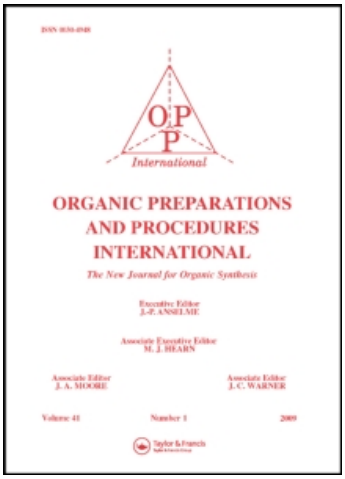

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AN IMPROVED SYNTHESIS OF 6-(O-PHOSPHORYLCHOLINE)HYDROXYHEXANOIC ACID

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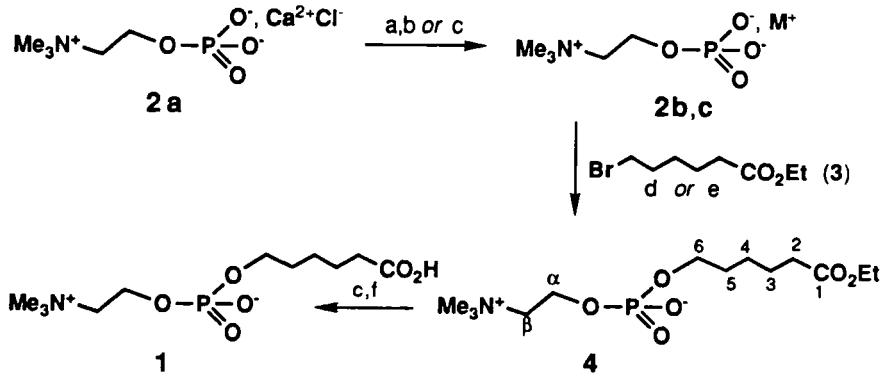
6-(Phosphorylcholine)hydroxyhexanoic acid (**1**) is a useful affinity ligand for the purification of calcium(II) ion-dependent phosphorylcholine binding proteins[1] and phosphorylcholine binding immunoglobulins.[2] The synthesis of this compound has been accomplished by a four-step procedure involving the substitution at phosphorus in 2-bromoethyl phosphorodichloridate with tert-butyl 6-hydroxyhexanoate followed by hydrolysis of the remaining phosphorochloridate, aminolysis of the bromoethyl derivative with trimethylamine and finally cleavage of the tert-butyl ester.[3] The hydroxyl component required for this synthesis is not readily available, and has to be prepared in a two-step process. The total yield of the synthesis based on bromohexanoic acid was 47%.[3]

We have found that the synthesis of **1** can be significantly improved by using an alkylation approach[4] to the synthesis of the phosphodiester bond. However, instead of preparing the troublesome silver salt of phosphorylcholine, it was decided to use either crown-ether catalysis or tetrabutylammonium salts. Thus, the calcium salt of phosphorylcholine (**2a**) was first converted into corresponding potassium salt **2b** by removing calcium as insoluble calcium oxalate and titrating the free acid with potassium hydroxide until pH 9 was reached. The salt **2b** was then alkylated with ethyl 6-bromohexanoate (**3**) in formamide in the presence of 10 molar % of dibenzo-18-crown-6 at 110-120° during 3 hrs to give ethyl 6-(phosphorylcholine)hydroxyhexanoate (**4**); in this step, conversions up to 85% were estimated by means of ^{31}P NMR. Alternatively, the acid form of phosphorylcholine



a) oxalic acid b) aq. KOH c) aq. $Bu_4N^+ OH^-$ d) dibenzo-18-crown-6/formamide
e) acetonitrile f) aq. HCl; $M^+ = K^+$ or Bu_4N^+

was titrated with tetrabutylammonium hydroxide and the resulting tetrabutylammonium salt **2c** was alkylated with **3** in acetonitrile at room temperature. In the latter case, the yield of alkylation was lower (50%), most likely due to the competing elimination of ethyl 6-bromohexanoate, generating hydrogen bromide and thus precipitating the acid form of phosphorylcholine from the reaction mixture. Finally, the resulting ethyl ester **4** was hydrolyzed in the presence of tertrabutylammonium hydroxide giving **1** in 80% yield.

EXPERIMENTAL SECTION

All reagents employed were obtained from commercial sources unless otherwise stated. Organic solvents were reagent grade and dried before use by routine methods. All NMR measurements were carried out with JEOL FX60 spectrometer operating at 60 and 24.3 MHz for ^{1}H and ^{31}P, respectively. Chemical shifts were referenced indirectly to tetramethylsilane and 85% phosphoric acid, correspondingly. Ethyl 6-bromohexanoate (**3**) was obtained by the treatment of the corresponding acid (Aldrich) with $EtOH/H_2SO_4$, 89%, bp. 82°/1 mm Hg. tetra-n-Butylammonium hydroxide was obtained from the corresponding bromide by passing its aqueous solution through the OH^- Dowex anion exchange resin.

Potasium Salt of Phosphorylcholine (**2b**).- The calcium salt of phosphorylcholine (Sigma, 10.3 g, 44 mmol) in water (60 mL) was treated with aqueous oxalic acid (3.96 g, 44 mmol). The white precipitate was centrifuged, and the solution was titrated with 1 M KOH to pH 9. The resulting solution was evaporated and the residue was dried under vacuum over P_2O_5 to yield 9.1 g (96%) of **2b** as white, highly hygroscopic crystals.

tetra-*n*-Butylammonium Salt of Phosphorylcholine (**2c**).- The acid form of phosphorylcholine was titrated with tetra-n-butylammonium hydroxide to pH 9. The product was rendered anhydrous by repeated evaporation of its benzene solution and stored under vacuum over P_2O_5.

Ethyl 6-(O-Phosphorylcholine)hydroxyhexanoate (**4**).- a) **2b** (2.8 g, 12.9 mmol) and dibenzo-18-crown-6 (Aldrich, 0.450 g, 1.25 mmol) in formamide (Fluka, 12 mL) were treated with **3** (2.9 g, 12.9 mmol). The resulting mixture was heated at 110-120° for 3 hrs, and then was subjected to column chromatography on silica gel. After formamide had been eluted with methanol, MeOH-H_2O (4:1) mixture was used for elution. The diester **4** (1.9 g, 46%) was isolated as a clear viscous oil which solidified upon storing at 4°.

b) **2c** (4.7 g, 11.2 mmol) in anhydrous acetonitrile (10 mL) was reacted with **3** (2.5 g, 11.2 mmol) at 23° during 15 hrs followed by refluxing for 3 hrs. Phase separation occurred and the ^{31}P NMR spectra of the light acetonitrile phase showed the absence of phosphorylcholine. The mixture was evaporated and the residue was dissolved in water (20 mL). The aqueous solution was washed three times with methylene chloride. The aqueous layer was concentrated and the product was purified as described above giving 1.8 g (50%) of **4**.

^{31}P NMR (MeOH): δ -0.7 ppm; ^{13}C NMR (MeOH-d_4): δ 176.1 (C-1), 67.9 (C_α), 66.9 (5.7 Hz, C-6), 60.5 (5.7 Hz, C_β), 55.1 (NMe), 52.2 (OCH_2), 35.0 (C-2), 31.7 (7.2 Hz, C-5), 26.7, 26.0 (C-3, C-4).

6-(O-Phosphorylcholine)hydroxyhexanoic acid (**1**).- Phosphodiester **4** (6.1 mmol) in water (6 mL) was treated with tetra-n-butylammonium hydroxide (12.2 mmol) and the resulting mixture was left at 23° for 12 hrs. The product was neutralized with dilute aq. HCl to pH 3.5 and extracted three times with methylene chloride. The aqueous phase was evaporated and the residue was dried under vacuum. The crude product was chromatographed on silica gel using MeOH-H_2O (4:1), yielding 1.55 g (80%) of **1** as white plates, mp. 8°.

Anal. Calcd. for $C_{11}H_{24}NO_6P \cdot H_2O$: C, 41.90; H, 8.25; N, 4.44

Found: C, 41.88; H, 8.30; N, 4.40

TLC: R_f 0.35 (MeOH-H_2O, 4:1); ^{31}P NMR (MeOH): δ -0.7 ppm; ^{13}C NMR (MeOH-d_4): δ 177.7 (C-1), 67.9 (C_α), 66.9 (8.0 Hz, C-6), 60.5 (5.0 Hz, C_β), 55.1 (NMe), 35.2 (C-2), 31.7 (7.3 Hz, C-5), 26.8, 26.0 (C-3, C-4); ^1H NMR (MeOH-d_4): δ 4.44 (m, 2H, H_α), 4.07 (q, 6.5 Hz, H-6), 3.82 (m, 2H, H_β), 2.49 (tr, 7.3 Hz, 2H, H-2), 1.83 (m, 4H), 1.65 (m, 2H).

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