Synthesis and Antiherpes Virus Activity of Phosphate and Phosphonate Derivatives of 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine[1]

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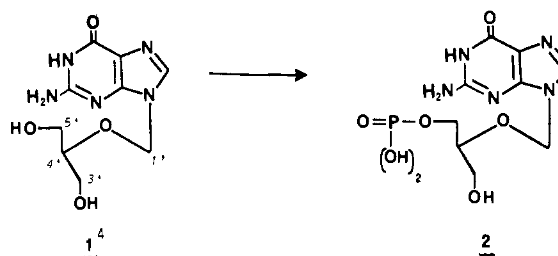
A series of phosphate esters of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, 1) were synthesized and evaluated for antiherpes virus activity. The cyclic phosphate esters were made by a new, efficient method utilizing stannic chloride as a solubilizing agent. Monophosphate **2** and bisphosphate **4** showed comparable activity to DHPG and probably acted as prodrugs of DHPG. On the other hand, the cyclic phosphate of DHPG **3** was taken up by cells and bypassed the virus-specified thymidine kinase. As a result, **3** was active against DHPG-resistant HSV mutants that lacked the viral-specified thymidine kinase and was more toxic than DHPG to uninfected cells. The phosphonate **5**, the least toxic of the derivatives tested, was only marginally active against HSV but showed substantial activity against human cytomegalovirus in vitro.

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (DHPG, 1),[3,4] an acyclic analogue of 2′-deoxyguanosine, is exceptionally potent against a broad range of herpes viruses including herpes simplex virus types 1 (HSV-1) and 2 (HSV-2),[3a,b,5–7] human cytomegalovirus (HCMV),[3b,5,6,8] Epstein-Barr virus (EBV),[3b,6,9] and varicella zoster virus (VZV).[3b,10] In HSV- and VZV-infected cells, DHPG is activated by phosphorylation by a virus-specified thymidine kinase to give DHPG monophosphate (**2**).[5,11] Inasmuch as DHPG is not phosphorylated very effectively by host enzymes, some selectivity is realized at this activation step. The monophosphate **2** is next converted by host-cell enzymes to DHPG triphosphate,[11] which, in turn, exerts the antiviral effect by inhibition of the viral DNA polymerase.[12,13] Because host polymerases are less sensitive than the viral polymerase to the triphosphate, selectivity is also realized at this stage.[12]



Numerous thymidine kinase deficient strains of herpes simplex virus have been characterized[6] and, in general, are resistant to DHPG. Cytomegalovirus (CMV) does not encode a thymidine kinase[14] but induces a higher level of host deoxyguanosine kinase.[15] Whether or not the Epstein-Barr virus (EBV) encodes a thymidine kinase is still unclear. What seems evident is that in the case of CMV and EBV a viral enzyme is not responsible for the phosphorylation of acyclic nucleosides[16] and the activity of DHPG against these viruses appears to be due to the low level of phosphorylation of DHPG by host enzymes. We have synthesized a number of phosphate esters and a phosphonate derivative of DHPG with the hope of bypassing the phosphorylation activation step. If this could be achieved, a broader spectrum antiviral agent could be discovered. However, inasmuch as most of the selectivity of DHPG is due to this activation in only infected cells, this new antiviral agent could be more toxic. We chose as target compounds the monophosphate **2**, cyclic phosphate **3**, bisphosphate **4**, and phosphonate **5**. Additionally the cyclic phosphates **6** and **7** were synthesized. Acyclic adenosine analogue **8** is not a substrate for the viral kinase,[18] but derivative **6** could bypass this step and continue

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(3) (a) Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759. Verheyden, J. P. H.; Martin, J. C. U.S. Patent 4355032, October 19, 1982. (b) Ashton, W. T.; Karkas, J. D.; Field, A. K.; Tolman, R. L. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1716. (c) Ogilvie, K. K.; Cheriyan, U. O.; Radatus, B. K.; Smith, K. O.; Galloway, K. S.; Kennell, W. L. *Can. J. Chem.* **1982**, *60*, 3005. (d) Schaeffer, H. J. In "Nucleosides, Nucleotides and their Biological Applications"; Rideout, J. L., Henry, D. W., Beacham, L. M., Eds.; Academic Press: New York, 1983; pp 1–17.
(4) The structural formulas of DHPG (1) and the related acyclic nucleoside analogues have been depicted in a "ribose-like" conformation only to draw attention to the similarity in structure between these compounds and 2′-deoxynucleosides. In accordance with this representation, the two terminal carbons of the glycerol moiety are referred to as the 3′- and 5′-positions.
(5) Smee, D. F.; Martin, J. C.; Verheyden, J. P. H.; Matthews, T. R. *Antimicrob. Agents Chemother.* **1983**, *23*, 676.
(6) Cheng, Y-C.; Huang, E-S.; Lin, J-C.; Mar, E-C.; Pagano, J. S.; Dutschman, G. E.; Grill, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2767.
(7) Smith, K. O.; Galloway, K. S.; Kennel, W. L.; Ogilvie, K. K.; Radatus, B. K. *Antimicrob. Agents Chemother.* **1982**, *22*, 55.
(8) Mar, E-C.; Cheng, Y-C.; Huang, E-S. *Antimicrob. Agents Chemother.* **1983**, *24*, 518.
(9) Lin, J-C.; Smith, M. C.; Pagano, J. S. *J. Virol.* **1984**, *50*, 50.
(10) Bryson, Y. J., UCLA School of Medicine, Los Angeles, CA, personal communication.
(11) (a) Cheng, Y-C.; Grill, S. P.; Dutschman, G. E.; Nakayama, K.; Bastow, K. F. *J. Biol. Chem.* **1983**, *258*, 12460. (b) Field, A. K.; Davies, M. E.; DeWitt, C.; Perry, H. C.; Liou, R.; Germershausen, J.; Karkas, J. D.; Ashton, W. T.; Johnston, D. B. R.; Tolman, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4139.
(12) St. Clair, M. H.; Miller, W. H.; Miller, R. L.; Lambe, C. U.; Furman, P. A. *Antimicrob. Agents Chemother.* **1984**, *25*, 191.
(13) Frank, K. B.; Chiou, J-F.; Cheng, Y-C. *J. Biol. Chem.* **1984**, *259*, 1566.
(14) Estes, J. E.; Huang, E-S. *J. Virol.* **1977**, *24*, 13.
(15) Meijer, H.; Bruggeman, C. A.; Dormans, P. H. J.; van Boven, C. P. A. *FEMS Microbiol. Lett.* **1984**, *25*, 283. Lewis, R. A.; Watkins, L.; St. Jeor, S. *Mol. Cell. Biochem.* **1985**, *65*, 67.
(16) Datta, A. K.; Pagano, J. S. *Antimicrob. Agents Chemother.* **1983**, *24*, 10.
(17) (a) Ashton, W. T.; Karkas, J. D.; Field, A. K.; Tolman, R. L. (Merck & Co., Inc.) Eur. Pat. Appl. EP 74306, 1983. (b) While this manuscript was in preparation, the antiviral activity of DHPG cyclic phosphate (3) was reported. Tolman, R. L.; Field, A. K.; Karkas, J. D.; Wagner, A. F.; Germershausen, J.; Crumpacker, C.; Scolnick, E. M. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 1329.
(18) Martin, J. C.; Jeffrey, G. A.; McGee, D. P. C.; Tippie, M. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1985**, *28*, 358.

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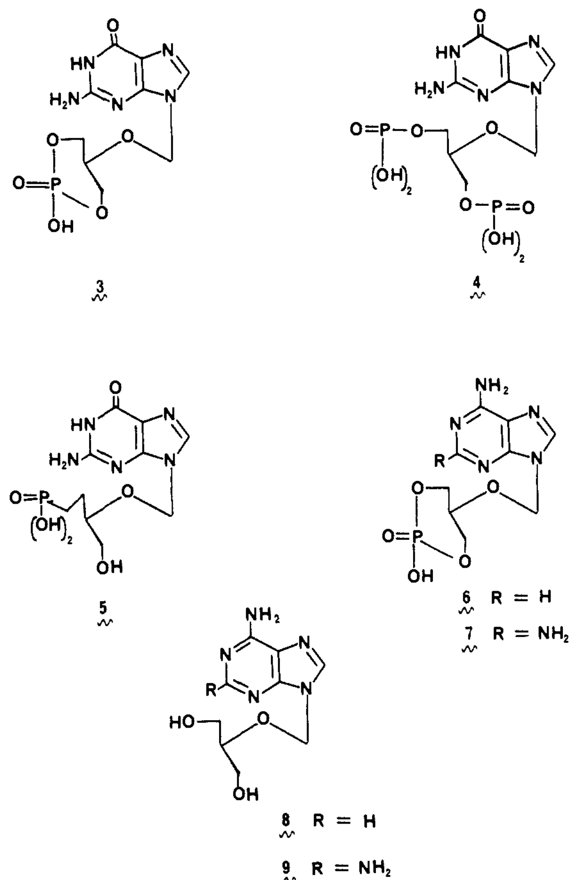
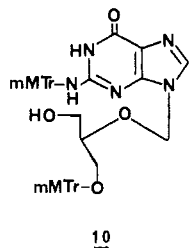
on to inhibit the viral DNA polymerase. Derivative **9** is a prodrug of DHPG[18] whose cyclic phosphate **7** might be converted to DHPG monophosphate by phosphodiesterase-catalyzed hydrolysis followed by deamination by adenylate deaminase.



Chemistry

The syntheses of DHPG monophosphate (**2**) and DHPG cyclic phosphate (**3**) have been reported in the patent literature.[17a,b] These esters were isolated in very low yield when DHPG (**1**) was treated directly with a mixture of phosphorus oxychloride and triethyl phosphate. We devised new syntheses that are more efficient and require less tedious purifications.

In order to prepare monophosphate **2**, DHPG (**1**) was first protected as the bis(monomethoxytrityl) derivative **10** in 43% yield. Phosphorylation of **10** with cyanoethyl phosphate/dicyclohexylcarbodiimide followed by treatment with ammonium hydroxide and then aqueous acetic acid gave **2** as the diammonium salt in 66% yield. This is actually a racemic mixture of what we refer to as the 3'- and 5'-monophosphates.

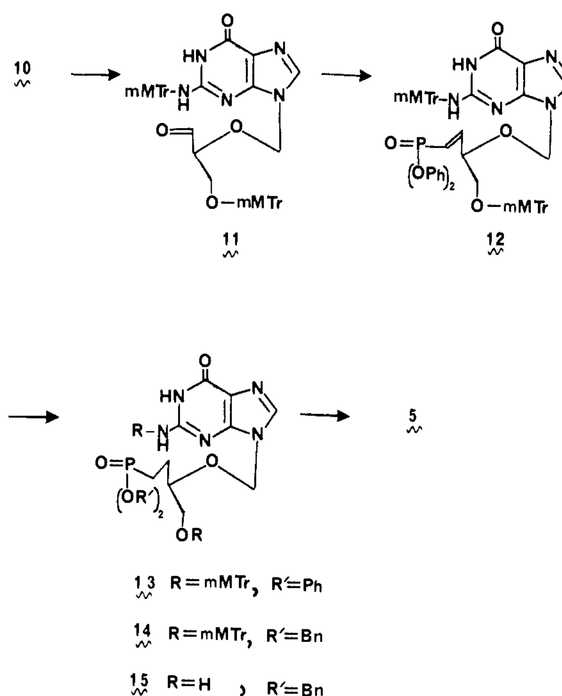


DHPG cyclic phosphate (**3**) was prepared from monophosphate **2** by a standard procedure[19] using dicyclohexylcarbodiimide in refluxing pyridine. Also, **3** was prepared directly from DHPG (**1**) by a new method. DHPG was first solubilized in a dilute acetonitrile solution with stannic chloride and then phosphorylated by slow addition of pyrophosphoryl chloride. After quenching and desalting using a charcoal column, the cyclic phosphate was isolated in 50% yield. (The previously reported yield of this compound was 7%[17b].) The stannic chloride procedure was also used to synthesize cyclic phosphates **6** and **7** from the adenine and 2,6-diaminopurine acyclic nucleosides **8** and **9**, respectively. The use of Lewis acids to solubilize nucleosides or their analogues prior to derivatization is heretofore unprecedented although we have noted in earlier work[20] that stannic chloride will facilitate the dissolution of many heterocycles in nonpolar solvents. This enhanced solubility is probably due to the formation of a σ-complex between the stannic chloride and the heterocycle in a similar fashion as was reported for σ-complex formation between silylated nucleoside bases and Friedel–Crafts catalysts.[21]

If the same phosphorylation is attempted without stannic chloride present, the mostly insoluble DHPG is first slowly converted to the monophosphate, which then enters solution where it is quickly phosphorylated again to form a bisphosphate derivative. Accordingly, the bisphosphate **4** was prepared in 74% yield by the treatment of a suspension of DHPG in ethyl acetate with pyrophosphoryl chloride.

Deoxy-DHPG phosphonate **5** was synthesized from alcohol **10** as starting material (Scheme I). Oxidation of **10** gave aldehyde **11**, which was converted by the procedure of Jones and Moffatt[22] to the unsaturated phosphonate **12** in 43% overall yield. Next, diimide reduction of **12** gave a 99% yield of saturated phosphonate **13**. Brief reaction of **13** with sodium benzyl oxide in Me_2SO furnished dibenzyl ester **14** (78%), which in turn was treated with 80% acetic acid at 80 °C to give **15** (65%). Catalytic transfer

Scheme I



(19) Smith, M.; Drummond, G. I.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 698.

(20) (a) Prisbe, E. J.; Smejkal, J.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1976**, *41*, 1836. (b) Prisbe, E. J.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1978**, *43*, 4774.

(21) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256.

(22) Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.

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

virus or cell	ID_{50},[a] μM						
	1	2	3	4	5	6	7
HSV-1 (F)[b]	0.2	0.2	2	0.4	28	600	125
HSV-2 (G)	1.6	1.7	6	5	>100	>1000	>100
HCMV (AD-169)	5	12	6	8	10		
HSV-1 (FΔ305)[c]	10	6	0.5	13	50		80
HSV-1 (MP-PAA^r/DHPG^r)[c]	125	>200	7	>200	>200		
Vero cells	450	700	40	800	>1000		

[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 parentheses. [c] Thymidine kinase mutants.

Table II. Effects of Oral Treatment with Acyclic Nucleotide Analogues on HSV-2-Induced Mortality in Mice

compd	mg/kg[a]	survivors/total	survivor increase[b]	mean survival time, days	mean survival time increase[c]
saline		2/20 (10)[d]		9.3 ± 2.8[e]	
1	20	11/20 (55)	0.005	11.9 ± 2.3	<0.01
	10	6/20 (30)	NS	10.9 ± 2.0	<0.025
	5	0/19 (0)	NS	11.3 ± 2.3	<0.01
2	20	12/20 (60)	0.002	10.5 ± 2.4	<0.001
	10	2/20 (10)	NS[f]	11.3 ± 2.6	<0.01
	5	1/19 (5)	NS	10.0 ± 1.8	NS
3	20	10/20 (50)	0.01	11.6 ± 1.7	<0.01
	10	3/20 (15)	NS	10.6 ± 3.0	NS
	5	8/20 (40)	NS	10.4 ± 1.9	NS
4	20	6/20 (30)	NS	10.6 ± 1.5	<0.02
	10	4/20 (20)	NS	10.8 ± 2.5	<0.05
	5	8/20 (40)	NS	9.6 ± 1.2	NS
5	20	3/20 (15)	NS	8.3 ± 0.9	NS
	10	0/20 (0)	NS	8.3 ± 1.3	NS
	5	3/20 (15)	NS	8.2 ± 0.9	NS

[a] Mice were infected intraperitoneally, and an oral dose was administered at 24-h intervals for 4 days, starting 24-h postinfection. [b] Probability (Fisher exact text). [c] Probability (Mann-Whitney U test). [d] Percent survivors. [e] Standard deviation. [f] Not statistically significant ($p > 0.05$).

Table III. Effect of Subcutaneous Treatment with 7 and DHPG (1) and DHPG (1) on HSV-2-Induced Mortality in Mice

compd	mg/kg[a]	survivors/total	survivor increase[b]	mean survival time, days	mean survival time increase[c]
saline		1/15 (7)[d]		8.6 ± 2.1[e]	
1	15	15/15 (100)	<0.001	>21	<0.001
	5	10/15 (67)	<0.001	13.8 ± 3.1	<0.001
	1.5	8/15 (53)	0.01	12.4 ± 2.3	<0.001
7	30	2/15 (13)	NS[f]	12.2 ± 3.1	0.01
	15	0/15 (0)	NS	11.5 ± 3.2	0.025
	5	2/15 (13)	NS	8.7 ± 1.5	NS

[a] Mice were infected intraperitoneally, and a subcutaneous dose was administered at 24-h intervals for 4 days, starting 24-h postinfection. [b] Probability (Fisher exact text). [c] Probability (Mann-Whitney U test). [d] Percent survivors. [e] Standard deviation. [f] Not statistically significant ($p > 0.05$).

hydrogenation (20% $Pd(OH)_2/C$, cyclohexene, ethanol, water, reflux) gave 5 in 88% yield. Without the water cosolvent, this reduction did not go to completion because of the insolubility of the intermediate monobenzyl ester. As was the case for the monophosphate 2, the phosphonate 5 is a racemic mixture.

Biological Results and Discussion

The acyclic nucleotide derivatives were first evaluated in vitro (Table I). The phosphate esters of DHPG (2–4) showed comparable activity to DHPG (1) against HSV-1, HSV-2, and HCMV. However, the phosphonate 5 was only active against HCMV. This is consistent with the observation that the triphosphate derivative of 5 is a poor inhibitor of HSV-1 and HSV-2 DNA polymerase but a moderate inhibitor of the HCMV DNA polymerase.[23] The cyclic phosphate 3 was the only ester active against thymidine kinase negative HSV-1 strains (FΔ305 and MP-PAA^r/DHPG^r). This result indicates that only 3 penetrates the cell membrane and bypasses the kinase, whereas 2 and 4 probably act only as prodrugs of DHPG (i.e., they are hydrolyzed before entering the cell). Cyclic phosphates 6 and 7 showed only marginal in vitro activity against HSV. The activity of DHPG cyclic phosphate parallels reports of other antiviral nucleosides maintaining good activity when derivatized as their 3′,5′-cyclic phosphates.[24]

In connection to its ability to bypass the viral thymidine kinase, cyclic phosphate 3 is also 10 times more toxic than DHPG to Vero cells. The low toxicity for 2 and 4 is probably indicative of the low permeability of the cell membrane to these charged species. The least toxic analogue was the phosphonate 5.

The phosphates 2–4 were also effective against HSV-2-induced mortality in mice (Table II). Both the monophosphate 2 and the cyclic phosphate 3 were comparable to DHPG in giving approximately a 50% survival rate at a dose of 20 mg/kg per day. The bisphosphate 4 did not

(23) Duke, A. E.; Smee, D. F.; Chernow, M.; Boehme, R.; Matthews, T. R., manuscript submitted for publication.

(24) Beres, J. B.; Bentrude, W. G.; Kruppa, G.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* 1985, *28*, 418 and references therein.

significantly increase the number of survivors but did prolong the mean survival time. Paralleling its low activity against HSV-2 in vitro, the phosphonate **5** was also inactive in vivo even at the 20 mg/kg per day dose.

Previous work has demonstrated that although diaminopurine analogue **9** is substantially less active than DHPG (**1**) in vitro, it is nearly equally potent in vivo.[18] For this reason, phosphate **7** was also evaluated in the above mouse HSV-2 model (Table III). Analogue **7** was much less effective than DHPG in increasing the number of survivors but did significantly increase the mean survival time.

Experimental Section

General Methods. Nuclear magnetic resonance spectra were recorded on a Bruker WM-300 (^{1}H NMR, 300 MHz) spectrometer, and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. For peak assignment, the carbons of the side chain are numbered like guanosine. UV spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. All chromatographic purifications were carried out on silica gel. Melting points were determined on a hot-stage microscope and are corrected.

***N*^{2}-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]guanine (10).** A mixture of **1** (8.18 g, 32 mmol), *p*-anisylchlorodiphenylmethane (21.7 g, 70 mmol), triethylamine (13.3 mL, 95 mmol), and 4-(dimethylamino)pyridine (0.08 g, 0.7 mmol) in DMF (100 mL) was stirred under anhydrous conditions at 40 °C for 2 h and then methanol (10 mL) was added, and the solvents were evaporated. The residue was dissolved in ethyl acetate, washed with saturated, aqueous $NaHCO_3$, and water, dried over $MgSO_4$, and evaporated. The resulting oil was chromatographed (1:14 methanol/dichloromethane) and the product crystallized from ethanol to give 11.2 g (43%) of **10**: mp 159–160 °C; UV λ_{max} (methanol) 279 nm (ϵ 13 000), 260 (12 000); ^{1}H NMR (Me_2SO-d_6) δ 7.75 (br s, 1 H, H-8), 7.35, 7.20, 7.03, 6.83, 6.64 (m, 28 H, aromatic), 5.03, 4.94 (AB, J = 11 Hz, 2 H, H-1′), 3.77, 3.59 (s, 3 H, OCH_3), 3.47 (m, 1 H, H-4′), 2.95, 3.05 (ABM, 2 H, J = 1, 3, 11 Hz, (CH_2OH), 2.77, 2.60 (ABM, 1 H, J = 1, 5, 11 Hz, CH_2OMTr). Anal. ($C_{49}H_{45}N_5O_6 \cdot 0.5H_2O$) C, H, N.

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine Monophosphate Diammonium Salt (2). A mixture of **10** (1.70 g, 2.1 mmol) and dicyclohexylcarbodiimide (2.68 g, 13 mmol) in 0.13 M cyanoethyl phosphate in pyridine (50 mL) was stirred at room temperature for 2.5 days and then quenched by the addition of water (10 mL). The solution was evaporated to dryness, and the residue was treated with concentrated ammonium hydroxide (80 mL) for 2 h at 60 °C and then evaporated to dryness. The residue was dissolved in 80% aqueous acetic acid and heated at 80 °C for 2 h and then left at 21 °C for 16 h before evaporation to dryness. The residue was taken in H_2O (80 mL) and the solution extracted with dichloromethane (3 × 50 mL). The aqueous phase was filtered, concentrated to ~10 mL, basified with concentrated ammonium hydroxide, and applied onto a column (5.5 cm × 40 cm) of Sephadex G-10 gel. The product was eluted with water and crystallized from H_2O/EtOH to give 520 mg (66%) of **2**. An analytical sample was obtained by conversion of **2** to the barium salt: mp >300 °C; UV λ_{max} (H_2O) 252 nm (ϵ 10 600), 272 (7600); ^{1}H NMR (D_2O) δ 7.97 (br s, 1 H, H-8), 5.62 (s, 2 H, H-1′), 3.87 (m, 3 H, H-4′, H-5′), 3.63 (ABX, 1 H, J = 3.5, 12.5 Hz, H-3′A), 3.55 (ABX, 1 H, J = 5.5, 12.5 Hz, H-3′B). Anal. ($C_9H_{12}N_5O_7P$-$Ba \cdot 6H_2O$) C, N; H: calcd, 4.18; found, 3.47.

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine Cyclic Phosphate (3). Monophosphate **2** (520 mg, 1.4 mmol) was converted to the free acid by passage through a column of Dowex 50-X8 cation-exchange resin (H^+ form) (45 mL) with water (1 L) as eluent. The residue, left on evaporation of the water, was dissolved in a mixture of pyridine (25 mL) and water (10 mL) containing *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine (350 mg) and then evaporated in vacuo. The residue was coevaporated three times with 10-mL portions of pyridine. A solution of the resulting foam in pyridine (150 mL) was added dropwise over 1 h to a refluxing solution of dicyclohexylcarbodiimide (500 mg, 2.4 mmol) in pyridine (120 mL). When addition was complete, the mixture was refluxed an additional 2.5 h before being evaporated in vacuo to a white solid. A solution of the solid in water (100 mL) was extracted three times with 10% butanol in dichloromethane (v/v, 30 mL) and then once with diethyl ether (30 mL). The aqueous solution was concentrated in vacuo to ~5 mL and applied onto a column (2.5 cm × 40 cm) of DEAE-Sephadex A-25 gel in the carbonate form. The column was eluted with a linear gradient consisting of 1 L of water and 1 L of 0.6 M triethylammonium bicarbonate (pH 7.6). Eluent containing pure product was lyophilized, leaving 282 mg of amorphous solid. The solid was dissolved in water (2 mL) and applied on a column of Dowex 50-8X cation-exchange resin (5 mL), which was then washed with water until the eluent was devoid of UV absorbance (~200 mL). The washings were basified with concentrated ammonium hydroxide and then evaporated in vacuo. The residue was crystallized from water/ethanol to give 203 mg (43%) of **3** as the ammonium salt: mp 230–233 °C dec; UV λ_{max} (H_2O) 252 nm (ϵ 13 300), 270 sh (9400); ^{1}H NMR (D_2O) δ 7.94 (s, 1 H, H-8), 5.61 (s, 2 H, H-1′), 4.18–4.39 (m, 4 H, H-3′, H-5′), 3.82 (br s, 1 H, C-4′). Anal. ($C_9H_{15}N_6O_6P \cdot 1.5H_2O$) C, H, N.

Synthesis of 3 from 1. To a stirred suspension of DHPG (2.0 g, 7.9 mmol) in dry acetonitrile (1.5 L) was added stannic chloride (1.3 mL, 11 mmol). The mixture was stirred at room temperature for 1 h during which time all DHPG dissolved. To this stirred solution was then added, dropwise over 1.5 h, a solution of pyrophosphoryl chloride (3.4 mL, 23 mmol) in acetonitrile (0.6 L). When addition was complete, the solution was stirred an additional 2 h at room temperature then neutralized (pH ~6) by the addition of saturated aqueous $NaHCO_3$ solution and filtered. The filtrate was concentrated in vacuo to ~50 mL and applied to a column of activated charcoal (Aldrich) (115 g). The column was first washed with 4 L of water to remove inorganic salts and then with 5% ammonium hydroxide in 50% aqueous ethanol to elute the desired cyclic phosphate. Eluent containing pure DHPG cyclic phosphate was pooled and evaporated in vacuo. The residue was crystallized from water/ethanol to give pure DHPG cyclic phosphate ammonium salt **3** (1.30 g) (50%), which was identical with the product derived from **2**.

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine Bisphosphate (4). To a 0 °C, stirred suspension of **1** (500 mg, 2.0 mM) in ethyl acetate (20 mL) was added pyrophosphoryl chloride (1.5 mL, 10 mM) dropwise over 3 min. After 2 h, the now clear reaction mixture was quenched by the addition of ice (~10 g) and then neutralized with solid $NaHCO_3$. The aqueous phase was isolated and purified on a column of Sephadex G-10 gel, using water as eluent. Evaporation of appropriate fractions gave a syrupy residue, which crystallized from dimethylformamide. Rinsing the crystals with methanol and drying in vacuo gave 725 mg (74%) of the pure bisphosphate **4** as the tetrasodium salt. An analytical sample was obtained by conversion of **4** to the barium salt: mp >300 °C; UV λ_{max} (H_2O) 251 nm (ϵ 12 900), 270 sh (ϵ 9220); ^{1}H NMR (D_2O) δ 8.04 (s, 1 H, H-8), 5.69 (s, 2 H, H-1′), 4.03 (br s, 1 H, H-4′), 3.85 (m, 4 H, H-3′, H-5′). Anal. ($C_9H_{11}N_5O_{10}P_2Ba_2 \cdot H_2O$) C, H, N.

2,6-Diamino-9-[(1,3-dihydroxy-2-propoxy)methyl]purine Cyclic Phosphate (7). A mixture of **9**[18] (345 mg, 1.36 mmol), acetonitrile (100 mL), and stannic chloride (222 μL, 1.90 mmol) was stirred at room temperature until dissolution had occurred (0.5 h). To this solution $POCl_3$ (416 mg, 252 μL, 2.70 mmol) in acetonitrile (30 mL) was added dropwise over 0.5 h. The reaction was quenched after 5 h with saturated aqueous $NaHCO_3$, the slurry filtered through Celite, and the filtrate evaporated to dryness. The residue was chromatographed over charcoal, eluting with water and then 5% NH_4OH in 1:1 water/ethanol, to give the crude cyclic phosphate, which crystallized in two crops from ethanol/water to give 160 mg (35%) of **7**. An analytical sample, as the free acid, was crystallized from water: mp >300 °C; UV λ_{max} (0.1 N HCl) 252 nm (ϵ 10 500), 292 (8990); UV λ_{max} (0.1 N NaOH) 256 (ϵ 9390), 280 (10 100); ^{1}H NMR (Me_2SO-d_6) δ 7.84 (s, 1 H, H-8), 6.72 (br s, 2 H, NH_2), 5.85 (br s, 2 H, NH_2), 5.42 (s, 2 H, H-1′), 3.98 (ddd, 2 H, J = 9, 9, 2.5 Hz, H-3′ eq, H-5′ eq), 3.83 (ddd, 2 H, J = 15, 9, 4 Hz, H-3′ ax, H-5′ ax), 3.57 (br s, 1 H, H-4′). Anal. ($C_9H_{13}N_6O_5P \cdot 0.5H_2O$) C, H, N.

9-[(1,3-Dihydroxy-2-propoxy)methyl]adenine Cyclic Phosphate Sodium Salt (6). A mixture of **8**[18] (400 mg, 1.67 mmol), stannic chloride (0.30 mL, 2.67 mmol), and acetonitrile

(300 mL) was stirred until dissolution occurred (0.5 h). A solution of pyrophosphoryl chloride (0.34 mL, 2.3 mmol) in acetonitrile (120 mL) was added dropwise over 1 h. After an additional 2 h, the reaction was neutralized with saturated aqueous $NaHCO_3$, filtered, and evaporated to dryness. The residue was chromatographed over charcoal, eluting with water and then 5% NH_4OH in 1:1 water/ethanol, to give after crystallization from water/ethanol 307 mg (57%) of **6**: mp 239–240 °C; UV λ_{max} (0.1 N HCl) 257 nm (ϵ 13400), (0.1 N NaOH), 260 (13800); ^{1}H NMR (Me_2SO-d_6) δ 8.29 (s, 1 H, H-2), 8.17 (s, 1 H, H-8), 7.30 (br s, 2 H, NH_2), 5.61 (s, 2 H, H-1′), 3.97 (ddd, 2 H, J = 9, 9, 2.5 Hz, H-3′ eq, H-5′ eq), 3.87 (ddd, 2 H, J = 15, 9, 4 Hz, H-3′ ax, H-5′ ax), 3.60 (br s, 1 H, H-4′). Anal. ($C_9H_{11}N_5O_5PNa{\cdot}H_2O$) C, H, N.

N^2-(*p*-Anisyldiphenylmethyl)-9-[[[4-(*p*-anisyldiphenylmethoxy)-1(*E*)-(diphenoxyphosphinyl)but-1-en-3-yl]oxy]methyl]guanine (12). A solution of **10** (9.5 g, 11.6 mmol), dicyclohexylcarbodiimide (15.0 g, 73 mmol), and methylphosphonic acid (0.50 g, 5.8 mmol) in Me_2SO (300 mL) was stirred at 18 °C for 5 h and then at room temperature for 16 h. The mixture was cooled in an ice–water bath and oxalic acid dihydrate (0.25 g) in methanol (15 mL) was added. The mixture was filtered and the Me_2SO was removed from the filtrate by kugelrohr distillation [60 °C (1 mm)]. The residue was chromatographed (1:19 methanol/dichloromethane) to give 9.5 g (quantitative) of aldehyde **11**.

A solution of **11** (9.5 g, 11.6 mmol), diphenyl [(triphenylphosphoranylidene)methyl]phosphonate (5.6 g, 11.5 mmol), and triethylamine (2.2 mL, 17 mmol) in THF (150 mL) was stirred at room temperature for 7 days. The solution was then diluted with ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed (1:24 methanol/dichloromethane) and the product crystallized from ethanol to give 5.17 g (43%) of **12**: mp 189–191 °C; UV λ_{max} (methanol) 261 nm (ϵ 16500), 232 (30000); ^{1}H NMR ($CDCl_3$) δ 6.5–7.3 (m, 39 H, aromatic, H-8), 6.22 (ddd, J = 3, 17, 23 Hz, 1 H, H-5′), 5.95 (ddd, J = 1.5, 17, 23 Hz, 1 H, H-6′), 4.94 and 4.78 (AB, J = 12 Hz, 2 H, H-1′), 4.06 (m, 1 H, H-4′), 3.72 (s, 3 H, OCH_3), 3.50 (s, 3 H, OCH_3), 2.77 (m, 2 H, H-3′). Anal. ($C_{62}H_{54}N_5O_8P$) C, H, N, P.

N^2-(*p*-Anisyldiphenylmethyl)-9-[[4-(*p*-anisyldiphenylmethoxy)-1-(diphenoxyphosphinyl)-3-butoxy]methyl]guanine (13). A solution of **12** (4.78 g, 4.6 mmol) and potassium azodicarboxylate (9.2 g, 4.7 mmol) in pyridine (120 mL) and acetic acid (5.3 mL) was stirred at room temperature for 6 days and then evaporated to dryness. A solution of the residue in dichloromethane was washed with water, dried over Na_2SO_4, and then evaporated to dryness. The product was crystallized from ethyl acetate to give 4.21 g (88%) of **13**: mp 202–204 °C; UV λ_{max} (methanol) 261 nm (ϵ 16600), 232 (30200); ^{1}H NMR (Me_2SO-d_6) δ 7.80 (s, 1 H, H-8), 7.69 (br s, 1 H, NH), 6.6–7.4 (m, 38 H, aromatic), 5.12 and 4.82 (AB, J = 12 Hz, 2 H, H-1′), 3.77 (s, 3 H, OCH_3), 3.56 (s, 3 H, OCH_3), 3.25 (m, 1 H, H-4′), 2.71 and 2.59 (m, 2 H, H-3′), 1.30–1.70 (m, 4 H, H-5′, 6′). Anal. ($C_{62}H_{56}N_5O_8P$) C, H, N, P.

N^2-(*p*-Anisyldiphenylmethyl)-9-[[4-(*p*-anisyldiphenylmethoxy)-1-[bis(benzyloxy)phosphinyl]-3-butoxy]methyl]guanine (14). To a solution of NaH (0.74 g, 50%, 15 mmol; prewashed with hexane) and benzyl alcohol (3.2 mL, 31 mmol) in dry Me_2SO (40 mL) was added with stirring a solution of **13** (3.94 g, 3.8 mmol) in Me_2SO (20 mL). After 3 min, the mixture was diluted with ethyl acetate, washed twice with 1% aqueous NH_4Cl, dried (Na_2SO_4), and evaporated. The residue was chromatographed (1:14 methanol/dichloromethane) and the product crystallized from ethanol to give 3.18 g (78%) of **14**: mp 152–155 °C; ^{1}H NMR (Me_2SO-d_6) δ 10.65 (br s, 1 H, NH), 7.78 (s, 1 H, H-8), 7.68 (br s, 1 H, NH), 6.6–7.4 (m, 38 H, aromatic), 4.82–5.08 (m, 6 H, H-1′, benzylic), 3.76 (s, 3 H, OCH_3), 3.55 (s, 3 H, OCH_3), 3.18 (m, 1 H, H-4′), 2.66 and 2.32 (m, 2 H, H-3′), 1.15–1.46 (m, 4 H, H-5′, 6′). Anal. ($C_{64}H_{60}N_5O_8P$) C, H, N, P.

9-[[1-[Bis(benzyloxy)phosphinyl]-4-hydroxy-3-butoxy]methyl]guanine (15). A solution of **14** (3.14 g, 3 mmol) in 80% aqueous acetic acid (100 mL) was heated at 80 °C for 2 h and then evaporated to dryness. The residue was triturated with 1:2 ethyl acetate/hexane and then chromatographed (1:6 methanol/dichloromethane). The product was crystallized from ethanol to give 0.99 g (65%) of **15**: mp 152–153 °C, UV λ_{max} (methanol) 270 sh nm (ϵ 10000), 255 (14100); ^{1}H NMR (Me_2SO-d_6) δ 7.80 (s, 1 H, H-8), 7.28–7.40 (m, 10 H, phenyl), 6.50 (br s, 2 H, NH_2), 5.37, 5.39 (AB, J = 11 Hz, 1 H, H-1′), 5.84–5.97 (m, 4 H, benzylic), 4.77 (t, J = 5 Hz, 1 H, OH), 3.54 (m, 1 H, H-4′), 3.35 (m, 2 H, H-3′), 1.40–1.78 (m, 4 H, H-5′, H-6′). Anal. ($C_{24}H_{28}N_5O_6P$) C, H, N, P.

9-[[1-(Dihydroxyphosphinyl)-4-hydroxy-3-butoxy]methyl]guanine (5). A suspension of **15** (740 mg, 1.44 mmol) and 20% $Pd(OH)_2/C$ (0.50 g) in cyclohexene (7.5 mL), ethanol (15 mL), and water (15 mL) was heated at reflux for 4 h and then filtered through Celite. After evaporation of the solvent, the residue was crystallized from water/ethanol to give 423 mg (88%) of **5**: mp >300 °C; UV λ_{max} (0.1 N HCl) 280 sh nm (ϵ 7890), 256 (11600); UV λ_{max} (0.1 N NaOH) 267 nm (ϵ 7890); ^{1}H NMR (Me_2SO-d_6) δ 7.87 (s, 1 H, H-8), 5.44 and 5.45 (AB, J = 11 Hz, 1 H, H-1′), 3.60 (m, 1 H, H-4′), 3.30–3.47 (m, 2 H, H-3′), 1.20–1.75 (m, 4 H, H-5′, H-6′). Anal. ($C_{10}H_{16}N_5O_6P{\cdot}H_2O$) C, H, N.

Plaque Assays. Experiments were conducted with Vero cells infected with HSV-1 (F-strain) and HSV-2 (G-strain) or MRC-5 cells with HCMV (AD 169) and then treated with the nucleoside analogue as described previously.[5] 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 (Simonsen Laboratories, Gilroy, CA), weighing ca. 20 g each, were infected intraperitoneally with 5×10^4 plaque forming units of HSV-2 (strain G). This challenge was approximately equivalent to ten 50% lethal doses. DHPG and derivatives were administered subcutaneously once a day for 4 days, starting 24 h postinfection. Deaths were recorded for 21 days after infection.

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