^1J_{CP}$ = 133 Hz), 28.2, 29.9, 79.9 (d, $^3J_{CP}$ = 15.2 Hz), 217.5. Anal. calcd for $C_5H_9O_5PBa$: 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**. ^{31}P NMR (D_2O) δ 12.0; ^1H NMR (D_2O) δ 1.46 (s, 3H), 3.1 (d, $^2J_{PH}$ = 20 Hz, 2H) 3.8–4.1 (m, 4H); ^{13}C NMR (D_2O) 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 $C_6H_9O_6PBa$: 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**. ^{31}P NMR (D_2O) δ 23.1; ^1H NMR (D_2O) δ 1.48 (s, 3H), 1.5–1.7 (m, 2H), 2.65 (m, 2H), 3.85–4.1 (m, 4H); ^{13}C NMR (D_2O) 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 $C_7H_{11}O_6PBa$: 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 ^{31}P and ^{13}C NMR. The title compound, did not show decomposition, was used for enzymatic studies without further purification. ^{31}P NMR ($D_2O + H_2O$) δ 16.1; ^{13}C NMR ($D_2O + H_2O$) δ 25.8, 38.5 (d, $^1J_{CP}$ = 129 Hz, CH_2-P), 90.6 (C2, hydrated form), 208.2 (d, $^3J_{CP}$ = 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**. ^{31}P NMR ($D_2O + H_2O$) δ 26.5; ^{13}C

NMR ($D_2O + H_2O$) δ 22.7 (d, $^1J_{CP} = 139$ Hz), 26.3, 32.9, 98.9 (d, $^3J_{CP} = 21.0$ Hz, C3, hydrated form), 209.0.

(±)-Glyceraldehyde diethyl acetal (10). To a stirred solution of acroleine diethylacetal (10 g, 77 mmol) in 85 mL of distilled water was added dropwise 13 g (84.6 mmol) of $KMnO_4$ in 300 mL of water over 1.5 h. The reaction mixture was stirred for 3 h at room temperature then refluxed for 1 h. After cooling, the precipitate was filtered off and K_2CO_3 (170 g) was added to the filtrate and the aqueous solution was extracted with ethyl acetate (3×100 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The remaining residue was purified by flash chromatography (CH_2Cl_2:MeOH, 95:5) to give **10** as a colourless oil (6.9 g, 54%). ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 6H), 2.6 (m, 2H, D_2O exchangeable), 3.4–3.9 (m, 7H, CH−O, CH_2O), 4.44 (d, $^3J_{HH} = 7.0$ Hz, 1H, O−CH−O); ^{13}C NMR ($CDCl_3$) δ 15.3, 62.5, 63.6, 64.2, 71.8, 103.4. Anal. calcd for $C_7H_{16}O_4$: C, 51.21; H, 9.82; O, 39.02. Found: C, 51.17; H, 10.01; O, 39.20.

(±)-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 2 h, then at room temperature overnight. The solvent was removed under reduced pressure and the remaining residue dissolved in 20 mL of CH_2Cl_2. The organic solution was washed with saturated $NaHCO_3$ (3×50 mL), brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure to yield the 3-*O*-mesylate of **10** as a colourless oil (3.0 g, 90%), which was used in the next step without further purification; the ^{1}H and ^{13}C 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 (CH_2Cl_2:MeOH, 98:2). The reaction mixture was extracted with ethylacetate (3×20 mL). The organic layer was washed with brine, dried over $MgSO_4$ 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 $KHCO_3$ (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 $MgSO_4$ 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 ($CDCl_3$) δ 1.29 and 1.33 (2t, $J = 7$ Hz, 2 CH_3), 2.53 (m, 2H, $CHCH_2$), 2.86 (m, 1H, CH−O), 3.2–3.6 (m, 4H, CH_2CH_3) 4.1 (d, $J = 4.3$ Hz, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 15.0, 43.5, 51.6, 62.0, 62.6, 101.4. Anal. calcd for $C_7H_{14}O_3$: 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 2 h in a round-bottomed flask equipped with a Dean–Stark system. The mixture reaction was cooled to room temperature, the resin filtered off and $NaHCO_3$ (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%). ^{1}H NMR ($CDCl_3$) δ 0.89 (t, $J = 7$ Hz, 2 CH_3), 1.5 (m, 8H, CH_2), 3.6 (m, 4H, CH_2O), 4.5 (d, $J = 2.1$ Hz, 1H, O−CH−O), 9.4 (d, $J = 2.1$ Hz, 1H, CHO); ^{13}C NMR ($CDCl_3$) δ 13.8, 19.2, 31.8, 67.3, 101.2, 196.9. Anal. calcd for $C_{10}H_{20}O_3$: 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. ^{31}P NMR ($CDCl_3$) δ 30.8; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 6H, CH_3 acetal), 1.31 (t, 6H, CH_3 ester), 1.9–2.1 (m, 2H, CH_2−P), 3.15 (s, 1H D_2O exchangeable), 3.5–3.8 (m, 5H, CH−O, OCH_2 acetal), 4.0–4.15 (m, 4H, CH_2O ester), 4.37 (d, 1H, CH acetal); ^{13}C NMR ($CDCl_3$) δ 15.3, 16.4, 28.0 (d, $^1J_{CP} = 141$ Hz, CH_2−P), 61.8, 63.5, 64.0, 67.7, 104.1 (d, $^3J_{CP} = 17.2$ Hz, CH acetal). Anal. calcd for $C_{11}H_{25}O_6P$: 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. ^{31}P NMR ($CDCl_3$) δ 30.8; ^{1}H NMR ($CDCl_3$) δ 1.15 and 1.17 (2t, 6H, CH_3 acetal), 1.27 (t, 6H, CH_3 ester), 1.4–2.1 (m, 4H, CH_2−CH_2−P), 2.5 (m, 1H D_2O exchangeable), 3.3–3.8 (m, 5H, CH−O, OCH_2 acetal), 4.0–4.15 (m, 4H, CH_2O ester), 4.21 (d, 1H, CH acetal); ^{13}C NMR ($CDCl_3$) δ 15.4, 16.5, 21.8 (d, $^1J_{CP} = 142$ Hz, CH_2−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.

(±)-Diethyl (3,3-dibutoxy-2-hydroxy-propyl)-1-phosphonate (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 1 h at −20 °C then overnight at room temperature and quenched with saturated NH_4Cl (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 $MgSO_4$ and concentrated. The resulting residue was purified by flash chromatography (CH_2Cl_2:MeOH, 96:4) to yield **13c** as a colourless oil (9.5 g, 93%). ^{31}P NMR ($CDCl_3$) δ 30.8; ^1H NMR ($CDCl_3$) δ 0.84 (t, 6H, CH_3 acetal), 1.25 (t, 6H, CH3 ester), 1.3–1.6 (m, 8H, CH_2), 1.65–1.95 (m, 2H, CH_2−P), 3.12 (s, 1H D_2O exchangeable), 3.4–3.7 (m, 4H, OCH_2 acetal), 3.95 (m, 1H, CH−O), 3.97–4.1 (m, 4H, CH_2O ester), 4.29 (d, 1H, CH acetal); ^{13}C NMR ($CDCl_3$) δ 13.8, 16.4, 19.3, 28.0 (d, $^1J_{CP}$ = 142 Hz, CH_2−P), 31.9, 61.7, 67.6, 67.7, 68.2, 104.3 (d, $^3J_{CP}$ = 17.4 Hz, CH acetal). Anal. calcd for $C_{15}H_{33}O_6P$: C, 52.9; H, 9.70; O, 28.2. Found: C, 52.8; H, 9.75; O, 28.3.

(±)-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. ^{31}P NMR ($CDCl_3$) δ 21.3; ^1H NMR ($CDCl_3$) δ 0.9 (t, 6H, CH_3 acetal), 1.31 (t, 6H, CH_3 ester), 1.3–1.5 (m, 4H, CH_2), 1.5–1.7 (m, 4H, CH_2), 2.9 (dd, $^3J_{HH}$ = 5.5 Hz, $^3J_{HP}$ = 14.5 Hz, 1H D_2O exchangeable), 3.45–3.8 (m, 4H, OCH_2 acetal), 3.85–4.0 (td, $^3J_{HH}$ = 5.0 Hz, $^2J_{HP}$ = 5.0 Hz, 1H, CH−O), 4.05–4.3 (m, 4H, CH_2O ester), 4.73 (dd, $^3J_{HH}$ = 5.1 Hz, $^3J_{HP}$ = 14.5 Hz, 1H, CH acetal); ^{13}C NMR ($CDCl_3$) δ 13.9, 16.8, 19.3, 31.8, 63.0, 63.7, 67.4, 68.4, 69.0 (d, $^1J_{CP}$ = 157 Hz, CH−P), 101.1 (d, $^2J_{CP}$ = 7.2 Hz, CH acetal). Anal. calcd for $C_{14}H_{31}O_6P$: 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. ^{31}P NMR ($CDCl_3$) δ 20.2; ^1H NMR ($CDCl_3$) δ 1.22 (t, 6H, CH_3 acetal), 1.31 (t, 6H, CH_3 ester), 3.25 (d, $^2J_{HP}$ = 21.8 Hz, 2H, CH_2−P), 3.5–3.7 (m, 4H, OCH_2 acetal), 4.0–4.2 (m, 4H, CH_2O ester), 4.71 (s, 1H, O−CH−O); ^{13}C NMR (CDCl3) δ 13.6, 16.3, 35.7 (d, $^1J_{CP}$ = 132 Hz, CH_2−P), 62.5, 63.6, 101.7 (d, $^3J_{CP}$ = 9.8 Hz, CH acetal), 201.6. Anal. calcd for $C_{11}H_{23}O_6P$: 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**. ^{31}P NMR ($CDCl_3$) δ 31.6; ^1H NMR ($CDCl_3$) δ 1.23 (t, 6H, CH_3 acetal), 1.32 (t, 6H, CH_3 ester), 1.8–2.2 (m, 2H, CH_2−P), 2.8–3.0 (m, 2H, CH_2C=O), 3.5–3.75 (m, 4H, OCH_2 acetal), 4.0–4.15 (m, 4H, CH_2O ester), 4.60 (s, 1H, CH acetal); ^{13}C NMR ($CDCl_3$) δ 15.1, 16.4, 18.9 (d, $^1J_{CP}$ = 145 Hz, CH_2−P), 30.2, 61.6, 63.5, 102.4, 202.4 (d, $^3J_{CP}$ = 15.4 Hz, C=O). Anal. calcd for $C_{12}H_{25}O_6P$: C, 48.65; H, 8.44; O, 32.4. Found: C, 48.8; H, 8.2; O, 32.6.

(±)-3,3-Dibutoxy-2-hydroxy-propyl-1-phosphonic acid, dilithium salt (15a). A mixture of acetal **13c** (2 g, 5.88 mmol), LiOH monohydrate (1.72 g, 41 mmol) and $NaBH_4$ (0.2 g, 5.1 mmol) in 30 mL of distilled water was warmed for 12 h 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%). ^{31}P NMR (D_2O) δ 20.0; ^1H NMR (D_2O) δ 0.89 (t, 6H, CH_3 acetal), 1.25–2.0 (m, 10H, CH_2, CH_2−P), 3.6–3.8 (m, 4H, OCH_2 acetal), 3.8–4.0 (m, 1H, CH−O), 4.38 (d, 1H, O−CH−O); ^{13}C NMR (D_2O) δ 15.8, 21.4, 33.2 (d, $^1J_{CP}$ = 127 Hz, CH_2−P), 33.8, 70.7, 71.1, 71.2, 108.0 (d, $^3J_{CP}$ = 18.0 Hz, CH acetal). Anal. calcd for $C_{11}H_{23}O_6PLi_2$: 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**. ^{31}P NMR (D_2O) δ 22.9; ^1H NMR (D_2O) δ 1.22 (t, 6H, CH_3 acetal), 1.3–2.0 (m, 4H, CH_2−CH_2−P), 3.5–3.9 (m, 5H, CH−O, OCH_2 acetal), 4.46 (d, $^3J_{HH}$ = 4.5 Hz, 1H, O−OH−O); ^{13}C NMR (D_2O) δ 17.1, 27.9 (d, $^1J_{CP}$ = 121 Hz, CH_2−P), 29.2, 66.7, 67.2, 75.3 (d, $^3J_{CP}$ = 14.0 Hz, CH−O) 107.3. Anal. calcd for $C_8H_{17}O_6PLi_2$: 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**. ^{31}P NMR (D_2O) δ 3.97; ^1H NMR (D_2O) δ 0.92 (t, 6H, CH_3 acetal), 1.25–1.5 (m, 4H, CH_2), 1.5–1.7 (m, 4H, CH_2), 3.5–3.85 (m, 4H, OCH_2 acetal), 3.85–4.0 (m, 1H, CH−O), 4.70 (m, 1H, O−CH−O); ^{13}C NMR (D_2O) δ 15.9, 21.5, 33.9, 70.5, 72.8 (d, $^1J_{CP}$ = 154 Hz, CH−P), 102.4. Anal. calcd for $C_{10}H_{21}O_6PLi_2$: 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. ^{31}P NMR (D_2O) δ 20.6; ^1H NMR (D_2O) δ 1.6–1.95 (m, 2H, CH_2−P), 3.8–3.95 (m, 1H, CH−O), 4.88 (d, $^3J_{HH}$ = 4.4 Hz, 1H, HC=O, hydrated form); ^{13}C NMR (D_2O) δ 32.3 (d, $^1J_{CP}$ = 136 Hz, CH_2−P), 72.4, 94.4 (d,

$^3J_{CP}$ = 16.4 Hz, C=O, hydrated form). Anal. calcd for $C_3H_5O_5PNa_2$, H_2O: 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**. ^{31}P NMR (D_2O) δ 23.7; ^{1}H NMR (D_2O) δ 1.4–1.9 (m, 4H, CH_2-CH_2-P), 3.75–3.85 (m, 1H, CH−O), 4.92 (d, $^3J_{HH}$ = 5.1 Hz, 1H, HC−O, hydrated form); ^{13}C NMR (D_2O) δ 25.75 (d, $^1J_{CP}$ = 135 Hz, CH_2-P), 27.65, 76.5 (d, $^3J_{CP}$ = 16.0 Hz, CH−O) 94.2 (C=O, hydrated form. Anal. calcd for $C_4H_7O_5PNa_2$, H_2O: 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. ^{31}P NMR (D_2O) δ 6.6; ^{1}H NMR (D_2O) δ 3.8–4.0 (m, 1H, CH−O), HC=O (hydrated form) mixed up with H_2O; ^{13}C NMR (D_2O) δ 71.5 (d, $^1J_{CP}$ = 146 Hz, CH−P), 93.4 (C=O, hydrated form). Anal. calcd for $C_2H_3O_5PLi_2$, H_2O: 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 (D_2O) δ 11.4; ^{1}H NMR (D_2O) δ 1.20 (t, 6H, CH_3), 3.10 (d, $^2J_{HP}$ = 19.5 Hz, 2H, CH_2-P), 3.5–3.65 (m, 4H, CH_2-O), 5.25 (s, 1H, O−CH−O); ^{13}C NMR (D_2O) δ 15.45, 32.2 (d, $^1J_{CP}$ = 131 Hz, CH_2-P), 63.1, 101.3 (d, $^3J_{CP}$ = 8.9 Hz, CH acetal), 200.9. Anal. calcd for $C_7H_{13}O_6PBa$: 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**. ^{31}P NMR (D_2O) δ 22.2; ^{1}H NMR (D_2O) δ 1.22 (t, 6H, CH_3), 1.7–2.1 (m, 2H, CH_2-P), 2.8–2.95 (m, 2H, $CH_2C=O$), 3.45–3.65 (m, 4H, OCH_2), 5.17 (s, 1H, O−CH−O); ^{13}C NMR (D_2O) δ 14.9, 19.35 (d, $^1J_{CP}$ = 139 Hz, CH_2-P), 33.6, 63.4, 103, 201 (d, $^3J_{CP}$ = 12.9 Hz, C=O). Anal. calcd for $C_8H_{15}O_6PBa$: 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. ^{31}P NMR ($D_2O + H_2O$) δ 14.6; ^{13}C NMR ($D_2O + H_2O$) δ 34.9 (d, $^1J_{CP}$ = 127 Hz, CH_2-P), 92.9 (d, $^3J_{CP}$ = 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**. ^{31}P NMR ($D_2O + H_2O$) δ 25.2; ^{13}C NMR ($D_2O + H_2O$) δ 18.9 (d, $^1J_{CP}$ = 125 Hz, CH_2-P), 33.6, 92.3 (HC=O, hydrated form), 201.2 (d, $^3J_{CP}$ = 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 2 h and monitored by TLC (CH_2Cl_2:MeOH, 9:1); the solution was freeze-dried. The resulting residue was purified by flash chromatography (CH_2Cl_2:MeOH, 9:1) to yield **19a** as a colourless oil (0.502 mg, 54%). ^{31}P NMR ($CDCl_3$) δ 19.4; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, CH_3), 3.14 (d, $^2J_{HP}$ = 26.9 Hz, CH_2-P), 3.51 (br, 1H, D_2O exchangeable), 4.10 (m, 4H, CH_2O) 4.27 (s, 2H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 18.4, 38.7 (d, $^1J_{CP}$ = 127 Hz, CH_2-P), 62.1, 63.0, 69.0, 202.6 (d, $^2J_{CP}$ = 6.5 Hz, C=O). Anal. calcd for $C_7H_{15}O_5P$: 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**. ^{31}P NMR ($CDCl_3$) δ 30.7; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, CH_3), 1.8–2.2 (m, 2H, CH_2-P), 2.6–2.8 (m, 2H, $CH_2-CH_2C=O$), 4.04 (m, 4H, CH_2O-P), 4.2 (s, 2H, CH_2O); ^{13}C NMR ($CDCl_3$) δ 16.4, 19.1 (d, $^1J_{CP}$ = 145 Hz, CH_2-P), 31.3, 61.8, 66.0, 208.1 (d, $^3J_{CP}$ = 13.4 Hz, C=O). Anal. calcd for $C_8H_{17}O_5P$: C, 42.8; H, 7.6; O, 35.7. Found: C, 43.1; H, 7.7; O, 35.4.

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