Synthesis, Antiproliferative, and Antiviral Activity of 4-Amino-1-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one and Related Derivatives

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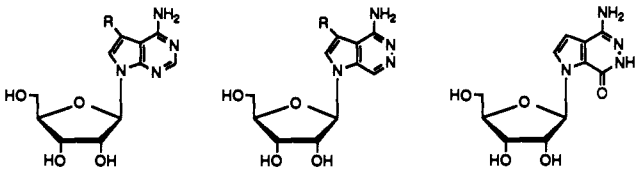
The synthesis of 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**3**) from the reaction of ethyl 3-cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (**10**) and hydrazine is described. The 5:6 pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one structure of **3** was established *via* a three-step conversion of **3** into 1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-4,7(5*H*,6*H*)-dione (**14**). 4-Amino-3-chloro-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**16**) and 4-amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**18**) were prepared *via* *N*-chlorosuccinimide or *N*-bromosuccinimide treatment of 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**7**) followed by a removal of the benzyl groups with boron trichloride. Direct treatment of **3** with *N*-iodosuccinimide furnished 4-amino-3-iodo-1-β-D-ribofuranosylpyrrolo-[2,3-*d*]pyridazin-7(6*H*)-one (**19**). The antiproliferative activity of the compounds was determined in L1210, H. Ep. 2 and several additional human tumor cell lines. In L1210 cells, the 3-halo-substituted compounds **16**, **18**, and **19** exhibited significant cytotoxicity (IC_{50} = 0.2, 0.1, 0.08 μM, respectively), in contrast to the 3-unsubstituted compound **3**, which had only slight activity. The greater antiproliferative activity of **18** and **19** in contrast to **3** was confirmed in H. Ep. 2 cells and KB cells. The antiviral evaluation of these compounds revealed that compounds **16**, **18**, and **19** were active against human cytomegalovirus in both plaque- and yield-reduction assays. However, this activity was only partially separated from cytotoxicity in human cell lines.

Introduction

Significant biological activity has been observed for the nucleoside antibiotic tubercidin (**1a**) (7-deazaadenosine)



and certain structurally related analogs which have been isolated from naturally occurring sources or prepared by synthetic methods. Tubercidin and especially certain 5-substituted analogs have shown antineoplastic activity in a variety of assays both *in vitro* and *in vivo*.[1] The 5-carboxamido derivative of tubercidin, sangivamycin, has undergone phase I clinical trials as an antineoplastic agent.[2] Although the 7-deazaadenosine analogs showed significant antiproliferative activity, their anticancer potential was tempered by the fact that these analogs also had significant host toxicity. Therefore, there is a continuing need to synthesize other structurally related nucleoside analogs which possess less host toxicity.

As part of an ongoing program to investigate other base-modified adenosine analogs in which the 7-nitrogen atom has been replaced by a carbon atom,[3-8] we have prepared the novel 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine (**2a**), an isomer of tubercidin (**1a**). Surprisingly this analog showed no antineoplastic or antiviral activity.[9] During our attempts to establish a facile preparation of **2a**, we prepared another novel adenosine analog, 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**3**). This prompted us to investigate its biological activity and to synthesize related derivatives. The 3-halo (Cl, Br, I) analogs of **3** were of particular interest because the 3-bromo derivative (**2b**) of **2a** had shown modest antiproliferative and antiviral activity while **2a** itself was completely inactive.[9] Also, the corresponding 5-halo analogs in the pyrrolo[2,3-*d*]pyrimidine series demonstrated significant antiproliferative and antiviral activities.[6,7]

Results and Discussion

Chemistry. Our initial synthetic efforts were focused on the preparation of 4-amino-1-β-D-ribofuranosylpyrrolo-[2,3-*d*]pyridazin-7(6*H*)-one (**3**). After a comprehensive literature search, we chose to use ethyl 3-cyanopyrrole-2-carboxylate (**4**)[10] as our starting material. This pyrrole had substituents in the proper juxtaposition and in addition these substituents had different chemical reactivities which could be exploited at a later stage of the synthesis. We elected to use 2,3,5-tri-*O*-benzyl-D-ribofuranosyl chloride (**5**)[11] as the sugar for the glycosylation procedure with the pyrrole **4**. Even though **5** had not been used previously in the sodium salt mediated coupling of pyrroles, the benzyl protecting groups were considered necessary for further transformations in the synthetic sequence. The sodium salt mediated coupling of **4** and **5** was initially attempted in acetonitrile, the usual solvent for the pyrrole coupling reaction.[12] However the reaction was quite sluggish in this solvent. Substitution of acetonitrile by dimethylformamide overcame this problem and DMF became our solvent of choice for this particular

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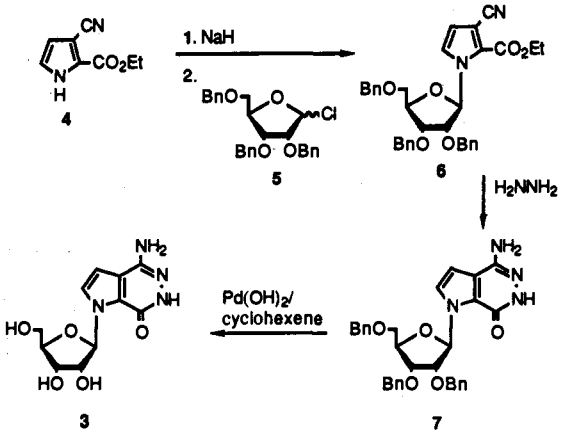
Scheme I

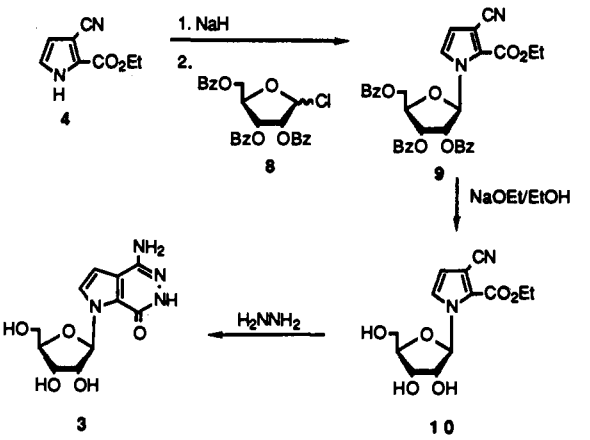


reaction (Scheme I). The coupling reaction in dimethylformamide afforded 36–40% yields of the desired ethyl 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxylate (**6**), after column chromatography. That the pyrrole had been alkylated at N-1 and not at a carbon atom was evident from the ^{1}H NMR of **6**, which revealed the presence of two doublets corresponding to the pyrrole H's. The anomeric configuration of **6** could be assigned from the ^{1}H NMR spectrum since the peak at 6.70 ppm corresponding to the anomeric proton appeared as a singlet. The coupling constant for the H-1′ and H-2′ protons was therefore less than 1 Hz, and this corresponds to a β configuration for **6**.[13]

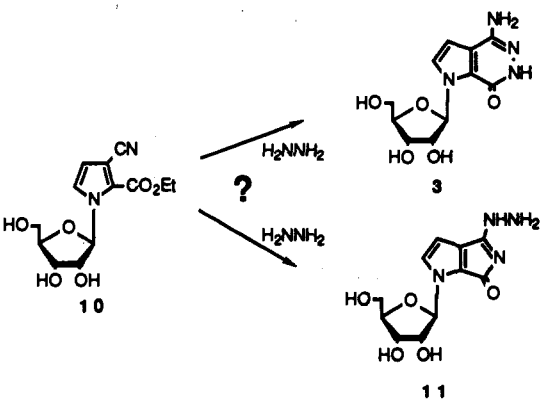
The pyridazine portion of the desired heterocyclic moiety was obtained *via* a ring annulation reaction of **6** using anhydrous hydrazine in refluxing EtOH (Scheme I). From the IR spectrum which revealed the loss of the absorbances at 2200 and 1708 cm^{-1} for the nitrile ester, respectively, it was determined that ring closure had occurred to form 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**7**). In the FAB+ mass spectrum of **7** a peak at *m/z* 553 was observed which corresponded to the M + 1 ion expected for a molecular formula of $C_{32}H_{32}N_4O_5$. Finally as further structure proof, **7** was deprotected using transfer hydrogenation conditions (Pearlman's catalyst/cyclohexene) to afford 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**3**) in 23% yield. In addition to the D_2O-exchangeable peaks corresponding to the sugar hydroxyls in the ^{1}H NMR spectrum of **3**, there were D_2O-exchangeable singlets at δ 11.23 and 5.69. The downfield singlet which integrated for one proton was assigned to the N*H*-6 proton and the upfield signal, which integrated for two protons, was assigned to the 4-amino protons.

The rather low overall yield for this particular synthetic sequence prompted an investigation for a higher yielding procedure for the preparation of **3**. Compound **3** was prepared more efficiently by a slightly modified procedure (Scheme II). Ethyl 3-cyano-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrole-2-carboxylate (**9**) was readily obtained by the sodium salt glycosylation of **4** with 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**).[7] Treatment of **9** with a solution of sodium ethoxide in ethanol effected debenzoylation and provided ethyl 3-cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (**10**). A ring annulation of **10** with hydrazine then furnished **3** in 62.5% yield. The compound obtained *via* this procedure had the same physical properties as those of the compound (**3**) obtained by the earlier procedure.

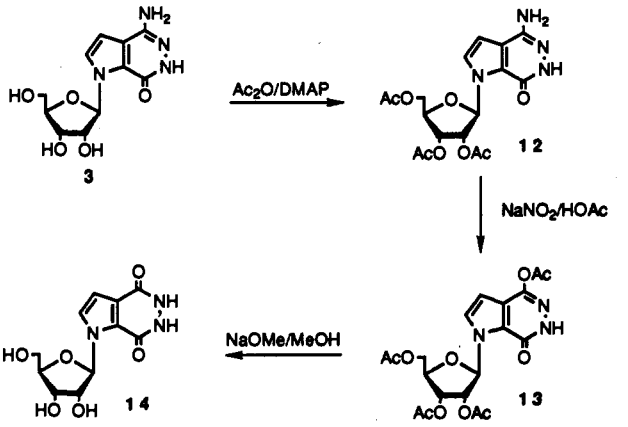
Scheme II



Scheme III

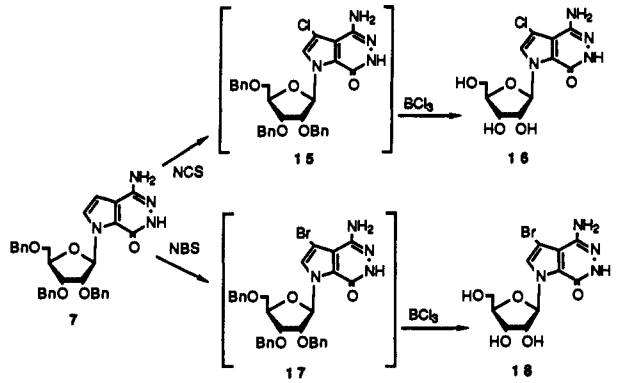


Scheme IV

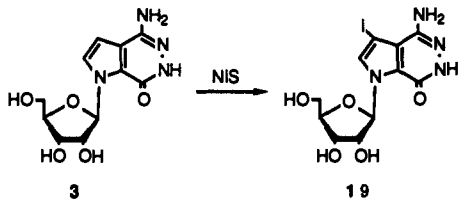


The elemental analyses and the electron impact mass spectrum of **3** suggested that compound **3** possessed a molecular formula of $C_{11}H_{14}N_4O_5$. These data were consistent with the proposed 5:6 pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one structure. Also consistent with this structure were D_2O-exchangeable singlets at δ 11.23 integrating for one proton and at δ 5.69 integrating for two protons. However, the data did not completely rule out the possibility of the isomeric 5:5 structure 4-hydrazino-1-β-D-ribofuranosylpyrrolo[3,4-*b*]pyrrol-6(1*H*)-one (**11**) (Scheme III). In order to establish the 5:6 pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one structure unequivocally, **3** was further derivatized (Scheme IV). Compound **3** was first acetylated with exactly 3 equiv of acetic anhydride to afford 4-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyridazin-7(6*H*)-one (**12**). This modification furnished a compound which was soluble in organic solvents and

Scheme V



Scheme VI



facilitated the workup of the next reaction. Upon diazotization of **12** using sodium nitrite and acetic acid, the tetraacetylated compound **13** was isolated. Four singlet peaks each integrating for three protons assigned to the methyl protons of the acetyl groups were evident in the ^{1}H NMR of **13**. The electron impact mass spectrum of **13** (with a molecular ion of 451) and elemental analyses were in agreement with a compound with a molecular formula of $C_{19}H_{21}N_3O_{10}$. Deprotection of **13** using a sodium methoxide/methanol solution yielded 1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-4,7(5*H*,6*H*)-dione (**14**). The mass spectrum of **14** showed the presence of a peak corresponding to the molecular ion at 283 and an intense peak at 151 corresponding to the B + 1. Elemental analyses were also consistent with a molecular formula of $C_{11}H_{13}N_3O_6$. Further proof was derived from the observation of a bathochromic shift in the UV spectrum at pH 11, which was consistent with the inosine-like structure for **14**. Had the structure of **3** been the isomeric **11**, the expected product from the diazotization would have contained an azido function.[14] The observed product (**14**) of this sequence was therefore in agreement with a 5:6 pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one structure for **3**.

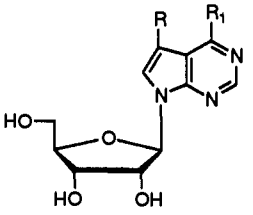
With the free nucleoside **3** and a protected analog, 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**7**), available as intermediates, the preparation of the 3-halogenated analogs was initiated. The chloro and bromo analogs were prepared by the treatment of **7** with N-chlorosuccinimide or N-bromosuccinimide, respectively, in dichloromethane (Scheme V). The presumed intermediates **15** and **17** were then deprotected using boron trichloride to furnish 4-amino-3-chloro-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**16**) and 4-amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyridazin-7(6*H*)-one (**18**), respectively. 4-Amino-3-iodo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (**19**) was obtained by a direct treatment of **3** with N-iodosuccinimide in glacial acetic acid (Scheme VI). That the halogen had added at the 3 position was established by the ^{1}H NMR and ^{13}C NMR spectra. In the fully coupled ^{13}C NMR spectrum of **16** the peaks corresponding to the pyrrole carbon attached directly to a hydrogen appeared (centered at δ 124.35) as a doublet of doublets. This pattern of peaks was expected for C-2, as it was coupled to both H-2 (^1J) and H-1′ (^3J). Upon irradiation of **16** with the frequency corresponding to the anomeric proton, these same peaks collapsed to a clean doublet. This observation was in agreement with the fact that chlorination of the heterocycle had occurred at the 3 position. The spectra of the analogous 3-bromo (**18**) and 3-iodo (**19**) compounds showed similar patterns in their ^{13}C NMR spectra.

Biology. *In Vitro* Antiproliferative Activity. The four pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one analogs of adenosine (**3**, **16**, **18**, and **19**) and the inosine analog **14** were evaluated for their antiproliferative activity in L1210 murine leukemia cells (Table I). Data for tubercidin (**1a**) and its bromo analog **1b** (both pyrrolopyrimidines), plus two pyrrolopyridazine analogs described previously[9] (**2a** and **2b**), also are included in the table for comparison. The well-established antiproliferative activity of tubercidin was readily detected while 5-bromotubercidin was 100-fold less active. In contrast to these pyrrolo[2,3-*d*]-pyrimidine nucleosides, halogenation of the new pyrrolo-[2,3-*d*]pyridazine nucleosides at the 3-position (analogous to the 5-position of the pyrrolo[2,3-*d*]pyrimidine nucleosides) conferred increased cytotoxic activity. The 4-amino-3-bromopyrrolo[2,3-*d*]pyridazine nucleoside **2b** was moderately cytotoxic, whereas the corresponding 3-unsubstituted adenosine analog **2a** was not growth inhibitory at 100 μM, the highest concentration tested. The new, 3-halogenated 4-aminopyrrolo[2,3-*d*]pyridazin-7(6*H*)-one nucleosides **16**, **18**, and **19** were highly cytotoxic, while the corresponding 3-unsubstituted adenosine analog **3** was only marginally growth inhibitory. It is of considerable interest that the 3-bromo (**18**) and 3-iodo (**19**) analogs appear to be almost as potent as the naturally occurring pyrrolo-[2,3-*d*]pyrimidine nucleoside tubercidin.

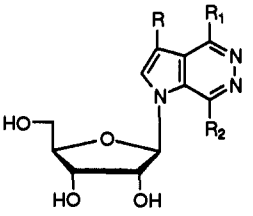
The new compounds also were evaluated in H. Ep. 2 human epidermoid carcinoma cells, and selected compounds from this series also were evaluated in nine additional human tumor cell lines (Table II) for their ability to inhibit cell growth (Table I). The structure–activity relationships were consistent with the pattern observed for L1210 cells. Introduction of either a 3-halo (**2b**) or 7-one (**3**) substituent provided moderate cytotoxic activity, in contrast to the unsubstituted adenosine analog (**2a**), which was completely inactive at 100 μM. The compounds with both 3-halo and 7-one substituents (**16**, **18**, **19**) were by far the most active. Their IC_{50}'s were lower than that of compound **2b** by a factor of 100 or more.

***In Vitro* Antiviral Activity.** All compounds also were tested for activity against two herpes viruses, human cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1), because halogenated analogs in the pyrrolopyrimidine series were very active against these viruses.[6,7] As a means of determining the selectivity of this activity, the compounds also were evaluated for cytotoxicity to uninfected cells used to propagate HCMV and HSV-1, human foreskin fibroblasts (HFF cells) and KB cells, respectively (Table III). The structural requirements for antiviral activity in this series of compounds were similar to those discussed above for antiproliferative activity. Only those analogs which contained both 3-halo and 7-one substituents (**16**, **18**, **19**) exhibited significant activity against HCMV in the plaque-reduction assay (Table III). These three compounds were more active than the

Table I. *In Vitro* Cell Growth Inhibition by 4-Aminopyrrolo[2,3-*d*]pyridazin-7-one and Related Nucleosides



1a,1b



2a–19

				IC_{50} (μM)[a]											
no.	R	R_1	R_2	L1210	H. Ep. 2	UMSCC-10A	CALU-1	NCI-H1437	NCI-H460	NCI-H146	NCI-N417	DLD-1	HT-29	SW-620
1a[b]	H	NH_2		0.043	0.06									
1b[b]	Br	NH_2		4										
2a[c]	H	NH_2	H	–[d]	–			–	–					
2b[c]	Br	NH_2	H	24	5.2		11		11					
3	H	NH_2	OH[e]	74	–		–			–	–			
14	H	OH	OH	–										
16	Cl	NH_2	OH	0.23										
18	Br	NH_2	OH	0.10	0.20	1.3	0.23	0.50	0.50					
19	I	NH_2	OH	0.08	0.076	2.5	0.07	0.13	0.20	0.42	0.28	0.07	0.50	0.23

[a] Concentration required to decrease growth rate to 50% of control. See Experimental Section for complete definition. [b] Results for these pyrrolopyrimidines have been reported previously[7]. [c] Results for these pyrrolopyridazines have been reported previously.[9] [d] A dash indicates that 100 μM, the highest concentration tested, had no significant effect on cell growth. [e] Shown as OH for convenience in presenting the structures.

Table II. Human Tumor Cell Lines Used for *in Vitro* Evaluation of Antiproliferative Activity

name	tumor site	histological type	source	plating cells/well	assay day	medium
UMSCC-10A	larynx	squamous cell carcinoma	Carey[a]	2000	5	MEM-5[b]
CALU-1	lung	squamous cell carcinoma	ATCC[c]	2000	5	R-5[d]
NCI-H1437	lung	adenocarcinoma	Gazdar[e]	2000	5	R-5
NCI-H460	lung	large cell carcinoma	Gazdar[f]	1000	4	R-5
NCI-H146	lung	classic small cell carcinoma	Gazdar[g]	20000	7	R-5
NCI-N417	lung	variant small cell carcinoma	Gazdar[g]	20000	7	R-5
DLD-1	colon	adenocarcinoma	ATCC	1000	5	R-5
HT-29	colon	adenocarcinoma	ATCC	2000	5	R-5
SW-620	colon	adenocarcinoma	ATCC	7500	4	R-5

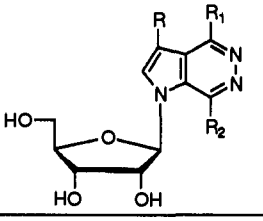
[a] Thomas E. Carey, University of Michigan, Ann Arbor, MI.[15] [b] MEM-5, Minimal Essential Medium with Earle's salts and 5% fetal bovine serum. [c] ATCC, American Type Culture Collection, Rockville, MD. [d] R-5, RPMI 1640 with 5% fetal bovine serum, 2 mM L-glutamine, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL neomycin. [e] Adi F. Gazdar, Simmons Cancer Center, Dallas, TX.[16] [f] Adi F. Gazdar, Simmons Cancer Center, Dallas, TX.[17] [g] Adi F. Gazdar, Simmons Cancer Center, Dallas, TX.[18]

marketed antiviral drug ganciclovir (Table III). Furthermore, they were highly active against HCMV in a yield-reduction assay, which is a more rigorous measure of antiviral activity. This assay also showed that the brominated pyrrolopyridazine **2b** was active against HCMV, even though this activity was not apparent in the plaque assay.[9] Compounds **16**, **18**, **19**, and **2b** also appeared to have some activity against HSV-1, but this activity was not separated from cytotoxicity in uninfected HFF and KB cells (Table III). Because this apparent activity occurred at cytotoxic concentrations, the activity observed in the HSV-1 assay probably was a consequence of cytotoxicity and not specific antiviral activity. Likewise, activity against HCMV of tubercidin and possibly bromotubercidin most likely was a consequence of cytotoxicity.

In contrast, activity of **16**, **18**, **19**, and **2b** against HCMV was separated to some extent from cytotoxicity. A comparison of IC_{90}'s in the HCMV yield assay to IC_{50}'s for cytotoxicity in HFF, KB and H. Ep. 2 cells (Table IV) illustrates that the bromo and iodo analogs **18** and **19** were more selective than the Cl analog **16**, due to their greater potency against HCMV. Depending upon which cell line and assay was used for determination of cytotoxicity, differences in antiviral selectivity were noted. In general, compounds appeared to be more selective on the basis of their cytotoxicity in HFF cells than in KB or H. Ep. 2 cells. This is probably a consequence of using visual inspection as a measure of cytotoxicity in nonproliferating HFF cells compared to measurement of growth in KB and H. Ep. 2 cells. Because growing cells are more susceptible to effects of antimetabolites than are stationary cells, determinations of cytotoxicity and antiviral selectivity are most meaningful using results obtained in proliferating cells. Apparent differences in cytotoxicity and antiviral selectivity between the KB and H. Ep. 2 cells shown in Table IV are more difficult to understand but may reflect the more rigorous assay used to determine the growth rate of H. Ep. 2 cells. Regardless of which of these two cell lines is used, the results support the conclusion that real but not extensive separation exists between anti-HCMV activity and cytotoxicity for all of the halogenated compounds. The mechanism for this selectivity has not been explored nor has the mechanism by which the compounds act. By analogy to the pyrrolopyrimidine analog of compounds **2b** and **18**, we speculate that the halogenated compounds are converted to the respective triphosphates which inhibit viral and cellular DNA polymerases. However, we have no data to substantiate these speculations.

In summary, the effect of placing a carbonyl group at C-7 of **2** to afford **3** has led to an increase in the biological activity of the latter nucleoside. As was the case in the

Table III. Antiviral Activity and Cytotoxicity of Pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one and Related Nucleosides



				IC_{50} or IC_{90} (μM)				
				antiviral activity[a]				
	substituent			HCMV			cytotoxicity[c]	
no.	R	R_1	R_2	plaque	yield	HSV-1:[b] EIA	HFF	KB
1a (tubercidin)[d]				0.5[e]			0.4[e]	1[e]
1b (5-bromotubercidin)[d]				0.6[e]	3.5	4	1.2[e]	3[e]
2a[f]	H	NH_2	H	>100[g]		>100	>100	
2b[f]	Br	NH_2	H	121[e]	1.4[e]	30[e]	100[e]	12[e]
3	H	NH_2	OH	>100		90	>100	
16	Cl	NH_2	OH	0.2[e]	0.7[e]	6.8[e]	2.1[e]	6.1[e]
18	Br	NH_2	OH	0.1	0.04[e]	2.0[e]	2.5[e]	<1.2[e]
19	I	NH_2	OH	0.4[e]	0.02[e]	4.5[e]	1.0[e]	1.2
14	H	OH	OH	>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] As described previously,[6,7] plaque assays were used to determine activity of tubercidin and bromotubercidin against HSV-1. New compounds were assayed by enzyme immunoassays (EIA). [c] Visual cytotoxicity was scored on HFF cells at time of HCMV plaque enumeration. Inhibition of KB cell growth was determined as described in the text. Results are presented as IC_{50}'s. [d] Results for tubercidin and bromotubercidin have been reported previously.[4,6,7] [e] Average concentration derived from two to four experiments. [f] Results reported previously.[9] [g] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [h] Average of 88 and 31 experiments, respectively, for plaque and yield experiments.

Table IV. Selectivity of Compounds against Human Cytomegalovirus

		IC_{50} in cytotoxicity assay (μM)[b] and antiviral selectivity[c]		
compound (3-substituent)	IC_{90} in yield assay (μM)[a]	HFF cells	KB cells	H. Ep. 2 cells
2b (Br)	1.4	100 (71)	12 (9)	5.2 (4)
16 (Cl)	0.7	2.1 (3)	6.1 (9)	
18 (Br)	0.04	2.5 (63)	<1.2 (<30)	0.2 (5)
19 (I)	0.02	1.0 (50)	1.2 (60)	0.08 (4)

[a] Data from Table III. [b] Data from Tables I and III. [c] Ratio, in parentheses, of cytotoxicity IC_{50} to HCMV yield reduction assay IC_{90}.

4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine nucleosides,[9] the addition of a 3-halo substituent significantly increased the activity of the 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one nucleosides.

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 (ν, 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 volume:volume ratios. Compounds of interest were detected by either ultraviolet lamp (254 nm), iodine vapors, or treatment with 10% sulfuric acid in methanol 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.

Ethyl 3-Cyano-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-pyrrole-2-carboxylate (6). A solution of ethyl 3-cyanopyrrole-2-carboxylate (4)[10] (6.86 g, 41.8 mmol) in DMF (200 mL) was treated with sodium hydride (3.00 g, 50% mineral oil dispersion, 1.5 equiv) and then 15-crown-5 (0.84 mL, 0.10 equiv). The resulting solution was allowed to stir under an argon atmosphere for 20 min and then a solution of 1-chloro-2,3,5-tri-*O*-benzyl-D-ribofuranose (5)[11] (1.3 equiv, prepared from 30.95 g of 1-*O*-(*p*-nitrobenzoate) precursor) in 160 mL of DMF was added in one portion. This suspension was allowed to stir at room temperature for 21 h under an argon atmosphere. The reaction mixture was then filtered through a thin pad of Celite, and the resulting filtrate was concentrated *in vacuo* (30 °C, oil pump) to furnish a dark oil. The oil was dissolved in EtOAc (400 mL) and the resulting solution was washed with H_2O (2 × 300 mL). The organic layer was dried over $MgSO_4$ and then concentrated *in vacuo* to a brown oil. The oil was dissolved in a minimal amount of EtOAc, and this solution was applied to a column (15 × 20 cm sintered glass funnel) packed with silica gel (878 g) and hexane/EtOAc 9:1. The column was eluted with the same solvent system, collecting 200 mL-sized fractions. The fractions containing a product with an R_f = 0.14 were pooled and concentrated *in vacuo* to afford an oil. The oil was dried *in vacuo* (25 °C, oil pump) for 2 h, at which point the oil began to crystallize. The semicrystalline residue was then triturated with a solution of pentane/ether 1:1. The resulting white solid was collected by filtration and dried *in* vacuo (25 °C, oil pump) for 15 h to afford 9.23 g (39%) of **6**. Mp: 68–69.5 °C. Mass spectrum (FAB+): 567 (M + 1). IR ($CHCl_3$): 2917.2, 2868.0, 2235.2, 1707.8 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.72 (d, 1H, H-5), 7.34–7.22 (m, 15H, phenyl H's), 6.70 (s, 1H, H-1′), 6.20 (d, J = 2.6 Hz, 1H, H-4), 4.89–3.69 (m, 13H, OCH_2CH_3, $C_6H_5CH_2$, H-2′, H-3′, H-4′, H-5′), 1.43 (t, 3H, CH_2CH_3). Anal. ($C_{34}H_{34}N_2O_6$) C, H, N.

4-Amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-[2,3-*d*]pyridazin-7(6*H*)-one (7). A solution of **6** (4.18 g, 7.4 mmol) in EtOH (100 mL) was treated with anhydrous hydrazine (8.4 mL) by pipet in one portion. The resulting solution was heated at reflux for 11 h and then concentrated *in vacuo* to an oil. The oil was dissolved in $CHCl_3$/MeOH 20:1 and applied to a column (12 × 14 cm sintered glass funnel) packed with silica gel (231 g) in the same solvent system. The column was eluted

with this same solvent system, with collection of fractions (15-mL sized). The fractions containing a product with an $R_f = 0.30$ were pooled and concentrated *in vacuo* to a white foam. The foam was dried further *in vacuo* (oil pump, 25 °C) for 15 h to afford 2.89 g (71%) of **7** as a white foam. Mass spectrum (FAB+): 553 (MH^+). IR ($CHCl_3$): 3191.4, 3015.6, 2917.2, 1658.6, 1637.5, 1553.1, 1454.7, 1271.9, 1215.6, 1117.2, 1089.1, 1053.9, 758.0 cm^{-1}. UV (MeOH): λ max nm (ε) 291 (4200). ^1H NMR ($CDCl_3$): δ 10.10 (s, 1H, NH-6, D_2O exchangeable), 7.84 (d, $J = 3.2$ Hz, 1H, H-2), 7.33–7.18 (m, 15H, phenyl H's), 7.04 (d, 1H, H-1'), 6.13 (d, $J = 3.2$ Hz, 1H, H-3), 4.97–3.65 (m, 15H, phenyl CH_2, NH_2, H-2', H-3', H-4', H-5'). Anal. ($C_{32}H_{32}N_4O_5$) C, H, N.

4-Amino-1-β-D-ribofuranosylpyrrole[2,3-*d*]pyridazin-7(6*H*)-one (3). A solution of **7** (2.18 g, 3.9 mmol) in EtOH (80 mL) was treated with $Pd(OH)_2$ on carbon (20% by wt, 2.18 g) and the resulting suspension was heated at reflux temperature for 6 h. The suspension was then filtered through a thin pad of Celite, and the resulting filtrate was concentrated *in vacuo* to afford a white powder. The powder was recrystallized from boiling MeOH, collected by filtration, and dried *in vacuo* (56 °C, oil pump) for 15 h to afford 260 mg (23%) of **3** as a white solid. Mp: 216–217.5 °C. Mass spectrum (EI): 282, 179 (B + 30). 150 (B + 1). IR (KBr pellet): 3670–2400 broad valley, 1658.6, 1637.5, 1546.1, 1440.6, 1278.9, 1201.6, 1103.1, 1053.9, 990.6, 779.7 cm^{-1}; UV (H_2O): (pH = 7) λ max nm (ε) 260 (5230), 288 (4740), (pH 1) 273 (6060); (pH 11) 260 (5230), 288 (4700). ^1H NMR (DMSO-d_6): δ 11.23 (s, 1H, NH-6, D_2O exchangeable), 7.71 (d, $J = 2.9$ Hz, 1H, H-2), 6.77 (d, $J = 5.3$ Hz, 1H, H-1'), 6.66 (d, $J = 2.9$ Hz, 1H, H-3), 5.69 (s, 2, NH_2, D_2O exchangeable), 5.29 (d, 1H, OH, D_2O exchangeable), 5.07 (d, 1H, OH, D_2O exchangeable), 5.02 (d, 1H, OH, D_2O exchangeable), 4.16–4.07 (m, 2H, H-2', H-3'), 3.59 (m, 2, H-5'). Anal. ($C_{11}H_{14}N_4O_5$) C, H, N.

Ethyl 3-Cyano-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrole-2-carboxylate (9). A solution of compound **4** (4.50 g, 27.0 mmol) in CH_3CN (200 mL) was treated with NaH (97% reagent, 0.87 g, 1.3 equiv). The suspension was stirred under an argon atmosphere for 30 min and then a solution of 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**)[12] prepared from 17.71 g of the 1-*O*-acetyl precursor (1.3 equiv) in CH_3CN (100 mL) was added in one portion. The reaction mixture was heated at 70 °C for 17 h and then allowed to cool to room temperature. 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 a solution of toluene/EtOAc 25:1 and applied to a column (12 × 15 cm sintered glass funnel) packed with silica gel (500 g) in the same solvent system. The column was eluted with the same solvent system, with collection of 200-mL sized fractions. Fractions containing a product with an $R_f = 0.21$ were pooled and concentrated *in vacuo* (<45 °C) to a white foam. The foam was dried further *in vacuo* (45–50 °C, oil pump) for 7 h to afford 15.83 g of **9** (96%). IR ($CHCl_3$): 3023.8, 2233.6, 1728.9, 1269.1, 1217.6, 1091.4 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.10–7.32 (m, 16H, phenyl H's, H-5), 7.10 (d, $J = 3.7$ Hz, 1H, H-1'), 6.50 (d, $J = 3.1$ Hz, 1H, H-4), 5.86–5.76 (m, 2H, H-2', H-3'), 4.90–4.66 (m, 3H, H-4', H-5'), 4.33 (q, 2H, OCH_2CH_3), 1.37 (t, 3H, OCH_2CH_3). Anal. ($C_{34}H_{28}N_2O_9$) C, H, N.

Ethyl 3-Cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (10). Compound **9** (32 g, 53.0 mmol) was added to a solution of NaOEt in EtOH (prepared by dissolving 0.60 g of Na in 500 mL of EtOH). The mixture was stirred at room temperature until a clear solution resulted (4 h). The solution was treated with sufficient Dowex 50×8–100 ion-exchange resin to effect a pH = 6. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to a sticky solid. The solid was suspended in ether (160 mL) and the resulting solid was collected by filtration, washed with an additional 200 mL of fresh ether, and dried *in vacuo* (78 °C, oil pump) to afford 2.12 g (77%) of **10** as an off-white solid. Mp: 157–159 °C.

An analytical sample of **10** was prepared by recrystallizing 272 mg of **10** from EtOH to afford 151 mg of **10** as a fluffy white solid. Mp: 158–159.5 °C. IR (KBr): 3600–3000 valley, 2937.5, 2240.2, 1715.6, 1416.8, 1257.4, 1191.0, 1051.6, 1025.0 cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.85 (d, $J = 3.0$ Hz, 1H, H-5), 6.76 (d, $J = 3.0$ Hz, 1H, H-4), 6.36 (d, $J = 2.6$ Hz, 1H, H-1'), 5.40 (br s, 1H, OH, D_2O exchangeable), 5.17 (br s, 2H, OH's, D_2O exchangeable), 4.31 (q, 2H, OCH_2CH_3), 4.02 (m, 2H, H-2', H-3'), 3.89 (m, 1H, H-4'), 3.74–3.56 (m, 2H, H-5'), 1.31 (t, 3H, OCH_2CH_3). Anal. ($C_{13}H_{16}N_2O_6$) C, H, N.

4-Amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (3). A mixture of ethyl 3-cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (**10**) (12.05 g, 40.7 mmol) and anhydrous hydrazine (24 mL) in EtOH (300 mL) was heated at reflux for 28 h. The resulting solution was concentrated *in vacuo* to a gum which was then coevaporated with EtOH (4 × 100 mL). The concentrate was dissolved in boiling H_2O (200 mL) and the solution was allowed to stand at room temperature for 15 h to effect crystallization. Additional H_2O (50 mL) was added and the resulting crystalline mass was collected by filtration. The solid was washed with EtOH and then ether and dried *in vacuo* (100 °C, oil pump) for 28 h to furnish 7.18 g (62.5%) of **3** as a white solid. Mp: 210.5–213 °C.

4-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyridazin-7(6*H*)-one (12). A mixture of **3** (2.50 g, 8.86 mmol), acetic anhydride (2.50 mL, 3.0 equiv), and 4-(dimethylamino)pyridine (105 mg, 0.1 equiv) in pyridine (30 mL) was stirred at room temperature for 5 h. The solution was then concentrated *in vacuo* (25 °C, oil pump) for 12 h to afford a yellow oil. The oil was dissolved in EtOAc (100 mL) and extracted with 2% aqueous acetic acid (1 × 100 mL) and then with H_2O (2 × 100 mL). The organic layer was dried over $MgSO_4$ and then concentrated *in vacuo* to a foam. The foam was dissolved in $CHCl_3$/MeOH 20:1 (a minimal amount) and applied to a column (3.2 × 20 cm) packed with silica gel (45 g) in the same solvent system. The column was eluted with this same solvent system, with collection of fractions (10-mL sized). The fractions containing a product with an $R_f = 0.26$ were pooled and concentrated *in vacuo* to a white foam which was dried further *in vacuo* (25 °C, oil pump) for 1 h to afford 1.69 g (47%) of **12**. IR (neat): 3198.4, 1743.0 1630.5, 1553.1, 1222.6, 758.6 cm^{-1}. UV (MeOH): λ max nm (ε) 296 (3480). ^1H NMR ($CDCl_3$): δ 10.68 (s, 1H, H-6, D_2O exchangeable), 7.47 (d, $J = 3.2$ Hz, 1H, H-2), 7.16 (d, $J = 5.1$ Hz, 1H, H-1'), 6.48 (d, $J = 3.2$ Hz, H-3), 5.54 (t, 1H, H-2'), 5.40 (t, 1H, H-3'), 4.94 (br. s., 2H, NH_2, D_2O exchangeable), 4.42–4.38 (m, 3H, H-4', H-5'), 2.18 (s, 3H, $C(O)CH_3$), 2.14 (s, 3H, $C(O)CH_3$), 2.10 (s, 3H, $C(O)CH_3$). Anal. ($C_{17}H_{20}N_4O_8 \cdot H_2O$) C, H, N.

4-Acetoxy-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (13). A solution of **12** (2.62 g, 6.4 mmol) in acetic acid (40 mL) was treated with $NaNO_2$ (70 mg, 1.6 equiv) and allowed to stir at room temperature for 2 h. The solution was concentrated *in vacuo* and the resulting orange residue was coevaporated with toluene (1 × 30 mL). The concentrate was diluted with $CHCl_3$ (50 mL), washed with H_2O (1 × 50 mL), dried over $MgSO_4$, and then concentrated *in vacuo* to an oil. The oil was dissolved in $CHCl_3$ (a minimal amount) and applied to a column (3.2 × 20 cm) packed with silica gel (48 g) in $CHCl_3$/MeOH 36:1. The column was eluted with the same solvent system, with collection of fractions (10-mL sized). The fractions containing a product with an $R_f = 0.31$ were pooled and concentrated *in vacuo* to a foam which was dried *in vacuo* (25 °C, oil pump) for 15 h to afford 1.86 g (64.4%) of **13**. Mass spectrum (EI): 451, 409, 349, 259, 230, 194, 180, 151, 139, 43. IR (neat): 3022.6, 1750.0, 1665.6, 1370.3, 1243.7, 1180.5, 758.6 cm^{-1}. UV (MeOH): λ max nm (ε) 269 (6100). ^1H NMR ($CDCl_3$): δ 10.20 (s, 1H, NH-6, D_2O exchangeable), 7.54 (d, $J = 3.2$ Hz, 1H, H-2), 7.11 (d, $J = 5.1$ Hz, 1H, H-1'), 6.44 (d, $J = 3.2$ Hz, 1H, H-3), 5.51 (t, 1H, H-2'), 5.39 (t, 1H, H-3'), 4.45–4.38 (m, 3H, H-4', H-5'), 2.41 (s, 3H, $C(O)CH_3$), 2.17 (s, 3H, $C(O)CH_3$), 2.14 (s, 3H, $C(O)CH_3$), 2.11 (s, 3H, $C(O)CH_3$). Anal. ($C_{19}H_{21}N_3O_{10}$) C, H, N.

1-β-D-Ribofuranosylpyrrolo[2,3-*d*]pyridazin-4,7(5*H*,6*H*)-dione (14). Compound **13** (680 mg, 1.51 mmol) was added to a solution of NaOMe (2 equiv) in MeOH and the resulting mixture was allowed to stir at room temperature for 24 h. The solution was treated with sufficient Dowex 50×8–100 ion-exchange resin to effect a pH = 6. The resin was removed by filtration and the filtrate was concentrated *in vacuo* to an amorphous solid. The solid was dissolved in hot H_2O (~20 mL) and the resulting solution was lyophilized in a freeze-drying apparatus for 12 h to afford 380 mg (89%) of **14** as a white fluffy solid. Mass spectrum (EI): 283, 265, 265, 217, 210, 180 (B + 30), 151 (B + 1), 139. IR (KBr pellet): 3670–2600 broad valley, 1651.6, 1560.2, 1496.9, 1440.6, 1103.1, 1053.9 cm^{-1}. UV (H_2O): (pH 7) λ max nm (ε) 278

(6070); (pH 1) 277 (6170); (pH 11) 289 (4770). ^{1}H NMR (DMSO-d_6): δ 11.25 (br s, 2H, D_2O exchangeable), 7.79 (d, J = 3.0 Hz, 1H, H-2), 6.72 (d, J = 5.1 Hz, 1H, H-1′), 6.54 (d, J = 3.0 Hz, 1H, H-3), 5.13–5.07 (m, 3H, OH's, D_2O exchangeable), 4.20 (t, 1H, H-2′), 4.06 (m, 1H, H-3′), 3.87 (m, 1H, H-4′), 3.64–3.51 (m, 2H, H-5′). Anal. ($C_{11}H_{13}N_3O_6$) C, H, N.

4-Amino-3-chloro-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (16). A solution of 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (7, 865 mg, 1.57 mmol) in CH_2Cl_2 (20 mL) was treated with *N*-chlorosuccinimide (236 mg, 1.1 equiv) in one portion. The solution was stirred for 10.5 h and then more N-chlorosuccinimide (43 mg, 0.2 equiv) was added. The reaction mixture was stirred for an additional 24 h and then concentrated *in vacuo* to ca. one-third of its original volume. The solution was applied to a column (3.2 × 20 cm) packed with silica gel (40 g) in $CHCl_3$/MeOH 30:1. The column was eluted with the same solvent system, with collection of fractions (10-mL sized). The fractions containing a product with an R_f = 0.18 were pooled and concentrated *in vacuo* to a reddish oil. The oil was dried further *in vacuo* (oil pump, 25 °C) over P_2O_5 for 20 h to afford 377 mg of 15.

The oil (15) was dissolved in CH_2Cl_2 (5 mL) and the resulting solution was chilled by an external 2-propanol–CO_2 bath. To this chilled, stirred solution was added a solution of BCl_3 in CH_2Cl_2 (1 M, 9.0 mL, 14 equiv) dropwise, by addition funnel over the course of 1 h. The external dry ice–2-propanol bath was then exchanged for a CH_3CN–CO_2 bath and the reaction mixture was allowed to stir in this bath for 30 min. A chilled solution (chilled *via* an external CH_3CN–CO_2 bath) of CH_2Cl_2/MeOH 1:1 (6 mL) was then added and the resulting mixture was allowed to warm to room temperature over the course of 1 h.

The reaction mixture was then concentrated *in vacuo* to an oil which was coevaporated with MeOH (5 × 15 mL). The oil was then dissolved in MeOH (ca. 20 mL) and the resulting solution was neutralized with sufficient concentrated NH_4OH to effect a pH = 6. Na_2SO_4 (3.5 g) was added and the resulting suspension was concentrated *in vacuo* to a yellow powder. The powder was layered onto the top of a column (3.2 × 20 cm) packed with silica gel (12 g) in $CHCl_3$/MeOH 4:1. The column was eluted with the same solvent system, with collection of fractions (5-mL sized). The fractions that contained a product with R_f = 0.18 were pooled and concentrated *in vacuo* to an off-white solid. The solid was triturated with MeOH, collected by filtration, washed with ether, and dried *in vacuo* (78 °C, oil pump) over P_2O_5 for 12 h to afford 72 mg of 16 (14.5% overall yield from 7). Mp > 193 °C dec. IR (KBr pellet): 3600–2500 valley, 1660, 1630, 1545, 1440, 1305, 1110, 1052, 1015 cm^{-1}. UV (H_2O): (pH 7) λ max nm (ε) 296 (6210); (pH 1) 291 (6500); (pH 11) 297 (5890); ^{1}H NMR (DMSO-d_6): δ 11.53 (s, 1H, NH-6), 7.98 (s, 1H, H-2), 6.83 (d, J = 4.1 Hz, 1H, H-1′), 5.45–5.11 (m, 5H, OH's, NH_2), 4.14 (m, 1H, H-2′), 4.05 (m, 1H, H-3′), 3.87 (apparent s, 1H, H-4′), 3.60 (m, 2H, H-5′). ^{13}C NMR (DMSO-d_6): δ 153.32, 143.99, 125.41, 124.35, 114.51, 105.04, 89.01, 85.06, 75.79, 69.92, 61.01. Anal. ($C_{11}H_{13}N_4O_5Cl\cdot H_2O$) C, H, N.

4-Amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (18). A solution of 7 (0.86 g, 1.6 mmol) in CH_2Cl_2 (15 mL) was treated with a solution of N-bromosuccinimide (389 mg, 1.4 equiv) in CH_2Cl_2 (20 mL) at room temperature, dropwise *via* an addition funnel, over the course of 30 min. The solution was allowed to stir at room temperature for 2 h; then the solution was concentrated *in vacuo* to a brown residue. The residue was suspended in toluene/EtOAc 4:1 and applied to a column (2.5 × 52 cm) packed with silica gel (66 g) in toluene/EtOAc/2-propanol 18:1:1. The column was eluted with the same solvent system, with collection of fractions (6-mL sized). The fractions containing a product with an R_f = 0.38 were pooled and concentrated *in vacuo* to afford a purple solid. The solid was further dried *in vacuo* (25 °C, oil pump) for 1 h. The solid was recrystallized from boiling MeOH, collected by filtration, and dried *in vacuo* (25 °C, oil pump) over P_2O_5 for 10 h to afford 268 mg of 17.

A solution of 17 (200 mg, 0.32 mmol) in CH_2Cl_2 (4 mL) was cooled by an external acetone–CO_2 bath. The solution was then treated with a solution of BCl_3 in CH_2Cl_2 (1 M in BCl_3, 4.4 mL, 1.4 equiv) dropwise by an addition funnel over the course of 25 min. The solution was allowed to stir at −78 to −74 °C for another 2 h and then the acetone–CO_2 bath was exchanged for an acetonitrile–CO_2 bath. The solution was allowed to stir at −45 to −40 °C for another 2 h. The reaction mixture was quenched with 6 mL of a prechilled solution of CH_2Cl_2/MeOH 1:1 (chilled via an external CH_3CN–CO_2 bath), and the resulting mixture was allowed to warm to room temperature. The reaction mixture was concentrated *in vacuo* and then coevaporated with MeOH (5 × 5 mL) to remove the excess BCl_3 as methyl borate. The resulting solid was dissolved in MeOH (ca. 20 mL) and then neutralized with NH_4OH (30% aqueous solution). The resulting suspension was concentrated *in vacuo*. Attempts to crystallize the product from MeOH were unsuccessful and so the solution was treated with Na_2SO_4 (2.2 g) and concentrated *in vacuo* to a solid. The solid was applied to the top of a column (2.2 × 9.5 cm) packed with silica gel (7 g) in $CHCl_3$/MeOH 4:1. The column was eluted with the same solvent system, with collection of fractions (3-mL sized). The fractions containing a product with an R_f = 0.33 were pooled and concentrated *in vacuo* to afford a gel. The gel was redissolved in MeOH and concentrated *in vacuo* to an off-white solid. The solid was slurried with MeOH, collected by filtration, and washed with ether to afford 18 as a white solid (72 mg, 62.6%). Mp > 176° dec. IR (KBr pellet): 3680–2500 valley, 1658.6, 1616.4, 1539.1, 1447.6, 1384.4, 1110.2, 1053.9 cm^{-1}. UV (MeOH): (pH 7) λ max nm (ε) 301 (5270); (pH 1) 292 (4540); (pH 11) 298 (6600). ^{1}H NMR (DMSO-d_6): δ 11.50 (s, 1H, NH-6, D_2O exchangeable), 8.01 (s, 1H, H-2), 6.84 (d, J = 5.4 Hz, 1H, H-1′), 5.41–5.08 (m, 5H, OH's, NH_2, D_2O exchangeable), 4.15 (q, 1H, H-2′), 4.04 (q, 1H, H-3′), 3.87 (dd, 1H, H-4′), 3.60 (m, 2H, H-5′). ^{13}C NMR (DMSO-d_6): δ 153.34, 144.02, 126.77, 126.12, 115.82, 89.36, 89.02, 85.06, 75.81, 69.89, 60.99. Anal. ($C_{11}H_{13}N_4O_5Br$) C, H, N.

4-Amino-3-iodo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazin-7(6*H*)-one (19). A solution of 3 (5.00 g, 18 mmol) in glacial acetic acid (700 mL) was treated with a solution of *N*-iodosuccinimide (6.29 g, 1.5 equiv) dropwise *via* an addition funnel over the course of 4 h. Powdered sodium sulfate (140 g) was added to the resulting solution and the suspension was concentrated *in vacuo* to a solid. The solid was coevaporated with toluene (2 × 200 mL) to remove the residual acetic acid. The solid was layered onto the top of a column (9.5 × 9 cm sintered glass funnel) packed with silica gel (108 g) in $CHCl_3$/MeOH 4:1. The column was eluted with this same solvent system collecting 75-mL-sized fractions. The fractions containing a product with an R_f = 0.29 were pooled and concentrated *in vacuo* to a yellow solid. The solid was swirled with hot H_2O (75 mL) and the resulting suspension was allowed to cool to room temperature. The precipitate was collected by filtration and dried *in vacuo* (100 °C, oil pump) for 20 h. The solid was recrystallized from boiling H_2O and collected by filtration. The solid was washed with EtOH and then ether and dried *in vacuo* (100 °C, oil pump) for 22 h to furnish 3.43 g (47.4%) of 19 as a white solid. Mp: 218–220 °C. IR (KBr pellet): 3600–2500 br valley, 1622.6, 1536.3, 1124.6, 1051.6 cm^{-1}; UV (H_2O): (pH 7) λ max nm (ε) 249 (15640), 298 (6960); (pH 1) 253 (10880), 294 (7850); (pH 11) 251 (13330), 298 (6590); ^{1}H NMR (DMSO-d_6): δ 11.53 (s, 1H, NH-6); 8.00 (s, 1H, H-2); 6.84 (d, J' = 5.1 Hz, 1H, H-1′); 5.34, 5.10 (2s, 5H, OH's, NH_2); 4.14 (m, 1H, H-2′); 4.03 (m, 1H, H-3′); 3.86 (m, 1H, H-4′); 3.59 (m, 2H, H-5′). ^{13}C NMR (DMSO-d_6) δ 153.49, 144.20, 131.90, 127.05, 118.45, 88.77, 85.03, 75.78, 69.95, 61.04, 55.82. Anal. ($C_{11}H_{13}N_4O_5I$) C, H, N.

***In Vitro* Antiproliferative Studies.** The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[19] 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 was defined as the slope of the semilogarithmic plot of cell number against time for the treated culture as a percent of the slope for the control culture. This parameter was determined experimentally by calculating the ratio of the population doubling time (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} was defined as the concentration required to decrease the growth rate of 50% of the control.

H. Ep. 2 cells were grown as described previously[9] in Minimal Essential Medium (MEM) (with Earle's Salts) (Gibco, Grand Island, NY) with 15% heat-inactivated (56 °C, 30 min) bovine calf serum (Hyclone Laboratories, Logan, UT) in a 5% CO_2–95% air atmosphere. For growth inhibition evaluations, 2000 cells were plated in each well of 96-well microtiter plates with MEM with Earle's Salts with 5% bovine calf serum. The test compounds were added 1 day later, and cell growth was assayed using crystal violet staining[9] on the day of compound addition and 2 and 5 days thereafter. The $A_{560-570}$ of each well was determined using a Model v_{max} microplate reader (Molecular Devices, Menlo Park, CA). The $A_{560-570}$ was found to be proportional to cell number and was therefore used in place of cell number to calculate T_d, growth rate, and IC_{50} as described above for L1210 cells. The control T_d was constant throughout the 5-day evaluation period, and the average value was 24 h.

The evaluation of antiproliferative activity in the remaining human tumor cell lines (Table II) was performed using the assay conditions as optimized for each cell line, to provide exponential growth of control cultures throughout the assay period. The cells were grown in the media shown in Table II, harvested and the number of cells shown was plated in each well of 96-well microtiter plates. For all cell lines except the small cell lung carcinomas NCI-H146 and NCI-N417, the compounds to be evaluated were added to the wells 1 day after the cells were plated. For NCI-H146 and NCI-N417, which grow in suspension, the compounds were added simultaneously with the cells. After the number of days specified in Table II, the extent of cell growth was assayed in control and compound-treated wells. The tetrazolium reduction assay[20] was used for NCI-H-146 and NCI-N417, and the sulforhodamine B dye binding assay[21] was used for the remaining lines in Table II. The optical densities were linearly related to cell number over the ranges used in the assays. Therefore, the optical densities were used in place of cell numbers in calculating the growth rate and IC_{50}'s as described for L1210 cells.

***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.[4] A plaque-purified isolate, P_O, of the Towne strain of HCMV as used and was a gift of Dr. M. F. Stinksi, of the 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.[4]

(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 approximmatey 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.[22] HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[23]

(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.[4] 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. Details of the procedure are in ref 23.

(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 or ganciclovir for HSV-1 and HCMV, 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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