and 25-fold less potency, respectively. Since it is unlikely that 7 can be biooxidized to an *o*-quinone, and because it does not inhibit purified topo II (vide supra), we postulate that its DNA breakage, cytotoxicity, and in vivo P388 antitumor activity may be due to a previously undescribed third mechanism of action.

Preliminary data from our laboratory indicate that etoposide binds to purified calf thymus DNA.[20] Subsequent to our findings, a recent report by Ross[21] also describes DNA binding by VP-16. The cytotoxicity and in vivo antitumor activity of these new analogues, and presumably etoposide as well, cannot be entirely due to the *o*-quinone mechanism. While topo II inhibition certainly is part of the in vitro mechanism of action, the comparable P388 activity of 6 (3′-DesOMeVP) and etoposide, coupled with the diminished activity of 7 (4′-DesOHVP) and 9 (3′,5′-DidesMeOVP), which are not capable of easy bioactivation to *o*-quinones, implicates the in vivo importance of the *o*-quinone mechanism. Furthermore, a free 4′-OH does not appear to be essential for DNA breakage or cytotoxicity but seems to be critical for topo II inhibition. Since 7 does not inhibit purified topo II and yet still exhibits good in vitro cytotoxic potency and ability to overcome the VP-16 aquired resistance of a topo II deficient cell line, it is likely that a third mechanism of action for 7 at least, and probably for VP-16 as well, that could relate to direct DNA binding, may be operative in the complete pharmacological profile of these agents.[22]

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(22) The DNA binding by etoposide may itself contribute to the overall cytotoxicity, perhaps via inhibition of DNA polymerase. Consideration of such possibilities should be the subject of future research, the results of which may contribute to the design of second-generation etoposide analogues endowed with better activity against resistant tumors.

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Articles

Synthesis, Cytotoxicity, and Antiviral Activity of Certain 7-[(2-Hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine Nucleosides Related to Toyocamycin and Sangivamycin

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A number of 7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine derivatives related to the nucleoside antibiotics toyocamycin and sangivamycin were prepared and tested for their biological activity. Treatment of the sodium salt of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (1) with (2-acetoxyethoxy)methyl bromide (2) afforded a mixture of 4-amino-6-bromo-5-cyano-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (3) and the corresponding N_1 isomer. Debromination of this mixture gave the corresponding 4-amino-5-cyano-7-[(2-acetoxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (4) and 4-amino-5-cyano-1-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (5). Deacetylation of 4 and 5 furnished 4-amino-5-cyano-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (6) and the corresponding N_1 isomer (7), respectively. The sites of attachment for the acyclic moiety for 6 and 7 were assigned on the basis of UV spectral studies as well as ^{13}C NMR spectroscopy. Conventional functional group transformation of 6 provided a number of novel 5-substituted derivatives (8–10), including the sangivamycin derivative 8. The methyl formimidate derivative 10 was converted to the thioamide derivative 11 and the carbohydrazide derivative 12. Compounds 6 and 8–12 were tested for cytotoxicity to L1210 murine leukemic cells in vitro. None of these compounds caused significant inhibition of cell growth. Evaluation of compounds 4 and 6–12 for activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) revealed that only the thioamide (11) was active. It inhibited HCMV but not HSV-1 at concentrations producing only slight cytotoxicity in human foreskin fibroblasts (HFF cells) and KB cells.

The pyrrolo[2,3-*d*]pyrimidine ribonucleoside antibiotics toyocamycin and sangivamycin have exhibited an interesting range of biological effects, including antitumor[1-7] and antiviral activity.[7-12] The antineoplastic activity of toyocamycin appears to be mediated by its incorporation

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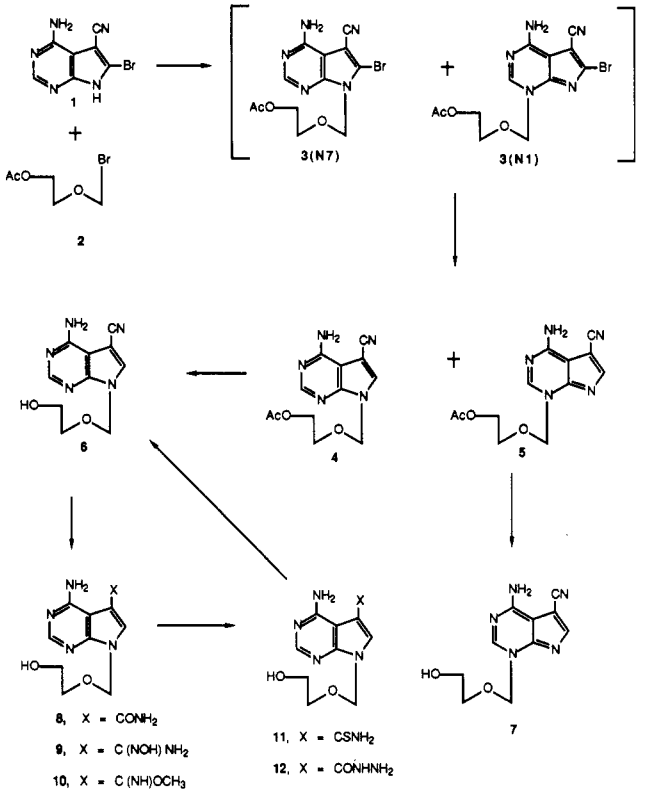
Scheme I



into RNA.[11a] Although incorporation may produce selective antiviral activity for RNA viruses, we have found little or no differential between the activity of toyocamycin against herpes viruses and its cytotoxicity in uninfected cells. These results led us to conclude that these ribosyl compounds do not have potential as anti-herpes agents.[13]

In contrast, acyclonucleosides such as acyclovir[14-16] are an important class of compounds having a broad range of antiviral properties. Intensive efforts to develop other acyclic nucleoside[17-20] analogues have produced ganciclovir (DHPG, 2′-NDG),[21,22] which is more active than acyclovir, especially against HCMV. Both acyclovir and ganciclovir are phosphorylated by herpes virus encoded thymidine kinase to their corresponding monophosphates, which are further phosphorylated by cellular kinases into the triphosphate derivatives. These inhibit virus-encoded DNA polymerase and also are incorporated into viral DNA.

The synthesis and evaluation of acyclic analogues of adenosine-type nucleosides are timely since all of the active compounds discussed above contain a guanine base. Furthermore, the utility of pyrrolo[2,3-*d*]pyrimidine nucleosides is enhanced by their stability to biodegradation. These compounds are not subject to deamination by adenosine deaminase, the major route to inactivation of adenosine analogues.[23] These considerations prompted us to synthesize acyclo analogues of the pyrrolo[2,3-*d*]pyrimidine adenosine nucleoside antibiotics toyocamycin and sangivamycin, in an effort to determine if they act as selective agents against DNA viruses.

Chemistry

After a careful evaluation of several synthetic approaches, designed to provide the desired compounds, we elected to use 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine[26] (**1**; see Scheme I) as our starting material. Treatment of the sodium salt of **1** (generated in situ by the treatment of **1** with NaH in DMF) with (2-acetoxyethoxy)methyl bromide[25] (**2**) afforded a mixture of two major nucleoside products. Separation of these two compounds proved to be very difficult, although a small amount of one compound was isolated after tedious column chromatography. This compound was assigned the structure 4-amino-6-bromo-5-cyano-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**3**) on the basis of UV and ^{1}H NMR spectroscopy. A mixture of the apparent positional isomers (**3** and the corresponding N_1 isomer, based on UV spectroscopy) was debrominated and the resulting mixture then separated on a silica gel column to afford 4-amino-5-cyano-7-[(2-acetoxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (**4**, 43.8%) and 4-amino-5-cyano-1-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**5**, 29%). These structural assignments for **4** and **5** were made on the basis of ^{1}H NMR and UV characteristics. The glycosylation reaction of **1** with (2-acetoxyethoxy)-methyl bromide (**2**) appears to be a thermodynamically controlled reaction. If this reaction was conducted at room temperature, the N_1 isomer predominates (virtually no isolable N_7 isomer, **3**, was observed). Formation of the N_7 isomer (**3**) increases with an increase of reaction temperature. In our hands, the best yield of **3** was obtained when

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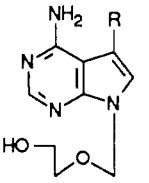
the reaction was conducted in dry DMF at 100 °C. Any further increase in temperature resulted in the decomposition of products and a concomitant decrease in the yield of **3**, which eventually resulted in a lower yield of **4** and **5**.

When the blocked N_7 and N_1 derivatives of 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine (**4** and **5**) were treated separately with methanolic ammonia in a pressure bottle, they yielded 4-amino-5-cyano-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**6**, 43%) and 4-amino-5-cyano-1-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**7**) (40%), respectively. The deblocking of **4** with methanolic ammonia also furnished a small amount of methyl 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-formimidate (**10**, 7%), along with the desired compound **6**. The structure of **6** was assigned on the basis of ^{1}H NMR and UV spectroscopy. The ^{1}H NMR spectrum revealed singlets at δ 8.25 and 8.34 which were assigned to the C-6 and C-2 protons. A broad singlet at δ 6.87 (D_2O exchangeable) was assigned to the amino function. The UV spectral data obtained for **6** were similar[26] to that reported for toyocamycin, thus confirming the site of glycosylation at N_7. The structural assignment for **7** was made by an examination of the ^{13}C NMR long-range C–H coupling constants (gated coupling technique). The ^{13}C NMR spectral data of toyocamycin is well documented in the literature,[24] and this allowed us to make a facile assignment of the carbon signals for the aglycon portion of compound **7**. The C-4 carbon is the most downfield carbon, appearing at δ 157.2. In the gated coupled spectra the C-4 carbon appears at δ 157.28 as a doublet due to coupling with the proton in the 2-position. Therefore, this confirmed the site of attachment for the hydroxymethyl group at the N_1 position in compound **7**. If the site of attachment had been at N_3, then the signal for the C-4 carbon would have been a multiplet due to the coupling of C-4 with the proton in the 2-position and the protons of the C'_1–CH_2–. In the gated spectra of **7**, the C-2 carbon appears as a pair of triplets centered at δ 146.66 and 144.36 due to coupling with the protons of the C'_1-CH_2, and the C-7a carbon appears as a multiplet, as expected.

To prepare the sangivamycin analogue 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**8**), **6** was treated with ammonium hydroxide containing H_2O_2, which gave a good yield of compound **8**. Treatment of **6** with hydroxylamine in ethanol gave 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (**9**) in 36% yield. Attempts to prepare 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-thiocarboxamide (**11**) by the treatment of **6** with liquid H_2S and 4-(dimethylamino)pyridine afforded a complex reaction mixture from which the isolation of **11** was not accomplished. On the basis of a previous report, it seemed apparent that the methyl formimidate derivative **10** might be used in a more successful manner to obtain compound **11**. Treatment of **6** with sodium methoxide in anhydrous methanol gave methyl 4-amino-7-[2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-formimidate (**10**) in low yield (yield 24%). Compound **10** was then treated with liquid H_2S in a sodium methoxide solution to afford compound **11** in 20% yield. Treatment of **10** with 85% hydrazine hydrate furnished 4-amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbohydrazide (**12**) instead of the expected carboxamidrazone. Compound **12** was identified by IR and ^{1}H NMR spectroscopy and elemental analysis. This rather unexpected result may be due to a hydrolysis of the reaction intermediate, since a conversion of **6** to the carboxamidine derivative was unsuccessful due to the instability of the carboxamidine derivative, which is structurally similar to the carboxamidrazone derivative.

Table I. Effect of 5-Substituted 4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidines on Growth of L1210 Murine Leukemic Cells in Vitro



compd	R	concn, μM	growth rate, % of control
6	CN[a]	100	100
8	$CONH_2$[a]	100	100
9	$C(NOH)NH_2$	100	89
10	$C(NH)OCH_3$	10	108
11	$CSNH_2$	10	93
12	$CO(NH)NH_2$	100	92

[a] For the corresponding ribonucleosides, the IC_{50}'s for inhibition of L1210 cell growth were 4 nM for toyocamycin [4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine] and 3 nM for sangivamycin [4-amino-5-carboxamido-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine].[13c]

Treatment of **11** with mercuric(II) chloride in the presence of the base triethylamine afforded the toyocamycin analogue 4-amino-5-cyano-7-[(2-hydroxyethoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**6**) in 41% yield. This conversion of the thioamide group to a nitrile group was confirmed by the presence of a nitrile absorption peak in the IR spectrum at 2205 cm^{-1} and the disappearance of the thioamide protons at δ 9.6 and 9.75 in the ^{1}H NMR spectrum. The physicochemical data of compound **6** prepared by this route are the same as those observed for compound **6** prepared earlier by the treatment of **4** with methanolic ammonia.

Biological Results and Discussion

In Vitro Antiproliferative Testing. The potential of compounds **6** and **8**–**12** as antitumor agents was evaluated by determining whether they were able to inhibit the growth of L1210 murine leukemic cells in vitro. As shown in Table I, none of these acyclic 4,5-disubstituted pyrrolo[2,3-*d*]pyrimidine nucleosides caused any significant inhibition. All were tested at 100 μM, or at the next highest concentration attainable in the tissue culture medium, as indicated in Table I.

The lack of cytotoxicity of these compounds is remarkable, particularly in view of the fact that compounds **6** and **8**, respectively, are direct acyclic analogues of the highly potent cytotoxic nucleoside antibiotics toyocamycin and sangivamycin. Thus, replacement of the ribosyl moiety of toyocamycin or sangivamycin by the acyclic moiety (2-hydroxyethoxy)methyl (HEM) caused a complete loss of cytotoxic activity. This loss might be accounted for by loss of the substrate activity of the analogues for activating cellular enzymes such as adenosine kinase,[27] and/or a loss of the ability of metabolites of the analogue to interact with their biochemical targets. The lack of cytotoxic activity of acyclo analogues of tubercidin, toyocamycin, and sangivamycin was also demonstrated in

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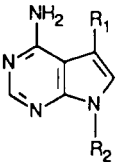
Table II. Antiviral Activity and Cytotoxicity of Acyclic Pyrrolo[2,3-*d*]pyrimidine Nucleosides



compd	substituent R_1	substituent R_2	50% inhibitory concentration, μM: plaque-reduction assay HCMV	plaque-reduction assay HSV-1	cytotoxicity HFF[a]	cytotoxicity BSC[a]	cytotoxicity KB[b]
1a	H	7-HEM[c]	>100[d]	>100	>100	>100	>320
4	CN	7-AEM	>100[e]	>100	>100[e]	>100	
6	CN	7-HEM	>100	>320	>100	>320	>100
7	CN	1-HEM	>320[e]	>100	>320[e]	>100	
8	$CONH_2$	7-HEM	>320[e]	>100	>320[e]	>100	>100
9	$HON{=}CNH_2$	7-HEM	>100	>100	>100	>100	
10	$HN{=}COCH_3$	7-HEM	>100	>100	>100	>100	
11	$CSNH_2$	7-HEM	11[e]	>100	100[e]	>100	>100[f]
12	$CONHNH_2$	7-HEM	>100	>100	>100	>100	
acyclovir			63[e]	3.9[g]	>100	>100	>100
ganciclovir (DHPG)			7.9[h]	2.3[e]	>100	>100	1000

[a] Visual cytotoxicity scored on uninfected HFF or BSC-1 cells at time of HCMV or HSV-1 plaque enumeration. [b] Average percent inhibition of DNA, RNA, and protein synthesis determined in KB cells as described in the text. [c] Abbreviations: AEM, 7-[(2-acetoxyethoxy)methyl]; 1-HEM, 1-[(2-hydroxyethoxy)methyl]; 7-HEM, 7-[(2-hydroxyethoxy)methyl]. [d] ">" indicates IC_{50} concentration not reached at noted (highest) concentration. [e] Average of two to four experiments. [f] Effect on RNA and protein synthesis only. The IC_{50} for effect on [^{3}H]dThd incorporation was 10 μM. [g] Average of 11 experiments. [h] Average of 45 experiments.

work from this laboratory reported previously.[13c] Thus, it appears that in general acyclo pyrrolo[2,3-*d*]pyrimidine adenosine analogues have a decreased cytotoxic activity in comparison with the corresponding ribonucleosides.

Antiviral Activity. Several HEM analogues of toyocamycin, sangivamycin, and tubercidin were evaluated for activity against HCMV and HSV-1. Cytotoxicity of each compound was determined visually in normal human diploid cells (HFF cells) and in monkey kidney cells (BSC-1 cells). In some cases cytotoxicity was also measured in a human neoplastic cell line (KB cells) by use of labeled precursor uptake. As we reported previously,[28] acyclotubercidin (**1a**, Table II) was inactive against HCMV and HSV-1 and was not cytotoxic. In addition, we now have found that it did not affect the plating efficiency of KB cells but it did reduce the plating efficiency of HFF cells (IC_{50} = 18 μM), indicating the possibility of biological activity in the series.

Examination of the new compounds (**4** and **6–12**, Table II) revealed that nearly all these compounds were inactive against both viruses. Surprisingly, compound **11** was active against HCMV but not against HSV-1. This compound inhibited HCMV at an IC_{50} concentration of 11 μM in plaque-reduction assays. In yield-reduction experiments, the IC_{90} concentration was 70 μM and a 2-log decrease in titer was achieved at 100 μM. Little or no visual cytotoxicity was observed with any of the target compounds, nor did nay compound inhibit RNA or protein synthesis. Compound **11**, however, did inhibit the incorporation of [^{3}H]dThd into acid-precipitable material at 10 μM (Table II, footnote *f*). Thus the reduction in HCMV titer achieved at 10 μM in the plaque-reduction assay may have been related to inhibition of cellular DNA synthesis.

The activity of compound **11** against HCMV is surprising because the closely related toyocamycin and sangivamycin analogues (compounds **6** and **8**) were inactive against the virus at concentrations as high as 320 μM (Table II). Interestingly, we found in a related study[13c] that the closely related thioamide analogue with a (dihydroxypropoxy)methyl group in the 7-position also was active. In contrast to most of these pyrrolopyrimidines, acyclovir was weakly active against HCMV at noncytotoxic concentrations. Ganciclovir (DHPG) was more active and less cytotoxic (Table II), producing a 5-log decrease in virus titer at 32 μM.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 270 MHz with a IBM WP 270 SY spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent (DMSO-d_6). Ultraviolet spectra were recorded on a Hewlett-Packard 0450A spectrophotometer, and the infrared spectra were measured on a Perkin-Elmer 281 spectrophotometer. Elemental analysis as performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was on silica gel 60 F-254 plates (Analtech, Inc.). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. Detection of components on TLC was made by UV light (254 nm). Rotary evaporations were carried out under reduced pressure with the bath temperature below 30 °C unless specified otherwise.

4-Amino-6-bromo-5-cyano-7-[(2-acetoxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine (3) and the Corresponding N_1 Isomer ($3N_1$). 4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine[26] (**1**, 2.38 g, 10 mmol) was dissolved in dry DMF (40 mL). NaH (97%; 0.25g, 10 mmol) was added to this solution at 100 °C, under a N_2 atmosphere, and the solution was stirred for 30 min. (2-Acetoxyethoxy)methyl bromide[25] (**2**, 1.98 g, 10 mmol) was then added to the solution with stirring. The reaction mixture was then heated at 100 °C for an additional 6 h. The solution was concentrated in vacuo, and the residue was partitioned in a mixture of EtOAc/H_2O (3:1; 150 mL). The ethyl acetate layer was collected, washed with H_2O (2 × 20 mL), and dried over Na_2SO_4. The ethyl acetate was evaporated, the residue was adsorbed onto silica gel (8.0 g) and then chromatographed on a column (4 × 80 cm; prepacked in CH_2Cl_2), using silica gel (120 g; 60–200 mesh). Elution of the column with CH_2Cl_2/CH_3OH (98:2 v:v) gave the 7-substituted compound along with the N_1 isomer as an oil (2.3 g; combined yield 65%). All attempts to separate the N_7 from its N_1 isomer by preparative-scale chromatography were unsuccessful. A small portion of the crude nucleoside mixture was purified by a column of silica gel. A very slow elution of the column with CH_2Cl_2/CH_3OH (98:2) gave the N_7 compound; ^{1}H

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NMR (DMSO-d_6) δ 5.65 (s, 2, C'$_1$-CH_2), 7.05 (br s, 2, C_4-NH_2), 8.30 (s, 1, C_2-H).

Further elution of the column furnished the N_1 isomer, which was crystallized from MeOH. ^{1}H NMR (DMSO-d_6) δ 5.72 (s, 2, C'$_1$-CH_2), 8.65 (s, 1, C_2-H).

4-Amino-5-cyano-7-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (4) and 4-Amino-5-cyano-1-[(2-acetoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (5). A mixture of **3** and the N_1 isomer (1.77 g, 5 mmol) was dissolved in a mixture of absolute EtOH and EtOAc (1:2 v:v; 60 mL). Palladium–carbon (5%; 2.0 g) and basic MgO (1.7 g) were added to this solution, and the mixture was hydrogenated at 48 psi for 12 h. The reaction mixture was filtered through a Celite pad. The filtrate was evaporated, adsorbed onto silica gel (5.0 g), and then chromatographed on a silica gel column (2 × 60 cm; prepacked in CH_2Cl_2) using silica gel (80 g; 60–200 mesh). Elution of the column with CH_2Cl_2/CH_3OH (96:4 v:v) gave the desired N_7 isomer, which was crystallized from EtOH to afford a pure compound (0.6 g; yield 44%): mp 138 °C; IR (KBr) ν 1740 (C=O), 2220 (CN) cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1), 233 (15 370), 272 (11 946); (MeOH) 228 (10 420), 278 (13 650); (pH 11) 230 (9900), 278 (14 030); ^{1}H NMR (DMSO-d_6) δ 1.95 (s, 3 H, $COCH_3$), 3.70 (m, 2 H, CH_2), 4.05 (m, 2 H, CH_2), 5.6 (s, 2, C'$_1$-CH_2), 6.95 (br s, 2, C_4-NH_2), 8.30 (s, 1, C_6-H), 8.42 (s, 1, C_2-H). Anal. ($C_{12}H_{13}N_5O_3$) C, H, N.

Further elution of the column with CH_2Cl_2/CH_3OH (95:5 v:v) afforded another compound which was crystallized from EtOH and characterized as the pure N_1 isomer (0.39 g; yield 29%); mp 165–166 °C; IR (KBr) ν 2220 (CN), 1740 (C=O) cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 283 (8151); (MeOH) 227 (13 900), 277 (13 320); (pH 11) 229 (8130), 277 (9630); ^{1}H NMR (DMSO-d_6) δ 1.92 (s, 3 H, $COCH_3$), 3.82 (m, 2 H, CH_2), 4.10 (m, 2 H, CH_2), 5.80 (s, 2 H, C_1-CH_2), 7.15 (br s, 2 H, C_4-NH_2), 7.85 (s, 1 H), 8.55 (s, 1 H). Anal. ($C_{12}H_{13}N_5O_3$) C, H, N.

4-Amino-5-cyano-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (6). Method 1. Compound **4** (0.69 g, 2.5 mmol) was stirred with methanolic ammonia (previously saturated at 0 °C; 30 mL) in a pressure bottle at 5 °C for 6 h. At this point, TLC showed the absence of any starting material. The solution was adsorbed onto silica gel (5.0 g) and purified by passing through a column (2 × 80 cm; prepacked in CH_2Cl_2) of silica gel (80 g; 60–200 mesh). Elution of the column with CH_2Cl_2/CH_3OH (95:5 v:v) gave a compound which was crystallized from absolute EtOH to afford pure **6** (0.25 g; yield 43%): mp 178 °C; IR (KBr) ν 2205 (CN), 3460 (OH) cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 235 (6200) 270 (6200); (MeOH) 278 (4610); (pH 11) 216 (28 800) 277 (4644); ^{1}H NMR (DMSO-d_6) δ 3.47 (m, 4, CH_2), 4.65 (t, 1, D_2O exchangeable, OH), 5.57 (s, 2, C'$_1$-CH_2), 6.87 (br s, 2, C_4-NH_2), 8.25 (s, 1 H, C_6-H), 8.34 (s, 1 H, C_2-H). Anal. ($C_{10}H_{11}N_5O_2$) C, H, N.

Method 2. Mercuric chloride (0.046 g) and triethylamine (0.1 mL) were added to a solution of compound **11** (0.046 g, 0.02 mmol) in dry DMF (5.0 mL). This mixture was stirred at room temperature for 4 h and then filtered through a Celite pad to remove the black mercuric sulfide that had formed. The filtrate was concentrated in vacuo to afford a solid residue, and the residue was purified by a small column (2 × 15 cm) of silica gel (5 g; 60–200 mesh; prepacked in CH_2Cl_2). Elution of the column with CH_2Cl_2/CH_3OH (96:4 v:v) gave the desired compound which was crystallized from absolute EtOH to afford pure **6** (0.025 g; 63% yield); mp 177 °C. The physicochemical data of this compound is essentially identical with the data obtained for **6** prepared by method 1 (vide supra).

4-Amino-5-cyano-1-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (7). Compound **5** (0.345 g, 1.25 mmol) was added to methanolic ammonia (saturated previously at 0 °C; 20 mL) in a pressure bottle, and the mixture was stirred at 5 °C for 6 h. At this point, TLC shows the absence of any starting material. The solution was adsorbed onto silica gel (5.0 g) and purified by a column (2 × 80 cm; prepacked in CH_2Cl_2) of silica gel (70 g; 60–200 mesh). Elution of the column with CH_2Cl_2/CH_3OH (95:5 v:v) gave a compound which was crystallized from absolute EtOH to afford **7** (0.116 g; yield 40%): mp 117–118 °C; IR (KBr) ν 2210 (CN), 3500 (OH) cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 282 (9672); (MeOH) 277 (11 410); (pH 11) 217 (26 400) 276 (10 490); ^{1}H NMR (DMSO-d_6) δ 3.50 and 3.65 (m, 2 each, CH_2), 4.75 (t, 1, D_2O exchangeable, OH), 5.80 (s, 1 C'$_1$-CH_2), 7.85 (s, 1 H), 8.60 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 157.18 (C-4), 147.17 (C-7a), 146.45 (C-2), 145.46 (C-6), 117.20 (CN), 102.87 (C-4a), 81.6 (C-5), 78.05 (C'$_1$-CH_2), 71.55 (C'$_4$-CH_2), and 59.93 (C'$_5$-CH_2). Anal. ($C_{10}H_{11}N_5O_2$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8). Compound **6** (0.1 g, 0.43 mmol) was dissolved in H_2O (5 mL) by the addition of 28% NH_4OH (5 mL). Hydrogen peroxide (30%) (1.0 mL) was added, and the solution was stirred at room temperature for 1 h. At this point TLC showed a complete absence of starting material. The solution was evaporated in vacuo and the residue coevaporated with EtOH twice. This semisolid mass was then crystallized from absolute EtOH/Et_2O to give compound **8** (0.06 g; yield 56%): mp 231 °C; IR (KBr) ν 3360 (NH, OH), 1630 ($CONH_2$) cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 275 (9400); (EtOH) 280 (11 230); (pH 11) 216 (26 600) 279 (9211); ^{1}H NMR (DMSO-d_6) δ 3.45 (m, 4, CH_2), 4.66 (t, 1, D_2O exchangeable, OH), 5.55 (s, 1, C'$_1$-CH_2), 7.36 (br s, 2, C_4-NH_2), 7.95 (br s, 2, $CONH_2$), 8.08 (s, 1 H), 8.11 (s, 1 H). Anal. ($C_{10}H_{13}N_5O_3$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (9). Compound **6** (0.1 g, 0.43 mmol) was dissolved in EtOH (10 mL) containing hydroxylamine (0.15 g; 50% aqueous hydroxylamine). The resulting solution was heated at reflux temperature for 3 h and concentrated in vacuo, and the residue was then coevaporated with toluene (2 × 3 mL). This residue was crystallized from a mixture of absolute EtOH/Et_2O to give pure **9** (0.04 g; yield 36%): mp 232–233 °C; IR (KBr) ν 3180–3470 (HN, OH), 1630 cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 274 (4650); (EtOH) 276 (5130); (pH 11) 219 (25 100) 277 (4747); ^{1}H NMR (DMSO-d_6) δ 3.50 (m, 4, CH_2), 4.67 (t, 1, OH), 5.58 (s, 2, C'$_1$-CH_2), 5.95 (s, 2, C(NOH)NH_2) 7.81 (s, 1 H), 8.06 (s, 1 H), 9.65 (s, 1 H, D_2O exchangeable, NO*H*). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

Methyl 4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-formimidate (10). Compound **6** (0.5 g, 2.15 mmol) was dissolved in dry MeOH (15 mL). A sodium methoxide (1 M) solution (5 mL) was added, and the mixture was stirred at room temperature for 3 h. This solution was adjusted to pH 7 by adding small portions of Dowex 50 (H^+ form, prewashed with dry CH_3OH). The solution was then quickly filtered to remove the ion-exchange resin followed by concentration of the filtrate in vacuo to a semisolid mass. The residue was purified by a column (2 × 60 cm; prepacked in CH_2Cl_2) of silica gel (15 g; 60–200 mesh). Elution of the column with CH_2Cl_2/CH_3OH (96:4 v:v), and a removal of the solvent afforded the desired compound. This compound was crystallized from dry CH_3OH/Et_2O to afford the pure imidate **10** (0.13 g; yield 23%): mp 124 °C; IR (KBr) ν 3350 (OH), 1600 cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 237 (14 700), 280 (12 710); (MeOH) 278 (15 770); (pH 11) 217 (26 100), 278 (13 880); ^{1}H NMR (DMSO-d_6) δ 3.50 (m, 4, CH_2), 3.75 (s, 3, OCH_3), 5.58 (s, 2, C'$_1$-CH_2), 7.25 (br s, 1, NH_2), 7.75 (s, 1, C_6-H), 8.06 (s, 1, C_2-H), 8.20 (br s, 1, C=NH), 9.98 (br s, 1, NH_2). Anal. ($C_{11}H_{15}N_5O_3$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (11). Dry H_2S was passed, with magnetic stirring, for 10 min through a sodium methoxide (1 M) solution (12 mL). The methyl imidate **10** (0.26 g, 1 mmol) was then added, in one portion, to this stirred solution of sodium hydrogen sulfide (generated in situ by the action of H_2S and sodium methoxide). The mixture was stirred at room temperature for 4 h and then allowed to stand at 0 °C for an additional 12 h. The mixture was filtered and the filtrate concentrated in vacuo to a solid mass. The residue was purified by a column (2 × 60 cm) of silica gel (25 g; 60–200 mesh; prepacked in CH_2Cl_2). Elution of the column with CH_2Cl_2/CH_3OH (96:4 v:v) gave a compound which was crystallized from H_2O to obtain pure **11** (0.05 g; yield 20%): mp 186–188 °C; IR (KBr) ν 3380 (OH), 1620 cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 240 (7400), 289 (5600); (MeOH) 284 (5095); (pH 11) 215 (26 400), 280 (5730); ^{1}H NMR (DMSO-d_6) δ 3.50 (m, 4, CH_2), 5.75 (s, 2, C'$_1$-CH_2), 7.95 (s, 1 H), 8.02 (br s, 2, D_2O exchangeable, NH_2), 8.20 (s, 1), 9.60 and 9.75 (br s, 1 each, D_2O exchangeable, $CSNH_2$). Anal. ($C_{10}H_{13}N_5O_2S$) C, H, N.

4-Amino-7-[(2-hydroxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbohydrazide (12). Hydrazine hydrate (85%) (2.0 mL) was added to a solution of compound **10** (0.52 g, 2 mmol) in absolute EtOH (10 mL). This mixture was heated at reflux temperature for 3 h and then allowed to stand at 5 °C for an

additional 12 h. The solid that had separated was collected by filtration and crystallized from MeOH to afford pure **12** (0.32 g; yield 60%): mp 269-270 °C; IR (KBr) ν 3280, 1615 cm^{-1}; UV λ_{max} [nm (ϵ)] (pH 1) 231 (7023) 276 (6101); (MeOH) 280 (6192); (pH 11) 218 (25 700) 279 (5937); ^1H NMR (DMSO-d_6) δ 3.50 (m, 4, CH_2), 4.50 (br s, 2, NH_2), 4.65 (t, 1, D_2O enchangeable, OH), 5.60 (s, 2, C'_1-CH_2), 7.98 (br s, 2, C_4-NH_2), 8.00 (s, 1 H), 8.15 (s, 1 H), 9.75 (br s, 1, CONH); MS m/e 266. Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

In Vitro Antiproliferative Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[29] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The IC_{50} was defined as the concentration required to reduce the growth rate to 50% of the control. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture as a percent of control and was experimentally derived as the population doubling time of control cells as the percent of the population doubling time of treated cells.

Antiviral Evaluation. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hank's salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[30] A plaque-purified isolate, P_0, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[13a]

(b) Assays for Antiviral Activity. HCMV plaque-reduction experiments were performed with monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. Protocols for HCMV titer reduction experiments have been described previously.[13a] HSV-1 plaque-reduction experiments were performed with monolayer cultures of BSC-1 cells. The assay was performed exactly as referenced above for HSV-1 titration assays except that the 0.2 mL of virus suspension contained approximately 100 PFU of HSV-1 and the compounds to be tested were dissolved in the overlay medium.

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF and BSC-1 cells was estimated by visual scoring of cells not affected by virus infection in the plaque-reduction assays described above. Drug-induced cytopathology was estimated at 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of radioactive precursors into DNA, RNA, and protein as detailed elsewhere.[13a]

(d) Plating Efficiency. A plating efficiency assay was used to confirm and extend the results of cytotoxicity testing. Briefly, KB cells were suspended in growth medium, and an aliquot containing 500–600 cells was added to a 140 × 25 mm Petri dish. Growth medium (40 mL) containing selected concentrations of test compounds was added, and the cultures were incubated in a humidified atmosphere of 4% CO_2–96% air at 37 °C for 14 days. Medium then was decanted, and colonies were fixed with methanol and stained with 0.1% crystal violet in 20% methanol. Macroscopic colonies >1 mm in diameter were enumerated. Effects were calculated as a percentage of reduction in number of colonies formed in the presence of each concentration of test compound compared to the number of colonies formed in their absence. Dose–response curves were generated, and IC_{50} concentrations for inhibition of plating/colony formation were calculated as follows.

(e) Data Analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentration. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. The three IC_{50}'s for inhibition of DNA, RNA, and protein synthesis were averaged to give the values reported in the table for KB cell cytotoxicity. Samples containing positive controls (acyclovir or ganciclovir) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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