Synthesis, Oral Bioavailability Determination, and *in Vitro* Evaluation of Prodrugs of the Antiviral Agent 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA)

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A series of phosphonate prodrugs were evaluated in an attempt to increase the oral bioavailability of the anti-HIV agent 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; 1). The majority of the bis(alkyl ester) and bis(alkyl amide) prodrugs were prepared by alcohol or amine displacement of dichlorophosphonate **2**. Basic hydrolysis of the bis(esters) or bis(amides) provided the corresponding monoesters or monoamides. Synthesis of bis[(acyloxy)alkyl] phosphonates **10a–c** was accomplished by alkylation of PMEA with the appropriate chloromethyl ether in the presence of *N,N'*-dicyclohexylmorpholinecarboxamidine. The systemic levels of PMEA following oral administration of a PMEA prodrug to rats were determined by measuring the concentration of PMEA in the urine for 48 h after administration of the prodrug. The oral bioavailability of PMEA employing this method was determined to be 7.8%. Oral dosing with bis(alkyl) phosphonates **3a,b** resulted in apparent absorption of the prodrugs (≥40%), although neither of the esters were completely cleaved to liberate the parent phosphonate PMEA. The mono(alkyl esters) **7a–e** and **8a,b** exhibited poor oral bioavailability (≤5%). Phosphonamides **5, 6,** and **9** were unstable under acidic conditions and provided levels of PMEA comparable to the parent compound after oral administration. Bis[(acyloxy)alkyl] phosphonates **10a–c** demonstrated significantly improved oral bioavailabilities of 17.6%, 14.6%, and 15.4%, respectively. When evaluated *in vitro* against HSV-2, (acyloxy)alkyl phosphonates **10a–c** were greater than 200-fold more active than PMEA.

Introduction

The adenine phosphonate 9-[(2-phosphonomethoxy)-ethyl]adenine (PMEA; 1) has demonstration broad antiviral activity against human immunodeficiency virus (HIV),[1] Rauscher murine leukemia virus (R-MuLV),[2] herpes simplex virus (HSV),[3] murine cytomegalovirus (MCMV),[2] Simian immunodeficiency virus (SIV),[4] and feline immunodeficiency virus (FIV).[5] In addition to *in vitro* activity, *in vivo* efficacy has been demonstrated when administered intravenously, intraperitoneally, or intramuscularly. Because of the interesting antiviral activity of PMEA, it is currently undergoing phase I/II trials for the evaluation of its toxicity and/or efficacy in AIDS patients. Recently presented clinical data indicate that PMEA has activity against HIV-1 *in vivo*.[6] However, the oral bioavailability of PMEA has been reported to be <1% in monkeys[4] and 11% in rats.[7] This very low oral bioavailability could limit the potential therapeutic uses of PMEA; current clinical trials with PMEA employ daily iv infusion.[6] We therefore undertook a study to prepare and evaluate prodrugs of PMEA in an attempt to increase its oral bioavailability.

Relatively few examples of phosphonate prodrugs or prodrugs of closely related analogues of phosphonates have appeared in the literature. Farquhar and co-workers have reported the use of (acyloxy)alkyl prodrugs of organophosphates to increase permeation across biological membranes.[8] The acyloxy alkyl ester of phosphonoformate has been prepared,[9] and Krapcho et al. have employed (acyloxy)alkyl prodrugs to improve the bioavailability of phosphinates.[10] A prodrug of PMEA has been synthesized by linking a synthetic polymer bearing mannosylated residues to PMEA.[11] In contrast to phosphonates, a much wider range of prodrugs have been successfully employed for preparation of carboxylic acid prodrugs. Acyloxy alkyl esters, as well as glycolamide esters, alkyl esters, and amides, have been extensively used.[12] The goal of the present study was to build on the carboxylic acid experience and evaluate a wide array of structural types as potential prodrugs of the phosphonate functionality. Preliminary results describing *in vitro* antiviral activity of the bis-[(pivaloyloxy)methyl] prodrug of PMEA (**10a**) against HIV, HCMV (human cytomegalovirus), HSV-1, and HSV-2 have recently been published in communication form.[13] We herein report on the synthesis, oral bioavailability, and antiviral activity of several different classes of phosphonate-derived prodrugs of PMEA.

Chemistry

For purposes of rapidly evaluating a diverse number of PMEA prodrugs, an easily accessible intermediate was desired. Stowell demonstrated that dichlorophosphonates, generated by oxalyl chloride treatment of phosphonic acids, react with alcohols or amines to give the corresponding bis(alkyl esters) or bis(alkylamines).[14] Quast has treated methylphosphonic acid with PCl_5 to prepare the corresponding dichlorophosphonate.[15] We found that dichlorophosphonate **2** can be conveniently prepared by refluxing PMEA (1) with thionyl chloride and a catalytic amount of DMF followed by cooling and concentration of the mixture to remove volatiles (Scheme 1). Intermediate **2** was very versatile and allowed us to prepare a majority of the prodrugs described in this study. Alcohols and amines which boil at ≤150 °C were employed as both the solvent and nucleophile and added directly to crude dichlorophosphonate **2** and heated (Table 1). With the higher boiling alcohols and amines (bp ≥150 °C), stoichiometric amounts were added to a suspension of

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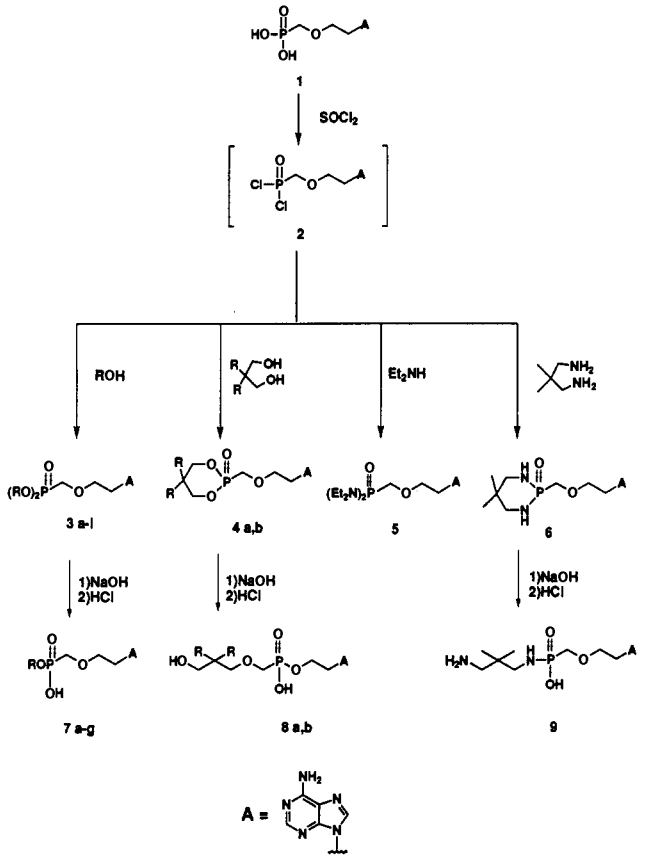
Scheme 1. Synthetic Scheme for Cyclic and Acyclic Ester and Amide Prodrugs of PMEA

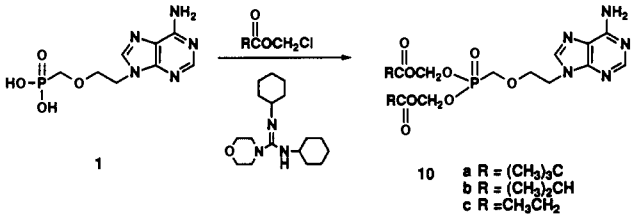


chlorophosphonate **2** in either dichloromethane or acetonitrile and then heated. (This eliminated the need to remove large volumes of high-boiling liquids.) Unless otherwise noted, the products were isolated by cooling the reaction, evaporating the mixture to dryness, and purifying the residue on a chromatography column. In general, there were large differences in R_f between the desired compounds and the unwanted side products. The bis(alkyl) phosphonate esters **3a–i**, cyclic esters **4a,b**, bis(alkylamide) **5**, and cyclic amide **6** were all prepared in this manner.

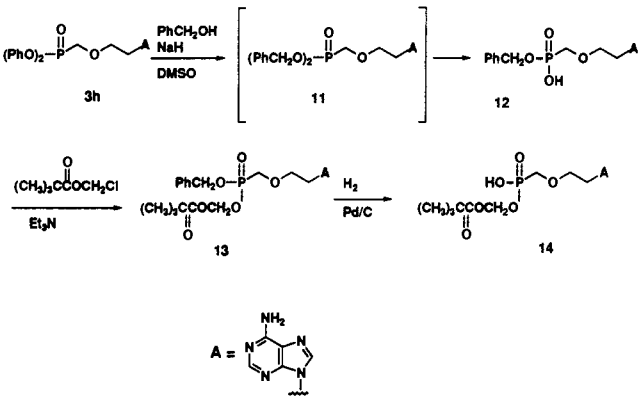
Saponification of esters **3a–c,f–i** with an excess of aqueous sodium hydroxide and heating (Table 1) followed by acidification and reverse-phase chromatography to remove the inorganic salts provided monoesters **7a–c** and **7d–7g**, respectively. These monoesters were remarkably resistant to further hydrolysis; little, if any PMEA was detected under the reaction conditions outlined. Similarly, cyclic esters **4a,b** provided monohydroxypropyl esters **8a,b**. Bis(*N,N*-dialkylamide) **5** proved to be extremely stable to basic conditions, and the corresponding mono-*N,N*-dialkylamide could not be prepared. In contrast to the bis(*N,N*-dialkylamide), cyclic *N*-alkylamide **6** was easily cleaved to mono-*N*-alkylamide **9**. Similar to monoesters **7** and **8**, mono-*N*-alkylamide **9** was stable to further hydrolysis to PMEA.

A variety of unsuccessful methods were employed in an attempt to synthesize bis[(pivaloyloxy)methyl] PMEA (**10a**). These attempts have been previously described in detail, as well as the successful method shown in Scheme 2.[13] Briefly, homogeneous reaction of PMEA (**1**) with chloromethyl pivalate in the presence of the hindered base *N,N'*-dicylohexylmorpholinecarboxamidine provided bis(pivaloyl ester) **10a** in 40% yield. Bis[(isobutyryloxy)methyl] PMEA (**10b**) and bis[(propionyloxy)methyl]

Scheme 2. Synthetic Scheme for (Acyloxy)alkyl Prodrugs of PMEA



Scheme 3. Synthetic Scheme for Mono(pivaloyloxy)methyl Prodrug of PMEA



PMEA (**10c**) were prepared in a corresponding manner. The monopivaloyl ester of bis(ester) **10a** could not be prepared by simple saponification since it was not stable to the reaction conditions and was quickly cleaved to afford PMEA. As a result, an alternate approach was employed (see Scheme 3). Ester exchange of diphenyl PMEA (**3h**) with the sodium salt of benzyl alcohol produced unstable dibenzyl PMEA (**11**) which cleaved under the reaction conditions to give monobenzyl ester **12**. Alkylation of the monoester with chloromethyl pivalate in the presence of triethylamine provided mixed alkyl phosphonate **13**. Subsequent removal of the benzyl group by hydrogenation with catalytic palladium on carbon provided monopivaloyl ester **14**.

The *N,N*-diethylacetamide was prepared as outlined in Scheme 4. In addition to determining the prodrug potential of the acetamide, we also studied the effects of mixed phosphonates containing acetamides, acyloxy alkyl esters, and alkyl esters. Acid chloride **15** was treated with sodium acetate to provide ester **16**. Hydrolysis of the ester with sodium methoxide gave hydroxyacetamide **17**. Acylation of dichloride **2** with alcohol **17** gave bis(ester) **18** which could not be isolated in pure form. The crude bis(ester) was saponified directly to provide mono-*N,N*-dialkylacetamide ester **19**. To prepare mixed (pivaloyloxy)methyl alkyl phosphonates, monoesters **19** and **7b** were alkylated with chloromethyl pivalate to give phosphonates **20a,20b**, respectively.

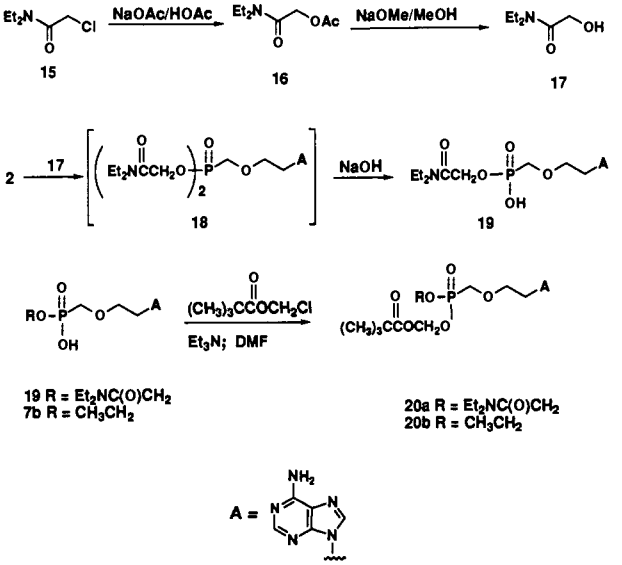
Cyclic esters **4a,b** were poor PMEA prodrugs (*vide infra*), possibly because of the high stability of the alkyl ester functionality. Studies with cyclic phosphates have shown that introduction of fluorine to give the cyclic difluorophosphate increased the rate of hydrolysis as compared to the unsubstituted cyclic phosphate.[16] In the present study, cyclic difluoro phosphonates were prepared to investigate the effect of fluorine on the stability of cyclic phosphonates. Fluorination of ketone **21** with (diethylamino)sulfur trifluoride (DAST) gave difluoride **22** which was hydrolyzed to afford dihydroxy difluoride **23** (Scheme

Table 1. Yields, Synthetic Methods, and Oral Bioavailability of PMEA Prodrugs

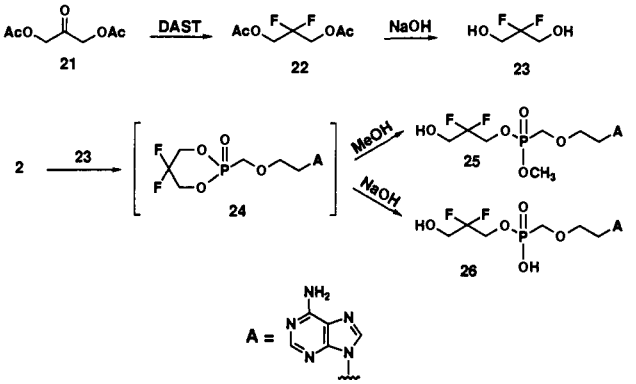
compd no.	R[a]	yield[b] (%)	synthetic method[c]	time (h)	temp (°C)	oral bioavailability
1	PMEA					7.8
3a	CH_3	47	A	4	60	0
3b	CH_3CH_2	69	A	3	60	(42)[d]
3c	$(CH_3)_2CH$	83	A	3	60	(44.4)[e]
3d	$(CH_3)_2CHCH_2$	75	A	3	60	0
3e	$(CH_3)_2CHCH_2CH_2$	77	A	1.5	80	0.5
3f	n-C_8H_{17}	40	B[f]	16	40	ND[g]
3g	Cl_3CCH_2	44	B	20	82	10.6
3h	Ph	38	B	20	22	11.1
3i	p-NO_2-$PhCH_2$	15	B	1	60	1.7
4a	H	9	C[h]	16	22	0.7
4b	CH_3	56	C	24	40	0
5		18	A	16	55	7.2
6		46	C	24	40	5.8
7a	CH_3	78	D	2	60	1.4
7b	CH_3CH_2	52	D	24	22	0
7c	$(CH_3)_2CH$	68	D	2	60	5.0
7d	n-C_8H_{17}	80	E[i]	1.5	60	0.8
7e	Cl_3CCH_2	69	E	1	60	2.5
7f	Ph	76	E	1	22	14.0
7g	p-NO_2-$PhCH_2$	78	E	20	60	1.9
8a	H	50	D	2	60	0
8b	CH_3	80	E	1.5	60	0
9		61	D	1.5	60	10.6
10a		32				17.3
10b		9				14.6
10c		9				15.4
14		12				6.5
19		19	B, E[j,k]	20; 0.3	0; 22	3.5
20a		4				2.2
20b		17				(34.9)[l]
21		44	B	1	82	5.8
26		18	B, E[j,m]	1;1	82; 22	3.5

[a] See schemes for general structures. [b] Yields are unoptimized starting from PMEA (1). Yields for compounds **7a–g**, **8a,b**, and **9** are from bis(ester) or bis(amide). [c] Synthetic methods are described in the Experimental Section of this paper. Where no method is given, see the Experimental Section for specific examples. [d] Detected as the diethyl ester. Oral bioavailability of PMEA was 0. [e] Detected as the monoisopropyl ester. Oral bioavailability of PMEA was 0. [f] The crude product obtained from column chromatography was recrystallized from CH_3CN. [g] The oral bioavailability could not be determined due to lack of solubility. [h] The impure product obtained from column chromatography was recrystallized from 25% MeOH/CH_3CN. [i] The crude product from acidification was recrystallized from water. [j] The crude product obtained from method B was employed directly in method E. Temperature and time are given for methods B and E, respectively. [k] See detailed Experimental Section for synthesis of hydroxyacetamide **17**. [l] Detected as the monoethyl ester. Oral bioavailability of PMEA was 0. [m] See detailed Experimental Section for synthesis of difluoro alcohol **23**.

Scheme 4. Synthetic Scheme for Glyoxamide Prodrugs of PMEA



Scheme 5. Synthetic Scheme for Difluoropropyl Ester Prodrug of PMEA



5). Crude cyclic difluoro ester **24** was obtained upon reaction of dichloride **2** with diol **23**. Introduction of the fluorine groups activated the phosphonate functionality to nucleophilic attack (as compared to cyclic ester **4a**) to such an extent that methoxy ester **25** was obtained upon column chromatography of the crude product when methanol was employed as an eluting solvent. Hydrolysis of crude cyclic difluoro ester **24** with sodium hydroxide gave monoester **26**.

Biological Evaluation and Discussion

To estimate the oral bioavailability of the prodrugs relative to that of PMEA, urinary concentrations of PMEA in male rats after oral administration of PMEA and each prodrug were compared to that of PMEA after iv administration at 30 mg/kg. Urine samples were collected for 48 h, and the amount of PMEA excreted in the urine

was measured by an HPLC method as previously reported.[17] As shown in Table 1, the absolute oral bioavailability of PMEA is estimated to be 7.8% in the rat. This value compares favorably with the previously reported oral bioavailability of 11% in the rat, which was obtained by measuring the concentration of PMEA in rat plasma after oral and iv administration of PMEA.[7] In the present study, the urinary method was chosen in preference to the plasma method because of the ease of sample analysis, thereby allowing rapid screening of a large number of compounds.

Table 1 shows that the oral bioavailability of PMEA after administration of bis(alkyl esters) **3a–e** was less than 1%, indicating that the bis(alkyl esters) were either not absorbed or absorbed but not cleaved completely to PMEA. The bioavailability value shown in parentheses after oral administration of diethyl ester **3b** (42%) is an estimate of the amount of *diester* **3b** excreted in the urine, based upon HPLC retention times. Similarly, the 44.4% value shown in parentheses after oral administration of bis(isopropyl ester) **3b** represents the amount of *monoester* **7c** detected in the urine, based upon HPLC retention times. These data suggest that both of these bis(alkyl esters) were well absorbed (>40%) but that the diethyl ester was excreted unchanged and that the bis(isopropyl ester) was cleaved to the mono(isopropyl) ester after absorption. Similar effects may be occurring with bis(secbutyl ester) **3d** and bis(isoamyl ester) **3e** (oral bioavailability of 0% and 0.5%, respectively) as well as with cyclic esters **4a,b** (oral bioavailability of PMEA of 0.7% and 0%); however, no attempt was made to assay for any of the mono- or bis-(esters). In the case of mixed alkyl (acyloxy)alkyl esters, following administration of ethyl (pivaloyloxy)methyl ester **20b**, a peak was observed that was consistent with the retention time of monoethyl ester **7b** (34.9% recovery). PMEA was not observed in this sample. This result indicates that mixed ester **20b** was absorbed but underwent incomplete hydrolysis to give the monoethyl ester. Bis-(trichloroethyl ester) **3g** was unique among the alkyl esters, demonstrating an oral bioavailability of 10.6%. The electron-withdrawing effect of the chlorines activate the phosphorus–oxygen bond and make it more susceptible to cleavage than that in the unactivated esters, such as diethyl ester **3b**.

In general, the monoesters **7a–g**, **8a,b**, **19**, and **26** exhibited very poor oral bioavailability, probably due to the polar nature of these compounds. The increased polarity relative to that of the diesters restricts their ability to permeate the gastrointestinal membrane. If monoesters **7b,7c** were absorbed but not cleaved, they would have been detected under the assay conditions (*vide supra*). In contrast to the low bioavailability of the alkyl esters, monophenyl ester **7f** provided an oral bioavailability of 14%. The reason for the elevated oral bioavailability of monophenyl ester **7f** is unclear at this time. The phenyl ester is less stable than the alkyl esters, and it may be cleaved to PMEA *in vivo;* however, this does not fully account for the 14% oral bioavailability of **7f**. It is also not well understood why PMEA is able to achieve oral bioavailability levels of 7.8%. The bioavailability data from the monoesters show that phosphonates bearing a negative charge are poorly absorbed (≤5%), and therefore, PMEA would be expected to have negligible bioavailability levels. It is possible that different mechanisms of absorption are used by the monoesters and PMEA.

Table 2. *In Vitro* Activity of Selected PMEA Prodrugs against HSV-2 (G strain)

compd no.	IC_{50} (μM)[a]
1 (PMEA)	119
3b	>300
7b	>300
3h	77
7f	95
10a	0.6
10b	<0.2
10c	0.4
14	22

[a] In HSV-infected vero cells, the concentration which gives a 50% reduction of plaque formation.

Amides **5**, **6**, and **9** were found to be extremely unstable under acidic conditions ($t_{1/2}$ at pH 2 < 10 min; data not shown) and therefore, would be expected to be cleaved to PMEA before absorption. This conclusion is supported by the oral bioavailability values (7.2%, 5.8%, and 10.6%, respectively). The (acyloxy)alkyl esters **10a–c** are the most promising PMEA prodrugs prepared to date. The oral bioavailability in rats of bis[(pivaloyloxy)methyl] PMEA (**10a**; 17.6%) more than doubled the oral bioavailability of PMEA (7.8%). Bis[(isobutyryloxy)methyl] PMEA (**10b**) and bis[(propionyloxy)methyl] PMEA (**10c**) also demonstrated improved oral bioavailabilities with values of 14.6% and 15.4%, respectively. The correct balance between lipophilicity and stability is obviously important, as mono(pivaloyloxy)methyl ester **14** had an oral bioavailability of PMEA of 6.5%. This value is lower than that of bis(ester) **10a** (17.3%) yet higher than that of any of the monoalkyl esters and higher than all but two of the bis(esters) (trichloroethyl ester **3g** and amide **5**).

The *in vitro* activity of selected PMEA prodrugs against HSV-2 is given in Table 2. Both diethyl ester **3b** and monoethyl ester **7b** did not inhibit plaque reduction at doses less than 300 μM, compared to PMEA which had an IC_{50} (dose which gives a 50% reduction of plaque formation) of 119 μM. The lack of activity with ethyl ester **3b** probably arises from the stability of the alkyl ester, which is then unable to undergo phosphorylation to the active diphosphate form of PMEA. Both diphenyl ester **3h** (IC_{50} = 77 μM) and monophenyl ester **7f** (IC_{50} = 95 μM) demonstrated similar efficacy to PMEA. This activity may reflect the instability of the esters, resulting in conversion to PMEA during the course of the assay. Mono(pivaloyloxy)methyl PMEA (**14**) had a 5-fold lower IC_{50} than PMEA (22 vs 119 μM), possibly due to improved cell penetration. The efficacy of PMEA was further enhanced through the masking of both charges with the use of bis[(acyloxy)alkyl] esters to give **10a–c** (IC_{50} = 0.6, <0.2, and 0.4 μM, respectively). The lower IC_{50} values observed with **10a–c** compared to that of PMEA can be attributed to an increase in the cellular uptake of the bis-[(acyloxy)alkyl] esters.[18] The toxicity of bis[(pivaloyloxy)-methyl] PMEA (**10a**) in stationary phase cultures and growing cells has been reported.[13] Similar toxic effects on stationary phase cultures were observed with all of the bis[(acyloxyalkyl)] esters; the majority of the cell monolayer remained intact when stationary-phase cultures of adherent cells were exposed to concentrations of **10a–c** as high as 200 μM.

In summary, (acyloxy)alkyl prodrugs of PMEA can be employed to increase the permeation of PMEA across biological membranes, both *in vitro* as well as *in vivo*. Recently published studies underscore the utility of bis-

[(pivaloyloxy)methyl)] PMEA as a prodrug; oral administration of bis[(pivaloyloxy)methyl)] PMEA (**10a**) to monkeys results in 30% bioavailability as compared to that of PMEA.[18] These data should provide the impetus for further exploration of this exciting class of compounds.

Experimental Section

Melting points were determined with a Fisher-Johns apparatus and are not corrected. Proton and carbon-13 magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Bruker AM 300 or Varian Gemini 300 spectrometer. Chemical shifts are expressed as parts per million (δ) relative to Me_4Si used as the internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; and dd, doublet of doublet. J values are expressed in hertz. Low-resolution mass spectra were recorded on a Kratos MS-50 or Finnegan 4500 instrument utilizing the fast atom bombardment (FAB) or direct chemical ionization (DCI) technique. High-resolution mass spectra were recorded on a Kratos MS-50 instrument. IR spectra were measured on a Perkin-Elmer 1800 FT-IR and are reported in cm^{-1}. IR, NMR, and mass spectral data of all compounds were consistent with reported structures. Elemental analyses indicated were within ±0.4% of the calculated values. Preparative chromatography was performed with flash chromatography on silica gel or octadecyl (C-18) from J. T. Baker Inc. 9-[2-(Phosphonomethoxy)ethyl]adenine (PMEA; 1) was prepared as described by Bronson et al.[2]

The compounds listed in in Table 1 were synthesized either by the general methods given below employing the time and temperature in the table or by the specific experimentals in this section when no general method appears in Table 1.

General Methods of Synthesis for Compounds in Table 1. A. A suspension of 1.00 g (3.66 mmol) of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA; 1) in 50 mL of thionyl chloride was refluxed for 1 h. The homogeneous, orange-red solution was cooled, and the solvents were removed *in vacuo* to afford crude dichlorophosphonate **2**. The dichloride was taken up in the appropriate alcohol or amine and stirred at the temperature and for the time given in Table 1. After the reaction was cooled to room temperature, the solvents were removed *in vacuo*. The residue was purified on a 30-mm flash chromatography column, eluting with 10% MeOH/CH_2Cl_2 to afford the bis(ester) or bis(amide).

B. This reaction was performed similarly to method A, except crude dichlorophosphonate **2** was suspended in 30 mL of acetonitrile before adding 4 equiv of alcohol or amine.

C. This reaction was performed similarly to method A, except crude dichlorophosphonate **2** was suspended in 30 mL of methylene chloride before adding 4 equiv of alcohol or amine.

D. An aqueous suspension of bis(ester) or bis(amide) was treated with 4 equiv of NaOH for the time and temperature given in Table 1. The mixture was cooled to room temperature and acidified until pH 8. The majority of the solvent was evaporated, and the residue was purified on a C-18 silica gel column, eluting with a gradient of 0–25% MeOH/H_2O. The fractions containing the product were combined and evaporated to give the mono(ester) or mono(amide).

E. This reaction was performed similarly to method D, except after cooling to room temperature, the reaction was acidified to pH 1.5.

Specific Experimental Details for Compounds in Table 1 Where No General Method Is Given. 9-[2-(Phosphonomethoxy)ethyl]adenine, Bis[(pivaloyloxy)methyl ester] (10a). To a solution of 1.00 g (3.66 mmol) of PMEA (1) in 15 mL of anhydrous DMF were added 2.08 g (7.32 mmol) of *N,N'*-dicyclohexyl-4-morpholinecarboxamidine and 2.75 g (18.3 mmol) of chloromethyl pivalate. The heterogeneous mixture became homogeneous after 15 min and was stirred at 22 °C for 36 h. The insolubles were filtered off, and the filtrate was concentrated *in vacuo*. The residue was partitioned between water (50 mL) and toluene (50 mL) and separated, and the water layer was extracted with toluene (2 × 50 mL). The toluene layers were combined and concentrated *in vacuo*. The residue was purified by silica gel chromatography, eluting with 5% MeOH/CH_2Cl_2 to give 0.59 g (32%) of the title compound.

^{1}H NMR ($CDCl_3$): 8.32 (1H, s, H-8), 7.91 (1H, s, H-2), 5.77 (2H, s, NH_2), 5.63 (4H, m, CH_2OP), 4.37 (2H, t, J = 5.0, H-1'), 3.92 (2H,t, J = 5.0,H-2'), 3.82 (2H, d, J = 7.7, H-4'), 1.18 (18H, s, CH_3). ^{13}C NMR ($CDCl_3$) 177.55 (C=O), 156.23 (C-6), 153.45 (C-2), 150.48 (C-4), 142.05 (C-8), 119.85 (C-5), 82.04 (CH_2OP, d, J = 6.0), 71.70 (C-2', d, J = 9.8), 65.86 (C-4', d, J = 167), 43.63 (C-1'), 38.95 (*C*C(=O)), 27.11 (CH_3). IR (KBr): 3366, 3178, 2976, 1754, 1660, 1600. MS (isobutane/DCI): m/z (rel intensity) 502 (M + H, 100). Anal. ($C_{20}H_{32}N_5O_8P_1$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis[(isobutyryloxy)methyl ester] (10b). To a mixture of 1.00 g (3.66 mmol) of PMEA (1) in 15 mL of anhydrous DMF were added 2.08 g (7.32 mmol) of *N,N'*-dicyclohexyl-4-morpholinecarboxamidine and 2.48 g (18.3 mmol) of chloromethyl isobutyrate. The heterogeneous mixture became homogeneous within 30 min and was stirred at 22 °C for 5 days. The mixture was concentrated *in vacuo* and partitioned between water (50 mL) and toluene (50 mL). The aqueous layer was extracted with toluene (250 mL), and the combined organic layers were concentrated *in vacuo*. The residue was purified by silica gel chromatography, eluting with 5% MeOH/CH_2Cl_2 to give 0.16 g (9%) of the title compound.

^{1}H NMR ($CDCl_3$): 8.31 (1H, s, H-8), 8.28 (1H, s, H-2), 5.68 (2H, s, NH_2), 5.59 (4H, m, CH_2OP), 4.33 (2H, t, J = 5.0, H-1'), 3.88 (2H, t, J = 5.0, H-2'), 3.78 (2H, d, J = 7.7H, H-4'), 2.52 (2H, apparent heptet, J = 7.0, CH), 1.11 (6H, d, J = 7.0, CH_3). IR (KBr): 3360, 2980, 1758, 1660, 1602. MS (isobutane/DCI) m/z (rel intensity) 474 (M + H, 100). Anal. ($C_{18}H_{28}N_5O_8P_1 \cdot 0.65\,H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis[(propionyloxy)methyl ester] (10c). To a rapidly stirred solution of 1.00 g (3.66 mmol) of PMEA (1) in 15 mL of anhydrous DMF were added 2.08 g (7.32 mmol) of *N,N'*-dicyclohexyl-4-morpholinecarboxamidine and 2.23 g (18.3 mmol) of chloromethyl propionate. The heterogeneous mixture became homogeneous within 30 min and was stirred at 22 °C for 5 days. The insolubles were filtered off, and the filtrate was concentrated *in vacuo*. The residue was purified twice by silica gel chromotography, eluting with 5% MeOH/CH_2Cl_2 to give 0.14 g (9%) of the title compound.

^{1}H NMR ($CDCl_3$): 8.29 (1H, s, H-8), 7.88 (1H, s, H-2), 5.65 (2H, s, NH_2), 5.60 (4H, m, CH_2OP), 4.35 (2H, t, J = 5.0, H-1'), 3.89 (2H, t, J = 5.0, H-2'), 3.80 (2H, d, J = 7.8, H-4'), 2.34 (4H, q, J = 7.5, CH_3CH_2), 1.10 (6H, t, J = 7.5, CH_3). IR (KBr): 3290, 3122, 1766, 1666, 1602. MS (FAB): m/z (rel intensity) 446 (M + H, 100). Anal. ($C_{16}H_{24}N_5O_8P_1$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono(pivaloyloxy)methyl Ester (14). To a mixture of 80% sodium hydride (0.95 g, 32 mmol) and benzyl alcohol (6.8 mL, 63 mmol) in anhydrous DMSO (50 mL) was added, with stirring, a solution of PMEA, bis(phenyl ester) (3.4 g, 8.0 mmol; **3h**) in DMSO (50 mL). The mixture was allowed to stir at 22 °C for 1 h and concentrated to a volume of approximately 25 mL. EtOAc (200 mL) was added, and the precipitate was collected by vacuum filtration. The precipitate was purified by C-18 chromatography, eluting with 20% MeOH/H_2O to give 2.09 g (68%) of PMEA, monobenzyl ester, sodium salt (**12**).

To 600 mg (1.56 mmol) of monobenzyl ester **12** in 14 mL of anhydrous DMF were added 2.16 mL (15.5 mmol) of Et_3N and 1.44 g (9.61 mmol) of chloromethyl pivalate. The mixture was allowed to stir at 22 °C for 2 days and concentrated *in vacuo* to provide crude mixed ester **13**.

To a stirred solution of the crude mixed ester **13** (300 mg) in 17 mL of EtOH and 17 mL of H_2O were added 3.45 mL of cyclohexene and 0.225 g of 20% $Pd(OH)_2/C$. The mixture was heated at reflux for 1 h and concentrated *in vacuo* and the residue purified by C-18 chromatography, eluting with 100% H_2O to give 270 mg (31% from PMEA, bis(phenyl ester), **3h**) of mono(pivaloyloxy)methyl ester (**14**).

^{1}H NMR (DMSO-d_6): 8.09 (2H, s, H-8, H-2), 7.17 (2H, s, NH_2), 5.44 (2H, m, CH_2OP), 4.26 (2H, t, J = 5.0, H-1'), 3.83 (2H, t, J = 5.0, H-2'), 3.47 (2H, d, J = 8.0, H-4'), 1.04 (9H, s, CH_3). ^{13}C NMR (DMSO-d_6): 176.70 (C=O), 155.98 (C-6), 152.39 (C-2), 149.55 (C-4), 141.30 (C-8), 118.59 (C-5), 83.14 (CH_2OP), 69.89 (C-2'), 64.5 (C-4'), 42.84 (C-1'), 38.13 ($(CH_3)_3C$), 26.69 (CH_3). IR (KBr): 3360, 1742, 1648, 1602. MS (FAB): m/z (rel intensity) 386 (M − H, 100). HRMS (M + H, $C_{14}H_{22}N_5O_6P$): calcd, 388.1386; found, 388.1377.

2-Hydroxy-*N,N*-diethylacetamide (17). To a solution of 10.5 g (0.0702 mol) of 2-chloro-*N,N*-diethylacetamide (**15**) in 75 mL of glacial acetic acid was added 11.5 g (0.140 mol) of sodium acetate. The mixture was refluxed for 16 h. After cooling, the solvents were removed *in vacuo*, the last traces of acetic acid being azeotropically removed with toluene. Crude ester **16** was dissolved in 125 mL of methanol and treated with 10.9 g (0.20 mol) of sodium methoxide. The reaction was stirred for 3 h and neutralized with Dowex 50X8–200 acidic ion exchange resin. The solvents were removed *in vacuo*, and the residue was purified on a flash chromatography column, eluting with hexane/ethyl acetate 1:1 to give 6.75 g (73%) of 2-hydroxy-*N,N*-diethylacetamide (**17**).

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-*N,N*-diethylacetamide Mono(pivaloyloxy)methyl ester (20a). To a suspension of 0.100 g (0.239 mmol) of PMEA, mono-*N,N*-diethylacetamide ester, sodium salt (**19**) in 2.5 mL of CH_3CN was added 0.25 mL of Et_3N, whereupon the reaction became homogeneous. To this mixture was added 0.17 mL (1.19 mmol) of chloromethyl pivalate. The reaction was stirred at 22 °C for 24 h, evaporated to dryness *in vacuo*, and purified on a 20 mm flash chromatography column. The title compound eluted with 10% $MeOH/CH_2Cl_2$ to provide 25 mg (21%) of a colorless oil. ^1H NMR ($CDCl_3$): 8.25 (1H, s, H-8), 7.94 (1H, s, H-2), 6.26 (2H, s, NH_2), 5.65 (1H, dd, J = 12.3, 5.4, OCH_2O), 5.60 (1H, dd, J = 12.3, 4.8, OCH_2O), 4.75 (1H, dd, J = 14.7, 10.8, $OCH_2C(O)$), 4.56 (1H, dd, J = 14.5, 14.3, $OCH_2C(O)$), 4.32 (2H, dd, J = 5.7, 4.4, H-1′), 3.97 (2H, d, J = 8.4, H-4′), 3.91 (2H, t, J = 4.8, H-2′), 3.28 (2H, q, J = 7.5, CH_2CH_3), 3.09 (1H, q, J = 7.2, CH_2CH_3), 1.12 (9H, s, $(CH_3)_3$), 1.07 (3H, m, CH_3CH_2), 1.05, (3H, t, J = 6.9, CH_3CH_2). ^{13}C NMR ($CDCl_3$): 177.85 (C(O)O), 166.25 (C(O)N), 156.34 (C-6), 153.48 (C-2), 150.49 (C-4), 142.22 (C-8), 119.79 (C-5), 81.94 ($(CH_3)_3C$), 81.71 (OCH_2O), 71.55 (C-2′, d, J = 10), 65.10 (C-4′, d, J = 165), 63.99 (CCH_2OP), 43.53 (C-1′), 41.03 (NCH_2), 40.78 (NCH_2), 27.00 ($(CH_3)_3$), 14.21 (CH_3CH_2), 13.00 (CH_3CH_2). MS (FAB): m/z (rel intensity) 501 (M + H, 100). IR: 3500–3000, 2978, 1750, 1654, 1600, 1480, 1250. Anal. ($C_{20}H_{33}N_6O_7P \cdot 0.5H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monoethyl Mono(pivaloyloxy)methyl Ester (20b). To a rapidly stirred solution of 400 mg (1.33 mmol) of monoethyl PMEA (**7b**) in 15 mL of anhydrous DMF were added 2.00 mL (14.3 mmol) of Et_3N and 1.0 g (6.7 mmol) of chloromethyl pivalate. The heterogeneous mixture became homogeneous after addition of Et_3N and was stirred at 22 °C for 2 days. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel chromatography, eluting with 10% $MeOH/CH_2Cl_2$ to give 180 mg (33%) of the title compound.

^1H NMR($CDCl_3$): 8.32(1H, s, H-8), 7.92 (1H, s, H-2), 5.74 (2H, s, NH_2), 5.62 (2H, m, OCH_2OP), 4.38 (2H, t, J = 5.0, H-1′), 4.10 1(2H, m, CH_3CH_2OP), 3.92 (2H, t, J = 5.0, H-2′), 3.79 (2H, d, J = 8.0, H-4′), 1.27 (3H, t, J = 7.0, CH_3CH_2), 1.18 (9H, s, $((CH_3)C)$. ^{13}C NMR ($CDCl_3$): 176.87 (C=0), 155.40 (C-6), 152.94 (C-2), 149.8 (C-4), 141.51 (C-8), 119.7 (C-5), 81.85 (CH_2OP, d, J = 6.2), 71.26 (C-2′, d, J = 10.2), 65.46 (C-4′, d, J = 167), 62.73 (CH_2CH_3, d, J = 7.0), 43.49 (C-1′), 38.70 ($(CH_3)_3C$), 26.84 ($(CH_3)_3C$), 16.27 (CH_2CH_3, d, J = 5.8), IR (KBr): 3288, 3120, 2982, 1752, 1666, 1600. MS (FAB): m/z (rel intensity) 416 (M + H, 100). Anal. ($C_{16}H_{26}N_5O_8P_1 \cdot 0.5H_2O$) C,H,N.

2,2-Difluoro-3-hydroxypropan-1-ol (23). A solution of 9.07 g (0.0521 mol) of 1,3-diacetylacetone in 20 mL of DAST was stirred at 22 °C for 2 days, diluted with ethyl acetate, washed with saturated $NaHCO_3$ and then water, dried over Na_2SO_4, and concentrated to yield 9.54 g of 1,3-diacetyl-2,2-difluoropropane. The diacetyldifluoropropane (7.53 g, 38.4 mmol) was dissolved in 300 mL of methanol and treated with 6.45 g (119 mmol) of sodium methoxide. After stirring at 22 °C for 2.5 h, the reaction was neutralized with Dowex 50X8–200 acidic ion-exchange resin and the mixture filtered and stripped to give 3.7 g (86%) of the title compound.

The following examples were prepared by the methods given in Table 1:

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(methyl ester) (3a). Mp: 133–135 °C. ^1H NMR (DMSO-d_6): 8.14 (1H, s, H-8), 8.10 (1H, s, H-2), 7.29 (2H, s, NH_2), 4.33 (2H, t, J = 5.0, H-1′), 3.90 (2H, d, J = 8.3, H-4′), 3.85 (2H, t, J = 5.0, H-2′), 3.57 (6H, d, J = 10.6, CH_3). ^{13}C NMR (DMSO-d_6): 155.87 (C-6), 152.87 (C-2), 149.59 (C-4), 141.27 (C-8), 118.65 (C-5), 70.40 (C-2′, d, J = 11.5), 63.17 (C-4′, d, J = 182), 52.79 (CH_3, d, J = 6.4), 42.48 (C-1′). IR (KBr): 3400, 3188, 1671, 1647, 1605. MS (methane/DCI): m/z (rel intensity) 302 (M + H, 100). Anal. ($C_{10}H_{16}N_5O_4P \cdot 0.6H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(isopropyl ester) (3c). Mp: 136–138 °C. UV_{max} (MeOH): 262 nm (ϵ = 14 360). ^1H NMR (DMSO-d_6): 8.15 (1H, s), 8.09 (1H, s), 7.21 (2H, br s, exch, NH_2), 4.50 (2H, apparent octet, J = 6.5, $P(OCH(CH_3)_2)_2$), 4.34 (2H, t, J = 5, NCH_2), 3.91 (2H, t, J = 5, CH_2OCH_2P), 3.79 (2H, d, J = 8, OCH_2P), 1.18 (6H, d, J = 6.5, $POCH(CH_3)_2$), 1.13 (6H, d, J = 6.5, $POCH(CH_3)_2$). ^{13}C NMR (DMSO-d_6): 155.86 (C-6), 152.23 (C-2), 149.46 (C-4), 140.90 (C-8), 118.57 (C-5), 70.22 ($POCH$, d, J = 10), 70.05 (CH_2OCH_2P, d, J = 12), 64.50 (OCH_2P, d, J = 165), 42.35 (NCH_2), 23.61 ($POCH(CH_3)_2$, d, J = 7), 23.52 ($POCH(CH_3)_2$, d, J = 7). MS (methane/DCI): m/z (rel intensity) 358 (M + H, 100), 344 (10), 316 (10). Anal. ($C_{14}H_{24}N_5O_4P$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(isobutyl ester) (3d). Mp: 109–110 °C. ^1H NMR (DMSO-d_6): 8.10 (1H, s, H-8), 8.05 (1H, s, H-2), 7.19 (2H, s, NH_2), 4.31 (2H, t, J = 5.0, H-1′), 3.87 (2H, t, J = 5.0, H-2′), 3.85 (2H, d, J = 8.5, H-4′), 3.61(4H, dt, J = 6.8, 1.4, CH_2OP), 1.72 (2H, apparent heptet, J = 6.7,CH), 0.77 (12H, d, J = 6.7, CH_3). ^{13}C NMR (DMSO-d_6): 156.04 (C-6), 152.42 (C-2), 149.60 (C-4), 141.05 (C-8), 118.69 (C-5), 71.42 (CH_2OP, d, J = 6.7), 70.36 (C-2′, d, J = 11.6), 63.65 (C-4′, d, J = 163), 42.52 (C-1′), 28.72 (CH, d, J = 5.7), 18.45 (CH_3). IR (KBr): 3286, 3104, 2960, 1670, 1600. MS (FAB): m/z (rel intensity) 386 (M + H, 100). Anal. ($C_{16}H_{28}N_5O_4P_1$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(isovaleryl ester) (3e). Mp: 94–98 °C. ^1H NMR ($CDCl_3$) 8.30 (1H, s, H-8), 7.94 (1H, s, H-2), 6.21 (2H, s, NH_2), 4.37 (2H, t, J = 5.0, H-1′), 4.01 (4H, dt, J = 6.8, 6.8, CH_2OP), 3.91 (2H, t, J = 5.0, H-2′), 3.75 (2H, d, J = 8.0, H-4′), 1.63 (2H, apparent heptet, J = 6.6, CH), 1.47 (4H, dt, J = 6.7, 6.7, CH_2CH_2OP), 0.84 (12H, d, J = 6.5, CH_3). ^{13}C NMR ($CDCl_3$): 155.28 (C-6), 152.38 (C-2), 150.38 (C-4), 141.70 (C-8), 119.76 (C-5), 71.13 (C-2′, d, J = 10.0), 65.17 (C-4′, d, J = 166), 65.02 (CH_2OP, d, J = 6.8), 43.46 (C-1′), 39.19 (CH_2CH_2OP, d, J = 5.7), 24.50 (CH), 22.31 (CH_3), 22.29 (CH_3). IR (KBr): 3282, 3106, 2958, 1672, 1600, 1478. MS (methane/DCI): m/z (rel intensity) 414 (M + H, 100). Anal. ($C_{18}H_{32}N_5O_4P_1 \cdot 0.75H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(2,2,2-trichloroethyl ester) (3g). Mp: 155–157 °C. ^1H NMR (DMSO-d_6): 8.11 (1H, s, H-8), 8.08 (1H, s, H-2), 7.16 (2H, s, NH_2), 4.68 (2H, d, J = 7, CCl_3CH_2), 4.67 (2H, d, J = 7, CCl_3CH_2), 4.34 (2H, t, J = 5, H-1′), 4.18 (2H, d, J = 8, H-4′), 3.95 (2H, t, J = 5, H-2′). ^{13}C NMR (DMSO-d_6): 156.09 (C-6), 152.59 (C-2), 149.71 (C-4), 141.28 (C-8), 118.75 (C-5), 95.42 (CCl_3, d, J = 8.6), 75.48 (CCl_3CH_2, d, J = 5.7), 70.92 (C-2′, d, J = 7), 63.99 (C-4′, d, J = 163), 42.72 (C-1′). IR (KBr): 3372, 3334, 3210, 1658, 1604, 1576. MS (methane/DCI): m/z (rel intensity) 536 (100), 534 (50), 192 (95). Anal. ($C_{12}H_{14}N_5O_{14}PCl_6$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(phenyl ester) (3h). Mp: 103–114 °C. ^1H NMR (DMSO-d_6): 8.15 (1H, s, H-8), 8.11 (1H, s, H-2), 7.40 (2H, s, NH_2), 7.34 (4H, t, J = 7, ArH), 7.20 (2H, t, J = 7, ArH), 7.04 (4H, t, J = 7, ArH), 4.38 (2H, t, J = 5, H-1′), 4.24 (2H, d, J = 8, H-4′), 3.98 (2H, t, J = 5, H-2′). ^{13}C NMR (DMSO-d_6): 155.51 (C-6), 151.77 (C-2), 149.57 (C-4), 141.46 (C-8), 130.02, 125.49, (ArC), 120.56 (ArC, d, J = 4), 118.71 (C-5), 70.58 (C-2′, d, J = 12), 63.52 (C-4′, d, J = 164), 42.68 (C-1′). IR (KBr): 3270, 3100, 1675, 1646, 1601, 1490. MS (FAB): m/z (rel intensity) 426 (M + H, 100). Anal. ($C_{20}H_{20}N_5O_4P \cdot 0.25H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(*p*-nitrophenyl ester) (3i). Mp: 190–193 °C. ^1H NMR (DMSO-d_6): 8.16 (4H, d, J = 8, ArH), 8.09 (1H, s, H-8), 8.08 (1H, s, H-2), 7.51 (4H, d, J = 8, ArH), 7.17 (2H, s, NH_2), 5.10 (4H, d, J = 8, $ArCH_2O$), 4.32 (2H, t, J = 5, H-1′), 4.07 (2H, d, J = 8, H-4′), 3.90 (2H, t, J = 5, H-2′). ^{13}C NMR (DMSO-d_6): 155.97 (C-6), 152.94 (C-2), 149.62 (C-4), 147.19, 143.96 (ArC), 141.13 (C-8), 128.15, 123.56 (ArC), 118.65 (C-5), 70.62 (C-2′, d, J = 7), 65.86 ($ArCH_2O$, d, J = 6), 63.75 (C-4′, d, J = 162), 42.49 (C-1′). IR (KBr): 3420, 3268, 3110, 1674, 1642, 1604. MS (FAB): m/z (rel intensity) 544 (M + H, 60). Anal. ($C_{22}H_{22}N_7O_8P$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Cyclic Propanyl Ester (4a). Mp: 195–199 °C. ^{1}H NMR (DMSO-d_6): 8.13 (1H, s, H-8), 8.12 (1H, s, H-2), 4.35 (2H, t, J = 4.8, H-1′), 4.2 (4H, m, CH_2OP), 3.95 (2H, d, J = 8.8, H-4′), 3.86 (2H, t, J = 4.8, H-2′), 1.98 (1H, m, $CH_2CH_2CH_2$), 1.55 (1H, m, $CH_2CH_2CH_2$). ^{13}C NMR (DMSO-d_6): 156.01 (C-6), 152.48 (C-2), 149.69 (C-4), 141.11 (C-8), 118.68 (C-5), 70.71 (C-2′, d, J = 13.8), 68.30 (CH_2OP, d, J = 6.9), 64.55 (C-4′, d, J = 158), 42.52 (C-1′), 25.85 ($CH_2CH_2CH_2$, d, J = 9.0). IR (KBr): 3351, 3169, 1660, 1601, 1256, 1063. MS (FAB): m/z (rel intensity) 314 (M + H, 100). Anal. ($C_{11}H_{16}N_5O_4P\cdot1.5\ H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Cyclic 2′,2′-Dimethylpropanyl Ester (4b). Mp 224–226 °C. ^{1}H NMR (DMSO-d_6): 8.11 (2H, s, H-8, H-2), 7.21 (2H, s, NH_2), 4.34 (2H, t, J = 5.0, H-1′), 3.99 (2H, d, J = 8.7, H-4′), 3.91 (2H, t, J = 5.0, H-2′), 3.95-3.75 (4H, m, $CH_2C(CH_3)_2CH_2$), 1.06 (3H, s, CH_3), 0.67 (3H, s, CH_3). ^{13}C NMR (DMSO-d_6, 50 MHz): 155.89 (C-6), 152.33 (C-2), 149.53 (C-4), 140.86 (C-8), 118.57 (C-5), 76.67 ($CH_2C(CH_3)_2CH_2$, d, J = 6.8), 70.44 (C-2′, d, J = 13.7), 64.43 (C-4′, d, J = 157), 42.43 (C-1′), 31.70 ($C(CH_3)_2$, d, J = 7.6), 21.05 (CH_3), 19.46 (CH_3). IR (KBr): 3417, 3324, 3152, 2970, 1668, 1650, 1602. MS (FAB): m/z (rel intensity) 342 (M + H, 100). Anal. ($C_{13}H_{20}N_5O_4P\cdot0.25\ H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Bis(diethylamide) (5). Mp: 93–96 °C. ^{1}H NMR (DMSO-d_6): 8.11 (1H, s, H-8), 8.07 (1H, s, H-2), 7.18 (2H, s, NH_2), 4.31 (2H, t, J = 4.8, H-1′), 3.85 (2H, t, J = 4.8, H-2′), 3.68 (2H, d, J = 8.1, H-4′), 2.70 (8H, m, CH_3CH_2), 0.86 (12H, t, J = 7.0, CH_3). ^{13}C NMR (DMSO-d_6): 155.98 (C-6), 152.33 (C-2), 149.63 (C-4), 141.04 (C-8), 118.75 (C-5), 70.30 (C-2′, d, J = 13.0), 66.30 (C-4′, d, J = 133), 42.63 (C-1′), 37.53 (CH_3CH_2), d, J = 4.1), 13.93 (CH_3, d, J = 1.9). IR (KBr): 3370–2935, 2875, 1680, 1649, 1605, 1211. MS (FAB): m/z (rel intensity) 384 (M + H), 100). Anal. ($C_{16}H_{30}N_7O_2P\cdot0.5\ H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Cyclic (2′,2′-Dimethylpropanyl)amide (6). ^{1}H NMR (DMSO-d_6): 8.11 (1H, s, H-8), 8.10 (1H, s, H-2), 7.18 (2H, s, NH_2), 4.30 (2H, t, J = 5.0, H-1′), 3.83 (2H, t, J = 5.0, H-2′), 3.63 (2H, d, J = 7.5, H-4′), 4.27 (2H, s, NH, NH), 2.65–2.40 (4H, m, $CH_2C(CH_3)_2CH_2$), 0.98 (3H, s, CH_3), 0.64 (3H, s, CH_3). ^{13}C NMR (DMSO-d_6): 156.01 (C-6), 152.42 (C-2), 149.60 (C-4), 141.24 (C-8), 118.68 (C-5), 70.35 (C-2′, d, J = 11.2), 68.53 (C-4′, d, J = 131), 52.72 ($CH_2C(CH_3)_2CH_2$, d, J = 2.3), 42.78 (C-1′), 30.54 ($C(CH_3)_2$, d, J = 5.6), 24.82 (CH_3), 23.25 (CH_3). IR (KBr): 3100, 2980, 2940, 1650, 1605. MS (FAB): m/z (rel intensity) 340 (M + H, 100). HRMS (M + H, $C_{13}H_{22}N_7O_2P$): calcd, 340.1651; found, 340.1647.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monomethyl Ester, Sodium Salt (7a). ^{1}H NMR (DMSO-d_6): 8.19 (1H, s, H-8), 8.11 (1H, s, H-2), 7.17 (2H, s, NH_2), 4.27 (2H, t, J = 5.0, H-1′), 3.77 (2H, t, J = 5.0, H-2′), 3.35 (2H, d, J = 8.0, H-4′), 3.24 (3H, d, J = 10.0, CH_3). ^{13}C NMR (DMSO-d_6, 90 MHz): 155.87 (C-6), 152.26 (C-2), 149.49 (C-4), 141.44 (C-8), 118.51 (C-5), 69.69 (C-2′, d, J = 9), 67.09 (C-4′, d, J = 152), 50.78 (CH_3, d, J = 5), 42.64 (C-1′). IR (KBr): 3421, 3195, 1649, 1605, 1578, 1516. MS (FAB): m/z (rel intensity) 310 (M + H, 23). Anal. ($C_9H_{13}N_5O_4P_1Na_1\cdot3H_2O\cdot NaCl$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monoethyl Ester, Sodium Salt (7b). ^{1}H NMR (DMSO-d_6): 8.19 (1H, s, H-8), 8.11 (1H, s, H-2), 7.21 (2H, br s, NH_2), 4.29 (2H, t, J = 4.8, H-1′), 3.74 (2H, t, J = 4.8, H-2′), 3.52 (2H, dq, J = 7.0, 7.0, $POCH_2$), 3.32 (2H, d, J = 8.5, H-4′), 0.93 (3H, t, J = 7.0, CH_3). ^{13}C NMR (DMSO-d_6, 90 MHz): 155.89 (C-2), 152.32 (C-6), 149.51 (C-4), 144.42 (C-8), 118.50 (C-5), 69.98 (C-2′, d, J = 9.2), 67.72 (C-4′, d, J = 153), 58.75 ($POCH_2$, d, J = 5.0), 42.65 (C-1), 16.87 (CH_3). IR (KBr): 3400, 2990, 1645, 1601, 1578. MS (FAB): m/z (rel intensity) 324 (M + H, 63). Anal. ($C_{10}H_{15}N_5O_4P_1Na_1\cdot2.2H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monoisopropyl Ester, Sodium Salt (7c). Mp: 77–85 °C, turned to glass and melted over next 40 °C. ^{1}H NMR (DMSO-d_6): 8.19 (1H, s, H-8), 8.13 (1H, s, H-2), 7.22 (2H, s, NH_2), 4.30 (2H, t, J = 4.4, H-1′), 4.10 (1H, m, OCH), 3.76 (2H, t, J = 4.4, H-2′), 3.31 (2H, d, J = 8.6, H-4′), 0.90 (6H, d, J = 6.0, CH_3). ^{13}C NMR (DMSO-d_6, 90 MHz), 155.90 (C-6), 152.35 (C-2), 149.54 (C-4), 141.39 (C-8), 118.53 (C-5), 70.23 (OCH, d, J = 10), 68.70 (C-4′, d, J = 192), 65.55 (C-2′, d, J = 5), 42.72 (C-1′), 24.43 (CH_3). IR (film): 3321, 3163, 1647, 1601, 1578. MS (FAB): m/z (rel intensity) 338 (M + H, 70). Anal. ($C_{11}H_{17}N_5O_4P_1Na_1\cdot H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monooctyl Ester, Sodium Salt (7d). ^{1}H NMR (pyridine-d_5): 9.47, 9.34 (2H, 2s, H-2, H-8), 5.46 (2H, t, J = 4.5), 5.3–5.1 (6H, m, H-2′, H-4′, $CH_2CH_2CH_2O$), 2.68 (2H, m, $CH_2CH_2CH_2O$), 2.33 (2H, m, CH_2CH2CH_2O), 2.1 (8H, m, $CH_3(CH_2)_4$)), 1.79 (3H, t, J = 6.5, CH_3). IR (KBr): 3416, 2928, 1690, 1065. MS (FAB): m/z (rel intensity) 386 (M + H, 100). Anal. ($C_{16}H_{28}N_5O_4P\cdot H_2O\cdot Na_1\cdot0.6NaCl$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-2,2,2-trichloroethyl Ester (7e). Mp: 218–225 °C. ^{1}H NMR (DMSO-d_6): 8.51 (2H, s, NH2), 8.30, 8.24 (2H, 2s, H-8, H-2), 4.36 (2H, t, J = 5, H-1′), 4.33 (2H, d, J = 6, Cl_3CCH_2), 3.72 (2H, d, J = 8, C-4′), 3.91 (2H, t, J = 5, H-2′). ^{13}C NMR (DMSO-d_6): 153.03 (C-6), 148.91 (C-2), 148.22 (C-4), 142.78 (C-8), 118.27 (C-5), 97.05 (CCl_3), 75.67 (CCl_3CH_2, d, J = 5), 69.99 (C-2′, d, J = 10), 66.17 (C-4′, d, J = 159), 43.12 (C-1′). IR (KBr): 3424, 1930, 1690, 1614, 1514, 1414. MS (methane/DCI): m/z (rel intensity) 404 (M + H, 1), 136 (40), 113 (100). Anal. ($C_{10}H_{13}N_5O_4PCl_3\cdot0.3\ Cl_3CCH_2OH$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Monophenyl Ester, Sodium Salt (7f). Mp: 223–228 °C. ^{1}H NMR (DMSO-d_6): 8.14 (1H, s, H-8), 8.13 (1H, s, H-2), 7.50 (2H, s, NH_2), 7.25 (2H, t, J = 8, ArH), 7.07 (1H, t, J = 8, ArH), 7.01 (2H, d, J = 8, ArH), 4.33 (2H, t, J = 5, H-1′), 3.89 (2H, t, J = 5, H-2′), 3.73 (2H, d, J = 8, H-4′). ^{13}C NMR (D_2O, partial spectrum): 131.46, 126.06 (ArC), 122.27 (ArC, d, J = 3.5), 72.27 (C-2, d, J = 12), 67.68 (C-4′, d, J = 160), 46.08 (C-1′). IR (KBr): 3389, 3068, 1693, 1594. MS (FAB): m/z (rel intensity) 350 (M + H, 40). Anal. ($C_{14}H_{16}N_5O_4P\cdot H_2O\cdot0.45$ Na) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-*p*-nitrobenzyl Ester, Sodium Salt (7g). Mp: 230–240 °C. ^{1}H NMR (DMSO-d_6): 8.19 (2H, d, J = 8.6, ArH), 8.12 (1H, s, H-8), 8.11 (1H, s, H-2), 7.54 (2H, d, J = 8.6, ArH), 4.93 (2H, d, J = 7.7, $ArCH_2O$), 4.63 (2H, t, J = 5, H-1′), 4.31 (2H, t, J = 5, H-2′), 3.72 (2H, d, J = 8.6, H-4′). IR (KBr): 3742, 1930, 1692, 1606, 1518. MS (FAB): m/z (rel intensity) 409 (M + H, 27). Anal. ($C_{15}H_{17}N_6O_6P\cdot0.75\ H_2O\cdot0.5$ Na) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-3′-hydroxypropanyl Ester, Sodium Salt (8a). ^{1}H NMR (DMSO-d_6): 8.17 (1H, s, H-8), 8.11 (1H, s, H-2), 7.20 (2H, s, NH_2), 5.11 (1H, t, OH), 4.28 (2H, t, J = 4.7, H-1′), 3.76 (2H, t, J = 4.7, H-2′), 3.64 (2H, q, J = 6.6, CH_2CH_2OP), 3.41 (2H, d, J = 8.0, H-4′), 3.35 (2H, t, J = 6.2, $HOCH_2$), 1.45 (2H, m, $HOCH_2CH_2$). ^{13}C NMR (DMSO-d_6, 50 MHz): 155.82 (C-6), 152.25 (C-2), 149.43 (C-4), 141.38 (C-8), 118.43 (C-5), 69.77 (C-2′, d, J = 10), 67.42 (C-4′, d, J = 152), 59.33 (CH_2CH_2OP, d, J = 6), 56.88 ($HOCH_2$), 42.60 (C-1′), 33.91 ($HOCH_2CH_2$, d, J = 4). IR (KBr): 3412, 2956, 1647, 1604, 1482, 1421. MS (FAB): m/z (rel intensity) 354 (M + H, 17). Anal. ($C_{11}H_{17}N_5O_5P_1Na_1\cdot2.5\ H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-2′,2′-dimethyl-3′-hydroxypropyl Ester (8b). ^{1}H NMR (DMSO-d_6): 8.14 (1H, s, H-8), 8.09 (1H, s, H-2), 7.16 (2H, s, NH_2), 5.84 (1H, t, OH), 4.27 (2H, t, J = 4.9, H-1′), 3.77 (2H, t, J = 4.9, H-2′), 3.33 (2H, d, J = 8.7, H-4′), 3.24 (2H, d, J = 10, $C(CH_3)_2CH_2OP$), 3.00 (2H, d, $HOCH_2$), 0.63 (6H, s, CH_3). ^{13}C NMR (DMSO-d_6, 50 MHz): 155.84 (C-6), 152.21 (C-2), 149.45 (C-4), 141.26 (C-8), 118.48 (C-5), 69.71 (C-2′, d, J = 9.2), 68.27 ($C(CH_3)_2CH_2OP$, d, J = 6.2), 67.48 (C-4′, d, J = 152), 65.93 ($HOCH_2$), 42.57 (C-1′), 36.71 ($C(CH_3)_2$, d, J = 2.5), 21.35 (CH_3). IR (KBr): 3426, 2960, 2883, 1645, 1478, 1417. MS (FAB): m/z (rel intensity) 360 (M + H, 100). Anal. ($C_{13}H_{22}N_5O_5P\cdot1.3\ H_2O$) C,H,N.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono(3′-amino-2′,2′-dimethylpropyl)amide, Sodium Salt (9). ^{1}H NMR (D_2O): 8.13 (1H, s, H-8), 8.11 (1H, s, H-2), 4.36 (2H, t, J = 5, H-1′), 3.90 (2H, t, J = 5, H-2′), 3.53 (2H, d, J = 8.5, H-4′), 2.71 (2H, s, NH_2CH_2), 2.07 (2H, d, J = 9.4, CH_2NH), 0.70 (6H, s, CH_3). ^{13}C NMR (D_2O): 157.25 (C-6), 154.19 (C-2), 150.78 (C-4), 144.73 (C-8), 120.03 (C-5), 72.24 (C-2′, d, J = 12.5), 69.63 (C-4′, d, J = 143), 50.05 (CH_2NH), 48.41 (H_2NCH_2), 45.53 (C-1′), 35.36 ($C(CH_3)_2$, d, J = 4), 24.09 (CH_3). IR (KBr): 3786, 3381, 1648, 1605, 1478. MS (FAB): m/z (rel intensity) 380 (M + H, 20). HRMS (M + H, $C_{13}H_{23}N_7O_3P_1Na_1$): calcd, 380.1576; found, 380.1567.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono-*N,N*-diethylacetamide Ester (19). Mp: 189–191 °C. ^{1}H NMR (DMSO-d_6) 8.16 (1H, s, H-8), 8.14 (1H, s, H-2), 7.55 (2H, s, NH_2), 4.80 (2H, d, J = 9.0, C(O)CH_2O), 4.31 (2H, t, J = 5.0, H-1′), 4.03 (2H, t, J = 5.0, H-2′), 3.74 (2H, d, J = 8.5, H-4′), 3.22 (2H, q, J = 7, CH_3CH_2), 3.16 (2H, q, J = 7, CH_3CH_2), 1.01 (3H, t, J = 7, CH_3), 1.01 (3H, t, J = 7, CH_3). ^{13}C NMR (CF_3CO_2D, 90 MHz): 166.10 (C=0), 150.04, 148.67 (C-6, C-4), 144.74, 144.55 (C-2, C-8), 117.96 (C-5), 70.05 (C-2′, d, J = 10), 65.37 (C-4′, d, J = 162), 62.87 (C(O)CH_2, d, J = 5), 43.44 (C-1′), 14.06 (CH_3), 12.91 (CH_3). IR (KBr): 3392, 3093, 1692, 1650, 1515. MS (methane/DCI): m/z (rel intensity) 500 (M + H, 30), 132 (100). HRMS (M + H, $C_{14}H_{23}N_6O_5P$): calcd, 387.1546; found, 387.1543.

9-[2-(Phosphonomethoxy)ethyl]adenine, Mono(2′,2′-difluoro-3′-hydroxypropyl ester) (26). ^{1}H NMR (DMSO-d_6) 8.20 (2H, s, H-8, H-2), 7.80 (2H, s, NH2), 4.34 (2H, t, J = 5.0, H-1′), 4.04 (2H, dt, J = 13.2, 7.9, CF_2CH_2OP), 3.87 (2H, t, J = 5.0, H-2′), 3.70 (2H, d, J = 8.0, H-4′), 3.60 (2H, t, J = 13, $HOCH_2$). ^{13}C NMR (D_2O/NaOD) 157.34 (C-6), 154.24 (C-2), 150.67 (C-4), 144.72 (C-8), 123.54 (CF_2, t, J = 30), 120.12 (C-5), 72.40 (C-2′, d, J = 12), 67.75 (C-4′, d, J = 159), 64.94 (CF_2CH_2OP, dt, J = 30, 5), 63.28 ($HOCH_2$, d, J = 27), 45.49 (C-1′). IR (KBr): 3310, 3112, 1694, 1602, 1514. MS (FAB): m/z (rel intensity) 368 (M + H, 55). HRMS (M + H, $C_{11}H_{16}N_5O_5F_2P$): calcd 368.0935; found, 368.0930.

Estimates of Oral Bioavailability in the Rat. All animals were fasted 12–18 h before dosing. For each route of administration of PMEA or the prodrug, three rats were used. For iv administration, PMEA (15 mg/mL in normal saline) was given as a bolus injection into the tail vein to provide a dose of 30 mg/kg. For oral administration, PMEA (3 mg/mL in 5% DMSO in water) was administered by gastric intubation to provide a dose of 30 mg/kg, whereas the prodrugs were administered as solutions in 5% DMSO in water to provide between 24 and 43 mg/kg equiv of PMEA. After administration of drug, the animals were placed into individual metabolism cages and a 0–24- and 24–48-h urine sample was collected from each animal. Concentration of PMEA in each urine sample was determined by a previously described reversed-phase HPLC method which employs fluorometric detection.[17] The absolute bioavailability of PMEA after administration of PMEA or prodrug was calculated from the equation below:

$$\text{percent bioavailability} = \frac{[B]_{0\rightarrow 48h}/\text{dose}}{[A]_{0\rightarrow 48h}/\text{dose}}$$

where A is the amount of PMEA excreted in the urine after iv administration of PMEA and B is the amount of PMEA excreted after oral administration of PMEA or the prodrug.

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