Synthesis of Two Novel Phosphorylcholine Esters for Probes in Immunological Studies

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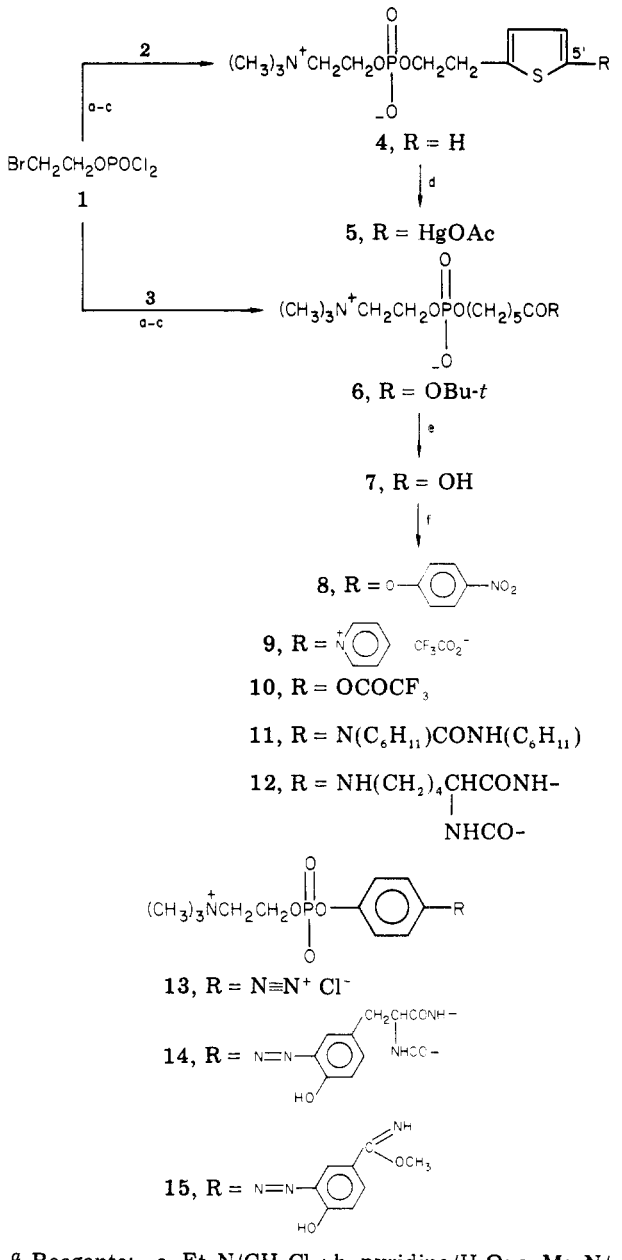
For use in immunological studies on phosphorylcholine (PC)-binding immunoglobulins, two PC esters were synthesized. Ester **5**, obtained in 65% overall yield, contained a covalently bound mercury atom at a specific site as required in an X-ray diffraction application; the other ester **8**, produced in 63% overall yield, was developed as an acylating reagent which would attach the PC group through an aliphatic spacer to protein amino groups under mild conditions. Modified procedures for the mercuration of the thiophene ring, hydrolysis of water-soluble *tert*-butyl esters, and the preparation of *p*-nitrophenyl esters are described.

In the context of immunological studies with phosphorylcholine (PC)-binding antibodies,[1] two rather unusual PC esters were required. X-ray diffraction work on the crystalline[2] myeloma antibody, Mc603, necessitated the synthesis of a heavy-metal-containing PC ester for use in locating and defining the PC-binding site. This heavy metal should, ideally be attached covalently to a single position uncomplicated by stereoisomerism. These specifications were met by the selective acetoxymercuration of the 2-(2-thienyl)ethyl PC ester **4** to produce **5**. Both **4** and **5** bound well to Mc603 and the use of **5** permitted, for the first time, the precise location and description by X-ray diffraction, of the antigen-binding site of an immunoglobulin.[3a]

The second synthesis was undertaken in response to the need for a PC ester which could be attached easily to proteins and solid supports for use in the production of synthetic PC antigens or for purifying anti-PC immunoglobulins, respectively. The diazonium salt **13** of Cheseboro and Metzger[4] is currently the only reagent available for such purposes; and while it continues to be extremely useful, it lacks specificity[5] in its reaction with proteins. This makes it impossible to quantitate the diazo coupling of **13** to most proteins by spectrophotometry alone and necessitates a separate destructive micro phosphate assay to determine the number of PC groups attached. For this reason and the following considerations, the PC ester **8** was synthesized. First, it was anticipated that reaction of **8** with a protein should lead to stable amide linkages involving only lysine ϵ-amino groups or N-terminals. Reaction with the former, the more plentiful target, should create a "spacer" (cf. **12**) of 11 contiguous atoms extending from the protein backbone. This should allow better penetration of the PC hapten in **12** vis-à-vis that of **13** into the binding sites of anti-PC antibodies, which are probably cleftlike.[3b]

Furthermore, the PC environment in **12** should more closely resemble that in such naturally occurring phosphorylcholine-containing substances such as the sphingomyelins and lecithins than that arising from the diazo-coupling reactions of **13** where a *p*-arylazo-substituted

Scheme I[a]



[a] Reagents: a, Et_3N/CH_2Cl_2; b, pyridine/H_2O; c, $Me_3N/CHCl_3$; d, $Hg(OAc)_2/CH_3OH$; e, Dowex-50 (H^+)/H_2O; f, CF_3COO-(C$_6$H$_4$)-NO_2/DMF/2,6-lutidine

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(3) (a) E. A. Padlan, D. M. Segal, T. Spande, D. R. Davies, S. Rudikoff, and M. Potter, *Nature (London) New Biol.*, **245**, 165 (1973); (b) D. M. Segal, E. A. Padlan, G. H. Cohen, S. Rudikoff, M. Potter, and D. R. Davies, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4298 (1974).

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(5) The following diazo-coupling reactions are possible with a typical protein: mono and bis azo coupling to tyrosine and reaction with histidine, tryptophan, and lysine, the latter reaction yielding acid-sensitive triazenes. A recent refinement[15] in the use of **13** circumvents this nonspecificity and directs the attachment of PC to protein amino groups by first coupling **13** to methyl *p*-hydroxybenzimidate and then reacting the resulting azo-substituted amidate **15** at pH 9 with proteins or cells.

phenyl PC ester (e.g., 14) would be generated. Finally, it was hoped that the *p*-nitrophenyl active ester (8) would be stable enough to be used by immunochemists "off-the-shelf" without having to prepare the reagent fresh with each use, as is the case with 13, and that the release of *p*-nitrophenolate (λ_{max}(pH 8–10) = 410 nm) during the acylation of proteins would provide a means of conveniently quantitating the reaction by UV spectrophotometry. Whether 8 will satisfy all these goals has yet to be thoroughly tested.[6]

Esters 5 and 8 were prepared in 65 and 63% overall yield, respectively, from 2-bromoethylphosphorodichloridate (1) and either 2-(2-thienyl)ethanol (2) or *tert*-butyl 6-hydroxyhexanoate (3) by the PC ester synthesis of Hirt and Berchtold[7] with hydrolysis[4] and aminolysis[8] modifications (see Scheme I). Overall yields of intermediates 4 and 6 were 70 and 74%, respectively. Acetoxymercuration of 4, avoiding excesses of reagent, proceeded in 94% yield to 5 which was conveniently freed of excess reagent, unreacted 4, and polymercurated products by chromatography on Sephadex LH-20. This mercuration procedure was found more satisfactory than the usual thiophene mercuration with buffered mercuric chloride.[9-11] The ^{1}H NMR spectrum of 5 (see Experimental Section) showed two one-proton doublets (J = 3 Hz) for the remaining aromatic protons and confirmed acetoxymercuration at the 5′-position.

The *tert*-butyl ester 6 was conveniently hydrolyzed (92–95%) by brief heating in water with Dowex-50 (H^+) resin. Brief contact of 6 with trifluoroacetic acid at room temperature also provided 7; however, it proved impossible to free it of substantial residual TFA.

The formation of the *p*-nitrophenyl ester 8 was accomplished (93%) by employing a variation of the Sakakibara–Inukai procedure.[12] The original procedure with 7 and *p*-nitrophenyl trifluoroacetate in pyridine gave no reaction at room temperature, while on brief heating, the pyridinium salt 9 resulted, probably via 8 or the mixed anhydride 10. The classic du Vigneaud procedure with *p*-nitrophenol and DCC in DMF gave 8 accompanied by 50–60% yields of acyl urea 11. Immunological studies using 8 will appear elsewhere (Moore and Segal).

Experimental Section

IR spectra were recorded with a Perkin-Elmer Model 137 spectrophotometer, UV spectra with a Cary Model 11 spectrophotometer, and ^{1}H NMR spectra with a Varian Associates A-60 or HA-100 spectrometer. Chemical shifts are reported in parts per million (δ) relative to internal Me_4Si in $CDCl_3$ or external sodium 2,2-dimethyl-2-silapentane-5-sulfonate in D_2O. Mass spectra were obtained with a Finnigan mass spectrometer 1015D with a model 6000 data system.

Elemental analyses were performed in the Microanalytical Services and Instrumentation Section of the Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, under the direction of Ms. Paula Parisius.

Thin-layer chromatograms (TLC) used Analtech silica gel-GF, 2.5 × 10 cm plates, and one or more of the following systems: toluene/ethyl formate/formic acid (5:3.5:1) (A), *n*-butanol/acetic acid/water (5:2:4) (B) or $CH_3OH/CHCl_3$ (1:9) (C). Detection used absorption of fluorescence (F), iodine vapor (I), or ammonia vapor (NH_3). Detections indicated as slow required several hours.

Reagent-grade acetonitrile, dimethylformamide (DMF), and dichloroethane (DCE) were dried several days with 4-Å molecular sieves (Davison). The reagent supplier was Aldrich, unless otherwise indicated.

2-Bromoethylphosphorodichloridate (1, bp 92–93 °C (5 mm)) was prepared from $POCl_3$ (Fisher, reagent) and 2-bromoethanol in 29% yield by following the procedure of Hirt and Berchtold[7] with one change. The 1-h addition of bromoethanol was made to a *refluxing* solution of $POCl_3$ in CCl_4, and liberated HCl was removed by periodic evacuation of the system and addition of more CCl_4 as needed. This was faster than the original 24-h procedure but did not materially affect the yield.

2-(2-Thienyl)ethyl 2-Bromoethyl Phosphate. To a stirred solution of 9.10 g (37.8 mmol) of 1 in 10 mL of DCA at 0 °C was added, dropwise over 30 min, a solution of 4.61 g (37.8 mmol) of 2-(2-thienyl)ethanol (2) and 3.94 g (39 mmol) of triethylamine (Eastman Organic Chemicals, dried over KOH pellets) in 10 mL of DCE. The addition funnel was rinsed with 5 mL of additional DCE. The reaction temperature was maintained at 10–15 °C during the addition and then allowed to warm to room temperature. After being stirred for 2.5 h, the reaction was cooled to 0 °C and a mixture of 5 mL of H_2O and 20 mL of pyridine was added dropwise with stirring over 2–3 min. This mixture was stirred at room temperature for 30 min and then stripped of solvents under vacuum. The residue was dissolved in water and the pH adjusted to 2.0 with 2 N HCl. The thick oil which precipitated was extracted with ethyl acetate and the extract was dried (Na_2SO_4) and evaporated to give 10.30 g (87%) of oil, showing no 2-(2-thienyl)ethanol present by TLC (C).

2-(2-Thienyl)ethylphosphorylcholine (4). To a solution of 7.14 g (22.7 mmol) of the above compound in 15 mL of reagent chloroform was added, with rapid stirring, 10 mL (ca. 100 mmol) of trimethylamine (Eastman Organic Chemicals). The solution was stirred until the reaction temperature had fallen to room temperature and then refluxed for 1 h. The reaction was cooled to 0 °C, another 10 mL of trimethylamine was added, and then reflux was resumed for 1 h.

This operation was repeated once more and then the solution refluxed for 3 days. The clear amber solution was stripped to dryness, the residue was dissolved in 10 mL of H_2O, applied to a 1.5 × 22 cm column of mixed bed resin[4] (Fisher, Rexyn I-300), and eluted with 1 L of H_2O. The initial 200 mL was slightly colored and was rechromatographed on another 2.0 × 22 cm column. A slowly crystallizing, colorless, highly hygroscopic oil (5.29 g, 80%) resulted after lyophilization: mp 174–179 °C; negative Beilstein (copper wire) test for bromine; IR (neat) 3350, 3020, 2950, 2880, 1650, 1480, 1240, 1090, 970, 920, 873 cm^{-1}; TLC, single spot at R_f 0.38 (B). Anal. Calcd for $C_{11}H_{20}NO_4PS\cdot H_2O$: C, 42.5; H, 7.10; N, 4.50; P, 10.0. Found: C, 44.36; H, 8.29; N, 4.77; P, 10.2.

2-(5-(Acetoxymercurio)-2-thienyl)ethylphosphorylcholine (5). To a rapidly stirred solution of 0.623 g (2.13 mmol) of 4 in 20 mL of absolute methanol was added dropwise over 10 min a solution of 0.750 g (2.35 mmol) of mercuric acetate in 20 mL of absolute methanol with the addition funnel being rinsed with small portions of methanol (5 mL total). After being stirred for 3.5 h at room temperature (UV λ_{max} (CH_3OH) shifts from 232 to 243 nm), the solution was concentrated (40 °C) to 5 mL and applied to a 2.9 × 51 cm column of Sephadex LH-20, equilibrated for 24 h with absolute methanol. Fractions (3.3 mL) were taken at a flow rate of 1 mL/min.

Mercurated product (instaneous black precipitate with $NaBH_4$) appeared in fractions 73–90, although fractions 73, 74, and 86–90

(6) Concentrated acetonitrile solutions of 8 have been stored at −70 °C for 6 months without appreciable decomposition. Acylations of bovine serum albumin (BSA) can be conducted over the pH range 7.5 to 9.0. At pH 8.5 (0.2 M borate) and room temperature, the acylation of BSA is essentially complete within 1 h with 55 ± 2 lysines (59 total) reacted, as determined by microphosphate analysis (J. J. Moore, personal communication). For roughly every 1.2 to 1.5 equiv of reagent expended, as determined by UV-spectrophotometry, one residue of lysine is acylated, the surplus being consumed by competing hydrolysis. The UV quantitation of the reaction of 8 with proteins at various pHs and the acylation of proteins, cells, and modified Sepharose with 8 will be reported elsewhere (J. J. Moore and D. M. Segal).

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(12) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Jpn.*, **37**, 1231 (1964).

(13) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).

were contaminated by byproducts absorbing in the UV at λ_{max} (EtOH) 260 and 235 nm. Fractions 75–85 were combined and stripped to dryness, leaving 1.10 g (94%) of hygroscopic clear oil.

^{1}H NMR (100 MHz). The thiophene six-line aromatic multiplet between δ 6.85–7.32 observed for **4** in CD_3OD (Me_4Si internal standard) changes on mercuration to two one-proton doublets (J = 3 Hz) at δ 6.95 and 7.15 (thiophene ring 3- and 4-protons, respectively) and confirms acetoxymercuration of the 5-position. Other signals observed for **5** are two overlapping triplets (J = 6 Hz) totaling four protons at δ 4.04 and 4.10 ($CH_2OPO_2^-$, OCH_2), a two-proton, five- or six-line multiplet at δ 3.5 (CH_2N^+),[14] a nine-proton singlet at δ 3.14 ($^+N(CH_3)_3$), and a three-proton singlet at δ 2.00 ($CH_3CO_2^-$). The SC(=CH)CH_2 signals are buried under impurity CHD_2OH (δ 3.34) and the NCH_3 signals. They appear as a triplet at δ 3.00 in 2-(5-(acetoxymercurio-2-thienyl)ethanol.

Compound **5** is a very hygroscopic material. Analysis of a sample allowed to equilibrate to ambient humidity was consistent with a trihydrate, $C_{13}H_{22}NO_6PSHg\cdot 3H_2O$. Anal. Calcd: C, 25.76; H, 4.66; N, 2.31; P, 5.11. Found: C, 25.90; H, 4.76; N, 2.21; P 5.09.

Stability of 5. Solutions of **5** in 50% saturated ammonium sulfate–imidazole (pH 7.0) showed no change in either extinction coefficient or λ_{max} up to 2 weeks. In unbuffered saturated ammonium sulfate (pH 5.2) on standing overnight, the spectrum of **5** (λ_{max} 243 nm) changes to that of **4** (λ_{max} 235 nm). A rough estimate of the molar extinction coefficient (6100) of **5** at 243 nm is permitted by this experiment and the extinction of the model 2-methylthiophene (ϵ_{235} 5000). Solutions of **5** in absolute methanol at 5 °C were stable for periods up to 3 months, as judged by no change in the ^{1}H NMR spectra and examination by TLC of an aliquot exposed to $NaBH_4$. The supernatant showed only **4** (R_f 0.38) and no phosphorylcholine (0.12, I_2 detection) or 2-(2-thienyl)ethanol (0.81) on silica gel-GF TLC (B).

***tert*-Butyl 6-Hydroxyhexanoate (3).** *tert*-Butyl 6-bromohexanoate was prepared by following the general procedure of Anderson and Callahan.[13] Isobutylene (Matheson) was passed slowly through a stirred solution of 20.0 g (100 mmol) of 6-bromohexanoic acid (Eastman Organic Chemicals) in 125 mL of CH_2Cl_2 containing 1 mL of concentrated H_2SO_4 for 2 h at room temperature. During the first 20 min the reaction temperature rose slightly and then fell. After standing an additional 2 h at room temperature, the mixture was extracted three times with portions of a mixture of 50 mL of saturated $NaHCO_3$ and 20 mL of saturated NaCl. The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated to afford 23.7 g (93%) of colorless oil; IR ($CHCl_3$) 1730 (sharp), 1390 (sharp), 1370 cm^{-1} (sharp). No CO_2H absorptions were found.

This was dissolved in 50 mL of DMF containing 20.4 g (300 mmol) of sodium formate, heated with stirring at 110 °C for 4 h, and then held at room temperature for 2 days. Then 7.3 g of 85% KOH (110 mmol) pellets in 25 mL of H_2O was added and the solution stirred at 30–40 °C for 4 h. After removal of the bulk of the DMF under vacuum at 35–40 °C, the residue in water was extracted three times with ethyl acetate and the ethyl acetate layer was dried (Na_2SO_4) and evaporated to give 18.6 g of oil which was shown by ^{1}H NMR to be 75% **3** and 25% DMF. The corrected yield (14 g) of **3** would be 73% and may reflect some losses by volatilization during the removal of the DMF.

A portion was distilled (77–79 °C (0.1–0.2 mm)) for the analytical data below. The remainder was used in the next step with no further purification: mass spectrum (chemiionization with NH_3), m/e 206 (P + 18, 28%), 189 (P + 1, 100%), 150 (206 – C_4H_8, 27%), 133 (189 – C_4H_8, 27%), 115 (133 – H_2O (?), 3%), 216 (3%, impurity); ^{1}H NMR δ 1.43 (s, 9, $C(CH_3)_3$), 1.4–1.8 (m, 6, CH_2), 2.22 (m, 2, CH_2CO), 2.60 (br, 1, OH, (exchanges with D_2O)), 3.70 (m, 2, CH_2OH); IR ($CHCl_3$) 3600 (sharp, OH unassociated), 3500 (br, OH associated), 1725 (ester C=O), 1390 and 1370 cm^{-1} (both sharp, *t*-Bu). Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.79, H, 10.71. Found: C, 63.50; H, 10.64.

***tert*-Butyl 6-(*O*-(2-Bromoethyl)phosphoryl)hydroxyhexanoate.** To a solution of 10.91 g (45.2 mmol) of **1** in 25 mL of DCE at 0 °C was added over 2 h a solution of 11.32 g (45.2 mmol) of **3** (prepared above, contains 25% DMF) in 25 mL of DCE containing 6.90 mL (50 mmol) of triethylamine (dried over KOH pellets). After warming to room temperature, the solution was stirred for 1 h and then cooled again to 0 °C and a mixture of 5 mL of water and 20 mL of pyridine added dropwise over 5 min with stirring. After warming to room temperature and being stirred overnight, the hydrolysis mixture was stripped of solvents and slurried between ethyl acetate and water in an ice bath as 22 mL of cold 2 N HCl was added gradually. The two phases were shaken well, and the organic layer was separated from the aqueous (pH 5.6) phase and dried (Na_2SO_4). The cold aqueous phase was adjusted to pH 1 with 2–3 mL of 2 N HCl and quickly extracted twice with ethyl acetate and washed with saturated NaCl. After being dried, this was combined with the first extract and evaporated to give 16.08 g (102%) of oil. A small amount of oily EtOAc-insoluble material deposited during the drying operation and, after being washed several times with EtOAc, was discarded: IR ($CHCl_3$) 1730, 1460, 1420, 1390 (sh), 1370 (sh), 1280, 1250, 1160, 1025, 850 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2–1.8 (m, 6, CH_2), 1.43 (s, 9, $C(CH_3)_3$), 2.0–2.5 (m, 2, CH_2CO_2-*t*-Bu), 3.4–3.75 (m, 2, CH_2Br), 3.9–4.7 (m, 4, CH_2OPOCH_2); TLC, R_f 0.20 (A, I), accompanied by minor impurities.

***tert*-Butyl 6-(*O*-Phosphorylcholine)hydroxyhexanoate (6).** The above product (46.4 mmol) was dissolved in 80 mL of reagent chloroform, cooled to 0 °C, and treated with 25 mL of anhydrous trimethylamine (Eastman Organic Chemicals). The mixture was allowed to warm to room temperature, stirred for 20 min with slight heating, and then cooled to 0 °C. Another 25 mL of trimethylamine was added, and a dry ice/2-propanol condenser fitted with a KOH drying tube was attached to the flask to return the refluxing (bp 3 °C) trimethylamine. After a total reflux period of 8 h, during which time two more 25-mL portions of trimethylamine were added, the excess trimethylamine and chloroform were evaporated and the product was dissolved in water and then extracted with 1:1 ether–ethyl acetate. The aqueous layer was concentrated to give 19 g of amber oil (contains water) which was applied in a small volume of water to a column (1.6 × 80 cm) of mixed bed resin (Amberlite MB-1, Fisher) and eluted with water to give 6.30 g of **6** (17.9 mmol, 40%) in the first 300 mL of eluate: IR (KBr) 3500, 1730, 1390, 1370 cm^{-1}; ^{1}H NMR (D_2O) δ 1.43 (s, 9, $C(CH_3)_3$), 1.15–1.8 (br, 6, CH_2), 2.27 (m, 2, CH_2CO_2-*t*-Bu), 3.19 (s, 9, $(CH_3)_3N^+$), 3.5–3.95 (m, 4, CH_2N^+ and POC(6)H_2 overlapping), 4.1–4.5 (br, 2, $(CH_3)_3N^+CH_2CH_2OP$); TLC, R_f 0.52 (B, I, slow).

During the purification of **6** on the mixed bed resin, heat and gas evolution were noted. This observation and the poor recovery of **6** suggested that substantial hydrolysis of the *tert*-butyl ester had occurred, catalyzed by the Dowex-50 (H^+) component of the resin. Small samples of **6** could be successfully purified on MB-1 without decomposition; however, local overheating with larger amounts evidently leads to hydrolysis.

Pure 6-(*O*-phosphorylcholine)hydroxyhexanoic acid (**7**) could be obtained by repeated washing of the resin in batches with HOAc–H_2O (1:4) to remove **7** now bound to the quaternary amine salt portion of the resin. After removal of the acetic acid by azeotropic distillation with *n*-butanol (15 mm), a total of 4.72 g (15.9 mmol, 34%) of slowly crystallizing material (mp 68–78 °C) was obtained: total yield of **6** + **7**, 74%; ^{1}H NMR δ 1.3–1.7 (m, 6, CH_2), 2.2–2.6 (m, 2, CH_2CO_2H), 3.20 (s, 9, $(CH_3)_3N^+$), 3.5–4.0 (m, triplet overlapping, 4, CH_2N^+ and POC(6)H_2), 4.1–4.5 (br, 2, $(CH_3)_3N^+CH_2CH_2OP$).

A 20-min exposure of **6** to anhydrous TFA at room temperature gave **7** in quantitative yield; however, it proved impossible to free it of substantial TFA, as detected by IR, even by exhaustive evacuation. The chromatography results with **6** suggested the use of Dowex-50 (H^+) resin with gentle heating to cleave the *tert*-butyl ester, and this worked well to afford 92–95% yields of pure **7**. A typical preparation follows.

6-(*O*-Phosphorylcholine)hydroxyhexanoic Acid (7). **6** (2.56 g) in 20 mL of water containing 1 g of wet Dowex-50 (H^+) resin (previously washed several times with decantation to remove colored impurities) was heated for 30 min on the steam bath and then filtered, and the resin was washed with portions of water (100 mL total). After removal of the water by evaporation under vacuum, 2.09 g (92%) of slowly crystallizing, clear oil resulted: IR (neat) 3700–2500 (CO_2H), 1720 (CO), 1470, 1220, 1080, 1079,

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(15) P. C. Isakson, J. L. Honegger, and S. C. Kinsky, *J. Immunol. Methods*, **25**, 89 (1979).

972, 935 cm^{-1}; ^1H NMR, see above section; TLC, R_f 0.37 (B, I, slow). Anal. Calcd for the monohydrate $C_{11}H_{24}NO_6P \cdot H_2O$ (mol wt 315): C, 41.90; H, 8.25; N, 4.44; P, 9.84. Found: C, 41.99; H, 8.46; N, 4.17; P, 9.77.

***p*-Nitrophenyl 6-(*O*-Phosphorylcholine)hydroxyhexanoate (8).** The acid 7 was converted to the *p*-nitrophenyl ester 8 as follows: 247 mg (0.79 mmol, as hydrate) of 7 in 5 mL of DMF was treated with 0.40 g (1.70 mmol) of *p*-nitrophenyl trifluoroacetate (Aldrich). After the mixture was stirred for 5 min, during which time 7 slowly dissolved, 0.10 mL (0.86 mmol) of 2,6-lutidine in 1.0 mL of DMF was added over 1 min. A TLC after 1 h showed extensive formation of 8. Another 0.20 g of *p*-nitrophenyl trifluoroacetate was added, followed by another 0.10 mL of lutidine in 1.0 mL of DMF. After the reaction mixture was at held at room temperature for 2 h more, ether was added to precipitate 8. The supernatant was withdrawn, and the oily precipitate (8) was redissolved in acetonitrile and then reprecipitated again with ether. This sequence was repeated once again to give, after removal of the last traces of solvents, 305 mg of 8 as a clear viscous oil (93% yield, assuming mol wt 418). Quantitative yields have sometimes resulted with this procedure: IR (KBr) 3400 (br), 2900, 1750 (s), 1670, 1590 (aromatic), 1560 (aromatic), 1520 (NO_2), 1480, 1340 (NO_2), 1200 (br), 1075 (br), 970, 920, 860, 820 cm^{-1}; ^1H NMR (D_2O) δ 1.2–1.8 (envelope, 6, CH_2), 2.2–2.7 (m, 2, CH_2CO), 3.19 (s, 9, $(CH_3)_3N^+$), 3.4–4.0 (m, 4, CH_2N and $POCH_2$, overlapping), 4.0–4.5 (m, 2, NCH_2CH_2OP), and two two-proton doublets (J = 9 Hz) at δ 7.08 ($H_{2,6}$) and 8.0 ($H_{3,5}$) for the aromatic protons; TLC, R_f 0.58 (B, F, I (slow)), immediate yellow color with NH_3 vapor, only traces of lutidine (0.50) and *p*-nitrophenol ($R_f \sim 1$) detected by fluorescence; UV λ_{max} (CH_3OH) 269 nm (log ϵ 3.81); λ_{max} [0.2 N NaOH–CH_3OH (1:1)] 403 nm (log ϵ 4.13).

Diisopropylethylamine or DBN worked well as nonnucleophilic bases to form 8; however, they could not be completely removed from the preparations and caused the slow decomposition of 8 in roughly a week. Traces of the weaker hindered base 2,6-lutidine are evidently not disadvantageous, as preparations of 8 have been kept for many months in the refrigerator. Traces of *p*-nitrophenol are easily removed by chromatography in CH_3OH on Sephadex LH-20 where it greatly lags behind either 7 or 8.

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Registry No. 1, 4167-02-6; 2, 5402-55-1; 3, 73839-20-0; 4, 73839-21-1; 5, 73839-22-2; 6, 73839-23-3; 7, 73839-24-4; 8, 73785-43-0; 2-bromoethanol, 540-51-2; 2-(2-thienyl)ethyl 2-bromoethyl phosphate, 73855-19-3; mercuric acetate, 1600-27-7; *tert*-butyl 6-(*O*-(2-bromoethyl)phosphoryl)hydroxyhexanoate, 73839-25-5; $POCl_3$, 10025-87-3.

α-Keto Mesylate: A Reactive, Thiol-Specific Functional Group

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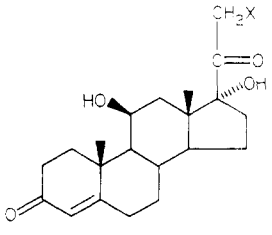
A systematic study of the reactivity of α-keto mesylates with various nucleophiles (i.e., carboxylate, –OH, imidazole, $-NH_2$, thiol acid anion, and –SH) under mildly basic conditions is reported. α-Keto mesylates do not react with imidazole or hydroxyl groups, react extremely slowly (if at all) with carboxylate and primary amines, and react several thousand times faster with thiols and thiol acid anions. The very rapid reaction with thiols occurs only with the dissociated thiolate anion. The addition of a β-hydroxyl group to α-keto mesylates accelerates the reaction with thiolate anions by a factor of 3–12 in acetone but has no effect on reactions run in dimethylformamide. α-Keto mesylates exhibit the same selectivity for thiolate anions, as compared to amines, as do α-keto chlorides. In view of this reactivity and selectivity, the α-keto mesylate appears to be a promising functional group for the electrophilic affinity labeling of biological macromolecules in weakly basic solutions.

α-Keto mesylates are relatively common in synthetic organic chemistry literature.[1-5] Since their main utility has been as a reactive intermediate in the preparation of other products,[1,3-6] we were surprised to find that no systematic study of the reactivity of α-keto mesylates exists.[4] During our preparation of several C_{21}-substituted glucocorticoids,[7-9] we found that α-keto mesylates possess very high, and selective, reactivity for thiols. Herein we describe the results of our studies on α-keto mesylates, including a comparison of the reactivity of α-keto mesylates with the closely related α-keto chlorides and with β-hydroxy-α-keto mesylates, which are even more reactive under some conditions.



1, X = $OSCH_3$ (O)

2, X = $SCCH_3$ (O)

3, X = $SCO\text{-}CH_2\text{-}CH_3$ (S)

Results

Reactivity of β-Hydroxy-α-keto Mesylates. Cortisol-21-mesylate (1) reacts with thiol acid anions such as potassium thiolacetate and potassium *O*-ethyl xanthate in acetone at 0 °C in less than 20 min to give high yields of 2 and 3, respectively.[9] Studies with another β-hydroxy-

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(1) British Patent 899 995 and 899 996, 1962; *Chem. Abstr.* **1962**, *57*, 13 842*f*.