Synthesis, Cytotoxicity, and Antiviral Activity of Some Acyclic Analogues of the Pyrrolo[2,3-*d*]pyrimidine Nucleoside Antibiotics Tubercidin, Toyocamycin, and Sangivamycin

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A number of 7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine derivatives that are structurally related to toyocamycin and sangivamycin and the seco nucleosides of tubercidin, 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 1,3-bis(benzyloxy)-2-propoxymethyl chloride (**2**) afforded compound **3**, which without isolation was debrominated to obtain 4-amino-5-cyano-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (**4**). Although catalytic hydrogenolysis failed, the benzyl ether functionalities of **4** were successfully cleaved by boron trichloride to afford 4-amino-5-cyano-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**6**). Conventional functional group transformation of the cyano group of **6** provided a number of novel 5-substituted derivatives. Tubercidin (**8a**), toyocamycin (**8b**), and sangivamycin (**8c**) were treated separately with sodium metaperiodate and then with sodium borohydride to afford the 2′,3′-seco derivatives **9a-c**, respectively. The acyclic nucleoside 4-chloro-2-(methylthio)-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (**10**) was aminated, desulfurized with Raney Ni, and then debenzylated to provide the tubercidin analogue **11**. Cytotoxicity evaluation against L1210 murine leukemic cells in vitro showed that although the parent compounds tubercidin (**8a**), toyocamycin (**8b**), and sangivamycin (**8c**) were very potent growth inhibitors, the acyclic derivatives **6**, **7a-c**, and **9a-c** had only slight growth-inhibitory activity. Evaluation of compounds **6**, **7a**, **7b**, **7c**, **9a**, **9b**, **9c**, **11** for cytotoxicity and activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) revealed that only the carboxamide (**7a**) and the thioamide (**7c**) were active. Compound **7c** was the more potent of the two, inhibiting HCMV but not HSV-1 at concentrations producing little cytotoxicity.

The discovery of 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) as a potent and selective inhibitor of herpes viruses[1-3] has led to extensive research into the preparation and evaluation of other novel acyclic analogues of nucleosides as potential antiviral agents. One of the most promising and potent agents in this class of compounds is 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, ganciclovir).[4-6] Like acyclovir, this drug is a potent and selective inhibitor of herpes simplex viruses types 1 and 2 (HSV-1, HSV-2)[4-6] varicella-zoster virus,[5] and Epstein-Barr virus.[5] Unlike acyclovir, it also is active against human cytomegalovirus (HCMV).[5,6] However, in animals[7] and in humans,[7,8] this compound has caused some adverse toxicological effects, thereby prompting additional research for a more effective agent.

In the search for more effective antiviral agents, nucleosides and nucleoside analogues have been explored extensively. One group of nucleoside analogues that has not been explored extensively are the pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin. The antineoplastic activity of these compounds is well established. For example, toyocamycin has shown significant antitumor activity[9] and sangivamycin exhibits antileukemic[10] and antitumor activity in vitro[11,12] and in vivo.[13,14] Unlike the nucleoside antibiotic tubercidin, which appears to be cytotoxic because of inhibition of a vital step in the glycolytic pathway,[15] the C-5 substituted analogue sangivamycin may act by inhibition of protein kinase C.[16] Although it has been reported that these ribosyl nucleosides also have antiviral activity,[17] there is little separation between antiviral activity and cytotoxicity,[18] leading us to conclude that this is not true antiviral activity. Even though tubercidin, toyocamycin, and sagivamycin, per se, have no potential as antiviral drugs, we[18] and others[19] have been investigating the antiviral potential of sugar-modified analogues. We have found that

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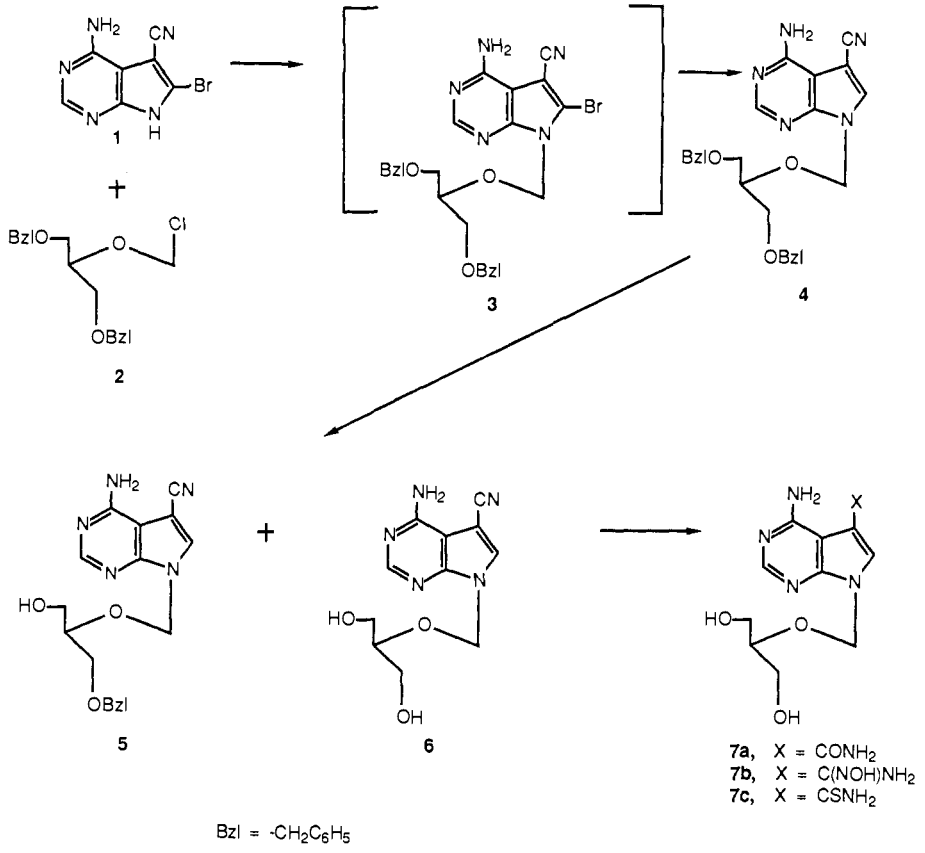
Scheme I



certain arabinosyl- and deoxyribosylpyrrolo[2,3-*d*]pyrimidines are active against HCMV and HSV-1.[18] In contrast, a series of acyclic 2,4-disubstituted pyrrolo[2,3-*d*]-pyrimidine analogues was not active.[20]

The observation that some pyrrolo[2,3-*d*]pyrimidine nucleosides, but not others, possess antiviral activity led us to initiate the synthesis of (dihydroxypropoxy)methyl (DHPG-like) analogues of toyocamycin, tubercidin, and sangivamycin. The antiherpes activity of other acyclic nucleosides such as 2′,3′-secoguanosine and (*R,S*)-9-[1-(2-hydroxyethoxy)-2-hydroxyethyl]guanine[21] also prompted us to prepare the seco nucleosides of tubercidin, toyocamycin, and sangivamycin. We now report on the synthesis and biological properties of DHPG-like and seco pyrrolo[2,3-*d*]pyrimidines.

Chemistry

1,3-Bis(benzyloxy)-2-propoxymethyl chloride (**2**) was prepared according to the literature method.[22] Compound **2** was then condensed with the sodium salt of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**1**), generated in situ by the treatment of **1** with NaH in dry DMF. This furnished 4-amino-6-bromo-5-cyano-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (**3**), as an oil after column chromatography. Compound **3** still contained some minor impurities, and further purification proved to be very difficult. Therefore, without further purification, **3** was debrominated with 5% Pd/C to afford 4-amino-5-cyano-7-[[1,3-bis(benzyloxy)-2-propoxy]-methyl]ypyrrolo[2,3-*d*]pyrimidine (**4**) in 48% yield. Although there may have been other compounds present in the filtrate, no attempt was made to isolate other positional isomers of compound **4**. Compound **4** was identified on the basis of ^{1}H NMR and UV[26] characteristics.

Cleavage of the benzyl ether functionalities of **4** was first attempted by standard hydrogenolysis conditions. However, standard catalytic reduction (10% Pd/C, 50 psi, H_2) or transfer hydrogenation (20% $Pd(OH)_2$/C, cyclohexene)[23] conditions were unsuccessful. The benzyl groups were removed successfully by the treatment of **4** with a 1 M BCl_3/CH_2Cl_2 solution to obtain 4-amino-5-cyano-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**6**) in 59% yield. The workup conditions for this reaction mixture appear to be crucial, since from this reaction, the monobenzylated compound **5** (7% yield) and the aglycon 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine (10% yield) were also isolated (Scheme I).

The UV spectrum of 4-amino-5-cyano-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (**6**) is essentially identical with that reported for toyocamycin.[26] This provides strong evidence that the acyclic moiety of compound **6** is residing at the N_7 position and not at N_1 or N_3. The ^{1}H NMR spectral data, two singlets at δ 8.32 and 8.25 for the C-2 and C-6 protons, a peak at δ 6.87 (D_2O exchangeable) for the C_4-NH_2, and a sharp singlet at δ 5.68 for the N7-CH_2, provide further support.

Compound **6** was treated with ammonium hydroxide containing H_2O_2 to obtain the sangivamycin derivative **7a**,

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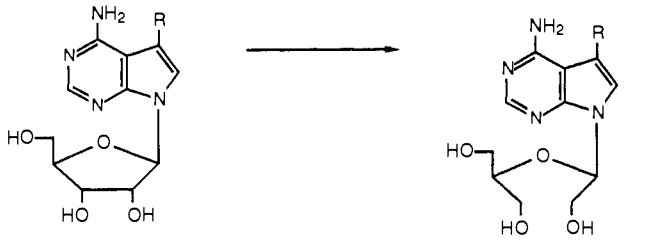
Scheme II



8a, R = H
8b, R = CN
8c, R = $CONH_2$

9a, R = H
9b, R = CN
9c, R = $CONH_2$

in 47% yield, while the treatment of **6** with hydroxylamine in ethanol gave the 5-carboxamidoxime derivative **7b** in 50% yield. These structural assignments for **7a** and **7b** were supported by ^1H NMR spectroscopy. The absence of a band in the IR for a cyano group provided additional proof that these specific conversions had indeed taken place.

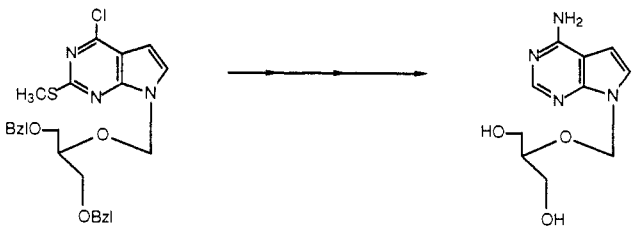
Compound **6** was treated with sodium hydrogen sulfide, generated in situ by the action of H_2S with sodium methoxide, to obtain the thiocarboxamide derivative **7c**, in 28% yield after purification on a SiO_2 column.

The action of $NaIO_4$ on an unprotected nucleoside is well known.[24,25] The resulting dialdehydes from these reactions exist as a complex mixture of hydrates, and are generally not isolated as such but converted to the respective acyclic triols by the action of $NaBH_4$. The purification of the acyclic triols from the residual salts is difficult and has usually been limited to the use of ion-exchange chromatography.[24]

For our synthesis of the seco nucleosides, the nucleoside antibiotics tubercidin, toyocamycin, and sangivamycin (**8a–c**) (Scheme II) were treated with $NaIO_4$ followed by $NaBH_4$. The resulting triols were purified by passing the mixture through an activated charcoal column.[25] The charcoal column was first eluted with water to remove the iodate and borate salts. The desired products (**9a–c**) were then eluted with 3% NH_4OH in a 1:1 ethanol–water mixture. Compounds **9a–c** were obtained by lyophilization to afford powders in yields of 23–44%. The structures of **9a–c** were confirmed on the basis of UV, IR, and ^1H NMR spectroscopy. In the ^1H NMR spectrum, the N7-CH_2 appears as a triplet ca. δ 6.0, which proves that the ring opening and conversion have indeed taken place. Three broad triplets (D_2O exchangeable, 1 proton each), ranging from δ 4.2 to 3.3, were assigned to three OH. The UV spectra of compounds **9a–c** were essentially identical with those of the corresponding parent compounds **8a–c**, which is supportive of the assigned structures.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (**11**) was synthesized in a one-pot reaction sequence via amination of 4-chloro-2-(methylthio)-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]-pyrimidine (**10**) with methanolic ammonia at 130 °C in a sealed reaction vessel followed by desulfurization with Raney Ni and subsequent debenzylation with BCl_3/CH_2Cl_2 at −78 °C. This gave compound **11** in a 33% overall yield (Scheme III).

Scheme III



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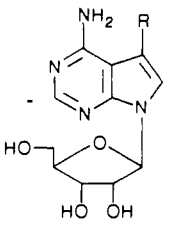
Biological Results

In Vitro Antiproliferative Testing. The pyrrolo-[2,3-*d*]pyrimidine ribonucleoside antibiotics tubercidin (**8a**), toyocamycin (**8b**), and sangivamycin (**8c**) were extremely potent inhibitors of the growth of L1210 cells in vitro (Table I). They decreased the growth rate to 50% of the control rate at concentrations of 40, 4, and 3 nM,

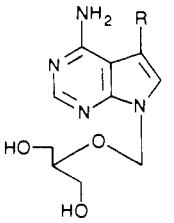
Table I. Cytotoxicity of 5-Substituted 4-Aminopyrrolo[2,3-*d*]pyrimidine Nucleoside Antibiotics against L1210 Murine Leukemic Cells in Vitro



compd	R	IC_{50},[a] M
8a	H (tubercidin)	4×10^{-8}
8b	CN (toyocamycin)	4×10^{-9}
8c	$CONH_2$ (sangivamycin)	3×10^{-9}

[a] IC_{50} is the concentration required to decrease the growth rate to half of the control rate.

Table II. Cytotoxicity of 5-Substituted 4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines against L1210 Murine Leukemic Cells in Vitro



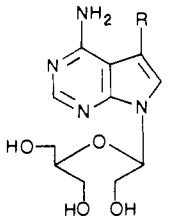
compd	R	initial screen:[a] growth rate, % of control	IC_{50},[b] M
6	CN	87	$>10^{-4}$
7a	$CONH_2$	84	$>10^{-4}$
7b	$C(NOH)NH_2$	80	$>10^{-4}$
7c	$CSNH_2$	71	$>10^{-4}$

[a] The effect of each compound at 10^{-4} M on the growth rate of L1210 cells. [b] See Table I, footnote *a*.

respectively, and all caused total growth inhibition at 100 nM. The replacement of the ribosyl moiety with (1,3-dihydroxy-2-propoxy)methyl (Table II) (DHPG analogues) or with 1-(1,3-dihydroxy-2-propoxy)-2-hydroxyethyl (Table III) (seco nucleosides), led to a dramatic loss of cytotoxic activity. Of the three seconucleosides, only the toyocamycin analogue caused a significant decrease in growth rate at 100 μM, and that was less than 50% (Table III). All the DHPG analogues also caused less than 50% decrease in growth rate when added to L1210 cell cultures at 100 μM (Table II). In an interesting parallel with the antiviral data, the thioamide derivative **7c** showed a slightly greater degree of growth inhibition than the other DHPG analogues (Table II).

Antiviral Activity. Target compounds as well as tubercidin, toyocamycin, and sangivamycin were evaluated for activity against HCMV and HSV-1. Cytotoxicity of each compound was determined visually in normal human

Table III. Cytotoxicity of 5-Substituted 4-Amino-7-[1-(1,3-dihydroxy-2-propoxy)-2-hydroxyethyl]pyrrolo-[2,3-*d*]pyrimidines against L1210 Murine Leukemic Cells in Vitro



compd	R	initial screen:[a] growth rate, % of control	IC_{50},[b] M
9a	H	93	-
9b	CN	80	$>10^{-4}$
9c	$CONH_2$	90	-

[a] See Table II, footnote *a*. [b] See Table I, footnote *a*. A dash indicates that no significant cytotoxicity was observed.

diploid cells (HFF cells) and in monkey kidney cells (BSC-1 cells). In some cases cytotoxicity also was measured in a human neoplastic cell line (KB cells) with labeled precursor uptake. As we have reported previously,[18] the three naturally occurring compounds (**8a–c**) were potent inhibitors of both viruses but did not show selectivity compared to uninfected cells (Table IV). The table also illustrates that although nearly all target compounds were inactive against both viruses, the carboxamide (**7a**) and the thioamide (**7c**) were active against HCMV but not against HSV-1. These compounds inhibited HCMV at IC_{50}'s of 144 and 8 μM, respectively, in plaque-reduction assays. In yield reduction experiments, IC_{90}'s of 85 and 25 μM, respectively, were noted; a 320 μM concentration of **7a** gave a 2-log reduction in virus titer whereas 100 μM of **7c** gave a 3-log reduction of virus titer (data not shown). Although little visual cytotoxicity was observed with any of the target compounds, compound **7c** did inhibit the incorporation of [^{3}H]dThd into acid precipitable material at 23 μM (Table IV, footnote *f*). Thus the 3-log reduction in HCMV titer achieved at 100 μM was not fully separated from cytotoxicity. The activity of compound **7c** against HCMV is surprising because the closely related sangivamycin analogue (**7a**) was weakly active and the toyocamycin analogue (**6**) inactive at 320 μM (Table IV). In contrast to the pyrrolopyrimidines, DHPG was more active and less cytotoxic (Table IV), producing a 5-log decrease in virus titer at 32 μM.

Discussion

The lack of cytotoxic activity for most of these acyclic nucleosides may be due to the inability of cellular enzymes to phosphorylate them, and/or an insensitivity of the cellular enzyme targets to their phosphorylated derivatives. Tubercidin, toyocamycin, and sangivamycin are known to be phosphorylated by adenosine kinase.[27] Because this enzyme is relatively specific regarding the carbohydrate moiety,[27b] we speculate that the adenosine-type DHPG analogues and seco nucleosides reported herein may not be phosphorylated by adenosine kinase. The possibility that mammalian thymidine kinase could be responsible for phosphorylating the analogues is remote because this enzyme has a narrow substrate specificity. Of the natural ribo- and deoxynucleosides, it accepts only thymidine and deoxyuridine as substrates.[28] Thus, it would not be expected to phosphorylate adenosine-type analogues. However, viral thymidine kinases are significantly less specific;[29] e.g. they are primarily responsible for selectively phosphorylating acyclovir and DHPG in virus-infected cells compared to uninfected host cells.[2,6] The lack of antiviral activity for most of the adenosine-type analogues and seco nucleosides reported herein suggests further that these compounds may not be phosphorylated by viral thymidine kinase. We propose that this lack of substrate activity may possibly be due to the absence of a proton on the N-3 (purine N-1) position. In contrast, as discussed above, all the compounds reported to be substrates for both the viral and the mammalian thymidine kinases possess protons at an analogous position: N-3 of pyrimidines, N-1 of purines, N-3 of pyrrolo[2,3-*d*]pyrimidines. Thus, viral thymidine kinases, in contrast to the mammalian enzyme, have sufficiently flexible substrate binding parameters to accommodate certain guanosine analogues, which have bicyclic bases, in contrast to the natural substrate which has a monocyclic base. However, they appear to retain a specificity for an NH at a position in the pyrimidine ring analogous to that in the natural substrate thymidine. These speculations indicate that the reported compounds would be useful in future investigations of the substrate specificities of viral thymidine kinase and of mammalian adenosine kinase.

Results presented herein show that changing the guanine moiety of ganciclovir (DHPG) to the pyrrolo[2,3-*d*]pyrimidine analogue of adenine (**11**) resulted in a total loss of antiviral activity. Thus, further studies on the requirements for the N-1 proton, the 2-amino, the 6-oxo, and the N-7 of guanine would delineate which of these structural features of guanine are required for antiviral activity. The potent activity of DHPG[1-3] and the selective activity of the thioamide derivative **7c** against HCMV but not HSV-1 suggests the possibility that kinases may be active in HCMV-infected cells which are not active in HSV-1-infected cells. In fact, it has been reported that HCMV is different from HSV-1 in that it does not induce a viral thymidine kinase[29] but it may induce a novel nucleoside kinase.[30] Thus HCMV-infected cells appear to have a different spectrum of kinase activities than HSV-1-infected cells.

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 were 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 35 °C specified otherwise.

4-Amino-5-cyano-7-[[1,3-bis(benzyloxy)-2-propoxy]-methyl]pyrrolo[2,3-*d*]pyrimidine (4). 4-Amino-6-bromo-5-

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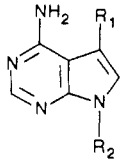
Table IV. Antiviral Activity and Cytotoxicity of Selected Pyrrolo[2,3-*d*]pyrimidine Nucleosides



compound	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]
11	H	DHPM[c]	>100[d,e]	>100	>100[e]	>100	
6	CN	DHPM	>320[e]	>100	>320[e]	>100	
7a	$CONH_2$	DHPM	144[e]	>100	>320[e]	>100	
7b	$HON{=}CNH_2$	DHPM	>100	>100	>100	>100	
7c	$CSNH_2$	DHPM	8[e]	>100	>100[e]	>100	>100[f]
8a	H	ribose	0.5[e]		0.3[e]		0.7[e]
8b	CN	ribose	0.05		0.03		0.04
8c	$CONH_2$	ribose	0.03[e]		0.08[e]		0.2[e]
9a	H	seco-ribose	>100	>100	>100	>100	>100
9b	CN	seco-ribose	>100	>100	>100	>100	
9c	$CONH_2$	seco-ribose	>100	>100	>100	>100	
acyclovir			63[e]	2.6[e]	>100	>100	>100
ganciclovir (DHPG)			8.8[g]	3.0[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] Abbreviation used: DHPM for (dihydroxypropoxy)methyl. [d] > indicates I_{50} concentration not reached at noted (highest) concentration. [e] Average of two to five experiments. [f] Effect on RNA and protein synthesis only. The I_{50} for effect on [^{3}H]dThd incorporation was 23 μM. [g] Average of 23 experiments.

cyanopyrrolo[2,3-*d*]pyrimidine[26] (1; 4.76 g, 20 mmol) was dissolved in dry DMF (80 mL) at 80 °C under N_2. Sodium hydride (dry 97%; 0.5 g, 20 mmol) was added in small portions to this stirred solution. When all hydrogen evolution had ceased (~0.5 h), 1,3-bis(benzyloxy)-2-propoxymethyl chloride[22] (2; 6.4 g, 20 mmol) was added in one batch to this solution, at 80 °C under a N_2 atmosphere. The reaction mixture was then stirred at 80 °C for 5 h. The solvent (DMF) was removed at 40 °C under a high vacuum to give a thick syrup. Water (100 mL) and EtOAc (300 mL) were added to this syrup, and the reaction mixture was heated on a steam bath and then transferred to a separatory funnel. The organic layer was separated, washed with a saturated solution of $NaHCO_3$ (3 × 30 mL) and H_2O (3 × 30 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated at 40 °C under reduced pressure to give a thick mass. This mass was rotary evaporated with silica gel (5.0 g) and then applied on the top of a column (4 × 60 cm) packed with wet silica gel, using $CHCl_3$ as the solvent. Elution of the column with a $CHCl_3/CH_3OH$ (99:1) mixture afforded a thick viscous oil (3) after evaporation of all the desired UV-absorbing fractions (5.6 g, yield 55%). Compound 3 (without further purification) was dissolved in a mixture of EtOH and EtOAc (1:2 v/v; 200 mL). To this solution, Pd/C (5%, 5.4 g) and basic MgO (5.4 g) was added and the mixture was hydrogenated at 48 psi for 6 h. The reaction mixture was filtered through a Celite pad. The filtrate was evaporated, and the residue was absorbed onto silica gel (5.0 g) and chromatographed on a silica gel column (3 × 60 cm; prepacked with CH_2Cl_2; silica gel, 120 g, 70–230 mesh). Elution of the column with a CH_2Cl_2/CH_3OH (96:4) mixture gave the desired nucleoside 4, which was crystallized from EtOH to afford pure 4 (2.24 g, yield 48%): mp 115 °C; IR (KBr) ν 2220 (CN), 3430 cm^{-1}; UV λ_{max} nm pH 1, 230 (9288) and 276 (7124), MeOH, 278 (8460), pH 11, 216 (26 800) and 278 (4876); ^{1}H NMR (DMSO-d_6) δ 3.51 (m, 4 H, H-3′, H-5′), 4.05 (m, 1 H, H-4′), 4.40 (s, 4 H, benzylic), 5.71 (s, 2 H, H-1′), 6.88 (br s, 2 H, NH_2), 7.18–7.38 (m, 10 H, aromatic), 8.25 (s, 1 H, C_6-H), 8.33 (s, 1 H, C_2-H). Anal. ($C_{25}H_{25}N_5O_3$) C, H, N.

4-Amino-5-cyano-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (6) and 4-Amino-5-cyano-7-[[1-(benzyloxy)-3-hydroxy-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (5). 4-Amino-5-cyano-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (4; 1.2 g, 2.71 mmol) was dissolved in dry CH_2Cl_2 (70 mL) and cooled to −78 °C with use of a dry ice/acetone bath. A solution of 1 M BCl_3/CH_2Cl_2 (20 mL) was then added through a dropping funnel to the cooled solution under a N_2 atmosphere. After the addition was completed, the reaction mixture was stirred at −78 °C under a N_2 atmosphere for 2 h and then at −60 °C for 4 more h. TLC (solvent system, 10% MeOH in CH_2Cl_2) showed a complete conversion of the starting material into one product. Cold MeOH (10 mL) was added to the solution at −60 °C, and the pH of the solution was immediately adjusted to 7 with 14% NH_4OH solution. The reaction mixture was then brought to room temperature and stirred for 1 h. The solvent was evaporated at 40 °C under reduced pressure to give a thick mass, which was rotary evaporated with silica gel (2.0 g) and then applied to the top of a column (2 × 40 cm) packed with wet silica gel with CH_2Cl_2 as an eluant. Elution of the column with CH_2Cl_2/CH_3OH (95:5) and evaporation of the desired UV-absorbing fractions afforded the monobenzyl compound, which was crystallized from MeOH to afford pure monobenzyl compound (0.066 g, yield 7%): mp 133–134 °C; IR (KBr) ν 3440 (OH), 2220 (CN) cm^{-1}; UV λ_{max} nm, pH 1, 234 (8120), 275 (6590), MeOH, 278 (6660), pH 11, 216 (26 000), 278 nm (8260); ^{1}H NMR (DMSO-d_6) δ 3.3–3.45 (m, 4 H, H-3′, H-5′), 3.85 (m, 1 H, H-4′), 4.38 (s, 2 H, CH_2), 4.75 (t, 1 H, OH), 5.70 (s, 2 H, H-1′), 6.88 (br s, 2 H, NH_2), 7.18–7.35 (m, 5 H, C_6H_5), 8.22 (s, 1 H, C_5-H), 8.32 (s, 1 H, C_2-H). Anal. ($C_{18}H_{19}N_5O_3$) C, H, N.

Further elution of the column with CH_2Cl_2/CH_3OH (93:7) afforded 4-amino-5-cyano-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (6), which was crystallized from MeOH to afford analytically pure 6 (0.42 g, yield 59%): mp 195 °C; IR (KBr) ν 2230 (CN), 3330 and 3440 (NH_2 and OH) cm^{-1}; UV λ_{max} nm, pH 1 232 (5000), 273 (3780), MeOH, 278 (5074); pH 11, 216 (27 600) 277 (6430); ^{1}H NMR (DMSO-d_6) δ 3.33–3.40 (m, 4 H, H-3′, H-5′), 3.60 (m, 1 H, H-4′), 4.63 (t, 2 H, D_2O exchangeable, OH), 5.68 (s, 2 H, H-1′), 6.87 (br s, 2 H, D_2O exchangeable, NH_2), 8.25 (s, 1 H, C_6-H), 8.32 s, 1 H, C_2-H). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7a). Compound 6 (0.2 g, 0.77 mmol) was dissolved in H_2O (10 mL) by the addition of 28% NH_4OH (10 mL). Hydrogen peroxide (30%, 2 mL) was added to this solution and the solution was stirred at room temperature for 3 h. At this point, TLC showed a complete disappearance of starting material. The solution was evaporated in vacuo, and the residue coevaporated with EtOH (2 × 5 mL). Finally, the semisolid mass was purified by chromatography with a column (2 × 20 cm prepacked in CH_2Cl_2) of silica gel (15 g, 70–230 mesh). Elution of the column with CH_2Cl_2/CH_3OH (92:8) and evaporation of the desired UV-absorbing fractions afforded the desired compound 7a, which was crystallized from H_2O to afford pure 7a (0.1 g, yield 47%): mp 188 °C; IR (KBr) ν 3460 and 3420 (NH_2, OH),

1640 ($CONH_2$) cm^{-1}; UV λ_{max} nm, pH 1, 276 (9500), MeOH, 280 (9353), pH 11, 216 (27 200), 279 (9344); ^1H NMR (DMSO-d_6) δ 3.35–3.40 (m, 4 H, H-3′, H-5′), 3.52 (m, 1 H, H-4′), 4.63 (t, 2 H, D_2O exchangeable, OH), 5.64 (s, 2 H, H-1′), 7.35 (br s, 2 H, D_2O exchangeable, C_4-NH_2), 7.92 (br s, 2 H, $CONH_2$), 8.09 (s, 1 H, C_6-H), 8.11 (s, 1 H, C_2-H). Anal. ($C_{11}H_{15}N_5O_4{}^{3}/_{2}H_2O$) C, H, N.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-carboxamidoxime (7b). Compound **6** (0.13 g, 0.5 mmol) was dissolved in EtOH (5 mL) containing NH_2OH (0.15 g, 50% aqueous NH_2OH) and the resulting solution was heated at reflux temperature for 4 h. The solution was concentrated in vacuo, and the residue was coevaporated with toluene (2 × 3 mL). The semisolid mass was then crystallized from MeOH to afford pure **7b** (0.06 g, yield 50%): mp 168–169 °C; IR (KBr) ν 3240–3520 (NH, OH), 1630 cm^{-1}; UV λ_{max} nm, pH 1, 274 (8210), MeOH, 277 (9312), pH 11, 218 (25 800) 276 (6583); ^1H NMR (DMSO-d_6) δ 3.38 (m, 4 H, H-3′, H-5′), 3.52 (m, 1 H, H-4′), 4.60 (t, 2 H, D_2O exchangeable, OH), 5.62 (s, 2 H, H-1′), 5.93 (br s, 2H, C(NOH)NH_2), 7.85 (s, 1 H, C_6-H), 8.08 (s, 1 H, C_2-H), 9.68 (s, 1 H, D_2O exchangeable, NOH). Anal. ($C_{11}H_{16}N_6O_4$) C, H, N.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine-5-thiocarboxamide (7c). Dry H_2S was passed through a $NaOCH_3$ solution (1 M) (6 mL) with magnetic stirring and cooling at 0 °C for 10 min. Compound **6** (0.13 g, 0.5 mmol) was then added in one portion to the stirred solution of NaSH which had been generated in situ as described above. 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. The residue was purified by a column (2 × 40 cm; prepacked in CH_2Cl_2) of silica gel (20 g, 70–230 mesh). Elution of the column with CH_2Cl_2/CH_3OH (94:6) gave the title compound, which was crystallized from H_2O to afford pure **7c** (0.04 g, yield 28%): mp 180–181 °C; IR (KBr) ν 3400 and 3180 (NH_2, OH), 1640 cm^{-1}; UV λ_{max} nm, pH 1, 241 (2750) 301 (1220), MeOH, 283 (5387), pH 11, 216 (26 200) 278 (5806); ^1H NMR (DMSO-d_6) δ 3.3–3.45 (m, 4 H, H-3′, H-4′), 3.60 (m, 1 H, H-4′), 4.62 (t, 2 H, OH) 5.65 (s, 2 H, H-1′), 7.9 (s, 1 H, C_6-H), 7.98 (br s, 2 H, D_2O exchangeable, NH_2), 8.15 (s, 1 H, C_2-H), 9.45 and 9.60 (br s, 1 each, D_2O exchangeable, $CSNH_2$). Anal. ($C_{11}H_{15}N_5O_3S{\cdot}H_2O$) C, H, N.

4-Amino-7-[2-hydroxy-1-(1,3-dihydroxy-2-propoxy)-ethyl]pyrrolo[2,3-*d*]pyrimidine (9a). Tubercidin (**8a**; 0.266 g, 1 mmol) was suspended in H_2O (15 mL) and $NaIO_4$ (0.32 g, 1.46 mmol) was added to the stirred solution at 0 °C. The mixture was stirred for 3.5 h; during this period the reaction temperature rose from 0 °C to 20 °C. Sodium borohydride (0.07 g, 1.85 mmol) was added and the mixture was stirred for an additional 1.5 h. The pH of the solution was adjusted from 9.5 to 7.0 with a 50% HCl solution. The solution was then applied to a carbon column (column size 2 × 40 cm; 10 g of activated carbon, Darco G-60). The column was first eluted with distilled H_2O (1 L) to remove the salts. The product was then eluted with 3% NH_4OH in 1:1 (H_2O/C_2H_5OH). Selected UV-absorbing fractions were concentrated in vacuo. The residue was dissolved in a small amount of H_2O and lyophilized to obtain a white powder of **9a** (0.06 g, yield 23%): mp 68–73 °C; IR (KBr) λ 3220–3400 (NH_2, OH), 1640 cm^{-1}; UV λ_{max} nm, pH 1, 227 (17 970), 272 (9166), MeOH, 270 (10 110), pH 11, 220 (25 300) 270 (9257); ^1H NMR (DMSO-d_6) δ 3.1–3.68 (m, H-3′, H-4′, and H-5′), 3.85 (m, 2 H, H-2′), 4.4, 4.60, and 5.02 (t, 1 H, each, D_2O exchangeable, OH), 6.01 (t, 1 H, H-1′), 6.60 (d, 1 H, C_5-H), 7.02 (br s, 2 H, D_2O exchangeable, NH_2), 7.28 (d, 1 H, C_6-H), 8.10 (s, 1 H, C_2-H). Anal. ($C_{11}H_{16}N_4O_4{\cdot}{}^{1}/_{2}H_2O$) C, H, N.

4-Amino-5-cyano-7-[2-hydroxy-1-(1,3-dihydroxy-2-propoxy)ethyl]pyrrolo[2,3-*d*]pyrimidine (9b). Toyocamycin (**8b**; 0.293 g, 1 mmol) was dissolved in H_2O (20 mL) and treated with $NaIO_4$ (0.32 g, 1.46 mmol) and $NaBH_4$ (0.074 g, 1.86 mmol). Chromatography with a carbon column (2 × 40 cm; 15 g of activated carbon, Darco G-60) then furnished selected UV-absorbing fractions, which were concentrated in vacuo. The residue was dissolved in a small amount of H_2O and lyophilized to obtain a white powder of **9b** (0.072 g, yield 25%): mp 66–88 °C; IR (KBr) ν 3220–3342 (NH_2, OH), 2226 (CN) cm^{-1}; UV λ_{max} nm, pH 1, 232 (15 260) 274 (10 740), MeOH, 229 (12 700), 278 (13 060), pH 11, 218 (26 400), 278 (11 890); ^1H NMR (DMSO-d_6) δ 3.2–3.6 (m, H-3′, H-4′, and H-5′), 3.80 (m, 2 H, H-2′), 4.45, 4.72, and 5.12 (br s, 1 H each, D_2O exchangeable, OH), 6.10 (t, 1 H, H-1′), 6.85 (br s, 2 H, NH_2), 8.22 (s, 1 H, C_6-H), 8.31 (s, 1 H, C_2-H). Anal. ($C_{12}H_{15}N_5O_4{\cdot}H_2O$) C, H, N.

4-Amino-7-[2-hydroxy-1-(1,3-dihydroxy-2-propoxy)-ethyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (9c). Sangivamycin (**8c**; 0.31 g, 1 mmol) was suspended in H_2O (20 mL), and after treatment with $NaIO_4$ (0.32 g, 1.46 mmol) and $NaBH_4$ (0.075 g, 1.85 mmol), chromatography with a carbon column (2 × 40 cm; 15 g of activated carbon, Darco G-60) furnished the desired UV-absorbing fractions, which were concentrated in vacuo. The residue was dissolved in a small amount of H_2O and lyophilized to obtain a brown solid of **9c** (0.135 g, yield 44%): mp 118–120 °C; IR (KBr) ν 3320–3450 (NH_2, OH), 1650 (C=O) cm^{-1}; UV λ_{max} nm, pH 1, 276 (6680), MeOH, 280 (7160), pH 11, 217 (26 500), 280 (6317); ^1H NMR (DMSO-d_6) δ 3.15–3.60 (m, H-3′, H-4′, and H-5′), 3.85 (m, 2 H, H-4′), 4.43, 4.70, and 5.13 (br s, 1 H each, D_2O exchangeable, OH), 6.10 (t, 1 H, H-1′), 7.35 (br s, 2 H, D_2O exchangeable, NH_2), 7.90 (br s, 2 H, D_2O exchangeable, $CONH_2$), 8.12 (s, 1 H, C_6-H), 8.20 (s, 1 H, C_2-H). Anal. ($C_{12}H_{17}N_5O_5{\cdot}{}^{3}/_{2}H_2O$) C, H, N.

4-Chloro-2-(methylthio)-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (10). Sodium hydride (0.48 g, 60% in mineral oil) was added, in small portions, to a stirred solution of 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (2.0 g) in dry DMF under a nitrogen atmosphere. When all hydrogen evolution has ceased, 1,3-bis(benzyloxy)-2-(chloromethoxy)propane (3.2 g) was added and the reaction mixture was stirred at room temperature for 20 h under a N_2 atmosphere. Water (20 mL) was added and the pH was adjusted to 7 with glacial acetic acid. The product was extracted with $CHCl_3$ (3 × 50 mL), and the extracts were combined, washed with water, and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a thick syrup, which was subjected to column chromatography over silica gel. The column (2 × 20 cm) was packed with wet silica with $CHCl_3$ as the eluent. Elution of the column with $CHCl_3$ yielded compound **10** (3.65 g, 75.4%) as a colorless syrup: ^1H NMR (DMSO-d_6) δ 7.3 (m, 10 H, aromatic), 7.2 (d, 1 H, C6-H), 6.5 (d, 1 H, C5-H), 5.7 (s, 2 H, N7-CH_2), 4.5 (s, 4 H, CH_2), 4.0 (m, 1 H, CH), 3.5 (d, 4 H, OCH_2), 2.6 (s, 3 H, SCH_3). Anal. ($C_{25}H_{26}ClN_3O_3S$) C, H, N.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyrimidine (11). 4-Chloro-2-(methylthio)-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine (**10**; 0.33 g) was covered with methanolic ammonia (20 mL) and the reaction mixture was heated in a steel reaction vessel at 130 °C for 10 h. The solvent was removed in vacuo to give a semisolid mass, which was dissolved in absolute EtOH (20 mL). To this solution was added a 15% aqueous NH_4OH solution (20 mL) followed by the addition of Raney Ni (1.0 g). The reaction mixture was heated at reflux temperature for 1.5 h. The reaction mixture was then filtered and the Raney Ni was washed (3 × 30 mL) with hot EtOH. The filtrate and washings were combined, and the solvent was removed in vacuo to give a thick syrup which was chromatographed on a SiO_2 column (2 × 10 cm) packed with wet silica gel (30 g). Elution of the column with $MeOH/CHCl_3$ (1:99 v/v) furnished a colorless syrup (0.15 g, 53%): ^1H NMR (DMSO-d_6) δ 8.4 (s, 1 H, C2-H), 7.35 (m, 10 H, aromatic), 7.15 (d, 1 H, C6-H), 6.35 (d, 1 H, C5-H), 5.8 (s, 2 H, N7-CH_2), 5.65 (br s, 2 H, exchangeable with D_2O, NH_2), 4.52 (s, 4 H, OCH_2), 3.55 (d, 4 H, CH_2), 4.0 (m, 1 H, CH). On the basis of the ^1H NMR spectrum, this compound was assigned the structure 4-amino-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine and used without further purification. It (0.11 g) was dissolved in dry CH_2Cl_2 (8 mL), and to the solution was added a 1 M solution of BCl_3/CH_2Cl_2 (2 mL) at −78 °C under a N_2 atmosphere. The reaction mixture was then stirred at −78 °C for 3 h. MeOH (40 mL) was added, the temperature of the reaction mixture was raised to room temperature and stirred for 1 additional h. The pH of the reaction mixture was then adjusted with aqueous NH_4OH (58%, w/v) to pH 7. The precipitate of NH_4Cl was removed by filtration and the solvent was removed in vacuo to give a colorless oil, which was chromatographed on a SiO_2 column (2 × 10 cm) packed with wet silica gel (30 g). Elution of the column with 15% MeOH in $CHCl_3$ yielded a colorless syrup, which was crystallized from MeOH to afford 11 as cream-colored crystals (0.02 g, 33%): mp 197–198 °C; ^1H NMR (DMSO-d_6) δ 8.07 (s, 1 H, C2-H), 7.24

(d, 1 H, C6-H), 7.02 (br s, 2 H, exchangeable with D_2O, NH_2), 6.58 (d, 1 H, C5-H), 5.59 (s, 2 H, N7-CH_2), 4.6 (t, 2 H, OH), 3.9–4.0 (m, 5 H, CH_2); UV λ_{max} nm, pH 7, 214 (13 000), 271 (11 000), pH 1, 225 (18 000), 272 (10 000), pH 11, 225 (6000), 270 (11 000). Anal. ($C_{10}H_{14}N_4O_3$) C, N, H.

In Vitro Antiproliferative Studies. The in vitro cytotoxicity against L1210 cells was evaluated as described previously.[31] L1210 cells were grown in static suspension culture with 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 plot of the log of the cell number against time for a treated culture, as a percentage of the slope for the control culture. Experimentally, this parameter was determined by calculating the ratio of the population doubling time of control cells to 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 Hanks salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and dipoloid 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.[32] A plaque-purified isolate, P_O, 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.[18]

(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 experiment have been described previously.[18] 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.[18]

(d) Data Analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. Fifty percent inhibitory concentrations (IC_{50}) 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 tables for KB cell cytotoxicity. Samples containing positive controls (acyclovir, 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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Registry No. 1, 19393-83-0; **2**, 74564-16-2; **3**, 118043-66-6; 4, 118043-67-7; **5**, 118043-68-8; **6**, 118043-69-9; **7a**, 118043-70-2; **7b**, 118043-71-3; **7c**, 118043-72-4; **8a**, 69-33-0; **8b**, 606-58-6; **8c**, 18417-89-5; **9a**, 118043-73-5; **9b**, 118043-74-6; **9c**, 118043-75-7; **10**, 118043-76-8; 11, 118043-78-0; 4-chloro-2-(methylthio)pyrrolo-[2,3-*d*]pyrimidine, 57564-94-0; 4-amino-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidine, 118043-77-9.

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