# Nucleic Acid Related Compounds. 47. Synthesis and Biological Activities of Pyrimidine and Purine "Acyclic" Nucleoside Analogues[1]

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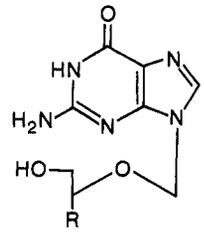
Various acyclic, i.e., (2-hydroxyethoxy)methyl and (2-acetoxyethoxy)methyl, analogues of pyrimidine and purine nucleosides have been prepared and evaluated for their antiviral, antimetabolic, and cytotoxic properties. All of the pyrimidine analogues, including (*E*)-5-(2-bromovinyl)-1-[(2-hydroxyethoxy)methyl]uracil (**12**) and its *O*-acetyl derivative (**13**), were virtually devoid of antiviral, cytotoxic, and antimetabolic activities. However, several of the 8-substituted derivatives of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) had higher antiviral specificity in vitro than the parent drug. The 8-methyl-, 8-amino-, 8-bromo-, and 8-iodoacyclovir derivatives have activities worthy of further investigation.

The "acyclic nucleoside" analogue of guanosine, 9-[(2-hydroxyethoxy)methyl]guanine (Ia) (acyclovir, Zovirax), is a highly potent and selective antiherpes agent that inhibits the replication of both type 1 (HSV-1) and type 2 (HSV-2) herpes simplex viruses at levels far lower than the cytotoxic concentrations.[2-6] Acyclovir derives its anti-



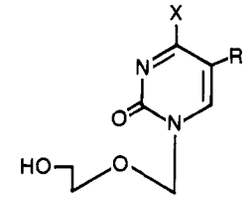
Ia R = H
b R = $CH_2OH$

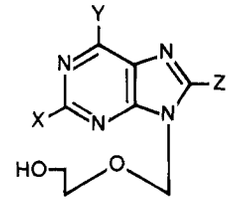
herpes selectivity from phosphorylation by the virus-encoded deoxythymidine (dThd) kinase.[7] The resulting acyclovir monophosphate is phosphorylated further by host cell enzyme(s), e.g., GMP kinase.[8] Acyclovir triphosphate inhibits viral DNA synthesis by preferential binding to the virus-encoded polymerase[9] and by incorporation at the growing 3′-end and tight complexing of this terminated DNA fragment with the viral polymerase.[10]

It is remarkable that an acyclic analogue of *guanosine* is recognized as a substrate by the viral-specified dThd kinase. The effective lack of such recognition by host cell enzymes provides the favorable low level of toxicity to uninfected cells. Hydroxylated acyclic alkyl analogues of adenosine also have been prepared and found to have biological properties.[11] One of these, (*S*)-9-(2,3-dihydroxypropyl)adenine, has broad-spectrum activity against DNA and RNA viruses.[12] A hydroxymethyl side-chain derivative of acyclovir, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (Ib) (also designated as BW 759, BIOLF-62, 2′-nor-2′-deoxyguanosine or 2′-NDG, and DHPG), has been reported from several laboratories.[13-18] This closely related analogue resembles acyclovir in its antiviral spectrum but clearly supersedes the parent drug in its antiviral potency in animal models. The empirical discovery of such potent agents with structures that are widely divergent from the "normal" substrate for the viral dThd kinase enzyme (and whose more closely related analogues often show no activity) suggested that evaluation of further acyclic nucleoside derivatives might prove to be useful.

In this report we describe the synthesis and biological properties of both purine- and pyrimidine-type acyclic nucleoside analogues with the (2-hydroxyethoxy)methyl side chain. A number of publications have appeared re-

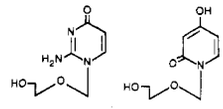
Chart I. Compound Structures[a]





| | X | R |
|---|---|---|
| 1 | OH | H |
| 2 | OH | $CH_3$ |
| 3 | OH | F |
| 4 | OH | Cl |
| 5 | OH | Br |
| 6 | OH | I |
| 7 | OH | $NO_2$ |
| 8 | $NH_2$ | H |
| 9 | $NH_2$ | $NO_2$ |
| 12 | OH | CH=CHBr (*E*) |
| 13 | *O*-acetyl-12 | |

| | R | X | Y | Z |
|---|---|---|---|---|
| 14 | H | H | OH | H |
| 15 | H | H | $NH_2$ | H |
| 16 | H | H | $N(CH_3)_2$ | H |
| 17 | H | $NH_2$ | Cl | H |
| 18 | Ac | H | Cl | H |
| 19 | Ac | Cl | Cl | H |
| 20 | Ac | $NH_2$ | Cl | H |
| 21 | H | $NH_2$ | OH | $CH_3$ |
| 22 | H | $NH_2$ | OH | Cl |
| 23 | H | $NH_2$ | OH | Br |
| 24 | H | $NH_2$ | OH | I |
| 25 | H | $NH_2$ | OH | OH |
| 26 | H | $NH_2$ | OH | $NH_2$ |
| 27 | H | $NH_2$ | OH | $NHCH_3$ |
| 28 | H | $NH_2$ | OH | $N(CH_3)_2$ |
| 29 | H | $NH_2$ | OH | $N(CH_2)_5$ |



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[a] The cyclic amide tautomers of the indicated hydroxy (lactim) heterocycles predominate almost exclusively in aqueous solution.

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cently describing syntheses (and biological studies) of related compounds.[19-25] We have previously reported a facile route to the useful reagent (2-acetoxyethoxy)methyl bromide and high-yielding procedures for introduction of this "acyclovir side chain" onto both purine- and pyrimidine-type bases.[26] This allowed convenient access to experimental quantities of acyclovir. We now report syntheses of some ring-substituted acyclovir derivatives with somewhat greater in vitro antiviral selectivity than the parent drug.

**Chemistry.** Compounds **1–3**, **5**, **6**, **8**, and **15** have been reported by others[21b,22,27,28] and ourselves.[26] Syntheses of compounds **4**, **7**, **11–13**, and **17–20** also were described by us previously[26] (see Chart I). Trimethylsilylation of 5-nitrocytosine and isocytosine (2-aminopyrimidin-4-one) and coupling of the resulting protected bases with (2-acetoxyethoxy)methyl bromide was effected by our earlier noted procedure.[26] Deacylation of these *O*-acetyl acyclic analogues of 5-nitrocytidine and isocytidine gave **9** and **10**, respectively.

The acyclic adenosine analogue **15** was known to function as a substrate of adenosine deaminase.[27] Enzymatic conversion of 100 mg of **15** gave 88 mg of the purified inosine analogue **14**. Treatment of 9-[(2-acetoxyethoxy)-methyl]-6-chloropurine (**18**) with dimethylamine in methanol gave 9-[(2-hydroxyethoxy)methyl]-6-(dimethylamino)purine (**16**) in 83% yield.

Of the series of 8-substituted acyclovir derivatives, only the 8-bromo compound **23** has been noted previously.[28] Bromination of acyclovir (**Ia**) was effected by using saturated bromine/water[29] to give **23** in 83% yield. Treatment of **23** with sodium acetate/acetic acid according to the general method of Ikehara et al.[30] normally gave 8-hydroxyacyclovir (**25**). However, in some experiments, the acetylated derivative 9-[(2-acetoxyethoxy)methyl]-2-aminopurine-6,8-dione was obtained. Deacetylation of this product with methanolic ammonia gave **25**. Treatment of **23** with aqueous hydrazine at reflux[31] gave 8-aminoacyclovir (**26**). Good yields of 8-(methylamino)- (**27**), 8-(dimethylamino)- (**28**), and 8-*N*-piperidinylacyclovir (**29**) were obtained by heating **23** with the respective aqueous amine solutions at elevated temperatures in a pressure vessel.

Treatment of acyclovir (Ia) with *tert*-butyl hydroperoxide in aqueous sulfuric acid in the presence of ferrous sulfate[32] gave 8-methylacyclovir (**21**). Repetition of the free-radical methylation procedure on the initially isolated mixture of Ia and **21** was required to effect an overall yield of 64% of **21**.

The novel chlorination procedure of Ryu and MacCoss[33] using dry hydrogen chloride and *m*-chloroperbenzoic acid in *N*,*N*-dimethylformamide converted acyclovir into its 8-chloro derivative **22** in 52% yield.

Attempted application of a reported procedure for the direct iodination of guanosine using *N*-iodosuccinimide[34] was not successful with acyclovir in our hands. We recently had observed that iodine monochloride smoothly converted uracil compounds to their 5-iodo derivatives.[35] Treatment of acyclovir with iodine monochloride in aqueous methanol gave the 8-iodo derivative **24** in 51% yield.

**Antiviral Activity.** The antiviral effects of these acyclic nucleoside analogues were assessed in primary rabbit kidney (PRK) cell cultures infected with herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), vaccinia virus (VV), and vesicular stomatitis virus (VSV). These assay systems have proven useful in previous studies on the comparative efficacy of antiherpes agents.[6] The data obtained in the present tests (Table I) for acyclovir, 5-iodo-2′-deoxyuridine (IDU), and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) are in accord with previously reported values.[6] Acyclovir and BVDU are seen to inhibit HSV-1 cytopathogenicity at concentrations of 0.05 and 0.007 μg/mL, respectively. However, the pyrimidine acyclonucleoside analogues were devoid of antiviral activity even at concentrations of 400 μg/mL (Table I), except for the cytosine compound. Analogous results were noted previously by Kelley et al.[21b] for compounds **1–3**, **5**, **6**, and **8**. It is noteworthy that compound **12**, which incorporates the pyrimidine and (*E*)-5-(2-bromovinyl) side chain of BVDU and the acyclic substituent of acyclovir,


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**Table I.** Antiviral Activity of Pyrimidine and Purine Acylic Nucleosides in Primary Rabbit Kidney (PRK) Cell Cultures

| no. | compd | $ID_{50}$,[a] μg/mL | | | |
|---|---|---|---|---|---|
| | | HSV-1 | HSV-2 | VV | VSV |
| | Pyrimidine Acyclic Nucleosides | | | | |
| 1 | 1-[(2-hydroxyethoxy)methyl]uracil | >400 | >400 | >400 | >400 |
| 2 | 1-[(2-hydroxyethoxy)methyl]thymine | >400 | >400 | >400 | >400 |
| 3 | 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil | >400 | >400 | >400 | >400 |
| 4 | 1-[(2-hydroxyethoxy)methyl]-5-chlorouracil | >400 | >400 | >400 | >400 |
| 5 | 1-[(2-hydroxyethoxy)methyl]-5-bromouracil | >400 | >400 | >400 | >400 |
| 6 | 1-[(2-hydroxyethoxy)methyl]-5-iodouracil | >400 | >400 | >400 | >400 |
| 7 | 1-[(2-hydroxyethoxy)methyl]-5-nitrouracil | >400 | >400 | >400 | >400 |
| 8 | 1-[(2-hydroxyethoxy)methyl]cytosine | 70 | 100 | >400 | >400 |
| 9 | 1-[(2-hydroxyethoxy)methyl]-5-nitrocytosine | >400 | >400 | >400 | >400 |
| 10 | 1-[(2-hydroxyethoxy)methyl]isocytosine | >400 | >400 | >400 | >400 |
| 11 | 1-[(2-hydroxyethoxy)methyl]-3-deazauracil | >400 | >400 | >400 | >400 |
| 12 | 1-[(2-hydroxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil | >400 | >400 | >400 | >400 |
| 13 | 1-[(2-acetoxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil | >400 | >400 | >400 | >400 |
| | Purine Acyclic Nucleosides | | | | |
| 14 | 9-[(2-hydroxyethoxy)methyl]hypoxanthine | >400 | >400 | >400 | >400 |
| 15 | 9-[(2-hydroxyethoxy)methyl]adenine | 10 | 10 | 20 | >400 |
| 16 | 9-[(2-hydroxyethoxy)methyl]-6-dimethyladenine | >400 | >400 | >400 | >400 |
| 17 | 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine | 2 (400)[b] | 20 | 70 | >400 |
| 18 | 9-[(2-acetoxyethoxy)methyl]-6-chloropurine | 70 (400)[b] | 70 | 20 | >400 |
| 19 | 9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine | 2 (40)[b] | >10 | >10 | >10 |
| 20 | 9-[(2-acetoxyethoxy)methyl]-2-amino-6-chloropurine | 20 (400)[b] | 20 | 70 | >400 |
| 21 | 9-[(2-hydroxyethoxy)methyl]-8-methylguanine | 0.5 | 0.5 | >400 | >400 |
| 22 | 9-[(2-hydroxyethoxy)methyl]-8-chloroguanine | 70 | >400 | >400 | >400 |
| 23 | 9-[(2-hydroxyethoxy)methyl]-8-bromoguanine | 0.5 | 0.5 | >400 | >400 |
| 24 | 9-[(2-hydroxyethoxy)methyl]-8-iodoguanine | 0.4 | 0.4 | >400 | >400 |
| 25 | 9-[(2-hydroxyethoxy)methyl]-8-hydroxyguanine | 15 | 25 | >400 | >400 |
| 26 | 9-[(2-hydroxyethoxy)methyl]-8-aminoguanine | 0.7 (200)[b] | 0.3 | >100 | >100 |
| 27 | 9-[(2-hydroxyethoxy)methyl]-8-monomethylaminoguanine | 7 | 3 | >400 | >400 |
| 28 | 9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine | 30 | 250 | ≥300 | >400 |
| 29 | 9-[(2-hydroxyethoxy)methyl]-8-*N*-piperidinylguanine | 70 | 150 | >400 | >400 |
| | Reference Compounds | | | | |
| acyclovir | 9-[(2-hydroxyethoxy)methyl]guanine | 0.05 | 0.04 | 50 | >400 |
| BVDU | (*E*)-5-(2-bromovinyl)-2′-deoxyuridine | 0.007 | 2 | 7 | >400 |
| IDU | 5-iodo-2′-deoxyuridine | 0.1 | 0.3 | 0.2 | >400 |

[a] Inhibitory $dose_{50}$ = concentration required to reduce cytopathogenicity of HSV-1 (strain KOS), HSV-2 (strain G), VV or VSV by 50%. [b] In parentheses: minimum cytotoxic concentration causing a microscopically detectable alteration of normal cell morphology.

had no antiviral activity. The 3-deazauridine (4-hydroxy-2-pyridinone) acyclic analogue **11** also was inactive.

Several of the 2- and 6-substituted purine analogues (**15**, **17**, **19**, and **20**) exhibited significant antiviral activity, although at concentrations 40–400-fold higher than required with acyclovir for inhibition of HSV replication (Table I). The $ID_{50}$ values for **15** for HSV-1 and HSV-2 correspond to the $ID_{50}$ for HSV-1 reported by Keller et al.[28] In contrast, the deamination product (inosine analogue **14**) had no antiherpes effect. The chloropurine analogues had antiviral activity but also displayed cytotoxic effects at 400 μg/mL. The 2,6-dichloropurine derivative **19** was cytotoxic even at 40 μg/mL.

The 2-amino-6-chloropurine analogue (**17**) had the largest margin between antiviral potency and cytotoxicity (Table I) of the chloro derivatives examined. Since this compound (**17**) was demonstrated to be a substrate for adenosine deaminase (hydrolytic dechlorination),[26] it was reasoned that it might function as an effective prodrug (for release of acyclovir) whose action might be dependent on the level of adenosine deaminase and the nature of the cell line. The antiviral potency of acyclovir had been shown to vary considerably from one cell line to another.[36] Direct comparison of acyclovir and **17** in a variety of cell lines of simian (BS-C-1 and Vero), murine (MO, BALB/3T3), feline (feline lung), and human (HeLa, human 21-trisomic fibroblast) origin[36] infected with HSV-1 was made. The antiviral potency of both compounds varied considerably in these test systems (data not shown), but the ratio of $ID_{50}$ values for **17** to acyclovir remained in the range of 20–200 throughout. An analogous approach using the 2,6-diaminopurine prodrug analogue has been reported very recently.[37]

Of the nine 8-substituted derivatives of acyclovir tested, the 8-methyl (**21**), 8-bromo (**23**), 8-iodo (**24**), and 8-amino (**26**) compounds inhibited HSV-1 and HSV-2 replication at concentrations only 10-fold higher than required for acyclovir (Table I). The antiviral potency of the 8-amino analogue (**26**) decreased upon substitution of alkyl groups for the amino hydrogens. It is noteworthy that the potent level of activity observed with the 8-bromo (**23**) and 8-iodo (**24**) analogues was absent with the 8-chloro (**22**) compound. A second preparation of **22** was purified, analyzed, and subjected to the biological test systems with comparable results. It is remarkable that substitution of the large and polarizable iodine atom at C8 of acyclovir is tolerated so well in the herpes-1 and -2 systems. In contrast to the 2- and 6-substituted chloropurine analogues, which retained some activity against VV, the 8-substituted acyclovir derivatives were devoid of anti-VV effect even at high concentrations.

**Antimetabolic Activity.** The antimetabolic effects of the acyclic nucleoside analogues were monitored by inhibition of incorporation of 2′-deoxythymidine (dThd) or 2′-deoxyuridine (dUrd) into DNA of PRK cell cultures (Table II). None of the pyrimidine compounds were inhibitory toward incorporation of either dThd or dUrd. The

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**Table II.** Antimetabolic Activity and Antiviral Index of Pyrimidine and Purine Acyclic Nucleosides in Primary Rabbit Kidney (PRK) Cell Cultures

| no. | compd | $ID_{50}$,[a] μg/mL dThd incorp | $ID_{50}$,[a] μg/mL dUrd incorp | antiviral index[c] |
|---|---|---|---|---|
| | Pyrimidine Acyclic Nucleosides | | | |
| 1 | 1-[(2-hydroxyethoxy)methyl]uracil | >400 | >400 | |
| 2 | 1-[(2-hydroxyethoxy)methyl]thymine | >400 | >400 | |
| 3 | 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil | >400 | >400 | |
| 4 | 1-[(2-hydroxyethoxy)methyl]-5-chlorouracil | >400 | >400 | |
| 5 | 1-[(2-hydroxyethoxy)methyl]-5-bromouracil | >400 | >400 | |
| 6 | 1-[(2-hydroxyethoxy)methyl]-5-iodouracil | >400 | >400 | |
| 7 | 1-[(2-hydroxyethoxy)methyl]-5-nitrouracil | >400 | >400 | |
| 8 | 1-[(2-hydroxyethoxy)methyl]cytosine | >400 | >400 | >5 |
| 9 | 1-[(2-hydroxyethoxy)methyl]-5-nitrocytosine | >400 | >400 | |
| 10 | 1-[(2-hydroxyethoxy)methyl]isocytosine | >400 | >400 | |
| 11 | 1-[(2-hydroxyethoxy)methyl]-3-deazauracil | >400 | >400 | |
| 12 | 1-[(2-hydroxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil | >200 | >400 | |
| 13 | 1-[(2-acetoxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil | >200 | >400 | |
| | Purine Acyclic Nucleosides | | | |
| 14 | 9-[(2-hydroxyethoxy)methyl]hypoxanthine | >100 | >100[b] | |
| 15 | 9-[(2-hydroxyethoxy)methyl]adenine | 49 | 22 | 2.2 |
| 16 | 9-[(2-hydroxyethoxy)methyl]-6-dimethyladenine | 400 | 350 | <0.9 |
| 17 | 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine | 50 | >100[b] | 25 |
| 18 | 9-[(2-acetoxyethoxy)methyl]-6-chloropurine | 60 | 62 | 3 |
| 19 | 9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine | 10 | 2 | 1 |
| 20 | 9-[(2-acetoxyethoxy)methyl]-2-amino-6-chloropurine | 55 | 71[b] | 2.7 |
| 21 | 9-[(2-hydroxyethoxy)methyl]-8-methylguanine | >400 | >400 | >800 |
| 22 | 9-[(2-hydroxyethoxy)methyl]-8-chloroguanine | >400 | >400[b] | >6 |
| 23 | 9-[(2-hydroxyethoxy)methyl]-8-bromoguanine | 340 | 225 | 450 |
| 24 | 9-[(2-hydroxyethoxy)methyl]-8-iodoguanine | ≥350 | ≥250 | 625 |
| 25 | 9-[(2-hydroxyethoxy)methyl]-8-hydroxyguanine | 48 | ≥100[b] | 3.2 |
| 26 | 9-[(2-hydroxyethoxy)methyl]-8-aminoguanine | 290 | 200 | 667 |
| 27 | 9-[(2-hydroxyethoxy)methyl]-8-monomethylaminoguanine | >400 | >400 | 133 |
| 28 | 9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine | >400 | >400 | 13 |
| 29 | 9-[(2-hydroxyethoxy)methyl]-8-*N*-piperidinylguanine | 250 | 333 | 3.5 |
| | Reference Compounds | | | |
| acyclovir | 9-[(2-hydroxyethoxy)methyl]guanine | 12 | 13 (21)[b] | 300 |
| BVDU | (*E*)-5-(2-bromovinyl)-2′-deoxyuridine | 73 | 27 | 3857 |
| IDU | 5-iodo-2′-deoxyuridine | 2 | 0.3 | 3 |

[a] Inhibitory $dose_{50}$ = concentration required to reduce [$^3$H-*methyl*]dThd or [$^3$H-1′,2′]dUrd incorporation into cellular DNA by 50%. Mean values for three or four separate experiments. [b] [$^3$H-6]dUrd instead of [$^3$H-1′,2′]dUrd. [c] Ratio of $ID_{50}$ for dThd or dUrd incorporation (whatever was lowest) to $ID_{50}$ for HSV-1, HSV-2, or VV (whatever was lowest; see Table I).

acyclic inosine (**14**) and 6-(dimethylamino)purine (**16**) compounds did not affect host cell DNA synthesis. However, the 2- and 6-substituted chloropurine compounds (**17–20**) were relatively active antimetabolites. The 2,6-dichloropurine compound (**19**) was the most active and inhibited dThd and dUrd incorporation into host cell DNA at concentrations of 10 and 2 μg/mL, respectively. Whereas acyclovir inhibited incorporation of dThd and dUrd at a concentration of 12–13 μg/mL, the 8-substituted derivatives exhibited little or no metabolic effects (Table II). The absence of antimetabolic activity is particularly striking for the 8-methyl derivative (**21**) which failed to inhibit incorporation of dThd and dUrd at a concentration of 400 μg/mL.

Consequently, the 8-methyl (**21**), 8-bromo (**23**), 8-iodo (**24**), and 8-amino (**26**) compounds are highly specific in their antiherpes activities. The antiviral index (as determined by the ratio of $ID_{50}$ for host cell DNA synthesis of the $ID_{50}$ for HSV-1 or HSV-2 replication) for each of these compounds is greater than that of the parent acyclovir (Table II). The antiviral index values for the other 8-substituted derivatives (**22**, **25**, **27–29**) are substantially lower than that of acyclovir.

**Antitumor Cell Activity.** Although acyclovir is considered to be essentially nontoxic to uninfected host cells,[2,4] it was inhibitory to the growth of murine leukemia L1210 cells at a relatively low concentration (13 μg/mL) (Table III). Compounds **15** and **19** inhibited the proliferation of L1210 cells at similar concentrations to that of acyclovir, and the purine analogues **17**, **18**, and **20** did so at higher concentrations. The hypoxanthine (**14**) and 6-(dimethylamino)purine (**16**) compounds were inactive.

With the exception of the iodo analogue **24**, none of the 8-substituted derivatives of acyclovir inhibited proliferation of L1210 cells at concentrations below 300 μg/mL. This further emphasizes their specificity as antiherpes agents. The pyrimidine compounds exhibited little or no cytotoxicity toward L1210 cells even at concentrations up to 1 mg/mL. In previously reported tests,[22] compounds **1–3** and **8** were found to be inactive against L1210 cells at concentrations up to $10^{-4}$ M.

Inhibition of incorporation of dThd and dUrd into the DNA of L1210 cells was monitored (as well as inhibition of cell proliferation) as parameters of cytotoxicity. The doses required to inhibit incorporation of dThd and dUrd were substantially higher than the $ID_{50}$ values for cell proliferation for all the cytotoxic acyclonucleoside analogues tested (Table III). The $ID_{50}$ for dUrd incorporation may reflect the potency for inhibition of thymidylate synthetase by nucleoside analogues of the 5-substituted 2′-deoxyuridine class such as 5-fluoro-dUrd, 5-(trifluoromethyl)-dUrd, 5-ethynyl-dUrd, etc.[38] The $ID_{50}$ for dUrd incorporation correlates well with the $ID_{50}$ for tumor cell proliferation for that type of compounds. It would not be expected that acyclic analogues of purine nucleosides would exert an inhibitory effect on thymidylate synthetase.

(38) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* **1981**, *19*, 321–330.

**Table III.** Inhibitory Effects of Pyrimidine and Purine Acyclic Nucleosides on the Growth and Metabolism of Murine Leukemia L1210 Cells

| no. | compd | $ID_{50}$,[a] μg/mL | | |
|---|---|---|---|---|
| | | cell growth | dThd incorp | dUrd incorp |
| | Pyrimidine Acyclic Nucleosides | | | |
| 1 | 1-[(2-hydroxyethoxy)methyl]uracil | >1000 | >1000 | >1000 |
| 2 | 1-[(2-hydroxyethoxy)methyl]thymine | >1000 | >1000 | >1000 |
| 3 | 1-[(2-hydroxyethoxy)methyl]-5-fluorouracil | >1000 | >1000 | >1000 |
| 4 | 1-[(2-hydroxyethoxy)methyl]-5-chlorouracil | >1000 | >1000 | >1000 |
| 5 | 1-[(2-hydroxyethoxy)methyl]-5-bromouracil | >1000 | >1000 | >1000 |
| 6 | 1-[(2-hydroxyethoxy)methyl]-5-iodouracil | >1000 | >1000 | >1000 |
| 7 | 1-[(2-hydroxyethoxy)methyl]-5-nitrouracil | 447 ± 82 | >1000 | >1000 |
| 8 | 1-[(2-hydroxyethoxy)methyl]cytosine | 675 ± 108 | >1000 | >1000 |
| 9 | 1-[(2-hydroxyethoxy)methyl)-5-nitrocytosine | 71 ± 21 | >500 | 374 |
| 10 | 1-[(2-hydroxyethoxy)methyl]isocytosine | >500 | >500 | >500 |
| 11 | 1-[(2-hydroxyethoxy)methyl]-3-deazauracil | >1000 | >1000 | >1000 |
| 12 | 1-[(2-hydroxyethoxy)methyl)-(*E*)-5-(2-bromovinyl)uracil | >1000 | >1000 | >1000 |
| 13 | 1-[(2-acetoxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil | >1000 | >1000 | >1000 |
| | Purine Acyclic Nucleosides | | | |
| 14 | 9-[(2-hydroxyethoxy)methyl]hypoxanthine | >1000 | >1000 | >1000 |
| 15 | 9-[(2-hydroxyethoxy)methyl]adenine | 19 ± 4 | >1000 | >1000 |
| 16 | 9-[(2-hydroxyethoxy)methyl]-6-dimethyladenine | >500 | >500 | >500 |
| 17 | 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine | 69 ± 31 | >1000 | ≥1000 |
| 18 | 9-[(2-acetoxyethoxy)methyl]-6-chloropurine | 44 ± 2 | 475 | 233 |
| 19 | 9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine | 12 ± 10 | 412 | 98 |
| 20 | 9-[(2-acetoxyethoxy)methyl]-2-amino-6-chloropurine | 91 ± 7 | 1000 | 1000 |
| 21 | 9-[(2-hydroxyethoxy)methyl]-8-methylguanine | 323 ± 52 | >500 | >500 |
| 22 | 9-[(2-hydroxyethoxy)methyl]-8-chloroguanine | 350 ± 40 | >1000 | >1000 |
| 23 | 9-[(2-hydroxyethoxy)methyl]-8-bromoguanine | 355 ± 131 | >500 | >500 |
| 24 | 9-[(2-hydroxyethoxy)methyl]-8-iodoguanine | 39 ± 3 | >1000 | >1000 |
| 25 | 9-[(2-hydroxyethoxy)methyl]-8-hydroxyguanine | >500 | >500 | >500 |
| 26 | 9-[(2-hydroxyethoxy)methyl]-8-aminoguanine | >500 | >500 | >500 |
| 27 | 9-[(2-hydroxyethoxy)methyl]-8-monomethylaminoguanine | >500 | >500 | >500 |
| 28 | 9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine | >500 | >500 | >500 |
| 29 | 9-[(2-hydroxyethoxy)methyl]-8-*N*-piperidinylguanine | 368 ± 69 | >500 | >500 |
| | Reference Compounds | | | |
| acyclovir | 9-[(2-hydroxyethoxy)methyl]guanine | 13 ± 2.5 | 367 | 50 |
| IDU | 5-Iodo-2′-deoxyuridine | 61 ± 6[b] | 4.3[b] | 0.82[b,c] |
| FDU | 5-Fluoro-2′-deoxyuridine | 0.001[b] | 80[b] | 0.003[b,c] |

[a] Inhibitory $dose_{50}$ = concentration required to reduce cell growth, or [$^3$H-*methyl*]dThd or [$^3$H-1′,2′]dUrd incorporation into cellular DNA by 50%. Mean values for four separate experiments. [b] As reported previously. [c] [$^{14}$C-2]dUrd instead of [$^3$H-1′,2′]dUrd.

The observed much lower $ID_{50}$ values for L1210 cell proliferation relative to the $ID_{50}$ concentrations for dUrd incorporation are in harmony with that expectation. The inhibition of cell proliferation without effect on incorporation of dUrd into DNA was particularly striking for the adenine compound **15**.

The inactive acyclic analogues of uridine were of interest for evaluation of potentiation of the antitumor activity of 5-fluoro-dUrd and its congeners. It has been reported that 5-benzyl-1-[(2-hydroxyethoxy)methyl]uracil functions as a potent inhibitor of uridine phosphorylase.[39] If the 5-substituted uracil acyclic nucleoside analogues interfere with phosphorylytic cleavage of uridines (and 2′-deoxyuridines) by uridine (or deoxythymidine) phosphorylases, an enhancing effect on the antitumor potency of 5-substituted 2′-deoxyuridines should result from combination treatment protocols. Four of the present acyclic compounds (**3**, **6**, **12**, and **13**) were examined in combinations with 5-fluoro-dUrd, 5-(trifluoromethyl)-dUrd, 5-nitro-dUrd (as its 5′-monophosphate), 5-ethyl-dUrd, (*E*)-5-(2-bromovinyl)-dUrd, 5-bromo-dUrd, and 5-propynyloxy-dUrd for inhibition of L1210 cell proliferation. Prior studies have demonstrated that the $ID_{50}$ inhibitory potency against L1210 cells ranged from 0.001 μg/mL for 5-fluoro-dUrd to >1000 μg/mL for 5-(propynyloxy)-dUrd (with the other dUrd compounds noted within these extremes).[38] These $ID_{50}$ values were not altered markedly by addition of any of the four acyclic uridine analogues (**3**, **6**, **12**, or **13**) (data not shown). Thus, the acyclic analogues did not potentiate (or antagonize) the cytotoxic effects of these 5-substituted 2′-deoxyuridines under the conditions employed in these preliminary experiments.

**Conclusions.** The salient features emerging from this study are (1) the remarkably high specificity of several of the 8-substituted acyclovir derivatives as inhibitors of HSV-1 and HSV-2 replication and (2) the absence of inhibitory activity of the pyrimidine acyclic nucleoside analogues. The latter point is exemplified by (*E*)-5-(2-bromovinyl)-1-[(2-hydroxyethoxy)methyl]uracil (**12**), which contains the structural features of the potent and selective antiherpes agents BVDU and acyclovir but is devoid of antiviral activity.

The 8-substituted acyclovir derivatives **21**, **23**, **24**, and **26** are about 10-fold less inhibitory than acyclovir against HSV-1 and HSV-2 in cell culture. However, they are more than 10-fold less toxic for the host cells and thus achieve a higher antiviral selectivity index. Further studies in animal models are in progress to evaluate the antiviral potential of certain of these new agents.

## Experimental Section

**Chemistry.** General procedures and instrumentation used were described in ref 26. $^1$H NMR spectral peaks (determined at 100 MHz in $Me_2SO$-$d_6$ with $Me_4Si$ as internal standard) for the "acyclic" side chain were similar as tabulated in ref 26. Complete spectral assignments are described for compounds **9** and **9a**, and heterocyclic proton peaks are given in Table IV. EI mass spectra were measured at 70 eV by direct sample introduction with a

(39) (a) Niedzwicki, J. G.; el Kouni, M. H.; Chu, S. H.; Cha, S. *Biochem. Pharmacol.* **1981**, *30*, 2097–2101. (b) Niedzwicki, J. G.; Chu, S. H.; el Kouni, M. H.; Rowe, E. C.; Cha, S. *Ibid.* **1982**, *31*, 1857–1861.

**Table IV.** Spectral Data for the Acyclic Nucleosides

| | ultraviolet spectral data | | | | mass spectra data: molecular ion | | |
|---|---|---|---|---|---|---|---|
| | 0.1 N HCl | | 0.1 N NaOH | | | | $^1$H NMR spectral data: |
| no. | max nm (ε) | min nm (ε) | max nm (ε) | min nm (ε) | calcd | found | base proton peaks |
| **9a** | 307 (9200) | 273 (3500) | 334 (17 100) | 255 (2900) | 273.0835[a] | 273.0844 | 8.06 + 8.56 (s + s, 1 + 1, $NH_2$), 9.27 (s, 1, H6) |
| | 253 (8500) | 230 (4600) | | | | | |
| **9** | 308 (9000) | 273 (3600) | 335 (17 200) | 255 (2800) | 231.0730[a] | 231.0737 | 8.04 + 8.53 (s + s, 1 + 1, $NH_2$), 9.26 (s, 1, H6) |
| | 253 (8600) | 232 (4700) | | | | | |
| **10a** | 255 (7500) | 236 (4700) | 252 (5100)[b] | | 227.0906 | 227.0902 | 5.96 (d, $J$ = 8 Hz, 1, H5), 7.86 (d, 1, H6), 8.20 (br s, 2, $NH_2$) |
| **10** | 255 (7900) | 237 (5400) | 250 (6100)[b] | | 185.0800 | 185.0794 | 5.56 (d, $J$ = 8 Hz, 1, H5), 6.48 (d, 1, H6), 6.95 (s, 2, $NH_2$) |
| | 216 (9700) | 210 (9400) | | | | | |
| **14** | 248 (12 400) | 217 (2600) | 252 (13 200) | 225 (3700) | 210.0753 | 210.0761 | 8.07 (s, 1, H2), 8.21 (s, 1, H8) |
| **16** | 267 (18 800) | 230 (1900) | 274 (19 500) | 235 (2800) | 237.1225 | 237.1222 | 3.46 (s, 6, $NMe_2$), 8.26 + 8.30 (s + s, 1 + 1, H2 + H8) |
| **21** | 255 (14 200) | 225 (3900) | 260 (13 400) | 230 (6200) | 239.1019 | 239.1014 | 2.40 (s, 3, $CH_3$), 6.47 (s, 2, $NH_2$), 10.55 (s, 1, NH) |
| | 275 (10 100)[b] | | | | | | |
| **22** | 256 (15 200) | 221 (2200) | 268 (13 100) | 232 (4400) | 259.0472[c] | 259.0470 | 6.66 (s, 2, $NH_2$), 10.81 (s, 1, NH) |
| **23** | 259 (16 700) | 223 (6400) | 270 (13 700) | 234 (5700) | 304.0045[a,d] | 304[e] | 6.66 (s, 2, $NH_2$), 10.77 (s, 1, NH) |
| **24** | 260 (17 300) | 230 (5200) | 270 (15 100) | 235 (6100) | 351.9829 | 351.9854 | 6.58 (s, 2, $NH_2$), 10.66 (s, 1, NH) |
| **25** | 293 (9700) | 269 (4200) | 281 (10 200) | 273 (9900) | 241.0811 | 241.0812 | 6.51 (s, 2, $NH_2$), 10.67 (br d, 2, NH1 + NH7) |
| | 247 (11 300) | 221 (3500) | 256 (10 800) | 230 (4500) | | | |
| **26** | 285 (13 600) | 270 (12 000) | 255 (20 100) | 240 (17 000) | 240.0971 | 240.0970 | 6.08 + 6.40 (s + s, 2 + 2, $NH_2$'s), 10.89 (s, 1, NH) |
| | 248 (21 500) | 223 (15 000) | 277 (16 100)[b] | | | | |
| **27** | 287 (9500) | 270 (7300) | 259 (13 900) | 228 (4500) | 254.1127 | 254.1125 | 2.81 (d, $J$ = 5 Hz, 3, $NCH_3$), 6.15 (q, 1, N*H*Me), 6.26 (s, 2, $NH_2$), 10.30 (s, 1, NH) |
| | 248 (16 900) | 223 (4300) | 280 (12 000)[b] | | | | |
| **28** | 288 (10 400) | 280 (10 000) | 268 (15 200) | 231 (3100) | 268.1283 | 268.1281 | 2.86 (s, 6, $NMe_2$), 6.42 (s, 2, $NH_2$), 10.48 (s, 1, NH) |
| | 258 (17 500) | 231 (3100) | | | | | |
| **29** | 265 (15 500) | 235 (3800) | 272 (13 100) | 222 (3800) | 308.1597 | 308.1594 | 1.60 (br s, 6, $(CH_2)_3$), 3.14 (s, 4, $N(CH_2)_2$), 6.40 (s, 2, $NH_2$), 10.40 (s, 1, NH) |
| | 285 (10 700)[b] | | | | | | |

[a] $MH^+$· (M + 1) ion. [b] Shoulder. [c] $^{35}$Cl-containing ion. [d] $^{79}$Br-containing ion. [e] Chemical-ionization ($NH_3$) low-resolution molecular ion.

computer-coupled AEI MS-50 instrument. Thin-layer chromatography (TLC) was performed on silica plates and Barnebey-Cheney AU-4 charcoal was purified and conditioned as described previously[40] for carbon column chromatography. Solvents for chromatography (volume:volume) were as follows: (A) MeOH/$CHCl_3$ (5:95), (B) MeOH/$CHCl_3$ (10:90), (C) upper phase of EtOAc/*n*-PrOH/$H_2O$ (4:1:2). Evaporations were effected in vacuo at room temperature with a Buchler rotary evaporator equipped with a Dewar dry ice condenser using aspirator or mechanical oil pump vacuum. Elemental analyses agreed within ±0.4% of theory.

**1-[(2-Acetoxyethoxy)methyl]-5-nitrocytosine (9a).** A drop of trimethylsilyl chloride was added to a magnetically stirred suspension of 468 mg (3 mmol) of 5-nitrocytosine in 10 mL of hexamethyldisilazane. This mixture was heated at reflux with exclusion of moisture until a clear solution was obtained. Volatile materials were evaporated in vacuo with protection against moisture, and the residue was dissolved in 25 mL of dry acetonitrile. This solution was stirred in an ice bath and treated slowly with a solution of 394 mg (2 mmol) of (2-acetoxyethoxy)methyl bromide[26] in 5 mL of dry acetonitrile. Stirring was continued for 2 h with warming to room temperature. TLC (solvent B) indicated that reaction was complete. Volatile materials were evaporated, and the residual yellow oil was chromatographed on a silica column (solvent A). Appropriately combined fractions were evaporated and, the residue was crystallized from MeOH with diffusion of $Et_2O$[40] to give 407 mg (75%) of **9a**: mp 108–109 °C; $^1$H NMR δ 2.01 (s, 3, OAc), 3.57 and 4.10 (AA'BB' multiplets, 2 and 2, $OCH_2CH_2OAc$), 5.29 (s, 2, $OCH_2N$), 8.06 and 8.56 (s and s, 1 and 1, $NH_2$), 9.27 (s, 1, H6). Anal. ($C_9H_{12}N_4O_6$) C, H, N.

**1-[(2-Hydroxyethoxy)methyl]-5-nitrocytosine (9).** To 15 mL of dry MeOH was added 30 mg (1.3 mmol) of sodium. After evolution of hydrogen was complete, 220 mg (0.81 mmol) of **9a** was added and stirring was continued for 2 h at room temperature. TLC (solvent B) indicated deprotection was complete. Amberlite IR-120($H^+$) resin was added until the solution gave a neutral test on moist pH paper. The resin was filtered and washed with MeOH and the combined filtrate evaporated. The colorless residual powder was crystallized from MeOH to give 170 mg (91%) of **9**: mp 192–193 °C; $^1$H NMR δ 3.52 (m, 4, $OCH_2CH_2O$), 4.66 (t, $J$ = 6 Hz, 1, OH), 5.26 (s, 2, $OCH_2N$), 8.04 and 8.53 (s and s, 1 and 1, $NH_2$), 9.26 (s, 1, H6). Anal. ($C_7H_{10}N_4O_5$) C, H, N.

**1-[(2-Acetoxyethoxy)methyl]-2-aminopyrimidin-4-one (10a).** Trimethylsilylation of 760 mg (6.85 mmol) of 2-aminopyrimidin-4-one (isocytosine) and coupling with 1.23 g (6.2 mmol) of (2-acetoxyethoxy)methyl bromide was effected as described above for the synthesis of **9a**. Chromatography of the crude material and diffusion crystallization gave 1.19 g (87%) of **10a**, mp 132–133 °C. Anal. ($C_9H_{13}N_3O_4 \cdot 0.5H_2O$) C, H, N.

**1-[(2-Hydroxyethoxy)methyl]-2-aminopyrimidin-4-one (10).** An 800-mg (3.5 mmol) sample of **10a** was deacetylated by stirring for 7 h at room temperature in 100 mL of $NH_3$/MeOH (presaturated at −10 °C and tightly stoppered). Evaporation of the solution and crystallization of the resulting solid from MeOH gave 625 mg (96%) of **10**, mp 187–189 °C. Anal. ($C_7H_{11}N_3O_3 \cdot 0.25H_2O$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]hypoxanthine (14).** A solution of 100 mg (0.48 mmol) of 9-[(2-hydroxyethoxy)methyl]-adenine[26] (**15**) in 30 mL of aqueous sodium hydrogen phosphate buffer (0.05 M, pH 7.5) was stirred at room temperature with 30 mg of Sigma Chemical Co. type II adenosine deaminase. When deamination was complete by TLC (solvent C) analysis, the solution was applied to a column of AU-4 carbon (10 g). The column was washed thoroughly with $H_2O$ and the product was eluted with $CH_3CN/H_2O$ (1:1). Evaporation of appropriately pooled fractions and crystallization of the residue from 98% EtOH gave 88 mg (88%) of **14**, mp 217–219 °C. Anal. ($C_8H_{10}N_4O_3$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-6-(dimethylamino)purine (16).** A 2.5-g (9.24 mmol) sample of 9-[(2-acetoxyethoxy)-methyl]-6-chloropurine[26] (**18**) was stirred for 8 h at room temperature in 150 mL of $Me_2NH$/MeOH (4:6). Evaporation of volatile materials and crystallization of the residue from *i*-PrOH gave 1.83 g (83%) of **16**, mp 107–108 °C. Anal. ($C_{10}H_{15}N_5O_2$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-methylguanine (21).** A stirred solution of 200 mg (0.89 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine[26] (**Ia**) and 800 mg (2.9 mmol) of $FeSO_4 \cdot 7H_2O$ in 50 mL of 1 M $H_2SO_4/H_2O$ was deoxygenated thoroughly ($O_2$-free $N_2$) and treated slowly with a mixture of 1 g (11 mmol) of *tert*-butyl hydroperoxide in 5 mL of $H_2O$ over 30 min. The

(40) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. *J. Am. Chem. Soc.* **1976**, *98*, 8204–8213.

resulting solution was stirred for an additional 30 min and adjusted to neutral pH with 1 M $NaOH/H_2O$. A fine brown precipitate was removed by centrifugation and the supernatent solution was decanted onto a carbon column which was washed thoroughly with $H_2O$. Elution with $CH_3CN/H_2O$ (3:7) gave an approximately equal mixture of starting material and product (TLC, *i*-$PrOH/H_2O$/concentrated $NH_3$(aq), 7:2:1). These fractions were combined and evaporated, and the residue was resubmitted to the above reaction conditions. TLC now indicated essentially complete conversion of Ia to product (**21**). Purification on a second carbon column and crystallization from $H_2O$ gave 135 mg (64%) of **21**, mp 304–305 °C. Anal. ($C_9H_{13}N_5O_3$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-chloroguanine (22).** A solution of 200 mg (0.89 mmol) of Ia in 2.5 mL of 0.5 M HCl/DMF(dry) was treated slowly with a solution of 225 mg (1.3 mmol) of *m*-chloroperbenzoic acid in 1 mL of DMF. The solution was stirred for 20 min at room temperature and treated with a further 50 mg (0.29 mmol) of *m*-chloroperbenzoic acid in 0.5 mL of DMF. Stirring was continued for 2 h and then saturated $NaHCO_3/H_2O$ solution was added dropwise to neutral pH. The solution was evaporated and the residue was partitioned between $H_2O$ and $Et_2O$. The aqueous layer was evaporated and the residue was purified by silica column (50 g) chromatography (EtOH/$CHCl_3$, 2:8). The product was crystallized from $EtOH/H_2O$ to give 121 mg (52%) of **22**, mp dec from ~280 °C. Anal. ($C_8H_{10}ClN_5O_3$) C, H, Cl, N.

**9-[(2-Hydroxyethoxy)methyl]-8-bromoguanine (23).** A stirred solution of 450 mg (2 mmol) of Ia in 100 mL of $H_2O$ was treated slowly with $Br_2/H_2O$ until the color of $Br_2$ persisted in solution. This solution was allowed to stand at 0 °C for 2 h and the separated solid was filtered and recrystallized from $EtOH/H_2O$ to give 507 mg (83%) of **23**, mp dec from ~280 °C. Anal. ($C_8H_{10}BrN_5O_3$) C, H, Br, N.

**9-[(2-Hydroxyethoxy)methyl]-8-iodoguanine (24).** A solution of 214 mg (0.95 mmol) of Ia in 14 mL of $MeOH/H_2O$ (1:1) was stirred at 50 °C and a solution of 1.55 g (9.5 mmol) of ICl in 7 mL of MeOH was added slowly. After 18 h of stirring at 50 °C, TLC (solvent C) indicated depletion of starting material. Solid $K_2CO_3$ (138 mg, 1 mmol) was added, and volatile materials were evaporated. The residue was triturated repeatedly with $Et_2O$ to remove excess ICl and was recrystallized twice from $EtOH/H_2O$ to give 170 mg (51%) of **24**, mp dec from ~260 °C. Anal. ($C_8H_{10}IN_5O_3$) C, H, I, N.

**2-Amino-9-[(2-hydroxyethoxy)methyl]purine-6,8-dione [9-[(2-hydroxyethoxy)methyl]-8-hydroxyguanine, 25].** A 100-mg (0.33 mmol) sample of **23** was heated at reflux for 3 h with 270 mg (3.3 mmol) of NaOAc in 10 mL of glacial HOAc. Volatile materials were evaporated, and the residue was dissolved in 50 mL of $H_2O$, neutralized with 0.1 N $NaOH/H_2O$, and applied to a carbon column (15 g). The column was washed thoroughly with $H_2O$ and the product eluted with $CH_3CN/H_2O$ (2:8). Evaporation of appropriately pooled fractions and crystallization of the residue from $EtOH/H_2O$ gave 54 mg (65%) of **25**, mp dec from ~260 °C. Anal. ($C_8H_{11}N_5O_4 \cdot 0.5H_2O$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-aminoguanine (26).** A 200-mg (0.66 mmol) sample of **23** was heated at reflux for 48 h with 0.5 mL of 95% $N_2H_4$ in 10 mL of $H_2O$. The solution was cooled and the crude product was filtered and recrystallized from $H_2O$ to give 110 mg (67%) of **26**, mp dec from ~300 °C. Anal. ($C_8H_{12}N_6O_3 \cdot 0.5H_2O$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-(methylamino)guanine (27).** A solution of 200 mg (0.66 mmol) of **23** in 5 mL of $CH_3NH_2/H_2O$ (1:1) was heated for 48 h at 120 °C in a small Parr pressure vessel. The solution was cooled and evaporated. The residue was dissolved in warm $H_2O$ and applied to a carbon column which was washed with $H_2O$. Product was eluted with $CH_3CN/H_2O$ (3:7). Evaporation of appropriate fractions gave 119 mg (71%) of a solid that was crystallized from $H_2O$ to give **27**, mp 216–220 °C dec. Anal. ($C_9H_{14}N_6O_3$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-(dimethylamino)guanine (28).** A 148-mg (0.49 mmol) sample of **23** was treated with 5 mL of $(CH_3)_2NH/H_2O$ (1:1) and processed as described above for the conversion of **23** → **27**. Crystallization from $H_2O$ gave 110 mg (85%) of **28**, mp dec from ~270 °C. Anal. ($C_{10}H_{16}N_6O_3$) C, H, N.

**9-[(2-Hydroxyethoxy)methyl]-8-*N*-piperidinylguanine (29).** A 150-mg (0.49 mmol) sample of **23** was heated at 160 °C for 48 h in 5 mL of piperidine/$H_2O$ (1:1) and processed as described above for the conversion of **23** → **27**. Further purification by preparative TLC (silica, solvent C), after the carbon column, was required before crystallization of the product from $H_2O$ to give 110 mg (72%) of **29**, mp dec from ~250 °C. Anal. ($C_{13}H_{20}N_6O_3 \cdot 0.5H_2O$) C, H, N.

**Antiviral and Antitumor Assays.** The methodology for measuring the inhibition of virus-induced cytopathogenicity and [$^3$H-*methyl*]dThd or [$^3$H-1′,2′]dUrd incorporation in PRK cell cultures has been described previously.[6] The procedures for monitoring the inhibition of L1210 cell growth and incorporation of [$^3$H-*methyl*]dThd and [$^3$H-1′,2′]dUrd into L1210 cell DNA also have been described.[38]

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