Notes

Synthesis and Evaluation of Certain Thiosangivamycin Analogs as Potential Inhibitors of Cell Proliferation and Human Cytomegalovirus

Steven H. Krawczyk,† Thomas E. Renau, M. Reza Nassiri, Allison C. Westerman, Linda L. Wotring, John C. Drach, and Leroy B. Townsend*

Departments of Medicinal Chemistry and Pharmaceutical Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Arts, Department of Biologic and Materials Sciences, School of Dentistry, The University of Michigan, Ann Arbor, Michigan 48109-1065

Received November 7, 1994®

A series of 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidines related to the nucleosides toyocamycin and thiosangivamycin were prepared and tested for their activity against human cytomegalovirus (HCMV). The nucleosides 2′-deoxytoyocamycin (**1**), *xylo*-toyocamycin (**2**), 3′-deoxytoyocamycin (**3**), 2′,3′-dideoxy-2′,3′-didehydrotoyocamycin (**4**), 2′,3′-dideoxytoyocamycin (**5**), *ara*-toyocamycin (**6**), 2′-deoxy-2′-amino-*ara*-toyocamycin (**7**), and 5′-deoxytoyocamycin (**8**) were treated with sodium hydrogen sulfide generated *in situ* to afford the corresponding thiosangivamycin analogs (**9**–**16**). The cyano derivatives **1**–**8** were synthesized by modifications of literature procedures. All of the thioamide derivatives (**9**–**16**) were active against HCMV with IC_{50}'s ranging from 0.5 to 6 μM. Most also were active against herpes simplex virus type 1 (HSV-1) but at higher concentrations. The antiviral activity was not completely separated from cytotoxicity in two human cell lines. The antiproliferative activity was strongly influenced by the position of the modification on the carbohydrate moiety. The xylosyl and 3′-deoxy derivatives were significantly more potent than those with modifications at the 2′, 5′, or 2′,3′ position(s). Interestingly, 5′-deoxythiosangivamycin (**16**) possessed both antiviral and antiproliferative activity suggesting that phosphorylation of the 5′-hydroxyl may not be required for these compounds to have biological activity.

Introduction

Although human cytomegalovirus (HCMV) is innocuous in the immunocompetent individual, it is a dangerous pathogen in immunocompromised individuals.[1] Individuals with the acquired immunodeficiency syndrome (AIDS) are vulnerable to the effects of this virus making the ability to control replication of HCMV an integral part of any program aimed at suppressing the proliferation of HIV in afflicted individuals.

The drugs currently approved for the treatment of HCMV are the nucleoside analog ganciclovir (DHPG)[2] and the pyrophosphate derivative foscarnet (PFA).[3] However, the clinical use of these compounds is limited because of host toxicity,[1-3] and recent reports suggest that strains of HCMV resistant to both drugs are emerging.[4]

In the search for a novel and more effective class of compounds for the treatment of HCMV infections, we have been evaluating various analogs of the pyrrolo[2,3-*d*]pyrimidine nucleosides toyocamycin, sangivamycin, and thiosangivamycin.[5] For example, we have reported that good activity against HCMV coupled with a reduction in cytotoxicity occurred with two acyclic 5-thioamide-substituted pyrrolo[2,3-*d*]pyrimidines whereas similar acyclic analogs of the corresponding 5-cyano derivatives were inactive and nontoxic.[6] Separate studies by Smee and co-workers[7] established broad antiviral activity for *ara*-thiosangivamycin and 2′-deoxythiosangivamycin, although cytotoxicity and antiviral activity were not well-separated. These data suggest that other 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamides with various carbohydrate substituents at the N-7 position should be synthesized and evaluated for activity against HCMV. This report describes such a study.

Chemistry

The synthesis of 2′-deoxytoyocamycin (**1**) was accomplished by coupling the sodium salt of 3,4-dicyano-2-(ethoxymethyleneamino)pyrrole, synthesized by a reductive debromination of 2-amino-5-bromo-3,4-dicyanopyrrole,[8] with 3,5-di-*O*-*p*-toluyl-2-deoxy-α-D-*erythro*-pentofuranosyl chloride[9] followed by a concomitant ring closure and deblocking of the intermediate glycosylated pyrrole with methanolic ammonia to afford the desired 2′-deoxytoyocamycin (**1**). The identity of compound **1** was confirmed by a comparison of UV and ^{1}H NMR spectral data with the data reported for 2′-deoxytoyocamycin (**4**) which was synthesized by two independent routes.[10,11] The synthesis of *xylo*-toyocamycin (**2**) was accomplished by a coupling of the sodium salt of 3,4-dicyano-2-(ethoxymethyleneamino)pyrrole with 2,3,5-tri-*O*-benzyl-D-xylofuranosyl bromide.[12a] This was followed by a ring closure of the anomeric mixture of the glycosylated pyrroles, chromatographic separation of the anomers of 2,3,5-tri-*O*-benzyl-D-*xylo*-toyocamycin, and deblocking of the desired β anomer with boron trichloride to yield the free nucleoside **2**. The anomeric configuration of **2** was confirmed by a conversion of the

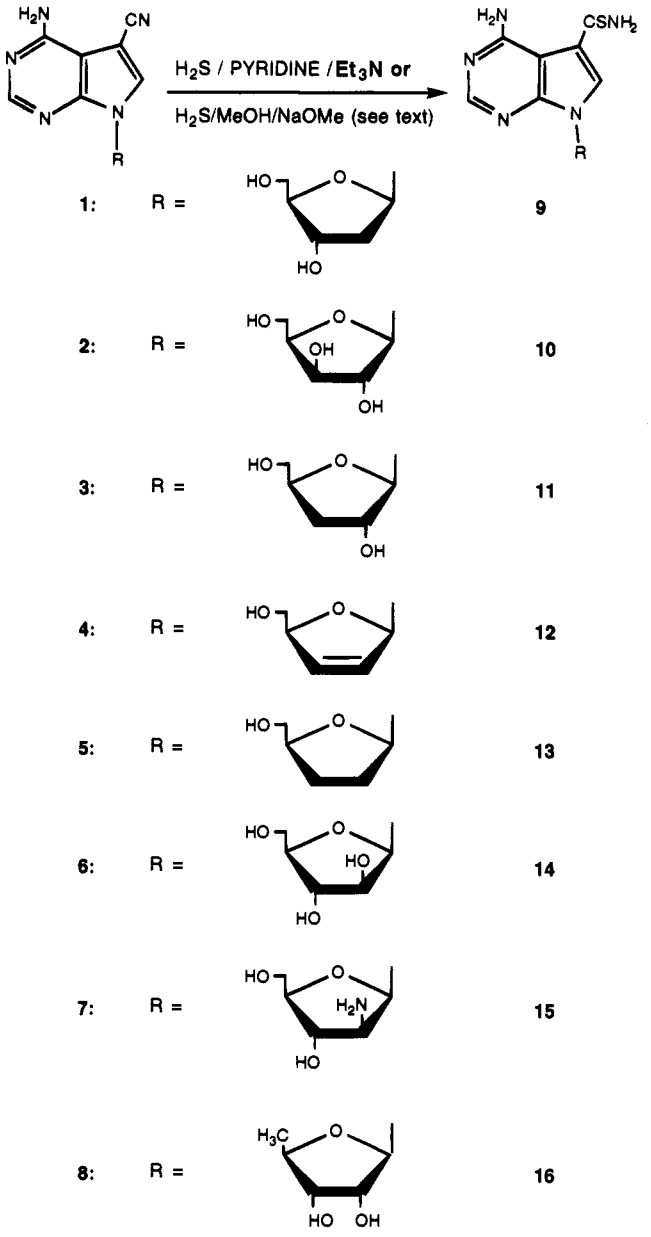
† Present address: Gilead Sciences, Foster City, CA 94404.
® Abstract published in *Advance ACS Abstracts,* May 1, 1995.

0022-2623/95/1838-4115$09.00/0

nitrile to the carboxamide moiety with hydrogen peroxide in ammonium hydroxide and comparison of the ^{1}H NMR spectrum of the resulting *xylo*-sangivamycin with that of an authentic sample which had been prepared by an alternate[12a] procedure from sangivamycin. The synthesis of 3′-deoxytoyocamycin (**3**) was accomplished by the modification of a known procedure.[13] Synthesis of the other 7-substituted toyocamycin derivatives was accomplished as follows: the 2′,3′-dideoxy-2′,3′-didehydro- and 2′,3′-dideoxytoyocamycin analogs (**4** and **5**) were obtained as previously described by us;[14] the synthesis of *ara*-toyocamycin (**6**) was accomplished by following a literature procedure;[15] the 2′-deoxy-2′-amino-*ara*-toyocamycin analog (**7**) was prepared by our previously reported procedures;[16] and the synthesis of 5′-deoxytoyocamycin (**8**) was accomplished in two steps from toyocamycin by a published procedure.[17]

The syntheses of the 5-thiocarboxamide substituted derivatives (**9**−**16**) were relatively straightforward and involved a conversion of the 5-cyano moiety to the 5-thiocarboxamide moiety (Scheme 1). This conversion was accomplished by the treatment of a pyridine solution of the appropriate carbonitrile (**1**−**7**) with hydrogen sulfide in the presence of triethylamine, followed by crystallization of the products. In the case of **16**, 5′-deoxytoyocamycin (**8**) was treated with hydrogen sulfide in methanol in the presence of sodium methoxide to generate 5′-deoxythiosangivamycin (**16**). The lack of a cyano absorption band in the IR spectrum of each of the products as well as their characteristic UV absorption spectra confirmed the success of each conversion.

Scheme 1. Synthesis of 7-Substituted Thiosangivamycin Analogs



Biological Evaluations

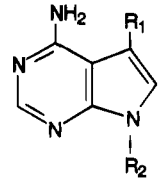
We have recently shown[18] that 7-substituted 4-aminopyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamides are converted to their corresponding 5-carbonitrile derivatives *in vitro*. Although we did not examine **9**−**16** for this phenomenon in the present study, we expect they would undergo this conversion in cell culture media. Hence, the biological data for compounds **9**−**16** may be the result of a mixture of 5-thioamide and 5-carbonitrile derivatives. Recent evidence has shown, however, that many acyclic 5-thioamide pyrrolo[2,3-*d*]pyrimidines have completely different biological properties when compared to their corresponding 5-nitrile analogs.[6,19] These are similar to the differences shown below for compounds **1/9**, **5/13**, and **6/14**.

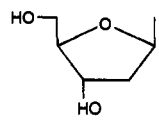
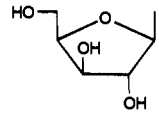
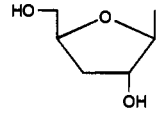
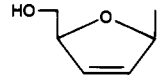
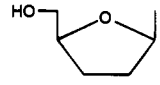
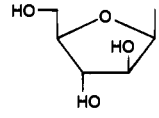
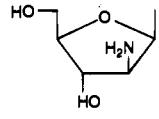
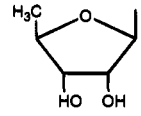
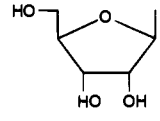
Antiproliferative Activity. The anticancer potential of this series of compounds was studied by evaluating their ability to inhibit the proliferation of L1210 murine leukemic cells *in vitro* (Table 1). The modifications introduced in the ribosyl moiety of toyocamycin (**A**) and thiosangivamycin (**B**) uniformly led to a decrease in cytotoxic potency. Among the analogs, there was a clear pattern of greater cytotoxic potency for those with 3′-modifications, i.e., xylosyl (**2** and **10**) or 3′-deoxy (**3** and **11**), than for those with 5′, 2′, or 2′,3′ modifications. Similar structure−activity patterns have been observed previously for the pyrrolo[2,3-*d*]pyrimidine nucleosides having a $CONH_2$ group (sangivamycin analogs)[12b,13] or a H (tubercidin analogs)[13,20] at the 5-position.

Antiviral Activity. The series of compounds was evaluated for activity against HCMV and HSV-1. As we reported previously,[5] toyocamycin (**A**) appeared to be active against HCMV and HSV-1, but this was a result of high cytotoxicity and not specific antiviral activity. The same appears to be the case for thiosangivamycin (**B**, Table 1). Replacement of ribose with the other sugars reported herein provided compounds that had reduced cytotoxicity and retained activity against HCMV and, to a lesser extent, against HSV-1. These modifications, however, did not completely separate antiviral activity from cytotoxicity (Table 1).

In general, there was excellent agreement between the results of cytotoxicity assays performed in HFF and KB cells as part of antiviral evaluations and those described above for antiproliferative activity (cytotoxicity) in L1210 cells. The activity and lack of separation of antiviral and antiproliferative activity with 5′-deoxythiosangivamycin (**16**) is particularly interesting because it establishes that phosphorylation of the 5′-position is not required for activity, confirming our recent report.[19] Our continuing investigations with non-

Table 1. Antiviral and Antiproliferative Activity of 4-Amino-7-substitutedpyrrole[2,3-*d*]pyrimidine-5-carbonitriles and -5-thiocarboxamides



			50 or 90% Inhibitory Concentration (μM)					
			Antiviral activity[a]			Antiproliferative activity[b]		
			HCMV		HSV-1			
Cmpd	R_1	R_2	plaque	yield	ELISA	HFF	KB	L1210
1	CN		35	13	4.5	66	25[d]	80
9	$CSNH_2$		5.7	15	8.2	41	7[d]	3.9
2	CN		0.3	0.3	0.4	0.3	0.1	0.1
10	$CSNH_2$		0.6	0.4	1.9	0.6	1.0[d]	0.5
3	CN		0.4	1.4	2.6	3.0	4	0.1
11	$CSNH_2$		0.5	3[d]	58	2.1	3[d]	0.6
4	CN		5.1[d]	>100[d,e]	41[d]	>100[d,e]	176	---[f]
12	$CSNH_2$		2.7	5[d]	14[d]	18	5	3
5	CN		19	90	21[d]	>100[e]	88[d]	---[f]
13	$CSNH_2$		2.5	12[d]	6	29	11	24
6	CN[g]		1.7	14	29	8	17	---[f]
14	$CSNH_2$		0.9	1.9[d]	4.7[d]	29	2	5
7	CN[g]		14	110	320	>100[e]	105[d]	100
15	$CSNH_2$		1.6	41[d]	28	73	2.5	2
16	$CSNH_2$		3.1	---[f]	2.5[d]	3.2	0.5	3
A[c]	CN		0.1		---[f]	0.03	0.03[d]	0.004
B	$CSNH_2$		0.4	0.1[d]		0.32	0.03	0.03
	Ganciclovir (DHPG)[h]		7.7	1.8	3.5	>100[e]	>100[e]	---[f]

[a] Plaque and yield reduction assays were performed as described in the text. Results from plaque assays are reported as IC_{50}'s, those for yield reduction experiments as IC_{90}'s. [b] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell and L1210 cell proliferation was determined as described in the text. [c] Portions of the results for toyocamycin (**A**) and thiosangivamycin (**B**) have been reported previously.[5,19] [d] Results from single experiment. All other results are averages from two or four experiments. [e] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [f] Not done. [g] Results reported previously in reference 5 and/or 16. [h] Average of >50 experiments in which DHPG was used as a positive control.

phosphorylatable derivatives of thiosangivamycin will be described in several forthcoming reports.

Experimental Section

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo-[2,3-*d*]pyrimidine-5-thiocarboxamide (9). Hydrogen sulfide gas was passed through a solution of 4-amino-5-cyano-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1, 0.4 g, 1.45 mmol) in pyridine (25 mL) containingtriethylamine (1 mL) over a period of 3 h. The solution was evaporated, the residue was coevaporated with water (10 mL), and the resulting solid was dissolved in boiling water (25 mL).

The hot solution was treated with decolorizing carbon and filtered, and the product was allowed to crystallize. The resulting solid was recrystallized from water/ethanol (9:1, v:v, 20 mL) to afford 0.17 g of product **9**: mp 210–211 °C dec (lit.[10] mp >230 °C); ^{1}H NMR (DMSO-d_6) δ 9.62, 9.41 (2bs, 2H, $CSNH_2$), 8.10 (s, 1H, H-2), 7.90 (bs, 3H, H-2, NH_2), 6.53 (dd, 1H, H-1′, $J_{1',2'a} = J_{1',2'b} = 6.6$ Hz), 5.27 (d, 1H, 3′-OH), 4.98 (t, 1H, 5′-OH), 4.35 (m, 1H, H-3′), 3.83 (m, 1H, H-4′), 3.54 (m, 2H, H-5′ab), 2.41, 2.23 (2m, 2H, H-2′ab); IR (KBr) no cyano absorption; TLC R_f = 0.41, chloroform/methanol, 4:1, v:v. Anal. ($C_{12}H_{15}N_5O_3S$) C, H, N.

4-Amino-7-β-D-xylofuranosylpyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (10). Hydrogen sulfide gas was passed through a solution of 4-amino-5-cyano-7-β-D-xylofuranosylpyrrolo[2,3-*d*]pyrimidine (**2**, 0.4 g, 1.37 mmol) in pyridine (25 mL) containing triethylamine (1 mL) over a period of 3 h. The solution was evaporated, the residue was coevaporated with water (10 mL), and the resulting solid was dissolved in boiling water (25 mL). The hot solution was treated with decolorizing carbon and filtered, and the product was allowed to crystallize to afford 0.34 g (69%) of product **10**: mp 119–120 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.57, 9.50 (2bs, 2H, $CSNH_2$), 8.13 (s, 1H, H-6), 7.91 (s, 1H, H-2), 7.84 (bs, 2H, NH_2), 6.02 (d, 1H, H-1′, $J_{1',2'} = 1.7$ Hz), 5.81 (d, 1H, 2′-OH), 5.57 (d, 1H, 3′-OH), 4.67 (t, 1H, 5′-OH), 4.25–4.00 (3m, 3H, H-2′,3′,4′), 3.73 (m, 1H, H-4′), 3.54 (m, 2H, H-5′ab), 2.41, 2.23 (2m, 2H, H-2′ab); UV λ_{max} (nm) (log ϵ) (MeOH) 287 (4.10), 247 (4.06), 209 (4.36); (pH 1) 293 (4.04), 243 (4.18), 206 (4.29); (pH 11) 281 (4.11), 260 (4.05); IR (KBr) no cyano absorption; TLC R_f = 0.31, chloroform/methanol, 4:1, v:v. Anal. ($C_{12}H_{15}N_4O_3S$) C, H, N.

4-Amino-7-(3-deoxy-β-D-erythro-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (11). Hydrogen sulfide gas was passed through a solution of 4-amino-5-cyano-7-(3-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**3**, 0.4 g, 1.45 mmol) in pyridine (25 mL) containing triethylamine (1.5 mL) over a period of 4 h. The flask was stoppered, and the mixture was stirred at room temperature for an additional 14 h. The solution was then evaporated, and the residue was triturated with methanol (10 mL). The solid which formed was recrystallized twice from water (100 mL) to yield 0.21 g (47%) of **11**: mp 234–235 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.58, 9.39 (2bs, 2H, $CSNH_2$), 8.11, 7.89 (2s, 2H, H-6, H-2), 7.88 (bs, 2H, NH_2), 6.08 (d, 1H, H-1′, $J_{1',2'} = 1.6$ Hz), 5.61 (d, 1H, 2′-OH), 4.95 (t, 1H, 5′-OH), 4.38–4.30 (2m, 2H, H-2′, H-4′), 3.66, 3.55 (2m, 2H, H-5′ab), 2.15, 1.91 (2m, 2H, H-3′ab); UV λ_{max} (nm) (log ϵ) (MeOH) 288 (4.12), 247 (4.09), 206 (4.47); (pH 1) 294 (4.11), 243 (4.24), 205 (4.38); (pH 11) 282 (4.09), 260 (4.01); IR (KBr) no peak for a cyano group; TLC R_f = 0.45, chloroform/methanol, 4:1, v:v. Anal. ($C_{12}H_{15}N_5O_3S$) C, H, N.

4-Amino-7-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (12). Hydrogen sulfide gas was passed through a solution of 4-amino-5-cyano-7-(2,3-dideoxy-2,3-didehydro-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**, 0.23 g, 0.9 mmol) in pyridine (10 mL) containing triethylamine (0.2 mL) for 5 h. The solution was purged with argon gas for 10 min and then evaporated to dryness. The residue was coevaporated with ethanol (3 × 10 mL), and the residue was dissolved in hot ethanol (*ca.* 80 °C, 15 mL) and treated with Norit, the solution was filtered, and the filtrate was allowed to stand at room temperature for 16 h. The product which crystallized was collected by filtration and washed with ethanol (5 mL) to afford 0.14 g (53%) of **12** as a yellow solid: mp 186–187 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.58, 9.42 (2bs, 2H, $CSNH_2$), 8.13 (s, 1H, H-2), 7.90 (bs, 2H, NH_2), 7.73 (s, 1H, H-6), 7.16 (m, 1H, H-1′), 6.49 (d, 1H, H-3′, $J_{2',3'} = 5.8$ Hz), 6.03 (d, 1H, H-2′), 4.92 (t, 1H, 5′-OH), 4.83 (bs, 1H, H-4′), 3.55 (m, 2H, H-5′ab); UV λ_{max} (nm) (log ϵ) (MeOH) 286 (4.36), 245 (4.34), 223 (4.34), 208 (4.69); (pH 1) 290 (4.41), 241 (4.59), 205 (4.74); (pH 11) 281 (4.41), 242 (4.37); TLC R_f = 0.21 (chars blue), chloroform/methanol, 9:1, v:v. Anal. ($C_{12}H_{13}N_5O_2S$) C, H, N.

4-Amino-7-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (13). Compound **13** was prepared by the same method as compound **12**. From 0.32 g (1.2 mmol) of 4-amino-5-cyano-7-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**5**) was obtained 0.18 g (50%) of **13**: mp 188–190 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.57, 9.41 (2bs, 2H, $CSNH_2$), 8.10 (s, 1H, H-2), 7.97 (bs, 3H, H-6, NH_2), 6.40 (dd, 1H, H-1′), 4.88 (t, 1H, 5′-OH), 4.06 (q, 1H, H-4′), 3.58 (m, 2H, H-5′ab), 2.37, 2.19 (2m, 2H, H-2′ab), 1.97 (m, 2H, H-3′ab); UV λ_{max} (nm) (log ϵ) (MeOH) 287 (4.42), 247 (4.39), 206 (4.74); (pH 1) 292 (4.41), 242 (4.55), 204 (4.71); (pH 11) 282 (4.44), 260 (4.40), 244 (4.39); TLC R_f = 0.24 (chars pink), chloroform/methanol, 9:1, v:v. Anal. ($C_{12}H_{15}N_5O_2S$) C, H, N.

4-Amino-7-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (14). This compound was prepared by the same method as **12**, except that after treating the solution with hydrogen sulfide gas for 5 h the solution was stirred in a sealed flask for 16 h, and the residue obtained after evaporation of the solution was crystallized from water/ethanol (9:1, v:v, 10 mL). Thus, from 0.113 g (0.93 mmol) of 4-amino-5-cyano-7-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**) was obtained 0.06 g (48%) of **14**: mp 243–244 °C (lit.[7] mp 260–262 °C); ^{1}H NMR (DMSO-d_6) δ 9.51, 9.38 (2bs, 2H, $CSNH_2$), 8.09 (s, 1H, H-2), 7.91 (bs, 2H, NH_2), 7.85 (s, 1H, H-6), 6.45 (d, 1H, H-1′, $J_{1',2'} = 4.56$ Hz), 5.46 (2m, 2H, 2′-OH, 3′-OH), 4.93 (t, 1H, 5′-OH), 4.08 (2m, 2H, H-2′, H-3′), 3.76, 3.66 (2m, 3H, H-4′ and H-5′ a,b), 3.49 (dd, 1H, H-2′); UV λ_{max} (nm) (log ϵ) (MeOH) 288 (4.35), 207 (4.72); (pH 1) 291 (4.40), 242 (4.53), 206 (4.66); (pH 11) 282 (4.38), 260 (4.32); TLC R_f = 0.29, ethylacetate/cyclohexane 1:3, v:v. Anal. ($C_{12}H_{15}N_5O_4S$) C, H, N.

4-Amino-7-(2-deoxy-2-amino-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (15). This compound was prepared by the same method as for **14**. From 0.4 g (1.4 mmol) of 4-amino-5-cyano-7-(2-deoxy-2-amino-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**7**) was obtained 0.39 g (87%) of **15**: mp 223–225 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.55, 9.43 (2bs, 2H, $CSNH_2$), 8.08 (s, 1H, H-2), 7.86 (bs, 2H, NH_2), 7.83 (s, 1H, H-6), 6.42 (d, 1H, H-1′, $J_{1',2'} = 6.45$ Hz), 5.37 (d, 1H, 3′-OH), 3.99 (dd, 1H, H-3′), 3.7–3.6 (m, 3H, H-4′ and H-5′a,b), 3.49 (dd, 1H, H-2′); UV λ_{max} (nm) (log ϵ) (MeOH) 286 (4.38), 247 (4.34), 207 (4.74); (pH 1) 289 (4.41), 240 (4.52), 205 (4.68); (pH 11) 282 (4.41); TLC R_f = 0.14, ethylacetate/cyclohexane, 1:3, v:v. Anal. ($C_{12}H_{16}N_6O_3S \cdot 0.5H_2O$) C, H, N.

4-Amino-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-thiocarboxamide (5′-Deoxythiosangivamycin, 16). H_2S(g) was passed through a solution of dry MeOH (25 mL) containing NaOMe (59 mg, 1.1 mmol) for 30 min, at which time the solution was transferred to a pressure bottle containing **8** (150 mg, 0.55 mmol). The bottle was sealed and heated to 88 °C for 1.5 h in an oil bath. The pH of the solution was adjusted to 7 with 1 N HCl, and the compound was purified by column chromatography (13% MeOH/$CHCl_3$), yielding 138 mg (83%) of **16**. This sample was recrystallized from aqueous ethanol to furnish pure **16**: mp 144 °C (foams); UV λ_{max} (nm) (ϵ mM) (MeOH) 240 (17.7), 296 (11.7); (pH 1) 241 (15.2), 291 (11.2); (pH 11) 290 (11.9); IR (KBr) no cyano absorption; ^{1}H NMR (DMSO-d_6) δ 9.65 and 9.47 (2brs, 1H each, $CSNH_2$), 8.11 (s, 1H, H-2), 7.96 (br s, 2H, NH_2), 7.82 (s, 1H, H-6), 6.07 (d, 1H, H-1′), 5.43 (d, 1H, 2′-OH), 5.12 (d, 1H, 3′-OH), 4.25 (q, 1H, H-2′), 3.86 (m, 2H, H-3′, H-4′), 1.30 (d, 3H, CH_3). Anal. ($C_{12}H_{15}N_5O_3S \cdot 0.5H_2O$) C, H, N.

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was evaluated as described previously,[21] except that cell number was determined only at 0, 48, and 96 h. When the growth rate in the treated cultures decreased during the experiment, the rate used was that between 48 and 96 h.

***In Vitro* Antiviral Evaluation. (1) Cells and Viruses.** Cell lines were subcultured according to conventional procedures previously described.[5] Stock preparations of HCMV and HSV-1 were prepared as described elsewhere.[5] **(2) Assays for Antiviral Activity.** The effect of compounds on the replication of HCMV and HSV-1 has been measured using plaque reduction assays,[5] ELISA's,[22] and a yield reduction assay.[6]

Cytotoxicity Assays. Two different assays were used to explore cytotoxicity of selected compounds using methods we have detailed previously. (i) Cytotoxicity produced in stationary HFF cells was determined by microscopic inspection of cells

not affected by the virus used in plaque assays.[5] (ii) The effect of compounds on proliferation of KB cells during two population doublings of the controls was determined by crystal violet staining and spectrophotometric quantification of dye eluted from stained cells as described earlier.[19]

Acknowledgment. The authors thank Lisa A. Coleman, Sandra L. Engelhardt, James S. Lee, and Mary Ann Walters for their expert technical assistance, Jack Hinkley for his large-scale preparation of starting materials, and Patti Compton and Marina Savic for manuscript preparation. This study was supported by Health and Human Services Contracts NO1-AI42554 and AI72641 and Grant U01-AI31718 from the National Institute of Allergy and Infectious Diseases and by Research Grant DHP-36 from the American Cancer Society.

Supporting Information Available: The preparation of the 5-cyano-substituted compounds **1**–**8** (8 pages). Ordering information is given on any current masthead page.

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JM940735C