Synthesis of Non-nucleoside Analogs of Toyocamycin, Sangivamycin, and Thiosangivamycin: The Effect of Certain 4- and 4,6-Substituents on the Antiviral Activity of Pyrrolo[2,3-*d*]pyrimidines

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A number of 4-substituted 7-(ethoxymethyl)- and 7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile and -5-thiocarboxamide derivatives and several 7-substituted 4,6-diaminopyrrolo[2,3-*d*]pyrimidine-5-carbonitrile, -5-carboxamide, and -5-thiocarboxamide analogs related to the nucleoside antibiotics toyocamycin and sangivamycin were prepared and tested for activity against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1). Biologically, modifications at the 4-position were not well tolerated in cell culture, and in almost all cases no activity against HCMV or HSV-1 was observed. Furthermore, none of the compounds inhibited the growth of L1210 murine leukemic cells *in vitro*. In sharp contrast to the 4-substituted compounds, all of the 4,6-diamino 5-nitrile and the 5-thioamide analogs were active against HCMV, whereas the 5-carboxamides were inactive. The corresponding 4-amino 6-methylamino and 6-dimethylamino 5-nitrile analogs were inactive against HCMV, establishing that an amino group at both C-4 and C-6 is a likely requirement for antiviral activity. Overall, our results demonstrate that an amino group at C-4 and a thioamide moiety at C-5 of a 7-substituted pyrrolo[2,3-*d*]pyrimidine are essential for activity against HCMV, whereas a 4,6-diamino analog does not necessarily require a thioamide group at C-5 for activity against HCMV.

Introduction

As part of a study examining the potential of non-nucleoside analogs of the nucleoside antibiotics toyocamycin and sangivamycin as antiviral agents, we previously described the synthesis, cytotoxicity, and antiviral activity of certain 7-substituted 4-aminopyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile, -5-carboxamide, and -5-thiocarboxamide analogs.[1] The study concentrated on the identification of new compounds to treat infections caused by human cytomegalovirus (HCMV) because of the problems associated with use of the clinically approved anti-HCMV agents ganciclovir (GCV) and foscarnet (PFA).[2−5] The results established that the thiosangivamycin analogs, which bear a $CSNH_2$ moiety at C-5 of the pyrrolopyrimidine, have selective activity against HCMV without the requirement for activation to an active metabolite by phosphorylation of a side chain hydroxyl group residing at the N-7 position.

To determine whether the 4-amino functionality is necessary for antiviral activity and to extend the structure−activity relationships, the synthesis, cytotoxicity, and antiviral activity of 4-substituted and 4,6-disubstituted 5-nitrile, 5-carboxamide, and 5-thiocarboxamide non-nucleoside analogs related to nucleosides toyocamycin, sangivamycin, and thiosangivamycin are described in this report.

Chemistry

The synthetic route used to prepare the C-4-modified non-nucleoside analogs **2a**,**b**−**20b** is illustrated in Scheme 1. Because of the good separation of antiviral activity from toxicity with the ether-substituted compounds previously described,[1] two of the more active 7-ether substituents, namely, ethoxymethyl (EM) and (methoxyethoxy)methyl (MEM) substituents, have been chosen for this study. Aqueous diazotization of the exocyclic amino group of 4-amino-7-(ethoxymethyl)-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**1a**)[1] and 4-amino-7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**1b**)[1] was accomplished with nitrous acid to afford the 5-cyano 7-substituted pyrrolo[2,3-*d*]pyrimidin-4-ones **2a**,**b** in good yields. The IR spectrum of **2a** confirmed the success of the transformation showing a strong absorbance at 1670 cm^{-1} for the C=O group. Treatment of **2a**,**b** with hydrogen sulfide for 24 h at 100 °C furnished the thioamides **3a**,**b**. A lack of absorbance in the 2200 cm^{-1} region of the IR spectrum of **3a** confirmed the absence of a nitrile group. The key intermediates, 4-chloro-7-(ethoxymethyl)pyrrolo[2,3-*d*]-pyrimidine-5-carbonitrile (**4a**) and 4-chloro-7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (**4b**), were generated in good yields by the treatment of **2a**,**b** with $POCl_3$ at reflux for 10 min followed by silica gel chromatography. The attempted reduction of **4a** with H_2 over a Pd catalyst in EtOH and 1 equiv of 1 N NaOH resulted in the nucleophilic substitution of an ethoxy group at C-4 rather than a removal of the halogen group. To avoid this, the reduction of **4a**,**b** was accomplished using 1 equiv of $NaHCO_3$ rather than NaOH, thereby furnishing analogs **5a**,**b** in good yields.

Treatment of **4a**,**b** with various nucleophiles at room temperature gave the 4-substituted derivatives **6a**,**b**−**12a**,**b**. We found the 4-chloro derivatives **4a**,**b** to be highly reactive to a host of O, N, and S nucleophiles

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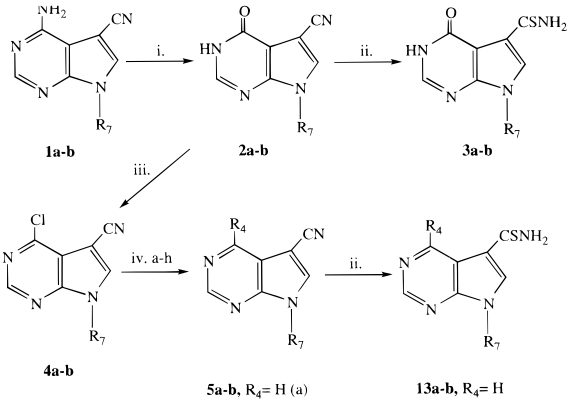
Scheme 1. Synthesis of 4-Substituted 5-Cyano- and 5-Thioamide Pyrrolo[2,3-*d*]pyrimidine Analogs Related to Toyocamycin and Thiosangivamycin[a]



[a] (i) $NaNO_2$, H_2O/AcOH; (ii) MeOH, H_2S/NaOMe; (iii) $POCl_3$; (iv) (a) Pd/C. H_2, EtOH, $NaHCO_3$, (b) NaOMe, MeOH, (c) EtOH, 1 N NaOH, (d) H_2NMe, (e) H_2NEt, (f) $H_2NC_3H_5$, (g) $HN(CH_3)_2$, (h) H_2NCSNH_2. **a**, $R_7 = CH_2OCH_2CH_3$; **b**, $R_7 = CH_2OCH_2CH_2OCH_3$.

with typical reaction times being <10 min at room temperature. The nitriles **5a**,**b**−**12a**,**b** were converted to the corresponding thioamide derivatives **13a**,**b**−**20b** by treatment with NaSH generated *in situ*. Supporting previous work in this area, we found that the reaction times for this conversion appeared to depend on the substituent at the 4-position.[6] For example, conversion of the nitrile of the 4-ethoxy analog **7a** to the $CSNH_2$ derivative **15a** took 3 days at 80 °C. In contrast, reaction of the 4-HNMe analog **8a** with NaSH furnished the corresponding thioamide **16a** in only 5 h at 95 °C. Comparison of the UV spectra of analogs **2a**−**19a** with their corresponding ribosyl-substituted compounds demonstrated similar spectral profiles.[7,8]

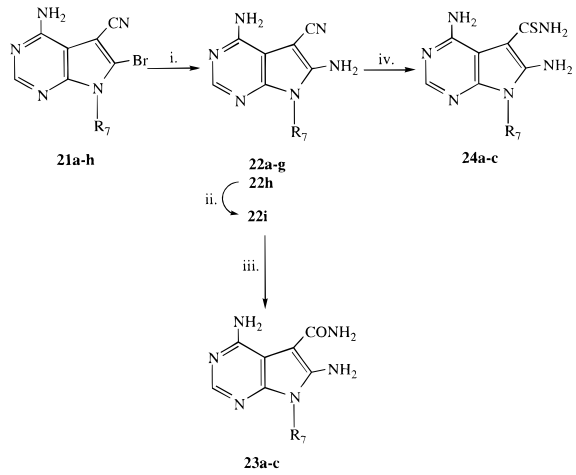
The 4,6-diamino 7-substituted pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide derivatives **24a**−**c** were generated from the requisite nitriles **22a**−**c** (Scheme 2). Compounds **22a**−**h** were synthesized in good yields by the treatment of **21a**−**h**[1] with liquid NH_3 for 16 h at 100 °C. Reduction of **22h** furnished the propyl derivative **22i** in modest yield. Treatment of the acyclic substituted nitriles **22a**−**c** with hydroxylamine hydrochloride in sodium methoxide in methanol furnished the carboxamide derivatives **23a**−**c**. Comparison of the UV spectra of analogs **22a** and **24a** with their corresponding ribosyl-substituted compounds demonstrated similar spectral profiles.[6]

The 4-amino, 6-methylamino and 4-amino, 6-dimethylamino analogs of **22b** (**25** and **26**, respectively) were prepared from **21b** (Scheme 3) with either methylamine or dimethylamine. The absorbance in the 2200 cm^{-1} region of the IR spectrum of **25** and **26** confirmed the presence of the nitrile group. Synthetic and physical data for all of the compounds examined in this study are presented in Table 1.

Biological Results and Discussion

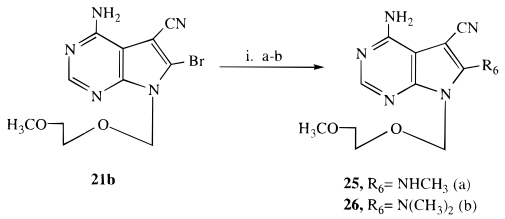
***In Vitro* Antiproliferative Testing.** A number of the 4-substituted 5-nitrile and 5-thioamide analogs (**2b**−**3b**, **5b**−**6b**, **8b**, **10b**, **13b**−**14b**, **16b**, and **19b**) were examined as potential antitumor agents by determining their effect on L1210 murine leukemic cells *in vitro*. None of the compounds examined displayed any significant inhibition with IC_{50}'s not reached at the highest concentration tested (100 μM).

Scheme 2. Synthesis of 4,6-Disubstituted 5-Cyano-, 5-Thioamide, and 5-Carboxamide Pyrrolo[2,3-*d*]pyrimidines Related to Toyocamycin, Sangivamycin, and Thiosangivamycin[a]



[a] (i) NH_3(l); (ii) Pd/C, H_2; (iii) NH_2OH·HCl/NaOMe, EtOH/H_2O; (iv) MeOH, H_2S/NaOMe. **a**, $R_7 = CH_2OCH_2CH_3$; **b**, $CH_2OCH_2CH_2OCH_3$; **c**, $R_7 = CH_2OCH_2C_6H_5$; **d**, $R_7 = CH_2C_6H_5$; **e**, $R_7 = CH_2$-3-$CH_3C_6H_5$; **f**, $R_7 = CH_2$-4-$CH_3C_6H_5$; **g**, $R_7 = CH_2$-4-$OCH_3C_6H_5$; **h**, $R_7 = CH_2CHCH_2$; **i**, $R_7 = CH_2CH_2CH_3$.

Scheme 3. Synthesis of 4-Amino, 6-Substituted Analogs of **21b**[a]



[a] (i) (a) NH_2CH_3, EtOH, (b) $NH(CH_3)_2$, EtOH.

***In Vitro* Antiviral Activity.** In the series of 4-substituted 5-nitrile and 5-thioamide derivatives (compounds **2**−**20**), only compounds **16a**,**b**, substituted with a methylamino group at the 4-position (R_4) and a thioamide group at the 5-position (R_5), had moderate antiviral activity (IC_{50} = 17−20 μM). All of the other compounds were inactive against HCMV with IC_{50}'s > 100 μM. The activity of the 7-ether-substituted derivatives **16a**,**b** was less potent than the activity for the corresponding 4-amino, 5-thioamide derivatives (compounds **III** and **IV**, Table 2). Of compounds **2**−**20**, only **16b** had moderate activity against HSV-1 (IC_{50} = 30 μM). The 4-methylamino derivative of thiosangivamycin[6] was more active than both **16a**,**b**, but it was also more toxic to uninfected HFF cells (IC_{50} = 5.0 μM, CC_{50} = 100 μM). The 4-unsubstituted thioamide derivatives **13a**,**b** were inactive at concentrations up to 100 μM, establishing that both the amino group at C-4 and a thioamide at C-5 are essential for activity against HCMV in this series of 7-substituted pyrrolo[2,3-*d*]-pyrimidines. Like the 4-amino, 5-nitrile analogs **I** and **II** (Table 2), none of the 4-substituted nitrile derivatives demonstrated activity against HCMV at concentrations

Table 1. Synthetic and Physical Data of the Pyrrolo[2,3-*d*]pyrimidines Prepared for This Study

compd	method of preparation[a]	yield (%)	mp (°C)	formula (C, H, N)[b]
2a	*c*	90	209−211	$C_{10}H_{10}N_4O_2$
2b	Similar to **2a**	73	155	$C_{11}H_{12}N_4O_3$
3a	*c*	67	240 dec	$C_{10}H_{12}N_4O_2S$
3b	Similar to **3a**	74	238 dec	$C_{11}H_{14}N_4O_3S$
4a	*c*	80	125−126	$C_{10}H_9N_4OCl$
4b	Similar to **4a**	63	105−106 dec	$C_{11}H_{11}N_4O_2Cl$
5a	*c*	80	89	$C_{10}H_{10}N_4O$
5b	Similar to **5a**	76	104−107	$C_{11}H_{12}N_4O_2$
6a	*c*	77	210−211	$C_{11}H_{12}N_4O_2$
6b	Similar to **6a**	86	123−124	$C_{12}H_{14}N_4O_3$
7a	*c*	56	151−152	$C_{12}H_{14}N_4O_2$
7b	Similar to **7a**	73	106−107	$C_{13}H_{16}N_4O_3$
8a	*c*	70	175−177	$C_{11}H_{13}N_5O$
8b	Similar to **8a**	77	134−135	$C_{12}H_{15}N_5O_2$
9a	*c*	77	152−154	$C_{12}H_{15}N_5O$
9b	Similar to **9a**	94	129−131	$C_{13}H_{17}N_5O_2$
10b	*c*	92	89−90	$C_{14}H_{17}N_5O_2$
11a	*c*	37	90−92	$C_{12}H_{15}N_5O$
11b	Similar to **11a**	75	103−104	$C_{13}H_{17}N_5O_2$
12a	*c*	93	218−220	$C_{10}H_{10}N_4OS$
12b	Similar to **12a**	93	179−181	$C_{11}H_{12}N_4O_2S$
13a	A	61	176−177	$C_{10}H_{12}N_4OS$
13b	A	53	151−153	$C_{11}H_{14}N_4O_2S$
14a	A	72	149−150	$C_{11}H_{14}N_4O_2S$
14b	A	52	128−129	$C_{12}H_{16}N_4O_3S$
15a	A	46	139	$C_{12}H_{16}N_4O_2S$
16a	A	94	157−158	$C_{11}H_{15}N_5OS$
16b	A	64	139−141	$C_{12}H_{17}N_5O_2S$
17a	A	91	143−144	$C_{12}H_{17}N_5OS$
17b	A	85	150−152	$C_{13}H_{19}N_5O_2S$
18b	A	92	180−182	$C_{14}H_{19}N_5O_2S$
19a	A	23	148−150	$C_{12}H_{17}N_5OS$
19b	A	41	103−105	$C_{13}H_{19}N_5O_2S$
20b	A	27	221−224	$C_{11}H_{14}N_4O_2S_2$
22a	B	87	229−230	$C_{10}H_{12}N_6O$
22b	B	74	190−192	$C_{11}H_{14}N_6O_2$
22c	B	84	218−219	$C_{15}H_{14}N_6O$
22d	B	20	285−288	$C_{14}H_{12}N_6$
22e	B	28	299−301	$C_{15}H_{14}N_6$
22f	B	41	286−289	$C_{15}H_{14}N_6 \cdot 0.25H_2O$
22g	B	34	261	$C_{15}H_{14}N_6O$
22h	B	86	293−295	$C_{10}H_{10}N_6$
22i	*c*	57	222−225	$C_{10}H_{12}N_6$
23a	C	24	176	$C_{10}H_{14}N_6O_2$
23b	C	61	243−244	$C_{11}H_{16}N_6O_3$
23c	C	80	212−214	$C_{15}H_{16}N_6O_2$
24a	D	43	209−210	$C_{10}H_{14}N_6OS$
24b	D	61	199−200	$C_{11}H_{16}N_6O_2S$
24c	D	42	199−200	$C_{15}H_{16}N_6OS$
25	*c*	28	168−169	$C_{12}H_{16}N_6O_2$
26	Similar to **25**	37	147−148	$C_{13}H_{18}N_6O_2$

[a] See the Experimental Section for methods A−D. [b] Letters in parantheses refer to those elements analyzed. [c] See the Experimental Section for the preparation of this compound.

at or below 100 μM. Of the 4-substitued derivatives, only the 4-chloro analogs **4a**,**b** were cytotoxic below 100 μM (65 μM ≤ CC_{50} ≤ 95 μM). The slight toxicity of **4a**,**b** may be caused by some modification of the compounds *in vitro* since both compounds were amenable to mild chemical nucleophilic substitutions.

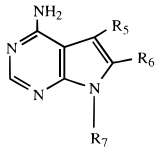
In the 6-substituted series (compounds **21**−**26**, Table 2), the thioamide derivatives **24a**−**c** demonstrated modest activity against HCMV and were essentially nontoxic in their antiviral dose range. These three compounds also were evaluated for activity in HCMV yield reduction assays. Compound **24c** was the most active (IC_{90} = 7 μM), whereas compounds **24a**,**b** were less active (IC_{90}'s = 45 and 100 μM, respectively). Compounds **24a**−**c** were relatively inactive against HSV-1. Interestingly, the corresponding 7-substituted (hydroxyethoxy)methyl (HEM) and (dihydroxypropoxy)methyl (DHPM) 4,6-diamino, 5-thioamide derivatives were inactive against HCMV up to 100 μM.[9] Like the HEM and DHPM analogs, though, the corresponding carboxamide derivatives **23a**−**c** were inactive and nontoxic. Similarly, the bromo-substituted analogs **21a**−**c**[1] were also inactive and nontoxic against HCMV.

The most surprising aspect is the observed activity against HCMV of the corresponding nitrile derivatives **22a**−**c**. These compounds typically were more active than the thioamide analogs **24a**−**c** in both the plaque reduction assay and ELISA. The activity of **22a**−**c** against HCMV was well separated from the toxicity in uninfected cells. More extensive studies of the cytotoxicity of **22b**, for example, by the incorporation of labeled precursors into protein, RNA, and DNA, supported the cytotoxicity data in Table 2. Specifically, IC_{50}'s for the incorporation of [^{3}H]Leu and [^{3}H]Urd were >400 μM, while the IC_{50} for the inhibition of [^{3}H]dThd incorporation was 200 μM. Clearly, in the case of the 4,6-diamino derivatives, a thioamide moiety at C-5 (R_5) is not essential. This is not the case for the 7-ether-substituted 4-aminopyrrolo[2,3-*d*]pyrimidines (compounds **I**−**IV**) where a thioamide at the 5-position is required for antiviral activity (see Table 2). This is the first case in our study of non-nucleoside pyrrolo[2,3-*d*]pyrimidine derivatives where a thioamide moiety at position 5 is not necessary for antiviral activity. To extend these results, we prepared and evaluated a variety of other 4,6-diamino 7-substituted derivatives of **22a**−**c** (Table 2). Like the acyclic analogs, compounds **22d**−**i** were all relatively active against HCMV (0.3 μM ≤ IC_{50} ≤ 32 μM). The compounds, however, were generally more toxic than the corresponding acyclic analogs **22a**−**c** in both uninfected HFF and KB cells. In general, the activity of compounds **22a**−**i** against HSV-1 was less than the activity of the compounds against HCMV.

To demonstrate that an amino group at both C-4 and C-6 is a requirement for the activity of **22b**, we prepared and evaluated two compounds (**25** and **26**) where the amino group at C-6 was replaced with either a methylamino (**25**) or a dimethylamino (**26**) group (Table 2). Both compounds were inactive against HCMV and HSV-1, establishing that amino groups at C-4 and C-6 are necessary for the antiviral activity of **22b** and a likely requirement for the other compounds in this series, **22a**,**c**−**i**.

We have recently shown that the 5-thioamide moiety of several structurally dissimilar 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidines, including thiosangivamycin, is unstable in cell culture medium and converted to the corresponding 5-nitrile.[10] In contrast, structural modifications of the 4-position had a definite effect on the stability of the 5-thioamide moiety. For example, replacement of the 4-amino group of **IV** with a methylamino (**16b**) decreased the stability of the thioamide. Specifically, the half-life decreased from 50 h for **IV** to 20 h for **16b**. In contrast, replacement with a hydrogen (**13b**) or a dimethylamino (**19b**) increased the stability of the thioamide ($t_{1/2}$ = 175 and 150 h, respectively). The 5-thioamide moiety of **24a**,**b** was similarly unstable and converted to the 5-nitrile following the appearance of an unidentified peak by HPLC.[11] Hence, the biological data for compounds **3a**,**b**, **13a**−**19a**, **13b**−**20b**, and

Table 2. Antiviral Activity and Cytotoxicity of 7-Substituted 4,6-Disubstituted 5-Cyano-, 5-Carboxamide, and 5-Thioamide Pyrrolo[2,3-*d*]pyrimidines



				50% inhibitory concentration[a] (μM)				
				antiviral activity				
	substituent			HCMV			cytotoxicity	
compd	R_5	R_6	R_7[b]	plaque	ELISA	HSV-1 (ELISA)	HFF (visual)	KB (growth)
21a	CN	Br	EM	>100			>100	>100
21b	CN	Br	MEM	>100			>100	>100
21c	CN	Br	BOM	>100			>100	>100
22a	CN	NH_2	EM	7.2	12	68	>100	138
22b	CN	NH_2	MEM	8.2	9	>100	>100	210
22c	CN	NH_2	BOM	2.8	9	12.5	>100	118
22d	CN	NH_2	$CH_2C_6H_5$	3.3		10	100	34
22e	CN	NH_2	CH_2-C_6H_4-3-CH_3	32		>100	100	>100
22f	CN	NH_2	CH_2-C_6H_4-4-CH_3	0.3		60	32	>100
22g	CN	NH_2	CH_2-C_6H_4-4-OCH_3	9		10	100	50
22h	CN	NH_2	CH_2CHCH_2	1.9		40	100	>100
22i	CN	NH_2	$CH_2CH_2CH_3$	0.5		2.5	100	15
23a	$CONH_2$	NH_2	EM	>100	>100		>100	>100
23b	$CONH_2$	NH_2	MEM	>100	>100		>100	>100
23c	$CONH_2$	NH_2	BOM	>100	>100		>100	>100
24a	$CSNH_2$	NH_2	EM	16	45	100	>100	>320
24b	$CSNH_2$	NH_2	MEM	5	20	100	>100	>320
24c	$CSNH_2$	NH_2	BOM	16	19	60	>100	225
25	CN	$HNCH_3$	MEM	>100		>100	>100	>100
26	CN	$N(CH_3)_2$	MEM	>100		>100	>100	>100
I[c]	CN	H	EM	>100			>100	>100
II[c]	CN	H	MEM	90		>100	>100	>100
III[c]	$CSNH_2$	H	EM	2.7	7		>32	60
IV[c]	$CSNH_2$	H	MEM	1.3	1.9	85	>100	183
ganciclovir (GCV)				8.7	22	3.0	>100	>320
acyclovir (ACV)				>100		3.4	>100	>100

[a] Results are the average of two or more experiments. A greater than sign (>) indicates IC_{50} not reached at highest concentration tested. [b] EM = ethoxymethyl; MEM = (methoxyethoxy)methyl; BOM = (benzyloxy)methyl. [c] The synthesis and evaluation of compounds **I**−**IV** are described in ref 1 and included in this table for comparative purposes.

24a−**c** may be the result of a mixture of 5-thioamide and 5-carbonitrile derivatives. However, since several 5-thioamide pyrrolo[2,3-*d*]pyrimidines such as **17b** and **18b** are inactive against HCMV but still undergo conversion to the nitrile,[11] we feel that the conversion of the 5-thioamide to the 5-nitrile is a secondary chemical event unrelated to the biological activity. Certainly, the activity of the analogs **22a**−**i**, which are stable in cell culture medium,[10,11] is due exclusively to the 4,6-diamino, 5-nitrile-substituted derivatives.

In summary, the synthesis and biological evaluation of 4-substituted and 4,6-disubstituted 5-cyano-, 5-carboxamide, or 5-thiocarboxamide non-nucleoside pyrrolo[2,3-*d*]pyrimidines related to toyocamycin and thiosangivamycin have revealed that an amino group at position 4 and a thioamide group at C-5 are essential for activity against HCMV, whereas the 4,6-diamino analogs do not necessarily require a thioamide group at C-5 for antiviral activity.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was run on silica gel 60F-254 plates (Analtech, Inc.). Detection of components on TLC was made by UV light absorption at 254 nm. Ultraviolet spectra were recorded on a Kontron-Uvikon 860 spectrophotometer. Infrared (IR) spectra were taken on a Perkin-Elmer infrared (IR) spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were determined at 200, 300, or 360 MHz with a Bruker WP 200/300/360 SY instrument. The chemical shift values are expressed in δ values (ppm) relative to the internal standard tetramethylsilane. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. E. Merck silica gel (230−400 mesh) was used for gravity or flash column chromatography. Evaporations were carried out on a rotary evaporator under reduced pressure (water aspirator) with the bath temperature at 37 °C. Acetonitrile and methanol were dried over activated molecular sieves (4 Å).

5-Cyano-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidin-4-one (2a). Compound **1a**[1] (7.97 g, 36.7 mmol) was suspended in distilled H_2O (414 mL) and AcOH (33%, 36 mL). This suspension was heated to 50 °C, and $NaNO_2$ (17.73 g, 256.9 mmol) was added in six batches over a period of 4.5 h. Following the additions of $NaNO_2$, the reaction mixture was heated to 70 °C for 16 h. The resulting reaction mixture was allowed to stand at 4 °C for 24 h. The solid was collected by filtration and dried at 60 °C for 16 h to yield 7.21 g (90%) of pure **2a**: mp 209−211 °C; IR (KBr) ν 2220 (CN), 1670 (C=O), 1590 (NH) cm^{-1}; UV λ_{max} [nm (ϵ, mM)] (pH 1) 264 (12.5), (MeOH) 263 (12.5), (pH 11) 274 (13.0); ^{1}H NMR (DMSO-d_6) δ 12.5 (1H, br s, NH, D_2O exchangeable), 8.23 (1H, s, H-2), 8.09 (1H, s, H-6), 5.50 (2H, s, NCH_2), 3.42−3.52 (2H, q, CH_2), 1.02−1.09 (3H, t, CH_3). Anal. ($C_{10}H_{10}N_4O_2$) C, H, N.

7-(Ethoxymethyl)-4-oxopyrrolo[2,3-*d*]pyrimidin-5-thiocarboxamide (3a). NaOMe (149 mg, 2.7 mmol) in dry MeOH (75 mL) was saturated with H_2S (g) for 30 min. This solution was transferred to a steel vessel containing **2a** (300 mg, 1.4

mmol) which was sealed and heated at 100 °C in an oil bath for 24 h. After this time the solution was allowed to cool to room temperature, and the pH was adjusted to 7 with 1 N HCl (2 mL). The resulting solution was heated on a steam bath and filtered and the filtrate cooled at 4 °C for 16 h. The resulting solid was collected to yield pure **3a** as a yellow powder (235 mg) in 67% yield: mp 240 °C dec; IR (KBr) ν 3210 (NH), 1660 (C═O), 1590 (NH) cm^{-1}; UV λ_{max} [nm (ϵ, mM)] (pH 1) 322 (11.7), 270 (13.4), (MeOH) 324 (13.0), 272 (16.3), (pH 11) 329 (12.1), 271 (18.4); ^{1}H NMR (DMSO-d_6) δ 12.79 (1H, br s, NH, D_2O exchangeable), 11.37 and 9.66 (1H each, br s, $CSNH_2$, D_2O exchangeable), 8.14 (2H, m, H-2, H-6), 5.58 (2H, s, NCH_2), 3.45−3.60 (2H, q, CH_2), 1.04−1.09 (3H, t, CH_3). Anal. ($C_{10}H_{12}N_4O_2S$) C, H, N.

4-Chloro-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (4a). Compound **2a** (1.14 g, 5.2 mmol) was dissolved in $POCl_3$ (10 mL) and heated at reflux for 12 min. The hot solution was poured onto ice water (100 mL), and the pH of the resulting mixture was adjusted to 7 with NH_4OH (38%, 15 mL). The title compound was extracted into CH_2Cl_2 (2 × 75 mL) from distilled H_2O (300 mL total) and $NaHCO_3$ (1 × 5 mL). The organic layer was collected, dried over $MgSO_4$, and filtered and the filtrate evaporated to dryness to produce a yellow solid (987 mg, 80%). A small sample (50 mg) was recrystallized from MeOH/H_2O and decolorizing charcoal to furnish pure **4a** as a white powder: mp 125−126 °C; IR (KBr) ν 2210 (CN), 1100 (C−Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.87 and 8.88 (1H each, s, H-2, H-6), 5.68 (2H, s, NCH_2), 3.60−3.45 (2H, q, CH_2), 1.04−1.09 (3H, t, CH_3). Anal. ($C_{10}H_9N_4OCl$) C, H, N.

7-(Ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (5a). Compound **4a** (900 mg, 3.8 mmol), $NaHCO_3$ (386 mg, 4.6 mmol), and 10% Pd/C (90 mg, 10% by wt) was dissolved in absolute EtOH (200 mL). The reaction was hydrogenated at room temperature at 45 psi for 4.5 h, filtered, and washed with hot EtOH (2 × 10 mL). The filtrate was evaporated to dryness and the residue dissolved in CH_2Cl_2 (2 × 50 mL) and then extracted with distilled H_2O (100 mL). The organic fractions were collected (total vol = 100 mL), dried over $MgSO_4$, filtered, and evaporated to afford 617 mg (80%) of crude **5a** as a light yellow solid. A small sample (150 mg) was recrystallized from H_2O/EtOH and decolorizing charcoal to furnish pure **5a**: mp 89 °C; UV λ_{max} [nm (ϵ, mM)] (MeOH) 271 (6.9), (pH 11) 270 (7.1); ^{1}H NMR (DMSO-d_6) δ 9.25, 9.03, and 8.76 (1H each, s, H-2, H-4, H-6), 5.68 (2H, s, NCH_2), 3.47−3.54 (2H, q, CH_2), 1.04−1.08 (3H, t, CH_3). Anal. ($C_{10}H_{10}N_4O$) C, H, N.

4-Methoxy-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (6a). Compound **4a** (100 mg, 0.4 mmol) was added to dry MeOH (20 mL) and stirred under argon. NaOMe (114 mg, 2.1 mmol) was then added, and the reaction mixture was heated at reflux for 1.5 h. At this time it was allowed to cool to room temperature, the pH adjusted to 7 with AcOH, and the solution evaporated to dryness. The resulting solid was dissolved in EtOAc (30 mL) and extracted with distilled H_2O (50 mL). The organic layer (50 mL) was collected, dried over $MgSO_4$, and filtered and the filtrate evaporated *in vacuo* to yield 72 mg (77%) of a tan solid. This solid was recrystallized from H_2O/MeOH to furnish pure **6a**: mp 210−211 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 264 (12.5), (MeOH) 264 (12.3), (pH 11) 264 (12.4); ^{1}H NMR (DMSO-d_6) δ 8.60 and 8.55 (1H each, s, H-2, H-6), 5.62 (2H, s, NCH_2), 4.10 (3H, s, OCH_3), 3.45−3.51 (2H, q, CH_2), 1.03−1.07 (3H, t, CH_3). Anal. ($C_{11}H_{12}N_4O_2$) C, H, N.

4-Ethoxy-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (7a). Compound **4a** (100 mg, 0.4 mmol) was dissolved in absolute EtOH (30 mL), and to this solution was added 1 N NaOH (400 μL, 0.4 mmol). The reaction mixture was stirred at room temperature for 2 h 45 min, at which time no starting material was observed by TLC. The solution was cooled at 4 °C for 2 days, at which time the solid was collected by filtration to furnish 55 mg (56%) of **7a** as a white powder: mp 151−152 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 264 (13.8), (MeOH) 264 (14.4), (pH 11) 264 (14.2); ^{1}H NMR (DMSO-d_6) δ 8.57 and 8.54 (1H each, s, H-2, H-6), 5.61 (2H, s, NCH_2), 4.55−4.61 (2H, q, CH_2), 3.45−3.50 (2H, q, CH_2), 1.38−1.42 (3H, t, CH_3), 1.03−1.07 (3H, t, CH_3). Anal. ($C_{12}H_{14}N_4O_2$) C, H, N.

4-(Methylamino)-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (8a). Compound **4a** (800 mg, 3.4 mmol) was dissolved in methylamine (100 mL, 33% in absolute EtOH) and stirred at room temperature for 2.5 h. The solution was cooled at 4 °C for 16 h and filtered to furnish 548 mg (70%) of pure **8a** as a white powder: mp 175−177 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 275 (19.8), 235 (17.4), (MeOH) 281 (21.0), (pH 11) 282 (20.4), 234 (10.3); ^{1}H NMR (DMSO-d_6) δ 8.33 and 8.32 (1H each, s, H-2, H-6), 6.73−6.74 (1H, br q, NH), 5.53 (2H, s, NCH_2), 3.43−3.49 (2H, q, CH_2), 2.98−3.00 (3H, d, CH_3), 1.01−1.06 (3H, t, CH_3). Anal. ($C_{11}H_{13}N_5O$) C, H, N.

4-(Ethylamino)-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (9a). Compound **4a** (800 mg, 3.4 mmol) was dissolved in $EtNH_2$ (30 mL, 70% in H_2O) and stirred at room temperature. Within 5 min a white precipitate was observed. The white suspension was poured into a 250 mL extraction flask, and distilled H_2O and EtOAc were added. The organic layer (total vol = 150 mL) was collected, dried over Na_2SO_4, and filtered and the filtrate evaporated to dryness. The resulting white solid was suspended in 100 mL of EtOH, heated to boiling, and then stored for 16 h at 4 °C. The resulting precipitate was filtered to collect 636 mg (77%) of **9a** as a white solid: mp 152−154 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 276 (21.2), 236 (18.1), (MeOH) 283 (23.5), (pH 11) 283 (22.7), 235 (10.8); ^{1}H NMR (DMSO-d_6) δ 8.33 and 8.31 (1H each, s, H-2, H-6), 6.66−6.68 (1H, br t, NH), 5.53 (2H, s, NCH_2), 3.50−3.56 (2H, q, NCH_2), 3.43−3.49 (2H, q, OCH_2), 1.16−1.20 (3H, t, CH_3), 1.03−1.06 (3H, t, CH_3). Anal. ($C_{12}H_{15}N_5O$) C, H, N.

4-(Cyclopropylamino)-7-[(2-methoxyethoxy)methyl]-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (10b). Compound **4a** (500 mg, 1.9 mmol) was dissolved in EtOH (50 mL), and cyclopropylamine (749 mg, 13.1 mmol) was added. The reaction mixture was heated at reflux for 1.5 h and then cooled to room temperature. The solution was evaporated to dryness and the resultant oil dissolved in water and extracted from CH_2Cl_2. The organic layer (total vol = 150 mL) was collected, dried over Na_2SO_4, filtered, and evaporated to dryness. The resulting oil was triturated with hexanes to reveal **10b** as a white solid (500 mg, 92%): mp 89−90 °C; ^{1}H NMR (DMSO-d_6) δ 8.35 (2H, m, H-2, H-6), 6.90 (1H, m, NH), 5.57 (2H, s, NCH_2), 3.52−3.57 (2H, m, OCH_2CH_2), 3.32−3.37 (2H, m, OCH_2CH_2), 3.17 (3H, s, OCH_3), 2.89−2.93 (1H, m, CH), 0.79−0.81 (2H, m, CH_2), 0.61 (2H, m, CH_2). Anal. ($C_{14}H_{17}N_5O_2$) C, H, N.

4-(Dimethylamino)-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (11a). Compound **4a** (500 mg, 2.1 mmol) was dissolved in dimethylamine (30 mL, 33% in absolute EtOH). The reaction mixture was stirred at room temperature for 30 min, at which time no starting material was observed by TLC. The solution was evaporated *in vacuo*, and the resultant brown solid was suspended in H_2O (50 mL) and MeOH (5 mL) and heated to boiling. To this mixture was added decolorizing charcoal. The boiling solution was filtered over Celite and the filtrate cooled for 16 h at 4 °C. The resulting white solid was collected by vacuum filtration and dried for 16 h at 60 °C to yield pure **11a** (193 mg, 37%): mp 90−92 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 281 (15.3), 244 (11.0), (MeOH) 290 (19.8), (pH 11) 290 (18.5); ^{1}H NMR (DMSO-d_6) δ 8.46 and 8.30 (1H each, s, H-2, H-6), 5.56 (2H, s, NCH_2), 3.44−3.51 (2H, q, CH_2), 3.31 (6H, s, CH_3 × 2), 1.03−1.08 (3H, t, CH_3). Anal. ($C_{12}H_{15}N_5O$) C, H, N.

5-Cyano-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-4-thione (12a). Compound **4a** (800 mg, 3.3 mmol) and thiourea (515 mg, 6.8 mmol) were dissolved in absolute EtOH (110 mL) and the reaction mixture heated at reflux for 60 min. The reaction mixture was allowed to cool to room temperature and then evaporated at 37 °C to $^1/_4$ vol. The resulting suspension was cooled at 4 °C for 16 h and then filtered to furnish **12a** as an off-white solid (720 mg, 93%): mp 218−220 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 330 (24.0), (MeOH) 331 (23.0), (pH 11) 321 (21.5), 231 (13.8); ^{1}H NMR (DMSO-d_6) δ 13.8 (1H, br s, NH), 8.40 and 8.22 (1H each, s, H-2, H-6), 5.52 (2H, s, NCH_2),

3.43−3.50 (2H, q, CH_2), 1.03−1.08 (3H, t, CH_3). Anal. ($C_{10}H_{10}N_4OS$) C, H, N.

General Method A for the Synthesis of Compounds 13a−20b: 7-(Ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (13a). H_2S(g) was passed through a solution of NaOMe (162 mg, 3.0 mmol) in MeOH (75 mL) for 30 min. This solution was transferred to a steel vessel containing **5a** (300 mg, 1.5 mmol). The vessel was sealed, stirred at room temperature for 24 h, at which time the pH was adjusted to 7 with 1 N HCl (1 mL), heated on a steam bath for 5 min, and then evaporated to dryness. The crude solid was directly recrystallized from H_2O and a small amount of MeOH to furnish 214 mg (61%) of **13a** as a light yellow powder: mp 176−177 °C; UV λ_{max} [nm (ϵ, mM)] (MeOH) 266 (16.2), (pH 11) 268 (14.9); ^{1}H NMR (DMSO-d_6) δ 9.80 (1H, s), 9.49 and 9.27 (1H each, br s, $CSNH_2$), 8.90 (1H, s), 8.39 (1H, s), 5.67 (2H, s, NCH_2), 3.46−3.52 (2H, q, CH_2), 1.04−1.08 (3H, t, CH_3). Anal. ($C_{10}H_{12}N_4OS$) C, H, N.

General Method B for the Synthesis of Compounds 22a−h: 4,6-Diamino-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (22a). A 125 mL steel vessel containing **21a**[1] (2.5 g, 8.4 mmol) was charged with 75 mL of NH_3(l). The vessel was sealed, and the reaction mixture was heated to 100 °C for 16 h. At this time, the vessel was allowed to cool to room temperature and further cooled to −75 °C, at which time the vessel was vented. The resulting solid was suspended in H_2O and heated to boiling. Following filtration the filtrate was cooled at 4 °C for 16 h, and pure **22a** was collected (1.70 g, 87%): mp 229−230 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 293 (15.9), (MeOH) 295 (16.0), (pH 11) 290 (19.9); ^{1}H NMR (DMSO-d_6) δ 8.00 (1H, s, H-2), 7.22 (2H, br s, NH_2, D_2O exchangeable), 6.10 (2H, br s, NH_2, D_2O exchangeable), 5.42 (2H, s, NCH_2), 3.44−3.48 (2H, q, CH_2), 1.03−1.07 (3H, t, CH_3). Anal. ($C_{10}H_{12}N_6O$) C, H, N.

4,6-Diamino-7-propylpyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (22i). Compound **22h** (300 mg, 1.41 mmol) was dissolved in 100 mL of EtOAc/EtOH (2:1, v:v), and 10% Pd/C (10% by wt, 30 mg) was added. The reaction mixture was hydrogenated at 50 psi for 1 h and the resultant red solution filtered through Celite. The filtrate was evaporated and the crude solid recrystallized from water and charcoal to furnish 170 mg (57%) of **22i**: 222−225 °C; ^{1}H NMR (DMSO-d_6) δ 7.98 (1H, s, C-2), 7.13 (2H, s, 4-NH_2), 6.02 (2H, s, 6-NH_2), 3.96 (2H, t, NCH_2), 1.60 (2H, sextet, CH_2), 0.82 (3H, t, CH_3). Anal. ($C_{10}H_{12}N_6$) C, H, N.

General Method C for the Synthesis of Compounds 23a−c: 4,6-Diamino-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (23a). NH_2OH hydrochloride (382 mg, 5.5 mmol) and NaOMe (297 mg, 5.5 mmol) were suspended in EtOH (40 mL), and the suspension was stirred for 30 min. Compound **22a** (250 mg, 1.1 mmol) and 10 mL of distilled H_2O were then added. This mixture was heated at reflux for 9 h and then evaporated to dryness. The resulting solid was suspended in MeOH and allowed to stand at 4 °C for 16 h. The solid was collected by filtration and recrystallized from aqueous EtOH to furnish pure **23a** (67 mg, 24%): mp 176 °C; ^{1}H NMR (DMSO-d_6) δ 7.92 (1H, s, H-2), 7.42, 6.97, and 6.22 (2H each, br s, 2 × NH_2, $CONH_2$), 5.48 (2H, s, NCH_2), 3.42−3.49 (2H, q, CH_2), 1.04−1.09 (3H, t, CH_3). Anal. ($C_{10}H_{14}N_6O_2$) C, H, N.

General Method D for the Synthesis of Compounds 24a−c: 4,6-Diamino-7-(ethoxymethyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (24a). NaOMe (194 mg, 3.6 mmol) in dry MeOH (60 mL) was saturated with H_2S(g) for 30 min. This solution was transferred to a steel vessel containing **22a** (400 mg, 1.8 mmol) which was sealed and heated to 100 °C in an oil bath for 16 h. After this time the solution was allowed to cool to room temperature and the pH adjusted to 7 with 1 N HCl (2.5 mL). The resulting solution was evaporated to dryness and recrystallized from H_2O/MeOH and decolorizing charcoal to furnish pure **24a** (204 mg, 43%): mp 209−210 °C; UV λ_{max} [nm (ϵ, mM)] (pH 1) 366 (13.6), 277 (15.5), (MeOH) 364 (11.7), 279 (14.6), (pH 11) 360 (12.3), 269 (17.4); ^{1}H NMR (DMSO-d_6) δ 8.15 and 8.02 (2H each, br s, 4-NH_2, $CSNH_2$, D_2O exchangeable), 8.06 (1H, s, H-2), 6.39 (2H, br s, NH_2, D_2O exchangeable), 5.49 (2H, s, CH_2), 3.45−3.52 (2H, q, CH_2), 1.06−1.10 (3H, t, CH_3). Anal. ($C_{10}H_{14}N_6OS$) C, H, N.

4-Amino-6-(methylamino)-7-[(2-methoxyethoxy)methyl]pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile (25). Compound **21b** (300 mg, 0.9 mmol) was dissolved in methylamine (33% in absolute alcohol) and stirred for 24 h in a pressure bottle at room temperature. The solution was evaporated to dryness and the crude solid recrystallized from MeOH. The white solid was collected by filtration to furnish 71 mg (28%) of pure **25**: mp 168−169 °C; IR (KBr) ν (cm^{-1}) 2175; ^{1}H NMR (DMSO-d_6) δ 8.02 (1H, s, H-2), 7.3−7.4 (1H, m, NH), 6.14 (2H, s, NH_2), 5.41 (2H, s, NCH_2), 3.52−3.56 (2H, m, OCH_2CH_2), 3.30−3.40 (2H, m, OCH_2CH_2), 3.19 (3H, s, OCH_3), 3.12−3.10 (3H, d, NCH_3). Anal. ($C_{12}H_{16}N_6O_2$) C, H, N.

Antiviral Evaluation: (a) Cells and Viruses. Human foreskin fibroblasts (HFF cells) and MRC-5 cells, a human embryonic lung cell line, were grown in minimum essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum (FBS). KB cells, an established human cell line derived from an epidermoid oral carcinoma, were grown in MEM with Hank's salts [MEM(H)] supplemented with 10% calf serum (CS). These cell lines were subcultured according to conventional procedures as described previously.[10] All cell lines were screened periodically for mycoplasma contamination and were negative. A plaque-purified isolate, P_o, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinski, University of Iowa. Stock preparations of HCMV and HSV-1 were prepared as described elsewhere.[12]

(b) Assays for Antiviral Activity. HCMV plaque-reduction assays were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exception that the virus inoculum (0.2 mL) contained ca. 100 plaque-forming units (PFU) of HCMV and the compounds to be assayed were dissolved in the overlay medium. HCMV also was assayed using an enzyme immunoassay in MRC-5 cells by a procedure described by Prichard and Shipman to assay HSV-1[13] and modified by us.[1] HSV-1 ELISA assays were performed as previously described.[13] Protocols for HCMV yield reduction experiments have been previously described.[14] Briefly, monolayer cultures of HFF cells in 96-well culture dishes (Costar, Cambridge, MA) were infected at an moi of 0.5, 0.05, or 0.005 PFU/cell and incubated in the presence of the test compounds for 7 days. Following one cycle of freezing at −76 °C and thawing at 36 °C, the resulting lysates were diluted, and the amount of infectious virus was quantified on new cultures of HFF cells.[14]

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque-reduction assay described above. Drug-induced cytopathology was estimated at 35-fold magnification and scored on a zero to four basis on the day of staining for plaque counting. Cytotoxicity in KB cells was determined by a staining method previously described.[15]

(d) Data Analysis. Dose−response relationships were used to quantitate drug effects. These were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentrations. The 50% inhibitory (IC_{50}) concentrations were calculated from the regression lines. Ganciclovir (GCV) was used as a positive control in all antiviral assays.

***In Vitro* Antiproliferative Studies**. The *in vitro* cytotoxicity against L1210 was determined in an L1210 cell growth assay described previously.[16] L1210 murine leukemic cells were grown in Fischer's medium supplemented with 10% heat-inactivated (56 °C, 30 min) horse serum and were subcultured by serial dilution. Growth rates were calculated from determinations of the number of cells at 0, 24, 48, 72, and 96 h in the presence of selected concentrations of the test compound. The IC_{50} was defined as the concentration required to decrease the growth rate to 50% of the untreated control rate. Growth inhibition was calculated as the slope of a semilogarithmic plot

of cell number against time for the treated culture as a percent of the control.

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References

(1) Renau, T. E.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Design of non-nucleoside analogs of toyocamycin, sangivamycin and thiosangivamycin: the influence of 7-substituents on the antiviral activity of pyrrolo[2,3-*d*]pyrimidines. *J. Med. Chem.* **1996**, *39*, 873−880.

(2) Rubin, R. H. Impact of cytomegalovirus infection on organ transplant recipients. *Rev. Infect. Dis.* **1990**, *12* (Suppl. 7), 754−766.

(3) Stratta, R. J.; Shaeffer, M. S.; Markin, R. S.; Wood, R. P.; Langnas, A. N.; Reed, E. C.; Donovan, J. P.; Woods, G. L.; Bradshaw, K. A.; Pillen, T. J.; Shaw, B. W., Jr. Cytomegalovirus infection disease after liver transplantation. *Dig. Dis. Sci.* **1992**, *37*, 673−688.

(4) Mills, J.; Masur, H. AIDS-related infections. *Sci. Am.* **1990**, *263*, 50−57.

(5) Peters, B. S.; Beck, E. J.; Anderson, S.; Coleman, D.; Coker, R.; Main, J.; Migdal, C.; Harris, J. R. W.; Pinching, A. J. Cytomegalovirus infection in AIDS. Patterns of disease, response to therapy and trends in survival. *J. Infect.* **1991**, *23*, 129−137.

(6) Schram, K. H.; Townsend, L. B. Pyrrolopyrimidine nucleosides. Part XI. Influence of amino groups at C-4 and C-6 or an amino group at C-6 on the reactivity of a cyano group in pyrrolo[2,3-*d*]pyrimidine nucleosides. *J. Chem. Soc., Perkin Trans. I* **1975**, 1253−1257.

(7) Hinshaw, B. C.; Leonoudakis, O.; Schram, K.; Townsend, L. B. Pyrrolopyrimidine nucleosides. Part X. Synthesis of certain 4,5-disubstituted 7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidines related to toyocamycin and sangivamycin. *J. Chem. Soc., Perkin Trans. I* **1975**, 1248−1253.

(8) Hinshaw, B. C.; Gerster, J. F.; Robins, R. K.; Townsend, L. B. Pyrrolopyrimidine nucleosides. V. A study on the relative chemical reactivity of the 5-cyano group of the nucleoside antibiotic toyocamycin and desaminotoyocamycin. The synthesis of analogs of sangivamycin. *J. Org. Chem.* **1970**, *35*, 236−241.

(9) Swayze, E. E.; Shannon, W. M.; Buckheit, R. W.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. Synthesis, antiproliferative and antiviral evaluation of certain acyclic 6-substituted pyrrolo[2,3-*d*]pyrimidine nucleoside analogs related to sangivamycin and toyocamycin. *Nucleosides Nucleotides* **1992**, *11*, 1507−1527.

(10) Renau, T. E.; Lee, J. S.; Kim, H.; Young, C. G.; Wotring, L. L.; Townsend, L. B.; Drach, J. C. Relationship between cytotoxicity and conversion of thiosangivamycin analogs to toyocamycin analogs in cell culture medium. *Biochem. Pharmacol.* **1994**, *48*, 801−807.

(11) Unpublished data from our laboratories.

(12) Turk, S. R.; Shipman, C., Jr.; Nassiri, M. R.; Genzlinger, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. Pyrrolo[2,3-*d*]pyrimidine nucleosides as inhibitors of human cytomegalovirus. *Antimicrob. Agents Chemother.* **1987**, *31*, 544−550.

(13) Prichard, M. N.; Shipman, C., Jr. A three dimensional model to analyze drug-drug interactions. *Antiviral Res.* **1990**, *14*, 181−206.

(14) Prichard, M. N.; Turk, S. R.; Coleman, L. A.; Engelhardt, S. L.; Shipman, C., Jr.; Drach, J. C. A microtiter virus yield reduction assay for the evaluation of antiviral compounds against human cytomegalovirus and herpes simplex virus. *J. Virol. Methods* **1990**, *28*, 101−106.

(15) Prichard, M. N.; Prichard, L. E.; Baguley, W. A.; Nassiri, M. R.; Shipman, C., Jr. Three-dimensional analysis of the synergistic cytotoxicity of ganciclovir and zidovudine. *Antimicrob. Agents Chemother.* **1991**, *35*, 1060−1065.

(16) Wotring, L. L.; Townsend, L. B. Study of the cytotoxicity and metabolism of 4-amino-3-carboxamido-1-(β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine using inhibitors of adenosine kinase and adenosine deaminase. *Cancer Res.* **1979**, *39*, 3018−3023.

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