of the corrected 1-propanol and mitomycin C fragments then gave a net log P of -0.283 for **2**. The contribution of one hydrogen to the 7-NH_2 group was determined by a calculation for aniline using CLOGP. For the substituted N^7-phenylmitomycin C analogues, the experimentally determined log P for N^7-phenylmitomycin C (**7**) was used and π values for the substituents were taken from the literature. The $E_{1/2}$ values used were previously published, except for those of **10**, **11**, **15**, **16**, and **17**, which were unknown. For the first four of these compounds, $E_{1/2}$ was calculated using an equation based on the linear relationship between $E_{1/2}$ and σ values for substituents on the benzene rings of N^7-arylmitomycin C analogues previously published.[5] This equation, derived by statistical analysis of the earlier data using SPSSX, was $E_{1/2} = -0.34 + 0.072\sigma$. In the calculations for σ in compounds with disubstituted N^7-phenyl groups, σ_p and σ_m values for each substituent are taken from the literature and it is assumed that they can be added together to obtain a combined σ for all substituents. Using compound **15** as an example, $E_{1/2} = -0.34 + 0.072(\sigma_{p\text{-OH}} + \sigma_{m\text{-}1}) = -0.34 + 0.072(-0.37 + 0.35) = -0.34$ V. Following this procedure, the $E_{1/2}$ value for **16** is -0.32 V. The $E_{1/2}$ for N^7-(5-indolyl) derivative **17** was estimated to be -0.36 V by comparing the nearly equal calculated electron densities (partial atomic charges) on 5-aminoindole (-0.436) and 4-aminophenol (-0.474), as determined by quantum mechanics using GAUSSIAN-80 UCSF.[23]

Molecular Modeling. New mitomycin analogues were obtained by displaying the mitosene forms of mitomycin C and mitomycin A with no substituent on C1, which has been previously subjected to energy refinement with the molecular mechanics program of AMBER,[24] and docking the new substituent groups onto them using MIDAS.[25] These substituent groups were constructed using CHEMLAB II, and their partial atomic charges (ESP) were calculated with GAUSSIAN-80 UCSF.[23] The resulting structures were then brought to minimum energy conformations using AMBER. Parameters previously outlined were used for the mitomycin part of the structure. Parameters for the new substituents were based on those already in AMBER.[26] For example, the indole substituent and the *p*-hydroxyphenyl substituent were taken from tryptophan and tyrosine, respectively. The united atom force field of AMBER 2.0 was used and all structures were refined until the root mean square gradient was less than 0.1 kcal/Å. A distance-dependent dielectric constant was used, and all nonbonded pairs were included in the calculations. These same parameters and conditions were used for the decanucleotide duplexes and their covalent complexes with the mitomycins described below.

The decanucleotide $d(GCGCGCGCGC)_2$ (referred to henceforth as GC10) was generated in the B form with Arnott's geometry[27] and brought to a minimum-energy conformation. Mitomycin A and mitomycin C were docked onto it near the 2-amino group of the fifth guanine residue in the first strand using MIDAS. The coordinates were captured, and the structures of the resulting complexes, made covalent by defining a 1.47 Å bond between C1 of the mitomycin and N2 of the guanine, were subjected to energy refinement using AMBER. Models for other covalently bound mitomycin analogues were derived from those of mitomycin A and mitomycin C by docking the new substituent onto the mitomycin A 7-CH_3O or mitomycin C 7-NH_2, removing hydrogens, and carrying out energy minimization on the resulting structures. Using the ANALYSIS mode of AMBER, the energies for interactions between the mitomycin analogues and GC10, as well as the internal energies of each, were calculated. Distortion energies in the GC10, resulting from induced fits with the mitomycins, were calculated by subtracting energies of GC10 minimized alone from those of GC10 in the covalent complex. In the same manner, distortion energies in the mitomycins were obtained by subtracting energies of the mitomycins minimized alone from their energies in the covalent complex. Net binding energies, which are used in the comparison of relative binding strengths of mitomycins to GC10, were obtained by adding the calculated intermolecular binding energies (electrostatic + van der Walls) and the two distortion energies. These data are given in Table II.

Concerning the helix distortion energies, their absolute values cannot be used to compare the distortion in a complexed polynucleotide relative to uncomplexed polynucleotide because they are chemically different molecules. It is, however, meaningful to compare the distortion energies among various complexes based on the same drug and polynucleotide and to draw inferences on their relative stabilities. The dominant components in the relative distortions are van der Waals and electrostatic interactions. Bond length and bond angle contributions make little difference. Previous publications discuss the scope of application of AMBER to drug-macromolecule complexes and the validity of parameters in its force fields.

Acknowledgment. We gratefully acknowledge the Medical Research Council of Canada for a fellowship to K.R.K. Dr. S. M. Sami (University of Arizona) generously provided some of the mitomycins used in this project. The CLOGP program was made available by Dr. S. H. Yalkowsky (University of Arizona). AMBER was a gift of Professor Peter A. Kollman (University of California, San Francisco).

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A New Class of Acyclic Phosphonate Nucleotide Analogues: Phosphonate Isosteres of Acyclovir and Ganciclovir Monophosphates as Antiviral Agents[1]

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Novel phosphonate isosteres of acyclovir (ACV) and ganciclovir (DHPG) monophosphates (**20** and **32**) were found to be potent and selective antiherpesvirus agents. In the series of phosphonate analogues of ACV monophosphate, only the guanine analogue **20** exhibited activity against herpesviruses, similar to the structure-activity relationship observed for base modification of ACV analogues. The phosphonate isostere of ACV monophosphate (**20**) was more effective than ACV in the HSV-1 infected mouse model. The 3'-carba analogues of 9-[3-hydroxy-2-(phosphonomethoxy)propyl]purines/pyrimidines (adenine, HPMPA; guanine, HPMPG; cytosine, HPMPC) are devoid of antiherpesvirus activity. This result confirms that the β-oxygen atom of the phosphonomethyl ether functionality in HPMP-purines/pyrimidines plays a critical role for activity against herpesviruses.

Some acyclic nucleoside analogues have achieved considerable success as antiviral agents.[3] Acyclovir (ACV, **1**)[4] and ganciclovir (DHPG, **2**)[5] exhibit potent and selective activity against herpesviruses including herpes simplex

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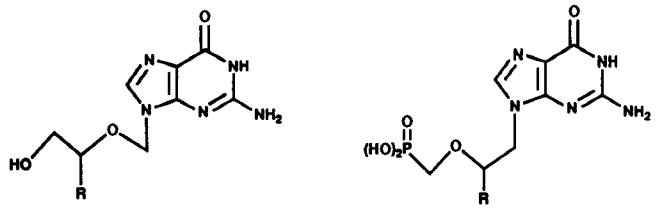
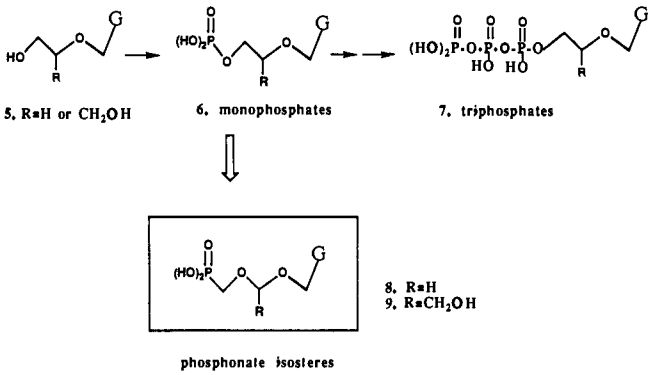
Chart I



1, R=H (ACV)
2, R=CH_2OH (DHPG)

3, R=H (PMEG)
4, R=CH_2OH (HPMPG)

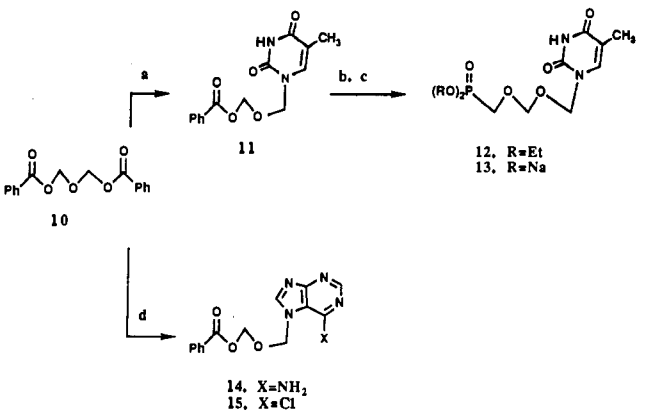
Scheme I



virus type 1 (HSV-1) and 2 (HSV-2), human cytomegalovirus (HCMV), and varicella zoster virus (VZV). Both compounds are believed to act by the same general mechanism. ACV and DHPG are selectively converted to monophosphates 6 by HSV (or VZV) specified thymidine kinase.[4,5b,6] HCMV does not encode a thymidine kinase[7] but induces a high level of host deoxyguanosine kinase.[8] Further phosphorylation of monophosphates 6 by host cellular kinases generates the acyclic nucleoside triphosphates 7 (Scheme I), which exert the antiviral effect by inhibition of herpesviral DNA polymerase.[4,5b,6]

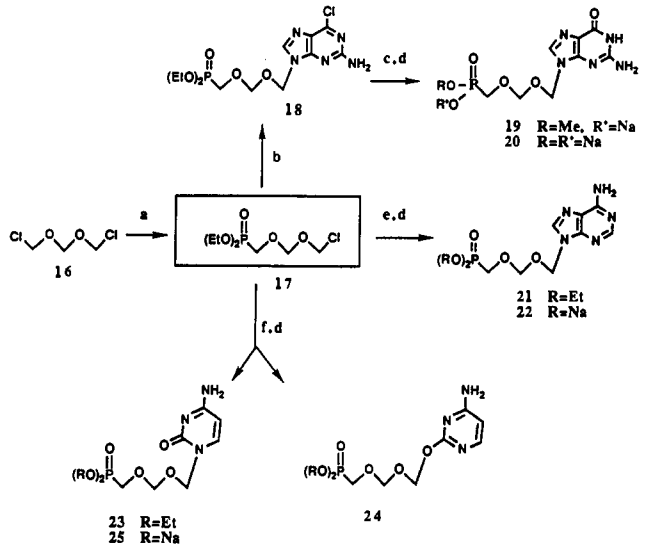
Recently new [(phosphonomethoxy)alkyl]purine/pyrimidine derivatives have emerged as potent antiviral

Scheme II[a]



[a] (a) Silylated thymine, $CF_3SO_3SiMe_3$; (b) $(EtO)_2P(O)CH_2OH$, $CF_3SO_3SiMe_3$; (c) Me_3SiBr, $NaHCO_3$; (d) silylated adenine (for 14) or silylated 6-chloropurine (for 15), $CF_3SO_3SiMe_3$.

Scheme III[a]



[a] (a) $(EtO)_2P(O)Na$, *n*-pentane; (b) 2-amino-6-chloropurine sodium salt; (c) MeONa, MeOH; (d) Me_3SiBr, then $NaHCO_3$; (e) adenine sodium salt; (f) cytosine sodium salt.

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(2) Present address: Gilead Sciences, 344 Lakeside Drive, Foster City, CA 94404.

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agents.[9] These analogues effectively inhibited a wide array of DNA viruses and retroviruses. The characteristic phosphonomethyl ether functionality present in [(phosphonomethoxy)alkyl]purine/pyrimidine derivatives is expected to be chemically and metabolically stable. This factor is probably responsible for the intrinsic in vivo antiviral activity of some of [(phosphonomethoxy)alkyl]-purine/pyrimidine derivatives.[10] The β-oxygen atom in the phosphonomethyl ether functionality enhances the acidity of the phosphonate due to the electron-withdrawing effect of the oxygen atom, thus bringing the second pK_a of phosphonate derivatives closer to that of phosphate ester.[11] In addition to this isoelectronic nature, the 3′-oxygen atom in 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG, 3) has been demonstrated to play a critical role for the enzymatic phosphorylation and thus for antiviral activity.[11] Since these phosphonate analogues are structural mimics of acyclic nucleoside monophosphates, they are expected to bypass the first enzymatic phosphorylation. Consequently, these compounds have been shown to be effective against thymidine kinase-deficient strains of HSV and VZV.[2a] Despite the apparent structural similarities of PMEG (3) and 9-[3-hydroxy-2-(phosphonomethoxy)-propyl]guanine (HPMPG, 4) to ACV monophosphate and DHPG monophosphate, they are not isosteric due to different length of the side chain. Therefore, in order to more fully evaluate structure–activity relationships (SAR) for PME- and HPMP-purine/pyrimidine analogues, the phosphonate isosteres (8 and 9) of ACV and DHPG monophosphates were synthesized and evaluated for antiviral activity. Furthermore, in a series of HPMP-purine/pyrimidine analogues, the 3′-carba analogues were also prepared to examine the role of the 3′-oxygen atom for their antiviral activity.

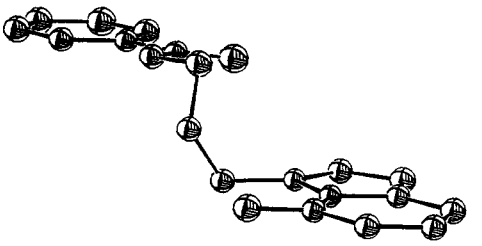
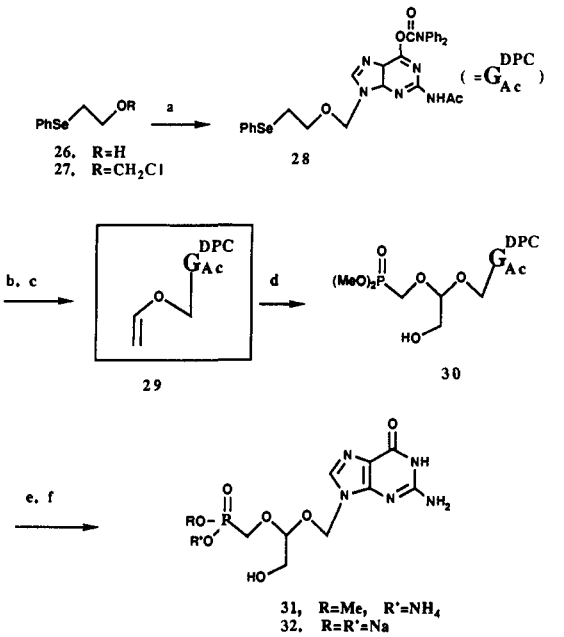


Figure 1. ORTEP drawing of compound 14.

Scheme IV[a]



[a] (a) 2-(Acetylamino)-6-(diphenylcarbamoyl)purine, $Hg(CN)_2$; (b) H_2O_2, $NaHCO_3$; (c) dioxane (80 °C); (d) $(MeO)_2P(O)CH_2OH$, MCPBA; (e) NH_4OH; (f) Me_3SiBr then $NaHCO_3$.

Chemistry

First, we developed a new methodology to assemble the acyclic acetal functionality as shown in the synthesis of the thymine analogue 13 (Scheme II). The Vorbruggen

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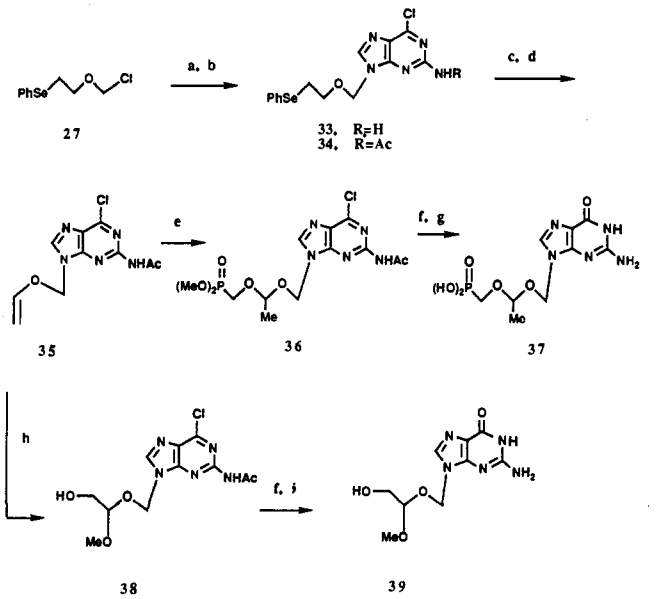
type coupling[12] of bis[(benzoyloxy)methyl] ether (10) and silylated thymine in the presence of trimethylsilyl trifluoromethanesulfonate (0.2 equiv) in CH_2Cl_2 at 0 °C provided the acyl acetal 11 (45%). Reaction of 11 with diethyl(hydroxymethyl)phosphonate[13] (1.2 equiv) in the presence of trimethylsilyl trifluoromethanesulfonate (1.2 equiv) in CH_2Cl_2 at 25 °C afforded the phosphonate 12 (65%), which upon treatment with trimethylsilyl bromide (5 equiv) in DMF at 25 °C gave the acetal phosphonate 13 (75%). Unfortunately, when the above reaction sequence was applied to the synthesis of purine analogues, undesired N-7 isomers were formed exclusively. For example, coupling of 10 with silylated adenine or silylated 6-chloropurine in the presence of trimethylsilyl trifluoromethanesulfonate gave the N-7 isomers (14) (39%) and (15) (42%) only. The attachment of the side chain at the N-7 position in 14 and 15 was confirmed by the ^{13}C–^{1}H two-dimensional long-range heteronuclear correlation spectroscopy, in which the C_5 and the 1′-H exhibited a strong interaction. This regioselectivity was further ascertained by the X-ray crystallography as shown in Figure 1. Although the highly regioselective kinetic formation of a N-7 (pentofuranosyl)guanine was reported,[14] the exclusive formation of the N-7 adenine acyclonucleotide 14 appears to be a first case.

In contrast to the Vorbruggen type coupling, the S_N2 displacement reaction of the purine sodium salt on the chloromethyl ether (17) produced only N-9 isomers as illustrated in Scheme III. Addition of bis(chloromethoxy)methane (16)[15] to the solution of sodium diethyl phosphite in THF at −70 °C produced the chloromethyl ether, which was used promptly in the subsequent transformations. Reaction of 17 with 2-amino-6-chloropurine sodium salt in DMF at 25 °C to give the N-9 isomer 18 (42% from 16), action of sodium methoxide followed by saponification to form 19 (60%), and deprotection with trimethylsilyl bromide provided the phosphonate 20 (68%). Despite the acetal linkage between the phosphonate functionality and the guanine base, 20 was quite acid stable. Thus, a pH 2 solution of 20 showed no sign of degradation after 24 h as evidenced by HPLC and NMR analysis. Likewise, the N-9 adenine phosphonate 22 was also prepared in 47% yield. The side-chain attachment at the N-9 position in 20 was ascertained by its ^{13}C NMR (δ 118.194 for the C_5 signal) and UV (λ_{max} 252 and 274 nm) spectra, which were consistent with the published data of N-9 alkylated guanine derivatives.[16] Coupling of 17 with cytosine sodium salt gave the desired N-alkylated isomer 23 (22%) and an O-alkylated byproduct 24 (10%). The phosphonate ester cleavage of 23 by trimethylsilyl bromide gave rise to the cytosine analogue 25.

Scheme V[a]



[a] (a) Silylated 2-amino-6-chloropurine, $Hg(CN)_2$; (b) $(MeCO)_2O$; (c) $NaIO_4$, $NaHCO_3$; (d) dioxane (80 °C); (e) $(MeO)_2P(O)CH_2OH$, CH_3SO_3H; (f) MeONa, MeOH; (g) Me_3SiBr; (h) MCPBA; (i) H_2O (100 °C).

The synthetic strategy for the assembly of the hydroxymethyl-substituted acetal functionality shown in 9 is depicted in Scheme IV. In this sequence, the key step involves the regiospecific addition of dimethyl (hydroxymethyl)phosphonate[13] to the enol ether 29 under oxidative conditions. Preparation of the requisite enol ether 29 was readily carried out. Thus, treatment of 2-(phenylselenyl)ethanol (26)[17] with 1,3,5-trioxane (1.1 equiv) in the presence of HCl gas in CH_2Cl_2 resulted in the production of the corresponding chloromethyl ether 27, which was used for the next reaction without purification. Addition of 27 to a preheated mixture of 6-[(diphenylcarbamoyl)oxy]purine[18] and $Hg(CN)_2$ (1.05 equiv) in benzene provided 28 (67%). Among many protecting groups used for the protection of the 6-oxo function of guanine, the diphenylcarbamoyl (DPC) was found to be the most effective for the synthesis of 32. Oxidation of 28 with excess H_2O_2 followed by thermolysis gave the enol ether 29 (65%). Addition of *m*-chloroperbenzoic acid (1.1 equiv) to a solution of 29 and dimethyl (hydroxymethyl)phosphonate[13] (10 equiv) in CH_2Cl_2 afforded 30 in 42% yield. Saponification of 30 with ammonium hydroxide gave the monoester 31 (65%). Further deblocking of the monoester 31 with trimethylsilyl bromide followed by neutralization with sodium bicarbonate produced the disodium salt 32 (62%).

Other acetal analogues are more conveniently prepared from the enol ether 35, which was in turn derived from

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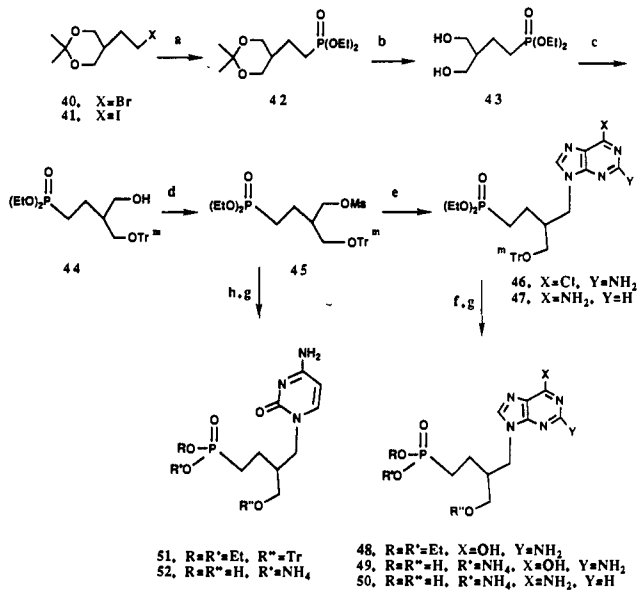
Scheme VI[a]



[a] (a) $(EtO)_3P$, 140 °C; (b) 2 N HCl, 25 °C; (c) (4-methoxyphenyl)diphenylmethyl chloride, TEA; (d) $MeSO_2Cl$, TEA; (e) 2-amino-6-chloropurine, Cs_2CO_3 (for **46**), adenine, Cs_2CO_3 (for **47**); (f) 2 N HCl, 100 °C; (g) Me_3SiBr then NH_4OH; (h) cytosine, Cs_2CO_3.

coupling of **27** and 2-amino-6-chloropurine in a similar manner described for the synthesis of **29**. Addition of dimethyl (hydroxymethyl)phosphonate to **35** in the presence of acid furnished **36**, from which the guanine derivative **37** was prepared by action of sodium methoxide followed by treatment with trimethylsilyl bromide (Scheme V). Under similar reaction conditions, addition of methanol to **35** led to **39** via **38**. The work described herein illustrates a ready access to the synthesis of new acyclic acetal nucleoside derivatives. Derivatives in which the 3'-oxygen atom of HPMP-purine/pyrimidine analogues was replaced with the carbon atom were synthesized as outlined in Scheme VI. Conversion of the iodide **41**[19] to the phosphonate **43** was conveniently achieved in an one-pot procedure. Treatment of **41** with triethyl phosphite gave **42**, which was subsequently hydrolyzed with 2 N HCl to provide **43**. A critical step in this sequence was the selective monoprotection of the diol **43**. Selection of the trityl protecting group enabled us to prepare the monotrityl intermediate **44** in moderate yield. Mesylation of **44** gave **45**, which was further converted to **46** by a nucleophilic displacement with 2-amino-6-chloropurine. Conversion of **46** to the guanine analogue **49** was completed by the sequence (1) hydrolysis with 2 N HCl to give **48**; (2) cleavage of the phosphonate ester by trimethylsilyl bromide; (3) adjustment of pH with ammonium hydroxide. Similarly, the adenine and cytosine analogues (**50** and **52**) were prepared from the mesylate **45**.

Biological Results and Discussion

Results of antiviral activity testing by the plaque reduction assay against herpesvirus are listed in Table I. The phosphonate isostere of ACV monophosphate (compound **20**) exhibited potent HSV-1, HSV-2, and HCMV activity (Table I). Other purine and pyrimidine analogues

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Table I. Antiviral and Anticellular Activities of Acyclic Nucleoside and Nucleotide Derivatives in Tissue Culture

	ID_{50},[a] μg/mL			
compd	HSV-1 (BWS)[b]	HSV-2 (G)	HCMV (AD-169)	vero cells
3	0.08	0.69	0.04	5
13	>100	>100	>100	>100
20	2.6	11.0	5.0	>100
22	>100	>100	>100	>100
25	>100	>100	>100	>100
32	3.3	8.2	0.9	>100
37	>100	>100	>100	>100
39	>100	>100	>100	>100
49	N.T.[c]	>100	>100	>100
50	N.T.	>100	>100	>100
52	N.T.	>100	>100	>100
1 (ACV)	0.5	2.4	38.4	>100

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

Table II. Antiviral Effect of Compound **20** Against HSV-1-Induced Mortality in Mice

	mg/kg[a]	survival (%)	MST[b]
compound **20**	300	10/10/(100)	21.0
	100	9/10 (90)	20.4
ACV	300	6/7 (86)	20.0
	100	7/10 (70)	18.4
Saline		3/10 (30)	13.1

[a] Mice were inoculated intraperitoneally with HSV-1 (BWS). Treatment was initiated 3 h postinfection and continued BID for 5 consecutive days. [b] Mean survival time, days: Experiments were terminated at day 21. Survival times of all treated groups are significantly different from the placebo treated control group ($P < 0.05$).

in this series (compounds **13**, **22**, and **25**) are devoid of antiviral activity. This result is consistent with SAR observed in ACV analogues in which only the guanine base showed antiherpes activity.[3] Although activity of **20** against HSV-1 and -2 is 5-fold less potent than that of ACV, its activity against HCMV is about 8-fold more potent than that of ACV. The phosphonate isostere of DHPG monophosphate (compound **32**) is also a potent inhibitor against herpesviruses. Similar to ACV and DHPG triphosphates, the diphosphates of some of PME- and HPMP-purine/pyrimidine analogues have been demonstrated to be selective inhibitors of viral DNA polymerase.[9f,k,m] It is assumed that a prerequisite for **20** and **32** to have antiherpetic activity is assumed to be that they are phosphorylated further in cells to their diphosphates. The efficiency of cellular phosphorylation and the inhibitory effect of diphosphates on viral DNA polymerases are remained to be investigated for **20** and **32**. Although PMEG was the potent antiviral agent it also has high cellular toxicity. By contrast, both compounds **20** and **32** showed no sign of toxicity to vero cells up to 100 μg/mL.

Lack of antiviral activity of **37**, which is a 3'-methyl analogue of **20**, indicates that few changes are allowed in the acyclic side chain to retain the antiherpetic activity. The 3'-methoxy ACV analogue **39** was also devoid of activity against herpesviruses. The carba analogues (**49**, **50**, and **52**) of HPMP (G, A, and C) did not exhibit antiherpetic activity except marginal HCMV activity of **49**. This result clearly demonstrates the 3'-oxygen atom in the side chain of HPMP-purine/pyrimidine analogues plays a critical role for activity against herpesviruses. Lack of herpesvirus activity of carba analogues may be due to the inability of cellular or virally induced kinase to catalyze their conversion to the mono- and diphosphate forms.

Alternatively, the respective diphosphates, if formed, may be poor inhibitors of viral DNA polymerase.

The phosphonate **20** is also protective against the HSV-1-induced mortality in mice (Table II). The number of survivors for **20** at a dose of 100 mg/kg is comparable to ACV. Since **20** is about 5-fold less active in vitro against HSV-1 compared to ACV, the in vitro potency does not predict potency in vivo. This result extends previous findings with HPMPC[9i,l,10] and other phosphonate nucleotide analogues,[9d,h,j] which demonstrate better efficacy in vivo than predicted from the potency in the in vitro assays.

In conclusion, the conceptual operation of incorporating the phosphonomethoxy ether functionality into structures of ACV and DHPG monophosphates gave rise to a novel class of acetal phosphonate nucleotide analogues (**20** and **32**). The potent antiherpesvirus activity exhibited by compounds **20** and **32** clearly demonstrated that not only these phosphonates may act as biologically equivalent isosteres of corresponding nucleoside monophosphates, but they also are taken up enough by cells to exert antiviral activity. The synthetic approach described herein should be applicable for other phosphonate mimics of many biologically interesting phosphates.[20]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Gemini-300 spectrometer at 300 MHz (^{1}H) and 75.5 MHz (^{13}C) with Me_4Si as internal standard. UV spectra were obtained on a Perkin-Elmer 552 spectrometer. All reactions were performed under nitrogen.

Bis[(benzoyloxy)methyl] Ether (10). To a suspension of sodium benzoate (5.0 g, 34.7 mmol) in DMF (70 mL) was added bis(chloromethyl) ether (10 g, 70.0 mmol), and the mixture was heated at 70 °C for 16 h. The insoluble material was removed by filtration. The filtrate was concentrated in vacuo to give a white crystal, which was recrystallized from ether–pentane to give 4.5 g (91%) of **10**: mp 39 °C; ^{1}H NMR ($CDCl_3$) δ 5.66 (s, 4 H), 7.75–8.05 (m, 10 H). Anal. ($C_{18}H_{14}O_5$) C, H, N.

1-[[(Benzoyloxy)methyl]oxy]thymine (11). A suspended solution of thymine (12.6 g, 0.1 mol), ammonium sulfate (300 mg), and trimethylsilyl chloride (2.5 mL) in hexamethyldisilazane (150 mL) was heated at 140 °C for 16 h. The volatiles were removed in vacuo at 50 °C, and the residual oil was dissolved in xylene (30 mL) and concentrated to dryness. To a solution of the silylated thymine in CH_2Cl_2 (200 mL) was added bis[(benzoyloxy)methyl] ether (30 g, 0.1 mol) and trimethylsilyl trifluoromethanesulfonate (50 mL). The solution was stirred at 25 °C for 8 h. The reaction was diluted with ethyl acetate (400 mL) and washed with aqueous sodium carbonate and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude oily material was purified by silica gel column chromatography using CH_2Cl_2–5% MeOH as eluent to give 14.5 g (50%) of **11** as white crystals: mp 141–143 °C; ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3 H), 5.38 (s, 2 H), 5.62 (s, 2 H), 7.10 (s, 1 H), 7.4–8.0 (m, 5 H). Anal. ($C_{14}H_{14}N_2O_5$) C, H, N.

7-[[[(Benzoyloxy)methyl]oxy]methyl]adenine (14). A suspended solution of adenine (2.52 g, 20 mmol), trimethylsilyl chloride (1 mL), and ammonium sulfate (130 mg) in hexamethyldisilazane (20 mL) was heated at 140 °C for 18 h. The volatiles were removed in vacuo at 50 °C, and the residual oil was dissolved in xylene (20 mL) and concentrated to dryness. To a solution of the silylated adenine in CH_2Cl_2 (20 mL) was added bis[(benzoyloxy)methyl] ether (4.5 g, 15 mmol) and trimethylsilyl trifluoromethanesulfonate (3 mL). The solution was stirred at 25 °C for 8 h. The reaction mixture was then diluted with ethyl acetate (50 mL) and washed with aqueous sodium carbonate and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude oily residue was chromatographed on silica gel to give 1.75 g (39%) of **14** as a white needle: mp 162–164 °C; UV max (EtOH) 230 (ϵ 12438), 278 nm (ϵ 9678); ^{1}H NMR ($CDCl_3$) δ 5.61 (s, 2 H), 5.83 (s, 2 H), 6.0 (br s, 2 H), 7.4–7.9 (m, 5 H), 8.20 (s, 1 H), 8.42 (s, 1 H). Anal. ($C_{14}H_{13}N_5O_3$) C, H, N.

In a similar manner, **15** was obtained from silylated 6-chloropurine as a white oil (42%): ^{1}H NMR ($CDCl_3$) δ 5.58 (s, 2 H), 5.89 (s, 2 H), 6.05 (br s, 2 H), 7.4–7.9 (m, 5 H), 8.54 (s, 1 H), 8.82 (s, 1 H).

2-Amino-6-chloro-9-[[[(diethoxyphosphinyl)methoxy]methoxy]methyl]purine (18). To a suspension of 60% sodium hydride in mineral oil (1.4 g, 34.5 mmol) in *n*-pentane (100 mL) at 0 °C was added dropwise diethyl phosphite (4.4 mL, 34.5 mmol). After the mixture was stirred for 1 h at 0 °C, a solution of bis(chloromethoxy)methane (25 g, 172 mmol) in *n*-pentane (50 mL) was added at −70 °C. The mixture was stirred for 90 min at 0 °C, and then the solvent was evaporated under reduced pressure. The residual oil was dissolved in xylene, and the volatiles were removed in vacuo to give crude (chloromethoxy)[(diethoxyphosphinyl)methoxy]methane (**17**). Without further purification, this material was used for the next reaction.

To a suspension of 60% sodium hydride in mineral oil (1.4 g, 34.5 mmol) in DMF (100 mL) was added 2-amino-6-chloropurine (5.78 g, 34.2 mmol), and the mixture was stirred for 1 h at 25 °C. To the resulting yellow solution was added dropwise a solution of above (chloromethoxy)[(diethoxyphosphinyl)methoxy]methane in DMF (20 mL). After the mixture was stirred for 15 h at 25 °C, the volatiles were removed in vacuo. The residual oil was suspended in ethyl acetate (100 mL), washed with water (30 mL) and brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil was chromatographed on silica gel using CH_2Cl_2–3% MeOH as eluent to give 3.0 g (23%) of **18** as a white oil: ^{1}H NMR δ 1.39 (t, J = 6.9 Hz, 6 H), 3.85 (d, J = 9.0 Hz, 2 H), 4.1–4.2 (m, 4 H), 4.69 (s, 2 H), 5.32 (br s, 2 H), 5.58 (s, 2 H), 7.89 (s, 1 H). Anal. ($C_{13}H_{18}N_5O_5PCl$) C, H; N: calcd 18.44; found 17.99.

9-[[[(Methoxyhydroxyphosphinyl)methoxy]methoxy]methyl]guanine Sodium Salt (19). A solution of **18** (325 mg, 0.84 mmol) in 1 N sodium methoxide (15 mL) was heated at 80 °C for 1 h. Volatiles were removed under reduced pressure. The residual oil was then dissolved in water (10 mL), and the solution was heated at 100 °C for 1 h. The pH of the solution was carefully adjusted to 8.0 at 0 °C by dropwise addition of 1 N HCl. Water was then evaporated in vacuo, and the residual oil was purified by C_{18} reverse-phase column using water as eluent to give 185 mg (60%) of **19** as a white powder: UV max (H_2O) 254 (ϵ 14372), 274 nm (ϵ 9788); ^{1}H NMR (D_2O) δ 3.68 (d, J = 10.3 Hz, 3 H), 3.62 (d, J = 90 Hz, 2 H), 4.82 (s, 2 H), 5.54 (s, 2 H), 7.88 (s, 2 H); ^{13}C NMR (D_2O) δ 51.87, 60.46, 63.63, 70.01, 94.71, 94.95, 116.17, 139.93, 151.67, 154.43, 159.28. Anal. ($C_8H_{13}N_5O_6PNa\cdot4H_2O$) C, H, N.

9-[[(Phosphonomethoxy)methoxy]methyl]guanine Disodium Salt (20). To a solution of **19** (1.5 g, 4.4 mmol) in DMF (5 mL) was added trimethylsilyl bromide (5 mL). After stirring for 3 h at 25 °C, the volatiles were removed in vacuo and the residue was neutralized to pH 8.0 by addition of aqueous saturated sodium bicarbonate. Water was then evaporated in vacuo, and the residue was purified by C_{18} reverse-phase column using water as eluent under 8 psi pressure to give 900 mg (59%) of **20** as a white powder: UV max (H_2O) 252 (ϵ 12113), 274 nm (ϵ 8201); ^{1}H NMR (D_2O) δ 3.53 (d, J = 8.9 Hz, 2 H), 4.77 (s, 2 H), 5.54 (s, 2 H), 7.89 (s, 1 H); ^{13}C NMR (D_2O) δ 67.02, 69.01, 70.75, 95.68, 95.83, 118.19, 141.81, 153.57, 157.39, 162.49. Anal. ($C_8H_{10}N_5O_6PNa\cdot3H_2O$) C, H, N.

9-[[[(Diethoxyphosphinyl)methoxy]methoxy]methyl]adenine (21). To a suspension of 60% sodium hydride in mineral oil (1.4 g, 34.5 mmol) in DMF (100 mL) was added adenine (4.7 g, 34.5 mmol), and the mixture was stirred at 80 °C for 1 h. To the resulting yellow solution was added dropwise a solution of **17**, prepared from diethyl phosphite (4.4 mL, 34.5 mmol) and bis(chloromethoxy)methane (35 g, 172 mmol) as described in the previous experiment, in DMF (20 mL). After the mixture was stirred at 25 °C for 15 h, the volatiles were removed in vacuo, and the resulting oily residue was purified by silica gel column chromatography using CH_2Cl_2–10% MeOH as eluent to give 6.0 g (50%) of **21** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 6.7 Hz, 6 H), 3.82 (d, J = 9.2 Hz, 2 H), 4.10–4.18 (m, 4 H), 4.79

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(s, 2 H), 5.69 (s, 2 H), 6.20 (br s, 2 H), 7.92 (s, 1 H), 8.30 (s, 1 H).

9-[[(Phosphonomethoxy)methoxy]methyl]adenine Disodium Salt (22). To a solution of **21** (600 mg, 1.7 mmol) in DMF (4 mL) was added trimethylsilyl bromide (5 mL) under nitrogen. After the mixture was stirred for 3 h at 25 °C, the volatiles were removed in vacuo and the residue was neutralized to pH 8.0 by addition of aqueous saturated sodium bicarbonate. Water was then evaporated in vacuo, and the residue was purified by C_{18} reverse-phase column using water as eluent under 8 psi pressure to give 280 mg (50%) of **22** as a white powder: UV max (H_2O) 260 nm (ε 12016); ^1H NMR (D_2O) δ 3.49 (d, J = 8.9 Hz, 2 H), 4.78 (s, 2 H), 5.71 (s, 2 H), 8.18 (s, 1 H), 8.27 (s, 1 H); ^{13}C NMR (D_2O) δ 66.91, 68.92, 71.03, 95.79, 94.94, 120.29, 144.51, 150.75, 154.76, 157.37. Anal. ($C_8H_{10}N_5O_5PNa_2 \cdot 3H_2O$) H, N; C: calcd 24.81; found 24.22.

1-[[[(Diethoxyphosphinyl)methoxy]methoxy]methyl]cytosine (23). To a suspension of 60% sodium hydride in mineral oil (700 mg, 17 mmol) in DMF (50 mL) was added cytosine (1.9 g, 17 mmol), and the mixture was heated at 80 °C for 2 h. To the resulting yellow solution was added dropwise a solution prepared from diethyl phosphite (2.4 g, 17 mmol) and bis(chloromethoxy)methane (12.5 g, 86 mmol) as described in the previous experiment, in DMF (10 mL). After the mixture was stirred for 15 h at 25 °C, the volatiles were removed in vacuo. The residue was dissolved in ethyl acetate (120 mL) and water (30 mL). The organic phase was washed with brine and dried ($MgSO_4$). After removal of the solvent in vacuo, the residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 1.2 g (22%) of **23** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.39 (t, J = 6.9 Hz, 6 H), 1.90 (br s, 2 H), 3.82 (d, J = 9.0 Hz, 2 H), 4.05–4.20 (m, 4 H), 4.75 (s, 2 H), 5.20 (s, 1 H), 5.86 (d, J = 7.4 Hz, 1 H), 7.31 (d, J = 7.4 Hz, 1 H).

1-[[(Phosphonomethoxy)methoxy]methyl]cytosine Disodium Salt (25). To a solution of **23** (1.2 g, 3.7 mmol) in DMF (5 mL) was added trimethylsilyl bromide (5 mL). After the mixture was stirred for 3 h at 25 °C, the volatiles were removed in vacuo, and the residue was neutralized to pH 8.0 by addition of aqueous saturated sodium bicarbonate. Water was then evaporated in vacuo, and the residue was purified by C_{18} reverse-phase column using water as eluent under 8 psi pressure to give 460 mg (47%) of **25** as a white solid: UV max (H_2O) 268 nm (ε 8245): ^1H NMR (D_2O) δ 3.56 (d, J = 9.0 Hz, 2 H), 4.85 (s, 2 H), 5.31 (s, 2 H), 6.03 (d, J – 7.3 Hz, 1 H), 7.71 (d, J = 7.3 Hz, 1 H); ^{13}C NMR (D_2O) δ 66.87, 68.87, 77.71, 96.14, 96.28, 98.18, 148.36, 160.41, 162.54. Anal. ($C_7H_{11}N_3O_6PNa_2 \cdot 3H_2O$) C, H, N.

[2-(Phenylselenyl)ethoxy]methyl Chloride (27). To a solution of 2-(phenylselenyl)ethanol (4.0 g, 20 mmol) in CH_2Cl_2 (15 mL) was added paraformaldehyde (620 mg, 20 mmol). HCl gas was then bubbled into the solution at 5 °C for 2 h. The solution was dried ($MgSO_4$), and the solvent was removed under reduced pressure to give **27** as a colorless oil in quantitative yield: ^1H NMR ($CDCl_3$) δ 3.06 (t, J = 7.0 Hz, 2 H), 3.88 (t, J = 7.0 Hz, 2 H), 5.45 (s, 2 H), 7.2–7.5 (m, 5 H).

2-(Acetylamino)-6-[(diphenylcarbamoyl)oxy]-9-[[2-(phenylselenyl)ethoxy]methyl]purine (28). A mixture of 2-(acetylamino)-6-(diphenylcarbamoyl)purine (36.7 g, 94.6 mmol) and *N,O*-bis(trimethylsilyl)acetamide (47.6 mL, 193 mmol) in dry dichloroethane (700 mL) was heated at 80 °C for 60 min. Volatiles were removed in vacuo, and the residue was evaporated with toluene twice. The silylated purine and mercuric cyanide (29.6 g, 117 mmol) in benzene (800 mL) were heated at reflux for 60 min, and then a solution of **27** (34 g, 94.5 mmol) in benzene (100 mL) was added dropwise. The mixture was refluxed for 4 h, and then allowed to stir for 15 h at 25 °C. The reaction was diluted with CH_2Cl_2 (500 mL) and quenched with aqueous saturated bicarbonate (1 L). The organic phase was washed with 2 N potassium iodide (200 mL) and dried, ($MgSO_4$) and the solvents were removed in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–5% MeOH as eluent to give 22 g (39%) of **28** as a slightly yellow powder: ^1H NMR ($CDCl_3$) δ 2.46 (s, 3 H), 2.95 (t, J = 6.9 Hz, 2 H), 3.72 (t, J = 6.9 Hz, 2 H), 5.48 (s, 2 H), 7.0–7.7 (m, 15 H), 8.0 (s, 1 H), 8.17 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.13, 26.19, 69.19, 72.60, 120.36, 126.97, 127.01, 129.17, 141.69, 143.73, 150.24, 152.49, 155.23, 156.28, 156.29, 170.79. Anal. Calcd for $C_{29}H_{25}N_6O_4Se$: C, 57.91; H, 4.36; N, 13.98. Found: C, 57.76; H, 4.46; N, 13.48.

2-(Acetylamino)-6-[(diphenylcarbamoyl)oxy]-9-[(vinyloxy)methyl]purine (29). To a solution of **28** (4.92 g, 8.16 mmol) in dioxane (80 mL) was added 30% H_2O_2 (4 mL, 35 mmol) and sodium bicarbonate (2.1 g, 24.5 mmol). The mixture was heated at 60 °C for 20 min. The reaction mixture was then concentrated to about 10 mL, diluted with ethyl acetate (100 mL), and dried ($MgSO_4$), and the solvents were removed in vacuo. The residue was dissolved in dioxane (40 mL), diisopropylethylamine (1.27 g, 10 mmol) was added, and the solution was heated at 80 °C for 30 min. The solvent was evaporated in vacuo, and the residual oil was chromatographed on silica gel using CH_2Cl_2–ethyl acetate (1:1) as eluent to give 2.3 g (65%) of **29** as a yellowish powder: ^1H NMR ($CDCl_3$) δ 2.49 (s, 3 H), 4.17 (dd, J = 2.7, 6.6 Hz, 1 H), 4.47 (dd, J = 2.7, 14.1 Hz, 1 H), 5.71 (s, 2 H), 6.39 (dd, J = 6.6, 14.1 Hz, 1 H), 7.0–7.5 (m, 10 H), 7.96 (s, 1 H), 7.99 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.12, 70.57, 92.43, 126.27, 126.34, 126.44, 126.50, 126.64, 141.63, 143.23, 148.93, 152.55, 170.51. Anal. ($C_{23}H_{20}N_6O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(Acetylamino)-6-[(diphenylcarbamoyl)oxy]-9-[[2-hydroxy-1-[(dimethoxyphosphinyl)methoxy]ethoxy]methyl]purine (30). To a suspension of **29** (1.0 g, 2.25 mmol) and dimethyl (hydroxymethyl)phosphonate (6 mL) in CH_2Cl_2 (6 mL) was added 80–85% *m*-chloroperbenzoic acid (611 mg, 3 mmol). After being stirred for 18 h at 25 °C, the clear solution was diluted with CH_2Cl_2 (100 mL) and washed with ice-cold 1 N NaOH (4 mL) and brine (20 mL). The organic phase was washed again with brine (20 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–5% MeOH as eluent to give 560 mg (42%) of **30** as a colorless oil: ^1H NMR ($CDCl_3$) δ 2.38 (s, 3 H), 3.58 (dd, J = 4.5, 12.5 Hz, 1 H), 3.72 (dd, J = 4.5, 12.5 Hz, 1 H), 3.77 (dd, J = 2.9, 10.7 Hz, 6 H), 3.80 (dd, J = 8.9, 14.0 Hz, 1 H), 4.0 (dd, J = 8.9, 14.0 Hz, 1 H), 4.91 (t, J = 4.9 Hz, 1 H), 5.65 (d, J = 10.8 Hz, 1 H), 5.72 (d, J = 10.8 Hz, 1 H), 7.0–7.4 (m, 10 H), 8.03 (s, 1 H), 8.66 (s, 1 H). Anal. Calcd for $C_{28}H_{29}N_6O_9P \cdot {}^1/_2CH_2Cl_2$: C. 49.22; H, 4.64; N, 13.00. Found: C, 49.87; H, 4.47; N, 12.76.

9-[[2-Hydroxy-1-[(methoxyhydroxyphosphinyl)methoxy]ethoxy]methyl]guanine Ammonium Salt (31). A solution of **30** (2.9 g, 4.8 mmol) in methanol (300 mL) and 28% NH_4OH (300 mL) was heated at 60 °C for 90 min. The solution was concentrated in vacuo, and the residual oil was purified by C_{18} reverse-phase column chromatography using water as eluent under 8 psi of pressure. The fractions having ultraviolet absorption were checked with HPLC, combined, and lyophilized to give 1.15 g (65%) of **31** as a white powder: UV max (H_2O) 252 nm (ε 13871); ^1H NMR (D_2O) δ 3.55 (d, J = 1.05 Hz, 3 H), 3.57 (dd, J = 4.8, 13.2 Hz, 1 H), 3.68 (dd, J = 9.3, 13.2 Hz, 1 H), 3.4–3.6 (m, 2 H), 4.84 (t, J = 3.9 Hz, 1 H), 5.57 (d, J = 11.4 Hz, 1 H), 5.64 (d, J = 11.4 Hz, 1 H), 7.93 (s, 1 H). Anal. ($C_{10}H_{19}N_6O_7P \cdot H_2O$) C, H, N.

9-[[2-Hydroxy-1-(phosphonomethoxy)ethoxy]methyl]guanine Disodium Salt (32). To a solution of **31** (780 mg, 2.0 mmol) in dry DMF (20 mL) was added at 5 °C trimethylsilyl bromide (8 mL, 60 mmol). After the mixture was stirred for 3 h at 5 °C, the volatiles were removed in vacuo and the residue was dissolved in aqueous saturated bicarbonate and reevaporated in vacuo to a solid. Purification of this material by C_{18} reverse-phase column chromatography using water as eluent under 8 psi pressure and lyophilization of combined fractions gave 520 mg (62%) of **32** as a white powder: UV max (H_2O) 252 nm (ε 15150); ^1H NMR (D_2O) δ 3.4–3.50 (m, 2 H), 3.61 (dd, J = 5.4, 11.6 Hz, 1 H), 3.70 (dd, J = 9.0, 11.6 Hz, 1 H), 4.88 (t, J = 4.5 Hz, 1 H), 5.67 (d, J = 11.3 Hz, 1 H), 5.73 (d, J = 11.3 Hz, 1 H), 7.99 (s, 1 H); ^{13}C NMR (D_2O) δ 63.27, 65.83, 67.83, 71.64, 104.56, 104.71, 117.98, 141.82, 153.52, 156.32, 161.13. Anal. ($C_8H_{12}N_5O_7PNa_2 \cdot 2H_2O$) C, H, N.

2-Amino-6-chloro-9-[[2-(phenylselenyl)ethoxy]methyl]purine (33). A mixture of 2-amino-6-chloropurine (20 g, 118 mmol) and ammonium sulfate (400 mg) in hexamethyldisilazane (400 mL) and chlorotrimethylsilane (6 mL) was heated at 145 °C for 5 h. Volatiles were removed in vacuo, and the residue was evaporated with xylene twice and further dried in vacuo for 3 h. The crude silylated 2-amino-6-chloropurine (15 g, 62 mmol) and mercuric cyanide (15 g, 59 mmol) in benzene (900 mL) was heated at reflux for 30 min, and then a solution of **27** (17 g, 68 mmol)

in benzene (100 mL) was added. The mixture was refluxed for 3 h and then allowed to stir for 15 h at 25 °C. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and quenched with aqueous saturated bicarbonate (2 L). The organic phase was washed with 2 N potassium iodide (200 mL) and dried ($MgSO_4$), and the solvents were removed in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–5% MeOH as eluent to give 15.0 g (63%) of **33** as a slightly yellow foam: ^1H NMR ($CDCl_3$) δ 2.96 (t, J = 6.9 Hz, 2 H), 3.70 (t, J = 6.9 Hz, 2 H), 5.20 (br s, 2 H), 5.42 (s, 2 H), 7.1–7.4 (m, 5 H), 7.81 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 26.04, 68.14, 72.71, 127.01, 128.65, 128.85, 131.23, 136.06, 144.95, 151.19, 151.83, 152.30. Anal. ($C_{14}H_{14}N_5OClSe \cdot {}^1/_2H_2O$) C, H, N.

2-(Acetylamino)-6-chloro-9-[[2-(phenylselenyl)ethoxy]methyl]purine (34). A solution of **33** (8 g, 21 mmol) in acetic anhydride (80 mL) was heated at 55 °C for 40 h. Volatiles were removed in vacuo, and the residual oil was purified by silica gel column chromatography using CH_2Cl_2–40% EtOAc as eluent to give 5.8 g (60%) of **34** as a yellow powder: ^1H NMR ($CDCl_3$) δ 2.49 (s, 3 H), 2.96 (t, J = 6.9 Hz, 2 H), 3.75 (t, J = 6.9 Hz, 2 H), 5.54 (s, 2 H), 7.2–7.4 (m, 5 H), 8.01 (s, 1 H). Anal. ($C_{16}H_{16}N_2O_2ClSe$) C, H; N: calcd 16.49; found 15.99.

2-(Acetylamino)-6-chloro-9-[(vinyloxy)methyl]purine (35). To a solution of **34** (424 mg, 1 mmol) in methanol (20 mL) were added sodium bicarbonate (92 mg, 1.1 mmol) and sodium periodate (320 mg, 1.5 mmol). After being stirred at 25 °C for 30 min, the mixture was filtered and evaporated to dryness. The residue was dissolved in dioxane (20 mL), and the solution was heated at 80 °C for 20 min. The solution was evaporated in vacuo, and the residual oil was chromatographed on silica gel using CH_2Cl_2–20% MeOH as eluent to give 220 mg (60%) of **35** as a slightly yellow foam: ^1H NMR ($CDCl_3$) δ 2.49 (s, 3 H), 4.19 (dd, J = 1.8, 4.8 Hz, 1 H), 4.51 (dd, J = 1.8, 10.6 Hz, 1 H), 5.24 (s, 2 H), 6.41 (dd, J = 4.8, 10.6 Hz, 1 H), 8.02 (s, 1 H), 8.20 (br s, 1 H). Anal. ($C_{10}H_{10}N_5O_2Cl$) C, H; N: calcd 26.17; found 26.65.

2-(Acetylamino)-6-chloro-9-[[1-[(dimethoxyphosphinyl)methoxy]ethoxy]methyl]purine (36). To a solution of **35** (2.2 g, 6.0 mmol) and dimethyl (hydroxymethyl)phosphonate (1.67 g, 12.0 mmol) in chloroform (100 mL) was added 120 mg of methanesulfonic acid. After the mixture was heated at 60 °C for 2 h, the solvent was removed in vacuo and the residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 1.2 g (50%) of **36** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.35 (d, J = 8.1 Hz, 3 H), 2.52 (s, 3 H), 3.85 (d, J = 16.2 Hz, 6 H), 5.03 (q, J = 8.1 Hz, 1 H), 5.65 (d, J = 15.6 Hz, 1 H), 5.79 (d, J = 15.6 Hz, 1 H), 7.28 (s, 1 H), 8.20 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 18.79, 25.05, 53.06, 53.22, 55.74, 59.06, 68.07, 99.25, 99.50, 127.92, 144.58, 151.60, 152.65, 170.30.

9-[[1-(Phosphonomethoxy)ethoxy]methyl]guanine Disodium Salt (37). To a solution of **36** (1.2 g, 2.95 mmol) in methanol (5 mL) was added 1 N sodium methoxide in methanol (10 mL). After the mixture was stirred at 25 °C for 1 h, water (10 mL) was added and the solution was heated at 90 °C for 1 h. Volatiles were removed in vacuo, and the residual oil was purified by C_{18} reverse-phase column chromatography using water as eluent under 8 psi of pressure. Each 10-mL fraction was assayed by high-pressure liquid chromatography. The combined fractions were lyophilized to give a white solid. This material was dissolved in DMF (20 mL) followed by trimethylsilyl bromide (5 mL). After the mixture was stirred for 2 h at 25 °C, the volatiles were removed in vacuo, and the residue was purified by C_{18} reverse-phase column chromatography using water as eluent under 8 psi of pressure to give 245 mg (24%) of **37** as a white powder: UV max (H_2O) 252 nm (ϵ 9751); ^1H NMR (D_2O) δ 1.20 (d, J = 6.3 Hz, 3 H), 3.31 (dd, J = 8.9, 8.4 Hz, 1 H), 3.50 (dd, J = 8.9, 8.4 Hz, 1 H), 4.87 (q, J = 6.3 Hz, 1 H). 5.48 (dd, J = 14.0. 11.1 Hz, 1 H), 5.52 (dd, J = 14.0, 11.1 Hz, 1 H), 7.79 (s, 1 H); ^{13}C NMR (D_2O) δ 20.86, 64.08, 66.09, 70.42, 102.05, 102.24, 119.11, 140.29, 153.11, 162.11, 168.71. Anal. ($C_9H_{12}N_5O_6PNa_2 \cdot 4H_2O \cdot 0.35NaBr$) C, H, N.

9-[(2-Hydroxy-1-methoxyethoxy)methyl]guanine (39). To a solution of **35** (1.29 g, 5.0 mmol) in CH_2Cl_2–MeOH (2:1, 60 mL) was added 80% 3-chloroperoxybenzoic acid (1.1 g, 5.1 mmol), and the mixture was stirred at 25 °C for 18 h. Volatiles were remove in vacuo, and the residue was chromatographed on silica gel using CH_2Cl_2–5% MeOH as eluent to give 1.5 g of **38** as a white powder. This material was dissolved in 1 N sodium methoxide (15 mL), and the solution was heated at reflux for 4 h. After adjusting the pH of the solution to 7.0 with 1 N HCl, the solution was heated at reflux for 2 h. All volatiles were removed in vacuo, and the residue was crystallized from hot water to give 497 mg (39%) of **39** as a colorless solid: mp >210 °C dec; UV max (H_2O) 252 (ϵ 12113), 276 nm (ϵ 8036); ^1H NMR (DMSO-d_6) δ 3.23 (dd, J = 5.3, 11.6 Hz, 1 H), 3.34 (dd, J = 5.3, 11.6 Hz, 1 H), 4.59 (t, J = 5.3 Hz, 1 H), 4.57 (d, J = 11.1 Hz, 1 H), 5.45 (d, J = 11.1 Hz, 1 H), 7.94 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 53.47, 61.72, 69.01, 103.08, 116.42, 137.59, 151.31, 154.16, 156.86. Anal. ($C_8H_{13}N_5O_4 \cdot {}^1/_2H_2O$) C, H, N.

Diethyl [4-Hydroxy-3-(hydroxymethyl)butyl]phosphonate (43). To a solution of 5-(2-bromoethyl)-2,2-dimethyl-1,3-dioxane (**40**) (6.7 g 30 mmol) in acetone (120 mL) was added sodium iodide (9.0 g, 60 mmol), and the mixture was stirred for 16 h at 25 °C. Acetone was evaporated in vacuo, and the residue was taken up in CH_2Cl_2–water. The organic layer was dried ($MgSO_4$) and evaporated to give **41** as a yellow oil. Without further purification, this material was dissolved in triethyl phosphite (8.3 g, 50 mmol), and the solution was heated at 140 °C for 40 min. The reaction mixture was then diluted with acetone (40 mL) and 2 N HCl (1 mL). After the mixture was stirred at 25 °C for 60 min, all volatiles were removed in vacuo. The crude residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 3.4 g (47%) of **43** as a colorless oil: ^1H NMR (CD_3OD) δ 1.28 (t, J = 7.2 Hz, 6 H), 1.4–2.1 (m, 5 H), 3.55 (d, J = 5.7 Hz, 4 H), 4.02–4.15 (m, 4 H). Anal. ($C_9H_{21}O_5P$) C, H, N.

Diethyl [4-Hydroxy-3-[diphenyl(4-methoxyphenyl)methoxy]butyl]phosphonate (44). To a solution of **43** (4.75 g, 198.8 mmol) in pyridine (20 mL) was added 4-methoxytriphenylmethyl chloride (7.6 g, 25 mmol), followed by addition of TEA (5.0 g, 50 mmol) and 4-(dimethylamino)pyridine (122 mg, 1 mmol). After the mixture was heated at 60 °C for 2 h, all volatiles were removed in vacuo. The residual oil was taken in CH_2Cl_2 and washed with water and brine. The CH_2Cl_2 was dried ($MgSO_4$), and concentrated to a brown oil, which was chromatographed on silica gel using CH_2Cl_2–3% MeOH as eluent to give 3.4 g (30%) of **44** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.31 (t, J = 6.9 Hz, 3 H), 1.39 (t, J = 6.9 Hz, 3 H), 1.63–1.83 (m, 5 H), 2.1–2.3 (br s, 1 H), 3.01 (dd, J = 6.3, 9.3 Hz, 1 H), 3.21 (dd, J = 4.5, 9.3 Hz, 1 H), 3.63 (m, 2 H), 3.77 (s, 3 H), 3.99–4.09 (m, 4 H), 6.8–7.4 (m, 14 H).

Diethyl [3-[[(Methanesulfonyl)oxy]methyl]-3-[diphenyl(4-methoxyphenyl)methoxy]butyl]phosphonate (45). To a solution of **44** (2.75 g, 5.36 mmol) and TEA (1.3 g, 13 mmol) in CH_2Cl_2 (35 mL) at –20 °C was added a solution of methanesulfonyl chloride (734 mg, 6.4 mmol) in CH_2Cl_2 (4 mL). After being stirred at –20 °C for 30 min and at 25 °C for 1 h, the reaction mixture was diluted with CH_2Cl_2, washed with 10% H_3PO_4, aqueous sodium bicarbonate, and brine, dried ($MgSO_4$), and evaporated in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–1% MeOH as eluent to give 3.0 g (93%) of **45** as a slightly yellow oil: ^1H NMR ($CDCl_3$) δ 1.30 (t, J = 6.9 Hz, 6 H), 1.4–1.8 (m, 4 H), 2.02 (m, 1 H), 2.89 (s, 3 H), 3.05 (dd, J = 6.2, 9.3 Hz, 1 H), 3.20 (dd, J = 4.2, 9.3 Hz, 1 H), 3.77 (s, 3 H), 3.95–4.09 (m, 4 H), 4.31 (d, J = 5.4 Hz, 2 H), 6.8–7.4 (m, 14 H). Anal. ($C_{30}H_{39}O_6PS$) C, H, N.

9-[2-(Hydroxymethyl)-4-phosphonobutyl]guanine Ammonium Salt (49). To a mixture of 2-amino-6-chloropurine (350 mg, 2.1 mmol) and **45** (1.18 g, 2.0 mmol) in DMF (40 mL) was added cesium carbonate (1.3 g, 4 mmol). After the mixture was stirred at 115 °C for 2 h, the volatiles were removed in vacuo. The residue was taken in CH_2Cl_2 (100 mL), washed with water and brine, dried ($MgSO_4$), and evaporated to dryness. The residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 780 mg (59%) of **46** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.22 (t, J = 6.7 Hz, 6 H), 1.5–1.9 (m, 4 H), 2.17 (m, 1 H), 2.90 (dd, J = 3.9, 7.0 Hz, 1 H), 3.02 (dd, J = 4.7, 7.0 Hz, 1 H), 3.74 (s, 3 H), 3.9–4.2 (m, 6 H), 5.24 (br s, 2 H), 6.8–7.5 (15 H).

To a solution of **46** (766 mg, 1.15 mmol) in EtOH (20 mL) was added 2 N HCl (20 mL), and the solution was heated at reflux for 5 h. All volatiles were removed in vacuo, and the residue was chromatographed on silica gel using CH_2Cl_2–15% MeOH as eluent to give 300 mg (70%) of **48** as a colorless oil. This material was dissolved in DMF (8 mL), followed by trimethylsilyl bromide (4 mL). After the mixture was stirred at 35 °C for 4 h, the pH of

the reaction was adjusted to 11 by addition of concentrated NH_4OH. All volatiles were removed in vacuo, and the residual solid was purified by C_{18} reverse-phase column chromatography using water as eluent to give 125 mg (55%) of **49** as an ammonium salt: UV max (H_2O) 254 (ε 12 140), 270 nm (ε 9058); ^1H NMR (D_2O) δ 1.4–1.8 (m, 4 H), 2.13 (n, 1 H), 3.51 (d, J = 6.1 Hz, 2 H), 4.14 (m, 2 H), 8.31 (s, 1 H); ^{13}C NMR (D_2O) δ 24.06, 27.66, 29.36, 42.95, 42.96, 47.10, 62.59, 113.82, 141.14, 152.50, 156.41, 159.05. Anal. ($C_{10}H_{19}N_6O_5P\cdot 4H_2O$) C, H, N.

9-[2-(Hydroxymethyl)-4-phosphonobutyl]adenine Ammonium Salt (50). To a mixture of adenine (600 mg, 4.4 mmol) and **45** (2.4 g, 4 mmol) in DMF (25 mL) was added cesium carbonate (1.5 g, 4.5 mmol). After the mixture was stirred at 110 °C for 2 h, the volatiles were removed in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 1.75 g (68%) of **47** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.21 (t, J = 6.7 Hz, 6 H), 1.5–1.8 (m, 4 H), 2.21 (br s, 1 H), 2.38 (dd, J = 3.7, 7.5 Hz, 1 H), 3.05 (dd, J = 3.0, 7.5 Hz, 1 H), 3.92 (s, 3 H), 3.9–4.3 (m, 6 H), 5.83 (br s, 2 H), 7.2–7.5 (m, 14 H), 7.55 (s, 1 H), 8.26 (s, 1 H). Deblocking of **47** in a similar manner described for **49** gave **50** (45% yield) as an ammonium salt: UV max (H_2O) 262 nm (ε 9328); ^1H NMR (D_2O) δ 1.4–1.8 (m, 4 H), 2.11 (br s, 1 H), 3.43 (dd, J = 4.5, 11.8 Hz, 1 H), 3.60 (dd, J = 4.5, 11.8 Hz, 1 H), 4.10 (dd, J = 6.7, 14.3 Hz, 1 H), 4.18 (dd, J = 7.6, 14.3 Hz, 1 H), 8.03 (s, 1 H), 8.06 (s, 1 H); ^{13}C NMR (D_2O) δ 24.28, 26.01, 27.77, 42.92, 43.14, 46.81, 62.61, 119.99, 144.67, 150.76, 153.82, 156.98. Anal. ($C_{10}H_{19}N_5O_4P\cdot 2H_2O$) C, H, N.

9-[2-(Hydroxymethyl)-4-phosphonobutyl]cytosine Ammonium Salt (52). To a mixture of cytosine (222 mg, 2.0 mmol) and **45** (880 mg, 1.4 mmol) in DMF (25 mL) was added cesium carbonate (652 mg, 2 mmol). After the mixture was stirred at 110 °C for 2 h, volatiles were removed in vacuo. The residual oil was chromatographed on silica gel using CH_2Cl_2–10% MeOH as eluent to give 700 mg (78%) of **51** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.26 (t, J = 7.2 Hz, 6 H), 1.5–1.9 (m, 4 H), 2.10 (br s, 1 H), 2.95 (dd, J = 4.2, 11.5 Hz, 1 H), 3.12 (dd, J = 4.5, 11.5 Hz, 1 H), 2.62 (dd, J = 7.5, 14.5 Hz, 1 H), 3.75 (s, 3 H), 3.92 (dd, J = 6.7, 14.5 Hz, 1 H), 3.95–4.15 (m, 4 H), 5.26 (d, J = 7.2 Hz, 1 H), 6.8–7.3 (m, 14 H), 7.37 (d, J = 7.2 Hz, 1 H). Deblocking of **51** in a similar manner described for **49** gave **52** (55% yield) as an ammonium salt: UV max (H_2O) 276 nm (ε 9084); ^1H NMR (D_2O) δ 1.4–1.8 (m, 4 H), 1.99 (br s, 1 H), 3.50 (dd, J = 4.6, 11.8 Hz, 1 H), 3.59 (dd, J = 4.6, 11.8 Hz, 1 H), 3.81 (d, J = 7.1 Hz, 2 H), 6.11 (d, J = 7.2 Hz, 1 H), 7.75 (d, J = 7.2 Hz, 2 H), 6.11 (d, J = 7.2 Hz, 1 H), 7.75 (d, J = 7.2 Hz, 1 H); ^{13}C NMR (D_2O) δ 24.20, 26.06, 27.82, 41.97, 53.03, 62.79, 96.75, 151.22, 154,37, 163.66. Anal. ($C_9H_{19}N_4O_5P\cdot H_2O$) C, H, N.

Plaque Assays. Experiments were conducted with vero cells infected with HSV-1 (BWS) and HSV-2 (G) or MRC-5 cells infected with HCMV (AD 169) and then treated with the phosphonate analogues as described previously.[11] Fifty percent inhibitory doses (ID_{50}) are defined as doses causing a 50% reduction in plaque numbers compared to untreated controls.

Animal Studies. Swiss–Webster female mice (Charles River, MA), weighing ca. 20 g each, were infected intraperitoneally with 5×10^4 plaque-forming units of HSV-1 (BWS). ACV and compound **20** were administered intraperitoneally twice a day for 5 days, starting 3 h postinfection. Deaths were recorded for 21 days after infection.

Crystallography of 14. A colorless platelet crystal of **14** having approximate dimensions of 0.06 × 0.22 × 0.35 mm was mounted on a glass fiber with its long axis roughly parallel to the phi axis of the goniometer. Preliminary examination and data collection were performed with Cu Kα radiation (λ = 1.541 84 Å) on an Enraf-Nonius CAD4 computer controlled κ axis diffractometer equipped with a graphite crystal, incident beam monochromator.

Cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 12 reflections in the range $10 < \theta < 20°$, measured by the computer-controlled diagonal slit method of centering. The monoclinic cell parameters and calculated volume are: a = 6.495 (2), b = 26.448 (4), and c = 8.217 (1) Å, β = 97.14 (1)°, V = 1400.6 (7) Å. For Z = 4 and FW = 299.29 the calculated density is 1.42 g/cm^3.

A total of 2489 reflections were collected, of which 2058 were unique and not systematically absent. As a check on crystal and electronic stability, three representative reflections were measured every 60 min. The slope of the least-squares line through a plot of intensity versus time was 0 (19) counts/hour which corresponds to a total gain in intensity of 0.1%. An anisotropic decay correction was applied. The correction factors on I ranged from 0.989 to 1.042 with an average value of 1.008.

The structure was solved by direct methods. Using 262 reflections (minimum E of 1.54) and 3474 relationships, a total of 10 phase sets were produced. A total of 22 atoms were located from an E map prepared from the phase set with probability statistics: absolute figure of merit = 1.10, residual = 15.30, and ψ_0 = 1.114. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were not included in the calculations. The structure was refined in full-matrix least-squares when the function minimized was $\Sigma W(|F_o| - |F_c|)^2$. Unit weights were used for all observed reflections.