Phosphoryloxymethyl Carbamates and Carbonates—Novel Water-Soluble Prodrugs for Amines and Hindered Alcohols

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Phosphoryloxymethyl carbonates and carbamates of the type R_1R_2X-CO-O-CH_2-O-PO_3^{-2} (X = O or N) were evaluated as potentially novel water-soluble collapsible prodrugs for alcohols and amines. These were prepared by reaction of α-chloromethyl chloroformate with the starting alcohol or amine to give the corresponding α-chloromethyl carbonate or carbamate, respectively. Reaction with silver dibenzyl phosphate followed by debenzylation by hydrogenolysis gave the desired products. The aqueous chemical stability of the phosphoryloxymethylcarbonyl derivatives of 2-indanol (**3a**), β-(3,4-dimethoxyphenyl)ethylamine (**3b**), and benzocaine (**3c**) were evaluated. The aqueous hydrolysis of **3a–3c** resulted in regeneration of the parent alcohol or amines. As expected, the hydrolytic behaviors of these derivatives were found to differ from that of simple alkyl and aryl phosphomonoesters. The rates of hydrolysis were extremely rapid, with the dianionic phosphate species possessing a higher reactivity than the monoanionic species. This was attributed to the proximity of the phosphate group to the carbonyl moiety. The carbamate derivatives, **3b** and **3c**, displayed greater chemical stability compared to the carbonate derivative, **3a**. Alkaline phosphatases-mediated hydrolysis of the phosphate ester bond in **3c** led to a rapid cascade reaction resulting in regeneration of the parent amine, benzocaine. Although the alcohol derivative described here appeared to be too chemically unstable to be ideal as a prodrug, the derivatives of the amines might have some use. They are expected to be cleaved *in vivo* by alkaline phosphatases.

KEY WORDS: phosphoryloxymethyl carbonates; phosphoryloxymethyl carbamates; water-soluble prodrugs; amines; hindered alcohols.

INTRODUCTION

The applicability of phosphomonoester prodrugs in circumventing various barriers to drug utilization have been examined and evaluated (1–7). The phosphomonoester prodrugs derived from primary alcohols and unhindered phenols display excellent chemical stability and high enzymatic and *in vivo* lability. Phosphomonoester prodrugs of sterically hindered secondary and tertiary alcohols suffer from a slow rate of bioconversion (8). This was recently confirmed by Kearney and Stella (9). The *in vivo* significance of these findings can be seen with the bioconversion of estramustine phosphate, a phosphate ester of sterically hindered secondary alcohol (10) whose dephosphorylation half-life of 1.3 hr contrasts with that of 8 min for phosphoryloxymethylphenytoin, which is a phosphate ester of a primary alcohol (4); both studies were carried out in human subjects.

A viable solution to enhance the rate of bioconversion of phosphomonoester prodrugs derived from sterically hindered alcohols is the incorporation of a less sterically hindered spacer group between the hydroxyl group and the phosphate functionality. Upon dephosphorylation, the spacer group must spontaneously decompose to generate the parent alcohol. This approach might also be utilized with drug molecules which do not possess a hydroxyl functionality (5).

The oxymethyloxycarbonyl spacer group has previously been applied in attempt to improve the characteristics of various amine containing drugs (11). The objective of the current study was to evaluate the oxymethyloxycarbonyl spacer group combined with a phosphate ester in designing novel water-soluble phosphoryloxymethyloxycarbonyl derivatives (Scheme I, **I**). Phosphatase-mediated hydrolysis of **I** should result in the formation of **II**, which should spontaneously decompose to **III** and subsequently the parent drug. The reaction products are the inorganic phosphate, an aldehyde, and carbon dioxide (Scheme I). This concept should be applicable to aliphatic alcohols (*x*, –O–), phenols, and primary and secondary aliphatic and aromatic amines (*x*, –NH–, or –NR–).

MATERIALS AND METHODS

Materials

All chemicals used were of reagent or analytical grade, obtained from Aldrich Chemical Co. Milwaukee, WI, and Fluka Chemika-Biochemika, Switzerland. Isolated human placenta alkaline phosphatase (Type XVII) was obtained from the Sigma Chemical Co., St. Louis, MO.

Analytical Procedure

The HPLC system consisted of a Gilson 115 UV detector, variable wavelength, operating at a fixed wavelength; a Gilson programmable 305 metering pump; a Rheodyne injector; and an IBM-based controller with Gilson 715 HPLC software. The HPLC studies were conducted using a reverse-phase analytical column C-18 (15 cm × 4.6 mm) with mean particle diameter of 5 μm. All the analyses were performed under isocratic conditions at ambient temperature. Flow rate was set at 1.5 mL/min. The chromatographic conditions and retention characteristics used for both nonenzymatic and enzymatic hydrolysis studies of the prodrugs are summarized in Table I. Melting points were determined in capillary tubes on a Mel-Temp II apparatus and are uncorrected. Elemental microanalyses were performed by Midwest Microlab, Indianapolis, IN. Infrared spectra were determined on a Beckman Acculab 3 instrument. Nuclear magnetic resonance spectra were obtained on a GE 300 or Varian XL-300 instruments. Chemical shifts are reported as parts

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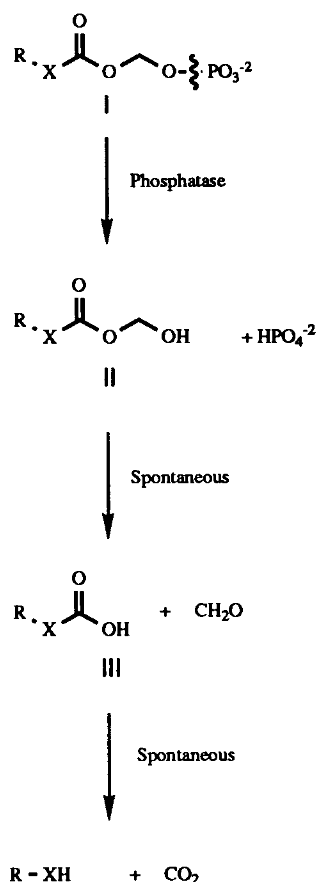


Scheme I. Enzymatic and nonenzymatic catalyzed steps involved in the generation of the parent drug from phosphoryloxymethyloxycarbonyl prodrug system.

per million downfield from tetramethylsilane or 3-(trimethylsilyl)-1-propane-sulfonic acid as internal standards for ^{1}H-NMR spectra and from 85% H_3PO_4 as external standard in ^{31}P spectra. Positive chemical shifts are at low field with respect to the standard. Precoated silica gel 60 F_{245} (E. Merck) plates were used for thin-layer chromatography.

Table I. Chromatographic Conditions and Retention Volumes for the Prodrugs 3a–c and the Respective Parent Compounds

Compound	Mobile phase (v/v)	L[a] (nm)	Retention volume (mL)
3a	25% acetonitrile/ 75% aqueous[b]	270	9.2
2-Indanol	"	"	7.4
3b	10% acetonitrile/ 90% aqueous[c]	278	20.0
β-(3,4-dimethoxy-phenyl) ethylamine	"	"	4.8
3c	25% acetonitrile/ 75% aqueous[b]	266	4.8
Benzocaine	"	"	15.3

[a] At an eluent flow rate of 1.5 mL/min.
[b] 25 m*M* KH_2PO_4, 1 m*M* tetrabutylammonium phosphate, pH 4.0.
[c] 25 m*M* KH_2PO_4, 0.025 m*M* tetrabutylammonium phosphate, pH 4.0.

Hydrolysis Studies

The pH of aqueous buffer solutions of varying buffer concentrations (10–30 m*M*) were adjusted at 25°C using an Accumet 915 Fisher pH meter which was standardized with NBS buffer solutions. The ionic strength of these solutions was adjusted to 0.15 *M* using potassium chloride. The following buffer systems were employed: acetate (pH 3.7–5.0) and phosphate (pH 6.5–7.5).

The hydrolysis reactions were initiated by the addition of 100 μL of the aqueous stock solution of **3a–c** (1–2 mg/mL) to 10 mL of the buffer solution, which was preequilibrated at 25°C in a circulating water bath. Aliquots were withdrawn at appropriate time intervals and analyzed by HPLC for the disappearance of the parent compound. The pseudo-first-order rate constants, k_{obs}, were obtained by following the disappearance of the prodrug for at least two half-lives.

The preliminary data indicated that the hydrolysis of **3b–c** was not significantly catalyzed by the buffer system employed. No attempts were made to determine the buffer catalysis on the degradation of **3a**. The observed rate constants determined at a 0.03 *M* buffer concentration were used to construct the pH–rate profiles.

The hydrolysis of *p*-NPP and **3c** in the presence of human placental alkaline phosphatase were conducted under conditions similar to those described elsewhere (9).

Synthesis

Chloromethyloxycarbonyl 3,4-Dimethoxyphenylethylamine (1b). To a stirred solution of β-(3,4-dimethoxyphenyl)ethylamine (1.81 g, 10 mmol) and Proton Sponge (2.41 g, 10 mmol) in dry dichloromethane (100 mL) at 0°C was added dropwise a solution of chloromethyl chloroformate (1.29 g, 10 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at room temperature overnight, washed with 1 *N* HCl (2 × 50 mL) and water (2 × 50 mL), dried over $MgSO_4$, filtered, and concentrated. The solid product, m.p. 103°C, was crystallized from dichloromethane/hexane in an 80% yield, 4.41 g. IR (nujol mull) 3300, 1740, 1715, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 6.80–6.70 (m, 3H, Ar–H); 5.73 (s, 2H, O–CH_2CL); 4.98 (bm, 1H, NH); 3.86 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 3.45 (m, 2H, CH_2NH); 2.79 (t, 2H, CH_2CH_2NH). *Anal.* Calcd for $C_{12}H_{16}NO_4Cl$: C, 52.65; H, 5.85; N, 5.11. Found: C, 52.80; H, 5.80; N, 5.10.

N-*[Dibenzylphosphoryloxymethyloxycarbonyl] 3,4-dimethoxyphenylethylamine (2b).* A solution of chloromethyloxycarbonyl 3,4-dimethoxyphenylethylamine (2.73 g, 10 mmol) and silver dibenzylphosphate (5.77 g, 15 mmol) in dry benzene was refluxed (100 mL) overnight. The reaction mixture was filtered, extracted with a saturated Na_2CO_3 solution, dried over Na_2SO_4, filtered, evaporated, triturated with ether overnight, and filtered to give 2.65 g of the product (m.p. 81°C) as a white powder. IR (nujol mull): 3300, 1740, 1250, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.33 (s, 10H, OBzl-H); 6.75–6.66 (m, 3H, Ar–H); 5.59 (d, 2H, O–CH_2-Cl, J = 13 Hz); 5.04 (d, 4H, J = 8 Hz, Ph–CH_2); 4.86 (t, 1H, NH); 3.83 (s, 3H, OCH3); 3.84 (s, 3H, OCH3); 3.64 (m, 2H, CH_2NH);

2.70 (t, 2H, CH_2CH_2NH). ^{31}P-NMR (CDCl3): H-coupled, 1.63 ppm (m); H-decoupled, 1.63 ppm (s). *Anal.* Calcd for $C_{26}H_{30}NO_8P$: C, 60.85; H, 5.83; N, 2.72; P, 6.02. Found: C, 60.67; H, 5.88; N, 2.77; P, 6.19.

[Dihydrogen Phosphoryloxymethyloxycarbonyl]-3,4-dimethoxyphenylethylamine (3b). *N*-[Dibenzylphosphoryloxymethyloxycarbonyl]-3,4-dimethoxyphenylethylamine (2 g, 3.88 mmol) was dissolved in ethyl acetate (100 mL), and 10% Pd/C (1 g) was added. After the mixture was purged with nitrogen for 5 min, it was subjected to hydrogenation for 45 min at atmospheric pressure and ambient temperature. The reaction mixture was filtered and evaporated to half of the initial volume. The resultant solid (m.p. 121°C) was then filtered to give 1.20 g (92.2%) of the product. ^{1}H-NMR (CD_3OD): 6.84–6.70 (m, 3H, Ar–H); 5.50 (d, 2H, O–CH_2–Cl, J = 13 Hz); 3.78 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.27 (m, 2H, CH_2NH); 2.70 (m, 2H, CH_2CH_2NH). ^{31}P-NMR (CD_3OD): −1.14 ppm (t, J = 13 Hz). *Anal.* Calcd for $C_{12}H_{18}NO_8P$: C, 42.98; H, 5.37; N, 4.17; P, 9.25. Found: C, 42.97; H, 5.41; N, 4.21; P, 8.94.

[Phosphoryloxymethyloxycarbonyl]-3,4-dimethoxyphenylethylamine Disodium Salt. To the solution of [dihydrogen phosphoryloxymethyloxycarbonyl]-3,4-dimethoxyphenylethylamine **3b** (335 mg, 1 mmol) in methanol (5 mL) was added 2 equiv of NaOH (20 mL of 0.1 *N* NaOH.). The aqueous methanol solution was evaporated to remove most of the methanol and lyophilized to give the disodium salt as a white powder (370 mg, 0.97 mmol, 97%). IR (nujol mull): 1735, 1705, 1250, 1040. ^{1}H-NMR (D_2O): 6.95–6.80 (m, 3H, Ar–H); 5.29 (d, 2H, O–CH_2–Cl, J = 13 Hz); 3.79 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.32 (m, 2H, CH_2NH); 2.71 (m, 2H, CH_2CH_2NH). ^{31}P-NMR (D_2O): 1.74 ppm (t, J = 13 Hz). *Anal.* Calcd for $C_{12}H_{16}NO_8PNa_2$. 1.5 H_2O: C, 35.46; H, 4.67; N, 3.45. Found: C, 35.50; H, 4.29; N, 3.49.

All the other products were prepared by the general procedures described above. The analytical properties of these compounds are as follows.

Ethyl 4-Chloromethylcarbonyl-aminobenzoate (1c). Yield, 80%; m.p. 145°C. IR (nujol mull): 1755, 1695 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.95 (d, 2H, Ar-H, J = 9 Hz); 7.44 (d, 2H, Ar–H, J = 9 Hz); 7.18 (m, 1H, NH); 5.75 (s, 2H, O–CH_2–Cl); 4.29 (q, 2H, CO_2CH_2, J = 7 Hz); 1.32 (t, 3H, $CO_2CH_2CH_3$, J = 7 Hz). *Anal.* Calcd for $C_{11}H_{12}NO_4Cl$: C, 51.26; H, 4.66; N, 5.43. Found: C, 51.34; H, 4.68; N, 5.39.

Ethyl 4-[Dibenzylphosphoryloxymethyloxycarbonyl]-aminobenzoate (2c). Yield, 47%; m.p. 80°C. ^{1}H-NMR ($CDCl_3$): 7.91 (d, 2H, Ar–H, J = 9 Hz); 7.40 (d, 2H, Ar–H, J = 9 Hz); 7.22 (s, 10H, O–Bzl–H); 5.60 (d, 2H, O–CH_2–Cl, J = 13 Hz); 4.98 (d, 4H, J = 7 Hz, Ph–CH_2); 4.29 (q, 2H, CO_2CH_2, J = 7 Hz); 1.32 (t, 3H, $CO_2CH_2CH_3$, J = 6 Hz). *Anal.* Calcd for $C_{25}H_{26}NO_8P$: C, 60.12; H, 5.20; N, 2.80; P, 6.21. Found: C, 60.12; H, 5.34; N, 2.85; P, 6.30.

Ethyl 4-[Dihydrogen Phosphoryloxymethyloxycarbonyl]-aminobenzoate (3c). Yield, 69%; m.p. 120°C. IR (nujol mull): 1760, 1735, 1705, 1250, 1040 cm^{-1}. ^{1}H-NMR (CD_3OD): 7.84 (d, 2H, Ar–H, J = 9 Hz); 7.40 (d, 2H, Ar–H, J = 9 Hz); 5.53 (d, 2H, O–CH_2–Cl, J = 13 Hz); 4.23 (q, 2H, CO_2CH_2, J = 7 Hz); 1.27 (t, 3H, $CO_2CH_2CH_3$, J = 7 Hz). ^{31}P-NMR (DMSO): 3.41 (t, J = 13 Hz). *Anal.* Calcd for $C_{11}H_{14}NO_8P.H_2O$: C, 39.16; H, 4.24; N, 4.15. Found: C, 39.06; H, 4.14; N, 4.07.

2-Chloromethyloxycarbonyloxyindane (1a). Yield, 73%; m.p. 45°C. IR (nujol mull): 1770 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 7.12 (m, 4H, Ar–H); 5.63 (s, 2H, O–CH_2–Cl); 5.43 [m, 1H, $(CH_2)_2$–CH–O]; 3.30 (d, 1H, CH_2, J = 6 Hz); 3.25 (d, 1H, CH_2, J = 6 Hz); 3.07 (d, 1H, CH_2, J = 3 Hz); 3.01 (d, 1H, CH_2, J = 3 Hz). *Anal.* Calcd for $C_{11}H_{11}O_3Cl$: C, 58.27; H, 4.85. Found: C, 58.09; H, 4.84.

2-Hydrogen Phosphoryloxymethyloxycarbonyloxyindane Anilinium Salt. IR (nujol mull): 1760, 1250, 1030. cm^{-1}. ^{1}H-NMR (DMSO): 7.01–7.22 (m, 9H, Ar–H); 5.39 (d, 2H, O–CH_2–Cl, J = 13 Hz); 5.35 [m, 1H, $(CH_2)_2$–CH–O]; 3.28 (d, 1H, CH_2, J = 6 Hz); 3.22 (d, 1H, CH_2, J = 6 Hz); 2.97 (s, 1H, CH_2); 2.91 (s, 1H, CH_2). ^{31}P-NMR (DMSO): 2.78 (t, J = 13.23). *Anal.* Calcd for $C_{17}H_{20}NO_7P.0.5H_2O$: C, 52.30; H, 5.38; N, 3.58; P, 7.95. Found: C, 52.45; H, 5.39; N, 3.60; P, 7.98.

RESULTS AND DISCUSSION

Chemistry

2-Indanol was chosen as a secondary alcohol-containing model compound since its phosphomonoester prodrug has displayed a slow rate of dephosphorylation in the presence of alkaline phosphatase (12). Benzocaine and β-(3,4-dimethoxyphenyl)ethylamine were chosen as model compounds for aromatic amines and aliphatic amines, respectively.

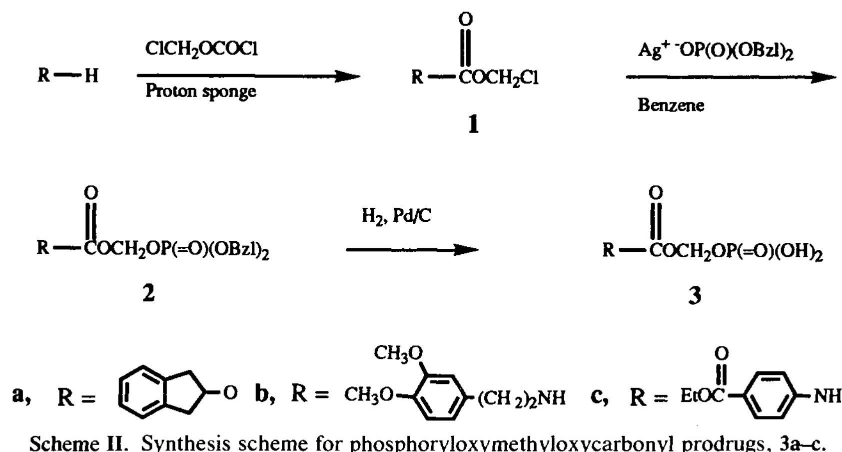
The promoiety group was introduced by reacting the corresponding alcohol or amines with chloromethyl chloroformate in the presence of Proton Sponge to form the chloromethyl carbonate (Scheme II, **1a**) or carbamate (Scheme II, **1b** and **1c**), respectively. The chloride was subsequently displaced by refluxing **1a–c** with silver dibenzyl phosphate in benzene to afford the phosphate triester derivatives **2a–c**. The final step involved the deprotection of the phosphate moiety by hydrogenolysis of **2a–c** to yield the dihydrogen phosphate monoesters **3a–c**.

These products were characterized by infrared and NMR spectroscopy. The IR spectrum of **3a** showed an absorption at 1760 cm^{-1} which corresponds to the carbonate carbonyl band. For **3b** and **3c** the IR spectra contained absorption bands at 1705–1735 cm^{-1} which correspond to the carbonyl carbamate moiety. In addition, bands at 1250 cm^{-1} (P=O) and 1030–1040 cm^{-1} (P–O–C) were observed for all three compounds (**3a–c**). The ^{1}H-NMR spectrum showed the appearance of a characteristic doublet corresponding to the P–O–CH_2 group as well as the anticipated ratios of the methylene protons with the various hydrogens in the molecules. The shape of the phosphorus signal (triplet) in the ^{31}P-NMR spectrum were also consistent with the anticipated structure.

Under aqueous alkaline conditions, the 2-dihydrogen phosphoryloxymethyloxy carbonyloxyindane was found to be quite unstable and therefore could not be isolated as the disodium salt. However, the monoanilinium salt was readily isolated and purified.

Chemical Stability of 3a–c.

To be therapeutically effective as a prodrug, phosphoryloxymethyloxycarbonyl derivatives must possess ade-



Scheme II. Synthesis scheme for phosphoryloxymethyloxycarbonyl prodrugs, 3a–c.

quate chemical stability for formulation purposes. In the present study, the dilute solution kinetics of hydrolysis of 3a–c were determined at 25°C and μ = 0.15 M as a function of pH.

Under the experimental conditions utilized, indanol was found to be the predominant degradation product of 3a, with no apparent accumulation of decomposition intermediate II or III (Scheme I). The hydrolysis of 3a to indanol followed apparent first-order kinetics for at least two half-lives in the pH range of 3.7–7.5. Figure 1 shows the partial pH–rate profile for the hydrolysis of 3a at 25°C, where k_{obs} is the apparent first-order rate constant. The resulting pH–rate profile displayed a maximum above pH 7, where 3a mainly exists in its dianionic form. This is in clear contrast with reported pH–rate profiles for conventional alkyl phosphomonoesters, where the pH of maximum reactivity is below 5.0 (13,14). Additionally, the hydrolysis rates of 3a in the pH range of 3.7–7.5 are much greater compared to the conventional alkyl phosphomonoesters. A mechanistic explanation of these observations follows.

The shape of the partial pH–rate profile for the hydrolysis of 3a can be adequately described by the following equation:

$$k_{obs} = k_o \frac{K_a}{[H^+ + K_a]} \quad (1)$$

where k_o is the apparent first-order rate constant for spontaneous or water-catalyzed hydrolysis of the dianionic species and K_a is the apparent second dissociation constant of the phosphate moiety. The kinetic rate constant and the dissociation constant parameters used to generate the solid line in Fig. 1 were $k_o = 1.20 \times 10^{-2}$ min^{-1} and $K_a = 1.84 \times 10^{-6}$ (pK_a = 5.70). This pK_a value of 5.7 was assigned to the second dissociation constant of phosphate moiety. This value is in good agreement with the second dissociation constant of structurally related phosphomonoesters.

The disparity in the pH dependency and the magnitude of the hydrolytic rate constants of 3a and those of conventional alkyl phosphomonoesters may be simply explained in terms of the reactive functional group(s). Generally, in the case of simple phosphomonoesters, dephosphorylation (P–O bond cleavage) is the governing route of degradation. The magnitude and the shape of the pH–rate profile for the hydrolysis of 3a are consistent with an alternative hydrolytic pathway, most likely the hydrolysis of the carbonate component of the spacer group (C–O bond cleavage).

In order to discern the chemical moiety responsible for the instability characteristics of 3a, compounds 3b and 3c, which are prodrugs of their corresponding amines, were designed and synthesized. In the case of 3b and 3c, the carbonate component of the spacer group is replaced by a more chemically stable carbamate functionality, while the phosphate group remains intact. Examining the chemical reactivity of 3b (Fig. 1) reveals that the substitution of the carbamate functionality for the carbonate significantly affected the relative chemical reactivity profiles of the spacer group to a degree consistent with carbamates having greater chemical stability than the carbonates. For example, prodrug 3b has a half-life of 48 days at 25°C and pH 7.5, which is substantially longer than the half-life of the corresponding carbonate prodrug, 3a (58 min). The observed increase in the chemical stability of the prodrug upon substitution reinforces our original assumption that the hydrolysis of the carbonate moiety is rate-limiting in the overall degradation of 3a. This assumes

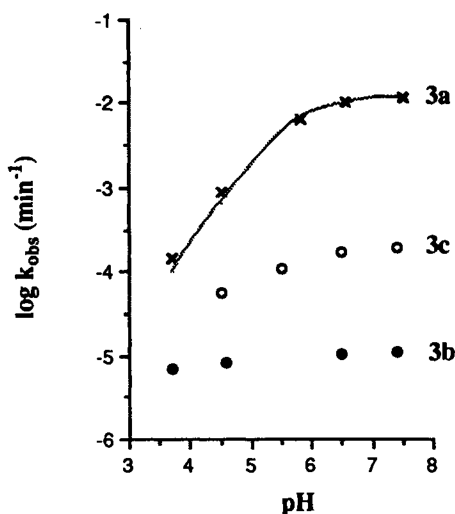


Fig. 1. Partial pH–rate profiles for the hydrolysis of 3a–c at 25°C and μ = 0.15 M. The solid line for 3a represents the theoretical profile based on Eq. (1) and values of 1.2×10^{-2} min^{-1} for k_o and 1.84×10^{-6} for K_a.

that other structural differences between the model compounds do not play a significant role in controlling the hydrolytic reactivity of spacer groups.

Compound **3b** was predominantly hydrolyzed to form the parent amine, β-(3,4-dimethoxyphenyl)ethylamine, as the only observable degradation product. Similarly, benzocaine was the predominant degradation product of **3c**. The compound **3c** displays a greater chemical stability and a depressed pH dependency compared to **3a** (Fig. 1). However, **3c** is more reactive than **3b**, consistent with the observation that carbamates derived from aliphatic amines are more stable than carbamates derived from aromatic amines (15).

It is informative to examine the pH–rate profile for the hydrolysis of **3a** (Fig. 1) in greater detail. The shapes of the pH–rate profiles for **3a**, as well as **3b** and **3c** (Fig. 1), indicate that the ionization of the phosphate moiety significantly perturbs the hydrolytic potential of the carbonate functionality and, to a lesser extent, that of the carbamate group. This observation may suggest that the reactive proximity of the phosphate group allows it to participate as a catalyst in the hydrolysis of the carbonate or the carbamate moiety of the spacer groups. The apparent enhanced chemical reactivity of the dianionic species of **3a** compared to its monoanionic form is consistent with phosphate catalysis via either an intramolecular general base (Scheme III, IV) or an intramolecular nucleophilic (Scheme III, V) mechanism. The nucleophilicity of phosphates, including neighboring phosphate group participation, in the hydrolysis of esters and amides has been observed by others (16–18).

The rapid hydrolysis of **3a** at neutral pH values where it should exhibit maximal solubility precludes the use of this spacer group in producing therapeutically useful prodrugs of alcohols. On the other hand, the data for **3b** and **3c** suggested that this technique might be useful for aliphatic and aromatic amines. Not studied here were sterically hindered aliphatic and aromatic amines, which might prove even more stable.

Hydrolysis of 3c in the Presence of Isolated Enzyme

To be potentially useful as a promoiety, the phosphoryloxymethyloxycarbonyl group must readily break down in the body to generate the parent drug (19). Due to the poor chemical stability of **3a** and the lack of general interest in water soluble prodrugs of aliphatic amines, no attempts were made to examine the biological lability of **3a** and **3b**.

The concept illustrated by **3c**, as a model for other aromatic amines, may have some pharmaceutical applicability, hence, its enzymatic lability was examined. Therefore, the hydrolytic reactivity of **3c**, in the presence of isolated human placental alkaline phosphatase at 25°C and pH 10.4, was studied and compared to that of *p*-nitrophenyl phosphate. The observed kinetic data were analyzed using a Lineweaver–Burk plot (20) (Fig. 2).

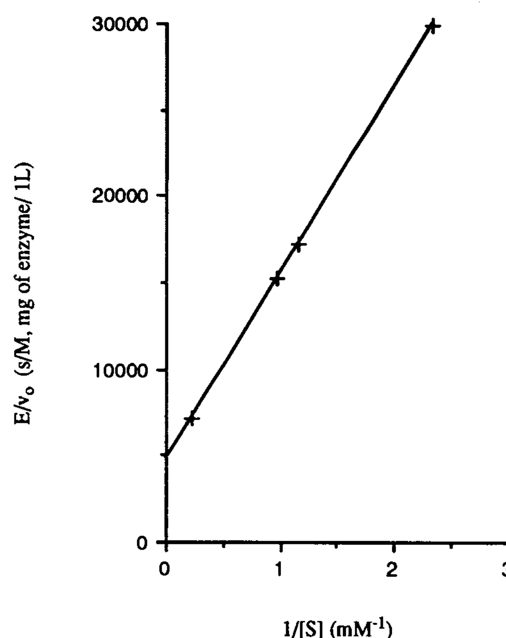
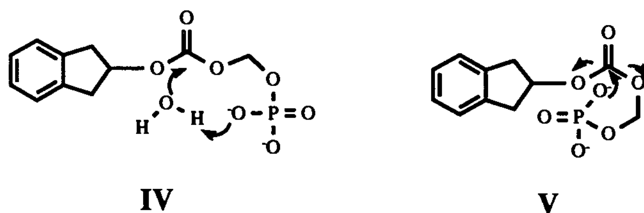


Fig. 2. Lineweaver–Burk plot for the hydrolysis of **3c** in the presence of human alkaline phosphatase (1×10^{-3} mg/mL) in pH 10.4 glycine buffer at 25°C. E represents the total enzyme concentration; vo is the initial rate of dephosphorylation; [S] is the initial substrate concentration.

The catalytic constants K_m and k_{cat} for the apparent dephosphorylation of *p*-nitrophenyl phosphate and **3c** are listed in Table II. The values of k_{cat} and k_{cat}/K_m for **3c** were found to be 10-fold larger than those found for *p*-nitrophenyl phosphate. This observation suggests that **3c** is an excellent substrate for alkaline phosphatases and the high specificity may be translated into rapid bioconversion of the prodrug.

CONCLUSION

In summary, the phosphoryloxymethyloxycarbonyl prodrugs of alcohols, aliphatic amines, and aromatic amines were successfully synthesized. These spacer group-containing prodrugs were found to possess a relatively poor chemical stability for formulation purposes. The chemical instability of these derivatives may be attributed to the hydrolytic lability of the carbamate or carbonate component of the spacer group, probably facilitated by the neighboring phosphate group. One of the phosphoryloxymethyl-



Scheme III. Proposed mechanisms for the hydrolysis of the carbonate component of 2-phosphoryloxymethyloxycarbonyl indanol via an intramolecular general base (IV) or an intramolecular nucleophilic catalysis (V).

Table II. Lineweaver–Burk-Generated Kinetic Constants for Human Placental Alkaline Phosphatase-Catalyzed Hydrolysis of *p*-Nitrophenyl Phosphate (*p*-NPP) and **3c** at pH 10.4 and 25°C

Compound	$K_m \times 10^2$ (m*M*)	$k_{cat} \times 10^4$ (sec^{-1})	$k_{cat}/K_m \times 10^4$ (mM^{-1}/sec^{-1})
p-NPP	89.0 ± 0.7	0.0800 ± 0.0003	0.09
3c	230.00 ± 28	2.10 ± 0.10	0.91

oxycarbmate prodrugs, **3c**, was an excellent substrate for alkaline phosphatase. The synthesis and evaluation of alternative spacer groups with improved chemical stability are under evaluation.

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REFERENCES

1. L. E. Hare, K. C. Yeh, C. A. Ditzler, F. G. McMahon, and D. E. Duggan. Bioavailability of dexamethasone II. Dexamethasone phosphate. *Clin. Pharmacol. Ther.* **18**:330–337 (1975).
2. H. Mollmann, P. Rohdewald, J. Barth, M. Verho, and H. Derendorf. Pharmacokinetics and dose linearity testing of methylprednisolone phosphate. *Biopharm. Drug. Disp.* **10**:453–464 (1989).
3. K. I. Plaisance, G. L. Drusano, A. Forrest, R. J. Townsend, and H. C. Standiford. Pharmacokinetic evaluation of two dosage regiments of clindamycin phosphate. *Antimicrob. Agents Chemother.* **33**:618–620 (1989).
4. N. Gerber, D. C. Mays, K. H. Donn, A. Laddu, R. M. Guthrie, P. Turlapaty, C. Y. Quon, and W. K. Riverburg. Safety, tolerance and pharmacokinetic of intravenous doses of the phosphate ester of 3-hydroxymethyl-5,5-diphenylhydantoin: A new prodrug of phenytoin. *J. Clin. Pharmacol.* **28**:1023–1032 (1988).
5. S. A. Varia, S. Schuller, K. B. Sloan, and V. J. Stella. Phenytoin prodrugs IV: Hydrolysis of various 3-(hydroxymethyl)-phenytoin esters. *J. Pharm. Sci.* **73**:1074–1080 (1984).
6. G. L. Amidon, D. Fleischer, and B. Stewart. Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting. In K. J. Widder and R. Green (eds.), *Drug and Enzyme Targeting,* Academic, New York, 1985, p. 360.
7. H. Mollmann, P. Rohdewald, E. W. Schmidt, V. Salomon, and H. Derendorf. Pharmacokinetics of triamcinolone acetonide and its phosphate ester. *Eur. J. Clin. Pharmacol.* **29**:85–89 (1985).
8. A. Williams and R. A. Naylor. Evidence for $S_N2(P)$ mechanism in the phosphorylation of alkaline phosphatase by substrate. *J. Chem. Soc. B* 1973–1979 (1971).
9. A. S. Kearney and V. J. Stella. The in vitro enzymatic labilities of chemically distinct phosphomonoester prodrugs. *Pharm. Res.* 9:497–503 (1992).
10. P. O. Gunnarsson, S. B. Andersson, S. A. Johansson, T. Nilsson, and G. Plym-Forshell. Pharmacokinetics of estramustine phosphate. *Eur. J. Clin. Pharmacol.* **26**:113–119 (1984).
11. J. Alexanders, R. Cargill, S. R. Michelson, and H. Schwam. (Acyloxy)alkyl carbamates as novel bioreversible prodrugs for amines: Increased permeation through biological membranes. *J. Med. Chem.* **31**:318–322 (1988).
12. A. S. Kearney and V. J. Stella. Unpublished results.
13. A. J. Kirby and A. G. Varvoglis. The reactivity of phosphate esters. Monoester hydrolysis. *J. Am. Chem. Soc.* 89:415–423 (1967).
14. F. H. Westheimer. Monomeric metaphosphates. *Chem. Rev.* 81:313–326 (1981).
15. M. N. Nassar, B. J. Agha, and G. A. Digenis. Effects of structural variation on the rates of enzymatic and nonenzymatic hydrolysis of carbonates and carbamates esters. *J. Pharm. Sci.* 81:295–298 (1992).
16. V. J. Arai. Über die spontane spaltung der salolphosphorsäure und phenylphosphosalizysäure. *J. Biochem. (Tokyo)* **20**:465–480 (1934).
17. R. Kluger and C.-H. Lam. General base catalysis in acidic solutions. Acceleration of intramolecular phosphonate-assisted amide hydrolysis. *Can. J. Chem.* **55**:640–645 (1977).
18. H. Bundgaard and J. Hansen. Nucleophilic phosphate-catalyzed degradation of penicillins: Demonstration of a penicilloyl phosphate intermediate and transformation of ampicillin to a piperazinedione. *Int. J. Pharm.* **9**:273–283 (1981).
19. V. J. Stella. Prodrugs: An overview and definition. In T. Higuchi and V. J. Stella (eds.), *Prodrugs as Novel Drug Delivery Systems,* American Chemical Society, Washington, DC, 1975, pp. 1–XX.
20. H. Lineweaver and D. Burk. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56**:658–666 (1934).