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Biologically-Cleavable Phosphate Protective Groups: 4-Acyloxy-1,3,2-Dioxaphosphorinanes as Neutral Latent Precursors of Dianionic Phosphates.

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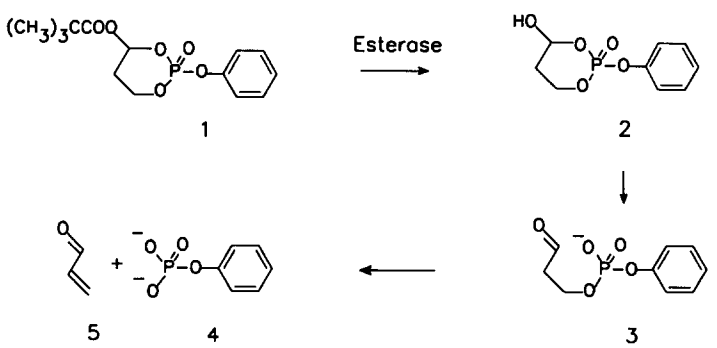
Abstract: 2-Phenoxy-2-oxo-4-pivaloyloxy-1,3,2-dioxophosphorinane (**1**) was prepared as a stable, neutral precursor of phenyl phosphate. In the presence of 20% human plasma, **1** was converted quantitatively to phenyl phosphate with a half-life of 15 min.

Dianionic phosphates play an important role in cellular metabolism. However, they have little or no potential as therapeutic agents because they are unable to penetrate into cells.[1,2] In an attempt to overcome this limitation, we sought to develop stable, neutral derivatives of dianionic phosphates that would revert to the parent compounds in the presence of human enzymes. To explore the feasibility of this approach, we selected phenyl phosphate as a model dianionic phosphate and prepared 2-phenoxy-2-oxo-4-pivaloyloxy-1,3,2-dioxophosphorinane, **1**, as a potential stable, neutral precursor. The anticipated mechanism of reversion of **1** to phenyl phosphate is shown in Scheme 1.

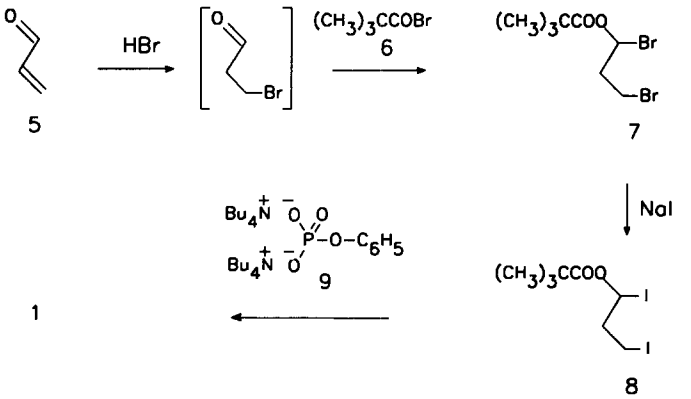
Scheme 1



In the presence of carboxylate esterases, enzymes that are ubiquitous in plasma and other tissues,[3] **1** should be hydrolyzed to the hydroxy analogue, **2**. After entering cells by passive diffusion, hemiacetal **2** should ring-open to the free aldehyde, **3**. Spontaneous elimination of acrolein (**5**) from **3** then generates the

dianionic phosphate, **4**. It is also conceivable that **1** might penetrate cells directly and undergo the same degradation sequence after hydrolysis by cellular esterases. 2-Phenoxy-2-oxo-4-pivaloyloxy-1,3,2-dioxo-phosphorinane was synthesized as shown in Scheme 2.

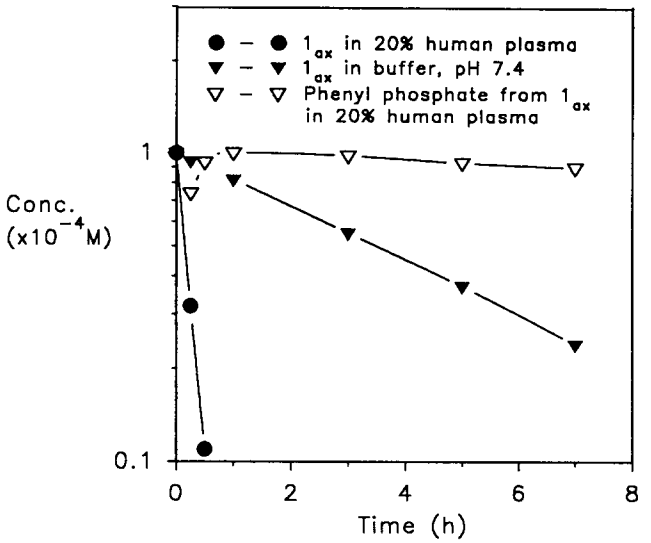
Scheme 2



A solution of acrolein (**5**) (6.72 g, 8.01 mL, 0.12 mmol) in anhydrous $CHCl_3$ (50 mL) was saturated with dry HBr gas at 0 °C. Pivaloyl bromide (**6**) (28.6 g, 0.13 mole) was added, followed by $ZnBr_2$ (0.2 g), and the mixture was stirred at room temperature for 5 days. The product was fractionated under reduced pressure to yield 1-pivaloyloxy-1,3-dibromopropane, (**7**)[4] (bp 85 °C/1.5mm Hg); yield, 16.4 g (45%). **7** (2.00 g, 6.60 mmol) in acetone (6.0 mL) was added to a solution of anhydrous NaI (2.48 g, 16.54 mmol) in dry acetone (40 mL) under a dry N_2 atmosphere. The mixture was stirred for 3 h and then poured into dry hexane (150 mL). Insoluble salts were removed by filtration, and the filtrate was concentrated under reduced pressure at < 30 °C. The remaining oil was taken up in dry hexane (30 mL) and again filtered to remove insoluble residue. Evaporation of the filtrate yielded 1-pivaloyloxy-1,3-diiodopropane (**8**)[5] as a light yellow oil (2.26 g, 86%). On attempted distillation, **8** decomposed. Since the ^1H NMR spectrum of the compound indicated that it was approx. 95% pure, it was used in the subsequent reaction without further purification. A solution of **8** (1.2 g; 3.03 mmol) in dry ethylene glycol dimethyl ether (10 mL) was added with stirring, under a dry N_2 atmosphere, to a solution of bis(tetrabutylammonium) phenyl phosphate[6] (1.99 g, 3.03 mmol) in dry ethylene glycol dimethyl ether (200 mL). The mixture was refluxed for 2 h, then cooled to room temperature, filtered, and concentrated. The residue was preadsorbed on silica gel (20 g), and the free-flowing powder was transferred to a column of silica (75 cm x 2.5 cm) made up in hexane. The products were eluted with EtOAc-hexane (1:1). Two diastereomers of **1** were obtained. The first to elute from the column was

obtained as a crystalline solid (76 mg, 8%), mp 125-126 °C.[7] The second was also obtained as a crystalline solid (126 mg, 13%), mp 99-100 °C.[8] Because of the complexity of the ^{1}H NMR spectra, it was not possible to unambiguously characterize the configurational and conformational properties of these stereeoisomers as has been reported for some 2-substituted 2-oxo-1,3,2-dioxaphosphorinanes.[9,10]

Figure 1



Stability Studies of 1

1 (fast eluting isomer) decomposed with a half-life of 3.5 h (Fig. 1) when incubated at a concentration of 10^{-4} M in 0.05 M phosphate buffer, pH 7.4, at 37 °C. In the presence of 20% mouse plasma, however, the compound was rapidly and quantitatively hydrolyzed[11] ($t_{1/2}$ = 15 min) to phenyl phosphate. No intermediates were detected in the incubation mixture. Similar results were obtained with the slower moving isomer (data not shown). Both diastereomers were also degraded rapidly in the presence of commercial (Sigma Chemical Co.) hog liver carboxylate esterase (data not shown). To the best of our knowledge, this is the first demonstration that a dianionic phosphate can be converted to a neutral derivative that is moderately stable in neutral aqueous media, yet reverts quantitatively to the parent dianionic phosphate in a single metabolic step in the presence of human plasma. The potential of this strategy to introduce dianionic phosphates and phosphonates into cells is under investigation.

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4. ^{1}H NMR (Varian 60 MHz; $CDCl_3$): δ 6.62 (t, 1 H, $CH_2C\underline{H}Br$), 3.47 (t, 2 H, CH_2Br), 2.72 (m, 2 H, $CH_2C\underline{H}_2CH$), 1.22 (s, 9 H, $C(CH_3)_3$). MS EI *m/z* 300/302/304 (1:2:1)
5. ^{1}H NMR (Varian 60 mHz; $CDCl_3$): δ 6.70 (t, 1 H, $CH_2C\underline{H}I$), 3.13 (t, 2 H, CH_2I), 2.63 (m, 2 H, $CH_2C\underline{H}_2CH$), 1.30 (s, 9 H, $C(CH_3)_3$).
6. Bis(tetrabutylammonium) phenyl phosphate was prepared by adding phenyl phosphate (1 equivalent) to a solution of bis(tetrabutylammonium) hydroxide (2 equivalents) in distilled water. The solution was lyophilized, and then dried by repeated evaporation with ethanol and chloroform. It was stored over P_2O_5 in vacuo.
7. TLC, Silica; EtOAc-hexane, 1:1; R_f = 0.64. ^{1}H NMR (Bruker 200 MHz; $CDCl_3$): δ 7.2-7.4 (m, 5 H, C_6H_5), 6.56-6.63 (m, 1 H, H-4), 4.5-4.7 (m, 2 H, H-6), 2.1-2.4 (m, 2 H, H-5), 1.28 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) (ppm): 176.76 ($O\underline{C}OC(CH_3)_3$), 150.13 (C-1'), 129.87 (C-3',5'), 125.39 (C-4'), 119.99 (C-2',6', J_{CP} = 4.5 Hz), 92.99 (C-4, J_{CP} = 5.5 Hz), 64.74 (C-6, J_{CP} = 6.0 Hz), 38.78 ($OCO\underline{C}(CH_3)_3$), 30.06 (C-5, J_{CP} = 6.0 Hz), 26.77 ($OCOC(\underline{C}H_3)_3$). ^{31}P NMR ($CDCl_3$): δ -14.33. MS (CI, CH_4) *m/z* 315 (MH^+). Anal. ($C_{14}H_{19}O_6P$) C, H.
8. TLC, Silica; EtOAc-hexane, 1:1; R_f = 0.41. ^{1}H NMR (Bruker 200 MHz; $CDCl_3$): δ 7.1-7.4 (s, 5 H, C_6H_5), 6.5-6.7 (m, 1 H, H-4), 4.3-4.9 (m, 2 H, H-6), 1.9-2.7 (m, 2 H, H-5), 1.16 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (50 MHz, $CDCl_3$) (ppm): 175.62 ($O\underline{C}OC(CH_3)_3$), 150.47 (C-1') 129.85 (C-3',5') 125.09 (C-4'), 119.59 (C-2',6', J_{CP} = 6.0 Hz), 93.14 (C-4, J_{CP} = 10.0 Hz), 64.52 (C-6, J_{CP} = 7.0 Hz), 38.90, $CO\underline{C}(CH_3)_3$), 29.72 (C-5, J_{CP} = 8.5 Hz), 26.76 ($OCOC(\underline{C}H_3)_3$). ^{31}P NMR ($CDCl_3$): δ -15.54. MS (CI, CH_4) *m/z* 315 (MH^+). Anal. ($C_{14}H_{19}O_6P$) C, H.
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11. 20 μL of a stock solution (10^{-2} M) of **1** in EtOH was added to 1.98 mL of 20% fresh human plasma in 0.05 M phosphate buffer, pH 7.4, contained in a 5-mL vial. The mixture was agitated for 15 sec on a Vortex shaker, then incubated in a water bath at 37 °C. Samples (100 μl) were withdrawn at 0, 5, 15, 30, 60, 180, 360, and 1,440 min intervals and added to 4 volumes of MeOH in a 1 mL vial. The mixtures were agitated on a Vortex shaker for 1 min, then centrifuged at 10,000 rpm for 10 min to sediment precipitated protein. The supernatants were analyzed for **1** on a μC-18 reversed phase column (Phenomenex, Torrance, CA; 150 mm x 3.9 mm) using MeOH-0.01 M potassium phosphate, pH 5.5 (55:45) as the mobile phase at a flow rate of 1.5 mL per min; the retention time was 8.6 min. Phenyl phosphate was analyzed on a SAX ion-exchange column (Whatman) using 0.02 M ammonium phosphate buffer, pH 3.5, as mobile phase at a flow rate of 2.0 mL per min; the retention time was 8.3 min.

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