Synthesis and Antiproliferative and Antiviral Activity of Carbohydrate-Modified Pyrrolo[2,3-*d*]pyridazin-7-one Nucleosides

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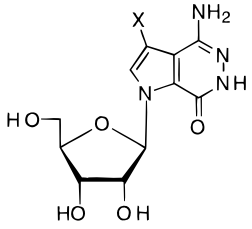
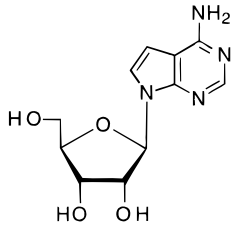
Sugar-modified analogs of 4-amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**1**) and 4-amino-3-bromo-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**3**) were prepared in an effort to obtain selective antiviral agents. Treatment of ethyl 3-cyano-1-(2,3,5-tri-*O*-benzyl-1-β-D-arabinofuranosyl)pyrrole-2-carboxylate (**6**) with hydrazine afforded 4-amino-1-(2,3,5-tri-*O*-benzyl-1-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**7**). Treatment of **7** with bromine afforded 4-amino-3-bromo-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one hydrobromide (**9**). The benzyl ether functions of **7** and **9** were removed with boron trichloride to afford 4-amino-1-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**8**) and its 3-bromo analog **10**. 4-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**13**) was prepared by the sodium salt condensation of ethyl 3-cyanopyrrole-2-carboxylate (**5**) with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**11**) followed by ring annulation with hydrazine. Deprotection of ethyl 3-cyano-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrole-2-carboxylate (**12**) using sodium ethoxide furnished ethyl 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3-cyanopyrrole-2-carboxylate (**14**) which served as the starting material for the preparation of 4-amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**20**). Selective protection of the 5′-hydroxyl group of **14** with *tert*-butyldimethylsilyl chloride followed by a Barton type deoxygenation sequence of the 3′-hydroxyl groups afforded ethyl 3-cyano-1-[2,3-dideoxy-5-*O*-*tert*-butyldimethylsilyl)-β-D-*glycero*-pentofuranosyl]pyrrole-2-carboxylate (**18**). Deprotection of **18** with tetra-*n*-butylammonium fluoride and ring annulation with hydrazine afforded **20**. The acyclic analog 4-amino-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyridazin-7-one (**24**) was prepared *via* the sodium salt glycosylation of **5** with (1,3-dihydroxy-2-propoxy)methyl bromide (**22**) followed by a ring annulation with hydrazine. *N*-Bromosuccinimide treatment of **13**, **20**, and **25** afforded the 3-bromo derivatives **15**, **21**, and **25**. Evaluation of these compounds in L1210, HFF, and KB cells showed that the sugar-modified analogs all were less cytotoxic than their corresponding ribonucleoside analogs. The compounds also were less active against human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1). The 3-bromo derivatives were much more active than the 3-unsubstituted analogs in both the cytotoxicity, and antiviral assays. However, there was only modest separation between activity against HCMV and cytotoxicity and there was virtually no selectivity for activity against HSV-1.

Introduction

As reported previously from these laboratories,[1−3] a novel series of 4-amino-3-halo-1-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyridazin-7-ones **2**−**4** have significant antiviral and antiproliferative activity. However, the antiviral potential of these compounds was tempered by their cytotoxicity to uninfected cells. In an effort to decrease the cytotoxic effects of the compounds and obtain more selective antiviral (human cytomegalovirus) agents, an investigation of 4-aminopyrrolo[2,3-*d*]pyridazin-7-one nucleosides with modified glycosyl moieties was undertaken.



1, X=H
2, X-Cl
3, X=Br
4, X=I



5 tubercidin

We initiated this investigation on the basis of the antiviral selectivity obtained previously when sugar modifications were introduced in a series of pyrrolo[2,3-*d*]pyrimidine nucleosides and some structurally related compounds. The ribonucleosides showed potent cytotoxic and antiviral effects but with little selectivity. The antiviral selectivity was improved by replacing the 7-ribofuranosyl moiety with a xylofuranosyl,[4] a 2-deoxyribofuranosyl or an arabinofuranosyl moiety.[5] More recently the replacement of the carbohydrate moiety of several 4,5-disubstituted pyrrolo[2,3-*d*]pyrimidine nucleosides with the acyclo side chains hydroxyethoxymethyl[6] or (1,3-dihydroxy-2-propoxy)methyl[7] (DHPM) also led to compounds with greater antiviral selectivity.

Using the above rationale, we have adopted a similar strategy for the synthesis of 4-aminopyrrolo[2,3-*d*]-

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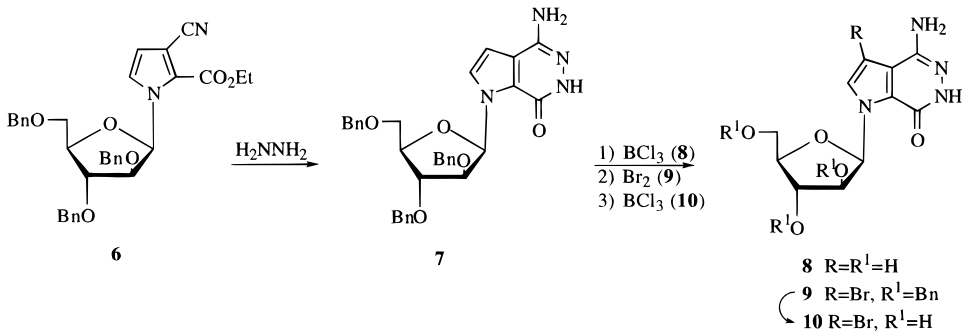
Scheme 1



pyridazin-7-one nucleosides in an effort to obtain more selective antiviral agents by replacing the 1-ribosyl moiety with 1-(arabinofuranosyl), 1-(2-deoxyribofuranosyl), 1-(2,3-dideoxyribofuranosyl), or 1-[(2,3-dihydroxy-2-propoxy)methyl]. We report herein the synthesis and biological evaluation of these compounds.

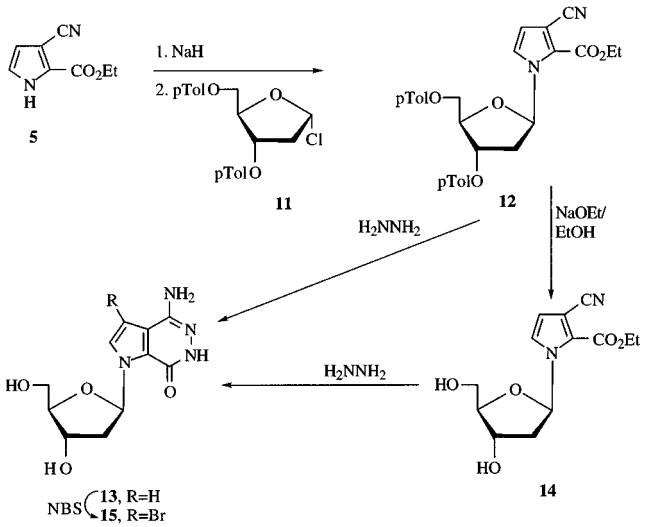
Results and Discussion

Chemistry. In order to obtain various sugar-modified analogs of the 4-aminopyrrolo[2,3-*d*]pyridazin-7-one nucleosides, we elected to use pyrrole glycosides as intermediates. The various pyrrole glycosides were prepared *via* the sodium salt glycosylation[8] of ethyl 3-cyanopyrrole-2-carboxylate[9] with the appropriate 1-haloglycoside. The various pyrrole glycosides were then ring annulated with hydrazine to furnish the 4-amino-1-glycosylpyrrolo[2,3-*d*]pyridazin-7-one nucleosides.

For the synthesis of 4-amino-1-(β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyridazin-7-one (**8**) and the 3-bromo analog **10**, ethyl 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole-2-carboxylate[3] (**6**) served as the starting material. Ring annulation of **6** using hydrazine in ethanol at reflux temperature proceeded smoothly to afford 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**7**) in 73% yield. The deprotection of **7** was accomplished using boron trichloride. The bromination of **7** using bromine in dichloromethane followed by deprotection afforded 4-amino-3-bromo-1-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**10**). An examination of the ^{1}H NMR spectrum of **10** and 4-amino-3-bromo-1-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyridazin-7-one (**3**) allowed us to assign the anomeric configuration for **8** and **10**. In **10**, a doublet corresponding to the anomeric proton was observed at δ 7.05 which was downfield from the peaks at δ 6.84 corresponding to the anomeric proton of the ribofuranosyl analog **3**. The loss of the shielding effect of the neighboring 2′-hydroxyl group in **10** was consistent with this observation.[10]

The preparation of the 2′-deoxyribofuranosyl analogs of the pyrrolo[2,3-*d*]pyridazin-7-ones was initiated from the sodium salt condensation of **5** with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride (**11**)[11] which furnished ethyl 3-cyano-1-(3,5-di-*O*-*p*-toluoyl-2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carboxylate (**12**). The β-configuration for **12** was determined from the ^{1}H NMR spectrum. The peaks corresponding to the anomeric proton appeared as a pseudotriplet which was in accord with similar observations for other β-2′-deoxyribonucleosides.[12] The direct treatment of **12** with hydrazine in ethanol effected ring annulation with a concomitant deprotection of the toluoyl blocking

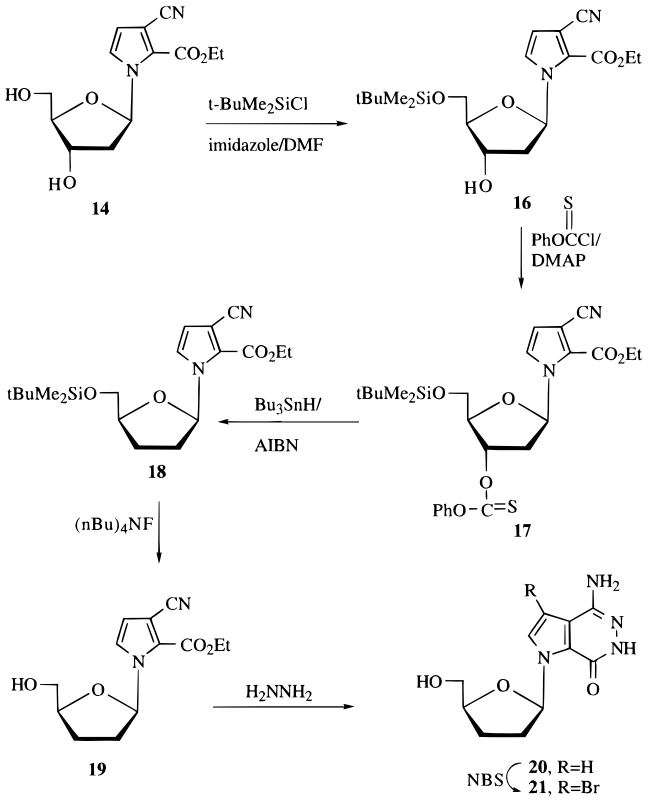
Scheme 2



groups to furnish 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**13**). For larger scale preparations of **13**, it proved to be more convenient to use a two-step procedure. Using this modification the *p*-toluoyl groups of **12** were removed with a solution of sodium ethoxide in ethanol to afford ethyl 3-cyano-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrole-2-carboxylate (**14**), which was then ring annulated using hydrazine to provide **13**. Treatment of **13** with *N*-bromosuccinimide in acetic acid furnished a good yield of 4-amino-3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyridazin-7-one (**15**). From the ^{1}H NMR spectrum of **15** it was apparent that monobromination had occurred at the 3-position. In the ^{1}H NMR spectrum of **13** the peaks corresponding to H-2 and H-3 appeared as doublets at δ 7.70 and 6.63, respectively. In the ^{1}H NMR spectrum of the bromo analog **15**, we observed a disappearance of the upfield peak corresponding to H-3 and a small downfield shift to δ 7.96 of the singlet corresponding to H-2. This observation was in good agreement with similar peak patterns observed in the ^{1}H NMR spectra of the ribofuranosyl analogs **1** and **3**, whose site of bromination had been established unequivocally by ^{13}C NMR.[3]

Compound **14** also served as a useful starting material for the preparation of the 2′,3′-dideoxy analogs 4-amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**20**) and 4-amino-3-bromo-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]-pyridazin-7-one (**21**), respectively. In order to selectively manipulate the 3′-hydroxyl, the 5′-primary hydroxyl group had to be protected. This manipulation was accomplished by a selective silylation of **14** using *tert*-butyldimethylsilyl chloride and imidazole in dimethyl-

Scheme 3



formamide. That the 5′-hydroxyl group was selectively silylated was determined by the loss of the D_2O exchangeable triplet corresponding to the 5′-OH in the ^1H NMR spectrum of ethyl 3-cyano-1-[5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-*β*-D-*erythro*-pentofuranosyl]pyrrole-2-carboxylate (**16**). The reductive deoxygenation of **16** was accomplished using a Barton type procedure.[13] Ethyl 3-cyano-1-[5-*O*-*tert*-butyl-3-*O*-(phenoxythiocarbonyl)-2-deoxy-*β*-D-*erythro*-pentofuranosyl]pyrrole-2-carboxylate (**17**) was prepared by the treatment of **16** with phenyl chlorothionoformate in the presence of 4-(dimethylamino)pyridine. A reduction of the thiocarbonate moiety of **17** to yield ethyl 3-cyano-1-[5-*O*-*tert*-butyldimethylsilyl)-2,3-dideoxy-*β*-D-*glycero*-pentofuranosyl]pyrrole-2-carboxylate (**18**) was then accomplished by heating **17** and tri-*n*-butyltin hydride in the presence of AIBN. Treatment of **18** with tetra-*n*-butylammonium fluoride successfully removed the 5′-silyl protecting group and furnished ethyl 3-cyano-1-(2,3-dideoxy-*β*-D-*glycero*-pentofuranosyl)pyrrole-2-carboxylate (**19**). The ring annulation of **19** with hydrazine provided 4-amino-1-(2,3-dideoxy-*β*-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]-pyridazin-7-one (**20**). The λ_{max} of 288 nm in the UV spectrum of **20** was identical with the λ_{max} of its corresponding ribofuranosyl analog **1**,[3] supporting the fact that ring closure to the pyrrolo[2,3-*d*]pyridazin-7-one heterocycle had occurred. Finally, 4-amino-3-bromo-1-(2,3-dideoxy-*β*-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**21**) was obtained by the bromination of **20** with *N*-bromosuccinimide. A comparison of the chemical shift patterns for the H-2 and H-3 protons in the ^1H NMR spectra of **20** and **21** with the corresponding patterns in the ribofuranosyl analogs **1** and **3** established the 3-position as the site of bromination *(vide supra)*.

The acyclo analog 4-amino-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyridazin-7-one (**24**) was again obtained using methods similar to those described above. Alkylation of the sodium salt of **5** with (1,3-diacetoxy-2-propoxy)methyl bromide (**22**)[14] afforded ethyl 3-cyano-1-[(1,3-diacetoxy-2-propoxy)methyl]pyrrole-2-carboxylate (**23**). The ring annulation and deprotection of **23** was accomplished using hydrazine in ethanol at reflux temperature to furnish 4-amino-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyridazin-7-one (**24**). The UV spectrum of **24** with a λ_{max} at 287 nm was in good agreement with the pyrrolo[2,3-*d*]pyridazin-7 one structure *(vide supra)*. 4-Amino-3-bromo-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo[2,3-*d*]pyridazin-7-one (**25**) was prepared *via* *N*-bromosuccinimide treatment of **24**. A comparison of the shift patterns for the H-2 and H-3 protons in the ^1H NMR spectra of **24** and **25** was in accord with the 3-position being the site of bromination *(vide supra)*.

Antiviral and Antiproliferative Studies. The antiproliferative activity of the compounds was evaluated in L1210 murine leukemic cells. The results of these evaluations, along with those reported previously[3] for the corresponding ribosyl derivatives **1**−**4**, are shown in Table 1. The 3-halo-substituted nucleosides **2**−**4**, **10**, **15**, and **21** all had more potent antiproliferative activity than their 3-unsubstituted counterparts. The carbohydrate moiety also had a strong influence on antiproliferative activity in this series of compounds. The 3-Br-pyrrolo[2,3-*d*]pyridazin-7-ones with a 1-arabinosyl, 1-(2-deoxyribosyl), or 1-(2,3-dideoxyribosyl) substituent were on the order of 100-fold less potent than the corresponding 1-ribosyl derivatives. Furthermore, replacing the furanosyl moiety with the acyclic (1,3-dihydroxy-2-propoxy)methyl side chain virtually abolished antiproliferative activity.

The antiviral effects of the compounds were investigated using human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1). To determine their selectivity, the cytotoxicities of compounds were investigated in uninfected stationary human foreskin fibroblasts (HFF cells) and growing KB cells (Table 2). Only compounds having a 3-halo substituent had significant antiviral activity. For these compounds the effect of replacing the ribofuranosyl moiety by a modified sugar decreased cytotoxicity to HFF and KB cells. Antiviral activity, however, was significantly lowered as well. It was of considerable interest that, in this series of compounds, replacing the ribofuranosyl moiety with the DHPM moiety (**25**) completely abolished the antiviral activity. In contrast, the arabinofuranosyl analog **10** was the most promising antiviral compound in this study with approximately a 10-fold difference between activity against HCMV and cytotoxicity in host HFF cells. Little or no selectivity for HSV-1 was observed (Table 2).

The goal of this investigation was to find more selective antiviral agents bearing the 4-amino-3-bromopyrrolo[2,3-*d*]pyridazin-7-one heterocycle. The findings in this investigation revealed that the sugar-modified derivatives were much less cytotoxic than their corresponding ribonucleoside **3**. Unfortunately there was a corresponding or even greater loss of activity against HCMV and HSV-1 leading to the conclusion that modification of the sugar did not lead to more selective antiviral agents.

Scheme 4

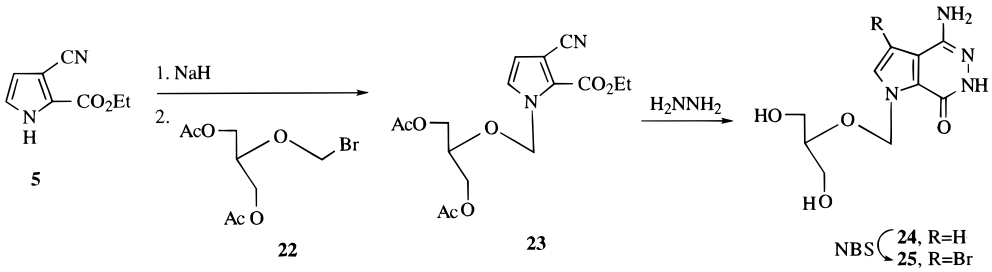
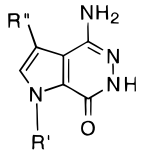


Table 1. Cytotoxicity of Pyrrolo[2,3-*d*]pyridazin-7-one Nucleosides in L1210 Cell Cultures



compd	substituent R′	substituent R″	IC_{50} (μM)[a]
1[b]	ribosyl	H	74
2[b]	ribosyl	Cl	0.23
3[b]	ribosyl	Br	0.10
4[b]	ribosyl	I	0.02
8	arabinosyl	H	100
10	arabinosyl	Br	18
13	2-deoxyribosyl	H	>100[c]
15	2-deoxyribosyl	Br	9.2
20	2,3-dideoxyribosyl	H	>100[c]
21	2,3-dideoxyribosyl	Br	38
24	DHPM	H	>100[c]
25	DHPM	Br	>100[c]
5 (tubercidin)[b]			0.043

[a] Concentration required to decrease growth rate to 50% of control. [b] Data for **1**−**4**[3] and **5**[7] have been reported previously.[c] At 100 μM, the highest concentration tested, cell proliferation was inhibited 0−50%.

Experimental Section

Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WP270SY spectrometer (solutions in dimethyl sulfoxide-d_6 or deuteriochloroform with tetramethylsilane as internal standard), with chemical shift values reported in δ, parts per million, relative to the internal standard. Carbon magnetic resonance (^{13}C NMR) spectra were obtained at 90 MHz in DMSO-d_6 with an IBM WM-360 spectrometer. Ultraviolet spectra were recorded on a Hewlett-Packard model 8450A UV/vis spectrophotometer. Infrared spectra were recorded on a Nicolet 5DXB FTIR spectrophotometer (μ, cm^{-1}). Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. E. Merck silica gel (230−400 mesh) was used for column chromatography. Thin layer chromatography was performed on silica gel GHLF-254 plates (Merck Reagents). R_f's were determined using the solvent system used to elute the column unless otherwise specified. Solvent systems are reported in vol:vol ratios. Compounds of interest were detected by either ultraviolet lamp (254 nm), iodine vapors, or treatment with 10% sulfuric acid in MeOH followed by heating. Evaporations were performed under reduced pressure with a bath temperature <35 °C, with a rotary evaporator using a water aspirator unless otherwise specified. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

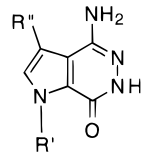
4-Amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyridazin-7-one (7). A solution of ethyl 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxylate[3] (**6**) (789 mg, 1.4 mmol) in EtOH (15 mL) was treated with anhydrous hydrazine (1.5 mL) in one portion. The resulting solution was heated at reflux under an argon atmosphere for 48 h. The reaction mixture was concentrated *in vacuo* to an oil which was coevaporated repeatedly with EtOH (3 × 10 mL) to remove the excess hydrazine. The resulting oil was dissolved in a minimal amount of a solution of $CHCl_3$:MeOH:acetic acid, 97:2:1, and applied to a column (3.2 × 20 cm) packed with silica gel (19.7 g) in the same solvent system. The column was eluted with the same solvent system, collecting 10 mL fractions. The fractions containing a product with an R_f = 0.42 ($CHCl_3$:MeOH, 20:1) were pooled and concentrated *in vacuo* to an oil. The oil was coevaporated with toluene (2 × 10 mL) and then ether (2 × 10 mL). The gum was further dried *in vacuo* (25 °C, oil pump) over P_2O_5 for 2 h to afford 558 mg (72.6%) of **7**. IR (neat): 3500−2700 valley, 1660, 1630, 1545, 1440, 1215, 745 cm^{-1}. UV (MeOH): λ_{max} nm (ϵ) 294 (3220). ^{1}H NMR ($CDCl_3$): δ 10.46 (s, 1H, H-6, D_2O exchangeable), 7.68 (d, J = 3.0 Hz, 1H, H-2), 7.42−6.93 (m, 16H, phenyl H's, H-1′), 6.30 (d, J = 3.2 Hz, 1H, H-3), 4.75−3.69 (m, 11H, $C_6H_5CH_2$, H-2′, H-3′, H-4′, H-5′). Anal. Calcd ($C_{32}H_{32}N_4O_5$) C, H, N.

4-Amino-1-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one Hydrochloride (8). A solution of **7** (573 mg, 1.1 mmol) in CH_2Cl_2 (6 mL), chilled by an external 2-propanol-CO_2 bath, was treated with a solution of BCl_3 (13 mL of 1 M solution in CH_2Cl_2) over the course of 40 min. After the addition was complete, the reaction mixture was stirred for an additional 1 h at −78 °C. An CH_3CN-C0_2 bath was then exchanged for the 2-propanol−CO_2 bath, and the solution was stirred for an additional 2.5 h. A solution of CH_2Cl_2:MeOH, 1:1 (16 mL, prechilled *via* an external CH_3CN-CO_2 bath), was added in three separate portions to quench the reaction. The reaction mixture was allowed to warm to room temperature at which point it became a white suspension. The suspension was concentrated *in vacuo*, and the resulting concentrate was coevaporated with MeOH (4 × 20 mL) to remove the excess BCl_3 as methyl borate. The resulting solid concentrate was suspended in MeOH (3 mL), allowed to stand for 15 h, collected by filtration, and then washed with ether to afford **8** as a white solid, 256 mg (75.4%), mp >216 °C dec. IR (KBr pellet): 3670−2500, 1679.7, 1651.6, 1560.2, 1433.6, 1215.6, 1060.9 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ϵ) 288 (5600); (pH 1) 281 (5600); (pH 11) 288 (6020). ^{1}H NMR (DMSO-d_6): δ 7.84 (d, 1H, H-2), 6.95 (br s, 1H, H-1′), 6.80 (d, 1H, H-3), 5.70−4.50 (br s, 4H, OH's, NH_2, D_2O exchangeable), 4.12 (t, 1H, H-2′), 4.00 (t, lH, H-3′), 3.79−3.60 (m, 3H, H-4′, H-5′). Anal. Calcd for ($C_{11}H_{15}N_4O_5$-$Cl\cdot{}^1/_2CH_3OH$) C, H, N.

4-Amino-3-bromo-1-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (10). A solution of 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (**7**; 481 mg, 0.87 mmol) in CH_2Cl_2 (50 mL) was treated with a solution of Br_2 (1.5 equiv) in CH_2Cl_2 (25 mL) by an addition funnel, dropwise, at room temperature, over the course of 3 h. The resulting mixture was allowed to stir at room temperature for 2 days. The yellow solid which precipitated from the mixture was collected by filtration to furnish 370 mg of the presumed HBr salt **9**.

A suspension of **9** in CH_2Cl_2 (10 mL) was chilled by an external 2-propanol−CO_2 bath. A solution of 1 M BCl_3 in CH_2Cl_2 (7 mL, 14 equiv) was added to the chilled reaction mixture over the course of 30 min. After the addition was complete, the external bath was removed and the reaction mixture was allowed to stir at room temperature over the course of 2 h. The reaction mixture was then rechilled by the external 2-propanol−CO_2 bath and a prechilled solution of MeOH: CH_2Cl_2,1:1 (8 mL, chilled *via* an external 2-propanol−CO_2 bath), was added to the mixture in one portion. The reaction mixture was again allowed to warm to room temperature over the course of 30 min. The mixture was concentrated *in vacuo*, and

Table 2. Antiviral Activity and Cytotoxicity of Pyrrolo[2,3-*d*]pyridazin-7-one Nucleosides



			50% or 90% inhibitory concentration (μM)				
			antiviral activity[a]				
	substituent		HCMV		HSV-1[b]	cytotoxicity[c]	
compd	R′	R″	plaque	yield	ELISA	HFF cells	KB cells
1[d,e]	ribosyl	H	>100[f]		90	>100	
2	ribosyl	Cl	0.2	0.7	6.8	2.1	6.1
3	ribosyl	Br	0.1	0.04	2.0	2.5	<1.2
4	ribosyl	I	0.4	0.02	4.5	1.0	1.2
8	arabinosyl	H	>100		90	>100	
10	arabinosyl	Br	2.7	6.3	20	32	15
13	2′-deoxyribosyl	H	>100		128	>100	>100[e]
15	2′-deoxyribosyl	Br	19	6.3	15	10	6.7
20	dideoxyribosyl[g]	H	>100		>100	>100	>100[e]
21	dideoxyribosyl	Br	12	10[e]	27	32	10[e]
24	DHPM[g]	H	>100		>100[e]	>100	>100[e]
25	DHPM	Br	>100		>100[e]	>100	>100[e]
ganciclovir (DHPG)			7.7[h]	1.8[h]	3.5[e]	>100[h]	>100[e]

[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] Assayed by ELISA in quadruplicate wells; given as average IC_{50}. [c] Visual cytotoxicity scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text in quadruplicate wells. Results are presented as IC_{50}'s. [d] Results for compounds **1**−**4** reported previously as compounds **3**, **16**, **18**, and **19** in ref 3. [e] Data from a single experiment; all other data except as indicated for DHPG are averages from two to four separate experiments. [f] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [g] Abbreviations: dideoxyribosyl, 2′,3′-dideoxyribosyl; DHPM, (1,3-dihydroxy-2-propoxy)methyl. [h] Average of 88 and 31 experiments, respectively, for plaque and yield experiments.

the concentrate was coevaporated with MeOH (1 × 20 mL). The resulting concentrate was redissolved in MeOH, and the solution was neutralized with sufficient NH_4OH to effect a pH = 8. The resulting solution was treated with powdered Na_2SO_4 (4.12 g), and the suspension was concentrated *in vacuo* to a powder. The powder was applied to the top of a column (3.2 × 20 cm) packed with silica gel (12.2 g) in $CHCl_3$:MeOH,4:1. The column was eluted with the same solvent system, collecting 5 mL fractions. The fractions containing a product with an R_f = 0.28 were pooled and concentrated *in vacuo* to an off-white solid. The solid was suspended in a small amount of MeOH, collected by filtration, washed with ether, and dried *in vacuo* (100 °C, oil pump) for 12 h to afford 58 mg of **10**.

The fractions containing the product with an R_f = 0.28 and an additional product with a lower R_f were pooled and concentrated *in vacuo* to furnish a sticky solid. The solid was heated on a steam bath with a minimal amount of H_2O and then allowed to cool to room temperature. The resulting white solid was collected by filtration, washed with MeOH and then ether, and dried *in vacuo* (100 °C, oil pump) to afford an additional 16 mg of pure **10**. The combined yield of **10** was 23.6% from **7**, mp >244 °C dec. IR (KBr pellet): 3600−2500 br valley, 1635.9, 1622.6, 1563.6, 1064.8 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ϵ) 290 (6230); (pH 1) 290 (6780); (pH 11) 296 (5970). ^{1}H NMR (DMSO-d_6): δ 11.47 (s, 1H, NH-6), 7.81(s, 1H, H-2), 7.05 (d, J = 4.9 Hz, 1H, H-1′), 5.45−5.15 (m, 5H, OH's, NH_2), 4.08 (dd, 1H, H-2′), 3.98 (dd, 1H, H-3′), 3.76−3.62 (m, 3H, H-4′, H-5′). Anal. Calcd ($C_{11}H_{13}N_4O_5Br$) C, H, N.

Ethyl 3-Cyano-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)pyrrole-2-carboxylate (12). A solution of ethyl 3-cyanopyrrole-2-carboxylate (**5**; 2.0 g, 12.2 mmol)[9] in CH_3CN (40 mL) was treated with sodium hydride (50% mineral oil dispersion, 702 mg, 1.2 equiv). The resulting suspension was allowed to stir under an argon atmosphere for 30 min before 2-deoxy-3,5-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl chloride (**11**; 4.98 g, 1.2 equiv)[11] was added in two portions. The resulting reaction mixture was further diluted with CH_3CN (80 mL) and then allowed to stir at room temperature for 4 h. The reaction mixture was filtered through a thin pad of Celite, and the resulting filtrate was concentrated *in vacuo* to an oil. The oil was dissolved in a minimal amount of EtOAc and applied to a column (5 × 60 cm) packed with silica gel (320 g) in hexane:EtOAc, 4:1. The column was eluted with the same solvent system, collecting 50−75 mL fractions. The fractions containing a product with R_f = 0.34 were pooled and concentrated *in vacuo* to a white solid. The solid was suspended in an ether:pentane, 1:1, mixture, allowed to stand at 5 °C for 30 min, and then collected by filtration. The solid was dried *in vacuo* (25 °C, oil pump) over P_2O_5 for 15 h to afford 5.21 g (82.7%) of **12** as a white solid, mp 122−124 °C. IR (KBr pellet): 3149.2, 2980.5, 2917.2, 2228.1, 1714.8, 1609.4 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 7.91−7.81 (m, 4H, Ph H's), 7.58 (d, J = 3.0 Hz, 1H, H-5), 7.37−7.29 (m, 4H, Ph H's), 6.84 (t, 1H, H-1′), 6.75 (d, J = 3.0 Hz, 1H, H-4), 5.57 (m, 1H, H-3′), 4.60 (s, 3H, H-4′, H-5′), 4.30 (q, 2H, OCH_2CH_3), 2.80−2.50 (m, 2H, H-2′), 2.39−2.36 (2s, 6H, C_6H_4-CH_3), 1.29 (t, 3H, OCH_2CH_3). Anal. Calcd ($C_{29}H_{28}N_2O_7$) C, H, N.

4-Amino-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)pyrrolo-[2,3-*d*]pyridazin-7-one (13). Method A. A suspension of **12** (4.40 g, 8.5 mmol) in EtOH (100 mL) was treated with anhydrous hydrazine (3 mL). The reaction mixture (which became a solution when heated) was heated at reflux temperature for 15 h under an argon atmosphere. The solution was allowed to cool to room temperature, and then an additional 5 mL of hydrazine was added. The solution was heated at reflux temperature for another 24 h, and then it was concentrated *in vacuo*. The concentrate was coevaporated with EtOH (5 × 100 mL) to remove the excess hydrazine to afford an off-white solid. The solid was suspended in acetone (60 mL), warmed on a steam bath for 5 min, and then stirred at room temperature for 20 min. The resulting off-white solid was collected by filtration and recrystallized from boiling MeOH to afford an off-white solid. The solid was dried *in vacuo* (56° C, oil pump) over P_2O_5 for 18 h to afford 1.23 g (54.4%) of **13** as a white solid, mp 235−237.5 °C.

An analytical sample of **13** was prepared by recrystallizing 159 mg of **13** from boiling MeOH. The resulting crystals were dried *in vacuo* (56 °C, oil pump) over P_2O_5 for 18 h, mp 238−239 °C. IR (KBr pellet): 3600−2700 valley, 1625, 1540, 1432, 1275, 1220, 1110, 772 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ϵ) 288 (5080); (pH 1) 273 (5720); (pH 11) 288 (4830). ^{1}H NMR (DMSO-d_6): δ 11.22 (s, 1H, NH-6, D_2O exchangeable), 7.70 (d, J = 3.0 Hz, 1H, H-2), 7.18 (t, J = 6.8 Hz, 1H, H-1′), 6.63 (d, J = 3.0 Hz, 1H, H-3), 5.68 (s, 2H, NH_2, D_2O exchangeable), 5.24 (d, 1H, OH-3′, D_2O exchangeable), 4.94 (t, 1H, OH-5′, D_2O

exchangeable), 4.29 (m, 1H, H-3′), 3.80 (m, 1H, H-4′), 3.55 (m, 2H, H-5′), 2.24 (q, 2H, H-2′). Anal. Calcd ($C_{11}H_{14}N_4O_4$) C, H, N.

Method B. A solution of **14** (1.21 g, 4.3 mmol) in EtOH (25 mL) was treated with H_2NNH_2 (anhydrous, 1.2 mL), and the resulting mixture was heated at reflux under an argon atmosphere for 22 h. The reaction mixture was allowed to cool to room temperature, and another portion (1.2 mL) of hydrazine was added. The resulting mixture was reheated at reflux for an additional 6 h, and then it was allowed to stand at room temperature for 15 h to effect the precipitation of a white solid. The solid was collected by filtration, washed with EtOH and then ether, and dried *in vacuo* (oil pump) to afford 0.87 g (75%) of **13**, mp 229−232 °C. A sample of **13** prepared by this method comigrated on TLC with a sample of **13** prepared by method A ($CHCl_3$:MeOH, 4:1; $R_f = 0.18$).

Ethyl 3-Cyano-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrole-2-carboxylate (14). Compound **12** (13.72 g, 26.6 mmol) was added to a solution of sodium ethoxide (0.5 equiv) in EtOH (300 mL). The reaction mixture was allowed to stir at room temperature for 11 h. The mixture was adjusted to pH = 6 with Dowex 50 × 8-100 ion exchange resin. The resin was removed by filtration, and the resulting filtrate was concentrated *in vacuo* (<40 °C, water aspirator) to an oily solid. The oily solid was suspended in ether (50 mL) and stirred with a spatula and the solid collected by filtration to afford 5.92 g (79.5%) of **14** as a light pink solid, mp 118−120 °C.

A small amount (290 mg) of **14** was recrystallized from EtOH and dried *in vacuo* (oil pump, 100 °C) for 6 h to afford 190 mg of **14**, mp 119−121 °C. IR (KBr pellet): 3600−2700 valley, 2228.1, 1707.8, 1581.2, 1426.6, 1053.9, 646.1 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.75 (d, J = 3.0 Hz, 1H, H-5), 6.73 (d, J = 3.0 Hz, lH, H-4), 6.64 (t, J = 5.0 Hz, 1H, H-l′), 5.26 (d, 1H, OH-3, D_2O exchangeable), 5.05 (t, 1H, OH-5′, D_2O exchangeable), 4.34−4.22 (m, 3H, OCH_2CH_3, H-3′), 3.82 (dd, 1H, H-4′), 3.64−3.54 (m, 2H, H-5′), 2.50−2.08 (m, 2H, H-2′), 1.31 (t, 3H, OCH_2CH_3). Anal. Calcd ($C_{13}H_{16}N_2O_5$) C, H, N.

4-Amino-3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (15). To a solution of **13** (0.41 g, 1.54 mmol) in glacial acetic acid (25 mL) was added *N*-bromosuccinimide (0.41 g, 1.5 equiv) in one portion. The reaction mixture was stirred at room temperature for 20 h and then concentrated *in vacuo* (<40 °C, oil pump). The resulting concentrate was dissolved in MeOH, and then powdered Na_2SO_4 (11 g) was added. The resulting suspension was concentrated *in vacuo* to afford a yellow solid. The solid was layered onto the top of a column (3.2 × 20 cm) packed with silica gel (26 g) in $CHCl_3$:MeOH, 5:1. The column was eluted with the same solvent system, collecting 10 mL fractions. The fractions containing a product with $R_f = 0.42$ were pooled and concentrated *in vacuo* to a gel. The resulting gel was dissolved in H_2O and lyophilized on a freeze-drying apparatus (0.2 atm) for 32 h to yield 0.31 g (57%) of **15** as a fluffy, white solid. IR (KBr pellet): 3650−2500 valley, 1665.9, 1635.9, 1536.3, 1091.4, 1058.2 cm^{-1}. UV (MeOH): λ_{max} nm (ϵ) 300 (5100). ^1H NMR (DMSO-d_6): δ 11.53 (s, 1H, NH-6), 7.96 (s, 1H, H-2), 7.19 (t, J = 6.5 Hz, 1H, H-1′), 5.41 (br s, 2H, NH_2), 5.26 (d, 1H, OH-3′), 5.04 (t, 1H, OH-5′), 4.29 (m, 1H, H-3′), 3.82 (m, 1H, H-4′), 3.57 (m, 2H, H-5′), 2.25 (m, 2H, H-2′). Anal. Calcd ($C_{11}H_{13}N_4O_4Br \cdot {}^1/_2H_2O$) C, H, N.

Ethyl 3-Cyano-1-[5-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]pyrrole-2-carboxylate (16). A solution of **14** (3.17 g, 11.3 mmol), imidazole (1.85 g, 2.4 equiv), and *tert*-$BuMe_2SiCl$ (1.87 g, 1.1 equiv) in DMF (80 mL) was stirred under an argon atmosphere for 5 h at room temperature. The reaction mixture was concentrated in *vacuo* (<30 °C, oil pump) to an oil. The oil was dissolved in EtOAc (150 mL) and the resulting solution extracted with H_2O (2 × 100 mL). The organic layer was dried over $MgSO_4$ and then concentrated in *vacuo* to an oil. The oil was dissolved in a minimal amount of a mixture of $CHCl_3$: MeOH,50:1, and applied to a column (9 × 7 cm) packed with silica gel (110 g) in the same solvent system. The column was eluted with the same solvent system, collecting (10 mL fractions). The fractions containing a product with an $R_f = 0.50$ were pooled and concentrated *in vacuo* to an oil. The oil was further dried *in vacuo* (25 °C, oil pump) over P_2O_5 for 7 h to afford **16** as a colorless oil, 3.03 g (67.9%). IR (neat): 3479.7, 2931.2, 2860.9, 2228.1, 1707.8, 1665.6, 1412.5, 1257.8, 1201.6, 1103.1 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.56 (d, J = 3.0 Hz, 1H, H-5), 6.76 (t, J = 5.9 Hz, 1H, H-1′), 6.46 (d, J = 3.0 Hz, 1H, H-4), 4.47 (m, 1H, H-3′), 4.34 (q, 2H, OCH_2CH_3), 4.00 (dd, 1H, H-4′), 3.87 (m, 2H, H-5′), 2.56 (m, 1H, H-2′), 2.22 (m, 1H, H-2′), 1.89 (d, 1H, OH-3′, D_2O exchangeable), 1.41 (t, 3H, OCH_2CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 0.09 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$). Anal. Calcd ($C_{19}H_{30}N_2O_5Si$) C, H, N.

Ethyl 3-Cyano-1-[5-*O*-(*tert*-butyldimethylsilyl)-3-*O*-(phenoxythiocarbonyl)-2-deoxy-β-D-*erythro*-pentofuranosyl]pyrrole-2-carboxylate (17). A solution of **16** (3.03 g, 7.68 mmol) and 4-(dimethylamino)pyridine (4.69 g, 5 equiv) in CH_3CN (60 mL) was chilled by an external ice water bath. Phenyl chlorothionoformate (3.3 mL, 3.1 equiv) was added in one portion by pipet. The resulting yellow suspension was stirred in the ice bath for 10 min, and then it was stirred at room temperature for 2.5 days. The yellow suspension was concentrated *in vacuo* to a residue which was suspended in EtOAc (125 mL) and extracted with 2% aqueous acetic acid (2 × 100 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to an oil. The oil was dissolved in a minimal amount of toluene:EtOAc, 30:1, and applied to a column (9.5 × 9 cm sintered glass funnel) packed with silica gel (160 g) in the same solvent system. The column was eluted with this same system, collecting 40 mL fractions. The fractions that were homogeneous in containing a product with an $R_f = 0.42$ were pooled and concentrated *in vacuo* to a colorless oil, which was dried *in vacuo* (25 °C, oil pump) to afford 1.94 g of **17**.

The fractions containing the product with an $R_f = 0.42$ and an additional impurity with a higher R_f were combined and concentrated *in vacuo* to a colorless gum (1.1 g). The gum was dissolved in a minimal amount of a solution of toluene:EtOAc, 30:1, and applied to a column (3.2 × 20 cm) packed with silica gel (39 g) in the same system. The column was eluted with the same solvent system collecting fractions (ca. 10 mL sized). The fractions containing a product with an $R_f = 0.42$ were still contaminated with the impurity. These fractions were pooled and concentrated *in vacuo* to a colorless residue (0.59 g). The residue was dissolved in a minimal amount of toluene and applied to a column (3.2 × 20 cm) packed with silica gel (23 g) in toluene (neat). The column was first eluted with toluene (neat) to remove the nonpolar impurity and then with toluene: EtOAc/30:1, to elute the desired product. The product fractions containing the product with an $R_f = 0.42$ were pooled and concentrated *in vacuo* to a colorless oil. The oil was dried further *in vacuo* (25 °C, oil pump) to afford an additional 0.41 g of **17**. The combined yield of **17** was 61%. IR (neat): 2958.2, 2932.2, 2858.9, 2232.8, 1708.5, 1492.3, 1420.2, 1263.0, 1210.5, 1118.8, 837.1, 771.2, 758.4 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.62 (d, J = 3.2 Hz, H-5), 7.47−7.10 (m, 5H, phenyl H's), 6.94 (dd, J = 5.7, 6.7 Hz, 1H, H-1′), 6.53 (d, J = 3.1 Hz, 1H, H-4), 5.77 (d, J = 6.1 Hz, 1H, H-3′), 4.48−4.37 (m, 3H, OCH_2CH_3, H-4′), 4.01 (d, J = 2.0 Hz, 2H, H-5′), 2.96 (m, 1H, H-2′), 2.38 (m, 1H, H-2′), 1.45 (t, 3H, OCH_2CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H, $SiCH_3$). Anal. Calcd ($C_{26}H_{34}N_2O_6SSi$) C, H, N.

Ethyl 3-Cyano-1-[5-*O*-(*tert*-butyldimethylsilyl)-2,3-dideoxy-β-D-*glycero*-pentofuranosyl]pyrrole-2-carboxylate (18). A solution of **17** (1.13 g, 2.3 mmol) in toluene (35 mL) was treated with AIBN (74 mg, 0.2 equiv) and then tributyltin hydride (1.24 mL, 2 equiv). The solution was lowered into an oil bath preheated to 83 °C, heated at this temperature for 2.5 h under an argon atmosphere, and then concentrated *in vacuo* (<35 °C, water pump and then oil pump) to afford a yellow oil. The oil was dissolved in a minimal amount of $CHCl_3$:EtOAc, 1:1, and applied to a column (2.5 × 52 cm) packed with silica gel (95 g) in cyclohexane:EtOAc: $CHCl_3$, 10:1:1. The column was eluted with the same solvent system, collecting 10 mL-sized fractions. The fractions containing a product with $R_f = 0.28$ were pooled and concentrated *in vacuo* to an oil. The oil was dried *in vacuo* (25 °C, oil pump) over P_2O_5 for 3 h to afford 0.53 g (61%) of **18** as a colorless oil. IR (neat): 3142.2, 2953.3, 2931.2, 2860.9, 2228.1, 1707.8, 1475.8, 1412.5, 1257.8, 1208.6, 1131.2, 835.9, 793.8 cm^{-1}. ^1H NMR ($CDC1_3$): δ 7.79 (d, J = 2.9 Hz, 1H, H-5), 6.61 (dd, J = 1.6, 6.0 Hz, 1H, H-1′), 6.44 (d, J = 2 Hz, 1H, H-4), 4.38 (q, 2H,

OCH_2CH_3), 4.17 (m, 1H, H-4′), 4.08 (dd, 1H, H-5′), 3.75 (dd, 1H, H-5′), 2.52−1.86 (m, 4H, H-2′, H-3′), 1.43 (t, 3H, OCH_2CH_3), 0.93 (s, 9H, $C(CH_3)_3$), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$). Anal. Calcd ($C_{19}H_{30}N_2O_4Si$) C, H, N.

Ethyl 3-Cyano-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrole-2-carboxylate (19). A solution of **18** (562 mg, 1.2 mmol) in THF (10 mL) was treated with *n*-Bu_4NF (1.0 M solution in THF, 1.3 mL, 1.1 equiv). The resulting yellow solution was allowed to stir at room temperature for 1 h. The reaction mixture was then concentrated *in vacuo* to a yellow oil. The oil was dissolved in CH_2Cl_2:MeOH, 1:1 (2 mL), and applied to a column (3.2 × 20 cm) packed with silica gel (32 g) in CH_2Cl_2:MeOH, 35:1. The column was eluted with the same solvent system, collecting 6 mL sized fractions. The fractions containing a product with an R_f = 0.22 were pooled and concentrated *in vacuo* to afford a white solid. The solid was suspended in ether, and the resulting suspension was chilled *via* an external ice−water bath. The resultant white crystalline precipitate was collected by filtration and dried *in vacuo* (25 °C, oil pump) to afford 194 mg (61.1%) of **19**, mp 98−100 °C. IR (neat): 3142.2, 2952.3, 2931.2, 2860.9, 2228.1, 1707.8, 1475.8, 1412.5, 1257.8, 1208.6, 1131.2, 835.9, 793.8 cm^{-1}. ^1H NMR (DMSO-d_6): δ 7.86 (d, J = 3.0 Hz, 1H, H-5), 6.70 (d, J = 3.0 Hz, 1H, H-4), 6.49 (d, J = 6.6 Hz, 1H, H-1′), 5.09 (t, 1H, OH-5′, D_2O exchangeable), 4.29 (q, 2H, OCH_2CH_3), 4.08 (m, 1H, H-4′), 3.73−3.55 (m, 2H, H-5′), 2.46−1.78 (m, 4H, H-2′, H-3′), 1.30 (t, 3H, OCH_2CH_3). Anal. Calcd ($C_{13}H_{16}N_2O_4$) C, H, N.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-pyrrolo[2,3-*d*]pyridazin-7-one (20). A solution of **19** (168 mg, 0.64 mmol) in EtOH (8 mL) was treated with anhydrous hydrazine (0.34 mL). The resulting solution was heated at reflux under an argon atmosphere for 24 h. The reaction mixture was allowed to cool to room temperature, and then another 0.34 mL of hydrazine was added by pipet. The solution was again heated at reflux for 24 h. The resulting reaction mixture was concentrated *in vacuo* to an oil which was coevaporated with EtOH (5 × 5 mL). The resulting concentrate was a white solid which was suspended in boiling EtOH (5 mL). The EtOH suspension was allowed to stand at room temperature for 6 h, and then the solid was collected by filtration. The solid was dried *in vacuo* for 16 h over P_2O_5 to afford 99 mg (62%) of **20** as a white solid, mp >200 °C dec. IR (KBr pellet): 3600−2700 valley, 1640, 1540, 1440, 1280, 1220, 1090, 1072, 1045, 1030, 980, 920, 820, 775 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ε) 287 (5280); (pH 1) 274 (6590); (pH 11) 286 (5400). ^1H NMR (DMSO-d_6): δ 11.18 (s, 1H, NH-6, D_2O exchangeable), 7.73 (d, J = 3.0 Hz, 1H, H-2), 6.99 (dd, J = 2.7, 6.8 Hz, 1H, H-1′), 6.59 (d, J = 3.0 Hz, 1H, H-3), 5.67 (s, 2H, NH_2, D_2O exchangeable), 4.95 (t, 1H, OH-5′, D_2O exchangeable), 4.04 (m, 1H, H-4′), 3.58 (m, 2H, H-5′), 2.42−2.35, 2.02−1.88 (2m, 4H, H-2′, H-3′). Anal. Calcd ($C_{11}H_{14}N_4O_3$) C, H, N.

4-Amino-3-bromo-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)pyrrolo[2,3-*d*]pyridazin-7-one (21). To a solution of **20** in glacial acetic acid (8 mL) was added *N*-bromosuccinimide (232 mg, 1.4 equiv). The resulting reaction mixture became a solution and was stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo* to a reddish-colored residue which was dissolved in MeOH. To this methanolic solution was added powdered Na_2SO_4 (6 g), and the resulting suspension was concentrated *in vacuo* to a powder. The powder was layered onto the top of a column (3.2 × 20 cm) packed with silica gel (16 g) in $CHCl_3$:MeOH, 12:1. The column was eluted with this same solvent system, collecting 5 mL fractions. The fractions that contained a product with an R_f = 0.22 were pooled and concentrated *in vacuo* to a gel. The gel was dissolved in a small amount of hot MeOH, and the solution was allowed to stand at room temperature for 6 h. The resulting precipitate was collected by filtration, washed with ether, and dried *in vacuo* (78 °C, oil pump) over P_2O_5 for 24 h to afford 94 mg (30.7%) of **21**, mp 213−215 °C. IR (KBr pellet): 3600−2700 valley, 1635, 1535, 1455, 1285, 1170, 1110, 1080, 985 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ε) 296 (5800); (pH 1) 291 (6690); (pH 11) 296 (5290). ^1H NMR (DMSO-d_6): δ 11.48 (s, 1H, NH-6) 8.05 (s, 1H, H-2) 6.98 (d, J = 6.2 Hz, 1H, H-1′) 5.40 (br s, 2H, NH_2) 5.09 (t, 1H, OH-5′) 4.07 (m, 1H, H-4′) 3.63 (m, 2H, H-5′) 2.40, 1.95 (2m, 4H, H-3′, H-2′). Anal. Calcd ($C_{11}H_{13}N_4O_3Br$) C, H, N.

Ethyl 3-Cyano-1-[(1,3-diacetoxy-2-propoxy)methyl]-pyrrole-2-carboxylate (23). A solution of **5** (2.48 g,15.1 mmol) in CH_3CN (60 mL) was treated with NaH (523 mg of 97% reagent, 1.5 equiv) in one portion. The resulting suspension was stirred under an argon atmosphere for 25 min, and then it was treated with (1,3-diacetoxy-2-propoxy)methyl bromide[14] (**22**; 4.5 mL, 1.5 equiv) by a syringe. The reaction mixture was allowed to stir at room temperature for 30 min, and then more **22** (0.33 equiv) was added. The reaction mixture was allowed to stir for an additional 10 min after the second addition and then filtered through a thin pad of Celite. The filtrate was concentrated *in vacuo* to an oil which was dissolved in toluene:EtOAc, 8:1, and applied to a column (4.5 × 24 cm) packed with silica gel (135 g) in the same solvent system. The column was eluted with this same solvent system and then with toluene:EtOAc, 5:1, collecting fractions. The fractions containing a product with an R_f = 0.28 (toluene:EtOAc, 5:1) were pooled and concentrated *in vacuo* to a colorless oil. The oil was dried *in vacuo* (100 °C, oil pump) for 48 h to afford 4.74 g (89.1%) of **23**. IR (neat): 3121.1, 2987.5, 2235.2, 1743.0, 1489.8, 1419.5, 786.7 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.07 (d, J = 2.9 Hz, 1H, H-5), 6.57 (d, J = 2.9 Hz, 1H, H-4), 5.85 (s, 2H, NCH_2O), 4.42 (q, 2H, OCH_2CH_3), 4.21−3.96 (m, 5H, OCH_2O, CH), 2.04 (s, 6H, $C(O)CH_3$), 1.45 (t, 3H, OCH_2CH_3). Anal. Calcd ($C_{16}H_{20}N_2O_7$) C, H, N.

4-Amino-1-[(1,3-dihydroxy-2-propoxy)methyl]pyrrolo-[2,3-*d*]pyridazin-7-one (24). A mixture of **23** (4.67 g, 13.2 mmol) and anhydrous H_2NNH_2 (9.6 mL) in EtOH (50 mL) was heated at reflux for 4 days. The reaction mixture was concentrated *in vacuo* (<40 °C) to an oil which was coevaporated with EtOH (3 × 40 mL). The oil was redissolved in boiling EtOH (50 mL), and the resulting solution was allowed to stand at room temperature to effect crystallization. The off-white solid was collected by filtration. This solid was recrystallized from a boiling solution of EtOH:H_2O, 9:1. The resulting off-white solid was collected by filtration, washed with a small amount of EtOH, and dried *in vacuo* (100 °C, oil pump) to afford 1.82 g (54%) of **24**, mp 186−188.5 °C.

An analytical sample of **24** was prepared by recrystallizing 200 mg of **24** in EtOH:H_2O, 4:1. The resulting crystals were collected by filtration and dried *in vacuo* (100 °C, oil pump) to afford 80 mg of **24** as white needles, mp 189−191 °C. IR (KBr pellet): 3389.1, 3276.2, 3209.8, 1635.9, 1543.0, 1443.4, 1410.1, 1290.6, 1224.2, 1104.7, 1051.6, 991.8, 779.3, 746.1 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ε) 262 (7040), 287 (6450); (pH 1) 272 (8750); (pH 11) 262 (6840), 287 (6710). ^1H NMR (DMSO-d_6): δ 11.27 (s, 1H, NH-6, D_2O exchangeable), 7.50 (d, J = 3.0 Hz, 1H, H-2), 5.89 (s, 2H, NCH_2O), 5.74 (s, 2H, NH_2, D_2O exchangeable), 4.59 (s, 2H, OH's, D_2O exchangeable), 3.56−3.22, (m, 5H, CH, $CHCH_2OH$). Anal. Calcd ($C_{10}H_{14}N_4O_4$) C, H, N.

4-Amino-3-bromo-1-[(1,3-dihydroxy-2-propoxy)methyl]-pyrrolo[2,3-*d*]pyridazin-7-one (25). To a solution of **24** (248 mg, 0.97 mmol) in glacial acetic acid (8 mL) was added a solution of NBS (280 mg, 1.6 equiv) in acetic acid (25 mL) dropwise, *via* an addition funnel over the course of 1.5 h. The resulting reaction mixture was concentrated *in vacuo* (<30 °C, oil pump) to an oil. The oil was dissolved in hot MeOH (40 mL), and the resulting solution was treated with powdered Na_2SO_4 (7 g). The suspension was concentrated *in vacuo* to a powder which was applied to the top of a column (3.2 × 20 cm) packed with silica gel (16.6 g) in $CHCl_3$:MeOH, 6:1. The column was eluted with this same solvent system and then with $CHCl_3$:MeOH, 4:1, collecting 5 mL fractions. The fractions containing a product with an R_f = 0.38 were pooled and concentrated *in vacuo* to a solid. The solid was suspended in a minimal amount of MeOH, collected by filtration, washed with ether, and dried *in vacuo* (100 °C, oil pump) over P_2O_5 for 18 h to afford 119 mg of (36.8%) **25** as a peach-colored solid, mp 195−196.5 °C. IR (KBr pellet): 3428.9, 3316.0, 3110.1, 3023.8, 2970.7, 2942.2, 1655.9, 1616.0, 1529.7, 1463.3, 1436.7, 1284.0, 1191.0, 1118.0, 1044.9, 1005.1, 845.7, 752.7, 659.8 cm^{-1}. UV (H_2O) (pH 7): λ_{max} nm (ε) 246 (7090), 294 (3050); (pH 1) 250 (5460), 290 (3500); (pH 11) 246 (6760), 294 (2940). ^1H NMR (DMSO-d_6): δ 11.54 (s, 1H, NH-6, D_2O exchangeable),

7.80 (s, 1H, H-2), 5.92 (s, 2H, OCH_2N), 5.44 (s, 2H, NH_2, D_2O exchangeable), 4.59 (t, 2H, OH's, D_2O exchangeable), 3.57−3.22 (m, 5, $CHCH_2OH$, $CHCH_2OH$). Anal. Calcd ($C_{10}H_{13}N_4O_4$-Br) C, H, N.

***In Vitro* Antiproliferative Studies**. The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[15] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice with 10% heat-inactivated (56 °C, 30 min) horse serum. The growth rate was calculated from determinations of cell number at 0, 48, and 96 h in the presence of various concentrations of the test compound. Growth rate is 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 (T_d) of control cells to the T_d of treated cells. When the growth rate decreased during the experiment, the rate used was that between 48 and 96 h. The IC_{50} is defined as the concentration required to decrease the growth rate to 50% of the control.

***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 MEM with Hank's salts supplemented with 5% fetal bovine serum. Diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[5,7] 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.[5,16]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed using 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 the HCMV yield reduction assay have been described previously.[16] HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[17]

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely 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 assays described above. Drug-induced cytopathology was estimated at 30-fold magnification and scored on a 0−4+ basis on the day of staining for plaque enumeration.[5] Cytotoxicity in KB cells was determined by measuring the effects of compounds on the growth of cells. Growth was measured spectrophotometrically by staining cells with crystal violet 2 days after drug treatment.[18]

(d) Data Analysis. Dose−response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log of drug concentration. Fifty percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (acyclovir, ganciclovir, or 2-acetylpyridine thiosemicarbazone for HSV-1, HCMV, and cytotoxicity, respectively) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

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