Novel Prodrug Approach for Tertiary Amines: Synthesis and Preliminary Evaluation of *N*-Phosphonooxymethyl Prodrugs

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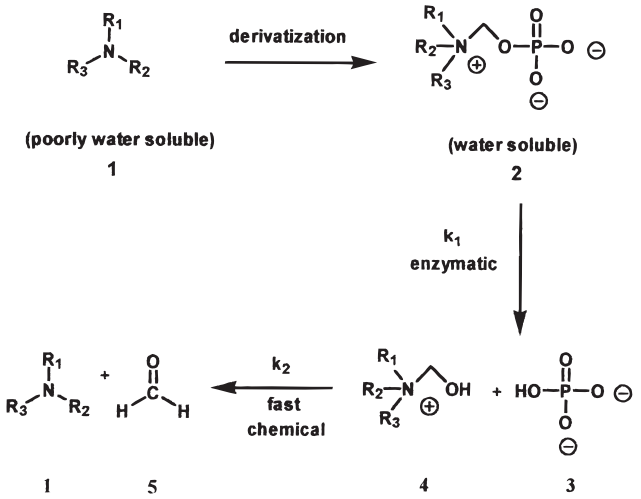
The synthesis and preliminary evaluation of a novel prodrug approach for improving the water solubility of drugs containing a tertiary amine group are reported. The prodrug synthesis involves a nucleophilic substitution reaction between the parent tertiary amine and a novel derivatizing reagent, di-*tert*-butyl chloromethyl phosphate, resulting in formation of the quaternary salt. The tertiary butyl groups are easily removed under acidic conditions with trifluoroacetic acid giving the *N*-phosphonooxymethyl prodrug in the free phosphoric acid form, which can subsequently be converted to the desired salt form. The synthesis was successfully applied to a model compound (quinuclidine) and to three tertiary amine-containing drugs (cinnarizine, loxapine, and amiodarone). The prodrugs were designed to undergo a two-step bioreversion process. The first step was an enzyme-catalyzed rate-determining dephosphorylation followed by spontaneous chemical breakdown of the *N*-hydroxymethyl intermediate to give the parent drug. Selected prodrugs were shown to be substrates for alkaline phosphatase in vitro. A preliminary in vivo study confirmed the ability of the cinnarizine prodrug to be rapidly and completely converted to cinnarizine in a beagle dog following iv administration.

Introduction

The synthesis and preliminary evaluation of a novel prodrug strategy for improving the water solubility of tertiary amine-containing drugs is described. A representation of the prodrug strategy is shown in Scheme 1. The tertiary amine **1** is chemically derivatized to produce the polar, water-soluble prodrug **2**. The prodrug is designed to release the parent tertiary amine in vivo through a two-step bioreversion process. The first step (k_1, rate-determining step) in prodrug bioreversion involves a phosphatase-catalyzed dephosphorylation to give the resultant hydroxymethyl quaternary ammonium intermediate **4** and inorganic phosphate (**3**). The intermediate **4**, at physiological pH, is highly unstable and spontaneously breaks down (k_2) to give the parent tertiary amine **1** plus formaldehyde (**5**).

Many drugs, including tertiary amines, have poor aqueous solubilities, which can create obstacles to their safe and effective delivery. Formulation-related toxicities can occur when a drug is given parenterally.[1,2] In addition, poor and/or erratic bioavailability can occur when a drug is given orally.[3] Prodrugs of tertiary amines have received little attention in the literature. Bodor et al. has previously described acyloxyalkyl quaternary ammonium derivatives of tertiary amines as "soft drugs",[4−7] while others, albeit few, have explored quaternary ammonium derivatives as water-soluble prodrugs for tertiary amines.[8−11] Quaternary amine prodrugs resulting from *N*-phosphonooxymethyl derivitization of the tertiary amine functionality of problematic drugs represent a novel approach for improving the water solubility.

Scheme 1. Illustration of the Prodrug Strategy



Three drugs (loxapine, cinnarizine, and amiodarone) and one model compound (quinuclidine), each containing a tertiary amine group, were chosen to demonstrate the synthetic feasibility of this prodrug strategy. Cinnarizine is a calcium channel blocker that is clinically used as a vasodilator and antihistaminic and antiallergic agent.[12] Cinnarizine has a pK_{a1} of 1.95[13] and pK_{a2} of 7.47.[14] The poor, pH-dependent, aqueous solubility of cinnarizine is believed to be responsible for the erratic oral bioavailability observed in dogs and in humans.[15,16] Loxapine is a tricyclic dibenzoxazepine antipsychotic agent, which is used in the treatment of schizophrenic and psychotic disorders.[17] The loxapine pK_{a2} is 7.5, and the intrinsic solubility of the free base is 12.6 μg/mL.[18] The drug is currently formulated for im administration

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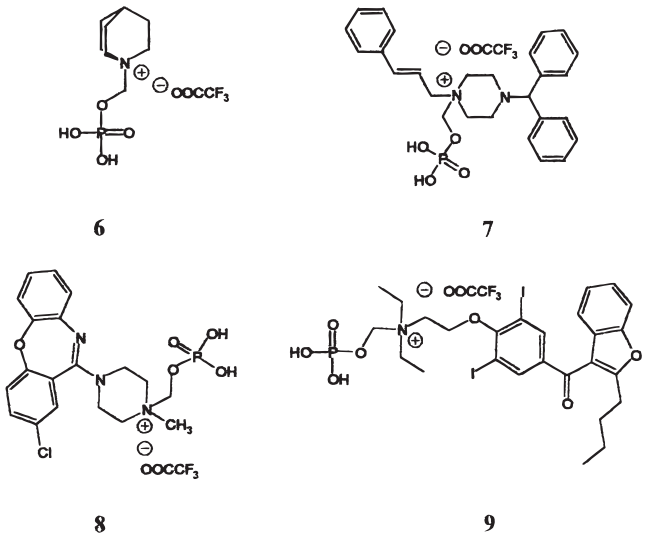
Chart 1. *N*-Phosphonooxymethyl Derivatives of Quinuclidine (**6**), Cinnarizine (**7**), Loxapine (**8**), and Amiodarone (**9**)



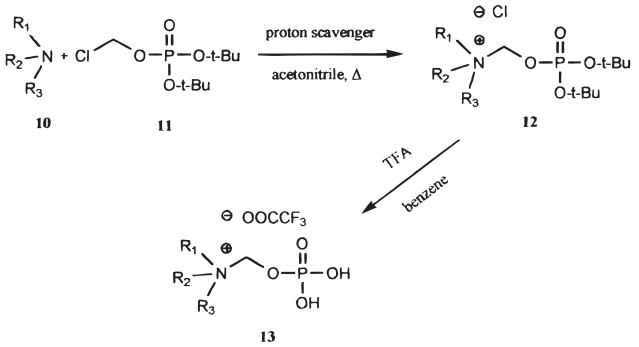
in a cosolvent consisting of propylene glycol (70% v/v) and polysorbate 80 (5% v/v) which has been shown to cause muscle damage.[19] Amiodarone is a widely used antiarrhythmic agent which is currently approved for life-threatening ventricular arrhythmias.[20−22] Amiodarone has a pK_a of 6.56 and also has poor aqueous solubility.[23] This necessitates an iv formulation to have lowered pH and nonaqueous cosolvent addition.[24] The development of venous phlebitis has been reported with the iv infusion of this formulation which is thought to be caused through precipitation of amiodarone at the injection site.[25] When given orally, the absolute bioavailability of amiodarone ranges from 22−86% which has been partially attributed to the poor water solubility.[26]

The purpose of the current studies was to evaluate the potential usefulness of the novel prodrug strategy in improving the water solubility of these drugs. To meet this goal the prodrug must have ample water solubility, sufficient chemical stability, and a rapid and complete bioreversion process. These issues were topics of separate reports.[18,27] At pH 7.4, the loxapine prodrug had over a 15 000-fold improvement in solubility relative to loxapine, and the predicted shelf life of an aqueous formulation was nearly 2 years. Both the loxapine and cinnarizine prodrugs appeared to be rapidly and quantitatively converted to their respective parent compound following iv dosing. The specific aim of this work was to describe the method of synthesis of the prodrugs. Also included are preliminary in vitro reversion kinetics of **7** and **8** in the presence of isolated human placental alkaline phosphatase and an in vivo evaluation of **7** in a beagle dog.

Results

Synthesis. The structures of the quaternary *N*-phosphonooxymethyl prodrugs of quinuclidine (**6**), cinnarizine (**7**), loxapine (**8**), and amiodarone (**9**) are shown in Chart 1. The general mechanism for the synthesis of prodrugs is depicted in Scheme 2. The parent tertiary amine **10** undergoes a nucleophilic substitution reaction

Scheme 2. General Synthetic Scheme for *N*-Phosphonooxymethyl Prodrugs of Tertiary Amines



with di-*tert*-butyl chloromethyl phosphate (**11**), which results in the formation of the quaternary ammonium phosphate-protected prodrug **12**. The solvent selection for this quaternization reaction was important. It was found that a relatively polar aprotic solvent such as acetonitrile worked best. The derivatizing reagent, di-*tert*-butyl chloromethyl phosphate (**11**), was not completely stable under the elevated temperature conditions required by less reactive amines, such as cinnarizine, and would slowly degrade to produce hydrochloric acid (HCl). The generation of HCl protonated any unreacted tertiary amine and catalyzed the removal of tertiary butyl protecting groups from both the product (**12**) and the unreacted derivatizing reagent (**11**). This led to the formation of multiple polar products, which made the purification of some prodrugs difficult. To limit this occurrence, a nonnucleophilic proton scavenger (1,2,2,6,6-pentamethylpiperidine) was used in excess in all reactions, except for quinuclidine which was sufficiently reactive to proceed at 37 °C in a relatively short time. Even in the presence of the proton scavenger, cinnarizine and loxapine reaction products were isolated as the monotertiary butyl-protected derivatives as opposed to the ditertiary butyl-protected intermediates that were isolated with quinuclidine and amiodarone. The mono- or diester prodrugs **12** were then treated with trifluoroacetic acid in benzene at room temperature to remove the tertiary butyl group(s). The resulting prodrugs in their free acid form, **13**, were isolated and characterized. In the cases of cinnarizine and loxapine, more than one molecule of trifluroacetic acid might have been associated with the products since they contain other basic centers. The prodrugs can be converted to their sodium salt by neutralization; however, the compounds were somewhat hygroscopic, so materials were stored in the quaternary, free acid form and all initial studies were on these materials.

The synthesis of derivatives of **11** with an improved leaving group, compared to chlorine (i.e., *p*-toluenesulfonate, triflate), were attempted; however, these compounds were too unstable for isolation. Derivatives of **11** with phosphate protecting groups other than *tert*-butyl may allow for these substitutions. A derivatizing reagent with improved reactivity could potentially lead to enhanced yields and less difficult purifications (particularly important when derivatizing weakly basic tertiary amines).

In Vitro and in Vivo Evaluation. In order for the derivatives to behave as prodrugs they must undergo a chemical or enzymatic bioreversion process. Alkaline

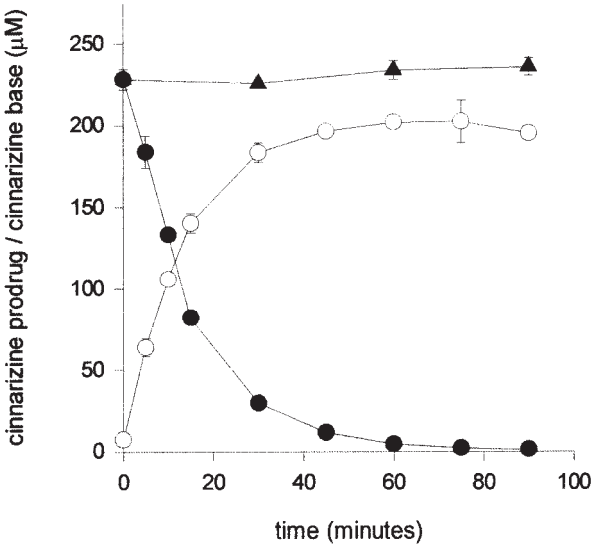


Figure 1. In vitro alkaline phosphatase-catalyzed reversion of **7** (●) to cinnarizine free base (○) at 37 °C and pH 10.4. As a control, levels of **7** (▲) are plotted as a function of time under the same conditions in which the buffer is devoid of alkaline phosphatase.

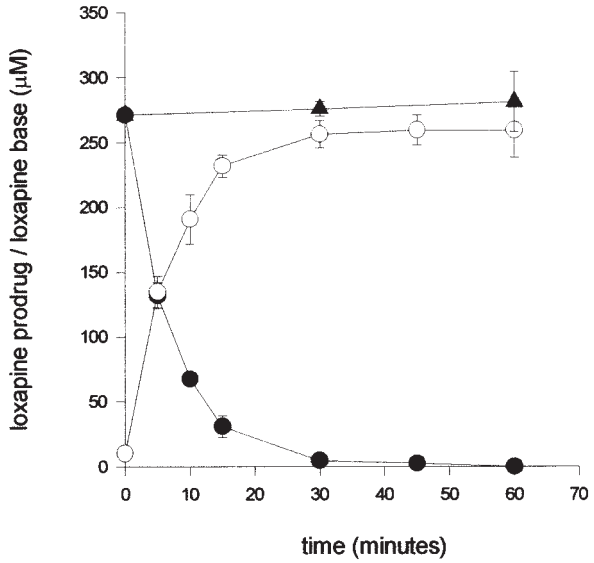


Figure 2. In vitro alkaline phosphatase-catalyzed reversion of **8** (●) to loxapine free base (○) at 37 °C and pH 10.4. As a control, levels of **8** (▲) are plotted as a function of time under the same conditions in which the buffer is devoid of alkaline phosphatase.

phosphatase is an enzyme that is found throughout various tissues, membranes, etc.[28] The described prodrugs were designed to be substrates for this enzyme, and the ability of the prodrugs to generate parent drug in the presence of the enzyme was assessed.

Figures 1 and 2 show the depletion of **7** and **8** in the presence of alkaline phosphatase along with the simultaneous formation of cinnarizine and loxapine, respectively. The closed triangles represent the prodrug levels under identical conditions without the addition of enzyme, which serves to demonstrate the chemical stability of the prodrugs under the conditions. As seen in Figure 1, complete disappearance of **7** led to approximately 88% apparent molar recovery of cinnarizine free base. The 12% not recovered could be partially accounted for by a 3.7% water content (Karl Fisher

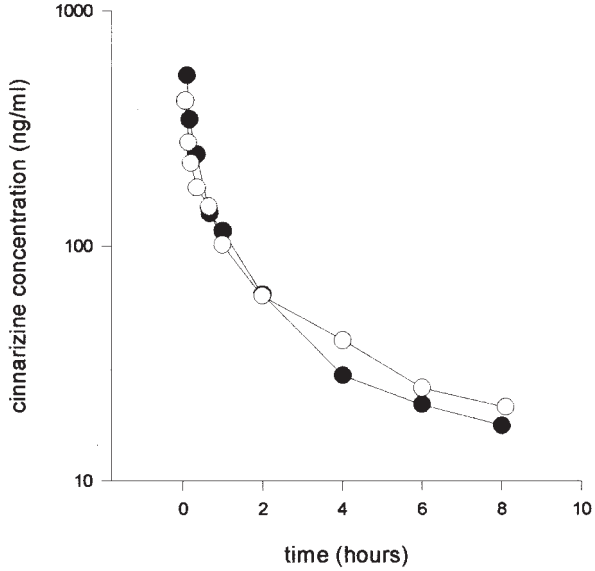


Figure 3. Plot of cinnarizine plasma concentration versus time following equimolar (3 μmol/kg) iv doses of cinnarizine and **7** to a beagle dog: (●) cinnarizine levels following cinnarizine administration; (○) cinnarizine levels following administration of **7**.

titration) and some minor impurities based on relative peak area HPLC analysis of the sample. Similarly, in Figure 2, the disappearance of **8** led to near-quantitative 94% molar recovery of loxapine. The 6% not recovered could be accounted for by a 4.2% water content and 2−3% impurities based on relative peak area HPLC analysis of the sample. NMR spectra (hydrogen, carbon, and phosphorus) and MS as well as HPLC chromatography all suggested that the prodrugs were quite pure; however, in hindsight the less than quantitative recovery from the alkaline phosphatase study suggested the presence of some unaccounted for impurities in the samples. The most likely impurities are small quantities of chloride ion and trifluroacetic acid species.

The ability of the prodrugs to revert to parent compound in the presence of alkaline phosphatase clearly indicates that these prodrugs are substrates for the enzyme. The promising in vitro reversion profiles of the prodrugs warranted further investigation in animals. In vivo, ideally, these prodrugs should be nontoxic and be rapidly and quantitatively converted to the parent compound. The ability of **7** to revert to cinnarizine was evaluated in a single beagle dog, crossover study. Neither injection (cinnarizine or **7**) caused observable signs of discomfort or toxicity to the dog. Figure 3 shows the concentration versus time profile for cinnarizine following equimolar (3 μmol/kg) iv doses of cinnarizine and **7**. A comparison of the cinnarizine area under the plasma concentration versus time curve was used to assess the extent of bioreversion in the dog. The $[AUC]_{0-8h}$ values were 456.0 and 449.5 ngh/mL after cinnarizine and **7**, respectively. Dividing the resulting cinnarizine $[AUC]_{0-8h}$ after administration of **7** by the $[AUC]_{0-8h}$ following cinnarizine administration gives a value of 0.98, which represents the extent of reversion of the prodrug to parent drug. This, along with the superimposability of the profiles, suggests that the prodrug is both rapidly and quantitatively converted to parent drug in dog.

Conclusion

The reaction of selected tertiary amine-containing drugs with di-*tert*-butyl chloromethyl phosphate followed by deprotection led to the formation of the *N*-phosphonooxymethyl prodrugs. When the parent tertiary amine is highly nucleophilic (e.g., quinuclidine) the prodrug can be obtained in high yield with little purification necessary. When the amine is less nucleophilic (e.g., cinnarizine), the required elevated temperature and long reaction times led to complex product formations, difficult purification, and reduced yields. Prodrugs **7** and **8** were shown to be substrates for alkaline phosphatase, an enzyme ubiquitous to the human body. In a preliminary in vivo dog study, **7** was shown to produce cinnarizine rapidly and quantitatively following intravenous administration. Further in-depth studies evaluating this prodrug concept are reported in separate papers.[18,27] Ongoing chemical studies include improved synthesis and purification procedures for these polar molecules.

Experimental Section

Synthetic Materials. Anhydrous acetonitrile, chloroiodomethane, anhydrous dimethoxyethane, 1,2,2,6,6-pentamethylpiperidine, quinuclidine, tetramethylammonium hydroxide 10% (w/v) aqueous solution, trifluoroacetic acid, magnesium chloride hexahydrate, tetrabutylammonium dihydrogen phosphate, and zinc chloride were obtained from Aldrich Chemical Co. (Milwaukee, WI). Cinnarizine and amiodarone hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO). Loxapine succinate was obtained from Research Biochemicals Inc. (Natick, MA). Di-*tert*-butyl phosphite was obtained from Lancaster Synthesis, Inc. (Windham, NH), while potassium bicarbonate and glycine were obtained from Fischer Scientific (Pittsburgh, PA). Potassium permanganate was purchased from Mallinckrodt Chemical Works (St. Louis, MO). Normal phase silica gel, particle size 32−63 μm, was obtained from Selecto Scientific (Norcross, GA), while preparative TLC plates (1000-μm thickness, 20 × 20 cm) were obtained from Alltech Associates, Inc. (Deerfield, IL). Amiodarone hydrochloride and loxapine succinate were converted to their free base form prior to use. All water was distilled in an all-glass still prior to use. All other chemicals and solvents were of reagent grade obtained from conventional sources and used without further purification.

Analytical Equipment. Nuclear magnetic resonance spectra were obtained using either a Bruker AM 500 or a Varian XL 300 instrument. Chemical shifts are reported as parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)-propanesulfonic acid as internal standards for ^{1}H NMR spectra and from 85% H_3PO_4 as an external standard in ^{31}P NMR spectra. *J* values are given in hertz (Hz). Elemental microanalyses were performed by Desert Analytics, Tuscon, AZ. Melting points obtained were determined in capillary tubes on a Mel-Temp II apparatus. Mass spectral analyses were performed by the Mass Spectral Laboratory at The University of Kansas (Lawrence, KS). Water content was determined on a Metrohm AG Karl Fischer coulometer (Herisam, Switzerland).

Di-*tert*-butyl Chloromethyl Phosphate (11). The conversion of di-*tert*-butyl phosphite into the corresponding phosphate was performed using a modification of the method reported by Zwierzak and Kluba.[29] To a stirred solution, in an ice bath, of di-*tert*-butyl phosphite (7.84 g, 40.36 mmol) and potassium bicarbonate (2.42 g, 24.20 mmol) in water (35 mL) was added, in three equal portions, finely powdered potassium permanganate (4.46 g, 28.30 mmol) over 1 h followed by continued stirring at room temperature for an additional 30 min. Decolorizing carbon (0.6 g) was added, and the resulting mixture was stirred at 60 °C for 15 min and filtered. The filter cake was washed three times with water (5 mL); all filtrates were combined, mixed with decolorizing carbon (1.0 g), and again stirred at 60 °C for 20 min. After filtration, the resulting colorless solution was cooled to 0 °C (ice bath) and carefully acidified with concentrated hydrochloric acid (7 mL) with stirring. The precipitated di-*tert*-butyl phosphate was filtered, washed with ice-cold water (10 mL), and dissolved in acetone (100 mL). To this stirred solution in an ice bath was added 1 mol equiv of a 10% water solution of tetramethylammonium hydroxide (24.2 mmol), and the resulting, homogeneous solution was evaporated under reduced pressure to give 7.16 g of solid. Crystallization from refluxing dimethoxyethane gave 6.52 g of the pure tetramethylammonium di-*tert*-butyl phosphate (6.52 g, 57%) as white hygroscopic crystals. To a refluxing solution of this phosphate (3.59 g, 12.70 mmol) in dimethoxyethane (70 mL) was added chloroiodomethane (25 g, 141.74 mmol), and the resulting reaction mixture was refluxed for 1.5 h. The mixture was filtered, and the filtrate was placed under reduced pressure to remove excess chloroiodomethane and solvent to yield a viscous yellow oil, which was a mixture of **11** and bis-di-*tert*-butyl methyl phosphate. The bis-di-*tert*-butyl methyl phosphate product was identified by NMR and mass spectral analysis (data not shown). The two products were easily separated via flash column chromatography (eluent 30% ethyl acetate and 70% hexane). **11** was isolated as a pale-gold oil (2.07 g, 35%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.51 (s, 12H), 5.63 (d, 2H, J = 15); ^{31}P NMR ($CDCl_3$, 300 MHz) δ −13.9 (s); MS (FAB+, GLY) *m/z* 259 (M + 1). Anal. ($C_9H_{20}ClO_4P$) C, H: calcd 41.79, 7.79; found 41.60, 7.68.

***N*-(Phosphonooxymethyl)quinuclidinium Trifluoroacetate (6).** To a solution of **11** (0.16 g, 0.64 mmol) in anhydrous acetonitrile (5 mL) maintained under argon was added quinuclidine (0.07 g, 0.636 mmol). The reaction mixture was capped with a rubber septum and stirred at 37 °C for 12 h. The mixture was then placed under reduced pressure to remove solvent. To the residue was added anhydrous ethyl ether (5 mL) with stirring to create a suspension that was centrifuged, and the supernatant was removed. This process was repeated three times. The remaining solid was dried under vacuum to give di-*tert*-butyl *N*-(phosphonooxymethyl)quinuclidinium chloride (0.18 g, 78%) isolated as a white amorphous solid: mp 88−105 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.54 (s, 18H), 2.07 (m, 6H), 2.27 (m, 1H), 3.86 (t, 6H, J = 7.9), 5.36 (d, 2H, J = 8.4); ^{31}P NMR ($CDCl_3$, 500 MHz) δ −14.97 (t, J = 19.4); MS (FAB+, GLY) *m/z* 334 (M^+).

Deprotection of di-*tert*-butyl *N*-(phosphonooxymethyl)quinuclidinium chloride (0.17 g, 0.46 mmol) was by the addition of trifluoroacetic acid (40 μL, 0.52 mmol) in benzene (5 mL) with stirring at room temperature for 24 h. The reaction vessel was then placed under reduced pressure to remove excess trifluoroacetic acid, HCl, and benzene to afford the title compound (0.14 g, 94% yield) as a white amorphous solid: ^{1}H NMR (D_2O, 300 MHz) δ 1.97 (m, 6H), 2.18 (m, 1H), 3.40 (t, 6H, J = 7.9), 4.68 (d, 2H, J = 6.8); ^{19}F NMR (D_2O, 500 MHz) δ −77.98 (s); ^{31}P NMR (D_2O, 500 MHz) δ −2.6 (s); MS (FAB+, GLY) 222 (M^+).

***N*-(Phosphonooxymethyl)cinnarizinium Trifluoroacetate (7).** To a solution of **11** (0.178 g, 0.74 mmol) in anhydrous acetonitrile (4 mL) maintained under argon were added cinnarizine (0.227 g, 0.62 mmol) and 1,2,2,6,6-pentamethylpiperidine (125 μL, 0.74 mmol). The reaction mixture was capped with a rubber septum and stirred at 70 °C for 6 days. The mixture was then placed under reduced pressure to remove the solvent. To the residue was added anhydrous ethyl ether (5 mL) with stirring to create a suspension, which was then centrifuged, and the supernatant was removed. This process was repeated three times. The product was then purified using preparative thin-layer chromatography (eluent 75% methylene chloride and 25% methanol). The R_f value of the intermediate was 0.7. The mono-*tert*-butyl *N*-(phosphonooxymethyl)cinnarizinium chloride (0.033 g, 8%) was isolated as a white amorphous solid: ^{1}H NMR (acetonitrile-d_3, 300 MHz) δ 1.35

(s, 9H), 2.70 (m, 4H), 3.39 (m, 2H), 3.56 (m, 2H), 4.12 (d, 2H, $J = 7.8$), 4.46 (s, 1H), 5.01 (d, 2H, $J = 8.4$), 6.4 (m, 1H), 6.95 (d, 1H, $J = 16$), 7.3 (m, 15H); ^{31}P NMR (acetonitrile-d_3, 500 MHz) δ −4.9 (s); MS (FAB+, GLY) *m/z* 535 (M^+).

This product (0.027 g, 0.048 mmol) was mixed with trifluoroacetic acid (20 μL, 0.26 mmol) in benzene (1 mL) with stirring at room temperature for 24 h to remove the *tert*-butyl protecting group. The reaction was then placed under reduced pressure to remove excess trifluoroacetic acid, HCl, and benzene to yield **7** (0.025 g, 87%) as a white amorphous solid (sample had 3.7% residual water): mp 140−146 °C; ^{1}H NMR (D_2O, 300 MHz) δ 2.98 (m, 4H), 3.58 (m, 4H), 4.23 (d, 2H, $J = 7.7$), 4.72 (s, 1H), 4.98 (d, 2H, $J = 6.2$), 6.3 (m, 1H), 7.01 (d, 1H, $J = 15$) 7.2−7.6 (m, 15H); ^{31}P NMR (acetonitrile-d_3, 500 MHz) δ 2.1 (s); MS (FAB+, GLY) *m/z* 479 (M^+). HPLC analysis showed a single major peak accounting for the majority of the total peak peak. There was a small peak corresponding to cinnarizine which accounted for <1% of the total peak area.

***N*-(Phosphonooxymethyl)loxapinium Trifluoroacetate (8).** To a solution of **11** (0.24 g, 0.91 mmol) maintained under argon in anhydrous acetonitrile (1 mL) were added loxapine (0.20 g, 0.61 mmol) and 1,2,2,6,6-pentamethylpiperidine (500 μL, 2.76 mmol), and the reaction mixture was capped with a rubber septum and stirred at 50 °C for 64 h. The mixture was then placed under reduced pressure to remove the solvent. To the residue was added anhydrous ethyl ether (5 mL) with stirring to create a suspension that was centrifuged, and the supernatant was removed. This process was repeated three times. The product was then purified using preparative thin-layer chromatography (eluent 90% methylene chloride and 10% methanol). The product had an R_f value of 0.3. The mono-*tert*-butyl *N*-(phosphonooxymethyl)loxapinium chloride was isolated as a white solid (0.08 g, 25%): ^{1}H NMR (D_2O, 300 MHz) δ 1.41 (s, 9H), 3.22 (s, 3H), 3.4−3.8 (m, 6H), 3.85 (m, 2H), 5.02 (d, 2H, $J = 7.6$) 7.20 (m, 5H), 7.42 (m, 2 H); ^{31}P NMR (D_2O, 500 MHz) δ −5.7 (t, $J = 17.5$); MS (FAB+, NBA) *m/z* 494 (M^+).

The mono-*tert*-butyl-protected *N*-(phosphonooxymethyl)loxapinium chloride (0.08 g, 0.153 mmol) was treated with a solution of trifluoroacetic acid (60 μL, 0.78 mmol) in benzene (4 mL) at room temperature for 24 h to remove the remaining *tert*-butyl protecting group. The reaction was then placed under reduced pressure to remove excess trifluoroacetic acid, HCl, and benzene to yield **8** (0.066 g, 76%) as a white amorphous solid (sample had 4.2% residual water): mp 114−145 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.27 (s, 3H) 3.4−4.2 (m, 8H), 5.08 (d, 2H, $J = 7.2$), 7.10−7.45 (m, 7H); ^{31}P NMR (D_2O, 500 MHz) δ −1.77 (m); MS (FAB+, TG) *m/z* 438 (M^+). HPLC analysis showed a single major peak accounting for 97% of the total peak area.

***N*-(Phosphonooxymethyl)amiodaronium Trifluoroacetate (9).** To a solution of amiodarone (0.275 g, 0.42 mmol), **11** (0.217 g, 0.84 mmol), and 1,2,2,6,6-pentamethylpiperidine (152 μL, 0.84 mmol) maintained under argon in anhydrous acetonitrile (3 mL) was added sodium iodide (5 mg, 33 μmol), and the resulting reaction mixture was capped with a rubber septum and stirred at 40 °C for 24 h with protection from light. The mixture was then placed under reduced pressure to remove the solvent. To the residue was added anhydrous ethyl ether (5 mL) with stirring to create a suspension that was centrifuged, and the supernatant was removed. This process was repeated three times. After vacuum-drying the di-*tert*-butyl-protected *N*-(phosphonooxymethyl)amiodaronium chloride was obtained as a white amorphous solid (0.180 g, 47%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, 3H, $J = 7.3$), 1.30−1.85 (m, 28H), 2.89 (t, 2H, $J = 7.7$), 3.88 (q, 4H, $J = 4.3$), 4.4−4.6 (c, 4H), 5.47 (d, 2H, $J = 7.4$), 7.3 (m, 2H), 7.49 (d, 2H, $J = 8.1$), 8.21 (s, 2H); ^{31}P NMR ($CDCl_3$, 500 MHz) δ −12.34 (t, $J = 17.2$); MS (FAB+, NBA) *m/z* 868 (M^+).

The di-*tert*-butyl-protected *N*-(phosphonooxymethyl)amiodaronium chloride (0.154 g, 0.17 mmol) was treated with trifluoroacetic acid (60 μL, 0.78 mmol) in benzene (5 mL) at room temperature for 24 h to remove the *tert*-butyl groups. The reaction was then placed under reduced pressure to remove excess trifluoroacetic acid, HCl, and benzene to yield **9** as a yellow oil. This was dissolved in water (5 mL) containing a two molar excess of sodium bicarbonate (0.026 g, 0.31 mmol) to form the disodium salt. The aqueous solution was then lyophilized to remove the water to yield *N*-(phosphonooxymethyl)amiodaronium sodium salt (0.16 g, 94%) as a white hygroscopic solid. Residual water content was variable due to the hygroscopic nature of the sample but corresponded approximately to a pentahydrate: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.84 (t, 3H, $J = 7.2$), 0.98 (m, 2H), 1.31 (m, 6H), 1.68 (m, 2H), 2.74 (m, 2H), 3.54 (m, 4H), 3.84 (m, 2H), 4.36 (m, 2H), 4.95 (d, 2H, $J = 8.7$), 7.22−7.65 (m, 4H), 8.18 (s, 2H); ^{31}P NMR (D_2O, 500 MHz) δ 4.77 (s); MS (FAB+, NBA) *m/z* 756 (M^+).

In Vitro/in Vivo Evaluation. Materials: **7** and **8** were synthesized using the previously described procedure. Human placental alkaline phosphatase, type XVII (14 units/mg), and meclizine were obtained from Sigma Chemical Co. (St. Louis, MO). The synthesis and characterization for $(SBE)_{4M}$-β-CD has been previously described.[30] Magnesium chloride hexahydrate, tetrabutylammonium dihydrogen phosphate, and zinc chloride were obtained from Aldrich Chemical Co. (Milwaukee, WI). Glycine was obtained from Fisher Scientific (Pittsburgh, PA). All other chemicals and solvents were of reagent grade and were used without further purification.

In Vitro Enzymatic Evaluation. 1. Procedure: All experiments involving alkaline phosphatase were performed in a pH 10.4 glycine buffer at 37 °C. The glycine buffer solution contained 1 mM $ZnCl_2$, 1 mM $MgCl_2$, and 0.1 M glycine. The final pH of the buffer was adjusted to pH 10.4 with additions of a 2 N NaOH solution. An alkaline phosphatase stock solution was prepared at a concentration of 1.54 units/mL in the glycine buffer. The enzyme solution was brought to 37 °C and spiked with a prodrug stock solution to give an initial prodrug concentration of 264 and 230 μM for **8** and **7**, respectively. Aliquots (0.2 mL) were separated into individual 4-mL glass tubes, capped, and placed in a 37 °C shaking water bath. Samples were removed from the bath at predetermined time points (0, 5, 10, 15, 30, 45, 60, 75, and 90 min) and spiked with acetonitrile (0.2 mL) which served to quench the enzymatic reaction and to solubilize the reaction products. As a test for the chemical stability of the prodrugs under these experimental conditions, the previously described procedure was repeated with the removal of enzyme from the media. All samples were analyzed by HPLC for both parent and prodrug concentrations.

2. Analytical: The HPLC system for all compounds consisted of a Waters model 510 pump (Milford, MA), a Waters 717 autosampler (Milford, MA), an LDC analytical spectromonitor 3100 variable wavelength detector, a Waters C18 symmetry column (3.9 × 150 mm; Milford, MA), a Shimadzu CR6A integrator (Kyoto, Japan), and a column heater (Timberline Instruments Inc., Boulder, CO).

For the analysis of cinnarizine and **7**, the mobile phase consisted of acetonitrile (55% v/v) and a 10 mM ammonium dihydrogen phosphate buffer adjusted to pH 3 with phosphoric acid (45% v/v) and was pumped at a flow rate of 0.9 mL/min. The injection volume was 20 μL, and the detection was ultraviolet using a wavelength of 254 nm. Under these conditions, the retention times were 5 and 9 min for **7** and cinnarizine, respectively.

For the analysis of loxapine and **8**, the mobile phase consisted of acetonitrile (28% v/v) and a 10 mM ammonium dihydrogen phosphate buffer adjusted to pH 3 with phosphoric acid (72% v/v) and was pumped at a flow rate of 0.9 mL/min. The injection volume was 20 μL, and the detection was ultraviolet using a wavelength of 251 nm. Under these conditions, the retention times were 8 and 12 min for **8** and loxapine, respectively.

In Vivo Dog Study. 1. Preparation of formulations: Cinnarizine for iv injection was prepared in a 10 mM phosphate buffer solution at pH 4.5 at a concentration of 12.5 mg/10 mL (3.39 mM) along with 37.5 mM $(SBE)_{4M}$-β-CD as a

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