g), ethanol (5 mL), and ethyl acetate (5 mL) was stirred under a hydrogen atmosphere until a black solid precipitated from the colorless solution (15 min). The mixture was filtered through Celite and the filtrate was evaporated to dryness. Trituration with ether gave pure **12** (0.15 g), 71%) as colorless crystals: mp 218-220 °C; $[\alpha]^{25}_D$ = +122.9° (c = 1.2 × 10^{-3}, THF); ^{1}H NMR (DMSO-d_6) δ 0.81 (s, 3 H, C(18)H), 1.09-1.62 (m, 8 H), 1.73-2.08 (m, 3 H), 2.36-2.76 (m, 5 H), 3.50 (m, 3 H, C(17)H, CH_2O), 4.26 (t, J = 5.5 Hz, CH_2OH), 4.48 (d, J = 5.0 Hz, CHOH), 6.41 (d, J = 2.5 Hz, 1 H, C(4)H), 6.52 (d, d, J = 2.5, 8.3 Hz, 1 H, C(2)H), 6.94 (d, J = 8.5 Hz, 1 H, C(1)H), 8.93 (s, 1 H, C(3)OH). Anal. ($C_{20}H_{28}O_3$·0.25H_2O) C, H.

X-ray Crystal-Structure Analysis of 3-Acetoxy-11β-(1-ethenyl)estra-1,3,5(10)-trien-17-one (4). A crystal of **4** ($C_{22}H_{26}O_3$) suitable for X-ray diffraction studies (0.20 × 0.20 × 0.80 mm) was grown from an ether solution. The compound crystallized in the orthohombic space group $P2_12_12_1$ with cell dimension of a = 10.407 (3) Å, b = 11.155 (3) Å, c = 15.773 (4) Å and with Z = 4; the calculated density was 1.228 g cm^{-3}. Of the 1468 reflections collected ($1 \le 2\theta \le 45°$) on a Syntex $P2_1$ automated diffractometer (λ_{MoK} = 0.71069 Å) using a $\theta/2\theta$ scan, 849 were considered observed ($I > 2\sigma(I)$). The structure was solved with the direct methods package MITHRIL and refined by using full-matrix least-squares techniques.[29] Hydrogen atoms were assigned isotropic thermal factors of 6.5 Å^3. The function $w(|F_o| - |F_c|)^2$ was minimized with $w^{1/2}$ = 1 for $|F_o| \le 15$ and $15/|F_o|$ for $|F_o| > 15$; R = 0.062; w_R = 0.070; $(\Delta/\sigma)_{max}$ = 0.23; $(e/Å^3)_{max}$ = 0.3. No short intermolecular contacts were observed. Tables II-V, containing final atomic positional parameters, anisotropic thermal parameters for the non-hydrogen atoms, and bond lengths and angles, are available as supplementary material.

(29) The following crystallographic programs were used: MITHRIL (Gilmore, C. J., Department of Chemistry, University of Glasgow, Glasgow, Scotland), FMLS (Ganzel, P. L.; Sparks, R. A.; Trueblood, K. N., UCLA, Los Angeles, CA), and ORTEP (Johnson, C. K., Oak Ridge National Laboratory, Oak Ridge, TN, 1976).

Competitive Receptor Binding Assay. All cytosols for the estrogen receptor were prepared and stored in TEA buffer (0.01M Tris-Hcl-0.0015 M EDTA-0.02% sodium azide pH 7.4 at 25 °C). Rat uterine cytosol was prepared from Holtzman rats (21-25 day oil females) and stored in liquid nitrogen. Lamb uterine cytosol was prepared and stored as described by Katzenellenbogen et al.[25] The competitive receptor binding assays were performed as previously described[25,26] and the results were tabulated as relative binding affinities RBA relative to estradiol (RBA = 100).

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Registry No. 4, 129000-33-5; 5, 68599-01-9; 6, 129000-34-6; 7, 126559-84-0; 8, 126559-85-1; 9, 129000-35-7; 11, 64109-72-4; 12, 129000-36-8; benzyl chloride, 100-44-7.

Supplementary Material Available: Tables of the atomic positional parameters for non-hydrogen atoms and for hydrogen atoms, anisotropic thermal parameters for the non-hydrogen atoms, and bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

Arabinofuranosylpyrrolo[2,3-*d*]pyrimidines as Potential Agents for Human Cytomegalovirus Infections

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Protection of the 3′- and 5′-hydroxyl groups of the nucleoside antibiotic toyocamycin (1) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane was followed by (trifluoromethyl)sulfonylation of the 2′-hydroxyl group. A displacement of the resulting triflate ester moiety with lithium chloride, lithium bromide, sodium iodide, and lithium azide in hexamethylphosphoramide was followed by a removal of the disilyl moiety with tetra-*n*-butylammonium fluoride to afford the appropriate (2′-deoxy-2′-substituted-arabinofuranosyl)toyocamycin analogues **6a-d**. Hydrolysis of the carbonitrile moieties of **6a-d** with hydrogen peroxide gave the corresponding sangivamycin analogues (**7a-d**). A reduction of the azido moiety of **6a** and **7a** with 1,3-propanedithiol furnished the corresponding amino derivatives (**6e** and **7e**). The antiproliferative activity of **6a-e** and **7a-e** was evaluated in L1210 cell cultures. None of these compounds caused significant inhibition of cell growth. Evaluation of these compounds for antiviral activity showed that all the toyocamycin analogues were active against human CMV, but of the sangivamycin analogues, only (2′-deoxy-2′-azidoarabinosyl)sangivamycin (**7a**) was active against this virus. None of the compounds were active against HSV-1 or HSV-2. (2′-Deoxy-2′-aminoarabinofuranosyl)toyocamycin (**6e**) was studied more extensively and showed some separation between antiviral activity and cytotoxicity as measured by effects on DNA synthesis, cell growth, and cell-plating efficiency. Although **6e** also was active against murine CMV in vitro, it was not active against this virus in infected mice. We conclude that arabinosylpyrrolopyrimidines have potential as antivirals, but no members of the current series are potent enough to show significant activity in vivo.

The development of agents to control human cytomegalovirus (HCMV) has become increasingly important because of its significant contribution to the mortality of AIDS patients.[1-10] The potential of currently available

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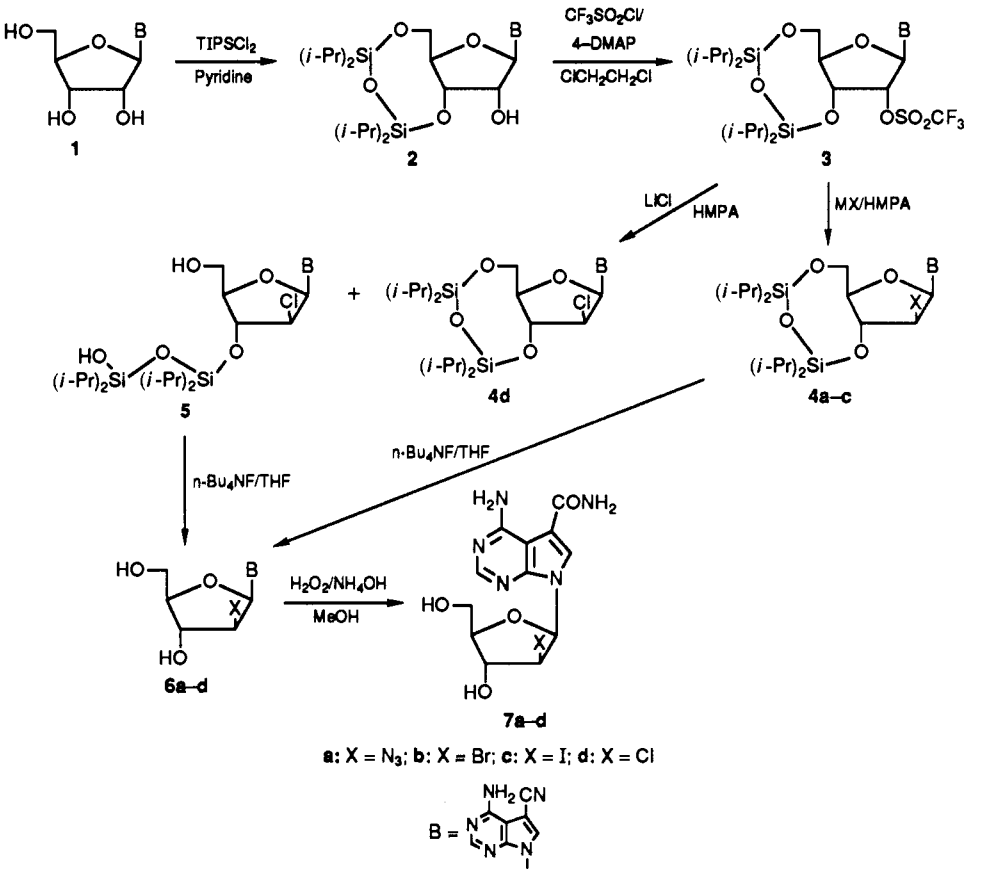
0022-2623/90/1833-3160$02.50/0

Scheme I



antiviral agents such as vidarabine (arabinofuranosyladenine, *ara*-A), acyclovir([(hydroxyethoxy)methyl]guanine), and ganciclovir([(dihydroxypropoxy)methyl]guanine, DHPG)[11-16] for treatment of HCMV is limited at least in part by their toxicity to the immune system.[15,17-19] Furthermore, the oral availability of these drugs is low.[20] In our search for more effective, less toxic, and orally available compounds for the treatment of HCMV infections, we have recently discovered that a number of 5-substituted pyrrolo[2,3-*d*]pyrimidine ribofuranosides, arabinofuranosides, and acyclic derivatives are active in vitro against HCMV.[21-26] Although these particular compounds did not exhibit sufficient potency and selectivity to warrant further development as antiviral agents, an important structure–activity correlation emerged from these studies. Namely, the arabinofuranosides showed the greatest potency. Therefore, we have undertaken the synthesis and evaluation of some closely related adenosine-type analogues, incorporating other substituents in the 2′-position.

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Catabolism by adenosine deaminase (ADA) is a significant limitation on the usefulness of many adenosine analogues, for example, *ara*-A,[27] which shows in vitro antitumor and antiviral activity but limited activity in vivo.[28] Coadministration of an inhibitor of ADA, such as deoxycoformycin, is an effective strategy for avoiding this catabolism in vivo and in vitro[28-31] but in clinical studies the resulting toxicity has proved unacceptable.[32-35]

A different approach to prevent catabolism is to make a chemical modification of the drug which imparts resistance to ADA. For example, the 2-fluoro derivative of *ara*-A (2-F-*ara*-A) is resistant to ADA and has a level of antitumor activity similar to that of the combination of *ara*-A and deoxycoformycin.[36] Previously studied pyrrolo[2,3-*d*]pyrimidine adenosine analogues are also resistant to ADA.[37] Thus, the compounds reported here should not be substrates for ADA and consequently should not require coadministration of an ADA inhibitor to achieve optimal activity.

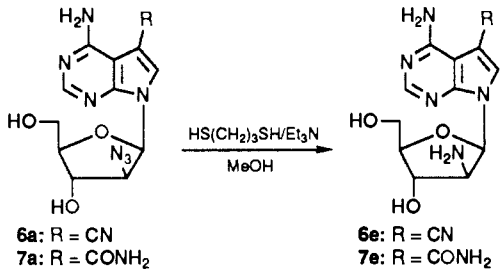
We had another biochemical parameter to consider when designing base-modified arabinofuranosyl derivatives to possess antiviral activity, but not cytotoxicity; namely, the resulting derivative should be a substrate neither for a nucleoside phosphorylase nor any other enzyme which could cleave the glycosidic bond. If heterocyclic bases were released, they could be metabolized to potentially cytotoxic ribonucleoside phosphates. This conversion would constitute a loss of antiviral selectivity in cases where the arabinosyl nucleotide possessed antiviral activity, whereas the ribonucleotide of the base was cytotoxic. For example, 2-F-*ara*-A is metabolized to 2-F-adenine, which is then converted to 2-fluoro-AMP.[38] This metabolite has been implicated in bladder toxicity in 2-F-*ara*-A-treated dogs.[38] The compounds reported here, 4-amino-5-substituted-7-(2-deoxy-2-substituted-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidines, are expected to avoid this potential problem because pyrrolo[2,3-*d*]pyrimidine nuclosides are resistant to glycosidic bond cleavage.[37] Thus, these analogues have the potential to be antiviral compounds with good therapeutic indices for the treatment of HCMV infections.

Results and Discussion

Synthesis. The synthesis of the target compounds was accomplished by minor variations of known[39-41] methodologies (Scheme I). The known[42] compound **2** was treated[38] with trifluoromethanesulfonyl chloride in 1,2-dichloroethane in the presence of 3 equiv of 4-(dimethylamino)pyridine (4-DMAP) to yield compound **3** in 84% yield as a crisp foam. Triflate **3** was characterized by a significant downfield shift of the 2′-proton as compared with the starting material **2**, as well as an absence of the 2′-hydroxyl proton. While it has been reported[39] that the sulfonylation can be accomplished satisfactorily with triethylamine and only 1 equiv of 4-DMAP, in our hands, it was found that the reaction proceeded much more reliably using 3-equiv of 4-DMAP as the only base. The advantage of this modification has been previously noted.[40] Treatment of a solution of this foam in hexamethylphosphoramide with lithium azide furnished compound **4a** as a crystalline solid in 78% yield. The displacement of the triflate moiety was confirmed by the upfield shift of the 2′-proton as compared with the starting material **3**, as well as the presence of an azide band in the IR spectrum of the product. Removal of the disiloxanyl blocking group was accomplished by the treatment of a THF solution of this compound with tetra-*n*-butylammonium fluoride[43] to yield compound **6a** in 60% yield. Treatment of compound **6a** with hydrogen peroxide in concentrated ammonium hydroxide[44] afforded compound **7a**, in 84% yield. Treatment of a methanolic solution of compound **6a** with 1,3-propanedithiol and triethylamine[45] yielded compound 6e in 56% yield (Scheme II). Reaction at the cyano moiety was ruled out by the observation of a nitrile absorption in the IR spectrum of the product. Since **7e** could not be readily purified by HPLC, it was prepared from an analytically pure sample of compound **7a** by a reduction of the azido moiety, as performed on compound **6a**. As expected, compound 7e displayed neither a cyano nor azido band in its IR spectrum.

The synthesis of the corresponding ara-halo derivatives was complicated by the lability of the disiloxanyl blocking group to lithium chloride, and the tendency of iodo derivative **6c** to form the ribo epoxide. Treatment of triflate **3** with lithium chloride in HMPA afforded both fully blocked derivative **4d** and partially deblocked derivative

Scheme II



6a: R = CN
7a: R = $CONH_2$
6e: R = CN
7e: R = $CONH_2$

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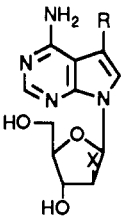
Table I. Antiviral Activity, Cytotoxicity, and Antiproliferative Activity of Arabinosyl Analogues of Toyocamycin and Sangivamycin



compound			50 or 90% inhibitory concentration, μM							
	substituent		HCMV[a]		HSV-1[a]		cytotoxicity[b]			L1210 growth rate,[c] % of control
no.	R	X	plaque	yield	plaque	yield	HFF	BSC	KB	
a[d]	CN	OH	1.7[e]	14	29	3.5	8	46	17	-
6a	CN	N_3	34	37	>100[f]	-	55	>100	151	14 (d)[g]
6e	CN	NH_2	14[e]	110[e]	>100	-	>100[e]	>100	105[h]	48 (d)
6d	CN	Cl	21[e]	75[e]	>100	-	140	>100	-	24 (d)
6b	CN	Br	162[e]	90	>100	-	181	>100	-	16 (d)
6c	CN	I	40[e]	20	>100	-	100	>100	-	24 (d)
b[d]	$CONH_2$	OH	0.6	1	13	0.9	8	>100	30	0
7a	$CONH_2$	N_3	28	100	>100	-	66[e]	>100	179	12 (d)
7e	$CONH_2$	NH_2	>100	-	>100	-	>100	>100	>100	46 (d)
7d	$CONH_2$	Cl	>100[e]	-	>100	-	>100[e]	>100	-	73
7b	$CONH_2$	Br	>100[e]	-	>100	-	>100[e]	>100	-	92
7c	$CONH_2$	I	>100[e]	-	>100	-	100[e]	>100	-	38 (d)
acyclovir			63	90	4[e]	7[e]	>100	>100	>100	
ganciclovir (DHPG)			9.2[i]	1.8[e]	4.5	1.2[e]	>100[e]	>100	>100	

[a] Plaque- and yield-reduction assays were performed as described in the text. Results from plaque assays are reported as IC_{50}'s, those for yield-reduction experiments as IC_{90}'s. [b] Visual cytotoxicity was scored on HFF and BSC cells at the time of HCMV or HSV-1 plaque enumeration. Average percent inhibition of DNA, RNA, and protein synthesis was determined in KB cells as described in the text. Results are presented as IC_{50}'s. [c] Growth rate during the first 48 h in the presence of 100 μM test compound was determined as described in the Experimental Section. [d] Compound a = *ara*-toyocamycin, compound b = *ara*-sangivamycin, some results have been reported previously.[21] [e] Average concentration derived from two to five experiments. [f] >100 indicates IC_{50} or IC_{90} not reached at 100 μM. [g] (d): delayed growth inhibition. Growth rate is reported for the 48–96-h period. [h] Little or no effect on the growth rate of KB cells was seen at concentration up to 32 μM, while at 100 μM, growth rate was reduced by 80–90%. [i] Average of 60 experiments.

5. The identification of **5** was based upon the observation of two exchangable peaks in the ^1H NMR spectrum. The low-field singlet was attributed to the silanol moiety, while the high-field triplet was assigned to the 5′-hydroxyl group. Because compound **5** was formed in higher yield and could be purified more readily, it was deemed the intermediate of choice for the next step. Deprotection of this compound with tetra-*n*-butylammonium fluoride in THF afforded chloro derivative **6d**. In anticipation of the possible formation of the ribo epoxide derivative, hydrolysis of the cyano moiety of **6d** was performed in methanol/water because of the solubility considerations and to avoid the harsh basic conditions encountered when neat concentrated ammonium hydroxide was used as the solvent. Reaction of triflate **3** with lithium bromide in HMPA furnished ara-bromo derivative **4b** with no evidence of deprotection of the disiloxanyl moiety. This product was then deprotected, under the same conditions as those used for compound **4d**, to afford free nucleoside **6b**. Compound **6b** was similarly hydrolyzed to afford carboxamide **7b**.

Likewise, the reaction of triflate **3** with sodium iodide in HMPA afforded ara-iodide derivative **4c**. Deprotection of **4c**, to yield **6c**, had to be performed very carefully because the product readily formed the ribo epoxide[41] if it was not isolated from the reaction mixture as soon as the reaction was deemed to be complete as evidenced by TLC analysis. The conversion of free nucleoside **6c** to carboxamide derivative **7c** also had to be performed carefully because of the above mentioned considerations. It was found that the hydrolysis could be accomplished using only a trace of ammonium hydroxide in the reaction medium, hence again omitting the necessity of using neat concentrated ammonium hydroxide as originally used for the conversion of toyocamycin to sangivamycin.[44] But the tendency of the two halo derivatives **6b** and **6c** to form the riboepoxide derivative upon treatment with base confirms the trans relationship between the 2′- and 3′-substituents. Thus, we have been able to obtain variously substituted arabinofuranoside analogues of toyocamycin and sangivamycin.

In Vitro Antiproliferative Evaluation. The effects of compounds **6a–e** and **7a–e** (100 μM) on the growth rate of L1210 cells are shown in Table I. The most cytotoxic of these arabinofuranosyl analogues, **6a** and **7a**, are about 10000 times less cytotoxic than the parent ribonucleosides, toyocamycin and sangivamycin. These highly cytotoxic ribonucleoside antibiotics decrease the growth rate of L1210 cells to 50% of that of the control at concentrations of only 3–4 nM.[23] In view of the extremely high potency of toyocamycin and sangivamycin, great care must be exercised in purifying derivatives prepared from them, because trace contaminants of the parent compounds can cause false positive tests for antiproliferative effect. Compounds **6a**, **6e**, and **7a** were purified by high-performance liquid chromatography as described in the Experimental Section,[46] before the evaluations reported in Table I were carried out. Nevertheless, subsequent HPLC analysis (see the Experimental Section) of the samples of **6a** and **7a** used for these evaluations, revealed trace contaminants that coeluted with toyocamycin and sangivamycin, respectively. Assuming these were the contaminants, the amounts found were sufficient to account for the observed growth inhibition. Compound **6a** contained 0.024% contaminant, and compound **7a** contained 0.08% contaminant. No contaminants (detection limit =

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0.01%) were detected in the samples of **6e** and **7e** which were an additional synthetic step away from the parent compounds. Therefore, it appears that the approximately 50% inhibition of cell growth reported in Table I for compounds **6e** and **7e** is due to the reported compounds. However, this level of growth inhibition could be produced by a 0.004% contaminant of sangivamycin or toyocamycin which is below the limit of detection. Thus, the greater than 85% growth inhibition reported in Table I for compounds **6a** and **7a** may be at least partially due to the possible contaminants of toyocamycin and sangivamycin as described above. Since **6a** and **7a** are the most potent antiproliferative compounds, it appeared that none of the compounds **6a–e** and **7a–e** showed significant antiproliferative activity against L1210 cells. The inactivity of these 2′-substituted-2′-deoxy derivatives of sangivamycin (**7a–e**) is particularly striking in contrast to the parent compound *ara*-sangivamycin (compound b in Table I). *ara*-Sangivamycin caused total growth inhibition at 100 μM and had an IC_{50} of 6 μM. Thus, even this compound, in turn, was 3 orders of magnitude less potent in inhibiting growth than its parent compound sangivamycin (IC_{50} = 3 nM).[23] This drastic loss of cytotoxic activity upon the conversion of a pyrrolo[2,3-*d*]pyrimidine riboside to its corresponding arabinoside was also noted by Cass and co-workers[47] and by us[21] for tubercidin and *ara*-tubercidin.

In contrast, the cytotoxicity of **6a** and **7a** was not evident in the studies with HFF and KB cells (Table I). The difference between these cells and L1210 cells may be accounted for by the difference in sensitivity to sangivamycin and toyocamycin. IC_{50}'s for HFF and KB cells, with the assays described below under antiviral evaluations, were 30–50 nM,[21] about 10-fold higher than for growth inhibition of L1210 cells. Alternatively, if the antiproliferative activity of **6a** and **7a** is due to the reported compounds and not to contaminants, this difference would reflect a difference in sensitivity to the reported compounds.

In Vitro Antiviral Activity. Target compounds (**6a–e**, **7a–e**) were evaluated for activity against HCMV and HSV-1 in plaque-reduction assays. 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) using labeled-precursor uptake or by measuring cell growth. Data in Table I show that the toyocamycin analogues (**6a–e**) were active against HCMV whereas all but one of the sangivamycin analogues (**7b–e**) were inactive. Surprisingly all the new compounds were inactive against HSV-1 even though the unmodified parent compounds *ara*-toyocamycin and *ara*-sangivamycin were active (compounds a and b, respectively, Table I and ref 21). Among the toyocamycin analogues, 2′-amino analogue **6e** was most active against HCMV. Such was not the case with the sangivamycin analogues—of compounds **7a–e** only azido analogue **7a** possessed weak activity. Sangivamycin analogues **7a–e** were, in general, less active than the toyocamycin analogues (**6a–e**) in L1210 cells and in uninfected and HCMV-infected HFF and KB cells.

In yield reduction assays with HCMV, differences among the active compounds became more clear. Although I_{90} concentrations for yield-reduction experiments presented in Table I appear to show only modest activity for the toyocamycin analogues (**6a–e**), the dose–response curves

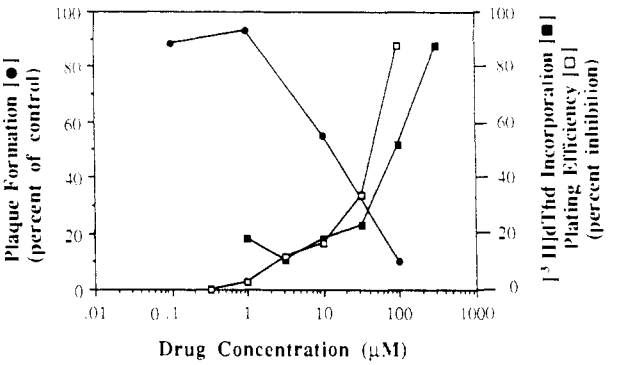

Figure 1. Comparison of antiviral and cytotoxic activities of (2′-deoxy-2′-aminoarabinosyl)toyocamycin (**6e**). Effects on HCMV plaque formation (●) are compared to effects on two measures of cytotoxicity, [³H]dThd incorporation into DNA (■) and plating efficiency of KB cells (□).

Table II. Activity of (2′-Deoxy-2′-aminoarabinosyl)toyocamycin (**6e**) against Selected Herpesviruses

virus[a]	cell line[b]	50% inhibitory concentration[c,d] μM: plaque assay	50% inhibitory concentration[c,d] μM: visual cytotoxicity
HCMV[e]	human foreskin fibroblasts	14	>100
MCMV	mouse embryo fibroblasts	13	>350
HSV-1	rabbit kidney cells	117	350
HSV-1[e]	monkey kidney cells	>100	>100
HSV-2	human foreskin fibroblasts	114	350

[a] Abbreviations used: HCMV, human cytomegalovirus; MCMV, murine cytomegalovirus; HSV-1, HSV-2, herpes simplex virus type 1 or 2, respectively. [b] Used to propagate the virus and assess visual cytotoxicity. [c] Assayed as described in the text, average of two or more experiments. [d] Except as noted in footnote *e*, techniques and virus strains are as described under Additional in Vitro Antiviral Evaluations in the text. [e] Data from Table I.

(not presented) which illustrated concentrations required to give multiple log reductions in HCMV titer were more revealing. Only compounds **6a** and **6e** produced five log reductions (100 000-fold) in virus titer at the highest concentration tested. Concentrations of 100 and 320 μM, respectively, were required.

Compound **6e** [(2′-deoxy-2′-aminoarabinosyl)toyocamycin] was chosen for additional study because it produced five log reductions in virus yield reduction assays, was most active against HCMV in plaque reduction assays, was less cytotoxic than azido analogue **6a** (Table I), and had no detectable toyocamycin contaminant (<0.01%). Cytotoxicity was explored in more depth by examining the effect of **6e** on the growth and plating efficiency of KB cells. Table I (footnote h) shows that concentrations up to 32 μM had little or no effect on cell growth but that 100 μM appeared to produce a cytostatic effect. Figure 1 illustrates that concentrations up to 32 μM had only modest effects on plating efficiency but that 100 μM was 90% inhibitory. A parallel but slightly lesser effect was observed on DNA synthesis ([³H]dThd incorporation) in uninfected cells (Figure 1). This figure also compares these two measures of cytotoxicity (shown as percent inhibition) with the antiviral effect (shown as percent of control). The fact that the respective curves cross at approximately 30% illustrates that compound **6e** is partially selective against HCMV.

The activity of compound **6e** against other herpesviruses was explored in more detail to determine the suitability of examining the compound in vivo. Data in Table II show that the compound was as active against murine cytomegalovirus (MCMV) as it was against HCMV. It had but

(47) Cass, C. E.; Selner, M.; Tan, T. H.; Muhs, W. H.; Robins, M. J. *Cancer Treat. Rep.* **1982**, *66*, 317.

Table III. Effect of Treatment with (2′-Deoxy-2′-aminoarabinosyl)toyocamycin (**6e**) on the Mortality of Mice Inoculated with MCMV

treatment[a]	mortality number	mortality %	P value	MDD[b]	P value
control	12/15	80		5.2	
placebo at 24 h	28/30	93	NS[c]	4.4	NS
compound **6e**					
50 mg/kg at 12 h	11/15	73	NS	4.1	NS
50 mg/kg at 24 h	15/15	100	NS	4.4	NS
50 mg/kg at 48 h	13/15	87	NS	4.9	NS
50 mg/kg—toxicity[d]	0/10	0			
16.7 mg/kg at 12 h	14/15	93	NS	4.6	NS
16.7 mg/kg at 24 h	15/15	100	NS	4.7	NS
16.7 mg/kg at 48 h	15/15	100	NS	4.6	NS
16.7 mg/kg—toxicity[d]	0/10	0			
5.6 mg/kg at 12 h	15/15	100	NS	4.5	NS
5.6 mg/kg at 24 h	14/15	93	NS	4.6	NS
5.6 mg/kg at 48 h	15/15	100	NS	5.6	<0.05
5.6 mg/kg—toxicity[d]	0/10	0			

[a] Animals were treated ip twice daily for 5 days with the doses stated above. Treatment was initiated at the times indicated following virus inoculation. [b] MDD = mean day of death. [c] NS = not significant. [d] Drug toxicity control. No virus administered.

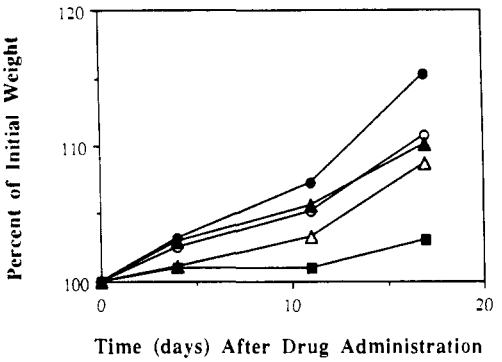

Figure 2. Effect of (2′-amino-2′-deoxyarabinosyl)toyocamycin (**6e**) on weight gain in albino mice. The compound was given as a single intraperitoneal injection in physiological saline at 0 (●), 1 (○), 10 (▲), 100 (△), or 320 (■) mg/kg. The latter two doses were administered as a suspension. Each data point is the average of three mice. The average initial weight was approximately 30 g.

little activity against HSV-1 and HSV-2. Little or no visual cytotoxicity was observed in cells used to propagate the viruses.

In Vivo Antiviral Activity. Compound **6e** was evaluated by using a murine model for cytomegalovirus infection.[48] Animals were treated intraperitoneally twice daily for 5 days beginning 6, 24, or 48 h after infection with MCMV. Table III shows that the compound had no activity in this model at doses up to 50 mg/kg twice daily for 5 days even though no deaths occurred from drug administration alone. In order to help determine if another experiment at higher doses was warranted in MCMV-infected animals, a more sensitive measure of in vivo drug toxicity was used. Compound **6e** was administered in single intraperitoneal doses to weanling mice in doses from 1 to 320 mg/kg and weight gain was noted. Figure 2 shows that even the lowest doses reduced weight gain in uninfected animals. Therefore, we decided against additional animal experiments in MCMV-infected animals. We conclude that even though compound **6e** has some separation between antiviral activity and cytotoxicity, there is neither adequate separation nor sufficient antiviral potency to justify further investigations.

(48) (a) Kern, E. R.; In *Antiviral Drug Development*; de Clerq, E., Walker, R. T., Eds.; Plenum Press: New York, 1988; p 149. (b) Glasgow, L. A.; Richards, J. T.; Kern, E. R. *Am. J. Med.* **1982**, *73*, 132.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The silica gel used for chromatography was silica gel 60 (230–400 mesh, E. Merck, Darmstadt, West Germany). Thin-layer chromatography (TLC) was performed on prescored SilicAR 7GF plates (Analtech, Newark, DE). Compounds were visualized by illumination under UV light (254 nm) and by spraying with 20% methanolic sulfuric acid followed by charring by means of a Bunsen burner. The designation "hexanes" refers to a commercially available mixture of hexane isomers. The following solvent designations are used throughout the paper: solvent A, ethyl acetate/hexanes (1:1, v/v); solvent B chloroform/methanol (9:1, v/v); solvent C, chloroform/methanol (4:1, v/v); solvent D, chloroform/methanol (16:1, v/v). All evaporations were carried out with a rotary evaporator connected to a water aspirator pump. The water bath temperature was maintained between 40 and 50 °C unless otherwise noted. High-performance liquid chromatography (HPLC) was performed on a Varian Vista 5000 instrument equipped with a UV-50 optical absorbtion detector, and the data were processed with a CDS-450 data station. A Whatman Magnum-20 ODS-3 preparative column (22.5 mm × 50 cm) was used for purifying biological test samples, as described below for the individual compounds. A Whatman Partisil 5 ODS-3 analytical column (4.6 mm × 25 cm) was used for analytical determinations with the same elution conditions as described for the preparative purification of each compound (**6a–e** and **7a**). The analysis of compound **7e** was performed with the same eluant as for **6e**. The purity of the biological test samples was determined either by comparing the area of the peaks of impurities with those of the target compounds, or by a comparison of peak heights of the impurities with those obtained by the injection of standard solutions of the parent nucleosides, toyocamycin and sangivamycin. Determination of UV spectra was performed on a Hewlett-Packard 8450-A UV/VIS spectrophotometer. IR spectra were obtained on a Perkin-Elmer 281 spectrophotometer. ^{1}H NMR spectra were obtained using an IBM WP 270-SY spectrometer operating in the FT mode at 270 MHz. Where necessary, deuterium exchange and homonuclear decoupling experiments were used to obtain proton-shift assignments of the ribose moiety.

4-Amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)disiloxan-1,3-diyl]-2-*O*-[(trifluoromethyl)sulfonyl]-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine (3). Trifluoromethanesulfonyl chloride (9.7 g, 57 mmol) was added, dropwise, with stirring and ice/salt bath cooling to a cold (0 °C) suspension of 4-amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)disiloxan-1,3-diyl]-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine (**2**; 25.7 g, 48.2 mmol) and 4-(dimethylamino)pyridine (21.5 g, 176 mmol) in 1,2-dichloroethane (600 mL). The mixture was then stirred at 0 °C until no more starting material could be detected by TLC analysis (solvent A, R_f = 0.50) (ca. 2 h). The reaction was then quenched with ice (200 mL). The mixture was extracted with chloroform (100 mL), the organic layer was collected, dried over magnesium sulfate, and filtered, and the filtrate was evaporated under reduced pressure to afford a syrup. This syrup was immediately dissolved in hexanes/THF (3:1, v/v, 200 mL) and the resulting solution was absorbed on a cylindrical pellet of silica gel (7.5 cm × 7.5 cm). The pellet was eluted with hexanes/THF (3:1, v/v, 1.2 L) and the eluate was then evaporated under reduced pressure to afford, after drying for 24 h in vacuo at room temperature, 27 g of a crisp foam (84%). An analytical sample was obtained by chromatography of a sample of the foam (0.3 g) on a silica gel column (22 × 300 mm) using hexanes/THF (3:1, v/v, 10 mL/min). The elution was monitored by TLC, and the fractions containing the product were evaporated under reduced pressure. The residue was kept in vacuo for 24 h at room temperature to afford a crisp foam. ^{1}H NMR (DMSO-d_6): δ 8.20, 8.14 (2 s, 2 H, H-2 and H-6); 7.02 (bs, 2 H, aromatic amino); 6.42 (s, 1 H, H-1′); 5.98 (d, 1 H, H-2′, $J_{2',3'}$ = 4.8 Hz); 5.07 (m, 1 H, H-3′); 4.01 (m, 3 H, H-4′ and H-5′a,b); 1.03 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ε): methanol, 278 (4.13); pH 1, 274 (4.05), 230 (4.12); pH 11, 281 (4.11), 235 (4.03). IR (KBr): 2220 cm^{-1} (cyano).

TLC: R_f = 0.81, solvent A. Anal. Calcd for $C_{25}H_{38}F_3N_5O_7Si_2S$: C, H, N.

4-Amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)-disiloxan-1,3-diyl]-2-deoxy-2-azido-β-D-arabinofuranosyl]-pyrrolo[2,3-*d*]pyrimidine (4a). A solution of compound **3** (26.0 g, 39 mmol) in dry (distilled in vacuo from calcium hydride) hexamethylphosphoramide (50 mL) was treated with powdered anhydrous lithium azide (5.0 g, 100 mmol) and then stirred rapidly (magnetic stirrer) at room temperature for 4 h. Water (500 mL) was added, and the resulting suspension was extracted with ethyl acetate/cyclohexane (1:1, v/v, 3 × 200 mL). The upper layers were collected, combined, washed with water (500 mL) and brine (2 × 100 mL), dried over magnesium sulfate, filtered, and then evaporated under reduced pressure to afford a sticky foam. Ethyl acetate (1 mL) was added to a solution of the resulting foam in hexanes (100 mL), at reflux. The filtrate was then allowed to stand at room temperature for 1 h and then at 4 °C for 2 h. The sticky solid which precipitated was collected by vacuum filtration, washed with hexanes (100 mL), and dried for 0.5 h under reduced pressure at room temperature. The still-moist solid was then dissolved in boiling methanol (350 mL) and the solution was filtered by gravity. This solution was allowed to cool to room temperature and then kept at 4 °C for 18 h. The crystallized product was collected by vacuum filtration to yield 14.08 g, after air-drying at room temperature. An additional 2.88 g could be obtained by evaporation of the hexanes mother liquor and crystallization of the residue from methanol (50 mL) to give a total yield of 16.96 g (78%). Mp: 94–97 °C. ^{1}H NMR (DMSO-d_6): δ 8.22, 8.03 (2 s, 2 H, H-2 and H-6); 6.96 (bs, 2 H, NH_2); 6.54 (d, 1 H, H-1′, $J_{1',2'}$ = 6.7 Hz); 4.88 (dd, 1 H, H-2′); 4.44 (dd, 1 H, H-3′); 4.19 (d, 1 H, H-5′a); 3.94 (m, 2 H, H-4′ and H-5′b); 1.1–1.00 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ε): methanol, 289 (4.01), 280 (4.19), 231 (4.02); pH 1, 274 (4.10), 234 (4.22); pH 11, 293 (4.08), 284 (4.16), 234 (4.08). IR (KBr): 2220, 2110 cm^{-1} (cyano, azido). TLC: R_f = 0.35, solvent A. Anal. Calcd for $C_{24}H_{38}N_8O_4Si_2$: C, H, N.

4-Amino-5-cyano-7-(2-deoxy-2-azido-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6a). A solution of compound **4a** (12.5 g, 22.4 mmol) in THF (100 mL) was treated with a solution of tetra-*n*-butylammonium fluoride in THF (50 mL, 1 M). The mixture was stirred at room temperature until no more starting material could be detected by TLC analysis (solvent A, R_f = 0.35) and only one product could be discerned by TLC analysis (solvent C, R_f = 0.74) (ca 2 h). The mixture was then evaporated under reduced pressure to afford a syrup which was dissolved in methanol (200 mL). Silica gel (50 g) was added to the methanolic solution and the mixture was evaporated to dryness under reduced pressure at 50 °C then kept in vacuo at room temperature for 18 h. The resulting solid was then gently ground with mortar and pestle until it was free flowing. The resulting powder was placed on the top of a dry-packed silica gel column (150 mm wide, 90 mm deep) and the column was then eluted with solvent B until no more product could be detected in the eluant by TLC (solvent C). This fraction (1.3 L) was evaporated under reduced pressure at 50 °C, and the sticky residue was allowed to cool to room temperature and then triturated with chloroform (150 mL). The residual solid was collected by vacuum filtration and the filter cake was washed with additional chloroform (50 mL). The solid as dried briefly under reduced pressure at room temperature and then dissolved in boiling water (100 mL). Charcoal was added (300 mg) and the hot mixture was vacuum filtered through a thin pad of Celite. The filtrate was heated to boiling and then allowed to stand at room temperature for 18 h. The crystallized product was then collected by vacuum filtration and washed with water (10 mL) and ethanol (20 mL). The solid was dried under reduced pressure at 60 °C overnight before being redissolved in boiling water (100 mL). The solution was allowed to stand for 18 h at room temperature and the product was collected by vacuum filtration, washed with water (10 mL) and ethanol (20 mL), and then dried under reduced pressure at 80 °C for 28 h to afford 4.2 g (60%) of a solid which was homogeneous by TLC and ^{1}H NMR analyses. An analytical sample was obtained by reverse-phase chromatography as follows: A sample of the solid (ca 0.15 g) was dissolved in water/methanol (2 mL, 70:30, v/v) by gentle heating and the solution was injected onto a Whatman Magnum 20 ODS-3 column (20 mm × 50 cm). The column was eluted with water/methanol (70:30, v/v, 15 mL/min). The elution was followed by observing the UV absorption at 280 nm and the fraction containing the product (225 mL, eluting between 30 and 45 min, K' = 3.36) was collected and pooled with the fractions obtained by repeating the above cited chromatographic procedure four more times. The pooled fractions were evaporated in vacuo at 50 °C and the product was collected by vacuum filtration and washed with water (10 mL) to yield 0.5 g of a white solid after drying under reduced pressure at 80 °C for 24 h. Mp: 186–187 °C dec. ^{1}H NMR (DMSO-d_6): δ 8.37, 8.21 (2 s, 2 H, H-2 and H-6); 6.84 (bs, 2 H, NH_2); 6.54 (d, 1 H, H-1′, $J_{1',2'}$ = 6.57 Hz); 6.00 (d, 1 H, 3′-OH); 5.23 (t, 1 H, 5′-OH); 4.54 (dd, 1 H, H-2′); 4.22 (dd, 1 H, H-3′); 3.8–3.6 (m, 3 H, H-4′ and H-5′a,b). UV λ_{max} (nm) (log ε): methanol, 280 (4.32); pH 1, 272 (4.38), 231 (4.52); pH 11, 279 (4.40), 232 (4.26). IR (KBr): 2220, 2120 cm^{-1} (cyano, azido). TLC: R_f = 0.74, solvent C. Anal. Calcd for $C_{12}H_{12}N_8O_3$ (H_2O): C, H, N.

4-Amino-5-cyano-7-(2-deoxy-2-amino-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6e). Triethylamine (2.5 mL, 0.018 mmol) and 1,3-propanedithiol (7.5 g, 0.07 mmol) were added to a hot solution of compound **6a** (2.3 g, 7 mmol) in methanol (50 mL) under a nitrogen atmosphere. The mixture was stirred under a nitrogen atmosphere for 3 h and then evaporated under reduced pressure to afford a white residue. This residue was triturated with hexanes (4 × 20 mL) and the residual solid was collected by vacuum filtration. The solid was recrystallized twice from hot water (30 mL), with the product then being washed with ether (60 mL), to yield 1.2 g (56%) of **6e** as a solid which was homogeneous by TLC and ^{1}H NMR analyses. An analytical sample was obtained by reverse-phase chromatography as follows: A sample of the solid was dissolved in aqueous 0.005 M triethylammonium formate buffer, pH = 3.0/methanol (2 mL, 77:23, v/v) by gentle heating and the solution was injected onto a Whatman Magnum 20 ODS-3 column (20 mm × 50 cm). The column was eluted with aqueous 0.005 M triethylammonium formate buffer, pH = 3.0/methanol (77:23, v/v, 10 mL/min). The elution was followed by UV (at 280 nm) and the fraction containing the product (110 mL, eluting between 24 and 35 min, K' = 1.3) was collected and pooled with the fractions obtained from six additional chromatographic runs. The pH of the pooled fractions was adjusted to pH = 10 with concentrated ammonium hydroxide, and the solution was evaporated under reduced pressure to a volume of 3 mL. The solution was then allowed to stand at 4 °C for 24 h. The crystallized product was collected by vacuum filtration and washed with cold water (2 × 25 mL) to afford 0.5 g of **6e**. Mp: 230 °C dec., ^{1}H NMR (DMSO-d_6): δ 8.35, 8.20 (2 s, 2 H, H-2 and H-6); 6.80 (bs, 1 H, NH_2); 6.37 (d, 1 H, H-1′, $J_{1',2'}$ = 6.15 Hz); 5.42 (d, 1 H, 3′-OH); 5.20 (bs, 1 H, 5′-OH); 3.97 (dd, 1 H, H-3′); 3.8–3.6 (m, 3 H, H-4′ and H-5′a,b); 3.50 (dd, 1 H, H-2′); 1.45 (bs, 2 H, 2′-NH_2). UV λ_{max} (nm) (log ε): methanol, 280 (4.47), 232 (4.32); pH 1, 272 (4.43), 232 (4.53); pH 11, 279 (4.50), 233 (4.32); IR (KBr): 2220 cm^{-1} (cyano). TLC: R_f = 0.23, solvent C. Anal. Calcd for $C_{12}H_{14}N_6O_3$ (H_2O): C, H, N.

4-Amino-7-(2-deoxy-2-azido-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7a). Hydrogen peroxide (2.0 mL, 30%) was added to a rapidly stirred suspension of compound **6a** (1.5 g, 4.5 mmol) in concentrated ammonium hydroxide (50 mL). The solution was stirred for an additional 1 h, during which time a clear solution was effected and then a solid precipitated. The solid was collected by vacuum filtration and then recrystallized from hot water (175 mL). The resulting solid was dried under reduced pressure at 80 °C to yield 1.2 g (84%) of a solid product which was homogeneous by TLC and ^{1}H NMR analyses. An analytical sample was obtained by reverse-phase chromatography as follows: A sample of the solid (ca. 0.15 g) was dissolved in DMF/water/methanol (5:14:1, v/v/v, 2 mL) by gentle heating and the solution was injected onto a Whatman Magnum 20 ODS-3 column (20 mm × 50 cm). The column was eluted with aqueous 0.005 M triethylammonium formate buffer, pH = 3.0/methanol (77:23, v/v, 10 mL/min). The elution was followed by UV (280 nm) and the fraction containing the product (140 mL, eluting between 36 and 50 min, K' = 2.3) was collected and pooled with the fractions obtained by repeating the above cited chromatographic procedure three more times. The pH of the pooled fractions was adjusted to pH 10 with concentrated ammonium hydroxide, and the solution was evaporated

under reduced pressure to a volumn of 30 mL. The product was then allowed to stand for 24 h, the solid was collected by vacuum filtration, washed with water (10 mL), and dried under reduced pressure at 80 °C to yield 0.4 g of product. Mp: 227 °C dec. ^{1}H NMR (DMSO-d_6): δ 8.08, 7.99 (2 s, 2 H, H-2 and H-6); 7.97, 7.36 (2 bs, 2 H, $CONH_2$); 6.58 (d, 1 H, H-1′, $J_{1',2'}$ = 6.55 Hz); 5.95 (d, 1 H, 3′-OH); 4.94 (t, 1 H, 5′-OH); 4.53 (dd, 1 H, H-2′); 4.22 (dd, 1 H, H-3′); 3.8–3.6 (m, 3 H, H-4′ and H-5′ a, b). UV λ_{max} (nm) (log ε); methanol, 281 (4.37); pH 1, 276 (4.33), 230 (4.40); pH 11, 280 (4.37), 235 (4.19). IR (KBr): 2120 cm^{-1} (azido). TLC: R_f = 0.47, solvent C. Anal. Calcd for $C_{12}H_{14}N_8O_4$: C, H, N.

4-Amino-7-(2-deoxy-2-amino-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7e). To a solution of compound **7a** (0.26 g, 0.78 mmol) in methanol/DMF (60 mL, 5:1, v/v) were added, under nitrogen, 1,3-propanedithiol (2.16 g, 20 mmol) and triethylamine (0.73 g, 7.2 mmol). The solution was stirred under nitrogen for 3 h at room temperature and then evaporated to dryness in vacuo at 50 °C. The residue was triturated with hot ethanol (20 mL), the suspension was filtered, and the filter cake was washed with ethanol (20 mL) and then ether (20 mL). The resulting powder was taken up in DMF (1 mL); the solution was diluted with water (9 mL) and allowed to stand for 18 h. The product was collected by filtration, washed with water (5 mL), and dried in vacuo at 80 °C to afford 120 mg of a solid. Mp: 283–285 °C. ^{1}H NMR (DMSO-d_6): δ 8.05, 7.99 (2 s, 2 H, 2-H and H-6); 7.91, 7.29 (2 bs, 2 H, $CONH_2$); 6.38 (d, 1 H, H-1′, $J_{1',2'}$ = 6.24 Hz); 5.39 (d, 1 H, 3′-OH); 3.94 (dd, 1 H, H-3′); 3.7–3.5 (m, 3 H, H-4′ and H-5′a,b); 3.48 (dd, 1 H, H-2′); 1.42 (bs, 2 H, 2′-NH_2). UV λ_{max} (nm) (log ε): methanol, 282 (4.36); pH 1, 274 (4.33), 228 (4.37); pH 11, 281 (4.38), 235 (4.19). IR (KBr): no cyano or azido absorption observed. TLC: R_f = 0.08, solvent C. Anal. Calcd for $C_{12}H_{16}N_6O_4$ (H_2O): C, H, N.

4-Amino-5-cyano-7-[3-*O*-[1,1,3,3-tetrakis(methylethyl)-3-hydroxydisiloxanyl]-2-deoxy-2-chloro-β-D-arabinofuranosyl]pyrrolo[2,3-*d*]pyrimidine (5) and 4-Amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)disiloxan-1,3-diyl]-2-deoxy-2-chloro-β-D-arabinofuranosyl]pyrrolo[2,3-*d*]pyrimidine (4d). Compound **3** (5.0 g, 7.5 mmol) was added to a cold (10 °C) solution of lithium chloride (2.0 g, 47 mmol) in hexamethylphosphoramide (30 mL). The ice bath was removed, the mixture was diluted with hexamethylphosphoramide (30 mL) and then stirred for 40 min. Ethyl acetate (125 mL) was added, and after 15 min of stirring, the mixture was partitioned between ethyl acetate (50 mL) and water (500 mL). The aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were then washed with water (4 × 250 mL) and brine (100 mL). The organic solution was dried over sodium sulfate, filtered, and evaporated. The residue was coevaporated with methanol (3 × 50 mL), the resulting syrup was dissolved in methanol (30 mL), and the solution was allowed to stand at 4 °C for 24 h. The solid which precipitated was collected by filtration and recrystallized from methanol (20 mL) to afford compound **4d** (0.26 g, 6%). Mp: 173–176 °C. ^{1}H NMR (DMSO-d_6): δ 8.19, 8.16 (2 s, 2 H, H-2 and H-6); 6.94 (bs, 2 H, NH_2); 6.61 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz); 5.04 (dd, 1 H, H-2′); 4.68 (dd, 1 H, H-3′); 4.22 (dd, 1 H, H-4′); 3.95 (m, 2 H, H-5′a,b); 1.1–1.0 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ε): methanol, 279 (4.11), 229 (3.95); pH 1, 274 (4.07), 232 (4.22); pH 11, 276 (4.11), 236 (4.11). IR (KBr): 2226 cm^{-1} (cyano). TLC: R_f = 0.46, solvent D. Anal. Calcd for $C_{24}H_{38}ClN_5O_4Si_2$ (H_2O): C, H, N.

The mother liquors were combined and evaporated. The residue was coevaporated with chloroform (3 × 50 mL) and finally dissolved in chloroform (20 mL). The solution was applied to a silica gel column (50 × 230 mm) which had been slurry packed in chloroform. The column was then eluted with chloroform/methanol (19:1, v/v) and the fractions containing the product (R_f = 0.31, solvent D, 230 mL) were pooled and evaporated. The residue was coevaporated with methanol (3 × 20 mL) to afford a gum which was crystallized from methanol/water (35:10, v/v, 45 mL) to afford 1.26 g (29%) of **5**. Mp 91–94 °C. ^{1}H NMR (DMSO-d_6): δ 8.38, 8.21 (2 s, 2 H, H-2 and H-6); 6.92 (bs, 2 H, NH_2); 6.65 (d, 1 H, H-1′, $J_{1',2'}$ = 5.6 Hz); 6.18 (s, 1 H, SiOH); 5.38 (t, 1 H, 5′-OH); 4.84 (dd, 1 H, H-2′); 4.73 (dd, 1 H, H-3′); 3.93 (dd, 1 H, H-4′); 3.78 (m, 2 H, H-5′a,b); 1.1–1.0 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ε): methanol, 279 (4.19), 230 (4.01); pH 1, 276 (4.10), 233 (4.18); pH 11, 296 (4.16), 286 (4.20), 236 (4.14). IR (KBr): 2228 cm^{-1} (cyano). TLC: R_f = 0.31, solvent D. Anal. Calcd for $C_{24}H_{40}ClN_5O_5Si_2$: C, H, N.

4-Amino-5-cyano-7-(2-deoxy-2-chloro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6c). To an ice-cold solution of compound **5** (1.2 g, 2.1 mmol) in THF (40 mL) was added, dropwise, a solution of tetra-*n*-butylammonium fluoride in THF (2.5 mL, 1 M). The mixture was stirred until starting material could not be detected by TLC analysis (R_f = 0.46, solvent D) and only one product could be discerned (R_f = 0.63, solvent C). The mixture was then evaporated at ambient temperature to a thick slurry which was coevaporated with 95% ethanol (2 × 50 mL). The resulting residue was triturated with 95% ethanol (10 mL) and the slurry was chilled (ice bath) for 15 min. The solid material was collected by filtration and washed with 95% ethanol (10 mL) to yield 0.34 g of crude product. The mother liquors were evaporated and coevaporated with methanol (2 × 30 mL), and the resulting residue was dissolved in methanol (50 mL). The solution was treated with Dowex 50X H^+ resin (1.5 g wet with methanol) and filtered, and the resin bed was washed with methanol (3 × 10 mL). The combined filtrates were evaporated and coevaporated with ethanol (2 × 10 mL), and the residue was triturated with ether (20 mL). The resulting solid was collected by filtration and washed with ether (20 mL) to afford an additional 0.31 g of product. The combined solids were dissolved in boiling 90% ethanol (30 mL). The solid which deposited after the solution was allowed to stand at room temperature for 3 h and then at 4 °C for 24 h was collected by filtration and washed with ethanol (10 mL) and ether (5 mL) to afford 0.453 g of material. Concentration of the mother liquor to ca. 10 mL and then allowing the solution to stand at 4 °C for 24 h provided an additional 0.063 g for a total yield of 0.516 g (79%). The analytical sample was obtained by recrystallizing the material three times (0.5 g) by dissolving the solid in dimethylformamide (1 mL) and diluting the solution with hot water (ca. 90 °C, 9 mL). The solid thus obtained was washed with water (5 mL) and ether (15 mL) each time, to finally afford 0.4 g of pure material. Mp: 247–248 °C. ^{1}H NMR (DMSO-d_6): δ 8.42, 8.21 (2 s, 2 H, H-2 and H-6); 6.89 (bs, 2 H, NH_2); 6.63 (d, 1 H, H-1′, $J_{1',2'}$ = 6.1 Hz); 6.08 (d, 1 H, 3′-OH); 5.25 (t, 1 H, 5′-OH); 4.74 (dd, 1 H, H-2′); 4.35 (dd, 1 H, H-3′); 3.8–3.7 (2 m, 3 H, H-4′ and H-5′a,b); UV λ_{max} (nm) (log ε): methanol, 279 (4.20), 230 (4.03); pH 1, 273 (4.14), 233 (4.27); pH 11, 278 (4.21), 232 (4.02). IR (KBr): 2221 cm^{-1} (cyano). TLC: R_f = 0.63, solvent C. Anal. Calcd for $C_{12}H_{12}ClN_5O_3$: C, H, N.

4-Amino-7-(2-deoxy-2-chloro-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7d). A rapidly stirred solution of compound **6d** (0.2 g, 0.646 mmol) in methanol/water (1:4, v/v, 50 mL) was warmed (steam bath) to effect dissolution. The solution was then cooled to room temperature, and concentrated ammonium hydroxide solution was added (1 mL). A fine precipitate formed, and the resulting rapidly stirred suspension was treated with three portions of hydrogen peroxide (0.2 mL, 30%) at 10-min intervals. After the last portion was added, the mixture was stirred for 5 min and then warmed (steam bath) just until a clear solution developed. The solution was immediately chilled (ice bath) and evaporated with a room temperature water bath. The residue was coevaporated with methanol (2 × 5 mL), and finally the residue was dissolved in methanol (1 mL) by gentle warming (steam bath). The solution was cooled to room temperature and then held at 4 °C for 2 h. The solid which precipitated was collected by filtration and washed with cold methanol (2 × 1 mL) to afford 0.183 g (86%) of product. The analytical sample was obtained by recrystallizing a portion of the material (0.17 g) from water (10 mL) to afford 0.11 g of material. Mp: 242–244 °C. ^{1}H NMR (DMSO-d_6): δ 8.07, 8.06 (2 s, 2 H, H-2, H-6); 7.99, 7.36 (bs, 2 H, $CONH_2$); 6.64 (d, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz); 6.06 (d, 1 H, 3′-OH); 4.97 (t, 1 H, 5′-OH); 4.68 (dd, 1 H, H-2′); 4.32 (dd, 1 H, H-3′); 3.8–3.7 (2 m, 3 H, H-4′, H-5′a,b). UV λ_{max} (nm) (log ε): methanol, 281 (4.14), 231 (3.96); pH 1, 275 (4.10), 230 (4.15); pH 11, 280 (4.15), 234 (3.96). IR (KBr): no cyano absorbtion observed. TLC: R_f = 0.63, solvent C. Anal. Calcd for $C_{12}H_{14}ClN_5O_4$ (H_2O): C, H, N.

4-Amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)-disiloxan-1,3-diyl]-2-deoxy-2-bromo-β-D-arabinofuranosyl]-pyrrolo[2,3-*d*]pyrimidine (4b). To a cold (ice bath) solution of lithium bromide (2.0 g, 23 mmol) in HMPA (30 mL) was added compound **3** (3.0 g, 4.5 mmol). The ice bath was then removed

and the solution was stirred for 3 h. The mixture was diluted with ethyl acetate (50 mL) and the solution was partitioned between ethyl acetate (50 mL) and water (200 mL). The organic layer was then washed with water (4 × 200 mL) and brine (100 mL), dried over sodium sulfate, filtered, and then evaporated to dryness. The residue was coevaporated with methanol (50 mL) and then dissolved in hot methanol (100 mL). The solution was treated with decolorizing charcoal, filtered, and evaporated. The residue was crystallized from methanol/water (47:3, v/v, 50 mL). The product was collected and washed with cold methanol (10 mL) to afford 1.59 g of material, after air-drying. Mp: 95–97.5 °C. ^{1}H NMR: (DMSO-d_6) δ 8.20, 8.12 (2 s, 2 H, H-2, H-6); 6.95 (bs, 2 H, NH_2); 6.58 (d, 1 H, H-1′, $J_{1',2'}$ = 7.0 Hz); 5.04 (dd, 1 H, H-2′); 4.77 (dd, 1 H, H-3′); 4.21 (dd, 1 H, H-4′); 3.95 (m, 2 H, H-5′a,b); 1.2–1.0 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ϵ): methanol, 280 (4.23), 230 (4.04); pH 1, 275 (4.16), 233 (4.28); pH 11, 296 (4.22), 236 (4.22). IR (KBr): 2228 cm^{-1} (cyano). TLC: R_f = 0.49, solvent D. Anal. Calcd for $C_{24}H_{38}BrN_5O_4Si_2$ (0.5 H_2O): C, H, N.

4-Amino-5-cyano-7-(2-deoxy-2-bromo-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6b). To an ice-cold solution of compound **4b** (1.3 g, 2.3 mmol) in THF (30 mL), was added, dropwise, a solution of tetra-*n*-butylammonium fluoride in THF (0.5 mL, 1 M) every 10 min for a total of four additions. After the last portion was added, the solution was allowed to stir for 10 min at ice-bath temperature, and then it was evaporated, without a water bath, to afford a thick syrup. This syrup was dissolved in methanol (20 mL) and kept at 4 °C for 3 h. The solid product was collected and washed with methanol (2 × 5 mL) to afford 0.6 g (73%) of a white solid. Mp: 228–230 °C dec. ^{1}H NMR (DMSO-d_6): δ 8.42, 8.22 (2 s, 2 H, H-6, H-2); 6.90 (bs, 2 H, NH_2); 6.60 (d, 1 H, H-1′, $J_{1',2'}$ = 6.4 Hz); 6.07 (d, 1 H, 3′-OH); 5.26 (t, 1 H, 5′-OH); 4.78 (dd, 1 H, H-2′); 4.44 (dd, 1 H, H-3′); 3.76 (m, 3 H, H-4′, H-5′a,b). UV λ_{max} (nm) (log ϵ): methanol, 279 (4.25), 230 (4.09); pH 1, 273 (4.19), 233 (4.31); pH 11, 279 (4.24), 233 (3.99). IR (KBr): 2221 cm^{-1} (cyano). TLC: R_f = 0.66, solvent C. Anal. Calcd for $C_{12}H_{12}BrN_5O_3$: C, H, N.

4-Amino-7-(2-deoxy-2-bromo-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7b). A rapidly stirred suspension of compound **6b** (0.2 g, 0.646 mmol) in methanol/water (1:4, 50 mL) was warmed (steam bath) to effect dissolution and the solution was then chilled (ice bath) to ca. room temperature. The solution was treated with concentrated ammonium hydroxide (1 mL) and a fine precipitate formed. The rapidly stirred suspension was then treated with three portions of hydrogen peroxide (0.2 mL, 30%) at 8-min intervals. The mixture was stirred for 25 min and a fourth portion was added (0.2 mL). After stirring for 10 min, the clear solution was evaporated with a room temperature water bath. The residue was coevaporated with methanol (3 × 15 mL), ethanol (2 × 15 mL), and 2-propanol (1 × 15 mL). The resulting syrup was dissolved in water (15 mL), and then slowly evaporated without a water bath until a thick paste had formed. This paste was suspended in water (5 mL), the solid was collected by filtration and washed with water (5 mL) and ether (10 mL) to yield 0.18 g (86%) of a solid. The analytical sample was obtained by recrystallizing this solid from water (5 mL) and washing the product with cold water (1 mL) to afford 0.12 g of white needles. Mp: 214–215 °C. ^{1}H NMR (DMSO-d_6): δ 8.07, 8.04 (2 s, 2 H, H-2, H-6); 7.98, 7.36 (2 bs, 2 H, $CONH_2$); 6.59 (d, 1 H, H-1′, $J_{1',2'}$ = 6.35 Hz); 6.05 (d, 1 H, 3′-OH); 5.00 (t, 1 H, 5′-OH); 4.73 (dd, 1 H, H-2′); 4.41 (dd, 1 H, H-3′); 3.76 (m, 3 H, H-4′ and H-5′a,b). UV λ_{max} (nm) (log ϵ): methanol, 2.81 (4.17), 233 (3.99); pH 1, 274 (3.88), 233 (3.81); pH 11, 280 (4.17), 247 (3.88), 234 (3.93). IR (KBr): no cyano absorbtion observed. TLC: R_f = 0.45, solvent C. Anal. Calcd for $C_{12}H_{14}BrN_5O_4$: C, H, N.

4-Amino-5-cyano-7-[3,5-*O*-[1,1,3,3-tetrakis(methylethyl)disiloxan-1,3-diyl]-2-deoxy-2-iodo-β-D-arabinofuranosyl]-pyrrolo[2,3-*d*]pyrimidine (4c). Compound **3** (5.0 g, 7.5 mmol) was added to a cold (ice bath) solution of sodium iodide (4.7 g, 32 mmol) in HMPA (30 mL). The ice bath was removed, and the mixture was stirred for 12 h. The resulting solution was partitioned between ethyl acetate (200 mL) and water (300 mL). The organic layer was washed with sodium thiosulfate solution (5%, 200 mL), water (3 × 300 mL), and brine (100 mL), dried over sodium sulfate, filtered, and then evaporated. The residue was coevaporated with methanol (50 mL) and then dissolved in hot methanol (50 mL). The solution was allowed to stand at room temperature for 2 h. The solid which precipitated was collected and washed with cold methanol (2 × 10 mL). Recrystallization of this solid was accomplished by dissolving the solid in hot ethanol (10 mL), diluting the resulting solution with methanol (25 mL), heating the solution to its boiling point for 5 min, and allowing it to stand at room temperature for 2 h. The product was collected by filtration and washed with methanol (5 mL) to afford 2.0 g of a white solid (41%). Mp: 164–165 °C. ^{1}H NMR (DMSO-d_6): δ 8.20, 8.09 (2 s, 2 H, H-2, H-6); 6.95 (bs, 2 H, NH_2); 6.47 (d, 1 H, H-1′, $J_{1',2'}$ = 6.85 Hz); 4.91 (dd, 1 H, H-2′); 4.80 (dd, 1 H, H-3′); 4.24, 4.03 (m, 2 H, H-5′a,b); 3.85 (m, 1 H, H-4′); 1.2–1.0 (m, 28 H, tetraisopropyl). UV λ_{max} (nm) (log ϵ): methanol, 280 (4.23), 231 (4.06); pH 1, 278 (4.17), 234 (4.24); pH 11, 295 (4.29), 286 (4.30), 237 (4.28). IR (KBr): 2228 cm^{-1} (cyano). TLC: R_f = 0.50, solvent D. Anal. Calcd for $C_{24}H_{38}IN_5O_4Si_2$: C, H, N.

4-Amino-5-cyano-7-(2-deoxy-2-iodo-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6c). To a cold solution (ice bath) of compound **4c** (1.0 g, 1.55 mmol) in THF (25 mL) was added, dropwise, a solution of tetra-*n*-butylammonium fluoride solution (2.0 mL, 1 M) in THF. The cold mixture was stirred for 30 min and then evaporated with a room temperature water bath. The residue was coevaporated with methanol (10 mL) and dissolved in methanol (15 mL) by gentle heating (steam bath), and the resulting solution was allowed to stand at 4 °C for 16 h. The crude solid was collected and recrystallized twice from ethanol (4 mL) to afford 0.15 g of product (24%). Mp: 202–203 °C dec. ^{1}H NMR (DMSO-d_6): δ 8.39, 8.22 (2 s, 2 H, H-2, H-6); 6.89, (bs, 2 H, NH_2); 6.47 (d, 1 H, H-1′, $J_{1',2'}$ = 6.75 Hz); 5.98 (d, 1 H, 3′-OH); 5.24 (t, 1 H, 5′-OH); 4.70 (dd, 1 H, H-2′); 4.47 (dd, 1 H, H-3′); 3.73 (m, 3 H, H-4′ and H-5′a,b). UV λ_{max} (nm) (log ϵ): methanol, 289 (4.01), 280 (4.19), 231 (4.03); pH 1, 274 (4.13), 234 (4.24); pH 11, 279 (4.19), 233 (4.01). IR (KBr): 2221 cm^{-1} (cyano). TLC: R_f = 0.65, solvent C. Anal. Calcd for $C_{12}H_{12}IN_5O_3$: C, H, N.

4-Amino-7-(2-deoxy-2-iodo-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (7c). To a solution of compound **6c** (0.2 g, 0.50 mmol) in methanol/water (2:1, v/v, 15 mL) was added a concentrated ammonium hydroxide solution (0.01 mL). The solution was then treated with three portions of hydrogen peroxide solution (0.2 mL, 30%) at 1-h intervals. After the last portion was added, the mixture was stirred for 1 h and evaporated without using a water bath. The residue was coevaporated with ethanol (3 × 10 mL), and then recrystallized twice from ethanol (5 mL, 3 mL) to afford 0.12 g (52%) of material. Mp: 162–165 °C. ^{1}H NMR: (DMSO-d_6): δ 8.07, 7.99 (2 s, 2 H, H-2, H-6); 7.97, 7.36 (2 bs, 2 H, $CONH_2$); 6.43 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz); 5.94 (d, 1 H, 3′-OH); 4.95 (t, 1 H, 5′-OH); 4.68 (dd, 1 H, H-2′); 4.41 (dd, 1 H, H-3′); 3.75 (m, 3 H, H-4′, H-5′a,b). UV λ_{max} (nm) (log ϵ): methanol, 282 (4.18), 233 (3.99); pH 1, 276 (4.13), 232 (4.19); pH 11, 281 (4.19), 236 (3.99). IR (KBr): no cyano absorbtion observed. TLC: R_f = 0.45, solvent C. Anal. Calcd for $C_{12}H_{14}IN_5O_4$ (C_2H_5OH): C, H, N.

Biological Evaluations. In Vitro Antiproliferative Studies. The in vitro cytotoxicity against L1210 cells was evaluated as described previously.[49] L1210 cells were grown in static suspension culture with Fischer's medium for leukemic cells of mice and the growth rate over a 4-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 that 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.

The growth rates were determined for two distinct growth phases: 0–48 h, during which the control cells were in exponential growth (T_d = 11–12 h), and 48–96 h, during which the control cells were growing more slowly (average T_d = 22 h). The values reported in Table I represent the lower of the two values for growth rate.

(49) Wotring, L. L.; Townsend, L. B. *Cancer Res.* **1979**, *39*, 3018–3023.

Primary in Vitro 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 10% calf 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.[21] A plaque-purified isolate, P_O, of the Towne strain of HCMV was used and was a gift of Dr. M. F. 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.[21]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed with monolayer cultures of HFF cells as detailed earlier[21] by using a procedure similar to that 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.[21] 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 0 to 4+ 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.[21]

(d) Cell-Growth Rates. Population doubling times were measured in uninfected KB cells. Cells were planted in replicate six-well plastic tissue culture dishes at 75 000 cells per well. Following an incubation period during which cells attached to the substrate, medium was decanted, the sheet was rinsed once with PBS, and fresh medium was added. The medium consisted of MEM(H) with 1.1 g of $NaHCO_3$/L and 10% calf serum plus appropriate log or half-log concentrations of drug. After additional periods of incubation from 1 to 48 h, at 37 °C, cells were harvested by means of 0.05% trypsin plus 0.02% EDTA in a HEPES-buffered salt solution. Cells were enumerated using a Coulter counter.

(e) 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 containing 10% fetal bovine serum 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 culture incubated in a humidified atmosphere of 4% CO_2–96% air at 37 °C for 14 days. Medium then was decanted and colonies 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.

(f) 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 Table II 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.

Additional in Vitro Antiviral Evaluation. The susceptibility of MCMV (Smith strain) and HCMV (AD 169 strain) to compound **6e** was explored in more detail. Primary mouse embryo fibroblasts (MEF) and low-passaged human foreskin fibroblasts (HFF cells), respectively, were used in plaque-reduction assays. The sensitivity of HSV-1 (E-377 strain) and HSV-2 (MS strain) to both compounds was determined in MEF, primary rabbit kidney (RK), and HFF cells, also by the following plaque-reduction assay. Confluent cell monolayers in six-well plates were inoculated with 20–50 PFU of the appropriate virus and incubated at 37 °C and 90% humidity for 1 h. Serial 5-fold dilutions of each drug were prepared in twice concentrated MEM(E) and mixed with an equal volume of a 1% agarose, and 2 mL of the mixture was added to the monolayer cultures. An additional overlay mixture without drug (1 mL) was added on day 4 for MCMV and on days 4 and 8 for HCMV. At the appropriate time (HSV, 3 days; MCMV, 7 days; HCMV, 13 days) monolayers were stained with neutral red and plaques were enumerated either visually or with the aid of a stereo dissecting microscope. Drug-treated cultures were compared to untreated control cultures and IC_{50} values were calculated by using a dose–effect analysis software program (Elsevier-Biosoft, Cambridge, U.K.).

Experimental MCMV Infection. As detailed elsewhere,[48] 3 week old Swiss Webster female mice (Simonsen Laboratories, Gilroy, CA) were inoculated intraperitoneally (ip) with 2×10^5 PFU of the Smith strain of MCMV. All animals were held for 21 days and checked daily for mortality. Groups of 15 animals were treated ip twice daily for 5 days with various concentrations of compound **6e** beginning 6, 24, or 48 h after viral inoculation. At the end of 21 days, final mortality rates and mean day of death for animals that died were calculated. For statistical analysis, the final mortality of drug-treated and placebo-treated mice were compared by using the Fisher exact test and differences in the mean day of death were evaluated by the Mann–Whitney *U* rank test.

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