Synthesis, Antiproliferative, and Antiviral Activity of Certain 4-Substituted and 4,5-Disubstituted 7-[(1,3-Dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines

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The sodium salts of 4-chloro- and several 4-chloro-5-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidines were treated with [1,3-bis(benzyloxy)-2-propoxy]methyl chloride (**6**) to provide the corresponding 4-chloro- and 4-chloro-5-substituted-7-[[1,3-bis(benzyloxy)-2-propoxy]methyl]pyrrolo[2,3-*d*]pyrimidines (**7–11**). Debenzylation with boron trichloride at −78 °C furnished 4-chloro- and several 4-chloro-5-substituted-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines (**12–16**). Subsequent amination of **12–16** yielded the 4-amino-5-substituted-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines (**17–21**). Treatment of **14** with methylamine and **13** and **14** with ethylamine yielded the 4-(alkylamino)-5-halo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines (**22–24**). Treatment of **12–15** with hydroxylamine in refluxing 2-propanol yielded the 5-substituted-4-(hydroxyamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidines (**25–28**). Treatment of compound **12** with Pd/C under a hydrogen atmosphere has furnished the nebularine analogue **31**. The antiproliferative activity of compounds **17–28** and **31** was studied using L1210 cells in vitro. The 4-amino- and 4-(hydroxyamino)-5-halogenated derivatives (compounds **18–20**, **26–28**) inhibited cell growth. Although the effect of compounds **18–20** and **27** on final growth rate was pronounced (IC_{50} = 2.3, 0.7, 2.8, and 3.7 μM, respectively), cells underwent at least one doubling before cell division stopped. The remaining compounds were less cytotoxic, with IC_{50}'s > 30 μM for **21**, **23**, **26**, and **28**, whereas no inhibition of L1210 cell growth was observed with compounds **17**, **22**, **24**, **25**, and **31** at 100 μM. The antiviral activity of these compounds also was tested. Compounds **18–20** and **26–28** were active against human cytomegalovirus and herpes simplex type 1. The 4-amino derivatives (**18–20**) were more active than the 4-hydroxyamino derivatives (**26–28**), the 4-amino-5-bromo and 4-amino-5-iodo derivatives produced more than five log reductions in virus titer at concentrations of 10–100 μM. Although some cytotoxicity was observed at these concentrations, compound **19** was active against murine cytomegalovirus in vivo. At 5.6 mg/kg, 14/15 animals survived compared to 10/15 treated with 5.6 mg/kg of ganciclovir or 1/15 treated with placebo.

Synthesis of the potent antiviral agent 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir, also known as DHPG, BIOLF-62, BW B759U, and 2′-NDG) has been reported by several groups.[1-4] The compound is active against herpes simplex virus (HSV) types 1 and 2,[1a,2,5] varicella-zoster virus,[2b] Epstein–Barr virus,[1a,2b,5] and human cytomegalovirus (HCMV).[1a,2b,5] Although a number of nucleoside analogues such as vidarabine,[6] acyclovir,[7] (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU),[8] and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC)[9] are active against herpes viruses, ganciclovir is the most promising agent against HCMV.

The mode of antiviral action for ganciclovir against HSV and HCMV is similar to that of acyclovir in that both compounds are phosphorylated to a greater extent in virus-infected cells than in uninfected cells.[10] The triphosphates of these two nucleosides then act as potent and selective inhibitors of the virus-encoded DNA polymerases.[10] Ganciclovir differs from acyclovir in the manner of its initial phosphorylation to the monophosphate. Acyclovir is almost exclusively dependent upon the action of a virus-encoded pyrimidine deoxynucleoside kinase (thymidine kinase) for phosphorylation. Thus, it is relatively inactive against viruses such as HCMV which lack this enzyme.[11] In contrast, ganciclovir is phosphorylated by a number of different enzymes, including an HSV-encoded thymidine kinase,[2b] a cellular deoxyguanosine kinase,[12] and possibly another nucleoside kinase hypothesized to be HCMV-encoded.[13]

The success of ganciclovir as an antiviral agent has prompted intensive efforts by several groups to prepare and evaluate many structurally related acyclic analogues.[14] In our laboratory, the synthesis of pyrrolo[2,3-*d*]pyrimidine

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nucleosides as potential antitumor agents has been under active investigation for a number of years.[15] We also have been investigating this class of nucleosides as potential antiviral agents and have found that arabinosyl- and deoxyribosylpyrrolo[2,3-*d*]pyrimidines are active against HCMV and HSV-1.[16] In addition, we have found that certain 2,4- and 4,5-disubstituted pyrrolo[2,3-*d*]pyrimidine analogues of acyclovir containing the (2-hydroxyethoxy)-methyl (HEM) moiety have good activity against HCMV with little visual cytotoxicity.[17,18] These observations led us to initiate the synthesis of selected 4-substituted and 4,5-disubstituted pyrrolo[2,3-*d*]pyrimidine nucleosides in which the sugar was replaced by a (1,3-dihydroxy-2-propoxy)methyl (DHPM) group. We report herein the synthesis and evaluation of such compounds for antiproliferative and antiviral activity.

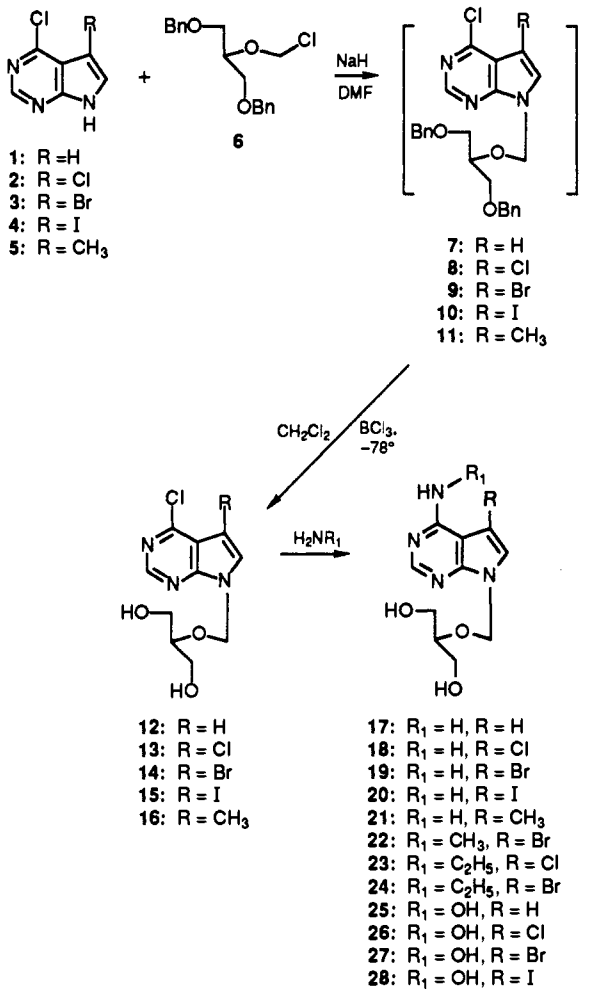
Chemistry

In the present work, we elected to use the sodium salt glycosylation method,[19] which was recently developed for the synthesis of various 2′-deoxynucleosides of several heterocycles including pyrrolo[2,3-*d*]pyrimidines. It was found that the sodium salt glycosylation method provided superior yields when compared to alternative glycosylation methods. The sodium salts of certain substituted pyrrolo[2,3-*d*]pyrimidines[17,18] were generated by the treatment of the free base with sodium hydride in dimethylformamide. These salts were then condensed with [1,3-bis-(benzyloxy)-2-propoxy]methyl chloride[1] (**6**) to give the corresponding blocked acyclic nucleosides (**7–11**) (Scheme I).

Although removal of the benzyl groups from purine and certain purine-like compounds has been reported to occur with BCl_3,[23] this method proved to be ineffective in this instance and resulted in intractable mixtures. On the assumption that the strongly acidic conditions generated upon the workup of these BCl_3 reactions were responsible for product decomposition, a Pd/C-catalyzed debenzylation was attempted. This method also failed and once again resulted in an intractable reaction mixture. We subsequently found that the benzyl groups could be successfully removed with boron trichloride/methylene chloride at −78 °C by using a modified literature preparation.[23] The cold (−78 °C) reaction mixture was neutralized *immediately* after methanol addition to furnish a good yield of the corresponding diols (**12–16**). The site of alkylation in **12–16** was confirmed to be at N-7 on the basis of ^{1}H NMR[21] and ultraviolet absorption[22] spectroscopic studies. The λ_{max} observed in the UV spectra of **12–16** showed no bathochromic shift relative to the λ_{max} observed in the UV spectrum of compounds **1–5**, which would have been indicative of alkylation on one of the pyrimidine ring nitrogens. This finding supported the N-7 assignment rather than the possibility of either N-1 or N-3 substitution. Further proof of alkylation at N-7 was provided by ^{1}H NMR spectroscopy. The multiplicity observed for the C-5 and C-6 protons appeared as two separate doublets for compound **12** and the C-6 proton appeared as a singlet for compounds **13–16**. If a proton resided at N-7, a more complicated splitting pattern would have been present.

Amination of 4-chloro-7-[(1,3-dihydroxy-2-propoxy)-methyl]pyrrolo[2,3-*d*]pyrimidine (**12**) was achieved with saturated methanolic ammonia in a sealed reaction vessel at 130 °C. Amination of compounds **13–16** was achieved in a similar fashion. Compounds **13** and **14** were also aminated with methylamine and ethylamine to determine if alkyl substitution on the 4-amino group would have an influence on biological activity. These compounds (**22–24**)

Scheme I[a]



[a] Bn= $CH_2C_6H_5$

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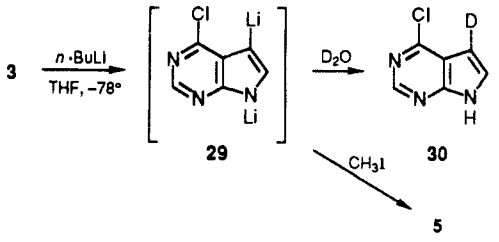
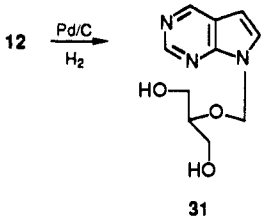
Scheme II



Scheme III

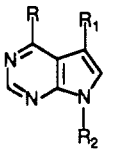


were obtained by heating the 4-chloro-5-halo compounds (**13** and **14**) in a sealed reaction vessel with methylamine (50% in H_2O) or ethylamine (70% in H_2O) at 135 °C. Finally, substitution of the C-4-amino group with an hydroxyl group was investigated in order to determine the effect upon biological activity. The hydroxyamino compound **25** was obtained by treating compound **12** in refluxing 2-propanol with hydroxylamine[21] (50% in H_2O). Compounds **18–20** were treated similarly to yield compounds **26–28**. Preliminary evaluation of these aminated compounds revealed that an halo substituent at the 5-position of the pyrrolo[2,3-*d*]pyrimidine system enhanced activity and selectivity toward certain virally infected cells. In an effort to ascertain whether the enhanced activity was due to the stereoelectronic effect or the increased lipophilicity of the halogen substituent, the 5-methyl compound (**21**) was synthesized. Unlike the electrophilic substitution at C-5 of the 4-chloropyrrolo[2,3-*d*]pyrimidine system to yield the corresponding 4-chloro-5-halo compounds (**2–4**),[20] the 5-methyl compound[24] (**5**) was obtained by an alternative method. Studies in our laboratories have revealed that treatment of **3** with *n*-butyllithium in THF at −78 °C resulted in a selective lithio–bromo exchange to yield the thermally unstable intermediate **29** (Scheme II). Quenching of **29** with D_2O produced a compound that contained deuterium at C-5. ^1H NMR evaluation of **30**[25] revealed that the spectral properties were identical with those of a known sample of **1** except for the absence of a signal corresponding to the C-5 proton and a decrease in the splitting multiplicity of C-6. It was envisioned that methylation of **29** would result in an alkylation at the 5-position. To this end **29** was generated and treated with an excess of MeI to afford **5**. Finally, the 4,5-unsubstituted compound **31** was prepared by treating the 4-chloro compound (**12**) with Pd/C at 50 psi of hydrogen (Scheme III). Compounds **17–28** and **31** were evaluated for antiproliferative activity against L1210 cells and antiviral activity against HCMV and HSV.

Biology

In Vitro Antiproliferative Testing. The potential of these 7-DHPM-pyrrolo[2,3-*d*]pyrimidines for antiproliferative cancer chemotherapy was evaluated by determining their ability to inhibit the growth of L1210 cells in vitro (Table I). The maximal growth inhibitory activity

Table I. Inhibition of L1210 Cell Growth in Vitro by 4,5-Disubstituted 7-[(1,3-Dihydroxy-2-propoxy)methyl]- and 7-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines



compound				initial screen: growth rate,[a] % of control	IC_{50},[b] μM
	substituent				
no.	R	R_1	R_2		
17	NH_2	H	DHPM[c]	106	-
18	NH_2	Cl	DHPM	6	2.3
19	NH_2	Br	DHPM	0	0.71
20	NH_2	I	DHPM	6	2.8
21	NH_2	CH_3	DHPM	73	>100
22	$NHCH_3$	Br	DHPM	100	-
23	NHC_2H_5	Cl	DHPM	78	>100
24	NHC_2H_5	Br	DHPM	95	-
25	NHOH	H	DHPM	100	-
26	NHOH	Cl	DHPM	41	75
27	NHOH	Br	DHPM	0	3.7
28	NHOH	I	DHPM	11	39
31	H	H	DHPM	100	-
Br-Tub[d,e]	NH_2	Br	ribose	9	4
d	NHOH	Br	ribose	10	25

[a] Final growth rate of L1210 cells in the presence of compound at 100 μM, determined as described in the text. [b] The concentration required to decrease the final growth rate of L1210 cells to half of the control rate. A dash indicates no significant growth inhibition at 100 μM. [c] Abbreviation used, DHPM: (1,3-dihydroxy-2-propoxy)methyl. [d] Prepared as described previously; see ref 21b. [e] 5-Bromotubercidin.

for this series of compounds was obtained for those having an NH_2 substituent in the 4-position (**18–20**). Conversion of the NH_2 to an NHOH (**26–28**) led to about a 10-fold decrease in activity, whereas derivatization to an alkylamino (**22–24**) virtually abolished activity. With either the NH_2 or the NHOH substituent, the activity was further modulated by variations of the 5-substituent. All three halogens investigated provided active compounds, with the ranking Br (**19** and **27**) > I (**20** and **28**) = Cl (**18** and **26**). Compounds having the 5-position unsubstituted (**17** and **25**) or having a 5-CH_3 (**21**) substituent were essentially inactive.

Two 5-bromo-7-ribosyl analogues prepared previously by us[21b] were studied for comparison with the new 5-bromo-7-DHPM compounds. Results with this limited series showed the same structure–activity relationship for the 4-substituent, namely that the 4-NH_2 derivative (bromotubercidin) was more strongly growth-inhibitory than was the NHOH compound (Table I). Both of these ribosyl nucleosides were less cytotoxic than the related nucleoside antibiotics toyocamycin and sangivamycin, which have 4-NH_2 and 5-CN or 5-$CONH_2$ substituents, respectively.[16,18,26] The surprising element in these results was that the ribosyl compounds were also somewhat less cytotoxic than their respective DHPM analogues (**19** and **27**).

The results reported in Table I are based on final growth rates. The initial effects of the 5-halogen DHPM analogues on the growth of L1210 cells were considerably less pronounced. For example, the 4-amino-5-bromo compound (**19**) at 0.7 μM caused a 50% decrease in the final growth rate, and at 10 μM it ultimately stopped cell division

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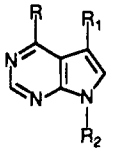
(25) Compound **30** was prepared by the method described for compound **5** by quenching **29** at −78 °C with D_2O instead of MeI.
(26) Gupta, P. K.; Daunert, S.; Nassiri, M. R.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med Chem.* **1989**, *32*, 402.

Table II. Antiviral Activity and Cytotoxicity of 4,5-Disubstituted 7-[(1,3-Dihydroxy-2-propoxy)methyl]- and 7-(β-D-Ribofuranosyl)pyrrolo[2,3-d]pyrimidines



compound	substituent			50 or 90% inhibitory concentration, μM						
				HCMV[a]		HSV-1[a]		cytotoxicity[b]		
no.	R	R_1	R_2	plaque	yield	plaque	yield	HFF	BSC	KB
17	NH_2	H	DHPM	>100[c,d]		>100		>100[d]	>100	
18	NH_2	Cl	DHPM	9.8[d]	8[d]	16	22[d]	>100[d]	>100	>100[d,e]
19	NH_2	Br	DHPM	1.6[d]	1.9[d]	2	17	>100[d]	>100[c]	>100[d,e]
20	NH_2	I	DHPM	3.1[d]	0.9	4		>100[d]		
21	NH_2	CH_3	DHPM	>100[d]	>100	>100		>100[d]		
22	$NHCH_3$	Br	DHPM	>100		>100		>100	>100	
23	NHC_2H_5	Cl	DHPM	>100		>100		>100	>100	
24	NHC_2H_5	Br	DHPM	>100		>100		>100	>100	
25	NHOH	H	DHPM	>100				>100		
26	NHOH	Cl	DHPM	56[d]	24	50		>100[d]		
27	NHOH	Br	DHPM	1.2[d]	10[d]	2.9	>100	>100[d]		320[d,e]
28	NHOH	I	DHPM	18[d]	3.2	30		>100[d]		
31	H	H	DHPM	>100[d]				>100[d]		
Br-Tub[f]	NH_2	Br	ribose	0.5[d]	3.4			4.5[d]		2.3[d]
g	NHOH	Br	ribose	5.6[d]	3.0[d]			>100		>100[d,e]
acyclovir				63	90	4[d]	7[d]	>100	>100	>100
ganciclovir (DHPG)				8.7[h]	1.8[d]	4.5	1.2[d]	>100[d]	>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 are reported 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] A greater than sign indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [d] Average concentration derived from two to five experiments. [e] Effect on RNA and protein synthesis only. The IC_{50} for effect on [^{3}H]dThd incorporation into DNA was 17, 2.4, 59, and 63 μM, respectively, for compounds **18**, **19**, **27**, and *g*. [f] Bromotubercidin, data also presented in ref 18 as compound **1n**; synthesis is in ref 21b. [g] Synthesis in ref 21b. [h] Average of 54 experiments.

completely. Nevertheless, cells treated with 10 μM compound **19** underwent more than two population doublings during the first 40 h of treatment.

In Vitro Antiviral Activity. The target compounds (**17–28**, **31**) as well as 5-bromotubercidin and its 4-NHOH analogue were evaluated for activity against HCMV and HSV-1. Cytotoxicity of each compound was determined visually in normal human diploid fibroblasts (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) by labeled-precursor uptake. Data in Table II show that activity against HCMV and HSV-1 paralleled antiproliferative activity (Table I). The 5-halogen compounds in both the 4-amino (**18–20**) and 4-hydroxyamino series (**26–28**) were active whereas the 5-unsubstituted (**17**,**25**,**31**) the 5-methyl (**21**), the 4-methylamino (**22**) and 4-ethylamino derivatives (**23**,**24**) were completely inactive. On the basis of plaque-reduction assays, the 5-bromo compounds (**19**,**27**) appeared to be the most active of all compounds tested, including acyclovir and ganciclovir. The corresponding ribosyl nucleosides also showed activity against HCMV which was of the same order of magnitude as that exhibited by the DHPM derivatives **19** and **27** (Table II).

In yield-reduction assays with HCMV, differences among the active compounds became more clear. Although IC_{90}'s presented in Table II appear somewhat anomalous when compared to IC_{50}'s for plaque reduction, examination of dose–response curves (not presented) for concentrations required to give multiple log reductions in HCMV titer were more revealing. For example, in the 4-amino series, the 5-halogen analogues produced five log reductions (100,000-fold reductions) in virus titer at 100, 10, and 32 μM, for compounds **18**, **19**, and **20**, respectively. Compounds in the 4-hydroxylamino series were less active; the 5-bromo (**27**) and 5-iodo (**28**) compounds produced five log reductions at 100 μM, whereas the 5-chloro compound (**26**) produced only a two to three log reduction at 100 μM. The capacity of the 4-amino compounds (**18–20**) to produce at least five log reductions in HCMV titer is directly related to the DHPM group in the 7-position: Analogues comprised of the same heterocycles but with a (2-hydroxyethoxy)methyl (HEM) group in the 7-position reduced HCMV titer by only 2–3 log units.[18] Interestingly, the two ribosyl compounds also produced five log reductions in HCMV titer although the activity of bromotubercidin was linked to cytotoxicity.

The activity of compound **19** against other herpes viruses was explored in more detail to determine the suitability of evaluating the compound in vivo. Data in Table III show that the compound was active against HCMV, MCMV, HSV-1, and HSV-2 at micromolar concentrations. Murine CMV had the lowest IC_{50}, and thus was the most sensitive of the viruses tested. Little or no visual cytotoxicity was observed in cells used to propagate the viruses.

Visual examination of uninfected cells used to test other compounds also revealed little evidence of cytotoxicity. This is in sharp contrast with results on final growth rates, which show significant inhibition (Table I). An examination of the effects of selected compounds on labeled precursor incorporation into DNA, RNA, and protein in KB cells showed no inhibition of RNA and protein synthesis but significant inhibition of DNA synthesis. This was particularly true for compound **19** (Table II, footnote *e*). To determine if this inhibition was related to that seen with L1210 cells, a more stringent test was performed with KB cells. Plating-efficiency experiments were performed and gave an IC_{50} of 0.3 μM for cells to plate and grow in clones in the presence of compound **19**. There was, therefore, a correlation between effects of the compound

Table III. Activity of 4-Amino-5-bromo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,-3-*d*]pyrimidine against Selected Herpes Viruses

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	1.6	>100
HCMV[f]	human foreskin fibroblasts	0.6	>158
MCMV	mouse embryo fibroblasts	0.03	
HSV-1	human foreskin fibroblasts	0.2	
HSV-1[e]	monkey kidney cells	2.0	>100
HSV-1	mouse embryo fibroblasts	1.9	>158
HSV-2	human foreskin fibroblasts	0.3	
HSV-2	mouse embryo fibroblasts	1.9	

[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 Evaluation in the text. [e] Data from Table II, Towne strain of HCMV. [f] AD169 strain of HCMV.

on final growth rate, plating efficiency, and DNA synthesis. These effects, however, were much more pronounced than effects on initial growth rates, RNA and protein synthesis. (The biochemical basis for the activity of this compound in uninfected cells will be reported in more detail elsewhere.) Nonetheless, because both L1210 cells and KB cells grew and underwent at least two doublings in the presence of a concentration of **19** (10 μM) which produced over a 100,000-fold decrease in virus titer, evaluation in an animal model was initiated.

In Vivo Antiviral Activity. Compound **19** was evaluated using a murine model for cytomegalovirus infection.[27] Animals were treated intrapertitoneally twice daily for five days beginning 6, 24, or 48 h after infection with MCMV. Table IV compares the activity of compound **19** to that of ganciclovir. At doses of 5.6 mg/kg, compound **19** produced highly significant decreases in MCMV-induced mortality even when administered 48 h after the virus. Ganciclovir also produced significant decreases in mortality but was less efficacious than **19** at this dose. Ganciclovir was slightly more effective at 16.7 mg/kg, but surprisingly, compound **19** was less effective although it did produce significant extensions of life span at 16.7 and 50 mg/kg (MDD column, Table IV). Because there was only one death (at 16.7 mg/kg) in 60 animals in toxicity controls, we conclude that compound **19** was not overtly toxic. The inverse dose–response effect noted between 5.6 and 50 mg/kg suggests, however, that when **19** was administered to virally immunocompromised animals, higher doses caused toxicity which was not seen in uninfected animals. This may have been a manifestation of the cytotoxicity noted in vitro (Tables I and II). Nevertheless, the high cure rate observed at 5.6 mg/kg suggests that there is significant separation between antiviral activity and toxicity.

Discussion

The synthesis of 4-amino-5-halo-7-DHPM-pyrrolo[2,3-*d*]pyrimidines reported here and the demonstration of their antiviral activity and anticancer potential create several new leads. Of particular interest is 5-bromo derivative **19**, which represents the first acyclic analogue of adenosine to show significant antiviral activity both in vitro and in vivo. It is also the first selective antiviral pyrrolo[2,3-*d*]pyrimidine.

The antiviral activity and cytotoxicity of compounds **18–20** is in sharp contrast to the results reported previously from this laboratory for other 4-amino-5-substituted-7-DHPM-pyrrolo[2,3-*d*]pyrimidines.[26] DHPM analogues of the highly cytotoxic nucleoside antibiotics toyocamycin, sangivamycin, and several related compounds were prepared and found to be virtually inactive as antiviral or antiproliferative agents. Thus, with the exception of the antiviral activity of the 5-$CSNH_2$ analogue, it appears that the lack of activity of the 5-CN, 5-$CONH_2$ and other derivatives of 4-amino-7-DHPM-pyrrolo[2,3-*d*]pyrimidine reported in ref 26 was comparable to results with the 5-CH_3 derivative in the present study.

The differences in cytotoxic activity among these compounds might be accounted for by the substrate specificity of activating enzymes such as kinases and/or the sensitivities of the relevant biochemical targets, which are unknown at this time. For simplicity, the following integrated picture of the structure–activity requirements for these compounds is hypothesized in terms of kinases. Since the 4-NH_2- and 4-NHOH-7-DHPM-pyrrolo[2,3-*d*]pyrimidines with 5-halo substituents are cytotoxic (Table I), it appears that they can be phosphorylated, possibly by a cellular deoxyadenosine/deoxyguanosine kinase.[28] In contrast, 7-DHPM analogues of toyocamycin and sangivamycin are inactive,[26] presumably because the 5-CN and 5-$CONH_2$ substituent prevent their being substrates for this or any cellular kinase. In the 7-ribosyl series, activation of the 4-NH_2- and 4-NHOH-pyrrolo[2,3-*d*]pyrimidines appears to involve a different kinase with different substrate specificities. In this case, the 4-NH_2 compounds with 5-CN or 5-$CONH_2$ (toyocamycin or sangivamycin, respectively) are readily phosphorylated by adenosine kinase,[29] and are extremely cytotoxic, with IC_{50}'s = 3 and 4 nM.[26] On the other hand, the 4-NH_2- and 4-NHOH-5-Br-7-ribosyl derivatives in Table I appear to be less readily phosphorylated since they are much less cytotoxic. Thus it appears that the different kinase(s) involved in phosphorylation of the 7-ribosyl vs the 7-DHPM derivatives possess opposite substrate specificities regarding the 5-CN and 5-$CONH_2$ vs 5-halo derivatives. In summary, the 5-halo substitution, particularly 5-Br (**19**), confers on the 4-amino-7-DHPM-pyrrolo[2,3-*d*]pyrimidines the capacity to act as substrates for an activating enzyme and/or to interact with a biochemical target, which is not shared by the compounds having 5-substituents such as CN or $CONH_2$.

The biological activity of the 5-halo compounds in the DHPM series (Table I and II) is further supported by the antiviral activity of directly related analogues in which the 7-position is substituted by an HEM substituent as opposed to the DHPM substituent. In that series, the 5-bromo compound also was the most active followed by the iodo and chloro derivatives.[18] Thus, in both series, the presence of a 5-halogen substituent conferred activity upon the molecule which was not shared by analogues with other 5-substituents such as CN and $CONH_2$.[17,26,30]

The antiviral activity of these compounds, particularly the potent activity against HCMV and MCMV, may be

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Table IV. Effect of Treatment with 4-Amino-5-bromo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine or Ganciclovir on the Mortality of Mice Inoculated with MCMV

treatment[a]	mortality				
	number	percent	*P* value	MDD[b]	*P* value
control	12/15	80		4.6	
placebo at 24 h	14/15	93	NS[c]	4.4	NS
compound 19					
50 mg/kg at 6 h	9/15	60	NS	10.8	<0.001
50 mg/kg at 24 h	14/15	93	NS	8.8	<0.001
50 mg/kg at 48 h	13/15	87	NS	7.5	<0.01
50 mg/kg—toxicity[d]	0/15	0			
16.7 mg/kg at 6 h	9/15	60	NS	8.7	<0.001
16.7 mg/kg at 24 h	12/15	80	NS	7.1	<0.01
16.7 mg/kg at 48 h	13/15	87	NS	8.4	<0.001
16.7 mg/kg—toxicity[d]	1/15	7		5.0	
5.6 mg/kg at 6 h	1/15	7	<0.001	8.0	NS
5.6 mg/kg at 24 h	3/15	20	<0.001	6.3	<0.01
5.6 mg/kg at 48 h	6/15	40	<0.01	5.5	NS
5.6 mg/kg—toxicity[d]	0/15	0			
1.9 mg/kg at 6 h	5/15	33	0.001	6.2	<0.01
1.9 mg/kg at 24 h	9/15	60	NS	4.8	NS
1.9 mg/kg at 48 h	10/15	67	NS	4.8	NS
1.9 mg/kg—toxicity[d]	0/15	0			
ganciclovir					
16.7 mg/kg at 6 h	4/15	27	<0.001	3.8	NS
16.7 mg/kg at 24 h	7/15	47	<0.01	5.3	NS
16.7 mg/kg at 48 h	8/15	53	<0.05	5.5	<0.05
5.6 mg/kg at 6 h	5/15	33	0.001	4.2	NS
5.6 mg/kg at 24 h	7/15	47	<0.01	5.3	NS
5.6 mg/kg at 48 h	10/15	67	NS	5.5	<0.01
1.9 mg/kg at 6 h	5/15	33	0.001	5.4	NS
1.9 mg/kg at 24 h	12/15	80	NS	4.9	NS
1.9 mg/kg at 48 h	11/15	73	NS	4.9	NS
0.6 mg/kg at 6 h	14/15	93	NS	4.6	NS
0.6 mg/kg at 24 h	12/15	80	NS	5.4	0.01
0.6 mg/kg at 48 h	14/15	93	NS	5.3	0.01

[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.

a consequence of preferential phosphorylation by a virus-induced or -specified enzyme. It is well established that in HSV-1-infected cells a viral deoxypyrimidine nucleoside kinase phosphorylates compounds such as acyclovir and ganciclovir to their monophosphates.[2b,11] However, HCMV does not encode a nucleoside kinase with substrate specificity similar to the one encoded by HSV;[31,32] therefore, the activity of acyclovir against HCMV may be a consequence of the action of a cellular deoxyguanosine kinase.[33] In contrast, studies with a ganciclovir-resistant mutant of HCMV do raise the possibility that HCMV encodes an as yet unidentified nucleoside kinase.[13] Our results (ref 18 herein and additional unpublished data) show that 4-NH_2-5-halogen-7-DHPM-pyrrolo[2,3-*d*]pyrimidines are 100–1,000-fold more active against HCMV as measured in a virus yield reduction assay, than are the corresponding 7-HEM-pyrrolo[2,3-*d*]pyrimidines. These results are consistent with the possible existence of a distinct, HCMV-encoded nucleoside kinase. This hypothesis is consistent with the observation that HCMV shows a greater sensitivity to ganciclovir (with a DHPM moiety) than to acyclovir (with HEM), whereas HSV-1 shows approximately equal sensitivity to both drugs. (Table II).

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 an IBM WP 270-SY or at 300 MHz with an IBM AM-300, or at 360 MHz with an IBM WM-360. The chemical shift values are expressed in δ values (part per million) relative to the standard chemical shift of DMSO-d_6. Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer. Elemental analyses were performed by M-H-W laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was performed on silica gel GHLF-254 plates (Merck Reagents). E. Merck Silica gel (230–400 mesh) was used for flash column chromatography. Detection of components on thin-layer chromatography was made by UV light (254 nm). Evaporations were carried out under reduced pressure (water aspiration) with the bath temperature below 50 °C unless specified otherwise.

Alkylation with 6. In a typical reaction, 25 mmol of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (1) was dissolved in dry DMF (40 mL) and NaH (1.5 equiv, 1.5 g, 60% oil dispersion) was added. This solution was stirred until no further H_2 evolution was detected (20 min) and [1,3-bis(benzyloxy)-2-propoxy]methyl chloride (6, 1.3 equiv, 10.4 g) was added dropwise. After complete addition, the solution was stirred for an additional 40 min and water (75 mL) was added. The pH of the solution was adjusted to 7 with glacial acetic acid. The aqueous solution was extracted with EtOAc (1 × 100 mL, 2 × 50 mL); the EtOAc extracts were combined and washed with water (3 × 50 mL). The EtOAc extracts were then dried over $MgSO_4$ (anhydrous), filtered, and reduced in vacuo at 40 °C to yield a yellow oil. This oil (7) was used without further purification for the debenzylation reaction.

4-Chloro-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (12). To a 1-L flask was added 7 (24.38 g, 0.056 mol) and dry CH_2Cl_2 (550 mL) and the reaction mixture was cooled to −78 °C under an argon atmosphere. BCl_3 (1 M, 210 mL) was then added dropwise while the temperature was maintained below −70 °C (internal). After complete addition, the solution was stirred for 15 min, MeOH (300 mL, 0 °C) was added, and the cold solution was neutralized *immediately* (pH = 7) with

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concentrated NH_4OH. The solution was then allowed to warm to room temperature, during which time a white precipitate formed. The white solid was collected by filtration, the solid was discarded, and the filtrate was reduced to a yellow oil. The oil was then suspended in Et_2O and MeOH was carefully added until the oil dissolved and a off-white solid formed. This suspension was then refrigerated (0 °C) for 12 h, after which time the solid was collected by filtration and recrystallized from Et_2O to yield 5.60 g (39%) of **12**: mp 113–114 °C; ^1H NMR δ 3.18–3.56 (m, 5), 4.67 (t, 2, exchanges with D_2O, OH), 5.73 (s, 2, C-1′), 6.68 (d, 1, C-5), 7.89 (d, 1, C-6), 8.67 (s, 1, C-2). Anal. ($C_{10}H_{12}ClN_3O_3$) C, H, N.

4,5-Dichloro-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (13). Compound **13** was prepared from **8** (12.95 g, 27.4 mmol) by the method described for **12** to yield 2.72 g (34%): mp 142.5–143 °C; ^1H NMR δ 3.22–3.55 (m, 5), 4.58 (t, 2, exchanges with D_2O, OH), 5.74 (s, 2, C-1′), 8.10 (s, 1, C-6), 8.72 (s, 1, C-2). Anal. ($C_{10}H_{11}Cl_2N_3O_3$) C, H, N.

5-Bromo-4-chloro-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (14). Compound **14** was prepared from **9** (9.05 g, 17.5 mmol) by the method described for **12** to yield 2.42 g (41%) of **14**: mp 152–152.5 °C; ^1H NMR δ 3.23–3.54 (m, 5), 4.58 (t, 2, exchanges with D_2O, OH), 5.74 (s, 2, C-1′), 8.12 (s, 1, C-6), 8.70 (s, 1, C-2). Anal. ($C_{10}H_{11}BrClN_3O_3$) C, H, N.

4-Chloro-5-iodo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (15). Compound **15** was prepared from **10** (14.18 g, 25.2 mmol) by the method described for **12** to yield 3.67 g (38%): mp 155–156 °C; ^1H NMR δ 3.22–3.55 (m, 5), 4.57 (t, 2, exchanges with D_2O, OH), 5.72 (s, 2, C-1′), 8.10 (s, 1, C-6), 8.67 (s, 1, C-2). Anal. ($C_{10}H_{11}ClIN_3O_3$) C, H, N.

4-Chloro-5-methyl-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (16). Compound **16** was prepared from **11** (7.23 g, 16.0 mmol) by the method described for **12** to yield 1.92 (44%) of **16**: mp 119.5–120 °C; ^1H NMR δ 2.41 (d, 3, CH_3), 3.23–3.49 (m, 5), 4.57 (t, 2, exchanges with D_2O, OH), 5.69 (s, 2, C-1′), 7.60 (d, 1, C-6), 8.59 (s, 1, C-2). Anal. ($C_{11}H_{14}ClN_3O_3$) C, H, N.

4-Amino-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (17). Compound **12** (0.41 g, 1.6 mmol) was suspended in saturated methanolic ammonia (20 mL) in a steel reaction vessel. The vessel was then heated at 130 °C for 9 h, after which time the vessel was cooled and the solvent was removed in vacuo at 40 °C. The resulting solid was recrystallized from methanol to yield 0.25 g (66%) of **17**: mp 202–203 °C; ^1H NMR δ 3.15–3.51 (m, 5); 4.59 (t, 2, exchanges with D_2O, OH), 5.58 (s, 2, C-1′), 6.56 (d, 1, C-5), 7.02 (bs, 2, exchanges with D_2O, NH_2), 7.23 (d, 1, C-6), 8.06 (s, 1, C-2). Anal. ($C_{10}H_{14}N_4O_3$) C, H, N.

4-Amino-5-chloro-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (18). Compound **18** was prepared from **13** (0.48 g, 1.6 mmol) by the method described for **17** to yield 0.28 g (61%) of **18**: mp 175–176.5 °C; ^1H NMR δ 3.22–3.50 (m, 5), 4.58 (t, 2, exchanges with D_2O, OH), 5.57 (s, 2, C-1′), 6.88 (b s, 2, exchanges with D_2O, NH_2), 7.47 (s, 1, C-6), 8.11 (s, 1, C-2). Anal. ($C_{10}H_{13}ClN_4O_3$) C, H, N.

4-Amino-5-bromo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (19). Compound **19** was prepared from **14** (3.43 g, 10.2 mmol) by the method described for **17** to yield 1.51 g (47%) of **19**: mp 171–172 °C; ^1H NMR δ 3.15–3.50 (m, 5), 4.59 (t, 2, exchanges with D_2O, OH), 5.57 (s, 2, C-1′), 6.80 (bs, 2, exchanges with D_2O, NH_2), 7.53 (s, 1, C-6), 8.11 (s, 1, C-2). Anal. ($C_{10}H_{13}BrN_4O_3$) C, H, N.

4-Amino-5-iodo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (20). Compound **20** was prepared from **15** (0.41 g, 1.1 mmol) by the method described for **17** to yield 0.20 g (51%) of **20**: mp 177–177.5 °C; ^1H NMR δ 3.22–3.52 (m, 5), 4.58 (t, 2, exchanges with D_2O, OH), 5.57 (s, 2, C-1′), 6.66 (b s, 2, exchanges with D_2O, NH_2), 7.55 (s, 1, C-6), 8.11 (s, 1, C-2). Anal. ($C_{10}H_{13}IN_4O_3$) C, H, N.

4-Amino-5-methyl-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (21). Compound **21** was prepared from **16** (1.37 g, 5.04 mmol) by the method described for **17** to yield 0.73 g (57%) of **21**: mp 175–176 °C; ^1H NMR δ 2.33 (s, 3, CH_3), 3.23–3.49 (m, 5), 4.56 (t, 2, exchanges with D_2O, OH), 5.51 (s, 2, C-1′), 6.57 (bs, 2, exchanges with D_2O, NH_2), 6.98 (s, 1, C-6), 8.02 (s, 1, C-2). Anal. ($C_{11}H_{16}N_4O_3$) C, H, N.

5-Bromo-4-(methylamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (22). Compound **14** (0.84 g, 2.5 mmol) was suspended in methylamine (50% in H_2O, 15 mL) in a steel vessel. The vessel was sealed and heated at 135 °C for 90 min, after which time the vessel was cooled and the solvent was removed in vacuo to yield a yellow oil. This oil was dissolved in a small amount of MeOH and the compound was crystallized by the addition of $CHCl_3$ to yield 0.31 g (38%) of **22**: mp 137.5–139 °C; ^1H NMR δ 2.98 (d, 3, CH_3), 3.23–3.51 (m, 5), 4.58 (t, 2, exchanges with D_2O, OH), 5.58 (s, 2, C-1), 6.64 (q, 1, exchanges with D_2O, NH), 7.51 (s, 1, C-6), 8.21 (s, 1, C-2). Anal. ($C_{11}H_{15}BrN_4O_3$) C, H, N.

5-Chloro-4-(ethylamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (23). Compound **23** was prepared from **13** (0.52 g, 0.18 mmol) by the method described for **22** using ethylamine (70% in H_2O, 15 mL) to yield 0.26 g (48%) of **23**: mp 100–101 °C; ^1H NMR δ 1.17 (t, 3, CH_3), 3.22–3.55 (m, 7), 4.58 (t, 2, exchanges with D_2O, OH), 5.57 (s, 2, C-1′), 6.75 (t, 1, exchanges with D_2O, NH), 7.46 (s, 1, C-6), 8.19 (s, 1, C-2). Anal. ($C_{12}H_{17}ClN_4O_3 \cdot 0.25H_2O$) C, H, N.

5-Bromo-4-(ethylamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (24). Compound **24** was prepared from **14** (0.55 g, 1.6 mmol) by the method described for **22** using ethylamine (70% in H_2O, 15 mL) to yield 0.21 g (38%) of **24**: mp 108–109 °C; ^1H NMR δ 1.18 (t, 3, CH_3), 3.24–3.56 (m, 7), 4.58 (t, 2, exchanges with D_2O, OH), 5.58 (s, 2, C-1′), 6.59 (t, 1, exchanges with D_2O, NH), 7.51 (s, 1, C-6), 8.19 (s, 1, C-2). Anal. ($C_{12}H_{17}BrN_4O_3$) C, H, N.

4-(Hydroxyamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (25). Compound **12** (0.69 g, 2.68 mmol) was dissolved in 2-propanol (35 mL) and hydroxylamine (50% in H_2O, 1 mL) was added. This solution was heated at reflux until no starting material was detected by TLC (7 h). The solvent was then removed in vacuo to yield a clear, colorless oil that was crystallized from MeOH to yield 0.40 g (59%) of **25**: mp 153–154.5 °C; ^1H NMR δ 3.22–3.53 (m, 5), 4.57 (bs, 2, exchanges with D_2O, OH), 5.59 (s, 2, C-1′), 6.49 (bs, 1, C-5), 7.16 (s, 1, C-6), 7.93 (b s, 1, C-2), 9.23 (s, 1, exchanges with D_2O, NOH), 10.28 (bs, 1, exchanges with D_2O, NH). Anal. ($C_{10}H_{14}N_4O_4$) C, H, N.

5-Chloro-4-(hydroxyamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (26). Compound **26** was prepared from **13** (0.55 g, 1.9 mmol) by the method described for **25** to yield 0.30 g (59%) of **26**: mp 178–179 °C dec; ^1H NMR δ 3.22–3.50 (m, 5), 4.56 (t, 2, exchanges with D_2O, OH), 5.43 (s, 2, C-1′), 7.13 (s, 1, C-6), 7.46 (d, 1, C-2), 9.68 (s, 1, exchanges with D_2O, NOH), 10.85 (d, 1, exchanges with D_2O, NH). Anal. ($C_{10}H_{13}ClN_4O_4$) C, H, N.

5-Bromo-4-(hydroxyamino)-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (27). Compound **27** was prepared from **14** (0.70 g, 2.1 mmol) by the method described for **25** to yield 0.42 g (61%) of **27**: mp 180–181 °C dec; ^1H NMR δ 3.23–3.49 (m, 5), 4.56 (t, 2, exchanges with D_2O, OH), 5.43 (s, 2, C-1′), 7.16 (s, 1, C-6), 7.45 (d, 1, C-2), 9.70 (s, 1, exchanges with D_2O, NOH), 10.84 (d, 1, exchanges with D_2O, NH). Anal. ($C_{10}H_{13}BrN_4O_4$) C, H, N.

4-(Hydroxyamino)-5-iodo-7-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (28). Compound **28** was prepared from **15** (0.44 g, 1.2 mmol) by the method described for **25** to yield 0.21 g (49%) of **28**: mp 166–167 °C; ^1H NMR δ 3.22–3.50 (m, 5), 4.56 (t, 2, exchanges with D_2O, OH), 5.43 (s, 2, C-1′), 7.19 (s, 1, C-6), 7.45 (d, 1, C-2), 9.70 (s, 1, exchanges with D_2O, NOH), 10.81 (d, 1, exchanges with D_2O, NH). Anal. ($C_{10}H_{13}IN_4O_4 \cdot 0.25MeOH$) C, H, N.

4-Chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (4). Compound **1** (7.30 g, 47.5 mmol) was suspended in dry CH_2Cl_2 (400 mL), and *N*-iodosuccinimide (11.8 g, 52.5 mmol) was added. This suspension was stirred at room temperature for 30 min, during which time a white precipitate formed. The solid was then filtered and recrystallized from MeOH to yield 10.61 g (80%) of **4**: mp 196–199 °C dec; ^1H NMR: δ 7.93 (s, 1, C-6); 8.58 (s, 1, C-2); 12.9 (bs, 1, N-7). Anal. ($C_6H_3ClIN_3$) C, H, N.

4-Chloro-5-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (5).[24] Compound **3** (4.86 g, 25.5 mmol) was dissolved in dry THF (200 mL) and cooled to −78 °C under an argon atmosphere. *n*-BuLi (1.6 M in hexanes, 57.6 mmol) was then added dropwise. After complete addition, the solution was stirred for 40 min and MeI (2.5 mL, 40 mmol) was added. The solution was then allowed

to slowly reach room temperature and water (2 mL) was added. The solvent was then removed in vacuo at 40 °C to yield a yellow slurry. This slurry was dissolved in EtOAc (150 mL) and washed with H_2O (2 × 75 mL). The organic layer was then decanted, dried over $MgSO_4$ (anhydrous), and filtered, and the solvent was removed in vacuo to yield an off-white powder that was recrystallized from MeOH to yield 2.94 g (69%) of 5: mp 221–222 °C (lit. mp 227–228 °C); ^1H NMR δ 2.40 (s, 3, CH_3), 7.42 (s, 1, C-6), 8.49 (s, 1, C-2), 12.25 (bs, 1, N-7).

7-[(1,3-Dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyrimidine (31). Compound 12 (1.13 g, 4.38 mmol), EtOH (50 mL), concentrated NH_4OH (2.5 mL), and Pd/C (5%, 0.51 g) were added to a flask and 12 was hydrogenated at 40 psi for 4 h. The suspension was then filtered through Celite and the solvent was removed in vacuo at 40 °C to yield a clear, colorless oil. This oil was dissolved in MeOH and Na_2SO_4 (anhydrous) was added. This suspension was reduced to a white powder and applied to the top of a silica column (40 g of silica, 1.5 in. i.d.) packed in EtOAc. The product was then eluted with 10% MeOH in EtOAc and all UV-absorbing fractions were combined and concentrated to furnish a clear, colorless oil. This oil was dissolved in a small amount of acetone and crystallized by the careful addition of hexanes to yield 0.50 g (51%) of 31: mp 84.5–85 °C; ^1H NMR δ 3.23–3.54 (m, 5), 4.57 (t, 2, exchanges with D_2O, OH), 5.75 (s, 2, C-1′), 6.67 (dd, 1, C-5), 7.73 (d, 1, C-6), 8.81 (s, 1), 9.02 (s, 1). Anal. ($C_{10}H_{13}N_3O_3$) C, H, N.

Biological Evaluations. In Vitro Antiproliferative Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[34] 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. Growth rate was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture as a percent of the slope for the control culture. This parameter was determined experimentally by calculating the ratio of the population doubling time of control cells to the population doubling time of treated cells. When the growth rate changed during the experiment, the rate used was the final rate attained at the end of the 4-day period. For example, in the presence of many of the compounds reported here, the cells initially grew and then stopped after 1–2 days. Such results were recorded as growth rate = 0. The IC_{50} was defined as the concentration required to reduce the growth rate to 50% of that of the control.

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 Hank salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[16] 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.[16]

(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.[16] HSV-1 plaque reduction experiments were performed using 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.[16]

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

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

Additional in Vitro Antiviral Evaluation. The susceptibility of MCMV (Smith strain) and HCMV (AD 169 strain) to compound 19 and ganciclovir was explored in more detail. Primary mouse embryo fibroblasts (MEF) and low-passage 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 6-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 using Dose–Effect Analysis software program (Elsevier-Biosoft, Cambridge, U.K.).

Experimental MCMV Infection. Three 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 19 or ganciclovir 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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Synthesis and Antiviral Activity of Metabolites of Rimantadine

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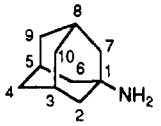
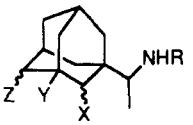
The hydroxy metabolites of rimantadine (3–5) were synthesized and compared to amantadine (1) and rimantadine (2) for their ability to inhibit the replication of influenza viruses in vitro. All three metabolites were inhibitory to wild-type influenza A viruses (H3N2 and H1N1). In particular, 2-hydroxyrimantadine (3) showed similar activity to amantadine, but the 3- and 4-hydroxy metabolites (4 and 5, respectively), both of which are found in rimantadine-treated patients, showed only modest inhibitory activity. A rimantadine-resistant isolate of influenza A virus exhibited cross-resistance to amantadine and to each of the metabolites 3–5. None of the compounds were effective against influenza B virus.

Amantadine (1) and rimantadine (2) are adamantane derivatives that show similar efficacy when administered orally to patients for the prophylaxis and treatment of influenza, although there are fewer side effects associated with the use of rimantadine.[1,2] However, peak plasma levels of rimantadine are 2–3-fold less than those achieved with amantadine when given at the same dose.[3] It has been proposed that the equivalence in activity in treated patients may be the consequence of selective concentration of rimantadine in the tissues of the respiratory system.[3] Alternatively, or in addition, the superior intrinsic antiviral activity of rimantadine compared to amantadine may be important.[4]



1



2: R = X = Y = Z = H
3: R = Y = Z = H, X = OH (α, β)
4: R = X = Z = H, Y = OH
5: R = X = Y = H, Z = OH (α, β)
6: R = Ac, X = Y = Z = H
7: R = Ac, X = Z = H, Y = Br

In treated patients, amantadine is, for the most part, excreted without metabolism.[5] In contrast, rimantadine is extensively metabolized by hydroxylation before excretion in the urine.[3,6] We were interested in synthesizing each of the hydroxy metabolites (3–5) of rimantadine and to determine whether they exhibited any antiviral activity. The presence in vivo of a metabolite of rimantadine with significant inhibitory activity against influenza A virus could contribute to the observed therapeutic efficacy of the parent compound.

Chemistry

The synthesis of 2-, 3-, and 4-hydroxyrimantadines 3, 4, and 5, respectively, followed a general route. Reduction of the 1-carboxyadamantanones 8[7] and 10 with sodium borohydride gave the hydroxy acids 11 and 12 as diastereomeric mixtures. It should be noted that although 1-carboxy-4-adamantanone (10) had been prepared by the Koch carboxylation of adamantanone[8] and by the oxidation of 1-carboxyadamantane,[9] we found it more expedient to prepare 10 by the ruthenium tetroxide oxidative degradation of 1-phenyl-4-adamantanone (19).[10] Treatment of the hydroxy acids 9,[11] 11, and 12 with a large excess of methyllithium afforded the methyl ketones 13, 14, and 15, from which the oximes 16, 17, and 18 were prepared with hydroxylamine. Reduction of the oximes 16 and 18 with lithium aluminum hydride gave the amines 3 and 5. To

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