Synthesis, Antiproliferative, and Antiviral Activity of Certain 4-Aminopyrrolo[2,3-*d*]pyridazine Nucleosides: An Entry into a Novel Series of Adenosine Analogues

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The preparation of novel heterocyclic base modified adenosine analogues, the 4-aminopyrrolo[2,3-*d*]pyridazine nucleosides, is described. Crucial to their successful preparation was the use of the pyrrole glycoside intermediates 3-cyano-2-formyl-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole (11) and 3-cyano-2-formyl-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole (17). Treatment of 11 and 17 with hydrazine dihydrochloride followed by treatment with boron trichloride provided 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine (2) and 4-amino-1-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyridazine (3), respectively. 4-Amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyridazine (4) was prepared by a bromination of 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine (12) and subsequent removal of the benzyl groups with boron trichloride. Compounds 2–4 were evaluated for antiproliferative and antiviral activity. The tubercidin analogue (2) and its arabinosyl derivative (3) were virtually inactive in all assays. In contrast, the 3-bromo analogue 4 inhibited growth of L1210 and H. Ep. 2 cells. Compound 4 was also active against human cytomegalovirus and herpes simplex virus type 1, but the antiviral activity was not completely separated from cytotoxicity.

Analogues of the naturally occurring nucleoside adenosine have proven to be important leads for therapeutically useful agents. Sugar-modified analogues of adenosine such as 6-amino-9-β-D-arabinofuranosylpurine (ara-A, vidarabine) have been used clinically for the treatment of certain herpes virus infections.[1] Another adenosine analogue, 2',3'-dideoxyadenosine, has recently undergone phase I clinical trials for the treatment of HIV.[2] Modifications of adenosine in the heterocyclic base moiety have afforded many other biologically active molecules.[3] The naturally occurring pyrrolo[2,3-*d*]pyrimidine analogue of adenosine, tubercidin (1), which contains a carbon atom in place of the 7-nitrogen atom, exhibited significant antineoplastic[4] and antiviral properties.[5] Unfortunately, antiviral activity is closely associated with cytotoxicity, particularly for the herpesviruses.[6] In addition to tubercidin, a number of other biologically active pyrrolo-[2,3-*d*]pyridmidine analogues of adenosine have been isolated or synthesized. This class of compounds has been under investigation as antineoplastic[7] and antiviral[6,8] agents for a number of years in our laboratories. We found that bromotubercidin and acyclic analogues of bromotubercidin were less cytotoxic than tubercidin and retained activity against herpesviruses, particularly human cytomegalovirus (HCMV).[9,10]

Although the chemical and biological effects of exchanging a carbon atom for the N-7 atom of the adenine base have been studied, the effects of an additional interchange involving the N-3 and C-2 atoms of adenosine remained to be investigated. This prompted us to initiate a synthesis of 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyridazine (2), a structural isomer of the nucleoside antibiotic tubercidin (1), in order to investigate the effects which these chemical changes would have on biological activity. The antiviral activity reported for vidarabine and 5-bromotubercidin[1,5] prompted us to undertake the preparation of 4-amino-1-β-D-arabinofuranosylpyrrolo[2,3-*d*]-pyridazine (3) as well as 4-amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine (4).

Results and Discussion

Chemistry. A literature search revealed that a number of syntheses which generate the pyrrolo[2,3-*d*]pyridazine

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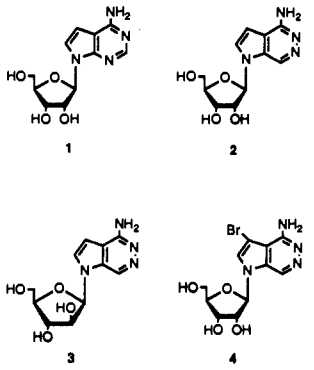
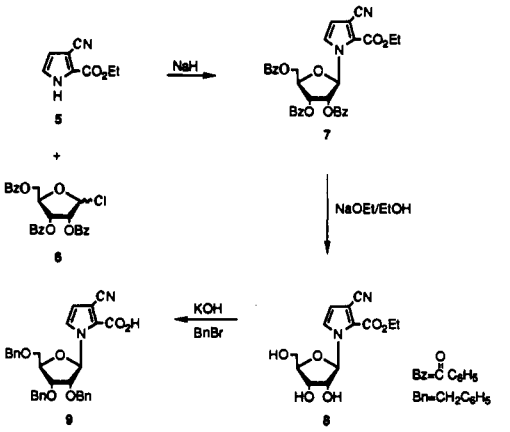


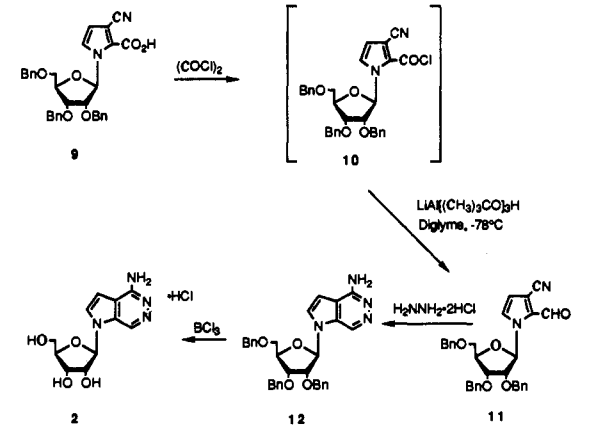
heterocyclic nucleus had been reported;[11] however, there were no reports involving the synthesis of pyrrolo[2,3-*d*]-pyridazine nucleosides. The ring annulation of a 2,3-disubstituted pyrrole precursor, as described by Bisagni and co-workers,[12] appeared to be the most relevant to our study. Using an appropriately substituted pyrrole with a sugar moiety at position 1 would allow us a facile entry into the pyrrolo[2,3-*d*]pyridazine nucleoside system. This approach was especially appealing since this would eliminate the problem of regioselectivity which is usually associated with a direct glycosylation of a bicyclic heterocycle. In addition, a convenient method for the stereoselective glycosylation of pyrroles with a number of different 1-halo sugars has been reported.[13,14] Thus, if the synthetic strategy was successful, it would provide a route for the regioselective introduction of other sugars to this heterocyclic system.

With this strategy chosen, our initial synthetic efforts were focused on the preparation of 4-amino-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine (**2**). After a comprehensive literature search, we chose to use ethyl 3-cyanopyrrole-2-carboxylate (**5**)[15] as our starting material because of the favorable juxtaposition of substituents and also because different chemical reactivity of these substituents would allow us to accomplish the requisite manipulations. The sodium salt glycosylation of **5** with 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribose (**6**)[16] in acetonitrile (Scheme I) provided a 95% yield of a single anomer of ethyl 3-cyano-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrole-2-carboxylate (**7**) as judged by ^{1}H NMR. In contrast to a previous report, we did not observe any product which would have resulted from an attack of the pyrrole anion on the 2′-carbonyl moiety of the benzoyl-protected sugar.[14]

Scheme I



Scheme II



In order to prevent unwanted side reactions due to deblocking during the modification of the pyrrole substituents, the base-labile benzoyl blocking groups were replaced by the more stable benzyl blocking groups. In order to effect this interchange, the benzoyl blocking groups of **7** were first removed with sodium ethoxide in ethanol to provide **8** in 77% yield. The sugar hydroxyl groups of ethyl 3-cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (**8**) were then benzylated with potassium hydroxide/benzyl bromide with a simultaneous saponification of the ester moiety of the pyrrole to provide 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxylic acid (**9**, 70%). A peak at 2235 cm^{-1} in the IR spectrum confirmed that the nitrile moiety in **9** was still present.

In order to reduce the carboxylic acid function to an aldehyde function, a selective procedure that would not affect the nitrile function was required. Lithium tri-*tert*-butoxyaluminohydride[17] has been used to selectively reduce an acyl chloride to an aldehyde moiety, without further reduction to the alcohol, while nitrile moieties are resistant to this hydride reagent.[18] This procedure was applied to our problem by first converting **9** to 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxylic acid chloride (**10**) by treatment with oxalyl chloride (Scheme II). Compound **10** was used, without further

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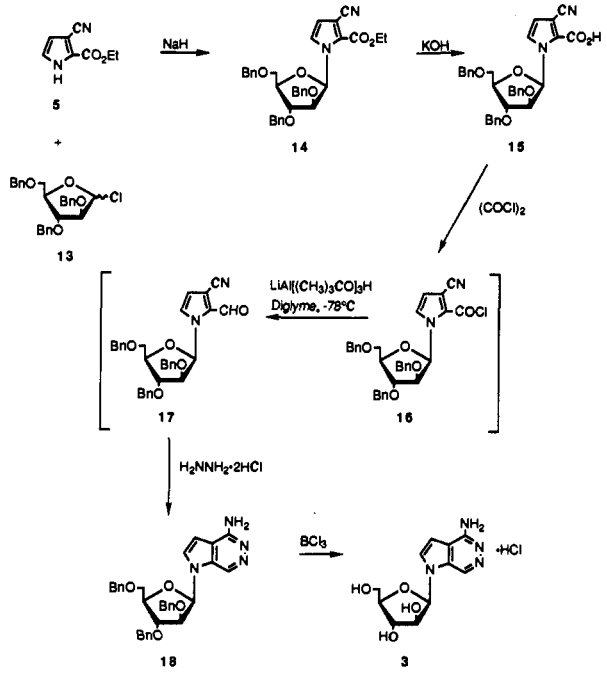
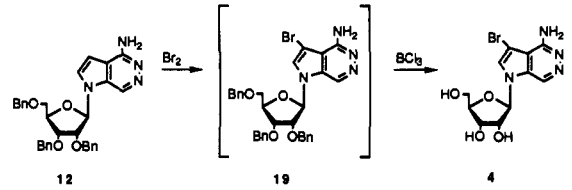
Scheme III



Scheme IV



isolation, in the reaction with lithium tri-*tert*-butoxyaluminohydride in diglyme at −78 °C to afford 3-cyano-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxyaldehyde (11). For analytical purposes, a sample of 11 was purified by column chromatography, and a ^{1}H NMR spectrum revealed a peak at 9.84 ppm corresponding to the aldehydic proton. The IR spectrum of 11 contained a peak at 2230 cm^{-1}, confirming the presence of the nitrile moiety. It was also at this stage that the anomeric configuration of the pyrrole nucleoside was determined. In the ^{1}H NMR spectrum of 11, the peak assigned to the anomeric proton appeared as a singlet at 6.61 ppm. A coupling constant of <1 Hz is possible only for a trans relationship of the H-1′ and H-2′ in the ribofuranosyl ring,[19] which established the β configuration for nucleoside 11.

Annulation of crude 11 with hydrazine dihydrochloride in ethanol at reflux proceeded smoothly to provide, after column chromatography, 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine (12) in a 33% overall yield from 9. Evidence that ring closure had occurred was confirmed by the loss of the absorbance due to the nitrile in the IR spectrum and by the molecular ion at 536 in the electron-impact mass spectrum of 12. The benzyl groups of 12 were removed with boron trichloride to provide the target compound 2, isolated as its monohydrochloride salt (59% yield).

The next goal of this investigation was to extend this methodology toward the synthesis of the adenosine analogue 4-amino-1-β-D-arabinofuranosylpyrrolo[2,3-*d*]-pyridazine (3). The sodium salt method for the glycosylation of pyrroles permits the attachment of a number of different sugars including the arabinofuranosyl moiety. The sodium salt method for pyrroles has been used previously for the successful synthesis of some arabinofuranosylpyrrolo[3,2-*d*]pyridine nucleosides[20] and some arabinofuranosylpyrrolo[2,3-*d*]pyrimidine nucleosides.[13] Extension of the above synthetic route to the arabinofuranosyl analogue of our heterocyclic system required the use of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (13).[21] Glycosylation of the sodium salt of 5 with 13 in acetonitrile proceeded smoothly to afford ethyl 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole (14) in 59% yield after chromatography (Scheme III). In this case, the appropriate benzyl protecting groups were already in place, and no interchange of protecting groups was necessary. The nucleoside 14 was converted into the 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole-2-carboxylic acid chloride (16) via a saponification with potassium hydroxide to form 3-cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole-2-carboxylic acid (15) followed by treatment with oxalyl chloride as before. The nucleoside 16 was then reduced with lithium tri-*tert*-butoxyaluminohydride to afford 3-cyano-2-formyl-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole (17). Compound 17 was ring annulated with hydrazine dihydrochloride to afford 4-amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazine (18) in 27% overall yield from compound 14. Deprotection of 18 with boron trichloride afforded the target compound 4-amino-1-(β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyridazine (3). This compound was isolated as its monohydrochloride salt in 53% yield from compound 18.

In order to synthesize the 3-bromo derivative of 2, the protected analog 12 was used as the starting material (Scheme IV). Bromination of 12 was accomplished by treating the compound with bromine to afford 4-amino-3-bromo-1-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyridazine (19). The benzyl groups were removed with boron trichloride to afford 4-amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]pyridazine (4) in 27% yield from 12. That monobromination had occurred at the expected 3-position was evident from an analysis of the 90-MHz ^{13}C NMR spectrum. In the spectrum of 4, the peaks corresponding to the pyrrole carbon attached directly to a hydrogen appeared as a doublet of doublets centered at δ 126.26. Upon irradiation at the frequency corresponding to the anomeric proton, these peaks collapsed to a clean doublet. Three-bond coupling of the anomeric proton to C-2, which was attached directly to a hydrogen atom, was consistent with this observation. This was in agreement with bromination having occurred at the C-3 position.

In summary, a synthetic route that provides an entry into a novel series of adenosine analogues has been established. These synthetic routes permit structural variations which were crucial for the successful synthesis of

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Table I. Antiproliferative Effects of Pyrrolo[2,3-d]pyridazine Adenosine Analogues on L1210 and H. Ep. 2 Cells

	L1210		H. Ep. 2	
compound	growth rate[a] at 100 μM, % of control	IC_{50},[b] μM	growth rate[a] at 100 μM, % of control	IC_{50},[b] μM
1 (tubercidin)[c]	0	0.04	0	0.06
5-bromotubercidin	9	4		
2	100	-	93	-
3	88	>100		
4	9	24	6	5.2

[a] Growth rate is defined in the Experimental Section. [b] Concentration required to decrease the growth rate to 50% of control. [c] Results for tubercidin and bromotubercidin have been reported previously.[10]

4-amino-1-β-D-ribofuranosylpyrrolo[2,3-d]pyridazine (2), 4-amino-1-β-D-arabinofuranosylpyrrolo[2,3-d]pyridazine (3), and 4-amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-d]pyridazine (4).

Biology. In Vitro Antiproliferative Testing. The potential of these pyrrolo[2,3-d]pyridazine nucleosides for antiproliferative cancer chemotherapy was evaluated by determining their ability to inhibit the growth of L1210 and H. Ep. 2 cells in vitro (Table I). In both cell lines, the tubercidin analogue in this series (2) showed a startling loss of cytotoxic activity in comparison to tubercidin itself. The analogue of 5-bromotubercidin (4), in contrast was only slightly less cytotoxic than its parent compound, with an IC_{50} of 24 μM as compared to 4 μM. Thus, it is of interest to note that among these pyrrolo[2,3-d]pyridazine nucleosides, the most cytotoxic is 4, the analogue of 5-bromotubercidin, while among the pyrrolo[2,3-d]pyrimidine nucleosides, tubercidin itself is by far the more cytotoxic. This reversal of structure–activity requirements may reflect a different enzymatic pathway for activation and/or a different biochemical target.

In Vitro Antiviral Activity. The target compounds (2–4) as well as tubercidin (1) and 5-bromotubercidin were evaluated for activity against HCMV and HSV-1. Cytotoxicity of each compound was determined visually in normal human diploid fibroblasts (HFF cells) and by assaying cell growth in a human neoplastic cell line (KB cells). Data in Table II show that activity against HCMV and HSV-1 are similar to antiproliferative activity. Neither of the 3-unsubstituted compounds (2 and 3) were active against HCMV or HSV-1, nor were they cytotoxic. This is somewhat surprising considering that compound 2 is a close analogue of tubercidin.

These results with pentofuranosyl tubercidin analogues are somewhat similar to previous observations that acyclic tubercidin analogues were inactive whereas the 5-halogen analogues were very active against herpesviruses.[9,10] This fact led to the synthesis of the 3-bromo analogue in the current work. Interestingly, the 3-bromo analogue (4) was active in all assays. It was weakly active in HCMV plaque reduction assays as well as in EIA assays for HSV-1; it also showed some cytotoxicity in HFF and KB cells. Surprisingly, it was most active in HCMV yield-reduction assays (Table II). The compound reduced HCMV titers by nearly four logs (10 000-fold) at 32 to 100 μM (data not shown). Because yield assays measure the presence of infectious virus particles compared to measurement of virus-induced alterations in cell morphology in plaque reduction assays, we believe these results are most significant. However, compound 4 did inhibit the growth of L1210, H. Ep. 2, and KB cells in the micromolar range (Tables I and II); thus the activity against HCMV was not fully separated from cytotoxicity. At this time we have no explanation for the observation that compound 4 was most active in yield reduction assays other than to raise the possibility that the compound acts late in HCMV replication cycle, after viral effects on cell morphology already have occurred.

Table II. Antiviral Activity and Cytotoxicity of Pyrrolo[2,3-d]pyridazine Nucleosides

	50 or 90% inhibitory concentration (μM)				
	antiviral activity[a]				
	HCMV		HSV-1[b]	cytotoxicity[c]	
compound	plaque	yield	plaque	HFF	KB
1 (tubercidin)[d]	0.5[e]			0.4[e]	1[e]
5-bromotubercidin	0.6[e]	3.5	4	1.2[e]	3[e]
2	>100[f]		>100	>100	
3	>100		>100	>100	
4	90[e]	2.2[e]	30[e]	100[e]	12[e]
acyclovir	63	90	3.4	>100	>100
ganciclovir (DHPG)	8.4[g]	1.8[e]	4.5	>100[e]	>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 and those for yield reduction experiments as IC_{90}'s. [b] As described previously,[9,10] 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.[6,9,10] [e] Average concentration derived from two to seven experiments. [f] >100 indicates IC_{50} or IC_{90} not reached at the noted (highest) concentration tested. [g] Average of 60 experiments.

Experimental Section

Proton magnetic resonance (^1H NMR) spectra were obtained with a Bruker WP270SY spectrometer (solutions in dimethyl-d_6 sulfoxide 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). The R_f values were determined using the solvent system used to elute the column, unless otherwise specified. Solvent systems are reported in v/v 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*-benzoyl-β-D-ribofuranosyl)-pyrrole-2-carboxylate (7). A solution of ethyl 3-cyanopyrrole-2-carboxylate[15] (5, 4.50 g, 0.027 mol) in acetonitrile (200 mL) was treated with sodium hydride (97% reagent, 0.87 g, 1.3 equiv). The suspension was stirred under an argon atmosphere for 30 min. A solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[16] (6, 1.3 equiv) in acetonitrile (100 mL) was then 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 the minimal amount of a toluene/ethyl acetate 25:1 mixture 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 while collecting 200-mL fractions. Fractions containing a product with an R_f = 0.21 were

combined 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 **7** (96%): IR (chloroform) ν_{max} 3024, 2234, 1729, 1269, 1218, 1091 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.10–7.32 (m, 16 H, phenyl H's, H-5), 7.10 (d, 1 H, J = 3.7 Hz, H-1′), 6.50 (d, 1 H, J = 3.1 Hz, H-4), 5.86–5.76 (m, 2 H, H-2′, H-3′), 4.90–4.66 (m, 3 H, H-4′, H-5′), 4.33 (q, 2 H, OCH_2CH_3), 1.37 (t, 3 H, OCH_2CH_3). Anal. ($C_{34}H_{28}N_2O_9$) C, H, N.

Ethyl 3-Cyano-1-β-D-ribofuranosylpyrrole-2-carboxylate (8). Compound **7** (32 g, 0.053 mol) was added to a solution of sodium ethoxide in ethanol (prepared by dissolving 0.60 g of sodium in 500 mL of ethanol). The mixture was stirred at room temperature until a clear solution resulted (4 h). The solution was treated with sufficient Dowex 50X8-100 ion exchange resin to effect a pH = 6. The resin was removed by filtration, and the filtrate was concentrated in vacuo to afford a sticky solid. The solid was triturated with ether (160 mL), and the resulting solid was collected by filtration, washed with additional ether (200 mL), and dried in vacuo (78 °C, oil pump) to afford 2.12 g (77%) of **8** as an off-white solid: mp 157–159 °C.

An analytical sample of **8** was prepared by recrystallizing 272 mg of **8** from ethanol to afford 151 mg of **8** as a fluffy white solid: mp 158–159.5 °C; IR (potassium bromide) ν_{max} 3600–3000 valley, 2938, 2240, 1716, 1417, 1257, 1191, 1052, 1025 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.85 (d, 1 H, J = 3.0 Hz, H-5), 6.76 (d, 1 H, J = 3.0 Hz, H-4), 6.36 (d, 1 H, J = 2.6 Hz, H-1′), 5.40 (br s, 1 H, OH, deuterium oxide exchangeable), 5.17 (br s, 2 H, OH's, deuterium oxide exchangeable), 4.31 (q, 2 H, OCH_2CH_3), 4.02 (m, 2 H, H-2′, H-3′), 3.89 (m, 1 H, H-4′), 3.74–3.56 (m, 2 H, H-5′), 1.31 (t, 3 H, OCH_2CH_3). Anal. ($C_{13}H_{16}N_2O_6$) C, H, N.

3-Cyano-1-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrrole-2-carboxylic Acid (9). A solution of **8** (0.50 g, 1.69 mmol) in tetrahydrofuran (20 mL) was treated with potassium hydroxide (8.57 mg, 9 equiv) and then benzyl bromide (1.22 mL, 6 equiv). The resulting suspension was heated at reflux for 21 h and then concentrated in vacuo. The concentrate was poured into ice water (40 mL), and this suspension was slowly neutralized with concentrated hydrochloric acid (1.3 mL, 9 equiv). The suspension was extracted with ether (2 × 30 mL). The ether extracts were combined, dried over magnesium sulfate, and concentrated in vacuo to an oil. The oil was dissolved in chloroform and applied to a column (3.2 × 20 cm) packed with silica gel (30 g) in chloroform (neat). The column was eluted with chloroform (neat) and then chloroform/methanol/acetic acid 96:3:1 while collecting 10-mL fractions. The fractions containing a product with an R_f = 0.41 were combined and concentrated in vacuo to an oil which was dried in vacuo (25 °C, oil pump) for 18 h to afford **9** (660 mg, 73%): IR (neat) ν_{max} 3650–2500, 2235, 1715, 1680, 1434 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.82 (d, 1 H, J = 3.0 Hz, H-5), 7.36–7.22 (m, 15 H, $C_6H_5CH_2$), 6.68 (d, 1 H, J = 1.6 Hz, H-1′), 6.31 (d, 1 H, J = 3.0 Hz, H-4), 4.84 (m, 11 H, $C_6H_5CH_2$, H-2′, H-3′, H-4′, H-5′). Anal. ($C_{32}H_{30}N_2O_6$) C, H, N.

4-Amino-1-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyridazine (12). A solution of **9** (12.13 g, 22.5 mmol) and oxalyl chloride (13 mL, 7 equiv) in toluene (250 mL) was heated at reflux temperature for 16 h. The solution was concentrated in vacuo to an oil, which was dried further in vacuo (25 °C, oil pump) for 9 h to afford the acid chloride **10** as a viscous oil (13.25 g); IR (neat) ν_{max} 3125, 3035, 2925, 2870, 2230, 1745, 1705 cm^{-1}. The acid chloride **10** was dissolved in diglyme (35 mL) and chilled by an external 2-propanol/dry ice bath. A solution of lithium tri-*tert*-butoxyaluminohydride was added dropwise, by an addition funnel, to this chilled solution over the course of 1.5 h. After the addition was complete, the reaction mixture was allowed to warm to room temperature over the course of 1 h. The mixture was then poured onto crushed ice (250 mL). When the ice had melted, the resulting suspension was filtered onto a thick pad of Celite. The resulting oily solid was removed from the top of the Celite pad, dried in vacuo (25 °C, oil pump) for 15 h, and then stirred with chloroform (250 mL). The chloroform suspension was filtered through a thin pad of Celite. The filtrate was dried over magnesium sulfate and then concentrated in vacuo to afford the crude aldehyde **11** (11.67 g) as an oil: IR (neat) ν_{max} 3120, 3020, 2920, 2860, 2225, 1675, 1355 cm^{-1}.

The crude aldehyde **11** was dissolved in ethanol (270 mL) and treated with hydrazine dihydrochloride (2.31 g). The resulting mixture was heated at reflux temperature for 20 h and then allowed to cool to room temperature. The insoluble material was removed by filtration, and the filtrate was concentrated in vacuo to a gum. The gum was dissolved in a mixture of chloroform/methanol/acetic acid 100:4:1 and applied to a column (12 × 14 cm sintered glass funnel) packed with silica gel (375 g) in the same solvent system. The column was eluted with the same solvent system and then chloroform/methanol/acetic acid 100:8:1 while collecting 100-mL fractions. The fractions containing a product with an R_f = 0.11 (chloroform/methanol/acetic acid 100:4:1) were combined and concentrated in vacuo to an oil, which was coevaporated with toluene (1 × 75 mL). The resulting concentrate was dissolved in ethyl acetate (300 mL) and extracted with sodium bicarbonate solution (2 × 250 mL). The organic layer was dried over magnesium sulfate and then concentrated in vacuo to afford 4.0 g of **12** (33% from **9**) as a foam: MS m/e 536 (M^+), 428, 181, 105, 91; 77, 66; IR (neat) ν_{max} 3580–2720 broad valley, 1630, 1580, 1490 cm^{-1}; UV λ_{max} nm (ε) (methanol) 264 (4640), 295 (5550); ^{1}H NMR ($CDCl_3$) δ 8.97 (s, 1 H, H-7), 7.34–7.00 (m, 16 H, phenyl H's, H-2), 6.35 (d, 1 H, J = 3.2 Hz, H-3), 6.08 (d, 1 H, J = 6.0 Hz, H-1′), 4.91 (br s, 2 H, NH_2, deuterium oxide exchangeable) 4.71–4.09 (m, 9 H, H-2′, H-3′, H-4′, $C_6H_5CH_2$), 3.75–3.54 (m, 2 H, H-5′). Anal. ($C_{32}H_{32}N_4O_4$) C, H, N.

3-Cyano-2-formyl-1-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)pyrrole (11). A solution of **10** in diglyme (8 mL) was chilled by an external 2-propanol/dry ice bath while a solution of lithium tri-*tert*-butoxyaluminohydride (0.5 M) in diglyme (4.8 mL, 0.96 equiv) was added dropwise, by addition funnel, over the course of 1 h. After the addition was complete, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature over the course of 1 h. The reaction mixture was poured onto 25 mL of crushed ice. After the ice had melted, the resulting suspension was filtered onto a thick pad of Celite. The gummy precipitate was scraped from the top of the Celite pad and dried in vacuo (25 °C, oil pump) for 4 h. The precipitate was stirred with chloroform (45 mL) for 20 min at room temperature, and the resulting suspension was then filtered through a thin pad of Celite. The filtrate was concentrated in vacuo to an oil, which was dissolved in toluene/ethyl acetate 10:1 (a minimal amount) and applied to the top of a column (3.2 × 20 cm) packed with silica gel (20 g) in the same system. The column was eluted with the same solvent system while collecting 5-mL fractions. The fractions containing a product with an R_f = 0.37 were combined, concentrated in vacuo, and dried in vacuo (25 °C, oil pump) for 12 h to afford **11** as an oil (402 mg, 31% yield): IR (neat) ν_{max} 3121, 3065, 3037, 2931, 2861, 2228, 1679 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.84 (s, 1 H, CHO), 7.95 (d, 1 H, J = 2.9 Hz, H-5), 7.37–7.23 (m, 15 H, phenyl H's), 6.61 (s, 1 H, H-1′), 6.28 (d, 1 H, J = 2.9 Hz, H-4), 4.97–3.68 (m, 11 H, H-2′, H-3′, H-4′, H-5′, $C_6H_5CH_2$). Anal. ($C_{32}H_{30}N_2O_5$) C, H, N.

4-Amino-1-β-D-ribofuranosylpyrrolo[2,3-d]pyridazine Hydrochloride (2). A solution of **12** (104 mg, 0.19 mmol) in dichloromethane (5 mL) was chilled via an external 2-propanol/dry ice bath. A solution of boron trichloride in dichloromethane (1 M, 2.2 mL, 14 equiv) was added to the chilled, stirring reaction mixture via an addition funnel over the course of 10 min. The reaction mixture was stirred while being cooled by the external bath for 1.25 h, and then a prechilled solution (chilled via an external 2-propanol/dry ice bath) of dichloromethane/methanol 1:1 (7 mL) was added in one portion. The external ice bath was removed, and the reaction mixture was allowed to warm to room temperature over the course of 1 h. The reaction mixture was concentrated in vacuo to a white solid, which was coevaporated with methanol (5 × 5 mL). The solid was triturated with a minimal amount of methanol, and the resulting solid was collected by filtration and washed with ether to furnish 34 mg (59%) of **2**: mp 231.5–233°; IR (potassium bromide) ν_{max} 3600–2400 valley, 3431, 3198, 2931, 1659, 1384, 1103, 1054, 977, 618 cm^{-1}; UV λ_{max} nm (ε) (methanol) 267 (6900), 285 (7400), (pH 1) 266 (5400), 286 (6200), (pH 11) 261 (5200), 294 (7600); ^{1}H NMR (DMSO-d_6) δ 14.45 (br s, 1 H, deuterium oxide exchangeable), 9.08 (s, 1 H, H-7), 8.77 (br s, 2 H, NH_2, deuterium oxide exchangeable), 8.04 (d, 1 H, H-2), 7.27 (d, 1 H, H-3), 6.04 (d, 1 H, H-1′), 5.95–5.28 (m, 3 H, OH's, deuterium oxide exchangeable), 4.16–4.02 (m, 3 H, H-2′, H-3′, H-4′), 3.65 (m, 2 H, H-5′). Anal. ($C_{11}H_{15}N_4O_4Cl$) C, H, N.

Ethyl 3-Cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole-2-carboxylate (14). A solution of **5** (10 g, 60.9 mmol) in acetonitrile (400 mL) was treated with sodium hydride (4.68 g, 50% mineral oil dispersion, 1.6 equiv) in two portions over the course of 5 min. The resulting suspension was allowed to stir at room temperature for 40 min. A solution of 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride (**13**)[21] (1.7 equiv) in acetonitrile (200 mL) was added over the course of 3 min. The resulting suspension was allowed to stir at room temperature for 27 h. An attempt to remove the fine precipitate by filtration of the reaction mixture through a pad of Celite was unsuccessful. The precipitate was removed by filtration through a pad of silica gel (150 g). The resulting filtrate was concentrated in vacuo to furnish a brown oil. The oil was dissolved in toluene/ethyl acetate 30:1 (50 mL) and applied to a column (15 × 20 cm sintered glass funnel) packed with silica gel (985 g) in the same solvent system. The column was eluted with the same solvent system while collecting 200-mL fractions. The fractions that were homogeneous in containing a product with a R_f = 0.32 (toluene/ethyl acetate 20:1) were combined and concentrated in vacuo (<40 °C, water pump) to a light green oil. The oil was dried further in vacuo (40–45 °C, oil pump) to afford 14.80 g of **14**.

The fractions containing **14**, which were contaminated with a second compound having a higher R_f as judged by TLC, were pooled and concentrated in vacuo (<40 °C, water pump). The resulting oil was redissolved in toluene/ethyl acetate 35:1, and applied to a column (15 × 20 cm sintered glass funnel) packed with silica gel (600 g) in the same solvent system. The column was eluted with the same solvent system while collecting 150-mL fractions. The fractions that contained only **14** [R_f = 0.32 (toluene/ethyl acetate 20:1)] were pooled and concentrated in vacuo to afford a light green oil. The oil was dried further in vacuo (40–45 °C, oil pump) for 2.5 h to afford an additional 5.40 g of **14**. The combined yield of **14** was 20.2 g (58.5%): IR (neat) ν_{max} 3010, 2940, 2220, 1710, 1600, 1480, 770 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.41 (d, 1 H, J = 3 Hz, H-5), 7.38–6.90 (m, 15 H, phenyl H's), 6.82 (d, 1 H, J = 4.6 Hz, H-1′), 6.46 (d, 1 H, J = 3 Hz, H-4), 4.55 (q, 2 H, OCH_2CH_3), 4.32–4.07 (m, 9 H, $C_6H_5CH_2$, H-2′, H-3′, H-4′), 3.68 (d, 2 H, J = 5.1 Hz, H-5′), 1.38 (t, 3 H, OCH_2CH_3). Anal. ($C_{34}H_{34}N_2O_6$) C, H, N.

3-Cyano-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-pyrrole-2-carboxylic Acid (15). A solution of **14** (29.68 g, 52.4 mmol) in ethanol/water 55:1 (560 mL) was treated with powdered potassium hydroxide (5.88 g, 2 equiv). The reaction mixture was heated at reflux for 1.5 h and then cooled via an external ice bath. The chilled, stirring reaction mixture was treated with concentrated hydrochloric acid (9 mL, 2 equiv) slowly via pipet. The resulting suspension was concentrated in vacuo to a gum, which was taken up in ethyl acetate (600 mL). The ethyl acetate layer was extracted with water (1 × 700 mL) and then brine (1 × 700 mL). The organic layer was dried over magnesium sulfate and then concentrated in vacuo. The resulting concentrate was coevaporated with ether (1 × 100 mL) and then dried in vacuo (25 °C, oil pump) for 1 h to afford **15** (28.78 g) as a gum: IR (neat) ν_{max} 3690–2740 valley, 2225, 1710, 1680, 1430, 1205, 1090, 730, 695 cm^{-1}. Anal. ($C_{32}H_{30}N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

3-Cyano-2-formyl-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)pyrrole (17). A solution of **15** (28.78 g) in toluene (500 mL) was treated with oxalyl chloride (32 mL). The resulting orange solution was heated at reflux temperature for 7 h and then allowed to cool to room temperature. The resulting solution was concentrated in vacuo (<40 °C, water pump) to an oil. The oil was dried further in vacuo (25 °C, oil pump) for 14 h to afford **16** (30.41 g) as a dark orange oil: IR (neat) ν_{max} 2235, 1743, 1708 cm^{-1}. A solution of **16** (30.41 g) in diglyme (50 mL) in a three-necked round-bottomed flask equipped with a mechanical stirrer and addition funnel was cooled to −78 °C via a dry ice/acetone bath. A solution of lithium tri-*tert*-butoxyaluminohydride in diglyme (95 mL of 0.5 M solution) was added dropwise to the chilled solution over the course of 2 h. After the addition was complete, the external cooling bath was removed, and the reaction mixture was allowed to warm to room temperature over the course of 1 h. The reaction mixture was then poured onto crushed ice (400 mL). After the ice had melted, the resulting suspension was filtered onto a thick pad of Celite. The resulting oily precipitate was carefully removed from the pad of Celite and dried in vacuo (25 °C, oil pump) over P_2O_5 for 17 h. The precipitate was then suspended in chloroform (500 mL), and the suspension was stirred by a magnetic stirrer at room temperature for 40 min. The suspension was then filtered through a thin pad of Celite, and the resulting filtrate was dried over magnesium sulfate. The filtrate was concentrated in vacuo to afford a brown gum which was dried further in vacuo (25 °C, oil pump) for 14 h to furnish 27.79 g of crude **17** as a brown gum: IR (neat) ν_{max} 3030, 2931, 2868, 2235, 1673, 1455, 1356, 1089 cm^{-1}.

4-Amino-1-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyridazine (18). A solution of **17** (27.79 g) in ethanol (575 mL) was treated with hydrazine dihydrochloride (4.95 g, 47.2 mmol). The reaction mixture was heated at reflux temperature under an argon atmosphere for 23 h. The reaction mixture was then allowed to cool to room temperature and then neutralized with a sufficient quantity of concentrated ammonium hydroxide to effect a pH = 8. The resulting suspension was filtered through a thin pad of Celite. The filtrate was concentrated in vacuo to an oily residue, which was suspended in chloroform (600 mL) and extracted with water (1 × 500 mL). The organic layer was dried over magnesium sulfate and then concentrated in vacuo to an oil. The oil was dissolved in a solution of chloroform/methanol 12:1 (30 mL) and applied to a column (15 × 20 cm sintered glass funnel) packed with silica gel (800 g) in the same solvent system. The column was eluted with the same solvent system while collecting 200 mL fractions. The fractions containing a product with an R_f = 0.33 (chloroform/methanol 10:1) were combined and concentrated in vacuo to afford a brownish foam. The foam was dried in vacuo (25 °C, oil pump) for 6 h to yield 13.88 g of **18** (49.4% overall yield from **17**): IR (chloroform) ν_{max} 3575–2778 broad valley, 3024, 1663, 1616, 1497, 1457, 1211, 1091 cm^{-1}; UV λ_{max} nm (ε) (methanol) 258 (4990), 264 (4970), 292 (5960); ^{1}H NMR ($CDCl_3$) δ 8.72 (s, 1 H, H-7), 7.47–6.80 (m, 17 H, phenyl H's, H-2, H-3), 6.59 (br s, 2 H, NH_2, deuterium oxide exchangeable), 6.17 (d, 1 H, J = 4.5 Hz, H-1′), 4.56–4.02 (m, 9 H, $C_6H_5CH_2$, H-2′, H-3′, H-4′), 3.71 (d, 2 H, J = 4.2 Hz, H-5′). Anal. ($C_{32}H_{32}N_4O_4$) C, H, N.

4-Amino-1-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyridazine Hydrochloride (3). A solution of **18** (573 mg, 1.1 mmol) in dichloromethane was chilled to −78 °C by an external dry ice/acetone bath. The chilled solution was treated with a solution of boron trichloride in dichloromethane (1 M in boron trichloride, 14.8 mL, 14 equiv) dropwise via an addition funnel over the course of 1.5 h. The dry ice/acetone bath was replaced by a dry ice/acetonitrile bath and the solution was allowed to stir for 1.5 h. A chilled solution of dichloromethane/methanol 1:1 (10 mL, chilled via an external 2-propanol/dry ice bath) was added to the reaction mixture, and then this mixture was allowed to warm to room temperature over the course of 1 h. The reaction mixture became a suspension as it warmed up. The suspension was concentrated in vacuo to afford an off-white solid. The solid was coevaporated with methanol (4 × 10 mL). The resulting concentrate was dissolved in hot methanol (ca. 4 mL). Ether was added to the solution, and a white solid precipitated upon cooling which was collected by filtration and washed with ether. The solid was dried in vacuo (78 °C, oil pump) for 17 h to afford 169 mg (52.6%) of **3**: mp 212–214 °C; IR (potassium bromide) ν_{max} 3500–2700 broad valley, 1660, 1620, 1390, 1215, 1135, 1095, 1050 cm^{-1}; UV λ_{max} nm (ε) (pH 7) 267 (4440), 286 (5170), (pH 1) 267 (4500), 286 (5180), (pH 11) 264 (3430), 293 (5380); ^{1}H NMR (DMSO-d_6) δ 9.93 (s, 1 H, H-7), 8.68 (br s, 2 H, NH_2, deuterium oxide exchangeable), 8.04 (d, 1 H, J = 2.9 Hz, H-2), 7.20 (d, 1 H, J = 3 Hz, H-3), 6.40 (d, 1 H, J = 5.2 Hz, H-1′), 5.60 (m, 2 H, OH's, deuterium oxide exchangeable), 5.25 (br s, 1 H, OH, deuterium oxide exchangeable), 4.21 (m, 1 H, H-2′), 4.04 (m, 1 H, H-3′), 3.81–3.70 (m, 3 H, H-4′, H-5′). Anal. ($C_{11}H_{15}N_4O_4Cl$) C, H, N.

4-Amino-3-bromo-1-β-D-ribofuranosylpyrrolo[2,3-*d*]-pyridazine (4). A solution of **12** (1.018 g, 1.89 mmol) in dichloromethane (200 mL) was treated with a solution of bromine (1.6 equiv) in dichloromethane (3 mL) dropwise over the course of 2 h by an addition funnel. After the addition was complete, the reaction mixture was concentrated in vacuo to an orange foam. The foam was dissolved in chloroform and applied to a column (3.2 × 20 cm) packed with silica gel (32 g) in chloroform (neat). The column was eluted with chloroform (neat) then chloroform/methanol/acetic acid/100:4:1 while collecting 10-mL frac-

tions. The fractions containing a product with an $R_f = 0.22$ were pooled and concentrated in vacuo to a light brown foam, which was coevaporated with toluene (2 × 15 mL) and then dichloromethane (2 × 10 mL). The foam was dried further in vacuo (25 °C, oil pump) to afford 604 mg of **19**. The foam (**19**) was dissolved in dichloromethane (10 mL) and chilled via an external 2-propanol/dry ice bath. To this chilled solution was added a solution of boron trichloride in dichloromethane (1 M, 13.7 mL, 14 equiv) dropwise by an addition funnel over the course of 1 h. After the addition was complete, the cooling bath was removed, and the reaction mixture was allowed to warm to room temperature over the course of 1.5 h. The reaction mixture was then recooled by the external 2-propanol/dry ice bath, and a prechilled solution of methanol/dichloromethane 1:1 (via an external 2-propanol/dry ice bath) was added in one portion. The resulting mixture was allowed to warm to room temperature and then concentrated in vacuo to an oil. The oil was coevaporated with methanol (3 × 10 mL) and then redissolved in methanol (40 mL). The resulting solution was treated with a sufficient amount of Dowex 1X-8 (OH^-) ion exchange resin to effect a pH = 7. The resin was removed by filtration, and the filtrate was concentrated in vacuo to a solid. The solid was suspended in a few milliliters of methanol, collected by filtration, and washed with ether to afford 179 mg of **4** (27% from **12**) as an off-white solid: mp 116–118 °C; IR (potassium bromide) ν_{max} 3451, 3128, 2917, 1631, 1588, 1427, 1391, 1286, 1096, 1061, 1026 cm^{-1}; UV λ_{max} nm (ϵ) (pH 7) sh 270 (5840), 300 (8320), (pH 1) 295 (8240), (pH 11) sh 266 (5570), 302 (8370); ^1H NMR (DMSO-d_6) δ 9.00 (s, 1 H, H-7), 7.91 (s, 1 H, H-2), 6.18 (s, 2 H, NH_2, deuterium oxide exchangeable), 5.88 (d, 1 H, J = 6.0 Hz, H-1'), 5.51–5.15 (m, 3 H, OH's, deuterium oxide exchangeable), 4.18 (dd, 1 H, H-2'), 4.05 (m, 1 H, H-3'), 3.95 (m, 1 H, H-4'), 3.62 (m, 2 H, H-5'); ^{13}C NMR ($CDCl_3$) δ 153.91, 132.21, 131.51, 126.26, 109.95, 89.49, 87.40, 85.89, 75.13, 70.16, 61.16. Anal. ($C_{11}H_{13}N_4O_4Br$) C, H, N.

Biological Evaluations. In Vitro Antiproliferative Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[22] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice. 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 to 50% of that of the control.

H. Ep. 2 cells were grown as described previously,[23] except that the medium was minimal essential medium (Earle's Salts) (Gibco, Grand Island, NY) with 15% bovine calf serum (Hyclone Laboratories, Logan, UT), and the cells were incubated in a 5% CO_2/95% air atmosphere. For growth inhibition evaluations, 2000 cells were plated in each well of 96-well plates with minimal essential medium (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 on the day of compound addition and 2 and 5 days thereafter. For crystal violet staining, the medium was decanted from the plates, and the cells were washed twice with Puck's Saline A (KCl, 0.40 g/L; NaCl, 8.0 g/L; $NaHCO_3$, 0.35 g/L; D-glucose, 1.0 g/L; phenol red, 0.005 g/L). Then 200 μL of 95% ethanol was added to each well, allowed to stand 5–10 min at room temperature, and decanted. One hundred microliters of 0.1% crystal violet (in 20% methanol) was added to each well, allowed to stand 5 min at room temperature, and decanted. Then the cells in each well were washed four times with tap water and extracted with 100 μL/well of acidic ethanol (ethanol/H_2O/1 N HCl 95:5:1) by agitating 5 min at room temperature. The $A_{560-570}$ of each well was determined using a model ν_{max} microplate reader (Molecular Devices, Menlo Park, CA). $A_{560-570}$ 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 previously for L1210 cells. The control T_d was constant throughout the 5-day evaluation period, and the average value was 24 h.

In Vitro Antiviral Evaluation. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hanks' salts [MEM(H)] supplemented with 5% fetal bovine serum. Diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[6] 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.[6]

(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.[24] HSV-1 was assayed using an enzyme immunoassay described by Prichard and Shipman.[25]

(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 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the growth of cells. Growth was measured spectrophotometrically by staining cells with crystal violet two days after drug treatment. Details of the procedure are in reference 25.

(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) 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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reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Registry No. 1, 69-33-0; 2 free base, 136947-20-1; 2 HCl salt, 135362-83-3; 3 free base, 64526-34-7; 3 HCl salt, 136947-12-1; 4, 136947-13-2; 5, 7126-44-5; 6, 5991-01-5; 7, 135362-84-4; 8, 135362-85-5; 9, 135362-86-6; 10, 135362-87-7; 11, 135362-88-8; 12, 135362-89-9; 13, 4060-34-8; 14, 136947-14-3; 15, 136947-15-4; 16, 136947-16-5; 17, 136947-17-6; 18, 136947-18-7; 19, 136947-19-8; 5-bromotubercidin, 21193-80-6.

Synthesis and Anti-HIV Activity of 4′-Thio-2′,3′-dideoxynucleosides†

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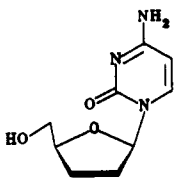
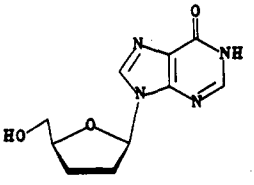
A series of 2′,3′-dideoxy-4′-thionucleoside analogues of purines and pyrimidines, including 4′-thioddI (17), 4′-thioddC (27), and 4′-thioAZT (34), were synthesized and evaluated for their inhibitory activity against human immunodeficiency virus (HIV). A stereospecific synthesis of the 2,3-dideoxy-4-thioribofuranosyl carbohydrate precursor 11 starting with L-glutamic acid is described. 2′,3′-Dideoxy-4′-thiocytidine (27) displayed significant, but modest activity in vitro against human immunodeficiency virus.

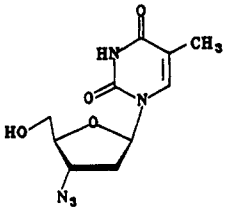
The 2′,3′-dideoxynucleosides are among the most potent and selective agents for the treatment of AIDS.[1,2] Within this series of compounds dideoxycytidine (1) and dideoxyinosine (2) have emerged as the most promising analogues and are currently undergoing clinical trials. The suggested mode of action of these analogues and 3′-azido-3′-deoxythymidine (AZT, 3), the present drug of choice, requires that they be metabolized sequentially via the monophosphate to the triphosphate, and the triphosphate then inhibits reverse transcriptase and/or terminates the growing DNA chain.[3]



1



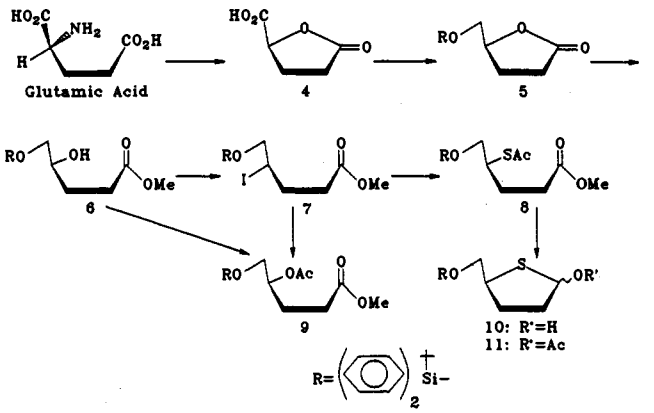
2



3

In hopes of finding better therapeutic agents for AIDS, a wide variety of sugar-modified nucleosides have been prepared. Thionucleosides in which oxygen of the sugar ring has been replaced by sulfur have shown interesting biological activities.[4-7] It has been demonstrated that 4′-thioadenosine, which is a potent inhibitor of *S*-adenosylhomocysteine hydrolase,[8] rapidly undergoes phosphorylation to its triphosphate. The resistance of several 4′-thioribonucleosides to bacterial cleavage[8] and of 4′-thioinosine to cleavage by purine nucleoside phosphorylase[9] has been reported. No 2′,3′-dideoxy-4′-thionucleosides have been reported, but it is logical to assume

Scheme I



that such nucleosides would also be resistant to phosphorolytic cleavage. As part of an ongoing effort in our

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