Design of Novel Derivatives of Phosphonoformate (Foscarnet) as Prodrugs and Antiviral Agents

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Introduction

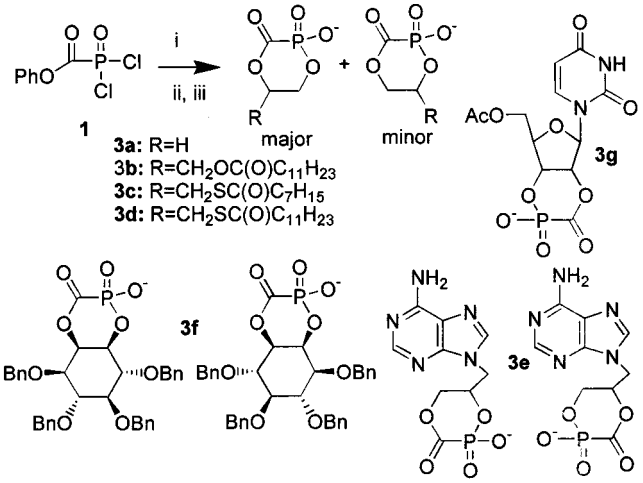
Phosphonoformate (PFA) is an effective antiviral agent that is used clinically in AIDS chemotherapy under the names of Foscarnet and Foscavir.[1] Although the primary indication is AIDS-related human cytomegalovirus (HCMV) infection, PFA is also effective against HIV itself. PFA is proposed to act via inhibition of reverse transcriptase in HIV and DNA polymerase in herpes simplex virus (HSV) and HCMV, by blocking the pyrophosphate binding site in these enzymes. PFA is remarkable in being an analogue of the ubiquitous pyrophosphate, yet acting as a drug with a relatively high therapeutic index. However, the efficacy of PFA is seriously impeded by poor bioavailability due to its polyanionic nature at physiological pH.

Prodrug approaches have been investigated to circumvent problems associated with the bioavailability and permeability of PFA.[2] These prodrug strategies have ranged from simple triester approaches to use of inventive bioreversible groups, but have largely applied the tactics used for simple phosphonate prodrugs to PFA. However, PFA is not a simple phosphonate. The juxtaposition of carbonyl and phosphoryl groups leads to complex reactivity that remains to be fully explored. Mechanistic studies have demonstrated competitive nucleophilic attack at P or C at neutral pH, which may lead to P−O, C−O, and P−C bond cleavage.[3] Reactivity at phosphorus in PFA esters is orders of magnitude greater than for simple phosphonates. In PFA triesters, nucleophilic substitution at the carbonyl carbon can lead to P−C bond cleavage and ester group migration between C and P. The idiosyncratic reactivity of PFA esters strongly influences synthetic strategies and pro-drug design. Herein, we report design rationale, synthesis, and antiviral activity for a novel family of PFA diesters that allows inclusion of the PFA moiety within a variety of biomolecules.

Results & Discusion

The potential for P−C bond cleavage in PFA triesters, following hydrolysis at carbonyl carbon, and hence the destruction of the drug moiety, leads us to focus prodrug strategies on PFA diesters and monoesters.[3a,b,4,5] A recent report from our laboratory demonstrated a novel one-pot synthesis of acyclic PFA diesters from phosphonochloridate (**1**) employing *vic*-trimethylsilyl ethers.[5] Extension of this methodology allows for synthesis of cyclic PFA diesters (**3a**−**g**), the first reported synthesis of such heterocycles (Scheme 1).

Scheme 1



The first step in this procedure, synthesis of PFA esters via TMS-ethers, offers some advantages over the traditional method of coupling the phosphonodichloridate with an alcohol, in the presence of a nitrogen base. First, the sole byproduct is TMSCl, which is easily removed under vacuum. Second, cyclic PFA esters, such as **3**, can be quantitatively prepared; this is not feasible by the traditional method, which leads to mixtures of cyclic and acyclic esters. Third, selective addition of two TMS ethers to form mixed esters was demonstrated.

The rationale for use of 1,2-bis-TMS-ethers lies in the clean formation of a five-membered cyclic phosphonate, especially reactive toward subsequent ring-opening at P due in large part to relief of ring strain.[6] Thus, ring-cleavage leads to cyclic precursors **2**, which may be prepared and isolated according to the published procedure.[5] Alternatively, the intermediate five-membered cyclic phosphonate, prepared from phenoxycarbonyl phosphonodichloridate (**1**), may be ring-opened by addition of 1 equiv of water and recyclized in situ to form a six-membered cyclic phosphonate. Use of the phenyl ester, **1**, as precursor, provides a sufficiently good leaving group at C to allow facile base-catalyzed cyclization from **2**. The entire synthetic procedure from reaction of **1** to isolation of products **3**, can be performed without isolation or purification of cyclic or acyclic synthetic intermediates (Scheme 2).

The acyclic PFA diester intermediates underwent cyclization to compounds **3a**−**f** at room temperature in

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Scheme 2

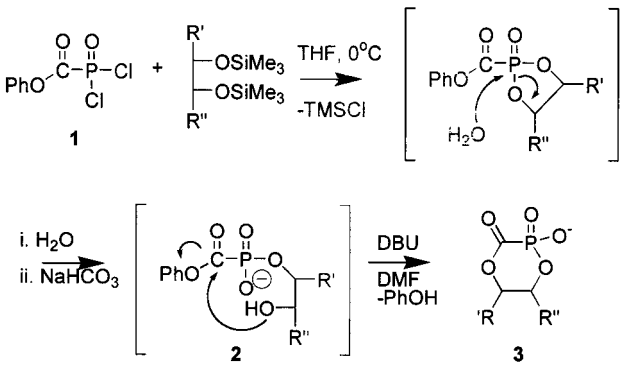


Table 1. Synthetic and Antiviral Data for 3a-g as Na Salts[a]

compd	% yield[b]	% major isomer[c]	^{31}P NMR[d] (ppm)	% minor isomer	^{31}P NMR[d] (ppm)	EC_{50} HSV-1[e,f] (μM)
3a	81	n/a	−7.84	n/a	n/a	
3b	72	84	−9.54	16	−11.48	
3c	72	82	−8.15	18	−11.37	547 (218)
3d	84	>95	−9.57	<5	n/a	256 (218)
3e	16[a]	59	−9.54	41	−12.29	738 (227)
3f	35[a]	61	−8.95	39	−14.7	
3g	12[a]	(100)	−14.4	(0)	n/a	395 (227)

[a] All compounds characterized by (−)FAB-MS (as M^- ions) or (+)FAB-MS (as $M^- + 2H^+$ ions). [b] Percentage yield calculated directly from **1** without isolation of intermediates. [c] Isomers are readily identified by ^{31}P-coupling in ^{1}H and ^{13}C NMR. [d] At 162 MHz in D_2O, ref. 85% H_3PO_4. [e] Values in brackets refer to PFA EC_{50}.[8a] [f] No PFA was observed in intestinal and liver homogenate assays except for **3c** (<2%) and **3d** (<7%).[8b]

DMF containing a catalytic amount of DBU (Scheme 2). Interestingly, cyclization to **3g** occurred without added base. Compounds **3b**−**f** were isolated as mixtures of two regioisomers, with the major isomer constituting 59−95% of the mixture, while **3g** was isolated as a single isomer. The compounds are distinctive by their upfield ^{31}P NMR chemical shifts relative to acyclic dialkyl PFA diesters (a shift of approximately −4 ppm) (Table 1).

Cyclic PFA diesters are attractive targets as synthetic prodrugs for a number of reasons. First, the protection of two oxyanion positions in PFA by one ester moiety leads to lower molecular weight prodrugs compared with acyclic counterparts. Second, the PFA moiety may be incorporated within a number of biomolecules, for example, cyclic nucleotides. But most importantly, ring strain in the six-membered ring leads to increased rates of hydrolytic ring cleavage and more facile breakdown of the prodrug.

Simple PFA alkyl diesters are relatively stable toward hydrolysis at C in neutral aqueous solution. Nonenzymic hydrolytic breakdown of a PFA diester prodrug requires a good leaving group at C (e.g., a phenol) or use of a bioreversible group. The six-membered, PFA-containing ring, **3a**, would be expected to possess torsional and bond angle bending strain owing to the presence of an sp^2 center, exacerbated by unfavorable electrostatic and steric interactions between vicinal, exocyclic oxygens. In simile with nucleophilic addition to the carbonyl C of cyclohexanones, relief of strain is anticipated in the tetrahedral transition state for ring cleavage of cyclic PFA diesters.

Relief of ring strain can be quantified by ab initio MO calculations on **3a**, an acyclic analogue **4**, and the

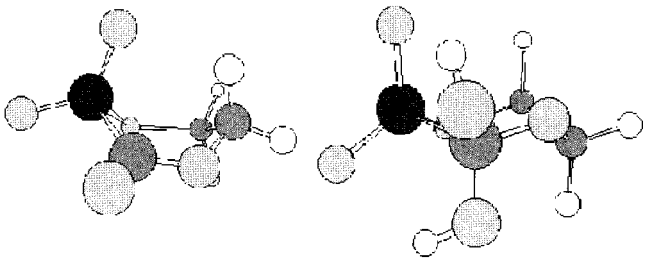
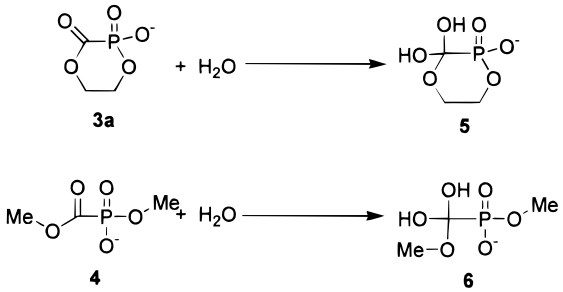


Figure 1. Minimum energy structures of **3a** (left; twist-boat) and **5** (right; chair) obtained by geometry optimization at the MP2/6-31+G*//HF/3-21+G(*) level.

Scheme 3



corresponding tetrahedral intermediates (**5** and **6**) for nucleophilic addition of water (Scheme 3). Using a level of calculation and size of basis set suitable for such calculations,[3d,7] the ring strain free energy ($\Delta G°$) intrinsic to the six-membered PFA is quantified as 6.1 kcal/mol. Indeed, in its lowest energy structure, **3a** adopts a strained boat conformation, while **5** is in a chair conformation (Figure 1). If all of the strain energy was released in the transition state for hydrolysis, which resembles the tetrahedral intermediate, an increased reactivity of cyclic over comparable acyclic PFA diesters of a factor of 10^5 would be predicted. To provide comparative experimental data, the half-life for hydrolysis, at pH 6.9 in 200 mM imidazole at 25 °C, was measured by ^{31}P NMR for **3a** and compared to the acyclic analogue, ethyl (ethoxycarbonyl) phosphonate (**7**). The acyclic analogue, **7**, showed <3% hydrolysis after 6 days at 25 °C, compared to $t_{1/2}$(**3a**) = 38min. This corresponds to a rate acceleration of $\geq 10^4$ for hydrolysis of **3a** over **7**, compatible with data on relief of ring strain from the MO calculations.

To test the potential of cyclic diesters **3a**−**g** as PFA prodrugs, antiviral assays on HSV-1-infected confluent human fibroplast cells were performed.[8a] Diester **3d** showed comparable activity to PFA itself, with **3c**,**e**,**g** showing antiviral activity somewhat less than PFA. Studies on the metabolism of the prodrugs in intestinal

(7) For example: Tole, P.; Lim, C. *J. Am. Chem. Soc.* **1994**, *116*, 3922.

(8) (a) Cells were incubated with drugs (3−800 μM) at 37 °C in a humidified atmosphere of 5% CO_2 in air until control wells showed characteristic cytopathic effects (24−48 h). Cells were lysed with Triton X-100 and viral antigen content of the supernatants measured by ELISA. (b) The liver and portion of the small intestine were removed from male Sprague-Dawley rats, thoroughly cleaned with deionized water, and homogenized in three (liver) or two (intestine) times their weight in Tris buffer (50 mmol, pH 7.5). Portions of the homogenates (15−30 mL) were incubated at 37 °C, the reactions were initiated by the addition of test substances in buffer (1 mM final concentration), and the mixtures were shaken. Samples (0.5 mL) were removed after 0, 5, 15, 60, and 240 min, added to methanol (0.5 mL), and centrifuged. The supernatant was then analyzed for PFA by HPLC (Pettersson, K. J.; Nordgren, T.; Westerlund, D. *J. Chromat.* **1989**, *488*, 447.). Bioassays performed by Astra Arcus, Sodertalje, Sweden.

and liver homogenates showed almost no conversion to PFA,[8b] although the half-life for ring cleavage is 14 min (for **3a**) in buffered aqueous solution, at 37 °C, physiological pH, and considerably less in the presence of amine biomolecules.[9] Thus, either, homogenate incubation does not accurately represent the inter-or intracellular metabolism of the PFA esters, or the PFA esters themselves, could be exerting antiviral activity through an alternate mechanism.

In conclusion, a series of novel cyclic PFA diesters were synthesized as potential PFA prodrugs and antiviral agents, in which the PFA moiety is incorporated within biomimetics of nucleotides, carbohydrates, and phospholipids. Cyclic PFA esters are much more reactive toward hydrolysis than their acyclic analogues due to relief of ring strain on hydrolysis and several show antiviral activity. This approach holds promise, in particular, when combined with a strategy for activating the prodrug to subsequent de-esterification at phosphorus.

Experimental Section

Materials. All solvents were used as supplied (BDH, Toronto, or Aldrich, Milwaukee), except DMF, which was dried over CaH_2 for at least 2 days before use, and THF and dioxane, which were dried by distillation from Na/benzophenone.

Sodium 2-Hydroxy-1,4,2-dioxaphosphorinane-2,3-dioxide 3a. To a solution of **1** (4.6 g, 20 mmol) in dry THF (20 mL) under Ar, cooled to 0 °C, was added a solution of 1,2-bistrimethylsiloxyethane (24.2 g, 20 mmol) in THF (20 mL) dropwise. The mixture was allowed to warm and stirred for 30 min. The solvent and TMSCl were removed under reduced pressure and the residue dissolved in dioxane (10 mL) containing water (0.36 g, 20 mmol) and then neutralized with a solution of $NaHCO_3$ (1.68 g, 20 mmol) in water (25 mL). The solvents were evaporated to yield the sodium salt of **2a** (δ_P (D_2O) −5.53), which was dissolved in DMF (40 mL) followed by addition of 10 drops of DBU. The colorless solution was stirred at room temperature until the reaction was observed to be complete by ^{31}P NMR. The product was poured into ether (200 mL), forming a white precipitate that was filtered and washed with acetone (2 × 10 mL) to yield the product **3a** (3.07 g, 87%). δ_C(D_2O): 66.59 (d, J_{CP} = 5.4 Hz), 70.82 (d, J_{CP} = 5.9 Hz), 176.31 (d, J_{CP} = 202 Hz). δ_P (D_2O): −7.84 (s). δ_H(D_2O): 4.27 (m, $J_{A'B}$ = 4.6 Hz, J_{PH} = 13.9 Hz), 4.43 (m, $J_{B'A}$ = 4.6 Hz). FAB-MS *m*/*z*: found 152.9913 $(M - Na + 2H)^+$, calcd for $C_3H_6PO_6$ 152.9953.

General Procedure. Acyclic precursors to **3a**−**g**, derived from phenoxycarbonyl phosphonodichloridate (**1**), can be prepared according to the published procedure,[5] in which an intermediate five-membered cyclic phosphonate is ring opened in situ by 1 equiv of water. However, the general procedure for synthesis of the cyclic diesters, **3a**−**g**, does not require isolation of the acyclic precursor. A general procedure for in situ synthesis follows: To a solution of **1** (5 mmol) in dry THF (20 mL) under Ar cooled to 0 °C was added a solution of the desired bis-TMS-ether (5 mmol) in THF (20 mL) dropwise. The mixture was stirred at room temperature for 30 min, and then the solvent and TMSCl were removed under reduced pressure. The residue was dissolved in dioxane (10 mL) containing water (90 mg, 5 mmol) and neutralized with a solution of $NaHCO_3$ (0.42 g, 5 mmol) in water (5 mL), and the solvents were evaporated. The crude diester was dissolved in DMF (20 mL), and to it were added 10 drops of DBU. The mixture was stirred at room temperature until the reaction was observed to be complete by ^{31}P NMR. The product was poured into ether (200 mL) forming a white precipitate, which was filtered and washed with acetone (2 × 10 mL).

Sodium 2-Hydroxy-5-octanoylthiomethyl-1,4,2-dioxaphosphorinane-2,3-dioxide 3c. δ_C(D_2O): 12.98 (s, CH_3); 21.58, 24.56, 27.83, 28.03, 28.22, 30.83 (6s); 42.92 (s); 66.11 (d, J_{PC} 4.5 Hz); 77.73 (s); 172.79 (d, J_{PC} 205.7); 199.21 (s). δ_P (D_2O): −8.15 (s). δ_H(D_2O): 0.98 (t, 3H); 1.38 (br. s, 8H); 1.77 (m, 2H); 2.75 (t, 2H); 3.42 (d, 2H); 4.43−4.68 (m, 2H); 4.96 (m, 1H). FAB-MS *m*/*z*: found 309. 9 $(M - Na)^-$, calcd for $C_{11}H_{18}O_6PS$ 309.1.

Sodium 2-Hydroxy-5-dodecanoylthiomethyl-1,4,2-dioxaphosphorinane-2,3-dioxide 3d. δ_C(DMSO-d_6): 13.91 (s); 22.06, 24.96, 28.17, 28.41, 28.59, 28.66, 28.78, 28.93, 29.00, 31.26 (10s); 42.94 (s); 66.19 (d, J_{PC} 4.5 Hz); 77.78 (s); 172.82 (d, J_{PC} 205.7 Hz); 199.25 (s). δ_P (DMSO-d_6): −9.57 (s). δ_H (DMSO-d_6): 0.87 (t, 3H); 1.22 (br, 16H); 1.54 (m, 2H); 2.62 (t, 2H); 3.13 (t, 2H); 3.96−4.18 (m, 2H); 4.63 (m, 1H). FAB-MS *m*/*z*: found 379.9 $(M - Na)^-$, calcd for $C_{16}H_{28}O_6PS$, 379.1.

Sodium 2-Hydroxy-4/5-(aden-9-yl)-methyl-1,4,2-dioxaphosphorinane-2,3-dioxide 3e. 9-(2,3-Dihydroxy)propyladenine (1.05 g, 5 mmol) was added to phosphonodichloridate **1** (1.2 g, 5 mmol) in trimethyl phosphate (10 mL) at 0 °C, under Ar. The reaction mixture was stirred until dissolution of solid and then for a further 16 h at room temperature. The reaction mixture was poured into dry ether (200 mL) with stirring, the precipitate was filtered, sodium bicarbonate (0.42 g, 5 mmol) was added to this solution, and the resulting mixture was stirred for 2 h. Water was removed under vacuum and the solid residue thoroughly dried. The resulting glassy solid was dissolved in dry DMF (10 mL), DBU (30 mg) added, and the reaction mixture stirred for 12 h. The resulting precipitate was removed by filtration and the filtrate poured into ether (200 mL) with stirring. The resulting precipitate was filtered and washed with acetone (2 × 50 mL) to give the product as a mixture of regioisomers (0.25 g, 16%). δ_P(DMSO-d_6): −9.54 (s); −12.29 (s). FAB-MS *m*/*z*: found 297.9 $((M - Na)^-$, calcd for $C_9H_9N_5O_5P$, 298.0), 272.0 $(M - Na - CO)^-$.

Sodium 2-Hydroxy-6-(urid-1-yl)-8-acetyloxymethyltetrahydrofurano[3,4-*e*]-1,4,2-dioxaphosphorinane-2,3-dioxide 3g. Phosphonodichloridate **1** (2.39 g, 10 mmol) was added dropwise to a stirred solution of 5′-acetyluridine (2.86 g, 10 mmol) in trimethyl phosphate (20 mL) at 0 °C under Ar and stirred for 1 h. The reaction mixture was allowed to warm and stirred for a further 2 h, before being poured into dry ether (200 mL) with stirring. The precipitate was filtered and washed with ether (2 × 100 mL), yielding an amorphous white hygroscopic solid (3.12 g) that was dissolved in dioxane (20 mL) and treated with sodium bicarbonate (1 M, 10 mL). After being stirred for 2 h at room temperature, the reaction mixture was concentrated. Ethyl acetate (200 mL) was added with stirring over 2 h, yielding a precipitate which was filtered off. The filtrate was concentrated, and the resulting clear, glassy solid crystallized from acetone to yield a white crystalline product (0.42 g, 12%). δ_C-(DMSO-d_6): 20.6 (s); 62.46 (s); 72.73 (d, J_{CP} 5.1 Hz); 74.59 (s); 75.34 (s); 89.09 (s); 102.36 (s); 140.78 (s); 150.26 (s); 163.10 (s); 172.87 (d, J_{CP} 205.5 Hz). δ_P (DMSO-d_6): −14.44 (s). δ_H(DMSO-d_6): 2.08 (s, 3H); 4.12−4.36 (m, 3H); 4.79 (ddd, 1H, J_{HH} 1.7, 6.2 Hz, J_{HP} 12.2 Hz); 5.28 (d, 1H, J_{HH} 3.5, 5.7 Hz); 5.68 (d, 1H, J_{HH} 7.0); 5.94 (d, 1H, J_{HH} 3.5 Hz); 7.67 (d, 1H, J_{HH} 7.0 Hz); 11.4 (br.s., 1H). FAB-MS *m*/*z*: found 375.4 $((M - Na)^-$, calcd for $C_{12}H_{12}N_2O_{10}P$, 375.02), 349.1 $(M - Na - CO)^-$.

Computational Methods. Calculations were performed using Gaussian 94 running in parallel on IBM SP2 RISC processors at the MP2/6-31+G*//HF/3-21+G(*) level.[10] Geometry optimizations were carried out on **3a**, **4**, **5**, and **6** to obtain minimum energy structures at the HF/3-21+G(*) level. Thermochemical data were obtained from normal-mode analysis at the lower level of calculation, from a geometry optimized at this level, using the following standard relationships: $H = T + V + R + PV$ and $\Delta G = \Delta H - T\Delta S$.[11] Errors deriving from various

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sources in MO calculations have been discussed previously and are estimated as ±1 kcal/mol for molecular energies and ±0.01 Å and ±1° for bond lengths and angles, respectively.[11] The enthalpy and free energy of reaction for conversion of **3a** plus water to **5** were calculated as −1.93 and +10.05 kcal/mol, respectively. The enthalpy and free energy of reaction for conversion of **4** plus water to **6** was calculated as 4.10 and 16.16 kcal/mol, respectively. The ring strain enthalpy in **3a** relieved on addition of water is therefore 6.03 kcal/mol.

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Supporting Information Available: ^{31}P NMR for compounds **3a**−**g** and representative FAB-MS for **3g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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