105-36-2; $NCCH_2CO_2H$, 372-09-8; (Z)-(n-Bu)$_3$SnCH=CHOEt, 64724-29-4; 2,4-dichlorobenzaldehyde, 874-42-0; N-((S)-α-methylbenzyl)-3(R),5(S)-dihydroxy-7-(2,4-dichlorophenyl)-6-(E)-heptenamide, 93922-56-6; N-((S)-α-methylbenzyl)-3S,5R-dihydroxy-7-(2,4-dichlorophenyl)-6-(E)-heptenamide, 93922-57-7; 6-(2,4-dichlorophenyl)-3,5-hexadien-2-one, 93863-86-6; diketene, 674-82-8; 4-phenanthrenecarboxaldehyde, 41498-43-5; HMG-CoA reductase, 9028-35-7.

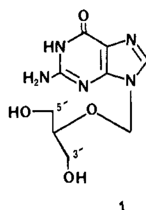
Acyclic Analogues of 2′-Deoxynucleosides Related to 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine as Potential Antiviral Agents[1,2]

John C. Martin,*† Gary A. Jeffrey, Danny P. C. McGee, Michael A. Tippie, Donald F. Smee, Thomas R. Matthews, and Julien P. H. Verheyden

Syntex Research, Palo Alto, California 94304. *Received June 11, 1984*

A series of acyclic analogues of 2′-deoxynucleosides related in structure to 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, 1) have been synthesized and evaluated for antiviral activity against herpes simplex virus type 1 (F strain). Additionally, the ability of these analogues to function as substrates for the virus-specified thymidine kinase was examined. Phosphorylation by this kinase is essential for antiviral activity. Although the acyclic 4-oxopyrimidine nucleosides were substrates for the kinase, they were devoid of antiviral activity. In the purine series, most analogues similar in structure to DHPG did exhibit significantly lower antiviral activity, indicating that even small modifications in the purine substituents substantially reduce the antiviral potency. The most active agent, 2,6-diaminopurine **27**, was only poorly phosphorylated by the viral kinase; therefore, its activity was most likely due to a prior enzymatic deamination to give DHPG. Evaluation of **27** in a mouse encephalitis model has shown it to be nearly as potent as DHPG (1).

The synthesis of 9-[(1,3-dihyroxy-2-propoxy)methyl]-guanine (DHPG, 1),[3] a potent antiherpetic agent, was recently reported independently by us[4] and others.[5] DHPG



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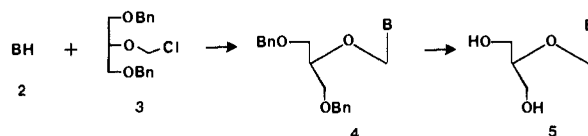
is a member of a class of selective antiherpetic nucleoside analogues[6] which includes 9-[(2-hydroxy-1-ethoxy)-methyl]guanine (acyclovir),[7] (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU),[8] and 1-(2-deoxy-2-fluoro-β-D-*arabino*-furanosyl)-5-iodocytosine (FIAC).[9] Of this class, DHPG appears exceptionally promising, being found to be effective against not only herpes simplex virus types 1 and 2[10] but also cytomegalovirus,[10,11a] varicella-zoster,[11b] and Epstein–Barr virus.[11a] The selectivity of these antiviral agents 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 5′-monophosphates. 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.[6b]

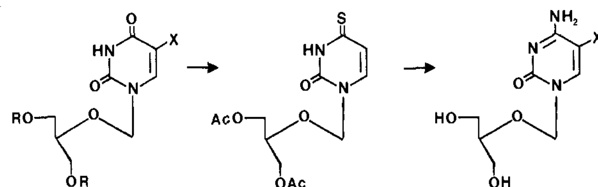
The potent and broad activity of DHPG against herpes viruses prompted us to synthesize other members of this class of acyclic deoxynucleosides. In this report we describe the synthesis of a number of pyrimidine and purine analogues of DHPG, some of which were recently disclosed by Ogilvie and co-workers in a series of publications.[12] This paper details the synthesis of new compounds and

Scheme I



B
a 1-uracilyl
b 1-thyminyl
c 1-(5-fluoro)uracilyl
d 9-(6-chloro)purinyl
e 9-adeninyl
f 9-hypoxanthinyl

Scheme II



6 X=H, R=Ac
9 X=F, R=Ac
11 X=Br, R=Ac
12 X=Br, R=H
8 X=H
10 X=F

our somewhat differing approaches to the recently reported analogues. Additionally, we present new in vitro and in

†Present address: Bristol-Meyers Co., Pharmaceutical Research and Development Division, Syracuse, NY 13221-4755.

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(2) Presented in part at the 185th National Meeting of the American Chemical Society, Seattle, WA; CARB 43; March 24, 1983.
(3) 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 are referred to as the 3′- and 5′-positions.

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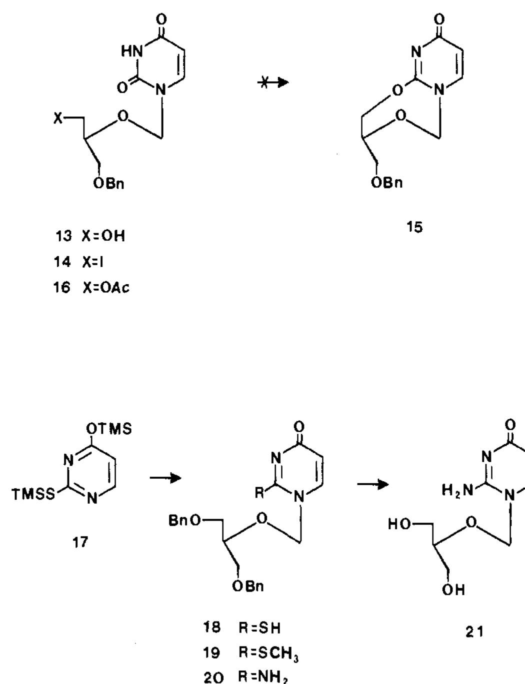
Table I. Phosphorylation of Nucleoside Analogues by Purified HSV-1 (F Strain) Thymidine Kinase, Antiviral Activities against HSV-1 (F Strain) in Cell Culture and Physical Data

compd	velocity (%) relative to thymidine	ID_{50}, μM	mp (lit.), °C	formula
thymidine	100			
DHPG (1)	98	0.2		
4a			68–70 (oil)[12b]	$C_{22}H_{24}N_2O_5$
4b			93–94 (96.5–97.5)[12b]	$C_{23}H_{26}N_2O_5$
4c			86–88 (87.5–88.5)[12b]	$C_{22}H_{23}N_2O_5F$
4d			oil (oil)[12c]	$C_{23}H_{23}N_4O_3Cl$
4e			127–129 (129–131)[12c]	$C_{23}H_{25}N_5O_3$
4f			129–130	$C_{23}H_{24}N_4O_4$
5a	14	>100	117–118 (120–122)[12b]	$C_8H_{12}N_2O_5$
5b	23	>100	156–157 (155–156)[12a,b]	$C_9H_{14}N_2O_5$
5c	16	>100	128–129 (130–131)[12b]	$C_8H_{11}N_2O_5F$
5e	<5	>100	195–197 (193–195)[12c]	$C_9H_{13}N_5O_3$
5f	15	>100	193–195 (193–195)[12c]	$C_9H_{12}N_4O_4$
6			108–109 (106–108)[12b]	$C_{12}H_{16}N_2O_7$
7			oil	$C_{12}H_{16}N_2O_6S$
8	<5	>100	147–148 (140–141)[12d]	$C_8H_{13}N_3O_4 \cdot 0.5H_2O$
9			99–100	$C_{12}H_{15}N_2O_7F$
10	<5	>100	165–166 (134–135)[12d]	$C_8H_{12}N_3O_4F$
11			92–94	$C_{12}H_{15}N_2O_7Br$
12	42	>100	145–147 (145–147)[12b]	$C_8H_{11}N_2O_5Br$
13			105-106	$C_{15}H_{18}N_2O_5$
14			80–81	$C_{15}H_{17}N_2O_4I$
18			93–94	$C_{22}H_{24}N_2O_4S$
19			oil	$C_{23}H_{26}N_2O_4S$
20			134–136	$C_{22}H_{25}N_3O_4$
21	15	>100	182–184	$C_8H_{13}N_3O_4$
22			240–242	$C_{13}H_{17}N_5O_6$
23			122–123	$C_{13}H_{16}N_5O_5Cl$
24			235–237	$C_{13}H_{17}N_5O_5S$
25	38	9	155–157	$C_9H_{13}N_5O_3S$
26	31	16	152–154 (156–157)[12c]	$C_9H_{13}N_5O_3$
27	11	3.3	176–177 (183–185)[12c]	$C_9H_{14}N_6O_3$
acyclovir	23	0.5		

vivo antiherpes virus activity data on this class of acyclic nucleosides. Also, the ability of these analogues to function as substrates for the virus-specified thymidine kinase was determined, since phosphorylation by this kinase is the essential first step for antiviral activity.

Scheme III



Chemistry. The compounds synthesized are listed in Table I. A variety of pyrimidines (silylated) or purines, represented by **2** (Scheme I), were reacted with chloromethyl ether **3** in a mercuric cyanide catalyzed condensation to give intermediates **4**. Catalytic hydrogenolysis afforded the desired analogues **5**.[12a–c]

Because of the susceptibility of cytidine derivatives to overreduction,[13] acyclic analogues of cytidine were not prepard by the above route requiring benzyl ether hydrogenolysis. Instead uridine analogues **5a** and **5c** were

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(12) (a) Acyclic thymidine **5b** and adenosine **5e** were reported without complete experimental or characterization several years ago in a preliminary communication. Ogilvie, K. K.; Gillen, M. F. *Tetrahedron Lett.* **1980**, *21*, 327. (b) Very recently the synthesis of thymidine analogue **5b** was reported along with preparations of other pyrimidine derivatives **5a**, **5c**, and **12**. Ogilvie, K. K.; Hamilton, R. G.; Gillen, M. F.; Radatus, B. K.; Smith, K. O.; Galloway, K. S. *Can. J. Chem.* **1984**, *62*, 16. (c) In a companion paper, a complete characterization of **5e** was reported along with a different synthesis of analogues **5f**, **26**, and **27**. Ogilvie, K. K.; Nguyen-ba, N.; Gillen, M. F.; Radatus, B. K.; Cheriyan, U. O.; Hanna, H. R.; Smith, K. O.; Galloway, K. S. *Can. J. Chem.* **1984**, *62*, 241. (d) Recently a different synthesis of cytidine analogues **8** and **10** was reported which utilized transfer reduction with cyclohexene for a final hydrogenolysis step. Ogilvie, K. K.; Dixit, D. M.; Radatus, B. K.; Smith, K. O.; Galloway, K. S. *Nucleosides Nucleotides* **1983**, *2*, 147.
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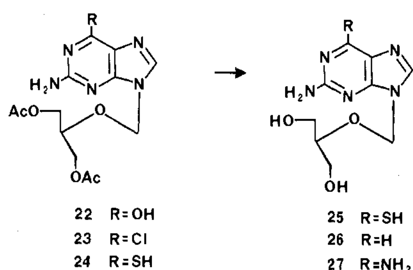
Scheme IV



used as a starting point (Scheme II). Acetylation of **5a** to the diacetate **6** followed by treatment with P_4S_{10} gave in 29% yield the acyclic 4-thio derivative **7**. Reaction of **7** with methanolic ammonia at 100 °C gave cytidine analogue **8** in 24% yield. The newer procedure of Sung [(a) acetic anhydride, (b) *p*-chlorophenyl phosphorodichloridate, triazole, (c) ammonia] was used to convert **5c** via diacetate **9** to fluorocytidine **10** in 35% overall yield.[14] This latter method was considerably more convenient for the preparation of cytidine derivatives. Ogilvie et al.[12d] synthesized **8** by utilizing a palladium-catalyzed transfer reduction with cyclohexene of a dibenzyl ether precursor under carefully controlled conditions. Due to overreduction, this procedure failed to give **10**, and consequently they prepared **10** by a different approach starting from 1,3-dichloro-2-propanol. Although our melting point for **10** is 31 °C higher than that reported,[12d] we have extensive spectroscopic data which confirm the structure. Additionally, the reported combustion analysis for **10** was 0.82% low for carbon, which could explain their lower melting point. Uridine analogue **5a** was also brominated (Br_2, acetic anhydride, acetic acid) to give **11** in 72% yield which was treated with methanolic ammonia to furnish, in 88% yield, the corresponding 5-bromo substituted uridine **12**.

The positions of the functional groups of isocytosine resembles that of guanine; therefore, isocytidine analogue **21** was synthesized. Two approaches were explored (Scheme III). In one, the monobenzyl ether **13** (obtained by partial hydrogenolysis of **4a**) was treated with methyltriphenoxyphosphonium iodide[15] to give iodide **14** in 61% yield. Attempted intramolecular cyclization of **14** catalyzed with silver acetate[16] failed to afford cyclonucleoside **15**. Only a small amount of acetate **16** was produced. Anhydro derivative **15**, if formed, could have been reacted with ammonia to give an isocytidine derivative. The alternative approach proved successful. The bis(trimethylsilyl)pyrimidine derivative **17** was condensed with **3** with use of mercuric cyanide to give 2-thiopyrimidine derivative **18** in 23% yield. Methylation of **18** (methyl iodide, $NaHCO_3$) gave a quantitative yield of **19** which in turn was treated with methanolic ammonia to furnish **20** (60%). Dibenzyl ether **20** was hydrogenolyzed by transfer hydrogenation[17] (20% $Pd(OH)_2/C$, cyclohexene, ethanol) to give isocytidine analogue **21** in 66% yield. Standard catalytic hydrogenolysis with H_2 over Pd/C led to overreduction of the pyrimidine.

DHPG (**1**) was used as a substrate for the preparation of other purine derivatives (Scheme IV). Selective acylation[18] of **1** with acetic anhydride and 4-(dimethylamino)pyridine gave diacetate **22** in 86% yield. The 2-amino-6-chloro derivative **23** was obtained in 65% yield by chlorination of **22**.[19] Treatment of **23** with thiourea yielded **24** which was deacetylated (73%) to give, in 63% overall yield, the 6-thio analogue **25** of DHPG. Desulfurization of **25** with Raney nickel afforded a 38% yield of the acyclic 2-aminopurine nucleoside **26**. Finally, the 2,6-diamino analogue **27** was prepared in 86% yield by the reaction of **23** with ammonia in methanol at 90 °C.[12c]

The structures of the condensation products were supported by spectroscopic comparisons (^1H NMR, ^{13}C NMR, UV) with the natural nucleosides. The chemical shifts of the heterocyclic moieties in the ^{13}C NMR spectra are in very close agreement with those reported for the various nucleosides.[20] Carbons 1′ and 4′ absorb at similar frequencies in the ^{13}C NMR spectra but were easily assigned by examination of peak multiplicity.

Biological Results and Discussion

The antiviral effectiveness of the acyclic nucleoside analogues was determined in a plaque reduction assay in Vero cells against herpes simplex virus type 1 (F strain). Also, the ability for these analogues to function as substrates for the virus-specified thymidine kinase was measured[21] and compared with the antiviral effect (Table I).

The data in the table indicate that the acyclic pyrimidine nucleosides exhibited negligible in vitro antiviral activity at 100 μM in spite of the fact that those analogues with a carbonyl functionality at the 4-position are modest substrates for the virus-specified thymidine kinase. Interestingly, although deoxycytidine is a good substrate for the kinase,[21a] acyclic cytidine analogues **8** and **10** are not. Because they lack activity, those pyrimidine analogues which are phosphorylated either are not converted to the triphosphates, or once at that level, they do not inhibit the viral DNA polymerase. This result is similar to that of Keller et al., who showed that a number of inactive acyclovir analogues were also substrates for the virus kinase.[21a]

The purine series of acyclic nucleosides, which includes DHPG, does contain active members. Hypoxanthine derivative **5f**, which corresponds to DHPG without the 2-amino functionality, is inactive even though it is phosphorylated by the thymidine kinase at 15% of the rate of thymidine. In contrast, the 2-aminopurine analogue **26** does inhibit herpes virus replication as does the 6-thio derivative **25**. Although **25** and **26** are better substrates than acyclovir for the thymidine kinase, they are substantially less active against herpes virus. The adenosine analogue **5e** is neither a substrate for the kinase or an inhibitor of herpes virus.

Other than DHPG, the 2,6-diamino derivative **27** with an ID_{50} of 3.3 μM is the most active member of this class of acyclic nucleosides. Since it is slowly phosphorylated by the virus thymidine kinase (at 11% of the rate of thymidine), **27** most likely exerts an antiviral effect by first being enzymatically deaminated to give DHPG. Precedent

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Table II. Effects of Oral Treatment with DHPG and **27** on an HSV-2 Encephalitis Infection in Mice

compd	dose, mg/kg	survivors/total	mean survival time, days
placebo		0/28 (0)[a]	9.1 ± 1.7[b]
DHPG	20	8/20 (40)[c]	13.8 ± 2.7[d]
	10	5/20 (25)[c]	10.9 ± 2.6[d]
	5	8/20 (40)[c]	9.9 ± 2.7
27	40	7/20 (35)[c]	11.5 ± 1.8[d]
	20	5/20 (25)[c]	10.9 ± 2.9[d]
	10	4/20 (20)[c]	10.8 ± 2.4[d]

[a] Percent survival. [b] Standard deviation. [c] Statistical significance of $p < 0.05$ by two-tailed Fisher extract test. [d] Statistical significance of $p < 0.05$ by two-tailed Mann–Whitney U test.

exists for the enzymatic deamination of 2,6-diaminopurine nucleosides. Baer et al. first showed that 2-aminoadenosine was converted to guanosine by adenosine deaminase isolated from calf intestinal mucosa.[22] More recently 2,6-diamino-9-[(2-hydroxyethoxy)methyl]purine was shown to exert an antiherpes effect by conversion to 9-[(2-hydroxyethoxy)methyl]guanine by adenosine deaminase.[23] Support for this mechanism comes from the fact that addition of 1 μM coformycin, an adenosine deaminase inhibitor, leads to a threefold reduction in the in vitro antiviral potency of **27**. An additional study confirmed that **27** is a substrate for calf intestinal mucosa adenosine deaminase, being converted to DHPG, which was detected by HPLC, but at less than 1% of the rate of the conversion of adenosine to inosine.

Because of the promising in vitro activity of **27**, it was evaluated in a mouse HSV-2 encephalitis model (Table II).[10] The data indicate that oral treatment with the diamino analogue **27** is nearly as effective as with DHPG (**1**) in preventing death in mice following HSV-2 infection. In a related experiment in mice in which the drug was administered subcutaneously, **27** and **1** were equally potent, and a dose of 20 mg/kg per day led to 60% survival. These data demonstrate that, although **27** is substantially less potent than DHPG in vitro, both compounds exert a similar antiherpetic effect in vivo.

Experimental Section

Nuclear magnetic resonance spectra were recorded on a Varian EM-390 (^{1}H NMR, 90 MHz), a Varian HA-100 (^{1}H NMR, 100 MHz), a Bruker WH-90 (^{1}H NMR, 90 MHz; ^{13}C NMR, 22.62 MHz), and a Bruker WM-300 instrument (^{1}H NMR, 300 MHz; ^{13}C NMR, 75.453 MHz), and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Ultraviolet spectra were recorded on a Cary-14 or a Hewlett Packard 8450A spectrometer. Spectroscopic data and elemental analyses were obtained by Syntex Analytical Research. All chromatographic purifications were carried out on silica gel. Melting points were determined on a hot-stage microscope and are corrected.

1-[[1-(Benzyloxy)-3-hydroxy-2-propoxy]methyl]uracil (13). A solution of **4a** (10.8 g, 27.2 mmol) in methanol (1 L) was vigorously stirred with 10% Pd/C (2.7 g) under 1 atm of H_2 for 24 h. The solution was then filtered, 10% Pd/C (2 g) was added to the filtrate, and vigorous stirring was continued under H_2 for an additional 24 h. The suspension was filtered, the filtrate evaporated to dryness and the residue chromatographed to give in order of elution 1.67 g (15%) of **4a**, 2.97 g (36%) of **13** and 1.44 g (25%) of **5a**. An analytical sample of **13** was obtained by recrystallization from acetone/dichloromethane: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 6 H, phenyl, H-6), 5.68 (d, J = 8 Hz, 1 H, H-5), 5.31, 5.26 (AB, J = 10 Hz, 2 H, H-1′), 4.51 (s, 2 H, benzylic), 3.91 (m, 1 H, H-4′), 3.50–3.74 (m, 4 H, H-3′, H-5′).

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1-[[1-(Benzyloxy)-3-iodo-2-propoxy]methyl]uracil (14). A solution of **13** (1.70 g, 5.6 mmol) and methyltriphenoxyphosphonium iodide (2.91 g, 6.4 mmol) in pyridine (2 mL) and DMF (10 mL) was stirred at room temperature for 1.5 h and then evaporated to dryness. The residue was chromatographed (1:20 methanol/dichloromethane) to give 1.42 g (61%) of **14** as an oil. An analytical sample was crystallized from diethyl ether: ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.71 (d, J = 8 Hz, 1 H, H-6), 7.30 (m, 5 H, phenyl), 5.60 (d, J = 8 Hz, 1 H, H-5), 5.20 (s, 2 H, H-1′), 4.48 (s, 2 H, benzylic), 3.81 (p, J = 5 Hz, 1 H, H-4′), 3.48 (d, J = 5 Hz, 2 H, CH_2O), 3.41, 3.27 (ABX, J = 5, 11 Hz, 2 H, CH_2I).

1-[[1,3-Bis(benzyloxy)-2-propoxy]methyl]-2-thiouracil (18). A mixture of bis(trimethylsilyl)-2-thiouracil (**17**) (9.5 g, 39 mmol), chloromethyl ether **3** (12.5 g, 39 mmol), and $Hg(CN)_2$ (9.9 g, 39 mmol) in benzene (60 mL) was stirred at room temperature for 16 h and then evaporated to dryness. The residue was dissolved in dichloromethane, filtered, and then washed with 30% aqueous KI and dilute aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and evaporated to a crude product (16 g) which was chromatographed (1:20 methanol/dichloromethane) and then crystallized from diethyl ether to give 3.7 g (23%) of **18**: ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.83 (d, J = 8 Hz, 1 H, H-6), 7.30 (m, 10 H, phenyl), 5.88 (d, J = 8 Hz, 1 H, H-5), 5.70 (s, 2 H, H-1′), 4.48 (s, 4 H, benzylic), 4.14 (p, J = 5 Hz, 1 H, H-4′), 3.53, 3.49 (ABX, J = 5, 11 Hz, 4 H, H-3′A, 5′A; H-3′B, 5′B).

1-[[1,3-Bis(benzyloxy)-2-propoxy]methyl]-*S*-methyl-2-thiouracil (19). A suspension of **18** (1.8 g, 4.4 mmol), $NaHCO_3$ (0.55 g, 6.5 mmol), and methyl iodide (0.81 mL, 13 mmol) in methanol (20 mL) was stirred at room temperature for 16 h and then evaporated. The residue was partitioned between dichloromethane and dilute aqueous HCl. The organic phase was dried (Na_2SO_4) and evaporated to an oil (1.9 g, quantitative). An analytical sample was purified by chromatography (3% methanol/dichloromethane) to give **19** as an oil: ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.80 (d, J = 8 Hz, 1 H, H-6), 7.30 (m, 10 H, phenyl), 5.83 (d, J = 8 Hz, 1 H, H-5), 5.38 (s, 2 H, H-1′), 4.47 (s, 4 H, benzylic), 3.97 (p, J = 5 Hz, 1 H, H-4′), 3.53, 3.49 (ABX, J = 5, 11 Hz, 4 H, H-3′A, 5′A, H-3′B, 5′B), 2.46 (s, 3 H, SCH_3).

1-[[1,3-Bis(benzyloxy)-2-propoxy]methyl]isocytosine (20). A solution of **19** (1.78 g, 4.17 mmol) in methanolic ammonia (40 mL saturated at 0 °C) was stirred for 16 h at room temperature and then for 24 h at 50 °C in a stoppered flask. The solution was evaporated to a white solid which was crystallized from ethyl acetate to give 1.0 g (60%) of **20**: ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.43 (d, J = 8 Hz, 1 H, H-6), 7.29 (m, 10 H, phenyl) 6.88 (br s, 2 H, NH_2), 5.51 (d, J = 8 Hz, 1 H, H-5), 5.22 (s, 2 H, H-1′), 4.47 (s, 4 H, benzylic), 3.93 (p, J = 5 Hz, 1 H, H-4′), 3.53, 3.49 (ABX, J = 5, 11 Hz, 4 H, H-3′A, 5′A, H-3′B, 5′B).

1-[(1,3-Dihydroxy-2-propoxy)methyl]isocytosine (21). A suspension of **20** (500 mg, 1.26 mmol) and 20% $Pd(OH)_2$/C (0.80 g) in cyclohexene (10 mL) and ethanol (30 mL) was heated at reflux for 16 h and then filtered through Celite and the filtrate evaporated to dryness. The residue was crystallized from ethanol/ethyl acetate to give 181 mg (66%) of **21**: UV λ_{max} (0.1 N HCl) 255 nm (ε 6050); (0.1 N NaOH), 256 (4400), 230 (11 900); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 7.42 (d, J = 8 Hz, 1 H, H-6), 6.86 (br s, 2 H, NH_2), 5.52 (d, J = 8 Hz, 1 H, H-5), 5.16 (s, 2 H, H-1′), 4.86 (br s, 2 H, OH), 3.45 (m, 5 H, H-3′, H-4′, H-5′); ^{13}C NMR (22.62 MHz, Me_2SO-d_6) δ 170.28 (C-4), 155.49 (C-2), 142.46 (C-6), 106.11 (C-5), 79.32 (C-1′, C-4′), 60.82 (C-3′, C-5′).

9-[(1,3-Diacetoxy-2-propoxy)methyl]guanine (22). A suspension of DHPG (**1**) (5.0 g, 20 mmol) and 4-(dimethylamino)pyridine (0.50 g, 4 mmol) in acetic anhydride was vigorously stirred at room temperature for 4 days and then evaporated to dryness. The residue was crystallized from methanol to give 5.7 g (86%) of **22**: ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 10.65 (br s, 1 H, NH), 7.82 (s, 1 H, H-8), 6.49 (br s, 2 H, NH_2), 5.42 (s, 2 H, H-1′), 4.13–3.95 (m, 5 H, H-3′, H-4′, H-5′), 1.91 (s, 6 H, OAc).

2-Amino-6-chloro-9-[(1,3-diacetoxy-2-propoxy)methyl]purine (23). A solution of **22** (2.98 g, 8.8 mmol), tetraethylammonium chloride (2.92 g, 17.6 mmol), *N,N*-dimethylaniline (1.12 mL, 8.8 mmol), and phosphorous oxychloride (4.79 mL, 51.4 mmol) in acetonitrile (25 mL) was heated at reflux for 10 min and then evaporated to dryness. The residue was chromatographed (1:15 methanol/dichloromethane) to give 2.15 g (65%) of **23**. Crystallization from ethyl acetate/hexane yielded 1.25 g (38%): ^{1}H

NMR (300 MHz, Me_2SO-d_6) δ 8.28 (s, 1 H, H-8), 7.01 (br s, 2 H, NH_2), 5.54 (br s, 2 H, H-1′), 4.14–3.93 (m, 5 H, H-3′, H-4′, H-5′), 1.85 (s, 6 H, OAc).

2-Amino-9-[(1,3-diacetoxy-2-propoxy)methyl]-6-thiopurine (24). A solution of **23** (1.25 g, 3.5 mmol) and thiourea (0.33 g, 4.3 mmol) in 2-propanol (100 mL) was heated at reflux for 18 h and then evaporated to dryness. The residue was crystallized from ethanol to give 1.07 g (86%) of **24**: ^1H NMR (300 MHz, Me_2SO-d_6) δ 8.17 (s, 1 H, H-8), 5.46 (s, 1 H, H-1′), 4.15–3.93 (m, 5 H, H-3′, H-4′, H-5′), 1.90 (s, 6 H, OAc).

2-Amino-9-[(1,3-dihydroxy-2-propoxy)methyl]-6-thiopurine (25). A solution of **24** (0.72 g, 2.0 mmol) and concentrated NH_4OH (7.5 mL) in methanol (75 mL) was kept in a stoppered flask at room temperature for 24 h and then evaporated to dryness. The residue was crystallized from water/methanol to give 0.40 g (73%) of **25**: UV λ_{max} (0.1 N HCl) 344 nm (ε 21 000), 262 (8110); (0.1 N NaOH) 319 (19 500), 270 (6870), 250 (14 100); ^1H NMR (300 MHz, Me_2SO-d_6) δ 10.80 (br s, 1 H, NH), 7.99 (s, 1 H, H-8), 6.83 (br s, 2 2 H, NH_2), 5.46 (s, 2 H, H-1′), 4.62 (t, J = 5 Hz, 2 H, OH), 3.54 (p, J = 5 Hz, 1 H, H-4′), 3.48–3.26 (m, 4 H, H-3′, H-5′).

2-Amino-9-[(1,3-dihydroxy-2-propoxy)methyl]purine (26). A suspension of Raney nickel (1.3 g) in a solution of **25** (254 mg, 0.94 mmol) in water (40 mL) and ethanol (20 mL) was heated at reflux for 24 h and then filtered and evaporated to dryness. The residue was crystallized from ethanol to give 84 mg (38%) of **26**: ^1H NMR (300 MHz, Me_2SO-d_6) δ 8.59 (s, 1 H, H-6), 8.18 (s, 1 H, H-8), 6.58 (br s, 2 H, NH_2), 5.56 (s, 2 H, H-1′), 4.70 (br m, 2 H, OH), 3.57 (m, 1 H, H-4′), 3.40 (m, 4 H, H-3′, H-5′).

2,6-Diamino-9-[(1,3-dihydroxy-2-propoxy)methyl]purine (27). A solution of **23** (200 mg, 0.63 mmol) in methanolic ammonia (15 mL saturated at 0 °C) was heated in a Parr bomb at 90 °C for 18 h. The solution was evaporated to dryness and the residue crystallized from methanol to give 136 mg (86%) of **27**: UV λ_{max} (0.1 N HCl) 291 nm (ε 9680), 251 (11 100); (0.1 N NaOH) 279 (9610), 255 (8810); ^1H NMR (300 MHz, Me_2SO-d_6) δ 7.88 (s, 1 H, H-8), 7.04 (br s, 2 H, NH_2), 6.10 (br s, 2 H, NH_2), 5.48 (s, 2 H, H-1′), 3.56 (p, J = 5 Hz, 1 H, H-4′), 3.45, 3.32 (ABX, J = 5, 11 Hz, 4 H, H-3′A, 5′A; H-3′B, 5′B); ^{13}C NMR (75.453 MHz, Me_2SO-d_6) δ 159.35 (C-2), 155.30 (C-6), 151.65 (C-4), 138.08 (C-8), 112.64 (C-5), 79.88 (C-4′), 71.28 (C-1′), 60.81 (C-3′, C-5′).

Plaque Assays. Experiments were conducted with Vero cells infected with HSV-1 (F strain) and then treated with the nucleoside analogue as described previously.[10] 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,[24] using HSV-1 (strain F)-affinity-purified kinase.[25] Reaction mixtures contained 100 mM Tris-Cl (pH 7.5), 50 mM KCl, 2 mM $MgCl_2$, 1 mM dithiothreitol, 25 mM NaF, 1 mg of BSA/mL, 0.5 mM [γ-^{32}P]ATP (20 μCi/μM), 0.2 mM nucleoside analogue, and enzyme in a 0.1-mL volume. The [γ-^{32}P]ATP required for the assay was from ICN Chemical and Radioisotope Division, 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,[26] and the results from the deoxynucleoside kinase assays were expressed as relative phosphorylation rates, a method that has been used by others.[25,27]

Adenosine Deaminase Assay. Adenosine deaminases from calf intestinal mucosa and bovine spleen were purchased from Sigma. Adenosine or **27** at a concentration of 100 μM was incubated with sufficient enzyme to convert 10–20% of substrate in 5 min at 37 °C. Enzyme reactions were stopped by the addition of 3.5% perchloric acid (final concentration), followed 10 min later by neutralization with 1 M KOH containing 0.2 M imidazole. Determination of substrate converted was done by separating products by high-pressure liquid chromatography (HPLC) using a Whatman SAX 10/25 column. To separate adenosine and inosine, a 0.01 M KH_2PO_4 buffer (pH 3.5) was used. DHPG and **27** were separated with use of deionized water at pH 7. In both assays 100-μL injections were made and 1 mL/min flow rates used. A Hewlett Packard Model 3390A integrator calculated peak areas. In order to determine molarity of the test sample, standard curves of inosine and DHPG were made by injecting 1–100 μM samples into the HPLC. Relative substrate conversion rates were based upon the amounts of adenosine and **27** converted per milligram of protein.

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 ten 50% lethal doses. DHPG and **27** were administered orally or subcutaneously once a day for 4 days, starting 24-h postinfection. Deaths were recorded for 21 days after infection.

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Registry No. 1, 82410-32-0; **2a**, 66-22-8; **2b**, 65-71-4; **2c**, 51-21-8; **2d**, 87-42-3; **2e**, 73-24-5; **2f**, 68-94-0; **3**, 74564-16-2; **4a**, 90056-91-0; **4b**, 90056-89-6; **4c**, 82410-25-1; **4d**, 74554-14-6; **4e**, 74554-15-7; **4f**, 93503-19-6; **5a**, 90067-88-2; **5b**, 74554-16-8; **5c**, 82410-26-2; **5e**, 74564-17-3; **5f**, 84960-04-3; **6**, 90057-04-8; **7**, 93503-20-9; **8**, 87892-46-4; **9**, 93503-21-0; **10**, 87892-55-5; 11, 93503-22-1; **12**, 90056-95-4; **13**, 93503-23-2; 14, 93503-24-3; **16**, 93503-25-4; **17**, 32865-74-0; **18**, 93503-26-5; **19**, 93503-27-6; **20**, 93503-28-7; **21**, 93503-29-8; **22**, 86357-19-9; **23**, 88110-70-7; **24**, 88110-69-4; **25**, 93503-30-1; **26**, 89419-23-8; **27**, 86629-59-6; $Hg(CN)_2$, 592-04-1; Pd, 7440-05-3; $Pd(OH)_2$, 12135-22-7.

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