

radiation chemical yield measured in mol J⁻¹).

γ-Radiolysis. Quinone solutions were first saturated with N₂O gas in syringes as above, before being irradiated by a ⁶⁰Co source. Dose rates of 36 Gy min⁻¹ were used, as measured by Fricke dosimetry.³⁰ Ion-selective electrodes for bromide (Russell pH Ltd.) and chloride (E.I.L.) were then used to monitor the release of leaving groups from quinones 1 and 2. Upon completion of irradiation, 20-mL aliquots of quinone solution were mixed with

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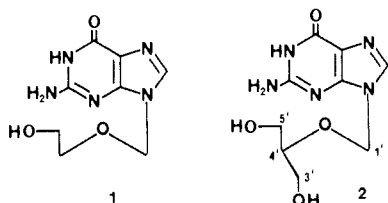
Synthesis and Anti-Herpes-Virus Activity of Acyclic 2'-Deoxyguanosine Analogues Related to 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine^{1,2}

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Several "sugar" modified acyclic nucleoside analogues related to 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, 2) were synthesized and evaluated for antiviral activity. The preparation generally involved the condensation of the acetoxymethyl ether of alcohols 6c-g and 10-12a with diacetylguanine to give adducts 7c-g and 14-16, which were then deprotected to afford analogues 9c-g and 17-19. Alternatively, alcohols 12a and 13a were converted to iodides via their tosylates 12b and 13b and then reacted with the sodium salt of guanine to afford, after deprotection, analogues 22 and 23. A crossed aldol-Cannizzaro reaction on aldehyde 27 readily afforded 28, which was deprotected to give analogue 29. An in vitro assay against HSV-1 showed that all compounds tested were less active than DHPG, though several were good substrates for the viral thymidine kinase. The more promising acyclic nucleosides 9c, 19, and 29 were evaluated in a mouse encephalitis model and proved ineffective at preventing death at a dose of 20 mg/kg.

The report of the anti-herpes-virus activity of acyclovir (1)⁴ followed more recently by the discovery of the substantially greater in vivo potency of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, 2)^{5,6} has stimulated a



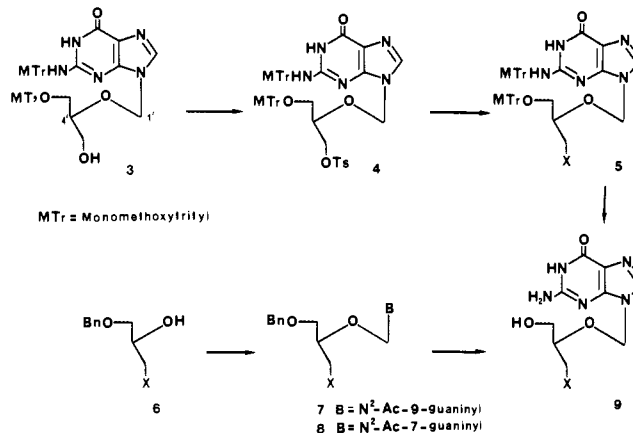
substantial research effort in the synthesis of acyclic guanosine analogues.⁷

- (1) Contribution 206 from the Institute of Bio-Organic Chemistry, Syntex Research.
- (2) Presented in part at the 185th National Meeting of the American Chemical Society, Seattle, WA; March 24, 1983; CARB 43.
- (3) Current address: Pharmaceutical Research and Development, Bristol-Myers Co., Wallingford, CT 06492-7660.
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- (6) The structural formulas of DHPG (2) 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 are referred to as the 3' and 5' positions.

an ionic strength adjuster (20 mL of 0.2 M KNO₃ in the case of bromide ion detection, and 2 mL of 0.5 M ammonium acetate with 0.5 M acetic acid for chloride ions), and electrode measurements were carried out with continuous N₂ gassing to minimize re-oxygenation. Results were compared with predetermined calibration measurements.

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Scheme I

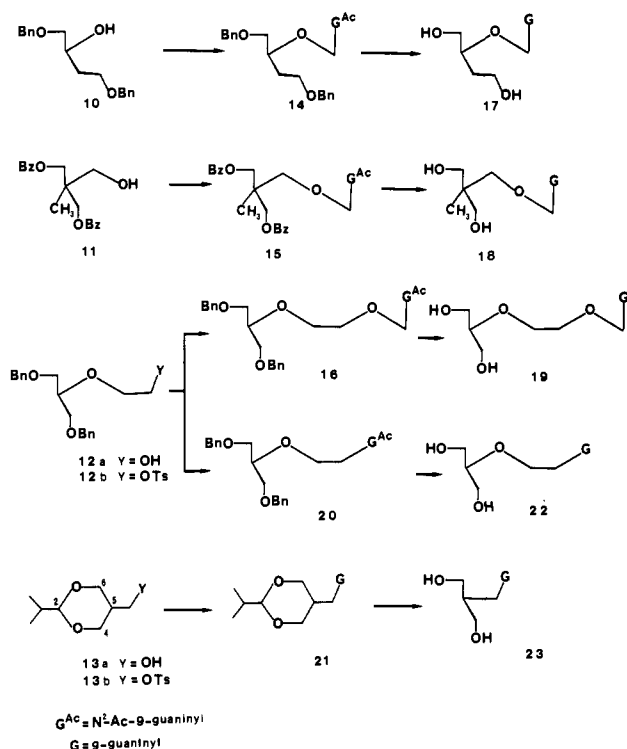


- a X = N₃
 b X = NH₂
 c X = F
 d X = Cl
 e X = OCH₃
 f X = OCH₂H₃
 g X = H

Both acyclovir and DHPG are unusually selective as compared to other nucleoside antiviral agents.⁸ The se-

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Scheme II



lectivity of these analogues is due in part to the fact that they are appreciably phosphorylated only in virus-infected cells, where a virus-specified thymidine kinase of low substrate specificity converts the nucleoside analogues to monophosphates.⁹ The monophosphates are next converted to diphosphates and then to the corresponding nucleoside triphosphates by cellular enzymes. The triphosphates prevent virus replication by inhibition of the viral DNA polymerase. Additional selectivity is realized at this stage because the host polymerase is less sensitive than the viral polymerase to the nucleoside triphosphate analogue.

We have been synthesizing a number of analogues of DHPG in order to further study the effects of structural modifications on antiviral activity and toxicity and now report the preparation and antiviral activity of a number of "sugar" modified DHPG analogues.

Chemistry. The synthesis of the DHPG analogues with one of the alcohol functionalities on the side chain replaced by another functionality is depicted in Scheme I. DHPG was converted to *N*²,*O*-bis(monomethoxytrityl) derivative **3** in 43% yield. Tosylation of **3** gave **4**, which was converted to azide **5a** in 96% overall yield. Deprotection of **5a** with aqueous acetic acid gave azide **9a** (88%). Alternatively, reduction of **5a** with Raney nickel afforded **5b**, which was deprotected to furnish amine **9b** (78% overall).

Substituted glycerol derivatives **6c-g** were used for the synthesis of other analogues. Halohydrins **6c,d** were prep'd. by the BF_3 etherate catalyzed opening of epifluorohydrin

Table I. Physical Data

compd	% yield	mp, °C/solvent	formula
3	43	159-161/ethanol	$\text{C}_{49}\text{H}_{45}\text{N}_5\text{O}_6 \cdot 0.5\text{H}_2\text{O}$
4	99	126-128/ethanol	$\text{C}_{66}\text{H}_{51}\text{N}_5\text{O}_8\text{S}$
5a	96	190-192/ethyl acetate-hexane	$\text{C}_{49}\text{H}_{44}\text{N}_5\text{O}_5 \cdot \text{H}_2\text{O}$
5b	80	206-210 dec/methanol-dichloro- methane	$\text{C}_{49}\text{H}_{46}\text{N}_5\text{O}_5 \cdot 2\text{H}_2\text{O}$
6c	33	oil	$\text{C}_{10}\text{H}_{13}\text{O}_2\text{F}$
6e	75	oil	$\text{C}_{11}\text{H}_{16}\text{O}_3$
6f	64	oil	$\text{C}_{15}\text{H}_{22}\text{O}_3$
6g	46	oil	$\text{C}_{10}\text{H}_{14}\text{O}_2$
7c	18	167-170/methanol-ethyl acetate	$\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_4\text{F}$
7d	20	174-175/methanol-ethyl acetate	$\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_4\text{Cl}$
7e	16	140-141/ethyl acetate-hexane	$\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_5$
7f	19	146-148/ethyl acetate-hexane	$\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_5$
7g	15	173-175/ethanol	$\text{C}_{18}\text{H}_{21}\text{N}_5\text{O}_4$
8c	11	127-133/ethanol	$\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_4\text{F}$
8d	6	151-153/ethyl acetate-hexane	$\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_4\text{Cl}$
8e	3	94-95/ethyl acetate-hexane	$\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_5 \cdot 0.5\text{H}_2\text{O}$
8f	5	102-103/ethyl acetate-hexane	$\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_5$
9a	88	207-208/water-methanol	$\text{C}_9\text{H}_{12}\text{N}_8\text{O}_3$
9b^a	78	157-158/water-ethanol	$\text{C}_9\text{H}_{14}\text{N}_8\text{O}_3 \cdot \text{H} \cdot \text{Cl} \cdot 0.25\text{H}_2\text{O}$
9c	32	255-256/water-methanol	$\text{C}_9\text{H}_{12}\text{N}_5\text{O}_3\text{F} \cdot 0.5\text{H}_2\text{O}$
9d	50	206-208/water-methanol	$\text{C}_9\text{H}_{12}\text{N}_5\text{O}_3\text{Cl}$
9e	67	208-210/water-methanol	$\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_4 \cdot 0.5\text{H}_2\text{O}$
9f	78	224-226/water-methanol	$\text{C}_{14}\text{H}_{21}\text{N}_5\text{O}_4$
9g^b	91	275 dec/water/ethanol	$\text{C}_9\text{H}_{13}\text{N}_5\text{O}_3 \cdot \text{H}_2\text{O}$
10	87	oil	$\text{C}_{18}\text{H}_{22}\text{O}_3$
11	50	oil	$\text{C}_{19}\text{H}_{20}\text{N}_5$
12a	50	oil	$\text{C}_{19}\text{H}_{24}\text{O}_4$
12b	92	oil	$\text{C}_{26}\text{H}_{30}\text{O}_6\text{S}$
13b	66	89-90/diethyl ether-petroleum ether	$\text{C}_{15}\text{H}_{22}\text{O}_5\text{S}$
14	11	122-124/ethyl acetate-hexane	$\text{C}_{26}\text{H}_{29}\text{N}_5\text{O}_5$
15	30	184-186/ethyl acetate-hexane	$\text{C}_{27}\text{H}_{27}\text{N}_5\text{O}_7$
16	25	133-135/ethyl acetate-ether	$\text{C}_{27}\text{H}_{31}\text{N}_5\text{O}_6$
17	55	222-223/water-methanol	$\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_4 \cdot 0.5\text{H}_2\text{O}$
18	81	230-232/methanol	$\text{C}_{11}\text{H}_{17}\text{N}_5\text{O}_4 \cdot \text{H}_2\text{O}$
19	67	167-169/ethanol	$\text{C}_{11}\text{H}_{17}\text{N}_5\text{O}_5$
20	4	144-146/ethyl acetate-hexane	$\text{C}_{26}\text{H}_{29}\text{N}_5\text{O}_5$
21	29	293-295/methanol	$\text{C}_{13}\text{H}_{19}\text{N}_5\text{O}_3$
22	50	220-222/methanol	$\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_4$
23	77	297-298/water-methanol	$\text{C}_9\text{H}_{13}\text{N}_5\text{O}_3$
24	98	243-244/methanol	$\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_4$
26	78	179-181/ethanol	$\text{C}_{28}\text{H}_{27}\text{N}_5\text{O}_4 \cdot 0.5\text{H}_2\text{O}$
28	53	159-161/ethanol	$\text{C}_{30}\text{H}_{31}\text{N}_5\text{O}_6 \cdot 1.5\text{H}_2\text{O}$
29^c	85	>300/water	$\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_5$

^a Mp reported 158-160 °C. ^b Previously disclosed without physical data. ^c Mp reported >300 °C.¹⁵

and epichlorohydrin with benzyl alcohol.¹⁰ Ethers **6e,f** were synthesized by the treatment of **6d** with the appropriate sodium alkoxide. Propylene glycol derivative **6g** was prepared by the reaction of propylene oxide with sodium benzoxide. Alcohols **6c-g** were chloromethylated, treated with sodium acetate, and then reacted with *N*²,9-di-acetylguanine to give **7c-g** and **8c-g** (Table I). The isomers were separated by chromatography. The structures of the *N*⁹-isomers **7** and **9** and the *N*⁷-isomer **8** were con-

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Scheme III

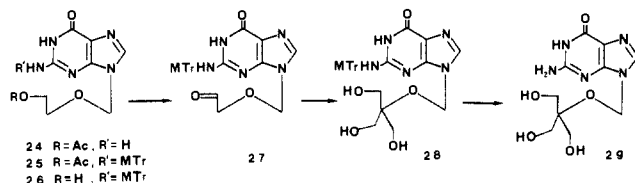


Table II. Phosphorylation of Nucleoside Analogues by Purified HSV-1 (F Strain) Thymidine Kinase and Antiviral Activities against HSV-1 (F Strain) in Cell Culture

compd	vel, % rel to thymidine	ID ₅₀ , μM	compd	vel, % rel to thymidine	ID ₅₀ , μM
1	23	0.5	9g	(28) ^b	(2.3) ^b
2	98	0.2 (0.5) ^a	17	111	95
9a	25	>100	18	100	>100
9b	<10	32 (100) ^a	19	23	7 (>100) ^a
9c	67	7 (>100) ^a	22	35	>100
9d	33	60	23		>100
9e	32	70	29	77	3.5 ^c (55) ^a
9f	21	40			

^a Antiviral activities against HSV-2 (G strain) in cell culture. ^b Biological data previously reported.^{9b} ^c ID₅₀ previously reported 54 μM.¹⁵

firmed by spectroscopic comparisons (¹³C NMR,¹¹ UV) with the natural nucleosides. The N⁹-isomers 7c-g were deprotected by normal or transfer hydrogenation¹² and then ammonolysis to give 9c-g.

Alcohols 10-13a were used as starting materials for the synthesis of chain-length-modified analogues of DHPG (Scheme II). Alcohol 10 was synthesized by the treatment of 4-benzyloxy-1-butene oxide¹³ with sodium benzoxide. Dibenzoate 11 was synthesized by the reaction of the corresponding triol with 2 equiv of benzoyl chloride. Hydroxyethyl derivative 12a was prepared by reacting the sodium salt of 1,3-di-O-benzyl glycol with bromoacetaldehyde diethyl acetal followed by hydrolysis and reduction.

Alcohols 10-12a were converted as described for the preparation of 7c to their corresponding acetoxyethyl ethers and then condensed with diacetylguanine to give 14-16. Standard deprotection afforded analogues 17-19. Alternatively, alcohols 12a and 13a¹⁴ were converted to their corresponding iodides via the tosylate intermediates 12b and 13b and then reacted with the sodium salt of guanine to give intermediates 20 (includes acetylation to aid purification) and 21. Deprotection gave the free acyclic nucleosides 22 and 23.

The trihydroxy analogue 29, recently reported by Ogilvie and co-workers,¹⁵ was synthesized by a method developed in this laboratory for the synthesis of 4'-hydroxymethylated nucleosides.¹⁶ Acyclovir (1) was acetylated to give 24, which in turn was monomethoxytritylated to 25 and then deacetylated to furnish 26. Moffatt oxidation

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Table III. Effects of Subcutaneous Treatment with 9c, 19, and 29 on an HSV-2 Encephalitis Infection in Mice

compd	dose, mg/kg	survivors/total	mean survival time, days
placebo		2/20 (10%) ^a	9.2 ± 1.9 ^b
DHPG	20	10/16 (62%) ^c	13.5 ± 1.8 ^d
9c	20	2/10 (20%) ^e	8.5 ± 1.1 ^e
19	20	3/10 (30%) ^e	10.3 ± 1.5 ^d
29	20	3/10 (30%) ^e	9.3 ± 0.75 ^e

^a Percent survival. ^b Standard deviation. ^c Statistical significance of $p < 0.05$ by two-tailed Fisher exact test. ^d Statistical significance of $p < 0.05$ by two-tailed Mann-Whitney test. ^e Not statistically significant.

of 26 afforded intermediate aldehyde 27, which was hydroxymethylated by treatment with paraformaldehyde and NaOH to give 28. Deprotection of 28 with aqueous acetic acid afforded the desired analogue 29 (Scheme III).

Biological Results and Discussion

The antiviral activities of the "sugar" modified DHPG analogues were determined against herpes simplex virus type 1 (F strain) by plaque reduction in Vero cells. The ability of these analogues to function as substrates for a viral-specified thymidine kinase was also measured and compared with the above in vitro antiviral effect (Table II). The data in the table indicate that of the C-3' (C-5') substituted analogues, 9a-f, the fluoro analogue 9c is the more active member and is phosphorylated by the viral kinase at 67% the rate of thymidine. Although analogues 9a-f are phosphorylated by the viral kinase at rates comparable to acyclovir, the attenuated antiviral activity exhibited by these compounds can possibly be attributed to the inefficient conversion of the substrate to the triphosphate or, once at that level, failure to inhibit the viral DNA polymerase. This is further evinced by the low antiviral activity of homologues 17 and 18 in spite of their phosphorylation by the viral kinase at a rate comparable to that of thymidine (100%). In addition, it has previously been demonstrated that certain analogues of DHPG^{7e} and acyclovir^{9b} were inactive as antivirals despite being substrates for the viral kinase.

Examination of the series of compounds 17-19, 22, 23, and 29 clearly demonstrates that a change of one or two carbons or branching at various points on the DHPG "sugar" portion generally leads to decreased antiviral activity, although some affinity for the viral kinase is retained. Prominent among these are compounds 19 and 29, which retained moderate in vitro antiviral activities with ID₅₀ of 7 and 3.5 μM, respectively, against HSV-1.

It should be noted that compounds 9a-g and 17, due to the method of preparation, are in fact racemic mixtures. The two enantiomeric forms need not necessarily have the same antiviral activity.¹⁷

The in vivo efficacies of the more promising analogues 9c, 19, and 29 were compared with DHPG in a mouse encephalitis model (Table III). All three compounds tested showed no activity at a dose of 20 mg/kg in terms of survivor increase, but analogue 19 did statistically increase the mean survival time. In a related experiment in mice (HSV-2 encephalitis), compound 9b showed a 31% increase in survivors at a dose of 500 mg/kg as compared to an equipotent dose of 200 mg/kg for acyclovir. Compound 9g was inactive when tested at this high dose.

Experimental Section

Nuclear magnetic resonance spectra were recorded on samples dissolved in deuteriodimethyl sulfoxide unless otherwise stated.

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For convenience and where applicable, the peak assignments of the two terminal carbons of the glycerol side chain are numbered 3' and 5'. 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 (3).** A mixture of 2 (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 magnetically stirred under a drying tube at 40 °C for 2 h; then methanol (10 mL) was added, and the solvents were evaporated. The residue was dissolved in ethyl acetate, washed with aqueous NaHCO₃ and water, dried over MgSO₄, and evaporated. The resulting oil was chromatographed (1:14 methanol/dichloromethane) and the product crystallized from ethanol to give 11.2 g (43%) of 3: UV λ_{max} (methanol) 279 nm (ε 13 000), 260 (12 000); ¹H NMR δ 10.63 (br s, 1 H, NH), 7.75 (s, 1 H, H-8), 7.66 (s, 1 H, NH), 7.24, 7.13, 7.05, 6.85, 6.70 (m, 28 H, aromatic), 5.04, 4.96 (AB, *J* = 11 Hz, 2 H, H-1), 4.39 (t, 1 H, OH), 3.77, 3.59 (s, 6 H, OCH₃), 3.47 (m, 1 H, H-4'), 3.05, 2.95 (ABM, 2 H, *J* = 1, 3, 11 Hz, CH₂OH), 2.77, 2.60 (ABM, 2 H, *J* = 5, 11 Hz, CH₂OMTr).

***N*²-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-(*p*-toluenesulfonyloxy)-2-propoxy]methyl]guanine (4).** A solution of 3 (11.5 g, 14.1 mmol) and *p*-toluenesulfonyl chloride (10.5 g, 55 mmol) in pyridine (100 mL) was kept at room temperature for 2 days, and then water (10 mL) was added. The solution was evaporated to dryness. The residue was dissolved in ethyl acetate, washed with water, dried over Na₂SO₄, and evaporated to give 11.6 g (100%) of 4 as a foam. An analytical sample was obtained by crystallization from ethanol: UV λ_{max} (methanol) 273 nm (ε 15 100), 260 (16 400); ¹H NMR δ 10.69 (br s, 1 H, NH), 7.76 (s, 1 H, H-8), 7.74 (br s, 1 H, NH), 7.66–6.67 (m, 32 H, aromatic), 5.03 and 4.84 (AB, *J* = 12 Hz, 2 H, H-1'), 3.77 (s, 3 H, OCH₃), 3.57 (s, 3 H, OCH₃), 3.60–3.30 (m, 3 H, H-4', CH₂OTs), 2.63 (m, 2 H, CH₂OMTr), 2.45 (s, 3 H, CH₃).

***N*²-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-azido-2-propoxy]methyl]guanine (5a).** A solution of 4 (11.6 g, 14.1 mmol) and NaN₃ (5.0 g, 77 mmol) in DMF (100 mL) was heated at 105 °C for 3 days. The solution was diluted with ethyl acetate, washed with water, dried over Na₂SO₄, and evaporated to give 11.1 g (96%) of 5a as a foam. An analytical sample was obtained by crystallization from ethyl acetate/hexane: UV λ_{max} (methanol) 276 nm (ε 13 900), 260 (14 900); IR 2100 (N₃) cm⁻¹; ¹H NMR δ 10.68 (br s, 1 H, NH), 7.78 (s, 1 H, H-8), 7.68–7.35 (m, 28 H, aromatic), 5.03 and 4.95 (AB, *J* = 12 Hz, 2 H, H-1'), 3.77 (s, 3 H, OCH₃), 3.59 (s, 3 H, OCH₃), 3.54 (m, 1 H, H-4'), 3.06–2.55 (m, 4 H, H-3', H-5').

9-[[3-Amino-1-(*p*-anisyldiphenylmethoxy)-2-propoxy]methyl]-*N*²-(*p*-anisyldiphenylmethyl)guanine (5b). A mixture of 5a (11.1 g, 13.5 mmol) and Raney nickel¹⁸ (60 g) in THF (150 mL) and ethanol (200 mL) was magnetically stirred under H₂ (1 atm) for 4 days. The mixture was filtered through Celite and the filtrate evaporated to dryness. The residue was chromatographed (1:9 methanol/dichloromethane) to give 8.63 g (80%) of 5b as a foam: UV λ_{max} (methanol) 276 nm (ε 14 700), 260 (16 400), 232 (30 300); ¹H NMR δ 7.79 (br s, 1 H, NH), 7.76 (s, 1 H, H-8), 7.37–6.68 (m, 28 H, aromatic), 5.03 and 4.80 (AB, *J* = 12 Hz, 2 H, H-1'), 3.77 (s, 3 H, OCH₃), 3.59 (s, 3 H, OCH₃), 3.40 (m, 1 H, H-4'), 2.82 (m, 2 H, CH₂OMTr), 2.40 (m, 2 H, CH₂NH₂).

9-[(1-Azido-3-hydroxy-2-propoxy)methyl]guanine (9a). A solution of 5a (1.24 g, 1.5 mmol) in 80% aqueous acetic acid (20 mL) was heated at 80 °C for 2 h and then evaporated to dryness. The residue was triturated with 1:3 ethyl acetate/hexane and then crystallized from water/methanol to give 0.37 g (88%) of 9a: UV λ_{max} (0.1 N HCl) sh 275 nm (ε 8000), 256 (11 100), (0.1 N NaOH) 265 (10 400); IR 2110 (N₃) cm⁻¹; ¹H NMR δ 10.69 (br s, 1 H, NH), 7.83 (s, 1 H, H-8), 6.51 (br s, 2 H, NH₂), 5.47 (s, 2 H, H-1'), 4.87 (t, *J* = 5 Hz, 1 H, OH), 3.79 (m, 1 H, H-4'), 3.40 (m, 2 H, CH₂OH), 3.35, 3.27 (ABX, *J* = 6 and 12 Hz, 2 H, CH₂N₃); ¹³C NMR (75.453 MHz) δ 156.77 (C-6), 153.76 (C-2), 151.30 (C-4), 137.53 (C-8), 116.38

(C-5), 78.04 (C-4'), 71.12 (C-1'), 60.59 (CH₂OH), 50.98 (CH₂N₃).

9-[(1-Amino-3-hydroxy-2-propoxy)methyl]guanine Hydrochloride (9b). A solution of 5b (8.63 g, 10.3 mmol) in 0.1 N methanolic HCl (500 mL) was kept at room temperature for 15 h and then evaporated to dryness. The residue was crystallized from water/ethanol to give 2.34 g (78%) of 9b: UV λ_{max} (0.1 N HCl) sh 276 nm (ε 8300), 257 (12 000), (0.1 N NaOH) 266 (10 800); ¹H NMR δ 10.85 (br s, 1 H, NH), 8.00 (br s, 3 H, NH₃Cl), 7.87 (s, 1 H, H-8), 6.69 (br s, 2 H, NH₂), 5.50 and 5.43 (AB, *J* = 11 Hz, 2 H, H-1'), 3.87 (m, 1 H, H-4'), 3.52 and 3.42 (after D₂O added, ABX, *J* = 5 and 11 Hz, 2 H, CH₂OH), 3.05 and 2.90 (after D₂O added, ABX, *J*_{AB} = 12 Hz, *J*_{AX} = 8 Hz, *J*_{BX} = 3 Hz, 2 H, CH₂NH₂).

1-Benzyloxy-3-fluoro-2-propanol (6c). A solution of epifluorohydrin (10 g, 0.13 mol), benzyl alcohol (35 mL, 0.34 mol), and BF₃ etherate (1.5 mL, 15 mmol) was kept at 0 °C for 2 h and then at room temperature for 17 h. The solution was diluted with ethyl acetate, washed with saturated aqueous NaHCO₃, dried over Na₂SO₄, and evaporated to an oil. The residual oil was chromatographed with 3:10 ethyl acetate/hexane to give 7.94 g (33%) of 6c: ¹H NMR (CDCl₃, 100 MHz) δ 7.35 (s, 5 H, aromatic), 4.75, 4.20 (dd, *J* = 55, 3 Hz, 2 H, H-3), 4.57 (s, 2 H, benzylic), 4.0 (m, 1 H, H-2), 3.58 (m, 2 H, H-1), 2.40 (br s, 1 H, OH); ¹³C NMR (CDCl₃, 22.62 MHz) δ 137.74, 128.61, 127.86 (aromatic), 86.97 (d, *J* = 169 Hz, C-F), 73.67 (benzylic), 70.07 (d, *J* = 6.6 Hz, C-1), 69.43 (d, *J* = 22 Hz, C-2).

1-Benzyloxy-3-methoxy-2-propanol (6e). A solution of 6d (20 g, 100 mmol) in 0.2 N methanolic NaOCH₃ was stirred for 16 h at room temperature then refluxed for 9 h. The resulting suspension was left at room temperature 16 h and was then concentrated and the residue partitioned between ethyl acetate and 5% aqueous HCl. The organic phase was washed successively with NaHCO₃ and brine, dried over Na₂SO₄, and concentrated to an oil, which was distilled at 101–105 °C/1 torr to afford 14.7 g (75%) of 6e: ¹H NMR (CDCl₃, 300 MHz) δ 7.35–7.28 (m, 5 H, aromatic), 4.56 (s, 2 H, benzylic), 4.0 (m, 1 H, CHOH), 3.55 and 3.50 (ABX, 2 H, *J*_{AX} = 4.4 Hz, *J*_{BX} = 6.3 Hz, *J*_{AB} = 6.8 Hz, CH₂OR), 3.47 and 3.43 (ABX, 2 H, *J*_{AX} = *J*_{AB} = 4.4 Hz, *J*_{BX} = 6.2 Hz, CH₂OR), 3.38 (s, 3 H, OCH₃), 2.55 (d, 1 H, OH); ¹³C NMR (CDCl₃, 75.453 MHz) δ 138.23, 128.59, 127.88 (aromatic), 73.98 (CH₂OCH₃), 73.54 (benzylic), 71.50 (CH₂OBn), 69.54 (CHOH), 59.20 (OCH₃).

***N*²-Acetyl-9-[(1-benzyloxy-3-fluoro-2-propoxy)methyl]guanine (7c).** Hydrogen chloride gas (dried through concentrated H₂SO₄) was bubbled into a stirred mixture of 6c (6.0 g, 33 mmol) and paraformaldehyde (2.0 g, 67 mmol) in dichloroethane (100 mL) at 0 °C until all the solids dissolved (3 h). The resulting solution was stored at 0 °C for 16 h, dried over MgSO₄, and evaporated to a clear oil. A solution of the oil and sodium acetate (4.0 g, 49 mmol) in DMF (75 mL) was kept at room temperature for 16 h and then evaporated to dryness. The residual oil was dissolved in ethyl acetate, washed with water and brine, dried over Na₂SO₄, and evaporated to a clear oil. A mixture of the oil, diacylguanine (13.0 g, 55 mmol), bis(*p*-nitrophenyl)phosphate (0.10 g, 0.3 mmol), and sulfolane (22 mL) was heated at 95 °C with stirring for 5 days. The mixture was diluted with methanol and filtered through Celite. The filtrate was evaporated to dryness and chromatographed (1:19 methanol/dichloromethane) to give after crystallization from ethyl acetate 2.30 g (18%) of 7c: UV λ_{max} (methanol) 279 nm (ε 11 500), 258 (16 000); ¹H NMR (300 MHz) δ 8.14 (s, 1 H, H-8), 7.35–7.18 (m, 5 H, aromatic), 5.59 and 5.58 (AB, *J* = 3 Hz, 2 H, H-1'), 4.62–4.30 (m, 4 H, CH₂F, benzylic), 4.08 (m, 1 H, H-4'), 3.61–3.38 (m, 2 H, CH₂O), 2.18 (s, 3 H, CH₃); ¹³C NMR (22.62 MHz) δ 173.60 (COCH₃), 154.91 (C-6), 148.80 (C-4), 148.08 (C-2), 139.99 (C-8), 137.97, 128.18, 127.70, 127.21 (aromatic), 120.22 (C-5), 82.76 (d, *J* = 168.4 Hz, CH₂F), 76.16 (d, *J* = 18.4 Hz, C-4'), 72.24 (benzylic) 72.04 (C-1'), 68.19 (d, *J* = 8.1 Hz, CH₂OBn), 23.67 (COCH₃).

***N*²-Acetyl-7-[(1-(benzyloxy)-3-fluoro-2-propoxy)methyl]guanine (8c).** From the above chromatographic purification of 7c, 1.39 g (11%) of 8c was isolated after crystallization from ethyl acetate: UV λ_{max} (methanol) 264 nm (ε 13 800); ¹H NMR (300 MHz) δ 8.38 (s, 1 H, H-8), 7.31–7.23 (m, 5 H, aromatic), 5.78 (s, 2 H, H-1'), 4.60–4.10 (m, 5 H, CH₂F, H-4', benzylic), 3.45 (m, 2 H, CH₂O), 2.18 (s, 3 H, COCH₃); ¹³C NMR (75.453 MHz) δ 173.32 (COCH₃), 157.44 (C-4), 152.44 (C-6), 147.13 (C-2), 144.96 (C-8), 137.94, 128.09, 127.54, 127.28 (aromatic), 110.90 (C-5), 82.70

(18) Raney nickel was prepared as described by Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. 1, p 729.

(d, $J = 168.5$ Hz, CH_2F), 75.87 (d, $J = 17.8$ Hz, C-4'), 74.53 (C-1'), 72.18 (benzylic), 68.18 (d, $J = 8.4$ Hz, CH_2OBN), 23.63 (COCH_3).

9-[3-Fluoro-1-hydroxy-2-propoxy)methyl]guanine (9c). A mixture of **7c** (1.05 g, 2.7 mmol), 2 drops of concentrated HCl, and 10% Pd/C in methanol (200 mL) and water (50 mL) was treated on a Parr hydrogenator with H_2 (50 psi) for 4 days. The mixture was filtered and the filtrate evaporated to dryness. A solution of the residue in concentrated NH_4OH (5 mL) and methanol (25 mL) was kept at room temperature for 18 h and then evaporated to dryness. The residue was crystallized from water/methanol to give 0.22 g (32%) of **9c**: UV λ_{max} (methanol) sh 273 nm (ϵ 9030), 253 (13 600); $^1\text{H NMR}$ (300 MHz) δ 7.83 (s, 1 H, H-8), 6.53 (br s, 2 H, NH_2), 5.45 (s, 2 H, H-1'), 4.88 (t, $J = 5$ Hz, 1 H, OH), 4.60–4.25 (m, 2 H, CH_2F), 3.91–3.76 (m, 1 H, H-4'), 3.40 (m, 2 H, CH_2OH); $^{13}\text{C NMR}$ (22.62 MHz) δ 156.79 (C-6), 153.77 (C-2), 151.36 (C-4), 137.68 (C-8), 116.38 (C-5), 82.68 (d, $J = 167.7$ Hz, CH_2F), 77.49 (d, $J = 18.4$ Hz, C-4'), 71.23 (C-1'), 59.15 (d, $J = 8.1$ Hz, CH_2OH).

1,3-Di-O-benzyl-2-O-(2-hydroxyethyl)glycerol (12a). A mixture of NaH (2.0 g, 50%, 42 mmol, prewashed with hexanes) and 1,3-di-O-benzylglycerol¹⁰ (10.0 g, 37 mmol) in DMF (25 mL) was heated at 50 °C for 0.5 h then cooled to 0 °C. Bromoacetaldehyde diethyl acetal (6.3 mL, 42 mmol) was added and the resulting solution heated at 50 °C for 17 h then evaporated. The residue was dissolved in ether, washed with water and brine, dried over Na_2SO_4 , and evaporated. The resulting clear oil was chromatographed (2:8 ethyl acetate/hexane) to give 8.9 g (62%) of 1,3-di-O-benzyl-2-O-(2,2-diethoxyethyl)glycerol as a clear oil: $^1\text{H NMR}$ (CDCl_3) δ 7.30 (s, 10 H, phenyl), 4.64 (t, $J = 5$ Hz, 1 H, acetal-H), 4.54 (s, 4 H, benzylic), 3.77 (m, 1 H, CHO), 3.71–3.51 (m, 10 H, CH_2O), 1.18 (t, $J = 7$ Hz, 6 H, CH_3).

To the above adduct (25 g, 64.3 mmol) in THF (60 mL) and H_2O (5 mL) was added *p*-toluenesulfonic acid (300 mg), and the solution was refluxed 7 h then evaporated. The residual oil was partitioned between toluene and dilute KHCO_3 , and the organic phase was dried with Na_2SO_4 and evaporated to a yellow oil. The crude aldehyde was redissolved in MeOH (60 mL) at 0 °C to which was added NaBH_4 (2.0 g, 52.8 mmol). The reaction was stirred for 30 min then evaporated. The residue was partitioned between toluene and dilute HCl (1×), dilute KHCO_3 (1×), and H_2O (1×); the organic phase was dried with Na_2SO_4 and evaporated to a golden oil. Fractional distillation afforded 9.93 g (48%) of pure **12a**: bp 195–200 °C/0.5 torr; $^1\text{H NMR}$ (CDCl_3) δ 7.4 (s, 10 H, phenyl), 4.60 (s, 4 H, benzylic), 3.75 (m, 5 H, H-4' and ethoxy), 3.55 (d, 4 H, H-3', H-5'), 3.05 (br, 1 H, OH).

5-[*p*-Toluenesulfonyloxy)methyl]-2-isopropyl-1,3-dioxane (13b). A solution of alcohol **13a** (1 g, 6.17 mmol)¹⁴ and *p*-toluenesulfonyl chloride (1.43 g, 7.5 mmol) in pyridine (10 mL) was stirred for 16 h at room temperature and then evaporated. The residue was redissolved in dichloromethane and partitioned with aqueous Na_2CO_3 , dried (MgSO_4), and evaporated to a yellow oil. Chromatography (CH_2Cl_2) followed by crystallization from diethyl ether–petroleum ether yielded 1.3 g (66%) of **13b**: $^1\text{H NMR}$ δ 7.78 (d, 2 H, $J = 8.4$ Hz, aromatic), 7.37 (d, 2 H, $J = 8.6$ Hz, aromatic), 4.10 (d, 1 H, $J = 5.2$ Hz, H-2), 4.05, 3.45 (ABX, 4 H, $J_{\text{AX}} = 5$ Hz, $J_{\text{BX}} = 11.2$ Hz, $J_{\text{AB}} = 11.4$ Hz, H-4, H-6), 3.80 (d, 2 H, $J = 5.7$ Hz, CH_2OTs), 2.46 (s, 3 H, aromatic CH_3), 2.32 (m, 1 H, H-5), 1.77 (m, 1 H, CH), 0.90 (d, 6 H, $J = 6$ Hz, CH_3).

9-[2-Isopropyl-1,3-dioxan-5-yl)methyl]guanine (21). A suspension of the sodium salt of guanine (1.24 mmol), **13b** (3.9 g, 1.24 mmol), and NaI (0.6 g, 4 mmol) in DMF (15 mL) was heated at 100–110 °C for 16 h then cooled, diluted with 20% methanol–dichloromethane (70 mL), filtered through Celite, and evaporated. The residue was chromatographed (15% methanol– CH_2Cl_2), and crystallization from methanol afforded 1.12 g (29%) of **21**: UV λ_{max} (0.1 N HCl) 281 nm (ϵ 7740), 254 (10 900), (0.1 N NaOH) 268 (10 300), sh 254 (9450); $^1\text{H NMR}$ δ 7.63 (s, 1 H, H-8), 6.47 (br s, 2 H, NH_2), 4.16 (d, 2 H, H-2'), 4.87, 3.43 (ABX, 4 H, $J = 4.7$, 11 Hz, H-4', H-6'), 3.75 (d, 2 H, $J = 7.3$ Hz, $\text{CH}_2\text{-G}$), 2.40 (m, 1 H, H-5'), 1.69 (m, 1 H, CH), 0.83 (d, 6 H, $J = 6$ Hz, CH_3).

9-[3-Hydroxy-2-(hydroxymethyl)prop-1-yl]guanine (23). A solution of **21** (254 mg, 0.87 mmol) in 90% aqueous trifluoroacetic acid was stirred at room temperature for 20 min and then evaporated. The residue was coevaporated 3 times with ethanol, triturated twice with acetone, and the resulting solid crystallized

from methanol/water to afford **23** (159 mg, 77%) in two crops: UV λ_{max} (0.1 N HCl) 278 nm (ϵ 7300), 253 (11 000), (0.1 N NaOH) 268 (9990), 254 (9160); $^1\text{H NMR}$ δ 7.59 (s, 1 H, H-8), 6.46 (br s, 2 H, NH_2), 4.6 (t, 2 H, OH's), 3.96 (d, 2 H, $J = 6.8$ Hz, CH_2), 3.34 (m, 4 H, CH_2OH), 2.02 (m, 1 H, CH).

9-[(2-Acetoxyethoxy)methyl]guanine (24). A mixture of **1** (3.0 g, 13.3 mmol) and 4-(dimethylamino)pyridine (0.30 g, 2.5 mmol) in acetic anhydride (100 mL) was vigorously stirred at room temperature for 5 days and then evaporated to dryness. The residue was crystallized from methanol to give 3.48 g (98%) of **24**: UV λ_{max} (methanol) sh 275 nm (ϵ 10 000), 254 (14 000); $^1\text{H NMR}$ δ 10.69 (br s, 1 H, NH), 7.83 (s, 1 H, H-8), 6.53 (br s, 2 H, NH_2), 5.37 (s, 2 H, NCH_2O), 4.08 (t, $J = 5$ Hz, 2 H, CH_2OAc), 3.68 (t, $J = 5$ Hz, 2 H, CH_2O), 1.97 (s, 3 H, COCH_3).

***N*'-(*p*-Anisyl)diphenylmethyl)-9-[(2-hydroxyethoxy)methyl]guanine (26).** A solution of **24** (2.96 g, 11.1 mmol), *p*-anisylchlorodiphenylmethane (4.35 g, 15.6 mmol), triethylamine (4.0 mL, 29 mmol), and 4-(dimethylamino)pyridine (0.30 g, 2.5 mmol) in DMF (50 mL) was heated at 50 °C for 23 h, and then methanol (5 mL) was added. The solution was evaporated to dryness. The residue was dissolved in dichloromethane, washed with saturated aqueous NaHCO_3 , dried over Na_2SO_4 , and evaporated to dryness. The residue was chromatographed (1:15 methanol/dichloromethane) to give 5.09 g (85%) of a foam. A solution of **25** (3.97 g, 7.35 mmol) and concentrated NH_4OH (10 mL) in methanol (50 mL) was kept at room temperature for 3 days and then evaporated to dryness. The residue was crystallized from ethanol to give 2.85 g (78%) of **26**: UV λ_{max} (methanol) 277 nm (ϵ 14 000), 260 (15 400); $^1\text{H NMR}$ δ 10.65 (br s, 1 H, NH), 7.69 (br s, 1 H, NH), 7.68 (s, 1 H, H-8), 7.17–7.35 (m, 12 H, aromatic), 6.86 (d, $J = 9$ Hz, 2 H, aromatic), 4.87 (s, 2 H, NCH_2O), 4.51 (t, $J = 5$ Hz, 1 H, OH), 3.72 (s, 3 H, OCH_3), 3.21 (m, 2 H, CH_2OH), 2.93 (t, $J = 5$ Hz, 2 H, CH_2O).

***N*'-(*p*-Anisyl)diphenylmethyl)-9-[[1,3-dihydroxy-2-(hydroxymethyl)-2-propoxy]methyl]guanine (28).** A solution of **26** (1.63 g, 3.28 mmol), dicyclohexylcarbodiimide (2.60 g, 12.6 mmol), and methylphosphonic acid (0.20 g, 2.3 mmol) in dry Me_2SO (150 mL) was magnetically stirred at 18 °C for 4 h and then at room temperature for 18 h. The resulting suspension was cooled to 18 °C, and a solution of oxalic acid dihydrate (90 mg) in methanol (9 mL) was added. The suspension was filtered and the filtrate evaporated to dryness on a Kugelrohr apparatus (60 °C/1 torr). The residue was chromatographed (1:12 methanol/dichloromethane) to give 1.32 g (82%) of **27** as a white foam. A solution of **27** (1.07 g, 2.17 mmol), 37% aqueous formaldehyde (7.8 mL), and 1 N sodium hydroxide (7.8 mL) in THF (50 mL) and water (50 mL) was kept at room temperature for 18 h. Ammonium chloride (1.5 g) was then added and the solution evaporated to approximately 40 mL. The resulting solution plus the precipitated gum was partially dissolved in a mixture of water (200 mL) and dichloromethane (300 mL). The dichloromethane phase was combined with the residual gum and the mixture evaporated to dryness. The residue, preabsorbed onto silica gel (25 g), was chromatographed (1:6 methanol/dichloromethane) to give 0.64 g (53%) of **28** as a white solid. An analytical sample was crystallized from ethanol: UV λ_{max} (methanol) 277 nm (ϵ 13 150), 260 (14 800); $^1\text{H NMR}$ δ 10.67 (br s, 1 H, NH), 7.70 (br s, 1 H, NH), 7.65 (s, 1 H, H-8), 7.15–7.35 (m, 12 H, aromatic), 6.85 (d, $J = 9$ Hz, 2 H, aromatic), 5.01 (s, 2 H, NCH_2O), 4.35 (t, $J = 5$ Hz, 3 H, OH), 3.72 (s, 3 H, OCH_3), 3.25 (d, $J = 5$ Hz, 6 H, CH_2OH).

9-[[1,3-Dihydroxy-2-(hydroxymethyl)-2-propoxy]methyl]guanine (29). A solution of **28** (272 mg, 0.49 mmol) in 80% aqueous acetic acid was heated at 80 °C for 2 h and then evaporated to dryness. The residue was triturated with ethyl acetate and then recrystallized from water to give 119 mg (85%) of **29**: UV λ_{max} (methanol) sh 273 nm (ϵ 9300), 253 (13 300), (0.1 N HCl) sh 276 (8500), 256 (12 100), (0.1 N NaOH) 266 (11 200), 256 (11 100); $^1\text{H NMR}$ δ 10.62 (br s, 1 H, NH), 7.77 (s, 1 H, H-8), 6.46 (br s, 2 H, NH_2), 5.53 (s, 2 H, NCH_2O), 4.66 (t, $J = 5$ Hz, 3 H, OH), 3.51 (d, $J = 5$ Hz, 6 H, CH_2OH).

Plaque Assays. Experiments were conducted with Vero cells infected with HSV-1 (F strain) and then treated with the nucleoside analogue as described previously.^{9a} Fifty percent inhibitory doses (ID_{50}) are defined as doses causing a 50% reduction in plaque numbers compared to untreated controls.

Thymidine Kinase Assay. The assay was performed by the methods of Doberson and Greer,¹⁹ using HSV-1 (strain F)-affinity-purified kinase.²⁰ Reaction mixtures contained 100 mM Tris-HCl (pH 7.5), 50 mM KCl, 2 mM MgCl₂, 1 mM dithiothreitol, 25 mM NaF, 1 mg BSA/mL, 0.5 mM [γ -³²P]ATP (20 μ Ci/ μ M), 0.2 mM nucleoside analogue, and enzyme in a 0.1 mL volume. The [γ -³²P]ATP required for the assay was from ICN Chemical and Radioisotope Div., Irvine, CA; thymidine came from Sigma Chemical Co., St. Louis, MO. The thymidine kinase affinity gel required for enzyme purification was prepared by the Cheng procedure.²¹ and the results from the deoxynucleoside kinase assays were expressed as relative phosphorylation rates, a method

that has been used by others.^{20,22}

Animal Studies. Swiss-Webster female mice (Simonsen Laboratories, Gilroy, CA), weighing approximately 20 g each, were infected intraperitoneally with 5×10^4 PFU of HSV-2 (strain G). This challenge was approximately equivalent to 10 50% lethal doses. DHPG and the nucleoside analogues 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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Synthesis and Antiviral Activity of Certain Nucleoside 5'-Phosphonofosphate Derivatives

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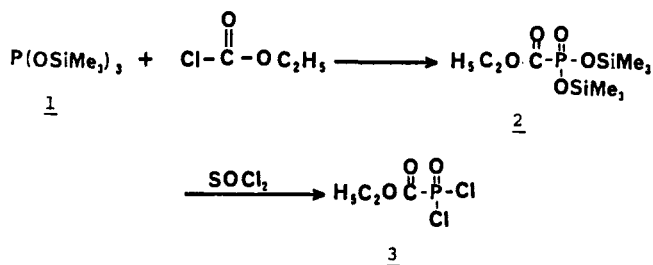
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(Ethoxycarbonyl)phosphonic dichloride (3) was synthesized by chlorination of bis(trimethylsilyl) (ethoxycarbonyl)phosphonate with thionyl chloride. Adenosine 5'-(ethoxycarbonyl)phosphonate (4), guanosine 5'-(ethoxycarbonyl)phosphonate (5), 2'-deoxyadenosine 5'-(ethoxycarbonyl)phosphonate (18) and 2'-deoxyguanosine 5'-(ethoxycarbonyl)phosphonate (19) were synthesized by coupling of compound 3 with adenosine, guanosine, 2'-deoxyadenosine, and 2'-deoxyguanosine, respectively. Alkaline treatment of 4, 5, 18, and 19 gave the corresponding adenosine 5'-(hydroxycarbonyl)phosphonate (14), guanosine 5'-(hydroxycarbonyl)phosphonate (15), 2'-deoxyadenosine 5'-(hydroxycarbonyl)phosphonate (20), and 2'-deoxyguanosine 5'-(hydroxycarbonyl)phosphonate (21). Treatment of 4 and 5 with methanolic ammonia resulted in the production of adenosine 5'-(aminocarbonyl)phosphonate (12) and guanosine 5'-(aminocarbonyl)phosphonate (13), respectively. The nucleotide analogue 20 exhibited the most potent antiviral activity of this group of nucleotide tested in vitro and was most active against herpes viruses especially HSV-2. The nucleotide analogue 21 had lower, but significant, activity against HSV-2. All of the compounds tested were nontoxic to confluent Vero cells at concentrations as high as 5000 μ M.

Compounds which selectively inhibit virus specific functions but have little effect on host cells have been the target of many recent investigations.^{1,2} Phosphonoformate (Foscarnet), an analogue of pyrophosphate, has been found to be a potent antiviral agent.³ Phosphonoformate inhibits viral reverse transcriptase⁴ and DNA polymerase⁵ and is active against herpes simplex virus (HSV-1)^{6,7} and certain other viruses.⁸⁻¹⁰ Recently, elimination of replication of human T-cell lymphotropic virus type III (HTLV-III) with phosphonoformate at a nontoxic dose has been reported.^{11,12} This study showed that the activity of reverse transcriptase of HTLV-III has been reduced drastically by phosphonoformate. Beldekas and co-workers studied the effect of foscarnet on the nature and frequency of T-cell differentiation defect seen in AIDS and in certain people with LAS.¹³ These investigators reported that foscarnet permits T-cell colony growth and expansion in the cell culture.

Phosphonoformate has been used topically for treatment of HSV-1 infection in laboratory animals with satisfactory results.¹⁴ Phosphonoformate has also been used in a 3% cream formulation, which is not irritating to the skin, and in clinical testing shortens the time mucocutaneous HSV-1 and HSV-2 lesions require to reach the stage of crusting and reduces the appearance of new vesicles.¹⁵ Topical treatment of recurrent genital herpes with foscarnet cream has also been claimed to accelerate healing in man.¹⁶

Scheme I



It appears that phosphonoformate binds to the pyrophosphate "well" of the viral DNA and RNA polymerase.^{4,5}

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