7.6 (dd, 1 H), 7.1 (d, 1 H), 6.75 (dd, 1 H), 4.4 (q, 2 H), 4.0 (dd, 2 H), 3.9 (s, 3 H), 3.3 (dd, 2 H), 2.7 (m, 2 H), 1.45 (t, 3 H); IR 2940, 1670 cm^{-1}. Anal. ($C_{15}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

Ethyl 1-Acetoxy-2,3-dihydro-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxylate (25). A mixture of **21** (646 mg, 2.5 mmol), freshly prepared lead tetraacetate (2.43 g, 4.4 mmol),[20] and acetic acid (60 mL) was stirred under N_2 for 10 h. Water was added and the mixture was filtered and extracted with ether. The organic layer was washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The oily residue was chromatographed on silica gel with chloroform–ethyl acetate (4:1) as solvent. This procedure gave 91 mg of **24**, which was used directly in the next step.

A mixture of **24** (60 mg), acetic acid (3 mL), and concentrated nitric acid (0.1 mL) was stirred for 1 h, cooled in an ice bath, and treated with 20 mL of ice water. The precipitate that formed was dissolved in 80 mL of methylene chloride and this solution was washed with brine, dried over $MgSO_4$, and concentrated. This procedure gave 66 mg (7% overall) of **25** as yellow solid: mp 169–173 °C; NMR ($CDCl_3$) δ 7.4 (dd, 1 H), 7.1 (dd, 1 H), 6.5 (dd, 1 H), 4.3 (m, 4 H), 3.95 (s, 3 H), 2.9 (m, 2 H), 2.1 (s, 3 H), 1.3 (t, 3 H); IR 1740, 1700, 153, 1370 cm^{-1}. Anal. ($C_{17}H_{18}N_2O_7 \cdot 0.05CH_2Cl_2$) C, H, N.

Ethyl 1-Acetoxy-2,3-dihydro-5,8-dioxo-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-9-carboxylate (22). A mixture of **25** (160 mg, 0.44 mmol), tin (630 mg, excess), ethanol (60 mL), and 3 N HCl (15 mL) was stirred for 3 h and then treated with dilute $NaHCO_3$ solution. The resulting mixture was extracted with chloroform and this extract was washed with brine, dried over Na_2SO_4, and concentrated. The oily residue (160 mg) containing **26** was used directly in the next step.

A solution of crude **26** in 60 mL of acetone was treated with a solution of potassium nitrosodisulfonate (1.34 g) in 85 mL of pH 6 potassium dihydrogen phosphate. After 4 h, water and methylene chloride were added. The organic layer was washed with 5% Na_2CO_3 solution, dried over $MgSO_4$, and concentrated. Purification of the residue by chromatography on silica gel with methylene chloride containing 5% acetone as solvent gave 78 mg (51%) of **22** as yellow solid: mp 81 °C; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.7 (s, 1 H), 4.4 (m, 4 H), 3.9 (s, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H), 1.3 (t, 3 H); IR 1730, 1720, 1635 cm^{-1}. Anal. ($C_{17}H_{17}NO_7 \cdot 0.1CH_2Cl_2$) C, H, N.

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Registry No. 1, 4792-58-9; **2**, 96000-33-8; **3**, 96000-34-9; **4**, 96000-35-0; **5**, 96000-36-1; **5** (phenylcarbonate ester), 96000-53-2; **6**, 96000-37-2; **7**, 96000-38-3; **8**, 96000-39-4; **9**, 96000-40-7; **10**, 96000-41-8; **11**, 96000-42-9; **12**, 96000-43-0; **13**, 96000-44-1; **14**, 96000-45-2; **16**, 96000-46-3; **17**, 96000-47-4; **18**, 96000-48-5; **19**, 35150-22-2; **20**, 31676-32-1; **21**, 71948-62-4; **22**, 96000-49-6; **24**, 96000-50-9; **25**, 96000-51-0; **26**, 96000-52-1; 1-acetoxy-7-methoxydecarbamoylmitosene, 40863-76-1; methyl isocyanate, 624-83-9.

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Synthesis and Antiherpetic Activity of (*S*)-, (*R*)-, and (±)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine, Linear Isomers of 2′-Nor-2′-deoxyguanosine[1]

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Racemic 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(±)-iNDG], a new analogue of acyclovir (ACV) and a structural analogue of 2′-nor-2′-deoxyguanosine (2′NDG), was synthesized and found to inhibit the replication of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2). Subsequently, its optical isomers, (*R*)- and (*S*)-iNDG, were prepared from chiral intermediates. The chloromethyl ethers of 1,2-di-*O*-benzyl-D- and -L-glycerol were made and reacted with tris(trimethylsilyl)guanine to give the 9-alkylated guanines, which were deprotected by catalytic hydrogenolysis. Against HSV-1 and HSV-2 in cell culture, (*S*)-iNDG was approximately 10- to 25-fold more active than the *R* enantiomer and had an ED_{50} comparable to those for ACV and 2′NDG. The inferior activity of (*R*)-iNDG paralleled the poor inhibition of viral DNA polymerase by its phosphorylation products. In mice infected intraperitoneally or orofacially with HSV-1 or intravaginally with HSV-2, (*S*)-9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(*S*)-iNDG] was less efficacious than 2′NDG but comparable to or more active than ACV.

One of the most promising approaches to the chemotherapy of herpes simplex virus (HSV) infections has been to utilize the broad specificity of the HSV-coded thymidine kinase to generate antiviral phosphorylated nucleosides or nucleoside analogues in infected cells. Thus, the high therapeutic ratio of acyclovir (ACV, 1), an acyclic analogue of 2′-deoxyguanosine (2), is attributed to selective phosphorylation in infected cells by the HSV thymidine kinase and further phosphorylation by host cell enzymes to the triphosphate, a potent inhibitor of the viral DNA polymerase.[2]

In order to more closely mimic the structure of 2′-deoxyguanosine, we[3] and, independently, others[4-6] synthesized an analogue, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (3) or 2′-nor-2′-deoxyguanosine (2′NDG;

† West Point, PA.

(1) Presented in part at 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, NV, Oct 24–26, 1983, Abstr. 146. The compound 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine was originally given the trivial appellation "382."
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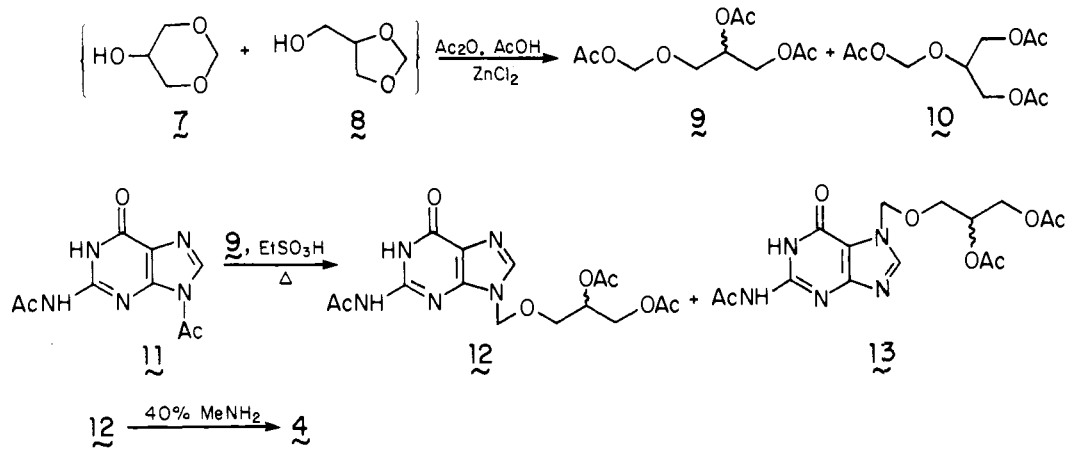
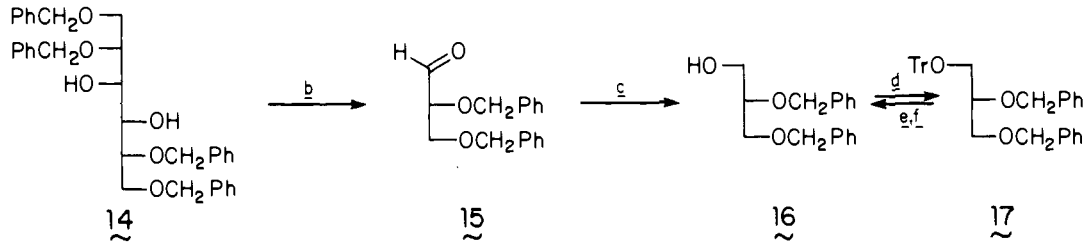
0022-2623/85/1828-0926$01.50/0

Scheme I

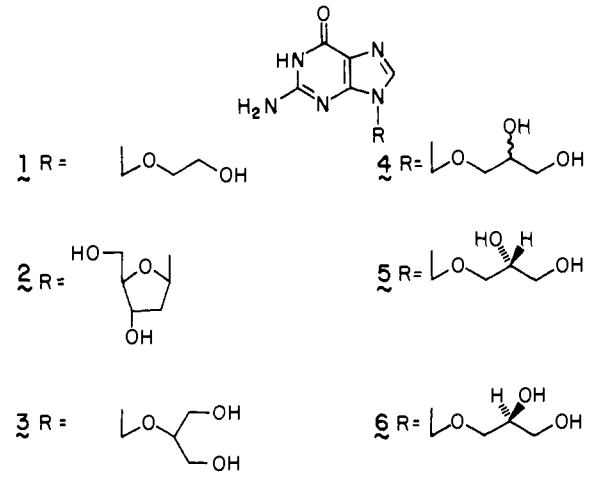


Scheme II[a]



[a] Configuration is represented by Fischer projection convention. [b] $NaIO_4$, MeOH. [c] $NaBH_4$, MeOH. [d] TrCl, pyridine. [e] AcOH-H_2O. [f] NaOMe-MeOH.

also known as BIOLF-62,[4] DHPG,[5] and BW-759U[7]), which showed superior efficacy in vivo. We now report the synthesis and biological activity of the racemic linear isomer of 2'NDG, 9-[(2,3-dihydroxy-1-propoxy)methyl]-guanine (4) or "iso-NDG" (iNDG). We also describe the synthesis of the enantiomers of 4, (*R*)- and (*S*)-iNDG (5, 6), from chiral starting materials. The antiherpetic efficacy of 5 and 6 is interpreted in terms of actions at the enzyme level. Subsequent to the completion of this work, an independent synthesis of 4 was reported by Lin and Liu[8] using a different route (which, based on our experience, runs the risk of migration of the acyl protecting groups during chloromethylation, although this possibility was not addressed by the authors).



Chemistry. In the synthesis of (±)-iNDG (Scheme I), as in our original preparation of 2'NDG,[3] the side chain was derived from glycerol formal, consisting of a mixture of isomers 7 and 8 in a ratio of approximately 3:1. Conversion of cyclic formals to acetoxymethyl ethers with acetic anhydride in the presence of strong acid is well-known.[9-11] We found that glycerol formal reacted readily with acetic anhydride in the presence of zinc chloride to give a mixture of the acetoxymethyl ethers 9 and 10.[3] The major isomer 9, isolated by preparative HPLC, was fused with diacetylguanine (11)[12] in the presence of acid catalyst under conditions similar to those reported by Schaeffer.[11] This led to a mixture of N^9- and N^7-alkylated guanine derivatives (12, 13), from which the major isomer 12 was isolated by chromatography. Deacetylation of 12 with 40% methylamine[11] afforded (±)-iNDG (4).

To prepare (*R*)- and (*S*)-iNDG, a different approach was adopted. The key chiral intermediates were the enantiomers of 1,2-di-*O*-benzylglycerol, each of which was derived from D-mannitol by modifications of literature methods. Because of difficulties in reproducing some of these literature procedures and incomplete characterization of several of the reported intermediates with regard to spectra and/or optical rotations, full experimental details are presented here.

For the synthesis of (*R*)-iNDG, 1,2,5,6-tetra-*O*-benzyl-D-mannitol (14)[13-15] was cleaved with sodium periodate (Scheme II) to give aldehyde 15.[13-15] This material was

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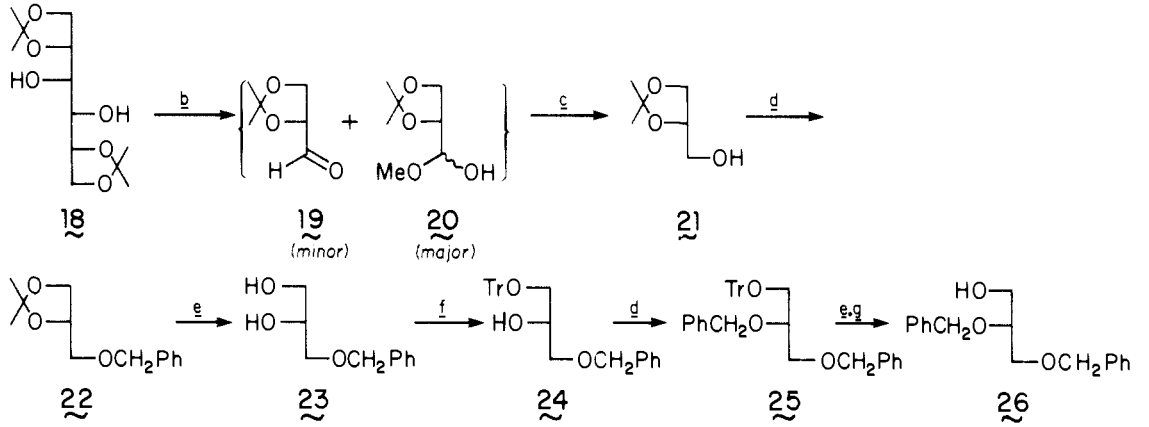
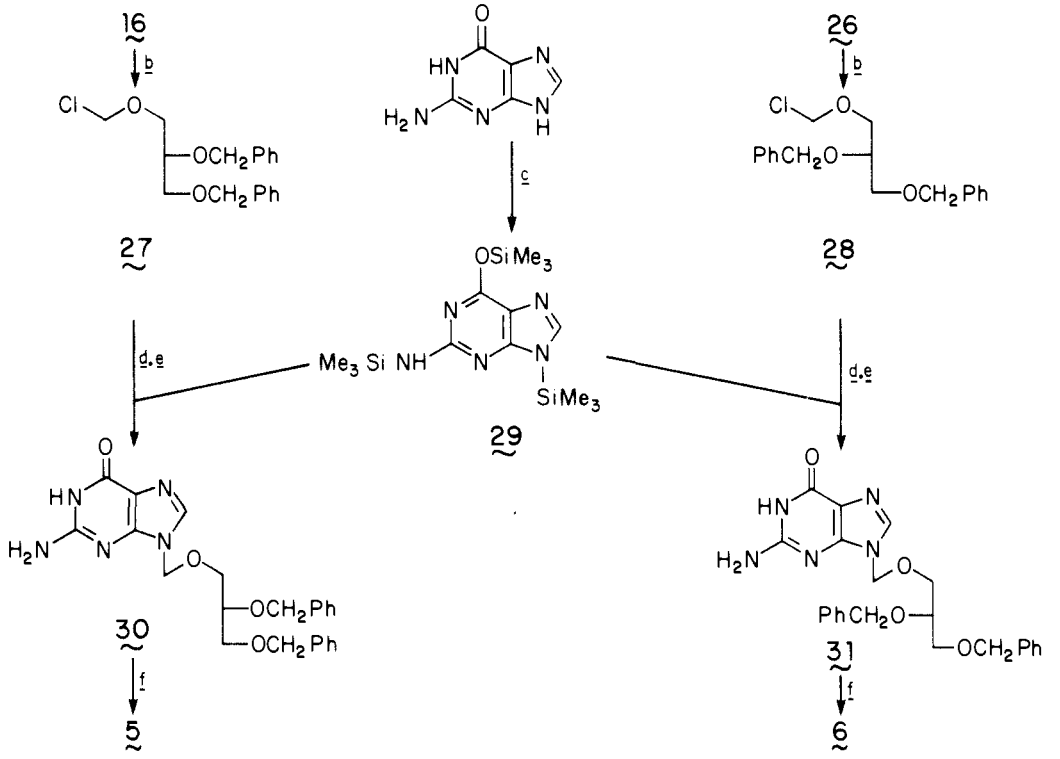
Scheme III[a]



[a] See footnote *a* to Scheme II. [b] $NaIO_4$, MeOH. [c] $NaBH_4$, 0.2 M Na_2HPO_4. [d] NaH, $PhCH_2Cl$, Me_2SO. [e] AcOH-H_2O. [f] TrCl, pyridine. [g] NaOMe, MeOH.

Scheme IV[a]



[a] See footnote *a* to Scheme II. [b] $(CH_2O)_x$, HCl, CH_2Cl_2. [c] BSA, Me_3SiCl, Et_3N. [d] Xylene, Δ. [e] *n*-PrOH-AcOH. [f] H_2, 20% $Pd(OH)_2/C$, TsOH, MeOH.

immediately reduced wtih sodium borohydride to 1,2-di-*O*-benzyl-L-glycerol (**16**, alternatively named as 2,3-di-*O*-benzyl-D-glycerol),[13,15] which was purified via the trityl derivative **17**.[13]

The dibenzylglycerol intermediate of opposite configuration was prepared according to Scheme III. Conversion of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**18**)[16,17] to the aldehyde **19** has been accomplished by lead tetraacetate[16] or aqueous sodium periodate.[17] We found that when the periodate cleavage was carried out in methanol, the major product was the hemiacetal **20**. This was in contrast to the synthesis of **15**, in which little if any hemiacetal was obtained under similar conditions. As **19** is subject to polymerization,[18] trapping it as the hemiacetal may be advantageous. In any case, the unpurified mixture of **20** and **19** was smoothly reduced to 1,2-*O*-isopropylidene-L-glycerol (**21**) with borohydride with use of Wickberg's conditions.[18] Because **21** is very susceptible to racemization, strictly acid-free conditions are required to maintain its optical integrity. The remaining steps in the conversion of **21** to 1,2-di-*O*-benzyl-D-glycerol (**26**, alternatively named as 2,3-di-*O*-benzyl-L-glycerol) correspond to the general route of Wickberg,[18] although many of the reaction conditions were significantly modified.

The chiral dibenzylglycerols **16** and **26** were treated with paraformaldehyde and anhydrous HCl to give the corresponding chloromethyl ethers **27** and **28** (Scheme IV). These were reacted at elevated temperature with the extremely moisture-sensitive tris(trimethylsilyl)guanine (**29**),[19,20] which was prepared by reaction of guanine with

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Table I. Staggered Enzyme Assay: Phosphorylation and DNA Polymerase Inhibition[a]

compd	step I:[b,c] MP, %	step II[c,d] MP, %	DP, %	TP, %	DNA polymerase inhibn,[e] % HSV-1	HeLa
(*R*)-iNDG	44	2	41	54	7	0
(*S*)-iNDG	61	2	6	91	57	10
2′NDG	85	1	3	93	55	13
ACV	9	2	1	52	85	34

[a] Average of three experiments. MP, DP, TP = mono-, di-, triphosphate. [b] Incubation with HSV-1 thymidine kinase. [c] Calculated percentages based on total phosphorylated and unphosphorylated compound. [d] Continued incubation of the mixture from step I with GMP kinase and crude extract of HSV-1 infected HeLa cells. [e] Following further incubation of the mixture from step II as described in the Experimental Section.

O,N-bis(trimethylsilyl)acetamide in the presence of trimethylsilyl chloride.[21] After workup and desilylation, the N^9-alkylated products **30** and **31** were isolated in approximately 50% yield. Debenzylation was accomplished by hydrogenolysis over palladium hydroxide on carbon in the presence of 2 equiv of *p*-toluenesulfonic acid.[21] The (*R*)- and (*S*)-iNDG (**5**, **6**) thus obtained gave optical rotations which, albeit small, were opposite in sign and equal in magnitude.

Biological Studies. Enzymatic phosphorylation of the acyclic nucleoside analogues was investigated by using a "staggered" assay. First, the compound was incubated with HSV-1 thymidine kinase, and the amount of monophosphate formed was measured after 4 h. Second, the incubation was continued overnight after adding GMP kinase and crude extract of HSV-infected cells; conversion to mono-, di-, and triphosphate was determined. Finally, an aliquot of the assay mixture containing the phosphorylated derivatives was incubated for 1 h with a DNA polymerase assay mixture to determine their inhibitory activity. As shown in Table I, both (*R*)- and (*S*)-iNDG were relatively good substrates for the HSV-1 thymidine kinase compared to ACV, although neither isomer matched the substrate activity of 2′NDG. However, (*S*)-iNDG was converted to triphosphate about as effectively as 2′NDG, while (*R*)-iNDG and ACV produced lesser amounts of triphosphate under these conditions. In the case of the *R* enantiomer, a significant amount of diphosphate accumulated. The ACV phosphorylation mixture was the most potent inhibitor of viral DNA polymerase. The (*S*)-iNDG and 2′NDG phosphorylation products were comparable in inhibition and in selectivity for the viral over the mammalian DNA polymerase. In contrast, the (*R*)-iNDG phosphorylation mixture was a very ineffective polymerase inhibitor, although more than 50% of the acyclonucleoside was at the triphosphate level.

Antiviral activities in vitro were determined for (*S*)-, (*R*)-, and (±)-iNDG in comparison with 2′NDG and ACV (Table II). (*S*)-iNDG was comparable in activity to 2′NDG and ACV against HSV-1 and HSV-2, while the *R* enantiomer required concentrations at least tenfold higher for equivalent inhibition. Consistent with the disparity in activity between the optical isomers, the racemate was no more than half as active as (*S*)-iNDG. In marked contrast to the potency of 2′NDG against human cytomegalovirus (HCMV) and varicella-zoster virus (VZV), (*S*)-iNDG was inactive against both viruses at 25 μg/mL.

In mice infected intraperitoneally with HSV-1, (*S*)-iNDG given subcutaneously or orally at 12.5 mg/kg per day provided significant protection, as measured by increase in survival time relative to placebo-treated controls (Table III). Subcutaneous treatment using the *R* enantiomer was not effective. In these experiments, the calculated relative potency of 2′NDG to (*S*)-iNDG (for comparable subcutaneous treatment) was 11.8, while the relative potency of (*S*)-iNDG to ACV (for comparable oral treatment) was 2.8. These values were statistically significant.

Table II. In Vitro Antiviral Activities[a]

virus	ED_{50}, μg/mL (*S*)-iNDG	(*R*)-iNDG	(±)-iNDG	2′NDG	ACV
HSV-1 (Schooler)	0.5–1.5	25	3–6	1–3	1–3
HSV-2 (Curtis)	1–3	25–50	6–12	3–6	1–3
HCMV (AD_{169})	NA[b]	NA	ND[c]	0.4–1.6	20–25
VZV (KMcC)	NA	ND	ND	1-2	1–2

[a] Assays for antiviral activity against HSV-1 and HSV-2 were done either by determining the drug concentration required to give 100% inhibition of viral cytopathic effect in half of infected primary rabbit kidney cell cultures or the drug concentration required to inhibit plaque development by 50% on MRC-5 cell monolayers. Assays for antiviral activity against HCMV and VZV were done by 50% plaque reduction on MRC-5 cell monolayers. [b] NA = not active at 25 μg/mL. [c] ND = not done.

Table III. Comparative Efficacy of Acyclic Nucleosides against Systemic HSV-1 Infection

treatment regimen[a] compd	route	daily dose, mg/kg	survivors: no./total	av survival time, days
(*S*)-iNDG	sc	12.5	4/10*[c]	10.7* (8.2, 15.0)[b]
		3.1	1/10	7.6 (6.3, 9.2)
		0.8	0/10	6.7 (5.9, 7.6)
(*R*)-iNDG	sc	12.5	1/10	7.5 (6.1, 9.2)
		3.1	1/10	6.5 (5.4, 7.9)
		0.8	0/10	6.3 (6.0, 6.6)
2′NDG	sc	12.5	7/10*	14.6* (11.5, 20.8)
		3.1	5/10*	12.6* (9.7, 18.0)
		0.8	3/10	9.8* (7.6, 13.2)
placebo			0/10	6.2 (5.3, 7.3)
(*S*)-iNDG	oral	50	3/10	12.9* (10.3, 17.2)
		12.5	0/9	8.1* (7.0, 9.4)
		3.1	0/10	6.9 (6.3, 7.6)
ACV	oral	50	2/10	8.7* (6.7, 11.7)
		12.5	1/10	7.6* (6.3, 9.3)
		3.1	0/10	6.5 (6.0, 6.9)
placebo			0/10	6.5 (5.3, 6.8)

[a] Groups of 10–20-g ICR/Ha mice (mixed sex) were infected intraperitoneally with 50–100 LD_{50} HSV-1 and treated twice daily subcutaneously for 4 days or orally for 7 days, starting immediately following infection. Animal survival was recorded daily for 15 days. [b] 95% confidence interval. [c] Asterisk indicates significantly different from placebo-treated infected mice ($p < 0.05$).

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(21) Conditions for the silylation, alkylation, and subsequent debenzylation were patterned after procedures of S. Karady, L. M. Weinstock, and J. S. Amato (Process Research, Merck and Co., Inc.), unpublished work.

Table IV. Efficacy against Orofacial HSV-1 Infection

treatment regimen[a]				
compd	route	dose, mg/kg per day or (concn)	mice with lesions: no./total[b]	av lesion score[b]
(S)-iNDG	oral	50	1/10*[d]	0.15* (−0.19, 0.49)[c]
		12.5	5/10*	0.75* (−0.05, 1.55)
		3.1	8/10	2.70 (1.57, 3.83)
		0.8	9/10	3.45 (2.52, 4.38)
ACV	oral	50	6/10*	1.45* (0.47, 2.43)
		12.5	8/10	2.95 (1.75, 4.15)
		3.1	10/10	3.75 (3.36, 4.14)
placebo			10/10	3.75 (3.40, 4.10)
(S)-iNDG	topical	(1.0%)	2/10*	0.40* (0.29, 1.09)
		(0.25%)	5/10*	1.35* (0.21, 2.49)
		(0.06%)	6/10	1.40* (0.36, 2.44)
		(0.02%)	9/10	2.75* (1.72, 3.78)
placebo			10/10	3.90 (3.67, 4.13)

[a] Oral treatment was by gavage administration of half-daily doses 8 h apart beginning 3 h after infection for a span of 7 days. Topical treatments were administered at 3-h intervals beginning 3 h after infection with three treatments on the first day and four treatments on the next 2 days. [b] Evaluated on day 7 after infection. [c] 95% confidence interval. [d] Asterisk indicates significantly different from placebo-treated infected mice ($p < 0.05$).

Oral delivery of (S)-iNDG at 12.5 mg/kg per day was also effective in preventing lesion development in mice infected with HSV-1 in the orofacial area and in reducing the severity of lesion involvement in those animals that developed lesions (Table IV). By contrast, 50 mg/kg per day of ACV was required to confer a significant reduction in lesion rate and lesion score.

(S)-iNDG was also effective against orofacial HSV-1 when applied topically (Table IV). Treatment using 0.25% (S)-iNDG significantly reduced the proportion of mice that developed orofacial lesions and lessened lesion severity in lesion-bearing animals. Treatment using as little as 0.02% (S)-iNDG resulted in significantly reduced lesion severity compared to placebo-treated infected control animals.

In mice infected intravaginally with 10 lethal doses (LD_{50}) of HSV-2, strain Curtis, (S)-iNDG administered orally, twice daily for 10 days at 50 mg/kg per day resulted in a significant increase in survival time (13.1 days) compared to similarly infected placebo-treated controls (8.3 days). This protection was comparable to that conferred by ACV (13.9 days) at 50 mg/kg per day.

(R)- and (S)-iNDG at 100 μg/mL produced no cytotoxicity in cell culture monolayers. Likewise, no overt toxicity was seen in mice when the iNDG enantiomers were used orally at 50 mg/kg per day for 10 days or subcutaneously at 12.5 mg/kg per day for 4 days. The solubility of (±)-iNDG in 0.15 M phosphate buffer (pH 7.2) at 25 °C was 3.6 mg/mL vs. 2.8 mg/mL for 2′NDG and 1.3–1.5 mg/mL for ACV.

Discussion

The discovery in these laboratories of antiherpetic activity for (±)-9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(±)-iNDG, 4], a linear isomer of 2′NDG (3), prompted the synthesis of the enantiomers of 4 from optically active starting materials. The importance of the chiral center in relation to antiviral activity is demonstrated by the fact that (S)-iNDG (6) inhibited HSV-1 and HSV-2 in vitro at levels comparable to those of 2′NDG and ACV, while (R)-iNDG (5) required concentrations at least 10-fold higher. In several tests against HSV-1 infections in mice, (S)-iNDG proved to be significantly more effective than ACV, although substantially less effective than 2′NDG, while the R enantiomer was ineffective at the same dose levels. Against HSV-2 infection in mice, (S)-iNDG and ACV were comparably active.

The mechanism of action for (S)-iNDG against herpes simplex virus infection is presumed to be similar to that of ACV.[2] (S)-iNDG was an excellent substrate for HSV-1 thymidine kinase, and the resulting monophosphate was converted efficiently to the triphosphate by GMP kinase and HSV-1 infected HeLa cell extracts. The less efficient phosphorylation of the R enantiomer and, in particular, the weak inhibition of HSV DNA polymerase by the (R)-iNDG phosphorylation products paralleled the inferior antiherpetic activity of this isomer in vitro and in vivo.

Although (S)-iNDG had activity in vitro against HSV-1 and HSV-2 comparable to 2′NDG and ACV, its antiherpetic spectrum appears narrower. Whether the inactivity of (S)-iNDG against HCMV and VZV is due to inadequate phosphorylation in cells infected by these viruses or to ineffective inhibition of the respective viral DNA polymerases is not presently known.

As a new analogue of ACV with excellent activity in murine herpes simplex infection models, apparently low toxicity, and enhanced aqueous solubility, (S)-9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(S)-iNDG, 6] is a promising candidate for further study.

Experimental Section

^1H NMR spectra were obtained with a Varian T-60A, XL-200, or SC300 spectrometer, using tetramethylsilane as internal standard. UV and IR spectra were run on Perkin-Elmer Lambda 5 and 727B spectrophotometers, respectively. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover apparatus. Preparative HPLC separations were performed on a Waters Prep 500 instrument using Prep Pak silica gel cartridges. Analytical HPLC determinations were carried out on a Varian Model 5060 liquid chromatograph. Compounds showed satisfactory purity by TLC on Analtech silica gel GF plates in the indicated solvent systems. Elemental combustion analyses, where indicated only by the elements, were within ±0.4% of theoretical values.

Solubilities were determined by suspending an excess amount of the compound in approximately 0.15 M phosphate buffer (pH 7.2) and shaking overnight in a water bath at 25 °C to give a saturated solution. The concentration of this compound in the filtered solution was calculated on the basis of spectrophotometric measurements, i.e., comparison of the UV absorbance at the λ_{max} for the saturated solution with the absorbance value observed for a known concentration of the compound.

(±)-1-(Acetoxymethoxy)-2,3-diacetoxypropane (9). To a stirred mixture of 17.1 mL (20.8 g, 0.2 mol) of glycerol formal, 60 mL of Ac_2O, and 6.7 mL of glacial AcOH maintained under N_2 was added 2.0 g of anhydrous $ZnCl_2$. All of the $ZnCl_2$ soon dissolved, followed within a few minutes by a fairly strong exotherm. After stirring at ambient temperature for 4.5 h, the solution was concentrated in vacuo. The residual oil was taken up in 700 mL of Et_2O and washed with 2 × 100 mL of saturated $NaHCO_3$, followed by 100 mL of H_2O. The organic phase was dried over $MgSO_4$, decolorized with charcoal, and concentrated in vacuo to give 45.8 g (92%) of colorless oil. By analytical HPLC on silica gel (7:3 hexane–EtOAc), this material appeared to consist exclusively of a mixture of the two product isomers (9, 10). The two peaks ran very close together, the major isomer having the shorter retention time. The ratio of major to minor isomer was estimated at approximately 3:1. The product mixture was divided into four nearly equal portions for purification by preparative HPLC. All runs were performed under identical conditions [two packs, elution with 7:3 hexane–EtOAc, flow rate 250 mL/min, with recycling (three cycles), refractive index detection]. Fractions were cut in the same place each run, so identical fractions from the various runs were combined as they were collected. No clear separation of peaks was observed from the chart trace. Fractions which contained only the major isomer by analytical HPLC were

combined and concentrated in vacuo to yield 17.0 g (34%) of colorless oil identified as **9**: 60-MHz NMR ($CDCl_3$) δ 2.15 (s, 9 H, $COCH_3$), 3.82 (d, J = 5.5 Hz, 2 H, CH_2OCH_2CH), 4.25 (center of m, 2 H, $CHCH_2OAc$), 5.0–5.4 (m, 1 H, CH_2CHCH_2), 5.32 (s, 2 H, OCH_2O).

Fractions highly enriched in the minor isomer were combined, concentrated, and rechromatographed under the same conditions as above to give 5.0 g of almost colorless oil identified as **10**:[3] 60-MHz NMR ($CDCl_3$) δ 2.10, 2.13 (overlapping s, 9 H, $COCH_3$), 4.0–4.3 [m, including apparent s at 4.17, 5 H total, $OCH(CH_2OAc)_2$], 5.36 (s, 2 H, OCH_2O).

(±)-N^2-Acetyl-9-[(2,3-diacetoxy-1-propoxy)methyl]guanine (12). A mixture of 2.61 g (11.1 mmol) of diacetylguanine (**11**),[12] 5.50 g (22.2 mmol) of **9**, and 55 mg of ethanesulfonic acid in a pear-shaped flask equipped with distillation adapter was heated in vacuo (≥100 mm) in an oil bath at 155–160 °C. The mixture gradually became homogeneous, and some distillate was collected. After stirring for 1.25 h, the melt was cooled and diluted with 100 mL of EtOAc. Crystallization was induced, leading to isolation of 1.23 g (29%) of the N^9-alkylated isomer as white crystals: mp 162.5–165 °C; homogeneous by TLC (9:1 $CHCl_3$–MeOH); UV λ_{max} (MeOH) 258 nm (ε 16 000), 277 (sh, 11 600); 60-MHz NMR (Me_2SO-d_6) δ 1.97 (s, 6 H, $COCH_3$), 2.19 (s, 3 H, $COCH_3$), 3.71 (d, J = 5 Hz, 2 H, CH_2OCH_2CH), 4.08 (distorted d, J = 6 Hz, 2 H, $CHCH_2OAc$), 4.8–5.2 [m, 1 H, $CH_2CH(OAc)CH_2$], 5.50 (s, 2 H, NCH_2O), 8.13 (s, 1 H, C8-H), 11.80, 12.10 (s, each 1 H, NH). An analytical sample (mp 173.5–176 °C) was obtained by recrystallization from n-PrOH. Anal. ($C_{15}H_{19}N_5O_7$) C, H, N.

Attempts to obtain a second crop from the mother liquor led to material contaminated with a substantial amount of the N^7-alkylated isomer **13** (slightly greater R_f on TLC) as well as some **11**. A previously isolated sample of **13** (contaminated only with some of the (1,3-diacetoxy-2-propoxy)methyl side-chain isomer) had been assigned as the 7-isomer on the basis of characteristic downfield shifts relative to **12** for the C8-H and NCH_2O resonances as well as the shift of the major UV absorption to longer wavelength and lower intensity: UV λ_{max} (MeOH) 263 nm (ε 13 700); 200-MHz NMR (Me_2SO-d_6) δ 5.90 (s, 2 H, NCH_2O), 8.39 (s, 1 H, C8-H). These shifts are consistent with spectral data recently reported for 7- and 9-(alkoxymethyl)-N^2-acetylguanines.[5]

(±)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine (4). A solution of 1.14 g (3.0 mmol) of **12** in 11.4 mL of 40% methylamine (aqueous) was stirred at reflux under N_2 for 1 h. The cooled, light orange solution was decolorized with charcoal and then concentrated in vacuo. Recrystallization of the residual solid twice from H_2O (adjusted to approximately pH 6 with AcOH) yielded 547 mg (73%) of white crystals: mp 246–247 °C dec; homogeneous by TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; UV λ_{max} (pH 1) 255 nm (ε 12 200), 274 (sh, 8250), λ_{max} (pH 7) 251 nm (ε 13 700), 269 (sh, 9560), λ_{max} (pH 13) 264 nm (ε 11 200); 60-MHz NMR (Me_2SO-d_6) 3.2–3.7 (m, 5 H, CH_2CHCH_2), 4.3–4.8 (v br mound, 2 H, OH), 5.35 (s, 2 H, NCH_2O), 6.47 (br s, 2 H, NH_2), 7.78 (s, 1 H, C8-H), 10.67 (br s, 1 H, N1-H). Anal. ($C_9H_{13}N_5O_4 \cdot 0.67H_2O$) C, H, N. These physical and spectral properties are in general agreement with those recently reported for **4** by an independent synthesis.[8]

2,3-Di-O-benzyl-D-glyceraldehyde (15). A mechanically stirred suspension of 10.3 g (48 mmol) of pulverized $NaIO_4$ in 100 mL of MeOH was adjusted to pH 6–6.5 by addition of 1 M NaOMe in MeOH. To this was added a solution of 19.0 g (36 mmol) of **14**[13–15] in 50 mL of MeOH. Vigorous stirring under N_2 at room temperature was continued for 24 h. The mixture was filtered and the filtrate was concentrated in vacuo to give 18.5 g (95%) of very pale yellow oil: TLC in 2:1 hexane–EtOAc; IR (Nujol) 1740 (s), 1710 (w) cm^{-1}; 60-MHz NMR ($CDCl_3$) δ 3.6–4.1 (m, 3 H, CH_2CH), 4.51, 4.68 (s, each 2 H, OCH_2Ar), 7.31 (m, 10 H, ArH), 9.68 (s, ≤ 1 H, CHO), in addition to minor contaminant peaks. The material was used directly without further purification.

2,3-Di-O-benzyl-D-glycerol (Equivalent to 1,2-Di-O-benzyl-L-glycerol) (16). To a solution of 18.35 g (68 mmol) of **15** in 200 mL of MeOH stirred in an ice bath under N_2 was added cautiously 1.36 g (36 mmol) of $NaBH_4$ in small portions. Immediately after the addition, no remaining starting material was observed by TLC (2:1 hexane–EtOAc). Therefore, the cold solution was neutralized with 1 M H_2SO_4 and filtered. Concentration of the filtrate gave 17.2 g of residual oil, which was divided into two equal portions and purified by preparative HPLC (two packs, elution with 5:2 hexane–EtOAc, 200 mL/min, refractive index detection). Fractions showing satisfactory purity by TLC were combined and concentrated in vacuo, yielding 13.2 g (71%) of colorless oil: $[\alpha]^{20}_D$ −18.8° (c 1.0, $CHCl_3$). This material was purified further via the trityl derivative as described below.

2,3-Di-O-benzyl-1-O-trityl-D-glycerol (17). A mixture of 6.80 g (25 mmol) of partially purified **16**, 6.97 g (25 mmol) of trityl chloride, and 40 mL of pyridine was stirred at room temperature under N_2 for 4 days and then concentrated in vacuo with mild warming. The residue was partitioned between 250 mL of Et_2O and 100 mL of H_2O. The Et_2O phase was washed with an additional 100 mL of H_2O and then quickly with 2 × 50 mL of cold 0.5 N H_2SO_4, followed immediately by 50 mL of half-saturated Na_2CO_3. The Et_2O solution was dried over Na_2SO_4, filtered, and concentrated in vacuo. Two recrystallizations from hexane–CH_2Cl_2 yielded 9.08 g (71%) of off-white crystals: mp 83.5–84.5 °C; $[\alpha]^{20}_D$ +8.8° (c 2.5, $CHCl_3$) [lit.[13] mp 84–84.5 °C; $[\alpha]_D$ + 9.2° (c 1, $CHCl_3$)]; 200-MHz NMR ($CDCl_3$) δ 3.29 (d, J = 5 Hz, 2 H, $CHCH_2OTr$), 3.67 (m, 2 H, $CHCH_2OCH_2Ar$), 3.78 (m, 1 H, CH_2CHCH_2), 4.53, 4.69 (s, each 2 H, OCH_2Ar), 7.25–7.5 (m, 25 H, ArH).

Regeneration of 2,3-Di-O-benzyl-D-glycerol (16). A mixture of 9.00 g (17.5 mmol) of **17**, 63 mL of glacial AcOH, and 44 mL of H_2O was stirred in an oil bath at 95–100 °C overnight. The mixture was cooled (refrigerator), and the solid trityl alcohol was removed by filtration. Concentration of the filtrate gave 5.1 g of amber oil. As the product had undergone partial acetylation, the oil was dissolved in 50 mL of MeOH and treated with 1 M NaOMe in MeOH until the solution was strongly basic. The solution was stirred at room temperature for 6 h, then neutralized with glacial AcOH, and concentrated in vacuo. The residue was partitioned between 50 mL of Et_2O and 5 mL of H_2O. The organic phase was washed with an additional 5 mL of H_2O, dried ($MgSO_4$), filtered, and concentrated. The residual oil was purified by preparative HPLC (one pack, elution with 5:2 hexane–EtOAc, 200 mL/min), yielding 4.21 g (88%) of a colorless oil: $[\alpha]^{20}_D$ −21.0° (c 1.0, $CHCl_3$); homogeneous by TLC (2:1 hexane–EtOAc); 60-MHz NMR ($CDCl_3$) δ 2.20 (s, 1 H, OH), 3.6–3.7 (m, 5 H, CH_2CHCH_2), 4.53, 4.65 (s, each 2 H, OCH_2Ar), 7.30 (s, 10 H, ArH).

1,2-O-Isopropylidene-L-glycerol (21). A mechanically stirred suspension of 81.3 g (0.38 mol) of pulverized $NaIO_4$ in 1.14 L of MeOH was adjusted to approximately pH 6 by addition of 1 M NaOMe in MeOH. To this mixture was added 75.0 g (0.287 mol) of 1,2:5,6-O-isopropylidene-D-mannitol (**18**),[16,17] and vigorous stirring under N_2 at room temperature was continued for 42 h, by which time TLC (1:1 hexane–EtOAc) indicated complete conversion to a product with a greater R_f value than that of the starting material. The insoluble salts were removed by filtration, and the filtrate was concentrated in vacuo. The residue was taken up in Et_2O, again filtered, and concentrated to give 83.0 g of colorless oil: IR (thin film) 3420 (s), 1740 (w); 60-MHz NMR ($CDCl_3$) 1.36, 1.44 (overlapping s, total 6 H, isopropylidene CH_3), 2.95 (v br s, ≤1 H, OH), 3.43 (s, ≤3 H, OCH_3), 3.8–5.0 (m, ≤4 H, glyceryl CH's), 9.67 (d, J = 1.5 Hz, ≤0.2 H, HC=O). This material, which appeared to consist mainly of the hemiacetal **20** along with a lesser amount of free aldehyde **19**, was dissolved directly in 830 mL of 0.2 M Na_2HPO_4. The solution was stirred mechanically in an ice bath as 5.0 g of $NaBH_4$ was added portionwise, followed by an additional 5.0 g of $NaBH_4$ 30 min later. Stirring in the ice bath was continued for 1 h. At this time, TLC (1:1 hexane–EtOAc) indicated complete conversion of starting material to a product having a slightly smaller R_f value. After the solution had warmed to room temperature, it was treated with solid NaCl until saturated. The mixture was decanted to remove undissolved salt, and the aqueous solution was extracted with 4 × 500 mL of $CHCl_3$. The combined organic fractions were dried over a mixture of K_2CO_3 and Na_2SO_4, filtered, and concentrated in vacuo. The residual oil was distilled through a Vigreux column to give 62.6 g (83% from **18**) of colorless liquid: bp 76–79.5 °C (10 mm); $[\alpha]^{22}_D$ +14.1° (neat) [lit.[18] bp 82–83 °C (14 mm); $[\alpha]^{23}_D$ +14.2° (neat)]; 60-MHz NMR ($CDCl_3$) δ 1.36, 1.43 (overlapping s, total 6 H, CH_3), 2.57 (t, J = 6 Hz, 1 H, OH), 3.5–4.4 (m, 5 H, CH_2CHCH_2).

3-O-Benzyl-1,2-di-O-isopropylidene-L-glycerol (22). To a suspension of 7.26 g (181 mmol) of NaH (60% in mineral oil) in 250 mL of dry Me_2SO stirred under N_2 in an ice bath was added

dropwise 21.8 g (165 mmol) of **21**. After H_2 evolution ceased, the mixture was warmed to room temperature, and 31.3 g (247 mmol) of benzyl chloride was added dropwise. Stirring at ambient temperature was continued overnight. The mixture was then partitioned between 1.8 L of H_2O and 1 L of Et_2O. The organic phase was dried ($MgSO_4$), filtered, and concentrated. Column chromatography of the residue on silica gel (elution with 95:5 hexane–EtOAc) afforded 20.2 g (55%) of colorless oil having $[\alpha]^{22}_D$ +19.4° (neat) and an additional 10.3 g from slightly less pure earlier fractions having $[\alpha]^{22}_D$ +17.9° (neat) [lit.[22] $[\alpha]_D$ +16.8° (neat)]; 60-MHz NMR ($CDCl_3$) δ 1.33, 1.38 (overlapping s, total 6 H, CH_3), 3.4–4.4 (m, 5 H, CH_2CHCH_2), 4.50 (s, 2 H, OCH_2Ar), 7.27 (s, 5 H, ArH).

3-*O*-Benzyl-L-glycerol (Equivalent to 1-*O*-Benzyl-D-glycerol) (23). A solution of 20.2 g (91 mmol) of **22** in 200 mL of 70% AcOH was stirred in an oil bath at 65 °C for 30 min, by which time TLC (4:1 hexane–EtOAc) indicated complete conversion to product. Concentration of the solution in vacuo yielded 15.7 g (95%) of colorless oil: α^{22}_D +6.3° (neat) [lit.[18] α^{21}_D +6.6° (neat); lit.[22] α_D +6.1° (neat)]; 200-MHz NMR ($CDCl_3$) δ 2.20, 2.69 (v br s, each 1 H, OH), 3.5–3.8 (m, 4 H, CH_2CHCH_2), 3.93 (m, 1 H, CH_2CHCH_2), 4.57 (s, 2 H, ArH), 7.37 (m, 5 H, ArH).

3-*O*-Benzyl-1-*O*-trityl-L-glycerol (24). This material was obtained from **23** according to the procedure of Wickberg,[18] who reported that it failed to crystallize. However, purification of the syrupy product by preparative HPLC (two packs, elution with 85:15 hexane–EtOAc, 200 mL/min, refractive index detection) gave a 54% yield of white crystals: mp 71–73.5 °C; 200-MHz NMR ($CDCl_3$) δ 2.42 (d, J = 6 Hz, 1 H, OH), 3.24 (m, 2 H, $CHCH_2OTr$), 3.59 (m, 2 H, $CHCH_2OCH_2Ar$), 4.00 [m, 1 H, $(CH_2)_2CHOH$], 4.56 (s, 2 H, OCH_2Ar), 7.3–7.5 (m, 20 H, ArH).

2,3-Di-*O*-benzyl-1-*O*-trityl-L-glycerol (25). Alkylation of **24** under the conditions used for the preparation of **22** yielded, after recrystallization from EtOH–$CHCl_3$, 29% of white crystals: mp 80–81 °C; $[\alpha]^{24}_D$ –9.4° (c 2.5, $CHCl_3$) [lit.[18] mp 84.5–86 °C; $[\alpha]^{18}_D$ –9° (c 2.5, $CHCl_3$)]. A subsequent run using DMF as solvent and preparative HPLC (elution with 95:5 hexane–EtOAc) for purification gave a 65% yield of white crystals: mp 81–83 °C; $[\alpha]^{22}_D$ –8.6° (c 2.5, $CHCl_3$); 200-MHz NMR ($CDCl_3$) identical with that of **17**.

2,3-Di-*O*-benzyl-L-glycerol (Equivalent to 1,2-Di-*O*-benzyl-D-glycerol) (26). Hydrolytic detritylation of **25** was accomplished by the procedure of Wickberg.[18] After purification on a silica gel column (elution with 4:1 hexane–EtOAc), there was obtained 68% of colorless oil: $[\alpha]^{20}_D$ +20.3° (c 1.1, $CHCl_3$); 200-MHz NMR ($CDCl_3$) δ 2.08 (v br s, 1 H, OH), 3.6–3.8 (m, 5 H, CH_2CHCH_2), 4.57 (s, 2 H, OCH_2Ar), 4.69 (dd, J = 12 Hz, 2 H, OCH_2Ar), 7.35 (s, 10 H, ArH). Inclusion of NaOMe treatment to regenerate any acetylated product, as in the conversion of **17** to **16**, afforded 89% of material with similar rotation. Wickberg[18] reported $[\alpha]^{23}_D$ +3.8° (neat), which in our hands was not reproducible.

1-*O*-(Chloromethyl)-2,3-di-*O*-benzyl-D-glycerol (27). A mixture of 2.26 g (8.3 mmol) of **16**, 0.25 g (8.3 mequiv) of paraformaldehyde, and 8 mL of CH_2Cl_2 was stirred vigorously in an ice bath under protection from moisture as HCl gas was bubbled in rapidly for 5 min, resulting in a clear solution, and then at a slower rate for 4 h. (**Caution**: As the carcinogen bis(chloromethyl) ether is a potential byproduct, due care should be exercised during this reaction and its workup.) The solution was removed from the ice bath, purged with N_2 to remove excess HCl, dried with Na_2SO_4, and filtered. Concentration of the filtrate in vacuo yielded 2.57 g (96%) of very pale yellow oil: 60-MHz NMR ($CDCl_3$) δ 3.7–3.9 (m, 5 H, CH_2CHCH_2), 4.47, 4.60 (s, each 2 H, OCH_2Ar), 5.40 (s, 2 H, OCH_2Cl), 7.23 (s, 10 H, ArH).

1-*O*-(Chloromethyl)-2,3-di-*O*-benzyl-L-glycerol (28). This material was prepared from **26** as described for the synthesis of **27** to give a 96% yield of **28** as a light yellow oil, identical with **27** by NMR.

(*R*)-9-[[2,3-Bis(benzyloxy)-1-propoxy]methyl]guanine (30). A mixture of 755 mg (5.0 mmol) of guanine, 5 mL of *O,N*-bis(trimethylsilyl)acetamide, 90 μL of Me_3SiCl, and 17 μL of Et_3N was stirred under N_2 in an oil bath at 115 °C for 5 h. The resulting solution was cooled and concentrated in vacuo (1 mm) over a hot water bath (raised to 87 °C). The viscous, amber residual oil (**29**) was removed from the rotary evaporator under N_2 and immediately covered with 6 mL of dry xylene. This solution was immediately transferred to an oil bath and stirred under N_2 at 115 °C as a solution of 2.02 g (≥5.7 mmol based on minimum purity of 90%) of **27** in 3 mL of xylene was added dropwise through a septum over 30 min. The bath temperature was then raised to 125 °C, and stirring was continued at this temperature for 12 h. The cooled solution was concentrated in vacuo. The residue was treated with 15 mL of *n*-PrOH and 2 mL of glacial AcOH, and the mixture was stirred under reflux for 1 h. Then the solution was filtered while hot and allowed to cool slowly, resulting in crystallization. The solid was collected on a filter and washed with *n*-PrOH followed by Et_2O, yielding 1.12 g (51%) of light golden-yellow powder: mp 192–197 °C; TLC in 9:1 $CHCl_3$–MeOH; UV λ_{max} (MeOH) 254 nm (ε 14 300), λ_{max} (MeOH + H^+) 259 nm (ε 13 000), λ_{max} (MeOH + OH^-) 264 nm (ε 12 400); 60-MHz NMR (Me_2SO-d_6) δ 3.4–3.8 (m, 5 H, CH_2CHCH_2), 4.44, 4.54 (s, each 2 H, OCH_2Ar), 5.38 (s, 2 H, NCH_2O), 6.51 (br s, 2 H, NH_2), 7.25 (s, 10 H, ArH), 7.81 (s, 1 H, C8-H), 10.69 (br s, 1 H, N1-H). Recrystallization from *n*-PrOH containing a little AcOH furnished an analytical sample: mp 191–195 °C. Anal. ($C_{23}H_{25}N_5O_4$) C, H, N.

(*S*)-9-[[2,3-Bis(benzyloxy)-1-propoxy]methyl]guanine (31). A solution of 135 mmol of tris(trimethylsilyl)guanine (**29**), prepared as above, in 150 mL of xylene was reacted with 53.5 g (≥155 mmol based on minimum purity of 93%) of **28** according to the conditions described for **30**. The viscous residual oil obtained upon concentration of the reaction solution was covered with 300 mL of *n*-PrOH, and the mixture was stirred under reflux. Initially, a clear solution was obtained, but precipitation soon began. After 1 h, the mixture was cooled. The solid was collected on a filter and washed successively with *n*-PrOH, a small volume of Me_2CO, more *n*-PrOH, and finally Et_2O. Recrystallization from *n*-PrOH–AcOH yielded 29.4 g (50%) of light yellow crystals: mp 198.5–200.5 °C; TLC in 9:1 $CHCl_3$–MeOH; UV λ_{max} (MeOH) 254 nm (ε 13 500), λ_{max} (MeOH + H^+) 259 nm (ε 12 200), λ_{max} (MeOH + OH^-) 264 nm (ε 12 200); 60-MHz NMR (Me_2SO-d_6) identical with that of **30**. A portion of the material was recrystallized from *n*-PrOH containing a little AcOH to give an analytical sample: mp 193–195.5 °C. Anal. ($C_{23}H_{25}N_5O_4$) C, H, N.

(*R*)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine (5). A mixture of 324 mg (0.74 mmol) of **30**, 281 mg (1.48 mmol) of *p*-toluenesulfonic acid monohydrate, 86 mg of 20% palladium hydroxide on carbon, and 5 mL of MeOH was shaken with H_2 (initial pressure 41 psi) for 17 h. The mixture was diluted with 1.75 mL of H_2O, neutralized with 0.60 mL (1.5 mmol) of 2.5 N NaOH, and then concentrated in vacuo (mild warming) to near dryness. The wet residue was treated with 2.5 mL of H_2O. The mixture was heated to boiling and filtered while hot through Celite. The filter cake was suspended in an additional 1.5 mL of H_2O, heated to boiling, and again filtered. The combined filtrates were reheated and then allowed to cool slowly, resulting in crystallization. The solid was collected on a filter and washed with small volumes of cold H_2O, followed by Me_2CO, to give 96 mg (47%) of white crystals: mp 238–239 °C dec; TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; $[\alpha]^{20}_D$ –1.4° (c 2, 0.1 N NaOH); UV λ_{max} (pH 1) 255 nm (ε 12 400), 275 (sh, 8410); λ_{max} (pH 7) 251 nm (ε 13 600), 268 (sh, 9580), λ_{max} (pH 13) 264 nm (ε 11 200); 300-MHz NMR (Me_2SO-d_6) δ 3.24–3.56 (m, 5 H, CH_2CHCH_2), 4.55 (t, J = 6 Hz, 1 H, CH_2OH), 4.78 (d, J = 5 Hz, 1 H, $CHOH$), 5.36 (s, 2 H, NCH_2O), 6.54 (br s, 2 H, NH_2), 7.85 (s, 1 H, C8-H), 10.67 (br s, 1 H, N1-H). Anal. ($C_9H_{13}N_5O_4 \cdot H_2O$) C, H, N.

(*S*)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine (6). Hydrogenation of **31** under the conditions described for the synthesis of **5** gave, after recrystallization from H_2O, a 49% yield of white crystals: mp 244–245 °C dec; homogeneous by TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; $[\alpha]^{20}_D$ +1.5° (c 2, 0.1 N NaOH); UV λ_{max} (pH 1) 255 nm (ε 13 600), 275 (sh, 9170), λ_{max} (pH 7) 251 nm (ε 13 800), 268 (sh, 9170), λ_{max} (pH 13) 264 nm (ε 11 000); NMR identical with that of **5**. Anal. ($C_9H_{13}N_5O_4 \cdot H_2O$) C, H, N.

Enzyme Assays. Step I (Thymidine Kinase Assay). The test compound (40 μg in 40 μL of 50% Me_2SO) was incubated in a mixture containing in a final volume of 200 μL 50 mM Tris-HCl, pH 7.5; 5 mM $MgCl_2$; 5 mM ATP; 2 mM dithiothreitol; 2.5 mM NaF; 15 mM phosphocreatine; 5 units of creatine kinase; 100 μg of bovine serum albumin; and 1 unit of HSV-1 thymidine kinase.[23] After 4 h of incubation at 37 °C, a 10-μL aliquot was

examined by HPLC (MicroPac AX-10 column, linear gradient from 0.01 to 1.0 M KH_2PO_4 in 7 and 5 min at 1.0 M, 3 mL/min) for the presence of monophosphate.

Step II (Phosphorylation to the Di- and Triphosphate). Twenty-five microliters of crude extract of HSV-1 infected HeLa cells was added to the rest of the step I assay mixture, followed by 2 μL (0.04 unit) of GMP kinase from hog brain (Boehringer-Mannheim). Incubation was continued overnight at 30 °C. Another 10-μL aliquot was examined by HPLC (same conditions), and the amounts of mono-, di-, and triphosphate were determined.

Polymerase Inhibition Assay. Twenty-microliter samples of the incubation mixture from step II above were incubated in a mixture containing in a final volume of 100 μL: 50 mM Tris-HCl, pH 8; 5 mM $MgCl_2$; 1 mM dithiothreitol; 13 μg of bovine serum albumin; 30 μg of activated salmon sperm DNA; 0.05 mM each of the four deoxyribonucleoside triphosphates, dATP, dGTP, dCTP, and dTTP, the latter labeled with ^3H in the methyl group; 10 μL of crude extract of HSV-1 infected HeLa cells; and 0.1 M $(NH_4)_2SO_4$. The $(NH_4)_2SO_4$ was omitted when the cellular polymerases were assayed. After 1 h of incubation at 37 °C, the reaction was stopped with 1 mL of 10% trichloroacetic acid (Cl_3CCOOH), and the precipitates were collected in glass fiber filters (Whatman GFC) and washed three times with 5% Cl_3CCOOH and once with EtOH. The radioactivity of the precipitates on the filter disks was counted in Aquasol 2.

In Vitro Antiviral Assays. A. Inhibition of Virus-Induced Cytopathology. Confluent monolayers of primary rabbit kidney cell cultures preincubated with serial dilutions of acyclic nucleoside in maintenance medium (EMEM plus 2% fetal calf serum plus neomycin) were challenged with use of ca. 10 $TCID_{50}$ of either HSV-1, strain Schooler, or HSV-2, strain Curtis. Cultures were incubated at 37 °C and evaluated for viral-induced cytopathology on day 5.

B. Inhibition of Virus Plaque Development. Confluent monolayers of MRC-5 cells were incubated for 1 h at 37 °C with HCMV, strain AD_{169}, VZV, strain KMcC, HSV-1, strain Schooler, or HSV-2, strain Curtis, prior to addition of the test acyclic nucleoside diluted in maintenance medium. Following further incubation at 37 °C (7 days for HCMV, 5 days for VZV, and 3 days for HSV-1 and HSV-2), monolayers were stained for plaque visualization.

The ED_{50} was determined as the drug concentration required to prevent virus cytopathology in half of the infected cell cultures or to reduce virus plaque numbers by 50%.

In Vivo Anitviral Evaluations. Intraperitoneal infection using HSV-1 (Schooler), vaginal infection using HSV-2 (Curtis), and orofacial infection using HSV-1 (strain S) were performed as described previously.[24,25] Subcutaneous and oral treatments were initiated immediately following infection and continued twice daily for 4 or 7 days, respectively, with 0.1-mL doses. Topical treatments were by application of 10 μL of compound at the concentrations indicated in HAV, a hydroalcoholic vehicle described by Davies et al.[25] Treatments were begun 3 h after orofacial infection and continued at 3-h intervals for three treatments on the first day and four treatments on the subsequent 2 days.

Statistical analyses were performed on transformed survival times for the intraperitoneal and intravaginal infection experiments according to the methods of Liddel.[26] Parallel-line analyses were performed on survival times to generate dose–response curves and calculate relative potencies. Statistical significance was based on a 95% confidence interval.

Orofacial lesions were evaluated on day 7 after infection. Lesion severity was evaluated on a graded scale of 0, for no evidence of infection, to 4.0, for total lesion involvement of the orofacial area.[25] Orofacial lesion scores were compared for different treatment groups by Duncan analysis[27] in which pairwise comparisons were performed at a level of significance based on a 95% confidence interval.

Note Added in Proof: An alternative synthetic route to (*S*)-iNDG has been reported in a recent communication from this laboratory.[28]

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