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A Novel Strategy for the Preparation of Naturally Occurring Phosphocitrate and Its Partially Esterified Derivatives

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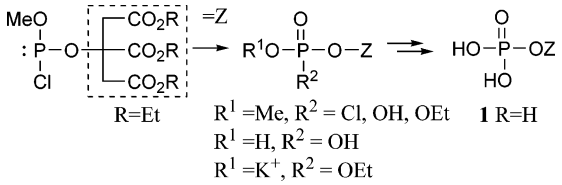

A novel method for the synthesis of phosphocitrate (**1**, PC) starting from triethyl ester of citric acid and $MeOPCl_2$ is described. The method is based on selective stepwise hydrolysis of ester moieties from the intermediate Me−O−P(O)(Cl)(Z) (Z = triethylcitrate), **4a**, which also allows one to prepare partially esterified derivatives of PC with good yield and purity without chromatographic purifications.

Osteoarthritis and rheumatoid arthritis are two of the most widespread diseases in the elderly population. In 2002, TIME magazine dedicated a "cover story" on these diseases in order to emphasize their significance for society and medicine.[1] According to the data presented, 20 million Americans today suffer from arthritis-related issues, a number likely to climb to 40 million in the coming years, mainly due to changes in lifestyle of modern humans in developed societies. Some of the well-recognized factors that result in osteoarthritis in humans are genetic disposition, joint trauma, and joint disuse. An inadequately investigated aspect of articular cartilage and other articular tissue is their tendency to form Ca^{2+}-containing crystals. Deposition of these crystals may be triggered by tissue trauma or abnormal fluctuations in intracellular Ca^{2+} concentrations. Deposits may initially accumulate in a "soft" and amorphous form, which subsequently transforms into a hard, crystalline Ca^{2+} salt of greatly reduced solubility. These crystals then act to activate further events for the development of the specific pathological disease state. This scenario prevails not only in osteoarthritis and rheumatoid arthritis but also in a broad range of related diseases, such as heart valve calcification, renal calcinosis, soft tissue tumor calcification, urinary lithiasis, arteriosclerosis, and chondrocalcinosis. Ca^{2+}-containing crystals form in the extracellular matrix of the cartilage and can then be released into joint space[2−4] where they can promote cartilage degeneration.[5] Ca^{2+}-containing crystal deposition in cartilage as a feature of osteoarthritis is widely recognized.[4b] For example, data indicate that in degenerative joints calcium pyrophosphate dihydrate and basic calcium phosphate crystals are more common than in normal joints. In addition, osteoarthritis is more common and severe in patients with detected Ca^{2+}-containing crystals.[4c]

A therapeutic strategy should include chemical compounds that possess no or low toxicity and block Ca^{2+}-containing crystal formation or inhibit intracellular responses stimulated by crystal formation and growth.[6] Phosphocitrate (PC, Figure 1) is a naturally occurring biomolecule found in mammalian mitochondria and crab hepatopancreas,[7] likely produced by cytosolic phosphorylation of citrate.[8] PC does not produce any significant toxic side effects in rats or mice when given in doses up to 150 μmol/kg/day.[8] Furthermore, in vitro studies suggested that PC concentrations up to 1.5 mM (4.5 mg/mL) do not have any effect on normal cellular functions such as DNA and protein synthesis.[9−11]

PC was found to inhibit crystal-induced matrix metalloproteinase synthesis and mitogenesis in cells, but it has no effect on similar processes induced by growth factors or serum.[10,12,13] This inhibitory action can be explained by the influence that PC exerts on the interaction of Ca^{2+}-containing crystals with biomembranes, as studied by computational methods.[14]

PC was also found to be a powerful inhibitor of hydroxyapatite crystal formation in vitro.[15] The precise mechanism of action of PC has been an issue of intense speculation. Tew et

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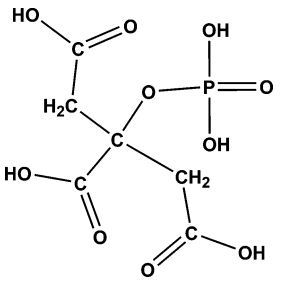

FIGURE 1. Schematic structure of PC in its protonated form.

al. proposed that PC inhibits hydroxyapatite ($Ca_5(PO_4)_3(OH)$) precipitation in cells or cellular compartments by maintaining high hydroxyapatite supersaturation.[8b] Sequestration/complexation of serum Ca^{2+} resulting in formation of Ca−PC complexes at pH 7.4 was disproven.[8b] As proposed before, PC acts as a powerful hydroxyapatite growth inhibitor by strongly binding to its surface, thus poisoning its growth.

The available literature synthetic procedures for PC report 7−61% overall yields, while laborious chromatographic purifications are needed for isolation of pure final product.[16] Also, the methods reported thus far do not allow access to preparation of partial ester PC derivatives. Our approach involves a synthetic strategy to improve overall yields for PC preparation, as well as to give access to several partial ester derivatives of PC. These modified PC molecules are needed for more complete in vivo and in vitro testing and detailed structure/function anticalcification relationships.

The difficulty in efficient synthesis of PC is phosphorylation of the sterically hindered tertiary alcohol group of citric acid ester **2** (Scheme 1). Previously, PC had been prepared by several methods starting from trialkyl esters of citric acid and powerful phosphorylating agents such as *o*-phenylene phosphorochloridate,[16a] 2-cyanoethyl phosphate,[16b] and diphenyl chlorophosphate.[16c] Also, partially substituted 1,3-dibenzyl-2-phosphonooxy citrate had been synthesized starting from tribenzyl citrate and PCl_5.[16d]

Our strategy to prepare PC and its derivatives is based on utilization of $MeOPCl_2$, which is a readily available, inexpensive, very reactive, and sterically nonhindered phosphitylating agent of trivalent P. Our key intermediate **3** (produced when 1.5 equiv of $MeOPCl_2$ is used, but not isolated) is proposed to form almost in quantitative yield according to ^{31}P NMR data (procedure i in Scheme 1; ^{31}P spectrum was measured after addition of methanol to **3** and was 132.1 ppm). The next step (procedure ii in Scheme 1) leads to formation of **4a** after addition of methanol and oxidation. **4a** is purified on dry silica, thus further chromatographic purifications are avoided. The absence of triethyl citrate starting material (**2**) in the reaction mixture is crucial for an efficient purification step. This is because of simultaneous elution of **4a** and **2** under these chromatographic conditions. Use of 1.5 equiv of $MeOPCl_2$ in our procedure caused none of the above-mentioned issues, ensuring depletion of all triethyl citrate.

We also attempted to oxidize **3** directly to the five-coordinated phosphorus derivative **4a**, but complicated mixtures resulted. However, after treatment of **3** with methanol, **4a** can be obtained after oxidation and purification with 68% yield (procedure ii in Scheme 1). Oxidation by sulfuryl chloride yielded **4a**, which contains a P−Cl moiety. This is expected according to results published earlier[17] (note, the reaction mechanism in ref 17 is incorrect). The P-bound chlorine substituent in **4a** is easily removed by addition of ethanol in the presence of $NaHCO_3$ leading to **4c** at 92% yield and ≥95% purity after isolation (procedure iv in Scheme 1). Triethylamine was also tested as HCl scavenger to prepare **4c** from **4a**, but the purified yield is only ∼10%. The main reason for this low yield is the ability of triethylamine, according the ^{31}P NMR spectrum, to dealkylate phosphorus esters[18] since unsymmetrical dimers and two monomers are detected in crude product. Compound **4a** is an interesting intermediate since it allows one to prepare (1) monomethyl ester **4b** after water addition (procedure iii), (2) mixed esters, such as **4c** (procedure iv), (3) phosphates **4d**, first by treatment with trimethylsilyl bromide, followed by desilylation with methanol (procedure v), and (4) **5a** by reaction with 2 M HCl at 38−40 °C (above 42 °C the PO−Z bond also starts to hydrolyze) for 20 h (procedure vii). The high selectivity (94%) of demethylation versus removal of the phosphate ethyl group is observed when KI in acetone is used as the dealkylation agent when compound **4e** is prepared from **4c** (procedure vi).

Compound **4a** seems to be quite stable since no degradation was observed during a 2-month storage period (without N_2) at −18 °C. Compound **5a**, which is a new type of partially esterified phosphocitrate, is a challenge to obtain in pure form since it is typically obtained in ∼66% yield with 83−90% purity and contains **5c** (R^1 = H), diethyl monomethyl PC, and a trace of **1** as impurities (according to ^1H and ^{31}P NMR spectra).

Alkaline hydrolysis was also tested with poor success since the PO−Z bond tends to hydrolyze as well under the conditions used. However, when **5a** is treated with acetone and KI, as described elsewhere for the demethylation of bisphosphonate and phosphonate compounds,[19] **5b** is obtained in quantitative yield and ≥95% purity (procedure viii).

For the last step (from **5b** to **1**, procedure ix), we resorted to a modification of a previously published method, in which an excess of NaOH and $CaCl_2$ was used at 4 °C for 4 h. New modification was used to avoid the aforementioned chromatographic purification, with precipitation of the final product.[16] Thus, in our approach, exactly 4.0 equiv of NaOH and 1.2 equiv $CaCl_2$ were added to **5b** at 65 °C and reacted for 2 h. A white precipitate formed almost instantaneously. On the basis of ^1H and ^{31}P NMR spectra, the organic portion of the precipitate contains exclusively **1** as the mixed K^+/Ca^{2+} salt ($C_6H_4O_{10}PKCa_2 \cdot 2.5\ H_2O$) at 92% yield according to elemental analysis. We also tried to use 2.0 equiv of $CaCl_2$, which in theory allows preparation of **1** in quantitative yield, but the inorganic phosphate impurity formed co-precipitated as insoluble calcium phosphate. Similar results were obtained when 1.5 equiv of $CaCl_2$ was used. As mentioned above, the best results were obtained upon addition of 1.2 equiv of $CaCl_2$, yielding the final compound **1**, with *no* co-precipitation of calcium phosphate. Compound **1** is insoluble in water but can be easily converted to a water-soluble Na^+ salt of PC in quantitative yield by treatment with the Dowex

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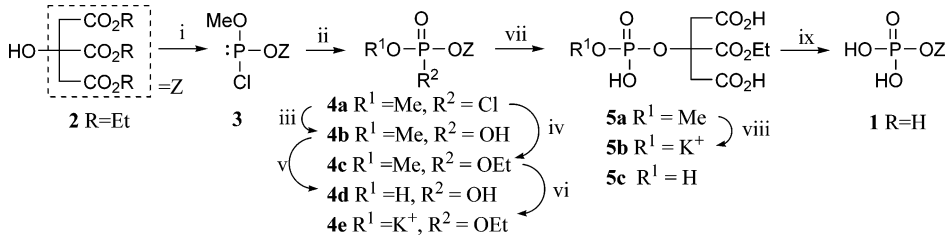
SCHEME 1. Preparation of PC (1) and Its Derivatives (3−5)[a]



[a] Conditions: (i) 1.5 equiv of $MeOPCl_2$, triethylamine, 24 h, rt, ca. 100%; (ii) MeOH, triethylamine, 3 h, rt then SO_2Cl_2, −5−0 °C and 1 h, rt, 68%; (iii) H_2O, $NaHCO_3$, 1 h, rt, 100%; (iv) abs. EtOH, $NaHCO_3$, 2 h, reflux, 92%; (v) $(CH_3)_3SiBr$, CH_3CN, $NaHCO_3$, 2 h, rt, then MeOH, 1 h, rt, 100%; (vi) KI, acetone, 48 h, 60 °C, 63%; (vii) 2 M HCl, 20 h, 38−40 °C, 66%; (viii) KI, acetone, 24 h, 60 °C, 100%; (ix) 4 equiv of 1 M NaOH, 1.2 equiv of $CaCl_2$, H_2O, 2 h, 65 °C, 92%.

50W cation-exchange resin (H^+ form) followed by partial neutralization with NaOH (see Experimental Section).

All compounds are identified by ^1H, ^{13}C, and ^{31}P NMR spectroscopy. Elemental analyses were determined for the compounds **1** and **5b**; ESI-MS spectra were recorded for the rest of the compounds. Assignment of the ^{31}P NMR spectra of the compounds described herein is straightforward since only one signal for each compound is observed. In the ^{13}C NMR spectra, signals were assigned based on $^{2,3}J_{CP}$ couplings. Symmetrical $-CH_2CO_2$ moieties in **4c** show different chemical shifts due to prochirality. Due to the same reason, ^1H NMR spectra of all PC compounds contain a typical AB quartet. Furthermore, in the case of **4a** and **4c**, the ester $O-CH_2$ protons are found at different chemical shifts.

In conclusion, the novel method described herein opens a new route to prepare PC and several new partial ester derivatives not reported earlier. The overall yield to prepare **1** (calculated based on the composition $C_6H_4O_{10}PKCa_2 \cdot 2.5H_2O$) starting from triethyl citrate **2** is 41.3%, which is quite reasonable for a four-step reaction procedure and is over 5 times higher than that published earlier.[16b] Final product **1** is easily separated and collected from the reaction mixture without further chromatographic purifications in contrast to the methods reported earlier[16] and can be converted quantitatively to water-soluble Na^+ salt of PC. Availability of PC and its derivatives will allow further and more systematic testing of these anticalcification agents in vivo and in vitro.[20] PC derivatives of various solubilities may allow control of bioavailability of "active" PC, as has been reported for $CaNa(PC)_2(H_2O)$.[20]

Experimental Section

Synthetic Procedures. Preparation of 1. Compound **5b** (200 mg, 0.52 mmol) was dissolved in distilled H_2O (2.5 mL), 1 M NaOH (2370 μL, ∼4 equiv) was added, and reaction mixture was stirred until a clear solution was obtained. $CaCl_2$ (79 mg, 1.2 equiv) was then added, and the reaction mixture was stirred for 2 h at 65 °C until a white precipitate appeared. The solid was filtered by suction, washed with very small portions of ice-cold water, and finally washed with a small volume of acetone. The precipitate was dried in vacuo, and **1** was obtained as white "felting like" solid in 92% yield based on the composition $C_6H_4O_{10}PKCa_2 \cdot 2.5\ H_2O$. K and Ca contents were quantitatively analyzed by flame AAS and CHN and were quantified by elemental analysis. These results confirmed the aforementioned formula. **1** was converted to a water-soluble Na^+ salt by the following method: **1** (30 mg, 0.071 mmol) was suspended in distilled H_2O (3 mL), Dowex 50W × 8 cation-exchange resin (H^+-form) was added (∼300 mg), and the mixture was stirred until no white solids were observed in the reaction mixture (this takes a few minutes). The resin was filtered off, 1 M NaOH (142 μL, 2 equiv) was added to the filtrate, and the reaction mixture was further stirred for a few minutes. Then, the solution was taken to dryness in vacuo. The PC Na^+ salt was obtained as a white solid in quantitative yield. ^1H, ^{13}C, and ^{31}P NMR spectra for the PC Na^+ salt were comparable to those of **1**.

Typical Preparation of 5a. 4a (600 mg, 1.54 mmol) was dissolved in 2 M HCl (5 mL), and the reaction mixture was stirred for 20 h at 38−40 °C (above 42 °C, the P−O−C bridge tends to hydrolyze!) followed by evaporation to dryness in vacuo. Diethyl ether (∼10 mL) was added to the residue, and the mixture was stirred until a fluffy white suspension was obtained. The precipitate (**5a**) was isolated by filtration, dried in vacuo, and was finally obtained as a white powder in 66% yield and 83−90% purity (containing diethyl monomethyl PC, monoethyl PC, and traces of PC as impurities).

Preparation of 5b. 5a (330 mg, 1.05 mmol) was dissolved in acetone (10 mL), KI (oven dried, 178 mg, 1.07 mmol) was added, and the reaction mixture was stirred for 24 h at 60 °C. The precipitate that formed was separated by centrifugation, washed with acetone, and dried in vacuo. **5b** was obtained quantitatively as white powder.

Preparation of 4a. Triethyl citrate (6.3 mL, 25.9 mmol) and dry triethylamine (5.4 mL, 38.7 mmol) were dissolved in diethyl ether (220 mL). $MeOPCl_2$ (3.8 mL, 40.0 mmol) was then added slowly to the reaction mixture, which was then stirred under nitrogen (in a glovebox) at room temperature for 24 h. The precipitate that formed was filtered off under nitrogen, and excess $MeOPCl_2$ was removed under vacuum. The residue was redissolved in diethyl ether (∼50 mL), and a mixture of dry MeOH (1.1 mL, 27.0 mmol) and dry triethylamine (4.0 mL, 28.7 mmol) in diethyl ether (∼25 mL) was added in portions to the reaction mixture under stirring in a nitrogen glovebox for 3 h at room temperature. Stirring was continued, and diethyl ether was added to dilute the reaction mixture if it was too viscous. A precipitate formed and was removed under a nitrogen atmosphere, and the volume was reduced to ∼70−80 mL and cooled to −5−0 °C. Then, distilled sulfuryl chloride (2.08 mL, 25.9 mmol) was added slowly and ***carefully***. The reaction mixture was then stirred for 1 h at room temperature. After filtration to remove any formed solids, diethyl ether was removed from the filtrate by evaporation. The crude product was purified by silica column chromatography using ethyl acetate/hexane (1:1) as eluent (silica was oven-dried at 120 °C and ethyl acetate over $MgSO_4$ before use). The final product was obtained as a colorless oil in 68% yield. Compound **4a** seems to be quite stable since no degradation was observed during a 2-month storage period (without N_2) at −18 °C.

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Preparation of 4b. 4a (200 mg, 0.51 mmol) was dissolved in H_2O (1 mL), and $NaHCO_3$ (43 mg, 0.51 mmol) was added to it. The reaction mixture was then stirred for 1 h at room temperature, and the solvent was removed in vacuo. The residue was dissolved in chloroform (~5 mL), dried over $MgSO_4$, and subsequently evaporated in vacuo. **4b** was obtained as a colorless oil in quantitative yield.

Preparation of 4c. 4a (200 mg, 0.51 mmol) was dissolved in absolute EtOH (1 mL), $NaHCO_3$ (44 mg, 0.52 mmol) was added to it, and the reaction mixture was refluxed for 2 h. A residue was obtained after evaporation of EtOH in vacuo and was then suspended in diethyl ether. The solid was removed by filtration, and the filtrate was evaporated to dryness in vacuo. **4c** was obtained as slightly yellow oil in 92% yield.

Preparation of 4d. 4b (177 mg, 0.48 mmol) was dissolved in CH_3CN (3 mL), $NaHCO_3$ (41 mg, 0.49 mmol) and trimethylsilyl bromide (140 μL, 1.06 mmol) were added to it, and the reaction mixture was stirred for 2 h at room temperature. A solid formed that was filtered off, and the filtrate was evaporated to dryness in vacuo. The residue was redissolved in MeOH (1 mL) and stirred for 1 h at room temperature and then evaporated to dryness in vacuo. **4d** was obtained quantitatively as an orange oil.

Preparation of 4e. 4c (186 mg, 0.47 mmol) was dissolved in acetone (4 mL), KI (oven-dried, 76 mg, 0.46 mmol) was added to it, and the reaction mixture was stirred for 48 h at 60 °C. It was then evaporated to dryness in vacuo, diethyl ether was added, and the resulting suspension was stirred for 15 min at room temperature. After centrifugation, the solids were separated and dried in vacuo. **4e** was obtained as a slightly yellow amorphous solid in 63% yield and 94% purity (contained triethyl monomethyl PC monopotassium salt as impurity).

Characterization of Compounds. 3-Carboxy-3-phosphonooxy Pentanedioic Acid, Phosphocitrate (1): ^{1}H NMR (D_2O + 1 drop of 6 M DCl) δ 3.33 (d, 2H, $^2J_{HH}$ = 16.5 Hz), 3.18 (d, 2H, $^2J_{HH}$ = 16.5 Hz); ^{13}C NMR (D_2O + 1 drop of 6 M DCl) δ 178.0 (d, $^3J_{CP}$ = 11.2 Hz), 175.6 (s, 2C), 81.2 (d, $^2J_{CP}$ = 6.3 Hz), 44.9 (t, 2C); ^{31}P NMR (D_2O + 1 drop of 6 M DCl) δ −4.91. Anal. Calcd for $C_6H_4O_{10}PKCa_2 \cdot 2.5\ H_2O$: C, 16.71; H, 2.10. Found: C, 17.04; H, 2.10. Mp > 310 °C (did not melt at 310 °C, so the exact mp could not be reasonably identified).

3-(Chloromethoxyphosphoryloxy)-3-ethoxycarbonyl Pentanedioic Acid Diethyl Ester (4a): ^{1}H NMR ($CDCl_3$) δ 4.31 (q, 2H, J = 7.0 Hz), 4.17 (q, 2H, J = 7.0 Hz), 4.16 (q, 2H, J = 7.0 Hz), 3.90 (d, 3H, $^3J_{HP}$ = 14.0 Hz), 3.37 (d, 1H, $^2J_{HH}$ = −16.1 Hz), 3.33 (s, 2H), 3.30 (d, 1H, $^2J_{HH}$ = −16.1 Hz), 1.33 (t, 3H, J = 7.0 Hz), 1.273 (t, 3H, J = 7.0 Hz), 1.268 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 168.4 (d, $^3J_{CP}$ = 5.2 Hz), 168.2 (s), 168.1 (s), 84.0 (d, $^2J_{CP}$ = 8.8 Hz), 62.9 (t), 61.2 (t), 61.1 (t), 56.0 (qd, $^3J_{CP}$ = 7.8 Hz), 41.3 (td, $^3J_{CP}$ = 5.0 Hz), 41.1 (td, $^3J_{CP}$ = 3.9 Hz), 14.1 (q, 2C), 13.9 (q); ^{31}P NMR (D_2O) δ −1.16; ESI-MS 389.0 (M + 1; 100%).

3-Ethoxycarbonyl-3-(hydroxymethoxyphosphoryloxy) Pentanedioic Acid Diethyl Ester (4b): ^{1}H NMR ($CDCl_3$) δ 4.27 (q, 2H, J = 7.0 Hz), 4.15 (q, 4H, J = 7.0 Hz), 3.75 (d, 3H, $^3J_{HP}$ = 11.5 Hz), 3.30 (d, 2H, $^2J_{HH}$ = −15.9 Hz), 3.29 (d, 2H, $^2J_{HH}$ = −15.9 Hz), 1.31 (t, 3H, J = 7.0 Hz), 1.25 (t, 6H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 169.3 (d, $^3J_{CP}$ = 5.8 Hz), 168.9 (s, 2C), 80.5 (d, $^2J_{CP}$ = 6.9 Hz), 62.5 (t), 61.0 (t, 2C), 54.4 (qd, $^3J_{CP}$ = 6.2 Hz), 40.9 (td, 2C, $^3J_{CP}$ = 3.8 Hz), 14.1 (q, 2C), 13.9 (q); ^{31}P NMR ($CDCl_3$) δ −5.86; ESI-MS 371.0 (M + 1; 100%).

3-Ethoxycarbonyl-3-(ethoxymethoxyphosphoryloxy) Pentanedioic Acid Diethyl Ester (4c): ^{1}H NMR ($CDCl_3$) δ 4.21 (q, 2H, J = 7.2 Hz), 4.09−4.04 (m, 2H), 4.07 (q, 4H, J = 7.1 Hz), 3.68 (d, 3H, $^3J_{HP}$ = 11.5 Hz), 3.258 (d, 1H, $^2J_{HH}$ = −16.21 Hz), 3.258 (d, 1H, $^2J_{HH}$ = −16.15 Hz), 3.242 (d, 1H, $^2J_{HH}$ = −16.15 Hz), 3.238 (d, 1H, $^2J_{HH}$ = −16.21 Hz), 1.26 (td, 3H, $^4J_{HP}$ = 1.0 Hz, J = 7.1 Hz), 1.25 (t, 3H, J = 7.2 Hz), 1.19 (t, 6H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 168.2 (d, $^3J_{CP}$ = 6.1 Hz), 167.7 (s, 2C), 79.4 (d, $^2J_{CP}$ = 6.5 Hz), 63.3 (td, $^2J_{CP}$ = 6.3 Hz), 61.3 (t), 59.8 (t, 2C), 53.5 (qd, $^3J_{CP}$ = 6.5 Hz), 39.96 (td, $^3J_{CP}$ = 1.1 Hz), 39.93 (td, $^3J_{CP}$ = 1.2 Hz), 14.9 (qd, $^3J_{CP}$ = 7.1 Hz), 13.1 (q, 2C), 12.9 (q); ^{31}P NMR ($CDCl_3$) δ −7.89; ESI-MS 399.1 (M + 1; 100%).

3-Ethoxycarbonyl-3-phosphonooxy Pentanedioic Acid Diethyl Ester (4d): ^{1}H NMR (CD_3OD) δ 4.23 (q, 2H, J = 7.0 Hz), 4.12 (q, 4H, J = 7.0 Hz), 3.35 (s, 2H), 3.28 (s, 2H), 1.29 (t, 3H, J = 7.0 Hz), 1.24 (t, 6H, J = 7.0 Hz); ^{13}C NMR (CD_3OD) δ 171.2 (d, $^3J_{CP}$ = 6.5 Hz), 170.5 (s, 2C), 80.7 (d, $^2J_{CP}$ = 5.0 Hz), 63.3 (t), 61.9 (t, 2C), 42.0 (td, 2C, $^3J_{CP}$ = 2.5 Hz), 14.4 (q, 2C), 14.2 (q); ^{31}P NMR (CD_3OD) δ −4.19; ESI-MS 357.1 (M + 1; 75%).

3-Ethoxycarbonyl-3-(ethoxyhydroxyphosphoryloxy) Pentanedioic Acid Diethyl Ester Monopotassium Salt (4e): ^{1}H NMR (D_2O) δ 4.22 (q, 2H, J = 7.0 Hz), 4.16−4.07 (m, 4H), 3.89 (qv, 2H, J = 7.0 Hz), 3.40 (d, 2H, $^2J_{HH}$ = 16.5 Hz), 3.17 (d, 2H, $^2J_{HH}$ = 16.5 Hz), 1.27 (t, 3H, J = 7.0 Hz); ^{13}C NMR (D_2O) δ 172.8 (d, $^3J_{CP}$ = 9.7 Hz), 171.3 (s, 2C), 78.7 (d, $^2J_{CP}$ = 6.3 Hz), 63.0 (t), 62.6 (td, $^2J_{CP}$ = 5.9 Hz), 61.8 (t, 2C), 42.3 (td, 2C, $^3J_{CP}$ = 2.3 Hz), 16.8 (qd, $^3J_{CP}$ = 7.9 Hz), 14.4 (q, 2C), 14.3 (q); ^{31}P NMR (CD_3OD) δ −3.51; ESI-MS 383.4 (M; 18%).

3-Ethoxycarbonyl-3-(hydroxymethoxyphosphoryloxy) Pentanedioic Acid (5a): ^{1}H NMR (D_2O) δ 4.27 (q, 2H, J = 7.2 Hz), 3.55 (d, 3H, $^3J_{HP}$ = 11.1 Hz), 3.33 (d, 2H, $^2J_{HH}$ = 16.4 Hz), 3.20 (d, 2H, $^2J_{HH}$ = 16.4 Hz), 1.28 (t, 3H, J = 7.2 Hz); ^{13}C NMR (D_2O) δ 175.65 (d, $^3J_{CP}$ = 10.9 Hz), 175.61 (s, 2C), 81.0 (d, $^2J_{CP}$ = 6.1 Hz), 66.3 (t), 56.1 (qd, $^3J_{CP}$ = 6.1 Hz), 44.8 (td, 2C, $^3J_{CP}$ = 1.7 Hz), 16.0 (q); ^{31}P NMR (D_2O) δ −3.53; mp 146−148 °C; ESI-MS 315.2 (M + 1; 22%).

3-Ethoxycarbonyl-3-phosphonooxy Pentanedioic Acid Monopotassium Salt, Phosphocitrate Monoethyl Ester Monopotassium Salt (5b): ^{1}H NMR (D_2O) δ 4.26 (q, 2H, J = 7.2 Hz), 3.35 (d, 2H, $^2J_{HH}$ = 16.2 Hz), 3.17 (d, 2H, $^2J_{HH}$ = 16.2 Hz), 1.28 (t, 3H, J = 7.2 Hz); ^{13}C NMR (D_2O) δ 176.1 (s, 2C), 175.8 (d, $^3J_{CP}$ = 10.6 Hz), 80.7 (d, $^2J_{CP}$ = 5.9 Hz), 66.3 (t), 45.0 (td, 2C, $^3J_{CP}$ = 1.9 Hz), 16.0 (q); ^{31}P NMR (D_2O) δ −4.21. Anal. Calcd for $C_8H_{12}O_{10}PK \cdot 0.5(CH_3)_2CO$: C, 31.07; H, 4.12. Found: C, 30.98; H, 4.15. Mp 242−244 °C (at 147 °C, a gas boiled off, most likely acetone).

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of key intermediate **4a** and final compound **1**, ^{1}H NMR spectra of compounds **4b**−**e** and **5a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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