Synthesis and Biological Activity of Certain 6-Substituted and 2,6-Disubstituted 2′-Deoxytubercidins Prepared via the Stereospecific Sodium Salt Glycosylation Procedure

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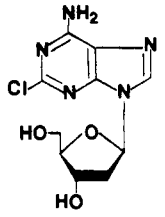
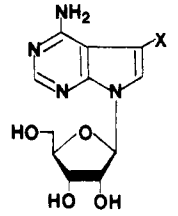
A number of 6-substituted and 2,6-disubstituted pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides were prepared by the direct stereospecific sodium salt glycosylation procedure. Reaction of the sodium salt of 4-chloro-6-methyl-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**6a**) or 4,6-dichloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**6b**) with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**9**) provided the corresponding N_7 2′-deoxy-β-D-ribofuranosyl blocked derivatives (**8a** and **8c**) which, on ammonolysis, gave 4-amino-6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11a**) and 4-amino-6-chloro-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11b**), respectively. Dethiation of **11a** and **11b** afforded 6-methyl-2′-deoxytubercidin (**10a**) and 6-chloro-2′-deoxytubercidin (**10b**), respectively. Dehalogenation of **10b** provided an alternate route to the reported 2′-deoxytubercidin (**3a**). Application of this glycosylation procedure to 4,6-dichloro and 4,6-dichloro-2-methyl derivatives of pyrrolo[2,3-*d*]pyrimidine (**15a** and **15b**) gave the corresponding blocked 2′-deoxyribonucleosides (**18a** and **18b**), which on ammonolysis furnished **10b** and 4-amino-6-chloro-2-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**17**), respectively. This stereospecific attachment of the 2-deoxy-β-D-ribofuranosyl moiety appears to be due to a Walden inversion at the C_1 carbon by the anionic heterocyclic nitrogen. Controlled deacylation of 4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**20a**) gave 4-chloro-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**20b**). Dehalogenation of **20b** gave the 2′-deoxynebularin analogue 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**19**), and reaction of **20b** with thiourea gave 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-thione (**21**). All of these compounds were tested in vitro against certain viruses and tumor cells. Only compounds **12a**, **20b**, and **21** showed significant activity against measles in vitro, and the activity is comparable to that of ribavirin. Although compounds **3a** and **12b** are slightly more active than ribavirin against HSV-2 in vitro, they are relatively more toxic to Vero cells. Compounds **3a** and **20b** exhibited moderate cytostatic activity against L1210 and P388 leukemia in vitro but are considerably less active than 2-chloro-2′-deoxyadenosine (**1**).

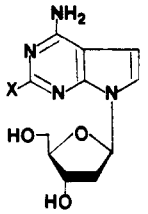
Derivatives of 2′-deoxyadenosine are of considerable current interest due to the potent anticancer effects of 2-chloro-2′-deoxyadenosine[1-4] (**1**). The naturally occurring



1 (2-chloro-2′-deoxyadenosine)



2a, X = H (tubercidin)
b, X = CN (toyocamycin)
c, X = $CONH_2$ (sangivamycin)
d, X = I



3a, X = H
b, X = Cl

cytotoxic[5] nucleoside antibiotic tubercidin (**2a**) is a structural analogue of adenosine and closely related to pyrrolo[2,3-*d*]pyrimidine ribonucleosides toyocamycin (**2b**) and sangivamycin (**2c**), which also exhibit antitumor properties.[6-8] From a biochemical viewpoint, these pyrrolo[2,3-*d*]pyrimidine nucleosides present potential advantages, since tubercidin is neither deaminated by adenosine deaminase[9] nor subject to glycosidic cleavage by purine nucleoside phosphorylase.[10] These observations provide a good rationale for the synthesis of halogen-substituted 2′-deoxytubercidins as potential chemotherapeutic agents. Moreover, 5-iodotubercidin (**2d**) has been found to be a potent inhibitor of adenosine kinase.[11] 5-Iodo-5′-deoxytubercidin, which has recently been isolated from an extract of the marine red alga *Hypnea valendiae*,[12] has been shown to produce muscle relaxation and hypothermia in mice besides being a potent inhibitor of the enzyme adenosine kinase.

Prior glycosylation procedures introducing the 2-deoxy-β-D-ribofuranosyl (2-deoxy-β-D-*erythro*-pentofuranosyl) moiety into an azole heterocycle reported from

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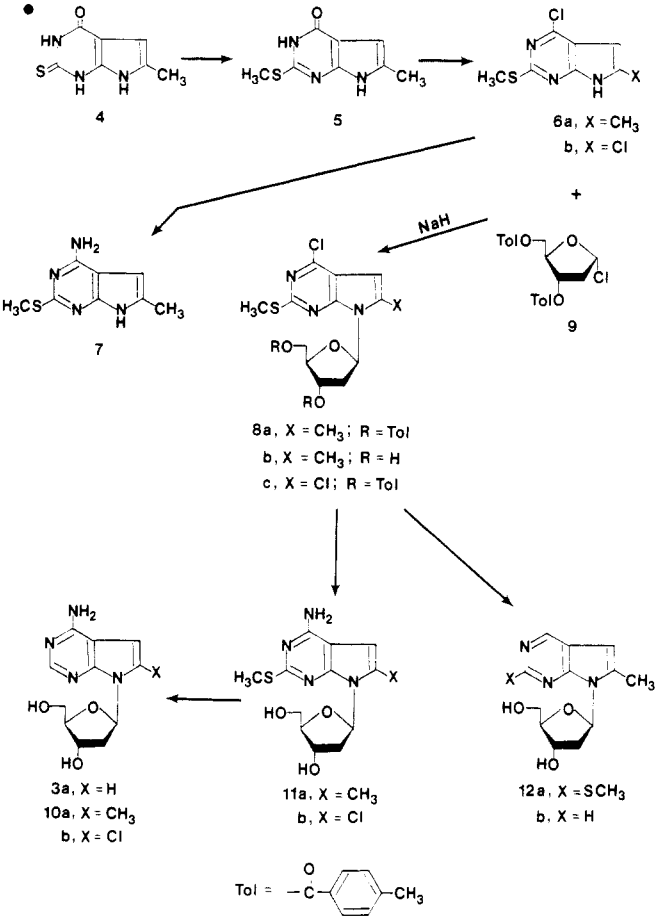
our laboratory[13-16] and by others[17-20] invariably provide anomeric mixtures as well as positional isomers that after tedious separation result in low yields of the desired 2′-deoxyribonucleoside. In only one instance has the β anomer of the 2′-deoxyribonucleoside been claimed exclusively;[21] however, the yield in this instance was only 40%, and the authors did not account for the remainder of the products. In view of these difficulties, a four-step deoxygenation procedure using phenoxythiocarbonylation[22-24] or imidazolylthiocarbonylation[25,26] of the 2′-hydroxy group of the corresponding 3′,5′-diprotected β-D-ribonucleoside has recently been developed to provide the requisite 2′-deoxynucleoside. These latter procedures, however, require the availability of the preformed ribonucleoside and are not applicable in the presence of halogenated azole derivatives,[27] which are very useful for further nucleophilic displacement reactions. Although the synthesis of a number of analogues of 2′-deoxyadenosine by an enzymatic procedure has recently been reported,[28] this approach is not applicable to the pyrrolo[2,3-*d*]pyrimidine ring system.[29] We have recently employed very successfully the sodium salt of several chloropyrrolo[2,3-*d*]pyrimidines and 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose or 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[30] (**9**) to obtain ribo-[31] and 2′-deoxyribo- (**3**)[32] nucleosides, respectively. Use of this simple single-phase sodium salt glycosylation procedure for the synthesis of hitherto inaccessable 6-substituted and 2,6-disubstituted 2′-deoxytubercidins is the subject of current report.

Chemistry. In the present work we elected to use 2,6-disubstituted 4-chloropyrrolo[2,3-*d*]pyrimidines for glycosylation studies to obtain the corresponding *N*-glycosyl intermediates, which could eventually be converted into the desired 6-substituted and 2,6-disubstituted 2′-deoxytubercidins by direct nucleophilic displacement reactions. In an effort to prepare 4-chloro-6-methyl-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**6a**), the readily available 6-methyl-2-thiopyrrolo[2,3-*d*]pyrimidine-4-(1*H*,3*H*)-dione[33] (**4**) was methylated with methyl iodide to obtain the corresponding 2-methylthio derivative (**5**) (Scheme I). Chlorination of **5** with phosphorus oxychloride in the presence of *N*,*N*-dimethylaniline afforded **6a**. Ammonolysis of **6a** with MeOH/NH_3 gave 4-amino-6-methyl-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**7**). Compounds **6a** and 4,6-dichloro-2-(methylthio)pyrrolo-[2,3-*d*]pyrimidine[31] (**6b**) served as versatile starting materials for these 2′-deoxyribosylation studies. The sodium salt of **6b**, produced in situ by NaH in anhydrous acetonitrile, was treated with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[30] (**9**) at ambient temperature. A clean reaction was observed, and the desired 4,6-dichloro-2-(methylthio)-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8c**) was precipitated out from the reaction mixture and was isolated in 87% yield. When **8c** was treated with MeOH/NH_3 at 120 °C for 16 h, deprotection of the glycon moiety with concomitant nucleophilic displacement of the 4-chloro function to an amino group occurred to give 4-amino-6-chloro-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11b**) in 82% yield. Dethiation of **11b** by the treatment with Raney nickel (W-4) catalyst provided 4-amino-6-chloro-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6-chloro-2′-deoxytubercidin, **10b**). The observed

Scheme I



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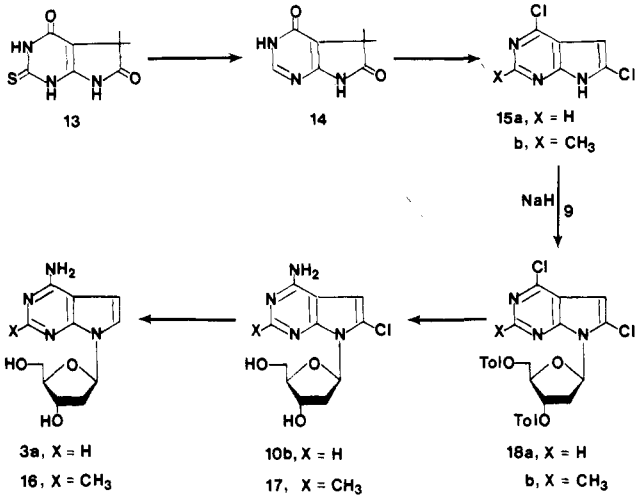
Scheme II



ultraviolet absorption spectra (in pH 1, 7, and 11) of **10b** was found to be virtually identical with that of 6-chlorotubercidin, reported recently from our laboratory,[31] thus confirming the site of glycosylation in **8c** and the 2'-deoxyribonucleosides derived therefrom as N_7. The structural assignment of **10b** was further corroborated by dehalogenation with Pd/C in a hydrogen atmosphere, which readily gave 4-amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2'-deoxytubercidin, **3a**), whose structure has been unequivocally established previously from our laboratory.[32]

This elegant 2'-deoxyribosylation procedure was also found to proceed well with **6a**. Thus, treatment of the sodium salt of **6a** with **9** in acetonitrile gave an excellent yield of 4-chloro-6-methyl-2-(methylthio)-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8a**). When compound **8a** was allowed to react with MeOH/NH_3 at 100 °C for 5 h, only the deprotected nucleoside 4-chloro-6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8b**) was obtained. However, further ammonolysis of either **8b** or **8a** with MeOH/NH_3 at 120 °C for 16 h furnished 4-amino-6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11a**) in more than 78% yield. The essentially identical ultraviolet absorption spectra of **11a** and the aglycon **7** (see Experimental Section) indicated the site of glycosylation in **11a** to be N_7. Dethiation of **11a** by the treatment with Raney nickel catalyst gave 4-amino-6-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6-methyl-2'-deoxytubercidin, **10a**). Dehalogenation of **8b** with Pd/C in a hydrogen atmosphere provided 6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**12a**), which on subsequent dethiation afforded 6-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**12b**).

The glycosylation studies were further extended to 4,6-dichloro and 4,6-dichloro-2-methyl derivatives of pyrrolo[2,3-*d*]pyrimidine (**15a** and **15b**). In an effort to prepare **15a**, diethyl α-cyanosuccinate[14] was converted to the intermediate imino ether with anhydrous HCl gas in absolute EtOH which, without isolation, was ring closed with thiourea in the presence of sodium ethoxide to give 4,6(3*H*,5*H*)-dioxopyrrolo[2,3-*d*]pyrimidine-2(1*H*)-thione (**13**) (Scheme II). Dethiation of **13** by treatment with freshly prepared Raney nickel catalyst gave pyrrolo[2,3-*d*]pyrimidine-4,6(3*H*,5*H*)-dione (**14**), which on subsequent

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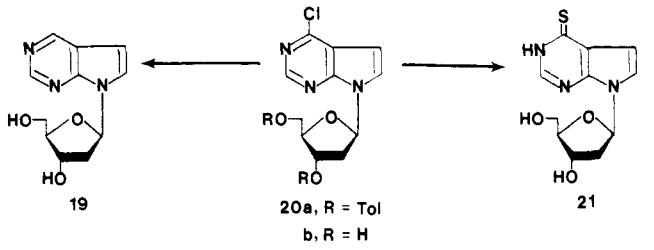
Scheme III



chlorination with phosphorus oxychloride in the presence of *N*,*N*-dimethylaniline afforded the desired **15a**. Treatment of the sodium salt of **15a**, generated in situ by NaH in anhydrous acetonitrile, with **9** gave a mixture of two nucleosidic products. After flash silica gel column chromatography, a 63% yield of crystalline 4,6-dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentafuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**18a**) was isolated. A minor product, which was isolated as light yellow needles in 19% yield, was presumed to be the corresponding N_1-glycosyl isomer on the basis of ultraviolet spectral data. It has been shown previously[35,36] that glycosylation of 4-chloropyrrolo[2,3-*d*]pyrimidine or other trisubstituted pyrrolo[2,3-*d*]pyrimidines at N_7 results in a small hypsochromic shift as compared to the ultraviolet spectrum of the corresponding aglycon, whereas glycosylation at N_1 exhibits a bathochromic shift relative to the aglycon. The observed 13-nm bathochromic shift (in pH 7) lends support to the tentatively assigned structure of the minor product as N_1-glycosyl isomer. Subsequent treatment of **18a** with MeOH/NH_3 at 120 °C for 16 h provided an alternate route to the synthesis of 4-amino-6-chloro-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**10b**), which was found to be identical with 6-chloro-2'-deoxytubercidin prepared from **11b**. Furthermore, dehalogenation of **10b** gave the reported[32] 2'-deoxytubercidin (**3a**), thus confirming the structural assignment of **18a**.

For the synthesis of 2-methyl-2'-deoxytubercidin (**16**) (Scheme II), 4,6-dichloro-2-methylpyrrolo[2,3-*d*]pyrimidine[37] (**15b**) was found to be a suitable starting material. Direct glycosylation of the sodium salt of **15b** with **9** gave 4,6-dichloro-2-methyl-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**18b**) as the sole product in 59% yield, after silica gel column chromatography. Treatment of **18b** with MeOH/NH_3 at elevated temperature gave 4-amino-6-chloro-2-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**17**). The ultraviolet absorption spectrum of **17** was found to be essentially identical with 6-chloro-2-methyltubercidin, reported recently from our laboratory,[31] thus confirming the site of glycosylation in **17** and the nucleosides derived thereform as N_7. Dehalogenation of **17** with Pd/C in a hydrogen atmosphere provided **16**, the ultaviolet absorption spectral characteristics of which are identical with that reported for 2-methyltubercidin.[31]

4-Chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**20a**), reported recently from our laboratory,[32] was employed for further functional group transformation studies (Scheme III). Controlled deacylation of **20a** by treatment with MeOH/NH_3 at 0–5 °C gave 5-chloro-7-(2-deoxy-β-D-

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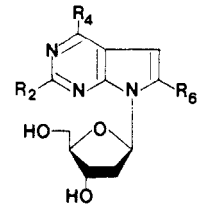
Table I. Comparative in Vitro Antiviral Activity of 2-Chloro-2′-deoxyadenosine (1), Ribavirin, and Certain Substituted Pyrrolo[2,3-*d*]pyrimidine 2′-Deoxyribonucleosides



compd	R_2	R_4	R_6	ED_{50},[a] M				toxic level, M
				Para 3	measles	VV	HSV-2	
20b	H	Cl	H	9.6×10^{-4}	8.9×10^{-5}	$>5.0 \times 10^{-3}$	6.5×10^{-4}	1.6×10^{-3}
21	H	SH	H	$>5.0 \times 10^{-3}$	2.7×10^{-5}	1.1×10^{-3}	1.6×10^{-4}	5.0×10^{-3}
3a	H	NH_2	H	6.6×10^{-4}	2.1×10^{-4}	1.6×10^{-4}	5.0×10^{-5}	1.6×10^{-3}
3b	Cl	NH_2	H	$>5.0 \times 10^{-3}$	$>5.0 \times 10^{-3}$	1.1×10^{-3}	$>5.0 \times 10^{-3}$	5.0×10^{-4}
10b	H	NH_2	Cl	1.6×10^{-3}	3.6×10^{-4}	3.3×10^{-4}	1.1×10^{-3}	1.6×10^{-3}
16	CH_3	NH_2	H	$>5.0 \times 10^{-3}$	9.2×10^{-4}	3.1×10^{-3}	1.6×10^{-3}	1.6×10^{-3}
10a	H	NH_2	CH_3	5.0×10^{-4}	5.0×10^{-4}	1.0×10^{-3}	2.1×10^{-3}	5.0×10^{-3}
17	CH_3	NH_2	Cl	$>1.6 \times 10^{-3}$	3.6×10^{-4}	8.4×10^{-4}	5.0×10^{-4}	5.0×10^{-4}
11b	SCH_3	NH_2	Cl	3.4×10^{-4}	1.0×10^{-4}	$>5.0 \times 10^{-4}$	5.8×10^{-4}	5.0×10^{-4}
8b	SCH_3	Cl	CH_3	*b*	*b*	*b*	*b*	
11a	SCH_3	NH_2	CH_3	$>5.0 \times 10^{-3}$	2.5×10^{-4}	1.0×10^{-3}	1.3×10^{-3}	1.6×10^{-3}
19	H	H	H	$>5.0 \times 10^{-3}$	2.5×10^{-4}	$>5.0 \times 10^{-3}$	1.4×10^{-3}	5.0×10^{-3}
12b	H	H	CH_3	1.1×10^{-3}	3.0×10^{-4}	1.0×10^{-3}	7.4×10^{-5}	5.0×10^{-3}
12a	SCH_3	H	CH_3	1.1×10^{-3}	9.5×10^{-5}	8.4×10^{-4}	2.9×10^{-3}	$>5.0 \times 10^{-3}$
1[32]				$>5.0 \times 10^{-4}$	$>5.0 \times 10^{-4}$	5.0×10^{-6}	5.0×10^{-6}	5.0×10^{-6}
ribavirin[44]				1.6×10^{-4}	3.3×10^{-5}	8.5×10^{-5}	1.9×10^{-4}	none

[a] The concentration of compound that resulted in a 50% reduction of viral CPE as compared with nondrug controls. [b] Too insoluble to test.

erythro-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**20b**) in 74% yield. Dehalogenation of **20b** with 5% Pd/C in the presence of K_2CO_3 gave the 2′-deoxynebularin analogue 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**19**). Reaction of **20b** with thiourea in boiling 1-propanol gave 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-thione (**21**) in good yield.

The anomeric configuration of the newly synthesized pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides was assigned by NMR spectroscopy, wherein the β anomers exhibited the characteristic triplet for the anomeric proton. The pattern of the anomeric proton signal was identical with that published for 2′-deoxytubercidin,[38] as well as for other 2′-deoxyribonucleosides.[14] Since the starting halosugar **9** has the α configuration[39] in the solid state, the exclusive formation of the blocked 2′-deoxy-β-nucleosides in the present study is believed to be due to a direct Walden inversion (S_N2) at the C_1 carbon by the anionic heterocyclic nitrogen.

This simple single-phase glycosylation via the sodium salt of a preformed pyrrolo[2,3-*d*]pyrimidine appears to be considerably superior to previously reported glycosylations of this ring system[35,36,40,41] including the phase-transfer procedure.[21,42,43] This synthesis of pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides provides a total synthesis starting with an appropriate aglycon, which appears to be supieror to published multistep procedures[23,36] requiring first the corresponding ribonucleoside as in the recently described six-step synthesis of 2′-deoxysangivamycin[29] from toyocamycin.

Biological Evaluations

A. Antiviral Activity. The pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides synthesized during this study were tested against herpes simplex type 2 (HSV-2), vaccinia (VV), parainfluenza type 3 (Para 3), and measles viruses in vitro in parallel with 2-chloro-2′-deoxyadenosine (**1**) and ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)[44] (Table I). Of all the compounds, **12a**, **20b**, and **21** exhibited significant activity against measles; and the activity was comparable to that of ribavirin with some toxicity to Vero cells at higher concentrations. Although compounds **3a** and **12b** do exhibit some toxicity to Vero cells, they are slightly more active than ribavirin against HSV-2 in vitro but are less active than the reference compound **1**. Compounds **3a**, **10a**, **11b**, and **20b** all showed moderate activity, which is accompanied by toxicity against Para 3 in vitro, but ribavirin is comparatively more active and nontoxic. Compounds **3a** and **10b** are 2-fold and 4-fold less active than ribavirin against VV in vitro, respectively, whereas the reference compound **1** is considerably more active. However, **17** and **12a** are 10-fold less active than ribavirin against VV in vitro. Compounds **3a**, **10b**, **11a**, **11b**, **12b**, **17**, and **19** were found to inhibit the growth of measles virus moderately in vitro. Similarly compound **21** is moderately active against HSV-2 in vitro. Because of the low solubility, compound **8b** could not be tested. All other pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides synthesized during this study were found to be devoid of significant antiviral activity in vitro.

B. Cytostatic Activity. The pyrrolo[2,3-*d*]pyrimidine 2′-deoxyribonucleosides synthesized during this study were also tested for their inhibitory effects on the growth of L1210 and P388 leukemic cell lines in vitro (Table II). Compounds **3a** and **20b** were found to be moderately active against these cell lines but considerably less active than the reference compound 2-chloro-2′-deoxyadenosine (**1**).

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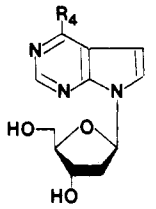
Table II. Comparative in Vitro Cytostatic Activity of 2-Chloro-2'-deoxyadenosine (1) and Certain Substituted Pyrrolo[2,3-*d*]pyrimidine 2'-Deoxyribonucleosides



compd	R_4	ID_{50},[a] M L1210	ID_{50},[a] M P388
3a	NH_2	8.0×10^{-5}	4.9×10^{-5}
20b	Cl	5.9×10^{-5}	7.3×10^{-5}
1[32]		2.6×10^{-8}	3.2×10^{-8}

[a] Inhibitory dose 50 (ID_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. Compound **8b** is insoluble to test, and all other 2'-deoxynucleosides synthesized during this study are inactive at 10^{-4} M.

All other compounds under study were devoid of cytostatic activity in vitro.

Experimental Section

General Procedures. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of solvent as indicated by elemental analysis was verified by NMR. Ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Labs, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C.

6-Methyl-2-(methylthio)pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (5). A solution of 6-methyl-2-thiopyrrolo[2,3-*d*]pyrimidine-4(1*H*,3*H*)-one[33] (**4**; 9.06 g, 50 mmol) in absolute EtOH (200 mL) containing 1.75 N NaOH (55 mL) was stirred at room temperature while CH_3I (7.10 g, 50 mmol) was added dropwise. Ten minutes after the addition was complete, the purple sodium salt of the product began to precipitate. This mixture was stirred an additional 30 min, and the sodium salt was collected by filtration. The sodium salt was dissolved in a minimum amount of water and acidified with 2 N H_2SO_4. The precipitated product was separated by filtration and crystallized from aqueous ethanol to yield 8.5 g (87%) of **5**: mp 260 °C dec; UV λ_{max} (pH 1) 219 nm (ϵ 15 200), 285 (11 900); UV λ_{max} (pH 7) 219 nm (ϵ 16 100), 285 (12 500); UV λ_{max} (pH 11) 227 nm (ϵ 17 400), 282 (13 100); NMR (Me_2SO-d_6) δ 2.24 (s, 3, CH_3), 2.51 (s, 3, SCH_3), 6.05 (s, 1 C_5 H), 11.61 and 11.98 (2 br s, 2, N_3 H, N_7 H). Anal. ($C_8H_9N_3OS$) C, H, N, S.

4-Chloro-6-methyl-2-(methylthio)pyrrolo[2,3-*f*]pyrimidine (6a). A mixture of **5** (19.5 g, 100 mmol), $POCl_3$ (200 mL), and *N*,*N*-dimethylaniline (20 mL) was heated at reflux for 4.5 h, at which time a clear solution was obtained. The excess $POCl_3$ was removed in vacuo, and the residual syrup was added slowly to crushed ice (750 g) with good stirring. The light orange crystals were collected by filtration, washed with water (2 × 50 mL), and air-dried. The combined filtrate and washings were extracted with $CHCl_3$ (5 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. The combined solids crystallized from *n*-heptane to yield 15.5 g (72.7%) of analytically pure **6a**: mp 230–231 °C; UV λ_{max} (pH 1) 251 nm (ϵ 3600), 283 (1300), 315 (1100); UV λ_{max} (pH 7) 250 nm (ϵ 5400), 283 (1800), 314 (1800); UV λ_{max} (pH 11) 251 nm (ϵ 8000), 282 (2600), 314 (2500); NMR (Me_2SO-d_6) δ 2.40 (s, 3, CH_3), 2.56 (s, 3, SCH_3), 6.26 (s, 1, C_5 H), 11.5 (br s, 1, NH). Anal. ($C_8H_8ClN_3S$) C, H, Cl, N.

4-Amino-6-methyl-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (7). Compound **6a** (3.0 g, 14.0 mmol) was combined with MeOH/NH_3 (75 mL, saturated at 0 °C) and the resultant mixture heated in a steel bomb at 135 °C for 48 h. The residue, after evaporation of the solvents, was crystallized from aqueous ethanol as needles to yield 1.64 g (60%): mp 207 °C; UV λ_{max} (pH 1) 231 nm (ϵ 11 700), 285 (9600); UV λ_{max} (pH 7 and 11) 233 nm (ϵ 15 300), 286 (10 200), 302 (sh 8700); NMR (Me_2SO-d_6) δ 2.25 (s, 3, CH_3), 2.41 (s, 3, SCH_3), 6.08 (s, 1, C_5 H), 6.77 (s, 2, NH_2), 11.20 (s, 1, N_7 H). Anal. ($C_8H_{10}N_4S$) C, H, N, S.

4,6-Dichloro-2-(methylthio)-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (8c). 4,6-Dichloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine[31] (**6b**; 3.0 g, 12.8 mmol) was dissolved in dry CH_3CN (200 mL) with heating. Sodium hydride (60% in oil, 0.62 g, 15.5 mmol) was added, and the mixture was stirred for 30 min at room temperature before 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose[30] (**9**; 5.0 g, 12.9 mmol) was added in one lot. After about 30 min, a flocculent off-white crystalline solid appeared. The reaction mixture was evaporated to dryness, and the residue was suspended in hot EtOAc (450 mL). The mixture was filtered hot to remove inorganic salts. Upon concentration of the filtrate, crystallization occurred. The off-white crystals were collected by filtration, washed with cold EtOAc (2 × 50 mL), and recrystallized from EtOAc/EtOH to yield 6.6 g (87%) of **8c**: mp 139–140 °C; UV λ_{max} (EtOH) 243 nm (ϵ 40 500), 280 (11 400), 312 (8200); NMR (Me_2SO-d_6) δ 2.31 and 2.36 (2 s, 6, toluoyl methyls), 2.57 (s, 3, SCH_3), 6.54 (t, 1, $C_{1'}$ H), 6.80 (s, 1, C_5H), 7.12–7.92 (m, 8, toluoyl aromatic protons), and other sugar protons. Anal. ($C_{28}H_{25}Cl_2N_3O_5S$) C, H, Cl, N, S.

4-Chloro-6-methyl-2-(methylthio)-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (8a). The title compound was prepared in the same manner as described for **8c** with **6a** (6.4 g, 30 mmol), NaH (60% in oil, 1.44 g, 36 mmol), and the α-chloro sugar **9** (12.0 g, 30 mmol). The product was crystallized from EtOH to yield 13.6 g (80%): mp 161 °C; UV λ_{max} (EtOH) 244 nm (ϵ 46 700), 280 (10 900), 314 (7600); NMR (Me_2SO-d_6) δ 2.32 and 2.38 (2s, 6, toluoyl methyls), 2.48 (s, 3, CH_3), 2.57 (s, 3, SCH_3), 6.32 (d, 1, J = 2 Hz, C_5 H), 6.48 (t, 1, $C_{1'}$ H, peak width 14.78 Hz), 7.18–8.0 (m, 8, toluoyl aromatic protons), and other sugar protons. Anal. ($C_{29}H_{28}ClN_3O_5S$) C, H, N.

4-Chloro-6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (8b). Compound **8a** (4.0 g, 7.1 mmol) was combined with MeOH/NH_3 (80 mL, saturated at 0 °C) and heated in a steel bomb at 100 °C for 5 h. The reaction mixture was evaporated to dryness, adsorbed onto silica gel (60 g), and placed atop a silica gel column (5 × 40 cm). The column was eluted with a $CHCl_3$/MeOH gradient. The desired product (**8b**) was crystallized from EtOAc to yield 1.35 g (58%): mp 155 °C; UV λ_{max} (pH 2, 7, and 12) 252 nm (ϵ 23 500); 285 (10 400), 313 (7700); NMR (Me_2SO-d_6) δ 2.46 (s, 3, CH_3), 2.54 (s, 3, SCH_3), 6.36 (d + t, 2, C_5 H and $C_{1'}$ H, J = 2.0 Hz, triplet peak width 14.0 Hz), and other sugar protons. Anal. ($C_{13}H_{16}ClN_3O_3S$) C, H, Cl, N.

4-Amino-6-chloro-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11b). Compound **8c** (5.0 g, 8.53 mmol) was combined with MeOH/NH_3 (75 mL, saturated at 0 °C) and heated in a steel bomb at 120 °C for 16 h. The reaction mixture was evaporated to dryness, adsorbed onto silica gel (40 g), and placed atop a flash silica gel column. The column was eluted with CH_2Cl_2/acetone (3:1, v/v). The appropriate fractions were pooled and evaporated to provide 2.3 g (82%) of **11b** after crystallization from a small amount of water: mp 101 °C (softens), resolidifies, 160–161 °C; UV λ_{max} (pH 1) 217 nm (ϵ 13 200), 282 (10 600), 302 (sh, 7900); UV λ_{max} (pH 7 and 11) 234 nm (ϵ 13 900), 284 (11 200); NMR (Me_2SO-d_6) δ 2.41 (s, 3, SCH_3), 6.31 (t, 1, $C_{1'}$ H, peak width 14.21 Hz), 6.58 (s, 1, C_5 H), 7.19 (s, 2, NH_2), and other sugar protons. Anal. ($C_{12}H_{15}ClN_4O_3S \cdot {}^1/_2H_2O$) C, H, Cl, N, S.

4-Amino-6-methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (11a). The title compound was prepared in the same manner as described for **11b** using instead **8a** (8.0 g, 14.1 mmol) and MeOH/NH_3 (90 mL):

yield, after crystallization from water, 3.4 g (78%): mp 205 °C dec (softens at 110 °C); UV λ_{max} (pH 1) 232 nm (ϵ 12 600), 284 (9600); UV λ_{max} (pH 7 and 11) 232 (ϵ 15 500), 286 (11 800), 302 (sh, 7800); NMR (Me_2SO-d_6) δ 2.35 (s, 3, CH_3), 2.42 (s, 3, SCH_3), 6.27 (t, 1, $C_{1'}$ H, peak width 12.63 Hz), 6.36 (s, 1, C_5 H), 6.91 (s, 2, NH_2), and other sugar protons. Anal. ($C_{13}H_{18}N_4O_3S\cdot H_2O$) C, H, N.

4-Amino-6-chloro-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6-Chloro-2′-deoxytubercidin, 10b). Method 1. To a solution of **11b** (0.5 g, 1.5 mmol) in 50% aqueous EtOH (50 mL) was added Raney nickel (W-4, 2.0 g, wet weight), and the mixture was heated under reflux for 2 h. An additional quantity of Raney nickel (2.0 g, wet weight) was added, and heating was continued for further 30 min. The mixture was filtered hot through a Celite pad to remove the catalyst, which was washed with hot EtOH (3 × 10 mL). The combined filtrate and washings were evaporated to dryness, adsorbed onto silica gel (5 g), and placed atop a flash silica gel column (4 × 25 cm). The column was eluted with $CHCl_3$/MeOH (6:1, v/v), and the faster moving compound was isolated and identified as 6-chloro-2′-deoxytubercidin. After crystallization from water, the title compound was obtained as needles, 0.28 g (66%): mp 178–180 °C; UV λ_{max} (pH 1) 226 nm (ϵ 15 400), 274 (11 500); UV λ_{max} (pH 7 and 11) 274 nm (ϵ 12 800); NMR (Me_2SO-d_6) δ 6.33 (t, 1, $C_{1'}$ H), 6.61 (s, 1, C_5H), 7.14 (s, 2, NH_2), 7.98 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{11}H_{13}ClN_4O_3$) C, H, Cl, N.

The slower moving compound was also isolated and identified as 2′-deoxytubercidin (**3a**): yield 70 mg (19%); mp 218 °C.

Method 2. Compound **10b** was also prepared from **18a** (6.0 g, 11.1 mmol) and MeOH/NH_3 (150 mL, saturated at 0 °C) at 120 °C for 16 h. After flash silica gel column (4 × 25 cm) chromatography using $CHCl_3$/MeOH (10:1, v/v) as the eluent, there was obtained 2.5 g (79%) of the title compound, mp 178 °C. This product was found to be identical with that prepared by method 1.

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2′-Deoxytubercidin, 3a). To a solution of **10b** (0.57 g, 2 mmol) in 80% aqueous 1-propanol (50 mL) containing K_2CO_3 (0.10 g) was added Pd/C (10%, 50 mg), and the mixture was hydrogenated at 2 atm for 8 h. The mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness. Crystallization of the residue from water gave 0.45 g (90%) of 2′-deoxytubercidin, mp 218 °C [lit.[31] mp 218 °C; all other physicochemical properties of **3a** are identical with those for 2′-deoxytubercidin previously reported].

4-Amino-6-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6-Methyl-2′-deoxytubercidin, 10a). To a solution of **11a** (2.0 g, 6.44 mmol) in 50% aqueous EtOH (100 mL) was added Raney nickel (W-4, 10 g, wet weight), and the mixture was heated under reflux for 1 h. The mixture was filtered hot through a Celite pad, and the filtrate was evaporated to dryness to yield a colorless foam that was crystallized from water to provide **10a**, 1.4 g (82%): mp 166–168 °C; UV λ_{max} (pH 1) 228 nm (ϵ 13 000), 276 (7400); UV λ_{max} (pH 7 and 11) 275 nm (ϵ 9500); NMR (Me_2SO-d_6) δ 2.38 (s, 3, CH_3), 6.28 (t, 1, $C_{1'}$ H, peak width 14.89 Hz), 6.29 (d, 1, J = 2.0 Hz, C_5 H), 6.91 (s, 2, NH_2), 7.95 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{12}H_{16}N_4O_3$) C, H, N.

6-Methyl-2-(methylthio)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12a). A mixture of **8b** (0.66 g, 2.0 mmol), K_2CO_3 (0.26 g), and Pd/C (5%, 0.60 g) in 90% aqueous 1-propanol (60 mL) was hydrogenated at 30 psi for 40 h. The mixture was filtered through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in hot water (50 mL) and applied to a Dowex 1-X8 (OH^-) column (1 × 10 cm). Elution with water (300 mL) followed by H_2O/MeOH (6:4, v/v, 400 mL) provided **12a**, which was crystallized from water to yield 0.36 g (61%): mp 182 °C; UV λ_{max} (pH 1) 224 nm (ϵ 15 400), 257 (21 300), 295 (10 000); UV λ_{max} (pH 7 and 11) 250 nm (ϵ 20 400), 278 (7300), 310 (5900); NMR (Me_2SO-d_6) δ 2.48 (s, 3, CH_3), 2.56 (s, 3, SCH_3), 6.36 (d, 1, J = 2.0 Hz, C_5 H), 6.42 (t, 1, $C_{1'}$ H, peak width 14.7 Hz), 8.70 (s, 1, C_4 H), and other sugar protons. Anal. ($C_{13}H_{17}N_3O_3S$) C, H, N.

6-Methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (12b). To a solution of **12a** (0.44 g, 1.5 mmol) in 1-propanol (25 mL) was added Raney nickel (W-4, 2.8 g, wet weight), and the mixture was refluxed for 3 h. The mixture was filtered hot through Celite, and the filtrate was evaporated to dryness. The residue was dissolved in hot water (50 mL) and applied to a Dowex 1-X8 (OH^-) column (1 × 10 cm). Elution with water (200 mL) followed by H_2O/MeOH (7:3, v/v, 300 mL) provided **12b**, which was crystallized from EtOAc to yield 0.29 g (78%): mp 214–216 °C; UV λ_{max} (pH 1) 231 nm (ϵ 29 400), 283 (3200); UV λ_{max} (pH 7 and 11) 227 nm (ϵ 26 100), 273 (4100); NMR (Me_2SO-d_6) δ 2.56 (s, 3, CH_3), 6.45 (d, 1, J = 2.0 Hz, C_5 H), 6.52 (t, 1, $C_{1'}$ H, peak width 14.8 Hz), 8.76 (s, 1, C_4 H), 8.94 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

4,6(3*H*,5*H*)-Dioxopyrrolo[2,3-*d*]pyrimidine-2(1*H*)-thione (13). An ice-cooled solution of diethyl α-cyanosuccinate (50.0 g, 0.25 mmol) in absolute EtOH (25 mL) was saturated with dry HCl gas (14.0 g). The resulting mixture was allowed to stand at 0–5 °C for 24 h, during which time the imino ether formed as a thick syrup. The syrup was dissolved in dry EtOH (50 mL) and the resultant mixture added dropwise to a refluxing solution of NaOEt in EtOH (prepared by dissolving 29 g of Na metal in 400 mL of absolute EtOH), to which was added dry, finely ground thiourea (38.0 g, 0.50 mol) during 15 min. After the reaction mixture was heated to reflux for 2.5 h, it was cooled to room temperature and stored in the refrigerator overnight. The pale yellow precipitate was collected by filtration, dissolved in water (500 mL), and acidified to pH 2 with concentrated hydrochloric acid. After cooling (0–5 °C) for an h, the resulting precipitate was collected, washed with cold water (2 × 25 mL), and dried to yield 27.8 g (59%): mp >300 °C; UV λ_{max} (pH 1) 237 nm (ϵ 5000), 278 (13 800); UV λ_{max} (pH 7) 261 nm (ϵ 11 700), 312 (6600); UV λ_{max} (pH 11) 263 nm (ϵ 16 200), 312 (5900); NMR (Me_2SO-d_6) δ 3.32 (s, 2, C_5 H_2), 10.75 (br s, 1, N_1 H), 12.0 (s, 1, N_3 H). Anal. ($C_6H_5N_3O_2S$) C, H, N, S.

Pyrrolo[2,3-*d*]pyrimidine-4,6(3*H*,5*H*)-dione (14). Compound **13** (15.0 g, 81.9 mmol) was dissolved in water (150 mL) containing concentrated NH_4OH (150 mL) and NaOH (3.0 g). To the resulting deep red solution was added Raney nickel (W-4, 35 g, wet weight), and the mixture was heated under reflux for 2 h. The reaction mixture was filtered hot, and the catalyst washed with a hot NH_4OH/H_2O mixture (1:1, 750 mL). The combined filtrate and washings were evaporated to dryness. The suspension of the residue in water (100 mL) was adjusted to pH 2 with concentrated HCl. After cooling, the precipitated product was collected, washed with cold water, and crystallized from water to yield 10.1 g (82%) of **14**: mp >300 °C; UV λ_{max} (pH 1) 262 nm (ϵ 13 500); UV λ_{max} (pH 7) 262 nm (ϵ 11 600); UV λ_{max} (pH 11) 260 nm (ϵ 7300); mass spectrum m/e 151 (M^+, 100%); NMR (Me_2SO-d_6) δ 3.27 (s, 2, C_5 H_2), 7.95 (s, 1, C_2 H), 10.80 (s, N_7 N, H). Anal. ($C_6H_5N_3O_2\cdot 1^1/_4H_2O$) C, H, N.

4,6-Dichloropyrrolo[2,3-*d*]pyrimidine (15a). A mixture of compound **14** (4.0 g, 26.3 mmol), $POCl_3$ (100 mL), and freshly distilled *N*,*N*-dimethylaniline (12 mL) was heated under reflux for 3 h with the exclusion of moisture. The excess $POCl_3$ was removed (to about one-fourth the original volume), and the syrupy residue was slowly poured onto crushed ice (300 g) with stirring. The cold aqueous mixture was extracted with EtOAc (3 × 250 mL). The combined organic layers were washed with cold, 5% aqueous $NaHCO_3$ solution (2 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized from EtOAc to yield off-white needles, 2.3 g (47%): mp >234 °C dec; UV λ_{max} (pH 1 and 7) 226 nm (ϵ 15 400), 288 (4300); UV λ_{max} (pH 11) 235 nm (ϵ 11 700), 282 (4100); NMR (Me_2SO-d_6) δ 6.71 (s, 1, C_5 H), 8.68 (s, 1, C_2 H). Anal. ($C_6H_3Cl_2N_3$) C, H, Cl, N.

4,6-Dichloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (18a). A suspension of **15a** (1.5 g, 8.0 mmol) in dry CH_3CN (225 mL) was heated to boiling. When most of the solid had dissolved, NaH (60% in oil, 0.35 g, 8.8 mmol) was added and the mixture was stirred for 30 min before the chloro sugar **9** (3.1 g, 8.0 mmol) was added. The reaction mixture was allowed to stir at room temperature overnight with the exclusion of moisture and then filtered to remove inorganic salts. The filtrate was evaporated, adsorbed onto silica gel (15 g), and chromatographed on a flash silica gel column (4 × 25 cm) with petroleum ether (bp 30–60 °C)/EtOAc (7:1, v/v). Two nucleoside products were isolated. The major product eluted first and was obtained as a colorless syrup that crystallized upon prolonged storage in the refrigerator. Recrystallization from

MeOH yielded **18a**, 2.7 g (63%): mp 108–110 °C; UV λ_{max} (EtOH) 242 nm (ϵ 27 800), 275 (25 400); NMR (Me_2SO-d_6) δ 2.45 and 2.47 (2 s, 6, toluoyl methyls), 6.70 (t, 1, $C_{1'}$ H, peak width 15 Hz), 7.02 (s, 1, C_5 H), 8.67 (s, 1, C_2 H), and other aromatic and sugar protons. Anal. ($C_{27}H_{23}Cl_2N_3O_5$) C, H, Cl, N.

The minor product, which eluted subsequently, was obtained as pale yellow needles upon concentration of the appropriate fractions: yield 0.81 g (19%); mp 134–136 °C; UV λ_{max} (EtOH) 243 nm (ϵ 39 200), 301 (8100); NMR (Me_2SO-d_6) δ 2.35 and 2.40 (2 s, 6, toluoyl methyls), 6.68 (s, 1, C_5 H), 6.87 (t, 1, $C_{1'}$ H, peak width 13.5 Hz), 8.94 (s, 1, C_2H), and other aromatic and sugar protons. Anal. Calcd for ($C_{27}H_{23}Cl_2N_3O_5$): C, 60.01; H, 4.29; Cl, 13.12; N, 7.78. Found: C, 60.28; H, 4.30; Cl, 13.40; N, 7.64.

On the basis of the ultraviolet spectral data,[35,36] the minor product was tentatively assigned the structure 4,6-dichloro-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo-[2,3-*d*]pyrimidine.

4,6-Dichloro-2-methyl-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (18b). The title compound was prepared in the same manner as described for **18a** using 4,6-dichloro-2-methylpyrrolo[2,3-*d*]pyrimidine (**15b**; 0.61 g, 3.0 mmol), NaH (60% in oil, 0.17 g, 3.4 mmol), and **9** (1.2 g, 3.1 mmol). The crude product was purified on a silica gel column (4 × 30 cm) using toluene/EtOAc (95:5, v/v) as eluent. The product was crystallized from EtOH to yield 0.98 g (59%): mp 167 °C; NMR (Me_2SO-d_6) δ 6.70 (d + t, 2, C_5 H and $C_{1'}$ H), and other sugar protons. Anal. ($C_{28}H_{25}Cl_2N_3O_5$) C, H, N.

4-Amino-6-chloro-2-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (17). Compound **17** was prepared in the same manner as described for the preparation of **10b** (method 2) using **18b** (3.0 g, 5.4 mmol) and MeOH/NH_3 (60 mL). After silica gel column (5 × 40 cm) chromatography, using $CHCl_3$/MeOH gradient elution, the product was crystallized from 50% aqueous EtOH to yield 1.24 g (72%): mp 121–123 °C; UV λ_{max} (pH 1) 226 nm (ϵ 17 600), 273 (12 600); UV λ_{max} (pH 7 and 11) 275 nm (ϵ 13 400); NMR (Me_2SO-d_6) δ 2.36 (s, 3, CH_3), 6.40 (t, 1, $C_{1'}$ H), 6.66 (s, 1, C_5 H), 7.20 (s, 2, NH_2), and other sugar protons. Anal. ($C_{12}H_{15}ClN_4O_3 \cdot H_2O$) C, H, N.

4-Amino-2-methyl-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (16). To a solution of **17** (0.90 g, 2.85 mmol) in 90% aqueous EtOH (60 mL) containing K_2CO_3 (0.36 g) was added Pd/C (5%, 0.30 g), and the mixture was hydrogenated at 2 atm for 6 h. The reaction mixture was filtered through a Celite pad and the filtrate evaporated to dryness. Crystallization of the residue from 80% aqueous EtOH gave 0.68 g (90%) of **16**: mp 213–215 °C dec; UV λ_{max} (pH 1) 227 nm (ϵ 19 200), 272 (9500); UV λ_{max} (pH 7 and 11) 271 nm (ϵ 8900); NMR (Me_2SO-d_6) δ 2.36 (s, 3, CH_3), 6.40 (d + t, 2, C_5 H and $C_{1'}$ H), 6.98 (s, 2, NH_2), 7.26 (d, 1, J = 4.0 Hz, C_6 H), and other sugar protons. Anal. ($C_{12}H_{16}N_4O_3$) C, H, N.

4-Chloro-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (20b). A suspension of 4-chloro-7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrrolo-[2,3-*d*]pyrimidine[31] (**20a**; 2.4 g, 4.7 mmol) in MeOH/NH_3 (60 mL, saturated at 0 °C) was stirred at 0–5 °C for 48 h in a pressure bottle (after 24 h the solid had dissolved). The clear solution was evaporated, adsorbed onto silica gel (50 g), placed atop a silica gel column (3 × 40 cm), and eluted with $CHCl_3$/MeOH gradient to obtain 0.95 g (74%) of **20b** after crystallization from EtOAc: mp 162–163 °C; UV λ_{max} (pH 1, 7, and 11) 224 nm (ϵ 26 300), 273 (4600); NMR (Me_2SO-d_6) δ 6.70 (d + t, 2, C_5 H and $C_{1'}$ H), 8.06 (d, 1, J = 2.0 Hz, C_6 H), 8.72 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{11}H_{12}ClN_3O_3$) C, H, Cl, N.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (19). Compound **19** was prepared in the same manner as described for the preparation of **16** using **20b** (0.22 g, 0.8 mmol), Pd/C (5%, 0.15 g), and K_2CO_3 (0.10 g) in 90% aqueous EtOH (25 mL). After hydrogenation, the residue was dissolved in water (50 mL) and applied to a Dowex 1-X8 (OH^-) column (1 × 10 cm). Elution with water (200 mL) and then H_2O/MeOH (3:1, v/v, 300 mL) gave 94 mg (50%) of the title compound after crystallization from EtOAc: mp 131 °C; UV λ_{max} (pH 1) 227 nm (ϵ 31 200), 266 (3000); UV λ_{max} (pH 7 and 11) 222 nm (ϵ 26 700), 270 (4000); NMR (Me_2SO-d_6) δ 6.76 (d + t, 2, C_5 H and $C_{1'}$ H), 7.88 (d, 1, J = 4.0 Hz, C_6 H), 8.84 and 9.06 (2 s, 2, C_2 H and C_4 H), and other sugar protons. Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-4(3*H*)-thione (21). A solution of **20b** (0.35 g, 1.3 mmol) and thiourea (0.19 g, 2.5 mmol) in 1-propanol (15 mL) was heated under reflux for 1 h. The reaction mixture was evaporated to dryness, and the residue was crystallized twice from MeOH to give 0.19 g (53%) of **21**: mp 222–223 °C; UV λ_{max} (pH 1 and 7) 278 nm (ϵ 7300), 323 (29 100); UV λ_{max} (pH 12) 310 nm (ϵ 23 800); NMR (Me_2SO-d_6) δ 6.56 (t, 1, $C_{1'}$ H), 6.74 (d, 1, J = 4.0 Hz, C_5 H), 7.64 (d, 1, J = 4.0 Hz, C_6 H), 8.16 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{11}H_{13}N_3O_3S$) C, H, N.

Antiviral Activity Evaluation. Test compounds were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by measles, herpes simplex virus type 2 (HSV-2, 333), vaccinia virus (VV), and parainfluenza virus type 3 (Para-3) in African green monkey kidney (Vero) cells (American Type Culture Collection, Rockville, MD). Vero cells were maintained in antibiotic free Eagle minimum essential medium (EMEM) with Earle's salts supplemented with 10% heat-inactivated newborn bovine serum (Grand Island Biological Co., Grand Island, NY). For antiviral experiments, cells were inoculated into 96 well tissue culture plates (Corning Glassworks, Corning, NY) at a concentration of 4×10^4 cells/0.2 mL per well and cultured for 24 h at 37 °C in 5% CO_2 to confluency.

Monolayers were inoculated with a predetermined number of TC ID_{50} (50% tissue culture infective dose) units of virus that will produce complete destruction of the cell monolayer in 72 h. The number of TC ID_{50} units in 0.1 mL/well were as follows: measles, 80; HSV-2, 100; VV, 200; para-3, 60. After 30-min adsorption at 37 °C, test compounds were added (0.1 mL/well) in seven 0.5 log concentrations ranging from 1×10^{-5} to 1×10^{-2} M, resulting in final well concentrations of 5×10^{-6} to 5×10^{-3} M. At each concentration, duplicate wells were used for evaluation of antiviral activity and single uninfected wells for cytotoxicity evaluation.

The degree of inhibition of viral-induced CPE and compound cytotoxicity were observed microscopically after 72-h incubation at 37 °C in 5% CO_2. CPE was scored numerically from 0 (normal control cells) to 4 (100% cell destruction as in virus controls), and the dose of test compound that inhibits viral CPE by 50% (ED_{50}) was calculated.

Cytostatic Activity Evaluation. Compounds were evaluated for their ability to inhibit growth of murine leukemia L1210 and lymphoid neoplasm P388 (American Type Culture Collection, Rockville, MD) maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 20 mM HEPES buffer. For growth experiments, cells were adjusted to 1×10^5 cells/mL and distributed into 24 well tissue culture plates (0.5 mL/well). Test compounds were dissolved in growth medium, sterilized by passage through a 0.22-μm membrane filter, and added to cells (0.5 mL/well). Compounds were tested in duplicate at log concentrations ranging from 1×10^8 to 1×10^{-4} M. Following 48-h incubation at 37 °C, cell counts were determined on a Coulter Model ZF cell counter. Cell growth in the presence of test compounds was expressed as a percentage of growth in untreated control wells, and the concentration of compound producing 50% inhibition of cell growth was determined (ID_{50}).

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