Acyclic Purine Phosphonate Analogues as Antiviral Agents. Synthesis and Structure–Activity Relationships[1]

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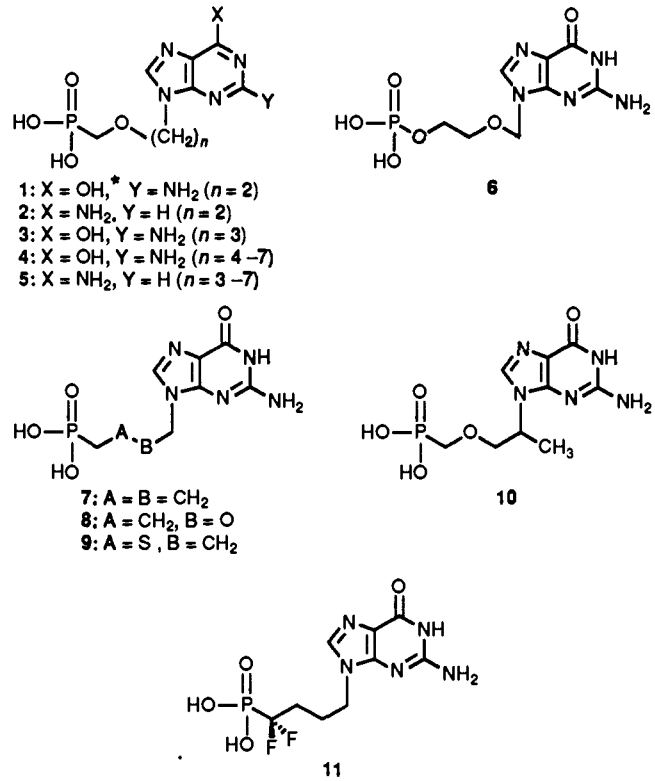
A series of 9-(phosphonoalkyl)purines, which are analogues of 9-[2-(phosphonomethoxy)ethyl]purines (guanine, PMEG, 1; adenine, PMEA, 2), were synthesized. The analogues were tested for activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), human cytomegalovirus (HCMV), Rauscher murine leukemia virus (R-MuLV), and human immunodeficiency virus type 1 (HIV-1). With variations in the length of the alkyl chain, the optimal activity was achieved with two carbons between the purine base and the phosphonomethoxy functionality. Despite the structural similarity and the close pK_{a_2} value of 8 to that of PMEG, no phosphorylation of 8 was observed by the bovine brain guanylate kinase. Since all isosteric analogues of PMEG (7–9) were not inhibitory against HSV-1 and HSV-2, the presence of the 3′-oxygen atom in the PME purines proved critical for anti-HSV activity. Introduction of the 1′-methyl group on the PMEG side chain significantly reduced its anti-HSV activity. Analogue 11, which is a mimic of the phosphate by incorporation of the α,α-difluoro carbon, was ineffective against HSV-1 and HSV-2. These results suggest that the structural requirements of PME purines for anti-HSV activity appear to be very strict.

A new class of (phosphonomethoxy)alkyl purine and pyrimidine derivatives has emerged as potent antiviral agents. Two series of these potent and broad spectrum antiviral phosphonate nucleotide analogues are 9-[2-(phosphonomethoxy)ethyl]purines (guanine, PMEG, 1; adenine, PMEA, 2) and (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]purines/pyrimidines (adenine, HPMPA; guanine, HPMPG; cytosine, HPMPC).[2] These analogues effectively inhibit a wide array of DNA viruses (Herpes, Adeno, Irido, and Poxviruses) and retroviruses (MSV, MuLV, and HIV). Since the activity of these phosphonate analogues is not dependent on an initial phosphorylation step by viral thymidine kinases (TKs), they are also active against TK-deficient mutants of HSV-1 which are resistant to acyclovir (ACV).[2a] The mechanism of action of these phosphonates is under investigation. However, it is postulated that (*S*)-HPMPA[3] and (*R,S*)-HPMPG[2f] as such are taken up by cells, and phosphorylated intracellularly to the diphosphoryl derivatives, which are selective viral DNA polymerase inhibitors.

An important initial objective of the present study was to examine the phosphonate side chain to establish optimal antiviral activity of the PME purine analogues and to better define the structural requirements for the rational design of potent antiviral agents. Our target structures in this series are summarized in Chart I. The longer alkyl chain phosphonate derivatives (3–5) were synthesized to examine the importance of the distance between the phosphorus atom and the purine base for antiviral activity. Furthermore, in order to study the structure–activity relationship of PME purine analogues, isosteric analogues of PMEG were prepared. Those include the 3′-carba (7), 2′-oxa (8), and 3′-thia (9) analogues. To investigate the effect of substitution on the phosphonate side chain, 1′-methyl-PMEG (10) was also prepared. Finally, α,α-difluorophosphonate analogue (11) was synthesized as a potential mimic of a phosphate. These compounds were evaluated for their inhibitory effect against herpesviruses (HSV-1, HSV-2, HCMV) and retroviruses (M-MuLV, HIV-1). Since these phosphonate analogues probably require further phosphorylation for their antiviral activity,

Chart I



*This designation is intended only to indicate substitution and not favored tautomeric form.

(1) Presented in part at the 8th International Round Table Meeting on Nucleosides, Nucleotides, and their Biological Applications, Orange Beach, AL, October 1988, Abstract No. 41.

(2) (a) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. *Nature* **1986**, *323*, 464. (b) De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holy, A. *Antiviral Res.* **1987**, *8*, 261. (c) Baba, M.; Mori, S.; Shigeta, S.; De Clercq, E. *Antimicrob. Agents Chemother.* **1987**, *31*, 337. (d) Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holy, A.; De Clercq, E. *Antimicrob. Agents Chemother.* **1988**, *32*, 1025. (e) Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839. (f) Terry, B. J.; Mazina, K. E.; Tuomari, A. V.; Haffey, M. L.; Hagen, M.; Feldman, A.; Slusarchyk, W. A.; Young, M. G.; Zahler, R.; Field, A. K. *Antiviral Res.* **1988**, *10*, 235. (g) De Clercq, E.; Holy, A.; Rosenberg, I. *Antimicrob. Agents Chemother.* **1989**, *33*, 185. (h) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332. (i) Bronson, J. J.; Ghazzouli, I.; Hitchcock, M. J. M.; Webb, R. R.; Martin, J. C. *J. Med. Chem.* **1989**, *32*, 1457.

(3) Votruba, I.; Bernaerts, R.; Sakuma, T.; De Clercq, E.; Merta, A.; Rosenberg, I.; Holy, A. *Mol. Pharmacol.* **1987**, *32*, 524.

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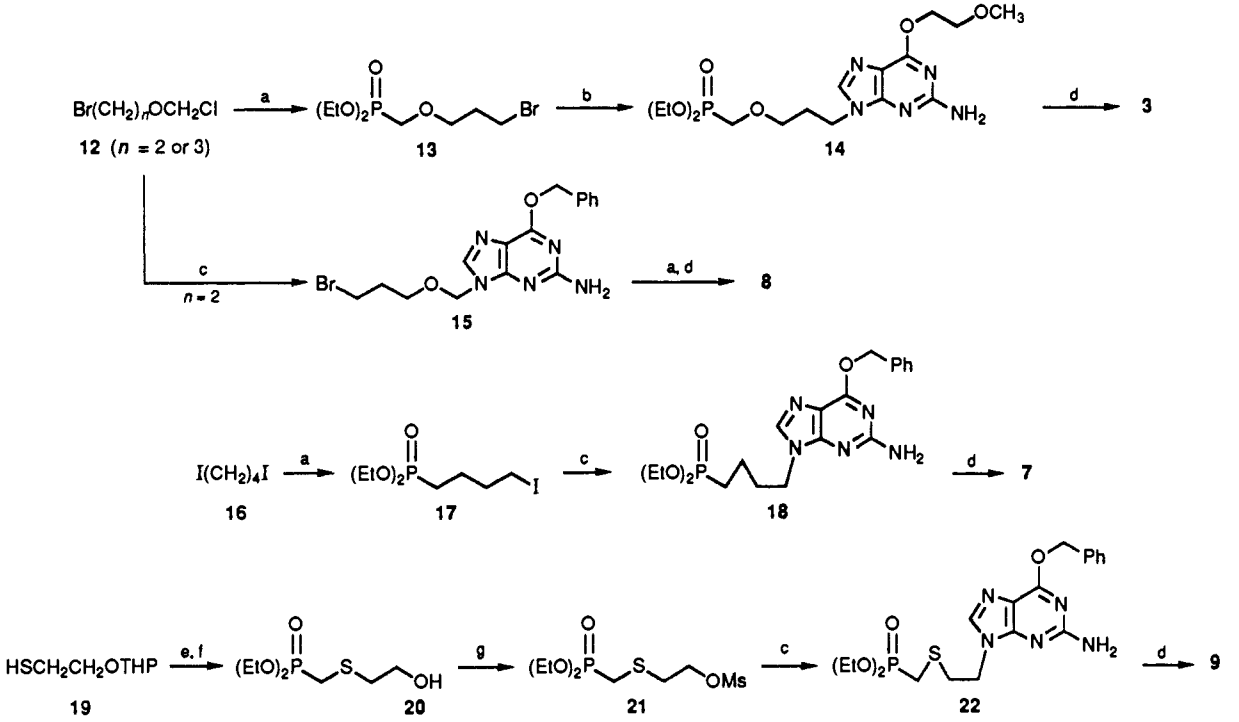
Scheme I[a]



[a] (a) $(EtO)_2P(O)Na$; (b) 2-amino-6-(methoxyethoxy)purine/NaH; (c) 2-amino-6-(benzyloxy)purine/NaH; (d) TMSBr/DMF; (e) $(EtO)_2P(O)CH_2OTf$/TEA; (f) 5% HCl; (g) CH_3SO_2Cl/TEA.

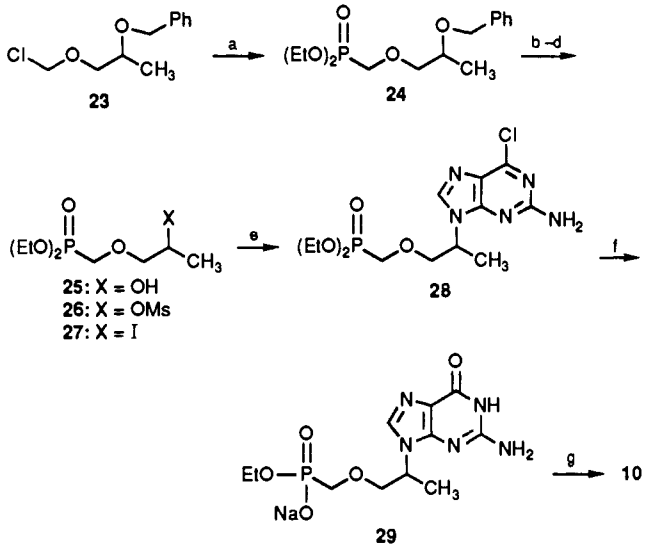
some of these derivatives were studied in an assay to measure their enzymatic phosphorylation by bovine brain guanylate kinase.

Chemistry

The syntheses of (phosphonomethoxy)alkyl purine analogues (**3–5**) and PMEG isosteric isomers (**7–9**) are outlined in Scheme I. The preparation of 9-[3-(phosphonomethoxy)propyl]guanine (**3**) is described as a typical example of the straight-chain alkyl derivatives. The general strategy for the synthesis of **3** involves the selective displacement of the chlorine atom of the chloromethyl ether functionality by the phosphite anion over the alkyl bromide. Thus, chloromethyl ether **12** (n = 3) was condensed with one equiv of sodium diethyl phosphite to give **13**. Coupling of **13** with 2-amino-6-(methoxyethoxy)purine sodium salt readily afforded a mixture of **14** and its N-7 isomer in a ratio of approximately 2:1 in 65% combined yield. Although a higher N-9/N-7 ratio was reported in a recent paper[4] for the similar alkylation of 2-amino-6-(methoxyethoxy)purine, we are unable to improve the ratio of **14** and its N-7 isomer by following the published procedure. The UV spectra and ^{13}C NMR were very useful to assert the site of alkylation. As described in the literature,[5] the N-7 isomer of **3** exhibited a bathochromic shift of the long-wavelength UV absorption maximum relative to **3**. In the ^{13}C NMR, the upfield shift of the C-5 signal of **3** at δ 117.665 relative to that of the N-7 isomer at δ 108.212 was also consistent with the assigned structure.[6]

For the synthesis 9-[(phosphonoethoxy)methyl]guanine (**8**), chloromethyl ether **12** (n = 2) was also used as a starting material. In this case, the purine base was first coupled with **12** (n = 2) and the resulting bromide **15** was then converted to **8** with sodium diethyl phosphite followed by bromotrimethylsilane. Similarly, 4-iodobutyl diethyl phosphonate **17**, prepared by condensation of sodium diethyl phosphite with 1,4-diiodobutane was successfully coupled with 2-amino-6-(benzyloxy)purine sodium salt to give **18**, from which 9-(4-phosphonobutyl)guanine (**7**) was obtained in good yield. To prepare 9-[2-[(phosphonomethyl)thio]ethyl]guanine (**9**), a different approach was adapted. Recently (diethylphosphono)methyl triflate[7] has been demonstrated to readily react with a variety of heteroatom nucleophiles. We found that (diethylphosphono)methyl triflate condenses smoothly with the

Scheme II[a]



[a] (a) $(EtO)_3P$; (b) Pd–C/H_2; (c) CH_3SO_2Cl/TEA; (d) NaI; (e) 2-amino-6-chloropurine/NaH; (f) NaOH; (g) TMSBr/DMF.

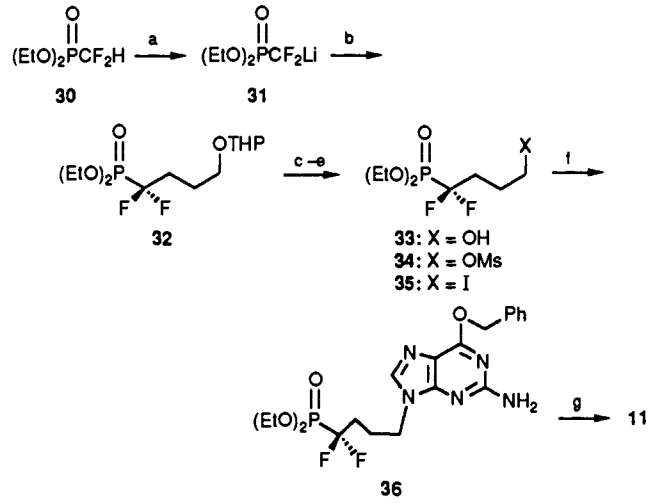
(4) (a) Kjellberg, J.; Liljenberg, M.; Johansson, N. G. *Tetrahedron Lett.* **1986**, *27*, 877. (b) Kjellberg, J.; Johansson, N. G.; *Nucleosides Nucleotides* **1989**, *8*, 225.

(5) (a) Zemlicka, J. *Nucleosides Nucleotides* **1984**, *3*, 245. (b) Montgomery, J. A.; Temple C., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 630.

(6) Kjellberg, J.; Johansson, N. G. *Tetrahedron* **1986**, *42*, 6544.

(7) Phillion, D. P.; Andrew, S. S. *Tetrahedron Lett.* **1986**, *27*, 1477.

Scheme III[a]



[a] (a) LDA/HMPA; (b) $I(CH_2)_3OTHP$; (c) 3 N HCl; (d) CH_3SO_2Cl/TEA; (e) NaI; (f) 2-amino-6-(benzyloxy)purine/NaH; (g) TMSBr/DMF.

thiol **19** in the presence of triethylamine at 0 °C. Intermediate **20** was then converted into **9** in a similar manner to that described above and shown in Scheme I.

Scheme II illustrates the synthetic sequence used for 9-[1-methyl-2-(phosphonomethoxy)ethyl]guanine (**10**). Phosphonate intermediate **24** was prepared from the known chloromethyl ether **23**[8] in the same manner as that of compound **13**. Conversion of mesylate **26** to the more reactive iodide **27** was necessary for the successful coupling with 2-amino-6-chloropurine sodium salt. Secondary mesylate **26** reacted with the purine base very poorly even at the elevated temperature. Purine derivative **28** was deprotected by saponification and subsequent treatment with bromotrimethylsilane.

To examine the effect of the acidity of the phosphonate functionality on the antiviral activity, α,α-difluorophosphonate derivative **11** was prepared. In the light of work by Blackburn[9] and others[10] on the α-fluorophosphonate chemistry, we considered that the most expedient route to **11** might be the alkylation of readily accessible diethyl (difluoromethyl)phosphonate (**30**).[10,11] When anion **31** was reacted at −25 °C with 3-(tetrahydropyranyloxy)propyl iodide in THF in the presence of hexamethylphosphoramide (HMPA), the desired product **32** was obtained in 23% yield. The poor yield observed in this reaction was probably due to the poor nucleophilicity of the highly stabilized anion **31** and the poor stability of **31** above −25 °C. Without HMPA, no alkylation was observed. In the coupling of iodide **35** with 2-amino-6-(benzyloxy)purine sodium salt, considerable dealkylation of the phosphonate ester was observed. It appeared that the strongly electron-withdrawing difluoro group considerably activated the phosphonate ester toward nucleophilic attack.

Biological Results and Discussion

The (phosphonoalkyl)purine analogues (**3–5**, **7–11**) prepared in this study were tested against herpesviruses (HSV-1, HSV-2, HCMV) and retroviruses (M-MuLV, HIV-1), and their potency is expressed as the 50% inhibitory concentration (Table I and II). Acyclovir (ACV) is representative of the acyclic nucleoside analogues, which exhibit more selective activity against the herpesviruses.[12] Acyclovir is initially phosphorylated at 4′-hydroxyl by viral thymidine kinase[13] to monophosphate **6**, which is subsequently converted to a triphosphate by host cell kinases.[14] This triphosphate selectively inhibits viral-specified DNA polymerase and thus virus replication. Despite the apparent structural resemblance of 9-(3-phosphonopropyl)-guanine (**3**) to ACV monophosphate (**6**), compound **3** showed no significant activity against HSV-1 and HSV-2, but it exhibited a substantial activity against HCMV and retroviruses. This result indicates that a subtle change in the phosphonate structure drastically effects the antiviral activity profile of these phosphonate derivatives. In view of the strict structural requirement of phosphonate derivatives for their antiviral activity (vida infra), it was not surprising that other derivatives of the straight-chain phosphonates (**4**, **5**) did not exhibit any significant activity against HSV-1 and HSV-2.[15] Some of these derivatives possess the same number of spacer atoms that link the purine base and the phosphorus atom as the di- or triphosphate of ACV. However, their lack of anti-HSV activity suggests that they are incapable of mimicking the di- or triphosphate of ACV. As for the phosphonate chain length of PME purine analogues, two carbons between the purine base and the phosphonomethoxy functionality appears to be optimal for antiviral activity.

Table I. Antiherpes and Anticellular Activities of the Acyclic Phosphonates in Tissue Culture

	ID_{50},[a] μg/mL					
virus or cell[b]	1	2	3	10	4, 5, 7, 8, 9, 11	ACV
HSV-1 (BWS)	0.08	21.0	65.5	2.92	>100	0.5
HSV-2 (G)	0.69	9.4	>100	3.29	>100	2.4
HCMV (AD-169)	0.04	0.93	6.0	0.4	NT[c]	38.4
vero cells	5	>100	76	60	100	100

[a] Determined by plaque reduction assays in vero (HSV) or MRC-5 cells (HCMV) or cell proliferation assays in uninfected cells. [b] The strain is given in parentheses. [c] NT, not tested.

Table II. Antiretroviral and Anticellular Activities of the Acyclic Phosphonates in Tissue Culture

	ID_{50}, μg/mL				
virus or cell	1	2	3	10	AZT
R-MuLV[a]	0.001	0.05	0.04	NT[d]	0.001
HIV-1[a]	>10	3.0	7.8	8.4	0.05
CEM cells[c]	10	>100	>100	>100	>50

[a] Rauscher-murine leukemia virus. [b] Human immunodeficiency virus type 1. [c] Determined by the dye-exclusion method. [d] NT, not tested.

(8) Karkas, J. D.; Ashton, W. T.; Canning, L. F.; Liou, R.; Germerhausen, J.; Bostedor, R.; Arison, B.; Field, A. K.; Tolman, R. L. *J. Med. Chem.* **1986**, *29*, 842.

(9) Blackburn, G. M.; Parratt, M. J. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1417.

(10) Obayashi, M.; Ito, E.; Matsui, K.; Kondo, K. *Tetrahedron Lett.* **1982**, *23*, 2323.

(11) Bergstrom, D. E.; Shum, P. W. *J. Org. Chem.* **1988**, *53*, 3953.

(12) (a) Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature (London)* **1978**, *272*, 583. (b) Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, No. 12, 5716. (c) Collins, P.; Bauer, D. J. *J. Antimicrob. Agents Chemother.* **1979**, *5*, 431.

(13) Fyfe, J. A.; Keller, P. M.; Furman, P. A.; Miller, R. L.; Elion, G. B. *J. Biol. Chem.* **1978**, *253*, 8721.

(14) (a) Furman, P. A.; St. Clair, M. H.; Spector, T. *J. Biol. Chem.* **1984**, *259*, 9575. (b) Derse, D.; Cheng, Y.-C.; Furman, P. A.; St. Clair, M. H.; Elion, G. B. *J. Biol. Chem.* **1981**, *256*, 11447.

(15) A similar result has been observed for other acyclic phosphonate nucleotide analogues: Holy, A.; Rosenberg, I.; Dvorakova, H.; De Clercq, E. *Nucleosides Nucleotides* **1988**, *7* (5 and 6), 667.

Table III. Kinetic Parameters with Bovine Brain Guanylate Kinase[a]

compd	K_m, mM	rel V_{max}	HSV-1 ID_{50}, μg/mL
GMP	3.3	100	
dGMP	1.2	14	
1	2.7	0.11	0.1
10	3.9	0.073	3
3	4.7	0.0024	65.5
4		NR[b]	>145
8		NR	>145

[a] Compounds were evaluated as potential substrates for bovine brain guanylate kinase. Briefly, the utilization of ATP was coupled to production of pyruvate from phosphoenol pyruvate and the reduction of pyruvate to lactate by lactate dehydrogenase was coupled to oxidation of NADH. The latter was measured by the change in absorbance at 340 nm with time. Rates were measured for several different concentrations of GMP or its analogues, and K_m and V_{max} were derived from a Lineweaver–Burk plot. [b] NR, no reaction at 16 mM.

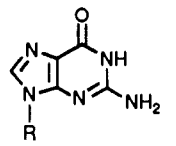
None of the PMEG isosteric isomers (7–9) exhibited any significant inhibitory activity against herpes simplex viruses. Since PMEG is such a potent antiviral agent and the molecular structures of 7–9 are so similar to that of PMEG, their total lack of activity against herpes simplex virus is quite striking. It was reported that cellular kinases converted HPMPA and HPMPG to the diphosphates, which are selective inhibitors of the viral specified DNA polymerase.[2f,3] To further investigate the mechanism of antiherpes activity of phosphonates, phosphorylation kinetics of some phosphonate analogues with bovine braine guanylate kinase were determined (Table III). Compounds inactive against herpes simplex virus (4, 8) were not phosphorylated by this enzyme. Among the compounds (1, 3, and 10) which could be phosphorylated, their K_m's were similar to those of GMP and dGMP, suggesting they all bound to the enzyme equally well. However, the rates of phosphorylation (rel V_{max}) for these compounds were different. The rank order of phosphorylation rate and HSV-1 activity are the same for the compounds tested. However, the rate of phosphorylation does not correlate directly with the observed HSV-1 activity. Therefore, in addition to the phosphorylation rate, other factors, e.g., effectiveness of herpes DNA polymerase inhibition by corresponding diphosphate derivatives of these phosphonates, are probably involved in HSV-1 activity.

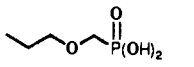
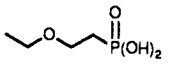
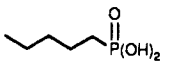
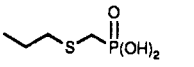
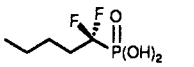
Although PMEG exhibits excellent antiviral activity, it is also very cytotoxic in vitro (vero cell, ID_{50} = 5 μg/mL). Introduction of the 1′-methyl group on the PMEG structure (compound 10) reduced the cell toxicity more than 10 times (ID_{50} of 10 = 60 μg/mL), but the HSV activity was also reduced significantly. Thus, overall therapeutic index of 10 did not exceed that of PMEG. As for retroviral activity, compounds 1–3 exhibited excellent activity against R-MuLV almost equal to that of AZT. Against HIV-1, these compounds also showed good activity, except PMEG, which was again quite cytotoxic (Table II). However, the anti-HIV activity of these phosphonates is much weaker than the anti-R-MuLV activity. Possibly this is a result of the very different types of antiviral assays used for these two viruses. Alternatively, there may be differences in the kinases in human and mouse cells responsible for activation of the phosphonates to their putative active diphosphates.

The characteristic phosphonomethyl ether functionality present in PME purines is expected to be chemically and enzymatically stable.[16] In addition to this, it is expected

(16) Holy, A. *Nucleosides Nucleotides* **1987**, *6*, 147.

Table IV. Second Dissociation Constants for Some Phosphonate Analogues



no.	R	pK_{a_2}[a]
1		6.52
8		6.76
7		7.08
9		6.59
11		5.52
thymidine monophosphate		6.50

[a] The analyses were performed on a Brinkman Potentiograph Model E 336A.

that the β-oxygen atom in the phosphonomethyl ether functionality enhances the acidity of the phosphonate due to its electron negativity. As expected, the pK_{a_2} of PMEG was very close to that of thymidine monophosphate (Table IV). The isosteric analogues (8, 9) had also very similar pK_{a_2} values to that of PMEG. Despite the close pK_{a_2} value of 8 compared to that of PMEG, compound 8 does not appear to be phosphorylated by bovine brain guanylate kinase (Table III). These results indicate that the position of the oxygen atom in the phosphonates plays a critical role for the compounds to be substrates for phosphorylation by kinases. The CF_2 group has been proposed as a reasonable isosteric and isoelectronic replacement for oxygen atom in many biologically active compounds.[17] Therefore α,α-difluoro phosphonate 11 was prepared as a mimic of a phosphate functionality. However, the pK_{a_2} of 11 was significantly lower than that of PMEG and compound 11 was inactive against HSV-1 and HSV-2.

In conclusion, as demonstrated in the present study, it is now apparent that isosteric and isoelectronic similarity to PMEG are insufficient for the phosphonate analogues of PMEG to be good anti-HSV compounds. It is possible that lack of HSV activity of those phosphonates (4–7, 8–11) is due to the inability of cellular or virally induced kinases to catalyze their conversion to the mono- and diphosphate forms. Alternatively, the respective diphosphates, if formed, may not be inhibitors of viral DNA synthesis.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All ^1H, ^{19}F, and ^{13}C NMR spectra were run on a Varian Gemini 300-MHz spectrometer. The internal reference for ^1H and ^{13}C spectra was TMS. For ^{19}F NMR, CF_3CO_2H was used as the internal reference. UV measurements were done on a Perkin-Elmer 552 spectrophotometer. Analytical results for compounds indicated by their molecular formula were within ±0.4% of the calculated values.

Testing and Evaluating of Compounds against Herpesvirus. The titer of HSV strains was determined by a plaque titration method (Roizman; Roane *Virology* **1961**, *15*, 75–79).

(17) (a) Blackburn, G. M.; Eckstein, F.; Kent, D. E.; Perree, T. M. *Nucleosides Nucleotides* **1985**, *4*, 165. (b) Blackburn, G. M.; Parratt, M. J. *J. Chem. Soc., Chem. Commun.* **1982**, 1270; **1983**, 886.

Tissue-culture 24-well petri dishes were seeded with cells and used for assays when approximately 75% confluent. Volumes (0.1 mL) of logarithmic dilutions of the virus strain were inoculated onto each of triplicate wells and adsorbed for 1 h with intermittent shaking. The inoculum was then removed, and 1 mL of 5–10% EMEM containing 0.3% human immune serum globulin was added. After a 48-h incubation period at 37 °C in a 5% CO_2 atmosphere, the overlay medium was removed, and the cell sheets were stained with Giemsa stain. The number of plaques was counted, the triplicate determinations were averaged, and the number of plaque-forming units/mL was calculated.

The compounds were tested for activity against the herpes simplex virus strains with a stock solution of each compound freshly prepared. Appropriate dilutions of each compound were made in 10% EMEM before use. The antiviral efficacy of each compound is determined by using the plaque reduction assay described above. Briefly, tissue-culture 24-well plates were inoculated with approximately 50 plaque-forming units of HSV/0.1 mL, and the virus was adsorbed for 1 h, with intermittent shaking. After removal of the inoculum, 1 mL of 10% EMEM containing 2-fold dilutions of the test drug were added in triplicate. Three wells in each plate received no drug and were used as a virus control. After incubation for 48 h, at 37 °C in a 5% CO_2 atmosphere, the overlay medium was removed, the cells were stained as described above, and plaques were counted. The number of plaques in the presence of each drug dilution was calculated as the average from the triplicate wells.

The antiviral potency of the drug (ID_{50}) was calculated as the drug concentration necessary to reduce the number of plaques by 50% of those in the virus control cultures.

Testing and Evaluating of Compounds against Human Cytomegalovirus. Human cytomegalovirus (HCMV) (strain AD169) was grown and titered at 37 °C in human embryonic lung (diploid) cells, MRC-5.

The activity of compounds against HCMV was determined by using the procedure for the plaque reduction assay described above.

Testing and Evaluating of Compounds against Retroviruses. The compounds were evaluated for antiviral activity against murine leukemia virus (MuLV) strains by using the UV-XC plaque assay (Rowe; et al. *Virology* **1970**, *42*, 1136).

The HIV in vitro assay was done as follows. The anti-HIV/LAV activity was measured in cultures of CEM-F cells. The CEM cells were infected with approximately 30 $TCID_{50}$ (50% tissue culture infectious dose) of HIV (LAV strain). The cells were then incubated for 45 min at 37 °C. The test compounds in culture medium were added at various concentrations to the infected cells and then incubated for a further 8 days. The antiviral activity was then evaluated in the culture media supernatant for p 24 gag protein by an enzyme capture assay (ELISA). The antiviral activity was expressed as the dose that inhibits 50% of the virus expression (ID_{50} in $\mu g/mL$) as detected by the assay described.

1-Bromo-3-(chloromethoxy)propane (12, *n* = 3). A solution of bromopropanol (42 g, 0.3 mol) and 1,3,5-trioxane (10 g, 0.11 mol) in 1,2-dichloroethane (40 mL) was saturated with HCl gas at 0 °C. After stirring for 3 h at 0 °C, magnesium sulfate was added to the solution. The mixture was filtered and the filtrate was evaporated in vacuo to give **12** as a colorless oil. This material was used without further purification: ^1H NMR ($CDCl_3$) δ 2.156 (dddd, J = 6.0 Hz, 2 H), 3.492 (t, J = 6.0 Hz, 2 H), 3.799 (t, J = 6.0 Hz, 2 H), 5.50 (s, 2 H).

1-Bromo-3-[(diethylphosphono)methoxy]propane (13). To a suspension of 57% sodium hydride in mineral oil (620 mg, 15.4 mmol) in pentane (20 mL) was added at 0 °C a solution of diethyl phosphite (2.1 g, 14.8 mmol) in pentane (3 mL) and the mixture was stirred for 30 min under nitrogen. To this solution was added at −78 °C a solution of **12** (2.6 g, 15 mmol) in pentane (5 mL). The mixture was then allowed to stir for 2 h without a cooling bath and any insoluble material was removed by filtration. The filtrate was concentrated to dryness, and the residual oil was purified by silica gel column chromatography using methylene chloride as eluent to give **13** (3.9 g, 90%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.40 (t, J = 6.8 Hz, 6 H), 2.20 (m, 2 H), 3.522 (t, J = 6.6 Hz, 2 H), 3.724 (t, J = 5.4 Hz, 2 H), 4.199 (m, 2 H).

9-[3-(Phosphonomethoxy)propyl]adenine (5, *n* = 3). To a suspension of 57% sodium hydride in mineral oil (962 mg, 22.8 mmol) in dry DMF (150 mL) was added adenine (3.4 g, 24.9 mmol) in one portion under nitrogen and the mixture was heated at 80 °C for 1 h. A solution of **13** (6.0 g, 20.7 mmol) in DMF (5 mL) was added at 25 °C and the reaction was heated at 60 °C for 15 h. The mixture was then concentrated in vacuo, taken up in methylene chloride–water, and adjusted to pH 7 with concentrated HCl. The methylene chloride was washed with saturated NaCl, dried over $MgSO_4$, and evaporated to a residual oil, which was purified by silica gel column chromatography using methylene chloride–5% methanol as eluent to give the diethyl ester of **5** (n = 3). This material was dissolved in DMF (30 mL) and treated with bromotrimethylsilane (15 mL). After stirring for 4 h at 25 °C, the solution was evaporated in vacuo and the resulting oil was treated with water (20 mL) to give **5** (n = 3, 3.9 g, 55%) as white crystals: mp 240 °C dec; UV_{max} (H_2O) 260 nm (ϵ 13976); ^1H NMR (DMSO-d_6) δ 2.135 (m, 2 H), 3.519 (t, J = 5.5 Hz, 2 H), 3.649 (d, J = 8.2 Hz, 2 H), 4.233 (t, J = 6.5 Hz, 2 H), 7.604 (s, 1 H), 8.201 (s, 1 H). Anal. ($C_9H_{14}N_5O_4P \cdot {}^1/_4H_2O$) C, H, N.

In a similar manner to that described above, compounds **5** (n = 4–7) were prepared.

2-Amino-6-(methoxyethoxy)-9-[3-[(diethylphosphono)methoxy]propyl]purine (14). To a suspension of lithium hydride (560 mg, 60 mmol) in dry DMF (200 mL) was added 2-amino-6-(methoxyethoxy)purine (8.0 g, 40 mmol) and the mixture was stirred for 60 min at 25 °C. To this solution was added over 3 min a solution of **13** (12.0 g, 41.5 mmol) in DMF (5 mL). After heating at 55 °C for 4 h, the reaction mixture was cooled to room temperature and water (50 mL) was added dropwise. Insoluble material was removed by filtration, and the filtrate was concentrated in vacuo. Silica gel chromatography of the residual oil using CH_2Cl_2–5% MeOH as eluent gave **14** (5.2 g, 31%) as white crystals: mp 78–79 °C; ^{13}C NMR (DMSO-d_6) δ 113.73, 140.99, 152.99, 159.43, 160.43; ^1H NMR (DMSO-d_6) δ 1.34 (t, J = 6.0 Hz, 6 H), 2.12 (m, 2 H), 3.51 (s, 3 H), 3.62 (t, J = 5.6 Hz, 2 H), 3.80 (t, J = 5.0 Hz, 2 H), 3.88 (d, J = 9.0 Hz, 2 H), 4.02 (q, J = 6.5 Hz, 4 H), 4.16 (t, J = 5.0 Hz, 2 H), 4.75 (t, J = 6.6 Hz, 2 H), 5.0 (brs, 2 H), 7,83 (s, 1 H). Anal. ($C_{16}H_{28}N_5O_6P$) C, H, N.

The silica gel column was continuously eluted with CH_2Cl_2–5% MeOH to obtain the N-7 isomer of **14** (2.5 g, 15%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.32 (t, J = 6.0 Hz, 6 H), 2.09 (m, 2 H), 3.37 (s, 3 H), 3.43 (t, J = 5.6 Hz, 2 H), 3.69 (d, J = 9.0 Hz, 2 H), 3.80 (t, J = 5.0 Hz, 2 H), 4.10 (t, J = 5.0 Hz, 2 H), 4.12 (q, J = 6.5 Hz, 4 H), 4.59 (t, J = 6.7 Hz, 2 H), 5.0 (br s, 2 H), 7.81 (5, 1 H).

9-[3-(Phosphonomethoxy)propyl]guanine Disodium Salt (3). A solution of **14** (1.4 g, 3.4 mmol) in 3 N HCl (10 mL) was heated at 85 °C for 4 h. The clear solution was evaporated to dryness, taken up in toluene (20 mL), and evaporated again. The resulting yellow oil was dissolved in DMF (5 mL) and treated with bromotrimethylsilane (5 mL). After being stirred at 25 °C for 4 h, the solution was concentrated in vacuo to a viscous yellow oil, which was then taken up in aqueous $NaHCO_3$ (5 mL). Lyophilization of the solution gave a slightly yellow solid, which was purified by C-18 reverse-phase column chromatography using water as eluent under 8 psi of pressure to give **3** (1.0 g, 85%) as a white, amorphous powder: UV_{max} (H_2O) 253 (ϵ 12778), 269 nm (ϵ 9525); ^{13}C NMR (D_2O) δ 117.665, 142.065, 147.896, 151.550, 154.584; ^1H NMR (D_2O) δ 2.09 (m, 2 H), 3.57 (t, J = 5.6 Hz, 2 H), 3.61 (d, J = 8.0 Hz, 2 H), 4.17 (t, J = 6.6 Hz, 2 H), 7.84 (s, 1 H). Anal. ($C_9H_{12}N_5O_5PNa_2 \cdot H_2O$) C, H, N.

The N-7 isomer of **14** was also treated with bromotrimethylsilane to give the N-7 isomer of **3** as a white foam: UV_{max} (H_2O) 249 (ϵ 6622), 281 nm (ϵ 7129); ^{13}C NMR (D_2O) δ 108.212, 144.513, 153.316, 155.945, 159.021; ^1H NMR (D_2O) δ 2.10 (tt, J = 6.6 Hz, 2 H), 3.514 (t, J = 6.6 Hz, 2 H), 3.536 (d, J = 8.8 Hz, 2 H), 4.321 (t, J = 6.6 Hz, 2 H), 7.923 (s, 1 H). Anal. ($C_9H_{12}N_5O_5P \cdot H_2O$) C, H, N.

2-Amino-6-(benzyloxy)-9-[(2-bromoethoxy)methyl]purine (15). To a suspension of 57% sodium hydride in mineral oil (1.3 g, 32 mmol) in dry DMF (50 mL) was added 2-amino-6-(benzyloxy)purine (6.9 g, 29 mmol) and the mixture was stirred for 1 h at 25 °C. To this solution was added at 0 °C a solution of **12** (n = 2, 5 g, 29 mmol) in DMF (10 mL). After stirring for 16 h at 25 °C, the mixture was filtered and the filtrate was evaporated in vacuo. The residual oil was purified by silica gel column chromatography using methylene chloride–10% methanol as

eluent to give **15** (4.0 g, 36%) as a white foam: ^{13}C NMR ($CDCl_3$) δ 31.93, 67.09, 68.74, 71.74, 113.69, 128.12, 128.47, 136.67, 140.10, 154.71, 160.17; ^1H NMR ($CDCl_3$) δ 3.421 (t, J = 5.9 Hz, 2 H), 3.849 (t, J = 6.0 Hz, 2 H), 4.913 (s, 1 H), 5.518 (s, 2 H), 5.572 (s, 2 H), 7.27–7.54 (m, 5 H), 7.749 (s, 1 H).

2-Amino-6-(benzyloxy)-9-[[2-(diethylphosphono)ethoxy]methyl]purine (8). To a suspension of 57% sodium hydride in mineral oil (170 mg, 4.3 mmol) in THF (20 mL) was added at 0 °C a solution of diethyl phosphite (582 mg, 4.2 mmol) in THF (1 mL) and the mixture was stirred for 30 min under nitrogen. To this solution was added at −78 °C a solution of **15** (1.6 g, 4.2 mmol) in THF (10 mL). The mixture was stirred for 4 h at −20 °C and then allowed to warm to 25 °C. After stirring for 16 h, insoluble material was removed by filtration. The filtrate was diluted with EtOAc (150 mL)–water (150 mL) and adjusted to pH 8 with concentrated HCl. The EtOAc was washed with saturated NaCl, dried over $MgSO_4$, and evaporated in vacuo. The residual oil was purified by silica gel column chromatography using CH_2Cl_2–10% MeOH as eluent to give the diethyl ester of **8** (660 mg, 36%) as a white foam: ^1H NMR ($CDCl_3$) δ 1.279 (t, J = 7.2 Hz, 6 H), 2.050 (tt, J = 7.3, 18.6 Hz, 2 H), 3.731 (tt, J = 7.3, 12.6 Hz, 2 H), 4.0 (m, 6 H), 5.426 (s, 2 H), 5.535 (s, 2 H), 7.2–7.5 (m, 5 H), 7.691 (s, 1 H).

The diethyl ester of **8** (550 mg, 1.26 mmol) was dissolved in DMF (15 mL) and treated with bromotrimethylsilane (4 mL) under nitrogen. After stirring for 4 h at 25 °C, the solution was evaporated in vacuo. The residual oil was taken up in aqueous, saturated $NaHCO_3$ (2 mL) and this solution was chromatographed on a C-18 reverse column under 8 psi of pressure to give **8** (260 mg, 56%) as a white solid: UV_{max} (H_2O) 252 nm (ε 13 784); ^{13}C NMR (D_2O) δ 28.990, 31.526, 66.823, 73.262, 116.809, 140.665, 152.372, 154.904, 159.609; ^1H NMR (D_2O) δ 1.882 (m, 2 H), 3.803 (m, 2 H), 5.492 (s, 2 H), 5.954 (s, 1 H).

1-Iodo-4-(diethylphosphono)butane (17). To a suspension of 57% sodium hydride in mineral oil (2.0 g, 50 mmol) in dry THF (70 mL) was added at −20 °C a solution of diethyl phosphite (5.5 g, 40 mmol) in THF (10 mL) under nitrogen. After stirring at −20 °C for 1 h, 1,4-diiodobutane (40.0 g, 100 mol) was added and the mixture was stirred at −10 °C for 18 h. The mixture was then concentrated in vacuo, taken up in EtOAc and water, and adjusted to pH 8 with concentrated HCl. The EtOAc was washed with brine, dried over $MgSO_4$, and evaporated to a colorless oil, which was purified by silica gel column chromatography using CH_2Cl_2 as eluent to give **17** (6.9 g, 54%) as a white oil: ^1H NMR ($CDCl_3$) 1.342 (t, J = 7.0 Hz, 6 H), 1.7–2.1 (m, 6 H), 3.199 (t, J = 6.6 Hz, 2 H), 4.112 (dt, J = 7.0, 14.8 Hz, 4 H).

2-Amino-6-(benzyloxy)-9-[4-(diethylphosphono)butyl]purine (18). To a suspension of lithium hydride (112 mg, 14 mmol) in dry DMF (30 mL) was added 2-amino-6-(benzyloxy)purine and the mixture was stirred at 25 °C for 1 h under nitrogen. To this solution was added a solution of **17** (3.4 g, 11 mmol) in DMF (4 mL). After heating at 55 °C for 4 h, the mixture was evaporated in vacuo, taken up in EtOAc, and washed with 20% H_3PO_4. The EtOAc was washed with brine, dried over $MgSO_4$, and concentrated to a brown oil, which was purified by silica gel column chromatography using CH_2Cl_2–5% MeOH as eluent to give **18** (1.9 g, 42%) as a colorless, hard oil: ^1H NMR ($CDCl_3$) δ 1.370 (t, J = 6.5 Hz, 6 H), 1.8–2.0 (m, 6 H), 4.071 (t, J = 6.7 Hz, 2 H), 4.117 (dt, J = 6.5, 14.7 Hz, 4 H), 4.96 (s, 2 H), 5.573 (s, 2 H), 7.34–7.51 (m, 5 H), 7.870 (s, 1 H).

9-(4-Phosphonobutyl)guanine (7). To a solution of **18** (900 mg, 2.1 mmol) in DMF (5 mL) was added bromotrimethylsilane (2 mL) under nitrogen. After stirring for 16 h at 25 °C, the volatiles were removed in vacuo and the residue was neutralized to pH 8 by aqueous $NaHCO_3$. Water was then evaporated in vacuo and the residue was purified by C-18 reverse-phase column under 8 psi of pressure using water as eluent to give **7** (305 mg, 43%) as a white solid: UV_{max} (H_2O) 254 nm (ε 11 880); ^{13}C NMR (D_2O) δ 21.858, 21.933, 27.808, 30.434, 31.506, 31.837, 44.402, 116.835, 140.990, 152.239, 154.565, 159.853; ^1H NMR (D_2O) δ 1.55–1.97 (m, 6 H), 4.071 (t, J = 6.8 Hz), 7.830 (s, 1 H). Anal. ($C_9H_{12}O_4N_5P$) C, H, N.

2-[[(Diethylphosphono)methyl]thio]ethanol (20). To a solution of (diethylphosphono)methyl triflate (15.0 g, 50 mmol) and **19** (8.1 g, 50 mmol) in CH_2Cl_2 (150 mL) was added at 0 °C a solution of triethylamine (6.8 g, 68 mmol) in CH_2Cl_2 (20 mL) under nigrogen. After stirring at 0 °C for 3 h, the CH_2Cl_2 was washed with water and 10% H_3PO_4 and evaporated in vacuo. The crude residue was dissolved in EtOH (40 mL)–3 N HCl (20 mL) and the solution was allowed to stir for 6 h. The solution was then concentrated in vacuo and the residual oil was purified by silica gel column chromatography using CH_2Cl_2 as eluent to give **20** (7.9 g, 70%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.366 (t, J = 7.2 Hz, 6 H), 2.80–2.82 (m, 4 H), 2.825 (d, J = 12.8 Hz, 2 H), 3.855 (t, J = 5.4 Hz, 2 H), 4.206 (m, 4 H).

1-[(Methylsulfonyl)oxy]-2-[[(diethylphosphono)methyl]thio]ethane (21). To a solution of **20** (5.0 g, 22 mmol) and methanesulfonyl chloride (2.8 g, 23 mmol) in CH_2Cl_2 (50 mL) was added at 0 °C a solution of triethylamine (4.5 g, 44 mmol) in CH_2Cl_2 (5 mL). After stirring at 0 °C for 30 min, the CH_2Cl_2 was washed with 10% H_3PO_4, dried over $MgSO_4$, and evaporated to give **21** as a colorless oil. This material was used for the next step without any further purification: ^1H NMR ($CDCl_3$) δ 1.359 (t, J = 7.0 Hz, 6 H), 2.781 (d, J = 12.8 Hz, 2 H), 2.80–2.82 (m, 2 H), 3.082 (m, 2 H), 3.082 (s, 3 H), 4.442 (t, J = 5.6 Hz, 2 H), 4.187 (m, 4 H).

2-Amino-6-(benzyloxy)-9-[2-[[(diethylphosphono)methyl]thio]ethyl]purine (22). To a suspension of lithium hydride (65 mg, 8 mmol) in DMF (20 mL) was added 2-amino-6-(benzyloxy)purine (1.2 g, 5 mmol) and the mixture was stirred for 60 min at 25 °C. To this solution was added a solution of **21** (1.5 g, 5 mmol) in DMF (3 mL). After heating at 55 °C for 8 h, the mixture was evaporated in vacuo, taken up in CH_2Cl_2 and water, and adjusted to pH 8 with concentrated HCl. The CH_2Cl_2 was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by silica gel column chromatography using CH_2Cl_2–5% MeOH as eluent to give **22** (800 mg, 35%) as a hard oil: ^1H NMR ($CDCl_3$) δ 1.356 (t, J = 7.2 Hz, 6 H), 2.705 (d, J = 12.8 Hz, 2 H), 3.196 (t, J = 6.4 Hz, 2 H), 4.145 (m, 4 H), 4.647 (t, J = 6.4 Hz, 2 H), 5.595 (s, 2 H), 7.2–7.5 (m, 5 H), 7.905 (s, 1 H).

9-[2-[(Phosphonomethyl)thio]ethyl]guanine (9). To a solution of **22** (400 mg, 0.9 mmol) in DMF (5 mL) was added bromotrimethylsilane (1.5 mL) under nitrogen. After stirring for 16 h at 25 °C, the volatiles were removed in vacuo and the residue was crystallized from water–ethanol to give **9** (90 mg, 33%) as white crystals: mp 282–285 °C; UV_{max} (H_2O) 254 nm (ε 11 048); ^{13}C NMR (D_2O) δ 28.867, 31.423, 33.924, 34.069, 43.803, 116.955, 141.171, 152.374, 155.173, 160.468; ^1H NMR (D_2O) δ 2.567 (d, J = 11.0 Hz, 2 H), 3.108 (t, J = 6.4 Hz, 2 H), 4.271 (t, J = 6.4 Hz, 2 H), 7.877 (s, 1 H). Anal. ($C_8H_{12}N_5O_4PS$) C, H, N.

2-(Benzyloxy)-1-[(diethylphosphono)methoxy]propane (24). A solution of 2-(benzyloxy)-1-(chloromethoxy)propane (9.0 g, 42 mmol) and triethyl phosphite (7.2 mL, 42 mmol) in toluene (10 mL) was heated at 110 °C for 11 h. The toluene was evaporated and the residual oil was purified by silica gel column chromatography using CH_2Cl_2–5% MeOH as eluent to give **24** (12 g, 90%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.332 (t, J = 7.2 Hz, 6 H), 2.195 (d, J = 6.4 Hz, 3 H), 3.5–3.8 (m, 3 H), 3.857 (d, J = 8.0 Hz, 2 H), 4.1–4.3 (m, 4 H), 4.606 (s, 2 H), 7.2–7.4 (m, 5 H).

2-Hydroxy-1-[(diethylphosphono)methoxy]propane (25). A mixture of **24** (12 g, 38 mmol) and 10% palladium on activated carbon (6 g) in MeOH (250 mL) was hydrogenated in the Parr hydrogenator at 50 psi for 7 h. The mixture was filtered through Celite and washed with MeOH, and the combined MeOH was evaporated in vacuo to give **25** (7.3 g, 85%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.146 (d, J = 6.4 Hz, 3 H), 1.354 (t, J = 7.0 Hz, 6 H), 3.3–3.6 (m, 2 H), 3.857 (d, J = 8.2 Hz, 2 H), 4.0 (m, 1 H), 4.186 (m, 4 H).

2-Iodo-1-[(diethylphosphono)methoxy]propane (27). To a solution of **25** (10.3 g, 46 mmol) and methanesulfonyl chloride (7.8 g, 68 mmol) in CH_2Cl_2 (200 mL) was added at 0 °C a solution of triethylamine (14 mL, 100 mmol) in CH_2Cl_2 (20 mL). After stirring at 0 °C for 2 h, the CH_2Cl_2 was washed with 30% H_3PO_4 and brine, dried over $MgSO_4$, and evaporated to give **26** as a slightly yellow oil. A mixture of crude **26** and sodium iodide (30 g, 200 mmol) in 2-butanone (200 mL) was heated at 90 °C for 10 h. Any insoluble material was removed by filtration and the clear filtrate was evaporated in vacuo. The residual oil was purified by silica gel column chromatography using CH_2Cl_2–5% MeOH as eluent to give **27** (11.3 g, 74%) as a colorless oil: ^1H NMR

($CDCl_3$) δ 1.330 (t, J = 7.0 Hz, 6 H), 1.906 (d, J = 7.2 Hz, 3 H), 3.6–3.9 (m, 3 H), 3.865 (d, J = 8.9 Hz, 2 H), 4.13–4.28 (m, 4 H).

2-Amino-6-chloro-9-[1-methyl-2-[(diethylphosphono)-methoxy]ethyl]purine (28). To a suspension of 57% sodium hydride in mineral oil (1.6 g, 40 mmol) in dry DMF (160 mL) was added 2-amino-6-chloropurine (6.78 g, 40 mmol) and the mixture was stirred for 1 h at 20 °C. To this solution was added a solution of **27** (11.3 g, 34 mmol) in DMF (10 mL). The resulting mixture was heated at 95 °C for 10 h. The mixture was then concentrated in vacuo, taken up in CH_2Cl_2 and water, and adjusted to pH 8 with concentrated HCl. The CH_2Cl_2 was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residual oil was purified by silica gel column chromatography using CH_2Cl_2–10% MeOH as eluent to give **28** (3.5 g, 31%) as a white foam: ^{13}C NMR ($CDCl_3$) δ 16.135, 16.583, 50.413, 62.231, 62.364, 63.453, 66.752, 74.394, 74.588, 124.906, 141.259, 150.792, 153.418, 158.895; ^1H NMR ($CDCl_3$) δ 1.277 (t, J = 7.2 Hz, 3 H), 1.296 (t, J = 7.2 Hz, 3 H), 1.613 (d, J = 7.0 Hz, 3 H), 3.75–4.16 (m, 8 H), 4.754 (m, 1 H), 5.157 (s, 2 H), 7.925 (s, 1 H).

9-[1-Methyl-2-[(ethoxyhydroxyphosphinyl)methoxy]-ethyl]guanine (29). A solution of **28** (3.4 g, 910 mmol) in 1 N NaOH (100 mL) was heated at 100 °C for 5 h. The cooled solution (5 °C) was adjusted to pH 2 with concentrated HCl. The resulting solution was evaporated in vacuo and the residual solid was purified by a C-18 reverse-phase column using water–10% MeOH as eluent under 8 psi of pressure to give **19** (1.5 g, 50%) as a white solid: UV_{max} (H_2O) 252 nm (ε 12 192); ^1H NMR (D_2O) δ 1.023 (t, J = 6.5 Hz, 3 H), 1.508 (t, J = 6.8 Hz, 3 H), 3.50–3.92 (m, 6 H), 4.823 (m, 1 H), 8.630 (s, 1 H). Anal. ($C_{11}H_{18}N_5O_5P·1.5H_2O$) C, H, N.

9-[1-Methyl-2-(phosphonomethoxy)ethyl]guanine (10). To a solution of **29** (1.4 g, 4.2 mmol) in DMF (50 mL) was added bromotrimethylsilane (6.4 g, 42 mmol) and the solution was stirred at 25 °C for 8 h. The solution was evaporated in vacuo and the residual solid was purified by C-18 reverse-phase column using water–5% CH_3CN as eluent under 8 psi of pressure to give **10** (850 mg, 67%) as a white solid: UV_{max} (H_2O) 252 nm (ε 12 015); ^{13}C NMR (D_2O) δ 17.394, 51.253, 67.052, 70.139, 75.315, 75.528, 116.726, 139.144, 152.201, 154.422, 159.675; ^1H NMR (D_2O) δ 1.641 (d, J = 7.0 Hz, 3 H), 3.769 (d, J = 8.9 Hz, 2 H), 4.002 (m, 3 H), 9.065 (s, 1 H). Anal. ($C_9H_{14}N_5O_5P·H_2O$) C, H, N.

4-(Tetrahydropyranyloxy)-1,1-difluoro-1-(diethylphosphono)butane (32). To a solution of diisopropylamine (6.1 g, 60 mmol) in dry THF (30 mL) was added at −20 °C 2.5 M n-BuLi (22 mL, 55 mmol) and the solution was stirred at 0 °C for 30 min under nitrogen. To this solution was added at −78 °C a solution of diethyl (difluoromethyl)phosphonate (9.4 g, 50 mmol) in HMPA (10 mL). After stirring for 1 h at −78 °C, a solution of 3-(tetrahydropyranyloxy)-1-iodopropane (14 g, 50 mmol) in THF (10 mL) was added and the mixture was allowed to stir for 6 h at −78 °C. The reaction mixture was then poured into ether (200 mL)–20% H_3PO_4 (70 mL) and the aqueous layer was extracted with ether (100 mL × 2). The combined ether was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by silica gel column chromatography using CH_2Cl_2–30% EtOAc as eluent to give **32** (6.2 g, 23%) as a yellow oil: ^1H NMR ($CDCl_3$) δ 1.40 (t, J = 6.8 Hz, 6 H), 1.5–2.4 (m, 10 H), 3.50 (m, 2 H), 3.82 (m, 2 H), 4.26 (dt, J = 7.4, 15.2 Hz, 4 H), 4.60 (m, 1 H); ^{19}F NMR ($CDCl_3$) δ −35.677 (dt, J_{HF} = 20.14 Hz, J_{PF} = 108.04 Hz).

4-Iodo-1,1-difluoro-1-(diethylphosphono)butane (35). To a solution of **32** (6.3 g, 19 mmol) in EtOH (30 mL) was added 3 N HCl (15 mL) and the solution was stirred at 25 °C for 6 h. The solution was adjusted to pH 7.0 with 20% NaOH and concentrated in vacuo. The residue was taken up in CH_2Cl_2 (120 mL), dried over $MgSO_4$, and evaporated to a white oil. To a solution of the crude product (3.0 g, 12 mmol) and methanesulfonyl chloride (2.0 g, 17 mmol) in CH_2Cl_2 (40 mL) was added at 0 °C a solution of triethylamine (2.1 g, 21 mmol) and the mixture was stirred at 0 °C for 4 h. The reaction was diluted with CH_2Cl_2 (50 mL), washed with 10% H_3PO_4 and brine, and evaporated to dryness. This material was dissolved in acetone (40 mL), sodium iodide (3.0 g, 20 mL) was added, and the mixture was heated at 50 °C for 18 h. The acetone was evaporated and the residue was taken up in CH_2Cl_2, washed with water and brine, and evaporated in vacuo. The residue was purified by silica gel column using CH_2Cl_2–30% EtOAc as eluent to give **35** (1.6 g, 67% from 33) as a white oil: ^1H NMR ($CDCl_3$) δ 1.392 (t, J = 6.8 Hz, 6 H), 2.05 (m, 2 H), 2.15 (m, 2 H), 3.27 (t, J = 6.5 Hz, 2 H), 4.282 (dt, J = 6.6, 15.0 Hz, 4 H); ^{19}F NMR ($CDCl_3$) δ −35.047 (dt, J_{HF} = 19.20 Hz, J_{PF} = 116.Z Hz).

2-Amino-6-(benzyloxy)-9-[4,4-difluoro-4-(diethylphosphono)butyl]purine (36). To a suspension of lithium hydride (180 mg, 22 mmol) in DMF (30 mL) was added 2-amino-6-(benzyloxy)purine (2.5 g, 10 mmol) and the mixture was stirred at 25 °C for 50 min under nitrogen. To this solution was added a solution of **35** (3.3 g, 10 mmol) in DMF (5 mL). After heating at 60 °C for 5 h, the mixture was concentrated in vacuo, taken up in EtOAc, and washed with 20% H_3PO_4. The EtOAC was washed with brine, dried over $MgSO_4$, and evaporated. The crude oil was purified by silica gel column chromatography using CH_2Cl_2–10% MeOH as eluent to give **36** (1.1 g, 25%) as a white oil: ^1H NMR ($CDCl_3$) δ 1.325 (t, J = 6.7 Hz, 6 H), 2.1–2.2 (m, 4 H), 4.15 (t, J = 6.2 Hz, 2 H), 4.260 (dt, J = 6.5, 15.0 Hz, 4 H), 5.0 (s, 2 H), 5.625 (s, 1 H), 7.45–7.50 (m, 5 H), 7.645 (s, 1 H); ^{19}F NMR ($CDCl_3$) δ 35.230 (dt, J_{HF} = 18.0 Hz, J_{PF} = 107.5 Hz).

9-(4,4-Difluoro-4-phosphonobutyl)guanine (11). To a solution of **36** (280 mg, 0.63 mmol) in DMF (3 mL) was added bromotrimethylsilane (1 mL). After stirring for 15 h at 25 °C, the volatiles were removed in vacuo, and the residue was purified by a C-18 reverse-phase column using water–10% CH_3CN as eluent to give **11** (75 mg, 37%) as a white solid: UV_{max} (H_2O) 252 nm (ε 11 137); ^1H NMR (D_2O) δ 2.1–2.2 (m, 4 H), 4.289 (t, J = 6.6 Hz, 2 H), 8.735 (s, 1 H); ^{19}F NMR ($CDCl_3$) δ −36.268 (dt, J_{HF} = 20.03 Hz, J_{PF} = 96.5 Hz). Anal. ($C_9H_{12}N_5O_4PF_2·H_2O$) C, H, N.

Registry No. 1, 32668-92-1; **2**, 36901-80-1; **3**, 125330-93-0; **4** (n = 4), 117087-00-0; **4** (n = 5), 117087-35-1; **4** (n = 6), 117087-37-3; **4** (n = 7), 117087-38-4; **5** (n = 3), 107021-13-6; **5** (n = 3) diethyl ester, 121149-86-8; **5** (n = 4), 107021-14-7; **5** (n = 5), 117087-34-0; **5** (n = 6), 117087-36-2; **5** (n = 7), 117105-79-0; **7**, 125330-94-1; **8**, 125330-95-2; **8** diethyl ester, 125331-18-2; **9**, 125330-96-3; **10**, 117087-03-3; **11**, 125330-97-4; **12** (n = 3), 54314-83-9; **13**, 125330-98-5; **14**, 125330-99-6; **15**, 125331-00-2; **16**, 628-21-7; **17**, 125331-01-3; **18**, 125331-02-4; **19**, 77597-08-1; **20**, 125331-03-5; **21**, 125331-04-6; **22**, 125331-05-7; **23**, 125331-06-8; **24**, 125331-07-9; **25**, 125331-08-0; **26**, 125331-09-1; **27**, 125331-10-4; **28**, 125331-11-5; **29**, 125331-12-6; **30**, 1478-53-1; **31**, 94993-99-4; **32**, 125331-13-7; **33**, 125331-14-8; **34**, 125331-15-9; **35**, 125331-16-0; **36**, 125331-17-1; 3-bromopropanol, 627-18-9; diethyl phosphite, 762-04-9; adenine, 73-24-5; 2-amino-6-(methoxyethoxy)purine, 105797-60-2; 2-amino-6-(benzyloxy)purine, 19916-73-5; (diethylphosphono)methyl triflate, 106938-62-9; triethyl phosphite, 122-52-1; 2-amino-6-chloropurine, 10310-21-1; 3-(triiodopyrryloxy)-1-iodopropane, 52103-12-5; chloromethyl 2-bromoethyl ether, 1462-35-7.